

**FAST, DIRECT AND RELIABLE ANALYSIS OF MINOR COMPONENTS IN OLIVE OIL BY DIRECT INJECTION INTO THE PATENTED HPLC-GC SYSTEM****Abstract**

The system KONIK K2 HPLC-HRGC instrument has been successfully applied as a direct method for the analysis of minor components of olive oil.

**Introduction**

Olive oil is characterised by its delicate and unique flavour. The uniqueness of the flavour and aroma is due to a variety of constituents that are present in very low concentrations. While the major part (>95%) of the oil consists of fatty acids bound to glycerol (the so-called triglycerides), there is a large number of constituents which are present only in small amounts. Nevertheless, these so-called minor components are of great importance; some of them have been reported to be beneficial to human health, others improve the stability of the oil and, not least, some are responsible for the unique flavour of the oil.

The minor constituents of olive oil can be subdivided into tocopherols, phenols, flavour compounds, hydrocarbons, and sterols. These substances are responsible for the unique taste and flavour of the oil, increase its stability and are beneficial to human health by preventing injurious or deleterious processes such as oxygen radical-induced oxidation of lipids. Therefore, the presence of these compounds in the oil is, in addition to its favourable fatty acid composition, a further reason to recommend olive oil as a main source of fat in our daily diet.

In the present application a method for direct on-line HPLC-HRGC determination of minor components of olive oil is proposed. The method allows the analysis of all minor components included in this fraction together or the analysis of two different fractions separately (erythrodiol, uvaol and squalene, sterols and tocopherols).

**Experimental**

The innovative KONIK K2 HPLC-HRGC system (Figure 1) marries in synergy the separation and fractionation potential of normal and reversed HPLC, to the separation and selective detection of HRGC.

Figure 1: KONIK K2 HPLC-HRGC System



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Certified Company

**LC-GC Transfer**

The flow diagram (Figure 2) describes the interface operating principles. This patented interface allows the trapping of the analytes in the modified injector of the HRGC system. The trap is held at the chosen temperature while a digitally controlled continuous flow of Helium maintains the columns flow and eliminates the solvent from the trap.

Figure 2-a:  
Stabilization step

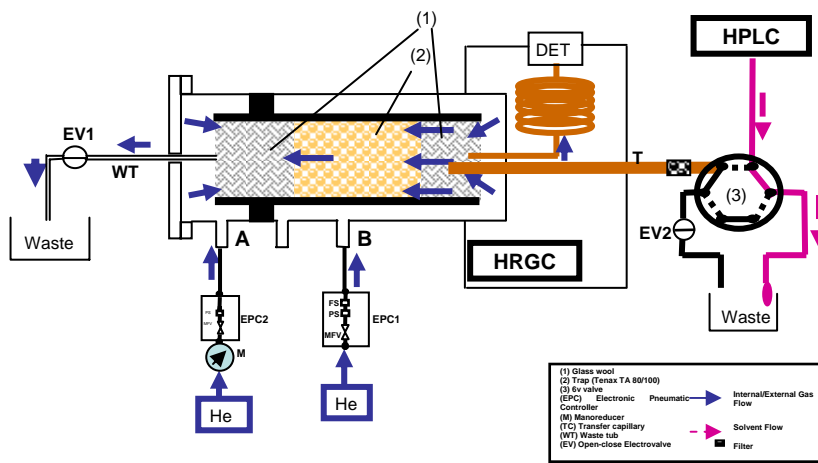
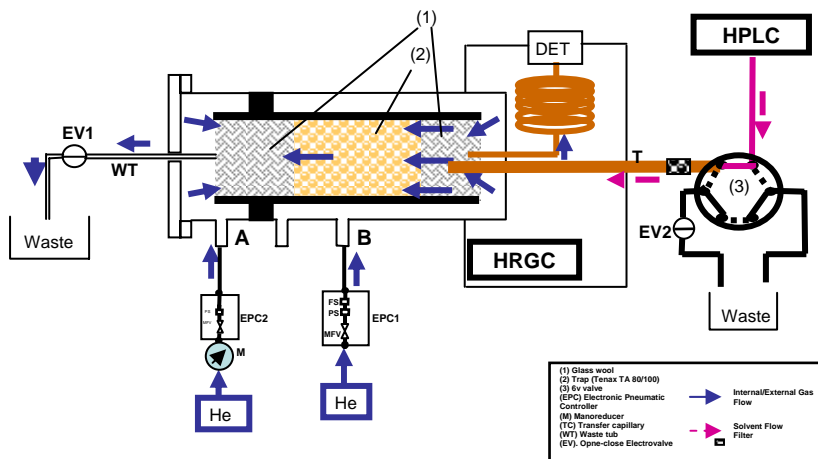


Figure 2-b:  
Transfer step



### 1- Stabilization

Helium enters the packed liner through both the external flow (500 mL/min) and the internal flow (500 mL/min) (A and B in Figure 2). Eluent coming from HPLC pump is sent to waste. K2 Injector temperature stabilizes at 80°C. Oven temperature is set at 40°C. EV1 is opened and EV2 is closed.

