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

This study demonstrates a simple, rapid, and reliable method for the simultaneous quantitation of 13 tricyclic antidepressants and their metabolites, as well as two atypical neuroleptics, in serum using the Bruker Elute UHPLC coupled to the EVOQ LC-TQ Elite MS/MS 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 anxiety or pain relief. Various classes of antidepressant drugs are prescribed today, including selective serotonin and serotonin-noradrenalin-reuptake inhibitors, monoamine oxidase inhibitors, and tricyclic compounds. Tricyclics were the first group of antidepressants, developed in the 1950s, and have a common tricyclic ring structure with different substituents.

Antidepressants are usually taken for an extended period of time, with the best effects observed at a stable blood concentration. Due to the narrow therapeutic range of tricyclic antidepressants, it is important to monitor their concentration in blood. Overdosing can lead

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to severe effects, ranging from mild agitation to delirium, coma, or death.

The method described in this manuscript focuses on the rapid and reliable quantification of tricyclic antidepressants in human serum by UHPLC-triple quadrupole mass spectrometry.

Experimental

The analysis was performed on an EVOQ LC-TQ Elite MS/MS system coupled to an Elute UHPLC using the ClinMass® TDM Platform (RECIPE Chemicals + Instruments GmbH, Munich, Germany) which included the mobile phases, 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 tricyclic antidepressants (MS9100), 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	
Instrument	Bruker 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	0.00 – 0.05 min 15% B 0.05 – 0.06 min to 30% B 0.06 – 2.10 min 30% B 2.10 – 2.11 min to 38% B 2.11 – 2.70 min 38% B 2.70 – 3.00 min 75% B 3.00 – 3.35 min 75% B 3.35 – 3.40 min to 15% B 3.40 - 3.80 min 15% B
Flow Rate	800 µL/min
Injection Volume	5 µL
Column Oven	40°C
Mass Spectrometry	
Instrument	EVOQ LC-TQ Elite MS/MS system
Ion Source	VIP H-ESI positive, 4500 V
Probe Gas	50 units at 400°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

Results and Discussion

Following fast and simple sample preparation requiring only 50 µL of serum, the chromatographic separation of the 15 analytes was performed within 3.8 minutes using the new Elute UHPLC system. Figure 1 illustrates an overlay of the MRM traces for all measured analytes. The quantitation of the analytes was performed using 12 isotopic labelled internal standards. Calibration curves included three calibrator levels and provided excellent linearity with R^2 values from 0.9985 – 1.0000 (Table 2). The calibration curve of doxepin is shown as an example in Figure 2. Quality controls (QC) in serum with a low (QC I) and a high (QC II) concentration were measured four times. The RSD of < 6% for all analytes underlines the very good precision of the instrumentation used. The experiments also showed a high accuracy with a bias within ± 9% for all measured analytes. 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.

Table 2: Retention times, MRM transitions, calibration ranges, and coefficient of determination R^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
Amitriptyline	3.25	278.1	233.1	14	105.0	22	14.9 – 305	1.0000
Clomipramine	3.44	315.0	86.2	15	58.3	21	18.8 – 393	0.9999
Clozapine	3.04	327.1	270.0	19	192.0	41	54.9 – 1166	0.9989
Desipramine	2.29	267.0	72.2	12	44.4	24	16.2 – 348	0.9999
Doxepin	2.22	280.0	107.1	23	235.0	14	13.4 – 270	0.9999
Imipramine	2.87	281.1	58.3	19	86.2	14	16.2 – 340	0.9997
Maprotiline	2.68	278.1	250.1	16	178.1	36	21.2 – 434	1.0000
Norclomipramine	3.22	301.0	72.3	13	242.0	22	20.7 – 431	1.0000
Norclozapine	1.50	313.0	192.0	41	270.0	21	46.0 – 942	0.9999
Nordoxepin	1.58	266.0	107.1	18	235.0	12	12.5 – 275	1.0000
Normaprotiline	2.27	264.0	169.0	15	219.1	21	30.6 – 677	0.9997
Nortrimipramine	2.80	281.1	44.4	20	55.0	25	14.1 – 315	0.9985
Nortriptyline	2.66	264.1	233.1	11	91.2	20	16.4 – 344	1.0000
Protriptyline	2.32	264.1	155.1	19	233.0	14	14.8 – 313	0.9993
Trimipramine	3.37	295.1	100.2	14	58.3	19	16.4 – 345	0.9987

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

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 [%]**
Amitriptyline	59.2	60.0	1.4	1.2	135	137	1.6	1.1
Clomipramine	73.9	76.3	3.3	5.2	171	171	0.0	0.5
Clozapine	217	220	1.2	3.1	510	497	-2.5	1.9
Desipramine	64.3	65.5	1.9	1.6	152	145	-4.9	1.1
Doxepin	50.8	54.7	7.8	1.2	117	122	4.3	0.9
Imipramine	63.9	65.3	2.2	2.0	148	146	-1.3	1.4
Maprotiline	82.7	82.0	-0.8	3.6	193	186	-3.4	2.4
Norclomipramine	79.9	82.5	3.3	2.0	187	187	0.2	1.1
Norclozapine	179	178	-0.5	3.1	418	403	-3.5	3.6
Nordoxepin	49.4	52.1	5.4	1.9	116	117	0.5	1.4
Normaprotiline	121	132	8.9	1.7	280	301	7.6	0.6
Nortrimipramine	55.1	58.3	5.7	3.2	133	134	0.5	1.6
Nortriptyline	64.5	64.5	0.0	0.9	145	150	3.2	1.5
Protriptyline	58.1	62.3	7.3	2.4	143	142	-0.5	2.3
Trimipramine	64.2	69.4	8.1	1.1	155	157	1.1	1.7

