

Application of a Triggered MRM Database and Library for the Quantitation and Identification of Pesticides in Food Extracts

Application Note

Food

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Abstract

This application note describes the development of a triggered MRM database and library for more than 300 pesticides. It illustrates its use to analyze a range of food commodities with an LC/MS method developed for a specific suite of 120 pesticide residues. An Agilent 1290 Infinity LC System was coupled to an Agilent 6460 Triple Quadrupole LC/MS System and operated in positive electrospray using Agilent Jet Stream Technology. The triggered MRM acquisition mode was used for quantitation and verification and to eliminate potential false detects. A short, in-house, validation done for three commodity groups with five representative matrices showed that the developed triggered MRM method was appropriate for the analysis of pesticides in food extracts with regards to the required limits of quantitation (LOQs), linearity, and reproducibility. Several examples are shown where a high risk of an interfering matrix peak being incorrectly assigned as a pesticide, was mitigated through triggered MRM. Automatic reference library matching, displayed alongside quantitation results allows data to be reviewed efficiently and for suspect cases to be flagged automatically.



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Introduction

Food commodities, designated for human consumption need to be analyzed for pesticide residues. Commission regulation (EC) 396/2005 and its annexes which were implemented in September 2008 set maximum residue limits (MRLs) for more than 170,000 matrix-pesticide combinations for food and feed products produced in, or imported into the European Union [1]. Criteria for method validation and quality control procedures for pesticide residue analysis in food and feed are set in the SANCO/12495/2011 guideline [2].

Multiresidue methods based on LC/MS are used to monitor and quantify an increasing number of pesticides. It is generally accepted that, due to their selectivity and sensitivity, triple-quadrupole instruments are the best instruments for the quantitation of hundreds of pesticide residues in a wide variety of food matrices at very low levels. However, in complex matrices, several cases have been reported where matrix constituents were interfering on the MRM traces of pesticides with similar or identical retention times, eventually resulting in false positives [3]. In some examples, the identification criteria laid down in SANCO/12495/2011 requiring two MRM transitions with a constant ion ratio, and the retention time have not been selective enough for an unambiguous identification. Consequently, it is desirable to acquire additional transitions or a full compound spectrum. Typically, this does compromise the number of compounds which can be included in the method.

Triggered MRM enables both, fast cycle times allowing for methods with hundreds of compounds and full spectrum acquisition. Based on the response of one or more primary transitions, the acquisition of up to nine additional transitions can be triggered resulting in a full compound spectrum which

can be compared against a spectral library. Therefore, confirmation of an analyte is not only based on the area ratio of the two primary MRM transitions but also on a reference library match score. Since each fragment is acquired with the optimized collision energy, and since dwell times for a fragment in triggered MRM are considerably longer than in a full scan cycle, spectral quality of the triggered MRM spectra is significantly better than data dependent product ion scans [4]. Furthermore, triggered MRM is managed by the Agilent dynamic MRM algorithm ensuring constant cycle times for the primary (quantitative) transitions. Therefore, the collection of triggered MRM spectra does not affect the data collection rate and area of the quantitative chromatographic peak.

This application note shows the development of a triggered MRM database and library for more than 300 of the most important pesticides amenable to LC/MS according to the "Check-your-Scope" list of the European reference laboratory (EURL) for pesticide residues. The triggered MRM acquisition mode was applied to the analysis of 120 pesticide residues in different food commodities (lemon, tomato, green tea, chamomile, and ginger). QuEChERS extracts of the selected matrices were spiked in several levels ranging from 1 µg/kg to 100 µg/kg. An in-house validation according to SANCO/12495/2011 was done for three commodity groups using tomato, lemon, and green tea as representative commodities. Method performance was characterized for the triggered MRM method and a corresponding dynamic MRM method including the evaluation of matrix effects due to signal suppression or enhancement (SSE), the determination of linearity, limits of quantitation (LOQs) based on the signal-to-noise ratio, and repeatability derived from five replicates on different spiking levels. Several examples of complex matrices are shown which show spectral interferences in either one or both primary MRM traces and for which triggered MRM adds valuable information to avoid false positives.

Experimental

Reagents and Chemicals

All reagents and solvents were HPLC or LCMS grade. Acetonitrile and methanol were purchased from Baker (Mallinckrodt Baker, Deventer, Netherlands). Ultrapure water was produced using a Milli-Q Integral system equipped with a 0.22 μm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid was from Fluka (Fluka AG, Buchs, Switzerland) and ammonium formate solution (5 M) was from Agilent (p/n G1946-85021). The majority of pesticide analytical standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Solutions and standards

The individual pesticide standard solutions were combined to eight mixtures containing 30 to 40 compounds of similar physicochemical properties at a concentration of 10 $\mu\text{g}/\text{mL}$ in acetonitrile and were stored at $-20\text{ }^{\circ}\text{C}$. Immediately before use, the eight sub-mixes were combined to a final pesticide mixture containing more than 300 pesticides at a concentration of 1 $\mu\text{g}/\text{mL}$ in acetonitrile. This solution was used for spiking the QuEChERS extracts and for the preparation of the calibration samples. Eight calibration samples in a concentration range from 0.1 to 100 ng/mL were prepared in pure acetonitrile.

Sample preparation

Fruit and vegetables, dried chamomile flowers, and green tea were obtained from a local greengrocery. Samples were prepared according to the official citrate buffered QuEChERS method [5] using an Agilent BondElut QuEChERS kit (p/n 5982-5650). Ten grams of homogenized fruit and vegetable samples or 2 g of chamomile flowers or green tea were weighed in 50-mL plastic tubes. The chamomile flowers and the green tea were wetted with 10 mL ultrapure water. All samples were extracted with 10 mL acetonitrile for 1 minute while shaking vigorously by hand. Only the lemon

homogenate was neutralized afterwards by adding 600 μL of a 5 M sodium hydroxide solution. An extraction salt packet containing 4 g anhydrous MgSO_4 , 1 g NaCl, and 1.5 g buffering citrate salts was added to each tube for partitioning. The tube was again shaken for 1 minute by hand. Sample tubes were centrifuged at 3,000 rpm for 5 minutes.

A 6-mL aliquot of the upper acetonitrile layer was transferred into an Agilent BondElut QuEChERS EN dispersive SPE tube (p/n 5982-5256) containing 150 mg primary secondary amine (PSA) and 15 mg graphitized carbon black (GCB) for sample cleanup, and 900 mg MgSO_4 for water removal. The tubes were closed and vortexed for 1 minute. Afterwards, the tubes were centrifuged at 3,000 rpm for 5 minutes. The clear extracts were transferred in glass vials and 10 μL of 5% formic acid in acetonitrile were added to each mL extract to improve the stability of the target pesticides.

For the evaluation of repeatability and matrix effects, blank tomato, ginger, lemon, green tea, and chamomile samples were extracted and matrix-matched standards were prepared in three concentration levels by adding the required amount of the final pesticide mixture to an aliquot of the final QuEChERS extract. The matrix matched standards were prepared immediately before injection and were measured with five technical replicates.

Equipment

Separation was carried out using an Agilent 1290 Infinity UHPLC system consisting of an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity High Performance Autosampler (G4226A), and an Agilent 1290 Infinity Thermostatted Column compartment (G1316C). The UHPLC system was coupled to an Agilent G6460A Triple Quadrupole LC/MS System equipped with an Agilent Jet Stream electrospray ionization source. MassHunter workstation software was used for data acquisition and analysis.

