Adnan Menderes University, 4th AACD Congress, 29 Sept-3 Oct.2004, Kuşadası-AYDIN, TURKEY
Proceedings Book 143.DOC

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METOD FOR DETERMINATION OF COCAINE AND BENZOYLECGONINE IN HUMAN URINE

Valentina Pavlova, Simka Petrovska-Jovanović and Vera Trajkovska

Institute of Chemistry, Faculty of Natural Sciences and Mathematics,
"Sts. Cyril and Methodius" University, Arhimedova 5, P.O. Box 162 1000 Skopje, Macedonia
e-mail: vpavlova@iunona.pmf.ukim.edu.mk

Abstract

A reversed-phase high-performance liquid chromatographic (RP-HPLC) method for the determination of cocaine (COC) and benzoylecgonine (BZE) in human urine is described. The sample preparation procedure developed here involves solid-phase extraction (SPE) utilizing carbon-based ENVI^{TM}-Carb cartridges. The separation of the drugs obtained after SPE was performed on a C-18 reverse-phase column using gradient elution with a mixture of acidified water (0.1 % H_3PO_4) and methanol as mobile phase. Ultraviolet detection was carried out at 234 nm for both analytes. The limits of detection for BZE and COC were 0.25 μg/mL and 0.26 μg/mL, respectively.

Introduction

Cocaine ($C_{17}H_{21}NO_4$) is a local anaesthetic agent and vasoconstrictor, but produces a sense of euphoria, which is the reason for its abuse. It is a vasoconstrictor of mucous membranes, but also a potent CNS^- stimulant that elicits a state of increased alertness and euphoria [1]. Nowadays, it is very difficult to detect and quantify cocaine in the body fluids of a drug abuser. This is primarily due to the rapid and extensive metabolism of cocaine in the body, resulting in transformation into the metabolites benzoylecgonine and ecgonine.

Recently, it has been demonstrated that, both cocaine and benzoylecgonine can be electrochemically detected by square-wave voltammetry at a mercury drop electrode, in phosphate buffer with pH = 8.5 [2].

The aim of our study was to develop a simple, rapid and sensitive HPLC-UV DAD method for simultaneous determination of cocaine and benzoylecgonine in human urine, using carbon-based solid phase extraction tubes for obtaining pure extracts without interfering compounds.

Material and Methods

A Varian HPLC system equipped with a ternary pump Model 9012 and UV-Diode Array detector Model 9065 was used. The chromatographic system was controlled by the software package Varian Star 4.50. All chemicals and reagents used were of a HPLC grade or an analytical grade. Cocaine and benzoylecgonine are products of Lipomed (Switzerland) and were kindly given by the Ministry of Internal Affairs, Department of Criminology Technique (R. Macedonia). H_3PO_4 (ortho-phosphoric acid) was obtained from Alkaloid (R. Macedonia). Columns for solid-phase extraction were obtained from Sigma-Aldrich (Germany). Other chemicals of the

reagent grade and solvents of analytical and HPLC grade were purchased from Merck (Germany).

Separations were performed on the reverse phase column Lichrospher 60 RP Select B (250 x 4.6 mm, 5 μm particle diameter), protected by a guard column of the same packing (4 mm x 4.6 mm, 5 μm) (Merck). Samples were injected through injector valve with a 20 μL sample loop. The identity of each compound was established by comparing the retention times and UV spectra in real samples with those obtained for standards. The wavelength of 234 nm was used for quantifying of both, cocaine and benzoylecgonine.

Stock solutions were prepared in methanol at a concentration of 1 mg/mL and stored at -20 ^{o}C. They are stable at least for six months. The working solutions were prepared by diluting appropriate portions of these solutions with methanol.

Typical calibration curves were constructed with six blank urine samples spiked with appropriate amounts of the standard solutions in the concentration range from 0.5 to 333.0 μg/mL. These spiked urine standards were stored in a refrigerator and kept at – 20 ^{o}C throughout the testing period. The calibration curves were obtained by plotting the peak area of cocaine and benzoylecgonine versus the concentration of cocaine and benzoylecgonine in μg/mL. The regression equations were calculated by the least-squares method.

The SPE phases containing mixed-mode phases (carbon-based ENVI^{TM}-Carb cartridges) were employed for the isolation of cocaine and benzoylecgonine from urine. This extraction procedure was derived from the generally accepted mixed-mode solid phase extraction protocol [3].The final eluate was evaporated under a gentle stream of nitrogen, the residue disolved in 0.5 mL methanol and 20 μL injected in the HPLC column.

