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PO-CON1777E

Fully automated platform for determination of Tricyclic Antidepressant in serum

ASMS 2017 ThP090

Davide Vecchietti[1], Claudio Ghilardi[1], Katharina Kern[2], Stephane Moreau[3], Isabel Cabruja[1]
1 Shimadzu Italia, Milano, Italy,
2 RECIPE Chemicals + Instruments GmbH, Munich, Germany,
3 Shimadzu Europe GmbH, Duisburg, Germany

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Introduction

Tricyclic antidepressants (TCAs) are a group of psychoactive drugs which are mainly used for the therapy of endogene depressions, anxiety and pain management. The therapeutic drug monitoring of these molecules needs to be accomplished by extremely accurate techniques due to the narrow therapeutic range and to the severe adverse effect caused by overdosing. Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) shows high sensitivity and specificity. Nevertheless, LC-MS/MS approaches mostly lack standardization and the necessary throughput for the application in routine analysis. We report a fully automated platform for the quantitation of more than 13 TCAs in serum samples with high throughput and no need for manual sample preparation (the present work is intended for Research use only).

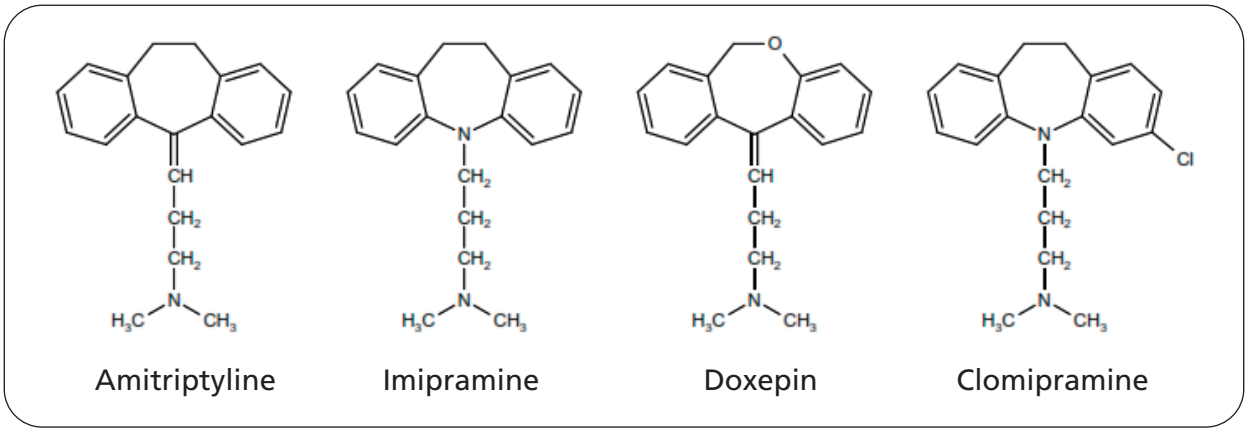

Figure 1: Some of the most common Tryciclic Antidepressants.

Methods and Materials

The analysis of TCAs was performed using a fully automatic LCMS preparation Unit (CLAM-2000, Shimadzu, for research use only) online with HPLC-LCMS (NexeraX2-LCMS8060, Shimadzu) starting from serum samples using the "ClinMass® TDM Kit System" (Recipe, MS9100). Samples (Reference material: human serum samples), calibrators and Internal standard mix (Recipe, ClinMass® MS9112) were loaded onto the CLAM-2000 (refrigerated at 8°C). The treated samples were separated by the analytical column (Recipe, MS9030) at 40°C with a binary gradient system at a flow rate of 0.6 ml/min in 3.8 min (Table 1). Quantification was performed using optimized MRM transitions and Internal standard calibration methods.

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Table 1: Analytical conditions and source parameters.

[LC] NexeraX2 System	
Column Temp.	: 40°C
Time Program	: gradient A-B 3.8 min
Injection Volume	: 0.5 µL
[MS] LCMS-8060	
Ionization	: ESI Positive
Nebulizer Gas	: 3 L/min
Interface temperature	: 300°C
Desolvation Line	: 250°C
Heat Block temperature	: 400°C
Drying Gas	: 10 L/min
Scan Type	: MRM

Analytical conditions

The TCAs were quantified using MRM transitions listed in Table 2.

