D'Elia, Valentina, Montalvo, Gemma & García-Ruiz, Carmen, 2016. Analysis of street cocaine samples in nasal fluid by Raman spectroscopy. Talanta (Oxford), 154, pp.367–373.

Available at https://doi.org/10.1016/j.talanta.2016.03.077

ANALYSIS OF STREET COCAINE SAMPLES IN NASAL FLUID BY RAMAN SPECTROSCOPY

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Talanta, 2016, 154, 367-373

DOI: 10.1016/j.talanta.2016.03.077

ABSTRACT

The principal objective of this work was to demonstrate the capability of Raman spectroscopy to detect small amounts of cocaine (COC) in nasal fluid, and to selectively identify the main drug and the most widely used cutting agents. Initially, standard samples were analyzed and sampling conditions were studied by comparing different swabs used for the sample collection. Once the most appropriate swab was selected, which permitted a relatively simple detection of the standard cocaine hydrochloride (COC.HCl), qualitative analyses of real samples were carried out. Three street COC samples were analyzed, and the presence of cutting substances was highlighted by the appearance of different bands not corresponding to the ones of the standard COC. To identify the substances present in each sample, the spectra of the street COC samples were collected and compared with a digital library created on purpose with the spectra of the most common cutting agents. In this case, correlation coefficients permitted to recognize the most important substances presumably present in the samples, and gave an estimation of the purity of the COC. However, when nasal fluid was present, its strong signal could overlap or interfere with the smaller signal of the cutting substances, hindering their identification.

Introduction

Today, the main objectives of drug testing are the detection and identification of substances of abuse. COC, in particular, is the second most commonly consumed drug overall, after cannabis [1]. COC is a natural organic substance of vegetable origin belonging to the family of alkaloids. It is extracted from the leaves of the coca plant (Erythroxylum coca), mainly cultivated in the tropical regions of South America such as Colombia, Peru and Bolivia. This drug could be consumed in different ways according to the form in which it is purchased. It could be ingested by chewing its leaves directly, but it is also sold as freebase (crack) or in its hydrochloride salt form (powder). When it is in the freebase form it is smoked. When it is in the hydrochloride salt form it could be injected after the dissolution in water, or inhaled by sniffing or snorting. The last way is the most commonly employed and often leads to the persistence of drug residues in the nostrils [2]. For this reason, even if up to date different body fluids have been successfully used to perform drug analyses [3], in the case of COC, the analysis in nasal fluid could be much more useful.

The main functions of nasal mucus are to trap small particles such as dust, particulate pollutants, and allergens, and make sure they do not enter the respiratory system. Another advantage of this fluid over other biological fluids is that the parent snorted compound is present instead of its metabolites [4]. The nasal fluid analysis offers different advantages because it can be collected in a simple, inexpensive and non-invasive manner also by nonmedical personnel. However, the principal drawback of the use of nasal fluid is the small quantity of sample collectable.

Until now, very few works concerning the analysis of illicit drugs in nasal fluid have been published [4-10]. Furthermore, most of them focused principally on the study of the physiological effects caused by the use of COC, in human [5-7] but also in experimental animals [8, 9]. As far as the authors know, up to date, only two articles have been published dealing specifically on the detection of COC in nasal fluid [4, 10]. Both of these studies, however, employed infrared spectroscopy (coupled with ion mobility spectrometry) due to its high selectivity. In this study, instead, we propose the use of Raman spectroscopy as rapid and non-destructive analytical technique, which require only small amounts of sample with no or minor pretreatment.

Raman spectroscopy is able to recognize even slight differences in chemical structures and provides a characteristic spectrum for each compound. In the case of COC, this is of fundamental importance considering that, once placed on the market, the drug is often “cut” with a variety of substances to stretch the amount of the product and increase profits. These substances, called cutting agents, could be classified according to their effect when mixed with pure COC [11]. The Active Cutting Agents are stimulants, with psychoactive effects similar to those of COC, but more perceptible physically and with more lasting effect. The Cosmetic Cutting Agents are substances that simulate some side effects of COC and are made with substances used in medicine, which can be very dangerous when taken intravenously. Most of them are local anaesthetics, but also benzodiazepines can be included in this group. Finally, the Inert Cutting Agents are substances with similar appearance to the one of COC, but without side effect, which only serve to increase the volume [11].

As a consequence, the main objective of this work was to demonstrate the capability of Raman spectroscopy to detect small amounts of COC in nasal fluid, and to distinguish the main drug from the most widely used cutting agents. To achieve this purpose, a spectroscopic study on

standard COC samples was initially performed. The influence of the swab used to collect the samples was subsequently studied by comparing different commercial swabs. Then, different samples of street COC were analyzed to identify the main drug and the most important cutting agents used, by comparison with a digital library created on purpose with the spectra of a great quantity of substances that may be added to pure COC.

Materials and methods

Reagents

All the reagents used for the analysis of the cutting agents were supplied by Sigma-Aldrich (St. Louis, MO, USA). COC.HCl was acquired from Lipomed, while minimal quantities of street COC were donated by volunteer consumers. The commercial swabs were provided from Seidden (SWAB 1 - DNA free), from Deltalab (SWAB 2) and from local supermarkets (SWAB 3 - Dr.SOS and SWAB 4 - Día) (Spain). Real nasal fluid samples were provided by non-consumer volunteer donors.

