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Journal of Chromatography A, 674 (1994) 217–224

JOURNAL OF CHROMATOGRAPHY A

Cocaine-related deaths

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Abstract

Cocaine availability has been increasing in Spain in the past few years. A review of all the toxicological analyses carried out at the Madrid Department of the Instituto Nacional de Toxicología, with subjects who had died of drugs from 1990 to 1992, found 533 persons who had cocaine in their blood and/or tissues; 450 (84%) deaths involved cocaine and heroin together whereas 83 (16%) deaths involved cocaine with an absence of heroin. This paper reports the circumstances, cocaine and benzoylecgonine concentrations in the blood and other toxicological findings for the two major groups of deaths where cocaine was found with an absence of heroin, *i.e.*, possible overdose cases (35 cases) and traffic accidents (23 cases).

1. Introduction

Spain has rapidly become a major port of entry into Europe for South American cocaine, owing not only to its geographical situation but also to its linguistic links and the historical bonds between the two countries. Although it is alleged that Spain is only the port for the introduction of cocaine into Europe, this traffic has resulted in cocaine becoming a leading abused drug in Spain. Cocaine is used not only along with heroin but also alone, resulting in an increase in cocaine-related deaths.

Fatalities from cocaine overdose were reported as early as 1972 [1] and 1973 [2]. Some studies tried to assess the blood concentration levels of cocaine in fatal cocaine overdoses [3]. Subsequent studies on cocaine-related deaths included the description of fatal cocaine poisonings [4], cocaine suicides [5] and cocaine deaths where the drug itself was not the primary cause but a contributory factor [6,7]. Other studies focused on cases where cocaine use was directly associated with cardiovascular and neurological deaths [8]. It has also been reported that the number of violent deaths involving cocaine was significant [9]. The association between cocaine and violence has been pointed out [10].

All but three of the above-mentioned studies reported blood concentrations of the parent drug, cocaine. One study calculated cocaine and benzoylecgonine together in the blood using radioimmunoassay [5] whereas only two of the studies calculated cocaine and benzoylecgonine in the blood separately, one of them suggesting that in the cases where the cause of death was not directly attributable to cocaine, only benzoylecgonine was found in the blood.

The major cocaine (COC) metabolites reported in the blood are benzoylecgonine (BE) and ecgonine methyl ester (EME), although relatively few quantitative data on these metabolites exist. Benzoylecgonine has a relatively long half-life (5–7 h) [11] in blood compared with cocaine (1 h) [12]. Consequently, BE is a much better marker for long-term exposure to cocaine

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0021-9673/94/$07.00
SSDI 0021-9673(93)E0034-R

and hence the amount of cocaine and benzoylecgonine in blood, calculated separately, may provide useful data for the interpretation of the cause of death. Ecgonine methyl ester is not considered so useful a marker as it is produced not only *in vivo* but also *in vitro* by hydrolysis of cocaine in the blood [13].

This paper presents the results of a systematic study of all the cases brought to the Madrid Department of the Instituto Nacional de Toxicologia, where cocaine and/or metabolites were found in the deceased upon toxicological analysis. The same methodology was applied to all the cases: after a preliminary immunoassay of blood and urine, a comprehensive screening of all the available samples was performed by gas chromatography with nitrogen–phosphorus detection (GC–NPD). The extracts of the samples used for GC were derivatized and subjected to gas chromatography–mass spectrometry (GC–MS) for confirmation and quantification of cocaine, its metabolites and heroin metabolites, if present. The major advantage of the method over other published methods is that only one extraction of the blood and one derivatization step are needed for the confirmation and quantification of heroin metabolites, cocaine and benzoylecgonine. The cases were classified into several groups according to the possible cause of death, and cases where cocaine and/or metabolites were found in the absence of heroin are described. We attempt to explain some of the cocaine-related deaths and try to present data that could aid in the forensic interpretation of those deaths.

2. Experimental

2.1. Samples

Blood, urine and tissues from all the cases suspected to be drug related were obtained during routine autopsies by various pathologists over a 3-year period. Information was provided by the police reports and the judicial inquests.

2.2. Drugs and chemicals

Cocaine hydrochloride, pentafluoropropionic anhydride and hexafluoro-2-propanol were obtained from Sigma. Benzoylecgonine and ecgonine methyl ester were prepared from cocaine according to a published procedure [14]. Columns packed with Extrelut silica were purchased from Merck (Scharlau, Germany) and Sep-Pak C_{18} cartridges from Waters. All other chemicals and solvents were of the purest grade available (Merck).