Method

The 1290 Infinity UHPLC conditions are summarized in Table 1 and the G6460A Triple Quadrupole parameters are summarized in Table 2 and Table 3. Analysis was carried out in positive electrospray ionization in dynamic MRM mode using two major transitions per compound and in triggered MRM mode using two primary transitions and up to eight confirmatory ions. The confirmatory ions were measured over five acquisition cycles once the primary transition set as the trigger (typically the quantifier trace) was over a given threshold. The thresholds were compound specific and were set to 50% of the lowest calibration standard. No trigger entrance delay or

trigger delay were set and the trigger window was set to the full width of the MRM window to allow for the multiple triggering in case of matrix interferences. The Pesticide MRM data base kit (p/n G1733AA) was used to populate the method with two major (primary) transitions and conditions [6]. Further transitions used as confirmatory ions in the triggered MRM method were optimized for each individual pesticide using the Mass Hunter Optimizer software. A Dynamic MRM method was then automatically produced from the primary transitions and was run both with and without triggered MRM. Table 5 summarizes the primary transitions and the number of additional confirmatory ions for all investigated pesticides.

Table 1. UHPLC Parameters

| | | |
|------------------|---|-----|
| UHPLC column | Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 μm (p/n 959758-902) at 30 °C | |
| Mobile phase | A: 0.1% formic acid + 5 mM ammonium formate in water B: 0.1% formic acid + 5 mM ammonium formate in methanol | |
| Gradient program | Min | %B |
| | 0 | 5 |
| | 0.5 | 5 |
| | 3 | 40 |
| | 17 | 100 |
| | 19 | 100 |
| | 19.1 | 5 |
| | Post time 2 minutes | |
| Flow rate | 0.4 mL/min | |
| Injection volume | 3 μL | |

Table 2. Agilent G6460A Triple Quadrupole Parameters Operated in Dynamic MRM Mode

| | |
|-----------------------------------|--------------------------------------|
| Ionization mode | Positive ESI with Agilent Jet Stream |
| Scan type | Dynamic MRM |
| Gas temperature | 300 °C |
| Gas flow | 9 L/min |
| Nebulizer pressure | 35 psi |
| Sheath gas temperature | 350 °C |
| Sheath gas flow | 12 L/min |
| Capillary voltage | +4,000 V |
| Nozzle voltage | 0 V |
| Cycle time | 800 ms |
| Total number of MRMs | 240 |
| Maximum number of concurrent MRMs | 36 |
| Minimum dwell time | 18.72 ms |
| Maximum dwell time | 396.5 ms |

Table 3. Agilent G6460A Triple Quadrupole Parameters Operated in Triggered MRM Mode

| | |
|-----------------------------------|--------------------------------------|
| Ionization mode | Positive ESI with Agilent Jet Stream |
| Scan type | Triggered MRM with five repeats |
| Gas temperature | 300 °C |
| Gas flow | 9 L/min |
| Nebulizer pressure | 35 psi |
| Sheath gas temperature | 350 °C |
| Sheath gas flow | 12 L/min |
| Capillary voltage | +4,000 V |
| Nozzle voltage | 0 V |
| Cycle time | 800 ms |
| Total number of MRMs | 818 |
| Maximum number of concurrent MRMs | 117 |
| Minimum dwell time | 3.34 ms |
| Maximum dwell time | 196.5 ms |

Results and Discussion

Development of a triggered MRM database and library

Most commercially available MRM databases as well as public domain collections of MRM transitions for pesticides typically contain only two major transitions. The value of triggered MRM arises from the availability of several MRM transitions per compound and a spectral library including those transitions acquired under optimized conditions. A major part of this work was the development of a triggered MRM database and library containing more than 300 pesticides. For each compound, all MRM transitions which showed a reasonable response were optimized using the Mass Hunter Optimizer software. The precursor and product ions as well as the fragmentor voltages and collision energies were optimized by flow injection of single analyte solutions into the

UHPLC-MS/MS system. In the default configuration, Optimizer automatically optimizes the four most abundant fragments per compound. If further fragments were observed in the product ion spectra which showed an intensity of more than 5% relative to the most abundant fragment, the additional fragments were optimized in a second Optimizer experiment. Figure 1 shows the sum of the spectra of napropamide resulting from collisionally induced dissociation (CID) and acquired at collision energies of 0, 15, 30, 45, and 60 eV. The fragments marked with a circle were automatically picked by the Optimizer algorithm; the fragments marked with a triangle were optimized in a second Optimizer experiment.

The optimized transitions were stored in an Optimizer database which finally contained more than 2,000 transitions and conditions for more than 300 pesticides. Depending on the fragmentation behavior of the individual compounds the triggered MRM database contained transitions and conditions of 2 to 10 fragments per pesticide.

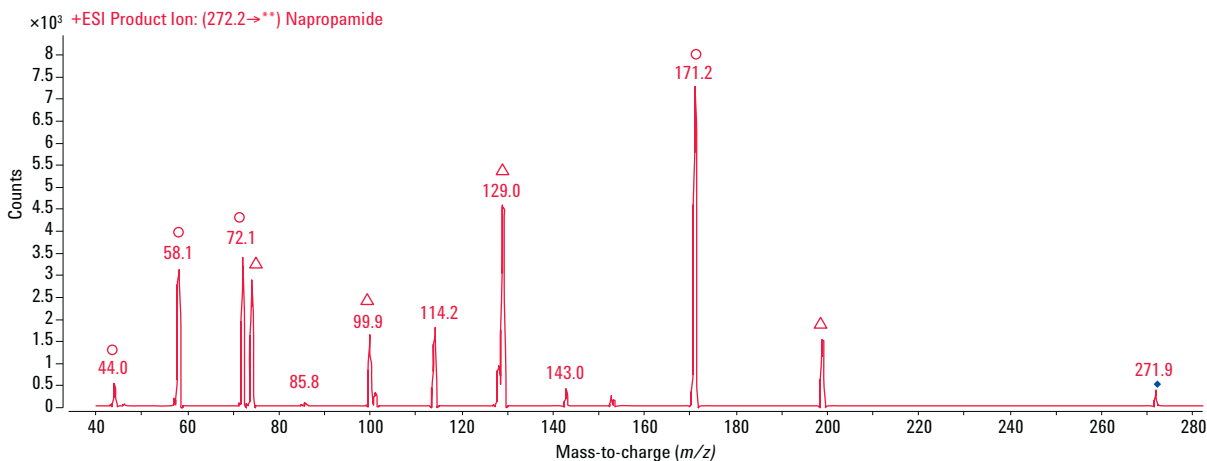


Figure 1. Sum of CID spectra of napropamide acquired at collision energies of 0, 15, 30, 45, and 60 eV. Fragments marked with a circle were optimized during the first optimization experiment, fragments marked with a triangle were optimized in a second experiment.

These optimized conditions were then used for the creation of a triggered MRM spectral library. Spectra were acquired under the UHPLC and Agilent Jet Stream conditions specified in Tables 1 and 3 for the eight pesticide sub-mixes diluted to a concentration of 100 ng/mL. Using Mass Hunter Quantitative Analysis software, the spectra could then be easily populated into a triggered MRM library. The library can be browsed with the Library Editor which shows the spectra along with the name, the CAS number, the molecular formula, weight, and the structure (an example is shown in Figure 2).

