

## Application Note

### ► Determination of pesticides by UHPLC-MS



Category	Environmental
Matrix	-
Method	UHPLC-ESI-MS
Keywords	Pesticides, herbicides, phenylurea pesticides, triazines, cyclodienes, chloroacetanilide pesticides
Analytes	Atrazine, Chlortolurone, Chloridazon, Diuron, Isoproturon, Linuron, Metazachlor, Methabenzthiazuron, Metobromuron, Metolachlor, Metoxuron, Monolinuron, Monuron, Parathion-ethyl, Propazine, Simazine, Terbutylazine
ID	VEV0040N, 08/11

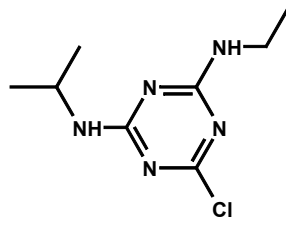
PLATINblue  
by Knauer

#### Summary

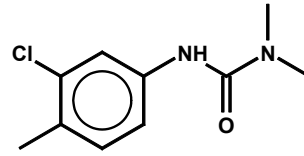
In this application note we present a method combining the rapidness of a UHPLC separation with the advantages of mass spectrometry detection. With the developed UHPLC-ESI-MS method it is possible to simultaneously qualify 17 pesticides in less than 5 minutes. A binary high pressure gradient is used at a flow rate of 0.2 ml/min. The high speed and reliability of this method applying the KNAUER PLATINblue UHPLC system equipped with a BlueOrchid 175-1.8 C18 column make it well-suited for routine analyses. The sensitive and exact detection of the pesticides is carried out using the MSQ Plus single quadrupole mass spectrometer.

#### Introduction

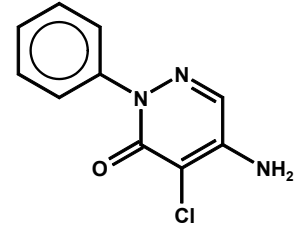
Pesticides are agriculturally used in great quantities today. In most cases they serve as herbicides, insecticides, nematocides, molluscicides, and acaricides.<sup>1</sup> The adopted substances can be of different chemical classes. Phenylurea herbicides, triazines, cyclodienes, and chloroacetanilides are most commonly used. Many of them are known for persistency and toxicological effects in the environment and to be potential endocrine disrupters.<sup>1</sup> As a conclusion, they have to be regarded as environmental pollutants so that their monitoring in ground and drinking water as well as in food becomes inevitable. As a reaction public authorities enacted laws and guidelines in this context. According to the European Union directive on water quality (98/83/CE), for example, the maximum concentration admissible for levels of pesticide residues in drinking and surface water is 0.10 µg/l for individual and 0.50 µg/l for the sum of pesticides.<sup>2</sup> It is obvious that the development of a reliable method for the analysis of pesticides with high sensitivity and selectivity is important to comply with these guidelines. Additionally the established method has to save time and money so that it can easily be used in routine laboratories. Most pesticides are polar and thermally labile substances which makes the classical analysis of environmental contaminants with gas chromatographic methods not suitable. As a conclusion, HPLC and UHPLC are the methods of choice.<sup>1</sup> UHPLC-MS equipped with an electrospray ion source (ESI) avoids the thermal degradation of labile pesticides that would occur using GC-MS. At the same time it also offers the benefits of mass sensitive detection. A UHPLC method that separates 17 commonly used pesticides in less than 5 minutes is presented in this application note. One great advantage of employing a UHPLC-MS method is to save analysis time and money caused by the high speed and minimized eluent consumption.



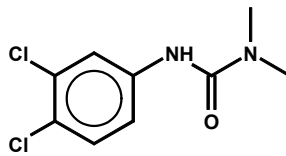
Atrazine



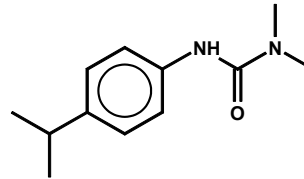
Chlortoluron



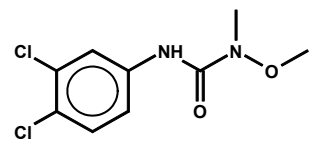
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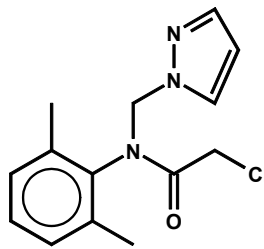
Diuron



