Cover: From left to right: a picture of Goma-2 ECO dynamite, the electrophoretic separation of atropine and scopolamine in an infusion of *Datura stramonium* L., and a detail of a portable capillary electrophoresis.

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LIST OF ABBREVIATIONS:

APCI, atmospheric pressure chemical ionization;
DAD, diode array detection
DBP, dibutyl phthalate
DNT, dinitrotoluene
C^4D, capacitively coupled contactless conductivity detector/detection
CWA, chemical warfare agent
CWC, chemical weapons convention
EDEA, N-ethyldiethanolamine
EGDN, ethylene glycol dinitrate or nitroglycol
$EGDN_M$, EGDN content in methanol
$EGDN_W$, EGDN content in water
EIC, extracted ion chromatogram
EOF, electro-osmotic flow
GC, gas chromatography
GC-MS, gas chromatography coupled to mass spectrometry detection
HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HCl, hydrochloric acid
HPLC, high performance liquid chromatography
HPLC-DAD-MS, high performance liquid chromatography with diode-array detection and mass spectrometry detection
HV, high voltage
I.D., inner diameter
IMS, ion mobility spectrometer
LEDs, light-emitting diodes
L_{eff}, effective length
LIF, laser-induced fluorescence
LOD, limit of detection
LOQ, limit of quantitation
L_{tot}, total length
MES, 2-(N-morpholino)ethanesulfonic acid
MDEA, N-methyldiethanolamine
MS, mass spectrometry
NaOH, sodium hydroxide
NM, nitrogen mustard
NG, nitroglycerin

LIST OF ABBREVIATIONS

NIR, near infra-red
PEEK, polyether ether ketone
P(VP-co-DMAEMA), poly(1-vinylpyrrolidone-co-2-dimethylaminoethyl methacrylate)
O. D., outer diameter
RSD, relative standard deviation
SI, sequential injection
TEA, triethanolamine
TIC, total ion chromatogram
TNT, trinitrotoluene
Tris, tris(hydroxymethyl)aminomethane

CONTENTS

SUMMARY

Evidence storage is a serious concern in forensic laboratories. If evidential items are not properly packaged and stored, important information can be lost. Specifically, packaging for evidence with volatile compounds is a sore point, since those components tend to volatilize, to leave the evidential item, and to escape from the container. There are many types of containers for evidence with volatile compounds, and all of them have advantages and disadvantages. During the development of this thesis, two types of plastic bags have been studied in order to analyze their suitability for the storage of dynamites, which are explosives with volatile compounds such as ethylene glycol dinitrate or dinitrotoluene.

Firstly, an extraction method to recover ethylene glycol dinitrate from dynamites was developed. Then, high performance liquid chromatography was used for the determination of ethylene glycol dinitrate in the extract. The analytical method was developed and optimized for the separations of seven compounds that may be present in dynamites and was the method also validated. Finally, the EGDN content of a sample of the Goma-2 ECO dynamite was determined obtaining a concentration of 30.29%, which was in accordance with the manufacturer's specifications for this dynamite (25.7% - 31.4%).

The same high performance liquid chromatography but using mass spectrometry detection, which was 2 orders of magnitude more sensitive than diode array detection, was subsequently applied, together with gas chromatography analyses, in a new study on the suitability of polyethylene bags for the storage of dynamites. The results proved that those polyethylene bags were not really appropriate for the storage of dynamites since they allowed the rapid

escape of volatile compounds as ethylene glycol dinitrate and dinitrotoluene from the bags. Moreover, it was demonstrated that the bags allowed the cross-contamination between dynamites of different compositions.

In a following study, the polyethylene bags were compared to a multilayer nylon/polyethylene bag, which have been designed to show high retentiveness for volatile compounds. The ethylene glycol dinitrate released from dynamites stored in the bags was followed by gas chromatography analysis in a comparative study over eleven weeks of storage. The results showed that the new bags were much more suitable for the storage of dynamites, since only a weak signal for ethylene glycol dinitrate was detected out of the new bags after eleven weeks, instead of the first week needed to be released from the polyethylene bags.

Furthermore, the use of portable analytical instrumentation has attracted attention in recent years due to its benefits. Portable analytical instrumentation allows *in situ* analyses, permitting the obtention of results and to make decisions directly at the place of sample collection, usually reducing the number of analyses needed, and reducing the chances of occurrence of sample degradation, alteration, misplacement or loss during its transportation and storage.

A new portable capillary electrophoresis with automated injection and contactless conductivity detection was developed. The system, of about 8 kg and able to operate continuously for 9 h in the battery-powered mode, relied on pressurized air for solution delivery and a micromembrane pump for sample aspiration. The injection system

allowed easy optimization for high separation efficiency, for fast separations, or for low limits of detection.

Another portable capillary electrophoresis, allowing to deliver up to four different solutions, was also built. The system was applied to the determination of degradation products of nitrogen mustards in different water samples, taken from a well and from three different rivers. The capillary was coated to avoid analyte-wall interactions and the coating procedure was optimized to get the best repeatability of the analyte migration times. The electrophoretic method was successfully applied to the determination of nitrogen mustards degradation products in the spiked water samples, without matrix interferences.

Finally, the same portable capillary electrophoresis was applied to the determination of scopolamine in different samples. Scopolamine has been commonly used in recent years for predatory and recreational uses. In fact, there are several cases in which people have died, have been raped and robbed, due to the abuse of this drug. The different evidential items analyzed were a infusion of *Datura stramonium* L., a spiked moisturizing cream, and six spiked beverages, since they have recently been used in different criminal actions or used with fatal consequences. An effort was made to develop easy and fast sample treatments which can be readily carried out at the place of sample collection. The developed electrophoretic method was applied to the determination of the drug in the three above-cited evidential items, without sample interferences.

RESUMEN

El almacenamiento de muestras es una preocupación importante en los laboratorios forenses. Si las muestras no se almacenan debidamente puede producirse una pérdida importante de información de las mismas. Concretamente, el almacenamiento de muestras con compuestos volátiles es crítico, ya que esos componentes tienden a volatilizarse, a abandonar las muestras y a escapar del contenedor. Existen muchos tipos de contenedores para muestras con compuestos volátiles y todos ellos tienen ventajas y desventajas. Durante el desarrollo de esta tesis se estudiaron dos tipos de bolsas de plástico para saber si eran apropiadas para el almacenamiento de dinamitas, que son explosivos con compuestos volátiles tales como el nitroglicol o el dinitrotolueno.

Primero, se desarrolló un método para la extracción de nitroglicol de dinamitas. A continuación, se optimizó un método de cromatografía líquida de alta eficacia para la separación de siete compuestos explosivos que pueden formar parte de ciertas dinamitas. El método fue validado y se aplicó a la determinación del contenido de nitroglicol en la dinamita Goma-2 ECO, obteniendo una concentración del 30.29%, la cual concordaba con la especificada por el fabricante (25.7–31.4%).

El mismo método de cromatografía liquida de alta eficacia pero con detección de espectrometría de masas, por ser dos ordenes de magnitud más sensible que la detección de matriz de diodos, se aplicó posteriormente, junto con análisis por cromatografía gaseosa, en un nuevo estudio sobre la idoneidad de bolsas de polietileno para el almacenamiento de dinamitas. Los resultaron demostraron que esas bolsas de polietileno no eran apropiadas para el almacenamiento de dinamitas ya que permitían la rápida liberación de las bolsas de

compuestos volátiles como el nitroglicol y el dinitrotolueno. Más aún, se demostró que dichas bolsas permitían la contaminación cruzada entre dinamitas de diferentes composición.

En un estudio posterior, se compararon las mismas bolsas de polietileno con otras bolsas multicapa de nylon/polietileno, diseñadas para proporcionar una mejor retención para compuestos volátiles que las bolsas de polietileno. El estudio se realizó siguiendo el nitroglicol liberado de las dinamitas almacenadas en las bolsas mediante cromatografía gaseosa. Los resultados mostraron que las nuevas bolsas eran mucho más apropiadas para el almacenamiento de dinamitas, ya que sólo se detectó una señal débil de nitroglicol fuera de las bolsas nuevas al cabo de once semanas, en lugar de en la primera semana como ocurría para las bolsas de polietileno.

Por otro lado, el empleo de instrumentación analítica portátil ha atraído una gran atención en los últimos años debido a sus beneficios. La instrumentación analítica portátil permite llevar a cabo análisis *in situ*, permitiendo obtener resultados y tomar decisiones directamente en el lugar de la recogida de la muestra, reduciendo, normalmente, el número de muestras necesarias y reduciendo las posibilidades de que la muestra se degrade, se altere, se traspapele, o se pierda durante su transporte y almacenamiento.

Se desarrolló un nuevo equipo de electroforesis capilar portátil con inyección automática y detección de conductividad sin contacto. El equipo, de alrededor de 8 kg y capaz de funcionar en modo constante durante 9 h trabajando con baterías, utilizaba gas comprimido para generar el flujo de las disoluciones y un bomba de micromembrana para la toma de muestra. El sistema de inyección se optimizó

fácilmente para conseguir una alta eficacia separativa, separaciones rápidas o para obtener bajos límites de detección.

También se construyó otro equipo de electroforesis capilar portátil, que permitía el trabajo con hasta cuatro disoluciones diferentes. El equipo se aplicó a la determinación de productos de degradación de mostazas de nitrógeno en muestras de agua diferentes, tomadas de un pozo y de tres ríos distintos. Se recubrió el capilar para evitar las interacciones entre la pared del capilar y los analitos y se optimizó el procedimiento de recubrimiento para obtener la mejor repetibilidad de los tiempos de migración. El método electroforético se aplicó con éxito a la determinación de los productos de degradación de las mostazas de nitrógeno en las muestras de agua sin interferencias de matriz.

Finalmente, se usó el mismo equipo de electroforesis capilar portátil para la determinación de escopolamina en diferentes muestras. La escopolamina se ha estado usando en los últimos años con fines depredadores y recreacionales. De hecho, hay muchos casos en los que ha muerto gente, o han sido violadas o atracadas mediante el uso de esta droga. Las diferentes muestras analizadas fueron una infusión de *Datura stramonium* L., una crema adulterada y seis bebidas adulteradas, ya que estas muestras se han utilizado recientemente en distintos actos criminales o utilizado con consecuencias fatales. Se hizo un esfuerzo en desarrollar tratamientos de muestra rápidos y sencillos que pudieran llevarse a cabo en el lugar de recogida de muestra. El método electroforético desarrollado permitió determinar la droga en las muestras analizadas sin interferentes debidas a las muestras.

PROLOGUE

The aim of this prologue is to contextualize this dissertation in the forensic field. The problems raised and the work carried out during the preparation of this thesis cannot be discussed without talking over some concepts such as *forensic science*, *forensic evidence*, and *forensic process*.

According to the Oxford Dictionary, the term *forensic* "relates to or denotes the application of scientific methods and techniques to the investigation of crime". Therefore, in accordance with this definition, *forensic science* (often shortened to *forensics*) may be defined as the application of basic sciences in the resolution of legal disputes. It is a multidisciplinary subject, which draws upon a variety of scientific principles, including biology, physics, chemistry, geology, psychology, social science, etc.

Forensic science plays a pivotal role in most criminal prosecutions, especially those of a more serious nature. Any forensic case follows a process that could be called *forensic process*. The term *forensic process* has been recently introduced by Pierre Margot as a process that encompasses many stages from the initial investigation of a crime scene to the presentation of expert witness testimony in court. It requires trace detection of evidence, which must be analyzed and interpreted and, finally, present the results in court. In fact, a forensic process can be long and complicated, depending on the aspects of the case to be investigated. In this thesis, the forensic process has been interpreted according to the different places where a forensic process is developed. As **Figure 1** illustrates, the forensic process always begins at the crime scene, progresses in the laboratory, and ends in court.

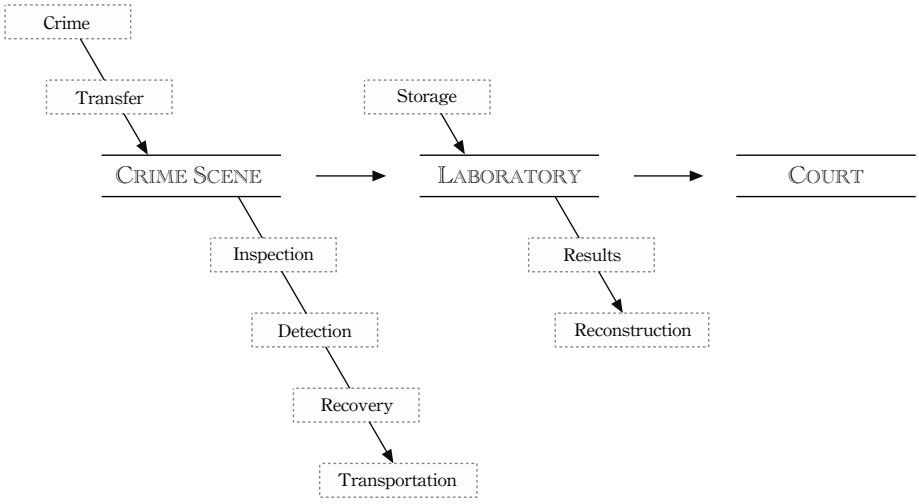

***Figure 1**. The forensic process*

1. The crime scene

When a crime occurs, a crime scene is created. A crime scene is the location where the crime took place. It is also the location where trace evidence of the crime may be found. Both places can be the same or different locations. Evidence is every fact, testimony, document, or object that can prove or disprove something. In the case of forensics, a criminal fact is what is intended to be proved or disproved. *Locard's exchange principle* holds that whenever two entities (be they people or objects) are in contact, there is a transfer of material between them. In other words: every contact leaves a trace. According to this principle, the perpetrator of a crime will bring something into the crime scene and take something from it, creating one or more crime scenes. The

material moved from or brought to the crime scene constitute the trace evidence of the crime.

The forensic investigators will move to the crime scene and will inspect it in search of evidence. When an evidential item is detected it must be properly recovered and then separately and appropriately packaged and labelled. The investigators in charge of the inspection of the crime scene must be highly trained operators. They cannot overlook any evidence and must know how to recover and package them in order to preserve their identities and avoid loss of information. At this point in time, the establishment of *chain of custody* is extremely important. It ensures a continuity of evidence from the point of its recovery through to its appearance in court. If continuity of an evidential item cannot be demonstrated, then the evidence may be deemed inadmissible in court because of the possibility of any alteration. Once recovered, packaged, and labelled for the chain of custody, evidence is transported to the next scenario in the progression of the forensic process.

2. The laboratory

In the laboratory, evidential items of potential forensic importance are subjected to forensic examination. However, the analysis might not occur immediately after the sample is delivered in the laboratory. Instead, it may be necessary to store the evidence until analysis.

When the evidence is examined and analyzed, the investigators must interpret the result by answering the following questions:

- where does this trace come from?

- why is the trace where it is?

- how might it relate to a proposed crime scenario?

The answers to these questions will reveal the identity of the evidential item and the mechanism of its appearance at the crime scene. These knowledges will help the investigators to reconstruct the events occurred at the crime scene.

3. The court

The forensic process comes to the end and the forensic investigator is, after all, required to transfer all the information obtained from the investigated case in an objective and readily understandable way to the final receiver: the court. The forensic investigator will present a report, in which his or her findings will be written up. Eventually, the forensic investigator will be required to give testimony of fact and of opinion about the case.

SCOPE AND OUTLINE OF THIS THESIS

Nowadays, evidence preservation is a big concern in the forensic laboratories. Forensic investigators and researchers have devised specific devices for packaging of evidence, new protocols for evidence management, and stricter safety measures in the laboratories. However, forensic cases are still being dismissed because evidential items are deemed inadmissible in court due to the possibility of evidence alteration or contamination. For this reason, researchers have put in practice a number of lines of investigation on the study of the suitability of packaging for evidence. On the other side, the employment of portable analytical instrumentation is having a growing interest since no storage is needed. In fact, many portable analytical instruments have been devised and commercialized in the recent years due to the advantageous features of *in situ* analysis.

The works described in the present thesis approach to both the problem of loss of information during storage and the recent tendency to use portable analytical instrumentation for *in situ* analysis. As consequence, the thesis is divided into two main parts, as follows:

- **The first part** of the thesis will introduce the reader to the loss of information that evidence may suffer throughout its storage and to the different packaging for evidence. A particular emphasis will be placed on the packaging for forensic evidence containing volatile compounds and the difficulties these constitute to the forensic laboratories. The study of dynamite evidence storage, since dynamites are commercial explosives containing volatile compounds, is the aim of this part. Chapter I.1 is focused to introduce the evidence packaging problematic and the most suitable packagings for samples with volatile compounds. Chapter I.2 is focused on the development and validation of an analytical method

for the analysis of certain components of dynamites. Chapter I.3 will discuss the possibility of cross-contamination between dynamites when a specific container is used for their storage. In chapter I.4 the suitability of an alternative container for the storage of dynamites will be studied in comparison to the previous container studied in chapter two.

- **The second part** will focus on the development of portable instrumentation and methods for the *in situ* analysis of evidence. Portable capillary electrophoresis will be considered as an alternative to the transport of evidence to fixed laboratories when evidence storage is not possible or not safe. In this case, the emphasis will be done on the current role of portable capillary electrophoresis for *in situ* analysis of different evidential items. Chapter II.1 is aimed to introduce the state of the art of portable capillary electrophoresis, which is a technique in a initial state of development. In chapter II.2 a new purpose-built portable capillary electrophoresis instrument is developed. In chapter II.3 a modified version of the portable capillary electrophoresis is used for the determination of nitrogen mustard degradation products in water. Chapter II.4 describes the employment of the portable instrument for the determination of scopolamine, a new emerging drug in Europe, in several evidential items.

PART ONE

DYNAMITE PACKAGING

CHAPTER I.1

INTRODUCTION

The management, control and protection of evidence during its warehousing must be focused to protect the integrity of evidence. Therefore, evidence storage is critically important and must be thought to avoid loss of information from evidence. Good storage practices must be a top priority in the forensic laboratory.

Evidential items are collected at the crime scene and they are packaged in specific containers, depending on the nature of the evidence. Typically, evidence storage is considered to begin once the evidential items are delivered to the laboratory and they are stored until its analysis. Oftentimes those evidential items remain stored directly in the same containers in which they were delivered to the laboratory. This is a good laboratory practice that prevents evidential items from being altered when changing containers. Therefore, in these cases, it can be stated that evidence storage did not begin when the evidence was delivered to the laboratory, but when it was collected and packaged at the crime scene, as the container packaging the evidence will remain the same throughout the entire storage process.

Packaging for evidence is of great importance during storage and this is often the source of extreme concern for forensic laboratories. In general terms, every packaging for evidence must fulfill some requirements, as follows:[1]

- It must be easy to handle;

- available in a wide range of sizes, so pieces of different sizes can be contained inside;

- resistant to shock, puncturing, cutting, and deterioration;

- secure, so no unauthorized person has access to the evidence and it cannot be tampered with, stolen, or substituted,

- able to be labeled to correctly identify the evidence with respect to description, source, chain of custody, and provide a unique identifier;

- contaminant-free;

- protect the evidence from damage, deterioration, partial loss, or any other type of alteration; and finally, it must

- avoid cross-transfer from item to item, person to item, item to person, and scene to scene.

Moreover, depending on the type of evidence, different containers will be needed because each one fulfills very specific requirements for the preservation of the contained evidence. For example, electronic devices must be protected from electrostatic discharges, blood and body fluids must be packaged in containers that prevent the accumulation of moisture, containers for fluid must avoid fluid leakages, flammable material must be protected from ignition, biological tissues should not decompose, and infection must be

prevented when containing infectious substances. Thus, anti-static container must be used for electronic devices, paper bags for blood and body fluid stains, glass stoppered bottles for liquid drugs, druggist folds for fibbers or hairs, fireproof containers for gasoline, strong boxes for glass fragments, refrigerated containers for biological tissues, and container that avoids the transmission of pathogens must be used for infectious substances (**Figure I.1.1**).[1-3]

***Figure I.1.1**. Specific containers for some evidential items. From left to right and from top to bottom: anti-static bags, paper bag, containers for gasoline or flammable liquids, druggist fold, refrigerated box, and glass stoppered bottles.*

In particular, packaging for evidence with volatile compounds such as accelerants, fire debris, or explosives is a sore point. The presence of

volatile compounds adds further complication to the storage of evidence because they tend to escape from both the evidential item and the container. The most direct consequence of the leakage of volatile compounds from evidence is the loss of qualitative and quantitative information. This fact should be taken into account by the forensic laboratories when analyzing evidence with volatile compounds.

The leakage of volatile compounds from evidence is an inevitable fact because the high vapor pressures of these compounds cause a large number of molecules to evaporate or sublimate into the air. The vapor pressure of a liquid (or a solid) is the force per unit area exerted by its vapor when the liquid (or the solid) and the vapor are in dynamic equilibrium.[4] The vapor pressures of a volatile compound indicates that, if released into air, it will exist solely as a vapor into the atmosphere, if we discard other mechanisms of condensation. In an ideal closed container, the pressure in the space above the volatile compound will increase until it stabilizes at a constant value, the vapor pressure. If the closed container is not able to contain this pressure, the vapor in its inside will start to be released to the outside.

It is possible to reduce the leakage of volatile compounds by reducing their vapor pressures during storage because vapor pressures increase with temperature. An easy example of the influence of temperature on the vapor pressures may be found in water. Water has a vapor pressure of 24.0 Torr at 25 ºC. At 4 ºC, the usual temperature in any domestic refrigerator, the vapor pressure is reduced down to 6.1 Torr, almost 4 times lower.[5] For this reason, keeping evidence with volatile compounds at low temperature is often recommended.

As explained above, volatile compounds can be released into the air, even when they are contained in closed compartments. There are many types of containers manufactured from a wide variety of materials. Retentiveness of packaging depends on both the design and the container material. Hence, the release of volatile compounds may be produced if the container is not hermetic due to the design and/or due to the material, which might not be impermeable to vapor and volatile substances. An example of a design flaw is the lid of a jar that does not close hermetically. An example of a permeable material might be a polymer with pores of large diameter, which allows the diffusion or leakage of small molecules. However, sometimes the mechanisms of release are not certain, and there are containers that, despite their hermetic and impermeable appearance, they allow the release of volatile substances.

An important requirement to be fulfilled by packaging for evidence with volatile compounds is, from the point of view of the chemical composition, that the components of containers must not interfere with the evidence analysis. Packaging must avoid substances in its composition that could chemically react with the analytes of the evidence. Moreover, containers must be free of substances which may hamper the results by background signals. It is necessary to perform blank analysis of containers in order to determine their suitability for containing evidence with volatile compounds. Finally, packaging for evidence with volatile compounds must be durable. Certain vapors may corrode some materials, which lose their physical properties and the container may lose its hermetic nature. Therefore, and considering all the above-mentioned characteristics, containers for evidence with volatile compounds must be:[6,8]

- Hermetically sealable

- Impermeable to vapor and volatile substances

- Inert

- Free of residues which may interfere with the results

- Durable

The perfect container does not exist and every container has advantages and disadvantages. Paint cans with compression lids and glass jars have traditionally been used for the storage of this kind of evidence (**Figure I.1.2**).[6,9,10]

***Figure I.1.2**. Metal paint cans and glass jars.*

Metal cans are more durable than glass jars or plastic containers and are not susceptible of breaking or tearing and spilling the contents. However, they are not transparent and must be opened in order to view what is in the inside. Moreover, they can corrode when stored in a moist atmosphere and their manipulation may result difficult due to their rigid form. Glass jars may also result difficult to manipulate, but they are transparent and there is no need to open the lid to view the content. However, they are fragile and may be easily broken. Moreover, the sealing or rubber gaskets in some glass jars may be damaged by the effect of the vapors of some accelerants, causing the vapors to escape from the container.[6,9,10]

On the other hand, polymer bags (**Figure I.1.3**) are transparent, resistant to shock, malleable, so the objects can be easily placed inside, and they occupy little volume when they are folded.

***Figure I.1.3**. Polymer bag for the evidence packaging.*

However, they can be poorly sealed leaving unclosed segments, can easily be damaged by sharp items, and they are afflicted with background interferences when they are analyzed. In spite of these disadvantages of plastic bags, they are used for the collection and storage of fire debris, accelerant, and other volatile substances.[7-9] In fact, in recent years, different polymer bags have been investigated to determine their suitability for the storage of accelerants, fire debris, and other volatile compounds.[7-12]

W. D. Kinard and C. R. Midkiff[7] studied the Kapak bags in terms of presence of contaminants and containment qualities. Their results concluded that the bags were free of contaminants, although they recommended that, prior their use as evidence container, a representative sample, should be tested in the laboratory. They also concluded that the bags had excellent containment qualities for Zippo lighter fluid, gasoline, and kerosene, as only minimal amounts of the materials tested were detected after 60 days of storage. From this study onwards the bags that were produced by Kapak were considered to be one of the best containers for fire debris.

Cryovac and Globus bags were tested by M. J. Kocisko.[11] The author placed diesel, kerosene, and gasoline in the bags. The bags were then heated for 24 h at 90 ºC to study if diesel, kerosene, or gasoline were absorbed in the bags. The results showed that both Cryovac and Globus bags absorbed components of the ignitable liquids. Moreover, the bags produced by-products that interfered the chromatographic results.

J. Pet'ka and col.[12] studied three types of bags in order to determinate which material was more suitable for the storage of volatile compounds. Saran, Tedlar, and Teflon materials were studied. This study concluded that Teflon was the most suitable, while Tedlar and Saran were unsuitable because they release a large number of volatile.

Kapak bags were also studied in comparison to metal paint cans and glass jars to study which container was more suitable for the storage of ignitable liquids.[10] This study demonstrated that the glass jars had the fastest leak rate of hydrocarbons followed by metal paint cans and the polymer bags with the slowest leak rate.

The production of Kapak bags were then discontinued from July 2010 and Ampac started offering a new packaging material. This Ampac bags were compared to the Kapak bags in a new study.[9] The results proved that the properties of the compared materials were similar. Moreover, Ampac bags showed higher retentiveness for flammable liquids, showing less risk of loss of analytes or cross-contamination.

A following study compared Ampac bags with Nylon, DUO, and ALU in terms of background volatiles, leak rate, cross-contamination, recovery, and sorption for ignitable liquids.[8] The Ampac bag showed low background signal, no leakage or cross-contamination, good recoveries and only traces of sorption. The DUO and ALU showed some background volatiles, sorption, and poor recoveries. Nylon bags showed relative high levels of leakage and cross-contamination, being the least suitable of the four bags compared.

The results of these studies showed that, to date, all the polymer bags for the storage of this kind of evidence show leakages, at least at trace

levels. Despite the disadvantages, some bags are better than others and they must be chosen in basis on the needs and the sample to be packaged. It is important to consider the evidence to be stored, since some bags may show better properties for certain material than for others. It is clear that the evidential item most commonly studied have been ignitable liquids. However, other materials with high volatile compounds, such as explosives, should be studied.

In this thesis, the storage of dynamites packaged in different bags will be studied. Thus, after chapter I.2, which is focused on the development of a method for the determination of the volatile compound ethylene glycol dinitrate in dynamites, two chapters are focused on the study of the suitability of two types of plastic bags for the storage of dynamites. Chapter I.3 aim to study the possibility of sample-to-sample cross-contamination between two dynamites, differing in their compositions, when they are stored in a certain type of plastic bags. Chapter I.4 compare the plastic bags studied in chapter I.3 with other type of bags, which are supposed to show better retentiveness. This chapter is focused to study an alternative packaging for dynamites.

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CHAPTER I.2

DETERMINATION OF ETHYLENE GLYCOL DINITRATE IN DYNAMITES USING HPLC: APPLICATION TO THE PLASTIC EXPLOSIVE GOMA-2 ECO

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Abstract

In this work, a sequential extraction method using water and methanol to recover ethylene glycol dinitrate contained in Goma-2 ECO dynamite was developed. After, a high performance liquid chromatography method was used for the determination of ethylene glycol dinitrate in the two extracted phases. The analytical method was validated by evaluating its selectivity, sensitivity, linearity and linear working concentration range, limit of detection and quantitation, precision (as repeatability and intermediate precision), accuracy, and robustness, providing appropriate values (i.e. RSD values for precision about 6% and accuracy about 100%). Finally, the ethylene glycol dinitrate content of a sample of the Goma-2 ECO dynamite studied was determined obtaining a concentration of 30.29 %, which is in accordance with the manufacturer's specifications for this dynamite (25.7-31.4 %).

