Application Note: 323

Simultaneous Detection of 88 Pesticides on the TSQ Quantum Discovery Using a Novel LC-MS/MS Method

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Key Words

- TSQ Quantum Discovery™
- Cross-talk
- EPA
- LC-MS/MS
- Pesticides

Overview

Pesticide residues in food are strictly regulated according to the provisions of US Environmental Protection Agency (EPA) CFR Title 40. Several hundred sections in Part 180 detail the maximum pesticide residue (tolerance) for a wide variety of foods. A pesticide's allowable tolerance (measured in ppm) can span several orders of magnitude, depending upon the food source. For example, the tolerance for captan in cattle fat is 0.05 ppm, while 100 ppm of captan is acceptable in lettuce and spinach.

To analyze the large numbers of samples whose pesticide treatment history is usually unknown, the US Food and Drug Administration (FDA) uses analytical methods capable of simultaneously determining a number of pesticide residues. These cost-effective multi-residue methods (MRMs) can determine about half of the approximately 400 pesticides and their metabolites with EPA tolerances. Most commonly, residues in extracts are separated by GC or HPLC, and then detected using UV absorbance, nitrogen phosphorus detection, or electron capture detection.

Due to its specificity in identifying compounds, LC-MS/MS is emerging as the technique of choice for identifying and quantifying pesticides. The most commonly used MRMs can also detect many metabolites, impurities, and alteration products of pesticides.

Conventional MS/MS methods generally require extensive optimization of operating parameters for each target analyte or even for compounds belonging to the same chemical class, significantly impacting analytical throughput. The objective of this work was to demonstrate the use of the Thermo Scientific TSQ Quantum Discovery in developing an automated, generic, high-throughput LC-MS/MS screening method to simultaneously detect and quantitate nearly 100 pesticides following minimal separation using an HPLC.

Goals

- Develop a multi-residue LC-MS/MS screening method to detect 88 analytes using a single, automated experiment with a short chromatographic time scale
- Demonstrate the utility of using different time segments and scan events
- Illustrate the large linear dynamic range for pesticide analysis in a multi-residue context
- Exhibit the absence of "cross-talk" between co-eluting components

Experimental Conditions

Chemicals and Reagents

Water, methanol, and acetic acid were HPLC grade and purchased from J. T. Baker Chemicals, France.

Samples

Pesticides listed in Table 1 were purchased from Sigma unless otherwise noted. Standards solutions of 0.1, 0.5, 1, 5, 10 and 50 pg/µL were prepared in methanol.

Sample Analysis

HPLC analysis was performed on the Thermo Scientific Surveyor™ HPLC System, using a Thermo Scientific AQUASIL C18 50×2.1 mm column. Mobile phase A was water/methanol 80/20 (v/v) and mobile phase B was methanol/water 90/10 (v/v) – both contained 0.05% acetic acid. Solvent was pumped at 200 µL/min and analytes eluted using a linear gradient of 100% A to 100% B over 11 minutes, holding at 100% B for 12 minutes, then returning to 100% A in 2 minutes.

Mass Spectrometry

Instrument: TSQ Quantum Discovery
Source: ESI
Ion polarity: Positive
Spray voltage: 3.5 kV
Sheath/Auxiliary gas: Nitrogen
Sheath gas pressure: 50 (arbitrary units)
Auxiliary gas pressure: 15 (arbitrary units)
Ion transfer capillary temperature: 350 °C
Scan type: SRM
CID conditions: Ar at 1.5 mTorr

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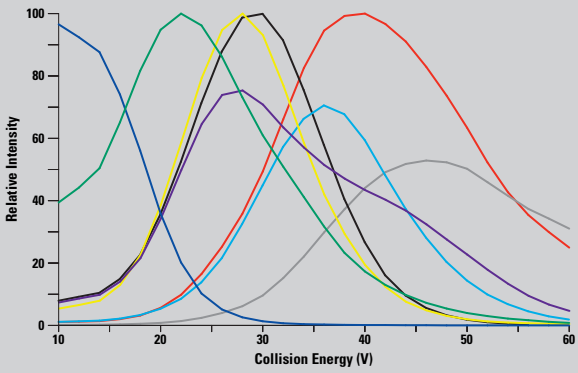


