

Original Paper

Identification of Heroin and its Metabolite in Visceral Tissues of a Habitual Drug User - A Case Report

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ABSTRACT

The global war being waged on drugs has so far not met with much success resulting in devastating consequences for individuals and societies around the globe. Large-scale drug abuse along with the problems associated with it affects the entire world and continues to thrive in certain regions, making drug addiction one of the biggest curses worldwide. Pharmacological, cultural, social and contextual issues are the four principal factors responsible for determining the pattern of drug use/abuse. Biological explanation is thought to be responsible for the substantial overlap that exists between drug addiction and mental illness. Addiction can be characterized as a tremendous attachment, thrust or desire to repeatedly experience the drug of choice. This pursuit to satisfy the need for the drug of choice occurs despite the fact that the drug is harmful and usually injurious to the bodily and mental functioning.

We report a case of death of an elderly male who was a chronic opium addict. Chemical tests in the acidic, basic and neutral extracts of the visceral tissues and blood were carried out for preliminary screening, which showed the presence of heroin. Thin layer chromatography (TLC) and gas chromatography-mass spectroscopy (GC-MS) were further used to confirm the presence of heroin and its metabolite 6-monoacetyl morphine. The presence of 6-monoacetyl morphine determines definite heroin exposure.

The aim of this study is to present the various analytical aspects of qualitative identification, and the

intricacies involved in interpretation of results, especially with reference to establishing the cause of death having forensic and legal implications.

Key Words: Drug abuse; Heroin; 6-Monoacetyl morphine; 6-MAM; Thin layer chromatography; TLC; Gas chromatography-mass spectrometry; GC-MS

INTRODUCTION

The illegal production and distribution of narcotic drugs and various psychotropic substances have led to crime and violence all over the world arising out of abuse of such substances. Abuse is the self-administration of any drug in a manner sufficient to cause adverse consequences to the user. As abuse continues, a pattern of addiction may develop. Addiction is a behavioural pattern characterized by an overwhelming, compulsive involvement with use of a drug and the securing of its supply as well as a high tendency toward relapse after use of the drug is discontinued.¹

In India, substance abuse is assuming alarming proportions and is eating into the fabric of the society. Increasing economic stress, decrease in cultural values and weak emotional supportive bonds are the main causes, which drive a person towards substance abuse.²

According to UNODC Report 2013, since 2009, the use of opioids (heroin, opium and prescription opioids) has increased in Asia, particularly in East, South East, Central and South-West Asia. The Russian Federation, the United States and China have the largest numbers of

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people who inject drugs. Combined, they account for an estimated 46%, or nearly one in two people who inject drugs globally.³ In India, the most frequently abused drugs are cannabis, heroin and Indian-produced pharmaceutical drugs. According to an International Narcotics Control Board Report, Indians addicted to opiates are shifting their drug of choice from opium to heroin. According to UN reports, one million heroin addicts are registered in India.² As per UNODC, there were between 99,000 and 253,000 deaths as a result of illicit drug use in 2010.³

Pharmacological, cultural, social and contextual issues are the four principal factors responsible for determining how a user experiences drugs. Biological explanation is thought to be responsible for the substantial overlap that exists between drug addiction and mental illness. Addiction can be characterized as a tremendous attachment, thrust or desire to repeatedly experience the drug of choice. This pursuit to satisfy the need for the drug of choice occurs despite the fact that the drug is harmful and usually injurious to the bodily and mental functioning.⁴

In the synthesis of heroin, morphine molecules are acetylated in an excess of acetic anhydride at high temperatures. The morphine ingredient is a natural alkaloid harvested from the latex of *Papaver somniferum* (poppy). Opium latex may contain many other alkaloids like papaverine, codeine, noscapine and thebaine. Heroin is very rapidly acting, highly addictive and one of the most abused opiates. Heroin [diacetylmorphine, (5 α , 6 α)-7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol diacetate (ester), diamorphine or Diagesil®] is a semi-synthetic morphine derivative and a powerful opioid analgesic. Heroin is manufactured from morphine, a natural alkaloid occurring in opium. The chemical addition of the ester groups renders lipophilicity. Heroin may pass the blood-brain barrier much faster than its precursor morphine.^{5,6,7} This contributes to a more intense pharmacodynamic effect with a more immediate onset of action compared to morphine. Opioid receptors are stereo-specific and heroin shows a lower opioid receptor affinity than its metabolites that lack conjugates at the 3-hydroxyl group, such as 6-monoacetylmorphine (6-MAM), morphine and morphine-6-glucuronide (M6G). Heroin is often considered as a pro-drug that mainly acts through its metabolites.^{8,9} Heroin is metabolized rapidly to 6-MAM, morphine and much less active 3-monoacetyl morphine (**Fig 1**).¹⁰

