Analysis of Early Eluting Pesticides in a C18-Type Column Using a Divert Valve and LC-MS/MS

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Application Note 572

Key Words
TSQ Quantum Access MAX, Divert Valve, Split Peaks, Reversed-Phase Liquid Chromatography, Pesticides

Goal
To demonstrate the ability to override the solvent effects from a sample extract using gradient solvents with liquid chromatography. Additionally, to increase injection volume without overloading the column.

Introduction
Many pesticide analyses are based on the QuEChERS extraction method, which uses acetonitrile (ACN) in the final extraction step. However, injecting a solvent stronger than the HPLC mobile phase can cause peak shape problems, such as peak splitting or broadening, especially for the early eluting analytes (low capacity factor, k). The common practice is to exchange the solvent of the final extraction step for one similar to the mobile phase, for example methanol / water, but this procedure is laborious and can lead to analyte losses.

There are several possible causes of peak splitting or broadening. This study presents the peak shape differences between acetonitrile and methanol / water [1:1 v/v] solutions due to the interaction of gradient and sample solvent, as indicated in Figure 1. The lowest detection limit is achieved when an analyte is in as compact a band as possible within the flow stream of mobile phase and with larger injection volumes. However, this is limited by maximum loop volume and column capacity.

Mobile phase composition and the use of a divert valve have been evaluated for the analysis of seven selected pesticides in acetonitrile solutions (Table 1). The sample solutions were chosen to represent both low and high analyte levels for compounds that elute either early or middle-early from a C18 column. Performance was evaluated in terms of linearity (injection volume range 1–8 µL), robustness (RSD), and sensitivity as measured by signal-to-noise ratio (S/N) and peak area reproducibility.

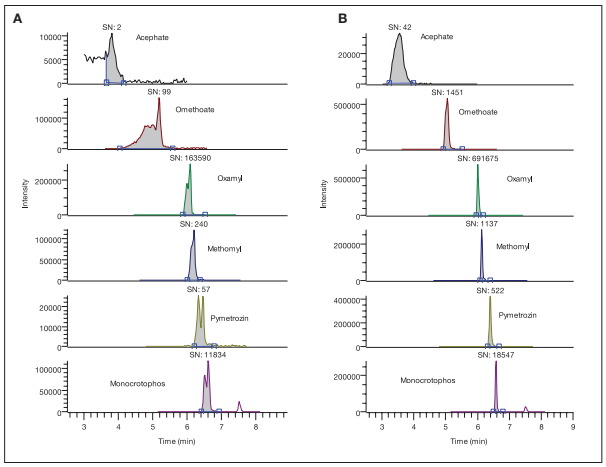

Figure 1. Chromatograms of 5 µL injections of acephate, omethoate, oxamyl, methomyl, pymetrozin, and monocrotophos in 50 µg/L acetonitrile (A) and methanol / water [1:1 v/v] solution (B), with no divert valve used

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Table 1. List of studied pesticides and their physicochemical properties

Name	Pesticide Class	Chemical Formula	Water Solubility [mg/L] / pKow	Vapor Pressure [Pa]	Molecular Weight [g/mol]
Acephate	Organophosphorous	$C_4H_{10}NO_3PS$	790,000 / -0.85	2.26 x 10^{-4} (24 °C)	183.165862
Aldicarb sulfone	Oxime carbamate	$C_7H_{14}N_2O_4S$	10,000 (25 °C) / -0.57 (calculated)	0.012 (25 °C)	222.26206
Metamitron	Triazinone	$C_{10}H_{10}N_4O$	1770 (25 °C; pH 5) / 0.85 (21 °C, not pH dependent)	7.44 x 10^{-7} (25 °C)	202.2126
Methomyl	Oxime carbamate	$C_5H_{10}N_2O_2S$	55,000 (25 °C, pH 7) / 0.09 (25 °C, pH 4-10)	7.2 x 10^{-4} (25 °C)	162.210100
Monocrotophos	Organophosphorous	$C_7H_{14}NO_5P$	water miscible	2.9 x 10^{-4} (20 °C)	223.163522
Omethoate	Organophosphorous	$C_5H_{12}NO_4PS$	water-miscible / -0.74 (20 °C)	3.3 x 10^{-3} (20 °C)	213.191842
Oxamyl	Oxime carbamate	$C_7H_{13}N_3O_3S$	148,100 (20 °C, pH 5) / -0.44 (25 °C, pH 5)	5.12 x 10^{-5} (25 °C)	219.26142

