Application NOTE

Waters

THE CONFIRMATION OF THE PRESENCE OF INCURRED PESTICIDE RESIDUES DETECTED USING A MULTI-RESIDUE SURVEILLANCE METHOD TO SCREEN FOR 81 TARGET ANALYTES

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Introduction

The legal enforcement of regulations governing pesticide use requires the regular monitoring of agricultural produce. Given the large number of pesticide residues that may be found in various crops it is advantageous to determine as many of them as possible during a single analysis. To meet this challenge multi-residue methods have been developed that target more than one analyte compound. Such a method, suitable for the surveillance monitoring of 81 pesticide compounds in a variety of fruit and vegetable types, has previously been described[1].

Once a surveillance analysis has indicated the occurrence of pesticide residues in a sample it is then necessary to verify their presence by the use of a confirmatory technique. In such a circumstance, a second MS method may be created, directed solely at the compounds suspected of being present, in which two or more MRM transitions are monitored for each analyte. When the sample is analysed a second time, these secondary and tertiary transitions provide effective proof that the compound is present.

Method

Details of the extraction and HPLC methods may be found in a separate Waters Application Note[1].

During the validation stage of the above multiresidue method, the blank extract of raisin, used to make up matrix matched standards, was suspected of containing incurred residues of certain target compounds. A second MS method was created that, in addition to the primary, contained two confirmatory MRM transitions for each of these compounds. Table 1 contains details of the residue compounds indicated, the primary MRM transitions and the estimated concentrations. It also shows the secondary and tertiary MRM transitions used for the confirmatory analysis. The concentrations of the incurred residues were estimated by dividing the Y intercept of each calibration line by the slope. This was done because the observed concentrations were below the range of the calibration curve.

Compound	Primary MRM Transition	Estimated Concentration (µg/kg)	Secondary MRM Transition	Tertiary MRM Transition
Methamidophos	142 > 94	10	142 > 125	142 > 112
Carbendazim	192 > 160	3	160 > 132	192 > 132
Carbaryl	202 > 145	40	202 > 127	202 > 117
Metalaxyl	280 > 220	8	280 > 192	280 > 160
Fluazifop-P-butyl	384 > 328	0.2	384 > 282	384 > 91

Table 1. Five residues detected in raisin sample

Matrix matched standards containing all five compounds were analysed at concentrations of 0.03, 0.1, 0.3, 1, 3, 10 and 30 pg/µL, corresponding to 0.06, 0.2, 0.6, 2, 6, 20 and 60 µg/kg levels. Each standard, and the sample, was analysed in duplicate. These standards were created using the extract of a sample of organic raisins that did not contain the five compounds listed in table 1.

The TargetLynx Application Manager, a new software tool designed for use in regulated quantification, was used to process all data. The ratios of ion abundance, between the primary and each of the two confirmatory MRM transitions, were automatically calculated. The Application Manager was set to flag any deviations by more than 20% from these expected ratios. In addition checks were automatically performed on peak retention times, signal to noise values, blank response values and calibration coefficients of determination.

Results

Figures 1 to 5 show calibration graphs for methamidophos, carbendazim, carbaryl, metalaxyl and fluazifop-P-butyl, respectively.

