

A Rapid Solution for Screening and Quantitating Targeted and Non-Targeted Pesticides in Water using the Exactive Orbitrap LC/MS

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Introduction

Within the field of environmental analysis, the demand for quick and simple techniques to analyze large numbers of samples is growing each year. While the limits of quantitation (LOQs) required by governmental authorities are lowered almost yearly, the number of analytes of interest is growing exponentially. By using high-resolution, accurate mass (HRAM) liquid chromatography-mass spectrometry (LC-MS) (at least 50,000 resolution) and full-scan experiments, compound identification, screening and quantitation for an unlimited number of compounds in a targeted or non-targeted screening approach can be accomplished with only one chromatographic run.

A very simple, easy-to-reproduce screening and quantitation method to identify pesticides in surface water, ground water, and drinking water is presented here. All samples were analyzed by using online solid phase extraction (SPE) coupled to a Thermo Scientific Exactive high performance benchtop mass spectrometer. The acquired HRAM data was processed by using Thermo Scientific ExactFinder software for unified qualitative and quantitative data processing. All targeted pesticides in the entire mixture were identified, and a number of non-targeted pesticides were found and confirmed by elemental composition. In the same workflow, all samples underwent quantitative analysis.

Goal

To demonstrate a screening and quantitation method for pesticides in water developed for the Thermo Scientific EQUAN MAX system utilizing ExactFinder™ software to process the HRAM data.

Experimental

Sample Preparation

A variety of water samples, including surface water, ground water, and drinking water, were spiked with 20 pesticides (Table 1) at different levels. The pesticide mixture consisted of very nonpolar analytes together with very polar metabolites, representing the full range of polarity characteristics, apart from ionic compounds, normally found in environmental analyses. A dilution series of the same pesticide mixture was provided in ultrapure water at six different levels for calculation of a calibration curve.

HPLC

All samples were injected onto the EQUAN MAX automated high throughput LC-MS system without further treatment (Figure 1). The EQUAN MAX system offers online-SPE for preconcentration of samples up to 20 mL. By using the EQUAN MAX system, the analysis of compounds in the ng/L or even lower concentrations are possible, saving time and capital by automation of the extraction and preconcentration process. To achieve a reliable extraction of all nonpolar analytes and polar metabolites in one run, two extraction columns with different polarity characteristics were coupled. A nonpolar column with C18 selectivity (Thermo Scientific Hypersil GOLD 20 x 2.1 mm, 12 µm particle size) was placed upstream of a very polar column (Thermo Scientific Hypercarb 10 x 2.1 mm, 5 µm particle size). Elution of the trapped analytes and the transfer to the analytical column (Hypersil™ GOLD PFP 100 x 2.1 mm, 1.9 µm particle size) were carried out in backflush mode to prevent retention of the nonpolar compounds trapped on the C18 column through contact with the Hypercarb™ material. The injection volume for all samples was 1000 µL.

Table 1. Pesticides and their metabolites spiked into water samples

Compound Name	Elemental Composition
Alachlor	C ₁₄ H ₂₀ NO ₂ Cl
Atrazine	C ₈ H ₁₄ N ₅ Cl
Atrazine Desethyl-	C ₆ H ₁₀ N ₅ Cl
Atrazine Desisopropyl-	C ₅ H ₈ N ₅ Cl
Carbamazepine	C ₁₅ H ₁₂ N ₂ O
Chloridazon	C ₁₀ H ₈ N ₃ OCl
Chloridazon Desphenyl-	C ₄ H ₄ N ₃ OCl
Chloridazon Methyl-desphenyl-	C ₅ H ₆ N ₃ OCl
Chlortoluron	C ₁₀ H ₁₃ N ₂ OCl
Diuron	C ₉ H ₁₀ N ₂ OCl ₂
Isoproturon	C ₁₂ H ₁₈ N ₂ O
Lenacil	C ₁₃ H ₁₈ N ₂ O ₂
Metalaxyl	C ₁₅ H ₂₁ NO ₄
Metamitron	C ₁₀ H ₁₀ N ₄ O
Metazachlor	C ₁₄ H ₁₈ N ₃ OCl
Metolachlor	C ₁₅ H ₂₂ NO ₂ Cl
Metribuzin	C ₈ H ₁₄ N ₄ OS
Quinoxifen	C ₁₅ H ₈ NOCl ₂ F
Simazine	C ₇ H ₁₂ N ₅ Cl
Terbuthylazine	C ₉ H ₁₆ N ₅ Cl

