U-SRM – Ultra Selective Detection of Analytes in Complex Matrix Samples on the TSQ Quantum XLS Ultra GC-MS/MS

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GC-MS/MS, U-SRM, Mass Resolution, Matrix, Selectivity, TSQ Quantum XLS Ultra

Introduction

The Thermo Scientific TSQ Quantum XLS Ultra triple quadrupole mass analyzer breaks the barrier of low level matrix interferences for a reliable compound quantitation. Where single quadrupole analyzers are limited to isobaric interferences on the same selected ion monitoring mass (SIM), a triple quadrupole mass spectrometer offers increased matrix selectivity. With this, triple quadrupole detection has provided a large step forward in our ability to detect at very low concentrations in complex matrix. But, the selectivity of this nominal mass analyzer can be challenged when some matrix/target combinations are considered. When analyzing at extremely low concentrations, the overwhelming intensity of matrix compounds can still provide interferences in some cases, as chemical noise, on the product ion mass traces. It is not surprising in this context that different matrices interfere with different impact. So, what is the technical solution the TSQ Quantum XLS Ultra™ triple quadrupole GC-MS/MS system is using to break these barriers and overcome these typical matrix limitations?

Delivering High Performance Triple Quadrupole Experiments

The TSQ Quantum XLS Ultra, as shown in Figure 1, provides enhanced mass resolution capabilities that are unique to GC-triple quadrupole systems. The specially designed high precision hyperbolic quadrupoles (Thermo Scientific HyperQuad quadrupoles), as pictured in Figure 2, provide enhanced mass resolution and ion transmission. This increased analyzer performances allows an extremely low background signal which simplifies quantification of trace compounds even when shadowed by hugely intense matrix components, of which a significant portion exhibit isobaric ions interfering with the selected reaction monitoring (SRM) process. For trace analysis in the ppt range, the increased radius of the HyperQuad™ quadrupole assembly used a 50% larger diameter between the rods for increased ion transmission and hence sensitivity.

Of course, high performance quadrupoles are a pre-requisite for a good GC triple quad system as the mass analyzers lie at the heart of the performance delivery. In a triple quadrupole analyzer, the first quadrupole (Q1) that sits directly behind the ion source and pre-filter, has a large influence on the target analyte selectivity of the mass spectrometer. The desired precursor ions, after being generated in the ion source, are selected by Q1 and are transmitted to the collision cell (Q2) for fragmentation. The third quadrupole (Q3) is set to transmit to the detector only preselected products ions generated in Q2 as a result of collision induced fragmentation (CID).

Without high-performing quadrupoles, mass selection capabilities become compromised and tend towards a lower resolution to transmit the same number of ions. Systems that do not employ HyperQuad technology have to open the mass transmission window (above unit mass resolution) to transmit the same number of ions as a HyperQuad analyzer in unit mass mode. This decreased selectivity for target ions can give rise to chemical

Figure 1. TSQ Quantum XLS Ultra enhanced mass resolution GC-MS/MS system

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interferences in the analysis. The same effect can be observed when mass resolution settings of 1.2 Da FWHM or higher are used during single quadrupole analysis. This can be seemingly effective when simple sample matrices are encountered. However, when facing a more complex sample matrix this strategy can cause problems in the analysis.

What are Isobaric Interferences?

The term "isobaric interferences" describes ions of the same nominal mass but of different chemical composition and structure. The "nominal mass" is typically the mass used in quadrupole instruments for programming SIM or SRM acquisition. This reflects the typical unit mass resolution capabilities of standard quadrupole instruments. The nominal mass, in this context, can be described as a 1 Da (1 *m/z*) resolution capability between mass peaks. The term "isobaric interference" means in practice that ion signals from other compounds than the target analyte appear at the same nominal mass in the scan spectrum, the SIM trace or, in some cases, the SRM trace.

