Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 852 (2007) 338–344
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

# Quantitative analysis of cocaine and its metabolites in whole blood and urine by high-performance liquid chromatography coupled with tandem mass spectrometry

Sys Stybe Johansen *, Helle Merete Bhatia

*Department of Forensic Chemistry, Institute of Forensic Medicine, University of Copenhagen, Frederik V's vej 11, DK-2100 Copenhagen OE, Denmark*

Received 13 July 2006; accepted 22 January 2007
Available online 1 February 2007

**Abstract**

*Aim:* In forensic toxicology it is important to have specific and sensitive analysis for quantification of illicit drugs in biological matrices. This paper describes a quantitative method for determination of cocaine and its major metabolites (ecgonine methyl ester, benzoylecgonine, norcocaine and ethylene cocaine) in whole blood and urine by liquid chromatography coupled with tandem mass spectrometry LC/MS/MS.
*Method:* The sample pre-treatment (0.20 g) consisted of acid precipitation, followed by centrifugation and solid phase extraction of supernatant using mixed mode sorbent columns (SPEC® MP1 Ansys Diag. Inc.). Chromatographic separation was performed at 30 °C on a reverse phase Zorbax C18 column with a gradient system consisting of formic acid, water and acetonitrile. The analysis was performed by positive electrospray ionisation with a triple quadropole mass spectrometer operating in multiple reaction monitoring (MRM) mode. Two MRM transitions of each analyte were established and identification criteria were set up based on the retention time and the ion ratio. The quantification was performed using deuterated internal analytes of cocaine, benzoylecgonine and ecgonine methyl ester. The calibration curves of extracted standards were linear over a working range of 0.001–2.00 mg/kg whole blood for all analytes. The limit of quantification was 0.008 mg/kg; the interday precision (measured by relative standard deviation—%RSD) was less than 10% and the accuracy (BIAS) less than 12% for all analytes in whole blood. Urine samples were estimated semi-quantitatively at a cut-off level of 0.15 mg/kg with an interday precision of 15%.
*Conclusion:* A liquid chromatography mass spectrometric (LC/MS/MS) method has been developed for confirmation and quantification of cocaine and its metabolites (ecgonine methyl ester, benzoylecgonine, norcocaine and ethylene cocaine) in whole blood and semi-quantitative in urine. The method is specific and sensitive and offers thereby an excellent alternative to other methods such as GC–MS that involves derivatisation.


*Keywords:* Cocaine and metabolites; Forensic samples; LC/MS/MS

## 1. Introduction

Cocaine (COC) is a potent central nervous system stimulant and a widespread drug of abuse that is high importance to identify and quantify its level in forensic toxicology. Cocaine is rapidly inactivated in blood by hydrolysis of the ester linkages to benzoylecgonine (BZE) and ecgonine methyl ester (EME), respectively—the two major metabolites, which are inactive but very useful in detection of cocaine abuse due to their longer half-lives in biological matrices (approx. five times longer than cocaine). To a lesser extent cocaine is hydrolysed to norcocaine (NCO), an active metabolite, or by presence of ethanol in the blood to the toxic ethylene cocaine (ECO) [1,2]. All metabolites are expected to be metabolites to the common product ecgonine, which is difficult to isolate from biological fluids and therefore only rarely done/determined [3]. Further, it is difficult to differentiate between in vivo and in vitro metabolism; cocaine present in blood is very unstable in vitro and undergoes enzymatic hydrolysis to EME and chemical hydrolysis to BZE [4,5]. It is therefore recommended for assessment of the severity of cocaine consumption to measure cocaine and its both inactive and active metabolites.

Several techniques for quantitative analysis of COC, BZE, EME, NCO and ECO in biological matrices are available. Gas chromatography (GC) is a frequently used technique in the last 15 years for the analysis of cocaine and its metabolites in whole

* Corresponding author. Tel.: +45 35326241; fax: +45 35326085.
*E-mail address:* SSJ@forensic.ku.dk (S.S. Johansen).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.01.033

blood and urine, typically including a derivatisation procedure (acylation or silylation) with MS detection to ensure a high specificity and GC applicable [5,6] but environmental problems with the derivatisation reagent occurs and the step is very sensitive in the analysis. However, newer techniques/alternatives have occurred such as liquid chromatography (LC) combined with MS detection, which offers a good separation and detection technique without need for derivatisation [7,8]. The use of tandem MS detection results in an excellent sensitivity and specificity. Several LC/MS/MS applications for cocaine have been published, but they have either been *qualitative* analysis of only COC and BZE in plasma [9,10] or quantitative analysis of the cocaine and metabolites *in other matrices* such as urine, oral fluid, hair and meconium [11–15]. Published methods in blood are mainly performed in plasma and typically limited to cocaine and one or two metabolites [3,8,16–18]. Cailleux et al. [19] published a LC/MS/MS method for whole blood where BZE and EME coeluted and the sample preparation involved the hazard trichloromethane. To our knowledge there have not been published LC/MS/MS applications of a quantitative method for determination of COC, EME, BZE, NCO and ECO in whole blood. This paper presents a validated method of confirmation and quantification of cocaine and its metabolites in whole blood by solid phase extraction (SPE) and LC/MS/MS analysis.

