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Nota de aplicación

An Enhanced LC-MS/MS Method for the Determination of 81 Pesticide Residues in Fruit and Vegetables Using the Quattro Premier Mass Spectrometer

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Abstract

This application note demonstrates a MRM technique, using which a method was developed for the quantification of 81 pesticides and pesticide metabolites.

Benefits

The limits of detection achieved for the pesticides analyzed are well below that required for surveillance monitoring in the European Union

Introduction

Worldwide, there are over 800 pesticides currently in use to control undesirable weeds, insects, rodents, and fungi. The legal enforcement of regulations governing pesticide use requires the regular monitoring of agricultural produce. Food produce used for human consumption must contain less than the statutory Maximum Residue Limit (MRL) of any given pesticide. Given the large number of pesticide residues that may be found in foodstuffs, it is advantageous to determine as many as possible during a single analysis.

81 pesticides including: carbamates, benzimidazoles, organophosphorus, oxime carbamates, sulfonylureas, triazines, cyclohexanedione oximes, and ureas were analyzed in this study. As the number and diversity of target analytes is increased, the selectivity of the clean-up stage of sample preparation is compromised, resulting in a more complex sample matrix. Significant improvements in analytical selectivity may be achieved using triple quadrupole mass spectrometry in the Multiple Reaction Monitoring (MRM) mode.

Using the MRM technique, a method was developed for the quantification of 81 pesticides and pesticide metabolites. A generic extraction procedure and clean-up was performed. The extraction and analytical methods were validated for five commodities (representative matrix): tomato (high water), avocado (high fat), lemon (low pH), raisin (high sugar), and wheat flour (dry).

Liquid chromatography separations were performed using a Waters Atlantis dC_{18} Column 2.1 mm i.d. x 100 mm. Experiments were performed on an Alliance HPLC System coupled to a Waters Micromass Quattro Premier Tandem Quadrupole Mass Spectrometer.

Waters Micromass Quattro Premier MS System.

Experimental

Extraction Procedure

The test sample is chopped avoiding loss of juice. 10 g is transferred into a blender cup. For the dry sample materials, e.g. raisin or wheat flour, a 5 g homogenized portion is weighed into the cup. Water is added to all samples to obtain 10 mL as a sum of natural and added water. To 10 g tomato (water content 95%), lemon (water content 90%) or avocado (water content 70%) 0.5 mL, 1 mL, and 3 mL of water are added, respectively. To 5 g of raisin (water content 20%) and wheat flour (water content 10%) 9 mL and 9.5 mL of water is added, respectively. In the case of dry sample materials, it is necessary to wait 10 minutes after the addition of water. After a further addition of 20 mL methanol, the sample is blended for 2 minutes. The total volume of supernatant extract is

30 mL. In the case of very turbid extracts, an aliquot is centrifuged at 6000 rpm for 5 minutes.

6 mL of the extract is mixed with 2 mL of a solution of NaCl (20 g in 100 mL water). An aliquot of 5 mL (which contains the pesticides residues of 1.25 g normal or 0.625 g dry sample material, respectively) is transferred to a column containing 5 mL of diatomaceous earth. After a 5 minute waiting period, the column is eluted with 16 mL of dichloromethane. The eluate is gently evaporated under a stream of dry nitrogen. The dry residue is redissolved in 250 µL methanol with the help of an ultrasonic bath and further diluted with 1000 µL water. The final extract is filtered through a 0.45 µm syringe filter into a glass HPLC vial and has a matrix equivalent of 1 g/mL for normal produce or 0.5 g/mL for dry produce.

LC Conditions

LC system:	Alliance 2795 HPLC System
Mobile phase A:	Methanol/water (1:9, v/v) + 5 mM $CH_3CO_2NH_4$
Mobile phase B:	Methanol/water (9:1, v/v) + 5 mM $CH_3CO_2NH_4$
Column:	Waters Atlantis dC_{18}, 2.1 x 100 mm, 3 µm at 30 °C
Guard column:	Waters Atlantis dC_{18}, 2.1 x 20 mm, 3 µm
Flow rate:	0.3 mL/min
Injection volume:	10 µL

Gradient

Time(min)	%B
0	0
15	100

Time(min)	%B
29	100
29.1	0
40	0

MS Conditions

MS system:	Waters Quattro Premier Mass Spectrometer Electrospray mode with positive polarity
Capillary voltage:	0.6 kV
Extractor:	5 V
RF lens:	0 V
Source temp.:	120 °C
Desolvation temp.:	450 °C
Cone gas flow:	450 °C
Desolvation gas flow:	850 L/hr
Collision gas pressure:	Argon at $3.2e^{-3}$mBar
Multiplier:	650 V

The MRM transitions, along with the cone voltages, collision energies and dwell times for each pesticide are listed

in Table 1. The MRM transitions were distributed into eleven function windows, based on analyte retention times. This system allows the flexible use of MRM dwell times, where the signal-to-noise (S/N) ratio of less intense peaks can be increased by the use of longer dwell times whilst a short overall scan cycle time is maintained.

Pesticide	MRM Transition	Cone Voltage	Collision Energy	Dwell Time/ms
Methamidophos	141.8 → 93.8	24	14	50
Acephate	183.8 → 142.8	16	9	50
Omethoat	213.9 → 182.8	20	12	50
Butoxycarboxim-sulfoxid	206.9 → 131.8	16	7	50
Aldicarb-sulfoxid	206.9 → 131.8	16	7	50
Butoxycarboxim	240.0 → 105.8	11	16	50
Aldoxycarb	240.0 → 85.8	13	22	50
Oxamyl	237.0 → 71.8	11	13	50
Propamocarb	189.0 → 101.8	26	20	50
Oxydemeton-methyl	247.0 → 168.8	18	15	50
Methomyl	162.8 → 87.8	10	10	50
Demeton-S-methyl-sulfon	263.0 → 168.8	26	18	50
Quinmerac	221.9 → 140.8	20	35	50
Pymetrozin	218.0 → 104.8	28	22	50
Nicosulfuron	411.0 → 181.9	26	20	200
Monocrotophos	223.9 → 126.7	18	18	50
Amidosulfuron	370.0 → 261.0	20	16	50
6-Cl-4-OH-3-phenylpyridazin	206.8 → 76.8	38	34	50
Ethiofencarbsulfon	275.1 → 106.8	12	22	50
Thiofanox-sulfoxid	252.1 → 103.8	10	14	50
Metsulfuron-methyl	382.0 → 166.8	22	18	50
Ethiofencarbsulfoxid	242.0 → 106.8	15	20	50
Thifensulfuron-methyl	388.0 → 166.8	20	18	50
Rimsulfuron	432.0 → 181.9	26	26	200
Imidacloprid	256.0 → 208.9	24	18	50
Thiofanox-sulfon	268.1 → 75.8	10	12	200
Clethodim-imin-sulfon	302.1 → 97.8	36	34	50
5-Hydroxy-clethodim-sulfon	408.0 → 203.9	22	24	200
Chlorsulfuron	357.9 → 140.8	26	21	50
Vamidothion	288.0 → 145.8	16	15	50
Clethodim-imin-sulfoxid	286.1 → 208.0	25	18	50
Carbofuran-3-hydroxy	220.0 → 162.9	26	12	50
Cinosulfuron	414.0 → 182.9	26	18	50
Metamitron	202.9 → 174.9	30	18	100
Dimethoat	229.9 → 124.7	16	22	50
Flazasulfuron	408.0 → 181.9	25	21	200
Triasulfuron	402.0 → 166.9	28	18	50
Clethodim-sulfon	392.1 → 300.1	22	15	50
Clethodim-sulfoxid	376.1 → 206.0	22	16	50
Thiacloprid	252.9 → 125.7	30	25	50

Table 1. MRM Method Parameters.

Pesticide	MRM Transition	Cone Voltage	Collision Energy	Dwell Time/ms
Carbendazim	191.8 → 159.8	26	20	200
Butocarboxim	212.9 → 74.8	24	16	50
Aldicarb	208.0 → 115.8	8	8	200
Propoxur	210.0 → 110.8	14	16	50
Carbofuran	221.9 → 164.8	19	14	50
Bendiocarb	224.0 → 108.8	16	20	50
Prosulfuron	420.0 → 140.8	26	22	50
Carbaryl	201.8 → 144.8	17	10	50
Ethiofencarb	225.9 → 106.8	15	18	50
Triflusulfuron-methyl	493.0 → 264.0	26	23	50
Pirimicarb	239.1 → 71.8	28	21	50
Thiodicarb	355.0 → 87.8	15	15	50
Bensulfuron-methyl	411.0 → 148.8	26	23	50
Atrazin	216.0 → 173.8	32	19	50
Metalaxyl	280.1 → 220.0	20	16	50
Isoproturon	206.9 → 71.8	26	20	50
Isoxaflutole	377.0 → 250.9	10	22	300
3,4,5-Trimethacarb	193.9 → 136.8	18	13	50
Diuron	232.9 → 71.8	25	20	50
Clethodim	360.0 → 163.8	22	23	50
Azoxystrobin	404.1 → 372.0	21	16	50
Linuron	249.0 → 159.8	26	20	50
Pyrimethanil	199.9 → 106.7	40	28	50
Methiocarb	243.1 → 120.8	8	26	50
Promecarb	208.0 → 150.8	18	10	50
Fenhexamid	302.1 → 96.9	36	26	50
Metolachlor	284.1 → 175.9	20	28	50
Fenoxycarb	302.1 → 87.8	22	22	50
Tebufenozid	353.1 → 132.8	12	20	50
Tebuconazol	308.1 → 69.8	30	24	50
Cyprodinil	226.0 → 92.8	44	35	50
Imazalil	297.0 → 158.8	32	25	200
Haloxyfop-methyl	376.0 → 315.9	30	20	50
Spiroxamine	298.2 → 143.9	30	22	50
Haloxyfop-ethoxyethyl	434.0 → 315.9	26	24	50
Fluazifop-P-butyl	384.1 → 282.0	30	24	50
Quizalofop-ethyl	373.0 → 299.0	34	21	50
Furathiocarb	383.1 → 194.9	22	21	50
Flufenoxuron	489.0 → 157.8	25	27	100
Pyridate	379.0 → 206.9	22	18	100
Fenpropimorph	304.2 → 146.9	44	30	100

Table 1. MRM Method Parameters.

