Highly Sensitive and Rugged GC/MS/MS Tool

For Pesticide Multiresidue Analysis in Food Samples

Agilent 7000 Series Triple Quadrupole GC/MS. The world's first MS/MS designed specifically for GC Analysis

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Introduction

Multi-residue methods are efficient and cost-effective for analysis of pesticide residues. For methods with a very wide scope, generic sample preparation procedures are usually employed. Inherent to this approach is that clean up of extracts is only possible to a limited extent[1]. When applying such methods to complex matrices like baby food, herbs, spices and tobacco, enhanced selectivity in detection is required to make up for the low selectivity in sample preparation.

Your Challenges

The analytical challenge is to maximize the number of pesticides, minimize the variety of methods, keep run times short and achieve limits of detection (LOD's) at or below the maximum residue limits (MRLs) which are specified for pesticides under EU legislation.

As regulations in the European Union require very low MRLs for pesticide residues, the latest challenge has been to reach part-per-billion level concentrations for hundreds of pesticides in complex matrices, which in turn has required greater sensitivity and efficiency in pesticide screening. Quantitation and confirmation of identity of trace level compounds can be complicated by the matrix, resulting in qualifier ion ratios out of range, or target ions buried in the high chemical background noise. With single quadrupole mass spectrometry, selected ion monitoring (SIM) is often used to improve the detection limit and quantitative reproducibility. In SIM mode, only a few ions are monitored for each target within the retention time (RT) range that the target elutes from the column. SIM may not work well for trace levels in matrix as the interferences in SIM are the same as in full scan mode.

Our Integrated Approach

Triple quadrupole mass spectrometry allows for drastic reduction or elimination of matrix interferences that limit the accuracy and detection limits of SIM methods. This process, referred to as Multiple Reaction Monitoring (MRM), has two fundamental advantages over SIM. First, detection is based secondary "product ion" produced by the collisional dissociation of an analyte "precursor ion". The analyte precursor ion (isolated in Q1 by a SIM mechanism) has the same selectivity as SIM, but there is a high probability that at least one of the resultant product ions will be unique to the precursor and not the interference. The increase selectivity of MRM is often apparent by the reduced offset of the baseline as compared to SIM. Secondly, during the mass filtering process in Q1, all lower m/z ions from the sample are eliminated. The unique product ions from the collisional dissociation are measured in this "zero" noise region of the spectrum. The combination of a unique product ions (more selectivity) and the elimination of background noise results in consistently low limits of detection even for complex matrices.

This application brief describes the analysis of pesticides in fruit and vegetable extracts using the Agilent 7000 Series Triple Quadrupole GC/MS system in MRM mode and in combination with Retention Time Locking[2] and Agilent Capillary Flow Technology to provide backflushing of high-boiling materials.[3]

Column backflushing is essential for the analysis of complex samples such as food extracts[4] because they usually contain high-boiling indigenous compounds. In just a few runs, these materials can collect on the head of the column, causing peak tailing, retention time shifts and increased chemical noise. Over time, they can migrate from the column to the ionization source, which would eventually have to be cleaned. Agilent's proprietary capillary flow technology makes column backflushing routine and easy to setup for non experts.

The method robustness is drastically increased and the analysis cycles are shortened[5]. In conclusion the system up-time is maximized allowing significant productivity gains. The need for maintenance is reduced by keeping the chromatographic system and MS ion source cleaner between each injection.

Figure 1
Agilent 7890A/7000A Triple Quadrupole GC/MS system with the new high capacity 7693 ALS.

Experimental

Samples were prepared using the QuEChERS[6] method. QuEChERS stands for Quick Easy, Cheap, Effective, Rugged and Safe and is a food sample preparation for multi-class, multi-residue pesticide analysis. See more at www.agilent.com/chem/Quechers

Instrumentation

The Triple quadrupole GC/MS system used for these experiments are described in Table 1 and shown in Figure 1.

