

Fast and Versatile Analysis of Desphenyl-Chloridazone and Methyl-Desphenyl-Chloridazone in Surface, Ground and Drinking Water Using LC-MS/MS and EQuan

N. Eßer, B. Preuß, F. Brille, Bergisches Wasser- und Umweltlabor der BTV GmbH, Wuppertal, Germany
O. Scheibner, Thermo Fisher Scientific, Dreieich, Germany

Introduction

Growing concerns about contaminants in water and food has resulted in governmental regulations with lower tolerance levels nearly every year. The number of samples has increased nearly at the same speed during the last few years. Thus the demand for shorter and less laborious techniques for residue analysis, with increased sensitivity levels, is higher than ever. The analysis of pesticides and other pollutant compounds such as pharmacological substances in water samples can be highly time-consuming due to the high volumes which need to be extracted. Direct injection of water samples is limited to a volume in the range of 100 μL , if one wishes to avoid affecting the peak shape. Automation of sample workup and use of fast HPLC methods for further method acceleration are the demands in modern residue analysis. The Thermo Scientific EQuan system provides a solution for these demands: injection of up to 5000 μL crude water sample onto an enrichment column with subsequent HPLC analysis results in full automation and high throughput. Methods for various pesticides, antibiotics and veterinary residues have been

described already, but still the automated analysis of highly polar compounds remains a major challenge since the reliable and quantitative trapping and re-elution during pre-concentration has not yet been satisfactorily resolved.

We describe a method for a fully automated analysis of metabolites of the well known herbicide Chloridazone, Desphenyl-Chloridazone and Methyl-Desphenyl-Chloridazone. Due to their polarity, quantitative trapping and sensitive HPLC analysis had not been achieved so far. Use of a Thermo Scientific Hypercarb trapping column (20 x 2.1 mm, 7 μm) in combination with a Hypercarb analytical column (100 x 2.1 mm, 3 μm) has resulted in a highly reliable and sensitive method for the direct analysis of surface, ground and drinking water.

Goal

The aim of our work was to establish a fast and versatile LC-MS/MS method using EQuan for both metabolites of Chloridazone providing a LOQ of less than 10 ng/L in all water samples.

