Ultra-Sensitive Forensic Analysis of Cocaine and its Metabolites in Hair Samples

Using the QTRAP® 6500+ LC-MS/MS System

Janna Anichina[1], Oscar G. Cabrices[2], Sean Orlowicz[3], Laura Snow[3], Pierre Negri[2]
[1]SCIEX, Concord, Canada; [2]SCIEX, Redwood City, USA; [3]Phenomenex, Torrance, USA

Cocaine is one of the most commonly abused recreational drugs, with an estimated 16.5 million people or 0.35% of the worldwide population abusing the substance.[1] Detection of its use can be performed in several biological matrices such as blood, urine, oral fluid and hair. While urine and oral fluid are very useful for determining cocaine use in short term, hair samples are becoming extremely valuable in testing the long-term use. Additional benefits of hair testing include but are not limited to (1) ease and non-invasive nature of sample extraction, (2) absence of storage requirement and (3) long term stability and little risk of sample degradation overtime.

Presence of cocaine and its metabolites in hair indicate active drug use. However, there are two major analytical challenges associated with detecting cocaine and its metabolites in hair sample: (1) low concentration of these compounds and (2) high abundance of matrix interferences associated with hair samples.

Herein, a sensitive and reliable analytical workflow is presented which combines the use of QTRAP 6500+ LC-MS/MS system with solid phase extraction (SPE) for picogram per mg of hair detection of cocaine and its metabolites. This method for quantification of cocaine and its metabolites was demonstrated to provide unique advantages in the ability to maximize selectivity when confirming and quantifying low level metabolites in hair.

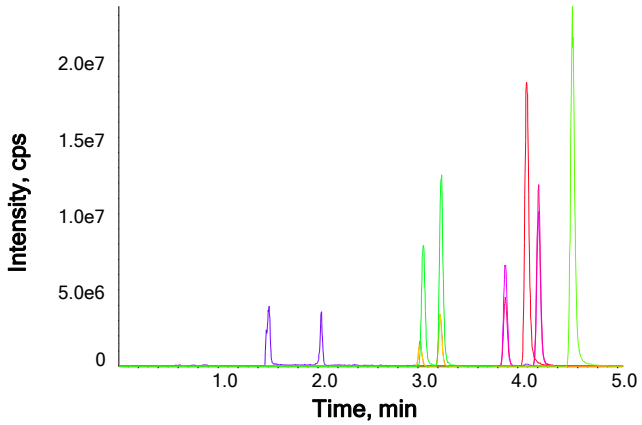

Figure 1: Sensitive Detection of Cocaine and its 10 Metabolites in Hair Using the QTRAP® 6500+ LC-MS/MS System. Detection of cocaine and its 10 metabolites in hair at 0.05 ng/mg of hair level.

Key Features of the QTRAP® 6500+ LC-MS/MS System with Optimized SPE

- IonDrive™ Technology on the QTRAP® 6500+ system provides improvements in ionization efficiency, ion sampling efficiency and detection dynamic range for very high sensitivity of detection (high fg/mL to low pg/mL).
- Simultaneous identification and confirmation of illicit drugs and their metabolites through the acquisition of full scan MS/MS data (Enhanced Product Ion (EPI) and automated MS/MS library searching).
- Improved sample preparation with SPE provides a robust and easily implemented method for selective analysis of trace levels of cocaine and its metabolites in hair.
- Optimized chromatography allows separation of cocaine and its metabolites in less than 5 minutes with a high level of selectivity.
- Automated generation of optimized linear curves using SCIEX OS Software 1.4 with less manual intervention and quick flagging of outliers.

