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Fully automatized LC-MS/MS analysis of neuroleptics using a novel sample preparation system

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Sigrid Baumgarten[1]; Stephane Moreau[1];
Daisuke Kawakami[2]
[1] Shimadzu Europa GmbH, Duisburg, Germany;
[2] Shimadzu Corporation, Kyoto, Japan

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Introduction

Neuroleptics also known as antipsychotics are a class of drug used for medication primarily used to manage psychosis. Their active principle is influencing the synaptic conduction in the central nervous system. Their precise quantitation for therapeutic purpose is necessary as for several class of drugs like antidepressants, benzodiazepines, anti-epileptics… Such analysis are mainly done by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with multi-analytes approach nowadays. In order to streamline the workflow, we demonstrate here the use of a novel fully automated sample preparation system coupled online with LC-MS/MS.

Method Development

The analysis of Neuroleptic was performed using the fully automated LCMS preparation Unit CLAM-2000 (Shimadzu, for research use only) online with HPLC-MS/MS (Nexera X2-LCMS-8045, Shimadzu), starting from plasma samples using the "MassTox® TDM Serie A Neuroleptic 1 and 2" (Chromsystems). Calibrators and Internal standard mix were loaded onto the CLAM-2000 (refrigerated at 8°C).

The treated samples were separated by the analytical column (Chromsystems, MassTox® TDM Serie A 92110) at 40°C with a binary gradient system (Mobile phase A and B Chromsystems, MassTox® TDM Serie A Basic Kit 92111) at a flow rate of 0.6 ml/min in 3.5min (Table 1). Quantification was performed using optimized MRM transitions and Internal standard calibration method.

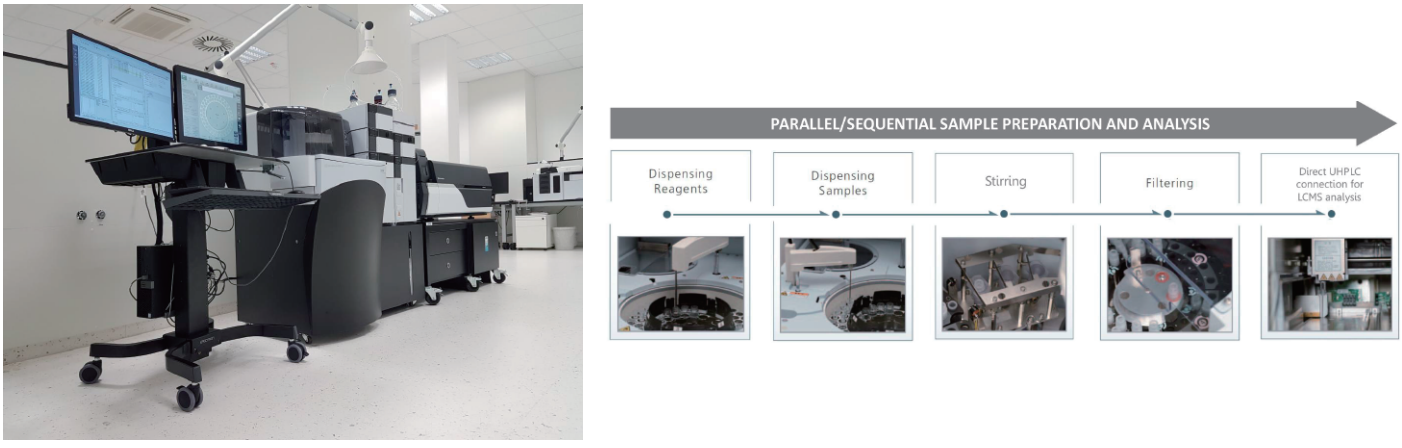

Figure 1: CLAM-2000 online with Nexera X2 system and LCMS-8045 triple quadrupole mass spectrometer.

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Table 1: Analytical conditions and source parameters.

