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Fast Scanning and Sensitivity for Real-Time Confirmation of Structural Isoforms Using a High-Performance Triple Quadrupole Mass Spectrometer

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ABSTRACT

Purpose: Demonstrate that Quantitation Enhanced Data-Dependent (QED) scanning can provide real-time, high-quality product ion spectra for compound confirmation and identification, with simultaneous quantitation.

Methods: Analysis performed on a Thermo Scientific™ TSQ Quantis™ triple quadrupole mass spectrometer using chromatography methodology from the Thermo Scientific™ Pesticide Explorer Collection. A mixture of pesticides in leek matrix (including some which were structural isomers of each other) were analyzed in QED mode. Data were processed using Thermo Scientific™ TraceFinder™ 4.1 software.

Results: Analysis of 260 pesticides in leek extract, demonstrating sensitivity at 10 ppb (10 mg/kg). In addition, we demonstrate automated, unambiguous identification of structural isomers, prometon & terbumeton (both $C_{10}H_{19}N_5O$) and prometryn & terbutryn (both $C_{10}H_{19}N_5S$) by matching MS/MS spectra against a spectral library.

INTRODUCTION

Analysis by high-performance liquid chromatography with triple quadrupole mass spectrometer detection (LC-MS/MS) in selected reaction monitoring (SRM) mode has become the gold standard for analysis in clinical,[1] forensic,[2] and environmental[3] applications, due to the technique's high throughput, sensitivity, and selectivity. As acceptance of LC-MS/MS has increased, the number of compounds and the variety of matrices to be analyzed has risen too, requiring additional levels of confirmation, while retaining high throughput, sensitivity, and selectivity.[1]

Although LC-MS/MS is a highly selective technique, some classes of compounds may require even higher levels of selectivity; steroid hormones are structurally similar to each other, with potential for in-source fragmentation, which may create isobars with non-unique SRM transitions.[1] Novel psychoactive substances are often derived from existing drugs that have been subtly modified,[4] and thus may share SRM transitions. Many pesticides are isomeric and require identification by both retention time and ion ratio.[5]

European legislation stipulates minimum reporting limits (MRLs) for pesticides in different food types. If no MRL is defined, a general default MRL of 0.01 mg/kg (10 ppb) applies.[6]

QED scanning, when used in SRM mode analysis, allows for acquisition of real-time MS/MS spectra, which can then be searched against spectral libraries to provide an additional level of compound confirmation and identification, while still providing high quality quantitative data.

We present data showing that isomeric pesticides can be quantitated in complex matrix at the default MRL of 10 ppb, with additional confirmation of identity by product ion scan.

MATERIALS AND METHODS

Sample Preparation

A QuEChERS leek extract was used as the matrix and was spiked with a 260 pesticide mixture at 1, 10, and 100 ppb.

Test Method

Analysis was performed on a TSQ Quantis triple-stage quadrupole mass spectrometer using chromatography methodology from the Thermo Scientific™ Pesticide Explorer Collection. In brief, a Thermo Scientific™ Accucore™ aQ column (2.1 x 100 mm, 2.6 µm) was used with mobile phases of 5 mM ammonium formate, 0.1% formic acid in water and methanol, and a run time of 15 minutes. A 1 µL aliquot of each spiked matrix sample was injected in triplicate.

Mass spectrometry conditions and SRMs were taken from the Thermo Scientific Pesticide Explorer Collection. The QED intensity trigger for selected compounds was set to 2e4 on two product ions for each precursor. The start and end collision energy for the QED scan were automatically calculated by the software.

Data Analysis

Data were processed using Thermo Scientific™ TraceFinder™ 4.1 software and Thermo Scientific™ mzVault™ 2.0 Library Management Tool.

RESULTS

Reported values of a selection of pesticides analyzed including two pairs of isomers (identified by color) showing accuracy (Amount Diff %) and precision (RSD %) of the assay. At 10 ppb accuracy and precision (n=3) are all less than 20%.

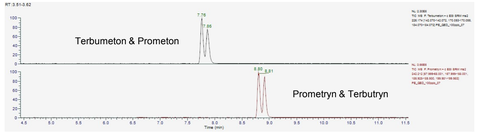
Table 1. Precision and accuracy for selection of 10 ppb pesticides.

