

Three-Fold Increase in Productivity for Pesticide Residue Analysis in Baby Food Using Fast Triple Quadrupole GC-MS/MS

Cristian Cojocariu,¹ Michael T. Hetmanski,² Paul Silcock,¹ and Richard J. Fussell²

¹Thermo Fisher Scientific, Runcorn, UK

²Food and Environment Research Agency (FERA), York, UK

Key Words

Pesticide Analysis, Baby Food, GC-MS/MS, TraceFinder, Food Safety

Goal

To assess the performance and productivity of the Thermo Scientific™ TSQ™ 8000 Evo GC-MS/MS for pesticide residues analysis.

Introduction

Pesticides include more than 1000 different substances used to control or eradicate pests. Strict regulatory controls are in place to ensure that these chemicals are used safely and effectively without harmful effects to humans, wildlife, and the environment. Maximum residue levels (MRLs) of pesticides in food and feed have been set by many international bodies including the EU.¹ Detection, quantification, and correct identification of pesticide residues at trace levels requires sensitive, selective, and robust analytical instrumentation. With ever-increasing pressure to analyze a greater number of samples of perishable commodities with shorter turnaround times, high throughput laboratories seek continuous improvements in analytical productivity. In recent times, substantial productivity gains have been achieved using the QuEChERS (quick, easy, cheap, effective, robust and safe) sample extraction approach in combination with gas or liquid chromatography (GC or LC) mass spectrometry (MS). Here, we report the possibility of further productivity gains using advanced, rapid GC-MS/MS technology in combination with new software developments to reduce the time needed to acquire and process the data.

Acetonitrile is commonly used as the extraction solvent for QuEChERS. Direct analysis of pesticide residues in acetonitrile is preferred to avoid the need for solvent exchange, which is time consuming and, hence, costly. However, the polar nature of acetonitrile results in poor focusing of chromatographic peaks and the high expansion coefficient limits the injection volume that can be used.



In this study, a fast, easy, and robust workflow was used to analyze pesticide residues in baby food. Accurate and sensitive detection, quantification, and identification of pesticides in baby foods is of particular importance because babies are more vulnerable to adverse health effects from these chemicals.

This work shows that laboratory productivity can be accelerated by direct injection of low sample volumes of QuEChERS acetonitrile extracts, in combination with fast temperature ramps to shorten GC run times. This is made possible using the innovative EvoCell collision chamber technology combined with the efficient selected reaction monitoring (SRM) scheduling of timed-SRM software in the TSQ 8000 Evo triple quadrupole GC-MS/MS.

A thorough assessment of the robustness of this fast GC analysis using acetonitrile was conducted following the SANCO guidelines.²

Instrument and Method Setup

A TSQ 8000 Evo triple quadrupole GC-MS/MS instrument coupled with a Thermo Scientific™ TRACE™ 1310 GC was used. Sample introduction was performed a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation using a Thermo Scientific™ TraceGOLD TG-5SilMS 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 below.

GC and Injector Conditions

TRACE 1310 GC

| | |
|------------------------|----------------------------------|
| 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 |
| Temperature 2 (°C): | 180 |
| Rate (°C/min) | 50 |
| Temperature 3 (°C): | 320 |
| Rate (°C/min) | 35 |
| Hold Time (min): | 4 |

Mass Spectrometer Conditions

TSQ 8000 Evo Mass Spectrometer

| | |
|------------------------------|-------|
| Transfer Line (°C): | 280 |
| Ionization Type: | EI |
| Ion Source (°C): | 320 |
| Electron Energy (eV): | 70 |
| Acquisition Mode: | t-SRM |
| Q2 Gas Pressure(argon)(psi): | 60 |
| Q1 Peak Width (Da): | 0.7 |
| Q3 Peak Width (Da): | 0.7 |

The TSQ 8000 Evo triple quadrupole mass spectrometer was operated in MS/MS mode using electron ionization (EI+). For each pesticide, two SRM transitions were chosen—one for quantification and one for identification purposes. A total 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 data using timed-selected reaction monitoring (t-SRM) with a minimum of 12 points/peak.

Sample Preparation

Baby food samples were extracted using the citrate buffered QuEChERS protocol. The homogenized sample was extracted (10 g) 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 dihydrate (1.0 g). Dispersive solid phase extraction [MgSO₄ (150 mg), C18 (50 mg), PSA (50 mg) and carbon (7.5 mg) per mL of extract] was used for sample clean-up. Final extracts (1 g/mL in acetonitrile) were spiked with a mixture of 132 pesticides at concentrations corresponding to 0.5–100 ng/g (ppb) and 1.0–200 ng/g (ppb) for some analytes.

Data Processing

Data were acquired and processed using the Thermo Scientific™ TraceFinder™ version 3.2 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 one for positive identification of the pesticide.

Results and Discussion

This study describes the methodology used for multi-residue pesticides analysis in baby food using fast GC for increasing laboratory productivity. The results described below were obtained with acetonitrile as the final extract solvent from the QuEChERS extraction and low-volume, hot splitless injection. The performance of the TSQ 8000 Evo GC-MS/MS system was evaluated by assessing the chromatography, sensitivity, linearity, and reproducibility of the target pesticides analyzed in the extracts of baby food samples.

