Application Note: ANCCSCETISOMER

Analysis of 14 Positional Isomers Using a Core Enhanced Technology Accucore HPLC Column

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Key Words

- Positional isomers
- Accucore PFP
- Fused core
- Superficially porous
- Enhanced Core Technology

Abstract

This application note demonstrates the selectivity of the Thermo Scientific Accucore PFP HPLC column for the fast analysis of 14 positional isomers.

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. Introduction of fluorine groups into the Accucore PFP (pentafluorophenyl) stationary phase causes significant changes in solute-stationary phase interactions. This can lead to extra retention and selectivity for positional isomers of halogenated compounds.

PFP Columns are also well suited to the selective analysis of non-halogenated compounds, in particular polar compounds containing hydroxyl, carboxyl, nitro, or other polar groups. High selectivity is often most apparent when the functional groups are located on an aromatic or other rigid ring system. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

The selectivity of the perfluorinated phase in comparison to a C18 phase is demonstrated here with the separation of 14 positional isomers.

Results

The analysis was carried out on an Accucore PFP and Accucore C18 2.6 μ m 100 x 2.1 mm column. As shown on Figure 1, 14 positional isomers are separated and eluted in less than 8 minutes using the PFP phase. Figure 2 demonstrates the same application on the C18 phase, however resolution is reduced and the run time is also greater, with the 14 isomers being eluted in 9 minutes.

Accucore PFP provides the optimum separation of 14 positional isomers. This is achieved without compromising peak shape or speed of analysis in comparison to a conventional stationary phase.



Sample Preparation

Primary standards of each of the positional isomers were prepared in acteonitrile to a concentration of 1 mg/mL. The working standard contained 50 µg/mL of each of the positional isomers

in mobile phase.

Thermo Scientific Column	Part Number
Accucore PFP 2.6 μm 100 x 2.1 mm	17426-102130
Accucore C18 2.6 µm 100 x 2.1 mm	17126-102130
Measured pressure: 250 bar	

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Column Temperature	50 °C
Injection volume	2.0 μL
Flow rate	0.6 mL/min
UV detection	270 nm

Mobile Phase

Mobile phase A: 0.1 % formic acid in water Mobile phase B: 0.1 % formic acid in acteonitrile Gradient: 15-30 %B in 7 minutes

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
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



Conclusions

The use of Accucore PFP column allowed to successfully separate 14 positional isomers in less than 8 minutes. Accucore PFP columns are therefore an excellent choice for the analysis of positional isomers, allowing greater selectivity and high sample throughput.

Position	Analyte name	Accucore PFP		Accucore C18	
		t _r (min)	Asymmetry	t _r (min)	Asymmetry
1	3,4 - Dimethoxyphenol	0.93	1.02	0.80	0.92
2	2,6 - Dimethoxyphenol	1.47	1.08	1.49	0.95
3	2,6 - Difluorophenol	1.80	N/A	1.68	N/A
4	3,5 - Dimethoxyphenol	1.90	N/A	1.68	N/A
5	2,4 - Difluorophenol	2.23	1.07	1.94	1.03
6	2,3 - Difluorophenol	2.58	1.06	2.27	1.04
7	3,4 - Difluorophenol	2.98	1.07	2.49	1.00
8	3,5 - Dimethylphenol	3.51	1.04	4.19	N/A
9	2,6 - Dimethylphenol	3.69	1.06	4.27	N/A
10	2,6 - Dichlorophenol	4.17	1.06	4.97	1.06
11	4 - Chloro-3-Methylphenol	5.08	1.07	5.80	1.07
12	4 - Chloro-2-Methylphenol	5.63	1.06	6.35	1.05
13	3,4 - Dichlorophenol	6.39	1.08	7.09	1.07
14	3,5 - Dichlorophenol	7.13	1.07	8.60	1.23

Table 1: Peak identity and position of the positional isomers including the results obtained from both Accucore PFP and Accucore C18







Figure 2: Separation of 14 positional isomers on Accucore C18 2.6 $\mu\text{m},$ 100 x 2.1 mm

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