1. **Introduction**

Nitro group containing compounds constitute the most important group of explosives, among them cyclotetramethylenetetranitramine (octogen), hexahydro-1,3,5-trinitro-5triazine (hexogen), 2,4,6,8,10,12-hexanitrohexaazaisowurtzitane (CL-20), nitrocellulose, pentaerythritoltetranitrate (pentrit), trinitrotoluene (TNT), dinitrotoluene (DNT), or dinitrobenzene are included.[1,2] The nitrate ester ethylene glycol dinitrate (EGDN), also known as nitroglycol, is used in the formulation of explosives such as dynamites.[3] EGDN is a yellowish liquid explosive, which replaces totally or partially the nitroglycerin (NG) in dynamites due to its higher resistance against knocks and friction. EGDN is a good solvent for low-grade nitrocellulose, more volatile and less viscous than NG. In addition, EGDN also decrease the freezing point of NG, which helps to the employment of dynamite in countries with a cold climate.[4] Most of the studies on EGDN are focused on its effects on health, since this compound is known as a potent vasodilating agent and may provoke adverse effects on cardiac muscle, blood flow or mitochondrial respiration among workers who are exposed to this substance.[5-9]

Goma-2 ECO is a dynamite containing EGDN. It is a Spanish-made high explosive for industrial use, primarily in mining, demolition and military applications. Goma-2 ECO is a gelatinous dynamite composed of EGDN, ammonium nitrate, nitrocellulose, dibutyl phthalate, calcium carbonate and flour or sawdust. This high explosive is commonly used in Spain and also exported abroad. However, there are several kinds of dynamites, differing in their compositions. For example, Goma-2 ECO is developed according with European regulations on the environment and does not contain DNT,

which is a highly toxic compound.[10] Nevertheless, some other dynamites manufactured before this regulation could contain DNT, like Goma-2 EC. Other dynamites can also incorporate other components, like NG, which is included, for example, in the formulation of Titadyne dynamite. Since EGDN is a component of the above-mentioned dynamites, the development of analytical methods for its determination in dynamites is important to achieve two main purposes: (i) its application to the determination of EGDN in explosives used in terrorist attacks in order to identify the explosive components and interpret the explosive used and (ii) its use for the quality control of the explosive composition by the manufacturer or the administration if it is required.

High performance liquid chromatography (HPLC) has been commonly used as a separation technique in several works concerning explosives.[11] A minority of these works are forensic studies and the great majority of them are focused on environmental matters,[12-19] contemplating explosives as contaminating agents, mainly in areas close to a manufacturing or ammunition plants[12-14] and/or paying attention to the extraction of the explosives from soils and waters.[17-19] Dealing with this, Gaurav and col. compiled a complete review on reported studies employing solid-phase micro-extraction as a technique for the sampling, isolation, enrichment, and analyte introduction to a HPLC equipment for the determination of explosives in environmental samples.[20] Up to date, published studies on explosives, either in forensic or environmental fields, cover the vast majority of known explosives and devices. Most of them are focused on explosives such as HMX, RDX and nitrobenzene and nitrotoluene derivatives and a minority are focused on dynamites. To our knowledge, the latest study on the development of extraction and

separation methods of the components of a dynamite was published by S. Baj and col. in 1993.[21] In this article, a ten-step extraction method was performed in order to achieve the total recovery of organic nitro derivative components (TNT, 2,4 DNT, NG, and EGDN) contained in the dynamite analysed. The best separation was obtained when LiChrosorb Si 60 5 μm column and isopropanol-hexane system as eluent were employed. However, since this last study there have not been new developments in the extraction and analysis of the components of dynamites. Recently, Tyrrell and col.[22] published a new work, in which a chromatographic based method has been developed for the determination of organic (and inorganic) based explosives, which also includes a sampling/extraction method for explosives targets including EGDN. However, in this work, homemade explosives are analysed, but not dynamite based explosives. Since dynamites are heterogeneous explosive materials, further improvements are needed in order to achieve best separations and more suitable extraction methods allowing the recovery of components of dynamites. This subject is of great importance in forensic science and for industrial laboratories.

Therefore, the aim of this study was to develop and validate an analytical method by HPLC for the determination of EGDN in dynamites. Due to the heterogeneity of the sample to be analyzed, an extraction method for all the components in dynamites was developed.

2. **Material and methods**

2.1. Apparatus

HPLC analyses were performed in a full-equipped Hewlett-Packard liquid chromatograph series 1100 (Hewlett-Packard, Pittsburgh, PA, USA) with diode-array detection (DAD). The separation was performed in a 250×4.6 mm ODS 5.0 μm Prontosil Hypersorb column (Scharlab S.L., Barcelona, Spain). The separation method used was optimized in this work and consisted of a one-step gradient from 25 to 95% B in 35 min, using a temperature of 40 ºC. Mobile phases were water (mobile phase A) and methanol (mobile phase B); the flow-rate was 1 mL /min, and the injection volume was 10 μL. UV detection at 230 nm and 550 nm as reference wavelength, with 4 nm and 100 nm bandwidth, respectively, were employed. Samples were prepared using a MC210P balance (Sartorius, Göttingen, Germany) with an accuracy of ± 10 μg and a precision of ± 20 μg and an ultrasound bath ULTRASONS-H (JP Selecta, Barcelona, Spain).

2.2. Reagents, samples, and materials

The extraction method was carried out with ultrapure water (18.2 Ω) from Milli-Q water system and HPLC-grade methanol, purchased from Sigma-Aldrich. EGDN (1000 mg/L) was from Teknokroma (Barcelona, Spain). Goma-2 ECO dynamite, with an EGDN content ranging from 25.7 to 31.4%, was purchased from MaxamCorp Holding, S.L. (Madrid, Spain). Standards of EGDN, NG, TNT, 3,4-DNT, 2,6-DNT, 2,4-DNT, 3,5-DNT, and dibutyl phthalate (1000 mg/L) were from Restek Co. (Bellefonte, PA, USA).

2.3 Extraction of EGDN from Goma-2 ECO dynamite

Figure I.2.1 shows the scheme of this extraction method.

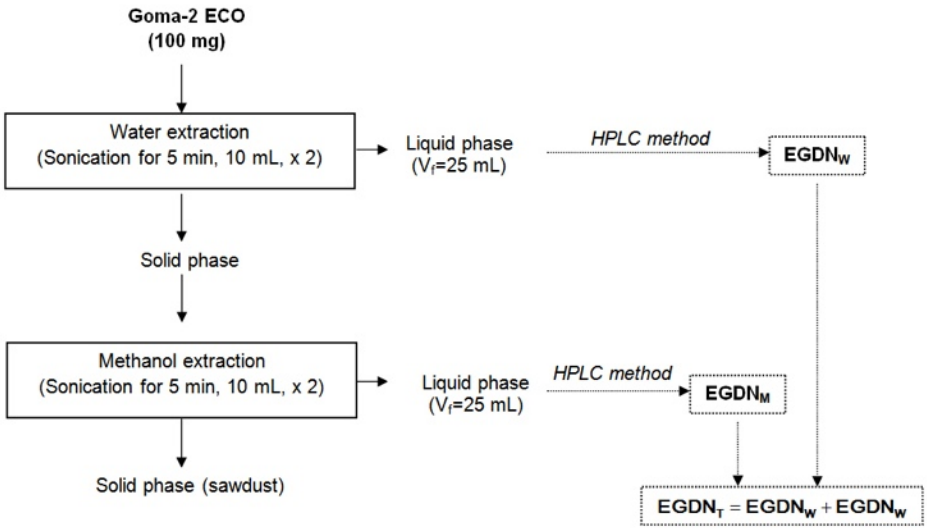

***Figure I.2.1**. Scheme of the method developed for the extraction of EGDN from Goma-2 ECO. Abbreviations used: V_f, final volume; $EGDN_W$, EGDN content after the two water extractions; $EGDN_M$, EGDN content after the two methanol extractions; $EGDN_T$, total EGDN content.*

10.0 milliliters of Milli-Q water were added to 100 mg of Goma-2 ECO sample and sonicated for 5 minutes in an ultrasound bath. The liquid phase was collected and stored for later use. Another 10.0 mL of Milli-Q water were added to the sample and the mixture was sonicated again for 5 minutes. The liquid phase was collected, added to the previous one and led to a final volume of 25.0 mL adding Milli-Q water. The final solution was stored at room temperature until its

analysis. Then 10.0 mL of methanol were added to the sample, the mixture was sonicated for 5 minutes, and the liquid phase was collected and stored for later use. Another 10.0 mL of methanol were added to the sample and the mixture was sonicated again for 5 minutes. The liquid phase was collected, added to the previous one and led to a final volume of 25.0 mL adding methanol. After this treatment, the entire Goma-2 ECO sample was dissolved, with the exception of sawdust.

3. **Results and Discussion**

3.1. Development of an HPLC method for the determination of EGDN in dynamites

An HPLC method to determine EGDN in dynamites was developed in this work. Since dynamites are heterogeneous explosives (see **Figure I.2.2**), the first step was to develop an easy and sustainable exhaustive extraction method of EGDN in dynamites.

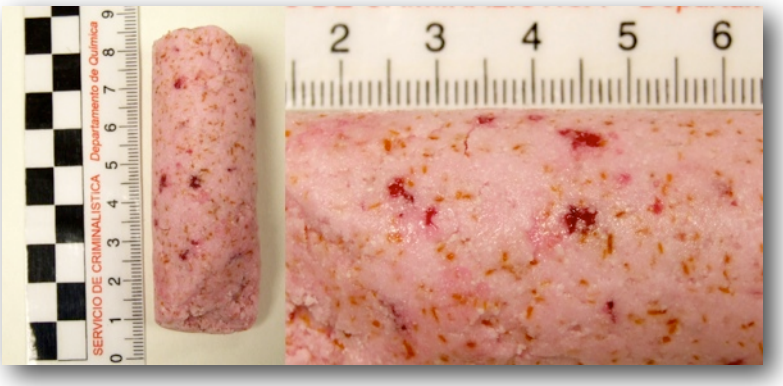

Figure I.2.2. *Picture of a Goma-2 ECO fragment, in which it can be appreciated the heterogeneity of this sample.*

Due to the solubility of EGDN in water and methanol these two solvents were tested to recover EGDN from the dynamite. Both solvents were also chosen because of their low price and toxicity in comparison with other organic solvents such as acetonitrile and chloroform previously used.[21] Different combinations and proportions were tested. Water and methanol were tested as separated solvents, but the complete dissolution of the dynamite could not be achieved. Then, water and methanol were mixed and used as extractive solvent in different proportions, from 30 to 70% of methanol /water (v/v). However, the complete dissolution of the dynamite was only achieved employing the same volume of both solvents separately, first water and, then, methanol and these conditions were chosen as the optimal conditions for the extraction. This extraction method ensured, not only the total dissolution of the EGDN contained in the dynamite used, also the dissolution of the whole dynamite, including salts and nitrocellulose, with the exception of sawdust. This is relevant because the last published study on the recovery of explosives compounds from dynamites only achieved the recovery of the organic nitro derivatives compounds.[21] In comparison with this previous work, this extraction method also reduces the time of sample treatment by reducing the number of steps from ten to only two. After the extraction, the EGDN content in both the water ($EGDN_W$) and methanolic ($EGDN_M$) extracts were measured by an HPLC method.

An HPLC method based on a gradient elution was also optimized. Different mobile phases and concentrations of them were tested. First, the separation was carried out under isocratic conditions, using methanol as mobile phase. However, since it is possible having explosives with different compositions a gradient was done as follows: from 25 to 40% in 10 min, of phases A (water) and B

(methanol), at 1 mL /min and 30 ºC. This separation method was designed in order to provide swiftness to those urgent forensic cases, being possible to apply the same method to different explosives. For this reason the method was slightly modified and, finally, a gradient elution from 25 to 95% in 35 min, of phases A (water) and B (methanol), at 1 mL /min and 40 ºC were chosen as the optimal conditions for the separation. This method was effective for the separation of seven standards (10 μg/mL) commonly used in the manufacture of dynamites (EGDN, NG, TNT, 3,4-DNT, 2,6-DNT, 2,4-DNT, and 3,5-DNT), as it is shown in **Figure I.2.3**.

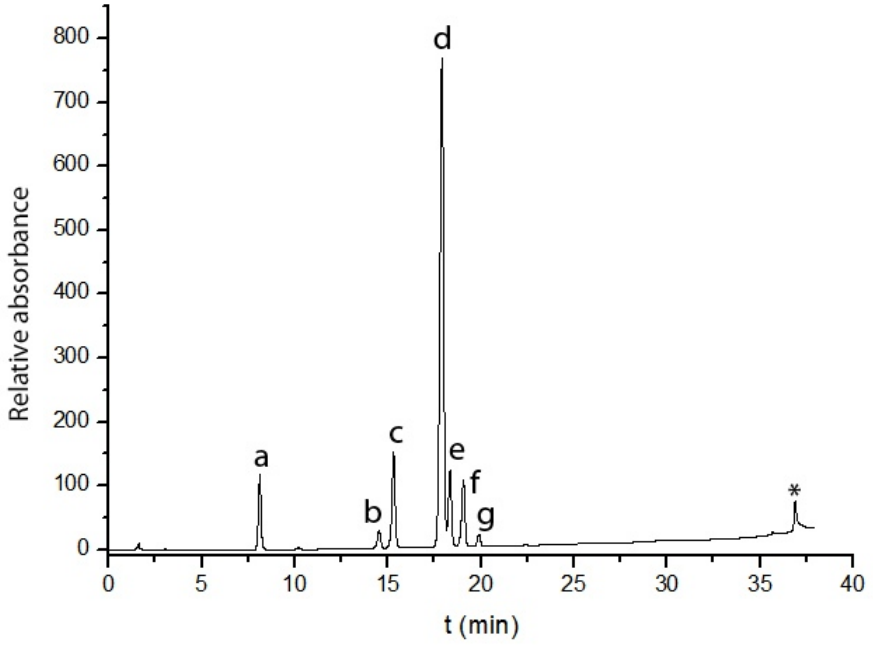

***Figure I.2.3**. Separation of a mixture of seven standards (10 μg/mL) of explosive compounds in methanolic solution. Identification of peaks: a, EGDN; b, NG; c, TNT; d, 3,4-DNT; e, 2,6-DNT; f, 2,4-DNT; g, 3,5-DNT; *, unknown peak.*

For this reason, it was applied to the water and methanolic extracts of the dynamite. The chromatograms obtained in this occasion are shown in **Figure I.2.4**.

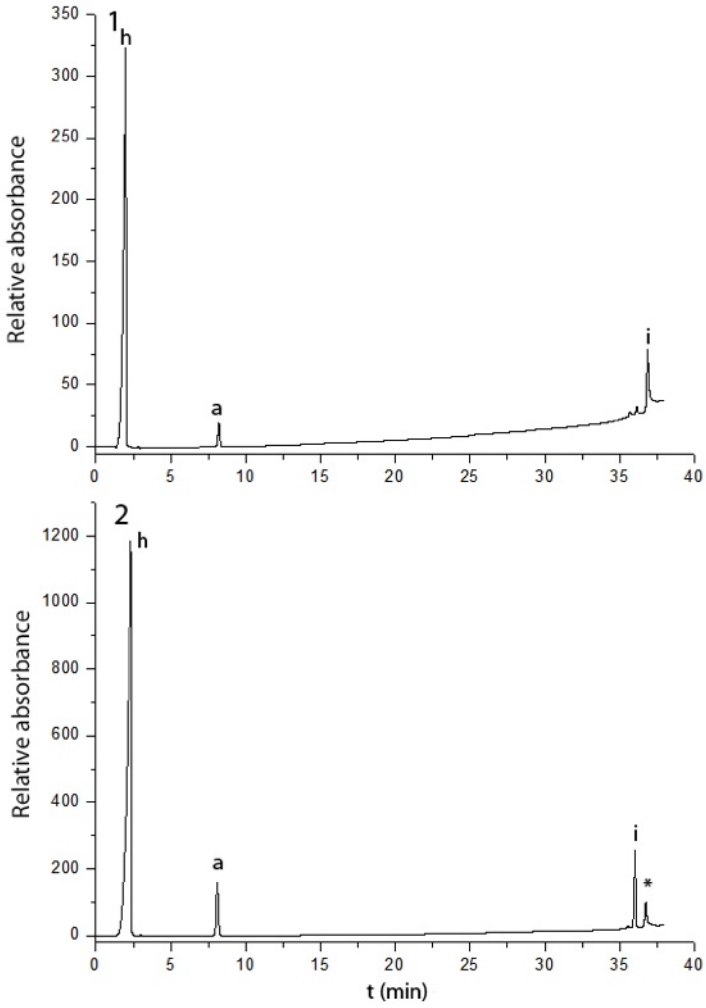

***Figure I.2.4**. Separation of the components of Goma-2-ECO after (1) the extraction with water and (2) the extraction with methanol. Both separations shown the good selectivity for the determination of EGDN in plastic explosives as Goma-2 ECO. Identification of peaks: a, EGDN; h, ammonium nitrate; i, dibutyl phthalate; *, unknown peak.*

These chromatograms also show that the method is also suitable for the determination of ammonium nitrate and dibutyl phthalate (peak identification was established from the correspondence of retention times with standards), which are two compounds that have not been taken into account so far in the analysis of dynamites because it was considered that the determination of organic nitro compounds was usually enough.[21]

3.3. Quantitative determination of EGDN in dynamites

For the quantitative determination of EGDN in dynamites, peak area of EGDN in the extracts was measured to determine the concentration of EGDN in the sample by external standard calibration. The total content of EGDN in Goma-2 ECO was calculated by summing the EGDN contents obtained for both extracts, as it has been explained in **Figure I.2.1**.

Calibration plots were obtained by analysis of nine standard solutions of EGDN at concentrations in the range 3 mg/L - 400 mg/L. Peak area was measured and plotted against EGDN concentration. Although a linear response was observed in the entire concentration range used for calibration, a linear working concentration range from 10 mg/L to 200 mg/L was selected, to remove those concentrations with higher associated errors.

Table I.2.1 compiles the values for the slope, intercept, correlation coefficient, R-squared, and standard error for the calibration plot obtained in the least-square fitting of these data (five concentration levels).

***Table I.2.1**. Calibration parameters for the determination of EGDN in the plastic explosive Goma-2 ECO.*

Calibration line parameters	**Value**
n[a]	5
Slope [b]	1.48 ± 0.01
Intercept [b]	1.0 ± 1.7
Correlation coefficient	0.999974
R-squared (%)	99.9949
Standard error of estimation ($S_{y/x}$)	0.833882
Intercept equals to 0,0?	YES [c]

[a] *Number of standard solutions of EGDN analyzed. Triplicate injections were made for each standard solution.*

[b] *95.0 % confidence intervals for coefficient estimates.*

[c] *p-value = 0.02*

A correlation coefficient above 0.9999 and an intercept including the zero value for a 95 % confidence level, were obtained. The effect of matrix interferences was studied with a homemade dynamite, prepared with ammonium nitrate, flour and sawdust, at which standard solutions of EGDN at 30 mg/L, 70 mg/L, and 150 mg/L were added. Water and methanolic extracts obtained from this sample were analyzed in quintuplicate and EGDN concentrations obtained from the calibration line were compared, by means of least-square fitting, with the standard concentrations added. A straight line with an intercept and slope equals 0.0 and 1.0, at the 90% or higher confidence level, was obtained (P-values: 0.966334 and 0.286526, respectively).

This analytical method was validated for the determination of EGDN in a plastic explosive as Goma-2 ECO in terms of selectivity, sensitivity, linearity and linear working concentration range, LOD, LOQ, precision (evaluated as repeatability and intermediate precision), accuracy, and robustness, according to the International Conference on Harmonization (ICH) Guidelines[23] and the National Association of Testing Authorities.[24] **Table I.2.2** summarizes the values obtained for the above-mentioned parameters.

***Table I.2.2**. Figures of merit for the validation of the analytical method developed for the determination of EGDN in the plastic explosive Goma-2 ECO.*[11, 12]

Validation parameters	**Value**
Sensitivity (mg/L)	1.48
Linearity (mg/L)	3.0 - 400
Linear working concentration range (mg/L)	10.0 - 200
Limit of detection (mg/L)	1.7 ± 0.2[a]
Limit of quantification (mg/L)	5.6 ± 0.2[a]
Precision (%)	
Repeatability at	
low-level (30 mg/L)	4.1
medium level (70 mg/L)	5.7
high-level (150 mg/L)	1.0
Intermediate precision (%)	6.5
Accuracy (%)	
Low-level (30 mg/L)	104
Medium level (70 mg/L)	101
High-level (150 mg/L)	104
Robustness (%) (3 mg/L)	
Peak area ± 2s	3.3 ± 0.4[a]
Retention time (min) ± 2s	8.34 ± 0.01[a]

[a] *95 % confidence.*

Selectivity. As it is shown in the chromatograms corresponding to the extracts obtained from Goma-2 ECO (**Figure I.2.4),** an adequate selectivity is obtained since EGDN can be determined without any interfering peak in the aqueous and methanolic phases.

Sensitivity. It was calculated as the slope of the calibration line. A value close to 1.5 mg/L was determined for EGDN standard.

Linear working concentration range. It was calculated by plotting relative response (EGDN peak areas divided by their respective standard concentrations) as a function of the corresponding concentrations, on a logarithmic scale; the method was considered linear up to the point where the plotted relative response line intersected the 95 % line. In this case, as stated above, a linear concentration range from 10 mg/L to 200 mg/L was obtained.

LOD and LOQ. These parameters were calculated, according to the ICH guideline on validation,[23] based on the standard error of regression ($S_{y/x}$) and the slope of the calibration line at levels approaching the limit. Thus, LOD, was calculated as ($3.3 \cdot S_{y/x}$ / slope), and LOQ estimated as ($10 \cdot S_{y/x}$ / slope). The values obtained were 1.7 ± 0.2 mg/L and 5.6 ± 0.2 mg/L, respectively. Taking into account that the injection volume was 10 μL, absolute LOD and LOQs values of 0.17 ng and 0.56 ng, respectively, corresponding to amounts of EGDN injected, were determined. The value of the LOD is slightly higher than that reached by Tyrrell at al.[22] in their recently published study, in which EGDN (beside other organic and inorganic explosives) was determined. In this work a Dionex Acclaim® Explosives E2 column (150 mm × 3 mm), 48/52 v/v methanol /Milli-Q water as mobile phase, and UV detection (210 nm) were used. However, in this work,

homemade explosives were analysed, but not dynamite based explosives.

Precision. Precision of the analytical method was evaluated as repeatability and intermediate precision. Repeatability (in peak area and in concentration) were expressed as the discordance among the measurements (RSD values in %) of five consecutive injection of solutions of 30 mg/L (low-level), 70 mg/L (medium-level), and 150 mg/L (high level) of EGDN standard. For the three concentration levels studied, RSD values lower than 5.7% were obtained. Intermediate precision was evaluated as the RSD value obtained for the injection, by different analysts on five different days, of a standard solution of whose EGDN corresponding to the medium-level concentration of the linear range (70 mg/L). An RSD value of 6.5% was obtained. According to the calculated values for repeatability and intermediate precision, a good precision of the analytical method was obtained since RSD values about 6% were measured.

Accuracy. The accuracy of the method was determined as the percentage of recovery of EGDN when known EGDN concentrations (30 mg/L, 70 mg/L, and 150 mg/L) were added to a sample of similar matrix to that of the plastic explosive studied. Recoveries obtained were 104%, 101%, and 106%, for the low-, medium-, and high-levels, respectively, of the linear working concentration range. Since these values are very close to the ideal recovery (100%), a good accuracy of the analytical method was found.

Robustness. Robustness of the method (%) was calculated by analyzing the peak areas and retention times for EGDN of ten injections of a standard solution of 3 mg/L, subjected to variation in the stability of

the flow-rate. The average peak area (± 2s) was 3.3 ± 0.4 and the average retention time (± 2s) was 8.34 ± 0.01 min. That is, an acceptable RSD value for peak area (~12%) and an excellent RSD value for retention time (0.1%) were obtained.

The determination of the main components of explosives is a subject of great importance in forensic chemistry and also for the industrial laboratories, being necessary to obtain reliable analytical data. As stated above, the dynamite analyzed in this work is characterized by a complex matrix consisting of diverse components mixed heterogeneously (see **Figure I.2.2**); however, since the method performance criteria were good enough for our practical purpose, the validated method was applied to the determination of the total EGDN content in Goma-2 ECO. The total EGDN content for the Goma-2 ECO analyzed was 30.29%, which is in accordance with the manufactureŕs specifications for this dynamite (25.7%-31.4%).

4. **Concluding remarks**

A selective, sensitive, and reliable analytical method was validated and applied for the first time for the determination of EGDN in a dynamite as Goma-2 ECO. First, an extraction method enabling to dissolve all the EGDN was developed. Then, an HPLC method providing good selectivity was applied. This method also allowed the determination of other components of dynamites, such as ammonium nitrate and dibutyl phthalate. The analytical method was validated for the reliable quantitative determination of EGDN in dynamites. The results obtained showed that EGDN in Goma-2 ECO was 30.29%, which is in accordance with the manufactureŕs specifications for this

dynamite (25.7%-31.4%). These results show the high potential of the proposed method to perform the quality control of plastic explosives containing EGDN.

Acknowledgements

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CHAPTER I.3

STUDY OF LOSSES OF VOLATILE COMPOUNDS FROM DYNAMITES. INVESTIGATION OF CROSS-CONTAMINATION BETWEEN DYNAMITES STORED IN POLYETHYLENE BAGS

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ABSTRACT

The purpose of this work was to study the appropriateness of polyethylene bags for the preservation of explosive specimens. To this end, specimens of two types of dynamites, Goma-2 EC, containing EGDN and DNT, and Goma-2 ECO, containing only EGDN, were placed individually inside bags and introduced into hermetically sealed glass jars, which were stored for a period of time. Losses of volatile compounds were studied by headspace analysis using gas chromatography coupled to mass spectrometry. The cross-contamination between dynamites was studied by using HPLC with mass spectrometry detection to analyze the extracts obtained after a sequential solvent extraction of these specimens. Polyethylene bags permit the loss of volatile compounds since EGDN and DNT were detected in the headspace of the jars. Moreover, cross-contamination between dynamites was also demonstrated since DNT content decreased in the dynamite containing this compound and increased in the dynamite that had not contained it.

1. **Introduction**

Explosive specimens are of great importance in cases of terrorism, in which dynamites and other kinds of explosives may be used. There are many types of explosives, which differ in their composition, appearance, explosive capability or even place of manufacture. These characteristics of the explosives may be used to identify the explosive that was used in the perpetration of a crime. Moreover, due to the fact that terrorists often use a single type of explosive, usually obtained in a robbery, these characteristics may be decisive when determining the source of the explosives used and the authorship of the attack, or at least, to offer some orientation to a forensic investigation.

Goma-2 ECO and Goma-2 EC are Spanish-made high explosives (dynamites) intended primarily for industrial use, such as mining and demolition, or military applications. Goma-2 ECO is a gelatinous dynamite composed of EGDN, ammonium nitrate, nitrocellulose, dibutyl phthalate (DBP), calcium carbonate, and flour or sawdust. This high explosive has commonly been used in Spain and also exported abroad. Unlike Goma-2 EC, Goma-2 ECO was developed in line with European environment regulations[1] and does not contain the highly toxic compound DNT in its composition. Unlike Goma-2 ECO, Titadyn contains NG and DNT in its formulation. In terms of their composition, Goma-2 ECO, Goma-2 EC, and Titadyn are heterogeneous materials.

Goma-2 ECO was allegedly used in the March 11, 2004 train bombings in Madrid (Spain). The authorship of the attack has not yet been clarified, the controversy still lingering today. At first, responsibility was assigned to ETA (the Basque terrorist organization); however, this

organization denied any connection with these attacks while the organization Al-Qaeda later acknowledged its responsibility for the outrage. ETA has used Titadyn repeatedly in terrorist attacks. Since trace amounts of NG and DNT were detected in some of the explosive evidences of the 11 March blast, the possibility ETA authorship was placed back on the table. Pursuant to the action protocol, explosive evidences collected at the scene of the bombings were put into official polyethylene bags and transported to a laboratory where many other explosives were kept. For this reason, the possibility that there might have been contamination between different specimens was considered.

