

Applying Fast GC-MS/MS Using Triple Quadrupole Technology to Increase Productivity for Pesticide Residue Analysis in QuEChERS Extracts

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Overview

Purpose: To demonstrate productivity gains using novel collision cell GC-MS/MS technology in combination with data processing capabilities, direct injection of low sample volumes of QuEChERS acetonitrile extracts, and a fast GC separation to increase sample throughput.

Methods: A Thermo Scientific™ TRACE™ 1310 GC coupled to a Thermo Scientific™ TSQ™ 8000 Evo triple quadrupole GC-MS/MS was used to analyze 132 pesticides in standards and baby food QuEChERS extracts. QuEChERS acetonitrile extracts were spiked with a mixture of 132 pesticides at concentrations corresponding to 0.5–100 ng/g (ppb).

Results: Using fast GC separation, run time was decreased to ~11 min from a typical run time of 40 minutes. A 73% gain in productivity resulted with no compromise in the number of data points acquired for each chromatographic peak.

Introduction

The detection, quantification, and confirmation of pesticide residues at trace levels requires sensitive, selective, and robust analytical instrumentation. With ever-increasing pressure to analyze a greater number of samples with shorter turnaround times, high-throughput laboratories seek continuous improvements in analytical productivity. Direct analysis of sample extracts in acetonitrile is desired to avoid the need for solvent exchange to a more GC-compatible solvent. Here, we report further productivity gains using novel collision cell GC-MS/MS technology in combination with data processing capabilities to increase sample throughput. These gains are achieved by the direct injection of low sample volumes of QuEChERS acetonitrile extracts in combination with a fast GC separation.

Methods

Sample Preparation

Baby food samples were extracted using the citrate buffered QuEChERS protocol. Homogenized samples (10 g) were extracted with acetonitrile (10 mL) followed by the addition of MgSO₄ (4 g), NaCl (1.0 g), disodium hydrogen citrate sesquihydrate (0.5 g), and trisodium citrate dehydrate (1.0 g). Sample clean-up was carried out using dSPE and 750 mg MgSO₄ + 250 mg C₁₈ + 250 mg PSA + 37.5 mg carbon. The final extracts (1 g/mL in acetonitrile) were spiked with a mixture of 132 pesticides at levels corresponding to 0.5–100 ng/g (ppb) and analyzed by GC-MS/MS using a timed-SRM detection method on the TSQ 8000 Evo instrument.

Mass Spectrometry

The instrumentation for all the experiments was a TSQ 8000 Evo GC-MS/MS coupled with TRACE 1310 GC. Sample introduction was performed using a Thermo Scientific™ TriPlus RSH™ autosampler, and chromatographic separation was achieved using a Thermo Scientific™ TraceGOLD™ TG-5SiIMS 15 m × 0.25 mm I.D. × 0.25 μm film capillary column (P/N: 26096-1300). Additional details of instrument parameters are displayed in Tables 1 and 2.

TABLE 1. GC and Injector Conditions.

TRACE 1310 GC Parameters	
Injection Volume (μL):	1.0
Liner	SSL single taper (P/N: 453A2342)
Inlet (°C):	240
Inlet Module and Mode:	Splitless
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program:	
Temperature 1 (°C):	60
Hold Time (min):	1.0
Temperature 2 (°C):	180
Rate (°C/min)	50
Temperature 3 (°C):	320
Rate (°C/min)	35
Hold Time (min):	4.0

TABLE 2. Mass Spectrometer Conditions.

TSQ 8000 Evo Mass Spectrometer Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source(°C):	320
Electron energy (eV):	70
Acquisition Mode:	SRM
Q2 Gas Pressure(argon)(psi):	60
Q1 Peak Width (Da):	0.7
Q3 Peak Width (Da):	0.7

The TSQ 8000 Evo mass spectrometer was operated in MS/MS mode using electron ionization (EI+). For each pesticide, two selected reaction monitoring (SRM) transitions were selected, one to quantify and one for identification purposes. A total number of 264 SRM transitions were acquired with dwell times varying from 1 ms to 52 ms, depending on the number of SRM transitions monitored simultaneously. Chromatographic data was acquired using timed-selected reaction monitoring (t-SRM) with a minimum of 12 points/peak.

