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**CERTIFICAN:**

Que el trabajo descrito en la presente memoria, titulado  
“ANALYSIS OF GLASS AND INKS IN FORENSIC CASEWORKS” ha  
sido realizado bajo su dirección por D. Francisco Alamilla  
Orellana en el Área de Química Analítica del Departamento de  
Química Analítica, Química Física e Ingeniería Química de esta  
Universidad. Asimismo, autorizan su presentación para que sea  
defendido como Tesis Doctoral.

Y para que conste y surta los efectos oportunos, firman  
el presente en Alcalá de Henares a 13 de Mayo de 2014.

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**CERTIFICA:**

Que el trabajo descrito en la presente memoria de tesis, titulado “ANALYSIS OF GLASS AND INKS IN FORENSIC CASEWORKS” ha sido realizado en este Instituto Universitario por D. Francisco Alamilla Orellana bajo la dirección de las Dras. Mercedes Torre Roldán y Carmen García Ruiz, Profesoras Titulares de la Universidad de Alcalá.

Dicha tesis reúne los requisitos necesarios para su presentación y defensa.

Y para que conste y surta los efectos oportunos, firma el presente en Alcalá de Henares a 13 de Mayo de 2014.

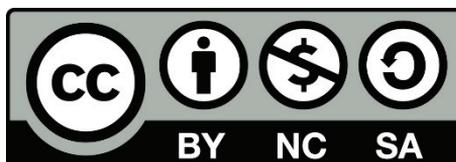
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# **analysis of glass and inks in forensic Caseworks**

**francisco alamilla Orellana | 2014**









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Profesionalmente estoy satisfecho con los objetivos que he alcanzado, sin embargo, desde mi Licenciatura en Química por la Universidad de Málaga en 1991, siempre he tenido en mente el reconocimiento a la investigación que supone la Tesis Doctoral. El motor que me impulsa a realizarla, es un sentimiento vocacional por la Ciencia que he tenido desde siempre.

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## ACRÓNIMOS Y ABREVIATURAS CIENTÍFICAS

### AAS

Spectroscopia de Absorción Atómica  
*Absorption Atomic Spectroscopy*

### ADN

Ácido Desoxirribonucleico  
*Deoxyribonucleic Acid*

### AICEF

Academia Iberoamericana de Criminalística y Estudios Forenses  
*Latin American Academy of Criminalistics and Forensic Studies*

### ANOVA

Análisis de Varianza  
*Analysis of Variance*

### ARN

Ácido Ribonucleico  
*Ribonucleic Acid*

### ASTM

Sociedad Americana para Ensayos y Materiales  
*American Society for Testing and Materials*

### BC

Capa Base, subcapa de la Capa Exterior de pintura de vehículos  
*Base Coat is a top coat sublayer from car paint*

### BKA

Servicio Central de Criminalística Alemán  
*Federal Criminal Police Office*

### BLR

Regresión Logística Binomial  
*Binomial Logistic Regression*

### CA

Análisis de Cluster  
*Cluster Analysis*

### CC

Capa Transparente o Barniz de Superficie, subcapa de la capa exterior de pintura de vehículos  
*Clear Coat is a top coat sublayer from car paint*

CCD

Dispositivo de Carga Acoplada  
*Charge-Coupled Device*

CS

Muestra Control o indubitada  
*Control Sample*

DART

Análisis Directo en Tiempo Real  
*Direct Analysis in Real Time*

DIC

Contraste Diferencial de Interferencia  
*Differential Interference Contrast*

DP

Potencia de Discriminación  
*Discrimination Power*

EDS

Espectroscopia de Energía Dispersiva  
*Energy-Dispersive Spectroscopy*

EDXRF

Fluorescencia de Energía Dispersiva de Rayos X  
*Energy-Dispersive X-Ray Fluorescence*

ENAC

Entidad Nacional de Acreditación  
*National Entity of Accreditation*

ENFSI

Red Europea de Institutos en Ciencias Forenses  
*European Network of Forensic Science Institutes*

EPMA

Microsonda Electrónica de Barrido  
*Electron Probe Micro Analyzer*

FGS 1-2

Estándar de Vidrio Forense 1 y 2 (BKA)  
*Forensic Glass Standard 1 and 2 (BKA)*





FI

Índice de Fraccionamiento

*Fractionation Index*

FP

Capa de Primera Imprimación en vehículos

*First Primer is a car paint layer*

HPLC

Cromatografía Líquida de Alta Eficacia

*High Performance Liquid Chromatography*

HSD

Test de Hotelling o de Diferencias Honestamente Significativas

*Hotelling test or Honestly Significant Difference*

ICP-MS

Espectrometría de Plasma de Acoplamiento Inductivo y Detección por Espectrometría de Masas

*Inductively Coupled Plasma Mass Spectrometry*

IPC-OES

Espectrometría de Plasma de Acoplamiento Inductivo y Detección por Espectrometría Óptica

*Inductively Coupled Plasma Optical Emission Spectroscopy*

IR

Espectroscopia de Infrarrojos

*Infrared Spectroscopy*

IRMS

Espectrometría de Masas de Relaciones Isotópicas

*Isotope Ratio Mass Spectrometry*

IS

Estándar Interno

*Internal Standard*

IUICP

Instituto Universitario de Investigación en Ciencias Policiales

*University Institute of Research in Forensic Sciences*

LA-ICP-MS

Ablación Láser con ICP-MS

*Laser Ablation ICP-MS*

LA-ICP-SF-MS

Ablación Láser acoplado a un ICP-MS de Sector Magnético  
*LA coupled to a magnetic Sector Field ICP-MS*

LA-ICP-TOF-MS

Ablación Láser acoplado a un ICP-MS de Tiempo de Vuelo  
*LA coupled to a Time Of Flight ICP- MS*

LA-MS

Ablación Láser con Espectrometría de Masas  
*Laser Ablation Mass Spectrometry*

LASER

Amplificación de luz por emisión estimulada de radiación  
*Light Amplification by Stimulated Emission of Radiation*

LDA

Análisis Discriminante Lineal  
*Linear Discriminant Analysis*

LDI-TOF-MS

Desorción por ionización con Láser y detección por TOF-MS  
*Laser Desorption Ionization TOF-MS*

LIBS

Espectroscopia de Plasma Inducido por Láser  
*Laser Induced Breakdown Spectroscopy*

LM-OES

Micro Laser con Espectrometría de Emisión Óptica  
*Laser Micro-Optical Emission Spectrometry*

LOD

Límite de Detección  
*Limit of Detection*

MANOVA

Análisis Multivariante de Varianza  
*Multivariate Analysis of Variance*

MASER

Amplificación de Microondas por Emisión Estimulada de Radiación  
*Microwave Amplification by Stimulated Emission of Radiation*





## NAA

Análisis de Activación Neutrónica

*Neutron Activation Analysis*

## Nd:YAG

Neodimio dopado con granate de óxido de aluminio e Ytrio

*Neodymium-doped Yttrium Aluminum Garnet; Nd:Y3Al5O12*

## NIST

Instituto Nacional de Estándares y Tecnología

*National Institute of Standards and Technology*

## NITE-CRIME

Isótopos Naturales y Elementos Traza en Criminalística y Forense-Medioambiente

*Natural Isotopes and Trace Elements in Criminalistics and Environmental Forensic*

## PC

Componente Principal

*Principal Component*

## PCA

Análisis de Componentes Principales

*Principal Component Analysis*

## PIXE

Espectroscopia de Rayos X Inducida por Partículas

*Particle-Induced X-ray Emission spectroscopy*

## PS

Capa de Imprimación de Superficie en Vehículos

*Primer Surface is a car paint layer*

## PyGC

Cromatografía de Gases con Pirólisis

*Pyrolysis-Gas Chromatography*

## RI

Índice de Refracción

*Refractive Index*

## RS

Muestra recuperada o dubitada

*Recovery Sample*

RSD

Coefficiente de Variación  
*Relative Standard Deviation*

SECRIM

Servicio de Criminalística  
*Criminalistics Service*

SEM-EDX

Microscopio Electrónico de Barrido con detector de Energía Dispersiva de Rayos X  
*Scanning Electron Microscope Energy Dispersive X-ray Spectroscopy*

SEM-WDS

Microscopio Electrónico de Barrido con Espectroscopia Dispersiva de Longitud de Onda  
*Scanning Electron Microscopy Wavelength Dispersive Spectroscopy*

SRM

Material de Referencia Certificado  
*Standard Reference Material*

TLC

Cromatografía de Capa Fina  
*Thin-Layer Chromatography*

TOF-SIMS

Sonda de Iones Secundarios y Espectrometría de Masas por Tiempo de Vuelo  
*Time Of Flight Secondary Ion Mass Spectrometry*

WDS

Espectroscopia Dispersiva de Longitud de Onda  
*Wavelength-Dispersive Spectroscopy*

XRF

Fluorescencia de Rayos X  
*X-Ray Fluorescence*









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# Resumen





Esta Tesis se estructura en tres partes, la primera es una revisión bibliográfica relacionada con aplicaciones exclusivamente forenses mediante la técnica combinada LA-ICP-MS, seguidamente se desarrollan metodologías de cotejo forense de vidrios, basadas en la medida del índice de refracción y en el análisis elemental. La última parte se dedica al análisis de cotejo de tintas con fines criminalísticos mediante LA-ICP-MS. En esta Tesis ha sido necesario emplear tratamientos estadísticos de los datos obtenidos, adaptándose a las características específicas del campo forense, como son la toma de muestras condicionadas a los vestigios hallados o el limitado número de réplicas que se pueden realizar debido al reducido tamaño de la muestra disponible. También se pone especial interés en la interpretación de resultados y en la forma de expresarlos en los informes periciales, ayudándonos de tablas y tratamientos gráficos, con el fin de hacerlos fácilmente comprensibles para los juristas a los que finalmente va dirigido.

El **CAPÍTULO I** se dedica a revisar las aplicaciones LA-ICP-MS publicadas en los últimos doce años, con objeto de adquirir una visión de las aplicaciones desarrolladas en el campo de la criminalística, conocer el estado actual de las mismas y obtener una guía práctica para estudiar casos forenses. Partiendo de este punto, se desarrollan nuevos trabajos de investigación en los capítulos II y III con el fin de aportar información útil a las demandas judiciales que habitualmente solicitan los Tribunales de Justicia.

En la revisión bibliográfica recogida en el Capítulo I, se pone de manifiesto que el mayor número de trabajos de investigación publicados está dedicado al análisis de vidrios por ser un material

omnipresente en nuestra sociedad y que, por tanto, aparece durante la investigación de muchos delitos. La aplicación forense más destacada es el cotejo, que consiste en relacionar restos de vidrios procedentes del lugar de los hechos con vidrios de vehículos, ventanas o fragmentos atrapados en prendas de vestir de sospechosos. Entre las causas más comunes, se encuentran los atropellos con resultado de homicidio, robos con fuerza en viviendas o locales comerciales, entre otros.

Debido a la escasa disponibilidad de materiales de referencia certificados adaptados a las necesidades de este campo, a nivel de la comunidad forense internacional, el Servicio Central de Criminalística Alemán (BKA) ha promovido la creación de dos materiales de referencia de vidrios flotados FGS1 y FGS2 con objeto de utilizarse como patrones de calibración y de control de calidad en el análisis forense de vidrios flotados. También ha propuesto un protocolo de trabajo adoptado finalmente por el grupo de pintura y vidrio de ENFSI en el que destaca como medida de normalización de los análisis, la recomendación del empleo de silicio como estándar interno, medido por los espectrómetros en su masa 29, así como estandarizar como concentración de calibración predeterminada el 72% m/m  $\text{SiO}_2$  en el análisis de todas las muestras de vidrio flotado con fines forenses. El desarrollo de aplicaciones con LA-ICP-MS ha dado lugar a varias publicaciones, entre las que destaca la propuesta de la BKA de un protocolo normalizado de trabajo, conjuntamente con un protocolo estadístico de discriminación-asociación de muestras de vidrios de interés criminalístico. Este último es basado en unos valores mínimos de RSD, combinados con una comparación por pares para dieciocho elementos analizados, en función de un intervalo de cuatro veces la desviación estándar. Este procedimiento ha obtenido los mejores resultados para los errores tipo I y II descritos hasta el momento. El





reconocimiento de la comunidad científica del alto grado de optimización alcanzado por la técnica de análisis LA-ICP-MS en vidrios, lo hace recomendable para que los laboratorios forenses siguiendo este protocolo descrito, den el paso de la validación interna, con vistas a la acreditación según norma UNE 17025.

Aunque el número de publicaciones ha sido menor, también se han desarrollado otras aplicaciones forenses de la técnica LA-ICP-MS, relacionados con otros materiales de uso común que también están presentes en la comisión de delitos como pintura, tintas y papel, fibras, cannabis, gemas, materiales cerámicos y metales preciosos. Estas aplicaciones aportan nuevas posibilidades para la aplicación de LA-ICP-MS a las investigaciones policiales.

LA-ICP-MS es una técnica prometedora para la caracterización de muestras de interés forense con un alto nivel de individualización por lo que su uso es ideal en el cotejo forense.

El **CAPÍTULO II** se dedica al análisis forense de vidrios mediante la medida del índice de refracción en vidrios y al análisis elemental mediante LA-ICP-MS.

La medida del índice de refracción mediante la variación del contraste de fase con la temperatura del vidrio inmerso en un aceite especial de silicona, es un método clásico de la criminalística ya que ofrece una buena capacidad de cotejo a un coste asequible. Además la relativa sencillez de su aplicación junto con su bajo coste de mantenimiento, lo han hecho un método muy extendido en los laboratorios forenses de todo el mundo. Hoy en día se usa como complemento no competitivo con otras técnicas como ICP-MS, ICP-OES, XRF, LIBS, SEM-EDX, PIXE, EPMA y LA-ICP-MS, aunque la

ausencia de la disponibilidad de estas otras técnicas en muchos laboratorios forenses hace que siga teniendo un papel relevante.

Dado que la validación con fines para la acreditación de la medida del índice de refracción no estaba descrita en la bibliografía y tampoco se había implantado en el SECRIM, al comienzo de esta Tesis se ha desarrollado una propuesta de validación para implantarla como análisis de rutina acreditada por ENAC.

El establecimiento de protocolos normalizados de trabajo debe incluir el uso de materiales de referencia certificados, al objeto de aportar la trazabilidad necesaria a las determinaciones analíticas y establecer los controles de calidad adecuados para garantizar que se mantienen las especificaciones de la validación, registrando parámetros como precisión y exactitud en el tiempo, para localizar tendencias y corregirlas antes de que la ejecución de la instrucción técnica quede fuera de especificaciones. Además, las medidas de índice de refracción han de ir acompañadas de su incertidumbre.

La interpretación con fines forenses de muestras de vidrio mediante la medida del índice de refracción se ha basado en la comparación por pares, mediante contraste de hipótesis de los resultados de los índice de refracción obtenidos. Inicialmente se seleccionó un conjunto de muestras de origen conocido, constituido por los tipos de vidrios que habitualmente se hallan durante las inspecciones oculares en las investigaciones Judiciales. Además de los anteriores, se analizaron materiales de referencia certificados, tanto para la calibración como para los controles de calidad y gráficos de control. Durante el estudio, se evaluó la precisión, exactitud, robustez y las rectas de calibración obtenidas. Después se realizó un estudio de errores





tipo I y II basándose en varios criterios de comparación. Finalmente se contrastaron los resultados con LA-ICP-MS en un caso real de atropello de un peatón con vehículo a motor.

En la segunda parte de este capítulo, se ha establecido una metodología de análisis de vidrios mediante LA-ICP-MS basada en la bibliografía existente. Esta metodología se ha validado intra-laboratorio con muestras procedentes en su mayoría de casos forenses, muestras de test interlaboratorios, muestras ciegas y un material de referencia certificado (NIST) de un vidrio flotado.

Se ha desarrollado una sistemática de análisis en la que se incluyen controles de calidad en cada serie de muestras. Controles previos que garantizan el adecuado estado de la instrumentación y controles durante el análisis que garantizan que la precisión y la capacidad de cotejo forense de muestras sean adecuadas para el cotejo de muestras de vidrios forenses.

Durante la validación se han estimado los parámetros de precisión y límites de detección del método, así como su capacidad para el análisis de vidrios de uso común. Además se ha desarrollado una hoja de cálculo para normalizar el tratamiento de resultados con el fin de comparar muestras forenses y obtener las conclusiones para el informe pericial, así como una presentación gráfica de los datos que facilite una interpretación visual intuitiva.

El método validado en este capítulo se ha diseñado para uso exclusivamente forense, por lo que busca una caracterización de las muestras para compararlas con un origen común y no de obtener resultados cuantitativos trazables, como se exige normalmente en un análisis cuantitativo, sino en obtener resultados comparables entre sí,

con una fiabilidad alta que lleve al mínimo de errores para que una persona inocente no sea culpada injustamente.

Además de lo anterior, se le da gran importancia a la expresión de resultados para que las conclusiones finales sean comprensibles para los juristas como clientes finales de los resultados forenses.

El **CAPÍTULO III** se dedica al análisis de tintas manuscritas mediante LA-ICP-MS con el fin de investigar la capacidad de individualización de esta técnica en el cotejo de tintas de documentos manuscritos.

En primer lugar se seleccionaron una serie de tintas azules, procedentes de bolígrafos conocidos. El conjunto de muestras permitió hacer tres tipos de grupos con características diferentes a nivel de fabricación. Un primer grupo con bolígrafos procedentes de diferentes marcas comerciales, un segundo grupo con bolígrafos de la misma marca comercial pero distintos lotes de fabricación y un último grupo con bolígrafos diferentes con tinta procedente de la misma marca y lote de fabricación.

La estrategia de análisis se ha basado en dos etapas jerarquizadas. Primero la identificación cualitativa de los elementos característicos de cada muestra de tinta (elementos target). Para ello, se caracterizó el perfil elemental del papel donde se había realizado la escritura manuscrita, evaluando el uso de tres elementos como estándar interno; Ca, Mg y Sr. De esta forma, se establecieron unos niveles de fondo a partir de los cuales, se puede identificar la presencia cualitativa de elementos pertenecientes a la tinta al analizar los trazos de tinta manuscrita sobre papel. Después de esta primera clasificación cualitativa, los grupos de bolígrafos que no se habían individualizado





se sometieron a un tratamiento estadístico multivariante, empleando como variables las relaciones entre elementos target, característicos de las tintas manuscritas, con el propósito de aumentar la capacidad discriminadora de la primera etapa cualitativa. Como resultado general, observamos que las casas comerciales de origen de los bolígrafos fueron fácilmente diferenciadas entre sí y en algunos casos, se encontraron además, claras diferencias entre lotes de la misma marca e incluso entre bolígrafos diferentes pertenecientes al mismo lote de fabricación.

Finalmente, este procedimiento se aplicó a un caso forense esclarecido por el Departamento de Grafística, comprobando que los resultados obtenidos fueron coherentes con las conclusiones obtenidas por los expertos grafísticos.

Las interpretaciones de los resultados obtenidos se han adoptado mediante tratamientos estadísticos, debido a que son objetivos y rigurosos. Sin embargo, presentan el inconveniente de que no son fácilmente comprensibles para los juristas a los que se dirige el informe pericial. Por este motivo, se propone expresar los resultados con apoyo de herramientas gráficas en las que se deduce fácilmente y de forma visual las conclusiones finales.



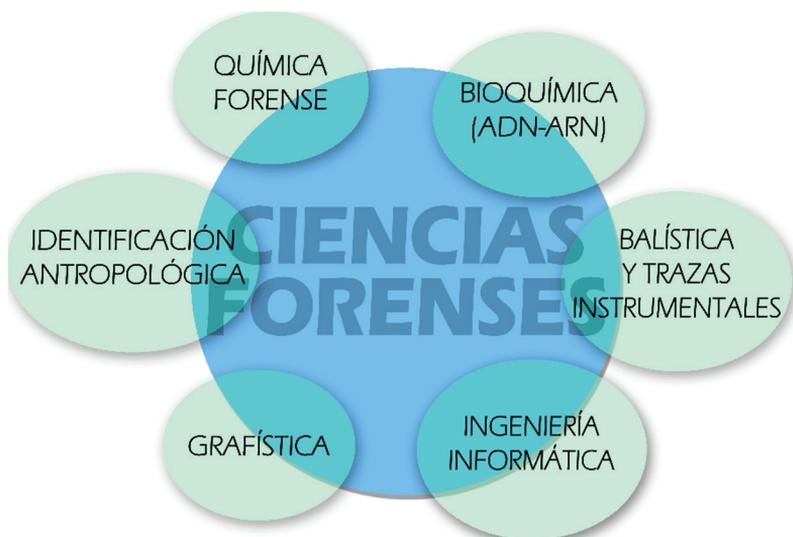
# Introducción





## LA QUÍMICA FORENSE EN EL CONTEXTO DE LAS CIENCIAS POLICIALES

La química forense es un pilar de las Ciencias Forenses que proporciona herramientas de ayuda en la investigación policial de delitos, principalmente en la confirmación o descarte de hipótesis reconstructivas de hechos (Fig. 1).



*Fig 1. Principales pilares de las Ciencias Forenses.*

Otras disciplinas forenses han sido también especialmente útiles, como el clásico revelado de huellas dactilares o la más reciente técnica de identificación de personas mediante el análisis del ADN o ARN de sus restos biológicos. Estos análisis han tenido una gran divulgación policial y social y se solicitan de rutina por los juzgados a través de los equipos de Policía Judicial en todo el mundo. Estos y otros ensayos similares no tan populares, se han convertido para las Ciencias Forenses en “casos tipificados”. Tanto es así, que la estructura orgánica de los

departamentos de química forense de muchos países se ha organizado en equipos de trabajo en función de estos “casos tipo”. Ejemplo de ello es la creación de grupos de trabajo dentro de instituciones internacionales de reconocido prestigio, como ENFSI (European Network of Forensic Science Institutes) [1] y AICEF (Academia Iberoamericana de Criminalística y Estudios Forenses) [2], los cuales se estructuran orgánicamente en torno a solicitudes de informes periciales en función de la matriz analizada o de los hechos delictivos. En la **TABLA 1**, se indican los grupos de trabajo creados en AICEF y ENFSI. Podemos ver que en 16 de los 22 grupos presentes, existe una participación total o parcial de la química forense (indicados con un asterisco), empleándose para ello la determinación cualitativa y cuantitativa de uno o varios analitos, tanto orgánicos como inorgánicos.

Tabla 1		
Países participantes y grupos de trabajo en ENFSI [1] y AICEF [2].		
Organismo	Países participantes	Grupos de trabajo
 <p><b>AICEF</b></p>	Argentina, Bolivia,	Balística Forense
	Brasil, Chile, Colombia,	Escena del Crimen *
	Costa Rica, Ecuador, El	Drogas de abuso *
	Salvador, España,	DNA Humano *
	Guatemala, Honduras,	Toxicología Forense *
	México, Nicaragua,	
	Panamá, Perú, Portugal,	
	Uruguay y Venezuela	





**Tabla 1 (continuación)**

Países participantes y grupos de trabajo en ENFSI [1] y AICEF [2].

<b>Organismo</b>	<b>Países participantes</b>	<b>Grupos de trabajo</b>
 <b>ENFSI</b>	Austria, Armenia,	Animales, plantas y trazas en suelos *
	Azerbaiyán, Bélgica,	Imagen digital
	Bosnia y Herzegovina,	DNA humano *
	Bulgaria, Croacia,	Documentos *
	Chipre, República	Drogas *
	Checa, Dinamarca,	Explosivos *
	Eslovenia, Eslovaquia,	Huellas dactilares *
	España, Estonia,	Armas y residuos de disparo *
	Finlandia, Francia,	Investigaciones de incendios y explosiones *
	Georgia, Alemania,	Tecnología de la información forense
	Hungría, Irlanda, Italia,	Análisis forense del habla y audio
	Letonia, Lituania,	Escritura manuscrita *
	Montenegro, Holanda,	Marcas
	Noruega, Polonia,	Pinturas y vidrios *
	Portugal, Reino unido,	Accidentes de tráfico
	Rumanía, Rusia, Serbia,	Escena del crimen *
	Suecia, Suiza, Turquía y	Fibras y pelos *
	Ucrania	

(\*) Grupos en los que de forma total o parcial está presente la química forense.

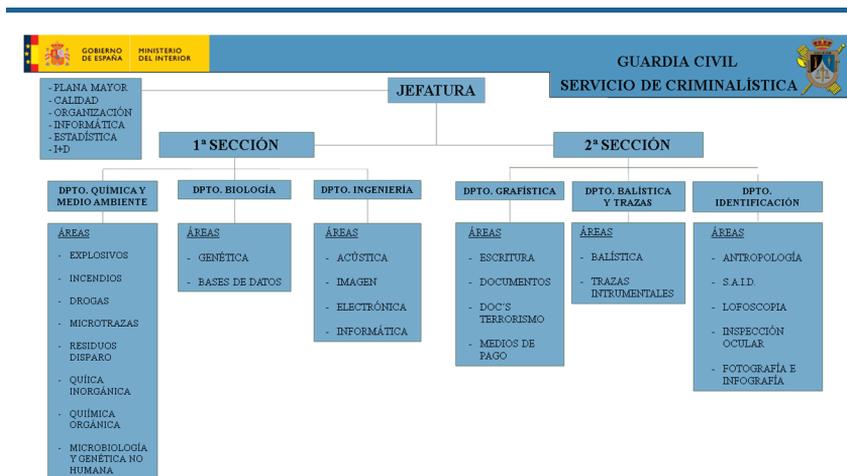
Es por tanto indudable la valiosa aportación de la química forense a las Ciencias Forenses. Además, esta disciplina sigue creciendo de forma continua con el desarrollo tecnológico y con ayuda de trabajos

de investigación, realizados por grupos mixtos de colaboración entre Universidades e Instituciones Policiales.

En esta línea, en España se establece el denominado IUICP (Instituto Universitario de Investigación en Ciencias Policiales) [3], que oferta el doctorado en Criminalística donde se realiza esta Tesis Doctoral. El citado Instituto emerge de un convenio de colaboración entre la Secretaría de Estado de Seguridad del Ministerio del Interior y la Universidad de Alcalá, celebrado el 28 de mayo de 2007. El artículo 96 de los Estatutos de la Universidad de Alcalá, aprobados por Decreto 221/2003, de 23 de octubre, define a los institutos mixtos como aquellos que han sido creados por convenio con otras instituciones. El IUICP se configura como un centro de investigación en materia policial que dirige sus líneas de acción a la realización de proyectos de investigación en las áreas relacionadas con la investigación en Ciencias Forenses, abarcando ámbitos como la química forense, la genética forense, la acústica e imagen forenses y el derecho procesal y penal, entre otras áreas.

El Servicio de Criminalística (SECRIM) de la Guardia Civil [4] es miembro del IUICP, está estructurado en secciones y éstas a su vez en departamentos. En la **FIG. 2** puede observarse dicha estructura. Estos últimos son unidades especializadas cuyo fin último es emitir informes periciales en diversas materias de interés policial y judicial.





*Fig 2. Estructura de la organización por Departamentos del SECRIM (Guardia Civil).*

El Departamento de Química y Medio Ambiente del SECRIM está organizado, a su vez, en áreas similares a algunos de los grupos de trabajo descritos anteriormente. La realización de informes de rutina, se desarrolla mediante procedimientos normalizados, ensamblados en el sistema de calidad, cuya competencia técnica está reconocida por la Entidad Nacional de Acreditación ENAC [5]. El alcance analítico del laboratorio es dinámico y todos los años sufre ampliaciones y cambios promovidos por la evolución de la demanda forense.

Entre la instrumentación analítica disponible actualmente, el Departamento de Química y Medio Ambiente cuenta con cromatografía iónica, líquida y de gases, electroforesis capilar, espectroscopia de emisión y absorción atómica, espectrometría de masas, difracción de rayos X, microscopía electrónica de barrido y espectroscopia de infrarrojos, visible y ultravioleta.

Por otra parte, el desarrollo tecnológico y la investigación en el campo de las ciencias forenses está en continuo crecimiento a nivel internacional, hecho que se pone de manifiesto en revistas especializadas como *Forensic Science International* [6], *Science and Justice* [7] o *Journal of Forensic Sciences* [8]. Actualmente, el SECRIM en general y el Departamento de Química y Medio Ambiente en particular, dedican parte de su trabajo a la formación de personal, al desarrollo y aplicación de nuevos métodos de ensayo y al desarrollo de nuevas herramientas estadísticas de interpretación de resultados.

Cuanto mayor es la especialización de los peritos, mejor es el aprovechamiento de los recursos y la calidad de los informes periciales. Sin embargo, para realizar un buen trabajo en conjunto, la labor de los especialistas debe estar ensamblada de forma global con la capacidad analítica del resto de las áreas. La implantación de un sistema de calidad y acreditación del alcance del laboratorio mediante norma UNE-EN ISO/IEC 17025 “Requisitos generales para la competencia de los laboratorios de ensayo y de calibración” [9], normaliza la forma de trabajar de todos y garantiza el reconocimiento de la competencia técnica del laboratorio a nivel internacional.

## EL COTEJO FORENSE

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El cotejo forense se caracteriza por la comparación de muestras dentro de dos entornos diferentes, la comparación de una muestra con una base de datos para buscar los candidatos que más se adapten al perfil obtenido, y la comparación de una muestra con otras de origen conocido, que la relaciona directamente con uno o varios sospechosos. La **FIG. 3**, presenta un ejemplo de muestras recibidas para cotejo.





**Muestra Recuperada (RS)**

Restos de vidrio hallados en una maza, supuestamente empleada para acceder a una entidad bancaria a través de la mampara de vidrio exterior.

**Muestra Control (CS)**

Mampara de vidrio exterior de la entidad bancaria.

De izquierda a derecha, en la parte superior de la imagen: Fragmento de la mampara, compuesto por dos láminas de vidrio muy fracturadas que aún conservan la lámina de plástico que las une y múltiples fragmentos de vidrio.

En la parte inferior de la imagen: Fragmento de cada lámina de vidrio del panel violentado.



*Fig 3. Ejemplo de muestras recibidas en el SECRIM para cotejo de vidrios.*

En el caso del ADN humano, las bases de datos son fácilmente accesibles y disponen de una cantidad de información muy amplia. Sin embargo, en química forense, la capacidad de generar bases de datos de tintas y vidrios es muy limitada por razones muy variadas.

- Por problemas de acceso, debido a la dificultad de obtener la fórmula original de los componentes, normalmente bajo patente, sobre todo en el caso de las tintas.
- Por dificultades en el control de la fabricación, debido a la continua evolución de materias primas y productos.

- Porque requiere disponer de un flujo continuo de muestras que aseguren el control de la ingente producción de vidrios y tintas y su distribución.
- Por la dificultad de coordinar los análisis necesarios para caracterizar dichas muestras.
- Por la problemática de mantener las bases de datos actualizadas.

Adicionalmente al esfuerzo que supone la elaboración y el mantenimiento de bases de datos de vidrios y pinturas, hay que tener en cuenta la utilidad forense que se puede obtener de ellas. En el caso de los vidrios de vehículos, no es posible relacionar un fragmento hallado en una Inspección Ocular con un modelo de vehículo, debido a que los vidrios originales que se montan en los vehículos no son trazables a los modelos de vehículos. Además, pueden sufrir cambios no controlados de las piezas originales durante su vida útil. Para el resto de los vidrios (arquitectónicos, ornamentales, envases, etc), la variabilidad dependerá de cada caso en concreto. Para las tintas, el principal problema es conseguir muestras con trazabilidad y composición conocida. A pesar de todos estos problemas, es de interés disponer de bases de datos, porque con ellas se puede estimar la variabilidad esperada en los casos forenses y por tanto, ver el grado de individualización de las técnicas de análisis frente a la población forense de muestras.

En esta Tesis se han abordado los estudios que implican la comparación por pares de muestras para relacionarlas con un origen común, práctica habitual en los laboratorios forenses de todo el mundo. Los estudios se han llevado a cabo tomando conjuntos de muestras representativos de vidrios y tintas comerciales, para demostrar que los

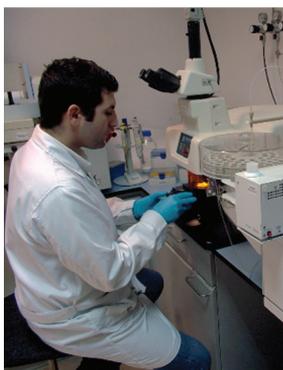
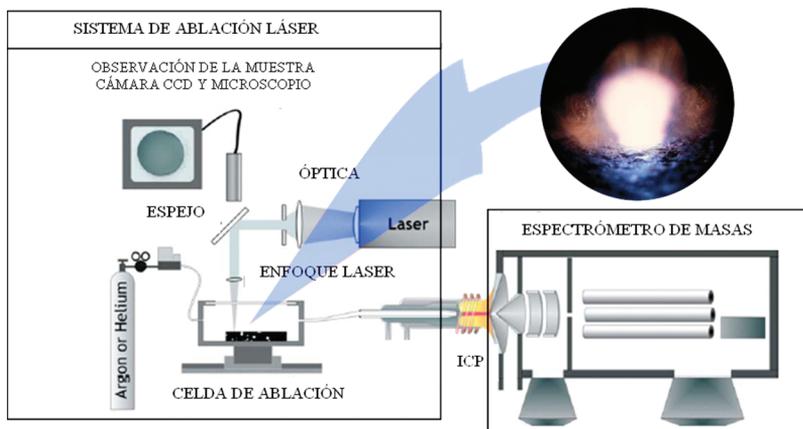




métodos son aptos para analizar muestras forenses habituales, pero sin la pretensión de generar una base de datos completa de vidrios y tintas actuales.

## LA-ICP-MS Y LA MEDIDA DEL ÍNDICE DE REFRACCIÓN EN VIDRIOS COMO HERRAMIENTAS PARA EL COTEJO FORENSE

LA-ICP-MS es una técnica de desarrollo reciente, ya que el primer ICP-MS comercial se desarrolló en el año 1983 y el primer acoplamiento con un LA fue descrito por Alan Gray en 1985. La **FIG. 4**, muestra la configuración del equipo de LA-ICP-MS, disponible en el SECRIM y utilizado en esta Tesis. La optimización de las características operacionales se está desarrollando actualmente con cambios significativos. Muestra de ello son los estudios de optimización del gas portador, en los que destaca el uso de He en lugar del Ar empleado inicialmente y el uso de láseres de menor longitud de onda, como los láseres de gases excímeros que pueden emitir por debajo de los 200 nm y el uso de pulsos cada vez menores como los láseres de femtosegundos, actualmente menos asequibles por su elevado precio respecto a los láseres de nanosegundos, que permiten minimizar los efectos térmicos en la extracción de la muestra, disminuyendo el efecto de fraccionamiento.



*Fig 4. Configuración de un sistema LA-ICP-MS. Esquema adaptado de [10].*

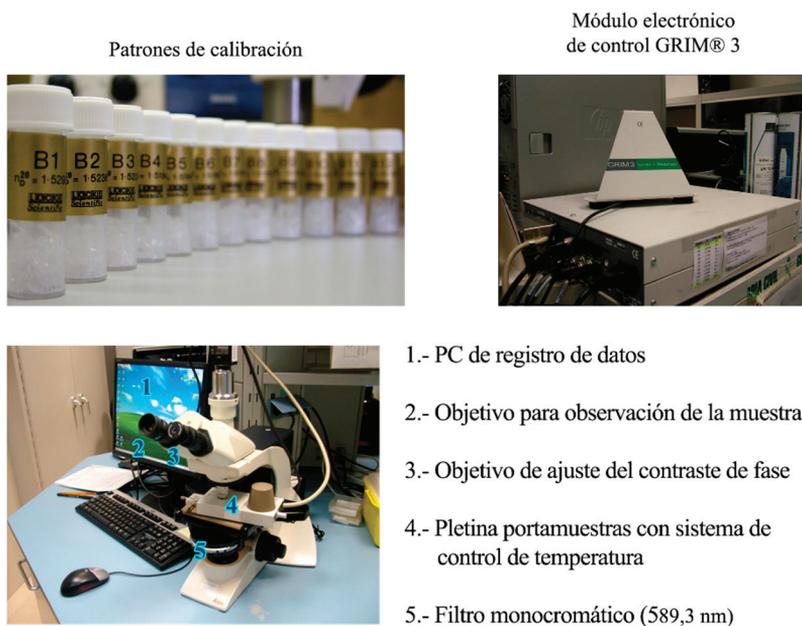
Las técnicas LA-ICP-MS e ICP-MS se emplean de rutina en el SECRIM en estudios medioambientales y forenses de aguas, suelos, aire, residuos sólidos y otras matrices. Sin embargo, dentro de la política de trabajo que persigue obtener el máximo rendimiento de cada equipo analítico y de su personal asociado, siempre es de interés ampliar la capacidad operativa de la instrumentación disponible. Por ello, uno de los propósitos de esta Tesis ha sido desarrollar una metodología para el análisis forense de tintas y vidrios mediante LA-ICP-MS, aprovechando la capacidad micro analítica y cuasi no destructiva de esta técnica, que permite obtener el perfil elemental de





muestras muy pequeñas y realizar posteriormente, otros estudios complementarios de la misma muestra.

La medida del índice de refracción en vidrios por cambio de contraste de fase con la temperatura en un aceite especial de silicona, comenzó a investigarse para uso forense en los años 80 y actualmente se usa de rutina en muchos laboratorios forenses. La **FIG. 5**, muestra el equipo de medida del Índice de Refracción del SECRIM. En esta Tesis se ha desarrollado y validado un método normalizado de trabajo, acompañado de los correspondientes controles de calidad necesarios para implantarlo como método acreditado por ENAC en el SECRIM.



*Fig 5. El equipo de medida del índice de refracción.*

Esta técnica presenta una precisión y exactitud adecuada para cotejar fragmentos de vidrio, requiere una cantidad de muestra muy

pequeña y supone un coste económico bajo, asequible a la mayoría de los laboratorios forenses de todo el mundo. Se la considera una técnica complementaria al análisis elemental. Al contrario que la técnica LA-ICP-MS, la instrumentación no ha cambiado significativamente a lo largo de los años, exceptuando la automatización del tratamiento de datos a través de un software específico, instalado en un PC.

## INTERÉS DEL ANÁLISIS FORENSE DE VIDRIOS Y TINTAS

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Entre las solicitudes de informes periciales que se reciben en el SECRIM, se encuentran las relacionadas con vidrios y tintas, ya que son materiales omnipresentes en la sociedad actual y por lo tanto, aparecen habitualmente como muestra de estudio durante la investigación de muchas actividades delictivas.

La solicitud más común de la Policía Judicial relacionada con las muestras de vidrio consiste en el cotejo de muestras dubitadas o recuperadas con muestras indubitadas o control para relacionarlas con un origen común, en delitos relacionados con el tráfico, robos en vivienda y comercios, entre otros. Del mismo modo, se solicita la comparación de la tinta manuscrita presente en escritos y firmas con el fin de comprobar la modificación de textos, números y la falsificación de firmas entre otros fraudes documentales. A modo de ejemplo, la **Fig. 6** presenta algunos escenarios en los que están presentes vidrios y tintas como evidencias forenses.





### Atropello y fuga del autor

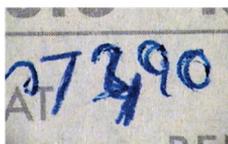


Escenario del hecho, estado del vehículo tras el atropello y fragmento de vidrio hallado en las prendas del ciclista.

### Robos en viviendas



### Modificación de documentos, firmas, falsificación papel moneda y documentos oficiales



*Fig 6. Algunos escenarios delictivos en los que están presentes vidrios y tintas.*

Los informes periciales relacionados con el análisis químico forense de vidrios y tintas, se emplean para contrastar las hipótesis de los hechos, actuando como complemento de la inspección ocular, declaraciones e interrogatorios, entre otras investigaciones del equipo de Policía Judicial. En la Instrucción Judicial, también se suelen incluir otros informes periciales de carácter grafístico, ADN o químico forense de otros restos hallados, como el análisis de restos de pintura. Los informes periciales se realizan de forma independiente por parte de los científicos forenses, los cuales no tienen conocimiento de los detalles de la investigación de la Policía Judicial, ni de los resultados del resto de pericias practicadas, con objeto de mantener la objetividad en la interpretación de los resultados obtenidos.





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# Objetivos





## OBJETIVOS

Esta Tesis surge de la continua necesidad de investigación y desarrollo en el campo de las Ciencias Forenses y pretende ser de aplicación directa a los informes periciales, dentro del marco de la alta especialización de los peritos y la optimización de los recursos del laboratorio forense.

El primer objetivo ha consistido en realizar una **revisión crítica de bibliografía relacionada con las aplicaciones forenses de la técnica LA-ICP-MS**, para conocer el estado actual de desarrollo de esta técnica y abordar una investigación que amplíe la capacidad operativa de este sistema disponible en el SECRIM, con el fin de dar respuesta a más cuestiones dentro de la demanda diaria recibida. Se ha observado que la aplicación más desarrollada de esta instrumentación en el campo exclusivamente forense es el análisis de vidrios, favorecido por varios motivos debido, por una parte, a que el vidrio está entre las evidencias recogidas en multitud de escenarios delictivos en los que están presentes vehículos a motor, viviendas, locales o naves industriales, tales como atropellos, accidentes, robos y atentados terroristas. Por otra parte, el vidrio es lo suficientemente homogéneo como para permitir que pequeños fragmentos recogidos en la escena del delito sean representativos del panel de vidrio original, además de ser una matriz sólida, ideal para el análisis por LA-ICP-MS. También se cuenta con la ventaja de que se han diseñado los materiales de referencia FGS1 y FGS2, específicos para la calibración y verificación en análisis forense, disponibles para los miembros de ENFSI a través de la BKA alemana, al igual que un protocolo de análisis aceptado por ENFSI y refrendado por publicaciones científicas. El resto de aplicaciones publicadas son menos numerosas y se han clasificado en dos grupos. Por una parte, el análisis forense de pinturas y, por otra, un grupo en el

que se reúnen publicaciones puntuales en matrices como tintas, papel, fibras, cannabis, gemas, cerámicas, porcelanas, ladrillos y metales preciosos.

A pesar de que el análisis, basado en la medida del índice de refracción en vidrios se realiza de rutina en el SECRIM desde hace unos 15 años, todavía no se ha incluido en el sistema de calidad con los requisitos que exige la norma UNE 17025. Por este motivo, otro objetivo de esta Tesis era revisar la validación de **la medida del índice de refracción en vidrios con el fin de acreditar el método por ENAC**. Además, para que esta determinación fuese aplicable en el ámbito de la criminalística, se hizo necesario realizar un estudio sobre los métodos de cotejo, basados en el contraste de hipótesis univariante, con el fin de comprobar la fiabilidad de los mismos en vestigios forenses reales y verificar su utilidad en un caso real de informe pericial.

La medida del índice de refracción en vidrios como aplicación forense está muy extendida, sin embargo solo aporta una variable de cotejo. Para caracterizar mejor los vestigios de vidrios y completar la sistemática de análisis forense de vidrios en el SECRIM, también se persiguió **desarrollar un método de cotejo de dieciocho elementos en vidrios mediante LA-ICP-MS con fines exclusivamente criminalísticos**, poniendo especial énfasis en obtener el máximo poder de individualización de los vestigios en el cotejo por pares. Con este objetivo, se procedió a la validación del método y al desarrollo del posterior tratamiento estadístico de contraste de hipótesis multivariante de resultados, ampliando así el poder de discriminación que ofrecía la medida del índice de refracción por si sola, con el consiguiente aumento de calidad y fiabilidad en la emisión de informes periciales relacionados con estos análisis.





Otra de las necesidades del SECRIM procede del Departamento de Grafística en el que se demanda, habitualmente el cotejo de tintas manuscritas en documentos, respecto a los bolígrafos y a los documentos manuscritos. Los métodos actualmente empleados se basan, en primer lugar, en análisis no destructivos mediante microscopía electrónica de barrido, observación por exposición a distintas longitudes de onda desde la región infrarroja del espectro electromagnético hasta la ultravioleta e incluso rayos X, para, posteriormente emplear técnicas destructivas, como cromatografía de capa fina o cromatografía líquida, micro espectrofotometría o electroforesis capilar, entre otras más sofisticadas y de elevado coste.

Otro objetivo de esta Tesis ha sido abordar el **cotejo forense de tintas en documentos manuscritos empleando LA-ICP-MS**. Esta técnica sólo se había empleado hasta entonces, en el SECRIM, para el análisis de vidrios. En este caso se perseguía la búsqueda de elementos característicos de la tinta que permitiera definir una huella dactilar lo más individualizada posible. Partimos de la principal ventaja de esta técnica que es cuasi no destructiva y permite analizar la muestra prácticamente sin manipulación. Por el contrario, nos enfrentamos con una matriz compleja en la que es imposible extraer la tinta con el láser sin extraer también parte del papel donde está impresa, por lo que ha sido necesario desarrollar un tratamiento de datos previo, con el propósito de filtrar la información útil antes de aplicar los tratamientos estadísticos de interpretación de resultados.

Estos dos últimos objetivos requieren un pretratamiento de los datos y la aplicación de tratamientos quimiométricos que permitan evaluar los resultados e interpretarlos de forma objetiva en los informes periciales. El cotejo de muestras dubitadas o recuperadas con muestras

indubitadas o control para relacionarlas con un origen común, es un tipo de comparativa muy habitual en criminalística. La mayor parte de las ocasiones, las muestras forenses procedentes de los sospechosos o de los lugares de los hechos están constituidas por pequeños fragmentos de un material original. Como ejemplos podemos citar, fragmentos de vidrio incrustados en una herramienta o pequeños trazos que constituyen sospechosas modificaciones manuscritas de un documento original en una hoja de papel. Este muestreo viene impuesto por las circunstancias de la inspección ocular, que pueden llevar a tener una cantidad insuficiente de muestras para realizar el número de réplicas necesarias, con el fin de alcanzar un elevado grado de certidumbre en la discriminación o asociación estadística de los vestigios. Por otra parte, por razones de economía y de tiempo, no se puede realizar un número elevado de réplicas por cada muestra, por lo que, los modelos estadísticos teóricos no siempre van a trabajar en condiciones ideales. Por estos motivos esta Tesis también persigue determinar los tratamientos estadísticos, que se adaptan mejor a la realidad de los casos forenses.

Es muy importante no perder de vista que los resultados químico forenses tienen su aplicación final en el ámbito judicial, el cual se caracteriza por un lenguaje no técnico. Por ello, es necesario hacer un esfuerzo para ser entendidos adecuadamente tanto por escrito en los informes periciales, como oralmente en las declaraciones judiciales. Para ello, se persigue el uso de herramientas gráficas y de expresiones escritas que excluyan en lo posible los términos científicos y con los que se visualicen fácilmente las conclusiones obtenidas por los miembros del tribunal.