Nasal fluid samples preparation

At first, four swabs were compared in order to select the most suitable to perform the analysis: two double-ended cotton tipped, and the other two with a unique cotton tip. The preparation procedure was the same in all cases. The swab was inserted into the nostril, approximately 1.5 cm, rotated three times (3 × 360° turns) to collect the fluid and then slowly removed.

Since there is not clear information about the quantity of COC that could remain in the nose after the snort of a line of COC (50-200 mg), the amount of COC used to dope the nasal fluid sample was arbitrarily selected. In this case, about 200 µg (estimated by weighing after deposition) of standard COC.HCl was used. The drug was deposited on a glass microscope slide and the nasal fluid was doped by rotating the swab on it until all the COC had been collected. Then, the swab with the doped nasal fluid sample was placed on the Raman microscope stage and directly analyzed.

To obtain the Raman spectroscopic signature of the undoped nasal fluid, instead, the sample was deposited directly on a glass support by sliding on it another wet swab and then immediately analyzed.

Instrumental

A Thermo Scientific DXRTM Raman Microscope (Waltham, MA, USA) was used to collect Raman spectra, by using a 532-nm laser excitation. The selected region of interest extended in the wavenumber range from 300 to 1800 cm-1 and a 50x objective was used. The laser power was set to 10 mW with an aperture of 50 µm. The exposure time for each scan was fixed to 15 s, while the number of scans was varied for the different analyses in order to observe the best spectrum, obtained by averaging all the spectra collected for each scan during a measurement.

All the spectra were collected without any automatic correction and then processed with the Thermo Scientific OMNICTM for dispersive Raman 8.3.103 software (Waltham, MA, USA): the fluorescence effect was corrected by means of a 3rd-order polynomial baseline, the noise interference was reduced by an 11-point smoothing and finally the spectra were normalized. In the case of the undoped nasal fluid sample, the glass background was also subtracted.

The same software was used to create the specific library of cutting agents and to carry out the matching among spectra by means of the "Library Search" function. The "correlation" algorithm was selected, since it removes any effect of offset in the unknown spectrum, thus eliminating the effects of baseline variation. All the graphs presented in this paper have been designed by using the OriginPro 8.6 software (Northampton, MA, USA).

Results and discussion

Reference spectra collection and peak assignment

Initially, the Raman spectra of the four swabs in comparison were collected and all of them showed identical spectra corresponding, as expected, to the one of the cotton (in figure 1, the spectrum of SWAB 1 has been represented as an example). Then, the spectra of standard COC.HCl and of undoped nasal fluid samples were measured as references and compared with the one of the swab. Figure 1 shows the collected spectra, in which all the most important bands have been indicated. As it can be seen, four of the characteristic bands of COC are also circled (1718, 1600, 1030 and 1003 cm-1), because they are the ones on which we will focus to verify if the drug is detectable in nasal fluid. The others (1280, 873, 789 and 617 cm-1¬), in fact, are less intense and could be overlapped by the large bands of the nasal fluid. Furthermore, a band of the nasal fluid spectrum has also been circled (1006 cm-1) because it is the only one that really could lead to misunderstanding in the identification of COC. On the contrary, the swab bands do not appear in the spectral fingerprint region of COC and, for this reason, none of them has been highlighted.

