

High-Throughput Analysis of Neuroleptic Drugs in Plasma Using Luxon Ion Source® MS/MS System

Serge Auger, Jean Lacoursière, and Pierre Picard
Phytronix Technologies, Québec, Canada

Keywords: High-Throughput, neuroleptics, Luxon-MS/MS

Introduction

Neuroleptics, also known as antipsychotic medication, are used to treat and manage symptoms of many psychiatric disorders. They can be divided into two classes: first-generation or "typical" antipsychotics and second-generation or "atypical" antipsychotics. Both generations of medication tend to block receptors in the brain's dopamine pathways, but atypical tend to act on serotonin receptors as well. To increase the analysis throughput of plasma samples, the Luxon Ion Source® coupled to tandem mass spectrometry (MS/MS) was used for the quantification of neuroleptic drugs.

For this project, we propose to perform a generic extraction method for neuroleptic drug analysis in plasma. Quantification using the Luxon coupled to a mass spectrometer (Luxon-MS/MS) is chosen as a fast-analytical technique.

Luxon Ionization Source

The Luxon Ion Source® (Figure 1) is the second-generation sample introduction and ionization source based on the LDTD® technology for mass spectrometry. Luxon Ion Source® uses Fiber-Coupled Laser Diode (Figure 2) to obtain unmatched thermal uniformity giving more precision, accuracy and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High-efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 1 second sample-to-sample and allows working with very small volumes.



Figure 1 - Luxon Ion Source®



Figure 2 - Schematic of the Luxon Ionization Source

Sample Preparation Method

Neuroleptics multilevel plasma calibrator set from Chromsystems™ containing the following drugs is used: Aripiprazole, Dehydroaripiprazole, Clozapine, Desmethylclozapine, Haloperidol, Olanzapine, N-desmethyloanzapine, Quetiapine, Norquetiapine, Risperidone and 9-Hydroxyrisperidone. These are used as calibration standards. Control Level I and Level II from Chromsystems™ are used as quality control samples.

Protein precipitation is performed by mixing 20 µL plasma, 4 µL internal standard solution and 200 µL acetonitrile. Vortex and centrifuge at 14000 rpm for 1 minute. Finally, 5 µL of the upper layer are spotted into 96-LazWell-DEC™ plates and evaporated to dryness. Luxon-MS/MS analysis is performed after a complete evaporation.

LDTD-MS/MS Parameters

LDTD

Model: Phytronix, Luxon S-960

Carrier gas: 6 L/min (air)

Laser pattern: 6 second ramp to 65% power and hold 2 seconds.

MS/MS

Model: Q-Trap System® 5500, Sciex

Ionization: APCI (Positive)

Table 1 – Mass spectrometer transitions

Sulfonamides	Transition	CE
Norquetiapine	296 → 253	31
N-Desmethyloanzapine	299 → 213	35
Desmethylclozapine	313 → 192	55
Olanzapine	313 → 256	31
Clozapine	327 → 270	32
Clozapine-D ₈	335 → 275	32
Haloperidol	376 → 165	25
Quetiapine	384 → 253	33
Quetiapine-D ₈	392 → 258	33
Risperidone	411 → 110	60
9-Hydroxyrisperidone	427 → 207	35
9-Hydroxyrisperidone-D ₄	431 → 211	35
Dehydroaripiprazole	446 → 285	22
Aripiprazole	448 → 285	22

Results and Discussion

Linearity

Calibrator set spiked at the following calibration ranges are extracted: 20.6 to 746 µg/L for Aripiprazole, 7.56 to 273 µg/L for Dehydroaripiprazole, 77.7 to 1149 µg/L Clozapine, 75.1 to 453 µg/L for Desmethyloanzapine, 2.03 to 30.03 µg/L for Haloperidol, 3.85 to 153 µg/L for Olanzapine, 3.92 to 160 µg/L for N-Desmethyloanzapine, 27.3 to 525 µg/L for Quetiapine, 11 to 221 µg/L for Norquetiapine, 2.04 to 30.7 µg/L for Risperidone and 3.68 to 138 µg/L for 9-Hydroxyrisperidone. Correlation coefficients are equal or greater than 0.99 (**Table 2**) for the quantification curve of each molecule. **Figure 3** shows the calibration curve of Clonidine. Similar results are obtained for the other Neuroleptic drugs. **Figure 4** shows the typical desorption peak of Clonidine with a 0.16-minute window.

Table 2– Calibration curve equations

Drug	Equation	R
Norquetiapine	$Y = 0.01130 X + 0.00782$	0.99585
N-Desmethyloanzapine	$Y = 2.01659 \cdot 10^{-4} X + 9.92306 \cdot 10^{-4}$	0.99597
Desmethyloanzapine	$Y = 0.00108 X + 0.00458$	0.99396
Olanzapine	$Y = 0.00262 X + 4.56504 \cdot 10^{-4}$	0.99875
Clozapine	$Y = 0.00619 X - 7.80539 \cdot 10^{-4}$	0.99914
Haloperidol	$Y = 0.06161 X + 0.02704$	0.99876
Quetiapine	$Y = 0.11178 X - 0.03033$	0.99925
Risperidone	$Y = 0.00888 X + 0.03699$	0.99452
9-Hydroxyrisperidone	$Y = 0.08996 X + 0.02258$	0.99980
Dehydroaripiprazole	$Y = 0.01801 X + 0.00193$	0.99347
Aripiprazole	$Y = 0.00484 X + 0.03608$	0.99844