LC	: NEXERA X2 system
Column Temperature	: 40°C
Flow rate	: 0.6 mL/min
Time Program	: Gradient A-B 3 min kit 2 Gradient A-B 3 min kit 1
Injection volume	: 2 µl

MS	: LCMS-8045
Ionization	: ESI positive
Nebulizer gas	: 3 L/min
Interface temperature	: 300°C
Desolvation line	: 200°C
Heat Block temperature	: 400°C
Drying Gas	: 5 L/min
Heating Gas	: 15 L/min
Analysis mode	: MRM

Table 2: MRM transitions for Neuroleptic Kit 1 (left) and Kit 2 (right)

Compounds	Quantifier ion	Qualifier ion
Aripiprazole	448.1>285.15	448.1>176.15
Clopazine	327.1>270.1	327.1>192.15
Dehydroaripiprazole	446.1>285.15	446.1>98.15
Desmethylclopazine	313.1>192.1	313.1>270.1
Haloperidol	376.1>123.1	376.1>165.15
N-Desmethylolanzapine	299.1>198.1	299.1>256.15
Norquetiapine	296.1>210.1	296.1>183.10
Olanzapine	313.1>256.15	313.1>198.05
Quetiapine	384.1>253.15	384.1>221.15
Risperidone	411.2>191.15	411.2>110.3
9-OH-Risperidone	427.2>207.15	427.2>110.15

Compounds	Quantifier ion	Qualifier ion
Amisulpride	370.1>242.1	370.1>196.05
Levomepromazine	329>100.2	329>58.2
Melperone	264.1>123.1	264.1>165.3
Perazine	340.1>141.2	340.1>113.15
Pipamperone	376.2>165.3	376.2>123.25
Promethazine	285.1>86.3	285.1>198.1
Sertindole	441.1>113.2	441.1>71.2
Sulpiride	342.1>112.15	342.1>214
Thioridazine	371.1>126.2	371.1>98.2
(Z)-Chorprothixene	316.1>231.1	316.1>271
Ziprasidone	413.1>194.15	413.1>166.1
Zotepine	332>72.15	332>70.2
Zuclopenthixol	401.1>221.1	401.1>231.5

Using the MassTox® TDM kit (Chromsystems) it is possible to perform both extraction and IS spike within a single step. Nevertheless, with this procedure, many manual steps are required in order to complete the sample preparation. This procedure is time consuming and could be affected by bias caused by the operator due to the liquid transfer steps that are required (Figure 2), moreover it is difficult to maintain the traceability of each steps for all the processed samples.
Using the CLAM-2000 it was possible to obtain a complete integration of sample preparation steps with the LC-MS/MS quantification.

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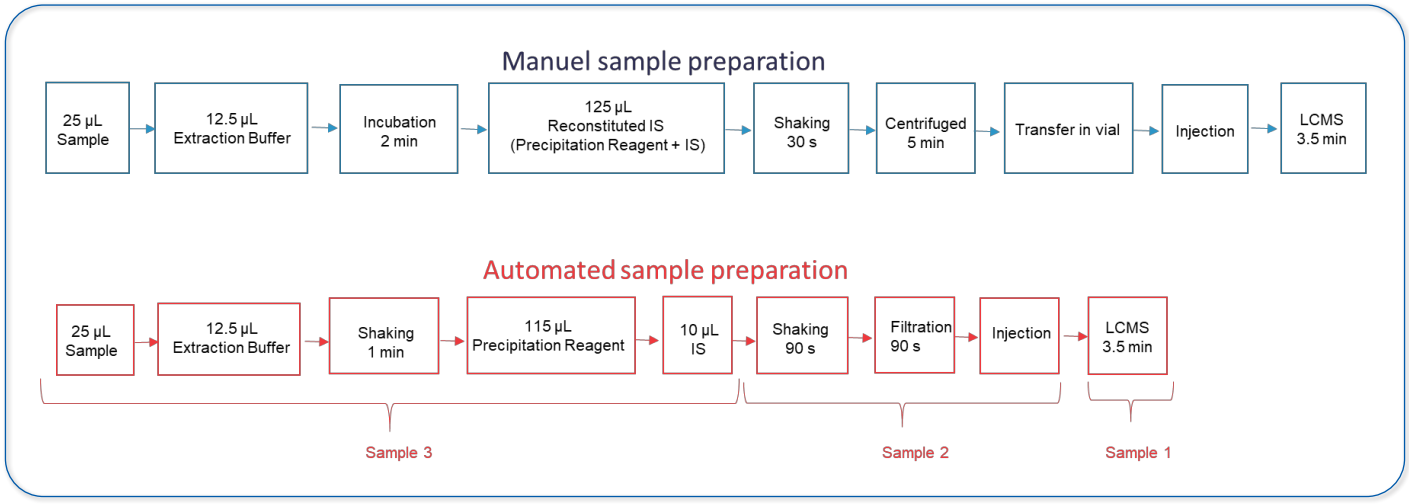

Figure 2: CLAM-2000 fully automated sample preparation and analysis - Due to the overlapped sample preparation, the throughput of the instrument was 1 result each 8 minutes for quantification of all Compounds.

