TECHNICAL NOTE 73392

Quantification of 28 neuroleptic drugs in human plasma or serum by LC-HRAM(MS) for clinical research

Authors: Claudio De Nardi[1], Katharina Kern[2], Steffen Peters[2]

[1]Thermo Fisher Scientific GmbH, Dreieich, Germany
[2]RECIPE Chemicals + Instruments GmbH, Munich, Germany

Keywords: Neuroleptics, offline sample preparation, plasma, serum, Orbitrap, high-resolution mass spectrometry

Application benefits

- Increased accuracy of method by implementation of a comprehensive ClinMass® kit for sample preparation
- High-resolution mass spectrometry for improved selectivity
- Robust, sensitive hardware enables increased confidence in data
- Simple offline sample preparation by protein precipitation
- 28 neuroleptic drugs in a single quantitative method

Goal

Implementation of an analytical method for the quantification of 28 neuroleptic drugs in human plasma or serum on a Thermo Scientific™ Q Exactive™ Plus hybrid quadrupole-Orbitrap™ mass spectrometer.

Introduction

Neuroleptics belong to the class of psychoactive drugs and are used to treat psychoses, in particular in the symptomatic treatment of schizophrenia. Since the antipsychotic drugs are very active, they are usually administered at low daily dosages. While most of the antipsychotic drugs are found in plasma at 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 the antipsychotics at low concentrations require a technology that can offer high resolution (to separately identify every analyte with confidence) along with high sensitivity. The utility of high-resolution accurate-mass (HRAM) mass spectrometers coupled to a UHPLC system can 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. A comprehensive list of analytes, corresponding internal standards and concentration ranges covered by the method is set forth in Table 1. While most reported LC-MS analyses of the above-mentioned neuroleptics involve triple quadrupole mass spectrometers, traditionally used for targeted, sensitive quantitation assays, in this report we present LC-MS data acquired using HRAM mass spectrometry leveraging Orbitrap technology. This report demonstrates the capability of HRAM mass spectrometry for routine quantitation analyses in addition to its use for performing in-depth qualitative investigations.

Table 1. Analytes, internal standards, and concentration ranges

Analyte	Internal standard	Concentration range (ng/mL)
Amisulpride	d_5-amisulpride	37.1–751
Aripiprazole	d_8-aripiprazole	54.8–1129
Chlorpromazine	d_6-chlorpromazine	18.5–375
Chlorprothixene	d_6-chlorprothixene	17.7–378
Clozapine	d_4-clozapine	57.1–1223
Dehydroaripiprazole	d_8-dehydroaripiprazole	10.5–217
Desmethylolanzapine	d_3-olanzapine	6.75–146
Flupentixol	d_4-flupentixol	0.648–13.2
Fluphenazine	d_8-fluphenazine	0.59–12.7
Haloperidol	d_4-haloperidol	0.63–12.5
Levomepromazine	d_3-levomepromazine	8.54–182
Melperone	d_4-melperone	11–220
Norclozapine	d_8-norclozapine	45.3–961
Norquetiapine	d_8-quetiapine	8.71–183
Olanzapine	d_3-olanzapine	7.14–145
Paliperidone	d_4-paliperidone	6.7–135
Perazine	d_8-perazine	23.9–487
Pipamperone	d_{10}-pipamperone	30.9–636
Promethazine	d_6-promethazine	20.8–416
Prothipendyl	d_6-prothipendyl	1.08–22
Quetiapine	d_8-quetiapine	30–571
Risperidone	d_4-risperidone	6.51–139
Sertindole	d_4-sertindole	10.3–222
Sulpiride	d_3-sulpiride	54.7–1154
Thioridazine	d_3-thioridazine	19.1–428
Ziprasidone	d_8-ziprasidone	16.8–335
Zotepine	d_8-aripiprazole	9.21–189
Zuclopenthixole	d_4-zuclopenthixole	3.85–81.8

Plasma or serum samples were extracted by offline internal standard addition and protein precipitation. Extracted samples were injected onto a Thermo Scientific™ Vanquish™ Flex Duo UHPLC system connected to a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer with a heated electrospray ionization (H-ESI II) source. Detection was performed by full scan coupled to data-dependent fragmentation (fullMS-ddMS^2) using 25 deuterated internal standards. The full scan experiment was used for quantification, and the fragmentation was used for confirmation. Quantitative method performance was evaluated using the ClinMass® TDM Platform with the ClinMass® Add-On Set for Neuroleptics from RECIPE Chemicals + Instruments GmbH (Munich, Germany) in terms of linearity of response within the calibration ranges, carryover, accuracy and intra- and inter-assay precision for each analyte.

