Ultra-Sensitive Forensic Analysis Workflow of Cocaine and Its Metabolites in Hair Samples Using LC-MS/MS

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ABSTRACT

A quantitative ultra-sensitive SPE-LC/MS/MS analysis workflow for simultaneous determination of cocaine and metabolites at picogram levels was developed and evaluated in hair matrix. The method was demonstrated to be sensitive, precise and accurate. Utilization of the SCIEX QTRAP® 6500+ LC-MS/MS system was demonstrated to provide unique advantages in the ability to maximize selectivity when confirming and quantifying low level metabolites in hair..

INTRODUCTION

Utilization of hair matrix in drug analysis has recently grown,[1-3 and ref. therein] Compared to other biological matrices, hair offers several advantages: its extraction is painless; there are no special requirements for the sample storage; incorporated in hair drugs are stable and are not metabolized; considering the rate of hair growth to be on average 1cm per month, multi-sectional hair analysis enables monitoring the history of drug use.[1]

Forensic analysis of cocaine in hair requires a sensitive and reliable analytical workflow. There are two major challenges for the detection of this compound and its metabolites in hair samples: low concentration and matrix interferences. In this presentation, an analysis workflow combining the use of triple quadrupole linear ion trap mass spectrometry with solid phase extraction (SPE) for picogram per mg of hair detection of Cocaine and its metabolites in hair is described.

MATERIALS AND METHODS

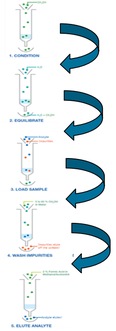
Sample Preparation:

Hair samples were washed, dried and cut into ~ 2 mm segments. The hair was digested with 0.1N HCl, extract aliquotte was mixed with the internal standards and underwent solid phase extraction procedure with Phenomenex Strata®-X-C, 30 mg/3 mL according to the following process:

MS/MS Conditions:

A SCIEX QTRAP® 6500+ LC-MS/MS system with IonDrive™ Turbo V source and Electrospray Ionization (ESI) probe was used. cocaine and its 10 metabolites were detected using two MRM transitions per compound to allow quantitation and identification based on the ratio of quantifier to qualifier MRM transitions.

Decontamination:	Dichlormethane and water wash followed by air dry.
Digest:	Incubate 20 mg hair with 1 mL 0.1 N HCl overnight at 45°C.
Pre-treatment:	Add 10 µL of IS to 800 µL of hair extract solution, vortex.
Cartridge:	Strata®-X-C, 30 mg/3 mL
Part No.:	8B-S029-TBJ
Condition 1	1 mL EtOAc/MeOH/28-30 % NH_4OH (70:20:10)
Condition 2	1 mL Methanol
Equilibrate	1 mL Water
Load	Pre-treated sample
Wash 1	1 mL 0.1N HCl
Wash 2	1 mL Methanol
Dry	10 min at high vacuum (~10" of Hg)
Elute	2 x 500 µL EtOAc/MeOH/28-30% NH_4OH (70:20:10)
Add	50 µL of 0.1N HCl to eluate
Dry Down	Evaporate to dryness under nitrogen at 40-45°C
Reconstitute	200 µL of initial mobile phase



RESULTS

A method for quantitation and identification of Cocaine and its 10 metabolites: benzoylecgonine (BZE), norcocaine, cocaine (COC), ecgonine, ecgonine methyl ester (EME), para-hydroxy-benzoylecgonine (p-OH-BZE), meta-hydroxy-benzoylecgonine (m-OH-BZE), cocaethylene, meta-hydroxy-cocaine (m-OH-COC), ortho-hydroxy-cocaine (o-OH-COC), para-hydroxy-cocaine was developed (Figure 1).

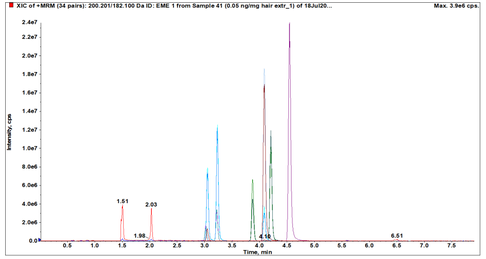


Figure 1. Detection of cocaine and its 10 metabolites in hair at 0.05 ng per mg of hair level.

In order to ensure correct quantitation of cocaine and possibility of hair sample contamination with cocaine easily available as powder when inhaled, detection and quantification of metabolites is a mandatory part of forensic analysis. Following administration of the drug, the main metabolites that are formed are benzoylecgonine and Ecgonine Methyl Ester while the minor ones are Norcocaine and meta- and para-hydroxycocaine.[2,3] The method discussed here demonstrated a good separation of isomeric compounds present in the mixture under analysis (Figure 2).

