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Quantification of 36 antidepressants in human plasma by LC-HRAM-MS for clinical research

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Application benefits

- Simple offline sample preparation by protein precipitation
- Increased accuracy of method by implementation of a comprehensive ClinMass® kit for sample preparation
- Robust, sensitive hardware enables increased confidence in data
- Fast acquisition time allows for increased productivity of the assay

Goal

Implementation of an analytical method for the quantification of 36 antidepressants in human plasma on a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer.

Introduction

Antidepressants are commonly prescribed to alleviate symptoms of depression and anxiety. There are different types of antidepressants, based on their mode of action. Therapeutic drug monitoring (TDM) research, i.e., the quantification of serum/plasma concentrations of medications for dose optimization, is required to ensure a matched psychopharmacotherapy and to avoid side effects.

Plasma samples were extracted by offline internal standard addition and protein precipitation. Extracted samples were injected onto a Thermo Scientific™ Vanquish™ Flex

Binary UHPLC system for chromatographic separation. Detection was performed on an Orbitrap Exploris 120 mass spectrometer with heated electrospray ionization (HESI) operated in positive ion mode. Method performance was evaluated using the ClinMass® TDM Platform with the ClinMass Add-On Set for Antidepressants in Serum/Plasma from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response, lower limit of quantitation (LLOQ), carryover, accuracy, and intra- and inter-assay precision for all analytes.

This report demonstrates the capability of HRAM mass spectrometry for routine quantitation analyses in addition to its well-known use for performing in-depth qualitative investigations.

Experimental

Target analytes

The complete list of analytes and corresponding internal standards is reported in Table 1. The retention times obtained and the concentration ranges covered by the calibrators used (MS9413 batch #1129) are reported in Table 2.