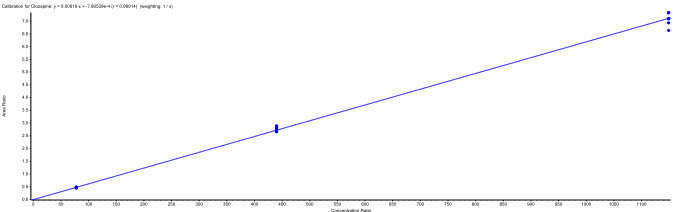


Figure 3 – Clozapine calibration curve

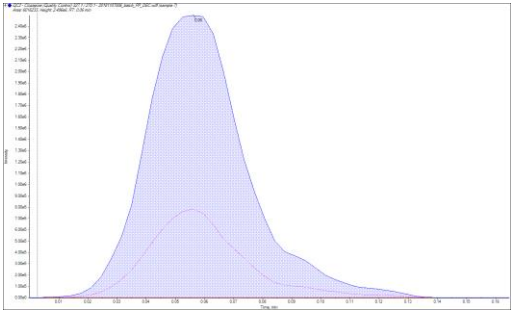


Figure 4 – Clozapine desorption peak

Precision and accuracy

Replicate extractions of control Level I and II are deposited on a LazWell™ plate and dried before analysis. Six replicas are analyzed for within-run evaluation. CV% was below 15% and accuracy within ±20% of the nominal concentration is reached. **Table 3** shows the intra-run precision/accuracy results.

For the inter-run precision/accuracy experiment, each control sample sets are analyzed in sextuplicate on three different days. **Table 3** shows the inter-run precision/accuracy results.

Table 3 – Intra and Inter-run precision/Accuracy

	Intra run		Inter-run	
	Level I	Level II	Level I	Level II
Norquetiapine				
Conc. (µg/L)	53.4	93.6	53.4	93.6
N	6	6	18	18
Mean (µg/L)	60.5	95.1	60.6	103.8
%CV	6.1	9.2	8.0	13.2
%NOM	113.3	101.6	113.5	110.9
N-Desmethyloanzapine				
Conc. (µg/L)	35.9	61.6	35.9	61.6
N	6	6	18	18
Mean (µg/L)	40.4	67.5	41.3	71.4
%CV	6.6	9.0	8.1	12.5
%NOM	112.6	109.6	115.1	115.9
Desmethyloanzapine				
Conc. (µg/L)	171	234	171	234
N	6	6	18	18
Mean (µg/L)	173.2	237.1	169.7	230.7
%CV	8.4	6.4	8.5	13.9
%NOM	101.3	101.3	99.3	98.6
Olanzapine				
Conc. (µg/L)	34.9	63.6	34.9	63.6
N	6	6	18	18
Mean (µg/L)	40.7	68.5	41.3	71.4
%CV	2.6	9.5	7.3	10.3
%NOM	116.6	107.7	118.2	112.2
Clozapine				
Conc. (µg/L)	337	567	337	567
N	6	6	18	18
Mean (µg/L)	352.0	565.2	355.9	576.1
%CV	3.5	2.3	3.2	4.5
%NOM	104.5	99.7	105.6	101.6
Haloperidol				
Conc. (µg/L)	3.7	15.7	3.7	15.7
N	6	6	18.0	18.0
Mean (µg/L)	4.3	18.4	4.4	18.5
%CV	4.5	5.5	5.2	5.6
%NOM	116.2	117.2	118.7	117.7
Quetiapine				
Conc. (µg/L)	147	256	147	256
N	6	6	18	18
Mean (µg/L)	152.5	251.3	153.7	258.0
%CV	3.5	3.3	3.2	5.6
%NOM	103.7	98.2	104.6	100.8
Risperidone				
Conc. (µg/L)	9.17	14.3	9.17	14.3
N	6	6	18	18
Mean (µg/L)	9.4	14.0	10.1	14.6
%CV	11.8	11.9	12.2	11.7
%NOM	102.5	97.9	110.2	102.4
9-Hydroxyrisperidone				
Conc. (µg/L)	35.8	64.2	35.8	64.2
N	6	6	18	18
Mean (µg/L)	36.1	61.4	36.4	63.5
%CV	1.7	2.7	3.8	6.5
%NOM	100.8	95.6	101.6	99.0
Dehydroaripiprazole				
Conc. (µg/L)	64.2	116	64.2	116.0
N	6	6	18.0	18.0
Mean (µg/L)	70.2	124.6	67.1	120.4
%CV	7.4	11.1	8.8	9.0
%NOM	109.3	107.4	104.5	103.8
Aripiprazole				
Conc. (µg/L)	172	314	172	314
N	6	6	18	18
Mean (µg/L)	194.1	346.2	193.1	349.0
%CV	6.5	4.4	7.6	10.6
%NOM	112.8	110.3	112.2	111.2

Conclusion

Luxon Ion Source® combined to Q-Trap 5500 mass spectrometer system allows ultra-fast (**10 seconds per sample**) quantification of neuroleptic drugs in plasma using a generic protein precipitation extraction procedure.