COMPOUND	PARENT M/Z	PRODUCT M/Z	COLLISION ENERGY V
Bendiocarb	224.1	167.1	10
Bendiocarb	224.1	109.0	22
Pyrimethanil	200.1	107.1	28
Pyrimethanil	200.1	182.1	28
Thiabendazol	202.0	175.0	30
Thiabendazol	202.0	131.1	36
Cyprodinil	226.1	93.1	40
Cyprodinil	226.1	77.1	46

Figure 1a: Simultaneous multicomponent optimization of MS/MS parameters

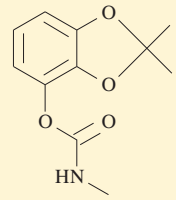
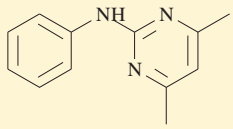
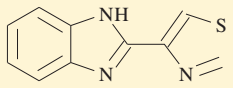
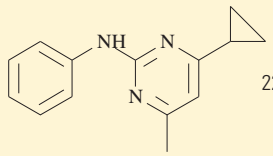
Pesticide	Structure	Mwt (da)	Use
Bendiocarb		223	Insect control against public health, industrial & storage pests
Pyrimethanil		199	Fungal control on vine/fruits, ornamentals
Thiabendazol		201	Fungal control on cotton, barley, bananas
Cyprodinil		225	Fungal control in cereals, grapes, strawberries

Figure 1b: Structures of the four pesticides used to generate the optimization graph of Figure 1a

Multi-residue Optimization

One of the most time consuming parts in the development of a large multicomponent assay is the optimization of MS/MS parameters for each analyte. The TSQ Quantum Discovery allows multicomponent optimization of MS/MS parameters to be carried out automatically, thus allowing for faster method development. Up to eight SRM transitions can be optimized simultaneously, either from a single parent component or from multiple components. In effect, this means the ability to carry out the optimization procedure 11 times for 88 pesticides (instead of 88 times if they were carried out singly), thus saving a significant amount of time in method development. An example of this is given in Figure 1a, displaying the simultaneous optimization of eight SRM transitions from four pesticides. The structures of these compounds are shown in Figure 1b.

MS Instrument Method

To accommodate such a large number of components over a short time range, the acquisition time was divided into two segments, each containing three scan events. Allowing for analyte overlap between the time segments, a total of 59 SRM transitions were performed in segment one and 56 SRM transitions in segment two, with dwell times of 20 ms for each transition. A graphical representation of the actual instrument method is shown in Figure 2.

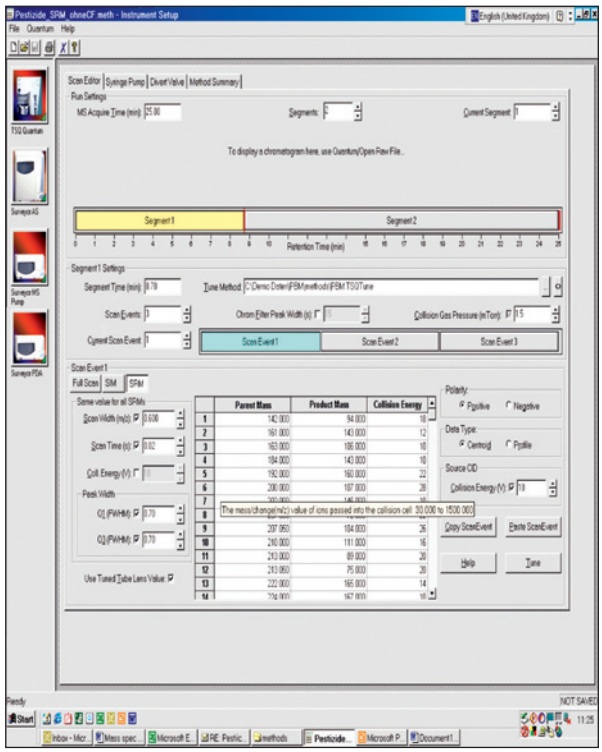


Figure 2: Splitting the acquisition time into two time segments and three scan events improves instrument performance for complex screening analyses

Results and Discussion

Figure 3a shows the LC-MS/MS chromatogram generated from the pesticide mix eluting over a chromatographic time scale of 16 minutes. The complexity of the chromatogram can be seen by expanding the area from 8 to 11 minutes (Figure 3b), where several different pesticides can typically be observed to co-elute.