Table 1. List of analytes and internal standards

Compound name	Chemical formula	Expected mass (*m/z*)	Internal standard name	Chemical formula	Expected mass (*m/z*)
Agomelatine	$C_{15}H_{17}NO_2$	244.1332	d_3-Agomelatine	$C_{15}H_{14}D_3NO_2$	247.1520
Atomoxetine	$C_{17}H_{21}NO$	256.1696	d_3-Atomoxetine	$C_{17}H_{18}D_3NO$	259.1884
Bupropion	$C_{13}H_{18}ClNO$	240.1150	d_9-Bupropion	$C_{13}H_9D_9ClNO$	249.1715
Citalopram	$C_{20}H_{21}FN_2O$	325.1711	d_6-Citalopram	$C_{20}H_{15}D_6FN_2O$	331.2087
Clomethiazole	C_6H_8ClNS	162.0139	d_9-threo-Dihydro-Bupropion	$C_{13}H_{11}D_9ClNO$	251.1871
Desmethylcitalopram	$C_{19}H_{19}FN_2O$	311.1554	d_3-Desmethylcitalopram	$C_{19}H_{16}D_3FN_2O$	314.1743
Desmethylfluoxetine	$C_{16}H_{16}F_3NO$	296.1257	d_5-Desmethylfluoxetine	$C_{16}H_{11}D_5F_3NO$	301.1571
Desmethylmianserine	$C_{17}H_{18}N_2$	251.1543	d_5-Reboxetine	$C_{19}H_{18}D_5NO_3$	319.2065
Desmethylmirtazapine	$C_{16}H_{17}N_3$	252.1495	d_{10}-Milnacipran	$C_{15}H_{12}D_{10}N_2O$	257.2433
Desmethylsertaline	$C_{16}H_{15}Cl_2N$	292.0654	d_4-Desmethylsertaline	$C_{16}H_{11}D_4Cl_2N$	296.0905
Dihydro-Bupropion	$C_{13}H_{20}ClNO$	242.1306	d_9-threo-Dihydro-Bupropion	$C_{13}H_{11}D_9ClNO$	251.1871
Dosulepin	$C_{19}H_{21}NS$	296.1468	d_3-Dosulepin	$C_{19}H_{18}D_3NS$	299.1656
Duloxetine	$C_{18}H_{19}NOS$	298.1260	d_7-Duloxetine	$C_{18}H_{12}D_7NOS$	305.1700
Fluoxetin	$C_{17}H_{18}F_3NO$	310.1413	d_5-Fluoxetin	$C_{17}H_{13}D_5F_3NO$	315.1727
Fluvoxamine	$C_{15}H_{21}F_3N_2O_2$	319.1628	d_3-Fluvoxamine	$C_{15}H_{18}D_3F_3N_2O_2$	322.1816
Guanfacine	$C_9H_9Cl_2N_3O$	246.0195	d_6-Tramadol	$C_{16}H_{19}D_6NO_2$	270.2335
Hydroxybupropion	$C_{13}H_{18}ClNO_2$	256.1099	d_6-Hydroxybupropion	$C_{13}H_{12}D_6ClNO_2$	262.1475
Methylphenidate	$C_{14}H_{19}NO_2$	234.1489	d_9-Methylphenidate	$C_{14}H_{10}D_9NO_2$	243.2054
Mianserin	$C_{18}H_{20}N_2$	265.1699	d_3-Mianserin	$C_{18}H_{17}D_3N_2$	268.1888
Milnacipran	$C_{15}H_{22}N_2O$	247.1805	d_{10}-Milnacipran	$C_{15}H_{12}D_{10}N_2O$	257.2433
Mirtazapine	$C_{17}H_{19}N_3$	266.1652	d_{10}-Milnacipran	$C_{15}H_{12}D_{10}N_2O$	257.2433
Moclobemide	$C_{13}H_{17}ClN_2O_2$	269.1051	d_8-Moclobemide	$C_{13}H_9D_8ClN_2O_2$	277.1554
Nefazodone	$C_{25}H_{32}ClN^5O_2$	470.2317	d_6-Nefazodone	$C_{25}H_{26}D_6ClN_5O_2$	476.2694
O-Desmethyltramadol	$C_{15}H_{23}NO_2$	250.1802	d_6-O-Desmethyltramadol	$C_{15}H_{17}D_6NO_2$	256.2178
O-Desmethylvenlafaxine	$C_{16}H_{25}NO_2$	264.1958	d_6-Venlafaxine	$C_{17}H_{21}D_6NO_2$	284.2491
Opipramol	$C_{23}H_{29}N_3O$	364.2383	d_4-Opipramol	$C_{23}H_{25}D_4N_3O$	368.2635
Paroxetine	$C_{19}H_{20}FNO_3$	330.1500	d_4-Paroxetine	$C_{19}H_{16}D_4FNO_3$	334.1751
Reboxetine	$C_{19}H_{23}NO_3$	314.1751	d_5-Reboxetine	$C_{19}H_{18}D_5NO_3$	319.2065
Ritalinic acid	$C_{13}H_{17}NO_2$	220.1332	d_6-O-Desmethyltramadol	$C_{15}H_{17}D_6NO_2$	256.2178

Table 1 (continued). List of analytes and internal standards

Compound name	Chemical formula	Expected mass (*m/z*)	Internal standard name	Chemical formula	Expected mass (*m/z*)
Sertraline	$C_{17}H_{17}Cl_2N$	306.0811	d_3-Sertraline	$C_{17}H_{14}D_3Cl_2N$	309.0999
Tinaeptine	$C_{21}H_{25}ClN_2O_4S$	437.1296	d_{10}-Milnacipran	$C_{15}H_{12}D_{10}N_2O$	257.2433
Tramadol	$C_{16}H_{25}NO_2$	264.1958	d_6-Tramadol	$C_{16}H_{19}D_6NO_2$	270.2335
Tranylcypromine	$C_9H_{11}N$	134.0964	d_5-Tranylcypromine	$C_9H_6D_5N$	139.1278
Trazodone	$C_{19}H_{22}ClN_5O$	372.1586	d_3-Sertraline	$C_{17}H_{14}D_3Cl_2N$	309.0999
Venlafaxine	$C_{17}H_{27}NO_2$	278.2115	d_6-Venlafaxine	$C_{17}H_{21}D_6NO_2$	284.2491
Vortioxetine	$C_{18}H_{22}N_2S$	299.1577	d_8-Vortioxetine	$C_{18}H_{14}D_8N_2S$	307.2079

Table 2. Concentration ranges covered by the calibrators (MS9413 batch #1129) and retention times

