Ultra-Fast Forensic Identification and Quantitation of Cocaine in Seconds

Using a Thermal Extraction Ionization Source (TEIS) Coupled with a SCIEX X500R QTOF System

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Recently, there has been increased interest in developing methods involving high resolution mass spectrometry for drug screening in forensic and toxicological environments. These screening methods need to be robust, rapid and detect an unlimited number of analytes, and ideally not require lengthy sample preparation procedures. Reducing the run times of chromatographic methods has been at the forefront of these investigations, as longer run times reduce throughput. While chromatographic run times have been greatly reduced, in some cases to below 3 minutes[1-2], it is beneficial to utilize screening methods which do not require chromatographic separation at all for highest throughput. The methods must also be non-targeted for screening, providing both MS and MS/MS information for higher confidence in identification.

In this technical note, a method combining a Thermal Extraction Ionization Source (TEIS) coupled with a SCIEX X500R QTOF system is used to establish a rapid screening method with minimal sample clean-up and no chromatography. The method for cocaine quantification resulted in real-time analysis times of only a few seconds per injection (Figure 1), coupled with the high resolution workflow to provide confident identification and quantification of cocaine and the deuterated standard.

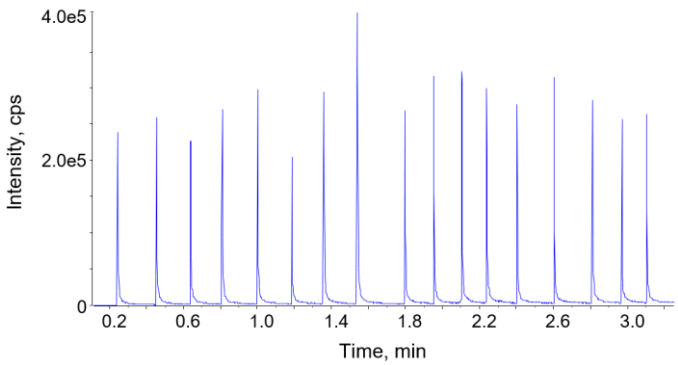

Figure 1: Extremely Fast Analysis of a Cocaine Sample using Flow Injection Analysis (FIA) with a Thermal Extraction Ionization Source. Data shows 17 x 2 µL manual injections (plotted against time) within 3.5 minutes of cocaine/deuterated cocaine mix for the ion transition *m/z* 304.15/182.1176. The time between the peaks is the time required to manually draw a sample with a syringe, inject into the instrument and obtain real-time data.

Key Feature of TEIS Coupled to X500R System

- SCIEX X500R system is an easy to use, rugged and versatile instrument, ideal fit for screening and confirmation applications.
  - Rapid switching between MS and MS/MS scans, for full mass spectral data of both the precursor ions and the product ion with very high resolving power.
  - SWATH® Acquisition workflow generates comprehensive MS and MS/MS, providing three characteristic ions such as the precursor ion and two product ions for confident ID, making it ideal for non-targeted screening workflows.
- Thermal Extraction Ionization Source (TEIS)[3] provides direct sampling without sample preparation or chromatography.
  - Provides real-time peak detection from swabs, vapors and direct injections, generating confident identification within seconds.
  - Self-purging source requires very little cleaning or maintenance, while providing minimal carry-over between samples (Figure 1).
  - Operates with an APCI needle, minimizing consumables such as solvents.
  - Compatible with Turbo V™ Source on SCIEX instruments (with firmware upgrade).

Methods

Sample Preparation: Cocaine and deuterated cocaine were obtained from Sigma Aldrich (Sigma-Aldrich Company Ltd, Dorset, England, UK) at 1000 ng/µL and 100 ng/µL in MeOH, respectively. Cocaine and deuterated cocaine were diluted together in MeOH to 500 pg/µL, 1 ng/µL, 5 ng/µL and 10 ng/µL concentrations for deuterated cocaine. Injections of the cocaine/deuterated cocaine mix were 2 µL.