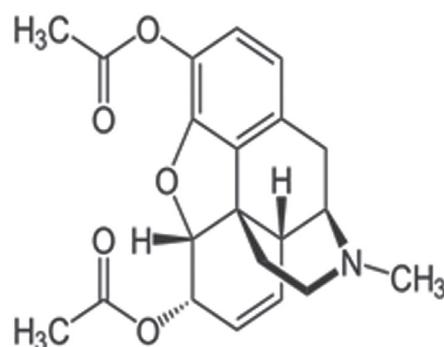


Fig 1: Molecular structure of heroin

In biological fluids, it is difficult to determine the accurate presence of heroin and its metabolites by various analytical procedures because of the thermodynamic instability of heroin and its principal metabolite 6-MAM.¹¹ (**Fig 2**). The presence of 6-MAM determines definite heroin exposure.¹²

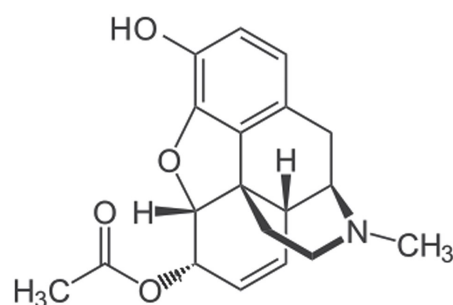


Fig 2: Molecular structure of 6-MAM

The case: A 60-year-old male of average build was admitted to hospital in an unconscious state. On clinical examination, the patient was responding to deep pain stimulus. There was history of occasional hallucinations and chronic opium addiction. During the course of treatment, the patient developed severe breathlessness and died after 15 hrs. Postmortem examination was conducted on the same day. Brain and both lungs were found to be congested. There was no abnormality in other viscera. 50 mL semi-digested fluid was found in stomach. The doctors opined that “No definite opinion about the cause of death can be given and the viscera have been preserved to rule out opium poisoning.” Preserved viscera were sent for toxicological examination to the Chemistry Division of the Forensic Science Laboratory (GNCT of Delhi) Rohini, Delhi, India.

Toxicological examination of the viscera and blood was performed. Viscera and blood were tested after ammonium sulphate digestion and screened using alkaline, acidic

and neutral ether extraction by chromatographic methods. Analysis of common poisons was undertaken.

MATERIALS AND METHODS

Reagents and Apparatus: The chemicals for digestion and extraction comprised the following: anhydrous ammonium sulphate, conc. acetic acid, sodium tungstate, diethyl ether, chloroform and ammonia of analytical grade. They were obtained from Merck. The solvents used for thin layer chromatography were chloroform, acetone, methanol, ethylacetate and ammonia of analytical grade, and were also obtained from Merck. Dragendorff's and acidified potassium iodoplatinate spray reagents were prepared according to Clarke.¹³

Extraction Procedure: 50 g of macerated viscera, i.e., stomach and intestines, liver, kidney and spleen were treated with 10 g of anhydrous ammonium sulphate and 10 mL of conc. acetic acid, then subjected to digestion on a water bath for 3 hrs at 100°C, and liquid-liquid extraction method was used. Extracts were further screened using alkaline, acidic and neutral ether extraction by chromatographic and spectrophotometric methods.¹⁴ For the identification of the basic group of drugs, the pH of the filtrate was adjusted to 8.5–9.0 and extracted with twice the volume of the following organic solvents: chloroform-isopropanol (9:1, v/v), dichloro-methane-isopropanol (9:1, v/v) and ethyl acetate. They were taken to allow the aqueous layer to separate completely the solvent layer before drawing off extract in order to avoid carrying over any water. The organic layer was separated and filtered through a small amount of dry sodium sulphate. The solution was concentrated to approximately 1–2 mL as the rest of the solvent was evaporated under a stream of nitrogen to dryness. The residue was re-dissolved in 0.1 mL methanol-chloroform (9:1) and used for TLC and GC analysis.

