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アプリケーションノート

A Multi-Residue LC-MS/MS Method for The Determination of 81 Pesticide Residues In Fruit and Vegetables: Part 1, Method Overview

Gordon Kearney, Lutz Alder, Anthony Newton, Jeannette Klein

Waters Corporation, Federal Institute for Risk Assessment

Abstract

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.

Introduction

There is currently a wide range of pesticides that may be applied to agricultural crops in order to control undesirable weeds, insects, mites and moulds. Over 800 such compounds exist and most countries have regulations governing their use. Produce that is to be used for human consumption must contain less than the statutory Maximum Residue Level (MRL) of a given pesticide. It is therefore necessary to monitor fruit, vegetable and cereal crops for the presence of these residues and, in order to maximize the efficient use of valuable analytical resources; it is desirable to test for as many compounds as possible during a single analysis.

Multi-residue methods are normally used to target members of a single class of compound. This enables any extraction and cleanup process to be optimized for a particular type of chemistry. The extraction and cleanup of a range of different compound types is less selective and results in a more complex extract, increasing the potential for matrix interference during the determinative step. Triple quadrupole mass spectrometry in the Multiple Reaction Monitoring (MRM) mode provides the analytical selectivity required for achieving low analyte detection levels in a complex sample matrix. Figure 1 shows the difference in selectivity between an MRM and a Single Ion Recording (SIR) type of experiment when analyzing flufenoxuron at a spiked concentration of 0.02 mg/kg in raisin matrix. The compound is poorly distinguished from the interfering noise when monitoring the ion at *m/z* 488.9 in an SIR experiment, whereas it can be clearly detected by monitoring the collision induced dissociation of *m/z* 488.9 to *m/z* 158.1 in an MRM analysis.

This Waters LC-MS/MS System combines the Waters Micromass Quattro micro Mass Spectrometer with the Waters Alliance 2795 Separations Module and 2996 Photodiode Array Detector using MassLynx Software.

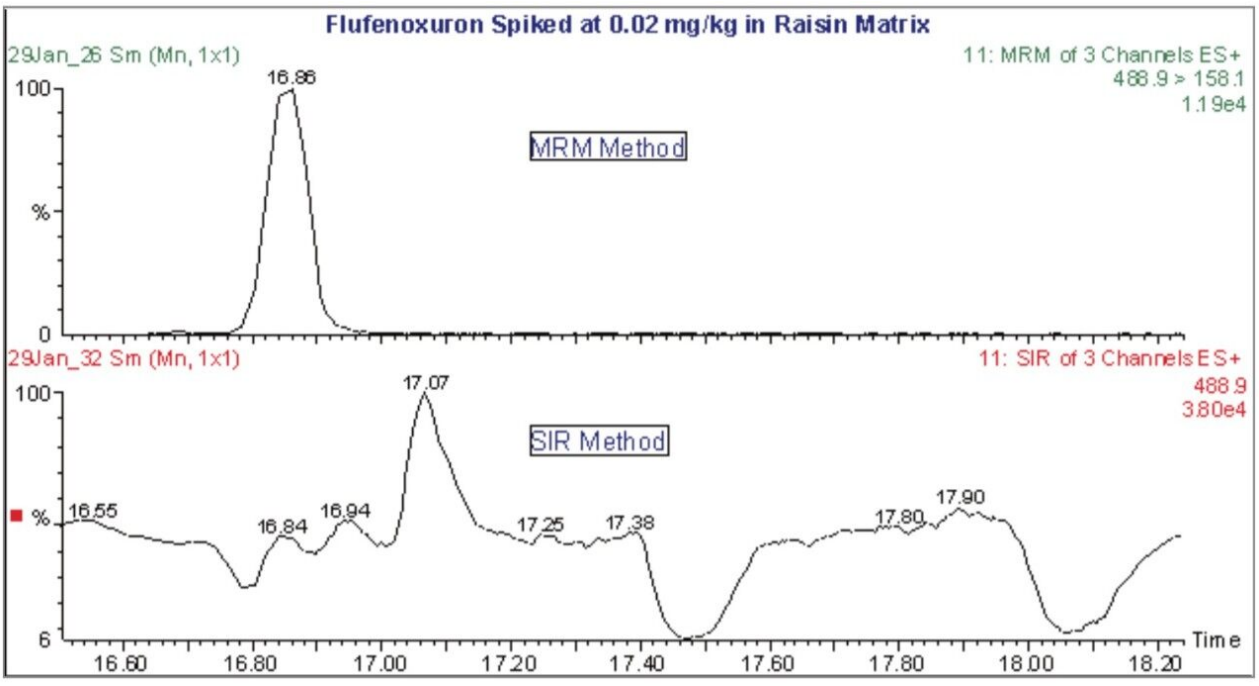

Figure 1. MRM v SIR analysis of flufenoxuron at 0.02 mg/kg in raisin matrix.

Using the MRM technique a method was developed for the quantification of 81 pesticides and pesticide

metabolites. A generic extraction and cleanup was performed. The method was validated for 5 commodities: - raisin, avocado, tomato, wheat flour, and lemon. These commodities represent high sugar (raisin), high fat (avocado) and high water (tomato), dry (wheat flour) and low pH (lemon) matrices. Target analytes include a number of compound classes such as carbamates, organophosphorous compounds, oximes and sulfonylureas.

Experimental

Extraction Procedure

The test sample is chopped avoiding loss of juice. An aliquot of 10 g is transferred into a blender cup. For dry sample materials like cereal grains, instant infant food or flour a homogenized portion of 5 g 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 raisins (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 min after the addition of water. After a further addition of 20 mL methanol the sample is blended for 2 min. The total volume of supernatant extract (taking into account the natural water content of the sample) is 30 mL. In the case of very turbid extracts an aliquot is centrifuged at about 3000 g.

Partition on an Extraction Cartridge

6 mL of the extract is mixed with 2 mL of a solution of sodium chloride (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 an extraction cartridge containing 5 mL of diatomaceous earth.

After a 5 min waiting period the cartridge is eluted with 16 mL of dichloromethane. The solvent of the collected eluate is gently evaporated. The dry residue is redissolved in 250 µL methanol with the help of an ultrasonic bath and further diluted with 1000 µL water. The resulting final extract contains the residues of 1 g normal or 0.5 g dry sample per millilitre. It is filtered through a 0.451 µm filter into a glass sample vial.

HPLC Method

HPLC:	Waters Alliance 2795 Separations Module
Mobile phase A:	MeOH/H_2O (1:4 v/v) + 5mM $CH_3CO_2NH_4$

Mobile phase B:	MeOH/H_2O (9:1 v/v) + 5mM $CH_3CO_2NH_4$
Column:	Waters Atlantis C_{18} 4.6 mm id 100 mm with 3 mm particle size
Flow:	1.0 mL/min
Injection volume:	20 mL

Approx 2:1 split of eluent before MS source

Gradient

Time 0	0% B
Time 15 mins	100% B
Time 29 mins	100% B
Time 29.1 mins	0% B
Time 40 mins	0% B

MS Method

A Waters Micromass Quattro micro API triple quadrupole mass spectrometer was operated in the positive ion electrospray mode. Nitrogen gas, at a flow rate of 850 L/hr and a temperature of 450 °C, was used for spray desolvation. The source block was maintained at 120 °C and the electrospray capillary voltage was 0.6 kV.

