TECHNICAL NOTE 73330

Quantification of 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 enables increased confidence in data
- Simple offline sample preparation by protein precipitation
- 13 tricyclic antidepressants in a single quantitative method

Goal

Implementation of an analytical method for the quantification of 13 different tricyclic antidepressants in human plasma or serum on a Thermo Scientific™ Q Exactive™ Plus hybrid quadrupole-Orbitrap™ mass spectrometer.

Introduction

Tricyclic antidepressants (TCAs) are commonly used to treat depression, anxiety reactions, and neuropathic pain, despite the risk of severe side effects. Monitoring serum concentrations of TCAs can assess suspected

toxicity, and/or drug-drug interactions. High-pressure liquid chromatography (HPLC) with ultraviolet (UV) spectrophotometric detection has been the recommended and most commonly used method for analyzing 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 the analysis of TCA.

An analytical method for clinical research for the quantification of 13 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, nortrimipramine, nortriptyline, and trimipramine. While most reported LC-MS analyses of these TCAs involve triple quadrupole mass spectrometers, traditionally used for targeted, sensitive quantitation assays, in this report we present LC-MS data acquired using high-resolution accurate-mass (HRAM) spectrometry leveraging Orbitrap technology. This report demonstrates the capability of HRAM for routine quantitation 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™ Duo UHPLC system connected to a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer with heated electrospray ionization. Detection was performed by full scan coupled to data-dependent fragmentation (fullMS-ddMS^2) using 12 deuterated internal standards. 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, carryover, accuracy, and intra- and inter-assay precision for each analyte.

Experimental

Target analytes

The concentration ranges covered by the calibrators (MS9113 batch #1308) used are reported in Table 1.

Sample preparation

Reagents included four calibrators (including blank) and two controls from RECIPE (MS9182 batch #1456), 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. Concentration ranges covered by calibrators

Analyte	Internal standard	Concentration range (ng/mL)
Amitriptyline	d_3-amitriptyline	15.6–316
Clomipramine	d_3-clomipramine	19.6–407
Clozapine	d_4-clozapine	60.4–1274
Desipramine	d_3-desipramine	17.2–363
Doxepin	d_3-doxepin	13.9–274
Imipramine	d_3-imipramine	17.0–362
Maprotiline	d_5-maprotiline	22.2–442
Norclomipramine	d_3-norclomipramine	22.4–458
Norclozapine	d_8-norclozapine	46.4–952
Nordoxepin	d_3-nordoxepin	14.1–301
Nortrimipramine	d_3-imipramine	12.7–253
Nortriptyline	d_3-nortriptyline	17.1–357
Trimipramine	d_3-trimipramine	17.0–360

Liquid chromatography

A Vanquish Duo UHPLC system, a dual-channel instrument configured for both LC-only and online SPE applications (Figure 1), was used for chromatographic separation. The LC-only channel was used in this case, utilizing mobile phases and analytical column provided by RECIPE. Details of the analytical method are reported in Table 2. Total runtime was 4.0 minutes.

Table 2. Liquid chromatography method description

Gradient profile			
Time (min)	**Flow rate (mL/min)**	**A (%)**	**B (%)**
0.00	0.8	85	15
0.05	0.8	85	15
0.06	0.8	70	30
2.10	0.8	70	30
2.11	0.8	62	38
2.70	0.8	62	38
3.00	0.8	25	75
3.35	0.8	25	75
3.40	0.8	85	15
4.00	0.8	85	15
Other parameters			
Injection volume (µL)		5	
Column temperature (°C)		40	

Mass spectrometry

Analytes and internal standards were detected by FullMS-ddMS^2 mode on a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer with heated electrospray Ionization (H-ESI II) operated in positive ion mode. A summary of the MS conditions is reported in Table 3.