Experimental Conditions

Sample Preparation

Individual stock solutions of pesticides were prepared at concentrations that were sufficient to evaluate the linearity of peak area versus injection volume at the same concentration e.g. 10 µg/L, but different injection volumes (e.g. 1, 2, 3, 4, 5, 6, 7 µL, etc.). Additional solutions with different concentrations (5, 10, 25, 50, 70, 100, 200 µg/L) were prepared to study the linearity of peak area versus compound concentration. Finally, solutions with different solvents (acetonitrile or methanol / water [1:1 v/v]) were prepared to study the solvent effect on the methanol / water gradient mobile phase during the injection.

HPLC

HPLC analysis was performed using a Thermo Scientific Accela UHPLC system. The chromatographic conditions were as follows:

HPLC Column	Thermo Scientific Hypersil GOLD, 100 mm x 2.1 mm, 1.9 µm particle size
Trap Column	Hypersil™ GOLD, 10 mm x 2.1 mm, 5 µm particle size
Column Temperature	40 °C
Mobile Phase A	Water with ammonium formate (5 mM) and formic acid (2 mM)
Mobile Phase B	Methanol with ammonium formate (5 mM) and formic acid (2 mM)

The trap column was used to trap the analytes, while the divert valve was switched to the waste position. A tee union between the trap column and the analytical column was connected to the divert valve. The two positions of the divert valve are shown in Figure 2.

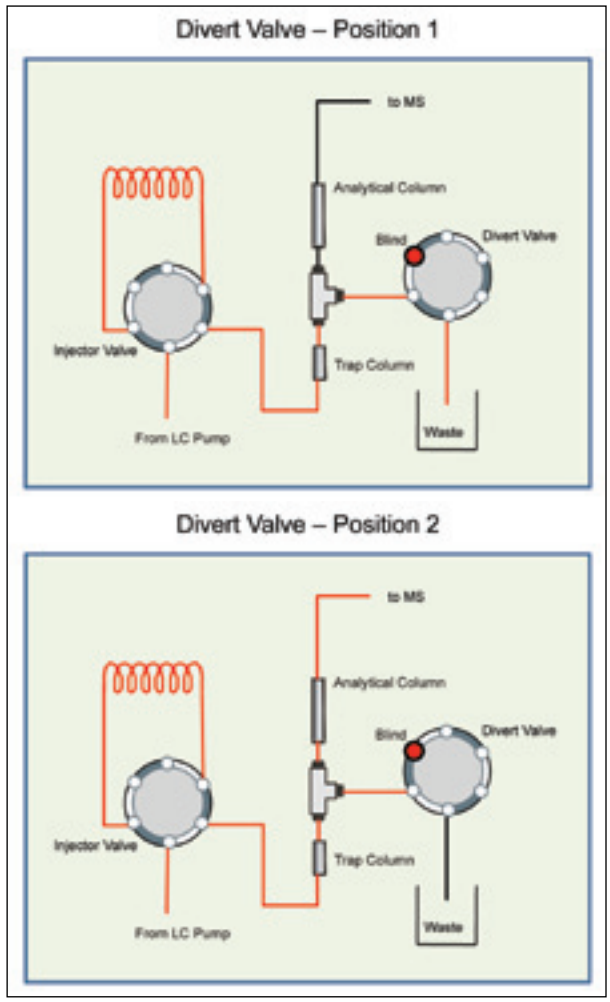

Figure 2. Divert valve positions

The gradient used is detailed in Table 2. The duration of the gradient was 21 minutes and the column equilibration time was 10 minutes. The flow rate increased at 21.10 min and decreased at 25.10 min to increase the speed of column equilibration for the next run (larger column volumes in less time). The maximum backpressure was 9,500 psi.

Table 2. HPLC Gradient. Mobile phase A is water with ammonium formate (5 mM) and formic acid (2 mM), and mobile phase B is methanol with ammonium formate (5 mM) and formic acid (2 mM).

