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On-line reversed–phase liquid chromatography-gas chromatography coupled to mass spectrometry for enantiomeric analysis of chiral compounds in fruit beverages

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Abstract

A method based on the on-line coupling of reversed phase liquid chromatography with gas chromatography/mass spectrometry (RPLC-GC-MS) for the chiral evaluation of characteristic constituents of fruit beverage aroma was investigated. The consideration of a variety of parameters involved in the transfer step allowed to achieve relative standard deviations ranging from 0.4 to 10% in most cases and detection limits from 0.2 to 2.5 mg/l. By applying the developed method to fruit beverages, racemic mixtures of ethyl 2-methylbutanoate and γ-nonalactone were found. This fact suggests the eventual addition of artificial aromas. The method proposed in the present work can be useful to assess reliably the authenticity of aqueous samples, such as fruit beverages.

Keywords: Enantiomer separation; Fruit beverages; Food analysis

1. Introduction

Over the past few years, there has been increasing interest in stereodifferentiation of chiral compounds in complex matrices, such as foodstuffs. This recent interest is due mostly to the possibility of relating the enantiomeric composition to certain technological processes, contamination, adulteration, ageing, etc. [1–3]. Also, it is well known that certain pairs of enantiomers exhibit different sensorial properties [4] so that slight variations in the enantiomeric composition may result in modifications of flavour perception. For that reason, the consideration of stereochemistry in food analysis may enable a more reliable evaluation to be accomplished.

However, the selection of chiral compounds to be used as suitable indicators in characterization studies may be a complicated task as it involves the analysis of trace levels in complex mixtures of a great number of compounds. Moreover, the sample preparation procedure prior to the chromatographic analysis may alter the characteristic enantiomeric composition of the selected chiral indicators leading, therefore, to incorrect results.

These problems can be efficiently addressed by using multidimensional techniques [5,6] as the sample pre-separation can be performed in the first dimension, what occasionally avoids the need for a major pre-treatment prior to the analysis. In this respect, the use of multidimensional techniques for food analysis has been mostly focused on multidimensional gas chromatography [7–9] and, to a far lesser extent, on the on-line coupling of high-performance liquid-chromatography (LC) with gas chromatography (GC) using normal phase in the pre-separation step [10–12]. Nonetheless, it is necessary to take into account that a very high percentage of LC separations are performed by reversed phase LC (RPLC) as the employment of hydrophobic packing materials and aqueous mobile phases enlarges the range of compounds which can be analysed. In fact, some specific applications concerning the usefulness of the combination of RPLC with GC (RPLC-GC) for the analysis of complex mixtures have already been reported in the literature [13–15], although, as far as food evaluation is concerned, in particular chiral studies, published reports on RPLC-GC have mostly centred on non-aqueous samples, such as vegetable oils [16,17].

With regard to the employment of mass spectrometry (MS) on-line coupled to a LC-GC system for food analysis, only a limited number of applications has been described

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by other researchers [18–21]. However, it is worth mentioning that up to date normal phase has been used in all cases as the employment of reversed phase in the LC-step not only makes it more difficult the solvent elimination during the LC-GC transfer but also the experimentation with MS.

On the other hand, fruit beverages are nowadays one of the most valuable products in the food industry. As a matter of fact, adulteration of this kind of foodstuffs has become a significant problem in the industry, resulting in the need for the development of new approaches which allow to guarantee fruit beverage authenticity.

Considering, on the one hand, the usefulness of the enantiomeric composition of chiral compounds for quality studies and, on the other, the necessity for extending the applicable range of on-line RPLC-GC for the analysis of aqueous matrices, the objective of the present work was to develop a method based on the use of on-line coupling RPLC-GC-MS to evaluate the authenticity of aqueous samples, such as fruit beverages, by means of the enantiomeric distribution of chiral compounds. Although we have recently applied RPLC-GC to the study of chiral compounds in beverages [22], in the present work we propose the use of a different experimental set-up for the RPLC-GC system. Specifically, we aimed to evaluate the employment of a vertically positioned programmed temperature vaporiser (PTV) as the interface of the system as it theoretically should allow a more efficient elimination of the solvent and, hence, the possibility of performing the direct MS analysis of the effluent coming from the RPLC-GC separation.