Methods

Hair Sample Preparation and Digestion: Hair samples were washed according to accepted laboratory procedure, dried and cut into segments of ~ 2 mm lengths. Approximately 20 mg of each hair sample was transferred into a suitable and sealable container with cap and 1 mL of 0.1 N HCl was added into each container. The containers were incubated overnight at 45ºC for complete digestion of the hair samples. The next day, the containers were removed from the incubator and allowed to cool down to room temperature. 10 µL of each internal standard (IS) were added to the 800 µL of hair extract solution and the containers were thoroughly mixed. The resulting solutions underwent solid phase extraction using the Phenomenex Strata®-X-C, 30 mg/3mL (Part No. 8B-S029-TBJ) according to the procedures shown in Figure 2.

HPLC Conditions: HPLC separation was performed on a Phenomenex Kinetex® Biphenyl column (100x3mm, 2.6µm, 00A-4723-AN) on the SCIEX ExionLC™ AC system. Mobile phase A (MPA) and mobile phase B (MPB) were 0.1% formic acid in water and methanol, respectively. The HPLC flow rate was 600 µL/min, column temperature was held at 30˚C, and the total HPLC runtime was less than 5 minutes.

MS/MS Conditions: A SCIEX QTRAP® 6500+ 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 quantification and identification based on the ratio of quantifier to qualifier MRM transitions (Table 3).

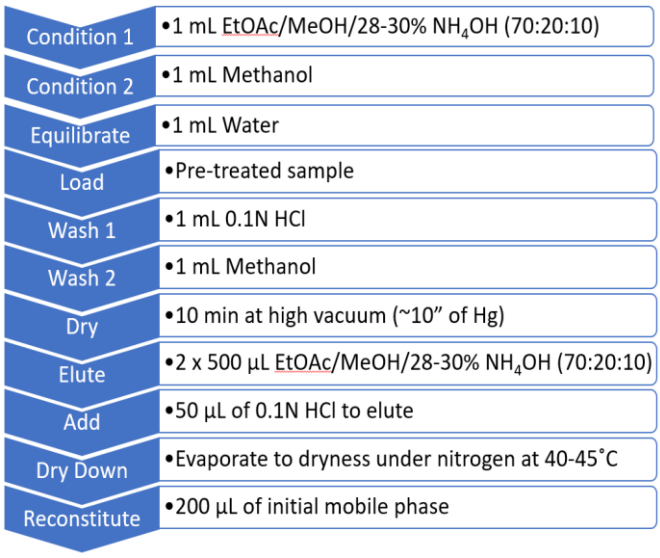

Figure 2: Strong Anion Exchange (SAX) Solid Phase Extraction (SPE) Workflow Using Phenomenex Strata®-X-C, 30 mg/3mL cartridges. An 11-step extraction protocol was developed and optimized for selectively extracting cocaine and its metabolites from hair samples for analysis with the QTRAP® 6500+ LC-MS/MS System.

The source parameters are provided in Table 1. The compound-dependent voltages of Declustering Potential (DP), Entrance Potential (EP), Collision Energy (CE) and Collision Cell Exit Potential (CXP) were optimized for each transition and are represented in Table 3.

Table 1. Source Conditions.

Parameter	Value
Curtain gas (CUR)	*30*
Collision gas (CAD)	*8*
IonSpray Voltage (IS)	*4500 V*
Temperature (TEM)	*600 ℃*
Ion Source Gas 1 (GS1)	*60*
Ion Source Gas 2 (GS2)	*20*

Separation of Isomers

Upon ingestion, cocaine is rapidly adsorbed and broken down in the body into several metabolites. Detection and identification of those metabolites is paramount to ensure correct quantification of cocaine. The existence of isomeric analogues within the panel of cocaine metabolites adds an additional level of complexity to the assay as these analogues have no unique fragments that can be used for detection. Therefore, chromatographic separation of these analogues from their isomers is critical for confident identification and quantitation and therefore was the focus of this study.

Optimal chromatographic separation was accomplished by using a Phenomenex Kinetex® Biphenyl column (100x3mm, 2.6µm, 00A-4723-AN) which allowed for better retention and selectivity of the more polar analytes throughout the gradient. The column was held at 30˚C during the course of the experiment. The column in conjunction with an optimized mobile phase composition produced the separation that was needed to correctly distinguish all isomers. This chromatographic separation was optimized for this MRM assay but can also be used on the SCIEX X500R QTOF System, for additional screening or confirmation techniques. Figure 3 shows the separation of three sets of isomeric metabolites of cocaine.

