Application Note: 52279

High Precision Pesticide Analysis in Produce using GC Triple Quadrupole and U-SRM Mode

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Key Words

- 1 ppb Levels
- Challenging Compounds
- Pesticide Analysis
- PTV Backflush
- U-SRM Mode
- Selected Reaction Monitoring
- Selectivity
- Timed-SRM

Introduction

Pesticides are widely used in agriculture to protect crops and to improve efficiency of production. Consequently, governments, food producers and food retailers have the duty to ensure that any residues occurring in foods for human consumption are at or below Statutory Maximum Residue Levels (MRLs). Regulation EC 396/2005 adopted in the European Union sets MRLs for more than 500 different pesticides in over 300 different food commodities.[1]

Many of these MRLs are set at a default value of 0.01 mg/kg,the typical limit of determination of routine analytical methods. Thus, there is a requirement for food safety laboratories to test a wide array of foods for a large number of pesticide residues at concentrations at or below 0.01 mg/kg, with low costs and fast turnaround times (often <48 hours). For the efficient control of the regulated MRL levels, the overall method sensitivity in matrix is required to be a factor of 10 lower. This is most often achieved using multi-residue methods based on the use of a combination of LC-MS/MS and GC-MS techniques to determine pesticide residues in a single generic solvent extract of the sample. One such example is the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure, which is based on acetonitrile extraction and dispersive solid phase extraction.[2] After the QuEChERS extraction, a solvent exchange was made to facilitate the GC injection.

The productivity benefit of using the QuEChERS extraction technique is the fast turnaround time for a large number of samples with small sample volumes in the range of 10 g. Limitations of this approach are typically arising from the heavy matrix load of QuEChERS extract requiring increased robustness of the GC inlet system and increased selectivity offered by using a MS/MS analyzer. This application note describes the high quality and low level analysis of pesticides in produce samples using the Thermo Scientific TSQ Quantum XLS Ultra GC-MS/MS system.

For most of the pesticide compounds included in the method, the complete list of the compounds with their respective SRM transitions have been downloaded from the Pesticides Method Reference CD (provided with the manual p/n 120390) into the instrument acquisition method. Each transition has been determined for optimal sensitivity and selectivity, with the complete list documented for TSQ Quantum XLS users.

Over 400 pesticides have been monitored in several matrices such as wheat, blackcurrants and cucumber; the results of the most challenging pesticides in terms of activity and response are highlighted, showing calibration curves, repeatability and ion ratio stabilities.

The TSQ Quantum XLS Ultra™ is able to perform SRM with a higher mass resolution (0.1 Da) setting thus allowing for better selectivity. Not all pesticides in all matrices benefit from a higher mass resolution setting, but depending on the matrix and the compound analyzed, there can be a significant improvement on the signal to noise ratio. Some examples are shown in the 'Advanced GC-MS/MS Experiment' section of this application note.

Experimental Conditions

All samples were prepared using the QuEChERS technique, and calibration was performed using a blank QuEChERS extract from cucumber. All target compounds were measured using at least two SRM transitions for each compound to a level of 0.001 mg/kg, which is ten times lower than the current maximum concentration limit.

All sample analyses were carried out using the TSQ Quantum XLS Ultra GC-MS/MS system, equipped with a Thermo Scientific TRACE GC Ultra gas chromatograph.

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The TRACE GC Ultra™ was configured with a B.E.S.T. PTV injector equipped with a backflush device. Sample introduction was performed using the Thermo Scientific TriPlus RSH autosampler. The capillary column was a Thermo Scientific TraceGOLD TG-5MS column (5% phenyl film) of 30 m length, 0.25 mm inner diameter and 0.25 µm film thickness (Table 1).

The pre-column used was a 1.2 m TG-5HT, 0.15 µm film thickness and 0.53 mm inner diameter (see Table 1).

