Identification and quantification of designer drugs in urine by LC-MS/MS

Using SCIEX QTRAP® LC-MS/MS Systems and technology

Recently, trends have been seen within the drugs of abuse arenas to suggest that attempts are being made to bypass controlled substance laws, with novel compounds appearing on the market that are similar in structure to current drugs of abuse. These "designer drugs" or "legal highs" have caused concern due to their unknown quantity in terms of potency, side effects, health consequences and potential for abuse. As the number of new designer drugs is constantly rising, methods which can be easily expanded and have a non-selective sample preparation are needed.

The method presented here uses a simple dilute and shoot sample preparation and using a Scheduled MRM™ Algorithm on a QTRAP System to quantify and identify a number of these compounds in urine, specifically ketamine, norketamine, dehydronorketamine, 3-methoxyeticyclidine (3-MeO-PCE), 3-methoxyphencyclidine (3-MeO-PCP) and methoxetamine.

To increase the reliability of identification by acquiring automatic triggering of MS/MS spectra and spectral library comparison, a second method was established utilizing QTRAP System technology. For this approach an information dependent acquisition (IDA) method was set up with one MRM transition per compound for detection and on-the-fly acquisition of EPI scans

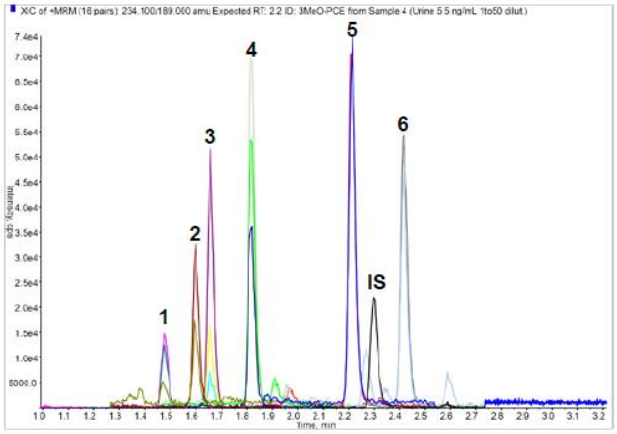


Figure 1. Chromatograph of drugs. Urine sample spiked with a concentration of 5 ng/mL for each analyte. 1: dehydronorketamine, 2: norketamine, 3: ketamine, 4: methoxetamine, 5: 3-MeO-PCE, 6: 3-MeO-PCP, IS: D5-PCP.

to obtain highly sensitive MS/MS spectra of the analytes for identification.

Key features of SCIEX QTRAP LC-MS/MS Systems for the identification and quantification of designer drugs

- The QTRAP System offers high sensitivity data acquisition strategies for both quantitative and qualitative experiments
- System sensitivity allows small sample volumes and simplified sample processing procedures
- High-throughput quantitative analysis is achieved using MRM (Multiple Reaction Monitoring) and the Scheduled MRM™ Algorithm
- MRM triggered EPI (Enhanced Product Ion) scans generate high sensitivity MS/MS spectra to enhance confidence of identification of analytes, even at low concentrations.

Experimental

Sample preparation: A simple dilution process has been chosen as sample preparation to enable a fast procedure without applying any selective extraction procedure:

- 20 µL of urine sample was added to 50 µL internal standard (D5-PCP in acetonitrile)
- Mixture was vortex mixed and centrifuged 10 minutes at 15000 rpm (21500 x g)
- Supernatant diluted with 930 µL of Mobile Phase A
- Solution vortex mixed and transferred to autosampler vial for injection

HPLC conditions: LC separation was achieved on an Agilent 1290 HPLC system by applying an 8 minute gradient on a Phenomenex Kinetex C18 100Å, 150 x 3 mm with KrudKatcher ULTRA HPLC In-Line Filter, 0.5 µm. Mobile phase A was water with formic acid and ammonium formate and mobile phase B was acetonitrile. The injection volume was 10 µL.

