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# Study of the adulteration of olive oil with hazelnut oil by on-line coupled high performance liquid chromatographic and gas chromatographic analysis of filbertone

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**Abstract**

The optimisation of the interface performance in the on-line coupling of reversed phase liquid chromatography and gas chromatography was intended to improve the sensitivity achievable in the direct analysis of olive oils adulterated with virgin and refined hazelnut oils. The efficient elimination of the eluent coming from the pre-separation was achieved by considering some experimental variables (i.e., transfer volume, interface temperature during transfer, helium flow during both transfer and purge, and purge time) affecting the operation of a vertically positioned programmed temperature vaporizer which acted as the interface of the system. The obtained results demonstrated the possibility of evaluating the genuineness of olive and hazelnut oils as well as of detecting adulterations of olive oil with percentages of around 5% and 10% of virgin and refined hazelnut oils, respectively, in less than 30 min by the method proposed.


*Keywords:* Enantiomers; Refined hazelnut oil; Olive oil adulteration; On-line coupled reversed phase liquid chromatography and gas chromatography

## 1. Introduction

Chirality has demonstrated to be an important aspect in food science owing to the fact that many of the compounds occurring in foodstuffs are characterized by a specific enantiomeric distribution (Armstrong, Chang, & Li, 1990; Ekborg-Ott & Armstrong, 1997). In this context, the separation of enantiomers present at low levels is obviously of great interest, specifically when identification of adulterated foods and beverages is demanded. However, despite the recognized relevance of the enantiomeric composition, its study in foods is at present rarely considered because of the complexity shown by food matrices in general. For oils, this fact is even more apparent as many of their components are fat-soluble, which can reduce considerably the efficiency of the isolation procedure. For this reason, the development of new analytical techniques that overcome the difficulties associated with the lipophilic nature of major compounds and, in turn, are able to isolate efficiently enantiomers from oils under non-racemization conditions is strongly required.

In this respect, on-line coupling LC-GC (Grob, 1991; Mondello, Dugo, & Bartle, 1996; Vreuls, de Jong, Ghijsen, & Brinkman, 1994) has proven to be an interesting alternative to traditional sample preparation methods for several reasons. It allows not only an effective sample fractionation in the LC step, even when reversed phase eluents are needed, but also an efficient and rapid separation by high-resolution capillary gas chromatography.

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Consequently, LC-GC enables sample manipulation to be minimized and overall analysis time to be shortened, enhancing the reliability of chiral analysis. In addition, the possibility of transferring large volumes from LC into GC may theoretically allow to increase the sensitivity and, thus, to detect compounds at trace level. This fact is particularly relevant when the presence of enantiomers to be determined in adulterated samples as they frequently occur at extremely low concentrations.

In previous work we have proposed the use of (*E*)-5-methylhept-2-en-4-one (filbertone) as a chiral marker to detect the adulteration of olive oil with hazelnut oil, and we have already reported its usefulness to study different aspects concerning this topic (Blanch, Caja, León, & Herraiz, 2000; Blanch, Caja, Ruiz del Castillo, & Herraiz, 1998; Blanch, Caja, Ruiz del Castillo, & Herraiz, 1999; Caja, Ruiz del Castillo, Herraiz, & Blanch, 1999; Caja, Ruiz del Castillo, Martínez Alvarez, Herraiz, & Blanch, 2000; Ruiz del Castillo, Caja, Herraiz, & Blanch, 1998). However, these works have been mainly focussed on the determination of filbertone when virgin hazelnut oil was involved in the adulteration. In this regard, the consideration of refined hazelnut oil implies an additional difficulty as the harsh conditions typically used over the refining process may result in partial or even total elimination of volatile compounds, such as filbertone. Consequently, it is expected that analytical methods developed to evaluate the occurrence of this chiral marker in virgin hazelnut oil are not suitable for determining its presence in hazelnut oil which has been submitted to a refining process and, thus, for detecting adulterations of olive oil performed by using refined oils.

The goal of this work was to evaluate the possibility of increasing the overall performance of filbertone determination in olive oils adulterated with both virgin and refined hazelnut oils by direct analysis (i.e., without sample preparation) using on-line coupled reversed phase liquid chromatography and gas chromatography (RPLC-GC). To this aim there was intended to modify the experimental set-up used up to now in our laboratory in such a way that the interface of the coupled system was vertically positioned to improve the efficiency of the eluent elimination coming from the pre-separation step as well as the sensitivity achievable by the method developed.