Table 2: MRM transitions for TCAs

Name	Quantifier ions	Qualifier ions	Name	Quantifier ions	Qualifier ions
d8-Norclozapine	321.20>192.10		d3-Imipramine	284.20>61.20	
Norclozapine	313.10>192.10	313.10>269.90	Nortimipramine	281.20>44.20	281.20>55.20
d3-Nordoxepin	269.20>107.10		Imipramine	281.20>58.20	281.20>86.20
Nordoxepin	266.20>107.10	266.20>44.20	d4-Clozapine	331.10>191.90	
d3-Doxepin	283.20>107.10		Clozapine	327.00>192.00	327.00>270.00
Doxepin	280.10>107.10	280.10>58.30	d3-Amitriptyline	281.20>105.10	
Desipramine	267.20>72.00	267.20>44.20	Norclomipramine	301.10>72.20	301.10>44.20
d3-Desipramine	270.20>75.20		Amitriptyline	278.10>105.10	278.10>233.00
Normaprotiline	264.20>169.20	264.20>219.10	d3-Norclomipramine	304.00>75.20	
Protriptyline	264.20>233.10	264.20>155.10	d3-Trimipramine	298.20>103.00	
d3-Nortriptyline	267.20>91.20		Trimipramine	295.20>100.20	295.20>58.20
Nortriptyline	264.20>233.10	264.20>91.10	d3-Clomipramine	318.20>89.20	
Maprotiline	278.20>250.20	278.20>178.10	Clomipramine	315.20>86.20	315.20>58.20
d5-Maprotiline	283.20>255.20				

The CLAM-2000 was programmed to perform sample extraction and protein precipitation followed by filtration and sample collection (Figure 2).

Figure 2: CLAM-2000 online with Nexera X2 system and LCMS-8060 triple quadrupole mass spectrometer.

Results

Fully Automated sample preparation

Prior to the LC-MS/MS analysis a sample preparation is carried out in order to remove the sample matrix and to spike the sample with an internal standard.
Using the ClinMass® MS9100 kit (Recipe) it is possible to perform both extraction and IS spike with 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 3), 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.
The samples were loaded onto the CLAM-2000 using microvials and/or primary sample collection vials. The fully automatic preparation/analysis procedure was performed as follows: I) 60 ul of IS std mix were dispensed in a filtration-collection vial (reagent for protein precipitation); II) 30 ul of serum sample were added;; III) stirring and incubation (1 min); IV) filtration for 0.45 min (deproteinization); the sample was finally transported to the LCMS system without human intervention (Figure 3). After LC-MS/MS analysis a data report was directly visualized by the CLAM-2000 control software.

