

Separation of Eighteen Pesticides Using Accucore RP-MS HPLC Column

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Key Words

- Accucore
- Core Enhanced Technology
- Pesticides
- Herbicides
- Triazines
- Phenylurea
- Environment

Abstract

This application shows the use of the Thermo Scientific Accucore RP-MS column for the separation of 18 pesticides. A 15 minute analysis time is demonstrated, with baseline resolution between all components. By operating at higher flow rates and backpressures a reduction in overall analysis time with little loss in column efficiency can be achieved.

Introduction

Environmental monitoring of chemicals used in the production of food is becoming more widespread due to the increased use of pesticides and the inevitable leaching from soil into water sources. European regulation DIN38-407 F12 covers the determination of 17 common pesticides in water, other legislation specifies different groups of pesticides. Typically methods have been carried out on long columns with extended run times in order to fully resolve the target analytes. New methods based on Accucore™ HPLC columns for the separation of these pesticides allow more rapid analysis with improved chromatographic resolution between components.

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. 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.



Experimental Details

Chemicals and Reagents	Part Number
Pesticide mix 18 obtained from Neochema (Bodenheim/Manz, Germany) containing the following pesticides at 10 µg/mL in acetonitrile: atrazine, desethylatrazine, metoxuron, hexazinone, simazine, cyanazine, methabenzthiazuron, chlorotoluron, monolinuron, diuron, isoproturon, metobromuron, metazachlor, sebuthylazin, propazine, terbuthylazine, linuron, metolachlor	
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17

Sample Handling Equipment	Part Number
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

Sample Preparation

An aliquot of the 18 pesticide mixture was diluted with an equal volume of water and then loaded into an autosampler vial.

Separation Conditions	Part Number
Instrumentation:	Thermo Scientific Accela UHPLC system
Column:	Accucore RP-MS 2.6 µm, 150 x 2.1 mm
Mobile phase:	A: water B: acetonitrile

Experiments were conducted at seven different flow rates with gradients scaled to deliver equal column volumes per gradient section. Data from three flow rates presented.

Flow rate	400 µL/min	700 µL/min	1000 µL/min
%B	Minutes		
20	0.00	0.00	0.00
40	11.50	6.60	4.70
80	15.00	8.60	6.00
80	16.50	9.40	6.60
20	17.25	9.90	6.90
20	22.50	15.00	11.00

Column temperature:	50 °C
Injection details:	2 µL partial loop
Injection wash solvent:	80:20 (v/v) water/acetonitrile
UV detector wavelength:	230 nm
Backpressure:	240 bar at 400 µL/min, 420 bar at 700 µL/min, 610 bar at 1000 µL/min

Results

The analysis was carried out on an Accucore RP-MS 2.6 µm 150 x 2.1 mm HPLC column. As shown in Figure 1, all eighteen pesticides eluted between 2 and 15 minutes with baseline resolution when using a flow rate of 400 µL/min. Of particular note is the separation achieved between diuron and isoproturon which have always been difficult compounds to fully resolve. Over seven replicate injections there was very little deviation in retention time for any of these molecules (Table 1) and the peak shapes are symmetrical.

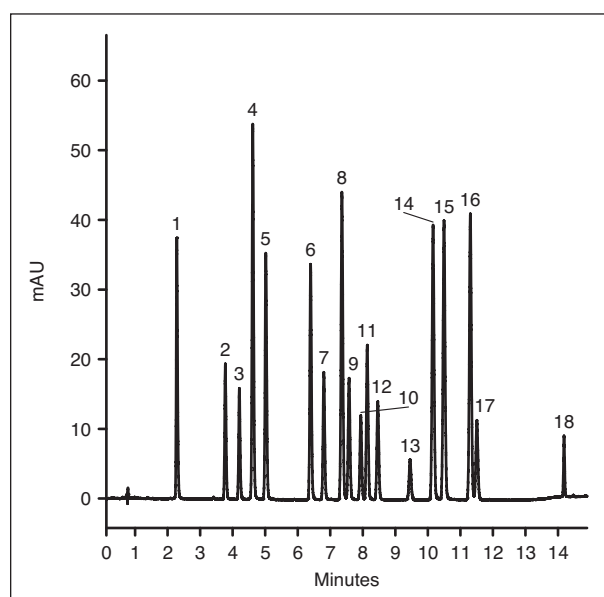


Figure 1: Example chromatography – analysis of eighteen pesticide mixture at 400 µL/min

1. desethylatrazine 2. metoxuron 3. hexazinone 4. simazine 5. cyanazine 6. methabenzthiazuron 7. Chlorotoluron 8. Atrazine 9. monolinuron 10. diuron 11. isoproturon 12. metobromuron 13. metazachlor 14. sebuthylazin 15. propazine 16. terbuthylazine 17. linuron 18. metolachlor

