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Offline and online sample extraction for the quantification of tricyclic antidepressants in human plasma or serum for clinical research

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Keywords
Tricyclic antidepressants, direct injection, offline sample preparation, plasma, serum, mass spectrometry

Application benefits

- Flexibility in sample preparation: direct injection of plasma or serum or offline sample preparation for improved sensitivity
- Analysis and quantitation of 12 tricyclic antidepressants in a single quantitative method

Goal

Development of a robust analytical method for the quantification of 12 different tricyclic antidepressants in human plasma or serum on a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer.

Introduction

An analytical method for clinical research for the quantification of 12 tricyclic antidepressants in human plasma or serum is reported; the analysis includes amitriptyline, clomipramine, clozapine, desipramine, doxepin, imipramine, maprotiline, norclomipramine, norclozapine, nordoxepin, nortriptyline, and trimipramine. Two different sample preparation steps were available: automated addition of the internal standards followed by direct injection of the unextracted plasma or serum sample for minimum human intervention or offline internal standard addition and protein precipitation for improved sensitivity. In both cases, samples were injected onto a Thermo Scientific™ Transcend™ II TLX-1 system coupled to a Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer with heated electrospray ionization.

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Detection was performed by selected-reaction monitoring (SRM) using a deuterated internal standard for each analyte in the panel. Method performance was evaluated using the MS9050 ClinMass® TDM Platform on-line with the MS9150 ClinMass Add-On Set for Tricyclic Antidepressants (for direct injection) and the MS9000 ClinMass TDM Platform with the MS9100 ClinMass Add-On Set for Tricyclic Antidepressants (for offline sample preparation), both from RECIPE®, to obtain limits of quantification, linearity ranges, accuracy, and intra- and inter-assay precision for each analyte.

Table 1. Concentration ranges covered by calibrators.

Analyte	Concentration Range (ng/mL)
Amitriptyline	23.7–422
Clomipramine	22.5–438
Clozapine	22.8–419
Desipramine	23.2–421
Doxepin	22.1–419
Imipramine	46.0–1177
Maprotiline	22.5–431
Norclomipramine	22.1–445
Norclozapine	36.1–1002
Nordoxepin	21.5–417
Nortriptyline	22.2–438
Trimipramine	21.6–436

Experimental

Target analytes

The analytes and corresponding concentration ranges covered by the calibrators used are reported in Table 1.

Sample preparation

Reagents included calibrators and controls from RECIPE at four (including blank) and two different levels, respectively, as well as 12 deuterated internal standards for the quantification.

Direct injection approach: No manual sample preparation was required. The autosampler of the Transcend II TLX-1 system was used for the automated addition of the internal standards prior to injection of the unextracted plasma or serum sample onto the LC system for online SPE.

Offline protein precipitation approach: 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.

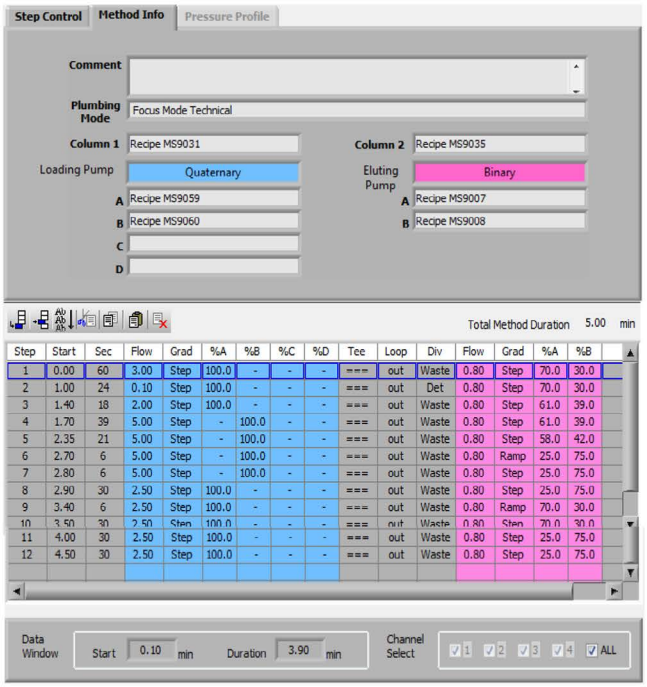
Liquid chromatography

The LC separation was achieved using mobile phases, an SPE cartridge (for direct injection only) and an analytical column provided by RECIPE. Details of the analytical methods for both approaches are reported in Figure 1. Total runtime was 5.0 minutes for direct injection and 2.9 minutes for offline protein precipitation.