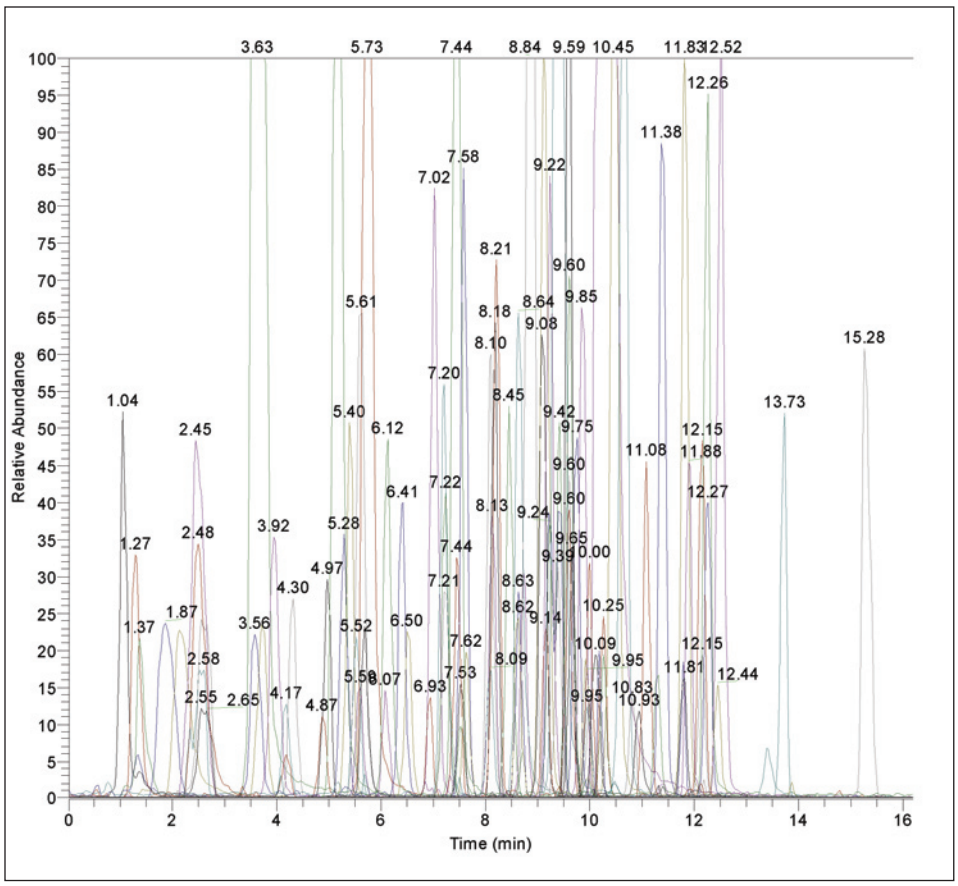


Figure 3a: LC-MS/MS chromatogram of 88 pesticides at 50 pg/µL

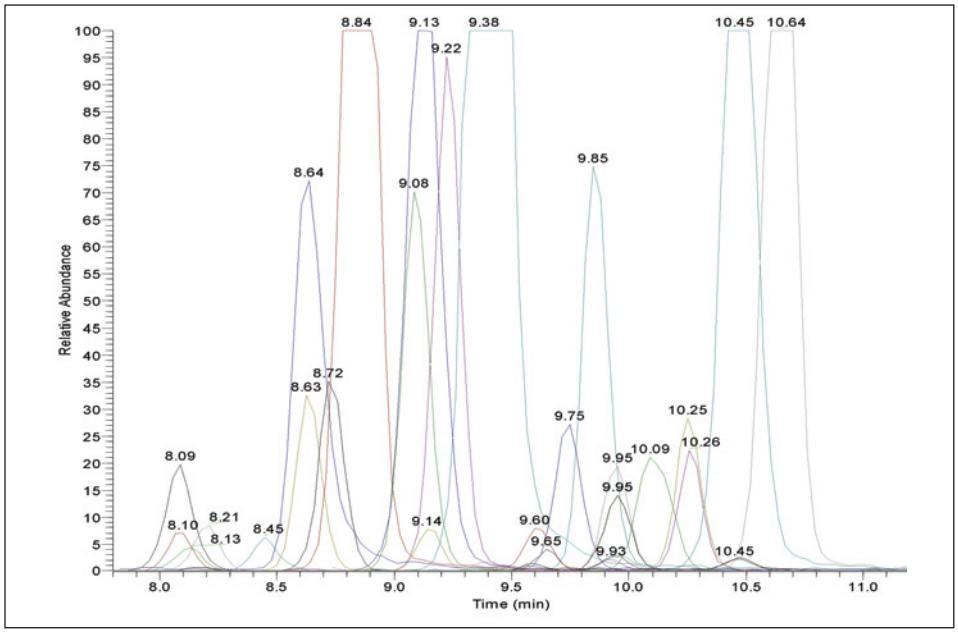


Figure 3b: Detection of minor components under the larger peaks

All compounds were mixed at the same concentration and the SRM method allows even those with low responses to be detected under other analytes. A summary of the results for these pesticides at 50 pg/µL is tabulated in Table 1. As is clearly evident, excellent linearity was observed with the coefficient of correlations of most components varying from 0.9900 to 0.9998.

