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Liquid Chromatograph Mass Spectrometer LCMS-8060

Application News

Analysis of Neuroleptics in Serum / Plasma Using RECIPE® ClinMass® TDM Kit System with Fully Automated Sample Preparation LC/MS/MS System

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User Benefits

◆ Full solution provided by Shimadzu and RECIPE®
◆ Fully automated sample preparation
◆ Verified method for RECIPE® ClinMass® TDM Kit System for Neuroleptics in Serum / Plasma

■ Introduction

Neuroleptics are psychoactive drugs used primarily for the treatment of psychoses, especially schizophrenia. They help reduce symptoms such as hallucinations and delusions, ultimately decreasing patient suffering. Neuroleptics are categorized into groups like phenothiazines, butyrophenones, dibenzazepines, and atypical neuroleptics. The effectiveness of these drugs correlates with their blood concentration, but higher doses can lead to adverse effects such as apathy, delirium, or even death. Therapeutic drug monitoring (TDM) is recommended to optimize drug levels and minimize side effects, particularly in cases of uncertain adherence, suboptimal tolerability, or drug interactions.

RECIPE's fully validated analytical method provides the quantification of 28 Neuroleptics and metabolites (Table 2) for TDM using LC-MS/MS.[1]

By addition of the Shimadzu CLAM (Clinical Laboratory Automated sample preparation Module) in front of the LC-MS/MS system (Figure 1) the required sample preparation could be fully automated which achieves results on a fast and high-precision analytical workflow.

To prove that the automated sample preparation leads to reliable results a method verification procedure was evaluated according to the CLSI Guidelines EP06-A, EP15-A3, EP17-A2.

Fig. 1 CLAM LCMS TQ

■ Materials and Methods

Fast, sensitive and robust LC-MS/MS systems provide the basis for routine analysis in clinical laboratories. For the described verification, a Shimadzu CLAM-2040 coupled with a Nexera X3 UHPLC system and a LCMS-8060 triple-quadrupole mass spectrometer was used.

Twenty-eight Neuroleptics in serum were verified using the ClinMass® TDM Platform (order no. MS9000) in combination with the ClinMass® Add-on Set for Neuroleptics in Serum / Plasma (order no. MS9300) (RECIPE®,Germany).

Lyophilized, matrix-based calibrator and control samples were reconstituted, aliquoted and stored until use.

Then the samples were loaded directly into the CLAM-2040. It was programmed to perform protein precipitation using Precipitant P including internal standards from the ClinMass® TDM Kit System for Neuroleptics followed by filtration and sample collection. The sample is then transported using an arm from the CLAM-2040 to the LC without human intervention for LC-MS/MS analysis. Due to overlapped sample preparation (Figure 2) and analysis the throughput was one complete analysis each 7.46 min. Analytical conditions are listed in Table 1. The optimized MRM transitions are summarized in Table 2.
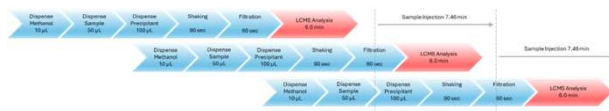

Fig. 2 Scheme fully automated sample preparation and analysis

Table 1 Analytical conditions

Mass Spectrometer	: LCMS-8060
Ionization	: Electrospray Ionization (ESI), positive
Interface Voltage	: 1 kV
Heating Gas	: 10 L/min
DL Temp.	: 250 ℃
Interface Temp.	: 300 ℃
Nebulizing Gas	: 3 L/min
Drying Gas	: 10 L/min
Heat Block	: 400 ℃
CID	: 270 kPa
UHPLC	: Nexera X3
Column Oven	: 40 ℃
Injection Volume	: 3.0 µL
Flow rate	: 0.65 mL/min
Time Programme	: Binary gradient

Time [min]	Flow [mL/min]	Mobile Phase A [%]	Mobile Phase B [%]
Initial	0.65	95	5
0.03	0.65	95	5
0.75	0.65	64	36
1.50	0.65	64	36
3.00	0.65	61	39
4.50	0.65	35	65
4.60	0.65	20	80
4.80	0.65	20	80
4.90	0.65	95	5
5.50	0.65	95	5
5.51	0.00	95	5
6.00	0.00	95	5

Table 2 MRM transitions and parameters of the analytes and isotope-labelled substances

