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Research article

Solid-Phase Extraction and GC/MS Confirmation of Heroin Abuse in Urine

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Keywords: morphine, heroin, urine, gas chromatography/mass spectrometry, solid-phase extraction, 6-monoacetylmorphine

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Abstract

A method for the analysis of urine for the heroin abuse is described. The analytical procedure uses solid-phase extraction (SPE), gas chromatography/mass spectrometry (GC/MS). It allows extraction, derivatization and analysis of 6-MAM and Morphine from urine. After solid-phase extraction was complete, the eluate was selectively derivatized with N-Methyltrimethylsilyltrifluoroacetamide (MSTFA). Analysis was performed using a GC/MS system operating in full scan mode. The mass spectrum of the derivatized metabolites was searched manually against reference libraries for positive identification and the retention time checked against that of the standard. This procedure has increased both the amount and the reliability of information given to analyst. The system has been in routine operation processing 100-150 urine samples per week. The results of the analysis of standard reference material and actual samples are presented and discussed. The developed method is sensitive enough to assess relevant 6-MAM and morphine levels in urine for forensic investigations.

INTRODUCTION

Heroin and its metabolites are both regulated by the Drug Enforcement Administration (DEA) of Jordan. The regulations require testing for both heroin and its metabolites, which can be found in urine including 6-Monoacetylmorphine and morphine. The presence of illicit drugs or their metabolites in urine is an evidence of the substance intake.[1,2] Heroin as one of the most widely abused drugs, rapidly metabolized to 6-monoacetylmorphine (6-MAM) once inside the human body. This specific heroin metabolite 6-MAM is detected at a higher concentration usually within 2 to 4 hours, and after six hours, has not been detected in the urine. In the absence of 6-MAM in urine, however, morphine as an important metabolite of heroin has relatively longer detection time.[3,4] Morphine, is the primary urinary metabolite of heroin. Morphine is a naturally occurring alkaloid in opioid plants, have long been used as a drug and is also a powerful narcotic analgesic and highly addictive.[5,6] Analysis of morphine in urine has been established as an effective approach for monitoring heroin intake.[7]

In recent years, the analytical instrument for measuring heroin metabolites in urine and other matrices was varied, such as gas chromatography (GC),[8] liquid chromatography (LC-DAD),[9] UV spectrophotometer,[10] LC-mass spectrometry,[11] etc. However, some of these techniques are time-consuming, have large fluid sample usage, etc. Among them, GC-MS have received much interest in recent years for pharmaceutical determination due to their advantages of its effectiveness, reproducibility, sensitivity, and selectivity.[12-15]

In the present study, urine samples screened positive for opiate were analyzed for 6-MAM and morphine. The analysis included solid-phase extraction method and GC-MS procedure operating in the electron impact mode and scan acquisition range from 50 to 500m/z. The analytical procedure was utilized to confirm positive immunoassay results in forensic samples.

MATERIALS AND METHODS

The work described in the present study is based on the results of analyses of urine samples provided by DEA, Amman-Jordan from a number of subjects, mostly adolescents suspected of drug abuse. Urine samples were received in labeled sterile plastic containers. In most cases, the samples obtained were processed for drug testing on the same day. If not, the samples were stored at 4°C until extraction and analysis.

All reagents used were of analytical or HPLC grade. Solid-phase extraction columns (ISOLUTE®) were supplied from International Sorbent Technology Ltd. Mid Glamorgan U.K. *ACON® - One Step Multi-Drug Screen Test Panel* was obtained from *ACON* Laboratories, Inc. CA, USA. Based on the regulation of DEA, and as primary indications of heroin use, an individual's urine is presumed positive for heroin by immunoassay cutoff level of 300ng/ml urine.

GAS CHROMATOGRAPHY

Gas Chromatography-Mass Spectrometry (GC-MS) was carried out on a Hewlett-Packard 5890 Series II gas chromatograph interfaced with 5972 MSD. Chromatographic separation was achieved on HP-5MS fused silica capillary column (W&J 30m × 0.25mm × 0.5µm) and helium 99.999 was used as a carrier gas (1.0ml/min).

