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應用手冊

Determination of Pesticide Residues in Complex Matrices Using the Waters Micromass Quattro Premier: A Method Combining Extreme Sensitivity and Unmatched Robustness

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Abstract

This application note focuses on the determination of seven pesticides in wheat, and evaluates matrix effects on the sensitivity and robustness of the method.

Benefits

LOD values are well below the EU requirements and allow routine quantitative determination at the 0.005–0.015 µg/kg level

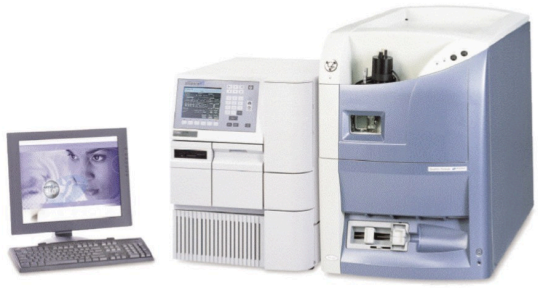
Introduction

The Food and Veterinary Office of the European Commission has published a report (2001 pesticide residues monitoring programme[1]) compiling EU wide analyses of pesticide residues in 46000 samples of fruits, vegetables and cereals. Detectable residues were found in 37% of the samples, 3.6% of the sample contained residue levels above the EC MRLs. The percentage of samples with no detectable residues is shown to have slightly decreased compared to previous years, whereas the percentage of samples with residues at or below the MRL has increased. These results do not reflect a real change in the residue situation, but rather an improvement in the analytical instrumentation used in modern laboratories. Techniques are becoming more and more efficient, allowing the detection and quantification of lower amounts and a higher number of various pesticides.

The detection of increasingly lower levels of residues in complex matrices is an on-going challenge for scientists, particularly since this has to be done on a day-to-day basis without compromising sensitivity, selectivity, and reproducibility.

In this study, a multi-residue method will be described. Previously developed[2,3] on a Waters Micromass Quattro micro, this method has now been adapted for use on the Quattro Premier.

This study focuses on the determination of seven pesticides in wheat, and evaluates matrix effects on the sensitivity and robustness of the method.



Waters Micromass Quattro Premier MS System.

Experimental

Sample Preparation

10 g of sample (wheat hay and wheat grain) were extracted with acetone using an ASE200 System. 40 mL of water were added, followed by a dichloromethane liquid/liquid extraction. After evaporation, the sample was purified using an Envirogel Column (aliquot 2/3). The sample was dried and then dissolved in 2 mL of a water/methanol (80/20) mixture containing the standards. Finally each extract was filtered through 0.45 µm microfilters.

LC Conditions

LC system:	Alliance 2695 HPLC
Mobile phase A:	MeOH/H_2O (1:4) + 5mM $CH_3CO_2NH_4$
Mobile phase B:	MeOH/H_2O (9:1) + 5mM $CH_3CO_2NH_4$
Column:	Waters Atlantis C_{18} 2.1 mm id, 100 mm with 3 µm particle size, 30 °C
Flow:	220 uL/min
Gradient:	100% A to 100% B in 15 min

	Primary Transition MRM	Cone Voltage (CV)	Collision Cell Energy (CE)
Methomyl	163.0 > 88.0	14 V	8 V
Metsulfuron-methyl	328.1 > 167.1	26 V	17 V
Imidacloprid	256.1 > 209.1	22 V	14 V
Dimethoat	230.0 > 125.0	17 V	20 V
Carbendazim	192.1 > 160.0	28 V	18 V
Linuron	249.0 > 160.0	25 V	18 V
Imazalil	297.1 > 159.0	31 V	18 V

*Table 1. MRM transitions for the seven analytes. *Collision cell operated at 4.5 x 10^{-3} mBar.*

Mass Spectrometry

The standard was analyzed using the parameters previously reported in the multi-residue method.[2] Two additional injections were made to determine the optimal cone voltage (CV) and collision cell energy (CE) values. These values varied slightly from those observed on a Quattro micro. MRM transitions defined in the original method were used.

Sensitivity and Linearity

Calibration curves were generated for the pure standard, for the grains matrix and for the hay matrix. Pure standard and matrix-matched standards containing all six compounds were analyzed at concentrations of 0.005, 0.02, 0.05, and 0.1 pg/µL, corresponding to 0.003, 0.012, 0.03, and 0.06 µg/kg levels, respectively.

Standard and sample analyses were duplicated and blanks were included at the beginning and at the end of the sample list sequence.

Method Robustness

The 0.06 µg/kg matrix-matched standard and the pure standard mixture were injected alternately over a period of 48 hours. Both matrix-matched and pure standards were analysed 48 times, with 30 minutes between each injection.

Results and Discussion

Figure 1 show the chromatogram for the “highest” standard concentration level (0.06 µg/kg) for the 7 pesticides. The signal to noise (S/N) ratio (calculated peak to peak) for all analytes exceeds 50 thus allowing determination of those pesticides in the 0.006 µg/kg level, which is far lower than the required EC MRLs.