## 2. Materials and methods

### 2.1. Samples and materials

The samples included in this study were obtained from different suppliers. They were received in two sets (i.e., Set 1 and Set 2), which included 12 and 9 oils, respectively, obtained from olives and hazelnuts of different varieties and geographical origins. As detailed below, different admixtures at various percentages of the mentioned oils were also analysed.

(*E*)-5-methyl-hept-2-en-4-one (filbertone) used for identification purposes was acquired from Haarman & Reimer (Holzminden, Germany). Methanol and water were obtained from Scharlau Chemie, S.A. (Barcelona, Spain) and from a Milli-Q water purification system (Millipore, Milford, MA), respectively. Tenax TA (80-100 mesh; Chrompack, Middelburg, The Netherlands) was placed in the silylated glass liner (70 mm × 1.8 mm i.d. × 2.0 mm o.d.) of a programmed temperature vaporizer (PTV) injector (Gerstel, Mülheim/Ruhr, Germany) of the gas chromatograph. Prior to its use, Tenax TA was conditioned under a helium stream at 350 °C for 120 min. All the oils were filtered through a 0.22-μm filter (Millipore, Bedford, USA) prior to its RPLC-GC analysis and no further pretreatment was required.

### 2.2. On-line coupled LC-GC system

The analyses were performed using an on-line LC-GC equipment. Initially, the coupled LC-GC device consisted of a liquid chromatograph linked to a gas chromatograph fitted with a horizontally positioned PTV injector that acted as the interface. To increase the system performance by achieving a more efficient elimination of the eluent coming from the LC-pre-separation, a different configuration of the on-line coupled LC-GC including a gas chromatograph equipped with a vertically positioned PTV was also used.

### 2.3. LC-pre-separation

The HPLC system was provided with a manual injection valve (Model 7125; Rheodyne, Cotati, CA) having a 20-μl sample loop and an ultraviolet detector (UV) operated at 205 nm. The previously filtered oils were injected onto a 50 mm × 4.6 mm Kromasil 100-10 $C_4$ column (Waters, MA, USA) and methanol:water (68:32, v/v) was used as the mobile phase, 2000 μl/min being the flow-rate (equal to the speed of sample transfer). After elution of the fraction-containing filbertone, the methanol percentage was increased up to 100% which was kept for 20 min. Acquisition of data from UV detector was performed using a HPChem Station (Hewlett-Packard).

### 2.4. LC-GC transfer

Transfer of the filbertone-containing fraction was performed by using a multiport valve placed immediately after the UV detector of the LC system. An 80-cm × 0.25-mm i.d. fused silica tube was used as the transfer line to connect the LC with the GC. Depending on the PTV configuration, either a 12-mg (equivalent to a 5-cm

length) or 30-mg (equivalent to a 4-cm length) weight of Tenax TA was used as retaining material in the glass liner (80 mm × 1 mm i.d. × 2 mm o.d. for the horizontal configuration and 70 mm × 2 mm i.d. × 3 mm o.d. for the vertical configuration) of the PTV injector between two plugs of glass wool to keep it in place. Upon elution in LC of the beginning of the target fraction, the multiport valve is switched from the waste to the transfer position and switched back again to waste after the transfer time. Elimination of the eluent coming from the LC-pre-separation is achieved through both the split exit (split ratio referred to the flow through the capillary column when connected; 50:1) and the injector bottom while maintaining disconnected the GC-column end. To promote solvent elimination, a helium flow was applied during transfer and it was maintained during an additional purge time once the transfer was completed. Subsequently, the column end from the injector body is again connected to carry out the GC analysis. Further variations of some experimental values (i.e., transfer volume, PTV temperature during transfer, helium flow during both transfer and purge, and purge time) were established as explained in Section 3.