Experimental

Target analytes

The concentration ranges covered by the calibrators (MS9313 batch #1368) used are reported in Table 1.

Sample preparation

Reagents included four calibrators (including blank) and two controls from RECIPE (MS9382 batch #1346), as well as 25 deuterated internal standards for the quantification. Samples of 50 µL of plasma or serum were protein-precipitated using 100 µL of precipitating solution containing the internal standards. Precipitated samples were vortex-mixed and centrifuged, and the supernatant was transferred to a clean plate or vial.

Liquid chromatography

A Vanquish Flex Duo UHPLC system, which comprises a dual-channel instrument configured for both LC-only and online SPE applications (Figure 1), was used for chromatographic separation. The LC-only channel was used in this case, utilizing mobile phases and analytical column provided by RECIPE. Details of the analytical method are reported in Table 2. Total runtime was 6.0 minutes.

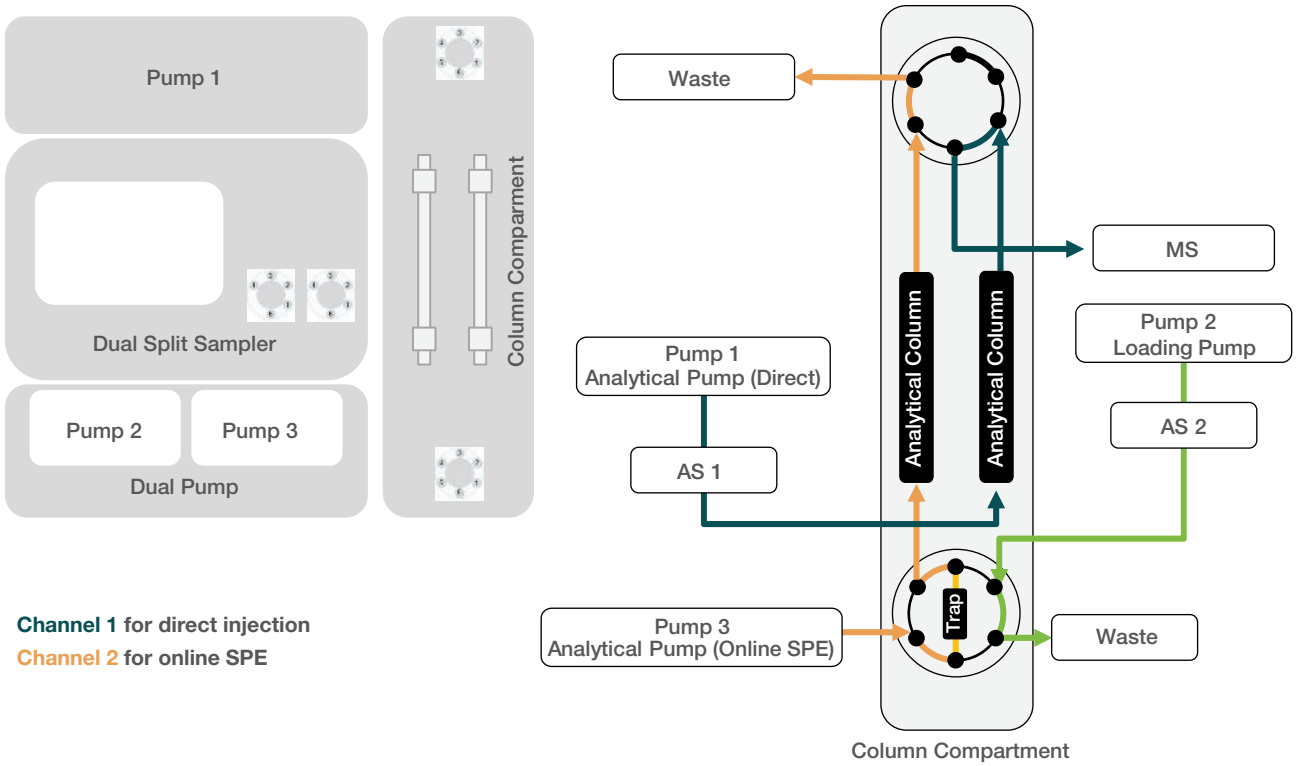

Figure 1. Schematic representation of the Vanquish Duo UHPLC system setup

Table 2. Liquid chromatography method description

Gradient profile			
Time (min)	Flow rate (mL/min)	A (%)	B (%)
0.00	0.65	95	5
0.30	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
5.51	0.00	95	5
6.00	0.00	95	5
Other parameters			
Injection volume (µL)		2	
Column temperature (°C)		40	

