

# A Rapid Solution for Combined Qualitative and Quantitative Analysis of Known and Unknown Pesticides in Water

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## Overview

A very simple and easy to reproduce screening and quantification method of pesticides in surface water, ground water and drinking water is presented. All samples were analyzed using online solid phase extraction coupled to Exactive mass spectrometer. Acquired high-resolution accurate mass (HRAM) data was processed using new and user friendly ExactFinder™ software. The entire mixture of targeted pesticides were identified and a number of untargeted pesticides were found and confirmed by elemental composition. In the same workflow all samples were expressed to quantitative analysis.

## Introduction

The demand for quick and simple analysis of large numbers of samples in environmental analysis is growing year by year. While the limits of quantification requested by governmental authorities are lowered nearly on a yearly basis, the number of analytes of interest is growing exponentially. Mass spectrometric detection with HRAM technology using full scan experiments can deliver the ability to detect as many analytes as necessary in combination with screening for an unlimited number of compounds in an targeted as well as untargeted approach, using only one chromatographic run. A single stage benchtop Orbitrap mass analyzer in combination with online solid phase extraction and a novel software application for unified qualitative and quantitative data processing fulfills these demands with higher confidence and precision.

## Methods

### Sample preparation

A variety of water samples (including surface water, ground water and drinking water) was spiked with 20 Pesticides at different levels. A dilution series of the same pesticide mixture was provided in ultrapure water at six different levels for calculation of a calibration curve. All samples were injected onto an online-SPE system (Thermo Scientific Equan MAX) without further treatment. Injection volume for all samples was 1000µL.

### Mass spectrometry

All experiments were performed on a Thermo Scientific Exactive benchtop LC-MS powered by Orbitrap™ technology (Bremen, Germany) using a heated electrospray source (HESI-II). The mass spectrometer was operated in positive/negative switching mode with full-scan setting using resolving power of 50,000 at *m/z* 200 (FWHM).

## Results

The pesticide mixture consisted of very unpolar analytes together with very polar metabolites, representing the full range of polarity characteristics normally found in environmental analyses, apart from ionic compounds. To achieve a reliable extraction of all compounds in one run, two extraction columns with different polarity characteristics were coupled, placing a unpolar C18 column (Hypersil GOLD™ 20x2.1 mm, 12µm particle size) upstream of a very polar one (Hypercarb™ 10x2.1mm, 5µm particle size (both columns from Thermo Fisher Scientific). Elution of the trapped analytes and the transfer to the analytical column were carried out in backflush mode to prevent the unpolar compounds trapped on the C18 column from getting into contact with the Hypercarb material (see Fig. 1). The analysis was run using conditions described earlier<sup>1,2</sup> without doing any application specific tuning of the instrument.

FIGURE 1. Equan MAX scheme

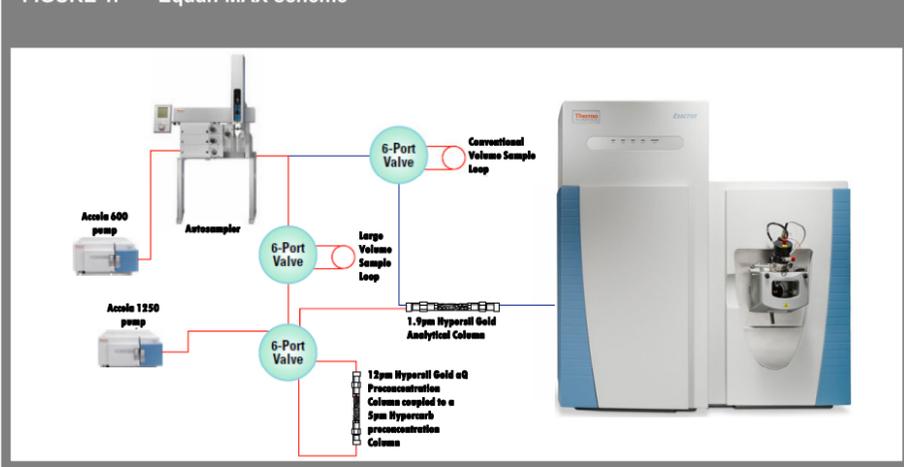
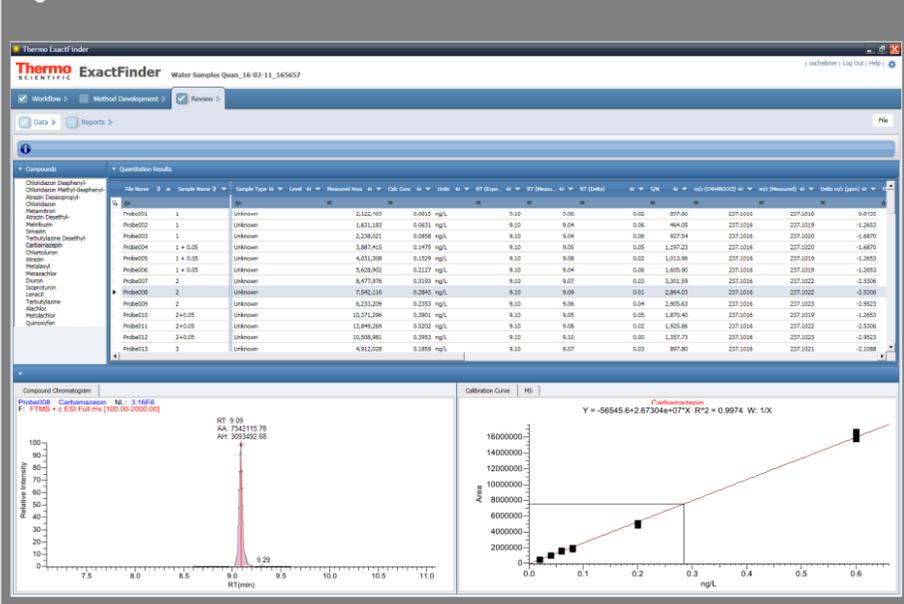