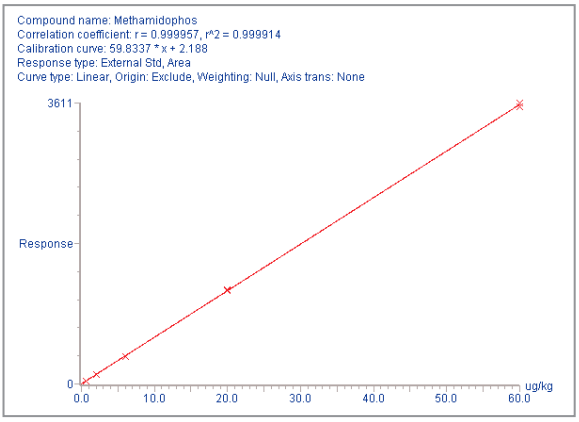

Figure 1

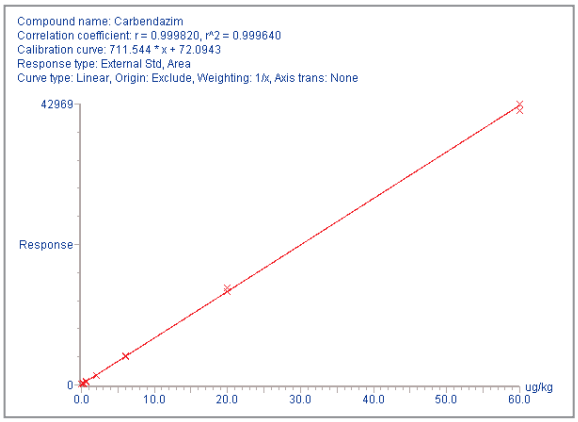

Figure 2

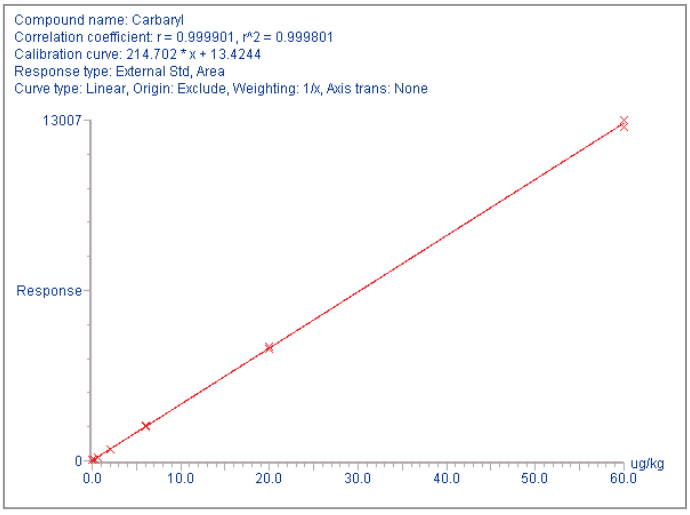

Figure 3

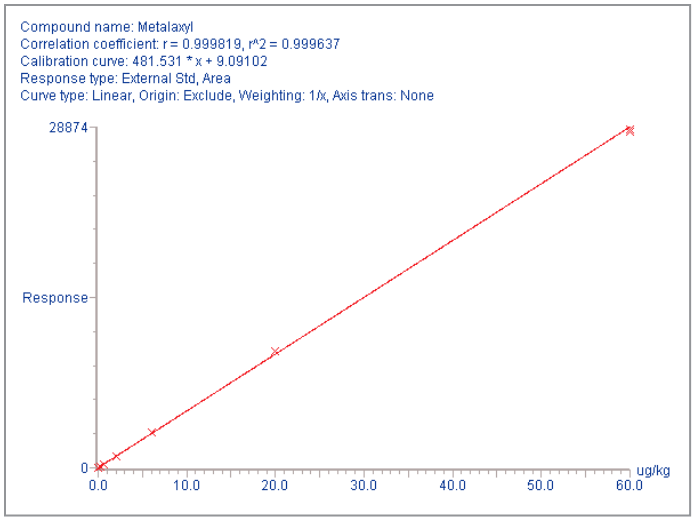

Figure 4

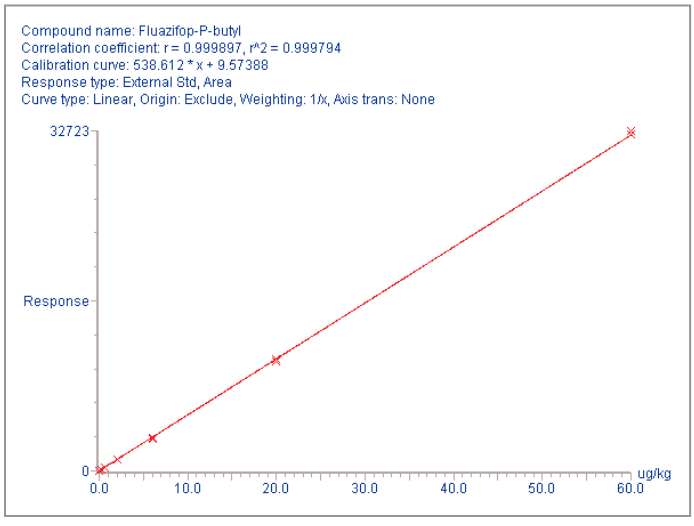

Figure 5

Figures 6 to 10 each show three chromatograms, corresponding to the three MRM transitions monitored for methamidophos, carbendazim, carbaryl, metalaxyl and fluazifop-P-butyl, respectively, taken from the analysis of the suspected sample.