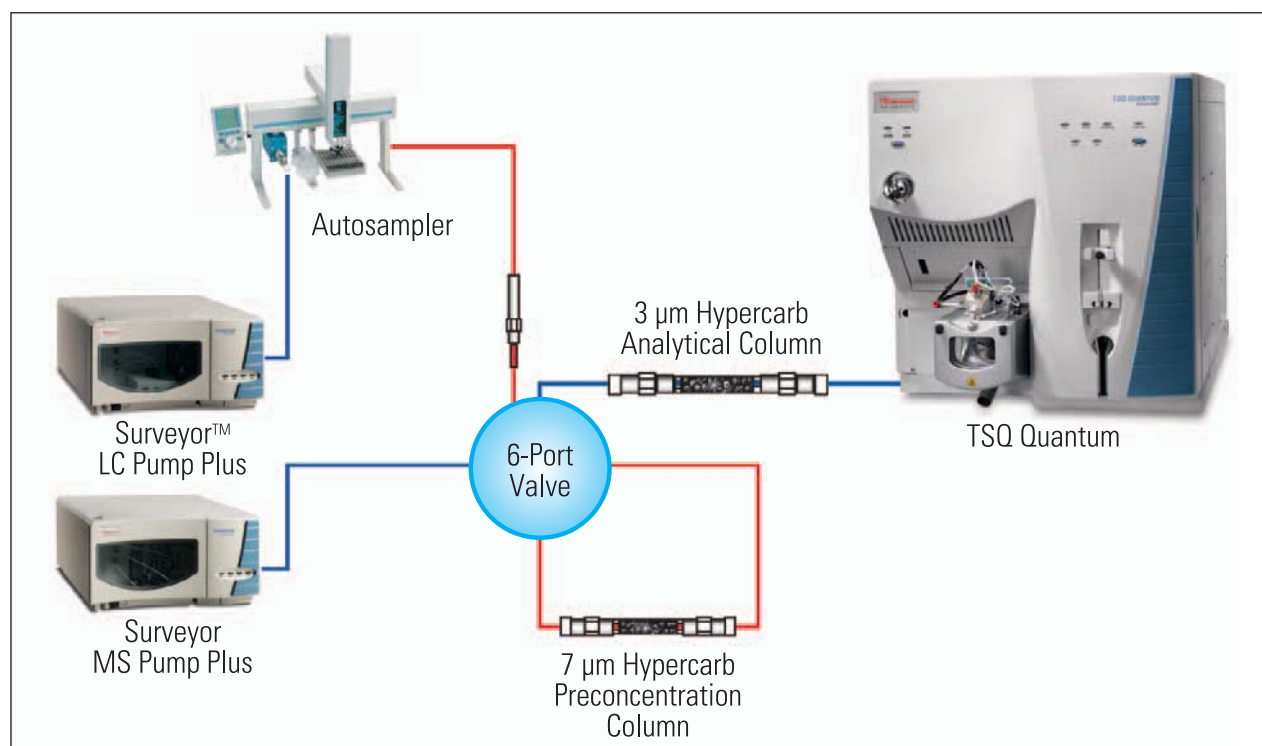


Figure 1: Schematic drawing of the EQuan system

Experimental

Sample Preparation

For the dilution series 10 g ultrapure water was passed through a syringe tip filter and spiked with the given levels of Desphenyl-Chloridazone and Methyl-Desphenyl-Chloridazone. Six levels with the following concentrations were prepared: 5, 6, 7, 8, 15 and 20 ng/L.

Samples were only passed through a syringe tip filter. No additional treatment of samples was necessary prior to measurement.

Instrumentation

Thermo Scientific Surveyor LC pump for sample concentration
Thermo Scientific Surveyor MS pump for sample analysis
CTC PAL autosampler for sample application
Thermo Scientific TSQ Quantum Access triple stage mass spectrometer.

Sample Pre-concentration

Hypercarb column 20 x 2.1 mm, 7 μ m
(part number 35007-022130)

A sample volume of 1 mL was transferred to the trap column with 20% methanol in water as solvent. Sample transfer and concentration was carried out with a flow rate of 500 μ L/min. The trap column was switched by means of the 6-port valve (Figure 1) into the flow of the analytical pump and followed by the introduction of an analytical gradient to both columns.

Chromatographic Conditions

Column: Hypercarb 100x2.1 mm, 3 μ m
(part number 35003-102130)

Mobile phase: A – Water +0.5% methanol; B – Methanol

Gradient: Time (min)	%A	%B
0	30	70
2	30	70
11	0	100
14	0	100
17	30	70
20	30	70

Flow rate: 200 μ L/min

The time for sample concentration was 1.9 min. The total cycle time for sample concentration, analysis and reconditioning was 20 minutes.

MS Conditions

Ionisation: ESI in positive mode
Spray voltage: 3500 V
Sheath gas pressure (N_2): 40 units
Auxiliary gas (N_2): 5 units
Ion transfer tube temperature: 350 °C
Collision gas pressure (Ar): 1.5 mTorr
Q1/Q3 peak resolution: 0.7 Da
Scan width: 0.01 u
Scan time: 0.6 s

The SRM transitions used are shown in Table 1. The product masses marked with * were used for quantification.

Analyte	Parent Ion (m/z)	Fragment Ions (m/z)	Collision Energy (V)
Desphenyl-Chloridazone	145.9	101.1	28
		117.1*	23
Methyl-Desphenyl-Chloridazone	159.9	88.2*	31
		117.0	23

Table 1: SRM conditions

Results and Discussion

The chromatogram of a real water sample spiked with 10 ng/L each of Desphenyl-Chloridazone and Desphenyl-Methyl-Chloridazone is shown in Figure 2. Thermo Scientific LCQuan 2.5 software was used to process the quantitative data. Figure 4 shows the calibration curves of Desphenyl-Chloridazone and Methyl-Desphenyl-Chloridazone and Table 2 summarizes the correlation coefficients, LODs and LOQs for both analytes.