Analyte	Concentration range (µg/L)	Retention time (min)
Agomelatine	5.09–727	1.6
Atomoxetine	151–2190	1.7
Bupropion	11.6–157	1.8
Citalopram	16.5–259	1.5
Clomethiazole	104–6773	1.4
Desmethylcitalopram	18.5–279	1.4
Desmethylfluoxetine	39.7–610	1.8
Desmethylmianserine	11.8–167	1.5
Desmethylmirtazapine	13.2–197	1.1
Desmethylsertaline	12.4–191	2.0
Dihydro-Bupropion	105.1–1568	1.2
Dosulepin	16.8–244	1.9
Duloxetine	18.5–284	1.8
Fluoxetin	35.1–553	2.0
Fluvoxamine	34.9–558	1.7
Guanfacine	0.911–15.0	1.0
Hydroxybupropion	145–2045	1.1
Methylphenidate	3.80–51.8	1.1

Analyte	Concentration range (µg/L)	Retention time (min)
Mianserin	10.3–168	2.4
Milnacipran	29.2–435	1.1
Mirtazapine	12.0–184	1.6
Moclobemide	156–2250	1.1
Nefazodone	34.9–491	2.7
O-Desmethyltramadol	83.8–1186	0.8
O-Desmethylvenlafaxine	37.4–554	0.9
Opipramol	41.5–611	1.6
Paroxetine	17.7–278	1.7
Reboxetine	48.0–753	1.5
Ritalinic acid	24.6–372	0.8
Sertraline	4.62–310	2.2
Tinaeptine	10.2–163	1.4
Tramadol	84.5–1138	1.0
Tranylcypromine	7.20–108	1.8
Trazodone	161–2752	1.7
Venlafaxine	22.9–369	1.2
Vortioxetine	8.96–119	2.4

Sample preparation

Reagents included four calibrators (including blank) and two controls from RECIPE (MS9482 batch #2040), as well as an internal standard mix (MS9412 batch #2120) for quantitation. Samples of 50 µL of plasma were protein precipitated using 100 µL of precipitating solution (MS9021) containing the internal standards. Precipitated samples were vortex-mixed and centrifuged for 5 minutes. 50 µL of the supernatant were transferred to a clean vial.

Liquid chromatography

The supernatant was injected via the autosampler of the Vanquish Flex Binary UHPLC system onto the analytical column and separated using the gradient shown in Table 3. Chromatographic separation was achieved using mobile phases and an analytical column provided by RECIPE. Total runtime was 3.70 minutes.

Table 3. LC conditions

Time (min)	Flow rate (mL/min)	B (%)
0.00	0.7	5
0.1	0.7	5
0.2	0.7	25
1.50	0.7	50
2.50	0.7	55
2.60	0.7	80
3.00	0.7	80
3.10	0.7	5
3.70	0.7	5
Phase A		MS9007
Phase B		MS9008
Autosampler washing solution		MS9005
Column temperature (°C)		40
Injection volume (µL)		2

Table 4. MS parameters

Ion source parameters	
Source type	Heated Electrospray Ionization (HESI)
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)	450
Settings	
Mild trapping	No
Internal mass calibration	RunStartEASY-IC™
Data acquisition mode	Full Scan – ddMS^2
Full scan parameters	
Resolution (at *m/z* 200)	60,000
Scan range (*m/z*)	100–500
Expected peak width (s)	6
RF lens (%)	90
AGC target	Standard (1e6)
Polarity	Positive
Data-dependent MS^2 scan properties	
Isolation window (*m/z*)	2
Collision energy type	Normalized
HCD collision energy (%)	30
Resolution (at *m/z* 200)	15,000
Scan range mode	Auto

Mass spectrometry

Analytes and internal standards were detected by Full Scan – data-dependent MS^2 acquisition mode on an Orbitrap Exploris 120 mass spectrometer using HESI operated in positive ionization mode. A summary of the MS conditions is reported in Table 4. The acquisition was performed in data-dependent MS^2 to confirm that the quantified molecule was the correct one.

