

Enantiomer Analysis of Chiral Lactones in Foods by On-Line Coupled Reversed-Phase Liquid Chromatography–Gas Chromatography

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Abstract

A new application is proposed for the on-line coupling of reversed-phase liquid chromatography to gas chromatography (RPLC–GC) that allows the GC chiroselective analysis of γ -lactones in fruits and commercially available fruit-containing products. The use of a programmed temperature vaporizer as an interface with the system makes the transfer of large volume fractions (i.e., 2520 μ L) of aqueous eluents from LC to GC possible (speed of sample transfer, 1800 μ L/min). Relative standard deviations obtained for the investigated lactones under the experimental conditions vary from 7 to 14%. The described system enlarges the LC–GC application field and overcomes the limitations reported thus far concerning the use of typical normal-phase eluents (i.e., the transfer of rather small volume fractions at low speeds of sample introduction).

Introduction

The significance of stereochemistry with respect to biological regulation and activity phenomena in various fields has been increasingly recognized over the past few years. Specifically, the relevance of enantiomeric separation has proven to be an important aspect in food science because many of the flavors, fragrances, preservatives, additives, etc. currently used in the food industry are characterized by a specific enantiomeric composition. In this respect, the ability to separate enantiomers at low levels is of great interest when identifying adulterated food and beverages, controlling fermentation processes and products, analyzing chiral metabolites of chiral and prochiral food and beverage components, and better characterizing flavor compounds (1,2).

Thus far, different analytical procedures have been reported for chiroselective studies in various matrices (3–7). Unfortunately, it may be rather difficult to develop new separation methods suitable for the assay of enantiomeric excesses of compounds occurring in complex mixtures (e.g., foods and beverages) and consisting of very many species with a wide range of molecular

sizes and polarities; these compounds may be present in very low concentrations and, moreover, may be chromatographically overlapped by other matrix components. For this reason, the enantiomeric composition of the chiral components is frequently not considered when analyzing food and beverage products.

On the other hand, the advantages of using coupled techniques in food composition studies have already been demonstrated (8–11). In this regard, the potential of the on-line coupling of liquid chromatography and gas chromatography (LC–GC) as a powerful separation technique that also allows effective sample preparation has been pointed out in the last few years (12–14). However, most of the work performed using coupled LC–GC actually employs a normal phase in the LC step, mainly because typical reversed-phase solvents bring about a number of problems (e.g., lack of wettability of retention gaps, low evaporation rates, large volumes of vapor per unit volume of liquid, etc.) that render the transfer of a fraction from the LC pre-separation to GC difficult. Taking into account that a high percentage of LC separations are actually performed by reversed-phase LC (RPLC), it is evident that the ability to use aqueous solvents in the LC step would result in a more versatile RPLC–GC approach and, consequently, would enlarge the LC–GC application field.

As far as food analysis is concerned, a number of practical applications of LC–GC have been described (15–18), although the fact that they refer almost exclusively to the use of a normal phase creates distinct problems. Specifically, the transfer technique in many cases does not allow high-volume fractions to be transferred so that a significant percentage of all the analytes available may be discarded. For this reason, the introduction of large volumes into a GC has recently been considered to be the key to on-line LC–GC (14). Previous experience concerning both large sample volume introduction into capillary GC (19,20) and the transfer of large volume fractions from LC into GC (21–23) has shown the merits of the RPLC–GC technique, although its usefulness for enantiomeric analysis has not yet been evaluated.

The aim of this work was to investigate the potential of RPLC–GC as a stereoselective separation method suitable for the easy and effective resolution and analysis of chiral compounds in fruits and fruit-containing products. A further aim of the investigation was to overcome the limitations reported thus far (i.e., use

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of normal-phase eluents, low transfer flow rates, and small volumes of transferred fractions) concerning the employment of LC–GC to analyze γ -lactones (24,25).

Experimental

Materials

The test solution contained 100 ppm of different γ -lactones (C_8 , C_9 , C_{10} , and C_{11}) obtained from Aldrich (Milwaukee, WI). Peach natural flavor and peach nature-identical flavor were supplied by Lucta S.A. (Barcelona, Spain). Different commercially available products including a raw fruit (i.e., peach) and products containing fruit (i.e., a strawberry flavor used in confection and a strawberry dressing) were also analyzed.

Tenax TA (80–100 mesh) was used as a packing material in the glass liner (80 mm \times 1-mm i.d. \times 2-mm o.d.) of the PTV injector.