Figure 2. HyperQuad quadrupole rods used in the TSQ Quantum XLS Ultra system

For triple quadrupole instruments operated in SRM mode, the selectivity is generally high. This is due to the MS/MS process. There are cases, however, where despite MS/MS being applied, selectivity is challenged. These cases appear more frequently when the matrix load of samples is very high. This is not unusual for a triple quadrupole, as often the most complex quantitative determinations are directed to this type of technology. The likelihood of encountering a full SRM interference increases as a function of the matrix complexity. The observed effects of isobaric interferences are also more apparent when targeting compounds in low or sub-ppb concentration ranges. This is because the target compound mass is more likely to be "shadowed" by interfering matrix ions (especially in the first stage of MS in Q1) that are typically orders of magnitude higher in concentration. The drive towards shorter clean-up procedures also pushes additional matrix to the detection system, adding to the problem.

When these intense interfering matrix ions successfully transmit through Q1 into the collision cell, there is a higher statistical probability that interfering product ion masses are formed. This gives rise to a higher occurrence of full SRM interferences and visibly reduced analyte selectivity. This often manifests as an increased chemical noise background and hence, low signal-to-noise detection. This is observed most frequently in matrix samples and is often unnoticed or absent in solvent only standards (see Figure 3). Because solvent standards are relatively clean, it is also possible (and sometimes practiced) to open Q1 above unit mass resolution (so called "wide" or "open" settings.) This creates the possibility to gain sensitivity, but does not help much when considering real backgrounds in complex samples. Sensitivity (and selectivity) achieved at wider Q1 resolution values can vary considerably between clean and dirty samples. With that in mind, it is sensible that any comparisons between instruments, especially those that are to face dirtier samples, are performed in matrix samples. If solvent standards are to be used, then the true instrument sensitivity should be compared using equivalent Q1 and Q3 resolution values.

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How Triple Quadrupole Analyzers Work

The idea of using three quadrupoles arranged in series in a triple quadrupole analyzer for structure elucidation ("an added dimension of mass spectral information") follows an idea first reported by Richard Yost and Chris Enke of Florida University in 1970. The analyzer should allow the detection of structure-related information and overcome the single quadrupole limitation of measuring a mass

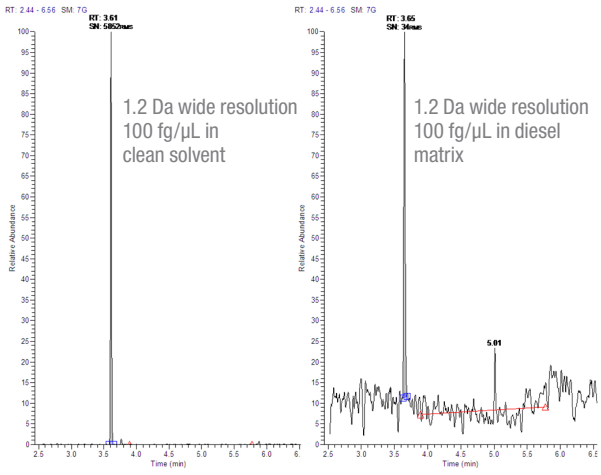

Figure 3. High occurrence of matrix interference on standard specification compound (octafluoronapthalene) with "Wide/Open" Q1 settings (1.2 Da FWHM) absent in solvent standard (left) but significant when in presence of complex matrix (right).

"only," working as a mass selective detector. The new capabilities have revolutionized all structure elucidation work at a time before quantitative triple quadrupole applications appeared.

Today, standard triple quadrupole analyzers still deliver selectivity through the same structure related mechanism. Advanced systems, such as the TSQ Quantum XLS Ultra, deliver selectivity via two mechanisms: structure-selective detection and enhanced mass resolution capabilities.

Structure-Selective Detection

The chemical composition and structure of a molecule determines the specific pathways of ion source fragmentation during ionization. During this process, energy is transferred to a compound, and it is distributed throughout the molecule and eventually breaks chemical bonds. This results in a spectrum of different mass fragments and relative intensities. A similar mechanism applies to collision induced dissociation (CID) in the collision cell (Q2) of a triple quadrupole analyzer.