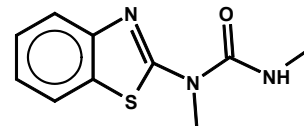
Isoproturon



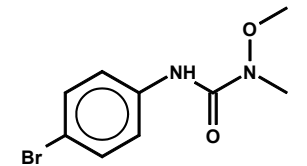
Linuron



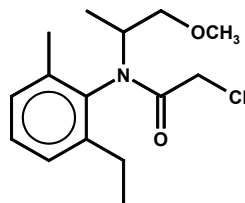
Metazachlor



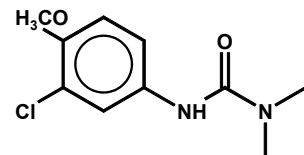
Methabenzthiazuron



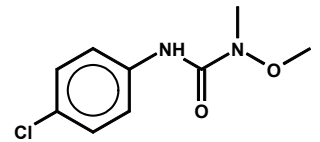
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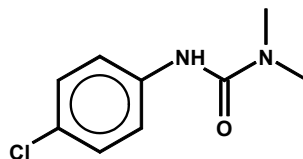
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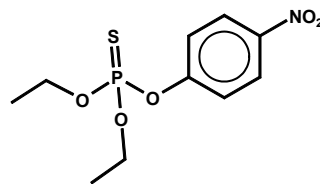
Metoxuron



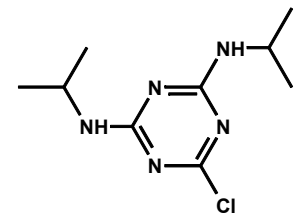
Monolinuron



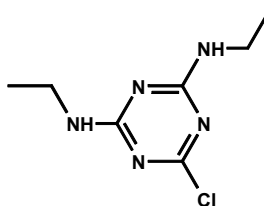
Monuron



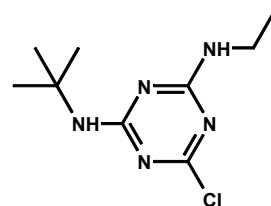
Parathion-ethyl



Propazine



Simazine



Terbutylazine

Fig. 2

Chemical structures of the analyzed pesticides

### Experimental preparation of standard solution

### Experimental sample preparation

### Method parameters

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.

The controlling of pesticide concentrations is most important in ground and drinking water as well as in 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 before analyses.<sup>3,4</sup> This step is needed because the admissible level of individual pesticides is only 0.10 µg/l according to the European Union directive on water quality. Hence a water sample has to be pre-concentrated to gain quantifiable results in this range.

<b>Column</b>	BlueOrchid 175-1.8 C18, 50 x 2 mm ID		
<b>Eluent A</b>	Water + 0.5 % Formic Acid		
<b>Eluent B</b>	Acetonitrile + 0.5 % Formic Acid		
<b>Gradient</b>	<b>Time [min]</b>	<b>% A</b>	<b>% B</b>
	0.00	65	35
	1.50	65	35
	3.50	60	40
	4.50	0	100
	6.00	0	100
<b>Flow rate</b>	0.2 ml/min		
<b>Injection volume</b>	1 µl		
<b>Column temperature</b>	40 °C		
<b>MS Detection Parameters</b>	Ionization mode	ESI, positive mode	
	Needle Voltage	3.5 kV	
	Cone Voltage	75 V	
	Probe temperature	200 °C	
	Mode	Full scan mode, m/z 180 – 300	

### Results

For MS detection, the characteristic mass spectra for every compound were figured out. The fragment with the highest intensity is chosen for quantification. Fragment ions with lower intensities can be used as qualifiers in real samples. Parameters important for MS detection are shown in table 1.

