Application Note: 10049

Trace Determination of Organo-Phosphorous Pesticides in Olive Oil by GC Analysis through PTV Backflush / FPD

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Key Words

- TRACE GC Ultra
- Olive Oil
- Organo-Phosphorus Pesticides
- ppb Levels
- PTV Backflush
- FPD Selectivity

Introduction

Organo-Phosphorous Pesticides (OPP) are widely used in agriculture, due to their relatively low cost, broad spectrum of activity, and high impact on insects compared to other pesticides. However, because the OPPs are well known to cause irreversible effects on the nervous system (reduced activity of neurotransmitters), their possible presence as trace residues in food must be strictly monitored. In this respect, one critical application is the control for OPPs in olive oil.

This class of compounds can effectively be analyzed by Gas Chromatography using a Programmable Temperature Vaporizing (PTV) injector and a Flame Photometric Detector featuring extremely high sensitivity and selectivity for phosphorus containing compounds.

The PTV injector is found to be particularly suitable for samples like edible oils, characterized by the presence of heavy fractions in potentially dirty matrices. The conventional Split-Splitless injector is advantageously able to be kept at a low temperature during the sample introduction phase. This prevents any sample evaporation from the syringe needle, hence eliminating a source of discrimination of higher boiling components. On the other hand, compared to the On-column injector, it allows non-volatile sample by-products to be retained in the vaporization chamber, thus preventing any decay of the column performance in time due to by-products accumulation.

This type of analysis requires high oven temperatures and short columns with a very thin film in order to allow complete elution of the main constituents of vegetable oil, triglycerides. Additionally, the sample must also be very diluted in order to avoid overloading the column with this primary fraction (for quantity) and consequent contamination of the detector. These two factors make trace analysis of contaminants even more complex. To overcome these problems, the heavier fraction is usually completely eliminated with an extended sample preparation step prior to GC analysis.

This paper describes an alternative way to effectively and rapidly analyze OPPs in oils eliminating any interference with the heavy fraction. The use of a special accessory vents the heavier components of the sample when these are not of interest.

Back-flush Device for PTV Injector

The Thermo Scientific TRACE GC Ultra™ equipped with a PTV inlet and a reverse flow device (back-flush) is used for this application. This accessory consists basically of a 3-way solenoid valve (back-flush valve) placed in the carrier gas line, a wide-bore pre-column, a high temperature "T" connector housed in the GC oven connecting the pre-column to the column, and a calibrated flow restrictor (Figure 1).

When the back-flush valve is off (Figure 2), the carrier gas flows in its normal direction through the inlet. A very small flow provided by the restrictor is able to constantly purge the "T" connector between the pre-column, analytical column, and back-flush inlet line. The pre-column consists of a 2 m x 0.53 mm i.d. uncoated fused silica tubing, and the purge flow is about 5 % of the column flow.

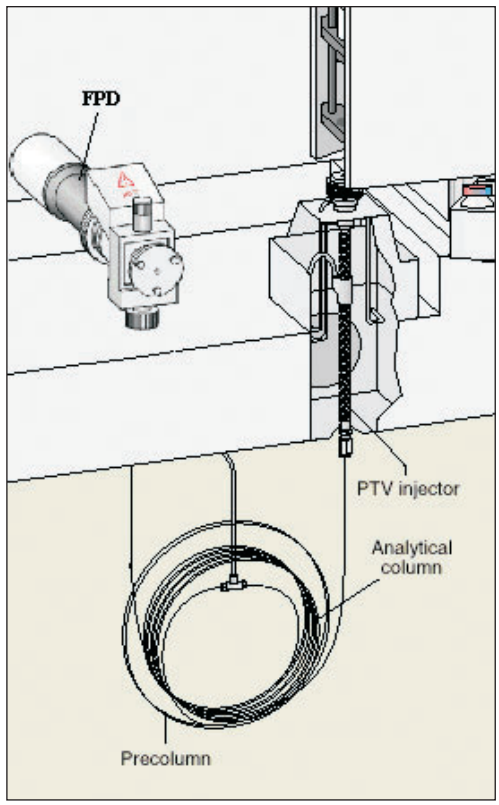

Figure 1: PTV-FPD configuration.

