An Executive Summary

Exceeding Requirements for Standard Environmental Methods using LECO Separation Science Gas Chromatography Time-of-Flight-Mass Spectrometer

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LECO

Concurrent Target and NonTarget Deconvolution® (**NTD**®) for High-Throughput Environmental Analysis

Overview

The use of gas chromatography (GC) coupled with high-resolution time-of-flight (TOF) mass spectrometry for environmental analysis exceeds the requirements for environmental analysis using standard methods. Leveraging the advances in new Deconvolution algorithms for target and NonTarget Deconvolution can greatly enhance the capability of the system. The addition of a robust ion source that does not require routine cleaning can improve the sample throughput.

Instrumentation

For all methods discussed here, a GC time-of-flight mass spectrometer (TOFMS) instrument (Pegasus® BT TOFMS, Reflectron, 1m total flight path, LECO™) was utilized to take advantage of its new hardware and software features that were designed to improve specificity, speed, and stability of the environmental analysis methods. An overview of these features is described below.

1. A **TOF** instrument is a high-resolution mass analyzer that provides analyte mass with two decimal points, which can enhance the selectivity and reliability of the library search.
2. The **StayClean® Ion Source** is an open ion source that minimizes/eliminates the time-consuming and laborious task of ion source cleaning, which reduces the instrument downtime and improves the productivity for samples with complex matrices containing pesticides, QuEChERS extract, oil, and others.
3. **New Target Analyte Find (TAF)** and **NonTarget Deconvolution (NTD)** algorithms can greatly enhance the detection and deconvolution of analytes with full mass range and library search capability along with the capability of concurrent target and non-target analysis. The ions of interest can be selected post-analysis, thus eliminating the need for reinjection for target analysis and improves productivity.
4. **Retrospective analysis** is intended to replace a physical sample archive with a data archive from past analysis that can be searched and compared to identify origin or fluctuating levels of analytes of interest to identify changes in the environment.
5. The instrument maintains a **continuous full mass range analysis** and offers response factor linearity across five orders of magnitude making it highly suited for environmental analysis with an array of applications.

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Analysis of Pesticides in Fruits and Vegetables

The European Commission has published a guidance document (SANTE/11945/2015) on analyzing the pesticide residues in food and feed (1). The compound identification requires a mass spectrometer, typically a quadruple ion-trap or TOF instrument. The acquisition can be a full scan with limited *m/z* range or a selected ion monitoring (SIM) mode. As per the guidelines, the following are the key identification requirements for the MS technique:

1. Have a minimum of three ions, each with S/N ≥ 3 (i.e., signal intensity [S] divided by noise width [N]).
2. The analyte peaks in the extracted ion chromatogram must completely overlap.
3. The relative ratio of ions must be within ±30% of the average calibration standards for a sequence.

The SANTE guidelines can be approached with this instrument in two ways.

- For qualitative analysis, **NTD** can be utilized by specifying the analyte retention time range, minimum S/N and the number of ions, and the mass tolerance limit for an automated library search. With this approach, a full mass spectrum was collected and compared with the NIST library (see **Figure 1**). In **Figure 1**, a library search for etridiazole returned a similarity score of 940/1.000. If desired, this approach can also be used for quantitative analysis.
- For a more specific quantitative analysis, **TAF** can be used with a target list for a specific analyte in a retention time range and mass within a tolerance limit. **Figure 1** shows an example of how 10 target ions were specified to obtain mass spectrum from a chosen peak. The tolerance limits were specified to search for quantitative and qualifier ions.

Indeed, both **NTD** and the **TAF** can be performed in a single injection, which can significantly enhance the throughput.

SANTE Guideline on Real Sample

Method: Cold splitless injections with TOFMS operating at 20 spectra/s over 35–650 *m/z* range.

Blueberry and spinach samples were spiked with a mixture of 203 pesticides to assess the effectiveness of **NTD** and **TAF** algorithms. **Figure 2a** shows the relative ion ratios of hexachlorobenzene in blueberry and spinach QuEChERS extracts. The M+2 and M+4 ions were within the required ±30% limit for all but one sample of 0.1 ng/g for blueberry. All concentrations from 0.1 to 500 ppb were within the tolerance limit for spinach extracts.