2. Experimental

2.1. Samples and materials

A standard solution containing ethyl 2-methylbutanoate, limonene, terpinen-4-ol, α-terpineol, γ-octalactone, γ-nonalactone, γ-decalactone, and γ-undecalactone (0.1 mg of each enantiomer in 10 ml of methanol) was used for identification purposes. All the standards were acquired from Aldrich (Milwaukee, WI) and used as racemic mixtures, except terpinen-4-ol, which was used as the pure (+)-enantiomer. Methanol (HPLC grade) was purchased from Scharlau Chemie, S.A. (Barcelona, Spain), and the water used was obtained from a Milli-Q water purification system (Millipore, Milford, MA). A 4 cm length plug of Tenax TA (80–100 mesh Chrompack, Middelburg, The Netherlands) was placed in the silylated glass liner (70 mm × 2 mm i.d. × 3 mm o.d.) of the GC-injector and maintained there with two plugs of glass wool. Prior to its use, Tenax TA was conditioned, under a stream of helium, for 120 min at 350 °C.

The fruit beverages analysed were purchased in the commercial market. Each beverage was centrifuged (10^4 rpm, 20 min at 10 °C) and the supernatant was filtered using a 0.20 μm filter and then submitted to the on-line coupled RPLC-GC-MS.

2.2. On-line coupling liquid chromatography-gas chromatography

The multidimensional system used in this study consisted of a liquid chromatograph and a gas chromatograph linked through a programmed temperature vaporizer (PTV) injector that acted as the interface. LC pre-separation was carried out with a Hewlett-Packard model 1050 (Wilmington, DE) chromatograph fitted with a manual injection valve (model 7125, Rheodyne, Cotati, CA) having a 100-μl sample loop and an ultraviolet (UV) detector operated at 205 nm. The GC step was accomplished with a Hewlett Packard model 6890 gas chromatograph coupled to an Agilent 5989A quadrupole instrument (Palo Alto, CA) as described further.

2.3. LC-analysis

The LC pre-separation was performed on a 100 mm × 4.6 mm i.d. Spherisorb S5C8 column (Waters, Milford, MA) operated at room temperature, methanol/water and 2000 μl/min being the eluent and the flow rate, respectively. The initial eluent composition (methanol/water, 35:65, v/v) was maintained for 5 min and subsequently a linear gradient was applied within 6 s up to 100% methanol. This latter mobile phase composition was kept over the transfer of the selected fraction from LC into GC-MS.

2.4. LC-GC transfer

The transfer into the gas chromatograph of the volume fraction previously selected from the LC pre-separation was accomplished using the PTV injector, a multi-port valve (Rheodyne, model 7060) positioned after the UV detector of the LC-system and a transfer line (a 90 cm × 0.25 mm i.d. fused silica tube) inserted into the septum of the PTV body. The solutes were then retained in the packing material and subsequently transferred to the GC column by increasing the PTV temperature at 12 °C/s to 320 °C (kept for 10 min). Between consecutive runs, satisfactory blanks (40–160 °C, 5 °C/min) were obtained. As earlier discussed [23] solvent elimination was promoted by detaching the GC column end from the injector body before starting the transfer procedure. During the transfer step, a helium flow was passed through the PTV which was, in turn, kept at low temperature. This helium flow was maintained once the transfer was finished, during the so-called purge time, to contribute to the elimination of the remaining solvent from the interface. Experimentation was carried out by considering different values of the following parameters: PTV temperature during the transfer, PTV temperature during the purge time, helium flow rate during the transfer, helium flow rate during the purge time, and the purge time (i.e. the time during which the purge is applied).

Finally, the column was connected again to start the chromatographic analysis under the experimental conditions specified further.

