

Determination of Beta Blockers from Urine Using SOLA CX and Accucore Core Enhanced Technology HPLC.

Joanne Jones and Joanna Denbigh, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words

- SPE
- SOLA CX Cartridges and Plates
- Beta Blockers

Abstract

Thermo Scientific SOLA CX cartridges are suitable for the extraction of four beta blockers from urine. High precision and recoveries were observed while using low loading volumes.

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.

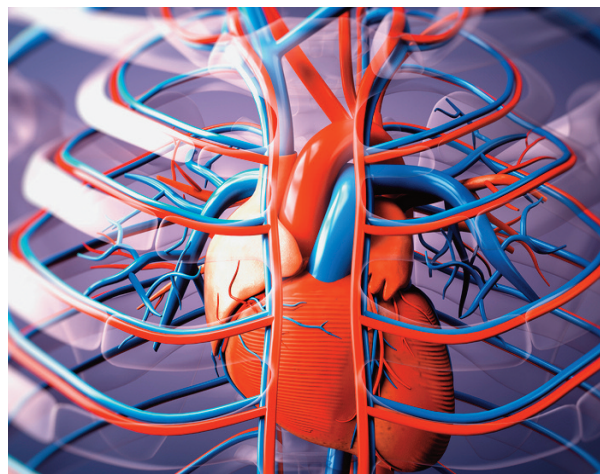
The increased performance from SOLA products provides higher confidence in analytical results and lowers cost without compromising ease of use or requiring complex method development.

Thermo Scientific 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 optimized phase bonding creates a series of high coverage, robust phases. The carbon loading of Accucore™ C18 provides high retention of non-polar analytes via a predominantly hydrophobic interaction mechanism. The tightly controlled 2.6µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Beta blockers (or beta antagonists) are a category of drugs used to treat a number of medical complaints, such as hypertension, angina, heart failure and heart attacks. Beta blockers are designed to stop the functioning of a naturally occurring compound, noradrenaline.

Noradrenaline is a chemical released in the body which can cause the arteries to narrow and an increased heart rate.

The extraction of five beta blockers from urine is demonstrated in this application.



Experimental Details

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade methanol	M/4056/17
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	Part Number
Thermo Scientific Ultra vap	CLS-229070
Thermo Scientific HyperSep glass block manifold	60104-232

Sample Pretreatment

An aliquot of 800 µL of urine was spiked into a clean tube. 100 µL of pindolol (IS) at 0.5 mg/mL was added along with 100 µL of mixed primary standard at 0.5 mg/mL. The contents were mixed well.

Sample Preparation - SOLA CX	Part Number
Compound(s):	atenolol, metoprolol, propranolol, alprenolol, pindolol (IS)
Matrix:	human male urine
Cartridge type:	SOLA CX 10 mg/1mL 60109-002
Conditioning stage:	500 µL methanol, 500 µL water
Application stage:	200 µL of spiked human urine
Washing stage:	250 µL water + 0.1% formic acid, 250 µL methanol + 0.1% formic acid
Elution stage:	250 µL DCM/IPA + 5% ammonia 80:20 (v/v)
Additional stage:	Dry down samples and reconstitute in 200 µL of 90:10 (v/v) water / acetonitrile (ensure the temperature is less than 40 °C when drying down samples)

Separation Conditions	Part Number	
Instrumentation:	Thermo Scientific Accela 1250	
Column:	Accucore C18, 2.6 μ m 50 x 2.1 mm	17126-052130
Mobile phase A:	water + 0.1 % formic acid	
Mobile phase B:	methanol + 0.1 % formic acid	
Gradient:	10-40 %B in 2.5 minutes	
Flow rate:	0.7 mL/min	
Column temperature:	45 °C	
Injection details:	1 μ L	
Injector wash:	water	
UV detector wavelength:	220 nm	

Solutions

Primary standards of each of the compounds were prepared at 2 mg/mL in methanol. Spiking standard contained 0.5 mg/mL of each compound and was spiked into urine to achieve a concentration of 0.05 mg/mL.

Results

The five beta blockers eluted in less than three minutes (Figure 1) with recovery levels greater than 80% for each component (Table 1).

Replicate extractions of the beta blocker mix (n=6) demonstrated that SOLA CX cartridges produce reproducible results with percentage relative standard deviation values of between 3.22% and 4.4% RSD.

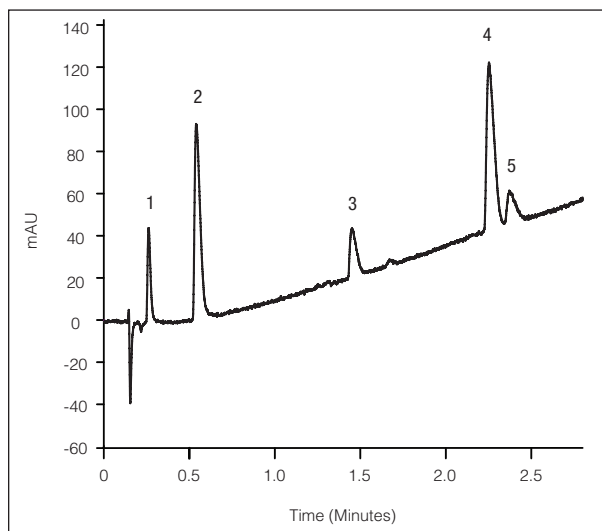


Figure 1: Chromatogram for atenolol (1), pindolol (2), metoprolol (3) propranolol (4) and alprenolol (5) separated on an Accucore C18, 2.6 μ m, 50 x 2.1 mm column.

	Atenolol	Pindolol (IS)	Metoprolol	Propranolol	Alprenolol
%RSD	4.2	3.2	3.6	3.8	4.4
%RECOVERY	87.7	79.4	93.7	88.3	88.5

Table 1: Method precision (%RSD) and recovery data for the beta blocker mix (data calculated from six replicate extractions).

Conclusion

SOLA CX cartridges yield high precision and recoveries for the extraction of beta blockers from human urine. SOLA cartridges require lower elution volumes in comparison to the conventional loose-packed products resulting in reduced solvent usage and analysis time.

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**North America
USA and Canada**
+1 800 332 3331

**Europe
France**
+33 (0)1 60 92 48 34

Germany
+49 (0) 2423 9431 -20
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United Kingdom
+44 1928 534110

**Asia
Japan**
+81 3 5826 1615

China
+86-21-68654588
or +86-10-84193588
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India
+91-22-6742 9494

**Thermo Fisher
Scientific Australia
Pty Ltd**
1300 735 292 (free call
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**Thermo Fisher
Scientific New
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0800 933 966 (free call
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All Other Enquiries
+44 (0) 1928 534 050

Technical Support

North America
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**Outside North
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