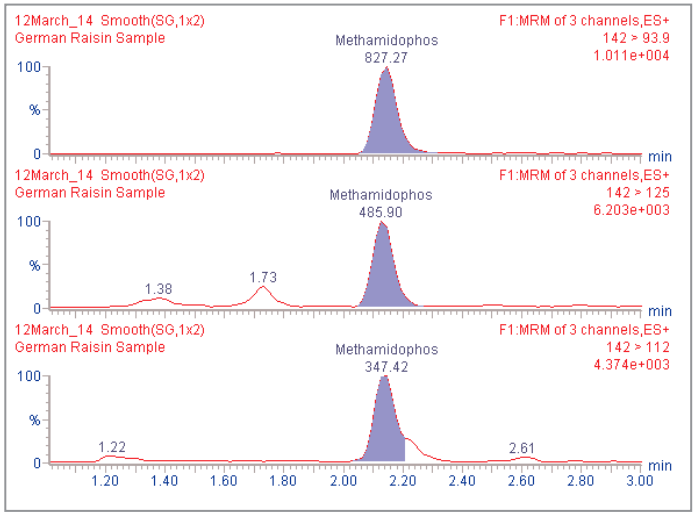

Figure 6

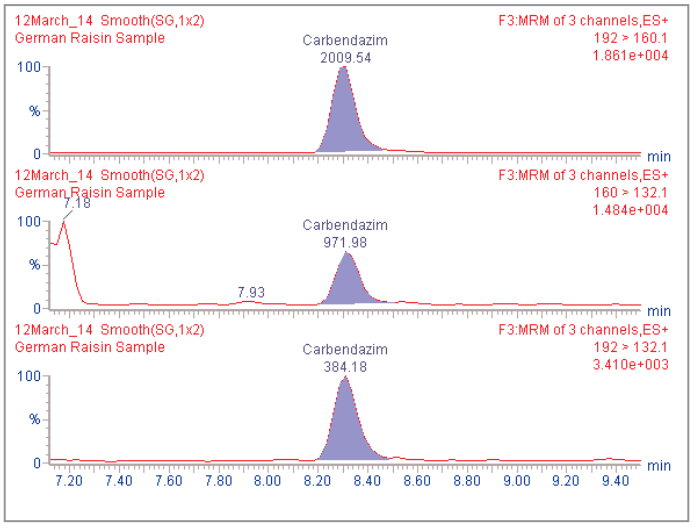

Figure 7

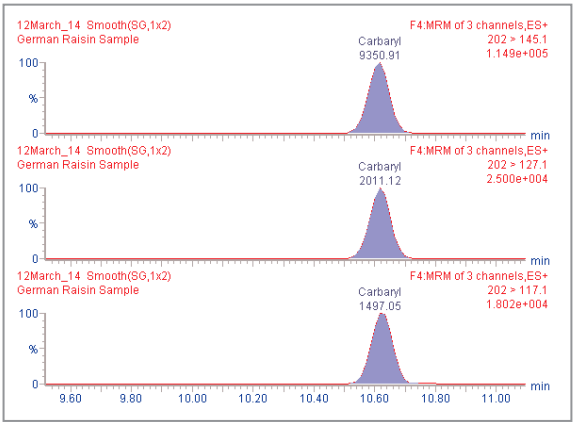

Figure 8

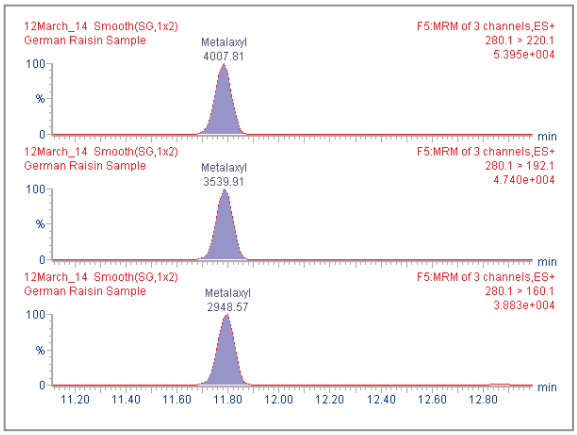

Figure 9

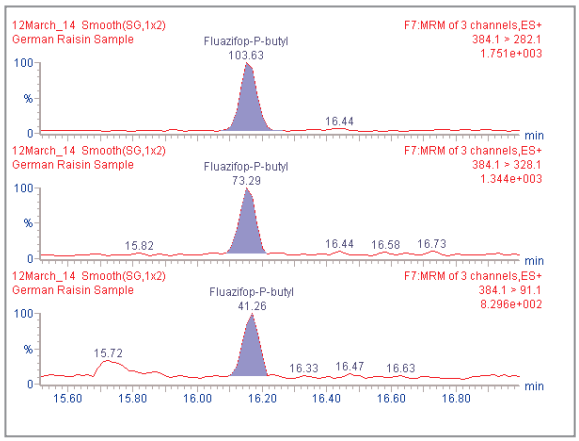

Figure 10

The chromatographic peak for methamidophos, monitored using the tertiary MRM transition, is not completely resolved from a peak corresponding to a co-extracted component of the sample matrix. At very low concentrations the analyte peak is obscured and, for future experiments, it may be necessary to choose a different MRM transition.

An image of the TargetLynx interactive browser window is shown in Figure 11. This graphical interface allows an operator to review data and manipulate quantification methods before producing comprehensive printed reports.

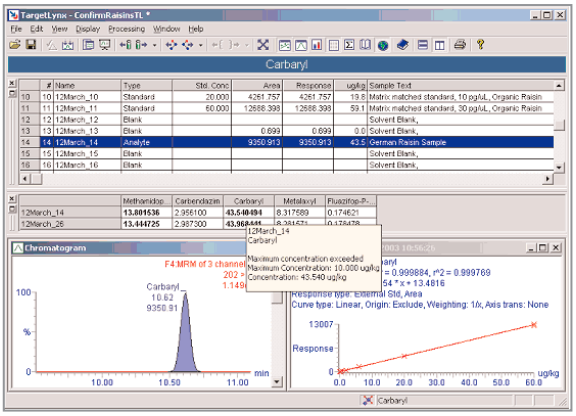

Figure 11

The quantification data passed all QC checks on retention times, signal to noise values, ion abundance ratios and minimum coefficients of determination, for all five residue compounds. The presence of the indicated compounds is effectively confirmed and Table 2 shows the precisely determined concentrations of each. This table also shows the expected ion abundance ratios for each secondary and tertiary MRM transition, determined from a solvent standard at 50 pg/mL, together with the observed ratios in the suspect sample.

Compound	Concentration of Incurred Residue (µg/kg)	Expected Secondary MRM Ion Ratio	Observed Secondary MRM Ion Ratio	Expected Tertiary MRM Ion Ratio	Observed Tertiary MRM Ion Ratio
Methamidophos	13.8	1.71	1.70	2.40	2.38
Carbendazim	2.8	2.16	2.07	5.63	5.23