This can be best illustrated having a closer look at a regular EI spectrum, for instance from the well known pesticide parathion. The parathion EI spectrum in Figure 4 shows the intense molecular ion as the base peak (nominal mass 291 *m/z* and accurate mass 291.03 *m/z*) and the dissociation of the molecular ion structure into a number of lower mass fragments, all of them contributing to a fingerprint of the given structure. For a low level SIM detection, the intense molecular ion 291 *m/z* is the signal of choice. When performing SRM on a triple quadrupole instrument the same 291 *m/z* is targeted as the precursor ion. After CID fragmentation in the collision cell, structurally selective product ions are formed and monitored using Q3. In the case of parathion, the product ions 109 and 97 *m/z* (which also occur during EI source ionization – Figure 4) are monitored. These product ions are formed in consistent ratios to each other.

Only structures eluting at the retention time of parathion with a parent ion of 291 *m/z* are expected to give a signal at the product ions 109 and 97 *m/z*. This filters out most of the unspecific background interference at 291 *m/z*, which limits the SIM detection in a single quadrupole instrument.

Adding Mass Selectivity with Enhanced Mass Resolution

In some matrices, the high structural selectivity of a triple quad analyzer can be impaired by a compromised selection of the precursor ion at Q1. The unique capability of the TSQ Quantum XLS Ultra is that it allows a much more selective isolation of a target pre-cursor ion at the first stage of MS (Q1). This allows further discrimination against intense matrix ions that appear at nominal precursor masses (isobaric interferences). As a general rule, in order to achieve this, precursor masses need to be specified more accurately to the instrument within 1 or 2 decimal places as well as the instrument mass resolution on Q1 being set to ≤ 0.2 Da FWHM. This is described as an "ultra-selective" mode for a quadrupole analyzer. Resolution settings can be increased for Q3 also for more selectivity in product ion experiments, although for SRM Q1, resolution is a more critical parameter.

Enhanced mass resolution with quadrupoles can be achieved by using precision machined hyperbolic quadrupoles of special length. Ultra-selective quadrupole mass resolution for SRM detection (U-SRM) is a novel acquisition mode for GC-MS/MS instruments. This mode allows the combination of increased mass resolution selectivity and structural selectivity when targeting compounds in complex matrix. The TSQ Quantum XLS Ultra allows this mode by incorporating extra long hyperbolic quadrupole rods (190 mm), with a wide internal radius of 6 mm, as shown in Figure 2. While the long rods deliver excellent mass peak form and resolution, the wide 6 mm radius accepts an increased number of ions from the source for increased ion transmission and sensitivity in the ultra selective SRM mode (U-SRM).

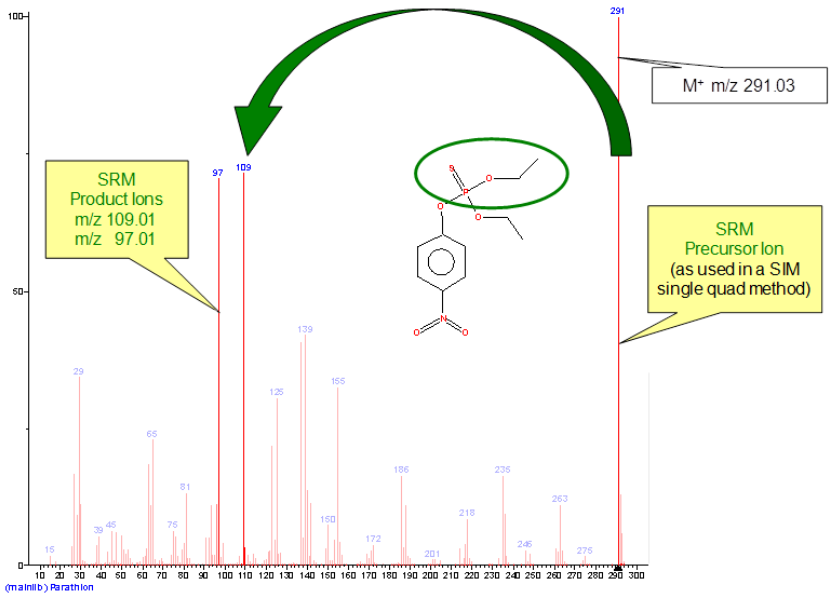

Figure. 4: EI mass spectrum of parathion simulating SRM precursor and product ions for MS/MS (NIST library)

Exact Mass and Mass Defect

Common target compounds for pesticides, drugs, or persistent organic pollutants (POPs) analyses typically contain a high number of heteroatoms or halogens in their structure. Typically hydrocarbon based or bio-organic compounds are forming common background matrix compounds. Examples of these include fuel oils, triglycerides, humic/fulvic substances, waxes, lignin structures or similar compound classes. In order to understand why ultra-selective precursor isolation increases selectivity in real applications when using U-SRM, it is necessary to visit the concept of exact mass and mass defect.