### 2.5. GC analysis of the fractions transferred from LC

The transferred fraction from the LC separation was analysed using a 25-m × 0.25-mm i.d. fused silica column coated with a 0.25-μm layer of Chirasil-β-Dex (Chrompack, Middelburg, The Netherlands), a flame ionisation detector (FID) operated at 250 °C and helium as the carrier gas. Desorption of the previously retained compounds and its subsequent transfer to the capillary column was achieved by increasing the injector temperature at 12–14 °C/s to 320 °C. The final PTV temperature was kept for 10 min. The column temperature was programmed at 3 °C/min from 40 °C (5 min) to 180 °C (maintained for 15 min). Acquisition of data from FID detector was performed using a HPChem Station (Hewlett-Packard).

## 3. Results and discussion

Initially, experimental conditions were established on the basis of our previous experience in the determination of filbertone by on-line coupling of reversed phase liquid chromatography and gas chromatography (Blanch et al., 1999; Ruiz del Castillo et al., 1998). As expected (Blanch et al., 1998), under these conditions, relative standard deviation (RSD) values of 1.4% and 3.4% for *R*- and *S*-filbertone, respectively, as well as detection limit (signal/noise = 5) data around 0.45 mg/l for each enantiomer, were obtained from three replicates. As a first step we tried to determine the absence or presence of filbertone in olive and hazelnut oils of different characteristics and geographical origins using the coupled LC-GC system previously developed in our laboratory (Fig. 1) in which the PTV acting as the interface is horizontally positioned. To this aim, the 10 olive oils and the 2 hazelnut oils included in Set 1 were analysed, as detailed in Section 2, under the initial conditions. As can be seen in Table 1, the presence of filbertone was detected in both refined and virgin hazelnut oils (samples 11 and 12) while their absence in all the olive oils analysed (samples 1–10) could be confirmed. It is important to mention that the occurrence of filbertone was always based on the presence of both enantiomers. Table 1 also shows the results of the analysis of some admixtures obtained from the adulteration at different levels of some of the olive oils with the hazelnut oils included in this set of samples. The method allowed to establish for certain the presence of both *R*- and *S*-filbertone in those admixtures of olive oil containing 20%, 17% and up to 14% of virgin and refined hazelnut oils. However, the detection of percentages of adulteration lower than 14% was not reliable in any sample due mainly to the difficulty that involved the determination of the minor enantiomer (i.e., the *R*-form). In this regard, it is also important to mention that in most olive oil samples adulterated with percentages lower than 14%, neither

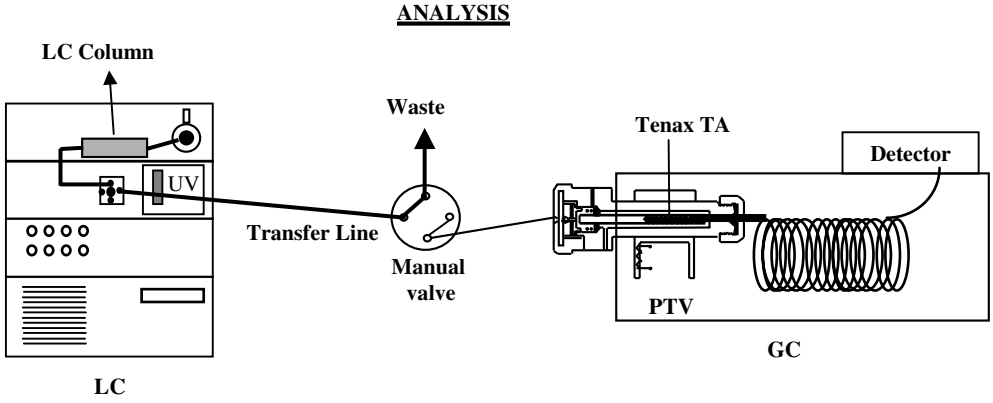


Fig. 1. Experimental set-up (GC analysis step) of the on-line coupled RPLC-GC system equipped with a manual transfer valve and a horizontally positioned PTV acting as the interface.

