Higher Confidence in Identification with QTRAP® LC/MS/MS Systems when Screening and Quantifying Pesticides in Fruit and Vegetable Samples

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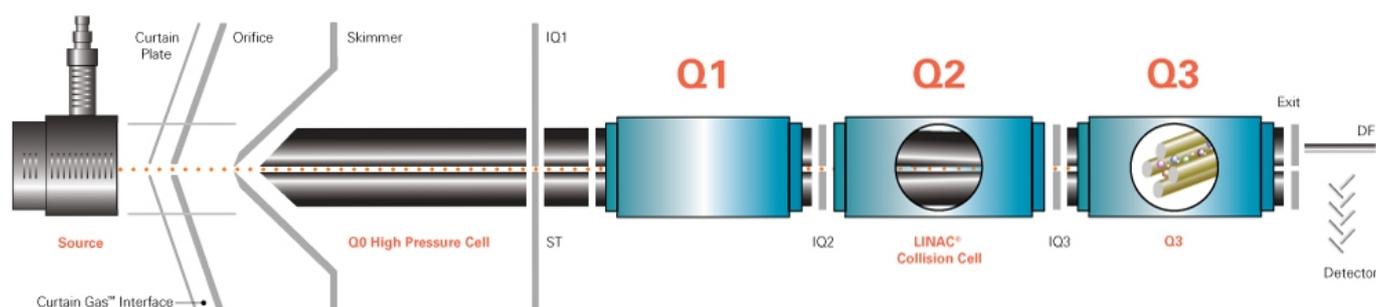*AB SCIEX, Concord, Ontario, Canada*

Figure 1. Ion path of a QTRAP® LC/MS/MS System

QTRAP® LC/MS/MS Systems provide in comparison to triple quadrupole systems:

- MRM selectivity and sensitivity for quantitation with the lowest limits of detection, excellent reproducibility and linear range

- Fast and high sensitivity Linear Ion Trap scan functions for qualitative analysis and identification

- Rich MS/MS spectra because of fragment ion generation in the LINAC® Collision Cell and the use of Collision Energy Spread

- Combination of triple quadrupole and QTRAP® System functions without delay time in IDA experiments allowing quantitation and identification with MS/MS spectra in a single LC run

- Higher confidence of identification based on mass spectral library search minimizing the risk of false positive and negative results

Overview

This paper describes the use of QTRAP® Systems coupled to liquid chromatography for the screening and identification of pesticides in fruit and vegetable samples. The selectivity, sensitivity, and accuracy of detection using QTRAP® System and conventional triple quadrupole mass spectrometers are compared. The higher confidence of identification provided by QTRAP® linear ion trap MS/MS scans followed by library searches which minimize the potential risk of false positive and negative results is highlighted.

Introduction

Liquid Chromatography (LC) coupled to tandem Mass Spectrometry (MS/MS) is a powerful analytical tool used in many food, environmental and forensic testing laboratories to detect targeted analytes, such as pesticides, drugs of abuse, steroids, pharmaceuticals, personal care products, and many other contaminants at trace levels.