2.5. Gas chromatography-mass spectrometric analysis

The GC separation was performed on a 25 m × 0.25 mm i.d. fused silica column coated with a 0.25-μm layer of Chirasil-β-Dex (Chrompack), and helium was used as the carrier gas at an initial flow rate of 1 ml/min. The GC-column was programmed at 3 °C/min from 40 °C (5 min) to 100 °C and subsequently at 5 °C/min to 150 °C (20 min). The PTV injector was operated in the split mode (split ratio, 10:1). The source and the quadrupole temperatures were set at 230 and 100 °C, respectively. Peak identification was carried out by comparison of the obtained mass spectra with those provided by the Wiley library and by the standards under the same experimental conditions. Data acquisition from the MS was performed with the HP-ChemStation system.

3. Results and discussion

The selection of the experimental conditions was accomplished using a standard solution containing the compounds described under the Section 2. Ethyl 2-methylbutanoate, some chiral terpenes (limonene, terpinen-4-ol and α-terpineol), and several γ-lactones (C_8, C_9, C_{10} and C_{11}), were included in the present study as they are important components of a variety of real-life aqueous samples, such as fruit beverages, whose complexity may require their analysis by RPLC-GC-MS.

Although a number of parameters affects the on-line RPLC-GC system, it can be highlighted those directly involved in the transfer step because of their greater influence. Specifically, both the interface (PTV) temperature and the helium flow rate during the transfer of the analytes from LC into GC have been demonstrated in earlier studies to affect significantly the recovery of a variety of solutes [23–25]. Nevertheless, it is worthy to mention that in these previous studies, an horizontally positioned PTV was used as the interface of the RPLC-GC system. In this work, however, we considered interesting to use a vertical PTV device to facilitate solvent elimination. Moreover, mass spectrometric detection directly coupled to the on-line RPLC-GC system was also used to enhance the reliability of the identification. For each set of the variables mentioned in the Section 2 under LC-GC transfer, the values were selected on the basis of those that provided the highest peak areas by RPLC-GC-MS. Other experimental values were established according to our previous experience concerning the transfer from LC into GC of selected cuts [25]. Specifically, a transfer volume equal to 600 μl was used at all times as this variable has been proved in earlier studies to affect particularly the absolute areas for γ-lactones [17]. In this regard, although the transfer of smaller fractions is preferable as it facilitates solvent elimination, in this specific case, the transfer of volumes <600 μl was not considered appropriate as it might imply the loss of some of the solutes of interest.

Concerning the PTV temperature, it has been previously proved to be a critical parameter to promote the retention of the compounds of interest in the packing material [24]. However, so far we had not studied the possibility of evaluating the effect of PTV temperatures during the transfer and the purge independently. For this reason, two different temperature values (20 and 10 °C) were tested during both the transfer and the purge. The use of the lowest interface temperature during the purge resulted in higher peak areas for all the compounds of interest and, therefore, the employment of 10 °C as a PTV temperature was considered advantageous over the use of 20 °C. On the contrary, a value of 10 °C during the transfer time did not contribute to improving the obtained results. Therefore, 20 and 10 °C were chosen as PTV temperatures during the transfer and the purge, respectively.

Once selected PTV temperatures, the effect of lowering the helium flow rate from 600 to 400 ml/min during the transfer and from 1000 to 500 ml/min during the purge was simultaneously evaluated. As a result, an improvement in the absolute areas of some of the studied compounds was apparent, although this did not seem to be a general trend. Subsequently, we intended to minimize the loss of the compounds of interest once retained in the packing material and prior to their transfer into the chromatographic column by lowering the purge time from 6 to 4 min. In this instance, all solutes showed a clear increase in their areas. Consequently, 4 min was eventually selected as the purge time.