## 2. Experimental

### 2.1. Chemicals and materials

Standard materials of EME, NCO, ECO and of internal standards (IS) EME-$d_3$ and COC-$d_3$ were purchased from Cambridge Isotope Laboratories, Andover, MA, USA whereas COC and BZE were from Lipomed, Artesheim, Switzerland and BZE-$d_8$ was Ceriliant, Round Rock, Texas, USA. All standard materials were of high purity. For extraction and analysis there were used solvents of chromatographic grade from Rathburn Chemicals Ltd., Walkerburn, UK and Merck, Darmstadt, Germany. The solid-phase extraction cartridges SPEC® MP1 were purchased from Kingo Diagnostics, Lake Forest, CA, USA. Authentic forensic samples of whole blood and urine from humans including post-mortem materials and whole blood from horse for quality control (QC) purposes were used in this study. Horse blood is used in the laboratory as a standard and is similar to human whole blood.

### 2.2. Stock solutions and standards

For simultaneous determination of cocaine and its metabolites, a 0.10 mg/l internal standard in acetonitrile was prepared as a mixed standard of the available deuterated analytes: COC-$d_3$, EME-$d_3$ and BZE-$d_8$. A 10.00 mg/l standard stock solution in acetonitrile was prepared as a mixed solution of COC, EME, BZE, NCO and ECO standards. In order to check the performance of the LC/MS/MS before doing the analysis, two 0.005 and 0.05 mg/l system controls with 0.05 mg/l IS were made in mobile phase. Five extracted standards (daily calibration curve) were made of 0.000–0.005–0.05–0.5–2.0 mg/kg whole blood with 0.05 mg/l IS. Blank urine and a 0.15 mg/l control sample of spiked urine (cut off) were made before forensic urine samples were to be analysed. Blood samples were weighed prior to analysis as a standard procedure in the laboratory with whole blood and post-mortem blood, while urine samples were dispensed.

### 2.3. Sample preparation

0.200 g whole blood or 50 μl urine were mixed with 200 μl water and 100 μl 0.10 mg/l IS in acetonitrile, thereafter with 2.5 ml 0.2 M HCl and afterwards centrifuged for 10 min at 3600 rpm at 5 °C. The supernatant was transferred to SPEC column for extraction. The SPEC column was first activated with 500 μl methanol and 500 μl 0.1 M HCl before applying the supernatant. The column was then washed with 1000 μl 0.1 M HCl and 1000 μl 20% methanol in $H_2O$, then dried for approx. 10 min and finally eluted with 500 μl freshly prepared solution of concentrated $NH_3$, dichloromethane and isopropanol (1:10:40, v/v). The eluate was evaporated to dryness at 50 °C under a steam of pure nitrogen. The remanence was reconstituted in 200 μl mobile phase and transferred to an autosampler vial of which 5 μl was injected into the chromatographic system.

### 2.4. Chromatography and MS/MS conditions

For separation an Agilent 1100 series HPLC system from Agilent Technologies, Waldbronn, Germany consisting of a binary pump, an autosampler and a thermo stated column compartment was used with a Zorbax SB-C18 column (2.1 mm, 30 mm, 3 μm). Gradient elution began with a mobile phase of 5% of solvent A (5% acetonitrile with 0.05% formic acid) and 95% of solvent B (100% acetonitrile with 0.05% formic acid). This composition was held for 2 min and then the percentage of solvent B was linearly increased to 60% over 10 min. A total analysis time of 17 min was used due to the re-equilibration time between injections. The flow rate was 0.2 ml/min at 30 °C.

