

LC-MS/MS Analysis of Triazine Pesticides in Drinking Water using a new software for streamlined method development

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Introduction

A software program, Thermo Scientific TraceFinder has been developed with built-in workflows for streamlining routine analysis in environmental and food safety labs. Incorporating an LC/MS methods database that can be customized by the user to include unique compounds, the analyst is able to access commonly-encountered contaminants found in the environment. To demonstrate the software capabilities, we analyzed a mixture of triazine compounds spiked into drinking water samples. 20mL direct injections (with online preconcentration) of the triazine samples were analyzed, allowing for the detection of low and sub pg/mL (ppt) level. The ability to analyze these drinking water samples with preconcentration saves considerable time and expense. These savings result from not using Solid Phase Extraction cartridges and the labor costs by eliminating sample preparation time.

Methods

Samples and LC-MS/MS

Water with 0.1% Formic Acid was spiked with a mixture of triazines ranging from 0.1 to 10.0 pg/mL. The following triazines were used: Atraton, Prometon, Secbumeton, Simetryn, Ametryn, Prometryn, Terbutryn, Simazine, Atrazine, Propazine, Terbutylazine (Ultra Scientific, North Kingstown, RI, USA). Using the Thermo Scientific EQuan environmental quantitation solution, 20mL of spiked water, commercial bottled water, diet soda and blank samples (reagent water) were injected directly onto a loading column (Thermo Scientific Hypersil GOLD 20X2.1mm 12 μ). After an appropriate time, depending on the volume injected, a multi-port valve was switched to enable the load column to be back flushed onto the analytical column (Hypersil™ GOLD 50X2.1mm 3 μ), where the compounds are separated prior to introduction into a triple stage quadrupole mass spectrometer. After all of the compounds are eluted, the valve was switched back to the starting position. The loading column was cleaned with a high organic phase and equilibrated, as is the analytical column. HPLC systems included a Thermo Scientific Surveyor Plus LC pump for loading the 20 mL sample, and a Thermo Scientific Accela U-HPLC pump for elution of the compounds. The autosampler was a HTC-Pal Autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a 20mL loop. Sequential 5 mL syringe fills were used to load the 20 mL loop in 4 steps by using a custom CTC Macro.

A Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer was used for the analysis of the triazines. The instrumental conditions are listed here:

Ion Source Polarity:	Positive ion mode, Electrospray
Spray Voltage:	4000 V
Ion Transfer Tube Temperature:	300 °C
Sheath Gas Pressure:	30 arbitrary units, Nitrogen
Auxiliary Gas Pressure:	5 arbitrary units
Collision Gas (Ar):	1.5 mTorr
Q1/Q3 Peak Resolution:	0.7 Da
Scan Width:	0.002 Da

Software

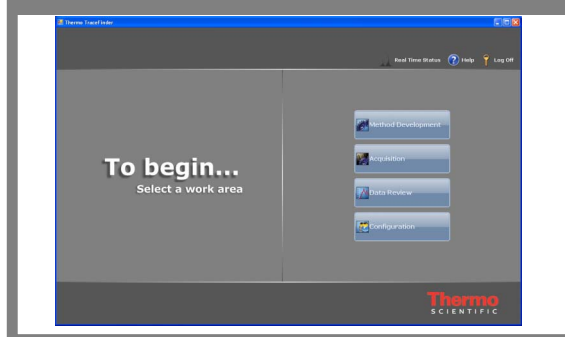
Data collection and processing was handled by TraceFinder™ Environmental and Food Safety software. TraceFinder includes several methods applicable to the Environmental and Food Safety markets, as well as a comprehensive Compound Data Store (CDS). The CDS includes SRM transitions and collision energies for several hundred pesticides, herbicides, personal care products, and pharmaceutical compounds that are of interest to the Environmental and Food safety field. A user may use one of the included methods in TraceFinder, or by using the CDS, quickly develop new or modified methods using the pre-existing SRM transition information eliminating time consuming compound optimizations.

The goal of this poster presentation is to demonstrate TraceFinder's ease-of-use for the analysis of triazine herbicides in water samples.

Results

To begin, the analyst chooses the area they would like to begin working (Figure 1). In this poster, the entire process will be illustrated, from Method Development to Reporting.

