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Direct injection determination of benzoylecgonine, heroin, 6-monoacetylmorphine and morphine in serum by MLC

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Abstract

A simple and sensitive direct injection chromatographic procedure is developed for the determination of heroin, two of its metabolites (morphine and 6-monoacetylmorphine (6-MAM)), and benzoylecgonine (a metabolite of cocaine) in serum samples. The proper resolution of the four substances is obtained with a chemometrics approach, where the retention is modelled as a first step using the retention factors obtained in a limited number of mobile phases. Afterwards, an optimisation criterion that takes into account the position and shape of the chromatographic peaks is applied. The mobile phase selected to carry out the analysis was 0.1 mol L^{-1} SDS–4% (v/v) butanol buffered at pH 7, in which the separation is performed in less than 18 min. The limits of quantification were in the 17–36 ng mL^{-1} range. Intra- and inter-day assay accuracy and precision (below 3%) were obtained following ICH guidelines. The method developed was applied to the determination of the drugs studied in serum samples with good recoveries (90–104%). Serum samples from subjects that have been ingested cocaine and heroin were also analysed. The samples were injected directly in the chromatographic system without any pretreatment.

Keywords: Benzoylecgonine; Heroin; Morphine; 6-Monoacetylmorphine; Micellar liquid chromatography; Direct injection; Serum

1. Introduction

Cocaine, a naturally occurring stimulant found in the leaves of coca plants (*Erythroxilon coca*), is one of the most widespread illicit drugs of abuse. In blood as well as in plasma, cocaine is rapidly and extensively broken down in vivo and in vitro [1–3]. Benzoylecgonine is one of the major hydrolysis products of cocaine in human plasma formed by the chemical hydrolysis of the methyl ester group [2,4]. Although benzoylecgonine is not a pharmacologically active metabolite, it is of great interest in pharmacological/toxicological and forensic studies because it has a longer half-life than cocaine itself [5–7].

Cocaine is often ingested with other drugs like heroin (speed ball) to enhance its effects. Heroin (3,6-diacetyl morphine) is a potent narcotic analgesic and is metabolised in serum or liver esterases, or spontaneously hydrolysed to 6-monoacetylmorphine (6-MAM), which is further hydrolysed to morphine and this in turn is conjugated to morphine glucuronides [8–10]. Morphine may be further metabolised in both the liver and the intestine to normorphine and codeine (Fig. 1) [11]. The half-life of heroin is 5–9 min, between 38 and 45 min for 6-monoacetylmorphine, and between 90 and 180 min for morphine. Heroin is rarely detected in biological samples except for those collected immediately after ingestion. Heroin ingestion is usually evidenced by the detection of morphine. However, codeine, which is often used as a cough medicine, can also be metabolised to morphine. Thus, the use of heroin can only be proved by detecting the presence of 6-monoacetylmorphine, the characteristic metabolite of heroin, in addition to morphine.

Gas chromatography coupled to mass spectrometry (GC–MS) is the most common method used to determine

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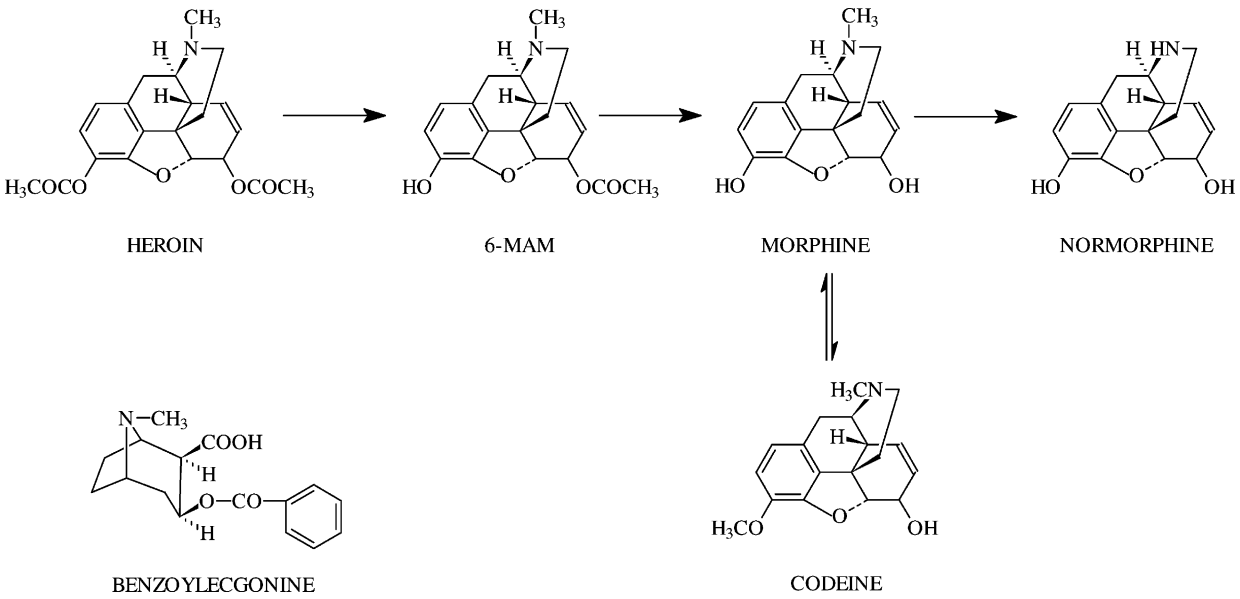