Table 1: Results of pesticide analysis at 50 pg/µL

Component Name	RT	Area	Specified Amount	Calculated Amount	Equation
Daminozoid	1.04	4674516	50.000	48.743	Y = 56652 + 94739.2 × R^2 = 0.9988
Methamidophos	1.27	3829646	50.000	49.984	Y = –27105.9 + 77160.3 × R^2 = 0.9992
Acephate	1.37	2535282	50.000	47.557	Y = 51553.4 + 52226.8 × R^2 = 0.9955
Omethoat	1.87	1516561	50.000	51.212	Y = –20184.5 + 30007.4 × R^2 = 0.9985
Propamocarb	2.13	4988264	50.000	49.355	Y = –31459.9 + 101707 × R^2 = 0.9997
Aldicarb-sulfoxid	2.45	15089	50.000	90.324	Y = 1383.55 + 151.735 × R^2 = 0.4642
Butocarboxim-sulfoxid	2.45	92079	50.000	59.539	Y = –5384.2 + 1636.97 × R^2 = 0.9372
Butoxycarboxim	2.62	30232	50.000	63.008	Y = –761.85 + 491.892 × R^2 = 0.8296
Aldoxycarb	2.55	210393	50.000	49.163	Y = –5547.18 + 4392.29 × R^2 = 0.9977
Pymetrozin	2.48	6401121	50.000	50.687	Y = –40420.7 + 127084 × R^2 = 0.9995
Carbendazim	3.63	67750507	50.000	49.534	Y = –61584.2 + 1.369e + 006 × R^2 = 0.9999
Methomyl	3.56	298259	50.000	57.081	Y = 9692.33 + 5055.42 × R^2 = 0.9934
Demeton-S-methyl-sulfon	3.71	3006988	50.000	49.419	Y = –13010.2 + 61109.5 × R^2 = 0.9995
Oxidemeton-methyl			50.000	N/F	Y = –38265.8 + 97760.8 × R^2 = 0.9989
Monocrotophos	4.17	1366861	50.000	52.403	Y = –16543.9 + 26399.4 × R^2 = 0.9964
Ethiofencarb-sulfon	4.30	277016	50.000	48.786	Y = –1760.21 + 5714.23 × R^2 = 0.9983
3-hydroxy-carbofuran	4.97	292068	50.000	51.737	Y = –3310.44 + 5709.23 × R^2 = 0.9871
Ethiofencarb-sulfoxid	4.87	1110901	50.000	48.253	Y = –24680.6 + 23533.9 × R^2 = 0.9964
Thiabenzadol	5.17	24648309	50.000	49.962	Y = –58379.8 + 494513 × R^2 = 0.9999
Dimethoat	5.28	3611384	50.000	50.512	Y = –18998 + 71871.1 × R^2 = 0.9997
Vamidothion	5.40	472217	50.000	51.537	Y = –5505.94 + 9269.53 × R^2 = 0.9982
Imidacloparid	5.59	1506719	50.000	52.085	Y = –9529.36 + 29111.3 × R^2 = 0.9969
Metamitron	5.52	2252454	50.000	49.143	Y = 1941.62 + 45795.4 × R^2 = 0.9993
Quinmerac	5.58	7922156	50.000	50.826	Y = –21139.4 + 156284 × R^2 = 0.9995
Clethodim-imin-sulfon	5.67	2327412	50.000	48.892	Y = –1206.9 + 47627.9 × R^2 = 0.9992
Pirimicarb	5.73	20969231	50.000	50.280	Y = –50637 + 418055 × R^2 = 0.9999
Clethodim-imin-sulfoxid	6.12	5154573	50.000	49.645	Y = 4532.14 + 103738 × R^2 = 0.9998
Butocarboxim	6.41	3903019	50.000	48.852	Y = –2919.87 + 79954.2 × R^2 = 0.9992
