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Original article

AN OPTIMIZED GC-MS METHOD FOR IDENTIFICATION OF COCAINE AND ITS MAJOR METABOLITES

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ABSTRACT

Purpose: To develop a simple yet sensitive, reliable and robust method for qualitative determination of cocaine and a series of its tropane metabolites in biological samples (blood, urine, etc.). The method should be optimized for routine forensic expertise in analytical toxicology.

Material/Methods: Gas chromatography coupled with mass spectrometry (GC-MS) has been used as the method for analytical identification. Certified analytical standards were used as a reference material for validation.

Results: Sample preparation procedure has been simplified and organized in such a way, that only common reagents are required. Initial matrix purification is done by means of solid phase extraction (SPE), during which a carefully chosen set of simple solvents is applied. Additional derivatization (BSTFA/MTBSTFA) has been shown to be of a major importance for benzoylecgonine determination. Limit of detection (LOD) has been determined to be as low as 50 ng/mL for cocaine and 25 ng/mL for benzoylecgonine. Results are given within 90 minutes.

Keywords: cocaine, benzoylecgonine, solid phase extraction, analytical toxicology, drugs of abuse, forensic expertise

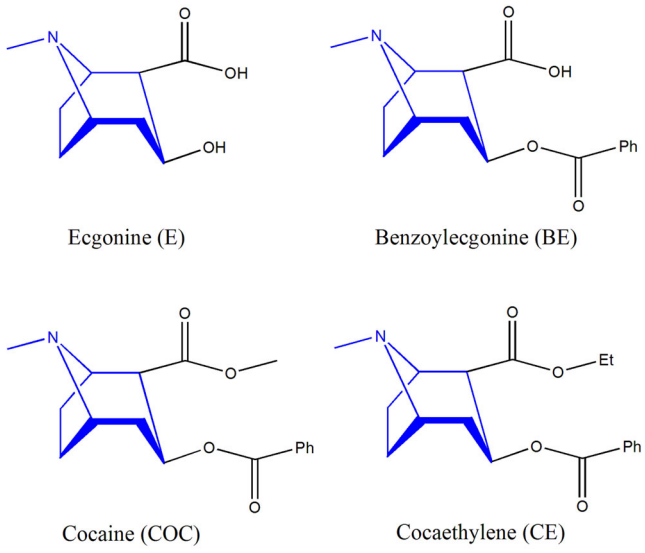
INTRODUCTION

Cocaine (COC) is powerful central nervous system stimulant. Chemically, it is an alkaloid of the tropane family, benzoylecgonine (BE) methyl ester. Although there are methods for its artificial synthesis, the major source of cocaine remains cultivated Coca (*Erythroxylum coca* and *Erythroxylum novogranatense*).

Cocaine is one of the most popular drugs nowadays, being on par with opioids and amphetamines, and clearly outweighed by cannabis only. According to United Nations Office on Drugs and Crime (UNODC) statistic report, there were more than 18 million people affected worldwide as of 2016 [1]. For the same period, the number of people in Europe who have used cocaine is estimated to be more than 4 million (0.7% of population) [ibid.].

As cocaine is an illegal drug in most countries (in Bulgaria it falls under Group II of controlled substances, and more specifically, "Substances of high risk and limited medical use"), the detection of cocaine as well as its metabolites is an often task in analytical toxicology. However, due to its pharmacokinetic properties (short biological half-life of about 1 hour [2] and low excretion rate) cocaine itself is rarely detected in body fluids. Instead, its major metabolite, benzoylecgonine, is usually monitored, as its bioavailability is considerably longer (half-life of 5-6 hours [ibid.]). Cocaethylene, an active metabolite, which is the byproduct of concurrent consumption of cocaine and alcohol, is also of forensic importance. Chemical structures of these compounds are shown in Fig 1.

Fig. 1. Chemical structure of cocaine and related compounds (the common tropane skeleton is given in blue).



From practical point of view, methods for cocaine determination in biological fluids could be separated into two groups: preliminary and confirmative. Preliminary methods are mostly based on immunoassay; they give express results (usually in order of minutes) and feature very good cut-off concentration levels (10-300 ng/mL) [3-7]. Such methods are of great importance to forensic work, e.g. crime scenes tests, workplace and driver tests, preliminary in-lab screenings, etc. However, due to low analytical specificity and non-zero risk of false positive / false negative results, a confirmative method for analysis is manda-

tory in most cases [5]. As a gold standard for this purpose, a gas chromatography mass-spectrometry technique is widely accepted and reviewed [8, 9]. Some of GS-MS methods are focused on best sensitivity to achieve excellent results, although that complicates the procedure and increases the lab time. Others use simplified techniques in order to cut down the price and shorten the time consumption, however purification of the sample is inevitably sacrificed resulting in overloaded chromatograms and loss of selectivity. Hence, the main task of the current research is to find out and validate the most efficient approach by combining the advantages of already known studies and optimizing the procedure to best fit the needs of Laboratory of analytical toxicology in Naval Hospital - Varna for routine forensic expertise on daily basis.