Mass spectrometry conditions: A SCIEX QTRAP LC-MS/MS System equipped with a Turbo V™ Ion Source was used. Source and gas parameters were optimized for best performance, and individual compound parameters were optimized using infusion. At least 2 MRM transitions per compound were optimized.

The Scheduled MRM™ Algorithm parameters used were a Ttrget scan time of 0.3 sec and a MRM detection window of 40 sec.

For the IDA method, a target scan time of 0.1 sec was used. The EPI spectra for identification were acquired using dynamic fill time and collision energy spread to obtain detailed spectral information.

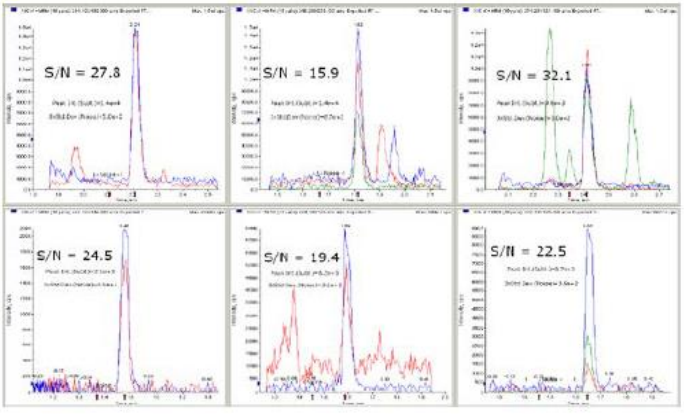


Figure 2. Chromatogram of all analytes in spiked urine at 1 ng/mL after 1:50 dilution. The signal to noise ratio w as calculated by dividing the average background signal intensity from the peak by 3 times the standard deviation of the noise region.

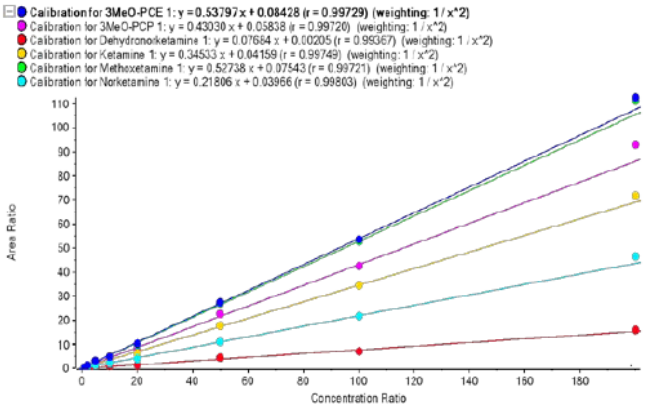


Figure 3. Calibration curves of all tested designer drugs in five different urine samples.

Generation of reference spectra: Reference EPI spectra were generated by analyzing the pure compounds and applying the same collision energy spread approach. This results in spectra which contain MS/MS fragments of a target compound at three different collision energies. The spectra acquired were combined into a library which was utilized for identification and confirmation.

Results

Using this method, it was possible to analyze the compounds ketamine, norketamine, dehydronorketamine, 3-methoxyeti-cyclidine, 3-methoxyphencyclidine and methoxetamine in a single method with an injection to injection time of 8 minutes (Figure 1). The method was found to be very specific as no interfering signals were observed in blank urine samples.

Quantitative performance has been demonstrated and shows accuracies within 15% of nominal at the LOQ and %CV of 15%, also at the LOQ and within 10% for the higher concentrations. However, for dehydronorketamine the performance was slightly lower with accuracies around 20% of nominal at the LOQ and %CV of 20%, also at the LOQ and within 15% for the higher concentrations.

An LOQ of 1 ng/mL after 1:50 dilution of the urine samples was estimated and additionally confirmed by signal to noise calculations (Figure 2). Excellent linearity was obtained for all analytes in the applied concentration range of 1 to 200 ng/mL with an applied weighting factor of $1/x^2$ (Figure 3).