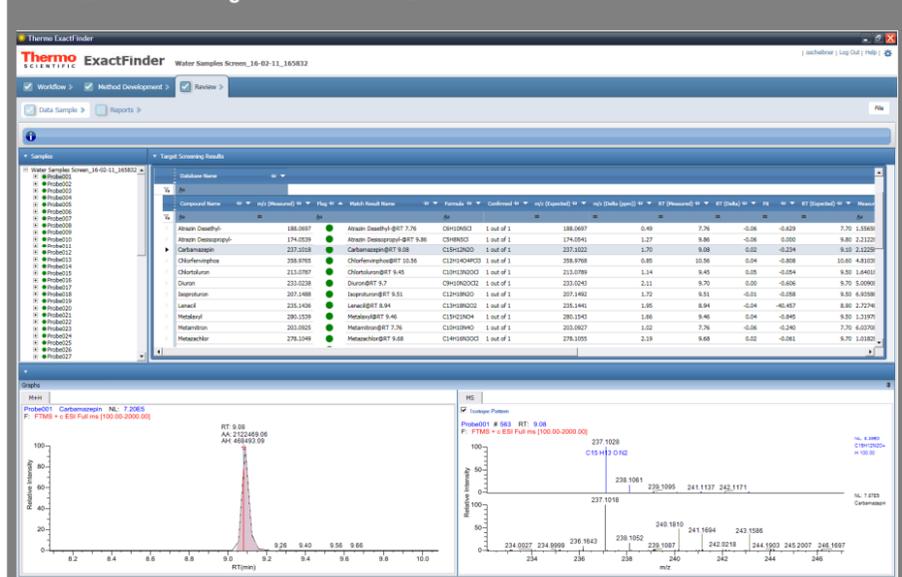
Figure 2. Quan result section of ExactFinder software



Data processing was carried out with Thermo Scientific ExactFinder software for qual/quan workflows.

All analytes gave very good linear response in the calibration range (0.02 to 0.6 µg/L) and didn't show any interference with other analytes or matrix components (see Fig. 2), the quantification data showed good reproducibility and good recovery rates, determined by standard addition to every sample.

FIGURE 3. Screening result section of ExactFinder software



Specificity of analysis was achieved by applying a mass window of 5ppm to the theoretical mass of the analytes.

In addition a targeted and untargeted screening was applied to all samples, using exact mass and retention time as identification criteria in the targeted screen. Confirmation of identity was achieved by automated matching of the given elemental composition with the isotopic pattern of the determined signal. Additional criteria, unused here, are occurrence of up to 5 fragment ions, library spectra match and internet database search via ChempSpider.

The remaining signals automatically occurred as unknowns, which again were screened against a larger compound list as untargeted screen. For all signals elemental compositions were calculated on base of the isotopic distribution after defining a list of elements to be used for this calculation.

The untargeted screen yielded additional compounds present in the samples. For most of the signals elemental compositions could be determined (see Fig.4).

All 20 analytes of interest could be easily quantified and were assigned as knowns in the automated screen. The unknown screen yielded additional identifications of analytes without additional analytical effort. Elemental compositions were assigned to most of the so far unknown signals, leading the path into a versatile and easy to do general unknown screening.

FIGURE 4. Unknown screening result table of ExactFinder software

## Conclusions

- The application of a combination of two different extraction columns proved to provide easy access to a wide range of environmental compounds in one general approach
- ExactFinder software provided easy access to full quantitative data
- Full qualitative data was provided from the same data set in one workflow, providing a wide range of confirmation tools for known analytes
- Additional unknown search led to the identification of a number of untargeted analytes and yielded a large number of unknown compounds, to which in most cases elemental compositions could be assigned

## References

- 1) Thermo Scientific Application Note: 54153
- 2) Thermo Scientific Application Note: 437

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