Data Analysis

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software, a single software package that integrates instrument control, method development functionality, and quantitation focused workflows. For each compound, one SRM transition was used for quantitation and the second for positive identification of the pesticide using the ion ratio.

Typically, a GC analysis of 132 target pesticides requires about 40 minutes to obtain a sufficient number of scans per chromatographic peak (Figure 1), especially in time windows containing many co-eluting peaks. In order to accurately integrate the peaks of interest, at least 10–12 scans across a chromatographic peak are needed. Until now, fast scan speeds compromised instrument sensitivity, especially when several SRM transitions were monitored simultaneously. Using the fast GC conditions shown in Table 1, the GC run time was decreased to ~11 min without compromising the number of data points acquired for each chromatographic peak (Figures 2 and 3). This performance is possible because the fast EvoCell technology in the TSQ 8000 Evo system allows fast clearance of ions from the collision cell and hence faster data acquisition without adversely affecting instrument sensitivity. Fast data acquisition leads to more information collected in less time, ultimately resulting in faster GC runs. This fast methodology resulted in a three-fold improvement in sample productivity. A typical overnight batch (16 hours) that allows around 24 injections now accommodates around 70 sample injections (including GC cycle times).

FIGURE 1. Typical GC-MS chromatographic run of 132 pesticides (full scan, TIC data) at 200 ng/g with a total run time of about 37 minutes. The first (dichlorvos, RT = 5.77 min) and the last (deltamethrin, RT = 29.61 min) eluting pesticides are highlighted.

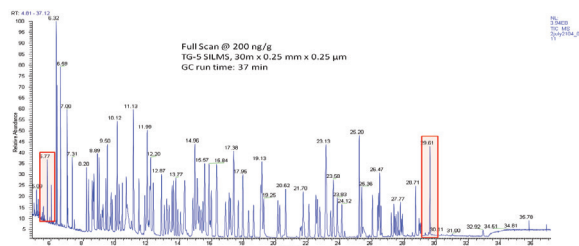


FIGURE 2. Fast GC-MS chromatographic run of 132 pesticides (SRM, TIC data) at 100 ng/g with a total run time of 11 minutes. The first (dichlorvos, RT = 4.33 min) and the last (deltamethrin, RT = 9.15 min) eluting pesticides are shown.

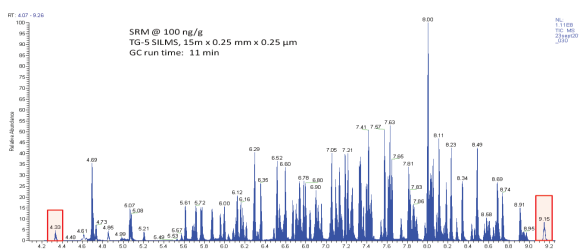
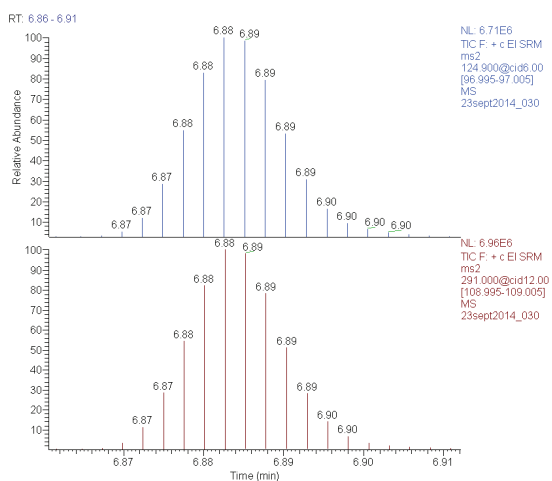