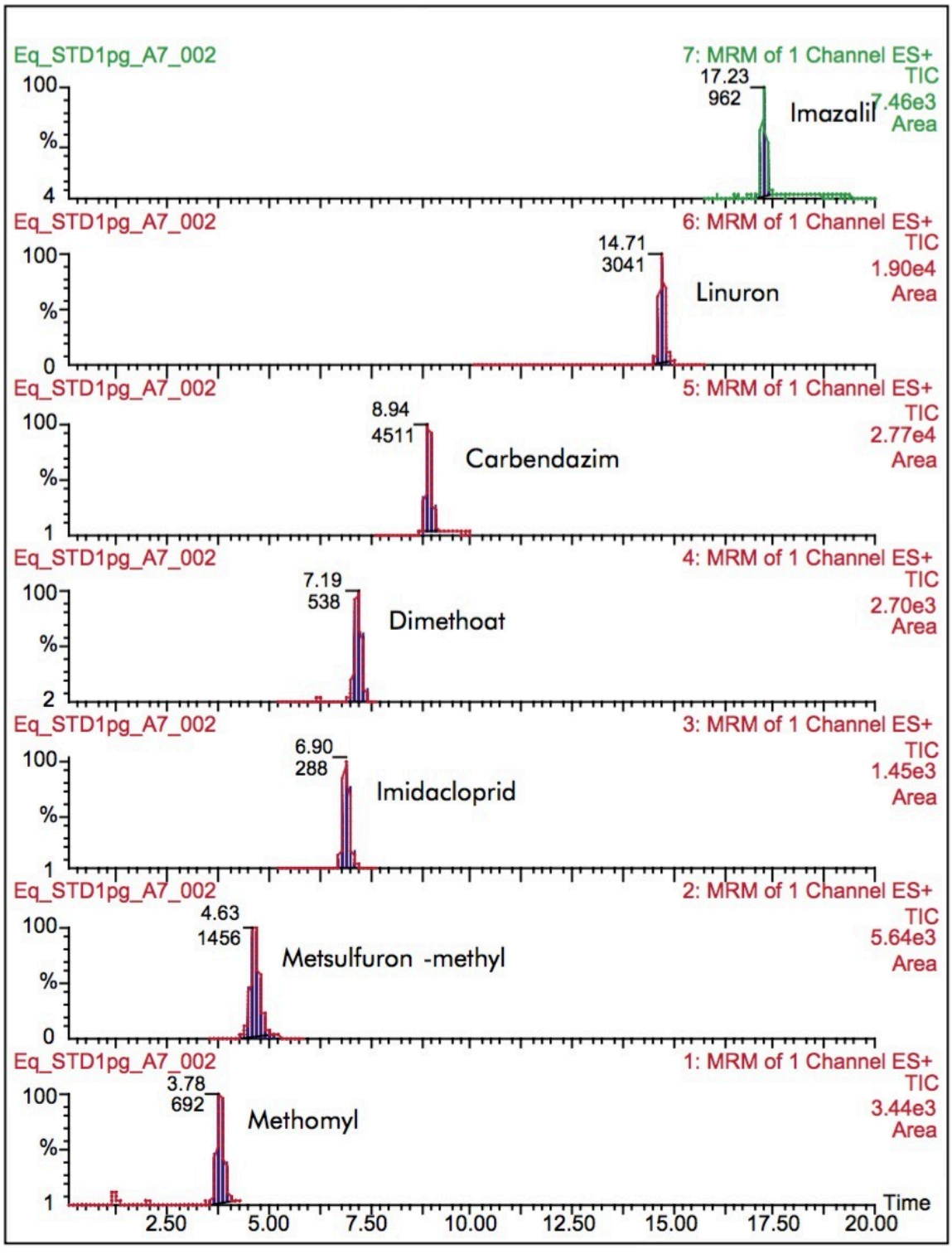


Figure 1. Chromatogram of the 0.06 µg/kg standard level.

Each pesticide in the standard mixture was successfully detected at the lowest (0.003 µg/kg) concentration level. Figures 2 and 3 show the calibration curve for the methomyl standards and the overlaid total ion chromatograms, respectively.

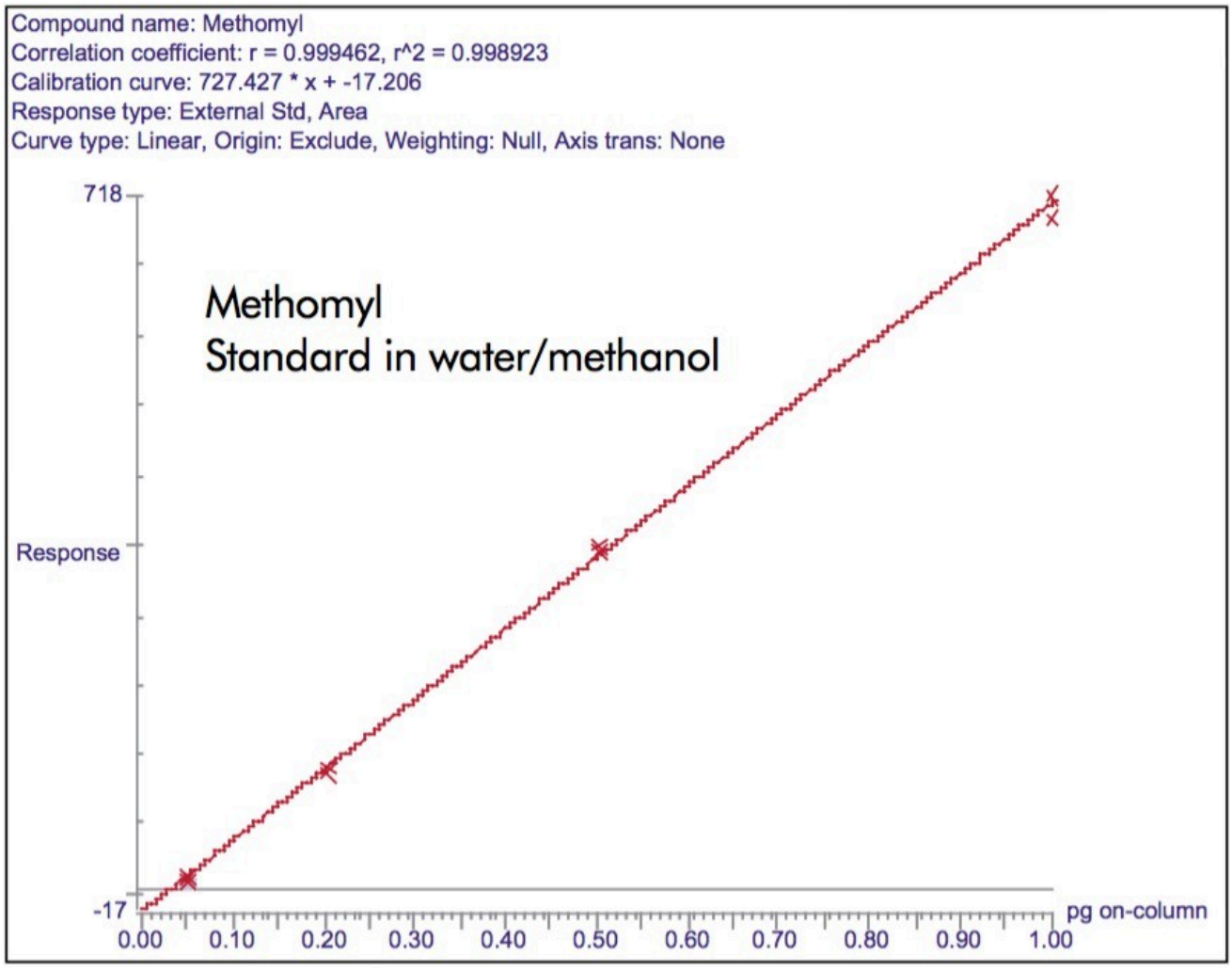


Figure 2. Methomyl calibration curve for duplicate injections of 0.005, 0.02, 0.05, and 0.1 pg/µL standard solutions, corresponding to 0.003, 0.012, 0.03, and 0.06 µg/kg levels, respectively.