When the back-flush valve is switched on, the system diverts the gas directly to the "T" connection at the end of the pre-column, therefore, sweeping both the latter and the inlet in the opposite direction, with a so called "reverse flow". In this configuration, the carrier gas is able to "flush" anything still in the pre-column or in the injector directly to the vent and through the injector's split line. The small flow provided by the restrictor in the other direction will prevent the back-flushed material to flow through the inlet liner.

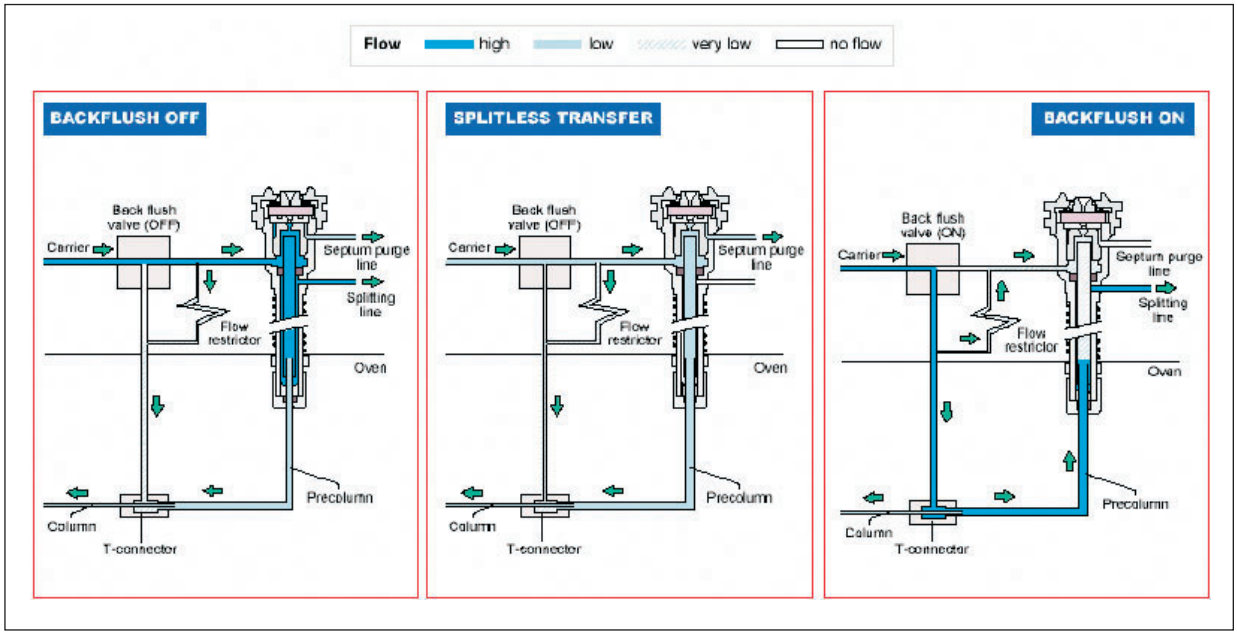

Figure 2: Reverse flow device

In order to clearly demonstrate the effect of the reverse flow device, 2 µL of virgin olive oil diluted 1:10000 in acetone are injected in a TRACE GC Ultra equipped with PTV injector and FID detector. An OV-5, 7 m long, 0.25 mm i.d., 0.25 µm f.t. column is used, together with a 2 m, 0.53 mm i.d. deactivated pre-column. The oven ramp is 60 °C (3 min) to 100 °C at 8 °C/min, then to 380 °C (10 min) at 20 °C/min. The PTV initial Temperature is 80 °C (hold 0.1 min) then ramped at 14.5 °C/sec up to 380 °C (held for all the analysis), with a splitless time of 3 minutes and a split flow of 50 mL/min. Helium is used as carrier gas at constant pressure (55 kPa). Finally the FID detector base body temperature is set at 350 °C.

