

# Fast LC-HRAM-MS quantification of 36 antidepressants in human plasma using the Orbitrap Exploris 120 mass spectrometer for clinical research

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## Application benefits

- Accurate and confident results, simple sample preparation, and rapid quantitation
- Robust, sensitive, and selective LC and HRAM MS platforms enable increased confidence in data
- Quantification of 36 antidepressants in human plasma in a single method

## Goal

Implementation of an analytical method for the quantification of 36 antidepressant drugs 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. In addition to the classical tricyclic antidepressants (TCAs), a variety of newer antidepressants (ADP) are available for the treatment of major depressive disorders (MDD). These



include selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs, NRIs) as well as tetracyclic antidepressants (TeCAs). 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.

In this study, a fast and robust analytical method for clinical research for the quantification of 36 antidepressants in human plasma is reported. Samples were extracted by protein precipitation followed by chromatographic separation on a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system. Detection was performed using an Orbitrap Exploris 120 mass spectrometer with heated electrospray ionization (HESI) operated in positive ion 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, accuracy, and intra- and inter-assay precision for all analytes.

## Experimental

### Target analytes

A list of the 36 antidepressant drugs, their internal standards, and their corresponding exact masses is presented in Table 1.

**Table 1. Compounds, internal standards, chemical formulas, and exact masses of the protonated ion [M+H]<sup>+</sup>**

Compound name	Formula	Exact mass [M+H] <sup>+</sup> (m/z)	Internal standard name	Exact mass [M+H] <sup>+</sup> (m/z)
Agomelatine	C <sub>15</sub> H <sub>17</sub> NO <sub>2</sub>	244.1332	Agomelatine-d <sub>3</sub>	247.1520
Atomoxetine	C <sub>17</sub> H <sub>21</sub> NO	256.1696	Atomoxetine-d <sub>3</sub>	259.1884
Bupropion	C <sub>13</sub> H <sub>18</sub> ClNO	240.1150	Bupropion-d <sub>9</sub>	249.1715
Citalopram	C <sub>20</sub> H <sub>21</sub> FN <sub>2</sub> O	325.1711	Citalopram-d <sub>6</sub>	331.2087
Clomethiazole	C <sub>6</sub> H <sub>8</sub> ClNS	162.0139	Threo-Dihydrobupropion-d <sub>9</sub>	251.1871
Desmethylcitalopram	C <sub>19</sub> H <sub>19</sub> FN <sub>2</sub> O	311.1554	Desmethylcitalopram-d <sub>3</sub>	314.1743
Desmethylfluoxetine	C <sub>16</sub> H <sub>16</sub> F <sub>3</sub> NO	296.1257	Desmethylfluoxetine-d <sub>5</sub>	301.1571
Desmethylmiansarine	C <sub>17</sub> H <sub>18</sub> N <sub>2</sub>	251.1543	Reboxetine-d <sub>5</sub>	319.2065
Desmethylmirtazapine	C <sub>16</sub> H <sub>17</sub> N <sub>3</sub>	252.1495	Mirtazapine-d <sub>3</sub>	269.1840
Desmethylsertraline	C <sub>16</sub> H <sub>15</sub> Cl <sub>2</sub> N	292.0654	Desmethylsertraline-d <sub>4</sub>	296.0905
Dosulepin	C <sub>19</sub> H <sub>21</sub> NS	296.1468	Dosulepin-d <sub>3</sub>	299.1656
Duloxetine	C <sub>18</sub> H <sub>19</sub> NOS	298.1260	Duloxetine-d <sub>7</sub>	305.1700
