TECHNICAL NOTE 000114

Quantification of 28 neuroleptics in human plasma by LC-HRAM-MS for clinical research

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Application benefits

- Simple offline sample preparation by protein precipitation
- Increased accuracy of method by implementation of a comprehensive ClinMass® kit for sample preparation
- Robust, sensitive hardware enables increased confidence in data
- Quantification of 28 neuroleptics in a single 6-minute runtime

Goal

Implementation of an analytical method for the quantification of 28 neuroleptics in human plasma on a Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer coupled with a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system.

Introduction

Neuroleptics belong to the class of antipsychotic drugs and are used to manage symptoms of psychoses, in particular schizophrenia. Since antipsychotic drugs are very potent, they are usually administered at low daily dosages. While most antipsychotic drugs are found in plasma in the low ng/mL range, concentrations for some antipsychotics are often found to be very low in brain tissue.

While high performance liquid chromatography (HPLC) with UV detection is one widely used technology for measurement of these drugs, identification and quantitation of antipsychotics at low concentrations require a technology that can offer high resolution (to separately identify every analyte with confidence) along with high

sensitivity. High-resolution accurate-mass (HRAM) mass spectrometers coupled to UHPLC offer the necessary selectivity and specificity, while providing the required sensitivity.

An analytical method for clinical research for the quantification of 28 neuroleptic drugs in human plasma or serum is reported in this study. This report demonstrates the capability of HRAM mass spectrometry for routine quantitation analyses in addition to its use for performing in-depth qualitative investigations.

Samples were processed by protein precipitation and injected onto a Vanquish Flex Binary UHPLC system for chromatographic separation. Detection was performed on an Orbitrap Exploris 120 mass spectrometer with heated electrospray ionization (HESI) operated in positive ion mode. Method performance was evaluated using the ClinMass™ TDM Platform with the ClinMass Add-On Set for Neuroleptics in Serum/Plasma from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response, lower limit of quantitation (LLOQ), carryover, accuracy, and intra- and inter-assay precision for all analytes.

Experimental

Target analytes

The complete list of analytes and corresponding internal standards is reported in Table 1. The retention times obtained and the concentration ranges covered by the calibrators used (MS9313 batch #2230) are reported in Table 2.