TRACE GC Ultra	
Injection Volume	2 µL injection
Liner	Siltec® baffled liner (part number 453T2120)
Carrier Gas	He, constant flow, 1.3 mL/min
Column Type	TraceGOLD™ TG-5MS column (5% phenyl film) of 30 m length, 0.25 mm inner diameter and 0.25 µm film thickness. (part number 26098-1420)
Precolumn	1.2 m of TraceGOLD TG-5HT column of 30 m length, 0.53 mm inner diameter and 0.15 µm film thickness (part number 26095-0620)
GC Method	Initial 65 °C, Hold 1.5 min, Ramp 30.0 °C/min–150 °C, Ramp 5.0 °C/min–290 °C, Ramp 30.0 °C/min–320 °C, Hold 5.0 min
Transfer Line	300 °C
TRACE GC Ultra PTV Program	
Injector Temperature	70 °C, splitless injection 1.5 min
PTV Inject	70 °C, 0.2 min, 8 °C/sec to transfer step
PTV Transfer	280 °C, 21 min, 10 °C/sec to clean step
PTV Clean	350 °C, 33 min, clean flow 30 mL/min
Transfer Time	21 min
TSQ Quantum XLS Ultra Mass Spectrometer	
Source Temperature	240 °C, CEI volume
Ionization	EI, 70 eV
Emission Current	50 µA
Resolution	0.7 Da Q1, Q3; 0.1 Da on Q1, 0.7 on Q3 for the wheat examples
Collision Gas	Argon, 1.5 mTorr

Table 1: Selected instrument conditions for the employed TRACE GC Ultra and TSQ Quantum XLS Ultra mass spectrometer

Maximizing Robustness

High boiling compounds in sample matrix have a negative effect on the analytical column's quality and lifetime, requiring a bake out process at high temperatures, thus limiting sample throughput. A backflush process was used to protect the column, allowing more samples to be injected before the phase attachment on the surface of the column becomes weak. Being able to inject more samples before necessary column replacement improves throughput and reduces costs per analyses.

During backflushing of the pre-column, the injector was set to a higher temperature and increased flow. This also allowed the injector liner to be swept of residual matrix contaminants during analysis time. This concurrent backflush operation results in the complete system staying clean and inert for a high number of injections, resulting in less maintenance frequencies.[3]

Method Setup

The method parameters for the PTV concurrent backflush operation, GC separation and TSQ Quantum XLS Ultra mass spectrometer setup are given in Table 1.

Each compound SRM transition was only monitored for a narrow time window around the established retention time (timed SRM). This led to a fully optimized instrument duty cycle for maximum analytical performance being handled automatically by the system. The complete list can be copied into the instrument method, thus saving time and avoiding entry errors.[4]

For data acquisition, the two most selective transitions were chosen after reviewing data from spiked matrix samples. Selection criteria were based on the absence of interferences from the matrix, along with signal generation of the transition.

Results and Discussion

Advanced GC-MS/MS Experiments – U-SRM

The patented HyperQuad™ technology in the TSQ Quantum XLS Ultra system offered high sensitivity by high ion transmission already found at the standard nominal mass resolution settings (0.7 Da FWHM). In addition, the HyperQuad technology allows the possibility to enhance the applied mass resolution for increased selectivity during analysis. The significantly increased selectivity further reduces the background caused by matrix components, thus giving a cleaner peak detection and high signal-to-noise results.

Some compound transitions are more susceptible to matrix interference than others. Standard SRM resolution (0.7 Da) can often provide enough selectivity to overcome most matrix interference challenges. In complex matrices, however, even with the structure-selective SRM acquisitions, removal of the isobaric matrix interference is insufficient.

By increasing the mass resolution (down to 0.1 Da) of the first quadrupole during SRM acquisitions, a more selective isolation of the compound pre-cursor ion is achieved. This acquisition mode is known as Ultra-Selective Reaction Monitoring (U-SRM).

Figure 1 gives examples of U-SRM acquisition of pentachloroanisole and isodrin at 10 ppb in wheat matrix.

