Quantitation of Antidepressants in Serum by UHPLC-Triple Quadrupole Mass Spectrometry

This study demonstrates a simple, rapid, and reliable method for the simultaneous quantitation of 26 antidepressants in serum using the Bruker Elute™ UHPLC coupled to the EVOQ™ LC-TQ Elite triple quadrupole mass spectrometry system. Sample preparation was performed via protein precipitation using the ClinMass® TDM kit system.

Introduction

Antidepressants are psychoactive drugs mainly used for the treatment of major depressive disorders, but also for mild depression, anxiety, eating disorders, and pain relief. They are the most prescribed psychotropic drugs, with a growing number of prescriptions throughout the world.

Keywords: Antidepressants, serum, quantitation, therapeutic drug monitoring, clinical research, forensic toxicology, EVOQ LC-TQ Elite, Elute UHPLC

Author: Rafaela Martin, Bruker Daltonik Applications Quadrupole MS, Bremen, Germany

Antidepressants are usually taken for an extended period of time, with the best effects observed at a stable blood concentration. As each individual metabolizes drugs at a different rate, it is important to regularly monitor drug (and often metabolite) concentrations in blood to ensure the correct dosage.

Beyond the classic tricyclic antidepressants, selective serotonin and serotonin-noradrenalin-reuptake inhibitors, tetracyclic antidepressants or monoamine oxidase inhibitors may be prescribed. The method described in this manuscript focuses on the rapid and reliable quantification of these classes of antidepressants in human serum by UHPLC-triple quadrupole mass spectrometry.

Experimental

The analysis was performed on an EVOQ LC-TQ Elite triple quadrupole MS/MS system coupled to an Elute UHPLC using the ClinMass TDM Platform (RECIPE Chemicals + Instruments GmbH, Munich, Germany) which included the mobile phase, autosampler washing solution, and precipitation reagent, as well as the HPLC column with prefilter. The serum calibrators and quality control samples were from the ClinMass Add-On Set for Antidepressants (MS9400), also provided by RECIPE.

Sample Preparation

Following the protocol of the ClinMass kit, 100 µL precipitation reagent containing the isotopically labelled internal standards were added to 50 µL serum samples and vortexed for 30 seconds. After centrifugation for 5 minutes at 10,000 x g, the supernatants were transferred to HPLC vials for analysis by UHPLC-MS/MS.

Table 1: Mass Spectrometry Method Conditions

Liquid chromatography Elute™ UHPLC			
Column	ClinMass TDM MS9030 with Prefilter MS9032 (RECIPE)		
Mobile Phase A	Included within the ClinMass MS9000 Kit (RECIPE)		
Mobile Phase B	Included within the ClinMass MS9000 Kit (RECIPE)		
Gradient	0.0 – 0.1 min		5% B
	0.1 – 0.2 min	to	25% B
	0.2 – 1.5 min	to	50% B
	1.5 – 2.5 min	to	55% B
	2.5 – 2.6 min	to	80% B
	2.6 – 2.8 min		80% B
	2.8 – 2.9 min	to	5% B
	2.9 – 3.5 min		5% B
Flow Rate	700 µL/min		
Injection Volume	3 µL		
Column Oven	40°C		
Mass Spectrometry Bruker EVOQ LC-TQ Elite MS/MS system			
Ion Source	VIP H-ESI positive, 4500 V		
Probe Gas	50 units at 350°C		
Cone Gas	20 units at 350°C		
Nebulizing Gas	50 units		
Active Exhaust	on		
Collision Gas	Argon, 1.5 mTorr		
MRM Transitions	see Table 2		