Cross-contamination occurs when certain compounds are transferred from one specimen to another.[2] It can take place between very different kinds of specimens and, if not detected, always leads to erroneous results in scientific studies. In forensic investigations, contamination between specimens as a result of incorrect preservation may lead to charging or arresting innocent people. Therefore, it is important to pay special attention when handling, sampling and storing specimens of explosive in order to avoid cross-contamination and incorrect interpretation of forensic cases.

Contamination between explosive specimens may occur through physical contact with contaminated surfaces, with other specimens or with particles or vapors. In fact, the contamination of explosives by vapors is possible because highly volatile compounds, such as EGDN, NG or DNT are usually present in many explosives. The vapor pressure of a given compound measures its capacity of volatilization; in general, the higher the vapor pressure, the higher the capacity of volatilization and the lower the capacity to be retained by certain

materials. EGDN has the highest vapor pressure (7.0×10^{-2} torr at 25 ºC), followed by DNT and NG (2.0×10^{-3} and 2.3×10^{-4} torr at 25 ºC, respectively).[3] Contamination by vapors is also dependent on the adsorption characteristics of the compounds, which are defined by their affinity with the material.[4] These compounds may exhibit a very strong affinity to be adsorbed on a variety of materials such as hair,[3] charcoal,[5] organic matter from soil,[6] other components of soils like sands or clays[7-9] and some polymers;[10-11] in most cases adsorption may occur in an almost irreversible manner.[4] In fact, volatile compounds may bind easily to porous materials with a high surface-area-to-volume ratio. It should be stressed that nowadays, adsorbent porous materials such as calcium carbonate are used as components of explosives.

As far as we know, there have been very few studies of the loss of volatile compounds due to the material of the container used for storing the specimens in the field of forensics.[12-15] Those studies that exist mainly focus on the capability of fire debris evidence receptacles to avoid the loss of vapors. However, both for fire debris and for specimens of explosives had the same objetives: to avoid the loss of volatile compounds and to prevent changes in the specimens. For this reason, the same containers used for specimens of fire debris can be used for specimens of explosives and their characteristics are similar. When dealing with specimens of fire debris the containers recommended are metal paint cans with compression lids, glass mason jars with standard pressure-canning flats and bands, and special polymer sample bags.[15-18] However, each type of container has its advantages and disadvantages and care must be taken when choosing the containers to be used for specimen storage. For example, containers made of glass are usually big and their handling and

transport requires special care because they are fragile. On the other hand, they are very simple to use and can be closed easily by screwing the tops of the jars. This is an advantage not shared by polyethylene bags. These are tough and not liable to breakage, but their handling is often complicated and great care is required when sealing the bag in order to ensure that there are no poorly closed segments that may allow the output of volatile compounds. Although the size, robustness and ease of use of containers are very important issues, when choosing a suitable container perhaps more importance should be attached to the capacity of containers to prevent loss of volatile compounds. Nowadays, polymer bags are being used to preserve explosive specimens because they are considered the best system to avoid vapor losses in comparison with metal paint cans and glass mason jars; however, these bags are not fully efficient and may allow losses of trace amounts of volatile compounds in specimens of fire debris.[15] In fact, recent discussion of the quality of the methods for sampling and preserving explosive specimens and other volatile substances in forensic laboratories[12-15] has led to the conclusion that current procedures are unsuitable or do not ensure the proper preservation of specimens.[12,14,15] This represents a huge problem that must always be borne in mind when interpreting the results of a forensic case. Some authors have developed and tested sampling kits for the collection of evidences as alternatives to those currently used,[12] paying special attention to avoid contact between the specimen and the walls of the container.

This work aimed, firstly, to study the dynamics of the loss of the highly volatile EGDN from Goma-2 ECO dynamite under different temperature and time conditions; and, secondly, to evaluate whether the official polyethylene bags used by the Spanish Security Forces to

transport and store specimens are suitable systems. For this latter purpose, the chances of losses occurring of volatile components (specifically, EGDN and DNT), as well as of cross-contamination occurring between different dynamite specimens kept in the official bags and stored together in a confined space, were investigated.

2. **Material and methods**

2.1. Chemicals and materials

The extraction of EDGN and DNT was carried out with ultrapure water (18.2 Ω) from a Millipore Milli-Q water system (Bedford, MA, USA) and HPLC-grade methanol purchased from Sigma-Aldrich (St. Louis, MO, USA). Mobile phases for HPLC were also prepared with Milli-Q water and HPLC-grade methanol. 2,4-DNT, 2,6-DNT, 3,4-DNT, and EGDN standards (1 μg/mL) were supplied by Restek Co. (Bellefonte, PA, USA).

Specimens of Goma-2 ECO dynamite were kindly donated by MaxamCorp Holding, S.L. (Madrid, Spain). Specimens of Goma-2 EC dynamite were provided by the Criminalistic Service of the Guardia Civil (Madrid, Spain). The composition of these specimens, together with that of another high explosive (Titadyn), is shown in **Table I.3.1**.

The official plastic bags used by Spanish Security Forces to transport and to store evidence specimens in forensic cases were provided by the Criminalistic Service of the Guardia Civil. These bags, supplied by Royal Pack, S.L. (Alcalá de Henares, Spain), are made from

polyethylene and have a rubber gasket that prevents the output and input of gases.

Table I.3.1*. Composition, indicated by the manufacturer, of high explosives Goma-2 ECO, Goma-2 EC and Titadyn.*

	Goma-2 ECO	**Goma-2 EC**	**Titadyn**
EGDN	✓	✓	✓
NG			✓
DNT		✓	✓
Nitrocellulose	✓	✓	✓
Ammonium nitrate	✓	✓	✓
DBP	✓		
Calcium carbonate	✓		
Flour/sawdust	✓		

2.2. Instrumentation

HPLC analyses of the extracts of dynamites were performed in a fully equipped, Agilent series 1100 liquid chromatograph (Pittsburgh, PA, USA) with a DAD and an atmospheric pressure chemical ionization (APCI) mass spectrometer (MS) detector (HPLC-DAD-MS). The separation was performed in an ODS ProntoSIL Hypersorb column (250 x 4.6 mm I.D.; 5.0 μm particle diameter) from Scharlab S.L. (Barcelona, Spain). The separation method used consisted in one-step gradient from 25% to 95% B in 35 min, using a temperature of 40 ºC. The mobile phases were water (mobile phase A) and methanol (mobile phase B); the flow-rate was 1 mL /min and the injection

volume was 10 µL.[19] UV detection at 230 nm as analytical wavelength (4 nm bandwidth) and 550 nm as reference wavelength (100 nm) was employed. For APCI, the instrumental conditions were: nebulizer pressure 40 psi; drying gas (N_2) at a flow-rate of 4 mL /min; drying gas temperature at 350 °C; vaporizer temperature at 325 °C; negative scan in the range m/z = 100–650; Vcap (positive) at 4000 V; Vcap (negative) at 1500 V; corona (positive) at 4 μA; and corona (negative) at 10 μA. Every specimen solution was injected by triplicate in the chromatographic system. Data were captured and processed using the LC-MSD ChemStation Software (Agilent Technologies) software package.

Gas chromatography coupled with mass spectrometry experiments to study volatile compounds in the headspaces of the glass jars were performed in an Agilent 7000A Triple Quad GC/MS (Pittsburgh, PA, USA). Headspace specimens from each jar was collected employing a solid-phase micro-extraction fiber (75 µm Carboxen™ PDMS) and was injected at 250 ºC with an Agilent 7683B injector (Pittsburgh, PA, USA) working in a pulsed splitless mode (at 6.5054 psi). Gas chromatography analysis was carried out with an Agilent 7890A (Pittsburgh, PA, USA) gas chromatograph with helium as the carrier gas and a HP-5MS (5 % Phenyl Methyl Siloxane) 30 m x 250 µm x 0.25 µm column, initially kept at 325 ºC. The separation method used consisted in a five-step gradient from 50 ºC to 260 ºC over 42 min: first, 50 ºC for 3 min; second, an increase of 2 ºC/min to 70 ºC; third, an increase of 4 ºC/min to 130 ºC; fourth, 130 ºC for 1 min; and fifthly, an increase of 10 ºC/min to 260 ºC. The flow-rate was 0.9 mL /min. Mass spectrometry detection was carried out using an Agilent 7000 A triple quadrupole mass spectrometer (Pittsburgh, PA, USA) operating in electronic impact mode. The temperature at 230 ºC, electron energy at

70 eV, electron multiplier voltage at 1449.0 V and scan in the range m/z = 30–550 over 260 ms, were used. Data were acquired and processed using the Agilent Masshunter version B.05.00.412 software (Agilent Technologies).

EGDN and DNT isomers in the chromatograms were identified by comparing the retention times of peaks in the chromatograms of the specimens with those for EGDN, 2,4-DNT, 2,6-DNT, and 3,4-DNT standards.

Specimens were weighed using a MC210P balance (Sartorius, Göttingen, Germany) with an accuracy of ± 10 μg and a precision of ± 20 μg. Solutions were prepared in an ultrasonic bath ULTRASONS-H (JP Selecta, Barcelona, Spain).

2.3. Experimental procedure

The dynamics of EGDN loss from Goma-2 ECO dynamite stored in plastic bags were tested in three parallel studies. In the first, a short-term study, six specimens of Goma-2 ECO dynamite, each weighing 6 g, were placed in official hermetic polyethylene bags that were properly closed. Then each bag with its specimen of Goma-2 ECO dynamite was placed in a glass jar special for gas chromatography (GC). The jars were stored at room temperature for 24 days in a cupboard and 2 specimen analyses per week were done. In the second, a temperature study, specimens were prepared as described above and jars were stored at 60 ºC for 24 days performing 2 analyses per week. For the third study, a long-term study, 34 specimens of Goma-2 ECO dynamite, each weighing 1.5 g were put into official

bags, which were sealed and stored for 121 days at room temperature in a cupboard. EGDN content (%) in the different specimens was monitored as a function of time (~9 specimens analyses per month). To this end, the bags were opened one by one and the EGDN remaining in Goma-2 ECO dynamite was extracted following the extraction method developed by Sáiz et al.[19] and analyzed by HPLC-DAD-MS. Briefly, 0.1 g of dynamite specimen was sequentially extracted with Milli-Q water and methanol (twice, 10 mL of solvent, sonication during 5 min, and adjustment of the two extracted phases with the same solvent to a final volume of 25 mL). Aqueous and methanolic extracts were finally analyzed in triplicate by HPLC-DAD-MS. The final concentration of EGDN in the specimen was calculated as the sum of EGDN concentrations in the methanolic and water extracts.

To study the loss of volatile compounds (EGDN and DNT) from two different dynamites (Goma-2 EC and Goma-2 ECO) stored at room temperature in plastic bags, eight specimens of Goma-2 EC dynamite and eight specimens of Goma-2 ECO dynamite of 0.25 g were weighed and put into one official plastic bag for transportation and storage of evidence specimens. Plastic bags were distributed by pairs, one containing Goma-2 EC dynamite and the other one containing Goma-2 ECO dynamite, into special glass jars for GC; a piece of paper was put between bags to avoid physical contact (see **Figure I.3.1**).

Because the plastic bags were larger than the jars, they were folded before being placed inside them. The jars were stored for a maximum period of 28 days. Every seven days the headspaces of two jars were analyzed by GC-MS in order to determine the presence of volatile components from the explosives.

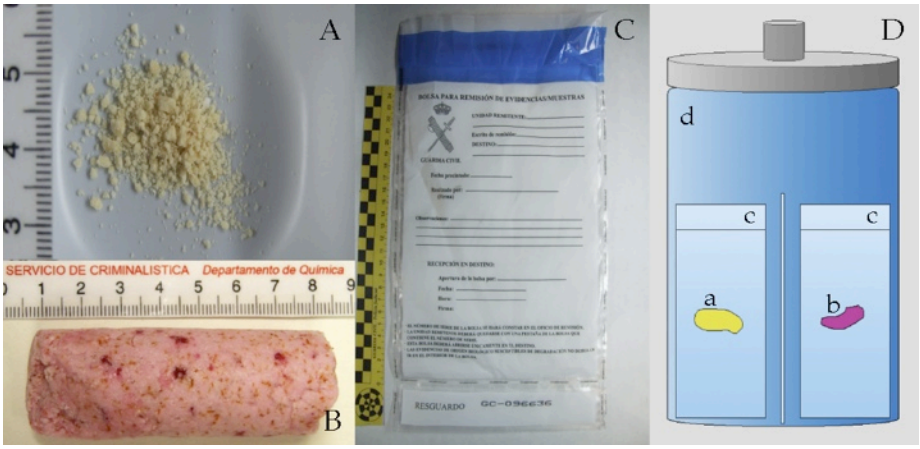

Figure I.3.1. *A) Goma-2 EC dynamite; B) Goma-2 ECO dynamite; C) Official plastic bags used by the Spanish Security Forces to transport and store specimens that are intended for forensic analysis; D) Diagram showing the experimental setup designed to study the losses of volatile compounds from polyethylene bags and the cross-contamination between dynamite specimens. Specimens of Goma-2 ECO (a) and Goma-2 EC (b) dynamites were introduced into official polyethylene bags (c) which were properly closed. They were placed in a glass jar for GC (d) separated by a piece of paper (e) and the glass jar was properly closed.*

After the analysis of vapors by GC-MS, the solid dynamite specimens were removed for the extraction of EGDN using the method explained above. Finally, aqueous and methanolic extracts were analyzed in triplicate by HPLC-DAD-MS in order to evaluate cross-contamination between explosive specimens kept in official plastic bags and stored in the same place.

LOD of the GC-MS, HPLC-DAD and HPLC-MS systems was calculated for a signal-to-noise ratio of 3:1. For this study, nine standard solutions of 2,6-DNT in methanol (2000 ng/mL, 1000 ng/mL, 500 ng/mL, 300 ng/mL, 100 ng/mL, 60 ng/mL, 12 ng/mL, 6 ng/mL, and 3 ng/mL) were injected into the chromatographic systems. The lowest concentration was used to calculate the LOD value.

3. Results and discussion

3.1. Study of the dynamics of EGDN loss from Goma-2 ECO during the storage of the dynamite

This study was devised in order to achieve a better understanding of the dynamics of the volatilization of EGDN from explosive materials, more specifically, Goma-2 ECO dynamite. As can be seen in **Figure I.3.2**, this study offers evidence that the loss of EGDN from the dynamite studied was progressive over time. In the short-term study and the long-term study performed at room temperature, the behavior of EGDN loss as a function of time was very similar: EGDN content diminished in a square-root-like way, according to the equations of the models that best fitted the experimental data (**Table I.3.2**).

It is worth noting that after approximately one month of storage, nearly 30% of this dynamite's EGDN content was lost, a figure which increased to 66% in two months.

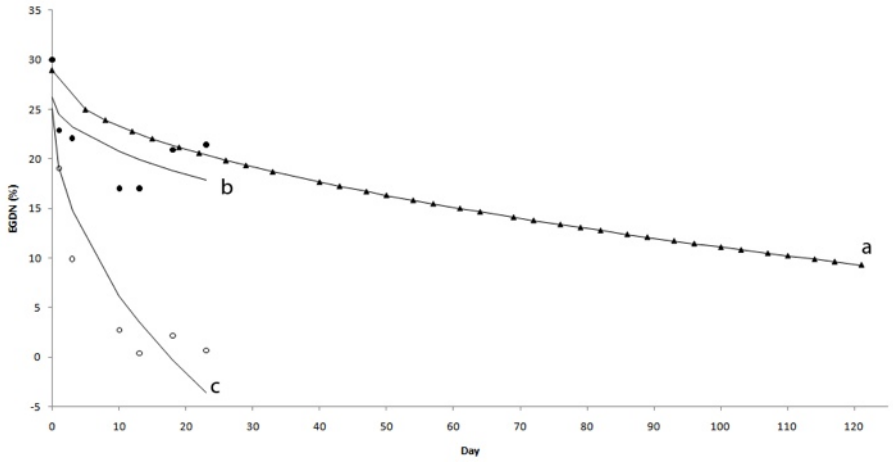

Figure I.3.2*. Evolution over time of EGDN content (%) in specimens of Goma-2 ECO stored in polyethylene bags. (a) Long-term study during 121 days at room temperature, (b) short-term study during 24 days at room temperature, (c) temperature study during 24 days at 60 ºC.*

Table I.3.2. *Best fitted equations to describe the loss of EGDN from a dynamite Goma-2 ECO.*

Study	Mathematical model	Correlation
Short-term study (room temperature)	$EGDN\ lost\ (\%) = 26.26 - 1.74 \times \sqrt{t\ (days)}$	-0.7079
Long-term study (room temperature)	$EGDN\ lost\ (\%) = 28.97 - 1.79 \times \sqrt{t\ (days)}$	-0.9377
Short-term study (60 ºC)	$EGDN\ lost\ (\%) = 25.14 - 5.99 \times \sqrt{t\ (days)}$	-0.9359

As was to be expected, in the short-term study carried out at higher temperature, a drastic loss of EGDN over time was observed (the square-root decay with time of EGDN content was, approximately, three times higher than in the previous studies). From all these data we may conclude that the EGDN content of Goma-2 ECO diminished substantially during the storage of this dynamite in a polymeric bag, at room temperature, the main reason for this being the easy diffusion of this compound through the pores of the packaging and its subsequent loss in the environment.

3.2. Study of losses of volatile compounds (EGDN and DNT) from two different dynamites stored in official plastic bags

The volatilization of both EGDN and DNT contained in two dynamites put into the official polyethylene bags used by the Spanish Security Forces to transport and store evidence specimens has been investigated. This study involves, firstly the analysis, at different times, of EGDN and DNT in the headspace of the jars containing the bags and, secondly, the determination of the EGDN concentration remaining in the dynamites. **Figure I.3.3** shows the chromatogram corresponding to the headspace of one glass jar, analyzed after one week of storage. It shows the peaks corresponding to EGDN and two isomers of DNT (2,6-DNT and 2,4-DNT). It is interesting to note that the LOD for DNT, to be precise, for the 2,6-DNT isomer, is 1.0 mg/mL, while the concentration of DNT isomers detected in the headspace of the jars is much higher than the LOD concentration.

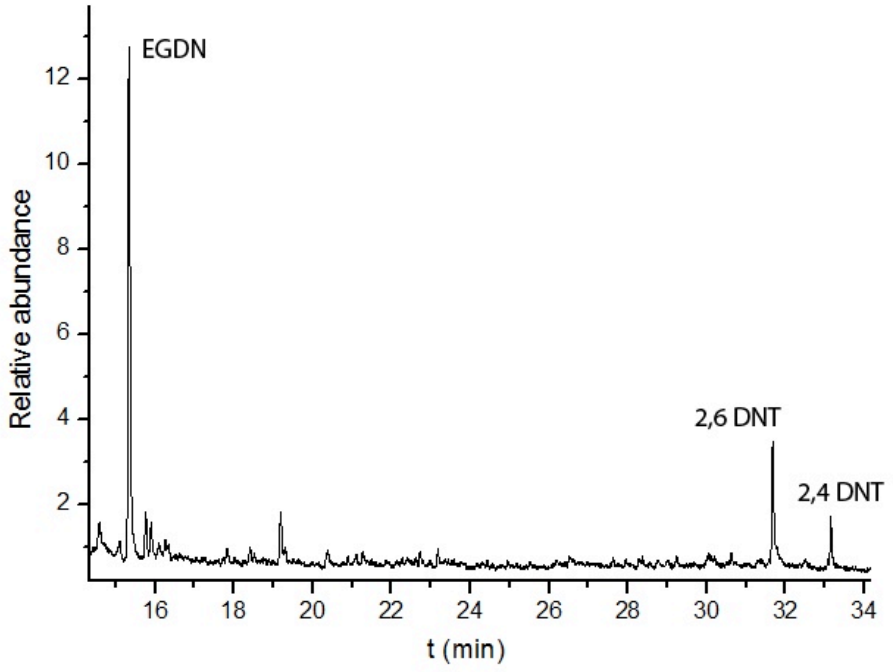

***Figure I.3.3**. GC-MS chromatogram of the headspace of a jar containing one polyethylene bag with a specimen of Goma-2 ECO and one polyethylene bag with a specimen of Goma-2 EC, after one week of storage. DNT, dinitrotoluene.*

According to these results, these storage bags allow the quick loss of volatile compounds. This leads to changes in the composition of the specimen during its storage time and may give rise to serious errors in the interpretation of the results obtained from explosive specimens in a given forensic case. Although the mechanism by which these volatile compounds are lost from the bags remains unclear, the main causes of this process may be the sealed areas of the bags and/or the pores in the polyethylene polymer bags. However that might be, this study demonstrates that the use of polyethylene bags for the transport and

storage of explosive evidence requires stringent oversight if cross-contamination due to volatile components is to be avoided.

3.3. Study of cross-contamination between explosive evidence stored in official plastic bags in a common space

This study has considered three different dynamites (Goma-2 ECO, Goma-2 EC, and Titadyn), which differed in their volatile components. According to **Table I.3.1**, a component common to all of them is EGDN. Goma-2 EC and Titadyn also have DNT in common, while only Titadyn contains NG. Since DNT is present in Goma-2 EC dynamite but not in Goma-2 ECO dynamite, the presence of DNT in this last dynamite was studied to evaluate the possibility of cross-contamination between these two dynamites. However, at the time of the study, dynamites containing both DNT and NG in their composition (like Titadyn, see **Table I.3.1** for further information) were not available. As a consequence, Goma-2 ECO and Goma-2 EC dynamites were placed in polyethylene bags stored in the confined space provided by glass jars hermetically closed. The evolution of EGDN and DNT content in both specimens over time was monitored by extracting these components with methanol and water, as described by Sáiz et al.,[19] and analyzing them by means of HPLC.

First of all, a comparison of the sensitivity of the two detection systems in HPLC was carried out to determine which was the best suited to detecting trace amounts of DNT. For DAD detection, the LOD for 2,6-DNT, the isomer mainly detected, were 300 ng/mL while, for MS detection, the LOD corresponding to 2,6-DNT was lower than 3 ng/mL; in other words, MS detection permitted the detection of

concentrations more than 2 orders of magnitude lower than DAD detection. Next, HPLC-MS was selected for the analysis of the extracts. The highest peak areas of volatile compounds in the extracts were always found in the methanolic phases, while their concentration in water was almost negligible. As a consequence, the methanolic extracts obtained by the extraction method were injected into the HPLC-MS system.

Figure I.3.4 shows the chromatograms obtained for the methanolic extracts of Goma-2 ECO dynamites before starting the study (**Figure I.3.4a**) and after one, two, three, and four weeks of storage in the conditions described above (**Figure I.3.4b-e**).

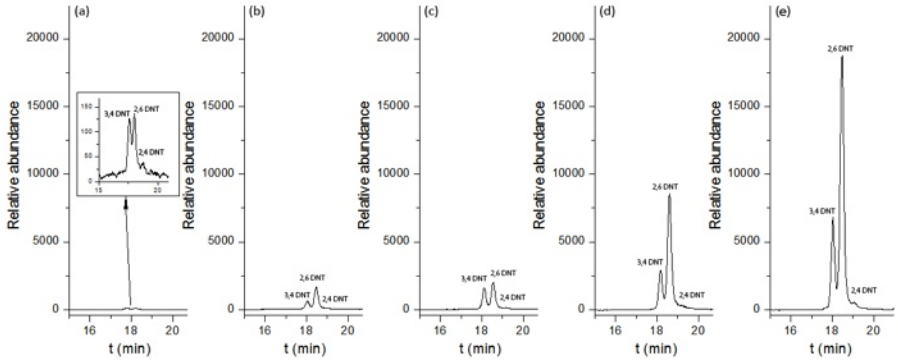

Figure I.3.4*. HPLC chromatograms of methanolic extracts of Goma-2 ECO specimens (a) before starting the study and after (b) one, (c) two, (d) three, and (e) four weeks of storage in polyethylene bags kept in hermetically closed glass jars containing also Goma-2 EC in polyethylene bags. DNT, dinitrotoluene.*

Curiously, in **Figure I.3.4a**, the chromatographic peaks of DNT, close to the LOD (3 ng/mL), were observed. This means that Goma-2 ECO specimens were contaminated before starting the study with trace amounts of DNT. For this reason, contamination should not be attributed only to the laboratory atmosphere because the specimens had been stored in the glass jars as soon as they had been received in the laboratory. The contamination probably begins during the manufacturing process of the explosive. This hypothesis was supported by two facts: the same reactors were used to make both Goma-2 EC and Goma-2 ECO dynamites; and cross-contamination during the storage of the dynamites in the warehouse must not be disregarded.

On the other hand, **Figure I.3.5** also shows how DNT peak areas in Goma-2 ECO dynamite grew over storage time. This implies that some transference of DNT from Goma-2 EC to Goma-2 ECO specimens occurred. Hypothetically, this transfer could occur in three stages. The first could be the volatilization of DNT from Goma-2 EC dynamite and its output through the plastic bag where it is kept in to the common space (jar compartment). The second stage could be the entrance of DNT into the interior of the bag containing the Goma-2 ECO dynamite. Finally, DNT could be retained by this dynamite, either by adsorption or some other process, given the strong affinity of this material towards volatile compounds.

Figure I.3.5 shows the evolution of DNT peak areas (calculated as the sum of the total DNT peak areas corresponding to 2,4-DNT, 2,6-DNT, and 3,4-DNT isomers in the aqueous and in the methanolic phases) in Goma-2 ECO and Goma-2 EC specimens.

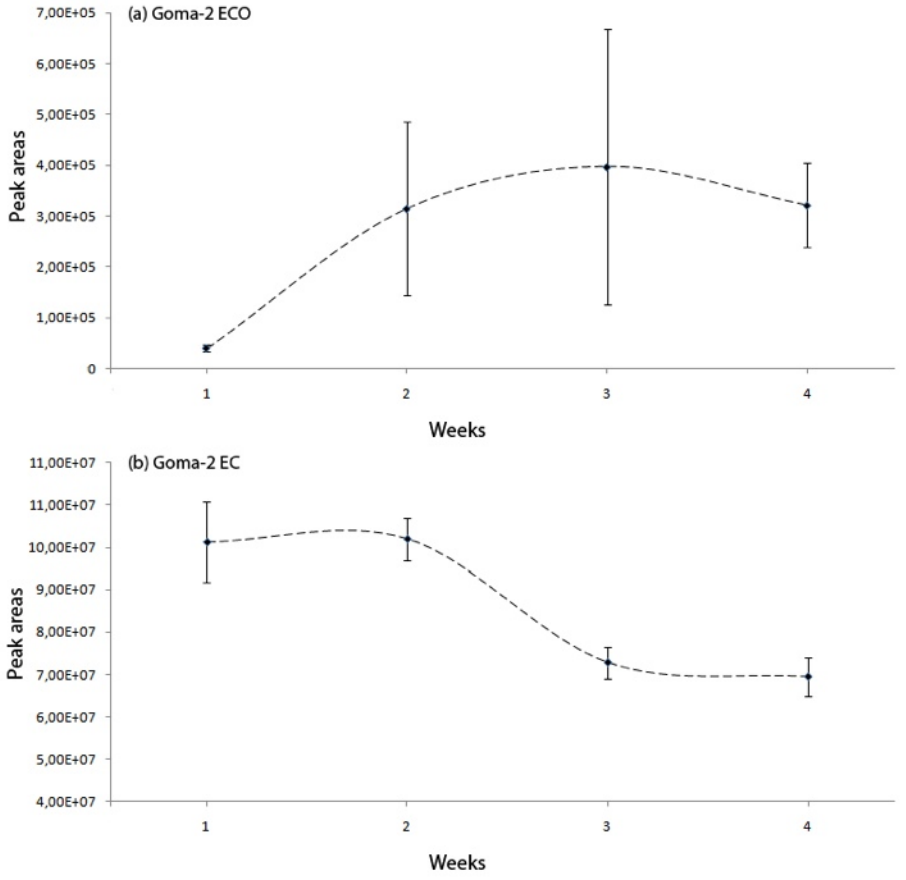

***Figure I.3.5**. Averages and standard deviations of DNT peak areas (sum of the total DNT areas corresponding to 2,4-DNT, 2,6-DNT, and 3,4-DNT isomers in the aqueous and methanolic extracts obtained from dynamites) in the dynamites stored during one, two, three, and four weeks in a common place (jars).*

An increase in DNT peak areas in Goma-2 ECO dynamite to the detriment of the DNT peak areas in Goma-2 EC dynamite was observed. Furthermore, the DNT peak area in Goma-2 ECO and in

Goma-2 EC dynamites reached a stationary state in which Goma-2 ECO seemed unable to retain more DNT while Goma-2 EC seemed to stop the DNT loss. Meanwhile, the high standard deviation values obtained for the two individual specimens analyzed (each by triplicate) may be explained because: a) it is impossible to standardize the manipulation of the bags, the different folding of each of which affected the area of the bag exposed to the environment and the contact area of the dynamite with the bag; b) the heterogeneity of the specimens, which affects the amount of DNT in Goma-2 EC specimens and the capacity to absorb DNT by Goma-2 ECO dynamite; and c) the DNT content in Goma-2 ECO dynamite measured during the study was 2-3 orders of magnitude smaller than that measured in Goma-2 EC dynamite, thus leading to higher relative standard deviation values for Goma-2 ECO in comparison with Goma-2 EC.