Fig. 1. Metabolic pathway of heroin and chemical structures of the compounds studied. 6-MAM is 6-monoacetylmorphine.

these drugs in different biological samples, including blood [12–15], saliva [14,15], urine [15,16] or human hair [13,15]. Other methods used to determine benzoylecgonine, heroin and its metabolites are high performance liquid chromatography (HPLC) [17,18], radioimmunoassay [17] or zonal capillary electrophoresis (CE) [19,20], electrochromatography [21], and electrospray ionisation mass spectrometry [22]. Most published procedures use HPLC with fluorescence [23], electrochemical [24], UV [25,26] or diode array detection [18,27]. GC, HPLC and CE methods for the analysis of biological samples like serum, blood, plasma, or urine require a previous step to eliminate proteins and other interferences from the matrix before injection into the column, which usually involves solid-phase or liquid–liquid extraction. This extraction step makes the procedure long, tedious and poorly reproducible.

Micellar liquid chromatography (MLC) allows biological samples to be analysed without needing to eliminate proteins and other interfering substances, thus considerably reducing the cost and analysis time. In MLC, the retention behaviour of compounds can be predicted with high accuracy [28]. This fact simplifies the optimisation of the mobile phase composition. In addition, one of the main applications of MLC is the possibility of direct sample injection of biological material into the column due to the ability of micellar aggregates to dissolve sample proteins and other compounds. MLC technique has proved to be a useful technique in the determination of diverse groups of drugs in serum and urine samples [29–34].

This study aims to develop a rapid, simple and selective MLC procedure for the screening of benzoylecgonine, heroin and two of its metabolites (6-monoacetylmorphine and morphine) in serum. The main advantage of the method lies in the direct injection of the serum samples, which largely simplifies the determination of these compounds.

2. Experimental

2.1. Reagents

Benzoylecgonine, heroin, 6-monoacetylmorphine and morphine were purchased from Sigma-Aldrich (Steinheim, Germany). The structures of these drugs are shown in Fig. 1. The mobile phases were prepared with the surfactant sodium dodecyl sulphate (99% purity, Merck, Darmstadt, Germany) and the alcohols: 1-propanol, 1-butanol or 1-pentanol (HPLC grade, Scharlab, Barcelona, Spain). The pH was buffered at 7 with disodium hydrogen phosphate (Panreac, Barcelona). Nanopure water (Barnstead, Sybron, Boston, MA, USA) was used throughout. Methanol (HPLC grade, Scharlab) was used to clean the column.

2.2. Apparatus and chromatographic conditions

An Agilent Technologies 1100 chromatograph (Palo Alto, CA, USA) equipped with a quaternary pump (Model G1311A), an autosampler with 2 mL vials (Model G1313A), a thermostated column compartment (Model G1316A) and a UV–vis detector (Model 1321A) was used. A PC workstation with HP^{3D} software was employed for instrumental control, acquisition of the chromatographic data and to measure peak properties, i.e. retention factor (k), efficiency (N) and asymmetry (B/A).

The analytical separation was accomplished using a Kromasil C18 column (Scharlab, 5 μm particle size, 250 mm × 4 mm i.d.). The flow-rate was 1 mL min^{-1} and the injection volume, 20 μL. The chromatographic runs were carried out at 25 ± 0.2 °C. Monitoring was performed at 230 nm. The column was washed with water and methanol before changing the mobile phase. The new mobile phase was then