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Validation of a gas chromatography—Ion trap tandem mass spectrometry for simultaneous analyse of cocaine and its metabolites in saliva

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Abstract

Cocaine (COC) is one of the most widely used drugs of abuse. Therefore numerous procedures are published in the literature to propose an analysis of this substance and related compounds in different matrixes. In the same way, the authors have described, in a previous work, the simultaneous analysis of COC and three of its metabolites in hair by gas chromatography–ion-trap tandem mass spectrometry (GC–MS/MS) using chemical ionization with isobutane. The present paper investigated the ability to transfer this convenient existing method for hair to another matrix, in occurrence saliva. The aim of this work was then to verify that the whole procedure (solid phase extraction (SPE) and analytical method) was also convenient to analyse simultaneously COC and three of its metabolites in this matrix. Therefore this sensitive GC–MS/MS method has been studied for the simultaneous analysis of COC, anhydroecgonine methylester (AEME), ecgonine methylester (EME) and cocaethylene (COET) in saliva samples. The method has been validated and its performances were evaluated in terms of trueness and precision using quality control (QC) samples. For quantification, the following ranges were found appropriate: 5–500 ng/ml for EME, 2–500 ng/ml for COC and COET; AEME could only be determined "semi-quantitatively" between 2 and 200 ng/ml according to our chosen acceptance criteria. Suggested dissociation pathways have also been proposed to interpret the obtained spectra.

Keywords: Chemical ionization; Cocaine; GC–MS/MS; Saliva; Ion-trap; Dissociation pathways

1. Introduction

Cocaine (COC), one of the most widely used drugs of abuse, is rapidly and almost completely metabolized to benzoylecgonine (BZE) by spontaneous chemical hydrolysis as well as to ecgonine methylester (EME) and ecgonine by esterase hydrolysis [1]. When COC is smoked, a pyrolysis product, anhydroecgonine methylester (AEME), is formed. COC is also frequently consumed together with alcohol; cocaethylene (COET), an active homologue, is formed arising through transesterification following concomitant intake of COC and ethanol. Fig. 1 displays the chemical structures of COC, and its metabolites COET, EME and AEME.

The traditional media for the quantitative measurement of most psychotropic drugs are blood and urine, because many substances and their metabolites are present in these biological matrices. Nevertheless, since the two past decades, the use of saliva for drug monitoring or pharmacokinetics studies has been developed. Oral fluid presents many advantages including the non-invasive and easy technique of collection, the low possibility of sample adulteration [2] and the presence of the parent drug as the principal analyte found. However, there are several disadvantages associated with saliva sampling like the limited sample volume, the concentration of target analytes which can be considerably low, the variable nature of salivary pH and the possibility of contamination with drug residues in the oral or nasal cavity [3]. Moreover, salivary pH and stimulated conditions of collection can affect the obtained results [4,5]. That is the reason why a great number of reviews focusing on the use of saliva in forensic drugs and other chemicals detection have been published [6–10]. There are also many articles reported for drugs concentrations in saliva [3,11,12] or correlation of oral fluid levels with plasma levels [12,13].

Saliva testing for COC and its metabolites has been reported in a number of publications and with many different

