LC/Q-TOF Workflows for Comprehensive Micropollutant Analysis

Targeted Quantification, Suspect Screening, and Unknown Compound Identification

Application Note

Environmental

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Abstract

This application note presents three complementary LC/Q-TOF workflows designed to provide comprehensive analysis of micropollutants in surface waters:

- Targeted quantification
- Suspect screening with and without MS/MS spectra
- Unknown compound identification

The first two workflows rely on the Agilent All Ions MS/MS accurate mass capabilities of the Agilent LC/Q-TOF system, Agilent MassHunter Qualitative Analysis software, and Agilent Personal Compound Database and Libraries (PCDLs) to detect and confirm compound identities, with or without reference standards. Agilent Molecular Structure Correlator (MSC) software is shown to aid in the identification of unknown compounds, in this case transformation products (TPs).

The targeted quantification workflow was validated using 32 reference standards. The workflow detected and quantified 25 compounds in at least one of the 51 surface water samples tested. The suspect screening workflow generated an expanded list of 85 possible pollutants, of which 73 were subsequently positively identified with an authentic standard (67 compounds) or by matching MS/MS spectra (six compounds). The unknown compound identification workflow identified five TPs that had not been identified using the targeted quantification or suspect screening workflows.

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Introduction

Comprehensive analysis of micropollutants in waste, surface, and drinking waters is necessary to confidently assess exposure and risk. Traditional targeted screening workflows, such as triple quadrupole MS methods, monitor and quantify a predefined list of compounds using analytical reference standards. However, a targeted approach will miss pollutants not on the target list, and may underestimate exposure when unexpected pollutants are present.

While triple quadrupole LC/MS systems are well suited for targeted analysis, their methods require standards that are not always easily obtained. In addition, they are not useful when trying to identify new or unknown compounds in the sample. High-resolution, accurate-mass (HRAM) quadrupole time-of-flight (Q-TOF) LC/MS analysis allows complementary suspect screening and unknown compound identification workflows, which, when used together with targeted quantification, provide a more complete picture of the chemical profile of the sample analyzed (Figure 1).

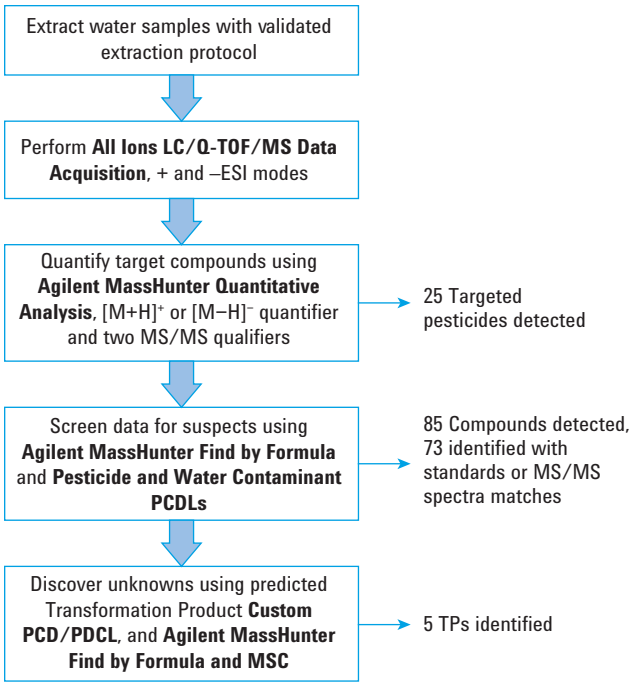

Figure 1. Complementary targeted quantification, suspect screening, and unknown compound identification LC/Q-TOF workflows for comprehensive micropollutant analysis.

