Application Note 02213

Added Robustness and Flexibility: Employing SelecTemp™ and SelecFlow™ for the Determination of Pesticides in a QuEChERS Matrix on the Varian 325-MS Triple Quadrupole Mass Spectrometer

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Introduction

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is a widely popular sample preparation technique used for rapid extraction of pesticides from fruits and vegetables. This high-throughput sample extraction procedure requires a robust LC/MS/MS method that minimizes downtime, maximizes productivity and meets the required method detection limits. Varian SelecTemp™ and SelecFlow™ are two features available on the 325-MS, which allow the analyst to modify the drying and vortex gas temperatures and pressures throughout an analytical run. As such, atmospheric pressure interface (API) conditions can be programmed to obtain the optimum conditions for a differentiated set of target compound chemistries. These features, along with the ability to divert eluent away from the ionization source, can greatly reduce source contamination, minimize ion suppression and maintain acquisition of high quality data.

Instrumentation

- Varian 325-MS triple quadrupole mass spectrometer with vortex ESI (vESI™) source
- Varian 212-LC binary solvent delivery system
- CTC Analytics HTS PAL™ autosampler with cooling option

Materials and Reagents

- Analytical standards of >94% purity were obtained from AccuStandard, Inc. (New Haven, CT).
- HPLC grade methanol and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA).
- Sodium chloride, magnesium sulphate anhydrous and trisodium citrate dihydrate were purchased from Sigma Aldrich (St. Louis, MO).

The extract was prepared from raw carrot and green beans using a modified QuEChERS extraction method.[1,2] The final cleanup steps dictated by the method were not used. The final contaminated extract represented a worst-case scenario QuEChERS matrix. This matrix was then diluted with solvent A 1:1, and spiked with standards to a final concentration of 500 ppb.

HPLC Conditions

Column: Pursuit™ XRs C18, 3 µm, 50 x 2.0 mm (Varian Part Number A6001050X020)

Guard Column: Pursuit XRs C18, 3 µm, MetaGuard™ 2.0 (Varian Part Number A6001-MG2)

Solvent A: 2 mM ammonium formate and 0.05% formic acid in water

Solvent B: Methanol

Column Heater: MetaTherm™ HPLC column heater (Varian Part Number A9540)

LC Program:

Time (min:sec)	%A	%B	Flow (µL/min)
0:00	98	2	300
2:00	98	2	300
12:00	10	90	300
14:00	10	90	300
14:05	98	2	300
15:00	98	2	300

Injection Volume: 20 µL

MS Parameters

Ionization Mode: vESI (positive)

API Nebulizing Gas: 70 psi

Detector: Extended Dynamic Range (EDR) Max

Needle: ±4000 V

Shield: ±600 V

CID Gas Pressure: 2.0 mTorr

Drying Gas: 300 °C at 35 psi

Vortex Gas: 250 °C at 25 psi

Table 1. 325-MS segment parameters.

CAS Number	Compound	MRM	Capillary Voltage (V)	Collision Energy (V)
35554-44-0	Imazalil	297 → 159 297 → 201 297 → 255	70	20 15.5 14.5
29232-93-7	Pirimiphos-methyl	306 → 95 306 → 108 306 → 125 306 → 164	65	24 26 24 19
5598-13-0	Chlorpyrifos-methyl	322 → 125 322 → 290 324 → 125 324 → 292	63	24.5 15.5 24.5 15.5
2921-88-2	Chlorpyrifos	350 → 97 350 → 198 352 → 97 352 → 200	55	24.5 15.5 24.5 15.5

Results and Discussion

The LC/MS/MS method was developed using Varian SelecTemp™ and SelecFlow™ features, along with the built-in divert valve on the 325-MS. Initially, the column flow was diverted to waste. During the divert time, a syringe pump containing a solution of 98:2 A/B at 20 µL/min was allowed to flow into the vESI™ source. This prevents arcing and helps bring the vESI assembly into equilibrium while the column flow is diverted to waste. During the final segment of the run, the source gas temperatures and pressures were increased. This procedure reduced contamination and ion suppression, while it increased reproducibility and robustness. Figure 1 shows a flow schematic of the valve configuration when used in divert mode.
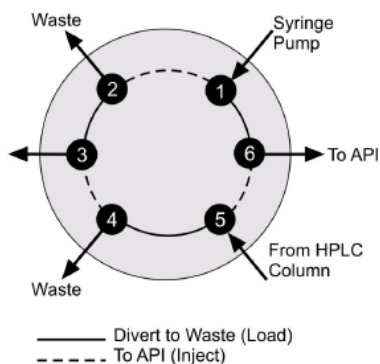