Results and Discussion

The Total Ion Chromatogram (TIC) for an avocado extract spiked at 10 µg/kg is illustrated in Figure 1.

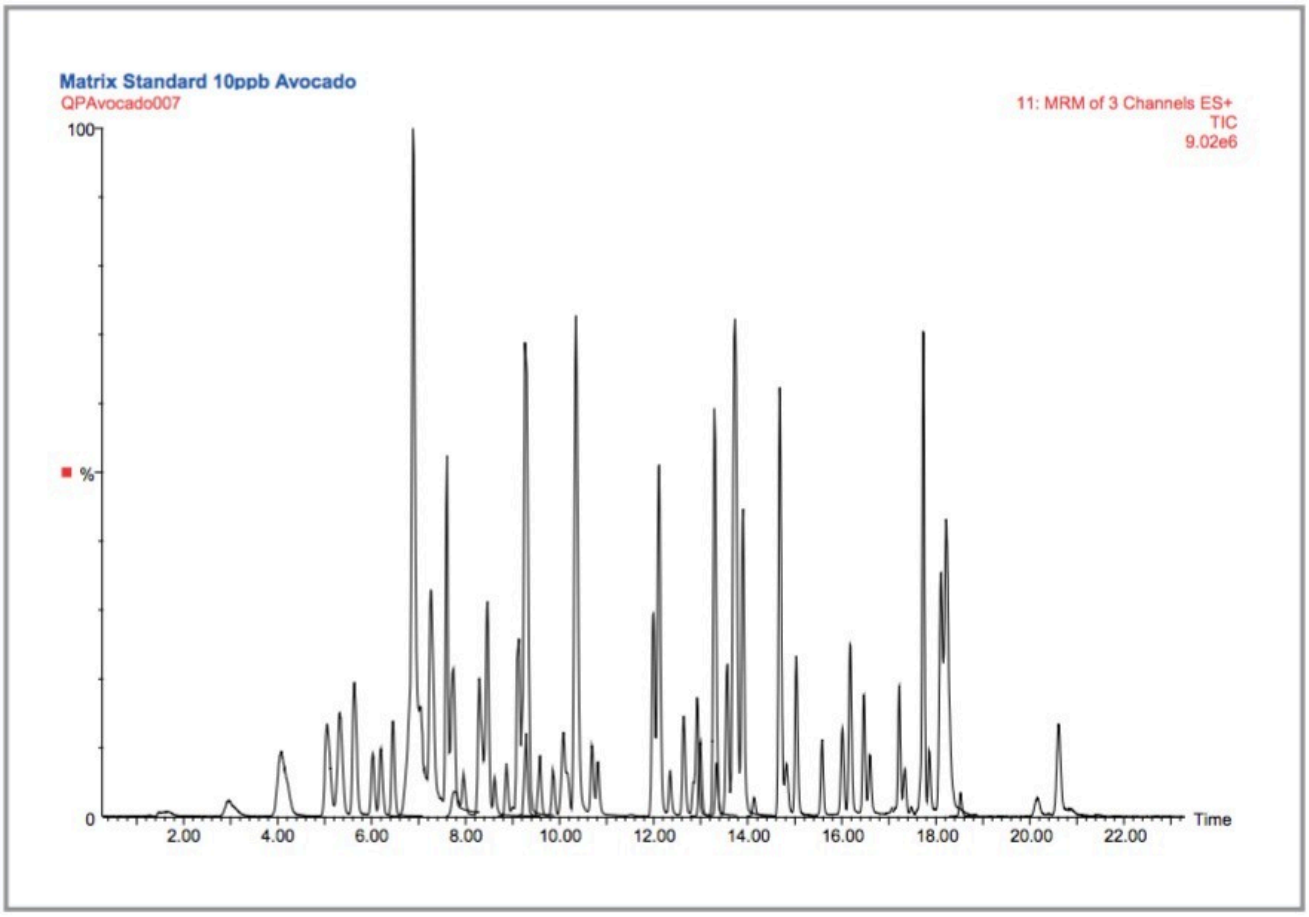

Figure 1. TIC of 81 pesticides divided into 11 MRM function windows, 10 µg/kg in avocado.

Matrix matched standards were generated at the 0.5, 1, 5, 10, 20, and 40 µg/kg levels for tomato, avocado and lemon, and at the 1, 2, 5 10, 20, and 40 µg/kg levels for raisin and wheat flour. These standards were each injected four times in a typical batch analysis and then processed using Waters QuanLynx Software. Representative calibration curves for quinmerac in tomato, carbaryl in avocado, monocrotophos in lemon, imazalil in raisin and aldicarb in wheat flour are illustrated in Figures 2, 4, 6, 8, and 10, respectively.

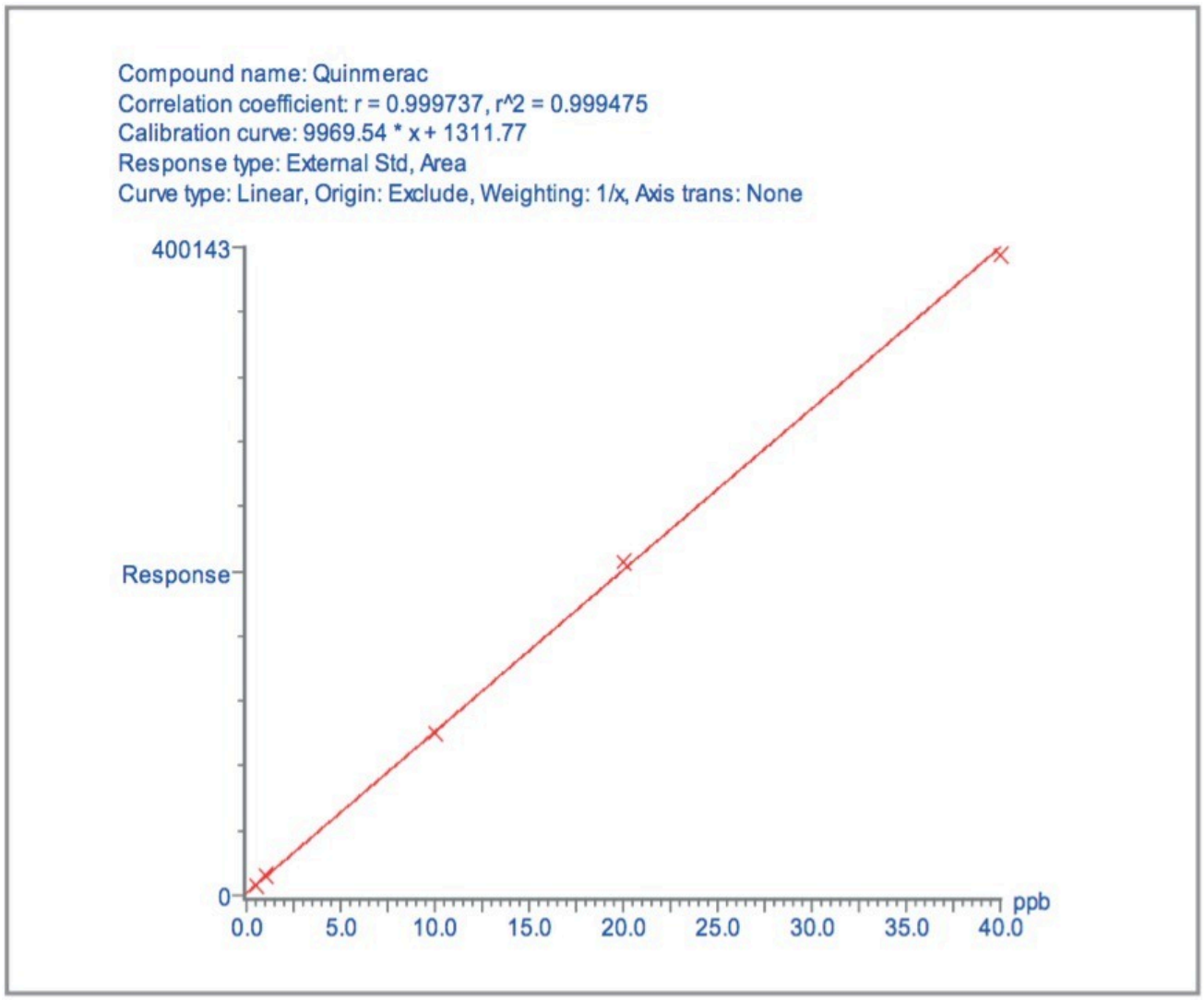

Figure 2. Calibration curve for quinmerac in tomato.