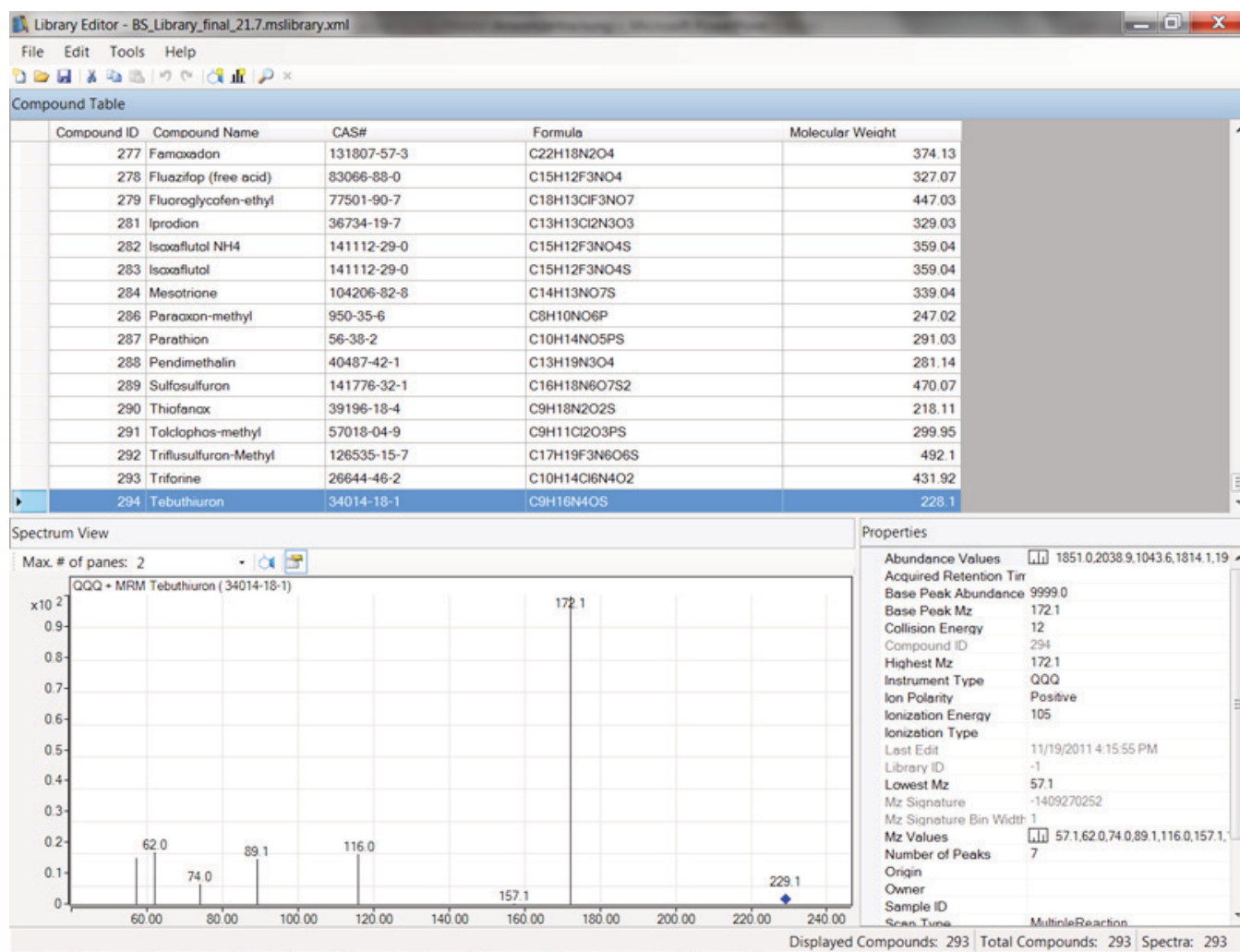


Figure 2. Mass Hunter Quantitative Analysis Library Editor showing a section of the triggered MRM library and the triggered MRM spectrum of tebuthiuron acquired in positive ESI.

Setup and optimization of the triggered MRM acquisition method

An analytical method using UHPLC and triggered MRM acquisition was set up for 120 relevant pesticides covering the full polarity range and the most important compound classes. The chromatographic method was optimized to fully resolve pesticides sharing isobaric transitions and the Agilent Jet Stream parameters were optimized to produce the highest abundance for the target compounds. Depending on the compounds, the $[M+H]^+$ or $[M+NH_4]^+$ species were used as the precursor ion. The two most abundant fragments were defined as primary transitions which were acquired over the full retention time window and were used as the quantifier and qualifier ion. The quantifier transition was typically used as the triggering transition and the threshold for the data dependent triggering of the additional four to eight fragment ions was set on a compound by compound basis between 100 and 5,000 counts corresponding to 50% of the response of the lowest calibration sample. This approach allowed the acquisition of product ion spectra for most compounds in all matrices and even at the lowest spiking levels.

Figure 3 shows the chromatogram of a lemon extract spiked with more than 120 pesticides at a concentration of 10 $\mu\text{g}/\text{kg}$ measured with triggered MRM with two primary transitions and up to eight additional confirmatory transitions (not shown).

While the primary transitions were measured during the whole observation window and were used for quantitation, the additional transitions were measured only for a certain number of repeats once the intensity of the triggering transition exceeded the given threshold. Triggered MRM is managed as part of the dynamic MRM algorithm so constant cycle times for the primary transitions are maintained throughout. Therefore, the acquisition of additional transitions does not compromise the peak shape of the quantifier or qualifier trace, and does not affect the signal intensities.

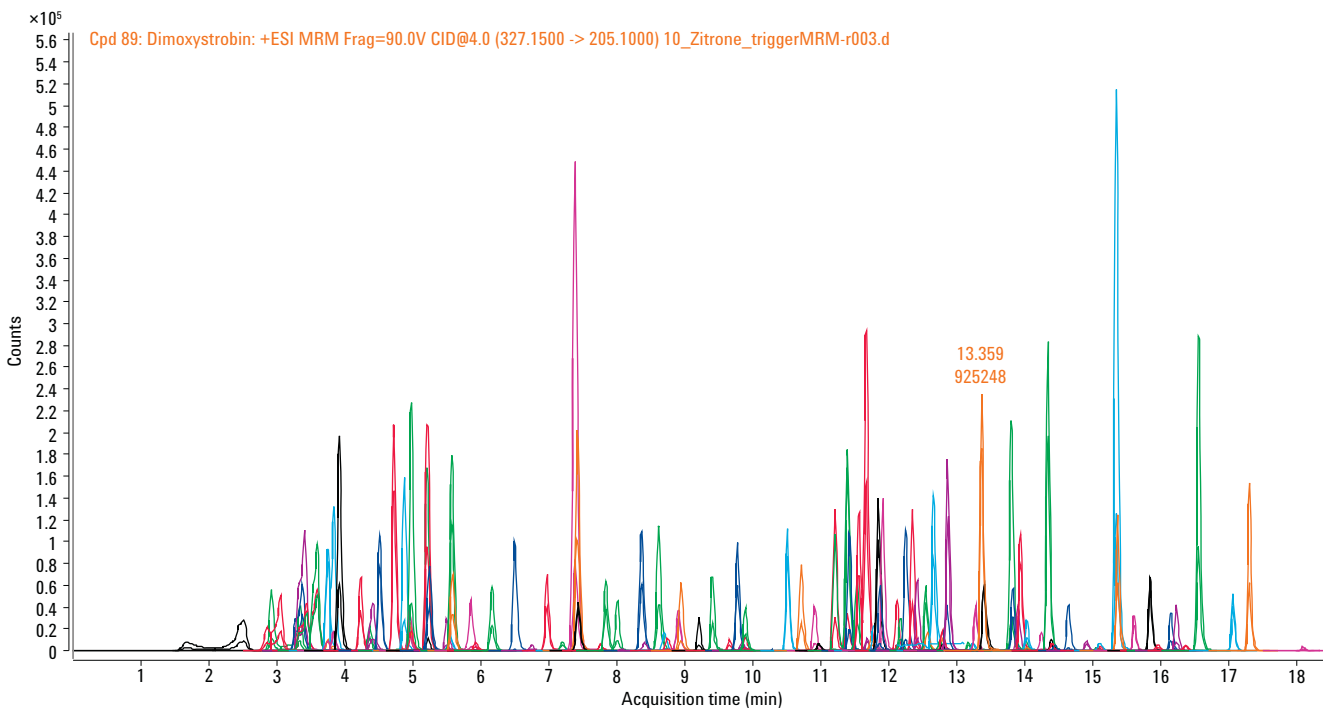


Figure 3. Chromatogram of the primary transitions of 120 pesticides spiked into lemon extract at a concentration corresponding to 10 $\mu\text{g}/\text{kg}$ and acquired with triggered MRM.