Ery-dihydro-bupropion	C <sub>13</sub> H <sub>20</sub> ClNO	242.1306	Threo-Dihydrobupropion-d <sub>9</sub>	251.1871
Fluoxetine	C <sub>17</sub> H <sub>18</sub> F <sub>3</sub> NO	310.1413	Fluoxetine-d <sub>5</sub>	315.1727
Fluvoxamine	C <sub>15</sub> H <sub>21</sub> F <sub>3</sub> N <sub>2</sub> O <sub>2</sub>	319.1628	Fluvoxamine-d <sub>3</sub>	322.1816
Guanfacine	C <sub>9</sub> H <sub>9</sub> Cl <sub>2</sub> N <sub>3</sub> O	246.0195	Tramadol-d <sub>6</sub>	270.2335
Hydroxybupropion	C <sub>13</sub> H <sub>18</sub> ClNO <sub>2</sub>	256.1099	Hydroxybupropion-d <sub>6</sub>	262.1475
Methylphenidate	C <sub>14</sub> H <sub>19</sub> NO <sub>2</sub>	234.1489	Methylphenidate-d <sub>9</sub>	243.2054
Mianserin	C <sub>18</sub> H <sub>20</sub> N <sub>2</sub>	265.1699	Mianserin-d <sub>3</sub>	268.1888
Milnacipran	C <sub>15</sub> H <sub>22</sub> N <sub>2</sub> O	247.1805	Milnacipran-d <sub>10</sub>	257.2433
Mirtazapine	C <sub>17</sub> H <sub>19</sub> N <sub>3</sub>	266.1652	Mirtazapine-d <sub>3</sub>	269.1840
Moclobemide	C <sub>13</sub> H <sub>17</sub> ClN <sub>2</sub> O <sub>2</sub>	269.1051	Moclobemide-d <sub>8</sub>	277.1554
Nefazodone	C <sub>25</sub> H <sub>32</sub> ClN <sub>5</sub> O <sub>2</sub>	470.2317	Nefazodone-d <sub>6</sub>	476.2694
O-Desmethyltramadol	C <sub>15</sub> H <sub>23</sub> NO <sub>2</sub>	250.1802	O-Desmethyltramadol-d <sub>6</sub>	256.2178
O-Desmethylvenlafaxine	C <sub>16</sub> H <sub>25</sub> NO <sub>2</sub>	264.1958	Venlafaxine-d <sub>6</sub>	284.2491
Opipramol	C <sub>23</sub> H <sub>29</sub> N <sub>3</sub> O	364.2383	Opipramol-d <sub>4</sub>	368.2635
Paroxetine	C <sub>19</sub> H <sub>20</sub> FNO <sub>3</sub>	330.1500	Paroxetine-d <sub>4</sub>	334.1751
Reboxetine	C <sub>19</sub> H <sub>23</sub> NO <sub>3</sub>	314.1751	Reboxetine-d <sub>5</sub>	319.2065
Ritalinic acid	C <sub>13</sub> H <sub>17</sub> NO <sub>2</sub>	220.1332	O-Desmethyltramadol-d <sub>6</sub>	256.2178
Sertraline	C <sub>17</sub> H <sub>17</sub> Cl <sub>2</sub> N	306.0811	Sertraline-d <sub>3</sub>	309.0999
Tianeptine	C <sub>21</sub> H <sub>25</sub> ClN <sub>2</sub> O <sub>4</sub> S	437.1296	Milnacipran-d <sub>10</sub>	257.2433
Tramadol	C <sub>16</sub> H <sub>25</sub> NO <sub>2</sub>	264.1958	Tramadol-d <sub>6</sub>	270.2335
Tranlycypromine	C <sub>9</sub> H <sub>11</sub> N	134.0964	Tranlycypromine-d <sub>5</sub>	139.1278
Trazodone	C <sub>19</sub> H <sub>22</sub> ClN <sub>5</sub> O	372.1586	Opipramol-d <sub>4</sub>	368.2635
Venlafaxine	C <sub>17</sub> H <sub>27</sub> NO <sub>2</sub>	278.2115	Venlafaxine-d <sub>6</sub>	284.2491
Vortioxetine	C <sub>18</sub> H <sub>22</sub> N <sub>2</sub> S	299.1577	Vortioxetine-d <sub>8</sub>	307.2079

## Sample preparation

Reagents included three calibrators (MS9413, lot no. 1129), two controls (MS9482 lot no. 1407), and a mix of 28 deuterated internal standards (MS9412 lot no. 1478) from RECIPE. The nominal concentrations of the calibrators are given in Table 2 together with the retention times of each compound.

Samples of 50  $\mu$ L plasma were protein precipitated using 100  $\mu$ L acetonitrile containing the internal standards. Precipitated samples were vortex-mixed and kept at room temperature for 5 minutes, vortex-mixed again, and centrifuged. The supernatant was transferred to a clean vial, and 2  $\mu$ L were injected onto the LC-MS system.