Aldicarb	6.50	2151719	50.000	49.942	Y = 11575.3 + 42852.7 × R^2 = 0.9998
PyridateXX	7.02	8244302	50.000	49.736	Y = –6792.58 + 165899 × R^2 = 0.9999
Thiacloprid	7.20	5497747	50.000	50.557	Y = –37790.7 + 109491 × R^2 = 0.9997
Propoxur	7.21	2762590	50.000	49.906	Y = –7780.97 + 55512 × R^2 = 0.9996
Thiophanat-methyl	7.53	1420464	50.000	50.642	Y = 731.145 + 28034.9 × R^2 = 0.9989
Bendiocarb	7.44	917258	50.000	49.829	Y = –4657.68 + 18501.5 × R^2 = 0.9991
Carbofuran	7.44	19195703	50.000	49.441	Y = –84755.6 + 389971 × R^2 = 0.9996
Cinosulfuron	7.58	794473	50.000	47.468	Y = –5092.19 + 16844.4 × R^2 = 0.9956
Triasulfuron	7.62	440622	50.000	48.954	Y = –7849.1 + 9161.13 × R^2 = 0.9973
5-hydroxy-clethodim-sulfon	8.13	356038	50.000	49.817	Y = –1197.11 + 7170.92 × R^2 = 0.9995
Ethiofencarb	8.09	1655866	50.000	51.049	Y = –2896.19 + 32493.3 × R^2 = 0.9989
Metsulfuron-methyl	8.10	534961	50.000	52.066	Y = –8787.37 + 10443.5 × R^2 = 0.9964
Nicosulfuron	8.18	55407	50.000	43.245	Y = –7332.14 + 1450.79 × R^2 = 0.9559
Carbaryl	8.21	665994	50.000	53.046	Y = –8256.25 + 12710.6 × R^2 = 0.9937
Chlorosulfuron	8.45	490049	50.000	49.618	Y = –7636.28 + 10030.3 × R^2 = 0.9977
Isoxaflutole	8.63	2483727	50.000	50.356	Y = –30166.7 + 49922.5 × R^2 = 0.9996
Amidosulfuron	8.58	44104	50.000	51.395	Y = 1474.67 + 829.433 × R^2 = 0.9605
Metalaxyl	8.72	2766611	50.000	50.460	Y = –19113 + 55207.1 × R^2 = 0.9998
Imazalil	8.64	7172377	50.000	51.281	Y = –146813 + 142726 × R^2 = 0.9951
Atrazin	8.84	22304226	50.000	49.756	Y = –79337.7 + 449862 × R^2 = 0.9998
3,4,5-Trimehacarb	9.08	5876561	50.000	49.047	Y = –31771.1 + 120463 × R^2 = 0.9994
Clethodim-sulfon	9.14	606123	50.000	47.974	Y = –6241.14 + 12764.5 × R^2 = 0.9963
Desmedipham	9.42	1513363	50.000	48.104	Y = –38755.8 + 32266.2 × R^2 = 0.9944
Phenmedipham	9.43	1349750	50.000	49.328	Y = –31936.5 + 28010.3 × R^2 = 0.9982
Pyrimethanil	9.13	11739201	50.000	49.761	Y = –72893 + 237379X × R^2 = 0.9996
Isoproturon	9.22	7708543	50.000	50.569	Y = –36587.8 + 153161 × R^2 = 0.9996
Fenpropimorph	9.38	41217540	50.000	50.340	Y = –1.58954e + 006+850358 × R^2 = 0.9877
Thiodicarb	9.39	131141	50.000	46.936	Y = –1721.31 + 2830.73 × R^2 = 0.9925
Flazasulfuron	9.65	275363	50.000	51.509	Y = –8454.51 + 5510.05 × R^2 = 0.9964
Bensulfuron-methyl	9.57	337368	50.000	44.644	Y = –60.8872 + 7558.17 × R^2 = 0.9836