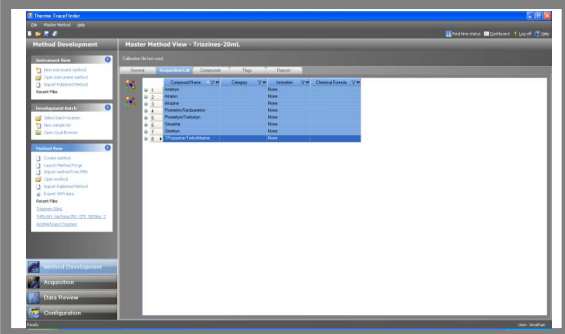
FIGURE 1. TraceFinder Welcome Screen.



Method Development

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

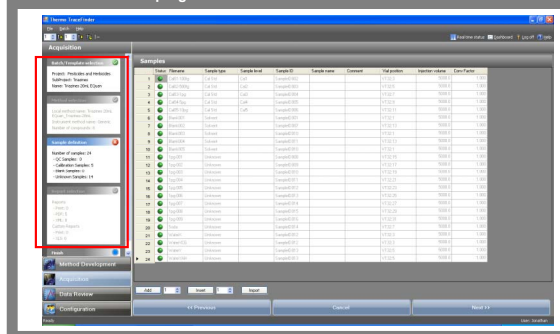
FIGURE 2. Compound Data Store showing the triazine compounds that will be monitored in this method.



Additionally, in the method development section, calibration and QC levels are defined, as well as peak detection settings. Additionally, the user has the option of defining flags, where the results are flagged based on different criteria. For example, the user may set a flag for a compound whose calculated concentration is beyond the upper limit of linearity, above a defined reporting limit, or below a limit of detection. This allows for faster reviewing of data after collection. Positive samples are quickly identified, saving time. Full support for ion ratios is also included, but was not used in this experiment.

Acquisition

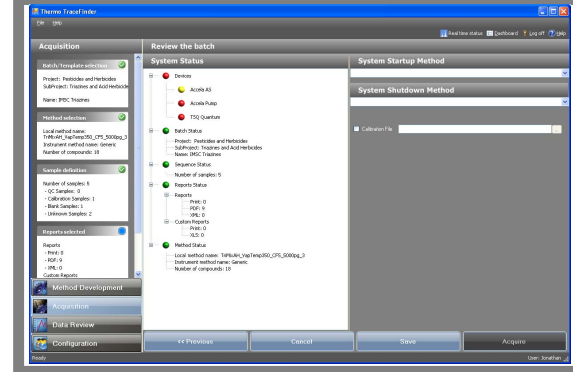
FIGURE 3. Acquisition section with the sample list being defined. Red box outlines the overall progress.



The Acquisition section provides a step-by-step process to begin data acquisition. The overall progress is followed in an overview section on the left-hand side of the screen, see red outline in Figure 3. The presence of a green checkbox notes that this step has been completed and that there are no errors. The steps include template selection (pre defined sample lists, helpful in routine analysis), method selection, sample list definition, report selection, and instrument status. In this experiment, calibrators, blanks replicate "unknowns" of a 1 pg/mL sample, and drinking water samples are shown in Figure 3.

The user is presented with a final status page (Figure 4) summarizing the method and all of the samples to be run, as well as giving an overall summary of the instrument status. In Figure 4, three (3) colored dots are shown: green indicating an 'ok' status, yellow indicating that the instrument module is in a standby condition, and red meaning that instrument module is either off or disconnected. From here, the batch can be acquired or saved to be run at a later date. The save function can be used to prepare for future batches in advance of sample preparation, for example. When the samples are ready to be run, the user, or another user, simply loads this previously saved batch and begins the acquisition.

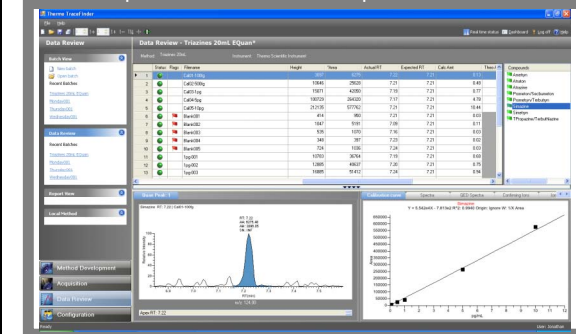
FIGURE 4. TraceFinder Acquisition status page. This is the final view before submitting a batch for analysis, providing the user instant instrument and method feedback.