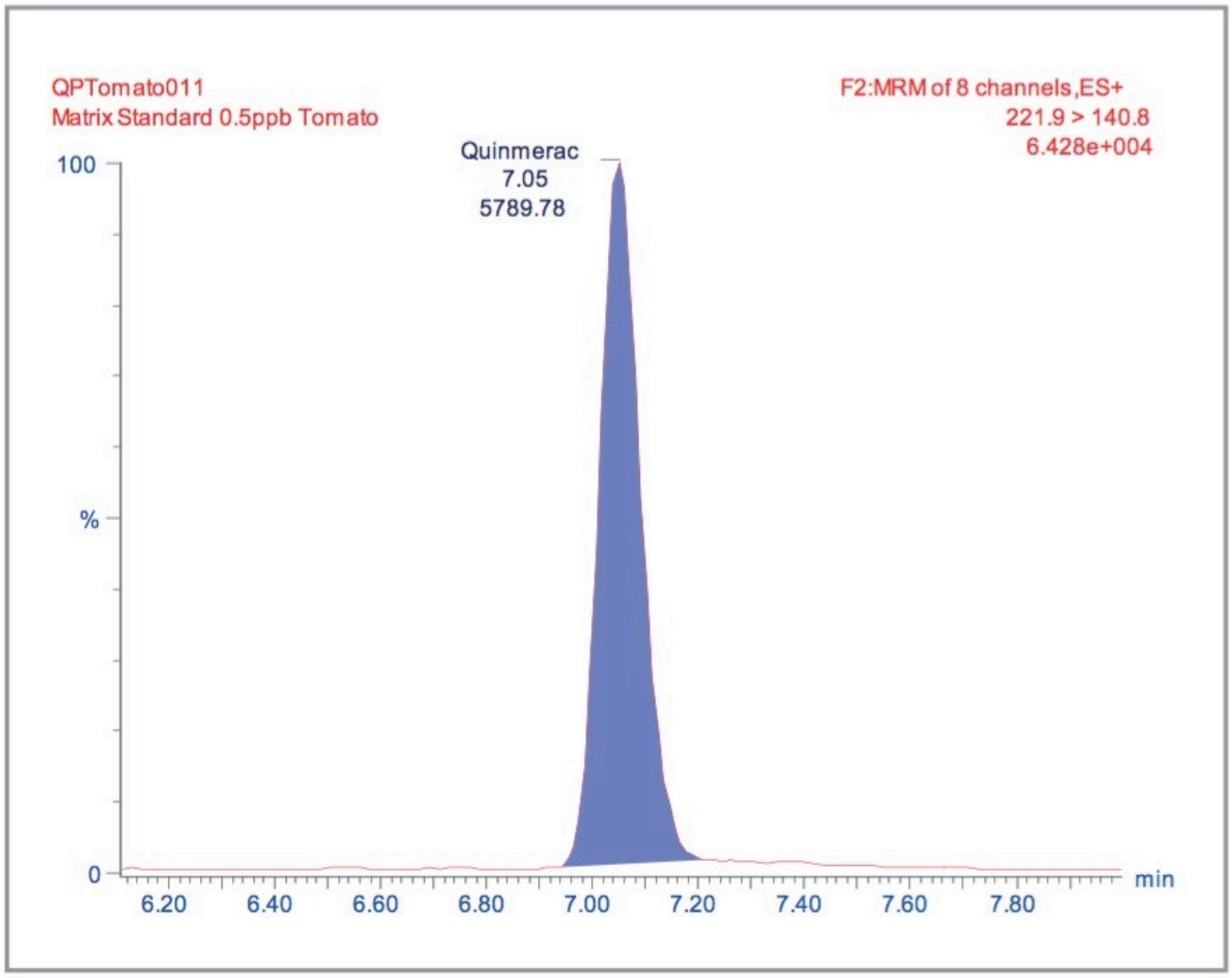

Figure 3. MRM chromatogram for quinmerac in tomato at 0.5 µg/kg.

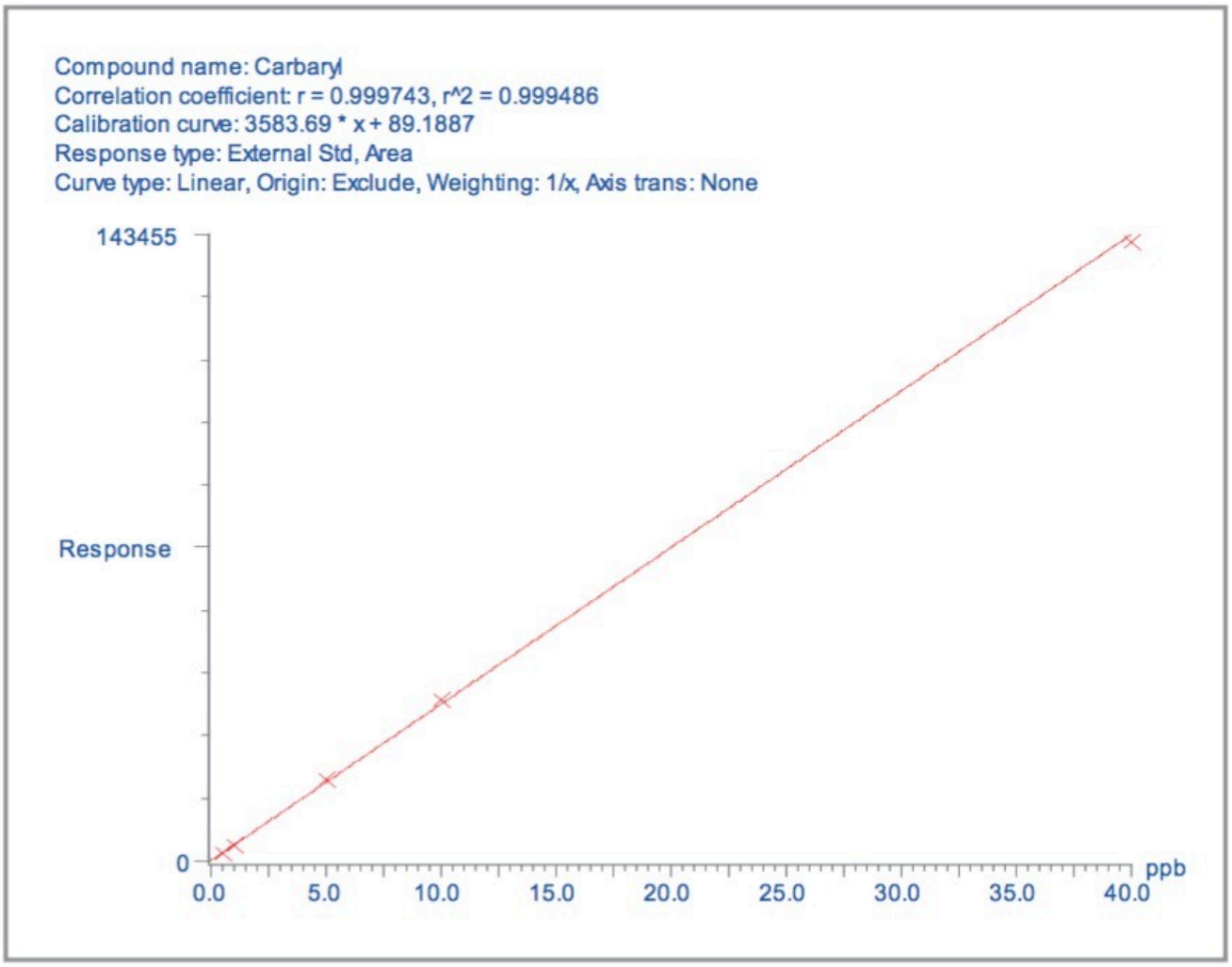

Figure 4. Calibration curve for carbaryl in avocado.