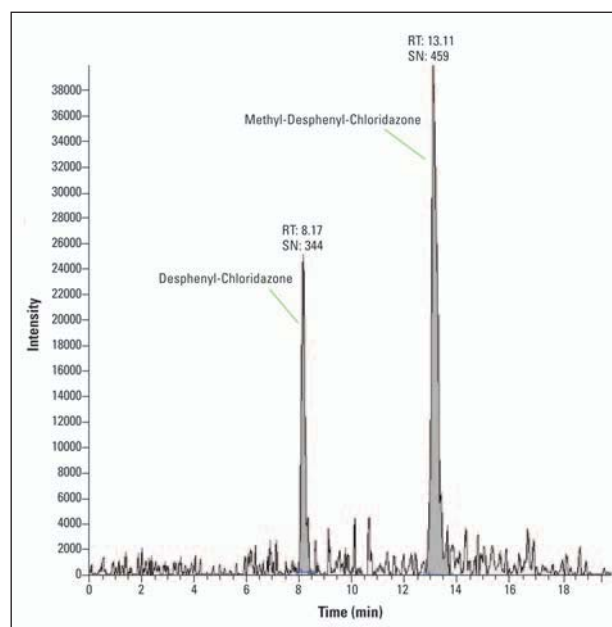


Figure 2: Chromatogram of a water sample spiked with a concentration of 10 ng/L each of Desphenyl-Chloridazone and Methyl-Desphenyl-Chloridazone

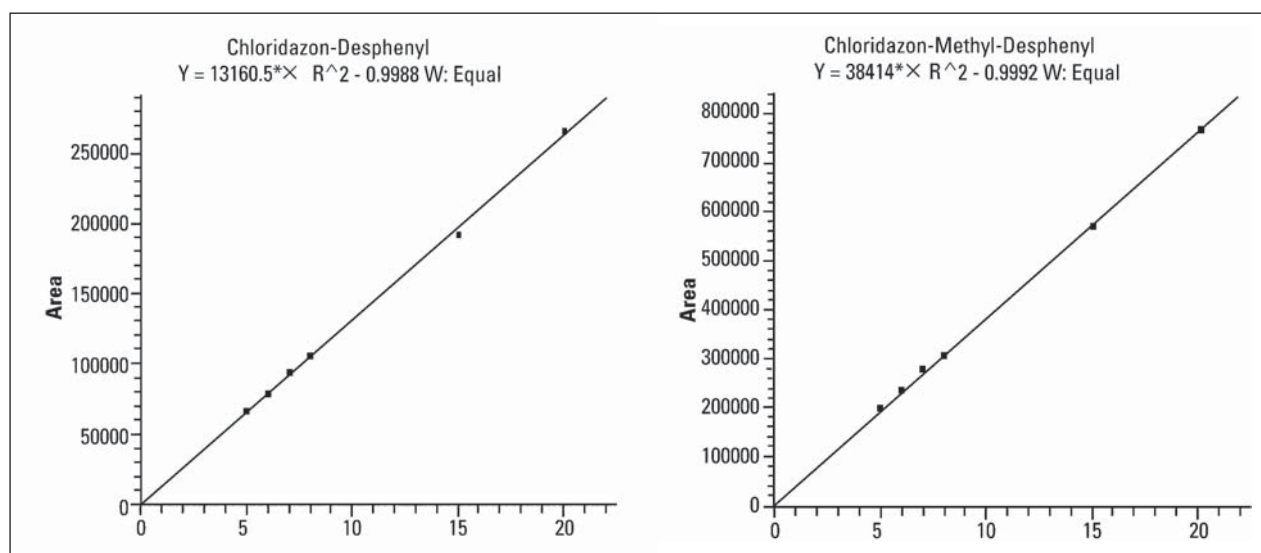


Figure 3: Calibration curves of Desphenyl-Chloridazone and Methyl-Desphenyl-Chloridazone

A concentration range from 5 to 20 ng/L was covered with pure standards, with focusing on the lower end of the range for quantitation in residue analysis from aqueous samples as specified in current legislation (Figure 3). A real sample containing 10 ng/L each of the two analytes shows peaks well suitable for quantitation which can be considered as limit of quantitation under such conditions. Less concentrated samples showed a limit of detection at less than 5 ng/L.

Analyte	Correlation Coefficient r^2	Limit of Detection (ng/L)	Limit of Quantitation (ng/L)
Chloridazone-desphenyl	0.9988	3.1	10
Chloridazone-methyl-desphenyl	0.9992	3.5	10

Table 2: LODs and LOQs for the two pesticide metabolites.

Conclusion

The online enrichment and analysis of highly polar compounds, especially polar metabolites of pesticides, is considered challenging due to insufficient trapping capabilities of available column materials. This application demonstrates that it is possible to build a system for online enrichment and automated LC-MS/MS analysis of polar metabolites of an important pesticide widely used in Europe.

A limit of quantitation of 10 ng/L, after pre-concentration from a sample volume of 1 mL, was demonstrated. Normally, sample volumes used for manual pre-concentration are much higher. The EQuan system provides capability to enlarge the sample volume up to 20 mL, thus opening the way to quantitation levels of 1 ng/L and below.