

Application Note

► High speed analysis of pesticides

Category	Environmental analysis
Matrix	-
Method	UHPLC
Keywords	Pesticides, herbicides, phenylurea pesticides, triazines, cyclodienes, chloroacetanilide pesticides
Analytes	Chloridazon, β -Endosulfan, Diuron, Linuron, Metolachlor, Metoxuron, Monuron, Parathion-ethyl, Propazine
ID	VEV6, 08/10



PLATIN blue

Summary

This application note describes a very fast gradient method for the simultaneous determination of nine pesticides commonly used in agriculture. The high speed and reliability of this method using the KNAUER PLATINblue HPLC/UHPLC System make it well-suited for routine analysis. Reduction of analysis time to less than 2 minutes is achieved by employing BlueOrchid C18 as the stationary phase with a 1.8 μm particle size filled in a 2 mm ID column. A binary high pressure gradient configuration was used at a flow rate of 0.6 ml/min in combination with UV detection.

Introduction

Pesticides are used in agriculture in great quantities. In most cases they act as herbicides, insecticides, nematocides, molluscicides and acaricides.¹ The substances used can be of different chemical classes. Most common are phenylurea herbicides, triazines, cyclodienes and chloroacetanilides. Many of them are known to persist in the environment and have toxicological effects, acting as potential endocrine disrupters.¹ Pesticides must therefore be regarded as environmental pollutants and must be monitored in ground and drinking water supplies, as well as in food.

Public authorities have reacted and enacted laws and guidelines to address the dangers of pesticides. For example, according to the European Union directive on water quality (98/83/CE) the maximum concentration admissible for levels of pesticide residues in drinking and surface water is 0.10 $\mu\text{g/l}$ for individual and 0.50 $\mu\text{g/l}$ for the sum of pesticides.² It is clear that a reliable method for the analysis of pesticides with high sensitivity and selectivity is important to comply with these guidelines. In addition, an ideal analytical method should be time efficient and inexpensive so that it can easily be used in routine laboratories. Most pesticides are polar and thermally labile substances so that the classical analysis methods for environmental contaminants with gas chromatographic methods are not suitable. HPLC and UHPLC are best suited for the analysis of a wide range of pesticides.¹

A UHPLC method that separates nine commonly used pesticides in less than 2 minutes is presented in this application note. The great advantage of employing a UHPLC instead of a HPLC method is that analysis time is shorter, running costs are reduced, and eluent consumption is minimized.

Experimental sample preparation

Experimental preparation of standard solution

Monitoring of pesticide concentrations is most important for groundwater, drinking water and food. Samples from drinking water can be collected, dechlorinated, preserved, shipped and stored as described by the EPA methods 532 or 535, for example. According to these methods, sample enrichment techniques like solid phase extraction or liquid-liquid-extraction must be used prior to analysis.^{3,4} This step is needed because the admissible level of individual pesticides according to the European Union directive on water quality is only 0.10 µg/l. Hence a water sample must be preconcentrated to produce quantifiable results in this range.

Standards were prepared by weighing out the exact masses for every pesticide compound and dissolving them in the mobile phase acetonitrile/water 35:65 (v/v) to the concentrations needed for calibration in the range of 0.015 – 0.5 µg/ml.