Figure 4A shows the non-smoothed MRM chromatograms of napropamide spiked into lemon extract to a concentration of 1 µg/kg acquired with the triggered MRM method. Although napropamide elutes in the most crowded region of the chromatogram with 34 primary transitions and additionally 86 confirmatory ions acquired at the tip of the peak, the peak shape of the quantifier and first qualifier is not impinged. Even at a concentration 50 times below the maximum residue limit (MRL) for lemons, the observed area ratio of the two primary transitions was in good agreement with the expected ratio. The triggered MRM spectra of napropamide in lemon extract acquired for spiking concentrations of 1, 10, and 100 µg/kg are shown in Figure 4B. Across the different concentration levels the in spectrum ratio of the fragments were extremely reproducible with RSDs well below 5% for five replicate injections. Consequently, Reference Library Match Scores above

90 were observed even for the lowest spiking levels. This was verified for several other pesticides within the test suite. The high quality spectra acquired with triggered MRM even at very low concentrations are a result of an improved ion statistics due to the use of optimized collision energies for each transition and reasonably long dwell times.

For standard dynamic MRM, the average dwell time of a transition is constant for different samples. When data dependent triggering is added to a method this will result in lower dwell times for the primary transitions when the confirmatory ions are triggered. This might be different for various samples or calibration standards. It is essential that these differences in the average dwell times are not reflected in the peak areas and do not have negative effects on the quantitation and the reproducibility.

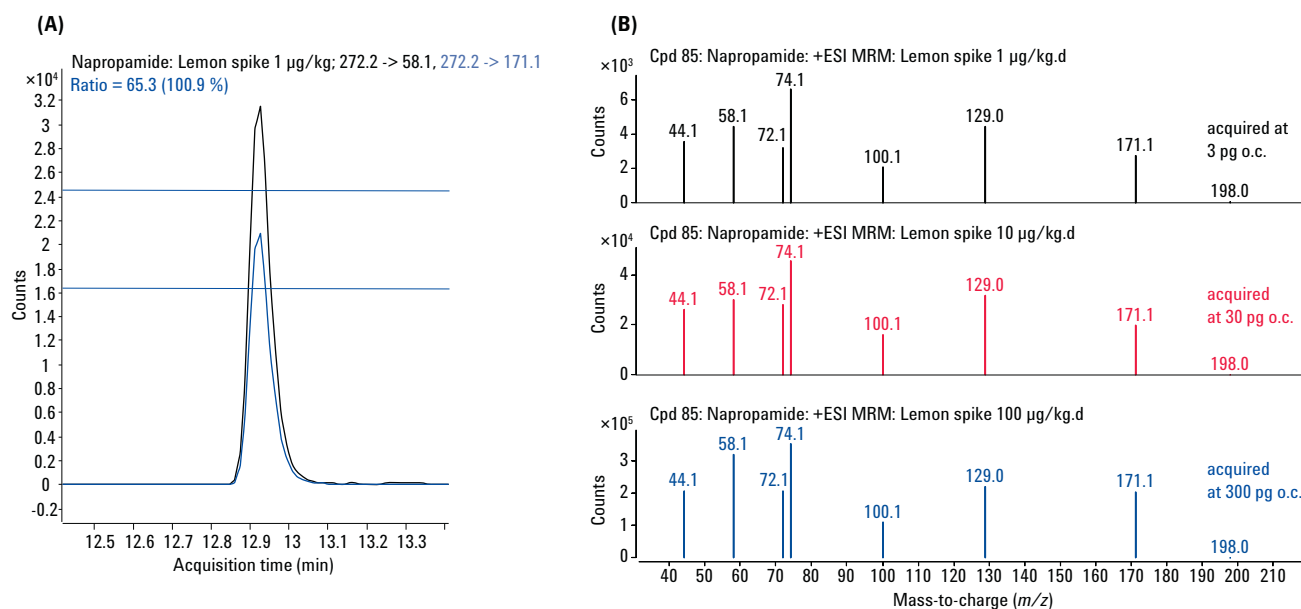


Figure 4. MRM chromatograms for the primary transitions for napropamide spiked into lemon extract at a concentration corresponding to 1 µg/kg (A) and triggered MRM spectra of napropamide spiked into lemon extract (B) at concentrations of 1 (black), 10 (red), and 100 µg/kg (blue).

To demonstrate this, a dynamic MRM method was compared to a triggered MRM method for the analysis of a complex standard (representing a worst case scenario). Figure 5 shows the calibration curves for oxamyl, a pesticide rated high in the Check-your-scope ranking of the EURL for pesticides acquired with dynamic MRM (A) and triggered MRM (B). The average dwell times for the transitions of oxamyl in the dynamic MRM method were 44 ms. In comparison, the dwell times for the primary transitions of oxamyl in the triggered MRM method was only 12 ms since product ion spectra

for all co-eluting target pesticides were triggered during this peak. Nevertheless, the calibration functions as well as the correlation coefficients were very similar.

Figure 6 compares the slopes of all targeted pesticides acquired with dynamic MRM and triggered MRM. For both acquisition modes, the calibration slopes are closely correlated with a slope of 0.9987 and a correlation coefficient of $R^2 = 0.9975$. This shows that peak areas for both acquisition modes were comparable at all levels and for differently

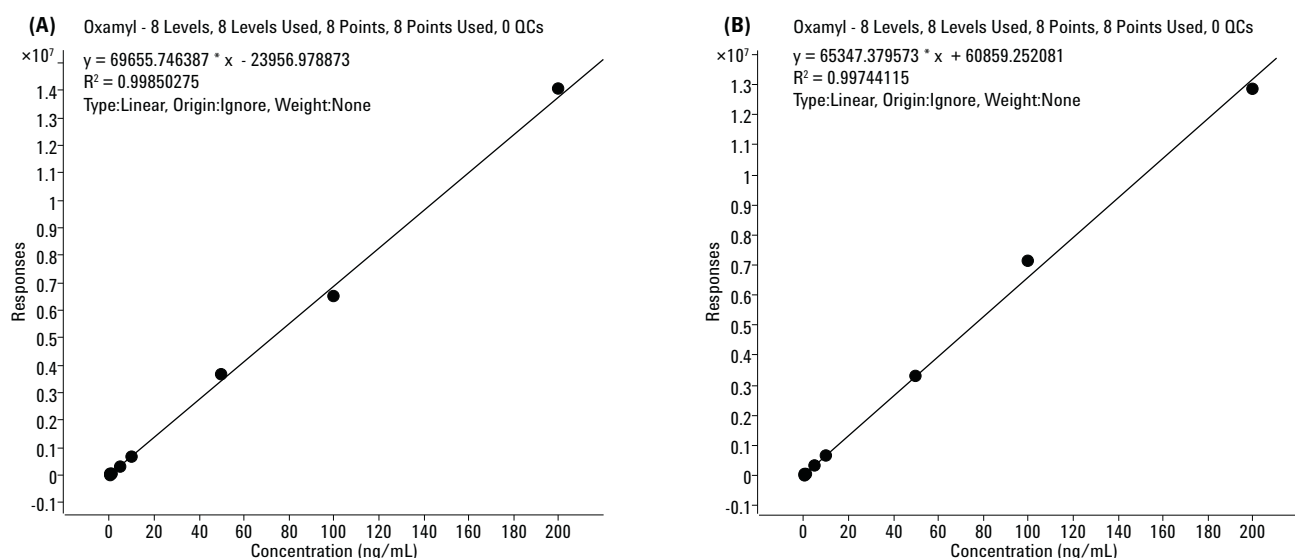


Figure 5. Calibration curves for the pesticide oxamyl acquired with dynamic MRM (A) and triggered MRM (B).

