Application Note: 355

Utility of H-SRM to Reduce Matrix Interference in Food Residue Analysis of Pesticides by LC-MS/MS Using the TSQ Quantum Discovery

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Key Words

- TSQ Quantum Discovery™
- Surveyor™ HPLC
- H-SRM
- Pesticides
- Zero Cross-talk

Introduction

With the recent trend of increased concern about food safety, the number of regulated pesticide residues in food has increased rapidly. In Japan, a new positive list system for monitoring pesticide residues will take effect in 2006. Consequently, an accurate high throughput multi-pesticide screening method which can quantitate high number of pesticide residues during a single analysis is required.

LC-MS/MS is fast becoming the technique of choice for the identification and quantitation of pesticide residues. This is due, in part, to the ease of sample preparation and chromatographic conditions that LC-MS/MS allows, when compared to other techniques such as GC or HPLC with UV absorbance, nitrogen phosphorus detection, or electron capture detection. However, it can be extremely challenging to quantitate multi-pesticide residues in food because of interference from complex sample matrices. Although matrix-related interferences can be decreased by various sample clean up procedures, the analytical instrument used for the quantitation also has to be highly selective and sensitive. The unique Highly-Selective Reaction Monitoring (H-SRM) detection method available with the Thermo Scientific TSQ Quantum has proven to be very useful for this purpose. The analytical results of 35 pesticide residues in food with the H-SRM detection method are reported in this application note.

Goals

- Illustrate the effectiveness of H-SRM for reducing background interference and improving s/n
- Develop a multi-residue LC-MS/MS screening method to detect 35 pesticides, and
- Exhibit the absence of “cross-talk” between co-eluting components.

Experimental Conditions

Sample Analysis

HPLC analysis was performed on the Thermo Scientific Surveyor HPLC System, using a Thermo Scientific HyPURITY™ C18 150 × 2.1 mm 5 µm column. Mobile phase A was water, mobile phase B was methanol, and mobile phase C was water containing 10 mM ammonium acetate. Solvent was pumped at 200 µL/min and analytes eluted using a linear gradient of 20% B to 99% B over 15 minutes, holding at 99% B for 3 minutes, and then returning to 20% B for 5 minutes. Mobile phase C was held at 1% throughout the run.

Mass Spectrometry

Instrument: TSQ Quantum Discovery

Positive ESI

Spray Voltage: 5kV
Sheath/Auxiliary gas: Nitrogen
Sheath gas pressure: 40 (arbitrary units)
Auxiliary gas pressure: 40 (arbitrary units)
Ion transfer capillary temperature: 380 °C
Scan type: SRM or H-SRM
CID conditions: Ar at 1.0 mTorr

Negative ESI

Spray Voltage: 4.25 kV
Sheath/Auxiliary gas: Nitrogen
Sheath gas pressure: 50 (arbitrary units)
Auxiliary gas pressure: 5 (arbitrary units)
Ion transfer capillary temperature: 350 °C
Scan type: SRM or H-SRM
CID conditions: Ar at 1.0 mTorr

MS Instrument Method

Thirty-five pesticide residue compounds were analyzed to find the product ion to be used for quantitation. Three of the compounds were ionized using negative electrospray, while the remaining 32 were ionized using positive electrospray in two different runs. A table of the compounds listing SRM transitions and the optimum collision energy are shown in Table 1.