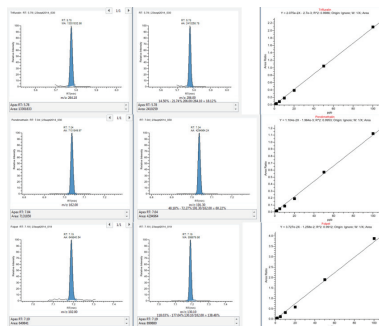
FIGURE 3. Parathion ethyl eluting at RT = 6.89 min showing 13 scans/peak (peak width 1.8 sec, dwell time of 1.7 ms).



Sensitivity

Almost all pesticides (97%) were detected at the 0.5 ng/g level. Examples of chromatography at this low level are shown in Figure 4. At 5 ng/g (half of MRL) all compounds were comfortably detected with all the ion ratios for compound confirmation within 15% of the average ion ratio values derived from the calibration curve across all concentrations.

FIGURE 4. Examples of chromatography and linearity (no internal standard correction) for trifluralin, pendimethalin, and folpet, at 0.5 pg absolute amount on column. Calibration curves indicate compound linearity across 0.5–100 ng/g.



Estimation of Instrument Detection Limit (IDL) and Peak Area Repeatability

The IDL of the target pesticides was determined empirically by injecting repeatedly ($n = 20$) the 5 ng/g matrix-matched standard and taking into account the Student's- t critical values for the corresponding degrees of freedom (99% confidence). The results of this experiment show an average %RSD for the peak area reproducibility of 7.3%, and the IDL values vary from 0.2 ng/g (for dimethamid) to 2.2 ng/g for cyanazine. Table 3 displays a subset of the results. By using internal standard correction to compensate for the injection errors it is possible that both %RSD for peak area repeatability values can be improved even further.

TABLE 3. Peak area reproducibility (% RSD, $n=20$) at 5 pg absolute amount on column and calculated instrument IDL₉₉ (in ng/g) for a subset of pesticides analyzed.

Compound	RT (min)	% RSD (n = 20)	IDL (ng/g)
Acetochlor	6.53	4.5	0.6
Aclonifen	7.64	8.3	1.1
Aldrin	6.90	5.9	0.7
Azinphos-ethyl	8.34	10.0	1.3
Benalaxyl	7.74	4.4	0.6
BHC, Alpha	5.96	5.3	0.7
BHC, Beta	6.11	8.7	1.1
BHC, gamma	6.18	6.6	0.8
Bifenox	8.09	10.3	1.3
Bifenthrin	8.00	4.3	0.5
Biphenyl	4.85	10.0	1.3
Bromophos-ethyl	7.21	6.8	0.9
Bromopropylate	8.04	5.7	0.7
Bupirimate	7.43	3.0	0.4
Buprofezin	7.45	4.9	0.6
Cadusafos	5.88	5.1	0.6
Captan	7.16	14.4	1.8
Carbetamide	6.90	9.9	1.3
Chlorbufam	6.09	7.6	1.0
Chlordane alpha-cis	7.25	6.4	0.8
Chlordane gamma-trans	7.32	5.7	0.7
Chlorothalonil	6.29	5.7	0.7
Chlorpropham	5.77	4.6	0.6
Chlorpyrifos-ethyl	6.85	4.3	0.5
Chlorpyrifos-methyl	6.55	4.2	0.5
Chlzolinate	7.07	7.9	1.0
Clomazone	6.12	4.1	0.5
Coumaphos	8.48	14.0	1.8
Cyanazine	6.85	17.0	2.2
Cycloate	5.72	8.8	1.1
Cyfluthrin peaks I-IV	8.59	10.9	1.4
Cyhalothrin-S	8.23	6.8	0.9
Cypermethrin peaks I-IV	8.68	10.2	1.3

