TECHNICAL NOTE 66063

Quantification of 15 tricyclic antidepressants in human plasma by LC-HRAM-MS using the Orbitrap Exploris 120 mass spectrometer for clinical research

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Keywords: Orbitrap Exploris 120 MS, TraceFinder software, Vanquish Flex UHPLC, high-resolution mass spectrometry, mass spectrometry, plasma, quantification, tricyclic antidepressants, TCAs, Orbitrap technology

Application benefits

- Accurate and confident results, simple sample preparation, and rapid quantitation
- Robust, sensitive LC and HRAM MS platforms enable increased confidence in data
- Quantification of 15 tricyclic antidepressants in human plasma in a single method

Goal

Implementation of an analytical method for the quantification of 15 tricyclic antidepressant drugs in human plasma on a Thermo Scientific™ Orbitrap Exploris 120™ mass spectrometer

Introduction

Antidepressants are commonly prescribed to alleviate symptoms of clinical depression and anxiety. There are different types of antidepressants, based on their mode of action. Tricyclic antidepressants (TCAs) are a group of psychoactive drugs that are mainly used for the therapy of endogene depressions, anxiety, and pain management.

Their name is derived from a common chemical structure with a tripartite ring system.

In this study, a fast and robust analytical method for clinical research for the quantification of 15 tricyclic antidepressants in human plasma is reported. Samples were processed by protein precipitation followed by chromatographic separation on a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system. Detection was performed on an Orbitrap Exploris 120 mass spectrometer with heated electrospray ionization (HESI) operated in positive ionization mode. Method performance was evaluated using the ClinMass™ LC-MS/MS calibrators, controls and internal standards from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response, lower limit of quantification (LLOQ), accuracy, and intra- and inter-assay precision for all analytes.

Experimental

Target analytes

The 15 tricyclic antidepressant drugs, their internal standards, and their corresponding exact masses are presented in Table 1.

Sample preparation

Reagents included three calibrators (MS9113, lot no. 1308), two controls (MS9182, lot no. 1456) and 12 deuterated internal standards (MS9112 lot no. 1199) from RECIPE. The nominal concentrations of the calibrators are given in Table 2 together with the retention times of each compound.

Table 1. Compounds, internal standards, chemical formulas, and exact masses of the protonated ion $[M+H]^+$

Compound name	Formula	Exact mass $[M+H]^+$ (*m/z*)	Internal standard name	Exact mass $[M+H]^+$ (*m/z*)
Amitriptyline	$C_{20}H_{23}N$	278.1903	Amitriptyline-d_3	281.2092
Clomipramine	$C_{19}H_{23}ClN_2$	315.1623	Clomipramine-d_3	318.1811
Clozapine	$C_{18}H_{19}ClN_4$	327.1371	Clozapine-d_4	331.1622
Desipramine	$C_{18}H_{22}N_2$	267.1856	Desipramine-d_3	270.2044
Doxepin	$C_{19}H_{21}NO$	280.1696	Doxepin-d_3	283.1884
Imipramine	$C_{19}H_{24}N_2$	281.2012	Imipramine-d_3	284.2201
Maprotiline	$C_{20}H_{23}N$	278.1903	Maprotiline-d_5	283.2217
Norclomipramine	$C_{18}H_{21}ClN_2$	301.1466	Norclomipramine-d_3	304.1654
Norclozapine	$C_{17}H_{17}ClN_4$	313.1215	Norclozapine-d_8	321.1717
Nordoxepin	$C_{18}H_{19}NO$	266.1539	Nordoxepin-d_3	269.1728
Normaprotiline	$C_{19}H_{21}N$	264.1747	Desipramine-d_3	270.2044
Nortrimipramine	$C_{19}H_{24}N_2$	281.2012	Imipramine-d_3	284.2201
Nortriptyline	$C_{19}H_{21}N$	264.1747	Nortriptyline-d_3	267.1935
Protriptyline	$C_{19}H_{21}N$	264.1747	Nortriptyline-d_3	267.1935
Trimipramine	$C_{20}H_{26}N_2$	295.2169	Trimipramine-d_3	298.2357

