

Doxepin in Human Plasma Using SOLA and Accucore Core Enhanced Technology HPLC Column

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Key Words

- SOLA Cartridges and Plates
- Tricyclic Anti-depressant
- Bioanalysis

Introduction

Thermo Scientific SOLA products are a revolutionary new Solid Phase Extraction (SPE) product range. This first in class SPE product range introduces next-generation, innovative technological advancements, giving unparalleled performance characteristics compared to conventional SPE, phospholipid and protein precipitation products.

This includes:

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

SOLA™ products have significant advantages for the analyst when processing compounds in complex matrices particularly in high throughput bioanalytical and clinical laboratories where reduced failure rate, higher analysis speed and lower sample/solvent requirements are critical.

The increased performance from SOLA products provides higher confidence in analytical results and lowers cost without compromising ease of use or requiring complex method development.

Accucore™ HPLC columns use Core Enhanced Technology to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimised phase bonding creates a series of high coverage, robust phases. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Doxepin is a drug used to treat depression and anxiety by raising the serotonin and norepinephrine levels and blocking the activity of acetylcholine in the brain. This is typically dosed at 75-300 mg daily, although 3-6 mg can also be used for the treatment of insomnia. A C_{max} value of the order of 15 ng/mL has been reported for 75 mg doses.

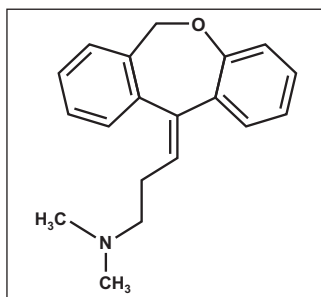
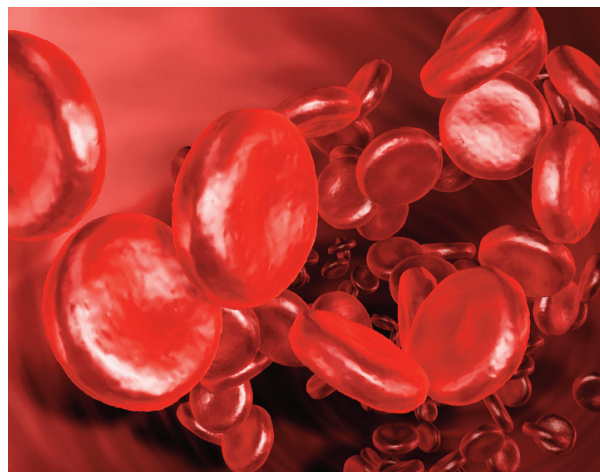


Figure1. Structure of Doxepin



Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific HPLC grade methanol	M/4056/17
Doxepin HCl, Sigma-Aldrich	
Protryptiline HCl, Sigma-Aldrich	

Sample Handling Equipment	Part Number
Thermo Scientific Finnpipettes	
HyperSep Glass Block Manifold, 16 port	60104-232
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

Sample and Calibration Preparation

Compound(s):	Doxepin HCl and Protryptiline HCl (IS)
Matrix:	Human Plasma (Seralab, UK)
	1000 µg/mL stock solutions of doxepin HCl and protryptiline HCl (IS) in methanol were prepared, of which 200 µL aliquots were diluted to 1000 µL with water to give the 200 µg/mL spiking solutions in water/methanol (80:20).
Samples:	190 µL of plasma was spiked with 10 µL of the doxepin spiking solution and 10 µL of the IS, which after SPE, drying and reconstitution in 200 µL resulted in a target concentration of 10 µg/mL.
Calibration:	180 µL of plasma was spiked with 5 µL of the doxepin spiking solution and 5 µL of the IS, which after SPE, drying and reconstitution in 200 µL resulted in a target concentration of 5 µg/mL.

Sample Preparation - SOLA

Part Number

Cartridge type:	SOLA 10mg/1mL cartridges	60109-001
Conditioning stage:	1 mL methanol	
Equilibration stage:	1 mL water	
Load:	200 µL plasma (spiked with IS)	
Wash:	1 mL water/methanol (95:5 v/v) + 0.5% ammonia solution	
Elute:	0.2 mL methanol + 0.1% formic acid	
Dry:	40 °C with nitrogen	
Reconstitute:	200 µL with water/methanol (80:20 v/v)	

Separation conditions

Instrumentation:	Thermo Scientific HPLC system	
Column(s):	Accucore C18 2.6 µm 50x2.1 mm	
Mobile phase:		
A:	Water + 0.1% formic acid	
B:	Acetonitrile + 0.1% formic acid	
T/min	% A	%B
0.01	75.00	25.00
2.00	5.00	95.00
2.50	5.00	95.00
2.60	75.00	25.00
10.00	75.00	25.00
Flow rate:	0.4 mL/min	
Column temperature:	40 °C	
Injection details:	10 µL	
Injection wash solvent:	water/methanol (80:20 v/v)	
UV detector wavelength:	245 nm	

Data processing

Software:	Thermo Scientific ChromQuest ver 5.0	
Integration parameters:		
Width:	0.1	
Threshold:	1000	

Additional manual integration was applied as necessary.

Results

The method resolved doxepin and the protryptiline internal standard in less than 2 minutes (Figure 2). A gradient was implemented after 2.5 minutes in order to wash the column, followed by a re-equilibration period. Instruments with lower dwell volumes will require less time for re-equilibration.

A single point solution calibration was used to provide an estimate of the recovery of the method. Based on five replicate injections, using the internal standard method, a mean recovery of 113 % was calculated (Table 1). The precision of the method was also estimated using the relative standard deviation, and calculated to be 5.6 %.

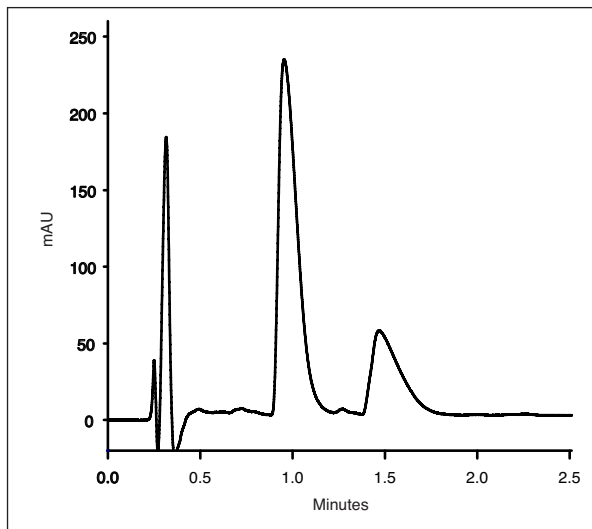


Figure 2. Chromatogram of doxepin (1) in extracted human plasma using protryptiline (2) as the internal standard

Extracted sample #	Doxepin Peak Area	Protryptiline (IS) Peak Area	Response ratio	Calculated Amount /µg.mL ⁻¹	Recovery
1	1596549	584194	2.7329089	9.01	1.18
2	1461480	491260	2.9749623	9.81	1.08
3	1650009	520862	3.1678429	10.44	1.22
4	1464907	469808	3.1180972	10.28	1.08
5	1526226	507463	3.0075611	9.92	1.12
Mean				9.89	1.13
RSD				5.6 %	
Calibration Standard (5.2 µg/mL)	678918	215242	3.1542078		

Table 1. Determination of doxepin in spiked plasma samples using an internal standard method with a single-point calibration

Conclusion

- SOLA cartridges provide good extraction reproducibility of doxepin from human plasma.
- Accucore can be used to separate doxepin from an internal standard in less than 2 minutes.

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