Table 3. Mass spectrometer source and scan settings

Parameter	Value
Source type	Heated electrospray ionization (H-ESI II)
Vaporizer temperature	450 °C
Capillary temperature	325 °C
Spray voltage (positive mode)	1500 V
Sheath gas	53 AU
Sweep gas	0 AU
Auxiliary gas	20 AU
S-Lens RF level	60
Data acquisition mode	FullMS-ddMS^2
FullMS resolution @ *m/z* 200	70,000
FullMS scan range	250–480 *m/z*
ddMS^2 resolution @ *m/z* 200	17,500
ddMS^2 isolation window	2.0 *m/z*
Stepped Normalized Collision Energy (NCE)	15, 25, 35

Mass spectrometry

Analytes and internal standards were detected by FullMS-ddMS^2 mode on a Q Exactive Plus mass spectrometer with a H-ESI II source operated in positive ion mode. A summary of the MS conditions is reported in Table 3.

Method evaluation

The method performance was evaluated in terms of linearity of response within the calibration ranges, lower limit of quantification (LLOQ), carryover, accuracy, and intra- and inter-assay precision for all the analytes.

To determine the LLOQ, the lowest calibrator was diluted 20-fold with blank matrix; a full set of calibrators (three levels), diluted calibrators (four levels) and controls (two levels) were extracted in replicates of five (n=5), 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 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 at two different levels using the quality control samples provided by RECIPE and prepared and analyzed in replicates of five on three different days.

Trueness of measurement was also evaluated as percentage bias using certified external quality controls (GTFCh - TDMA 1/19 and TDMC 1/19) prepared and analyzed in replicates of five on a single day.

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™ 4.1 software.

Results and discussion

A linear response with 1/x weighting was obtained for all the analytes, not only in the calibration range covered by the calibrators, but also down to a LLOQ reported in Table 4. The percentage bias between nominal and back-calculated concentration was always within ±10% for all the calibrators (±15% for the lowest calibrator) in all the runs. Representative chromatograms for the LLOQ for amisulpride, quetiapine, and the corresponding internal standards are depicted in Figure 2. Representative calibration curves for the same analytes in the concentration range covered by the kit (three calibrators) are shown in Figure 3.

No significant carryover was observed, with a maximum value of 0.02% for melperone.

Table 4. Analytes and corresponding LLOQ

Analyte	LLOQ ng/mL)
Amisulpride	3.71
Aripiprazole	5.48
Chlorpromazine	1.85
Chlorprothixene	3.54
Clozapine	2.86
Dehydroaripiprazole	2.10
Desmethylolanzapine	3.38
Flupentixol	0.648
Fluphenazine	0.590
Haloperidol	0.630
Levomepromazine	4.27
Melperone	1.10
Norclozapine	2.27
Norquetiapine	1.74
Olanzapine	3.57
Paliperidone	1.34
Perazine	12.0
Pipamperone	1.55
Promethazine	2.08
Prothipendyl	1.10
Quetiapine	3.00
Risperidone	0.651
Sertindole	2.06
Sulpiride	2.74
Thioridazine	19.1
Ziprasidone	3.36
Zotepine	4.61
Zuclopenthixole	1.93

