Application Note:
ANCCSBAS&NEUT

HPLC-UV Method for the Fractionation of Basic and Neutral Compounds Extracted from Human Plasma and Urine using SOLA CX

Eilidh MacRitchie, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words

- SOLA Cartridges and Plates
- Accucore RP-MS
- Core Enhanced Technology
- Solid Core
- Hydrocortisone
- Corticosterone
- Progesterone
- Procainamide
- Propranolol
- Amitriptyline

Abstract

This application note demonstrates the use of the Thermo Scientific SOLA CX cartridges for the extraction of neutral and basic compounds from human plasma and urine and subsequent HPLC-UV analysis using a Thermo Scientific Accucore RP-MS column.

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 a 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 of 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 optimized phase bonding creates a series of high coverage, robust phases. Accucore RP-MS uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

The extraction of both basic and neutral compounds from human plasma and urine has been demonstrated in this application note.

Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific HPLC grade ammonium acetate	A/3446/50
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17

Sample Handling Equipment	Part Number
Thermo Scientific HyperSep glass block manifold	60104-232
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

Sample Preparation - SOLA CX		Part Number
Compounds:	hydrocortisone, cortisone, progesterone (IS), procainamide, propranolol and amitriptyline	
Matrix:	human plasma and urine	
Cartridge type:	SOLA CX 10 mg / 1 mL	60109-002
Conditioning stage:	1 mL methanol, 1 mL water	
Application stage:	350 µL spiked human plasma or 350 µL spiked urine	
Washing stage:	350 µL water + 2 % formic acid	
Elution stage 1:	350 µL methanol	
Elution stage 2:	350 µL methanol + 5 % ammonia	
Additional stage:	Add 50 µL internal standard and 350 µL water to elution stage 1 and elution stage 2 samples	

Thermo SCIENTIFIC

Separation Conditions			Part Number
Instrumentation:	Thermo Scientific HPLC system		
Column:	Accucore RP-MS 2.6 µm, 50 x 3 mm		17626-053030
Mobile phase A:	20 mM ammonium acetate		
Mobile phase B:	Acetonitrile		
Gradient:	Time (minutes)	% B	
	0.0	5	
	0.5	5	
	5.0	95	
	5.5	95	
	5.6	5	
	7.5	5	
Flow rate:	0.8 mL/min		
Column temperature:	25 °C		
Injection details:	10 µL partial loop		
Injection wash solvent:	20 mM ammonium acetate		
UV detector wavelength:	254 nm		

Solutions

Stock solutions of each standard were prepared at 1 mg/mL in methanol.

Working solutions contained 10 µg/mL of each of the standards in human plasma and urine.

Results

The analysis was performed on an Accucore 2.6 µm, 50 x 3 mm column. When all six compounds are analysed together, hydrocortisone and propranolol co-elute at approximately 3.75 minutes (Figure 1). By using the SOLA CX cartridges, hydrocortisone can be analysed in the neutral fraction and propranolol analysed in the basic fraction. This allows accurate quantification of both hydrocortisone and propranolol. Figure 2a and 2b show all the compounds extracted from human plasma and Figure 3a and 3b show all the compounds extracted from urine

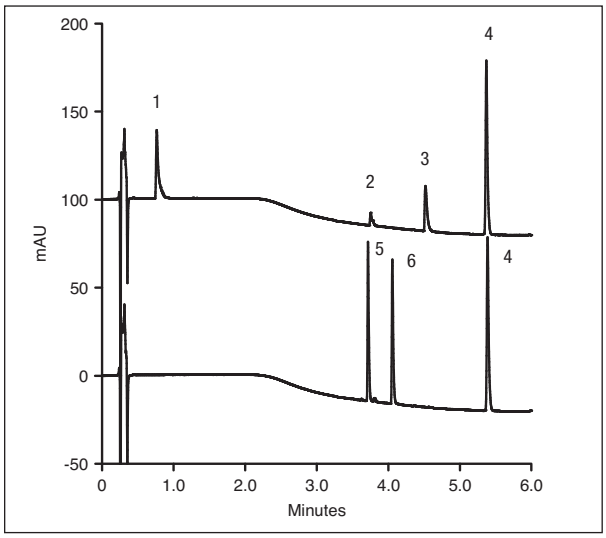

Figure 1. Chromatogram of a standard solution containing procainamide (1), hydrocortisone (2), propranolol (3), corticosterone (4), amitriptyline (5) and progesterone (6) prepared in water, separated using a Accucore RP-MS 2.6 µm, 50 x 3 mm column