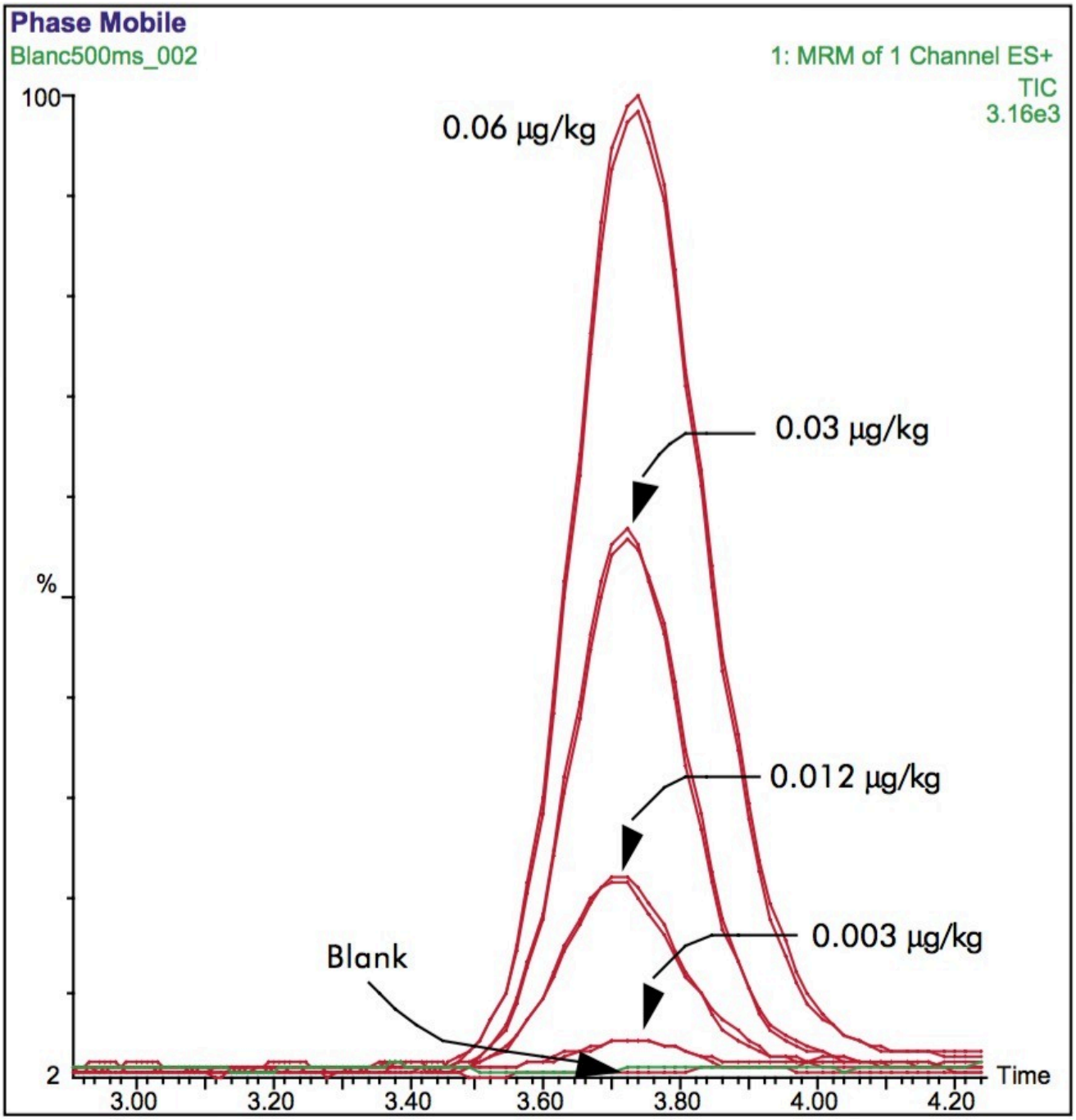


Figure 3. Methomyl standard chromatograms overlaid.

Wheat grains matrix

Calibration graphs for the lowest calibrants, Figures 4 and 5, show that the matrix is negligible and sensitivity levels remain similar.