Table 3. MS settings

Parameter	Value
Source type	Heated electrospray ionization (H-ESI II)
Vaporizer temperature	450 °C
Capillary temperature	350 °C
Spray voltage (positive mode)	3500 V
Sheath gas	50 AU
Sweep gas	0 AU
Auxiliary gas	15 AU
S-Lens RF level	60
Data acquisition mode	FullMS-ddMS^2
FullMS resolution @ *m/z* 200	70,000
FullMS scan range	250–350 *m/z*
ddMS^2 resolution @ *m/z* 200	17,500
ddMS^2 isolation window	4.0 *m/z*
Stepped Normalized Collision Energy (NCE)	15, 25, 35

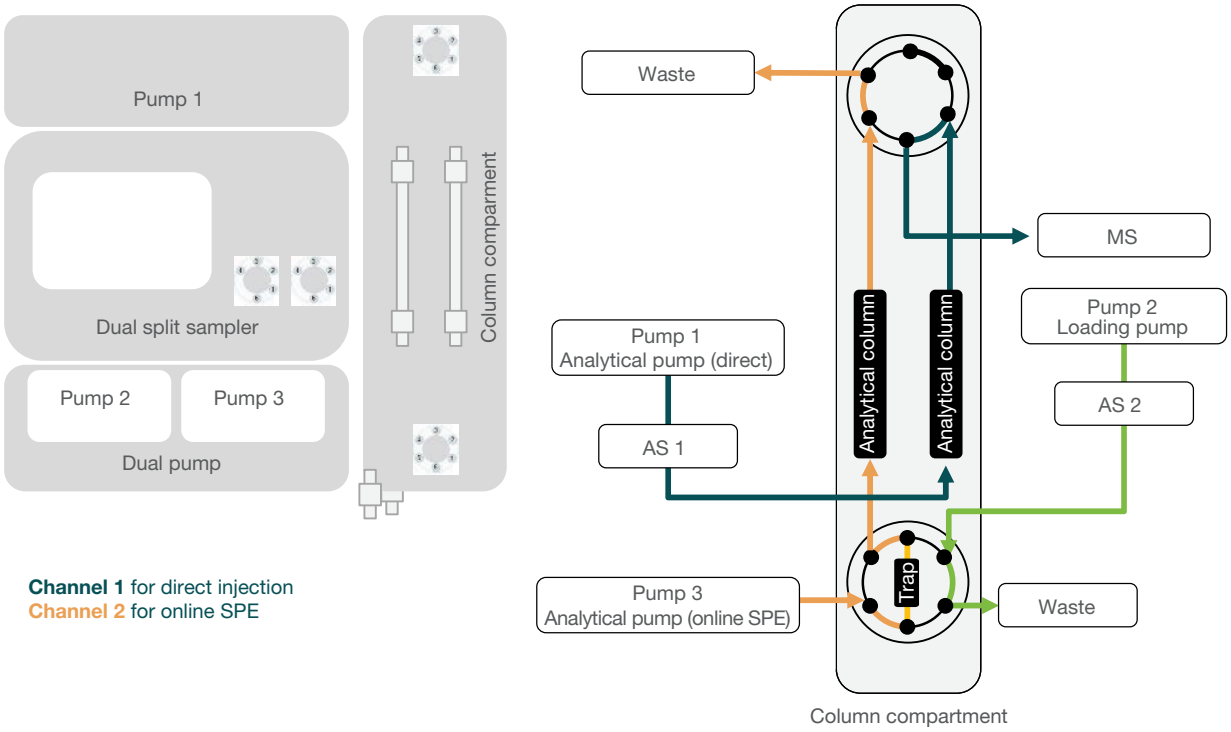

Figure 1. Schematic representation of the Vanquish Duo UHPLC system setup

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. To determine the LLOQ, the lowest calibrator was diluted down to 20-fold with blank matrix; a full set of calibrators (three levels), diluted calibrators (four levels), and controls (two levels) were extracted in replicates of five (n=5), 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™ 4.1 software. FullMS data were used for quantification, and ddMS^2 fragments were used for confirmation based on ion ratio.