Instrument conditions

Instrumentation	
GC/MS Triple Quadrupole:	Agilent 7000A
GC:	Agilent 7890A
Inlet:	PTV, in splitless mode, 1µL Injection, Multi-baffle liner 80 °C for 0.5 min, then 500 °C/min to 280 °C for 2 min
Capillary flow technology device:	3-way splitter with analytical column in and restrictor out to the triple quadrupole helium pressure provided by Aux EPC at 1 psi
Column:	Agilent J&W HP-5ms Ultra Inert 30 m x 0.25 mm ID, 0.25 µm HP-5MSUI
Restrictor:	80 cm x 0.180 mm deactivated fused silica
Carrier gas:	Helium 30.883 psi (constant pressure mode)
Oven temperature:	70 °C (1 min), 25 °C/min to 150 °C (0 min), 3 °C/min to 200 °C (0 min), 8 °C/min to 280 °C (10 min)
Backflush:	Time 5 min, inlet press. 1 psi, Aux EPC 80 psi, oven temp. 280 °C
Retention time locking:	Chlorpyrifos-methyl locked to 16.53 min
Collision cell gases:	N_2 2.60 psi and He 6.25 psi
Inert source temperature:	260 °C
Quadrupole temperature:	150°C

Table 1
Instrument conditions.

Method Development

In order to maximize the response of the instrument for each residue the choice of precursor ion, product ion and collision energy were optimized. The spectra of a typical pesticide in full scan mode (50-500 m/z) e.g. Dicloran (MW = 206) is displayed in Figure 2. The product ion scan spectra of 206 m/z at different collision energy are displayed in Figure 3.

Results

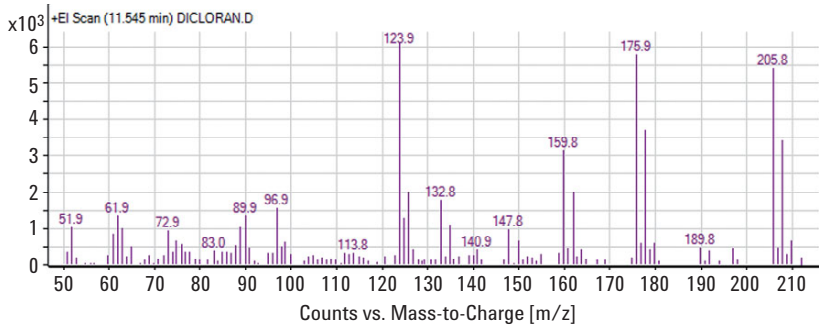

Figure 2
Full scan spectrum of Dicloran under EI ionization mode.