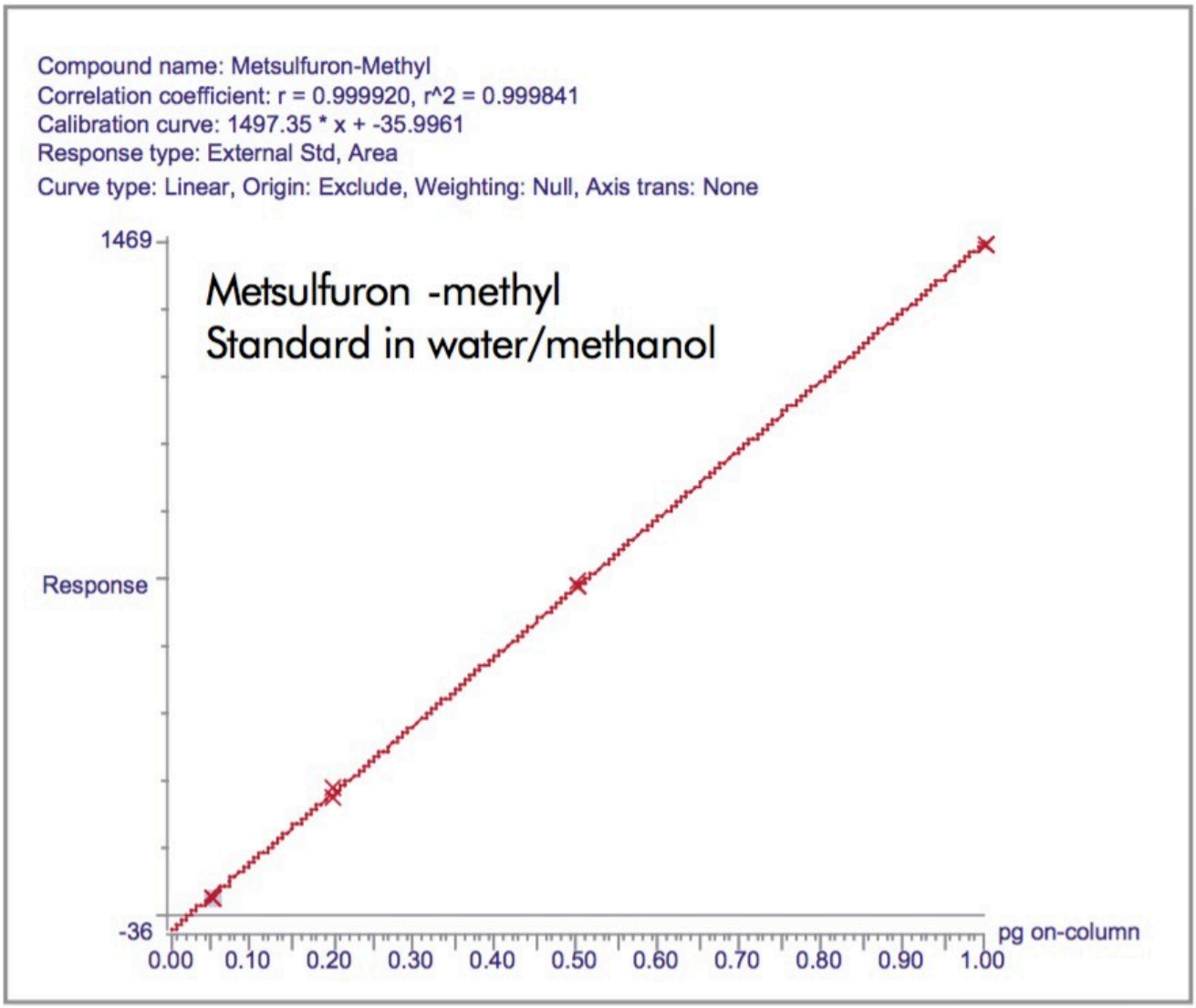


Figure 4. Metsulfuron-methyl calibration curve for duplicate injections of 0.005, 0.02, 0.05, and 0.1 pg/µL standard solutions, corresponding to 0.003, 0.012, 0.03, and 0.06 µg/kg levels, respectively.

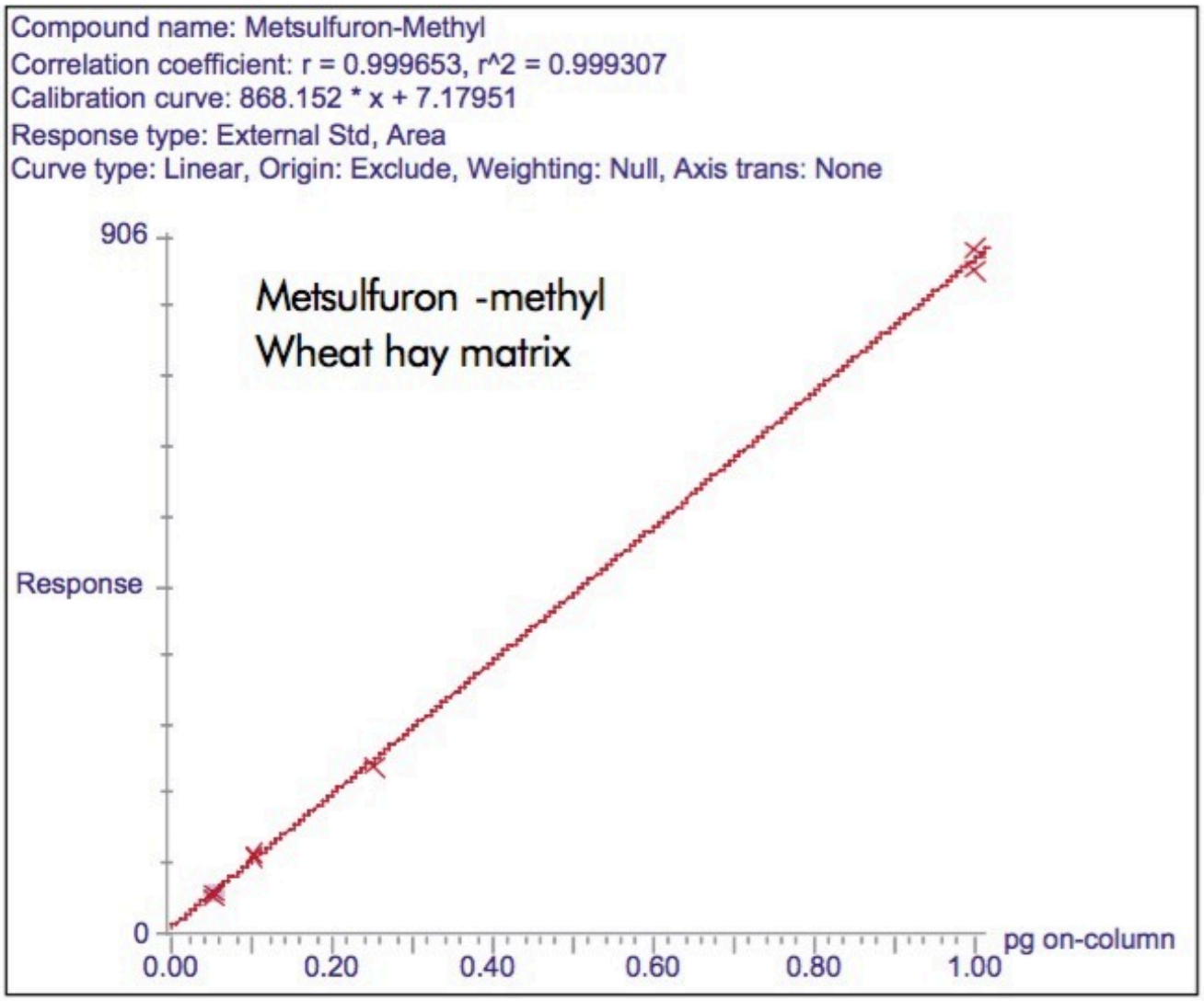


Figure 5. Metsulfuron-methyl calibration curve for duplicate injections of 0.005, 0.01, 0.025 pg/µL extracts, corresponding to 0.003, 0.006, and 0.015 µg/kg levels, respectively.

