Quantitation Target Compound Ion Extraction Matched with Unknowns Analysis Component Perception

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ASMS 2011
MP23 404

Agilent Technologies

Introduction

• Although quantitative analysis has proven adequate to determine concentrations of environmental contaminants, the additional need to report tentatively identified compounds has led to coupling Quantitative Analysis with Unknowns Analysis (UA).

• Quantitation uses a prescriptive ion extraction method, and is well-accepted; but its assessment in concert with Unknowns Analysis, which utilizes deconvolution and library search algorithms, is not well-documented.

• The study goal is to test a seamless joining of Unknowns Analysis to the Quantitation workflow, and to evaluate the degree to which these methodologies can provide both target and unknowns information from one scan.

• Target and non-target analyses in the complex trace pesticide batch with overlapping matrix peaks were evaluated by comparing spectral scores and ion peak shape metrics.

Experimental

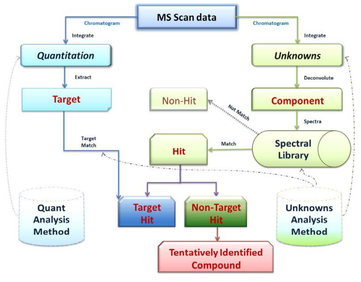
• The fruit juice extracts were prepared using the Agilent standard QuEChERs protocol. The extracts were evaporated to dryness and reconstituted in acetone.

• The pesticide standards were prepared in acetone at 10, 20, 50, 100, 200ng/mL with 2 ISTDs: 4,4′-Dibromooctafluorobiphenyl and Triphenyl phosphate at 0.5 ng/mL. Each standard was spiked with 50uL of the concentrated juice extract and analyzed in 3 replicates.

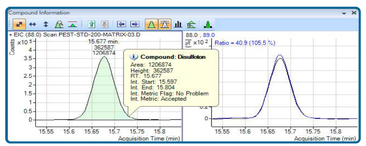
• The samples were analyzed by full-scan GC/MS using the instrumental arrangement as follows: 7890 GC with 5975C MSD utilizing the Rapid Universal GC/MS Backflushing kit (G1472A) and two 15-m (0.25mmx0.25µm) HP-5ms columns (part 19091S-431UI). The oven program was: 60°C (2.0 mins), 25°C/min to 150°C (0 mins), 3°C/min to 200°C (0 mins), 8°C/min to 325°C with constant flow of helium carrier at 1.2-mL/min.

• GC/MS analysis was performed by MassHunter Quant and Unknowns Analysis software.

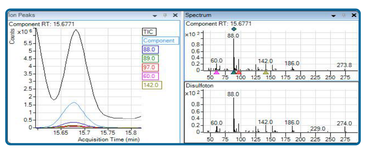
Quant → Unknowns Analysis



• **Quant Analysis** follows the steps of signal extraction, signal integration and quantitation using calibration curves to measure the concentration of targets. In Quant, the target ion is integrated for concentration calculation and the qualifier ratio serves as a confirmation metric.

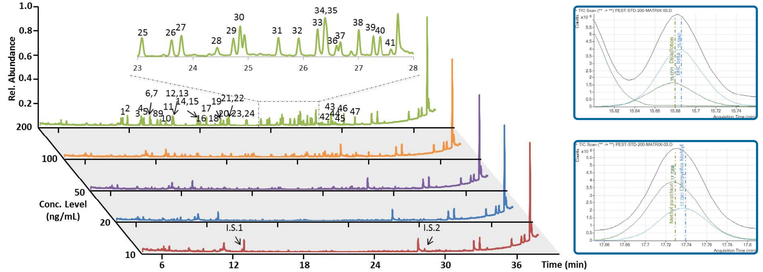


• **Unknowns Analysis** displays the deconvoluted ion peaks as well as the "clean" spectra compared to the library spectra to assist in evaluating the hit. Deconvolution may find more components than targets present. Reducing the list of components to isolate the tentatively identified compounds is the key operation.



Results and Discussion – Target Analysis

Figure. (Left) Total Ion Chromatograms (TICs) of the pesticide mix at 5 conc. levels with overlapping matrix. (Right) Two chromatographic views of the overlapping target pairs along with the TIC at the 200ng/mL sample.



No.	Target Name	R. T. (min)	CAS #
1	Demeton-O	11.402	298-03-3
2	Ethoprophos	11.797	13194-48-4
3	Sulfotep	12.003	3689-24-5
4	Phorate	13.045	298-02-2
5	BHC alpha isomer	13.182	319-84-6
6	Pentachloroanisole	13.702	1825-21-4
7	Dimethoate	13.765	60-51-5
8	BHC beta isomer	14.322	319-85-7
9	Lindane	14.584	58-89-9
10	Fonofos	15.013	944-22-9
11	Diazinon	15.588	333-41-5
12	Disulfoton	15.677	298-04-4
13	BHC delta isomer	15.684	319-86-8

No.	Target Name	R. T. (min)	CAS #
14	Methyl parathion	17.727	298-00-0
15	Chlorpyrifos Methyl	17.736	5598-13-0
16	Heptachlor	17.945	76-44-8
17	Fenchlorphos	18.466	299-84-3
18	Fenitrothion	19.194	122-14-5
19	Aldrin	19.668	309-00-2
20	Malathion	19.907	121-75-5
21	Chlorpyrifos	20.349	2921-88-2
22	Parathion	20.374	56-38-2
23	Heptachlor exo-epoxide B	21.835	1024-57-3
24	Oxychlordane	21.890	27304-13-8
25	trans-Chlordane	23.076	5103-74-2
26	Endosulfan (alpha isomer)	23.611	959-98-8