Table 2. Retention time and calibration levels

Compound name	Retention time (min)	Concentration ( $\mu$ g/L)		
		L1	L2	L3
Agomelatine	3.76	5.09	122	727
Atomoxetine	3.46	151	738	2190
Bupropion	2.36	11.6	54.1	157
Citalopram	3.28	16.5	86.4	259
Clomethiazole	2.75	146	2011	6773
Desmethylcitalopram	3.29	18.5	94.9	279
Desmethylfluoxetine	3.84	42.7	214	656
Desmethylmiansarine	3.19	11.8	52.7	167
Desmethylmirtazapine	2.26	13.2	65.0	197
Desmethylsertraline	4.09	12.4	62.5	191
Dosulepin	3.72	16.8	83.3	244
Duloxetine	3.79	18.5	92.5	284
Ery-dihydro-bupropion	2.54	105	520	1568
Fluoxetine	3.84	37.8	196	595
Fluvoxamine	3.73	34.9	185	558
Guanfacine	2.21	0.911	4.90	15.0
Hydroxybupropion	2.28	145	712	2045
Methylphenidate	2.17	3.80	18.4	51.8
Mianserin	3.18	10.3	55.0	168
Milnacipran	2.41	29.2	148	435
Mirtazapine	2.29	12.0	62.0	184
Moclobemide	2.00	156	841	2250
Nefazodone	4.14	34.9	176	491
O-Desmethyltramadol	1.78	83.8	422	1186
O-Desmethylvenlafaxine	1.98	37.4	189	554
Opipramol	3.73	41.5	205	611
Paroxetine	3.83	19.0	96.1	299
Reboxetine	3.42	48.0	254	753
Ritalinic acid	1.97	24.6	116	372
Sertraline	4.07	4.62	79.3	310
Tianeptine	3.49	10.2	54.5	163
Tramadol	2.10	84.5	414	1138
Tranlycypromine	3.84	7.20	36.4	108
Trazodone	2.99	161	1005	2752
Venlafaxine	2.51	22.9	120	369
Vortioxetine	4.08	8.96	41.1	119

## 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.

**Table 3. Gradient profile**

Time (min)	Flow rate (mL/min)	%B
0	0.5	10
0.2	0.5	10
1	0.5	50
2.5	0.5	55
3.5	0.5	95
5	0.5	95
5	0.5	10
6.5	0.5	10

## 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.

**Table 4. Ion source settings**

Parameter	Setting
Sheath gas	50 AU
Aux gas	10 AU
Sweep gas flow rate	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

## 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 concentrations. 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 ( $n=15$ ).

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 0.9932. The mean accuracies were within 92.9 to 106.6% and the precision was <7.3% for all compounds at all levels.

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

	Mean accuracy (n=3)			%CV		
	L1	L2	L3	L1	L2	L3
Agomelatine	101.5	98.2	100.3	4.3	5.1	0.8
Atomoxetine	99.9	100.1	100.0	3.0	4.3	1.2
Bupropion	101.9	97.4	100.8	3.1	4.4	1.3
Citalopram	100.4	99.4	100.2	3.5	4.9	1.4
Clomethiazole	97.8	103.0	99.2	5.3	7.3	2.1
Desmethylcitalopram	102.0	97.2	100.8	2.7	3.9	1.1
Desmethylfluoxetine	98.6	102.0	99.5	4.0	5.5	1.5
Desmethylmiansarine	97.2	104.3	98.8	3.2	4.5	1.2
Desmethylmirtazapine	100.9	103.1	99.0	5.8	4.7	1.4
Desmethylsertraline	103.6	94.4	101.6	4.1	5.7	1.6
Dosulepin	101.3	98.9	100.3	3.7	5.4	1.6
Duloxetine	99.7	98.2	100.6	5.8	6.1	1.7
Ery-dihydro-bupropion	101.4	101.3	99.5	3.0	4.5	1.4
Fluoxetine	101.5	100.7	99.7	3.6	4.7	1.4
Fluvoxamine	101.5	102.9	99.0	5.2	3.8	1.3
Guanfacine	104.7	98.3	100.3	7.3	6.8	2.0
Hydroxybupropion	101.9	99.7	100.0	3.5	4.8	1.5
Methylphenidate	100.8	97.5	100.8	4.1	4.4	1.3
Mianserin	102.9	100.6	99.7	5.6	5.6	1.7
Milnacipran	101.1	99.5	100.1	2.5	4.0	1.2
Mirtazapine	101.5	98.8	100.3	3.4	5.3	1.6
Moclobemide	104.7	93.9	102.0	2.8	4.3	1.4
Nefazodone	103.1	95.1	101.5	3.5	4.7	1.4
O-Desmethyltramadol	102.1	97.9	100.6	2.5	3.7	1.2
O-Desmethylvenlafaxine	100.8	102.8	99.1	4.4	5.3	1.7
Opi Pramol	100.9	99.8	100.0	3.0	4.7	1.4
Paroxetine	99.8	103.2	99.0	3.8	4.1	1.2
Reboxetine	101.3	100.3	99.8	3.2	4.4	1.3
Ritalinic acid	99.3	104.7	98.6	6.0	5.4	1.4
Sertraline	102.4	100.9	99.7	4.5	6.1	1.5
Tianeptine	102.8	100.9	99.6	5.5	4.6	1.5
Tramadol	102.2	96.7	101.0	3.2	4.6	1.4
Tranlycypromine	102.0	98.7	100.3	2.3	4.2	1.3
Trazodone	106.6	92.9	102.2	2.9	3.9	1.3
Venlafaxine	100.8	101.2	99.6	4.4	5.1	1.4
Vortioxetine	99.5	97.4	100.9	5.8	4.7	1.3

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% are presented in Table 7. For all compounds, there is a possibility to extend the LLOQ below the lowest calibrator.

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 85.2 and 113.9%, and the intra-assay precision was better than 6.5% for all compounds at all levels.

The inter-assay accuracy and precision results are reported in Table 10. The inter-assay accuracy was between 89.2 and 114.8%, and the inter-assay precision was better than 6.4% for all compounds at all levels.

**Table 7. Estimated lower limit of quantitation (LLOQ)**

Analyte	LLOQ (µg/L)
Agomelatine	0.509
Atomoxetine	15.1
Bupropion	1.16
Citalopram	1.65
Clomethiazole	48.7
Desmethylcitalopram	1.85
Desmethylfluoxetine	4.27
Desmethylniansarine	1.18
Desmethylmirtazapine	1.32
Desmethylsertraline	4.13
Dosulepin	1.68
Duloxetine	6.17
Ery-dihydro-bupropion	10.5
Fluoxetine	3.78
Fluvoxamine	3.49
Guanfacine	0.091
Hydroxybupropion	14.5
Methylphenidate	0.380
Mianserin	1.03
Milnacipran	2.92
Mirtazapine	1.20
Moclobemide	15.6
Nefazodone	3.49
O-Desmethyltramadol	8.38
O-Desmethylvenlafaxine	3.74
Opi Pramol	4.15
Paroxetine	1.90
Reboxetine	4.80
Ritalinic acid	2.46
Sertraline	0.462
Tianeptine	1.02
Tramadol	8.45
Tranylcypromine	1.44
Trazodone	32.2
Venlafaxine	2.29
Vortioxetine	0.896

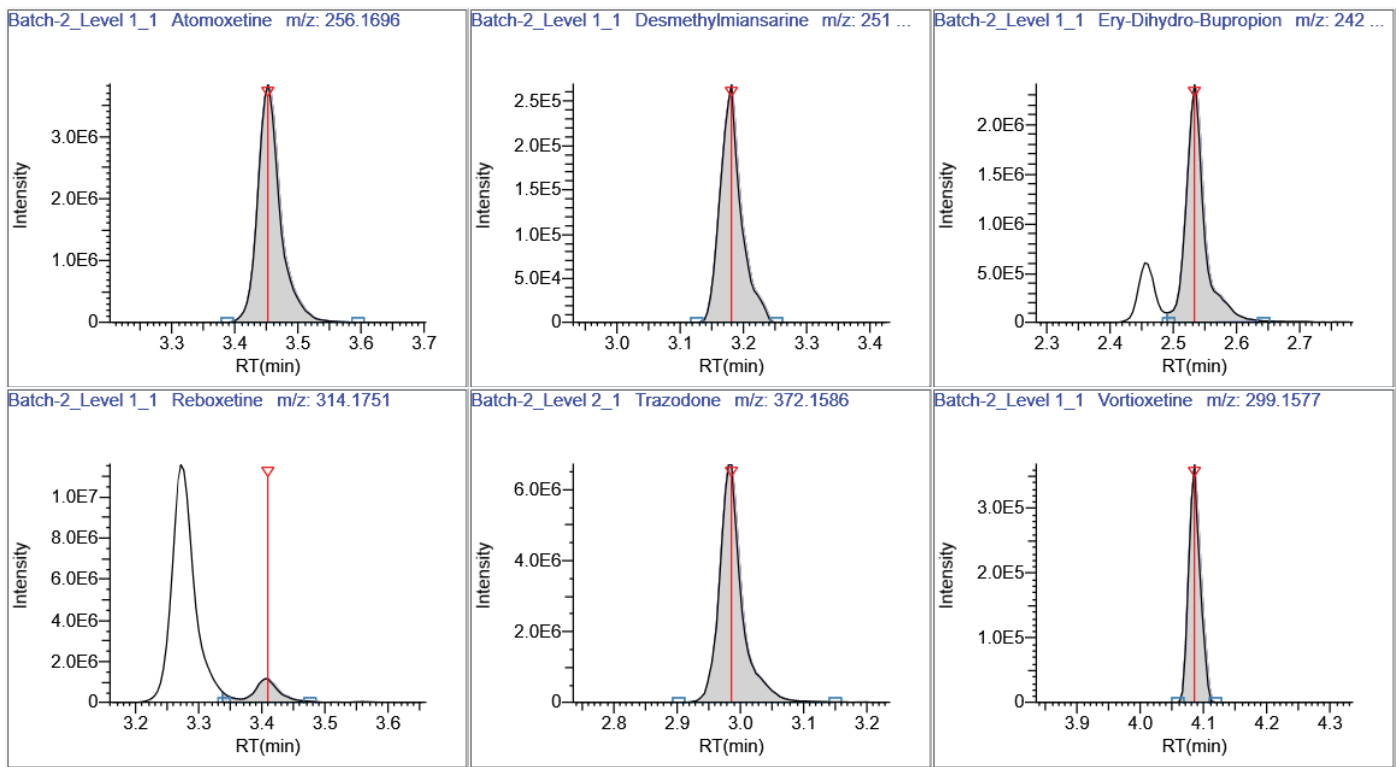


Figure 1. Representative chromatograms at the estimated LLOQ

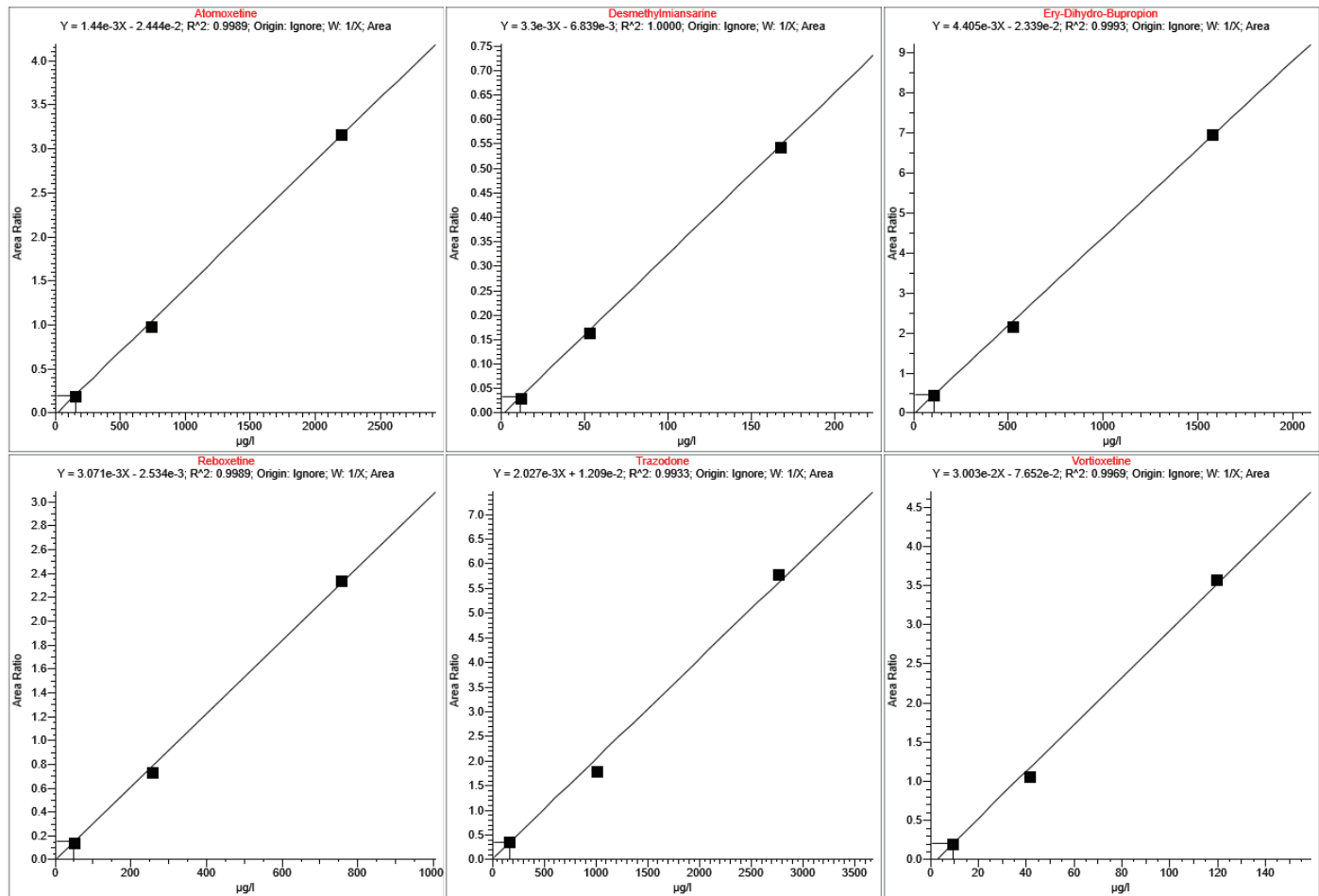


Figure 2. Representative calibration curves



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
Agomelatine	<b>26.9</b>	102.6	98.0	93.1	<b>290</b>	101.9	97.5	90.2
Atomoxetine	<b>407</b>	104.9	99.8	97.5	<b>952</b>	104.5	102.1	94.9
Bupropion	<b>35.3</b>	105.4	101.0	97.2	<b>78.7</b>	106.0	102.8	97.2
Citalopram	<b>49.0</b>	108.1	103.6	100.6	<b>116</b>	107.1	103.8	99.2
Clomethiazole	<b>518</b>	112.0	104.9	108.4	<b>2828</b>	114.8	111.7	101.4
Desmethylcitalopram	<b>24.7</b>	105.9	104.7	107.2	<b>58.9</b>	105.7	104.6	100.1
Desmethylfluoxetine	<b>118</b>	104.7	101.3	98.3	<b>275</b>	105.2	105.3	98.0
Desmethylmiansarine	<b>30.0</b>	102.3	99.3	97.5	<b>67.6</b>	104.8	104.3	98.3
Desmethylmirtazapine	<b>34.9</b>	104.4	106.1	100.5	<b>80.6</b>	104.2	107.8	100.2