Samples (volume 1-µL) were injected with an injection port temperature of 250°C. The column oven temperature was programmed from an initial temperature of 180°C (held for 1min) at 15°C/min to 270°C (held for 11min). The detector temperature was 280°C. Mass spectrometer was operated in positive electron ionization mode (EI) and scan mass spectra 50 to 500 m/z were obtained.

SAMPLE PRETREATMENT

Solid-phase extraction procedure was processed on spiked and subject's samples and carried out using ISOLUTE HCX (100mg/3mL) column. The extraction of morphine and 6-MAM involved the following steps:

I. SAMPLE PREPARATION:

1mL urine sample was pipetted into a glass tube with screw cap top. For a spiked sample; 300ng morphine/6-MAM (30µL of a 100µg/10mL Methanol) was added to the sample followed by 1mL acetonitrile and then 3mL of 0.1M phosphate buffer (pH 6) solution. The solution was vortex mixed and centrifuged for 10 min at 3000rpm.

II. COLUMN CONDITIONING:

The column was conditioned with 3mL methanol followed by 2mL DI water and finally with 2mL 0.1M phosphate buffer (pH 6). The column was not allowed to dry before the addition of the sample. The vacuum was then adjusted in such a way to achieve a flow rate of about 1.5mL/min.

III. APPLY SAMPLE:

Sample was then allowed to decanted onto a pretreated column at 1-2mL/minute.

IV. COLUMN WASHING:

The column was rinsed with 3mL deionized water followed by 1mL 0.1M acetic acid. The column was then dried and then washed with 2mL hexane. Acidic and neutral impurities were eluted with 3mL of hexane–ethyl acetate (50:50). The columns were washed with 3mL of methanol and dried under vacuum for 5min at~10mm of Hg.

V. SAMPLE ELUTION AND EXTRACT DRYING:

The column was eluted with 3mL of freshly prepared methylene chloride–isopropanol–ammonium hydroxide (78:20:2). The fraction collected was evaporated to dryness at <40°C under N_2.

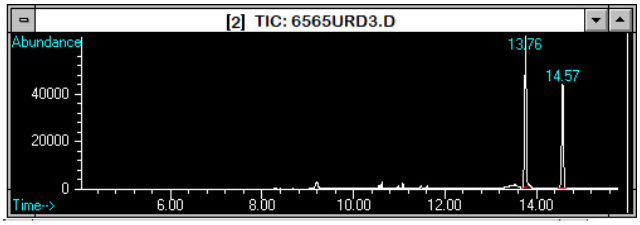

Figure 1. GC chromatogram for Morphine-TMS (13.76) and 6-MAM-TMS (14.57) from a urine extract.

VI. DERIVATIZATION:

The dried extract was dissolved in 50µl acetonitrile followed by addition of 50µl N-Methyltrimethylsilyltrifluoroacetamide (MSTFA) containing 1% Trimethylchlorosilane (TMCS). The tube content was mixed and flushed with nitrogen before it was transferred into GC vials. The sealed vial was incubated at 70°C for 20 min and cooled. 1µL of the final product was injected into GC/MS.

RESULTS AND DISCUSSION

This study was done to generate a standardized protocol for detecting heroin intake in urine samples of abusers. Urine is generally accepted as the sample of choice for drugs-of-abuse testing because urine drug testing is reliable, economical, widely utilized, and strictly regulated.[16] Immunoassays were used as a preliminary screening procedure to identify presumably positive heroin urine samples. Samples were presumed positive by immunoassays at a cutoff concentration equal to 300ng morphine/6-MAM per ml urine or at concentrations exceeded the threshold proposed by DEA. Urine with positive immunoassay was further processed to sample pretreatment by solid-phase extraction and analysis by GC-MS. The selectivity and sensitivity of GC-MS analysis implemented in this study has achieved the correlation with the immunoassay findings and the analysis results confirmed the positive immunoassay screening results for heroin -metabolite in urine. 78 out of 100 urine samples presumed positive by immunoassays were confirmed positive by GC-MS analysis. 22 samples with negative immunoassays were reported negative and not tested further.