Method evaluation

The method performance was evaluated in terms of linearity of response within the calibration ranges, LLOQ, carryover, accuracy, and intra- and inter-assay precision for all the analytes. To determine the LLOQ, the lowest calibrator was diluted down to 5, 10 and 20-fold with blank matrix. Thus, 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 percentage coefficient of variation (%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 immediately 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 interpolation with 1/× weighting was used for all analytes. The percentage bias between nominal and back-calculated concentration was always within ±11% for all the calibrators in all the runs. Chromatograms of representative analytes and their internal standards at their respective lowest limit of quantitation are reported in Figure 1. Representative calibration curves are reported in Figure 2.

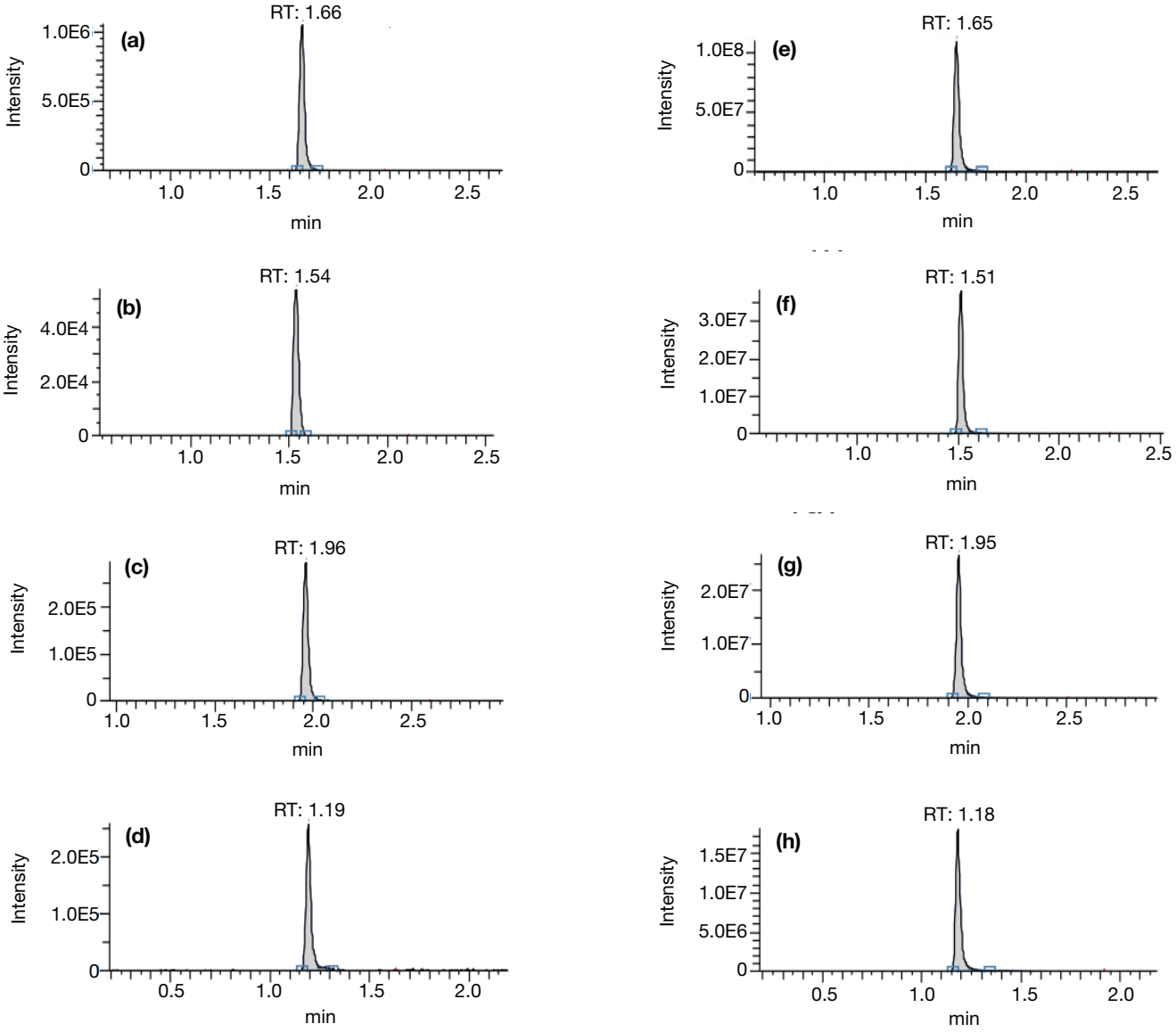

Figure 1. Representative chromatograms of the lower limit of quantification for (a) atomoxetine, (b) desmethylmianserine, (c) fluoxetine, (d) venlafaxine, (e) d_3-atomoxetine, (f) d_5-reboxetine, (g) d_5-fluoxetine, (h) d_6-venlafaxine

