Method for the Extraction of Cyclosporine from Whole Blood Using ISOLUTE® SI Columns and RapidTrace® Workstation

Introduction

This application note describes the extraction of Cyclosporine from whole blood using ISOLUTE columns and Rapid-Trace Workstation.

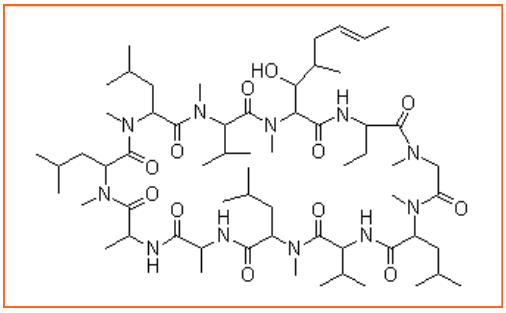

Figure 1. Structure of Cyclosporine

Cyclosporine (Figure 1) is an immunosuppressant drug most significantly used in post-operative organ transplants to reduce the chance of rejection of donor organs by decreasing the activity of the patient's immune system. Subsequent to an organ transplant, patients are tested at regular intervals for the presence of very low levels of the compound. Cyclosporine is a cyclic non-ribosomal peptide of 11 amino acids and contains a single D-amino acid.

Columns

ISOLUTE SI (3mL 500 mg part number 460-0050-B) is a silica-based polar sorbent efficient at extracting polar compounds from a non-polar sample matrix using a hydrogen bonding retention mechanism.

'The methods offered within this application are meant to represent a starting point and guide for method development and some amendment may be necessary.'

Analytes

Cyclosporine.

Sample pre-treatment

A 1 mL sample of blood is taken and 100 µL of Cyclosporine D internal standard in methanol is added. 8 mL of ethyl acetate: hexane (15:85) is added with mixing followed by centrifuging. The supernatant liquid above the blood is then transferred to the sample test tube.

The SPE steps were configured schematically using the RapidTrace software as follows:

RapidTrace procedure

	Step	Source	Destination	Volume (mL)	Flow (mL/min)
1	Condition	Methanol	Organic Waste	3.0	15.00
2	Condition	Ethyl Acetate: Hexane (15:85)	Organic Waste	3.0	15.00
3	Load	Sample	Biological Waste	5.0	1.00
4	Rinse	Ethyl Acetate: Hexane (60:40)	Biological Waste	3.0	3.00
5	Purge-Cannula	Methanol	Cannula Waste	2.0	30.00
6	Collect	Methanol	Fraction 1	2.0	1.00
7	Purge-Cannula	Methanol	Cannula Waste	5.0	30.00

Application Note

Reagent lines

Line 2: Methanol (sip speed = 30 mL/min)

Line 5: Ethyl acetate: hexane (v/v, 15:85) (sip speed = 30 mL/min)

Line 6: Ethyl acetate: hexane (v/v, 60:40) (sip speed = 30 mL/min)

Air push: 2 mL

Air push multiplier: 2

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

Ordering information

Part number	Description	Quantity
460-0050-B	ISOLUTE SI 3 mL 500 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	1000
C44651	Test Tubes - 12 x 75 mm	1000
C52006	RapidTrace Start-Up Kit	1
C52689	RapidTrace Notebook Controller	1

RapidTrace Overview

The RapidTrace has been designed to eliminate Sample prep bottlenecks and it is a modular, highly scalable, automated Sample Prep platform designed for high throughput. Units are available to accommodate 1 mL and 3 mL, or 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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