

# New Streamline Software for Screening to Determine 250 Pesticides in Orange Oil by LC-MS/MS

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## Introduction

A software program, TraceFinder (Thermo Scientific, San Jose, CA) has been developed with built-in workflows for routine analysis in environmental and food residue analysis. The software incorporates an LC-MS methods database that can be customized by the user to include unique compounds, in addition to preloaded contaminants commonly encountered in environmental and food safety samples. A NIST-format LC-MS/MS library of these commonly found contaminants helps confirm the compounds being analyzed. Data collection, analysis, and report generation can be performed using the same software program. To demonstrate capabilities of the software, we analyzed a mixture of 250 pesticides spiked into orange oil samples using both negative and positive ionization modes on a Thermo Scientific TSQ Vantage EMR mass spectrometer.

## Methods

Orange oil was spiked with a mixture of 250 pesticides to give a solution containing 1 and 10 ng/mL (ppb) of each pesticide. A 10  $\mu$ L sample of the spiked orange oil was injected directly onto the HPLC column. A simple gradient was used with a retention time of 18 minutes. Using Thermo Scientific TraceFinder software, we were able to use Timed SRMs (TSRMs) to create the instrument method and collect and process the data.

## Samples and LC-MS/MS

Samples were prepared by a modified QuEChERS procedure. Mixtures of 250 pesticides were prepared in acetonitrile at concentrations of 20 ng/mL and 200 ng/mL. For the 10 ng/mL experiment, a solvent standard was made by mixing 50  $\mu$ L of the 200 ng/mL pesticide mixture, 150  $\mu$ L of acetonitrile, and 800  $\mu$ L of buffer. The 10 ng/mL spiked orange oil sample was prepared by adding 50  $\mu$ L of the 200 ng/mL pesticide mixture, 50  $\mu$ L of acetonitrile, and 800  $\mu$ L of water to orange oil that had been extracted with 100  $\mu$ L of acetonitrile. The sample was filtered with a 0.2  $\mu$ m nylon membrane to remove any particulates. Similarly, for the 1 ng/mL experiment, the solvent standard was prepared by mixing 50  $\mu$ L of the 20 ng/mL pesticide mixture, 150  $\mu$ L of acetonitrile, and 800  $\mu$ L of buffer. The 1 ng/mL spiked orange oil sample was prepared by adding 50  $\mu$ L of the 200 ng/mL pesticide mixture, 50  $\mu$ L of acetonitrile, and 800  $\mu$ L of water to orange oil that has been extracted with 100  $\mu$ L of acetonitrile. The sample was filtered to remove any particulates.

Chromatographic analysis was performed using the Thermo Scientific Accela system with Thermo Scientific Hypersil GOLD PFP (100 x 2.1mm, 1.9 $\mu$ ) column with a 5 $\mu$ L injection size.

A TSQ Vantage™ EMR triple quadrupole mass spectrometer with HESI-II (Heated Electrospray Ionization) was used for the screening of 250 pesticides. The instrumental conditions are listed here:

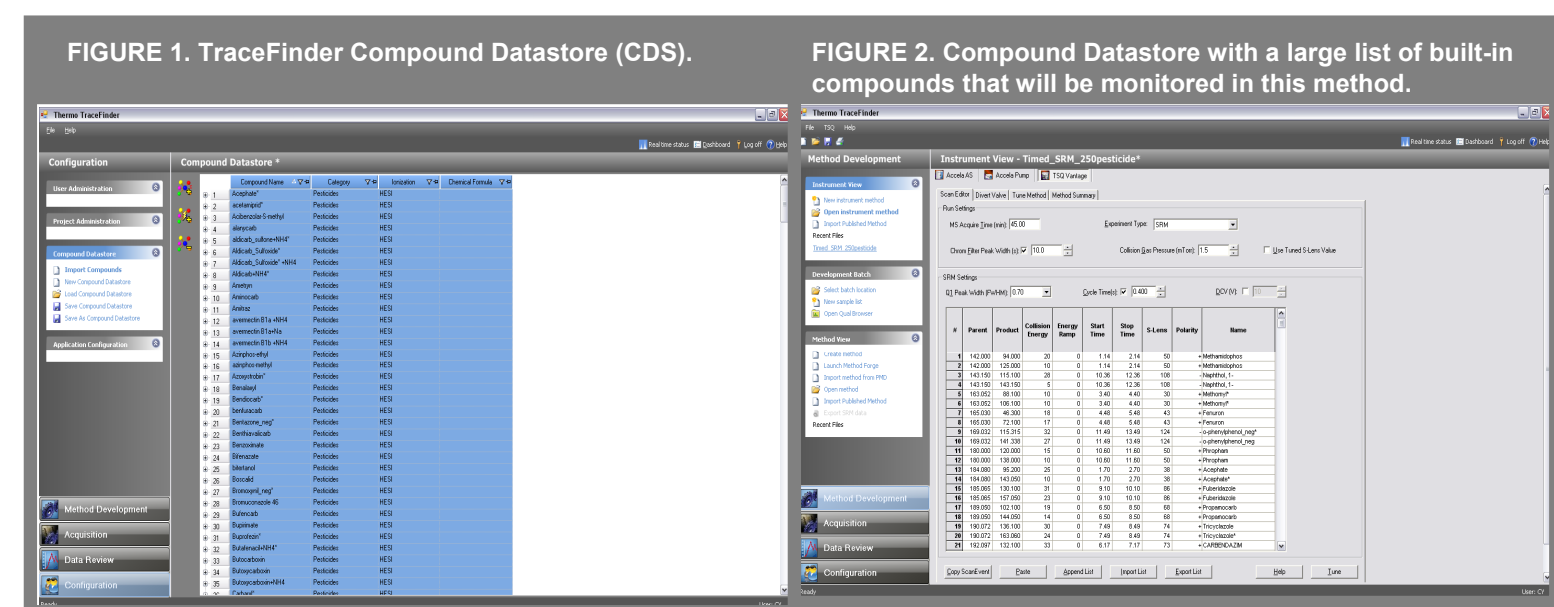
Ion Source Polarity: Positive and Negative ion mode, HESI  
Spray Voltage: 3500 V  
Ion Transfer Tube Temperature: 200 °C  
Vaporizer Temperature: 400°C  
Sheath Gas Pressure: 55 arbitrary units, Nitrogen  
Auxiliary Gas Pressure: 15 arbitrary units  
Ion Sweep Gas: 2.0 arbitrary units  
Collision Gas (Ar): 1.5 mTorr  
Q1/Q3 Peak Resolution: 0.7 Da  
Scan Width: 0.002 Da  
Cycle Time: 0.4s

## Software

Data collection and processing were 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 method development and analysis of 250 pesticides in orange oil.

