Rapid Analysis of Free Erythrodiol and Uvaol in Olive Oils by Coupled Reversed Phase Liquid Chromatography−Gas Chromatography

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On-line coupling of reversed phase liquid chromatography and gas capillary chromatography (RPLC−GC) using a programmed temperature vaporizer (PTV) as interface is used for the direct analysis of erythrodiol and uvaol of edible oils by direct injection (i.e., without the need for prior enrichment of the sample). The reported procedure allows 2000 μL volume fractions of aqueous methanol eluents to be transferred into the GC system, 2000 μL/min being the speed of sample introduction. Relative standard deviations lower than 1.5% and detection limits lower than 1 ppm were obtained. The proposed method can be used to determine extra virgin olive oil adulterations as detection of an addition (10% (w/w)) of a solvent extracted oil to a cold pressed oil was achieved in less than 40 min on the basis of the determination of erythrodiol and uvaol.

Keywords: *On-line coupled RPLC-GC; erythrodiol; uvaol; direct analysis; olive oils*

INTRODUCTION

Analysis of minor components of the unsaponifiable fraction found in edible oils is of extreme importance in establishing not only their origin but also the extraction technique, the acidity of the oil, the final treatment, and possible adulterations (Grob et al., 1990). The sterol fraction is usually analyzed for the identification of a fat or an oil (e.g., to distinguish between sunflower oil and other oils of similar fatty acid composition), for the detection of the addition of nondeclared cheap oils to more expensive oils (e.g., rapeseed oil in olive oil) or to distinguish between different qualities of the same oil. Analyses of other minor components (e.g., alkanols, triterpene dialcohols) are also very important as they are used as a reference for olive oil regulation and for the investigation of its quality. In this respect, the need to establish the physicochemical characteristics of the product and, consequently, to develop new analytical methods suitable for this type of investigation is generally admitted (Amelio et al., 1992). However, in some cases, rapid and reliable methods of some minor compounds are not yet available. Specifically, the analysis of some triterpene dialcohols (e.g., erythrodiol and uvaol) is recommended for the investigation of the quality of olive oil as these compounds are commonly used as indicators for extraction oils. In fact, it has been established already that absolute concentrations of erythrodiol and uvaol in pressed oils are clearly lower than those corresponding to the raw solvent extracted oils. For that reason, analysis of erythrodiol and uvaol is used for distinguishing among olive oils of different qualities such as cold pressed (extra virgin) and either solvent extracted or pressed residues oils.

The methods normally used for the analysis of different fractions, also including triterpene dialcohols, of edible oils involve several steps, namely, the removal of triglycerides, the fractionation of the unsaponifiable matter into several classes of compounds, and their subsequent analysis by gas chromatography (Official Journal of the European Communities). As widely recognized, however, the conventional methods are tedious and time-consuming and also may involve the loss of important information by saponification (Grob et al., 1990; Park et al., 1996).

A markedly more interesting approach based on direct coupling of high-performance liquid chromatography and high-resolution capillary gas chromatography (LC−GC) has also been reported (Biederman et al., 1996; Gallina Toschi et al., 1996; Grob et al., 1989b; Grob and Lanfranchi, 1989; Lanuzza et al., 1996). This latter method allows a more reliable and rapid determination of different minor components of edible oils as it uses the LC preseparation to circumvent saponification and extraction and to replace the subsequent cleanup steps.

Actually, the mentioned method offers the evident advantages of on-line coupling LC−GC (Grob, 1991, 1995; Vreuls et al., 1994) for the analysis of triterpene dialcohols (Grob et al., 1989a) although several limitations should be considered. First, uvaol was not positively identified, and second, the fact that the normal phase was used in the LC step may cause several problems affecting the performance of the column as triglycerides deactivate the silica gel. As a result, variability in retention times can be observed finally, especially when the triglyceride concentrations to be analyzed vary from one sample to another (Grob et al., 1991). For that reason, use of reversed phase liquid chromatography in the preseparation step seems to be an interesting alternative, although transfer to GC of

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polar solvents is rather difficult mainly due to poor wettability of retention gaps and a large volume of vapor which is produced per unit volume of liquid.

