Automated SPME for the Analysis of Environmental Contaminants in Milk by GC-MS/MS

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Introduction

Organophosphorus (OP) pesticides are a class of insecticides known for their acute toxicity towards a large variety of insects, and a relatively lower persistence in the environment compared to organochlorine pesticides. Gas chromatography mass spectrometry (GC/MS) instrumentation is a powerful tool for the separation and identification of volatile and semi-volatile compounds, including many OP pesticides. Gas chromatography coupled to a triple quadrupole mass spectrometer (GC-MS/MS) allows for even greater separation, as the mass spectrometer is operated in the Multiple Reaction Monitoring (MRM) mode with two stages of mass analysis. This mode of detection virtually eliminates background interference, making GC-MS/MS a powerful technique with improved sensitivity, selectivity, and specificity.

Solid Phase Micro-Extraction (SPME) has produced promising results when used as a sample preparation technique for complex matrices. The SPME technique has several desirable advantages; most importantly it does not require the use of harmful organic solvents, and it is easily automated.

This poster describes development of a fully automated SPME extraction method combined with GC-MS/MS analysis for detection and quantitation of OP pesticides in a complex milk matrix. The Shimadzu GCMS-TQ8040 triple quadrupole mass spectrometer used for this application note is shown in Figure 1. The SPME method development and validation were fully automated using the Shimadzu AOC-6000 Multifunctional Autosampler.

Figure 1: Shimadzu GCMS-TQ8040

Experimental

Headspace - Solid Phase Micro Extraction (HS-SPME)

The HS-SPME extraction was performed using a fiber coated with polydimethylsiloxane-divinylbenzene (PDMS-DVB, 65 µm) (Supelco, Bellefonte, USA). Individual aliquots of milk were placed in 20-mL SPME vials and spiked with the OP pesticide mix to yield a constant concentration of 1.25 µg/mL. To minimize the number of individual analyses required, a half factorial experimental

design was used to optimize the experimental variables affecting the SPME extraction. The half-factorial experimental design provides the maximum amount of information with the least number of analyses. It can be used to determine the effect from each independent parameter, and to identify any interaction effects when more than one parameter influences the observed result.

GC-MS/MS

The GC-MS/MS was operated in the Multiple Reaction Monitoring (MRM) mode. Three transitions were monitored for each target compound, one for quantitation, and two additional transitions for confirmation. The Shimadzu Smart Pesticide Database was used as the foundation for creating the MRM analysis method.

Results and Discussion

GC-MS/MS

The MRM method was established by using the three most prominent MRM transitions for the OP pesticides found in the Smart Database; compound retention times were predicted for the method using the principle of Retention Indices. Figure 2 shows an MRM chromatogram of the 8-pesticide mix at 10 µg/mL, with all peaks baseline resolved.
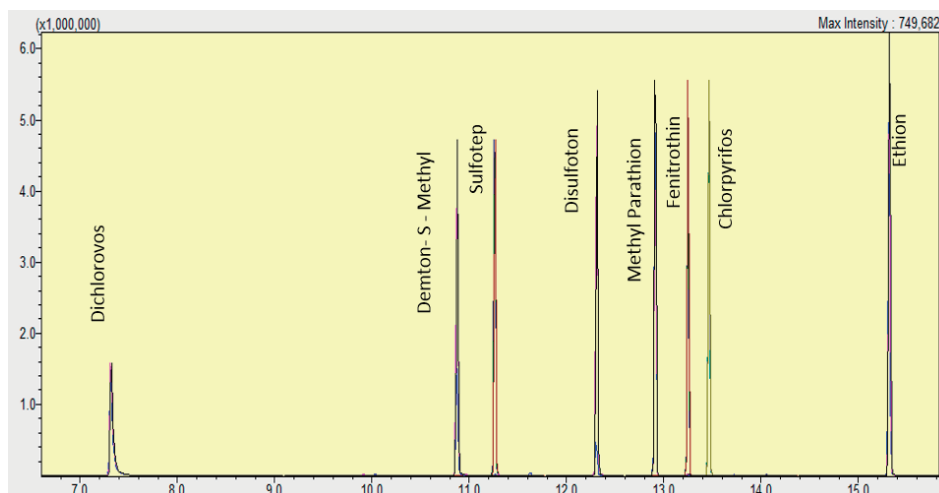
Figure 2: MRM Chromatogram of the Eight OP Pesticides at 10 µg/mL