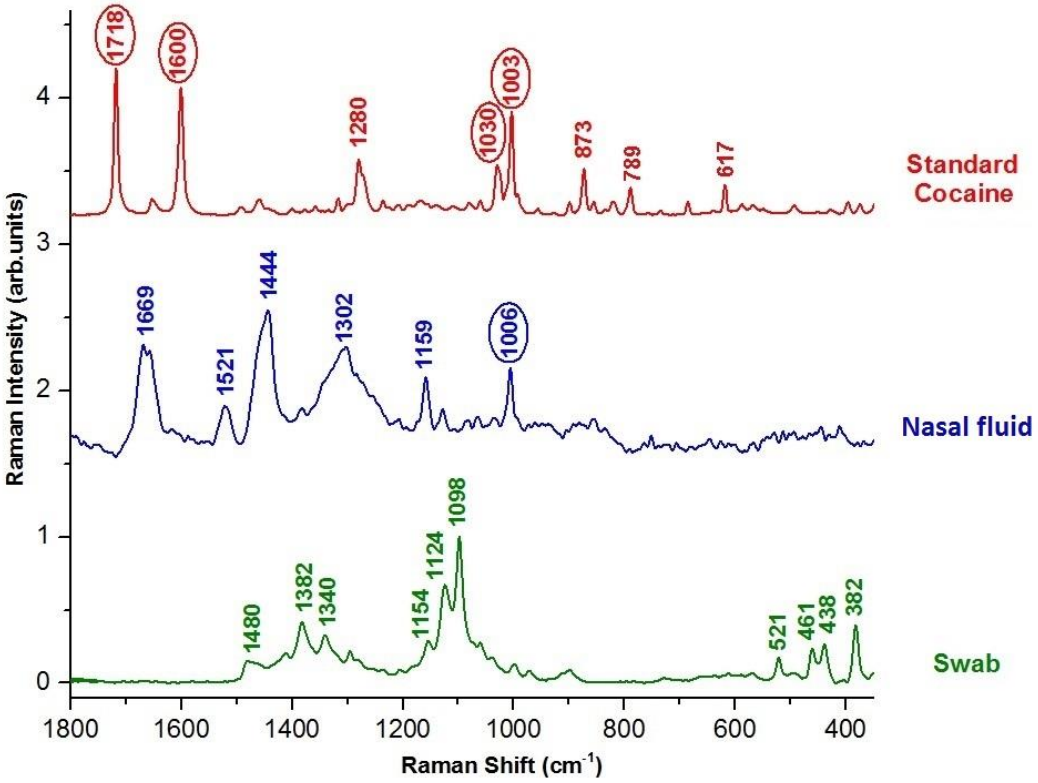

Figure 1. Spectra of standard COC, nasal fluid and swab (SWAB1). All the most important bands have been indicated. The ones circled in the first spectrum correspond to the characteristic bands of COC, the one in the second spectrum corresponds to the possible interfering band of the nasal fluid with the COC.

Considering the instrumental variability of the Raman spectrometer employed (about ± 4 cm-1), the bands of COC spectrum have been assigned as follows: the band at 617 cm-1, was attributed to the phenyl ring deformation; the band at 789 cm-1, corresponded to a piperidine ring C-C stretching; the band at 873 cm-1, was attributed to a tropine ring C-C stretching; the bands at 1003 and 1030 cm-1, corresponded respectively to the symmetric and asymmetric phenyl ring breathing modes; the band at 1280 cm-1, was attributed to the C-phenyl stretching; the one at 1600 cm-1, corresponded to the trigonal phenyl ring breathing mode and, finally, the band at 1718 cm-1, was attributed to the ester carbonyl C=O stretching [12, 13]. Also for the nasal fluid spectrum, the principal bands were highlighted, even though it was not possible assign them due to the complex composition of the fluid. However, the presence of antiseptic enzymes, glycosylated proteins and salts, could explain the great width of the bands. Finally, for the swab the strongest bands observed were at 1480, 1382, 1340, 1154, 1124, 1098, 521, 461, 438 and 382 cm-1. The bands at 1480, 1382 and 1340 cm 1, were associated with CH2 deformation vibrations, although the band at 1340 cm-1, could also be due to OH deformation vibrations. The bands observed at 1154, 1124 and 1098 cm-1, were attributed to the stretching vibrations of the β-1,4-glycosidic ring linkages between the D-glucose units in cellulose. The 1154 cm-1 band, was associated with C-C ring asymmetric stretching vibrations, while the 1124 and 1098 cm-1 bands, were due to the symmetric and asymmetric stretching modes, respectively, of the C-O-C glycosidic link. The 521 and 382 cm-1 bands were attributed to C-O-C glycosidic link deformation and C-C-C ring deformation vibrations, respectively. The bands at 461 and 438 cm-1, were associated to C-C-O ring deformation modes [14].

Comparison of the different swabs for the detection of cocaine in nasal fluid

As previously stated, since COC does not metabolize in nasal fluid, the parent compound instead of its metabolites was present in the sample. In fact, despite the presence of the nasal fluid, solid particles of COC were visible under the microscope. For this reason, independently of the amount of COC taken, the detection of residues of the drug in nasal fluid could be considered as a proof of its consumption by snorting.

The analyses of the nasal fluid samples were conducted monitoring the spectral changes over time, from the moment in which the sample was collected until it was completely dry. However, it should be underlined that the desiccation of the sample depended not only on the time but also on the heat emanated from the laser during the measurements.

Analyzing undoped samples, it was observed that initially only the characteristic bands of nasal fluid were visible in the spectra collected. Instead, when the sample began to dry out (over 20 min), its signal became less intense and some bands of the swabs appeared. Then, as expected, when doped samples were analyzed, the bands of COC appeared clearly visible only when the signal of the nasal fluid was relatively weak. Figure 2 shows the relative intensity of the four characteristic bands of COC, after 25 minutes from the collection, when the nasal fluid was dry enough to observe the COC, and after 7 days, when the sample on the swab was completely dried (and no more changes are observed over time).