A variety of ion sources including Electrospray Ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI), and Atmospheric Pressure Photo Ionization (APPI) facilitates the ionization of polar to non-polar compounds. Generated ions are transferred following ionization through a vacuum interface into the mass analyzer.
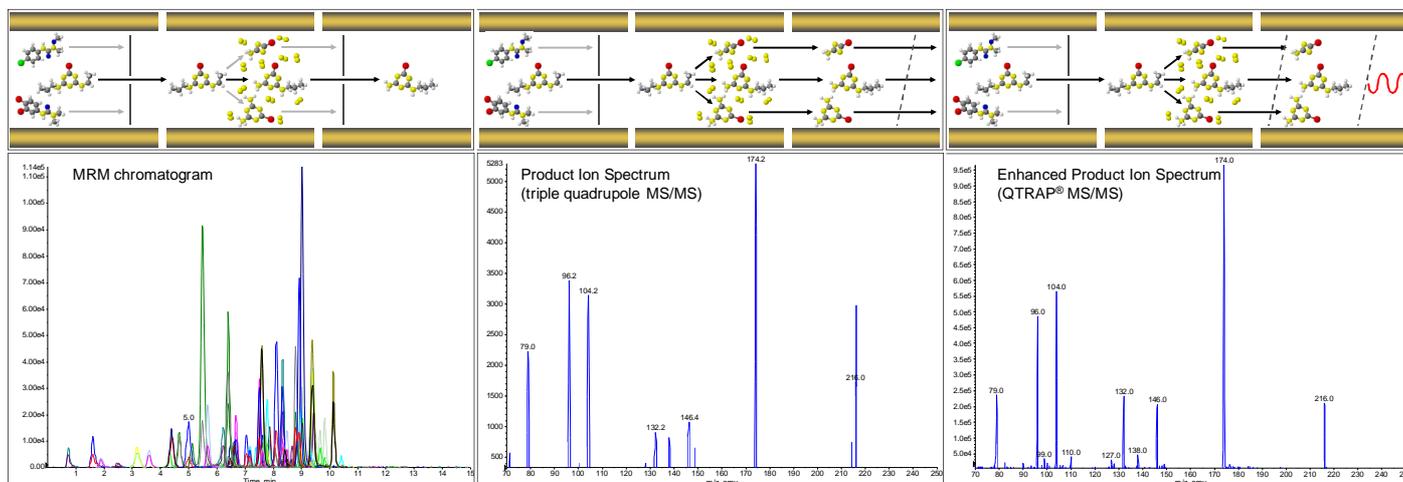
Figure 2. Screening and identification of pesticides using triple quadrupole and QTRAP® LC/MS/MS Systems. Left: detection 150 Multiple Reaction Monitoring transitions to screen for pesticides. Middle: Product Ion spectrum of a triple quadrupole MS/MS. Right: Enhanced Product Ion spectrum of a QTRAP® MS/MS to identify the presence of a detected pesticide. (Note that the sensitivity of the QTRAP® MS /MS spectrum is more than 100 times higher than that of the triple quadrupole spectrum.)

Triple quadrupole (QqQ) mass analyzers operating in Multiple Reaction Monitoring (MRM) are mostly used for the quantitation of targeted analytes. In MRM mode, the first quadrupole filters a specific precursor ion; the collision cell (second quadrupole) generates fragments (product ions) which are filtered in the third quadrupole (Figure 2).

In 2002, AB SCIEX introduced a new generation of LC/MS/MS systems: hybrid triple quadrupole linear ion trap mass spectrometers with axial ion ejection (QTRAP® System). QTRAP® Systems enable the unique capabilities provided by this technology by coupling targeted triple quadrupole experiments, such as MRM, precursor ion, and neutral loss scans, with high sensitivity ion trap scan functions, such as enhanced MS, enhanced product ion, enhanced resolution, enhanced multiple charge and MS/MS/MS scan.[2-4]

Having a variety of new scan types and the possibility to combine them in single LC runs enable completely new workflows for many LC/MS/MS applications, including food, environmental, and forensic testing, metabolite identification and proteomics.[5-15]

QTRAP® System Technology

The mass analyzer region of a QTRAP® System is based on the conventional ion path of a triple quadrupole mass spectrometer. In comparison to QqQ systems, the third quadrupole (Q3) of a QTRAP® System can be operated as a quadrupole or as a Linear Ion Trap (LIT). The dual functionality of Q3 provides the QTRAP® System complete functionality of QqQ mass spectrometer, including true Multiple Reaction Monitoring for selective and sensitive quantitation and additional LIT scan functions. These additional LIT scan functions greatly enhance the performance for screening, confirmation and identification applications.
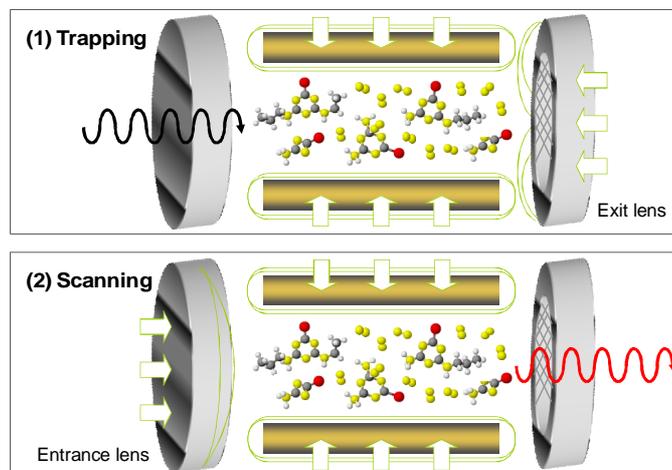