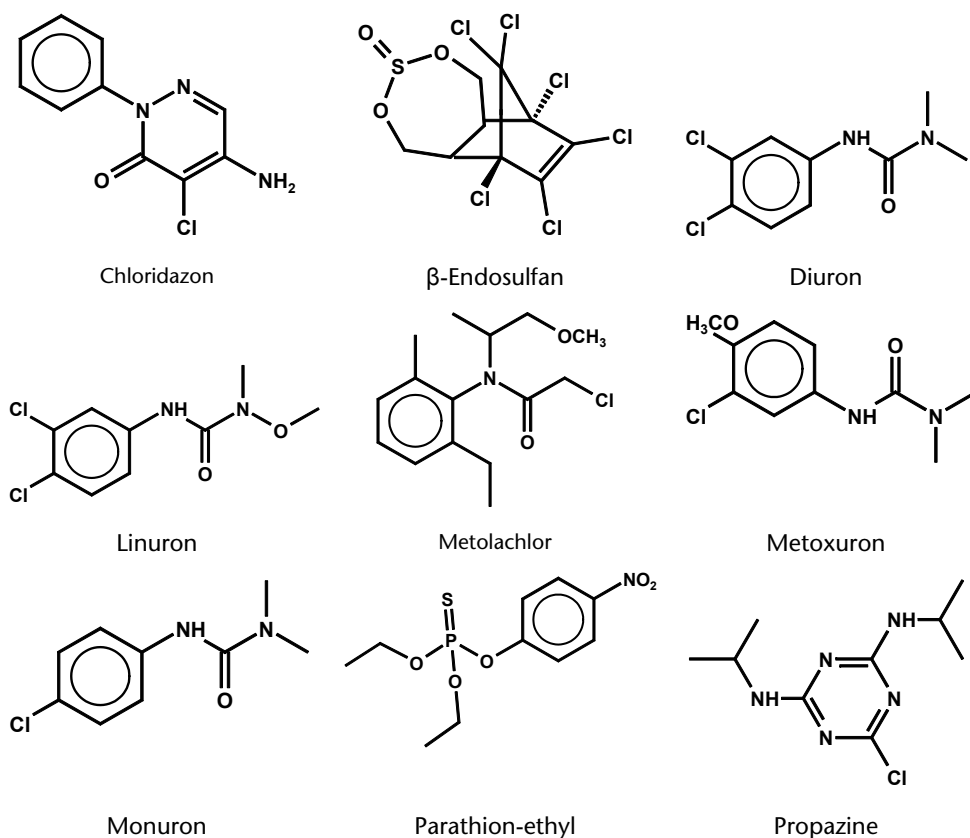


Fig. 1
Chemical structures

Method parameters

Column	BlueOrchid 1.8 C18, 50 x 2 mm		
Eluent A	Water		
Eluent B	Acetonitrile		
Gradient	Time [min]	% A	% B
	0.00	65	35
	0.50	65	35
	1.50	0	100
	2.00	0	100
Flow rate	0.6 ml/min		
Injection volume	1 µl		
Column temperature	40 °C		
System pressure	approx. 280 bar		
Detection	UV at 215 nm (50 Hz, 0.05 s)		
Analysis time	1.6 min		

Results

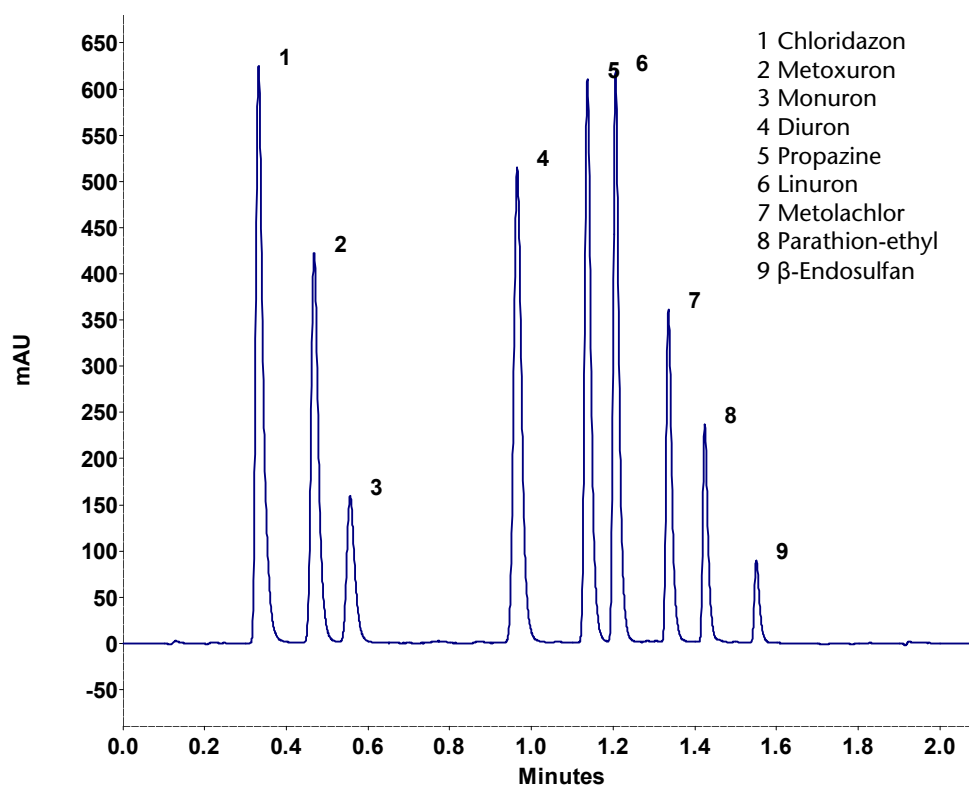


Fig. 2

Separation of the pesticide mix (0.5 mg/ml).

