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

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OVERVIEW

Purpose
- High-throughput screening of 7 Antidepressants in urine.

Method
- Basic pH Liquid-Liquid extraction followed by LDTD-MS/MS analysis
- Quantification:
  - Linearity: $r^2 > 0.995$ over the calibration range (15.6 to 2000 ng/mL)
- **Samples were analyzed with a run time of 9 seconds using LDTD-MS/MS system**

INTRODUCTION

According to a 2011 National Center for Health Statistics (NCHS) report, the rate of Antidepressants use in USA increased by almost 400% between 2005 and 2008 for people older than 12 years old. The federal government's health statisticians figure that about one in every 10 Americans takes at least one antidepressant. And by their calculations, antidepressants were the third most common prescribed medication taken by Americans. These numbers have consistently increased over the years. Using mass spectrometry combined with high-throughput LDTD ion source enhances specificity at equivalent of better speed for the quantification of 7 Antidepressants in human urine matrix. Using a basic liquid-liquid extraction for the sample preparation, we are able to achieve precision and accuracy at a speed of 6 seconds per sample.

LDTD® Ionization Source:

The LDTD uses a Laser Diode to produce and control heat on the sample support **(Figure 1)** which is a 96 wells plate**.** The energy is then transferred through the sample holder to the dry sample which vaporizes prior to being carried by a gas in a corona discharge region. High efficiency protonation and strong resistance to ionic suppression characterize this type of ionization, and is the result of the absence of solvent and mobile phase. This allows for very high throughput capabilities of 9 seconds sample-to-sample analysis time, without carry over.

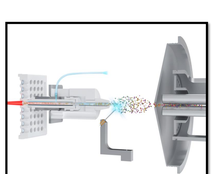

Figure 1 Schematic of the LDTD ionization source

METHOD

Glucuronide Hydrolysis procedure:
50 µL patient sample (or standard)
2.5 µL IS (Clomipramine-d3 10 µg/mL in MeOH:Water (1:1))
10 µL Purified b-Glucuronidase (IMCSzyme)
12.5 µL Hydrolysis buffer
Vortex. Incubation at 55°C for 30 minutes

Basic Liquid Liquid extraction procedure:
100 µL Na_2CO_3 buffer (0.5M pH 10)
800 µL Hexane/EtAC (25/75)
Vortex
Wait for phase separation
Transfer 5 µL of organic upper layer in a LazWell plate
Dry prior to analysis

Figure 2 LDTD system on Sciex 5500 QTrap®

Instrumentation
- LDTD model: S-960
- MS: Sciex 5500 QTrap®

LDTD Parameters
- Laser power pattern :
  - ➢ Increase laser power to 65 % in 3.0 sec
  - ➢ Maintain for 2 sec
  - ➢ Decrease laser power to 0 %
- Carrier gas flow (Air) : 3 L/min

MS Parameters
- APCI (+)
- Dwell: 5 msec
- Corona discharge: 3 µA
- DP: 80 V
- MRM mode (see **Table 1**)

Table 1 MRM method transitions

Compound	Q1	Q3	CE (V)
Amitriptyline (Quant)	278	233	25
Amitriptyline (Conf)	278	117	30
Clomipramine (Quant)	315	86	25
Clomipramine (Conf)	315	58	55
Clomipramine-d3	320*	61	55
Cyclobenzaprine (Quant)	276	215	50
Cyclobenzaprine (Conf)	276	231	25
Desipramine (Quant)	267	72	20
Desipramine (Conf)	267	44	55
Doxepin (Quant)	280	117	30
Doxepin (Conf)	280	235	25
Imipramine (Quant)	281	86	20
Imipramine (Conf)	281	236	25
Nortriptyline (Quant)	264	233	20
Nortriptyline (Conf)	264	117	30

*Chlorine isotope was used as primary mass

RESULT

Linearity results:

A standard calibration curve (with all 7 drugs) ranging from 15.6 to 2000 ng/mL has been prepared in blank urine matrix. All curves have 0.995 coefficients or better. **Figure 3** present typical calibration curves for Clomipramine. In Table 2, correlation coefficient (r) obtained for the intra-run assay.