Prior to its use, it was conditioned under a stream of helium at 350°C for 120 min. Different blanks (350°C, 20 min) were performed between successive runs.

Steam distillation solvent extraction

In those cases in which an isolation step was necessary, sample concentration was carried out using a commercial (Chrompack, Middelburg, The Netherlands) micro steam distillation–extraction (SDE) apparatus in the high-density solvent configuration. SDE extracts were obtained from different amounts of sample materials (100 g peach, 3 mL strawberry flavor, and 10 mL strawberry dressing). In each case, 100 mL of water purified in a Milli-Q system (Millipore, Milford, MA) was used, and 2 mL of distilled dichloromethane was employed as the extraction solvent. The sample material was heated by applying an oil bath at 140°C, and at the same time, the solvent was distilled by heating with a water bath maintained at 67°C. Vapors of the solvent and the sample were condensed by a cold finger refrigerated at 0°C. A continuous

reflux of water and solvent was maintained during the extraction time (60 min). The SDE apparatus was cleaned between successive extractions with acetone and Milli-Q purified water. Once the SDE operation was complete, a subsequent concentration step was not required, and a 20- μ L volume of the extract was directly injected into the LC–GC system.

Instrumentation

The injector of the GC (PTV programmed temperature injector, Perkin-Elmer, Norwalk, CT) was used as the interface of the LC–GC system, as shown in Figure 1. For the LC pre-separation, a Hewlett-Packard (Palo Alto, CA) model 1050 chromatograph equipped with a manual injection valve (Rheodyne model 7125) having a 20- μ L sample loop and an ultraviolet (UV) detector was used. In all cases, analyses were carried out on a column (50 \times 4.6-mm i.d.) which was slurry packed using Vydac 214 TPB 10 as packing material, following a previously reported procedure (26). The initial composition of the eluent (methanol–water, 35:65) was maintained for 18 s,

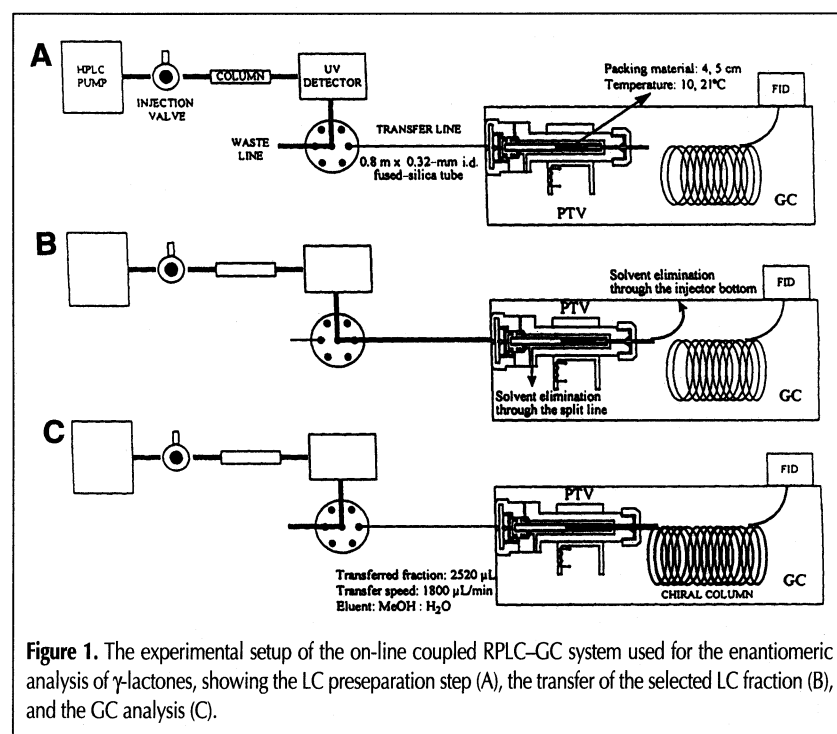


Figure 1. The experimental setup of the on-line coupled RPLC–GC system used for the enantiomeric analysis of γ -lactones, showing the LC pre-separation step (A), the transfer of the selected LC fraction (B), and the GC analysis (C).