Figure 2 shows the distribution of MRM functions into windows based on analyte retention times. Such a system allows the flexible use of MRM dwell times, with less intense peaks having their S:N values increased by the use of longer dwell times whilst a short overall scan cycle time is maintained. For each pesticide residue the precursor and product ion *m/z* values, the cone and collision voltages and the retention and dwell times are given in Table 1. The relative response of each analyte, with reference to the response of Imazalil, is also shown. The relative response values were determined using the analysis of a solvent standard at the 50 pg/µL level.

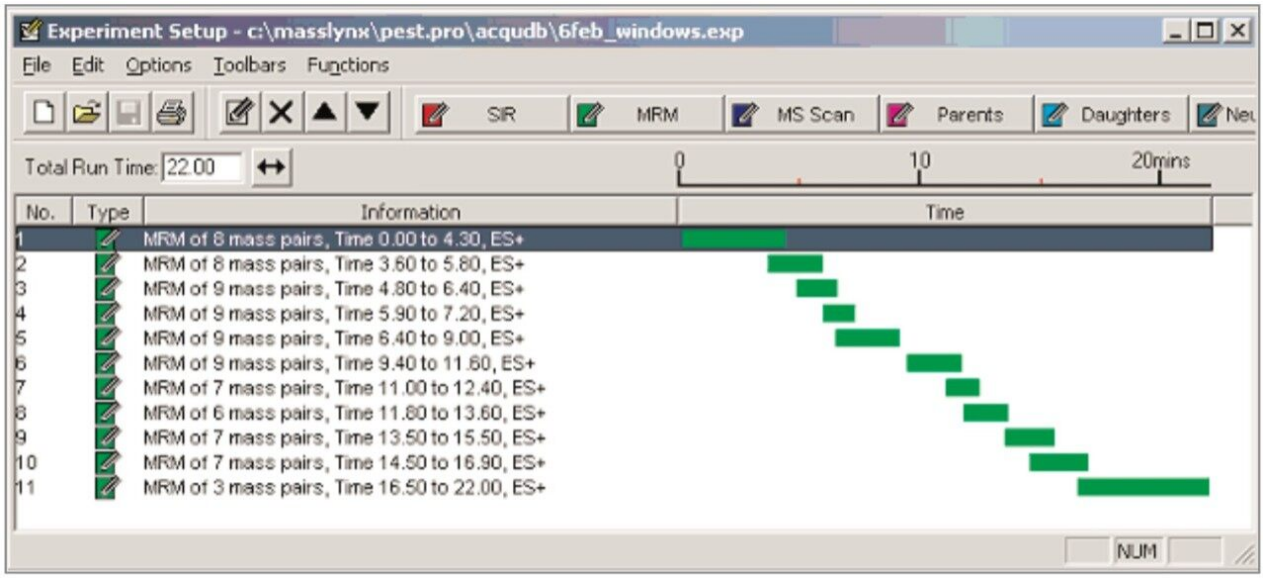

Figure 2. MRM functions arranged into time windows.

Pesticide Residue	Precursor Ion (m/z)	Product Ion (m/z)	Cone Voltage (V)	Collision Voltage (V)	Retention Time (min)	Dwell Time (ms)	Relative Response
Daminozid	161	143	20	12	1.19	50	0.89
Methamidophos	142	93.9	22	14	2.11	50	0.82
Acephate	184	143	15	8	2.51	50	2.29
Omethoat	214	183	20	12	2.91	50	2.19
Butoxycarboxim - sulfoxid	207	132	15	10	2.97	50	0.58
Aldicarb -sulfoxid	207	132	16	10	3.26	50	0.58
Butoxycarboxim	240	106	13	14	3.62	50	0.30
Aldoxycarb	240	86	15	22	3.78	50	0.53
Oxamyl	237	71.9	13	10	3.89	50	0.63
Propamocarb	189.1	102	25	17	4.06	30	10.98
Oxydemeton-methyl	246.9	169	20	13	4.24	30	2.43
Methomyl	163	87.9	12	8	4.66	50	0.68
Demeton-S-methyl -sulfon	263	169	25	16	4.72	30	1.88
Quinmerac	222	141.1	20	33	4.95	50	0.57
Pymetrozin	218	105	25	17	5.06	50	1.42
Nicosulfuron	411	182.1	25	20	5.12	200	0.30
Monocrotophos	224	127	20	15	5.23	30	1.50
6-chloro-4-hydroxy-3-phenyl pyridazin	207	76.9	35	30	5.58	200	0.90
Amidosulfuron	370	261	20	14	5.58	50	0.11
Thiofanox - sulfoxid	252.1	103.9	9	12	5.81	50	0.18
Ethiofencarbsulfon	275	107	13	18	5.81	50	0.97
Metsulfuron - methyl	381.9	167.1	22	15	5.89	30	0.31
Ethiofencarbsulfoxid	242	107	14	18	5.94	30	1.86
Thifensulfuron - methyl	387.9	167.1	22	14	5.94	50	0.31
Rimsulfuron	431.9	182.1	25	20	6.17	200	0.12
Imidacloprid	256	209	20	16	6.23	50	0.30
Thiofanox-sulfon	268.1	76	10	12	6.4	200	0.22
Clethodim-imin -sulfon	302.1	98	35	30	6.66	50	0.52
5-Hydroxy - clethodim -sulfon	408	204.1	20	16	6.75	200	0.19
Chlorsulfuron	357.9	141.1	25	16	6.79	30	0.19
Vamidothion	288	146.1	16	12	6.81	30	3.85
Clethodim-imin -sulfoxid	286.1	208.1	20	17	6.85	50	0.82
Carbofuran-3-hydroxy	220	163	25	10	6.91	30	2.62
Cinosulfuron	414	183.1	25	18	6.91	50	0.63
Metamitron	203.1	175.1	28	16	6.92	100	0.80
Dimethoat	229.9	124.9	15	20	7.09	50	0.80
Hazasulfuron	408	182.1	25	20	7.58	200	0.59
Triasulfuron	402	167.1	25	17	7.71	30	0.31
Clethodim-sulfon	392	300.1	25	13	7.95	50	0.35
Clethodim-sulfoxid	376	206.1	22	17	7.97	50	0.62
Thiacloprid	253	126	25	20	8.14	30	1.64
Carbendazim	192	160.1	25	18	8.38	200	6.84
Butocarboxim	213	74.9	25	14	8.53	50	0.77
Aldicarb	208.1	116	10	6	8.65	200	0.04
Propoxur	210.1	111	13	13	9.88	50	1.45
Carbofuran	222.1	165.1	20	12	10.06	50	4.46
Bendiocarb	224.1	109	18	18	10.06	50	1.23
Prosulfuron	420	141.1	25	20	10.45	50	0.63
Carbaryl	202.1	145.1	20	10	10.74	30	1.74
Ethiofencarb	226	107	15	15	11.01	50	1.34
Triflusulfuron - methyl	493	264.1	28	20	11.19	50	0.66
Pirimicarb	239.1	71.9	28	18	11.37	30	3.81
Thiodicarb	354.9	87.9	15	12	11.56	50	0.56
Atrazin	216	174.1	30	17	11.74	50	2.31
Metalaxyl	280.1	220.1	20	14	11.87	50	3.75
Isoproturon	207.1	71.9	25	15	11.93	30	3.02
Isoxaflutole	377	251	13	18	11.93	300	0.07
3,4,5 Trimethacarb	194.1	137.1	15	10	12.13	30	3.62
Diuron	233	71.9	25	18	12.19	50	1.10
Clethodim	360	164.1	20	19	12.83	50	1.35
Azoxystrobin	404	372	20	13	12.99	50	7.66
Pyrimethanil	200.1	107	45	25	13.12	50	0.28
Linuron	248.9	160	25	18	13.12	50	0.51
Methiocarb	243	121.1	10	25	13.18	50	0.29
Promocarb	208.1	151.1	18	9	13.38	50	1.94
Fenhexamid	302	97.1	35	25	14	50	1.35
Metolachlor	284	176.1	20	25	14.43	50	2.40
Fenoxycarb	302.1	87.9	20	20	14.61	50	2.01
Tebufenozid	353.1	133.1	14	18	14.61	50	0.64
Tebuconazol	308.1	69.9	30	18	14.98	50	1.64
Cyprodinil	226	93	40	33	15.13	50	0.61
Imazalil	297	159	30	18	15.16	200	1.00
Haloxyfop - methyl	376	316	30	16	15.74	50	3.75
Spiroxamine	298.2	144.1	30	20	16.03	50	7.95
Haloxyfop - ethoxyethyl	434	316	25	20	16.23	50	4.57
Fluazifop -P-butyl	384.1	282.1	30	22	16.29	50	5.18
Quizalofop-ethyl	373	299.1	30	20	16.35	50	3.65
Furathiocarb	383.1	195.1	20	16	16.48	50	2.89
Flufenoxur on	488.9	158.1	25	16	17.22	90	1.10
Pyridate	379.1	207.1	20	16	19.43	90	1.55
Fenpropimorph	304.2	147.1	40	30	19.74	90	3.11