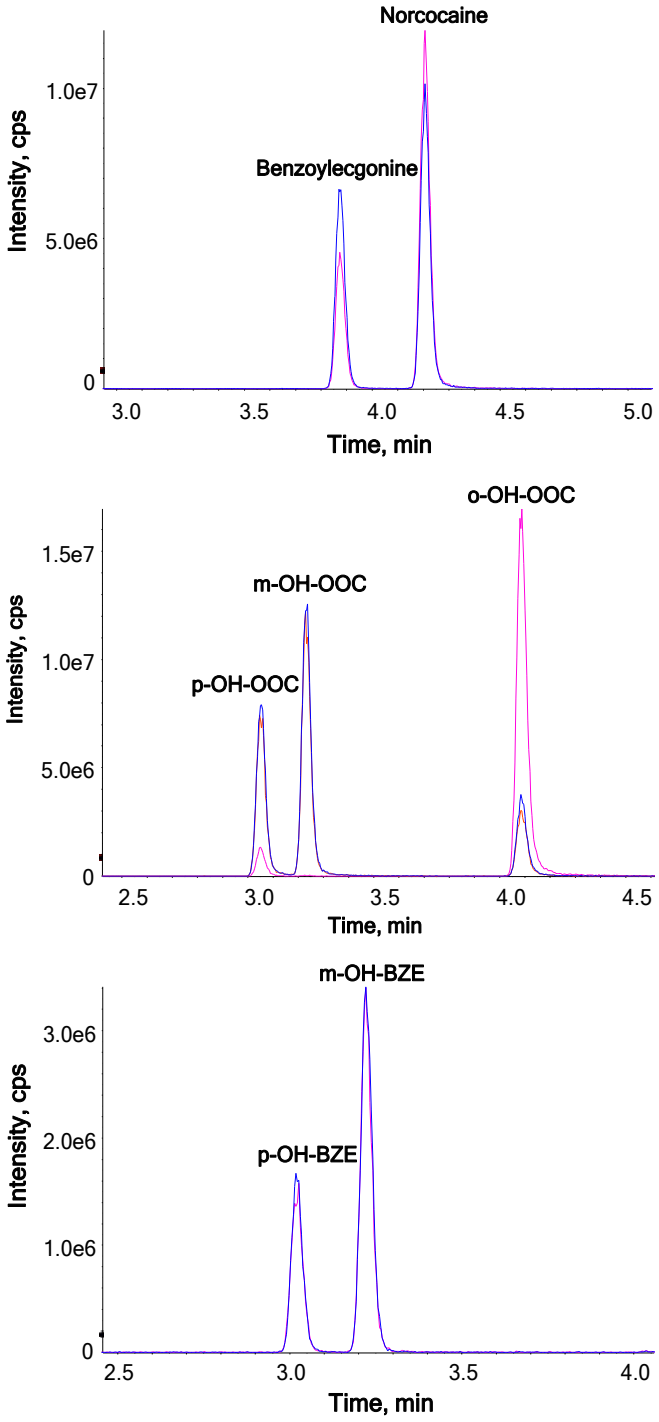

Figure 3: Examples of Separation of Specific Cocaine Metabolites Isomers. (Top) Separation of the isomers Benzoylecgonine and Norcocaine. (Middle) Separation of the isomers p-OH-OOC, m-OH-OOC and o-OH-OOC. (Bottom) Separation of the isomers p-OH-BZE and m-OH-BZE. These individual chromatograms showing isomeric separation were extracted from the full chromatogram shown in Figure 1 that includes the full panel of analytes used in this study.