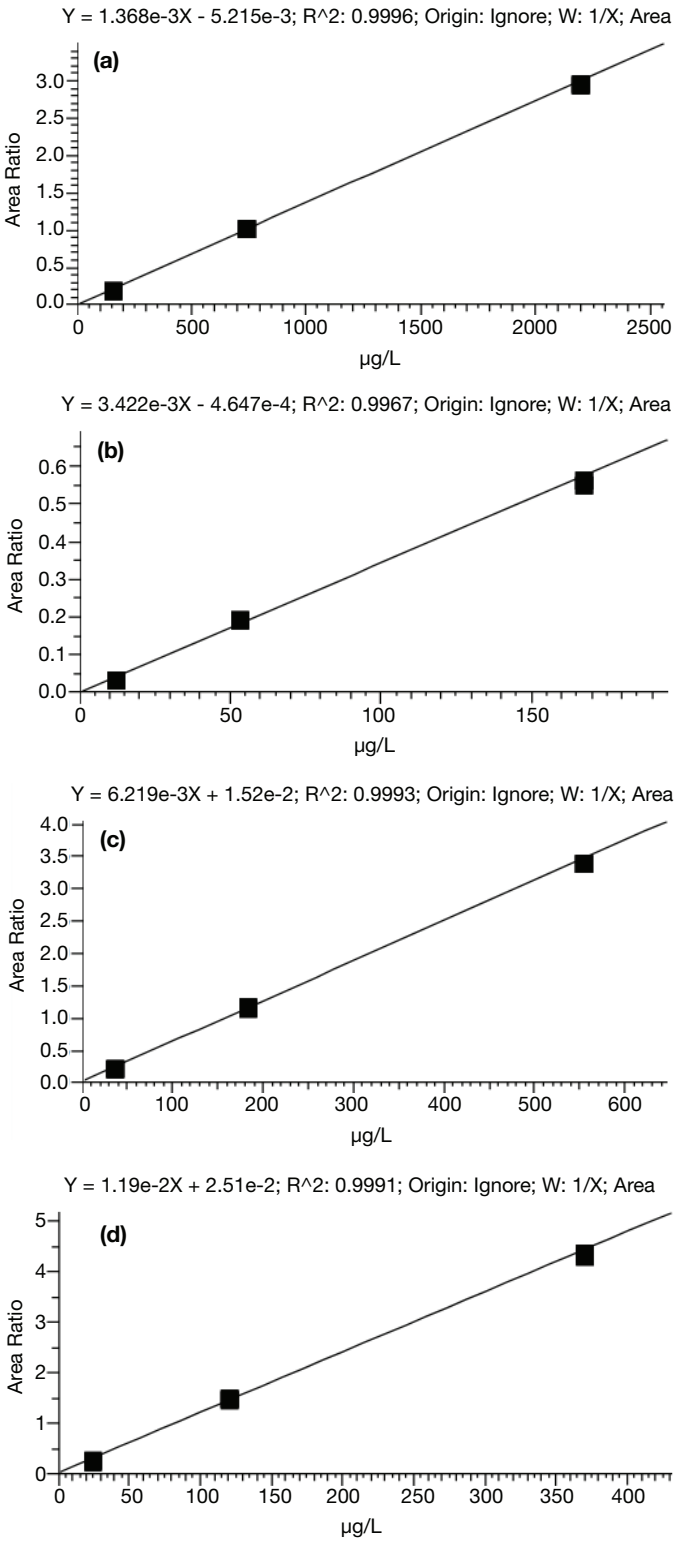

Figure 2. Representative calibration curves for (a) norclozapine, (b) norquetiapine, (c) pipamperone, (d) risperidone

No significant carryover was observed for any of the analytes, with no signal detected in the blank injected immediately after the highest calibrator.