1 mL of blood was diluted and treated with a pinch of sodium tungstate and 2 mL of conc. hydrochloric acid. The de-proteinised blood was also extracted by the above procedure and then analyzed by TLC and GC/MS.

Chemical Tests¹⁵: The preliminary identification of diacetyl morphine (heroin) in the visceral exhibits (stomach, intestines and liver) and blood was done based on the Marquis test, which was found to be positive.

Thin Layer Chromatography: Preliminary examination of samples was done using TLC plates (silica gel G60

F254 DC Kiesel gel 60 F254 CCM Gel silica gel 60 F254TLC silica gel 60 F254), which were placed at 105°C for 30 min for activation. The chamber was saturated for 30 min. The solvent systems used were: A - Chloroform/acetone (8:2), B - Chloroform/methanol (9:1) and C - Ethylacetate:methanol:ammonia (85:10:5). The developed plates were sprayed with Dragendorff's and potassium iodoplatinate reagents to visualize the location of heroin, other groups of drugs and pesticides. The test was positive for heroin for all the exhibits, while the tests were negative when screened for pesticides and other groups of drugs.

Gas Chromatography-Mass Spectrophotometry:

Agilent 6890 GC with mass spectrophotometer was used to analyze all samples. The instrument had Agilent 19091 J-333 HP-5 column packing with 5% phenyl methyl siloxane with MSD detector. The setting of the instrument was: oven temperature – 300°C, injection temperature – 280°C, injection volume – 1 µL, mode – split, carrier gas – helium, flow rate – 70.9 mL/min, detector temperature – 290°C. Total run time for a cycle was 25 min. The instrument accesses the various types of international library software.

RESULTS AND DISCUSSION

The preliminary identification of diacetyl morphine was done by chemical test, i.e., Marquis test as described above, resulting in the appearance of violet colour of the test solution, which was comparable in intensity and colour with that of working standard solution. The blank test was run simultaneously, which gave negative result confirming that our chemical test gave us a lead to move for confirmatory test of the target poison through other complimentary techniques like TLC and GC-MS. In our study, we used the ultraviolet method for detection of the spots. TLC was run along with a reference standard. The chromatographic spot of the biological extract showed coloured reaction with Dragendorff reagent and retention factor values 0.87 and 0.37 were identical with that of corresponding reference compounds of heroin and 6-MAM in the solvent system. Orange spots on a yellow background with the Dragendorff reagent and bluish-brown spots when sprayed with the iodoplatinate reagent were observed on the plates.¹⁶

The samples were further analysed by GC-MS, which is the unique identification and confirmatory technique, wherein the compounds of interest are confirmed for their presence on the basis of retention time and matching of

fragmentation pattern of the target molecule with that of standard compound present in the database of the instrumental library. In this study, the presence of heroin and its metabolite 6-MAM were confirmed by way of comparison of both retention time (min) (Fig 3), and the matching with MS fragmentation (Fig 4). Degradation of heroin to 6-MAM and then to morphine happens rapidly *in vivo* and *in vitro*. The rates of heroin and 6-MAM degradation depend on the type of biological samples, and the duration and conditions of storage. A study of the fragmentation is highly useful for specific fragment selection or for recognition of the compounds when interpreting MS-based analytical methods for the trace level or qualitative determination of these compounds in complex matrices. The loss of the ketone $H_2C=C=O$ and acetic acid (from heroin), acetic acid (from 6-MAM) and water (from morphine) in all three cases results in a fragment ion at m/z 268 ($C_{17}H_{18}NO_2^+$). Subsequent fragmentation involves cleavages in the morphinan backbone. This results in complex fragmentation patterns, in which heroin, 6-MAM and morphine show many similar features. In heroin, an initial cleavage of the piperidine ring occurs. The most abundant fragment results from the loss of $H_3C-CH_2=N-CH_3$ (57 Da) to fragment ion with m/z 211 ($C_{14}H_{11}O_2^+$).