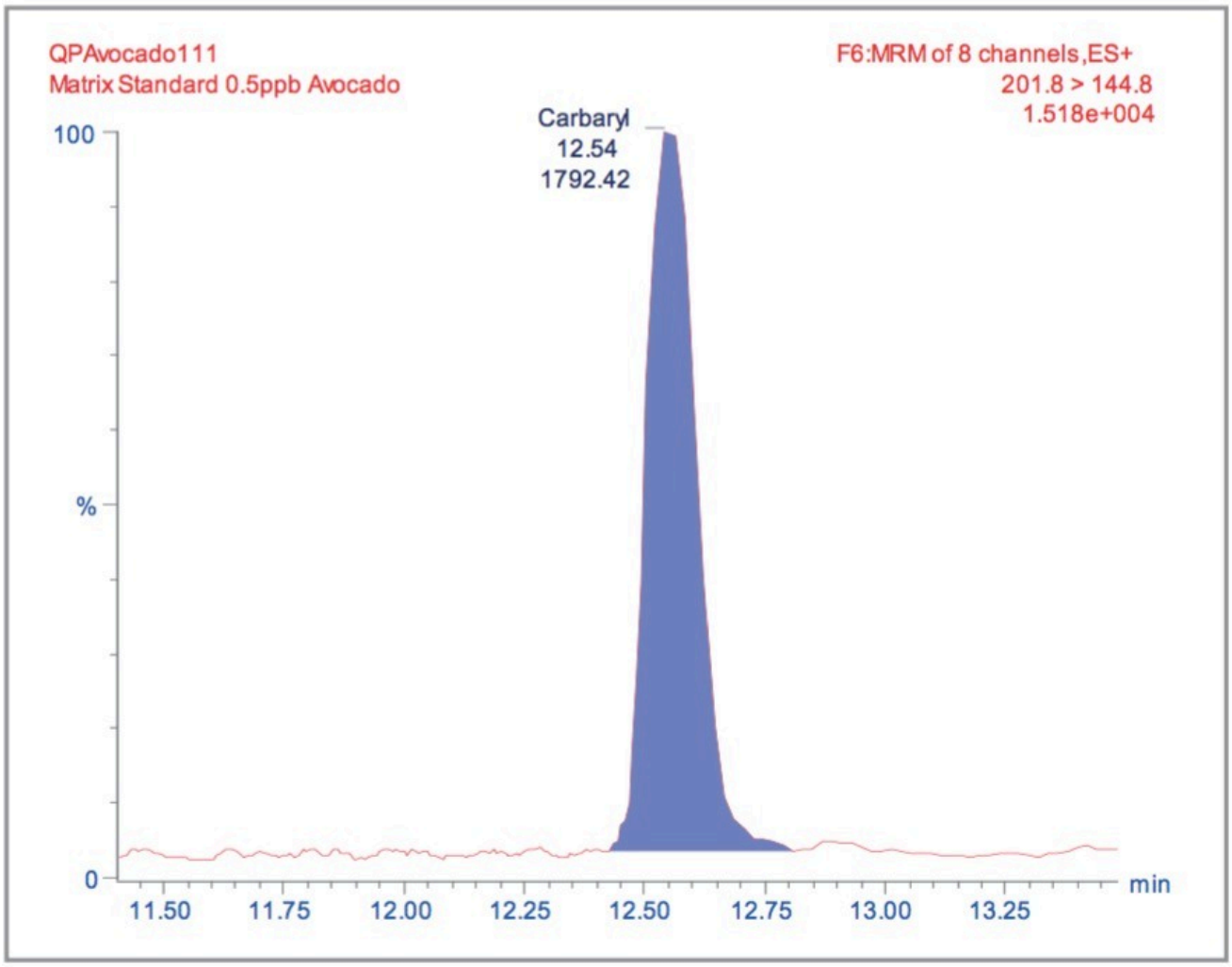

Figure 5. MRM chromatogram for carbaryl in avocado at 0.5 µg/kg.

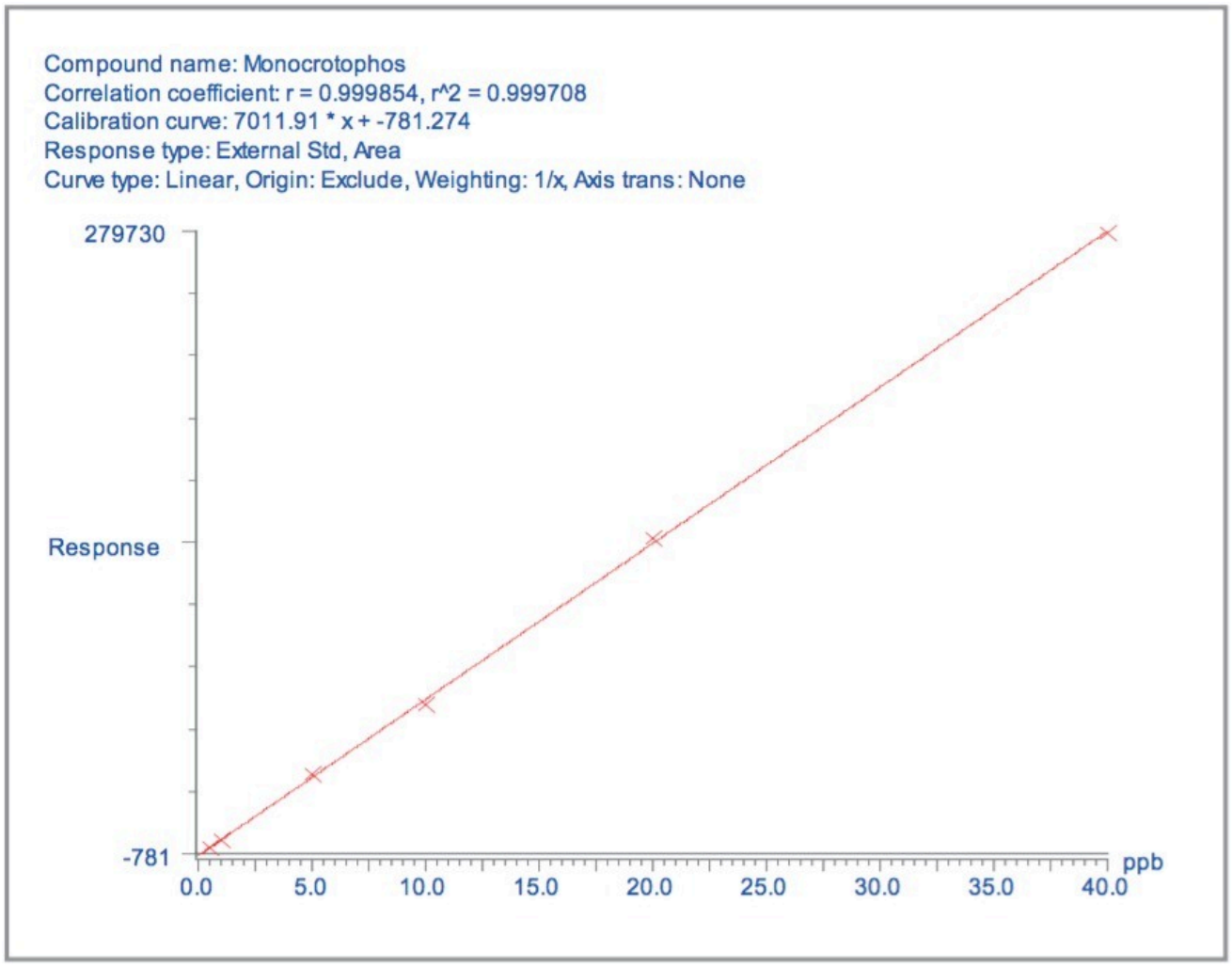

Figure 6. Calibration curve for monocrotophos in lemon.

The MRM extracted chromatogram for these compounds at the lowest calibrated level of 0.5 µg/kg in tomato, avocado and lemon, and 1 µg/kg in raisin and wheat flour are illustrated in Figures 3, 5, 7, 9, and 11, respectively.

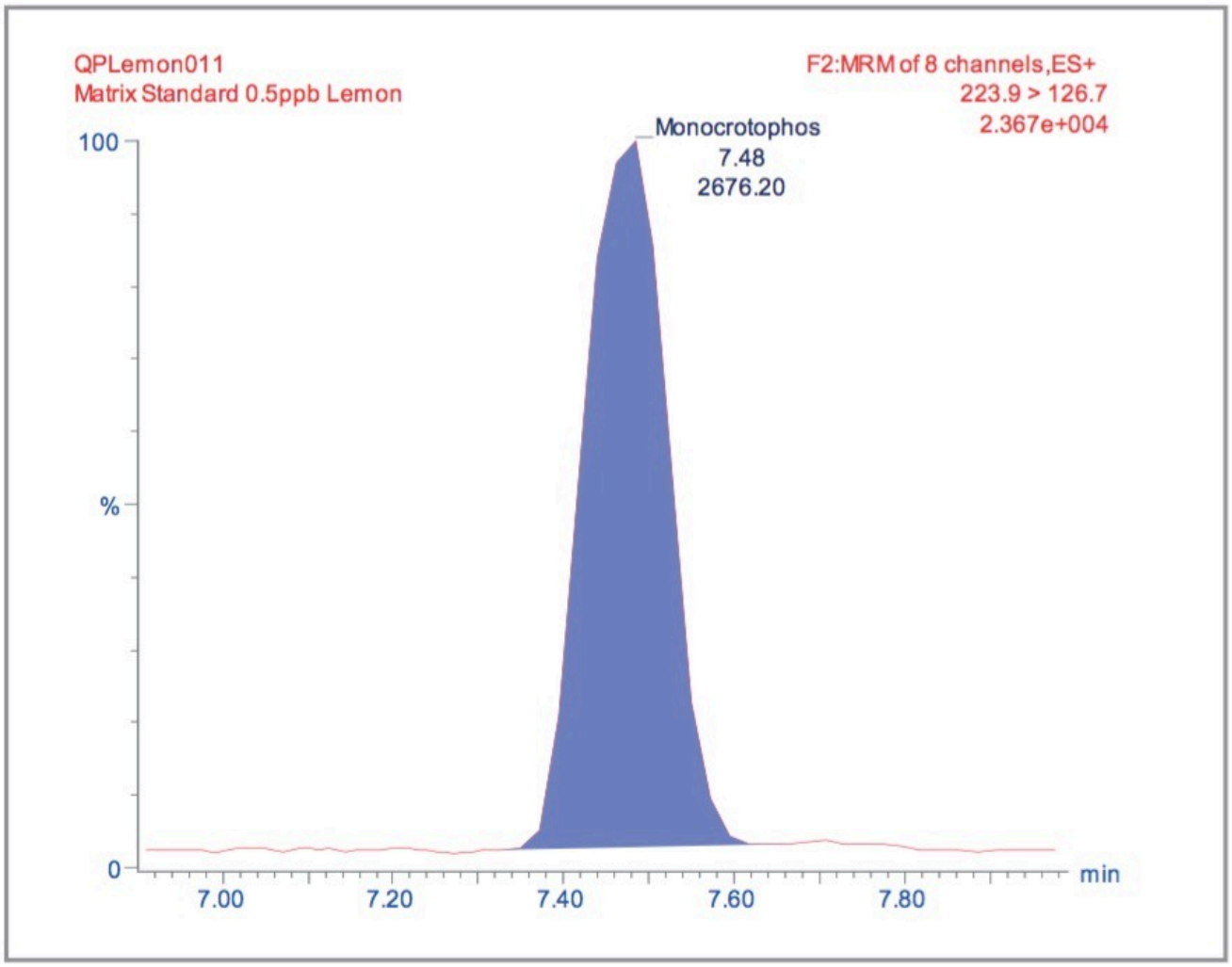

Figure 7. MRM chromatogram for monocrotophos in lemon at 0.5 µg/kg.