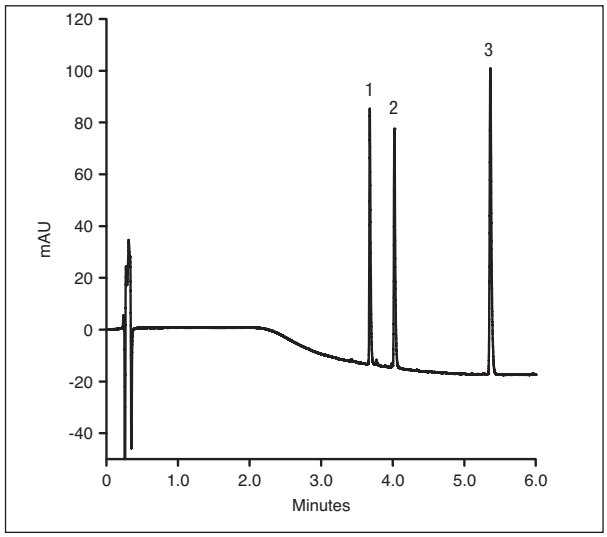

Figure 2a. Chromatogram of neutral compounds hydrocortisone (1), corticosterone (2) and the internal standard, progesterone (3) extracted from human plasma using SOLA CX 10 mg / 1 mL cartridges and separated using a Accucore RP-MS 2.6 µm, 50 x 3 mm column

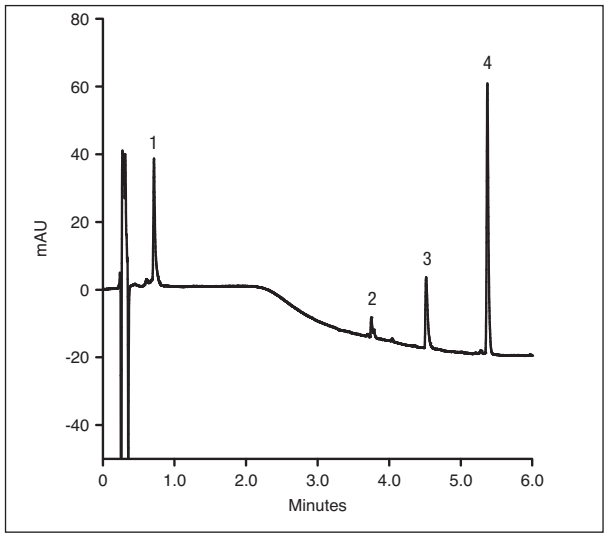

Figure 2b. Chromatogram of basic compounds procainamide (1), propranolol (2), amitriptyline (3) and the internal standard, progesterone (4) extracted from human plasma using SOLA 10 mg / 1 mL cartridges and separated using a Accucore RP-MS 2.6 µm, 50 x 3 mm column