Key Words

- EQUAN MAX
- Exactive
- ExactFinder
- Pesticide screening
- Water analysis



Figure 1. EQuan MAX system equipped with the Exactive mass spectrometer and ExactFinder software

Mass Spectrometry

All experiments were performed on an Exactive™ benchtop LC-MS powered by Thermo Scientific Orbitrap technology using a heated electrospray ionization source (HESI-II). The mass spectrometer was operated in positive/negative switching mode with a full-scan setting.

MS parameter settings:

Spray voltage:	4100 V in positive mode and 3100 V in negative mode
Sheath gas pressure (N ₂):	30 (arbitrary units)
Auxiliary gas pressure (N ₂):	5 (arbitrary units)
Capillary temperature:	250 °C
Heater temperature (HESI-II):	300 °C
Resolution:	50,000 (FWHM at <i>m/z</i> 200)
Acquisition time:	20.00 min
Polarity switching:	One full cycle in less than 1 sec

The analysis was run using conditions described earlier^{1,2} without doing any application-specific tuning of the instrument. Quantitative and qualitative data were collected in the same run and data file.

Results and Discussion

Data processing was carried out with ExactFinder software for qualitative and quantitative workflows. All analytes gave very good linear response in the calibration range (0.02 to 0.60 µg/L) and did not show any interference with other analytes or matrix components (Figure 2). The quantitation data showed good

reproducibility and good recovery rates, as determined by the addition of internal standard to every sample. The specificity of analysis was achieved by applying a mass window of 5 ppm to the theoretical mass of the analytes.

In addition, both targeted and non-targeted screening processes were applied to all samples. Exact mass and retention time were used as identification criteria in the targeted screen (Figure 3). Confirmation of identity was achieved by automated matching of the given elemental composition with the isotopic pattern of the determined signal. An example of isotopic pattern matching is given in Figure 4. ExactFinder software can also provide compound identification through the following criteria: occurrence of up to five fragment ions, library spectra match, and internet database search via ChemSpider®.

The remaining peaks were also screened against a larger compound list. For all signals, elemental compositions were calculated based on the isotopic distribution of a pre-defined list of elements.

The non-targeted screening yielded additional compounds present in the samples. For example, in addition to the targeted compounds, we found the presence of carbendazim in some of the samples and thiometoxam in one. For most of the signals, elemental compositions were determined. All 20 analytes of interest were easily quantified and assigned as knowns in the automated screen. The non-targeted screening yielded additional identifications of analytes without additional analytical effort.

To ensure maximum detection of all possible ions from the samples analyzed, the Exactive mass spectrometer was operated in positive/negative switching mode. This did not affect the mass accuracy or sensitivity of the system at

any time. The same results were achieved by performing the analysis in separate runs with the mass spectrometer operating in positive mode for one run and in negative mode for the other.