Clethodim-sulfoxid	9.60	673868	50.000	47.765	Y = –9675.27 + 14310.6 × R^2 = 0.9951
Diuron	9.75	2269026	50.000	50.361	Y = –9647.22 + 45246.5 × R^2 = 0.9996
Prosulfuron	9.93	166599	50.000	42.400	Y = –2469.03 + 3987.42 × R^2 = 0.9581
Azoxystrobin	9.85	5924744	50.000	50.076	Y = –109126 + 120494 × R^2 = 0.9973
Methiocarb	9.95	1164890	50.000	49.681	Y = –8196.01 + 23612.5 × R^2 = 0.9991
Promecarb	9.95	1530917	50.000	51.120	Y = –20134.2 + 30341.5 × R^2 = 0.9990
Iprovalicarb	10.09	2190910	50.000	50.988	Y = –17552.3 + 43313.4 × R^2 = 0.9994
Fenhaxamid	10.25	2163320	50.000	51.113	Y = –21459.1 + 42744.1 × R^2 = 0.9990
Linuron	10.26	1668513	50.000	48.797	Y = –26949.6 + 34745.1 × R^2 = 0.9980
Triflusulfuron-methyl	10.19	5971	50.000	54.571	Y = 2451.06 + 64.4984 × R^2 = 0.6233
Cyprodinil	10.45	14661158	50.000	50.662	Y = –222152 + 293778 × R^2 = 0.9982
Spiroxamine	10.39	75041736	50.000	50.560	Y = –2.9315e + 006 + 1.54218e + 006 × R^2 = 0.9880
Metolachlor	10.64	15042434	50.000	49.975	Y = –148631 + 303973 × R^2 = 0.9992
Tebufenzoid	10.83	1226295	50.000	55.610	Y = –14352.5 + 22309.7 × R^2 = 0.9985
Thiofanox	10.93	118596	50.000	-129.926	Y = 110983 – 58.591 × R^2 = 0.0058
Fenoxycarb	11.08	409691	50.000	49.579	Y = –27661.2 + 8821.35 × R^2 = 0.9956
Fentin-hydroxide	11.02	4603640	50.000	50.016	Y = –89525.3 + 93833.1 × R^2 = 0.9963
Diflubenzuron	11.30	700297	50.000	49.001	Y = –7945.49 + 14453.7 × R^2 = 0.9879
Tebuconazol	11.38	7987902	50.000	49.908	Y = –170977 + 163478 × R^2 = 0.9964
Rimsulfuron			50.000	N/F	N/A
Haloxyfop-methyl	11.83	8823982	50.000	48.905	Y = –344151 + 187469 × R^2 = 0.9885
Indoxacarb	11.88	370334	50.000	43.918	Y = –28514.8 + 9081.59 × R^2 = 0.9678
Triflumuron	11.81	1448162	50.000	50.860	Y = –72372.4 + 29896.2 × R^2 = 0.9902
Clethodim	12.15	856888	50.000	59.816	Y = –5040.92 + 14409.7 × R^2 = 0.9991
Fluzifop-P-butyl	12.26	8028262	50.000	51.498	Y = –365417 + 162992 × R^2 = 0.9866
Haloxyfop-ethoxyethyl	12.27	3551906	50.000	52.226	Y = –131575 + 70529.2 × R^2 = 0.9887
Flurathiocarb	12.44	1424774	50.000	48.972	Y = –65299.8 + 30427.4 × R^2 = 0.9828
Quizalofop-ethyl	12.52	9664875	50.000	51.959	Y = –380173 + 193325 × R^2 = 0.9886
Flufenoxuron	13.73	40186	50.000	44.371	Y = –10159.7 + 1134.66 × R^2 = 0.9607
Pyridate	15.28	66084	50.000	40.512	Y = –18878.2 + 2097.21 × R^2 = 0.8556