Mass spectrometry

Analytes and internal standards were detected by SRM on a TSQ Endura triple quadrupole mass spectrometer with heated electrospray ionization operated in positive mode. Two SRM transitions for each analyte were included in the acquisition method for quantification and confirmation, respectively.

A



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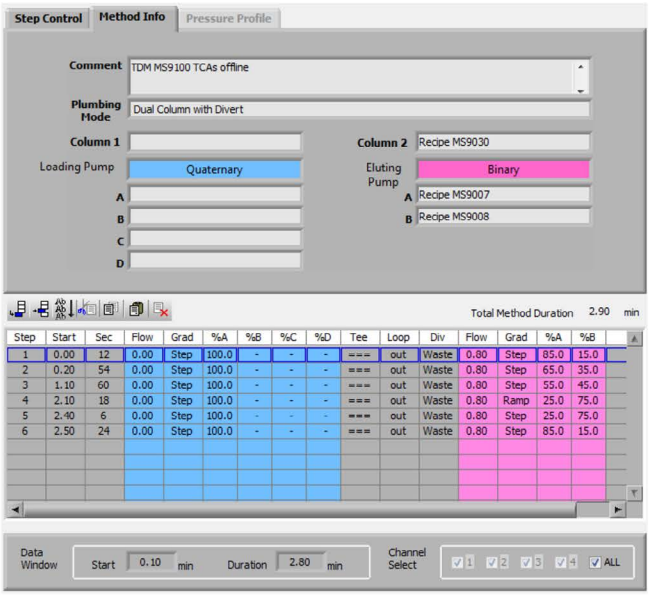

Figure 1. LC method description for (A) direct injection and (B) offline protein precipitation.

Method evaluation

The method performance was evaluated by obtaining limits of quantification, linearity ranges, accuracy, and intra- and inter-assay precision for each analyte. Analytical accuracy was evaluated in terms of percentage bias between nominal and average back-calculated concentrations using the quality control samples #863-41 and #863-42 from INSTAND e.V. prepared and analyzed on five different days in a single run each day. Intra-assay precision was evaluated in terms of percentage coefficient of variation (%CV) using the controls from RECIPE at two different levels in replicates of eight (n=8) prepared and analyzed in one batch. Inter-assay precision was evaluated on the same controls in replicates of three (n=3) prepared and analyzed on five different days.

Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ 3.3 software.

Results and discussion

The method proved to be linear not only in the calibration ranges covered by the calibrators but also in wider ranges obtained by diluting the lowest calibrator up to 10-fold; the obtained linearity ranges are reported in Table 2 for both approaches. Representative chromatograms for the lowest calibrator for imipramine, nordoxepin, and their corresponding calibration curves, are reported (Figures 2 and 3).

Table 2. Linearity ranges for both direct injection and offline protein precipitation approach.

Analyte	Concentration Range (ng/mL)	
	Direct Injection	Offline Protein Precipitation
Amitriptyline	2.37–422	0.95–422
Clomipramine	2.28–419	0.91–419
Clozapine	4.60–1177	1.84–1177
Desipramine	2.21–445	0.88–445
Doxepin	2.22–438	0.89–438
Imipramine	2.25–438	0.90–438
Maprotiline	2.32–421	0.93–421
Norclomipramine	2.25–431	4.50–431
Norclozapine	3.61–1002	1.44–1002
Nordoxepin	2.16–436	0.86–436
Nortriptyline	2.21–419	0.88–419
Trimipramine	2.15–417	0.86–417