A Quattro micro, tandem quadrupole mass spectrometer, from Waters, Manchester, UK, was coupled to the HPLC system. Data was acquired in the positive ion mode with an electrospray (ESI) source using MassLynx software 4.0, and calculations used extracted ion chromatograms by QuanLynx. Mass spectrometer conditions (cone, lens voltage, collision energy, etc.) were optimised by infusion of the standard solution through a T-piece into the Z-spray ion source in a continuous flow of mobile phase at 0.2 ml/min. Multiple reaction monitoring (MRM) analysis was used for data collection and the settings are listed in Table 1. For each analyte two MRMs were set up, one for quantification and one as qualifier using one parent ion and two daughter ions per analyte as shown in Table 1. The cone energy was optimised to 28 V, while the source and desolvation temperatures were 120 and 300 °C, respectively. As part of identification criteria for each analyte, the absolute retention time and ion ratio was applied according to international EU guidelines on environmental and food analyses [20]. The absolute

Table 1
MS/MS conditions for the Quattro micro

| | Retention time (min) | Collision 1 (V) | Transition 1 MRM1 | Collision 2 (V) | Transition 2 MRM2 | Ion ratio MRM2/MRM1 |
|---|---|---|---|---|---|---|
| EME | 0.60 | 25 | 200.0 → 81.9 | 17 | 200.0 → 182.0 | 2.0 |
| EME-$d_3$ | 0.60 | 25 | 203.0 → 84.9 | – | | |
| BZE | 3.45 | 20 | 290.0 → 168.0 | 28 | 290.0 → 104.9 | 0.33 |
| BZE-$d_8$ | 3.45 | 20 | 298.1 → 171.0 | – | | |
| COC | 6.92 | 20 | 304.0 → 182.0 | 30 | 304.0 → 81.8 | 0.24 |
| COC-$d_3$ | 6.92 | 20 | 307.0 → 185.0 | – | | |
| NCO | 7.27 | 15 | 290.0 → 168.0 | 20 | 290.0 → 135.9 | 0.65 |
| ECO | 8.14 | 20 | 318.0 → 196.0 | 30 | 318.0 → 81.8 | 0.35 |

retention time may have a maximum deviate of 0.1 min. Furthermore, the ratio of the responses for MRM2 against MRM1 may have a maximum deviation of 5% within the measuring range.

### 2.5. Assay characteristics for method validation

#### 2.5.1. Specificity

The selectivity is high, as a tandem MRM is used for detection of the analytes. Only drugs with same molecular weight and similarity of structure could be of interest to test, such as atropine and scopolamine. Furthermore, the pure chemicals used in sample preparation were also tested for contributing to positive blank values.

Matrix interferences for EME, BZE and COC were investigated by spiking negative forensic samples: six whole blood samples and four urine samples. The spiking level was 0.05 mg/kg in the blood samples and 0.15 mg/kg in urine samples.

Ion suppression experiment was performed by injecting cocaine and its metabolites through the syringe pump into a T-piece mixing it with mobile phase. Suppression was examined in extracts of control whole blood within an extended run time of 100 min.

#### 2.5.2. Calibration curve and linearity

The linearity was first established in pure standards of cocaine and its metabolites in mobile phase. Then a calibration curve of extracted whole blood standards was determined to confirm the linearity and define the measuring range. The calibration graphs were derived by plotting the peak area of analytes to IS versus the whole blood concentration of the analytes. The analytes EME, BZE and COC were calculated on basis of their deuterated IS, whereas NCO and ECO were related to COC-$d_3$. Linear regression with $1/x$ weighting was used and the slope, intercept and coefficient of determination ($R^2$) were determined in 22 series.

#### 2.5.3. Recovery

The recovery was determined as the amount of extracted standard compared to standards in pure solvent (system controls). The average of absolute recovery was calculated as percent recovery of each analyte of extracted against pure solvents standards. The relative recovery concurs with the accuracy and is described in the next section.

#### 2.5.4. Accuracy and precision

The intraday precision was determined at four levels (0.005, 0.05, 0.5 and 2.0 mg/kg whole blood) as duplicates in nine series. The interday precision was determined at eight levels of control standards (0.0025, 0.005, 0.025, 0.05, 0.25, 0.5, 1.0 and 2.0 mg/kg whole blood), of which two were used at the time. Precision was expressed as the relative standard deviation (%RSD). Accuracy was expressed in terms of bias, as the percent deviation of the mean determined concentration against the spiked concentration. A %RSD and %BIAS less than or equal to 15% in measuring range above LOQ and less than 20% at the level of LOQ were demanded.

#### 2.5.5. Determination of LOD and LOQ

LOD and LOQ are calculated on basis of interday precision of a standard at very low concentration close to the expected LOD. The LOD and the LOQ were defined respectively, as 3 and 10 times the standard deviation (SD) of the low standard including the blank level. Therefore, data from control standard (0.0025 mg/kg) of the analytes EME, BZE and COC was used to calculate the LOD and LOQ for these analytes, whereas NCO and ECO had to be calculated from intraday precision data. The intraday and interday precision data were compared for EME, BZE and COC and a derived factor (3.0) was used to estimate the interday precision for NCO and ECO.

#### 2.5.6. Stability

The stock solutions of standards and IS in acetonitrile were kept in refrigerator for a month and the response and the response systems controls made from this were compared to freshly made solutions.