No.	Time	A%	B%	µL/min
0	0.00	90.0	10.0	450.0
1	2.40	90.0	10.0	450.0
2	7.00	40.0	60.0	450.0
3	14.00	10.0	90.0	450.0
4	21.00	10.0	90.0	450.0
5	21.10	90.0	10.0	560.0
6	25.00	90.0	10.0	560.0
7	25.10	90.0	10.0	450.0
8	31.00	90.0	10.0	450.0

Mass Spectrometry

MS analysis was carried out on a Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer with an electrospray ionization (ESI) probe. The MS conditions were as follows:

Ion polarity	Positive
Q1 Resolution	0.7 Da
Spray Voltage	4000 V
Sheath/Auxiliary Gas	Nitrogen
Sheath Gas Pressure	40 (arbitrary units)
Auxiliary Gas Pressure	25 (arbitrary units)
Ion Transfer Tube Temperature	325 °C
Scan Type	Selected-Reaction Monitoring (SRM)
Collision Gas	Argon
Collision Gas Pressure	1.5 mTorr
Divert Valve	Rheodyne® model 7750E-185

The divert valve was connected to the front of the TSQ Quantum Access MAX™ and was fully controlled from the data system software.

Results and Discussion

The comparison of peak shapes between the acetonitrile and methanol / water sample solutions demonstrated that only early eluting analytes were altered by the mobile phase composition (Figure 3). Without the divert valve, the peak shape of omethoate, which elutes earlier than methomyl, was unacceptable in acetonitrile solution; whereas the peak shape of methomyl was better but not optimum (Figure 3a). The peak shape of metamitron, which elutes later than methomyl, was good in both acetonitrile and methanol / water sample solutions (Figures 3a, 3b). With the divert valve switched to the waste position for 1.30 minutes in the beginning of the run, the peak shapes of both omethoate and methomyl resembled those in the methanol / water sample solutions (Figure 3c).

The amount of time the valve was in the waste position affected the combination of peak shape and S/N ratio. As shown in Figure 4, the optimum combination of peak shape and RMS S/N ratio was achieved with a divert valve time of 1.30 minutes. Longer duration times were avoided, since the column equilibration was disturbed.

Figure 5 shows the range of injection volumes used. To assess the dependence between each compound peak area and the corresponding injection volume, eight injection volumes (1–8 µL) at a level of 10 µg/L were run three times each. The linear correlation coefficients (R^2 values) of the curve plots for all analytes studied were >0.99, and relative standard deviations were <20% (range 1%–14%). A S/N ratio greater than 10 for acephate and omethoate could not be achieved for injection volumes of 1 µL and 2 µL.

Figure 6 shows the curve of each compound's peak area versus concentration for a 5 µL injection volume. Seven different concentration levels (5, 10, 25, 50, 70, 100, 200 µg/L) with 5 µL injection volumes were run three times. The linear correlation coefficients (R^2 values) of the curve plots for all analytes studied were >0.99 and relative standard deviations were <20% (range 2%–16%). Using 5 µL injections of 5 µg/L acetonitrile solutions, RMS S/N ranged between 75 and 263,000.