At 0.5 ppb concentration, the peak apex for both matrices from extracted ion chromatogram overlap (**Figure 2b**). LOD values for hexachlorobenzene, endosulfan sulfate, and folpet were found to be 0.5, 0.5, and 5.0 ppb, respectively in blueberries. Similarly, for spinach extracts, the limit of detection (LOD) values of hexachlorobenzene, endosulfan sulfate, and folpet were 0.5 ppb, 1.0 ppb, and 5.0 ppb, respectively. The linearity correlation (R^2) was 0.99 or above in each case. These values far exceed the typical pesticide quantitation performed at 10 ppb levels.

A study of 30 pesticides in strawberry matrix (incurred pesticides excluded) found a LOD < 0.5 ppb for most pesticides, which makes the instrument a very powerful tool for sensitive detection of analytes in this matrix. To extract a single analyte from the matrix, a low tolerance limit (i.e., ±0.095 Daltons = 600 ppm) can be set to take advantage of the high-resolution

Figure 1: Data processing methods.

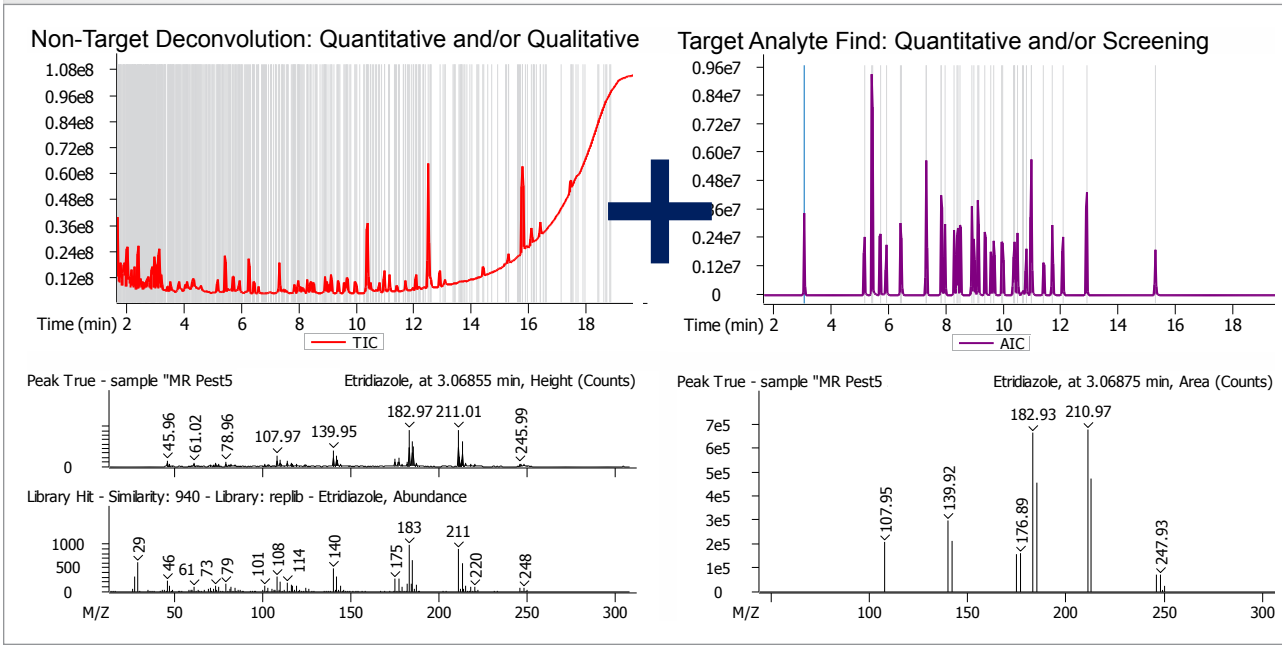


Figure 2a: MS criteria for SANTE/11945/2015.

