Application Note: 52102

Analysis of Pesticides in Citrus Oil using PTV Backflush with GC-MS/MS Triple Quadrupole for High Sample Throughput

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Key Words

- TSQ Quantum XLS
- Backflush
- Citrus Oil
- Food Safety
- Maximum Residue Levels (MRL)
- Pesticide Analyzer Reference
- Selected Reaction Monitoring (SRM)

Introduction

International regulations on maximum residue levels (MRLs) of pesticides in food cover hundreds of individual contaminants at the 10 ppb or below range. With more than 500 regulated pesticides that can be analyzed using gas chromatography-mass spectrometry (GC/MS) and the large number of food/agricultural samples there is a large number of samples that must be tested.

The analysis of citrus oil for pesticide contamination holds specific challenges. Pesticides that are used on the citrus crop are concentrated along with the matrix in this oil. This matrix is known for its high boiling point components. Complicated methods have been developed to deal with this. Many of these include long sample preparation or significant work on the instrumentation to deal with the contamination.

This application describes the analytical methodology for fast multi-residue determination using the unique timed selected reaction monitoring (timed-SRM) capabilities of the Thermo Scientific TSQ Quantum XLS triple quadrupole mass spectrometer. The function of timed-SRM allows the operator to easily setup methods and run samples while the instrument automatically determines the optimal time for SRM parameters. Using the unique and simple backflush capability of the Thermo Scientific TRACE GC Ultra with programmable temperature vaporizing inlet (PTV) removes all high boiling point contamination before it damages any part of the analytical system. The system of the TRACE GC Ultra™ and the TSQ Quantum XLS™ GC-MS/MS allows for the analysis of more than 40 pesticides in citrus oil with minimal sample preparation.

Method

Sample Preparation

The samples were simply diluted 20-to-1 using hexane. No other sample preparation was done. The samples for method development and calibration curve were made by spiking the list of pesticides into a known clean sample.

TRACE GC Ultra with Backflush

The TRACE GC Ultra PTV injector with backflush was the injector chosen due to the heavy contamination potential from the citrus oil matrix. During the method development process it was found that there was a significant amount of high boiling point matrix, Figure 1. Using backflush with the PTV provided a means to remove that from the system. The initial temperature of the PTV was set high enough to load the analytes of interest and light matrix while excluding the high boiling matrix. The temperature of the injector was increased to 310 °C and the gas flow through the injector was reversed to back flush the contaminants through the split vent. This provides the flow required for the analytical column and ensures contamination does not cause a problem for the column or the rest of the system.

The oven temperature profile used provided effective separation of the compounds of interest. The beginning temperature optimized the backflush performance for the method. It was found during the method development process that using a higher flow rate during the loading of the column provided slightly faster run times and allowed for more thorough removal of the high boiling point matrix, as seen in Figure 2.

Figure 1 shows the very heavy matrix effect of this assay without backflush at the beginning of the method development process. The marker labels show the characteristic compounds that can be followed through the method development process. Marker 5 shows the high-boiling-point contamination, without the use of backflush, which was a problem for the long-term performance of the system.

Figure 2 shows the same compounds labeled with markers 1 through 5. This chromatogram, of the same sample, shows the completed method with the use of backflush. The retention time difference in the two is due to an increased flow rate during the transfer of the compounds to the column. This transfer flow rate was increased to 4 mL/min for the final method. The method demonstrated in Figure 2 provides an increase in system robustness and good precision.

