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Determination of cocaine and benzoylecgonine by direct injection of human urine into a column-switching liquid chromatography system with diode-array detection

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Abstract

A method for the determination of cocaine (COC) and benzoylecgonine (BZE) in human urine using a column-switching liquid chromatography system is reported. A homemade precolumn (20 mm × 4.6 mm i.d.) dry-packed with Alltech ODS-C18 (35–750 μm) was employed as an extraction precolumn in order to extract and concentrate the COC and BZE from the human urine sample. The analytes were continuously transferred to the analytical column (Spherisorb-C8, 250 mm × 4.6 mm i.d.; dp = 5 μm) by means of the switching arrangement in the back-flush mode. Detection was carried out at 235 nm in a UV-diode array detector. The validation of the method revealed analytes quantitative recoveries (96–102%) at three concentrations in the range from 0.25 to 4.00 and from 0.5 to 12.0 μg/mL for COC and BZE, respectively. These values demonstrate the excellent extraction efficiency of the precolumn. The detection limits for COC and BZE at a signal-to-noise ratio of 3 were 0.08 and 0.15 μg/mL when a sample volume of 50 μL was injected. The overlap of sample preparation, analysis and recondition of the precolumn increases the sample throughput to four samples per hour. The proposed method has been applied to the determination of COC and BZE in human urine samples from 73 suspecting drug addicts. Urine concentrations of 1.0–118.10 μg of BZE/mL and 0.1–41.0 μg of COC/mL were found.

Keywords: Cocaine; Benzoylecgonine; Urine samples; HPLC; Solid-phase extraction; Column switching; Sample preparation

1. Introduction

The fast detection of illegal drugs is an important analytical task. Cocaine (COC) has become one of the most prominently abused drugs and its illicit uses have prompted considerable interest in the development of methods for the detection of users and abusers of the drug. Via distinct mechanisms, COC is rapidly metabolized in the body and its concentration in urine usually does not exceed 10% of its principal metabolite, benzoylecgonine (BZE). In this way, because of the short half-life of COC, both compounds can be analyzed in urine and plasma samples in order to monitor cocaine abuse. Several methods have been reported for the identification and quantification of COC and its metabolite in urine samples. These include immunoassay [1–3], gas chromatography [4–7], gas chromatography–mass spectrometry (GC–MS) [2,8–17], and time-of-flight secondary ion mass spectrometry (TOF-SIMS) [18]. Currently, immunoassays are frequently employed for the identification of cocaine and its metabolites in biological fluids but it cannot be considered as a reliable quantitative assay because normally all positive results must be confirmed by other quantitative methods. GC–MS is perhaps regarded as the method of choice; sev-

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eral applications reported so far testify its remarkable high sensitivity and specificity. However, derivatization of BZE is necessary to convert the polar functional groups into non-polar derivatives making the analyte suitable for gas chromatography and also to produce relatively intense high-mass molecular ions [8,13]. In the last decades, high-performance liquid chromatography (HPLC) technique is becoming more common and has been used successfully for determination of COC and BZE in human urine samples [19–25]. The use of diode-array detectors, improved the selectivity of the method by giving ultraviolet (UV) absorption profiles for each chromatographic peak [19,20,23]. With few exceptions, HPLC determinations use conventional sample preparation procedures prior to injection, such as liquid–liquid or solid-phase extraction (SPE) in cartridges with sorbents of different nature [19–23]. However, a direct injection technique is generally preferable, since the problems involved in off-line sample pretreatments, such as time-consuming procedures, errors and the risk of low recoveries, can be readily avoided. In this way, Larsen and colleagues [26] published a procedure for the determination of COC and BZE in urine using a cyano precolumn in a column switching system. On the other hand, we have reported [27] a method for the quantification of these analytes in human blood plasma samples using switching devices with an extraction precolumn packed with restricted access material. In that case, the high protein content of the blood samples forced the use of this type of precolumns that are very expensive for a routine analysis. However, considering that urine is a matrix with a smaller protein content, in this work we propose an on-line HPLC method with UV detection for the determination of COC and BZE in human urine with direct injection of the sample in a homemade C18 precolumn. The method was applied successfully to 73 urine samples from individuals suspected of cocaine abuse. Concentrations of BZE in urine determined by the method described in this paper correlated well with concentrations determined by GC–MS. Finally, when compared with the majority of classical methods described in the literature, our method offers several advantages which include simplicity and short analysis time, low cost, and high-sample throughput.

2. Experimental

2.1. Reagents and standards

HPLC-grade acetonitrile and methanol, analytical-grade monobasic and dibasic potassium phosphate and 85% (w/w) phosphoric acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). Double deionized and distilled water was processed through a Milli-Q water system (Millipore, Bedford, MA, USA).

Cocaine and benzoylecgonine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Stock solutions containing 100 μg/mL cocaine and benzoylecgonine as the free bases in methanol were prepared and stored at −24 °C. Standard aqueous solutions of both analytes at low concentration levels (<5.00 μg mL^{-1}) were prepared by diluting of stock solutions in phosphate buffer pH 6.

2.2. Urine samples

Drug-free urine obtained from 100 healthy volunteers was used for the optimization of the method and 73 urine samples from individuals suspected of cocaine abuse were analyzed using the proposed method. In all cases an informed consent was obtained from each person. The urine samples (30–40 mL) were collected in standard polyethylene bottles containing a saturated NaF solution (50 μL) and were stored at −70 °C until time of HPLC analysis in order to avoid the potential decay of cocaine. In this way, the enzymatic hydrolysis of COC to BZE is inhibited in vitro by NaF, while the non-enzymatic degradation of COC at low temperature is insignificant [28].

Prior to the analysis, the untreated urine samples were centrifuged at 3000 × *g* for 5 min in order to obtain clear supernatant, free from particle matter.

The high concentrations of BZE found in some samples required multiple dilutions in order to accommodate them within the range of the calibration graph.

2.3. Chromatographic instrumentation and conditions

The HPLC system consisted of a Perkin–Elmer LC-250 (Norwalk, CT, USA) binary solvent delivery pump (Pump 2), a Model 7125 Rheodyne injector (Cotati, CA, USA) (V1) with variable volume loops and a Perkin–Elmer Model 235 (Norwalk, CT, USA) diode-array UV absorbance detector operated at 235 nm. Data handling was performed by a VARIAN recorder/integrator Model 4290 (Palo Alto, CA, USA). A homemade precolumn (20 mm × 4.6 mm i.d.) dry-packed with Alltech ODS-C18 (35–750 μm) was employed as an extraction precolumn in order to extract and concentrate the COC and BZE from the human urine sample. Chromatographic separation was achieved on an Spherisorb RP-18 column (250 mm × 4.6 mm i.d., 5 μm particle size, Jones Chromatography) maintained at room temperature (20 °C). Both, the precolumn and analytical column were fitted to a 6-port Rheodyne 7000 switching valve controlled electronically by the binary pump. An inlet filter, with a 0.22 μm stainless-steel frit (Valco, Houston, TX, USA) was inserted in front of the precolumn to protect the analytical column further.

A solution of phosphate buffer 0.02 M (pH 6) at a flow rate of 1 mL/min was used as extraction mobile phase.

On the other hand, a gradient elution was used for optimal separation of COC and BZE in the analytical column; solvent A was 15% acetonitrile, 15% methanol in phosphate buffer 0.02 M, pH 3.0 (vvv) and solvent B was 50% acetonitrile, 45% methanol, 5% phosphate buffer 0.02 M, (pH 3) (vvv). The elution programme comprised a linear gradient from 0 to 20% of solvent B within 9 min and was then held for 9 min.