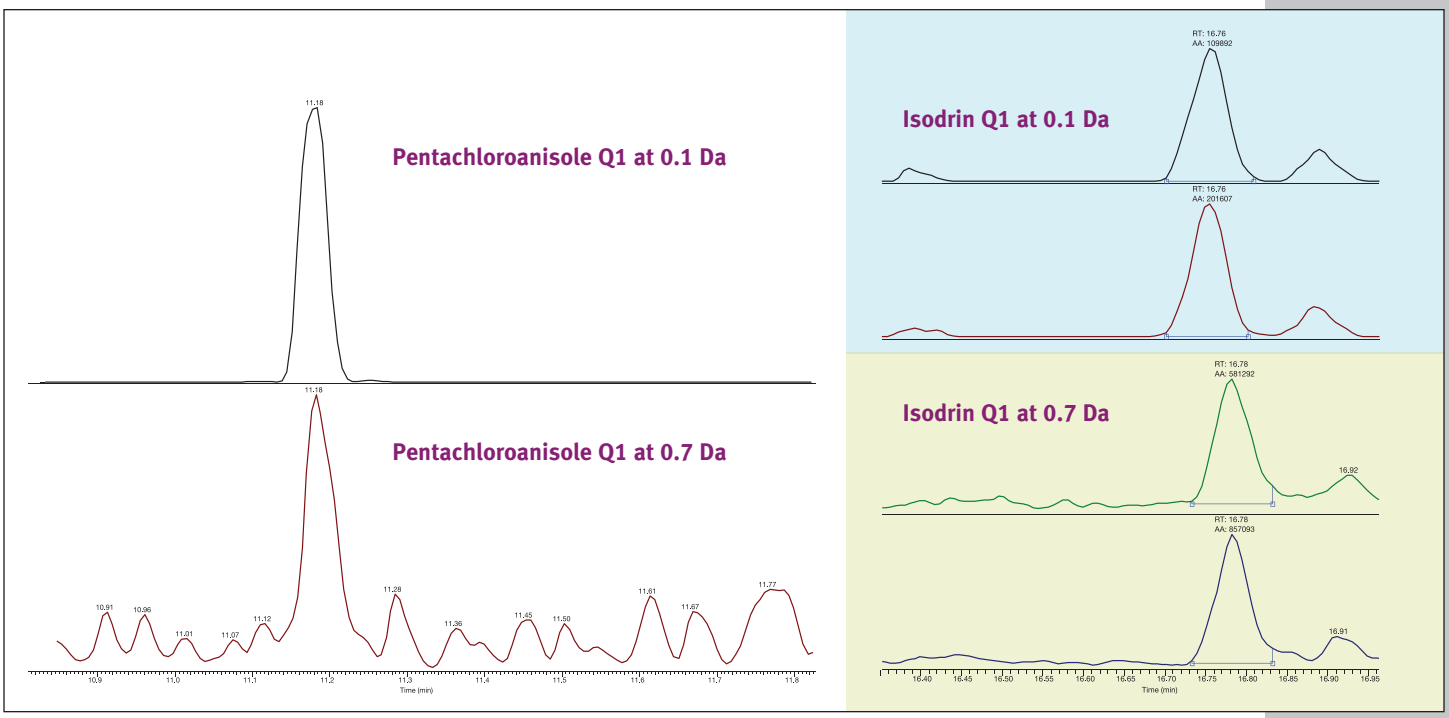
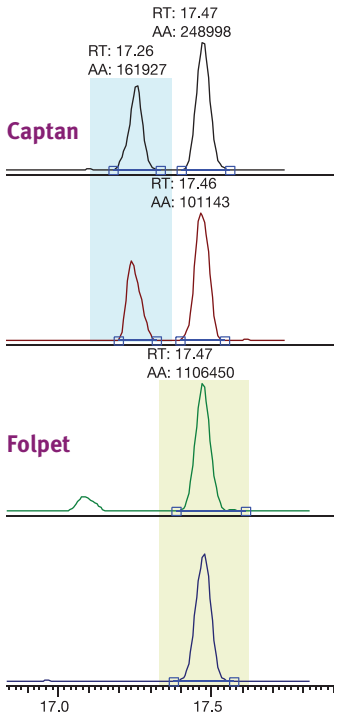