HS-SPME Method Development

The results obtained from the evaluation of the significant parameters by half factorial design are summarized in the Pareto Chart of Standardized Effects, shown in Figure 3.
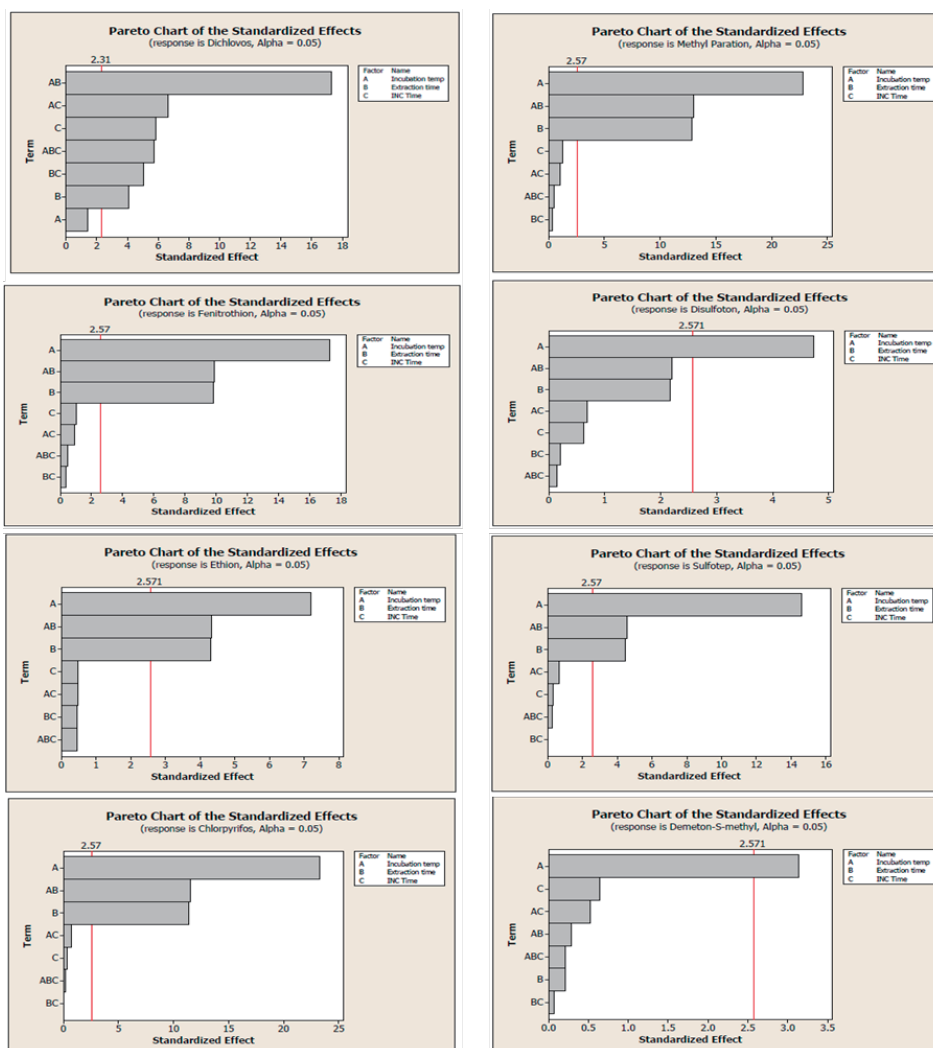
Figure 3: Pareto Chart of Standardized Effects for 8 OP Pesticides Extracted by HS-SPME

As can be seen by the Pareto charts in Figure 3, only two of the five test variables were found to consistently have a significant effect on extraction of the pesticides during the SPME method development, as indicated by extension of the bar beyond the vertical red line. The Incubation Temperature (parameter A) crosses the vertical red reference line for seven of the eight pesticides, indicating that it was a decisive factor for HS-SPME extraction experiment. This is in agreement with HS-SPME principle, as the incubation temperature is a key factor for driving pesticides from sample into the headspace during the equilibration step.

Extraction Time (parameter B) was significant for 6 of the 8 pesticides, indicating that it was also an important factor for SPME extraction of pesticides from the milk.

Method Validation

Calibration standards were prepared and analyzed using the optimized HS-SPME and GC-MS/MS parameters, with concentrations ranging from 20 to 1000 µg/L (ppb). Figure 5 shows the individual calibrations curves for all 8 pesticides, with the corresponding correlation coefficients illustrating linearity.