Tables 2 and 3 show that the concentrations recalculated from the calibration curves are in good agreement with the theoretical values, with residuals of around 5%. Figure 6 describes the chromatogram obtained by injecting 50 fg of metsulfuron-methyl oncolumn, which constitutes, more or less, the limit of detection for that compound.

	#	Name	Trace	Type	Std. Conc	RT	Area	Flags	pg on-column	%Dev
1	1	Eq_STD50fg_A7_001	382.1 > 167.1	Standard	0.05	4.58	34.3	bb	0.047	-6.2
2	2	Eq_STD50fg_A7_002	382.1 > 167.1	Standard	0.05	4.55	40.2	MM	0.051	1.8
3	3	Eq_STD200fg_A7_001	382.1 > 167.1	Standard	0.20	4.55	273.1	bb	0.206	3.2
4	4	Eq_STD200fg_A7_002	382.1 > 167.1	Standard	0.20	4.57	251.1	bb	0.192	-4.1
5	5	Eq_STD500fg_A7_001	382.1 > 167.1	Standard	0.50	4.58	714.3	bb	0.501	0.2
6	6	Eq_STD500fg_A7_002	382.1 > 167.1	Standard	0.50	4.58	721.7	bb	0.506	1.2
7	7	Eq_STD1pg_A7_001	382.1 > 167.1	Standard	1.00	4.60	1461.5	bb	1.000	0.0
8	8	Eq_STD1pg_A7_002	382.1 > 167.1	Standard	1.00	4.63	1456.6	bb	0.997	-0.3

Table 2. Metsulfuron-methyl standard: recalculated concentrations.

	#	Name	Trace	Type	Std. Conc	RT	Area	Flags	pg on-column	%Dev
1	1	A31600101E_50fg_oncol001	382.1 > 167.1	Standard	0.05	4.86	66.5	bd	0.053	5.0
2	2	A31600101E_50fg_oncol002	382.1 > 167.1	Standard	0.05	4.83	62.2	bb	0.049	-2.1
3	3	A31600101E_100fg_oncol001	382.1 > 167.1	Standard	0.10	4.79	122.9	bd	0.099	-1.0
4	4	A31600101E_100fg_oncol002	382.1 > 167.1	Standard	0.10	4.76	122.9	bb	0.099	-1.0
5	5	A31600101E_250fg_oncol001	382.1 > 167.1	Standard	0.25	4.76	313.4	bb	0.256	2.5
6	6	A31600101E_250fg_oncol002	382.1 > 167.1	Standard	0.25	4.74	299.1	bs	0.244	-2.3

Table 3. Metsulfuron-methyl in wheat grain matrix: recalculated concentrations.

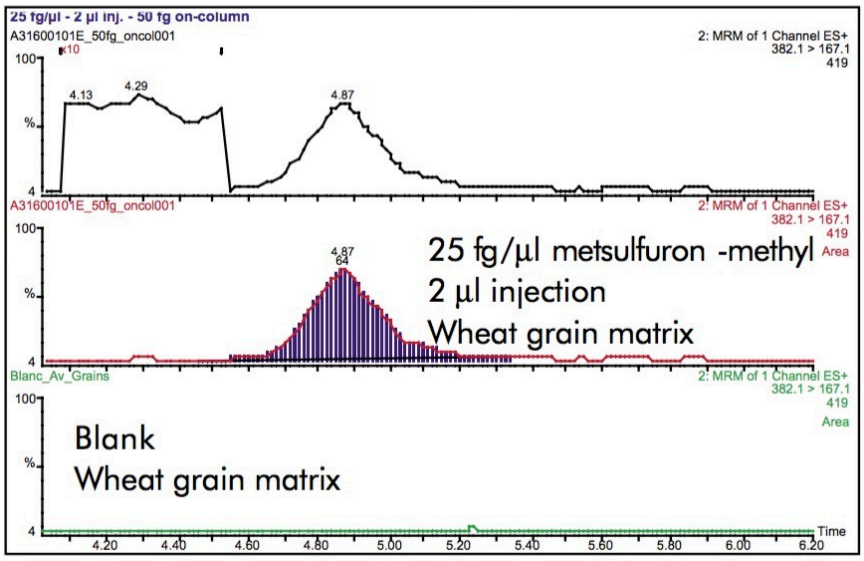


Figure 6. Chromatograms and limit of detection for Metsulfuron-methyl in wheat grain matrix at 0.025 pg/µL (0.0015 µg/kg), compared to blank wheat grain matrix.

Hay matrix is far more complex than grain matrix and is known to contain many compounds that may detrimentally affect the ionization and subsequently the sensitivity of the analysis. The advantage of using the MRM technique is that it is extremely selective, and even in highly complex matrices, allows the detection of very low level compounds with good S/N. Figure 7 shows a comparison of SIR vs MRM for the detection of methomyl in wheat hay matrix. Using SIR the compound is not detectable, however, the selectivity of MRM allows detection of methomyl with good S/N. Results obtained from wheat hay matrix analysis show good consistency and are presented in Figure 8 and Table 4.

