TECHNICAL NOTE 73480

Quantification of 36 antidepressants in human plasma or serum by LC-HRAM(MS) for clinical research

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Application benefits

- High accuracy with use 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
- Multiple analytes in a single quantitative method

Goal

Implementation of an analytical method for the quantification of 36 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. However, next generation antidepressants (ADPs), such as selective serotonin and norepinephrine reuptake inhibitors (SSRIs, SNRIs, NRIs), are available for the treatment of depression and result in fewer side effects.

An analytical method for clinical research for the quantification of 36 antidepressants in human plasma or serum is reported in this study. A comprehensive list of analytes, corresponding internal standards, and concentration ranges covered by the calibrators is reported in Table 1. While most reported LC-MS analyses of the

above-mentioned antidepressants 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.

Table 1. Analytes, internal standards, and concentration ranges

Analyte	Internal standard	Concentration range (ng/mL)
Agomelatine	d_3-agomelatine	5.09–727
Atomoxetine	d_3-atomoxetine	151–2190
Bupropion	d_9-bupropion	11.6–157
Citalopram	d_6-citalopram	16.5–259
Clomethiazole	d_9-dihydrobupropion	146–6773
Desmethylcitalopram	d_3-desmethylcitalopram	18.5–279
Desmethylfluoxetine	d_5-desmethylfluoxetine	42.7–656
Desmethylmianserine	d_5-reboxetine	11.8–167
Desmethylmirtazapine	d_3-mirtazapine	13.2–197
Desmethylsertraline	d_4-desmethylsertraline	12.4–191
Dihydrobupropion	d_9-dihydrobupropion	105–1568
Dosulepin	d_3-dosulepin	16.8–244
Duloxetine	d_7-duloxetine	18.5–284
Fluoxetine	d_5-fluoxetine	37.8–595
Fluvoxamine	d_3-fluvoxamine	34.9–558
Guanfacine	d_6-tramadol	0.911–15
Hydroxybupropion	d_6-hydroxybupropion	145–2045
Methylphenidate	d_9-methylphenidate	3.8–51.8
Mianserine	d_3-mianserine	10.3–168
Milnacipran	d_{10}-milnacipran	29.2–435
Mirtazapine	d_3-mirtazapine	12–184
Moclobemide	d_8-moclobemide	156–2250
Nefazodone	d_6-nefazodone	34.9–491
O-Desmethyltramadol	d_6-O-desmethyltramadol	83.8–1186
O-Desmethylvenlafaxine	d_{10}-milnacipran	37.4–554
Opipramol	d_4-opipramol	41.5–611
Paroxetine	d_4-paroxetine	19–299
Reboxetine	d_5-reboxetine	48–753
Ritalinic acid	d_6-O-desmethyltramadol	24.6–372
Sertraline	d_3-sertraline	4.62–310
Tianeptine	d_{10}-milnacipran	10.2–163
Tramadol	d_6-tramadol	84.5–1138
Tranylcypromine	d_5-tranylcypromine	7.2–108
Trazodone	d_4-opipramol	161–2752
Venlafaxine	d_6-venlafaxine	22.9–369
Vortioxetine	d_8-vortioxetine	8.96–119

Plasma or serum samples were extracted by protein precipitation and offline addition of internal standard. Extracted samples were injected onto a Thermo Scientific™ Vanquish™ Flex Duo UHPLC system connected to a Q Exactive Plus mass spectrometer with heated electrospray ionization (H-ESI II). Detection was performed by full scan MS coupled to data-dependent fragmentation (fullMS-ddMS^2) using 28 deuterated internal standards. The full scan data were used for quantification while fragmentation data was used for confirmation. Method performance was evaluated using the ClinMass® TDM Platform with the ClinMass® Add-On Set for Antidepressants from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of lower limit of quantification (LLOQ), 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 (MS9413 batch #1129) used are reported in Table 1.

Sample preparation

Reagents included four calibrators (including blank) and two controls from RECIPE (MS9482 batch #1407), as well as 28 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.

Liquid chromatography

A Vanquish Flex Duo UHPLC 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 3.7 minutes.