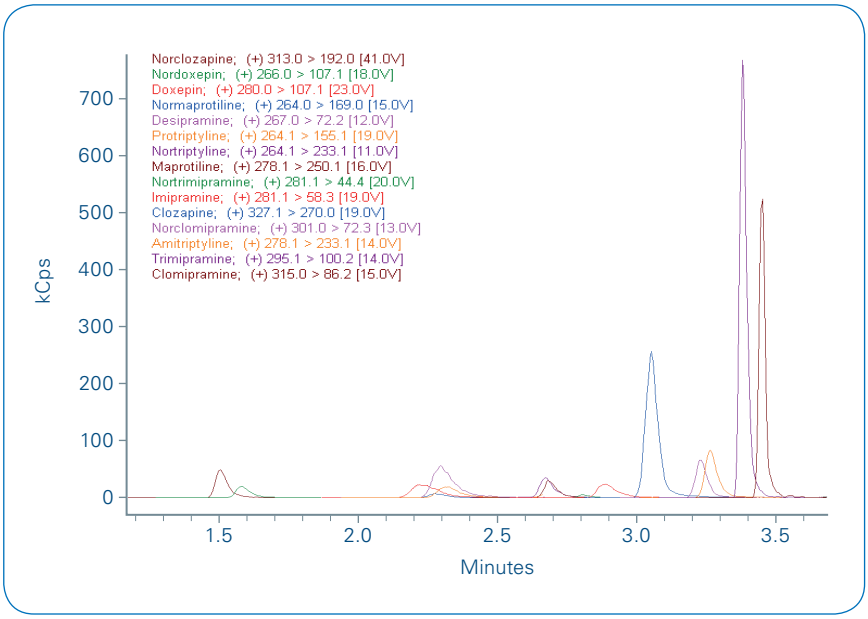

Figure 1: Overlaid MRM traces of all analytes (lowest calibrator level)

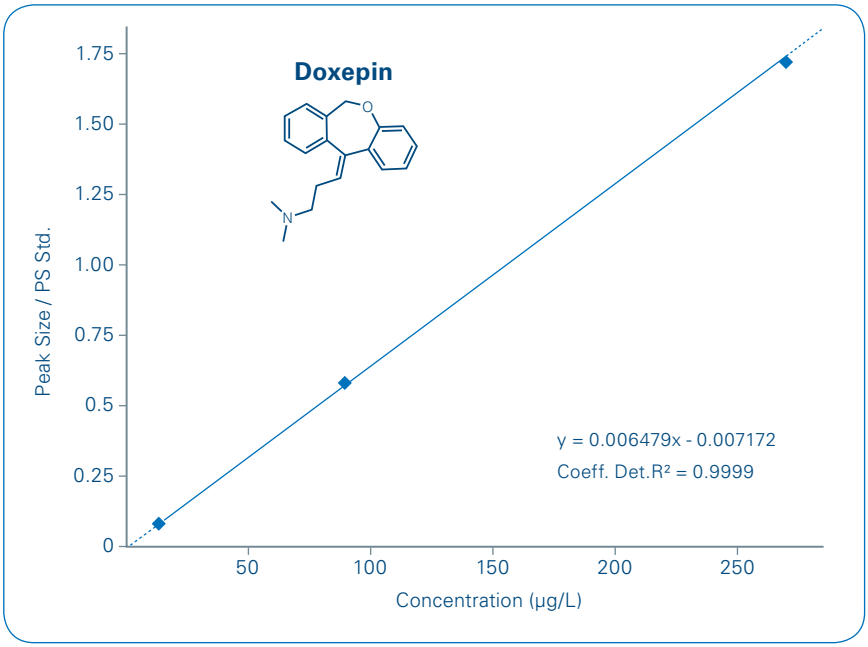

Figure 2: Calibration curve of doxepin. Calibration range 13.4 – 270 µg/L

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Further Reading

To learn more about the EVOQ LC-TQ Elite MS/MS and Elute LC systems, please see:
https://www.bruker.com/products/mass-spectrometry-and-separations/lc-ms/evoq/overview.html
https://www.bruker.com/products/mass-spectrometry-and-separations/lc-ms/liquid-chromatography/elute-lc-series/overview.html

Please see Application Notes LCMS-138, LCMS-139, LCMS-145, and LCMS-146 for additional examples of quantitation of clinically relevant drug panels using the ClinMass TDM platform.

Conclusion

- The Bruker Elute UHPLC coupled to the EVOQ LC-TQ Elite MS/MS system and the ClinMass TDM kit provide a quick and reliable method to easily detect and quantitate 15 triycyclic antidepressants and atypic neuroleptics in serum.
- Low sample requirements (50 µl serum), easy preparation, and short run time (3.8 minutes) support high sample throughput. Linearity of calibration, precision, and accuracy were outstanding, supporting the use of this combined system in clinical research workflows.

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