FAST, DIRECT AND RELIABLE ANALYSIS OF MINOR COMPONENTS IN OLIVE OIL BY ON –LINE REVERSED PHASE LIQUID CHROMATOGRAPHY-GAS CHROMATOGRAPHY USING A PATENTED AUTOMATED THROUGH OVEN TRANSFER ADSORPTION DESORPTION INTERFACE

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INTRODUCTION

A new application of the patented, automated, *Through Oven Transfer Adsorption Desorption* (TOTAD®) Interface for on-line coupling of reversed phase liquid chromatography and gas chromatography (RPLC-HRGC) is presented.

The TOTAD® interface coupling an HPLC to a HRGC is based on a modified Programmed Temperature Vaporizer (PTV) Injector of a KONIK 4000 HRGC packed with a suitable trapping material, but the way it operates is very different. All the system is automated, consequently, in order to obtain the gas chromatogram of any fraction of HPLC fractionation is only necessary to inject the chosen sample of the eluent from HPLC. The manual TOTAD interface has been previously described (J. Microcol. Sep. 1999, 11, 582-89).

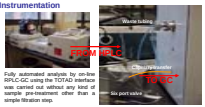
In the present application a method for direct on-line RPLC–HRGC determination of minor components of olive oil is proposed. The method allows the analysis of all minor components included in this fraction together or the analysis of two different fractions separately (erythrodiol and uvaol, sterols and tocopherols and squalene). The fraction containing the components of interest is transferred by using the automated TOTAD® interface.

MATERIAL & METHODS

Samples

Virgin olive oil was purchased from a local market . As a sample pretreatment prior to RPLC-GC using the TOTAD interface was carried out without any kind of. Hexane (Chromatography Research Supplies, Inc) and diluted in isopropanol 1:50. Standards of some minor components were uvaol, erythrodiol, cholesterol, stigmasterol, β-sitosterol, α-tocopherol, γ-tocopherol and squalene all obtained from different sources. Methanol and water (95/5 (v/v)) of HPLC grade used as mobile phase were purchased from LabScan (Dublin, Ireland).

Instrumentation



Fully automated analysis by on-line RPLC-GC using the TOTAD interface was carried out without any kind of sample pre-treatment other than a simple filtration step.

LC conditions

Preseparation: Start and end times of the fraction to be transferred from the LC to GC must be selected in the LC chromatogram. It is important to establish the correct experimental RPLC conditions to ensure majority components of the oils, especially the triglycerides are well separated from the minor components.

RPLC analysis:

Sample volume injected: 20µL

Column: C_{18} Kromasil 100/10 (50x4.6 mm i.d.)

Eluent: Methanol/Water (95:5)

Flow: 2 mL/min

LC-GC Transfer

When the beginning of the fraction of interest reaches the six-port valve, it is automatically switched and the pump flow changes from 2 to 0.1 mL/min. This flow is maintained until the end of the fraction of interest reaches the GC injector.

Solutes are retained in the packed bed of the liner. PTV temperature during transfer is 80ºC. Solvent is eliminated pushed by the helium flow (500 mL/min by A and by B) (Figure 1).

TOTAD is heated to 300ºC (5 ºC/min) to achieve the thermal desorption of the retained solutes and its subsequent transfer to the capillary GC column.

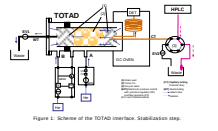
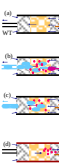


Figure 1: Scheme of the TOTAD interface. Stabilization step.

TOTAD Operation Mode



Stabilization:
Helium enters the packed liner through both the oven side (500 mL/min) and the opposite side (500 mL/min) (A and B). Helium flow is maintained until the system is ready to waste. TOTAD temperature stabilizes at RPC. Oven temperature is set at40ºC. EV1 and EV2 are open.

Transfer:
The solution reaches the liner at 0.1 mL/min. Helium pushes the solution through the corbeic. Analytes are retained, and the solvent is vented to waste through the waste tubing (W1 in Figures 1 and 2).

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

Thermal desorption:
Helium enters only through the usual gas inlet and exits only through the usual gas inlet (B in Figure 1). TOTAD interface is heated for 5 min and the retained analytes are desorbed and transferred to the capillary GC column. EV1 and EV2 are closed.