Table I. Absolute Peak Areas and Relative Standard Deviations ($n = 3$) for Different Plug Lengths and Initial Liner Temperatures Obtained for the RPLC–GC Transfer of a Standard Solution of γ -Lactones*

γ -Lactone	4 cm, 10°C		4 cm, 21°C		5 cm, 10°C		5 cm, 21°C	
	Area	RSD (%)	Area	RSD (%)	Area	RSD (%)	Area	RSD (%)
(R)-Octa	66968	9.9	19794	5.5	95142	8.3	52352	7.6
(S)-Octa	69904	2.5	20842	7.8	108379	7.5	50851	9.4
(R)-Nona	53004	9.9	17353	2.9	80348	12.7	46649	7.6
(S)-Nona	56485	30.2	19546	2.5	88079	12.1	54999	6.4
(R)-Deca	48042	21.4	17011	3.8	77777	12.9	41951	8.5
(S)-Deca	49244	9.9	17274	3.3	78359	14.3	62043	8.2
(R)-Undeca	55505	14.6	18619	6.2	93606	10.9	54952	7.7
(S)-Undeca	60068	9.8	19482	6.7	97779	7.6	80965	6.9

* Transferred fraction, 2520 μ L; speed of sample transfer, 1800 μ L/min; eluent, methanol–water.

followed by a linear gradient up to 90% methanol within 3 s and maintained during the remainder of the analysis. Throughout the experiment, the column was maintained at 45°C (flow rate, 1.8 mL/min). UV detection was carried out at 205 nm. An HP Chem Station (Hewlett-Packard) was used for the acquisition of data from the UV detector.

The GC system consisted of a Perkin-Elmer model 8500 instrument provided with a flame ionization detector (FID) and coupled to model 2600-rev 5.0 chromatography software. The temperature of the FID was 320°C. A fused-silica column (25 m × 0.25-mm i.d.) coated with a 0.25- μ m layer of Chirasil- β -Dex was used. The GC oven was maintained at 130°C for 10 min and programmed to 180°C at 2°C/min. The final temperature was maintained for 30 min. Helium was used as the carrier gas.

LC–GC transfer

As previously mentioned, the LC–GC transfer was carried out using the PTV injector of the GC system as an interface.

Table II. Limits of Detection (LODs) for Different Plug Lengths and Initial Liner Temperatures Obtained for the RPLC–GC Transfer of a Standard Solution of γ -Lactones*

γ -Lactone	4 cm, 21°C LOD (ng/mL)	5 cm, 10°C LOD (ng/mL)
(R)-Octa	230	24
(S)-Octa	230	27
(R)-Nona	340	44
(S)-Nona	350	47
(R)-Deca	330	63
(S)-Deca	350	70
(R)-Undeca	290	44
(S)-Undeca	300	52

* Transferred fraction, 2520 μ L; speed of sample transfer, 1800 μ L/min; eluent, methanol–water.

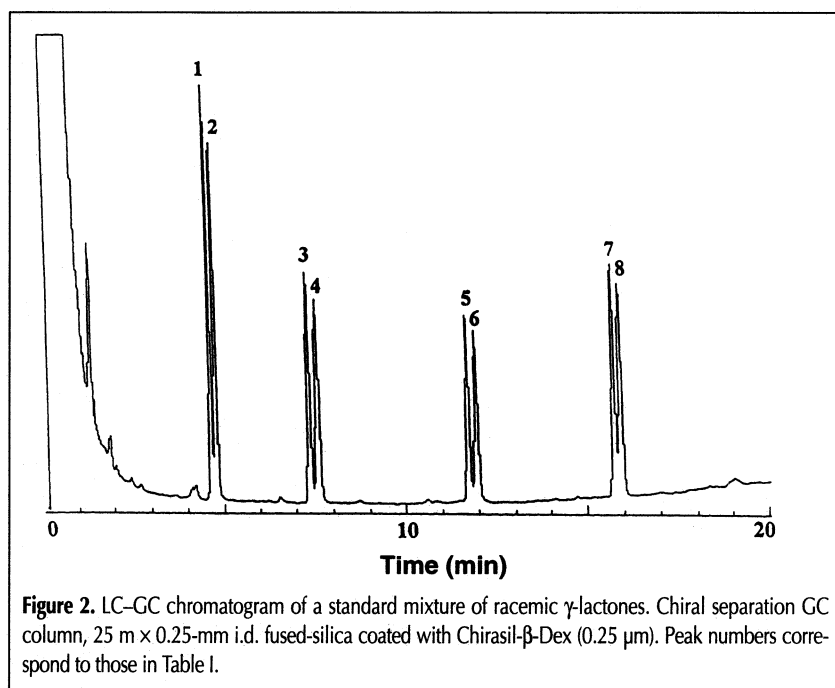


Figure 2. LC–GC chromatogram of a standard mixture of racemic γ -lactones. Chiral separation GC column, 25 m × 0.25-mm i.d. fused-silica coated with Chirasil- β -Dex (0.25 μ m). Peak numbers correspond to those in Table I.

