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CASE REPORT

A COCAINE AND HEROIN-RELATED SUDDEN DEATH IN SRI LANKA: TOXICOLOGICAL EVALUATION

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ABSTRACT

Cocaine is a prominent illicit substance associated with sudden mortality in individuals engaging in poly-drug use. We are presenting a case of a sudden death of a 37-year-old male. The autopsy failed to reveal the pathological cause of death, and the toxicological analysis of post-mortem samples was carried out. Quantitative analysis of ethanol was carried out by headspace gas chromatography with a flame ionization detector (FID). Solid-phase extraction was carried out to extract drugs and their metabolites from biological samples. After derivatization, these drugs and metabolites were then quantitatively analysed using gas chromatography-mass spectrometry. The analytical methods were validated before analysing the samples.

In the toxicology results, all biological samples, including blood, bile urine, and stomach contents, contained morphine, with concentrations ranging from 12 µg/mL to 55 µg/mL. 6-Monoacetylmorphine was detected in urine at a concentration of 8 µg/mL and in stomach contents at a concentration of 60 µg/mL. The concentrations of cocaine were detected in bile, urine, and stomach contents at 727 µg/mL, 202 µg/mL, and 39 µg/mL, respectively. Benzoylecgonine was detected in blood, urine, and stomach contents at concentrations of 6 µg/mL, 227 µg/mL, and 7 µg/mL, respectively. Additionally, ecgonine methyl ester was identified in urine at a concentration of 123 µg/mL. Furthermore, ethanol concentrations of 109 mg/100 mL in blood and 102 mg/100 mL in urine were also detected.

The presence of cocaethylene in the stomach contents and urine samples confirmed that cocaine and ethanol were co-administered, leading to a more pronounced effect. The presence of cocaine, its metabolites, ethanol, cocaethylene, and heroin metabolites in the biological samples reveals the simultaneous use of cocaine, heroin, and ethanol. This drug combination likely led to acute toxicity. Therefore, these findings provide a well-defined outline for the cause of death.

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INTRODUCTION

Forensic toxicology analysis involves evaluating toxicological data to understand its implications for legal and medical investigations[1]. Forensic toxicology qualitative analysis identifies the specific drugs or chemicals present, while quantitative analysis measures their concentrations[1]. Together, these methods provide crucial information on whether a substance could have contributed to a person's

death or impairment, aiding in the reconstruction of events and supporting legal decisions in cases such as overdose, poisoning, or substance-related incidents[1-4]. Furthermore, toxicological analysis revealed that the deaths were caused by overdoses of illicit drugs, with some cases involving the use of multiple drugs simultaneously[2-4]. These findings highlight the significant role of drug abuse, both singularly and in combination, in contributing to the fatalities[2-4].

In the literature, Joaquín Lucena *et al*. found that out of 668 cases of sudden death, 21 were related to cocaine use[2]. Additionally, the study reported that 62% of the sudden deaths were due to cardiovascular causes, while 14% were attributed to cerebrovascular causes. In a two-year toxicology study by Candia *et al*., 136 cases were analysed[3]. The results showed that 74.3% of the cases tested positive for substances, which included illicit drugs such as cocaine and morphine, along with ethanol. Furthermore, Rooney *et al*. stated that a significant risk of cocaine overdose occurs in cases of polydrug use, particularly when combined with opiates and/or alcohol[4].

Cocaine is one of the most common illicit drugs of abuse in many countries[4-7]. Heroin is a potent opioid that, when used concurrently with other substances like cocaine, increases the risk of adverse cardiovascular and respiratory events, often leading to fatal outcomes[8].

The World Drug Report 2022 by the United Nations Office on Drugs and Crime (UNODC) showed that the annual prevalence of cocaine and heroin use at the global level is approximately 0.45% and 1.15% of the general population, respectively. Cocaine is a central nervous system stimulant, and it blocks the re-uptake of neurotransmitters such as norepinephrine, dopamine, and serotonin[1,9]. Consumption of cocaine together with ethanol results in the formation of a pharmacologically active metabolite, cocaethylene[1,9-11]. Cocaine and cocaethylene structurally differ only in the substitution of an ethyl group in place of the methyl ester of cocaine however, it has stronger physiological effects compared to cocaine[12-13]. Cocaine is metabolized primarily into two major metabolites, biologically active benzoylecgonine and biologically inactive ecgonine methyl ester (EME) by plasma and liver enzymes[14]. In this study, we present a case of a sudden and tragic death of a male individual who ingested a combination of cocaine and heroin in conjunction with alcohol, which is reported in the literature for the first time in Sri Lanka.