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Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)	Retention Time (min)
Oxamyl	237.17	72.0	15	3.9
Imidacloprid	256.12	209.1	16	6.3
Acetamiprid	223.12	126.0	23	7.3
Aldicarb	208.17	116.0	8	9.0
Propoxur	210.16	111.0	14	10.3
Carbofuran	222.16	165.1	14	10.4
Bendiocarb	224.14	167.0	10	10.4
Carbaryl	202.15	145.0	10	11.0
Ethiofencarb	226.13	107.0	14	11.3
Pirimicarb	239.22	182.1	16	11.5
Methabenzthiazuron	222.12	165.0	17	11.9
MIPC	194.17	95.0	20	11.9
Diuron	233.06	72.1	19	12.4
Azoxystrobin	404.17	372.1	15	12.8
BPMC	208.19	152.0	10	13.1
Siduron	233.20	137.0	17	13.2
Linuron	249.09	182.0	18	13.2
Methiocarb	226.14	169.1	10	13.4
Daimuron	269.21	151.1	14	13.7
Cumyluron	303.14	185.0	14	13.9
Tebufenozide	353.24	133.0	19	14.7
Iprodione	330.07	245.1	15	14.7
Diflubenzuron	311.04	158.0	14	14.8
Etobenzanid	340.08	121.0	36	15.2
Cyprodinil	226.18	93.0	38	15.2
Phoxim	299.08	129.0	12	15.4
Bitertanol	338.21	269.2	10	15.6
Hexythiazox	353.13	228.0	16	16.8
Piperonyl butoxide	356.26	177.1	13	17.2
Flufenoxuron	489.09	158.0	20	17.4
Fenpyroximate	422.26	366.1	15	17.6
Chlorfluazuron	540.03	382.9	20	17.8
Teflubenzuron	379.00	339.0	12	17.08
Hexaflumuron	459.02	439.0	12	16.04
Lufenuron	509.00	326.0	18	16.77

(Positive in Black, Negative in Red)

Table 1: Summary of SRM transitions used for the analysis

Results and Discussion

Figure 1a shows the chromatogram of the 32 pesticides in positive ESI, and Figure 1b shows the three pesticides under negative ESI, all eluting over a chromatographic time scale of 18 minutes. While some compounds co-elute, the specificity of the H-SRM method allows for the individual quantitation and detection of each component, even at very low levels. A summary of the calibration range, linearity, and the reproducibility of each individual compound at 5 ppb (ng/mL) is tabulated in Table 2.

Effect of H-SRM on Detection Limits

H-SRM is an acronym for Highly-Selective Reaction Monitoring (H-SRM), which is a more advanced form of Selective Reaction Monitoring (SRM). Although traditional SRM is a selective technique by itself, it still can not completely eliminate the interference from some food matrix components. Sometimes, it is possible to get incorrect qualitative results or the quantitative analysis can not reach the required detection limits of targeted compounds due to matrix-related interferences. The traditional SRM experiment, using a triple quadrupole instrument, is usually conducted with unit resolution (0.7 FWHM) for the precursor ion. With the more advanced H-SRM, the precursor ion is selected with a peak width of 0.1-0.2 FWHM. The more stringent tolerance accounts for the higher selectivity, which can lower LOQs and increase precision and accuracy at the limits of detection. This can also, in effect help reduce the overall bench time required for sample preparation.