Specifically, a multi-port valve placed after the UV detector was used either to transfer the LC effluent directly into the GC via PTV or to waste (Figure 1). The transfer line (an 0.8-m × 0.32-mm-i.d. fused-silica tube) allowed the sample fraction to be transferred from the LC system to the GC injector by inserting it into the PTV body through the septum.

The choice of transfer conditions was based on previous experience concerning the use of experimental designs both in large sample introduction in capillary GC and on-line LC–GC transfer (21,22) and on the characteristics of the samples to be analyzed. As a result, experimental conditions were established as follows: fraction volume transferred from LC into GC, 2520 μ L; helium flow rate during the transfer, 1500 mL/min; speed of sample transfer (equal to the flow rate during the LC pre-separation step), 1800 μ L/min. The silylated glass insert from the PTV injector was packed with two different lengths (4 and 5 cm) of the trapping material (Tenax TA, 80–100 mesh), and two different PTV initial temperatures (10 and 21°C) were considered in order to evaluate the influence of the variation of the mentioned experimental conditions on the sensitivity achievable with the analysis.

The proposed experimental procedure allows solvent elimination to be achieved through both the split line and the injector bottom. In order to facilitate the elimination of the solvent that passes through the injector as a liquid, the GC column end was withdrawn from the injector body prior to the LC–GC transfer and it was again connected after 6 min, once the purge time (i.e., the time during which both the final PTV temperature and the helium flow rate were kept constant to promote elimination of the remaining solvent from the glass liner) was completed. At the end of the solvent elimination step, the transfer line was removed from the PTV injector. The oven program started when the column reached 130°C, and at that moment, the PTV temperature was raised to 350°C at 14°C/s and maintained for 11 min to achieve the thermal desorption of the retained material. Subsequently, the gas chromatographic analysis was performed (split ratio, 50:1) and the enantiomeric composition of the chiral compounds was evaluated (Figure 1).

Results and Discussion

With the aims of the method (i.e., transfer from LC into GC of high-volume fractions of aqueous eluents) in mind, it is evident that the achievement of complete solvent elimination may be rather difficult. In fact, it has already been shown (27) that large volumes can be sampled into capillary GC, provided that the speed of sample introduction equals the solvent elimination rate. This certainly involves a serious limitation for this specific RPLC–GC analysis, because the fraction of interest consists of several hundred microliters, and by no means can the solvent elimination rate equal the speed of sample introduction. Actually, complete solvent elimination may only be achieved if the experimental conditions allow both the evaporative and non-evaporative modes of solvent elimination.

For that reason, experimental conditions affecting the analysis were carefully established in such a way that the requirements of both solvent splitting and solvent-phase extraction could somehow be met. As a result, the experimental procedure presented in this work allows the speed of sample transfer to be higher than that demanded if the evaporative mode of solvent elimination were exclusively involved in the process, and consequently, large volume fractions can be transferred in relatively short periods of time.