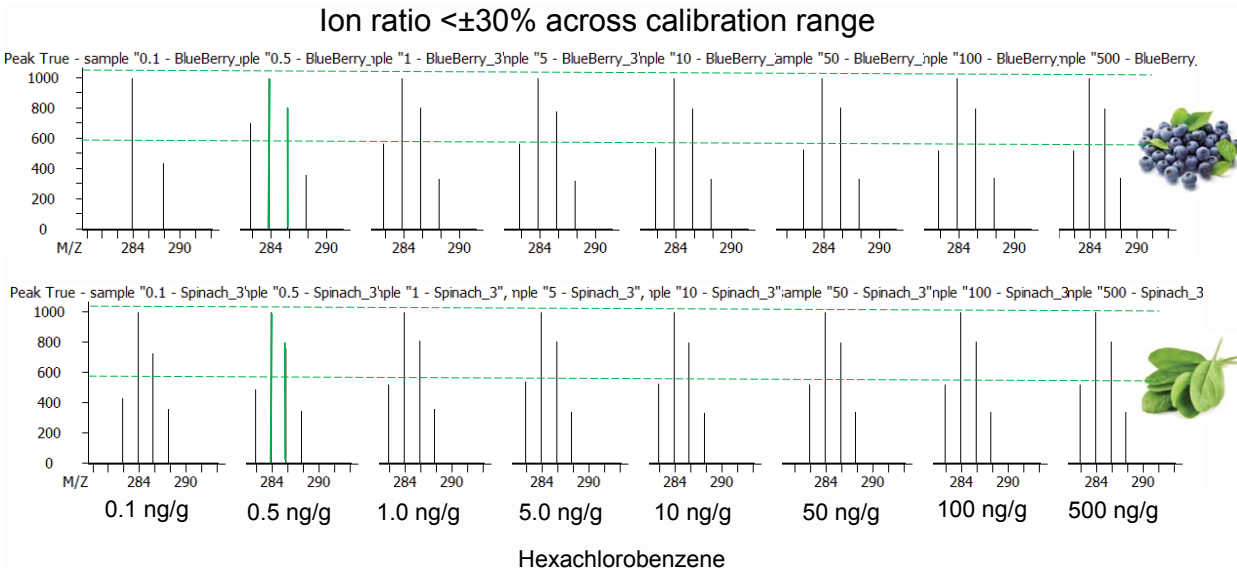
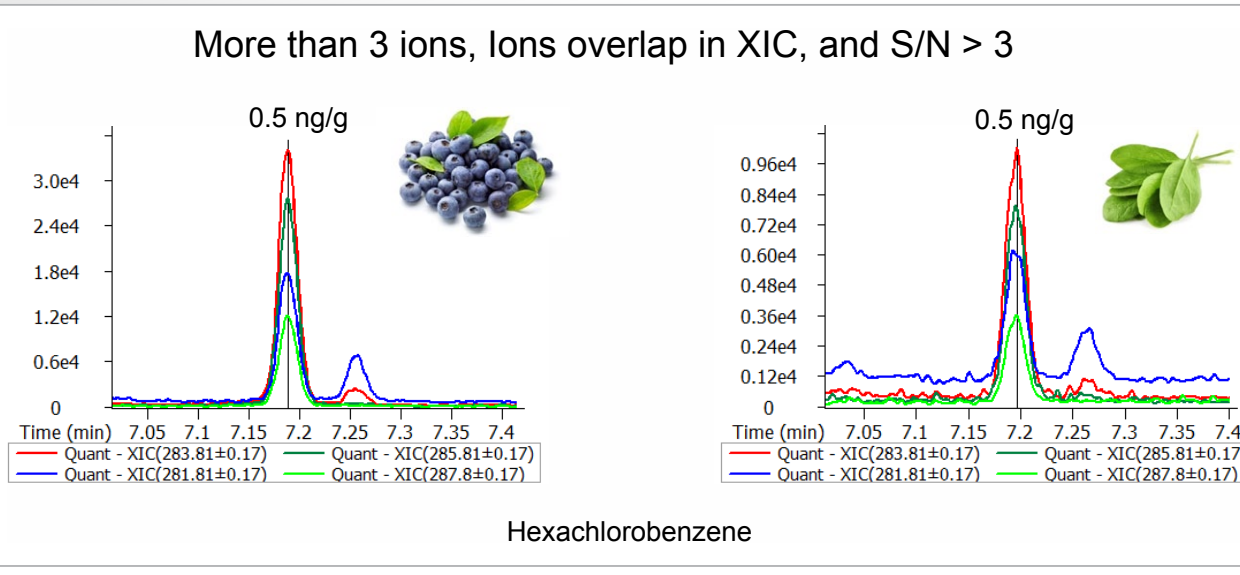