Table 2. Retention times and calibration levels

Compound name	Retention time (min)	Concentration (µg/L)		
		L1	L2	L3
Amitriptyline	5.11	15.6	103	316
Clomipramine	5.90	17.6	123	366
Clozapine	3.20	60.4	432	1274
Desipramine	4.65	17.2	117	363
Doxepin	3.20	13.9	91.1	274
Imipramine	4.70	15.3	105	326
Maprotiline	4.90	22.2	145	442
Norclomipramine	5.87	20.2	135	412
Norclozapine	3.00	46.4	318	952
Nordoxepin	3.20	14.1	97.4	301
Normaprotiline	4.80	33.4	230	737
Nortrimipramine	5.60	8.24	54.0	164
Nortriptyline	5.15	17.1	117	357
Protriptyline	4.55	15.1	102	315
Trimipramine	5.55	17.0	117	360

Samples of 50 µL of plasma were protein precipitated using 100 µL of acetonitrile containing the internal standards. Precipitated samples were vortex-mixed, kept at room temperature for 5 minutes, vortex-mixed again, and centrifuged. The supernatant was transferred to a clean vial, and 1 µL was injected onto the LC-MS system.

Liquid chromatography

LC separation was performed on a Vanquish Flex Binary UHPLC system using the following mobile phases:

Mobile phase A: 5 mM ammonium formate + 0.1% formic acid in water

Mobile phase B: 5 mM ammonium formate + 0.1% formic acid in methanol

Chromatographic separation was achieved by gradient elution on a Thermo Scientific™ Hypersil GOLD™ Phenyl 2.1 × 100 mm (1.9 µm) analytical column (P/N 25902-102130) run at 40 °C at a flow rate of 0.5 mL/min. The chromatographic conditions are given in Table 3.

Mass spectrometry

Detection was performed in Full Scan acquisition mode using a resolution setting of 60,000 (FWHM) at *m/z* 200 and a scan range of 100–500 using an Orbitrap Exploris 120 mass spectrometer, equipped with a HESI source operated in positive ionization mode. The ion source conditions and the mass spectrometry settings are presented in Tables 4 and 5, respectively.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 5.1 software.

Method evaluation

The parameters used to evaluate the performance of the method included linearity of response, lower limit of quantification (LLOQ), intra- and inter-assay accuracy and precision for all the analytes.

Accuracy was calculated as the percent of the nominal concentration. Precision was evaluated as the coefficient of variation (%CV). The intra-assay accuracy and precision were evaluated on two levels of QC samples extracted in replicates of five (n=5) on three different days. The inter-assay accuracy and precision were calculated using the same approach as for the intra-assay ones but using the full set of replicates (n=15).

Table 3. Gradient profile

Time (min)	Flow rate (mL/min)	%B
0	0.5	10
0.2	0.5	10
0.5	0.5	50
4.5	0.5	50
5.2	0.5	100
6.5	0.5	100
6.5	0.5	10
8	0.5	10

Table 4. Ion source settings

Parameter	Setting
Sheath gas	50 AU
Aux gas	10 AU
Sweep gas	0 AU
Spray voltage	3,500 V
Ion transfer tube temperature	300 °C
Vaporizer temperature	320 °C

Table 5. Mass spectrometer settings

Parameter	Setting
Resolution @ *m/z* 200	60,000
Scan range (*m/z*)	100–500
AGC target	Standard (1e6)
RF lens	70%
Maximum injection time mode	Auto
Data type	Profile
Polarity	Positive
Source fragmentation	Off

Table 6. Mean accuracy and CV% of back-calculated calibrators (n=3)