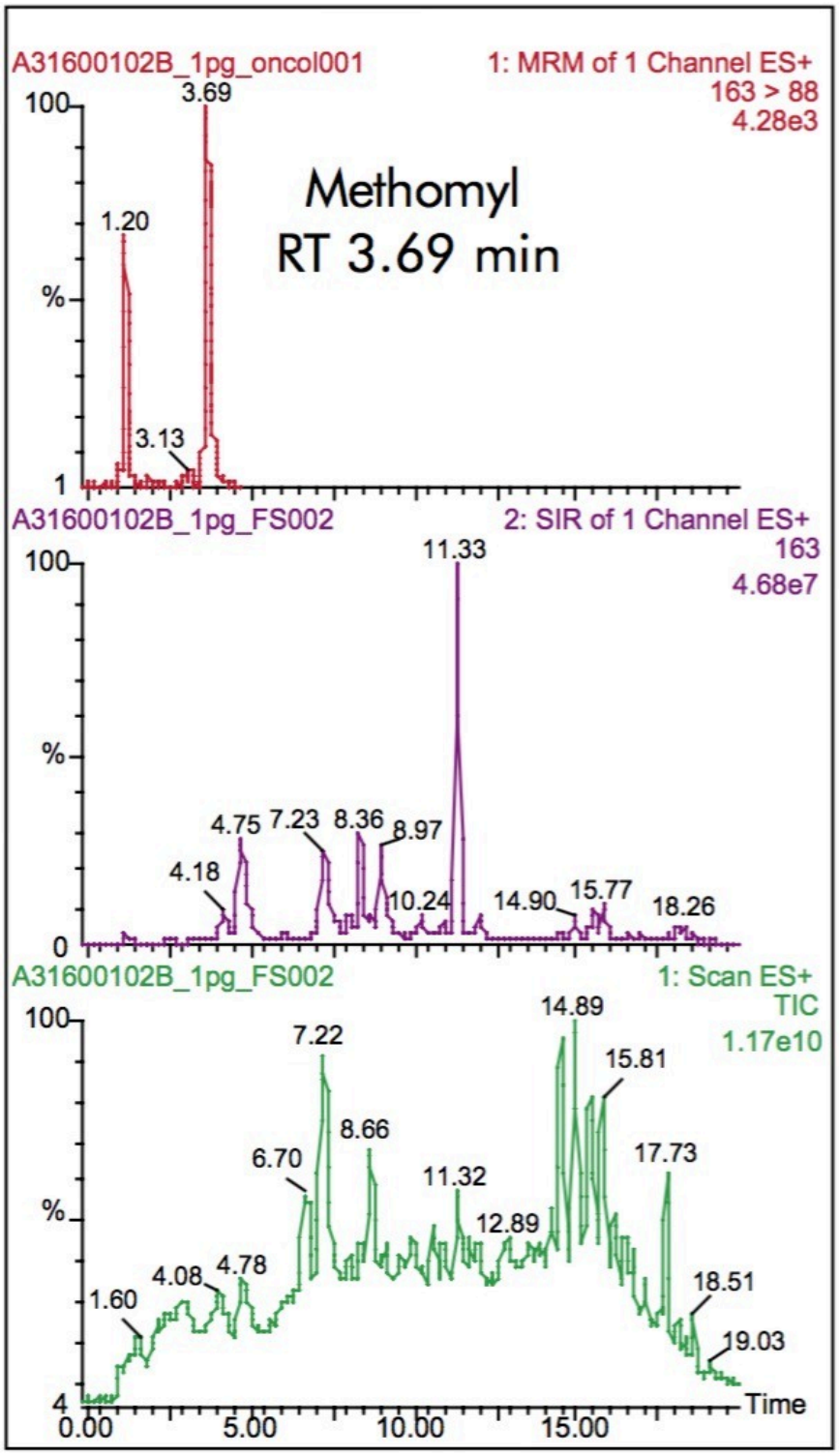


Figure 7. Methomyl in wheat hay matrix: 0.1 pg/µL extract corresponds to 0.06 µg/kg levels. Comparison of full

scan, SIR and MRM.