The same sample is then injected in the PTV equipped with the back-flush device. Since the heavier fraction is now vented out by the reversed flow, the sample is diluted only 1:1 in acetone.

Sensitivity towards the compounds of interest is simply increased by 4 orders of magnitude, and the absence of the predominant fraction allows both to eliminate the risk of column overloading and to target separation optimization on the lighter components only.

Figure 3 shows the two chromatograms obtained with and without back-flush valve activation respectively. The complete absence of the triglycerides in the second chromatogram proves the effective reliability of the reverse flow enabled after 3 minutes. This timing is proven to be sufficient to allow transfer of the compounds of interest into the analytical column, while diverting any residual heavy fraction into the pre-column for venting.

Analysis of OPPs in Olive Oil

The same equipment is used for the determination of Organo-Phosphorous Pesticides with exception of the detection system. A highly sensitive phosphorous-selective FPD detector is used in place of the FID. Performance and repeatability tests are performed by injecting 2 µL of virgin olive oil spiked with 50/100 ppb of OPPs mixture. Also, in this case, the sample is diluted only 1:1 with acetone, and the optimum conditions for the separation of OPPs are applied. An SE54, 10 m long, 0.25 mm i.d., 0.1 µm f.t. capillary column is used, together with a 2 m, 0.53 mm deactivated pre-column. The GC oven temperature starts with an isotherm at 60 °C (1 min) and is then raised to 350 °C (10 min) at 8 °C/min. The PTV Temperature ranges between 50 °C (0.1 min) and 400 °C (held for all the analysis) at 10 °C/sec, with a splitless time of 1 minute. Helium is used as carrier gas at constant flow (1.5 mL/min), and the FPD detector is set at 300 °C. A 300 mL/min back-flush flow is enabled after 16 minutes.

Figure 4 reports the related chromatogram, together with the repeatability of retention times and peak areas based on 10 consecutive injections, showing excellent separation and sensitivity. Three different commercial olive oils were tested under the same conditions (Figure 5): only Fenthion resulted present in Oil 1 and Oil 3 in different amounts, while Oil 2 was found to be completely destitute of such pesticides. A large number of injections of oil (over 100) were performed without replacing the liner or the pre-column, and no degradation of chromatographic performance was observed.

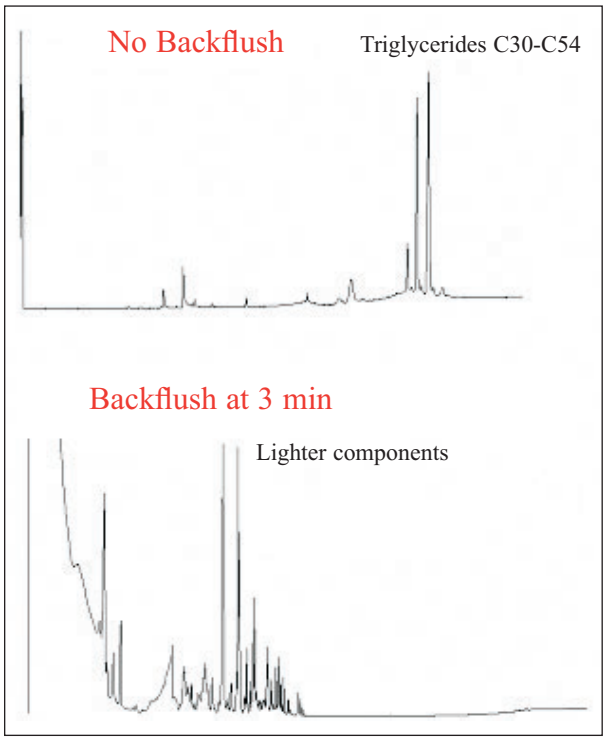

Figure 3: Olive Oil analysis with and without reverse flow; Detector: FID

PEAK NUMBER	SAMPLE COMPOUND	RETENTION TIMES AVERAGE (MIN)	RSD%	PEAK AREAS AVERAGE (COUNTS)	RSD%
1	Dimethoate	16.24	0.08	371681	3.1
2	Parathion-methyl	19.85	0.06	290948	2.6
3	Chlorphiriphos-methyl	18.97	0.05	134474	3.0
4	Malathion	20.04	0.08	174849	5.8
5	Fenthion	20.23	0.04	229989	2.5
6	Chlorphiriphos-ethyl	20.98	0.08	132520	3.7
7	Methidathion	21.89	0.04	826901	3.8