Figure 2b: MS criteria for SANTE/11945/2015.

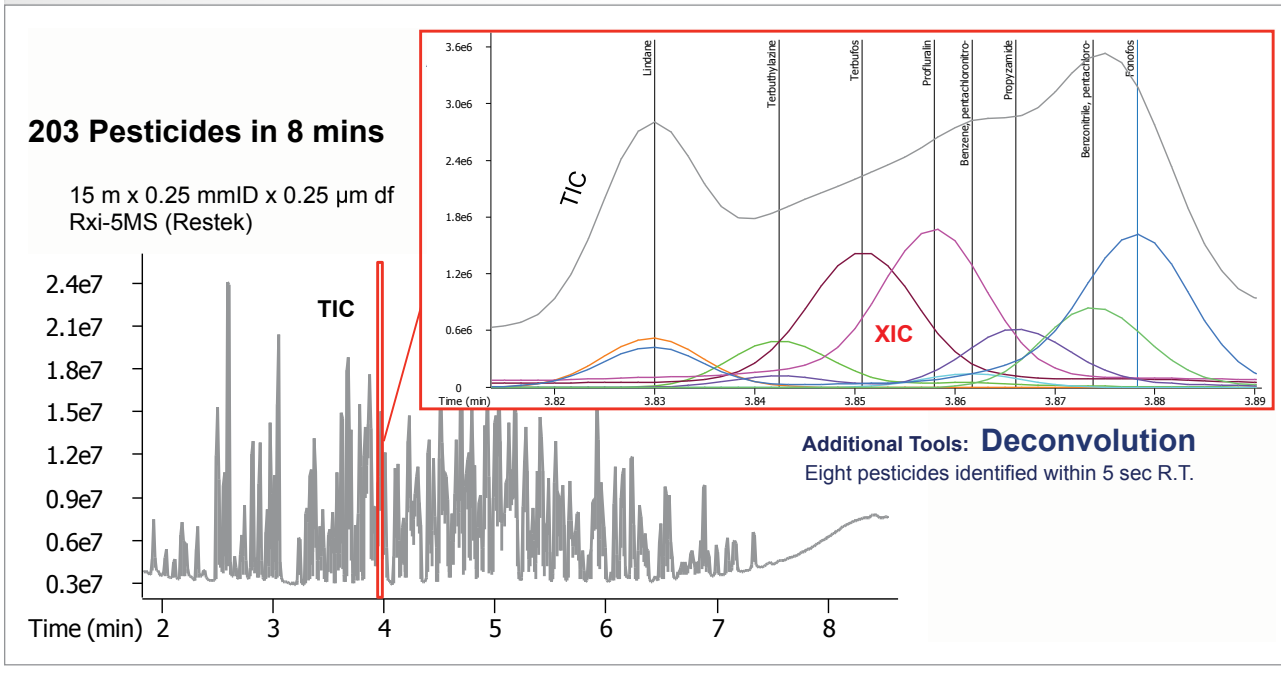


afforded by a TOF system. The high-resolution of the TOFMS instrument enhances the specificity and resolution of analytes over other mass analyzers such as a quadruple (and many triple quadruples), which only support the lowest tolerance limit of ±3 Daltons and would consequently have a much higher background noise. When using TOF, the mass profiles of the chosen analyte can be inspected using a relative mass tolerance to maintain the same mass profile for all masses in the spectra.

