Identification and Quantitation of Designer Drugs in Urine by LC-MS/MS

Sebastian Dresen[1], Daniel Blake[2], Adrian Taylor[3] and Keith Williams[4]
[1]AB SCIEX, Darmstadt, Germany, [2]AB SCIEX, Warrington, UK, [3]AB SCIEX, Concord, Canada and [4]LGC Standards, Teddington, UK

Overview

This application note describes the analysis of selected designer drugs in urine after a very simple, fast and non-selective dilution step. Besides identification of the analytes by two or three MRM transitions, an information dependent acquisition (IDA) approach was used for automatically triggering enhanced product ion (EPI) scans after detection with one MRM transition per analyte. EPI spectra can be submitted for an MS/MS library search for unambiguous identification of the analytes.

Introduction

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, using the QTRAP® 4500 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 using a *Scheduled* MRM™ Algorithm.

To increase the reliability of identification by acquiring automated triggered MS/MS spectra and spectral library comparison, a second method was established utilizing the QTRAP® 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 to obtain highly sensitive MS/MS spectra of the analytes for identification.

Figure 1: 4500 QTRAP® system for quantitation, identification and confirmation of analytes

Experimental

Standards

All compounds were provided by LGC GmbH, Luckenwalde, Germany (catalogue numbers in brackets):

3-MeO-PCE (as free base) (LGCAMP1366.10-01)
3-MeO-PCP (as free base) (LGCAMP1366.09-01)
Ketamine HCl (as free base) (LGCAMP0144.00-01)
Norketamine (as free base) (LGCAMP0144.06-01)
Methoxetamine (as free base) (LGCAMP1275.65-01)
D5-PCP (as free base) (LGCAMP1358.80-01)
Dehydronorketamine (as free base) (LGCAMP0144.07-12)

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

▼

+ 50 µL internal standard (D5-PCP in acetonitrile)

▼

Centrifuged 10 minutes 15000 rpm (21500 x g)

▼

Dilute supernatant with 930 µL solvent A

▼

Transfer to vial

HPLC Conditions

LC separation was achieved on an Agilent 1290 HPLC system applying an 8 minute gradient on a Phenomenex Kinetex 2.6µ C18 100Å, 150 x 3 mm with KrudKatcher ULTRA HPLC In-Line Filter, 0.5 µm at 50 °C. Mobile phase A was water with 0.1 % formic acid and 5 mM ammonium formate and mobile phase B acetonitrile.

The injection volume was 10 µL.

Time (min)	Flow (mL/min)	Solvent A (%)	Solvent B (%)
0	0.45	95	*5*
5	0.45	*5*	*95*
6	0.45	*5*	*95*
6,2	0.45	*95*	*5*
8	0.45	*95*	*5*

Table 1: HPLC gradient conditions

MS Conditions

An AB SCIEX Q TRAP® 4500 LC/MS/MS System equipped with Turbo V™ Source was used. The following parameters were kept constant during the whole acquisition:
Temp.: 600ºC; Curtain Gas: 30 psi; Gas 1: 40 psi; Gas 2: 70 psi. CAD Gas: 9 psi; Ionisation Voltage: +2500 V.

The following *Scheduled* MRM™ Algorithm parameters were used:
Target scan time: 0.3 sec, MRM detection window: 40 sec.

For the IDA method a target scan time of 0.1 sec was used. The EPI spectra for identification were acquired at a scan rate of 10000 Da/s using dynamic fill time at a collision energy spread of 35±15 V to obtain a maximum of spectral information.

The threshold intensity was set to 2000 cps. In addition to using dynamic background subtraction, a dynamic exclusion was applied after 3 occurrences for 5 sec.

The transitions and applied potentials, as well as the obtained retention times are shown in Table 2.