Table 1. List of analytes and internal standards

Compound name	Chemical formula	Expected mass (*m/z*)	Internal standard name	Chemical formula	Expected mass (*m/z*)
Amisulpride	$C_{17}H_{27}N_3O_4S$	370.1795	d_5-Amisulpride	$C_{17}H_{22}D_5N_3O_4S$	375.2109
Aripiprazole	$C_{23}H_{27}Cl_2N_3O_2$	448.1553	d_8-Aripiprazole	$C_{23}H_{19}D_8Cl_2N_3O_2$	456.2055
Chlorpromazine	$C_{17}H_{19}ClN_2S$	319.10302	d_6-Chlorpromazine	$C_{17}H_{13}D_6ClN_2S$	325.1407
Chlorprothixene	$C_{18}H_{18}ClNS$	316.0921	d_6-Chlorprothixene	$C_{18}H_{12}D_6ClNS$	322.1298
Clozapine	$C_{18}H_{19}ClN_4$	327.1371	d_4-Clozapine	$C_{18}H_{15}D_4ClN_4$	331.1622
Dehydro-Aripiprazole	$C_{23}H_{25}Cl_2N_3O_2$	446.1397	d_8-Dehydroaripiprazole	$C_{23}H_{17}D_8Cl_2N_3O_2$	454.1899
Desmethylolanzapine	$C_{16}H_{18}N_4S$	299.1325	d_3-Olanzapine	$C_{17}H_{17}D_3N_4S$	316.1670
Flupentixol	$C_{23}H_{25}F_3N_2OS$	435.1713	d_4-Flupentixol	$C_{23}H_{21}D_4F_3N_2OS$	439.1964
Fluphenazine	$C_{22}H_{26}F_3N_3OS$	438.1821	d_8-Fluphenazine	$C_{22}H_{18}D_8F_3N_3OS$	446.2324
Haloperidol	$C_{21}H_{23}ClFNO_2$	376.1474	d_4-Haloperidol	$C_{21}H_{19}D_4ClFNO_2$	380.1725
Levomepromazine	$C_{19}H_{24}N_2OS$	329.1682	d_3-Levomepromazine	$C_{19}H_{21}D_3N_2OS$	332.1870
Melprone	$C_{16}H_{22}FNO$	264.1758	d_4-Melperone	$C_{16}H_{18}D_4FNO$	268.2009
Norclozapine	$C_{17}H_{17}ClN_4$	313.1215	d_8-Norclozapine	$C_{17}H_9D_8ClN_4$	321.1717
Norquetiapine	$C_{17}H_{17}N_3S$	296.1216	d_8-Quetiapine	$C_{21}H_{17}D_8N_3O_2S$	392.2242
Olanzapine	$C_{17}H_{20}N_4S$	313.1481	d_3-Olanzapine	$C_{17}H_{17}D_3N_4S$	316.1670
Paliperidone	$C_{23}H_{27}FN_4O_3$	427.2140	d_4-Paliperidone	$C_{23}H_{23}D_4FN_4O_3$	431.2391
Perazine	$C_{20}H_{25}N_3S$	340.1842	d_8-Perazine	$C_{20}H_{17}D_8N_3S$	348.2344
Pipamperone	$C_{21}H_{30}FN_3O_2$	376.2395	d_{10}-Pipamperone	$C_{21}H_{20}D_{10}FN_3O_2$	386.3023
Promethazine	$C_{17}H_{20}N_2S$	285.1420	d_6-Promethazine	$C_{17}H_{14}D_6N_2S$	291.1797
Prothipendyl	$C_{16}H_{19}N_3S$	286.1372	d_6-Prothipendyl	$C_{16}H_{13}D_6N_3S$	292.1749
Quetiapine	$C_{21}H_{25}N_3O_2S$	384.1740	d_8-Quetiapine	$C_{21}H_{17}D_8N_3O_2S$	392.2242
Risperidone	$C_{23}H_{27}FN_4O_2$	411.2191	d_4-Risperidone	$C_{23}H_{23}D_4FN_4O_2$	415.2442
Sertindole	$C_{24}H_{26}ClFN_4O$	441.1852	d_4-Sertindole	$C_{24}H_{22}D_4ClFN_4O$	445.2103
Sulpiride	$C_{15}H_{23}N_3O_4S$	342.1482	d_3-Sulpiride	$C_{15}H_{20}D_3N_3O_4S$	345.1670
Thioridazine	$C_{21}H_{26}N_2S_2$	371.1610	d_3-Thioridazine	$C_{21}H_{23}D_3N_2S_2$	374.1799
Ziprasidone	$C_{21}H_{21}ClN_4OS$	413.1198	d_8-Ziprasidone	$C_{21}H_{13}D_8ClN_4OS$	421.1700
Zotepine	$C_{18}H_{18}ClNOS$	332.0870	d_8-Aripiprazole	$C_{23}H_{19}D_8Cl_2N_3O_2$	456.2055
Zuclopenthixol	$C_{22}H_{25}ClN_2OS$	401.1449	d_4-Zuclopenthioxol	$C_{22}H_{21}D_4ClN_2OS$	405.1700

Table 2. Concentration ranges covered by the calibrators (MS9313 batch #2230) and retention times