Results

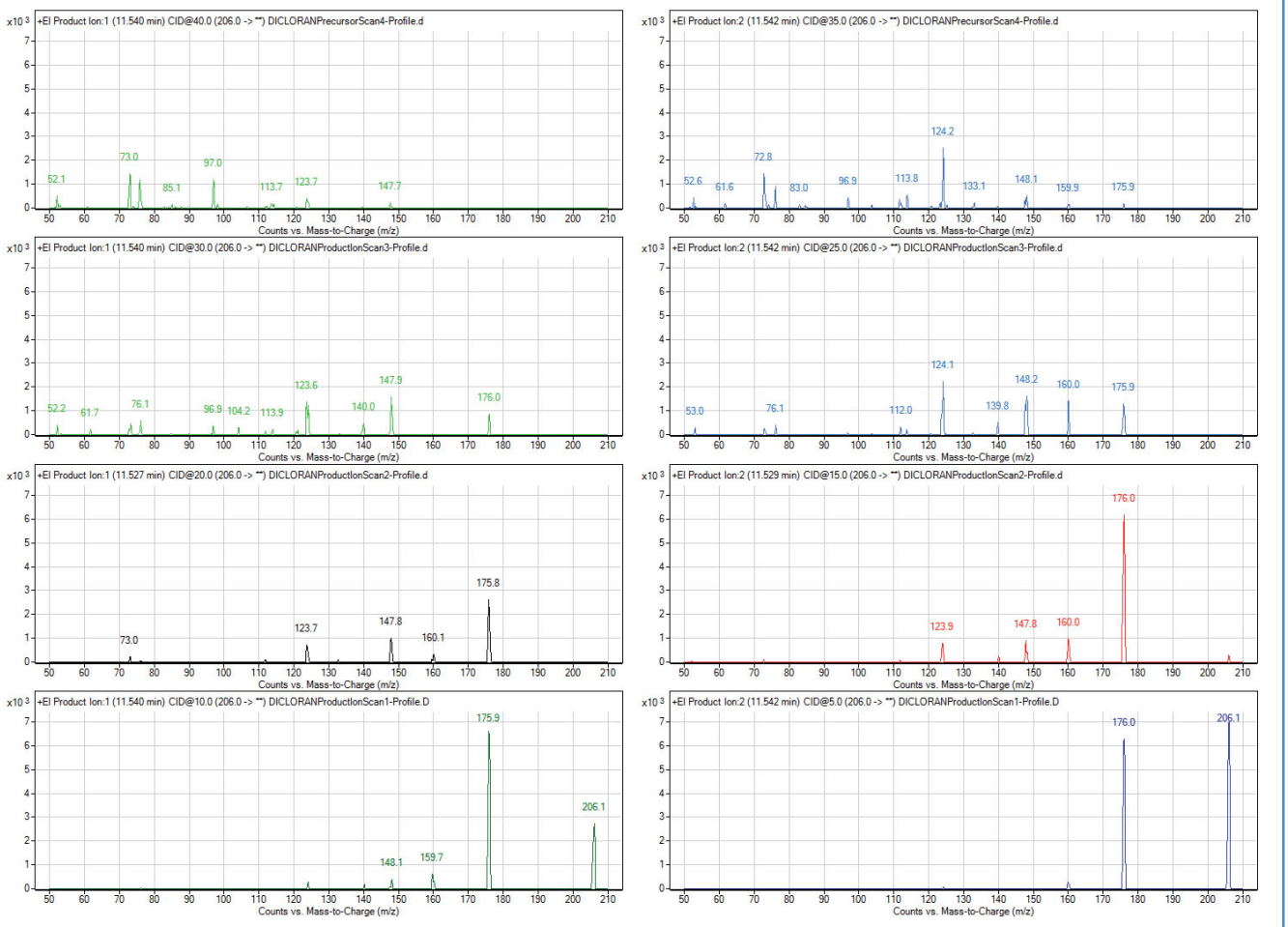

Figure 3
Product ion scan of Dicloran for the Precursor (206 m/z) at different collision energies (5-40 V).

The optimum collision energy for the 206>176 transition product ion was found to be at 10V and the resulting MRM chromatogram is shown in Figure 4.

Results and discussion

Figure 5 shows the TIC chromatogram acquired in MRM mode for 360 pesticides. Each MRM segment is indicated by a grey marker line. An enhanced view on a selected part of the analysis with an overlay of all MRM for the compounds in this part is shown Figure 6. The MRM mode allows for accurate quantification of many coeluting analytes as shown between 13.6 and 14.2 minutes. Figure 7 demonstrates the identity confirmation of Diclobenil in a peppermint extract at 10 pg on column using two MRM transitions. The dashed lines indicate the allowed range of the ion ratio as specified in the method.

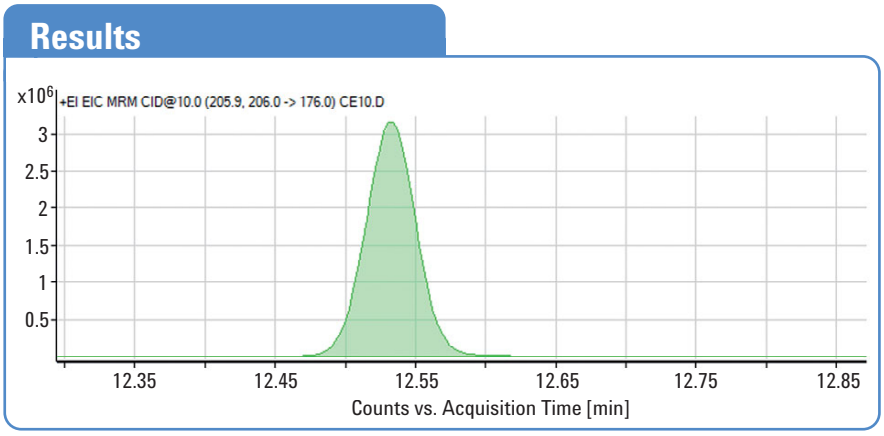

Figure 4
MRM chromatogram of Dicloran at 10ppb.