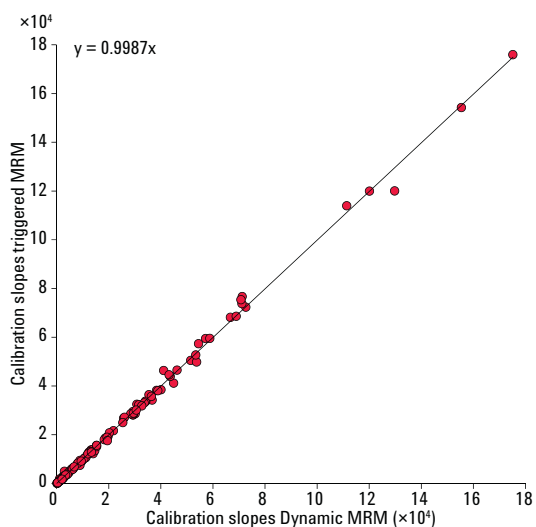


Figure 6. Comparison of the calibration curve slopes of triggered MRM versus dynamic MRM for all pesticides included in the methods. The linear range of the 7-point calibration curves were selected on a compound by compound basis and the equal calibration points were compared for both acquisition modes.

responding compounds. For 97% of all target compounds, the slope deviation was less than 20% which allows the quantitation of samples acquired with triggered MRM based on a calibration acquired with dynamic MRM.

In-house validation of triggered MRM for the quantitation of pesticides in different matrices

Method performance was characterized by the linear working range, limits of quantitation, and the repeatability of the method. Matrix effects were evaluated for spiked QuEChERS extracts of tomato, ginger, chamomile, green tea, and lemon. Validation experiments were done and evaluated based on the guideline SANCO/12495/2011.

LOQs of the triggered MRM method were derived from a signal-to-noise ratio of 10:1 (peak-to-peak noise algorithm; based on signal height) of the quantifier transition and were below 5 µg/kg for all target compounds. More than 100 compounds could be quantified in all tested matrices well below 1 µg/kg. Figure 7 shows the histogram of the LOQs in the solvent standard and in the lemon extract. As expected, LOQs in the lemon matrix were slightly higher due to matrix effects. A similar distribution was observed for the ginger, green tea, and chamomile matrix.

The repeatability was determined for all matrices at three different concentration levels (n = 5) and was below 5% for more than 80% of all compounds at a spiking level corresponding to 1 µg/kg independent of the matrix. At this concentration even in the lemon matrix, 95% of all compounds showed RSDs below 20% and could be successfully validated according to the SANCO guidelines.

Evaluation of matrix suppression and enhancement was done by comparing the response of the target compound in a solvent standard against a spiked sample extract. Depending on the commodity, up to 90% of the target compounds were affected by matrix effects. Primarily, signal suppression was observed, but for the ginger matrix, more than 10% of the target compounds showed an enhanced signal of more than 120% of the solvent response. When using matrix matched calibrations, accurate quantitation of the target compounds in each matrix could be achieved.

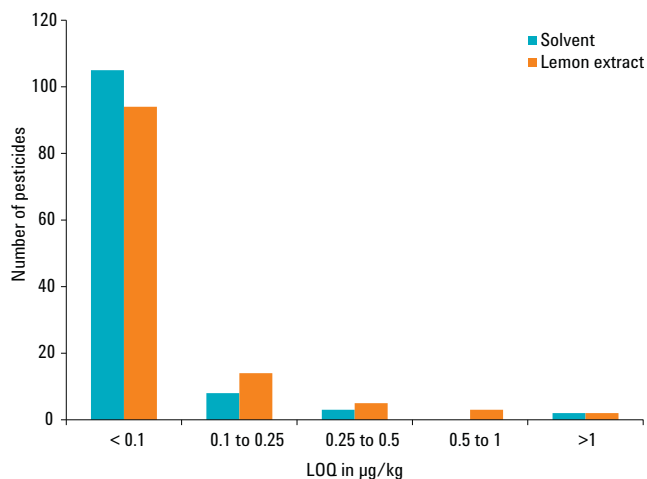


Figure 7. LOQ for the 120 evaluated pesticides in solvent and in the spiked lemon extracts. Results were classified in five relevant concentration ranges and are shown as histogram.

Analysis of real samples

During the validation runs several matrix-pesticide combinations were observed for which natural compounds showed high analogies to the targeted pesticides, as for example, identical precursors and fragments and similar retention times. By using a single qualifier/quantifier ratio, this could result in false detects, especially in a high-throughput environment. The key advantage of using triggered MRM is the acquisition of additional information allowing for the unequivocal verification of compounds by the comparison of a compound spectrum with spectra saved in a reference library.

Figure 8 shows the chromatograms and triggered MRM spectra of a natural compound in a QuEChERS extract of chamomile flowers (A) which has a similar retention time and qualifier/quantifier ratio as the herbicide tebuthiuron (B) in a solvent standard (10 ng/mL). The triggered MRM spectra are shown in comparison to the reference library spectrum. While the spectrum of the calibration sample (B) shows a perfect match and consequently results in a match score of 100.0, the fragment spectrum of the chamomile constituent (A) shows low abundances for the low mass fragments 57.1, 62.0, 74.0, and 89.1 (red arrows), the fragments 116.0 and 157.1 show high abundances (green arrows) compared to the quantifier transition.