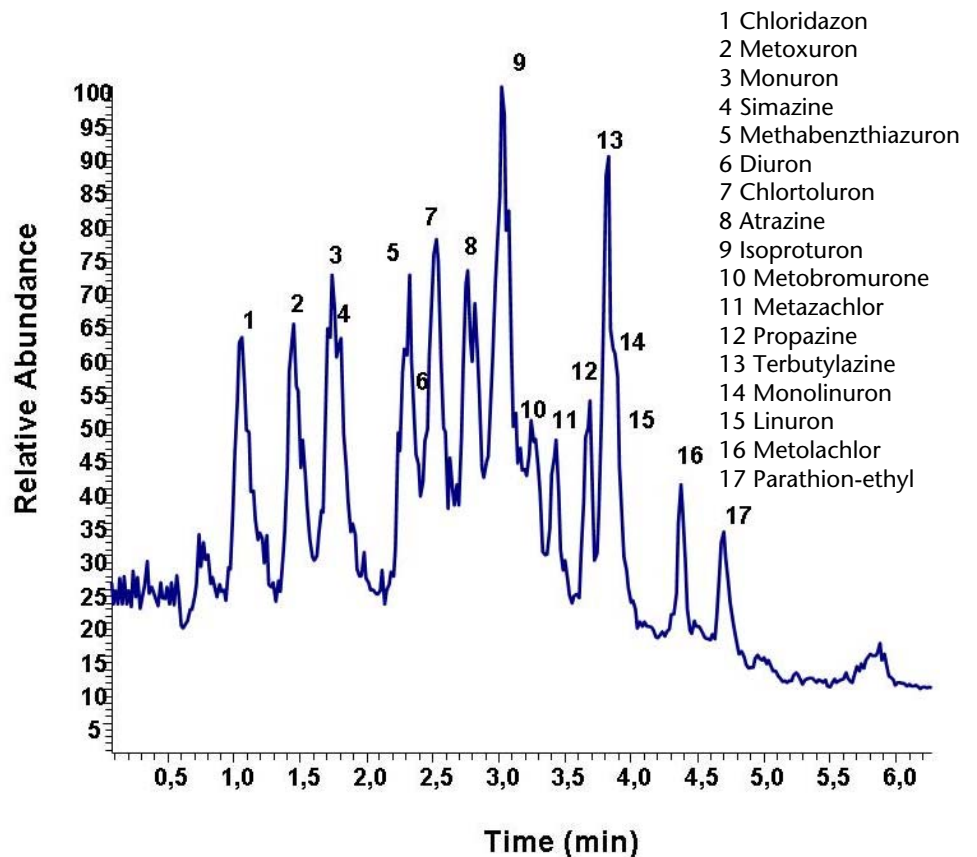
**Table 1**

Analyzed steroids and their MS Parameters

Compound	Ionisation	Mass [g/mol]	m/z expected [M+H] <sup>+</sup>	m/z found (highest intensity)
Atrazine	ESI +	215.7	216	216
Chlortolurone	ESI +	212.7	213	213
Chloridazon	ESI +	221.7	222	222
Diuron	ESI +	233.1	234	234
Isoproturon	ESI +	206.3	207	207
Linuron	ESI +	249.1	250	249
Metazachlor	ESI +	277.8	278	278
Methabenzthiazuron	ESI +	221.1	222	222
Metobromuron	ESI +	259.1	260	261
Metolachlor	ESI +	283.8	284	284
Metoxuron	ESI +	228.7	229	229
Monolinuron	ESI +	214.7	215	215
Monuron	ESI +	198.7	199	199
Parathion-ethyl	ESI +	291.3	292	292
Propazine	ESI +	229.7	230	230
Simazine	ESI +	201.7	202	202
Terbutylazine	ESI +	229.7	230	230

All analyzed pesticides were detected as expected as singly charged quasi-molecular ions [M+H]<sup>+</sup>. Therefore, this mass was chosen for further investigation in all cases.

For the separation, a BlueOrchid 175-1.8 C18 50 x 2 mm ID column was chosen. The scan range of  $m/z = 180 - 300$  was chosen as narrow as possible. The resulting total ion chromatogram (TIC) shown in figure 3 demonstrates that the baseline separation of the 17 pesticides could not be completed in the short analysis time.