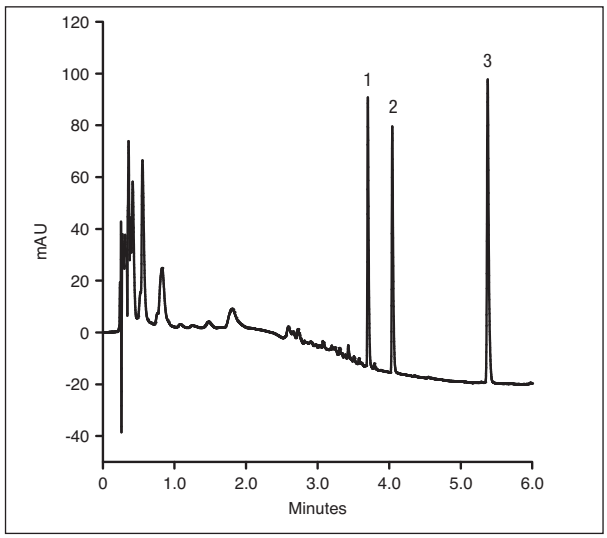

Figure 3a. Chromatogram of neutral compounds hydrocortisone (1), corticosterone (2) and the internal standard, progesterone (3) extracted from urine using SOLA CX 10 mg / 1 mL cartridges and separated using a Accucore RP-MS 2.6 µm, 50 x 3 mm column

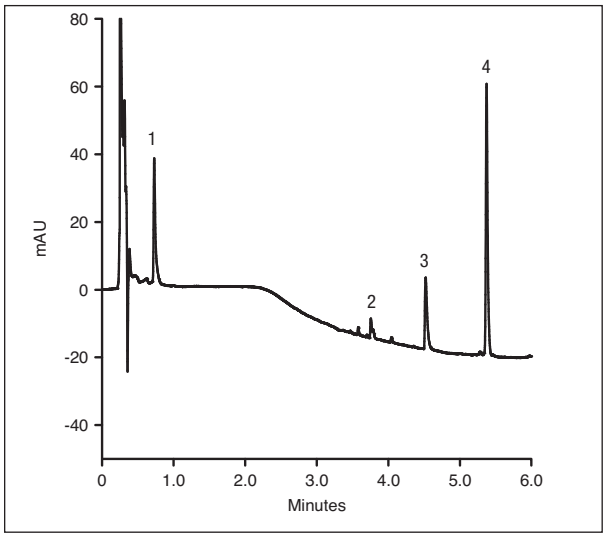

Figure 3b. Chromatogram of basic compounds procainamide (1), propranolol (2), amitriptyline (3) and the internal standard, progesterone (4) extracted from urine using SOLA CX 10 mg / 1 mL cartridges and separated using a Accucore RP-MS 2.6 µm, 50 x 3 mm column

Table 1 illustrates the excellent recoveries and reproducible results obtained by using the SOLA CX cartridges material for the extraction of basic and neutral compounds from both human plasma and urine.

	Hydro-cortisone	Corticosterone	Procainamide	Propranolol	Amitriptyline
% Recovery extracted standards using SOLA CX cartridges	96.7	95.9	91.6	102.3	95.4
% Recovery from plasma using SOLA CX cartridges	91.4	95.8	98.3	97.6	95.3
% Recovery from urine using SOLA CX cartridges	98.5	98.9	87.3	94.2	96.9
% RSD extracted standards using SOLA CX cartridges	2.72	2.86	2.29	3.39	2.78
% RSD from plasma using SOLA CX cartridges	4.58	6.44	11.8	3.71	5.18
% RSD from urine using SOLA CX cartridges	1.31	1.08	1.73	2.91	1.83

Table 1 Recovery and precision (% RSD) results for the mixture of basic and neutral compounds extracted from human plasma and urine using SOLA CX 10 mg / 1 mL cartridges (data calculated from six replicate injections)

Conclusion

This application note has shown that SOLA CX cartridges can be successfully used to extract basic and neutral compounds from both human plasma and urine. The results obtained show high recoveries with excellent precision for all compounds using SOLA CX cartridges.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

North America
USA and Canada
+1 800 332 3331

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

Germany
+49 (0) 2423 9431 -20 or -21

United Kingdom
+44 1928 534110

Asia
Japan
+81 3 5826 1615

China
+86-21-68654588 or +86-10-84193588
800-810-5118

India
+91-22-6742 9494

Thermo Fisher Scientific Australia Pty Ltd
1300 735 292 (free call domestic)

Thermo Fisher Scientific New Zealand Ltd
0800 933 966 (free call domestic)

All Other Enquiries
+44 (0) 1928 534 050

Technical Support

North America
800 332 3331

Outside North America
+44 (0) 1928 534 440

www.thermoscientific.com/chromatography

ANCCSBAS&NEUT 1211