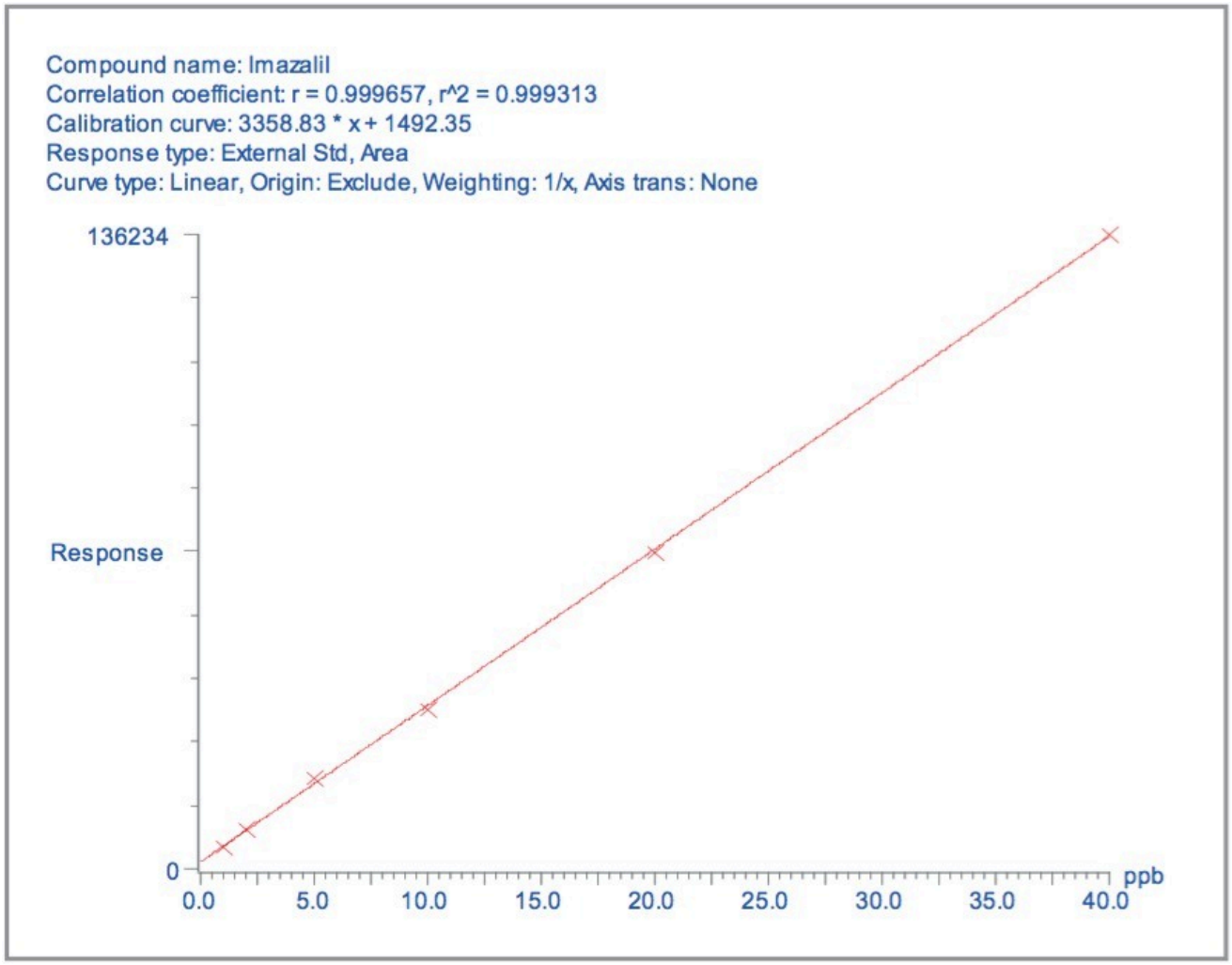

Figure 8. Calibration curve for imazalil in raisin.

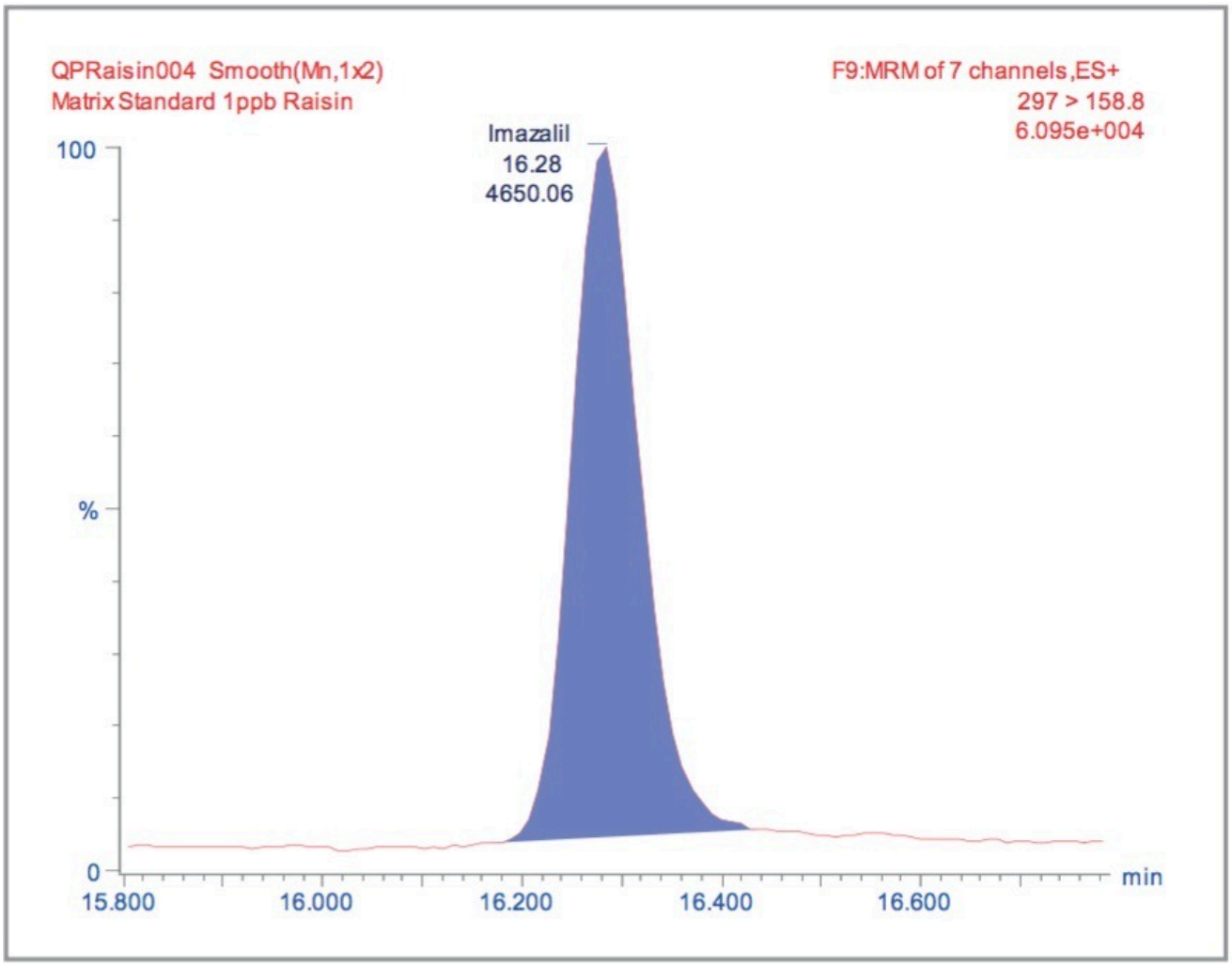

Figure 9. MRM chromatogram for imazalil in raisin at 1 µg/kg.