### 2- Transfer

The solvent with the analytes of interest reaches the liner at 0.1 mL/min through the transfer capillary (TC). Helium pushes the solvent through the adsorbent. Analytes are retained, and the solvent is vented to waste through the waste tubing (WT in Figure 2).

### 3- Remaining solvent elimination

LC solvent coming from the pump is sent to waste. Helium pushes the remaining solvent in the capillary tubing to waste. These conditions are maintained for 2 min in order to achieve complete elimination of the solvent. EV2 is opened.

### 4- Thermal desorption

Helium enters only through the external gas inlet (A in figure 2) to the column. K2 interface is heated for 5 min and the retained analytes are desorbed and transferred to the capillary GC column. EV1 and EV2 are closed.

## Material & Methods

Virgin olive oil was purchased from a local market. As sample pretreatment prior to HPLC-GC analysis, the oil samples were only filtered through a 0.22 µm filter (Chromatography Research Supplies, Inc) and diluted in isopropanol 1:50. Standards of some minor components were uvaol, erythrodiol, cholesterol, stigmaterol, β-sitosterol, α-tocopherol, δ-tocopherol and squalene obtained from different sources. Methanol and water (95:5 (v/v)) of HPLC grade used as mobile phase were purchased from LabScan (Dublin, Ireland).

## Chromatographic Conditions

Standards, samples and the necessary Konik HPLC-HRGC system conditions for this analysis are listed in Table 1. For this study, control of the GC, data acquisition, reduction, and analysis were done using Konikrom® Plus Chromatography Data System.

**Table 1: Chromatographic Conditions**

**Samples:** Olive Oil sample filtered with 0.22µm filter and diluted in Isopropanol 1:50.

**Standards :** Standards of some minor components were uvaol, erythrodiol, cholesterol, stigmaterol, β-sitosterol, α-tocopherol, δ-tocopherol and squalene obtained from different sources

### HPLC Conditions

**Column:** C4 Kromasil 100/10; 50x4.6 mm  
**Injection volume:** 20µL  
**Detector:** UV-VIS 220 nm  
**Mobil Phase:** Methanol/Water 95:5

### K2 Interface Conditions

**Adsorbent:** TENAX TA 80/100 mesh  
**Adsorption Temp:** 80 °C  
**Desorption Temp:** 300 °C  
**Transfer Flow:** 0.1 mL/min / 20 min  
**Carrier Flow:** 500 mL/min (A) + 500 mL/min (B)

### HRGC Conditions

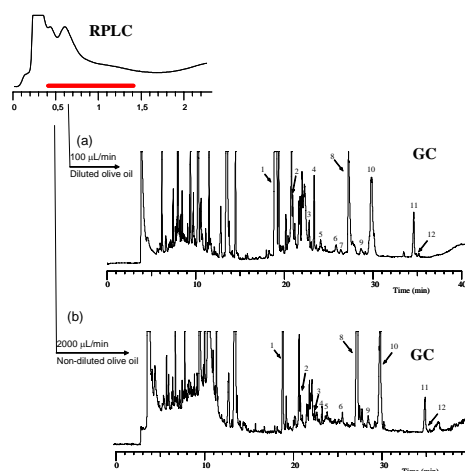
**Column:** KAP-5 (5% Phenyl Methyl Silicone); 30m x 0.32mm  
**Carrier:** Helium at 1.8 mL/min  
**Oven:** 40°C (1min); 40°C/min; 240°C (5min); 4°C/min; 290°C (5min); 5°C/min; 320°C (5min)  
**Detector:** FID at 320°C; Detector Gases: H2 at 38mL/min; Air at 220mL/min; N2 at 25mL/min

## Results

Different HPLC eluent flow during transfer can be used. The sensitivity will increase if lower flows (100  $\mu\text{L}/\text{min}$ ) are used in despite of higher flows (2000  $\mu\text{L}/\text{min}$ ), so, using a lower flow, a sample dilution is needed.