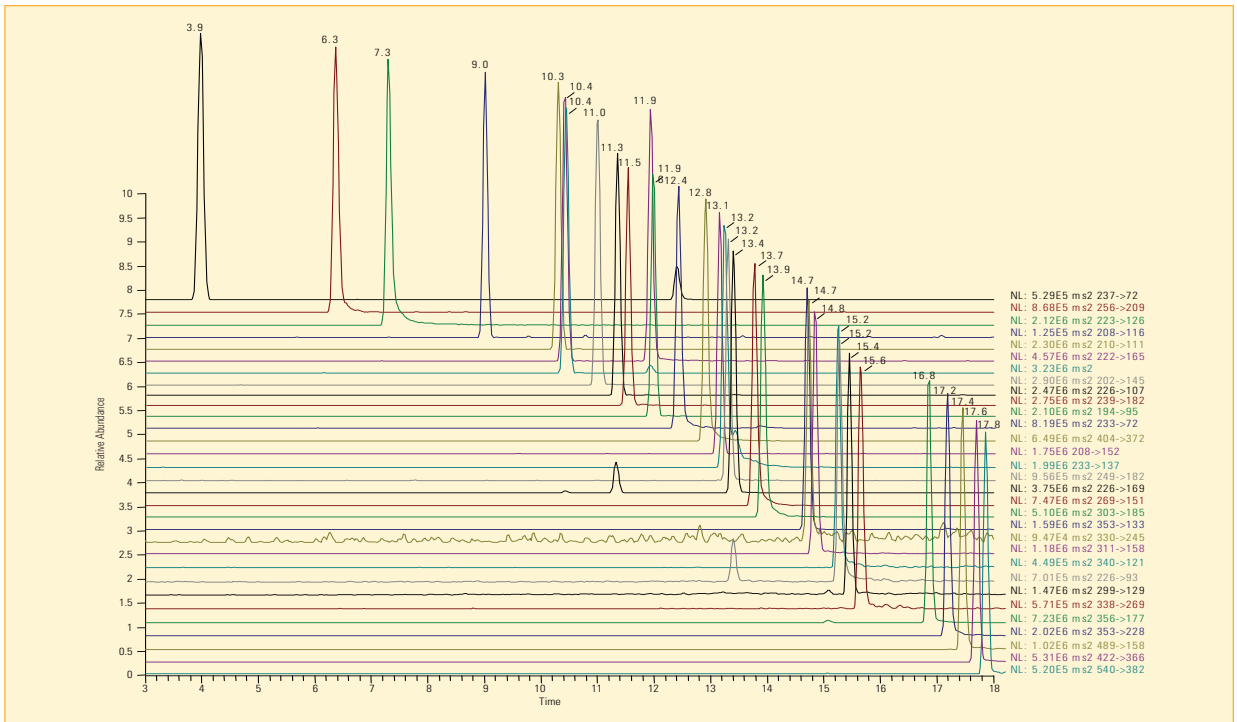

Figure 1a: LC-MS/MS chromatogram of 32 pesticides at 10 ng/mL, positive ESI

Compound	R^2	Range (ppb)	CV(%), n=5
Oxamyl	1.000	0.01-100	1.79
Imidacloprid	0.9994	0.05-100	2.84
Acetamiprid	0.9987	0.05-100	1.17
Aldicarb	0.9993	0.05-100	6.89
Propoxur	0.9997	0.01-100	1.70
Carbofuran	0.9996	0.05-100	0.95
Bendiocarb	0.9992	0.01-100	2.30
Carbaryl	0.9999	0.01-100	1.44
Ethiofencarb	0.9996	0.01-100	2.64
Pirimicarb	0.9995	0.01-100	3.55
Methabenzthiazuron	0.9989	0.01-100	1.73
MIPC	0.9987	0.01-100	1.26
Diuron	0.9987	0.05-100	2.23
Azoxystrobin	0.9989	0.01-100	2.60
BPMC	0.9999	0.05-100	1.57
Siduron	0.9989	0.05-100	1.59
Linuron	0.9989	0.05-100	4.04
Methiocarb	0.9997	0.01-100	1.88
Daimuron	0.9992	0.01-100	3.03
Cumyluron	0.9993	0.01-100	3.17
Tebufenozide	0.9995	0.05-100	1.83
Iprodione	0.9979	0.5-100	6.17
Diflubenzuron	0.9997	0.01-100	2.98
Etobenzanid	0.9997	0.05-100	1.82
Cyprodinil	0.9998	0.1-100	4.49
Phoxim	0.9997	0.05-100	3.14
Bitertanol	0.9996	0.05-100	3.54
Piperonyl butoxide	0.9996	0.01-100	1.65
Hexythiazox	0.9999	0.01-100	2.43
Flufenoxuron	0.9997	0.01-100	3.63
Fenpyroximate	0.9999	0.01-100	2.22
Chlorfluazuron	0.9987	0.01-100	2.77
Teflubenzuron	0.9986	0.01-100	2.35
Hexaflumuron	0.9973	0.01-50	1.58
Lufenuron	0.9998	0.01-10	2.56

Table 2: Calibration range and linearity of each compound, as well as the reproducibility of each compound at 5 ppb

The effects of H-SRM over SRM are clearly illustrated for the three pesticides Iprodione, Biteranol and Etobenzanid in Figures 2 a, b, and c.

Absence of Cross-talk

In order to quantitate mixtures of many compounds accurately, it is necessary to use short scan speed to ensure sufficient data points for integration. It is important that the system can maintain its sensitivity without cross-talk even at short scan speeds. Cross-talk occurs when ions from one scan event are still present in the collision cell when a second SRM transition is taking place. This leads to signal artifacts in the next transition's chromatogram. This can be especially problematic when different SRM events have the same product ions formed from different precursor ions. Thermo Fisher Scientific's patented design of the orthogonal collision cell used on the TSQ Quantum product line eliminates cross-talk. Figure 3a shows the absence of cross-talk between two different SRM transitions, pirimicarb and linuron. Both yield a product ion of 182, and no artifacts are seen in either chromatogram, even when magnified 100-1000 times. The same effect is shown in Figure 3b for diflubenzuron and flufenoxuron for a common product ion of 158 for dwell times of 20 msec.