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 operated in positive ion mode. A summary of the MS conditions is reported in Table 3.

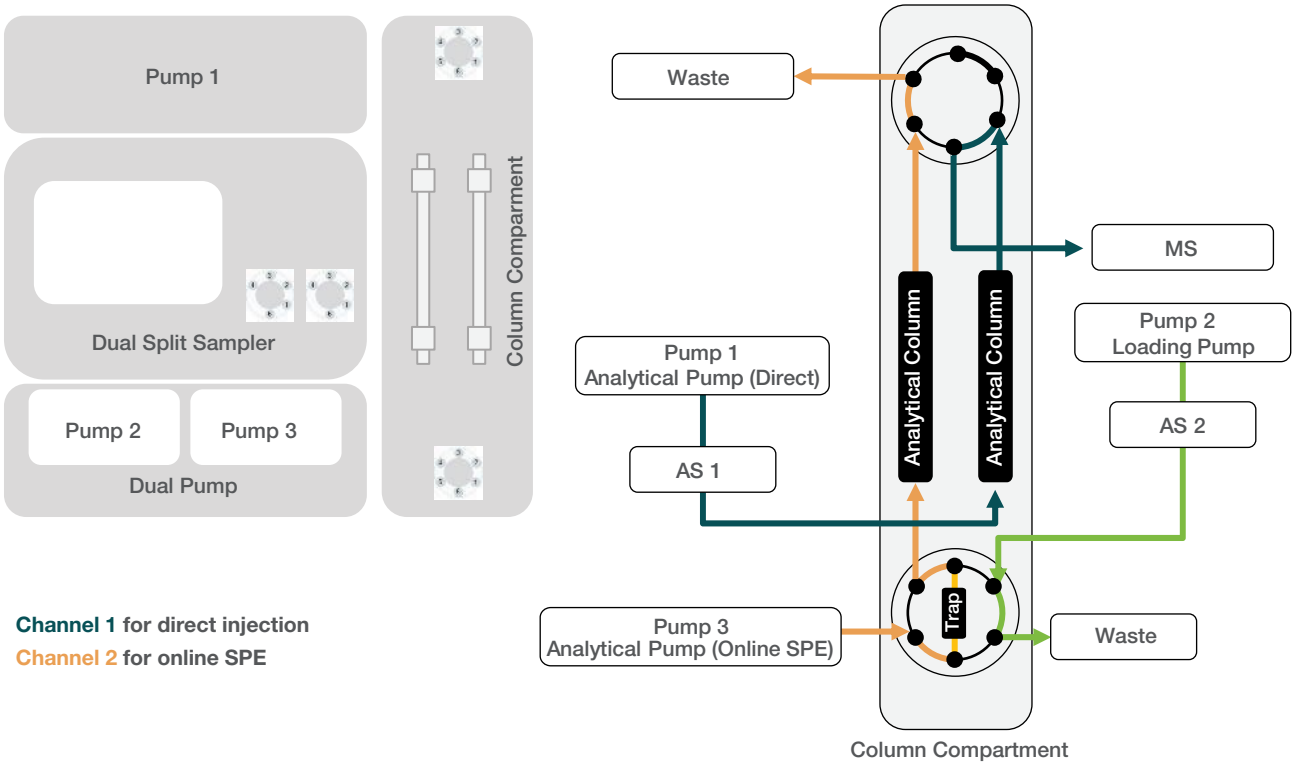

Figure 1. Schematic representation of the Vanquish Duo UHPLC system setup

Table 2. Liquid chromatography method description

Gradient profile			
Time (min)	Flow rate (mL/min)	A (%)	B (%)
0.00	0.7	95	5
0.01	0.7	95	5
0.20	0.7	75	25
1.50	0.7	50	50
2.50	0.7	45	55
2.60	0.7	20	80
3.00	0.7	20	80
3.10	0.7	95	5
3.70	0.7	95	5
Other parameters			
Injection volume (µL)		2	
Column temperature (°C)		40	