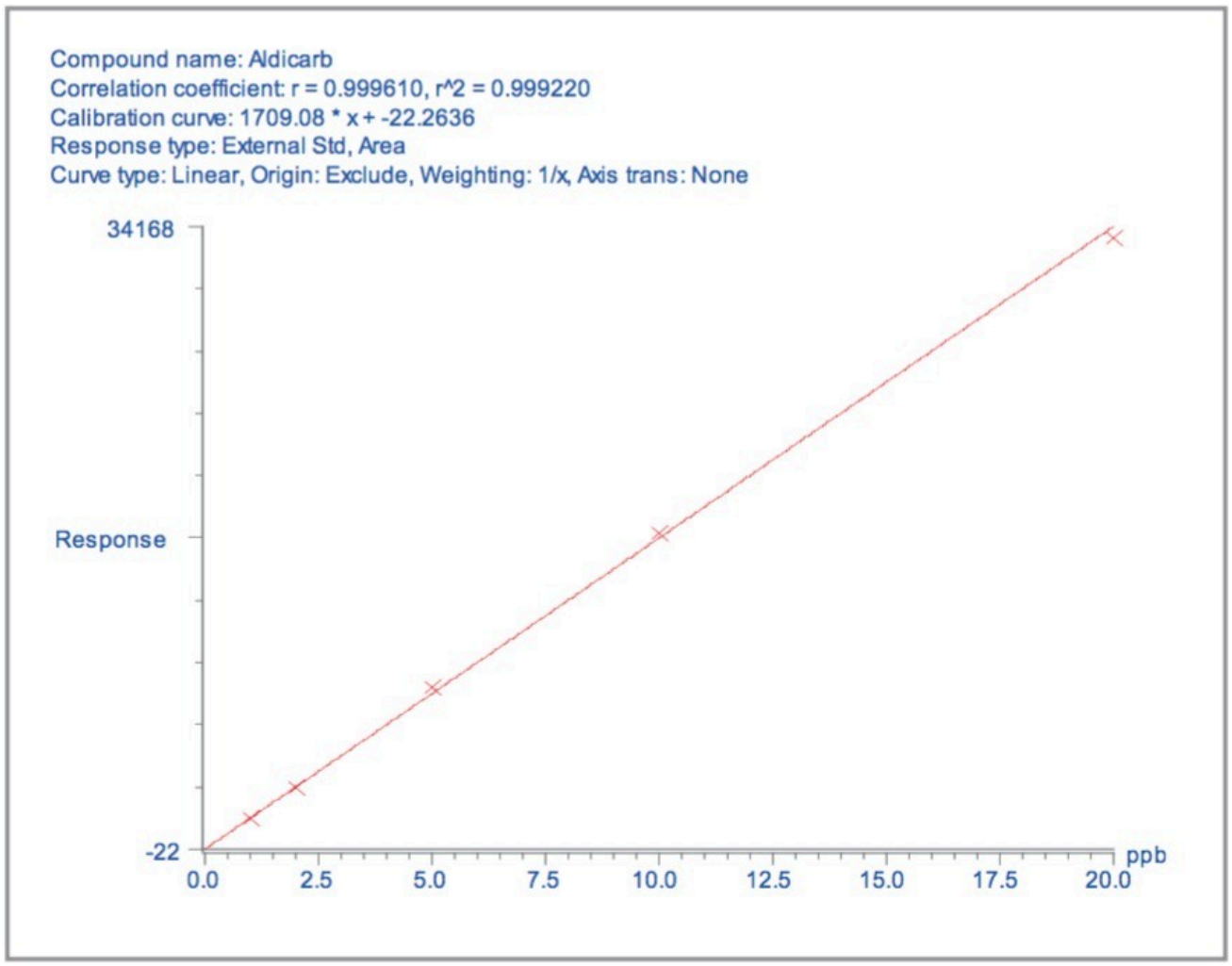

Figure 10. Calibration curve for aldicarb in wheat flour.

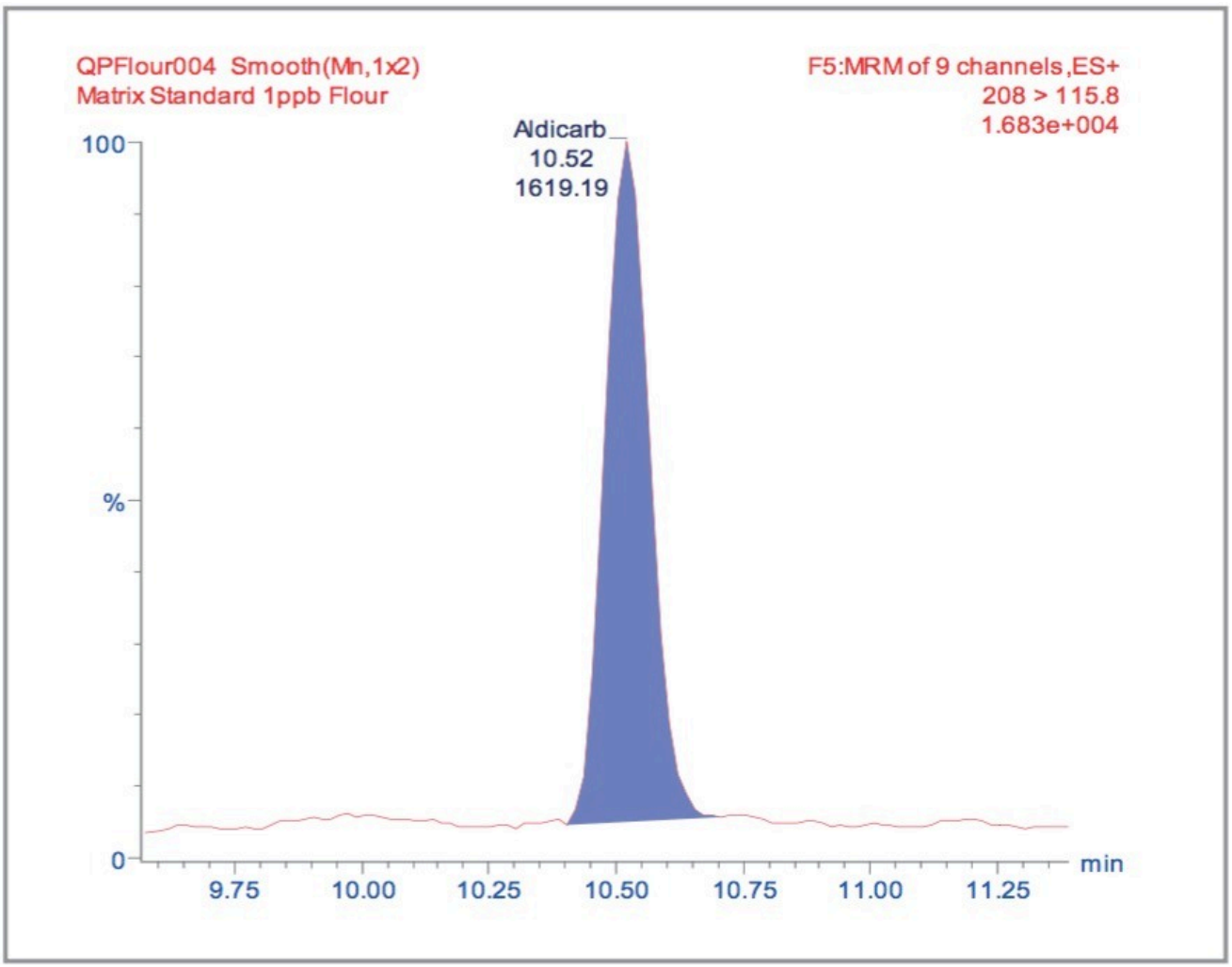

Figure 11. MRM chromatogram for aldicarb in wheat flour at 1 µg/kg.

For each matrix the lowest level calibration standard was used to estimate the Limits of Detection (LODs) for all 81 pesticides. The LODs are defined as the concentrations at which the S/N ratio is ≥3:1. The results are illustrated in Figure 12, with the least sensitive compounds in the most complex matrices giving LODs at least an order of magnitude less than those specified by the MRLs.

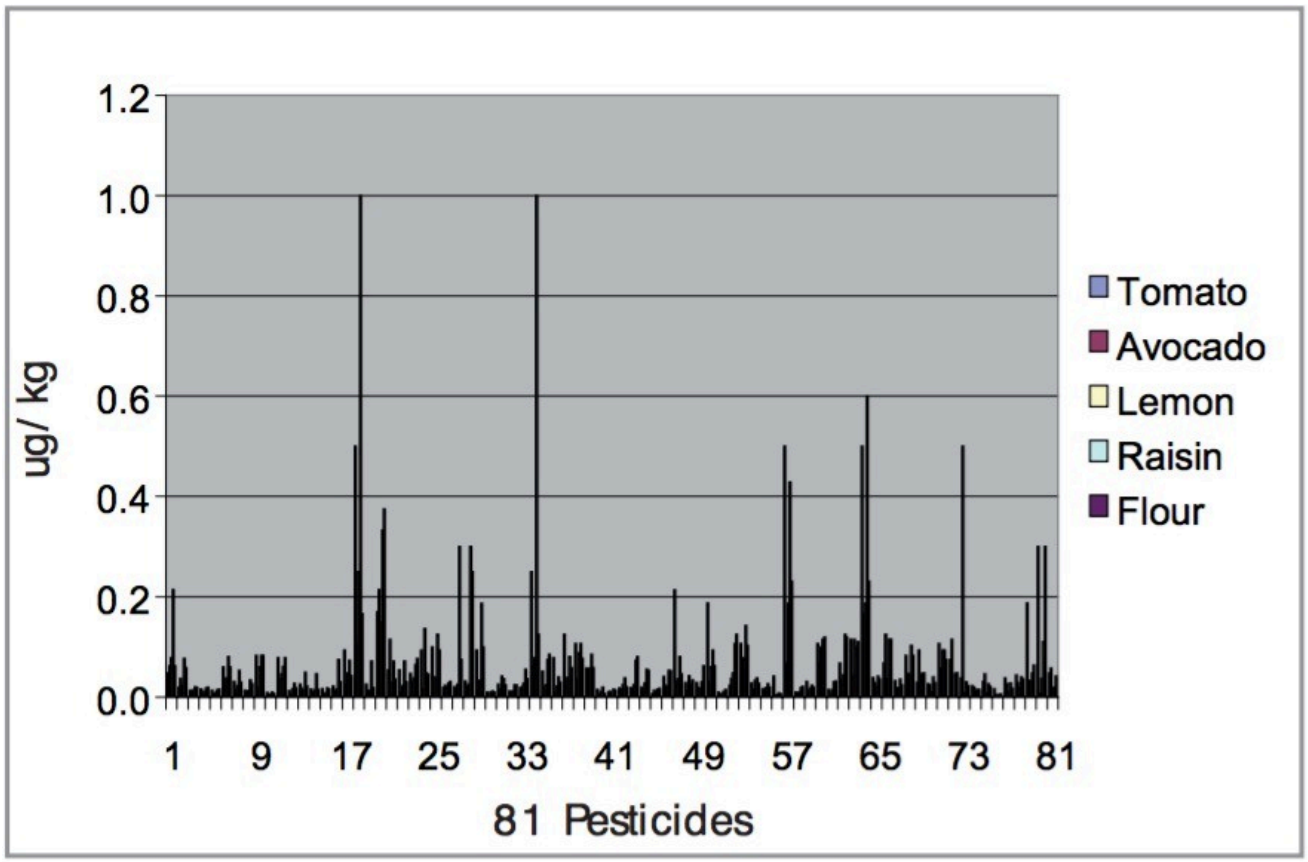

Figure 12. Estimated LODs for all 81 pesticides in all five matrices, µg/kg.

