

# UV Method for the Determination of Tricyclic Antidepressants' from Human Plasma Using SOLA CX and Hypersil GOLD HPLC

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## Abstract

This application note demonstrates the use of the Thermo Scientific SOLA CX cartridges for the extraction of four tricyclic antidepressants' from human plasma, while achieving high method precision.

## Introduction

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.

Thermo Scientific Hypersil GOLD columns offer excellent peak shape. Based on highly pure silica, Hypersil™ GOLD columns provide very symmetrical peaks, even when analyzing compounds that give notoriously poor peak shape on traditional silica-based chemistries. Hypersil GOLD media provides a stationary phase with C18 selectivity and a predictable elution order, but can provide new capabilities such as improved peak shape, increased peak capacity, and greater sensitivity, especially for trace compound analysis.

Doxepin, imipramine, amitriptyline and trimipramine belong to a class of antidepressants identified by the three ringed chemical structure commonly referred to as tricyclic antidepressants.

Tricyclic antidepressants dominated the market until the 1980s when antidepressants with fewer side effects were first introduced. Tricyclic antidepressants are used for:

- Depression and mood disorders such as bipolar disorder
- Medical disorders e.g. social anxiety disorder (SAD) and obsessive compulsive disorder (OCD)



- Eating disorders e.g. anorexia nervosa and bulimia nervosa
- Tourette syndrome
- Irritable bowel syndrome (IBS)

The extraction of four tricyclic antidepressants from human plasma is demonstrated.

## Experimental Details

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific Analytical grade formic acid	F/1900/PB08
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

## Sample Handling Equipment

SPE Positive pressure manifold Thermo Fisher Scientific	CLS-229070
Ultra vap, Thermo Fisher Scientific	

## Sample Pretreatment

Aliquot 121.4 µL of blank plasma into a clean tube. Add 13.6 µL of standard spiking solution, and 15 µL of internal standard spiking solution, for blanks add methanol, mix well.

## Key Words

- SPE
- SOLA CX Cartridges and Plates
- Tricyclic Antidepressants

### Sample Preparation - SOLA CX

### Part Number

Compound(s):	doxepin, imipramine, amitriptyline, trimipramine (IS)
Matrix:	human plasma
Cartridge type:	SOLA CX 10 mg/1mL 60109-002
Conditioning stage:	500 µL methanol, 500 µL water
Application stage:	450 µL of a 1:2 mix of spiked human plasma and 100 mM sodium phosphate buffer (pH 6.0)
Washing stage:	500 µL water + 0.1% formic acid, 500 µL methanol + 0.1 % formic acid
Elution stage:	500 µL acetonitrile + 5 % ammonia
Additional stage:	samples and were dried down (no heating) and reconstituted in 150 µL of 80:20 (v/v) water / acetonitrile

### Separation Conditions

### Part Number

Instrumentation:	Thermo Scientific Accela 600
Column:	Hypersil GOLD, 3 µm, 150 x 2.1 mm 25003-152130
Mobile phase:	water + 0.1% formic acid / acetonitrile + 0.1% formic acid 70:30 (v/v)
Flow rate:	0.4 mL/min
Column temperature:	30 °C
Injection details:	1 µL
Injection wash solvent:	water

### Solutions

Primary standards of each of the compounds were prepared at concentrations of 1 mg/mL in methanol. Working standard contained 0.02 g/mL of each compound in human plasma

### Results

The four tricyclic antidepressants eluted in less than eight minutes (Figure 1) with recovery levels greater than 70% for each compound (Table 1).

Replicate extractions of the tricyclic antidepressant mix (n=6) demonstrated that SOLA CX cartridge produces reproducible results with %RSD <5% (Table 1).

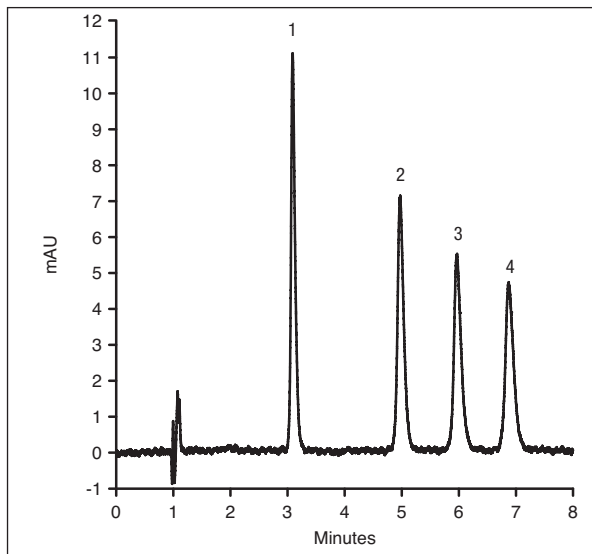


Figure 1. Chromatogram for doxepin (1), imipramine (2), amitriptyline(3) and trimipramine (4) separated on an Hypersil GOLD, 3 µm, 150 x 2.1 mm column.

### Conclusion

SOLA CX cartridges yield reproducible results for the extraction of tricyclic antidepressants from human plasma. SOLA CX cartridges require lower elution volumes in comparison to the traditional loose-packed material and therefore use less solvent. This reduces sample preparation time and increases overall sample throughput.

	Doxepin	Imipramine	Amitriptyline	Trimipramine (IS)
%RSD	5.0	4.8	4.0	5.1
%Recovery	78.9	73.4	74.3	69.7

Table 1: Method precision (%RSD) and recovery data for the tricyclic antidepressant mix.

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