

# Method for Extraction of Tricyclic Antidepressants from Whole Blood Using ISOLUTE<sup>®</sup> HCX Columns and RapidTrace<sup>®</sup>

## Introduction

This application note describes the Extraction of Tricyclic Antidepressants from Whole Blood Using ISOLUTE HCX Columns and RapidTrace Workstation.

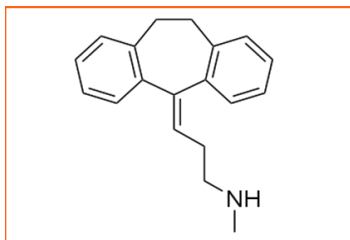


Figure 1. Nortriptyline

Tricyclic antidepressants (TCAs) are chemical compounds with a three ringed structure that were originally designed to be used, as the name suggests, in the treatment of depressive disorders. Their use as antidepressants has been significantly scaled down due to newer anti-depressants taking their place which have fewer and less severe side effects. TCAs have since been found to have significant efficacy in the treatment of sleep disorders, ADHD, IBS and pain management. TCAs work as neurotransmitter reabsorption blockers, significantly for serotonin and norepinephrine which allows these neurotransmitters to have a greater therapeutic effect on the user.

## Columns

ISOLUTE HCX (3mL 130mg part number 902-0013-B) is a silica-based mixed-mode sorbent optimized to extract basic drugs from biological fluids. The combination of non-polar and strong cation retention mechanisms allows for rigorous interference elution steps resulting in extremely clean extracts.

*The methods offered within this application are meant to represent a starting point and guide for method*

## Analytes

Amitriptyline, Nortriptyline, Imipramine and Desipramine

## Sample pre-treatment

Prior to extraction, 1 mL of serum is diluted with 2 mL of 0.1 M phosphate buffer and 0.1 mL of internal standard. The sample is vortexed and centrifuged and then decanted into a 13 X 100 mm test tube to remove particulates. The SPE steps were configured schematically using the RapidTrace software as follows:

## RapidTrace procedure

	Step	Source	Destination	Volume (mL)	Flow (mL/sec)
1	Condition	Methanol	Waste 2	3.0	0.25
2	Condition	Water	Waste 1	3.0	0.25
3	Condition	Buffer	Waste 1	1.0	0.25
4	Load	Sample	Waste 1	3.0	0.05
5	Rinse	Water	Waste 1	3.0	0.10
6	Purge-Cannula	Water	Cannula Waste	6.0	0.50
7	Rinse	Acetic acid	Waste 1	1.0	0.10
8	Rinse	Methanol	Waste 2	3.0	0.10
9	Dry	Time = 2.0 min			
10	Collect	Elution solvent	Fraction 1	3.0	0.03
11	Purge-Cannula	Methanol	Cannula Waste	6.0	0.50
12	Purge-Cannula	Water	Cannula Waste	6.0	0.50

Biotage recommends TurboVap Workstations post elution to blow down your samples.

### Reagent lines

Line 1: Water  
Line 2: Methanol  
Line 4: Acetic acid

### Results

From control samples tested over five consecutive days it can be seen that the recoveries achieved are very repeatable demonstrating this method to be both robust and reliable with desirable recoveries.

### Test to test variability

	Amitriptyline (ng/mL)	Nortriptyline (ng/mL)	Imipramine (ng/mL)	Desipramine (ng/mL)
Day 1	93.0	102.7	113.3	104.3
Day 2	95.6	104.2	112.2	104.6
Day 3	94.5	104.8	113.6	103.4
Day 4	92.8	106.7	115.2	108.6
Day 5	96.8	103.4	118.6	109.1
	mean = 94.5	mean = 104.4	mean = 114.6	mean = 106.0
Target value	95.9	102.7	112.5	106.3
Average recovery (%)	98.5	102.6	101.9	99.7
C.V. (%)	1.8	1.5	2.2	2.5

### Ordering information

Part number	Description	Quantity
902-0013-B	ISOLUTE HCX 3 mL 130 mg columns	50
C50000	RapidTrace 3 mL Workstation	1
C50974	Tube Rack (13 x 100 mm Sample Tubes & 12 x 75 Fraction Tubes)	1
C40707	Test Tubes - 13 x 100 mm, Uncap	1000
C44651	Test Tubes - 12 x 75 mm, Uncap	1000
C52006	RapidTrace Start-Up Kit	1
C52689	RapidTrace Notebook Controller	1

### RapidTrace Overview



The RapidTrace has been designed to eliminate sample preparation bottlenecks and it is a modular, highly scalable, automated platform designed for high throughput. Units are available to accommodate 1 mL, 3 mL and 6 mL SPE cartridges. Up to 10 modular units can be connected together and controlled through a simple, easy-to-use software package. The systems are widely used within analytical industry and are ideal both for industrial settings and for efficient SPE method development.

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