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

Author: Mariana Barcenas
Thermo Fisher Scientific, Les Ulis, France

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Application benefits
- Accurate results, simple sample preparation, and rapid quantitation

- High-resolution mass spectrometry for improved selectivity

- Robust, sensitive LC and MS platforms enable increased confidence in data

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

Introduction
Neuroleptics are a class of psychoactive drugs that are used to treat psychoses, a symptom of schizophrenia. Since antipsychotic drugs are very powerful and can cause adverse side effects, they are usually administered at low daily dosages. While most antipsychotic drugs are found in plasma in the low ng/mL range, some are found in very low concentrations in brain tissue. High performance liquid chromatography (HPLC) with UV detection is widely used for measurement of these drugs. However, identification and quantitation of antipsychotics at low concentrations require technology that can offer high resolution (to separately identify every analyte with confidence) and high sensitivity. The use of high-resolution accurate-mass (HRAM) mass spectrometers coupled to UHPLC systems can offer the necessary selectivity and specificity while providing the required sensitivity.

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In this study, a clinical research analytical method for the quantification of 28 neuroleptics in human plasma is reported. Plasma samples were extracted by internal standard addition and protein precipitation. Method performance was evaluated using calibrators and controls from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response within the calibration ranges, lower limit of quantification (LLOQ), carryover, accuracy, and intra- and inter-assay precision for the entire panel of analytes.

Experimental

Target analytes

The list of analytes, their corresponding internal standards, and concentration ranges covered by the calibrators (MS9313 batch #1368) are reported in Table 1.

Table 1. Analytes, internal standards, and concentration ranges covered by calibrators

Analyte	Internal standard	Retention time (min)	Concentration (ng/mL)		
			L1	L2	L3
Amisulpride	Amisulpride-d$_5$	2.46	37.1	255	751
Aripiprazole	Aripiprazole-d$_8$	3.66	54.8	362	1129
Chlorpromazine	Chlorpromazine-d$_6$	4.19	18.5	126	375
Chlorprothixene	Chlorprothixene-d$_6$	4.22	17.7	125	378
Clozapine	Clozapine-d$_4$	3.26	57.1	406	1223
Dehydro-Aripiprazole	Dehydro-Aripiprazole-d$_8$	3.59	10.5	70.9	217
Desmethylolanzapine	Olanzapine-d$_3$	2.29	6.75	49.3	146
Flupentixol	Flupenthixol-d$_4$	4.33	0.648	4.56	13.2
Fluphenazine	Fluphenazine-d$_8$	4.31	0.590	4.12	12.7
Haloperidol	Haloperidol-d$_4$	3.40	0.630	4.28	12.5
Levomepromazine	Levomepromazine-d$_3$	3.84	8.54	60.2	182
Melperone	Melperone-d$_4$	2.95	11.0	72.4	220
Norclozapine	Norclozapine-d$_8$	3.19	45.3	323	961
Norquetiapine	Quetiapine-d$_8$	3.38	8.71	61.3	183
Olanzapine	Olanzapine-d$_3$	2.31	7.14	50.2	145
Paliperidone	Paliperidone-d$_4$	2.96	6.70	45.3	135
Perazine	Perazine-d$_8$	3.87	23.9	160	487
Pipamperone	Pipamperone-d$_{10}$	2.59	30.9	218	636
Promethazine	Promethazine-d$_6$	3.58	20.8	139	416
Prothipendyl	Prothipendyl-d$_6$	3.43	1.08	6.93	22.0
Quetiapine	Quetiapine-d$_8$	3.39	30.0	200	571
Risperidone	Risperidone-d$_4$	3.01	6.51	44.0	139
Sertindole	Sertindole-d$_4$	4.27	10.3	71.0	222
Sulpiride	Sulpiride-d$_3$	1.90	54.7	382	1154
Thioridazine	Thioridazine-d$_3$	4.28	19.1	142	428
Ziprasidone	Ziprasidone-d$_8$	3.21	16.8	116	335
Zotepine	Sertindole-d$_4$	4.25	9.21	59.1	189
Zuclopenthixol	Zuclopenthixol-d$_4$	4.30	3.85	26.4	81.8

Sample preparation

Reagents included four calibrators (including blank) and two controls (MS9382 batch #1279) from RECIPE, as well as internal standards for quantification. Samples of 50 μL of plasma were protein precipitated using 100 μL of acetonitrile containing the internal standard. Precipitated samples were vortex-mixed and centrifuged. The supernatant was diluted 10-fold using mobile phase A and transferred to a clean vial.