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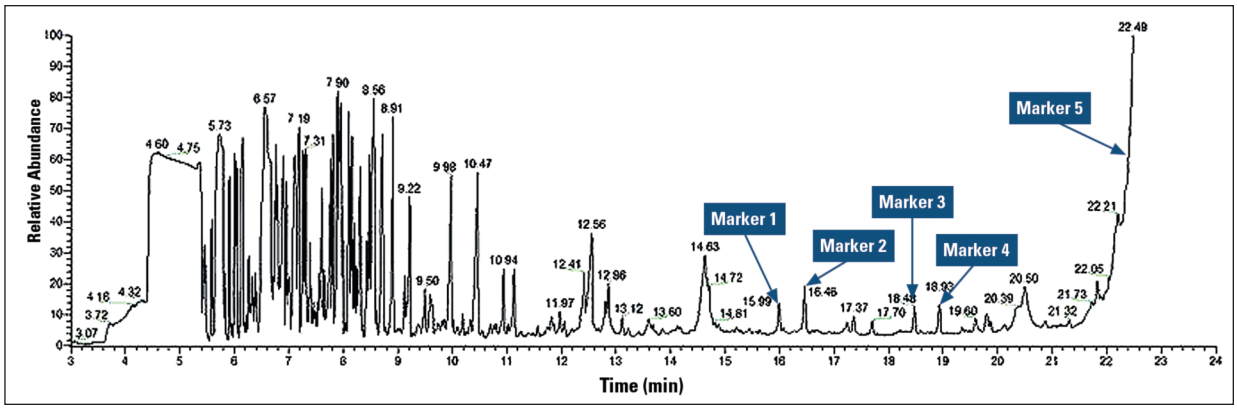

Figure 1: Chromatogram at the beginning of the method development process demonstrating no backflush

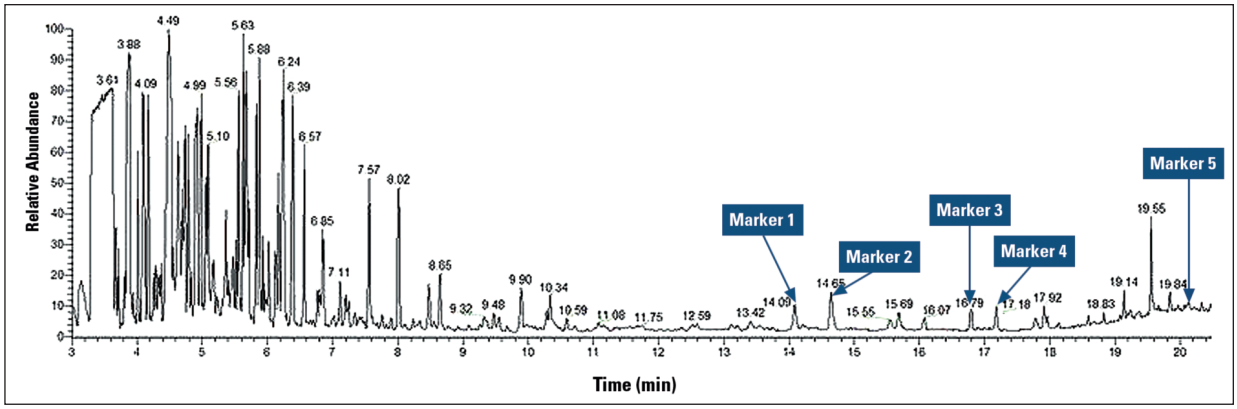

Figure 2: Chromatogram of the fully developed method. Backflush was implemented and a higher column flow rate was used. The markers in both chromatograms point out the common compounds. Marker 5 shows removal of the paraffins using backflush.

MS Timed-SRM Method

All selected reaction monitoring (SRM) transitions were optimized for best collision energy and full conversion from precursor to product ion for monitoring. Each analyte had at least one confirming ion. A portion of the transition list is seen in Table 1. The information for these transitions was provided by the Pesticide Analyzer Reference (PAR) version 2 (part number 120390). The function of timed-SRM was used to allow the instrument to provide an automatic means of determining the most efficient use of its time. Timed-SRM allows the instrument to spend no more time than is necessary for monitoring a specific SRM transition. This time is automatically determined and allows for partial overlap of SRM transition monitoring. This is significantly different from time-segmented SRMs. During the time-segmented SRMs the instrument is programmed to monitor all of the transitions in that time. The instrument cannot provide or compensate for partial overlaps. This means that the timed-SRM function of the TSQ Quantum XLS system provides more points across the peaks with better sensitivity and precision.