Analyte	Retention time (min)	Precursor Ion	Product Ion 1	CE 1 (V)	Product Ion 2	CE 2 (V)	Calibration Range [µg/L]	R^2
Atomoxetine	1.71	256.1	148.0	4	44.3	8	147 – 2295	0.9993
Bupropion	1.90	240.0	184.0	9	131.0	25	10.5 – 157	0.9964
Citalopram	1.58	325.0	109.1	23	261.9	17	15.7 – 242	0.9999
Clomethiazole	1.38	162.0	113.0	23	126.0	17	372 – 5661	0.9978
Desmethylfluoxetine	1.89	296.0	134.1	3	105.0	15	38.1 – 618	0.9989
Desmethylmirtazapine	1.16	252.0	195.0	21	209.0	21	11.7 – 181	0.9997
Desmethylsertraline	2.14	291.9	158.9	22	274.9	5	13.3 – 203	0.9993
Dihydro-Bupropion	1.32	242.0	168.0	15	115.1	31	97.2 – 1498	0.9999
Dosulepin	2.00	296.0	222.9	22	202.0	50	14.1 – 220	0.9998
Duloxetine	1.86	298.0	154.0	3	44.4	7	17.2 – 259	1.0000
Fluoxetine	2.01	310.0	148.0	4	44.4	8	36.8 – 577	0.9999
Fluvoxamine	1.83	319.0	71.2	12	200.0	18	34.1 – 547	1.0000
Hydroxybupropion	1.22	256.0	139.0	25	167.0	20	109 – 1639	0.9991
Mianserine	2.61	265.0	208.0	18	58.3	20	10.2 – 165	0.9998
Milnacipran	1.16	247.0	230.0	8	100.1	16	16.8 – 260	1.0000
Mirtazapine	1.81	266.1	195.0	23	72.2	15	11.7 – 184	0.9992
Moclobemide	1.15	269.0	181.9	16	138.9	30	147 – 2310	0.9980
O-Desmethylvenlafaxine	0.92	264.0	58.3	14	107.1	27	36.5 – 556	0.9996
Opipramol	1.70	364.1	171.1	19	143.0	26	36.0 – 565	0.9999
Paroxetine	1.74	330.0	192.0	18	70.2	23	16.5 – 262	0.9996
Reboxetine	1.62	314.0	176.0	10	91.1	23	50.2 – 802	1.0000
Ritalinic acid	0.78	220.0	84.2	17	56.3	36	24.8 – 377	0.9996
Sertraline	2.35	305.9	158.9	24	274.9	8	9.16 – 148	1.0000
Tranylcypromine	0.90	134.0	117.1	6	115.0	17	6.46 – 99.5	0.9995
Trazodone	1.84	372.0	176.0	21	148.0	30	189 – 2870	0.9998
Venlafaxine	1.28	278.0	58.3	14	260.1	7	21.5 – 350	0.9997

Table 2: Retention times, MRM transitions, calibration ranges, and coefficient of determination R^2

Results and Discussion

Following fast and simple sample preparation, which requires only 50 µL of serum, the chromatographic separation of the 26 antidepressants was performed within 3.5 minutes using the Elute UHPLC system. Figure 1 illustrates an overlay of the MRM traces for all measured analytes. The quantitation of the analytes was performed using 20 isotopic labelled internal standards. Calibration curves included three calibrator levels and provided excellent linearity with R^2 values from 0.9964 – 1.0000 (Table 2). Figure 2 shows exemplarily the calibration curve of fluoxetin. Quality controls (QC) in serum with a low (QC I) and a high (QC II) concentration were measured four times. The RSD of < 7% for all analytes underlines the very good precision of the instrumentation used. The experiments showed also a high accuracy with a bias within ± 10% for most of the measured analytes (highest value 12.9%). Detailed results are presented in Table 3. The values for precision and accuracy are well within the range required by common guidelines for quantitative results.