2.3. Immunoassays

Immunoassay screenings were performed on a Syva (Palo Alto, CA, USA) ETS system using original reagents for opiates, cocaine, benzodiazepines, methadone, propoxyphene and amphetamines.

Urine samples were filtered and the pH was adjusted to 6.5 with 0.1 *M* HCl or 0.1 *M* NaOH.

Blood samples (0.5 ml) were extracted in Sep-Pak C_{18} cartridges following procedures already described [15]. The residue was reconstituted with 0.5 ml of the buffer supplied with the EMIT reagents (0.055 *M* Tris buffer, pH 8). The results from both urine and blood provided rapid information on the possible presence of drugs, which was confirmed later.

2.4. Extraction procedure for blood and urine

To 5 ml of whole blood, 100 μl of nalorphine solution (8 μg/ml) were added as an internal standard. The pH was then adjusted to 9 with borate buffer. The sample was vortex mixed for 10 min and poured into an extraction column packed with Extrelut silica. After 10 min, elution was carried out with 20 ml of a 2-propanol–dichloromethane (10:90). The eluate was evaporated under nitrogen.

The same procedure was applied to 5 ml of urine, but adding 0.1 ml of nalorphine (80 μg/ml).

2.5. Extraction procedure for viscera

To 5 g of lung, liver or kidney, 0.5 ml of the nalorphine solution (80 μg/ml) was added as an internal standard, then 2.5 ml of 10% phosphoric acid and 15 ml of saturated ammonium chloride solution were added. The mixture was heated in a water-bath for 10 min. After cooling, it was filtered and the filtrate was extracted as for urine.

2.6. Gas chromatographic analysis

The extraction residues of blood, urine, etc., were reconstituted with 0.3 ml of methanol, shaken and poured into GC autosampler vials. They were injected into the chromatograph for general screening of drugs and metabolites [16].

The instrument was a Hewlett-Packard (HP) (Avondale, PA, USA) Model 5890 gas chromatograph equipped with a nitrogen–phosphorus detector, a Model 7673A automatic sampler and a split–splitless capillary inlet system. A 25 m × 0.20 mm I.D. fused-silica capillary column coated with cross-linked methylsilicone (0.11-μm film thickness) was used. The operating conditions were as follows: linear velocity of helium, 53 cm/s; detector and injection port temperatures, 300°C; and splitting ratio, 1:30. The column temperature was initially held at 180°C for 1 min and then increased to 300°C at 10°C/min.

2.7. Gas chromatographic–mass spectrometric analysis

The GC autosampler vials containing the extracts were evaporated to dryness under nitrogen. A 100-μl volume of pentafluoropropionic anhydride (PFPA) along with 50 μl of hexafluoro-2-propanol (HFIP) were added to each vial; after sealing, the vials were incubated for 30 min at 60°C. They were then decapped and carefully evaporated to dryness under nitrogen. The dried extracts were taken up in 100 μl of ethyl acetate before injection into the GC–MS system. The instrument was an HP Model 5890 gas chromatograph coupled to an HP Model 5791 mass selective detector, controlled by MS Chemstation (DOS series) software. The column was identical with that used for GC. The operating conditions for the GC–MS system were as follows: injection port, oven temperature, linear velocity of helium and splitting ratio as for GC; ionization energy, 70 eV; MS temperature, 190°C; and operating mode, selected-ion monitoring (SIM). A user macro was created to automate the analysis. The selected ions for cocaine (COC) and its derivatized metabolites ecgonine methyl ester pentafluoropropionyl derivative (EMEPFP), benzoylecgonine hexafluoroisopropyl ester (BEHFIP) and the heroin derivatized metabolites 3-monoacetylmorphine pentafluoropropionyl derivative (MAMPFP), codeine pentafluoropropionyl derivative (CODPFP) and morphine pentafluoropropionyl derivative (MORFPFP) are given in Table 1.

Cocaine and benzoylecgonine concentrations in the blood were determined in all cases where this sample was available. Quantification was performed by GC–MS in the SIM mode using calibration graphs obtained with nalorphine as internal standard and derivatization as described for the samples. The procedure was achieved by application of linear regression analysis to the peak-area ratios of ions of m/z 182 to 440 (for COC) and m/z 318 to 440 (for BEHFIP) for a blood sample and the three-point calibration graph prepared at the beginning of the batch.