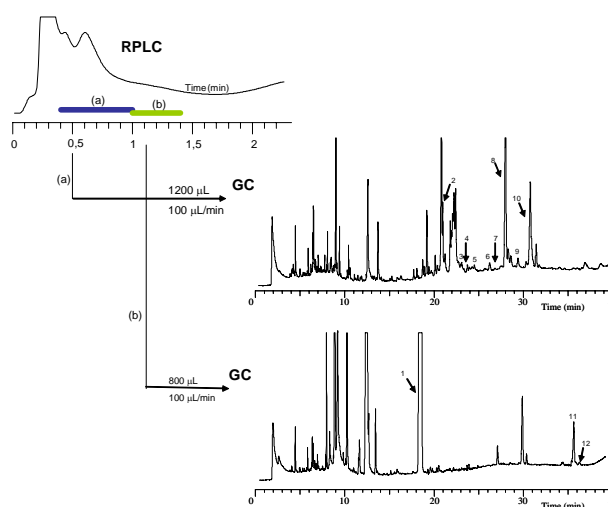
Figure 3 shows the HPLC+GC chromatogram obtained in the analysis of olive oil at 100  $\mu\text{L}/\text{min}$  (a) and at 2000  $\mu\text{L}/\text{min}$  (b) transfer flow. At lower flow the sample must be diluted to obtain the peaks into the linearity range.

Figure 3: HPLC-GC chromatogram:  
 (1) squalene  
 (2)  $\delta$ -tocopherol  
 (3)  $\gamma$ -tocopherol  
 (4) cholesterol  
 (5)  $\alpha$ -tocopherol  
 (6) campesterol  
 (7) stigmasterol  
 (8)  $\beta$ -sitosterol  
 (9)  $\Delta^7$ -stigmasterol  
 (10)  $\Delta^7$ -avenasterol  
 (11) erythrodiol  
 (12) uvaol



The HPLC fraction to be transferred to the HRGC system can be selected in order to analyze the desired compounds. Figure 4 shows an example of HPLC+GC chromatogram obtained by changing the chosen fraction in the HPLC system.

Figure 4: HPLC-GC chromatogram:  
 (1) squalene  
 (2)  $\delta$ -tocopherol  
 (3)  $\gamma$ -tocopherol  
 (4) cholesterol  
 (5)  $\alpha$ -tocopherol  
 (6) campesterol  
 (7) stigmasterol  
 (8)  $\beta$ -sitosterol  
 (9)  $\Delta^7$ -stigmasterol  
 (10)  $\Delta^7$ -avenasterol  
 (11) erythrodiol  
 (12) uvaol



### Quality Parameters

Table 2 shows the results of precision in retention times and areas:

MINOR COMPONENTS	TOTAL (0.4-1.4 min)		FRACTION a (0.4-1 min)		FRACTION b (1-1.4 min)	
	R.S.D. (Area)	R.S.D. (tr)	R.S.D. (Area)	R.S.D. (tr)	R.S.D. (Area)	R.S.D. (tr)
Squalene	3	0.1	--	--	4	0.09
δ-tocopherol	4	0.1	6	0.3	--	--
γ-tocopherol	9	0.1	7	0.3	--	--
Cholesterol	14	0.1	4	0.2	--	--
γ-tocopherol	12	0.2	11	0.2	--	--
Campesterol	6	0.2	6	0.3	--	--
Stigmasterol	8	0.1	9	0.3	--	--
β-sitosterol	5	0.2	8	0.3	--	--
Δ <sup>7</sup> -Stigmasterol	10	0.2	10	0.3	--	--
Δ <sup>7</sup> -Avenasterol	3	0.1	3	0.2	--	--
Erythrodiol	11	0.3	--	--	6	0.1
Uvaol	9	0.2	--	--	6	0.1

### Conclusions

- KONIK K2 HPLC-HRGC instrument have been proved to be suitable for the automated determination of minor components of edible oils.
- The method eliminates the time-consuming sample preparation step as no pretreatment is required other than a simple filtration step.
- K2 interface is highly suitable for the automatization of HPLC-GC systems avoiding errors caused by sample manipulation.
- The described method shows good precision and high sensitivity.
- HPLC as a sample preparation method is a good alternative to traditional techniques such liquid-liquid extraction or SPE.