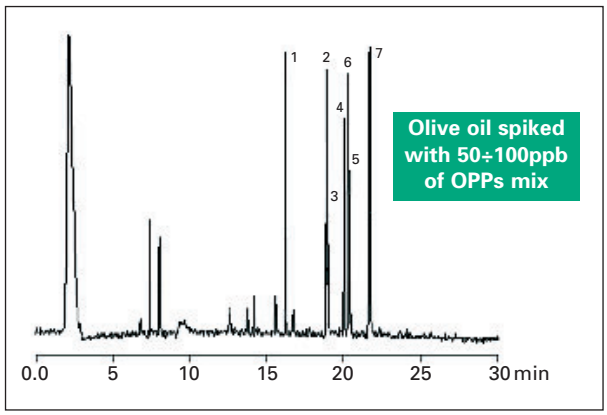

Figure 4: Repeatability Test based on 10 injections; Detector: FPD

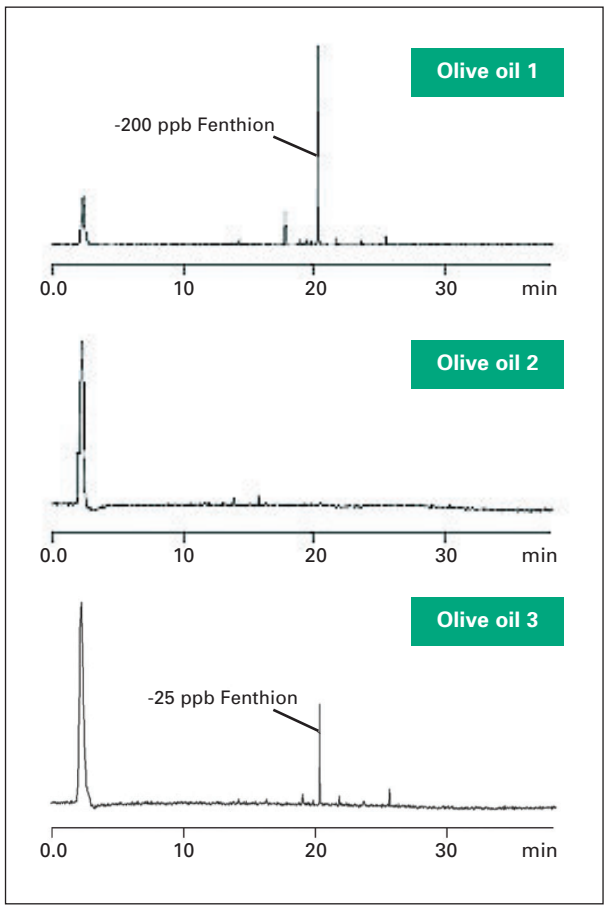

Figure 5: Detection of Fenthion in 3 commercial olive oils; Detector: FPD

Conclusions

OPPs in olive oil matrix can effectively be analyzed with PTV and FPD, provided that the triglycerides are vented out by a reverse flow device. Under these conditions, performance of the PTV injector is found to be greatly improved. The total analysis time is much shorter since no extra waiting time for complete elution of the high boiling components is now required. Sensitivity can be increased by four orders of magnitude (a few ppb) simply through the injection of a more concentrated sample.

Two additional important benefits obtained with the use of the back-flush are the highly extended column lifetime and the strongly simplified sample preparation procedure, which now only requires the dilution of the olive oil with acetone solvent.

Acknowledgement

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