## Results



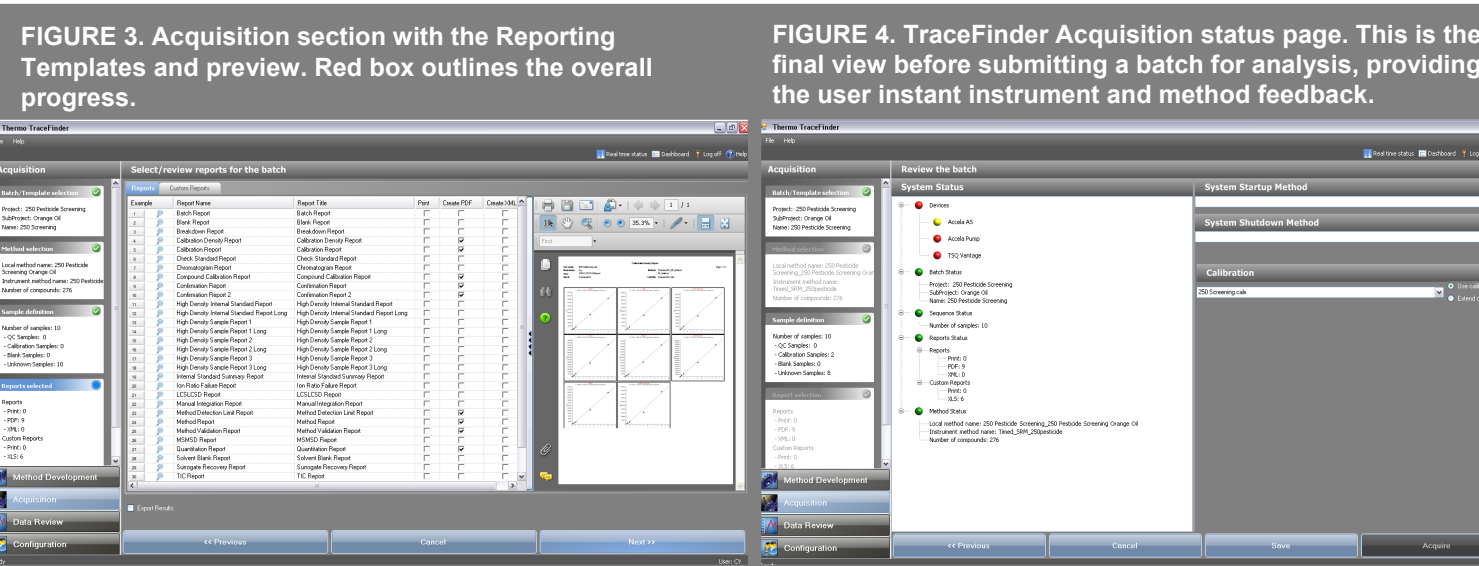
## Method Development

The flow of TraceFinder in the Method Development section allows the user to choose the compounds that will be analyzed in their method. In this experiment, the appropriate SRMs for the 250 pesticides are chosen from the CDS (Figure 1) and inserted into the instrument method for detection (Figure 2). No compound optimization is necessary for compounds already in the datastore.

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 as well as ion ratio. This allows for faster reviewing of data after collection. Positive samples are quickly identified, saving time.

## Acquisition

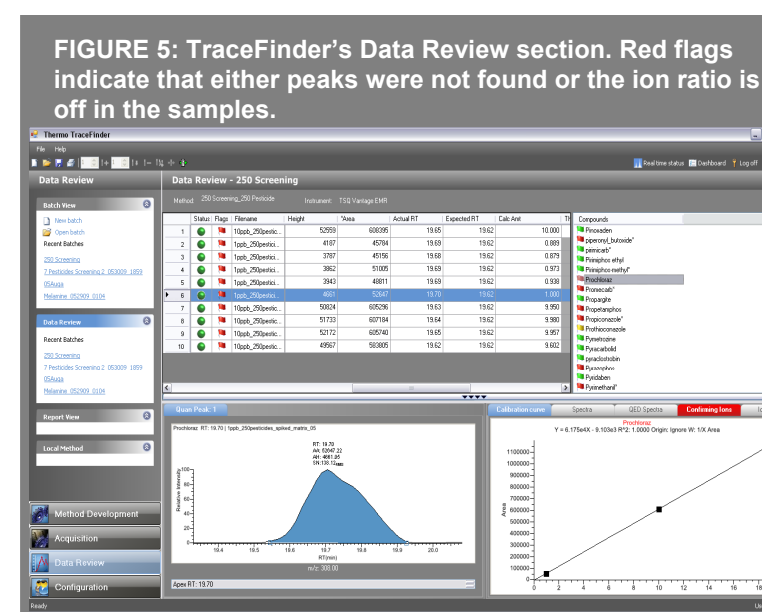
The Acquisition section provides a step-by-step process to begin data acquisition. The overall progress is followed in an overview section on the left-hand side of the screen (see red outline in Figure 3). The presence of a green checkbox notes that this step has been completed and that there are no errors. The steps include template selection (predefined sample lists, helpful in routine analysis), method selection, sample list definition, report selection, and instrument status. In the Reporting Section we are selecting and previewing the reports (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's status. In Figure 4, three 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. Also the user has the ability to choose a previous calibration curve that was run at the beginning of the week. So this way you don't have to run calibration every day but just put in a QC sample.

## Data Review

The targeted screening analysis of 250 pesticides in orange oil sample 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 viewed. In addition, the Data Review section flags samples that meet certain user-set criteria; for example, if the ion ratio is set with a tolerance. A green flag means that the user's 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 red flags for compounds that did not meet the Confirming ions (Ion Ratio), as they should be. Flags can also be set to alert for the presence of carryover in blank samples. In this experiment the two point calibration was sufficient enough to show the calculated amount of the different pesticides found in orange oil.