Analyte	Concentration range (µg/L)	Retention time (min)
Amisulpride	36.3–769	1.1
Aripiprazole	57.5–1234	4.5
Chlorpromazine	18.9–401	3.5
Chlorprothixene	19.1–420	4.0
Clozapine	59.5–1336	2.6
Dehydro-Aripiprazole	10.0–71.8	4.1
Desmethylolanzapine	7.31–154	1.4
Flupentixol	0.601–13.8	4.1
Fluphenazine	0.586–13.3	3.9
Haloperidol	0.632–12.9	2.1
Levomepromazine	13.0–282	3.1
Melprone	11.1–230	1.5
Norclozapine	44.8–995	1.6
Norquetiapine	18.6–376	1.8
Olanzapine	7.28–150	1.7
Paliperidone	6.77–143	1.4
Perazine	24.7–521	3.1
Pipamperone	30.3–632	1.5
Promethazine	5.99–125	2.8
Prothipendyl	2.61–56.9	1.9
Quetiapine	37.8–725	2.3
Risperidone	6.38–142	1.5
Sertindole	11.3–251	3.7
Sulpiride	59.6–1283	0.8
Thioridazine	19.5–476	4.1
Ziprasidone	17.8–367	3.2
Zotepine	10.3–215	4.5
Zuclopenthixol	3.83–82.4	3.5

Sample preparation

Reagents included four calibrators (including blank) and two controls from RECIPE (MS9382 batch #1279), as well as an internal standard mix (MS9312) for quantitation. Samples of 50 µL of plasma were protein precipitated using 100 µL of precipitating solution (MS9021) containing the internal standards. Precipitated samples were vortex-mixed and centrifuged for 5 minutes. 50 µL of the supernatant were transferred to a clean vial.

Liquid chromatography

The supernatant was injected via the autosampler of the Vanquish Flex Binary UHPLC system onto the analytical column and separated using the gradient shown in Table 3. Both mobile phase and analytical columns were provided by RECIPE. Data acquisition was done on the Orbitrap Exploris 120 mass spectrometer.

Details of the analytical method are reported in Table 3. Total runtime was 6 minutes.

Table 3. Liquid chromatographic conditions

Time (min)	Flow rate (mL/min)	B (%)
0.00	0.65	5
0.01	0.65	5
0.75	0.65	36
1.50	0.65	36
3.00	0.65	39
4.50	0.65	65
4.60	0.65	80
4.80	0.65	80
4.90	0.65	5
6.00	0.65	5

Phase A	MS9007
Phase B	MS9008
Column temperature (°C)	40
Injection volume (µL)	2

Mass spectrometry

Analytes and internal standards were detected by Full Scan – data-dependent MS^2 acquisition mode on an Orbitrap Exploris 120 mass spectrometer using a HESI ion source. The mass spectrometer was operated in positive ion mode. A summary of the MS conditions is reported in Table 4. Two fragments for each analyte were used for confirmation based on the average ion ratio of all samples.

Table 4. MS parameters

Ion source parameters	
Source type	Heated Electrospray Ionization (HESI)
Spray voltage – Positive (V)	3,500
Sheath gas (Arb)	60
Aux gas (Arb)	10
Sweep gas (Arb)	1
Ion transfer tube temp. (°C)	350
Vaporizer temp. (°C)	425
Settings	
Mild trapping	No
Internal mass calibration	RunStart EASY-IC™
Data acquisition mode	Full Scan – ddMS^2
Full scan parameters	
Resolution (at *m/z* 200)	60,000
Scan range *(m/z)*	200–500
Expected peak width (s)	6
RF lens (%)	80
AGC target	Standard (1e6)
Polarity	Positive
Data-dependent MS^2 scan properties	
Isolation window *(m/z)*	2
Collision energy type	Normalized
HCD collision energy (%)	30
Resolution (at *m/z* 200)	15,000
Scan range mode	Auto

Method evaluation

The method performance was evaluated in terms of linearity of response within the calibration ranges, LLOQ, carryover, accuracy, and intra- and inter-assay precision for all the analytes. To determine the LLOQ, the lowest calibrator was diluted down to 20-fold with blank matrix; a full set of calibrators (four levels), diluted calibrators (three levels), and controls (two levels) were extracted and injected in a single batch and all used for the linear interpolation.