Using the KNAUER PLATINblue UHPLC system and a BlueOrchid C18 1.8 µm column, nine commonly used pesticides were successfully separated in less than 2 min (Fig. 2). All compounds are baseline separated with resolution values in the range of 2.6 for Monuron to 11.7 for Diuron.

Calibration was realized for concentrations from 0.015 – 0.5 µg/µl and the goodness of linearity fit (r^2) was between 0.998145 and 0.999105 for all components. The limit of detection (LOD) was in the range of 0.15 – 1.0 ng for all compounds. The reliability of the UHPLC method is underlined by its retention time reproducibility in the range of 0.1 – 1.0 % RSD (n = 4) for all nine pesticides.

Method performance

Limit of detection	0.15 – 1.0 ng range (S/N = 3)
Linearity (r²)	> 0.998
Linearity range	0.015 – 0.5 µg
Resolution values	2.6 – 11.7
Retention time precision*	< 1 % RSD

*repeatability calculated over 4 replicate runs

Conclusion

This application note describes a very fast method for the determination of nine pesticides that are commonly used in agriculture. The easy separation in less than 2 minutes was possible by employing the KNAUER PLATINblue UHPLC system, a BlueOrchid C18 stationary phase and an acetonitrile gradient. The 2 mm inner diameter column resulted in a comparably smaller amount of required eluent than conventional ID columns. When compared with an optimized HPLC method using 3 µm particles and a 3 mm column ID, up to 78 % eluent can be saved and the analysis time can be reduced by 70 %.

References

1. J.M.F. Nogueira, Tom Sandra, Pat Sandra. Multiresidue screening of neutral pesticides in water samples by high performance liquid chromatography–electrospray mass spectrometry. *Analytica Chimica Acta* 505 (2004) 209–215.
2. EU Council, Directive on the Quality of Water Intended for Human Consumption, 98/83/EC, 1998.
3. Office of Research and Development, National Exposure Research Laboratory (NERL), US Environmental Protection Agency. Method 532. Determination of Phenylurea Compounds in Drinking Water by Solid Phase Extraction and High Performance Liquid Chromatography with UV Detection - Revision 1.0., June 2000.
4. Office of Research and Development, National Exposure Research Laboratory (NERL), US Environmental Protection Agency. Method 535: Measurement of Chloroacetanilide and Other Acetamide Herbicide Degradates in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Revision 1.1, issued in April 2005.

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Physical properties of recommended column



BlueOrchid C18 separates by using hydrophobic interactions and offers an extended pH range for analysis of acidic, basic and neutral analytes in reversed phase mode. All BlueOrchid phases feature exceptional peak symmetry and resolution. Due to the narrow particle size distribution, the column back pressure of all BlueOrchid columns is lower than other high speed column materials on the market.

Stationary phase	BlueOrchid 1.8 C18
USP code	L1
Pore size	180 Å
Particle size	1.8 µm
Form	spherical
Surface area	180 m ² /g
% C	10
Endcapping	yes
Dimensions	50 x 2 mm
Order number	05BI181BOE

Recommended Instrumentation



This application requires the PLATINblue binary high pressure gradient HPLC/UHPLC system equipped with degasser, autosampler, column thermostat, and PDA detector. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

Description	Order No.
PLATINblue UHPLC System	A69420
PLATINblue Pump P-1 with SmartMix mixer	
PLATINblue Pump P-1 with degasser	
PLATINblue Autosampler AS-1	
PLATINblue Column Thermostat T-1 Basic	
PLATINblue Detector PDA-1	
PDA-1 flow cell (10 mm, 2 µl)	
PLATINblue Modular Eluent Tray ET-1	
PLATINblue ChromGate Software	
PLATINblue ChromGate PDA license	

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