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New workflow for contaminants screening in strawberries using high-resolution GC/Q-TOF and expanded accurate mass library of pesticides and environmental pollutants

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Introduction

Strawberry is considered one of the most contaminated produce, and therefore, has been chosen to demonstrate a novel pesticide screening workflow using high-resolution GC/Q-TOF and an accurate mass library of pesticides and environmental pollutants, containing over 1000 unique compounds. The challenge of screening contaminants in food matrices is that it requires both high sensitivity to meet strict regulatory requirements for Maximum Residue Levels (MRLs), and a comprehensive scope. Here we demonstrate the new streamlined workflow for pesticides screening that is designed to comply with SANTE guidelines and offer high degree of flexibility for the data review process.

Experimental

Strawberry samples were extracted using the EN QuEChERS method with by the use of a dSPE cleanup for general fruits and vegetables (p/n 5982-6650 & 5982 5056). The samples were analyzed using GC with a mid-column backflush configuration, a 40 min retention time locked (RTL) method and a high-resolution Q-TOF as well as a Single Quadrupole (SQ) MS in full acquisition mode. The conditions are described in detail in Table 1.

GC and MS Conditions:	Q-TOF (7250)	SQ (5977)
GC	8890	
Column	2 x HP-5MS UI, 15 m, 0.25 mm, 0.25 µm	
Inlet	MMI, 4-mm UI liner single taper w wool	
Injection volume	1 µL	
Injection mode	Pulsed Splitless	
Inlet temperature	280°C	
Oven temperature program	60°C for 1 min; 40°C/min to 120°C, 5°C/min to 310°C	
Carrier gas	Helium	
Column 1 flow	~1.2 mL/min	
Column 2 flow	~ 1.4 mL/min	
Backflushing conditions	5 min (Post-run), 310 °C (Oven), 50 psi (Aux EPC pressure), 2 psi (Inlet pressure)	
Transfer line temperature	280°C	
Quadrupole temperature	150°C	
Source temperature	280°C	
Electron energy	70 eV	
Spectral acquisition rate	5 Hz	2.9 Hz
Mass range	45 to 650 m/z	45 to 550 m/z

Table 1. GC/Q-TOF and GC/MSD acquisition parameters.

Experimental

The GC/Q-TOF data were processed using new screening workflow available in MassHunter Quantitative Analysis Software 10 and an accurate mass Pesticide Personal Compound Database and Library (PCDL) (Figure 1A and Figure 2). The SQ data were also processed using MassHunter Quantitative Analysis Software 10 as well as Unknowns Analysis and a unit mass pesticide library (Figure 1B).