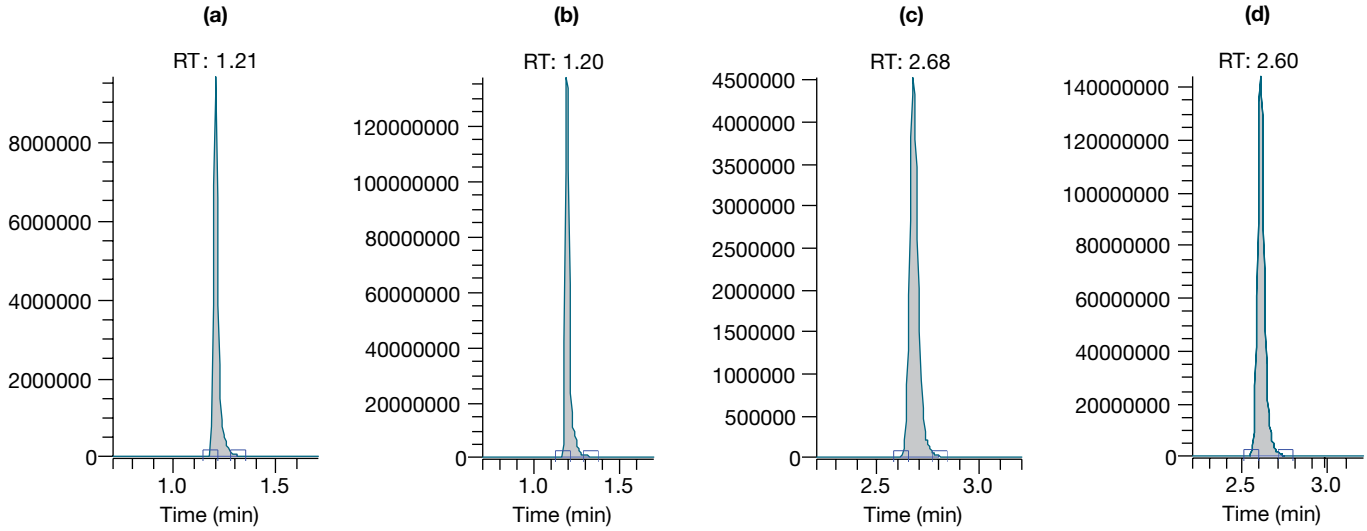

Figure 2. Representative chromatograms of the LLOQ for (a) amisulpride, (b) d_5-amisulpride, (c) quetiapine, and (d) d_8-quetiapine

