Application Note 20589

Rapid Separation of Analgesic Compounds Using a Solid Core HPLC Column

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Key Words
Accucore, HILIC, urea-HILIC, aspirin, acetaminophen, salicylic acid

Abstract
This application note demonstrates the use of a Thermo Scientific™ Accucore™ Urea-HILIC column for the rapid analysis of the analgesic compounds aspirin, acetaminophen and salicylic acid.

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 optimised phase bonding creates a series of high coverage, robust phases. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Accucore Urea-HILIC has an alternative selectivity and lower ion exchange activity than an unbonded silica phase such as Accucore HILIC. In HILIC mode the bonded hydrophilic stationary phase provides retention of broad range of polar analytes using up to 20% aqueous mobile phase.

The low hydrophobicity of the surface has been optimized 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.

Acetaminophen and aspirin are commonly used as analgesics, antipyretics, or both. These drugs are normally administered singly, although some commercial products combining these agents are on the market. Increasing numbers of accidental or deliberate poisonings from these drugs, either singly or in combination, make it important to know as quickly as possible whether a patient has been poisoned with one or both of these drugs, so that the appropriate clinical action may be taken.

This application note demonstrates a rapid HILIC based HPLC method that can be applied.

Experimental Details

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
Fisher Scientific LC-MS grade formic acid	A/3295/PB05
Fisher Scientific AR grade ammonium acetate	A/3440/53
Thermo Scientific Premium 2 mL vial convenience kit	60180-600
Acetaminophen, salicylic acid, aspirin – purchased from Sigma-Aldrich	

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Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Accela 1000 UHPLC system	
Column:	Accucore Urea-HILIC 2.6 µm, 100 mm x 2.1 mm	27726-102130
Mobile phase:	composition 10:80:10, A : B : C	
	A: water	
	B: acetonitrile	
	C: 100 mM ammonium acetate adjusted to pH 4.9	
Flow rate:	300 µL/min	
Column backpressure:	71 bar	
Run time:	2 minutes	
Column temperature:	35 °C	
Injection details:	2 µL into 10 µL partial loop mode	
Injection wash solvent:	water:acetonitrile 20:80	
UV detector wavelength:	230 nm	

Solutions

Buffer preparation: ammonium acetate 100 mM was prepared by dissolving 7.7 g of ammonium acetate in 1 liter of water, then adjusting the pH to 4.9 with formic acid.
Sample preparation: standards were prepared to nominal concentrations of 100 µg/mL by dissolution in the mobile phase.

Data processing	
Software:	Thermo Scientific Chromquest v 5.0 Chromatography Data System

Results

The analysis was carried out on an Accucore Urea-HILIC 2.6 µm, 100 x 2.1 mm column.

Aspirin, the related salicylic acid and acetaminophen all eluted in less than 1.5 minutes with baseline resolution.

Replicate injections of a mixture of the three compounds showed consistent performance.

	Acetaminophen		Salicylic Acid			Aspirin		
	t_R	A_s	t_R	A_s	R_s	t_R	A_s	R_s
Mean	0.760	1.474	0.908	1.303	2.359	1.100	1.318	3.264
CV %	0.00	1.17	0.48	0.92	0.49	0.00	0.63	0.48

Table 1: Data from eight replicate analyses of a mixture of acetaminophen, salicylic acid and aspirin
Retention time (t_R), peak asymmetry (A_s), peak resolution (R_s)

Additionally the standards were analyzed at increased flow rate of 600 µL/min, the data demonstrated that although peak resolution was slightly worse, baseline separation was still achieved. Column backpressure was 144 bar, well within the operating parameters of most conventional laboratory HPLC systems. This flexibility allows for selection of the best compromise between peak resolution and cycle time (Figure 1).

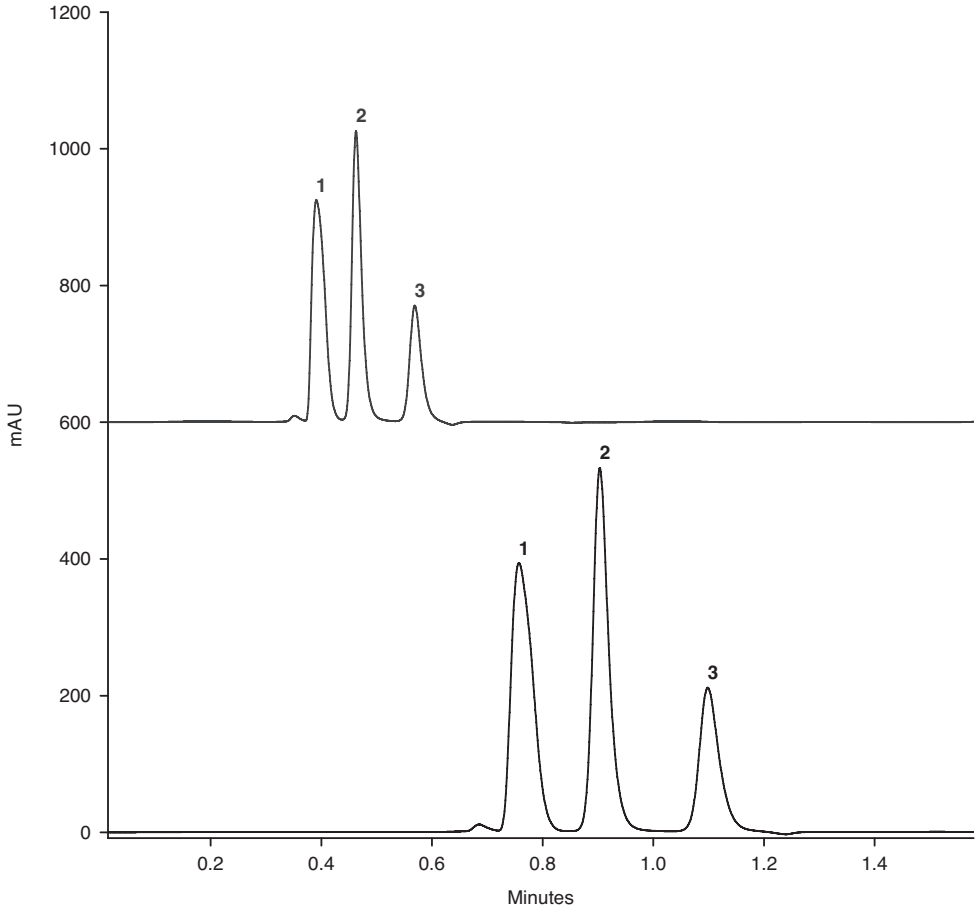

Figure 1: Chromatogram showing the separation of 1. acetaminophen 2. salicylic acid 3. aspirin on an Accucore Urea-HILIC, 2.6 µm, 100 x 2.1 mm column at two different flow rates. Lower trace 300 µL/min, upper trace 600 µL/min.

Conclusion

Rapid analysis of selected analgesic compounds was successfully achieved on an Accucore Urea-HILIC column, the performance of the column was maintained at elevated flow rates allowing flexibility in method choice or development.

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