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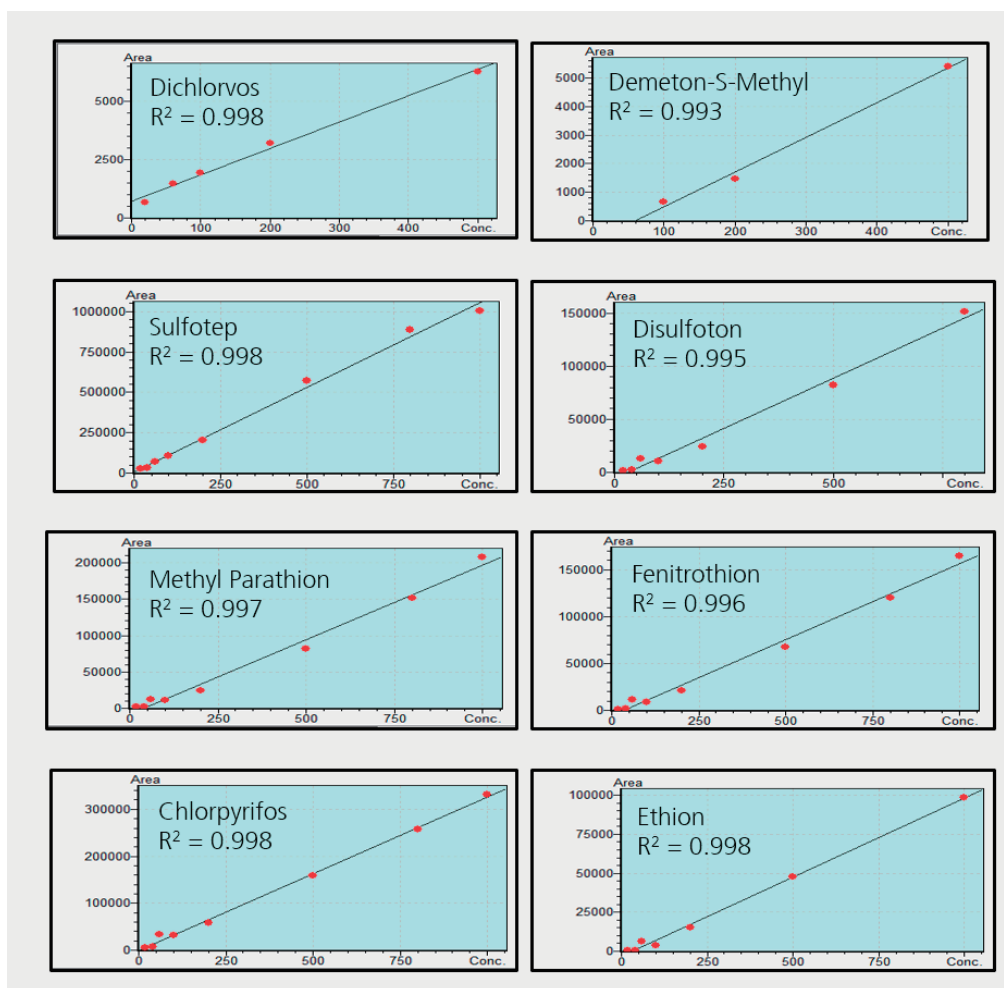

Figure 5: Calibration Plots for all 8 Pesticides in Milk using HS-SPME and GC-MS/MS

Table 1 lists the method validation statistics as defined by IUPAC guidelines, which include linearity, repeatability as % RSD, and accuracy as % Recovery for extraction of pesticides in milk by the HS-SPME method developed here. All pesticides had repeatability of less than 12.8 %, and recovery between 82 and 105 %, as calculated using a spiking level of 20 μg/L.

Table 1: Method Validation Statistics

Pesticide	Conc. in µg/L (R^2)	% RSD (20 µg/L)	% Recovery (20 µg/L)
Dicholorovos	20-600 (0.998)	5.3	87.7
Demeton-S-Methyl	100-600 (0.993)	12.8	82.4
Sulfotep	20-1000 (0.998)	3.5	88.8
Disulfoton	20-1000 (0.995)	8.4	105.1
Methyl Parathion	20-1000 (0.997)	3.1	92.9
Fenitrothin	20-1000 (0.996)	2.5	90.9
Chlorpyrifos	20-1000 (0.998)	1.6	88.8
Ethion	20-1000 (0.998)	4.3	93.1

Conclusion

A method based on HS-SPME, presented here for the analysis of organophosphorus pesticides in milk, was developed using a fully automated AOC-6000 Autosampler. SPME-GC-MS/MS in multiple reaction monitoring (MRM) mode was used for the trace analysis of pesticides in milk. The method was calibrated from 20 µg/L to 1000 µg/L, and all validation statistics were within an acceptable range.

References

1. Analysis of Organophosphorus Pesticides in Milk Using SPME and GC-MS/MS, Application News No. GCMS-1603.
2. The Smart Forensic Database, Shimadzu Corporation (Japan), is part of the Smart Database Series. The database contains 480 pesticides and approximately 2,680 transitions, and eliminates the need to configure complicated analysis conditions. Retention Indices (RI) are registered for all components, enabling easy updating of retention times via the AART function. Analysis by the internal standard method is also supported, with MRM transitions and RIs registered for compounds commonly used as internal standards for pesticides.
3. Antony,Jiju: Design of Experiments for Engineers and Scientists: Butterworth-Heinemann, Sep 5, 2003, Pages 36-42.
4. Volante, Marco; Pontello, Mirella: Application of Solid Phase Micro-Extraction (SPME) to the analysis of pesticide residues in vegetables: 56(7); 618–636, 2000.

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