Three-Fold Increase in Sample Throughput

Typically, a GC analysis of 132 target pesticides has a run time of around 42 minutes in order to obtain a sufficient number of scans per chromatographic peak (Figure 1), especially in time windows containing many co-eluting peaks. At least 10–12 scans across a chromatographic peak are needed in order to accurately integrate the peaks of interest.

Previously, fast scan speeds compromised instrument sensitivity, especially when several SRM transitions were monitored simultaneously. Using the fast GC conditions described above, the GC run time was decreased to

~11 min with no compromise in the number of data points acquired for each chromatographic peak (Figures 2 and 3). This advance is possible because the fast EvoCell technology allows fast clearance of ions from the collision cell and hence faster data acquisition, without adversely affecting instrument sensitivity. Fast data acquisition enables more information to be collected in a shorter time, ultimately resulting in faster GC runs. Using this fast methodology, sample productivity is improved by approximately three-fold, as around three times as many injections of sample/standard extracts can be carried out in an overnight sequence.

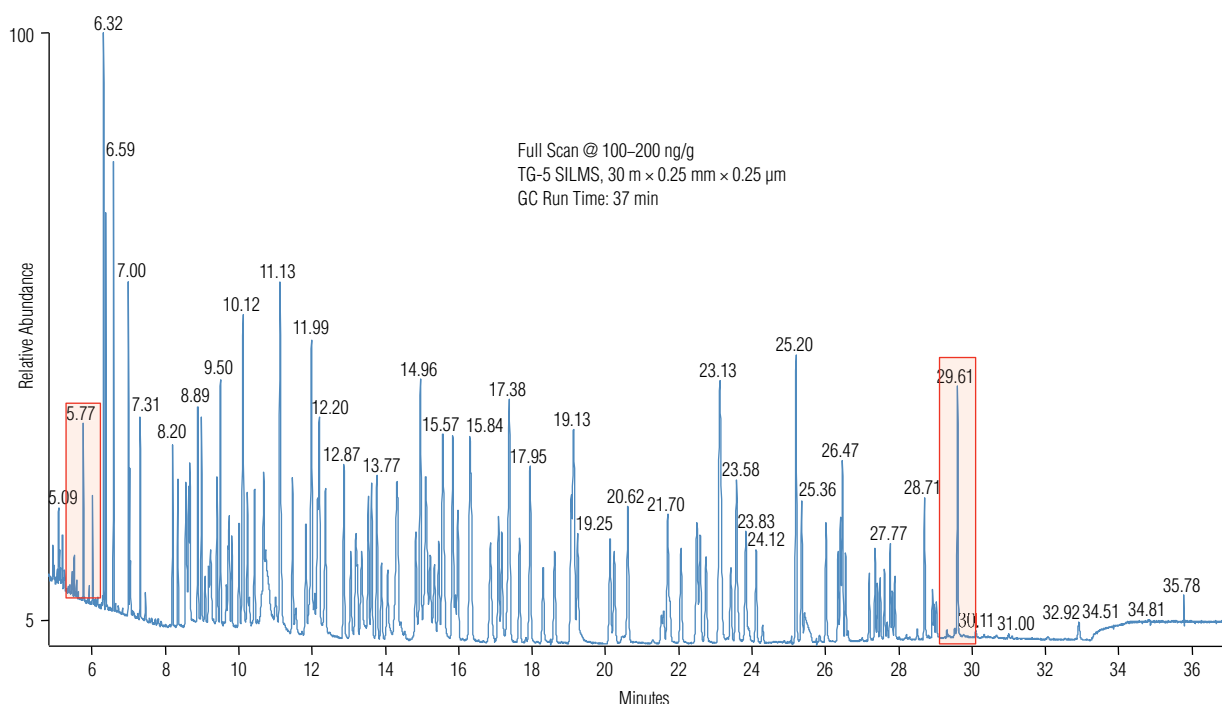


Figure 1. Total ion chromatogram (TIC, full scan) for a typical GC-MS chromatographic run of 132 pesticides at 100–200 ng/g with a total run time of approximately 40 minutes. The first (dichlorvos, RT = 5.77 min) and the last (deltamethrin, RT = 29.61 min) eluting pesticides are highlighted.