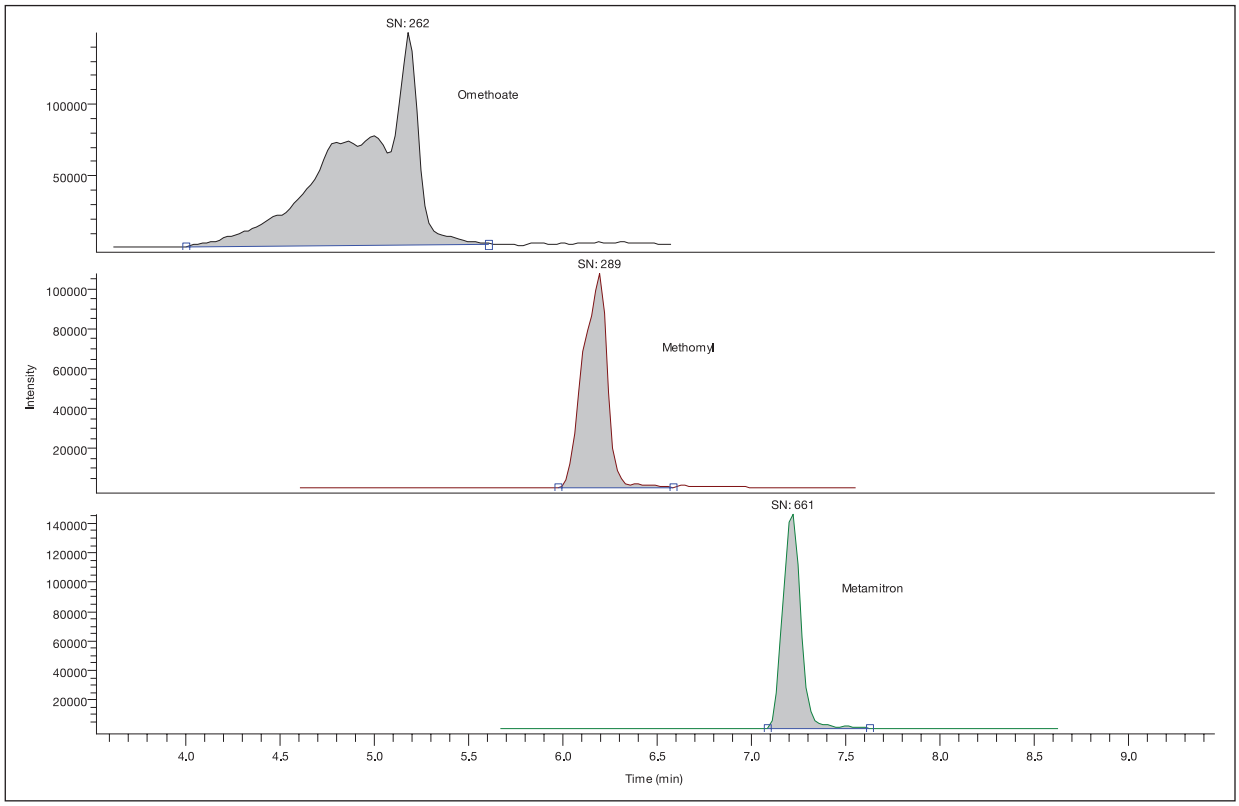

Figure 3a. Extracted chromatograms of 50 µg/L omethoate, methomyl, and metamitron in acetonitrile solution with no divert valve

