Application Note: 351

Zero Cross-talk on the TSQ Quantum

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Key Words

- TSQ Quantum™
- Surveyor™ HPLC
- AQUASIL™
- Environmental samples
- Quantitation
- SRM

Introduction

In assays involving closely eluting multi-target analytes, Selective Reaction Monitoring (SRM) is the most commonly used mass spectrometry technique for performing quantitative assays on triple quadrupole systems. Examples include DMPK assays, monitoring for drugs of abuse in urine extracts, and pesticide residues monitoring in environmental samples.

The SRM experiment consists of three distinct events. First, precursor ions of a specific mass to charge (*m/z*) ratio are transmitted through the first quadrupole, while all ions of different *m/z* values are filtered out. Secondly, the selected ions collide with a neutral gas present in the second quadrupole (collision cell) where they undergo collision induced decomposition (CID) reactions. Finally, product ions of specific *m/z* values are transmitted through the third quadrupole, after which they are detected.

When performing LC-MS/MS analyses of multi component mixtures by SRM, it is often necessary to carry out such assays using very short dwell times of 10–20 milliseconds. In certain instances, especially in environmental monitoring of pesticides, it is possible to get a large number of parent compounds of the same chemical class eluting very close to each other in a short chromatographic timescale. Oftentimes, these compounds also give rise to exactly the same product ions which are used to monitor the SRM transitions. For example, the compounds Triadimenol (mwt 295 amu), Tebuconazole (mwt 307 amu), Cyproconazole (mwt 291 amu), and Hexaconazol (mwt 314 amu) have different precursor ions, but all give rise to the same product ion at *m/z* 70.

One potential scenario in such assays is that when SRM dwell times and inter scan times are very small, and sample concentrations are high, storage of product ions can take place in the collision cell. In other words, fragment ions from the one transition can still be in the collision cell when the next SRM transition is monitored. This can result in '*Cross talk*,' which is used to describe the phenomenon when the fragment ions from one SRM transition are scanned out during another transition. Even a 0.01% cross-talk effect can result in false positives being observed in the resulting quantitative data.

It is a well known fact that Thermo Scientific triple quadrupole systems, ranging from the earlier TSQ70 series to the current TSQ Quantum range, have never suffered from any cross-talk issues due to the advanced design of the collision cell. The purpose of this application note is to demonstrate the absence of cross-talk on the TSQ Quantum using examples from the analyses of pesticides.

Goal

To illustrate the absence of cross-talk in a screening assay for 88 different pesticides with closely eluting multi-component compounds belonging to the same chemical classes.

Experimental Conditions

Chemicals and Reagents

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

Samples

Pesticides (3,4,5-Trimehacarb, 3-hydroxy-carbofuran, 5-hydroxy-clethodim-sulfon, Acephate, Aldicarb, Aldicarb-sulfoxid, Aldoxycarb, Amidosulfuron, Atrazin, Azoxystrobin, Bendiocarb, Bensulfuron-methyl, Butocarboxim, Butocarboxim-sulfoxid, Butoxycarboxim, Carbaryl, Carbendazim, Carbofuran, Chlorosulfuron, Cinosulfuron, Clethodim, Clethodim-imin-sulfon, Clethodim-imin-sulfoxid, Clethodim-sulfon, Clethodim-sulfoxid, Cyprodinil, Daminozoid, Demeton-S-methyl-sulfon, Desmedipham, Diflubenzuron, Dimethoat, Diuron, Ethiofencarb, Ethiofencarb-sulfon, Ethiofencarb-sulfoxid, Fenhaxamid, Fenoxycarb, Fenpropimorph, Fentin-hydroxide, Flazasulfuron, Flufenoxuron, Flurathiocarb, Fluzifop-P-butyl, Haloxyfop-ethoxyethyl, Haloxyfop-methyl, Imazalil, Imidacloparid, Indoxacarb, Iprovalicarb, Isoproturon, Isoxaflutole, Linuron, Metalaxyl, Metamitron, Methamidophos, Methiocarb, Methomyl, Metolachlor, Metsulfuron-methyl, Monocrotophos, Nicosulfuron, Omethoat, Oxidemeton-methyl, Phenmedipham, Pirimicarb, Promecarb, Propamocarb, Propoxur, Prosulfuron, Pymetrozin, Pyridate, PyridateXX,