Table 3. Mass spectrometer source and scan settings

Parameter	Value
Source type	Heated electrospray ionization (H-ESI II)
Vaporizer temperature	460 °C
Capillary temperature	280 °C
Spray voltage (positive mode)	3000 V
Sheath gas	58 AU
Sweep gas	0 AU
Auxiliary gas	16 AU
S-Lens RF level	60
Data acquisition mode	FullMS-ddMS^2
FullMS resolution @ *m/z* 200	70,000
FullMS scan range	120–490 *m/z*
ddMS^2 resolution @ *m/z* 200	17,500
ddMS^2 isolation window	2.0 *m/z*
Stepped Normalized Collision Energy (NCE)	15, 25, 35

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 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.

Trueness of measurement was also evaluated as percentage bias using certified external quality controls (GTFCh - TDMD 1/19 - Probe A and B, GTFCh - TCA 3/19 - Probe A and B and INSTAND 879 1/19 - Probe 11 and 12) prepared and analyzed in replicates of five on a single day.

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.

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 LLOQs reported in Table 4. The percentage bias between nominal and back-calculated concentration was always within ±10% for all the calibrators in all the runs. Representative chromatograms for the LLOQ for atomoxetine, fluvoxamine, 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.

No significant carryover was registered; no peak was detected in the blank sample following the highest calibrator.

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 -7.4% and 5.5% (Table 5).

Excellent results were obtained also from the evaluation of trueness of measurement, with a percentage bias between -8.6% and 13.2% (Table 6 and Table 7).

The %CV for intra-assay precision was always below 9.1%. The maximum %CV for inter-assay precision was 6.9%. Results for intra- and inter-assay precision reported in Table 8 and Table 9, respectively.

Table 4. Analytes and corresponding LLOQ

Analyte	LLOQ (ng/mL)
Agomelatine	0.51
Atomoxetine	7.6
Bupropion	1.2
Citalopram	0.83
Clomethiazole	73
Desmethylcitalopram	0.93
Desmethylfluoxetine	2.1
Desmethylmianserine	0.59
Desmethylmirtazapine	1.3
Desmethylsertraline	12.4
Dihydrobupropion	5.3
Dosulepin	0.84
Duloxetine	0.93
Fluoxetine	1.9
Fluvoxamine	1.7
Guanfacine	0.91
Hydroxybupropion	7.3
Methylphenidate	0.38
Mianserine	0.52
Milnacipran	1.5
Mirtazapine	0.6
Moclobemide	78
Nefazodone	1.7
O-Desmethyltramadol	4.2
O-Desmethylvenlafaxine	3.7
Opipramol	2.1
Paroxetine	0.95
Reboxetine	2.4
Ritalinic acid	12
Sertraline	0.46
Tianeptine	0.51
Tramadol	4.2
Tranylcypromine	0.36
Trazodone	161
Venlafaxine	1.1
Vortioxetine	4.5