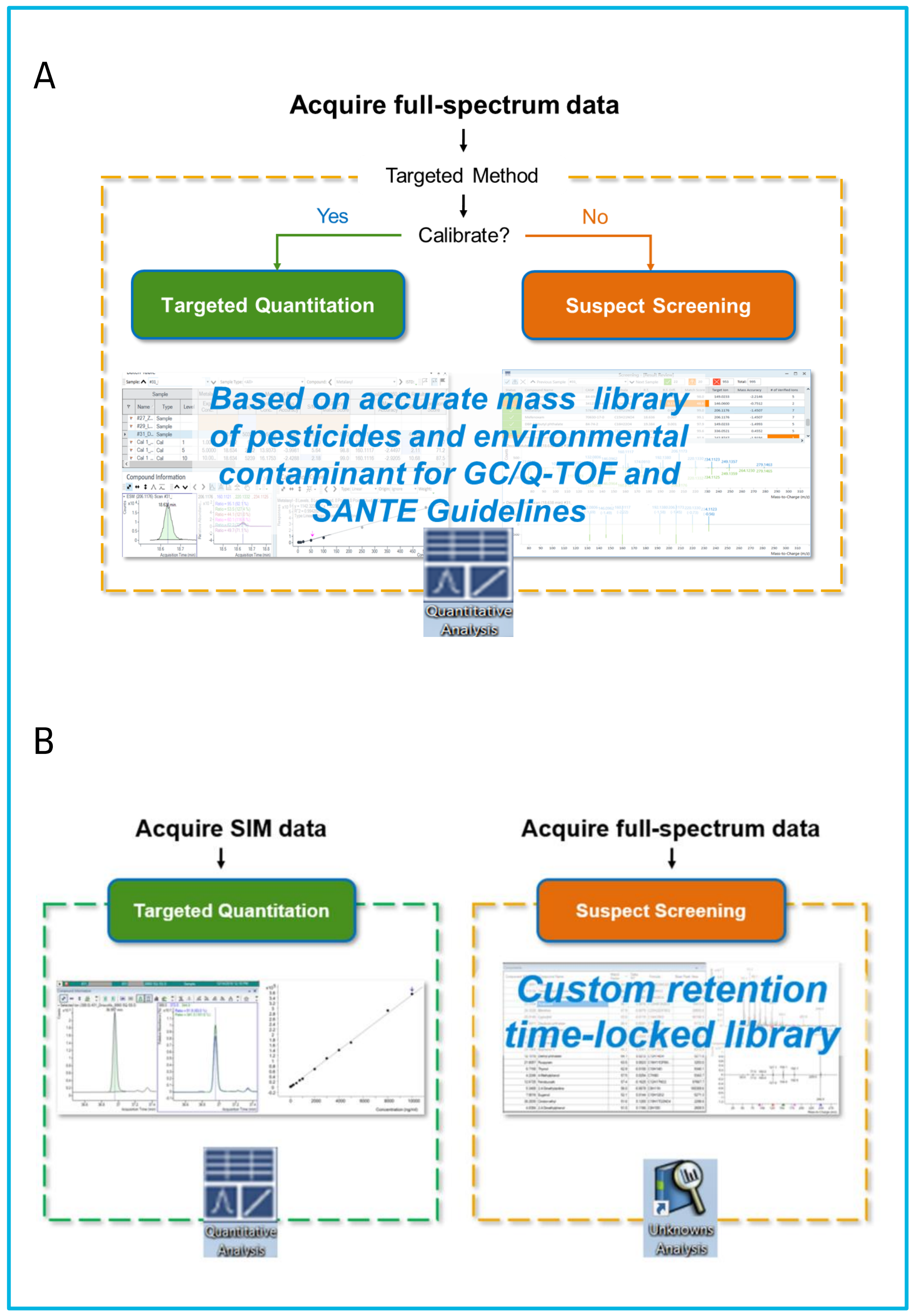


Figure 1. A) Combined contaminants screening and target quantitation workflow based on the Pesticides and Environmental Contaminants PCDL for GC/Q-TOF. B) Screening and target quantitation workflows using custom retention time-locked unit mass libraries for GC/MSD.

Suspect Screening Using GC/Q-TOF

Sixteen organic and non-organic strawberry samples were obtained from different vendors around the West Coast. The new accurate mass screening workflow for GC/Q-TOF was used simultaneously for quantitative analysis of pesticides as well as for the quick suspect screening of the incurred pesticides and environmental pollutants in strawberry extracts.