# Capítulo I

## Forensic applications on LA-ICP-MS





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## **Applications of laser-ablation inductively-coupled plasma-mass spectrometry in chemical analysis of forensic evidence**

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## ABSTRACT

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Since the first coupling of laser-ablation inductively-coupled plasma to mass spectrometry (LA-ICP-MS) in 1985, applications of this analytical technique in various scientific fields have been developed. LA-ICP-MS is an elemental and isotopic microanalytical technique that is increasingly used for routine analysis in forensic laboratories around the world due to its great discriminating power between samples and its capacity for analyzing solid evidence of small size. In addition, its quasi-non-destructive character is especially welcome for this type of sample.

The main forensic applications of LA-ICP-MS have been developed for glass and paint samples. However, applications to other samples have also been reported [e.g., documents (ink and paper), fibers, cannabis, gems, Australian ocher and porcelain, brick stones, and gold and silver]. In the forensic field, the adjustment of instrumental analytical parameters, the study of interferences produced by fractionation and polyatomic ions, and the statistical treatment of data are considered of great importance. This review is intended as a practical, useful guide to help forensic chemists incorporate this attractive, powerful technique into their laboratories.

# 1 Introduction

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After the first source of coherent microwave radiation, named microwave amplification by stimulated emission of radiation (MASER) [1], the first source of coherent light radiation, known as light amplification by stimulated emission of radiation (LASER), was developed in 1960. The capacity and the precision of LASER for the collection of data directly from solid materials attracted the attention of researchers [2]. Shortly after the creation of lasers, laser micro-optical emission spectrometry (LM-OES) and laser-ablation mass spectrometry (LA-MS) were investigated for identification and elemental quantitative analysis of solids [3]. More recently, the coupling of laser ablation with inductively coupled plasma-MS (LA-ICP-MS) has shown great potential for the direct elemental analysis of solid samples [4].

The first ICP-MS commercial instruments were available in 1983. They were initially employed on daily routine analysis of aqueous solutions. However, for the analysis of solid samples, the transformation of the sample in a solution was necessary, usually by sample digestion with strong acids and oxidizers. Obviously, any such sample preparation involves the risk of sample contamination, the loss of volatile elements, and, besides, it creates a complex aqueous matrix, which can be source of several spectroscopic and non-spectroscopic interferences. The coupling of ICP-MS with an LA system for sample introduction [4,5] enabled direct, reliable, sensitive, and quasi non-destructive analysis of major and trace elements of solid samples. In the past 15 years, there has been rapid development of this technique, which has been widely applied to several fields of science (e.g., geology,





medicine, science of materials and, more recently, to forensic sciences, where solid samples constitute a large part of the physical evidence submitted to forensic chemistry laboratories).

As a consequence of the increasing interest on LA-ICP-MS, several reviews have been written on the technique itself and its applications to the elemental analysis of different types of samples. Those that stand out are from the Günther Group [3,6,7], Durrant [4], Russo et al. [8], and Mokgalaka and Gardea-Torresdey [9]. Recently, Trejos and Almirall [10] published quite an interesting chapter in the Encyclopedia of Analytical Chemistry, entitled “Laser Ablation Inductively Coupled Plasma Mass Spectrometry in Forensic Science”, where they explain the technique, its main characteristics, and give a general view of its potential for the analysis of a wide number of forensic samples, ranging from glass to biological and environmental samples.

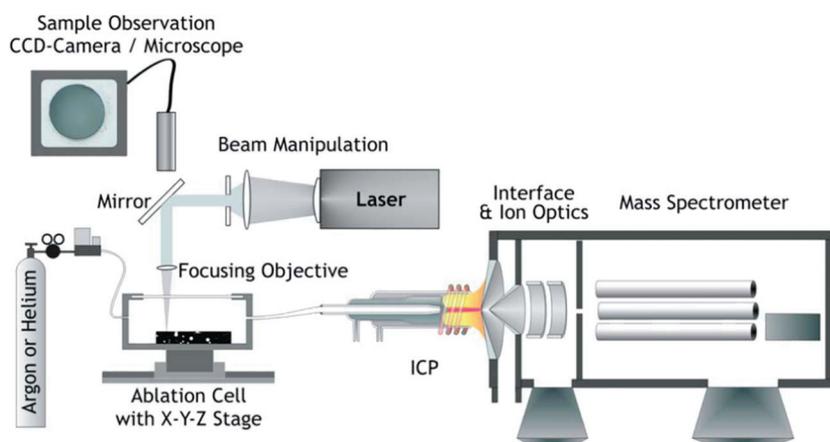
This review is a practical guide to help forensic scientists in the use of LA-ICP-MS as a versatile tool for the chemical investigation of common physical evidence found in forensic laboratories [e.g., glass, paints, documents (inks and papers), fibers, cannabis, gems, porcelains, brick stones, and gold and silver]. The review, based on the most important articles published in the past 12 years in forensic chemistry, comprises a basic introduction to the technique, a detailed overview of its applications to the analysis of these samples, and future perspectives. Biological and environmental-forensic samples do not fall within the scope of this review.

## 2 Basics of LA-ICP-MS

### 2.1 Instrumentation

The general configuration of an LA-ICP-MS instrument (**FIG. 1**) comprises:

- An LA system, for sample introduction into the plasma, coupled to
- ICP-MS equipment [3,6,11–13].



*Fig 1. Set-up of an instrument for LA-ICP-MS [3].*

A solid sample is placed into the ablation cell, which is mechanically adjusted, and a carrier gas (argon or helium) flows into this cell. Then, an energy-controlled pulsed laser is focused by the optic lens of the microscope onto the sample surface. The high-energy photons generated in the laser are converted into thermal energy, which is responsible of the ablation and the vaporization of the material. As a





consequence, an aerosol composed of vapor and microscopic particles and agglomerates from the ablated solid sample is generated and transferred by the continuous gas stream of the ablation cell into the plasma source. The ICP works as an atomization and ionization system. The ions formed are directed to the mass spectrometer and then separated according to their mass/charge ( $m/z$ ) ratio before being detected individually [3,11,14]. A more detailed description of each of these two blocks of the instrument is given next.

### 2.1.1 Laser-ablation system

The components of a typical LA system are: a laser, a microscope, an optical lens, a charge-coupled device (CCD), and a sample holder or ablation cell, mounted on an adjustable XYZ platform. The lens of the optical microscope can be utilized to visualize the sample and to focus the laser with the optical device; however, a CCD camera is very often used remotely to adjust the sampling position and to observe the spot size. The sample is located inside the ablation cell, which has a fused-silica window. The adjustable platform allows tridimensional positioning of sample (in the X, Y, and Z directions). Optimal depth profiling and spatial resolution when scanning can be achieved by adjusting individual laser pulses and platform movement [8].

In the LA process, a minimum mass amount of sample is removed by the energy from the laser beam. Obviously, laser parameters (e.g., pulse length and wavelength) affect in a decisive way the amount, the composition and the particle-size distribution of the aerosol formed [14]. In general, the shorter the laser wavelength and the

pulse length, the better the properties of the laser-generated aerosol [14] and, thus, a more efficient conversion of the atoms into ions within the ICP is achieved [3,4,8,14,15].

The pulsed lasers most often used for the LA process during the development of LA-ICP-MS have been ruby, Nd:YAG (neodymium-doped yttrium aluminum garnet;  $\text{Nd:Y}_3\text{Al}_5\text{O}_{12}$ ) and excimer lasers [3,4,8,14]. Ruby lasers (emitting at 694 nm) were the first used to ablate solids before being used in LA-ICP-MS for chemistry applications. Nd:YAG lasers have a fundamental wavelength of 1064 nm, in the infrared range; nevertheless by doubling, tripling, quadrupling and quintupling the optical frequency of this laser, wavelengths of 532, 355, 266, and 213 nm, respectively, can be obtained. These lasers have been widely used since they are cheap, require little maintenance and can be incorporated easily into commercial ablation instruments. Finally, excimer lasers utilize a gas-filled chamber containing an inert gas and a halogen (e.g., KrF and ArF) rather than solid-state crystals. The wavelength of excimer lasers depends on the type of excited molecules used: wavelengths at 308, 248, 193 and 157 nm are obtained using XeCl, KrF, ArF or  $\text{F}_2$ , respectively. With lasers working in the UV region, better analytical characteristics (precision and accuracy) compared with IR or visible regions are achieved [16]. Nowadays, lasers most commonly used are those working in the near UV region of the spectrum, as are Nd:YAG lasers (266 and 213 nm) and the excimer laser of 193 nm [8,9,14,17,18]. Excimer lasers that work at wavelengths below 200 nm require a complex design of the optical system [19,20]. Nevertheless, current developments in lasers employing short wavelengths and short pulses (ps and fs) of high photon intensity are





being performed to achieve better efficiency in the ablation process [14,21].

The sample cell must allow the perfect interaction of the laser beam with the sample. Basically, there are two types of ablation cells: the open and the hermetically closed chambers, the latter being used most often. In recent years, in order to achieve a perfect LA-ICPMS performance, most efforts have been directed towards:

- Improvement in the LA-cell design (geometry and volume), for quick, easy sample handling.
- Rapid, quantitative transport of the aerosol formed in the sample chamber to the ICP-MS [7,8,15,22,23].

Recently, computational fluid dynamics (CFD) simulations have been carried out to improve the capabilities of conventional LA cells [23]. The main innovations in the design of these devices are directed to sampling large samples that cannot be placed in conventional closed chambers. Examples of these designs are the open, noncontact cells, mounted on a planar platform without an outer containment enclosure (for large planar samples or samples <2 mm) [24], and large ablation cells, designed for high spatial-resolution analyses of multiple or large samples [22].

The mode for acquiring the ICP-MS time-dependent signal is of great importance, since it governs analytical performance characteristics (e.g., accuracy and precision) [25]. Laser pulses may be applied to the solid sample at a specific point, for a given period of time (spot sampling technique), or the sample chamber can be moved in such a way that the laser beam produces a trench (rastering, line or scanning

mode) [25,26]. The spot strategy is recommended for both depth-profile analyses and samples in which the elements are homogeneously distributed. By contrast, the scanning mode of ablation would better represent the average content of the element of interest and is generally used for elemental spatial distribution.

### 2.1.2 Inductively-coupled plasma-mass spectrometer

Since its marketing in 1980s, the ICP-MS technique has been expanding compared to several other instrumental techniques [e.g., neutron activation analysis (NAA), absorption atomic spectroscopy (AAS), X-ray fluorescence (XRF), and inductively-coupled plasma atomic emission spectroscopy (ICP-AES)]. Among the numerous advantages of the ICP-MS, we should emphasize its high sensitivity [limits of detection (LODs) less than  $\text{pg mL}^{-1}$ ] and capacity to perform rapid, simultaneous and multi-elemental analysis. Nowadays, this technique is considered a great tool for elemental analysis that, in addition, can accomplish isotope-ratio analysis [3,18,27].

In the current analytical chemistry market, there are several types of mass spectrometers based in different philosophies for ion selection and detection [6,8,12]. Double-focusing electrostatic-magnetic sector systems are characterized by their fast data-acquisition capability, low background levels and high resolution. Sector field instruments provide high sensitivity and can operate at higher mass-resolving powers. In time of- flight (TOF) systems, ions are sampled at exactly the same time, which means that multi-elemental data can be collected with no significant deterioration in quality; they can collect full mass spectra in less than  $50 \mu\text{s}$  [9]. Nevertheless, traditional quadrupole mass analyzers, cheaper and easier to use than others, are the most widely





used in the ICP-MS instruments (Q-ICP-MS) available in forensic laboratories, since they provide a good sensitivity/versatility ratio in elemental analysis. Although their resolution is 1 amu, they allow the analysis of most elements of the Periodic Table. They are also characterized by very low LODs and high speed scans, because they are able to achieve temporal resolution of several spectra per second.

A quadrupole mass filter consists of four parallel metal rods, connected in pairs. A radio-frequency voltage applied to these rod filters allows the separation of ions according to their  $m/z$  value, it being possible to transmit only ions within a very small  $m/z$  ratio. A characteristic of these mass analyzers is their ability to scan between selected masses: because the settling time at a particular mass is less than 1 ms, 1000 masses can be measured each second. Also, because the response time of LA sample-introduction systems is lower than that of these analyzers, they are well suited for the measurement of short, very transient signals typical of LA analysis [8,11,28,29]. However, a disadvantage when the sample is introduced into the ICP spectrometer by LA is that large particles may be present in the ablated vapor, leading to spatial changes in the ion cloud in the ICP. Therefore, enhancements or depressions in the signal level are produced [3,9]. Some Q-ICP-MS instruments have correction cells for polyatomic interferences, based on the collision or the reaction of interferent polyatomic ions with a gas introduced into the hexapole (Thermo-Scientific), quadrupole (PerkinElmer) or octopole (Agilent) mass filters, which are placed between the plasma and the quadrupole mass analyzer [8]. Usually, helium as collision gas and hydrogen (or other), as reaction gas, have been employed.

## 2.2 Advantages and limitations of LA-ICP-MS

There are numerous advantages of LA-ICP-MS for direct elemental analysis of solid samples that justify the growth of this technique compared with conventional solution techniques:

- A significant reduction of analysis time, since minimal or no sample and standards preparation is needed.
- Chemically inert elements (e.g., zirconium, hafnium, tantalum, niobium, titanium, thorium and uranium) can be analyzed.
- Contamination problems, which are very important for the determination of trace-level elements, are avoided.
- A minute quantity of solid sample, compared to that required for solution nebulization, is needed, and a micro-amount of material is ablated each laser pulse.
- Lower personal risks, because solvents and hazardous reagents needed for acid digestion are eliminated.
- Both spatially resolved (local analysis) and bulk sampling are possible.
- Large dynamic range.
- Versatility, since major and trace elements can be analyzed.
- Isotope-ratio analysis is possible.





Nevertheless, we should point out some disadvantages of using LA as the sample-introduction device for ICP-MS:

- Optimization of the laser parameters, depending on the matrix type, is required.
- The use of an internal standard (IS) is recommended to correct for variations in mass ablation between replicates. Selection of an appropriate IS is not always easy: it should be a time-consuming task and, in some occasions, it requires labor-intensive sample pretreatment.
- There are serious limitations in quantitative analysis.
  - The lack of many types of matrix-matching reference materials and well-established external calibration standards.
  - The impossibility of diluting the sample to optimize the amount of elements to be analyzed.
  - The impracticability to use simple calibration approaches typical of the analysis of aqueous solutions (single standard addition and isotope dilution).
- Long-term sensitivity drift due to the ICP-MS instrument, sample position in the cell and laser focusing.
- Reduced precision and accuracy, due to the nonstoichiometric representation of the elemental composition of the sample in the mass spectrum (fractionation process).

Fractionation is a major problem of LA-ICP-MS. There is much controversy about the term “fractionation”. This phenomenon is not easy to define, but it can be summarized as “the occurrence of non-stoichiometric effects in the transient signals” during LA-ICP-MS analysis [14]. Fractionation depends on [3,6–8,15,30–44]:

- The element itself, in view of fractionation mostly affecting both volatile and refractory elements and depending on thermodynamic properties of the elements (e.g., melting and boiling temperatures, vapor pressure, charge, and atomic radius).
- The sample matrix (also called “intrinsic fractionation”).
- The LA-ICP-MS steps (ablation stage, aerosol transport by the carrier gas, and atomization/ionization in the ICP).

With respect to the different steps of the analysis process, Guillon and Guenther [35] stated that what they defined as “time-dependent changes in elemental ratios” or “elemental fractionation” were ascribed significantly to processes occurring at the ablation site during the laser-sample interaction (ablation efficiency). This stage of the analysis is conditioned by, e.g. [3,7,8,19,25,30,35,40,42,45–49]:

- The laser irradiance, fluency, pulse length and wavelength.
- The depth-to-diameter ratio of the crater formed.
- Mineralogical factors.
- The chemical composition of the matrix.
- The ablation mode.





Fractionation may be minimized [3,8,21,24,30-35] by:

- The creation of clean, well-defined ablation craters.
- Assuring high absorption (low penetration depth) of laser light in the material.
- Using short laser wavelengths, in order to obtain low particles size distribution.
- Making a careful adjustment of gas flow.
- Avoiding particle re-deposition over the sample surface.
- Using a rapid-pulse laser (of the order of fs) that causes minimal thermal effect.

Finally, with respect to the other steps of the analysis [6,8,14,15,31,32,44], Figg et al. [32] already reported in 1998 that non-representative sampling originated in the transport of element-enriched particles from the ablation cell to the ICP and their later transfer into ions within the plasma. In this respect, both the instrumental design of the aerosol transport tubing and the type of carrier gas affect the transport efficiency and the signal profile. Once in the plasma, since the largest particles are not adequately handled in the ICP, the efficiency with which different elements are released from the particles leads to a non-stoichiometric representation of the sample.

Although mathematical correction for fractionation effects is yet not possible [10], the extent of the fractionation process can be estimated by using different fractionation indexes or elemental ratios (e.g., U/Th or U/Pb) [3,8,14,30,40-42,50]. The first fractionation index,

introduced by Fryer et al. [51], was calculated as the signal-intensity ratios of two isotopes during the transient signal acquisition, using calcium as IS element. This index reflected the effect of time (progressive ablation) on elemental ratios and, consequently, the efficiency of particle vaporization, transport and ionization in the plasma [30]. However, it did not account for the influence of other factors (e.g., the sample matrix). For this reason, other indexes that may better reflect the potential elemental fractionation between different matrices have been proposed [3,6,16,30,35,41,51].

### 3 Forensic applications of LA-ICP-MS

The forensic scientist must work with physical evidence of different types, shape, and size, whose origin must be ascertained and linked to the suspect in order to ensure a successful prosecution. For this reason, reliable analytical techniques are needed (e.g., LA-ICP-MS, which is capable of determining metals, metalloids and even nonmetals in solid samples, providing highly discriminating data and preserving the sample, practically, “as it is”). Furthermore, suitable chemometrics methods to evaluate the large amount of data obtained by these techniques must be used.

It was in 1994 when Watling et al. [52] emphasized the potential of LA-ICP-MS for identification of provenance of stolen gold. Between 1995 and 1999, Watling continued with his investigation on this technique as a useful tool to assist police forces [53–55]. Since then, the number and the type of forensic applications of this technique have increased considerably.





In this section, we describe in detail two of the major forensic applications of LA-ICP-MS – the elemental analysis of glass and paints. In addition, we also present other samples, whose forensic study by this technique has been developed to a lesser extent. Finally, we briefly review the usual chemometrics methods used in LA-ICPMS applications in the forensic field.

### 3.1 Glass

Glass is ubiquitous in our society since it is commonly used {e.g., buildings, containers, vehicles (e.g., headlights, windows and windshields) and ornaments}. As a result, glass debris is physical evidence frequently found at a large number of crime scenes and on suspects, so accurate analysis of these samples to establish the provenance of the glass is of great forensic importance [56].

When a glass is broken during a criminal offence (e.g., traffic accidents, burglaries, bombings or assaults), many fragments of various sizes are dispersed in all directions at the crime scene. Small glass fragments are the most prone to transfer (e.g., to clothing or shoes of the suspect). Glass generally represents primary transfer, resulting from contact or close proximity of the suspect to the broken glass product; moreover, this physical evidence usually persists with the suspect for an extended period of time. When these diminutive fragments are recovered and transported to the laboratory, they can be compared with control glass fragments (of a wellknown origin). If the fragments are considered to have a common origin, with a high degree of confidence, they can be used as strong scientific evidence to associate the suspect to the crime scene and thus to support a trial. A glass fragment is

therefore physical evidence that can be considered as a unique marker [56–58].

The two types of glass evidence most commonly received in forensic laboratories are:

- Architectural float glass (manufactured by flotation on molten tin) and used for fabricating windows.
- Glass from automobiles, which can be of different types.
  - Laminated (combination of two glass sheets with one or more interlayers of plastic or resin) for windshields;
  - Tempered (float and hardened glass) for left/right/back car windows; and,
  - Blown manufactured glass for bulbs and headlights.

Occasionally, glass containers, usually manufactured through blowing, must also be analyzed. All of them have the same common matrix, with silicon as a major component.

Traditional techniques for the forensic analysis of glass samples include the determination of physical and optical properties of glass (e.g., color, thickness, density or refractive index) [50]. However, over the years, advances in the process of glass manufacture, combined with improvements in its quality control, have made it possible to obtain glass samples of very similar physical and chemical composition, providing a narrow range of refractive index (RI) values among different glass samples. As a result, RI measurements or microscopy techniques [e.g., scanning electron microscopy (SEM) or optical microscopy] sometimes have not enough potential for forensic discrimination between glass fragments found at the crime scene and





others recovered from the suspicious source. It is therefore necessary to use other complementary techniques providing greater differentiation that may reinforce the value of the forensic analysis of the glass samples [31,57]. These techniques used to be characterized by a great discriminatory potential, including: neutron activation analysis (NAA); flame atomic absorption spectroscopy (FAAS); scanning electron microscopy, coupled with energy dispersive spectroscopy (SEM-EDS) or wavelength dispersive spectroscopy (SEM-WDS); particle-induced X-ray emission spectroscopy (PIXE); energy-dispersive X-ray fluorescence (EDXRF); inductively-coupled plasma atomic emission spectroscopy (ICP-AES), ICP-MS, and, laser-induced breakdown spectroscopy (LIBS). Each technique has both advantages and disadvantages that the analyst must take into account to proceed to proper selection [56,59].

In general, glass samples have silicon, calcium, sodium, aluminum, and oxygen as major components [43,58]. Because glass is produced by a continuous process, slight variations in the origin and the mixing of starting materials, in addition to degradation of the furnace itself, will lead to variations in the trace and ultra-trace element profile of the glass produced over time. Consequently, it would be possible to use these potential variations in trace-element profiles to differentiate among glass fragments. On some occasions, even a major element (e.g., magnesium) may also be used for this purpose [17]. Then, analytical techniques that allow the analysis of the major and trace elemental profile of these samples would be welcome.

LA-ICP-MS has gained strength in the past 10–15 years, to such an extent that forensic glass analysis is considered one of the main applications of this technique [37,55,60]. LA-ICP-MS has the capacity

to analyze directly very small glass fragments (up to 0.1–1 mm in length), which are typical debris found at a crime scene [58]. TABLE 1 shows the main glass elements, and their masses, which were determined by LA-ICP-MS.

**Table 1**

Elements commonly determined in glass samples by LA-ICP-MS<sup>a</sup>

<u>7Li</u>	<u>27Al</u>	<u>55Mn</u>	<sup>71</sup> Ga	<u>118Sn</u>	<u>139La</u>	<sup>151</sup> Eu	<u>178Hf</u>	<sup>207</sup> Pb
<sup>11</sup> B	<u>29Si</u>	<u>57Fe</u>	<u>85Rb</u>	<sup>121</sup> Sb	<u>140Ce</u>	<sup>159</sup> Tb	<sup>181</sup> Ta	<u>208Pb</u>
<u>23Na</u>	<u>39K</u>	<sup>59</sup> Co	<u>88Sr</u>	<sup>123</sup> Sb	<sup>144</sup> Nd	<sup>165</sup> Ho	<sup>182</sup> W	<sup>209</sup> Bi
<u>24Mg</u>	<u>42Ca</u>	<sup>65</sup> Cu	<u>90Zr</u>	<sup>133</sup> Cs	<u>146Nd</u>	<sup>169</sup> Tm	<sup>197</sup> Au	<sup>232</sup> Th
<sup>25</sup> Mg	<u>49Ti</u>	<sup>66</sup> Zn	<sup>93</sup> Nb	<u>137Ba</u>	<sup>147</sup> Sm	<sup>175</sup> Lu	<u>206Pb</u>	<sup>238</sup> U

<sup>a</sup> <sup>29</sup>Si is usually used as internal standard. Elements underlined are recommended in float glass analysis by ENFSI (European Network of Forensic Science Institutes).

Other important benefits of this forensic application are the following:

- The heterogeneity of trace elements in most bulk float glasses is small enough to make it possible to discriminate between different glasses, as already been reported [61,62].
- The high content of silica of all these samples makes <sup>29</sup>Si a good choice for internal standardization in order to overcome the instrumental drift typical of plasma techniques [60].
- The current availability of matrix-matched certified reference materials is a major advantage for quantitative analysis of glass samples [50].





Available standard reference materials (SRMs) for glass analysis are: NIST (National Institute of Standards and Technology) and P&H (Pulles & Hanique, Veldhoven, The Netherlands) glass standards, the former being used most. Moreover, the NITE-CRIME (Natural Isotopes and Trace Elements in Criminalistics and Environmental Forensic) European Network has distributed to its members two new glass (float) reference standards (FGS 1 and FGS 2) for quantitative analysis of major, minor and trace elements by LA-ICP-MS [60]. These standards are not yet certified and they can only be acquired from BKA (Bundeskriminalamt, Germany).

The elemental compositions (% m/m) of the NIST glass standards used most often (610 and 612) are 72 (SiO<sub>2</sub>), 14 (Na<sub>2</sub>O), 12 (CaO), 2 (Al<sub>2</sub>O<sub>3</sub>), and (MgO) only at mg/kg level [61]. FGS 1 and FGS 2 standards are sodalime type, although they have been enriched with some elements. Their approximate average matrix compositions (% m/m) are 72 (SiO<sub>2</sub>), 13 (Na<sub>2</sub>O), 8 (CaO) and 4 (MgO) [61]. As can be seen, NIST standards have lower magnesium concentration than the FSG standards; besides, in FGS materials, Al, K, and Fe are minor elements, while Li, Ti, Mn, Rb, Sr, Zr, Sn, Ba, La, Ce, Nd, Hf, and Pb are trace elements at varied concentrations. As a consequence, FSG standards seem to be the best option as calibration standard in float-glass analysis [60,61].

**TABLE 2** summarizes the main forensic applications of LA-ICP-MS to the analysis of glass samples, following a classification according to the glass-sample type (manufacturing process). Detailed information on the experimental conditions for the analysis of the different samples analyzed has also been included.

**Table 2**  
Experimental conditions of main applications of LA-ICP-MS on forensic analysis of glass samples.

Sample type	Elements / nuclides	Laser	ICP-MS	Instrumental parameters	Standard reference material	Chemometric processing	Ref.
Automobile glass (window and windshield)	Mg, Al, Mn, Ce, Ti, Zr, Ga, Ba, Rb, Sr, Hf, La, Pb	CETAC LSX-200 plus, Nd:YAG (266 nm)	Hewlett-Packard, 4500 Plus	<u>L/A:</u> D: 50 $\mu\text{m}$ , F: 10 Hz, V: 50.2 $\text{cm}^3$ ; AT: 50 s, AM: Depth profiling (LSX 200) and single point (LSX 500), SS: 1 $\mu\text{m}$ $\text{s}^{-1}$ (LSX 200). <u>ICP-MS:</u> RFP: 1302 W, RFM: 1.95 V, GTC: He (0.95 $\text{L min}^{-1}$ ) GAC: (Ar): 1.00 $\text{L min}^{-1}$ (LSX 200) and 0.95 $\text{L min}^{-1}$ (LSX 500), PG: 16.0 $\text{L min}^{-1}$	NIST 612, 610, 1831		[30]
Automobile glass (window and windshield)	$^{49}\text{Ti}$ , $^{85}\text{Rb}$ , $^{88}\text{Sr}$ , $^{90}\text{Zr}$ , $^{137}\text{Ba}$	New Wave UP-213	Perkin Elmer ELAN 6100 DRC II	<u>L/A:</u> D: 55 $\mu\text{m}$ , F: 10 Hz, E: 27.2 $\text{J cm}^{-2}$ , AM: Single point. <u>ICP-MS:</u> RFP: 1500 W, GTC: (He) 0.9 $\text{L min}^{-1}$ GAC: (Ar) 1.00 $\text{L min}^{-1}$ , PG: (Ar) 16.0 $\text{L min}^{-1}$ , AG: (Ar) 1.0 $\text{L min}^{-1}$ .	NIST 612 y 1831	ANOVA; GLM; Tukey's HSD test	[63]
Automobile glass (windows)	$^{7}\text{Li}$ , $^{23}\text{Na}$ , $^{24}\text{Mg}$ , $^{27}\text{Al}$ , $^{27}\text{Si}$ , $^{39}\text{K}$ , $^{44}\text{Ca}$ , $^{47}\text{Ti}$ , $^{52}\text{Cr}$ , $^{55}\text{Mn}$ , $^{56}\text{Fe}$ , $^{60}\text{Ni}$ , $^{66}\text{Zn}$ , $^{118}\text{Sn}$ , $^{232}\text{Th}$ , $^{238}\text{U}$	New Wave LUV 213; Nd:YAG (213 nm)	Hewlett Packard 7500s	<u>L/A:</u> D: 50 $\mu\text{m}$ , F: 10 Hz, V: 22 $\text{cm}^3$ ; AM: Raster, SS: 30 $\mu\text{m s}^{-1}$ . <u>ICP-MS:</u> RFP: 940 W, RFM: 1.81 V, GAC: (Ar) 1.00 $\text{L min}^{-1}$	NIST 612	ANOVA; Tukey's HSD test	[64]





**Table 2 (continuation)**

Experimental conditions of main applications of LA-ICP-MS on forensic analysis of glass samples.

Sample type	Elements / nuclides	Laser	ICP-MS	Instrumental parameters	Standard reference material	Chemometric processing	Ref.
Automobile glass (window, windshield and headlight)	Mg, Al, Ca, Mn, Ce, Ti, Zr, Sb, Ga, Ba, Rb, Sm, Sr, Hf, La, Pb	CETAC, LSX-200 <sup>+</sup> , Nd:YAG (266 nm)	Hewlett-Packard 4500 Plus Shield Toreh	<p><u>LA</u>:</p> <p>D: 50 <math>\mu\text{m}</math>, F: 10 Hz, E: 3.8 J <math>\text{cm}^{-2}</math>, V: 50.2 <math>\text{cm}^3</math>, AT: 50 s, AM: Depth profiling, (window) and raster (headlight), SS: 1 <math>\mu\text{m s}^{-1}</math> (window) and 3 <math>\mu\text{m s}^{-1}</math> (headlight)</p> <p>ICP-MS:</p> <p>RFP: 1302 W, RFM: 1.95 V, IT: 30 s, GTC: (He) 0.95 L <math>\text{min}^{-1}</math>, GAC: (Ar): 1.05 L <math>\text{min}^{-1}</math> (window) and 0.95 L <math>\text{min}^{-1}</math> (headlight).</p>	NIST 612, 1831, 1411 and NIST P6981.	ANOVA; GLM; Tukey's HSD test	[31]
Automobile glass (headlight and driving mirror)	<p><u>Headlight glass samples</u></p> <p><math>^{7}\text{Li}/^{63}\text{Cr}</math>, <math>^{23}\text{Na}/^{24}\text{Mg}</math>, <math>^{27}\text{Al}/^{47}\text{Ti}</math>, <math>^{57}\text{Fe}/^{55}\text{Mn}</math>, <math>^{66}\text{Zn}/^{60}\text{Ni}</math>, <math>^{85}\text{Rb}/^{232}\text{Th}</math>, <math>^{88}\text{Sr}/^{90}\text{Zr}</math>, <math>^{118}\text{Sn}/^{44}\text{Ca}</math>, <math>^{139}\text{La}/^{178}\text{Hf}</math>, <math>^{27}\text{Al}/^{29}\text{Si}</math></p> <p><u>Mirror glass samples</u></p> <p><math>^{7}\text{Li}/^{63}\text{C}</math>, <math>^{23}\text{Na}/^{24}\text{Mg}</math>, <math>^{27}\text{Al}/^{47}\text{Ti}</math>, <math>^{29}\text{Si}/^{59}\text{K}</math>, <math>^{55}\text{Mn}/^{238}\text{U}</math>, <math>^{57}\text{Fe}/^{65}\text{Mn}</math>, <math>^{66}\text{Zn}/^{60}\text{Ni}</math>, <math>^{118}\text{Sn}/^{44}\text{Ca}</math>, <math>^{232}\text{Th}/^{238}\text{U}</math>, <math>^{27}\text{Al}/^{29}\text{Si}</math></p>	New Wave LUV 213; Nd:YAG (213 nm)	Hewlett Packard 7500s	<p><u>LA</u>:</p> <p>AM: Raster</p>	NIST 612	ANOVA; Tukey's HSD test	[2]

**Table 2 (continuation)**  
 Experimental conditions of main applications of LA-ICP-MS on forensic analysis of glass samples.

Sample type	Elements / nuclides	Laser	ICP-MS	Instrumental parameters	Standard reference material	Chemometric processing	Ref.
Automobile glass (headlight)	$^{23}\text{Na}$ , $^{24}\text{Mg}$ , $^{27}\text{Al}$ , $^{44}\text{Ca}$ , $^{47}\text{Ti}$ , $^{55}\text{Cr}$ , $^{55}\text{Mn}$ , $^{57}\text{Fe}$ , $^{60}\text{Ni}$ , $^{66}\text{Zn}$ , $^{85}\text{Rb}$ , $^{88}\text{Sr}$ , $^{89}\text{Y}$ , $^{90}\text{Zr}$ , $^{95}\text{Mo}$ , $^{138}\text{Ba}$ , $^{140}\text{Ce}$ , $^{178}\text{Hf}$ , $^{208}\text{Pb}$ , $^{232}\text{Th}$	New Wave UP 213Al; Nd:YAG (213 nm)	Hewlett Packard 7500s	<u>LA</u> : D: 100 $\mu\text{m}$ , F: 10 Hz, AM: Raster, SS: 20 $\mu\text{m s}^{-1}$ . <u>ICP-MS</u> : RFP: 1200 W, IT: 10 ms, GTC: 0.8 L $\text{min}^{-1}$ , PG: 14 L $\text{min}^{-1}$	NIST 612, 620, 621, and 1831		[57]
Automobile glass (windshield)	Windshield glasses: Al/K, Ba/La, Ce/La, Co/Sr, Mg/Al, Mn/Rb, Hf/Zr, Pb/Th, Rb/Sr, Sr/Zr, Ti/Mn, U/Th	CETAC LSX-200 <sup>+</sup> Nd:YAG (266 nm)	Hewlett Packard, 4500 Plus	<u>LA</u> : D: 50 $\mu\text{m}$ , F: 10 Hz, V: 50.2 $\text{cm}^3$ , AT: 50s, AM: Single point. <u>ICP-MS</u> : RFP: 1302 W, RFM: 1.95 V, GTC: (He) 0.95 L $\text{min}^{-1}$ GAC: (Ar) 0.95 L $\text{min}^{-1}$ , PG: 16.0 L $\text{min}^{-1}$ .	NIST 612, 621 and 1831.	ANOVA;GLM ; Tukey's HSD test	[58]
Architectural glass	Architectural glasses: Al/K, Ba/La, Ce/La, Cs/Ba, Mg/Li, Mn/Rb, Pb/Ce, Zr/Sn, Sr/Zr, Ti/Mn, U/Th						
Container glass	Container glass samples: Al/K, Ba/La, Ce/La, Fe/Mn, Mg/Al, Mn/Rb, Pb/Hf, Rb/Sr, Sr/Zr, Ti/Mn, U/Th, Zr/Sn, B/Li						
Tempered glass	Tempered glass samples: Al/K, Ba/La, Ce/La, Co/Sr, Mg/Al, Mn/Rb, Hf/Zr, Pb/Hf, Rb/Sr, Sr/Zr, Ti/Mn, U/Th						



**Table 2 (continuation)**

Experimental conditions of main applications of LA-ICP-MS on forensic analysis of glass samples.

Sample type	Elements / nuclides	Laser	ICP-MS	Instrumental parameters	Standard reference material	Chemometric processing	Ref.
Tempered glass and residential window		MPB PSX-100, Excimer ArF (193 nm)	Thermo Finnigan Element 1	<u>LA</u> : F: 10 Hz, AM: Raster. <u>ICP-MS</u> : GAC: (Ar) 1.3 L min <sup>-1</sup> .	NIST 612	PCA	[65]
Float glass	<sup>39</sup> K, <sup>49</sup> Ti, <sup>55</sup> Mn, <sup>85</sup> Rb, <sup>88</sup> Sr, <sup>90</sup> Zr, <sup>137</sup> Ba, <sup>139</sup> La, <sup>140</sup> Ce, <sup>118</sup> Sn y <sup>206/207/208</sup> Pb	New Wave UP 213; Nd:YAG (213 nm)	Perkin Elmer ELAN DRC plus  Element 2	<u>LA</u> : D: 60 μm, F: 10 Hz, E: 12 J cm <sup>-2</sup> , V: 30 cm <sup>3</sup> . <u>ICP-MS</u> : RFP: 1200 W, GTC: (He) 0.75 L min <sup>-1</sup> GAC: (Ar) 0.90 L min <sup>-1</sup> , PG: (Ar) 15.0 L min <sup>-1</sup> , AG: (Ar) 0.90 L min <sup>-1</sup> .	NIST 610, 612, 614, 1830, 1831.  FGS 2	ANOVA/MAN OVA GLM y MSE; Tukey's HSD test	[66]
Float glass	<sup>23</sup> Na, <sup>25</sup> Mg, <sup>27</sup> Al, <sup>29</sup> Si, <sup>39</sup> K, <sup>42</sup> Ca, <sup>46</sup> Ti, <sup>55</sup> Mn, <sup>57</sup> Fe, <sup>7</sup> Li, <sup>11</sup> B, <sup>59</sup> Co, <sup>65</sup> Cu, <sup>66</sup> Zn, <sup>70</sup> Ga, <sup>75</sup> As, <sup>83</sup> Rb, <sup>88</sup> Sr, <sup>90</sup> Zr, <sup>93</sup> Nb, <sup>118</sup> Sn, <sup>123</sup> Sb, <sup>137</sup> Cs, <sup>137</sup> Ba, <sup>139</sup> La, <sup>140</sup> Ce, <sup>151</sup> Eu, <sup>159</sup> Tb, <sup>165</sup> Ho, <sup>169</sup> Tm, <sup>175</sup> Lu, <sup>178</sup> Hf, <sup>181</sup> Ta, <sup>182</sup> W, <sup>197</sup> Au, <sup>208</sup> Pb, <sup>209</sup> Bi, <sup>212</sup> Tl, <sup>238</sup> U	CETAC LSX-200. Nd:YAG (266 nm)  CETAC LSX 500 Nd:YAG (266 nm) VG  New Wave UP 213; Nd:YAG (213 nm).  GeoLasM Excimer ArF (193 nm)	Hewlett Packard 4500 ELAN 6100 DRC II. ELAN 6100 DRC+ Element2 VG PlasmaQuad 2+ VG PlasmaQuad 3	<u>LA</u> : D: 40–100 μm, F: 10 Hz, V: 30–140 cm <sup>3</sup> . <u>ICP-MS</u> : RFP: 1300–1400 W, GAC: (Ar) 0.85– 1.30 L min <sup>-1</sup> .	NIST 610, 612, 1831 and 621.  DGG 1.  FGS1, FGS2.		[61]

**Table 2** (continuation)

Experimental conditions of main applications of LA-ICP-MS on forensic analysis of glass samples.

Sample type	Elements / nuclides	Laser	ICP-MS	Instrumental parameters	Standard reference material	Chemometric processing	Ref.
Float, sheet and container glass	Mo, Ni, Y, Zr, Ba, Rb, Sr, Ce	VG Nd:YAG (1064 nm)	VG Turbo Plus	<u>LA:</u> F: 15 Hz, AT: 60 s.	NIST 610	Ternary plot	[55]
Container glass	<sup>7</sup> Li, <sup>23</sup> Na, <sup>24</sup> Mg, <sup>39</sup> K, <sup>44</sup> Ca, <sup>45</sup> Sc, <sup>49</sup> Ti, <sup>51</sup> V, <sup>53</sup> Cr, <sup>55</sup> Mn, <sup>59</sup> Co, <sup>60</sup> Ni, <sup>65</sup> Cu, <sup>66</sup> Zn, <sup>69</sup> Ga, <sup>70</sup> Ge, <sup>85</sup> Rb, <sup>8</sup> Sr, <sup>89</sup> Y, <sup>90</sup> Zr, <sup>93</sup> Nb, <sup>98</sup> Mo, <sup>118</sup> Sr, <sup>133</sup> Cs, <sup>138</sup> Ba, <sup>139</sup> La, <sup>140</sup> Ce, <sup>141</sup> Pr, <sup>146</sup> Nd, <sup>153</sup> Eu, <sup>165</sup> Dy, <sup>166</sup> Ho, <sup>1</sup> Er, <sup>178</sup> Hf, <sup>181</sup> Ta, <sup>208</sup> Pb, <sup>232</sup> Th, <sup>238</sup> U	New Wave UP-213	Hewlett Packard 7500CS	<u>LA:</u> D: 55 µm, F: 10 Hz, E: 17 J cm <sup>-2</sup> , AT: 60 s., AM: Single point.	NIST 610	LDA and PCA	[56]
Forensic glass	<sup>23</sup> Na, <sup>24</sup> Mg, <sup>27</sup> Al, <sup>44</sup> Ca, <sup>45</sup> Sc, <sup>48</sup> Ti, <sup>51</sup> V, <sup>52</sup> Cr, <sup>53</sup> Mn, <sup>56</sup> Fe, <sup>59</sup> Co, <sup>63</sup> Cu, <sup>66</sup> Zn, <sup>69</sup> Ga, <sup>75</sup> As, <sup>85</sup> Rb, <sup>88</sup> Sr, <sup>89</sup> Y, <sup>90</sup> Zr, <sup>93</sup> Nb, <sup>98</sup> Mo, <sup>118</sup> Sr, <sup>127</sup> Sb, <sup>138</sup> Ba, <sup>139</sup> La, <sup>140</sup> Ce, <sup>141</sup> Pr, <sup>178</sup> Hf, <sup>208</sup> Pb, <sup>209</sup> Bi, <sup>232</sup> Th, <sup>238</sup> U	CETAC LSX-100, Nd:YAG (266 nm)	Thermo Finnigan Element 1.	<u>LA:</u> D: 75 µm, F: 10 Hz, E: 80 J cm <sup>-2</sup> , AM: Raster, SS: 50 µm s <sup>-1</sup> . <u>ICP-MS:</u> GAC: (Ar) 1.3 L min <sup>-1</sup> .	NIST 1264a.	PCA	[43]
Forensic glass	<sup>7</sup> Li, <sup>25</sup> Mg, <sup>27</sup> Al, <sup>29</sup> Si, <sup>39</sup> K, <sup>42</sup> Ca, <sup>49</sup> Ti, <sup>55</sup> Mn, <sup>57</sup> Fe, <sup>85</sup> Rb, <sup>88</sup> Sr, <sup>90</sup> Zr, <sup>118</sup> Sn, <sup>121</sup> Sb, <sup>137</sup> Ba, <sup>139</sup> La, <sup>140</sup> Ce, <sup>146</sup> Nd, <sup>178</sup> Hf, <sup>208</sup> Pb	New Wave UP-213	Perkin Elmer ELAN@ DRC II	<u>LA:</u> D: 55 µm, F: 10 Hz, E: 27.25 J cm <sup>-2</sup> , AT: 60 s., AM: Single point and raster, SS: 15 µm. s <sup>-1</sup> (raster). <u>ICP-MS:</u> GTC: (He) 0.86 L.min <sup>-1</sup> GAC: (Ar) 0.90 L.min <sup>-1</sup> .	NIST SRM 612, 621 and 1831. FGS01 and FGS02.		[60]





**Table 2 (continuation)**

Experimental conditions of LA-ICP-MS on forensic analysis of glass samples.

Sample type	Elements / nuclides	Laser	ICP-MS	Instrumental parameters	Standard reference material	Chemometric processing	Ref.
NIST SRM	<sup>7</sup> Li, <sup>11</sup> B, <sup>24</sup> Mg, <sup>27</sup> Al, <sup>39</sup> K, <sup>29</sup> Si, <sup>49</sup> Ti, <sup>55</sup> Mn, <sup>57</sup> Fe, <sup>59</sup> Co, <sup>83</sup> Rb, <sup>88</sup> Sr, <sup>90</sup> Zr, <sup>118</sup> Sn, <sup>137</sup> Ba, <sup>139</sup> La, <sup>140</sup> Ce, <sup>178</sup> Hf, <sup>208</sup> Pb, <sup>232</sup> Th, <sup>238</sup> U	CETAC LSX-500 Nd:YAG, (266 nm)	Hewlett Packard, 4500 Plus	<u>LA</u> : D: 50 µm, F: 10 Hz, AT: 50 s, AM: Single point. <u>ICP-MS</u> : GTC: (He) 0.95 L min <sup>-1</sup> GAC: (Ar) 0.95 L min <sup>-1</sup> .	NIST 610 and 612.	ANOVA, Tukey's HSD test	[50]
Float glass	<sup>7</sup> Li, <sup>23</sup> Na, <sup>24</sup> Mg, <sup>27</sup> Al, <sup>29</sup> Si, <sup>39</sup> K, <sup>42</sup> Ca, <sup>49</sup> Ti, <sup>55</sup> Mn, <sup>57</sup> Fe, <sup>83</sup> Rb, <sup>88</sup> Sr, <sup>90</sup> Zr, <sup>118</sup> Sn, <sup>137</sup> Ba, <sup>139</sup> La, <sup>140</sup> Ce, <sup>146</sup> Nd, <sup>178</sup> Hf, <sup>208</sup> Pb	NEW WAVE UP213, Nd:YAG (213 nm)	Agilent 7500cs	<u>LA</u> : D: 80 µm, F: 10 Hz; AT: 60 s, AM: Spot, E: 10 J cm <sup>2</sup> <u>ICP-MS</u> : RFP: 1390 W, GTC: (He) 1.00 L min <sup>-1</sup> , GAC: (Ar): 1.00 L min <sup>-1</sup> , PG: 14.0 L min <sup>-1</sup>	NIST 612, FGG2, DGG1	Modified <i>n</i> sigma criterion with fixed standard deviations and others	[67]

AG, auxiliary gas flow; AM, ablation mode; ANOVA, analysis of variance; AT, ablation time; D, crater diameter; E, energy density; F, laser pulse frequency; GAC, gas flow after the ablation cell; GLM, general linear model; GTC, gas flow through ablation cell; IT, integration time; LDA, linear discriminant analysis; MANOVA, multivariate analysis of variance; Tukey's HSD, Honestly Significant Different test; MSE, mean square error; PCA, principal component analysis; PG, plasma gas flow; RFM, radio frequency matching; RFP, radio frequency power; SS, scan speed; V, ablation cell volume.