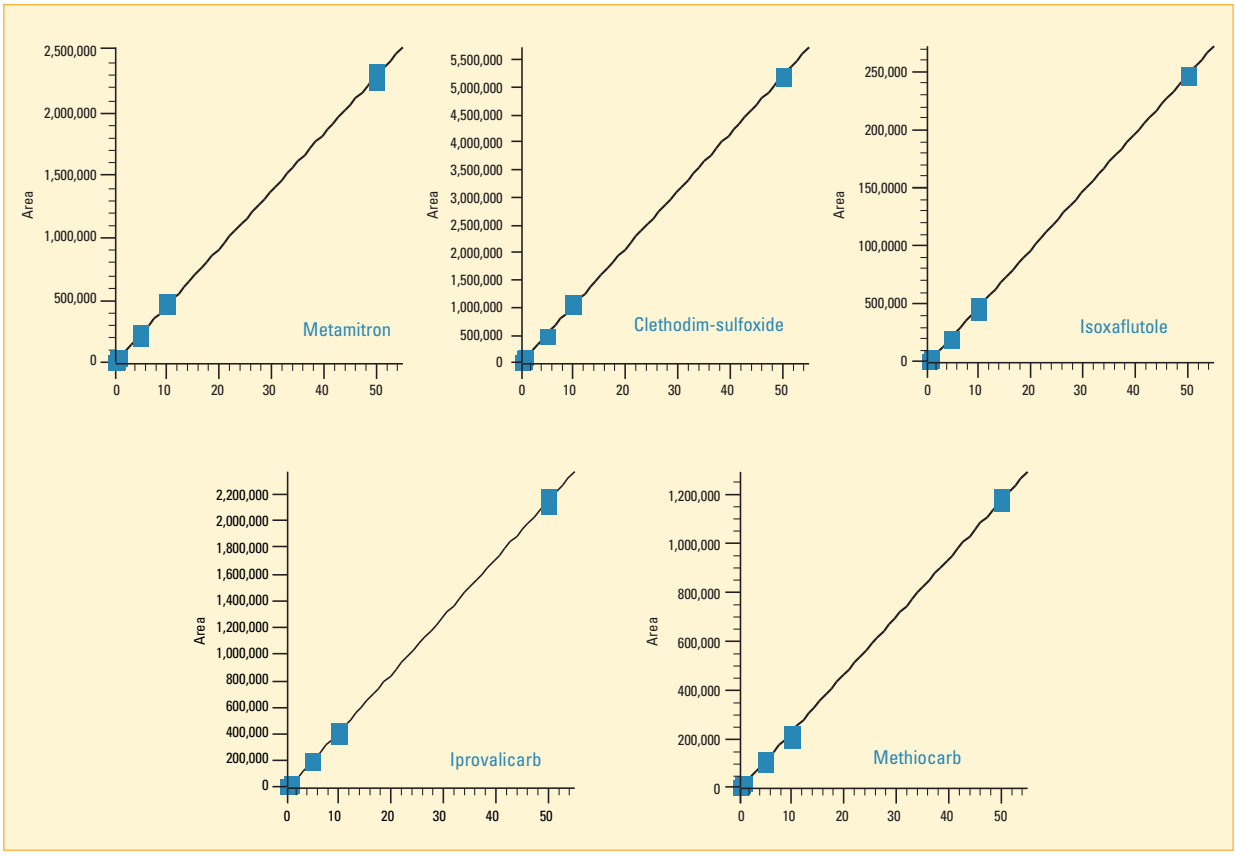


Figure 4. Linearity of response for the five components: metamitron, clethodim-sulfoxide, isoxaflutole, iprovalicarb, methiocarb

Linearity

Peak areas were used for quantitation and the resultant linearity of responses are plotted in Figure 4 for five different compounds. Although no internal standard was used during the assay, excellent correlation coefficient values were observed between 0.9990 and 0.9998 for the five components metamitron, clethodimin-sulfoxide, isoxaflutole, iprovalicarb, and methiocarb.

Absence of Cross-talk

High-throughput characterization of very complex mixtures requires rapid analysis of coeluting analytes. An effective way to accomplish this is by reducing the dwell and interscan times. However, cross-talk can occur in triple quadrupole instruments when short scan times are employed because the fragment ions from one SRM transition are often scanned out during another transition. This is due to some fragment ions from one transition still residing in the collision cell when the next transition starts, resulting in signal artifacts. However, the patented design of the orthogonal collision cell of the TSQ Quantum Discovery virtually eliminates cross-talk.

This was demonstrated during the pesticide assay by monitoring the SRM transitions of three components: triasulfuron, metasulfuron-methyl and chlorosulfuron. These compounds have different precursor ions but all generate a product ion at *m/z* 167 (see Table 2). The chromatograms in Figure 5 show the transitions for these compounds, with no evidence of any cross-talk.