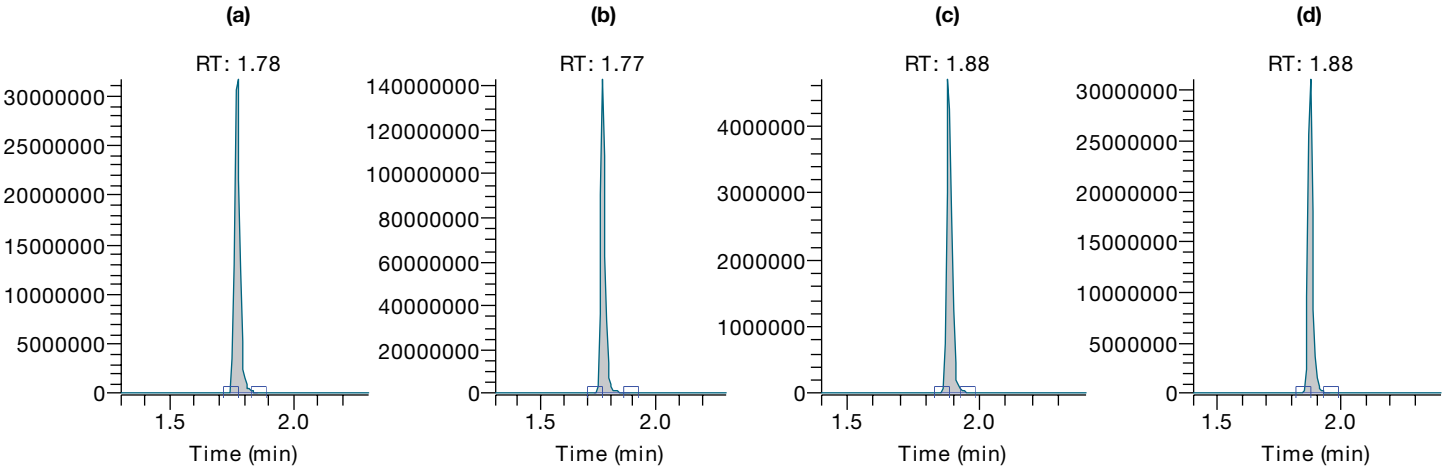

Figure 2. Representative chromatograms of the LLOQ for (a) atomoxetine, (b) d_3-atomoxetime, (c) fluvoxamine, and (d) d_3-fluvoxamine

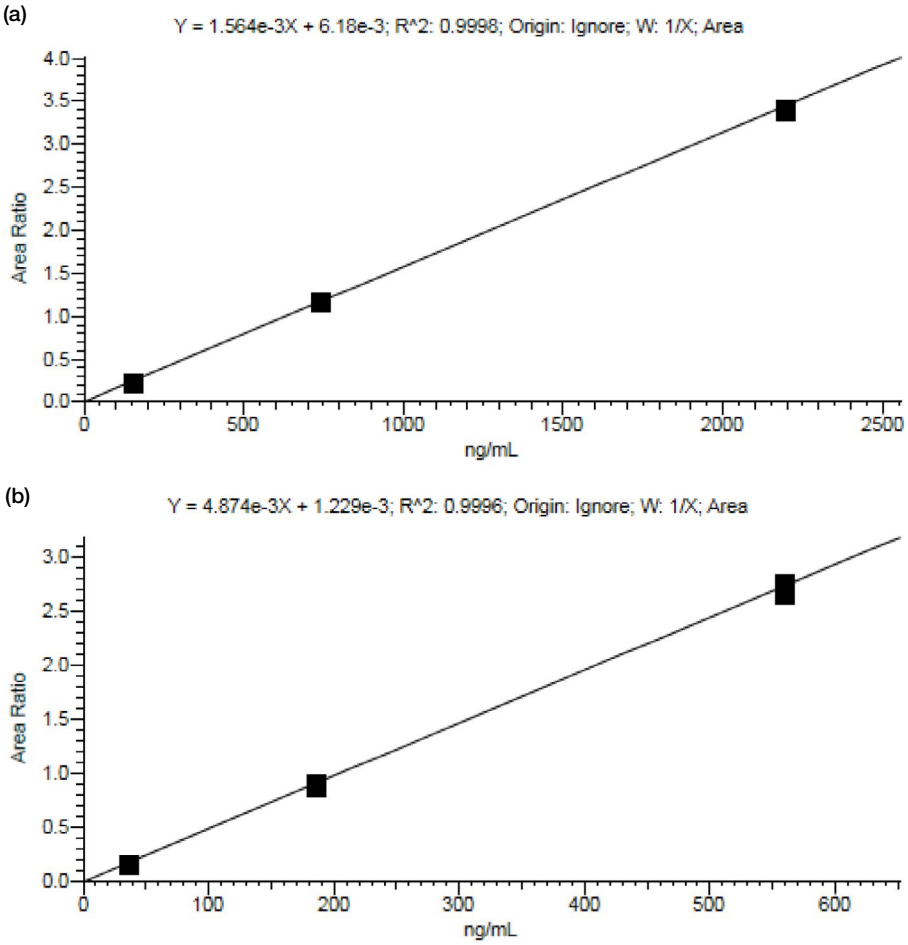

Figure 3. Representative calibration curves for (a) atomoxetine and (b) fluvoxamine – day 2