Table 1. MRM method parameters.

Results and Discussion

A chromatogram, showing the results from the analysis of a tomato extract spiked at 0.01 mg/kg, is shown in Figure 3.

Figure 3. A chromatogram corresponding to 81 MRM functions over 11 time windows. Tomato extract spiked at 0.01 mg/kg.

For each of the five crop types, matrix-matched standards were generated at the 5, 20, 40, 60, 80, and 100 pg/µL levels for all analytes. These correspond to 0.005, 0.02, 0.04, 0.06, 0.08, and 0.1 mg/kg in tomato, avocado and lemon and to 0.01, 0.04, 0.08, 0.12, 0.16, and 0.2 mg/kg in raisin and wheat flour. Figures 4, 6, 8, 10, and 12 show representative calibration graphs for butocarboxim (Oxime Carbamate Insecticide) in tomato, pyrimethanil (Pyrimidine Fungicide) in raisin, promecarb (Phenyl Methylcarbamate Insecticide) in avocado, cyprodinil (Pyrimidine Fungicide) in wheat flour and vamidothion (Organothiophosphate Insecticide) in lemon respectively. Figures 5, 7, 9, 11, and 13 show chromatograms for these compounds at

the lowest calibrated level of 0.005 mg/kg in tomato, lemon and avocado and 0.01 mg/kg in raisin and wheat flour.

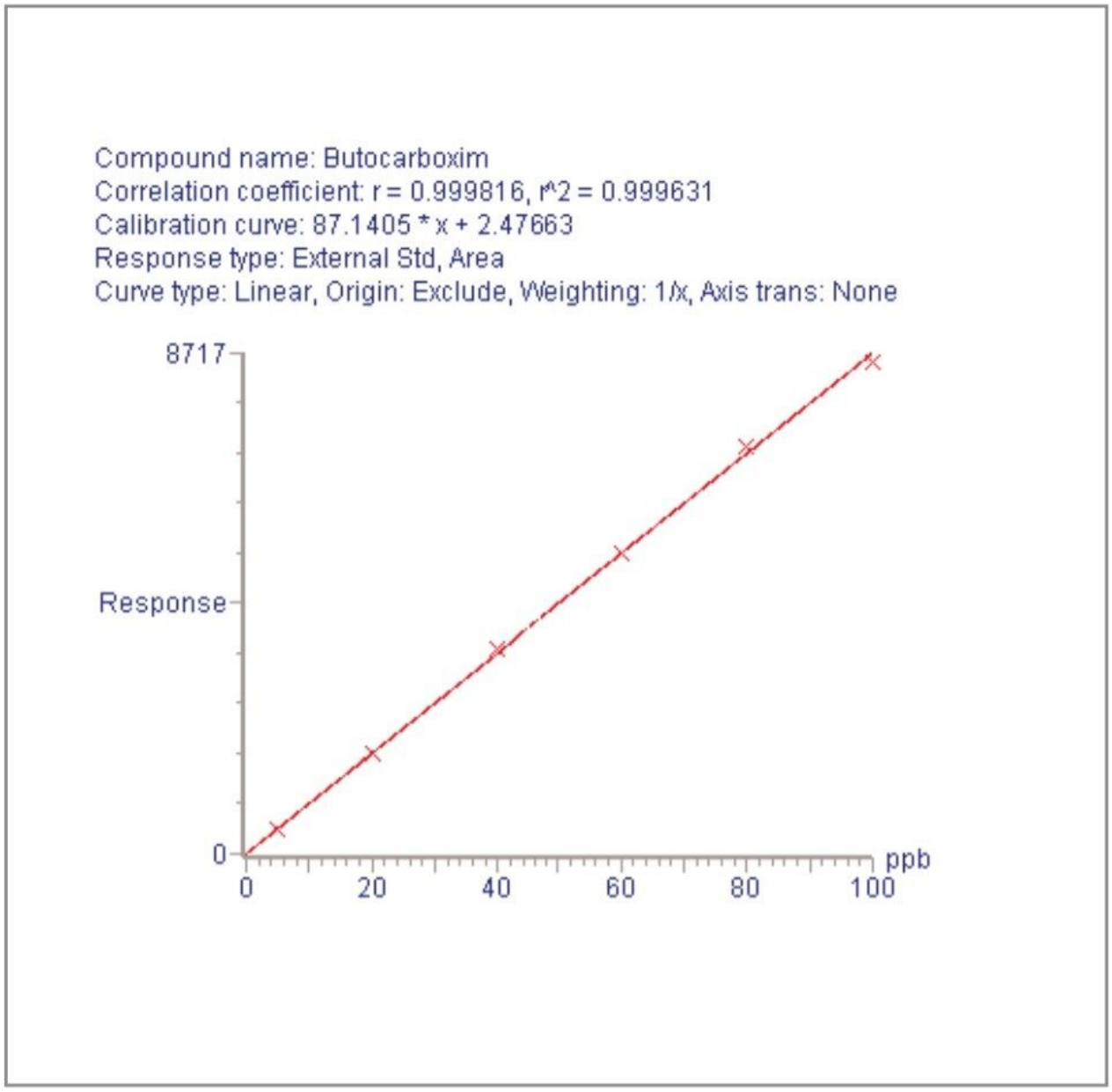

Figure 4. Calibration graph for butocarboxim in tomato matrix.