Authentic samples were kept in the freezer at −10 °C before and after analysis. The stability of six samples stored for half a year and 1 year were investigated by reanalysing these samples and calculating the bias.

Quality control samples (QCs) were produced at two levels (0.020 and 0.50 mg/kg) from whole blood from horse. The blank blood was acid stabilised at pH 5.0 with 5 M acetic acid before spiking with analytes and kept frozen at −20 °C. The stability for 1 year was investigated.

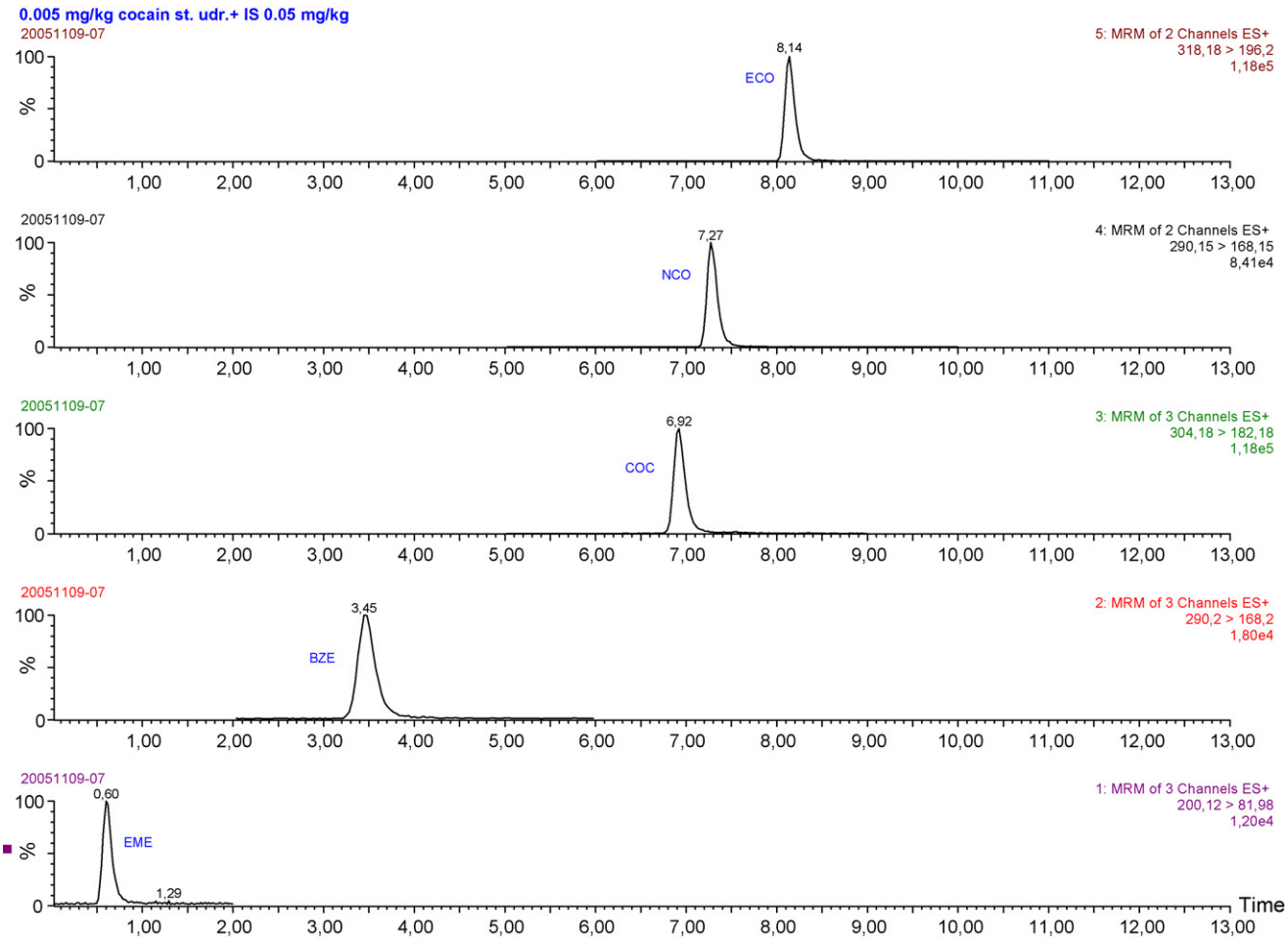


Fig. 1. Ion chromatograms of cocaine and metabolites at 0.005 mg/kg in whole blood—analysed by LC/MS/MS after SPE.

The vial content of a series was reanalysed 20 days after first analysis. It was stored at room temperature. Deviation of results should be within the method.