Table 5. Analytical accuracy results for controls MS9482 batch #1407

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 (%)
Agomelatine	26.9	25.5	-5.2	290	279	-3.7
Atomoxetine	407	404	-0.8	952	947	-0.5
Bupropion	35.3	34.8	-1.4	78.7	80.7	2.5
Citalopram	49.0	50.4	2.8	116	120	3.7
Clomethiazole	518	528	1.9	2828	2951	4.4
Desmethylcitalopram	24.7	25.7	3.9	58.9	60.8	3.3
Desmethylfluoxetine	118	116	-1.8	275	276	0.5
Desmethylmianserine	30.0	28.1	-6.4	67.6	66.4	-1.7
Desmethylmirtazapine	34.9	35.7	2.2	80.6	84.3	4.6
Desmethylsertraline	38.7	35.8	-7.4	91.7	85.3	-7.0
Dihydrobupropion	279	294	5.5	662	689	4.0
Dosulepin	44.6	43.9	-1.5	102	103	1.0
Duloxetine	50.3	49.4	-1.7	115	120	4.1
Fluoxetine	105	105	0.2	249	251	0.8
Fluvoxamine	101	101	-0.3	244	243	-0.6
Guanfacine	2.52	2.52	0.1	5.97	6.02	0.8
Hydroxybupropion	398	408	2.4	903	924	2.3
Methylphenidate	11.1	11.5	3.7	25.3	26.5	4.6
Mianserine	29.3	29.5	0.6	69.3	70.7	2.1
Milnacipran	77.4	77.9	0.7	180	188	4.3
Mirtazapine	33.9	33.1	-2.3	79.0	78.2	-1.0
Moclobemide	474	478	0.9	1089	1080	-0.8
Nefazodone	103	100	-3.0	232	227	-2.0
O-Desmethyltramadol	227	219	-3.3	518	507	-2.2
O-Desmethylvenlafaxine	104	106	2.0	239	246	3.0
Opipramol	108	109	0.5	257	253	-1.4
Paroxetine	50.6	50.6	0.0	121	122	0.6
Reboxetine	134	135	0.6	318	319	0.4
Ritalinic acid	63.2	62.8	-0.7	151	149	-1.3
Sertraline	28.9	28.6	-0.9	145	150	3.6
Tianeptine	31.7	31.0	-2.1	73.4	74.7	1.8
Tramadol	231	220	-4.9	501	500	-0.3
Tranylcypromine	19.6	19.5	-0.4	46.9	47.6	1.5
Trazodone	548	554	1.0	1224	1259	2.8
Venlafaxine	60.9	60.7	-0.3	145	147	1.1
Vortioxetine	22.4	22.4	0.1	51.1	51.3	0.5

Table 6. Analytical accuracy results for controls GTFCh - TDMD 1/19 and TCA 3/19

Analyte	TDMD 1/19 - Probe A			TDMD 1/19 - Probe B			TCA 3/19 - Probe A			TCA 3/19 - Probe B		
	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL	Bias (%)
Citalopram	74.5	82.5	9.7	155	177	12.6	N/A	N/A	N/A	N/A	N/A	N/A
Desmethylfluoxetine	63.3	70.2	9.8	93.1	107	13.2	N/A	N/A	N/A	N/A	N/A	N/A
Duloxetine	50.3	55.5	9.4	95.4	110	13.0	N/A	N/A	N/A	N/A	N/A	N/A
Fluoxetine	119	130	8.5	322	370	13.0	N/A	N/A	N/A	N/A	N/A	N/A
Fluvoxamine	77.6	80.4	3.5	155	172	9.7	N/A	N/A	N/A	N/A	N/A	N/A
Mianserine	33.2	36.1	7.9	58.4	66.6	12.4	N/A	N/A	N/A	N/A	N/A	N/A
Mirtazapine	46.5	46.3	-0.3	55.8	57.7	3.2	N/A	N/A	N/A	N/A	N/A	N/A
O-Desmethylvenlafaxine	50.5	51.3	1.6	131	147	10.6	N/A	N/A	N/A	N/A	N/A	N/A
Opipramol	N/A	N/A	N/A	N/A	N/A	N/A	79.6	73.3	-8.6	224	233	4.0
Paroxetine	55.0	58.5	6.0	103	118	12.4	N/A	N/A	N/A	N/A	N/A	N/A
Reboxetine	92.5	95.7	3.3	275	290	5.2	N/A	N/A	N/A	N/A	N/A	N/A
Sertraline	45.9	49.2	6.6	130	144	9.4	N/A	N/A	N/A	N/A	N/A	N/A
Venlafaxine	71.4	73.6	3.1	211	235	10.2	N/A	N/A	N/A	N/A	N/A	N/A