The LLOQ was set as the lowest level that could be determined with a percentage coefficient of variation (%CV) < 20% across the entire batch of samples. Carryover was calculated in terms of percentage ratio between peak area of the highest calibrator and a blank sample injected immediately after it. Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using the quality control samples at two different levels provided by RECIPE. They were prepared and analyzed in replicates of five on three different days. Intra-assay precision for each day was evaluated in terms of percentage coefficient of variation (%CV) using the controls at two different levels in replicates of five (n=5). Inter-assay precision was evaluated as the %CV on the full set of samples (control samples at two levels in replicates of five prepared and analyzed on three different days).

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 5.1 software.

Results and discussion

A linear interpolation with 1/x weighting was used for all analytes. The percentage bias between nominal and back-calculated concentration was always within ±10% for all the calibrators in all the runs. Chromatograms of representative analytes and their internal standards at their respective lowest limit of quantitation are reported in Figure 1. Representative calibration curves are reported in Figure 2.

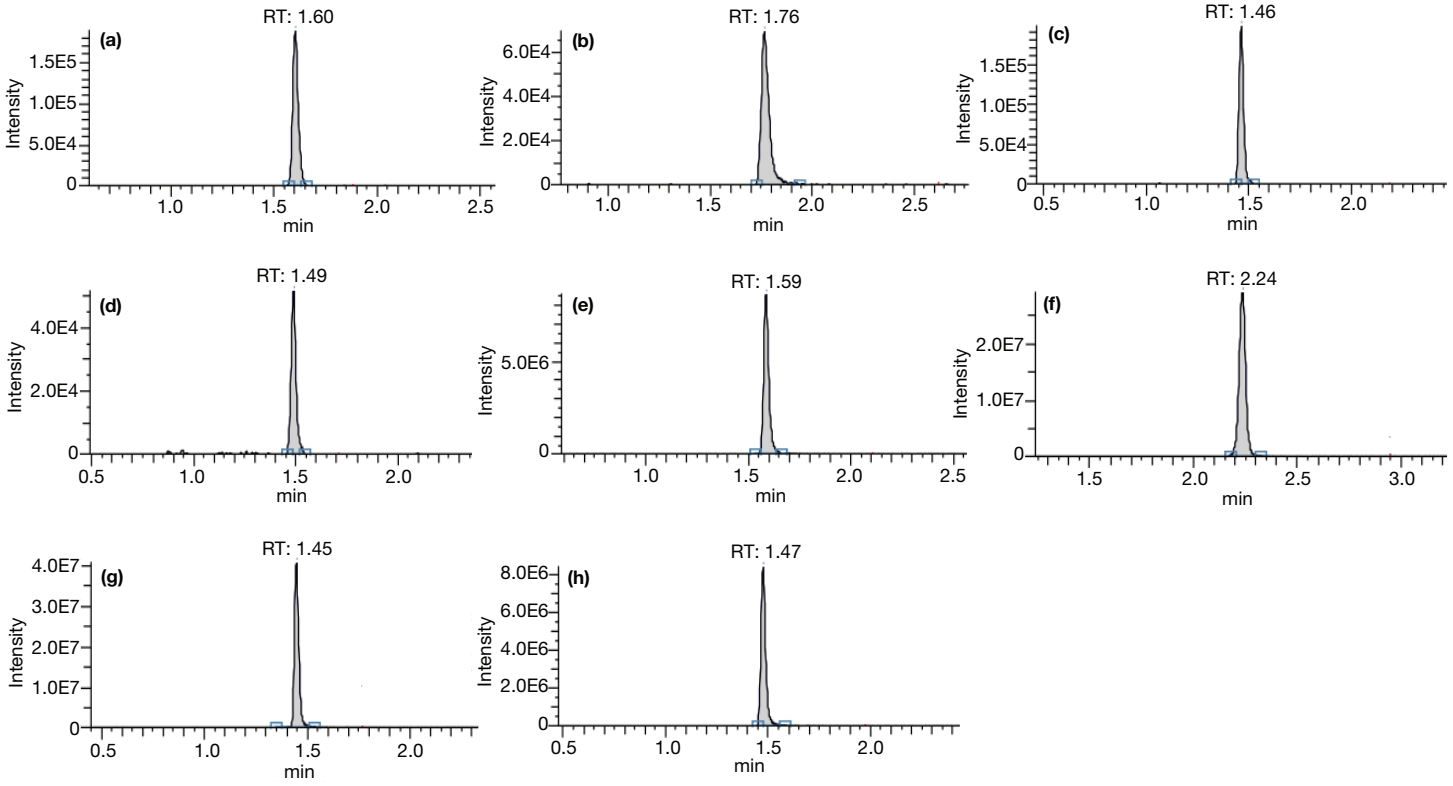

Figure 1. Representative chromatograms of the lower limit of quantification for (a) norclozapine, (b) norquetiapine, (c) pipamperone, (d) risperidone, (e) d_8-norclozapine, (f) d_8-quetiapine, (g) d_{10}-pipamperone, (h) d_4-risperidone

