TECHNICAL NOTE 66058

Quantification of 15 tricyclic antidepressants in human plasma or serum by LC-HRAM-MS for clinical research

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Application benefits

- Increased accuracy of method by implementation of a comprehensive ClinMass® kit for sample preparation
- High-resolution mass spectrometry for improved selectivity
- Robust, sensitive hardware enabling increased confidence in data
- Simple offline sample preparation by protein precipitation
- 15 tricyclic antidepressants in a single quantitative method

Goal

Implementation of an analytical method for the quantification of 15 tricyclic antidepressants in human plasma or serum on a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer.

Introduction

Tricyclic antidepressants (TCAs) are commonly used to alleviate depression, anxiety reactions, and neuropathic pain. Despite the risk of severe side effects, even in therapeutic doses, TCAs are still prescribed due to their effectiveness compared to other antidepressants. Monitoring serum concentrations of TCAs can improve therapeutic management of depression in recipients with questions of compliance, suspected toxicity, and/or drug-drug interactions. High-performance liquid chromatography (HPLC) with ultraviolet (UV) spectrophotometric detection has been the recommended and most commonly used method for therapeutic drug monitoring (TDM) research of TCA for over 30 years. However, with increasing demands

for higher sensitivity, selectivity, and specificity, several methods leveraging HPLC coupled to tandem mass spectrometry (LC-MS/MS) have been developed for TCA TDM research.

An analytical method for clinical research for the quantification of 15 tricyclic antidepressants in human plasma or serum is reported in this study; the analysis includes amitriptyline, clomipramine, clozapine, desipramine, doxepin, imipramine, maprotiline, norclomipramine, norclozapine, nordoxepin, normaprotiline, nortrimipramine, nortriptyline, protriptyline, and trimipramine. While most reported LC-MS analyses of the above-mentioned TCAs involve triple quadrupole mass spectrometers, which have been traditionally used for targeted, sensitive quantitation assays, in this report we present LC-MS data acquired using high-resolution accurate mass (HRAM) mass spectrometry leveraging Orbitrap technology. This report demonstrates the capability of HRAM for routine quantitative analyses in addition to its use for performing in-depth qualitative investigations.

Plasma or serum samples were extracted by offline internal standard addition and protein precipitation. Extracted samples were injected onto a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system connected to an Orbitrap Exploris 120 mass spectrometer with a heated electrospray ionization (HESI) source. Detection was performed by full scan coupled to data-dependent fragmentation (fullMS-ddMS^2) except for normaprotiline and protriptyline, which were acquired in targeted-MS^2 (tMS^2) mode. Method performance was evaluated using the ClinMass TDM Platform with the ClinMass Add-On Set for Tricyclic Antidepressants from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response within the calibration ranges, lower limit of quantification (LLOQ), carryover, accuracy and intra- and inter-assay precision for each analyte.

Experimental

Target analytes

A complete list of analytes and corresponding internal standards is provided in Table 1. The concentration ranges covered by the calibrators (MS9113 batch #1389) used are reported in Table 2.

Sample preparation

Reagents included four calibrators (including blank) and two controls from RECIPE (MS9182 batch #1389), as well as 12 deuterated internal standards for the quantification. Samples of 50 µL of plasma or serum were protein precipitated using 100 µL of precipitating solution containing the internal standards. Precipitated samples were vortex-mixed and centrifuged, and the supernatant was transferred to a clean plate or vial.