CONCLUSION

Illicit drug abuse remains a significant health problem all over the world, including India. Heroin-addicted patients constitute a significant population at risk. Both the liver and kidneys are involved in heroin metabolism and excretion. In human plasma, heroin is rapidly hydrolysed to 6-MAM and finally into morphine. Drug testing is divided into a two-step process — the screening test and the confirmatory test. The former test is designed to maximise the likelihood of finding a drug. Confirmatory tests, on the other hand, are designed to ensure that positive screens are in fact true positives, while also enabling the identification of the specific drug or metabolite in the biological specimens.

Heroin was identified in the visceral tissues and blood samples of the deceased through a classical chemical test and further its presence along with the metabolite 6-monoacetyl morphine was confirmed by sophisticated instrumental analysis. Under our working conditions, the mass spectrum of the biological extract displays a molecular peak for heroin and for 6-MAM corresponding to the adduct $[M+H]^+$ m/z 370 and 328, respectively. The other major peaks identified were 163, 205 and 215 for the morphinan backbone.

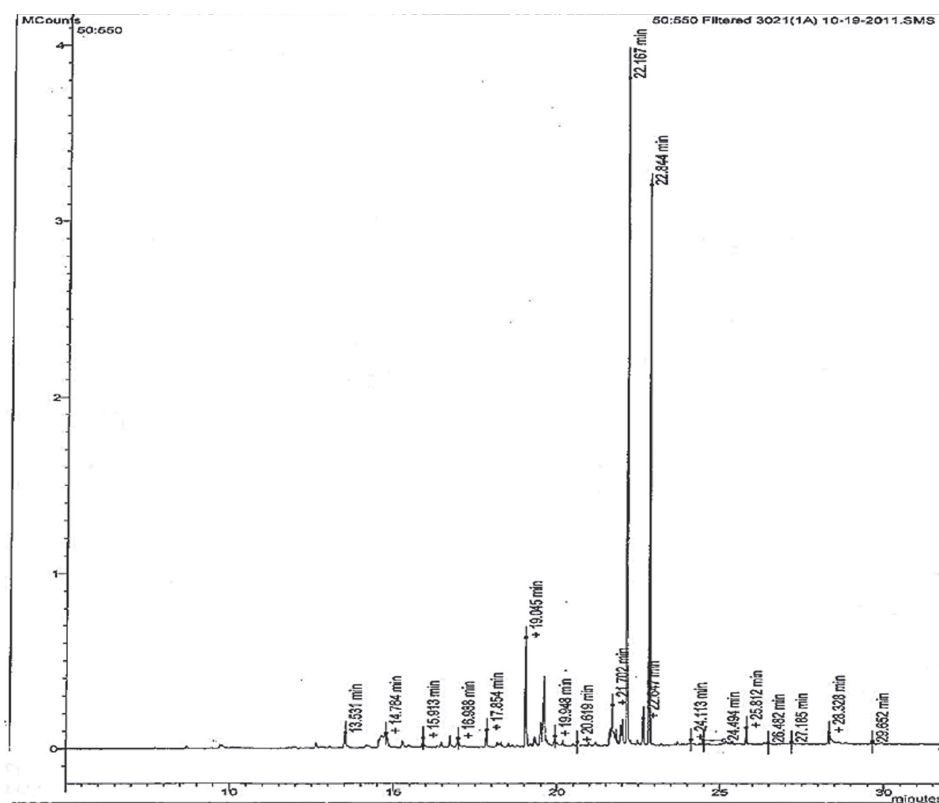


Fig 3: Gas chromatogram of exhibit (visceral tissue)