Figure 3. Trapping and scanning of ions in a Linear Ion Trap (LIT)

The operation of Q3 as LIT can be divided into two basic steps: (1) trapping and (2) scanning as shown in Figure 3. First precursor ions and/or product ions, depending on the selected scan mode, exit the collision cell and enter Q3 operating in LIT mode. The ions are trapped in Q3 by the RF voltage in radial direction and by a repulsive DC voltage applied to the 'exit lens' of Q3. After a fill time of typically a few milliseconds to accumulate ions, a repulsive 'entrance lens' is used to stop ions entering Q3. Trapped ions are then cooled for a short time and subsequently ejected axially from the LIT using an auxiliary voltage that is ramped in concert with the drive RF voltage.

The most important LIT scan modes for screening, confirmation and identification applications are Enhanced MS (EMS), Enhanced Resolution (ER) and Enhanced Product Ion (EPI) scans.

In EMS, the ion trap is filled with molecular ions generated in the ion source to identify unknown compounds. The ER scan measures the mass of a targeted ion more accurately and can be used to determine charge states and isotopic patterns. Finally, the EPI mode can be used to identify the presence of the detected analyte by interpretation of fragment ions or mass spectral library searching.

The Enhanced Product Ion scan of QTRAP® Systems has several advantages over product ion scanning of QqQ mass spectrometers, including:

- Much higher sensitivity of Enhanced Product Ion spectra because of ion accumulation in the LIT as shown in Figure 2
- More product ion information in a single MS/MS spectrum because of Collision Energy Spread
- Lower cycle time due to faster scanning of linear ion trap

The Enhanced Product Ion scan of QTRAP® Systems has also several advantages over product ion scan of traditional ion trap mass spectrometers, including:

- Fragment rich MS/MS spectra characteristic for Collision Induced Dissociation (CID) in comparison to resonance excitation spectra
- No inherent low mass cut-off due to fragment generation in the collision cell rather than in the ion trap
- Lower cycle time than traditional ion traps because of simultaneous precursor ion selection and fragmentation while QTRAP® System is filled
- Remarkable immunity of LIT from space charge effects which result in mass shift and incorrect isotopic pattern in the spectrum[2-3]

Experimental

A 3200 QTRAP® LC/MS/MS System with Turbo V™ Source and an ESI probe was used in both triple quadrupole and QTRAP® modes to investigate the use of these two mass spectrometers for the screening and identification of pesticides.

Samples

Fruit and vegetable samples (apple, apricot, banana, cucumber, grape, grapefruit, kiwi, lemon, orange, pear, pepper, raisin, strawberry, tea, and tomato) were extracted using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) procedure.[16]

HPLC

A Shimadzu Prominence LC system consisting of system controller CBM-20A, 2 pumps LC-20AD, degasser DGU-20A3, autosampler SIL-AC, and column oven CTO-20AC was used. 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 8 min was used on a Phenomenex Synergi 4u Fusion-RP 80A 50x2 mm column. The column temperature was set to 25°C. Sample volume of 20 µL was injected.

MS/MS Detection

In QqQ mode 75 pesticides were detected in positive polarity using 2 MRM transitions per analyte.

The detected quantifier and qualifier MRM with the respective MRM ratio are listed in Table 1. All compound dependent parameters were completely optimized for the detection of each pesticide with the highest sensitivity. A dwell time of 5 ms was used for all MRM transitions.