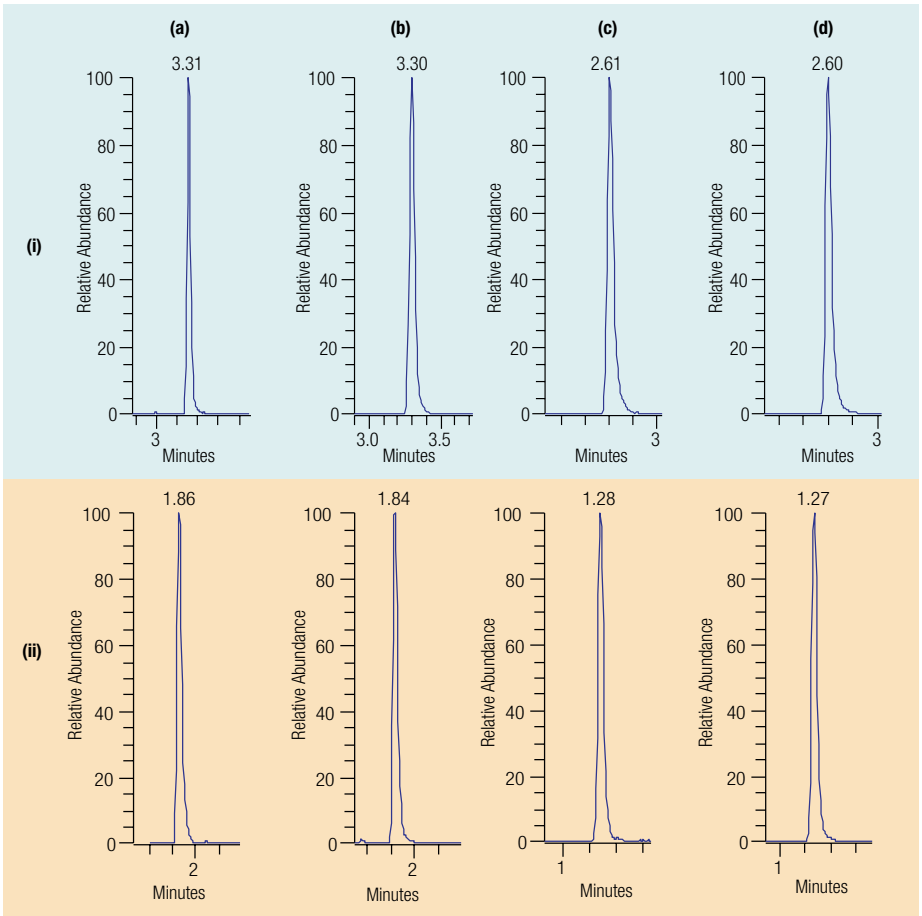

Figure 2. Representative chromatograms for the lowest calibrator for (a) imipramine, (b) d3-imipramine, (c) nordoxepin and (d) d3-nordoxepin for (i) direct injection and (ii) offline sample preparation.

