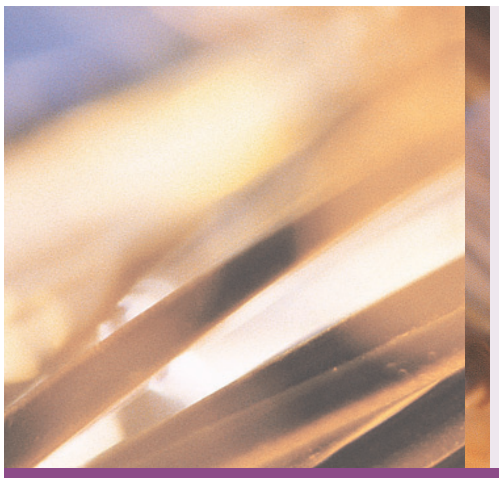


Food Analysis Feature

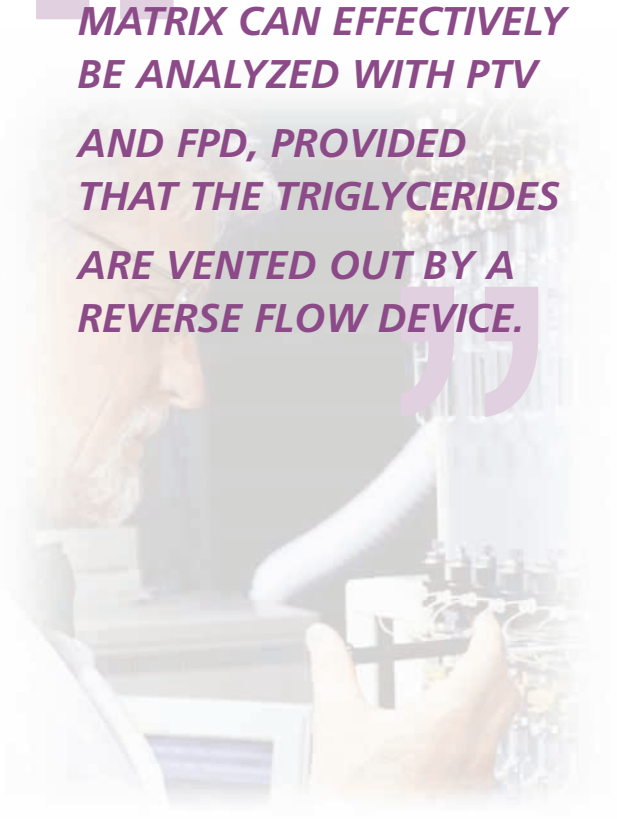
THE ROLE OF CHROMATOGRAPHY IN FOOD ANALYSIS

Thermo Electron Corporation

Whether it is natural, processed or cooked, food is consumed both for the preservation and enjoyment of human life. Food products are very complex mixtures that hold many nutrients of an organic and inorganic nature. In addition to their natural properties, they can contain xenobiotic substances that come mainly from technological processes, agrochemical treatments or packaging materials.

Most reasons for the analysis of food content result from nutrition and health concerns, process-control and quality-assurance purposes or flavour and fragrance issues. Other reasons include the identification of the origin of components, the checking for food adulteration and the "mining" of food for naturally occurring products. The main method that is employed for food analysis is chromatography. The aim of this article is to provide a brief overview of a number of uses of chromatography for food analyses.

"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."



GAS CHROMATOGRAPHY

Since the commercial introduction of gas chromatography (GC) over 50 years ago, GC has been used to help determine food composition and ensure food quality. The technique is commonly employed for analyzing non-polar and semi-polar, volatile and semi-volatile chemicals. The technique is also often used for the analysis of oils, sterols, flavour and fragrance components and many contaminants in food.

For example, 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 GC 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 able to be kept at a low temperature during the sample introduction phase, preventing 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-product 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 extremely 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. The following example 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.

METHOD

A TRACE™ GC Ultra (Thermo Electron Corporation) equipped with a PTV inlet and a reverse flow device (back-flush) was used. This accessory consists 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 precolumn 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 precolumn consists of a 2 m 0.53 mm i.d. uncoated fused silica tubing, and the purge flow is about 5% of the column flow.

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 "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 from flowing through the inlet liner.