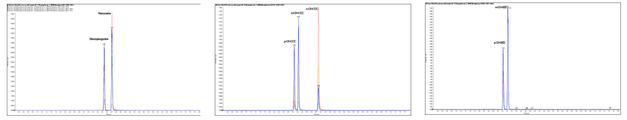


Figure 2. Separation of isomeric metabolites

Hair is a very complex matrix, which may represent a problem when detecting analytes at low concentration levels. Robust and reliable extraction procedures are of a great importance in achieving the desired reproducibility, good linear responses and limits of quantitation. Our extraction procedure demonstrated excellent recoveries of the analytes of interest

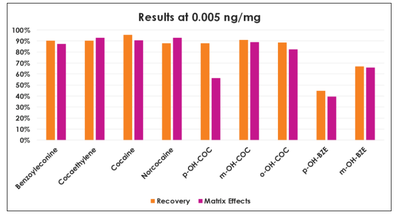


Figure 3. Recovery and Matrix Effects

Analyte	LLOQ (ng/mg)
Ecgonine	0.05
Ecgonine Methyl Ester	0.0025
Benzoylecgonine	0.001
Norcocaine	0.0005
Cocaine	0.0005
p-OH-Benzoylecgonine	0.01
m-OH-Benzoylecgonine	0.01
Cocaethylene	0.0001
m-OH-Cocaine	0.00005
o-OH-Cocaine	0.00005
p-OH-Cocaine	0.001

Table 1. Lower Limits of Quantitation for Cocaine and Metabolites Panel.

Our measurements also demonstrated excellent linearity of the generated regression curves covering linear dynamic range from 3 to 4 orders of magnitude; coefficients of variation (CVs) within 10% and good accuracies. Signal-to-Noise ratios at LLOQ were found to vary from 10 to 50. LLOQs are presented in Table 1.

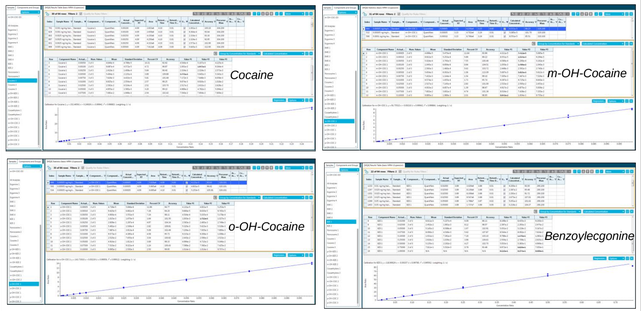


Figure 4. Calibration curves and statistics information for cocaine, m-OH-cocaine; o-OH-cocaine and benzoylecgonine.

Utilization of a SCIEX QTRAP® 6500+ LC-MS/MS system, a hybrid linear ion trap, enables generation of enhanced product ion spectra that contain information of the complete molecular fingerprint of cocaine and metabolites that were searched against relevant spectral libraries. This approach to compound confirmation significantly reduces the risk of false positives in the unknown samples.

To demonstrate these capabilities of the 6500+ system we have acquired the samples in MRM-IDA-2EPI experimental set-up. Figure 5 illustrates typical results of MS/MS library searching.

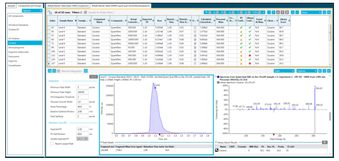


Figure 5. MS/MS library searching results for a cocaine in a standard solution prepared by spiking in blank hair extract.

CONCLUSIONS

- The data presented here demonstrate a complete method for analysis of cocaine and metabolites from hair, including sample extraction, chromatography, and MS detection across a wide analytical range.
- Analyte extraction recoveries were demonstrated to be greater than 80 % enabling the analytical workflow to obtain sub pg/mg Lower Limits of Quantitation (LLOQ) in hair matrix. The workflow showed excellent accuracy (>95%) and precision (< 15%), with excellent linearity resulting in R^2 values of 0.9990 for all analytes.
- Specifically, hydroxycocaine isomers demonstrated acceptable accuracy and precision down to 0.00005ng/mg .
- Overall, lower limits of quantitation for cocaine and metabolites were shown to be in low pg per mg of hair sample ranges.
- Linear dynamic ranges of the panel under analysis were found to be of 3 to 4 orders of magnitude.
- It is necessary to note that hydroxybenzoylecgonines suffered from sample stability issues during transport, having previously produced 92% (m-OH-BZE) and 82% (p-OH-BZE) recoveries. Recovery for ecgonine was 87% at 0.1 ng/mg. Ecgonine methyl ester (EME) suffered from high background and produced recoveries greater than 100%.
- In addition to quantitation, the SCIEX QTRAP® 6500+ LC-MS/MS system enabled simultaneous identification and confirmation of illicit drugs and their metabolites through utilization of Enhanced Product Ion Scan (EPI) by acquiring full scan MS/MS data. Forensic drug identification and confirmation was achieved using automated MS/MS library searching.

REFERENCES

1. P. Lopez, S. Martello, A .M. Bermejo. E. De Vincenzi, M.J. Tabernero, N. Chiarotti. Validation of ALISA screening and LC-MS/MS confirmation methods for cocaine in hair after simple extraction. Anal. Bioanal. Chem (2010), 397: 1539-1548.
2. Maria João Valente, Félix Carvalho, Maria de Lourdes Bastos, Márcia Carvalho and Paula Guedes de Pinho (2012). Chromatographic Methodologies for Analysis of Cocaine and Its Metabolites in Biological Matrices, Gas Chromatography - Biochemicals, Narcotics and Essential Oils, Dr. Bekir Salih (Ed.), ISBN: 978-953-51-0295-3, InTech, Available from: http://www.intechopen.com/books/gas-chromatography-biochemicalsnarcotics-and-essential-oils/chromatographic-methodologies-for-analysis-of-cocaine-and-its-metabolites-inbiological-matrices
3. K.B. Scheidweiler, M.A. Huestis. Simultaneous quantification of opiates, cocaine and metabolites in hair by LC-APCI-MS/MS. Anal. Chem. (2004), 76, 4358-4363

TRADEMARKS/LICENSING

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