The data demonstrated good accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the used control samples ranging between -8.3% and 10.3% (Table 5). The %CV for intra-assay precision was always below 10.1% for all the analytes. The maximum %CV for inter-assay precision including all the analytes was 9.0%. Results for intra- and inter-assay precision are reported in Table 6.

LLOQs of all compounds are reported in Table 7.

Table 5. Analytical accuracy results for control MS9482 batch#2040

Analyte	Control	Nominal conc. (µg/L)	Average calculated conc. (µg/L)	Bias (%)
Agomelatine	Level I	29.2	29.4	0.7
	Level II	305	296	-2.9
Atomoxetine	Level I	424	444	4.7
	Level II	955	992	3.9
Bupropion	Level I	23.7	22.6	-4.5
	Level II	53.1	50.5	-4.9
Citalopram	Level I	48.6	50.4	3.8
	Level II	114	115	0.8
Clomethiazole	Level I	511	491	-4.0
	Level II	3115	2890	-7.2
Desmethylcitalopram	Level I	52.5	54.5	3.8
	Level II	124	122	-1.2
Desmethylfluoxetine	Level I	113	118	4.6
	Level II	265	268	1.2
Desmethylmianserine	Level I	31.1	32.0	2.9
	Level II	69.3	71.8	3.7
Desmethylmirtazapine	Level I	35.5	36.9	3.9
	Level II	82.3	83.5	1.4
Desmethylsertaline	Level I	36.6	35.3	-3.6
	Level II	85.7	81.1	-5.4

Table 5 (continued). Analytical accuracy results for control MS9482 batch #2040

Analyte	Control	Nominal conc. (µg/L)	Average calculated conc. (µg/L)	Bias (%)
Dihydro-Bupropion	Level I	292	303	3.6
	Level II	689	682	-0.9
Dosulepin	Level I	44.6	44	-0.4
	Level II	104	100	-3.7
Duloxetin	Level I	52.7	54	3.4
	Level II	123	122	-0.8
Fluoxetine	Level I	103	108	4.5
	Level II	244	244	-0.1
Fluvoxamine	Level I	104	103	-0.6
	Level II	244	238	-2.3
Guanfacine	Level I	2.65	2.62	-1.2
	Level II	5.98	5.87	-1.8
Hydroxybupropion	Level I	422	414	-2.0
	Level II	957	919	-3.9
Methylphenidate	Level I	10.3	10.6	2.5
	Level II	21.9	21.5	-1.9
Mianserine	Level I	32.1	32.7	2.0
	Level II	76.7	75.1	-2.1
Milnacipram	Level I	69.1	70.1	1.5
	Level II	160	158	-1.1
Mirtazapine	Level I	36.3	37.6	3.4
	Level II	86.0	86.6	0.7
Moclobemide	Level I	477	526	10.3
	Level II	1102	1133	2.8
Nefazodone	Level I	99.7	101	0.9
	Level II	228	227	-0.6
O-Desmethyltramadol	Level I	167	169	1.3
	Level II	382	375	-1.7
O-Desmethylvenlafaxine	Level I	109	115	5.1
	Level II	253	257	1.7
Opipramol	Level I	114	116	1.5
	Level II	266	262	-1.7
Paroxetine	Level I	42.3	43.3	2.2
	Level II	98.4	98.4	0.0
Reboxetine	Level I	150	138	-8.3
	Level II	353	331	-6.2
Ritanalic acid	Level I	60.9	60.2	-1.1
	Level II	145	144	-0.8
Sertaline	Level I	27.8	29.1	4.5
	Level II	145	146	0.6
Tianeptine	Level I	32.8	32.6	-0.5
	Level II	77.7	76.7	-1.3
Tramadol	Level I	233	238	2.0
	Level II	529	528	-0.3
Tranylcypromine	Level I	20.4	21.1	3.5
	Level II	48.0	47.4	-1.2
Trazodone	Level I	572	571	-0.2
	Level II	1310	1271	-3.0
Venlafaxine	Level I	70.2	72.9	3.8
	Level II	167	167	0.3
Vortioxetine	Level I	22.8	24.1	5.6
	Level II	50.4	52.4	3.9