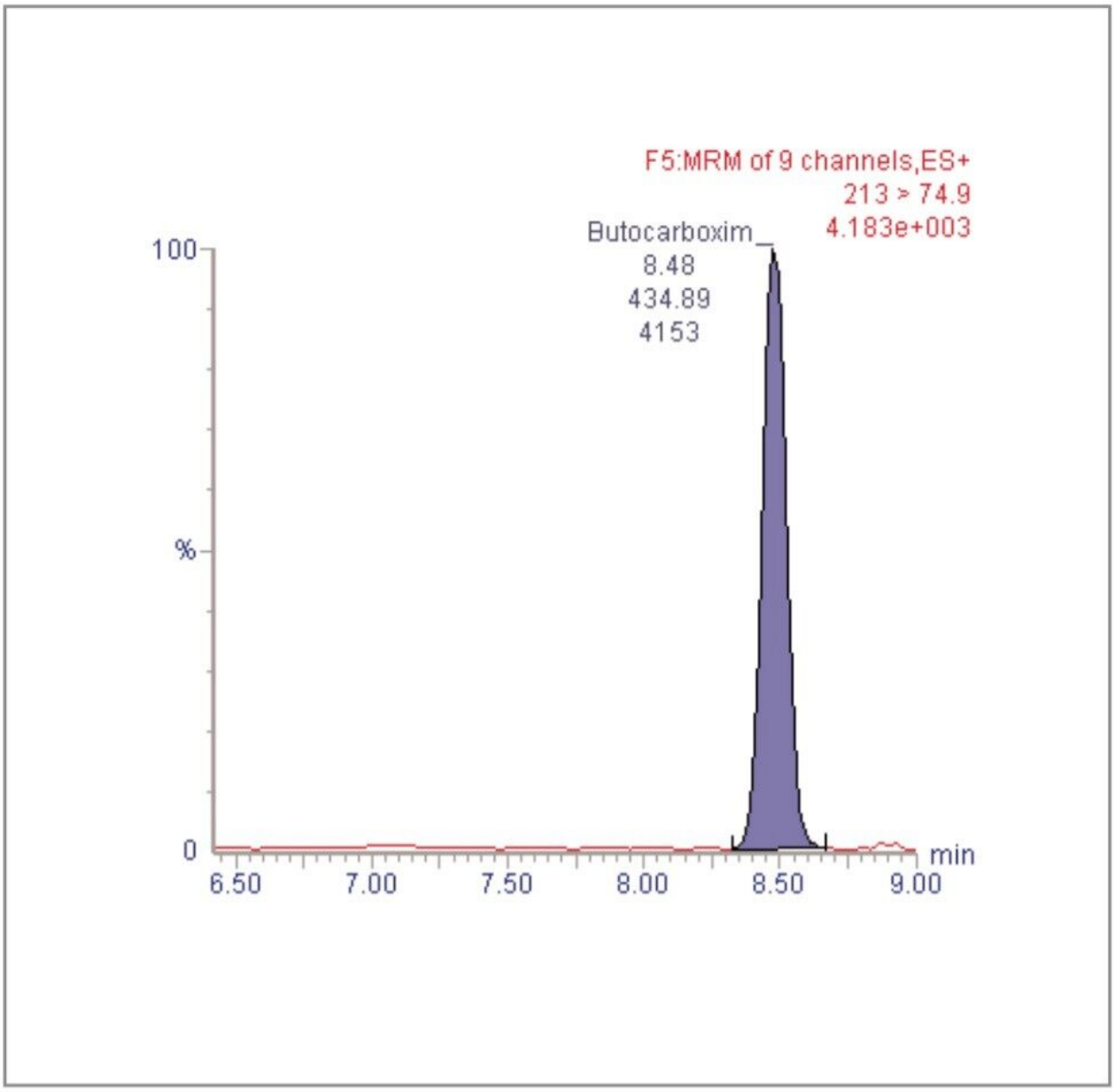

Figure 5. Chromatogram for butocarboxim at 0.005 mg/kg in tomato matrix.

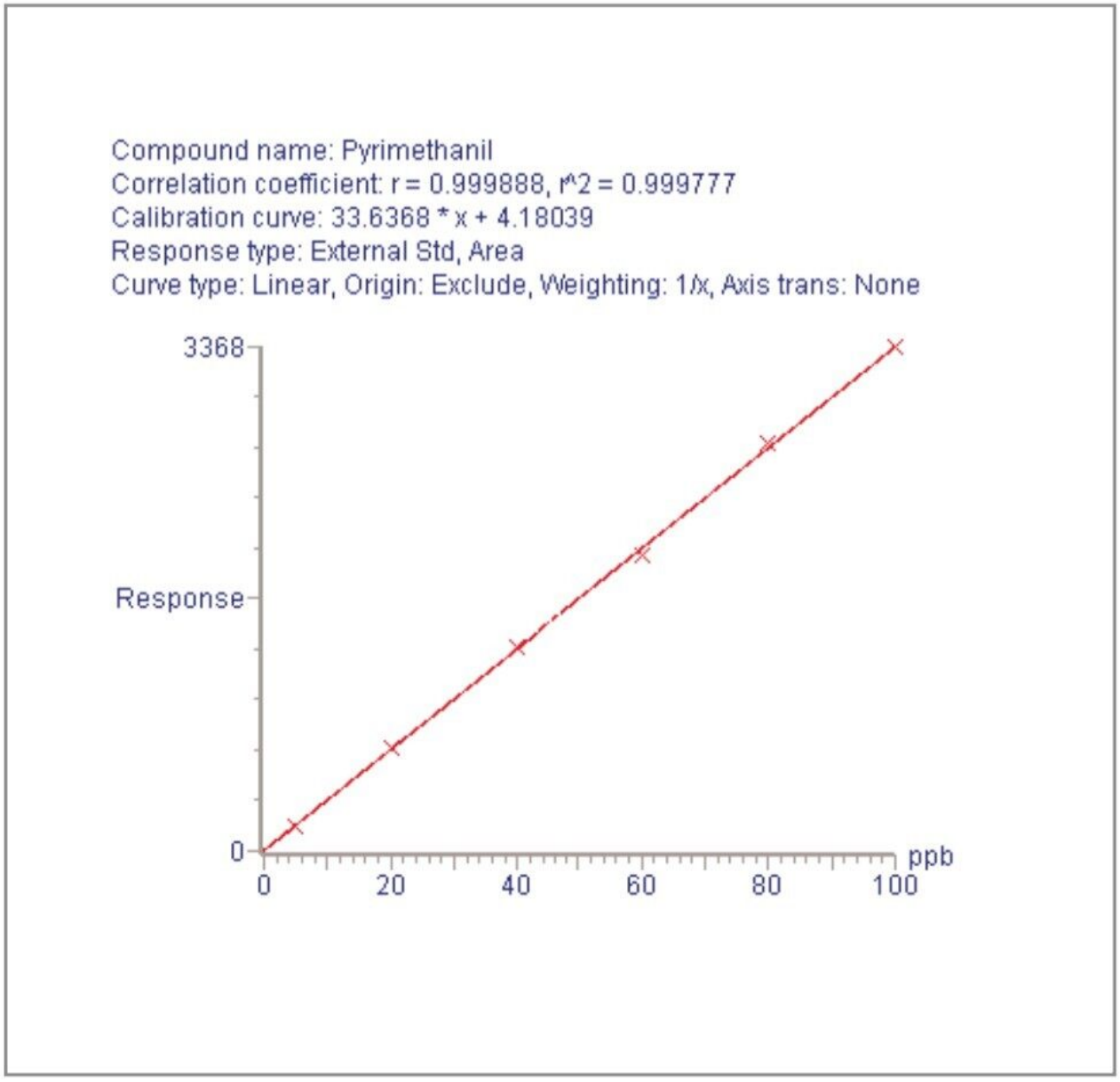

Figure 6. Calibration graph for pyrimethanil in raisin matrix.