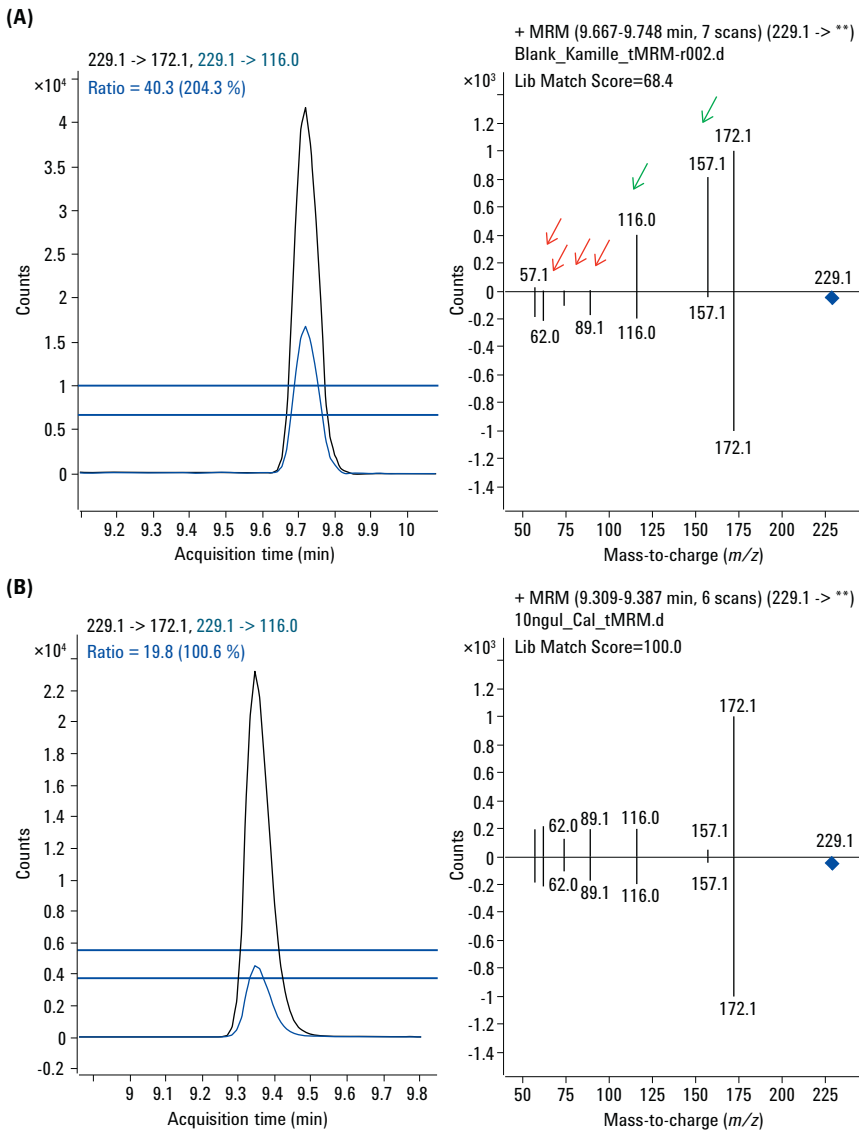


Figure 8. Chromatograms and triggered MRM spectra of a natural chamomile constituent (A) and the herbicide tebuthiuron (B). Spectra are shown in comparison to the reference library spectrum of tebuthiuron.

The resulting match score was only 68.4. From the validation results, it was shown that positive identification required a match score above 75.0 out of 100.0.

Without this additional qualitative filter there is a risk that, sooner or later, such a peak from a chamomile extract might be assigned as tebuthiuron. In this example, that would have produced a result of 0.67 mg/kg which would have been well over the default MRL of 0.01 mg/kg.

Within the tested matrix-pesticide combinations the example of tebuthiuron in chamomile extract was only one out of several where matrix interferences may appear as pesticides. Table 4 summarizes the commodities together with the suspected target analytes and the observed library match scores. In a high throughput environment, these interferences might result in false positives. Additional information such as product ion spectra and minimum required reference library match scores can help prevent reporting false detects.

Table 4. Reference Library Match Scores for Matrix Compounds Showing High Analogies to Targeted Pesticides

| Pesticide | Matrix | Reference Library Match Score | |
|-----------------------|-----------|-------------------------------|---------------------|
| | | Target compound | Matrix interference |
| Dichlorovos | Lemon | 94.5% | 78.1% |
| Thifensulfuron-methyl | Green tea | 96.6% | 71.5% |
| Tebufenpyrad | Ginger | 99.8% | 55.9% |
| Tebuthiuron | Chamomile | 97.8% | 58.0% |
| Imazalil | Chamomile | 99.8% | 58.1% |
| Terbutylazin | Chamomile | 99.6% | 82.1% |

Table 5. Primary Transitions and Number of Additional Confirmatory Ions Included in the Triggered MRM Method

| Compound name | CAS number | Precursor species | Primary transitions | Additional confirmatory ions |
|--|-------------|-------------------------------------|------------------------------|------------------------------|
| Acephate | 30560-19-1 | [M+H] ⁺ | 184.0 → 143.0; 184.0 → 49.1 | 6 |
| Acetamiprid | 135410-20-7 | [M+H] ⁺ | 223.0 → 126.0; 223.0 → 90.1 | 4 |
| Aclonifen | 74070-46-5 | [M+H] ⁺ | 265.0 → 182.1; 265.0 → 218.0 | 5 |
| Aldicarb | 116-06-3 | [M+NH ₄] ⁺ | 208.1 → 116.2; 208.1 → 89.1 | 7 |
| Aldicarb sulfone | 1646-88-4 | [M+H] ⁺ | 223.0 → 86.1; 223.0 → 76.1 | 7 |
| Aldicarb sulfoxide | 1646-87-3 | [M+H] ⁺ | 207.1 → 131.9; 207.1 → 89.1 | 7 |
| Alloxydim | 55634-91-8 | [M+H] ⁺ | 324.2 → 178.1; 324.2 → 234.1 | 6 |
| Amidosulfuron | 120923-37-7 | [M+H] ⁺ | 370.0 → 261.1; 370.0 → 218.1 | 4 |
| Amitraz | 33089-61-1 | [M+H] ⁺ | 294.2 → 163.1; 294.2 → 122.1 | 5 |
| Azinphos-ethyl | 2642-71-9 | [M+H] ⁺ | 346.0 → 77.0; 346.0 → 132.2 | 6 |
| Bifenazate | 149877-41-8 | [M+H] ⁺ | 301.1 → 198.2; 301.1 → 170.1 | 6 |
| Bispyribac | 125401-75-4 | [M+H] ⁺ | 431.1 → 275.1; 431.1 → 413.1 | 6 |
| Bitertanol | 55179-31-2 | [M+H] ⁺ | 338.2 → 99.1; 338.2 → 269.1 | 5 |
| Bromacil | 314-40-9 | [M+H] ⁺ | 261.0 → 205.0; 261.0 → 187.9 | 4 |
| Butocarboxim | 34681-10-2 | [M+NH ₄] ⁺ | 208.1 → 116.1; 208.1 → 75.0 | 7 |
| Butocarboxim sulfoxide | 34681-24-8 | [M+H] ⁺ | 207.1 → 132.0; 207.1 → 75.0 | 4 |
| Butoxycarboxim | 34681-23-7 | [M+H] ⁺ | 223.0 → 106.1; 223.0 → 166.1 | 6 |
| Buturon | 3766-60-7 | [M+H] ⁺ | 237.1 → 84.1; 237.1 → 53.1 | 6 |
| Cadusafos | 95465-99-9 | [M+H] ⁺ | 271.1 → 159.0; 271.1 → 97.0 | 5 |
| Carbaryl | 63-25-2 | [M+H] ⁺ | 202.1 → 145.1; 202.1 → 127.1 | 6 |
| Carbendazim | 10605-21-7 | [M+H] ⁺ | 192.1 → 160.1; 192.1 → 105.0 | 5 |
| Carbosulfan | 55285-14-8 | [M+H] ⁺ | 381.2 → 118.1; 381.2 → 76.0 | 5 |
| Chlorflurazurone | 71422-67-8 | [M+H] ⁺ | 539.9 → 158.0; 539.9 → 383.0 | 4 |
| Chloridazone | 1698-60-8 | [M+H] ⁺ | 222.0 → 77.0; 222.0 → 87.9 | 6 |
| Chlorsulfuron | 64902-72-3 | [M+H] ⁺ | 358.0 → 141.1; 358.0 → 167.0 | 5 |
| Clomazone | 81777-89-1 | [M+H] ⁺ | 240.1 → 223.1; 240.1 → 44.1 | 6 |
| Cyhexatin | 13121-70-5 | [M+H-H ₂ O] ⁺ | 369.2 → 205.0; 369.2 → 287.0 | 2 |
| Cymoxanil | 57966-95-7 | [M+H] ⁺ | 199.1 → 128.0; 199.1 → 110.9 | 2 |
| DEET | 134-62-3 | [M+H] ⁺ | 192.1 → 91.1; 192.1 → 119.0 | 4 |
| Desmedipham | 13684-56-5 | [M+NH ₄] ⁺ | 318.1 → 182.1; 318.1 → 108.0 | 8 |
| Dichlorvos | 62-73-7 | [M+H] ⁺ | 221.0 → 109.0; 221.0 → 127.0 | 3 |
| Diclofop-methyl | 51338-27-3 | [M+NH ₄] ⁺ | 358.1 → 281.0; 358.1 → 120.0 | 6 |
| Dicrotophos | 3735-78-3 | [M+H] ⁺ | 238.1 → 72.1; 238.1 → 112.1 | 6 |
| Diflubenzuron | 35367-38-5 | [M+H] ⁺ | 311.0 → 158.0; 311.0 → 141.0 | 2 |
| Dimethoate | 60-51-5 | [M+H] ⁺ | 230.0 → 125.0; 230.0 → 198.8 | 4 |
| Dimoxystrobin | 149961-52-4 | [M+H] ⁺ | 327.2 → 205.1; 327.2 → 116.0 | 4 |
| Diniconazole | 83657-24-3 | [M+H] ⁺ | 326.1 → 70.0; 326.1 → 159.0 | 7 |
| N,N-Dimethyl-N'-phenylsulfamide (DMSA) | 4710-17-2 | [M+H] ⁺ | 201.0 → 92.1; 201.0 → 65.1 | 5 |
| O-ethyl O-(4-nitrophenyl) P-phenylphosphonothioate (EPN) | 2104-64-5 | [M+H] ⁺ | 324.0 → 156.9; 324.0 → 296.1 | 5 |
| Ethiofencarb | 29973-13-5 | [M+H] ⁺ | 226.1 → 107.0; 226.1 → 77.0 | 5 |
| Ethiofencarb sulfone | 53380-23-7 | [M+H] ⁺ | 258.0 → 201.0; 258.0 → 106.9 | 6 |
| Ethiofencarb sulfoxide | 53380-22-6 | [M+H] ⁺ | 242.1 → 185.0; 242.1 → 107.0 | 6 |