Table 7. Analytical accuracy results for controls INSTAND 879 1/19

Analyte	Probe 11			Probe 12		
	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)
Atomoxetine	1146	1237	7.4	1191	1269	6.1
Methylphenidate	10.2	10.9	6.1	16.2	17.3	6.1
Ritalinic acid	113	109	-4.0	94.4	89.5	-5.4

Table 8. Intra-assay precision results for control MS9482 batch #1407

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 (%)
Agomelatine	25.3	1.4	25.2	2.5	26.1	4.5	280	2.5	281	1.1	277	2.3
Atomoxetine	404	1.2	397	1.2	410	0.5	951	1.5	944	0.4	946	1.0
Bupropion	34.2	2.4	35.5	1.8	34.7	3.1	79.4	2.3	81.9	2.0	80.7	0.5
Citalopram	50.6	3.8	50.1	3.1	50.4	4.8	119	2.6	118	1.5	123	2.1
Clomethiazole	526	2.6	533	2.3	525	2.9	2918	2.0	2921	2.3	3014	2.8
Desmethylcitalopram	25.8	0.7	25.6	1.0	25.6	0.5	60.8	1.5	60.9	0.7	60.8	0.9
Desmethylfluoxetine	116	1.2	115	1.8	116	1.2	275	1.3	278	1.3	276	1.5
Desmethylmianserine	28.0	1.3	28.0	1.0	28.2	1.5	64.6	1.8	68.0	3.6	66.7	4.0
Desmethylmirtazapine	36.3	2.6	34.4	1.2	36.3	3.4	85.5	4.9	83.6	3.3	83.8	3.3
Desmethylsertraline	35.6	2.1	35.8	1.1	36.1	2.2	84.3	1.2	85.8	1.5	85.7	1.9
Dihydrobupropion	295	1.7	291	2.1	296	1.7	696	2.2	682	2.7	688	0.9
Dosulepin	43.9	2.9	43.6	4.5	44.4	3.0	102	3.7	102	2.9	105	0.9
Duloxetine	47.9	1.6	49.9	2.5	50.5	2.9	119	2.0	119	1.7	121	2.0
Fluoxetine	105	1.4	105	1.5	106	1.2	251	1.8	248	1.9	254	2.0
Fluvoxamine	98.4	1.7	101	2.2	103	3.0	239	2.7	241	2.2	248	1.7
Guanfacine	2.60	5.0	2.53	9.1	2.44	5.9	6.03	4.8	6.04	6.1	5.99	4.9
Hydroxybupropion	411	2.3	401	0.7	411	2.4	941	1.7	921	1.7	910	2.0
Methylphenidate	11.9	1.1	11.2	5.4	11.4	5.7	26.9	1.2	26.6	4.6	25.8	2.8
Mianserine	29.5	1.7	29.5	3.0	29.4	1.3	70.4	1.8	70.6	2.7	71.2	3.1
Milnacipran	78.2	3.8	76.9	2.2	78.8	3.8	187	3.3	188	4.1	189	3.1
Mirtazapine	33.9	3.5	32.1	1.1	33.3	3.3	79.6	3.2	76.3	2.5	78.8	2.1
Moclobemide	494	2.1	467	3.9	472	5.0	1115	2.0	1055	2.5	1071	3.5
Nefazodone	98.8	4.7	100	2.8	101	2.5	227	3.2	229	2.9	226	3.1
O-Desmethyltramadol	219	1.0	217	2.0	222	1.6	509	0.9	504	1.3	506	1.8
O-Desmethylvenlafaxine	106	4.1	105	4.1	107	3.3	248	3.5	243	3.6	247	2.1
Opipramol	109	1.3	107	1.4	110	0.9	256	1.2	250	1.1	255	1.0
Paroxetine	50.4	1.8	50.6	2.6	50.9	2.6	122	1.4	119	1.6	124	2.4
Reboxetine	132	3.7	133	3.1	139	3.0	316	1.9	320	2.3	323	3.2
Ritalinic acid	62.4	4.0	62.3	3.9	63.6	3.9	148	3.8	154	2.3	145	2.4
Sertraline	29.4	2.3	27.2	1.1	29.4	2.3	155	1.9	146	1.5	150	1.1
Tianeptine	30.5	3.4	30.8	2.4	31.8	3.5	76.9	3.1	70.9	4.9	76.4	4.2
Tramadol	218	3.8	214	2.1	227	3.5	496	2.0	490	3.8	513	1.5
Tranylcypromine	19.6	4.0	19.5	4.2	19.5	4.4	46.5	3.7	46.6	2.6	49.6	2.3
Trazodone	578	1.1	522	2.2	561	4.7	1275	1.3	1228	2.7	1273	1.6
Venlafaxine	59.9	3.0	61.7	4.0	60.6	2.8	148	1.5	145	2.4	147	2.1
Vortioxetine	22.4	2.1	22.3	1.8	22.5	1.8	51.2	3.2	51.8	2.1	51.0	1.8