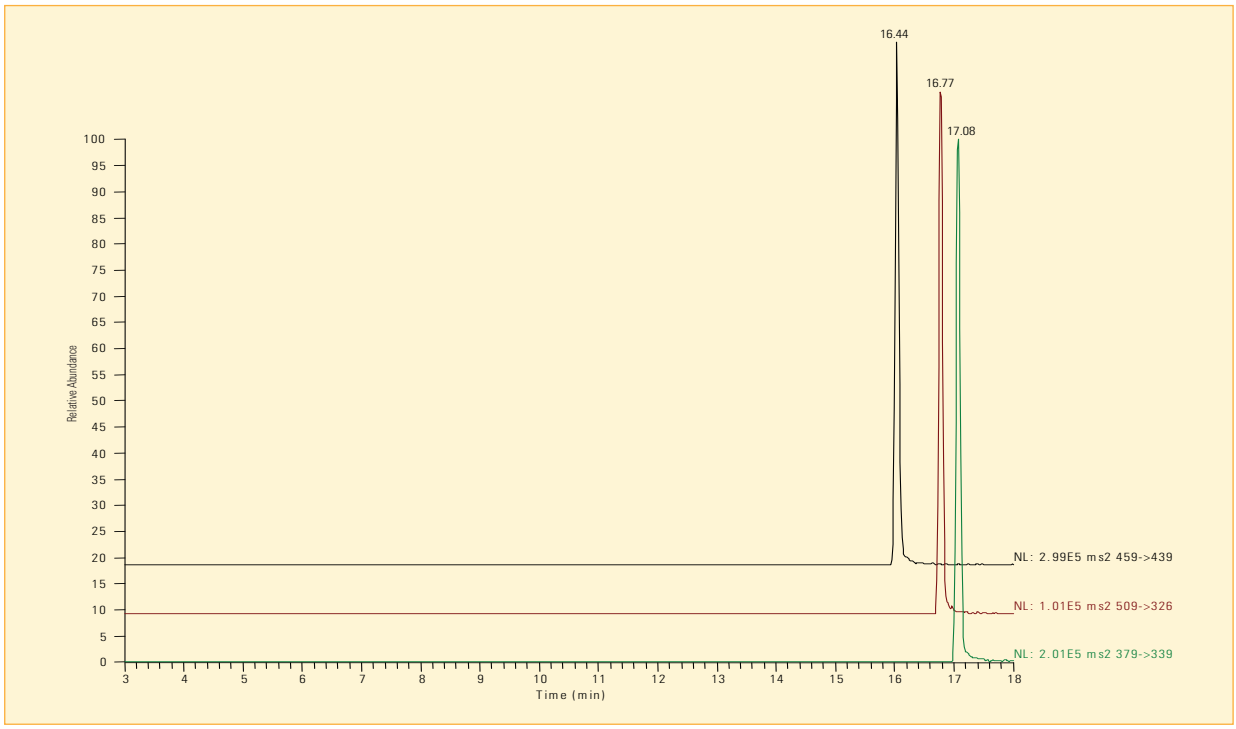

Figure 1b: LC-MS/MS chromatogram of 3 pesticides at 10 ng/mL, negative ESI

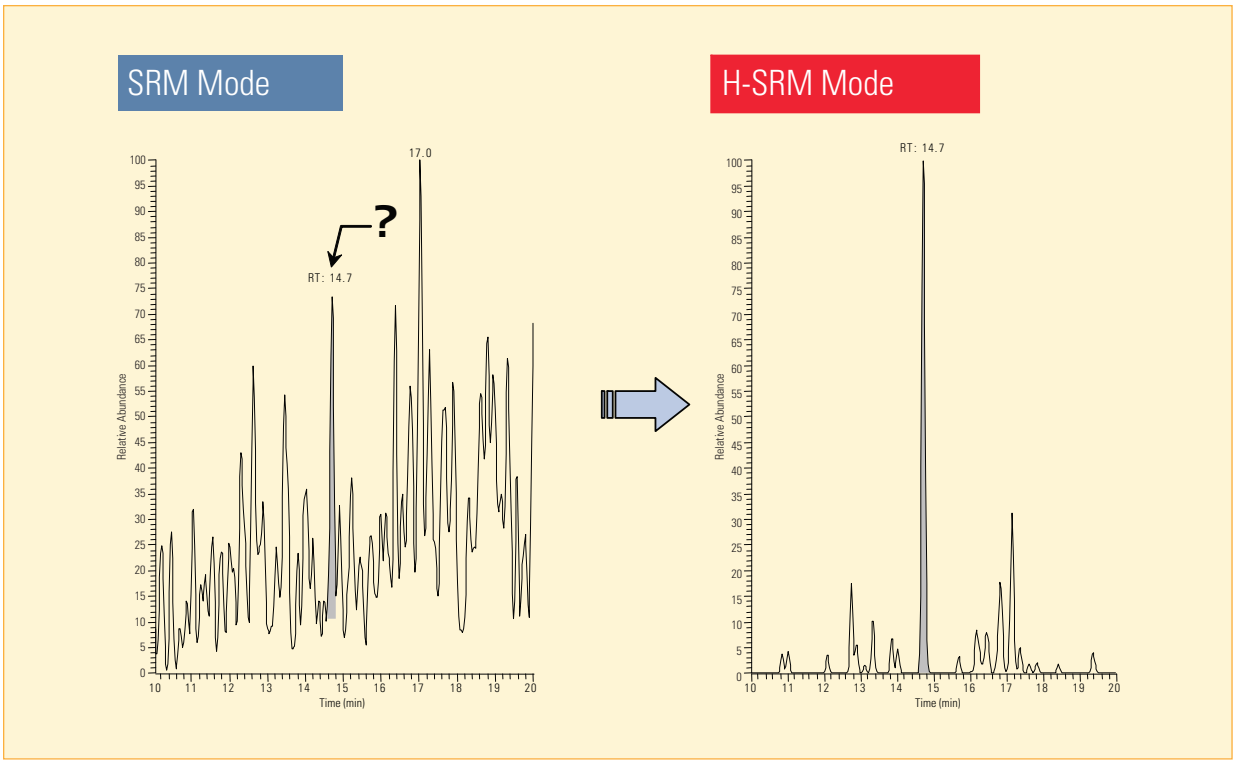

Figure 2a: Comparison of SRM mode and H-SRM mode for the analysis of the fungicide Iprodione