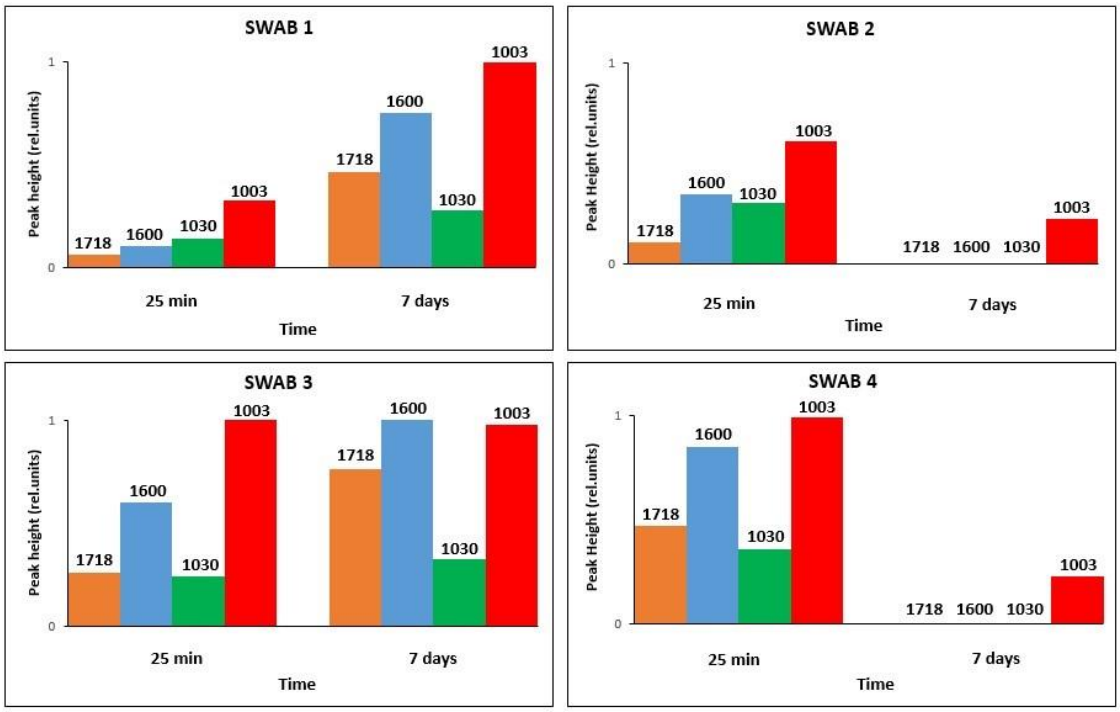

Figure 2. Relative intensity of the four characteristic bands of COC (1718, 1600, 1030, 1003 cm-1) after 25 minutes and after 7 days from the sample collection.

As it can be seen, only in two cases, for SWAB 1 and SWAB 3, it was possible to observe the characteristic bands of COC even after a relatively long time (7 days) from the sample collection. This is a great advantage considering that, in most of the cases, the analyses are not carried out immediately after the collection of the samples. For SWAB 2 and SWAB 4, instead, it was not possible to detect the COC when the nasal fluid on the swab was completely dry. Furthermore, it is possible that even the small band observed at 1003 cm-1 is not representative of COC. In fact, it could be also attributed to the characteristic band of the nasal fluid before highlighted (Fig. 1).

In order to understand the reason of this discrepancy, the patterns of the cotton swabs employed were carefully observed with the optical microscope of the Raman spectrometer (see comparison in Figure 3).

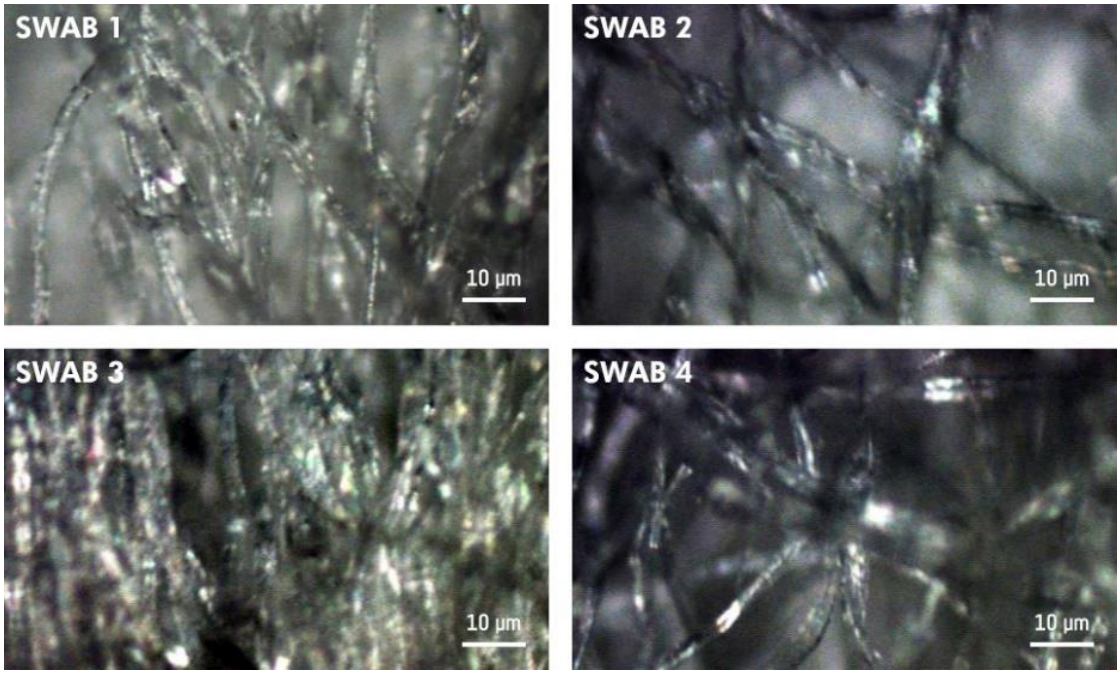

Figure 3. Comparison of the photomicrographs (10x magnification) of the fiber patterns of the four swabs employed.

It was evident that SWAB 1 and SWAB 3 presented a quite compact fiber pattern, while in SWAB 2 and SWAB 4 the cotton fibers are much more separated from each other. This latter conformation could then cause the entrapment of the particles of COC by part of the fibers so that, once dried the nasal fluid, the signal of the drug was overlapped by the one of the matrix.