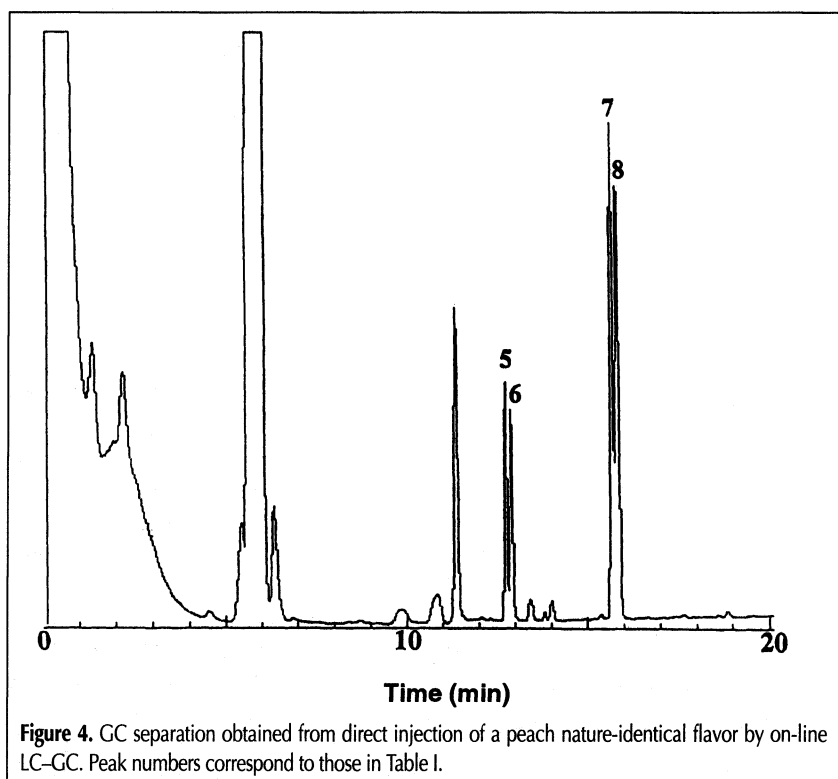
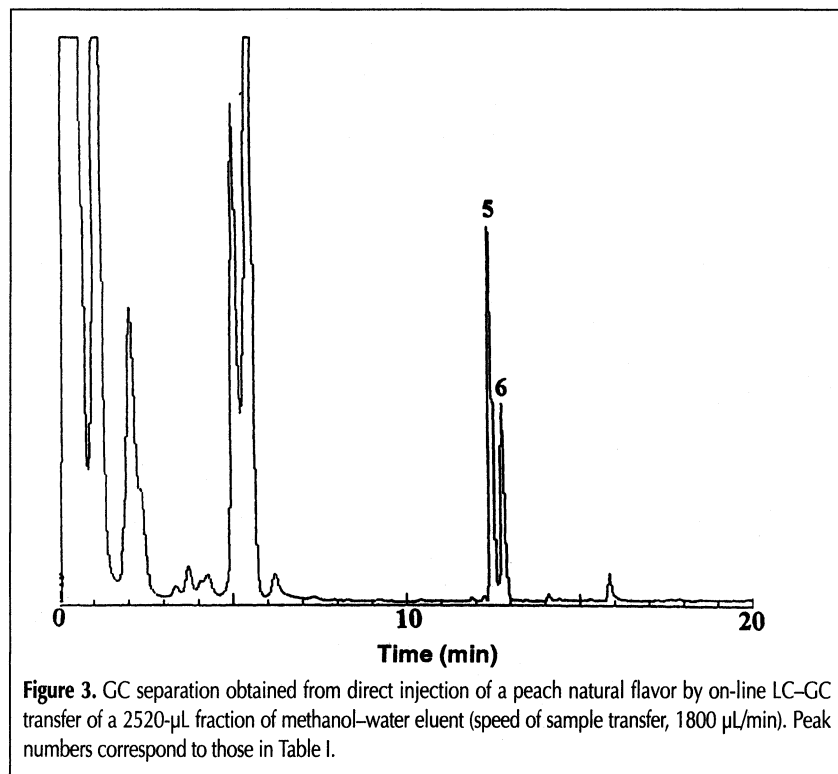


Table I shows the effect of both the length of the packing material in the glass-liner of the PTV and its initial temperature on the absolute peak areas obtained in the GC analysis of the transferred LC fraction. The repeatability, in relative standard deviation (RSD) values, obtained under the investigated conditions from a minimum of three replicates is also given in Table I. As can be seen, experimental conditions providing the highest peak areas (length of packing material, 5 cm; initial PTV temperature, 10°C) resulted in RSD values ranging from 7.5 to 14.3%.

The limits of detection (LOD), expressed as the amount of compound giving a signal equal to three times the background noise (established from the width of the base line), are shown in Table II. These values were determined for each analyte under the experimental conditions providing the highest sensitivity (according to Table I: length of packing material, 5 cm; initial PTV-temperature, 10°C). For the sake of comparison, LODs obtained under those experimental conditions yielding the lowest sensitivity (length of packing material, 4 cm; initial PTV temperature, 21°C) are also included in Table II. From these data, it is clear that the use of a 4-cm plug length maintained at 21°C does not efficiently retain the transferred solutes.

Figure 2 shows the chromatogram obtained (under experimental conditions providing the highest sensitivity) from the transfer of a 2520- μ L fraction resulting from the LC pre-separation of the standard mixture of γ -lactones mentioned in the Experimental section. Pairs of peaks show racemates with the (*R*)-enantiomer eluted first. The ability to transfer large volume fractions is of special importance to achieve the transfer of the LC fraction comprising the whole peak of interest. In fact, the transfer of fractions lower than 2520 μ L did not allow the GC analysis of the four lactones included in the test solution.