To assess the effectiveness of the **NTD** algorithm (which is more appropriate for qualitative analysis), a mixture of 203 pesticides were analyzed using a relatively short and unoptimized 8-min method (**Figure 3**). The zoomed-in region shown in **Figure 3** covers a 6-s time window, which appears to have three convoluted analytes in the total ion chromatogram. Upon deconvolution, the extracted trace reveals eight pesticides present in the 5-s time window with full mass range, which can be library searched for identification. The deconvolution performance of the algorithm in this case is notable because it could deconvolute peaks with significant overlap as well as perform a library search of the full spectra with **NTD**.

The availability of the full mass range with library search becomes particularly useful in the case of non-target incurred pesticides/fungicides that were not spiked in the sample. In the previous example with 203 pesticides, when comparing

Figure 3: Automatic peak find: NTD™.



the identification for blueberry, spinach, and tomato, an additional fungicide (i.e., boscalid) was automatically identified in blueberry though it was absent in spinach and tomato. Similarly, propoxur, an incurred pesticide, was automatically identified in cabbage samples.

In summary, even for unit mass resolution application, use of a high-resolution TOFMS instrument over other more traditional MS systems (e.g., quadruple) can provide enhanced specificity and more accurate mass, leading to fewer false positives. When paired together, the ability to concurrently perform target and **NTD** with even the most difficult matrix challenges can be overcome.

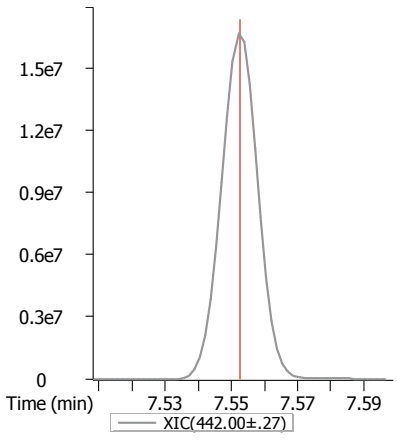
Semi-Volatile Organic Compounds

US Environmental Protection Agency (EPA) Method 8270 provides guidelines on the analysis of semi-volatile organic compounds by GC-MS instruments (2). With more than adequate sensitivity, split injection is typically used in this method to increase the inlet uptime and, consequently, the sample throughput. The evaluation of Method 8270 involves five main criteria below.

Method: Full mass range from 30–650 *m/z* is monitored at 10 spectra/s. An 84 compound spiked matrix sample is used.

1. **Decafluorotriphenylphosphine tuning**. For a typical analysis, the baseline noise is subtracted from the chromatogram and the mass spectra at the peak apex searched in NIST library for a similarity match. **Figure 4** shows an overview of 10 replicate analyses (50 ng/µL, split 20:1) with their statistical parameters such as mean, max, min, and standard deviation. As indicated in the final column of the table, all analysis criteria passed for all replicates, individually and as an average.
2. **GC-MS check solution**. A quality control check solution is necessary to verify that the parameters are within required limit, such as DDT breakdown < 10% and the peak tailing factor (or asymmetry factor) < 2. With the chosen GC TOFMS instrument, a 10-replicate analysis (50 ng/µL concentration) led to < 1% breakdown of DDT and tailing factors of 1.13 and 1.30 for pentachlorophenol and benzidine, respectively, both well below the required criteria (data not shown). A 20:1 injection split in this case also helped to avoid column overload and severe departure of the peak shapes from the ideal Gaussian profile.
3. **Linearity of calibration**. A calibration curve can be constructed to determine the linearity of the response factors. **Figure 5** includes data from a 10-point calibration curve (0.05 to 50 ppm) showing the %RSD of the response factors (RF) for 84 compounds. In this case, 91% of the compound showed %RSD RF < 10% (threshold: %RSD RF < 20%). The method advocates primary usage of RF, followed by the equation of the calibration curve, if necessary. The only point above the 10% RSD RF in **Figure 5**, the compound benzanthracene, still showed a R^2 value of 0.995. Considering that this is the weakest performing of all calibration curves, it can be concluded that the system is sufficiently robust and well-suited for this analysis.
4. **Calibration verification**. A calibration verification can be performed for each analyte by comparing the %deviation between the theoretical and observed

Figure 4: USEPA method 8270 – 1. Decafluorotriphenylphosphine.