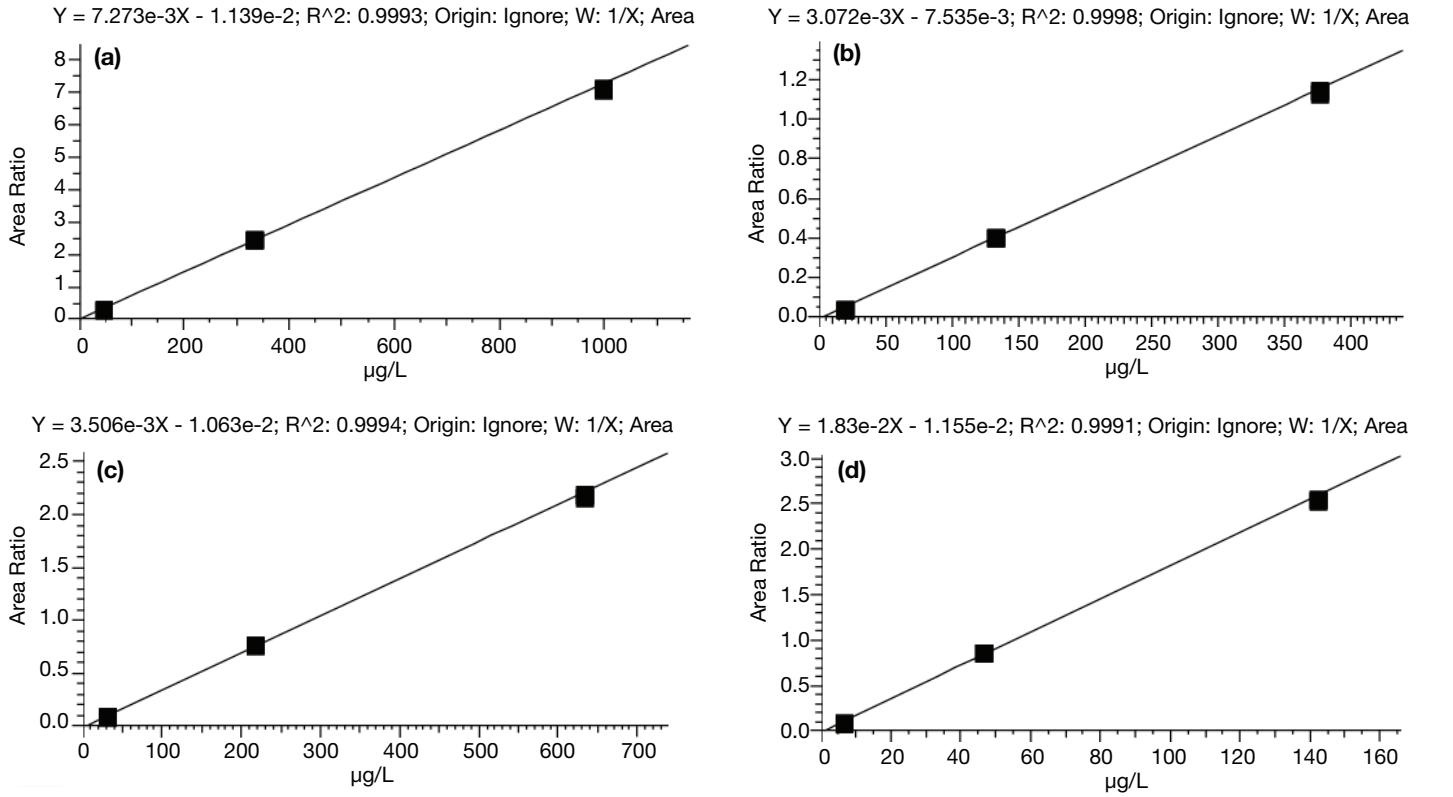

Figure 2. Representative calibration curves for (a) norclozapine, (b) norquetiapine, (c) pipamperone, (d) risperidone

No significant carryover was observed for any of the analytes, with no signal detected in the blank injected immediately after the highest calibrator.

The data demonstrated good accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the used control samples ranging between -6.7% and 9.0% (Table 5). The %CV for intra-assay precision was always below 12.5% for all the analytes. The maximum %CV for inter-assay precision including all the analytes was 8.3%. Results for intra- and inter-assay precision are reported in Table 6.

LLOQs of all compounds are reported in Table 7.

Table 5. Analytical accuracy results for control MS9382 batch #1279

Analyte	Control	Nominal conc. (µg/L)	Average calculated conc. (µg/L)	Bias (%)
Amisulpride	Level I	135	137	1.8
	Level II	317	320	1.0
Aripiprazole	Level I	212	231	9.0
	Level II	488	521	6.9
Chlorpromazine	Level I	74.3	74.2	-0.1
	Level II	172	174	1.0
Chlorprothixene	Level I	72.9	73.8	1.2
	Level II	170	171	0.7
Clozapine	Level I	235	247	5.2
	Level II	551	573	4.1
Dehydro-Aripiprazole	Level I	39.3	41.4	5.3
	Level II	91.7	97.4	6.2