GC-MS analysis has indicated the presence of a specific peaks at retention time around (13.76) for morphine-TMS and (14.57) for 6-MAM-TMS which was recorded and considered as a positive confirmation for heroin use. The identity of the analyte was confirmed by matching its MS spectrum to the MS spectrum of the derivatized standards [previously confirmed by matching with derivatized morphine and 6-MAM found in MS library data system] (Figure1).

Mass spectrum of morphine-TMS and 6-MAM-TMS prepared from the actual urine sample are shown in Figures 2 & 3.

The spectra provided qualitative indications that the "SPE-GC/MS analysis" scheme is a viable approach for the

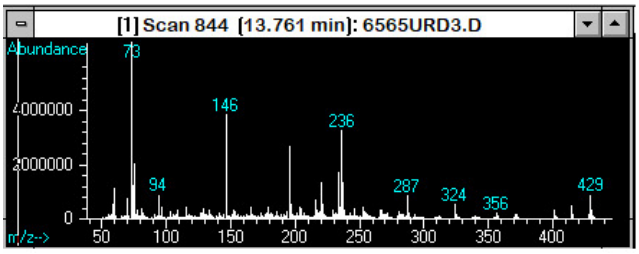

Figure 2. Mass spectrum of Morphine-TMS.

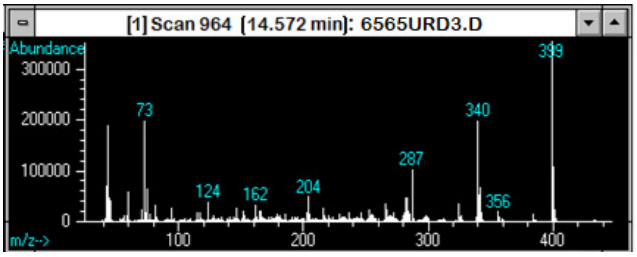

Figure 3. Mass spectrum of 6-MAM-TMS.

intended purpose. The scan range 50-500 m/z was chosen to preserve the spectrum abundance details for morphine-TMS primary peak ions at m/z = 73, 146, 236 & 429 and for 6-MAM-TMS, the primary peak ions at shown at m/z = 73, 287, 340 & 399. Extraction efficiency was studied to evaluate the effectiveness of the entire analytical protocol.

EXTRACTION EFFICIENCY

Solid-phase extraction procedure utilized a column from ISOLUTE. Recovery efficiency of the extraction column was studied by comparing the amount of the morphine (observed at the final GC/MS-measuring step) in two samples containing the same amount of the analyte. 100µl of morphine (in methanol concentrated stock) was spiked into a drug-free urine blank (Sample 1), while the same amount of stock was spiked into an empty tube at the same time (Sample 2). Sample 1, was first subjected to the solid-phase extraction process to the step ready for derivatization. Sample 2, was evaporated to dryness and processed in parallel for the derivatization and GC/MS analysis procedures. The amount of morphine spiked into both the tubes equivalent to 300ng/mL. The result derived from GC-MS analysis was 268 ng/mL, which represents a recovery of 89.3%. Urine samples for suspected heroin users have also been analyzed and found to generate reliable results.

In summary, the protocol developed in this study, in terms of the method of solid-phase extraction and GC-MS analysis provided a viable approach for the analysis of morphine and 6-MAM in urine for monitoring heroin intake.

CONCLUSION

The results show that a new competitive GC/MS method to detect heroin metabolites in human urine is successfully established. The analytical method described in this study is suitable for morphine and 6-MAM extraction from urine by SPE and detection by GC-MS. A reproducible, sensitive, and selective method for the analysis of these metabolites in human urine using GC-MS is developed. A run time of 18 minutes per sample and ability to use MS library searching for confirmation allow for high throughput of bioanalytical samples and makes full use of the GC-MS system.

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