Finally, the effect of the purge time and the helium flow rate was simultaneously evaluated. Thus, a value of 4 min as the purge time as well as of 400 and 500 ml/min as helium flow rates during the transfer and the purge, respectively, were combined and tested in the same experiment. As a result, a general improvement was achieved in most compounds. Only α-terpineol and γ-undecalactone displayed lower peak areas, however the substantial increase obtained for the rest of the compounds made it advisable the employment of the tested values. Evidently, from these results, it seemed convenient to test even smaller purge times and helium flows. Nevertheless, this possibility was ruled out as a reduction in both parameters might bring about irreversible damage in the mass spectrometer due to the eventual presence of large liquid volumes, resulting from the ineffective elimination of the eluent coming from the LC pre-separation.

In short, the experimental conditions selected to perform the determination of the target compounds in aqueous samples by on-line coupling RPLC-GC-MS were: PTV temperature during the transfer: 20 °C, PTV temperature during the purge: 10 °C; helium flow rate during the transfer: 400 ml/min; helium flow rate during the purge: 500 ml/min and purge time: 4 min.

The repeatability, expressed as relative standard deviation (R.S.D.), and the detection limits were estimated from a

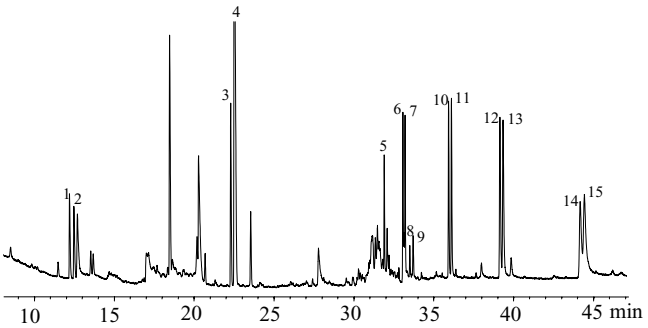

Fig. 1. Chromatogram obtained from a standard solution of chiral compounds by on-line RPLC-GC-MS analysis under the selected conditions: PTV temperature during the transfer: 20 °C, PTV temperature during the purge: 10 °C; helium flow rate during the transfer: 400 ml/min; helium flow rate during the purge: 500 ml/min; purge time: 4 min. Peak number identification: (1) (−)-ethyl 2-methylbutanoate, (2) (+)-ethyl 2-methylbutanoate, (3) (−)-limonene, (4) (+)-limonene, (5) (+)-terpinen-4-ol, (6) (−)-α-terpineol, (7) (+)-α-terpineol, (8) (+)-γ-octalactone, (9) (−)-γ-octalactone, (10) (+)-γ-nonalactone, (11) (−)-γ-nonalactone, (12) (+)-γ-decalactone, (13) (−)-γ-decalactone, (14) (+)-γ-undecalactone, (15) (−)-γ-undecalactone.

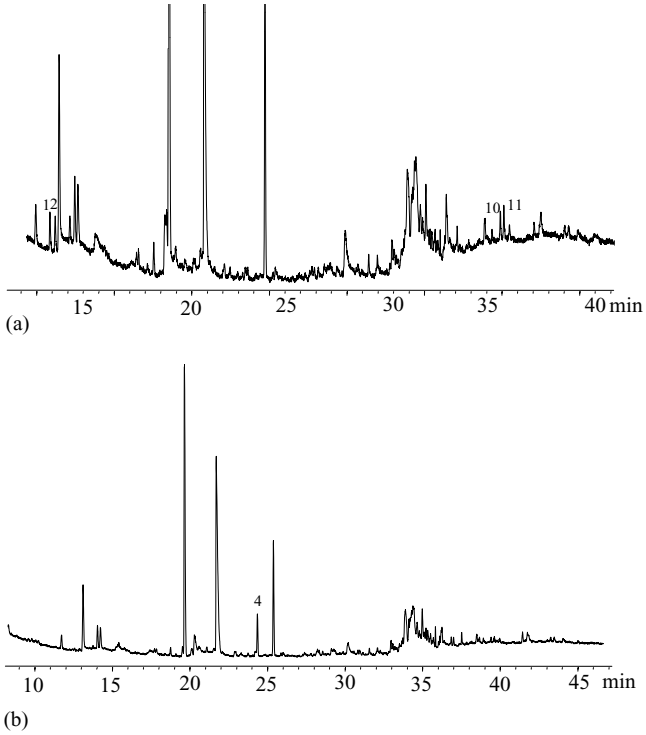

Fig. 2. Chromatogram obtained from two fruit beverages (a and b, respectively) by on-line RPLC-GC-MS analysis under the selected conditions. Experimental conditions and peak number identification as in Fig. 1.