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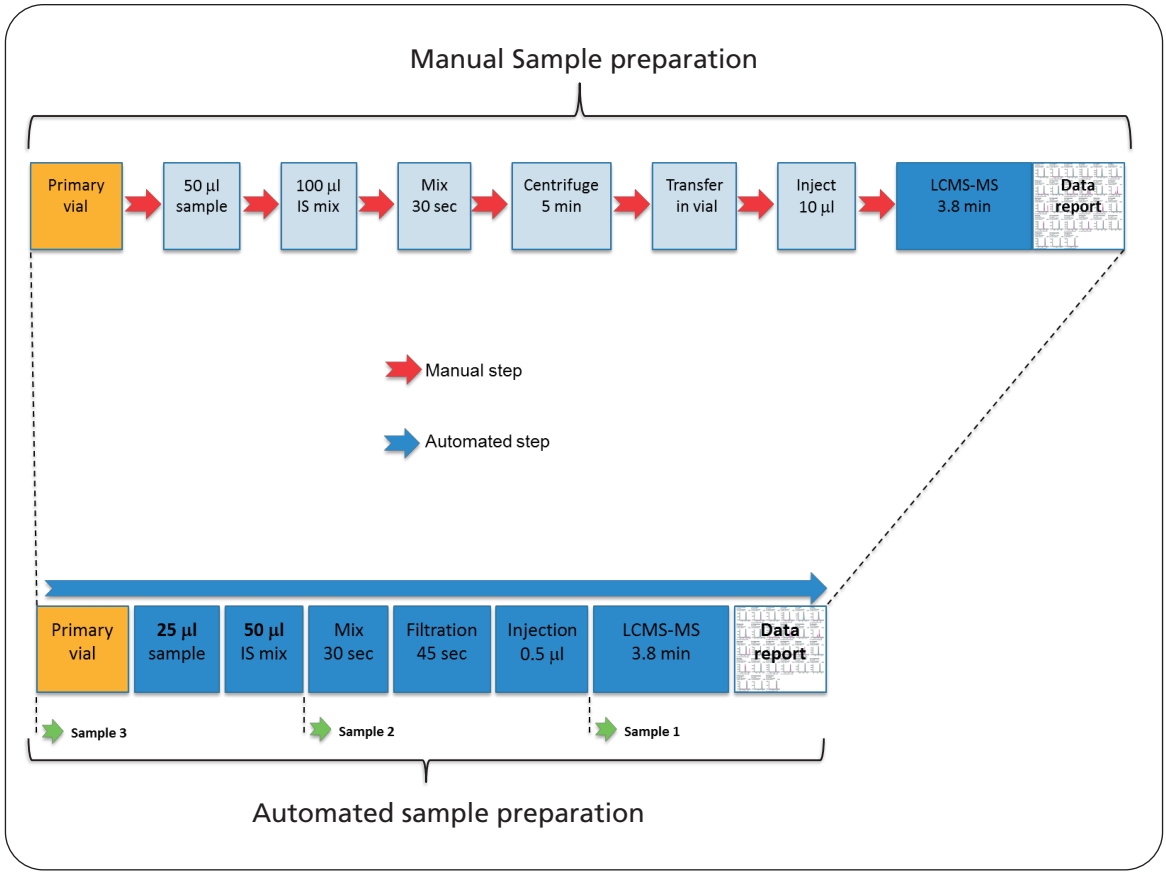

Figure 3: CLAM-2000 fully automated sample preparation and analysis. Due to the overlapped sample preparation the throughput of the instrument was 1 result each 5 minutes for quantification of all TCAs.

Linearity, Accuracy and Precision

The linearity and accuracy of the method was evaluated using 3 reference serum calibrators levels (Recipe MS9213). For all the analytes linearity and accuracy were within the analytical acceptable range (85%-115%). Furthermore in order to estimate the precision of the method, reference serum samples (Recipe MS9182) spanning from low concentration level (Figure 4) to high concentration level were analyzed several times (6 replicates). For all analytes the CV% values were within acceptable analytical ranges. The same experiment was repeated for 3 non-consecutive days in order to estimate the inter-day precision (Table 3).

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Table 3: Linearity, Accuracy, and precision evaluated using ClinChek ® MS9182 reference serum controls.
*n=6 replicates; **n=3 non-consecutive days.

	R^2	ACCURACY		PRECISION (%CV)				BIAS %			
				INTRADAY*		INTERDAY**		INTRADAY*		INTERDAY**	
		min	max	Low	High	Low	High	Low	High	Low	High
Amitriptyline	0.999	98.2	100	2.82	2.22	2.99	2.19	2.97	0.75	6.71	5.32
Clomipramine	0.999	99.3	100	1.80	1.09	2.94	1.46	0.82	2.93	1.86	3.23
Clozapine	0.999	96.9	101	2.45	2.17	2.70	2.71	2.71	4.96	2.13	5.42
Desipramine	0.999	96.9	104	2.07	2.80	2.65	2.49	2.17	0.90	2.60	2.06
Doxepin	0.999	88.8	102	4.29	2.21	2.39	1.70	3.87	1.50	5.56	3.37
Imipramine	0.999	96.1	105	2.47	1.88	2.59	2.40	0.83	0.11	2.95	1.95
Maprotiline	0.999	93.5	101	0.98	3.75	1.60	1.99	4.63	5.42	3.51	3.17
Norclomipramine	0.999	97.9	103	5.10	3.16	3.74	2.68	2.75	6.66	3.36	2.94
Norclozapine	0.999	95.8	101	2.96	3.12	2.68	2.41	7.58	3.86	4.19	4.04
Nordoxepin	0.999	91.4	102	2.47	2.74	2.06	2.18	1.64	0.65	1.76	2.22
Nortimipramine	0.999	97.4	104	3.69	3.45	5.59	5.13	3.03	1.77	8.21	3.31
Nortriptyline	0.999	92.8	110	7.84	5.50	6.42	5.49	5.85	4.88	5.77	6.46
Protriptyline	0.999	94.6	101	2.47	3.01	3.73	3.87	8.15	1.17	4.53	1.54
Trimipramine	0.999	97.6	103	2.91	3.47	2.89	2.65	13.60	10.46	11.30	6.89
Normaprotiline	0.999	96.5	105	4.51	4.71	7.87	4.97	8.22	10.37	7.01	12.79

