Application Note: 425

Determination of Different Classes of Pesticide Residues in Processed Fruits and Vegetables by LC-MS Using the TSQ Quantum Ultra According to EU Directive 91/414 EEC

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Key Words

- TSQ Quantum Ultra™
- Surveyor™ HPLC System
- H-SRM
- Food Safety
- Pesticide Residues
- Sensitivity

Introduction

A diet rich in fruits and vegetables is thought to reduce the risk of some types of cancer, atherosclerosis, and heart disease. However, commercially grown produce often contains high levels of pesticide residues that can lead to serious health problems when consumed. Due in large part to growing public concern over the amount of pesticide residues in foods, the European Union (EU) has enacted several directives to fix Maximum Residue Limits (MRLs) for different pesticide residues in food of plant origin. MRLs represent the maximum amount of pesticide residues that might be expected in a commodity produced under conditions of good agricultural practice and typically range between 0.01 mg/kg and 10 mg/kg[1]. Although MRLs are not maximum toxicological limits, care is taken to ensure that these maximum levels do not generate toxicological concerns. Thus far, MRLs have been set for approximately 250 active substances. To cover the full variety of agricultural raw commodities (approximately 260 products of plant and animal origin), MRLs must be established for more than 260,000 pesticide/commodity combinations[1,2].

In the EU, pesticides are regulated principally by Directive 91/414/EEC concerning the placing of plant-protection products on the market[3]. According to this legislation, chemical substances or micro-organisms in pesticides are approved for use only if they have undergone a peer-reviewed safety assessment. All foodstuffs intended for human consumption or animal feed in the EU are now subject to a maximum residue limit for pesticides to protect human and animal health. Regulation (EC) 396/2005[4] consolidates in a single act all the limits applicable to various types of food and feed. It establishes MRLs for products of plant and animal origin at the Community level, taking into account good agricultural practices. It was based on several substantial amendments in the Council Directives:

- 76/895/EEC[5], which relates to the fixing of maximum levels for pesticide residues in and on specific fruits and vegetables
- 86/362/EEC[6] for cereals and cereal products
- 86/363/EEC[7] for products of animal origin
- 90/642/EEC[8] for plant products

Additionally, more stringent legislation has been established concerning pesticides in baby food. Since 1999, the EU has introduced the Commission Directives 1999/39/EC[9] and 1999/50/EC[10], which limit all pesticide residues to an MRL value of 0.010 mg/kg in processed cereal-based foods and in fruit and vegetables intended for the production of baby foods. MRLs below 0.010 mg/kg have been established for a few pesticides of higher toxicity, while the use of certain very toxic pesticides has been completely prohibited in the production of baby foods, as underlined in Commission Directives 2003/13/EC[11] and 2006/125/EC[12].

New "active" ingredients entering the market to replace compounds banned by Directive 91/414/ EEC possess considerably different physicochemical properties, and thus demand the development of multi-residue analytical methods. Analytical methodologies used to determine pesticide residues in foods must be capable of quantifying very low levels of residues as well as confirming their identity. This task becomes more difficult as MRLs are decreased and the number of target pesticides and metabolites increases. Therefore, the challenge is to develop a sensitive, cost-effective, multi-residue analytical method that can quickly identify and confirm pesticide residues belonging to various chemical classes in food products. At the same time, the method must accurately quantify these residues at low levels, thus fulfilling the performance criteria described in "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed," European Commission Document SANCO 2007/3131[13].

Goal

To develop a multi-residue liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method for the detection and quantification of 45 pesticides, including parent compounds and their transformation products from different chemical classes, in various food matrices.

Experimental Conditions

LC-ESI-MS/MS is the analytical technique of choice to assay environmental and food matrices with high sensitivity and selectivity. The technique is especially well-suited for the identification and quantification of polar and thermally labile pesticides and metabolites down to mg/kg levels.

The pesticides included in this study are listed in Table 1.