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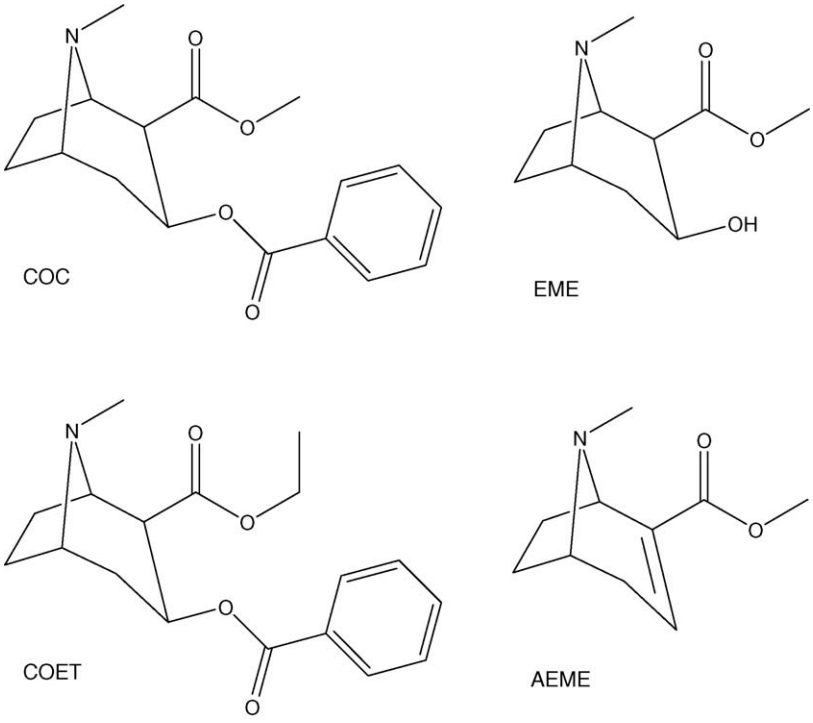

Fig. 1. Chemical structures of cocaine (COC), cocaethylene (COET), ecgonine methylester (EME) and anhydroecgonine methylester (AEME).

analytical techniques like immunoassays [14,15], spectrofluorimetry [2], liquid chromatography coupled with mass spectrometry (LC–MS) [16], gas chromatography using a nitrogen–phosphorus detector (GC–NPD) [17] or a mass spectrometer (GC–MS) [15,17,18].

In a previous work, we had developed and validated a complete procedure for the analysis of cocaine and three of its metabolites in hair by GC–CI/MS/MS using chemical ionization (CI) and ion-trap detection [19]. In hair matrix (like in saliva), the parent substance is present predominantly and only traces of metabolites are detectable. This is the main reason why ion-trap was preferred to a simple quadrupole detector such as those generally available in forensic laboratories. As a matter of fact, this spectrometric technique allows performing tandem mass spectrometry (MS/MS) at a cost much lower than triple quadrupole mass spectrometers and thus presents the advantage to permit a very selective and sensitive detection of traces. Chemical ionization stood out as the technique of choice because it carries out a "light" fragmentation (unlike electron impact) and allows to form abundant pseudo-molecular ions (MH^+) which generate characteristic fragment ions during the collision induced dissociation (CID) step of the MS/MS process. The combination of ion-trap MS/MS and CI qualities have allowed to obtained a powerful procedure for the quantitative analysis of cocaine and its metabolites in hair.

The present work consisted in adapting the analytical method mentioned above to make it suitable for analysis of COC and its metabolites in saliva, an alternative matrix. The same automated solid phase extraction (SPE) with the same analytical method (positive CI using isobutane as reagent gas with MS/MS detection) as in the previous work applied to hair were then considered. Therefore the simultaneous quantitative determination of COC and its related metabolites AEME, EME and COET in saliva by GC–MS/MS was validated. The strategy applied for the validation was based on the approach proposed by the "Société Française des Sciences et Techniques Pharmaceutiques" (SFSTP) and adapted to our specific case in forensic toxicology.

The present work also suggests dissociation pathways for interpreting the CID spectra.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile solutions of COC, COET, EME, and AEME, 1000 μg/ml, were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Acetonitrile solutions of deuterated cocaine (COC-d_3) and deuterated ecgonine methylester (EME-d_3), 100 μg/ml, 98% pure, were purchased from Cambridge Isotope Laboratories Inc. too. The methyl of the amino function is deuterated in both cases. Methanol, toluene, acetic acid (100%), hydrochloric acid concentrated solution (37%), ammonium hydroxide solution (25%), sodium hydroxide, potassium hydroxide, sodium hydrogenophosphate, and potassium dihydrogenophosphate were supplied by Merck (Darmstadt, Germany). Methylene chloride and isopropyl alcohol were obtained from Fluka (Buchs, Switzerland).

2.2. Instrumentation

Automated extraction was performed on an ASPEC apparatus (Gilson Medical Electronics, Villiers-le-Bel, France) and