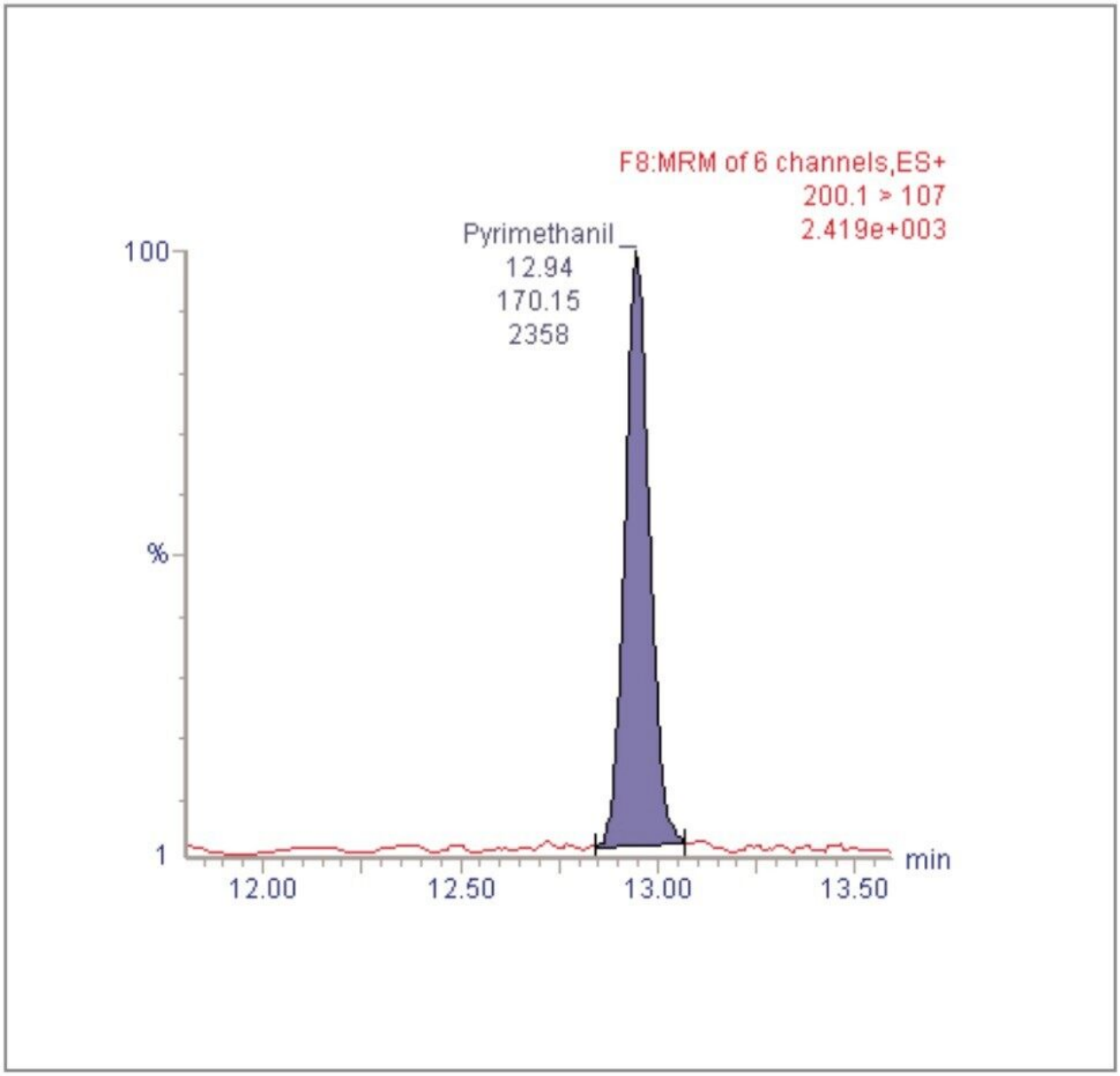

Figure 7. Chromatogram for pyrimethanil at 0.01 mg/kg in raisin matrix.