Recovery and Matrix Effects

Hair is a very complex matrix, which may represent a problem when detecting analytes at low concentration levels. Robust and reliable extraction procedures are critical in achieving the desired reproducibility, good linear response and limits of quantitation. To assess the recoveries of the analytes used in this experiment, recovery (RE) and the matrix effect (ME) were calculated using 0.005 ng/mg of each internal standard. If one depicts the peak areas obtained in neat solution standards as A, the corresponding peak areas for internal standard spiked after extraction into hair extracts as B, and peak areas for internal standards spiked before extraction as C, the RE and ME values can be calculated as follows:

$$RE\ (\%) = C/B \times 100 \quad (1)$$

$$ME\ (\%) = B/A \times 100 \quad (2)$$

The extraction procedures demonstrated excellent recoveries of the analytes of interest, as shown in Figure 4.

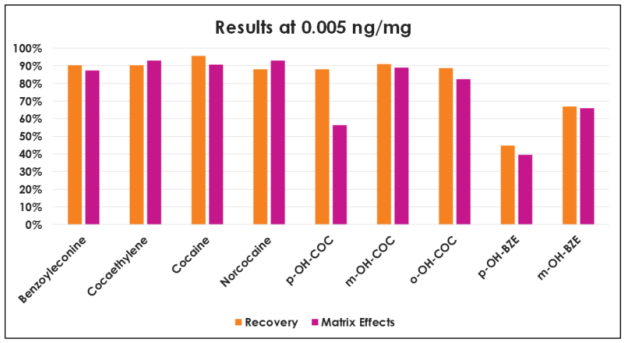

Figure 4: Recovery (RE) and Matrix Effects (ME) Calculated for Cocaine and Its Metabolites using 0.005 ng/mg of Each Internal Standard. (Top) Separation of the isomers Benzoylecgonine and Norcocaine. (Middle) Separation of the isomers p-CH-OOC, m-CH-OOC and o-CH-OOC. (Bottom) Separation of the isomers p-OH-BZE and m-OH-BZE.

Analytical Performance of the SCIEX QTRAP 6500+ LC-MS/MS System

Following the SPE procedure, 10 µL of the reconstituted solution were injected for each compound. Calibration curves were generated for each of the compounds to determine limits of quantitation (LOQ). The results demonstrated excellent linearity of the generated regression curves covering linear dynamic range from 3 to 4 orders of magnitude; coefficients of variations (Cs) within 10% and good accuracies. Signal-to-noise ratios (S/N) at LLOQ were found to vary from 10 to 50. Table 2 summarized the lower limits of quantitation (LLOQ) for cocaine and metabolites panel. Figure 5 shows a few representative calibrations curves and statistics of quantitation generated using this comprehensive method.