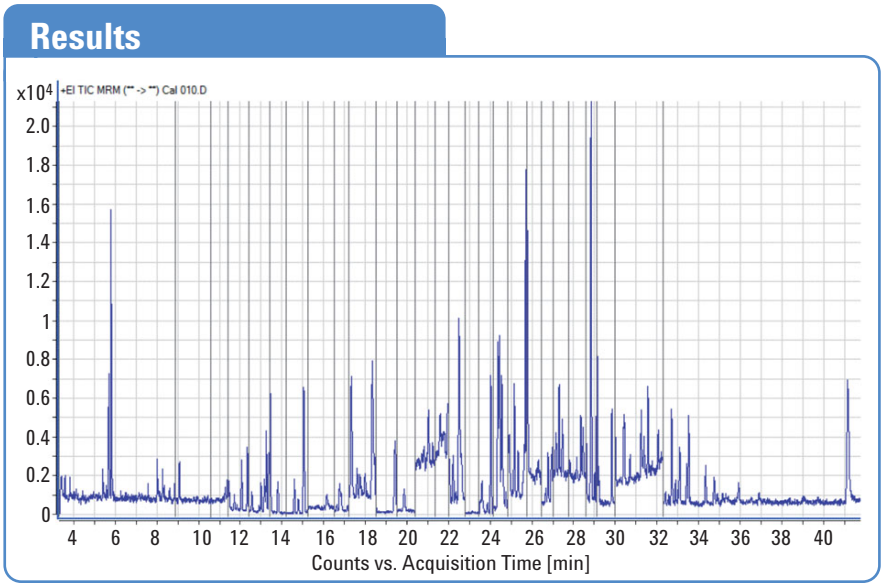

Figure 5
Total ion chromatogram of the vegetable extract by GC/MS/MS.

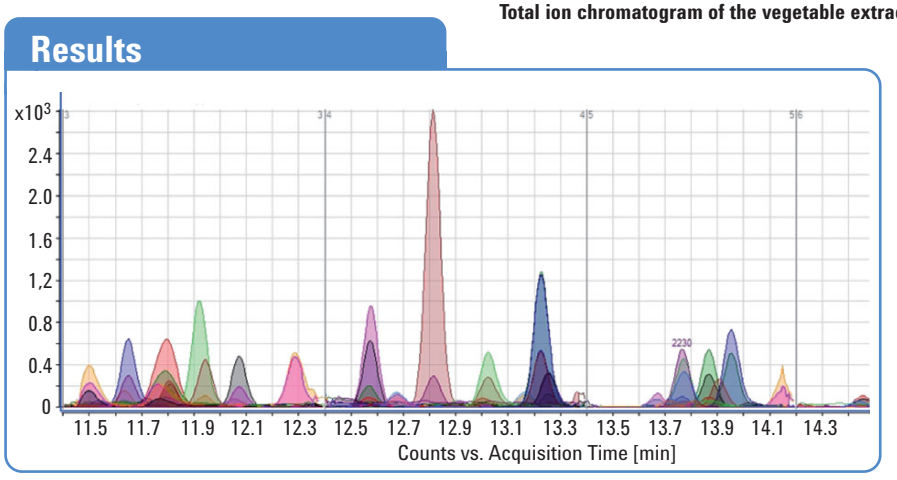

Figure 6
Overlay of extracted MRM transitions.

Results

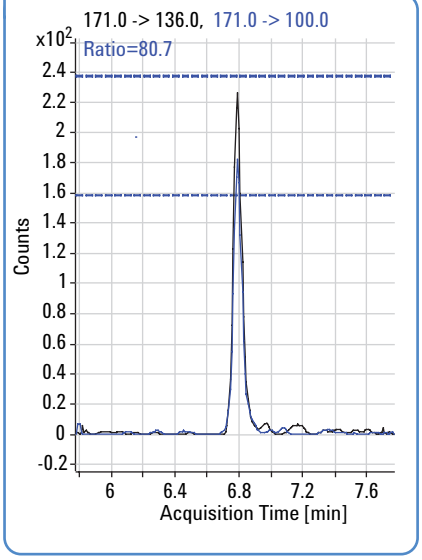

Figure 7
Two transitions identifying Diclobenil in a peppermint extract at 10 pg on column. The dashed lines indicate the allowed range of the ion ratio.

Linearity was also tested with five levels between 1 and 200 ppb for 360 pesticides and Figure 8 shows the calibration curves for Diclobenil and Chlormefos. The correlation coefficients of the external standard calibration curve were 0.99 on average. The LOD was estimated based on the calculated S/N of the 10 pg standard. For the majority of pesticides, LODs were below 2 pg on column (based on S/N >3:1 Peak to Peak).

Retention time reproducibility was also tested to demonstrate the robustness of the analytical method. Figure 9 shows the outstanding retention time stability of one representative compound: Trifluralin. Calculated %RSD is 0.0306 at 6.073 min for one hundred consecutive injections of lettuce extract into the GC/MS/MS system. Only 3 minute Backflush was necessary to remove all high boiling matrix compounds, the total cycle time for this stability test was 21 hours.