Liquid chromatography

LC separation was performed on a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system using the following mobile phases:

• Mobile phase A: 0.1% formic acid in water

• Mobile phase B: 0.1% formic acid in methanol

Chromatographic separation was achieved by gradient elution on a Thermo Scientific™ Hypersil GOLD™ 2.1 × 50 mm (1.9 μm) analytical column (P/N 25002-052130) maintained at 40 °C at a flow rate of 0.4 mL/min. Total run time was 5.5 minutes. The chromatographic conditions are given in Table 2.

Table 2. LC gradient profile

Time (min)	Flow (mL/min)	B (%)
0.00	0.4	2
0.80	0.4	2
2.50	0.4	55
3.40	0.4	55
3.50	0.4	98
4.50	0.4	98
4.51	0.4	2
5.50	0.4	2
Other parameters		
Column temperature		40 °C
Injection volume		5 μL

Mass spectrometry

Detection was performed on an Orbitrap Exploris 120 mass spectrometer equipped with a heated electrospray ionization (HESI) ion source operated in positive ionization mode. Data was acquired in Full MS-ddMS^2 mode using a resolution of 60,000 (FWHM) at m/z 200 on a scan range of m/z 100 to 500. The ion source conditions and the mass spectrometer settings are presented in Tables 3 and 4, respectively.

Table 3. Ion source settings

Parameter	Value
Source type	Heated Electrospray Ionization (HESI)
Vaporizer temperature	350 °C
Ion transfer tube	300 °C
Capillary voltage	3,500 V
Sheath gas	40 AU
Sweep gas	0 AU
Auxiliary gas	10 AU

Table 4. MS settings

Parameter	Value
Resolution at m/z 200	60,000
Scan range	100–500
AGC target	Standard
RF lens	70%
Maximum injection time mode	Auto
Data type	Profile
Polarity	Positive
Source fragmentation	Off
Mild trapping	Off

Method evaluation

The parameters used to evaluate the performance of the method included linearity of response, LLOQ, intra- and inter-assay accuracy and precision, and carryover for all the analytes. 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 quality control samples at two different levels provided by RECIPE, 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). The LLOQ was investigated by dilution of the lowest calibrator with blank matrix and was established as the lowest concentration with a mean accuracy and precision better than 20%.

Data analysis

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

Results and discussion

A summary of the LLOQs obtained for all the analytes is presented in Table 5. A linear regression with 1/× weighting down to the LLOQ value was obtained for all the analytes, except for norclozapine where a quadratic regression was used. The percentage bias between nominal and back-calculated concentration was always within ±10% for all the calibrators in all the runs.

Table 5. Lower limit of quantitation

| Analyte | Concentration (ng/mL) | |
	Lowest calibrator	LLOQ
Amisulpride	37.1	3.71
Aripiprazole	54.8	5.48
Chlorpromazine	18.5	3.70
Chlorprothixene	17.7	3.54
Clozapine	57.1	5.71
Dehydro-Aripiprazole	10.5	5.25
Desmethylolanzapine	6.75	2.25
Flupentixol	0.648	0.648
Fluphenazine	0.590	0.590
Haloperidol	0.630	0.630
Levomeprazine	8.54	2.85
Melperone	11.0	2.20
Norclozapine	45.3	9.06
Norquetiapine	8.71	2.90
Olanzapine	7.14	2.38
Paliperidone	6.70	2.23
Perazine	23.9	7.97
Pipamperone	30.9	6.18
Promethazine	20.8	6.93
Prothipendyl	1.08	1.08
Quetiapine	30.0	3.00
Risperidone	6.51	1.30
Sertindole	10.3	5.15
Sulpiride	54.7	5.47
Thioridazine	19.1	3.82
Ziprasidone	16.8	3.36
Zotepine	9.21	4.61
Zuclopenthixol	3.85	1.93

Representative chromatograms for the LLOQ for clozapine, quetiapine, and perazine, and their corresponding internal standards are reported in Figure 1. Representative calibration curves for the same analytes are reported in Figure 2.