signal equal to five times the base line noise and from a minimum of three replicates of the studied chiral compounds by RPLC-GC-MS under the experimental conditions selected. R.S.D. values were in all cases <18% and most of them did not overcome 10%. Consequently, all studied compounds, exhibited acceptable R.S.D.s, particularly, taking into account that the on-line system considered in the present work includes three analytical techniques. As far as detection limit is concerned, values ranging from 0.2 mg/l for (+)-limonene to 2.5 mg/l for (−)-γ-octalactone were obtained.

On the other hand, the linear range resulting from RPLC-GC-MS analysis of the standard solution carried out in the selected experimental conditions, varied from 0.5 to 100.0 mg/l for ethyl 2-methylbutanoate and limonene, from 1.0 to 100.0 mg/l for terpinen-4-ol and from 5.0 to 100.0 mg/l for α-terpineol and the studied γ-lactones. In all cases, regression coefficients were ≥0.998 and, hence, the linear ranges were considered satisfactory.

Fig. 1 depicts the chromatogram provided by the RPLC-GC-MS analysis of a standard solution of the target compounds under the experimental conditions selected. As can be seen, the solutes considered in this study could be successfully transferred from LC into GC and separated into their corresponding enantiomers by using the chiral column, except terpinen-4-ol which, as previously mentioned, was used as the pure (+)-enantiomer. As also illustrated, the achieved chromatographic resolution allowed to verify the identities of all compounds considered in this study through mass spectrometry. It is also interesting to point out that the overall analysis time, including LC pre-separation, GC analysis and MS identification, was approximately equal to 60 min.

In order to consider the matrix effect, a commercial beverage containing a mixture of different fruit juices spiked with a standard solution was analysed by RPLC-GC-MS under the proposed experimental conditions. In general, the obtained profile as well as chromatographic resolutions were highly similar to those observed for the standard solution. This fact proves that, in this specific case, no significant matrix influence could be established.

To evaluate the applicability of the proposed method, two commercial beverages (samples 1 and 2) containing a variety of fruit juices were analysed by RPLC-GC-MS under the selected experimental conditions. Fig. 2 represents the chromatograms resulting from these analyses. As shown, the occurrence of both enantiomers of ethyl 2-methylbutanoate and γ-nonalactone in sample 1 (Fig. 2a) as well as (+)-limonene in sample 2 (Fig. 2b), could be detected and positively identified by RPLC-GC-MS. It is interesting to highlight that ethyl 2-methylbutanoate and γ-nonalactone were found as racemic mixtures whereas limonene occurred as the pure (+)-enantiomer. Regarding the presence of racemates in sample 1, it can be stated that this is quite unusual in real samples. Consequently, this result might be indicative of the addition of artificial aromas to the fruit beverage referred as sample 1. Actually, synthetic aromas are frequently added to some foodstuffs with the purpose of enhancing their natural flavour and, thus, making the product more attractive to the consumer. In any case, it is clear that the label designation of the product should always be consistent with the result obtained from its analysis. On the contrary, the pure (+)-enantiomer of limonene was found in sample 2 (Fig. 2b).

This fact is in good agreement with data earlier published in other fruit beverages [26]. On the other hand, it is convenient to mention that the presence of limonene in sample 2 is associated with the occurrence of orange juice in the analysed sample. Equally, its absence in sample 1 suggests that this beverage was made from a mixture of fruits, other than orange [26].