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 MS Spectra (if applicable) can all be monitored. In addition, the Data Review section flags samples that meet certain user set criteria. For example, a limit on a calibration line's R² value. A green flag means that all user criteria have been met, while a red or yellow flag indicates something is not meeting the user's criteria. Flags can also be used to highlight "positive" or "negative" hits in a sample. Figure 5 illustrates the red flags for the absence of peaks in blank samples, as they should be. Flags can be set to alert the presence of carryover in blank samples. In this study, 20mL injections of the calibration standards, even at the highest level, resulted in no detectable carryover. Figure 5 shows the compound Simazine at its lowest calibration level, 100 fg/mL.

FIGURE 5. TraceFinder's Data Review section. Red flags for blank samples indicate that peaks were not found in these samples.



The Data Review pane allows for user adjustments, such as peak re-integration. The effect 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 Terbutylazine (co-eluting isomers, summed together for this analysis).

As mentioned previously, no carryover was observed in the blank samples, illustrating the ability to use a single loading column for multiple analyses of drinking water samples. No triazines were detected in the soda sample. One of the commercial drinking water samples tested positive for the triazine atrazine. The concentration of atrazine in the drinking water sample was calculated at 240 fg/mL, well below the regulatory levels in the US and EU. However, using standard injection techniques without sample preconcentration, it is unlikely that this amount of atrazine would be detected in a LC-MS/MS analysis of triazines.

In addition to 20mL injections, 1 and 5mL injections were analyzed in a separate experiment. The %RSD for replicate injections, without internal standards, at 20mL are shown, with all of the compounds exhibiting RSDs less than 12%. The area increase for the different injection volumes are also shown in Figure 6.

FIGURE 6. Reproducibility and peak area enhancement for 1, 5, and 20mL injections for the mixture of triazines at the 1 pg/mL level.

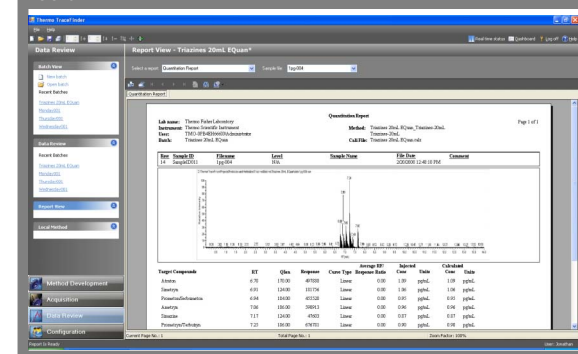
Compound	Area, 1 mL	Area, 5 mL	Area, 20 mL	Factor 1 mL to 5 mL	Factor 5 mL to 20 mL	%RSD (n = 6)
Atraton	ND	1.18E+07	5.42E+07	N/A	4.69	11.15
Simetryn	ND	4.27E+06	1.94E+07	N/A	4.56	8.93
Prometon/Secbumeton	3.25E+06	1.07E+07	4.85E+07	3.30	4.47	9.99
Ametryn	4.34E+06	1.42E+07	5.95E+07	3.27	4.22	11.59
Simazine	3.18E+05	1.28E+06	5.75E+06	4.03	4.44	5.22
Prometryn/Terbutryn	6.19E+06	1.89E+07	7.61E+07	3.05	4.02	3.99
Atrazine	1.26E+06	4.46E+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 them in an XML format, 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 setup methods and reports, lock the method, and make it non-editable by technicians. In this way, the integrity of a method is preserved, especially useful in controlled environments.

An example of one of the reports generated by TraceFinder is shown in Figure 7. This view shows the on-screen preview function of the report. This chromatogram is for a 1pg/mL "unknown" spiked water sample. The sample's chromatogram is at the top of the page, and the quantitated results follow beneath the chromatogram. At the very top of the page is a sample summary. TraceFinder can generate the entire batch's results with the click of a button, or the user can choose to view reports individually, printing only those of interest.

FIGURE 7. Screen capture of the report preview section of TraceFinder. In this report, the results of a water sample spiked with 1 pg/mL of the triazine mixture is shown.



Conclusions

A new software, TraceFinder, was used in conjunction with an online preconcentration setup, 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, that being the compound atrazine. The combination of TraceFinder's method development capabilities including compound data stores allowed for the quick creation of a method for the analysis of these compounds.

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