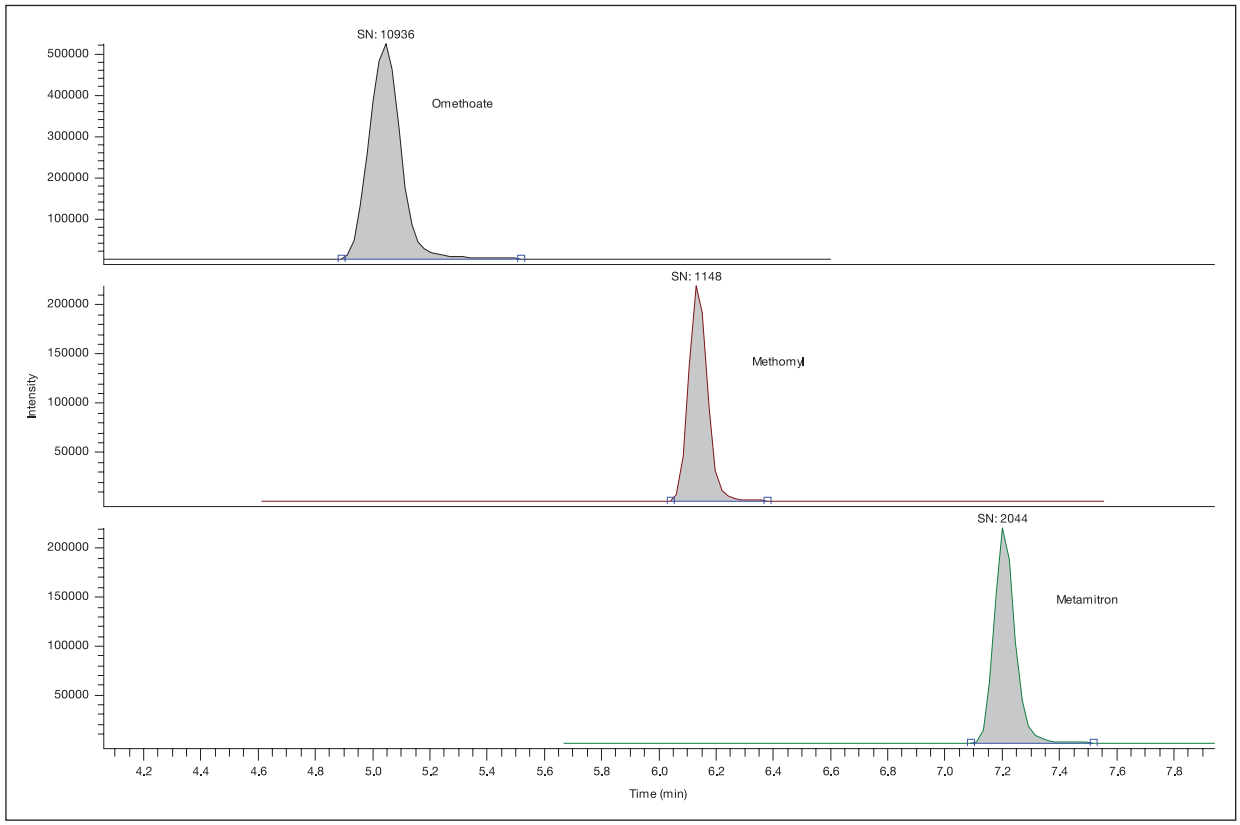

Figure 3b. Extracted chromatograms of 50 µg/L omethoate, methomyl, and metamitron in methanol / water [1:1 v/v] solution with no divert valve

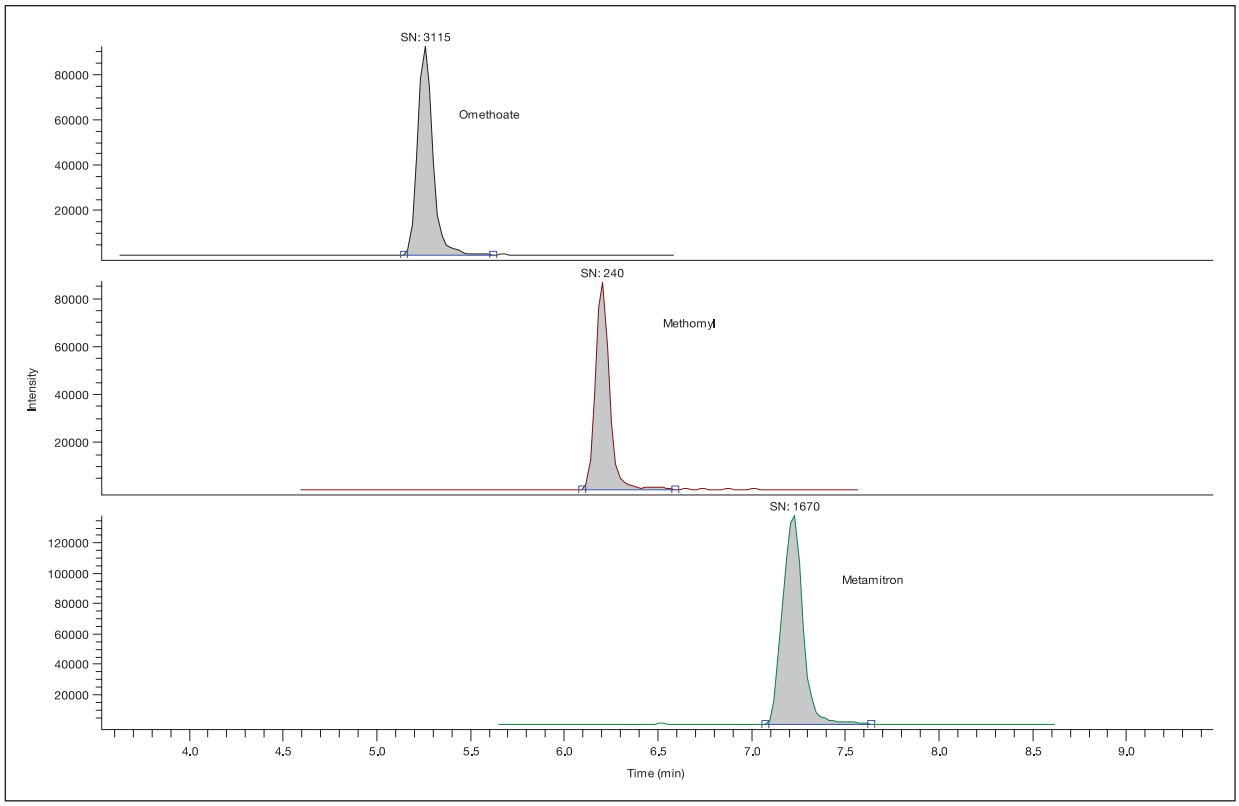

Figure 3c. Extracted chromatograms of 50 µg/L omethoate, methomyl, and metamitron in acetonitrile solution with divert valve open for 1.30 minutes