As a conclusion, chiral evaluations in aqueous matrices may be faced by on-line RPLC-GC-MS. However, to that aim, a selection of the experimental variables to be used, specially those involved in the transfer of selected cuts, must be previously accomplished. The method proposed in the present work enables both efficient chromatographic separation and high sensitivity for chiral compounds to be achieved, avoiding, in turn, the sample handling.

References

[1] D.W. Armstrong, Ch.-D. Chang, W.Y. Li, J. Agric. Food Chem. 38 (1990) 1674.
[2] R. Marchelli, A. Dossena, G. Palla, Trends Food Sci. Tech. 7 (1996) 113.
[3] A.M. Stalcup, K.H. Ekborg, M.P. Gasper, D.W. Armstrong, J. Agric. Food Chem. 41 (1993) 1684.
[4] D. Lehmann, A. Dietrich, U. Hener, A. Mosandl, Phytochem. Anal. 6 (1995) 255.
[5] W. Bertsch, J. High Resolut. Chromatogr. 23 (2000) 167.
[6] K. Grob, On-Line Coupled LC-GC, Hüthig, Heidelberg, 1991.
[7] S. Asche, T. Beck, U. Hener, A. Mosandl, Front. Flav. Sci. (2000) 102.
[8] A. Wanikawa, K. Hosoi, T. Kato, K. Nakagawa, Flav. Fragr. J. 17 (2003) 207.
[9] E. Jagerdeo, S. Dugar, G.D. Foster, H. Schenck, J. Agric. Food Chem. 50 (2002) 5797.
[10] L. Mondello, G. Dugo, K.D. Bartle, J. Microcol. Sep. 8 (1996) 275.
[11] W. Kamm, F. Dionisi, L.-B. Fay, C. Hischenhuber, H.-G. Schmarr, K.-H. Engel, J. Am. Oil Chem. Soc. 79 (2002) 1109.
[12] W. Kamm, F. Dionisi, C. Hischenhuber, H.-G. Schmarr, K.-H. Enge, Eur. J. Lipid Sci. Technol. 104 (2002) 756.
[13] E.C. Goosens, I.M. Beerthuizen, D. de Jong, G.J. de Jong, U.A.Th. Brinkman, Chromatographia 40 (1995) 267.
[14] M. Pérez, J. Alario, A. Vázquez, J. Villén, Anal. Chem. 72 (2000) 846.
[15] T. Hyoetylaeinen, H. Keski-Hynnilae, M.-L. Riekkola, Anal. Chem. 73 (2001) 21.
[16] M.L. Ruiz del Castillo, M.M. Caja, M. Herraiz, G.P. Blanch, J. Agric. Food Chem. 46 (1998) 5128.
[17] M.L. Ruiz del Castillo, M. Herraiz, G.P. Blanch, J. Agric. Food Chem. 48 (2000) 1186.
[18] W. Meier, A. Artho, P. Naegeli, Mitt. Geb. Lebensm. Unter. Hyg. 87 (1996) 118.
[19] L. Mondello, P. Dugo, K.D. Bartle, Flav. Fragr. J. 10 (1995) 33–42.
[20] A.J. Bulterman, J.J. Vreuls, R.T. Ghijsen, U.A.Th. Brinkman, J. High Resolut. Chrom. Commun. 16 (1993) 397.
[21] M. Hartmann, J. Ammon, H. Berg, Ann. Falsif. Expert Chim. Toxicol. 90 (1997) 381.
[22] M.L. Ruiz del Castillo, M.M. Caja, G.P. Blanch, M. Herraiz, J. Food Sci. 68 (2003) 770.
[23] G.P. Blanch, M.L. Ruiz del Castillo, M. Herraiz, J. Chromatogr. A 818 (1998) 77.
[24] G.P. Blanch, M.L. Ruiz del Castillo, M. Herraiz, J. Chromatogr. Sci. 36 (1998) 589.
[25] M.L. Ruiz del Castillo, E. Gómez Caballero, M. Herraiz, J. Chromatogr. Sci. 41 (2003) 26.
[26] M. Jia, Q.H. Zhang, D.B. Min, J. Agric. Food Chem. 46 (1998) 2744.