Pesticide	Retention time (min)	SRM transition (*m/z*)
Triasulfuron	7.62	402 > 167
Metasulfuron-methyl	8.10	382 > 167
Chlorosulfuron	8.45	358 > 167

Table 2: Characteristics of the three pesticides – triasulfuron, metasulfuron-methyl, and chlorosulfuron – used to demonstrate zero cross-talk on the TSQ Quantum Discovery

Conclusions

An LC-MS/MS screening assay to monitor 88 pesticides using minimal LC separation was developed using the TSQ Quantum Discovery. It was possible to detect all components within a chromatographic time scale of 16 minutes by performing SRM transitions during two user-determined time segments. Even with dwell times of only 20 ms, no cross-talk interference was observed during the analysis.

Typical food monitoring applications require screening for tens to hundreds of pesticides. Although conventional detection is accomplished using UV absorbance, nitrogen phosphorus, or electron capture detection, LC-MS/MS provides superior sensitivity, and more importantly, specificity of identification as compared with these other commonly used techniques. The LC-MS/MS-based method described here, with its speed, sensitivity, and specificity, is highly applicable to both the environmental monitoring and agrochemical industries operating within EPA and FDA criteria.

CD-ROM

The data generated for this application note, along with the instrument and processing methods, are available on a CD-ROM from Thermo Fisher Scientific at www.thermo.com/quantum.

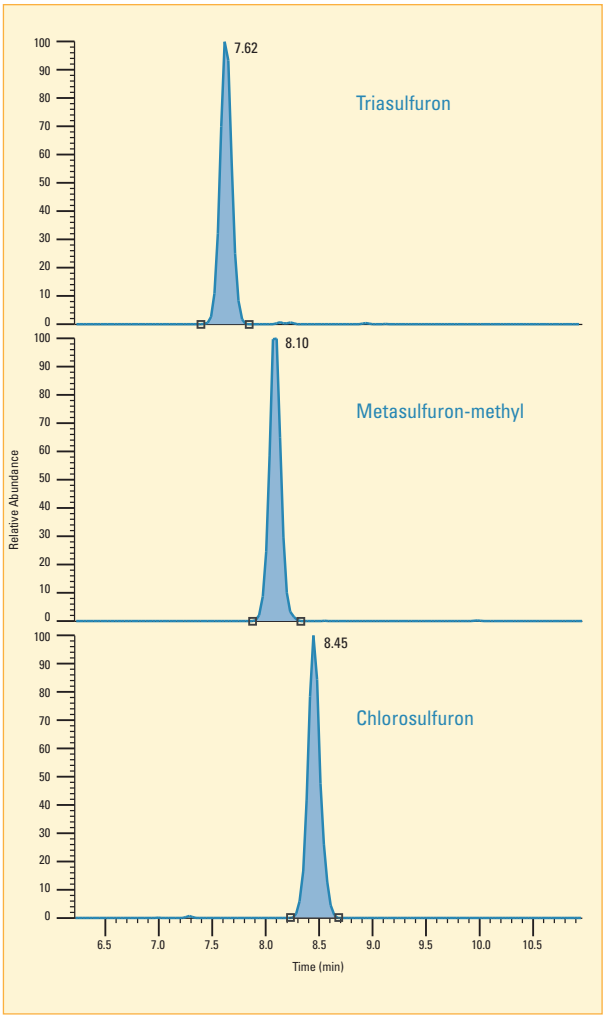


Figure 5: No cross-talk was observed when the pesticides triasulfuron, metsulfuron-methyl, and chlorosulfuron were detected

References

U.S. Environmental Protection Agency website at www.epa.gov: US Environmental Protection Agency Code of Federal Regulations 40. Chapter I, Subchapter E, Part 180 details the tolerances and exemptions from tolerances for pesticide chemicals in food.

Pesticide Analytical Manual Volume 1, Sections 605-606 (describes MS applications and benefits). Transmittal No. 94-1 (1/94), Form FDA 2905a (6/92). Available on the FDA website at www.cfsan.fda.gov. Chapter 3 describes multi-class multi-residue methods, while Chapter 4 provides selective multi-residue methods.

View additional Thermo Scientific LC/MS application notes at: **www.thermo.com/appnotes**

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