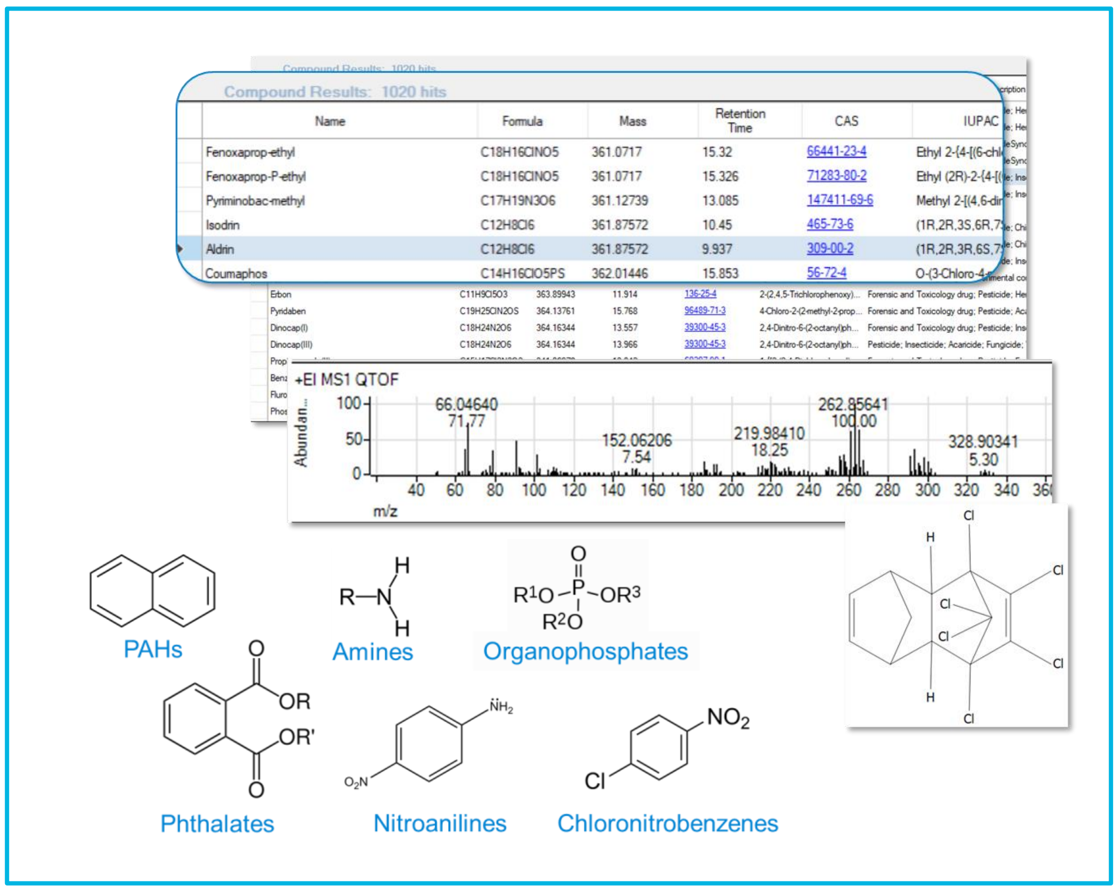


Figure 2. Updated GC/Q-TOF Accurate Mass Library of Pesticides and Environmental Contaminants containing accurate mass spectra for over 1000 compounds.

A few examples of contaminants identified in strawberry extract by GC/Q-TOF using the suspect screening workflow are shown in Figure 3A-C. Typically, 10-20 pesticides were identified in each non-organic extract (Table 2). **Flonicamid, pyrimethanil, cyprodinil, fluopyram, novaluron, captan** and **bifenthrin** were among pesticides most frequently identified in non-organic strawberry extracts. Most organic extracts contained only few trace levels pesticides, including some legacy pesticides. The lowest pesticide concentration detected in strawberry extracts was 1.2 ppb for both Cyprodinil and p,p'-DDE.

In addition, few environmental pollutants, including E&L compounds, flame retardants and disinfectants were also identified.

