

Advantages of Using Triple Quadrupole over Single Quadrupole Mass Spectrometry to Quantify and Identify the Presence of Pesticides in Water and Soil Samples

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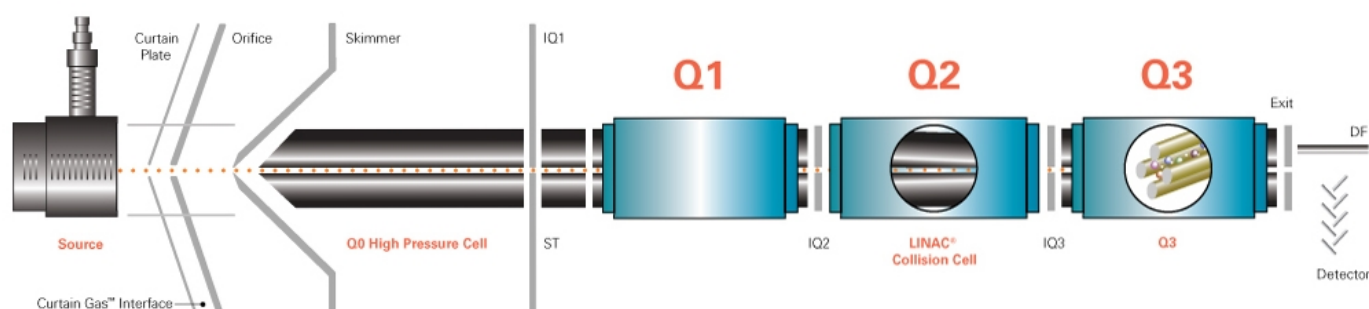


Figure 1. Schematic of Triple Quadrupole Configuration

Triple quadrupole (MS/MS) systems provide in comparison to single quadrupole (MS) systems:

- Higher selectivity resulting in less interference of co-eluting compounds and matrix, thus less HPLC separation is required
- Better Signal-to-Noise (S/N) allowing quantitation with lower limits of quantitation
- More reliable identification of detected analytes using Multiple Reaction Monitoring (MRM) in comparison to Selected Ion Monitoring (SIM)
- Wider linear range of quantitation
- Better accuracy and reproducibility especially at low concentrations

Overview

This paper describes the use of single quadrupole and triple quadrupole Mass Spectrometry coupled to Liquid Chromatography for the analysis of 17 pesticides in drinking water and soil samples. Both detection technologies are compared with respect to selectivity, sensitivity, identification, linear range, accuracy, and reproducibility for quantitative analysis.

Introduction

The coupling of Liquid Chromatography (LC) and tandem Mass Spectrometry (MS/MS) is a widely used analytical technique for quantitative and qualitative analysis. Electrospray Ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI), or Photo Ionization (APPI) allow the ionization of various semi-volatile, thermally labile, and polar to nonpolar compounds, such as pharmaceuticals, pesticides, personal care products, steroids, explosives, drugs of abuse etc., in trace levels. Generated ions will be transferred after ionization through a vacuum interface into the mass analyzer.

Quadrupoles are mass analyzers which consist of four rods with DC and RF voltages applied. An ion of a specific mass-to-charge ratio (m/z) will be stable and can pass through the quadrupole only when a specific DC/RF voltage combination is applied. Quadrupoles are therefore called mass filters.