Ionization Source: The Thermal Extraction Ionization Source (TEIS) was heated to 285 °C to volatilize the solvent injection and a sample pump was used to draw the gaseous molecules towards the ionization region with a flow of 25 L/min. A schematic of the TEIS is depicted in Figure 2, showing the heated brass blocks, ceramic transfer line and APCI region in relation to the orifice. Samples were injected via an injection port in the top block.

Mass Spectrometry: Analysis was performed on the SCIEX X500R QTOF system using SCIEX OS software version 1.2. System required upgrade with custom firmware to recognize a third-party ionization source; the TEIS. The method of acquisition used was MRM^{HR} workflow.

The source conditions are described in Table 1. Although an ESI source was not used, the spray voltage parameter was used to control the APCI needle voltage. The TOF-MS/MS conditions for cocaine and deuterated cocaine using an accumulation time of 0.025 s, declustering potential of 20 V and collision energy of 20 V with a spread of 20 V. All four bins from channels 1-4 were set to sum. These values could be further optimized which may result in improved results.

Data Processing: Data processing was performed using MultiQuant™ Software 3.0. Global integration parameters were selected for all peaks within the run and the integration parameters used are shown in Table 2.

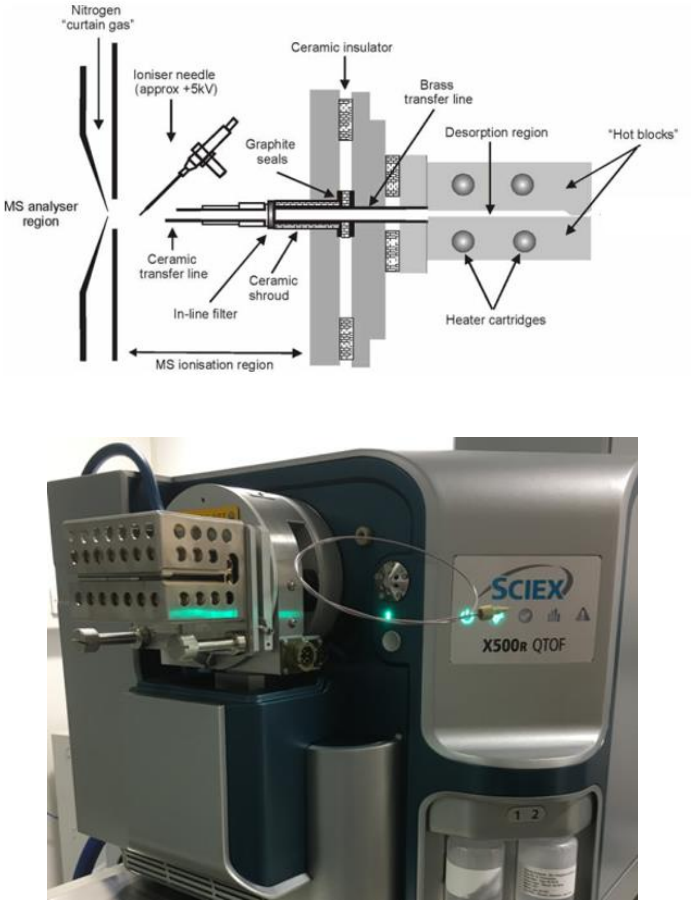

Figure 2: Schematic of the Thermal Extraction Ionization Source (TEIS). (Top) This source sits in front of the MS orifice and consists of two heated blocks, transfer line and location of the APCI needle. This configuration provides real-time peak detection and direct sample introduction. The bottom pane shows the source coupled to the SCIEX X500R QTOF System.

Table 1. Source Parameters.

Parameter	Setting
Polarity	*Positive*
Curtain Gas (psi)	*25*
CAD Gas (psi)	*7*
Ionspray voltage (V)	*5500*
GS1 (psi)	*0*
GS2 (psi)	*0*

Table 2. Integration Parameters.

