

Analysis of Melamine and Cyanuric Acid Using a Core Enhanced Technology Accucore HILIC HPLC column

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Abstract

This application note demonstrates the use of the Thermo Scientific Accucore HILIC HPLC column for the fast analysis of melamine and cyanuric acid without compromising backpressure.

Introduction

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 low hydrophobicity of the surface has been optimised for HILIC separations, where an aqueous rich layer of the mobile phase interacts with the surface and the analytes partition between this layer and the organic rich layer of the mobile phase. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

In recent years there have been a number of food recalls relating to melamine adulteration, most notably that in China in 2008 relating to infant milk formula which led to many cases of serious kidney damage. Cyanuric acid is a degradation product of melamine. Food safety regulatory bodies including the U.S. Food and Drug Administration and the Japanese Ministry of Health, Labor and Welfare recommend the analysis of melamine and cyanuric acid using hydrophilic interaction (HILIC) HPLC separation prior to Mass Spectrometry (MS) detection. In addition all products imported to the EU from China containing greater than 15% milk product require melamine concentration analysis. The implementation of AccuCore HILIC HPLC analysis allows for fast analysis of these polar food contaminants at standard HPLC backpressures.

Experimental

LC-MS method separating an acid and a base using positive – negative switching.



Sample Preparation

A melamine standard solution of 3 mg/mL and a cyanuric acid standard solution of 3 mg/mL were prepared in dimethyl sulfoxide (DMSO). These solutions were mixed and diluted in 1:3 DMSO:mobile phase to give a final concentration of 70 µg/mL each.

Thermo Scientific Column	Part Number
Accucore HILIC 2.6 µm 150 x 4.6 mm	17526-154630
Measured pressure: 117 bar	

Thermo Scientific Accela HPLC system

Column Temperature	40 °C
Injection volume	5.0 µL
Flow rate	1.0 mL/min
MS detection	negative mode 0-3 mins, positive mode 3-10 mins Cyanuric acid: m/z 128.1 [M-H] ⁻ Melamine: m/z 127.1 [M+H] ⁺ , 168.1 [M+41+H] ⁺ (acetonitrile adduct)

Mobile Phase

90:10 acetonitrile:50 mM ammonium acetate, pH 5

Consumables	Part Number
Fisher Scientific LC-MS grade water	W/0112/17
Thermo Scientific Pierce LC-MS grade acetonitrile	TS-51101
Fisher Scientific dimethyl sulfoxide (DMSO)	D/4121/PB08
Fisher Scientific ammonium acetate	A/3440/50
Fisher Scientific acetic acid	A/0400/PB08
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

Key Words

- Accucore HILIC
- Fused core
- Superficially porous
- Food contaminants
- Core Enhanced Technology

Results

The analysis was carried out on an Accucore HILIC 2.6 μm 150 x 2.6 mm column. As shown on Figure 1, cyanuric acid eluted at 2.15 min and melamine at 4.61 min. The percentage relative standard deviation in the retention times for six replicate injections for cyanuric acid and melamine were 0.4 and 0.3 respectively.

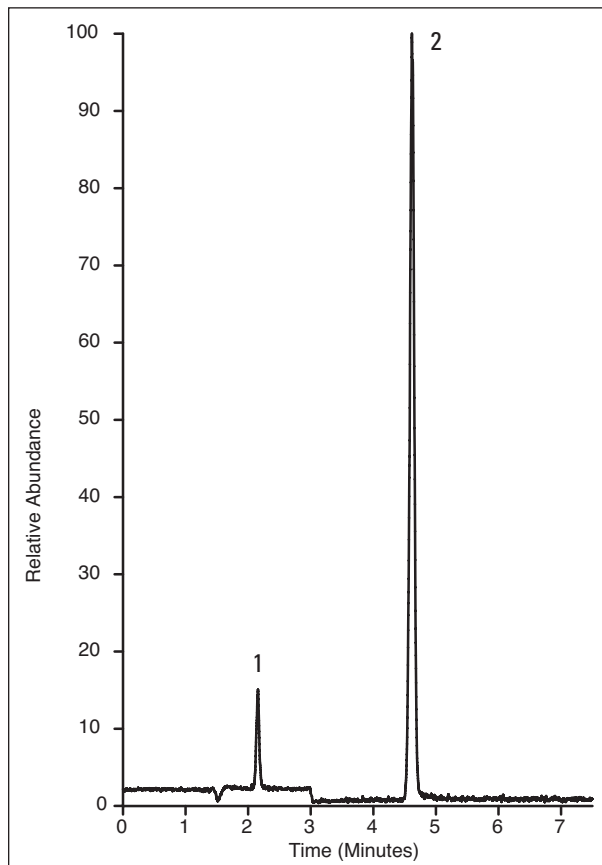


Figure 1: Chromatogram for 1. cyanuric acid and 2. melamine separated on an Accucore HILIC 2.6 μm 150 x 4.6 mm column

	Cyanuric Acid	Melamine
Retention time (min)	2.15	4.61
%RSD	0.4	0.3

Table 1: Method precision (% RSD) for cyanuric acid and melamine (data calculated from six replicate injections)

Conclusions

The use of Accucore HILIC column allowed the successful analysis of melamine and cyanuric acid giving good retention and separation at a back pressure suitable for use in a conventional HPLC system. Accucore HILIC columns are therefore an excellent choice for the fast analysis of melamine and cyanuric acid, allowing high sample throughput.

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