Finally, to select which of the two best working swabs was the most appropriate for the analysis, the attention was focused on the intensity of the characteristic COC bands. As it can be seen in figure 2, either the sample was partially or completely dry, the bands observed with SWAB 3 were more intense than the ones observed with SWAB 1. For this reason, SWAB 3 was chosen and employed for the successive analyses.

With this comparison it was also demonstrated that no significant differences were observed between “DNA free” (SWAB 1) and common-use (SWAB 3) swabs. Furthermore, the choice of SWAB 3 was advantageous from an economic point of view, since its cost was very much lower than the one of SWAB 1.

Analysis of street cocaine samples

Once the swab to be used was chosen, three samples of street COC declared from Colombia were qualitatively analyzed. At first, the spectra of the three drug powders were collected by depositing them, as well as they had been provided, on a glass microscope slide and directly analyzed by Raman spectroscopy. As expected, even if all the most intense band of standard COC were clearly observable, some bands were characteristic of each sample (see Figure 4).

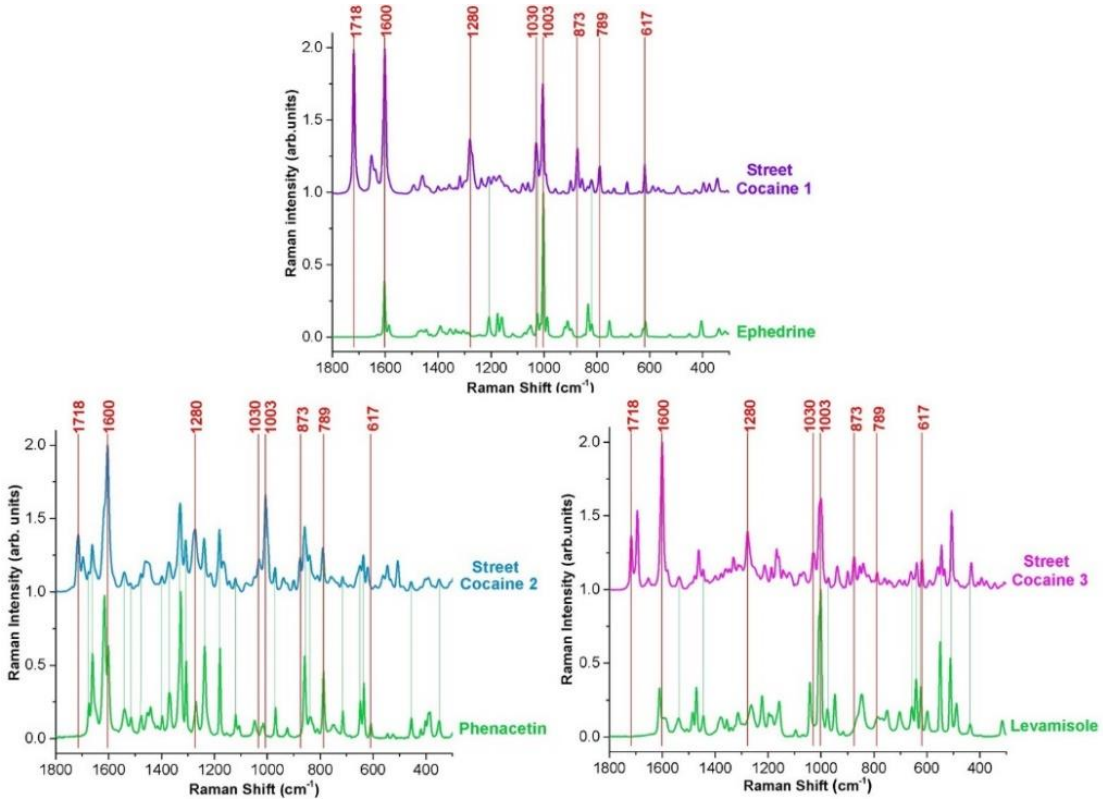

Figure 4. Correspondence between the spectra of the street COC samples and the respective most relevant cutting agent spectra identified by matching with the digital library created. Raman shifts of the characteristic bands of standard COC have also been marked.

The appearance of these bands was surely due to the presence in the analyzed samples of one or more cutting agents. Sometimes these substances, in addition to diluting the active compound, can modify the pharmacological effect of the final product, so they may themselves be drugs of abuse, such as amphetamines, narcotics, or psychedelics [15, 16].

After an extensive research about all the cutting agents employed so far to cut the pure COC, the 40 most widely used were selected and their spectra were collected and treated mathematically with the OMNICTM Software. In Table 1 the studied cutting agents have been listed and classified according to their effect in the groups described in the introduction section (Active, Cosmetic and Inert). The most important bands of each substance have also been indicated. The bands framed and highlighted in bold text correspond to bands which have Raman shifts very similar to the ones of the most important bands of the standard COC. The presence of these bands, in fact, could make it difficult the identification of COC.

Later, a digital library was created on purpose, collecting the spectra of the selected cutting agents, in order to identify which of them had been used for cutting the street COC samples analyzed. In fact, by using the “Library Search” function of the OMNICTM Software, with the “correlation” algorithm, it was possible to compare the spectrum of each street COC sample with those included in the library, and then identify the cutting agents presumably employed. It should be remarked that, even if Raman spectroscopy is able to distinguish the different substances present in a sample, it is not a very sensitive technique. For this reason, only the substances with a relatively high signal intensity could be clearly identified.