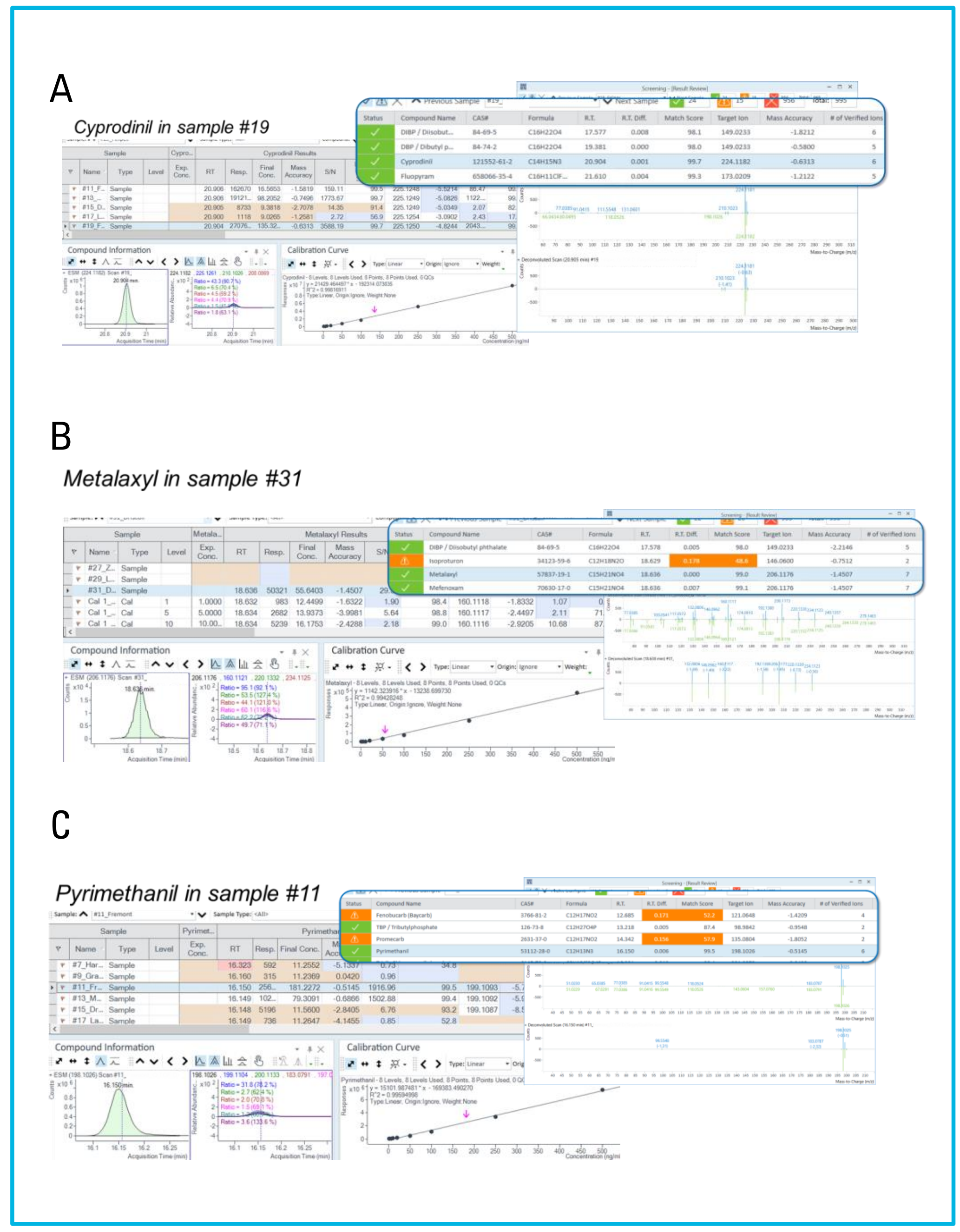


Figure 3. Screening Results Review.