This typical triple quadrupole experiment was compared to a unique QTRAP® Information Dependent Acquisition (IDA) where only a single MRM transition was monitored with a dwell time of 10ms per compound. This MRM survey automatically triggered an Enhanced Product Ion scan if the MRM signal was above a threshold of 500 cps. EPI spectra were acquired from 50 to 550 amu with a scan speed of 4000 amu/s and a Collision Energy (CE) of 35 V with Collision Energy Spread (CES) of 15 V. Also dynamic exclusion was activated to minimize the risk of missing co-eluting compounds.
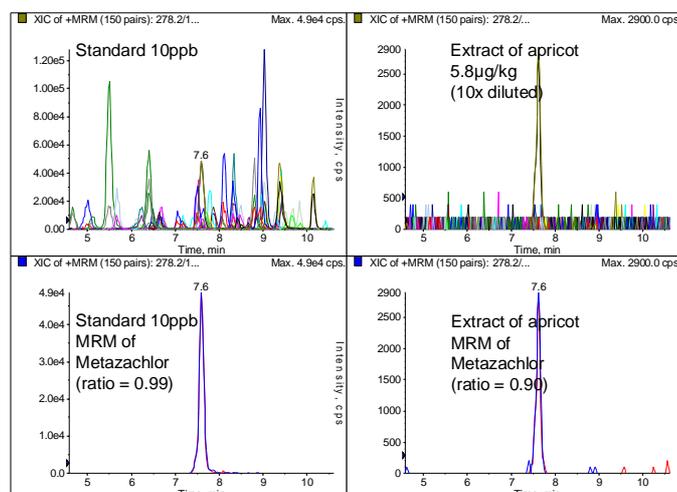

Figure 4. Quantitation and MRM ratio identification of 5.8 µg/kg Metazachlor in an extract of apricot (278/134 and 278/210)

Table 1. MRM transitions for the 75 studied pesticides, MRM ratios, Limits of Detection (S/N=6) and coefficients of variation at 10ng/mL (n=3)

Pesticide	MRM 1	MRM 2	Ratio	LOD (ng/mL)	%CV (n=3)	Pesticide	MRM 1	MRM 2	Ratio	LOD (ng/mL)	%CV (n=3)
Acetamiprid	223/126	223/99	0.20	0.2	4.7	Irgarol	254/198	254/83	0.14	0.05	2.20
Aldicarb	208/89	208/116	0.60	1.0	8.5	Isoproturon	207/72	207/46	0.25	0.20	3.90
Atrazine	216/174	216/104	0.51	0.1	5.5	Lenacil	235/153	235/136	0.24	0.20	5.00
Azoxystrobin	404/372	404/344	0.45	0.1	1.5	Linuron	249/160	249/182	0.98	1.00	8.50
Benalaxyl	326/148	326/91	0.97	0.1	3.3	Lufenuron	511/141	511/158	0.50	5.00	11.90
Bendiocarb	224/109	224/167	0.95	0.5	7.9	Malathion	331/127	331/99	0.42	0.20	2.70
Bromuconazole	378/159	378/161	0.77	0.5	4.5	Mebendazole	296/164	296/105	0.26	0.20	1.10
Buprofezin	306/201	306/116	0.71	0.2	4.9	Metalaxyl	280/220	280/192	0.79	0.50	4.90
Carbaryl	202/145	202/127	0.40	0.2	5.4	Metamitron	203/175	203/104	0.72	0.20	4.30
Carbendazim	192/160	192/132	0.17	0.1	3.1	Metazachlor	278/134	278/210	0.98	0.10	2.40
Carbofuran	222/165	222/123	0.44	0.2	3.3	Methabenzthia-zuron	222/165	222/150	0.35	0.10	3.50
Chlorfenvinphos	359/99	359/155	0.90	0.5	5.5	Methidathion	303/145	303/85	0.93	0.50	4.70
Chlorotoluron	213/72	213/46	0.21	0.2	3.2	Methiocarb	226/169	226/121	0.96	0.50	3.30
Chlorpyrifos-methyl	322/125	324/125	0.71	1.0	8.4	Methomyl	163/88	163/106	0.77	1.00	7.00
Cyanazine	241/214	241/104	0.24	0.5	6.9	Metolachlor	284/252	284/176	0.29	0.10	2.20
Cyproconazole	292/125	292/70	0.95	0.5	7.2	Metolcarb	166/109	166/94	0.30	0.50	3.80
Cyromazine	167/85	167/68	0.45	0.5	4.2	Metribuzin	215/187	215/84	0.70	1.00	8.20
Desethyl-atrazine	188/146	188/104	0.40	0.5	7.7	Molinate	188/126	188/55	0.52	2.00	7.90
Desisopropyl-atrazine	174/104	174/96	0.77	1.0	6.7	Monolinuron	215/126	215/99	0.68	0.50	6.00
Diazinon	305/169	305/97	0.94	0.1	3.1	Monuron	199/72	199/126	0.16	0.10	1.60
Dichlorvos	221/109	223/109	0.55	1.0	4.6	Nitenpyram	271/126	271/237	0.78	0.50	4.10
Difenoconazole	406/251	408/253	0.56	0.2	2.8	Oxadixyl	279/219	279/132	0.51	0.50	0.70
Difenoxuron	287/72	287/123	0.65	0.5	2.0	Pendimethalin	282/212	282/194	0.18	1.00	8.70
Diflubenzuron	311/141	311/158	0.70	1.0	6.6	Prochloraz	376/308	376/70	0.26	0.20	2.80
Dimethenamide	276/244	276/168	0.24	0.1	3.2	Profenofos	375/305	373/303	0.87	1.00	1.90
Dimethoate	230/125	230/199	0.76	0.2	2.8	Promecarb	208/109	208/151	0.94	1.00	6.40
Dimethomorph	388/301	388/165	0.52	0.5	4.3	Prometon	226/142	226/86	0.29	0.10	3.20
Diuron	233/72	235/72	0.70	0.5	5.1	Prometryn	242/158	242/200	0.43	0.20	0.20
Ethiofencarb	226/107	226/77	0.62	0.5	6.7	Propachlor	212/170	212/94	0.72	0.20	3.70
Fenamiphos	304/217	304/202	0.59	0.1	2.6	Propanil	218/127	218/162	0.96	1.00	3.60
Flufenacet	364/194	364/152	0.71	0.2	3.4	Propiconazole	342/159	342/69	0.25	0.50	1.00
Flufenoxuron	489/141	489/158	0.51	1.0	7.6	Prosulfocarb	252/91	252/128	0.21	0.20	7.30
Fluroxypyr	255/209	255/181	0.74	5.0	10.4	Simazine	202/132	202/124	0.90	0.50	3.10
Hexaflumuron	461/158	461/141	0.59	5.0	9.0	Spiroxamine	298/144	298/100	0.66	0.20	3.40
Imazalil	297/159	299/161	0.37	0.5	8.5	Terbutryn	242/186	242/68	0.18	0.02	2.00