CASE HISTORY

A 37-year-old male was found suddenly unresponsive at a party. There is evidence that the deceased used alcohol at the party, along with others. Post-mortem samples, including blood, urine, bile, vitreous humour, and stomach contents, were sent to the forensic toxicology laboratory at the Government Analyst's Department, Sri Lanka, for toxicology analysis. Used vodka bottles, food, and beverage samples from the scene were also sent to the said department. All samples were stored and preserved in a cold room at 2-8°C.

METHOD OF ANALYSIS

Reference standards of ethanol, benzoylecgonine, cocaine, 6-monoacetylmorphine (6-MAM), morphine, and ecgonine methyl ester were purchased from Lipomed (Switzerland). Solid phase extraction (SPE) cartridges (Bond Elute Certify Columns 10 mg) were purchased from Agilent Technologies (USA). Other common chemicals: methanol (99.5%), hydrochloric acid (HCl 37%), dichloromethane (98%), isopropyl alcohol (99.5%), n-propanol (99.8%), ammonium hydroxide (25%), ethyl acetate (99.5%) and phosphoric acid (88%); all were analytical grade and purchased from Sigma-Aldrich Laborchemikalien GMBH, Germany. The two derivatization agents, N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and Trimethylchlorosilane (TMCS), were also purchased from Sigma-Aldrich Laborchemikalien GMBH, Germany.

The analysis of ethanol and methanol in blood, urine, and vitreous humour was conducted

using n-propanol as an internal standard, employing headspace gas chromatography with a flame ionization detector (HS-GC/FID). A bond Elute certify column (10 mg) was used for solid-phase extraction (SPE) to extract benzoylecgonine, ecgonine methyl ester, cocaine, 6-monoacetylmorphine (6-MAM), and morphine from biological samples. These drugs and metabolites were then quantitatively analysed using gas chromatography-mass spectrometry (GC-MS) after derivatization.

Alcohol analysis- HS-GC/FID apparatus and conditions

HS-GC/FID analysis was performed on a headspace autosampler, Thermo Scientific Triplus 300, and Thermo Scientific Trace 1300 GC with flame-ionization detector (FID). The analysis was carried out using Restek Rtx -BAC 1 capillary column (30 m x 0.32 mm ID x 1.8 µm gf).

Blood/urine/vitreous humor samples were prepared by adding a sample (0.1 mL) and internal standard (0.02% n-propanol, 0.7 mL) into a headspace vial and rapidly sealed with a septum and an aluminium crimp cap to prevent any loss of alcohols. The sealed vials were introduced to the Triplus 300 headspace auto-sampling system for volatilization (70 ^{0}C for 13 min) and GC cycle time of 30 min. The transfer line temperature was set to 200 ^{0}C, and the carrier gas flow rate was 3 mL/min. Split mode injection was done with a split ratio of 10:1, and the front inlet temperature was set to 200 ^{0}C, pressure 12.1 psi. The oven temperature was held at 70 ^{0}C. The total run time for gas chromatography was 7 min. Quantification of ethanol was performed using a calibration plot of the area ratio of ethanol to the internal standard versus ethanol concentrations.

Solid phase extraction[15]

0.1 M potassium phosphate buffer (5 mL) was added to the autopsy urine/bile sample (1 mL) followed by the addition of phosphoric acid till the pH of the sample was around 5.8-6.0. The sample was then subjected to SPE. The cartridges were activated with methanol (2 mL) and 0.1 M potassium phosphate buffer (2 mL) before use. Then the above prepared sample was applied to the SPE cartridge and slowly passed through it. The cartridges were rinsed with de-ionized water (6 mL), 0.1 M HCl (3 mL), methanol (9 mL) and vacuum-dried for 5 min. The retained drugs of abuse were eluted with a mixture of dichloromethane, isopropyl alcohol, and ammonium hydroxide (78.4:19.6:2) (2.5 mL) under gravity. Each elute was evaporated to dryness under a stream of nitrogen using a nitrogen evaporator.

