Rapid, Automated Extraction and LC-MS/MS Analysis of Tricyclic Antidepressants from Plasma using Strata®-X-Drug B SPE and a Kinetex® Core-Shell HPLC/UHPLC Column

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Using an automated SPE method that requires no condition or equilibration steps, time and solvent are saved during the analysis of 9 tricyclic antidepressant (TCA) compounds from human plasma. The rapid automated procedure proved to be reproducible and provided absolute recoveries >75 % for all TCA compounds studied. Coupled to a rapid LC-MS/MS method using a Kinetex core-shell HPLC/UHPLC column, this method can instantly provide time and solvent savings to high-throughput laboratories.

Introduction

As the name implies, TCAs are heterocyclic chemical compounds consisting of 3 ring structures and a variety of carbon chain functional groups. TCAs are basic in nature and typically have a pK_a of 8 or higher. A basic pK_a combined with a hydrophobic ring structure makes these compounds excellent targets for extraction via a strong cation-exchange SPE sorbent. After extraction, TCAs can be separated using a reversed phase C18 HPLC column, relying on their functional groups to provide separation and resolution of each compound.

Materials and Methods

Sample Pretreatment

500 µL of plasma was first diluted with 1 mL of 100 mM Sodium acetate buffer (pH 5.0) spiked with TCAs. The diluted sample was then subjected to an automated SPE protocol (below) which was run on a PerkinElmer® MultiPROBE® II.

Solid Phase Extraction	
96-Well Plate:	Strata-X-Drug B 30 mg/well
Part No.:	8E-S128-TGB
Condition:	NOT REQUIRED
Equilibrate:	NOT REQUIRED
Load:	500 µL pretreated plasma
Wash 1:	0.8 mL 100 mM Sodium acetate (pH 5.0)
Wash 2:	0.8 mL Methanol
Dry:	8 to 10 minutes under maximum vacuum
Elute:	0.4 mL of Methanol/Acetonitrile (50:50) plus 2 % Ammonium hydroxide
Blow Down:	To dryness under a slow stream of nitrogen @ 45 °C
Reconstitute:	500 µL of initial mobile phase

HPLC Conditions

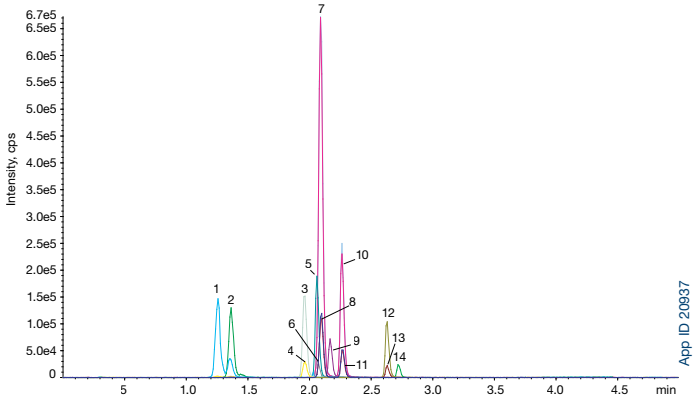
Column: Kinetex 2.6 µm C18, 100 Å
Dimensions: 50 x 2.1 mm
Part No.: 00B-4462-AN
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Methanol

Gradient:

Time (min)	% B
0	40
3.5	80
4	80
4.01	40
5	40

Flow Rate: 0.5 mL/min
Temperature: Ambient
Detection: API 4000™ MS/MS, ESI Positive (ESI+)
Injection: 10 µL
Sample:
1. Doxepine
2. DM-Doxepine
3. Imipramine-D3 (IS)
4. Imipramine
5. Desipramine-D3 (IS)
6. Desipramine
7. Nortriptyline-D3 (IS)
8. Nortriptyline
9. Amitriptyline
10. Protriptyline-D3 (IS)
11. Protriptyline
12. Clomipramine-D3 (IS)
13. Clomipramine
14. DM-Clomipramine

Figure 1.
LC-MS/MS chromatogram of 9 TCA compounds using a Kinetex 2.6 µm C18 core-shell HPLC/UHPLC column



MS/MS Conditions

Analysis is performed with an Agilent® 1200 HPLC system (Agilent Technologies, Inc., Santa Clara, CA USA) coupled to a SCIEX API 4000™ triple-quadrupole tandem mass spectrometer equipped with an ESI probe operating in positive polarity mode. Under an MRM mode, two channels were monitored for 9 TCAs (**Table 1**).