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 a lower limit of quantification 20-fold lower than the lowest calibrator (10-fold for doxepin). 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 for the LLOQ for clozapine, maprotiline, and the corresponding internal standards are depicted in Figure 2. Representative calibration curves for the same analytes in the concentration range covered by the kit (three calibrators) are shown in Figure 3. A maximum carryover of 0.09% was reported for clozapine.

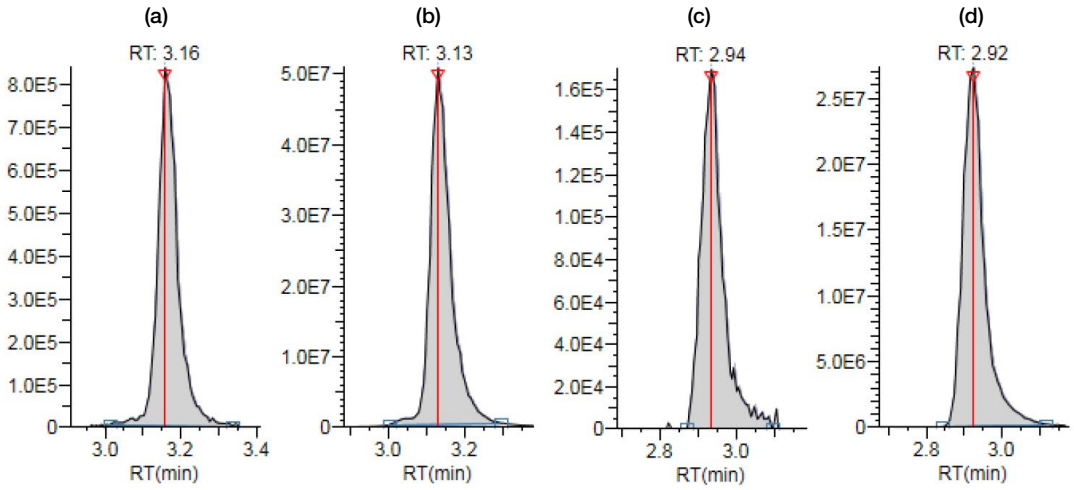

Figure 2. Representative chromatograms of the LLOQ for (a) clozapine, (b) d_4-clozapine, (c) maprotiline, and (d) d_5-maprotiline

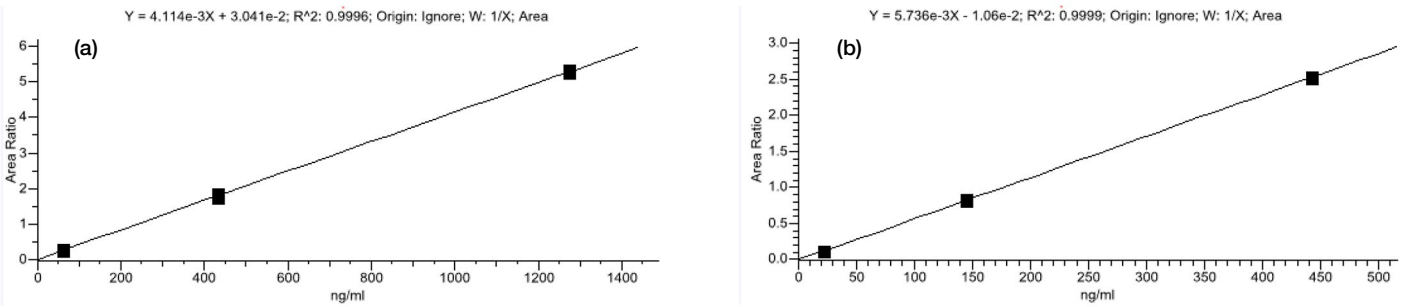

Figure 3. Representative calibration curves for (a) clozapine and (b) maprotiline – day 1

The data presented in this report demonstrate the outstanding accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the used control samples ranging between -3.9% and 9.1% (Table 4). The %CV for intra-assay precision was always below 12.1%. The maximum %CV for inter-assay precision was 6.9%. Results for intra- and inter-assay precision are reported in Table 5 and Table 6, respectively.