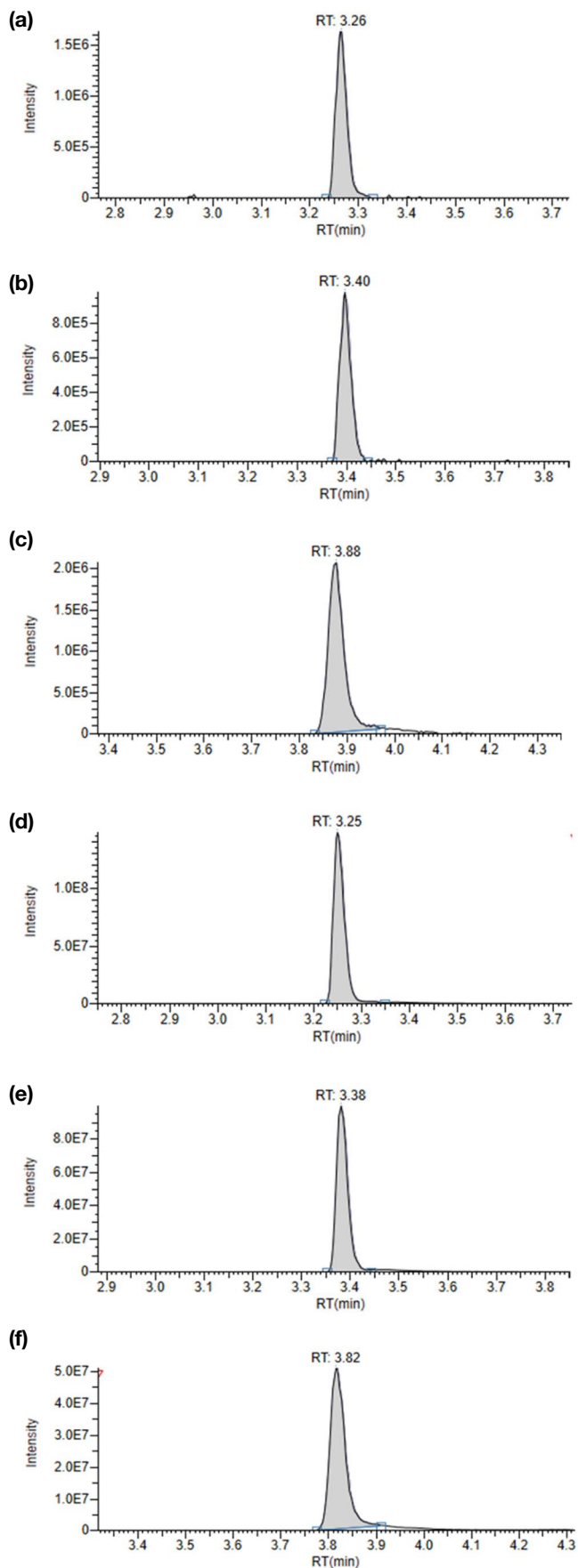No significant carryover was observed for any of the analytes.