For compounds not identified through targeted analysis, the suspect screening workflow, which uses Agilent All Ions MS/MS and Personal Compound Database and Libraries (PCDLs), allows accurate mass determination and MS/MS fragment confirmation of compounds. The screening workflow allows the analyst to presumptively identify compounds without analytical reference standards with a high degree of confidence. The unknown compound identification workflow attempts to identify compounds for which there are not MS/MS fragment entries in the selected PCDLs, nor are analytical standards readily available. In this workflow, Agilent Molecular Structure Correlator (MSC) software compares MS/MS fragments for compounds of interest to plausible candidate structures in additional databases such as Chemspider, Pubchem, or custom databases. MSC calculates scores based on the quality of the matches of the experimental fragments with those predicted.

This application note describes the use of targeted quantification, suspect screening, and unknown compound identification with LC/Q-TOF data acquisition and analysis workflows to achieve broader, more confident characterization of micropollutants, including transformation products (TPs), in surface water samples. The study *LC- and GC-QTOF-MS as Complementary Tools for a Comprehensive Micropollutant Analysis in Aquatic Systems* provides a detailed description of the analytical results and their implications for environmental monitoring [1]. Because not all of the compounds studied are amenable to LC/MS analysis, the research also describes a complementary GC/Q-TOF workflow that provides a comprehensive chemical profile of the sample.

Experimental

Target compounds and standards

For the targeted quantification workflow, 32 LC/MS-amenable pesticides were chosen for analysis of the surface water samples (Table 1). The target compounds were chosen to include compounds used in the area of the waters sampled, and to represent pesticides of different classes and physicochemical properties. Seventeen of the targets had better sensitivity in positive ESI mode, while 15 had better sensitivity in negative ESI mode. Eleven isotopically-labeled internal standards were used for accurate quantification.

For method validation and quality control analyses, reference standards for the target compounds were prespiked before extraction and post-spiked before injection. Procedural blanks were also used to look for blank contamination.

Table 1. Target Pesticides and Targeted Quantification Results

Target analyte	ESI mode	Matrix factor[1]	Method detection limit (ng/L)[2]	Absolute recovery (%)[3]	Accuracy (%)[4]	Precision (%)[5]
2,4-D	–	2.6	2.6	95 %	160 %	18 %
2-Phenylphenol	–	1.2	1.2	75 %	170 %	6 %
Azoxystrobin	+	1.7	0.2	95 %	128 %	1 %
Boscalid	–	1.6	0.3	97 %	111 %	3 %
Chlorantraniprole	+	2.7	2.7	95 %	87 %	3 %
Clomazone	+	2.7	1.3	76 %	190 %	1 %
Cyprodinil	+	3.1	0.3	91 %	118 %	3 %
DEET	+	2.2	0.2	76 %	78 %	4 %
Difenoconazole	+	1.7	0.9	95 %	104 %	1 %
Dimethoate	+	3.8	0.9	92 %	62 %	5 %
Diuron	–	1.7	0.2	92 %	102 %	1 %
Fipronil	–	1.2	0.1	97 %	96 %	6 %
Fipronil-desulfinyl	–	1.2	0.1	101 %	78 %	4 %
Fipronil-sulfide	–	1.1	0.1	97 %	74 %	18 %
Fipronil-sulfone	–	1.2	0.1	96 %	113 %	1 %
Hexazinon	+	3.0	0.3	91 %	117 %	3 %
Imidacloprid	–	4.2	2.1	93 %	152 %	5 %
MCPA	–	2.9	1.1	96 %	112 %	1 %
Methomyl	+	2.7	13	93 %	104 %	2 %
Methoxyfenozide	–	1.3	0.1	99 %	72 %	4 %
Metolachlor	+	1.7	0.2	80 %	108 %	2 %
Novaluron	–	1.2	0.6	74 %	91 %	2 %
Pendimethalin	+	1.8	1.8	71 %	74 %	3 %
Propanil	–	1.1	1.2	98 %	138 %	4 %
Propoxur	+	2.6	1.3	76 %	83 %	1 %
Pyriproxyfen	+	2.3	0.2	89 %	98 %	7 %
Simazine	+	7.2	1.8	89 %	77 %	3 %
Thiacloprid	+	4.1	1.0	93 %	97 %	6 %
Thiamethoxame	+	2.0	1.0	92 %	108 %	3 %
Thiobencarb	+	1.6	1.6	77 %	99 %	2 %
Triclocarban	–	1.4	0.1	92 %	97 %	1 %
Triclosan	–	1.2	1.3	89 %	89 %	2 %