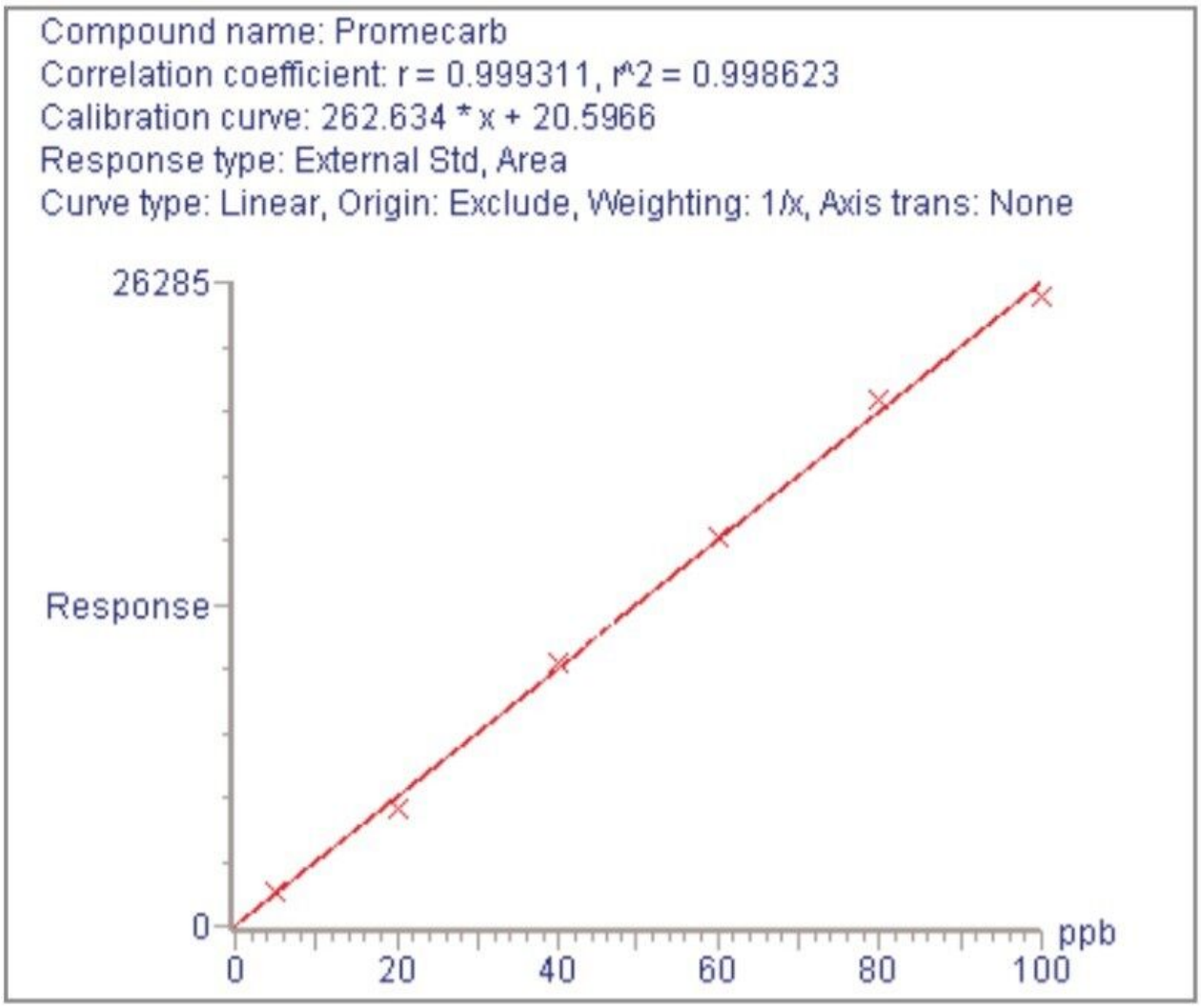

Figure 8. Calibration graph for promecarb in avocado matrix.

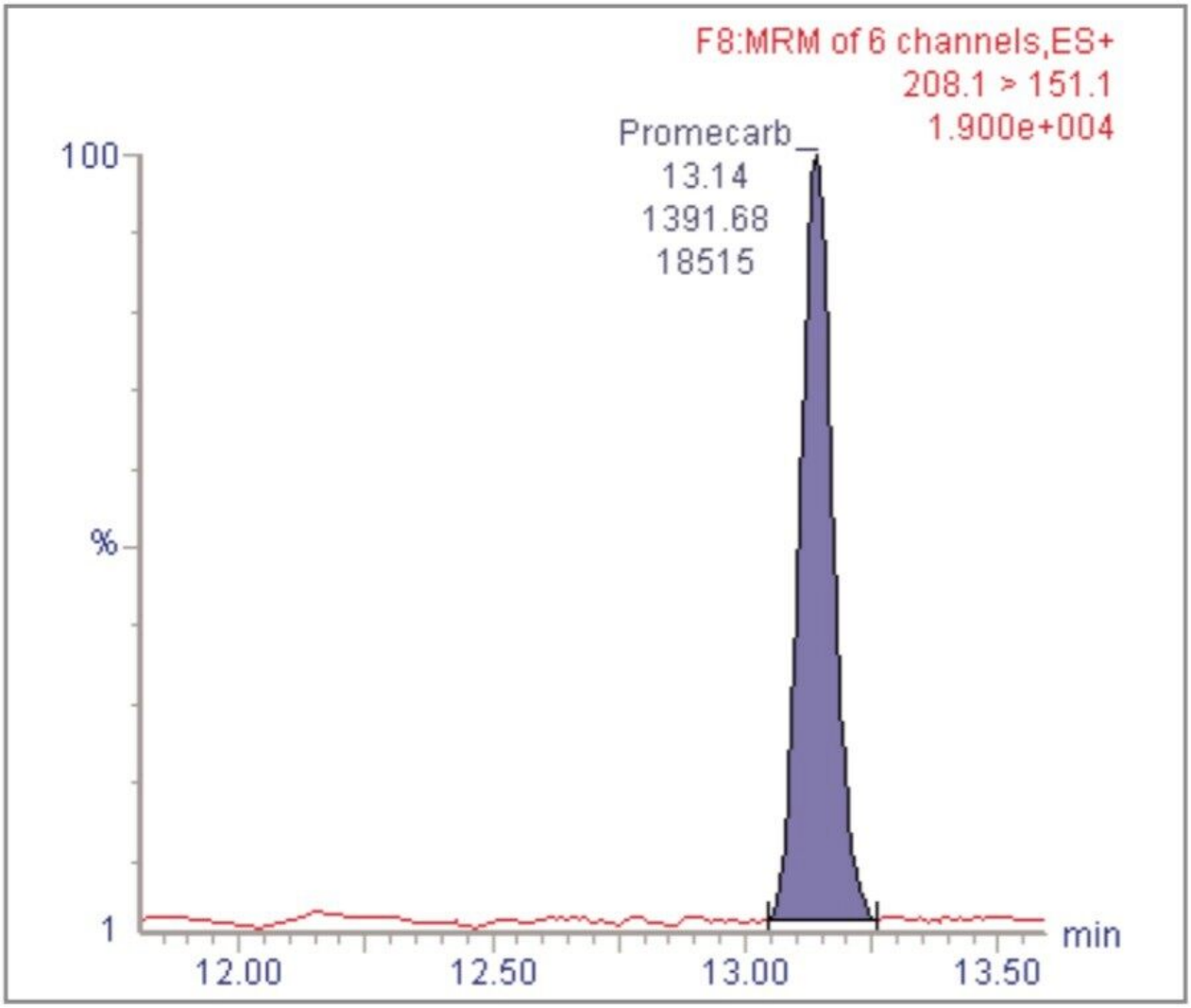

Figure 9. Chromatogram for promecarb at 0.005 mg/kg in avocado matrix.