Figures 3 and 4 show the LC-GC separation obtained (in less than 20 min) from a peach natural flavor and a peach nature-identical flavor, respectively. The term nature-identical refers to a synthetic or an extracted compound whose chemical structure is identical to that of a chemical compound naturally occurring in a vegetal or animal matrix. Therefore, nature-identical is used to differentiate naturally occurring aromas from synthesized aromas, the latter including artificial and nature-identical flavor substances. The enantiomeric excess of the γ -decalactone detected in the natural flavor was 32%, and racemic mixtures of γ -decalactone and γ -undecalactone were found in the nature-identical flavor. Consequently, the knowledge of the optical purity of a chiral molecule may be used to distinguish between naturally occurring and nature-identical aromas in food. On the other hand, because enantiomeric excesses reported for natural γ -lactones in fruits

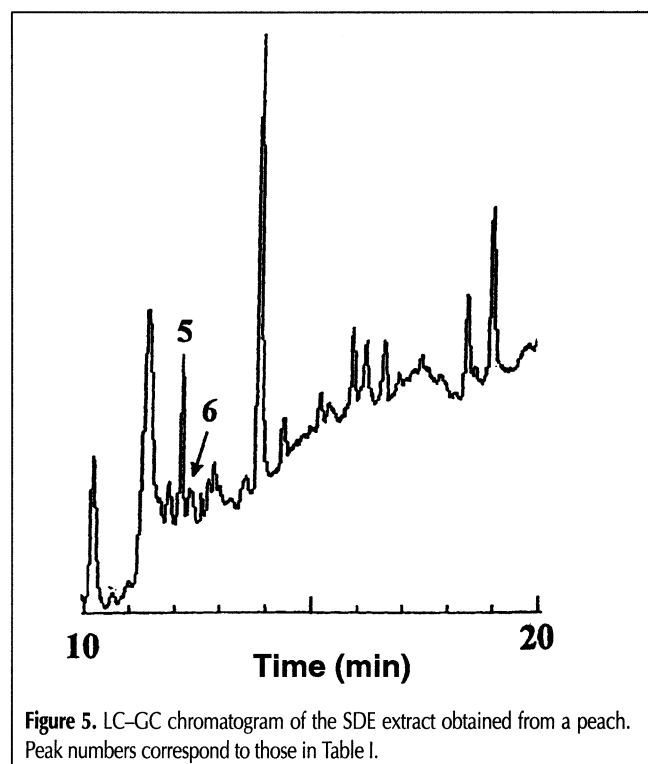
are usually higher than 80%, the low value found in this work suggests that the natural aroma has not been obtained from the peach but from another matrix in which the lactones of interest are present.

The LC-GC analysis of the SDE extract of a native fruit (Figure 5) allowed the assay of the enantiomeric excess, revealing the fruit-specific distribution of the corresponding chiral compound (*[R]* and *[S]*- β -decalactone) from a natural source. Taking into account the signal and noise level, the identification of *S*-decalactone was confirmed by adding the standard compound to the sample and performing a new analysis under identical experimental conditions.

When performing a similar analysis (i.e., SDE and LC-GC) using both a strawberry flavor used in confection and a strawberry dressing (Figure 6), racemic γ -decalactones were observed. because the natural occurrence of racemic γ -lactones has not yet been reported, their detection in a specific sample may indicate the addition of synthetic flavorings.