Desmethylolanzapine	Level I	29.7	27.7	-6.7
	Level II	68.5	65.6	-4.3
Flupentixol	Level I	2.69	2.68	-0.3
	Level II	6.12	6.35	3.8
Fluphenazine	Level I	2.44	2.52	3.2
	Level II	5.55	5.94	7.0
Haloperidol	Level I	2.56	2.57	0.5
	Level II	6.04	5.92	-1.9
Levomepromazine	Level I	52.1	50.8	-2.5
	Level II	122	120	-1.6
Melprone	Level I	41.1	40.5	-1.4
	Level II	93.4	94.5	1.1
Norclozapine	Level I	184	195	5.8
	Level II	424	446	5.1
Norquetiapine	Level I	70.2	73.4	4.6
	Level II	168	166	-1.3
Olanzapine	Level I	29.9	28.8	-3.8
	Level II	70.2	66.7	-4.9
Paliperidone	Level I	26.3	26.9	2.5
	Level II	61.7	62.8	1.9
Perazine	Level I	94.5	94.4	-0.1
	Level II	217	219	1.1
Pipamperone	Level I	125	128	2.7
	Level II	288	300	4.1
Promethazine	Level I	22.9	22.4	-2.3
	Level II	51.7	51.7	0.1
Prothipendyl	Level I	11.9	12.1	1.8
	Level II	28.1	28.4	1.0
Quetiapine	Level I	142	138	-2.5
	Level II	322	321	-0.4
Risperidone	Level I	25.8	26.6	3.3
	Level II	59.9	62.7	4.7
Sertindole	Level I	43.4	43.7	0.7
	Level II	102	103	1.0
Sulpiride	Level I	225	229	1.6
	Level II	529	535	1.2
Thioridazine	Level I	80.6	86.7	7.5
	Level II	194	202	4.3
Ziprasidone	Level I	64.7	67.1	3.7
	Level II	154	155	0.7
Zotepine	Level I	38.4	40.9	6.5
	Level II	87.9	92.4	5.1
Zuclopenthixol	Level I	16.6	16.7	0.6
	Level II	39.0	38.8	-0.5