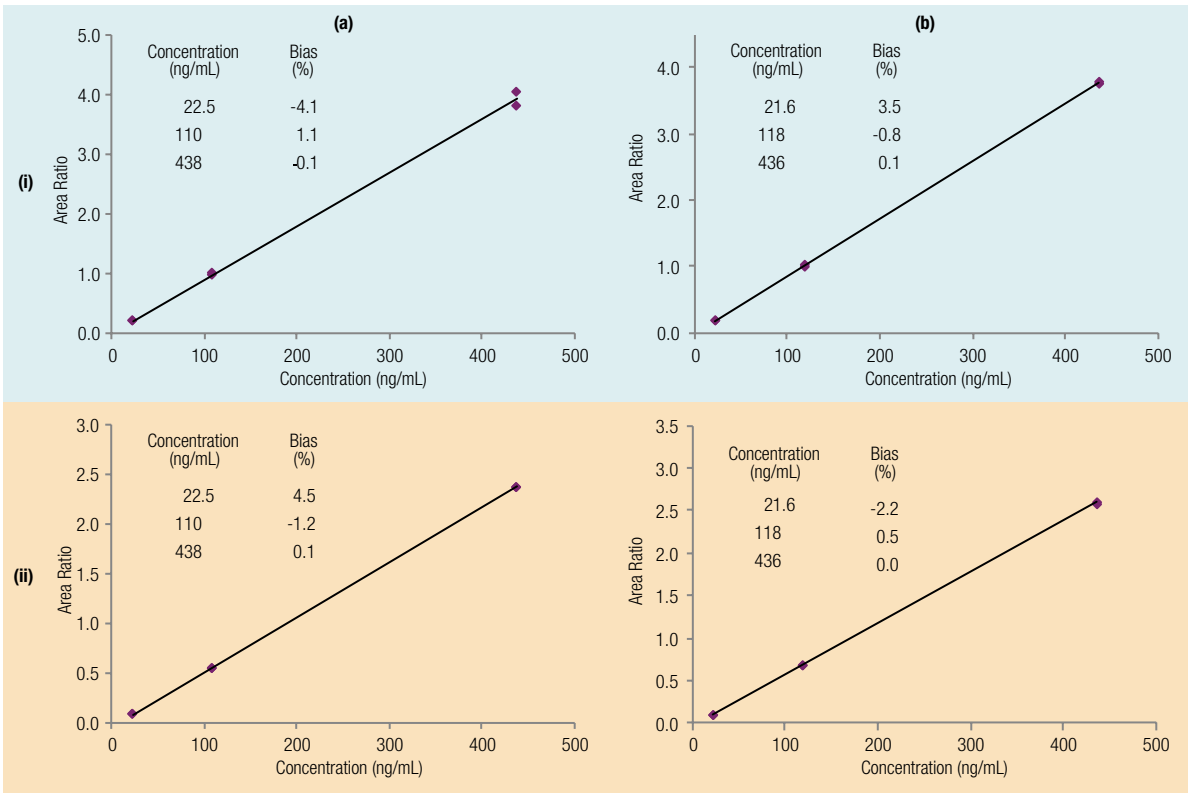

Figure 3. Representative calibration curves for (a) imipramine and (b) nordoxepin for (i) direct injection and (ii) offline sample preparation.

The analytical accuracy was exceptional, with the percentage bias between nominal and average back-calculated concentration for the used control samples ranging between -7.2% and 17.1% for direct injection and between -7.6% and 9.2% for offline sample preparation. Results are reported in Table 3 and Table 4.

Table 3. Analytical accuracy results for control #863-41.

Analyte	Nominal Concentration (ng/mL)	Direct Injection		Offline Protein Precipitation	
		Measured Concentration (ng/mL)	Bias (%)	Measured Concentration (ng/mL)	Bias (%)
Amitriptyline	261	258	-1.3	263	0.6
Clomipramine	221	211	-4.5	241	9.2
Clozapine	715	711	-0.6	748	4.6
Desipramine	272	269	-1.0	278	2.0
Doxepin	243	232	-4.4	251	3.3
Imipramine	270	255	-5.5	270	-0.1
Maprotiline	240	244	1.6	257	7.2
Norclomipramine	264	251	-4.9	257	-2.8
Norclozapine	465	465	0.0	471	1.3
Nordoxepin	254	253	-0.2	258	1.5
Nortriptyline	269	251	-6.7	266	-1.1
Trimipramine	234	221	-5.5	243	3.9

Table 4. Analytical accuracy results for control #863-42.

Analyte	Nominal Concentration (ng/mL)	Direct Injection		Offline Protein Precipitation	
		Measured Concentration (ng/mL)	Bias (%)	Measured Concentration (ng/mL)	Bias (%)
Amitriptyline	57.0	63.5	11.5	58.0	1.7
Clomipramine	89.2	104	17.1	91.1	2.2
Clozapine	330	322	-2.5	331	0.3
Desipramine	115	117	1.5	115	-0.1
Doxepin	48.8	48.7	-0.3	47.1	-3.5
Imipramine	76.9	71.3	-7.2	76.5	-0.5
Maprotiline	76.1	86.1	13.2	77.2	1.5
Norclomipramine	94.9	97.5	2.7	87.7	-7.6
Norclozapine	144	145	0.7	135	-6.4
Nordoxepin	48.2	54.1	12.3	46.7	-3.0
Nortriptyline	79.4	79.4	0.0	74.7	-5.9
Trimipramine	147	160	8.8	147	0.3

The %CV for intra-assay precision was less than 7.2% for all the analytes (Table 5). The maximum %CV for inter-assay precision including all the analytes was 9.4% (Table 6).