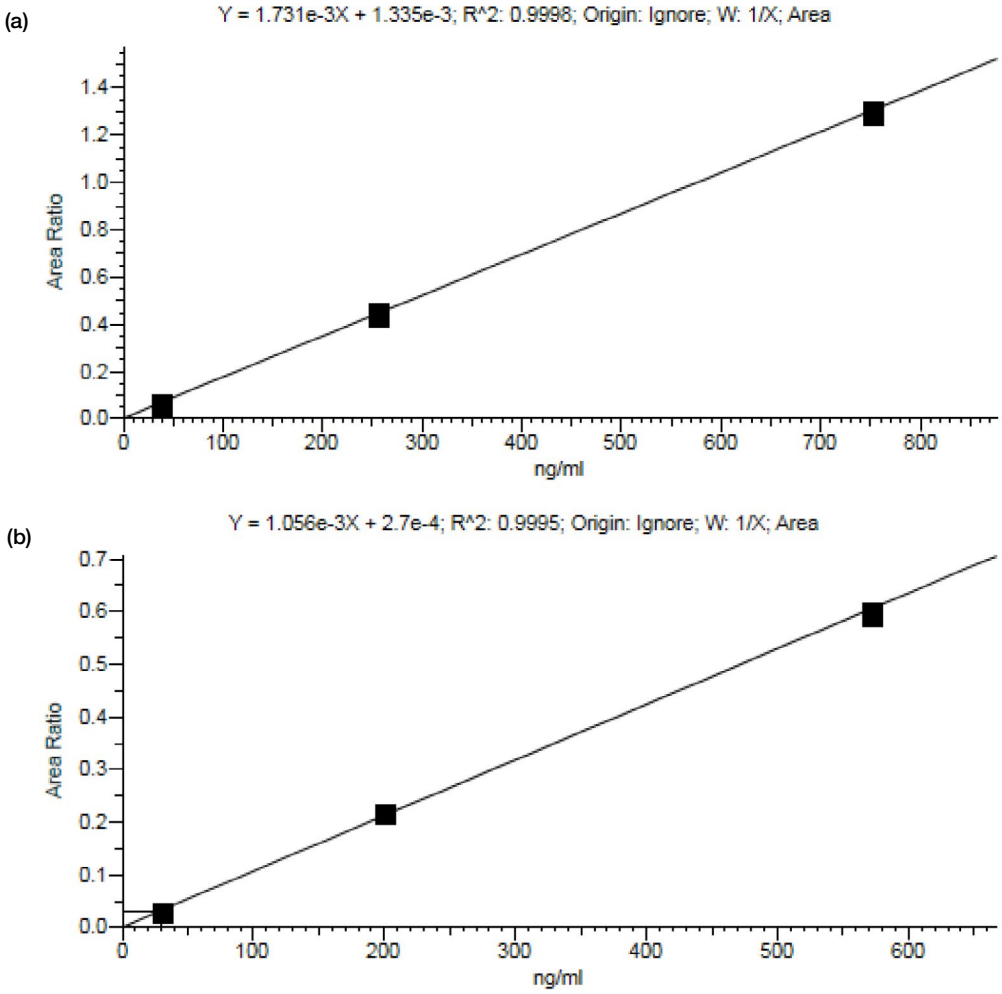

Figure 3. Representative calibration curves for (a) amisulpride and (b) quetiapine – day 1

The data presented in this report demonstrate the outstanding accuracy of the method with the percentage bias between nominal and average back-calculated concentration for the control samples from RECIPE ranging between -6.8% and 5.8% (Table 5).

Excellent results were also obtained from the evaluation of trueness of measurement using the external controls, with a percentage bias between -4.5% and 13.7% (Table 6 and Table 7).

The %CV for intra-assay precision was always below 6.6%. The maximum %CV for inter-assay precision was 6.2%. Results for intra- and inter-assay precision are reported in Table 8 and Table 9, respectively.

Table 5. Analytical accuracy results for controls MS9382 batch #1346

	Control 1			Control 2		
Analyte	Nominal concentration (ng/mL)	Average calculated concentration (ng/mL)	Bias (%)	Nominal concentration (ng/mL)	Average calculated concentration (ng/mL)	Bias (%)
Amisulpride	138	138	-0.1	323	321	-0.6
Aripiprazole	216	219	1.5	494	502	1.6
Chlorpromazine	69.4	68.5	-1.3	167	165	-1.4
Chlorprothixene	66.0	68.4	3.7	156	158	1.0
Clozapine	224	230	2.7	524	536	2.3
Dehydroaripiprazole	37.4	38.3	2.5	86.8	88.8	2.3
Desmethylolanzapine	28.3	28.4	0.4	66.8	66.6	-0.3
Flupentixol	2.34	2.45	4.7	5.65	5.95	5.4
Fluphenazine	2.34	2.34	0.0	5.56	5.72	2.9
Haloperidol	2.52	2.49	-1.1	6.08	5.88	-3.3
Levomepromazine	36.8	34.6	-6.1	89.2	83.1	-6.8
Melperone	38.3	36.8	-3.8	91.0	90.2	-0.9
Norclozapine	188	194	3.5	445	453	1.7
Norquetiapine	34.9	35.0	0.3	81.9	81.1	-1.0
Olanzapine	28.0	29.1	3.8	66.5	66.8	0.4
Paliperidone	25.3	25.6	1.4	60.2	59.7	-0.8
Perazine	99.4	93.5	-6.0	233	218	-6.4
Pipamperone	120	123	2.3	283	286	1.1
Promethazine	75.9	77.8	2.5	180	184	2.3
Prothipendyl	4.00	4.13	3.3	9.42	9.49	0.7
Quetiapine	117	116	-1.0	269	264	-1.8
Risperidone	25.5	25.9	1.6	58.1	61.3	5.5
Sertindole	40.9	41.2	0.8	96.1	97.1	1.0
Sulpiride	214	218	1.7	512	512	0.0
Thioridazine	81.4	79.8	-1.9	199	190	-4.5
Ziprasidone	65.2	66.6	2.2	157	156	-0.8
Zotepine	34.1	34.5	1.1	78.0	82.1	5.3
Zuclopenthixole	14.6	15.3	5.0	33.4	35.4	5.8