Pyrimethanil, Quinmerac, Quizalofop-ethyl, Rimsulfuron, Spiroxamine, Tebuconazol, Tebufenzoid, Thiabenzadol, Thiacloprid, Thiodicarb, Thiofanox, Thiophanat-methyl, Triasulfuron, Triflumuron, Triflusulfuron-methyl, Vamidothion) were purchased from Sigma unless otherwise noted. A standard solution containing the above 88 pesticides was prepared at 50 pg/mL in methanol.

Sample Analysis

HPLC analysis was performed on the Thermo Scientific Surveyor HPLC system, using a 50×2.1 mm Thermo Scientific AQUASIL C18 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 mL/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. MS analyses was carried out using a Thermo Scientific TSQ Quantum Discovery™ mass spectrometer.

Mass Spectrometry

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

MS Instrument Method

To accommodate the analysis of 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 msec for each transition.

Results and Discussion

Figure 1 shows the LC-MS/MS chromatogram generated from the pesticide mix eluting over a chromatographic time scale of 16 minutes.

Absence of Cross-talk

A number of compounds from the list of pesticides belonging to the same class are highlighted in Tables 1, 2 and 3. Although they have different parent molecular weights, they belong to the same, or similar, chemical class and have a common basic carbon skeleton. This results in them giving the same structural product ions (i.e., identical mass product ions), which are being monitored for the SRM transition.

This was demonstrated during the pesticide screening assay by extracting three examples of compound classes containing different precursor ions but all generating the same product ion mass.