The results obtained in this work demonstrate that polyethylene bags used by Spanish Security Forces are not ideal systems for transporting and storing explosive specimens because they do not offer a physical barrier for some volatile molecules present in explosives, which can enter and leave the bags easily. In addition, the high capacity of other explosive materials to retain these molecules is a significant factor. Therefore, the chances of cross-contamination between explosive specimens stored in polyethylene bags in a common space are very high in a short period of time.

4. **Conclusions**

In this work the capacity of the polyethylene bags used by the Spanish Security Forces to preserve explosive evidences has been investigated. GC-MS results showed that the volatilization of EGDN and DNT from dynamites occurs gradually over time, happening faster at higher temperatures. The mechanism by which these volatile molecules may leave the bags is still unclear and further investigations are needed.

In addition, the results obtained by HPLC-DAD-MS demonstrated cross-contamination of volatile compounds between dynamites stored in the same small closed space. In fact, DNT was found in Goma-2 ECO dynamite while simultaneously the concentration of this compound decreased in Goma-2 EC dynamite. This process continues over the storage time until a stationary state is achieved. As a result, this work demonstrates that:

a) Polyethylene bags are not ideal for transporting and storing specimens of dynamites at room temperature. Since the volatilization process diminishes at low temperatures, the storage of explosive specimens in refrigerated chambers or refrigerators is recommended. Another solution could be the substitution of bags by other kinds of packaging containers;

b) Cross-contamination, which has an important impact on the interpretation of results, should be taken into account when explosive specimens have been stored in the same room at room temperature;

c) Further studies are required to collect information about the loss of volatile compounds under the proposed conditions.

Acknowledgements

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CHAPTER I.4

STUDY OF THE SUITABILITY OF DUO PLASTIC BAGS FOR THE STORAGE OF DYNAMITES

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Forensic Sci. Int., submitted, April 2013.

ABSTRACT

A comparative study on the retentiveness of two plastic bags (DUO and Royal Pack) has been carried out by gas chromatography with mass spectrometry detection. Two types of dynamites were packed in both plastic bags. The bags were placed into glass jars and headspace analyses were performed over 11 weeks to detect whether the volatile constituents of the dynamites were released from the bags. DUO plastic bags showed much better retentiveness than Royal Pack plastic bags. EGDN was quickly detected in the headspace of the glass jars containing Royal Pack plastic bags after 1 week of storage. On the contrary, only a weak signal of EGDN, which was not detectable in the total ion chromatogram, was detected after 11 weeks of storage. Moreover, DUO plastic bags have shown less background signals than the Royal Pack bags, being the former bags much more suitable for the storage of dynamites.

1. **Introduction**

Packaging for evidence must be convenient to store and transport evidential items to and from the crime scene. It must be available in a range of sizes, water resistant, and easy to seal. Packaging for evidence with volatile compounds must be also effective retaining vapors.[1] Metal cans, glass jars, and plastic bags are the containers more frequently used as packaging for fire debris and explosive evidence.[1,2] However, the perfect container does not exist and every container shows advantages and disadvantages. The advantages and disadvantages of metal cans and glass jars are discussed in the second edition of Practical Fire and Arson Investigation.[3] Regarding plastic bags, as they are transparent it is possible to see the contents without opening the bags, they can accept larger pieces of evidence than cans or jars, may easily be sealed at the site of collection, and they are available in a wide range of sizes.[2] However, plastic bags may allow losses of trace amounts of volatile compounds, are afflicted with background interferences, and can be damaged by sharp items.[2-5]

Retentiveness in packaging is essential since it avoids losses of information in the evidence. In recent years, there has been a growing interest in the study of the suitability of certain plastic bags commonly used for the storage of specimens with volatiles, either fire debris, accelerants, explosives, or other specimens.[1-7] The retentiveness of a bag should be studied for the kind of bag used and the kind of evidence stored. The users need to know the properties for an specific container and the risks for the evidence storage in order to treat properly the sample collection and storage. Recently, Grutters and col.[7] studied the DUO bags for the storage of fire debris in terms of background volatiles, leak rate, cross-contamination, recovery, and

sorption. The authors infused a filter paper with commercial gasoline inside the bags and stored them for 56 days. They observed that, within one day, the bags permitted the leakage of volatiles in an amount less than 1% of the gasoline stored. This amount increased up to more than 3% in some compounds within 14 days.

After an important terrorism case in which the cross-contamination of the samples was suspected, our research group studied the retentiveness of the Royal Pack plastic bags used by the Spanish Security Forces to transport and store specimens that are intended for forensic analysis. Our conclusion was that those bags were not ideal for transporting and storing specimens of dynamites since they allowed dynamite-to-dynamite cross-contamination with volatiles in a easy and fast way.[4] Previously the Spanish Security Forces changed them by DUO plastic bags, supposed to show better retentiveness for volatile compounds. The aim of this work was to compare both plastic bags and to study whether DUO bags were really suitable for the storage of dynamites containing highly volatile compounds.

2. **Material and methods**

2.1. Samples and materials

Specimens of Goma-2 ECO and Goma 1 were kindly provided by MaxamCorp Holding, S.L. (Madrid, Spain). The compositions of these dynamites are shown in **Table I.4.1**.

***Table I.4.1**. Composition, indicated by the manufacturer, of Goma-2 ECO and Goma 1.*

	Goma-2 ECO	Goma 1
EGDN	✓	✓
TNT		✓
Nitrocellulose	✓	✓
Calcium carbonate	✓	
DBP	✓	
Ammonium nitrate	✓	✓
Others	✓	✓

The plastic bags used by the Spanish Security Forces to transport and store evidence specimens in forensic cases were provided by the Criminalistic Service of the Guardia Civil. The Royal Pack plastic bags were made from polyethylene, have a rubber sealing and were supplied by Royal Pack S.L. (Alcalá de Henares, Spain). The DUO plastic bags were made from nylon/polyethylene must be heat-sealed and were supplied by Duotec A/S - Handels & Ingeniørfirma (Copenhagen, Denmark).

The glass jars used for GC studies were provided by the Criminalistic Service of the Guardia Civil and had a security seal, which ensures that the glass jars had not been previously opened.

Specimens were weighted using a MC210P balance (Sartorius, Götingen, Germany) with an accuracy of ± 10 μg and a precision of ± 20 μg.

2.2. Instrumentation

GC coupled with mass spectrometry (GC-MS) experiments to study the headspace of the glass jars were performed in an Agilent Technologies 6890/5973 gas chromatography/mass selective detector system (Pittsburgh, PA, USA). A solid-phase micro-extraction fiber (75 μm CarboxenTM PDMS) was used for the extraction in the headspace (30 min at room temperature). The injection was carried out at 250 ºC working in a pulsed splitless mode at 8.98 psi. Helium was used as the carrier gas and a HP-1MS (100% dimethylpolysiloxane) 30 m x 250 μm x 0.50 μm column was employed. The separation method consisted in a three-step gradient from 70 ºC to 270 ºC over 32 min: first, 70 ºC for 1 min; second, an increase of 4 ºC/min to 130 ºC; and third an increase of 10 ºC/min to 270 ºC. The total flow was 23.9 mL /min, the purge flow to split vent was set at 19.9 mL /min and the flow of the gas saver was 20 mL /min. Mass spectrometry detection settings were: MS Source Setpoint, 230ºC; MS Quad Setpoint, 150 ºC; electron multiplier voltage at 400 V; time window, 10 min; scan in the range m/z = 20-600 amu. Data were acquired and processed using the MSD Chemstation Build 75 software (Agilent Technologies).

2.3. Experimental procedure

The study of the loss of volatile compounds from the two types of plastic bags was carried out in a long term study for 11 weeks. **Figure I.4.1** shows an image of the experimental design. The design was similar to that in our previous publication.[4] Two series of glass jars suitable for GC headspace analysis were prepared. The good condition of the seal was checked in all the glass jars and they were

only opened once to introduce the specimens and then were closed and stored until their analysis. In the first series of glass jar, two Royal Pack plastic bags were introduced, one containing 250 ± 10 mg of Goma-2 ECO, and the other one containing 250 ± 10 mg of Goma 1. The other series of glass jars were prepared in the same way, but using DUO plastic bags. DUO plastic bags were double heat-sealed.

***Figure I.4.1**. Image of the experimental design showing a set of two glass jars, one containing two Royal Pack plastic bags (a) and the other one containing two DUO plastic bags (b). A bag containing Goma 1 and a bag containing Goma-2 ECO were contained together inside the same glass and stored.*

A total of 20 glass jars were prepared, which were stored until their analysis at room temperature. Two glass jars of each series were analyzed each day of analysis. Four glass jars more were prepared, two with the DUO plastic bags and the other two with the Royal Pack

plastic bags, without dynamites inside. Those glass jars were analyzed the first day as blank samples in order to know the background signal of the bags. Empty glass jars were also analyzed as blank samples in order to know the background signal of the glass jars. The 20 glass jars stored were analyzed after one, two, four, seven, and eleven weeks of storage at room temperature. After this study, the glass jars of the last week were subjected to heating, first for 2 h at 50 ºC and then for 24 h at 50 ºC in order to simulate the aging of the bags.

3. Results and discussion

The retentiveness of DUO plastic bags was studied in comparison with the Royal Pack plastic bags. The study involved the analysis of volatiles from dynamites in the headspace of the glass jars containing the bags at different times, during 11 weeks. Two different dynamites, named Goma 1 and Goma-2 ECO, were used. **Table I.4.1** shows the compositions of Goma-2 ECO and Goma 1. The most volatile compounds in these dynamites are EGDN and TNT. EGDN posses a vapor pressure of 2.8 x 10^{-2} Torr at 25 ºC and TNT of 7.1 × 10^{-6} Torr at 25 ºC.[8] Both dynamites were distributed in the plastic bags according to the experimental design and stored.

After 1 week of storage, EGDN was detected in the headspace of one of the glass jars containing the dynamites in the Royal Pack bags, as is shown in **Figure I.4.2**. The detection of EGDN agrees with the results of our previous study in which EGDN was also detected in the headspace of the glass jars after only 1 week of storage. **Figure I.4.2** shows the comparison of two chromatograms: one for the headspace of the jar in which EGDN was detected and the other one for the

headspace of one jar containing dynamites inside DUO bags. It can be noted that the background interferences of the Royal Pack bags were bigger than the background signal in the DUO bags. As can be seen in **Figure I.4.2**, EGDN contain characteristic ions at m/z 30 (NO^+), 46 (NO_2^+), and 76 ($CH_2ONO_2^+$). However, EGDN does not show molecular ions.[9]

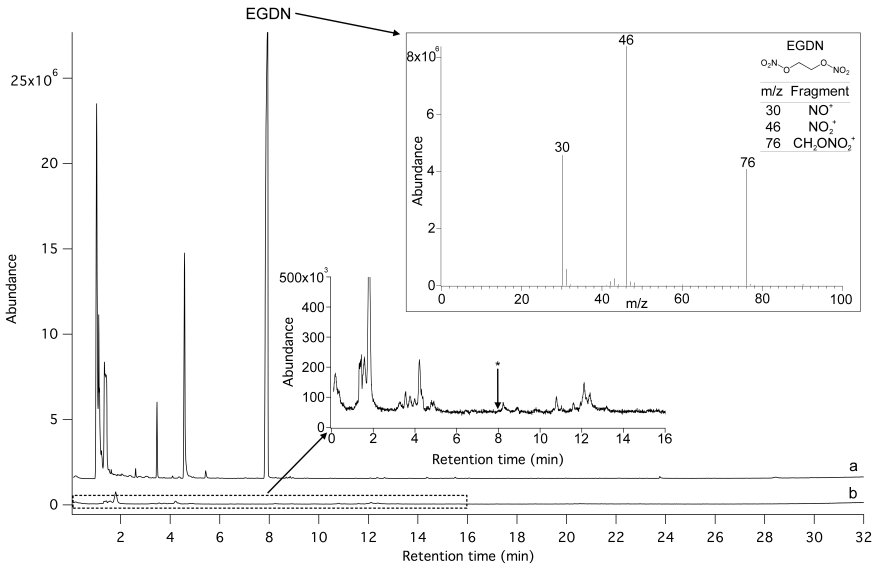

***Figure I.4.2**. GC/MS chromatograms for the headspace of glass jars containing (a) Royal Pack plastic bags and (b) DUO plastic bags after 1 week of storage. EGDN was only detected in Royal Pack plastic bags. EGDN characteristic MS spectra with its typical fragments is also shown.*

After the second week, EGDN was detected in the headspace of all the glass jars analyzed containing the Royal Pack bags. **Figure I.4.3** shows the average peak areas of EGDN with the standard deviation of the measurements in the long term study (11 weeks) for each set of glass jars.

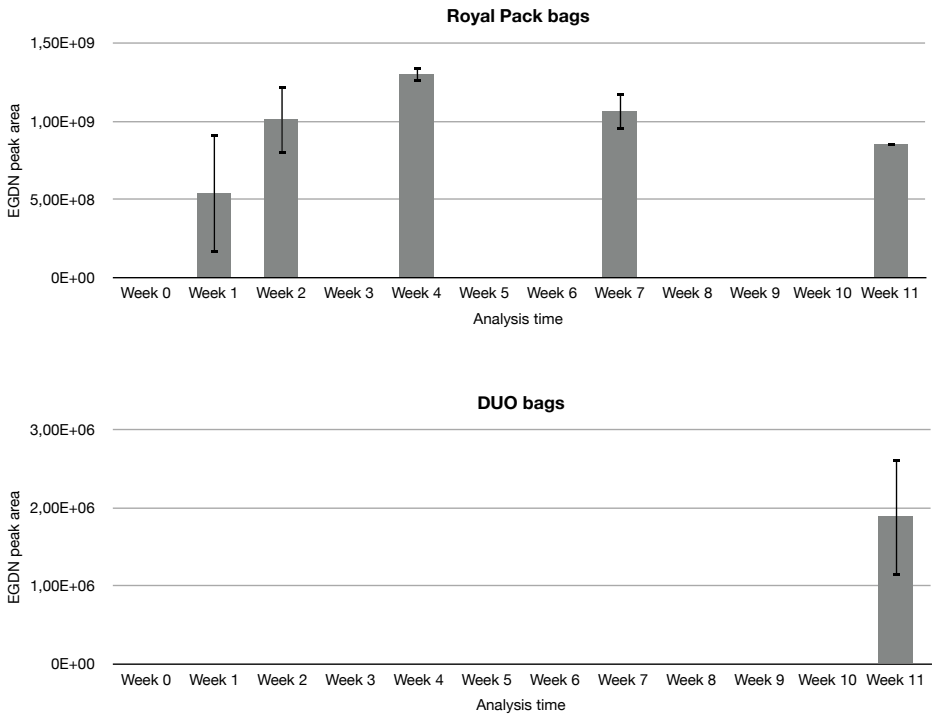

***Figure I.4.3**. Averages and standard deviations of EGDN peak areas (sum of the areas corresponding to the characteristic ion fragments NO^+ (m/z = 30), NO_2^+ (m/z = 46), and $CH_2ONO_2^+$ (m/z = 76) of EGDN) of two independent samples in the glass jars stored during one, two, four, seven, and eleven weeks. Note the different scales on the y-axis.*

On the other side, the DUO plastic bags are, apparently, more suitable than the Royal Pack plastic bags for the storage of dynamites with highly volatile compounds, such as EGDN. No EGDN peaks were detected during the first 7 weeks of study (see **Figure I.4.3**). Note that the small peak after the minute 8 does not correspond to EGDN but a hydrocarbon. However, a weak signal of EGDN was detected after 11

weeks of storage in the two glass jars stored when the characteristic ions were extracted in the extracted ion chromatogram (EIC) but no signal for EGDN was detected in the total ion chromatogram (TIC). Those signals were under the limit of detection in the TIC. **Figure I.4.4** shows the TIC and the EICs for the ions at m/z 30, 46 and 76, for the headspace of a glass jars at the week 11, showing the weak signal of EGDN detected.

TNT was not detected in the headspace of any of the 20 glass jars analyzed throughout the 11 weeks of study. The vapor pressure of TNT was not high enough to be released from the bags. In order to force the output of TNT from the bags, the last 4 glass jars were subjected to heating in a temperature study, although the heating of the bags was not recommended by the manufacturer. The 4 glass jars were firstly heated for 2 h at 50 ºC and then for 24 h more at 50 ºC. This study was carried out immediately after analyzing the samples of the week 11. **Figure I.4.5** shows how, after heating at 50 ºC for 2 h and 24 h, similar contents to those in **Figure I.4.4** were observed. That is, heating had not effect in the concentration of EGDN neither in the glass jars containing dynamites in Royal Pack bags nor in those using DUO plastic bags. Then, we can state that the heating of dynamites that have been stored for a long time had not a significant effect on the release of TNT from the studied bags.

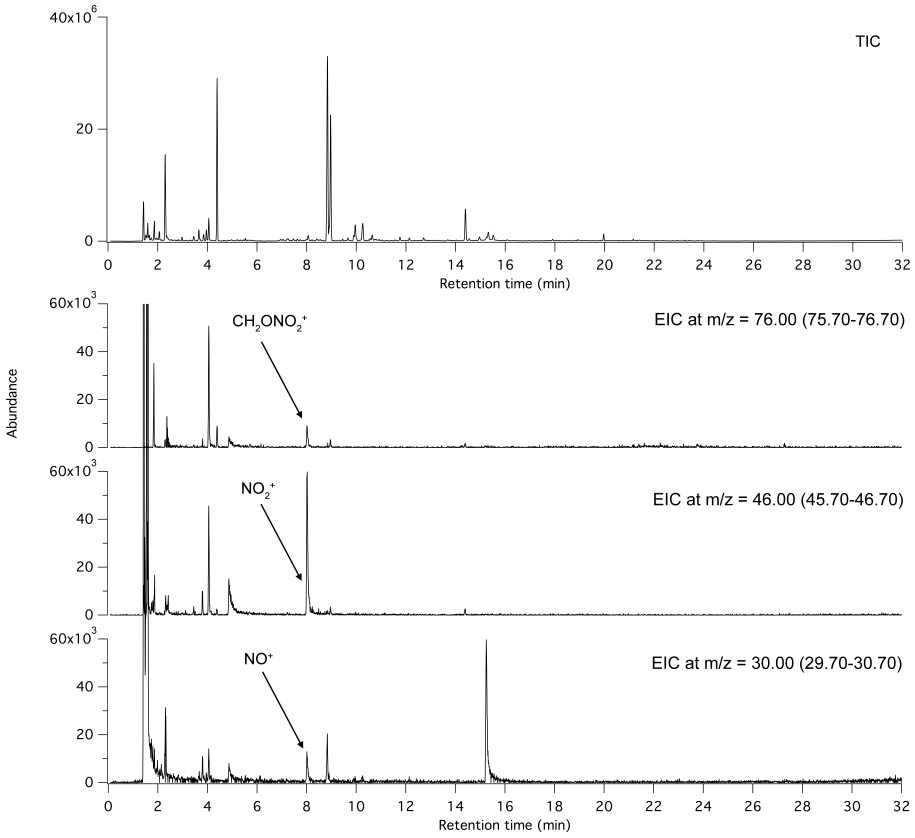

Figure I.4.4*. GC/MS chromatograms for the headspace of a glass jar containing the dynamites inside DUO plastic bags after 11 weeks of storage. The TIC did not show an recognizable EGDN peak. However, the EIC with the extraction of the characteristic EGDN fragments at m/z = 30, 46, and 76 revealed the presence of EGDN in the headspace of the glass jar.*

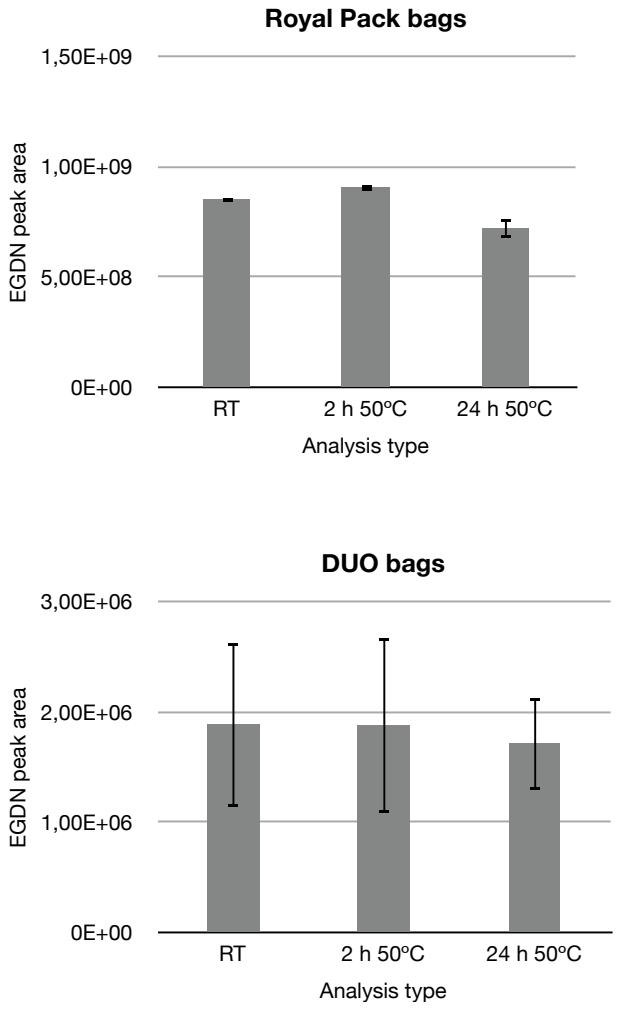

***Figure I.4.5**. Averages and standard deviations of EGDN peak areas (sum of the areas corresponding to the characteristic ion fragments NO^+ (m/z = 30), NO_2^+ (m/z = 46), and $CH_2ONO_2^+$ (m/z = 76) of EGDN) of two independent samples stored for 11 weeks in the glass jars after heating at 50ºC for 2 h and 24 h. Note the different scales on the y-axis.*

DUO plastic bag has been proved to show much better retentiveness for the volatile constituents present in dynamite evidence than the Royal Pack plastic bags. However, plastic bags may be used as packaging for many other kind of evidence, such as accelerants, fire debris, or drugs, and therefore, they should be studied depending on the kind of evidence as well.

4. **Conclusions**

DUO plastic bags have shown much better retentiveness than Royal Pack plastic bags for the volatile dynamite constituents of the dynamites studied. Only a weak signal of EGDN was detected after 11 weeks of storage. DUO bags have also been proved to show less background signal than Royal Pack plastic bags. The temperature study showed that the heating does not affect the release and leakage of volatile compounds of the dynamites from the plastic bags. Ultimately, the authors recommend the employment of DUO plastic bags, instead of Royal Pack plastic bags, for the proper storage of dynamites at least for 11 weeks. However, we suggest the study of the retentiveness of the bags when storing other kind of volatile evidence.

Acknowledgements

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PART TWO

PORTABLE CAPILLARY ELECTROPHORESIS

CHAPTER II.1

INTRODUCTION

Miniaturization and portability are closely related terms which have become important trends in modern society. Nowadays, small and portable devices can be found in different areas such as medicine, telephony, computer science, astronomy, music, or gaming. In chemistry, portability of analytical instrumentation shows several advantages in comparison with the commonly used bench-top instrumentation. T. Kappes and P. C. Hauser summarized these advantages in 1998.[1] Among them, the most important advantage is that these instruments are independent from the laboratory infrastructure and, therefore, can be operated at any place, preferably as close as possible to the point where the sample is taken. This gives two additional advantages. On the one hand, the degradation of the sample during its transport and storage is avoided, removing the need for sample preservation. On the other hand, as the data are immediately available, it is possible to make decisions readily at the place of sample collection. Moreover, portable analytical instrumentation reduces number of the necessary samples and therefore reduces cost and time spent.[1] As a general rule, a portable object is defined as "easily movable, convenient for carrying, and capable of being transferred or adapted in altered circumstances".[2] Moreover, it must me able to be transferred and placed in any altered location. In terms of scientific instrumentation, a portable instrument can be used outside the laboratory and does not need to be connected to the main power. Besides, it must be miniaturized in some way and easily to be placed at the site of analysis.[3]

Many efforts have been made to develop portable analytical instruments in the recent years and several techniques have been miniaturized. The most commonly commercialized portable instruments are X-Ray fluorescence analyzers, GC combined with MS detectors, HPLC with UV detector, ion mobility spectrometers, near infra-red spectrometers, and Raman spectrometers.

On the other hand, there are many research groups with a number of publications on the development of miniaturized techniques. I could highlight the ultra-small and lightweight ion chromatograph with contactless conductivity detector recently published by I. K. Kiplagat.[4] A high effort has been made on the development of instruments of capillary electrophoresis (CE) and microchips CE. This is because only a capillary or a microchip and a high voltage (HV) source is required for separation. Moreover, the consumption of sample and buffer solution is negligible.[5] However, microchips are expensive in comparison to the silica capillaries used in traditional CE. Nevertheless, despite the benefits of CE, the industry of portable CE (P-CE) is almost inexistent.

Concerning portable capillary-based (non-microchip) CE, the first instrument was reported in 1998 and was built by T. Kappes and P. C. Hauser.[1] The instrument consisted on a PVC box of 240 x 175 x 175 mm with a total weight of 7.5 kg (**Figure II.1.1**). The instrument was fitted with a HV supply able to provide up to 30 kV in both negative and positive polarities. The instrument could work up to 5 h with two lead acid batteries, depending on the working conditions. This P-CE had a purpose-built potentiometric detector, which was connected to the computer through an analog-digital convertor with a RS232 connector. Subsequently, the instrument was upgraded with an

amperometric[5] and a conductimetric[6] purpose-built detectors. In that way, the most appropriate detector could be chosen depending on the analytes of interest. It can be noted that this first P-CE relied on electrochemical detectors. Moreover, in the latest work, the recently reported capacitively contactless conductivity detector (C^4D) was implemented as the most versatile detection technique applicable, specially for small inorganic ions. Optical absorption detection was not chosen because it is not ideal for a portable instrument as the power consumption of the required lamps is too high.

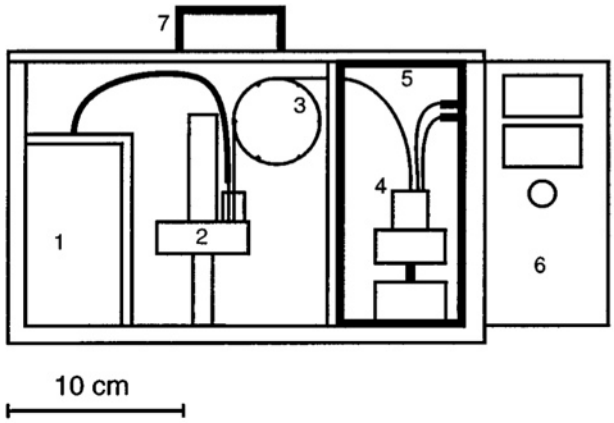

***Figure II.1.1**. P-CE designed by T. Kappes and P. C. Hauser.[1] 1, HV supply; 2, turntable for sample vials; 3, capillary rolled on spool; 4, detection cell with electrical connections for detector electrode and ground; 5, Faraday cage; 6, electronics case; 7, handle.*

In 1998, Gerhardt and col.[7] built an elegant miniaturized 42 × 76 × 101 mm P-CE. It counted with an addressable rotary tray with a vial lifting for the automated selection of the inlet vial. As in the previous system, end-capillary electrochemical detection was used, in this case voltametric and amperometric detection.