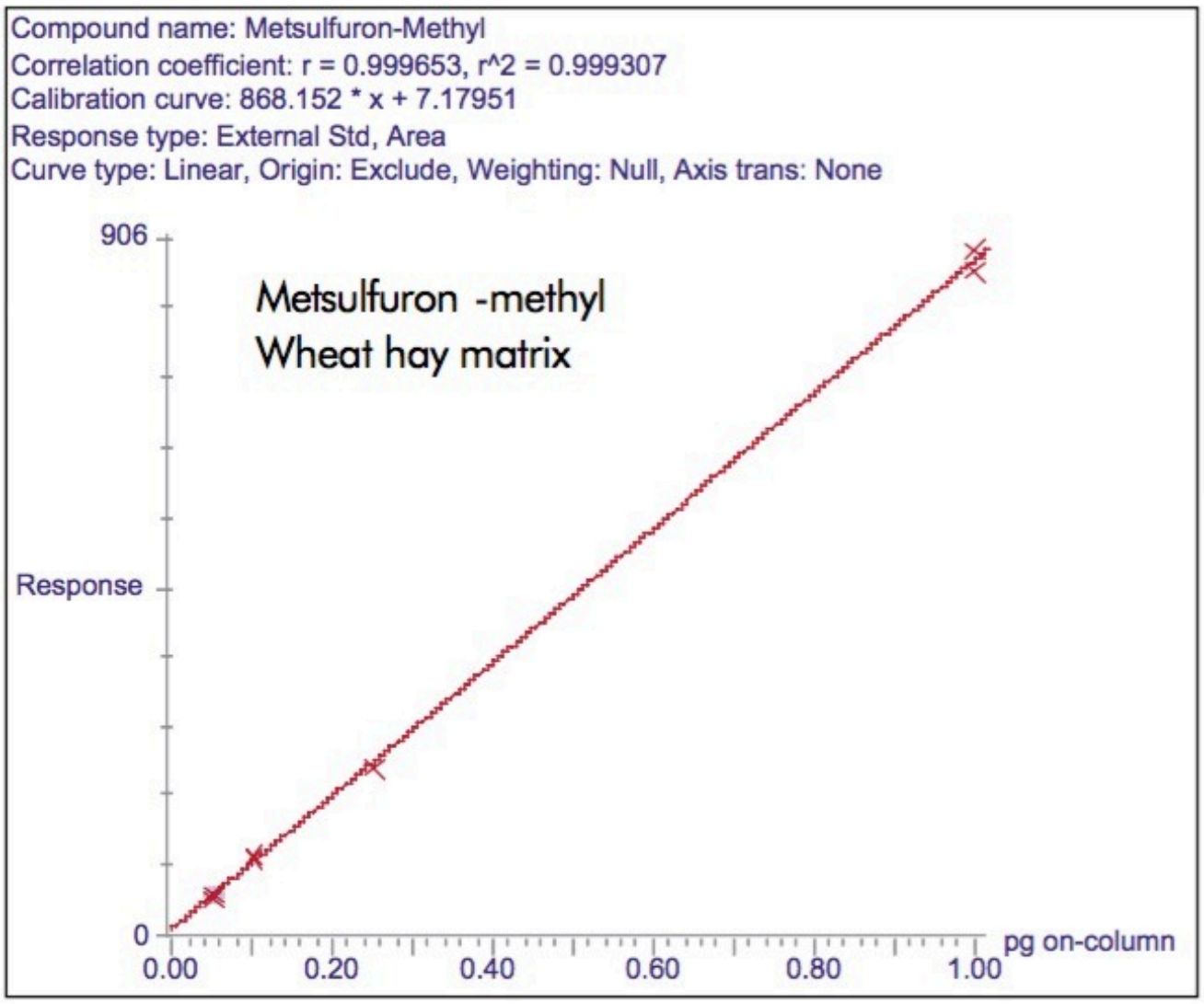


Figure 8. Metsulfuron-methyl calibration curve for duplicate injections of 0.005, 0.01, 0.025 pg/µL extracts, corresponding to 0.003, 0.006 and 0.015 µg/kg levels in wheat hay matrix, respectively.

	#	Name	Trace	Type	Std. Conc	RT	Area	Flags	pg on-column	%Dev
1	1	A31600102C_50fg_oncol001	382.1 > 167.1	Standard	0.05	4.49	52.3	MM	0.052	4.0
2	2	A31600102C_50fg_oncol002	382.1 > 167.1	Standard	0.05	4.51	47.7	bd	0.047	-6.7
3	3	A31600102C_100fg_oncol001	382.1 > 167.1	Standard	0.10	4.51	98.0	bb	0.105	4.6
4	4	A31600102C_100fg_oncol002	382.1 > 167.1	Standard	0.10	4.49	103.4	bb	0.111	10.8
5	5	A31600102C_250fg_oncol001	382.1 > 167.1	Standard	0.25	4.49	216.1	bb	0.241	-3.7
6	6	A31600102C_250fg_oncol002	382.1 > 167.1	Standard	0.25	4.49	217.7	bb	0.243	-3.0
7	7	A31600102B_1pg_oncol001	382.1 > 167.1	Standard	1.00	4.47	862.6	bb	0.985	-1.5
8	8	A31600102B_1pg_oncol002	382.1 > 167.1	Standard	1.00	4.47	890.4	bb	1.017	1.7

Table 4. Metsulfuron-methyl in wheat hay matrix: recalculated concentrations.

Repeatability and Robustness

To evaluate the robustness of the analytical system, 96 samples of pure standard and spiked wheat hay extracts were injected alternately over a period of 48 hours. The results from the 0.06 µg/kg wheat hay extract was used, representing injections of 1 pg of each analyte onto the column. Figures 9 and 10 show the stability graphs obtained for methomyl, which give a similar response for both samples. Calculated standard deviations for the pure standard and for the hay matrix extracts were 2.7% and 3.5%, respectively.