The S/N ratios for a 5 pg/µL standard in solvent were compared to a 50 pg/µL standard injected on the Quattro micro API using the conditions specified in Waters Application Note 720000686EN < https://www.waters.com/nextgen/us/en/library/application-notes/2003/a-multi-residue-lc-ms-ms-method-for-the-determination-of-81-pesticide-residues-in-fruit-and-vegetables-part-1-method-overview.html> . An example of the sensitivity difference between the Quattro micro API and the Quattro Premier for propoxur is illustrated in Figure 13. In this example, 500 pg was injected on column with the Quattro micro API, compared to 50 pg with the Quattro Premier – the S/N ratios are comparable. The average increase in sensitivity across all 81 pesticides between the two instruments was calculated to be 7.2.

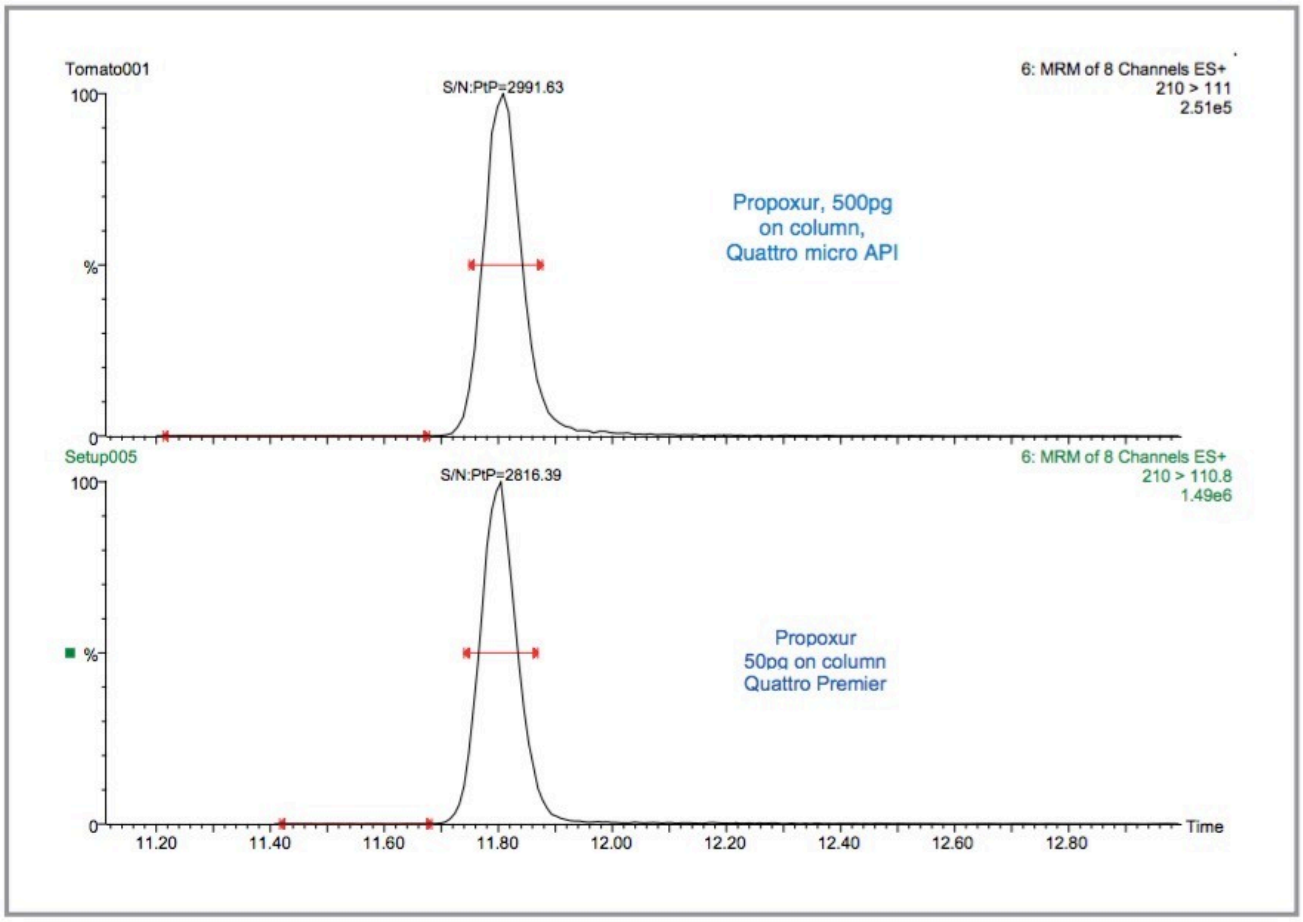

Figure 13. Sensitivity difference between the Quattro Micro API and the Quattro Premier for propoxur.

Four replicate batch analyzes were carried out on matrix-matched samples spiked with all 81 pesticides. The calibration curves were overlaid and a representative curve for methiocarb from the wheat flour matrix is illustrated in Figure 14. For the concentrations of 1, 2, 5, and 10 µg/kg, the mean values for all 81 pesticides were calculated to be 0.92, 1.97, 5.38, and 10.44 µg/kg, with percent relative standard deviations of 7.7, 5.2, 4.0, and 3.5, respectively. These results indicate that the method is reproducible at concentration levels that are significantly lower than the detection limits required by law.

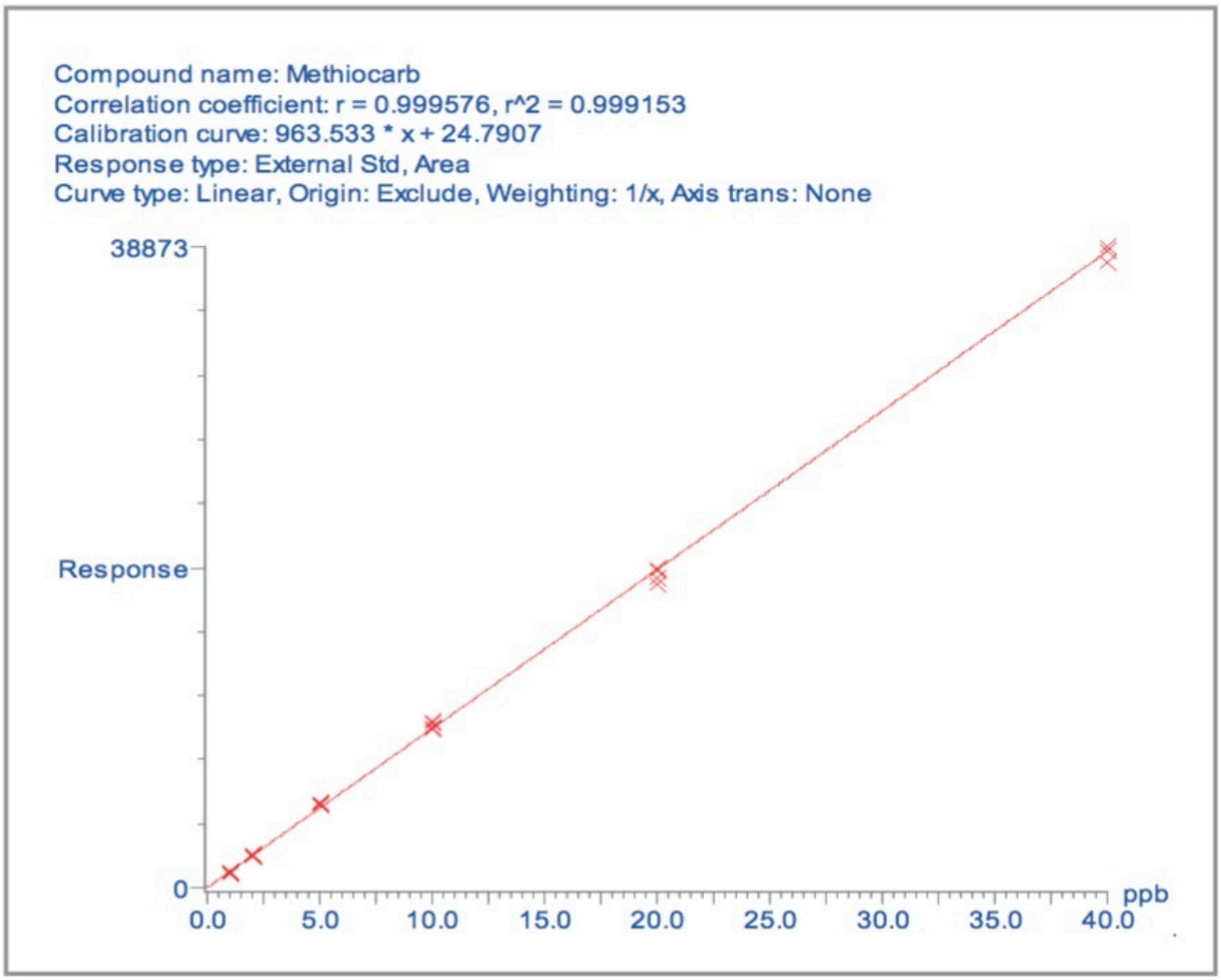

Figure 14. Overlaid calibration curves for methiocarb in wheat flour.