Figure 1: Comparison of U-SRM and standard SRM for pentachloroanisole and isodrin in wheat at X µg/mL levels;
Top: The chromatogram in U-SRM SRM (Q1 FWHM at 0.1 Da); Bottom: The same sample in standard mode (Q1 FWHM at 0.7 Da).



Injections (10 ppb blackcurrant)	Captan (area)	Folpet (area)
1	221347	247021
2	180365	229513
3	196336	273992
4	189745	277547
5	199317	273831
6	176386	270323
7	174745	296082
8	203117	231265
9	210897	248086
10	231017	234245
11	193543	264083
12	208722	292804
13	184633	246250
14	175942	285853
15	204764	295590
Average	196725.1	264432.3
Standard Deviation	16988.97	23534.93
RSD	**8.64%**	**8.90%**

Figure 2 and Table 2: Captan (RT 17.26) and Folpet (RT 17.47) in blackcurrant extract spiked at 10 ppb level, showing both transitions

Analytical Performance

The complete method validation was performed using standard mass resolution settings at 0.7 Da.

A very comfortable detection of virtually all pesticides was achieved at the 1 ppb level. Excellent linearity was also observed with correlation values exceeding 0.995 for the linear calibration. In addition to this, the residual errors for each calibration point were less than 10% for all compounds (RSD). This included a calibration point at the 1 ppb level.

Also, more difficult compounds such as Captan and Folpet showed excellent peak signal and repeatability when using this method.