Parameter	Setting
Quantitation method	*MQ4*
Min Peak Width	*3 points*
Min Peak Height	*100.00*
XIC Width	*0.02 Da*
Gaussian smooth width	*3.0 points*
Peak Splitting	*2 points*

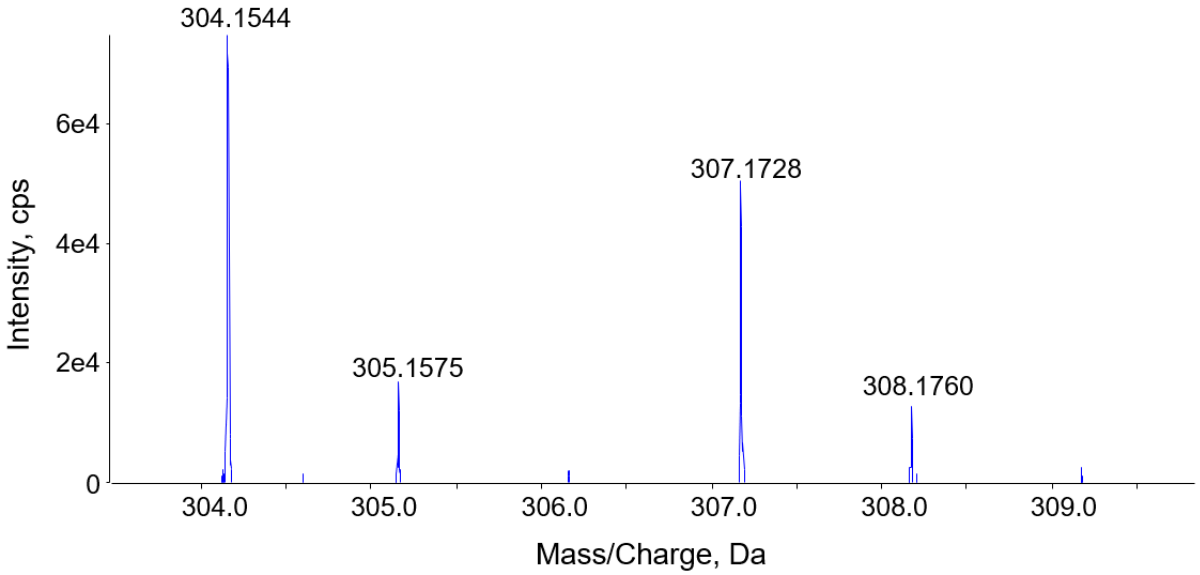

Figure 3: High Resolution MS Spectra for Confident Compound ID. Mass Spectra of cocaine and deuterated cocaine for the $[M+H]^+$ ions at *m/z* 304.1544 and 307.1728, respectively, and the ^{13}C isotopes at *m/z* 305.1575 and 308.1760.

Obtaining High Resolution, High Mass Accuracy Data

One advantage of using an accurate mass MS system such as the X500R QTOF is that additional information can be obtained from the full scan MS data that would not usually be seen using a targeted analysis. In addition, the accurate mass of the precursor ions can be generated au automatically processed using the SCIEX OS™ Software to predict elemental compositions and match isotopic pattern data to any extracted peaks. For example, the accurate mass of the precursor ion as well as the carbon 13 isotopes of compounds can be measured, and the mass accuracy and isotope ratio can be used to confirm identification of compounds. Further confirmation can be obtained through full scan MS/MS data using multiple fragment ions for compound identification.

To get started with the analysis, the instrument was first calibrated using the standard protocol with the Turbo V™ Source, then the TEIS was installed. MS analysis was performed on the cocaine sample mixed with a deuterated internal standard of cocaine (Figure 3). The recorded and theoretical accurate masses for the $[M+H]^+$ ions for cocaine and deuterated cocaine are shown in Table 3. Figure 3 also shows the ^{13}C isotopes of cocaine and deuterated cocaine at *m/z* 305.1575 and 308.1760 for computation of isotope ratios. MS/MS analysis was included in the same experiment and the XIC of fragment ion 182.1176 m/z was used for quantitation (Figure 1).