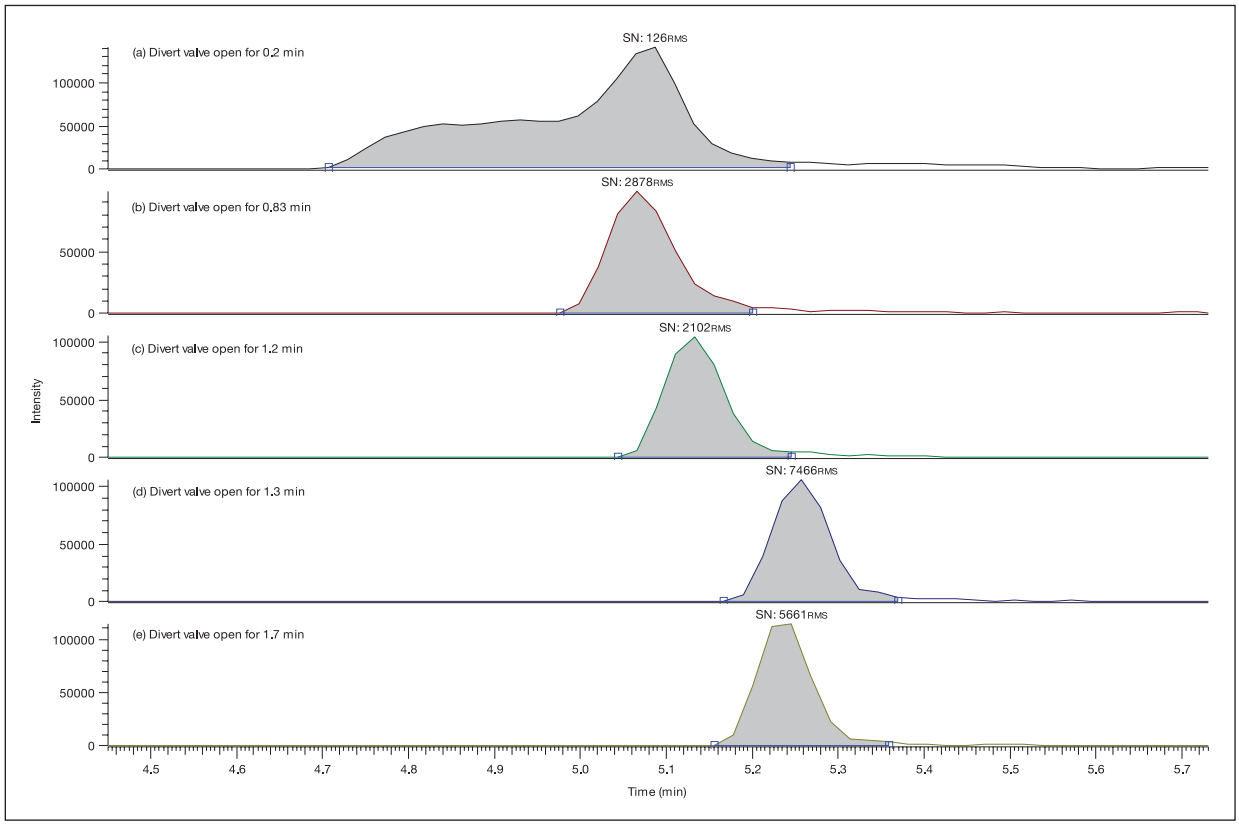

Figure 4. Extracted chromatograms of 5 µL injections of omethoate in 50 µg/L acetonitrile solution with various divert valve duration times used