Table 6. Analytical intra- and inter-assay precision results for control MS9482 batch #2040

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 (%)
Agomelatine	Level I	29.7	3.9	30.0	3.6	28.5	2.0	29.4	2.6
	Level II	297	2.8	306	6.4	286	4.7	296	3.3
Atomoxetine	Level I	447	2.7	453	3.9	432	3.5	444	2.5
	Level II	993	3.5	1007	8.4	976	4.0	992	1.6
Bupropion	Level I	22.7	3.3	23.2	3.9	22.0	4.3	22.6	2.6
	Level II	50.5	3.5	52.8	8.6	48.3	5.2	50.5	4.4
Citalopram	Level I	51.3	3.8	51.4	4.4	48.6	1.8	50.4	3.1
	Level II	115	2.3	119	7.9	111	4.6	115	3.4
Clomethiazole	Level I	495	3.2	500	4.3	477	4.6	491	2.5
	Level II	2947	2.1	2896	9.2	2827	4.4	2890	2.1
Desmethylcitalopram	Level I	55.1	2.8	55.8	3.8	52.7	3.3	54.5	3.0
	Level II	123	3.3	126	7.4	119	4.9	123	2.9
Desmethylfluoxetine	Level I	121	2.5	120	4.3	114	4.0	118	3.1
	Level II	273	3.3	276	8.7	256	4.9	268	3.9
Desmethylmianserine	Level I	32.2	2.5	32.6	3.3	31.2	3.1	32.0	2.2
	Level II	71.2	3.3	72.8	6.7	71.5	4.2	71.8	1.2
Desmethylmirtazapine	Level I	37.5	3.7	37.8	4.3	35.4	3.6	36.9	3.6
	Level II	84.3	2.5	84.9	8.6	81.2	4.3	83.5	2.4
Desmethylsertaline	Level I	35.4	3.1	35.9	5.8	34.6	1.5	35.3	1.8
	Level II	78.5	3.6	82.8	9.4	81.9	3.0	81.1	2.8
Dihydro-Bupropion	Level I	306	2.3	307	4.0	296	3.4	303	2.1
	Level II	682	2.7	698	8.3	668	4.8	683	2.2
Dosulepin	Level I	44.7	3.1	45.4	3.3	43.2	4.0	44.4	2.5
	Level II	100	3.1	102	9.2	97.9	5.0	100	2.2
Duloxetine	Level I	55.7	2.0	54.8	6.7	52.9	3.3	54.5	2.6
	Level II	121	3.3	125	7.0	120	8.2	122	2.3
Fluoxetin	Level I	109	2.4	109	5.4	105	3.7	108	1.8
	Level II	245	2.0	247	7.7	239	4.2	244	1.6
Fluvoxamine	Level I	103	4.1	106	3.9	101	4.5	103	2.7
	Level II	237	3.0	247	7.9	232	4.0	239	3.2
Guanfacine	Level I	2.65	3.9	2.72	6.3	2.48	7.4	2.62	4.8
	Level II	5.85	2.8	6.10	9.5	5.68	10.1	5.87	3.5
Hydroxybupropion	Level I	414	2.8	420	4.3	406	3.4	414	1.7
	Level II	916	3.5	942	7.1	899	4.0	919	2.3
Methylphenidate	Level I	10.8	3.1	10.8	4.4	10.2	4.9	10.6	3.3
	Level II	21.5	2.8	22.1	8.2	20.8	3.0	21.5	3.1
Mianserin	Level I	33.3	3.4	33.4	4.3	31.6	4.4	32.8	3.2
	Level II	75.5	3.7	76.7	9.2	73.1	4.5	75.1	2.4

Table 6 (continued). Analytical intra- and inter-assay precision results for control MS9482 batch #2040