Quantitation of Cocaine

Using deuterated cocaine as an internal standard to account for any matrix effects, cocaine was quantified in methanol from matrix with no chromatography using the TEIS on the X500R system configuration.

First, the responses of the MS/MS quantifier ion for 17 manual injections (2 µL volume) within 3.5 minutes of the cocaine/deuterated cocaine mix at 1 ng/µL was shown in Figure 1, highlighting the speed of analysis possible. As the TEIS is a third-party source, it is not currently possible to use an autosampler for direct injection and therefore, all injections were performed using a manual, 2 µL direct injection, which contributed to some variance observed in peak responses.

Table 3. Accurate Masses Used for Quantitation.

Analyte	Theoretical Mass (m/z)	Observed Precursor Mass (m/z)	MS/MS Fragment (m/z)
Cocaine	*304.15433*	*304.15*	*182.1176*
Deuterated cocaine	*307.17316*	*307.17*	*185.1336*

Next, four concentrations of cocaine were used, 550 pg/µL, 1.1 ng/µL, 5.5 ng/µL and 11 ng/µL; deuterated cocaine was added as an internal standard at 500 pg/µL, 1 ng/µL, 5 ng/µL and 10ng/µL. The mass of the precursor ion and fragment ions used for quantitation are listed in Table 3. Good linearity was observed across the 4 concentrations even with the manual injection process, with the calibration curve for both the cocaine and the deuterated cocaine showing R^2 values greater than 0.993. The calibration curve of these samples is shown in Figure 4. The ion ratios between cocaine and the internal standard measured across the concentration range also found to be very consistent, with an RSD of 3.68%.

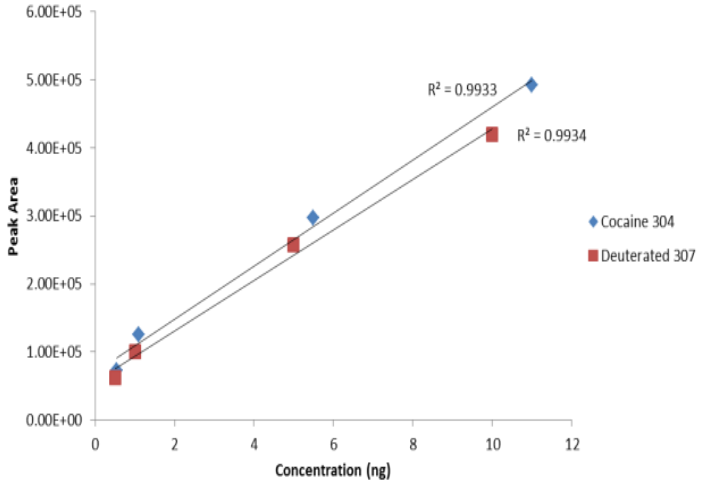

Figure 4: Quantitation of Cocaine from Matrix with No LC or Sample Preparation. Calibration curve of cocaine (blue) and deuterated cocaine (red) across four different concentrations for the quantifier ion transition *m/z* 304.15/182.1176 is shown.

Conclusion

These results suggest that quantitation using the Thermal Extraction Ionization Source coupled with a SCIEX X500R for forensic applications is feasible without chromatography or sample preparation, with very fast acquisition times. This technique would afford a higher laboratory throughput and reduced consumable needs when compared to LC-MS approaches.

The approach was demonstrated through development of a fast screening and identification method for cocaine. While sample injection was done manually which added variance to the data, good quantitation results were observed, suggesting workflow extensions such as autosampler based flow injection could reduce variance.

References

1. SWGDRUG. Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Recommendations. US, 2016.
2. SCIEX Forensics Compendium. 2018, RUO-MKT-03-7423-A.
3. For more information on the TEIS source, see www.msaltd.co.uk.

Document number: RUO-MKT-02-8257-A

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