Table 6. Analytical accuracy results for controls GTFCh - TDMA 1/19

Analyte	Probe A			Probe B			Probe C			Probe D		
	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL	Bias (%)
Amisulpride	95.0	101	5.5	224	242	7.4						
Chlorprothixene	87.8	84.8	-3.6	199	195	-2.0						
Clozapine	155	161	3.7	365	396	7.8						
Levomepromazine							55.6	56.4	1.5	181	185	2.0
Norclozapine	132	136	2.7	336	361	7.0						
Olanzapine	40.7	44.1	7.8	78.3	85.0	7.9						
Paliperidone							25.5	25.8	1.0	87.1	90.0	3.2
Perazine							75.5	75.8	0.3	339	326	-4.1
Promethazine							71.9	72.2	0.5	226	231	2.2
Quetiapine	66.6	72.6	8.3	158	170	6.8						
Risperidone							5.19	4.97	-4.5	14.4	14.6	1.3
Sertindole												
Thioridazine	110	106	-4.2	235	245	4.1						
Ziprasidone							64.3	65.7	2.1	123	125	1.7

Table 7. Analytical accuracy results for controls GTFCh - TDMC 1/19

Analyte	Probe A			Probe B			Probe C			Probe D		
	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL)	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL	Bias (%)	Nominal conc. (ng/mL)	Average calculated conc. (ng/mL	Bias (%)
Aripiprazole	203	225	9.7	321	355	9.6	N/A	N/A	N/A	N/A	N/A	N/A
Dehydroaripiprazole	115	116	1.2	164	162	-1.5	N/A	N/A	N/A	N/A	N/A	N/A
Flupentixol	2.89	3.18	9.0	4.72	5.41	12.8	N/A	N/A	N/A	N/A	N/A	N/A
Fluphenazine	4.51	4.38	-2.9	7.97	7.96	-0.1	N/A	N/A	N/A	N/A	N/A	N/A
Haloperidol	4.73	4.85	2.5	12.7	12.9	1.4	N/A	N/A	N/A	N/A	N/A	N/A
Melperone	N/A	N/A	N/A	N/A	N/A	N/A	92.1	99.3	7.3	241	267	9.7
Pipamperone	N/A	N/A	N/A	N/A	N/A	N/A	167	172	3.0	307	327	6.0
Sertindole	57.4	61.3	6.4	87.7	94.3	7.0	N/A	N/A	N/A	N/A	N/A	N/A
Sulpiride	230	245	6.0	491	537	8.5	N/A	N/A	N/A	N/A	N/A	N/A
Zotepine	N/A	N/A	N/A	N/A	N/A	N/A	22.1	25.4	13.1	102	118	13.7

Table 8 (part 1). Intra-assay precision results for control MS9382 batch #1346

Analyte	Control 1						Control 2					
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3	
	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)
Amisulpride	137	0.9	138	1.0	139	0.8	318	0.6	323	1.0	323	1.3
Aripiprazole	220	3.0	218	2.9	220	2.1	527	1.4	469	2.3	510	1.1
Chlorpromazine	71.1	1.2	66.6	2.4	67.9	3.1	165	1.0	165	3.1	164	1.6
Chlorprothixene	68.9	2.0	68.6	1.7	67.7	1.5	159	0.7	152	4.5	162	1.5
Clozapine	227	2.7	231	0.6	232	1.8	528	0.7	536	1.3	543	1.4
Dehydroaripiprazole	38.8	1.5	38.3	4.8	37.9	1.3	92.0	2.0	87.6	5.0	86.9	2.6
Desmethylolanzapine	27.9	3.1	28.9	1.7	28.5	2.7	66.3	1.5	66.8	1.7	66.7	2.5
Flupentixol	2.43	4.5	2.46	1.6	2.46	4.3	5.99	2.3	5.97	2.2	5.90	0.7
Fluphenazine	2.35	3.4	2.25	1.8	2.41	2.8	5.78	4.6	5.64	2.5	5.73	2.9
Haloperidol	2.36	0.9	2.46	5.7	2.66	3.4	5.81	3.0	5.77	3.0	6.05	3.6
Levomepromazine	34.3	1.4	35.1	2.7	34.3	0.9	81.7	1.4	85.7	2.4	82.1	1.0
Melperone	37.7	2.2	35.6	1.0	37.2	1.3	88.3	1.2	90.5	4.5	91.6	2.8
Norclozapine	191	1.8	197	1.0	195	1.8	446	2.1	458	2.1	454	1.9
Norquetiapine	35.4	1.9	34.4	2.2	35.2	1.8	81.4	3.5	80.2	1.2	81.6	0.6