### 3.1.1 Sampling of glass fragments

Due to the minute amount of material removed during the LA process (as little as 0.3  $\mu\text{g}$  of glass per analysis), the forensic chemist must design a suitable strategy for sampling of glass evidence found at a crime scene. To achieve this purpose, it is desirable to have in mind two questions:

- Will the sample be representative of the original source?
- Will the size and the form of the analyzed glass fragments influence the obtained results?

In order to answer the first question, Trejos et al. [58] evaluated the micro-homogeneity of the elemental composition of different types of glass samples (from different manufacturing process) by analyzing the following types of glass: architectural window, windshields of vehicles, containers and tempered glass.  $^{29}\text{Si}$  was used, as IS and NIST 612 was employed as the single point external calibrator. Due to the similarity of matrices between the standards and the samples of interest, NIST 621 was used for glass containers, and NIST 1831 for architectural-window and vehicle-windshield glass samples. Based on the results obtained, several recommendations were established for a good sampling, as follows.





- In principle, due to the inherent heterogeneity that glass pieces could have, sampling of different points of the material is advisable.
- Architectural float glass is considered homogeneous at the micro level, so sampling of five different fragments of this type of glass should be enough to establish comparisons.
- In windshield glass pieces, differences in the elemental composition of both sides (inner and outer) suggest the collection of samples of at least five glass pieces of each side.
- Glass samples from containers are characterized by differences in their elemental composition, attributed to the manufacturing process. For this reason, it is suggested to take samples at the top, the middle and the bottom area of the container. If the container is broken, then it is recommended to take at least five fragments of the original material.
- Attention should be paid when analyzing tin in a glass submitted to float processing, since the concentration of this element in the glass side directly exposed to the tin pool is much greater (25–50 times) than that of the rest of the glass fragment. For recovered glass pieces large enough, this face should be identified by fluorescence emission by illuminating with UV radiation (at 254 nm). If such identification is impossible, careful interpretation of the Sn concentration found in the sample should be made.
- Each sample must be analyzed at least in triplicate in order to accomplish proper data treatment.

The influence of the size and the form of the glass fragments on the quantitative elemental analysis (second question) was studied by

Trejos et al. [50], using two standard reference materials [NIST 612 and NIST 610 (seven fragments of sizes 6–0.2 mm length)]. These standards had different quantitative elemental composition and distinct opacities (the standard SRM 612 is more transparent than SRM 610). The experimental results obtained evidenced that the proposed LA-ICP-MS method was not affected by variability in the sample size, being a reliable analytical method to accomplish routine analysis of glass fragments of quite different sizes. In addition, this method provided similar analytical performance to that of the well-accepted acid-digestion ICPMS methods. Nevertheless, these conclusions could not be generalized when analyzing glass fragments smaller than 0.2 x 0.1 mm.

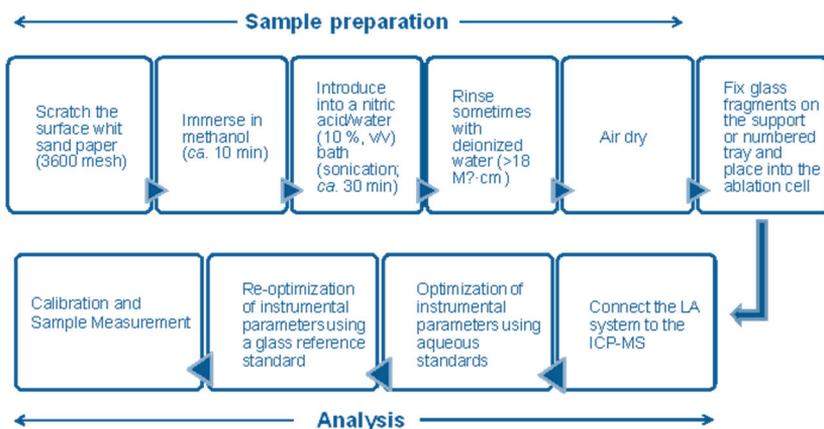
### 3.1.2 Sampling preparation and optimization of the instrumental parameters for the analysis of glass samples

As already mentioned, one of the advantages of elemental analysis of glass samples by LA-ICP-MS over conventional dissolution techniques is the minimal sample preparation before the analysis. Some authors simply recommend cleaning the glass surface under study with a paper free of lint, rejecting the ablation information of the superficial first layer to ensure the analysis of a completely clean surface [2]. However, most researchers follow some guidelines (see FIG. 2) for treatment and analysis of glass samples [30,51,55,56,58,61,65,66]. This stage of sample preparation involves scratching the surface of glass, followed by several cleaning steps (e.g., with methanol and nitric acid) and air-drying. Next, the sample is placed into the ablation cell, on a support [e.g., plastic, resin, or methacrylate, with the face of the glass submitted to flotation down (in the case of float glasses)]. Then, the glass is fixed with cyanoacrylate, epoxy resin or a transparent self-





double-faced adhesive sheet. It is recommended to put the glass fragment just in the center of the ablation cell, in order to get better transport efficiency of ablated material by the carrier gas (helium is recommended).



*Fig 2. General guidelines for the treatment and analysis by LA-ICP-MS of glass samples.*

At the second stage, analysis of glass samples by LA-ICP-MS requires preliminary optimization of instrumental parameters, using aqueous standards for an initial quality control of the instrumental performance. Afterwards, a second optimization with glass standards (NIST 612 or 610) must be accomplished. It is strongly recommended that at least 3–4 replicates (i.e. different spots) per sample be analyzed, in order to apply appropriate statistical analyses. Moreover, the Nite-Crime network recommends analyzing the NIST 612 standard at the beginning and the end of the sequence of sample analysis to correct for drift over time [60], and to analyse six replicates of each sample. It should be stressed that, since NIST standards contain Ga ( $m/z$  69) in

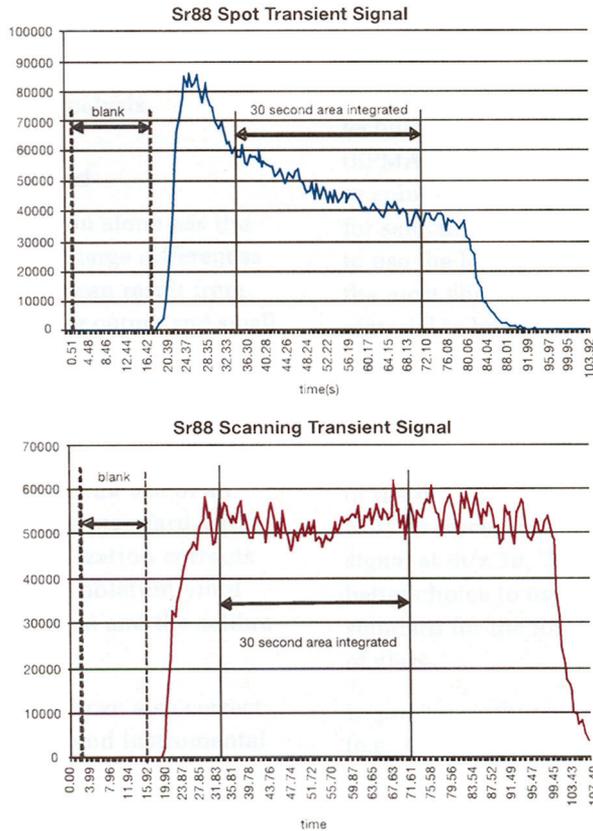
their composition, they are not recommended for the determination of the doubly-charged percentage using Ba (% Ba<sup>++</sup>/Ba). If there is no other option, a correction for <sup>69</sup>Ga should be made by measuring <sup>71</sup>Ga, or the European glass standard FGS 2, which does not contain Ga, may be used [60].

Careful optimization of the ablation strategy prior to LA-ICP-MS analysis is of great importance. FIG. 3 shows the typical transient signal of <sup>88</sup>Sr obtained in the analysis of glass by ablating with single spot and line scanning. Both signals consist of three components:

- A background blank component (approximately, 20 s).
- The time-dependent signal corresponding to sample ablation (~60 s).
- A post-ablation signal (20 s), necessary to reach the background level [58].

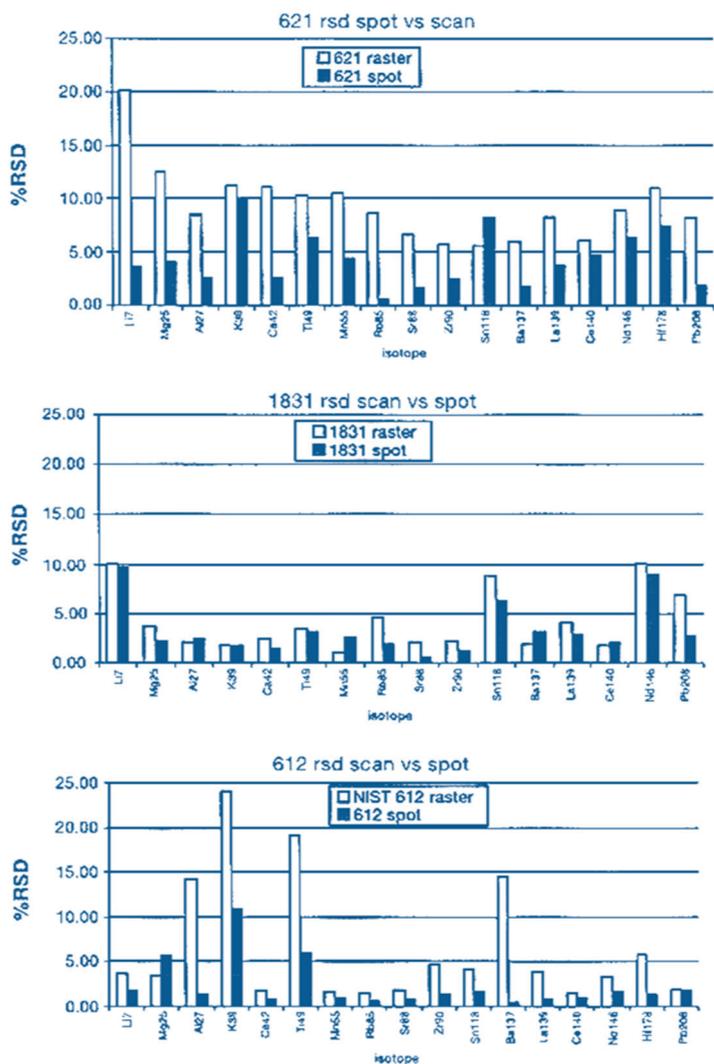
In the spot mode, after firing the laser, an increase in the signal intensity is observed, followed by a slow signal decay when increasing the crater depth; this steady decrease in the signal intensity is not observed with scanning analysis, since the laser beam has various impacts on the sample. The first coupling of the laser with the sample produces large particles that enter into the plasma and are not uniformly ionized. Afterwards, small, uniform particles are formed and transported to the ICP. This explains why the scanning mode leads to greater noise in the transient signal, more pronounced matrix effects, and, probably, an increase in fractionation, compared with spot ablation [60,63].





*Fig 3. Typical transient signals for 88Sr obtained by spot ablation (top) and scan ablation (bottom) of a forensic glass sample analyzed by LA-ICP-MS [60].*

The lack of homogeneity of the sample itself also influences the noise of the intensity signal obtained with line-scanning ablation. The effect of the ablation mode on the precision [relative standard deviation, RSD (%)] is clearly illustrated in **FIG. 4** for the analysis of different elements in NIST glass SRMs 612, 621, and 1831.



**Fig 4.** Precision (RSD, %) of signal intensities obtained in the analysis by LA-ICP-MS of glass SRMs (NIST 621, 1831 and 612) [61]. Scan mode in white bars; single-spot mode in grey bars. The laser used for this work was a New Wave UP-213 LA system (New Wave Research, Fremont, California, USA).





In this respect, the general recommendations to analyze glass samples by LA-ICP-MS can be summarized as follows [60,63]:

- Use single-spot ablation mode, with an optimum spot diameter of 50  $\mu\text{m}$ , approximately.
- Disregard the first 15 s of the signal obtained, to eliminate the effects of non-uniform particle sizes in the plasma.

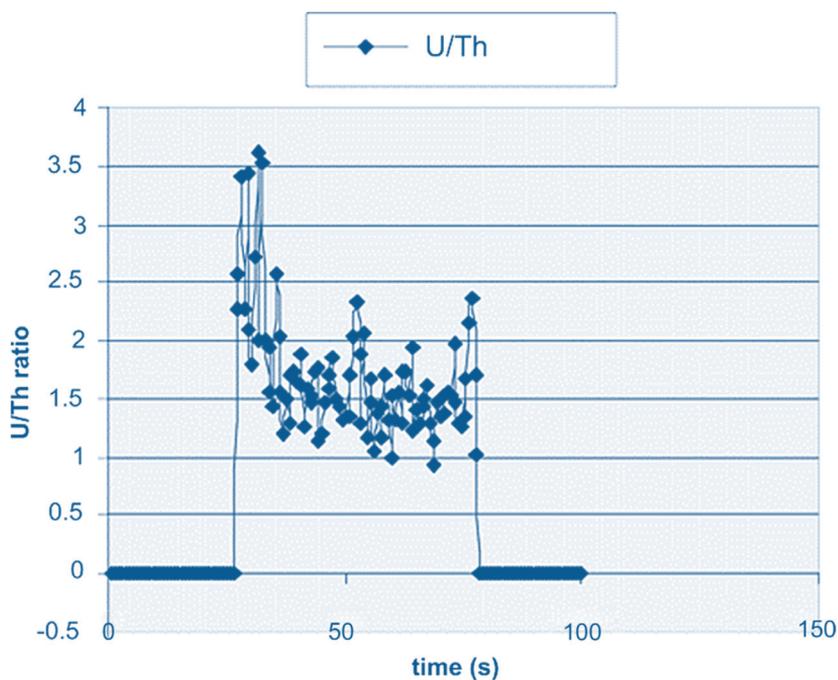
The effect of fractionation in comparing forensic glass samples by LA-ICP-MS has also been studied, using NIST standards 612 and 610 [30]. The degree of fractionation was measured using both a fractionation index (FI) and the U/Th ratio.  $^{238}\text{U}$  and  $^{232}\text{Th}$  have very close ionization potentials and their concentrations in NIST standards 612 and 610 are similar, the true ratio being close to 1. The FI was calculated according to the following expression:

$$FI = \frac{\left[ \frac{I_e}{I_{29\text{Si}}} \right]_{t_2}}{\left[ \frac{I_e}{I_{29\text{Si}}} \right]_{t_1}} \quad (1)$$

Where  $I_e$  is the intensity of the element of interest,  $I_{29\text{Si}}$  the intensity of  $^{29}\text{Si}$  (taken as IS in most glass studies),  $t_1$  the first half of the ablation signal, and  $t_2$  the second half of the ablation signal.

When calculating both parameters for SRM 610 and all elements studied in glass samples, FI values close to 1.0 (0.8–1.2) were obtained, indicating a low incidence of the fractionation phenomenon. However, as shown in **FIG. 5** for reference glass SRM 610, values of the U/Th ratio are not in good agreement with those of the FI, as we already explained in sub-section 2.2. The value of the U/Th ratio was close to 3 during the

first 10 s of the transient signal (acquired for 50 s). The authors attributed this result to the first interaction of the laser with the glass surface [30]. Then, a sharp drop of this ratio to about 1.5 was observed, followed by an almost constant tendency in its variation with time during the last 40 s of ablation. Based on these results, integration of the signal in its “stable area” was recommended by the authors.



*Fig 5. Variation of the U/Th ratio, as a function of time, obtained in the analysis of glass SRM NIST 610 during 50 s of ablation [30].*

Whether the particle-size distribution due to the ablation mode affects fractionation and quantitative analysis of glass samples has also been studied with glass reference materials, using two CETAC LA systems (model LSX-200 Plus and model LSX 500), with Q-switched Nd:YAG lasers and operating at 266 nm [30]. The first laser system had





a Gaussian-beam profile and worked with depth profile ablation mode (ablation from top to bottom), while the second had a flat beam profile and worked with a single-spot ablation mode. Better coupling of the laser with the surface of the glass was observed with the LSX 500 system, improving the symmetry of the crater and removing, approximately, double the amount of glass material with respect to the LSX 200. No significant differences in fractionation were observed for both systems (FI values in the range 0.8–1.2 for the NIST 610 SRM). This could be explained taking into account that about a 98% of the particles generated by the laser have a diameter of 0.1–0.2  $\mu\text{m}$  and only those particles with diameters greater than 0.7  $\mu\text{m}$  are responsible of the fractionation process. In addition, it was proved that, for both ablation systems, precision in the quantitative analysis of 10 glass samples was good and the elemental concentrations determined by LA-ICPMS were in good correlation with those obtained by bulk-solution methods. However, since fractionation depends on the matrix, these conclusions cannot be applied to other samples.

### 3.1.3 Quantitative analysis of glass samples

Different strategies have been described for the quantitative analysis of glass samples [61], as reported in **TABLE 3**.

**Table 3**

Different strategies for the quantitative analysis of glass samples by LA-ICP-MS [8,12,31,44,45,48,61,63,65,66,68,69]

**Calibration line and equation**

- External standardization with matrix-matched standards (single point or multipoint calibration)

$$(C_A)_{sample} = (C_A)_{Ref} \times \frac{(I_A)_{sample}}{(I_A)_{Ref}}$$

- Internal standardization

100% normalization technique

One single standard element

- External standardization with matrix-matched standards, together with internal standardization

$$(C_A)_{sample} = (C_A)_{Ref} \times \left( \frac{I_A}{I_{IS}} \right)_{sample} \times \left( \frac{I_{IS}}{I_A} \right)_{Ref} \times \frac{(C_{IS})_{sample}}{(C_{IS})_{Ref}}$$

- On-line solution-based calibration with internal standard

$$(C_A)_{solid} = (C_{IS})_{solid} \times \left( \frac{I_A}{I_{IS}} \right)_{solid} \times \left( \frac{I_{IS}}{I_A} \right)_{liquid} \times \left( \frac{C_A}{C_{IS}} \right)_{liquid}$$

- Calibration with nebulized standard solutions

With a mono gas sample introduction

With a dual gas sample introduction

- On-line isotope dilution

$$(C_A)_{solid} = (C_A)_T \times \left( \frac{T-X}{X-S} \right) \times \left( \frac{m_T}{m_S} \right)$$

$C_A$ : analyte concentration;  $C_{IS}$ : internal standard concentration;  $I_A$ : background-corrected signal intensity (counts  $s^{-1}$ ) of the analyte;  $I_{IS}$ : background-corrected signal intensity (counts  $s^{-1}$ ) of the internal standard;  $(C_A)_T$ : analyte concentration in the highly enriched tracer;  $X$ : isotopic ratio of the two selected isotopes in the mixture;  $T$ : isotopic ratio of the two selected isotopes in the tracer;  $S$ : isotopic ratio of the two selected isotopes in the sample;  $m_S$  and  $m_T$ : atomic mass of the element in nature and of the isotopically enriched element, respectively.





External standardization with matrix-matched standards alone assumed that the ablation behavior of the standards and the sample is identical [44]. Both single-point calibration and multiple-point calibration need standards with a composition in major elements similar to that of the sample, at concentrations above the LOD of the method [69]. Besides, instrumental interferences must be subtracted by running a “gas blank” (analysis of the carrier gas without LA) before analyzing each sample [16,68]. However, these standardization techniques present disadvantages, e.g.:

- The correction of signal drifts caused by differences in laser interaction with the sample and the standard is required [30,44,68].
- Appropriate and well-characterized reference materials are usually lacking.

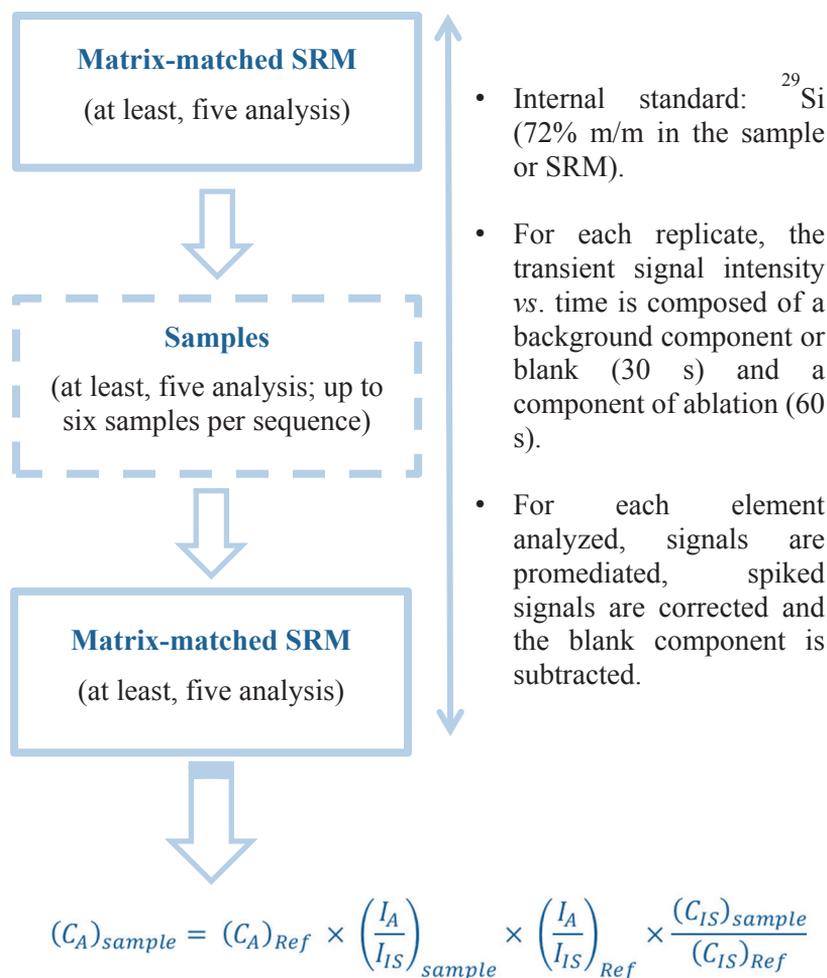
Internal standardization may be accomplished by means of only one IS or using all the main elements together (100% normalization technique) [30]. With this strategy, matrix effects and instrumental drift are minimized, any variation in the amount of ablated material is taken into account, systematic errors are corrected, and better precision is achieved. Although not strictly necessary, the following characteristics are desirable for an ideal IS [60,63,68,69]:

- Closely match the chemical and physical properties of the analytes in the sample.
- Be a major component of the material, of known concentration.

- Be the lowest-abundance isotope of the most abundant element in the sample, to avoid saturation of the detector.
- The concentration of the IS must be known independently (by means of other analytical techniques) in all reference standards and sample unknowns. For glass analysis,  $^{29}\text{Si}$  is very often used as IS, since silicon is a major component of the glass and its concentration is well known (72% of silicon, as  $\text{SiO}_2$ ) [61]. The least abundant isotope of silicon is  $^{30}\text{Si}$  (3.09%); nevertheless, because  $\text{NO}^+$  produces a background noise at  $m/z$  30,  $^{29}\text{Si}$  (4.7%) is the best option as IS in forensic analysis of glass samples [61]. There exist other major elements that can also be used as ISs (e.g., calcium). The choice will depend on the similarity between the external standard and the sample, as well as on the possibility to determine its concentration. In practice, to analyze an unknown material, it is advisable to use two or more ISs to verify parameters (e.g., the consistency, the accuracy and the precision) [60].

The ideal mode of quantification, which is used most often, is a combination of external standardization, using matrix-matched standards, with internal standardization. In this case, a typical sequence followed in the analysis of glass by LA-ICP-MS is shown in **Fig. 6**.





**Fig 6.** Steps followed in the quantitative analysis of glass samples by laser-ablation inductively-coupled plasma-mass spectrometry (LA-ICP-MS), using external standardization with matrix-matched standards and internal standardization. Terms in the equation defined are given in TABLE 3.

Another strategy of calibration in forensic glass analysis by LA-ICP-MS is liquid calibration, using internal standardization with Na (all elements) and Yb (rareelements only), with either a mono gas-sample-introduction system or a dual gas-flow technique [45]. In the

configuration of mono gas-sample introduction, the nebulizer is directly coupled to the LA cell (the nebulizer gas flow is used as the carrier gas for LA), while dual gas flow system is used for simultaneous dried solution aerosol and laser-induced aerosol-introduction system [45]. Both techniques are good alternatives for simultaneous solution nebulization and LA sample introduction, and to overcome the problems of low transport efficiencies in both introduction systems (the gas flow of the liquid nebulizer and the LA system) [70].

Finally, on-line isotope dilution has scarcely been used for element quantification in glass samples of forensic interest [12,44,45]. In this quantification strategy, only one isotope ratio per analyte must be measured in the isotope-diluted sample [44]; Th has been used as IS element in the case of NIST 612 [45].

## 3.2 Paint

Very often, paint samples from crime scenes arrive at forensic laboratories because they may be involved in criminal acts (e.g., thefts, hit-and-run cases, homicides, falsification of art pieces, or injuries). [71]. Nevertheless, one of the principal reasons of death in the world is the traffic accidents. Therefore, the majority of the paint samples in a forensic laboratory are related to this type of event. Paint evidence may provide a link between victims and suspect vehicles and throw light on such incidents. The forensic chemist must be prepared to analyze this type of evidence with the whole scientific rigor that Justice requires.

There are different types of car paint available in the market: “solid paints” and “effect paints”. The general composition of both paints is shown in **TABLE 4**.



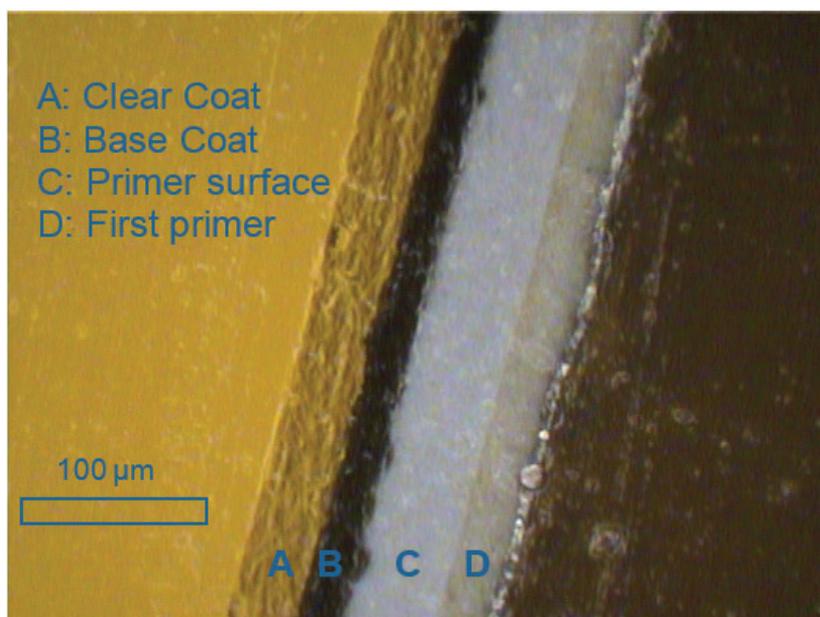


**Table 4**  
Composition of car paints. [71].

<b>Component</b>	<b>Function</b>
Pigments (organic and inorganic compounds)	Give the paint its color
Aluminium flakes, mica pigments and/or other effect pigments	Give the vehicle its metallic sheen
Modifiers	Control certain properties of the paint (gloss, flexibility, toughness and durability)
Extenders	Give bulk and covering capacity to the paint
Binders	Natural or synthetic resin that stabilize the mixture of components and form a film when it is spread

<sup>a</sup> Only in effect paints.

In addition, they are composed of a first primer (FP), a primer surface (PS) and a top coat, all of different chemical compositions. The FP attaches the paint to the underlying steel substrate of the vehicle and protects the steel against weathering; the second layer (PS) protects the first layer against ultraviolet radiation; and, the third layer (top coat) gives the color of the vehicle and contains pigments. In “effect paints”, this top coat is divided into a base coat (BC), which contains the pigments, and a protective clear coat (CC) [71,72], as can be seen in the FIG. 7.



*Fig 7. Transverse section of an ablated effect paint sample.*

When car-paint fragments found at crime scenes are sent to a forensic laboratory, the aim of the analysis may be:

- Comparison of the evidence recovered from the crime scene with a paint fragment of the suspect vehicle.
- Comparison of the layer-specific elemental composition of a car paint sample with a database.

In the first case, qualitative information might be sufficient to draw conclusions. However, in the second case, quantitative information is necessary [72]. The sample characterization includes the study of its chemical characteristics (identification of polymer type, pigments and additives) and physical properties (color, structure, sequence and thickness of the layers). These characteristics can be





compared with existing databases and provide relevant information (e.g., identification of the manufacturer, year of production, and brand and model of the vehicle). In order to avoid mistakes, the forensic chemist must take into account that many factors (e.g., composition or color of the paint of the vehicle) can be altered due to the prolonged exposure to the external environment [72,73].

Afterwards, other tests may obtain an analytical profile of the paint, depending on the polymers, pigments and additives. These tests include analysis of the sample by infrared (IR) spectroscopy and Raman spectroscopy and pyrolysis–gas chromatography (PyGC), the last usually combined with MS. The characterization of the metallic composition of car paints can be carried out by electron-probe microanalysis (EPMA), with either energy-dispersive spectroscopy (EDS) or wavelength-dispersive spectroscopy (WDS), and by SEM/EDS and XRF. EPMA provides information on the major elements of the sample, while the other two techniques are suitable for both major and minor components, but they have some disadvantages [68,70]:

- SEM–EDS is mainly used for qualitative analysis, due to its low sensitivity (LOD of 0.1%) for quantification of trace elements.
- XRF analysis of one layer is difficult because of the deep penetration of X-ray radiation, which provides data from several or all the layers of a paint sample.
- When using SEM–EDS and XRF techniques, an overlap of the lines of elements commonly present in car paints (Ba and Ti, S and Pb) impairs interpretation of the results obtained.

Thanks to the appearance of a powerful technique for elemental analysis (e.g., LA-ICP-MS), useful information about the composition of car paints can be achieved in terms of major, minor and trace elements. Besides, depth-profiling analysis of these samples by LA-ICP-MS gives valuable analytical information about individual layers. However, the main drawback found when applying this technique to car paints comes from the complex nature of these samples, as they contain organic components and high concentrations of inorganic compounds that may induce the formation of polyatomic or doubly-charged interfering ions. These spectral interferences, shown in **TABLE 5**, affect, above all, the transition elements and must be taken into account when a quadrupole-based mass spectrometer is used as the detection system. Then, for database matching of car-paint samples, LA coupled to a magnetic sector field ICP-MS (LA-ICP-SF-MS) is recommended [72].

**Table 5**

The most important spectral interferences in forensic analysis of car paints by LA-ICP-MS [72]

Main compounds	Interfering ions <sup>b</sup>	Nuclides affected
Organic <sup>a</sup>	$^{12}\text{C}_2^+$	$^{24}\text{Mg}^+$
	$^{12}\text{C}^{14}\text{N}^+$	$^{26}\text{Mg}^+$
	$^{12}\text{C}^{16}\text{O}^+$ , $^{14}\text{N}_2^+$	$^{28}\text{Si}^+$
	$^{13}\text{C}^{16}\text{O}^+$	$^{29}\text{Si}^+$
	$^{14}\text{N}^{16}\text{O}^+$	$^{30}\text{Si}^+$
	$^{40}\text{Ar}^{12}\text{C}^+$	$^{52}\text{Cr}^+$
	$^{40}\text{Ar}^{14}\text{N}^+$	$^{54}\text{Cr}^+$ , $^{54}\text{Fe}^+$
	$^{40}\text{Ar}^{16}\text{O}^+$	$^{56}\text{Fe}^+$
	$^{40}\text{Ar}^{14}\text{N}^{14}\text{N}^+$ ,	$^{68}\text{Zn}^+$
	$^{40}\text{Ar}^{12}\text{C}^{16}\text{O}^+$	
Inorganic	$^{24}\text{Mg}^{2+}$	$^{12}\text{C}^+$
	$\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2 \cdot 2\text{H}_2\text{O}$	$^{13}\text{C}^+$
	$3\text{MgO} \cdot 4\text{SiO}_2 \cdot \text{H}_2\text{O}$	$^{23}\text{Na}^+$
	$\text{BaSO}_4$	$^{24}\text{Mg}^+$
	$\text{TiO}_2$	$^{25}\text{Mg}^+$
	$^{46}\text{Ti}^{2+}$ , $^{46}\text{Ca}^{2+}$	
	$^{48}\text{Ti}^{2+}$ , $^{48}\text{Ca}^{2+}$	
	$^{50}\text{Ti}^{2+}$	





**Table 5 (continuation)**

The most important spectral interferences in forensic analysis of car paints by LA-ICP-MS [72]

Main compounds	Interfering ions <sup>b</sup>	Nuclides affected
CaCO <sub>3</sub>	<sup>40</sup> Ar <sup>+</sup> , <sup>24</sup> Mg <sup>16</sup> O <sup>+</sup>	<sup>40</sup> Ca <sup>+</sup> , <sup>40</sup> K <sup>+</sup>
Fe-containing pigments	<sup>25</sup> Mg <sup>16</sup> O <sup>+</sup> , <sup>24</sup> Mg <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>41</sup> K <sup>+</sup>
	<sup>26</sup> Mg <sup>16</sup> O <sup>+</sup> , <sup>25</sup> Mg <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>42</sup> Ca <sup>+</sup>
	<sup>27</sup> Al <sup>16</sup> O <sup>+</sup> , <sup>26</sup> Mg <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>43</sup> Ca <sup>+</sup>
	<sup>27</sup> Al <sup>16</sup> O <sup>1</sup> H <sup>1</sup> , <sup>28</sup> Si <sup>16</sup> O <sup>+</sup>	<sup>44</sup> Ca <sup>+</sup>
	<sup>28</sup> Si <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>45</sup> Sc <sup>+</sup>
	<sup>46</sup> Ti <sup>+</sup>	<sup>46</sup> Ca <sup>+</sup>
	<sup>32</sup> S <sup>16</sup> O <sup>+</sup>	<sup>48</sup> Ti <sup>+</sup> , <sup>48</sup> Ca <sup>+</sup>
	<sup>32</sup> S <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>49</sup> Ti <sup>+</sup>
	<sup>50</sup> Ti <sup>+</sup>	<sup>50</sup> Cr <sup>+</sup> , <sup>50</sup> V <sup>+</sup>
	<sup>54</sup> Fe <sup>+</sup>	<sup>54</sup> Cr <sup>+</sup>
	<sup>40</sup> Ar <sup>16</sup> O <sup>+</sup> , <sup>40</sup> Ca <sup>16</sup> O <sup>+</sup>	<sup>56</sup> Fe <sup>+</sup>
	<sup>40</sup> Ar <sup>16</sup> O <sup>1</sup> H <sup>+</sup> , <sup>40</sup> Ca <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>57</sup> Fe <sup>+</sup>
	<sup>42</sup> Ca <sup>16</sup> O <sup>+</sup>	<sup>58</sup> Fe <sup>+</sup> , <sup>58</sup> Ni <sup>+</sup>
	<sup>43</sup> Ca <sup>16</sup> O <sup>+</sup> , <sup>42</sup> Ca <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>59</sup> Co <sup>+</sup>
	<sup>44</sup> Ca <sup>16</sup> O <sup>+</sup> , <sup>43</sup> Ca <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>60</sup> Ni <sup>+</sup>
	<sup>44</sup> Ca <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>61</sup> Ni <sup>+</sup>
	<sup>46</sup> Ti <sup>16</sup> O <sup>+</sup> , <sup>46</sup> Ca <sup>16</sup> O <sup>+</sup>	<sup>62</sup> Ni <sup>+</sup>
	<sup>47</sup> Ti <sup>16</sup> O <sup>+</sup> , <sup>46</sup> Ti <sup>16</sup> O <sup>1</sup> H <sup>+</sup> , <sup>46</sup> Ca <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>63</sup> Cu <sup>+</sup>
	<sup>48</sup> Ti <sup>16</sup> O <sup>+</sup> , <sup>48</sup> Ca <sup>16</sup> O <sup>+</sup> ,	<sup>64</sup> Ni <sup>+</sup> , <sup>64</sup> Zn <sup>+</sup>
	<sup>40</sup> Ar <sup>24</sup> Mg <sup>+</sup> ,	
	<sup>47</sup> Ti <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	
	<sup>130</sup> Ba <sup>2+</sup> , <sup>49</sup> Ti <sup>16</sup> O <sup>+</sup> ,	<sup>65</sup> Cu <sup>+</sup>
	<sup>46</sup> Ti <sup>16</sup> O <sup>1</sup> H <sup>+</sup> , <sup>48</sup> Ca <sup>16</sup> O <sup>1</sup> H <sup>+</sup> ,	
	<sup>40</sup> Ar <sup>25</sup> Mg <sup>+</sup>	
	<sup>132</sup> Ba <sup>2+</sup> , <sup>50</sup> Ti <sup>16</sup> O <sup>+</sup> ,	<sup>66</sup> Zn <sup>+</sup>
	<sup>40</sup> Ar <sup>26</sup> Mg <sup>+</sup> , <sup>49</sup> Ti <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	
	<sup>134</sup> Ba <sup>2+</sup> , <sup>40</sup> Ar <sup>27</sup> Al <sup>+</sup> ,	<sup>67</sup> Zn <sup>+</sup>
<sup>50</sup> Ti <sup>16</sup> O <sup>1</sup> H <sup>+</sup>		
<sup>136</sup> Ba <sup>2+</sup> , <sup>40</sup> Ar <sup>28</sup> Si <sup>+</sup> ,	<sup>68</sup> Zn <sup>+</sup>	
<sup>32</sup> S <sup>16</sup> O <sub>2</sub> <sup>+</sup>		
<sup>138</sup> Ba <sup>2+</sup>	<sup>69</sup> Ga <sup>+</sup>	
<sup>54</sup> Fe <sup>16</sup> O <sup>+</sup>	<sup>70</sup> Zn <sup>+</sup>	
<sup>54</sup> Fe <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>71</sup> Ga <sup>+</sup>	
<sup>54</sup> Fe <sup>16</sup> O <sup>+</sup> , <sup>40</sup> Ar <sup>32</sup> S <sup>+</sup>	<sup>72</sup> Ge <sup>+</sup>	
<sup>54</sup> Fe <sup>16</sup> O <sup>+</sup> , <sup>54</sup> Fe <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>73</sup> Ge <sup>+</sup>	
<sup>58</sup> Fe <sup>16</sup> O <sup>+</sup> , <sup>57</sup> Fe <sup>16</sup> O <sup>1</sup> H <sup>+</sup>	<sup>74</sup> Ge <sup>+</sup> , <sup>74</sup> Se <sup>+</sup>	

**Table 5 (continuation)**

The most important spectral interferences in forensic analysis of car paints by LA-ICP-MS [72]

Main compounds	Interfering ions <sup>b</sup>	Nuclides affected
	$^{58}\text{Fe}^{16}\text{O}^+\text{H}^+$	$^{75}\text{As}^+$
	$^{40}\text{Ar}_2^+$ , $^{40}\text{Ar}^{40}\text{Ca}^+$	$^{80}\text{Se}^+$
	$^{40}\text{Ar}^{42}\text{Ca}^+$	$^{82}\text{Se}^+$
	$^{40}\text{Ar}^{44}\text{Ca}^+$	$^{84}\text{Sr}^+$
	$^{40}\text{Ar}^{46}\text{Ca}^+$ , $^{40}\text{Ar}^{46}\text{Ti}^+$	$^{86}\text{Sr}^+$
	$^{40}\text{Ar}^{47}\text{Ti}^+$	$^{87}\text{Sr}^+$ , $^{87}\text{Rb}^+$
	$^{40}\text{Ar}^{48}\text{Ca}^+$ , $^{40}\text{Ar}^{48}\text{Ti}^+$	$^{88}\text{Sr}^+$
	$^{40}\text{Ar}^{49}\text{Ti}^+$	$^{89}\text{Y}^+$
	$^{40}\text{Ar}^{50}\text{Ti}^+$	$^{90}\text{Zr}^+$
	$^{40}\text{Ar}^{54}\text{Fe}^+$	$^{94}\text{Mo}^+$ , $^{94}\text{Zr}^+$
	$^{40}\text{Ar}^{56}\text{Fe}^+$	$^{96}\text{Mo}^+$ , $^{96}\text{Ru}^+$ , $^{96}\text{Zr}^+$
	$^{40}\text{Ar}^{57}\text{Fe}^+$	$^{97}\text{Mo}^+$
	$^{40}\text{Ar}^{58}\text{Fe}^+$	$^{98}\text{Ru}^+$ , $^{98}\text{Mo}^+$
	$^{130}\text{Ba}^+$	$^{130}\text{Te}^+$
	$^{136}\text{Ba}^+$	$^{136}\text{Ce}^+$
	$^{138}\text{Ba}^+$	$^{138}\text{Ce}^+$ , $^{138}\text{La}^+$
	$^{130}\text{Ba}^{16}\text{O}^+$	$^{146}\text{Nd}$
	$^{135}\text{Ba}^{16}\text{O}^+$ , $^{134}\text{Ba}^{16}\text{O}^+\text{H}^+$	$^{151}\text{Eu}^+$
	$^{136}\text{Ba}^{16}\text{O}^+$ , $^{135}\text{Ba}^{16}\text{O}^+\text{H}^+$	$^{152}\text{Sm}^+$
	$^{137}\text{Ba}^{16}\text{O}^+$ , $^{136}\text{Ba}^{16}\text{O}^+\text{H}^+$	$^{153}\text{Eu}^+$
	$^{138}\text{Ba}^{16}\text{O}^+$ , $^{137}\text{Ba}^{16}\text{O}^+\text{H}^+$	$^{154}\text{Sm}^+$
	$^{40}\text{Ar}^{132}\text{Ba}^+$	$^{172}\text{Yb}^+$
	$^{40}\text{Ar}^{134}\text{Ba}^+$	$^{174}\text{Yb}^+$
	$^{40}\text{Ar}^{135}\text{Ba}^+$	$^{175}\text{Lu}^+$
	$^{40}\text{Ar}^{137}\text{Ba}^+$	$^{177}\text{Hf}^+$
	$^{40}\text{Ar}^{138}\text{Ba}^+$	$^{178}\text{Hf}^+$

<sup>a</sup>For example, melamine, styrene, polyurethane, nitrocellulose, epoxy-resin.

<sup>b</sup>Non-restrictive list (REE nuclides with an isotopic abundance  $\leq 15\%$  were not taken into account).

To date, there are not many applications of LA-ICP-MS to the forensic analysis of car paints. The most important works, with the exception of a paint sample extracted from a wood door, are compiled in **TABLE 6**.



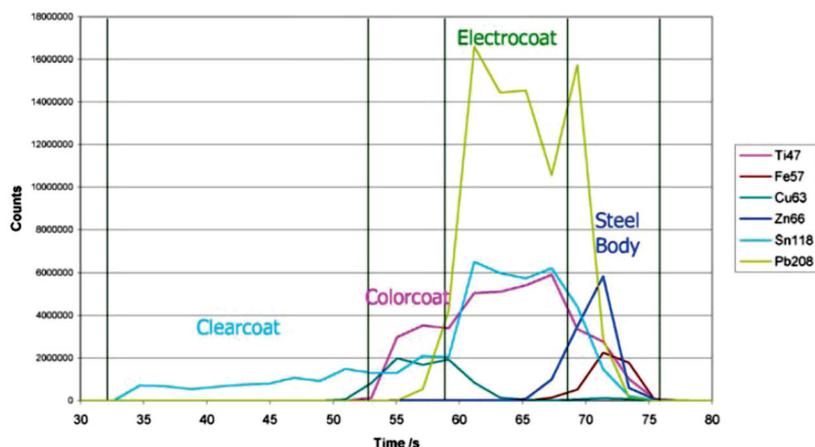


**Table 6**  
Experimental conditions of main application of LA-ICP-MS in forensic analysis of car paints

Sample Type	Element/isotopes	Laser	ICP-MS	Instrumental parameters	Standard reference material	Chemometric processing	Ref.
Car Paint	<sup>13</sup> C, <sup>23</sup> Na, <sup>24</sup> Mg, <sup>25</sup> Mg, <sup>26</sup> Mg, <sup>27</sup> Al, <sup>28</sup> Si, <sup>29</sup> Si, <sup>30</sup> Si, <sup>31</sup> P, <sup>32</sup> S, <sup>34</sup> S, <sup>44</sup> Ca, <sup>47</sup> Ti, <sup>48</sup> Ti, <sup>50</sup> V, <sup>51</sup> V, <sup>52</sup> Cr, <sup>53</sup> Cr, <sup>54</sup> Cr, <sup>55</sup> Mn, <sup>56</sup> Fe, <sup>57</sup> Fe, <sup>58</sup> Co, <sup>59</sup> Ni, <sup>60</sup> Ni, <sup>61</sup> Ni, <sup>62</sup> Ni, <sup>63</sup> Ni, <sup>64</sup> Cu, <sup>65</sup> Cu, <sup>66</sup> Zn, <sup>67</sup> Zn, <sup>68</sup> Zn, <sup>69</sup> Zn, <sup>70</sup> Zn, <sup>71</sup> Ga, <sup>73</sup> Ga, <sup>83</sup> Rb, <sup>86</sup> Sr, <sup>88</sup> Sr, <sup>111</sup> Cd, <sup>118</sup> Sn, <sup>120</sup> Sn, <sup>134</sup> Ba, <sup>135</sup> Ba, <sup>136</sup> Ba, <sup>137</sup> Ba, <sup>138</sup> Ba, <sup>139</sup> La, <sup>139</sup> La, <sup>140</sup> Ce, <sup>142</sup> Ce, <sup>200</sup> Hg, <sup>201</sup> Hg, <sup>202</sup> Pb, <sup>203</sup> Pb, <sup>204</sup> Pb, <sup>205</sup> Pb	MicroLas GeolLas, ArF excimer, (193 nm)	Perkin Elmer Sciex	<p>LA: D: 120 µm, F: 3 Hz, E: 11.7-15.8 J cm<sup>-2</sup>, V: 42 cm<sup>2</sup>, AM: Single point ablation</p> <p>ICP-MS: RFP: 1200 W, GTC: 1.4 L min<sup>-1</sup>, PG: 17 L min<sup>-1</sup>, AG: (Ar): 1.2 L min<sup>-1</sup>.</p>	NIST 612	Hotelling T <sup>2</sup> test	[72]
Car Paint	Mg, Ti, Cr, Mn, Fe, Cu, Zn, Rb, Sr, Sn, Cs, Ba, La, Ce, Pb, Bi	CETAC LSX 200 Plus, Nd:YAG, (266 nm)	Agilent (HP) 4500 Plus Shield Torch	<p>LA: D: 100 µm, F: 5 Hz, AM: Single point ablation</p> <p>ICP-MS: RFP: 1302 W, VRF: 1.95 V, GTC: (He) 0.95 L min<sup>-1</sup>, GAC: (Ar): 0.95 L min<sup>-1</sup>, PG: 16.2 L min<sup>-1</sup></p>	NIST 2570, 2574, 2575	Pairwise comparison	[73]
Car Paint	Mg, Ti, Cr, Mn, Fe, Cu, Zn, Rb, Sr, Sn, Cs, Ba, La, Ce, Pb, Bi	CETAC LSX-200 Plus, Nd:YAG, (266nm)	Agilent (HP) 4500 Plus Shield Torch	<p>LA: D: 100 µm, F: 5 Hz, V: 50.2 cm<sup>2</sup>, AM: Single point ablation</p> <p>ICP-MS: RFP: 1302 W, VRF: 1.95 V, GTC: (He) 0.95 L min<sup>-1</sup>, GAC: (Ar): 0.95 L min<sup>-1</sup></p>	NIST 2570, 2574, 2575	ANOVA, GLM, Tukey's HSD test	[74]
Wooden door paint	<sup>201</sup> Pb, <sup>202</sup> Pb, <sup>203</sup> Pb, <sup>204</sup> Pb, <sup>205</sup> Pb, <sup>206</sup> Hg, <sup>208</sup> Hg	New Wave-MerchanteK, Nd:YAG, (213 nm)	Finnigan MAT	<p>LA: D: 100 µm, AM: Single point ablation</p>	NIST 612		[75]

AG, auxiliary gas flow; AM, ablation mode; ANOVA, analysis of variance; AT, ablation time; D, crater diameter; E, energy density; F, laser pulse frequency; GAC, gas flow after the ablation cell; GLM, general linear model; GTC, gas flow through ablation cell; IT, integration time; LDA, linear discriminant analysis; MANOVA, multivariate analysis of variance; Tukey's HSD, Honestly Significant Different test; MSE, mean square error; PCA, principal component analysis; PG, plasma gas flow; RFM, radio frequency matching; RFP, radio frequency power; SS, scan speed; V, ablation cell volume.

