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On-line coupled reversed phase liquid chromatography and gas chromatography: A new sealing design for the TOTAD interface

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ABSTRACT

Total elimination of the eluent resulting from the pre-separation step is the critical point when coupling LC to GC. As a helium flow is applied during transfer to eliminate the solvent, the interface used for linking the two chromatographic systems must be properly sealed to prevent gas leaks and to achieve an effective evaporation of the eluent. The aim of this work was to improve the performance of the Through Oven Transfer Adsorption Desorption (TOTAD) interface to remove the eluent coming from LC by modifying the way in which the injector sealing system is held in place. As with the original design, the new approach makes it possible to transfer high volumes at a high rate, but the proposed modification also simplifies the experimental work because the displacement risk of the sealing system is reduced. Analyses of an ester mixture by RPLC–GC were performed to confirm the applicability of the system modification. In this work, volumes of up to 5 ml, at flow rates as high as 2 ml/min, were transferred from LC to GC with almost complete solvent removal even when working in reversed phase mode in the LC step.

1. Introduction

Analytical techniques evolve in response to the need for methods to accurately determine the chemical composition of samples through the separation and identification of those compounds which are of relevance for characterizing a specific product.

Chromatographic systems have proved effective for the analysis of different types of matrices, but one-dimensional techniques may be unsuitable when very complex mixtures are studied and high resolution chromatographic separations are required.

Multidimensional chromatography techniques involving the use of two columns coupled in series are able to handle these problems since the first dimension of the system permits selection of the fraction of interest and its transfer to the second one, thus attaining high separation efficiency and suitable selectivity for analyzing complex matrices [1–5].

On-line coupled liquid chromatography with gas chromatography (LC–GC) [6] is a multidimensional system that presents important advantages, especially replacement of the sample preparation step (*e.g.*, clean-up and sample enrichment), which is necessary in many methods, by pre-separation in different fractions or the isolation of minor components present in complex mixtures using LC. In this way, analyte loss is minimized and the reliability of the analysis is improved. This technique reduces sample handling and decreases the overall analysis time needed, while the possibility of transferring large volumes from LC to GC may lead to an increase of the sensitivity, allowing the possible detection of compounds that occur at low concentrations [7]. The use of coupled LC–GC requires the selective removal of a large amount of solvent, leaving the solute in a sharp band at the entrance of the GC column [8].

Initially, most works that referred to the on-line coupling LC–GC reported the use of normal phase in the LC step, because the eluents using this approach are more easily removed [9–14]. However, LC separations are largely performed in the reversed mode (RPLC), so it is important to use polar solvents in the pre-separation step, making it necessary to develop procedures avoiding the main problem involved in the transfer of polar eluents from LC to GC, which is the high volume of vapor produced per unit volume of liquid.

Some reports have been published on the different interfaces that can be used for on-line coupled RPLC–GC [15–18] and, more specifically, the use of the programmable temperature vaporizer (PTV) injector of a gas chromatograph as interface for LC–GC has already demonstrated its effectiveness for analyzing complex matrices [19–24].

The Through Oven Transfer Adsorption Desorption (TOTAD) interface has been proposed for the on-line coupling of LC–GC. This interface is a significantly modified PTV injector incorporating changes that affect the pneumatics, sample introduction, solvent

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elimination and operation mode [25–27]. With this approach, solvent removal is achieved using an auxiliary carrier gas stream applied in the opposite direction to that of the carrier gas passing through the GC column, providing an effective solution for solving the main troubles which arise when coupling LC–GC.

The TOTAD interface has been successfully used for dealing with different aspects related to the study of complex matrices such as foodstuffs or essential oils. Specifically, pesticide residues in different food samples have been analyzed by means of the on-line coupling of RPLC–GC [28–32] and using large volume injection (LVI) [33]. In the same way, this technique has been found highly effective for testing edible oils by NPLC–GC [34,35], and for the detection of irradiation markers in irradiated fat-containing foods by RPLC–GC, using a mass spectrometer (MS) detector in GC [36]. As regards essential oils, RPLC–GC–MS coupling makes it possible to determine enantiomeric excesses for chiral compounds [37].

Aqueous samples have also been efficiently analyzed by LVI–GC–MS using the TOTAD interface [38].

Despite the valuable results obtained so far with the on-line coupling of LC–GC via the TOTAD interface, problems may still occur in the experimental procedure caused by failure of the injector sealing system. In such cases, insufficient eluent elimination may result in flooding of the GC system and, consequently, both the GC column and the detector can be damaged. This is especially relevant when working with an MS detector in GC.

