Application Note: 478

Analysis of Triazine Herbicides in Drinking Water Using LC-MS/MS and TraceFinder Software

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Introduction

 TSQ Quantum Access MAX

Key Words

- TraceFinder software
- Water analysis
- Environmental

Thermo Scientific TraceFinder software includes built-in workflows for streamlining routine analyses in environmental and food safety laboratories. By incorporating a database of liquid chromatography-mass spectrometry (LC/MS) methods that can be customized to include unique compounds, TraceFinder[™] allows the analyst to access commonly encountered contaminants found in the environment. To demonstrate the capabilities of this software, a mixture of triazine compounds spiked into drinking water samples was analyzed. Using direct injections of 20 mL samples (with on-line preconcentration), low- and sub-pg/mL (ppt) levels were detected. The ability to analyze these drinking water samples with on-line preconcentration saves considerable time and expense compared to solid phase extraction techniques.

Goal

To demonstrate the ease-of-use of TraceFinder software for the analysis of triazine herbicides in water samples.

Experimental Conditions

Sample Preparation

Water with 0.1% formic acid was spiked with a mixture of triazines ranging from 0.1 pg/mL to 10.0 pg/mL. The following triazines were used: ametryn, atraton, atrazine, prometon, prometryn, propazine, secbumeton, simazine, simetryn, terbutryn, and terbuthylazine (Ultra Scientific, North Kingstown, RI).

HPLC

HPLC analysis was performed using the Thermo Scientific Surveyor Plus LC pump for loading the samples and a Thermo Scientific Accela UHPLC pump for the elution of the compounds. The autosampler was an HTC-Pal Autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a 20 mL loop.

Sequential 5 mL syringe fills were used to load the 20 mL loop in 4 steps by using a custom CTC macro. Using the Thermo Scientific Equan online sample enrichment system, 20 mL samples of spiked water, commercial bottled water, diet soda, and blanks (reagent water) were injected directly onto a loading column (Thermo Scientific Hypersil GOLD 20 × 2.1 mm, 12 µm).

After an appropriate time, depending on the volume injected, a multi-port valve was switched to enable the loading column to be back-flushed onto the analytical column (Hypersil GOLD^m 50 × 2.1 mm, 3 µm), where the compounds were separated prior to introduction into a triple stage quadrupole mass spectrometer. After all of the compounds were eluted, the valve was switched back to the starting position. The loading column and the analytical column were cleaned with a high organic mobile phase and equilibrated.

MS

MS analysis was carried out on a Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) source. The MS conditions were as follows:

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Software

Data collection and processing was handled by TraceFinder software. TraceFinder includes methods applicable to the environmental and food safety markets, as well as a comprehensive Compound Datastore (CDS). The CDS includes selective reaction monitoring (SRM) transitions and collision energies for several hundred pesticides, herbicides, personal care products, and pharmaceutical compounds that are of interest to the environmental and food safety fields. A user can select one of the included methods in TraceFinder, or quickly develop new or modified methods by using the preexisting SRM transition information in the CDS, thus eliminating time-consuming compound optimizations.



Results and Discussion

The analyst can select in which area to begin working (Figure 1). In this application note, the entire process will be illustrated, from method development to reporting. peak detection settings are defined in the Method Development section. Results can be flagged based on user-defined criteria. For example, the user can set a flag for a compound whose calculated concentration is beyond



Figure 1. TraceFinder Welcome screen

Method Development

The Method Development section of the software allows the user to select the compounds that will be analyzed in the method. In this experiment, the appropriate SRM transitions for the triazine mixture were chosen from the CDS and inserted into the method for detection (Figure 2). No compound optimization is necessary for compounds already in the data store.

Additionally, the calibration standards, QC levels, and

the upper limit of linearity, above a defined reporting limit, or below a limit of detection. This allows for faster data review after collection, and quick identification of positive samples. Full support for qualifier SRM ion ratios is also included but was not used in this experiment.

Acquisition

The Acquisition section provides a step-by-step process to acquire data. The progress is followed in an overview section on the left side of the screen (Figure 3). A green checkbox indicates that the step has been completed and there are no errors. The steps include template selection (pre-defined sample lists, which are helpful in routine analysis), method selection, sample list

definition, report selection, and instrument status. Figure 3 shows calibrators, blanks, replicate "unknowns" of a 1 pg/mL sample, and drinking water samples for this experiment.

A final status page summarizes the method and all of the samples to be run and gives an overall summary of the status of the instrument (Figure 4). Three color-coded dots are shown: green indicates an 'ok' status; yellow indicates the instrument module is in standby; and red indicates the

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Figure 2. Master Method View, showing the triazine compounds that will be monitored in this method.

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Figure 3. Acquisition section with the sample list being defined. The red box at left outlines the overall progress.

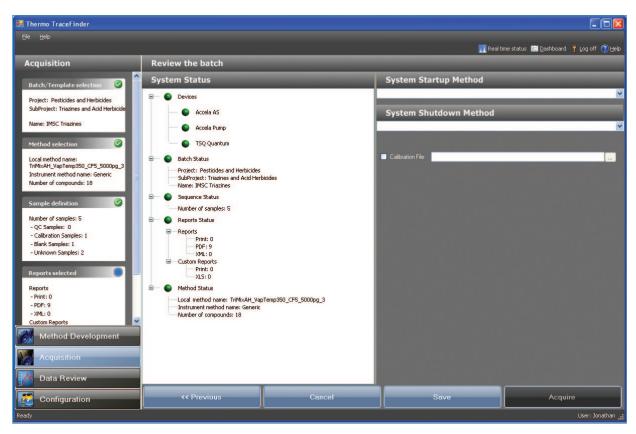


Figure 4. Acquisition status section. This is the final view before submitting a batch for analysis, providing the user instant instrument and method feedback.

instrument module is either off or disconnected. From the final status page, the batch can be acquired or saved to be run at a later date. A previously saved calibration curve can be used, so that a calibration need not be run every day. For example, the save function can be used to prepare for future batches in advance of sample preparation. When the samples are ready to be run, the previously saved batch is loaded and acquisition is begun.