Results

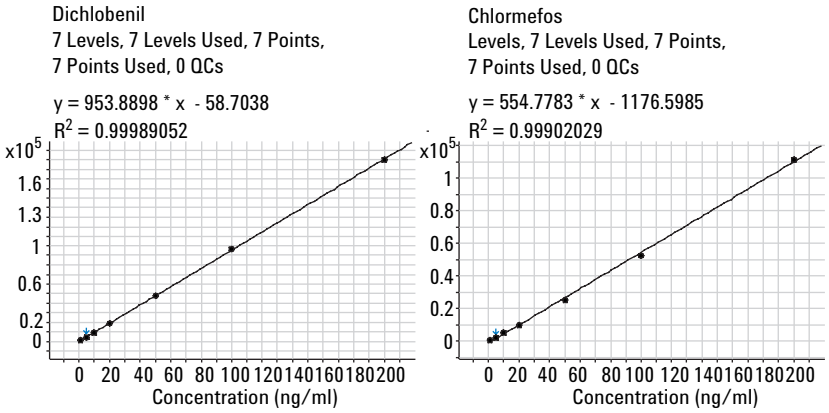

Figure 8
Calibration curves showing excellent linearity over the concentration from 1 ppb to 200 ppb range R^2= 0.999 respectively for Dichlobenil and Chlormefos.

Results

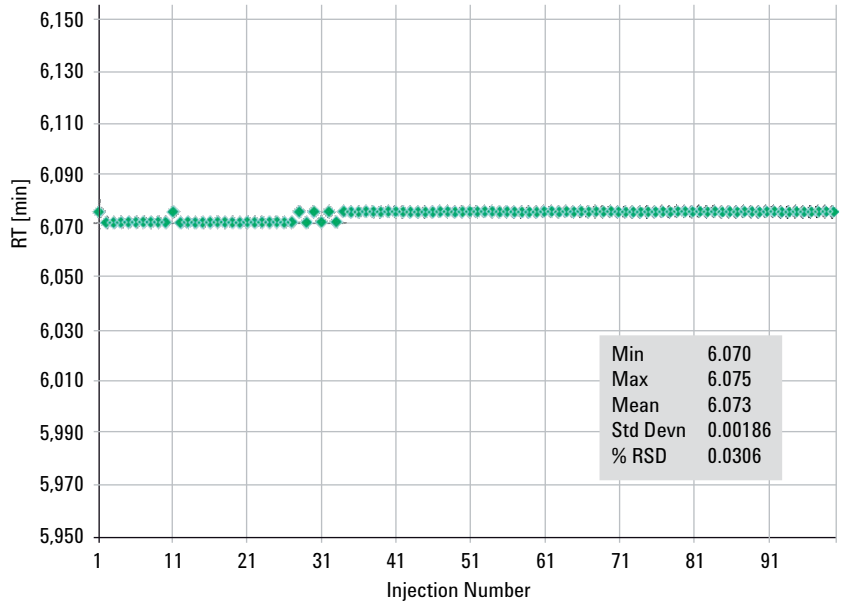

Figure 9
Exceptional retention time stability of Trifluralin with 100 injections of lettuce extract thanks to column backflushing.

Conclusion

- Agilent's 7000 Series Triple Quadrupole GC/MS in combination with the 7890 GC is a sensitive and rugged tool for target pesticide analysis in complex matrices. The single multi-residue method we developed also meets the performance and identity confirmation criteria defined by the stringent EU regulations.

- Excellent selectivity has been achieved to allow unambiguous confirmation of identity for these 360 pesticides even in very complex food matrices and generic sample clean up. Agilent SampliQ QuEChERS kits enable you to prepare food samples for multiresidue, multi-class pesticide analysis with just a few simple steps.

- For this new GC/MS/MS method, the Agilent Retention Time Locking (RTL) database was used to calibrate the retention times of all pesticides. Therefore, the presented GC/MS/MS method can be easily transferred to other Agilent 7000 Series Triple Quadrupole systems with minimum effort and time.

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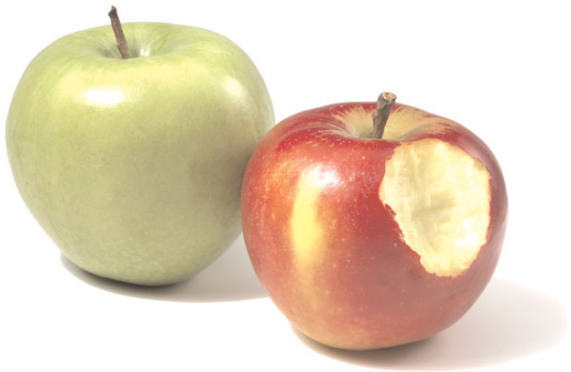

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