Conclusion

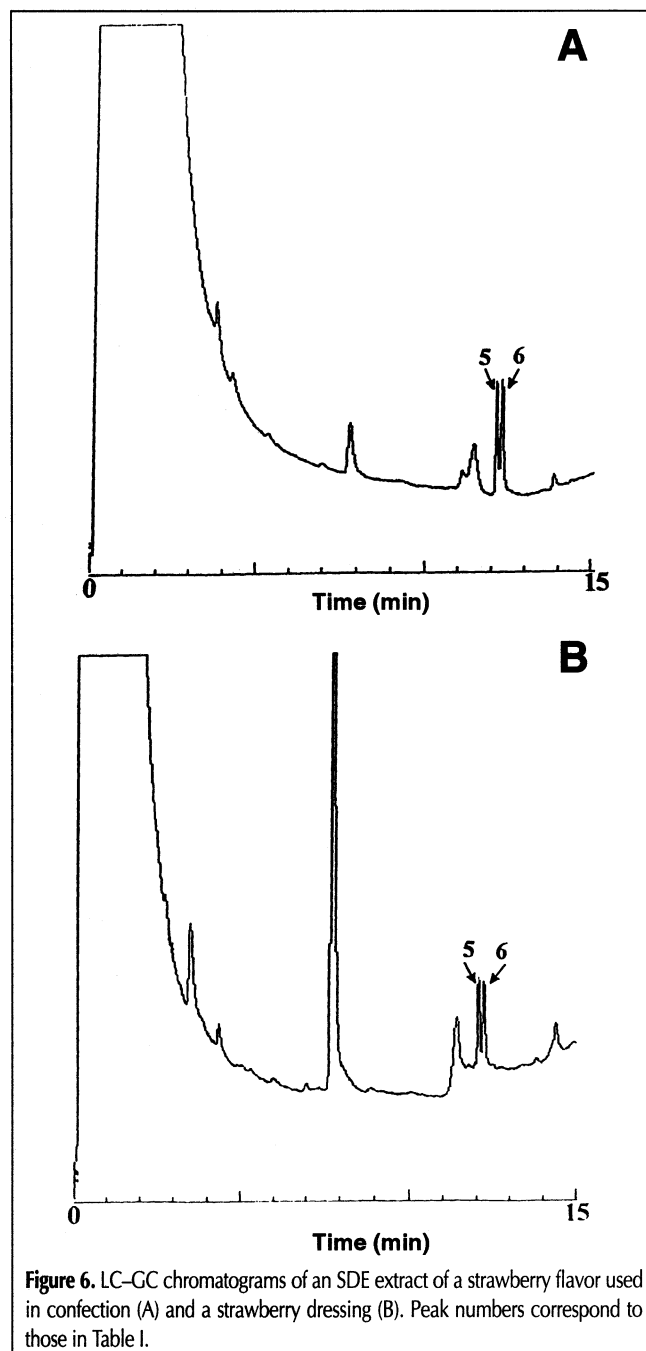
The ability to transfer high-volume fractions of aqueous solvents (e.g., 2520 μ L) at high speeds of sample transfer (e.g., 1800 μ L/min) may significantly enlarge the LC-GC application field concerning chirality evaluation in food composition studies. Specifically, the enantiodifferentiation method reported in this paper makes it possible to distinguish between γ -lactones of natural and synthetic (racemic) origin. Consequently, the declaration of additional flavors contained in commercially available products as natural or nature-identical compounds may be easily checked, even when the complexity of the sample to be analyzed



demands a rapid, sensitive, and reliable analytical method as an indispensable prerequisite for the chiroselective analysis of γ -lactones from flavor matrices.