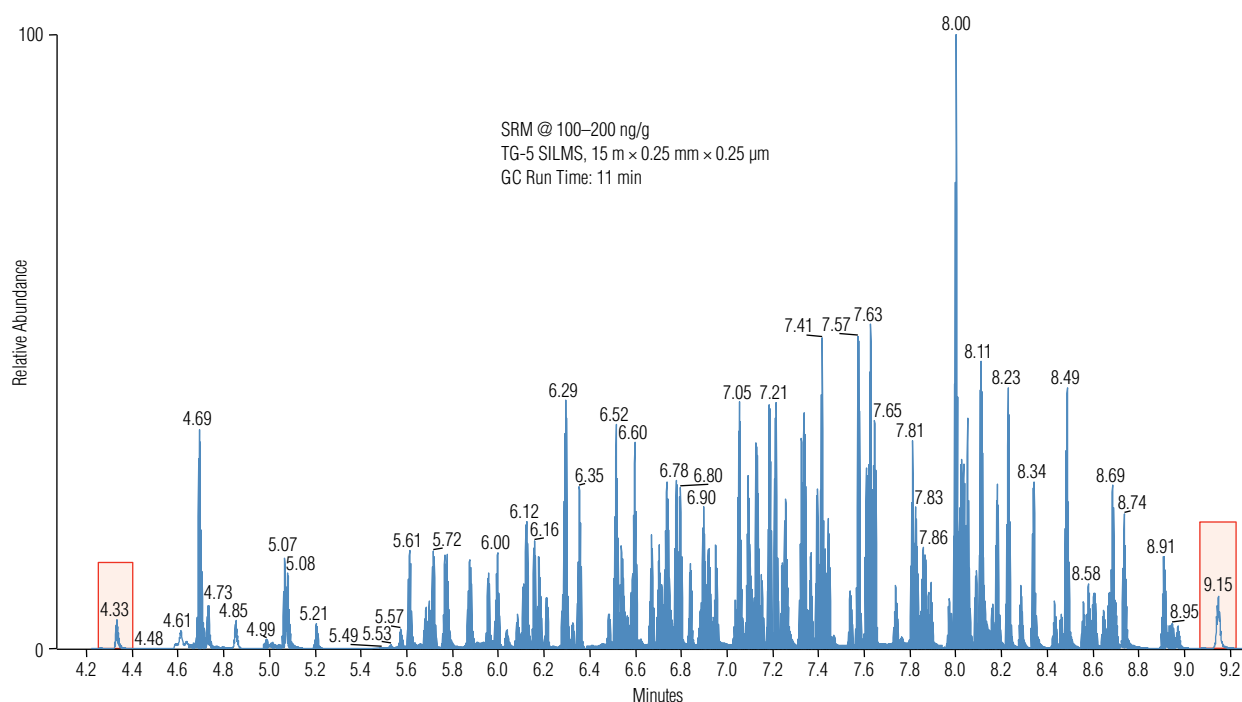


Figure 2. SRM chromatogram for a fast GC-MS chromatographic run of 132 pesticides at 100–200 ng/g with a total run time of 11 minutes. The first (dichlorvos, RT = 4.33 min) and the last (deltamethrin, RT = 9.15 min) eluting pesticides are highlighted.

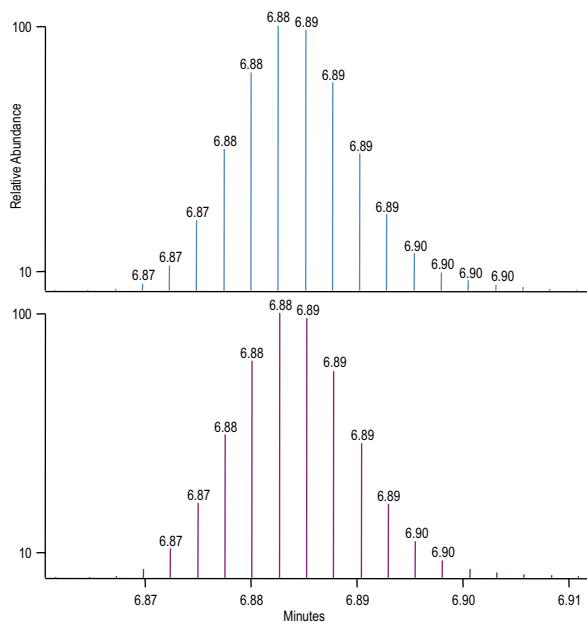


Figure 3. SRM chromatogram for parathion ethyl eluting at RT = 6.89 min showing 13 scans/peak (peak width 1.8 sec, dwell time of 1.7 ms).

Sensitivity

Almost all pesticides (97%) were detected at a concentration of 0.5 or 1.0 ng/g (ng/mL) and calibration curves were linear over the range 0.5–100 ng/g (or 1.0–200 ng/g). Examples of chromatography at this low concentration and calibration curves are shown in Figure 4. At the lowest calibration concentration of 5–10 ng/g ($0.5\text{--}1 \times$ default MRL), all compounds were comfortably detected with all the ion ratios for compound identification within 15% of the average ion ratio values derived from the calibration curve across all concentrations.

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

The IDL of the target pesticides was determined empirically by repeatedly injecting ($n=20$) the 5 ng/g (and 10 ng/g) matrix-matched standard and taking into account the Student's-t critical values for the corresponding degrees of freedom (99% confidence).