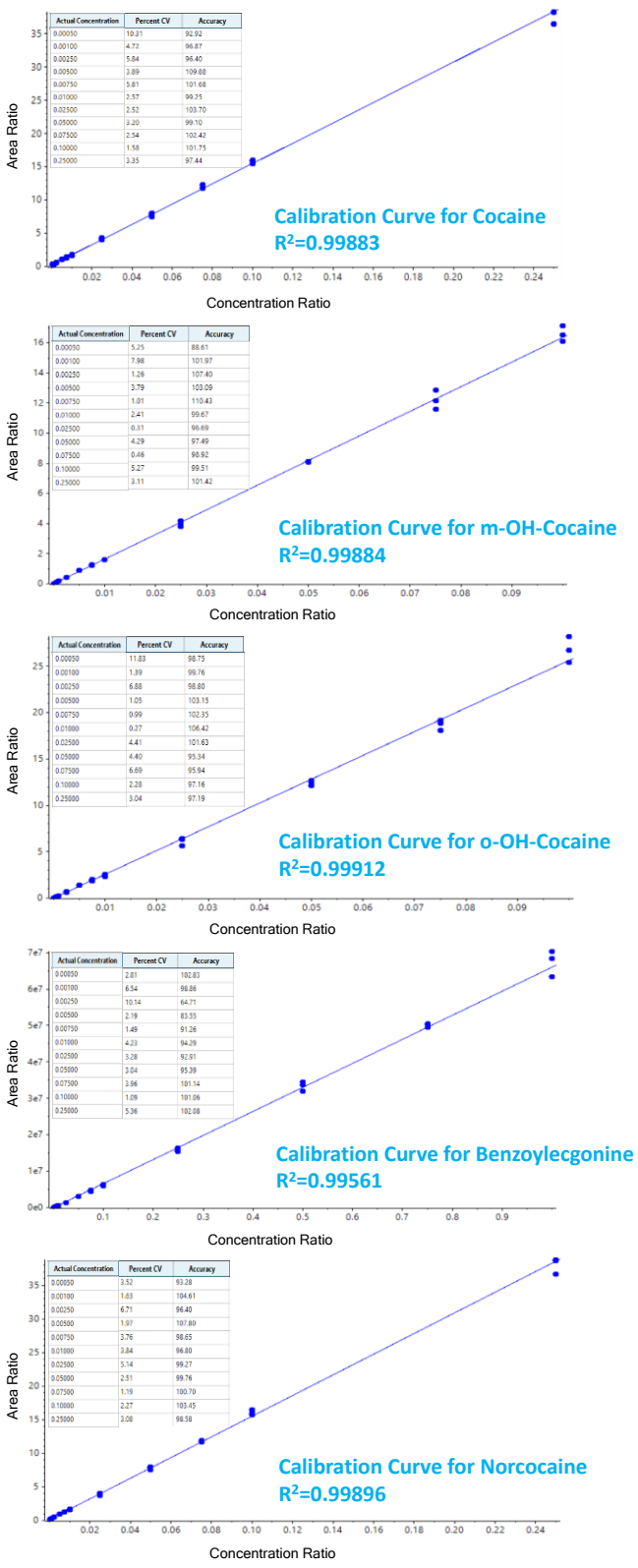

Figure 5: Excellent Linearity Was Achieved for Cocaine and Its Metabolites. Calibration curves and statistic information are shown for Cocaine, m-OH-Cocaine, o-OH-Cocaine, Benzoylecgonine and Norcocaine. R^2 values were equal to or higher than 0.9990 for all the analytes used in the comprehensive method.

Table 2. Lower Limits of Quantitation (LLOQ) for Cocaine and Metabolites Panel.

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*

Full Scan MS/MS for Confirmation

The QTRAP 6500+ System is a hybrid triple quadrupole linear ion trap mass spectrometer which allows to easily switch between quantitative MRM scans and qualitative trap scans. For this assay, the method uses an MRM survey scan (Table 3) followed by two EPI scans (full scan MS/MS) which are triggered when signal is detected for each specific MRM transition. The acquired full scan MS/MS spectra contain the complete molecular fingerprint of cocaine and its metabolites and can be searched against relevant spectral libraries for confirmation of detection. This approach provides both high sensitivity quantitation with compound confirmation which significantly reduces the risk of false positives in the unknown samples.

Using this comprehensive method, cocaine and its metabolites were identified, and confirmation was achieved using MS/MS library searching. Figure 6 illustrates typical results of MS/MS library searching.

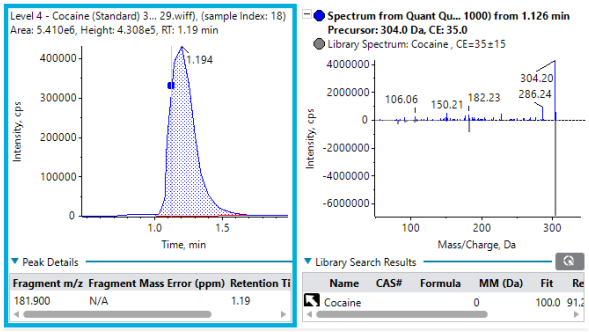

Figure 6: Typical Results of MS/MS Library Searching Using SCIEX OS Software 1.4. MS/MS library searching for Cocaine in a standard solution prepared by spiking in blank hair extract.