Table 8 (part 2). Intra-assay precision results for control MS9382 batch #1346

Analyte	Control 1						Control 2					
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3	
	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)
Olanzapine	30.1	2.6	28.1	0.9	29.0	1.2	68.6	1.2	66.1	1.3	65.6	1.5
Paliperidone	25.6	2.4	25.7	0.6	25.6	3.0	60.3	3.5	58.9	2.0	59.9	2.2
Perazine	94.2	0.8	93.7	1.7	92.5	1.5	218	0.8	217	0.6	219	1.0
Pipamperone	121	3.4	121	1.5	125	1.3	283	1.6	285	2.6	290	2.7
Promethazine	79.4	0.9	76.9	1.8	77.1	1.8	183	2.8	186	1.7	183	2.3
Prothipendyl	4.15	2.9	4.16	1.5	4.09	1.6	9.55	6.6	9.18	5.7	9.73	2.6
Quetiapine	115	1.6	115	1.8	118	2.2	265	1.2	258	1.3	269	1.4
Risperidone	25.9	2.0	26.1	2.6	25.7	1.8	61.2	2.7	61.5	1.3	61.1	1.6
Sertindole	40.0	1.3	42.1	1.6	41.5	1.5	93.8	2.8	99.6	1.2	97.9	2.4
Sulpiride	216	1.1	218	0.8	220	1.2	509	0.7	514	1.2	514	1.1
Thioridazine	80.8	1.5	78.7	4.4	80.0	0.6	189	1.8	191	1.8	190	1.7
Ziprasidone	66.3	2.0	66.0	2.2	67.5	0.8	153	1.6	156	1.9	158	3.6
Zotepine	35.6	1.1	32.3	2.2	35.5	3.7	83.0	1.6	81.7	1.0	81.7	1.7
Zuclopenthixole	15.3	2.5	15.5	1.7	15.2	1.0	35.5	1.2	35.3	1.5	35.2	0.6

Table 9. Inter-assay precision results for control MS9382 batch #1346

Analyte	Control 1		Control 2	
	Average calculated concentration (ng/mL)	CV (%)	Average calculated concentration (ng/mL)	CV (%)
Amisulpride	138	1.0	321	1.3
Aripiprazole	219	2.5	502	5.3
Chlorpromazine	68.5	3.6	165	1.9
Chlorprothixene	68.4	1.8	158	3.8
Clozapine	230	2.0	536	1.6
Dehydroaripiprazole	38.3	2.9	88.8	4.1
Desmethylolanzapine	28.4	2.8	66.6	1.8
Flupentixol	2.45	3.5	5.95	1.9
Fluphenazine	2.34	3.9	5.72	3.4
Haloperidol	2.49	6.2	5.88	3.7
Levomepromazine	34.6	2.0	83.1	2.7
Melperone	36.8	2.9	90.2	3.3
Norclozapine	194	1.9	453	2.2
Norquetiapine	35.0	2.2	81.1	2.2
Olanzapine	29.1	3.3	66.8	2.4
Paliperidone	25.6	2.1	59.7	2.7
Perazine	93.5	1.5	218	0.8
Pipamperone	123	2.6	286	2.5
Promethazine	77.8	2.1	184	2.3
Prothipendyl	4.13	2.1	9.49	5.5
Quetiapine	116	2.0	264	2.2
Risperidone	25.9	2.1	61.3	1.8
Sertindole	41.2	2.7	97.1	3.3
Sulpiride	218	1.3	512	1.1
Thioridazine	79.8	2.7	190	1.7
Ziprasidone	66.6	1.9	156	2.7
Zotepine	34.5	5.2	82.1	1.6
Zuclopenthixole	15.3	1.9	35.4	1.2

thermo scientific

Conclusions

An LC-HRAM mass spectrometry-based method using a Vanquish Flex Duo UHPLC system connected to a Q Exactive Plus hybrid quadrupole-Orbitrap MS is reported here, demonstrating the power of Orbitrap technology in performing accurate qualitative analyses and routine quantitation with high efficiency. A liquid chromatography-HRAM mass spectrometry method for clinical research was developed and implemented for the quantification of 28 neuroleptic drugs in human plasma or serum. The ClinMass TDM Platform with the ClinMass Add-On Set for Neuroleptics from RECIPE was used. The method incorporates a quick and simple offline protein precipitation step with concomitant internal standard addition. The described method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.

Find out more at **thermofisher.com/clinicalresearch**

ThermoFisher SCIENTIFIC