In 2007, J. P. Hutchinson and col.[8] published the first work in which the CE-P2 (**Figure II.1.2**), the first commercially available P-CE, was used. This instrument has the dimensions of 320 x 230 x 150 mm, a weight of about 8 kg and can work continuously for 3 h. It can be operated connected to the main power. The system allows both the hydrodynamic (from 0 to 25 psi) and the electrokinetic injection and it is able to work at 15 kV in negative and positive polarity. The P-CE can be connected to the computer throughout RS232, USB and TCP/IP ports and can be fitted with most of the commercially available detectors.

***Figure II.1.2**. The CE-P2, the first commercially available P-CE. CE Resources Pte Ltd.*

In 2009, A. Seiman and col.[9] developed a new P-CE. The dimension of this instrument were 330 x 180 x 130 mm and the weight less than 4 kg. It had two HV supplies of +25 kV and -25 kV and could work in a continuous mode for 4 h. The P-CE was fitted with a purpose-built C^4D. The connection with the computer was through a RS232 port.

The injection was carried out manually, by using a syringe, in an interface called "cross-sampler" (**Figure II.1.3**).

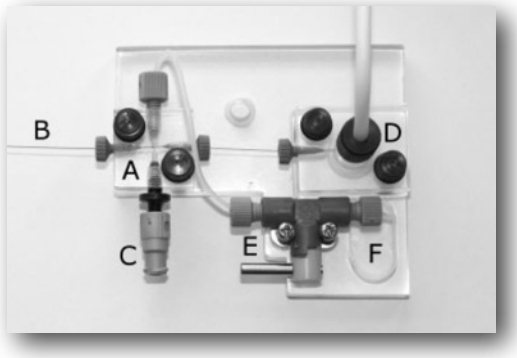

***Figure II.1.3**. Cross-sampler. A, cross- sampler; B, separation capillary; C, socket for syringe; D, buffer vessel with electrode lead; E, shut-off valve; and F, waste reservoir.*

The cross sampler consisted of a piece of PMMA with two perpendicular channels. Two capillaries were fitted in one channel, slightly separated in the cross point. The distance between both capillaries determined the volume of sample to be injected. The introduction of the sample in the separation capillary was carried out by the effect of the electro-osmotic flow (EOF), generated when the HV supply was turned on. The sample delivery was carried out manually, and the authors recommended the use of internal standards to overcome the problems derived of the instability of the manual flushing injection.

The same group developed a digital microfluidic sampler for the P-CE the same year.[10] The injection was carried out by immersing the capillary in drops of sample, which were grounded, applying voltage.

The separation took place between another grounded drop of buffer and the outlet vial. One year later, in 2010, the same research group published an article in which three types of injectors were compared.[11] The previously described "cross-sampler"[9] was among them. The other two injection systems varied in the length and position of the channels. The study concluded that the "cross-sampler" was the most appropriate injector for field work, because less manipulation was required. However, no quantitative studies were carried out because none of the three devices provided a good reproducibility of the peaks area.

In 2010, M. Lee and col.[12] presented a new P-CE of 440 x 270 x 130 mm and 8 kg. It was the first P-CE with a LIF detector system built in the laboratory. It was controlled using LabView 8.2 through a USB connector. The sample injection was carried out manually with a syringe and a HV supply allowing to work between 0.01 and 10 kV was used for the separations.

In 2011, P. Kubáň and col.[13] developed a new 300 x 300 x 150 mm and 5 kg P-CE with a HV supply capable of working at -25 kV. The capillary endings were introduced in two polyimide interfaces. These interfaces design were based on CE microchips and were used to control the sample injection and buffer introduction in the capillary. The flow of the sample/buffer, which was carried out manually, could be regulated using a valve placed at the interface outlet and thereby control the different operation modes (sample injection, buffer delivery into the interface or buffer introduction into the capillary).

The above-summarized works used P-CE instrumentation and are compiled in two reviews reported by A. P. Lewis[14] and M. Ryvolová[3].

Other works based on microchip CE were collected in the review reported by A. P. Lewis[14] which are out of the scope of this thesis.

It could be inferred that many efforts have been made on the improvement of several common elements of P-CEs. Here, the detection, the injection system, the autonomy provided by the batteries, and the total weight and dimensions of the instrument have been studied in the search of a global performance improvement.

Concerning the detection, low-power consuming systems were employed in order to prolong the system autonomy. Electrochemical detectors, such as voltametric, amperometric, potentiometric, and conductimetric detectors, have been mostly used. Those based on end-capillary ("off-column") detection systems present two well known problems. The first problem is the corrosion of the electrodes, since they are in direct contact with the solution. The second problem is the band broadening created due to dead volumes between the end of the capillary and the measuring electrode. Contactless conductivity detection has been stablished as a good alternative to the end-capillary detection techniques because it overcomes those above-mentioned problems. C^4D employs electrodes arranged along the axis of the fluid channel, being the fluid channel a capillary in traditional CE or a engraved channel in microchip CE (see **Figure II.1.4**). Further information about the principle of C^4D can be found in the review reprinted by P. Kubáň and P. C. Hauser.[15] This configuration allow the actuator electrode and the pick-up electrode not come in contact with the solution and they are free of any potential interference from the electrophoretic separation voltage. Therefore the electrodes do not corrode.

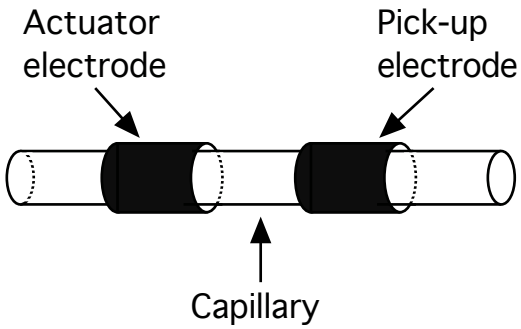

***Figure II.1.4**. Schematic representation of the arrangement of the actuator and the pick-up electrodes to the capillary.*

Moreover, there is no band broadening due to dead volume and it is not necessary to remove the protective capillary coating from fused silica capillaries. Furthermore, the fabrication and alignment of a C^4D cell is easier and simpler than other cells for end-capillary electrochemical detection and the cell can be moved along the capillary if is needed to change the detection point. Finally, C^4D systems are not heavy, so fitting it to a P-CE does not suppose a serious increase of the total system weight, and the novel systems are not very power consuming, so the autonomy of the system is not compromised. For all these reasons, C^4D has widely substituted other end-capillary detection systems in P-CE. However, C^4D still need improvement to get higher sensitivities and smaller sizes.

As stated before, a variety of injection systems have been devised for improve the systems' performance. Some of them did not show the expected performance, such as the "cross-sampler",[9] due to the instabilities of the manual sample flushing. Other P-CE system also included manual injection systems, such as the systems published by

Lee and col.[12] and P. Kubáň and col.[13] The main problem of manual restricted operations is, in spite of the difficulty of carry out reproducible injections, the impossibility to perform unattended operations. Moreover, automated systems allows use by non-experienced personnel. Some automated sample injection has been reported, such as the digital microfluidic sampler developed by Gorbatsova,[10] however, the evaporation of the buffer and sample drops leaded to possible low efficiencies. Therefore, there is a current need to develop new injection systems enabling reproducible injections. A good starting point to continue with the instrument development on P-CE in this thesis would be an instrument with a fully automated sample injection system, as can be observed in chapters II.2 and II.3.

One of the requirements that a portable system must fulfill is that it must be able to be operated without a main power source. Nowadays, there is a requirement of batteries with higher capacities and usually battery-powered devices do not last as long as one would wish. However, there is a increase of the number of low-power consuming components. Therefore, a system developer must be aware of the new available technologies and try to incorporate those with lower power consumption. Specifically, in CE the HV supply is the component with highest power requirements.

The total weight and dimensions of the up-to-date published P-CE are acceptable. However, they could be lighter and smaller. In any case, portable analytical systems should accomplish two important requirements:

- They must be light, but also robust.

- They must be small, but easy to handle as well.

All the above mentioned considerations regarding the detection and injections systems, the autonomy and system automation, and system designs were considered in this thesis for the development of P-CEs (chapters II.2 and II.3). Moreover, the system developed in chapter II.3 was applied to the forensic field in chapter II.4.

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CHAPTER II.2

PORTABLE CAPILLARY ELECTROPHORESIS INSTRUMENT WITH AUTOMATED INJECTOR AND CONTACTLESS CONDUCTIVITY DETECTION

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Abstract

A portable capillary electrophoresis instrument featuring an automated, robust, valve-based injection system was developed. This significantly facilitates operation in the field compared to previous injection approaches. These generally required delicate manual operations which are difficult to perform outside the laboratory environment. The novel system relies on pressurized air for solution delivery and a micromembrane pump for sample aspiration. Contactless conductivity detection was employed for its versatility and low power requirement. The instrument has a compact design, with all components arranged in a briefcase with dimensions of 45 × 35 × 15 cm (w × d × h) and a weight of about 8 kg. It can operate continuously for 9 h in the battery-powered mode. Depending on the task at hand, the injection system allows easy optimization for high separation efficiency, for fast separations, or for low limits of detection. To illustrate these features, the separation of four anions within 16 s is demonstrated as well as the determination of nitrite below 1 μM. The determination of phosphate at a sewage treatment plant was carried out to demonstrate a field application.

Introduction

The use of portable instrumentation for field analysis is of interest due to the rapid availability of results, elimination of complications with sample storage and transport, and better cost effectiveness than conventional bench-top analytical systems. A mobile analytical instrument should satisfy requirements of compact size, light weight, robustness and low power consumption. Automation of operation is also desirable. CE, with its advantageous properties of a wide range of accessible analytes, high separation efficiency, short analysis time, low power requirements, limited consumption of chemicals, ease of installation, operation and maintenance, is a particularly interesting candidate for portable analytical instrumentation.

One challenge for a portable CE system is detection. Optical detection methods can only be implemented with non-standard light sources such as light-emitting diodes or laser diodes because of the high power requirement of conventional UV or visible sources, and these are not ideal for non-light absorbing inorganic or organic ions. Electrochemical detection methods, on the other hand, are better suited for portable CE as their fully electronic configuration can easily be miniaturized and translated into the compact, low power format. Of the variants of electrochemical detection methods, C^4D is very attractive as it can be considered universal for all ionic species, which includes the non-UV/Vis-active ones, and the axial tubular arrangement of the electrodes positioned outside the capillary offers ease in construction and operation. Publications on fundamental aspects of C^4D are available[1-9] and discussions of general applications of C^4D for CE can be found in different recent reviews.[10-14]

To our knowledge, the first portable CE instrument was reported by our group in 1998 and was based on potentiometric and amperometric detection.[15-16] The addition of contactless conductivity detection was then reported in 2001[17] and this was later followed by a version with an improved detector in 2007.[18] Li and co-workers introduced a portable CE instrument with potential gradient detection,[19] which was later also fitted with a contactless conductivity detector.[20-21] The instrument is commercially available, and Haddad and co-workers reported its use for the determination of residues from improvised explosive devices using an optical detector based on a light-emitting diode[22] as well as a contactless conductivity detector.[23] Kaljurand and co-workers developed a system for the on-site determination of chemical warfare agent degradation products.[24] Lee et al. described a system with LIF detection based on a solid-state laser.[25] A detailed discussion of the portable CE instruments based on conventional capillaries up to 2010 can be found in a review by Macka and coworkers.[26] Also have been reported portable systems based on microchip-CE devices.[27-29]

A weak point of the field-portable CE instruments reported thus far has been the injection systems. These have generally been very delicate, requiring careful manual operations. From our experience with previous instruments,[15-18] it has become clear that a robust, automated injection system is necessary to make the instruments amenable to routine use in field analysis. The difficulty arises from the fact that in capillary electrophoresis very small volumes in the nanoliter range must be injected into tiny capillaries. Because of the low volumes and the high voltages involved, it is not possible to use rotary injection valves for direct injection as ordinarily employed for column chromatography. Therefore, usually the capillary itself is

placed temporarily into the sample container. Two modes of injection are then possible. For electrokinetic injection, high voltage is applied, while for hydrodynamic injection, siphoning or pressurization is used, before the capillary is moved back to a buffer container for application of the separation voltage. While electrokinetic injection is easier to implement, the hydrodynamic mode is preferred, as it avoids a sampling bias. Commercial bench-top instruments for the laboratory feature a robotic system for movement of sample and buffer vials and/or capillary end and pneumatic pressurization or application of a vacuum. Little effort has been spent to date on the development of injection systems for portable instruments. The commercially available unit[19] has a turntable and an automated hydrodynamic injection arrangement similar to conventional bench-top instruments. However, this is relatively complex and fairly delicate. The research instruments reported have relied on electrokinetic injection or improvised hydrodynamic injection, typically by manually elevating the injection end of the capillary for a few seconds timed with a wristwatch.[15-18,25] Kaljurand and co-workers have addressed this general weakness by developing different versions of split injectors and used this approach in their instrument developed for the determination of chemical warfare agents.[24,30,31] Injection was carried out by emptying a sample into the splitting device using a syringe. This is easier to perform than manual injection directly into capillaries and was essential for the reported field work on chemical warfare agents where the operator had to wear full body protective clothing.[24] However, a limitation of this system was the fact that the injection relied largely on the reproducibility of the pressure created by hand when emptying the syringe.

The aim of the project reported herein was the development of a further improved injection system for a portable instrument which is fully automated and thus eliminates the operational difficulties as well as any measurement bias of manual injections. The arrangement employed is based on a split injector which had been used in previous stationary instruments based on sequential injection manifolds employing a syringe pump and a multiposition valve.[32,33] It takes the approach reported by Kaljurand and co-workers[31] for their portable system further, in that the sample is passed through the splitter automatically. The use of fixed pressurization and computer-controlled timing precludes the variations of manual operation. The injected volume can be set readily over a large range, which allows easy optimization for different tasks. The sample is drawn into the system automatically by using a small membrane pump.

2. Experimental section

2.1. Chemicals, sample collection, and preparation

All chemicals were of analytical or reagent grade and purchased from Fluka (Buchs, Switzerland) or Merck (Darmstadt, Germany). Stock solutions (10 mM) of chloride, nitrate, sulfate, nitrite, fluoride, phosphate, oxalate, malonate, citrate, succinate, phthalate, acetate, lactate, benzoate, vanillate, ascorbate, and gluconate were used for the preparation of the standards of inorganic and organic anions, using their respective sodium or potassium salts. Before use, the capillary was preconditioned with 1 M NaOH for 10 min and deionized water for 10 min prior to flushing with buffer. Deionized water purified using a system from Millipore (Bedford, MA) was used for the

preparation of all solutions and for sample dilution if required. The soft drinks were passed through 0.45 μm membrane filters and diluted 10 times before analysis. The orange juice sample was first centrifuged for 10 min at 6000 rpm and filtered to remove the flesh content and then diluted 50 times due to the high concentration of citrate. For the analysis of phosphate in wastewater, samples were filtered with 0.45 μm membrane filters and injected directly into the system for analysis. No further treatment was carried out.

2.2. Instrumentation

The injection interface accommodating the capillary and the ground electrode was machined in a plexiglass block (3 × 2 × 2 cm) according to a previously reported design.[34] This was fitted with a micro-graduated needle valve obtained from IDEX (P-470, Oak Harbor, WA) and solenoid valves from NResearch (product nos. 116T021 and 116T031, West Caldwell, NJ). Pressurization was achieved using a steel cylinder (Swagelok 304L-HDF4- 150), a regulating valve (Swagelok 1ELA2C1000BK), and a pressure gauge (Swagelok PGI-40M-BG6-LANX-0) (Arbor, Niederrohrdorf, Switzerland). The miniature membrane pump (NF-5-DCB) for sample aspiration was purchased from KNF (Balterswil, Switzerland). All fluidic connections were made with 0.02 in. inner diameter (I.D.) and 1/16 in. outer diameter (O.D.) Teflon tubing and with polyether ether ketone (PEEK) flangeless nuts and ferrules 1/4-28 UNF (IDEX). Two high voltage modules (DX250 and DX250N) capable to provide a maximum of 25 kV of either polarity were obtained from EMCO (Sutter Creek, CA). Polyimide-coated fused silica capillaries of 50 μm I.D. and 365 μm O.D., and capillaries of 25 μm I.D. and 365 μm O.D. (from Polymicro,

Phoenix, AZ), were used for separation. The high voltage end of the capillary was isolated with a safety cage made from Perspex, which was equipped with a microswitch to interrupt the high voltage upon opening.

The purpose-made contactless conductivity detector was based on a design reported previously[9] and used an integrated circuit oscillator from Exar (XR-2206, Fremont, CA) to create a sine wave of 300 kHz, an OPA627 operational amplifier (Texas Instruments, Dallas, TX) to bring the amplitude to ± 10 V, an OPA602 operational amplifier (Texas Instruments) fitted with a 1.5 MΩ feedback resistor to convert the pick-up current to voltage, and a monolithic AD630 synchronous detector (Analog Devices, Norwood, MA) for rectification. The voltage signal was then amplified, low-pass filtered,[9] and passed to an ADC-20 from Pico Technology (St. Neots, UK) connected to a notebook class personal computer for data acquisition. Most of the parts, i.e., valves, high voltage modules, and membrane pump, were controlled from the same computer using an Arduino Nano microcontroller board (RS Components, Wädenswil, Switzerland) programmed using the Arduino integrated development environment and appropriate interface circuitry. The instrument features built-in rechargeable lithium ion batteries. A battery pack of 14.8 V and a capacity of 6.6 Ah with the dimensions of 73 × 55 × 67 mm (CGR 18650CG 4S3P, Contrel, Hünenberg, Switzerland), which was fitted with a voltage regulator to produce a 12 V output, was used to provide power to the valves, membrane pump, and the high voltage modules. A separate pair of smaller Li-ion batteries with a capacity of 2.8 Ah each (CGR 18659CG 4S1P, Contrel), which was fitted with positive and negative 12 V regulators, provided the split ±12 V supply

for the detector circuitry. Alternatively, mains power can be utilized when available via appropriate external adaptors.

3. Result and discussion

3.1. System design

A schematic drawing of the system is shown in **Figure II.2.1**. Precise propulsion of fluids through the system is made possible by pressurizing a reservoir of background electrolyte with compressed air. This is provided from a small metal cylinder which is filled with a manual pump (normally used to pressurize shock absorbers of bicycles). The pressure delivered can be set with a regulating valve and monitored with a small gauge. The sample is loaded into a sample loop which is extended between two three-way valves as described previously by Sweileh and Dasgupta.[35] Note that it would also be possible to use a rotary valve as is customary for flow-injection analysis or column chromatography, but the use of the solenoid valves is simple and less expensive. The loop is filled by using a small membrane pump to aspirate a sample directly through a thin tube. If preferred, manual filling of the loop with a syringe is also possible. Subsequently, the sample is moved to the injector block by switching the three-way valves 1 and 2 (V1 and V2) to allow background electrolyte to flow from the pressurized reservoir. A fraction of the sample is pushed into the capillary for hydrodynamic injection as the plug is located at its front end while applying a back-pressure for a determined period of time. The back-pressure is set by adjustment of the needle valve (a bleeding type which splits the flow into two paths) and applied for the desired duration by closure of gate valve V3

(while V4 stays open). Flushing of the interface and the manifold ahead of the interface, as well as of the capillary, is possible by either opening or closing V3 and V4 at the same time. All components were integrated into an aluminum briefcase with the dimensions of 35 × 45 × 15 cm (w x d x h), and the system had a weight of 8kg.

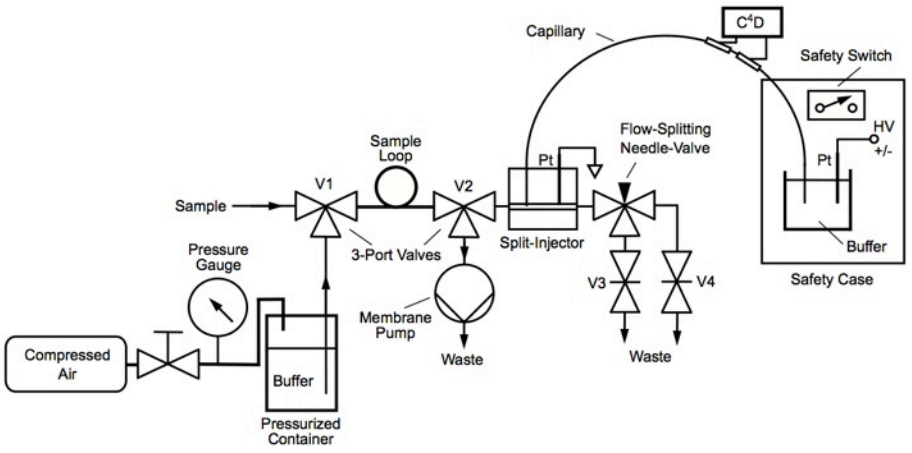

***Figure II.2.1**. Diagram of the fluidic connections of the instrument. Pt denotes the two platinum electrodes for application of the high voltage (HV) for separation. The injector/interface is grounded, while the voltage is applied from the detector end.*

A photograph of the assembly is shown in **Figure II.2.2**. The fluidic parts are seen on the left. The plexiglass cage to the right contains the high voltage electrode, and the small metal box sitting on top is the C^4D cell. The pneumatic parts for pressurization of the buffer reservoir are seen to the far right. The control and detector electronics as well as the rechargeable batteries are contained in the back of the

instrument, and some manual switches and connectors are mounted on the panel. The internal batteries were found to provide sufficient power for typically about 9 h of operation before recharging was necessary.

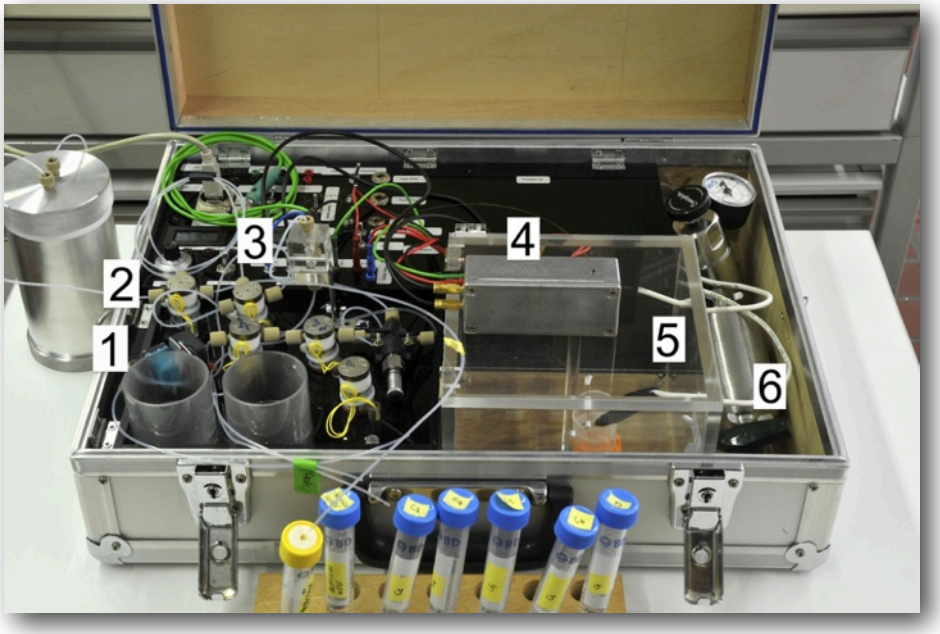

***Figure II.2.2**. Photograph of the instrument. (1) Membrane pump, (2) valves, (3) splitter, (4) detector, (5) safety cage for application of high voltage, (6) pressurized air.*

3.2. Performance

3.2.1. Standard separation of some common inorganic cations and anions

To demonstrate the versatility of the system in analyzing different target analytes, the separation of some common inorganic anions and cations was carried out using a background electrolyte consisting of 12 mM histidine adjusted to pH 4 with acetic acid in the presence of 2 mM 18-crown-6, which is commonly used for the separation of inorganic cations and anions by CE-C^4D.[32] The crown ether facilitates the separation of $NH4^+$ and K^+. The separations of these cations and anions were carried out by switching the polarity of the system between negative and positive modes. The relatively low pH-value of the buffer leads to a limited EOF so that the anions can be determined without surface modification of the capillary. Separations of standard solutions of these cations (NH_4^+, K^+, Ca_2^+, Na^+, Mg_2^+, and Li^+) and anions (Cl^-, NO_3^-, SO_4^{2-}, NO_2^-, F^-, and $H_2PO_4^-$) at 50 μM for each ion are shown in **Figure II.2.3**. The quantitative performance data for the conditions used is given in **Table II.2.1**. The limits of detection were in the lower micromolar range. The linear ranges depended on the species. Baseline separation between NH_4^+ and K^+ as well as the peaks of Ca_2^+ and Na^+ were still achieved at the concentration of 100 μM for each cation. However, at higher concentrations, baseline separation of these peaks was lost. In the case of Mg^{2+} and Li^+, linear ranges extended to 200 and 1000 μM, respectively. The reproducibilities of peak areas and migration times were determined over a period of 8 h.

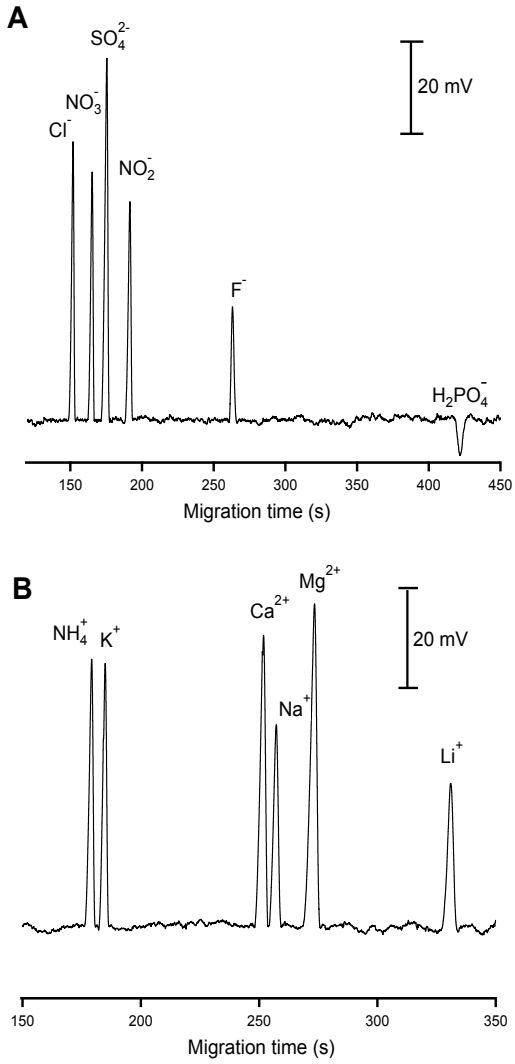

Figure II.2.3. *Typical separation of a standard solutions containing: (A) inorganic anions; (B) inorganic cations; 50 μM for each ion. Background electrolyte: His 12 mM adjusted to pH 4 with acetic acid in the presence of 2 mM of 18-crown-6. Capillary: 50 μm I.D., 36 cm effective length (L_{eff}), and 50 cm total length (L_{tot}). Separation voltage: +15 kV for anions and −15 kV for cations. Injection: pressure, 1 bar; sample loop, 150 μL; splitting valve set to 0.15; injection time, 4 s.*

Table II.2.1. *LODs, correlation coefficients (r^2) and reproducibility for the determination of inorganic cations and anions.*

Ion	Linear Range (μM)	r^2	LOD[a] (μM)	Intraday Reproducibility of Peak Area (%RSD)[b]	Intraday Reproducibility of Migration Time (% RSD)[b]
NH_4^+	6-100	0.9994	2	1.1	0.5
K^+	6-100	0.9996	2	1.7	0.4
Ca^{2+}	6-100	0.9983	2	0.6	0.4
Na^+	10-100	0.9994	3	1.4	0.5
Mg^{2+}	10-200	0.9992	3	1.0	0.3
Li^+	10-1000	0.9999	5	1.8	0.3
Cl^-	10-1000	0.9999	3	0.9	0.6
NO_3^-	10-450	0.9999	3	1.0	0.4
SO_4^{2-}	5-450	0.9999	1.5	0.6	0.3
NO_2^-	10-1000	0.9999	3	0.7	0.6
F^-	27-2000	0.9998	8	2.1	0.5
$H_2PO_4^-$	57-500	0.9991	17	2.4	0.3

[a]*Peak heights corresponding to 3 x baseline noise*

[b]*Determined for 50 μM, n = 9, over a period of 8 hours*

The system was programmed to autonomously carry out repeated injections and separations of the standard mixture of 50 μM every 10 min throughout this duration, i.e., a total of 48 measurements. The standard deviations for peak areas were well acceptable, being about 1%, and the stability of migration times was also excellent. Note, that the standard deviations were calculated from the nine data points acquired after each hour.

