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

This study demonstrates a simple, rapid, and reliable method for the simultaneous detection and quantitation of 28 neuroleptics in serum using the Bruker Elute™ UHPLC coupled to the EVOQ LC-TQ Elite triple quadrupole MS/MS system. Sample preparation was performed with protein precipitation using the ClinMass® TDM kit system.

Introduction

Neuroleptics are psychoactive drugs used for the treatment of psychoses such as hallucinations or paranoia, as well as schizophrenia and bipolar disorder. They are also applied as sedatives against restlessness or anxiety, especially for elderly people in continuous care facilities. They are the second most prescribed class of psychotropic drugs following antidepressants. Several substance groups, including phenothiazines, butyrophenones and dibenzazepines, are classified as neuroleptics.

Keywords:
Neuroleptics, serum, quantitation, therapeutic drug monitoring Clinical Research, Forensic Toxicology, EVOQ LC-TQ MS/MS, Elute UHPLC

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The acute onset effect of neuroleptic drugs correlates with blood level concentrations. To ensure the correct dosage, it is important to accurately monitor the concentration of neuroleptics in blood. Due to the potential for side effects such as sedation and extrapyramidal symptoms (drug-induced movement disorder), neuroleptics can have a negative effect on everyday activities, including driving ability. Thus, they are also a class of drugs of interest in criminal and forensic toxicology.

This manuscript describes a UHPLC-triple quadrupole mass spectrometry method to rapidly and reliably detect and quantify 28 neuroleptics in human serum.

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, auto-sampler washing solution, precipitation reagent, and the HPLC column with prefilter. Serum calibrators and quality control serum samples were from the ClinMass Add-On Set for 28 Neuroleptics, also provided by RECIPE.

Sample Preparation

Following the ClinMass kit protocol, 100 µL of precipitation reagent containing 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 – 0.3 min		5 % B
	0.3 – 0.75 min	to	36 % B
	0.75 – 1.5 min		36 % B
	1.5 – 3.0 min	to	39 % B
	3.0 – 4.5 min	to	65 % B
	4.5 – 4.6 min	to	80 % B
	4.6 – 4.8 min		80 % B
	4.8 – 4.9 min	to	5 % B
	4.9 – 5.5 min		5 % B
Flow Rate	650 µL/min		
Injection Volume	5 µL		
Column Oven	40°C		

Mass Spectrometer 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	25 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
Amisulprid	1.09	370.1	241.9	22	195.9	37	36.1 – 721	1.0000
Aripiprazol	4.67	448.0	285.0	24	176.0	29	54.7 – 1105	0.9998
Chlorpromazin	3.71	319.0	86.1	16	58.3	21	17.7 – 375	0.9988
Chlorprothixen	4.22	316.0	270.9	16	230.9	26	17.6 – 356	0.9976
Clozapin	2.93	327.0	269.9	19	192.0	41	56.9 – 1185	0.9987
Dehydroaripiprazol	4.38	446.1	284.9	19	98.1	34	9.85 – 191	1.0000
Desmethylolanzapin	1.40	299.0	255.9	19	197.9	34	7.07 – 152	0.9994
Flupentixol	4.28	435.1	304.9	27	264.8	37	0.622 – 13.1	0.9986
Fluphenazin	4.10	438.1	171.0	22	143.1	27	0.605 – 12.6	1.0000
Haloperidol	2.18	376.0	165.0	20	123.0	31	0.646 – 13.0	1.0000
Levomepromazin	3.29	329.0	100.1	16	58.3	20	9.49 – 203	0.9997
Melperon	1.54	264.1	165.1	16	123.0	29	10.4 – 212	0.9987
Norclozapin	1.68	313.0	192.0	38	269.9	20	45.9 – 1020	0.9997
Norquetiapin	1.85	296.0	209.9	27	139.0	52	9.16 – 185	1.0000
Olanzapin	1.83	313.0	256.0	18	197.9	38	7.26 – 151	0.9999
Paliperidon	1.42	427.1	207.0	24	110.1	36	6.67 – 136	1.0000
Perazin	3.49	340.1	141.1	17	113.1	23	25.5 – 535	0.9998
Pipamperon	1.52	376.1	98.1	23	291.0	15	30.9 – 636	0.9985
Promethazin	3.07	285.0	86.2	13	197.9	21	20.4 – 406	0.9986
Prothipendyl	1.98	286.0	240.9	13	212.9	26	1.06 – 22.0	0.9998
Quetiapin	2.52	384.1	252.9	19	221.0	33	29.4 – 571	1.0000
Risperidon	1.62	411.1	191.0	25	110.1	40	6.60 – 142	0.9995
Sertindol	3.88	441.1	113.1	26	70.2	41	10.5 – 215	0.9998
Sulpirid	0.89	342.0	112.1	22	213.9	29	55.6 – 1172	0.9991
Thioridazin	4.21	371.0	126.1	19	98.1	22	20.5 – 475	0.9968
Ziprasidon	3.57	413.0	193.9	25	166.0	42	17.5 – 350	1.0000
Zotepin	4.69	331.9	72.2	9	44.3	35	8.73 – 178	0.9991
Zuclopenthixol	3.81	401.0	230.9	34	100.1	22	3.66 – 77.8	0.9996