For autopsy samples of blood (1 mL) and stomach contents (1 g), preparation of the samples was needed. Methanol (5 mL) was added to autopsy blood/ stomach contents, and the sample mixture was vortexed. This was centrifuged for 15 minutes at 5000 rpm. The above steps were repeated for the infranatant, and the resulting supernatants were combined. The combined supernatants from the above centrifugations were evaporated to 1 mL and followed by the above-mentioned procedure, which was used for urine/bile.

Derivatization[16]

For the GC-MS analysis, extracted samples were derivatized using a mixture of the derivatizing agents BSTFA and TMCS (99:1, 50 µL). As the final step, ethyl acetate (50 µL) was added, followed by 15 minutes in the oven at 90 °C. This was then injected into GC-MS.

GC-MS apparatus and conditions

GC-MS analysis was performed on Agilent Technologies, USA 8890-GC with Agilent 5977B-MS. The analysis was carried out using a capillary column HP-5 MS (30 m x 0.250 mm x 0.25µm). The oven temperature program had an initial temperature of 150°C held for 1 min, then 10°C/min to 270 °C held for 4 mins. The final temperature was held for 12 min. Helium was used as the carrier gas at a flow rate of 0.6 ml/min. The GC injector and transfer line temperatures were set at 270°C and 280 °C, respectively. The mass spectrophotometer was operated in the electron impact ionization (EI)

mode. The mass detector was linked to a data handling system with Agilent MSD Chem-Station integration software (E.02.02.1431) for data acquisition.

RESULTS, INTERPRETATION AND DISCUSSION

The gas chromatography detection process had a total run time of 17 minutes. The method was validated according to Eurochem guidelines, and all validated parameters fell within the accepted range of the validating method. (Table 1)

Table 1: Method validation parameters for 6-MAM, morphine, cocaine, benzoylecgonine and ecgonine methyl ester

Sample	RT (mins)	Calibration Range (µg/mL)	R^2	LOD (µg/mL)	LOQ (µg/mL)
6-MAM	15.123	5-100	0.9976	1.5	2.0
Morphine	14.407	10-100	0.9936	1.1	1.3
Cocaine	11.76	10-100	0.9957	1.1	1.3
Benzoylecgonine	12.31	5-100	0.9996	1.2	1.6
Ecgonine methyl ester	5.52	5-100	0.9975	1.2	1.6

RT- Retention Time LOD-Limit of detection; S/N=3 LOQ- Limit of quantification; S/N=10

According to the toxicology results (Table 2), all biological samples contained morphine, with concentrations ranging from 12 µg/mL to 55 µg/mL. Further, the post-mortem urine and stomach contents contained 8 µg/mL and 60 µg/mL, respectively. Diacetylmorphine (heroin) is rapidly metabolized to 6-MAM and then to morphine after administration[4]. 6-MAM is a biomarker for heroin use; therefore, the presence of 6-MAM in urine and stomach contents and morphine in the deceased's blood, bile, urine, and stomach contents confirmed the use of heroin[17].

Baselt R *et al* in 2008 reported that morphine concentrations in blood ranged from 0.2 µg/mL to 2.3 µg/mL, with an average of 0.7 µg/mL, and in urine from 14 µg/mL to 81 µg/mL, with an average of 52 µg/mL in fatal cases[18]. Furthermore, Poklis *et al* in 1995 found concentrations of 0.128 µg/mL, 135 µg/mL, and 16 µg/mL in the blood, bile, and stomach contents, respectively in a fatal case of morphine overdose[19]. Hence, it is evident that the morphine levels found in the post-mortem samples in our case have exceeded those reported in the literature.

Table 2: Toxicological results

Alcohol detection						
Blood	Ethyl alcohol-109 mg/100 mL					
Urine	Ethyl alcohol-102 mg/100 mL					
Vitreous humor	Ethyl alcohol- Not detected					
Drugs/metabolite concentration (µg/mL)						
	6-MAM	Morphine	Cocaine	Benzoylecgonine	Ecgonine methyl ester	Cocaethylene
Blood	N/D	55	N/D	6	N/D	N/D
Bile	N/D	46	727	N/D	N/D	N/D
Urine	8	12	202	227	123	Detected
Stomach content	60	42	39	7	N/D	Detected

The analytical results revealed the presence of cocaine in bile, urine, and stomach contents at concentrations of 727 µg/mL, 202 µg/mL, and 39 µg/mL, respectively. Cocaine metabolites, including benzoylecgonine were detected in the blood, urine, and stomach contents at concentrations of 6 µg/mL, 227 µg/mL, and 7 µg/mL, respectively. Ecgonine methyl ester was also detected in the urine at a concentration of 123 µg/mL. The above results confirmed that the deceased used cocaine along with heroin, which resulted in a fatal outcome. This is confirmed by the literature, where Rooney *et al*. in 2003 found that cases of morphine-cocaine co-administration had the highest proportion of fatalities during their research period from 2000 to 2019 in England[4].