Figure 1. Schematic flow diagram for the 6-port valve in divert mode. The valve and syringe pump can be controlled through MS Workstation software.
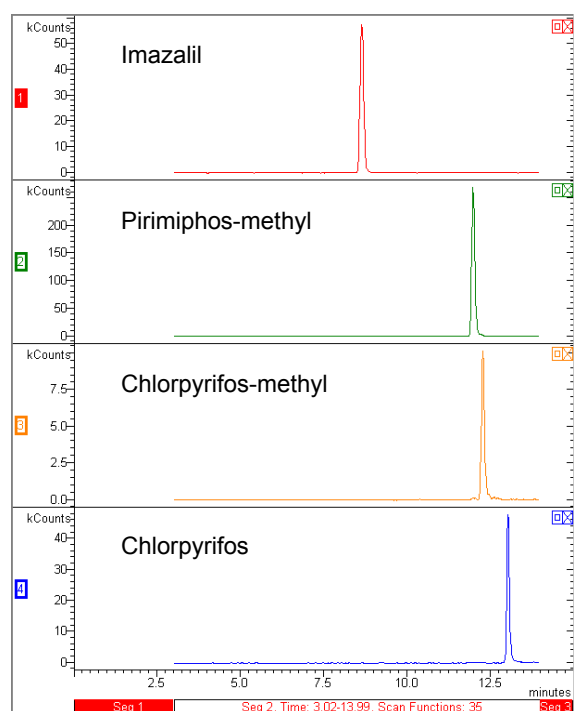

Figure 2. Extracted ion chromatograms at a concentration of 500 ppb in the QuEChERS extract.

Table 2. %RSD values for 500 on-column injections.

Compound	%RSD
Imazalil	4.6
Pirimiphos–methyl	5.4
Chlorpyrifos–methyl	6.2
Chlorpyrifos	6.5

Figure 2 shows the stacked extracted ion chromatograms for four pesticides: Imazalil, Pirimiphos-methyl, Chlorpyrifos-methyl and Chlorpyrifos. Figures 3-6 show graphs of area versus injection number for the four pesticides. The calculated RSD values ranged between 4-7% for the 500 injections. The %RSD values are shown in Table 2.
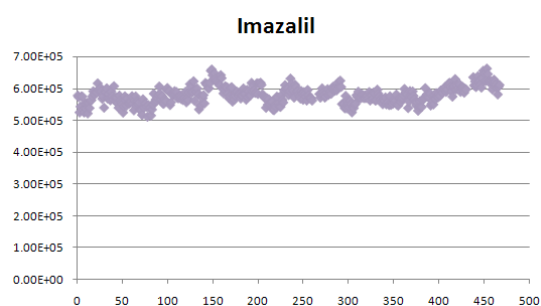

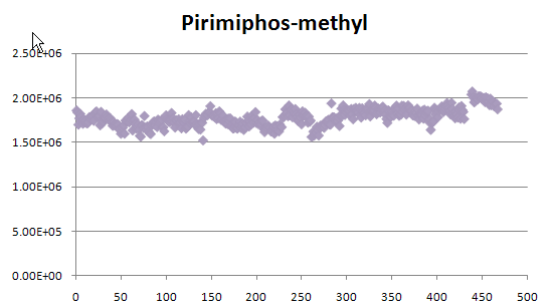Figure 3. Peak area versus injection number for Imazalil.

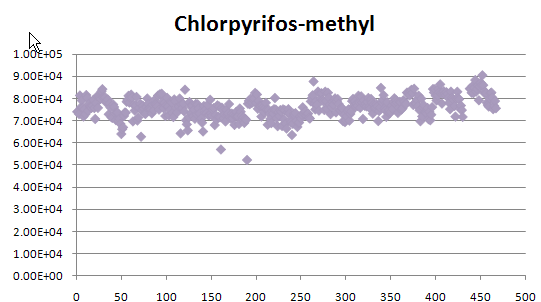Figure 4. Peak area versus injection number for Pirimiphos-methyl.

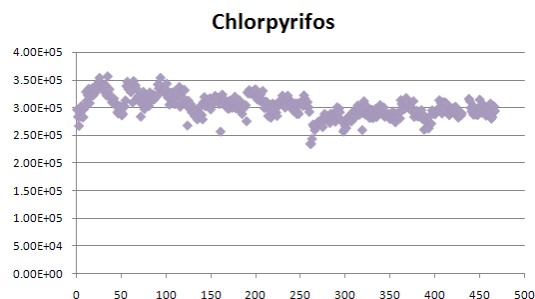Figure 5. Peak area versus injection number for Chlorpyrifos-methyl.

Chlorpyrifos

Figure 6. Peak area versus injection number for Chlorpyrifos.

Conclusion

The Varian 325-MS LC/MS/MS was used with the divert valve in conjunction with the SelecTemp™ and SelecFlow™ features to reduce source contamination, minimize ion suppression and maintain acquisition of high quality data. Four pesticides spiked into a crude QuEChERS extract were evaluated, showing excellent %RSD of raw peak area for 500 injections.

These instrument features are unique to Varian, and combine with Varian HPLC columns and consumables for a complete Varian solution.

References

1. http://www.quechers.com/docs/quechers_en_oct2005.pdf

2. M. Anastassiades, S. J. Lehotay, D. Stajnbaher, F. J. Schenck, Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, *J. AOAC Int.*, 86 (2003) 412-431.

These data represent typical results.
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