Table 1. Most important bands of the forty selected cutting substances classified according to their effect. Raman bands which could interfere with the most characteristic bands of the cocaine have been framed and highlighted in bold text. Bands are labelled as: w, weak; m, medium; s, strong; and vs, very strong according to their relative intensity.

EFFECT	SUBSTANCES	Raman Shifts of the characteristic bands																								
ACTIVE	Aminopyrine	1672w	1635m	**1599vs**	1478w	1453w	1441w	1424w	1350m	1317m	1179w	1106w	**1027w**	**1003vs**	887w	823w	656m	646m	622m	609m						
	Amphetamine	**1606w**	1584w	1456w	1207w	1181w	**1031m**	**1001vs**	972m	841w	816w	622w	449w	342w												
	Buflomedil	1704m	1612m	1461m	1420w	1347vs	1301w	1209m	1198w	1168w	1090w	1061m	**1037s**	922w	952w	927w	917m	797m	632m	615m	469s	378vs				
	Caffeine	1703m	1662w	**1605m**	1462w	1435w	1413w	1365m	1334vs	1290m	1246w	1076w	**1027w**	933w	807w	746m	649w	561vs	489w	450w						
	Chlorpromazine	1591m	1566vs	1451w	1294w	1248w	1165w	1140w	1107m	1039m	931w	723w	678m	430w	378w	314m										
	Diltiazem	1686w	1614s	1589vs	1576w	1455m	1420m	1369m	1309w	1254s	1223s	1184m	1165m	1444w	1065w	1041vs	918m	824m	697w	644w	383w					
	Diuron	1652w	**1599vs**	1306m	1242s	1138w	**1033m**	907w	895w	730w	696w	677m	638w	533m	338m											
	Levamisole	1610m	1471m	1264w	1223w	1158w	1042m	**1001vs**	976w	948w	846w	641m	623m	550s	511s	489w										
	Noscapine	1770vs	1612m	1504w	1456m	1443m	1391m	1358w	1322w	1284m	1227w	1199w	**1035w**	983w	760w	722w	685w	590w	449m	398m	361w					
	Papaverine	1635w	1609m	1511m	1489w	1453m	1442m	1409vs	1390vs	1350m	1332w	770w	745w	717w	364w											
	Paracetamol	1652s	1614s	1564m	1375m	1328vs	1281m	1260w	1241s	1172m	862s	838w	801m	714w	655m	395w	332w									
	Phenacetin	1675w	1661m	1615s	**1601m**	1540w	1453w	1441w	1370m	1327vs	1307m	1270w	1237s	1179s	969w	859m	788m	714w	648w	635m	385w					
	Piracetam	1682w	1650s	1495w	1473m	1451m	1438s	1414m	1307m	1225m	1193m	1138m	1074w	**1034m**	953m	924m	859vs	789w	718m	617s	547w	508s	380m	345m		
	Strychnine	1655s	**1598s**	1479m	1443m	1388m	1329m	1253w	1206w	1191w	1157w	**1023m**	983w	770m	678w	650m	531w	492w	407w	392m	366w	326w				
	Taurine	1610w	1588w	1510w	1457w	1426w	1387w	1342w	1304w	1258w	1220w	1179w	1109w	1049m	**1031vs**	963w	895w	847w	735m	592w	529m	473w	367w	325w		
COSMETIC (Benzodiazepines)	Alprazolam	**1607m**	1594vs	1562w	1485w	1445w	1423m	1319w	1301w	1265w	1160m	1126w	**1026w**	994vs	681m	632w	610w	451w	398w	342m	311m					
	Bromazepam	1686m	**1606s**	1592m	1580s	1563m	1476m	1331m	1322m	1311m	1293w	1162s	1144w	1089w	1042m	990vs	943w	861w	678m	563w	407w	374w	330m			
	Diazepam	1677w	1586vs	1568w	1554w	1334w	1307m	1161m	1135w	1121w	1021w	992m	980w	805w	756w	683m	622w	610w	445w	355w						
	Flunitrazepam	1610m	1579m	1343vs	1335vs	1287w	1168w	1128w	1099w	1074w	883w															
	Lorazepam	1615s	**1596s**	1587s	1562m	1494w	1474m	1326m	1316m	1289m	1165vs	1092m	**1025s**	935w	856w	742w	669m	631m	465w	448s	429w	378s	318w			
	Nitrazepam	1612w	1591w	1566w	1333vs	1300w	1150w	1090w	992w	734w	372w	328w	311w													

Table 1. (Continuation)