Conclusions

The combination of a solid phase extraction (SPE) procedure and optimized chromatography with the highly sensitive QTRAP 6500+ System allowed the efficient and sensitive detection of trace levels of cocaine and its metabolites (0.05 pg/mg) in hair samples, making the workflow easily adaptable into a forensic toxicology laboratory.

- An 11-step extraction protocol using SAX SPE can be rapidly implemented and optimized for selective analysis of cocaine and it metabolites.
- Optimized chromatographic separation was achieved with a high level of selectivity for cocaine and its metabolites using Phenomenex's Kinetex Biphenyl column for a total HPLC runtime of less than 5 minutes.
- Analyte extraction recoveries were demonstrated to be greater than 80% enabling the analytical workflow to obtain sub pg/mg lower limits of quantification (LLOQ) in hair matrix for the two hydroxycocaine isomers.
- Successful quantitation of cocaine and its metabolites was performed using SCIEX OS Software 1.4 allowing streamlined and accurate data processing of trace level concentrations (0.05 pg/mg) in hair samples.
- The workflow showed excellent accuracy (>95%) and precision (<15%), with excellent linearity resulting in R^2 values of 0.9990 for all analytes.
- In addition to quantitation, the QTRAP 6500+ System enabled simultaneous identification and confirmation of cocaine and its metabolites by acquiring full MS/MS data and using automated MS/MS library searching.

References

1. Peacock A, Leung J, Larney S, Colledge S, Hickman M, Rehm J, et al. Global statistics on alcohol, tobacco and illicit drug use: 2017 status report. Addiction. 2018. DOI: 10.1111/add.14234.

Table 3. MRM Transitions for Cocaine and its Metabolites, Including Optimized Compound Dependent Parameters.

Analyte	Q1	Q3	DP	EP	CE	CXP
BZE_1	*290.2*	*168.1*	*65*	*10*	*25*	*10*
BZE_2	*290.2*	*105*	*65*	*10*	*36*	*10*
Norcocaine_1	*290.2*	*168*	*50*	*10*	*21*	*10*
Norcocaine_2	*290.2*	*136.1*	*50*	*10*	*30*	*10*
Cocaine_1	*304.2*	*182.2*	*70*	*10*	*26*	*10*
Cocaine_2	*304.2*	*82.1*	*70*	*10*	*35*	*10*
Cocaine_3	*304.2*	*105*	*70*	*10*	*37*	*10*
Ecgonine_1	*186.2*	*168.1*	*60*	*10*	*23*	*10*
Ecgonine_2	*186.2*	*100.3*	*60*	*10*	*31*	*10*
EME_1	*200.201*	*182.1*	*45*	*10*	*23*	*10*
EME_2	*200.201*	*82.1*	*45*	*10*	*32*	*10*
p-OH-BZE_1	*306.1*	*168.1*	*70*	*10*	*26*	*10*
p-OH-BZE_2	*306.1*	*186.1*	*70*	*10*	*27*	*10*
m-OH-BZE_1	*306.102*	*168.1*	*80*	*10*	*27*	*10*
m-OH-BZE_2	*306.102*	*121.1*	*80*	*10*	*35*	*10*
Cocaethylene_1	*318.2*	*196.1*	*50*	*10*	*26*	*10*
Cocaethylene_2	*318.2*	*82.1*	*50*	*10*	*37*	*10*
m-OH-COC_1	*320.1*	*182.1*	*70*	*10*	*27*	*10*
m-OH-COC_2	*320.1*	*82.1*	*70*	*10*	*42*	*10*
o_OH-COC_1	*320.101*	*200.1*	*50*	*10*	*27*	*10*
o_OH-COC_2	*320.101*	*182.1*	*50*	*10*	*37*	*10*
p_OH-COC_1	*320.102*	*182.2*	*80*	*10*	*26*	*10*
p_OH-COC_1	*320.102*	*82.1*	*80*	*10*	*44*	*10*

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices