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TECHNICAL NOTE 65133

Quantification of antidepressants in human plasma or serum by liquid chromatography-tandem mass spectrometry for clinical research

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Keywords

Antidepressants, offline sample preparation, plasma, serum, mass spectrometry

Goal

Implementation of an analytical method for the quantification of 23 antidepressants in human plasma or serum on a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer.

Application benefits

- Simple offline sample preparation by protein precipitation
- 23 antidepressants in a single quantitative method

Introduction

An analytical method for clinical research for the quantification of 23 antidepressants in human plasma or serum is reported; the analysis includes atomoxetine, bupropion, citalopram, desmethylfluoxetine, desmethylmirtazapine, desmethylsertraline, dosulepin, duloxetine, threo-dihydrobupropion, fluoxetine, fluvoxamine, mianserin, milnacipran, mirtazapine, moclobemid, hydroxybupropion, opipramol, paroxetine, reboxetine, sertraline, tranylcypromine, trazodone, and venlafaxine. 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 system connected to a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer with heated electrospray ionization. Detection was performed by selected-reaction monitoring (SRM) using 20 deuterated internal standards. 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 linearity of response within the calibration ranges, accuracy, and intra- and inter-assay precision for each analyte.

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Experimental

Target analytes

The analytes and corresponding concentration ranges covered by the calibrators used are reported in Table 1.

Table 1. Concentration ranges covered by calibrators.

Analyte	Concentration (ng/mL)
Atomoxetine	147–2295
Bupropion	10.5–157
Citalopram	15.7–242
Desmethylfluoxetine	38.1–618
Desmethylmirtazapine	11.7–181
Desmethylsertraline	13.3–203
Dosulepin	14.1–220
Duloxetine	17.2–259
Fluoxetine	17.2–259
Fluvoxamine	36.8–577
Hydroxybupropion	78.3-–1206
Mianserin	10.2–165
Milnacipran	16.8–260
Mirtazapine	11.7–184
Moclobemid	147–2310
Opipramol	36.5–556
Paroxetine	36–565
Reboxetine	16.5–262
Sertraline	24.8–377
threo-Dihydrobupropion	36–565
Tranylcypromine	6.46–9 .5
Trazodone	189–2870
Venlafaxine	21.5–350

Sample preparation

Reagents included four calibrators (including blank) and two different quality control (QC) levels from RECIPE, as well as 20 deuterated internal standards for 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

Chromatographic separation was achieved using mobile phases and analytical column provided by RECIPE. Details of the analytical method are reported in Table 2. Total runtime was 3.2 minutes.

Table 2. Liquid chromatographic method description.

Gradient profile:

Time (min)	*Flow Rate (mL/min)*	*A (%)*	*B (%)*
0.0	0.8	95	5
0.1	0.8	75	25
1.4	0.8	50	50
2.4	0.8	45	55
2.5	0.8	20	80
2.7	0.8	20	80
2.8	0.8	95	5
3.2	0.8	95	5

Injection volume: 5 µL

Column temp.: 40 °C

Mass spectrometry

Analytes and internal standards were detected by SRM on a TSQ Quantis triple quadrupole mass spectrometer with heated electrospray ionization operated in polarity switching mode. A summary of the MS conditions is reported in Table 3. Two SRM transitions for each analyte were included in the acquisition method for quantification and confirmation, respectively.

Table 3. MS settings.

Source type:	Heated electrospray ionization (HESI)
Vaporizer temperature:	450 °C
Capillary temperature:	300 °C
Spray voltage (positive/negative):	4500/2500 V
Sheath gas:	60 AU
Sweep gas:	0 AU
Auxiliary gas:	12 AU
Data acquisition mode:	Selected-reaction monitoring (SRM)
Collision gas pressure:	1.5 mTorr
Cycle time:	0.300 s
Q1 mass resolution (FWMH):	0.7
Q3 mass resolution (FWMH):	0.7

Method evaluation

The method performance was evaluated in terms of linearity of response within the calibration ranges, accuracy, and intra- and inter-assay precision for each analyte. Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using quality control samples at two different levels provided by RECIPE (MS9482 batch #1247), prepared and analyzed in replicates of five on three different days. Intra-assay precision was evaluated for each day on the same set of runs (control samples at two levels, replicates of five each day, three days) in terms of percentage coefficient of variation (%CV). Inter-assay precision was evaluated on the same controls including all the 15 replicates of the three days.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 4.1 software.

Results and discussion

The method proved to be linear in the calibration ranges covered by the calibrators. Representative chromatograms for the lowest calibrator for mirtazapine, opipramol, and their internal standards are reported in Figure 1. Representative calibration curves for the same analytes are reported in Figure 2.

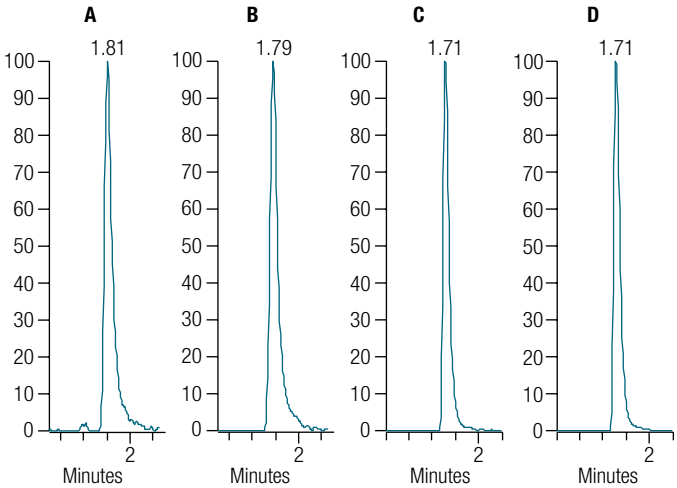

Figure 1. Representative chromatograms of the lowest calibrator for (A) mirtazapine, (B) d3-mirtazapine, (C) opipramol, and (D) d4-opipramol.

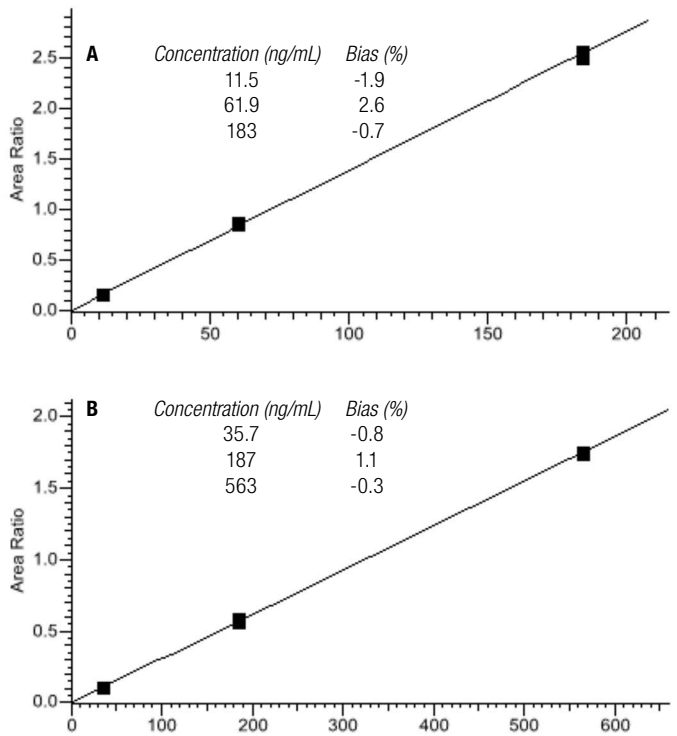

Figure 2. Representative calibration curves for (A) mirtazapine and (B) opipramol – day 3.

The data demonstrated outstanding accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the control samples ranging between -15.7% and 8.5%. Results are reported in Table 4.

Table 4. Analytical accuracy results for control MS9482 batch #1247.

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 (%)
Atomoxetine	469	509	8.5	1131	1133	0.2
Bupropion	35.1	36.5	4.0	79.9	86.2	7.9
Citalopram	48.6	45.7	-5.9	114	109	-4.4
Desmethylfluoxetine	121	118	-2.1	287	282	-1.7
Desmethylmirtazapine	35.9	36.0	0.3	84.6	79.2	-6.4
Desmethylsertraline	37.3	38.5	3.3	89.2	87.4	-2.0
Dosulepin	43.2	40.3	-6.7	103	92.0	-10.6
Duloxetine	47.9	47.8	-0.2	117	112	-4.2
Fluoxetine	109	113	3.4	256	272	6.2
Fluvoxamine	106	104	-1.9	252	247	-2.0
Hydroxybupropion	338	302	-10.5	788	698	-11.5
Mianserin	30.3	30.8	1.7	71.4	71.8	0.6
Milnacipran	54.8	53.8	-1.9	127	125	-1.5
Mirtazapine	34.6	32.2	-6.8	81.8	78.2	-4.4
Moclobemid	454	449	-1.0	1068	1018	-4.7
Opipramol	101	103	1.6	239	241	1.0
Paroxetine	50.3	52.0	3.4	119	122	2.1
Reboxetine	196	190	-2.9	462	446	-3.5
Sertraline	26.6	27.6	3.8	64.5	65.9	2.2
threo-Dihydrobupropion	237	229	-3.2	568	558	-1.8
Tranylcypromine	26.5	22.3	-15.7	61.7	54.3	-11.9
Trazodone	538	562	4.4	1283	1314	2.4
Venlafaxine	65.1	63.1	-3.0	153	150	-2.2

The %CV for intra-assay precision was always below 12.6% for all the analytes (Table 5). The maximum %CV for inter-assay precision including all the analytes was 10.8% (Table 6).

Table 5. Intra-assay precision results for control MS9482 batch #1247.

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 (%)
Atomoxetine	529	6.4	470	11.7	528	1.1	1129	4.5	1021	6.8	1247	2.6
Bupropion	34.3	8.9	37.3	9.6	38.2	10.3	79.9	7.3	88.1	7.6	90.6	2.7
Citalopram	47.6	2.5	44.3	5.9	45.3	2.2	111	1.3	110	2.0	106	6.6
Desmethylfluoxetine	113	7.4	118	8.2	124	4.0	271	5.7	282	6.8	293	2.8
Desmethylmirtazapine	35.6	4.7	36.1	9.7	36.4	8.5	73.2	5.3	82.5	11.3	82.5	11.3
Desmethylsertraline	37.2	7.5	39.8	3.4	38.6	8.0	83.0	8.1	87.7	6.4	91.5	8.2
Dosulepin	39.5	7.9	41.2	7.1	40.3	3.9	90.3	3.7	92.1	11.1	93.7	5.4
Duloxetine	48.2	5.5	45.9	8.7	49.5	12.2	107	10.4	115	8.2	114	8.7
Fluoxetine	119	6.7	107	7.0	112	5.1	275	6.0	273	2.9	267	5.4
Fluvoxamine	104	1.9	102	3.0	107	5.6	247	1.7	232	7.7	262	3.3
Hydroxybupropion	317	4.7	287	3.8	304	8.0	734	2.8	659	2.4	699	4.6
Mianserin	32.2	4.8	29.6	6.2	30.7	2.9	70.9	6.1	70.3	7.6	74.3	8.3
Milnacipran	54.8	2.5	53.0	3.8	53.5	1.1	127	2.9	123	2.5	125	2.7
Mirtazapine	32.4	5.1	32.1	7.4	32.2	8.0	77.2	3.8	77.9	2.6	79.4	4.2
Moclobemid	464	2.0	437	4.3	447	3.4	1013	2.5	1013	2.0	1028	3.0
Opipramol	104	2.3	103	3.5	101	6.4	240	1.5	239	2.5	245	0.9
Paroxetine	52.1	8.5	51.7	5.4	52.2	7.6	115	7.5	125	5.0	125	6.0
Reboxetine	196	2.3	187	4.3	188	2.3	462	1.7	434	7.5	442	2.7
Sertraline	26.7	5.6	28.2	6.0	27.9	5.0	64.1	4.0	66.3	4.3	67.3	2.6
threo-Dihydrobupropion	242	3.1	219	6.1	226	5.8	597	2.9	530	2.6	546	1.8
Tranylcypromine	21.9	2.5	22.3	1.6	22.8	8.5	51.6	2.8	51.9	5.5	59.5	7.7
Trazodone	578	12.6	565	7.9	537	7.4	1345	3.3	1249	9.2	1355	10.5
Venlafaxine	64.8	6.3	59.3	8.7	65.4	3.2	152	2.9	140	2.2	157	3.3

Table 6. Inter-assay precision results for control MS9482 batch #1247.

Analyte	Control 1		Control 2	
	Average Calculated Concentration (ng/mL)	CV (%)	Average Calculated Concentration (ng/mL)	CV (%)
Atomoxetine	509	8.8	1133	9.5
Bupropion	36.5	10.0	86.2	7.9
Citalopram	45.7	4.7	109	4.1
Desmethylfluoxetine	118	7.3	282	6.0
Desmethylmirtazapine	36.0	7.4	79.2	10.8
Desmethylsertraline	38.5	6.6	87.4	8.2
Dosulepin	40.3	6.3	92.0	7.1
Duloxetine	47.8	9.4	112	9.0
Fluoxetine	113	7.4	272	4.8
Fluvoxamine	104	4.1	247	6.7
Hydroxybupropion	302	6.9	698	5.5
Mianserin	30.8	5.7	71.8	7.4
Milnacipran	53.8	2.9	125	2.9
Mirtazapine	32.2	6.5	78.2	3.5
Moclobemid	449	4.0	1018	2.4
Opipramol	103	4.3	241	1.9
Paroxetine	52.0	6.8	122	7.0
Reboxetine	190	3.6	446	5.0
Sertraline	27.6	5.7	65.9	4.0
threo-Dihydrobupropion	229	6.4	558	5.7
Tranylcypromine	22.3	5.2	54.3	8.9
Trazodone	562	9.6	1314	8.3
Venlafaxine	63.1	7.4	150	5.5

Conclusions

A liquid chromatography-tandem mass spectrometry method for clinical research for the quantification of 23 different antidepressants in human plasma or serum was implemented. The ClinMass TDM Platform with the ClinMass Add-On Set for Antidepressants from RECIPE was used. The method was analytically evaluated on a Vanquish Flex Binary system connected to a TSQ Quantis triple quadrupole mass spectrometer. The method 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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