MATHERIALS AND METHODS

All of the used chemical reagents were of analytical grade or better. The necessary solutions are prepared using HPLC grade solvents and purified deionized water (0.067-0.100 μS cm^{-1}, TKA™ Pacific water purification system). Certified reference material (Quick-Check™ Drug Solutions of 1 mg mL^{-1} COC in MeOH and 1 mg mL^{-1} BE in MeOH) were purchased from f Alltech Associates, Inc., USA. SPE Phenomenex® cartridges (Strata® Screen-C, 55μm, 70Å, 150 mg/3mL tubes) were used. Human blood and plasma from controlled stationary patients of Naval Hospital – Varna, available in Clinical Laboratory, were taken at random and used for preparation of spiked samples.

GC-MS analysis was done on Agilent Technologies 7890B GC System & 5977A MSD module. Data acquisition and processing were controlled by Agilent MassHunter software package. Reference data from mass spectral library NIST version 2.0 g was used for comparison.

Statistical analysis was done using OriginPro® software.

RESULTS AND DISCUSSION

Reference data

Prior to analysis of biological matrices, reference GC-MS data were collected, using appropriately diluted standard cocaine and benzoylecgonine solutions. The general screening procedure used on daily basis in Laboratory was applied; analysis conditions are listed in Table 1. All GC-MS parameters have been intentionally left unoptimized, as it has been proved inconvenient to switch software settings over and over again in an environment of frequent emergency.

Tabl. 1. GC/MS Analysis conditions, SCAN mode.

Parameter	Value
Initial oven temp.	50°C
Initial time	0.5 min
Oven ramp rate	3°Cmin^{-1}
Oven final first ramp	200°C
Final time first ramp	0 min
Oven ramp rate	4°Cmin^{-1}
Oven final temp.	320°C
Final time	10 min
Total run time	90.5 min

Parameter	Value
GC Column	HP-5ms
Column dimensions	30 m × 0.25 mm
Film thickness	0.25 μm
Inlet mode	splitless
Flow mode	constant flow
Flow rate	1.5 mL min^{-1}
Carrier gas	He
Ion source temp.	230°C
Inlet temp.	250°C

Cocaine solution (1 μL 50 μg mL^{-1} in MeOH) was directly injected into GC column, yielding a clear, single-peak chromatogram. Cocaine was identified at R_t = 25.62 min, mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 82 (100), 94 (41), **182*** (87), **198** (10), **303** (18). Qualifying ions are given in bold, first of which is routinely used for quantitation purposes. The result is in excellent agreement with both software NIST library and reference MS spectrum data [10].

Benzoylecgonine was analyzed only after chemical derivatization by silylation. Two commercially available agents were used and compared: BSTFA (*N*,*O*-bis (trimethylsilyl) trifluoroacetamide + 1% TMCS (trimethylchlorosilane)) and MTBSTFA (*N*-methyl-*N*-*tert*-butyldimethylsilyltrifluoroacetamide + 1% TBDMCS (*tert*-butyldimethylchlorosilane)). Derivatization procedure included initial evaporation of aliquot BE solution (10 μL 100 μg mL^{-1} in MeOH) under gentle stream of nitrogen (at < 60ºC), reconstitution in 30 μL of ethyl acetate, addition of 50 μL silylation mixture and heating for 20 min at 70°C. After cooling, 1 μL of solution was injected into GC column, without evaporation. Using BSTFA a benzoylecgonine derivate BE × TMS was identified at R_t = 25.97 min, mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 82 (100), 105 (32), **240*** (39), **256** (6), **361** (12). Using MTBSTFA a benzoylecgonine derivate BE × TBDMS was identified at R_t = 28.31 min, mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 82 (100), 105 (32), **282*** (25), **346** (17), **403** (11).

Other cocaine-related tropane-type substances had been occasionally identified in real blood samples as well. However, as analytical grade standards were not available at the time of the study, results given bellow should not be treated as reference data. Cocaethylene: R_t = 26.17 min, mass spectrum (EI, 70 eV), *m/z*: 82, 94, 105, 196, 318; Methylecgonidine: R_t = 14.20 min, mass spectrum (EI, 70 eV), *m/z*: 122, 152, 181; Methylecgonine: R_t = 16.13 min, mass spectrum (EI, 70 eV), *m/z*: 82, 96, 199; Ethylecgonine:

R_t = 17.04 min, mass spectrum (EI, 70 eV), *m/z*: 82, 96, 168.

Experimental procedure

Purification of biological matrices before GC-MS analysis was done by mean of solid-phase extraction using silica-based strong type cation exchanger. Procedure description follows:

(1) Condition the SPE column applying 2 mL MeOH followed by 2 mL water.

(2) Prepare the sample by diluting 1 mL blood serum/plasma with 2 mL of water, *or* by diluting 1 mL urine with 2 mL of 0.1 M phosphate buffer pH 6.0. Load the sample at rate as low as 1 drop per second.

(3) Wash the column consequently by 2 mL 0.1 M HCl and 2 mL MeOH.