Table 1
Detection of *R*-filbertone and *S*-filbertone in oil samples included in the Set 1 by direct coupling RPLC-GC via horizontally positioned PTV

| Samples | Characteristics[a] | | Direct LC-GC analysis |
|---|---|---|---|
| | Olive oil | Hazelnut oil | |
| 1 | RSpPi | | Negative |
| 2 | LSpPi | | Negative |
| 3 | RGrKo | | Negative |
| 4 | LGrKo | | Negative |
| 5 | RMo– | | Negative |
| 6 | LMo– | | Negative |
| 7 | LTunCh | | Negative |
| 8 | RSpHo | | Negative |
| 9 | LItCo | | Negative |
| 10 | RItPo | | Negative |
| 11 | | RTuHaz | Positive |
| 12 | | VSpHaz | Positive |
| 13 | 80%RSpPi | 20%RTuHaz | Positive |
| 14 | 80%LGrKo | 20%VSpHaz | Positive |
| 15 | 80%RMo– | 20%RTuHaz | Positive |
| 16 | 83%LSpPi | 17%VSpHaz | Positive |
| 17 | 83%RGrKo | 17%RTuHaz | Positive |
| 18 | 83%LMo– | 17%VSpHaz | Positive |
| 19 | 86%RSpPi | 14%RTuHaz | Positive |
| 20 | 86%LItCo | 14%VSpHaz | Positive |
| 21 | 86%RItPo | 14%RTuHaz | Positive |
| 22 | 86%LGrKo | 14%VSpHaz | Positive |

*Geographical origin* Gr: Greece; It: Italy; Mo: Morocco; Sp: Spain; Tun: Tunisia; Tu: Turkey.
*Variety* Co: Coratina; Ch: Chemlali; Ko: Koroneiki; Ho: Hojiblanca; Pi: Picual; –: Unknown.
The positive identification of filbertone was based on the presence of both *R*- and *S*-enantiomers.
[a] S: Set; V: Virgin; L: Lampante virgin olive oil; R: Refined; Haz: Hazelnut; Po: 40% Olive pomace + 60% olive oil.

the *R*- nor the *S*-enantiomer could be detected when refined hazelnut oil was involved in the adulteration. This fact suggested the difficulties to be faced when trying to detect low percentages of adulteration, especially if refined hazelnut oils are to be used. Specifically, some of the system limitations, such as the difficulty of achieving complete eluent evaporation as well as the limited maximum amount of Tenax TA, which can be employed as retaining material in the system interface, did not allow both filbertone enantiomers to be detected in those samples in which their concentrations were particularly low.

For that reason, we considered mandatory to carry out a further optimisation of some critical variables (mainly those affecting the interface operation) to increase the overall system performance. First of all, to facilitate the evaporation of the eluent coming from the LC step, the PTV was changed from the horizontal to the vertical position. The new design also allowed the amount, and as a consequence the surface, of adsorbent placed into the glass liner to be increased 2.5 times (from 12 to 30 mg) with respect to that used in previous work. For that purpose, the internal diameter of the liner was increased twice in relation with that used with the horizontal configuration. Also, the manual multi-