Time-resolved plots are very useful for quantitative analysis and comparative qualitative evaluation of results obtained by LA-ICP-MS. For example, **FIG. 8** shows changes in the signal intensity, due to differences in concentrations of each element when the laser crosses the different layers of car paints in depth profiling or single-spot mode. Comparison of signal intensity and the type of elements for the different layers, help to identify different paint samples [73,74]. As is shown in **FIG. 8**, when the laser is ablating from top to bottom, the elemental intensities of the deepest layers begin to be mixed together, so it is difficult to differentiate between them.



*Fig 8. Time-resolved plot chart of the ablation of three layers of paint. The separation of the layers is marked by the vertical lines [73].*

To overcome this problem, two solutions are proposed:

- To turn all the paints upside down and to ablate them again.
- To mount the automobile paints directly upon silver foil (99.95% purity), monitoring the  $^{109}\text{Ag}$  signal intensity; a huge





increase in this signal indicates that the bottom of the paint has been reached. The first part of the two times resolved plots has a good layer resolution and information from all layers of the paint sample.

However, the in situ determination of concentrations of Pb and Hg in an environmental sample in a form of layered paint chip has been carried out by LA-ICP-SF-MS. This is an interesting sample from a forensic point of view, since Pb-based paints are one of the major sources of lead poisoning. Isotope compositions of  $^{206}\text{Pb}/^{204}\text{Pb}$  versus  $^{207}\text{Pb}/^{206}\text{Pb}$  are used to find the source of the pollutants. LA-ICP-SF-MS proved to be a powerful technique for rapid, accurate and relatively low-cost elemental and isotopic ratio analysis of trace elements in environmental samples [75].

The main limitation on the quantitative analysis of paints by LA-ICP-MS is the lack of certified reference standards to use in a matrix matching calibration.  $^{49}\text{Ti}$  has been used as IS and NIST glass standards and Pb paint film have been used as calibration standards [72–75].

### 3.3 Other forensic applications

In spite of the main forensic applications of the LA-ICP-MS technique having been developed for glass and paint samples, there are some papers on forensic analysis by LA-ICP-MS of other samples (e.g., ink and paper, fiber, cannabis, gems, Australian ocher and porcelain, brick stones, and gold and silver). **TABLE 7** compiles the main studies appearing in recent years; like **TABLES 2 AND 3**, **TABLE 7** also includes other relevant information (e.g., elements and ions, instrumental parameters, reference materials and chemometric processing used in the different samples of forensic interest).



**Table 7**  
Experimental conditions of main applications of LA-ICP-MS to the analysis of other samples of forensic interest

Sample type	Elements/isotopes	Laser	ICP-MS	Instrumental parameters	Standard reference material	Chemometric processing	Ref.
Inkjet inks	C, Na, Mg, K, Ca, Cu, Ga, Ge, Sr, Y, Zr, Nb, Mo, Ba, La, Hf	CETAC, LSX-500, (266 nm)		LA: AM: Raster.			[76]
Inks and papers	<sup>7</sup> Li, <sup>13</sup> C, <sup>23</sup> Na, <sup>24,25</sup> Mg, <sup>27</sup> Al, <sup>39</sup> K, <sup>42</sup> Ca, <sup>45</sup> Se, <sup>47,49</sup> Ti, <sup>51</sup> V, <sup>52,53</sup> Cr, <sup>55</sup> Mn, <sup>57</sup> Fe, <sup>59</sup> Co, <sup>60,65</sup> Ni, <sup>63,65</sup> Cu, <sup>64,66</sup> Zn, <sup>85</sup> Rb, <sup>88</sup> Sr, <sup>89</sup> Y, <sup>90</sup> Zr, <sup>107</sup> Rh, <sup>118,120</sup> Sn, <sup>137</sup> Ba, <sup>139</sup> La, <sup>149</sup> Ce, <sup>142,143</sup> Nd, <sup>180</sup> Hf, <sup>206,207,208</sup> Pb	CETAC, LSX-500, (266 nm)	Perkin Elmer, Elan DRC II	LA: D: 200 µm, F: 10 Hz, AM: Raster SS: 25 µm/s	In house standard	PCA, ANOVA Tukey's HSD test, t-test Bonferroni correction	[68]
Document papers	<sup>24</sup> Mg, <sup>27</sup> Al, <sup>55</sup> Mn, <sup>56</sup> Fe, <sup>88</sup> Sr, <sup>89</sup> Y, <sup>138</sup> Ba, <sup>140</sup> Ce, <sup>146</sup> Nd		Thermo Fisher Scientific (hexapole collision cell)	ICP-MS: RFP: 1350 W, PG: 13 L min <sup>-1</sup> , Carrier: 0.71-0.73 L min <sup>-1</sup>	Aqueous calibration standards	Two-way ANOVA, Tukey's HSD test, t-test and F-test	[77]
Document papers	<sup>7</sup> Li, <sup>11</sup> B, <sup>23</sup> Na, <sup>24</sup> Mg, <sup>25</sup> Mg, <sup>27</sup> Al, <sup>28</sup> Si, <sup>29</sup> Si, <sup>39</sup> K, <sup>42</sup> Ca, <sup>49</sup> Ti, <sup>51</sup> V, <sup>52</sup> Cr, <sup>53</sup> Cr, <sup>55</sup> Mn, <sup>57</sup> Fe, <sup>58</sup> Ni, <sup>59</sup> Co, <sup>60</sup> Ni, <sup>61</sup> Ni, <sup>63</sup> Cu, <sup>66</sup> Zn, <sup>71</sup> Ga, <sup>75</sup> As, <sup>83</sup> Rb, <sup>88</sup> Sr, <sup>90</sup> Zr, <sup>95</sup> Nb, <sup>97</sup> Mo, <sup>118</sup> Sn, <sup>123</sup> Sb, <sup>133</sup> Cs, <sup>137</sup> Ba, <sup>139</sup> La, <sup>140</sup> Ce, <sup>143</sup> Nd, <sup>147</sup> Sm, <sup>151</sup> Eu, <sup>159</sup> Tb, <sup>165</sup> Ho, <sup>167</sup> Tm, <sup>173</sup> Lu, <sup>175</sup> Hf, <sup>181</sup> Ta, <sup>183</sup> W, <sup>193</sup> Pt, <sup>197</sup> Au, <sup>208</sup> Pb, <sup>209</sup> Bi, <sup>232</sup> Th, <sup>238</sup> U.	New Wave UP-213, Nd:YAG (213 nm)	Perkin Elmer ELAN DRC plus	LA: D: 140 µm, F: 5 Hz, E: 4.5 ml cm <sup>-2</sup> , AM: Raster, SS: 50 µm s <sup>-1</sup> . ICP-MS: RFP: 1200 W, GTC: 1.0 L min <sup>-1</sup> , PG: 15 L min <sup>-1</sup> , AG: 0.8 L min <sup>-1</sup> .		LDA; PCA; CA	[78]
Black and colored toners	<sup>7</sup> Li, <sup>25</sup> Mg, <sup>44</sup> Ca, <sup>51</sup> V, <sup>60</sup> Ni, <sup>66</sup> Zn, <sup>75</sup> As, <sup>86</sup> Sr, <sup>93</sup> Nb, <sup>103</sup> Rh, <sup>114</sup> Cd, <sup>121</sup> Sb, <sup>133</sup> Ba, <sup>141</sup> Pr, <sup>153</sup> Eu, <sup>164</sup> Dy, <sup>169</sup> Tm, <sup>180</sup> Hf, <sup>187</sup> Re, <sup>195</sup> Pt, <sup>205</sup> Tl, <sup>206,207,208</sup> Pb, <sup>208</sup> Bi.	CETAC LSX-500 (266 nm)	Optimass 8000 ICP-TOF-MS	LA: D: 150 µm, F: 20 Hz, AT: 60 s, E > 9 mJ; SS: 100 µm s <sup>-1</sup> ICP-MS: RFP: 800 W, GAC: (A) 0.79 L min <sup>-1</sup> , PG: 10 L min <sup>-1</sup> , AG: 1.0 L min <sup>-1</sup>		CA, PCA	[79]
Cotton filters	<sup>25</sup> Mg, <sup>27</sup> Al, <sup>55</sup> Mn, <sup>57</sup> Fe, <sup>88</sup> Sr, <sup>137</sup> Ba	CETAC LSX-200, (266 nm)	Perkin Elmer, Elan DRC II	LA: D: 200 µm, F: 10 Hz, AT: 45 s, AM: Single spot. ICP-MS: GTC: (A) 0.80-0.95 L min <sup>-1</sup> GAC: (He) 0.90 L min <sup>-1</sup> .	SRM cotton: IAEA V-9.	ANOVA; GLM; Tukey's HSD test PCA, CA	[80]
Cannabis	<sup>49</sup> Ti, <sup>51</sup> V, <sup>52</sup> Cr, <sup>59</sup> Co, <sup>60</sup> Ni, <sup>64</sup> Zn, <sup>65</sup> Cu, <sup>69</sup> Ga, <sup>73</sup> Ge, <sup>75</sup> As, <sup>82</sup> Se, <sup>83</sup> Rb, <sup>86</sup> Sr, <sup>89</sup> Y, <sup>93</sup> Nb, <sup>98</sup> Mo, <sup>111</sup> Cd, <sup>115</sup> In, <sup>117</sup> Sn, <sup>121</sup> Sb,	VG LaserLab, Nd:YAG (1064 nm)	VG Turbo Plus	LA: D: 100 µm.	NIST 610, 612, 614.	Ternary diagrams	[81]



**Table 7 (continuation)**

Experimental conditions of main applications of LA-ICP-MS to the analysis of other samples of forensic interest

Sample type	Elements/isotopes	Laser	ICP-MS	Instrumental parameters	Standard reference material	Chemometric processing	Ref.
	<sup>129</sup> Te, <sup>133</sup> Ba, <sup>139</sup> La, <sup>140</sup> Ce, <sup>141</sup> Pr, <sup>146</sup> Nd, <sup>151</sup> Eu, <sup>152</sup> Sm, <sup>158</sup> Gd, <sup>159</sup> Tb, <sup>163</sup> Dy, <sup>166</sup> Hg, <sup>169</sup> Er, <sup>169</sup> Tm, <sup>172</sup> Yb, <sup>175</sup> Lu, <sup>178</sup> Hf, <sup>181</sup> Ta, <sup>182</sup> W, <sup>202</sup> Hg, <sup>203</sup> Tl, <sup>208</sup> Pb, <sup>209</sup> Bi, <sup>232</sup> Th, <sup>238</sup> U				In-house reference cannabis samples		
Sapphires	<sup>7</sup> Li, <sup>11</sup> B, <sup>23</sup> Na, <sup>39</sup> K, <sup>27</sup> Al, <sup>38</sup> Si, <sup>39</sup> K, <sup>49</sup> Ti, <sup>51</sup> V, <sup>52</sup> Cr, <sup>55</sup> Mn, <sup>56</sup> Fe, <sup>59</sup> Co, <sup>60</sup> Ni, <sup>65</sup> Cu, <sup>66</sup> Zn, <sup>75</sup> Ga, <sup>72</sup> Ge, <sup>85</sup> Rb, <sup>88</sup> Sr, <sup>89</sup> Y, <sup>90</sup> Zr, <sup>97</sup> Nb, <sup>98</sup> Mo, <sup>120</sup> Sn, <sup>133</sup> Cs, <sup>138</sup> Ba, <sup>139</sup> La, <sup>140</sup> Ce, <sup>141</sup> Pr, <sup>159</sup> Tb, <sup>165</sup> Ho, <sup>169</sup> Tm, <sup>172</sup> Yb, <sup>178</sup> Hf, <sup>181</sup> Ta, <sup>182</sup> W, <sup>208</sup> Pb, <sup>232</sup> Th, <sup>238</sup> U	Compex 110i ArF excimer, (193 nm)	Perkin Elmer Elan 6100 DRC	LA: D: 120 µm, F: 10 Hz, E: 6 mJ cm <sup>-2</sup> , V: 20 cm <sup>3</sup> , AT: 2 s, AM: Single spot ICP-MS: RFP: 1500 W, GTC: (He) 1.2 L min <sup>-1</sup> , GAC: (Ar) 0.85 L min <sup>-1</sup> , PG: 14.8 L min <sup>-1</sup>	NIST 612	Ternary diagrams; PCA	[82]
Rubies and Sapphires	Be, Ti, V, Cr, Mn, Fe, Ga, Al	New-Wave UP-213 (213 nm)	Agilent 7500a	LA: D: 120 µm, F: 20 Hz, E: 200 mJ, AM: Single spot, ablation	NIST 612		[83]
Diamonds	<sup>13</sup> C, <sup>202</sup> Hg, <sup>208</sup> Pb, <sup>58</sup> Ni, <sup>64</sup> Zn, <sup>121</sup> Sb, <sup>23</sup> Na, <sup>120</sup> Sn, <sup>27</sup> Al, <sup>47</sup> Ti	GeoLas ArF excimer (193 nm)	Perkin Elmer 6100	LA: D: 120 µm, F: 20 Hz, V: 30 cm <sup>3</sup> , AT: 30 s ICP-MS: RFP: 1200 W, GAC: (Ar) 1.3 L min <sup>-1</sup> , PG: (Ar) 17 L min <sup>-1</sup> , AG: (Ar) 1.2 L min <sup>-1</sup>	NIST 612	Ternary diagrams; PLS; CA.	[84]
Diamonds	<sup>7</sup> Li, <sup>9</sup> Be, <sup>11</sup> B, <sup>13</sup> C, <sup>23</sup> Na, <sup>27</sup> Al, <sup>29</sup> Si, <sup>31</sup> P, <sup>39</sup> K, <sup>45</sup> Sc, <sup>47</sup> Ti, <sup>51</sup> V, <sup>55</sup> Mn, <sup>59</sup> Co, <sup>60</sup> Ni, <sup>63</sup> Cu, <sup>66</sup> Zn, <sup>69</sup> Ga, <sup>85</sup> Rb, <sup>88</sup> Sr, <sup>89</sup> Y, <sup>90</sup> Zr, <sup>93</sup> Nb, <sup>95</sup> Mo, <sup>107</sup> Ag, <sup>111</sup> Cd, <sup>115</sup> In, <sup>118</sup> Sn, <sup>121</sup> Sb, <sup>133</sup> Cs, <sup>137</sup> Ba, <sup>139</sup> La, <sup>140</sup> Ce, <sup>141</sup> Pr, <sup>140</sup> Nd, <sup>147</sup> Sm, <sup>152</sup> Eu, <sup>157</sup> Gd, <sup>163</sup> Dy, <sup>166</sup> Hg, <sup>169</sup> Er, <sup>172</sup> Yb, <sup>175</sup> Lu, <sup>178</sup> Hf, <sup>181</sup> Ta, <sup>182</sup> W, <sup>208</sup> Pb, <sup>209</sup> Bi, <sup>232</sup> Th, <sup>238</sup> U	GeoLas <sup>IM</sup> 193 nm ArF excimer	Thermo Element 2	LA: D: 17, 250, 333 µm, F: 6, 10 Hz, E: 1, 5, 6.1 cm <sup>2</sup> , V: 22.2 cm <sup>3</sup> , AM: Single spot ICP-MS: RFP: 950-1200 W, PG: 16 L min <sup>-1</sup> , AG: 0.7 L min <sup>-1</sup> , GTC: (He) 0.70 L min <sup>-1</sup> , GAC: (Ar) 1.035 L min <sup>-1</sup>	CRM-S2150 oil and NIST 625 glass	LDA, BLR	[85]
Ocher	Sc, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Y, Zr, Nb, Mo, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, S, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Tl, Pb, Bi, Th, U	CETAC LSX 200. (266 nm)	VG Plasma Quad III	LA: D: 100 µm, F: 10 Hz, AT: 90 s, AM: Raster.	NIST 610	Ternary diagrams; PCA; CNP.	[86]

**Table 7 (continuation)**

Experimental conditions of main applications of LA-ICP-MS to the analysis of other samples of forensic interest

Sample type	Elements/isotopes	Laser	ICP-MS	Instrumental parameters	Standard reference material	Chemometric processing	Ref.
Oriental porcelain	<sup>90</sup> Yb, <sup>35</sup> Cr, <sup>66</sup> Zn, <sup>88</sup> Sr, <sup>100</sup> Sn, <sup>121</sup> Pr, <sup>139</sup> Pb, <sup>172</sup> Yb, <sup>205</sup> Tl, <sup>44</sup> Ca, <sup>55</sup> Mn, <sup>69</sup> Ga, <sup>89</sup> Y, <sup>121</sup> Sb, <sup>146</sup> Nd, <sup>162</sup> Dy, <sup>175</sup> Lu, <sup>208</sup> Pb, <sup>45</sup> Sc, <sup>39</sup> Co, <sup>73</sup> Ge, <sup>90</sup> Zr, <sup>137</sup> Ba, <sup>152</sup> Sm, <sup>165</sup> Ho, <sup>176</sup> Hf, <sup>209</sup> Bi, <sup>90</sup> Tl, <sup>69</sup> Ni, <sup>72</sup> As, <sup>93</sup> Nb, <sup>139</sup> La, <sup>151</sup> Eu, <sup>166</sup> Er, <sup>181</sup> Ta, <sup>232</sup> Th, <sup>51</sup> V, <sup>65</sup> Cu, <sup>85</sup> Rb, <sup>88</sup> Mo, <sup>140</sup> Ce, <sup>158</sup> Gd, <sup>169</sup> Tm, <sup>184</sup> W, <sup>238</sup> U	CETAC LSX-200 (266 nm)	VG Plasma Quad III	LA: D: 100 µm, F: 10 Hz, E: 32 J cm <sup>-2</sup> , AT: 60 s, SS: 30 µm s <sup>-1</sup>	NIST 610 In house Spode Italian Olive Dish (2004/SP1)	PCA	[87]
Brick stone	Al, Ba, Ca, Ce, Co, Cr, Hf, K, Mg, Mn, Na, Rb, Si, Sr, Ti, Th, Zn	New-Wave UP-213 (213 nm)	Agilent 7500cs	LA: D: 80 µm, F: 10 Hz, E: 10 J cm <sup>-2</sup> . ICP-MS: RFP: I390 W, GTC: (He) 1 L min <sup>-1</sup> , GAC: (Ar) 1 L min <sup>-1</sup> , PG: (Ar) 14 L min <sup>-1</sup> , AG: (Ar) 0.9 L min <sup>-1</sup>	NIST SRM 679, 98b, 97b	PCA; CA.	[88]
Silver antiques	<sup>66</sup> Zn, <sup>107</sup> Ag, <sup>197</sup> Au, <sup>208</sup> Pb, <sup>110</sup> Cd, <sup>117</sup> Sn, <sup>125</sup> Sb, <sup>209</sup> Bi	Spectra-Physics (532 nm)	Perkin Elmer ELAN 6000	LA: D: 100 µm, F: 10 Hz, AM: Single spot. ICP-MS: RFP: 1050 W, PG: 15 L min <sup>-1</sup> , AG: 0.8 L min <sup>-1</sup> , GTC: (Ar) 1.0 L min <sup>-1</sup>	Three silver calibration standards: VEB Bergbau- und Hüttenkombinat (Freiberg, Germany) NIST 681 (Pt metal)		[89]
Platinum	Co, Ni, Cu, Zr, Ru, Rh, Ag, Pd, In, Sn, Hf, Ir, Pb, U	CETAC LSX-200 (266 nm)	Perkin Elmer ELAN 6000	LA: D: 300 µm, F: 20 Hz, E: 1.9x10 <sup>8</sup> W cm <sup>-2</sup> , AM: Single line scan. ICP-MS: RFP: 1000 W, PG: 14 L min <sup>-1</sup> , AG: 0.8 L min <sup>-1</sup> , GTC: (Ar) 0.78 L min <sup>-1</sup>			[90]

AG, auxiliary gas flow; AM, ablation mode; ANOVA, analysis of variance; AT, ablation time; D, crater diameter; E, energy density; F, laser pulse frequency; GAC, gas flow after the ablation cell; GLM, general linear model; GTC, gas flow through ablation cell; IT, integration time; LDA, linear discriminant analysis; MANOVA, multivariate analysis of variance; Tukey's HSD, Honestly Significant Different; MSE, mean square error; PCA, principal component analysis; PG, plasma gas flow; RFP, radio frequency matching; RPP, radio frequency power; SS, scan speed; V, ablation cell volume.



### 3.3.1 Ink and paper

The elemental analysis of inks and document papers is of forensic interest in crimes committed (e.g., falsified or counterfeit documents, threatening letters and ransom notes) and signature authentication and identification. The questions to answer may be quite different, depending on the information required:

- To determine the authenticity of these documents.
- To identify the source of questionable ink or paper.
- To compare the ink and paper of different documents.
- To establish a common origin of different documents or inks.
- To discriminate between different ink manufacturers.
- To determine the printer used to print the document.
- To date documents [77].

Forensic document examination usually involves:

- Study of gross physical properties of paper (e.g., strength, thickness, weight, fiber content, color and fluorescence) [73].
- Visual and microscopic examination of the questionable paper, and chemical analysis, usually by SEM-EDX and IR spectroscopy or by acid digestion-ICP-MS (for studying the elemental composition).

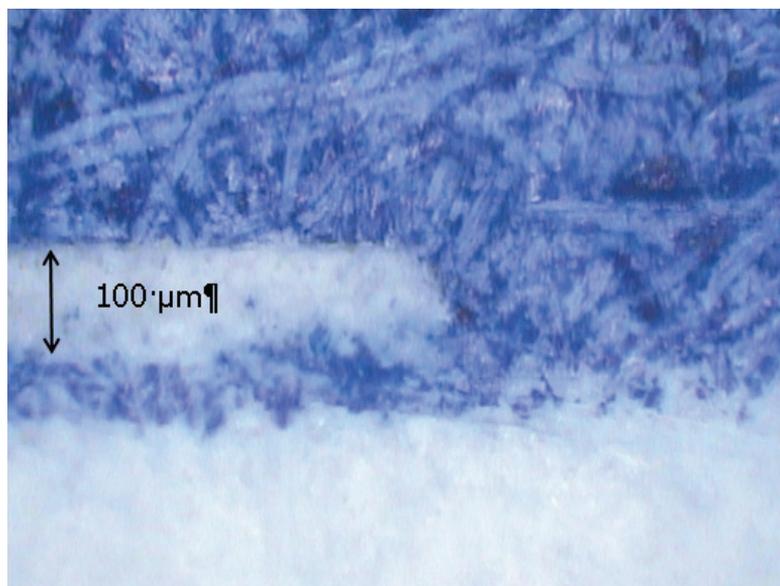
Inks of writing instruments are mainly composed of colorants (mainly organic), a vehicle (organic solvents or water) and additives

(e.g., resins and viscosity adjusters). Ink-chemical analysis may be accomplished by nondestructive or minimally destructive techniques of the extracted organic dyes [e.g., spectrophotometric methods (e.g., FTIR, Raman spectroscopy, or micro-spectrophotometry), thin-layer chromatography (TLC) or high performance liquid chromatography (HPLC)]. In both cases, a particular problem is found: the difficulty to definitively match one document paper/ink to another, due to the similar characteristics of inks and papers available on the market.

XRF seems to be a very suitable technique for the nondestructive analysis of historic inks. Despite its lower sensitivity, XRF is characterized by a good discriminating capability. However, the elemental profiling of inks and paper by LA-ICP-MS constitutes nowadays an effective, practical and robust alternative for comparison, identification and discrimination of document papers and inks. As stated for other forensic samples, two of the most valuable characteristics of this technique for analyzing this type of evidence are:

- There is no need for document destruction or ink extraction from the document.
- Minimum mass removal of samples during LA. This latter characteristic is illustrated in [Fig. 9](#), which shows how the laser may remove only 9–15 µg of paper from microscopic areas, leaving the rest of paper almost intact after the analysis [\[68\]](#).





*Fig 9. Outline of ink in paper image, after ablation.*

The best ablation mode for ink sampling, without ablating through the document, has been the raster or scan mode since the penetration depth of the laser is limited to few microns. In such experimental conditions, the analysis of inks and papers exhibits good sensitivity and precision (<10–15% RSD, depending on the elemental concentration level) [68]. However, as for other forensic samples, ink analysis is limited by the lack of matrix-matched standards with certified element concentrations. For this reason, in some studies, in-house matrix-matched standards have been created, using the Whatman 42 filter paper as the support matrix in which the ink was embedded [68].

Van Es et al. [78] evaluated the potential of XRF, LAICP-MS and isotope-ratio MS (IRMS), using multivariate statistical techniques for the discrimination of document papers. With this purpose, three

different areas of each paper were selected for analysis. For LA-ICP-MS analysis (51 elements and nuclides determined), relative intensities were calculated using  $^{88}\text{Sr}$  as the IS, since this isotope is found in all paper samples in a similar concentration and it is uniformly distributed over a sheet of paper. The authors concluded that, in spite of the high discriminant power and great sensitivity of LA-ICP-MS, the combination of the three studied elemental techniques was more effective for differentiating inks.

Qualitative and quantitative LA-ICP-MS and LIBS methods have been developed and optimized for the elemental analysis of multi-use paper, ballpoint ink and gel-pen inks [68]. More than 200 pens and 20 different types of paper (seven different brands, manufactured at 10 different plants, all in USA) were studied. To simplify the analyses, elements with higher discrimination power were selected according to the following criteria:

- Good precision between replicates (<15%).
- Homogeneous distribution within the sample.
- Concentration above the LOD.
- Low percentage of error (false inclusions or exclusions).
- Lower variability within a source (package) than between sources (brands).

These elements were present at minor and major concentration levels in paper while, in the case of inks, discriminators were present at trace level. External calibration and standard-addition methods were used to characterize the ink standards. Internal standardization with the





low-abundance carbon isotope of cellulose ( $^{13}\text{C}$ , 1.1%) and background correction from the paper were required. In these conditions, significant, detectable differences (>98%) were observed for multi-purpose papers from different sources. Also, discrimination of ~96–99% of pen markings from different brands and models was possible.

Finally, a recent work of Szyrkowska et al. [79] described the application of ICP-time-of-flight MS (LA-ICP-TOF-MS) for the forensic discrimination of photocopy and printer toners. The samples studied were black (201 samples) and colored (23 samples) toners produced by different manufacturers. The samples analyzed were both powdered and printed in documents. With the aid of chemometric analysis, it was possible to find the characteristic elements of the toners of different manufacturers and to discriminate between them.

### 3.3.2 Fibers

Fibers are physical trace evidence that may be recovered due to primary transfer (suspect's clothing) or secondary or tertiary transfer (suspect's home or environment). Usually, they are a few mm long and  $\mu\text{m}$  in diameter.

Micro-spectrophotometry is the technique routinely used to analyze fibers. However, this technique presents some drawbacks:

- Fibers containing polymers (e.g., silk, polyester and acrylics, acetates, or nylon) are not suitable for UV analysis below 300 nm, due to the polymer absorbance at this spectral region.
- The spectra of color fibers must be compared to the reference spectra of non-color fiber to test if the dye contributes to the absorbance.

In 2009, Gallo et al. [80] used microwave-digestion ICP-MS and LA-ICP-MS to develop and to validate new methods capable of increasing the discrimination among cotton fibers, since cotton plants grown in different geographic regions may present different trace-metal profiles due to soil nutrients, water content and type of fertilizers used. A cotton standard reference material (IAEA V-9) was used to validate the two methods and 24 raw cotton samples and five white cotton T-shirts were analyzed. Preparation of cotton samples for LA-ICP-MS analysis implied the formation of sample pellets, with the addition of Ge as IS.

According to the results, LA-ICP-MS offered the following advantages over the dissolution prior to an ICPMS method: better accuracy (<10% bias); higher precision (typically <5% RSD) for  $^{25}\text{Mg}$ ,  $^{27}\text{Al}$ ,  $^{55}\text{Mn}$ ,  $^{57}\text{Fe}$ ,  $^{88}\text{Sr}$  and  $^{137}\text{Ba}$ ; shorter analysis time; low sample consumption; and its pseudo non-destructive character.

With the optimized LA-ICP-MS method, cotton sources from different origins were distinguishable from each other. Also, it was possible to associate cotton sources having a common origin.

### 3.3.3 Cannabis

In 1998, Watling [81] developed an LA-ICP-MS method to obtain the location of cannabis crops based on their elemental composition. Cannabis samples were ground to a fine powder under liquid nitrogen and pressed to obtain a pellet of micro-homogeneous composition. Reference glasses from NIST were used to optimize the instrument and four in-house reference standards were prepared by the authors, since commercial matrix-matched cannabis standards are not





available. The matches between samples were accomplished by means of ternary plots, profiles of signal intensity, index percentage fit and histogram plots obtained in the same analytical conditions. The main innovation of this work was that the authors used the “fingerprint” of the elemental association patterns rather than the traditional element concentrations to determine the provenance of cannabis samples. This strategy was successfully applied to samples from bulk batches of cannabis obtained during police drugs raids.

### 3.3.4 Gems

Two of the most famous gemstones, ruby and sapphire, are varieties of corundum ( $\text{Al}_2\text{O}_3$ ). Red corundum is called ruby, while all other colors are considered sapphire. Sapphires contain 97–99% of  $\text{Al}_2\text{O}_3$  and the remaining components are several trace elements, which can be used to distinguish the origin of these gemstones [82].

Rubies and sapphires can be heat treated to enhance their color and thus their value. This treatment has been accepted by the trade, and, currently, there are analytical methods to differentiate treated gemstones from untreated natural or synthetic ones [83]. A new treatment of these gemstones applied in the industry adds the element beryllium during the heating process, mainly for sapphire. The gemstone is modified to resemble one of the most valuable colored stones: *padparadscha* sapphire. Some manufacturers do not disclose the gems as “Be-treated”, referring to them only as “heat treated”, which represents a fraud [83]. All these aspects have applications in the forensics field to identify falsifications.

In 2001, Guillong et al. [82] compared traditional optical microscopic techniques and XRF with LA-ICP-MS to establish the source of sapphires from different locations (25 sapphire samples from five different locations). The results showed that the XRF method had limitations, due to its low sensitivity and its inability to determine some light elements. For LA-ICP-MS, in order to increase the reproducibility of the analysis, the surface of the samples was cleaned prior to their analysis with three single laser pulses. A total of 20 laser pulses in the same pit were sufficient to characterize the important trace elements (Mg, Si, Ti, V, Cr, Fe, and Ga) used to distinguish the different groups of sapphires. The mean amount of sample ablated in each analysis was about 55 ng. Calibration was carried out using NIST 612 glass reference material as external standard and Al, the major element in sapphire, as IS. The chemometric analysis of the results by PCA and the use of ternary plots made it possible to distinguish the five different groups of sapphires.

Resano et al. [84] evaluated the performance of LA-ICP-MS in fingerprinting 31 diamonds originating from four different deposits. The authors used a homogenized 193 nm excimer laser with a flat-top beam profile and ablated each diamond on eight different spots for 30 s. The total mass ablated from each diamond was 16  $\mu\text{g}$ . Nine elements (Al, Hg, Na, Ni, Pb, Sb, Sn, Ti and Zn) were used for fingerprinting purposes, and, with the aid of chemometric techniques, the authors obtained promising results in classifying the samples.

Watling et al. [53] suggested the use of the LA-ICP-MS technique to help police forces in tracing the origin of stolen material and establishing the trace-element distribution as an indicator for diamond exploration. Statistical analysis of data obtained from the





semiquantitative determination of 43 trace elements in diamonds from five countries gave distinctive patterns for each source.

Dalpé et al. [85] applied LA-ICP-MS for analyzing ultra-trace impurities in rough diamond for profiling. With this aim, the authors analyzed diamonds from two sources and applied two statistical techniques – linear discriminant analysis (LDA) and binomial logistic regression. The main conclusion of this study was the need to obtain a good reference material to compare diamond pools analyzed over an extended time period or to compare results between laboratories.

### 3.3.5 Australian ocher and porcelain

Pigments used in Australian indigenous art, known as Australian ochers, consist primarily of oxides and hydrated oxides of Fe, Si, Al and Mn with alumino-silicate inclusions of K, Na, and Li; rare earths are also present in their composition. The distribution pattern of these elements used to be specific of the region of provenance. This type of art involves large amounts of money and its growth in popularity correlates with both its growth in the “Black Market” and the production of fraudulent copies [86].

In 2007, Green et al. [86] developed an LA-ICP-MS method for elemental differentiation and a definitive, accurate establishment of the provenance of Australian ochers and the artworks created using them. They studied 36 samples of ocher, collected from across northern Western Australia and Northern Territory and 36 samples of ocher obtained from 11 different artworks. The data obtained were processed using statistical and chemometrics procedures as ternary diagrams, PCA, search/match procedure developed by Watling [55]. The results

showed that the trace-metal distribution patterns alone were sufficient evidence to establish the provenance of specific ochers.

The sale of fraudulent oriental ceramics constitutes a large proportion of the illegal artifact and antique trade. Due to the increase in the quality and the quantity of falsifications, it is necessary to develop scientific and robust methods for the authentication of ceramics [85]. In 1997, Bartle et al. [87] evaluated the potential of LA-ICP-MS for determining the provenance of Chinese, English and Japanese porcelain with respect to geographical origin. Isotope-distribution patterns were established and used to classify the pieces of art according to their country of manufacture. Detailed statistical analyses of data by PCA confirmed statistically significant differences in trace-element composition of porcelains from different geographical origins.

### 3.3.6 Brickstones

Building materials (e.g., brick stones) are also of forensic interest, since they can be used as evidence to associate a crime scene to a suspect or to another crime scene. Scheid et al. [88] showed the potential of elemental analysis of brick stones in forensic examinations. The techniques used were: LA-ICP-MS, instrumental NAA (INAA) and XRF. The samples studied were several brick stones from one production batch, several brick stones from different producers, and brick stones of unknown origin. Sample preparation utilizing borate fusion, followed by matrix matching, was used for XRF and LA-ICP-MS analyses. Three standard reference materials, brick clay (NIST 679), plastic clay (NIST 98b) and flint clay (NIST 97b), were used to evaluate the performance of the methods developed. As for LA-ICP-MS analysis, recoveries of 96–105% were obtained for 16 elements;





worse recoveries were obtained for Zn (64%), Hf (89%) and Na (113%). These techniques made possible the characterization of brick stones in order to compare them with each other.

### 3.3.7 Gold and silver

Gold and silver have long been regarded as a measure of the economic power, for both individuals and nations. Moreover, most jewels and antique objects are made of these metals. From a forensic point of view, it is important to verify the authenticity of these objects and to identify forgeries. In addition to visual and physical examination of these samples, which is accomplished by trained experts, the determination of minor and trace element patterns is very interesting. These elements come from the metallurgical manufacturing and refinement processes [89].

Modifications of the technique of LA-ICP-MS and the use of appropriate software for interpreting the results have aided the establishment of the sources of modern and ancient gold. By comparing elemental associations, it has been possible to relate gold to a specific mineralizing event, mine or bullion sample or to facilitate the identification of the provenance of stolen gold [91,52,92].

Devos et al. [89] have proposed the use of an alternative cell design for LA-ICP-MS that is placed upon antique silver objects too large to fit into a normal ablation cell. In this way, small amounts of sample (about 3  $\mu\text{g}$  per crater; three craters per sample) were ablated. Quantitative analyses of  $^{66}\text{Zn}$ ,  $^{107}\text{Ag}$ ,  $^{111}\text{Cd}$ ,  $^{117}\text{Sn}$ ,  $^{121}\text{Sb}$ ,  $^{197}\text{Au}$ ,  $^{208}\text{Pb}$  and  $^{209}\text{Bi}$  were carried out by calibration with external standards and  $^{107}\text{Ag}$  as IS. This method dated antique silverware.

## 4 Graphical and statistical processing of data

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As stated above, LA-ICP-MS provides a chemical fingerprint of the analyzed materials in forensic applications. Given the great amount of information (variables) obtained with this technique for different samples (objects), raw data must be treated statistically. Analytical signals for every element can be expressed as an average value and its standard deviation. However, even in this simplified form, the matrix of average values is large enough to obtain detailed information on samples by direct data visualization. For this reason, statistical data handling is necessary to assist forensic decision-making. We briefly describe the graphical and statistical techniques most often employed in LA-ICP-MS.

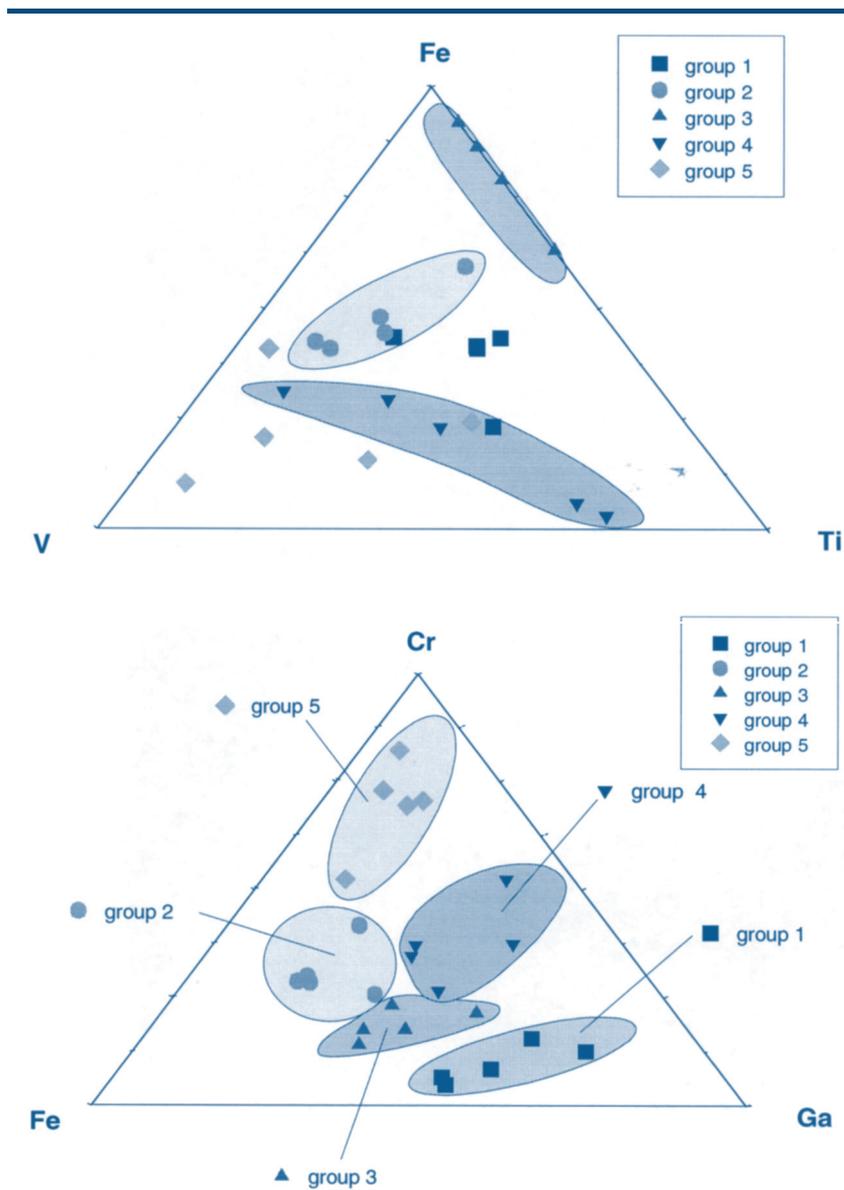
### 4.1 Ternary diagrams as graphical tools

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A ternary diagram is a triangular flat representation of three variables (in LA-ICP-MS, three isotopes) on the three axes of an equilateral triangle. Before constructing these diagrams, the data should be scaled. With these graphs, it is possible to group samples with similar characteristics. The choice of these three elements is fundamental to establish subtle differences in sample populations, especially when a multiple data-point display is obtained [17,55]. For this reason, the main drawback of these diagrams is the large number of possible combinations of elements that must be tested in order to find the most interesting information [82].

This graphical technique has been applied to the interpretation of results corresponding to float, sheet and container glass samples [55], cannabis [81], diamonds [84], Australian ocher [86] and sapphires [82].





*Fig 10. Ternary plots for 25 sapphires from five different origins, analyzed by LA-ICP-MS. The shadow regions show the predicted areas in which samples from specific origins are expected to be found [82].*

The important information given by these graphical tools is illustrated in **FIG. 10**, which shows how ternary diagrams Ga-Fe-Cr and Ti-V-Fe, corresponding to LA-ICP-MS results obtained for 25 sapphires, allow separation of these samples into five different groups (Ga-Fe-Cr plot), in agreement with their five different origins. Interestingly, the Ti-V-Fe diagram clearly separates groups 2, 3 and 4 from each other in a better way than the other diagram. In this work, raw data were normalized using the standard deviation of the concentration of the element.

## 4.2 Basic statistical analysis

Traditional statistical tools applied to data obtained in LA-ICP-MS analysis of forensic samples are:

- The well-known statistical t-test.
- One-way analysis of variance (ANOVA) or multiple analysis of variance (MANOVA).
- The honestly significant difference post-hoc test (Tukey's HSD), which is a single-step multiple-comparison procedure and statistical test, generally used in conjunction with ANOVA to find which means are significantly different from one another.

These statistical tools have widely been employed to discriminate between forensic samples {e.g., glass [2,50,58,63,64,66], cotton fibers [80], inks and papers [68], and document papers [77]} with low rates of false inclusions.





Recently, Weis et al. [67] published a new matching criterion useful for forensic comparison of elemental analysis of two sets of float-glass samples. Two dataset samples were analyzed by LA-ICP-MS:

A. dataset A, containing 34 pieces from one float glass pane with the size of 122 cm 122 cm; and,

B. dataset B, with 62 float glass samples from different countries, manufacturers and production lines, as well as glass samples from the same production line with different production dates.

For all these samples, 18 elements were determined: Li, Na, Mg, Al, K, Ca, Ti, Mn, Fe, Rb, Sr, Zr, Ba, La, Ce, Nd, Hf, and Pb. Weis et al. [67] selected a modified sigma criterion with fixed relative standard deviations, which minimized the type 1 (false positive) and type 2 (false negative) error rates in their analytical conditions. This new method was presented as an alternative to other statistical treatments, which were not good enough for comparison of two glass samples of unknown origin or for reduced replicate measurements of each of two samples [e.g., multivariate techniques such as PCA and cluster analysis (CA), and Hotelling's  $T^2$ -test (a multivariate equivalent of Student's t-test)]. In this work, the fixed relative standard deviation filter was established between 3.0% and 8.9%, depending on the element determined. These values were taken from the 90 determinations of the quality standard float glass DGG1 (Deutsche Glastechnische Gesellschaft, Germany). Good compromise values were found using a modified 4 sigma match criterion. Low relative standard deviations (1.04% for type 1 error and 0.11% for type 2 error) were calculated.

## 4.3 Multivariate statistical analysis

### 4.3.1 Principal Component Analysis (PCA)

PCA is an unsupervised pattern-recognition technique (no labeling or class information is available) aimed at data reduction. PCA is useful for a set of data with a large number of variables somewhat redundant. After applying this technique, a smaller number of variables [termed principal components (PCs)], uncorrelated and representing maximal variance of the overall data, are obtained. Then, the dataset for each sample may be explained by a reduced number of new variables (PCs) [93]. Each PC is a linear combination of the original variables, the PC loadings being the coefficients representing the size of the contribution of each variable to the PC and the scores, the coordinates of samples in terms of PCs. Very often, two or three PCs account for maximal variance of data; in this way, 2D or 3D graphs, obtained by representing two or three PCs can be constructed. These graphs, in which clouds of points identifying samples with similar characteristics are displayed, aid visualization of groups of multivariate data sets. Moreover, the most discriminant variables of the dataset are also identified. Data preprocessing (e.g., mean centering and autoscaling) used to be recommended to eliminate interferences and noise in raw data. In LA-ICP-MS analysis, these approaches to data pre-processing commonly consist of peak-area integration and background subtraction [43,65].

PCA has been widely applied to the interpretation of results corresponding to the analysis of glass samples [43,56,65], document papers [78], cotton fibers [80], sapphires [82], diamonds [84], ocher





[86], oriental porcelain [87], brick stones [88], and inks and papers [68].

Bajic et al. [65] have emphasized the importance of this multivariate technique in forensic glass analysis for positive association of glass samples being questioned based on their elemental composition.