Compound Name	Peak Area	Calculated Amount	Theoretical Amount	Amount Diff (%)	RSD (%)
Aldicarb_sulfone+H	5709	8.87	10	-11.29	8.39
Aminocarb	678756	11.31	10	13.07	9.19
Ancymidol	236384	9.60	10	-3.98	3.51
Anilofos	599677	10.71	10	7.06	9.04
Aramite+NH4	1001827	10.02	10	0.2	1.69
Atrazine	496830	10.10	10	1.02	2.51
Azaconazole	782437	10.29	10	2.89	4.03
Azamethiphos	345271	9.99	10	-0.09	0.61
azoxystrobin	1106093	10.42	10	4.21	5.3
Bendiocarb	152807	11.04	10	10.36	7.84
Benodanil	833159	9.63	10	-3.66	2.9
Benoxacor	62288	10.43	10	4.31	17.7
bentazon	67323	10.43	10	4.26	3.47
Benzoximate	288425	9.08	10	-9.18	7.28
Benzoylprop-ethyl	420616	9.47	10	-5.31	5.39
bioscalid	445474	9.65	10	-3.52	7.98
Brodifacoum	67746	10.48	10	4.8	4.62
Bromacil	230107	9.92	10	-0.8	3.56
bromuconazole	115856	9.08	10	-9.25	7.63
bupirimate	343905	9.22	10	-7.82	6.08
buprofezin	1536617	10.69	10	6.88	6.63
Butafenacil+NH4	623116	10.01	10	0.13	8.05
Carbaryl	27223	8.83	10	-11.73	13.14
Carbendazim	832605	10.40	10	3.97	3.68
Carbetamide	275585	9.45	10	-5.49	4.95
Promecarb	53682	8.04	10	-19.56	18.31
Prometon	1223170	9.72	10	-2.84	4.64
Prometryn	1535180	9.54	10	-4.6	5.86
Propamocarb	694050	9.35	10	-6.46	5.42
Propazine	482257	9.52	10	-4.79	3.8
Propiconazole	104448	8.15	10	-18.5	17.81
Propoxur	228946	9.27	10	-7.28	5.97
Propyzamide	254092	9.03	10	-9.72	12.7
Prosulfocarb	776408	9.45	10	-5.53	4.71
Pymetrozine	157642	10.91	10	9.08	7.04
Pyraclostrobin	338810	9.28	10	-7.2	8.58
Pyrimethanil	230110	10.16	10	1.61	4.94
Pyroxsulam	650894	10.16	10	1.6	2.75
Quinoxyfen	362724	9.61	10	-3.87	5.03
Quizalofop-ethyl	845100	10.20	10	2.02	11.77
Rotenone	152735	8.77	10	-12.35	14.45
Schradan	624488	10.20	10	2.03	2.96
Sethoxydim	150232	9.06	10	-9.42	17.72
Simeconazole	540507	10.71	10	7.09	7.4
Simetryn	667752	9.61	10	-3.87	4.51
Spinosad A	233472	10.00	10	-0.03	8.05
Spiromesifen	11804	9.42	10	-5.82	16.26
Spirotetramat	298885	10.15	10	1.53	6.31
Spiroxamine	1390770	10.19	10	1.94	6
Sulfotep	565260	10.57	10	5.71	4.9
Sulprofos	238751	10.85	10	8.5	6.29
Tebuconazole	595111	10.76	10	7.58	7.38
Tebufenozide	206839	9.31	10	-6.92	5.67
Tebufenpyrad	368387	9.54	10	-4.58	7.69
Tebuthiuron	1571935	10.16	10	1.62	1.86
Tepraloxydim	95656	9.40	10	-5.98	7.59
Terbumeton	1523486	9.88	10	-1.18	2.22
Terbuthylazine	1277004	9.89	10	-1.06	1.93
Terbutryn	2157803	10.14	10	1.42	6.1
Tetraconazole	318539	9.17	10	-8.34	15.51
Thiabendazole	427700	10.19	10	1.87	3.51
Thiacloprid	663824	9.98	10	-0.22	1.12

Potential Ambiguity of Detection of Isomeric Pesticides

Isomeric pesticides may produce the same fragment ions, which without careful consideration of selective product ions, may mean multiple peaks are observed for the same SRM transitions.