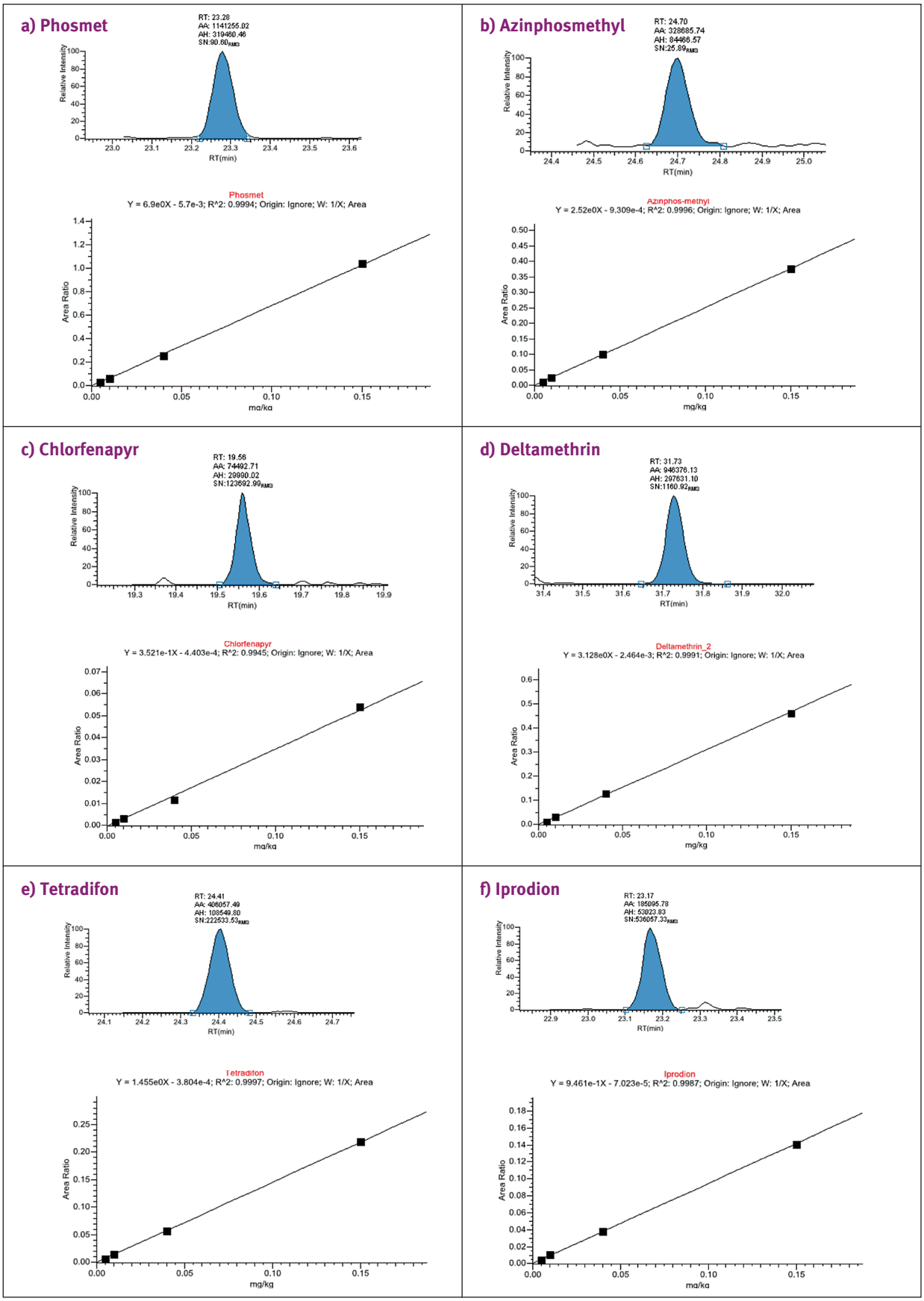


Figure 3: Calibration curves and peaks at 1 ppb level with 2 µL injection

As an additional test, the ion ratio at different levels has been monitored and the deviation of the transitions has been calculated.

Compound	Ion Ratio Deviation RSD in % (n=5)
Phosmet	0.79
Azinphosmethyl	3.65
Chlorfenapyr	15.08
Deltamethrin	0.88
Iprodion	5.34
Alfa Endosulfan	3.63
Methidathion	0.84
Carbaryl	3.64
Cyfluthrin	3.55
Pyrimifos	3.83

Table 3: Ion ratio deviation of some challenging pesticides in cucumber matrix at several levels of concentration

Figure 3 (a.) through (f.) show a 1 ppb matrix spike and calibration data obtained for select targeted pesticides in cucumber matrix.

Conclusions

- Advances in HyperQuad technology offers increased analytical performance for routine applications such as pesticide analysis.
- A true multi-compound method was developed for over 400 pesticides using timed SRM; easily transferable from a spreadsheet.
- A high level of accuracy and precision was reached during data evaluation, on several cornerstones of analysis, such as repeatability, linearity and ion ratio stability.
- Furthermore, all examples shown are the more challenging pesticides faced analytically in terms of stability, activity and response.
- This resolution technology development allows for advanced GC-MS/MS operations to be performed, such as U-SRM to further increase selectivity in complex matrices. This not only improves quantitative measurements, but it is also amenable when using a reduced sample clean-up which is typical for QuEChERS methodologies.

References

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2. M. Anastassiades, S. Lehotay D. Steinbacher, F. Schenck, *J. AOAC Int.* 86 (2) (2003) 412.
3. Munari, F., Huebschmann, H.J., Eliminating High Boiling Matrix in GC and GC/MS by Using PTV Backflush Injection Technique for Increased Productivity and Reliability, Application Note 51888, Thermo Fisher Scientific, 2010.
4. Pesticides Method Reference, 2nd ed. 2011, Thermo Fisher Scientific, Austin, TX, USA, (part number 120390).

For reference on Thermo Scientific QuEChERS products, please see our catalog, Thermo Scientific HyperSep Dispersive SPE Products (part number BRGSCQUECHERS 1109).

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

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