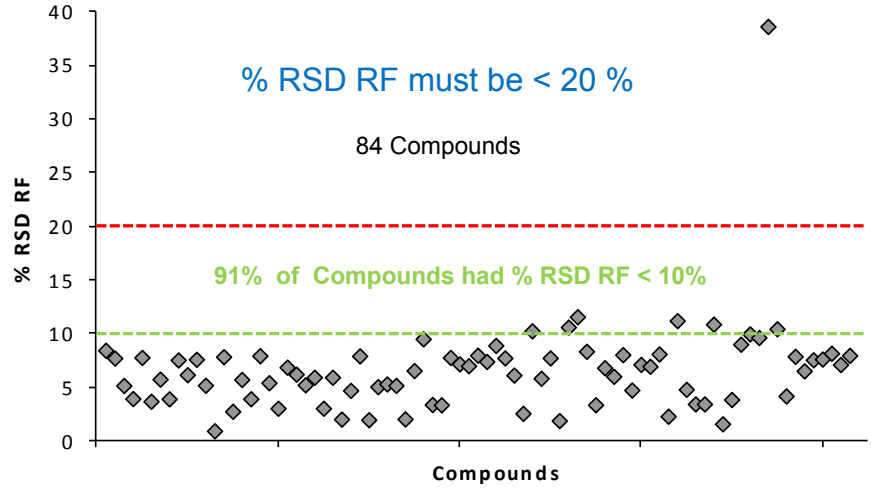
Average of 10 replicate injections of 50 ng/µL, Split 20:1

Mass	Ion Abundance Criteria	Mean	STDEV	Min	Max	Criteria
51	10-80% of Base Peak	64.5	0.25	64.1	64.9	PASS
68	< 2% of mass 69	1.93	0.04	1.87	1.98	PASS
70	< 2% of mass 69	0.38	0.01	0.37	0.40	PASS
127	10-80% of Base Peak	52.1	0.18	51.9	52.4	PASS
197	< 2% of mass 69	0.22	0.03	0.19	0.28	PASS
198	Base Peak, or > 50% of Mass 442		Base Peak			PASS
199	5-9% of mass 198	6.73	0.04	6.68	6.77	PASS
275	10-60% of Base Peak	23.0	0.08	22.9	23.1	PASS
365	> 1% of mass 198	1.90	0.02	1.86	1.92	PASS
441	Present but < 24% of mass 442	13.2	0.03	13.15	13.25	PASS
442	Base Peak, or > 50% of mass 198	76.5	0.3	76.1	76.9	PASS
443	15-24% of mass 442	19.5	0.08	19.4	19.6	PASS

concentrations. **Figure 6** shows an example of 2,4-dimethylphenol calibration verification curve with %deviation for 0.05–50 ppm concentrations. All %deviations are less than 15% with the average %deviation being 5%, which is well below the required upper limit of < 20%.

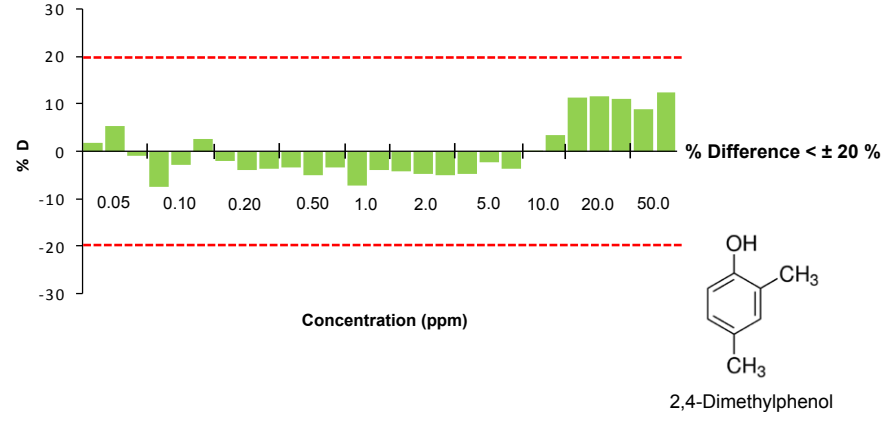
5. **QC check standard.** The final criteria are to compare the values to a check standard. A third-party standard is typically used that is at a mid-point concentration, and the quantitation of the analytes is carried out based on the calibration curve generated in previous steps. When this step is performed for 84 compounds (74 analytes and 8 ISTD), a %deviation less than 20% with absolute average %deviation being only 6% was observed, which shows that the system is very robust for all compounds in EPA Method 8270.