	Mean accuracy (n=3)			%CV		
	L1	L2	L3	L1	L2	L3
Amitriptyline	98.6	102.0	99.4	2.1	3.0	0.9
Clomipramine	100.6	99.2	100.2	1.4	2.1	0.6
Clozapine	101.0	98.5	100.5	1.5	2.1	0.7
Desipramine	98.0	102.8	99.2	1.6	2.3	0.7
Doxepin	99.6	100.6	99.8	1.3	1.8	0.5
Imipramine	98.1	102.7	99.2	1.7	2.4	0.7
Maprotiline	98.8	101.8	99.5	1.2	1.6	0.5
Norclomipramine	98.9	101.5	99.6	1.7	2.4	0.7
Norclozapine	98.5	102.1	99.4	0.3	0.5	0.1
Nordoxepin	98.7	101.9	99.5	1.7	2.4	0.7
Normaprotiline	98.4	102.2	99.4	1.9	2.6	0.7
Nortrimipramine	98.6	102.0	99.4	1.4	2.0	0.6
Nortriptyline	99.2	101.1	99.7	1.4	1.9	0.6
Protriptyline	98.4	102.3	99.3	1.5	2.2	0.6
Trimipramine	98.2	102.6	99.2	1.7	2.4	0.7

The limit of quantitation (LLOQ) used in this study was set to the level of the lowest calibrator, L1. The possibility to use a lower concentration as LLOQ was investigated by dilution of the lowest calibrator.

Linearity was investigated on three calibration curves prepared and extracted on three different days by evaluation of the accuracy of the back-calculated concentration for the provided calibrators.

Results and discussion

A linear regression with 1/x weighting was used for all compounds. The mean accuracy and precision of the back-calculated calibrators are presented in Table 6. The linearity was good for all compounds in the calibrated range, with a coefficient of determination (R^2) above 0.9989. The mean accuracies were within 98.0 to 102.8% and the precision was <3.0% for all compounds at all levels.

The lowest concentration of the diluted calibrators that had a mean back-calculated accuracy within 80 to 120% and a precision (CV) better than 20% (1 replicate for 3 days) are presented in Table 7. For all compounds, there is a possibility to extend the LLOQ below the lowest calibrator.

Table 7. Estimated extension of LLOQ for all compounds

Analyte	LLOQ (µg/L)
Amitriptyline	1.56
Clomipramine	1.76
Clozapine	6.04
Desipramine	1.72
Doxepin	1.39
Imipramine	1.53
Maprotiline	2.22
Norclomipramine	2.02
Norclozapine	4.64
Nordoxepin	1.41
Normaprotiline	3.34
Nortrimipramine	0.824
Nortriptyline	1.71
Protriptyline	1.51
Trimipramine	1.70

Representative chromatograms at the estimated LLOQ are presented in Figure 1, and representative calibration curves are presented in Figure 2.

The data demonstrate good accuracy and precision of the method. The intra-assay accuracy and precision results are reported in Tables 8 and 9, respectively. The intra-assay accuracy was between 95.1 and 113.4%, and the intra-assay precision was better than 7.8% for all compounds at all levels.

The inter-assay accuracy and precision results are reported in Table 10. The inter-assay accuracy was between 98.1 and 111.6%, and the inter-assay precision was better than 6.1% for all compounds at all levels.