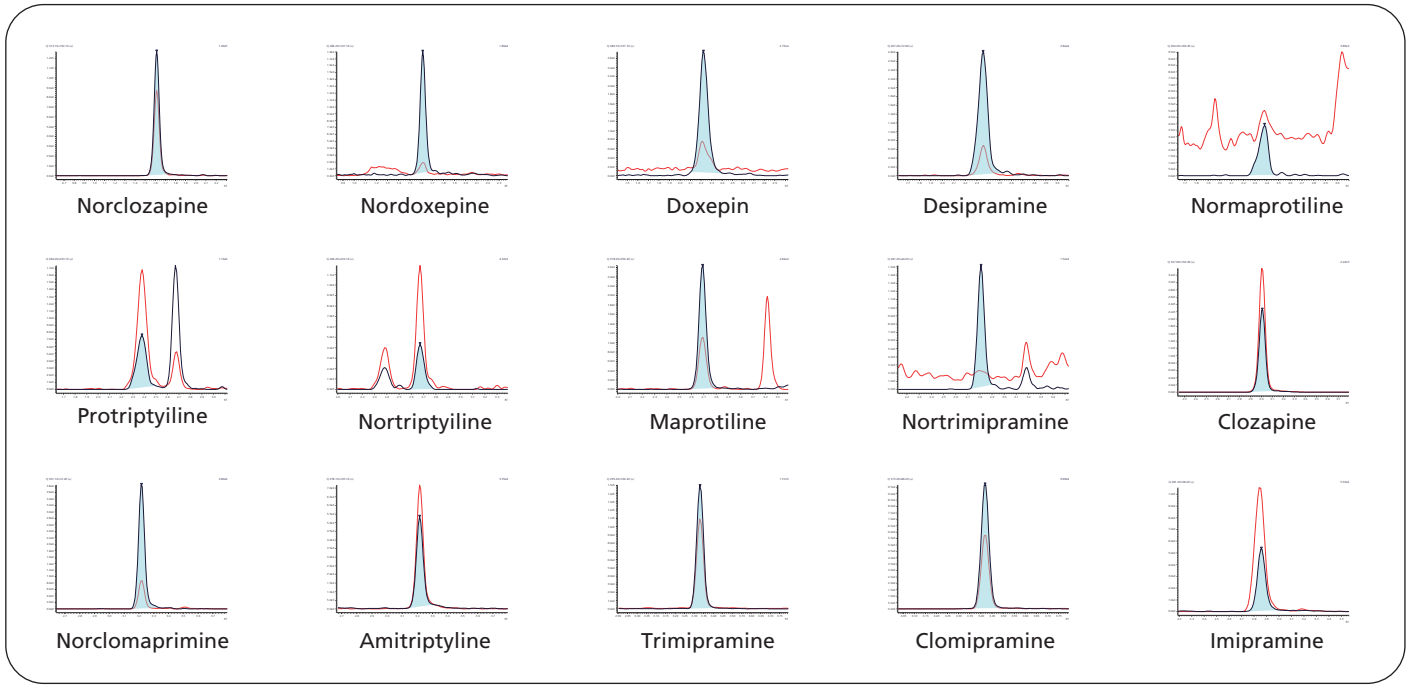

Figure 4: Chromatograms of TCAs at LLOQ.

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Conclusions

- Fully Automated sample preparation procedure resulted suitable for the quantitation of Tricyclic Antidepressant by elimination of all manual preparation steps.
- The automation of the method increases the analytical performance, reduces the risk for human operators and due to the reduced reagent consumption, reduces also the cost of the analysis.

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First Edition: June, 2017

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