Taking into account these possible problems, the aim of this work was to evaluate a modification of the interface which keeps the sealing system in position, thus facing the critical point of the technique, *i.e.*, the efficient removal of the eluent arriving from the LC.

2. Experimental

2.1. Materials

A standard mixture containing 8 ethyl esters in methanol (boiling points ranging from 120 to 269 °C; concentrations ranging from 1 to 100 mg/l) was used in this study. The solution was composed of ethyl butyrate (≥ 99.5%), ethyl hexanoate (≥ 98%), ethyl octanoate (≥ 98%) and ethyl decanoate (≥ 98%) provided by Fluka (Steinheim, Germany); ethyl pentanoate (99.5%) obtained from Dr. Ehrenstorfer (Augsburg, Germany); ethyl nonanoate (≥ 98%), ethyl undecanoate (≥ 97%) and ethyl dodecanoate (≥ 98%) supplied by Aldrich (Steinheim, Germany).

The HPLC grade methanol used as solvent for the test mixture and as eluent for liquid chromatography was from Lab Scan Ltd (Gliwice, Poland).

To assess the viability of the improvement proposed in this work, three real samples were injected into the LC–GC system. On the one hand, two flavorings, namely white grape and champagne grape pomace provided by IFF (International Flavors & Fragrances Inc. Madrid, Spain) and WILD Flavors (GmbH & Co. KG, Berlin, Germany) respectively, and on the other a white wine from Rueda (Valladolid, Spain). The wine must be filtered with a 0.2-μm, 13-mm filter (Pall Corporation, New York, USA) before being injected.

2.2. On-line coupled LC–GC system

The analyses were performed using on-line coupled LC–GC equipment including a TOTAD interface (U.S. patent 6,402,947 B1, exclusive rights assigned to KONIK-Tech, Sant Cugat del Vallés, Barcelona, Spain) which allows full automatic operation for the whole analysis. The LC system (Konik model 560) was provided with a manual injection valve (model 7725, Rheodyne, California, USA) with 20-μl loop volume and an ultraviolet (UV) detector operated a 205 nm. The gas chromatograph (Konik, model HRGC 4000B) was equipped with a flame ionization detector (FID). Data acquisition and processing were carried out with KoniKrom Plus (Konik, Sant Cugat del Vallés, Barcelona, Spain).

2.3. Chromatographic conditions for LC

To achieve LC pre-separations, a 100 mm × 4.6 mm i.d. C_4 HPLC column packed with modified silica (5 μm particle size, Hypersil, Phenomenex, Torrance, California, USA) was used. The LC column temperature was set at 30 °C throughout the experiment. In a first step, 20 μl of a methanolic solution containing 100 mg/l of each ester was tested in the LC system to determine the fraction to be transferred to the gas chromatograph. Once this fraction had been selected, a methanolic solution of 10 mg/l of each standard was injected into the LC and transferred to the GC as described below. 100% Methanol was used as the mobile phase and different initial flow rates were tested to optimize the pre-separation of the fraction of interest, namely, 1, 1.5 and 2 ml/min. The initial flow was maintained until the target fraction began to be eluted and then changed to different values as detailed bellow. After completing the transfer to GC, the initial flow rate was resumed and maintained for 20 min to ensure the complete elimination of the retained compounds.

2.4. LC–GC transfer

Throughout the transfer step, the TOTAD interface was thermostated at 50 °C. At the beginning of the analysis, the eluent from the LC was sent to waste. When the front of the target fraction reached the six-port valve placed immediately after the UV detector, it was switched, transferring the fraction to the modified PTV injector. A helium flow rate (ranging from 100 to 1000 ml/min) was applied to impel the eluent from the LC through the retaining material as well as to avoid solvent condensation in the external part of the interface body. The gas enters through both inlets B and A (Fig. 1A), while two electrovalves are responsible for directing the helium flow [28]. The heated cover of the interface body was set at 100 °C, which contributes to avoid eluent condensation. The liner (110 mm × 2 mm i.d. × 3 mm o.d.) was filled with a plug (1 cm) of Tenax TA (80–100 mesh), obtained from Supelco (Bellefonte, PA, USA), kept in place between two plugs of glass wool. Before use, Tenax TA was conditioned under a helium flow by successively raising the temperature to 10 °C every 5 min, beginning at 40 °C and finishing at 300 °C. The final temperature was maintained for 60 min.