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Compound	t_R (min)[a]	Parent ion (*m/z*)	Quantifier ion (*m/z*)	Quantifier ion (*m/z*)
Acephate	2.5	184	143 (10V)	125 (10V)
Aldicarb[b] (Na+)	19.7	213	89 (22 V)	116 (22 V)
Aldicarb-sulfoxide (Na^+)	2.7	229	166 (9V)	109 (15V)
Aldicarb-sulfone (Na^+)	4.1	245	109 (25V)	166 (25V)
Acetamiprid	17.3	223.0	126 (21 V)	90 (35 V)
Azoxystrobin	28.8	404.2	372 (16 V)	344 (26 V)
Carbaryl	24.0	202.1	145 (25 V)	127 (25 V)
Carbofuran	23.1	222.1	123 (24 V)	165 (24 V)
3-hydroxy-carbofuran (-H_2O)	15.8	220	135 (15V)	163 (15V)
Chlorpropham	29.6	214.0	172 (12 V)	154 (19 V)
Carbendazim + benomyl[b]	2.5	192.0	160 (22 V)	132 (31 V)
Cyprodinil	25.3	226.1	93 (40 V)	77 (46 V)
Demeton-S	26.0	259.0	89 (22 V)	116 (22 V)
Demeton-S-methyl	22.8	253.0	61 (40 V)	89 (20 V)
Demeton-S-methyl-sulfone	7.0	263.0	109 (30 V)	169 (20 V)
Demeton-S-methyl-sulfoxide	3.3	247.0	169 (17 V)	109 (29 V)
Dimethomorph A[c] / B[c]	26.8 27.3	388.1	301 (23 V)	165 (35 V)
Disulfoton	33.8	275.0	89 (15 V)	61 (30 V)
Disulfoton-sulfone	27.1	307.0	125 (20 V)	153 (20 V)
Disulfoton-sulfoxide	24.2	291.0	185 (15 V)	157 (25 V)
Ethoprofos	29.2	243.0	131 (21 V)	173 (21 V)
Fenhexamid[d]	29.0	302.0 304.0	97 (22 V)	 97 (26 V)
Flusilazole	29.8	316.1	247 (21 V)	165 (31 V)
Imazalil	21.4	297.0	159 (24 V)	255 (25 V)
Imidachloprid	15.3	256.1	209 (22 V)	175 (22 V)
Kresoxim-methyl	31.8	314.0	222 (14 V)	116 (19 V)
Metalaxyl	24.8	280.1	220 (15 V)	192 (25 V)
Methiocarb	27.6	226.0	169 (11 V)	121 (19 V)
Methiocarb sulfoxide (Na^+)	6.6	185.0	122 (23 V)	170 (23 V)
Methomyl	5.0	163.0	106 (12 V)	88 (12 V)
Myclobutanil	28.7	289.0	125 (35 V)	70 (25 V)
Oxamyl (Na^+)	4.2	242	70 (20V)	121 (20V)
Penconazole	30.0	284.0	159 (35 V)	70 (35 V)
Pirimicarb	7.3	239.1	182 (15 V)	72 (30 V)
Propiconazole	30.8	342.0	159 (31 V)	69 (31 V)
Propoxur	22.7	210.1	111 (17 V)	168 (10 V)
Pyrimethanil	21.2	200.0	182 (35 V)	168 (35 V)
Tetraconazole[d]	29.4	372.0 374.0	159 (38 V)	 161 (31 V)
Thiabendazole	2.5	202.0	131 (36 V)	175 (36 V)
Thiachloprid	21.0	253.0	99 (45 V)	126 (25 V)
Thiodicarb	23.3	355.0	88 (20 v)	108 (20 V)
Thiophanate-methyl	22.5	343.0	151 (23 V)	311 (15 V)
Triadimefon	28.9	294.1	197 (19 V)	225 (19 V)
Triadimenol A[c]/ B[c]	27.1/27.5	296.1	70 (16 V)	99 (16 V)
Triazophos	30.4	314.1	162 (19 V)	119 (33 V)

[a] Retention time

[b] Benomyl was measured as carbendazim[14]

[c] Dimethomorph and triadimenol exist as two isomers with different retention times

[d] For fenhexamid and tetraconazole, the isotopic parent ions were selected due to the lack of a second sound transition

Table 1: Retention times and compound-specific ESI(+)-MS/MS parameters

Sample Preparation

A stock mix solution of all the pesticides was prepared at a concentration of 1 mg/L. Calibration solutions in the concentration range 0.5-100 µg/L were prepared by serial dilution of the stock solution.

Samples were prepared for analysis using extraction with ethyl acetate. Individual samples of fruits and vegetables were first homogenized. After homogenization, a 10.0 g sample was extracted using ethyl acetate and anhydrous sodium sulfate. The mixture was ultra-sonicated for 20 minutes. The mixture was filtrated through a thin layer of anhydrous sodium sulfate and the filtrate was evaporated. The extracts were then reconstituted in 5 mL of methanol. The solution was diluted with water and then filtered through a 0.45 µm syringe filter[14].