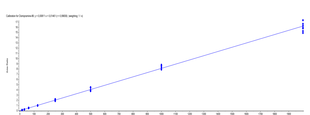

Figure 3 Clomipramine typical calibration curve

Table 2 Correlation coefficient (r)

Compound	r
Amitriptyline	0.99704
Clomipramine	0.99836
Cyclobenzaprine	0.99817
Desipramine	0.99674
Doxepin	0.99766
Imipramine	0.99637
Nortriptyline	0.99609

Ionization suppression / enhancement evaluation:

Ionization suppression/enhancement evaluation was performed by spiking ten different matrixes at low level QC. Bias and precision criteria were applied. In **Table 5,** results of ionization suppression/enhancement verification are reported for Clomipramine. Similar result are obtained for the other drugs (results not reported). All QCs have bias value within the acceptance criteria (±20%) and a precision value with a %CV ≤ 15%.

Table 5 Ionization suppression/enhancement result of Clomipramine

	Conc. (ng/ml)	N	Mean (ng/ml)	%CV	%Bias
Matrix 1	62.4	3	58.0	3.16	-7.07
Matrix 2	62.4	3	58.1	1.94	-6.86
Matrix 3	62.4	3	64.5	1.68	3.37
Matrix 4	62.4	3	63.2	2.00	1.28
Matrix 5	62.4	3	64.1	2.84	2.66
Matrix 6	62.4	3	62.4	4.74	0.00
Matrix 7	62.4	3	66.3	4.03	6.30
Matrix 8	62.4	3	57.6	4.27	-7.68
Matrix 9	62.4	3	59.0	4.26	-5.50
Matrix 10	62.4	3	63.2	4.56	1.31

Bias and precision results:

Bias and precision in an Intra-run assay (**Table 3**) and for the inter-run assay (**Table 4**) are reported for Clomipramine. Similar results are obtained for the other drugs (results not reported). All QCs have bias value within the acceptance criteria (±20%) and a precision value with a %CV ≤ 15%.

Table 3 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 4 Inter-run assay results for Clomipramine

	QC Low	QC Med	QC High
Conc. (ng/ml)	62.5	250	1000
N	24	24	24
Mean (ng/ml)	64.3	246.8	1005.1
%CV	6.1	4.9	4.0
%Bias	2.8	-1.3	0.5

Interference evaluations:

The carry-over is evaluated by the analysis of three blanks after the highest standard. The blank peak areas were evaluated against the mean peak area of the lower standard and internal standard to determine the interference percentage. In **Table 6,** carry over results for Clomipramine are reported.

Interference evaluation of 10 blank matrices and other commonly encountered analytes spiked in blank matrix were evaluated by concentration titer. Results for Clomipramine are reported in **Table 7**. No interferences were observed in 10 blank matrixes tested. No interferences were noted in blank matrix spiked with potential drug interferences at 1 µg/ml level (total of 35 drugs: benzodiazepines, barbiturates, amphetamines, cannabinoids and others were tested).

Similar results were obtained with the other Antidepressant drugs (not reported).

Table 6 Carry over evaluation of Clomipramine

Sample	%Interf. Drug (%)	%Interf. IS (%)
Blank 1	1,2%	1,0%
Blank 2	0,0%	0,8%
Blank 3	13,7%	4,2%

Table 7 Interference evaluation of Clomipramine

	Calc. Conc. (ng/ml)
BLK Matrix 1	<15.6
BLK Matrix 2	<15.6
BLK Matrix 3	<15.6
BLK Matrix 4	<15.6
BLK Matrix 5	<15.6
BLK Matrix 6	<15.6
BLK Matrix 7	<15.6
BLK Matrix 8	<15.6
BLK Matrix 9	<15.6
BLK Matrix 10	<15.6
Drug interf. G1	<15.6
Drug interf. G2	<15.6
Drug interf. G3	<15.6
Drug interf. G4	<15.6
Drug interf. G5	<15.6

CONCLUSION

- **Simultaneous quantification and confirmation evaluation of 7 Antidepressant drugs in urine is performed in 9 seconds sample-to-sample by LDTD-MS/MS**
- **Linearity range of 15.6 to 2000 ng/ml in urine.**
- **Good bias and precision are obtained for intra-run, inter-run and ionization suppression/enhancement assay.**
- **No carry over and no interference from different blanks and from potential concomitant drugs.**