Exact mass is simply the calculation of the mass of a compound to a greater degree of accuracy. This is typically identifiable when masses are shown carry multiple decimal places. When this is measured value on a mass spectrometer, we refer to this as the accurate mass within a specified tolerance.

A closer look at the elemental composition of common target compounds detected in trace residue analysis reveals that, relative to carbon (with its IUPAC defined atomic weight of exact 12.00 g/mol), only hydrogen and nitrogen show a significant positive shift of its exact mass from the nominal mass of 1 g/mol and 14 g/mol respectively (see Table 1). Because of the high hydrogen occurrence in organic molecules, the apex of MS-detected mass peaks of hydrocarbons shift significantly on the accurate mass scale to higher masses. The calculated "mass defect" (in this case positive), is commonly expressed as a percentage of the deviation of the exact mass from its nominal value normalized to 100 Da, is typically in the range of 100 mDa/100Da for hydrocarbons.

In contrast to hydrogen, most heteroatoms, predominantly halogens, sulfur, phosphorous and silicone shift the mass peak of compounds containing these elements to lower masses. This can be described as a "negative mass defect." This fine difference in exact mass is used by the TSQ Quantum XLS Ultra to select target analytes during ultra selective acquisitions whilst discriminating against coeluting isobaric matrix ions. An example to illustrate this effect can be made for the pesticide HCB at the nominal mass *m/z* 282, see Table 2. The HCB mass peak is separated more than 0.5 Da on the mass scale from a nominally isobaric hydrocarbon background compound. This mass difference can be exploited to cleanly separate the HCB precursor ion in Q1 from the hydrocarbon matrix on the TSQ Quantum XLS Ultra. This is a relatively extreme example with a large delta mass. Depending on analyte/matrix combinations encountered, the resolving power of the quadrupole may need to exceed 5000+ resolution (FWHM). This is not usually available on standard triple quadrupole instruments that do not benefit from HyperQuad technology (see Figure 8).

Table 1: Mass defect of major elements in common analytes

Element	Nominal M [Da]	Exact M [Da]	Delta abs [Da]	Rel. Mass Defect [mDa/100Da]
C	12	12	0	0
H	1	1.0078	0.0078	783
N	14	14.0031	0.0031	22
O	16	15.9949	-0.0051	-32
O	16	15.9949	-0.0051	-32
S	32	31.9721	-0.0279	-87
Si	28	27.9769	-0.0231	-82
F	19	18.9984	-0.0016	-8
Cl	35	34.9689	-0.0311	-89
Br	79	78.9183	-0.0817	-103
I	127	126.9045	-0.0955	-75

Table 2: Example of the impact of the mass defect on the accurate mass at nominal mass *m/z* 282

Compound	Nominal M [Da]	Exact M [Da]	Delta abs [Da]	Rel. Mass Defect [mDa/100Da]
HCB				
C_6Cl_6	282	281.8134	-0.1866	-66
Alkane				
$C_{20}H_{42}$	282	282.3276	0.3276	116
Difference on mass scale 0.5142				