Table 1. List of analytes and internal standards

Analyte	Molecular formula	Expected mass (*m/z*)	Internal standard	Molecula formula	Expected mass (*m/z*)
Amitriptyline	$C_{20}H_{23}N$	278.1903	d_3-Amitriptyline	$C_{20}H_{20}D_3N$	281.2092
Clomipramine	$C_{19}H_{23}ClN_2$	315.1623	d_3-Clomipramine	$C_{19}H_{20}D_3ClN_2$	318.1811
Clozapine	$C_{18}H_{19}ClN_4$	327.1371	d_4-Clozapine	$C_{18}H_{15}D_4ClN_4$	331.1622
Desipramine	$C_{18}H_{22}N_2$	267.1856	d_3-Desipramine	$C_{18}H_{19}D_3N_2$	270.2044
Doxepin	$C_{19}H_{21}NO$	280.1696	d_3-Doxepin	$C_{19}H_{18}D_3NO$	283.1884
Imipramine	$C_{19}H_{24}N_2$	281.2012	d_3-Imipramine	$C_{19}H_{21}D_3N_2$	284.2201
Maprotiline	$C_{20}H_{23}N$	278.1903	d_5-Maprotiline	$C_{20}H_{20}D_3N$	283.2217
Norclomipramine	$C_{18}H_{21}ClN_2$	301.1466	d_3-Norclomipramine	$C_{18}H_{18}D_3ClN_2$	304.1654
Norclozapine	$C_{17}H_{17}ClN_4$	313.1215	d_8-Norclozapine	$C_{17}H_9D_8ClN_4$	321.1717
Nordoxepin	$C_{18}H_{19}NO$	266.1539	d_3-Nordoxepin	$C_{18}H_{16}D_3NO$	269.1728
Normaprotiline*	$C_{19}H_{21}N$	169.1012	d_3-Doxepin*	$C_{19}H_{18}D_3NO$	235.1117
Nortrimipramine	$C_{19}H_{24}N_2$	281.2012	d_3-Imipramine	$C_{19}H_{21}D_3N_2$	284.2201
Nortriptyline	$C_{19}H_{21}N$	264.1747	d_3-Nortriptyline	$C_{19}H_{18}D_3N$	267.1935
Protriptyline*	$C_{19}H_{21}N$	155.0855	d_3-Nortriptyline*	$C_{19}H_{18}D_3N$	155.0855
Trimipramine	$C_{20}H_{26}N_2$	295.2169	d_3-Trimipramine	$C_{20}H_{23}D_3N_2$	298.2357

*Analytes acquired with targeted-MS^2 experiment

Table 2. Concentration ranges covered by the calibrators (MS9113 batch #1389)

Analyte	Concentration range (µg/L)
Amitriptyline	16.3–331
Clomipramine	18.3–361
Clozapine	58.5– 202
Desipramine	18.1–377
Doxepin	14.8–287
Imipramine	16.7–343
Maprotiline	24.2–465
Norclomipramine	21.1–416
Norclozapine	47.3–950
Nordoxepin	13.6–279
Normaprotiline	33.4–725
Nortrimipramine	11.0–220
Nortriptyline	18.2–371
Protriptyline	16.9–344
Trimipramine	27.3–579

Liquid chromatography

A Vanquish Flex Binary UHPLC system was used for chromatographic separation, using mobile phases and an analytical column provided by RECIPE. Details of the analytical method are reported in Table 3. Total runtime was 4.0 minutes.

Table 3. LC method description

Time (min)	Flow rate (mL/min)	B (%)
0.00	0.8	15
0.05	0.8	15
0.06	0.8	30
2.10	0.8	30
2.11	0.8	38
2.70	0.8	38
3.00	0.8	75
3.35	0.8	75
3.40	0.8	15
4.00	0.8	15

Mass spectrometry

Analytes and internal standards were detected using an Orbitrap Exploris 120 mass spectrometer with a HESI source operated in positive ionization mode. Targeted-MS^2 acquisition mode was used to distinguish between the coeluting isobaric analytes normaprotiline and protriptyline. Their internal standards were also included in order to take into consideration the variation from the fragmentation. A full scan approach with $ddMS^2$ for confirmation based on the ion ratio was applied for the detection of the remaining analytes and internal standards. A summary of the MS conditions is reported in Table 4.

Table 4. Description of the MS parameters

Ion source parameters	
Source type	High Electrospray Source Ionization (H-ESI)
Polarity	Positive
Spray voltage - Positive (V)	3,500
Sheath gas (Arb)	50
Aux gas (Arb)	10
Sweep gas (Arb)	0
Ion transfer tube temp. (°C)	300
Vaporizer temp. (°C)	320
Settings	
Mild trapping	No
Data acquisition mode	Experiment 1: Full scan - $ddMS^2$ Experiment 2: tMS^2 using a mass list inclusion
Internal mass calibration	RunStart Easy-IC™
Full scan parameters	
Orbitrap resolution (@ *m/z* 200)	60,000
Scan range	250–350
RF lens (%)	70
AGC target	Standard
Maximum injection time mode	Auto
Data-dependent MS^2 scan properties	
Orbitrap resolution (@ *m/z* 200)	15,000
Isolation window (*m/z*)	2
Collision energy type	Normalized
HCD collision energy (%)	30
Scan range mode	Auto
Targeted MS^2 scan properties	
Orbitrap resolution (@ *m/z* 200)	30,000
Isolation window (*m/z*)	2.0
Collision energy type	Normalized
HCD collision energy (%)	30

Method evaluation

The method performance was evaluated in terms of linearity of response within the calibration ranges, lower limit of quantification (LLOQ), carryover, accuracy, and intra- and inter-assay precision for all the analytes. A 20-fold serial dilution of the lowest calibrator using blank matrix was performed to evaluate the LLOQ. A full set of calibrators (four levels), diluted calibrators (three levels), and controls (two levels) were extracted and injected in a single batch and all used for the linear interpolation. The LLOQ was set as the lowest level that could be determined with a CV <20% across the entire batch of samples.