Table 1. continued

Pesticide	MRM 1	MRM 2	Ratio	LOD (ng/mL)	%CV (n=3)	Pesticide	MRM 1	MRM 2	Ratio	LOD (ng/mL)	%CV (n=3)
Imazapyr	262/217	262/149	0.54	0.2	3.0	*Thiabendazole*	202/175	202/131	0.62	0.10	1.60
Imazaquin	312/199	312/267	0.99	0.5	4.5	*Thiacloprid*	253/126	253/99	0.32	0.20	6.70
Imidacloprid	256/175	256/209	0.73	1.0	4.6						

Results

MRM Selectivity

Detection in Multiple Reaction Monitoring (MRM) using QTRAP® and triple quadrupole Systems offers superior selectivity because of double mass filtering in the mass analyzer. This selectivity greatly assists the screening for unknown and targeted analytes in complex samples such as extracts of fruits and vegetables. Figure 4 shows an example of MRM detection of 5.8 µg/kg Metazachlor in apricot extract. No matrix signals interfere with the quantitation of the identified contaminant because of the high selectivity of MRM detection.

MRM Sensitivity

QTRAP® and triple quadrupole Systems operated in MRM allow quantitation of trace levels with superior sensitivity. The Limits of Detection (LOD) of 75 pesticides are summarized in Table 1. Figure 5 gives example chromatograms of selected pesticides spiked into a tomato extract at a concentration of 10 ng/mL. All MRM transitions in the presented screening method were detected using a short dwell time of 5 ms. The Linear Accelerator (LINAC®) Collision Cell enables the use of such low dwell times without loss in sensitivity.