A systematic drift in these parameters over the time period is not apparent in the data. This demonstrates the inherent stability of the mechanical and electronic design of the system. However, under field conditions, due to temperature changes and other effects, larger fluctuations can be expected.

3.2.2. Fast separation

The system can be optimized differently to meet different objectives. Very fast separations are possible by using a capillary with a short effective length of only a few centimeters. Note, that this is not readily possible with standard bench-top instruments, as these are not designed accordingly. A further requirement is a fast and well reproducible automated injection system for small sample plugs, [36,37] which has not been available for portable CE instruments. The separation of four inorganic anions (Cl^-, NO_3^-, NO_2^-, SO_4^{2-}) within 17 s carried out on the current system is demonstrated in **Figure II.2.4**. To accelerate the migrations of anions, an elevated electric field was applied by introduction of a high voltage of +15 kV over a short capillary of only 25 cm. The detector was positioned 4.5 cm from the injection end. To inject only a short plug, the back-pressure was reduced compared to the test reported in the previous section and the injection time was shortened to 1 s only. While baseline separation was achieved in both cases, it is clear from a comparison of **Figures II.2.3** and **II.2.4** that a more complex sample would require the better separation possible in the longer capillary. Under the conditions for fast separation, the LODs for Cl^-, NO_3^-, and NO_2^- were 5 μM and the LOD for SO_4^{2-} was 2.5 μM, which is still acceptable and only approximately 2 times higher than those for normal conditions.

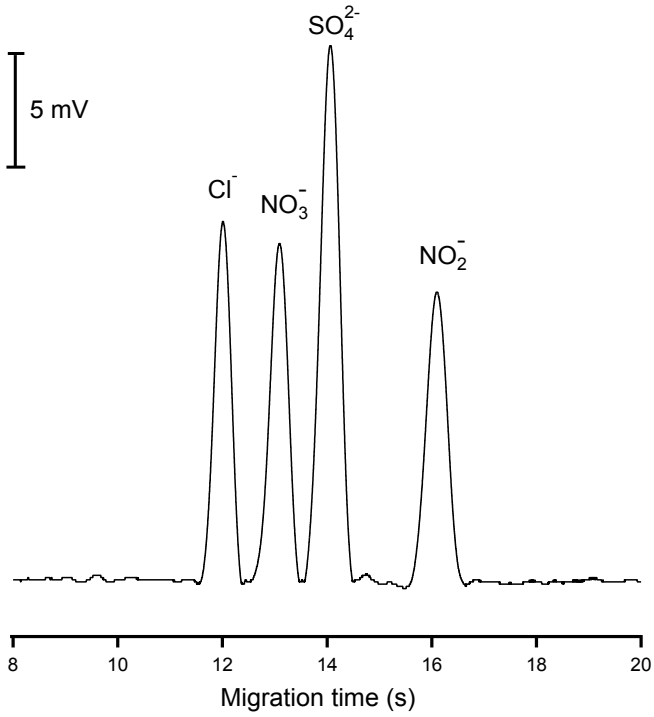

***Figure II.2.4**. Fast separation of Cl^-, NO_3^-, SO_4^{2-}, and NO_2^- at 50 μM. Background electrolyte: His 12 mM adjusted to pH 4 with acetic acid in the presence of 2 mM of 18-crown-6. Capillary: 25 μm I.D., Leff/Ltot = 4.5/25 cm. Separation voltage: +15 kV. Injection: pressure, 1 bar; sample loop, 150 μL; splitting valve set to 0.20; injection time, 1 s.*

3.2.3. Enhanced detection limit

When separation efficiency is not a limitation, LODs can be enhanced by introducing a large sample volume. This is illustrated in **Figure II.2.5** for the analysis of a tap water sample spiked with 1 μM NO_2^- as a potential analyte of interest which is well separated from other species. As can be seen from electropherogram (a) of

Figure II.2.5, for a normal injection volume, for which chloride, nitrate, and sulfate are well separated, a peak for nitrite is not visible, as its concentration is below the detection limit. When the injected volume is increased, by prolonging the injection time from 4 to 10 s and increasing the backpressure, nitrite becomes detectable as the LOD is lowered to 0.7 μM (electropherogram b). However, it is clear that the separation of the three major anions (Cl^-, NO_3^-, SO_4^{2-}) was not possible under these conditions.

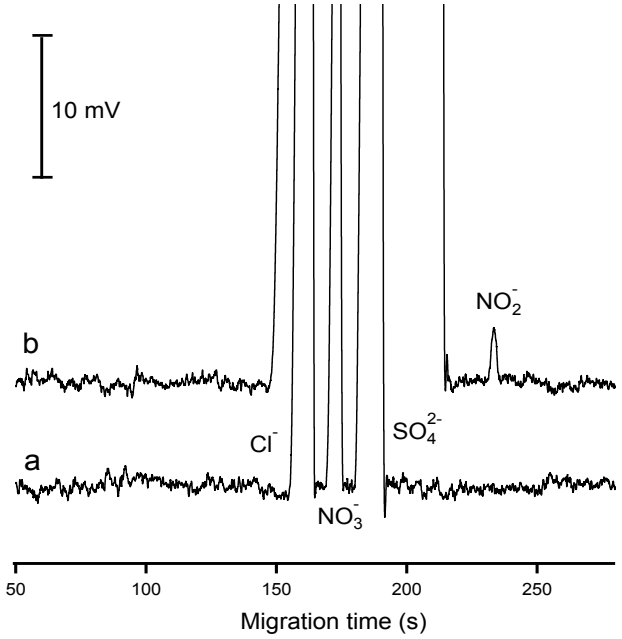

***Figure II.2.5**. Sensitive determination of a tap water sample spiked with 1 μM NO2⁻. (a) Normal injection volume: 1 bar, 150 μL, splitting valve set to 0.15, 4 s. (b) Large volume injection: pressure, 1 bar; sample loop, 150 μL; splitting valve set to 0.10; injection time, 10 s. Background electrolyte: His 12 mM adjusted to pH 4 with acetic acid in the presence of 2 mM of 18-crown-6. Capillary: 50 μm I.D., 36 cm L_{eff} and 60 cm L_{tot}. Separation voltage: +15 kV.*

3.2.4. High peak capacity

Complex samples containing a relatively large number of similar ions require conditions that give good peak capacities. This usually requires relatively long residence times with long capillaries if the sensitivity is not to be compromised.

Such an application is illustrated for the current system with the simultaneous separation of 11 slowly migrating organic anions, namely oxalate, malonate, citrate, succinate, phthalate, acetate, lactate, benzoate, vanillate, ascorbate, and gluconate. These compounds are found in various beverages either as major constituents or as additives. Separation was successfully achieved using a basic background electrolyte of Tris/CHES at a concentration of 70 mM for each compound and in the presence of 200 μM CTAB for reversal of the EOF. Three different soft drinks were analyzed as illustrative samples for this demonstration.

The electropherograms for a standard mixture and for soft drink samples are shown in **Figure II.2.6**. Electropherogram b is for a soft drink made from a byproduct of cheese production and for this reason contains a large concentration of lactate besides other anionic species. The cola beverage (electropherogram c) was found to contain phosphate, while the orange juice (electropherogram d), as expected, contained a high concentration of citrate.

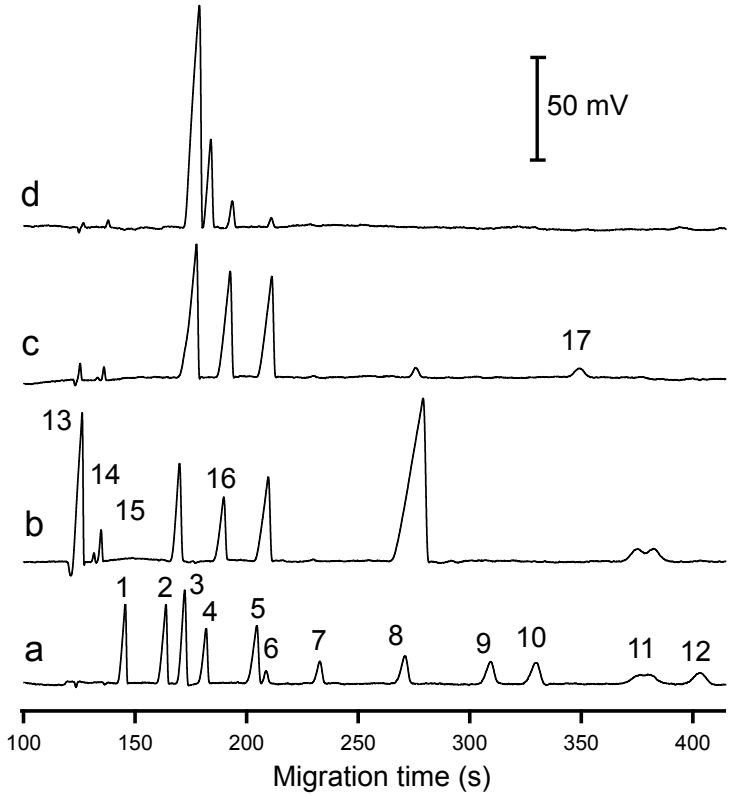

***Figure II.2.6**. High efficiency separations. (a) Eleven organic compounds often found in beverages and carbonate, (b) lactate-containing soft drink, (c) cola beverage, (d) orange juice. Background electrolyte: Tris/CHES 70 mM and CTAB 0.2 mM (pH 8.5). Capillary: 25 μm I.D.; l_{eff}/L_{tot} = 36/65 cm. Separation voltage: +15 kV. Injection: pressure, 1 bar; sample loop, 60 μL; splitting valve set to 0.15; injection time, 4 s. Peaks: (1) oxalate, (2) malonate, (3) citrate, (4) succinate, (5) phthalate, (6) carbonate, (7) acetate, (8) lactate, (9) benzoate, (10) vanillate, (11) ascorbate, (12) gluconate, (13) chloride, (14) nitrate, (15) sulfate, (16) phosphate, (17) unidentified compound.*

3.3. Application example

3.3.1. Field measurements of phosphate at a wastewater treatment plant

To demonstrate its suitability for field work, the instrument was taken to a local sewage treatment plant and set up for the determination of phosphate. A solution of 1 mM His/25 mM acetic acid (pH 3.5) was found to be an optimal background electrolyte for the determination of this species. Under this condition, the phosphate peak is very well separated from the very broad peak of the major anions (Cl^-, NO_3^-, and SO_4^{2-}) which are present in the sewage water at very high concentrations (ranging from 1 to 4.5 mM). An electropherogram for separation of phosphate in a sewage water sample is shown in **Figure II.2.7**. In **Table II.2.2** the phosphate concentrations (mg P/L) measured with the new instrument in several samples are given together with the results from the standard photometric molybdenum blue method for validation. The first six samples were determined in the field (single measurements), and the remainder back in the laboratory (in triplicate). As shown in **Table II.2.2**, the results from the CE method are in good agreement with those obtained from the molybdenum blue reference method (errors between the two methods were less than 10% for measurement done in the lab). However, the on-site measurement generally gave higher deviations which is ascribed to the fact that the freshly collected wastewater samples contained some bubbles of dissolved gases. As no degassing could be carried out in the field, these would have influenced the precision of injection. The detection limit of the method was 0.15 mg P/L (5 μM) (based on signal-to-noise ratio = 3), and its linear range extended from 0.5 mg P/L (16 μM) to 10 mg P/L (320 μM).

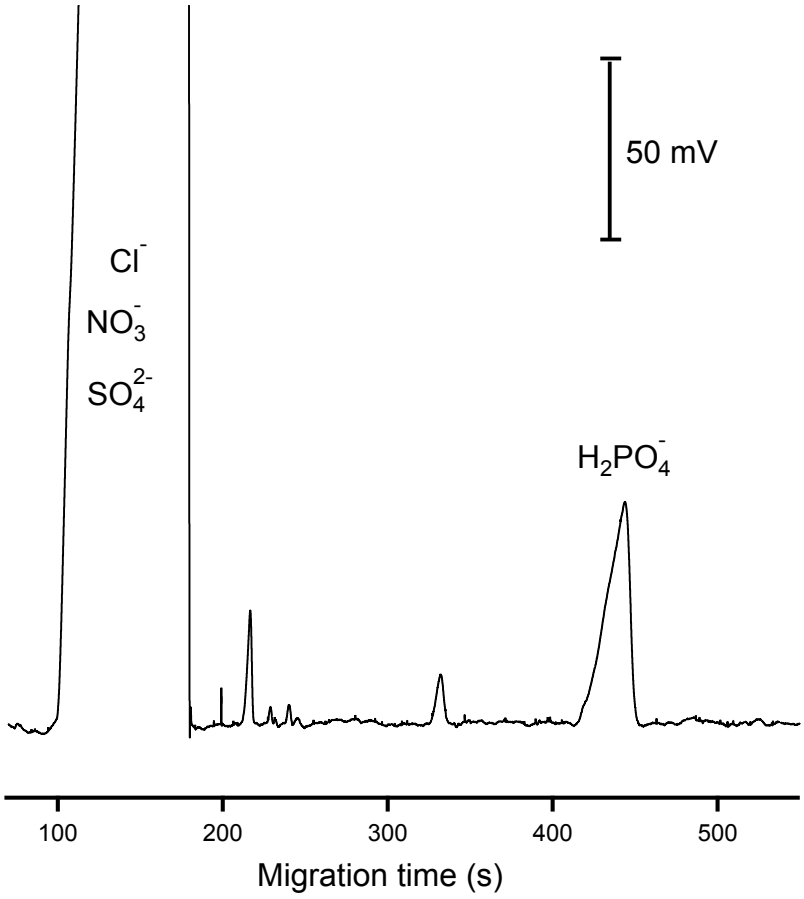

Figure II.2.7. *Detection of phosphate (1.6 mg P/L) in a sewage sample. Background electrolyte: His 1 mM/acetic acid 25 mM (pH 3.47). Capillary: 50 μm I.D.; l_{eff}/L_{tot} = 29/50 cm. Separation voltage: +15 kV. Injection: pressure, 1 bar; sample loop, 60 μL; splitting valve set to 0.12; injection time, 4 s.*

***Table II.2.2**. Concentrations of phosphate in sewage samples measured in the field and in the Llaboratory*

Sample	Capillary electrophoresis (mg P/L)	Molybdenum blue method (mg P/L)	% error
1	2.1	2.4	13
2	2.3	2.1	6
3	1.5	1.6	8
4	1.5	1.6	9
5	1.5	1.8	16
6	1.8	2.1	15
7*	3.6	3.6	1
8*	4.9	4.6	7
9*	3.5	3.4	2
10*	0.54	0.58	8
11*	0.76	0.78	2

**Samples measured in the laboratory.*

4. Conclusions

The portable CE-C^{4}D instrument with automated injection built in-house showed a good performance with high reproducibility. The results obtained confirm its suitability for on-site measurements. The system may be optimized for different compromise conditions with regard to detection limits, dynamic range, separation efficiency, and analysis time according to the task at hand. As demonstrated by the autonomous stability test, which extended over 8 h duration, the

instrument also has the potential to be set up for unattended monitoring operations. This is facilitated by the automated aspiration of the sample.

Acknowledgments

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CHAPTER II.3

DETERMINATION OF NITROGEN MUSTARD DEGRADATION PRODUCTS IN WATER SAMPLES USING A PORTABLE CAPILLARY ELECTROPHORESIS INSTRUMENT

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Abstract

In this work, a new purpose-made portable capillary electrophoresis instrument with a contactless conductivity detector was used for the determination of degradation products of nitrogen mustards in different water samples. The capillary was coated with poly(1-vinylpyrrolidone-co-2-dimethylaminoethyl methacrylate) to avoid analyte-wall interactions. The coating procedure was studied to obtain the best repeatability of the analytes migration time. Four different coating procedures were compared, while flushing the capillary with the co-polymer at 100 psi for 2 min at 60ºC provided the best RSD values (<4%). The analytical method was also optimized. The use of 20 mM of MES adjusted to pH 6.0 with His as running buffer allowed a good baseline separation of the three analytes in different water samples without matrix interferences. The method permitted the detection down to 5 μM of the three degradation products.

Introduction

Nitrogen mustards (NMs) were produced in the 1920s and the 1930s as potential chemical warfare agents. Fortunately, NMs have never been used in combat, although they were stockpiled by many countries during the Second World War for military purposes. They come as an oily liquid (at room temperature), vapor, or solid. NM are blister (or vesicant) and nonspecific DNA alkylating agents, similar to lewisites and sulphur mustards (or mustard gas).[1] NMs are powerful irritants that damage the skin, eyes, and the respiratory tract. They can enter the cells of the body very quickly and cause damage to the immune system and bone marrow. No antidote exists for nitrogen mustard exposure.[1] Nowadays, their use, development, production and stockpiling is prohibited by the Chemical Weapons Convention (CWC), and so they are listed in the Schedule 1, part A of the CWC.[2] NMs are also known by their military designations HN-1 (bis(2-chloroethyl)ethylamine), HN-2 (bis(2-chloroethyl)methylamine), and HN-3 (tris(2-chloroethyl)amine).

N-methyldiethanolamine (MDEA), N-ethyldiethanolamine (EDEA), and triethanolamine (TEA) are basic compounds. **Figure II.3.1** shows the structures, pKa-values, and the molecular mass of each of them. They are degradation products of NMs as long as NMs undergo fast hydrolysis in presence of water to form these three ethanolamines.[2] Because of this behavior, it could be possible to identify the employment of NMs if they were used as, for example, water contaminants in chemical warfare, by determining their degradation products MDEA, EDEA, and TEA. On the other hand, MDEA, EDEA, and TEA are also precursor reactants of the NMs and they can be used

in their manufacture. Therefore, they are listed in the Schedule 3, part B of the CWC,[3] and thus, they have to be controlled.

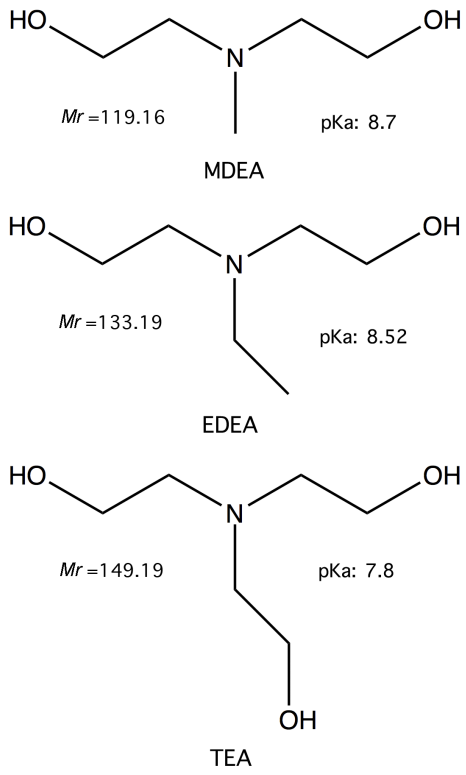

Figure II.3.1*. Structures, molecular masses (Mr), and pKa-values of MDEA, EDEA, and TEA.*

NM precursors analyses are usually performed in fixed-base laboratories, and so, samples require to be transported to the laboratory from the sample collection place. Then, they have to be analyzed in the laboratory to obtain information which will be transferred to the field units. NMs in particular undergo a fast hydrolysis becoming undetectable after a short period of time. HN-1

and HN-2 become untraceable faster than HN-3, which turns undetectable after 25 h. Nevertheless, some of them could be detected later thanks to a reversion process produced by some reaction intermediaries.[4] In this context, the availability of portable tools for field analysis is of great importance in order to achieve an effective and reliable detection of NMs. Their use in the very place of sample collection avoids loss of information of the samples during the transport and the storage, allows to take decisions on-site, reduces the number of the necessary samples, avoids multiple sampling trips, and therefore reduces cost and time spent.[5]

Up to date, NMs have been mainly studied as their degradation products. MDEA, EDEA, and TEA have essentially been examined in water samples.[4,6-12] The above-mentioned NM degradation products have also been determined in other samples, such as in urine,[8,13] blood,[8] or soil.[14] Generally, gas chromatography[6,8-11] and liquid chromatography[7,12,13] have been used for the analysis of these samples. However, the use of capillary electrophoresis is not so common for the determination of ethanolamines. CE with indirect UV detection was used for the determination of TEA and/or MDEA.[15-17] However, NMs degradation products were not determined in those works and EDEA was not included. As far as we know, there is only one work on the determination by microchip electrophoresis of MDEA, EDEA, and TEA, as degradation products of NMs.[18] Nevertheless, there are several publications on the use of CE for the analysis and screening of other chemical warfare agents (CWA). CE is arguably the technique that is best suited for field and on-site analysis, since it can easily be miniaturized, the start-up time is significantly short, and CE analysis times are the shortest of the available separation techniques.[19] Conductivity detection is best suited for

in situ analysis of CWA because it can be miniaturized and the power consumption is minimal.[19] In fact, in 2001 Kappes and col. fitted a conductimetric detector to a P-CE instrument, which was already fitted with both amperometric and potentiometric detectors.[20] However, contact conductivity detection was replaced in the recent years by C^4D due to its advantages.[21] Nevertheless, although conventional CE has been widely used for the determination of nerve agents as CWA,[22-33] to the knowledge of the authors, there are only four publications on the use of a P-CE with C^4D for the analysis of nerve agents.[19,34-36]

In this study, for the first time, a P-CE instrument with C^4D was developed and applied for the determination of MDEA, EDEA, and TEA in various water samples.

2. Materials and methods

2.1. Reagents and samples

All chemicals used were of analytical grade. L-Histidine (His), 2-(N-morpholino)ethanesulfonic acid (MES), acetic acid, sodium hydroxide (NaOH), MDEA, EDEA, TEA, and poly(1-vinylpyrrolidone-co-2-dimethylaminoethyl methacrylate) solution (P(VP-co-DMAEMA)) ≥ 19 % wt. in water, 1.047 g/mL at 25 °C were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (HCl) was from Scharlau (Barcelona, Spain). Ultrapure water was obtained from a Millipore Milli-Q water system (Bedford, MA, USA). Samples of water were taken from a well (Alcalá de Henares, Madrid, Spain) and from

three rivers: Pas (Vega del Pas, Cantabria, Spain), Pisuerga (Cervera de Pisuerga, Palencia, Spain) and Ebro (Valdenoceda, Burgos, Spain)

2.2. Instrumentation and experimental procedure

The CE experiments were performed on a purpose-made P-CE. The P-CE had similar characteristics to the one presented by Mai and col.,[37] but included some differences: the possibility of automated introduction of up to four different solutions in the capillary, the sample injection by hydrodynamic sample splitting, and the use of a normally open valve as V1. A block diagram of the instrument is given in **Figure II.3.2**. All the electronic components as well as the components of the fluidic system were fitted into an aluminum case with the dimensions of 450 x 150 x 350 mm (w x h x d). The total weight of the system was approximately 8 kg. A multiple port manifold (Upchurch Scientific, Oak Harbor, WA, USA) was used to distribute the pressurized gas to the pressurized fluid containers. The delivery of different solutions into the grounded interface, the flushing of the capillary, and the sample injection was controlled by a 2-way normally open isolation valve (Neptune Research & Development Inc., West Caldwell, NJ, U.S.A.), called V1 from now on. Besides, a 4 x 2-way normally closed manifold (Neptune Research & Development Inc., West Caldwell, NJ, U.S.A.), was added. This manifold can operate in two modes: four different inputs and one single output or one single input and four different outputs. When V1 was closed and the valves controlling the buffer or water flow were open, the capillary was rinsed with buffer or water.

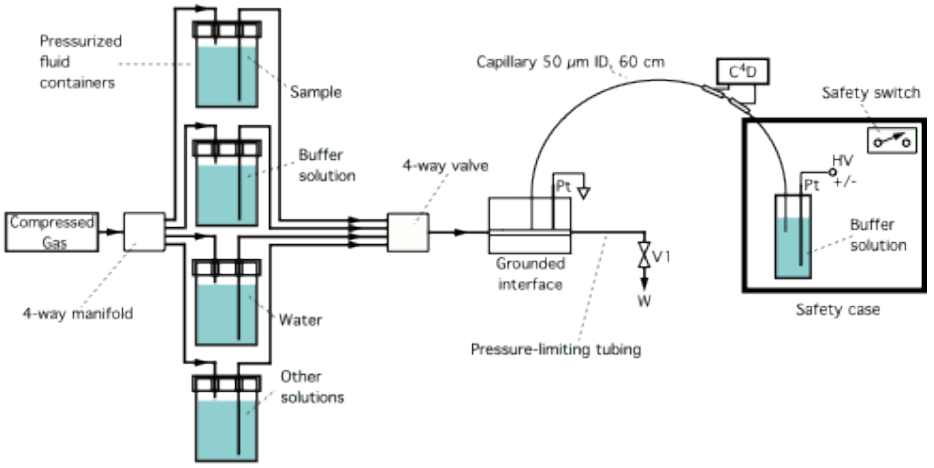

***Figure II.3.2**. Block diagram of the P-CE.*

If V1 was open as well as the valve controlling the buffer or water flow, the grounded interface was flushed. And when V1 was open as well as the valve controlling the sample flow, the sample injection into the capillary by hydrodynamic sample splitting was carried out. PFA tubings (Upchurch Scientific, Oak Harbor, WA, USA) were used to connect the components of the fluidic system. The inside diameter of all of them was 0.020 inches with the exception of the tubing connecting the grounded interface with V1, whose internal diameter was 0.010 inches. This tubing had a length of 12 cm. Two HV supplies, able to operate up to +25 kV and -25 kV, respectively, were incorporated. The device was controlled by an Arduino Nano microcontroller board (RS Components, Wädenswil, Switzerland). Further instrumental characteristics are detailed in the work published by Mai and col.[37] The instrument was fitted with a purpose-made C^4D detector or with a commercial contactless conductivity

detector (eDAQ, Denistone East, NSW, Australia). The commercially available contactless conductivity detector was used for the method optimization and the detector excitation frequency was set to 1200 kHz and the amplitude to 100%.

Bare fused silica capillaries of 50 μm I.D. and 365 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA) with a total length of 60 cm and an effective length of 45 cm were used. Capillaries were conditioned by flushing at 20 psi 1.0 M NaOH for 10 min, water for 5 min, 1.0 M HCl for 5 min, and finally water 5 min. The pre-treated capillary was coated by flushing a solution P(VP-co-DMAEMA) with a concentration of 1.5 % (m/m) in water as an EOF modifier at 100 psi for 2 min at 60 ºC, using a PA800 Capillary Electrophoresis (Beckman-Coulter, Fullerton, CA). Then the coated capillary was rinsed with the running buffer for 5 min, at 20 psi and at 25 ºC to completely remove any possible coating residues in the capillary. After each analysis run, and for maintaining the reproducibility of the analysis, the capillary was rinsed with the running buffer for 4 min. Afterwards, no further treatments were needed for the preparation of the capillary for the next run. A single run encapsulated a total runtime of less than 11 min (flushing time + running time). When a new buffer was used, the capillary was rinsed with water for 5 min and then 5 min with the running buffer. A PA800 Capillary Electrophoresis (Beckman-Coulter, Fullerton, CA) with DAD detection at 209 nm was used to study the performance of the coated capillary. In this case, the injection time was set at 0.5 psi for 10 s. The pH-values of the buffers, which were prepared fresh daily, were determined with a pH-meter model Crison GPL-21 (Crison Instruments, Barcelona, Spain). Samples were injected directly without dilution.

2.3. Data treatment

The electropherograms were treated in Origin (OriginLab Corporation, USA). LOD were determined as the concentrations giving peak heights three times the noise in a sample of ultrapure water. The noise value was measured as the maximum deviations of the baseline around the migration time of the analyte signals obtained for a concentration of 20 μM of MDEA, EDEA, and TEA.

3. Result and discussion

For the field determination of MDEA, EDEA, and TEA in various water samples by a P-CE with C^4D, the most appropriate operation conditions in the P-CE system were selected. Then the capillary coating procedures for avoiding the analyte-wall interactions at the inner surface of the capillary were studied, and finally the P-CE separation conditions prior to the analysis of different water samples were optimized.