Van Es et al. [78] applied PCA to compare document papers based on measurements of 51 elements and isotopes in 25 different paper sheets from European markets; in this case, intensities were normalized to  $^{88}\text{Sr}$ .

Trejos et al. [68] also applied PCA to the multielemental composition of paper sources. Thanks to this technique, the authors differentiated paper sheets from the same mill and fabricated at the same time.

Aeschliman et al. [43] used PCA and the Q-statistic for fault detection and diagnosis to compare glass samples with others (e.g., multi-stranded copper-wire fragments and NIST steels) analyzed by LA-ICP-MS. The Q-statistics used in this work was defined as the residual between isotopic signals from one sample and their projection into the PCA model. In addition, the authors applied this statistical technique as a tool for diagnosis and minimization of fractionation in homogeneous solid samples.

Guillon and Günther [82] have compared the grouping ability of ternary diagrams with that of PCA. Although they may be complementary data-treatment techniques, PCA had the advantage of reducing many variables to a few relevant variables. Aeschliman et al.

[43] also highlighted, as an advantage of PCA, that there is no need for trial-and-error selection of the diagnostic elements.

Finally, we should mention that, when Gallo and Almirall [80] compared PCA and the t-test to distinguish between different cotton samples and to associate samples originating from the same source, they found that the t-test worked better than PCA.

PCA was also used to interpret the results obtained in the isotopic analysis of photocopy and printer toners [79]. Mean values of five replicates of the strongest isotope signal for each element were considered the raw data, which were then standardized before applying chemometric techniques. According to the authors, only two PCs explained ~87% of the total variance of data and allowed satisfactory discrimination of toners of different producers.

### 4.3.2 Cluster Analysis (CA)

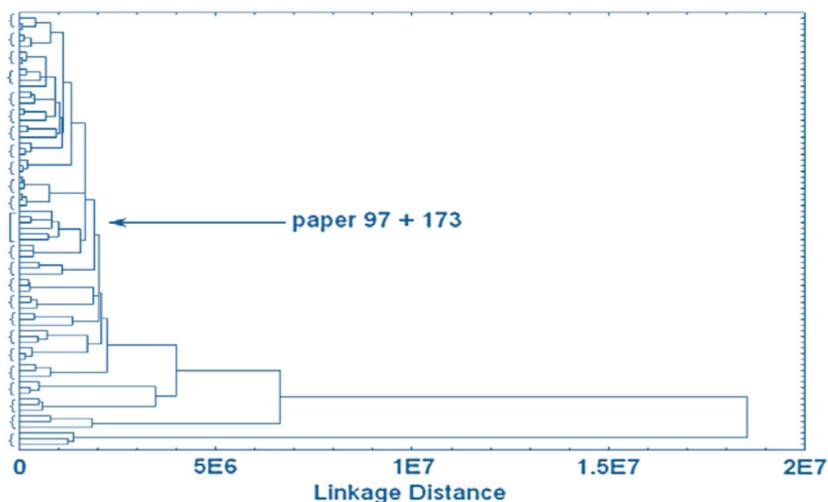
CA is a multivariate unsupervised pattern-recognition technique designed to form groups (clusters) of samples with similar characteristics in the profile of their variables (elements). Sometimes, PCA analysis is the first step and then CA is applied to the principal factors from PCA as input variables. The most used rule in the forensic field to determine when two clusters are similar enough to be linked together is Ward's method, based on minimizing the within-group variance. The CA can be shown as an easy, intuitive interpretation of results in a dendrogram (tree diagram). Dendrograms list all the samples in one axis while the other axis gives a measure of the similarity at which clusters join. These plots are very useful and accepted to present information in a Court of Justice.





CA has been employed for the analysis of samples of cotton fibers [80], paper documents [78], photocopy and printer toners, according to their producers [79], diamonds [85] and brick stones [88].

Van Es et al. [78] evaluated by CA (Euclidean distance, single linkage) the LA-ICP-MS results obtained for a set of 25 office papers from the European market, from which three areas (top, middle and bottom) were sampled and 51 elements and isotopes were determined. The resulting dendrogram (Fig. 11) shows that 23 of the 25 paper types (92%) could be separated as triplicates. Moreover, Scheid et al. [88] applied CA, together with PCA, for the interpretation of the elemental concentration data obtained in the forensic analysis of brick stones by LA-ICP-MS. The variability of elemental concentrations was significantly greater between stones from different producers than between stones from the same production batch.



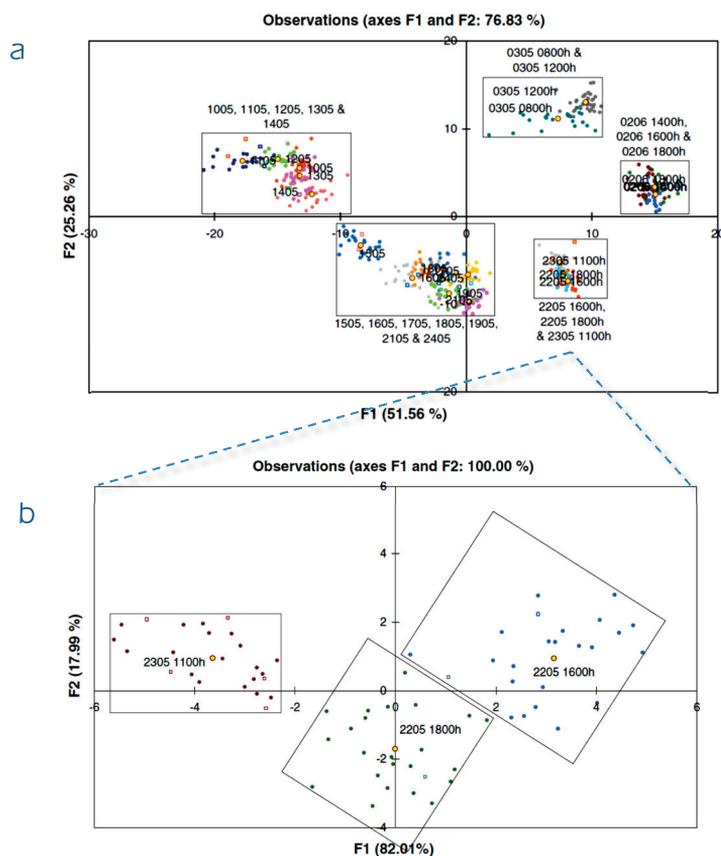
*Fig 11. Cluster Analysis applied to results obtained by LA-ICP-MS for 25 papers of different types that are separated as triplicates, according to measurements made at the top, middle and bottom of the A4 sheet [78].*

### 4.3.3 Linear Discriminant Analysis (LDA)

LDA is a supervised pattern-recognition technique that seeks to analyze and to classify a set of samples (with associated labels, the class types) into predefined classes. With this aim, new variables (linear discriminant functions) are formed as combinations of the original variables, in such a way that these functions provide the best discrimination among groups by maximizing the ratio of the “between-group dispersion” to the “within-group dispersion”. Once the discriminant functions are obtained, a new external sample can be assigned to one of the groups previously established. In LA-ICP-MS, the original variables are the intensity signals corresponding to each element analyzed.

This statistical technique has been applied to discriminate between two glass bottles manufactured in the same plant at different time and date of manufacture [56]; with this aim, 38 trace elements determined by LA-ICP-MS were used. With the model constructed, 77% of the overall samples used to validate the model were correctly classified. **FIG. 12** illustrates the separation using LDA of 20 glass bottles, of known time and date of manufacture, into five different groups (**FIG. 12a**); in this case, the data set consisted of 24 replicate analyses per bottle. Interestingly, for a given group (bottles manufactured on 22 and 23 May 2003), the separation in three different sub-groups (**FIG. 12b**), according to the time of manufacture (16:00 h and 18:00 h on 22 May 2003 and 11:00 h on 23 May 2003) was possible. The authors also emphasized the success of combining PCA and LDA for better interpretation of data.





**Fig 12.** Discriminant Analysis applied to results obtained by LA-ICP-MS for green glass bottles of five distinct groups, (a) according to time and date of manufacture, and (b) detailed separation of data associated with glass samples manufactured at 16:00 and 18:00 h on 22 May 2003 and at 11:00 h on 23 May 2003 [56].

Dalpé et al. [85] compared the validity of two statistical techniques [i.e. binomial logistic regression (BLR) and LDA] to achieve a good classification of diamonds from two different sources. According to these authors, BLR (>91% correct classification) was preferred to LDA (overall correct classification >86.7%). The main reason to explain this conclusion was that the BLR model does not

assume the normal distribution of variables, collinearity between variables and equal within-group variances.

Van Es et al. [78] applied this technique to discriminate among 25 different multi-purpose office papers by LA-ICP-MS, XRF and IRMS. They found that the combination of LA-ICP-MS and IRMS easily discriminated all 25 paper types. In addition, thanks to this statistical technique, the authors found the most discriminating elements for this type of samples,  $^{208}\text{Pb}$ ,  $^{55}\text{Mn}$ ,  $^{28}\text{Si}$ ,  $^{182}\text{W}$ ,  $^{97}\text{Mo}$ ,  $^{137}\text{Ba}$  and  $^{238}\text{U}$  being the most important.

## 5 Conclusions and future trends

LA-ICP-MS is an effective technique for the direct analysis of solids without requiring their dissolution. LA-ICP-MS is especially recommended to overcome the limitations regarding the sample size generally associated with forensic analysis. Its quasi non-destructive nature also allows that samples already analyzed by LA-ICP-MS to be available for complementary analysis. In addition, its excellent sensitivity, accuracy and precision, combined with its capacity for isotopic and multielemental analysis, offer an excellent power of discrimination to classify samples.

LA-ICP-MS shows great potential for the forensic analysis of glass samples. Colorless glass fragments, which are indistinguishable to the human eye and identical at the trace level in their elemental concentrations, can be identified with good accuracy and precision, even when they are of quite small dimensions. In addition, taking into account the availability in the market of reference standard materials [e.g., the NIST glass standards (series 600)], the quantitative analysis of unknown glass samples can be accurately performed. LA-ICP-MS is





also very promising technique for characterizing paint samples of forensic interest, especially those found after car accidents. However, some drawbacks are known (e.g., lack of appropriate standards, heterogeneity of samples, instrumental drift, and mixture of elements among paint layers).

Although the main forensic applications of LA-ICP-MS were developed for glass and paint samples, there are many other applications of forensic interest (e.g., ink and paper, fiber, cannabis, gem, Australian ocher and porcelain, brick stones, and gold and silver). As a consequence, the application of LA-ICP-MS to the sample types covered in this review and to others of forensic interest will be developed in the near future. In addition, the direct representation of results in simple graphical diagrams, making use of suitable software, allows easy discrimination of samples with similar physical and chemical characteristics. Also, the results can be evaluated using appropriate statistical analysis [e.g., basic statistical techniques and multivariate analysis (e.g., PCA, LDA, and CA)], which excludes the subjective judgments of interpretation required by a Court of Justice.

All these characteristics make LA-ICP-MS a useful technique to compare, to characterize and unequivocally to identify physical evidence found at a crime scene with a high level of confidence. Nevertheless, future trends in LA-ICP-MS should overcome the main problems of this technique in quantitative analysis and fractionation. Moreover, improvements in both the optics of the equipment and the design of the sample chamber are needed. Finally, the development of small-scale instruments and the coupling with other instrumental techniques would be also desirable in forensic analysis.

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## Capítulo II

### Forensic analysis of glass samples





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## **Validation of an Analytical Method for the Refractive Index Measurement of Glass Fragments. Application to a Hit-and-run Incident**

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## ABSTRACT

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A method to determine the refractive index (RI) of glass samples was validated in this research article. This validation was performed studying the working range, precision, robustness, and bias as analytical features. Locke silicone oil type B, glass standards type B and a sodium D source (589.3 nm) were chosen for a working RI range from 1.50225 to 1.52381, according to the typical RI values of glass samples of forensic interest. Robustness, calculated as Relative Standard Deviation (RSD) of slopes of ten calibration lines within a period of one month was 0.24%. Precision of the method (calculated for the two extreme glass standards B2 and B12 in the working range) was 0.21% and 0.14% (repeatability) and 0.12% and 0.09% (intermediate precision), respectively. A Certified Reference Material (CRM) of glass of known RI was employed to estimate the bias of the method, verifying a relative error of 0.00066%. The method was applied to eight validation samples (six glass fragments from different parts of an automobile, a glass container and an architectural tinted window), which were differentiated through their RI values. Finally, the procedure was applied to interpret the origin of glass evidence taken from a hit-and-run incident. A common origin of recovered and control glass fragments was supposed on the basis of the RI determination of these samples, together with the application of useful match criteria in forensic pairwise comparisons of glass fragments. Results were confirmed by means of a highly discriminatory technique such as Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS).

## 1 Introduction

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Glass features potential applications worldwide. As a consequence, it commonly appears in criminal acts, such as intended traffic accidents, reckless driving, robberies, murders, etc. The small fragments generated when glass breaks can adhere to the perpetrators clothing or tools used. If these fragments are collected, analyzed and compared, they can play an important role as evidence in court cases.

In the forensic study of glass samples, physical and chemical analyses are complementary tools, especially in those cases where morphological properties of collected glass samples do not allow their discrimination. The refractive index measurement using the oil immersion temperature variation method and a phase contrast microscope have been widely applied for the analysis of forensic glass samples [1-13]. Apart from early studies from the 80s and 90s, which confirmed the variation of RI within different samples, some research has been carried out in the last 10 years. For instance, the variation of RI within sheets of both toughened and non-toughened glass samples has been evaluated, being the RI more disperse in the first one [2]. Other works have concluded that RI values of recovered glass samples (from a suspect) show more dispersion than control glass samples (of known origin) [6]. Furthermore, debris in samples causes variation in their RI values [12]. The effect of glass annealing has also been investigated in glass samples submitted to a controlled heating-cooling program to compare RI before and after the process, being possible the differentiation between toughened and non-toughened glass samples [3-5]. In any case, glass fragments with sharp, fine, and high contrast edges provide the most precise and accurate RI values [13].





Study of glass samples through both RI determination and elemental analysis has also been performed [7-11]. Most of these studies have concluded that these analyses are complementary, not competitive. Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) and ICP-Mass Spectrometry (ICP-MS) allow analysis of metals in glass samples [9, 14-18]. However the main disadvantage is their destructive character, thus being partially replaced by Laser Ablation-ICP-MS (LA-ICP-MS). This quasi non-destructive technique allows the direct analysis of glass samples in a short time [8, 19, 20-25]. In other research X-Ray Fluorescence (XRF) techniques have been used [20, 26, 27]. In comparison with ICP-MS, XRF has a non destructive nature, is easier to use and less expensive. Recently, Laser Induced Breakdown Spectroscopy (LIBS) has become an emerging technique in this field, since it is fast, non destructive, and it needs minimal sample preparation, achieving a similar sensitivity to LA-ICP-MS [20, 21, 28, 29]. Scanning Electron Microscope-Energy Dispersive X-Ray Spectroscopy (SEM-EDX) [7, 10, 27], Particle Free Induced X-Ray Emission (PIXE) [15, 30], and Electron Probe Micro-Analyzer (EPMA) [27] have also been used for glass analysis, though in a lesser extent.

Despite the increased use of these techniques previously mentioned, Glass Refractive Index Measurement (GRIM<sup>®</sup>) is one of the most conventional equipments employed for RI determination of glass samples in forensic laboratories since it provides the sensitivity and accuracy needed to compare glass fragments and it is notable for its relatively low cost. This equipment is based on the determination of the match point, which is the temperature where the RI of the silicone oil

(which varies with temperature significantly) is equal to that from a glass sample (whose RI hardly changes with temperature). The technique requires then to perform an oil calibration through glass standards of known RI values, and subsequently, the RI values of real glass samples are determined by submerging them in the same oil.

Over the years, many studies on the RI determination have been made. Even ASTM International designed basic guidelines in order to facilitate reproducible RI measurements of glass samples (standard ASTM E 1967-98) [31]. However a specific work protocol for security forces together with a method validation had not been reported in detail. Bearing in mind the previous literature, this research manuscript focuses on a current vision of the technique. Firstly, a detailed work protocol was specifically designed and validated for a fast RI measurement of forensic glass samples. Then, the method was employed in a real casework (a hit-and-run incident) with the aim of showing the potential of the technique when it is complemented with conventional match criteria. These results were also confirmed through LA-ICP-MS elemental analyses.

## 2 Material and Methods

### 2.1 Instrumentation

Match temperature values were measured with GRIM<sup>®</sup>3 (Foster & Freeman Ltd, Evesham, UK), fitted to a phase contrast DMLB Leica microscope (DM2500) with x10 objective (D-35578 Wetzlar, Germany) with a Na light source (589.3nm). The GRIM<sup>®</sup>3 was connected to a Mettler Hot Stage Heating Plate FP 82 (1900 Polaris





Parkway, Columbus, USA) and an integrated videocamera Sony XC75 used to find the edges of the glass fragments. Elemental analysis in the real case was made through a LA system from New Wave Research UP 213 (Freemont, CA, USA) with a 213 nm fifth harmonic Nd-YAG solid state laser, coupled to a quadrupole ICP-MS system 7500cx (Agilent, Japan). Laser operational parameters optimized were taken from previous literature [32].

## 2.2 Materials, standards and samples

Eleven glass standards type B (Locke Scientific Hampshire, UK) with known RI values comprising the common range of forensic glass samples were used to calibrate the silicone immersion oil type B (Locke Scientific Hampshire, UK). Values of RI of the standards employed were as follows: B2 (1.52381), B3 (1.52249), B4 (1.51856), B5 (1.51862), B6 (1.51671), B7 (1.51467), B8 (1.51166), B9 (1.51048), B10 (1.50962), B11 (1.50566) and B12 (1.50225). As a result, temperature range comprised through these standards varied from 54.00 to 113.20 °C, (with a precision of 0.01 °C). A Certified Reference Material (CRM), called BKA-K5 Standard glass for RI measurements (KT13, Forensic Science Institute, Bundeskriminalamt, 65173 Wiesbaden, Germany), with a RI value of  $1.52241 \pm 0.00003$  was employed to test the suitability of the method. Different glass samples (validation samples) were analyzed in order to test the usefulness of the method: glass fragments from an automobile, corresponding to the outside and inside faces of a front windshield, side window, rear window, headlight, front car driver, a glass container and an architectural tinted window.

In the casework, recovered fragments (collected from the crime scene) of a rear window (RC1) and a side window (RC2), and control glass fragments taken from the rear window (RC3) and side window (RC4) of the suspected vehicle were collected, analyzed and compared. Glass evidence was collected at the crime scene by trained personnel in conducting ocular inspections, in such a way that glass contamination was minimised.

### 2.3 Experimental procedure

For both glass standards and validation samples a small piece of glass was taken and crushed in an agate mortar to obtain a glass powder which was mounted on a slide containing a drop of silicone oil type B. Glass fragments were not previously cleaned neither differentiated between surface and bulk fragments. In addition, the amount of sample analysed was the minimum possible (particle diameters  $< 0.1\text{mm}$ ) in order to make it similar to a real case where small particles are collected. Next, the slide was placed onto the hot stage. RI values were measured at a wavelength of 589.3 nm, reaching sharp, thin and high contrast glass edges. For this purpose, only fragments with edgecounts over 80 were chosen. In addition, since GRIM<sup>®</sup>3 allows four different simultaneous measurements, analyses were performed by searching four different particles from the same crushed fragment using a temperature ramp of  $4^\circ\text{C min}^{-1}$ , in order to cover as much as possible the RI variation within that fragment.

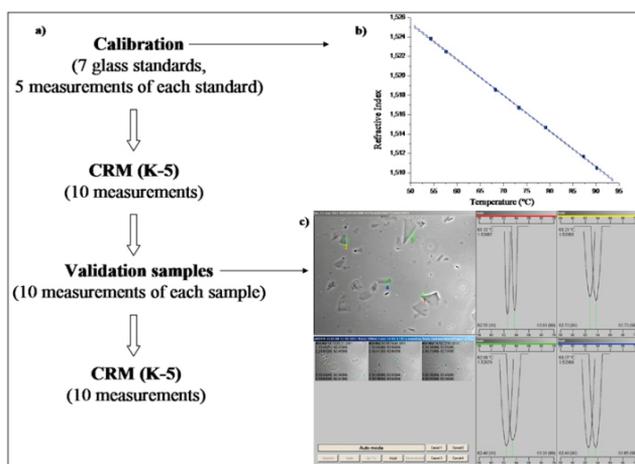
Taking into account the criteria of the National Entity of Accreditation (Entidad Nacional de Acreditación, ENAC, Spain) an appropriate analytical procedure was designed and validated in order to measure the RI of glass samples (**FIG. 1.a**). Ten calibrations of the





immersion oil type B were made over one month period, using seven of the eleven glass standards type B available (five measurements of each standard were performed in each calibration). **FIG. 1.b** presents an example of one of the ten calibrations performed. On one hand, RI measurements of the CRM BKA-K5 were made before and after the validation-sample analyses (a total of twenty measurements per calibration). Analyses of validation samples were carried out in order to assess the usefulness of the method (10 measurements for each sample per calibration). **FIG. 1.c** discloses a screenshot of one of the measurements performed for the side window.

In the casework, fragments were directly crushed (without cleaning) and only ten replicates were made for each fragment (RC1 to RC4), limited in this case by the available sample quantity.



*Fig 1. a) Scheme of the designed procedure to validate the method; b) One calibration performed; c) Screenshot of Glass 2.0.10r where one validation sample (side window) is being measured.*

## 2.4 Data treatment

An interpretation model is needed in forensic laboratories. The most appropriate Bayesian approach, which requires relatively large database to evaluate model parameters, could not be carried out as there was not enough information available. As consequence, other statistical approaches were employed to validate the method and compare samples.

Dixon and Grubbs tests were applied to the results for glass standards (B1-B12), the CRM K-5 and validation samples in order to identify possible outliers in data sets. Computed Chi-Square goodness-of-fit statistic, Shapiro-Wilks W statistic, Skewness and Kurtosis tests were applied to the validation-sample results to evaluate the normal distribution of the data. Cochran's C, Bartlett's, Hartley's and Levene's tests were employed to check the variance of data.

To assess frequencies of type I and type II errors in both the validation set and the casework, several statistical tests commonly used in forensic laboratories were applied [33]. Much controversy exists over the match criterion used to evaluate errors in RI comparisons between recovered and control samples. Depending on the number of known fragments and the number of measurements made from each known or questioned fragment different tests can be performed to evaluate rate errors:

$$CS - t_{0.05(0.01)}^{n-1} S_{CS} \leq RS \leq CS + t_{0.05(0.01)}^{n-1} S_{CS} \quad (1)$$

$$CS - 1(2)S_{CS} \leq RS \leq CS + 1(2)S_{CS} \quad (2)$$





$$CS - 0.00010(0.00020) \leq RS \leq CS + 0.00010(0.00020) \quad (3)$$

$$CS_{\min} - (0.00005) \leq RS \leq CS_{\max} + (0.00005) \quad (4)$$

Where, CS: either single RI value or RI mean value for control sample;  $S_{cs}$ : standard deviation of RI values for control sample; RS: either single RI value or RI mean value for recovered sample;  $t$  the Student's  $t$  parameter at  $n-1$  degrees of freedom (two-tailed confidence level);  $CS_{\min}$  and  $CS_{\max}$ : minimum and maximum RI values for control samples.

Each test led to two possibilities (a or b), in such a way b tests (numbers within parenthesis from eqn. (1)-(4)) implied wider intervals, thus being less restrictive. Tests 1a, 2a, 3a and 4a were expected to produce less type I error rates but more type II ones.

Test 1a and 1b, based on  $t$  times SD, are less dependent of the number of measurements performed. Tests 2a and 2b are based on a fixed multiple of SD. Tests 3a and 3b are the less restrictive tests (one order of magnitude higher intervals than the remaining ones) and finally, tests 4a and 4b are based on the range of the measurements. To evaluate the validation samples, tests 3a and 3b were not useful due to their wide intervals, and tests 4a and 4b were not used, in order to avoid the inherit RI variation of samples. As not many measurements were made, tests 1a and 1b were chosen to evaluate type I and type II errors. However, in the hit-and-run incident, CS from rear window and side window of the suspected car were compared to those at the crime scene by using the eight tests available in order to choose the most appropriate one for this case. Being our case a double comparison, more reliable results were expected.

Statistical treatment was performed with Statgraphics Plus 5.1 (Statpoint Technologies, INC, Warrenton, Virginia, USA) and SPSS (IBM Corp, Armonk, New York, USA)

## 3 Results and Discussion

### 3.1 Method optimization and validation

Seven of the eleven type B glass standards were randomly chosen to perform each one of the ten calibrations. The validity of the linear regression model was demonstrated by evaluating the homoscedasticity and normal distribution of residuals. A good linear relationship between the two fitted variables (RI vs match temperature, °C) was evidenced (excellent correlation coefficients obtained, higher than 0.9999). Glass standards with lowest and highest RI values (B-12, RI= 1.50225 and B-2, RI= 1.52381) were used to calculate the precision of the method. Five measurements of each standard were made each day, in ten different days, and the mean value of RI and the RSD (%) were calculated for both of them. Highest value of RSD within a day was given as repeatability. On the other hand, RSD (%) corresponding to the mean values of RI obtained in ten different days was given as intermediate precision. Analytical features can be consulted in **TABLE 1**. It should be noted the good robustness of the analytical method, since a value of 0.24% of RSD was obtained through the slopes of all calibrations.

The bias of the method was assessed analysing the BKA-K5 (RI= 1.52241±0.00003) as shown in **FIG. 1**. A total of 227 measurements of this CRM were performed overall the period of experimental phase. From all these measurements, Dixon and Grubbs tests only detected one discrepant value and it was removed for further processing of data.





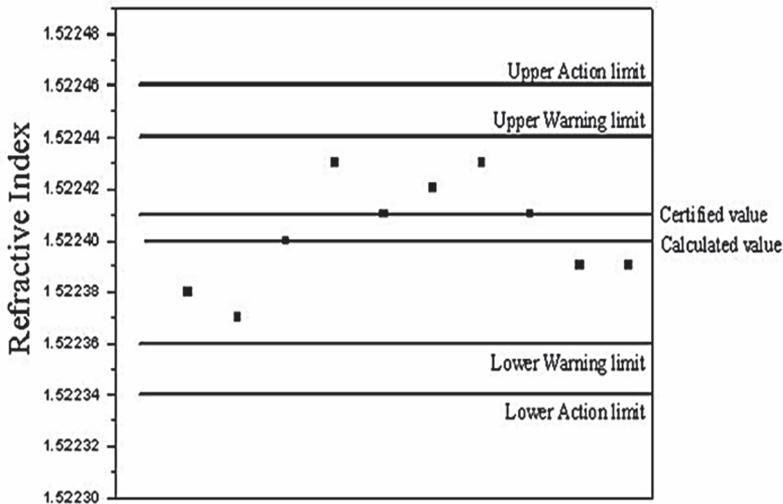
**Table 1**

Analytical features assessed during method validation<sup>a</sup>

Analytical feature	Method of calculation	Result
Calibration Equation	Least squares method	Slope (Sensitivity of the method): $-3.66 \cdot 10^{-4} (1 \cdot 10^{-7})$ °C <sup>b</sup> Intercept: 1.54381 (0.00007)
Working range	Calculated with the glass standards with the highest (B-12) and lowest (B-2) temperature and RI values of the calibration line.	RI: 1.52381-1.50225 T (°C): 54.0-113.2
Linearity	Lowest linearity curve correlation coefficient	R <sup>2</sup> >99.993%
Precision	RSD calculated for the glass standards with the highest (B-2) and lowest (B-12) RI values	Repeatability (1 day). B2 < 0.21%; B12 < 0.14% Intermediate precision (30 days). B2: 0.12%; B12: 0.09%
Robustness	Calculated as RSD of the slopes of ten independent calibrations performed in a thirty days period	0.24%
Bias	Calculated by comparison (t-test) of two homogeneous sample means, taking into account the certified and measured RI values of the CRM K-5, their standard deviations, and the number of replicas performed for each RI value.	Certified RI: 1.52241 (0.00003) Calculated RI: 1.52240 (0.00002) Relative error: -0.00066%

<sup>a</sup> Validated by checking the goodness-of-fit Chi-squared test. <sup>b</sup> In parenthesis is given the standard error of the slope and intercept of the straight line

A two-factor analysis of variance (day and sequence in a day in which this CRM was analysed) indicated that the factor ‘sequence’ did not have a statistically significant influence ( $p$ -value = 0.6783) in the results, that is, there were not statistical differences between RI values obtained before and after measuring glass samples (95% confidence). The control chart for CRM BKA-K5 measurements ( $1.52240 \pm 0.00002$ , 95% confidence) is shown in **Fig. 2**. Upper and lower warning limits were calculated as twice the SD (2SD), while upper and lower action limits were obtained as three times the SD (3SD). As can be seen, the values obtained for CRM (K-5) are within the range of the warning limits. Besides, long-term variability was not observed.



*Fig 2. Control chart of CRM (K-5). Each point corresponds to the average of the twenty RI measurements made for each calibration.*





## 3.2 Application to control glass samples (validation samples)

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While the method was being validated, the general procedure was applied to analyze and compare RI measurements from eight different control glass samples of forensic interest: outside and inside faces from a front windshield, side window, rear window, headlight, front car driver, architectural tinted window and glass container. A total of 100 analyses per sample were made, thus being measured 100 different glass particles for each fragment of approximately 2 cm<sup>2</sup>. **TABLE 2** summarizes the results obtained for this set of samples subjected to statistical analysis.

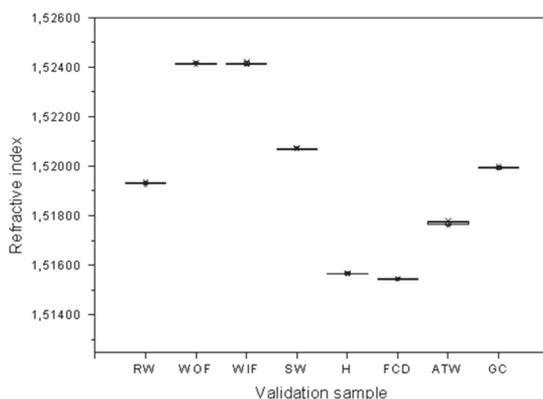
The sample with highest values of match point was the front car driver (10 measurements ranging from 77.43 to 77.6 °C) while lowest values of match point were found for the windshield (53.40 to 53.68 °C). The match temperature measured for the remaining samples ranged between these two extremes. Dixon and Grubbs tests confirmed the absence of outliers in all measurements. In addition, Computed Chi-Square goodness-of-fit statistic, Shapiro-Wilks *W* statistic and *Z* score tests verified normality of the data since p-values > 0.10 (95% confidence) in all cases, except in the front car driver. Deviations from normality in such a type of cases have been described in other studies; [2, 4, 6, 33] however statistical data tests based on the assumption of normality were applied in these cases. Comparison of the mean RI values for these samples by the Fisher's least significant difference (LSD) procedure showed statistically significant differences (95% confidence) for all glass samples, as shown in **FIG. 3**.


**Table 2**

Statistical results obtained for the control glass samples studied through the ten calibrations performed

Glass Sample	RI min	RI max	RI <sup>a</sup>	SD max <sup>b</sup>	RSD <sup>c</sup>	Normality tests (p-values)		Z-score
						Chi square	Shapiro-Wilks W	
Rear window	1.51925	1.51937	1.51931	<0.00006	0.0024	0.7798	0.9237	0.8620
Windshield (inside face)	1.52410	1.52417	1.52413	<0.00004	0.0014	0.7798	0.7982	0.5570
Windshield (outside face)	1.52402	1.52425	1.52414	<0.00004	0.0025	0.5347	0.9501	0.1361
Side window	1.52067	1.52071	1.52070	<0.00006	0.0024	0.1246	0.4429	0.4429
Headlight <sup>d</sup>	1.51563	1.51567	1.51566	<0.00004	0.0003	0.2381	0.5931	0.3123
Front car driver	1.51536	1.51542	1.51545	<0.00005	0.0015	0.0062	0.0690	0.8765
Architectural tinted window	1.51761	1.51777	1.51771	<0.00010	0.0035	0.1886	0.4823	0.4811
Glass container	1.51990	1.51993	1.51993	<0.00004	0.0021	0.7798	0.2234	0.2856

<sup>a</sup> Each data corresponds to the RI mean value of approximately 100 measurements obtained for each sample. <sup>b</sup> Maximum standard deviation found for each sample. <sup>c</sup> Maximum standard deviation found for each sample among the ten calculated (ten replicates for each calibration were made). <sup>d</sup> RSDs calculated from the RI average values obtained in the analysis of glass samples through each one of the ten calibrations performed. <sup>e</sup> Data shown corresponding to nine calibration lines (one outlier was obtained).



**Fig 3.** Boxplot showing RI values from the validation set under study. RW (rear window); WOF (Windshield outer face); WIF (Windshield inner face); SW (Side window); H (Headlight); FCD (Front car driver); ATW (Architectural tinted window); GC (Glass container).

However, windshield inside and outside samples were not differentiated between them. In fact, windshields are usually made of two sheets of glass separated by a polymer which could have been made by the same manufacturer and subsequently in the same form or with the same raw materials. On one hand, it is remarkable how front car driver and car headlight can be differentiated despite providing similar RI values, due to the high repeatability of the method (low variability within RI measurements for each sample). On the other, sample with the highest RSD (0.0035%) was the architectural tinted window (0.0035%), possibly due to the manufacturing process or raw materials used to fabricate this type of glass. Bearing in mind the precision of the method and the inherent RI variation within an entire sample one must take into account that fragments with different RI (statistically different) could come from the same sample, leading to the possibility of making type I errors. Then, criterion from eqn (1) (tests 1a and 1b)

described in the Materials and method section (data treatment) was employed to evaluate them. **TABLE 3** shows that average type I error percentages (9.2 and 4.4 % for a 95% and a 99% confidence level) were acceptable, in concordance with previous literature (6 and 2% respectively). To evaluate type II errors only 27 pairwise comparisons could be made. Considering the inherent RI differences among samples and the number of comparisons, no matches were found and 0% of type II errors were assigned.

**Table 3**

Percentage of type I error (Type I error) found in each sample by applying eqn (1) and (2)

	% Type I error	
	t= 0.05	t= 0.01
Rear window	4.4	0.0
Windshield inside face	8.9	0.0
Windshield outside face	20.0	8.9
Side window	2.2	0.0
Headlight	20.0	20.0
Front car drive	2.2	0.0
Architectural tinted window	4.4	2.2
Glass container	11.1	4.4
<b>TOTAL (Average)</b>	<b>9.2</b>	<b>4.4</b>

### 3.3 Application to a Hit-and-run Incident

Finally, the validated procedure was applied to a real forensic case in order to check its potential together with the application of current match criteria. A vehicle knocked down a pedestrian and glass





fragments from the rear window and one side window were collected on the crime scene. From the position of the glass on the ground and the testimony of witnesses, recovered fragments collected at area 1 (RC1) were assigned as glass fragments from the rear window, and fragments collected at area 2 (RC2) were attributed to the side window. Finally, the suspected vehicle was found and control samples from the rear window (RC3) and the side window (RC4) were taken and compared with the recovered samples (RC1 and RC2).

**TABLE 4** shows results obtained for each one of the samples studied. Rear window taken from the crime scene (RC1) presented a RI mean value of  $1.51938 \pm 0.00003$ , while RI of the rear window taken from the vehicle (RC3) was  $1.51941 \pm 0.00003$ . The other pair consisted on the side window taken from the scene (RC2) and the vehicle (RC4), and RIs obtained for these samples were  $1.52022 \pm 0.00003$  and  $1.52022 \pm 0.00002$  respectively. Although RI values of glass fragments collected from the crime scene are usually more dispersed than fragments from the control samples, [6] RI differences between control and recovered samples are minimal in this case. Normal distribution of data was assessed in samples RC1, RC2 and RC4 since  $p\text{-value} > 0.10$  in all tests (90% confidence). P-values of RC3 were  $< 0.10$  in two of the three tests applied. Variance check following the Cochran's C, Bartlett's, Hartley's and Levene's tests showed that there was not a statistically significant difference among the standard deviations of these samples at the 95% confidence level.

**Table 4**

RI values of samples CR1-CR4 corresponding to the knocking down case

Sample	RI min	RI max	RI <sup>a</sup>	SD average	RSD	p-value		
						Chi square	S-Wilks <i>W</i>	Z score
RC1	1,51932	1,51941	1,51938	0,00003	0,0017	0,1886	0,1062	0,1591
RC2	1,52017	1,52026	1,51022	0,00003	0,0019	0,7798	0,9330	0,9225
RC3	1,51936	1,51944	1,51941	0,00003	0,0021	0,0119	0,0170	0,8622
RC4	1,52019	1,52026	1,51022	0,00002	0,0016	0,3320	0,5738	0,8625

<sup>a</sup> Each data corresponds to the RI mean value of 10 measurements obtained for each sample.

As stated in Data treatment section, the real case was subjected to eight different well established tests from bibliography, in order to calculate Type I error percentages. Test 1a verified a 10% rate error in the rear window comparison, whereas all values of the recovered side window (RC2) entered within the interval of the criterion giving a 0% rate error. Applying test 1b, no values of both recovered samples were outside the range of the criterion. Test 2a is the most restrictive test, and rate errors of 30% (for rear window) and 20% (for side window) were found when comparing recovered with control samples. Applying test 2b, rate errors were reduced to 10% in both cases. When using tests 3a and 3b, RI values from both recovered samples were within the intervals and 0% rate errors were obtained in both comparisons and for both tests. Finally, Test 4a identified one value out of the range for both recovered samples giving a 10% rate error in both cases, while test 4b did not identify any type I error.

These rate errors obtained for the hit-and-run incident agree with results obtained by Garvin and Koons [33]. Tests 2a, 2b, 4a and 4b are strongly dependent on the number of measurements made, since this real case presents only 10 replicates from each sample, rate errors are

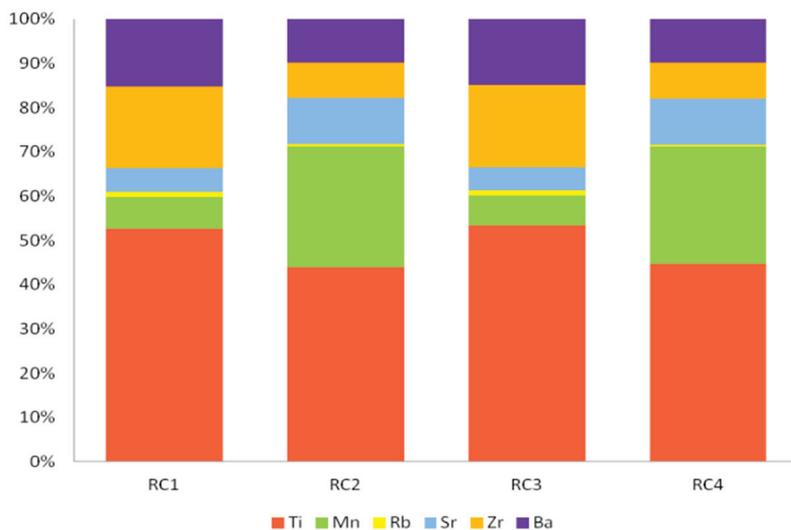




expected to be relatively high and they would not be recommended. Tests based on a fixed interval have not been quite useful due to their intervals, which are much larger than the ranges found in the samples RC1 to RC4, and 0% rate errors have been obtained. In these cases, the rate error type II could be too high. Tests based on a  $t$  times the standard deviation (1a and 1b) are independent of the number of replicates [33] thus being the most appropriate for this real case.

As a result, a significant difference among pairs implied in the real case was not found and RI from fragments attributed to the side window in the crime scene (RC2) was quite close to those from the side window of the suspected vehicle (RC4). The same observations were found for samples RC1 and RC3 from the rear window. To unequivocally confirm these results, samples from the casework were subjected to elemental analysis by LA-ICP-MS. Parameters were taken from bibliography [32] and Ti, Mn, Rb, Sr, Zr and Ba were the minor elements (between 40 and 500 mgK $g^{-1}$ ) selected for comparison and discrimination. In **FIG. 4** composition percentage of these elements from both pairs is depicted. Results confirmed that there was not a significant difference between RC1 and RC3 as well as for RC2 and RC4 which was in accordance with previous results from the validated method.

As Type II error is more insidious in forensics because it could involve an innocent in a crime scene, it would be more appropriate to choose test 1a (less Type II error) when only RI measurement are carried out. Nevertheless, test 1b could be used as well, if an elemental analysis complements the RI measurement



*Fig 4. Bar chart showing mg•Kg-1 (%) for the minor elements determined by LA-ICP-MS in glass fragments corresponding to the hit-and-run incident (RC1 to RC4).*

## 4 Conclusions

A standard analytical protocol for the RI determination of glass samples has been established and validated in this work, with the easy and rapid implementation in forensic laboratories being one of its main strengths. Calibration of the system (GRIM<sup>®</sup>3) followed a linear relationship and was fully reproducible (0.24 % RSD) in calibration slopes during one month. Besides, the precision of the method and accuracy of results are quite good. Application of the validated procedure to the analysis of control glass samples of forensic interest (glass fragments from different parts of a vehicle, glass container and architectural window) allowed their differentiation by means of their RI determination. A basic statistical data treatment for these results





allowed confirmation of similar type I and type II rate errors to those obtained by other authors.

The resolution of a real casework through the validated method, confirmed by a powerful technique such as elemental analysis, has proved that once the analytical features have been assessed and the limitations of the method are known, simple but effective analyses can be made and improved with statistical tools. Reliable results without carrying out complex sample treatments or studying the variation of RI within the sample have been achieved.

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# **The strength of pairwise comparison in forensic chemistry. Recommendations on the use of validated methodologies for the forensic analysis of glass samples by LA-ICP-MS**

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Carmen García-Ruiz; Mercedes Torre Roldán

Science & Justice, submitted March 2014

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## ABSTRACT

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This work focuses on the validation of an analytical method for the forensic comparison of glass samples using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), on the basis of previous research and standard tests on glass analysis by this technique. But more than an in-house validation, this article intends to: i) show the differences between analytical chemistry and forensic chemistry, ii) include possible tools to facilitate the routine analysis by technicians and the interpretation of results by judges, and iii) discuss typical concepts which may create confusion among the involved people during a trial and propose solutions to this handicap. To this aim, the in-house validation was firstly proposed by using two certified reference materials (CRMs) to obtain quantitative information. Different elemental concentrations were obtained for the same sample as a function of the CRM employed. Therefore, the validation was based on the correct pairwise comparison by using different statistical tools instead of employing the accuracy of the method. Different quality controls were proposed: precision control and limit of detection (LOD) control. After ensuring the analytical range as well, different samples from real caseworks were analysed. Elements which did not pass the quality controls were eliminated and the pairwise comparisons were made with the remaining elements (up to three elements from the 18 initially selected could be rejected without reliability problems). Significant differences between two samples were considered when one or more differences were found for any of the elements in the pairwise comparison. In addition, a criteria based on the terminology MATCH / NO MATCH was employed in order to facilitate the meaning of the interpretation of the results in expert valuation reports by non-scientific experts in a court. Throughout the manuscript, eye-friendly graphics and calculations are proposed for expert valuation reports with the aim of obtaining easy interpretations and avoiding in this way confusing results in court.

## 1 Introduction

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Undoubtedly, analytical chemistry is one of the pillars of forensic chemistry as, an important part of expert valuation reports are instrumental conditions, analytical methodologies and validation parameters. During this step, good practices in laboratories such as quality controls are applied in order to obtain results under controlled conditions and with known uncertainty. These results are analytical values which may characterize the evidence. However, this characterization might not be concluding enough for a court, and a comparison between two different samples are required to relate a scene, an object or a victim to a suspect. This is usually the case of glass samples, where big efforts are being made in order to standardize methods to carry out correct analysis, by using certified reference materials, interlaboratory tests, etc.

In order to evaluate the association between two samples with a common origin, one must take into account the grade of singularity achieved in the chemical profile of the sample, apart from the sample population. For this purpose, databases are helpful but sometimes are not available. DNA databases, for instance, can be easily consulted and provide enough information. However, in forensic chemistry these databases are usually more limited. In the case of glass samples some particular databases have been created, such as the one from Grainger et al. in New Zealand, where 243 samples of automobiles from 12 different countries manufactured between 2002 and 2006 were registered [1], or the one from Trejos et al. in US, who analysed 127 and 286 glass samples from architectural samples and vehicles respectively [2]. Nevertheless, a comprehensive glass database does not exist due to the need for a continuous flow of analyses of glass samples depending





on the manufacturing processes and the huge analysis capacity required for this purpose.

The study of glass samples has become a habitual task in forensic laboratories. Cases such as traffic offences, terrorist attacks, robberies to particulars or companies, etc involve glass samples as evidence. Classic assays like the refractive index (RI) measurement are advantageous due to its high precision, accuracy and speed, low cost and simplicity [3]. Nonetheless, only one variable (the RI) is determined to compare among glass samples, and taking into account the current and improved manufacturing processes which make glass with homogeneous RI among batches, individualization capacity is reduced and this analysis is usually not enough to interpret the evidence [4].

Due to this reason, new analytical techniques are emerging, such as laser induced breakdown spectroscopy or laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) which provide analytical profiles of glass samples with multiple variables (inorganic elemental concentration) which constitute a fingerprint useful for the forensic comparison of these samples [5].

Concerning the data treatment for these analytical techniques, statistic tools are necessary in most of the cases as huge datasets are obtained after analysis. Focusing on LA-ICP-MS, our group carried out a comprehensive review about the potential of this technique in forensic chemistry [6]. Taking into account this manuscript and publications based on the criteria of the statistic evaluation of the analytical results [1,2,7], multivariate hypothesis contrast based on statistic concepts but adapted to elemental composition variability of glass sample population, and also to the LA-ICP-MS analysis itself, has been

optimized and employed to minimise type I and II errors. This criterion was firstly proposed by Peter Weis [8] by comparing the average of the questioned or recovered sample (RS) to the comparison interval, calculated as  $\pm 4$  times the standard deviation (SD) interval ( $\pm 4SD$ ) around the average of the control sample (CS), with a minimum fixed relative standard deviation (RSD) of 3%. Afterwards, a modification to this interval was proposed by increasing the minimum value between 3 and 5% [2]. This methodology has been internationally accepted and even a normalized procedure has been proposed as ASTM standard test [9].