Table 1
Selected ions for GC–MS

Compound[a]	m/z[b]
Ecgonine methyl ester-PFP	182, 345, 314
Cocaine	182, 303, 272
Benzoylecgonine-HFIP	318, 439, 334
Morphine-$(PFP)_2$	414, 577, 430
Monoacetylmorphine-PFP	414, 473, 361
Codeine-PFP	282, 445, 388
Nalorphine-PFP	440, 456, 357

[a] PFP = pentafluoropropionic derivative; HFIP = hexafluoro-2-propyl ester;
[b] The first ions listed are selected for quantification.

The calibration standards were prepared from spiked negative blood to provide concentrations of 0.1, 0.8 and 2.5 μg/ml for each compound. The equations for the graphs were $y = 1.02x - 0.157$ ($r = 0.998$) for COC and $y = 3.88x - 0.18$ ($r = 0.999$) for BEHFIP, where y is response ratio (area of COC or BHFIP/area of nalorphine-PFP) and x is amount ratio (concentration of COC or BE/concentration of nalorphine). Two controls of 0.05 and 5 μg/ml were then analysed to verify the linearity of each graph. The limit of quantification was 0.01 μg/ml for both COC and BEHFIP. Samples with a higher concentration than the highest standard were diluted so as to fall within the stated levels before repeating the analysis. The precision of the method was determined by analysing quality control samples that contained COC and BE at known concentrations. At the level of 0.1 μg/ml, the relative standard deviations (R.S.D.s) were 7% for COC and 5% for BEHFIP ($n = 6$). At the level of 10 μg/ml, the R.S.D.s were 5% for COC and 3% for BEHFIP ($n = 6$). The recovery for COC and BE was established using spiked negative blood: for samples spiked at 0.1 μg/ml the mean recovery was 85% for COC and 72% for BE. At higher concentration (10 μg/ml) the mean recovery was 80% for COC and 68% for BE. Fig. 1 shows a typical SIM chromatogram for the determination of COC and BE in a sample of blood.

Table 2
Cocaine-related deaths (in absence of heroin)

Cause of death	No.
Possible cocaine overdose	35
Traffic accidents	23
Suicide by hanging	7
Gunshot (homicide or suicide)	6
Stab wound (homicide)	3
Accidental	9
Total	83

Ecgonine methyl ester was always investigated but not routinely determined.

3. Results and discussion

A total of 533 deaths involved cocaine in the period 1990–92. Of these, 450 (84%) were found to contain heroin plus cocaine and only 83 cases (16%) contained cocaine with no presence of heroin. Table 2 presents these cases classified according to the possible cause of death. Tables 3 and 4 show the circumstances of death, toxicological findings and cocaine and benzoylecgonine concentrations in the blood of the two major groups of deaths that involved cocaine in absence of heroin.

We purposely avoided the study of the 450 deaths involving cocaine plus heroin because it is

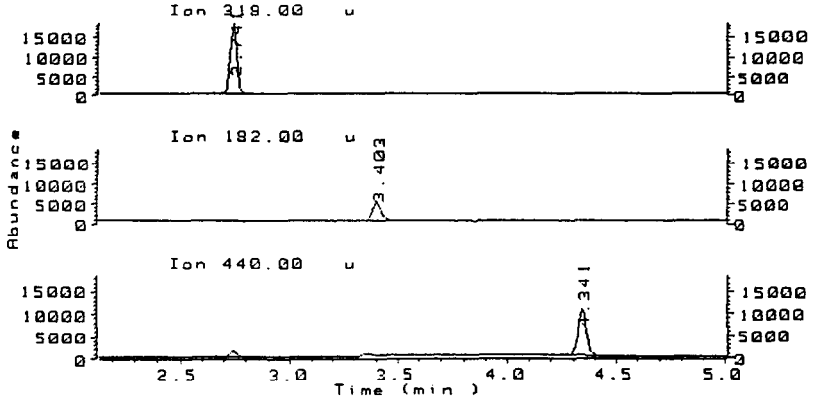

Fig. 1. Single-ion profile of the molecular ions for each drug in a 0.05 μg/ml blood control: benzoylecgonine hexafluoroisopropyl ester, m/z 318; cocaine, m/z 182; nalorphine pentafluoropropionyl derivative, m/z 440.