Results and Discussion

A series of parameters, including composition and pH of mobile phase (water and methanol; 5 % CH_3COOH and methanol; 1 % H_3PO_4 and methanol), isocratic and gradient elution were tested. Isocratic elution was found to be unsuitable for HPLC analysis of cocaine and benzoylecgonine because of the very long retention times obtained for cocaine compared to the ones obtained for benzoylecgonine.

The optimal resolution was obtained with mobile phases containing two solvents: 0.1 % H_3PO_4 (A) and CH_3OH (B), and the elution program was the following:

0 min.	75 % A:	25 % B;
10 min.	50 % A:	50 % B;
12 min.	20 % A:	80 % B;
13 min.	20 % A:	80 % B.

The flow rate was 1.0 mL/min. All experiments were carried out at room temperature. The elution was monitored in the whole UV range and 234 nm was selected for quantitation because both analytes exhibit absorption maximum at this wavelength.

Using the described HPLC conditions, benzoylecgonine (BZE) and cocaine (COC) were well resolved and the retention times were 8.87 min. and 10.82 min. (Fig. 1), respectively.

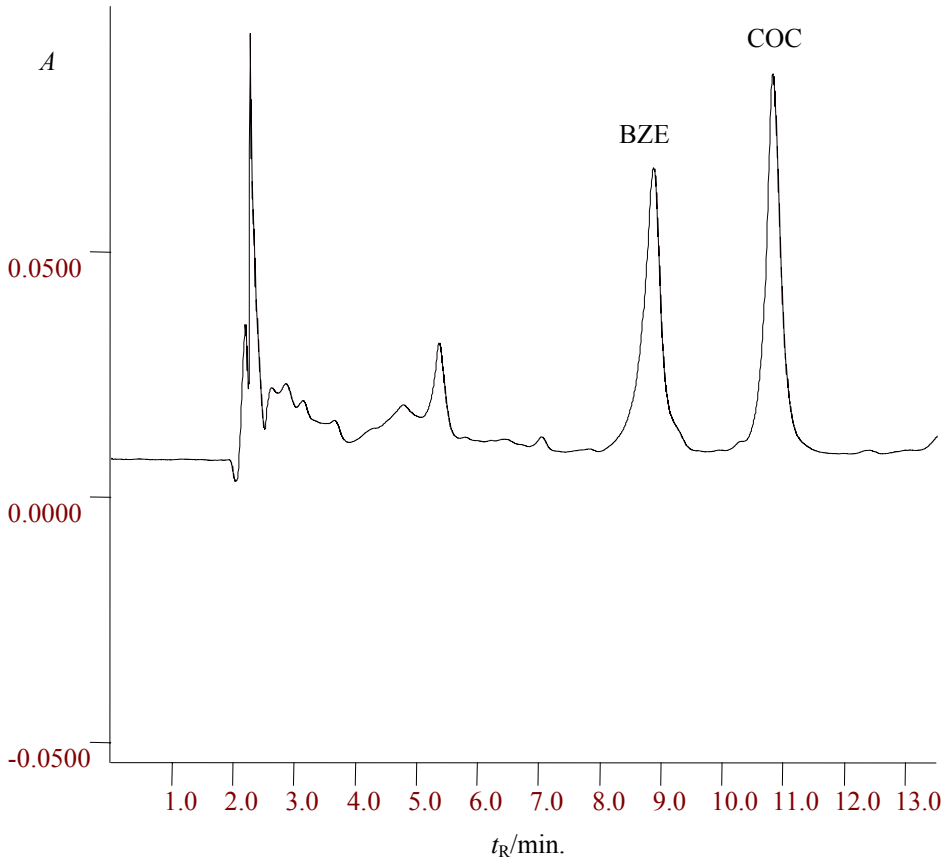

Fig. 1. Chromatogram obtained for urine containing 50 μg/mL benzoylecgonine and 50 μg/mL cocaine.

Conclusion

It was developed a simple, rapid and sensitive HPLC-UV DAD method for simultaneous determination of cocaine and benzoylecgonine in human urine, using carbon-based solid phase extraction tubes for obtaining pure extracts without interfering compounds.

References

1. Clauwaert, K.; Lambert W.; De Leenheer A. High Performance Liquid Chromatographic determina-tion of cocaine and its main metabolites in biological samples: a review. (1995): J. Liq. Chromatogr. 18, 2097.
2. Pavlova, V.; Mirčeski V.; Komorsky-Lovrić Š.; Petrovska-Jovanović S.; Mitrevski B. Studying electrode mechanism and analytical determination of cocaine and its metabolites at the mercury electrode using square-wave voltammetry. (2004): Anal. Chim. Acta. 512, 49.
3. Varian's Bond Elut Certify Instruction Manual; Varian Sample Preparation Products: Harbor City, CA, 1989.