Following these guidelines, the aim of this work was to develop an internal validation for the forensic analysis of glass samples by LA-ICP-MS ready to be used in the police laboratories. But far from the methodology employed and as important as its validation, this paper deals with some recommendations on the use of these methodologies with various purposes: testing the real strength of pairwise comparison and precision as key parameters in forensic method validation, useful tools to establish correct comparisons and carrying out quality controls easy to use for technicians, creating expert validation reports able to be understood in courts, and finally establish and discuss the differences among the inherent roles involved in trials, which include analytical chemistry, forensic chemistry and statistics, and propose real solutions to these handicaps.

## 2 Experimental Section

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Instrumental parameters and statistic treatments were taken from the ASTM standard test on the analysis of glass fragments by LA-ICP-MS [9]. This procedure was optimized to study samples such as float





glass, containers and ornaments, and therefore the following isotopes were analysed:  $^7\text{Li}$ ,  $^{23}\text{Na}$ ,  $^{24}\text{Mg}$ ,  $^{27}\text{Al}$ ,  $^{39}\text{K}$ ,  $^{42}\text{Ca}$ ,  $^{49}\text{Ti}$ ,  $^{55}\text{Mn}$ ,  $^{57}\text{Fe}$ ,  $^{85}\text{Rb}$ ,  $^{88}\text{Sr}$ ,  $^{90}\text{Zr}$ ,  $^{137}\text{Ba}$ ,  $^{139}\text{La}$ ,  $^{140}\text{Ce}$ ,  $^{146}\text{Nd}$ ,  $^{178}\text{Hf}$ ,  $^{208}\text{Pb}$ . In addition,  $^{29}\text{Si}$  was employed as internal standard and  $^{118}\text{Sn}$  was monitored to identify the glass surface which was exposed to the Sn bath during the manufacturing of float glass. Therefore,  $^{29}\text{Si}$  and  $^{118}\text{Sn}$  were not used as pairwise comparison variable in the statistic treatment. Note that throughout the entire manuscript, elements will refer to the isotopes that appear in the previous list.

## 2.1 Instrumentation and methodology

LA-ICP-MS analyses were conducted using a LA system from New Wave Research UP 213 (Freemont, CA, USA) with a 213 nm fifth harmonic Nd-YAG solid state laser, coupled to a quadrupole ICP-MS system 7500cx (Agilent, Japan). Laser operational parameters optimized were: ablation mode, spot; diameter, 80  $\mu\text{m}$ ; repetition rate, 10 Hz and fluence, 10  $\text{J cm}^{-2}$ . Total time 150 s: laser warm up, 30 s; laser ablation, 60 s; washout delay, 60 s. Instrumental parameters used were: carrier gas through the ablation cell, He 1.00  $\text{L min}^{-1}$ ; carrier gas flow after the cell, Ar 1.0  $\text{L min}^{-1}$ ; plasma gas Ar 14  $\text{L min}^{-1}$ ; auxiliary gas flow, Ar 0.9  $\text{L min}^{-1}$ ; rf power, 1400 W. Mass spectra were acquired in time resolved analysis mode (TRA), 120 s; acquisition time: 10 ms  $\text{mass}^{-1}$ .

As usually, a glass reference material NIST 612 from the National Institute of Standards and Technologies (USA) was used to establish the proper functioning of the equipment (instrumental calibration) through the following parameters: torch position, ion optics, fractionation index (measured as the ratio  $^{238}\text{U}/^{232}\text{Th}$ ) between

0.8 and 1.2, detector pulse/analog (P/A) factor calibration, doubly charged ion rates  $< 0.8\%$  ( $^{21}\text{Ca}^{+2}/^{42}\text{Ca}^{+}$ ) and oxide rates  $< 1.0\%$  ( $^{248}\text{ThO}^{+}/^{232}\text{Th}^{+}$ ). TRA signals were performed using the GLITTER software from GEMOC, Macquarie University (Australia), and v4.4.2. Graphics and statistical treatments were made using Excel 2013 from Microsoft, Corp (Redmon, Washington, USA), and SPSS Statistics v18 from IBM, Corp. (Armonk, New York, USA) software.

## 2.2 Standards, real samples and sample preparation

Glass certified reference materials (CRMs) employed were NIST 612-613 [10], and FGS1 and FGS2 (Bundeskriminalamt, Forensic Sciences Institute KT13, Wiesbaden, Germany) [11].

Real samples were collected during a period of two years. In total, 50 different samples from containers, vehicles and float architectural glass were collected for analysis. **FIG. 1.a** shows a graphic with the number of samples of each type, clustered according to their traceability. This traceability was needed to evaluate the errors type I and II obtained with our methodology. On this way, samples with RI traceability were previously analysed by using a recently published and certified methodology for the RI measurement of glass samples through GRIM<sup>®</sup> 3 [3], in order to know the common or different origin of the fragments. Blind and interlaboratory tests were samples of known origin and there were no need for extra analyses. CRM NIST 1830 was also taken as real sample to test the validated methodology. Only 3 automobile samples were not blind or interlaboratory samples, and they were not analysed by GRIM<sup>®</sup>3 due to the minimal quantity of sample collected on the crime scene. Taking into account that their origin was unknown, they were only used to perform pairwise comparisons with



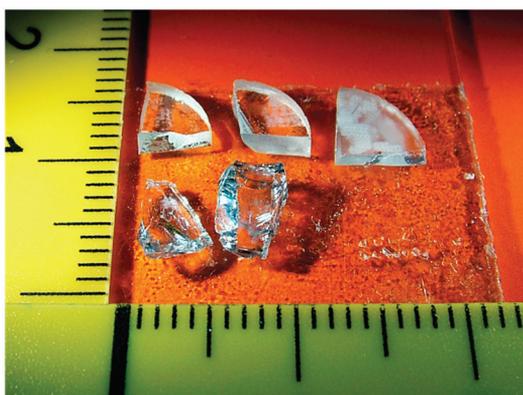


samples from other forensic caseworks to ensure that the fragments were not from the same origin.

#### a) Sample traceability

RI	CRM	Interlaboratory test	Blind sample	No traceability
Automobile 21 samples	NIST 1830 2 samples	Container 3 samples	Automobile 4 samples	Automobile 3 samples
Architectonic float 3 samples		Architectonic float 7 samples	Architectonic float 3 samples	
		Automobile 4 samples		

#### b) Sample preparation for analysis sequence



**Fig 1. a)** Real samples analysed through the LA-ICP-MS validated method. Note that samples are clustered as a function of their traceability. No traceable samples were 3 automobile glass fragments only analysed by LA-ICP-MS without knowing if they come from the same origin or not; **b)** Example of the whole system sample preparation to carry out the analysis sequence.

LA-ICP-MS sample preparation is minimal and fast. Only in the cases where samples showed debris in its surface, a previous cleaning treatment with ethanol, hydrogen peroxide or diluted nitric acid was made. Once cleaned, the glass fragment was put on the slide and fixed

with doubled adhesive tape. Two requirements were needed to overcome the analyses:

- The total number of fragments needed for an analysis (samples, quality controls and calibration controls) can be introduced in the analysis cell of the LA microscope.
- All fragments are analysed by the laser on their flattest fracture face.

### 2.3 Analysis sequence

Each sample was analysed 6 times in two sequences of 3 spots (fragments with 0.25 mm<sup>2</sup> surface or larger were enough to carry out the analysis). Between samples, calibration controls were also analysed in sequences of 3 spots. As quality controls, the following parameters were daily performed:

- Reproducibility control ( $N_{z\text{-rep}}$ ) to verify that the SD of the 6 replicates of each sample was inside the precision levels of the method. Any element with RSD higher than 3 times the RSD of the precision level for each element was discarded for the forensic pairwise comparison between two real samples.
- Limit of detection (LOD) control to verify that the elemental concentrations obtained were above the LODs achieved by the methodology. If any element from any sample had a concentration below the LOD, that element was discarded for the forensic pairwise comparison between two real samples.





- Positive pairwise control (two known samples from the same origin) and negative pairwise control (two known samples from different origin). If these controls were not passed, the analytical sequence was cancelled.

In **Fig. 1.b** a picture of the whole system preparation is showed as an example. As can be seen, each real sample analysis included also the analysis of both CRMs to have a double calibration, both a positive and a negative pairwise comparison control, precision of the repeatability and LOD control to ensure the correct comparison of each pair of fragments

## 3 Results and Discussion

### 3.1 In house validation of the method

#### 3.1.1 Accuracy of the method. NIST 612 vs. FGS2

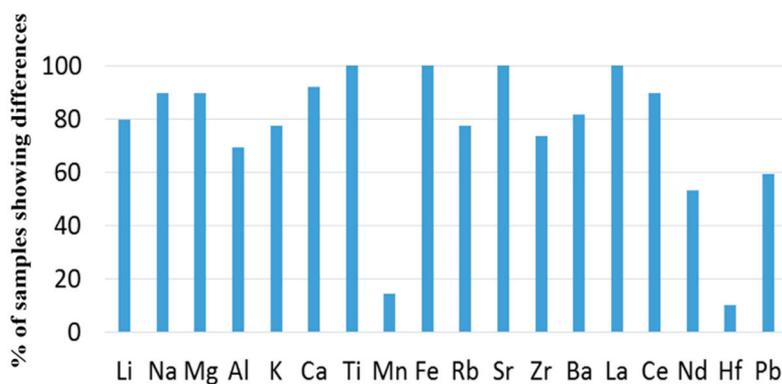
Accuracy plays a vital role in most of the analytical protocols, overall methodologies which deal with the quantification of the analyte under study, such as the case of pharmacology studies, drug analysis, food quality controls, etc. This parameter is also calculated in the ASTM standard test for the analysis of glass samples by LA-ICP-MS [9].

Two different but usually employed CRMs were used to test the accuracy of the method, NIST 612 and FGS2 [10,11]. On the one hand, FGS2 is a float glass designed specifically for the forensic pairwise comparison of samples which makes it ideal for our purpose. On the

other hand, NIST 612 is a glass which differs from the typical float glass composition and possesses an homogeneous fixed concentration of  $50 \text{ mg}\cdot\text{Kg}^{-1}$  of 58 different elements. Despite not having a composition similar to float glasses, NIST 612 possesses a siliceous nature and presents a specified and well-documented elemental composition which make this material widely used in studies related to the forensic analysis of glass [2,5,12,13].

Thus, taking the two CRMs, two different calibrations were obtained and employed to calculate the elemental concentration of all samples. Afterwards, the elemental concentrations obtained for each pair of results from the same sample were compared through a MANOVA analysis at 95% confidence level. **FIG. 2** depicts the percentage of samples which showed a significant difference when comparing their profiles by using the two calibrations. We found significant differences in all the samples for the elements Ti, Fe, Sr and La. For the remaining elements the percentage oscillated between 10% and 90% of samples. These differences indicated that the quantitative elemental concentrations of the samples depended on the calibration standard. Therefore, it is suggested that for the proposed methodology, one must perform the elemental quantification by using always the same calibration standard, as different CRMs could cause different concentrations from some elements and therefore errors in the forensic pairwise comparison and statistic treatment.





*Fig 2. Percentages of samples which show significant differences in their elemental concentration (when comparing the results from the two calibrations using FGS2 or NIST612 as calibration standards).*

Accuracy, as stated above, is an important analytical parameter. However, the aim of the forensic cases involving glass samples usually relies on answering the following question: are these two fragments from the same origin or not? Thus, ensuring a good pairwise comparison is the main objective in a forensic case, rather than knowing an accurate value of elemental concentration of a fragment by itself. To this aim, in our proposed methodology accuracy values were not evaluated on the basis of the standard CRMs. Instead, other parameters were calculated to ensure a good performance in the pairwise comparison. Quality controls were established as precision in each sample, accuracy controls were made through positive and negative comparison controls, and finally elemental concentration values were verified in order to discard the concentrations under the LODs of the methodology.

### 3.1.2 Analytical parameters checked to ensure the forensic method validation.

Working range, precision and LODs were the analytical parameters calculated to ensure the correct comparison of samples. In addition, useful tools were proposed to show the results as clear as possible, being in this way the interpretation of the results easy to be detected by non-expert personnel and non-scientific experts.

- Working range: the elemental concentrations found for each one of the 50 real samples were calculated and the working range obtained was compared to the two batches analysed in the ASTM standard test [2,9]. The range of these batches was in the same order than the one taken in our laboratory, thus the set employed in this study was adequate for the validation as it was representative of the typical glass samples found in forensic cases.
- Precision (designated as  $RSD_{rep}$ ) was obtained by calculating the root mean square of the SD from the 6 replicates of each one of the 50 samples contained in the batch under study. Two values were calculated for each element, one for each calibration standard employed (FGS2 or NIST 612). Table 1 shows the  $RSD_{rep}$  of each one of the elements under study. As can be seen,  $RSD_{rep}$  was similar for all elements independently of the calibration standard employed, except for K and Fe, where the  $RSD_{rep}$  was higher when employing the CRM NIST 612. This can be due to the low concentration these elements have in the CRM NIST 612 (51 and 62.3  $mg \cdot Kg^{-1}$  [10]) against the concentrations found in the CRM FGS2, 2600 and 4600  $mg \cdot Kg^{-1}$ , respectively [11]. This  $RSD_{rep}$  values were accepted as





the precision values for the method and are also the values employed to establish the precision quality control for real samples. To overcome this quality control, a parameter called  $N_{z\text{-rep}}$  [14], which is a normalized proportional RSD was employed:

$$N_{z\text{-REP}} = \frac{RSD \text{ sample}}{RSD \text{ rep}}$$

**Table 1**  
Repeatability obtained for each element under study and each calibration standard (FGS2 and NIST-612). Total number of replicates: 300; total number of samples: 50

	<b>FGS2</b> RSD <sub>rep</sub>	<b>NIST 612-13</b> RSD <sub>rep</sub>
Li	13.4	13.4
Na	2.7	1.9
Mg	1.7	1.8
Al	1.7	1.3
K	2.8	9.6
Ca	2.0	1.1
Ti	2.9	2.4
Mn	3.0	2.8
Fe	2.8	12.4
Rb	4.8	4.5
Sr	1.9	1.4
Zr	8.7	8.5
Ba	8.0	7.9
La	2.7	2.5
Ce	2.5	2.2
Nd	6.9	6.7
Hf	9.5	9.6
Pb	6.9	6.7

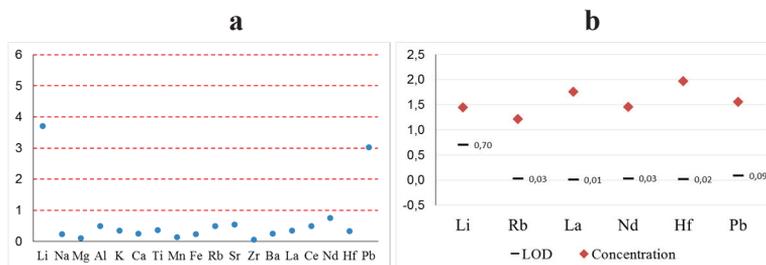
$N_{z\text{-rep}}$  reaches 1 when the RSD obtained for the six replicates is equal to the repeatability precision of the method (RSD<sub>rep</sub>). This parameter can be used as quality control to evaluate if the repeatability obtained for a sample is consistent with the one in the method. If  $N_{z\text{-rep}}$  values are below 3 (RSD of the sample is three times or lower than the RSD of the method) the element is accepted for pairwise comparison. If this value is reached, the

RSD of the sample is significantly higher than the RSD precision of the method, and therefore the element is discarded for the posterior pairwise comparison. High RSDs in a sample can be due to irregularities in the fragment surface, heterogeneity of the sample, irregular gas flow from the LA-ICP-MS system, or concentrations near the LODs. As example, Table 2 shows the average, SD, RSD and  $N_{z\text{-rep}}$  values for two samples from the same origin and from the batch under study. Li and Pb were discarded for the posterior pairwise comparison as high values ( $N_{z\text{-rep}} > 3$ ) were obtained for these elements. In order to show the results clearer, a graphic for this quality control was proposed. FIG. 3.a depicts the graphic proposed, where the elements Li and Pb presents a  $N_{z\text{-rep}}$  higher than 3 and therefore these elements are rejected for the posterior pairwise comparison. A possible reason for these high RSDs could be the low concentration in the case of Li and possible inhomogeneity in the case of Pb distribution across the sample.

**Table 2**  
Average (mg Kg<sup>-1</sup>), SD (mg Kg<sup>-1</sup>), CV and  $N_{z\text{-rep}}$  (precision quality control parameter) for two random samples from the same origin (control and recovered) taken as example

	Control Sample (CS)				Recovered Sample (RS)			
	Average	SD	RSD	$N_{z\text{-rep}}$	Average	SD	RSD	$N_{z\text{-rep}}$
Li	1.45	0.72	49.8	3.71	1.70	0.81	48.0	3.57
Na	101119	615	0.6	0.23	102150	800	0.8	0.29
Mg	19157	33	0.2	0.10	19031	171	0.9	0.53
Al	1742.2	15.2	0.9	0.50	1711.0	38.1	2.2	1.28
K	442.5	4.3	1.0	0.34	452.9	9.3	2.0	0.72
Ca	67682	326	0.5	0.24	66929	1173	1.8	0.88
Ti	237.0	2.5	1.1	0.37	231.0	4.5	2.0	0.68
Mn	70.98	0.27	0.4	0.13	71.30	0.19	0.3	0.09
Fe	7400	49	0.7	0.24	7447	77	1.0	0.37
Rb	1.22	0.03	2.4	0.49	1.26	0.06	4.7	0.97
Sr	118.1	1.2	1.0	0.54	116.6	1.9	1.6	0.84
Zr	75.01	0.38	0.5	0.06	72.95	1.94	2.7	0.31
Ba	22.83	0.46	2.0	0.25	22.93	0.39	1.7	0.21
La	1.76	0.02	0.9	0.34	1.74	0.06	3.6	1.32
Ce	5.80	0.07	1.3	0.50	5.90	0.09	1.6	0.63
Nd	1.46	0.08	5.2	0.75	1.48	0.04	2.9	0.42
Hf	1.98	0.06	3.1	0.33	1.94	0.08	3.9	0.41
Pb	1.56	0.33	21.0	3.03	1.47	0.22	15.0	2.17





**Fig 3.** Example of user-friendly graphics to observe the quality controls proposed in the method validation. **a)** elemental precision quality control ( $N_{z-rep}$ ) of CS from TABLE 2; **b)** elemental concentration found in CS from TABLE 2 and LODs obtained for the method

- LODs were calculated for each element and each calibration standard CRM through the software Glitter on the basis of Poisson statistic at 99% confidence level:

$$LOD = 2.3 (2B)^{1/2}$$

Were B is the total counts per second in the blank interval. Table 3 shows the LODs for each one of the elements from the CRMs employed as calibration standards, and also the LODs from the ASTM standard test [9]. As can be seen, LODs were similar for both calibration standards and in the same order as the ones obtained in the ASTM standard test. In addition, as can be seen in Table 3, these values were below the minimal concentrations found for our batch of 50 glass samples and therefore were accepted for the objective of the method. LODs from the FGS2 were the ones taken as the LODs of the method. **FIG. 3.b** shows a graphic with the elemental concentration of some elements from one sample taken as example and their respective LODs.

During the validation, none of the 50 samples and their elemental concentrations were below the LODs and therefore this quality control did not reject any elemental concentration.

Note that by applying these quality controls, some elements are rejected from the initially selected to perform the posterior pairwise comparison just to avoid a false interpretation of the results. However, we have tested that up to three elements can be rejected from the 18 initially selected with no reliability problems.

**Table 3**

LODs obtained for each calibration standard (FGS2 and NIST612), ASTM standard test and minimum concentrations of the set of samples under study in  $\text{mg Kg}^{-1}$

	Limits of detection			Minimum concentrations
	Std Cal: FGS2	Std Cal: NIST 612-13	ASTM test	
Li	0.70	0.60	0.75	1.2
Na	3.58	3.41	-	89459
Mg	0.42	0.34	0.52	203
Al	0.43	0.44	1.9	514
K	2.22	1.95	2.2	107
Ca	74.06	76.94	150	57341
Ti	0.54	0.62	3.2	41
Mn	0.22	0.22	0.77	3.7
Fe	6.71	1.34	9.2	55
Rb	0.03	0.03	0.19	0.3
Sr	0.01	0.01	0.072	22
Zr	0.02	0.02	0.13	25
Ba	0.09	0.10	0.30	5.4
La	0.01	0.01	0.050	1.1
Ce	0.01	0.01	0.028	2.0
Nd	0.03	0.04	0.17	0.8
Hf	0.02	0.02	0.090	0.7
Pb	0.09	0.09	0.16	0.7





## 3.2 Validation through pairwise comparisons

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The proposed methodology focuses on the correct pairwise comparison of glass fragments for forensic purposes through appropriate analytical parameters and useful tools which allow us to show the results in a useful way for technicians and non-scientific experts involved in a trial.

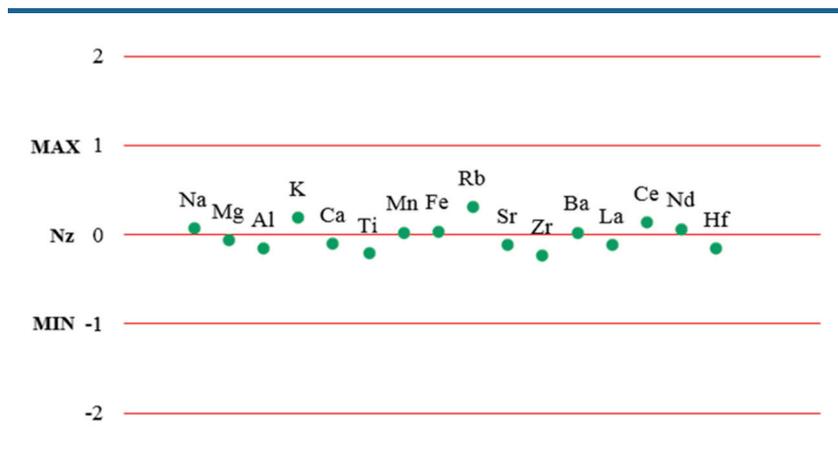
Firstly, only elements which passed the quality controls testing precision and LOD (assays described in the previous section) were taken to perform the pairwise comparison. After ensuring the correct selection of elements the pairwise comparison was carried out. The comparison was made for each element individualized, verifying if the average of the 6 replicates from the RS was inside the comparison interval of the average of the CS ( $\pm 4$  times the SD of the 6 replicates of the CS) with a minimum fixed relative standard deviation (RSD) of 3%. If all elements from RS remained inside the comparison interval, there were no significant differences while one or more elements outside the intervals imply that there were significant differences between the two fragments [8].

To clarify the results for the expert valuation report, the results of the pairwise comparisons were plotted by using a new parameter,  $N_{Zi}$  [14]. This parameter is calculated as:

$$N_{Zi} = \frac{\overline{RS}_i - \overline{CS}_i}{4 \times SD_i(\text{FixMin } 3\%)}$$

This parameter normalized the results from the pairwise comparison, in such a way that values of  $N_{Zi}$  outside the limits  $\pm 1$  imply that the element concentration present significant differences among the

samples under study and therefore the result on the interpretation is NO MATCH. **FIG. 4** shows a representative example of a comparison between two samples assumed from the same origin, observing that all elements compared were inside the limits and therefore the result of the interpretation was MATCH. This graphic together with graphics from **FIG. 3** intend to be useful tools for technicians in forensic laboratories and expert reports presented in trials. Note that the terminology employed for the interpretation of the results has been MATCH and NO MATCH which are explained in detail in the following section.



*Fig 4. Example of an element pairwise comparison graphic with a MATCH conclusion*

### 3.2.1 Useful and informative terminology recommended for forensic reports.

If focusing on the statistics field, we start with a hypothesis (Ho) which consists on the equality of the averages of all the variables (in our case, elements). Therefore, null hypothesis is stated as  $H_0: CS = RS$  [15-17]. The hypothesis contrast is defined to find significant differences between two average samples. Significant level is usually calculated at





5 or 1%. If the value obtained is higher, we can say that there are significant differences among the sample averages. Depending on the result, we may obtain 4 different possibilities: Positive, Negative, False positive (error type I or  $\alpha$ ) and false negative (error type II or  $\beta$ ). On this way, a result is positive when we correctly accept the null hypothesis, both samples come from the same origin and we do not find significant differences between the averages; a result is negative when we correctly reject the null hypothesis, samples come from different origin and we find significant differences between the averages; a result is false positive, error type I or  $\alpha$  when we incorrectly reject the null hypothesis, samples come from the same origin but we find significant differences between the averages; and finally false negative, error type II or  $\beta$ , when we incorrectly accept the null hypothesis, samples come from different origin but we do not find significant differences between the averages.

If focusing on the forensic field, other recent names have been designed for these four possible conclusions. But these terms are not totally implemented, which has finally created controversies, overall in the expressions ‘False positive’ and ‘False negative’ [8,16,18]. Being these expressions analytically correct, they sometimes create controversy in the meaning of the interpretation, overall for non-experts in statistics. However, if we focus on other forensic fields there are concepts which may be interesting in order to avoid this controversy. Genetic studies of DNA employ the terms MATCH / NO MATCH, terminology that is being useful and does not generate confusing results [19]. Therefore, we suggest using as hypothesis  $H_0$  the equality of the averages of the elements and the concept MATCH / NO MATCH for the final expression of results in the forensic comparison of glass samples. On this way, the term MATCH in the interpretation means

intuitively that the two samples have the same origin (although statistically a more correct conclusion would be that there are not significant differences between the averages). The term NO MATCH means intuitively that the samples have not the same origin (statistically, there are significant differences between the averages). When errors are committed in the interpretation of the results, the following terminology is proposed: when concluding from the results that two samples have different origin (NO MATCH) but in fact they have the same origin, a FALSE NO MATCH (type I error) will have been committed. When concluding from the results that two samples have the same origin (MATCH) and they do not, a FALSE MATCH (type II error) will have been committed. This terminology may be easily comprehensible by non-scientific experts during a trial and can be therefore used as final expressions in the interpretation of the results of glass pairwise comparisons in expert valuable reports, where statistical treatments are employed but confusing terms are avoided in order to eliminate erroneous interpretations. Table 4 summarizes the concepts assigned for each case, the consequences for the suspect and the new terms proposed for the forensic field to assign these cases.

**Table 4**  
Comparative on the different concepts employed to interpret the results from a forensic pairwise comparison of two glass fragments

Sample origin	Significant differences?	Forensic interpretation <sup>1</sup>	Statistic interpretation	Consequences for the suspect <sup>2</sup>
Same	No	<b>MATCH</b>	Positive	Justly accused
Different	Yes	<b>NO MATCH</b>	Negative	Justly absolved
Different	No	<b>FALSE MATCH</b>	Error type II ( $\beta$ )	Unjustly accused
Same	Yes	<b>FALSE NO MATCH</b>	Error type I ( $\alpha$ )	Unjustly absolved

<sup>1</sup>This alternative is the proposed in this in-house validation and therefore the one used throughout the manuscript.  
<sup>2</sup>Only for the cases where a sample from the crime scene is compared to another directly related to the accused.





### 3.2.2 Results on pairwise comparisons

Table 5 summarizes the results from all the possible pairwise comparisons of the 50 samples in repeatability conditions, employing the MATCH / NO MATCH / FALSE MATCH /FALSE NO MATCH criteria proposed, for each one of the calibration standards employed.

**Table 5**

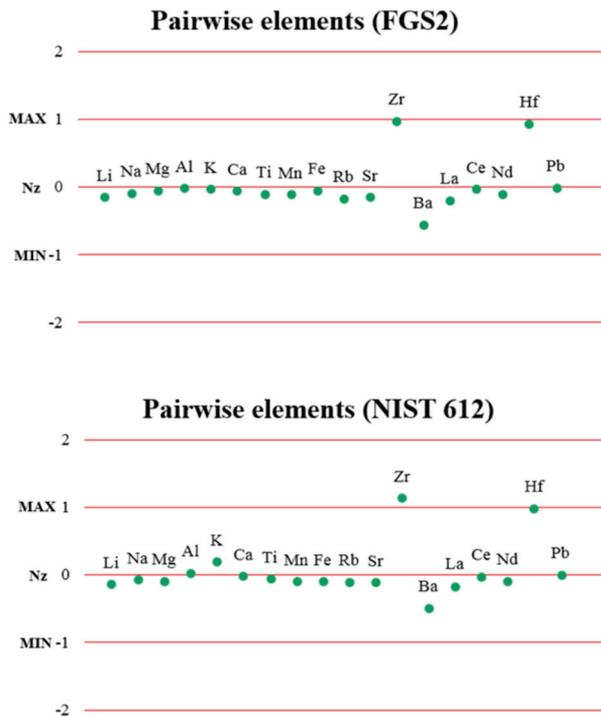
Type I and type II errors in the pairwise comparisons of the samples and elements in repeatability conditions

	StdCalib FGS2		StdCalib NIST 612-13	
	N° comparisons*	Error (%)	N° comparisons*	Error (%)
<b>MATCH</b>	33(594)	-	32(576)	-
<b>NO MATCH</b>	59(1062)	-	59(1062)	-
<b>FALSE MATCH (Error type II)</b>	0	0	0	0
<b>FALSE NO MATCH (Error type I)</b>	0	0	1	3(0,17)
<b>TOTAL</b>	92(1656)	-	92(1656)	-

\*In brackets is given the number of elemental comparisons.

As can be seen in Table 5, all results from the forensic interpretation were satisfactory when employing FGS2 as calibration standard, as FALSE MATCH or FALSE NO MATCH were not found considering the known traceability of the samples analysed. However, when using the CRM NIST 612 as calibration standard, one FALSE NO MATCH (Type I error) was obtained. Therefore, significant differences were found for these two samples assumed from the same origin. These samples come from a forensic casework (car accident) where fragments from the headlight of the suspected vehicle were compared to fragments on the road found at the crime scene. To explain the differences in the forensic interpretation of this specific case, **FIG. 5** was depicted. These

figures plotted the  $N_{zi}$  values (pairwise plot) and the limits MIN and MAX which establish the limits between MATCH and NO MATCH. When using NIST612 as calibration standard, Zr remained outside the limits contrary to the case of FGS-2, where Zr remained inside the limits. Note that in any case both elements Zr and Hf remained almost in the limit of the MATCH. These minimal differences can be attributed to minimal variability differences in the averages of the calibration standards, or the low concentration found for these elements in the real samples.



*Fig 5. Element pairwise comparison graphics of the pair of samples with differences in the forensic interpretation depending on the calibration standard employed. Differentiation is originated by the element Zr.*





From Table 5 we can say that the results obtained when applying the pairwise comparison methodology proposed are adequate and the results did not depend on the calibration CRM employed, which reinforces the idea that quantification and the accuracy attached to this value it is not as important as the correct pairwise comparison, where the most important procedures are the correct selection of elements to carry out the comparison and controlling the repeatability of the replicates for a sample (which are correctly selected thanks to the quality controls of precision and LOD purposed in the method).

### 3.3 Towards the understanding of the interdisciplinary found in forensic caseworks

Once presented and discussed the validated method, we considered that a call to the understanding of the roles in a trial is important and must be discussed. From the forensic standpoint, a comparative study on glass samples begins with the sample collection and ends with the respective report which is assessed in a trial. Therefore, obtaining scientific results and communicating them in an expert report able to be understood by non-scientific experts involved in that trial is one of the functions of a forensic scientist. Nonetheless, a total or partial incomprehensibility of the expert report by the non-scientific experts can exist due mainly to two reasons. The first, the result interpretation, where the conclusion may be ‘there are or there are not significant differences between two fragments’, or ‘these fragments may proceed from the same glass object’ (when there are databases at our disposal). The second, the scientific language and conclusion expressed in the expert report. Scientific debates about this issue are focused on the use of an appropriate statistic for the results interpretation and advise about the possible errors from using

automatized statistic software by non-experts, as not always the adequate model is employed or the initial data are suitable for a direct statistic treatment [20,21]. Howes et al. made a study to know the comprehension level of 110 conclusions from different laboratories by non-scientific experts, and more than 50% of the individuals considered the conclusions ‘difficult’ or ‘very difficult’ to be understood, being the possibilities: ‘very easy’, ‘easy’, ‘reasonably simple’, ‘medium’, ‘some difficulties’, ‘difficult’ and ‘very difficult’. Reasons for these answers involved text font, structure, phrase structures, vocabulary, coherence and unity [22].

It is important to note that in trials the association or no association of two fragments with a common origin is usually required. From the statistic standpoint this stage has been carried out with pairwise comparisons through Students T-test, ANOVA, MANOVA, Post hoc Hotelling or HSD. Other contrast hypothesis such as the one proposed in this article are used, as well as Bayesian statistics [23-30]. Mathematical tools are at our disposal, but the probability levels in which two samples are associated to the same origin are conditioned by the limited knowledge of the population variability of glass samples. Interpretation stages are therefore subjected to empiric data and expert opinions.

Therefore, in order to optimize the comprehensibility of the results the elaboration of conclusions in a clear text, avoiding data and using easy graphics and tools is recommended. The improvements of these aspects are subjected to the statistic formation and improvements in writing expression of the results by the scientific staff. Also, non-scientific experts in a trial need formation to familiarize about some scientific and statistic concepts. In addition, the development of





specialised software to interpret results in the forensic field and the effort to create comprehensive databases which allow the individualization of the samples and the objective assessment to a common origin is of great interest.

## 4 Conclusions

A methodology to analyse glass fragments by LA-ICP-MS has been critically analysed in order to be employed in forensic laboratories. The in-house validation has been developed on the basis of previous studies which have been finally proposed as normalized procedure by ENFSI and ASTM standard test. The validation of the method has been tested and a working protocol has been optimized, including quality controls to ensure the correct functioning of the validated methodology. Even rejecting some elements through the quality controls (up to 3/18 in some cases) results have been satisfactory. Focusing on the final results, FALSE MATCH and FALSE NO MATCH error rates are low enough to be suitable for a pairwise comparison methodology for the analysis and discrimination of glass samples found in forensic caseworks. Nevertheless, we propose forensic comparison in repeatability conditions in such a way that all samples involved in a casework should be analysed in the same analysis series and employing the same calibration standard. Taking into account that forensic chemistry has its peculiarities and differs in some aspects from analytical chemistry, a validation method based on the correct pairwise comparison instead of high levels of accuracy has proven to be suitable and effective to compare glass samples taken from forensic caseworks.

Finally, one should note that answering the questions from a court is the main objective of an expert valuation report and therefore we must make the effort to redact these reports with the appropriate language ready to be understood by all the roles present in the trial, and complement that information with understandable graphics. On the one hand, the concepts MATCH / NO MATCH / FALSE MATCH / FALSE NO MATCH proposed in this article for the forensic interpretation of statistic results can be of interest as they avoid the use of other definitions such as false positive or false negative errors which create controversy and confusion among the specialist involved in a case. On the other, the graphics proposed in this article with the parameters  $N_{z_{rep}}$ , LODs and  $N_{zi}$  may be useful in forensic expert reports.





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## SUPPLEMENTARY MATERIAL

The supporting information of this manuscript deals with a comprehensive example of a pairwise comparison between two glass fragments from a real casework. This detailed example is showed in order to clarify the concepts and tools proposed in the manuscript. In the casework, fragments recovered from the road (recovered sample, RS) were compared to fragments from a rear window from a vehicle, brand Jeep, and model Cherokee (Control sample, CS). Excel 2013 (Microsoft, Corp) was used to design a calculation sheet where all the graphics, quality controls and calculations are showed and depicted. Figures with the excel sheets are depicted for a better understanding of the process.

### **Real casework. Violent incident involving glass fragments on a road and a suspected vehicle**

Once obtained the elemental quantitative profile of the CS and RS, as well as the Quality controls described in the manuscript, calculations related to the quality controls and the pairwise comparison are made for each analytical series. This process is made through a calculation Excel sheet. In the following example FGS2 CRM is used as standard in the calibration. Quantitative composition in  $\text{mg}\cdot\text{Kg}^{-1}$  from the 6 replicates made for both the CS and RS are depicted in **FIG. S1**.

For both CS and RS samples the mean, standard deviation (SD) and relative standard deviation (RSD) are calculated. Normalized Z-score of repeatability is also calculated as the quotient between the RSD of the 6 replicates of each sample ( $\text{RSD}_{\text{sample}}$ ) and the RSD of the method ( $\text{RSD}_{\text{rep}}$ ):

$$N_{z\text{-rep}} = \frac{\text{RSD sample}}{\text{RSD rep}}$$





SAMPLE	CS=CONTROL SAMPLE; R=REPLICATE						RS=RECOVERED SAMPLE; R=REPLICATE						
	CS-R1	CS-R2	CS-R3	CS-R4	CS-R5	CS-R6	RS-R1	RS-R2	RS-R3	RS-R4	RS-R5	RS-R6	
Li	1,66	2,54	0,3	0,3	1,49	1,4	1,28	3,02	1,14	1,39	0,72	2,14	1,77
Na	101848,83	100908,28	101837,82	101094,89	100342,72	100681	102086,87	101703,77	101656,55	103722,34	101625,36	102103,41	
Mg	19135,84	19128,16	19203,9	19186,59	19127,06	19163,23	19237,79	19256,2	18953,51	18968,05	18879,43	18892,92	
Al	1752,84	1752,38	1753,96	1714,12	1739,3	1740,49	1764,95	1736,42	1706,64	1674,11	1720,29	1663,66	
K	443,6	444,95	447,3	444,33	435,25	439,64	463,59	453,93	461,3	453,39	446,96	438,46	
Ca	67520,85	67887,95	68085,14	67146,99	67791,77	67656,81	68230,02	67984,37	66374,92	65900,62	67641,78	65444,67	
Ti	238,42	236,54	240,95	233,89	235,08	237,03	235,87	233,27	229,3	223,6	234,57	229,41	
Mn	70,6	71	70,8	71,19	71,37	70,93	71,36	71,39	71,4	71,42	70,91	71,33	
Fe	7324,79	7407,24	7356,87	7428,51	7446,69	7438,47	7431,4	7462,24	7371,24	7582,44	7375,99	7460,55	
Rb	1,201	1,224	1,235	1,196	1,261	1,183	1,285	1,345	1,223	1,277	1,172	1,275	
Sr	118,39	119,22	119,49	116,64	118,02	116,71	118,52	118,58	116,2	114,69	117,26	114,18	
Zr	74,84	75,25	75,23	74,32	75,33	75,08	75,16	74,52	73,14	71,13	73,56	70,18	
Ba	23,22	23,12	23,37	22,32	22,4	22,53	22,98	23,49	23,2	22,53	22,48	22,92	
La	1,75	1,772	1,748	1,746	1,763	1,787	1,775	1,826	1,674	1,689	1,78	1,692	
Ce	5,81	5,8	5,91	5,8	5,82	5,68	5,93	6,01	5,84	6	5,77	5,86	
Nd	1,41	1,554	1,551	1,387	1,419	1,418	1,437	1,505	1,529	1,424	1,51	1,467	
Hf	1,92	1,97	2,08	1,97	1,91	2	1,96	2,03	1,87	1,87	2,02	1,88	
Pb	2,15	1,71	1,34	1,51	1,24	1,43	1,88	1,5	1,44	1,35	1,25	1,37	

**Fig S1.** Quantitative composition (mg·Kg<sup>-1</sup>) from the analysis of two glass samples (CS and RS) by LA-ICP-MS. 6 replicates are made for each sample.

Depending on the value that  $N_{z\text{-rep}}$  reaches, the element under study is rejected for the posterior pairwise comparison. On this way, if  $N_{z\text{-rep}}$  reaches a value of 3, this means that the  $RSD_{\text{sample}}$  of the element is more than three times the  $RSD_{\text{rep}}$  of the method for that element, being this variability above the limit ( $\pm 3 RSD_{\text{rep}}$ ) and rejecting the element for the following pairwise comparison. The usefulness of this parameter is to clearly detect excessive variability between replicates and eliminate the element or elements which cause these deviations for the following pairwise comparison. In addition, excel allows to include an automatic colour code which highlights the elements which present values outside the quality controls, as can be seen in **FIG. S2** (no color cells for  $N_{z\text{-rep}} < 2$ , yellow cells for near the limit values  $2 < N_{z\text{-rep}} < 3$  and red cells for values above the limit  $N_{z\text{-rep}} > 3$ ). **FIG. S2** shows the values of the parameters previously described for both samples.

In this case, Li and Pb are excluded for the posterior pairwise comparison because the replicates show an excessive variability for these elements. In the case of Li, low concentration (near the LOD,  $0.7 \text{ mg Kg}^{-1}$ ) may be the reason for this variability. In the case of Pb,  $N_{z\text{-rep}}$  a possible inhomogeneity could be the reason for the variability.

In addition to the precision quality control, LOD control is also included in the calculation sheet. If an element present a concentration under the LOD estimated in this in-house validation, it was rejected for the pairwise comparison and automatically a message in columns 3 and 4 from the calculation sheet (**FIG. S3**) would appear indicating the reason for rejection. This control does not require additional analyses and guarantees a normalized procedure where the LODs are controlled.





	CS (CONTROL SAMPLE)			QC			RS (RECOVERED SAMPLE)			QC		
	AVERAGE	SD	RSD	RSD rep	Nz-rep		AVERAGE	SD	RSD	RSD rep	Nz-rep	
Li	1,45	0,72	49,78	13,4	3,71		1,70	0,81	47,96	13,4	3,57	
Na	101118,92	614,62	0,61	2,7	0,23		102149,72	799,53	0,78	2,7	0,29	
Mg	19157,46	32,52	0,17	1,7	0,10		19031,32	170,58	0,90	1,7	0,53	
Al	1742,18	15,20	0,87	1,7	0,50		1711,01	38,12	2,23	1,7	1,28	
K	442,51	4,34	0,98	2,8	0,34		452,94	9,27	2,05	2,8	0,72	
Ca	67681,59	325,52	0,48	2,0	0,24		66929,40	1173,23	1,75	2,0	0,88	
Ti	236,99	2,50	1,05	2,9	0,37		231,00	4,51	1,95	2,9	0,68	
Mn	70,98	0,27	0,39	3,0	0,13		71,30	0,19	0,27	3,0	0,09	
Fe	7400,43	49,07	0,66	2,8	0,24		7447,31	77,18	1,04	2,8	0,37	
Rb	1,22	0,03	2,37	4,8	0,49		1,26	0,06	4,68	4,8	0,97	
Sr	118,08	1,21	1,03	1,9	0,54		116,57	1,88	1,61	1,9	0,84	
Zr	75,01	0,38	0,51	8,7	0,06		72,95	1,94	2,65	8,7	0,31	
Ba	22,83	0,46	2,02	8,0	0,25		22,93	0,39	1,69	8,0	0,21	
La	1,76	0,02	0,92	2,7	0,34		1,74	0,06	3,59	2,7	1,32	
Ce	5,80	0,07	1,26	2,5	0,50		5,90	0,09	1,61	2,5	0,63	
Nd	1,46	0,08	5,17	6,9	0,75		1,48	0,04	2,88	6,9	0,42	
Hf	1,98	0,06	3,12	9,5	0,33		1,94	0,08	3,88	9,5	0,41	
Pb	1,56	0,33	21,04	6,9	3,03		1,47	0,22	15,03	6,9	2,17	

**Fig S2.** Mean (mg Kg-1), SD (mg Kg-1), RSD and Nz-rep (precision quality control parameter) for both the CS and the RS.

Finally, the pairwise comparison is executed by employing the elements which have passed the precision and LODs controls. The pairwise comparison consists of estimating a comparison interval of 4 times the SD around the average from the CS, with a minimum of fixed relative standard deviation (RSD) of 3%. If the average of the RS presents a value inside this interval, we consider the comparison as MATCH, meaning that no significant differences have been found for that element in the comparison. In order to establish a MATCH between the RS and CS, none of the elements should present significant differences. In other words, all the elemental concentrations from the RS should be inside the comparison interval  $CS \pm 4SD$ , with a minimum of fixed relative standard deviation (RSD) of 3%. When one or more elements are not inside their respective intervals, samples are considered different and a NO MATCH is interpreted from the results. **FIG. S3** shows the final pairwise comparison carried out for the example casework in the calculation sheet.

As can be seen, in the sheet each row shows the calculation for each one of the elements analysed. If the element does not pass any of the quality controls proposed for the method the word “EXCLUDED” appears automatically in the column QC RESULTS. The reason for the exclusion can be seen in the calculation sheet in the following 4 columns. In this example, RSD of Li and Pb were higher than 3 times the RSD rep of the method and these elements are automatically excluded for the comparison.

For the remaining elements, the averages from the RS appear in the following column. If this value is inside the comparison interval (the two following columns) the result is a MATCH for that element. As can be seen in our example, all the comparisons gave a value of MATCH, and as a result no significant differences between their elemental concentrations have been found.





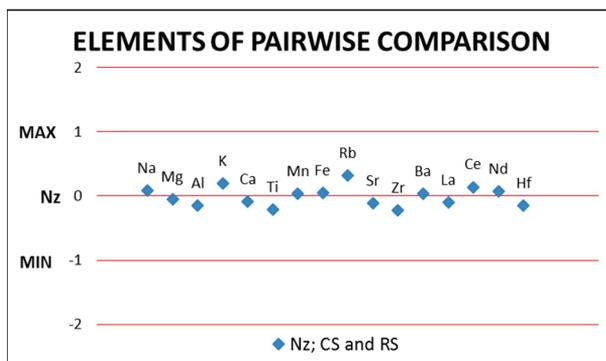
	QC RESULTS	DETAIL OF QC EXCLUSION				PAIRWISE COMPARISON AND RESULT:		
		LOD (CS)	LOD (RS)	QCREP (CS)	QCREP (RS)	RS (AVERAGE)	CS COMPARISON INTERVAL	RESULT
Li	EXCLUDED	-	-	HIGH RSD	HIGH RSD	-	-	-
Na	-	-	-	-	-	102150	88985	113253
Mg	-	-	-	-	-	19031	16859	21456
Al	-	-	-	-	-	1711	1533	1951
K	-	-	-	-	-	453	389	496
Ca	-	-	-	-	-	66929	59560	75803
Ti	-	-	-	-	-	231	208,5	265,4
Mn	-	-	-	-	-	71	62,46	79,50
Fe	-	-	-	-	-	7447	6512,4	8288,5
Rb	-	-	-	-	-	1	1,07	1,36
Sr	-	-	-	-	-	117	103,91	132,25
Zr	-	-	-	-	-	73	66,01	84,01
Ba	-	-	-	-	-	23	20,09	25,57
La	-	-	-	-	-	2	1,55	1,97
Ce	-	-	-	-	-	6	5,11	6,50
Nd	-	-	-	-	-	1	1,16	1,76
Hf	-	-	-	-	-	2	1,73	2,22
Pb	EXCLUDED	-	-	HIGH RSD	-	-	-	-

**Fig S3.** Results on the quality controls and automatic interpretation of the results in the pairwise comparison between the RS and CS (mg Kg-1).