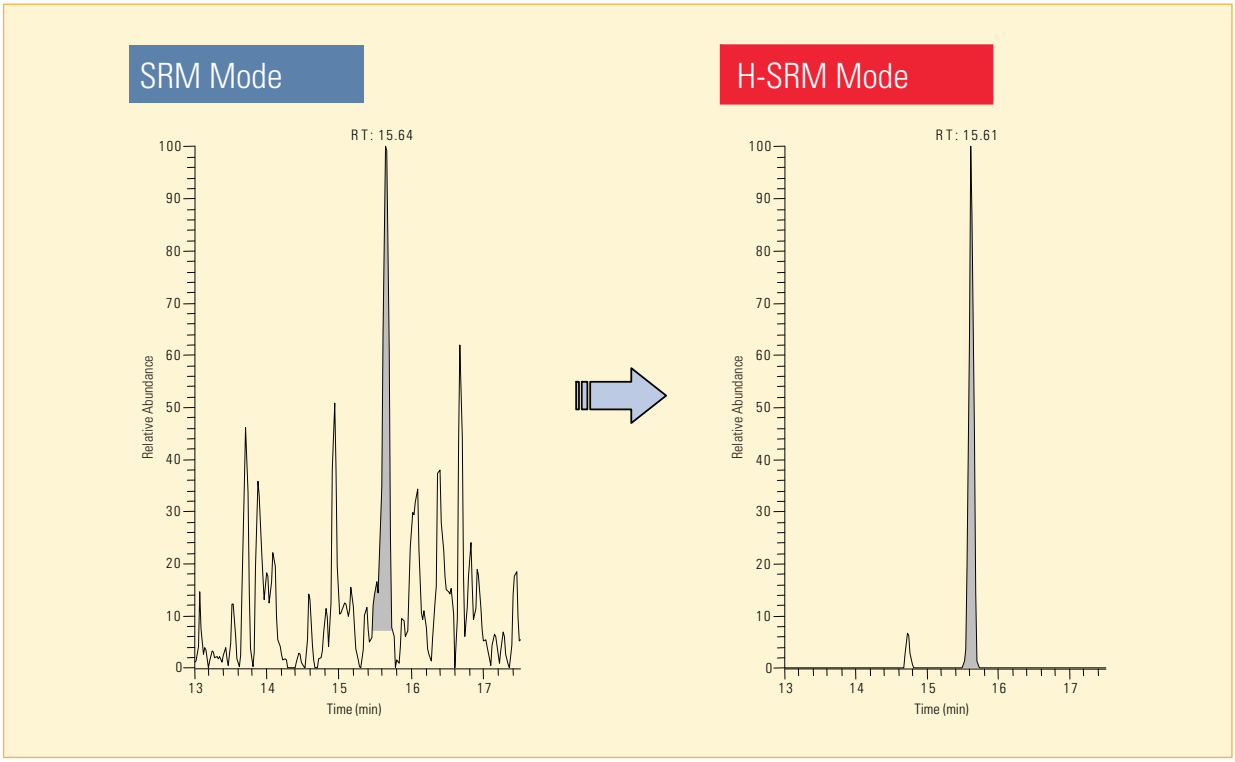

Figure 2b: Comparison of SRM mode and H-SRM mode for the analysis of the fungicide Bitertanol