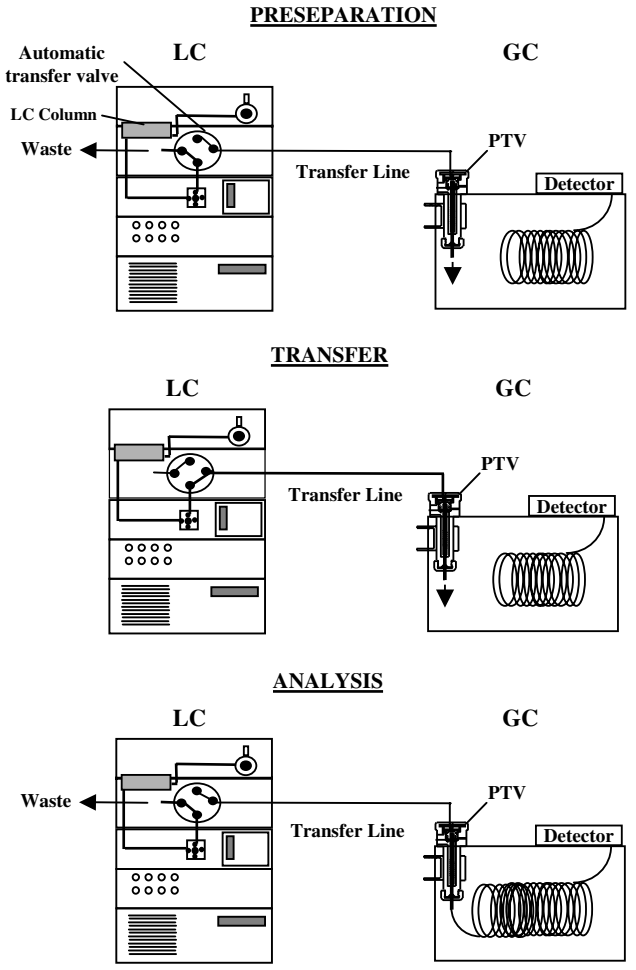


Fig. 2. Experimental set-up of the on-line coupled RPLC-GC system equipped with an automatic transfer valve and a vertically positioned PTV acting as the interface. The scheme shows the three steps which includes the experimentation, namely LC-pre-separation, transfer from LC into GC of the selected fraction and GC analysis of the compounds retained in the adsorbent material placed in the interface.

port valve placed after the UV detector of the LC system was housed into a termostated block to automate its operation through its computerized control. As shown in Fig. 2, the operation mode comprises three steps, namely LC-pre-separation, transfer from LC into GC and GC analysis. The evaluation of the new configuration was performed using standard solutions containing filbertone (1, 5 and 50 mg/l) to study the effect on the sensitivity achievable in the overall analysis of different transfer volumes (400, 600, 1000 and 1600 μl/min), PTV temperatures during transfer (0, 10 and 20 °C), helium flows during both transfer and purge (800, 1000 and 1500 ml/min), and purge times (2, 5 and 10 min).

The obtained results showed the usefulness of evaluating different sets of experimental values as it was finally possible to increase up to 15 times the sum of the peak areas corresponding to the two filbertone enantiomers when transferring a 400-μl volume fraction instead of a 1600-μl volume while setting the PTV temperature

during transfer at 10 °C. This is likely due to the fact that larger solvent volumes passing through the adsorbent sweeps in some extent the retained compounds and, consequently, losses of filbertone are finally observed. Concerning the helium flow to be applied during both transfer and purge as well as the time during which the helium flow is maintained upon completion of the transfer (i.e., the purge time), their effect on the efficient elimination of the solvent entering the GC column was also evaluated on the basis of the solvent peak area observed in the resulting chromatogram.

Summarising, the best results were obtained using 400 μl/min as transfer volume, 10 °C as PTV temperature during transfer, 1000 ml/min of helium flow during both transfer and purge, and 5 min of purge time. Under these experimental conditions, RSD values found for *R*-filbertone and *S*-filbertone ($n = 3$) were 3.4% and 0.7%, respectively, while detection limits of 0.03 mg/l were achieved for each enantiomer.

It is interesting to note that in our earlier work on filbertone determination the use of Tenax TA as adsorbent material in the PTV interface of a RPLC-GC system had provided significantly higher detection limits than those obtained in the present study. Actually, our previous papers concerning this topic have showed that filbertone results similar to those reported in the present work were only obtained if Gaschrom instead of Tenax TA was used in the interface (Ruiz del Castillo et al., 1998). However, Gaschrom exhibits important limitations with respect to Tenax TA for the direct oil analysis by RPLC-GC via PTV, namely its lower desorption temperature (i.e., 275 vs. 350 °C) and the difficulty of obtaining satisfactory blanks after having performed an analysis. This latter aspect demands an extensive and very time-consuming clean up of the chromatographic system between consecutive runs, making hence the experimental work difficult and tedious. For that reason, the possibility of achieving similar sensitivity using Tenax TA as adsorbent material instead of Gaschrom demonstrates the validity of the modifications proposed in this work concerning the PTV design and the overall system performance.

To test the sensitivity improvement attained, a new set of oil samples (Set 2), including various refined hazelnut oils from different varieties and geographical origins, was analysed by direct RPLC-GC applying the modified system. Table 2 gives the results obtained when analysing the samples included in Set 2 using the RPLC-GC system in which the PTV is in the vertical configuration. From the results obtained for samples 1–9 it is clear that the absence of filbertone was confirmed in both the lampante virgin olive oil and the refined olive oil (samples 4 and 9, respectively) while its presence could be detected in all the virgin and refined hazelnut oils, in spite of the minor enantiomer (i.e., *R*-filbertone) could not be unambiguously identified in some samples due to its co-elution with other compound.