Sample	QC I				QC II			
Analyte	**Specified Value [µg/L]**	**Actual Value [µg/L]**	**Bias [%]**	**RSD [%]**	**Specified Value [µg/L]**	**Actual Value [µg/L]**	**Bias [%]**	**RSD [%]**
Atomoxetine	404	436	7.8	3.1	969	1006	3.8	5.3
Bupropion	29.3	28.5	-2.8	4.1	66.8	66.4	-0.7	6.2
Citalopram	43.5	47.0	8.0	2.8	102	107	5.0	3.1
Clomethiazole	1027	1002	-2.4	2.6	2370	2351	-0.8	3.5
Desmethylfluoxetine	107	119	10.8	5.3	256	263	2.8	2.5
Desmethylmirtazapine	32.7	35.6	8.8	1.7	78.3	80.6	2.9	2.2
Desmethylsertraline	36.0	38.4	6.7	5.7	84.6	87.4	3.3	1.0
Dihydro-Bupropion	269	276	2.9	1.5	634	648	2.2	2.2
Dosulepin	39.2	42.3	7.9	2.5	92.6	95	2.5	1.3
Duloxetine	47.3	47.6	0.6	2.5	111	109	-2.2	1.0
Fluoxetine	102	111	8.5	2.5	247	251	1.5	1.1
Fluvoxamine	98.2	102	3.9	4.4	229	241.0	5.3	2.2
Hydroxybupropion	317	345	8.7	6.8	722	757	4.8	4.5
Mianserine	29.5	31.3	6.2	6.6	70.9	70.3	-0.9	1.3
Milnacipran	48.0	49.1	2.3	3.7	113	116	2.2	1.7
Mirtazapine	33.1	34.4	4.0	3.2	79.1	78.4	-0.9	1.9
Moclobemide	414	463	11.9	3.0	1010	1040	3.0	2.6
O-Desmethylvenlafaxine	102	115	12.9	2.7	238	260	9.4	4.2
Opipramol	102	105	2.8	5.6	238	240	1.0	0.7
Paroxetine	46.8	50.0	6.8	5.7	111	111	0.2	3.8
Reboxetine	143	145	1.5	1.1	338	338	0.0	4.4
Ritalinic acid	66.7	69.7	4.6	3.9	158	158	0.2	1.7
Sertraline	26.0	26.9	3.4	0.8	62.0	62.6	1.0	0.8
Tranylcypromine	18.1	18.5	2.0	3.5	40.7	45.4	11.5	2.9
Trazodone	534	581	8.8	4.5	1284	1257	-2.1	5.7
Venlafaxine	61.7	64.2	4.0	2.3	146	150	2.7	1.7

Table 3: Quantitative results, bias, and relative standard deviation of fourfold measurement of Quality Controls I and II