The method used for the analysis was a 1 µL injection of the citrus oil, diluted 20:1 in hexane. The TRACE GC Ultra PTV backflush time was set to the point just after the last compound of interest was loaded onto the analytical column. The TSQ Quantum XLS SRM transition information for the more than 40 pesticides was provided by PAR with specific adjustments made for the matrix. Timed-SRM provided the best possible precision. Matrix samples were used in method development and calibration curves. Each calibration curve ranged from 10 to 200 ppb.

#	Parent	Product	Collision Energy	Start Time	Stop Time	Polarity	Name
1	170.07	115.05	15	6.30	6.70	+	2-Phenyphenol_2
2	170.07	169.07	20	6.30	6.70	+	2-Phenylphenol
3	180.91	144.93	15	7.40	8.00	+	alplha-BHC_2
4	234.94	198.95	15	15.10	15.80	+	4,4'-DDT
5	236.94	164.96	17	15.10	15.80	+	4,4'-DDT_2
6	314.03	245.03	15	16.30	17.00	+	iprodione_2
7	316.03	247.03	15	16.30	17.00	+	iprodione

Table 1: A portion of the compound list for this method. The Pesticide Analyzer Reference was used to develop this method.

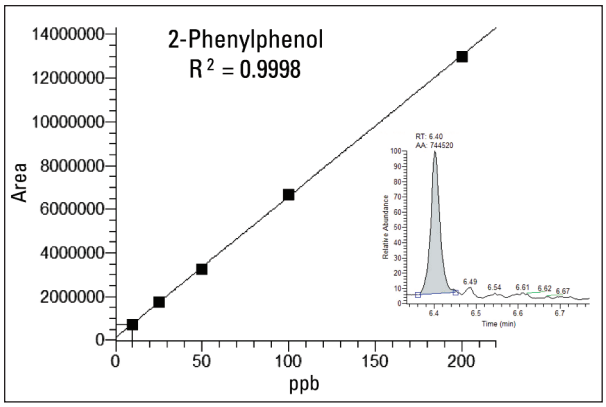

Figure 3: Calibration curve for 2-phenylphenol with the quantitation peak at 10 ppb

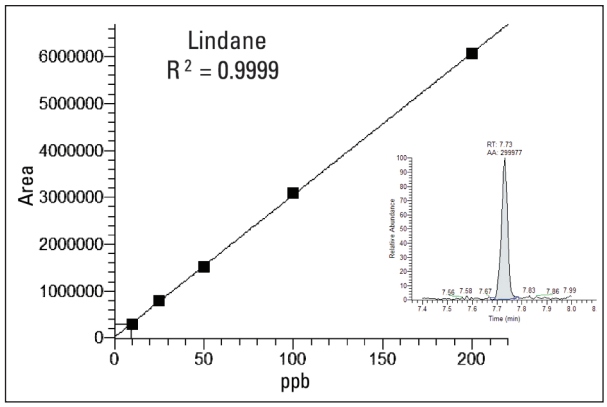

Figure 4: Calibration curve for α-lindane (BHC) with the quantitation peak at 10 ppb