## 3. Results and discussion

### *3.1. Chromatography and MS/MS conditions (see also Section 3.3)*

The LC/MS/MS technique cannot be applied to biological samples without a sample clean up and isolation of the drug of interest. The different methods reflect the variations of this approach. Cocaine and its metabolites are cumbersome as their chemistry goes from polar analytes (basic and acid) to rather nonpolar. Compromise must be made in order to keep all analytes in one method. The chromatographic separation of cocaine and metabolites were optimised with respect to analysis time and peak shape. Several column types were examined (Zorbax CN and C18, 2.1 mm, 30, 50, 150 mm, Agilent Technologies, Waldbronn, Germany and Atlantis Silica C18, 2.1 mm, 150 mm, Waters, Massachusetts, USA). However, none of the colomns were able to retain EME with solvents consisting of only 5% organic solvent (acetonitrile). The final column applied Zorbax C18, 2.1 mm, 30 mm were chosen due to its versatile applicability, short analysis time and symmetric peak shape of all analytes (Fig. 1). The gradient system was necessary in order to elute all analytes within an acceptable analysis time. Retention of EME has been described by Needham et al. [21] on PFPP stationary phase but only together with cocaine.

### *3.2. Sample preparation*

Several sample preparation techniques were considered in connection to published methods (see Section 1). Liquid–liquid extraction showed limited extraction efficiency (EE) of these very different analytes (polar, basic and acid, relatively non polar). Typically high EE of COC, NCO and ECO at basic pH and very low EE of BZE and EME were observed with organic solvents (several dichloromethane/isopropanol mixtures, butyl acetate) and the opposite at acidic pH. Therefore, two solid phase columns were also examined. With the newer Oasis HLB, Waters, Massachusetts, USA the eluate was not clean enough because when it was injected into the chromatographic system, the column lost its retention abilities. Several clean up steps with diluted acid, methanol, methyl-*t*-butyl ether were unsuccessful. The former GC/MS method for cocaine determination using traditional SPEC® MP1 column was therefore applied again with minor modifications before LC/MS/MS analysis.

### 3.3. Specificity

The method was found to be selective, as no chemicals or compounds of similar structure interfered with the determinations of cocaine and its metabolites for all transitions. Atropine and scopolamine eluted between EME and BZE at 3.00 and 1.43 min, respectively, and they did not interfere with any signals of the analytes. Chemicals and blanks of whole blood were not interfering either, apart from a minor signal (<1/4 of lowest standard) positive cocaine blank, which was adjusted by including the blank in the calibration curve.

Some overlaps between transitions of the analytes are observed (see Table 1) between BZE and NCO and have already been described by Van Bocxlaer et al. in 2000 [22]. However, in contrast to their work, the chromatographic system described here separates the analytes and eliminates the problem. In Fig. 1 the ion chromatogram of cocaine and its metabolites are shown.

Matrix interference caused by whole blood and urine were not detected for the analytes as recovery of spiked samples were within the accuracy of the method (±10%, see Section 3.7). No significant ion suppression was observed at the expected retention times of the targeted ions in blank blood samples or within the analysis time (0–8.4 min). Nevertheless, after the last metabolite, ion suppression was observed between 8.7 and 9.3 min for all transitions. As the total analysis time is 17 min, this suppression will not intervene with the quantification.

### 3.4. Linearity

A linear correlation was observed from 0.0001 to 4.00 mg/l in standards of COC, EME, BZE, NCO and ECO in mobile phase with acceptable coefficients of determination and residuals. Similarly, a linear correlation was observed in extracted whole blood standards, which was found linear over working range of 0.001–2.00 mg/kg for all ten transitions of cocaine and its four metabolites. The linear functions of the five quantitative curves for cocaine and its metabolites from 22 series are summarised in Table 2. All had acceptable $R^2$ (>0.99) and residuals.

### 3.5. Absolute recovery

The absolute recovery of the analyte expresses the efficiency of the extraction procedure and the results for all analytes are shown in Table 2. The recoveries of COC, BZE, NCO and ECO were about 70%, whereas EME was 32%, which for all are acceptable.

### 3.6. LOD and LOQ

The results are shown in Table 2 for each of the investigated analytes. The data are very similar, so an overall LOQ for the analysis was determined to 0.008 mg/kg. LOQ was therefore found to be around 1/10 of normal range for the drug of abuse (cocaine: 0.05–0.3 mg/kg [23]) revealing a sufficient sensitivity of the method. In Fig. 1 ion chromatograms of cocaine and metabolites at 0.005 mg/kg whole blood are shown.

### 3.7. Precision and accuracy

The intraday precision of nine duplicates and studied at four concentration levels in whole blood was less than 5% for all five analytes and the accuracy was less than ±10%.