Sample/concentration in extract, ppb		1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31
Compound Name	RT	(organic)		(organic)	(organic)	(organic)			(organic)								
Novaluron	8.28	117.8						122.8		100.7		159.8				181.5	119.3
Diphenyl ether (Diphenyl oxide)	8.61									N/A		N/A					
Tetrahydrophthalimide, cis-1,2,3,6-	9.90	197.9					715*	893*	55.3	37.8		520*	485.7	201.2	347.8	54.9	615*
Flonicamid	12.42	18						330.7	37.3	157.3	32.19	51	94.3	64.9	38.1	58.7	46
Pyrimethanil	16.16			11.4			181.2	79.3	11.6			233.5	12.6	11.4		11.5	11.3
Tri-(2-chloroisopropyl)phosphate	16.29	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Tris(3-Chloropropyl)phosphate	16.57		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pentachloroaniline	17.33			N/A													
Chlorpyrifos-methyl	18.11			N/A											N/A		
Carbaryl	18.23	34.8															
Metalaxyl	18.64						28.9										55.6
Anthraquinone	19.56											N/A					
Malathion	19.64	36.2					23.6			44						39.7	
Tetraconazole	20.37							36.2				27.9					68.1
Fthalide (Tetrachlorophthalide)	20.45																N/A
Cyprodinil	20.91			1.2			11.2	111.8	1.6		153.7	179.6	57.2	2.4	20.6	1.2	
Captan	21.43	151					105.3	16598*		58.7		5188*	11589*	812.5*	3600*	92.9	3294*
Fluopyram	21.62						N/A	N/A		N/A	N/A	N/A	N/A	N/A	N/A		
Folpet	21.67							N/A									
Flutriafol	22.75						21.6			17.3						18.2	
Fludioxonil	23.41						28.7	101.5	28.2		147.9	200.2	57.2		36.2		
p,p'-DDE	23.44		1.2		1.2				1.3		1.4			1.3	1.3		1.3
Myclobutanil	23.73										127.6	18.2				18	
Quinoxyfen	26.05												47.5			14.3	30.6
Fenhexamid	26.20							90.2			41.8	242.5					
Trifloxystrobin	26.50						21.2				45.1		54.9				
Piperonyl butoxide	27.22	273.9	19.7														
Acetamiprid	27.99							262.3									
Fluxapyroxad	28.32										N/A		N/A				
Bifenthrin	28.34						36.8	229.2		219.9	230.7		19.5	110.2	40.7	96.5	
Bifenazate	28.35							52				44					
Etoxazole	28.62										45.7						
Boscalid (Nicobifen)	33.36							N/A						N/A			
Azoxystrobin	37.00																198.3

Verified automatically

Verified after review

* Calculated concentration value outside of calibration

Table 2. Target quantitation and suspect screening results summary. Whenever a standard was available, the concentration of the contaminant in the strawberry extract is shown in the table.

Reducing False Negatives

Generally, GC/Q-TOF was able to identify higher number of pesticides in each sample as compared to the GC/MSD (Figure 4). This was especially evident for organic strawberry extracts where the levels of the detected pesticides were substantially lower as compared to non-organic extracts.

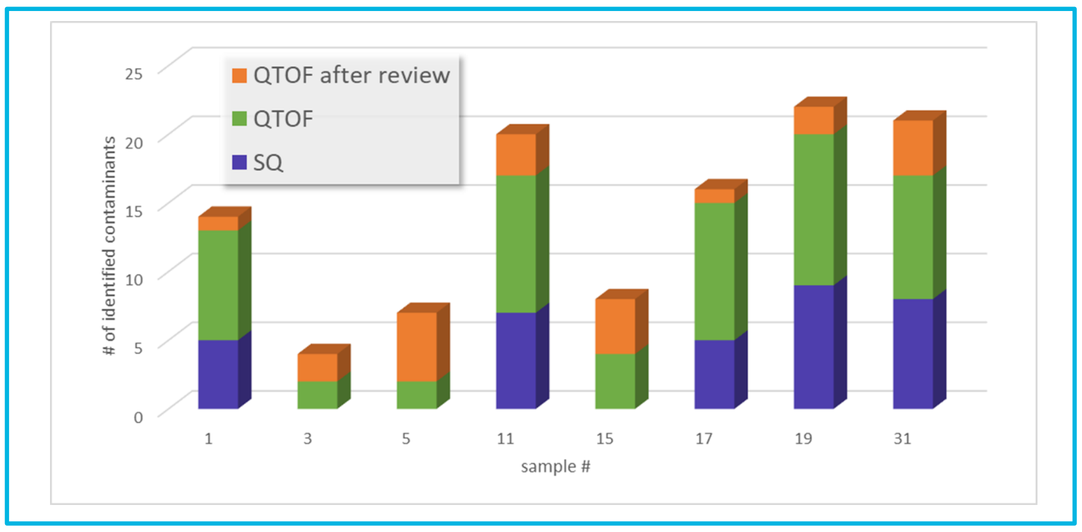
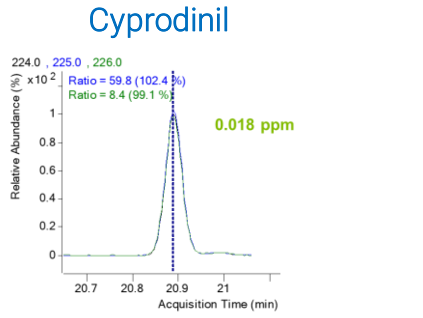