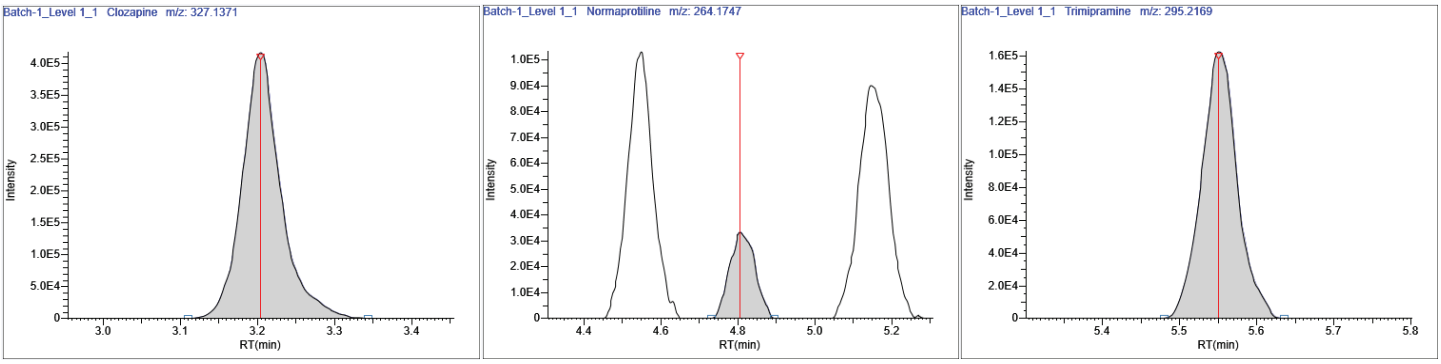

Figure 1. Representative chromatograms at the estimated LLOQ

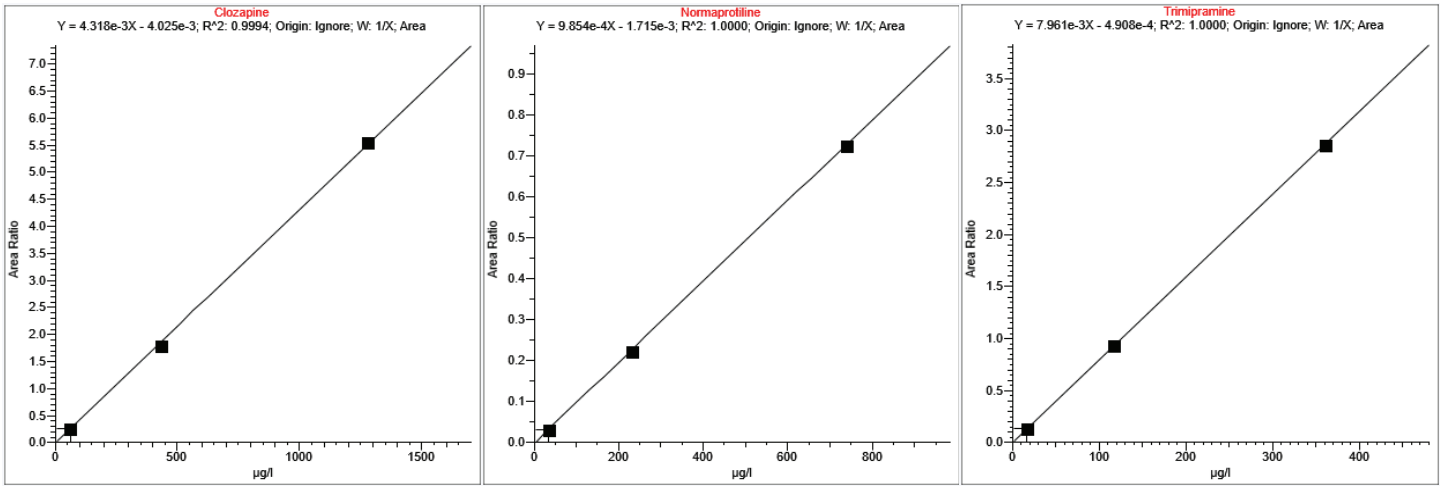

Figure 2. Representative calibration curves

Table 8. Intra-assay % accuracy of the QC samples (mean, n=5)