With the sensitivity and reproducibility achieved by the Quattro Premier, the results indicate either an increased number of pesticides could be analyzed in a single run, and/or confirmatory transitions for each pesticide could be added to the method. A suitable number of data points across any of the chromatographic peaks are still required for good quantification, but more pesticides or confirmatory transitions would lead to an increase in the number of MRM transitions in a function and the overall cycle time. To overcome this the dwell time of each transition and the interchannel delay must be decreased.

The Quattro Premier incorporates a T-Wave (Travelling Wave) collision cell that minimizes ion transit times and provides optimum performance for narrow chromatographic peaks or, in this case, multiple MRM transitions. This

T-Wave cell maintains signal intensity and minimizes interchannel crosstalk even with dwell and interchannel delay times of 5 ms.

To study this the original experiment, containing eleven MRM function windows, was changed to three and the inter-channel and inter-scan delays were standardised on 5 ms for each function. All the dwell times for all the pesticides were changed from those listed in Table 1 to 40, 30, 20, 10, and 5 ms. Therefore, each function contained approximately 27 MRM transitions so the overall cycle time was 1.22, 0.95, 0.68, 0.41, and 0.275 s, respectively. The results are illustrated in Figure 15 for thiacloprid where the overall cycle time has decreased by a factor of 4.4 but the peak area has only decreased by 5.5% between 40 and 5 ms. Similarly, the S/N ratios for the smoothed data between 40 and 5 ms have not significantly changed, as shown in Figure 16. This feature of the T-Wave collision cell allows fast switching between MRM transitions.

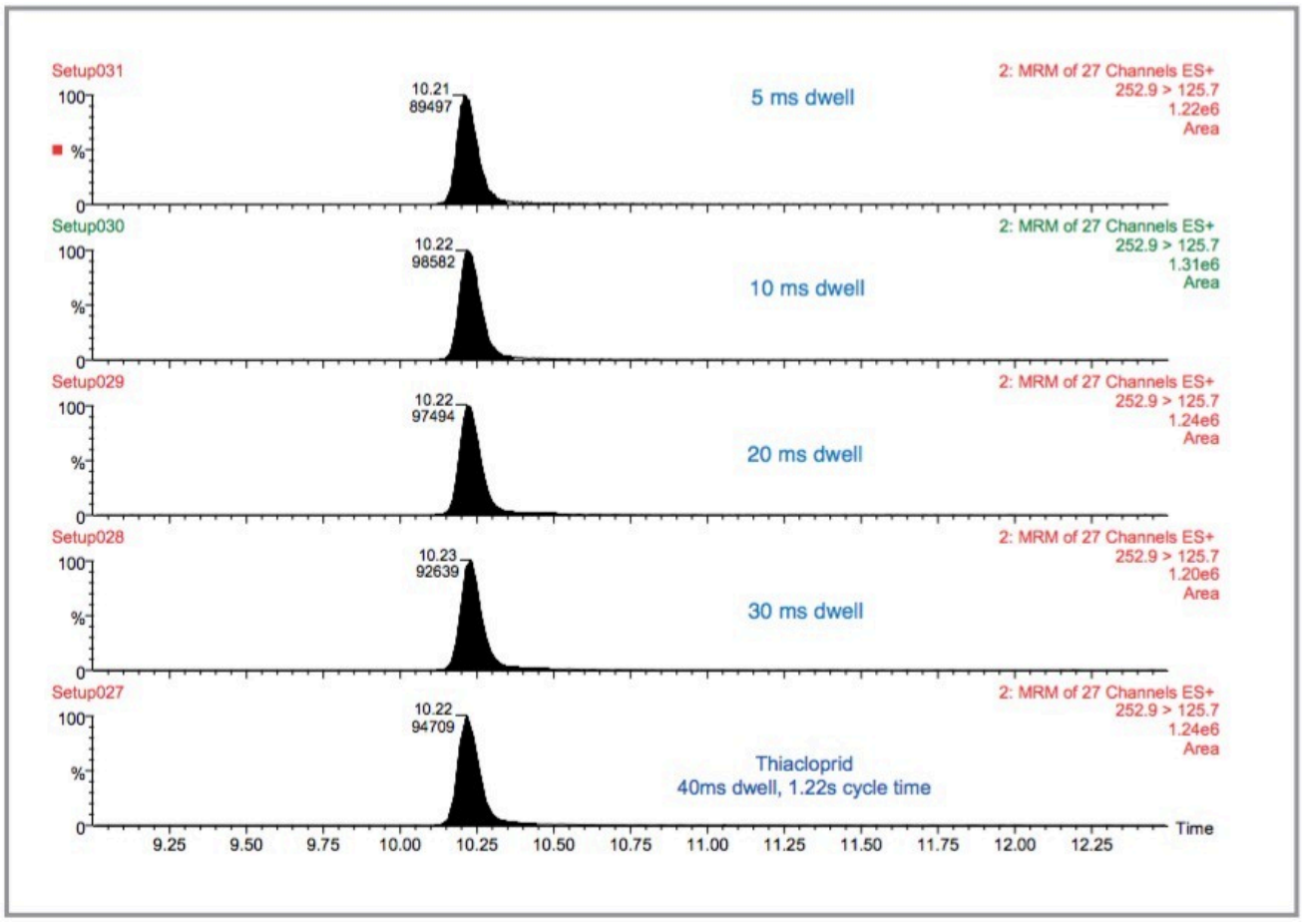

Figure 15. Peak area versus decreasing dwell time.

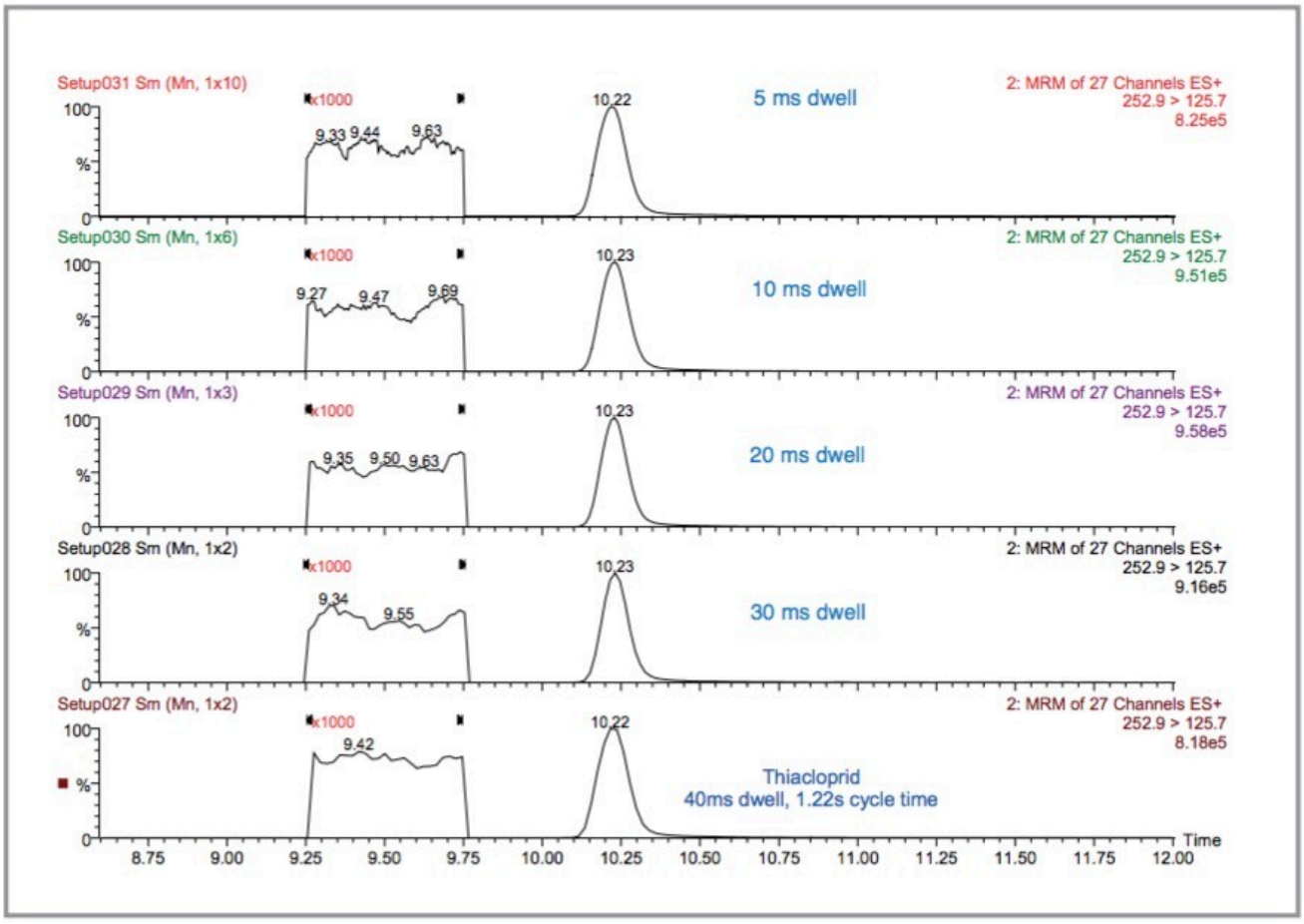

Figure 16. S/N of smoothed data versus decreasing dwell time.

Therefore, the method could be extended to further pesticides and/or confirmatory transitions for each.

Conclusion

A generic extraction and LC-MS/MS method, valid for a wide range of compound classes in a representative set of matrix types, was validated and shown to be suitable for the screening of 81 pesticide residue compounds in fruit and vegetables. The limits of detection achieved for the pesticides analyzed are well below that required for surveillance monitoring in the European Union. Therefore, the method is clearly extendable to greater numbers of pesticide targets and or confirmatory transitions in a single run.

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