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 (%)
Milnacipran	Level I	70.9	4.1	70.3	4.5	69.1	4.3	70.1	1.3
	Level II	159	2.1	161	8.2	155	3.7	158	1.9
Mirtazapine	Level I	38.4	3.1	38.2	4.1	36.1	4.7	37.6	3.4
	Level II	87.2	3.0	88.2	7.3	84.5	4.3	86.6	2.2
Moclobemide	Level I	525	2.8	533	4.0	520	3.7	526	1.3
	Level II	1117	4.7	1176	5.9	1106	2.9	1133	3.3
Nefazodone	Level I	102	3.0	102	4.6	97.2	3.1	101	3.0
	Level II	229	3.5	234	8.8	218	4.5	227	3.6
O-Desmethyltramadol	Level I	171	2.4	169	4.3	167	2.6	169	1.3
	Level II	374	2.9	383	6.8	369	4.2	375	1.9
O-Desmethylvenlafaxine	Level I	117	3.6	116	3.7	111	4.6	114	3.1
	Level II	259	3.2	265	7.0	249	5.3	257	3.1
Opipramol	Level I	117	3.0	118	3.8	112	3.1	116	2.9
	Level II	262	3.1	269	7.1	255	4.5	2612	2.7
Paroxetine	Level I	43.8	2.7	44.0	4.6	42.0	4.1	43.3	2.6
	Level II	99.2	3.1	101	8.2	94.9	4.3	98.4	3.2
Reboxetine	Level I	136	3.6	141.2	4.6	136	3.6	138	2.2
	Level II	324	3.6	339	5.6	330	4.8	331	2.2
Ritalinic acid	Level I	60.5	8.0	64.3	9.0	55.8	6.6	60.2	7.1
	Level II	141	5.1	158	6.0	132	9.5	144	9.0
Sertraline	Level I	29.4	2.2	29.8	3.7	28.0	5.0	29.1	3.3
	Level II	145	3.3	148	9.9	144	4.3	146	1.4
Tinaeptine	Level I	33.2	2.5	33.7	5.3	31.0	5.5	32.6	4.4
	Level II	77.4	3.8	78.8	9.8	73.9	6.5	76.7	3.3
Tramadol	Level I	239	2.5	242	6.4	231	3.3	238	2.4
	Level II	537	2.0	530	9.5	515	4.1	528	2.1
Tranylcypromine	Level I	21.5	3.4	21.5	4.9	20.4	4.2	21.1	3.0
	Level II	47.5	2.5	48.5	8.2	46.4	5.0	47.4	2.2
Trazodone	Level I	569	4.6	580	5.7	563	2.0	571	1.5
	Level II	1274	2.0	1300	7.4	1239	5.1	1271	2.4
Venlafaxine	Level I	73.7	2.6	74.3	4.1	70.5	3.5	72.9	2.8
	Level II	167	3.6	174	7.3	162	4.6	168	3.7
Vortioxetine	Level I	24.6	2.9	24.4	1.7	23.3	4.4	24.1	3.0
	Level II	52.2	2.7	53.6	7.0	51.4	4.1	52.4	2.1

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Table 7. LLOQs for all compounds

Analyte	LLOQ (µg/L)	Analyte	LLOQ (µg/L)
Agomelatine	0.509	Mianserin	0.515
Atomoxetine	7.55	Milnacipran	1.46
Bupropion	11.6	Mirtazapine	0.600
Citalopram	0.825	Moclobemide	156
Clomethiazole	20.8	Nefazodone	1.75
Desmethylcitalopram	0.925	O-Desmethyltramadol	4.19
Desmethylfluoxetine	1.99	O-Desmethylvenlafaxine	1.87
Desmethylmianserine	0.590	Opipramol	2.08
Desmethylmirtazapine	0.660	Paroxetine	0.885
Desmethylsertaline	1.24	Reboxetine	48.0
Dihydro-Bupropion	5.26	Ritalinic acid	24.6
Dosulepin	0.840	Sertraline	0.231
Duloxetine	0.925	Tinaeptine	0.510
Fluoxetin	1.76	Tramadol	4.23
Fluvoxamine	1.75	Tranylcypromine	0.360
Guanfacine	0.911	Trazodone	161
Hydroxybupropion	7.25	Venlafaxine	1.15
Methylphenidate	0.760	Vortioxetine	8.96

Conclusions

A robust, reproducible, and sensitive liquid chromatography-HRAM mass spectrometry method for clinical research for the quantification of 36 antidepressants in human plasma was developed. The method was analytically implemented and validated on a Vanquish Flex Binary UHPLC system coupled to an Orbitrap Exploris 120 mass spectrometer. The method described here offers quick 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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