EFFECT	SUBSTANCES	Raman Shifts of the characteristic bands																								
COSMETIC	Aspirin	1755w	1632m	1610vs	1433w	1298m	1263w	1227w	1195m	1158w	1049m	1019w	788w	755m	709w	645w	556w	483w	430w							
	Benzocaine	1682s	1635w	**1604vs**	1575m	1445w	1369w	1312w	1282m	1173w	1112w	863m	819w	640w	619w	506w	318w									
	Ebastine	1680s	**1606vs**	1296w	1280w	1215w	1190m	1109m	1072w	**1029w**	**1002s**	836w	806w	746w	713w	638w	620w									
	Ephedrine	**1603m**	1585w	1391w	1208w	1176w	1159w	1051w	**1025w**	**1002vs**	988w	910w	833w	820w	752w	616w	405w	339w								
	Hydroxyzine	**1608s**	1593w	1470w	1309w	1206m	1193m	1163w	1099s	1054w	1041w	**1007vs**	851w	835w	814m	785w	764w	697w	628m	424w	352w	334w				
	Lidocaine	1664m	**1595vs**	1482m	1453s	1375m	1295w	1264vs	1215m	1096m	992m	973w	959w	785w	754w	706s	620s	540m	489w	408w	327w					
	Metronidazol	1534m	1487m	1469w	1436w	1378s	1357s	1270s	1185vs	1160w	1077w	992w	865w	829w	739w	647w	559w	394w	309w							
	Phencyclidine	**1601m**	1582w	1452m	1430w	1378w	1313w	1296w	1273m	1204m	1158w	1119w	1077w	1048s	**1036s**	**1001vs**	894w	859m	797w	700m	619m	550w	510m	430w		
	Procaine	1695vs	1646w	**1606vs**	1575w	1522w	1265m	1168m	867w	849w	826w	641w														
	Quinidine	1643w	1621w	1591w	1566w	1511w	1466w	1443w	1420m	1368vs	1303w	1293w	1254w	1238w	1174w	1140w	903w	837w	798w	765w	565w	383w	354w			
INERT	Flour	1656w	1524w	1459m	1396m	1381m	1339s	1263w	1209w	1126s	1111m	1082m	1051w	**1004w**	940m	867w	770w	716w	613w	578w	521w	478vs	441w			
	Milk powder	1746w	1655m	1451vs	1441vs	1359m	1335m	1305s	1261m	1118m	1094m	1063m	1014w	**1003w**	949w	895w	879m	854w	360m	477w	439w	416w	360m	353m		
	Glucose	1465w	1437w	1411w	1377w	1351m	1336w	1278w	1155w	1125m	1114m	1080s	1060m	**1027w**	920m	847s	778w	658w	547vs	446w	429w	412s	369m			
	Sucrose	1462s	1378w	1367w	1348w	1239m	1161w	1126s	1105w	1089w	1038s	922w	850vs	641m	585w	550m	539m	522s	443m	418w	402s	366w				
	Lactose	1470w	1358m	1347m	1335m	1327m	1263m	1142m	1134m	1120m	1086s	1053m	1040w	1019m	916w	876m	851s	554w	477s	399m	377s	357vs				
	Inositol	1415w	1357w	1325w	1279w	1132w	1118m	1093w	1063w	1050w	**1008w**	932w	897m	734w	505vs	463w	431vs	390w	375w							
	Mannitol	1460s	1403w	1387w	1364w	1322w	1283w	1261w	1232w	1133m	1118w	1080w	**1037m**	935w	876vs	788w	648w	518w	494wm	477m	420w	368w				
	Talc (perfumed)	1102w	1056w	1023w	797w	681vs	522w	513w	474w	458w	438w	384w	367m	339w												
	Sodum hydroxide	1291w	1282w	1084vs	705w	491w	383w																			

In figure 4, the spectra of the cutting agents with the highest correlation coefficients, estimated for each street COC sample by matching their spectra with the library, are also shown.

As it can be observed, the spectrum of the first sample (Street Cocaine 1) is almost identical to the one of the standard COC.HCl (Fig. 1), which could mean that the COC was not or barely adulterated. In fact, by matching the spectrum with the library, we obtained a correlation coefficient of ~98 for standard COC.HCl and a smaller coefficient of ~48 for ephedrine.

For the second sample (Street Cocaine 2), a lot of bands which do not correspond to the ones of pure COC were observed, meaning that the signal of the cutting substances was very strong. Surprisingly, by matching the spectrum with the library, we obtained a correlation coefficient of ~65 for phenacetin, even higher than the one of ~49 for standard COC.HCl.

Finally, the matching of the third spectrum (Street Cocaine 3) with the library, provided a correlation coefficient of ~58 for standard COC.HCl and of ~40 for levamisole.

However, to avoid confusion, it should be underlined that the correlation coefficient does not reflect the quantity of a substance present in the sample. This value, in fact, corresponds to the percentage of match between the spectra obtained from the analysis of the street COC samples and those included in the library. In spite of that, after the comparison, the correlation coefficient could permit to obtain an estimation of the purity of COC.

Subsequently, samples of nasal fluid collected with SWAB 3 were doped with the three before mentioned street COC and stored in the dark at room temperature. Since a better signal was observed when nasal fluid was completely dried (Fig. 3), the spectra were collected after 7 days (Figure 5) and compared with those collected in the digital library.