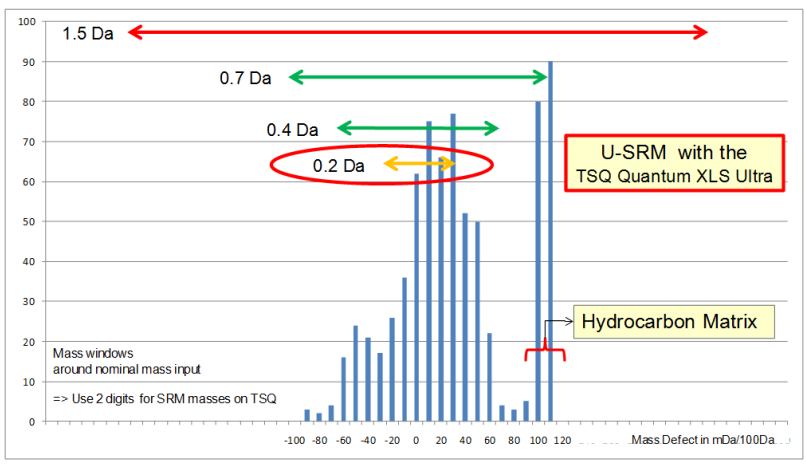

Figure 5: 700 Pesticides sorted according to frequency of their relative mass defect

This concept of enhanced mass resolution can be best visualized looking at the broad spectrum of pesticides with their inherent diversity of chemical compound classes. Figure 5 shows more than 700 pesticides and POPs compounds and their frequency distribution according to their "mass defect" values, see also the Thermo Fisher Scientific Pesticides Analyzer Reference Manual (Thermo Fisher Scientific, 2010). All of the compounds show a significant shift away from the organic hydrocarbon background to lower accurate masses due to their accurate mass.

The graphics in Figure 5 also shows the effect of increased mass resolution. The "selectivity window" with increasingly narrower mass peaks allows a good separation from hydrocarbon matrix interferences. Using a wider Q1 mass window beyond 0.7 Da with nominal or wide resolution setting of 1.5 Da will include the interfering matrix compounds into the fragmentation processes in the collision cell exhibiting high background noise. "Closing" the selectivity window to 0.2 Da using U-SRM on the TSQ Quantum XLS Ultra provides efficient selectivity for all shown compounds from matrix interferences.

Isolation of the Precursor Ion from Isobaric Matrix

Increased analyte selectivity is obtained during U-SRM with a narrow pre-selection of the precursor ion. The situation of having the pesticide lindane detected in a dirty matrix sample is shown in Figure 6 and Figure 7. During standard SRM operation, the Q1 nominal mass resolution of 0.7 Da (at the target mass *m/z* 219) transmits the lindane ion the collision cell for fragmentation along with a number of matrix ions. This can lead to isobaric interference and an increase in chemical noise. This problem can be exacerbated for systems that incorporated even wider precursor ion windows, such as the commonly seen "wide" or "open" resolution settings of > 1.0 Da FWHM. These wider settings can give an artificial impression of sensitivity, as the number of ions transmitted increases because noise can quickly appear in dirtier matrix samples.

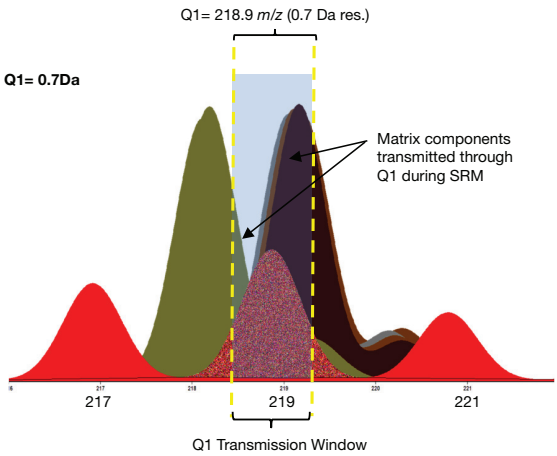

Figure 6: Precursor ion selection for lindane at 0.7Da FWHM (Q1) in standard SRM mode. Matrix components are transmitted to the collision cell during SRM acquisition.