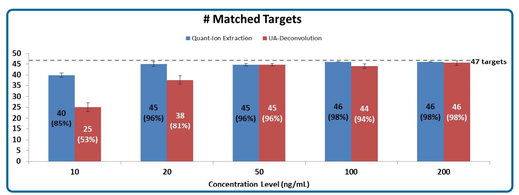
No.	Target Name	R. T. (min)	CAS #
27	cis-Chlordane	23.789	5103-71-9
28	Fenamiphos	24.439	22224-92-6
29	Dieldrin	24.729	60-57-1
30	p,p'-DDE	24.854	72-55-9
31	Endrin	25.545	72-20-8
32	Endosulfan (beta isomer)	25.909	33213-65-9
33	Fensulfothion	26.252	115-90-2
34	p,p'-DDD	26.381	72-54-8
35	Nonachlor, cis-	26.418	5103-73-1
36	Endrin aldehyde	26.598	7421-93-4
37	Ethion	26.664	563-12-2
38	Sulprofos	26.997	35400-43-2
39	Carbophenothion	27.263	786-19-6

No.	Target Name	R. T. (min)	CAS #
40	Endosulfan sulfate	27.386	1031-07-8
41	p,p'-DDT	27.589	50-29-3
42	Endrin ketone	28.758	53494-70-5
43	Phosmet	29.004	732-11-6
44	EPN	29.142	2104-64-5
45	Methoxychlor	29.340	72-43-5
46	Azinphos-methyl	30.082	86-50-0
47	Azinphos-ethyl	31.019	2642-71-9

No.	ISTD Name	R. T. (min)	CAS #
1	4,4'-Dibromooctafluorobiphenyl	12.803	10386-84-2
2	Triphenyl phosphate	28.266	115-86-6

• A mixture of 47 pesticide standards (targets) at 5 different concentration levels with 2 ISTD (top table) were spiked in fruit juice matrix and analyzed by GC/MS in replicate. There are 6 overlapping target pairs among the mixture where the TIC trace appears to show just a single peak and their retention time (RT) difference is less than 4 seconds. The figure above shows two example of the overlapping target pairs where the retention time differences are both less than 1 second.

• Two approaches were used for target analysis: 1) Quant ion extraction where the targets were confirmed based on retention time and qualifier ratios; 2) Unknown Analysis where the data were processed by spectral deconvolution and library search against a pesticide library containing 927 entries. The targets were the primary hits with match score over 50 and retention time difference to the library hit is less than 9 sec.



• At 10ng/mL, Quant confirmed 40 of 47 targets, but deconvolution only identified 25 targets. Low ID of both methods at 10ng/mL reflects the high influence of the matrix interference at low concentration.

• Deconvolution detects more targets as concentration increases. At 200ng/mL, both Unknowns Analysis and Quant confirmed 46 targets.

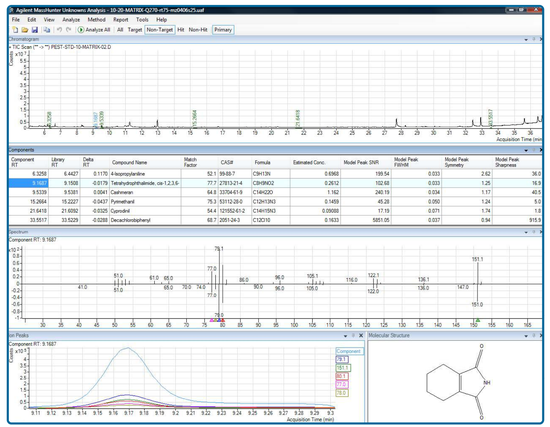
Results and Discussion – Non-Target Analysis

• Target Match step identifies both Target Hits and Non-Target Hits. Hits that are not target matched are labeled as non-target Hits. Estimation of contaminant concentrations leverages the Quant target response factors (RF) which are applied to Non-Target Hits. Estimation of response factors is flexible and can be adjusted to suit the particular analytical requirements. For example, the RF of the closest target or ISTD in retention time can be used to estimate the concentration of any hit.

Non-target Name	CAS#	R. T. (min)	RT Diff (min)	Match	Mean ppm
4-Isopropylaniline	99-88-7	6.325	0.117	52	0.69
Tetrahydrophthalimide, cis-1,2,3,6-	27813-21-4	9.168	-0.017	77	0.25
Cashmeran	33704-61-9	9.534	0.004	65	1.16
Pyrimethanil	53112-28-0	15.265	-0.042	70	0.16
Cyprodinil	121552-61-2	21.643	-0.034	56	0.08
Decachlorobiphenyl	2051-24-3	33.552	-0.029	68	0.16

• A total of 6 non-targets were identified in all samples by deconvolution. The estimated concentrations of each compound at different concentration level samples are consistent. The non-targets maybe the contaminants from the fruit juice matrix.

• The right figure displays the chromatogram of the six non-target compounds at 10ng/mL sample, which is highlighted in the top plots of the figure.



• The component information and the peak shape metrics of the non-targets are listed in the lower table.

• The non-target compound, Cis-1,2,3,6-tetrahydrophthalimide, is highlighted in Components. Its component spectrum along with the associated library hit is shown in the middle part of the figure.

• The Ion Peaks displayed in the lower part of the figure shows the peak shapes. The Molecular Structure is drawn for visual confirmation.

Conclusions

• The Quant → Unknowns Analysis workflow matches the library hits with the known targets so the user can focus on the Non-Target Hits. It also offers the opportunity to employ a number of criteria to speed up the classification of Non-target to Tentatively Identified Compounds.

• Batch review of both quantitative and unknowns results on one scale meets the escalating industry demands of food safety as well as other industries facing same productivity challenges. This workflow applied with Time-Of-Flight (TOF) mass spectrometers with their inherent advantages of scan speed and mass accuracy enriches that promise.