Carryover was calculated in terms of percentage ratio between peak area of the highest calibrator and a blank sample injected just after it. Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using the quality control samples at two different levels provided by RECIPE prepared and analyzed in replicates of five on three different days. Intra-assay precision for each day was evaluated in terms of percentage coefficient of variation (%CV) using the controls at two different levels in replicates of five (n=5). Inter-assay precision was evaluated as the %CV on the full set of samples (control samples at two levels in replicates of five prepared and analyzed on three different days).

Data analysis

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

Results and discussion

A linear response with 1/× weighting was obtained for all the analytes not only in the calibration range covered by the calibrators but also down to the LLOQ (Table 5). The percentage bias between nominal and back-calculated concentration was always within ±15% for all the calibrators (±20% for the lowest calibrator) in all the runs. Representative chromatograms at the LLOQ for clomipramine, nortrimipramine, imipramine, trimipramine and the corresponding internal standards are depicted in Figure 1. Representative calibration curves for the same analytes in the concentration range covered by the kit (three calibrators) are shown in Figure 2.

No carryover was observed, with no signal detected in the blank injected after the highest calibrator.

Good accuracy was obtained from the evaluation of this method with a percentage bias between nominal and average back-calculated concentration for the used control samples ranging between -2.0% and 11.9% (Table 6). Excellent results were also obtained in terms of reproducibility, with a maximum %CV always below 7.1% and 4.2% for intra- and inter-assay precision, respectively (Table 7).

Table 5. LLOQs for all compounds

Analyte	LLOQ (µg/L)
Amitriptyline	3.26
Clomipramine	18.3
Clozapine	5.85
Desipramine	1.81
Doxepin	14.8
Imipramine	3.34
Maprotiline	24.2
Norclomipramine	21.1
Norclozapine	9.46
Nordoxepin	0.680
Normaprotiline	3.34
Nortrimipramine	2.20
Nortriptyline	1.82
Protriptyline	3.38
Trimipramine	2.73

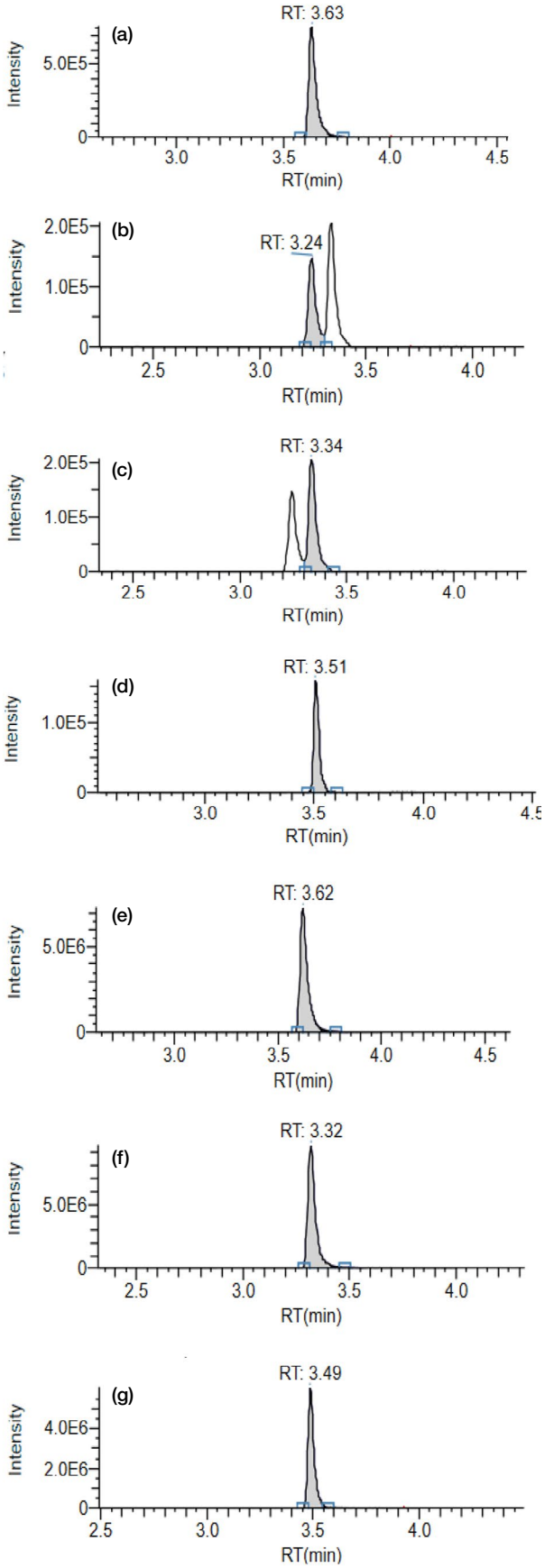

Figure 1. Representative chromatograms of the lower limit of quantification for (a) clomipramine, (b) nortrimipramine, (c) imipramine, (d) trimipramine, (e) d_3-clomipramine, (f) d_5-imipramine, (g) d_3-trimipramine, obtained with the exact mass and a precision of 5 ppm