HPLC

HPLC analysis was performed using the Surveyor HPLC System (Thermo Fisher Scientific, San Jose, CA). Each 20 µL sample was injected onto a 150×2.1 mm, 3.5 µm, C18 HPLC column equipped with a 10×2.1 mm, 3.5 µm, C18 HPLC guard column. A gradient LC method used mobile phases A (0.1% formic acid) and B (0.1% formic acid in acetonitrile) at a flow rate of 0.2 mL/min. The gradient was: 0–3 min A:B = 90:10 (v/v), 3–31 min A:B = 90:10 (v/v) to A:B = 10:90 (v/v), 31–36 min A:B = 10:90 (v/v), 36–36.5 min A:B = 10:90 (v/v) to A:B = 90:10 (v/v), 36.5–45 min A:B = 90:10 (v/v).

MS

MS analysis was carried out on a TSQ Quantum Ultra triple stage quadrupole mass spectrometer with an electrospray ionization source (Thermo Fisher Scientific, San Jose, CA).

The MS conditions were as follows:
Ion source polarity: Positive
Spray voltage: 4000 V
Sheath gas pressure (N_2): 40 units
Auxiliary gas pressure (N_2): 10 units
Ion transfer tube temperature: 350 °C
Collision gas pressure (Ar): 1.0 mTorr
Q1 resolution: 0.2 FWHM (H-SRM)
Q3 resolution: 0.7 FWHM
Scan Type: H-SRM
Dwell time: 20–50 ms

The LC-MS/MS method was developed according to the scheme shown in Figure 1. The run was divided into four time segments based on the retention times of the target compounds. Multiple scan events were included in each time segment. For each target compound, the protonated molecule $[M+H]^+$ was usually investigated, except in the cases of compounds where the adduct $[M+Na]^+$ was the base peak in the ESI(+) spectra. Two transitions were selected per compound in order to perform quantification and identification simultaneously.

The SRM transitions that were monitored are summarized in Table 1. Identification criteria for the target compounds were based on the LC retention time (t_R) and on the ratio of the two monitored transitions for each compound.[13,14]

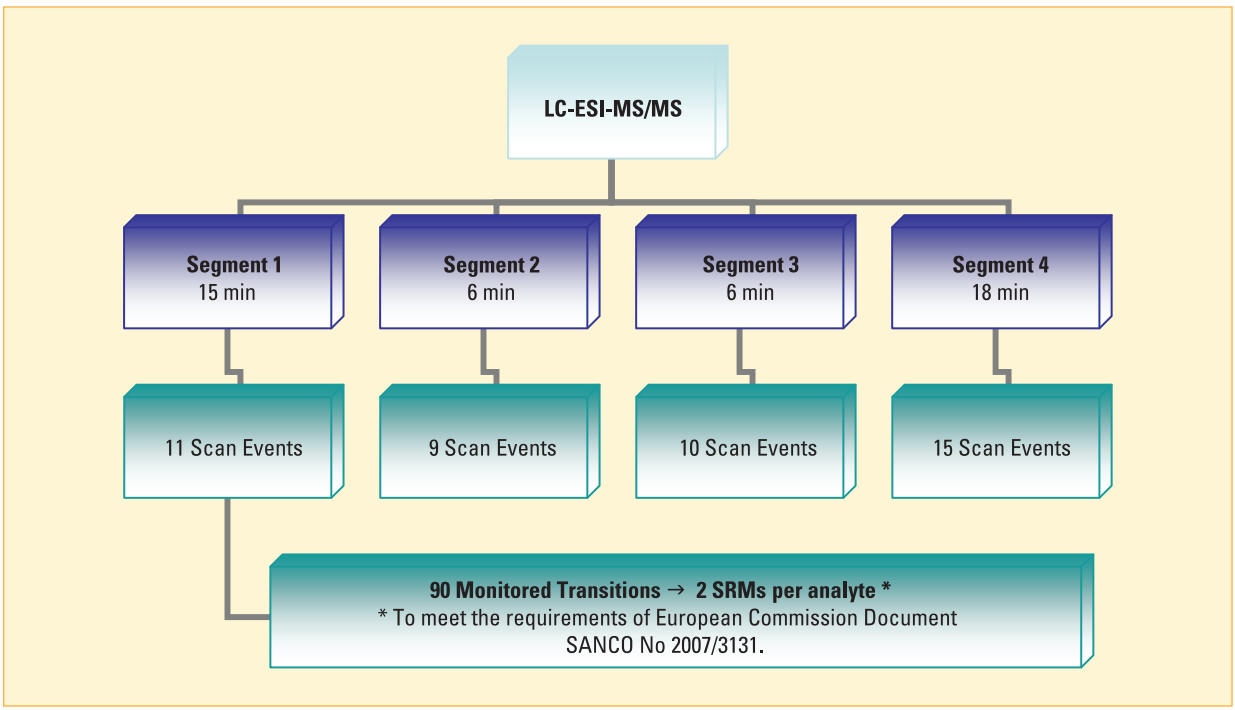

Figure 1: LC-ESI-MS/MS method

Results and Discussion

Although LC-MS/MS is a selective technique, interferences due to isobaric compounds can appear in chromatograms. These isobaric interferences increase the chemical background and can make it difficult to integrate the desired analyte peak reproducibly. Among the compounds included in this study were three sets of isobaric compounds and one set of compounds that share the same fragment ions, which increases the likelihood of cross-talk. Therefore, to eliminate the noise and lower the detection limits, all of the assays in this study were run in the Highly Selective Reaction Monitoring (H-SRM) mode with the Q1 FWHM peak width set at 0.2[14].