Equation 1.
$$\text{Matrix factor} = \frac{\text{Area STD 100 ng/mL}}{\text{Area extract post spiked 100 ng/mL} - \text{Area extract unspiked}}$$

Equation 2.
$$\text{Method detection limit (ng/L)} = \frac{\text{Instrument detection limit (ng/mL)} \times \text{Matrix factor (–)}}{\text{Concentration factor (mL/L)}}$$

Equation 3.
$$\text{Absolute recovery (\%)} = \frac{\text{Area prespiked extract} - \text{Area unspiked extract}}{\text{Area post spiked extract} - \text{Area unspiked extract}}$$

Equation 4.
$$\text{Accuracy (\%)} = \frac{\text{Concentration prespiked extract} - \text{Concentration unspiked extract}}{\text{100 ng/mL}}$$

Equation 5.
$$\text{Precision (\%)} = \frac{\text{Standard deviation concentration prespiked extract (triplicate)}}{\text{Average concentration prespiked extract (triplicate)}}$$

Sample preparation

Fifty-one 1-L surface water samples collected from the Sacramento-San Joaquin River Delta in Northern California were extracted using a mixed-mode solid phase extraction (SPE) cartridge. The cartridges were eluted sequentially with 6 mL of 50/50 methanol/ethyl acetate with 0.5 % ammonia, 3 mL of methanol/ethyl acetate with 1.7 % formic acid, and 2 mL of methanol, per Moschet; *et al.* [2]. The eluent was evaporated to 0.2 mL and reconstituted to 1 mL with nanopure water to obtain a 20 %/80 % methanol/water ratio for injection into the LC/MS system.

LC/Q-TOF analysis

LC/MS analysis of the reference standards and sample extracts was performed using an Agilent 1260 Infinity LC coupled to an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS system equipped with an Agilent Jet Stream dual electrospray ionization (ESI) source. The HPLC system included a binary pump, Agilent 1260 Autosampler, and an Agilent ZORBAX Eclipse Plus C-18, 2.1 × 100 mm, 1.8 µm column (959758-902). Table 2 lists the LC parameters.

Table 2. LC Parameters. (+): Positive ESI Mode; (-): Negative ESI Mode

Parameter	Value
Liquid chromatograph	Agilent 1260 Infinity Binary LC
Analytical column	Agilent ZORBAX Eclipse Plus C-18, 2.1 × 100 mm, 1.8 µm
Injection volume	20 µL
Column temperature	30 °C
Mobile phase	A) Water + 0.1 % formic acid (+)/ Water + 1 mM ammonium fluoride (–) B) Acetonitrile + 0.1 % formic acid (+)/ Acetonitrile (–)
Flow rate	0.35 mL/min
Gradient	Time (min) %A 0.0 98 1.5 98 16.5 0 21.5 0
Equilibration time	3.0 minutes

The Q-TOF mass spectrometer was operated in both positive and negative ESI modes to maximize compound detection. Agilent MassHunter Workstation software was used to acquire data (version B.07.00). The All Ions MS/MS acquisition mode with collision energies (CEs) of 0, 10, 20, and 40 V was used to obtain both precursor and fragment ion data for all species. The All Ions MS/MS acquisition mode simultaneously collects high- and low-CE scans. The low CE scans allow the user to obtain precursor information, while the higher CE scans provide fragment information that enhances compound identification and confirmation when comparing experimental spectra to those in the PCDL. Table 3 lists the Q-TOF mass spectrometer parameters.

To facilitate identification of TPs not identified in the targeted quantification or suspect screen workflows, the samples with the highest abundances of plausible TPs were rerun in targeted MS/MS mode with the collision energy set to 20 V.

