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Polar Basic Drugs in Environmental Samples; Improved Analysis Using a New High Efficiency UPLC Column for HILIC

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APPLICATION BENEFITS

- Improved separation for EPA 1694 basic compounds compared with prior HPLC methodology
- Solid-core particle UPLC® Column technology to maximize efficiency and speed compared with fully-porous particle columns

WATERS SOLUTIONS

ACQUITY UPLC®

Xevo® TQ MS Mass Spectrometer

CORTECS® UPLC HILIC Column

Oasis® HLB Cartridge for sample preparation

LC-MS Certified Vials

MassLynx® v4.1 data system with Quanpedia™ data base

KEY WORDS

Solid-core particle UPLC, polar basic drugs, environmental analysis, SPE, UPLC-MS/MS

INTRODUCTION

Highly polar basic compounds, such as albuterol, can be determined in environmental water samples by ion-pairing reversed-phase liquid chromatography or by HILIC (Hydrophilic Interaction LIquid Chromatography). Although volatile ion-pairing reagents are available for use with LC-MS, significant reduction in MS response is usually observed compared with response with no ion-pairing reagent. Therefore the HILIC approach with no ion-pairing reagents is preferred for trace-level LC-MS analysis.

The purpose of this application note is to demonstrate the CORTECS UPLC HILIC Column for determination of highly polar basic compounds. In this application, a number of these compounds are determined in a spiked river water sample. Sample preparation was performed using the EPA 1694 method modified for use with a smaller sample volume (100 mL rather than 1000 mL) and a smaller Oasis HLB Cartridge (150 mg compared with 1000 mg). The same enrichment factor was maintained and similar performance was observed compared with the protocol given in method 1694.[1] Sample preparation time was reduced from about 4 hours to about 1 hour.

EXPERIMENTAL

UPLC conditions

System:	ACQUITY UPLC
Column:	CORTECS UPLC HILIC, 2.1 x 100 mm, 1.6 µm
Injection volume:	30 µL
Temperature:	30 °C
Mobile phase A:	Ammonium Acetate Buffer (18 mM acetic acid/13 mM ammonium acetate)
Mobile phase B:	Acetonitrile
Flow rate:	0.45 mL/min
Gradient:	See Table 1

MS conditions

Instrument:	Xevo TQ MS
Mode:	Electrospray positive (ES+)
Source temp.:	150 °C
Desolvation temp.:	500 °C
Desolvation gas:	1200 L/hr
Collison gas (Argon):	0.18 mL/min
Cone gas:	30L/hr

MRM transitions

Compound	MRM (*m/z*)	Cone (V)	Collision (eV)
Cimitidine	253.0>116.9	30	20
	253.0>158.9	30	15
Clenbuterol	277.0>167.9	24	26
	277.0>202.9	24	18
Albuterol	240.2>148.0	28	15
	240.2>222.0	28	10
Metformin	130.9>59.9	25	15
	130.9>71.9	25	20
Ranitidine	315.0>129.9	25	25
	315.0>175.9	25	20

The ACQUITY UPLC® Columns Calculator was used to convert the original HPLC conditions to UPLC conditions

HPLC

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.250	2.0	98.0	Initial
5.00	0.250	30.0	70.0	6
12.00	0.250	30.0	70.0	6
12.50	0.250	2.0	98.0	6
16.00	0.250	2.0	98.0	6

UPLC

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.600	2.0	98.0	Initial
2.08	0.600	30.0	70.0	6
5.00	0.600	30.0	70.0	6
5.20	0.600	2.0	98.0	6
6.67	0.600	2.0	98.0	6

Table 1. Gradient conditions for HPLC (left) and UPLC (right); Note total run time of 16 minutes for HPLC, 6.67 minutes for UPLC.

Sample preparation

The surface water sample (100 mL taken from a local river) was adjusted to pH 11 with concentrated ammonium hydroxide. An Oasis HLB Cartridge (6 cc, 150 mg) was conditioned with 2 mL methanol and equilibrated with 2 mL pH 11 water. The sample was loaded at approximately 5 mL/min using a vacuum manifold. After loading, the cartridge was washed with 2 mL of pH 11 water and was eluted with 1 mL methanol followed by 2 mL of 2% formic acid in methanol. The eluent was evaporated and reconstituted in 0.4 mL 20:80 methanol/acetonitrile.