Linearity of Response

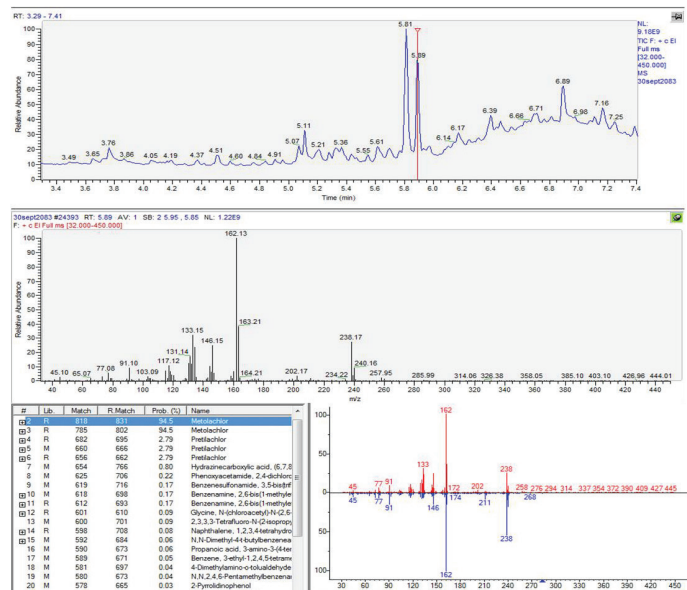
Linearity of the GC-MS/MS system was evaluated across a concentration range of 0.5–100 ng/g using matrix-matched standards. In all cases, the coefficient of determination (R^2) was higher than 0.999 with an average value of $R^2 = 0.997$. Moreover, individual residual values were <20% with an average value of 10%.

Comprehensive Analysis of Additional Pesticides

Targeted screening and quantification of a given number of pesticides is important, but there is an increased interest in screening the samples for additional compounds other than those in a target list. To answer the question "what else is in my sample" one will have to screen the samples for less common or new pesticides or for unsuspected metabolic/transformation products that could be present in the samples in addition to targeted compounds. The capability of fast analytical instrumentation enables simultaneous acquisition of full scan and SRM/SIM data.

Using the TSQ 8000 Evo system, the baby food samples were screened for additional compounds. The data was acquired in full scan and SRM mode simultaneously; an example of full scan/SRM chromatogram is shown in Figure 5. The extracted mass spectrum of the peak eluting at RT = 5.89 min was submitted to the NIST mass spectral library and identified as metolachlor (a compound not in the spiking solution) with a probability of 95%. This result shows the advantage of using such simultaneous data acquisition, which is possible only on fast instrumentation such as the TSQ 8000 Evo GC-MS/MS system.

FIGURE 5. Comprehensive analysis of baby food contaminants using simultaneous full scan/SRM data acquisition. Compound at RT = 5.89 min identified as metolachlor (using NIST) in the full scan acquisition window.



Conclusion

The results of this work show that by using the Thermo Scientific TSQ 8000 Evo triple quadrupole GC-MS/MS system, laboratory productivity can be tripled. Acceleration of sample analysis is made possible by:

- Direct analysis of acetonitrile extracts with no need for an additional solvent-swap step.
- Accelerating the GC run and fast data acquisition using EvoCell fast collision cell technology.
- Comprehensive detection of target pesticides and of additional unexpected or non-targeted pesticides using simultaneous full scan and SRM data acquisition. Additional pesticides were identified by searching the full scan data against the NIST library.

Excellent sensitivity was achieved with all pesticides detected and confirmed at the 5 ng/g level with IDL values from 0.2–2.2 ng/g. Moreover, these results demonstrate that for fast GC acquisition, the TSQ 8000 Evo delivers excellent peak area reproducibility and compound linearity.

References

1. Commission Regulation (EU) No 396/2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EC, 16.3.2005, p. 1–16.
2. SANCO/12571/2013 Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed, 19.11.2013 rev. 0, 2014.

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