Figure 1. Representative chromatograms of the LLOQ for (a) clozapine, (b) quetiapine, (c) perazine, (d) clozapine-d$_4$, (e) quetiapine-d$_8$, and (f) perazine-d$_8$
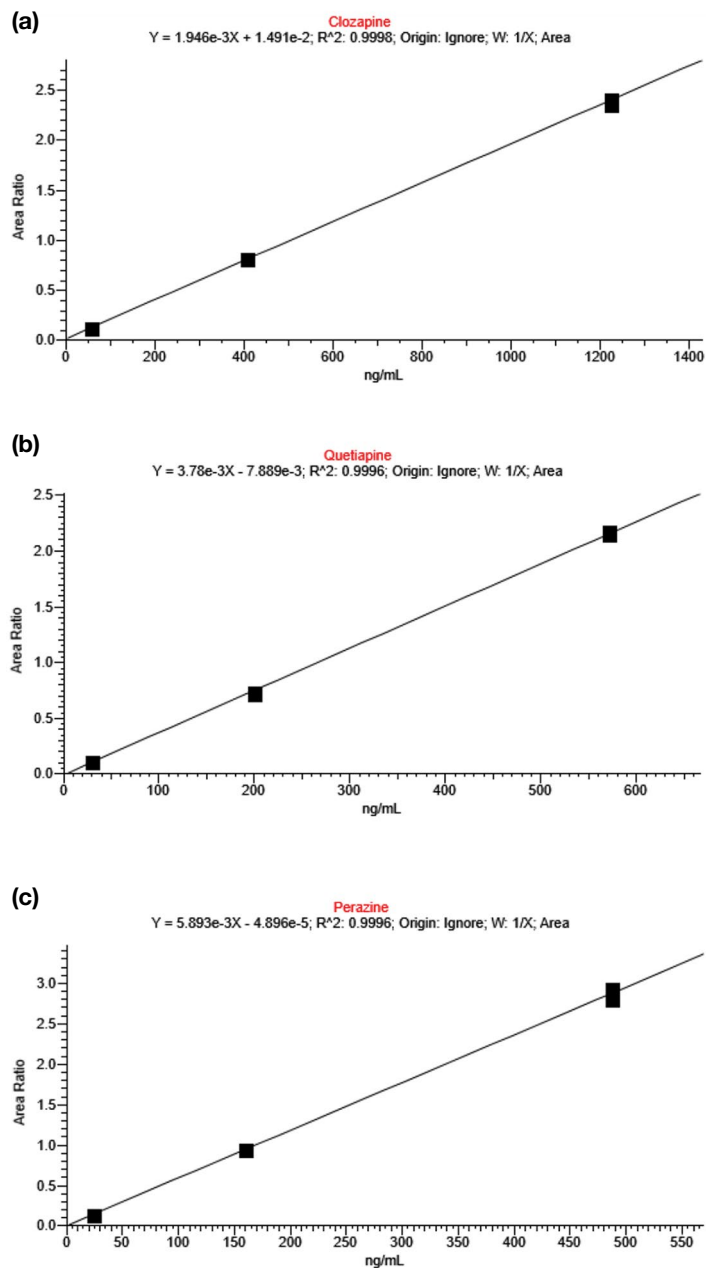

Figure 2. Representative calibration curves for (a) clozapine, (b) quetiapine, and (c) perazine

The data demonstrated good accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the control samples ranging between -8.0% and 5.1% (Table 6). The %CV for intra-assay precision was always below 8% for all the analytes, reported in Table 7. The maximum %CV for inter-assay precision including all the analytes was 7.9% (Table 8).