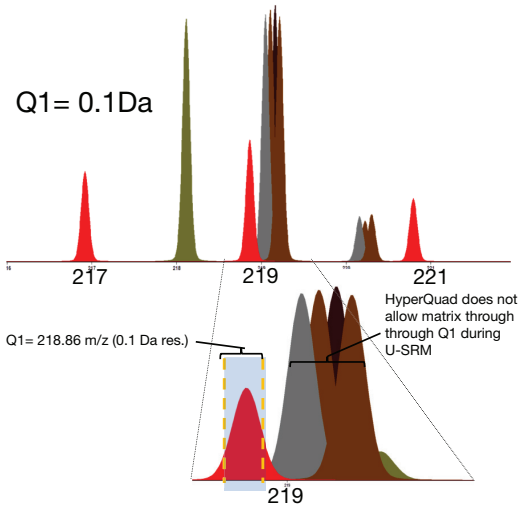

Figure 7: Precursor ion selection during U-SRM

In contrast to the standard or "wide" Q1 window setting, U-SRM "closes" the mass window in Q1 to < 0.2 Da, as seen in Figure 7. The increased resolution allows the lindane peak to be efficiently isolated for transmission to the collision cell in the absence of interfering matrix components. The resolution of the HyperQuad is such that any delta mass defect between target and matrix ions can be exploited. This is why U-SRM most efficiently eliminates isobaric interference effects on the precursor ion.

The difference in the mass resolution effect becomes even more evident comparing the resolution power of different triple quadrupole instrument types in Figure 8. The graph shows the calculated resolution over mass (peak width at half peak height, FWHM) for different mass peak width settings. Starting on the bottom from the "wide" setting with 1.2 Da peak width and the "standard" setting with

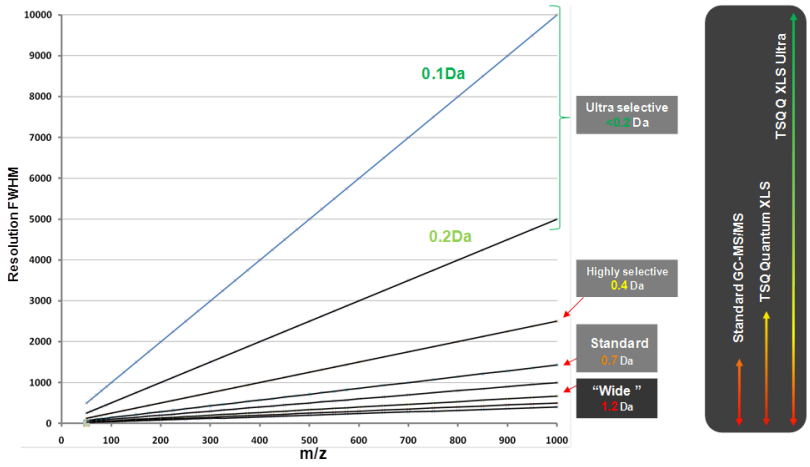

Figure 8: Mass resolution power of different instrument types

0.7 Da, there is already a remarkable resolution increase by using the highly selective setting with 0.4 Da peak with of the Thermo Scientific TSQ Quantum XLS. A significant increase in mass resolution is observed when progressing to the ultra selective mode with resolution settings of ≤ 0.2 Da when using the TSQ Quantum XLS Ultra.

Figure 9 extends the earlier example from Figure 3, octafluornapthalene in diesel, to compare the additional selectivity power of U-SRM. As already discussed, the use of increased resolution HyperQuads, operating in U-SRM mode, allows the possibility to further eliminate interference when moving to complex matrix. When compared to modes of operation that utilize "wide" or "open" quad resolution settings, it is clear that much higher confidence when addressing matrix samples can be taken with high sensitivity and high selectivity operation modes. In addition to this, it reminds us that we should endeavor to perform instrument evaluations in complex matrix samples with normalized instrument resolution settings. This allows both sensitivity and selectivity power to be observed.

Conclusions

Selectivity is a critical evaluation parameter for a GC-MS/MS system that is to face complex matrix samples. This is a key parameter for instrumental evaluation criteria alongside raw sensitivity and low-level precision performance.

GC-MS/MS using enhanced mass resolution mitigates the effect of surviving background interferences in SRM experiments, especially in complex sample matrices. High sensitivity, high selectivity analysis becomes possible, even with reduced clean-up procedures or direct Thermo Scientific Dionex ASE extracts for a large number of target compounds in one run.