Di Candia *et al*. in 2022 reported at the Bureau of Legal Medicine of the University of Milan in Italy that the most common drug detected in drug-related deaths was cocaine, with concentrations ranging from 0.33 µg/mL to 19.8 µg/mL in cardiac blood and 0.49 µg/mL to 18.78 µg/mL in femoral blood[3]. According to these data, the minimum levels detected are 0.33 µg/mL and 0.49 µg/mL in blood samples from different sites of the body. These values are below the limit of detection of the method used, which may explain the failure to detect cocaine in blood samples in our study.

Furthermore, the concentrations of ethanol in the blood and urine, which were 109 mg/100 ml and 102 mg/100 ml, respectively, indicate ethanol consumption by the deceased. Cocaethylene was detected in the urine and stomach contents samples; however, we were unable to quantify it due to the unavailability of reference standard material. The presence of cocaethylene indicates the co-administration of cocaine and ethanol, which resulted in a cumulative effect with a fatal outcome.

Cocaine use carries a significant overdose risk, particularly when consumed in large amounts or in combination with other substances, like opioids or alcohol. It can have a serious impact on the cardiovascular system, with elevation of the heart rate and blood pressure. With higher doses or more frequent use, there can be dangerous complications like seizures, strokes, and heart attacks. Chronic use may cause lasting damage to the heart, including conditions like cardiomyopathy and arrhythmias. Users of cocaine are also more prone to develop mental health issues, such as depression and suicidal thoughts[4]. When mixed with potent opioids like heroin, which has a quick action, the risk of respiratory depression and overdose is significantly heightened, even for those not regularly using opioids[20]. Additionally, cocaethylene, which is the toxic by-product of alcohol and cocaine, leads to an even greater increase in heart rate and cardiac toxicity than cocaine alone[4].

The observed variations in results can be attributed to the pharmacokinetics of the drugs, which are influenced by multiple factors. These factors may be related to the drug itself, such as the postmortem interval, route of administration, postmortem stability, redistribution, and concomitant use of other substances. Additionally, individual-specific factors, including body mass index (BMI), acute or chronic use, underlying health conditions, age, and sex, also play a significant role.

CONCLUSIONS

This case highlights a trend of polydrug abuse in Sri Lanka, especially for recreational purposes, where cocaine may be used combined with other substances. Therefore, medical professionals need to have awareness and vigilance when handling patients with sudden unconsciousness/ death.

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CONFLICTS OF INTEREST

The author declared no conflicts of interest.

ETHICAL ISSUES

None.

SOURCES OF SUPPORT

None.

AUTHOR CONTRIBUTIONS

LSH: Design of the work; the acquisition, analysis, and interpretation of data for the work; drafting the work or revising it critically for important intellectual content; and final approval of the version to be published. **WDVK:** Design of the work; and final approval of the version to be published. **SLRG:** Analysis, and interpretation of data for the work; and final approval of the version to be published. **AJW:** Analysis, and interpretation of data for the work; drafting the work and final approval of the version to be published.

REFERENCES

1. Moffat AC, Osselton MD, Widdop B. *Clarke's Analysis of drugs and poisons*. 4th ed. London: Pharmaceutical Press; 2011. p190-207.

2. Lucena J, Blanco M, Jurado C. et al. Cocaine-related sudden death: a prospective investigation in south-west Spain. *European Heart Journal*. 2010;31(3):318-329. https:// doi.org/10.1093/eurheartj/ehp557.

3. Di Candia D, Giordano G, Boracchi M, *et al*. Postmortem forensic toxicology cases: A retrospective review from Milan, Italy. *Journal of Forensic Science*. 2022;67:1640–1650. https://doi.org/10.1111/1556-4029.15050.

4. Rooney B, Sobiecka P, Rock K, Copeland C. From Bumps to Binges: Overview of Deaths Associated with Cocaine in England, Wales, and Northern Ireland (2000–2019). *Journal of Analytical Toxicology*. 2023;47(3):207-215. https://doi.org/10.1093/jat/bkad002.

