Ultra-Fast Identification of Drugs of Abuse

Using a Thermal Extraction Ionization Source (TEIS) Coupled with a SCIEX QTRAP 4500® System

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The abuse of opioids and other related drugs continue to pose serious public health and safety issues worldwide. According to the National Survey on Drug Use and Health (NSDUH), 19.7 million American adults (aged 12 and older) battled a substance use disorder in 2017.[1] As these substances continue to cause widespread intoxications resulting in fatalities worldwide, robust and comprehensive detection is critical to enable forensic laboratories to rapidly and accurately identify these substances. Analysis of seized powder and used syringes containing drug residues requires specific and sensitive screening methods. These analytical methods need to be rapid, robust and able to detect a variety of analytes. To that extent, the use of high sensitivity and selectivity MS-based screening technology providing both MS and MS/MS information is an ideal tool for higher confidence in identification.

In this technical note, a method combining a Thermal Extraction Ionization Source (TEIS)[2] coupled with a QTRAP 4500 System is used for ultra-fast screening of drugs of abuse without the need for chromatography or extensive sample preparation.[3] The combined system can quickly identify unknown drugs of abuse with a high level of confidence suitable for rapid forensic screening.

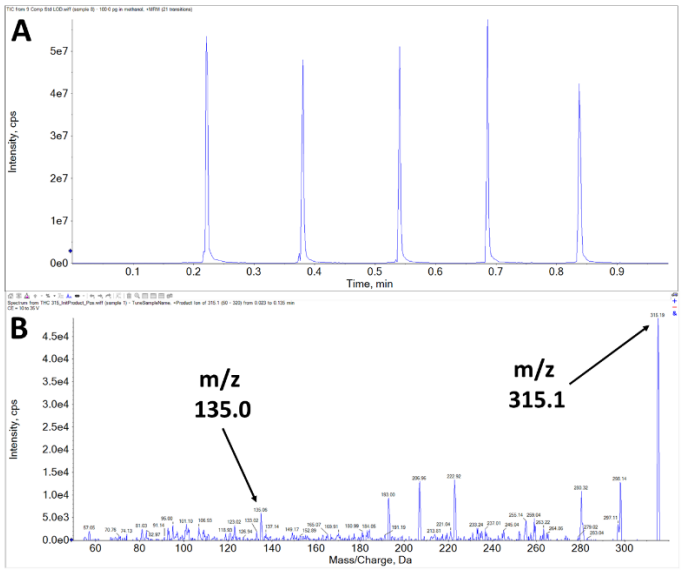
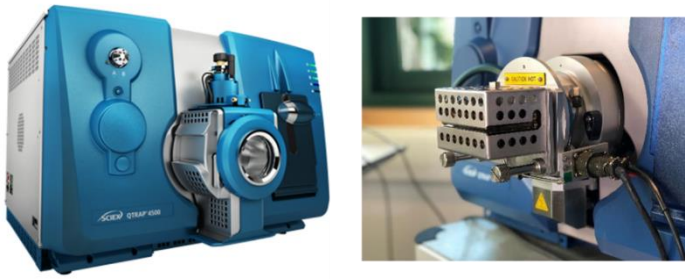


Figure 1: Extremely Fast and Reproducible Analysis of Tetrahydrocannabinol (THC) Using Direct Injection Analysis with the TEIS. A) Total Ion Chromatogram (TIC) showing 5 x 2 µL manual injections within a minute of 0.002 ng of THC for the ion transition *m/z* 315.1/135.0, B) Product ion scan for $[THC+H]^+$ ion at m/z 315.1.



Key Features of the TEIS Coupled to the QTRAP 4500 System For Direct Identification of Drugs of Abuse

- Streamlined targeted approach allows direct MS analysis of drugs of abuse without sample preparation or chromatography
- Direct sampling from cell phone screen swabs allows drug identification within seconds
- Robust MRM workflow allows illicit substance detection with improved selectivity and confidence in identification
- Precursor and product ion information using MRM transitions provides higher confidence in positive detection, reducing the rate of false positives for drug residues detection
- Additionally, QTRAP technology provides full scan MS/MS spectra that can be matched to library spectra for added confidence
- Quantitation method described provides an estimate of unknown drug residues present on a swab used to sample a cell phone screen
- Combination of the TEIS with QTRAP 4500 System for direct analysis is an ideal fit for screening and confirmation applications, with increased selectivity and confidence in identification for trace analysis