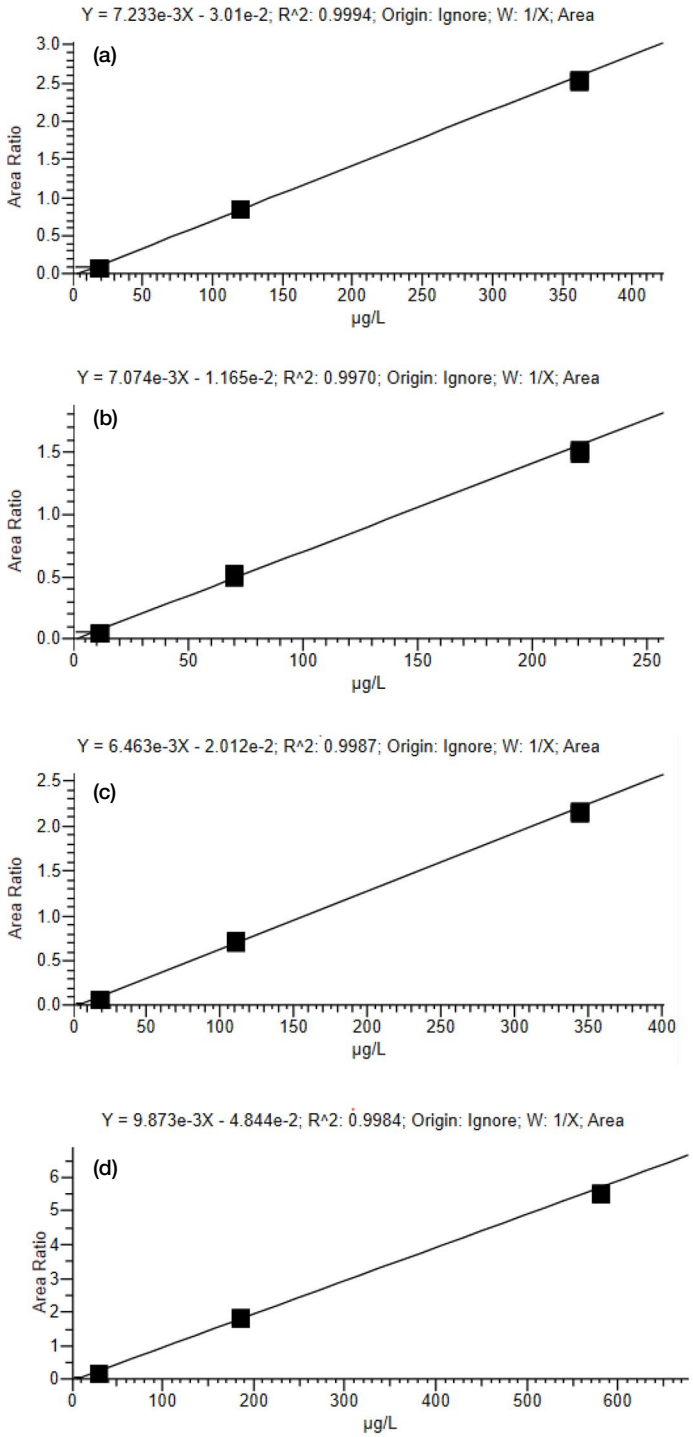

Figure 2. Representative calibration curves for (a) clomipramine, (b) nortrimipramine, (c) imipramine, (d) trimipramine