Analyte	Q1 Mass (amu)	Q3 Mass (amu)	CE (V)	RT (min)
3MeO-PCE 1	234.1	189	15	2.2
3MeO-PCE 2	234.1	121	31	2.2
3MeO-PCP 1	274.2	86.1	15	2.4
3MeO-PCP 2	274.2	189.1	19	2.4
3MeO-PCP 3	274.2	121.1	37	2.4
Dehydronorketamine 1	222.1	142	33	1.5
Dehydronorketamine 2	222.1	141	51	1.5
Ketamine 1	238.1	125	37	1.6
Ketamine 2	238.1	89	73	1.6
Ketamine 3	238.1	207	19	1.6
Methoxetamine 1	248.2	203.1	19	1.8
Methoxetamine 2	248.2	121.1	37	1.8
Methoxetamine 3	248.2	175.1	25	1.8
Norketamine 1	224.1	125	33	1.6
Norketamine 2	224.1	179	21	1.6
D5-PCP	249.2	96.2	45	2.4

Table 2: MRMs and retention times of the designer drugs. The first transition of each compound was used in the survey scan of the IDA method.

Reference Spectra

Reference EPI spectra have been recorded by analyzing the pure compounds applying a collision energy spread of 35 ± 15 V. This results in spectra which contain MS/MS fragments of 20, 35 and 50 V and were combined into a library which was utilized for identification and confirmation.

Results and Discussion

Using this method it was possible to analyze the compounds ketamine, norketamine, dehydronorketamine, 3-methoxyeticyclidine, 3-methoxyphencyclidine and methoxetamine with an injection - injection time of 8 minutes (Figure 2). The method showed to be very specific as no interfering signals could be observed in blank urine samples.

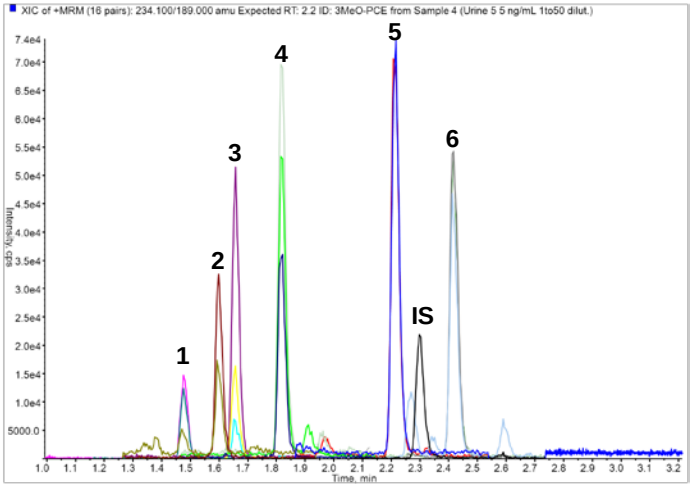


Figure 2. Chromatogram of a spiked urine sample with the concentration of 5 ng/mL for each analyte. 1: Dehydronorketamine, 2: Norketamine, 3: Ketamine, 4: Methoxetamine, 5: 3-MeO-PCE, 6: 3-MeO-PCP, IS: D5-PCP.

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.

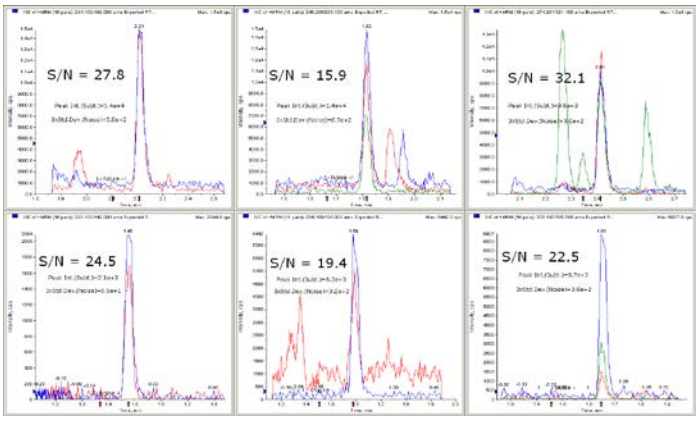


Figure 3. Chromatogram of all analytes in spiked urine at 1 ng/mL after 1:50 dilution. The signal to noise ratio was calculated by dividing the average background signal intensity from the peak by a 3 times the standard deviation of the noise region.

An LOQ of 1 ng/mL after 1:50 dilution of the urine samples was estimated and additionally confirmed by signal to noise calculations (see Figure 3). An excellent linearity was obtained for all analytes in the applied concentration range of 1 to 200 ng/mL with an applied weighting factor of $1/x^2$ (see Figure 4).