(4) Dry at full vacuum for 2 minutes.

(5) Elute drop wise using 2 mL 5% ammonium hydroxide in MeOH.

(6) Thoroughly evaporate under nitrogen stream (at < 60°C) to complete dryness.

If the explicit goal is cocaine identification, at this stage reconstitute the residue in 2-3 droplets of MeOH and inject 1 µL of solution into GC column. However, more often the benzoylecgonine is the primary target, and hence the procedure continues with derivatization steps. Note that cocaine (as well as cocaethylene) goes through derivatization unaffected and will be present in final chromatogram anyway.

(7) Reconstitute the residue in 30 µL ethyl acetate and add 50 µL silylating mixture (BSTFA + 1% TMCS or MTBSTFA + 1% TBDMCS).

(8) Heat for 20 min at 70°C.

(9) After cooling, inject 1 µL of solution into GC column, without any further manipulations/evaporation.

Method validation

Keeping in mind that COC/BE/CE confirmative GC-MS analysis is often used as a decision factor in criminal justice and forensic science, increased demands on results accuracy are applicable, according to Blackstone's law formulation. Declaring a sample positive is granted only after a rigorous set of acceptance criteria has been met. Strictly identical set of acceptance criteria were involved in procedure of LoD determination. Acceptance criteria were constructed *a priori*, applying unusually severe requirements, all of which to be *simultaneously* agreed:

(1) R_t of the sample signal differs less than 1% of the value, obtained from calibrating standard *and* qualifying ions ratios agree within ±20% relative to those obtained for a calibrating standard;

(2) Relative intensity of *any* qualifying ion at peak maximum exceeds mean background noise plus 3 standard deviations ($p < 0.01$).

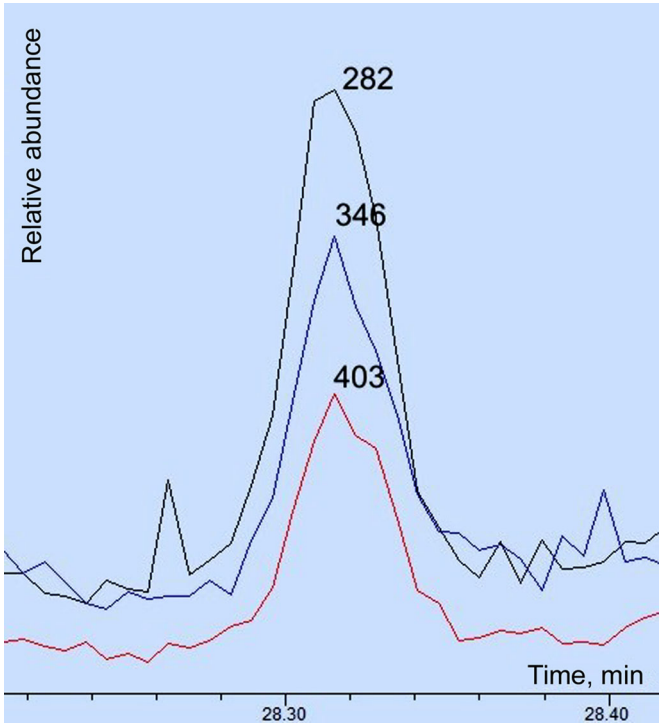
(3) Positive NIST library identification of the substance.

Here, set (1) represents the strongly recommended empirical approach [11, 12], while set (2) follows statistical approach. In order to achieve maximum reduction of type I (false positive) errors, we apply both.

Actual LoD determination was done by carefully preparing a set of spiked blood samples (at levels 400, 200, 100, 50, 25, 12.5 ng mL^{-1}) for every substance of interest. Spiked samples underwent described analytical procedure. LoD was associated with the lowest analyte concentration at which all of the acceptance criteria are still agreed. For cocaine LoD was estimated to 50 ng mL^{-1}, for benzoylecgonine (derivatized by BSTFA) to 100 ng mL^{-1}, and for benzoylecgonine (derivatized by MTBSTFA) – to 25 ng mL^{-1}. The advantage of MTBSTFA over BSTFA silylation is obvious.

Due to the severe requirements accepted, presence of benzoylecgonine in test samples even at LoD levels was proven to produce clear evidence of well-defined peak area, as it is shown on Fig. 2 for example.

Fig. 2. Fragment of extracted ion chromatogram representing a clear evidence of BE presence at LoD level (25 ng mL^{-1} BE in blood, MTBSTTFA derivatization).



CONCLUSION

After a critical consideration of available methods, an optimized approach to cocaine and its major metabolite identification in biological samples has been proposed. Validation of the method by means of certified analytical standards has proven it accurate and sensitive. It has been demonstrated that effectiveness of MTBSTFA as a derivatization agent is significantly greater than of BSTFA. The optimized analytical procedure is simple, relatively short (under 90 minutes), and needs maintaining routine set of reagents only (water, methanol, HCl, NH_3). The main purpose of this optimized method is application in forensic expertise analysis.

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