After completion of the transfer step, the six-port valve was switched again to send the LC eluent to waste. Additionally, both the temperature and helium flow rate applied during transfer were maintained for 2 min to facilitate elimination of the remaining solvent from the TOTAD interface. The flow through B was then interrupted and the helium flow through A was lowered to start the gas chromatographic analysis.

To achieve the thermal desorption of the analytes retained in the packing material inside the liner, the TOTAD interface was quickly heated to 250 °C, and maintained at this temperature for 5 min. The analytes were then transferred to the capillary column and the GC analysis was carried out. Between runs, the interface was kept at 250 °C under a helium stream (200 ml/min) for 10 min and then cooled to the initial working temperature to start a new run.

2.5. GC analysis

Esters were analyzed using a 30 m × 0.25 mm i.d. ZB-WAX (100% polyethylene glycol) capillary column with a 0.25-μm film

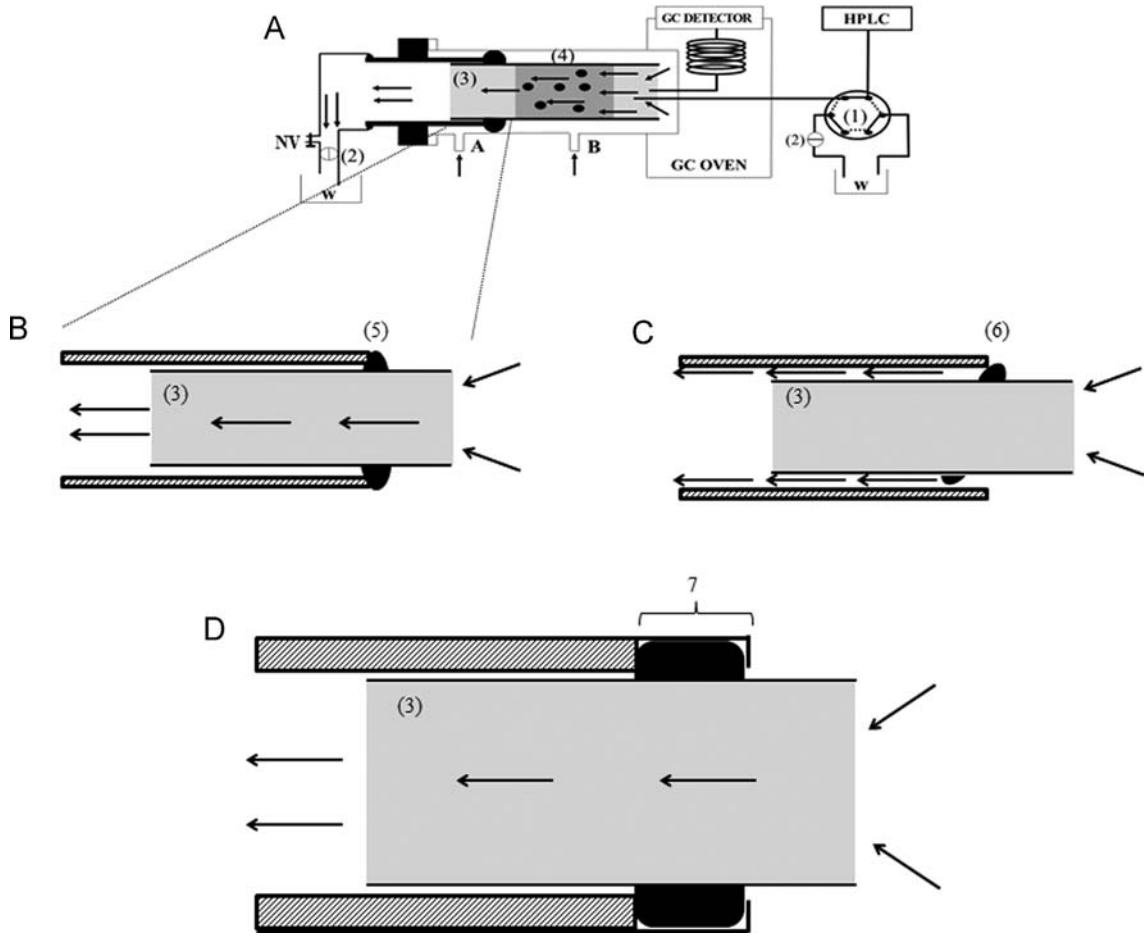

Fig. 1. Simplified scheme of the automated TOTAD interface for LC–GC coupling (A). A section of the original tubular metallic piece is expanded to show the toric joint correctly positioned (B), and to illustrate the possible displacement of the seal (C). An enlargement of the modified section is shown to give detail of the proposed improvement (D). Symbols: (1) six-port valve; (2) electrovalves; (3) glass liner; (4) Tenax TA; (5) original O-ring joint; (6) displaced original O-ring joint; (7) new O-ring joint housing; A and B: helium inlets; Arrows: gas flow; W: waste tube; NV: needle valve; Dots: analytes.