Identification using MRM ratios

Despite the high selectivity of MRM detection, there is always a risk of false positive or negative findings due to interfering matrix signals. Accordingly, screening results have to be confirmed. Typically a second MRM is monitored per analyte and the ratio of quantifier to qualifier transition is calculated for each unknown sample and compared to the MRM ratio of standards. Various guidelines such as the European Commission Decision 2002/657/EC define MRM ratio tolerance levels for identification (Table 2).[17] Figure 4 shows an example of positive identification based on MRM ratio of 5.8 µg/kg Metazachlor in apricot extract.
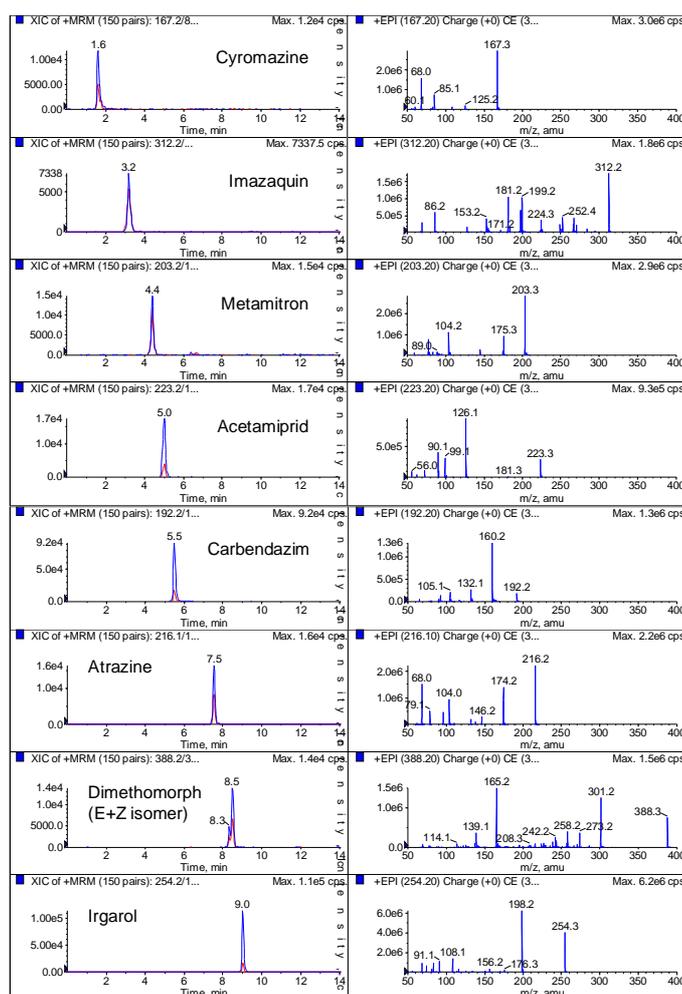

Figure 5. Example chromatograms of pesticides spiked at 10 ng/mL into a tomato extract (left) with corresponding Enhanced Product Ion spectra at the same concentration (right)

Identification using QTRAP® MS/MS Spectra and Library Searching

As an alternative, identification can be performed using full scan MS/MS experiments and library searching to compare the unknown with a standard spectrum. As shown in Figure 2, identification based on MS/MS spectra is more efficient using QTRAP® Systems because of higher sensitivity and faster

scanning in comparison to QqQ. In addition, QTRAP® Systems provide more characteristic MS/MS spectra and lower cycle times than traditional ion traps.

Figure 5 shows the Enhanced Product Ion (EPI) spectra of selected pesticides at a concentration of 10 ng/mL spiked into tomato extract. These spectra were acquired automatically using IDA with a survey scan containing 75 MRM transitions. The spectra shown illustrate that EPI spectra are highly selective and they contain the complete molecular fingerprint of the analyte for identification. Also EPI spectra have superior sensitivity and can be searched against existing mass spectral libraries.

Table 2. MRM ratio tolerances of 2002/657/EC[17]

Relative MRM Intensity	Maximum Tolerance
> 0.5	± 20%
0.2 – 0.5	± 25%
0.1 – 0.2	± 30%
< 0.1	± 50%

This higher degree of Identification can be used to minimize the potential risk of false positive and false negative detection based on MRM ratio calculation. An example is presented in Figure 6. The pesticides Thiabendazole and Imazalil were identified and quantified above the MRL in a banana extract. However, only Thiabendazole was identified by its MRM ratio. Imazalil could not be identified based on the MRM ratio of 0.50 and an MRM ratio tolerance of 0.28-0.46. In comparison, the EPI spectrum rich in fragment ions searched against a library clearly identifies the presence of both contaminants in the banana sample and the false negative detection for Imazalil was avoided.