The interday precision for EME, BZE and COC at eight levels in whole blood are shown in Table 3 together with the intraday precision data of NCO and ECO. The precisions were overall less than 10%, but around the LOQ the precisions were less than 15%. The accuracy was less than ±10% for all at concentration at and above LOQ. In urine the interday precision for EME, BZE and COC at cut-off level 0.15 mg/l was in the order of 15% ($n = 7$) and the accuracy was below ±6%. This is acceptable and all data accomplish the criteria set for the performance of the method.

### 3.8. Stability

No difference in response of system controls made from freshly and 1 month old stock solutions was seen for all analytes and internal standards; therefore, stock solutions made in acetonitrile can be stored for a month in a refrigerator.

In six whole blood forensic samples stored at −10 °C for half a year there were found a reduction of COC and EME of about 15–20%, whereas the content of BZE raised with 10%. This was most likely caused by a hydrolysis of COC to BZE and of EME to ecgonine, in spite of the blood was preserved (fluoride) and kept frozen. Results of endured storing for 1 year confirmed the first results, but now giving a reduction of COC and EME on 15–30%, and an increase of BZE on 15%. Data for NCO and ECO were too scarce to give any trend. The reduction might be explained by the age and experience of the samples, which are being thaw and freeze many times before all examinations for other analytes than cocaine that have been performed and perhaps reanalysed. However, this is the reality of a sample in the laboratory and it is important to know and it should be mentioned

Table 2
Calibration curves for cocaine and its metabolites in whole blood (function, $R^2$), absolute recovery, LOD and LOQ

| | Function curve, $n = 20$ | $R^2$, $n = 20$ | Recovery, $n = 20$ (%) | Recovery IS, $n = 20$ (%) | LOD, $n = 6$ | LOQ, $n = 6$ |
|---|---|---|---|---|---|---|
| EME | $17x - 0.036$ | 0.9983 | 32 | 27 | 0.001 | 0.005 |
| BZE | $19x - 0.037$ | 0.9983 | 76 | 65 | 0.002 | 0.008 |
| COC | $18x - 0.099$ | 0.9967 | 75 | 60 | 0.003 | 0.008 |
| NCO | $16x - 0.016$ | 0.9995 | 69 | | 0.002[a] | 0.008[a] |
| ECO | $18x - 0.006$ | 0.9992 | 67 | | 0.002[a] | 0.007[a] |

[a] LOD and LOQ are calculated from intraday precision data.

Table 3
Interday precision and accuracy of cocaine and its metabolites at different levels in whole blood

| Nominal concentration (mg/kg) | Precision | | | Accuracy |
|---|---|---|---|---|
| | $n$ | $M \pm$ SD | %RSD | %BIAS |
| EME | | | | |
| 0.0025 | 7 | 0.0021 ± 0.0005 | 23 | −16 |
| 0.0050 | 6 | 0.0051 ± 0.0005 | 9.9 | 2.0 |
| 0.025 | 8 | 0.025 ± 0.0029 | 12 | −0.8 |
| 0.050 | 6 | 0.052 ± 0.0032 | 6.2 | 4.4 |
| 0.25 | 7 | 0.24 ± 0.013 | 5.2 | −3.6 |
| 0.50 | 5 | 0.55 ± 0.056 | 10 | 9.9 |
| 1.0 | 7 | 0.90 ± 0.043 | 4.7 | −10 |
| 2.0 | 6 | 1.9 ± 0.060 | 3.1 | −3.1 |
| BZE | | | | |
| 0.0025 | 6 | 0.0023 ± 0.0008 | 36 | −8.0 |
| 0.0050 | 6 | 0.0045 ± 0.0004 | 9.9 | −10 |
| 0.025 | 8 | 0.026 ± 0.0036 | 14 | 2.5 |
| 0.050 | 5 | 0.048 ± 0.0031 | 6.3 | −3.5 |
| 0.25 | 7 | 0.26 ± 0.017 | 6.4 | 2.3 |
| 0.50 | 6 | 0.55 ± 0.040 | 7.2 | 9.9 |
| 1.0 | 5 | 0.96 ± 0.0032 | 3.3 | −5.6 |
| 2.0 | 7 | 2.0 ± 0.096 | 4.7 | 1.0 |
| COC | | | | |
| 0.0025 | 6 | 0.0021 ± 0.0008 | 42 | −16 |
| 0.0050 | 6 | 0.0052 ± 0.0006 | 11 | 4.9 |
| 0.025 | 8 | 0.026 ± 0.0025 | 9.7 | 3.8 |
| 0.050 | 8 | 0.048 ± 0.0033 | 6.9 | −3.7 |
| 0.25 | 7 | 0.25 ± 0.010 | 4.2 | −0.2 |
| 0.50 | 5 | 0.52 ± 0.013 | 2.5 | 3.5 |
| 1.0 | 6 | 0.93 ± 0.081 | 8.7 | −7.1 |
| 2.0 | 6 | 2.0 ± 0.085 | 4.3 | −1.2 |
| NCO[a] | | | | |
| 0.005 | 9 | 0.0053 ± 0.0003 | 4.9 | 6.0 |
| 0.050 | 9 | 0.049 ± 0.0016 | 3.3 | −1.4 |
| 0.50 | 9 | 0.49 ± 0.0080 | 1.6 | −2.3 |
| 2.0 | 9 | 2.0 ± 0.059 | 2.9 | 1.0 |
| ECO[a] | | | | |
| 0.005 | 9 | 0.0051 ± 0.0002 | 4.3 | 2.0 |
| 0.050 | 9 | 0.048 ± 0.0007 | 1.4 | −4.8 |
| 0.50 | 9 | 0.50 ± 0.0009 | 1.8 | 4.6 |
| 2.0 | 9 | 2.0 ± 0.031 | 1.6 | −0.4 |