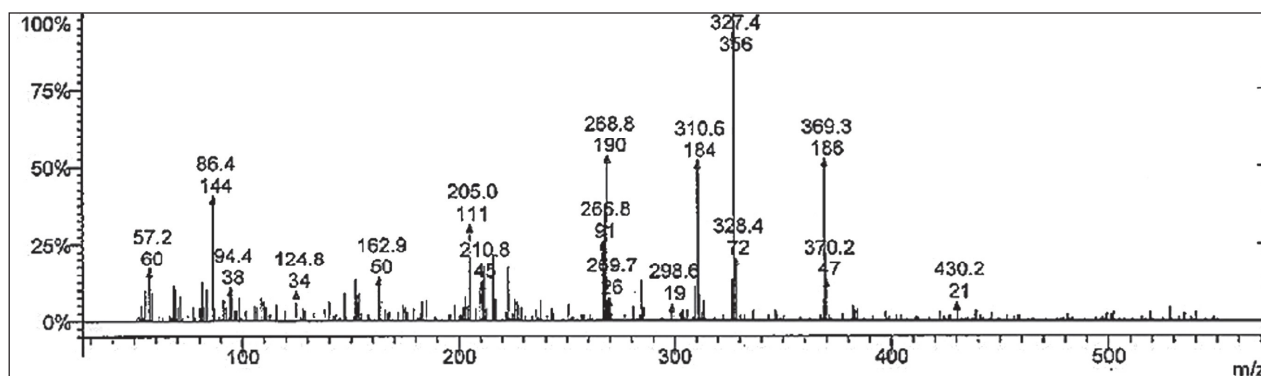


Fig 4: Mass spectra of exhibit (visceral tissue)

We detected the presence of heroin and its metabolite 6-MAM by versatile, reliable and rapid techniques like TLC and GC-MS along with the classical chemical tests without any complex sample clean-up steps. These analytical techniques are suitable for the rapid detection of such chemical species in whole blood and postmortem specimens. Presence of 6-MAM in visceral tissues confirms the definite exposure of heroin, on the basis of which we concluded this case was of chronic heroin addiction.

REFERENCES

1. Stahl SM. Essential Psychopharmacology: Neuroscientific Basis and Clinical Applications. New York: Cambridge University Press; 1996:332–370.
2. Ahmad N, Bano R, Agarwal VK, Kalakoti P. Substance abuse in India. *Pravara Med Rev* 2009;1(4):4–6.
3. World Drug Report 2013. United Nations Office on Drugs and Crime, Vienna. United Nations, New York.
4. Hanson G, Venturelli P. World Wide Web Enhanced Drugs and Society, 5th ed.; 1998:26.
5. Williams PE. Factors affecting the oral absorption of esterified antibiotics. *Biochem Soc Trans* 1985;13:511–513.
6. Oldendorf WH, Hyman S, Braun L, Oldendorf SZ. Blood-brain barrier: penetration of morphine, codeine, heroin, and methadone after carotid injection. *Science* 1972;178:984–986.
7. Cornford EM, Braun LD, Oldendorf WH, Hill MA. Comparison of lipid-mediated blood-brain-barrier penetrability in neonates and adults. *Am J Physiol* 1982;243:C161–C168.
8. Selley DE, Cao CC, Sexton T, Schwegel JA, Martin TJ, Childers SR. μ opioid receptor-mediated G-protein activation by heroin metabolites: evidence for greater efficacy of 6-monoacetylmorphine compared with morphine. *Biochem Pharmacol* 2001; 62:447–455.
9. Mignat C, Heber D, Schlicht H, Ziegler A. Synthesis, opioid receptor affinity, and enzymatic hydrolysis of sterically hindered morphine 3-esters. *J Pharm Sci* 1996;85:690–694.
10. Principles of Forensic Toxicology, 2nd ed. Washington DC: AACC Press; 2003:187–205.
11. Guillot JG, Lefebvre M, Weber JP. Determination of heroin, 6-acetylmorphine, and morphine in biological fluids using their propionyl derivatives with ion trap GC-MS. *J Analyt Toxicol* 1997;21:127–133.
12. Wyman J, Bultman S. Postmortem distribution of heroin metabolites in femoral blood, liver, cerebrospinal fluid, and vitreous humor. *J Analyt Toxicol* 2004;28:260–263.
13. Clarke EGC. Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids and Postmortem Material, 2nd ed. London: The Pharmaceutical Press; 1986:133–135.
14. Laboratory Procedure Manual: Forensic Toxicology. Directorate of Forensic Science, Ministry of Home Affairs, Govt of India, New Delhi.
15. Laboratory Procedure Manual: Narcotic Drugs. Directorate of Forensic Science, Ministry of Home Affairs, Govt of India, New Delhi.
16. Recommended Methods for Testing Heroin. Manual for Use by National Narcotics Laboratories. 1986. ST/NAR/6. United Nations.