The H-SRM chromatograms of a mix solution of certain pesticides at a concentration of 1 µg/L are shown in Figure 2. Linearity of the method was proven for all cases because the R^2 values were usually greater than 0.99 for the linear regression equations (1/x weighted) in the concentration ranges tested. The instrumental detection limits (IDLs) were, in most cases, below 0.5 µg/L. Figure 3 displays the linearity plots of selected compounds. Linearity data for certain compounds are summarized in Table 2.

Using the H-SRM mode reduced the matrix effects by minimizing the chemical noise caused by co-eluting isobaric compounds. Consequently, the signal-to-noise ratio was enhanced in the complicated food matrices. This effect can be observed in the chromatograms in Figure 4, which show the analysis of a peas sample in the SRM and H-SRM modes. The top two SRM chromatograms illustrate the background in a blank peas extract whereas the bottom two SRM chromatograms show the peaks for methomyl in a peas extract spiked with 1 ppb of methomyl. The narrower window of the Q1 set at 0.2 FWHM in the H-SRM mode improves the selectivity of the analysis and increases the signal-to-noise ratio.

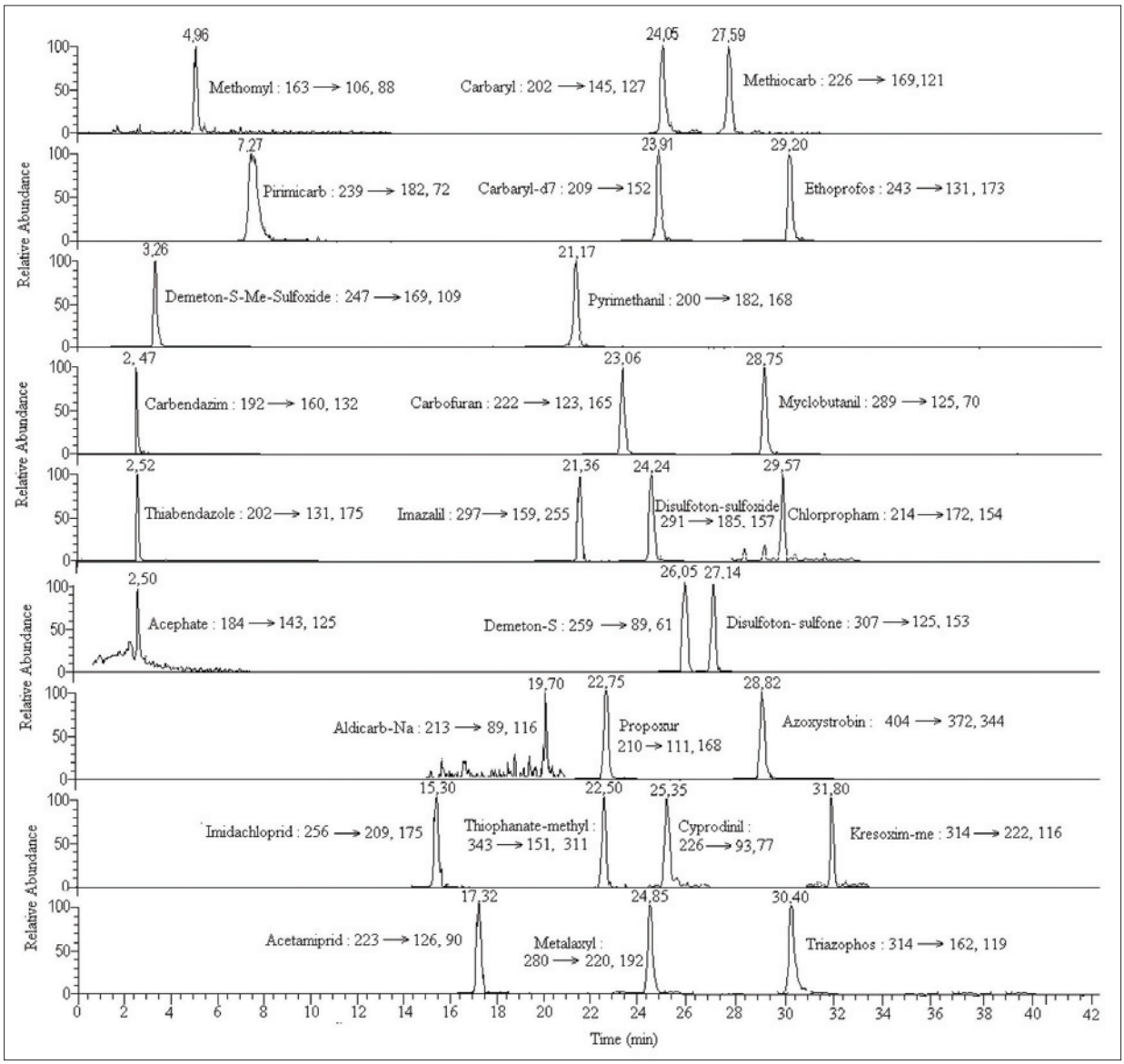

Figure 2: SRM chromatograms for certain pesticides of the standard mix solution

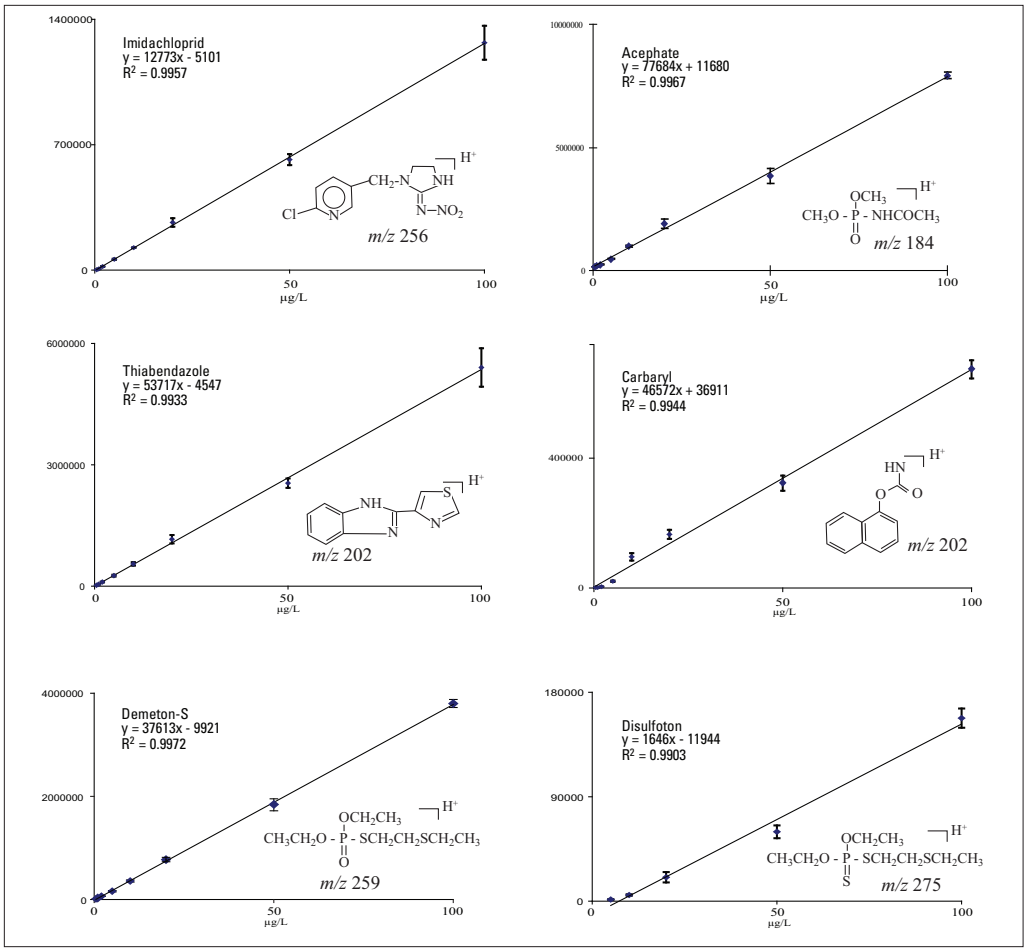

Figure 3: Linearity plots for certain compounds

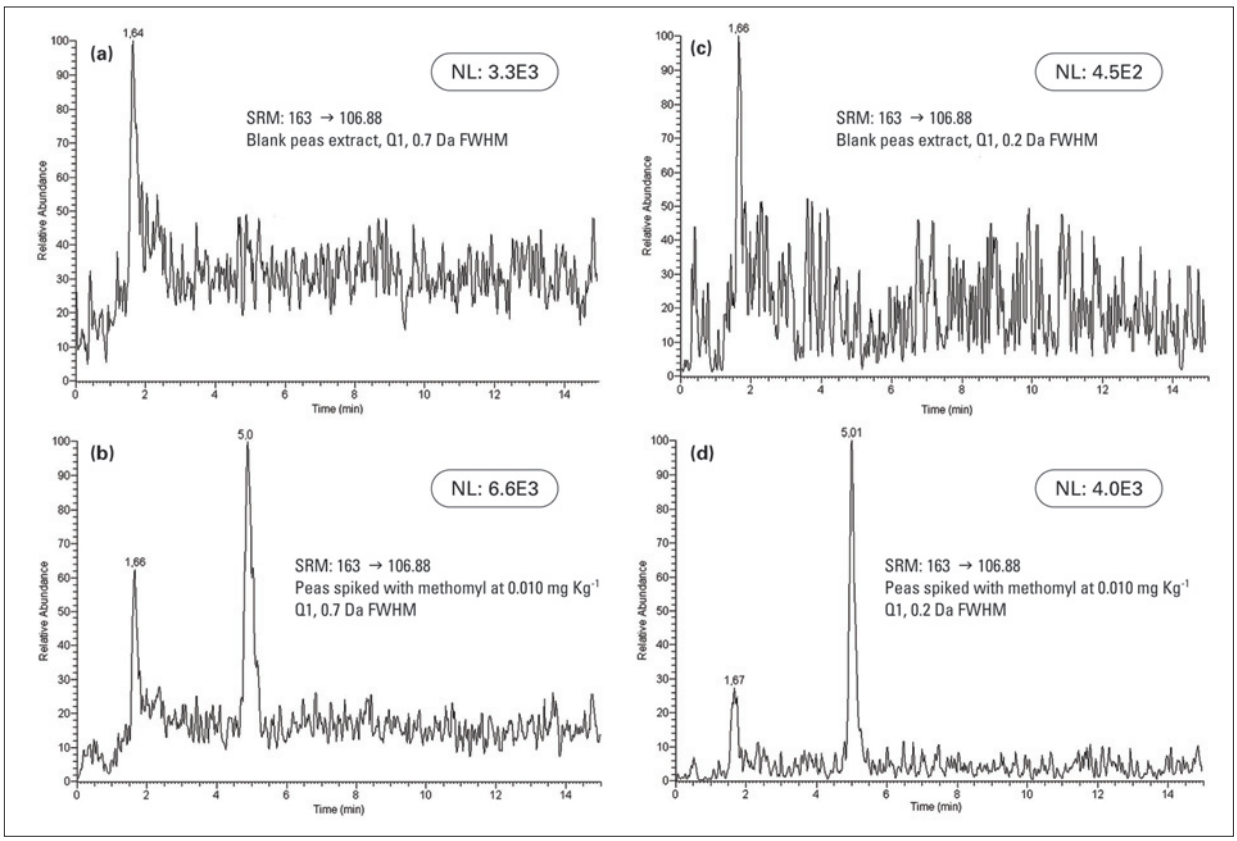

Figure 4: H-SRM and SRM chromatograms of methomyl in pea sample matrix

The matrix-matched calibration curves of methomyl at different Q1 settings are shown in Figure 5 and data of the calibration curves are listed in Table 3. The signal itself is reduced by a factor of two when the Q1 FWHM peak width is changed from 0.7 to 0.2, yet the linearity and accuracy are improved (as demonstrated by the correlation coefficients and back-calculated values of the matrix-matched standards at the low concentration levels, in Table 3).

Some samples were found to contain pesticide residues. Figure 6 displays SRM chromatograms of a sample of frozen peas that contained residues of triazophos and myclobutanil. The confirmation of identity was based on the ion ratio of monitored transitions in the sample and in the standard solution according to the EU Guidelines for pesticide residues monitoring.[13] The concentrations of the residues found in the sample were below the Maximum Residue Limits (MRLs)[1,2].