[a] Data for NCO and ECO are based on intraday precision determinations.

in the laboratory report when late reanalysis is performed on a sample.

Long-term stability of quality control material acidified at pH 5 and stored at −20 °C was established for all analytes (COC, EME, BZE, NCO and ECO) within a period of observation of 1 year. The control limits of the low QC (level 0.02 mg/kg) and the high QC (level 0.5 mg/kg) were respectively 10% and 5% (RSD) for EME, BZE and COC, whereas 10% for NCO and ECO, which were fulfilled for all analytes. For BZE, EME, COC the actual mean were between 5 and 8% at both levels within 35 series in a year.

The re-examined series of vials content stored at room temperature for 20 days showed no deviation between the determinations. Though vials can be stored and analysed after breakdown of equipment, etc.

### 3.9. Method performance

The method has been tried in several international quality assurance tests with good results. To be mentioned are the ring test BTMF of illicit drugs in blood arranged by the Gesellshaft für Toxikologische und Forensische Chemie [24] and a test NORDQUANT-Proficiency Testing Scheme arranged by the Norwegian Institute of Public Health [25]. In the BTMF 2/2004 and 2/2005 the analytes COC, BZE and EME were approved, the bias were less than 7% for all analytes. In the NORDQUANT 1/2004, 2/2004, 1/2005, 2/2005 and 1/2006 the analyte BZE passed all five times with a bias less than 10%.

This method is an improved alternative to liquid–liquid method of whole blood by among others Cailleux et al. [19]. Like Klingmann et al. [3] we could also not obtain an adequate recovery by liquid–liquid extraction for all analytes. Klingmann et al. [3] applied successfully solid phase extraction (SPE) of plasma, and now a whole blood SPE procedure combined with LC/MS/MS that only require 0.20 g sample are described here.

### 3.10. Application

The described method has been applied in the laboratory since 2005 and in Table 4 the typical concentration values of each analyte in forensic cases are reported. In 2005 100 cases were confirmed positive for cocaine and/or a metabolite. Of the 100 samples typically taken in connection with drug addiction 23 samples were from autopsies and the rest were from persons taken in connection with traffic or criminal offences. BZE was determined in all samples concurring with the screening procedure (EMIT) that is based on this analyte, while cocaine was found in 44% of the cases. On the other hand, there were only a few cases were NCO was found and its level was 1/10 of cocaine itself in these samples. ECO was also rare and the concentration level was low, although it is more toxic than cocaine itself, the level found in these cases did not contribute to the total assessment. The measured concentrations of all analytes were mainly low (see Table 4 median) probably due to the domination of samples from living persons and that the material only contained a few fatal poisonings of cocaine. It has been suggested that the molar concentration of all analyte may provide additional information in a given cases [4]. However, in the above 100 cases that was not applicable because BZE totally dominated the picture.

Table 4
Typical measured concentration levels of cocaine and metabolites in 100 forensic samples from autopsies and criminal cases

| Analyte | Measured conc. (mg/kg) (next highest) | Mean conc. (mg/kg) | Median conc. (mg/kg) | $N$ |
|---|---|---|---|---|
| Cocaine | 0.010–50[a] (−0.76) | 1.2 | 0.044 | 44 |
| BZE | 0.009–24[a] (−6.7) | 0.87 | 0.26 | 100 |
| EME | 0.008–21[a] (−1.6) | 0.44 | 0.057 | 65 |
| NCO | 0.008–0.56[a] (−0.026) | 0.10 | 0.015 | 6 |
| ECO | 0.009–0.088 | 0.035 | 0.021 | 16 |

[a] A fatal poisoning of cocaine in a body packer with cocaine.