Table 5. Primary Transitions and Number of Additional Confirmatory Ions Included in the Triggered MRM Method (Continued)

| Compound name | CAS number | Precursor species | Primary transitions | Additional confirmatory ions |
|----------------------|-------------|--|------------------------------|------------------------------|
| Ethion | 563-12-2 | [M+H] ⁺ | 385.0 → 199.1; 385.0 → 142.8 | 6 |
| Ethirimol | 23947-60-6 | [M+H] ⁺ | 210.2 → 140.1; 210.2 → 43.1 | 6 |
| Ethofumesate | 26225-79-6 | [M+NH ₄] ⁺ | 304.1 → 121.1; 304.1 → 161.2 | 5 |
| Etofenprox | 80844-07-1 | [M+NH ₄] ⁺ | 394.2 → 177.3; 394.2 → 107.1 | 2 |
| Fenazaquin | 120928-09-8 | [M+H] ⁺ | 307.2 → 57.1; 307.2 → 161.1 | 5 |
| Fenbutatin oxide | 13356-08-6 | [M+H-C ₃₀ H ₄₀ SnO] ⁺ | 519.2 → 91.1; 519.2 → 196.9 | 6 |
| Fenhexamid | 126833-17-8 | [M+H] ⁺ | 302.1 → 97.1; 302.1 → 55.1 | 4 |
| Fenobucarb | 3766-81-2 | [M+H] ⁺ | 208.1 → 95.0; 208.1 → 77.1 | 3 |
| Fenpyroximate | 111812-58-9 | [M+H] ⁺ | 422.2 → 366.2; 422.2 → 107.0 | 6 |
| Fluopicolid | 239110-15-7 | [M+H] ⁺ | 382.9 → 172.9; 382.9 → 144.9 | 6 |
| Fluroxypyr | 69377-81-7 | [M+H] ⁺ | 255.0 → 209.1; 255.0 → 181.1 | 7 |
| Flurtamone | 96525-23-4 | [M+H] ⁺ | 334.1 → 178.1; 334.1 → 247.1 | 6 |
| Formothion | 2540-82-1 | [M+H] ⁺ | 258.0 → 199.0; 258.0 → 125.0 | 5 |
| Fuberidazole | 3878-19-1 | [M+H] ⁺ | 185.1 → 157.1; 185.1 → 156.0 | 6 |
| Hexaconazole | 79983-71-4 | [M+H] ⁺ | 314.1 → 70.1; 314.1 → 159.0 | 7 |
| Hexythiazox | 78587-05-0 | [M+H] ⁺ | 353.1 → 168.1; 353.1 → 227.9 | 4 |
| Imazalil | 35554-44-0 | [M+H] ⁺ | 297.1 → 159.0; 297.1 → 201.0 | 6 |
| Indoxacarb | 144171-61-9 | [M+H] ⁺ | 528.1 → 150.0; 528.1 → 203.0 | 6 |
| Ipconazole | 125225-28-7 | [M+H] ⁺ | 334.1 → 70.0; 334.1 → 125.0 | 4 |
| Iprodione | 36734-19-7 | [M+H] ⁺ | 330.0 → 245.0; 330.0 → 56.1 | 4 |
| Mepanipirim | 110235-47-7 | [M+H] ⁺ | 224.1 → 77.0; 224.1 → 42.1 | 5 |
| Mesotrione | 104206-82-8 | [M+H] ⁺ | 340.0 → 228.0; 340.0 → 104.0 | 3 |
| Metamitron | 41394-05-2 | [M+H] ⁺ | 203.1 → 77.0; 203.1 → 175.1 | 4 |
| Methamidophos | 10265-92-6 | [M+H] ⁺ | 142.0 → 94.0; 142.0 → 125.0 | 5 |
| Methiocarb | 2032-65-7 | [M+H] ⁺ | 226.1 → 121.1; 226.1 → 169.0 | 6 |
| Methiocarb sulfone | 2179-25-1 | [M+H] ⁺ | 258.0 → 122.0; 258.0 → 201.1 | 7 |
| Methiocarb sulfoxide | 2635-10-1 | [M+H] ⁺ | 242.1 → 185.1; 242.1 → 122.1 | 7 |
| Methomyl | 16752-77-5 | [M+H] ⁺ | 163.1 → 88.0; 163.1 → 106.0 | 3 |
| Methoxyfenozide | 161050-58-4 | [M+H] ⁺ | 369.2 → 149.0; 369.2 → 313.1 | 6 |
| Metoxuron | 19937-59-8 | [M+H] ⁺ | 229.0 → 72.1; 229.0 → 46.1 | 5 |
| Monocrotophos | 6923-22-4 | [M+H] ⁺ | 224.1 → 127.0; 224.1 → 193.0 | 6 |
| Monuron | 150-68-5 | [M+H] ⁺ | 199.1 → 72.0; 199.1 → 46.1 | 2 |
| Myclobutanil | 88671-89-0 | [M+H] ⁺ | 289.1 → 70.1; 289.1 → 125.1 | 2 |
| Napropamide | 15299-99-7 | [M+H] ⁺ | 272.2 → 58.1; 272.2 → 171.1 | 5 |
| Neburon | 555-37-3 | [M+H] ⁺ | 275.1 → 88.1; 275.1 → 57.1 | 5 |
| Ofurace | 58810-48-3 | [M+H] ⁺ | 282.0 → 160.1; 282.0 → 148.1 | 6 |
| Omethoate | 1113-02-6 | [M+H] ⁺ | 214.0 → 125.0; 214.0 → 109.0 | 5 |
| Oxamyl | 23135-22-0 | [M+NH ₄] ⁺ | 237.1 → 72.0; 237.1 → 90.0 | 4 |
| Phenmedipham | 13684-63-4 | [M+NH ₄] ⁺ | 318.1 → 136.0; 318.1 → 168.0 | 8 |
| Phorate | 298-02-2 | [M+H] ⁺ | 261.0 → 75.1; 261.0 → 199.0 | 3 |
| Phosalone | 2310-17-0 | [M+H] ⁺ | 368.0 → 182.0; 368.0 → 110.9 | 3 |
| Phosmet | 732-11-6 | [M+H] ⁺ | 318.0 → 160.0; 318.0 → 133.0 | 8 |
| Phosphamidon | 13171-21-6 | [M+H] ⁺ | 300.0 → 127.1; 300.0 → 174.1 | 6 |
| Piperonyl butoxide | 51-03-6 | [M+NH ₄] ⁺ | 356.2 → 177.1; 356.2 → 119.1 | 2 |