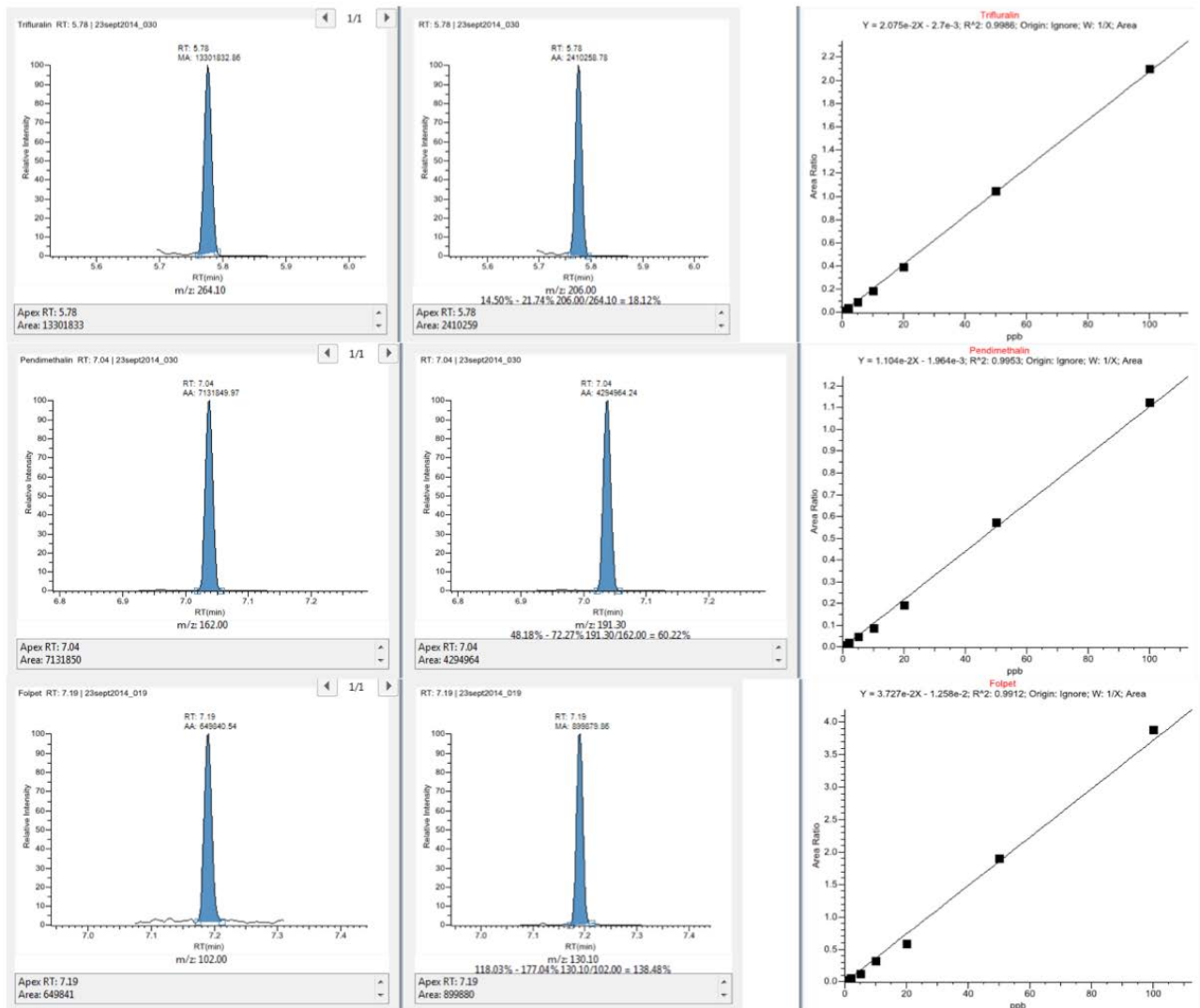


Figure 4. Examples of chromatography (0.5 pg on column) and linearity (no internal standard correction) for trifluralin, pendimethalin, and folpet.

The results of this experiment showed an average %RSD for the peak area reproducibility of 7.3 % and IDL values varying from 0.2 ng/g for dimethamid to 3.7 ng/g for

captan (Table 3). By using internal standard correction to compensate for the injection errors both %RSD for peak area repeatability values can be improved even further.

Table 1. Peak area reproducibility (% RSD, n=20) at 5 or 10 pg absolute amount on column and calculated instrument IDL99 (in ng/g).