Table 3. Q-TOF Mass Spectrometer Parameters

Parameter	Value
Mass spectrometer	Agilent 6530 Accurate Mass Q-TOF-LC/MS with Agilent Jet Stream Technology
Ionization mode(s)	Positive (+) and negative (–) ESI
Instrument mode	2 GHz extended dynamic range
Mass range	50–1,050 *m/z*
Gas temperature	300 °C
Drying gas flow	12 L/min
Nebulizer	25 psig
Sheath gas temperature	350 °C
Sheath gas flow	11 L/min
Capillary voltage	3,500 V (+), 3,000 V (–)
Fragmentor voltage	110 V
Scan speed	4.0 spectra/sec
Collision energies	Agilent All Ions MS/MS: 0, 10, 20, 40 V Targeted MS/MS: 20 V
Reference ions*	(+) 121.0509 and 922.0098 (–) 112.9855 and 1033.9881

*The positive mass reference ions were not used in some instances because of interferences in some of the samples. The user can check for interferences and use alternate reference ions if required.

Data processing and analysis

Target compounds were quantified using Agilent MassHunter Quantitative Analysis software (version B.07.00). The $[M+H]^+$ or $[M-H]^-$ ion within the exact mass window of ±10 ppm was used as the quantifier ion. The two most abundant unique MS/MS fragments for each compound were selected from the MS/MS library spectra in the Agilent PCDLs to use as qualifiers. The Agilent Pesticide PCDL for TOF or Q-TOF LC/MS systems (1,684 compounds, 914 with MS/MS spectra) and the Agilent Water Screening PCDL (1,451 compounds, 1,157 with MS/MS spectra) were used.

The suspect screening workflow used the Agilent Pesticide and Water Screening PCDLs, in combination with the MassHunter Qualitative Analysis software (version B.07.00) Find by Formula algorithm. Find by Formula automatically extracts precursor ions from the All Ions MS/MS data using the accurate-mass database in the PCDLs. When available in the accurate mass library, the corresponding MS/MS fragments are also extracted from the data. Precursor and corresponding fragment ion peaks are plotted to score the quality of their correlation for each compound. The suspect screening workflow data analysis parameters used are provided in Figure 2.