Results

The linearity and accuracy of the method was evaluated using 3 reference serum calibrators levels MassTox® TDM . For all the analytes linearity and accuracy were within the analytical acceptable range (91.4%-105.7%). Furthermore in order to estimate the precision of the method, reference plasma control MassTox® TDM were analyzed several times (10 replicates). For all analytes the CV% values were within acceptable analytical ranges.

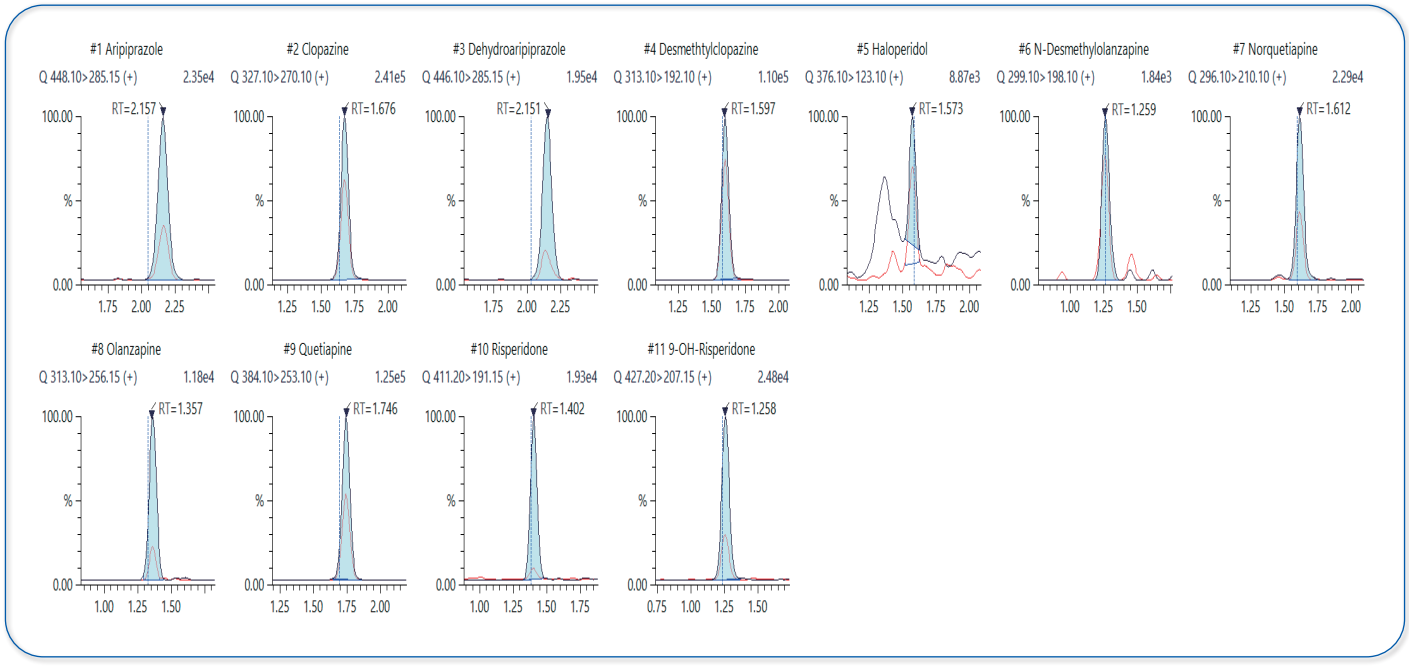

Figure 3: Chromatograms of the Neuroleptic Kit 1 at LLOQ.

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Table 3: Linearity, Accuracy, and precision evaluated using MassTox®
TDM plasma controls level 2 n=10 replicates (kit 1).

Compound	R2	Accuracy		CV (%) Control Level 2
		min	max	
Aripiprazole	0.999	96.8	102.1	5.27
Clopazine	0.998	91.4	105.7	3.75
Dehydroaripiprazole	0.999	95.1	103.3	6.99
Desmethylclopazine	0.999	99.2	102.5	5.98
Haloperidol	0.998	99.5	100.2	5.42
N-Desmethylolanzapine	0.999	98.5	101	4.52
Norquetiapine	0.999	98.3	100.6	4.10
Olanzapine	0.999	98.3	101.1	4.27
Quetiapine	0.999	97.8	100.7	4.79
Risperidone	0.999	98.3	101.1	8.06