Table 6. Analytical accuracy results for control MS9382 batch #1279

Analyte	CTRL1			CTRL2		
	Nominal concentration (ng/L)	Average calculated concentration (ng/L)	Bias %	Nominal concentration (ng/L)	Average calculated concentration (ng/L)	Bias %
Amisulpride	135	136	0.9	317	299	-5.7
Aripiprazole	212	222	4.8	488	449	-8.0
Chlorpromazine	74.3	75.3	1.3	172	161	-6.1
Chlorprothixene	72.9	73.5	0.9	170	157	-7.7
Clozapine	235	247	5.1	551	513	-7.0
Dehydro-Aripiprazole	39.3	40.7	3.6	91.7	86.8	-5.3
Desmethylolanzapine	29.7	28.8	-2.9	68.5	63.1	-7.9
Flupentixol	2.69	2.60	-3.2	6.12	5.72	-6.6
Fluphenazine	2.44	2.48	1.8	5.55	5.28	-4.8
Haloperidol	2.56	2.61	2.0	6.04	5.65	-6.4
Levomepromazine	52.1	53.2	2.1	122	112	-8.1
Melperone	41.1	42.6	3.7	93.4	87.0	-6.8
Norclozapine	184	193	4.8	424	404	-4.7
Norquetiapine	70.2	73.1	4.1	168	156	-7.3
Olanzapine	29.9	29.2	-2.3	70.2	66.2	-5.7
Paliperidone	26.3	26.7	1.5	61.7	57.9	-6.2
Perazine	94.5	93.9	-0.7	217	203	-6.5
Pipamperone	125	126	1.1	288	270	-6.2
Promethazine	22.9	23.0	0.5	51.7	48.3	-6.7
Prothipendyl	11.9	12.1	1.7	28.1	26.2	-6.9
Quetiapine	142	143	0.8	322	300	-6.8
Riperidone	25.8	27.1	4.9	59.9	55.3	-7.6
Sertindole	43.4	44.3	2.0	102	95.5	-6.4
Sulpiride	225	231	2.6	529	495	-6.5
Thioridazine	80.6	84.4	4.7	194	180	-7.1
Ziprasidone	64.7	64.4	-0.4	154	144	-6.2
Zotepine	38.4	38.2	-0.6	87.9	81.3	-7.5
Zuclopenthixol	16.6	16.4	-1.0	39.0	36.6	-6.0

Table 7 (part 1). Analytical intra-assay precision results for control MS9382 (CTRL1) batch #1279

Analyte	CTRL1					
	Day 1		Day 2		Day 3	
	Average calculated concentration (ng/L)	CV %	Average calculated concentration (ng/L)	CV %	Average calculated concentration (ng/L)	CV %
Amisulpride	135	5.6	144	1.4	129	3.1
Aripiprazole	218	5.2	225	1.9	224	1.1
Chlorpromazine	75.6	4.0	76.8	0.8	73.4	2.0
Chlorprothixene	73.9	3.3	75.8	1.5	71.0	2.1
Clozapine	245	3.2	255	0.6	241	1.9
Dehydro-Aripiprazole	40.6	3.7	41.9	3.5	39.7	4.3
Desmethylolanzapine	27.4	3.3	31.6	2.4	27.4	1.7
Flupentixol	2.51	4.7	2.82	4.7	2.53	2.4
Fluphenazine	2.41	5.6	2.62	1.5	2.43	3.1
Haloperidol	2.56	5.2	2.70	2.4	2.58	3.2
Levomepromazine	51.5	7.9	55.5	2.6	52.6	2.8
Melperone	42.3	4.2	43.9	0.7	41.6	2.7
Norclozapine	196	1.9	197	0.7	185	2.1
Norquetiapine	70.4	7.4	73.6	1.3	75.2	2.8
Olanzapine	27.9	3.6	32.3	1.0	27.5	1.2
Paliperidone	25.3	5.2	28.6	1.3	25.9	1.8
Perazine	92.4	4.0	98.3	1.8	90.9	3.3
Pipamperone	128	6.1	129	4.5	122	5.4
Promethazine	22.7	4.4	24.5	1.5	21.8	1.7
Prothipendyl	11.8	6.3	12.5	2.2	12.1	4.2
Quetiapine	141	5.4	147	0.8	141	2.8
Riperidone	26.4	3.8	27.5	1.2	27.4	4.5
Sertindole	43.2	3.7	46.5	1.3	43.1	1.5
Sulpiride	223	7.2	240	0.9	229	4.0
Thioridazine	83.6	6.0	85.7	1.9	83.8	2.1
Ziprasidone	62.9	4.8	67.0	0.8	63.4	2.0
Zotepine	37.1	3.7	40.8	1.4	36.6	1.8
Zuclopenthixol	16.1	3.9	17.5	6.9	15.7	2.3