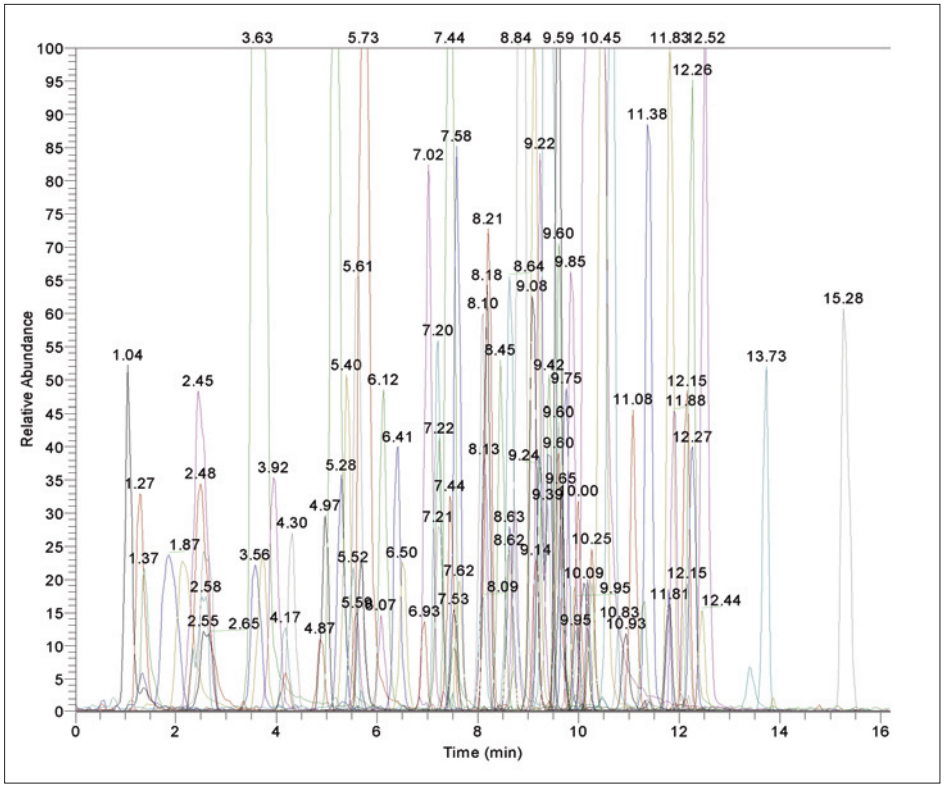


Figure 1 : LC-MS/MS chromatogram of 88 pesticides at 50 pg/µL

Example 1: Triasulfuron, Metsulfuron-methyl and Chlorosulfuron. These compounds have different precursor ions but all generate a product ion at *m/z* 167 (see Table 1 and Figures 1a and 1b). The chromatograms show the transitions for these compounds, with no evidence of cross-talk.

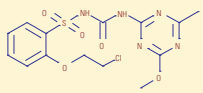
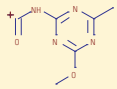
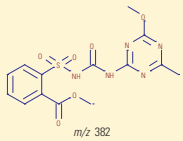
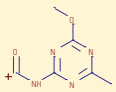
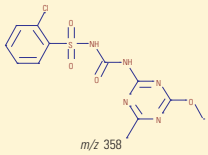
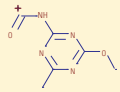
Compound	Retention Time (min)	Parent ion	Predicted product ion fragment
Triasulfuron	7.62	 *m/z* 402	 *m/z* 167
Metsulfuron-methyl	8.07	 *m/z* 382	 *m/z* 167
Chlorosulfuron	8.45	 *m/z* 358	 *m/z* 167

Table 1: Triasulfuron, Metsulfuron-methyl and Chlorosulfuron

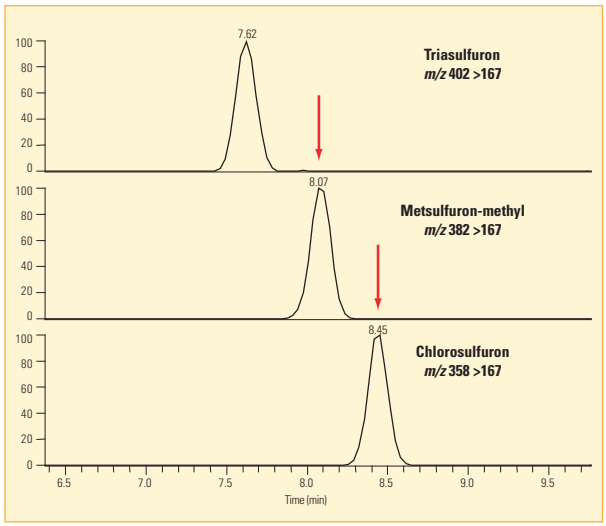


Figure 1a: Zero cross-talk was observed when the pesticides triasulfuron, metsulfuron-methyl and chlorosulfuron were detected. Peak height shown full scale. Arrows indicate position of potential cross-talk

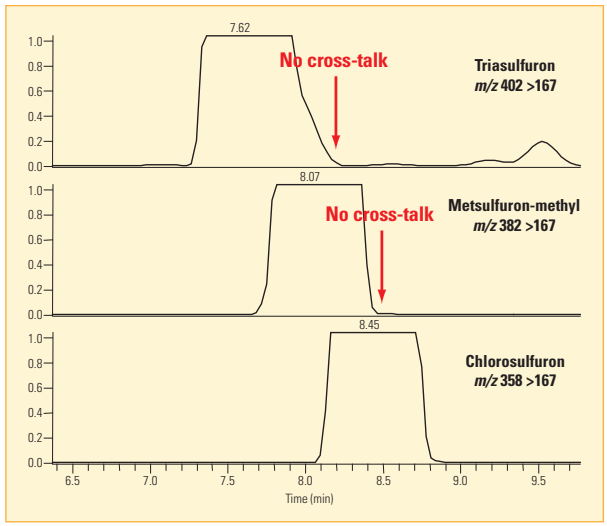


Figure 1b: Zero cross-talk (indicated by red arrows) was observed when the pesticides triasulfuron, metsulfuron-methyl and chlorosulfuron were detected. Baseline magnified x100

Example 2: Isoproturon and Diuron. These compounds have different precursor ions but both generate a product ion at *m/z* 72 (see Table 2 and Figures 2a and 2b). The chromatograms show the transitions for these compounds, with no evidence of cross-talk.

