CASE REPORT

Forensic Analysis of Fatal Heroin Overdose in Pakistan: Examination of Seized Materials and Postmortem Blood Sample

MUHAMMAD USMAN[1,2*], YAWAR BAIG[2], ABID NASEER[3]

[1]Department of Agriculture, Food, Natural resources and Engineering(DAFNE), University of Foggia, Italy.
[2]Narcotic Unit, Punjab Forensic Science Agency, Lahore, Pakistan.
[3]Narcotics Forensic Lab, Anti-Narcotic Force Academy, Islamabad, Pakistan.

Abstract

Introduction: The concentration of heroin in street drugs is highly uncertain. This uncertainty of drug purity is the major cause of overdose. If used in combination with other drugs, it can be fatal. The route of administration and drug tolerance are also important in this prospect.
Case presentation: This article represents a case study, in which the person was found dead in a hotel room. During the investigation by the Law Enforcement agencies, two pieces of forensic evidence, i.e., currency notes, cigarette butts and postmortem specimens were submitted to the author's laboratory for investigation about the cause of death. The aliquot from pieces of evidence was analyzed using a gas chromatography-mass spectrometer (GC-MS), gas chromatography-flame ionization detector (GC-FID), and FTIR.
Discussion & conclusions: The GC-MS analysis showed a high concentration of heroin, along with dextromethorphan and phenobarbital on currency notes, while analysis of cigarette butts showed the presence of phenobarbital mixed with cannabis constituents in Tobacco leaves. The purity of the heroin sample was found to be 65.5 %. Also, toxicological analysis of postmortem blood sample showed above the lethal levels of morphine (0.619 mg/L). Moreover, the synergistic effects of the other drugs may have caused the morbidity of the victim.

Keywords: Forensic Chemistry, Diacetylmorphine, Synergistic effects of Drugs, Illicit Drugs

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INTRODUCTION

Increasing mortalities due to prolonged abuse of heroin is a worldwide social problem. In Pakistan, nearly 8.9 million people abuse drugs of multiple types, i.e., hashish, heroin, opium, and alcohol. The main cause of the high addiction rate in Pakistan is the fact that it is located on a drug trafficking route [1-3].

Hashish, commonly known as Cannabis resin, is a common drug of abuse in Pakistan. The Cannabis resin constitutes several alkaloids, among which delta-9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD) and cannabinol (CBN) are most important. Δ^9-THC is the active component of hashish [4,5], which produces a hallucinogenic effect by acting on gamma amino butyric acid (GABA) protein-coupled receptors [6]. Heroin, a semi-synthetic opioid obtained by acetylation of Morphine, is a central nervous system depressant. It is commonly abused by snorting or intravenous injection. Opium is the dried poppy latex and its derivatives produces euphoric and analgesic effects by interacting with endorphins, enkephalins and dynorphins [7].

Heroin powder sold in streets is highly impure and adulterated with a variety of cutting agents, like acetaminophen, caffeine, phenobarbital, dextromethorphan and diazepam [8,9]. Several impurities and intermediates can also be found in heroin due to poor extraction and synthesis process in clandestine laboratories [10,11]. Phenobarbital is clinically used as an anti-epileptic drug, enhancing the GABA receptor's activity, while dextromethorphan is mainly used as a cough suppressant which binds to N-methyl-D-aspartate (NMDA) receptors [12]. Both agents have their mechanism of action, but as cutting agents in heroin powder, they potentiate severe CNS depression. Other adverse effects of this mixture are euphoria, convulsion, and mental distortion, along with physical dependence and social issues [13,14].

Heroin is more potent than morphine two-fold and the lethal dose of morphine is 0.021 mg/L in blood, but addicts can tolerate it up to 10 times [15]. Heroin crosses the blood–brain barrier within 20 seconds, and nearly 70 % of the dose reaches the brain. After absorption, heroin converts to morphine and 6-diacetylmorphine (6-AM),

*Correspondence to: Muhammad Usman, Ph.D, Department of Agriculture, Food, Natural resources and Engineering(DAFNE), University of Foggia, Italy.
Email: muhammad.usman@unifg.it, Tel: 0881589360

which depresses CNS activity [16]. The stability of 6-AM in postmortem blood depends on the preservative and the storage temperature [17,18]. The annual mortality rate of heroin overdose as per a recent review of international longitudinal studies is estimated to be 0.8% [19]. However, the data about the deaths due to heroin overdose is not reported in Pakistan.