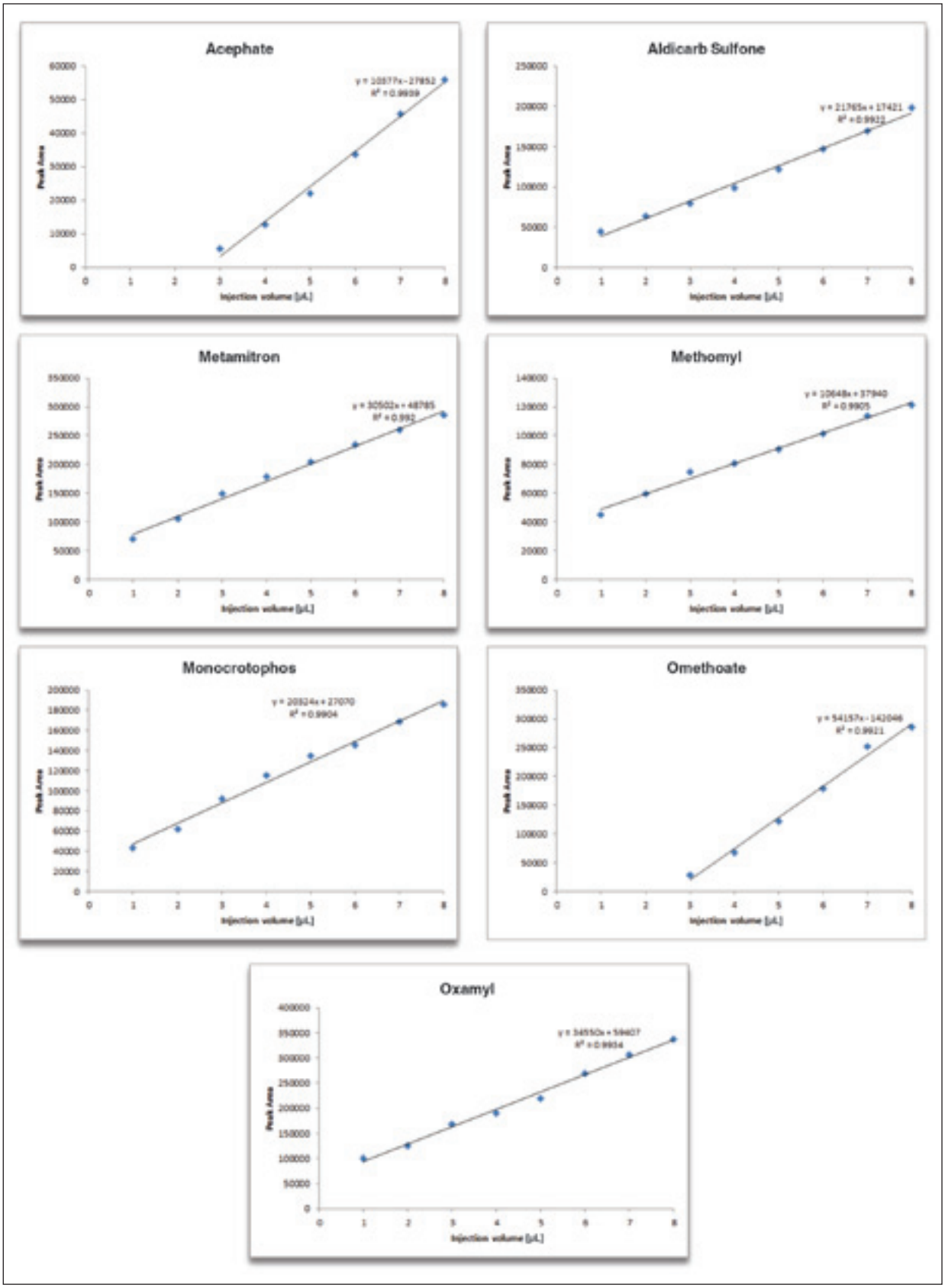

Figure 5. Curves for analyte peak area versus injection volumes 1-8 µL in 10 µg/L acetonitrile solution