Figure 5: USEPA method 8270 – 3. linearity of target analytes.



Concentration range from 0.05 to 50 ppm, 10 point curve in replicate

Figure 6: USEPA method 8270 – 4. calibration verification.



Organic Compounds in Drinking Water

The EPA Method 525.2 offers guidelines on the analysis of organics in drinking water (or water at any stage in drinking water) by liquid–solid extraction and capillary GC-MS techniques (3).

Method: 258 pesticides were spiked in extracted water samples. Cold splitless injection was used to minimize the degradation of analytes in the inlet. The mass range 35–650 *m/z* is used at 8 spectra/s. The ion source temperature is kept relatively high at 280 °C to accommodate the existence of certain polycyclic aromatic hydrocarbons in the sample as well as to minimize the tailing.

To determine the LOD, a fortified water sample is used with five replicate injections for on-column concentrations of < 0.5–25 ppb. Based on the statistical calculation (LOD=3.7469*RSD*concentration), the left side of **Figure 7a** shows the calculated LOD values were out of 258 analytes, 183 had LOD < 0.5 ppb; only three had an LOD of 25 ppb. When using field water samples (typically 1 L) (**Figure 7b**, right), LOD values are in the ppt range with only three analytes showing LOD > 10 ppt. With such a high sensitivity, it becomes easier to detect even the lowest quantities of organics in water samples.

Table 1a shows results from an analysis of 1 L river water samples using this highly sensitive method. The presence of pesticides such as atrazine and metolachlor indicate possible pesticide in runoff water from corn fields that were in proximity

Figure 7: USEPA method 525.2.

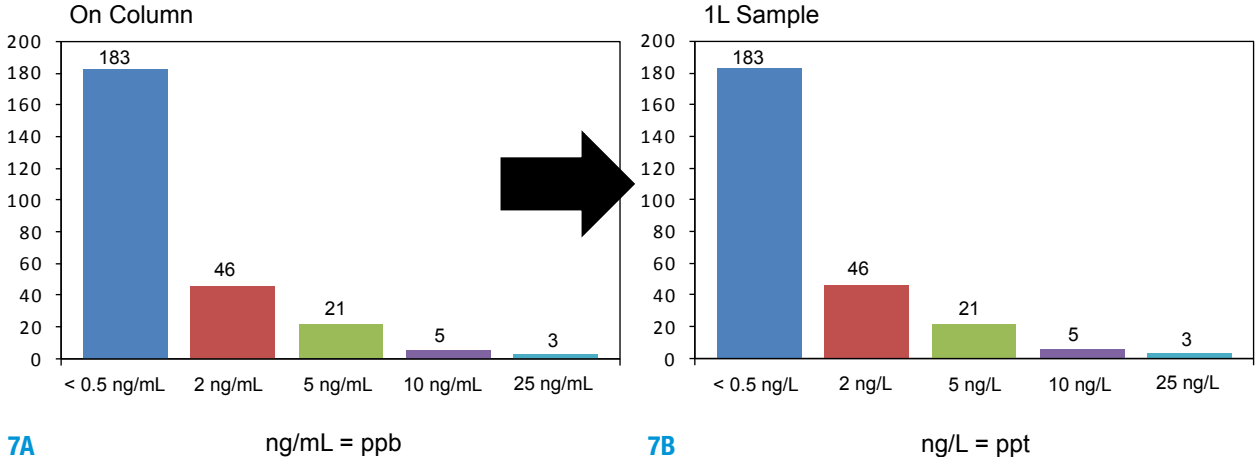


Table 1a: Concentration of organic compounds in a 1L river water sample.