Data Review

The targeted analysis of triazine compounds in drinking water samples was reviewed in the Data Review section of TraceFinder. In this section, calibration lines, ion ratios, peak integration, and mass spectra (if applicable) can be monitored. In addition, the Data Review section can flag samples that meet certain user-set criteria. For example, a limit can be set on the R² value of a calibration line. A green flag means that all user-set criteria have been met, while a red flag indicates that the sample exceeds or fails some user-set criteria and a yellow flag indicates that the compound was not found in the sample. Flags can also be used to highlight "positive" or "negative" hits in a sample. Figure 5 illustrates the red flags indicating the absence of peaks in blank samples for the compound simazine at its lowest calibration level, 100 fg/mL. In addition, flags can be set to alert for the presence of carryover in blank samples. In this study, 20 mL injections of the calibration standards, even at the highest level, resulted in no detectable carryover.

The Data Review pane allows user adjustments, such as peak reintegration. The effects of the changes on the results are instantly updated in the results grid. Excellent linearity was observed for all analytes, with R² values ranging from 0.9921 for atrazine to 0.9995 for propazine and terbuthlazine (co-eluting isomers, summed together for this analysis).

As mentioned previously, no carryover was observed in the blank samples, which illustrates the ability to use a single loading column for multiple analyses of drinking water samples. No triazines were detected in the soda sample, but one of the commercial drinking water samples tested positive for atrazine. The concentration of atrazine in the sample was calculated to be 0.24 pg/mL, well below the regulatory levels in the United States and Europe. However, using standard injection techniques without sample preconcentration, it is unlikely that this amount of atrazine would be detected in a typical LC-MS/MS analysis of triazines.

In addition to 20 mL injections, 1 mL and 5 mL injections were analyzed in a separate experiment. The %RSDs for replicate injections, without internal standards, at 20 mL are shown with all of the compounds in Table 1.



Figure 5. Data Review section. The red flags for blank samples indicate that peaks were not found in these samples.

Table 1. Reproducibility and peak area enhancement for 1, 5, and 20 mL injections for the mixture of triazines at the 1 pg/mL level (n=20).

Compound	Area, 1 mL	Area, 5 mL	Area, 20 mL	Factor 1 mL to 5 mL	Factor 5 mL to 20 mL	%RSD (n = 8)
Atraton	ND	1.16E+07	5.42E+07	N/A	4.69	11.15
Simetryn	ND	4.27E+06	1.94E+07	N/A	4.56	8.93
Prometon/Secbumeton	3.26E+06	1.07E+07	4.80E+07	3.30	4.47	9.89
Ametryn	4.34E+06	1.42E+07	5.99E+07	3.27	4.22	11.59
Simazine	3.18E+05	1.28E+06	5.70E+06	4.03	4.44	5.32
Prometryn/Terbutryn	6.19E+06	1.89E+07	7.61E+07	3.05	4.02	3.99
Atrazine	1.26E+06	4.45E+06	1.55E+07	3.53	3.49	4.97

Reporting

A large number of customizable report templates are included in TraceFinder. The user has the option of creating PDF reports, printing reports directly to the printer, or saving reports in an XML format, which is useful for LIMS systems. In each method, the user can decide which reports are most applicable to a given method. In this manner, a supervisor or lab director can set up methods and reports, lock the method, and make it non-editable by technicians. In this way, the integrity of a method is preserved, which is especially useful in controlled environments.

An example of one of the reports generated by TraceFinder is shown in Figure 6. This view shows the onscreen preview function available in TraceFinder. The chromatogram shown is for a 1 pg/mL "unknown" spiked water sample. The quantitated results follow beneath the chromatogram. At the very top of the page is a sample summary. TraceFinder can generate results for the entire batch with the click of a button, or the user can choose to view reports individually and print only those of interest.

Conclusion

In this application note, TraceFinder software was used in conjunction with an online preconcentration system, Equan[™], for the robust and reproducible analysis of large volumes of drinking water. Triazines were quantitated at the sub-ppt level, and several commercial bottled drinking water samples and one sugar-free soda sample were analyzed for the presence of triazines. Only one sample contained any traces of triazines: a commercial drinking water sample tested positive for atrazine. TraceFinder can also be used for traditional LC/MS applications, minimizing method development time. The method development capabilities and Compound Datastore of TraceFinder allowed for the quick creation of a method for the analysis of these compounds.

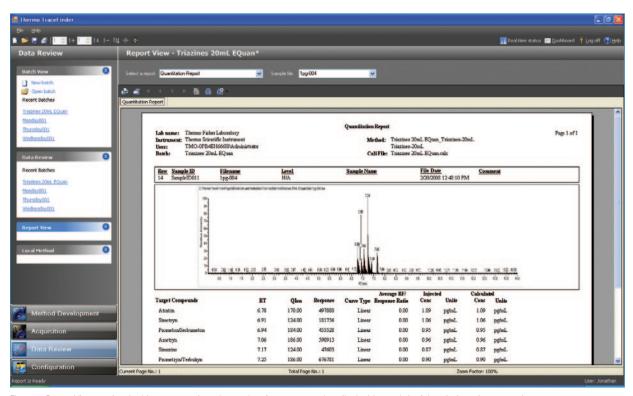


Figure 6. Report View section. In this report preview, the results of a water sample spiked with 1 pg/mL of the triazine mixture are shown.

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