Table 7 (part 2). Analytical intra-assay precision results for control MS9382 (CTRL2) batch #1279

| Analyte | CTRL2 | | | | | |
| | Day 1 | | Day 2 | | Day 3 | |
	Average calculated concentration (ng/L)	CV %	Average calculated concentration (ng/L)	CV %	Average calculated concentration (ng/L)	CV %
Amisulpride	299	2.2	296	1.5	302	1.8
Aripiprazole	441	0.5	454	1.1	451	1.1
Chlorpromazine	163	1.6	163	1.0	158	1.2
Chlorprothixene	160	1.7	156	1.5	155	0.7
Clozapine	520	2.0	505	1.7	513	1.4
Dehydro-Aripiprazole	85.0	3.1	90.8	4.4	84.5	1.2
Desmethylolanzapine	63.9	1.1	62.8	1.2	62.7	1.3
Flupentixol	5.77	2.0	5.84	1.8	5.54	1.3
Fluphenazine	5.15	2.5	5.56	3.1	5.14	1.3
Haloperidol	5.67	3.4	5.69	2.0	5.60	1.3
Levomepromazine	113	2.6	112	1.0	112	1.0
Melperone	86.8	1.4	86.3	1.3	87.9	2.1
Norclozapine	423	3.9	393	1.4	397	1.5
Norquetiapine	153	0.7	155	1.5	159	2.5
Olanzapine	66.5	2.5	65.3	1.2	66.7	2.5
Paliperidone	57.9	2.4	57.7	1.6	58.0	2.5
Perazine	201	1.3	205	2.2	203	1.8
Pipamperone	270	2.6	271	1.9	270	1.0
Promethazine	48.0	1.2	48.3	1.3	48.5	2.4
Prothipendyl	26.0	2.7	26.2	0.7	26.4	1.7
Quetiapine	302	1.1	301	0.7	297	0.9
Riperidone	54.6	0.7	56.0	1.1	55.4	1.7
Sertindole	94.6	2.1	96.0	2.4	95.7	1.9
Sulpiride	498	2.3	494	1.1	492	1.3
Thioridazine	183	2.9	179	1.2	179	1.8
Ziprasidone	143	2.1	144	2.1	147	2.0
Zotepine	80.6	1.4	81.6	0.7	81.6	1.1
Zuclopenthixol	36.3	1.7	37.2	2.3	36.5	1.1

Table 8. Analytical inter-assay precision results for control MS9382 batch #1279

Analyte	CTRL1		CTRL2	
	Average calculated concentration (ng/L)	CV %	Average calculated concentration (ng/L)	CV %
Amisulpride	136	6.0	299	1.9
Aripiprazole	222	3.3	449	1.5
Chlorpromazine	75.3	3.1	161	1.8
Chlorprothixene	73.5	3.6	157	1.9
Clozapine	247	3.1	513	2.0
Dehydro-Aripiprazole	40.7	4.2	86.8	4.5
Desmethylolanzapine	28.8	7.5	63.1	1.4
Flupentixol	2.60	6.7	5.72	2.8
Fluphenazine	2.48	5.2	5.28	4.4
Haloperidol	2.61	4.3	5.65	2.3
Levomepromazine	53.2	5.7	112	1.6
Melperone	42.6	3.6	87.0	1.7
Norclozapine	193	3.5	404	4.1
Norquetiapine	73.1	5.0	156	2.2
Olanzapine	29.2	7.9	66.2	2.2
Paliperidone	26.7	6.3	57.9	2.1
Perazine	93.9	4.5	203	1.9
Pipamperone	126	5.7	270	1.8
Promethazine	23.0	5.7	48.3	1.7
Prothipendyl	12.1	4.8	26.2	1.9
Quetiapine	143	3.9	300	1.2
Riperidone	27.1	3.7	55.3	1.5
Sertindole	44.3	4.3	95.5	2.1
Sulpiride	231	5.3	495	1.6
Thioridazine	84.4	3.7	180	2.2
Ziprasidone	64.4	4.0	144	2.3
Zotepine	38.2	5.6	81.3	1.2
Zuclopenthixol	16.4	6.8	36.6	2.0

Conclusions

A robust, reproducible, and sensitive liquid chromatography-high resolution mass spectrometry method for clinical research for quantification of 28 neuroleptics in human plasma was developed, implemented, and analytically validated. Sample preparation is based on a rapid and simple offline protein precipitation with concomitant internal standard addition. The described method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.

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