Name	CAS	Formula	R.T. (min)	Quant Masses	Peak S/N	Conc. (ng/L)
2,4-Dimethylphenol	105-67-9	$C_8H_{10}O$	4.052	XIC(107.06±0.06)	113	35.7
Diethyl Phthalate	84-66-2	$C_{12}H_{14}O_4$	7.122	XIC(222.09±0.13)	1265	8.7
Atrazine	1912-24-9	$C_8H_{14}ClN_5$	8.935	XIC(200.08±0.12)	164	11.6
Anthraquinone	84-65-1	$C_{14}H_8O_2$	12.02	XIC(208.05±0.12)	81	2.0
Aldrin	309-00-2	$C_{12}H_8Cl_6$	12.04	XIC(262.86±0.16)	113	6.0
Metolachlor	51218-45-2	$C_{15}H_{22}ClNO_2$	12.08	XIC(162.14±0.10)	82	4.4
Fluoranthene	206-44-0	$C_{16}H_{10}$	13.28	XIC(200.07±0.12)	280	2.1
Pyrene	129-00-0	$C_{16}H_{10}$	14.02	XIC(202.08±0.12)	262	1.9
Chrysene	218-01-9	$C_{18}H_{12}$	18.61	XIC(228.09±0.14)	41	1.3
Benzo[b]fluoranthene	205-99-2	$C_{20}H_{12}$	22.35	XIC(252.09±0.15)	138	1.1

Table 1b

Name	CAS	R.T. (min)	Formula	Area	Similarity	Peak S/N
2-Naphthalenol, 5,6,7,8-tetrahydro-	1125-78-6	5.87761	$C_{10}H_{12}O$	3.91E+08	920	17633
Dihydroactinidiolide	17092-92-1	6.71752	$C_{11}H_{16}O_2$	16861655	853	838
Diethyltoluamide	134-62-3	7.0222	$C_{12}H_{17}NO$	14883574	867	573
2,6-Dibromohydroquinone	3333-25-3	8.9611	$C_6H_4Br_2O_2$	1517919	830	271
Tris(1,3-dichloroisopropyl)phosphate	13674-87-8	16.8471	$C_9H_{15}Cl_6O_4P$	6366710	756	97
Dehydroabietic acid	1740-19-8	18.2731	$C_{20}H_{28}O_2$	97540876	902	2154

to the sampling location. The low concentrations of pesticides quantified in the sample had a range of 1.1–35.7 ppt. These low levels of quantifiable values are typically only observed in selected-ion monitoring mode. However, in this case, the high sensitivity also comes with the added benefit of the full mass range that can be library searched.

The real advantage of this technique becomes apparent when **NTD** is applied to the data to discover new compounds that were not specified (**Table 1b**). **Table 1b** shows non-target compounds discovered with a library search showing a similarity score as high as 920, which indicates a reliable identity match. The presence of the non-target analyte in the field sample can be confirmed with a blank water run. Interestingly, diethyltoluamide (DEET), a mosquito repellent, was also detected in water. The combined power of detecting and quantifying targeted pesticides as well as detecting and identifying non-target analytes makes this technique truly powerful.

References

(1) Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed. SANTE 11945/2015. EU Reference Laboratories for Residues and Pesticides. European Commission: 2016.

(2) USEPA Method 8270D: Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry. United States Environmental Protection Agency: 2014.

(3) Eichelberger, J. W.; Behymer, T. D.; Budde, W. L.; Munch, J. W.; Shoemaker, J. A., USEPA Method 525.2 Rev2.0: Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry. United States Environmental Protection Agency: 1995.

This executive summary is based on material presented in a webcast that can be viewed on demand here.