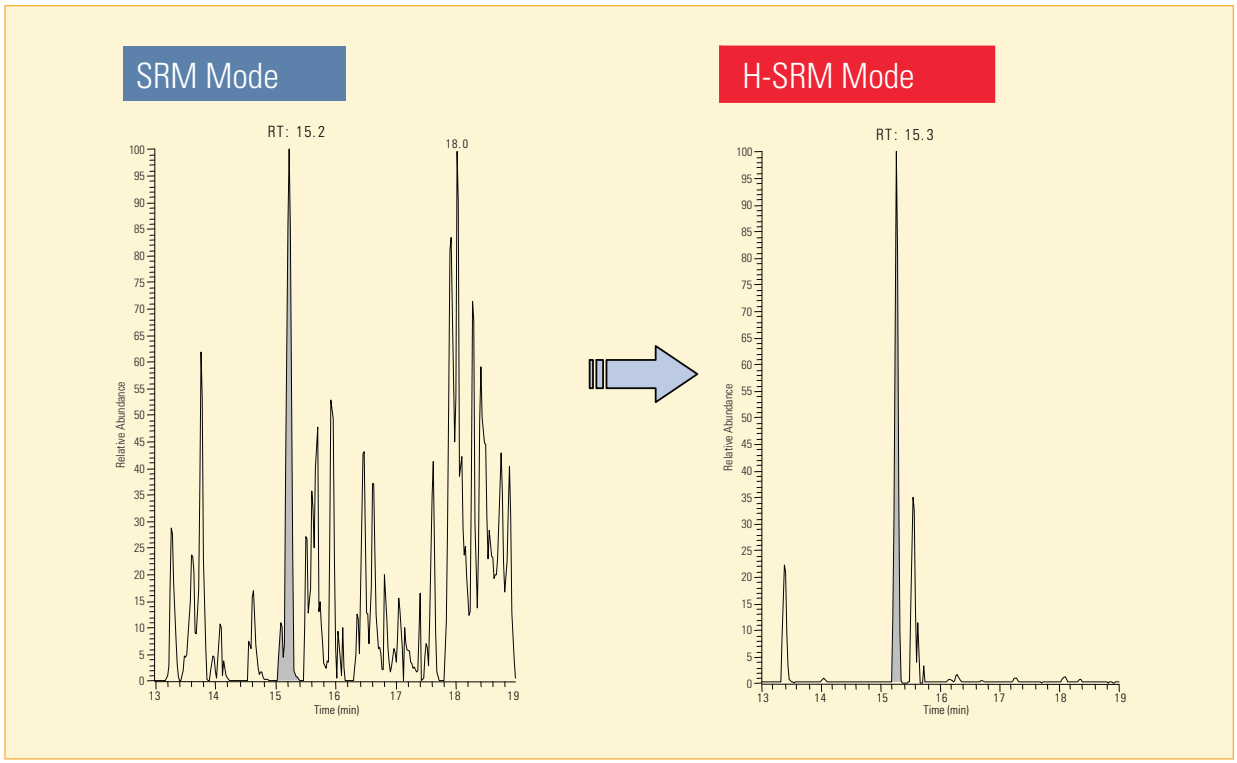

Figure 2c: Comparison of SRM mode and H-SRM mode for the analysis of the herbicide Etobenzanid