Carbaryl	43.5	4.87	4.65	6.42	6.25
Metalaxyl	8.3	1.14	1.13	1.39	1.36
Fluazifop-P-butyl	0.17	1.24	1.41	2.47	2.51

Table 2

Compound	Esti mated LoD Primary MRM (µg/kg)	Estimated LoD Secondary MRM (µg/kg)	Estimated LoD Tertiary MRM (µg/kg)
Methamidophos	0.12	0.62	0.67
Carbendazim	0.052	0.36	0.14
Carbaryl	0.016	0.060	0.090
Metalaxyl	0.016	0.016	0.028
Fluazifop-P-butyl	0.013	0.015	0.046

Table 3

Table 3 contains estimated LoD values for each compound using the primary, secondary and tertiary MRM transitions. These LoDs are the concentrations at which the S:N ratio of the chromatographic peak would be expected to be 3:1.

The tertiary MRM transition for carbendazim, although it is less abundant than the secondary, actually has a lower level of baseline noise leading to a lower estimated LoD.

Conclusions

Once the presence of incurred pesticide residues is detected by a multiresidue screening method it is a relatively straightforward task to build a confirmatory method that targets those analytes suspected of being present. Chromatographic conditions remain unchanged and it is only necessary to create a new MS acquisition method.

However, the initial multiresidue screening method has, for most compounds, the sensitivity to provide LoD values well below what is required for surveillance monitoring in the EU. Therefore it would be possible to insert confirmatory MRM transitions into the screening method, circumventing the need for the second analysis of a sample.

References

1 A Multi-Residue HPLC/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 Application Note, 2003

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