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

Results and Discussion

Following simple and rapid sample preparation using 50 µl of serum, the chromatographic separation of the 28 neuroleptics was performed in 5.5 minutes by the Elute UHPLC system. Overlaid MRM traces for all target drugs are shown in Figure 1. The quantitation of the analytes was performed using 25 internal standards.

Calibration curves included three calibrator levels and provided excellent linearity with R^2 values from 0.9976 – 1.0000 (Table 2). The calibration curve of fluphenazin is shown in Figure 2 as an example. 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 underscores the very good precision of the instrumentation used. The experiments showed also a high accuracy with a bias within ± 7% for all analytes, with the exception of dehydroaripiprazol, with a bias of ± 14%. 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 [%]**
Amisulprid	138	135	-1.9	1.0	323	320	-1.0	0.7
Aripiprazol	216	213	-1.4	1.8	494	528	6.9	4.0
Chlorpromazin	69.4	70.7	1.8	4.1	167	164	-1.7	1.8
Chlorprothixen	66.0	68.9	4.4	1.9	156	157	0.9	3.3
Clozapin	224	232	3.4	2.0	524	552	5.4	1.4
Dehydroaripiprazol	37.4	32.2	-13.9	2.2	86.8	77.3	-11.0	2.1
Desmethylolanzapin	28.3	29.0	2.6	3.0	66.8	68	1.8	1.1
Flupentixol	2.34	2.36	0.7	3.7	5.65	5.52	-2.3	2.2
Fluphenazin	2.34	2.22	-4.9	1.8	5.56	5.20	-6.5	3.6
Haloperidol	2.52	2.49	-1.2	1.1	6.08	5.87	-3.4	1.7
Levomepromazin	36.8	37.7	2.4	3.4	89.2	87.1	-2.3	1.8
Melperon	38.3	40.6	6.0	0.6	91.0	95.0	4.5	1.9
Norclozapin	188	190	0.9	2.0	445	455	2.4	2.6
Norquetiapin	34.9	33.4	-4.3	5.5	81.9	80.4	-1.8	5.0
Olanzapin	28.0	27.8	-0.7	0.2	66.5	66.1	-0.5	0.9
Paliperidon	25.3	25.5	0.9	1.4	60.2	61.3	1.8	0.8
Perazin	99.4	97.6	-1.8	1.1	233	240	2.9	3.3
Pipamperon	120	121	1.0	1.7	283	284	0.4	3.5
Promethazin	75.9	77.1	1.5	3.4	180	185	2.6	3.3
Prothipendyl	4.00	4.06	1.6	3.1	9.42	9.63	2.2	2.7
Quetiapin	117	109	-6.5	1.5	269	256	-4.7	2.1
Risperidon	25.5	25.3	-0.8	1.8	58.1	61.2	5.4	2.1
Sertindol	40.9	40.1	-2.0	0.9	96.1	96.9	0.8	1.3
Sulpirid	214	221	3.2	2.4	512	516	0.8	2.1
Thioridazin	81.4	84.5	3.8	2.1	199	201	1.1	1.7
Ziprasidon	65.2	67.3	3.2	3.7	157	159	1.0	5.7
Zotepin	34.1	32.6	-4.3	1.5	78.0	79.5	2.0	4.9
Zuclopenthixol	14.6	14.1	-3.7	3.4	33.4	33.9	1.4	1.5

