

Confirmation and Quantitation of Cocaine and Major Metabolites in Urine Using the ISQ Single Quadrupole GC-MS

Matthew Lambing, Eric Phillips, Trisa Robarge, Thermo Fisher Scientific, Austin, TX, USA

Overview

Cocaine (benzoyl methyl ecgonine, coke, blow, crack) is a central nervous system stimulant derived from the South American shrub *Erythroxylon coca*. It is commonly taken as the hydrochloride salt by nasal insufflation or intravenous injection, or as the free base by smoking. Cocaine is metabolized *in vivo* resulting in the formation of ecgonine methyl ester, norcocaine and benzoylecgonine. Cocaethylene is a substance formed when cocaine and ethanol are coadministered.¹

A forensic toxicology method for the confirmation and quantitation of ecgonine methyl ester (EME), benzoylecgonine (BE), cocaine (COC) and cocaethylene (CE) in human urine was developed using the Thermo Scientific ISQ single quadrupole GC-MS system. This method adheres to guidelines published by the United States Substance Abuse and Mental Health Services Administration (SAMHSA),² the College of American Pathologists (CAP), the Society of Forensic Toxicologists (SOFT) and the European Workplace Drug Testing Society (EWDTS).

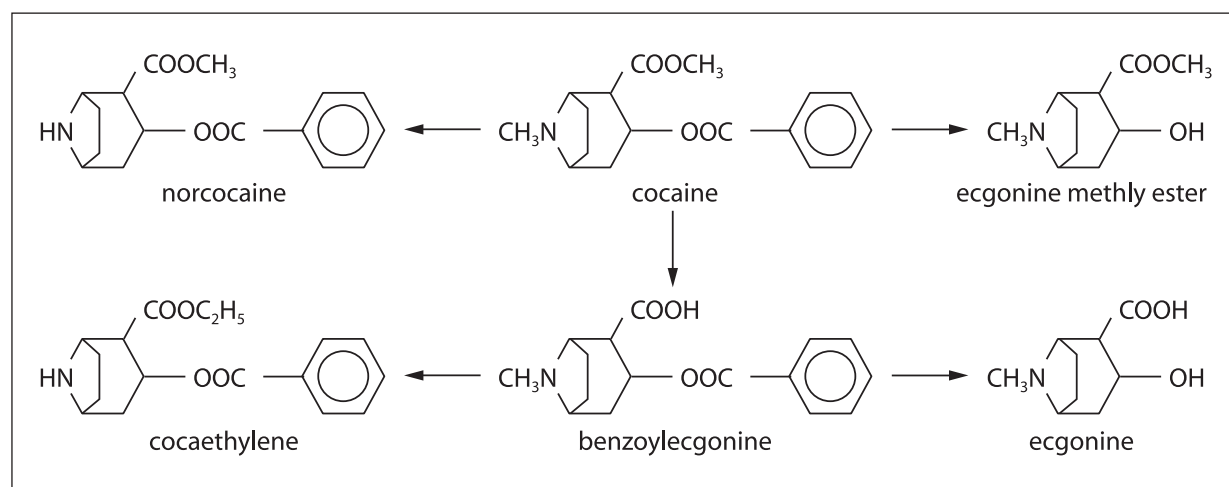
Methods

All validation samples were prepared as batches using a 2 mL sample size. Standard materials were obtained for calibration and separate sources of cocaine and metabolites were used as controls. Deuterated internal standards were employed. Batches included a matrix-matched single point calibrator (at 150 ng/mL), quality control samples set to contain each target compound at 40% and 125% of the calibrator (60 ng/mL and 187.5 ng/mL respectively) and a negative control, which was blank urine with internal



standard only. Thermo Scientific HyperSep Verify-CX solid phase extraction columns (200 mg, 10 mL, P/N: 60108-742) were used for sample extraction. Samples were derivatized with hexafluoroisopropanol (HFIP) and pentafluoropropionic acid (PFPA or PFAA).

The ISQ[™] mass spectrometer system was operated in selected ion monitoring mode (SIM), collecting 3 ions for each target compound and 2 ions for each deuterated internal standard (Table 1). A Thermo Scientific AS 3000 II autosampler and a Thermo Scientific TRACE GC Ultra gas chromatograph, equipped with a split/splitless injection port, provided sample introduction and separation. A 15 m × 0.25 mm ID × 0.25 μm film thickness Thermo Scientific TraceGOLD TG-5MS (P/N: 26098-1300) analytical column was used to enhance separation of the target cocaine class compound from each other and from matrix components (Figures 1 and 2). Thermo Scientific ToxLab Forms software automated the acquisition and processing of all data, including quantitation and ion ratio confirmation calculations.