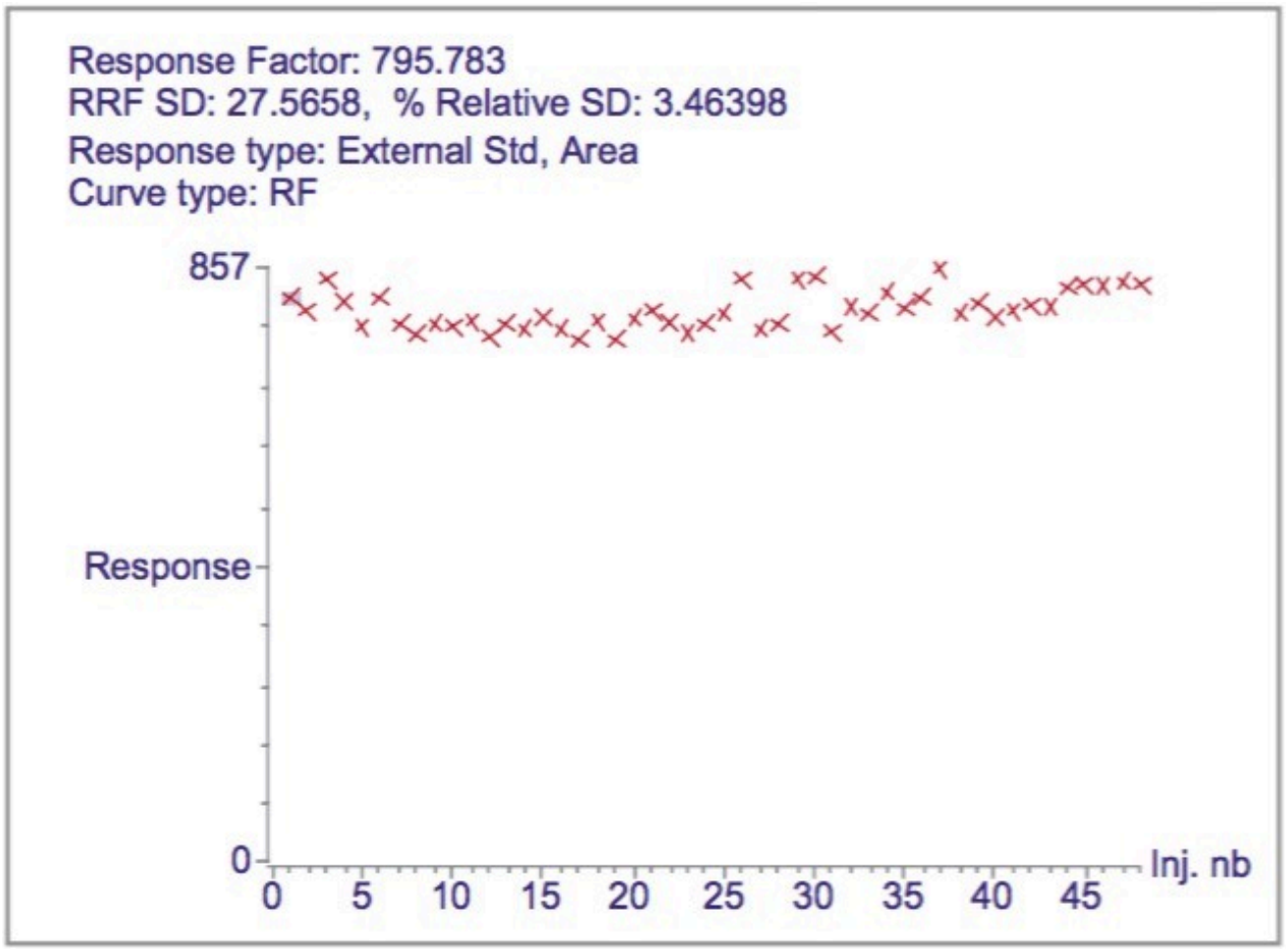


Figure 9. Stability graph for Methomyl standard in water/methanol over a period of 48 h (48 injections).

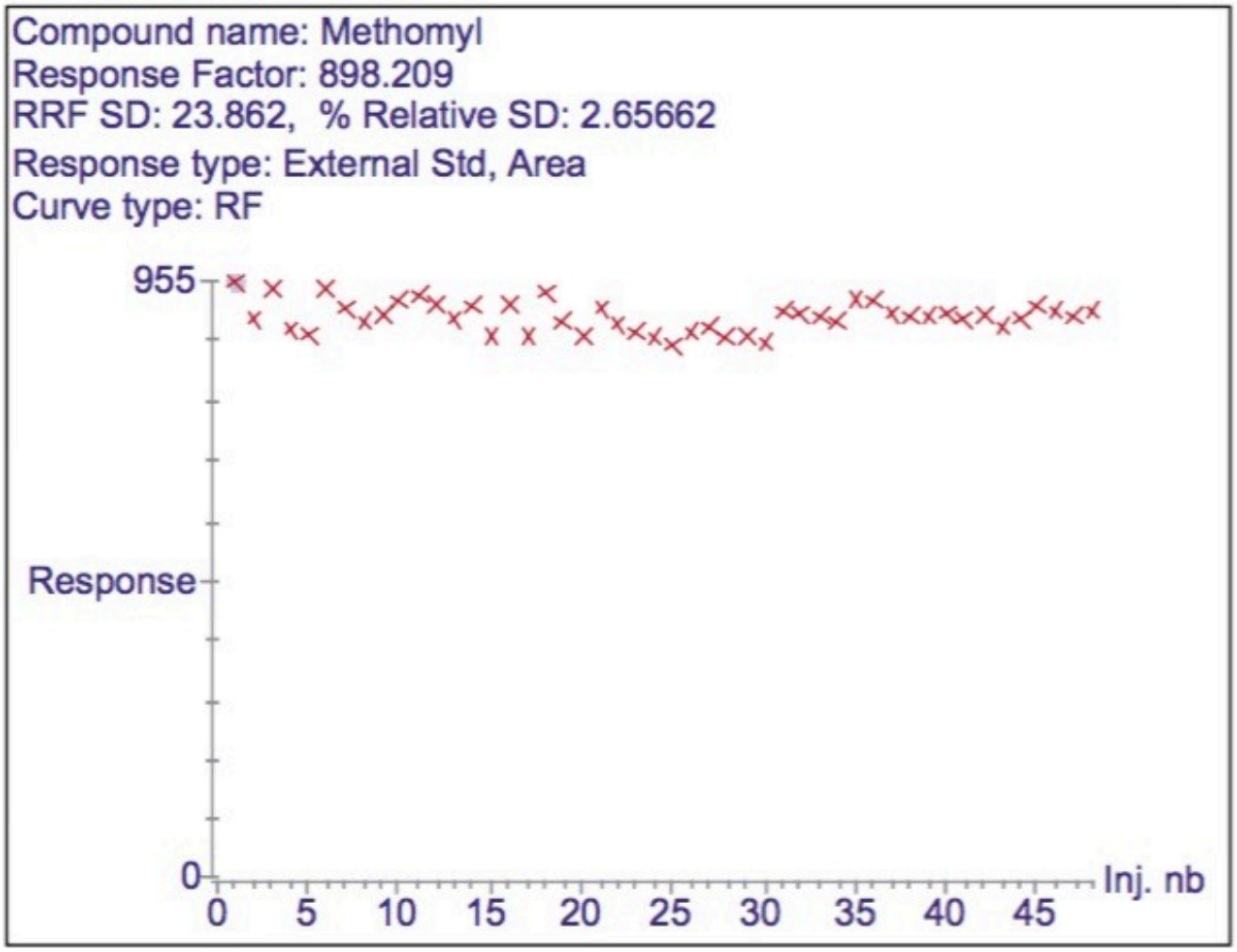


Figure 10. Stability graph for Methomyl in wheat hay extract in water/methanol over a period of 48 h (48 injections).

Conclusion

As it has been demonstrated in the previous application notes,[2,3] sensitivity and robustness of the analytical technique are crucial to fit within the EU regulatory requirements concerning the monitoring of agricultural produce. This application note demonstrates that similar levels of sensitivity can be achieved whatever the matrix and that the overall performance of the analytical system is maintained over a long period of time, even after dirty

matrix injections. LOD values are well below the EU requirements and allow routine quantitative determination at the 0.005–0.015 µg/kg level.

In order to decrease the impact of the matrix and thus improve the robustness further, a smaller amount of sample (*i.e.* 1 g instead 10 g) or greater dilution of the extract could be used. Importantly, the method could of course be applied to many other pesticides through the use of additional confirmatory MRM transitions, as described previously.[3]

References

1. European Commission, Health & Consumer Protection, Directorate F, Food and Veterinary Office. Report on "Monitoring of Pesticide Residues in Products of Plant Origin in the European Union, Norway, Iceland and Liechtenstein", 2001 report, published March 2003.
2. A Multi-Residue HPLC-MS/MS Method for the Determination of 81 Pesticides Residues in Fruits and Vegetables: Part 1, Method Overview. Gordon Kearney, Lutz Alder, Anthony Newton, Jeannette Klein. 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> (2003).
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