RESULTS

Figure 1 shows an LC-MS/MS chromatogram obtained from a spiked sample of river water. Figure 2 shows a comparison of the same sample analyzed by UPLC and by HPLC. The CORTECS UPLC HILIC Column gave much improved resolution for metformin and ranitidine in less than half the time.

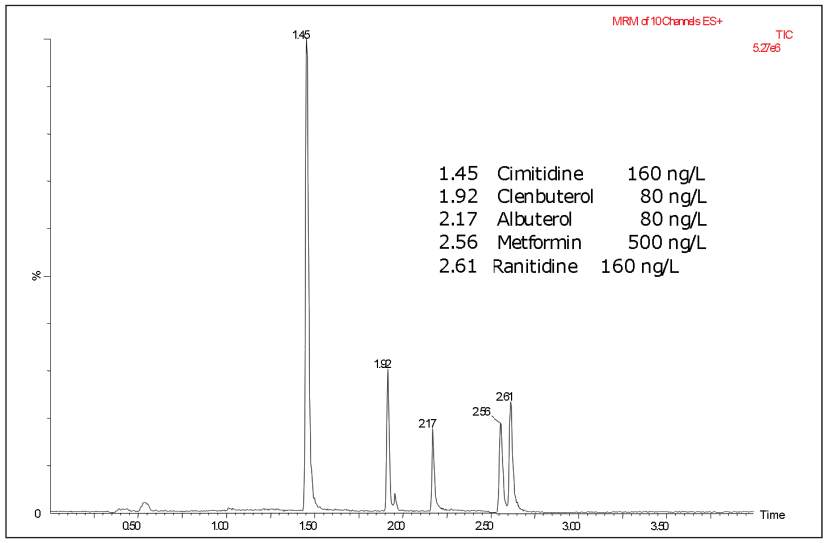

Figure 1. UPLC-MS analysis; five basic drugs in surface water at the concentrations shown.

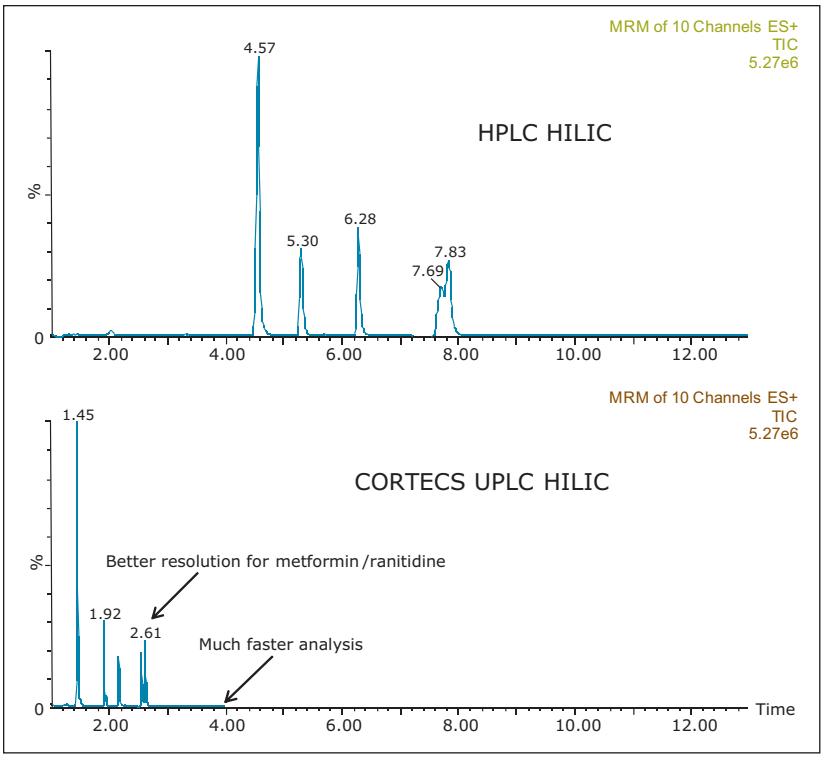

Figure 2. Comparison of HPLC (top) vs UPLC (bottom) for the analysis of five basic drugs in surface water

CONCLUSIONS

- Better resolution was achieved using the CORTECS UPLC Column compared with a traditional HPLC column
- Analysis time using the CORTECS UPLC Column was reduced by 60% compared with a traditional HPLC column
- CORTECS UPLC Columns provide exceptional efficiency and throughput based on advanced solid-core particle and column packing technology

Reference

1. EPA Method1694

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