Analyte / IS	Quantifier MRM		Dwell Time	Q1 Pre Bias	CE	Q3 Pre Bias
	Precursor [m/z]	Product [m/z]	msec	[V]	[V]	[V]
Amisulpride	370	242.1	10	-11	-50	-16
Aripiprazole	448	285.1	10	-13	-45	-19
Chlorpromazine	318.9	86.1	10	-17	-21	-15
Chlorprothixene	315.9	271	10	-16	-35	-18
Clozapine	326.9	192,1	10	-17	-47	-18
Dehydro-Aripiprazole	446	285.1	10	-13	-44	-13
Desmethylolanzapine	298.9	256.1	10	-15	-24	-17
Flupenthixol	435	305.1	10	-13	-29	-20
Fluphenazine	438	171.2	50	-13	-29	-17
Haloperidol	376	165.1	10	-11	-25	-16
Levomepromazine	329	100.1	50	-10	-21	-18
Melperone	264	165.1	10	-14	-35	-16
Norclozapine	313	192.1	10	-16	-27	-19
Norquetiapine	295.9	210.1	10	-15	-13	-21
Olanzapine	313	256.1	50	-16	-23	-17
Paliperidone (9-OH-Risperidone)	427	207.1	10	-13	-10	-21
Perazine	340	141.2	50	-18	-21	-14
Pipamperone	376	98.1	10	-19	-10	-17
Promethazine	285	86.1	50	-15	-18	15
Prothipendyl	286	241.1	50	-15	-17	-16
Quetiapine	384	221.1	10	-20	-10	-14
Risperidon	411	191.2	10	-12	-10	-19
Sertindole	441	113.1	10	-13	-17	-11
Sulpiride	342	112.1	10	-17	-10	-11
Thioridazine	371	126.2	50	-11	-23	-12
Ziprasidone	412.9	166.1	10	-21	-32	-17
Zotepine	331.9	72.1	10	-30	-50	-10
Zuclopenthixole	400.9	231	10	-12	-34	-23
d5-Amisulpride	375	242.1	10	-11	-50	-16
d8-Aripiprazole	456	293.1	10	-13	-45	-19
d6-Chlorpromazine	324.9	92.1	10	-17	-21	-15
d6-Chlorprothixene	321.9	271	10	-16	-30	-18
d4-Clozapine	330.9	192.1	10	-17	-20	-19
d8-Dehydroaripiprazole	454	293.1	10	-13	-35	-13
d4-Flupentixol	439	305.1	10	-13	-29	-20
d8-Fluphenazine	446	179.2	50	-13	-26	-17
d4-Haloperidol	380	165.1	10	-11	-25	-16
d3-Levomepromazine	332	103.1	50	-10	-21	-18
d4-Melperone	268	165.1	10	-14	-30	-16
d8-Norclozapine	321	192.1	10	-16	-40	-19
d3-Olanzapine	316	256.1	50	-16	-23	-17
d4-Paliperidone	431	211.1	10	-13	-50	-21
d8-Perazine	348	149.2	50	-18	-21	-14
d10-Pipamperone	386	98.1	10	-19	-17	-17
d6-Promethazine	291	92.1	50	-15	-18	-15
d6-Prothipendyl	292	241.1	50	-15	-17	-16
d8-Quetiapine	392	226.1	10	-20	-22	-14
d4-Risperidone	415	195.2	10	-12	-17	-19
d4-Sertindole	445	117.1	10	-13	-20	-11
d3-Sulpiride	345	112.1	10	-17	-10	-11
d3-Thioridazine	374	129.2	50	-11	-23	-12
d8-Ziprasidone	420.9	166.1	10	-21	-35	-17
d4-Zuclopenthixol	404.9	231	10	-12	-34	-23

■ Results

The trueness was determined by 4-fold analysis of two different quality control (QC) samples in a single analysis sequence. The results (precision in CV% and deviation from the target in % Bias) are summarized in Table 3.

To determine the precision two different levels of QC samples were prepared in 8-fold and analyzed in a single analysis sequence. The intraassay precision for each level is summarized in Table 4.

For determination of the linearity and the lower limit of quantification (LLOQ) several dilutions of the ClinCal® Serum Calibrator Set lyophil. for Neuroleptics (order no. MS9313, RECIPE® ,Germany) were prepared in 3-fold and analyzed in a single analysis sequence. The results for linearity evaluation and for the LLOQ are summarized in Table 5.