Previous research on RPLC−GC has focused on the development of different interfaces adequate for the transfer of aqueous eluents (Goosens et al., 1994; Mol et al., 1993; Staniewski et al., 1992; Vreuls et al., 1991), although several problems have avoided to extend the range of applicability of the technique. In this respect, our previous experience in RPLC−GC coupling using a programmed temperature vaporizer (PTV) as the interface of the system has already proved its usefulness for the analysis of minor components, i.e., free sterols of edible oils (Señoráns et al., 1996).

The objective of this work was to develop a new and rapid qualitative method suitable for the direct analysis of erythrodiol and uvaol in olive oils which allows one to circumvent problems associated with both off-line sample preparation techniques and limitations of previously proposed methods. In this regard, a further objective of our research was to extend the upper limit of the fraction volumes which so far have been adequately transferred to GC in analyzing the mentioned triterpene dialcohols. To this aim the proposed analytical method was developed on the basis of using an LC column having a large internal diameter (i.e., 4.6 mm).

EXPERIMENTAL PROCEDURES

Samples and Materials. The reference substances used in this study were obtained from Extrasynthèse (Genay, France; erythrodiol) and Sigma Chemical Co. (St. Louis, MO; uvaol). Olive oils were either purchased in the local market or obtained from an oil mill. As sample pretreatment prior to RPLC−GC analysis, oils were only filtered through a 0.22 μm filter. Methanol (HPLC grade) was purchased from Lab Scan (Dublin, Ireland), and the water used was collected from a Milli-Q water purification system (Millipore, Milford, MA). The silylated glass inserts (75 mm × 1 mm i.d. × 2 mm o.d.) of the PTV injector were obtained from Gerstel (Mülheim/Ruhr, Germany).

According to our previous experience in RPLC−GC (Señoráns et al., 1995a), a 4 cm plug length of Tenax TA, 80−100 mesh (Chrompack, Middelburg, The Netherlands) was used as packing material in the PTV, between two plugs of glass wool to keep it in place. In the liner there was a dent, 1 cm from the bottom side, to reduce the amount of glass wool needed to prevent the Tenax from being blown out of the liner.

Instrumentation. The on-line coupled equipment consisted of a Hewlett-Packard model 1050 liquid chromatograph involving a manual injection valve (Rheodyne 7125) having a 20 μL loop and a Perkin-Elmer model 8310 gas chromatograph equipped with a flame ionization detector (FID) and a PTV injector which actually acted as an interface for the system.

LC Conditions. All analyses were performed by injecting 20 μL of the filtered oil onto a 50 × 4.6 mm i.d. column slurry packed with 10 μm silica (C4, Vydac 214 TPB). The initial composition of the eluent (methanol−water, 70:30 (v/v)) was maintained for 3 min and then followed by a linear gradient of up to 22% water within 3 min. The latter water percentage was kept for 4 min and then was again modified up to 14% within 2 min and maintained there for 3 min. Finally, the gradient was varied by up to 0% water within 4 min.

All throughout the experimentation, the column temperature was maintained at 45 °C and the detector (UV) was operated at 205 nm, 2000 μL/min being the flow rate used during both LC preseparation and LC−GC transfer.

LC−GC Transfer. Transfer of the fraction of interest to the GC was performed by means of a multiport valve, placed between the detector of the HPLC and the PTV injector of the GC, as described elsewhere (Señoráns et al., 1995a,b). During LC−GC transfer both the PTV temperature and the helium flow rate which passes the injector were maintained, respectively, at 21 °C and 800 mL/min. An additional purge time (0.32 min) was established to efficiently eliminate the remaining solvent from the glass liner before transfer of the compounds to the GC column. Elimination of the extremely large volume of vapor resulting from the aqueous eluent during LC−GC transfer was promoted through the injector bottom by removing the GC column end from the injector body (Señoráns et al., 1995a,b, 1996). Once the transfer step was completed, subsequent thermal desorption of the trapped material was simply achieved by increasing the PTV temperature to 350 °C (at 14 °C/s). The final temperature was kept for 6 min.