Standard	Peak Number	Retention time (minutes)		
		mean	SD	%CV
desethylatrazine	1	2.29	0.01	0.3%
metoxuron	2	3.78	0.01	0.2%
hexazinone	3	4.21	0.01	0.1%
simazine	4	4.62	0.01	0.1%
cyanazine	5	5.02	0.01	0.1%
methabenzthiazuron	6	6.40	0.01	0.1%
chlorotoluron	7	6.80	0.01	0.1%
atrazine	8	7.35	0.01	0.1%
monolinuron	9	7.57	0.01	0.1%
diuron	10	7.94	0.01	0.1%
isoproturon	11	8.13	0.01	0.1%
metobromuron	12	8.46	0.01	0.1%
metazachlor	13	9.45	0.01	0.1%
sebuthylazin	14	10.16	0.01	0.1%
propazine	15	10.49	0.01	0.1%
terbuthylazine	16	11.30	0.01	0.1%
linuron	17	11.51	0.01	0.1%
metolachlor	18	14.19	0.00	0.0%

Table 1: Retention data of pesticide standards from seven replicate injections

The Core Enhanced Technology within Accucore columns provides opportunities to exploit the wide efficiency range of these columns. By increasing the flow rate it is possible to reduce overall analysis time with only a small deterioration in column efficiency.

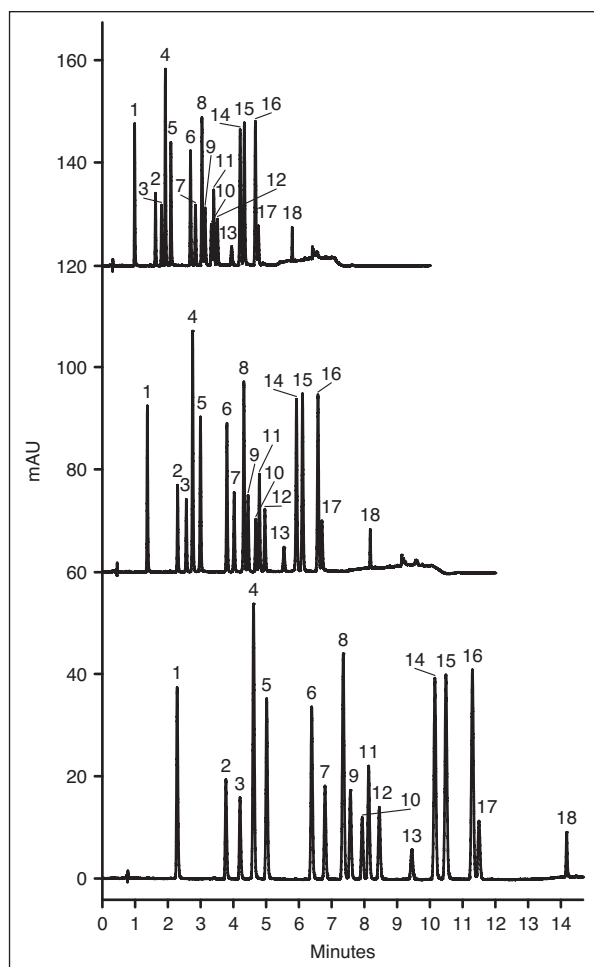


Figure 2: Example chromatography – analysis of eighteen pesticide mixture at 400, 700 and 1000 µL/min

Standard	Peak Number	Flow rate (µL/min)		
		400	700	1000
desethylatrazine	1	-	-	-
metoxuron	2	19.8	18.0	15.3
hexazinone	3	5.3	5.0	4.2
simazine	4	4.5	3.5	2.5
cyanazine	5	4.5	4.0	3.3
methabenzthiazuron	6	14.2	13.1	11.0
chlorotoluron	7	4.0	3.5	2.6
atrazine	8	5.2	4.4	3.3
monolinuron	9	1.9	1.8	1.5
diuron	10	3.3	3.4	3.0
isoproturon	11	1.9	1.6	1.2
metobromuron	12	2.8	2.4	1.8
metazachlor	13	8.4	7.6	6.2
sebuthylazin	14	5.7	4.9	3.8
propazine	15	2.8	2.5	1.9
terbutylazine	16	6.6	6.2	5.1
linuron	17	1.6	1.6	1.3
metolachlor	18	26.2	24.5	20.0

Table 2: Peak resolution values from analysis of eighteen pesticide mixture at 400, 700 and 1000 µL/min (taken from named peak to previous peak)

Conclusions

The use of an Accucore RP-MS column allowed the complete separation of eighteen pesticides in less than 15 minutes and at standard HPLC backpressures. If equipment compatible with higher backpressure is available, further gains can be made through reduced analysis time with little loss in column efficiency. Accucore RP-MS columns are therefore an excellent choice for the analysis of pesticides, allowing good sample throughput and analysis on both conventional and higher pressure HPLC systems.

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