Table 3 Trueness of measurement

Analyte	Sample	Target value [µg/L]	Measured value [µg/L]	CV	Bias
			Mean (n=4)	[%]	[%]
Amisulpride	MS9382, Level I	141	143	5.1	1.6
	MS9382, Level II	325	323	1.3	-0.5
Aripiprazole	MS9382, Level I	222	231	2.8	4.3
	MS9382, Level II	507	499	1.9	-1.5
Chlorpromazine	MS9382, Level I	71.1	74.7	6.4	5.0
	MS9382, Level II	169	170	1.5	0.7
Chlorprothixene	MS9382, Level I	69.3	71.2	4.7	2.8
	MS9382, Level II	162	164	1.8	1.0
Clozapine	MS9382, Level I	244	252	2.9	3.1
	MS9382, Level II	570	567	1.0	-0.6
Dehydro-Aripiprazole	MS9382, Level I	43.2	44.8	1.8	3.7
	MS9382, Level II	104	100	0.8	-3.5
Desmethylolanzapine	MS9382, Level I	25.3	22.5	2.3	-11.1
	MS9382, Level II	64.2	55.2	4.3	-14.0
Flupenthixol	MS9382, Level I	2.46	2.50	5.6	1.5
	MS9382, Level II	6.12	5.66	4.3	-7.5
Fluphenazine	MS9382, Level I	2.53	2.55	4.0	0.7
	MS9382, Level II	5.82	5.82	1.5	0.0
Haloperidol	MS9382, Level I	2.51	2.56	3.4	1.9
	MS9382, Level II	5.99	5.83	1.8	-2.7
Levomepromazine	MS9382, Level I	50.2	50.5	3.8	0.7
	MS9382, Level II	119	118	2.1	-0.5
Melperone	MS9382, Level I	45.8	45.5	4.0	-0.7
	MS9382, Level II	108	107	2.6	-1.3
Norclozapine	MS9382, Level I	182	192	4.8	5.6
	MS9382, Level II	425	428	2.6	0.6
Norquetiapine	MS9382, Level I	70.5	73.6	6.1	4.4
	MS9382, Level II	167	167	2.1	-0.1
Olanzapine	MS9382, Level I	30.1	28.9	2.2	-4.0
	MS9382, Level II	72.3	66.4	1.7	-8.2
Paliperidone	MS9382, Level I	27.7	28.3	5.9	2.1
	MS9382, Level II	63.7	63.3	1.7	-0.7
Perazine	MS9382, Level I	99.6	98.7	3.5	-0.9
	MS9382, Level II	226	227	1.5	0.6
Pipamperone	MS9382, Level I	120	131	4.3	8.8
	MS9382, Level II	288	299	4.4	3.8
Promethazine	MS9382, Level I	22.9	23.2	2.7	1.5
	MS9382, Level II	52.3	54.4	1.7	4.0
Prothipendyl	MS9382, Level I	10.8	10.9	2.6	0.7
	MS9382, Level II	25.2	25.0	0.4	-0.6
Quetiapine	MS9382, Level I	155	152	5.0	-2.0
	MS9382, Level II	354	337	1.9	-4.9
Risperidone	MS9382, Level I	27.3	28.5	2.4	4.5
	MS9382, Level II	65.7	63.7	1.8	-3.0
Sertindole	MS9382, Level I	40.4	41.5	3.6	2.7
	MS9382, Level II	94.7	95.1	1.4	0.4
Sulpiride	MS9382, Level I	222	232	4.7	4.5
	MS9382, Level II	529	521	1.9	-1.6
Thioridazine	MS9382, Level I	83.0	89.8	4.3	8.2
	MS9382, Level II	197	205	2.7	4.2
Ziprasidone	MS9382, Level I	66.2	69.2	3.6	4.5
	MS9382, Level II	156	158	2.0	1.4
Zotepine	MS9382, Level I	35.3	37.9	2.1	7.3
	MS9382, Level II	81.6	84.1	1.3	3.0
Zuclopenthixole	MS9382, Level I	14.4	14.1	3.3	-1.9
	MS9382, Level II	32.7	33.2	1.2	1.5

Table 4 Intraassay results [CV%]