Table 1.
MRM Transitions

Peak Name	MRM Channel
Protriptyline	264.3 ⇒ 191.1
Nortriptyline	264.3 ⇒ 191.2
DM-Doxepin	266.2 ⇒ 107.1
Desipramine	267.2 ⇒ 208.2
Protriptyline-D3	267.2 ⇒ 191.1
Nortriptyline-D3	267.3 ⇒ 191.2
Desipramine-D3	270.2 ⇒ 208.2
Amitriptyline	278.2 ⇒ 191.2
Doxepin	280.3 ⇒ 107.1
Imipramine	281.3 ⇒ 208.2
Imipramine-D3	284.2 ⇒ 208.1
DM-Clomipramine	301.2 ⇒ 242.2
Clomipramine	315.2 ⇒ 242.2
Clomipramine-D3	381.2 ⇒ 242.2

Results and Discussion

The goal of this study was to determine a rapid analysis for 9 TCAs from human plasma that was both sensitive and accurate. When working with human plasma, many endogenous interferences are present including proteins and phospholipids. In order to obtain a clean sample prior to LC-MS/MS analysis, the plasma sample was subjected to an SPE cleanup using Strata®-X-Drug B. Strata-X-Drug B was chosen as the ideal SPE sorbent for our plasma cleanup because it does not require a condition or equilibration step which saved both time and solvent. The sorbent also allowed us to develop a single, simplified method, rather than several different methods for the extraction of 9 TCAs, resulting in absolute recoveries of >75 % for all analytes of interest even at low concentration levels (**Table 2**).

After cleanup by SPE, the TCAs were analyzed by LC-MS/MS using a Kinetex® 2.6 µm C18 core-shell HPLC/UHPLC column. The Kinetex core-shell particles provided the performance, efficiencies and speed of a sub-2 µm column without the added backpressure that is associated with sub-2 µm particles, resulting in a run time of under 3 minutes for all 9 target compounds (**Figure 1**).

Table 2.
Absolute recoveries (%) of TCA compounds after extraction with Strata-X-Drug B SPE

	500 ng/mL		25 ng/mL	
Analyte	Absolute Recovery (%)	% CV	Absolute Recovery (%)	% CV
Amitriptyline	94	5.6	82	12.2
Nortriptyline	80	1.7	75	6.1
Protriptyline	87	2.1	94	4.3
Doxepin	94	7.3	93	3.0
DM-Doxepine	85	4.0	96	9.0
Imipramine	85	1.5	90	4.1
Desipramine	87	1.0	85	1.1
Clomipramine	87	3.4	92	0.2
DM-Clomipramine	84	4.2	89	0.4

Conclusion

Utilizing a single SPE method, 9 TCAs were extracted from plasma and analyzed by LC-MS/MS. The Strata-X-Drug B SPE sorbent did not require a condition or equilibration step which saved both time and solvent. The ability to extract all 9 TCA compounds at once also provided time savings. The extraction resulted in absolute recoveries of >75 % for all 9 analytes at low detection levels (25 ng/mL). Following the extraction, the TCAs were separated and further analyzed using a Kinetex 2.6 µm C18 core-shell HPLC/UHPLC column which provided excellent separation and a fast run time of < 3 minutes. Due to the time and solvent savings, this method could be beneficial in a high-throughput laboratory.

APPLICATION

Ordering Information
Strata®-X-Drug B SPE

Format	Sorbent Mass	Part Number	Unit
Tube			
	10 mg	8B-S128-AAK	1 mL (100/box)
	10 mg	8L-S128-AAK†	1 mL (100/box)
	30 mg	8B-S128-TAK	1 mL (100/box)
	30 mg	8L-S128-TAK†	1 mL (100/box)
	30 mg	8B-S128-TBJ	3 mL (50/box)
	60 mg	8B-S128-UBJ	3 mL (50/box)
	60 mg	8B-S128-UCH	6 mL (30/box)
	60 mg	8B-S128-UCL	6 mL (200/box)
Giga™ Tube			
	100 mg	8B-S128-EDG	12 mL (20/box)
96-Well Plate			
	10 mg	8E-S128-AGB	2 Plates/box
	30 mg	8E-S128-TGB	2 Plates/box
	60 mg	8E-S128-UGB	2 Plates/box

†Tab-less tube

Ordering Information
Kinetex® Core-Shell HPLC/UHPLC Columns

5 µm Columns (mm)		SecurityGuard™ ULTRA Cartridges‡					SecurityGuard ULTRA Cartridges‡
Phases	50 x 2.1	3/pk	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
C18	00B-4601-AN	AJ0-8782 for 2.1 mm ID	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768 for 4.6 mm ID

2.6 µm Analytical Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768 for 4.6 mm ID

2.6 µm MidBore™ Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
XB-C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775 for 3.0 mm ID

2.6 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782 for 2.1 mm ID

1.7 µm MidBore Columns (mm)			SecurityGuard ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	3/pk
C18	00B-4475-Y0	00D-4475-Y0	AJ0-8775 for 3.0 mm ID

1.7 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJ0-8782 for 2.1 mm ID

1.3 µm Minibore Columns (mm)	
Phase	50 x 2.1
C18	00B-4515-AN

‡SecurityGuard ULTRA cartridges require holder, Part No.: AJ0-9000

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