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Editors

Acta Medicinae Legalis et Socialis

IMPRENSA DA UNIVERSIDADE DE COIMBRA
COIMBRA UNIVERSITY PRESS

ACADEMIA INTERNATIONALIS MEDICINAE LEGALIS

COIMBRA 2010

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ALTERNATIVE MATRICES: A CASE REPORT

Abstract: A 36-year-old Portuguese male hit by a train was found dead on the tracks, with his limbs partially separated and several parts of his body exhibiting severe injuries. The precise circumstances of death were unknown. As in many other similar cases, blood and urine were unavailable. A toxicological study for drugs of abuse in the liver and kidney was performed by solid phase extraction after sonication and homogenization of the samples. The organic phases obtained were evaporated until dryness and derivatized with a mixture of MSTFA/TMCS for the analysis of opiates, cocaine and canabinnoids, and with MBTFA for amphetamines. Derivatized extracts were analyzed by gas chromatography/mass spectrometry in selected ion monitoring mode. Morphine, cocaine and its metabolite benzoilecgonine were found in the liver and kidney. No alcohol was found in the vitreous humor. Although blood and urine are the most common and preferred matrices used for toxicological studies involving drugs of abuse, sometimes the choice of specimen is dictated by the case being investigated.
Keywords: Drugs of abuse; gas chromatography-mass spectrometry; alternative matrices.

Introduction

Forensic toxicology includes an understanding of drug use in the immediate ante mortem setting, analytical methodologies and interpretation of results [1].

In recent years, there has been a growing interest in the development of methodologies for detecting drugs of abuse in alternative biological matrices, although the analysis of these specimens is limited by many factors including putrefaction, sample homogenization and complexity, time-consuming techniques and analytical and chromatographic problems [2-19]. Tissues, such as the liver and kidney have been long used in post mortem toxicology analysis, especially in those cases where blood is unavailable. The extraction of the compounds from within these matrices is the major problem in forensic toxicology, due to potentially interfering substances. The liver and kidney are suitable tissues to prepare homogenates but they contain high concentrations of lipids, which may interfere in analytical procedures [12, 14-19].

The liver is the largest organ in the human body and has been used extensively as an important specimen in postmortem toxicology analysis. As a specimen, the liver has the advantage of being relatively unaffected by postmortem redistribution compared to

blood, but drug concentrations in the lobe proximal to the stomach may be affected by post mortem diffusion in cases of oral overdose [3, 10, 14, 15]. The great impediment to using liver for the interpretation of routine positive drug findings is the lack of database information of liver concentrations. As with all drugs and specimens, the process of interpretation should include consideration of all aspects of the investigation into the death, including the analysis of multiple specimens [10, 20, 21].

This work presents a particular case where the liver and kidney are the only available matrices to perform the toxicological analyses of drugs of abuse.

Case Report

A 36-year-old Portuguese male hit by a train was found dead on the tracks, with his limbs partially separated and several parts of his body exhibiting severe injuries.

The precise circumstances of his death were unknown. At the scene of the accident there was a car with the key in the ignition. The local authorities managed to identify its owner and the family made a statement saying that he had not been seen for over 3 days. One of the parents mentioned the man's drug addiction.

Like in many other similar cases, blood and urine were unavailable. The vitreous humor, liver and kidney were collected for toxicological analysis of alcohol and drugs of abuse.

Materials and Methods

The toxicological study for drugs of abuse was performed in the liver and in the kidney according to the validated procedures for the analyses of opiates, cocaine, cannabinoids, amphetamines and related compounds in blood, routinely used in our Laboratory of Forensic Toxicology.

Ethanol was tested in vitreous humor, using a gas chromatograph/flame ionization detector system, model 6890N (Hewlett-Packard, Waldbronn,Germany) equipped with a headspace autosampler.

Portions of the tissues (2g) were placed in disposable plastic tubes, sonicated and centrifugated at 3000 rpm for 5 min. Appropriate trideuterated internal standards, purchased from Cerilliant (Round Rock, TX, USA), were added to the pre-treated samples and subsequently the extraction of the drugs of abuse were performed with a Vac-Elut system assembled with columns Oasis® MCX (3 mL, 60 mg) purchased from Waters (Milford, MA, USA). The obtained extracts were dried at 40ºC under a gentle stream of N2 and dissolved in the derivatization reagent (N-Methyl-Bis (trifluoroacetamide) for amphetamines and related compounds, and with a mixture of N-Methyl-N-(trimethylsilyl) trifluoroacetamide/chlorotrimethylsilane for cocaine and metabolites, opiates and cannabinoids). The extracts were transferred to autosampler vials, and a 1 µL aliquot was injected in a HP 6890 gas chromatograph (Hewlett-Packard, Waldbronn, Germany), equipped with a 5973 mass-selective detector (Hewlett-Packard), and a capillary column (30mx0.32mm i.d., 0.25mm film thickness) with 5% phenylmethylsiloxane (HP-5 MS) from J&W Scientific (Folsom, CA, USA).