Desmethylsertraline	<b>34.8</b>	113.9	108.9	113.3	<b>82.5</b>	109.6	106.4	111.2
Dosulepin	<b>44.6</b>	103.7	98.8	96.6	<b>102</b>	105.6	101.2	96.1
Duloxetine	<b>50.3</b>	100.1	99.2	95.3	<b>115</b>	105.4	103.5	95.4
Ery-dihydro-bupropion	<b>279</b>	111.1	101.6	103.8	<b>662</b>	108.7	102.7	100.3
Fluoxetine	<b>105</b>	108.9	104.2	100.2	<b>249</b>	107.0	104.5	98.3
Fluvoxamine	<b>101</b>	113.9	109.1	104.8	<b>244</b>	109.8	105.1	101.4
Guanfacine	<b>2.52</b>	104.4	103.0	95.4	<b>5.97</b>	106.0	105.3	95.7
Hydroxybupropion	<b>398</b>	104.8	99.8	97.2	<b>903</b>	106.1	102.3	96.8
Methylphenidate	<b>11.1</b>	110.9	104.9	110.4	<b>25.3</b>	111.4	106.8	109.2
Mianserin	<b>29.3</b>	108.7	104.0	98.7	<b>69.3</b>	107.6	106.0	98.7
Milnacipran	<b>77.4</b>	105.8	101.2	98.9	<b>180</b>	106.0	103.5	97.8
Mirtazapine	<b>33.9</b>	102.1	102.1	96.7	<b>79.0</b>	105.0	104.3	96.5
Moclobemide	<b>474</b>	94.9	91.6	88.6	<b>1089</b>	95.9	93.1	87.8
Nefazodone	<b>103</b>	98.1	95.1	92.4	<b>232</b>	102.3	98.7	92.6