Table 2
Detection of *R*-filbertone and *S*-filbertone in oil samples included in the Set 2 by direct coupling RPLC-GC via vertically positioned PTV

| Samples | Characteristics[a] | | Direct LC-GC analysis |
|---|---|---|---|
| | Olive oil | Hazelnut oil | |
| 1 | | VTu1Haz | Positive |
| 2 | | VItHaz | Positive |
| 3 | | VBeHaz | Positive[b] |
| 4 | LTuCe | | Negative |
| 5 | | VFrHaz | Positive |
| 6 | | RFrHaz | Positive[b] |
| 7 | | RTu2Haz | Positive[b] |
| 8 | | RTu3Haz | Positive[b] |
| 9 | RTuEr | | Negative |
| 10 | 91%LTuCe | 9%VTu1Haz | Positive[b] |
| 11 | 88%LTuCe | 12%VTu1Haz | Positive |
| 12 | 94%LTuCe | 6%VTu1Haz | Positive |
| 13 | 85%LSpPi | 15% VTu1Haz | Positive |
| 14 | 90%LMo– | 10%VItHaz | Negative |
| 15 | 85%LMo– | 15%VItHaz | Positive |
| 16 | 90%LItCo | 10%VFrHaz | Positive |
| 17 | 95%LItCo | 5%VFrHaz | Positive[b] |
| 18 | 88%RMo– | 12%RFrHaz | Positive[b] |
| 19 | 88%RGrKo | 12%RTu3Haz | Positive[b] |

*Geographical origin* Be: Belgium; Fr: France; Gr: Greece; It: Italy; Mo: Morocco; Sp: Spain; Tu: Turkey.
*Variety* Ce: Celebi; Co: Coratina; Er: Erkence; Ko: Koroneiki; Pi: Picual; –: Unknown.
The positive identification of fibertone was based on the presence of both *R*- and *S*-enantiomers.
[a] S: Training set; V: Virgin; L: Lampante virgin olive oil; R: Refined; Haz: Hazelnut.
[b] Coelution of *R*-filbertone with an unidentified compound.

As can also be observed in Table 2, the analysis of different admixtures obtained from the pure olive oils and the pure hazelnut oils showed the possibility of detecting filbertone in adulterated olive oils at levels as low as 5% of virgin hazelnut oil (sample 17) and 12% of refined hazelnut oil (samples 18 and 19) for sure since the identification of the chiral marker was based on the presence of both enantiomers. As an example, Fig. 3 shows the satisfactory enantiomeric resolution attained in the GC analysis of the fraction transferred from LC into GC for both a virgin hazelnut oil (a) and a refined hazelnut oil (b). In this respect, it is worthy to mention that, generally speaking, the possibility of transferring a small portion of the first column effluent containing the analytes of interest enabled clean gas chromatograms to be obtained by optimising the selectivity achievable in the overall analysis. As a consequence, it was possible the reliable confirmation of either the absence of filbertone in a lampante virgin olive oil (Fig. 4(a)) or the presence of both *R*- and *S*-filbertone in an olive oil adulterated with either 10% of a virgin hazelnut oil (Fig. 4(b)) or 12% of a refined hazelnut oil (Fig. 4(c)). It is convenient to point out that the *R*-enantiomer of filbertone co-eluted with other matrix component in Fig. 4(c) so that the identification of the chiral marker