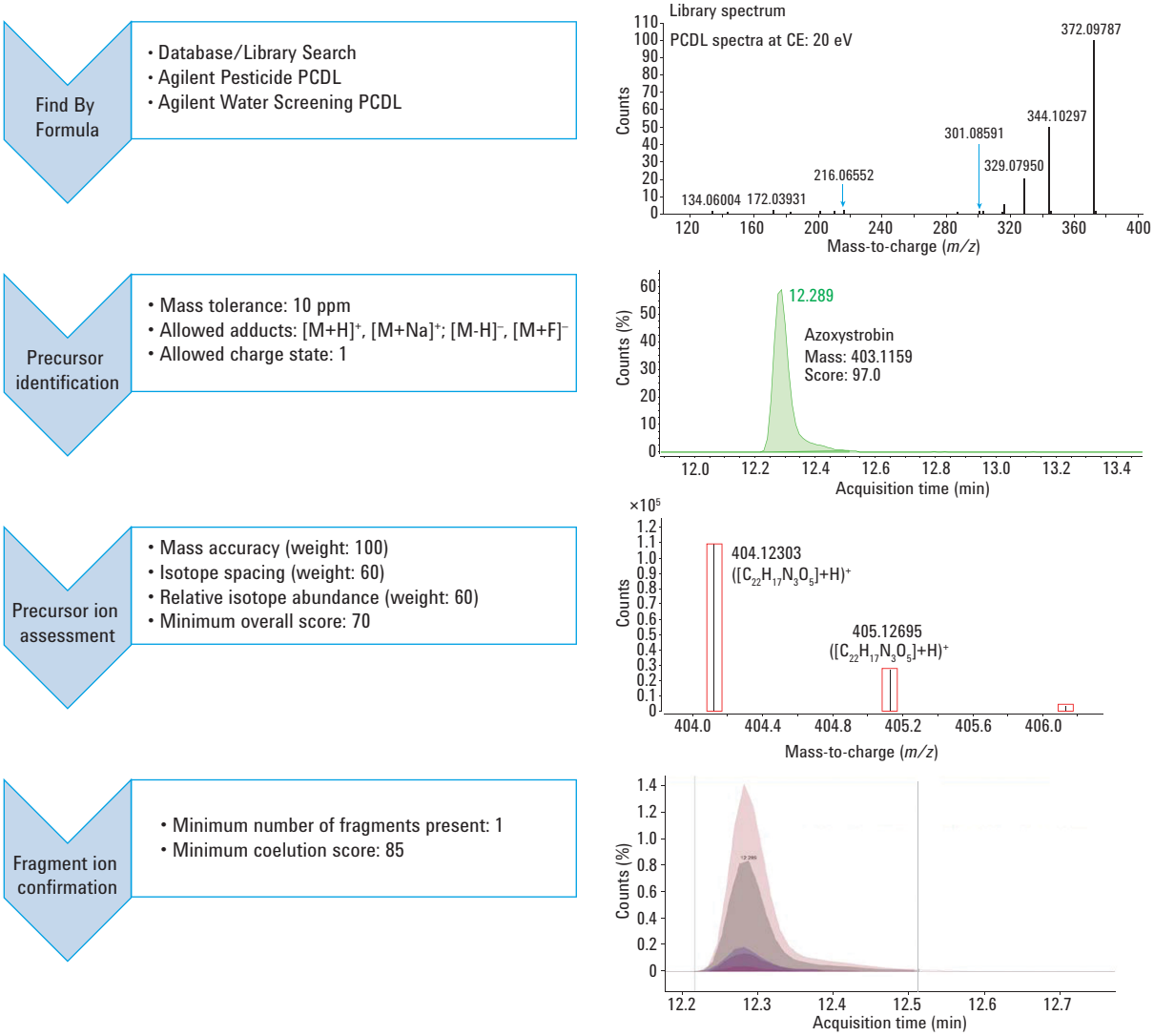

Figure 2. Suspect screening workflow: data analysis parameter settings and example. The precursor mass of the fungicide azoxystrobin (403.1168 m/z) is found at retention time 12.3, and its fragment ions are confirmed.

The unknown compound identification workflow can be used to identify compounds without *a priori* knowledge using accurate mass and fragment data along with *in silico* fragment prediction solfware. In this case, it relied on a custom PCD of potential TPs, which contained accurate masses and molecular formulas. The Eawag Pathway Prediction System (EAWAG-PPS) [3] was used to generate 1,409 possible TP structures for the pesticides detected in the study. After eliminating 71 structures due to the implausibility of being ionized by ESI, the molecular formulas of the plausible structures were added to build the custom PCD. The MassHunter Qualitative Analysis software Find by Formula feature was used to screen the data for these plausible molecular formulas. Because there were no MS/MS spectra for these compounds in the custom PCD, the exact mass, isotope score, number of detections across the 51 samples, and retention time (RT) were used to produce a short list of plausible matches.

Water samples with the highest abundances of the short list of plausible candidates were rerun in targeted MS/MS mode to obtain MS/MS spectra for processing by MSC software (version, B.07.00). MSC software was used to search the custom PCD for compounds with the same exact mass as the isolated mass. MSC software automatically compares predicted *in silico* fragments of the structures in the custom PCD (or in a web-based database such as ChemSpider or PubChem) with the measured MS/MS spectra. All measured MS/MS fragments that can be explained by each structure were listed and scored based on a weighted match. It is important to note that this workflow requires that the matching structures are present in the custom PCD. The structures can be manually uploaded into the PCD using mol-files.

To aid in the identification of unknown TPs, CFM-ID (http://cfmid.wishartlab.com/predict) was used to predict the MS/MS spectra of the plausible TPs [4].

Results and Discussion

Targeted quantification

Table 1 shows the LC/Q-TOF positive and negative ESI quantification results for the 32 target pesticide standards. The pesticide standards were run on the LC/Q-TOF system to obtain method validation parameters and to determine method suitability for targeted quantification. The method validation parameters, including method detection limits, absolute recovery, accuracy, precision, and matrix factors, are presented. The parameter details and their calculation have been described by C. Moschet; *et al.* [1].