Table 5. Intra-assay precision results.

Analyte	Direct Injection				Offline Protein Precipitation			
	8709 #411		8710 #411		8709 #411		8710 #411	
	Average Concentration (ng/mL)	CV (%)	Average Concentration (ng/mL)	CV (%)	Average Concentration (ng/mL)	CV (%)	Average Concentration (ng/mL)	CV (%)
Amitriptyline	63.3	2.9	146	4.7	68.8	1.4	157	1.8
Clomipramine	98.1	2.8	238	6.3	107	1.1	261	1.1
Clozapine	334	3.2	544	6.4	324	1.3	554	1.0
Desipramine	119	3.2	268	5.7	120	1.3	282	0.9
Doxepin	48.4	4.5	133	5.8	54.2	2.0	153	2.0
Imipramine	78.5	4.5	188	7.0	85.7	1.7	209	1.7
Maprotiline	81.6	3.2	117	4.3	83.9	2.3	124	2.4
Norclomipramine	104	3.8	150	5.8	95.8	1.7	236	2.3
Norclozapine	135	3.8	259	5.2	138	2.5	280	2.1
Nordoxepin	50.0	3.8	141	6.3	48.4	2.6	147	2.2
Nortriptyline	82.1	3.5	144	7.2	81.8	2.7	149	1.4
Trimipramine	146	3.9	246	5.3	160	1.7	281	2.1

Table 6. Inter-assay precision results.

Analyte	Direct Injection				Offline Protein Precipitation			
	8709 #411		8710 #411		8709 #411		8710 #411	
	Average Concentration (ng/mL)	CV (%)	Average Concentration (ng/mL)	CV (%)	Average Concentration (ng/mL)	CV (%)	Average Concentration (ng/mL)	CV (%)
Amitriptyline	60.8	4.1	143	9.4	59.4	6.9	151	6.3
Clomipramine	101	5.7	248	8.7	101	3.7	240	6.6
Clozapine	329	6.7	545	7.4	333	5.6	571	4.4
Desipramine	119	4.8	273	7.9	117	6.0	271	6.2
Doxepin	117	3.8	268	8.0	48.8	8.5	148	8.4
Imipramine	48.3	5.9	138	6.4	77.8	4.4	196	4.6
Maprotiline	75.1	4.4	184	8.9	80.1	4.8	117	5.2
Norclomipramine	83.4	3.2	117	8.3	91.7	3.6	233	4.6
Norclozapine	99.3	4.6	240	7.0	138	5.9	272	6.1
Nordoxepin	141	4.2	275	6.5	45.9	5.8	145	4.5
Nortriptyline	49.8	5.3	145	7.3	79.5	6.0	145	6.9
Trimipramine	80.9	4.3	145	8.7	150	6.7	277	7.7

Conclusions

A liquid chromatography-tandem mass spectrometry method for clinical research for the quantification of 12 different tricyclic antidepressants in human plasma or serum was developed in this study. Two different sample preparation techniques were implemented. For direct injection, the MS9050 ClinMass TDM Platform on-line with the MS9150 ClinMass Add-On Set for Tricyclic Antidepressants from RECIPE was used. For offline sample preparation, the MS9000 ClinMass TDM Platform with the MS9100 ClinMass Add-On Set for Tricyclic Antidepressants also from RECIPE was used. The method was analytically validated on a Transcend II TLX-1 system coupled to a TSQ Endura triple quadrupole mass spectrometer. The method offers the flexibility of two possible sample preparation approaches, with direct injection for minimal human intervention, and offline sample precipitation, for improved sensitivity. The described method meets research laboratory requirements in terms of sensitivity, linearity of response, accuracy, and precision.

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