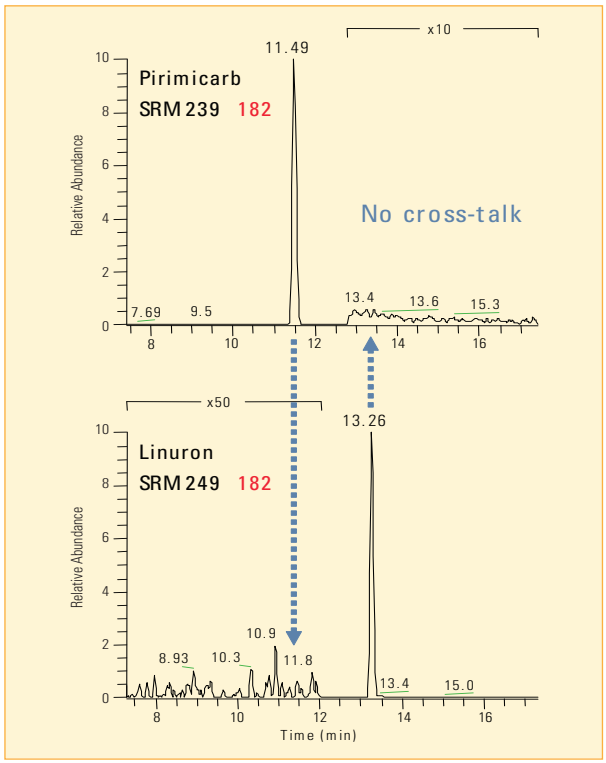

Figure 3a: No cross-talk is observed for the SRM transitions of primicarb and linuron

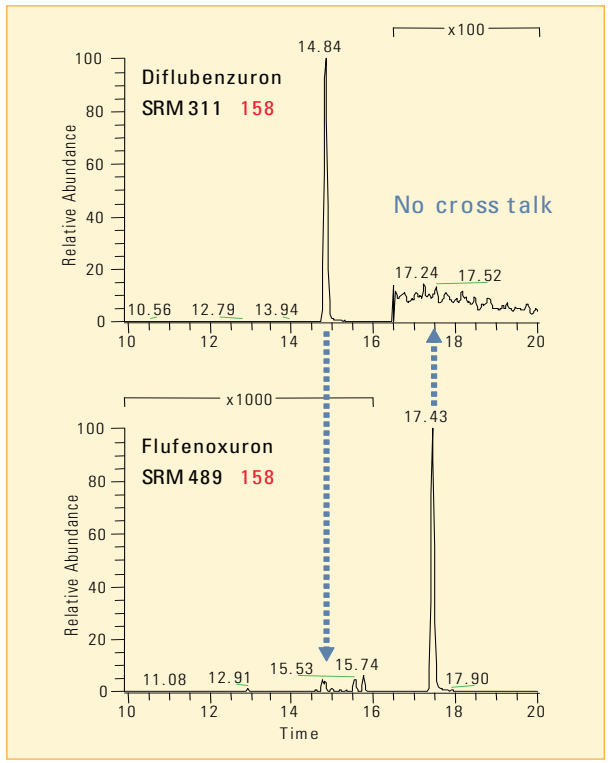

Figure 3b: No cross-talk is observed for the MRM transitions of diflubenzuron and flufenoxuron

Conclusions

An H-SRM LC-MS/MS method to monitor 35 pesticide residues was developed using the TSQ Quantum Discovery. All 35 pesticide residues were quantitated in 18 minutes. Using H-SRM, interferences from the sample matrix background were substantially reduced, leading to improved LOQs. Similarly, no cross-talk issues were detected for any of the tested analytes.

Compared with traditional single pesticide analysis methods, the sample preparation procedures are usually simplified in multi-pesticide analysis methods. This means more interference from the sample matrix may be present making H-SRM the technique of choice for improving detection limits.

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