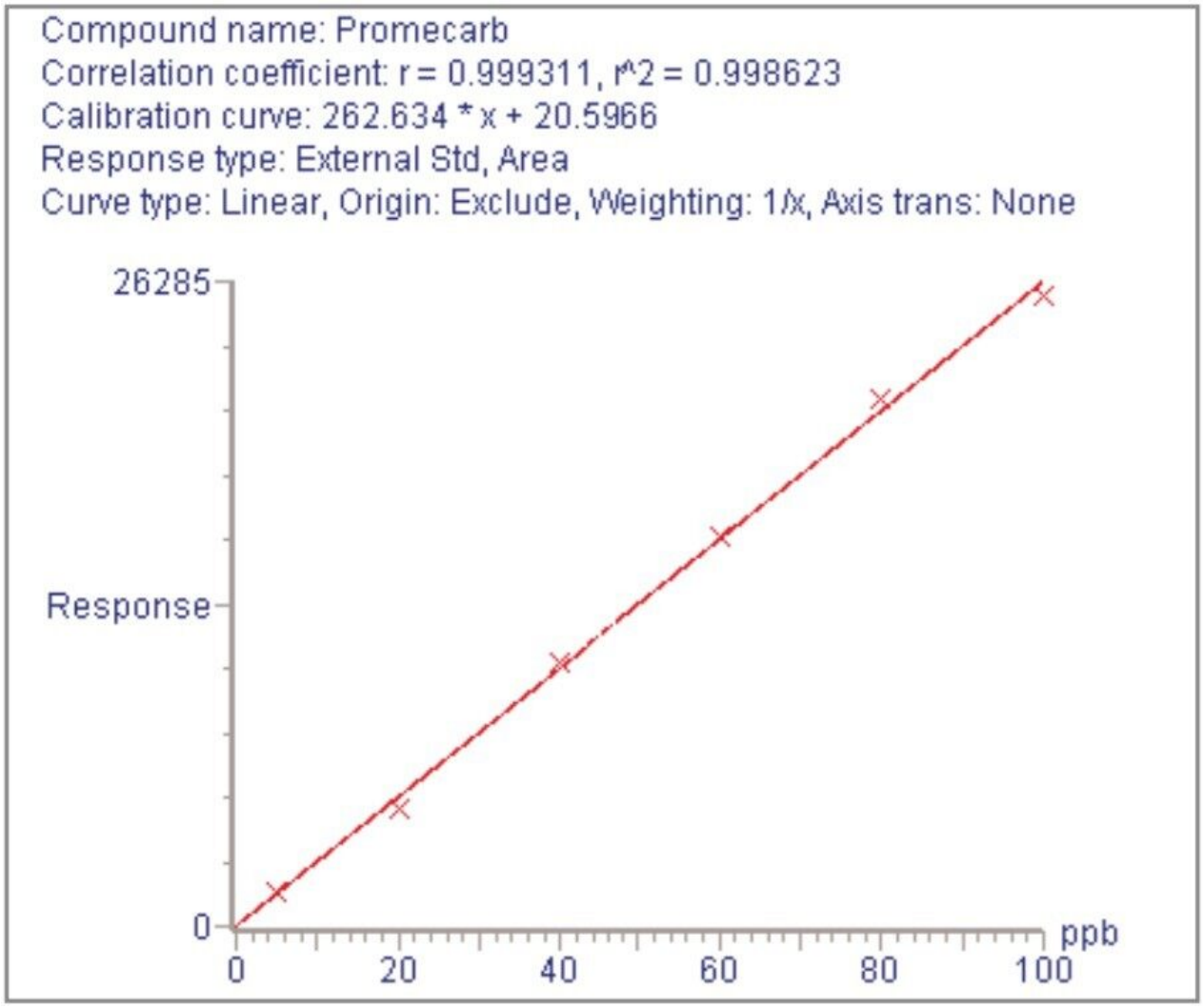

Figure 10. Calibration graph for cyprodinil in wheat flour matrix.

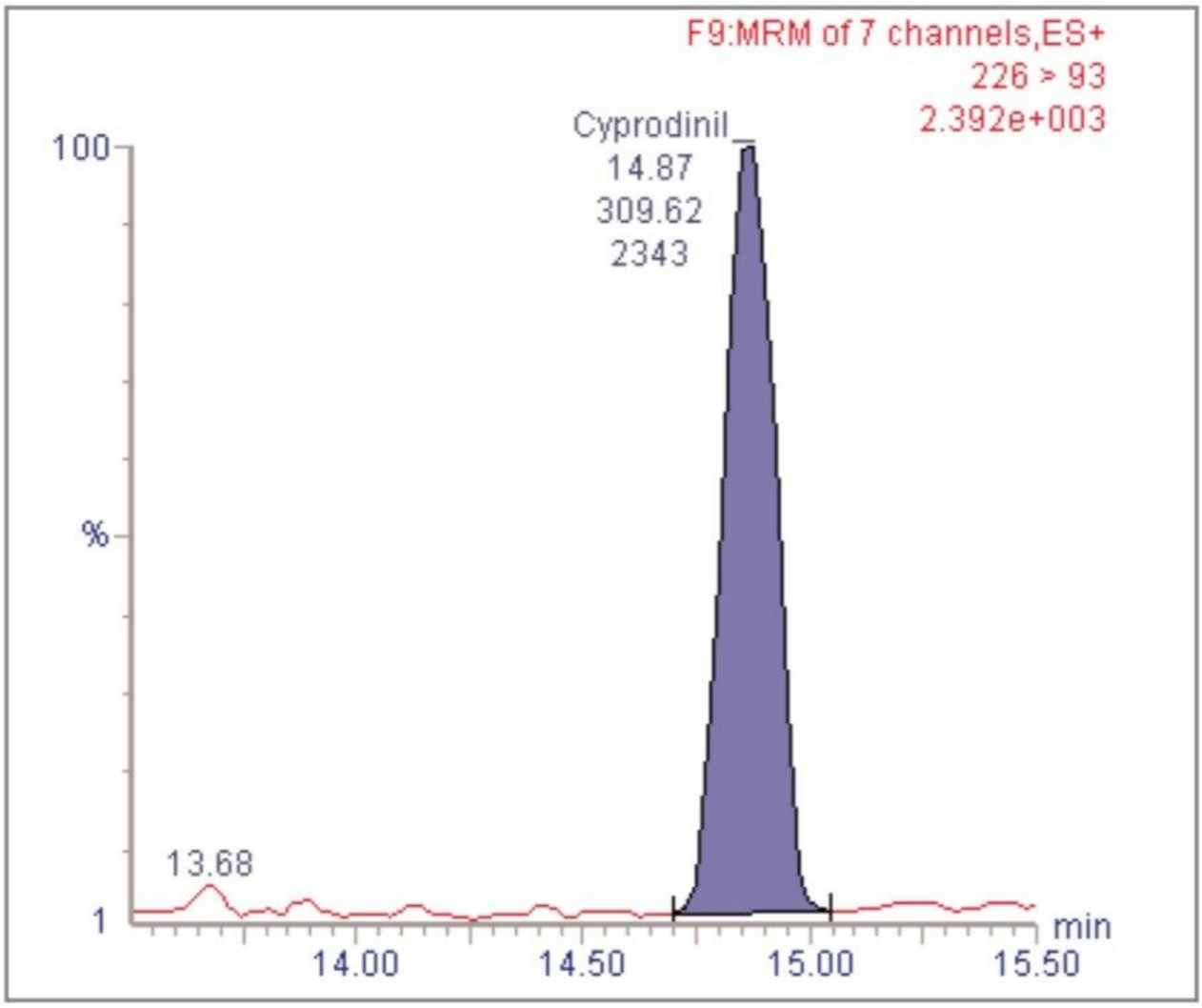

Figure 11. Chromatogram for cyprodinil at 0.01 mg/kg in wheat flour matrix.