Metabolism of cocaine

Chromatography

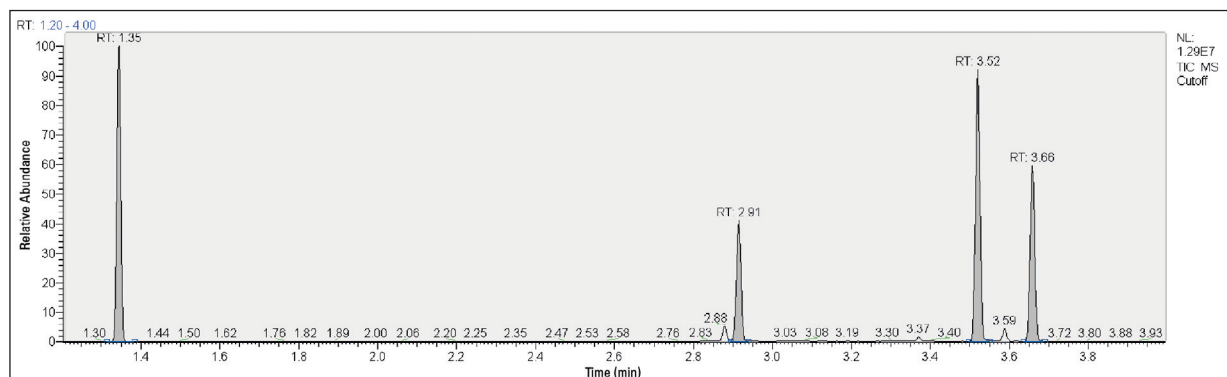


Figure 1: Total ion chromatogram of cocaine and its metabolites from an extracted urine sample at the cutoff (150 ng/mL)

Batches were reviewed for conformance to quality control criteria regarding both quantitative and qualitative performance, based on accrediting agency guidelines. All quality controls within a batch demonstrated quantitative results within $\pm 20\%$ of their expected (theoretical) concentration. Additionally, ion ratio ranges for qualifier ions for target compounds were established using $\pm 20\%$ of the ratios calculated for the 150 ng/mL calibration standard. These ranges were used to assess ion ratio performance. ToxLab™ Forms performed ion ratio confirmations, retention time checking and quality control conformance automatically as a part of batch acquisition and processing. For precision analyses, a coefficient of variation (CV) of $< 10\%$ of the average calculated quality control amounts were required for each analyte and inter-day percent differences of calculated amounts also had to be less than 10% .

Results

- Assay linearity ranged from 15 ng/mL to 12,500 ng/mL for BE, EME and CE, and 15 ng/mL to 5000 ng/mL for cocaine (Figure 3)
- Limits of detection and quantitation of 15 ng/mL using a 2 mL sample size
- Intra- and inter-day precision of $< 10\%$ CV at the quality control levels of 60 ng/mL and 187.5 ng/mL
- Correlation coefficients (R^2) better than 0.9990 for cocaine, benzoylecgonine, ecgonine methyl ester and cocaethylene based on a one point calibration
- Pseudoephedrine at a concentration of 20,000 ng/mL showed interference with EME at the 40% and 125% QC levels
- Norcocaine at 10,000 ng/mL demonstrated no interference with any analyte tested, but limited coelution was observed with cocaethylene. Relative retention time to CE = 1.005.

Analyte	Retention Time (min)	Quan Ion (m/z)	Qual Ion(s) (m/z)	Dwell Time (ms)
EME-d3	1.34	348	317	15
EME	1.35	182	345, 314	15
Benzoylecgonine-d3	2.91	321	442	15
Benzoylecgonine	2.91	318	334, 272	15
Cocaine-d3	3.51	185	306	15
Cocaine	3.52	82	182, 303	15
Cocaethylene-d3	3.65	199	275	15
Cocaethylene	3.66	196	317, 212	15

Table 1: Retention times and ions monitored for the cocaine and metabolite analytes and their deuterated internal standards