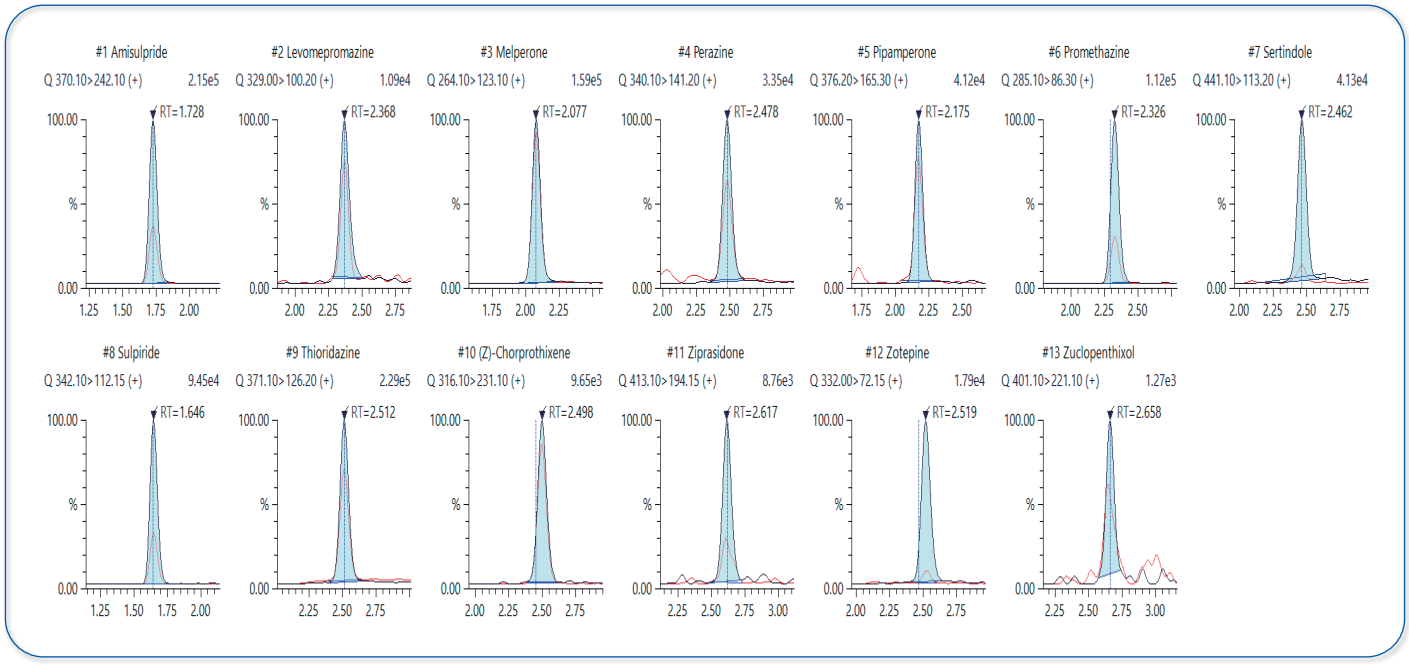

Figure 4: Chromatograms of the Neuroleptic Kit 2 at LLOQ.

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Table 4: Linearity, Accuracy, and precision evaluated using MassTox®
TDM plasma controls level 1 and 2 n=10 replicates.

Compound	R2	Accuracy		CV (%)	
		min	max	Control Level 1	Control Level 2
Amisulpride	0.999	99.7	100.6	3.70	5.73
Levomepromazine	0.999	97.3	101.4	5.56	8.76
Melperone	0.999	96.2	102	4.18	5.40
Perazine	0.999	96.1	101.7	5.39	5.76
Pipamperone	0.999	99.3	100.3	3.68	6.01
Promethazine	0.999	99.8	100.4	4.92	3.07
Sertindole	0.999	100	100.1	5.19	4.23
Sulpiride	0.999	99.4	100.3	4.55	4.29
Thioridazine	0.999	99.6	100.7	5.02	5.68
(Z)-Chorprothixene	0.999	98.9	100.2	7.29	5.81
Ziprasidone	0.999	97.6	101.2	4.63	4.76
Zotepine	0.999	99.1	101.9	3.56	6.19
Zuclopenthixol	0.999	97.7	101.2	6.11	6.39

Conclusion

Fully Automated sample preparation procedure led to suitable results for the quantitation of Neuroleptic thus eliminating all manual preparation steps
The novel system workflow results in easier and safer operation for users even without Chromatography and Mass Spectrometry experience, thus reducing risk of exposure. It allows to access and analyse hundreds of analytes on the same system without any modification thus improving the quality of service delivered to doctors for quick decision.

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