In this article, a case of death was reported, in which the possible cause of death was an adulterated heroin overdose. All cutting agents and heroin were detected using gas chromatography-mass spectrometry (GC-MS), while heroin was quantified using a gas chromatography-flame ionization detector (GC-FID). This analysis provides not only a pathway to determine the composition of street samples but also the chemical profiling of heroin samples.

CASE PRESENTATION

A male aged nearly 30 years was found dead in a hotel room. Law enforcement agency (LEA) responded to the crime scene and found two currency notes and a cigarette butt near the corpse. LEA submitted these two pieces of evidence to the author's laboratory for analysis. Also, a postmortem sample of blood was submitted for toxicological analysis. There was no visible injury on the dead body and the hyoid bone was intact, as well. As per findings of the crime scene, LEA suspected the drug overdose as a possible cause of death.

MATERIALS & METHODS

Methanol (MeOH) was purchased from Merck (USA). Moreover, reference standards of heroin, dextromethorphan, phenobarbital, caffeine, THC, CBN, CBD and monoacetylmorphine (MAM) were purchased from Sigma Aldrich (USA).

Presumptive Analysis

Colourimetric reactions were employed for presumptive analysis. Also, Mecke test was employed for opiate detection on powder, and the Modified Duquenois Levine Test was used for the detection of cannabinoids on cigarette butt [20].

Stereomicroscopy

Stereomicroscopy of currency notes was performed under the stereomicroscope (EZ4D, Leica), and photos were acquired in various magnifications ranging from 8x to 32x.

Sample Preparation

0.1 mg of sample dissolved per mL of methanol from powder particles adhered to the currency notes and vortexed for 1 min. In addition, a 20 mg part of the cigarette butt was soaked in 5 mL methanol for 1 hour, and the extract was used for qualitative analysis. Both samples were filtered before analyzing on GC-MS and GC-FID.

Gas Chromatography-Mass Spectrometry (GC-MS)

GCMS (7890B-5977, GC System and MSD Triple Axis Detector, Agilent technologies, Palo Alto, CA), along with ALS were used for the analysis of aliquots from both samples. 1 µL of the sample was injected in split mode (50:1) at 250°C in the DB-5MS column (20 m x180 µm x 0.18 µm), with a column flow rate of helium at 1 mL/min. Column programming was maintained at 150 °C for 1 min, then ramp up at the rate of 25°C/min up to 300°C and maintained there for 3.5 min. MS Source and MS Quad temperatures were set at 230°C and 150°C respectively at Scan mode for m/z from 43-550. Results were compared with both reference standards and the library (AAFS2010, NIST05). The drugs were confirmed on GCMS in SIM mode [11,21]. The analytical method was pre-validated for specificity/selectivity, precision under repeatability/reproducibility conditions, matrix effects and LOD, according to the guidelines by UNODC [22].

GC-FID Analysis

GC-FID (7890B, GC System, Agilent Technologies, Palo Alto, CA) with ALS was used for quantification of the aliquot drug of interest. 2 µL of the sample was injected in split mode (50:1) at 240°C in DB-5MS column (30 m x250 µm x 0.25 µm), with a column flow rate of N_2 at 1 mL/min. Column programming was maintained at 150 °C for 1 min, then ramp up at the rate of 25°C/min up to 300 °C, and maintained there for 3.5 min. Front Detector FID was maintained at 250°C with a flow rate of hydrogen of 40 mL/min, the airflow of 400 mL/min, and make-up gas nitrogen at 30.4 mL/min. The external standard method was used for quantification [21,23].

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis of the sample was performed on diamond ATR (iS10, Thermo Nicolet), where the aperture was set at 80, and wavenumber range from 4000 cm^{-1} to 525 cm^{-1} was taken under the resolution 4.000, sample gain 1.0 and optical velocity 0.4747. After the Background and Blank run, the sample was directly placed on diamond ATR, and a spectrum was acquired for 32 seconds [21].

Toxicological Analysis

Toxicological analysis of submitted biological evidence was performed according to the internal lab-validated method. The colourimetric technique was performed for screening poisonous gases, while immunoassay was used for screening benzodiazepines and opiates. Screening test for basic drugs was performed using GCMS on scan mode, while confirmation was performed on SIM mode, where nalorphine (1µg/L) was used as an internal standard.