Figure 2. Glass liner during the steps of the operation mode. (a) Stabilization, (b) Transfer, (c) Remaining solvent elimination and (c) Thermal desorption.

GC Analysis

Injector: PTV modified ◆ TOTAD interface

Glass liner (2 mm i.d.) filled with Tenax TA (1 cm length)

Column: 30 m x 0.32 mm i.d.; 5% phenyl methyl silicone

Column temperature: 40 ºC, 1 min, to 240 ºC at 40 ºC/min, 5 min, to 290 ºC at 4 ºC/min, 5 min, and finally to 320 ºC at 5 ºC/min, 5 min.

Carrier gas: Helium (Flow rate: 1.8 mL/min)

Detector: FID (320 ºC)

When the GC analysis is finished TOTAD interface is maintained at 350 ºC and helium flow at 500 mL/min. Afterwards, it is cooled to 80 ºC so that another analysis can be carried out.

RESULTS

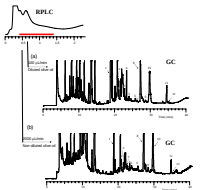


Figure 3. LC and GC chromatograms obtained from the direct RPLC-GC-FID analysis of a virgin olive oil sample. 20 µL of (a) olive oil diluted 1:50 in isopropanol or (b) non-diluted olive oil, were injected into LC. The thick line situated between the time axis and the LC chromatogram indicates the fraction that has been transferred from LC to GC. Speed of transfer: (a) 0.1 mL/min y (b) 2 mL/min. GC Scale: 70 mV.

Identified peaks: (1) squalene; (2) α-tocopherol; (3) γ-tocopherol; (4) cholesterol; (5) α-tocopherol; (6) campesterol; (7) stigmasterol; (8) β-sitosterol; (9) Δ7-stigmasterol; (10) Δ7-avenasterol; (11) erythrodiol; (12) uvaol.

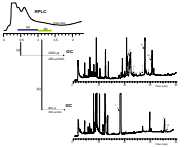


Figure 4. GC chromatograms obtained from the direct RPLC-GC-FID analysis of the two fractions indicated in the LC chromatogram. 20 µL of olive oil diluted 1:50 in isopropanol were injected into LC to obtain each fraction. Speed of transfer: 0.1 mL/min. GC Scale: 70 mV. Peak identification as in Figure 3.

PEAK COMPONENTS	TOTAL (0.1-2 mL/min)		FRACTION A (0.1 mL/min)		FRACTION B (1-2 mL/min)	
	R.S.D. (Area)	R.S.D. (RT)	R.S.D. (Area)	R.S.D. (RT)	R.S.D. (Area)	R.S.D. (RT)
Squalene	4	0.0	4	0.0		
α-tocopherol	4	0.0	3	0.0		
γ-tocopherol	4	0.0	4	0.0		
Cholesterol	1.9	0.0	2	0.0		
Erythrodiol	2.2	0.0	2.5	0.0		
Campesterol	5	0.0	5	0.0		
Stigmasterol	4	0.0	4	0.0		
β-sitosterol	3	0.0	3	0.0		
Δ7-stigmastenol	2.5	0.0	3.0	0.0		
Δ7-avenasterol	5	0.0	5	0.0		
Erythrodiol	3.5	0.0			3	0.0
Uvaol	5	0.0			5	0.0

Table 1. Relative standard deviation (RSD) for absolute peak areas and retention time resulting from the analysis of the fraction of minor components (n=3) and the delimited fractions (n=3).

CONCLUSIONS

- The method allows the automated analysis of minor components of edible oils.
- The method eliminates the time-consuming sample preparation step.
- TOTAD interface is highly suitable for the automatization of RPLC-GC systems avoiding errors caused by sample manipulation.
- The described method shows good repeatability and high sensitivity.
- Different LC eluent flow during transfer can be used. Lower flows (100 µL/min) shows higher sensitivity, so that a sample dilution is needed. The analysis is faster when higher flows (2000 µL/min) are used.
- The LC fraction to be transferred to GC can be selected in order to analyze the desired compounds.

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