Finally, in order to facilitate the visual interpretation of the results for the expert valuation inform, a parameter  $Nz_i$ , is calculated for each element involving the pairwise comparison. This parameter normalized the elemental concentrations and the comparison interval in the range of  $\pm 1$  as follows:

$$Nz_i = \frac{\overline{RS}_i - \overline{CS}_i}{4 \times SD_i(\text{FixMin } 3\%)}$$

Where  $RS_i$  and  $CS_i$  are the averages of the elemental composition of the recovered and control samples, and  $SD_i$  (FixMin 3%) the comparison interval for that element. These values are represented in a simple graphic where limits are depicted in such a way that any of the elements which gives a NO MATCH will appear outside these limits. **FIG. S4** shows the resulting graphic for the forensic casework, no significant differences between elemental concentrations were found. Therefore all of them appeared inside the limits and the final pairwise comparison between the RS and CS was interpreted as MATCH.



*Fig S4. Nz values for each one of the elements for the pairwise comparison of the RS and CS in casework example. MIN and MAX are the limits of comparison interval to conclude if a comparison is MATCH or NO MATCH.*







## Capítulo III

### Forensic analysis of inks samples





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## **Forensic discrimination of blue ballpoint pens on documents by laser ablation inductively coupled plasma mass spectrometry and multivariate analysis**

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## ABSTRACT

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The differentiation of blue ballpoint pen inks written on documents through an LA-ICP-MS methodology is proposed. Small common office paper portions containing ink strokes from 21 blue pens of known origin were cut and measured without any sample preparation. In a first step, Mg, Ca and Sr were proposed as internal standards (ISs) and used in order to normalize elemental intensities and subtract background signals from the paper. Then, specific criteria were designed and employed to identify target elements (Li, V, Mn, Co, Ni, Cu, Zn, Zr, Sn, W and Pb) which resulted independent of the IS chosen in a 98% of the cases and allowed a qualitative clustering of the samples. In a second step, an elemental-related ratio (ink ratio) based on the targets previously identified was used to obtain mass independent intensities and perform pairwise comparisons by means of multivariate statistical analyses (MANOVA, Tukey's HSD and T2 Hotelling). This treatment improved the discrimination power (DP) and provided objective results, achieving a complete differentiation among different brands and a partial differentiation within pen inks from the same brands.

The designed data treatment, together with the use of multivariate statistical tools represents an easy and useful tool for differentiating among blue ballpoint pen inks, with hardly sample destruction and without the need for methodological calibrations, being its use potentially advantageous from a forensic-practice standpoint. To test the procedure, it was applied to analyze real handwritten questioned contracts, previously studied by the Department of Forensic Document Exams of the Criminalistics Service of Civil Guard (Spain). The results showed that all questioned ink entries were clustered in the same group, being those different from the remaining ink on the document.

## 1 Introduction

Conventional substances such as inks become evidence in forensic laboratories because of the criminal activity associated to documents, such as threatening letters, fraudulent documents, authentication of signatures, insurance frauds, etc. Despite the increased use of printers and copiers, most of the documents under forensic examination are written with pen inks, more specifically with ballpoint pen inks [1].

Inks from writing instruments are mainly composed of colorants, a vehicle and additives. Colorants make up about a 20% (m/m) and can be divided into dyes and pigments. Carriers or vehicles make up approximately a 50% (m/m) and are usually organic solvents or water. Additives provide specific properties, being their nature quite varied (resins, viscosity adjusters, antioxidants, surfactants, softeners, corrosion inhibitors, lubricants, even tags as rare earth chelates). In this way, traditional ballpoint pens are usually oil-based and dyes are their principal colorants, while gel pens contain water and pigments remain dispersed in it. As a result, the number and chemical nature of all these components may be very different [1-3].

Some practices in the forensic analysis of ink evidence are: (1) pairwise comparisons of ink strokes related to specific writing instruments; (2) comparison and differentiation among strokes; (3) gathering information from the ink source such as the manufacturers, country, etc; and (4) dating different documents of forensic interest [3]. In order to carry out these practices, forensic protocols suggest firstly the use of non-destructive analytical techniques such as Scanning Microscopy, Infrared Reflectance and exposure to ultraviolet or X-ray





radiations. If classical methods do not solve the problem, destructive techniques may be used. Among them, Thin Layer Chromatography, High Performance Liquid Chromatography, Microspectrophotometry and Capillary Electrophoresis have been used [1,2,4,5]. However, these procedures imply sample extraction with organic solvents before characterizing or identifying the organic colorants and do not provide precise comparisons among inks. Contrary to separation techniques, spectrometric ones have demonstrated quantitative and more concrete results. Coumbaros et al. [6] demonstrated that Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS) is a useful technique to characterize dyes and inorganic substances of inks on documents without extraction or complicated sample preparations. Gallidabino et al. used Laser Desorption Ionization-TOF-MS (LDI-TOF-MS) in both positive and negative mode to classify and compare inks from 33 blue ballpoint pens analyzed directly on paper, achieving a discrimination power of 99.6% when considering unidentified compounds and relative peak areas [7]. In addition, Jones et al. [8] developed a non-destructive in situ method to differentiate writing inks on paper using Direct Analysis in Real Time (DART) for which forty two of forty three black and blue ballpoint, black fluid, and black gel inks were differentiated thanks to this relatively new ion source for MS.

More specifically, the use of laser-based elemental analytical techniques such as Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) or Laser Induced Breakdown Spectroscopy (LIBS) have been proposed as good discriminatory techniques for ink analysis. Recently, McGaw et al. [9] and van Es et al. [10] applied them to study the distribution of ink elements on paper, from a qualitative point of view. Also, Trejos et al. [11] applied LA-ICP-

MS and LIBS to analyze gel inks and paper, achieving a discrimination power of ~ 96–99% for three black ink sets and more than 98% between different paper sources. As can be seen in the literature, quantitative analyses can be made, but complex methodologies are needed for this purpose, as not only ink compounds age and degrade due to environmental factors, but no certified reference materials exist for ink analysis. Considering also that sample history is usually unknown, this elemental quantification may be subjected to different source errors.

Then, a question remains unanswered: would a halfway between qualitative and quantitative method be more appropriate for this kind of application? In a forensic laboratory, simple procedures have the advantage of providing fast conclusions, being relatively easy their use. Taking into account that a solid and well-established comparison of strokes is one of the most usually tasks in forensic cases, this research paper proposes a two-step method using a quasi-non-destructive technique (LA-ICP-MS) for the discrimination of blue inks with no need for methodological calibrations. Firstly, the procedure was optimized with blue ballpoint pens of known origin by using instrumental parameters reported in the literature [11]. Then, specific criteria were designed in order to obtain objective and robust results without the need for methodological calibrations. This method was applied to a case example arrived to the Criminalistics Service of Civil Guard (Spain).





## 2 Material and Methods

### 2.1 Inks of known origin

A set of twenty one blue ballpoint pens of ten known trademarks was studied with the aim of optimizing the method. A number code was assigned for each brand, while samples within the same brand or the same batch were codified with subscripts, as can be seen in **TABLE 1**. About 4.5 cm length parallel lines were drawn (one line for each pen) on a sheet of common office paper (white, A4 80 g·Kg<sup>-1</sup> density). Each sample was analyzed five times. Besides, a blank (paper without ink) was measured ten times lengthways the sheet of paper (top, middle and bottom) resulting in thirty different measurements.

**Table 1**

Manufacturing data and type of blue pens studied in the set

Sample	Brand	Model	Ink Type	Manufacturing data <sup>a</sup>		
1	PAPER MATE	Gel 2020	Gel	-		
2	Pentel	BK77	Oil-Based	2008	Nov	France
3	PILOT	G1-0.5	Gel	2011	Feb	Japan
4	Stabilo	pointVisco	Oil-Based	-		
5	Staedtler	Stick 430	Oil-Based	2011	Feb	Germany
6	Uniball	Jetstream	Gel	2009	Aug	Japan
7	Unipapel	Unipen	Oil-Based	-		
8	Pelikan	Stick	Liquid	-		

<sup>a</sup>Samples without manufacturing data were acquired from different shops in 2010

**Table 1 (continuation)**

Manufacturing data and type of blue pens studied in the set

Sample	Brand	Model	Ink Type	Manufacturing data <sup>a</sup>		
9A				2008	Nov	China
9B				2007	Jul	China
9C				2007	Aug	China
9D	BIC	Crystal	Oil-Based	2008	Jul	China
9E				2008	Aug	China
9F_1				2010	Aug	China
9F_2				2010	Aug	China
9F_3				2010	Aug	China
10A				2010		Spain
10B				2011		Spain
10C	Inoxcrom	MEDIUM	Oil-Based	2011	-	Spain
10D				2011		Spain
10E				2011		Spain

<sup>a</sup>Samples without manufacturing data were acquired from different shops in 2010

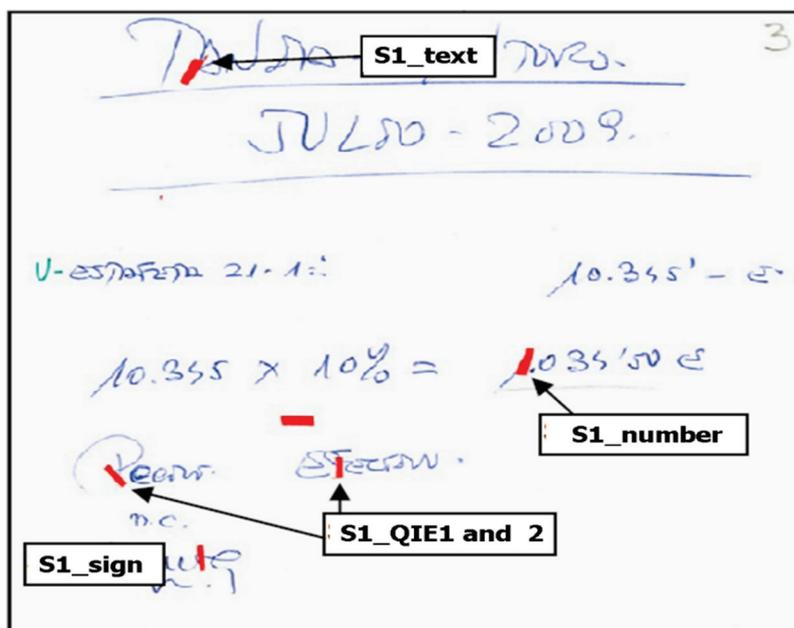
## 2.2 Questioned handwritten documents

Different documents (18 paper sheets) from a real case arrived to the Criminalistics Service of Civil Guard were subjected to LA-ICP-MS analysis in order to test the designed methodology. In all handwritten sheets there was a suspicion that some words (paid, effective and salary, in Spanish “recibí”, “efectivo” and “nómina” respectively) had been intentionally included for fraudulent purposes. Elemental analyses through LA-ICP-MS were carried out in order to identify possible differences in composition between the allegedly manipulated word and the remaining document. Taking into consideration the previous optical analyses carried out by the Department of Forensic Document Exams (different light contrast





analyses and graphology studies), which concluded that these words had been allegedly counterfeited, six sections (0.5 x 0.3 cm size) containing 0.5 cm length ink stroke and one blank (paper without ink) with the same dimensions were removed from each sheet and. Each one of these sections corresponded to different parts of the document: main text, financial calculations, the three questioned ink entries, and the signature. In some cases (sheets numbers 5, 7, 8, 12, 17 and 18) only five sections were collected since signatures belonging to these sheets were written with black ink and were directly discriminated. A reference number was allocated in all samples, according to the different parts of these documents. As a representative example, the **FIG. 1** shows a picture of sheet No. 1.



*Fig 1. Illustrated example of a questioned document (sheet No. 1) showing the different sections submitted to analysis by LA-ICP-MS. S1\_Text corresponds to the section from the main text, S1\_number to the financial calculations, S1\_QIE1 and S1\_QIE2 to the different questioned ink entries and S1\_sign to the signature.*

## 2.3 Instrumentation

LA-ICP-MS analyses were conducted using a LA system from New Wave Research UP 213 (Freemont, CA, USA) with a 213 nm fifth harmonic Nd-YAG solid state laser, coupled to a quadrupole ICP-MS system 7500cx (Agilent, Japan). Laser operational parameters optimized were: ablation mode, line; spot size, 100  $\mu\text{m}$ ; scan rate, 20  $\mu\text{m/s}$ ; repetition rate, 10 Hz and fluence, 2.00-3.00  $\text{J/cm}^2$ . Instrumental parameters used were: carrier gas through the ablation cell, He 1.00 L/min; carrier gas flow after the cell, Ar 1.0 mL/min; plasma gas Ar 14 L/min; auxiliary gas flow, Ar 0.9 L/min; rf power, 1400 W. Mass spectra were acquired in time resolved analysis mode (TRA); acquisition time: 20 ms/mass. As usually, a glass reference material NIST 612 (National Institute of Standards and Technologies, USA) was used to establish the proper functioning of the equipment (instrumental calibration) through the following parameters: torch position, ion optics, fractionation index (measured as the ratio  $^{238}\text{U}/^{232}\text{Th}$ ) [12] between 0.8 and 1.2, detector pulse/analog (P/A) factor calibration, doubly charged ion rates < 0.8 % ( $^{21}\text{Ca}^{+2}/^{42}\text{Ca}^{+}$ ) and oxide rates < 1.0% ( $^{248}\text{ThO}^{+}/^{232}\text{Th}^{+}$ ) [13]. TRA signals were performed using the GLITTER software (GEMOC, Macquarie University, Australia), v4.4.2. Graphics and statistical treatments were made using Excel 2007 (Microsoft, Corp.) and SPSS Statistics v18 (IBM, Corp.) software.

## 2.4 Methodology and data treatment

Elemental compositions from both the set of inks and the case example were obtained through a LA-ICP-MS equipment with parameters listed in section 2.3. Note that in the text of this manuscript elements will refer to their respective isotopes which are compiled in





**TABLE 2.** For the set, a total of 135 measurements were made, from which 42 isotopes of different elements could be collected and a total of 5670 intensity values were obtained. For the case example, different sections from eighteen questioned handwritten contracts (18 independent sheets) were analyzed. A total of 303 replicates and 22 elements per replicate were obtained, leading to 6666 values.

**Table 2**  
Raw and corrected intensities (using Sr as IS) for both the blank and sample 1 (taken as example) measurements, as well as the ink intensity ratios calculated for targets of sample 1.

	Blank intensities					Sample 1 intensities					Ink ratio <sup>c</sup>	
	Raw <sup>a</sup>		Corrected (Sr) <sup>b</sup>			Raw <sup>a</sup>		Corrected (Sr) <sup>b</sup>				
	$\bar{x}$	RSD (%)	$\bar{x}$	10SD	RSD (%)	$\bar{x}$	RSD (%)	$\bar{x}$	RSD (%)	$\bar{x}$	RSD (%)	
<sup>7</sup> Li	5	104	5	51	101	5	115	4	108			
<sup>23</sup> Na	255385	24	256570	515471	20	293873	10	240596	14			
<sup>24</sup> Mg	23394	21	23439	35570	15	32784	23	26967	30			
<sup>27</sup> Al	25747	24	25670	40940	16	42709	35	35351	43			
<sup>29</sup> Si	585	24	597	1890	32	833	19	671	6			
<sup>39</sup> K	7224	190	7013	126458	180	95913	55	81674	65			
<sup>42</sup> Ca	55408	21	55276	64699	12	71416	20	57421	6			
<sup>51</sup> V	61	67	60	385	64	57	24	47	31			
<sup>55</sup> Mn	462	20	462	516	11	6875	53	5839	64	211.4	4.3	
<sup>57</sup> Fe	777	43	768	2918	38	1065	13	867	12			
<sup>59</sup> Co	9	50	9	40	45	24	17	19	12			
<sup>60</sup> Ni	8	55	8	43	52	18	29	15	39			
<sup>63</sup> Cu	95	119	99	1310	132	330768	59	282036	69	-	-	
<sup>66</sup> Zn	17	83	17	150	87	13	64	12	73			
<sup>88</sup> Sr	57175	16	-	-	-	70869	16	-	-			
<sup>90</sup> Zr	18	36	18	56	31	750	72	651	82	21.9	29.3	
<sup>118</sup> Sn	3	152	3	46	141	67	79	58	88			
<sup>137</sup> Ba	189	101	196	2263	116	224	12	184	20			
<sup>140</sup> Ce	48	37	47	136	29	48	15	39	14			
<sup>182</sup> W	1	268	1	26	258	93	45	78	53			
<sup>208</sup> Pb	23	61	23	145	62	934	60	798	71	28.1	3.3	
<sup>238</sup> U	53	21	53	84	16	63	11	51	6			

<sup>a</sup> Average of 30 measurements (blank) or 5 measurements (sample 1).

<sup>b</sup> Corrected data average obtained by applying Eq. (1) to all replicates using Sr as IS.

<sup>c</sup> Ink intensity ratios obtained by applying Eq. (5) to raw intensities of targets (which satisfied criteria 2, 3 and 4).

The methodology employed for the dataset was based on two main steps:

**1. Data normalization:** which allowed to calculate background signals (interferences from the blank paper), and identify target elements (those whose intensities were high enough to differentiate from the background signals) in order to cluster samples into qualitative groups. In all cases, blank paper measurements were needed to subtract background from the elemental intensities from the strokes. This normalization was made by means of an internal standardization. Thus, to overcome this step the following correction to calculate the normalized intensity (cps) for each element and each sample ( $I_{CS}$ ) was raised:

$$I_{CS} = I_{rs} \cdot \frac{I_{ISp}}{I_{ISs}} \quad (1)$$

Where,  $I_{rs}$  is the raw elemental intensity (cps) for each replicate of each sample, and  $I_{IS}$  is the intensity of the isotope (cps) taken as IS, measured both in the blank or paper ( $I_{ISp}$ ) and in the ink samples ( $I_{ISs}$ ).

Mg, Ca and Sr were the elements proposed as ISs. Estimations were made for the three ISs in parallel in order to compare the possible differences for the targets depending on the IS selected. Once intensities had been normalized using the three ISs, a screening based on the following criteria was made in order to select target elements:

$$I_{CS} > 200 \text{ cps} \quad (2)$$

$$I_{CS} > I_{CP} + 10 \cdot SD_{I_{CP}} \quad (3)$$

$$No. Target replicates \geq 4 \quad (4)$$

On one hand criterion 2 imposed elemental intensities higher than 200 cps could be selected as target (to avoid possible contaminations among samples and selecting targets with relatively





high concentrations in the ink). On the other, criterion 3 (what we call “limit lines”) considered the background signals, in such a way that corrected intensities from the ink samples had to be higher than those from the blank ( $I_{CP}$ ) plus ten times their standard deviation (SD). Finally, according to criterion 4, elements whose intensities satisfied criteria 2 and 3 in more than four replicates within each sample were taken into account. This first approach is able to find characteristic elements from each pen, leading to the possibility of clustering them in qualitative groups and reduce the initial dataset to carry out easier and clearer statistical treatments.

**2. Semi-quantitative treatment:** dealt with the attempt to differentiate among samples within the groups qualitatively defined in the first step. For this purpose, only target elements were subjected to statistical treatment. This study was carried out through elemental correlations, what we called “ink ratios” (R), relating raw intensities from target elements measured for an ink stroke ( $I_{r, target}$ ) with Cu raw intensity from the same stroke ( $I_{r, Cu}$ ), as stated in equation 5:

$$R = \frac{I_{r, target}}{I_{r, Cu}} \quad (5)$$

Cu was selected as reference on the basis of the assumption that most blue ink samples present this element in their composition. It is important to emphasize that only raw intensities and not the corrected ones from the first step were used, as this ink ratio was capable of minimizing intensity variations related to the writing pressure and speed, irregularities of ink mass expelled from the pen and laser drift.

To accomplish an objective ink differentiation within groups, different multivariate statistical treatments were applied to these ink

ratios: Multivariate Analysis of Variance (MANOVA), post hoc Honestly Significant Difference (HSD) Tukey's test or  $T^2$  Hotelling. In all cases a 95% confidence interval was established considering some previous research [13]. MANOVA is a multivariate analysis of variance for multiple dependent variables, which in our case are the elements, and only one independent variable, which are the samples. This test considers both inter and intra-variability and it is based on the null hypothesis of the equality of multiple averages. Tukey's test establishes various groups by studying all significant differences from all possible combinations among samples (in our case,  $n(n-1)/2$ , being  $n$  the number of samples under study).  $T^2$  Hotelling could be treated as a special case of MANOVA as a direct extension to multivariate  $t$  contrast. MANOVA and Tukey's tests were used when more than two samples were within a group, while  $T^2$  Hotelling was used when groups contained only 2 samples.

Ink ratios together with their statistical treatment allowed carrying out differentiations among inks without the need for instrumental calibrations, which require home-made ink patterns since no ink certified reference materials are currently available.

This procedure was applied to the following workplan. Firstly, the set of inks of known origin was analyzed with the aim of evaluating the discrimination power of the proposed methodology. Afterwards, documents from the case example were subjected to the same methodology in order to test the usefulness of the method and compare the results with those obtained by the Department of Forensic Document Exams, whose practices are accredited by the National Entity of Accreditation (in Spanish "Entidad Nacional de Acreditación, ENAC").





## 3 Results and Discussion

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### 3.1 Discrimination of blue pen inks of known origin

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**TABLE 2** illustrates the procedure followed for data treatment of measured intensities both in the blank (paper) and in the ink strokes (sample 1 as a representative example), taking Sr as IS. Elements which did not provide information in any sample due to their extremely low intensities, almost undistinguishable from the background (Sc, Ti, Cr, Rb, Mo, Rh, Ag, Cd., Sb, Cs, La, Pr, both isotopes of Nd, Eu, Hf, Ta, Re, Os and Tl), were no longer taken into account and do not appear in the table. The non-uniform distribution of the ink on the paper fibers, the laser drift and the low intensities from some elements justified the poor reproducibility of the intensity values obtained for this sample, as RSDs ranged from 10 to 115%. Further information related to raw and corrected intensities with Sr as IS for each sample and replicate can be found in the Supplementary File of this manuscript.

Obviously a proper methodology for data interpretation should be optimized to use these values for discrimination purposes. As stated in the previous section, a two-step methodology was applied: 1) selection of an adequate IS to correct raw intensities and identify target elements for each sample to cluster them qualitatively; 2) Obtaining ink ratios and application of multivariate statistical treatments to confirm or improve the differentiation among inks. These two steps were first optimized with blue pen inks of known origin and, then, tested with questioned handwritten contracts from a forensic case example.

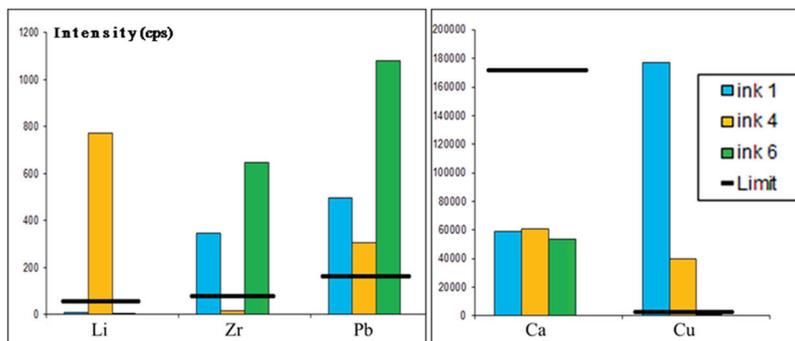
### 3.1.1 Selection of the Internal Standard and Qualitative Clustering Based on Target Elements

Raw data normalization through an IS is a critical step. A suitable internal standard (IS) would be an element found in the sheet of paper and homogeneously distributed within it. The selection of this IS seems a debated question since different elements have been proposed in the literature with not always successful results [9–11]. Mg, Ca and Sr were the elements chosen as IS in this case due to three main reasons: 1) raw intensity averages from these elements in the blank were in a great extent than others and with relatively low RSD values (Mg 21%, Ca 21% and Sr 16%) ; 2) Sr has been successfully chosen as IS in a previous research [10]; and 3) paper samples had been distinguished through Mg and Ca concentrations, although different pieces of paper from the same sheet had been indistinguishable [14], however, this problem may be solved if different paper sheets are treated as independent samples with independent blank measurements, as done in this work. The study was then made by taking the three IS and working in parallel in order to determine the variability of the targets depending on the selected IS. Target selection was made as shown in **FIG. 1**. This one illustrates graphically some corrected elemental intensities (Li, Zr, Pb, Ca and Cu) for samples 1, 4 and 6 as examples. As can be seen, despite the fact that intensities from Li, Zr and Pb were much lower than those from Ca, they sometimes fulfilled the requirements for being target elements (for instance the case of Li in ink No. 4) and potentially characterize inks. Ca intensities are relatively high for both analysis (blank and ink stroke on paper) so limit line (criterion 3) is above the eights of corrected Ca intensities for all samples, confirming that it is not a target and its composition comes from the sheet of paper. Contrary to this, intensities clearly above the limit have been confirmed in the case of Cu when analyzing ballpoint pens such as 1 and 4, but not for sample 6 (Uniball Jetstream gel). Other elements, such as Zr and Pb are





usually present in some samples, thus allowing a proper qualitatively clustering.



*Fig 2. Bar chart showing corrected intensities in cps of five different elements (Li, Zr, Pb, Ca and Cu) for inks 1, 4 and 6 (values corresponding to the first replicate using Sr as IS). Limit line (criterion 3) corresponds to the background limit level which establishes that elements are assigned as targets for each sample and replicate.*

**TABLE 3** summarizes target elements according to the sample and IS chosen. From the 231 possible cases, only in four of them different ISs led to different target elements (V for inks 2 and 5 and Pb for inks 10A and 10B), being the targets independent on the IS in a 98% of the cases. When Sr was selected as IS, V obeyed criteria 2 and 3 in three replicates from sample 5, but it could not be assigned a target because of the criterion 4. Similar behavior was evidenced for Pb in samples 10A and 10B: V and Pb intensities were extremely near the limit imposed by the criterion 3, leading to slightly differences depending on the IS. Since Sr has presented lower RSDs than Mg and Ca, it could be the best one to carry out the treatment. Nevertheless, the second step in section 3.1.2 was made in parallel with the three IS in order to evaluate final differences and observe their potential use as IS.



**Table 3**  
Target elements found for each ink sample<sup>a</sup>

	1	2	3	4	5	6	7	8	9A	9B	9C	9D	9E	9F1	9F2	9F3	10A	10B	10C	10D	10E	
<sup>7</sup> Li				T																		
<sup>51</sup> V		Ca, Sr			Mg, Ca*		T						T <sup>S</sup>				T <sup>X</sup>	T	T <sup>&amp;</sup>	T <sup>Z</sup>	T <sup>&amp;</sup>	T <sup>&amp;</sup>
<sup>55</sup> Mn	T		T	T													T <sup>X</sup>	T	T <sup>&amp;</sup>	T <sup>Z</sup>	T <sup>&amp;</sup>	T <sup>&amp;</sup>
<sup>59</sup> Co						T																
<sup>60</sup> Ni						T																
<sup>63</sup> Cu	T	T		T	T*		T	T	T	T <sup>S</sup>	T <sup>S</sup>	T <sup>S</sup>	T <sup>S</sup>	T <sup>V</sup>	T <sup>V</sup>	T <sup>V</sup>	T <sup>X</sup>	T	T <sup>&amp;</sup>	T <sup>Z</sup>	T <sup>&amp;</sup>	T <sup>&amp;</sup>
<sup>66</sup> Zn					T*		T	T	T	T <sup>S</sup>	T <sup>S</sup>	T <sup>S</sup>	T <sup>S</sup>	T <sup>V</sup>	T <sup>V</sup>	T <sup>V</sup>			T <sup>&amp;</sup>	T <sup>Z</sup>	T <sup>&amp;</sup>	T <sup>&amp;</sup>
<sup>90</sup> Zr	T					T																
<sup>118</sup> Sn									T													
<sup>182</sup> W					T*					T <sup>S</sup>	T <sup>S</sup>	T <sup>S</sup>	T <sup>S</sup>	T <sup>V</sup>	T <sup>V</sup>	T <sup>V</sup>		T				
<sup>208</sup> Pb	T	T	T	T	T*	T	T	T	T	T <sup>S</sup>	T <sup>S</sup>	T <sup>S</sup>	T <sup>S</sup>	T <sup>V</sup>	T <sup>V</sup>	T <sup>V</sup>	Ca, Sr <sup>X</sup>	Ca, Sr		T <sup>Z</sup>		

<sup>a</sup>T indicates that the element is a target regardless the IS chosen, different symbols on the right in some cells indicate same qualitative groups in scheme from Fig. 3.



It is also evident from **TABLE 3** that samples 1, 2, 3, 4, 6, 7, 8, 9A and 10B (cells with no color or symbol) were qualitatively individualized. Samples 9B, 9C, 9D and 9E (group 1, “\$”) and samples 9F1, 9F2 and 9F3 (group 2, “#”) were differentiated, independently of the IS selected. However, sample 5 fit with group 1 when Mg and Ca were the IS and with group 2 if Sr was the IS. Samples 10C and 10E formed the group 3 (“&”) and, similarly to groups 1 and 2, sample 10A (“X”) fit with group 3 when Mg was the IS, and with sample 10D when Ca or Sr were used, creating the group 4 (“Z”). Consequently, a screening among brands of blue ballpoint pen inks was possible, although discrimination between different batches from the same brand or different pens from the same batch was not.

Attending to the chemical composition, element frequencies can be discussed. Li, Co, Ni and Sn appeared in one brand, while Zr did it in two brands, W in three of them, Zn appeared in four brands, V and Mn in five samples, Cu in eight different inks and finally Pb which was detected in all the brands studied. Consequently, some elements such as Li, Co, Ni and Sn exhibit higher discrimination power (DP) than others such as Pb and Cu. Inks without Cu correspond to gel pens possibly due to the absence of Cu in their formulation which fits with the previous literature, though it is not obeyed in all the cases (sample 1 is a gel pen which contains Cu in its composition). Focusing on Bic samples, the six batches were clustered in three groups, independently of the IS chosen. However, Inoxcrom pens resulted in the most conflictive samples since slight differences were confirmed depending on the IS selected. All these inks contained V, Mn and Pb, while W was only present in sample 10B, and Pb in samples 10D, 10A and 10B. The Supplementary File can be consulted for further information of data obtained.

### 3.1.2 Statistical treatment of Ink Ratios. Improvement of the Discrimination Power

The presence of Cu in most blue ballpoint pens and results found in the set (which showed higher concentrations of Cu and its low RSD values in most of the samples) led to the selection of this element to calculate ink ratios. Gel pens which did not show Cu in their formulation were in this case qualitatively differentiated, but in the case of facing up various gel pens in the same group, another metal should be chosen to carry out that differentiation. As a general rule, an element present in all samples with a high concentration would be a possible candidate to calculate ink ratio.

Last two columns on **TABLE 2** show the ink ratios for sample 1 and their RSD values, which decreased dramatically. The remaining ink ratios for each sample can be consulted in the Supplementary File of this manuscript. Interestingly, in some cases RSD values were not reduced when applying these ratios, such as the case of W in Bic samples (9C-9F3). Low elemental intensities near to the LODs could cause these variations.

After obtaining the ink ratios statistical tests were applied. In this case only V, Mn, Zn, W and Pb ratios were necessary, as the remaining targets belonged to the samples which had been qualitatively individualized (see **TABLE 2**). Statistical treatments applied to these ratios were conducted in parallel with the three IS studied, to assure the robustness of the results.

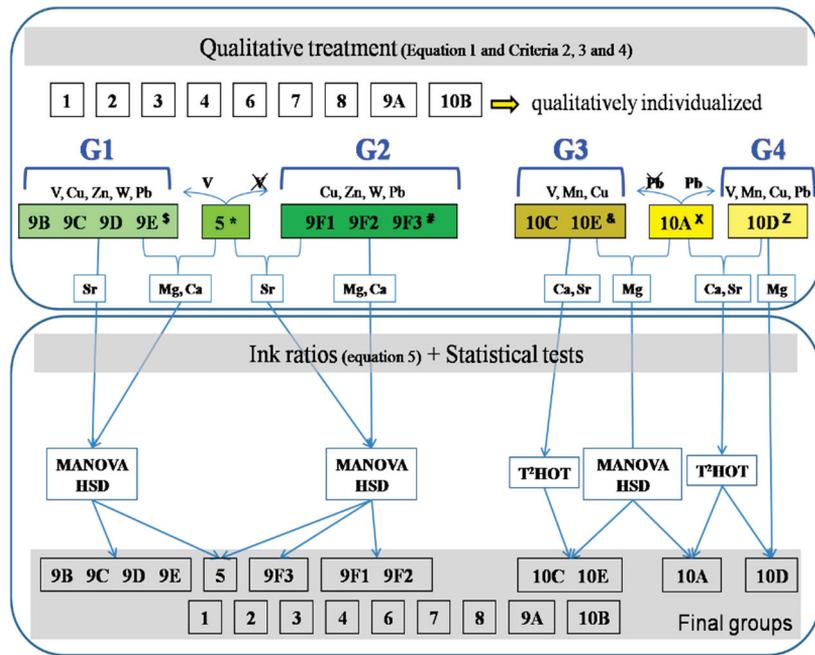
**FIG. 3** shows a summary of all data treatment used to differentiate the inks of known origin. The first qualitative treatment had led to nine samples qualitatively individualized and four different ink groups (G1, G2, G3 and G4) in which samples 5 and 10A belonged to different ones depending on the IS selected. In the second step, these groups were





statistically treated through their respectively ink ratios (Equation 5). In all cases groups 1 and 2 were subjected to MANOVA/HSD tests (more than 2 samples in each group). Despite of being sample 5 totally discriminated; four batches from Bic (9B, 9C, 9D and 9E) resulted indistinguishable, though batch 9F was statistically different. Within that batch, sample 9F3 could be also distinguished from 9F1 and 9F2. For groups 3 and 4, only samples 10C and 10E from the same brand were indistinguishable, while 10A and 10D were statistically different. These results were found independently on the IS selected and using different statistical tests.

Although a complete discrimination was not possible, the DP was improved by using statistical tests, leading to the differentiation of five batches from Innoxrom (10A and 10D), samples from Staedtler (5) and one of the three Bic pens from the same batch (9F3). As a result, the use of two consecutive treatments (internal standardization for qualitative clustering and ink ratios with statistical test for the semi-quantitative study) provided similar results to those previously published in LA-ICP-MS: a DP of 100% among samples from different brands and a partial discrimination among batches or samples within the same batch. This procedure has been capable of discriminating ink samples with similar DP to spectrometric techniques, but avoiding the use of home-made patterns to perform methodological calibrations, which reduces both analysis time and resources.



*Fig 3. Scheme showing the complete treatment carried out. The first frame deals with the treatment where samples were qualitatively clustered by means of their target elements (equation 1 and criteria 2, 3 and 4). The second frame shows the treatment carried out with the target elements (ink ratios from equation 5) in order to pass the statistical tests and obtain the final groups.*

### 3.2 Application to a questioned handwritten document

The methodology was also applied to the analysis of questioned handwritten contracts from a forensic case example. Starting from the results obtained from the Department of Forensic Document Exams of the Criminalistics Service of Civil Guard (Spain), the allegedly manipulated documents were taken and analyzed, in order to apply the



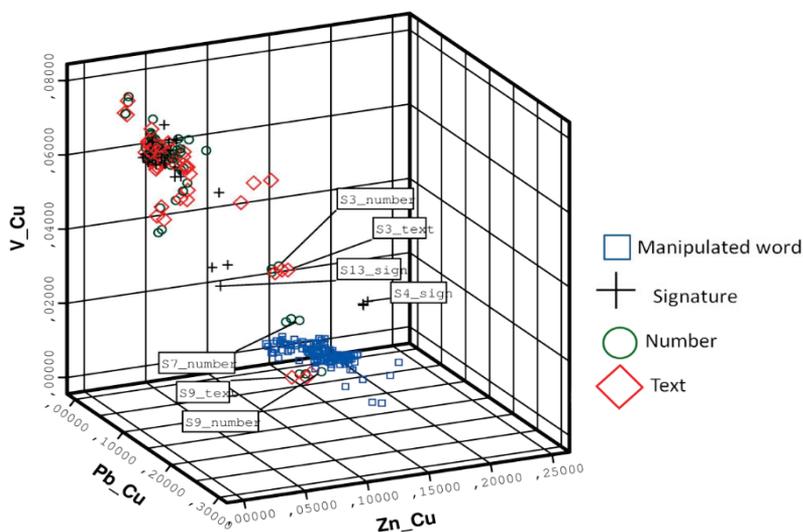


developed procedure and compare results obtained with those achieved by that Department.

On the first step, sections from different parts of the document and blanks from each one of the 18 independent paper sheets were taken and analyzed. Raw intensities from the samples were corrected using Sr as IS (equation 1). Applying the designed criteria (2, 3 and 4) a qualitative clustering in two groups was possible. On one hand, the majority of ink samples were characterized by the elements V, Cu, Zn, and Pb (group A). On the other, only text and numbers from sheet 11 contained Cu, Zn and Pb in their composition (group B; S11\_text and S11\_number). This may be explained considering that in most cases the documents could have been written with similar ballpoint pens and trademarks. As can be seen, in the case studied the qualitatively clustering was not as effective as in the set of 21 blue pens. This also fits with the conclusions of the qualitative method, where a differentiation among brands was a successful but a multivariate statistical treatment was necessary in order to improve the DP within the same brand.

On the second step, raw signals of the targets in group A (81 samples) were used to calculate the ink ratios (equation 5) using Cu as quotient. As this group was characterized by 4 elements, only 3 ink ratios for each replicate were calculated. A 3D plot is showed in **FIG. 4** to help out understanding this clustering. Three subgroups within the group A can be visualized. One of them (at the bottom of the plot) is composed of all questioned ink entries, text and numbers from sheets 9 and sheet 7, slightly differentiated among them. The second subgroup (at the middle of the plot) consist of the signatures of sheets 4 and 13 as well as text and the number of sheet 3. The remaining samples formed the third group, at the top of the plot.

When  $T^2$  Hotelling test was applied to the 81 samples of group A, the number of possible pairwise comparisons evaluated was 3240. This test confirmed the same 3 subgroups of samples described in **Fig. 4** (95% confidence). Inks from the questioned ink entries were identified in only one subgroup with other three samples (text and number from sheet 9 and number from sheet 7). As can be seen, the method did confirm that these questioned ink entries had been written with different pens to those used to write the remaining document. As a consequence, results obtained with the designed methodology in LA-ICP-MS agreed with those from the Department of Forensic Document Exams. And moreover, this methodology would allow the use of other analytical techniques after analyzing real documents if complementary results or data were needed to solve the case.



*Fig 4. Visual clustering of the ink samples from the case example studied according to the value of three discriminant variables (ink ratios of V/Cu, Zn/Cu, and Pb/Cu).*





## 4 Conclusions

An LA-ICP-MS methodology has been designed and applied to a set of blue inks of known origin and a case example. Firstly, the selection of target elements meant a reduction of trivial variables that would have hindered statistical treatment interpretations from the dataset. These targets were good discriminators for a first screening of inks into qualitative groups, according to their brand. Secondly, the use of “ink ratios” allowed a reduction of heterogeneities in the measurements and an easy application of multivariate statistical analysis (MANOVA, Tukey’s HSD and  $T^2$  Hotelling tests), leading to an improvement of the DP of the method. In this way, some differentiations within a batch or discrimination among batches from the same brand were possible. Finally, when this procedure was applied to interpret a case example, results obtained showed that inks used to write some questioned ink entries in the document were indistinguishable but different from the rest of the document, showing the potential of the methodology employed. In this work the optimization of the whole data treatment methodology has been developed but, once designed, analyses are fast enough to be useful in forensic laboratories.

This LA-ICP-MS methodology has provided higher DPs than other conventional methods or techniques, such as visual comparison that lead to more subjective results or separation techniques which require tedious sample preparation. Besides, it should be stressed the advantage of being a quasi-non-destructive technique which allows complementary analyses of the documents under study. Compared to spectrometric techniques, and specifically to other works focused on

LA-ICP-MS, this methodology has showed a different data treatment based on two steps, which permitted ink sample discrimination without performing methodological calibrations, the Achilles heel of this technique, due to the lack of ink certified reference materials.

Finally, these preliminary results confirm our proposed methodology as a proof of concept which in the future could be applied to overcome more complete sample sets and carry out studies on samples from the same brand and different batches.





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## SUPPLEMENTARY MATERIAL

The supplementary material of this work shows a background datasheet with the corrected intensities for each replicate of each sample analyzed from the set of inks of known origin (from 2 to 10E) taking Sr as IS. Each table contains the corrected intensities as well as the Target/Cu ratios calculated except in the cases where intensities did not satisfy the criteria. Note that Target/Cu ratios of samples 3 and 6 were not obtained since these ones did not present Cu in their formulation, although these ink ratios were not necessary because they were qualitatively individualized. Limits obtained by means of the Equation 1 can be obtained in **TABLE 3** of the manuscript, since all ink strokes were written in the same sheet of paper, thus being the same blank averages for all of them. The first **TABLE S-1** contains the notes which can be used for the rest of tables in this Supplementary-file appendix. The same treatment was applied when using Ca and Mg as IS, resulting in the target elements which can be consulted in **TABLE 3** of the manuscript.

**Table S-1**

Corrected intensities of sample 2 using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>					$\bar{x}$	RSD	T/Cu ratio <sup>3</sup>	
	Rep1	Rep2	Rep3	Rep4	Rep5			$\bar{x}$	RSD
<sup>7</sup> Li	11	22	9	0	6	10	84.1		
<sup>23</sup> Na	252740	253671	233611	228574	198476	233414	9.6		
<sup>24</sup> Mg	20913	23291	23068	36167	22723	25232	24.5		
<sup>27</sup> Al	24146	24615	28163	26952	23163	25408	8.2		
<sup>29</sup> Si	741	521	495	858	545	632	25.2		
<sup>39</sup> K	2551	3898	2155	2062	1495	2432	37.1		
<sup>42</sup> Ca	59239	59858	60961	61886	58380	60065	2.3		
<sup>51</sup> V	705(T)	749(T)	622(T)	627(T)	317	604	28.0	311.76	0.8
<sup>55</sup> Mn	569	576	610	620	601	595	3.7		
<sup>57</sup> Fe	860	729	825	913	843	834	8.0		
<sup>59</sup> Co	13	14	9	16	9	12	25.0		
<sup>60</sup> Ni	27	66	22	25	20	32	60.6		
<sup>63</sup> Cu	22436(T)	24278(T)	20012(T)	19959(T)	10189(T)	19375	28.1		
<sup>66</sup> Zn	68	140	90	67	34	80	49.0		
<sup>90</sup> Zr	16	27	31	26	14	23	33.4		
<sup>118</sup> Sn	4	0	1	0	0	1	170.3		
<sup>137</sup> Ba	154	138	180	158	136	153	11.6		
<sup>140</sup> Ce	40	51	35	51	37	43	17.4		
<sup>182</sup> W	18	15	22	15	18	18	17.2		
<sup>208</sup> Pb	444(T)	452(T)	432(T)	376(T)	210(T)	383	26.4	198.96	6.3
<sup>238</sup> U	56	57	59	59	55	57	3.1		

<sup>1</sup>Corrected data obtained by applying equation 1

<sup>2</sup>(T) indicates the intensity of the replicate satisfied expressions 2 and 3.

<sup>3</sup>Ink ratio was obtained by applying equation 5 and multiplying values per 10000 (only in the cases in which expression 4 was satisfied).