RESULTS & DISCUSSION

Particles were observed on the currency notes under the stereomicroscope using different magnifications ranging from 8x to 32x (figure 1). The result of the colourimetric reactions showed the presence of opiates on currency notes and cannabis in the cigarette butt (Supplementary file, table 1).

GC-MS data showed the presence of caffeine (R.T. 4.059), phenobarbital (R.T. 4.616), dextromethorphan (R.T. 5.346), 6-acetylmorphine (6-AM) (R.T. 6.720), and heroin (R.T 7.085) on currency notes (figure 2-A and table 2), while phenobarbital (R.T. 5.485), cannabidiol (R.T. 6.204), tetrahydrocannabinol (R.T. 6.517), and cannabinol (R.T. 6.714) were identified in cigarette butt (figure 2-B and table 2). Quantification of heroin was performed using GC-FID by external standard method, and its concentration was

Figure 1. Particles on currency notes observed under a stereomicroscope.

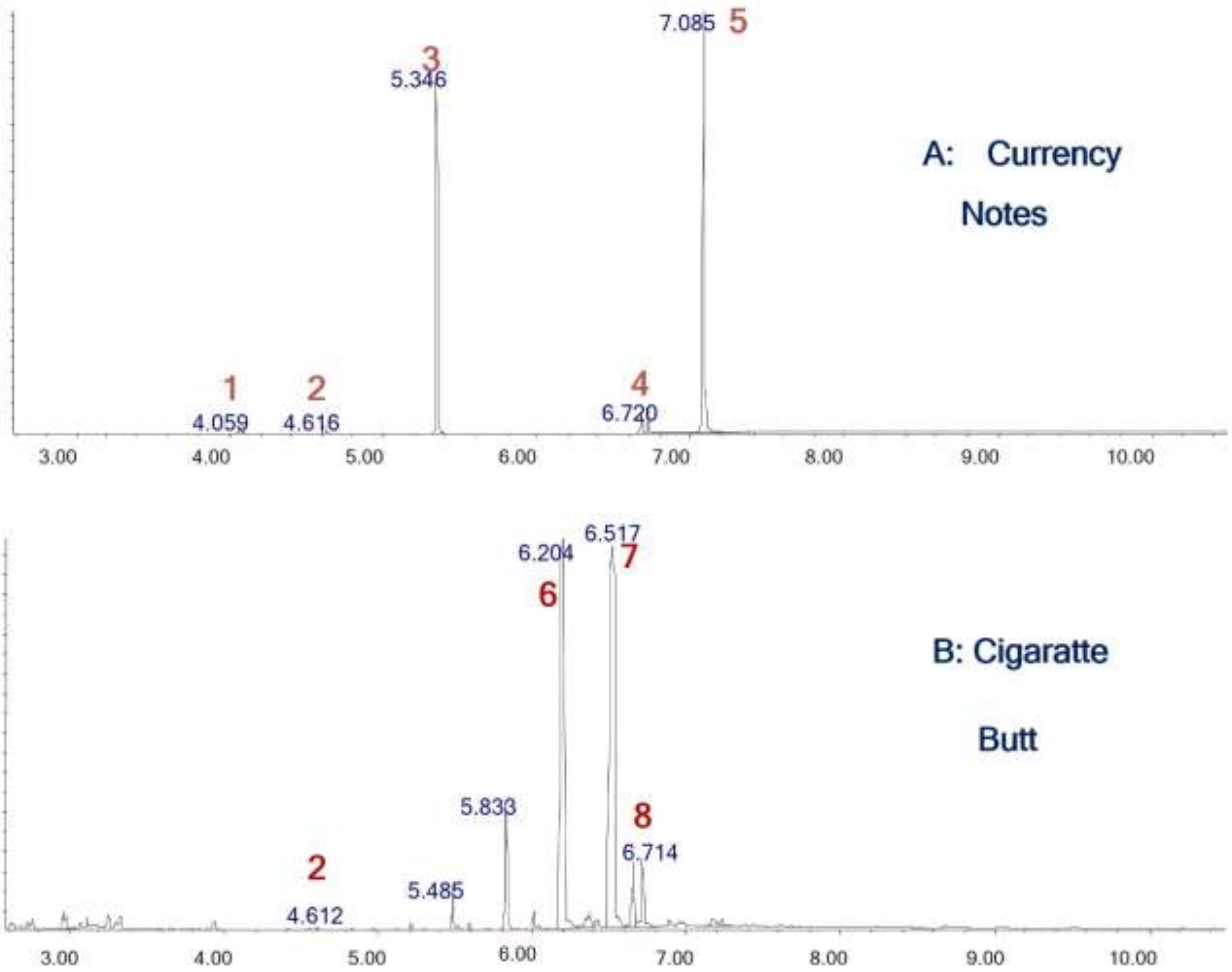

Figure 2. Total Ion Chromatogram (TIC) of an aliquot from Currency notes (A) and from cigarette butt (B), where presenting Caffeine (1), Phenobarbital (2), Dextromethorphan (3), Monoacetylmorphine (4), Diacetylmorphine (5), Cannabidiol (6), Tetrahydrocannabinol (7), Cannabinol (8).

Table 1. Showing major peaks w.r.t. m/z ions in TIC of currency notes and a cigarette butt.

No.	Compound	Retention Time (min)	Scanned ions (m/z)	Quality Match
1	Caffeine	4.08	194, 109, 55, 67, 82, 195, 42, 110	98%
2	Phenobarbital	4.61	204, 117, 146, 161, 77, 103, 115, 118	96%
3	Dextromethorphan	5.34	271, 150, 270, 31, 214, 42, 171	99%
4	Monoacetylmorphine	6.72	327, 268, 42, 43, 215, 44, 328, 269	99%
5	Heroin	7.08	327, 43, 369, 268, 310, 42, 215, 204	99%
6	Cannabidiol	6.20	231, 246, 314, 232, 121, 193, 74, 174	96%
7	Tetrahydrocannabinol	6.51	299, 231, 314, 43, 41, 295, 55, 271	98%
8	Cannabinol	6.71	295, 296, 238, 310, 119, 43, 251, 239	91%

found to be 65.5% (Supplementary file, figure 3).

FTIR analysis on extracted samples showed principal peaks of heroin at wave numbers, 1760 and 1734 cm^{-1} due to carbonyl stretching; while, 1243, 1176, 1215, and 909 cm^{-1} are due to C-O and O-H functional group absorption, which are consistent with reported values in literature [20,24,25] and with the reference standard as well (Supplementary file, figure 4).