Methods

Sample Preparation: Cocaine, benzocaine, mephedrone, amphetamine, diamorphine, methylenedioxymethamphetamine (MDMA), ketamine, 4-MEC and Delta9-THC were purchased from Sigma Aldrich (Sigma-Aldrich Company Ltd, Dorset, England, UK). Standard solutions were obtained at 100 ng/µL and diluted in MeOH to create a series of calibration solutions ranging from 0.0001 ng/µL to 1 ng/µL. A solution containing all nine drug analytes was created by mixing solutions of 1 ng/µL of all the components. 20 µL of this solution was placed onto a cleaned cell phone screen and allowed to completely evaporate before a passport-sized piece of plain paper was “rubbed” in the area where the sample was placed in order to transfer the drug residues. This procedure was intended to simulate a swab of a fingerprint containing drug residues. The paper was then passed through the TEIS source block using the slot between the two heated blocks. The entire process is shown in Figure 3.

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. Although an ESI source was not used, the spray voltage parameter was used to control the APCI needle voltage.

Sample Introduction: To generate calibration curves, 2 µL of liquid standards were injected via a microliter syringe into an injection port at the front of the top block. Residues from paper swabs were inserted in the slot between the two heated blocks.

Mass Spectrometry: Data was acquired on the SCIEX QTRAP 4500 system using Analyst® Software 1.6.3. Optimization of each compound was performed independently to determine the best source conditions. The DOA standards were optimized using a 1 ng/µL solution to determine the fragmentation of each analyte and to appropriately select two product ions. Using the determined precursor and product ions, a targeted Multiple Reaction Monitoring (MRM) method for each compound was developed and used for identification and quantification of the drug analytes.

Data Processing: *Data* processing was performed using MultiQuant™ Software 3.0. Global integration parameters were selected for all peaks within the run and the multiple fragments monitored were averaged together for each analyte calibration series. The integration parameters used are shown in Table 1.

Table 1. Integration Parameters.

Parameter	Setting
Quantitation Method	*IntelliQuan MQ4*
Min Peak Width	*3 points*
Min Peak Height	*500 cps*
Speak Split Factor	*8*
Gaussian Smooth Width	*3 points*
Noise Percentage	*80%*
Baseline Subtract Window	*2 min*

Method Development For Ultra-Fast Detection of Drug Residues

The QTRAP 4500 System was first calibrated using the standard protocol with a Turbo V™ Source. Compound optimization was performed for each drug compound in ESI mode. The TEIS source was then placed on the instrument to optimize source conditions. Figure 1A shows the typical signal response from the instrument in the form of a Total Ion Chromatogram (TIC) following five sequential 2 µL manual injections of 0.002 ng of THC using the TEIS, highlighting the speed of analysis possible and reproducibility of signal response from the instrument. Figure 1B shows the resulting product ion scan for $[THC+H]^+$ ion at m/z 315.1.

Generation of Calibration Curves Using MRM

The MRM targeted acquisition method used in this study allows quantification of the drug analytes through the detection of two ion pair transitions (one precursor and two product ions), meaning additional confidence in forensic analyte detection at low ng levels. To generate calibration curves, the series of calibrator solutions were manually injected into the TEIS source using a syringe via the front injection port for each of the drug analytes. Two MRM transitions were monitored per drug analyte across five sequential 2 µL manual injections at each concentration. The linear dynamic range was evaluated across 4 orders of magnitude with drug residue amounts ranging from 0.0002 to 2 ng.

Figure 2 shows the calibration curves for MDMA (A), diamorphine (B), and THC (C) using the MRM method as well as the XIC trace for MDMA (D) showing the five sequential 2 µL manual injections at the LOQ (0.002 ng). Excellent linear dynamic range was achieved across the targeted drug analytes. The use of MRM enabled sensitive quantitation of low concentration samples which improved selectivity and confidence in identification. As seen in Figure 2, the calibration curves for MDMA, diamorphine and THC transitions are showing

R^2 values greater than 0.980, 0.998 and 0.998, respectively, even with the manual injection process. Peak areas were plotted as a function of ng amount of drug to make the calibration curves compatible with trace testing.

These results suggest that quantification using the TEIS with a QTRAP 4500 System is feasible without the need for chromatographic separation. This screening approach would significantly decrease consumable needs when compared to LC-MS approaches while greatly increasing laboratory throughput. Consequently, this screening method would be ideal in a scenario where first responders are figuring out what a victim might be under the influence of by testing the syringe or powder residue, which can ultimately inform them so they can take the right course of action and administer the appropriate medical treatment.