| No | Compound | RT (min) | pg on Column | % RSD | IDL |
|-----|--------------------------|----------|--------------|-------|-----|
| 1 | Acetochlor | 6.53 | 5 | 4.5 | 0.6 |
| 2 | Aclonifen | 7.64 | 10 | 8.3 | 2.1 |
| 3 | Aldrin | 6.90 | 5 | 5.9 | 0.7 |
| 4 | Azinphos-ethyl | 8.34 | 10 | 10.0 | 2.5 |
| 5 | Benalaxyl | 7.74 | 5 | 4.4 | 0.6 |
| 6 | BHC, Alpha | 5.96 | 5 | 5.3 | 0.7 |
| 7 | BHC, Beta | 6.11 | 5 | 8.7 | 1.1 |
| 8 | BHC, gamma | 6.18 | 5 | 6.6 | 0.8 |
| 9 | Bifenox | 8.09 | 10 | 10.0 | 2.6 |
| 10 | Bifenthrin | 8.00 | 5 | 4.3 | 0.5 |
| 11 | Biphenyl | 4.85 | 5 | 10.0 | 1.3 |
| 12 | Bromophos-ethyl | 7.21 | 5 | 6.8 | 0.9 |
| 13 | Bromopropylate | 8.04 | 5 | 5.7 | 0.7 |
| 14 | Bupirimate | 7.43 | 5 | 3.0 | 0.4 |
| 15 | Buprofezin | 7.45 | 5 | 4.9 | 0.6 |
| 16 | Cadusafos | 5.88 | 5 | 5.1 | 0.6 |
| 17 | Captan | 7.16 | 10 | 14.0 | 3.7 |
| 18 | Carbetamide | 6.90 | 10 | 9.9 | 2.5 |
| 19 | Chlorbufam | 6.09 | 10 | 7.6 | 1.9 |
| 20 | Chlordane alpha-cis | 7.25 | 5 | 6.4 | 0.8 |
| 21 | Chlordane gamma-trans | 7.32 | 5 | 5.7 | 0.7 |
| 22 | Chlorothalonil | 6.29 | 5 | 5.7 | 0.7 |
| 23 | Chlorpropham | 5.77 | 10 | 4.6 | 1.2 |
| 24 | Chlorpyrifos-ethyl | 6.85 | 5 | 4.3 | 0.5 |
| 25 | Chlorpyrifos-methyl | 6.55 | 5 | 4.2 | 0.5 |
| 26 | Chlzolinate | 7.07 | 5 | 7.9 | 1.0 |
| 27 | Clomazone | 6.12 | 5 | 4.1 | 0.5 |
| 28 | Coumaphos | 8.48 | 5 | 14.0 | 1.8 |
| 29 | Cyanazine | 6.85 | 5 | 17.0 | 2.2 |
| 30 | Cycloate | 5.72 | 5 | 8.8 | 1.1 |
| 31 | Cyfluthrin peaks I-IV | 8.59 | 10 | 11.0 | 2.8 |
| 32 | Cyhalothrin-S | 8.23 | 10 | 6.8 | 1.7 |
| 33 | Cypermethrin peaks I-IV | 8.68 | 10 | 10.0 | 2.6 |
| 34 | DDD p,p | 7.63 | 5 | 5.6 | 0.7 |
| 35 | DDE p, p | 7.42 | 5 | 4.0 | 0.5 |
| 36 | DDT o,p | 7.65 | 5 | 8.2 | 1.0 |
| 37 | DDT p,p | 7.80 | 5 | 12.0 | 1.6 |
| 38 | Deltamethrin | 9.15 | 10 | 9.8 | 2.5 |
| 39 | Diazinon | 6.21 | 5 | 4.9 | 0.6 |
| 40 | Dichlobenil | 4.69 | 10 | 8.3 | 2.1 |
| 41 | Dichlofluanid | 6.80 | 5 | 3.7 | 0.5 |
| 42 | Dichloran | 6.04 | 5 | 13.0 | 1.7 |
| 43 | Dichlorvos | 4.33 | 5 | 12.0 | 1.5 |
| 44 | Dicrotophos | 5.79 | 5 | 6.8 | 0.9 |
| 45 | Dieldrin | 7.47 | 5 | 8.0 | 1.0 |
| 46 | Diflufenican | 7.86 | 5 | 5.0 | 0.6 |
| 47 | Dimethenamid | 6.52 | 5 | 1.9 | 0.2 |
| 48 | Diphenylamine | 5.68 | 10 | 6.8 | 1.7 |
| 49 | Endosulfan I | 7.36 | 5 | 5.6 | 0.7 |
| 50 | Endosulfan II | 7.62 | 5 | 4.3 | 0.5 |
| 51 | Endosulfan sulfate | 7.81 | 5 | 4.1 | 0.5 |
| 52 | Endrin | 7.58 | 5 | 13.0 | 1.6 |
| 53 | EPN | 8.03 | 5 | 6.0 | 0.8 |
| 54 | EPTC | 4.73 | 10 | 10.0 | 2.6 |
| 55 | Ethion | 7.61 | 5 | 4.2 | 0.5 |
| 56 | Ethofumesate | 6.74 | 5 | 8.6 | 1.1 |
| 57 | Ethoprop (Ethoprofos) | 5.70 | 5 | 7.1 | 0.9 |
| 58 | Etoxazole | 8.04 | 10 | 7.5 | 1.9 |
| 59 | Etridiazole | 5.07 | 10 | 1.0 | 2.6 |
| 60 | Etrimfos | 6.33 | 5 | 4.4 | 0.6 |
| 61 | Fenazaquin | 8.11 | 5 | 4.5 | 0.6 |
| 62 | Fenitrothion | 6.74 | 5 | 4.1 | 0.5 |
| 63 | Fenpropathrin | 8.05 | 10 | 8.9 | 2.3 |
| 64 | Fenvalerate I | 8.91 | 5 | 7.4 | 0.9 |
| 65 | Fenvalerate II | 8.98 | 5 | 7.6 | 1.0 |
| 66 | Flucythrinate I | 8.69 | 10 | 8.9 | 2.3 |