	QC Level 1				QC Level 2			
	Nominal conc. (µg/L)	Batch 1	Batch 2	Batch 3	Nominal conc. (µg/L)	Batch 1	Batch 2	Batch 3
Amitriptyline	59.2	108.6	102.8	104.6	135	104.4	105.9	108.8
Clomipramine	66.5	113.4	106.5	107.3	154	107.7	107.1	105.9
Clozapine	217	110.1	103.1	103.8	510	103.0	103.8	99.7
Desipramine	64.3	111.9	105.3	105.1	152	103.5	106.1	98.2
Doxepin	50.8	110.1	103.1	104.8	117	105.6	104.8	108.1
Imipramine	57.5	111.2	104.0	104.9	133	105.4	104.3	106.8
Maprotiline	82.7	112.4	104.8	105.8	193	105.5	104.3	98.8
Norclomipramine	71.9	113.2	107.0	108.1	169	105.4	105.9	100.4
Norclozapine	179	112.7	103.3	109.2	418	105.7	104.4	104.5
Nordoxepin	49.4	112.6	105.9	107.6	116	107.5	106.4	100.9
Normaprotiline	121	110.8	104.6	110.5	280	107.0	107.3	103.2
Nortrimipramine	35.8	109.9	112.3	112.6	86.4	107.2	106.9	107.5
Nortriptyline	64.5	111.3	104.4	105.5	145	108.9	107.2	103.0
Protriptyline	58.1	110.8	104.1	105.5	143	99.9	99.3	95.1
Trimipramine	64.2	112.5	105.3	107.4	155	102.2	102.8	105.8

Table 9. Intra-assay precision of the QC samples, CV% (n=5)

	QC Level 1			QC Level 2		
	Batch 1	Batch2	Batch 3	Batch 1	Batch 2	Batch 3
Amitriptyline	3.0	7.2	3.1	5.6	6.7	3.5
Clomipramine	3.3	7.8	3.9	5.8	4.6	4.5
Clozapine	3.3	7.1	3.7	5.9	7.4	4.7
Desipramine	3.5	7.3	3.1	5.3	6.5	4.2
Doxepin	3.5	7.2	5.3	6.2	6.9	4.6
Imipramine	3.6	6.9	3.2	5.5	4.9	4.4
Maprotiline	3.2	7.1	3.5	5.9	5.1	4.7
Norclomipramine	3.0	6.8	3.2	5.1	3.8	4.3
Norclozapine	3.6	6.8	2.9	5.6	4.5	6.1
Nordoxepin	2.8	6.2	3.4	5.5	4.3	4.7
Normaprotiline	2.8	5.3	3.3	4.0	1.5	2.6
Nortrimipramine	2.5	7.5	1.8	5.4	5.1	2.6
Nortriptyline	3.4	7.5	3.0	5.1	3.6	4.4
Protriptyline	2.8	7.5	2.8	5.1	6.2	4.6
Trimipramine	3.3	7.2	3.3	5.7	7.1	4.6

Table 10. Inter-assay accuracy and precision of the QC samples, CV% (n=15)

	Accuracy		Precision	
	QC1	QC2	QC1	QC2
Amitriptyline	105.3	106.4	5.0	5.3
Clomipramine	109.0	106.9	5.7	4.7
Clozapine	105.7	102.2	5.5	6.0
Desipramine	107.4	102.6	5.5	6.1
Doxepin	106.0	106.2	5.8	5.7
Imipramine	106.7	105.5	5.4	4.7
Maprotiline	107.7	102.9	5.6	5.7
Norclomipramine	109.4	103.9	5.0	4.8
Norclozapine	108.4	104.9	5.7	5.1
Nordoxepin	108.7	105.0	4.8	5.3
Normaprotiline	108.6	105.8	4.5	3.2
Nortrimipramine	111.6	107.2	4.5	4.2
Nortriptyline	107.1	106.4	5.4	4.8
Protriptyline	106.8	98.1	5.2	5.4
Trimipramine	108.4	103.6	5.3	5.7

Conclusion

A reproducible, accurate, and sensitive liquid chromatography-HRAM mass spectrometry method was implemented for the quantification of 15 tricyclic antidepressant drugs in human plasma in less than 8 min/sample. The method was analytically validated on a Vanquish Binary Flex UHPLC system coupled to an Orbitrap Exploris 120 mass spectrometer. It offers a rapid and simple offline protein precipitation with concomitant internal standard addition. The described method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.

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