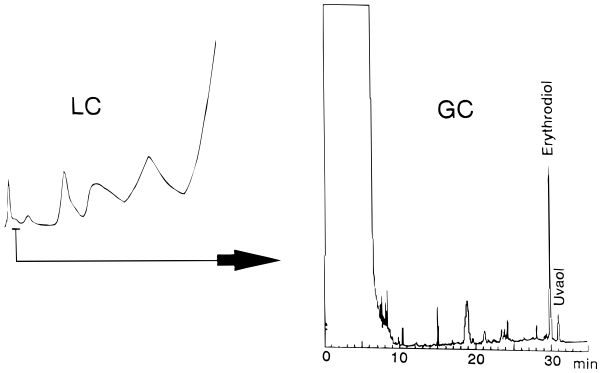

Figure 1. Liquid chromatogram of a solvent extracted olive oil and gas chromatogram (full range, 5 mV) obtained after transfer of the indicated fraction (2000 μL of methanol−water eluent, 70:30; HPLC flow rate, 2000 μL/min).

GC Analysis. GC separations were carried out on a 5%/95% diphenyl/dimethylpolysiloxane fused silica column (30 m × 0.250 mm i.d., 0.25 μm film thickness; Sugelabor, Madrid, Spain) with helium as the carrier gas. The GC oven was heated at 20°/min from 130 to 230 °C (2 min) followed by a temperature program of 3°/min to 320 °C. The FID temperature was kept at 320 °C and the 2600 Chromatography software (Perkin-Elmer Nelson Systems) was used for data acquisition.

RESULTS AND DISCUSSION

Figure 1 shows both the liquid chromatogram resulting from the preseparation obtained from the direct injection of a solvent extracted oil (from olive residues) and the gas chromatogram collected after transfer of the indicated fraction (2000 μL) as described under Experimental Procedures. Erythrodiol and uvaol were identified in oil samples by matching their relative retention with that obtained from the RPLC−GC analysis, performed under identical experimental conditions, of a standard solution containing the mentioned triterpene dialcohols in 2-propanol, 200 ppm being the concentration of each compound. The actual erythrodiol and uvaol concentrations in solvent extracted oil were estimated to be, respectively, 90 and 22 ppm, i.e., in the range of concentrations usually encountered for these compounds. This estimation was performed from the areas of the peaks obtained for the components of the standard mixture in the gas chromatogram resulting after transfer of the selected LC fraction and the corresponding areas obtained by LC−GC analysis of the oil.

It should be underlined that although the aim of the work was, as previously mentioned, to develop a qualitative screening method in order to evaluate the pos-

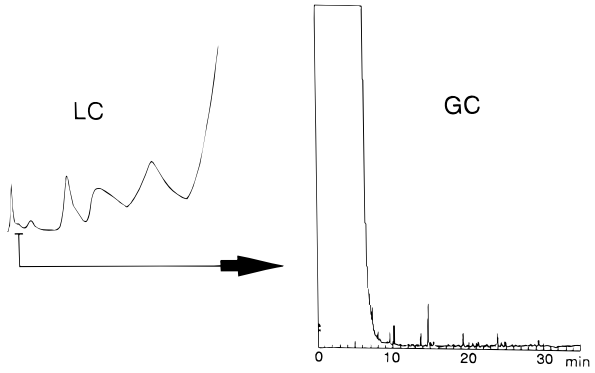

Figure 2. Liquid chromatogram of a cold pressed (extra virgin) olive oil and gas chromatogram (full range, 5 mV) resulting from transfer of the indicated fraction (2000 μL of methanol−water eluent, 70:30; HPLC flow rate, 2000 μL/min).