O-Desmethyltramadol	<b>227</b>	102.1	97.8	95.7	<b>518</b>	103.1	99.8	94.9
O-Desmethylvenlafaxine	<b>104</b>	108.0	101.4	99.0	<b>239</b>	109.2	106.4	97.8
Opipramol	<b>108</b>	105.1	100.6	96.5	<b>257</b>	103.1	99.8	93.1
Paroxetine	<b>50.6</b>	110.6	103.3	102.8	<b>121</b>	108.0	104.4	98.7
Reboxetine	<b>134</b>	104.8	102.1	98.2	<b>318</b>	104.6	103.9	96.7
Ritalinic acid	<b>63.2</b>	112.6	104.3	102.2	<b>151</b>	110.9	105.7	97.8
Sertraline	<b>28.9</b>	107.2	99.4	95.6	<b>145</b>	110.6	105.8	99.3
Tianeptine	<b>31.7</b>	107.1	102.8	98.6	<b>73.4</b>	109.7	104.9	97.7
Tramadol	<b>215</b>	104.1	101.6	98.2	<b>501</b>	102.2	99.6	93.8
Tranlycypromine	<b>19.6</b>	105.7	101.2	97.2	<b>46.9</b>	105.8	103.2	93.5
Trazodone	<b>548</b>	94.1	90.2	85.2	<b>1224</b>	100.1	98.6	91.8
Venlafaxine	<b>60.9</b>	107.7	102.3	98.4	<b>145</b>	107.1	103.4	98.0
Vortioxetine	<b>22.4</b>	104.6	101.5	98.7	<b>51.1</b>	105.3	101.3	97.0