Figure 1. 100 ppb terbumeton & prometon and prometryn & terbutryn with non-selective SRMs.



A Super-Fast Instrument is Required to Monitor All SRMs and MS/MS Scans

Even using scheduled time windows, the large number of concurrent SRMs when monitoring hundreds of pesticides places a huge burden on the mass spectrometer. Enough scans are required to adequately describe the chromatographic peak, while still maintaining excellent ion statistics, even at low concentrations. The TSQ Quantis MS is capable of running 600 SRMs per second. This super-fast scanning ability allows all SRMs to be monitored, as well as having sufficient scan time to generate QED product ion spectra, of high enough quality, that they can be searched against a spectral library (see Figure 2 and Figure 3)

Figure 2. 10 ppb prometon and terbumeton showing scans across the peak and QED product ion scans.

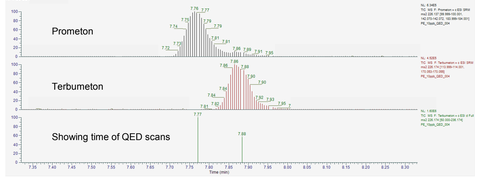
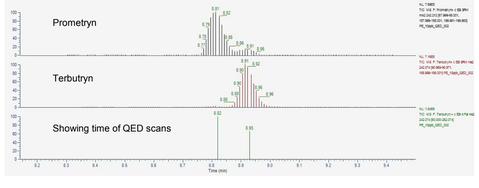


Figure 3. 10 ppb prometryn and terbutryn showing scans across the peak and QED product ion scans.



Confirmation by Library Search Using TraceFinder 4.1 Software

QED product ion spectra are searched against the mzVault database with TraceFinder 4.1 software. Even at 10 ppb levels, the number of fragment ion matches, mass accuracy, and relative intensities are very consistent, giving high confidence in identity confirmation.

Figure 4. Prometon at 10 ppb, Experimental product ion scan (top trace) match to Library scan (bottom trace).

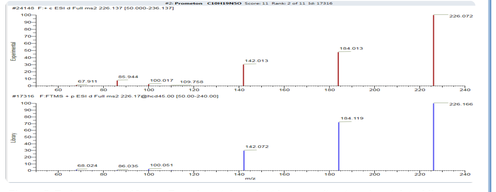


Figure 5. Terbumeton at 10 ppb, Experimental product ion scan (top trace) match to Library scan (bottom trace).

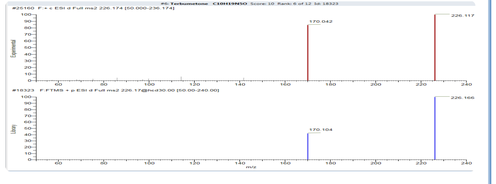


Figure 6. Prometryn at 10 ppb, Experimental product ion scan (top trace) match to Library scan (bottom trace).

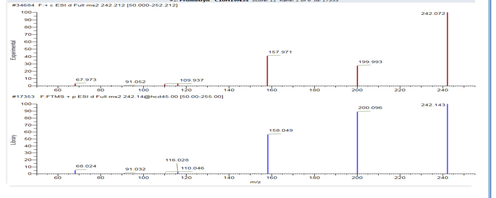
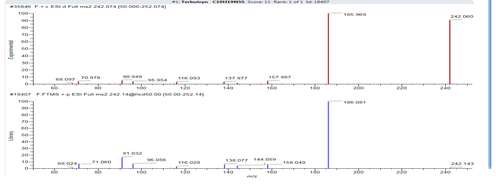


Figure 7. Terbutryn at 10 ppb, Experimental product ion scan (top trace) match to Library scan (bottom trace).



CONCLUSIONS

- QED can be used to give an additional level of confidence to the identification of pesticides, especially isomeric pesticides. Identification can now be made using SRMs, retention time, product ion ratios, and product ion scans searched against a spectral library. This reduces the possibility for false positives to be reported.
- QED methodology can be added to existing methods, with only the input of a threshold for triggering the product ion scan required from the user.
- Utilizing an instrument capable of targeting hundreds of SRMs per second, incorporating QED has little or no impact on quantitative performance. Both accuracy and precision remain excellent.

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TRADEMARKS/LICENSING

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