Analyte	Sample	Measured value [µg/L]	CV
		Mean (n=8)	[%]
Amisulpride	MS9382, Level I	143	4.0
	MS9382, Level II	326	1.6
Aripiprazole	MS9382, Level I	224	4.5
	MS9382, Level II	510	2.7
Chlorpromazine	MS9382, Level I	74.1	4.6
	MS9382, Level II	170	2.2
Chlorprothixene	MS9382, Level I	69.9	4.1
	MS9382, Level II	164	1.7
Clozapine	MS9382, Level I	250	2.9
	MS9382, Level II	573	2.0
Dehydro-Aripiprazole	MS9382, Level I	44.0	3.0
	MS9382, Level II	101.4	1.8
Desmethylolanzapine	MS9382, Level I	21.8	4.2
	MS9382, Level II	55.1	4.3
Flupenthixol	MS9382, Level I	2.44	5.1
	MS9382, Level II	5.72	3.3
Fluphenazine	MS9382, Level I	2.47	5.1
	MS9382, Level II	5.92	3.9
Haloperidol	MS9382, Level I	2.51	3.9
	MS9382, Level II	5.80	2.0
Levomepromazine	MS9382, Level I	49.2	4.2
	MS9382, Level II	120	4.0
Melperone	MS9382, Level I	45.8	4.0
	MS9382, Level II	107	2.2
Norclozapine	MS9382, Level I	192	3.5
	MS9382, Level II	435	2.7
Norquetiapine	MS9382, Level I	73.7	4.5
	MS9382, Level II	167	1.8
Olanzapine	MS9382, Level I	28.6	3.0
	MS9382, Level II	66.2	1.8
Paliperidone	MS9382, Level I	27.4	5.5
	MS9382, Level II	62.6	1.7
Perazine	MS9382, Level I	98.9	3.8
	MS9382, Level II	228	1.7
Pipamperone	MS9382, Level I	130.6	3.3
	MS9382, Level II	301.5	3.1
Promethazine	MS9382, Level I	23.3	2.5
	MS9382, Level II	54.2	1.7
Prothipendyl	MS9382, Level I	10.9	2.6
	MS9382, Level II	25.0	1.1
Quetiapine	MS9382, Level I	151	4.4
	MS9382, Level II	338	2.2
Risperidone	MS9382, Level I	28.3	2.4
	MS9382, Level II	64.5	3.4
Sertindole	MS9382, Level I	41.4	2.8
	MS9382, Level II	95.6	1.3
Sulpiride	MS9382, Level I	230	4.4
	MS9382, Level II	526	2.0
Thioridazine	MS9382, Level I	88.7	3.5
	MS9382, Level II	207	2.8
Ziprasidone	MS9382, Level I	69.1	3.3
	MS9382, Level II	158	2.1
Zotepine	MS9382, Level I	37.6	2.3
	MS9382, Level II	85.8	2.5
Zuclopenthixole	MS9382, Level I	14.0	3.7
	MS9382, Level II	32.7	2.2

Table 5 Linearity evaluation, including LLOQ / LOD

Analyte	Linear Range [µg/L]	R²	LLOQ [µg/L]	LOD [µg/L]
Amisulpride	18.1 - 760	0.997	18.1	6.02
Aripiprazole	51.6 - 1193	0.995	51.6	17.2
Chlorpromazine	18.0 - 404	1.000	18.0	6.00
Chlorprothixene	17.0 - 380	0.999	17.0	5.67
Clozapine	56.0 - 1320	0.999	56.0	18.7
Dehydro-Aripiprazole	10.5 - 246	0.997	10.5	3.50
Desmethylolanzapine	6.22 - 143	0.994	6.22	2.07
Flupenthixol	0.338 - 13.7	0.997	0.338	0.113
Fluphenazine	0.608 - 13.6	0.993	0.608	0.200
Haloperidol	0.604 - 13.8	0.997	0.604	0.200
Levomepromazine	6.00 - 280	0.999	6.00	2.00
Melperone	6.05 - 254	0.997	6.05	2.02
Norclozapine	44.5 - 971	0.998	44.5	14.8
Norquetiapine	18.3 - 379	0.996	18.3	6.10
Olanzapine	7.36 - 142	0.996	7.36	2.45
Paliperidone	3.32 - 147	0.997	3.32	1.11
Perazine	12.3 - 530	0.998	12.3	4.10
Pipamperone	31.5 - 686	0.996	31.5	10.5
Promethazine	2.88 - 128	0.999	2.88	0.96
Prothipendyl	2.65 - 59.8	0.998	2.65	0.883
Quetiapine	9.65 - 760	0.997	9.65	3.22
Risperidon	6.69 - 156	0.999	6.69	2.23
Sertindole	9.71 - 228	0.994	9.71	3.24
Sulpiride	54.3 - 1215	0.992	54.3	18.1
Thioridazine	19.8 - 483	0.998	19.8	6.60
Ziprasidone	16.9 - 363	0.999	16.9	5.63
Zotepine	8.80 - 195	0.998	8.80	2.93
Zuclopenthixole	3.38 - 79.1	0.993	3.38	1.13

■ Conclusion

The ClinMass® TDM Kit System for Neuroleptics in Serum / Plasma (order no. MS9000 and MS9300) was successfully verified on the CLAM-2040 with the analytical system LCMS-8060 from Shimadzu.

All 28 analytes passed the acceptance criteria for accuracy (trueness, precision) and linearity.

The lower limit of quantification (LLOQ) was below published clinical reference ranges.

■ References

1. Instruction Manual, ClinMass® TDM Kit System, Neuroleptics in Serum / Plasma, RECIPE® Chemicals + Instruments GmbH

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU Europa GmbH,
www.shimadzu.eu