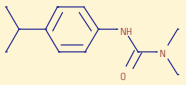
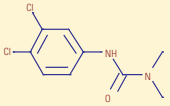
Compound	Retention Time (min)	Parent ion	Predicted product ion fragment
Isoproturon	9.22	 *m/z* 207	 *m/z* 72
Diuron	9.75	 *m/z* 233	 *m/z* 72

Table 2 : Isoproturon and Diuron

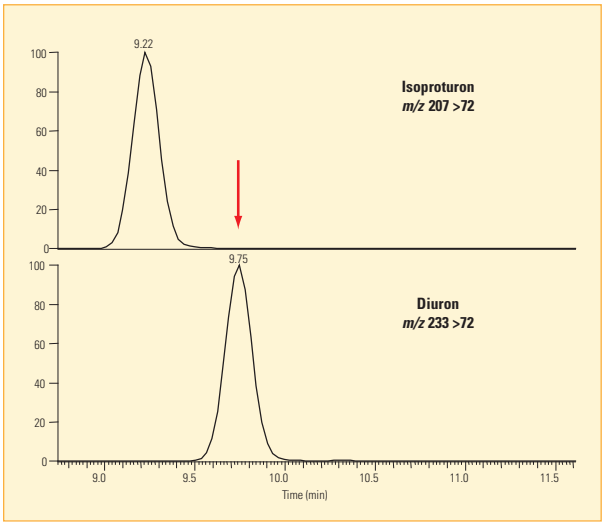


Figure 2a : Zero cross-talk was observed when the pesticides Isoproturon and Diuron were detected. Peak height shown full scale. Arrow indicates position of potential cross-talk

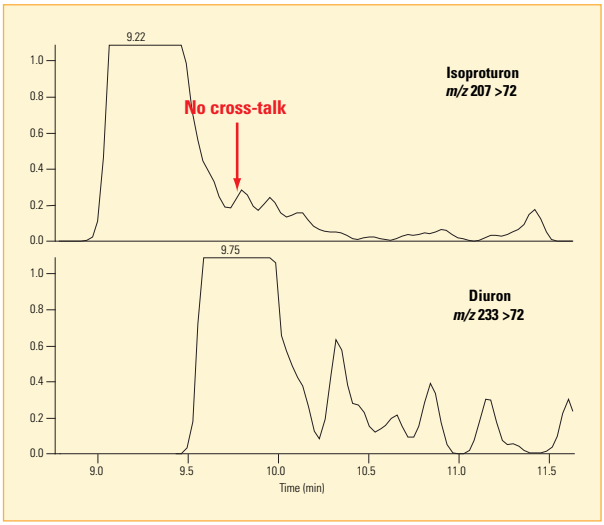


Figure 2b : Zero cross-talk (indicated by red arrow) was observed when the pesticides Isoproturon and Diuron were detected. Baseline magnified x100

Example 3: Nicosulfuron and Linuron. These compounds have different precursor ions but both generate a product ion at *m/z* 182 (see Table 3 and Figures 3a and 3b). The chromatograms show the transitions for these compounds, with no evidence of cross-talk.