Table 6. Analytical intra- and inter-assay precision results for control MS9382 batch #1279

Analyte	Control	Intra-assay						Inter-assay	
		Day 1		Day 2		Day 3			
		Average calculated concentration (µg/L)	CV (%)	Average calculated concentration (µg/L)	CV (%)	Average calculated concentration (µg/L)	CV (%)	Average calculated concentration (µg/L)	CV (%)
Amisulpride	Level I	141	1.1	130	8.1	142	2.9	137	4.5
	Level II	312	3.7	329	1.0	319	1.1	320	2.7
Aripiprazole	Level I	234	0.7	214	8.0	245	3.6	231	6.8
	Level II	513	4.3	530	1.2	521	1.5	521	1.6
Chlorpromazine	Level I	77.3	1.3	68.6	11.4	76.7	2.8	74.2	6.6
	Level II	167	4.6	180	0.9	175	1.6	174	3.8
Chlorprothixene	Level I	76.1	1.9	67.7	12.5	77.5	2.8	73.8	7.1
	Level II	162	4.9	179	1.2	173	1.8	171	4.9
Clozapine	Level I	253	0.9	232	8.5	257	2.5	247	5.5
	Level II	562	3.5	587	0.9	571	1.2	573	2.2
Dehydro-Aripiprazole	Level I	41.9	1.5	38.4	8.8	43.8	3.4	41.4	6.6
	Level II	94.0	3.4	99.5	1.8	98.6	1.2	97.4	3.1
Desmethylolanzapine	Level I	28.1	1.0	25.9	7.6	29.1	2.1	27.7	5.9
	Level II	64.4	3.7	66.7	1.7	65.6	1.7	65.6	1.7
Flupentixol	Level I	2.71	0.9	2.45	8.9	2.89	3.8	2.68	8.3
	Level II	6.01	4.3	6.41	3.1	6.64	2.4	6.35	5.0
Fluphenazine	Level I	2.56	2.9	2.30	8.6	2.69	2.5	2.52	7.9
	Level II	5.70	3.7	6.11	1.6	6.00	1.0	5.94	3.6
Haloperidol	Level I	2.63	0.6	2.41	8.7	2.68	2.1	2.57	5.6
	Level II	5.70	3.8	6.14	1.1	5.93	1.7	5.92	3.7
Levomepromazine	Level I	53.0	1.4	47.5	10.2	51.9	2.6	50.8	5.8
	Level II	116	4.7	125	1.4	120	1.2	120	3.7
Melperone	Level I	42.3	1.7	37.9	11.4	41.4	2.7	40.5	5.8
	Level II	90.1	5.1	99.3	0.6	94.0	1.0	94.5	4.9
Norclozapine	Level I	200	1.4	183	8.5	201	2.7	195	5.3
	Level II	438	3.6	458	1.0	441	1.0	446	2.4
Norquetiapine	Level I	75.8	1.9	69.7	8.8	74.7	3.0	73.4	4.4
	Level II	161	2.6	172	1.3	165	1.2	166	3.4
Olanzapine	Level I	29.4	1.5	27.4	7.5	29.5	2.7	28.8	4.1
	Level II	65.0	3.4	68.5	0.8	66.7	1.0	66.7	2.6
Paliperidone	Level I	27.9	1.2	25.6	8.2	27.4	2.9	27.0	4.5
	Level II	61.6	3.8	65.1	2.0	61.8	1.2	62.9	3.1
Perazine	Level I	98.3	1.9	87.9	8.6	96.9	2.6	94.4	6.0
	Level II	214	4.6	223	1.9	221	1.1	219	2.1
Pipamperone	Level I	132	1.3	121	7.6	132	2.9	128	5.1
	Level II	292	4.3	307	1.3	300	1.5	300	2.4
Promethazine	Level I	23.2	1.0	20.8	10.7	23.1	3.1	22.4	6.2
	Level II	49.4	4.1	53.7	0.9	52.2	1.2	51.7	4.2