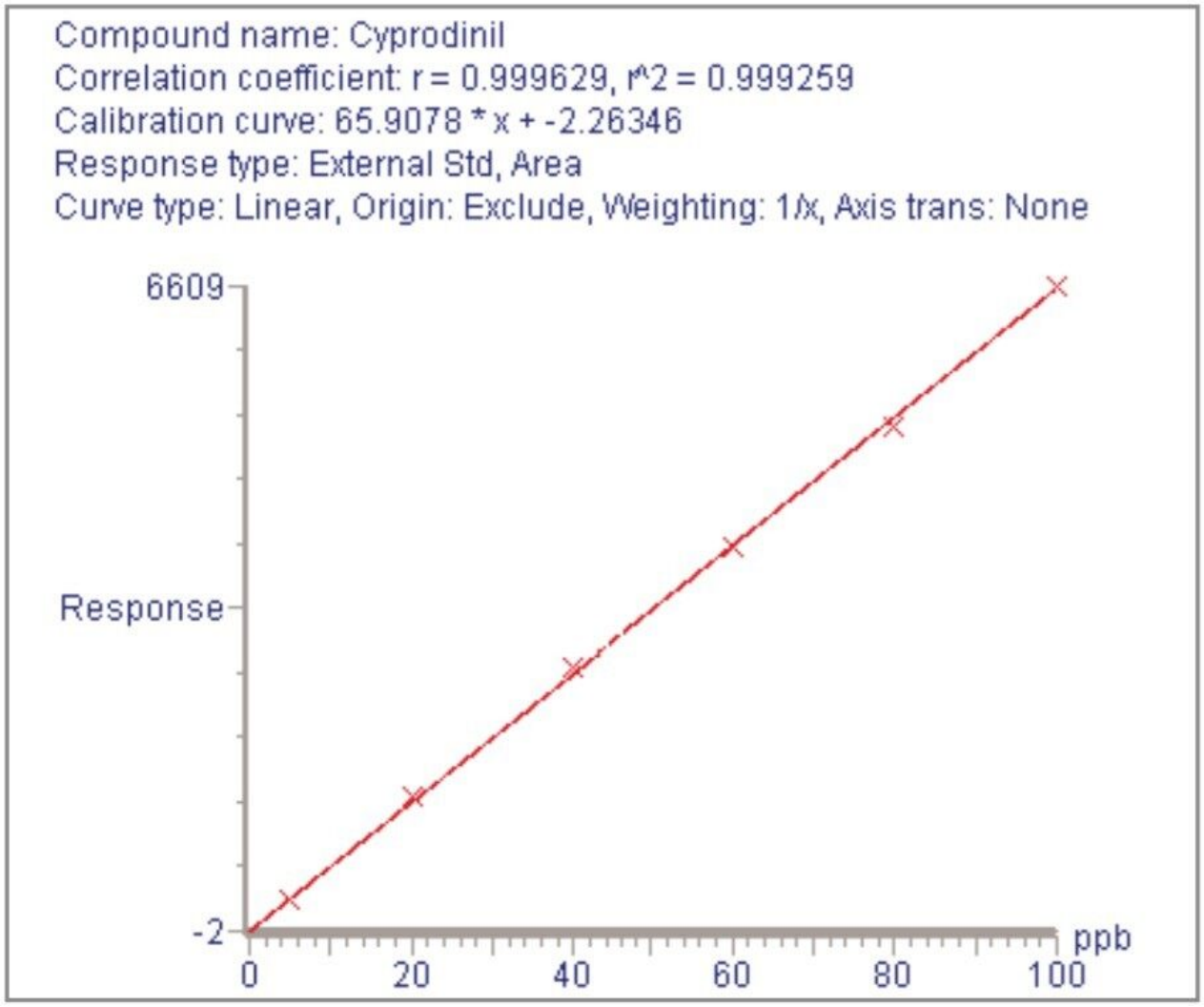

Figure 12. Calibration graph for vamidothion in lemon matrix.

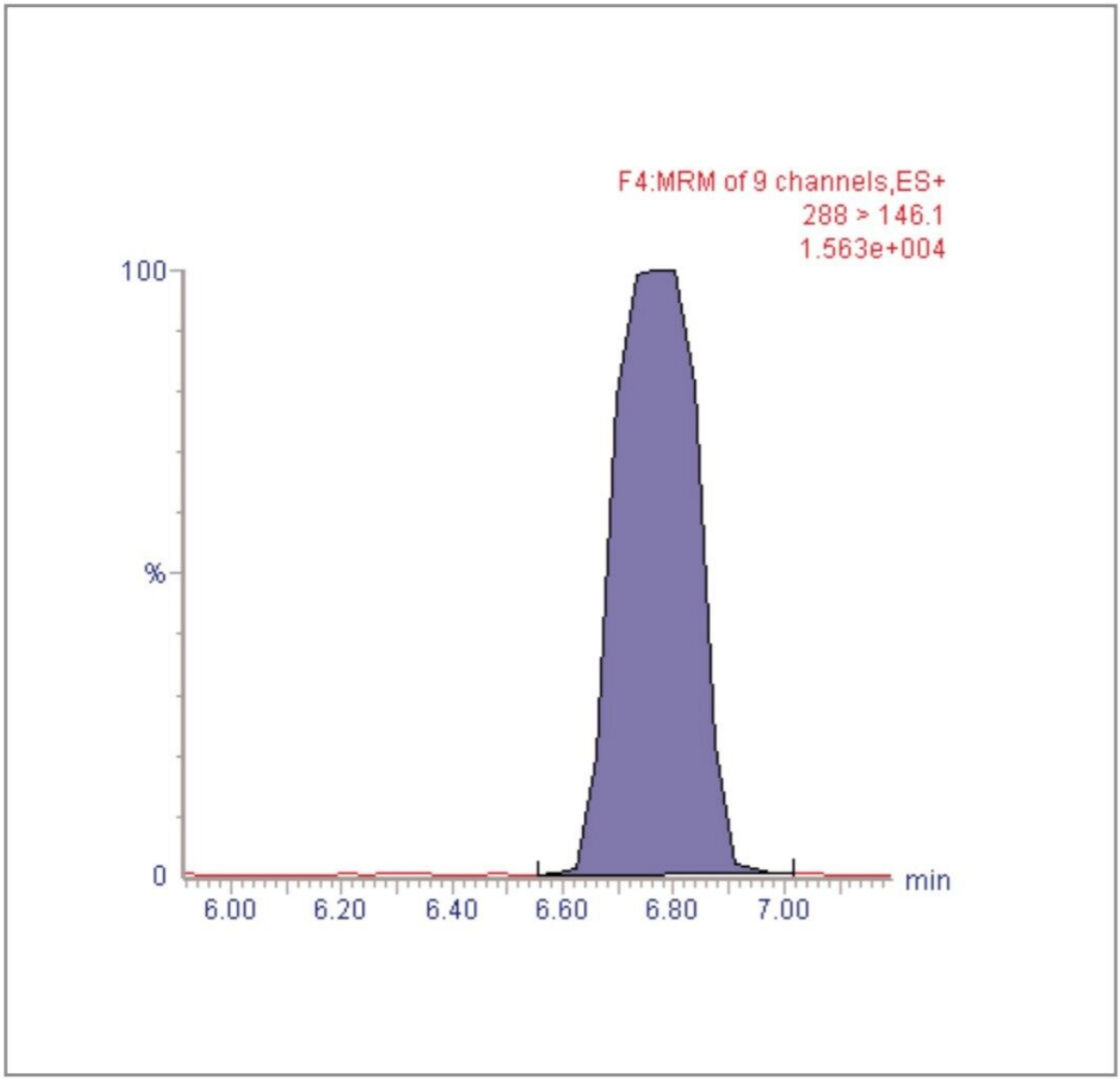

Figure 13. Chromatogram for vamidothion at 0.005 mg/kg in lemon matrix.

Five replicate analyses were performed on extracts, from each matrix type, spiked at levels equivalent to 0.01 and 0.05 mg/kg for tomato, lemon and avocado, and at 0.02 and 0.1 mg/kg for raisin and wheat. These analyses were interspersed between bracketing calibrations. Further application notes in this series, entitled "A Multi- Residue LC-MS/MS Method for the Determination of 81 Pesticide Residues in Fruit and Vegetables: Part 2···etc.", give details of method repeatability, at these levels, for each compound class. These application notes also give estimates of the Limits of Determination (LoD) for each compound.

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 determination achieved for the pesticides analyzed are generally well below that required for surveillance monitoring in the EU. Therefore the method is clearly extendable to greater numbers of pesticide targets within the compound classes examined.

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