3.1. Design of the P-CE system

Usually, due to instrumental limitations, capillaries used for field CE analyses have to be manually conditioned because the P-CE system does not allow the automatic introduction of more than one or two solutions, including the sample. This could pose a problem insofar sometimes it is required to condition the capillary between analyses with more solutions than just the running buffer, such as NaOH, HCl, or water. If the system does not allow the automatic flushing of the capillary with these solutions, it has to be carried out manually,

increasing the chances of human errors occurrence and decreasing the reproducibility of results. On the other hand, new bare capillaries must be cleaned before their first use. For field analyses, capillaries can be pre-cleaned in the lab or can readily be flushed manually using specific syringe adapters. In order to avoid manual operations, the P-CE system used in this work was designed with an automatically-controlled valve operated fluidic system that allows the ready flushing of the capillary with up to four different solutions, including the sample.

An effort was made in the design of the system to reduce the power consumption during the injection of the sample. The sample injection by flow splitting has already been used in some previous works.[38-41] Then, a pressure-limiting tubing with a narrow diameter was placed after the grounded interface (**Figure II.3.2**). The grounded interface and the pressure-limiting tubing create a back-pressure while the sample is been flushed through the grounded interface, which causes small volumes of sample going into the capillary. The volume of the sample injected can be adjusted by regulating the length and the inner diameter of the tubing, as well as the duration of sample flushing. Thus, for the injection the sample can be flushed through the grounded interface and no extra valves or power-consuming components are needed. This is only possible if the ending of the capillary is placed right in the center of the channel in the grounded interface (**Figure II.3.3**). In this way, when the sample goes through the grounded interface, it surrounds the capillary ending and, due to the back-pressure created in the pressure-limiting tubing, it diffuses inside the capillary.

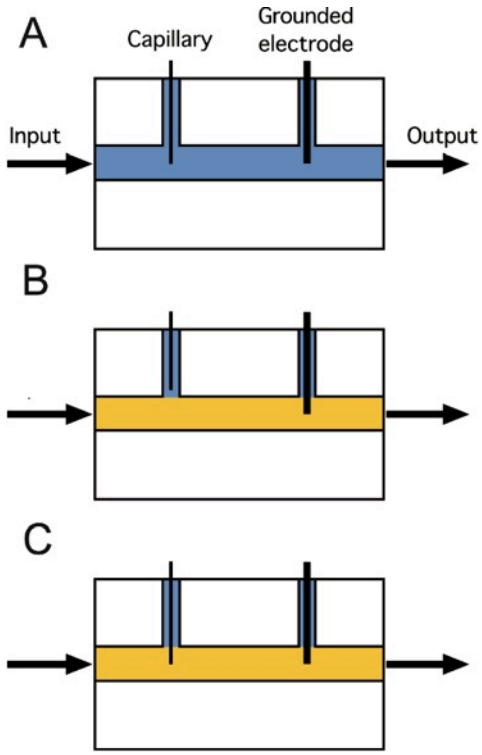

***Figure II.3.3**. Diagram of the grounded interface of the P-CE showing the placement of the capillary. A, grounded interface filled with buffer; B, sample delivered into the grounded interface with the capillary placed in wrong position (buffer injection); C, sample delivered into the grounded interface with the capillary placed in the correct position (sample injection).*

We noticed that, when V1 was closed, a small volume of the buffer inside the system moved from V1 towards both the waste and the grounded interface sides. This movement of liquid was attributed to the displacement of liquid caused by the motion of the solenoid core inside V1 when the valve was closed. The overpressure created in the grounded interface caused the introduction of buffer into the capillary reducing the separation migration time and decreasing the reproducibility between analyses. For this reason, a normally-open

valve was chosen as V1 which remained open during the separation in order to minimize the power consumption.

3.2. Study of the capillary coating conditions

The separation performance in CE can be impaired by the adsorption of analytes at the inner surface of the capillary. Basic compounds, such as amines, tend to interact with the negatively charged silanol groups above pH 2.0. These interactions lead to peak tailing, band broadening, peak distortion, poor reproducibility of the migration times, and decreased efficiency.[42]

Many polymer coatings have been used to control analyte-wall interactions and to avoid adsorption problems. P(VP-co-DMAEMA) is a copolymer which has been proved to avoid the wall-protein interactions.[43] P(VP-co-DMAEMA) has an "anchor part" (poly(DMAEMA)), which attaches to the capillary surface via electrostatic interactions and hydrogen bonding, and a "functional part" (poly(VP)) which acts as a non-biofouling interface creating a hydrophilic and non-ionic layer, keeping basic molecules at a sufficient distance from the capillary surface and so preventing biofouling.[43] Wang and col. developed a fast coating procedure of the capillary with P(VP-co-DMAEMA) in which no chemical reactions were required. After the pre-treatment of the capillary described above, they flushed the capillary with a solution of P(VP-co-DMAEMA) with a concentration of 1.5 % (m/m) in water during 2 min at high pressure (20 psi). In order to increase migration time repeatability, this coating procedure and three other methods (varying time, pressure, and temperature) were compared. The four different methods of coating involved rinsing the capillary with the copolymer

solution at (i) 20 psi for 2 min at 25 ºC, (ii) 20 psi for 10 min at 25 ºC, (iii) 100 psi for 2 min at 25 ºC, and (iv) 100 psi for 2 min at 60 ºC. 12 mM of His adjusted to pH 5.0 with acetic acid was used as running buffer. This preliminary separation buffer was used for the selection of the coating procedure and the separation conditions were subsequently optimized. **Table II.3.1** shows the repeatability, expressed as RSDs, of the migration times for the three analytes studied in this work recorded for nine consecutive electropherograms obtained using each coated capillary. The capillary coated with the copolymer solution at 100 psi for 2 min at 60 ºC showed the best RSD value (< 4 %). Thus, this capillary coating was chosen as the best coating conditions for MDEA, EDEA, and TEA separation.

Table II.3.1*. RSDs of the migration times of MDEA, EDEA, and TEA using capillaries coated with different coatings procedures. n=9.*

	20 psi for 2 min at 25ºC	**20 psi for 10 min at 25ºC**	**100 psi for 2 min at 25ºC**	**100 psi for 2 min at 60ºC**
MDEA	11.9	10.7	7.0	3.3
EDEA	12.1	10.2	7.2	3.6
TEA	12.1	10.1	6.0	3.7

To determine the viability of this approach for field measurements, a high number of run with the same coated capillary were carried out to study the capillary performance. A total number of 50 runs were consecutively performed without observing a significant variation of results. The RSD values for the migration times of MDEA, EDEA, and TEA were 1.5%, 1.3%, and 1.2%, respectively. The RSD values for the

peak areas for MDEA, EDEA, and TEA were 4.0%, 3.9%, and 2.7%, respectively.

Additionally, the best conditions for the storage of the coated capillary were studied to determine an appropriate capillary preservation. The capillary was stored filled with buffer for 3 days without a significant decrease in performance. However, when the capillary was stored filled with polymer or with air no reproducible result were obtained.

3.3. Optimization of the P-CE separation conditions and application to water samples

The main consideration for the selection of a suitable running buffer in this work was based on the ionic characteristics of the basic analytes studied (**Figure II.3.1**) and the conductivity of the buffer and the analytes. Buffers of low conductivity must be chosen for conductivity detection to minimize Joule heating. Moreover, buffers with very high conductivities result in baselines instabilities.[22] Four different buffers of pH-values below the pKa-values of the analytes were used for their separation. At these pH-values the species are in their cationic form and migrate to the cathode. His was selected as buffer co-ion in the buffers of pH 4.0, 5.0. MES was used as chosen counter-ion in the buffer of pH 6.0. Only acetic acid was used in the composition of the buffer of pH 3.0. According to the study performed by Wang and col.,[43] P(VP-co-DMAEMA) greatly suppresses the EOF at pH range from 3 to 10. The EOF approaches zero at about pH 5.7. Above and below this value, the direction of the EOF changes. At acidic pH the amine groups of poly(DMAEMA) chain are protonated and there is an anodic EOF because of the positive charges on the

capillary surface, whereas at basic pH the EOF migrates to the cathode because the surface is slightly negatively charged. Buffers at pH-values which provide a positive net charge in the inner surface of the capillary can, additionally, decrease the adsorption of the cationic analytes to the capillary wall. To avoid the presence of negative charges in the coated capillary surface and undesirables analyte-wall interactions, buffer at pH-values above 6.0 were not used.

We noticed that the capillary coating was quickly removed when using the buffer of pH 3.0. For this reason, this buffer was not considered for further analyses. **Figure II.3.4** shows the electropherograms for a mixture of MDEA, EDEA, and TEA using the three remaining buffers. As expected, the compounds were well separated according to their increasing size, making possible their identification in all buffers. It can be noted that the noise in the baseline was higher when using the buffer composed of His and acetic acid at pH 4.0 than when the same buffer at pH 5.0 was used. In turn, the baseline produced by the buffer composed of MES and His showed the lowest noise of the three compared buffers. This can be explained because the buffers with acetic acid have higher conductivities than MES buffers. In addition, in order to decrease the basic pH obtained with the His solution more acetic acid was added to the buffer at pH 4.0 than to the buffer at pH 5.0. In consequence, the baseline of the signal produced with the buffer at pH 4.0 showed the highest noise. As a result, MES/His at pH 6.0 was chosen as the running buffer for this study, because it provided the best signal to noise ratio and, therefore, the best detectability. A commercially available detector was used for the separation optimization while a purpose-made C^4D detector for the field application. Consequently, before the application was performed a comparison of the signal-to-

noise ratios obtained by both detectors was made. Similar signal-to-noise ratios were collected by both detector systems. As example, when the mixture of MDEA, EDEA, and DEA was injected at a concentration of 20 μM, the signal-to-noise ratio values were in the range of 6-6.5 for both detectors.

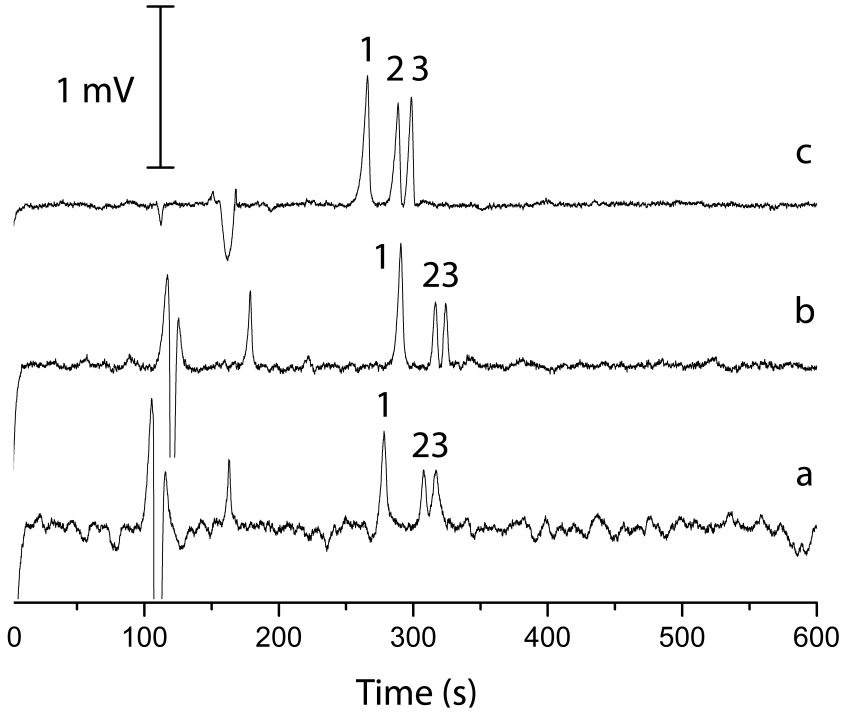

***Figure II.3.4**. Electropherograms for a mixture of 20 μM of (1) MDEA, (2) EDEA, and (3) TEA in a coated capillary using: (a) 12 mM His adjusted to pH 4.0 with acetic acid; (b) 12 mM His adjusted to pH 5.0 with acetic acid; and (c) 20 mM MES/His pH 6.0. Capillary total length: 60 cm. Length to the detector: 45 cm. Injection time: 5 s.*

Due to the characteristics of the target analytes, it was impossible to find sources of water contaminated with MDEA, EDEA, or TEA. For this reason, samples of water from different sources were spiked with the analytes and then analyzed to study the selectivity of the electrophoretic method. A total of 4 different samples were collected, namely water from three rivers and from a well. **Figure II.3.5** shows the electropherograms for each of the analyzed samples. MDEA, EDEA, and TEA peaks are well resolved among them and from the first big multiple peak. This peak corresponds to the bulk of the fast analytes in the matrix which do not interfere with the analyte signals. Calculated LODs for MDEA, EDEA, and TEA were 4.4, 5.7, and 5.5 μM, respectively.

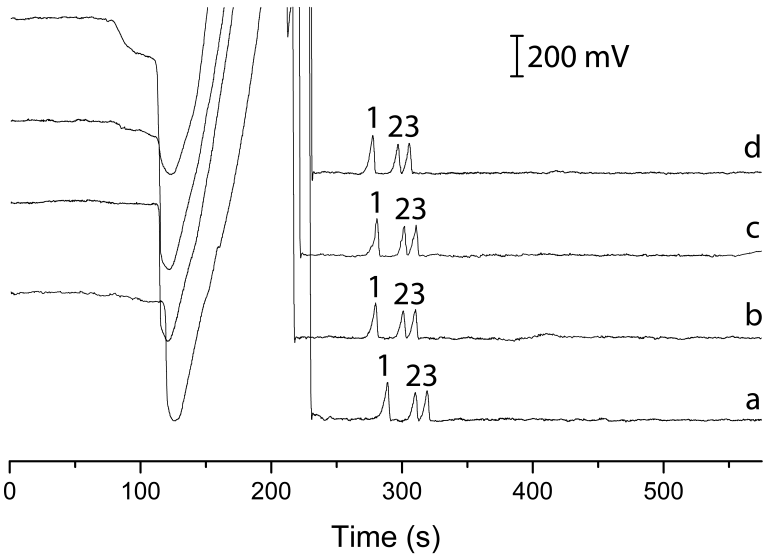

***Figure II.3.5**. Electropherograms of the samples of water spiked with 20 μM of (1) MDEA, (2) EDEA, and (3) TEA. Running buffer; 20 mM MES/His pH 6.0. (a) well water; (b) Pisuerga river water; (c) Pas river water; (d) Ebro river water. Other experimental conditions as in **Figure II.3.4**.*

4. Conclusions

In this work, NM degradation products have been determined in water samples with a P-CE system. The employment of portable instruments allow fast *in situ* analyses and is of great importance in cases of safety threats. The P-CE used included news features compared to the previous P-CE:[36] a) the possibility of introducing up to 4 different solutions, which allowed the fully automated pre-run capillary conditioning; b) a new redesigned grounded interface allowing the sample injection by hydrodynamic sample splitting with the minimum electric power consumption; and c) the use of a normally open valve as V1, which avoids the decrease of the migration times reproducibility obtained when a normally close valve was used before the separation step. A polymeric coating with P(VP-co-DMAEMA) was optimized to work with a capillary, avoiding undesirable analyte-wall interactions that affect the electrophoretic separation. Higher pressures and temperatures in the coating procedure showed better repeatability of the migration times, compared with the coating procedure found in literature. Finally, the optimized electrophoretic method permitted a clear determination of the NM degradation products in the different water samples analyzed (from a well and three rivers) without any matrix interferences and achieving LODs in the 5 μM range.

Acknowledgments

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CHAPTER II.4

IN SITU DETERMINATION OF SCOPOLAMINE IN EVIDENCE OF RECREATIONAL AND PREDATORY USE

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Abstract

In the recent years, scopolamine has become a drug of common use for predatory and recreational purposes and several ways of administration have been devised for its administration or abuse. Therefore, there is a need for new methodologies for the determination of scopolamine in the different evidential items used, which usually constitute highly complex matrices. In this work, a fast capillary electrophoresis method was firstly devised for the separation of scopolamine and atropine, which tend to co-migrate when they exist together in the same sample under certain electrophoretic conditions. For the first time, a contactless conductivity detector was used for the detection of both substances. Then, scopolamine was determined in the most commonly used evidential items, which have recently been employed in different criminal actions or abused with fatal consequences. First, an infusion of *Datura stramonium* L. used with recreational purposes, in which scopolamine and atropine exist together, and then a spiked moisturizing cream and 6 spiked alcoholic beverages, used with predatory purposes, were analyzed. In spite of the complex specimens analyzed, the sample pre-treatments developed were simple and fast and can be readily carried out at the place of evidence collection. For this reason, a portable capillary electrophoresis, allowing the *in situ* determination of the drug has been used. The method has been proved to be very highly selective for scopolamine, due to the characteristic signal of scopolamine produced by the contactless conductivity detection employed.

1. Introduction

Scopolamine is a tropane alkaloid extensively used for clinical purposes because of its strong parasympatolytic, anticholinergic and anti-emetic actions.[1,2] This alkaloid has an inhibitory effect on acetylcholine muscarinic receptors, having a more effective influence on neurotransmission pathways involved in memory, causing a box lock amnesia by basal nucleus of Meynert, which is an important structure for the amnesic functions, especially the fixing of memory.[3] Moreover, scopolamine can block free will. This is why scopolamine is used as an assault drug for drug-facilitated robberies and drug-facilitated sexual assaults. Scopolamine is also known as "burundanga" within the circles of people who abuse it. Scopolamine is tasteless and odorless and can be easily absorbed in the digestive tract, or orally, dermally, or by inhalation.[3] For these reasons, several ways of administration have been devised by the attackers to administrate the drug. For example, it has been reported that scopolamine powder can be blown onto the face of the victim, who will be under the drug effects within minutes. Recently, in Madrid (Spain), one person was arrested after a complaint from a woman who had been drugged with scopolamine. The suspect, who pretended to be a shaman, took the woman to his house and, after drugging her with a drink to which scopolamine had been added, he sexually assaulted her several times.[4] After this case, 38 more women reported having been raped by the same person.[5] Scopolamine has allegedly also been added to moisturizing creams, which are applied by the ingenuous victims themselves causing them to be under the influence of the drug. Scopolamine has also been added to drinks in night clubs to commit robberies or sexual assaults. Approximately, one in eight emergency room admissions by poisoning in Bogotá (Colombia) have

been attributed to scopolamine.[3] However, in spite of its characteristics, no mention of scopolamine can be found among the information about predatory drugs, for which typical examples are Ecstasy, Ketamine, Rohypnol, Gamma-Hydroxybutyrate (GHB), and Gamma-Butyrolactone (GBL).[6-9]

On the other hand, due to its hallucinogenic effects scopolamine is also used as a recreational drug. Like other tropane alkaloids, such as atropine, scopolamine is produced by plants of the Solanaceae family such as *Hyoscyamus albus* L, *Datura stramonium* L., *Mandragora autumnalis* Bertol., *Scopolia carniolica* Jacq., *Brugmansia candida* Pers. and other plants belonging to the same plant genera. Many species of this family are used as food plants, medicinal, or ornamental plants. These plants are naturally distributed along the world, mainly in temperate regions, and several of them are used in gardening. They can be smoked and much information can be found in Internet forums about how to prepare an infusion from the plants, either roots, leaves, stems, flowers, or fruits and seeds.[10,11] The ingestion of scopolamine is very risky, since its active dose is very close to the lethal dose. Recently, in Madrid (Spain), two 18 years old men died and one more was hospitalized in serious condition after drinking an infusion made with seeds.[12,13]

Additionally, the Security Forces of Spain have been removing plants of the Datura genus and other related species from the land surrounding some cities during the last years.[14] However, plants of Datura genus coexist with corn crops where the plants have found the optimal medium for living. Therefore, these plants have a wider distribution year on year.[15]

As above-explained, scopolamine is becoming a drug of common use in some countries, either for recreational use, predatory use, or for robbery purposes. However, it is eliminated very rapidly from the body, which precludes the detection of its presence in the organism after 24 hours.[3] Moreover, the victims usually do not report the assaults out of shame, or complain too late, when the drug has already been eliminated from the organism. Then, it becomes necessary to analyze the evidence used to administer the drug.

Different techniques have been used for the analysis of samples with scopolamine, such as gas chromatography.[16,17] and high performance liquid chromatography.[18–29] Most of these studies have been focused on the determination of scopolamine in plant material.[16,17,25,28,29] Scopolamine has also been determined in serum and plasma[18,19,27] and other biological samples, such as urine[22] or hair.[21,26] Additionally, scopolamine has been determined in pharmaceuticals.[20,23,24] CE has also been used for the determination of scopolamine in plant samples[1,29-37] and in pharmaceutical compositions.[2,38]

In the case of CE, a variety of detectors have been employed, such as time of flight-mass spectrometry and ion trap-mass spectrometry,[33] electrochemiluminescence,[1,30,37] electrochemistry,[1] and DAD or UV detectors.[2,31,32,34-3638] However, contactless conductivity detection has not been employed yet. C^4D is a highly sensitive tool in CE, which has been widely used in the recent years for many application (see, for example, the following review articles[39-41]). C^4D detectors are small, light, and do not consume much electrical power. Moreover, the placement of the capillary is easy and removal of the polyimide coating is not required. These advantages make C^4D significantly

suitable for portable analysis, which is required for the *in situ* analysis of evidential items.

Different CE methods have been devised for the simultaneous determination of scopolamine and other related compounds present in the same sample. For example, atropine and scopolamine have been separated in *Datura metel* L. samples to investigate the effect of space environment on the content of both substances.[34] Scopolamine and atropine derivatives have also been separated for their simultaneous determination in pharmaceutical formulations.[38]

With regard to their use as drugs, scopolamine can exist together with atropine in certain specimens and so the availability of methods allowing their separation is cardinal since both substances show different properties. Among others differences, scopolamine is much more likely to produce sedation and amnesia than atropine. Therefore, both substances could, in principle, be used for different purposes and, accordingly, their simultaneous determination is samples in which scopolamine and atropine are present is necessary.

The aim of this work was to apply a P-CE with C^4D for the separation and characteristic detection of atropine and scopolamine in evidential items used with recreational purposes where both analytes are present (infusion of *Datura stramonium* L.) or evidential items spiked with scopolamine used with predatory purposes (moisturizing cream and beverages). An effort was made on developing simple and fast sample treatments able to be carried out in the field.

2. Materials and methods

2.1. Reagents and samples

All the chemicals used were of analytical grade. Tris(hydroxymethyl)aminomethane (Tris), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), NaOH, atropine and scopolamine hydrobromide were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol was from Scharlau (Barcelona, Spain). Ultrapure water was obtained from a Millipore Milli-Q water system (Bedford, MA, USA). Soft drink and alcoholic beverages, as well as the moisturizing cream, were purchased in a local supermarket. Samples of *Datura stramonium* L. were collected in San Andrés del Congosto (Guadalajara, Spain) in mid-January and stored at room temperature.

2.2. Instrumentation and experimental procedure

CE experiments were performed on a purpose-built P-CE. The P-CE was previously described by Sáiz and col.[42] The instrument was fitted with a contactless conductivity detector (eDAQ, Deninstone East, NSW, Australia). The detector was modified to work at 12 V with the batteries of the P-CE. The detector excitation frequency was set to 1200 kHz and the amplitude to 100%.

Bare fused silica capillaries of 50 μm I.D. and 365 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA) with a total length of 80 cm and an effective length of 65 cm were employed. New capillaries were conditioned by flushing 1M NaOH for 40 min, water for 5 min, and running buffer for 30 min. The running buffers consisted on 10 mM

HEPES/Tris at different pH values comprised from 7.2 to 7.6. The pH-values of the running buffers, which were prepared daily, were determined with a pH-meter model Crison GPL-21 (Crison Instruments, Barcelona, Spain). Optimized conditions were achieved when the buffer at pH 7.6 was used. The injection was carried out by sample splitting in the interface for 3 s and the separations were carried out by applying -25 kV at the outlet of the capillary. After each analysis, the capillary was rinsed with the running buffer for 4 min to maintain the reproducibility of the analyses. When a new buffer was used, the capillary was rinsed with water for 5 min and then with the running buffer for 5 min.

2.3. Evidence preparation

Stock solutions of 1 mg/mL of atropine and scopolamine were prepared in methanol. For the identification of scopolamine and atropine peaks in the electrophoregrams in the electropherograms, the samples were spiked with the standards.

2.3.1. Infusion specimen

Fresh seeds of *Datura stramonium* L. were homogenized in a domestic grinder. 1 g (fresh weight) of the homogenate was added to 50 mL of water and heated on a hot plate until it just began to boil. After cooling down, the infusion was filtered by gravity through a Whatman filter grade GF/A (1.6 μm) in a conical funnel. Samples were then directly injected without dilution into the P-CE system.

2.3.2. Moisturizing cream specimen

Samples were prepared by dissolving 5 mg of scopolamine in 500 μL of methanol employing a vortex. The solution was added to 5 g of the moisturizing cream in a polypropylene tube, which was vortex-mixed for 2 min. The extraction of scopolamine from the spiked moisturizing cream was carried out by adding 5 mL of methanol to 5 g of the moisturizing cream evidence. The mixture was vortex-mixed for 2 min and then centrifuged 10 min at 4000 g. After centrifugation, three phases were clearly visible and the middle liquid phase was recovered by puncturing the tube with a syringe. Finally, the sample was diluted 10-fold (v/v) in water and injected in the P-CE system without filtration.

2.3.3. Beverage specimens

The beverages studied were prepared using 1/3 (v/v) of the alcoholic drink and 2/3 (v/v) of the soft drink because these proportions are usually utilized in mixed alcoholic beverages. The beverages prepared were: whisky with cola, rum with cola, rum with lemon, gin with tonic water, vodka with tonic water, and vodka with lemon. The total volume of the drink was considered to be 150 mL. Scopolamine (MW 438.31) was added to the beverages in a concentration of 1.3 mM. Then, samples were vortex-mixed for 2 min, diluted 5-fold (v/v) in water, and directly injected in the P-CE system without filtration. Corrected peak areas were calculated dividing the peak area by the migration time of the corresponding peak to correct the area deviation produced when peaks are delayed depending on the sample.

2.4. Data treatment

The electropherograms were treated in Origin (OriginLab Corporation, USA). LOD were estimated as three times the signal to noise ratio in the electropherograms obtained for the spiked beverages and the moisturizing cream. The noise value was measured as the maximum deviation of the baseline around the migration time of the analyte signal.

3. Results and discussion

After preparing the samples and developing evidence pre-treatments that can be readily and quickly carried out at the place of evidence collection, the separation conditions were optimized with the aim of obtaining the complete baseline separation of atropine and scopolamine. First, both compounds were determined in an infusion prepared with *Datura stramonium* L. seeds, normally used with recreational purposes. Then, two types of spiked specimens (a moisturizing cream and 6 commonly consumed beverages) were also investigated.

3.1. Preparation of the specimens

3.1.1. Infusion specimen

Seeds of *Datura stramonium* L. were homogenized and directly boiled to prepare the infusion. This drink has a increasing popularity for its use with recreational purposes.

3.1.2. Moisturizing cream specimen

Nowadays, Novartis™ is marketing Transderm Scōp® patches for people who are suffering from motion sickness. These patches contain 1.5 mg of scopolamine and are programmed to deliver in vivo approximately 1.0 mg of scopolamine over 3 days.[43] Considering this therapeutic dosage, a concentration at the rate of, approximately, 1 mg/mL of scopolamine was added to the moisturizing cream (5 mg of scopolamine in 5 g of moisturizing cream) since it can be used with predatory purposes.

3.1.3. Beverage specimens

The beverages studied were chosen for being commonly consumed. The amount of scopolamine added to the beverages was chosen considering the available data about lethal and active dosages of scopolamine. As example, the ingestion of 10 mg has been reported lethal in children, whereas adults survived more than 100 mg of scopolamine.[44-46] However, exact data on lethal and active doses of scopolamine are lacking.[44,47] For this reason, a concentration of 1.3 mM of scopolamine (MW 438.31), representing a total amount of 85.5 mg in 150 mL of drink, was chosen for the preparation of the beverage samples.

This concentration was chosen because high concentrations of scopolamine are required to induce the symptoms of amnesia, sedation, and loss of free will without risks of death.

3.2. Selection of evidence treatment

When samples are being analyzed *in situ*, it is important to be able to rely on treatment procedures which allow the recovery of the majority of the analyte in the shortest time possible, without many steps in the procedure. This prevents contamination of samples and allows for quick results, which usually are necessary when doing *in situ* analyses. In this context, evidence treatments as fast and simple as possible were designed.