Table 3
Cocaine-related deaths: possible cocaine overdose

Case/age/sex	Administration route	Circumstances	COC/BE concentration in blood (μg/ml)	Other drugs in blood (μg/ml)
1/23/M	Unknown	Emergency room Heart attack Cardiomegalia Hepatitis AIDS	0.95/0.5	None
2/30/M	Unknown	Emergency room Suspected heroin O.D.	N.D./0.18	None
3/30/M	Unknown	Emergency room Suspected heroin O.D.	9/16	Lidocaine: 0.01
4/30/M	Oral	Hospital Five packages in bowel	N.D./7	Dipyrone: 17.4 Theophylline: 1.7
5/35/M	Unknown	Emergency room Heart attack Chronic hepatopathy	N.D./0.4	Nordiazepam: 0.5 Ethanol: 0.4 g/l
6/28/M	Unknown	Emergency room	N.D./0.4	None
7/22/M	Unknown	Home Heart attack Hepatitis AIDS	0.1/1	Phenobarbital: 5 Naproxen: 0.13
8/30/M	Unknown	Prison Heart attack	1.5/1	Nordiazepam: 1
9/33/F	Unknown	Home Cocaine user Diabetic	N.D./4.6	None
10/37/M	Unknown	Emergency room Cocaine user Myocardial failure AIDS	0.5/5.18	None
11/28/M	Unknown	Sudden death Anoxia Cardiomegalia	N.D./0.10	None
12/29/M	Intravenous injection	Home Lung acute oedema	1.2/3.9	None
13/?/M	Intravenous injection	Open space Suspected heroin O.D.	0.25/5.7	None
14/50/M	Oral	Airport; 85 packages in stomach	10/1.2	None
15/35/M	Oral	Prison hospital; 98 packages in digestive tract	11.8/0.67	Dipyrone: 0.41
16/20/M	Unknown	Discoteque Sudden death after seizures	0.6/14	Ethanol: 0.4 g/l

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Table 3 *(continued)*

Case/age/sex	Administration route	Circumstances	COC/BE concentration in blood (μg/ml)	Other drugs in blood (μg/ml)
17/41/M	Inhaled	Home Heroin seller Brain and lung oedema	1.24/0.05	Ethanol: 0.84 g/l
18/40/M	Unknown	Transvestite Death after vomiting and abdominal cramps	N.D./0.21	Ethanol: 1.10 g/l
19/30/M	Unknown	Home Cardiorespiratory failure	B.N.A.	None
20/30/M	Unknown	Heroin and cocaine user Heart attack Lung oedema	N.D./0.2	Methadone: 0.1 Levomepromazine: 0.56 Nordiazepam: 1.8
21/?/M	Oral	Death in hospital after 4 days	N.D./4.5	Dipyrone: 4
22/28/F	Unknown	Recent injection	N.D./24	Naproxen: 45
23/36/M	Unknown	Home	6/24	None
24/35/M	Unknown	Sudden death after seizures	0.6/7.4	None
25/34/M	Snorted	Cocaine user Suspected cocaine O.D.	0.65/3.5	None
26/23/M	Unknown	Emergency room Vomiting, bronchoaspiration and heart attack	3.6/20	None
27/32/M	Unknown	Death on arrival at the hospital	0.7/4	None
28/25/M	Intravenous injection	Death with syringe affixed	1.7/16	None
29/32/M	Intravenous injection	Suspected cocaine O.D.	2.1/39	None
30/60/M	Oral	Hospital Packages in digestive tract	66/40	None
31/30/M	Oral	Police station. Ingested the drug	0.38/15	None
32/22/M	Oral	Police station. Ingested the drug	6/129	None
33/29/M	Intravenous injection	Home	B.N.A.	None
34/26/M	Unknown	Open space	N.D./0.02	MDMA: 0.41 Lidocaine: 1.9 Nordiazepam: 0.13
35/33/M	Intravenous injection	Death on arrival at the hospital after seizures	N.D./0.18	Nordiazepam: 0.18

Abbreviations: M = male; F = female; N.D. = not detected; B.N.A. = blood not available; O.D. = overdose