Figure 4. The number of identified contaminants in strawberry extracts, comparison between 7250 GC/Q-TOF and 5977 GC/MSD.

Eliminating False Positives

The GC/Q-TOF screening workflow was also found to less likely report false positives, due to both high-resolution accurate mass capability of the instrument as well as multiple parameters of the screening software with easy-to-review capabilities for verification.
In many cases, all the techniques provided correct identification as well as close concentration values, one of the examples is shown in Figure 5.
GC/Q-TOF also helped to reduce false positives.

Cyprodinil



Sample #27

Technique	Measured (ppb)
8860 GC/MSD w SS Ion Source	18
8890 GC/MSD w Extractor Ion Source	23
8890 GC/Q-TOF	21

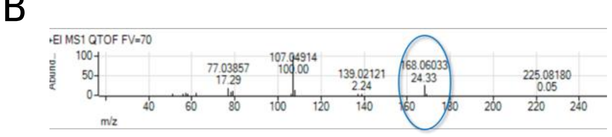
Figure 5. Overlay of quant and qualifier ions of Cyprodinil (GC/MSD) and its calculated concentrations in sample #27 by GC/MSD and GC/Q-TOF.

One of the examples is shown in Figures 6 and 7. Ethiofencarb was positively identified by GC/MSD but was not detected by the GC/Q-TOF screening workflow (Figure 6A). When accurate mass EIC (168.0603 +/- 20 ppm) was extracted manually, no peak was detected either. When a Q-TOF spectrum was extracted from the chromatographic region where ethiofencarb is expected to elute, two ions with 168 unit m/z were observed (Figure 6C). Accurate m/z of neither ion matched the theoretical m/z of ethiofencarb fragment 168.

A

Technique	Measured (ppb)
8860 GC/MSD w SS Ion Source	77
8890 GC/MSD w Extractor Ion Source	470
8890 GC/Q-TOF	ND

B



C

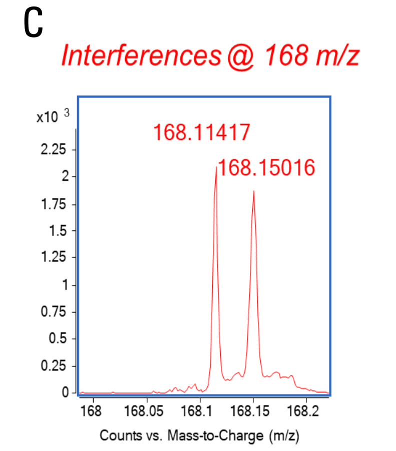


Figure 6. GC/Q-TOF correctly identifies false positive as interference. A) Measured concentrations of ethiofencarb. B) Accurate mass of ethiofencarb spectrum from GC/Q-TOF PCDL. C) A fragment of the GC/Q-TOF spectrum from chromatographic region corresponding to ethiofencarb RT.

Figure 7. GC/Q-TOF correctly recognizes ethiofencarb false positive as interference using a suspect screening workflow, which is evident from the low Library Match Score as well as poor spectra matching.

Conclusions

- New streamlined workflow for screening pesticides and environmental contaminants with high-resolution GC/Q-TOF and accurate mass pesticide and environmental pollutants library was demonstrated using organic and non-organic strawberry extracts.
- The comparison of GC/Q-TOF and GC/MSD screening results demonstrated that the GC/Q-TOF screening workflow is less likely to generate both false negatives as well as false-positives as compared to the GC/MSD.

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