Method detection limits for all target analytes ranged from 0.1 and 13 ng/L in water. All targets had absolute recoveries between 70–110 %. The precision, calculated as the relative standard deviation (%RSD) of triplicate injections on the LC/Q-TOF system, was <10 % for 30 of the target analytes, while the accuracy for 26 analytes was between 70 and 130 %.

Targeted screening detected 25 of the target compounds among the 51 water samples tested (Figure 1).

Suspect screening

The All Ions MS/MS sample data collected in both positive and negative ESI modes were screened for compounds present in the Agilent Pesticide and Water Screening PCDLs using the Find by Formula algorithm. Based on specified adducts (Figure 2), the software automatically searched the acquired data for the presence of the precursor ions of compounds stored in the PCDL, and assigned a cumulative score to matches based on mass accuracy, isotopic spacing, and relative isotope abundance.

A score threshold of >70 was chosen (Figure 2) for compounds to be considered for further evaluation. Compound matches with higher scores were subsequently evaluated for the presence of MS/MS fragment ions matching the compounds' MS/MS spectra in the PCDL (when MS/MS spectra were present in the PCDL). Compounds without MS/MS spectra in the PCDL were tentatively identified.

The validity of fragment ion matches was evaluated by scoring their coelution with their corresponding precursor ions (coelution score). Using the spiked analytes, it was determined that the presence of one fragment with a coelution score >85 was sufficient to identify compounds with a low false positive rate, while producing a manageable amount of data to process and review (Figure 2). Figure 2 also provides an example of the identification of the fungicide azoxystrobin using the suspect screening workflow. Figure 3 provides a more detailed comparison of the theoretical (in the PCDL) and measured isotope pattern, as well as the coelution of the main fragments (from the PCDL) of the herbicide fluridone.

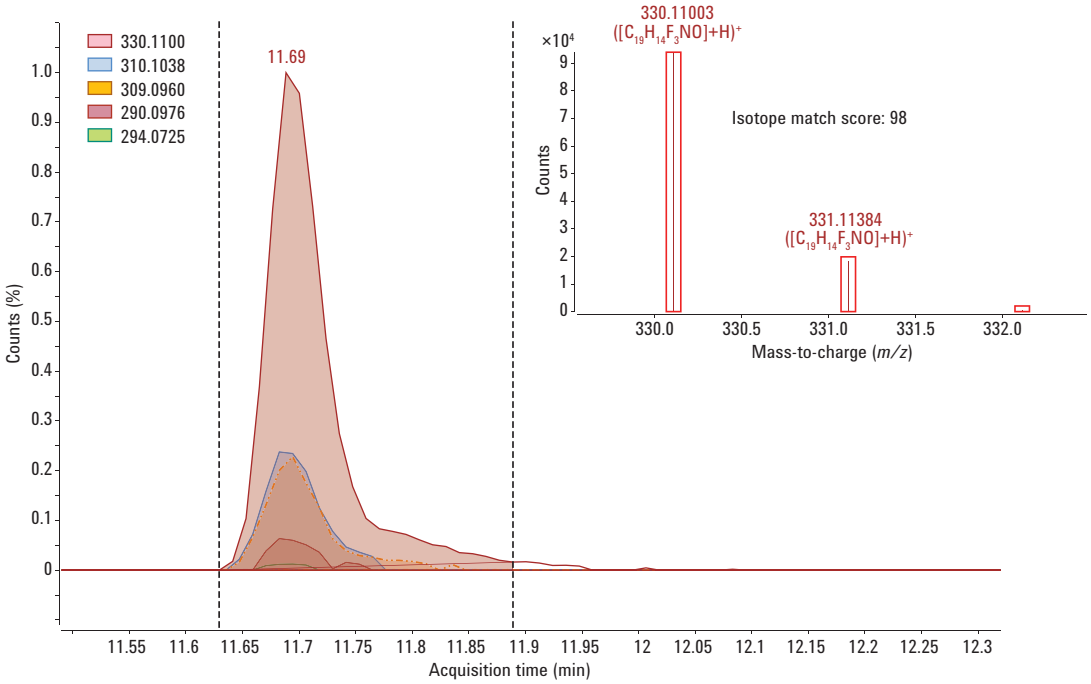

Figure 3. Details of the theoretical and measured isotope pattern, along with molecular and fragment ions, identified for fluridone using All Ions MS/MS and the PCDL.