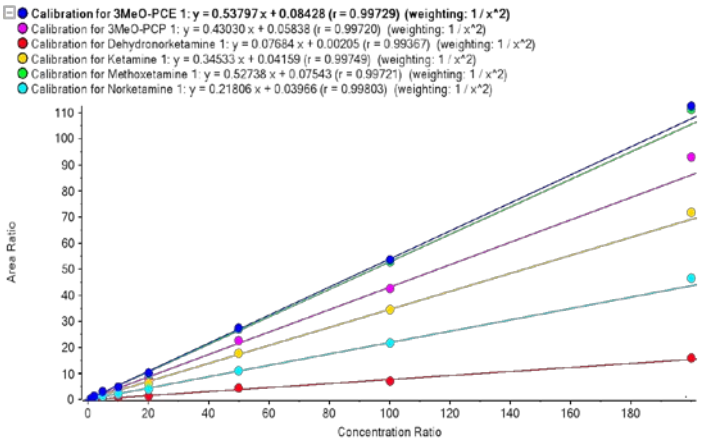


Figure 4. Calibration curves of all tested designer drugs in five different urine samples.

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 (Figure 5).

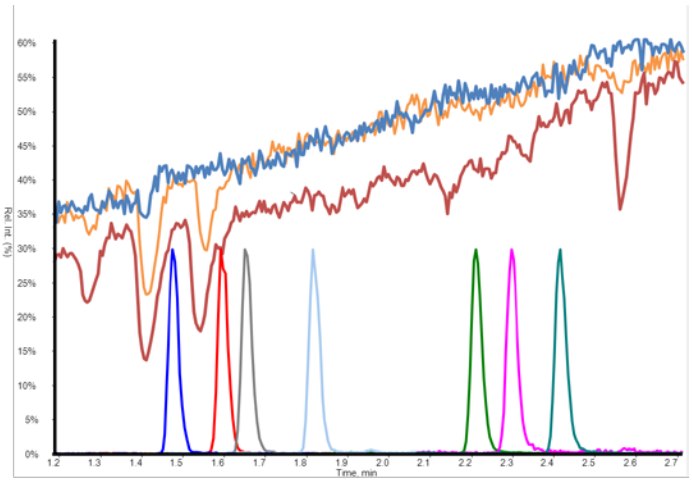
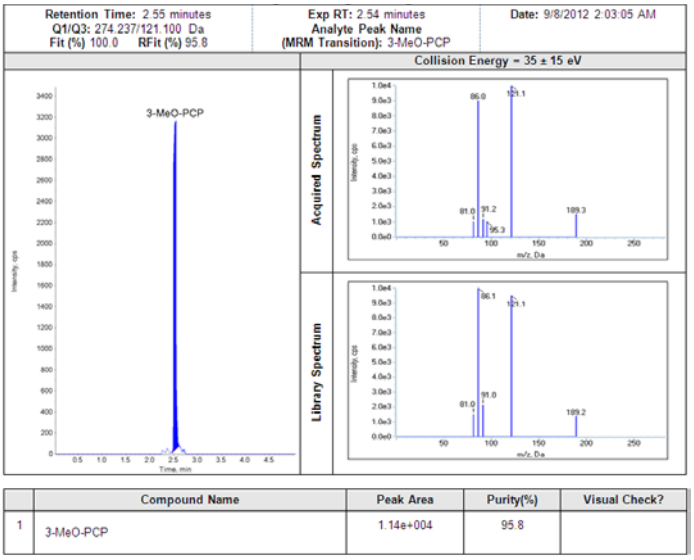


Figure 5. Results of an ion suppression test. All analytes were infused post column using a tee connector. The blue signal corresponds to the analyte signals after injection of pure solvent A. Dilution of urine by 1:10 (red signal) shows ion suppression which can almost completely be eliminated by diluting the urine samples 1:50 (orange signal). The peaks of the analytes were normalized and overlaid to demonstrate that the analytes do not elute at times where some ion suppression can be observed.

To increase confidence in identification full scan MS/MS experiments using the linear ion trap of the QTRAP® 4500can be performed and acquired spectra can be searched against mass spectral libraries. With this approach there is no need to monitor a second MRM transition as a qualifier for identification.



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

Figure 5. The 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 spectrums from the library (bottom right) are shown.

Conclusion

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

RUO-MKT-02-1407-A

AB SCIEX

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

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