The split injection mode was used at a ratio of 6:1, and helium was used as the carrier gas with a constant flow rate of 1.2 mL/min.

The mass spectrometer was operated with a filament current of 300 μA at electron energy of 70 eV in the electron ionization (EI) mode. The temperatures of the injector and detector were set at 250 and 280°C respectively. The chromatographic conditions were as follows: initial oven temperature was 90°C for 2 min, which was increased by 20°C/min to 300°C and held for 3 min.

Confirmation was done in the selected ion monitoring (SIM) mode, and the ions were monitored at *m/z* 236, 429 and 414 for morphine, at *m/z* 82, 182 and 303 for cocaine, and at *m/z* 82, 240 and 361 for benzoilecgonine. For the internal standards, only one ion was monitored for each compound, at *m/z* 432 for morphine-*d3* and at *m/z* 243 for benzoilecgonine-*d3*. The retention times were 12.24, 10.96 and 11.25 for morphine, cocaine and benzoilecgonine respectively.

Results and Discussion

Ethanol was not detected in the vitreous humor. Cannabinoids, amphetamines and related compounds were not detected either in the liver or kidney.

Morphine, cocaine and its main metabolite benzoylecgonine were detected and confirmed in the liver and kidney. It was not possible to determine 6-acetylmorphine in the liver or kidney, probably due to its instability and transformation to morphine in the liver. The results obtained in this study revealed evidence of cocaine intake [9-13]. Since both the illicit drug heroin and the prescription drug codeine are metabolized to morphine there is no evidence of heroin intake, which tends to complicate the interpretation of opiate-positive specimens in similar cases [15, 16, 18-20].

Conclusions

This work demonstrates that our validated methodologies used in the routine analysis to determine drugs of abuse in whole blood are suitable for application in other matrices such as the liver and kidney. However, in relation to the complexity of these alternative matrices, the samples must be pre-treated and homogenized before SPE cartridge application and thoroughly cleaned so that chromatographic analysis can be performed.

The lack of information provided both from the place where the victim was found and the circumstances of his death indicate the need for a toxicological analysis despite blood and urine not being available.

Lastly, as demonstrated by the results obtained, alternative specimens can provide important information about the intake of drugs of abuse. Forensic toxicologists must bear in mind that in some special circumstances the selection of matrices is dictated by the case under investigation.

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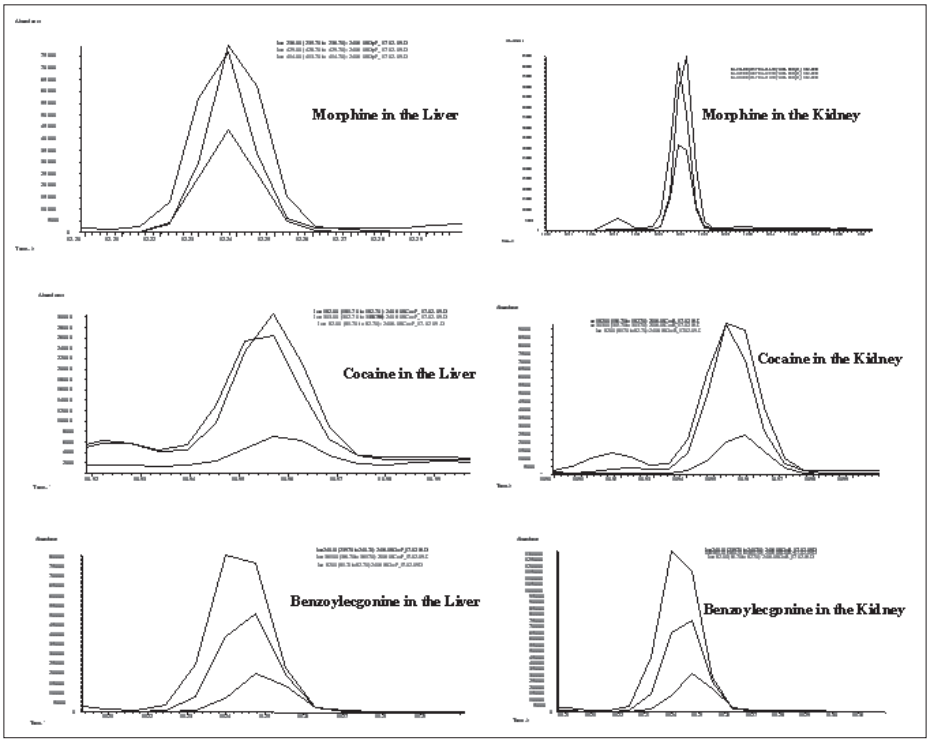

Figure 1 – Merged extracted ions chromatograms for morphine (*m/z* 236, 429, 414), cocaine (*m/z* 82, 182, 303) and benzoilecgonine (*m/z* 82, 240, 361) of liver and kidney.

	Morphine	Cocaine	Benzoilecgonine	Ethanol
Liver	D	D	D	
Kidney	D	D	D	
Vitreous Humor				ND

D – Detected
ND – Not Detected

Table 1 – Toxicological results of postmortem tissues.