Figure 7 shows another example demonstrating the advantage of MS/MS library searching for identification. The MRM ratio clearly identifies the presence of Metalaxyl in strawberry extract. The corresponding EPI spectrum and the library search result clearly identify a false positive finding. Metalaxyl was not present in this strawberry sample.

Reproducibility of quantitation and identification

Performing identification analysis using an automatically triggered EPI scan instead of MRM ratio calculation allows a reduction of the number of monitored MRM transitions. Only 75, instead of 150, MRM transitions have to be monitored using the QTRAP® screening method for detection and identification of 75 pesticides. The reduction in MRM transitions resulted in a shorter cycle time of the MRM experiment (QTRAP®: 1.1 s and

QqQ: 1.5 s) although a longer dwell time was used per transition. This shorter cycle improved reproducibility since more data points were acquired across the LC peaks.

A mix of pesticides was spiked into tomato extract and analyzed 10 times using the developed QqQ and QTRAP® methods. Data presented in Table 3 and example spectra in Figure 8 clearly show the better reproducibility of identification based on mass spectral library searching of EPI spectra in comparison to MRM ratio calculation.
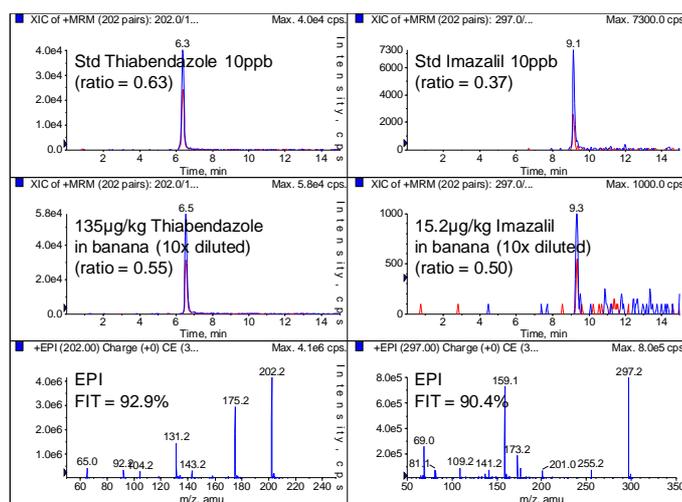

Figure 6. Quantitation of 135 µg/kg Thiabendazole and 15.2 µg/kg Imazalil in banana extract with positive identification of Thiabendazole (0.55 with tolerance of 0.50-0.76) and negative identification of Imazalil (0.50 with tolerance of 0.28-0.46). This false negative can be avoided by identification based on Enhanced Product Ion (EPI) scan and library search
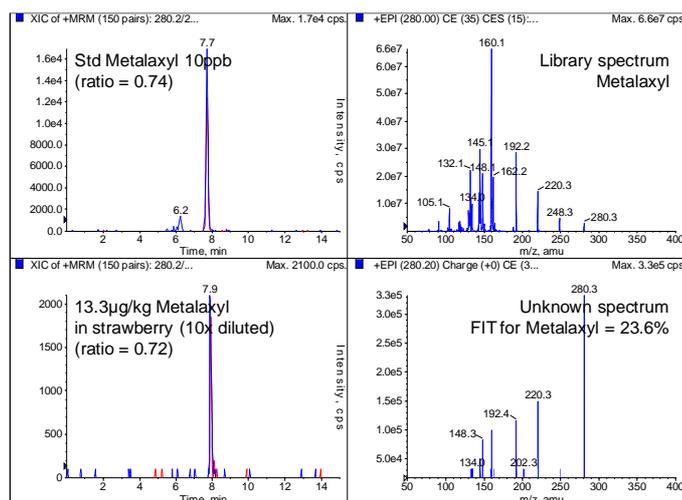

Figure 7. Quantitation of 13.3 µg/kg Metalaxyl in strawberry extract. This result was identified by MRM ratio calculation (0.72 with tolerance of 0.59-0.89), but the library search clearly indicates a false positive finding