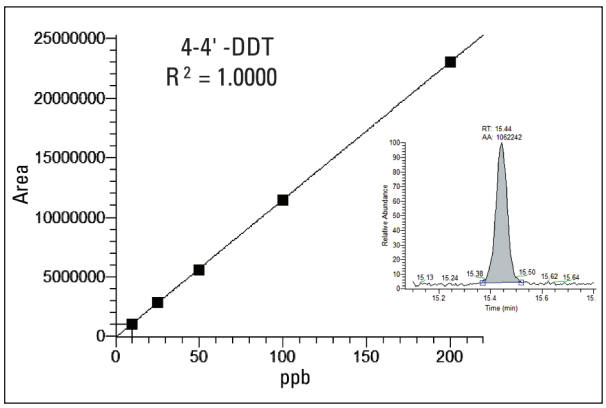

Figure 5: Calibration curve for 4,4'-DDT with the quantitation peak at 10 ppb

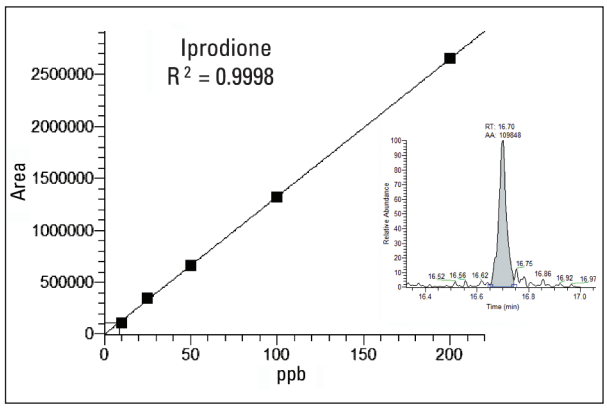

Figure 6: Calibration curve for iprodione with the quantitation peak at 10 ppb

Results

In the final developed method, the last peak of the more than 40 pesticides eluted in less than 20 minutes. A subset of the calibration curves are given below. The extracted standards showed better than 0.995 r^2 values for the correlation coefficient. The peaks shown with the curves in Figures 3 through 6 show the low point on the calibration curve, at 10 ppb, extracted from the citrus oil matrix.

Precision of the final method is demonstrated in Figure 7. Six matrix samples were spiked at 25 ppb level. These samples were analyzed and the areas were used to demonstrates stability of the final method, including backflush. The relative standard deviations for the areas ranged from 0.69% for α-lindane to 3.68% for iprodione.

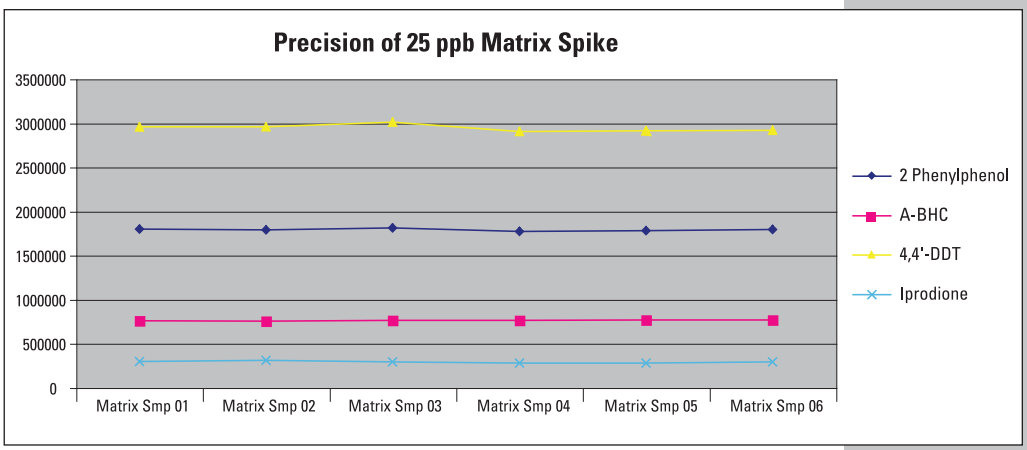

Figure 7: Demonstration of precision in matrix samples spiked to develop the final method

Conclusion

The use of PTV with backflush allowed the development of a method with very little sample preparation. Backflush of the PTV kept all of the high-boiling-point contamination from reaching any part of the analytical system, reducing maintenance. It also provided an increased sample throughput by decreasing time needed for sample or system cleanup. The timed-SRM function of the TSQ Quantum XLS provided the most efficient use of the instrument time, only monitoring the expected SRMs when needed. The timed-SRM function provided the precision needed for the low level analysis of pesticides in citrus oil.

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