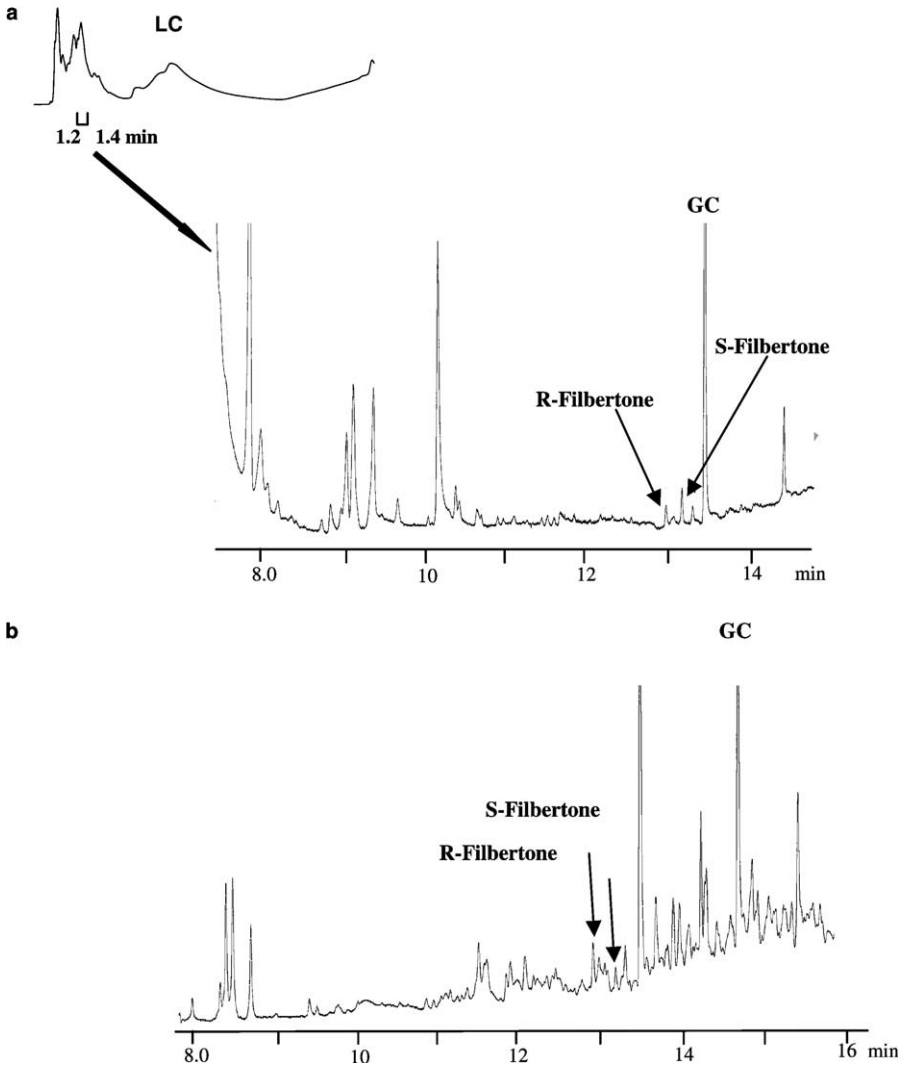


Fig. 3. Chromatograms obtained from the LC-pre-separation and the subsequent GC analysis of a 400-μl transferred fraction (helium flow rate, 2000 μl/min) from (a) a virgin hazelnut oil (Sample 3 in Table 2) and (b) a refined hazelnut oil (Sample 6 in Table 2). GC column: 25-m × 0.25-mm i.d. fused silica coated with Chirasil-β-Dex. See text for further details.

was mainly based on the occurrence of its major enantiomer (*S*-filbertone).

As expected, the percentage of adulteration to be finally detected depends strongly on the filbertone level in the pure hazelnut oil used to prepare the admixture. This is clearly reflected in sample 14 (Table 2), in which a percentage of 10% of virgin hazelnut oil (VItHaz) could not be detected whereas a 5% adulteration of VFr-Haz (sample 17 in Table 2) was easily identified. In the same way, it could be stated that the hazelnut oil labelled as RTuHaz (sample 11 in Table 1) contained reasonably high amount of filbertone since adulterations of up to 14% by using the refined oil could be observed by using the unmodified design of the on-line RPLC-GC system. In any case, it should be kept in mind that the high temperatures applied in the deodorisation step of the refining process produce losses of volatile compounds (e.g., filbertone) that can increase considerably depending on how drastic the conditions are, namely temperature and time under which the deodorisation has been performed. Moreover, other chemical structures can also be altered during the refining process so that significant interferences from other compounds, and hence very dirty chromatograms, can be eventually observed. As a result, the fraudulent addition of some refined hazelnut oil in particular might be specially complicated.