Compound	Linear regression equations	Concentration range (µg/L)	R^2	IDLs (µg/L)
Acephate	Y=116806+77683.7 X	(1-100)	0.9967	0.5
Aldicarb	Y=-590.6 +1115.4 X	(1-100)	0.9900	0.7
Azoxystrobin	Y=-363884 + 213698 X	(0.5-100)	0.9912	0.2
Carbaryl	Y=36911 + 46572 X	(0.5-100)	0.9903	0.3
Carbendazim	Y=10192 + 211684 X	(0.5-100)	0.9964	0.1
Carbofuran	Y=11107 + 161251 X	(0.5-100)	0.9920	0.2
Chlorpropham	Y=-5289 + 6893.5 X	(1-100)	0.9954	0.6
Cyprodinil	Y=-57425 + 30565.4 X	(0.5-50)	0.9931	0.3
Demeton-S	Y=-9921 + 37615 X	(0.5-100)	0.9972	0.3
Disulfoton	Y=-11944 + 1646.2 X	(5-100)	0.9903	1.5
Disulfoton Sulfoxide	Y=40274 + 141033 X	(0.5-100)	0.9961	0.4
Disulfoton Sulfone	Y=-1633.2 + 8994 X	(0.5-100)	0.9904	0.4
Ethoprofos	Y=-10106 + 40922 X	(0.5-100)	0.9940	0.3
Imidachloprid	Y=-5101.1 +12773.2 X	(0.5-100)	0.9957	0.3
Kresoxim-methyl	Y=-7877.4 + 3056.8 X	(2-100)	0.9900	1.0
Metalaxyl	Y=28427.5 +117245 X	(0.5-100)	0.9964	0.3
Methiocarb	Y=4861 + 48380.4 X	(0.5-100)	0.9921	0.3
Methomyl	Y=-2440.7 +13847.8 X	(0.5-100)	0.9990	0.4
Myclobutanil	Y=-16905.7 +10101.5 X	(0.5-100)	0.9953	0.4
Pirimicarb	Y=23403 +168260 X	(0.5-100)	0.9953	0.2
Propoxur	Y=9181 + 151300 X	(0.5-100)	0.9947	0.2
Pyrimethanil	Y=-4723.7 + 9197.2 X	(0.5-100)	0.9900	0.4
Thiabendazole	Y=-4546.8 + 53716.7 X	(0.5-100)	0.9933	0.3
Triazophos	Y=-18350 +134057 X	(0.5-100)	0.9954	0.3

Table 2: Linearity data and instrumental detection limits (IDLs) for certain pesticides

	Peas Matrix 0.1 g/mL Q1: 0.2 FWHM	Peas Matrix 0.1 g/mL Q1: 0.7 FWHM	Peas Matrix 0.2 g/mL Q1: 0.2 FWHM	Peas Matrix 0.2 g/mL Q1: 0.7 FWHM
1/x	Y = -2469.2 + 10863 X	Y = 4631.3 + 18381.3 X	Y = -3845.1 + 8212 X	Y = 10244 + 19142 X
R^2	0.9966	0.9851	0.9945	0.9861
Accuracy of Matrix-Matched Calibration Curves (1/x)				
1 µg/L	0.91 µg/L (91%)	0.68 µg/L (68%)	0.87 µg/L (87%)	1.24 µg/L (124%)
5 µg/L	4.89 µg/L (97%)	4.74 µg/L (94%)	5.26 µg/L (105%)	4.56 µg/L (91%)
10 µg/L	9.78 µg/L (97%)	11.5 µg/L (85%)	10.5 µg/L (95%)	9.05 µg/L (90%)