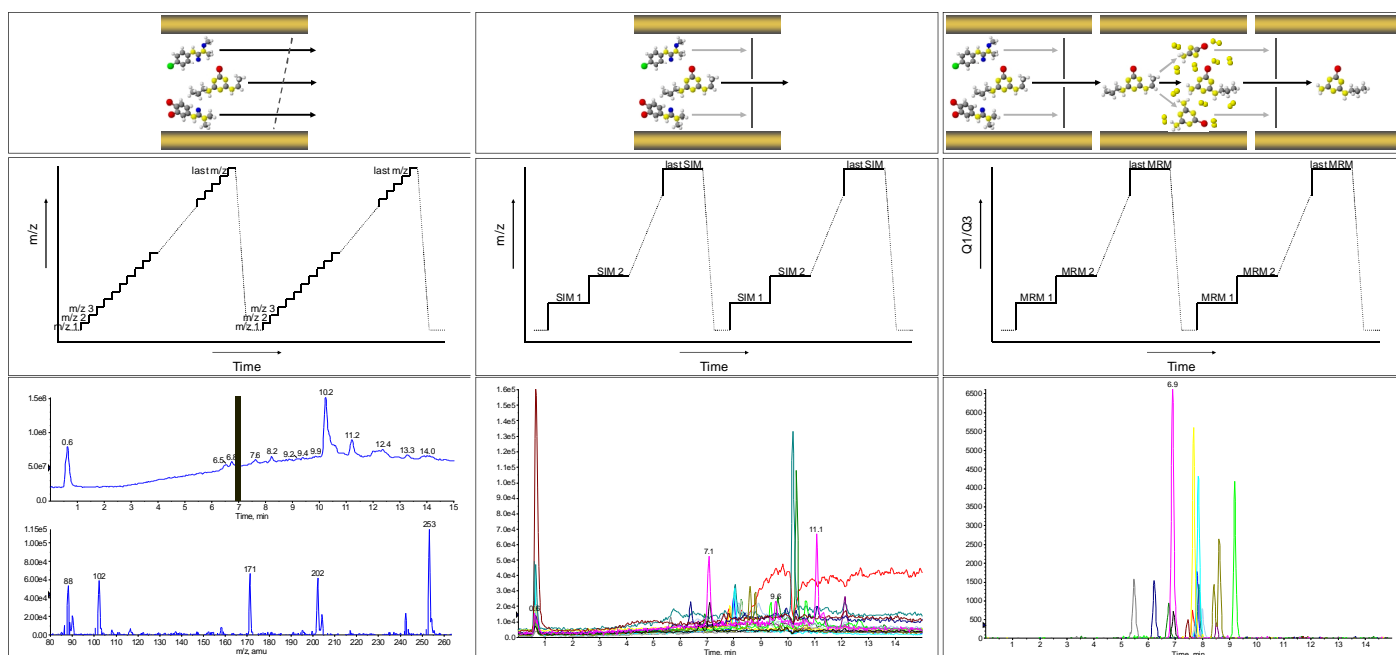


Figure 2. Pesticide in a soil extract detected in different single and triple quadrupole scan modes (left to right): Full scan with MS spectrum (50 µg/kg), Selected Ion Monitoring (5 µg/kg), and Multiple Reaction Monitoring (5 µg/kg)

Single quadrupole systems contain only one mass filtering quadrupole while triple quadrupole systems consist of three quadrupoles. Q1 and Q3 are working as mass filters while Q2 is acting as collision cell.

Quadrupoles can be used in scanning or filtering mode. During a mass scan, DC and RF voltages are ramped resulting in the acquisition of full scan mass spectra. Such spectra are typically used for qualitative data analysis. However, scanning a quadrupole suffers from low sensitivity and slow scan speed. Thus, quantitative studies are performed with quadrupoles working in filtering mode.

The most selective mode to use a single quadrupole MS is called Selected Ion Monitoring (SIM). Hereby, a fixed set of DC and RF voltages is applied to the quadrupole and thus only a single m/z can pass. Ions with different m/z are filtered out.

Multiple Reaction Monitoring (MRM) is the most common mode of using a triple quadrupole MS/MS for quantitative analysis, allowing enhanced sensitivity and selectivity. The first quadrupole filters a specific precursor ion of interest. Ions generated in the ion source having a different m/z can not pass Q1. The collision cell is optimized to produce a characteristic product ion by collision of the precursor ion with a neutral collision gas, such as nitrogen. This process is called Collision Induced Dissociation (CID). Generated product ions are transferred into the third quadrupole where only a specific m/z is allowed to pass. All other product ions are filtered out in Q3.

Thus MRM mode works like a double mass filter which drastically reduces noise and increases selectivity. The principle and resulting chromatograms of various scan modes analyzing a mix of pesticides spiked into soil is given in Figure 2. It illustrates how increasing selectivity of the mass spectrometric experiment reduces the noise of eluting background and matrix components.

Single quadrupole and triple quadrupole systems allow the detection of many SIM and MRM transitions, respectively. This enables quantitation of many targeted analytes in a single experiment. Typically, additional SIM and MRM transitions have to be detected to perform identification of quantified compounds. Hereby, the most intense ion is called the 'quantifier' and all additional ions are called 'qualifiers'. The EU Commission Decision 2002/657/EC defined performance criteria for confirmatory methods, such as MS and MS/MS, by introducing the concept of identification points. The required number of 4 identification points can be achieved by detecting 4 SIM on a single quadrupole MS or 2 MRM transitions on a triple quadrupole MS/MS.

