TECHNICAL NOTE

imcs
integrated micro-chromatography systems

A summary of "Ultra-Fast Quantification of Antidepressants in urine at 9 seconds per sample using LDTD-MS/MS"

Overview

According to the National Center for Health Statistics, antidepressants are the third most common prescription drug used, and roughly 10% of Americans are on antidepressants. With an increase in demand for antidepressants, a need for increase in sample throughput emerges. By using an LDTD® ion source coupled with mass spectrometry, quantification of 7 antidepressants in urine samples becomes faster and more specific. The LDTD® ion source uses a laser diode to vaporize dry samples from a 96-well plate, after which the sample is carried by a gas into a corona discharge region, resulting in high efficiency protonation and strong resistance to ionic suppression. The use of the LDTD® ion source enables samples to be processed at a run time of only 9 seconds.

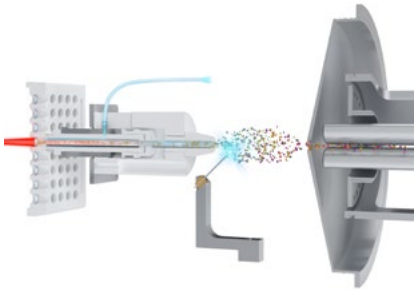

*Figure 1. Schematic of the LDTD® ionization source**

Materials and Methods

Antidepressant patient samples were hydrolyzed at 55 °C for 30 minutes. Fifty µL of each, patient urine or standard was combined with 2.5 µL of internal standard (10 µg/mL Clomipramine-d3 in 50% MeOH), 10 µL of purified β-glucuronidase (IMCSzyme®), and 12.5 µL of hydrolysis buffer. After hydrolysis a liquid-liquid extraction procedure was performed by adding 100 µL Na_2CO_3 buffer (0.5 M, pH 10) and 800 µL hexane/EtOAC (25/75). The samples were vortexed and then 5 µL of the upper organic layer was transferred to a LazWell™ plate and then dried.

For sample analysis, The LDTD® source model S-960 was used along with a Sciex 5500 QTrap® MS system in MRM mode. The laser power of the LDTD® source was ramped to 65% in 3 seconds, maintained for 2 seconds, and then decreased to 0%. Carrier gas flow (air) was at 3 L/min.

Results

Calibration curves with standard concentrations from 15.6 to 2000 ng/mL for each of the 7 drugs (amitriptyline, clomipramine, cyclobenzaprine, desipramine, doxepin, imipramine, and nortriptyline) had R^2 values equal to or exceeding 0.995 (Table 1). The % biases of the QC samples for each drug were within the acceptable range with ≤ 15 % CV.

To test ionization suppression/enhancement evaluation, ten different matrices were spiked at low level QC (62.4 ng/mL). Again, % biases of the detected concentrations were within the acceptable range and ≤ 15 % CV.

To assess carry-over, three blanks were analyzed after the highest standard, and to determine interference percentage, the peak areas were measured against the average peak areas of the lowest standard and internal standard (Table 2).

Interference evaluation was assessed by testing 10 blank matrices as well as blanks spiked with 35 other possibly interfering drugs. No interference was observed in any of the blanks or spiked samples after concentration evaluation.

Stability of the samples was evaluated in both their wet and dry states by testing them after 24 hours at 4 °C in their wet state, and after 24 hours at room temperature in their dry state. All bias and precision results for all antidepressant drugs were within the acceptable range.

Conclusion:

Accurate quantitation of 7 different antidepressant drugs following urine sample hydrolysis and extraction was accomplished by LDTD-MS/MS in a mere 9 seconds per sample. Even with such a short sample processing time, linearity of calibration curves was exceptional, as well as all bias and precision results. No carry-over or interference was observed, and stability results of wet and dry samples were good. The combination of IMCSzyme® and LDTD-MS/MS system significantly increases the throughput for processing antidepressant patient samples while maintaining accuracy and precision.

Table 1. Intra-run assay results for Clomipramine

	LLOQ	QC Low	QC Med	QC High	ULOQ
Conc. (ng/ml)	15.6	62.5	250	1000	2000
N	6	6	6	6	6
Mean (ng/mL)	16.1	63.6	253.3	1027.3	1971.5
%CV	10.7	5.9	7.1	4.6	4.8
% Bias	3.1	1.7	1.3	2.7	-1.4

Table 2. Carry-over evaluation of Clomipromine

Sample	% Inter. Drug	% Interf. IS
Blank 1	1.2%	1.0%
Blank 2	0.0%	0.8%
Blank 3	13.7%	4.2%

**This information was summarized by IMCS from the technical poster "Ultra-Fast Quantification of Antidepressants in urine at 9 seconds per sample using LDTD-MS/MS" presented by Alex Birsan - Phytronix at MSACL 2016.*