Table 4
Cocaine-related deaths: traffic accidents

Case/age/sex	COC/BE concentration in blood (μg/ml)	Other drugs in blood (μg/ml)
36/26/M	0.34/43	None
37/34/M	N.D./0.04	None
38/26/M	N.D./0.3	Ethanol: 1.2 g/l
39/?/M	0.01/1.6	Ethanol: 0.3 g/l
40/22/M	B.N.A.	None
41/?/M	0.02/2	None
42/27/M	0.07/3.5	None
43/25/M	N.D./0.13	Ethanol: 0.93 g/l THC: 4
44/26/M	N.D./0.3	Ethanol: 1.14 g/l
45/?/F	N.D./0.7	Ethanol: 0.56 g/l
46/36/M	N.D./0.05	Ethanol: 1.37/g/l
47/26/M	N.D./0.20	Ethanol: 0.51 g/l
48/26/M	N.D./0.20	None
49/24/M	B.N.A.	None
50/31/M	N.D./13	None
51/35/M	B.N.A.	None
52/33/M	N.D./0.07	None
53/25/M	N.D./0.05	Ethanol: 2.08 g/l
54/19/M	0.2/3.7	Ethanol: 1.55 g/l
55/27/M	N.D./0.03	Ethanol: 1.91 g/l
56/24/M	0.03/31	Ethanol: 1.7 g/l
57/29/M	B.N.A.	None
58/?/M	N.D./3.8	None

Abbreviations: M = male; F = female: N.D. = not detected; B.N.A. = blood not available

very difficult to evaluate the contributions of each of the two drugs to the deaths. The risk of acute overdose reaction increases with the mixture of both drugs [17]. We concentrated on deaths where only cocaine was involved as a major drug and we present data for the two largest groups: the first where cocaine appeared to be the cause of death and the second, traffic accidents, where cocaine may have contributed to the deaths.

In both groups, women made up a small number of cases (5%), which is in accordance with published data on the distribution of gender of cocaine users [18].

The median of age at the time of death was 31 years for the first and 27 years for the second group. The ages ranged from 20 to 60 years in the first group and from 19 to 36 years in the second group.

In the first group the major route of intake, when known, was by oral ingestion followed by intravenous injection. Of the seven cases of oral ingestion, five corresponded to body-packers. One of them died in the airport, two others lived for a short time until arrival at the hospital and the other two survived for 2 days (case 4) and 4 days (case 21) in the hospital. The other two cases of oral intake corresponded to cocaine sellers who after having been arrested and taken to the police station, ingested the drug to avoid being caught when searched. Except for cases 4 and 21 these cases showed high values of cocaine or benzoylecgonine in blood. Owing to the shorter half-life (1 h) of cocaine in blood compared with that of BE (5–7 h), the survival time determines which is higher, the level of the parent drug or the level of the metabolite in the blood. Fifteen more cases in this group also had both cocaine and benzoylecgonine in the blood: five died on arrival at the hospital or emergency

room, two died suddenly after seizures, three were suspected drug (cocaine or heroin) overdoses, two died of a heart attack, one died with a syringe still attached to the arm and two died at home of lung oedema. In the other thirteen cases in this group (35%), only benzoylecgonine was found in the blood, thus suggesting longer survival periods for most of them or the contribution of other drugs to the cause of death (cases 5, 18 and 35). A wide range was noted for both cocaine and benzoylecgonine concentrations in the blood: from 0.1 to 66 μg/ml for cocaine and from 0.02 to 129 μg/ml for benzoylecgonine. The cocaine concentrations are in agreement with previously published data [19–21]. For benzoylecgonine this wide variation has also been described [9]. Ecgonine methyl ester was found positive in all the cases, which confirms the *in vivo* or *in vitro* hydrolysis of cocaine in the blood [13]. Ethanol was found only in four of the 35 cases in this group and some of the other drugs found in blood could be attributed to medical treatment (dipyrone, theophylline).

In the second group, traffic accidents (Table 4), the presence of ethanol in the blood was very significant: eleven of the nineteen blood samples analysed (57%), at concentrations ranging from 0.3 to 2.08 g/l with a mean value of 1.21 g/l. Only six cases in this group (26%) had detectable levels of cocaine in the blood, ranging from 0.01 to 0.34 μg/ml. One of them (case 56) was found in the involved vehicle with a syringe still attached to the arm. For the rest, only benzoylecgonine was found at the time of the death at concentrations ranging from 0.03 to 43 μg/ml. Those ranges, albeit also wide, are lower than those obtained for the group in Table 3. In all the cases involving traffic accidents ecgonine methyl ester was also found.

4. Conclusions

In conjunction with the availability and popularity of cocaine, this drug is becoming responsible for a certain number of deaths, either directly (overdoses) or indirectly (traffic accidents, homicides, etc.). In this study it has been shown that the amounts of cocaine and benzoylecgonine in the blood should be calculated separately as their presence and relative concentrations prove useful in determining the cause of death and possible survival time after administration.

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