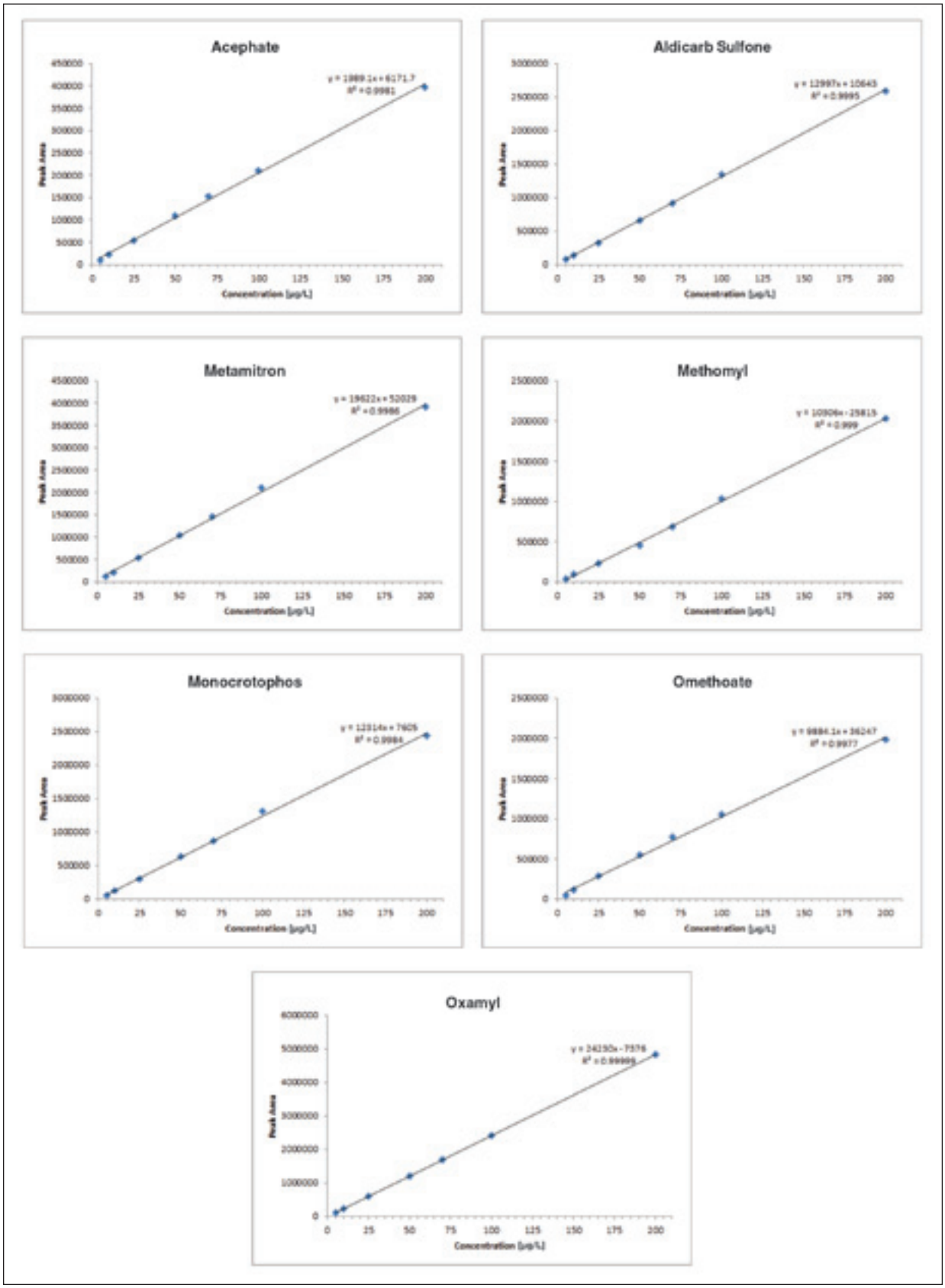

Figure 6. Curves for analyte peak area versus concentration 5-200 µg/L acetonitrile solution with 5 µL injection volume

Conclusion

The use of a divert valve proved suitable for the analysis of early eluting pesticides in acetonitrile solutions. Good peak shapes and S/N ratios were achieved and chromatographic problems, such as peak splitting or broadening, were overcome. In addition, the injection volume was increased up to 8 µL, reaching low detection limits with good linearity and repeatability, even for a sample concentration of 5 µg/L. It may be possible to increase the injection volume to 10 µL, and in some cases up to 15 µL, but with a larger loop volume. After the initial experiments, we concluded that a 5 µL injection volume is sufficient to achieve RMS S/N ratio greater than 10.

This technique resolves chromatographic issues involving interactions of gradient and sample solvent in a simple way and offers an increased laboratory sample capacity by avoiding solvent exchange in the final extract.

Reference

1. Jake L. Rafferty, J. Ilja Siepmanna, Mark R. Schure *Journal of Chromatography A*, **2011**, *1218*, 2203–2213.

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