Confident Screening and Quantification of Unknown Drug Residues Using MRM

The capability of the instrument to identify and quantify drug residues in real time was further tested by swabbing a cell phone screen onto which a 20 µL drop of a 1 ng/µL solution of cocaine, amphetamine and MDMA was placed. The paper swab used to wipe the cell phone screen was then passed through the slot between the two heated blocks. The whole process is summarized in Figure 3. Figure 3D shows the thermal desorption profile observed in real time from Explore Mode in Analyst Software in the form of the XIC for two MRM transitions for each of the three analytes monitored. This enables the positive identification of the three drug analytes (cocaine, amphetamine and MDMA) present in the 20 µL drop.

Quantification of the drug residues present on the cell phone screen was also performed. The amount of the drug residues transferred to the paper swab was calculated using the area value for each of the XIC peaks resulting from the thermal desorption profile for each of the two transitions shown in Figure 3D and solving for x using the linear regression equation. Table 2 shows the quantitative results of the targeted screen from the drug residues present on the cell phone screen. The averaged values for cocaine, amphetamine and MDMA were found to be 8.16, 7.84, and 5.53 ng, respectively. These values provide a fairly quantitative measure of the drug residues amounts detected from swabs and take into account the collection and desorption efficiencies. Overall this manual screening method allows confident detection of low levels of drug residues with improved selectivity and reduced false positive rates when compared to ion-mobility-based trace detection systems.

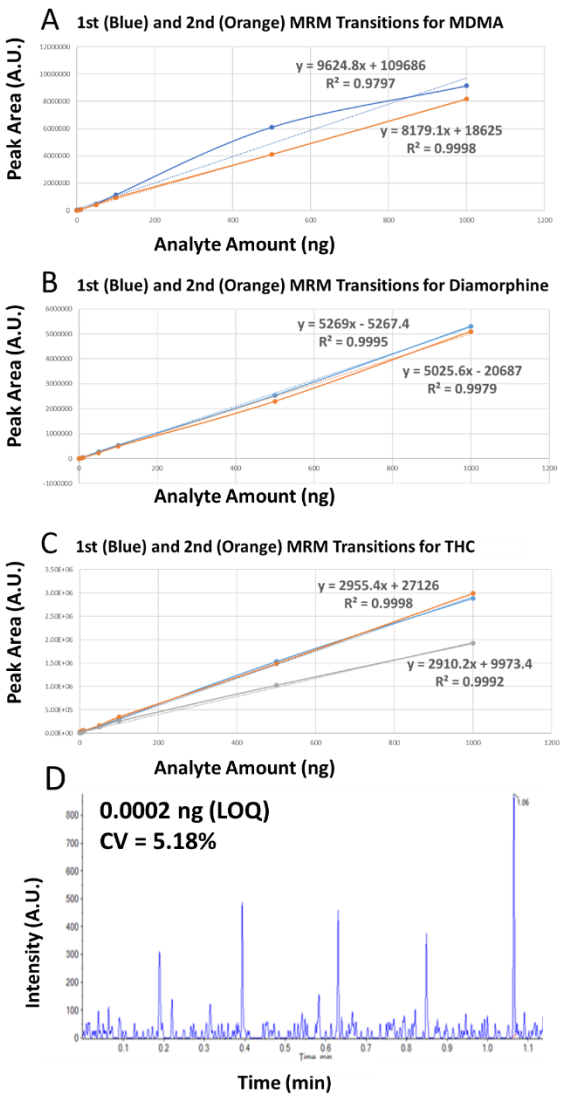


Figure 2: Good Linear Dynamic Range Achieved Across the Targeted Compounds. Calibration curve for MDMA (A), diamorphine (B), and THC (C) for both MRM transitions. (D) Extracted Ion Chromatogram (XIC) trace showing the five sequential 2 µL injections of MDMA at the LOQ (0.002 ng). Linear dynamic range averaged ~4 orders of magnitude with analyte amount range from 0.0002 to 2 ng.

Table 2. Quantitative Results From the Swab Analysis of the Drug Residues Using the QTRAP 4500 System.