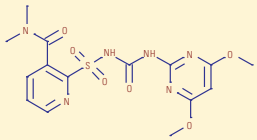
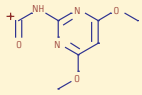
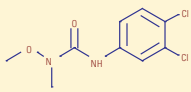
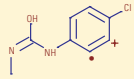
Compound	Retention Time (min)	Parent ion	Predicted product ion fragment
Nicosulfuron	9.59	 *m/z* 411	 *m/z* 182
Linuron	10.26	 *m/z* 249	 *m/z* 182

Table 3 : Nicosulfuron and Linuron

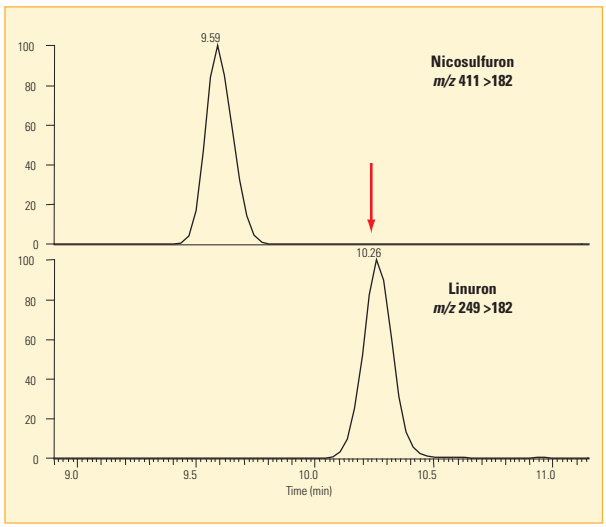


Figure 3a: Zero cross-talk was observed when the pesticides Nicosulfuron and Linuron were detected. Peak height shown full scale. Arrow indicates position of potential cross-talk

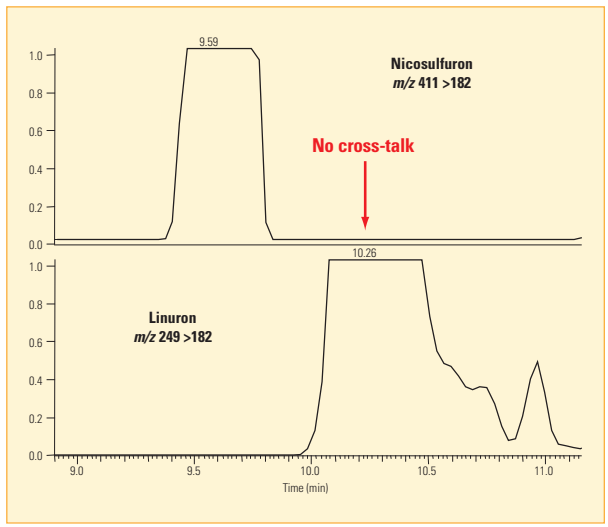


Figure 3b: Zero cross-talk (indicated by red arrow) was observed when the pesticides Nicosulfuron and Linuron were detected. Baseline magnified x100

Why is there Zero Cross-talk on the TSQ Quantum?

The reason for not observing any cross-talk on the TSQ Quantum can be ascribed to the way the collision cell (Q2) is operated. The ions are actively ejected radially by selecting Mathieu a and q operational parameters such that all ions of all masses become unstable. This instantaneously dumps all ions from the collision cell. The offset voltages are also operated to keep the cell empty while Q1 slews to the next precursor mass. Once Q1 is set, the cell (Q2) parameters are selected to accept and fragment the precursor ions for the next transition. As this procedure is not dependent upon ion residence times, each SRM transition becomes a discreet experiment, with no memory of the one before, thus totally eliminating any possibility of cross-talk.

It should be noted that for SRM and MRM assays, this technique is superior to clearing a collision cell using axial ejection with a supplementary field which is slower and less efficient. Indeed, axial techniques applied on this time scale always have some residual cross-talk.

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 timescale of 16 minutes by performing 88 SRM transitions. Even with dwell times of only 20 milliseconds no cross-talk interfered with the analysis. The absence of cross-talk was demonstrated by extracting and comparing the ion chromatogams of similar classes of compounds with different precursor ion masses but with the same product ion mass.

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.

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