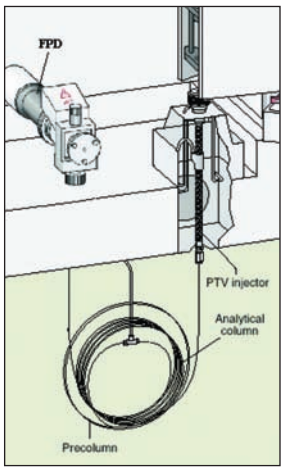


Figure 1. PTV-FPD configuration

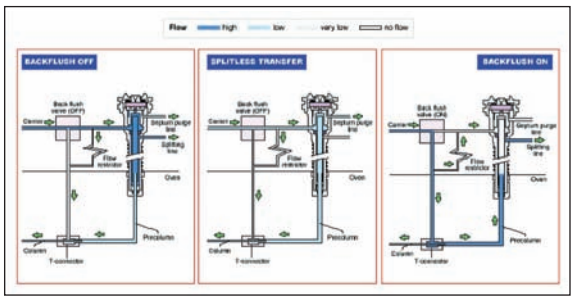


Figure 2. Reverse Flow Device

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, still maintaining any residual heavy fraction into the pre-column for venting.

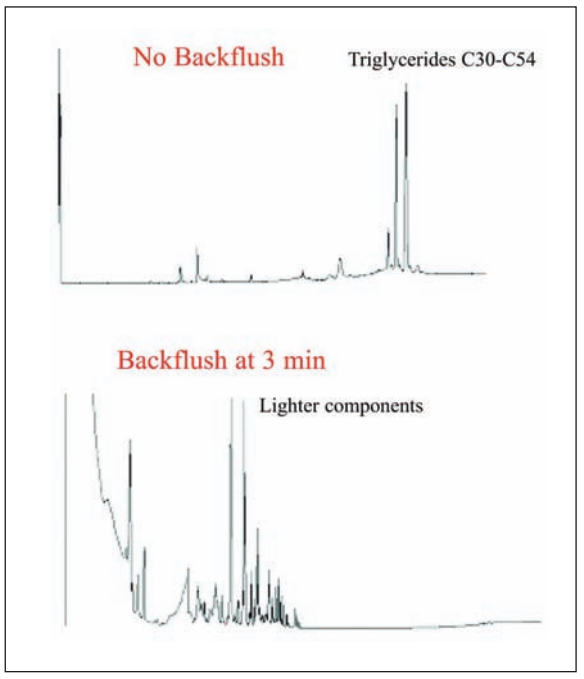


Figure 3: Olive oil analysis with and without reverse flow; Detector: FID

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.

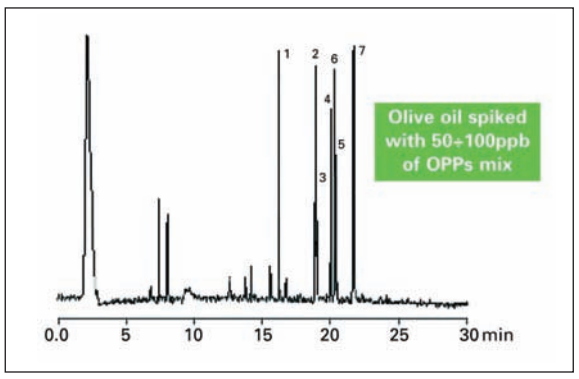


Figure 4: Chromatogram for olive oil spiked with OPP mixture. Detector: FPD

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. Repeatability of retention times and peak areas were evaluated based on 10 consecutive injections, and these tests showed 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.

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.

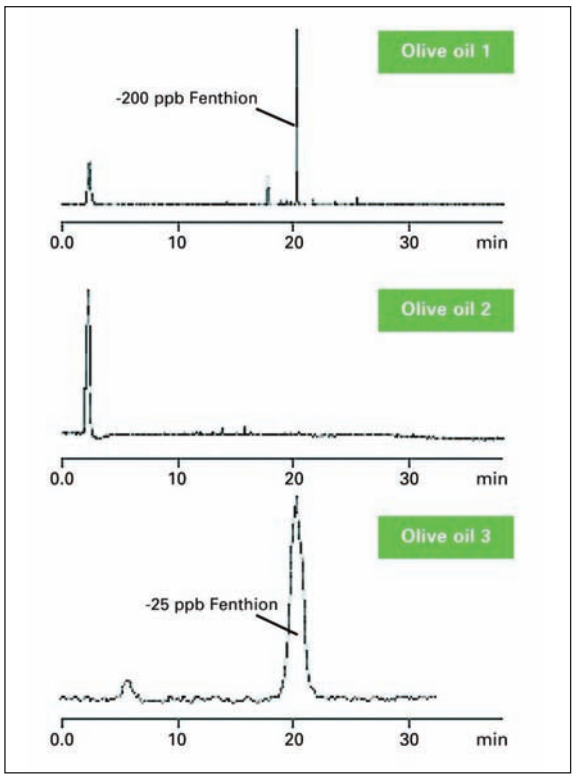


Figure 5: Detection of Fenthion in three commercial olive oils; Detector: FPD

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.