5. Magalhães EJ, de Queiroz MELR, de Oliveira Penido ML, *et al*. Determination of cocaine in postmortem human liver exposed to overdose. Application of an innovative and efficient extraction/clean-up procedure and gas chromatography–mass spectrometry analysis. *Journal of Chromatography A*. 2013;1309:15-21. https://doi.org/10.1016/j.chroma.2013.08.039.

6. European Monitoring Centre for Drugs and Drug Addiction. *European Drug Report 2020: Key issues*. Luxembourg: Publications Office of the European Union; 2020.

7. Wagner FA, Anthony JC. Male–female differences in the risk of progression from first use to dependence upon cannabis, cocaine, and alcohol. *Drug and Alcohol Dependence*. 2007;86:191-198. https://doi.org/10.1016/j.drugalcdep.2006.06.003.

8. Wunsch MJ, Nakamoto K, Behonick G, Massello W. Opioid deaths in rural Virginia: a description of the high prevalence of accidental fatalities involving prescribed medications. *American Journal on Addictions*. 2009;18(1):5-14. https://doi.org/10.1080/10550490802544938.

9. Levine B. *Principles of forensic toxicology*. 4th ed. Washington: AACC Press; 2013. p293-315.

10. Laizure S, Mandrell T, Gades N, Parker R. Cocatheylene metabolism and interaction with cocaine and ethanol: role of carboxylesterases. *Drug Metabolism and Disposition*. 2002;31:16 20. https://doi.org/10.1124/dmd.31.1.16.

11. Harris D, Everhart T, Mendelson J, Jones R. The pharmacology of cocaethylene in humans following cocaine and ethanol administration. *Drug and Alcohol Dependence*. 2003;72:169-182. https://doi.org/10.1016/S0376-8716(03) 00200-X.

12. Farooq M, Bhatt A, Patel M. Neurotoxic and cardio-toxic effects of cocaine and ethanol. *Journal of Medical Toxicology*. 2009;5(3):134-138. https://doi.org/10.1007/BF03161224.

13. Raven M, Necessary BD, Danluck DA, Ettenberg A. Comparison of the reinforcing and anxiogenic effects of intravenous cocaine and cocaethylene. *Journal of Experimental and Clinical Psychopharmacology*. 2000;8(1):117-124. https://doi.org/10.1037/1064-1297.8.1.117.

14. Parker R, Laizure C. The effect of ethanol on oral cocaine pharmacokinetics reveals an unrecognized class of ethanol-mediated drug interactions. *Drug Metabolism and Disposition*. 2009;38(2):317-322. https://doi.org/10.1124/dmd.109.030056.

15. Abusada GM, Abukhalaf IK, Alford DD, *et al*. Solid-phase extraction and GC/MS quantitation of cocaine, ecgonine methyl ester, benzoylecgonine, and cocaethylene from meconium, whole blood, and plasma. *Journal of Analytical Toxicology*. 1993;17(6):353-358. https://doi.org/10.1093/jat/17.6.353

16. Menotti SV, Scanferla DP, Ota K, Fratucci GF, Souza Alves G, Bando É, Nerilo SB, Oliveira M, Junior M, Mossini S. Validation of a method for simultaneous analysis of cocaine, benzoylecgonine, and cocaethylene in urine using gas chromatography-mass spectrometry. *Brazilian Journal of Pharmaceutical Sciences.* 2020;56. https://doi.org/10.1590/s2175-97902020000118664.

17. Al-Asmari, AI, Alharbi H, Zughaibi TA. Post-Mortem Analysis of Heroin Biomarkers, Morphine and Codeine in Stomach Wall Tissue in Heroin-Related Deaths. *Toxics*. 2022;10(8):473. https://doi.org/10.3390/toxics10080473.

18. Baselt R. *Disposition of toxic drugs and chemicals in man*. 8th ed. California: Biomedical Publications; 2008. p349-354.

19. Poklis A, Edinboro LE, Wohler AS, Presswalla F, Barron D. Fatal morphine poisoning in a child due to accidental oral ingestion. *Forensic Science International*. 1995;76:55-59. https://doi.org/10.1016/0379-0738(95)01797-6.

20. Jones CM, Baldwin GT, Compton WM. Recent increases in cocaine-related overdose deaths and the role of opioids. *American Journal of Public Health*. 2017;107(3):430-432. https:// doi.org/10.2105/AJPH.2016.303627.