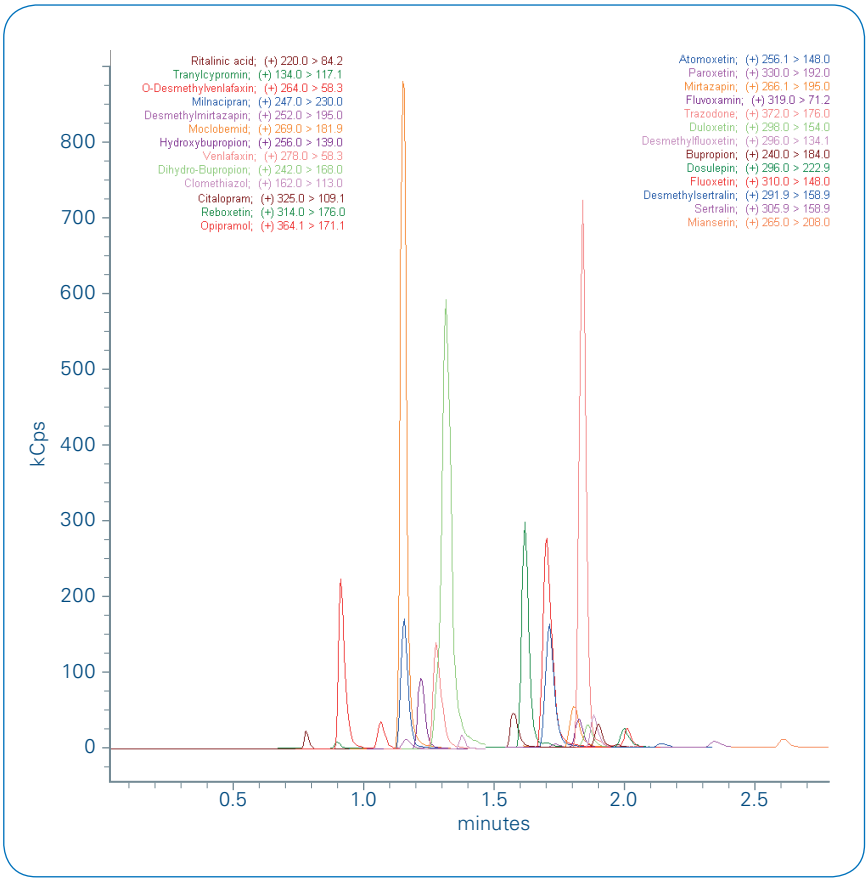

Figure 1: Overlaid MRM traces of all analytes (lowest calibrator level)

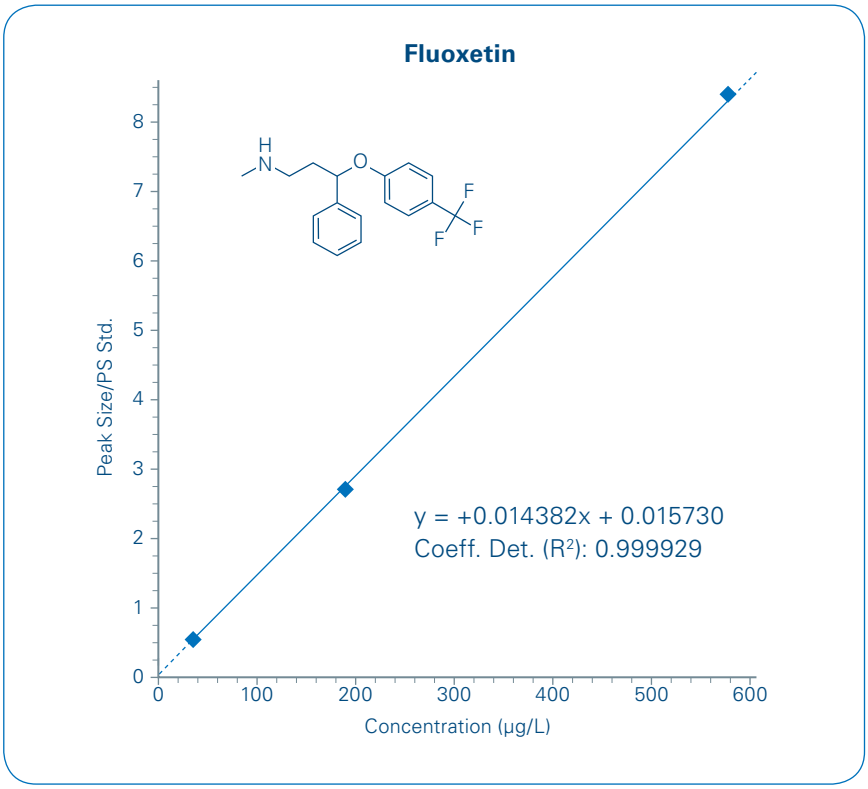

Figure 2: Calibration curve of fluoxetin. Calibration range 36.8 – 577 µg/L

Acknowledgement

The authors acknowledge RECIPE Chemicals + Instruments GmbH (Munich, Germany) for providing the ClinMass TDM kit.

Conclusion

The Bruker Elute UHPLC coupled to the EVOQ LC-TQ Elite triple quadrupole system and the ClinMass TDM kit system provide a quick and reliable method to easily detect and quantitate 26 antidepressants in serum.

Low sample requirements (50 µl serum), easy preparation, and short run time (3.5 minutes) support high sample throughput. Linearity of calibration, precision, and accuracy were outstanding, supporting the use of this combined system in clinical research workflows.

Learn More

You are looking for further Information?
Check out the link or scan the QR code for more details.

www.bruker.com/evoq-lc

For research use only. Not for use in diagnostic procedures.

Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0)421-2205-0

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com

Bruker Daltonics is continually improving its products and reserves the right to change specifications without notice. © Bruker Daltonics 03-2018, LCMS-139, 1859683 , Rev. 01