Table 5. Primary Transitions and Number of Additional Confirmatory Ions Included in the Triggered MRM Method (Continued)

| Compound name | CAS number | Precursor species | Primary transitions | Additional confirmatory ions |
|------------------------|-------------|-----------------------------------|------------------------------|------------------------------|
| Pirimicarb | 23103-98-2 | [M+H] ⁺ | 239.2 → 72.1; 239.2 → 182.1 | 4 |
| Pirimicarb, Desmethyl- | 30614-22-3 | [M+H] ⁺ | 225.1 → 72.1; 225.1 → 168.1 | 5 |
| Pirimiphos-methyl | 29232-93-7 | [M+H] ⁺ | 306.2 → 108.1; 306.2 → 164.1 | 4 |
| Propamocarb | 24579-73-5 | [M+H] ⁺ | 189.2 → 102.0; 189.2 → 144.0 | 2 |
| Propargite | 2312-35-8 | [M+NH ₄] ⁺ | 368.1 → 231.2; 368.1 → 175.2 | 3 |
| Propoxur | 114-26-1 | [M+H] ⁺ | 210.1 → 168.1; 210.1 → 153.1 | 5 |
| Proquinazid | 189278-12-4 | [M+H] ⁺ | 373.0 → 331.0; 373.0 → 289.0 | 6 |
| Pymetrozine | 123312-89-0 | [M+H] ⁺ | 218.1 → 105.0; 218.1 → 51.0 | 2 |
| Pyrifenoxy | 88283-41-4 | [M+H] ⁺ | 295.0 → 93.0; 295.0 → 66.1 | 4 |
| Pyrimethanil | 53112-28-0 | [M+H] ⁺ | 200.1 → 82.0; 200.1 → 106.9 | 8 |
| Pyroxsulam | 422556-08-9 | [M+H] ⁺ | 435.1 → 195.1; 435.1 → 124.1 | 4 |
| Quisqualoprop-ethyl | 76578-14-8 | [M+H] ⁺ | 373.1 → 271.2; 373.1 → 255.1 | 6 |
| Rimsulfuron | 122931-48-0 | [M+H] ⁺ | 432.1 → 182.0; 432.1 → 324.9 | 6 |
| Rotenone | 83-79-4 | [M+H] ⁺ | 395.0 → 213.1; 395.0 → 192.1 | 6 |
| Spinosad (Spinosyn A) | 131929-60-7 | [M+H] ⁺ | 732.5 → 142.1; 732.5 → 98.1 | 4 |
| Spirotetramat | 203313-25-1 | [M+H] ⁺ | 374.2 → 216.1; 374.2 → 302.2 | 6 |
| Spiroxamine | 118134-30-8 | [M+H] ⁺ | 298.3 → 144.1; 298.3 → 100.1 | 3 |
| Sulfosulfuron | 141776-32-1 | [M+H] ⁺ | 471.0 → 211.0; 471.0 → 261.0 | 5 |
| Tebufenpyrad | 119168-77-3 | [M+H] ⁺ | 334.2 → 117.0; 334.2 → 145.0 | 7 |
| Tebuthiuron | 34014-18-1 | [M+H] ⁺ | 229.1 → 172.1; 229.1 → 116.0 | 5 |
| Terbutylazine | 5915-41-3 | [M+H] ⁺ | 230.1 → 174.1; 230.1 → 104.0 | 4 |
| Tetraconazole | 112281-77-3 | [M+H] ⁺ | 372.0 → 70.0; 372.0 → 159.0 | 5 |
| Thiabendazole | 148-79-8 | [M+H] ⁺ | 202.0 → 175.0; 202.0 → 131.0 | 6 |
| Thiacloprid | 111988-49-9 | [M+H] ⁺ | 253.0 → 126.0; 253.0 → 186.0 | 3 |
| Thiamethoxam | 153719-23-4 | [M+H] ⁺ | 292.0 → 211.1; 292.0 → 181.1 | 4 |
| Thifensulfuron-methyl | 79277-27-3 | [M+H] ⁺ | 388.0 → 167.0; 388.0 → 205.0 | 5 |
| Thiofanox sulfone | 39184-59-3 | [M+H] ⁺ | 251.1 → 57.0; 251.1 → 75.9 | 5 |
| Thiofanox sulfoxide | 39184-27-5 | [M+NH ₄] ⁺ | 252.1 → 104.0; 252.1 → 57.2 | 6 |
| Topramezone | 210631-68-8 | [M+H] ⁺ | 364.1 → 334.1; 364.1 → 125.1 | 4 |
| Tralkoxydim | 87820-88-0 | [M+H] ⁺ | 330.2 → 216.1; 330.2 → 244.1 | 7 |
| Trichlorfon | 52-68-6 | [M+H] ⁺ | 256.9 → 109.0; 256.9 → 221.0 | 2 |
| Trinexapac-ethyl | 95266-40-3 | [M+H] ⁺ | 253.1 → 69.1; 253.1 → 207.1 | 3 |
| Triticonazole | 131983-72-7 | [M+H] ⁺ | 318.1 → 70.2; 318.1 → 125.2 | 2 |
| Zoxamide | 156052-68-5 | [M+H] ⁺ | 336.0 → 187.0; 336.0 → 159.0 | 4 |

Conclusion

False positive identifications of pesticides in food products are a major concern for official control labs. Several pesticide-matrix combinations exist for which false positive identifications could occur when only two MRM transitions are acquired. In this application note, we showed that triggered MRM in combination with library searching against a reference spectra library reliably eliminated potential false positives. Due to the use of optimized collision energies for each MRM and due to reasonably long dwell times per transition, triggered MRM produced authentic compound spectra even at very low concentrations and in complex matrices. Linear calibration curves and excellent precision data for replicate injections showed that quantitation was not compromised when triggering additional transitions for confirmation. Triggered MRM allowed the accurate quantification and confirmation of a large number of pesticides in a single analytical run. Using only one primary transition triggered MRM potentially extends the scope of multiresidue methods to up to twice as many compounds as currently possible when using identification criteria based on the concept of a quantifier and a qualifier transition.

The developed data base and library is available from Agilent as part of the Triggered MRM library and database. It contains transitions, conditions and spectra for more than 600 pesticides and is available as Agilent product p/n G1733CA or p/n G1733BA which also contains a column, a comprehensive pesticide standard and application support.

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