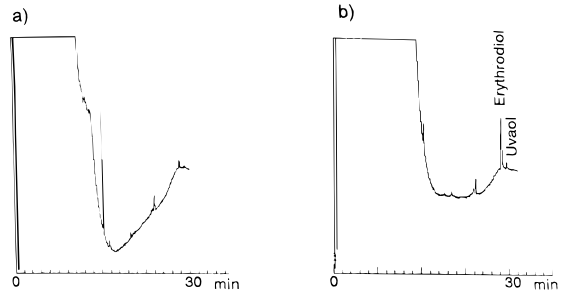

Figure 3. Gas chromatograms (full range, 2 mV) of an extra virgin oil (a) and the same oil containing 10% of solvent extracted oil (b) resulting from LC−GC transfer of 2000 μL of methanol−water eluent, 70:30 (HPLC flow rate, 2000 μL/min).

sibility of performing a rapid analysis of erythrodiol and uvaol in edible oils by RPLC−GC, it is also interesting to consider some aspects concerning the adequation of the procedure for an eventual quantitative analysis. Specifically, relative standard deviations (n = 3) obtained from the normalized peak areas were 1.0 and 1.5% for erythrodiol and uvaol, respectively, while detection limits (calculated as the amount of product giving a signal equal to three times the background noise) were 0.67 ppm (erythrodiol) and 0.62 ppm (uvaol).

Figure 2 depicts the LC and GC chromatograms resulting from the analysis of a cold pressed oil (extra virgin olive oil) which reveals the absence of erythrodiol and uvaol. The obtained results are in agreement with data previously reported by other authors as they establish that concentrations of triterpene dialcohols in solvent-extracted olive oils are far above those found for pressed oils.

As can be seen in Figure 3, the proposed method also allows the detection of both erythrodiol and uvaol in an admixed oil obtained from the addition of a solvent extracted oil (10% (w/w)) to a cold pressed oil.

In conclusion, the detection of adulterations of cold pressed (extra virgin) oil with solvent extracted oils can be achieved on the basis of the analysis of erythrodiol and uvaol in olive oils by direct injection, i.e., without the need for prior enrichment of the sample. As LC flow during transfer was 2000 μL/min throughout the experimentation, transfer of the selected fraction (2000 μL) only took 1 min, while the overall procedure (i.e., including LC preseparation, LC−GC transfer, and GC analysis) required less than 40 min.

LITERATURE CITED

Amelio, M.; Rizzo, R.; Varazini, F. Determination of sterols, erythrodiol, uvaol and alkanols in olive oils using combined solid-phase extraction, high performance liquid chromatographic and high-resolution gas chromatographic techniques. *J. Chromatogr.* **1992**, *606*, 179−85.

Biedermann, M.; Grob, K.; Mariani, C.; Schmidt, J. P. Detection of desterolized sunflower oil in olive oil through isomerized Δ7-sterols. *Z. Lebensm. Unters. Forsch.* **1996**, *202*, 199−204.

Gallina Toschi, T.; Bendini, A.; Lercker, G. Evaluation of 3,5-stigmastadiene content of edible oils: Comparison between the traditional capillary gas chromatographic method and the on-line high performance liquid chromatography-capillary gas chromatographic analysis. *Chromatographia* **1996**, *43*, 195−9.

Goosens, E. C.; de Jong, D.; de Jong, G. J.; Brinkman, U. A. Th. Reversed-phase liquid chromatography coupled on-line with capillary gas chromatography II. Use of a solvent vapor exit to increase introduction volumes and introduction rates into the gas chromatograph. *J. Microcolumn Sep.* **1994**, *6*, 207−15.

Grob, K. *On-Line Coupled LC-GC*; Hüthig: Heidelberg, Germany, 1991.