Consequently, it can be concluded that the proposed procedure based on the use of RPLC-GC coupled by means of a horizontally positioned PTV may be of great usefulness as a rapid screening method for the detection of olive oils adulterated with 5% or 12% of some virgin and refined hazelnut oils, respectively, as the overall RPLC-GC analysis of both enantiomers of filbertone only takes 30 min without demanding any kind of sample pre-treatment. Additional advantages of the method

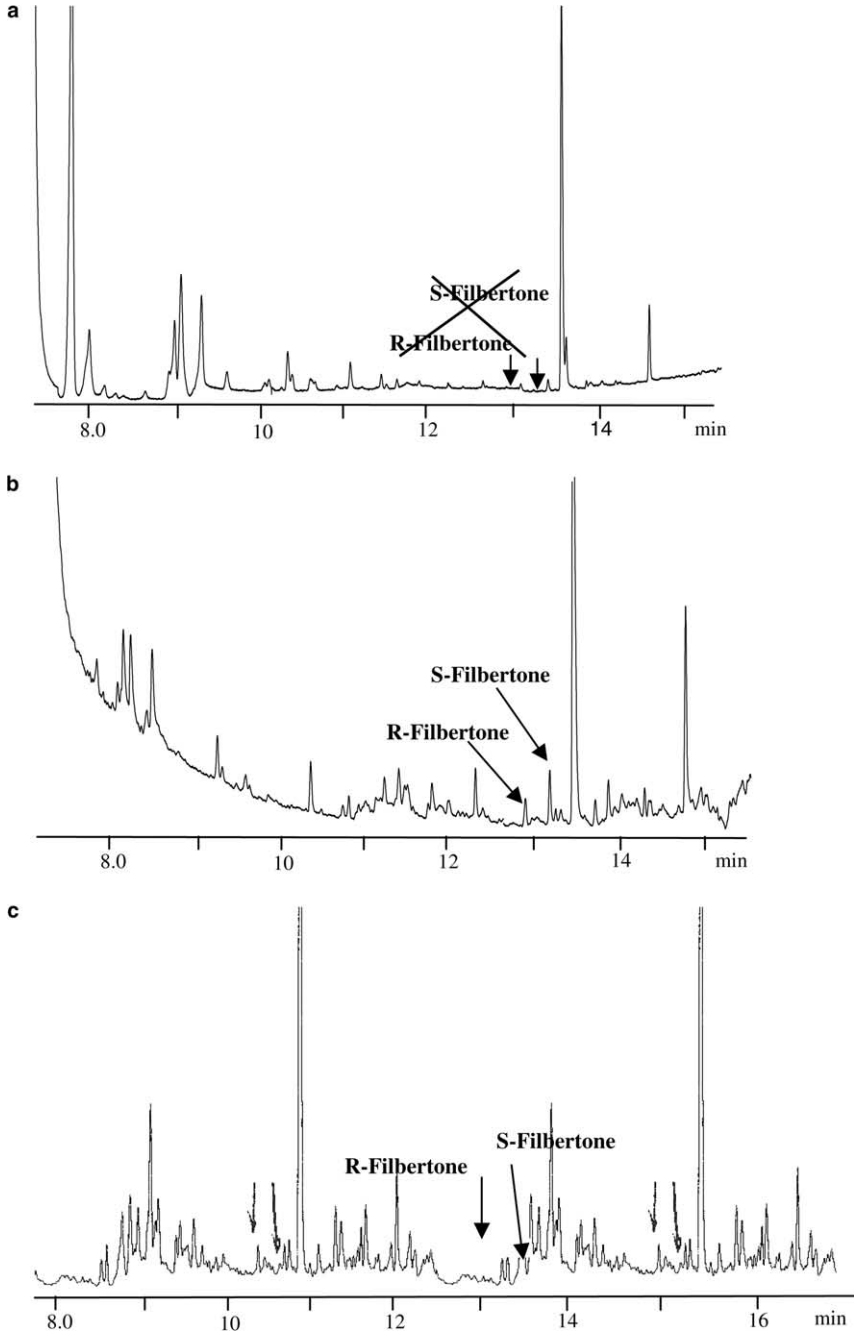


Fig. 4. Chromatograms obtained from LC-GC transfer of a 400-μl fraction from (a) a lampante olive oil (Sample 4 in Table 2), (b) an admixture of a lampante olive oil and 10% of a virgin hazelnut oil (Sample 16 in Table 2), and (c) an admixture of a refined olive oil and 12% of a refined hazelnut oil (Sample 19 in Table 2).

proposed are its selectivity and sensitivity when a vertically positioned PTV is used as the interface of the coupling.

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