| 67 | Flucythrinate II | 8.74 | 5 | 7.5 | 1.0 |
| 68 | Flurochloridone | 6.92 | 5 | 4.9 | 0.6 |
| 69 | Flutolanil | 7.33 | 5 | 10.0 | 1.2 |
| 70 | Fluvalinate | 8.93 | 5 | 11.0 | 1.4 |
| 71 | Folpet | 7.19 | 5 | 9.2 | 1.2 |
| 72 | Furalaxyl | 7.13 | 5 | 3.6 | 0.5 |
| 73 | Heptachlor | 6.67 | 5 | 3.7 | 0.5 |
| 74 | Heptachlor epoxide-cis | 7.12 | 5 | 6.3 | 0.8 |
| 75 | Heptachlor epoxide-trans | 7.14 | 5 | 9.4 | 1.2 |
| 76 | Hexachlorobenzene | 6.00 | 5 | 9.6 | 1.2 |
| 77 | Hexazinone | 7.83 | 5 | 6.9 | 0.9 |
| 78 | Iprodione | 7.99 | 10 | 12.0 | 3.1 |
| 79 | Malaoxon | 6.55 | 5 | 4.1 | 0.5 |
| 80 | Mephosfolan | 7.11 | 5 | 9.2 | 1.2 |
| 81 | Metazachlor | 7.06 | 10 | 6.4 | 1.6 |
| 82 | Methacrifos | 5.21 | 5 | 9.6 | 1.2 |
| 83 | Methidathion | 7.21 | 5 | 4.7 | 0.6 |
| 84 | Methoxychlor | 8.05 | 5 | 8.6 | 1.1 |
| 85 | Metribuzin | 6.54 | 10 | 3.8 | 1.0 |
| 86 | Napropamide | 7.34 | 10 | 6.2 | 1.6 |
| 87 | Nitrofen | 7.54 | 5 | 7.0 | 0.9 |
| 88 | Nitrothal-isopropyl | 6.92 | 5 | 4.3 | 0.5 |
| 89 | Oxadiazon | 7.39 | 5 | 4.7 | 0.6 |
| 90 | Oxychlordane | 7.12 | 5 | 5.9 | 0.7 |
| 91 | Oxyfluorfen | 7.42 | 10 | 7.7 | 2.0 |
| 92 | Paraoxon-methyl | 6.30 | 5 | 10.0 | 1.3 |
| 93 | Parathion (ethyl) | 6.88 | 5 | 6.5 | 0.8 |
| 94 | Parathion-methyl | 6.59 | 5 | 4.4 | 0.6 |
| 95 | Pendimethalin | 7.04 | 5 | 6.7 | 0.9 |
| 96 | Pentachloroaniline | 6.48 | 5 | 5.4 | 0.7 |
| 97 | Pentanochlor (Solan) | 6.78 | 5 | 5.0 | 0.6 |
| 98 | Permethrin I | 8.43 | 5 | 10.0 | 1.3 |
| 99 | Permethrin II | 8.46 | 5 | 9.7 | 1.2 |
| 100 | Phosalone | 8.18 | 5 | 8.5 | 1.1 |
| 101 | Phosmet | 8.02 | 5 | 6.8 | 0.9 |
| 102 | Pirimiphos methyl | 6.72 | 5 | 4.0 | 0.5 |
| 103 | Pirimiphos-ethyl | 6.95 | 5 | 4.0 | 0.5 |
| 104 | Procymidone | 7.15 | 5 | 5.7 | 0.7 |
| 105 | Propachlor | 5.61 | 5 | 6.9 | 0.9 |
| 106 | Propanil | 6.52 | 10 | 13.0 | 3.2 |
| 107 | Propargite | 7.86 | 5 | 10.0 | 1.3 |
| 108 | Propetamphos | 6.16 | 5 | 4.3 | 0.5 |
| 109 | Propham | 5.08 | 5 | 5.7 | 0.7 |
| 110 | Prosulfocarb | 6.71 | 10 | 4.2 | 1.1 |
| 111 | Prothiofos | 7.37 | 5 | 7.7 | 1.0 |
| 112 | Pyrazophos | 8.29 | 5 | 9.0 | 1.1 |
| 113 | Pyridaben | 8.49 | 5 | 10.0 | 1.3 |
| 114 | Pyridaphenthion | 7.97 | 5 | 16.0 | 2.0 |
| 115 | Pyrifeno-E | 7.11 | 5 | 11.0 | 1.4 |
| 116 | Quinalphos | 7.13 | 5 | 6.9 | 0.9 |
| 117 | Quinomethionate | 7.25 | 5 | 8.8 | 1.1 |
| 118 | Quintozene | 6.16 | 5 | 6.3 | 0.8 |
| 119 | Resmethrin | 7.89 | 5 | 9.6 | 1.2 |
| 120 | Spirodiclofen | 8.42 | 5 | 7.9 | 1.0 |
| 121 | Tecnazene | 5.57 | 5 | 9.6 | 1.2 |
| 122 | Tefluthrin | 6.30 | 5 | 4.5 | 0.6 |
| 123 | Terbutylazine | 6.18 | 10 | 5.1 | 1.3 |
| 124 | Terbutryn | 6.73 | 5 | 5.6 | 0.7 |
| 125 | Tetrachlorvinphos | 7.24 | 5 | 8.7 | 1.1 |
| 126 | Tetradifon | 8.16 | 5 | 8.0 | 1.0 |
| 127 | Tetramethrin | 8.01 | 5 | 6.2 | 0.8 |
| 128 | Tolclofos-methyl | 6.60 | 5 | 4.6 | 0.6 |
| 129 | Tolyfluanid | 7.09 | 5 | 5.3 | 0.7 |
| 130 | Triallate | 6.36 | 10 | 5.9 | 1.5 |
| 131 | Trifluralin | 5.78 | 5 | 7.5 | 1.0 |
| 132 | Vinclozolin | 6.57 | 5 | 7.1 | 0.9 |