## 4. Conclusion

In this paper a quantitative method for determination of cocaine and its major metabolites (EME, BZE, NCO and ECO—compounds of wide different polarities) in whole blood (0.20 g) and urine (0.05 ml) by solid phase extraction and LC/MS/MS analysis has been described. The method has been found specific and sensitive with a broad working range of 0.003–2.00 mg/kg of all analytes in forensic samples. The LOQ was 0.008 mg/kg for all analytes. The method has been fully validated and meets the international recommendations. In whole blood the interday precision was less than 10% and the accuracy less than 12% for all analytes. In urine the interday precision at a cut-off level of 0.15 mg/l was 15%. The procedure includes no derivatisation steps of the analytes and offers an excellent alternative to other methods such as GC–MS. Thus, the described LC/MS/MS method is very well suited as a quantitative analysis of cocaine and its major metabolites in forensic toxicology.

## Acknowledgements

The authors appreciate Jytte Lundsby Jensen and Hanne West Nielsen for excellent technical assistance.

## References

[1] R.C. Baselt, Disposition of Toxic Drugs and Chemicals in Man, vol. 256, seventh ed., Biomedical Publications, Foster city, USA, 2004.
[2] K. Srinivasan, P. Wang, A.T. Eley, C.A. White, M.G. Bartlett, J. Chromatogr. B: Biomed. Sci. Appl. 745 (2000) 287.
[3] A. Klingmann, G. Skopp, R. Aderjan, J. Anal. Toxicol. 25 (2001) 425.
[4] M.J. Bogusz, J. Chromatogr. B 748 (2000) 3.
[5] R.A. Jufer, W.D. Darwin, E.J. Cone, in: M.J. Bogusz (Ed.), Forensic Science, Handbook of Analytical Separations, vol. 2, Elsevier Science B.V., Amsterdam, The Netherlands, 2000.
[6] D. Smirnow, B.K. Logan, J. Anal. Toxicol. 20 (1996) 463.
[7] H.H. Maurer, Anal. Bioanal. Chem. 381 (2005) 110.
[8] K. Kudo, H. Tsuchihashi, N. Ikeda, Anal. Chim. Acta 492 (2003) 83.
[9] E.J. Rook, M.J.X. Hillebrand, H. Rosing, J.M. van Ree, J.H. Beijnen, J. Chromatogr. B 824 (2005) 213.
[10] M. Gergov, I. Ojanperä, E. Vuori, J. Chromatogr. B 795 (2003) 41.
[11] M. Wood, M. Laloup, F.M.M. Ramirez, K.M. Jenkins, M.S. Young, J.G. Ramaekers, G. De Boeck, N. Samyn, Forensic Sci. Int. 150 (2005) 227.
[12] Y. Xia, P. Wang, M.G. Bartlett, H.M. Solomon, K.L. Busch, Anal. Chem. 72 (2000) 764.
[13] M. Nishikawa, K. Nakajima, M. Tatsuno, F. Kasuya, K. Igarashi, M. Fukui, H. Tsuchihashi, Forensic Sci. Int. 66 (1994) 149.
[14] P.M. Jeanville, E.S. Estape, I. Torres-Negron, A. Marti, J. Anal. Toxicol. 25 (2001) 69.
[15] R.B. Paulsen, D.G. Wilkin, M.H. Slawson, K. Shaw, D.E. Rollins, J. Anal. Toxicol. 25 (2001) 490.
[16] S. Lin, D.E. Moody, G.E. Bigelow, R.L. Foltz, J. Anal. Toxicol. 25 (2001) 497.
[17] P. Marquet, Ther. Drug Monit. 24 (2002) 255.
[18] G. Singh, V. Arora, P.T. Fenn, B. Mets, I.A. Blair, Anal. Chem. 71 (1999) 2021.
[19] A. Cailleux, A. Le Bouil, B. Auger, G. Bonsergent, A. Turcant, P. Allain, J. Anal. Toxicol. 23 (1999) 620.
[20] SANCO, Off. J. Eur. Commun. 221 (2002) 8.
[21] S.R. Needham, P.M. Jeanville, P.R. Brown, E.S. Estape, J. Chromatogr. B 748 (2000) 77.
[22] J.F. Van Bocxlaer, K.M. Clauwaert, W.E. Lambert, D.L. Deforce, E.G. Van den Eeckhout, A.P. de Leenheer, Mass Spectrom. Rev. 19 (2000) 165.
[23] M. Schulz, A. Schmoldt, Pharmazie 58 (2003) 7.
[24] Institute of Forensic Medicine of Ruprecht Karls University in Heidelberg, Germany.
[25] Norwegian Institute of Public Health, P.O. Box 4404, Nydalen, Oslo, Norway.