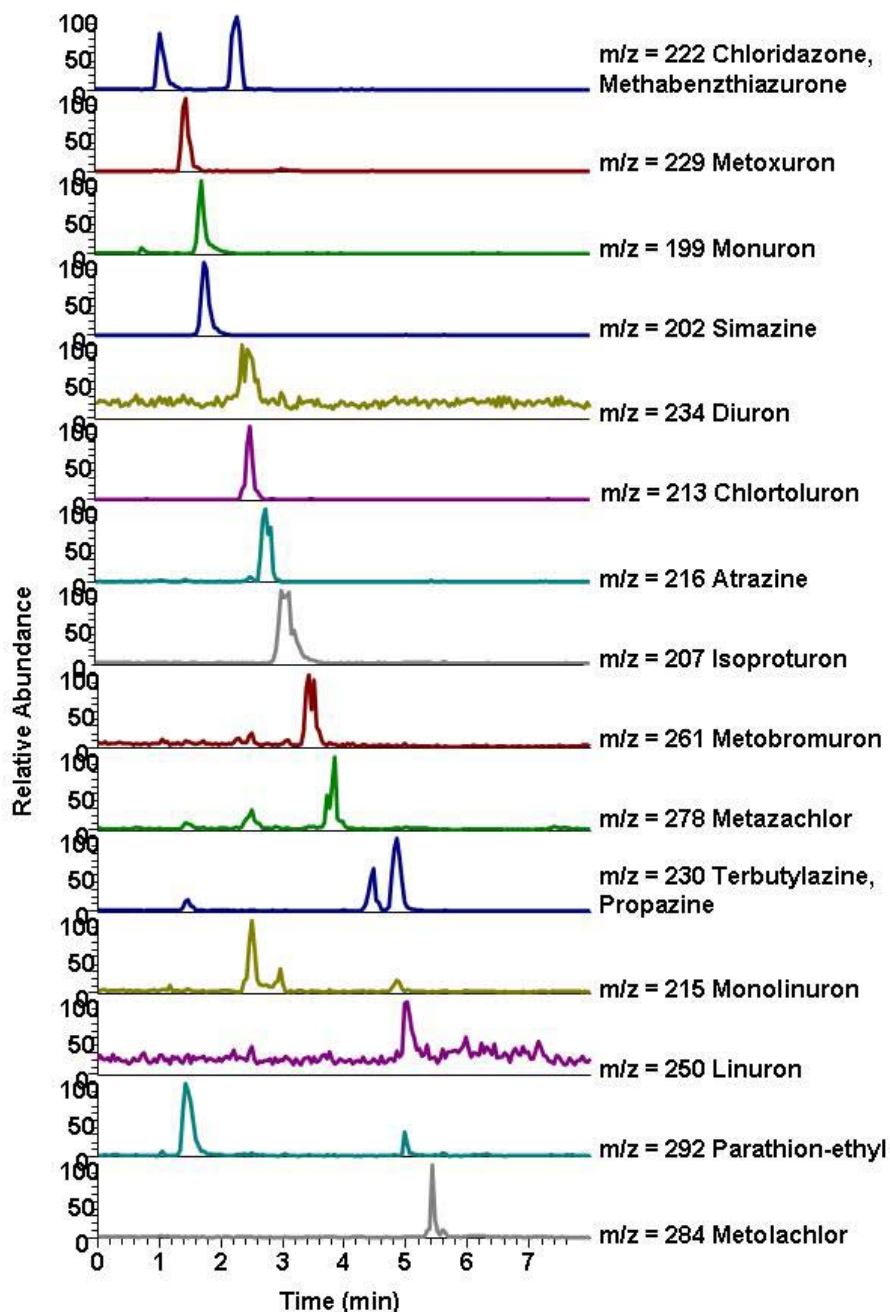


**Fig. 3**

Total Ion Chromatogram of the pesticide standard

One of the great advantages of MS detection is that the user is able to extract the single ion chromatograms (SICs) for every compound from the TIC. The Xcalibur software enables the user to evaluate much sharper and singly occurring peaks for selected  $m/z$  values even if they are not chromatographically separated. The SICs for the occurring masses are shown in figure 4. It becomes obvious that every peak can be integrated without a doubt using the SIC although the compounds are not chromatographically separated. This demonstrates the superiority of mass detection .. Additionally, sensitivity and selectivity are significantly better compared to the usage of an UV detector.

Only isomasses like Chloridazone and Methabenzthiazurone (or Terbutylazine and Propazine in this case) have to be separated by chromatography to reach quantifiable results.



**Fig. 4**

SICs extracted from the TIC of the pesticide standard

## Conclusion

The UHPLC-ESI-MS method presented in this application note describes the fast and simultaneous analysis of 17 pesticides. Less than 6 minutes are needed for the analysis of one sample making this method well-suitable for routine analyses. Due to the high separation speed and the low eluent flow rate, only about 1.2 ml eluent are needed per run, which is equivalent to less than 1 ml of acetonitrile. This makes the method both economical and environmentally friendly. Additionally, the method can be readily applied for the detection of a greater variety of pesticides, because of the mass sensitive detection method. If a higher resolution is required, the use of a 100 x 2 mm ID column is recommended.

The ability of the MSQ Plus for mass sensitive detection and therefore the separation of co-eluting peaks by their m/z values are demonstrated in this application note. Although a chromatographic separation is always recommended to avoid ion suppression in the ESI probe, co-eluting substances were successfully ionized using the method described here.

## 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.

## Authors

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## Physical properties of recommended column

BlueOrchid C18 uses hydrophobic interactions as the separation mechanism 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 BlueOrchid columns is lower than of most other fully porous sub-2 µm column materials on the market.



<b>Stationary phase</b>	BlueOrchid C18 1.8 µm
<b>USP code</b>	L1
<b>Pore size</b>	175 Å
<b>Pore volume</b>	0.98 ml/g
<b>Particle size</b>	1.8 µm
<b>Form</b>	spherical
<b>Surface area</b>	320 m <sup>2</sup> /g
<b>% C</b>	10
<b>Endcapping</b>	yes
<b>Dimensions</b>	50 x 2 mm
<b>Order number</b>	05BI181BOE

## Recommended Instrumentation

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



Description	Order No.
PLATINblue UHPLC-MS System	A69450
PLATINblue Pump P-1	
PLATINblue Pump P-1 with Degasser	
PLATINblue Autosampler AS-1	
PLATINblue Column Thermostat T-1 Basic	
PLATINblue modular eluent tray	
PLATINblue stainless steel capillary kit	
MSQ Plus MS, Single Quadrupole MS with Edwards vacuum pump, PC, Xcalibur SW, ESI and APCI ion sources	

## Contact information

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