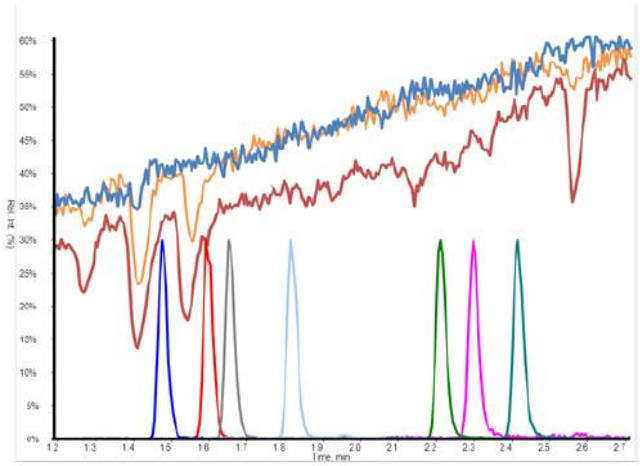


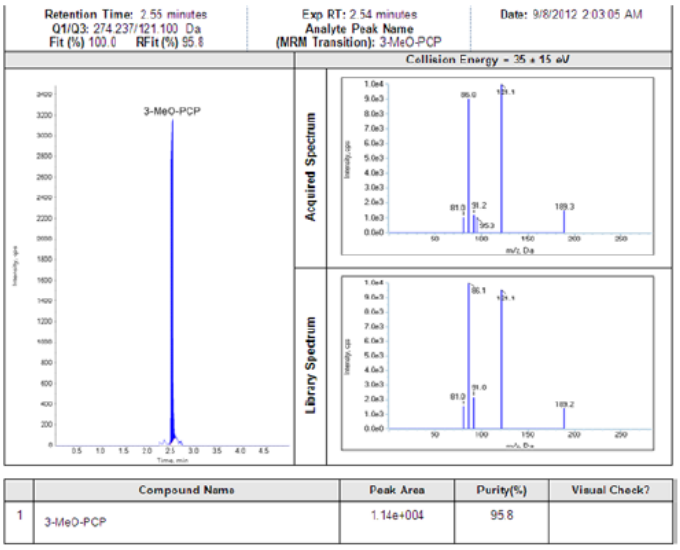
Figure 4. Ion suppression results. Blue = pure solvent, Red = 1:10 urine dilution, Orange = 1:50 urine dilution. Examples of compound retention times also shown.

All analytes were infused post column using a tee connector during injection of solvent A and a blank urine sample, which was diluted 1:50 and 1:10 respectively. Figure 4 clearly shows the benefit of a high dilution factor for urine analysis as this causes a significant decrease of ion suppression. At a dilution factor of 50, no ion suppression could be observed compared to the solvent injection and the injection of 1:10 diluted urine at relevant retention times.

To increase confidence in identification full scan MS/MS experiments using the linear ion trap of the QTRAP System can be performed and acquired spectra can be searched against mass spectral libraries (Figure 5). With this approach there is no need to monitor a second MRM transition as a qualifier for identification.

Conclusions

An LC-MS/MS method for the analysis of 6 designer drugs was developed utilizing a designer drug library for high confidence identification either by at least two MRM transitions per compound or by performing an information dependent acquisition to generate MS/MS spectra using the linear ion trap of the QTRAP System. The obtained MS/MS spectra can be



	Compound Name	Peak Area	Purity(%)	Visual Check?
1	3-MeO-PCP	1.14e+004	95.8	

Figure 5. Analyst® Reporter Software. Analyst Reporter Software was used to generate a report after automated library search. In this report the extracted and integrated MRM signal of 3-MeO-PCP (left) and the comparison of the acquired EPI spectrum (top right) and the spectra from the library (bottom right) are shown.

searched against spectral libraries using the Analyst Reporter Software for unambitious identification and confirmation.

The use of the Scheduled MRM Algorithm allows inclusion of further analytes and internal standards without sacrificing cycle time and therefore data points across the chromatographic peaks.

The very simple, fast and non-selective dilution step shows to be a reliable and robust sample preparation and ensures that no analytes are lost which can be the case if a selective extraction procedure is performed instead.

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