HPLC

High-performance liquid chromatography (HPLC) is also a chromatographic method that can be employed in food analysis. It is most often used for the separating of types of organic chemicals independent of polarity and volatility, although its primary application is for the analysis of more polar, thermolabile and non-volatile chemicals which are not easily separated by GC.

A recent investigation into the polar retention of vitamin C highlights the suitability of HPLC to analyze polar chemicals. Vitamin C is a water soluble vitamin which has an important role within living systems. It is vital in the production of collagen, a major structural protein which is fundamental to the production of connective tissue within the body, for example, tendons and cartilage. The addition of mineral crystals to collagen enables the formation of bones and teeth. Vitamin C prevents the oxidation of fatty acids and other fat-soluble vitamins within the body. It also has important anti-oxidant and immune system boosting properties and is promoted as a dietary supplement for this purpose. The recommended daily amount (RDA) of vitamin C for an average adult is 60 to 90 mg with a maximum dose of 2000 mg. Many foods, including processed products, contain relatively large quantities of vitamin C. Indeed, the compound is used as a natural preservative. Food nutritional labelling laws in the USA require that amounts of vitamin C are listed on processed foods.

The strong polar nature of the vitamin C molecule accounts for its high solubility in water and other high polarity solvents. Consequently, it possesses reduced solubility in less polar solvents and shows minimal retention on conventional reversed phase HPLC columns. HPLC is commonly used for the determination of this compound; however, inadequate retention often leads to overlap with the solvent front, thereby resulting in poor accuracy and reproducibility.

The separation of analytes on conventional alkyl chain chemistries is based primarily on dispersive and steric interactions only. The perfluorinated phenyl ring of Hypersil GOLDTM PFP enables TT-TT and dipole interactions that provide additional mechanisms of retention and selectivity with analyte species. Such interactions facilitate the increased retention of polar compounds on the perfluorinated phase compared with conventional chemistries. The Log P values of vitamin C and uracil are -1.8 and -0.87 respectively.

EXPERIMENTAL

The vitamin C analysis was carried out on a Finnigan™ Surveyor™ HPLC equipped with Xcalibur™ 1.3 software (Thermo). The column was a 5 µm Hypersil GOLD PFP 150 x 4.6 mm column.

Mobile Phase:	A: H_2O + 0.1% Formic Acid B: ACN + 0.1% Formic Acid 99:1
Flow Rate:	1.0 mL/min
Injection Volume:	5 µL
Detection:	UV at 254 nm
Temperature:	25 °C

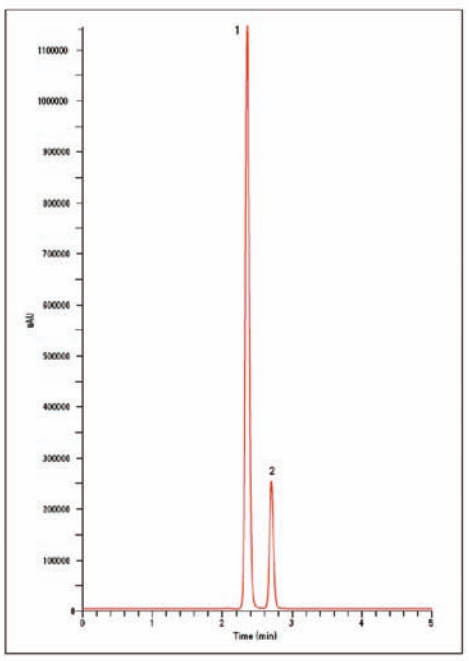


Figure 6: The analysis of vitamin C on a perfluorinated phase illustrates the enhanced retention of very polar compounds using Hypersil GOLD PFP columns. Anayltes: 1) vitamin C; 2) uracil.

RESULTS

The analysis was performed in less than three minutes using a simple isocratic mobile phase. The chromatogram shows the retention of vitamin C and uracil using the PFP column. The highly polar nature of the vitamin is illustrated by the fact that it elutes from the column *(Figure 6)* before uracil (a traditional t0 marker on alkyl chain chemistries). Effectively demonstrated is the ability of HPLC to retain these polar compounds.

CONCLUSION

The application of chromatography to food analysis has revolutionized the food industry, but the coupling of chromatographic techniques with the sensitive and selective detection techniques such as MS, the possibilities for the analysis of food are increasing.

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