Toxicological analysis of the postmortem blood revealed the presence of 0.619 mg/L of morphine (a metabolite of heroin) which is well above the lethal levels and confirmed the cause of death due to heroin intoxication (Supplementary file, Figure 5). Blood was not preserved in sodium fluoride, and 6-acetyl morphine might have been hydrolyzed to morphine [17]. Screening for cannabinoids showed the absence of drug metabolites in a blood sample.

CONCLUSION

In conclusion, the case study presented in this research article highlights the dangers associated with heroin abuse and the uncertainty of drug purity in street drugs. The high concentration of heroin found on the currency notes analyzed, along with the presence of other drugs like phenobarbital and dextromethorphan, suggests the use of a highly impure and adulterated form of the drug. The synergistic effects of these drugs, along with the high tolerance of addicts, can lead to fatal overdoses [26–28]. This toxicological analysis of the blood of the deceased person confirmed a high concentration of morphine level above the lethal dose leading to death.

The study also highlights the importance of forensic evidence in investigating drug-related deaths. The use of gas chromatography-mass spectrometry (GC-MS) and gas chromatography flame ionization detector (GC-FID) in the analysis of postmortem specimens and evidence like currency notes and cigarette butts provided important information about the drugs used and their concentrations.

Overall, this study is intended to highlight the need for better regulation of illicit drugs and improved drug purity testing methods. It is also important to educate the public and implement intervention programs to prevent drug abuse and overdose-related deaths.

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List of abbreviations:

GC-MS	Gas Chromatography-Mass Spectrometry
GC-FID	Gas Chromatography-Flame Ionization Detector
Δ^9-THC	Delta-9-Tetrahydrocannabinol
CBD	Cannabidiol
CBN	Cannabinol
GABA	Gamma Amino Butyric Acid
NMDA	N-Methyl-D-Aspartate
CNS	Central Nervous System
6-AM	6-Acetylmorphine
LEA	Law Enforcement Agency
MAM	Monoacetylmorphine