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References

1. D.W. Armstrong, C.D. Chang, and W.Y. Li. Relevance of enantiomeric separations in food and beverage analyses. *J. Agric. Food Chem.* **38**: 1674–77 (1990).
2. A.M. Stalcup, K.H. Ekborg, M.P. Gasper, and D.W. Armstrong. Enantiomeric separation of chiral components reported to be in coffee, tea, or cocoa. *J. Agric. Food Chem.* **41**: 1684–89 (1993).
3. R.W. Shouter. *Chromatographic Separations of Stereoisomers*. CRC Press, Boca Raton, FL, 1985.
4. W.A. König. *The Practice of Enantiomer Separation by Capillary Gas Chromatography*. Hüthig, Heidelberg, Germany, 1987.
5. S. Allenmark. *Chromatographic Enantioseparation*. Ellis Horwood, New York, NY, 1991.
6. W.A. König. *Enantioselective Gas Chromatography with Modified Cyclodextrins*. Hüthig, Heidelberg, Germany, 1992.
7. V. Schurig. Enantiomer separation by gas chromatography on chiral stationary phases. *J. Chromatogr. A* **666**: 111–29 (1994).
8. G. Schomburg, H. Husmann, L. Podmaniczky, and F. Weeke. *Analysis of Volatiles*, P. Schreier, Ed. Walter de Gruyter & Co, Berlin, Germany, 1984.
9. A. Mosandl, U. Hener, U. Hagenauer-Hener, and A. Kusterman. Direct enantiomer separation of chiral γ -lactones from food and beverages by multidimensional gas chromatography. *J. High Resolut. Chromatogr.* **12**: 532–36 (1989).
10. A. Mosandl, U. Hener, U. Hagenauer-Hener, and A. Kusterman. Stereoisomeric flavor compounds. Multidimensional gas chromatography direct enantiomer separation of γ -lactones from fruits, foods and beverages. *J. Agric. Food Chem.* **38**: 767–71 (1990).
11. G.P. Blanch, E. Ibáñez, M. Herraiz, and G. Reglero. Use of a programmed temperature vaporizer for off-line SFE/GC analysis in food composition studies. *Anal. Chem.* **66**: 888–92 (1994).
12. K. Grob. *On-Line Coupled LC–GC*. Hüthig, Heidelberg, Germany, 1991.
13. J.J. Vreuls, G.J. de Jong, R.T. Ghijssen, and U.A.Th. Brinkman. Liquid chromatography coupled on-line with gas chromatography: state of the art. *J. Ass. Off. Anal. Chem. Int.* **77**: 306–327 (1994).
14. K. Grob. Development of the transfer techniques for on-line high performance liquid chromatography–capillary gas chromatography. *J. Chromatogr. A* **703**: 265–76 (1995).
15. K. Grob, M. Lanfranchi, and C. Mariani. Evaluation of olive oils through the fatty alcohols, the sterols and their esters by coupled LC–GC. *J. Am. Oil Chem. Soc.* **67**: 626–34 (1990).
16. K. Grob and M. Bronz. On-line LC–GC transfer via a hot vaporizing chamber and vapor discharge by overflow; increased sensitivity for the determination of mineral oil in foods. *J. Microcol. Sep.* **7**: 421–27 (1995).
17. M. Biedermann, K. Grob, C. Mariani, and J.P. Schmidt. Detection of deuterolized sunflower oil in olive oil through isomerized Δ^7 -sterols. *Z. Lebensm. Unters. Forsch.* **202**: 199–204 (1996).
18. L. Mondello, G. Dugo, and K.D. Bartle. On-line microbore high performance liquid chromatography–capillary gas chromatography for food and water analyses. A review. *J. Microcol. Sep.* **8**: 275–310 (1996).
19. J. Villén, F.J. Señoráns, M. Herraiz, G. Reglero, and J. Tabera. Experimental design optimization of large volume sampling in a programmed temperature vaporizer. Application in food analysis. *J. Chromatogr. Sci.* **30**: 261–66 (1992).
20. F.J. Señoráns, J. Tabera, J. Villén, M. Herraiz, and G. Reglero. Variables affecting the introduction of large sample volumes in capillary gas chromatography using a programmed-temperature vaporizer. *J. Chromatogr.* **648**: 407–414 (1993).
21. F.J. Señoráns, M. Herraiz, and J. Tabera. On-line reversed-phase liquid chromatography capillary gas chromatography using a programmed temperature vaporizer as interface. *J. High Resolut. Chromatogr.* **18**: 433–438 (1995).
22. F.J. Señoráns, G. Reglero, and M. Herraiz. Use of a programmed temperature injector for on-line reversed-phase liquid chromatography–capillary gas chromatography. *J. Chromatogr. Sci.* **33**: 446–50 (1995).
23. F.J. Señoráns, J. Tabera, and M. Herraiz. Rapid separation of free sterols in edible oils by on-line coupled reversed phase liquid chromatography–gas chromatography. *J. Agric. Food Chem.* **44**: 3189–92 (1996).
24. A. Mosandl and A. Kustermann. Stereoisomere Aromastoffe: HRGC-Analyse chiraler γ -Lactone aus Getränken und Fruchtzubereitungen. *Z. Lebensm. Unters. Forsch.* **189**: 212–15 (1989).
25. H.-G. Schmarr, A. Mosandl, and K. Grob. Stereoisomeric flavour compounds. Direct chiroselective analysis of γ -lactones using on-line coupled LC–GC with a chiral separation column. *Chromatographia* **29**: 125–30 (1990).
26. M. de Frutos, A. Cifuentes, L. Amigo, M. Ramos, and J.C. Díez-Masa. Rapid analysis of whey proteins from different animal species by reversed-phase high-performance liquid chromatography. *Z. Lebensm. Unters. Forsch.* **195**: 326–31 (1992).
27. J. Staniewski and J.A. Rijks. Solvent elimination rate in temperature programmed injections of large sample volumes in capillary gas chromatography. *J. Chromatogr.* **623**: 105–13 (1992).

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