**Table S-2**

Corrected intensities of sample 3 using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>					$\bar{x}$	RSD	T/Cu ratio <sup>3</sup>	
	Rep1	Rep2	Rep3	Rep4	Rep5			$\bar{x}$	RSD
<sup>7</sup> Li	0	0	14	0	8	5	145.4		
<sup>23</sup> Na	362147	206601	402990	318321	450724	348157	26.7		
<sup>24</sup> Mg	21057	12625	19474	18841	20388	18477	18.3		
<sup>27</sup> Al	24993	14945	25333	23766	25638	22935	19.7		
<sup>29</sup> Si	521	278	409	529	587	465	26.3		
<sup>39</sup> K	5493	2887	6472	4773	6900	5305	29.9		
<sup>42</sup> Ca	66706	40468	59267	57174	63076	57338	17.6		
<sup>51</sup> V	54	30	64	58	42	50	27.3		
<sup>55</sup> Mn	2852(T)	1495(T)	2943(T)	2549(T)	3942(T)	2756	31.8		
<sup>57</sup> Fe	853	554	792	841	821	772	16.1		
<sup>59</sup> Co	99	45	94	96	110	89	28.3		
<sup>60</sup> Ni	8	7	10	9	14	9	27.6		
<sup>63</sup> Cu	46	42	120	14	53	55	71.3		
<sup>66</sup> Zn	6	3	7	4	3	4	40.1		
<sup>90</sup> Zr	23	11	23	18	20	19	25.0		
<sup>118</sup> Sn	12	5	11	5	5	7	50.0		
<sup>137</sup> Ba	163	80	131	125	144	129	24.1		
<sup>140</sup> Ce	38	23	41	38	46	37	22.5		
<sup>182</sup> W	142	64	143	133	143	125	27.7		
<sup>208</sup> Pb	544(T)	270(T)	528(T)	450(T)	693(T)	497	31.1		
<sup>238</sup> U	51	37	45	51	59	49	16.7		





**Table S-3**

Corrected intensities of sample 4 using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	772(T)	712(T)	604(T)	741(T)	672(T)	700	9.3	200.93	5.3
<sup>23</sup> Na	250043	205539	179459	202026	230313	213476	12.8		
<sup>24</sup> Mg	20825	18847	19357	23998	22901	21186	10.5		
<sup>27</sup> Al	24209	21513	20802	26075	23087	23137	9.1		
<sup>29</sup> Si	503	390	384	1300	503	616	62.8		
<sup>39</sup> K	105958	91523	79784	100854	93923	94408	10.6		
<sup>42</sup> Ca	60590	52790	54213	58062	61580	57447	6.7		
<sup>51</sup> V	41	45	43	44	48	44	5.6		
<sup>55</sup> Mn	1807(T)	1669(T)	2187(T)	1761(T)	2386(T)	1962	15.7	568.88	21.1
<sup>57</sup> Fe	784	676	704	777	745	737	6.3		
<sup>59</sup> Co	17	13	11	11	13	13	17.7		
<sup>60</sup> Ni	33	39	34	40	46	38	14.4		
<sup>63</sup> Cu	40031(T)	34388(T)	30418(T)	34286(T)	35358(T)	34896	9.9		
<sup>66</sup> Zn	4	11	9	10	7	8	33.4		
<sup>90</sup> Zr	18	12	17	15	14	15	14.4		
<sup>118</sup> Sn	3	2	3	0	0	2	100.0		
<sup>137</sup> Ba	130	111	123	137	135	127	8.4		
<sup>140</sup> Ce	45	38	37	46	34	40	12.7		
<sup>182</sup> W	123	97	132	152	174	135	21.3		
<sup>208</sup> Pb	307(T)	258(T)	920(T)	376(T)	688(T)	510	55.7	151.71	64.1
<sup>238</sup> U	49	43	45	49	51	47	6.8		

**Table S-4**

Corrected intensities of sample 5 using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	0	3	2	3	0	2	94.4		
<sup>23</sup> Na	216956	133798	190303	170016	177631	177741	17.1		
<sup>24</sup> Mg	24680	22029	18111	19563	16310	20138	16.3		
<sup>27</sup> Al	22554	22364	27919	23530	19592	23192	13.0		
<sup>29</sup> Si	582	444	729	418	371	509	28.7		
<sup>39</sup> K	2058	1402	1691	1779	2388	1864	20.1		
<sup>42</sup> Ca	58688	57282	57979	58400	53081	57086	4.0		
<sup>51</sup> V	512(T)	435	434	479(T)	467(T)	465	7.0		
<sup>55</sup> Mn	420	453	666	451	369	472	24.1		
<sup>57</sup> Fe	808	723	697	850	637	743	11.5		
<sup>59</sup> Co	218(T)	124	202(T)	134	205(T)	176	24.9		
<sup>60</sup> Ni	22	17	15	19	13	17	21.0		
<sup>63</sup> Cu	2192(T)	2126(T)	2124(T)	2315(T)	1945(T)	2140	6.2		
<sup>66</sup> Zn	819(T)	663(T)	732(T)	756(T)	745(T)	743	7.5	3478.87	8.7
<sup>90</sup> Zr	15	13	21	18	19	17	18.3		
<sup>118</sup> Sn	30	22	32	20	25	26	19.6		
<sup>137</sup> Ba	206	139	164	133	136	156	19.8		
<sup>140</sup> Ce	48	34	54	39	38	43	18.9		
<sup>182</sup> W	658(T)	364(T)	542(T)	313(T)	727(T)	521	34.5	2471.05	39.0
<sup>208</sup> Pb	2441(T)	1147(T)	1481(T)	1337(T)	1784(T)	1638	30.9	7690.72	31.5
<sup>238</sup> U	51	50	54	52	48	51	4.0		

Table S-5

Corrected intensities of sample 6 using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	3	1	1	6	3	3	69.7		
<sup>23</sup> Na	185239	287493	231146	178501	280253	232526	22.0		
<sup>24</sup> Mg	21205	24400	20082	20395	20903	21397	8.1		
<sup>27</sup> Al	22131	30661	22011	22390	27007	24840	15.6		
<sup>29</sup> Si	1970	2573(T)	1613	1418	2704(T)	2056	27.7		
<sup>39</sup> K	2032	3240	2082	1708	2744	2361	26.2		
<sup>42</sup> Ca	53538	68582	58180	55050	60270	59124	10.0		
<sup>51</sup> V	44	70	65	44	62	57	21.0		
<sup>55</sup> Mn	2646(T)	4831(T)	2878(T)	2278(T)	4039(T)	3335	31.9		
<sup>57</sup> Fe	687	840	1071	734	822	831	17.8		
<sup>59</sup> Co	1237(T)	2106(T)	1445(T)	1100(T)	2137(T)	1605	30.4		
<sup>60</sup> Ni	243(T)	405(T)	260(T)	212(T)	377(T)	299	28.7		
<sup>63</sup> Cu	96	169	125	100	177	133	28.5		
<sup>66</sup> Zn	4	11	10	5	3	7	55.8		
<sup>90</sup> Zr	648(T)	457(T)	288(T)	316(T)	251(T)	392	41.6		
<sup>118</sup> Sn	5	6	4	2	1	4	52.9		
<sup>137</sup> Ba	139	160	151	134	150	147	6.9		
<sup>140</sup> Ce	41	43	39	53	43	44	11.9		
<sup>182</sup> W	44	90	56	34	74	60	38.0		
<sup>208</sup> Pb	1081(T)	1741(T)	1231(T)	856(T)	1725(T)	1327	29.7		
<sup>238</sup> U	44	56	44	56	49	50	11.9		

Table S-6

Corrected intensities of sample 7 using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	13	24	8	0	7	10	84.6		
<sup>23</sup> Na	168613	206726	176252	256083	203224	202180	17.0		
<sup>24</sup> Mg	35758	19736	16867	20587	28519	24294	31.8		
<sup>27</sup> Al	21232	23474	20429	26901	24662	23340	11.2		
<sup>29</sup> Si	684	627	480	456	685	587	18.9		
<sup>39</sup> K	1416	2064	1487	2099	2137	1840	19.4		
<sup>42</sup> Ca	56733	60661	48753	55078	56882	55621	7.8		
<sup>51</sup> V	890(T)	1216(T)	1080(T)	1289(T)	1204(T)	1136	13.8	978.93	12.1
<sup>55</sup> Mn	385	430	395	482	441	427	9.1		
<sup>57</sup> Fe	810	732	582	776	692	719	12.3		
<sup>59</sup> Co	8	12	9	7	11	9	25.8		
<sup>60</sup> Ni	16	25	19	27	32	24	25.8		
<sup>63</sup> Cu	7981(T)	14895(T)	10393(T)	14290(T)	11787(T)	11869	23.9		
<sup>66</sup> Zn	1745(T)	2036(T)	2105(T)	2592(T)	2188(T)	2133	14.3	1849.85	16.6
<sup>90</sup> Zr	16	13	14	12	13	14	10.4		
<sup>118</sup> Sn	109	123	116	151	135	127	12.9		
<sup>137</sup> Ba	130	138	124	208	151	150	22.6		
<sup>140</sup> Ce	38	39	33	36	51	39	17.6		
<sup>182</sup> W	1	1	0	0	0	0	139.2		
<sup>208</sup> Pb	8570(T)	12332(T)	8991(T)	15084(T)	10955(T)	11187	23.7	9503.60	11.6
<sup>238</sup> U	46	50	40	47	48	46	8.6		





**Table S-7**

Corrected intensities of sample 8 using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	0	0	13	0	0	3	223.6		
<sup>23</sup> Na	192276	211717	215072	148332	224662	198412	15.3		
<sup>24</sup> Mg	18331	22911	22532	13871	26891	20907	23.7		
<sup>27</sup> Al	20690	21367	20831	16311	25616	20963	15.7		
<sup>29</sup> Si	547	485	565	416	546	512	12.1		
<sup>39</sup> K	1610	1641	2211	1356	1744	1713	18.3		
<sup>42</sup> Ca	54728	60316	53345	39016	61610	53803	16.7		
<sup>51</sup> V	178	44	43	34	60	72	83.8		
<sup>55</sup> Mn	431	443	420	336	573	440	19.3		
<sup>57</sup> Fe	803	739	666	476	741	685	18.5		
<sup>59</sup> Co	6	10	9	6	12	8	26.5		
<sup>60</sup> Ni	38	54	87	129	104	82	45.0		
<sup>63</sup> Cu	5622(T)	10692(T)	10957(T)	8263(T)	11627(T)	9432	26.3		
<sup>66</sup> Zn	917(T)	1823(T)	2035(T)	1343(T)	1811(T)	1586	28.5	1675.50	6.8
<sup>90</sup> Zr	15	15	11	12	25	15	35.3		
<sup>118</sup> Sn	210(T)	216(T)	285(T)	179	256(T)	208	33.8	254.41	27.5
<sup>137</sup> Ba	121	166	149	106	156	140	18.1		
<sup>140</sup> Ce	46	40	33	34	51	41	18.5		
<sup>182</sup> W	0	0	1	1	0	0	141.0		
<sup>208</sup> Pb	3654(T)	6768(T)	7565(T)	5335(T)	8565(T)	6378	30.2	6711.62	6.3
<sup>238</sup> U	60	60	57	42	68	57	17.0		

**Table S-8**

Corrected intensities of sample 9A using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	0	0	16	4	0	4	174.3		
<sup>23</sup> Na	259766	243176	249622	247741	188175	237696	11.9		
<sup>24</sup> Mg	22166	20812	20944	26526	19786	22047	12.0		
<sup>27</sup> Al	27670	24701	27263	23679	24802	25623	6.8		
<sup>29</sup> Si	480	516	487	555	434	494	9.1		
<sup>39</sup> K	1925	1689	3170	2321	2023	2226	25.8		
<sup>42</sup> Ca	65016	58080	55767	60260	57596	59344	6.0		
<sup>51</sup> V	109	94	109	108	107	105	6.0		
<sup>55</sup> Mn	515	434	498	517	509	495	7.0		
<sup>57</sup> Fe	892	831	852	840	845	852	2.8		
<sup>59</sup> Co	70	60	74	71	70	69	7.3		
<sup>60</sup> Ni	25	29	31	34	29	29	11.5		
<sup>63</sup> Cu	44887(T)	40962(T)	54059(T)	46029(T)	46330(T)	46453	10.2		
<sup>66</sup> Zn	1533(T)	1262(T)	1838(T)	1454(T)	1468(T)	1511	13.8	324.49	4.7
<sup>90</sup> Zr	16	16	22	18	20	18	14.4		
<sup>118</sup> Sn	15	7	21	16	16	15	33.7		
<sup>137</sup> Ba	149	156	166	169	301	188	33.9		
<sup>140</sup> Ce	40	44	50	82	54	54	30.3		
<sup>182</sup> W	71	110	198	99	67	109	48.7		
<sup>208</sup> Pb	4687(T)	4179(T)	5227(T)	5315(T)	5730(T)	5028	12.0	1084.56	10.1
<sup>238</sup> U	52	53	53	52	51	52	1.6		

Table S-9

Corrected intensities of sample 9B using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>					$\bar{x}$	RSD	T/Cu ratio <sup>3</sup>	
	Rep1	Rep2	Rep3	Rep4	Rep5			$\bar{x}$	RSD
<sup>7</sup> Li	8	9	0	7	10	7	57.8		
<sup>23</sup> Na	191106	204698	200852	211995	179665	197663	6.4		
<sup>24</sup> Mg	24720	18643	20809	21545	20257	21195	10.6		
<sup>27</sup> Al	19842	21010	24755	24082	22484	22435	9.1		
<sup>29</sup> Si	525	624	500	503	701	571	15.6		
<sup>39</sup> K	1656	1723	1746	1865	1682	1734	4.7		
<sup>42</sup> Ca	45626	52585	55616	60214	60557	54920	11.2		
<sup>51</sup> V	519(T)	534(T)	423	448(T)	538(T)	492	10.8	128.17	5.5
<sup>55</sup> Mn	377	424	438	485	468	438	9.6		
<sup>57</sup> Fe	545	567	680	772	691	651	14.5		
<sup>59</sup> Co	73	78	89	84	64	78	12.4		
<sup>60</sup> Ni	19	20	28	16	26	22	24.1		
<sup>63</sup> Cu	39058(T)	43928(T)	34044(T)	36184(T)	38855(T)	38414	9.7		
<sup>66</sup> Zn	1889(T)	2262(T)	1742(T)	1919(T)	2270(T)	2016	11.8	524.98	7.1
<sup>90</sup> Zr	13	13	16	20	24	17	28.6		
<sup>118</sup> Sn	3	7	8	8	8	7	32.9		
<sup>137</sup> Ba	124	118	154	139	134	134	10.4		
<sup>140</sup> Ce	51	42	39	43	41	43	10.5		
<sup>182</sup> W	231(T)	270(T)	214(T)	214(T)	262(T)	238	11.2	62.05	5.6
<sup>208</sup> Pb	4528(T)	4938(T)	3608(T)	4105(T)	4504(T)	4337	11.6	1127.35	3.6
<sup>238</sup> U	48	52	58	57	64	56	10.9		

Table S-10

Corrected intensities of sample 9C using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>					$\bar{x}$	RSD	T/Cu ratio <sup>3</sup>	
	Rep1	Rep2	Rep3	Rep4	Rep5			$\bar{x}$	RSD
<sup>7</sup> Li	12	8	0	34	3	11	116.2		
<sup>23</sup> Na	170551	236697	196792	156396	286091	209305	25.2		
<sup>24</sup> Mg	18639	18685	20727	13711	21706	18694	16.5		
<sup>27</sup> Al	24033	22387	24640	17981	25913	22990	13.4		
<sup>29</sup> Si	433	494	444	399	568	468	14.0		
<sup>39</sup> K	2002	1886	2102	1860	2563	2083	13.7		
<sup>42</sup> Ca	51098	56001	55310	40259	61636	52861	15.1		
<sup>51</sup> V	505(T)	592(T)	608(T)	455(T)	790(T)	590	21.7	83.72	2.4
<sup>55</sup> Mn	497	367	472	322	466	425	17.8		
<sup>57</sup> Fe	587	640	712	484	718	628	15.5		
<sup>59</sup> Co	57	92	74	71	70	73	17.4		
<sup>60</sup> Ni	36	35	38	31	51	38	20.3		
<sup>63</sup> Cu	59010(T)	70417(T)	72552(T)	56608(T)	93409(T)	70399	20.8		
<sup>66</sup> Zn	3319(T)	3392(T)	3452(T)	2770(T)	5092(T)	3605	24.3	510.90	7.8
<sup>90</sup> Zr	14	14	14	11	20	15	21.0		
<sup>118</sup> Sn	4	6	9	6	11	7	38.8		
<sup>137</sup> Ba	126	127	128	99	147	125	13.8		
<sup>140</sup> Ce	36	39	46	26	45	38	20.7		
<sup>182</sup> W	210(T)	351(T)	283(T)	242(T)	131	243	33.6	36.24	37.2
<sup>208</sup> Pb	7663(T)	9939(T)	9164(T)	7814(T)	12089(T)	9334	19.4	1329.52	4.7
<sup>238</sup> U	54	54	52	39	54	51	13.2		





**Table S-11**

Corrected intensities of sample 9D using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	0	12	0	8	18	7	103.7		
<sup>23</sup> Na	259508	247317	251491	223093	273075	250897	7.3		
<sup>24</sup> Mg	21510	18140	50098	21525	20551	26365	50.6		
<sup>27</sup> Al	24910	27677	21160	26165	24655	24914	9.7		
<sup>29</sup> Si	1168	409	1136	683	555	790	43.6		
<sup>39</sup> K	1978	1822	2270	2088	2309	2093	9.7		
<sup>42</sup> Ca	59430	57187	51951	55162	55475	55841	4.9		
<sup>51</sup> V	758(T)	756(T)	706(T)	884(T)	884(T)	798	10.2	140.85	3.9
<sup>55</sup> Mn	496	460	395	601	471	485	15.4		
<sup>57</sup> Fe	713	652	630	718	631	669	6.5		
<sup>59</sup> Co	143	116	115	167	126	133	16.5		
<sup>60</sup> Ni	44	41	41	63	45	47	19.4		
<sup>63</sup> Cu	51174(T)	55838(T)	51633(T)	63547(T)	61026(T)	56644	9.8		
<sup>66</sup> Zn	2711(T)	3137(T)	2789(T)	3004(T)	3335(T)	2995	8.5	530.18	6.4
<sup>90</sup> Zr	14	17	13	23	19	17	24.0		
<sup>118</sup> Sn	23	21	25	38	22	26	27.1		
<sup>137</sup> Ba	166	144	132	147	161	150	8.9		
<sup>140</sup> Ce	35	40	32	51	38	39	18.8		
<sup>182</sup> W	620(T)	659(T)	422(T)	656(T)	422(T)	556	22.2	98.63	23.1
<sup>208</sup> Pb	16068(T)	17230(T)	15809(T)	19823(T)	18426(T)	17471	9.6	3085.23	1.5
<sup>238</sup> U	57	51	51	56	44	52	10.2		

**Table S-12**

Corrected intensities of sample 9E using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	13	7	0	2	5	5	97.0		
<sup>23</sup> Na	214140	196589	192121	212404	228538	208758	7.0		
<sup>24</sup> Mg	18857	21951	17489	21004	19007	19661	9.1		
<sup>27</sup> Al	19832	23963	18867	22054	24035	21750	10.8		
<sup>29</sup> Si	562	753	440	428	485	533	25.0		
<sup>39</sup> K	1510	1375	1571	1864	1766	1617	12.2		
<sup>42</sup> Ca	54347	58020	46946	48682	52029	52005	8.5		
<sup>51</sup> V	737(T)	620(T)	702(T)	729(T)	681(T)	694	6.7	164.40	3.1
<sup>55</sup> Mn	383	451	379	394	437	409	8.1		
<sup>57</sup> Fe	601	737	593	534	560	605	13.0		
<sup>59</sup> Co	176	127	107	208(T)	167	157	25.7		
<sup>60</sup> Ni	34	34	33	40	39	36	9.2		
<sup>63</sup> Cu	43122(T)	38756(T)	41751(T)	44426(T)	42883(T)	42188	5.1		
<sup>66</sup> Zn	2002(T)	1664(T)	1754(T)	2430(T)	1970(T)	1964	15.1	464.03	10.8
<sup>90</sup> Zr	16	16	11	15	12	14	16.2		
<sup>118</sup> Sn	0	1	1	44	3	10	194.5		
<sup>137</sup> Ba	120	139	111	126	134	126	8.9		
<sup>140</sup> Ce	37	50	37	37	33	39	16.8		
<sup>182</sup> W	859(T)	427(T)	696(T)	1002(T)	1103(T)	818	32.6	191.80	29.4
<sup>208</sup> Pb	11057(T)	8395(T)	9719(T)	9858(T)	10397(T)	9885	10.0	2340.31	6.8
<sup>238</sup> U	50	43	43	63	54	51	16.9		

Table S-13

Corrected intensities of sample 9F\_1 using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>					T/Cu ratio <sup>3</sup>			
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	12	8	0	5	0	5	105.7		
<sup>23</sup> Na	232324	220785	161806	148735	194881	191706	18.9		
<sup>24</sup> Mg	21024	21269	19449	24087	17549	20676	11.7		
<sup>27</sup> Al	23360	28341	22713	19220	20284	22784	15.5		
<sup>28</sup> Si	528	514	445	380	306	434	21.4		
<sup>39</sup> K	1879	2523	1774	1204	1618	1800	26.6		
<sup>42</sup> Ca	59101	55522	56832	50609	49264	54266	7.7		
<sup>51</sup> V	66	78	81	54	82	72	16.7		
<sup>55</sup> Mn	428	429	438	362	359	403	9.8		
<sup>57</sup> Fe	653	676	779	524	547	636	16.3		
<sup>59</sup> Co	107	122	103	68	89	98	20.9		
<sup>60</sup> Ni	23	31	19	18	20	22	24.2		
<sup>63</sup> Cu	51662(T)	62651(T)	44305(T)	36489(T)	41190(T)	47260	21.6		
<sup>66</sup> Zn	812(T)	815(T)	594(T)	474(T)	546(T)	648	24.2	136.74	8.4
<sup>90</sup> Zr	17	15	21	17	13	17	17.4		
<sup>118</sup> Sn	7	0	6	7	3	5	67.9		
<sup>137</sup> Ba	147	167	131	114	137	139	14.0		
<sup>140</sup> Ce	38	40	39	32	46	39	12.3		
<sup>182</sup> W	398(T)	393(T)	420(T)	346(T)	372(T)	386	7.3	83.94	16.6
<sup>208</sup> Pb	5230(T)	6109(T)	4170(T)	3272(T)	4903(T)	4737	22.7	1003.11	11.3
<sup>238</sup> U	60	49	57	40	43	50	17.8		

Table S-14

Corrected intensities of sample 9F\_2 using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>					T/Cu ratio <sup>3</sup>			
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	4	9	1	10	0	5	95.0		
<sup>23</sup> Na	219772	152138	184159	213474	248294	203567	18.0		
<sup>24</sup> Mg	20536	18898	29786	16815	22380	21683	22.9		
<sup>27</sup> Al	20645	22272	22485	20958	26281	22528	10.0		
<sup>28</sup> Si	539	856	506	444	537	576	28.0		
<sup>39</sup> K	1967	1687	1965	1553	2558	1946	19.9		
<sup>42</sup> Ca	57016	60808	51842	53757	58909	56466	6.5		
<sup>51</sup> V	60	68	58	53	69	62	11.2		
<sup>55</sup> Mn	381	627	362	396	441	442	24.3		
<sup>57</sup> Fe	610	755	532	603	606	621	13.1		
<sup>59</sup> Co	76	62	77	83	71	74	11.0		
<sup>60</sup> Ni	12	22	15	14	26	18	32.3		
<sup>63</sup> Cu	37264(T)	30020(T)	37167(T)	34692(T)	52204(T)	38269	21.8		
<sup>66</sup> Zn	422(T)	312(T)	449(T)	359(T)	586(T)	426	24.5	110.74	6.5
<sup>90</sup> Zr	14	38	17	16	24	22	45.4		
<sup>118</sup> Sn	0	4	2	3	9	3	92.4		
<sup>137</sup> Ba	133	140	118	138	136	133	6.4		
<sup>140</sup> Ce	39	46	40	41	45	42	7.8		
<sup>182</sup> W	410(T)	232(T)	550(T)	223(T)	269(T)	337	41.8	90.24	43.1
<sup>208</sup> Pb	3678(T)	2702(T)	3838(T)	3109(T)	4634(T)	3592	20.5	940.67	6.9
<sup>238</sup> U	53	58	42	35	49	47	19.2		





**Table S-15**

Corrected intensities of sample 9F\_3 using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	10	5	9	12	9	9	29.6		
<sup>23</sup> Na	201613	221163	195289	210236	286146	222889	16.5		
<sup>24</sup> Mg	33787	21776	19869	23389	25538	24872	21.7		
<sup>27</sup> Al	23963	24512	23021	22207	24543	23649	4.3		
<sup>29</sup> Si	555	632	479	461	729	571	19.5		
<sup>39</sup> K	1824	1740	2122	1932	2118	1947	8.8		
<sup>42</sup> Ca	56141	63118	60459	55638	58965	58864	5.3		
<sup>51</sup> V	129	115	136	249	362	198	53.4		
<sup>55</sup> Mn	432	491	421	364	475	437	11.5		
<sup>57</sup> Fe	657	658	645	590	634	637	4.4		
<sup>59</sup> Co	248(T)	124	96	129	156	151	39.0		
<sup>60</sup> Ni	22	22	32	23	28	25	17.6		
<sup>63</sup> Cu	61990(T)	53888(T)	61668(T)	57030(T)	78019(T)	62519	14.9		
<sup>66</sup> Zn	786(T)	1699(T)	915(T)	770(T)	894(T)	1013	38.4	168.02	49.6
<sup>90</sup> Zr	20	17	16	15	18	17	10.8		
<sup>118</sup> Sn	6	6	3	8	6	6	28.0		
<sup>137</sup> Ba	144	138	143	136	150	142	4.0		
<sup>140</sup> Ce	50	44	31	45	42	42	16.6		
<sup>182</sup> W	435(T)	412(T)	270(T)	502(T)	349(T)	394	22.4	64.66	30.4
<sup>208</sup> Pb	12639(T)	12759(T)	14404(T)	17233(T)	15661(T)	14539	13.5	2354.29	17.3
<sup>238</sup> U	51	52	57	48	59	53	7.9		

**Table S-16**

Corrected intensities of sample 10A using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	7	0	0	2	0	2	171.4		
<sup>23</sup> Na	215525	229128	215648	224457	232061	223364	3.4		
<sup>24</sup> Mg	20212	24669	20009	24538	32489	24383	20.7		
<sup>27</sup> Al	22436	24939	22801	26761	24664	24320	7.2		
<sup>29</sup> Si	433	494	665	710	476	556	22.2		
<sup>39</sup> K	1677	2206	1763	4335	2354	2467	43.9		
<sup>42</sup> Ca	56551	57346	57893	58651	62897	58668	4.2		
<sup>51</sup> V	750(T)	731(T)	985(T)	981(T)	951(T)	879	14.5	294.49	1.5
<sup>55</sup> Mn	3458(T)	3500(T)	4253(T)	4095(T)	4196(T)	3900	10.0	1313.23	4.5
<sup>57</sup> Fe	623	638	617	689	693	652	5.6		
<sup>59</sup> Co	7	13	10	13	11	11	20.9		
<sup>60</sup> Ni	9	10	6	9	2	7	46.9		
<sup>63</sup> Cu	25445(T)	25209(T)	32825(T)	32989(T)	32738(T)	29841	13.8		
<sup>66</sup> Zn	7	16	29	8	5	13	77.3		
<sup>90</sup> Zr	20	17	16	16	22	18	14.8		
<sup>118</sup> Sn	0	0	5	0	0	1	223.6		
<sup>137</sup> Ba	150	150	132	161	149	149	6.9		
<sup>140</sup> Ce	38	48	46	44	47	45	9.2		
<sup>182</sup> W	25	22	21	31	29	25	17.7		
<sup>208</sup> Pb	203(T)	210(T)	254(T)	274(T)	267(T)	242	13.5	81.03	2.9
<sup>238</sup> U	48	49	48	59	62	53	12.9		

Table S-17

Corrected intensities of sample 10B using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>					T/Cu ratio <sup>3</sup>			
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	16	10	13	3	20	13	51.2		
<sup>23</sup> Na	271233	211939	293078	172327	284600	246635	21.2		
<sup>24</sup> Mg	26361	22126	31840	20992	22388	24741	18.0		
<sup>27</sup> Al	23817	24994	24662	27711	25644	25366	5.8		
<sup>29</sup> Si	575	488	501	841	405	562	29.7		
<sup>39</sup> K	2122	1657	2036	1743	2002	1912	10.5		
<sup>42</sup> Ca	55114	58968	55277	53060	52628	55009	4.6		
<sup>51</sup> V	1232(T)	1028(T)	1276(T)	941(T)	1472(T)	1190	17.7	193.01	2.8
<sup>55</sup> Mn	8675(T)	7213(T)	9102(T)	6671(T)	12872(T)	8907	27.3	1426.99	7.9
<sup>57</sup> Fe	845	893	826	974	768	861	9.0		
<sup>59</sup> Co	45	46	55	44	63	50	16.6		
<sup>60</sup> Ni	14	8	12	11	13	12	20.2		
<sup>63</sup> Cu	62827(T)	51819(T)	65114(T)	49802(T)	79216(T)	61756	19.1		
<sup>66</sup> Zn	13	5	5	9	10	8	39.1		
<sup>90</sup> Zr	17	11	16	462	15	104	192.0		
<sup>118</sup> Sn	0	1	8	5	4	4	86.6		
<sup>137</sup> Ba	134	135	146	227	164	161	24.0		
<sup>140</sup> Ce	38	39	43	87	40	50	42.7		
<sup>182</sup> W	258(T)	219(T)	292(T)	209(T)	347(T)	265	21.3	42.77	3.5
<sup>208</sup> Pb	219(T)	145	223(T)	208(T)	271(T)	213	21.2	34.62	14.2
<sup>238</sup> U	45	44	46	177	44	71	83.1		

Table S-18

Corrected intensities of sample 10C using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>					T/Cu ratio <sup>3</sup>			
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	2	8	10	5	2	5	63.3		
<sup>23</sup> Na	170990	183040	199560	240048	196684	198064	13.2		
<sup>24</sup> Mg	18443	51669	23470	20814	16230	26125	55.6		
<sup>27</sup> Al	20911	22183	32014	26502	17746	23871	23.2		
<sup>29</sup> Si	404	509	681	709	355	531	30.0		
<sup>39</sup> K	1247	1788	2092	2818	1863	1961	29.1		
<sup>42</sup> Ca	59093	56831	60115	57361	45202	55720	10.8		
<sup>51</sup> V	544	616(T)	744(T)	690(T)	595(T)	638	12.5	220.36	5.4
<sup>55</sup> Mn	2884(T)	3200(T)	4084(T)	4111(T)	3037(T)	3463	17.0	1190.77	6.2
<sup>57</sup> Fe	670	559	647	675	513	613	11.8		
<sup>59</sup> Co	15	12	14	14	13	13	8.9		
<sup>60</sup> Ni	8	8	6	7	6	7	13.7		
<sup>63</sup> Cu	22655(T)	28818(T)	33829(T)	33052(T)	27138(T)	29098	15.7		
<sup>66</sup> Zn	4	6	8	6	4	5	29.8		
<sup>90</sup> Zr	11	17	15	64	14	24	92.4		
<sup>118</sup> Sn	5	0	6	1	4	3	83.9		
<sup>137</sup> Ba	124	127	155	168	309	176	43.3		
<sup>140</sup> Ce	36	41	42	42	29	38	15.1		
<sup>182</sup> W	29	46	46	39	34	39	18.7		
<sup>208</sup> Pb	103	142	169	172	120	141	21.3		
<sup>238</sup> U	49	48	64	51	45	51	13.9		





**Table S-19**

Corrected intensities of sample 10D using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	6	5	1	8	0	4	87.8		
<sup>23</sup> Na	209581	289248	183344	185361	253202	224147	20.5		
<sup>24</sup> Mg	18202	19005	20552	20642	16982	19077	8.2		
<sup>27</sup> Al	23017	21387	23363	23675	20405	22369	6.3		
<sup>29</sup> Si	421	429	543	538	651	516	18.4		
<sup>39</sup> K	2008	2343	1756	1994	3285	2277	26.4		
<sup>42</sup> Ca	59504	49264	58804	60551	50411	55707	9.7		
<sup>51</sup> V	689(T)	766(T)	719(T)	704(T)	747(T)	725	4.3	215.71	3.0
<sup>55</sup> Mn	4164(T)	4418(T)	4438(T)	4482(T)	4583(T)	4417	3.5	1314.82	4.1
<sup>57</sup> Fe	651	589	651	678	664	647	5.2		
<sup>59</sup> Co	27	26	29	28	21	26	12.1		
<sup>60</sup> Ni	7	9	8	13	14	10	28.6		
<sup>63</sup> Cu	30310(T)	35956(T)	33799(T)	33302(T)	34917(T)	33657	6.3		
<sup>66</sup> Zn	3	8	6	5	13	7	52.6		
<sup>90</sup> Zr	17	18	21	17	16	18	11.7		
<sup>118</sup> Sn	2	2	0	2	2	1	57.0		
<sup>137</sup> Ba	149	137	151	162	218	163	19.4		
<sup>140</sup> Ce	47	37	39	42	42	41	9.3		
<sup>182</sup> W	43	53	65	64	69	59	17.8		
<sup>208</sup> Pb	206(T)	224(T)	247(T)	273(T)	196	229	13.7	68.32	14.6
<sup>238</sup> U	52	44	52	56	46	50	9.7		

**Table S-20**

Corrected intensities of sample 10E using Sr as IS and target/Cu ratios obtained.

	5 replicates, corrected data with Sr <sup>1</sup> (T) <sup>2</sup>						T/Cu ratio <sup>3</sup>		
	Rep1	Rep2	Rep3	Rep4	Rep5	$\bar{x}$	RSD	$\bar{x}$	RSD
<sup>7</sup> Li	14	0	12	7	12	9	62.8		
<sup>23</sup> Na	246923	181953	192570	203704	221159	209262	12.2		
<sup>24</sup> Mg	21378	18915	19138	22712	20189	20467	7.8		
<sup>27</sup> Al	23047	20610	19416	22242	23123	21688	7.5		
<sup>29</sup> Si	486	277	480	402	485	426	21.3		
<sup>39</sup> K	2443	2834	1902	1587	2704	2294	23.2		
<sup>42</sup> Ca	53044	54450	53033	56534	57239	54860	3.6		
<sup>51</sup> V	761(T)	419	734(T)	610(T)	490(T)	603	24.7	214.19	5.9
<sup>55</sup> Mn	3687(T)	2084(T)	3388(T)	3152(T)	2726(T)	3007	20.8	1075.67	4.9
<sup>57</sup> Fe	562	546	570	612	615	581	5.3		
<sup>59</sup> Co	37	22	33	30	25	29	20.9		
<sup>60</sup> Ni	10	11	7	6	10	9	27.4		
<sup>63</sup> Cu	32517(T)	19966(T)	33523(T)	29602(T)	24227(T)	27967	20.6		
<sup>66</sup> Zn	2	7	6	5	9	6	46.4		
<sup>90</sup> Zr	17	16	13	20	16	16	16.1		
<sup>118</sup> Sn	1	1	3	0	2	1	83.4		
<sup>137</sup> Ba	154	123	136	131	147	138	9.0		
<sup>140</sup> Ce	170	33	40	37	41	64	92.2		
<sup>182</sup> W	42	13	47	38	39	36	36.4		
<sup>208</sup> Pb	153	82	161	136	173	141	25.3		
<sup>238</sup> U	47	48	51	53	54	51	6.0		



# Conclusiones y perspectivas de futuro





En esta Tesis se lleva a cabo una revisión bibliográfica de las aplicaciones químico forenses por LA-ICP-MS, se desarrollan diferentes metodologías para el cotejo forense de muestras de vidrios y tintas y se estudia su interpretación estadística y expresión de resultados, teniendo en cuenta que serán presentadas ante los Tribunales de Justicia. Como consecuencia del trabajo del trabajo realizado en esta Tesis se proponen modificaciones a tener muy en cuenta en los métodos ya descritos para el análisis de vidrios y nuevas formas de abordar el cotejo de tintas.

Durante el desarrollo de esta Tesis, se estudian distintas herramientas estadísticas para evitar interpretaciones subjetivas y minimizar los errores.

Teniendo en cuenta que el objetivo final de los análisis forenses es elaborar informes periciales para responder a las preguntas de los Tribunales de Justicia, se propone hacer un esfuerzo en redactar los informes con un lenguaje comprensible por los miembros del Tribunal y complementar la información con datos gráficos que faciliten la interpretación de los resultados.

A continuación se indican las conclusiones principales obtenidas en cada capítulo y algunas tendencias de futuro.

## **CAPÍTULO I. APLICACIONES FORENSES MEDIANTE LA-ICP-MS**

LA-ICP-MS es una técnica efectiva de análisis directo de sólidos que no requiere pasos previos de digestión de muestra.

Esta especialmente recomendada para muestras muy pequeñas, características de los vestigios hallados en las investigaciones forenses.

Es cuasi no destructiva por lo que permite posteriores análisis complementarios de muestras muy pequeñas.

Tiene capacidad para realizar análisis tanto multielemental como de relaciones isotópicas por lo que tiene un gran poder de discriminación forense.

Es una técnica útil para caracterizar, comparar y discriminar de forma inequívoca los vestigios obtenidos en el escenario de un delito, con un alto nivel de confianza, por lo que presenta un gran potencial para el análisis forense de muestras de vidrio, así como tintas y papel, pintura, fibras, cannabis, gemas, materiales cerámicos y metales preciosos.

Los mayores inconvenientes actuales son la escasa disponibilidad de materiales de referencia para la cuantificación y el fraccionamiento.

## **CAPÍTULO II.**

### **MEDIDA DEL ÍNDICE DE REFRACCIÓN EN VIDRIOS**

Se ha desarrollado y validado intra-laboratorio un método estandarizado de medida del índice de refracción en vidrios que posteriormente se ha acreditado por ENAC en el expediente 383/LE 1151 de la Dirección General de la Guardia Civil. Servicio de Criminalística.

Se ha comprobado que la linealidad, precisión y exactitud son adecuadas. Se han establecido controles de calidad que aseguran la ejecución del método en condiciones controladas.





Se ha comprobado la aplicación del método validado para muestras de origen forense, asociando adecuadamente las muestras con su origen mediante las medidas de índice de refracción, comprobando que los errores tipo I y II son similares a los obtenidos por otros autores.

Además se han contrastado los resultados en un caso real, analizado también por LA-ICP-MS, confirmando la validación del método.

Es una técnica de aplicación sencilla y de bajo coste, que presenta resultados efectivos en un breve plazo de tiempo y que puede interpretarse con facilidad por herramientas estadísticas de contraste de hipótesis. Por este motivo, esta medida está muy extendida en los laboratorios forenses de todo el mundo.

#### **ANÁLISIS ELEMENTAL DE VIDRIOS MEDIANTE LA-ICP-MS**

Se ha desarrollado un protocolo normalizado de análisis elemental de vidrios mediante LA-ICP-MS con fines exclusivamente de cotejo forense, la validación intra-laboratorio se ha desarrollado basándose en la norma ASTM E-2927.

Se ha optimizado el procedimiento de trabajo, diseñando los controles de calidad necesarios para ejecutar el método estándar en condiciones controladas.

Se ha propuesto sustituir los términos “falso negativo” y “falso positivo”, que poseen significados opuestos en el ámbito forense y estadístico, por la adopción de los términos específicos “falso match” y “falso no match” en las comparaciones por pares de vestigios de vidrio procedentes de casos forenses.

Se ha observado una dependencia de los resultados cuantitativos con el estándar de calibración, por lo que se ha relativizado el concepto de exactitud para el cotejo forense de vidrios. El método de validación tiene como objetivo la asociación correcta de muestras por pares, en lugar de la obtención de altos niveles de exactitud.

Se propone analizar las muestras de los casos forenses en condiciones de repetibilidad y empleando el mismo estándar de calibración para las muestras que van a cotejarse.

Finalmente, tanto peritos forenses como juristas, tienen que hacer un esfuerzo para acercar los lenguajes y las mentalidades científico-jurídicas. Para ello los informes periciales deben transmitir la información a los juristas y a su vez, estos deben hacer un esfuerzo por entender las conclusiones emitidas en su justa medida. Como propuesta, sugerimos que se fomente la realización de cursos conjuntos de interpretación de resultados en informes periciales.

### **CAPÍTULO III. ANÁLISIS FORENSE DE TINTAS**

Se ha elaborado una metodología para el análisis directo de tintas de bolígrafos manuscritas en papel de uso comercial, mediante LA-ICP-MS. Se ha estudiado un conjunto de tintas de origen conocido que contiene bolígrafos de diez casas comerciales diferentes, además de distintos lotes de fabricación.

En la primera etapa del análisis, mediante el estudio de la relación señal/ruido del fondo que produce el papel, se han identificado los elementos target que son los que caracterizan cualitativamente a las tintas. Mediante la comparación de los elementos target característicos





de cada muestra del conjunto de tintas estudiado, se han diferenciado fácilmente marcas de fabricación.

En la segunda etapa, basándonos en las intensidades relativas de los elementos target y mediante un estudio MANOVA, se ha ampliado el poder de discriminación de la primera etapa, distinguiendo además, en algunos casos, entre tintas de diferentes lotes de fabricación e igual marca comercial.

Este estudio se ha llevado a cabo con muestras de papel y bolígrafos comerciales, por lo que es de aplicación directa en el campo forense.

Es un análisis sin preparación de muestra por lo que se evitan contaminaciones cruzadas en el laboratorio. Además al ser un análisis microscópico, necesita muy poca muestra y es cuasi no destructivo, lo que permite la realización de otros estudios complementarios.

Comparada con otras técnicas y específicamente con otros trabajos mediante LA-ICP-MS, esta metodología permite realizar el estudio sin necesidad de calibración, con el consiguiente ahorro de tiempo y evitando la falta de materiales de referencia certificados de tintas.

Esta metodología se aplica a un caso forense, obteniendo conclusiones que coinciden con las obtenidas por los expertos del Departamento de Grafística del SECRIM.

Por último, estos resultados confirman que la metodología propuesta es prometedora para uso forense y que, por tanto, interesa evaluarla en más profundidad, completando el estudio con otros conjuntos de tintas, sobre todo muestras de la misma marca y diferentes lotes.

## TENDENCIAS DE FUTURO

En esta Tesis se han estudiado métodos de análisis forense de vidrios y tintas y que la interpretación de los resultados se adapte específicamente para su uso en Tribunales de Justicia.

En el caso del análisis de vidrios, queda pendiente una mejor caracterización de los únicos materiales de referencia certificados disponibles por el momento para la calibración forense mediante LA-ICP-MS: FGS1 y FGS2, mediante un estudio interlaboratorio con mayor número de participantes. También es necesario elaborar un protocolo de interpretación estadística de resultados, que tenga en cuenta los resultados de todas las técnicas de análisis de vidrios, o al menos, las dos más discriminatorias: Índice de refracción y LA-ICP-MS, de forma conjunta.

Otra tendencia de futuro es la investigación de otras técnicas de análisis elemental como el estudio de las relaciones isotópicas de plomo en vidrios para su aplicación forense o el desarrollo de otros métodos ópticos, como la medida del Contraste Diferencial de Interferencia (DIC).

A pesar de la dificultad de elaborar y mantener actualizada una base de datos de vidrios y tintas, es de interés realizar estudios de muestras de origen conocido y crear una plataforma en la que esta información esté disponible para los investigadores. Una de las utilidades de esta plataforma puede ser la de establecer frecuencias poblacionales para las variables analizadas, de esta forma podemos estimar la rareza de un vidrio frente a la población de vidrios forenses y mediante la estadística bayesiana, ponderar el poder de





discriminación de las variables de acuerdo a su naturaleza y al valor que toman en cada vestigio de vidrio estudiado.

El cotejo de tintas mediante LA-ICP-MS también puede ampliarse estudiando un mayor número de tintas y de muestras de papel, sobre todo encaminado a incrementar la capacidad de discriminar muestras de distintos lotes del mismo fabricante, así como de tinta del mismo lote y fabricante, contenida en bolígrafos diferentes. Además de lo anterior, es de interés realizar estudios en los que se combinen el análisis elemental con otras técnicas de análisis prometedoras como la Espectroscopía Raman o el análisis hiperespectral.

Otra vía de investigación es el desarrollo de un software estadístico-forense que asesore en la interpretación de resultados, en el que tengan cabida todo tipo de análisis, tanto cualitativos como cuantitativos y que pueda manejar datos categóricos y numéricos continuos, además de estar enfocado tanto al cotejo de vestigios como a la comparativa de la información contenida en bases de datos.



## **Contribuciones del autor**





Las contribuciones del autor durante el periodo de tutorización de la Tesis son las siguientes:

## **PUBLICACIONES**

- **El análisis forense de vidrios como ayuda en la investigación de delitos.** Francisco Alamilla Orellana, César González Gálvez, Mercedes Torre Roldán y Carmen García Ruiz. Revista Guardia Civil Nº 806, Pag 82-87. Junio 2011.
- **Análisis forense de tintas azules de bolígrafo mediante espectrometría de masas con plasma de acoplamiento inductivo, equipada con sistema de ablación láser (LA-ICP-MS).** Francisco Alamilla Orellana, Carmen García Ruiz y Mercedes Torre Roldán. Revista Actualidad Analítica, nº 36, 17-18, 2011.
- **Applications of laser-ablation inductively-coupled plasma-mass spectrometry in chemical analysis of forensic evidence.** Francisco Alamilla Orellana, César González Gálvez, Mercedes Torre Roldán, Carmen García-Ruiz. Trends In Analytical Chemistry, Vol 42, 1-34, 2013.
- **Validation of an analytical method for the refractive index measurement of glass fragments. Application to a hit-and-run incident.** Francisco Alamilla Orellana, Matías Calcerrada Guerreiro, Carmen García Ruiz y Mercedes Torre Roldán. Analytical Methods 5, 1178-1184, 2013.

- **Forensic discrimination of blue ballpoint pens on documents by laser ablation inductively coupled plasma mass spectrometry and multivariate analysis.** Francisco Alamilla Orellana, Matías Calcerrada Guerreiro, Carmen García Ruiz y Mercedes Torre Roldán. Forensic Science International 228, 1-7, 2013.
- **The strength of pairwise comparison in forensic chemistry. Recommendations on the use of validated methodologies for the forensic analysis of glass samples by LA-ICP-MS.** Francisco Alamilla Orellana, Matías Calcerrada Guerreiro, Carmen García Ruiz y Mercedes Torre Roldán. Enviado a Science & justice en marzo de 2014.

#### **PARTICIPACIÓN EN CONGRESOS, SEMINARIOS, JORNADAS PROFESIONALES Y ENSAYOS INTERLABORATORIO**

- Presentación de poster en la 13ª Jornadas de Análisis Instrumental (JAI) “Forensic analysis of blue ballpoint pen inks using laser ablation-inductively coupled plasma-mass spectrometry”, Barcelona Nov 2011. Premio otorgado por la SEQA, Sociedad Española de Química Analítica, a las mejores comunicaciones presentadas en estas Jornadas de Análisis Instrumental.
- Presentación de poster en la 13ª Jornadas de Análisis Instrumental (JAI) “Refractive index measurements as tool for forensic glass analysis”, Barcelona Nov 2011.
- Tercera Reunión de usuarios de ICP-MS de Agilent Technologies, Madrid 16 Jun 2009.





- 16° Meeting European Paint and Glass Group. ENFSI. Krakow (Poland) 26-29 Sep 2010.
- I Workshop Nacional en ablación láser LA-ICP-MS. Universidad de Oviedo. 18-19 Nov 2010.
- IV Encuentro de Investigadores IUICP, Alcalá de Henares (Madrid) 3 Nov 2010.
- 17° Meeting European Paint and Glass Group. ENFSI. Vilnius (Lithuania) 26-28 Sep 2011.
- V Encuentro de Investigadores IUICP, Alcalá de Henares (Madrid), 17 Nov 2011.
- I Seminario “Análisis criminalístico de suelos”. IUICP. Universidad de Alcalá de Henares (Madrid) 12-13 Abr 2012.
- 11th European Workshop on Laser Ablation and II Workshop Nacional en LA-ICP-MS. Gijón, Universidad de Oviedo. 18-22 Jun 2012.
- VII Congreso Internacional de Peritos en Documentoscopia, Las Palmas de Gran Canaria. 23-25 Oct 2012.
- Reunión de usuarios de ICP-MS de Agilent Technologies, Madrid. 05 Feb 2013.
- 19° Meeting European Paint and Glass Group. ENFSI. Larnaca, (Chipre) 22-25 Sep 2013.

- II Seminario “Análisis criminalístico de suelos”. IUICP. Universidad de Alcalá de Henares (Madrid) 26-27 Nov 2013.
- Participación, con resultado satisfactorio en los test de aptitud: Collaborative Testing Services (CTS), Inc Forensic Testing Program nº 548, Glass Analysis Test. 2010, 2011, 2012 y 2013.
- Participación, con resultado satisfactorio en el test “Proficiency Test Association/differentiation of three glass samples”. ENFSI Expert working Group Paint and Glass: Glass Elemental Analysis 2013.