Grob, K. Development of the transfer techniques for on-line high-performance liquid chromatography-capillary gas chromatography. *J. Chromatogr.* **1995**, *703*, 265−76.

Grob, K.; Lanfranchi, M. Reproducibility of results from LC-GC of sterols and wax esters in olive oil. *J. High Resolut. Chromatogr.* **1989**, *12*, 624−6.

Grob, K.; Biedermann, M.; Läubli, T. Determination of free erytrodiol in olive oil by coupled LC-GC. *J. High Resolut. Chromatogr.* **1989a**, *12*, 49−50.

Grob, K.; Lanfranchi, M.; Mariani, C. Determination of free and esterified sterols and of wax esters in oils and fats by coupled liquid chromatography−gas chromatography. *J. Chromatogr.* **1989b**, *471*, 397−405.

Grob, K.; Lanfranchi, M.; Mariani, C. Evaluation of olive oils through the fatty alcohols, the sterols and their esters by coupled LC-GC. *J. Am. Oil Chem. Soc.* **1990**, *67*, 626−34.

Grob, K.; Kaelin, I.; Artho, A. Coupled LC-GC: The capacity of silica gel (HP)LC columns for retaining fat. *J. High Resolut. Chromatogr.* **1991**, *14*, 373−6.

Lanuzza, F.; Micali, G.; Calabrò, G. On-line HPLC−HRGC coupling and simultaneous transfer of two different LC fractions: determination of aliphatic alcohols and sterols in olive oil. *J. High Resolut. Chromatogr.* **1996**, *19*, 444−8.

Mol, H. G.; Staniewski, J.; Janssen, H. G.; Cramers, C. A.; Ghijsen, R. T.; Brinkman, U. A. Th. Use of an open-tubular trapping column as phase-switching interface in on-line coupled reversed-phase liquid chromatography-capillary gas chromatography. *J. Chromatogr.* **1993**, *630*, 201−12.

Official Journal of the European Communities. *Off. J. Eur. Communities* **1988** (Jan 29), *L22* 58 (EEC Regulation 183/93 of Jan 29, 1993).

Park, P. W.; Guardiola, F.; Park, S. H.; Addis, P. B. Kinetic evaluation of 3β- hydroxycholest-5-en-7-one (7-ketocholesterol) stability during saponification. *J. Am. Oil Chem. Soc.* **1996**, *73*, 623−9.

Señoráns, F. J.; Herraiz, M.; Tabera, J. On-line reversed-phase liquid chromatography-capillary gas chromatography using a programmed temperature vaporizer as interface. *J. High Resolut. Chromatogr.* **1995a**, *18*, 433−8.

Señoráns, F. J.; Reglero, G.; Herraiz, M. Use of a programmed temperature injector for on-line reversed-phase liquid chromatography-capillary gas chromatography. *J. Chromatogr. Sci.* **1995b**, *33*, 446−50.

Señoráns, F. J.; Tabera, J.; Herraiz, M. Rapid separation of free sterols in edible oils by on-line coupled reversed phase liquid chromatography-gas chromatography. *J. Agric. Food Chem.* **1996**, *44*, 3189−92.

Staniewski, J.; Janssen, H. G.; Cramers, C. A.; Rijks, J. A. Programmed-temperature injector for large-volume sample introduction in capillary gas chromatography and for liquid chromatography-gas chromatography interfacing. *J. Microcolumn Sep.* **1992**, *4*, 331−8.

Vreuls, J. J.; Brinkman, U. A. Th.; de Jong, G. J.; Grob, K.; Artho, A. On-line solid-phase extration−thermal desorption for introduction of large volumes of aqueous samples into a gas chromatograph. *J. High Resolut. Chromatogr.* **1991**, *14*, 455−9.

Vreuls, J. J.; de Jong, G. J.; Ghijsen, R. T.; Brinkman, U. A. Th. Liquid chromatography coupled on-line with gas chromatography: State of the art. *J. AOAC Int.* **1994**, *77*, 306−27.

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