Table 6. Analytical accuracy results for control MS9182 batch #1389

Analyte	Control	Nominal conc. (µg/L)	Average calculated conc. (µg/L)	Bias (%)
Amitriptyline	Level I	59.1	60.5	2.4
	Level II	137	146	6.7
Clomipramine	Level I	67.3	69.2	2.8
	Level II	161	164	1.7
Clozapine	Level I	229	225	-1.5
	Level II	529	546	3.2
Desipramine	Level I	68.1	68.9	1.2
	Level II	158	167	5.8
Doxepin	Level I	53.3	54.6	2.4
	Level II	123	130	5.3
Imipramine	Level I	62.2	63.3	1.7
	Level II	143	152	6.1
Maprotiline	Level I	86.6	87.2	0.7
	Level II	199	207	4.2
Norclomipramine	Level I	78.7	78.3	-0.5
	Level II	180	186	3.3
Norclozapine	Level I	183	179	-2.0
	Level II	413	427	3.3
Nordoxepin	Level I	50.6	51.5	1.8
	Level II	118	125	5.5
Normaprotiline	Level I	129	135	4.4
	Level II	297	326	9.8
Nortrimipramine	Level I	40.4	41.9	3.8
	Level II	90.8	102	11.9
Nortriptyline	Level I	69.1	68.4	-1.1
	Level II	160	166	3.5
Protriptyline	Level I	61.8	62.0	0.4
	Level II	146	153	4.9
Trimipramine	Level I	102	108	5.6
	Level II	240	260	8.1

Table 7. Analytical intra- and inter-assay precision results for control MS9182 batch #1389

Analyte	Control	Intra-assay						Inter-assay	
		Day 1		Day 2		Day 3			
		Average calculated concentration (µg/L)	CV (%)	Average calculated concentration (µg/L)	CV (%)	Average calculated concentration (µg/L)	CV (%)	Average calculated concentration (µg/L)	CV (%)
Amitriptyline	Level I	61.4	3.8	59.1	5.7	61.1	4.9	60.5	2.1
	Level II	145	2.2	147	2.5	147	2.5	146	0.5
Clomipramine	Level I	69.7	3.8	67.1	6.6	70.8	5.0	69.2	2.7
	Level II	164	2.8	164	2.5	163	2.9	164	0.3
Clozapine	Level I	229	4.1	222	3.8	226	4.7	226	1.6
	Level II	549	2.3	547	2.4	542	2.5	546	0.6
Desipramine	Level I	69.6	3.7	67.9	4.2	69.2	4.6	68.9	1.3
	Level II	168	2.7	168	2.9	166	3.0	167	0.8
Doxepin	Level I	54.9	3.7	53.0	4.6	55.8	4.9	54.6	2.6
	Level II	130	2.4	129	2.5	129	2.7	130	0.4
Imipramine	Level I	64.4	4.1	61.8	5.4	63.7	5.5	63.3	2.1
	Level II	152	2.5	152	2.3	152	2.6	152	0.1
Maprotiline	Level I	87.5	3.7	85.1	4.4	89.1	4.2	87.2	2.3
	Level II	208	2.3	208	2.0	207	2.9	207	0.2
Norclomipramine	Level I	78.7	3.6	76.3	4.6	79.9	4.7	78.3	2.3
	Level II	186	2.2	187	1.9	185	2.6	186	0.5
Norclozapine	Level I	180	2.9	175	2.6	184	3.9	179	2.5
	Level II	427	3.9	428	3.1	425	2.9	427	0.4
Nordoxepin	Level I	52.1	3.9	50.6	4.4	51.9	4.6	51.5	1.5
	Level II	125	2.5	125	2.4	124	2.7	125	0.4
Normaprotiline	Level I	137	2.6	132	5.5	136	4.0	135	2.1
	Level II	329	2.9	330	3.1	320	3.8	326	1.8
Nortrimipramine	Level I	41.4	2.7	41.2	6.0	43.2	4.5	41.9	2.6
	Level II	101	3.5	103	3.1	101	2.7	102	1.3
Nortriptyline	Level I	69.2	3.4	67.2	4.5	68.7	4.7	68.4	1.5
	Level II	166	2.4	166	2.2	165	2.8	166	0.3
Protriptyline	Level I	63.0	2.8	60.1	5.8	62.9	4.8	62.0	2.6
	Level II	154	2.2	155	3.5	152	2.8	153	1.1
Trimipramine	Level I	113	3.5	104	7.1	107	6.3	108	4.2
	Level II	261	3.2	261	2.8	256	3.1	260	1.1

Conclusions

The reported LC-HRAM method, based on a Vanquish Flex Binary UHPLC system connected to an Orbitrap Exploris 120 mass spectrometer, demonstrates the power of Orbitrap technology in performing accurate qualitative analyses and routine quantitation with high efficiency. A liquid chromatography-HRAM method for clinical research was developed and implemented for quantification of 15 different tricyclic antidepressants in human plasma or serum. The ClinMass TDM Platform with the ClinMass Add-On Set for Tricyclic Antidepressants from RECIPE was used. The method incorporates a quick and simple offline protein precipitation step 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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