After manual inspection, the suspect screening workflow detected 85 compounds (53 in positive ESI, 26 in negative ESI, and six in both positive and negative ESI). Of these, 67 could be confirmed unambiguously by a reference standard, six could be confirmed tentatively with high confidence by matching MS/MS spectra, and 12 compounds were rejected because they were not confirmed by a reference standard or due to implausible MS/MS fragments. The latter was the case when there were no MS/MS spectra in the PCDL for the suspected compound.

Unknown compound identification

The MassHunter Qualitative Analysis software Find by Formula feature was used to identify TPs in the water sample data using plausible TP structures that had been added to the custom PCD. This step yielded 110 matches. Inspection of peak shape, signal-to-noise ratio (S/N), RT plausibility, and whether the detected compound is theoretically ionizable in the selected ionization mode left 33 plausible compounds.

Comparison of the abundance pattern of the 33 plausible TPs with the concentration pattern of their potential parent compounds in the water samples reduced the list of those tentatively identified to 14.

After rerunning the samples in targeted MS/MS mode, evaluating the MS/MS spectra using the MSC software, comparing measured fragments to those predicted by CFM-ID and manually inspecting the results, seven compounds were eliminated for having implausible MS/MS spectra (that is, having fragments that could not be explained by the molecular structure). Thus, seven plausible TPs were identified using the workflow. Two of them were already detected and confirmed using the targeted quantification or suspect screening workflows. Therefore, five new TPs could uniquely be identified by the unknown compound identification workflow. Three of them were able to be confirmed unambiguously by a reference standard, and two of them remained tentatively identified because no reference standard was commercially available.

Figure 4 shows an example of a transformation product of the herbicide dithiopyr (CAS 128294-56-4), which was tentatively identified with high confidence in several samples. This compound, along with several MS/MS fragment ion structures, was identified using MSC (score of 92.4). MSC was able to identify and elucidate structures for 96.7 % of the ions in the MS/MS spectra for this TP.