Table 1. Pesticides detected in Multiple Reaction Monitoring (MRM) and Selected Ion Monitoring (SIM)

Pesticide	CAS	MRM 1	MRM 2	SIM 1	SIM 2	SIM 3	SIM 4	t _R (min)
<i>Atrazine</i>	1912-24-9	216/174	216/104	216	174	146	104	7.8
<i>Chlortoluron</i>	15545-48-9	213/72	213/46	213	72	140	168	7.6
<i>Cyanazine</i>	21725-46-2	241/214	241/104	241	214	104	132	6.7
<i>Desethylatrazine</i>	6190-65-4	188/146	188/104	188	146	104	110	5.5
<i>Diuron</i>	330-54-1	233/72	233/46	233	72	235	160	8.0
<i>Hexazinone</i>	51235-04-2	253/171	253/71	253	171	71	85	6.9
<i>Isoproturon</i>	34123-59-6	207/72	207/46	207	72	165*	134*	7.9
<i>Linuron</i>	330-55-2	249/160	249/182	249	160	251	182	8.5
<i>Metazachlor</i>	67129-08-2	278/134	278/210	278	134*	210	105	7.7
<i>Methabenzthiazuron</i>	18691-97-9	222/165	222/150	222	165*	150	124	7.8
<i>Metobromuron</i>	3060-89-7	259/170	259/148	259	261	148	170	7.7
<i>Metolachlor</i>	51218-45-2	284/252	284/176	284	252	176	134	9.2
<i>Metoxuron</i>	19937-59-8	229/72	229/46	229	72	156	106	6.2
<i>Monolinuron</i>	1746-81-2	215/126	215/99	215	126	99	148	7.4
<i>Sebuthylazine</i>	7286-69-3	230/174	230/104	230	174	104	146	8.4
<i>Simazine</i>	122-34-9	202/132	202/124	202	132	104	174	6.9
<i>Terbuthylazine</i>	5915-41-3	230/174	230/104	230	174	104	146	8.6

* Not enough HPLC separation to differentiate these in-source fragment ions

Experimental

HPLC

An Agilent 1200 standard HPLC system with binary pump, well plate autosampler, and column oven was used. Chromatographic separation was performed on a Phenomenex Synergi 4u Fusion RP-80 (50x2 mm) column. A gradient of eluent A (water with 5 mM ammonium formate) and eluent B (methanol with 5 mM ammonium formate) from 80/20 to 10/90 (A/B) over 8min was used. The column temperature was set to 25°C. A sample volume of 10 µL was injected.

MS/MS

MS and MS/MS detection was performed using an API 3200™ LC/MS/MS system with Turbo V™ source and an Electrospray ionization probe. All pesticides were detected in positive polarity using an ionization voltage of 5000V. The ion source temperature was set to 500°C. The detected quantifier and qualifier ions are listed in Table 1. A dwell time of 25 ms was used to detect 34 MRM transitions and 15ms to detect 68 SIM.

Results and Discussion

Selectivity

Detection on a triple quadrupole MS/MS results in higher selectivity due to the double mass filtering (Figure 3). In MRM typically a single signal per analyte is detected while SIM can result in multiple signals per compound. The reason for this is the generation of identical in-source fragment ions of pesticides of the same compound class. Thus more time consuming HPLC is required to separate such compounds to allow quantitation and identification. Needed HPLC development and analysis times are then comparable to traditional UV detection.

Sensitivity

Detection on a MS/MS system also results in better sensitivity. Detected Signal-to-Noise (S/N) is higher resulting in lower Limits of Quantitation (LOQ). The typical difference in sensitivity observed is at least one order of magnitude as illustrated in Figure 4.