Table 6 (continued). Analytical intra- and inter-assay precision results for control MS9382 batch #1279

Analyte	Control	Intra-assay						Inter-assay	
		Day 1		Day 2		Day 3			
		Average calculated concentration (µg/L)	CV (%)	Average calculated concentration (µg/L)	CV (%)	Average calculated concentration (µg/L)	CV (%)	Average calculated concentration (µg/L)	CV (%)
Prothipendyl	Level I	12.6	0.8	11.3	8.9	12.5	2.8	12.1	5.6
	Level II	27.4	4.1	29.4	1.0	28.3	1.3	28.4	3.5
Quetiapine	Level I	142	0.9	130	7.9	143	3.3	139	5.1
	Level II	315	2.9	329	1.4	318	0.4	321	2.3
Risperidone	Level I	27.5	1.5	25.1	9.1	27.3	3.0	26.6	5.0
	Level II	61.3	3.6	64.5	1.1	62.3	0.9	62.7	2.6
Sertindole	Level I	45.4	1.1	41.0	9.0	44.7	2.6	43.7	5.4
	Level II	101	3.8	106	1.0	102	1.3	103	2.4
Sulpiride	Level I	234	1.7	216	10.1	235	2.9	229	4.6
	Level II	519	3.6	554	1.5	534	1.2	535	3.3
Thioridazine	Level I	89.8	1.5	79.8	10.2	90.4	3.4	86.7	6.9
	Level II	197	2.8	208	1.1	202	0.8	202	2.7
Ziprasidone	Level I	69.1	1.3	62.7	9.5	69.5	3.3	67.1	5.6
	Level II	153	4.1	158	1.4	155	1.2	155	1.6
Zotepine	Level I	42.3	1.0	38.6	9.9	41.7	4.8	40.9	4.8
	Level II	89.1	5.1	95.5	1.0	92.4	1.6	92.4	3.5
Zuclopenthixol	Level I	17.1	1.0	15.6	9.4	17.4	3.0	16.7	6.0
	Level II	37.9	3.7	39.9	0.9	38.6	0.9	38.8	2.7

Table 7. LLOQs for all compounds

Analyte	LLOQ (µg/L)
Amisulpride	1.82
Aripiprazole	2.88
Chlorpromazine	0.945
Chlorprothixene	0.955
Clozapine	2.98
Dehydro-Aripiprazole	0.555
Desmethylolanzapine	7.31
Flupentixol	0.601
Fluphenazine	0.586
Haloperidol	0.126
Levomepromazine	1.30
Melprone	0.555
Norclozapine	2.24
Norquetiapine	0.930

Analyte	LLOQ (µg/L)
Olanzapine	0.728
Paliperidone	0.677
Perazine	1.24
Pipamperone	1.52
Promethazine	0.300
Prothipendyl	0.131
Quetiapine	7.31
Risperidone	0.319
Sertindole	1.13
Sulpiride	5.96
Thioridazine	3.90
Ziprasidone	3.56
Zotepine	0.515
Zuclopenthixol	0.766

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Conclusions

A robust, reproducible, and sensitive liquid chromatography-HRAM mass spectrometry method for clinical research for the quantification of 28 neuroleptics in human plasma was developed. The method was analytically implemented and validated on a Vanquish Flex Binary UHPLC system coupled to an Orbitrap Exploris 120 mass spectrometer. The method described here offers quick and simple offline protein precipitation with concomitant internal standard addition, enabled by the ClinMass TDM Platform with the ClinMass Add-On Set for Neuroleptics in Serum/Plasma. Finally, thanks to the use of HRMS, it is possible to obtain reliable and very precise results in terms of mass accuracy. The described method also enables meeting the laboratory requirements because the results obtained show good sensitivity, linearity of response, and precision.

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