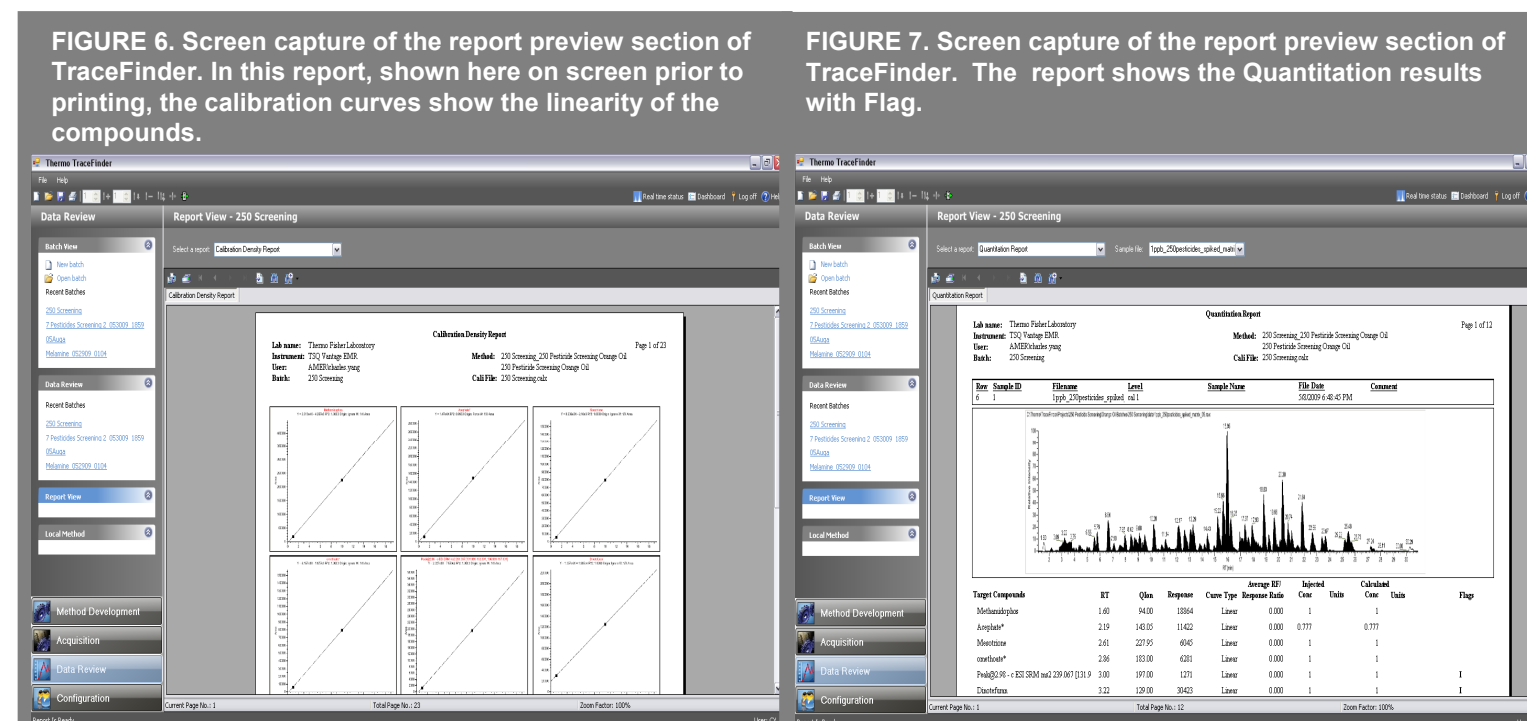


The Data Review pane allows user adjustments, such as peak re-integration. The effect of the changes on the results are instantly updated in the results grid. Excellent linearity was observed for all analytes, with R2 values 1.0000 with 1/X weighting.

## Reporting

A large number of 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 laboratory information management systems (LIMS). 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, especially useful in controlled environments.

Two examples of the reports generated by TraceFinder are shown in Figure 6 and Figure 7. This view shows the on-screen preview function available in TraceFinder. Figure 6 shows the calibration curve for each individual compound on one page while Figure 7 is a chromatogram for 1ppb level in orange oil. The sample's chromatogram is at the top of the page, and the quantified 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.



## Conclusions

A new software, TraceFinder, was used to simplify method development for the screening of 250 pesticides in orange oil. The results from this experiment showed positive confirmation of the pesticides by having set the tolerances in the method for quantitation and confirmation. The combination of TraceFinder's method development capabilities, including the Compound Datastore, allowed for the quick creation of a method for the analysis of these compounds.

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