Table 3. Reproducibility of identification based on MRM ratio calculation and mass spectral library search based on repeat injections of pesticides spiked into tomato extract (n=10)

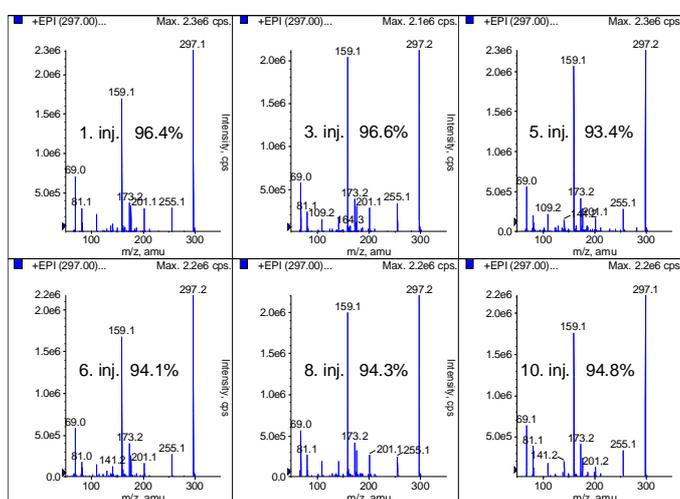| Pesticide	MRM ratio	%CV	Library FIT	%CV
Azoxystrobin	0.22	8.9	88.8	1.8
Dimethomorph	0.76	8.8	95.7	0.5
Flufenacet	0.90	11.8	91.2	1.3
Imazalil	0.52	13.6	94.7	1.3
Imidacloprid	0.39	25.3	78.1	5.5
Methabenzthiazuron	0.47	11.7	95.9	1.0
Thiabendazole	0.66	4.9	91.2	2.4

Figure 8. Repeated analysis of banana extract with detection of 15.2 μg/kg Imazalil – Library search FIT values of Enhanced Product Ion spectra indicate the high reproducibility of MS/MS identification using QTRAP® LC/MS/MS Systems

Summary

Multiple Reaction Monitoring (MRM) using triple quadrupole and QTRAP® LC/MS/MS Systems is widely used for targeted analyte screening and quantitation because of its unmatched selectivity and sensitivity. Double mass filtering in the mass analyzer allows for the best Signal-to-Noise (S/N) and thus the lowest Limits of Detection (LOD) with excellent reproducibility and linear range. In many applications, such as food, environmental and forensic testing, additional mass spectrometric information is required by guidelines for identification of detected analytes. This identification is typically performed by detecting two MRM transitions per compound and the calculation of an MRM ratio.

However, with an increasing demand to test for more and more contaminants in a larger variety of samples, the required number of MRM transitions exceeds the possibilities

of even state-of-the art triple quadrupole mass spectrometers, such as AB SCIEX systems using the patented Linear Accelerator (LINAC®) Collision Cell. This results in a limitation of the number of compounds that can be screened for in a single injection and/or less reproducibility and accuracy due to an insufficient number of data points across the LC peaks.

Here Hybrid Triple Quadrupole Linear Ion Trap (QTRAP®) Systems provide a novel workflow for the screening and identification of a multitude of targeted analytes by combining selective MRM detection with a highly sensitive MS/MS scan using Q3 as Linear Ion Trap. In Information Dependent Acquisition (IDA) experiments, the detection of an MRM signal above a specified threshold automatically triggers an Enhanced Product Ion (EPI) scan. These EPI spectra are as sensitive and selective as MRM signals and contain the complete molecular fingerprint because of precursor ion selection in Q1, product ion generation in the collision cell, and ion accumulation in the LIT. The rich product ion spectra are the result of the generation of fragment ions in the collision cell. These spectra can be searched against existing mass spectra libraries.

The information saved into a full scan MS/MS spectrum allows identification with a higher degree of confidence minimizing the risk of potential false positive and negative detection. In addition, the improved cycle time for all confirmatory MRM transitions can be used to increase the dwell time of all other MRM transitions to improve S/N, resulting in better reproducibility and accuracy. Alternatively, additional compounds can be screened in the MRM survey scan.

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