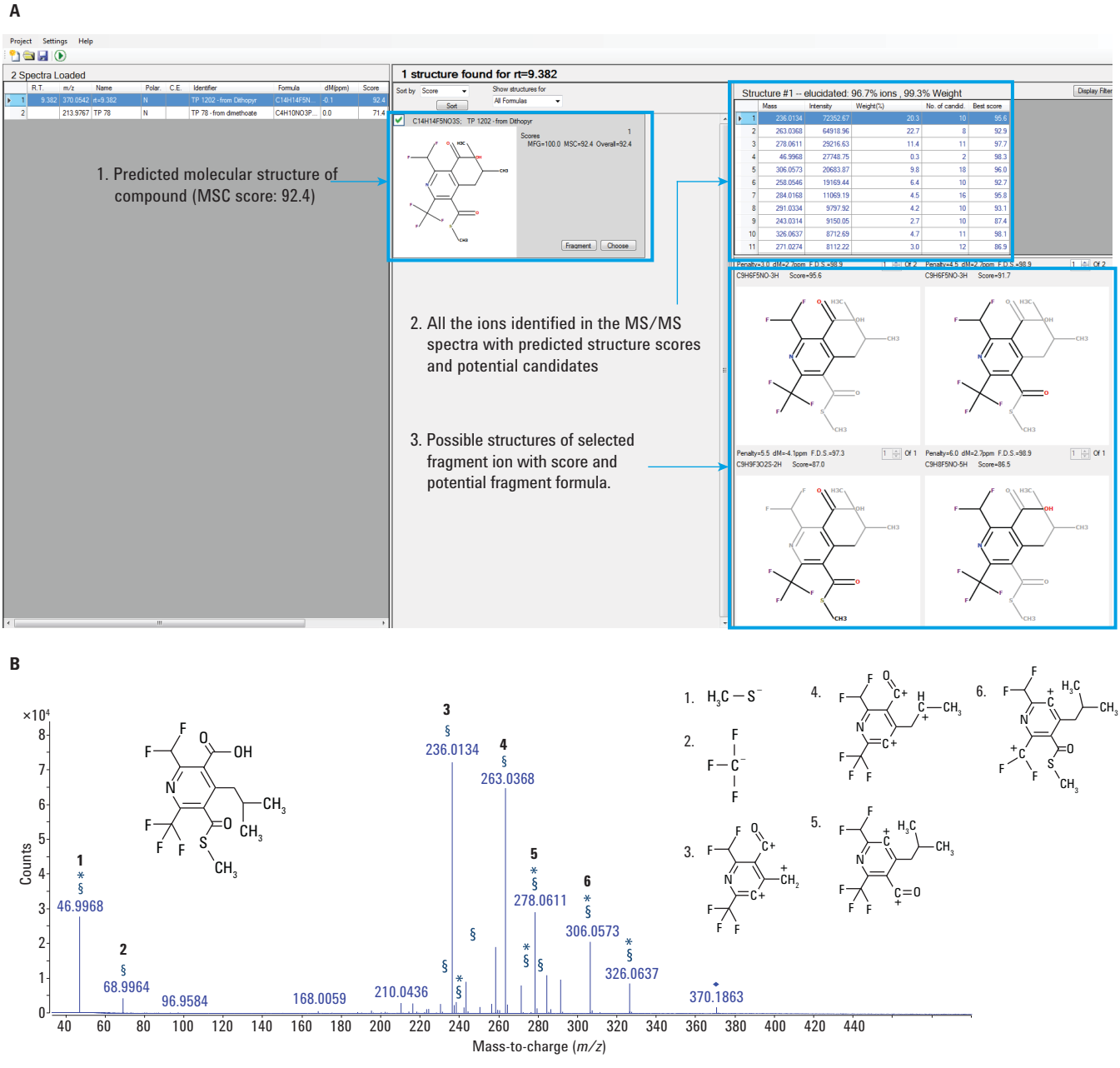

Figure 4A. Agilent MSC software window showing MS/MS fragments of a dithiopyr TP with CAS 128294-56-4 (MSC score 92.6). 1) predicted by MSC software; 2) list of all ions identified with predicted structure scores and candidates; 3) possible structures and formulas of selected fragment ions. B) Mass spectrum of a dithiopyr TP (§) predicted by MSC software; (*) predicted by CFM-ID.

Conclusions

LC/Q-TOF targeted quantification, suspect screening, and unknown compound identification workflows were applied to the analysis of surface water samples. The targeted quantification workflow was validated using 32 pesticide standards. Twenty-five of them were detected among the samples tested using the targeted quantification workflow. The suspect screening workflow generated an expanded list of 85 possible pollutants, 73 of which were subsequently positively identified. When applied to herbicide and pesticide TPs, the unknown compound identification workflow identified five plausible TPs not identified using the targeted quantification or suspect screening workflows. Compared to targeted analysis alone, the complementary workflows enabled by the Agilent 6530 Accurate-Mass Q-TOF LC/MS system provided more comprehensive, higher confidence characterization of the micropollutants present, including potential pesticide and herbicide TPs. Several more compounds were identified that would have been missed if only a targeted approach were used.

The Agilent Pesticide and Water-Screening PCDLs, combined with the Agilent All Ions MS/MS accurate mass capabilities of the Q-TOF LC/MS system and Agilent MassHunter Qualitative Analysis software, enabled presumptive matching of acquired spectra with library spectra to confirm compound identities, without the need to source standards. Agilent Molecular Structure Correlator software aided identification of TPs by correlating the unknown MS/MS spectrum against multiple candidate structures in compound databases.

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