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

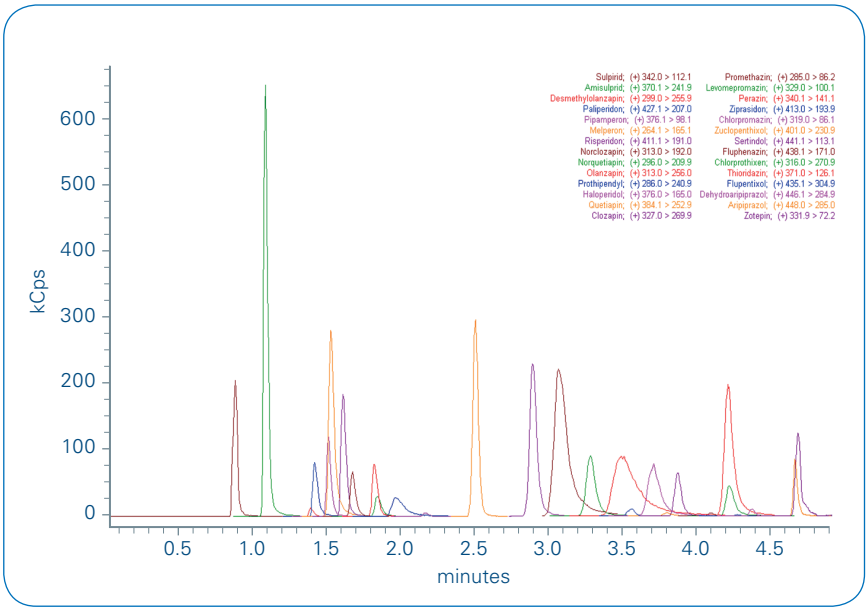

Figure 1: Overlaid MRM traces of all analytes (lowest calibrator level)

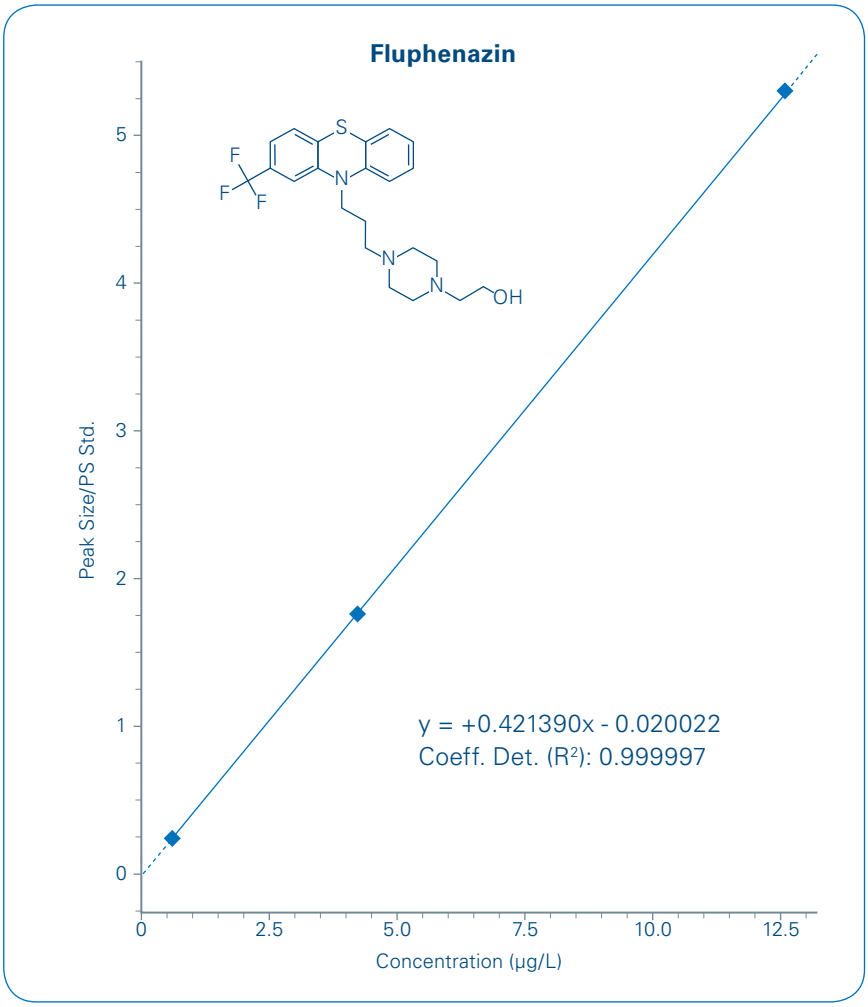

Figure 2: Calibration curve of fluphenazin. Calibration range 0.605 – 12.6 µg/L

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Conclusion

The Bruker Elute UHPLC coupled to the EVOQ LC-TQ Elite triple quadruple MS/MS system in conjunction with the ClinMass TDM kit system provides a quick and reliable method to easily detect and quantitate 28 neuroleptics in serum.

Low sample requirements (50 µl serum), easy preparation, and short run time (5.5 minutes) support high sample throughput. Linearity of calibration, precision and accuracy were outstanding, supporting the use of this combined system in clinical research and toxicological workflows.

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