The analytical advantages of using U-SRM on the TSQ Quantum XLS Ultra translate into increased productivity for routine analysis by the increasing data quality and increasing the possibility to save time with more generic sample preparation approaches. Reliable automatic peak integration becomes a regular feature of data analysis which allows a much reduced manual invention and faster time to result. This capability is particularly critical for laboratories with high sample throughput.

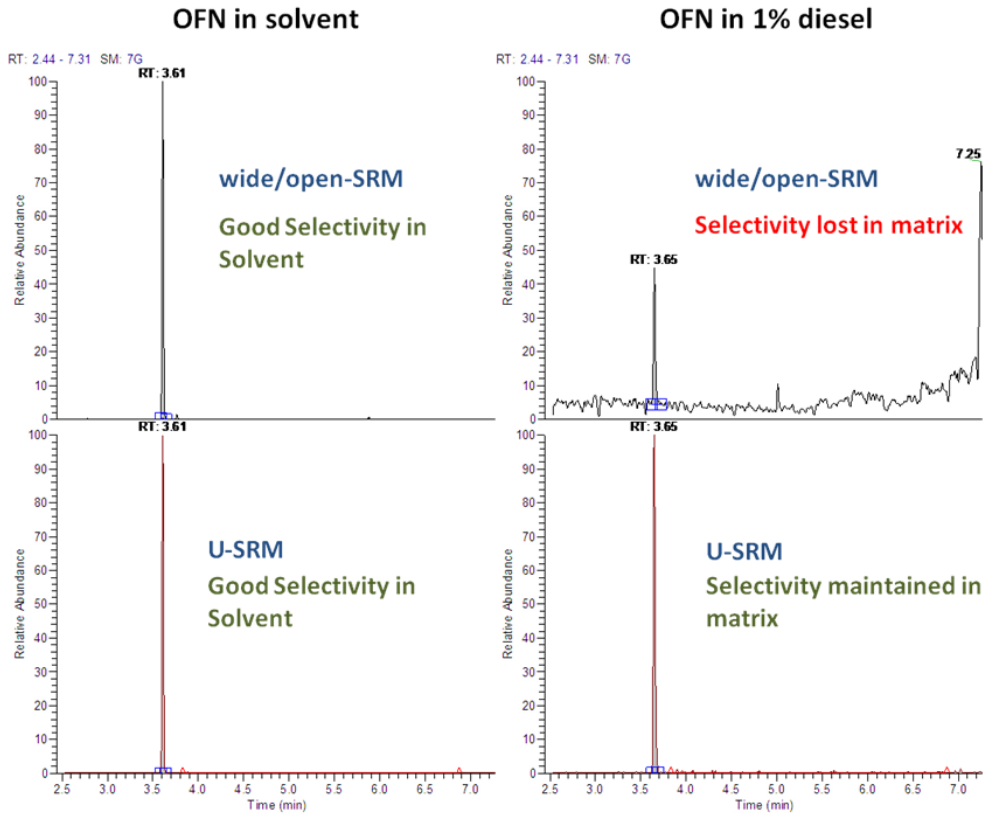

Figure 9. 100 fg/µL OFN in solvent (left) and 1% diesel (right) under "wide/open" SRM conditions (top) and ultra selective SRM mode (bottom)

Abbreviations

Da	International SI mass unit Dalton
CID	Collision-Induced Fragmentation (typically in the collision cell Q2)
FWHM	Full width at half maximum (a measure of the peak width at half peak height)
H-SRM	Highly selective SRM process (typically at 0.4 Da peak width)
HxCDD	Hexachlorodibenzodioxin
MRM	Multiple Reaction Monitoring (typically describing the analysis for multiple compounds)
OCP	Organochlorine pesticides
PCB	Polychlorinated biphenyl
PCDD/F	Polychlorinated dibenzodioxins and dibenzofurans
QuEChERS	Dispersive SPE extraction method, acronym for Quick Easy Cheap Efficient Rugged and Safe
SIM	Selected Ion Monitoring
SRM	Selected Reaction Monitoring (typically describing the technical process)
TSQ	Triple Stage Quadrupole instrument
U-SRM	Ultra selective SRM process (typically at peak widths at or below 0.2 Da peak widths)

Acknowledgement

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