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

	QC Level 1			QC Level 2		
	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
Agomelatine	2.1	2.7	3.1	3.9	2.2	2.9
Atomoxetine	2.6	2.5	2.8	3.5	2.0	3.4
Bupropion	2.4	2.6	2.9	3.5	1.6	3.1
Citalopram	2.5	2.6	3.2	3.1	2.6	3.5
Clomethiazole	2.7	2.3	4.0	2.8	2.8	4.4
Desmethylcitalopram	3.1	4.1	3.9	3.4	2.1	2.0
Desmethylfluoxetine	2.3	3.6	3.8	3.0	1.9	4.1
Desmethylmiansarine	3.0	2.6	3.2	3.7	2.4	3.3
Desmethylmirtazapine	2.1	3.0	3.8	3.2	3.6	3.5
Desmethylsertraline	4.1	5.9	6.0	6.4	5.5	4.2
Dosulepin	1.8	2.7	2.9	3.5	1.6	3.5
Duloxetine	3.0	3.6	2.8	2.1	3.4	3.9
Ery-dihydro-bupropion	2.3	3.0	3.5	3.0	2.8	3.6
Fluoxetine	2.9	3.1	3.8	3.4	2.3	3.6
Fluvoxamine	2.0	2.9	2.5	3.4	2.1	3.7
Guanfacine	3.0	3.9	4.6	4.4	3.2	3.7
Hydroxybupropion	2.5	2.7	2.9	3.4	2.1	3.4
Methylphenidate	3.3	2.8	3.4	3.0	2.5	3.9
Mianserin	2.3	2.5	2.8	3.4	2.2	4.4
Milnacipran	2.2	3.0	3.9	3.5	2.7	4.2
Mirtazapine	2.3	3.4	4.2	3.3	3.1	3.8
Moclobemide	1.8	2.2	2.2	3.5	2.1	2.9
Nefazodone	2.3	2.4	3.2	3.1	2.8	3.2
O-Desmethyltramadol	2.6	2.4	3.1	3.2	2.1	2.9
O-Desmethylvenlafaxine	3.8	3.3	3.3	3.8	2.3	4.4
Opipramol	1.9	2.5	3.2	3.0	2.5	3.4
Paroxetine	2.7	3.8	3.5	3.0	2.2	3.8
Reboxetine	1.9	3.0	3.1	3.3	2.6	3.4
Ritalinic acid	4.9	3.8	2.4	3.4	1.6	3.3
Sertraline	2.3	2.2	3.5	3.3	2.4	3.4
Tianeptine	3.2	2.8	3.7	3.8	3.0	3.8
Tramadol	2.2	2.4	3.6	2.9	2.6	3.1
Tranlycypromine	2.4	4.7	1.0	3.5	2.6	4.9
Trazodone	2.5	2.4	3.1	2.7	2.3	2.8
Venlafaxine	2.5	2.8	2.7	3.5	2.0	3.6
Vortioxetine	2.6	1.4	2.9	3.5	3.1	3.8

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

	Accuracy		Precision	
	QC1	QC2	QC1	QC2
Agomelatine	97.9	96.5	4.8	5.9
Atomoxetine	100.7	100.5	4.0	5.1
Bupropion	101.2	102.0	4.2	4.5
Citalopram	104.1	103.4	4.0	4.3
Clomethiazole	108.4	109.3	4.0	6.3
Desmethylcitalopram	106.0	103.5	3.6	3.4
Desmethylfluoxetine	101.4	102.9	4.0	4.5
Desmethylmiansarine	99.7	102.5	3.4	4.2
Desmethylmirtazapine	103.7	104.1	3.7	4.5
Desmethylsertraline	112.0	109.1	5.4	5.4
Dosulepin	99.7	101.0	3.9	4.9
Duloxetine	98.2	101.4	3.6	5.3
Ery-dihydro-bupropion	105.5	103.9	4.8	4.5
Fluoxetine	104.4	103.3	4.6	4.7
Fluvoxamine	109.3	105.5	4.2	4.5
Guanfacine	100.9	102.3	5.4	5.9
Hydroxybupropion	100.6	101.7	4.1	4.8
Methylphenidate	108.8	109.1	3.9	3.4
Mianserin	103.8	104.1	4.7	4.9
Milnacipran	101.9	102.4	4.1	4.7
Mirtazapine	100.3	101.9	4.1	5.0
Moclobemide	91.7	92.2	3.5	4.6
Nefazodone	95.2	97.9	3.5	5.1
O-Desmethyltramadol	98.5	99.3	3.8	4.4
O-Desmethylvenlafaxine	102.8	104.5	5.0	5.8
Opipramol	100.7	98.7	4.3	5.2
Paroxetine	105.6	103.7	4.7	4.7
Reboxetine	101.7	101.7	3.7	4.6
Ritalinic acid	106.4	104.8	5.7	6.0
Sertraline	100.7	105.2	5.6	5.4
Tianeptine	102.8	104.1	4.6	5.9
Tramadol	101.3	98.5	3.6	4.6
Tranlycypromine	101.4	100.8	4.6	6.4
Trazodone	89.8	96.8	4.9	4.5
Venlafaxine	102.8	102.8	4.6	4.8
Vortioxetine	101.6	101.2	3.3	4.7

## Conclusion

A fast, reproducible, accurate, and sensitive liquid chromatography-HRAM mass spectrometry method was implemented for the quantification of 36 antidepressant drugs in human plasma in less than 7 min/sample. The method was analytically validated on a Vanquish Flex Binary UHPLC system interfaced 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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