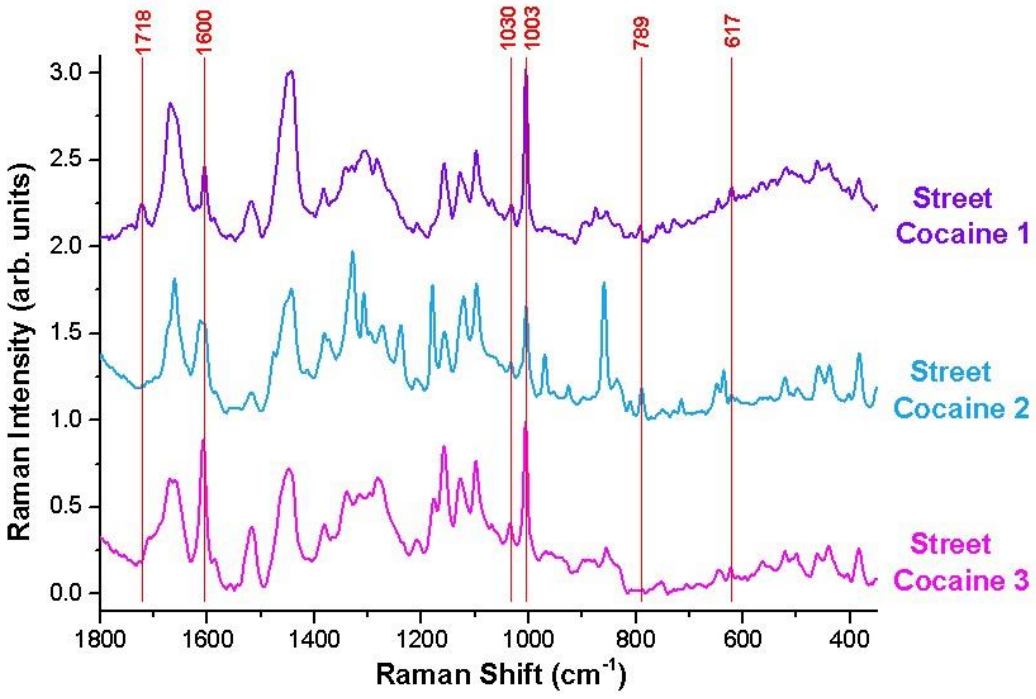

Figure 5. Spectra of nasal fluid samples collected with SWAB 3 and doped with the three street COC samples before analyzed. Raman shifts of the characteristic bands of standard COC have also been marked.

In this case, two different results were observed. For the first (Street Cocaine 1 in nasal fluid) and the third (Street Cocaine 3 in nasal fluid) samples, the software provided the highest correlation coefficients for a cutting substance, hydroxyzine, which is different from those before estimated for the corresponding street COC samples in powder form. For Street Cocaine 1, the correlation coefficient was of ~59 for hydroxyzine and of ~56 for ephedrine. For Street Cocaine 3, the correlation coefficient was of ~55 for hydroxyzine and only ~26 for levamisole. For the second sample (Street Cocaine 2), on the contrary, the main cutting substance identified was again the phenacetin (correlation coefficient of ~67). The difference of the detected cutting agents, for samples 1 and 3, could be mainly due to the presence of the nasal fluid and of the swab. In fact, by matching the spectrum of the undoped nasal fluid on the swab (used as control) with the library, the highest correlation coefficient (~15) was actually obtained for hydroxyzine. Evidently, unlike the ephedrine and levamisole signals, the signal of phenacetin in sample 2 is strong enough to not be affected by the presence of the matrix, so no differences are observed.

Therefore, it could be concluded that the signal of nasal fluid could affect the spectral identification of the cutting agents, because it could overlap or interfere with their signal, but does not prevent the detection of COC. It was thus demonstrated the capability of Raman spectroscopy to clearly identify COC in nasal fluid also when samples are adulterated.

Conclusions and future trends

In this work it has been demonstrated the capability of Raman spectroscopy to detect very small amounts of COC (~ 200 μg) in nasal fluid and to distinguish its signal from those of other cutting agents eventually present in street COC samples. Sampling conditions were studied by comparing different swabs and monitoring the times of spectra collections. The four swabs in comparison presented the same Raman spectrum, corresponding to the one of the cotton, even if their fibers patterns were different. Among these four swabs, SWAB 3 (Dr.SOS), which was a common-use swab bought in a local supermarket, was selected as the best to make the analyses. The choice was mainly due to its characteristic interlacement of cotton fibers that permitted to detect an intense signal for COC in nasal fluid. Furthermore, the identification was possible both if the sample was recently collected (after 25 minutes) or completely dry (after 7 days from the collection).

The qualitative analysis of street COC samples showed the presence of one or more different cutting agents other than COC. The collection of the spectra of the forty most widely used cutting agents allowed to create a digital spectral library. The comparison of the street COC spectra with those included in the digital library permitted to detect which were the major substances present in the analyzed sample. The analysis of doped nasal fluid samples, collected with the previously selected swab (SWAB 3), indicated that sometimes the signal of the matrix could affect the cutting agent recognition. Its signal, in fact, could overlap or interfere with the signal of the cutting agents, causing the identification of substances different than those actually present in the sample. On the other hand, the presence of the nasal fluid did not prevent the identification of COC. Consequently, this qualitative analytical method has a high potential as non-destructive screening tool, even if a separation technique implemented with mass spectroscopy is recommendable for quantitative purposes.

In this study a benchtop Raman spectrometer was used, but a future trend could be to employ portable instrumentation to conduct in situ screening analyses. This could be a very important

goal especially in forensic and clinical fields, permitting to perform roadside tests but also controls in emergency situations to recognize possible overdoses.

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