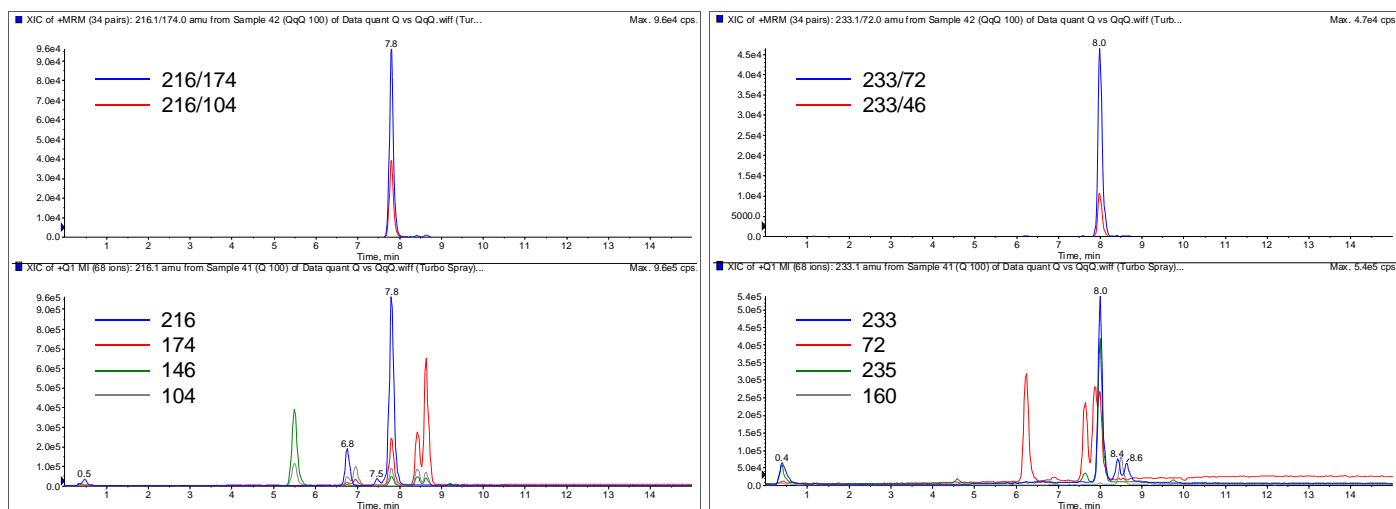


Figure 3. Comparison of selectivity detecting Atrazine (left) and Diuron (right) in MRM and SIM mode at 100 µg/L in drinking water

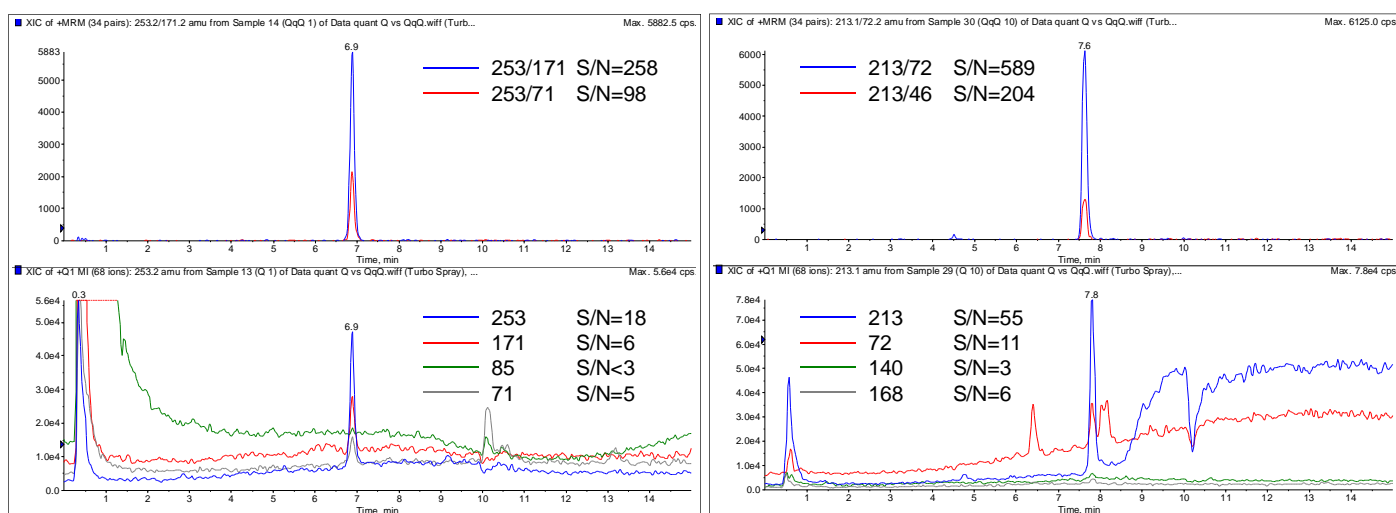


Figure 4. Comparison of sensitivity detecting 1 µg/L Hexazinone (left) and 10 µg/L Chlortoluron (right) in MRM and SIM mode in drinking water

If identification with 4 identification points is required and more than a single ion has to be detected the difference in LOQ based on quantifier MRM and SIM is significantly higher (Table 2).