Extracted Ions

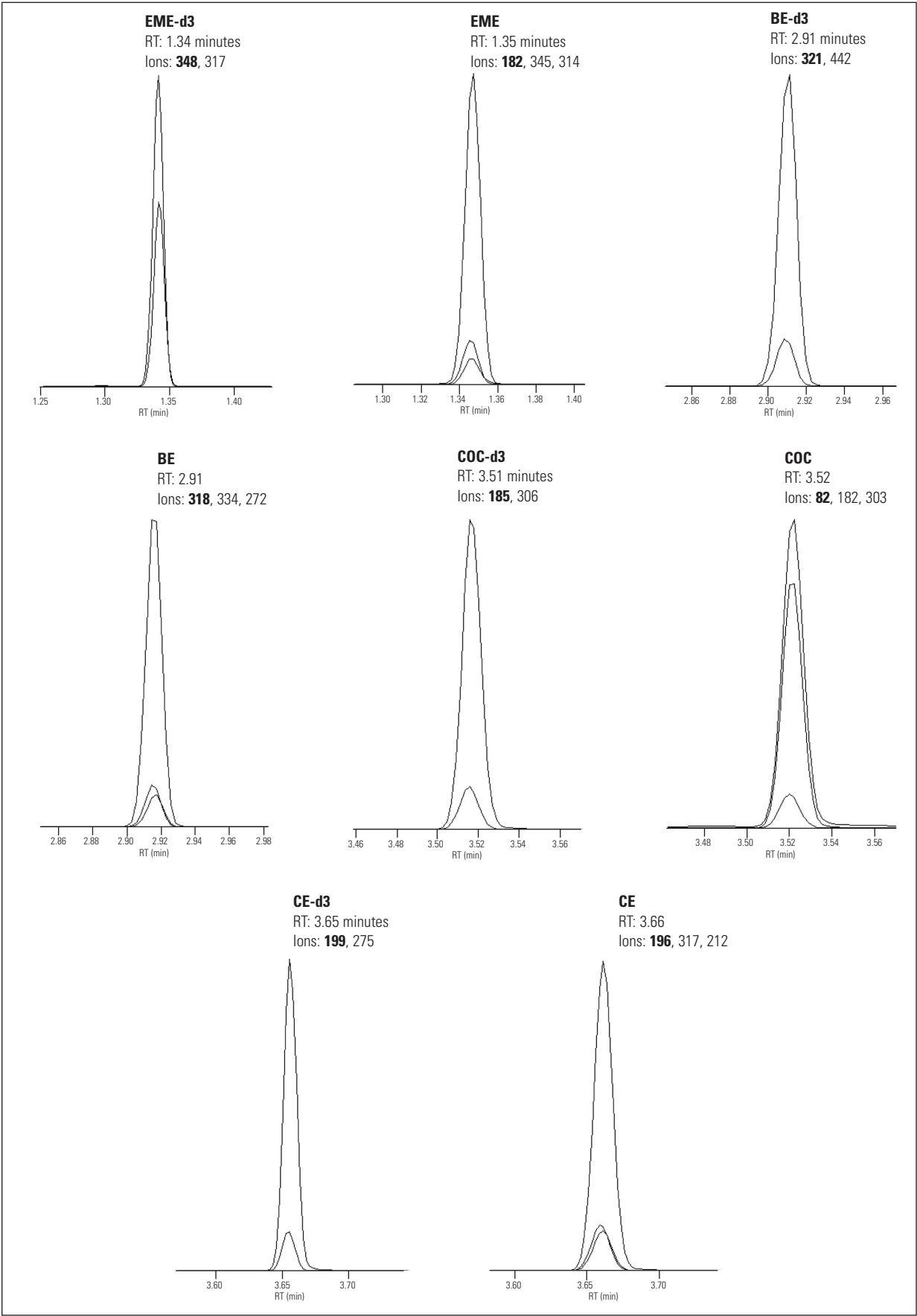


Figure 2: Extracted ion overlays of cocaine, its metabolites and corresponding internal standards at the cutoff (150 ng/mL). Note that no interference is seen from coeluting matrix ions.

