Development of the DPX-RAM / LC-MS method for analysis of cocaine in biological fluid

Ludmyla S. Tavares[1](PG), Boniek G. Vaz[1](PQ), Andréa R. Chaves[1]*(PQ)

[1]*Instituto de Química, Universidade Federal de Goiás, Vila Itatiaia, Avenida Esperança s/n, Goiânia - GO.*
**andrearchaves@gmail.com*

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Abstract

The RAM-BSA sorbent has been developed and used as extraction phase for DPX sample preparation technique. The DPX-RAM/LC-MS method was employed to analyze cocaine and its metabolite in saliva. The DPX-RAM variables were optimized.

Introduction

The analysis of drugs and their metabolites in biological fluids is of great importance in forensic chemistry, doping and toxicology research. However, these assays present many problems: drugs are frequently present in very low concentration; they are strongly bound to proteins and there are numerous endogenous compounds in these matrices[1]. Cocaine analysis in complex matrices such as saliva and hair is usually done by GC-MS (Gas Chromatography - Mass Spectrometry) with a derivatization step[2]. However, LC-MS (Liquid Chromatography - Mass Spectrometry) has come a promising alternative for drug analysis, showing adequate sensibility and precision without a derivatization step required.

To clean up the samples of cocaine for the LC-MS analysis, different sample preparation techniques can be used, such as solid phase extraction, liquid-liquid extraction, solid phase microextraction, and others. DPX (disposable pipette extraction) is a miniaturized sample preparation technique based on SPE devices. In DPX, a small amount of sorbent is placed into a pipette tip and used as extraction media. The first commercially available DPX extraction phases were based on chromatographic media, as C18 material.

The RAM-BSA sorbent (restricted access material with albumin serum) combines extraction by size exclusion and partition. The macromolecules present in the biological fluids are excluded by the surface groups and the analytes interact with the hydrophobic particle interior.

In this work RAM-BSA had been developed and employed as DPX extraction phase for the analysis of cocaine, its main metabolite (benzoylecgonine) and an adulterant methaqualone in saliva samples by DPX-RAM / LC-MS method.

Results and Discussion

In order to prepare the RAM-BSA phase, the synthetic route described in Figure 1 was followed.

An adsorption test was performed with standard solution and the saturation point of the extraction phase was found to be 0.015 mg/mL.

The RAM-BSA phase was placed in a pipette tip (1000 µL) to perform the extractions.

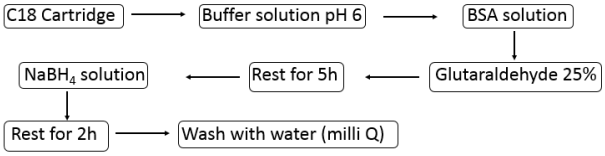

Figure 1. Schematic representation of preparation of the extraction phase RAM-BSA.

The parameters of the extraction, such as: pH (4, 7, 9), number of extraction cycles (1, 3, 5, 7, 10), volume of extractions (250, 500, 750 1000 µL), desorption solvent (mobile phase, methanol, acetonitrile) and desorption cycles (1, 3, 5, 7, 10) were optimized.

According to the optimized variables, adequate extraction efficiency was obtained in 3 draw/eject cycles (250 µL) in a solution containing 100 µL of the saliva sample in 900 µL of buffer solution (phosphate 0.05 M at pH 9); and desorption with 1 draw/eject cycle (500 µL) of methanol. A chromatogram obtained in an optimized condition is presented in Figure 2.

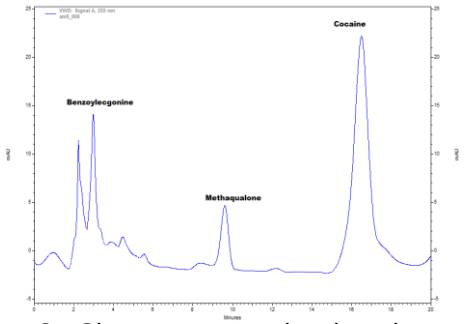

Figure 2. Chromatogram showing the separation of.benzoylecgonine, methaqualone and cocaine (Colum: C18; Mobile phase: water/methanol (30:70).

Conclusions

The DPX-RAM/LC-MS method for the analysis of cocaine benzoylecgonine and methaqualone showed to be very promising. The RAM-BSA phase excluded the endogenous and pre concentrated the analytes. The extraction and chromatographic conditions were optimized. In the next step the DPX-RAM/LC-MS developed method will be validated.

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[1] Pereira, A. G., et al., *Anal. Methods*, **2014**, *6*, 456–462
[2] Smith F. P., et al., *Forensic Sci Int.* **1966**, *83*, 179-189.