Linearity and Reproducibility

Generally a wider linear range can be used for quantitation if a triple quadrupole MS/MS is used because of increased selectivity allowing detection at lower LOQ (Figure 5). The upper limit of quantitation of SIM and MRM is typically comparable due to saturation of the ion source or detector. The typical linear range in MRM using Electrospray Ionization is 3 to 4 orders of magnitude while SIM provides only 2 to 3 orders of magnitude.

Additionally, MS/MS allows more accurate and reproducible quantitation. Table 3 compares Coefficients of Variation (%CV) of detected pesticides at different concentrations. Triple quadrupole MS/MS provides %CV below 10 at LOQ and below 5 at higher concentrations. The reproducibility on single quadrupole MS is significantly lower.

Table 2. Limits of Quantitation (LOQ) and Limit of Identification (LOI) of detected pesticides*

Pesticide	LOQ (MRM 1)	LOI (MRM 2)	LOQ (SIM 1)	LOI (SIM 4)	Enhanced Quantifier	Enhanced Qualifier
<i>Atrazine</i>	0.02	0.05	0.2	1	10x	20x
<i>Chlortoluron</i>	0.1	0.5	1	10	10x	20x
<i>Cyanazine</i>	0.1	0.2	0.5	2	5x	10x
<i>Desethylatrazine</i>	0.1	0.1	0.5	2	5x	20x
<i>Diuron</i>	0.2	0.5	1	10	5x	20x
<i>Hexazinone</i>	0.02	0.05	0.2	1	10x	20x
<i>Isoproturon</i>	0.05	0.1	0.5	N/A	10x	N/A
<i>Linuron</i>	0.1	0.2	2	10	20x	50x
<i>Metazachlor</i>	0.02	0.02	0.5	5	25x	250x
<i>Methabenzthiazuron</i>	0.02	0.05	0.5	2	25x	40x
<i>Metobromuron</i>	0.2	0.5	2	5	10x	10x
<i>Metolachlor</i>	0.02	0.1	0.5	50	25x	500x
<i>Metoxuron</i>	0.1	0.5	0.5	20	5x	40x
<i>Monolinuron</i>	0.1	0.2	2	5	20x	25x
<i>Sebuthylazine</i>	0.1	0.1	0.5	2	5x	20x
<i>Simazine</i>	0.1	0.2	0.5	1	5x	5x
<i>Terbuthylazine</i>	0.02	0.1	0.5	2	25x	20x

*using Multiple Reaction Monitoring (MRM) and Selected Ion Monitoring (SIM) in drinking water samples based on S/N=6

Table 3. Reproducibility (%CV) of detected pesticides in MRM and SIM at different concentrations

Pesticide	%CV at 1µg/L (MRM)	%CV at 10µg/L (MRM)	%CV at 100µg/L (MRM)	%CV at 1µg/L (SIM)	%CV at 10µg/L (SIM)	%CV at 100µg/L (SIM)
<i>Atrazine</i>	4.4	3.7	0.9	16.2	1.8	0.6
<i>Chlortoluron</i>	4.2	3.4	1.1	20.3	2.9	1.7
<i>Cyanazine</i>	9.0	2.1	1.8	16.6	4.6	0.9
<i>Desethyl-atrazine</i>	3.8	2.0	1.5	5.8	0.8	1.4
<i>Diuron</i>	4.4	2.5	1.2	23.7	2.9	1.6
<i>Hexazinone</i>	1.7	1.3	1.2	3.0	1.5	1.5
<i>Isoproturon</i>	8.7	0.9	1.3	17.2	3.0	1.3
<i>Linuron</i>	9.6	7.0	0.9	<LOQ	3.9	2.4
<i>Metazachlor</i>	3.6	1.9	0.9	5.4	1.5	1.1
<i>Methabenzthiazuron</i>	3.0	0.4	0.9	9.9	1.4	0.5
<i>Metobromuron</i>	4.0	3.9	1.4	<LOQ	8.5	2.5
<i>Metolachlor</i>	4.7	1.0	0.7	7.8	1.6	1.6
<i>Metoxuron</i>	5.9	2.3	0.9	13.5	2.7	1.5
<i>Monolinuron</i>	6.2	6.0	1.3	<LOQ	5.5	2.9
<i>Sebuthylazine</i>	7.9	2.3	1.0	7.5	1.3	2.5
<i>Simazine</i>	7.0	2.8	1.8	8.7	1.2	1.5
<i>Terbuthylazine</i>	6.3	3.4	1.2	8.5	3.9	2.4