Table 4. Analytical accuracy results for control MS9182 batch #1456

Analyte	Control 1			Control 2		
	Nominal concentration (ng/mL)	Average calculated concentration (ng/mL)	Bias (%)	Nominal concentration (ng/mL)	Average calculated concentration (ng/mL)	Bias (%)
Amitriptyline	59.2	57.9	-2.2	135	134	-0.7
Clomipramine	73.9	75.4	2.0	171	172	0.8
Clozapine	73.9	75.4	2.0	171	172	0.8
Desipramine	64.3	65.7	2.2	152	153	0.7
Doxepin	50.8	51.8	2.0	117	119	2.1
Imipramine	63.9	64.7	1.3	148	150	1.1
Maprotiline	82.7	84.4	2.0	193	195	0.9
Norclomipramine	79.9	82.3	3.0	187	193	2.9
Norclozapine	179	193.4	8.0	418	437	4.6
Nordoxepin	49.4	51.5	4.3	116	120	3.7
Normaprotiline	55.1	60.1	9.1	133	135	1.1
Nortrimipramine	64.5	65.7	1.9	145	153	5.4
Nortriptyline	64.2	67.1	4.5	155	149	-3.9

Table 5. Intra-assay precision results for control MS9182 batch #1456

Analyte	Control 1						Control 2					
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3	
	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)
Amitriptyline	60.6	1.5	57.6	1.7	55.5	0.8	134	1.6	131	1.5	137	2.2
Clomipramine	77.2	5.2	75.4	2.5	73.6	2.3	172	2.9	173	2.9	172	2.2
Clozapine	77.2	5.2	75.4	2.5	73.6	2.3	172	2.9	173	2.9	172	2.2
Desipramine	67.5	0.7	65.6	0.6	64.1	0.9	154	2.3	152	1.0	154	1.3
Doxepin	53.2	0.3	51.5	2.2	50.7	0.6	120	1.0	118	1.7	120	1.5
Imipramine	65.2	0.4	65.4	1.1	63.5	1.4	147	1.4	151	1.1	151	1.2
Maprotiline	85.8	0.3	84.0	1.7	83.4	0.8	194	1.1	192	0.9	197	2.1
Norclomipramine	83.8	0.8	82.6	1.5	80.6	0.9	192	0.6	191	1.3	195	1.5
Norclozapine	194.3	1.0	195.5	1.1	190.4	2.0	435	1.2	438	0.9	438	1.4
Nordoxepin	52.2	0.5	51.5	0.7	50.8	0.7	120	1.1	120	1.2	121	1.6
Normaprotiline	61.9	1.6	59.9	2.2	58.5	2.0	135	2.9	134	2.3	135	2.9
Nortrimipramine	66.9	0.8	66.0	1.1	64.2	1.3	152	0.8	153	0.9	154	1.5
Nortriptyline	68.5	4.8	65.5	8.3	67.2	4.7	150	3.6	149	2.6	147	12.1

Table 6. Inter-assay precision results for control MS9182 batch #1456

Analyte	Control 1		Control 2	
	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)
Amitriptyline	57.9	4.0	134	2.5
Clomipramine	75.4	3.9	172	2.5
Clozapine	75.4	3.9	172	2.5
Desipramine	65.7	2.3	153	1.6
Doxepin	51.8	2.4	119	1.6
Imipramine	64.7	1.7	150	1.9
Maprotiline	84.4	1.6	195	1.7
Norclomipramine	82.3	2.0	193	1.4
Norclozapine	193.4	1.8	437	1.2
Nordoxepin	51.5	1.3	120	1.3
Normaprotiline	60.1	3.0	135	2.6
Nortrimipramine	65.7	2.0	153	1.1
Nortriptyline	67.1	6.0	149	6.9

Conclusions

An HRAM-based method (a Vanquish Duo UHPLC system connected to a Q Exactive Plus hybrid quadrupole-Orbitrap MS) is reported here demonstrating 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 13 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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