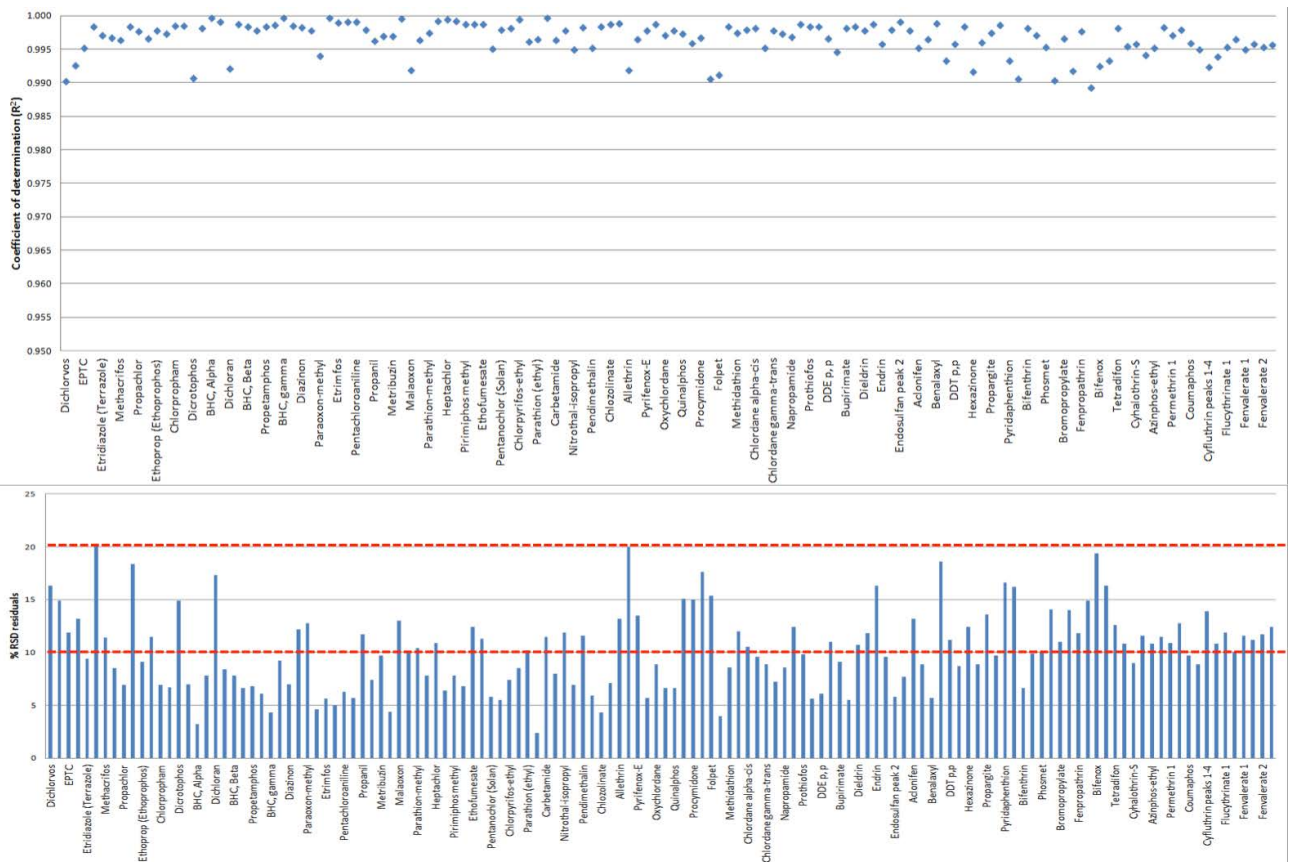


Figure 5. Coefficient of determination (R^2) and residuals values (%RSD) calculated for a linear range of 0.5–100 ng/g (or 1.0–200 ng/g). Dashed lines represent the 10% and 20% RSD residual limits.

Linearity of Response

Linearity of the GC-MS/MS system was evaluated across a concentration range of 0.5–100 ng/g (or 1–200 ng/g for some analytes) using matrix-matched standards. In all cases the coefficient of determination (R^2) was higher than 0.99 with an average value of $R^2 = 0.997$. Moreover, individual residual values were <20% with an average value of 10% (Figure 5).

Comprehensive Analysis of Additional Pesticides

Targeted screening and quantification of a given number of pesticides is important, but there is increasing interest in screening samples for compounds other than those in a target list. To answer the question, “What else is in my sample?”, samples have to be screened for unexpected or new pesticides or for metabolic/transformation products that could be present in the samples in addition to the targeted compounds. The capability of fast analytical instrumentation enables simultaneous acquisition of full scan and SRM/SIM data.