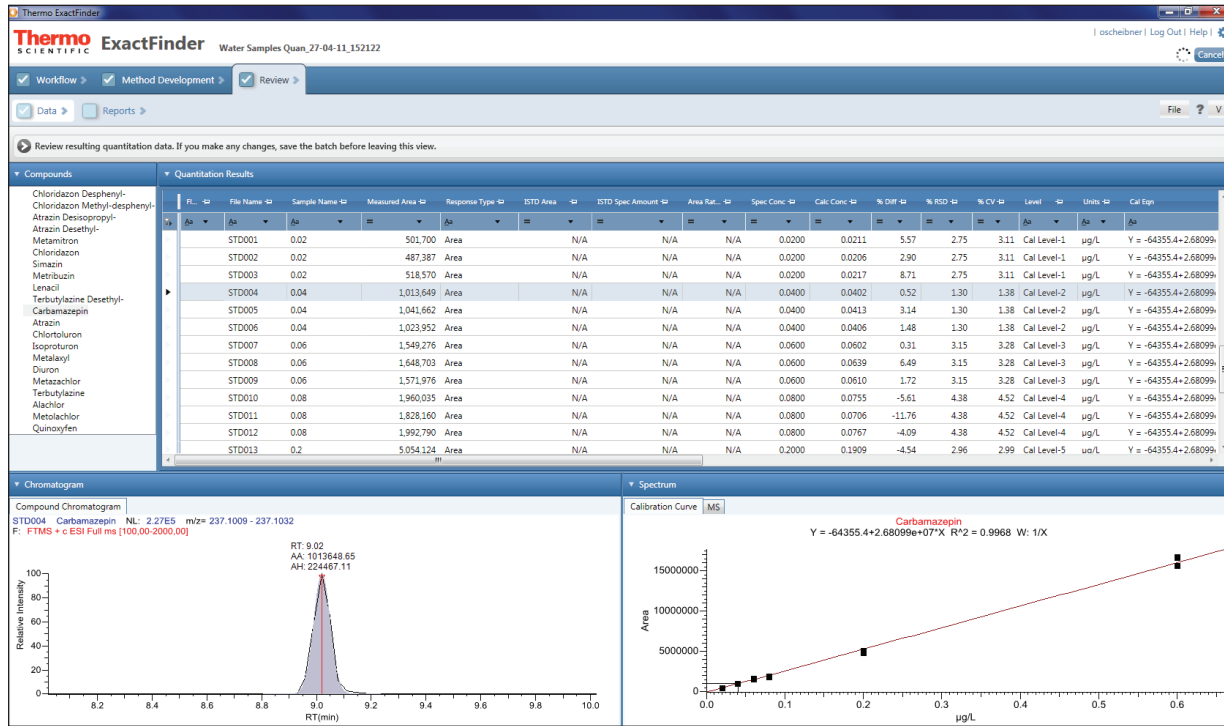


Figure 2. Quantitation Results section of ExactFinder software

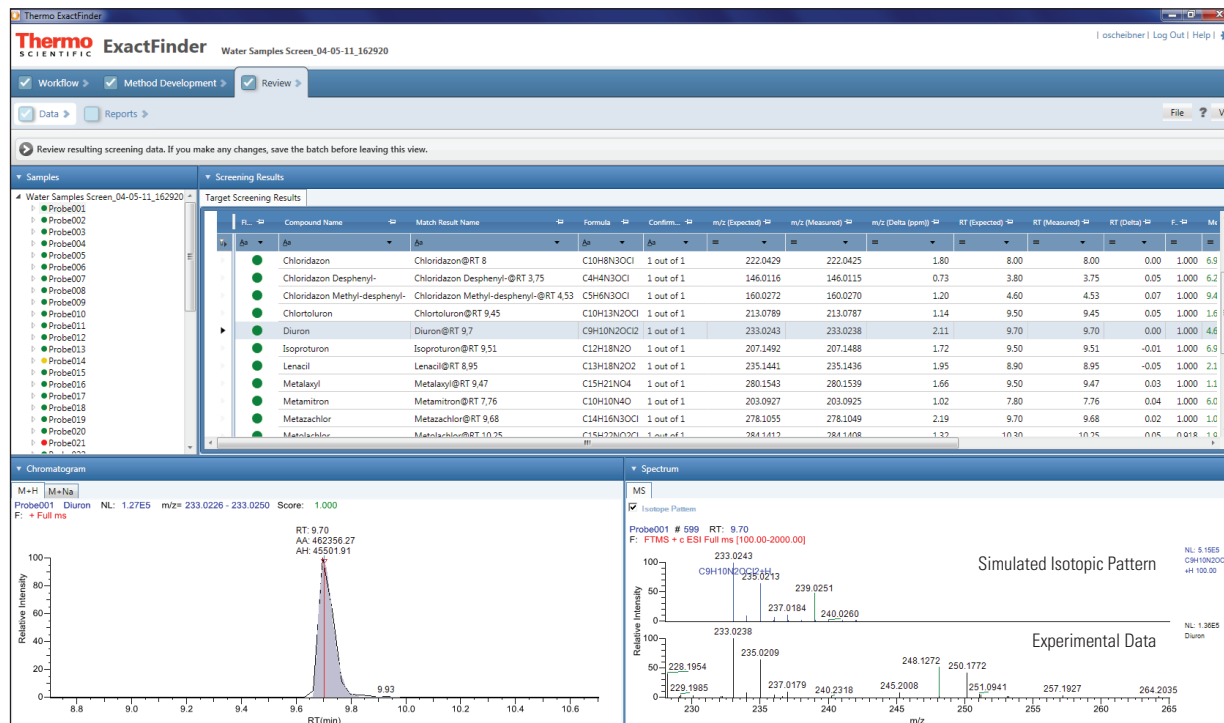


Figure 3. Target Screening Results section of ExactFinder software

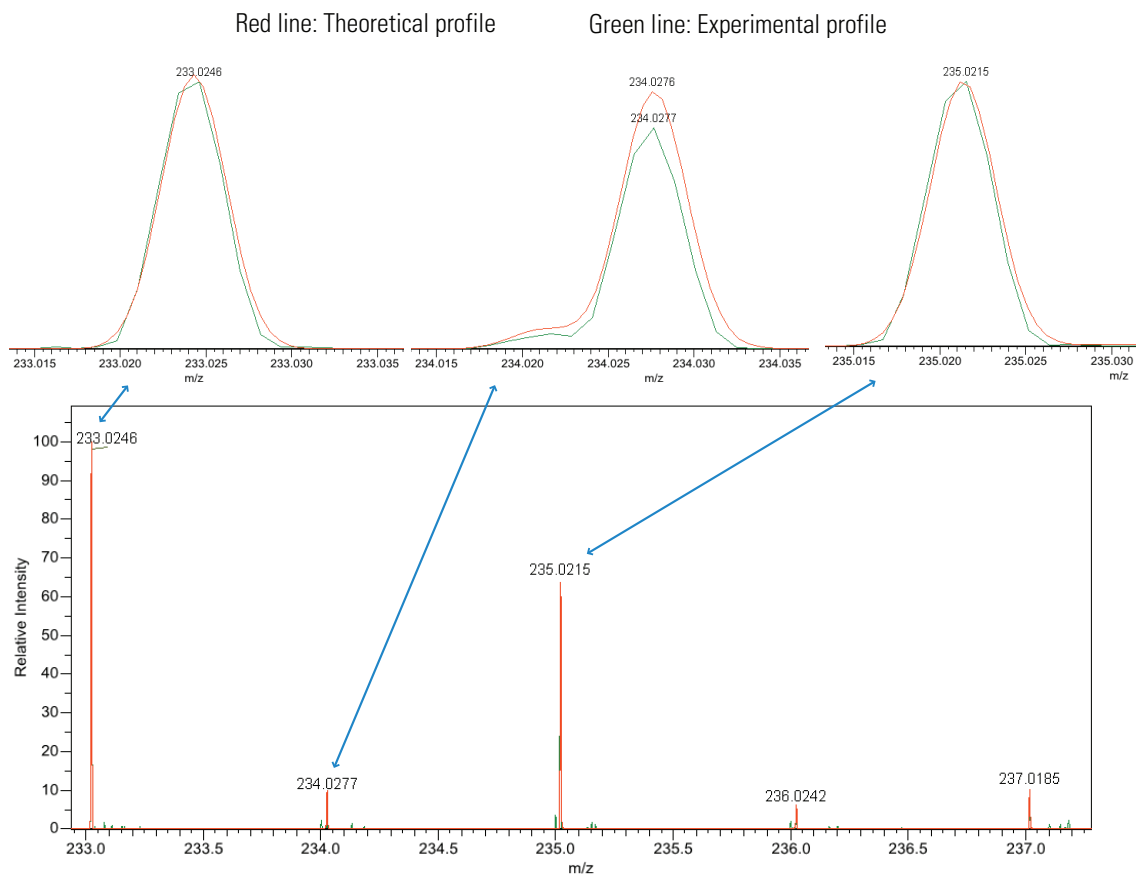


Figure 4. Isotopic pattern matching example

Conclusion

In this screening and quantitation method to identify pesticides in water, the combination of two different extraction columns yielded easy access to a wide range of environmental compounds in one general approach. ExactFinder software provided a single streamlined workflow with high productivity and confidence required for targeted and non-targeted screening experiments. Full qualitative data was attained from the same data set in one workflow, and a wide range of confirmation tools for known analytes were available. An additional search led to the identification of a number of non-targeted analytes and yielded a large number of compounds, to which elemental compositions can be assigned in most cases. Lastly, acquiring the data at 50,000 resolution reduces the likelihood of coeluting isobaric interferences and thus diminishes the likelihood of false positives.

References

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