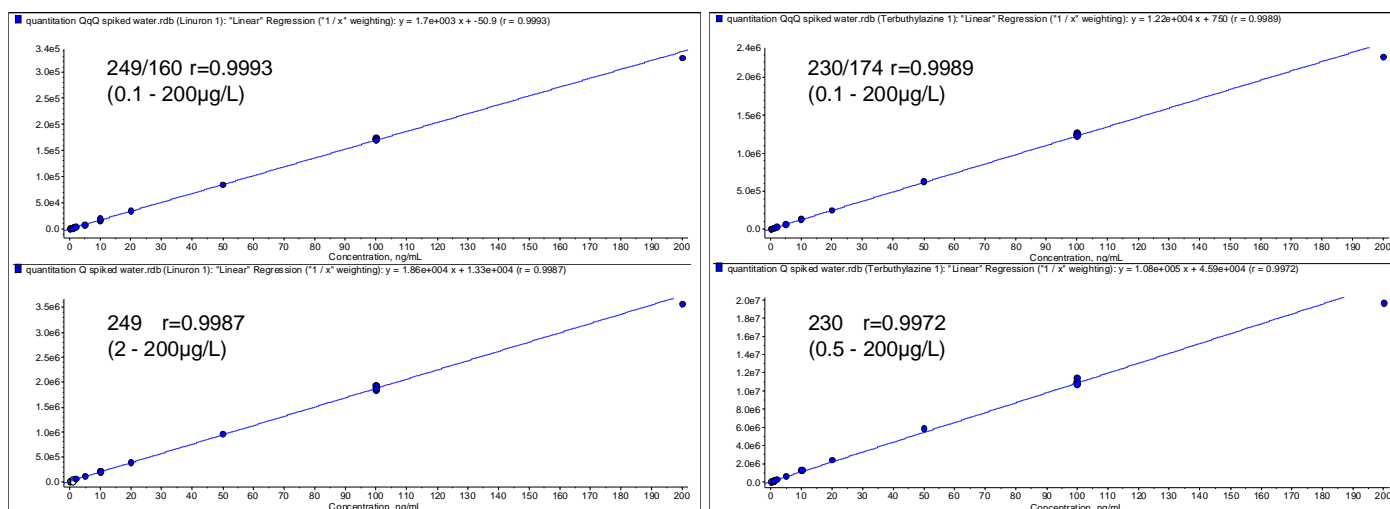


Figure 5. Comparison of linearity detecting Linuron (left) and Terbutylazine (right) in MRM (top) and SIM (bottom) mode in spiked drinking water samples

Summary

The detection in Multiple Reaction Monitoring (MRM) using a triple quadrupole MS/MS has a number of advantages in comparison to Selected Ion Monitoring (SIM) of a single quadrupole MS.

Due to double mass filtering MS/MS detection provides much higher selectivity with less interference of co-eluting compounds and matrix components, resulting in less time consuming method development and faster analysis times. Better Signal-to-Noise allows quantitation with lower Limits of Quantitation (LOQ).

Fewer ions have to be detected per compound on MS/MS in comparison to MS for confirmatory analysis. The concept of identification points introduced by the EU Commission Decision requires the detection of 2 MRM transitions and 4 SIM, respectively. If identification is required the detected qualifier signals have to be compared to specify LOQ. The difference between both technologies can be then at least two orders of magnitude. Finally, a wider linear range, higher accuracy, and reproducibility can be obtained on triple quadrupole MS/MS.

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