Table 3: Linearity and accuracy data for methomyl in pea matrix

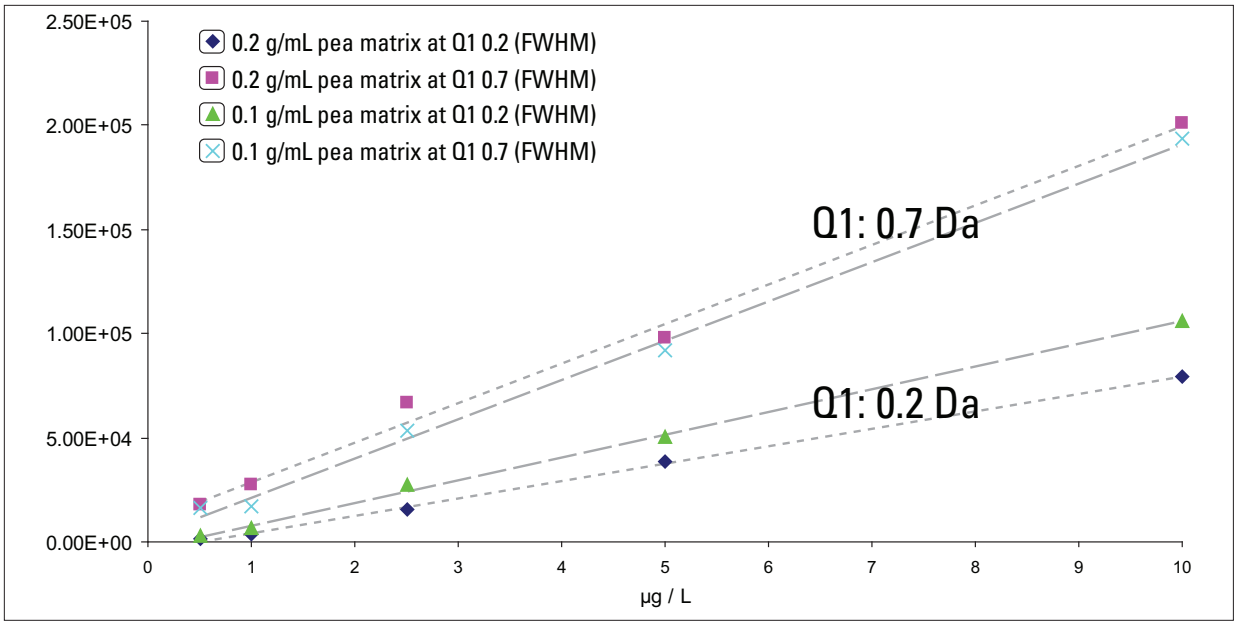

Figure 5: Matrix-matched calibration curves of methomyl in pea extract at Q1: 0.2 (FWHM) and 0.7 (FWHM)

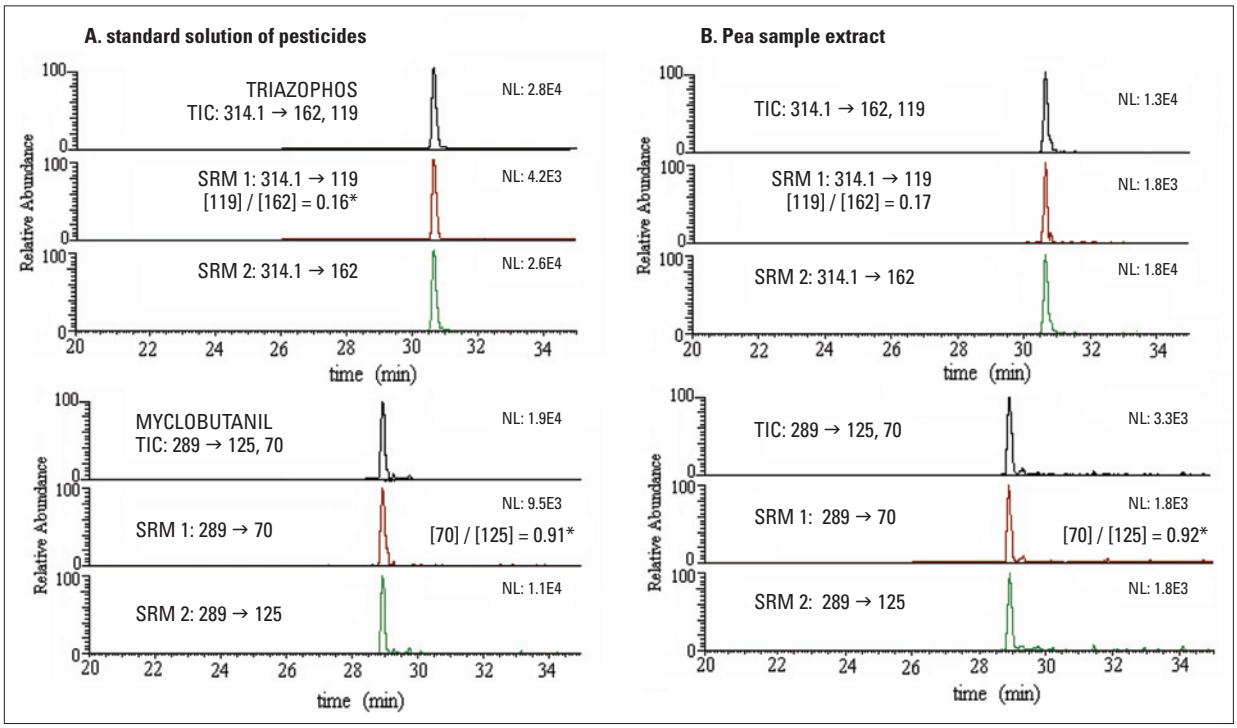

Figure 6: LC-ESI-SRM chromatograms of frozen pea sample extract, with residues of triazophos and myclobutanil

Conclusion

A multi-residue LC-ESI-MS/MS method was developed for the reliable confirmation and quantification of pesticides from different chemical classes at low ppb levels in food matrices. The method uses the Highly Selective Reaction Monitoring (H-SRM) mode of the TSQ Quantum Ultra triple quadrupole mass spectrometer to effectively reduce the background interference and improve the signal-to-noise ratios. For the pesticides investigated, satisfactory precision and accuracy were achieved and Limit of Quantitation (LOQ) values of 0.010 mg/kg were established. The method can be expanded to include more pesticides and their metabolites to improve the range of pesticide residues monitored in food commodities.

References

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