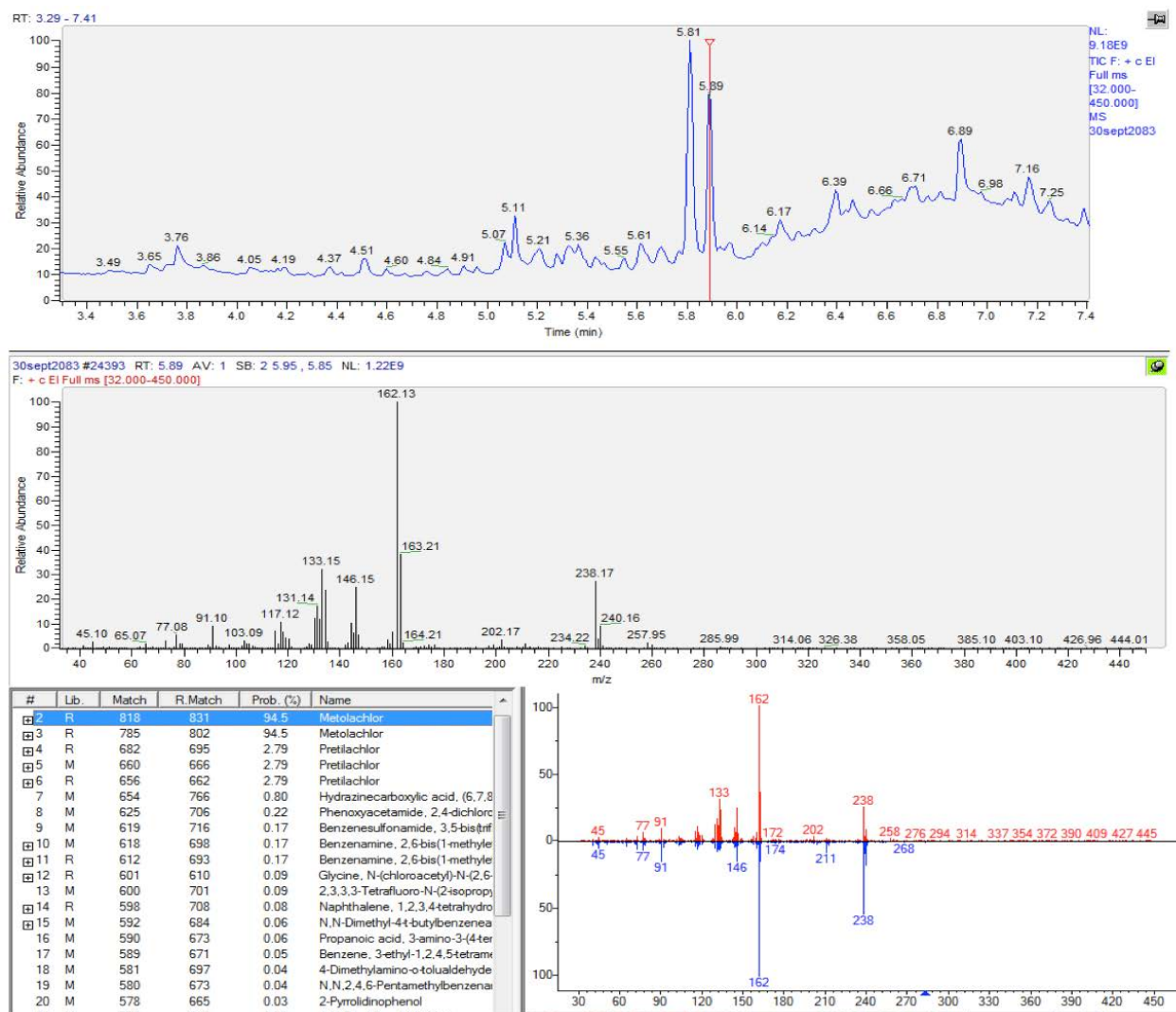


Figure 6. Comprehensive analysis of baby food contaminants using simultaneous full scan/SRM data acquisition. Compound at RT = 5.89 min identified as metolachlor (using NIST) in the full scan acquisition window.

Using the TSQ 8000 Evo GC-MS/MS system, the baby food samples were screened for additional compounds. Data was acquired in full scan and SRM modes simultaneously. An example of a full scan/SRM chromatogram is shown in Figure 6. The extracted mass spectrum of the peak eluting at RT = 5.89 min

was submitted to NIST mass spectral library and identified as metolachlor (a compound not in the spiking solution or the target list) with a probability of 95%. This result shows the advantage of using such simultaneous data acquisition, which is possible only using fast instrumentation such as the TSQ 8000 Evo GC-MS/MS.

Conclusion

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

- direct analysis of acetonitrile extracts with no need for an additional solvent exchange step.
- shorter GC run times using fast data acquisition with the EvoCell fast collision cell technology.
- comprehensive detection of target pesticides and nontargeted 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. All pesticides were detected and identified at a concentration of 5–10 ng/g with IDL values from 0.2–3.7 ng/g.

These results demonstrate that fast GC data acquisition using the TSQ 8000 Evo GC-MS/MS system delivers excellent peak area reproducibility and compound linearity.

References

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2. SANCO/12571/2013 (2014), Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed, 19.11.2013 rev. 0.
3. EN 15662 Version 2.2, Date: 2008-04, Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and cleanup by dispersive SPE – QuEChERS-method.

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