Beverage samples were just diluted 5-fold because of the high concentration of scopolamine in the drinks and no further sample treatment was needed before the analysis. This sample treatment was carried out within 1 min.

The infusion evidence was filtered by gravity through a membrane filter in a conical funnel and directly injected in the P-CE system. This step does not require specific instrumentation, such as vacuum filters, and was carried out manually in two minutes.

In the case of the moisturizing cream sample further treatment was needed due to the complexity of the evidence item, which was viscous and non-aqueous. However, an easy step of centrifugation with methanol followed by a 10-fold dilution in water of the middle phase was enough to detect the scopolamine contained in the moisturizing cream and to have a clean sample suitable for injection into the P-CE. This step can be carried out in a portable centrifuge. The whole process was accomplished in less than 15 min.

Therefore, the time needed for the treatments of the infusion and the beverages specimens was insignificant. The moisturizing cream needed more time to be treated, but considering the complexity of the sample, the time spent was reasonable short. Although, all the above-explained sample treatments can be readily carried out at the place of evidence collection, being possible the in situ analysis of these specimens.

3.3. Optimization of the separation conditions

Atropine and scopolamine are two tropane alkaloids present in Solanaceous plants. Both tropane alkaloids have very similar structures (**Figure II.4.1**).

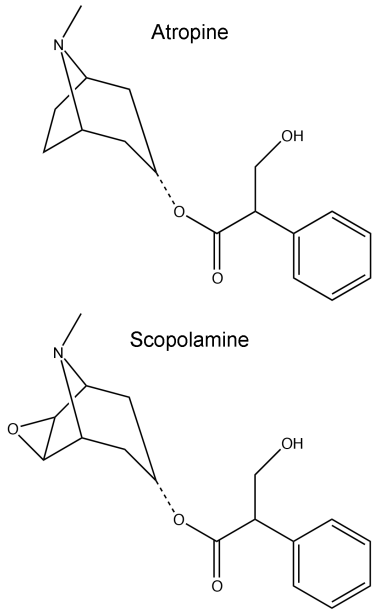

Figure II.4.1*. Structures of atropine and scopolamine.*

Hence their electrophoretic separation could be difficult at certain pHs. However, the separation of scopolamine and atropine is necessary for the determination of scopolamine in evidence in which both compounds are present, such as plant samples. The pKa values of atropine and scopolamine are 9.8 and 7.55, respectively. In theory, a pH close to the pKa value of scopolamine is expected to separate atropine and scopolamine because atropine has more net charge and would migrate faster than scopolamine, that is, at shorter migration time.

Buffers consisting of HEPES/Tris at pH 7.6 as running buffer was prepared at three different concentrations (10, 15, and 20 mM). The buffer consisting on 10 mM HEPES/Tris was chosen for showing the highest signal-to-noise ratio. Then, three pH values close to the pKa of scopolamine, namely 7.2, 7.4, and 7.6. Using a pH value of 7.6, a capillary with a total length of 80 cm and 65 cm to the detector was needed to achieve the initial separation of atropine and scopolamine. When shorter capillaries were used, a single peak was observed. The electropherograms for a mixture of 100 μM of scopolamine and 100 μM of atropine using the three above-mentioned buffers are shown in **Figure II.4.2**.

As expected, scopolamine migrated after atropine and the detection of both compounds at the three selected pHs was possible. At the working pHs, scopolamine and atropine have low charge and their equivalent conductivities are lower than the conductivity of the running buffers. Note that for this reason, the detector signals have been inverted in this work in order to show positive-going peaks for scopolamine and atropine.

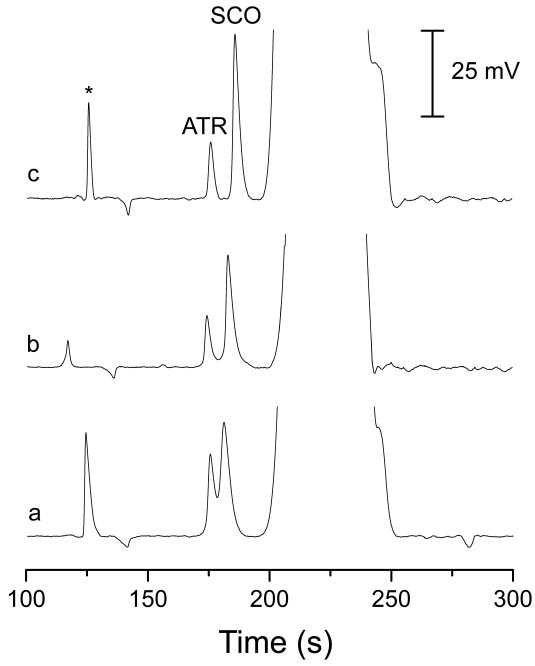

***Figure II.4.2**. Electropherogram for a standard mixture of 100 μM of scopolamine and atropine in (a) 10 mM HEPES/Tris pH 7.2; (b) 10 mM HEPES/Tris pH 7.4; and (c) 10 mM HEPES/Tris pH 7.6. CE conditions: capillary length, 80 cm (65 cm to the detector); applied voltage, -25 kV; hydrodynamic injection for 3 s; pressure in the system set at 1.5 bar. SCO, scopolamine; ATR, atropine; *, system peak.*

In **Figure II.4.2** it can also be observed that, when the pH values are increased from 7.2 to 7.6, scopolamine is loosing its net charge and getting closer to the peak attributed to the EOF signal migrating after scopolamine peak. The buffer at pH 7.6 was chosen as the running buffer because, as can be seen in **Figure II.4.2**, the complete baseline separation of scopolamine from atropine was achieved when this

buffer was used. Moreover, at this pH the scopolamine peak did not overlap to the peak attributed to the EOF signal.

Although the concentrations of scopolamine and atropine were the same, there are differences in their peak heights. This is due to the different net charges and limiting equivalent conductivities of the analytes, which are lower for scopolamine at the given pHs. For this reasons, the peak of scopolamine is higher than the peak of atropine. On the other side, the peak height of scopolamine increases with the pH value because scopolamine has lower limiting equivalent conductivity at pH 7.6 than at pH 7.4 or 7.2. Therefore, the buffer at pH 7.6 was chosen to allow the baseline separation of scopolamine and atropine and to show the highest signal-to-noise ratio for scopolamine. The strong EOF created at the working pH allowed the determination of scopolamine and atropine within 3.5 min.

3.4. Evidence analysis

Prior to the application of the developed method to the evidence analysis, its analytical performance in terms of LODs and precision, measured as repeatability and reproducibility, was studied. The LODs for scopolamine were calculated to be 1.2 μM in beverages and approximately 1.3 μM in the sample of moisturizing cream. Repeatability of 10 consecutive beverage analysis provided RSD values of 1.8% for migration times and 6.5% for corrected peak areas. The inter-day reproducibility for the same samples during 3 different days gave RSD values of 3.3% (n=18, 3-10 analysis per day) for migration times and 15.0% (n=18, 3-10 analysis per day) for corrected peak areas.

First, an infusion of seeds of *Datura stramonium* L. was analyzed because it may be consumed with recreational purposes. Then a moisturizing cream and 6 different alcoholic beverages, all spiked with commercially available scopolamine, were also analyzed. These evidential items were chosen for being commonly used for the administration of the drug with predatory purposes.

Figure II.4.3 shows the electropherogram for an infusion of seeds of *Datura stramonium* L. In this evidence, a group of fast compounds migrated first showing higher conductivities than the baseline. This group of compounds corresponded to the sample matrices. Following, atropine and scopolamine peaks were determined, as expected, for being part of the biological tissue. After scopolamine a large peak attributed to the EOF signal appeared. In this case, changes in the migration times of scopolamine and atropine, compared with those shown in **Figure II.4.2**, were attributed to the changes in mobility caused by the sample matrix because direct injection of the sample was performed without any dilution or purification.

The electropherogram for the moisturizing cream evidence spiked with scopolamine is shown in **Figure II.4.4**. As in the previous case, scopolamine is shown as a characteristic positive-going peak between the group of negative-going peaks belonging to the sample matrix and the large peak attributed to the EOF signal. In this case, a good resolution of scopolamine from other peaks was achieved, making possible its determination.

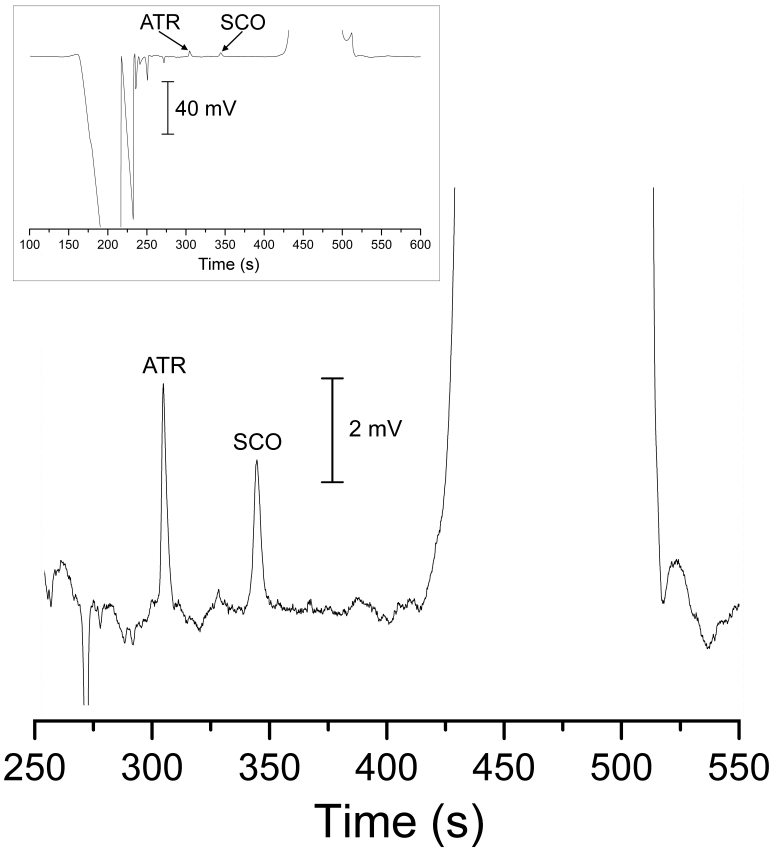

***Figure II.4.3**. Electropherogram for the determination of scopolamine and atropine in a infusion of seeds of Datura stramonium L. The reduced image shows the electrophoregram in which the big peaks produced by the sample matrix are shown. Other experimental conditions as in **Figure II.4.2**. SCO, scopolamine; ATR, atropine.*

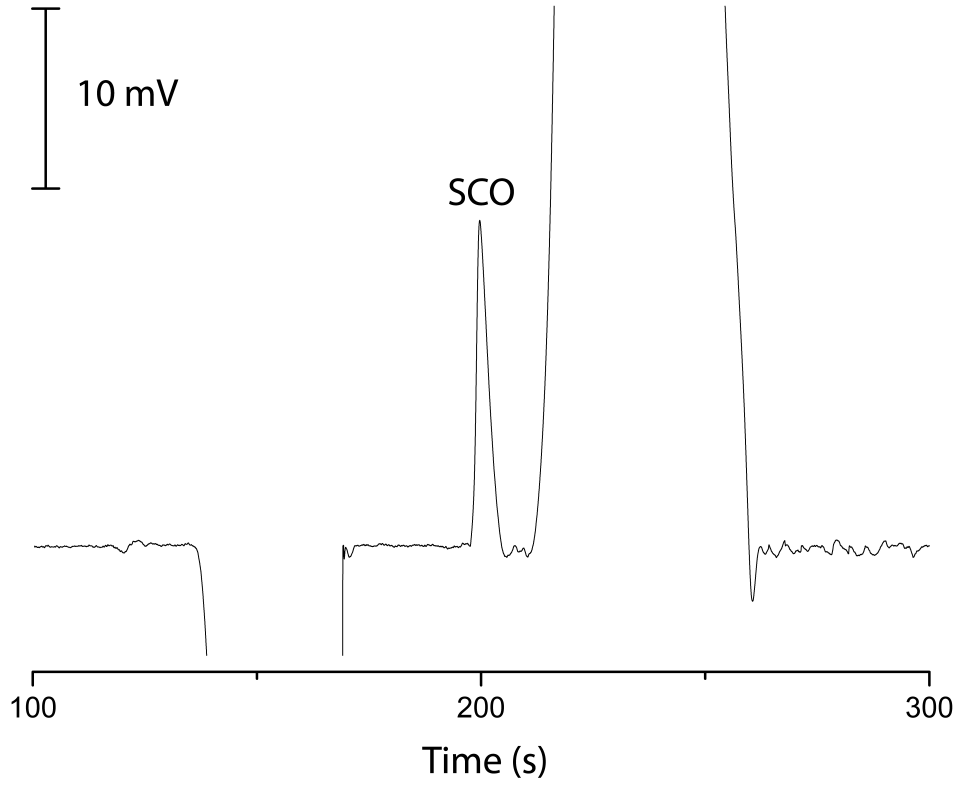

***Figure II.4.4**. Electropherogram for the extract of the moisturizing cream spiked with scopolamine diluted 10-fold in water. Other experimental conditions as in **Figure II.4.2**. SCO, scopolamine.*

The electropherograms for the six spiked alcoholic beverages analyzed are depicted in **Figure II.4.5**. All the beverage samples showed similar electropherograms. The scopolamine peak appeared after the sample matrix peaks and before the peak attributed to the EOF.

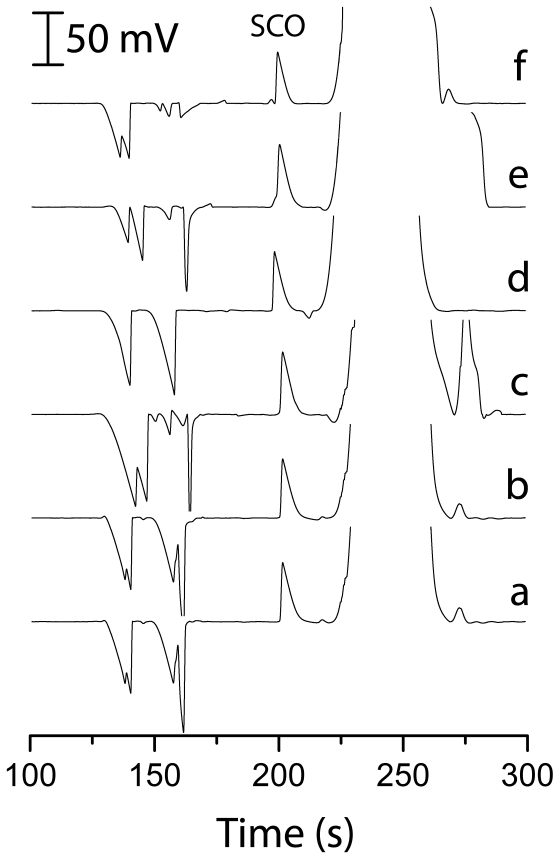

***Figure II.4.5**. Electropherograms for alcoholic beverages spiked with 1.3 mM of scopolamine. (a) whisky with cola, (b) rum with cola, (c) rum with lemon, (d) vodka with lemon, (e) vodka with tonic water, and (f) gin with tonic water. CE conditions: buffer, 10 mM HEPES/Tris pH 7.6; samples diluted 5-fold in water. Other electrophoretic conditions as in **Figure II.4.2**. SCO, scopolamine.*

As can be seen in **Figures II.4.3, II.4.4,** and **II.4.5**, the method has been proved to be highly selective for scopolamine and atropine due to the characteristics peaks showed in the electrophoregrams. C^4D allowed to detect the peaks corresponding to atropine and scopolamine very easily in all the evidential items analyzed. This can be extremely useful in cases of routine screening of evidential items. If the samples do not show the typical positive going peaks for scopolamine and atropine between the first negative-going peaks and the last positive-going peak, they can be discarded as positive to scopolamine and no further experiments will be needed. However, if those typical peaks are identified, the samples can be fortified with standards to confirm the presence of the drugs. This practice can reduce the time spent in screening operation in the search of evidence containing scopolamine when several evidential items are intended to be analyzed.

4. Conclusions

Nowadays, scopolamine has become a drug of increasing use in Europe, and several ways of administration and consumption have been devised. In this work, several commonly used evidential items for the administration or consumption of scopolamine for predatory, robbery, and/or recreational purposes have been analyzed. The developed sample treatments were simple and can be readily carried out within a few minutes at the place of evidence collection. The employment of a P-CE also allowed the analysis of evidence at the place of collection. A HEPES/Tris buffer at pH 7.6 allowed the baseline separation of atropine and scopolamine in samples in which both compounds are present, such an infusion of *Datura stramonium* L. Independently of the evidence analyzed, scopolamine was clearly

detected and, interestingly, showed a highly characteristic peak in the different evidential items studied (the infusion of seeds of *Datura stramonium* L., the moisturizing cream and the 6 alcoholic beverages spiked with scopolamine).

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CONCLUSIONS
AND
FUTURE OUTLOOK

This thesis addresses to the resolution of a variety of forensic issues. These issues include the study of suitable dynamite packaging and the *in situ* analysis of other forensic evidential items.

- On the one hand, the problematic of a proper dynamite packaging has been studied.

  - Firstly, an analytical method was designed for the determination of EGDN in dynamites. An extraction method was selected to achieve the total recovery of the EGDN contained in the Goma 2-ECO dynamite by means of the total dissolution of the whole dynamite. Then, a HPLC method was developed for the resolution of EGDN from other explosives that may be present in dymanites such as nitroglycerine, trinitrotoluene and dinitrotoluene isomers. This method was proved to be selective, sensitive, and reliable prior to its application to the quantitation of EGDN in the studied dynamite. The results obtained showed that EGDN quantified was 30.29%, which is in accordance with the manufacturer's specifications for the studied dynamite (25.7–31.4%). These results show the high potential of the proposed method to perform quality controls of plastic explosives containing EGDN.

  - Following, the suitability of the polyethylene bags used by the Spanish Security Forces to preserve explosive evidence was investigated. The results showed that the volatilization of EGDN and DNT from dynamites occurred gradually over time, happening faster at higher temperatures. Moreover, the

validated analytical method above-mentioned was applied to demonstrate cross-contamination due to DNT between dynamites stored together. The contamination by DNT was proved to occur gradually in time until a stationary state was reached. Therefore, polyethylene bags are not ideal for the transport and storage of specimens of dynamites at room temperature. In addition, the possibility of cross-contamination should be considered when interpreting results for dynamites which have been stored in these bags. It was recommended to store these explosives at low temperature, in refrigerated chambers, to diminish the volatilization process. The substitution of polyethylene bags by other fashion of more suitable packaging containers was also recommended.

- The polyethylene bags were then compared with DUO plastic bags in order to know which bags showed better retentiveness for the volatile dynamite constituents. DUO plastic bags were proved to be much more suitable than the polyethylene bags, although a weak signal of EGDN was detected outside the bags after eleven weeks of storage. Moreover, DUO bags showed less background signal than the polyethylene bags. After this study, the employment of DUO plastic bags, instead of Royal Pack plastic bags, for the storage of dynamites was recommended. The study of the retentiveness of these bags when storing other kind of evidence containing volatiles was suggested before their use.

As a general conclusion, it could be stated that the perfect container does not exists yet. More studies should be carried out on different

containers, and not only for dynamites, which only represent a small proportion of the overall evidential items with volatile compounds, but for other explosives and forensic specimens of interest, such as accelerants, fire debris, etc. Manufacturers should be aware of those studies on their containers and should continue manufacturing new containers which overcome the weaknesses of the present containers. The forensic laboratories must be aware of those weaknesses of containers and study them carefully for the different evidential items that may be stored in. Meantime in the search of the perfect container, all the weaknesses of packaging must be considered in the forensic reports.

- On the other hand, the development and employment of P-CE for the application in forensic analyses has been covered.

  - Firstly, a P-CE-C^4D instrument with automated injection was built in-house showing a good performance with high reproducibility. The system was suitable to perform *in-situ* measurements and was optimized for different compromise conditions with regard to limits of detection, dynamic range, separation efficiency, and analysis time according to the task at hand. As demonstrated by the autonomous stability test, which extended over 8 h duration, the instrument has also the potential to be set up for unattended monitoring operations.

  - Since the employment of portable instruments allows in situ analysis and it is of great importance in cases of safety threats, a second version of the above-mentioned system was applied to the determination of NM degradation products in water samples. A capillary coating with a polymer was studied

proving that higher pressures and higher temperatures in the coating procedure leaded to better migration times repeatability. Moreover, the electrophoretic method developed permitted the clear identification of the NM degradation products in the different water samples analyzed without any matrix interferences. Therefore, the analytical method can be used for the analysis of water samples in search of MDEA, EDEA, and TEA resulting from the contamination with NM.

- Finally, the same P-CE was applied to the determination of scopolamine, for being a drug of increasing use in Europe, in a variety of samples. The sample treatments selected were easy and can be readily carried out within a few minutes at the place of evidence collection allowing the in situ employment of the P-CE. The electrophoretic method also allowed the fast simultaneous determination of scopolamine and atropine in samples in which both compounds are present. It was also useful for the determination of scopolamine in spiked samples. The C4D detection of scopolamine showed a highly characteristic peak and, therefore, the method can be used for the rapid screening of suspicious samples containing own or spiked scopolamine.

As a general conclusion, it has been proved that portable instrumentation has interesting applications in forensics. The most valuable characteristic is that portable instrumentation can be readily used at the place of sample collection, being of great importance for certain evidential items. New and automated portable instrumentation should be demanded by the forensic laboratories in

order to increase their analytical potential. The forensic interest in this instrumentation would stimulate the manufacturers to collaborate with researchers in the production of new and improved instruments. In this context, the researchers should continue with the production of new systems, which must be more versatile, automated, and easily manipulated by non-expert operators.

CONCLUSIONES
Y
PERSPECTIVAS DE FUTURO

Esta tesis está dirigida a la resolución de varios problemas forenses. Entre estos problemas se incluye el almacenamiento de muestras forenses y el análisis *in situ* de muestras forenses.

- Por un lado, se han estudiado distintos contenedores para el almacenamiento de muestras de dinamitas.

  - Primero, se diseñó un método analítico para la determinación de EGDN en dinamitas. Se seleccionó un método de extracción para conseguir recuperar la totalidad de EGDN en la dinamita Goma-2 ECO a través de la completa disolución de la misma. Después, se desarrolló un método de HPLC para resolver el EGDN de otros compuestos explosivos que pueden estar presentes en las dinamitas como la nitroglicerina, el trinitrotolueno o isómeros de dinitrotolueno. Se probó que este método era selectivo, sensible y fiable antes de aplicarlo a cuantificación de EGDN en la dinamita estudiada. Se cuantificó el EGDN en un 30.29%, lo cual concuerda con las especificaciones del fabricante (25.7–31.4%). Estos resultados muestran el gran potencial del método propuesto para llevar cabo controles de calidad para llevar a cabo controles de calidad de explosivos plásticos que contengan EGDN.

  - A continuación, se investigó la idoneidad de las bolsas de polietileno que las Fuerzas de Seguridad del Estado usaban para almacenar muestras de explosivos. Los resultados mostraron que la volatización de de EGDN y DNT ocurría gradualmente en las dinamitas, siendo más rápida a altas temperaturas. Además, el método analítico validado anteriormente se aplicó para demostrar que podía ocurrir

contaminación cruzada por DNT en dinamitas almacenadas juntas. Se demostró que esta contaminación cruzada ocurría gradualmente en el tiempo hasta alcanzar un estado estacionario. Por lo tanto, las bolsas de polietileno no son ideales para transportar y almacenar muestras de dinamitas a temperatura ambiente. Además, la posibilidad de que una contaminación cruzada pueda ocurrir debe considerarse a la hora de interpretar los resultados de dinamitas que se han almacenado en estas bolsas. Este estudió concluyó recomendando almacenar los explosivos a baja temperatura, en cámaras refrigeradas, para reducir el proceso de volatilización. También se sugirió la sustitución de las bolsas de polietileno por otro tipo de contenedor más apropiado para dinamitas.

- Las bolsas de polietileno se compararon con las bolsas DUO para conocer cuales mostraban mejor retención para los constituyentes volátiles de las dinamitas. Se probó que las bolsas DUO eran mucho más apropiadas que las bolsas de polietileno, a pesar de que se detectase una señal débil de EGDN después de once semanas de almacenamiento. Además, las bolsas DUO mostraban menos señal de fondo que las bolsas de polietileno. Después de este estudio, se recomendó el empleo de las bolsas DUO, en lugar de las bolsas de polietileno. También se sugirió el estudio de la capacidad de retención de estas bolsas cuando se pretenda almacenar otro tipo de muestras que contengan volátiles.

Como conclusión general se puede establecer que hoy en día el contenedor perfecto no existe. Se deben llevar a cabo mas estudios

sobre tipos de contenedores diferentes, y no solo para dinamitas, que sólo representan una fracción pequeña del total de muestras con compuestos volátiles, sino para otros explosivos y muestras forenses de interés, como acelerantes, restos de incendios, etc. Los fabricantes deben ser conscientes de los estudios que se hacen sobres sus contenedores y deben continuar fabricando contenedores que superen las limitaciones de los actuales. Los laboratorios forenses deben, a su vez, ser conscientes de esas limitaciones y estudiar el comportamiento de los contenedores con diferentes muestras. Mientras se busca el contenedor perfecto, todas las limitaciones de los contenedores deben considerarse en los informes periciales forenses.

- Por otro lado, se ha cubierto el desarrollo y empleo de P-CE para su aplicación en análisis forenses.

  - Primero, se construyó un equipo CE-C^4D con inyección automática que mostró una buena eficacia con una alta reproducibilidad. El equipo se utilizó para llevar a cabo mediciones en el campo y se optimizó para cumplir una serie de condiciones en relación a límites de detección, rango dinámico, eficacia separativa y tiempo de análisis de acuerdo a diferentes necesidades. Las pruebas de autonomía, que duraron más de 8 h, demostraron el potencial del equipo para llevar a cabo operaciones de monitorización sin la necesidad de un operario.

  - Ya que los equipos portátiles permiten análisis *in situ* y que esto es de gran importancia en casos de amenazas de seguridad, se utilizó una segunda versión del ya mencionado equipo portátil para la determinación de los productos de

degradación de las NM en muestras de agua. Se estudió un recubrimiento del capilar con un polímero probando que altas presiones y altas temperaturas en el proceso de recubrimiento conseguían mejoras en la repetitividad de los tiempos de migración. El método electroforético permitió la identificación clara de los productos de degradación de las NM en las diferentes muestras de agua sin picos interferentes. Por lo tanto, el método analítico puede usarse para analizar muestras de agua en búsqueda de la presencia de MDEA, EDEA, y TEA como resultado de la contaminación con NM.

- Por último, el mismo P-CE se aplicó a la determinación de escopolamina en varias muestras, por ser una droga cuyo uso está aumentando en Europa. Los tratamientos de muestra seleccionados fueron fáciles y se pueden llevar a cabo en pocos minutos en el lugar de recogida de muestra, permitiendo el empleo *in situ* del P-CE. El método electroforético también permitió la determinación de escopolamina y atropina en muestras en las que ambas sustancias están presentes. Además fue útil para la determinación de escopolamina en muestras adulteradas. La detección de escopolamina con C^4D mostró picos muy característicos y, por lo tanto, el método puede ser utilizado para llevar a cabo muestreos rápidos de muestras que contengan escopolamina de manera natural o que hayan sido cebadas con esta sustancia.

Como conclusión general, se ha comprobado que el empleo de P-CE tiene interesantes aplicaciones en la ciencia forense. La característica más importante es que la instrumentación portátil puede utilizarse directamente en el lugar de recogida de la muestra, siendo de gran

importancia en ciertos delitos. Los laboratorio forenses deben demandar nuevos equipos portátiles automáticos para aumentar su potencial analítico. El interés interés forense en este tipo de instrumentación estimularía a los fabricantes a colaborar con los investigadores en la producción de nuevos y mejorados equipos portátiles. En este contexto, los investigadores deben continuar con la producción de nuevos sistemas, que sean más versátiles, automatizados y más fáciles de manejar por operarios sin experiencia.

LIST OF AUTHOR'S CONTRIBUTIONS

Articles:

- M. López López, M. A. Fernández de la Ossa, J. Sáiz, J. L. Ferrando, M. Torre, C. García-Ruiz. *New protocol for the isolation of nitrocellulose from gunpowders: Utility in their identification*, Talanta, 2010, 81, 1742-1749.

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