Sample ID	Drug Transition ID	Averaged Area (N=2)	Linear Regression Equation	Calculated Amount (ng)
Amphetamine	*136.1→91.0*	*1.75E+05*	*y=7800.5x+111259*	*8.17*
	136.1→119.1	*1.10E+05*	*y=5713.1x+63506*	*8.14*
Cocaine	*304.1→182.0*	*7.85E+05*	*y=16286+632016*	*9.39*
	304.1→105.0	*9.92E+04*	*y=7177.5x+54023*	*6.29*
MDMA	*194.1→163.0*	*1.66E+05*	*y=9624.8x+109686*	*5.85*
	194.1→105.0	*7.76E+04*	*y=8179.1x+18625*	*7.21*

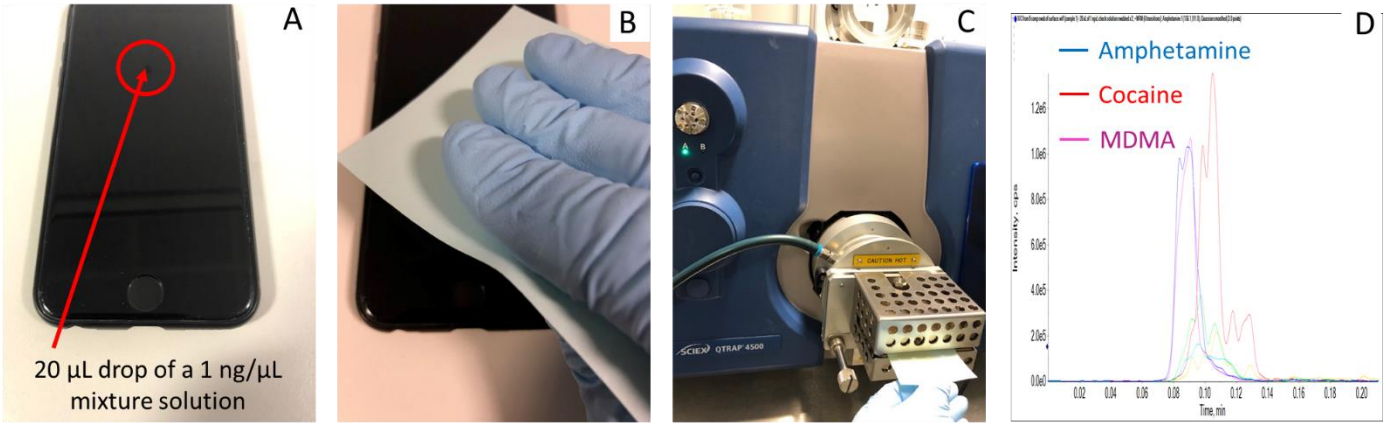


Figure 3: Process of Screening for Drug Residues from a Cell Phone Screen Using the TEIS. A) Sampling of drug residues by placing 20 µL of a solution containing 1 ng/µL of cocaine, amphetamine and MDMA on a clean cell phone screen, B) paper swab of cell phone screen before insertion in the slot between the two heated blocks, C) introduction of the paper swab containing drug residues into the thermal desorption source for sample extraction, and D) data acquisition and real-time monitoring of the thermal desorption profile of cocaine, amphetamine and MDMA in the Explore portion of Analyst Software 1.6.3.

Conclusions

A fast and streamlined targeted workflow was developed for the successful identification and quantification of drug residues. Acquisition of MRM data using the combination of the TEIS and the QTRAP 4500 System enabled accurate screening of drug residues compatible with high throughput screening. The acquisition of full scan product ion information can provide a substantial improvement for confident drug identification.

Using this optimized targeted workflow, confident identification of low levels of drug residues is also feasible using the extracted XICs based on the MRM transitions for the drug analytes. This demonstrates that the combination of atmospheric pressure chemical ionization (APCI) with thermal desorption is suitable as a fast sampling and screening method for trace analysis of drug residues. This approach shows potential application for trace analysis of drugs residues from containers and parcels for high throughput security screening.

References

1. Substance Abuse and Mental Health Services Administration. (2018). Key Substance Use and Mental Health Indicators in the United States: Results from the 2017 National Survey on Drug Use and Health.
2. Direct Sample Analysis Using a Thermal Extraction Ionization Source (TEIS) Combined with Mass Spectrometry, SCIEX Technical Note RUO-MKT-02-9913-A.
3. Rapid Identification of Seized Controlled Substances and Related Compounds by Tandem mass Spectrometry Without Chromatography. Carl M. Fletcher and Richard Sleeman. *Rapid Commun. Mass Spectrom*. (2016), **30**, 908-916.
4. For more information on the TEIS source, please visit: www.msaltd.co.uk

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