Table 9. Inter-assay precision results for control MS9482 batch #1407

Analyte	Control 1		Control 2	
	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)
Agomelatine	25.5	3.3	279	2.0
Atomoxetine	404	1.7	947	1.0
Bupropion	34.8	2.7	80.7	2.1
Citalopram	50.4	3.7	120	2.8
Clomethiazole	528	2.5	2951	2.7
Desmethylcitalopram	25.7	0.8	60.8	1.0
Desmethylfluoxetine	116	1.4	276	1.3
Desmethylmianserine	28.1	1.2	66.4	3.7
Desmethylmirtazapine	35.7	3.6	84.3	3.8
Desmethylsertraline	35.8	1.8	85.3	1.7
Dihydrobupropion	294	1.9	689	2.1
Dosulepin	43.9	3.4	103	2.9
Duloxetine	49.4	3.3	120	1.9
Fluoxetine	105	1.4	251	2.1
Fluvoxamine	101	2.9	243	2.6
Guanfacine	2.52	6.9	6.02	4.9
Hydroxybupropion	408	2.2	924	2.2
Methylphenidate	11.5	4.9	26.5	3.4
Mianserine	29.5	2.0	70.7	2.5
Milnacipran	77.9	3.3	188	3.3
Mirtazapine	33.1	3.6	78.2	3.1
Moclobemide	478	4.3	1080	3.5
Nefazodone	100	3.4	227	2.9
O-Desmethyltramadol	219	1.7	507	1.4
O-Desmethylvenlafaxine	106	3.7	246	3.0
Opipramol	109	1.6	253	1.5
Paroxetine	50.6	2.2	122	2.6
Reboxetine	135	3.9	319	2.5
Ritalinic acid	62.8	3.8	149	3.6
Sertraline	28.6	4.2	150	3.0
Tianeptine	31.0	3.4	74.7	5.3
Tramadol	220	4.1	500	3.1
Tranylcypromine	19.5	3.9	47.6	4.1
Trazodone	554	5.2	1259	2.6
Venlafaxine	60.7	3.3	147	2.1
Vortioxetine	22.4	1.8	51.3	2.4

Conclusions

An HRAM mass spectrometry-based method (utilizing a Vanquish Flex 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. This clinical research method was developed and implemented for quantification of 36 antidepressants in human plasma or serum. The ClinMass TDM Platform with the ClinMass Add-On Set for Antidepressants from RECIPE was used. The fast method employs 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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