Linearity Study

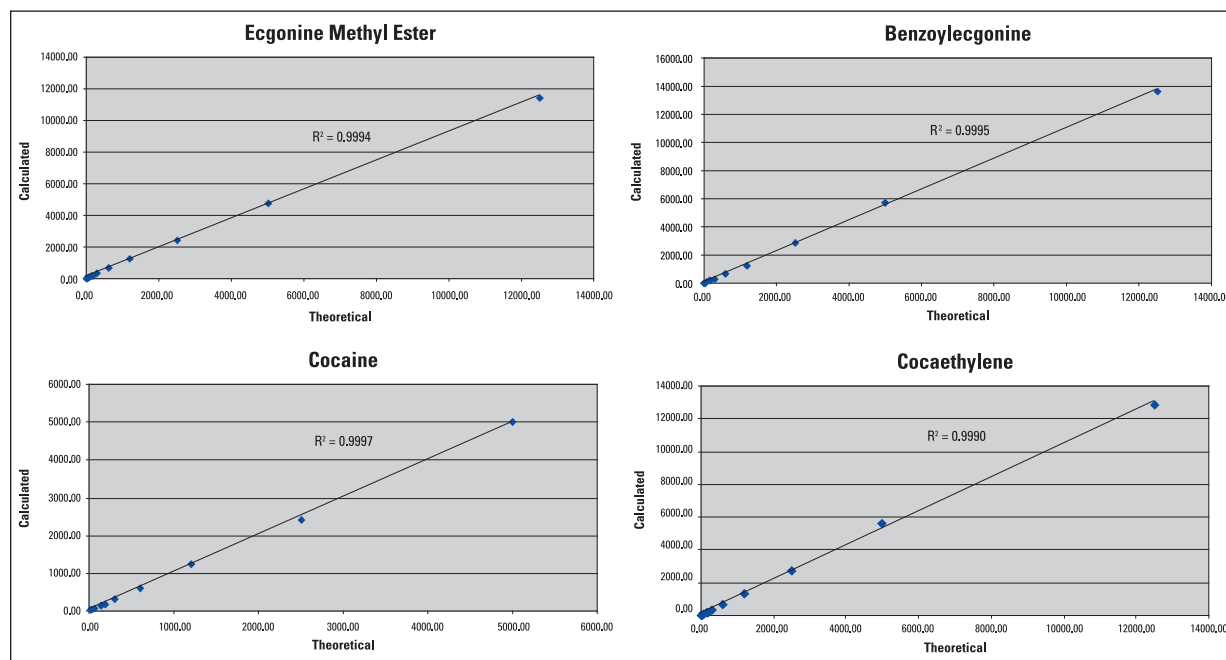


Figure 3: Linearity study results for cocaine and metabolites comparing calculated concentrations to the expected amounts at each level. The regression analysis for this study gave a correlation coefficient of 0.9990 or higher for each analyte tested.

Conclusions

A method was developed to demonstrate the performance of the ISQ GC-MS system for the confirmation and quantitation of cocaine and its major metabolites in a urine matrix. The assay described offers a broad linearity to cover a wide range of analyte concentrations, thus, reducing the need for dilutions or repeat extractions. Excellent precision was also demonstrated around the 150 ng/mL cutoff, with CV measurements of 10% or less over the study. Limits of detection and quantitation at 15 ng/mL ensure sensitive performance for retest and directed assay samples. The methodology described offers a means for a forensic toxicology laboratory to confirm and quantitate cocaine, benzoylecgonine, ecgonine methyl ester and cocaethylene in human urine.

References

- Disposition of Toxic Drugs and Chemicals in Man, Eighth Edition. Randall C. Baselt, Biomedical Publications, 2008.
- Mandatory Guidelines for Federal Workplace Drug Testing Programs, Revised Mandatory Guidelines, Fed. Reg. 71857-71907 (Nov. 25, 2008)

www.thermoscientific.com

Legal Notices: ©2010 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa-Other
+27 11 570 1840

Australia
+61 3 9757 4300

Austria
+43 1 333 50 34 0

Belgium
+32 53 73 42 41

Canada
+1 800 530 8447

China
+86 10 8419 3588

Denmark
+45 70 23 62 60

Europe-Other
+43 1 333 50 34 0

Finland/Norway/Sweden
+46 8 556 468 00

France
+33 1 60 92 48 00

Germany
+49 6103 408 1014

India
+91 22 6742 9434

Italy
+39 02 950 591

Japan
+81 45 453 9100

Latin America
+1 561 688 8700

Middle East
+43 1 333 50 34 0

Netherlands
+31 76 579 55 55

New Zealand
+64 9 980 6700

Russia/CIS
+43 1 333 50 34 0

South Africa
+27 11 570 1840

Spain
+34 914 845 965

Switzerland
+41 61 716 77 00

UK
+44 1442 233555

USA
+1 800 532 4752



Thermo Fisher Scientific,
Austin, TX USA is ISO Certified.

AN51997_E 08/10M