thickness (Zebron, California, USA). The oven temperature was kept at 30 °C until completion of the transfer step, then increased to 200 °C at a rate of 5 °C/min. The FID was maintained at 250 °C, and helium, with a column head pressure of 16 psi, was used as the carrier gas.

To determine the fraction of the sample recoverable by the proposed approach (LC-TOTAD-GC), the ester mixture was analyzed with a conventional GC injector operated in the splitless mode, using 1 μl of the solution in methanol containing 10 mg/l of each standard. The injector was held at 250 °C.

Satisfactory blanks between consecutive runs were obtained for the complete procedure under the experimental conditions applied in the overall analysis.

3. Results and discussion

3.1. General considerations

Since the elimination of the eluent from the LC is directly related with the quality of the injector sealing, any improvement in this aspect will contribute to the development and application of the technique. The TOTAD interface has already proved to be highly effective for on-line LC–GC coupling, as it provides a high rate of solvent removal, which allows working in both normal and reversed phase mode. Occasionally, however, some problems related to the injector sealing system have been observed, which may lead to ineffective eluent elimination.

In the TOTAD interface, a helium stream is applied during the transfer step to achieve eluent removal. Viton® O-ring (5 mm i.d. × 7 mm o.d.), also known as a toric joint, is used to make the injector airtight in order to prevent helium leaks. A tubular metallic piece (a common part of most injectors) presses the O-ring joint to divide the outer part of the glass liner into two chambers [39]. This tubular piece is integrated in the waste tube of the TOTAD interface, as indicated in Fig. 1A. In the original design (Fig. 1B), due to the higher pressure of helium during the transfer step, the O-ring seal can be displaced from its position because it is simply pressed by the metallic piece and, as a result, the helium, instead of passing through the glass liner, can be lost along the sides of the same (Fig. 1C). If this occurs, there is a clear possibility that the ineffectively removed solvent may flood the GC system. Consequently, the injector has to be occasionally opened to check the position of the toric seal, thus interrupting the experimental work in progress.

It is for this reason that we propose a modification, which should contribute to avoid these problems. The modification involves redesigning of the bottom end of the tubular metallic piece to house the toric joint, ensuring that the seal fits and remains in place, thus avoiding or, at least, significantly reducing the risk of displacement. This improved sealing prevents helium from escaping and ensures that the flow goes through the glass liner, optimizing eluent elimination (Fig. 1D). The new design

requires a smaller viton O-ring (3 mm i.d. × 5 mm o.d.) and, since the risk of displacement is reduced, injector performance is improved with respect to the original design.

When on-line coupling LC–CG is performed, numerous variables must be considered, such as the mobile phase used in HPLC (polar or non-polar eluent), initial HPLC flow rate, transferred volume, HPLC and helium flow rates during the transfer step, initial PTV temperature, additional time for solvent removal and length of packing material inside the glass liner [23,40].

Some of these aspects were tested in this study, as detailed below, in order to ascertain whether the proposed improvement prevents helium leaks, while also maintaining the interface capabilities of previous designs. Although LC–GC transfer is easier when using normal phase in the pre-separation step, mainly due to the lower evaporation volume produced per unit of liquid from non-polar eluents, we decided to use polar eluents because most LC separations are performed using reversed-phase. Moreover, the use of this operation mode is frequently mandatory (*e.g.*, when analyzing biological and aqueous samples).

To verify the feasibility of the proposed modification, an ethyl ester mixture was injected into the RPLC–GC system.

3.2. On-line coupled RPLC–GC analysis

As a previous step of the analysis, the fraction to be transferred from LC to GC must be established. This fraction must be sufficient to ensure that all the analytes of interest are sent to the GC, while keeping the transferred volume as small as possible, both to concentrate the sample and to reduce the overall analysis time, as well as to minimize difficulties due to eluent elimination in the interface. Hence, the experimental work was started by injecting the ester mixture into the LC with different initial flow rates, namely 1, 1.5 and 2 ml/min. Once obtained the LC profiles, and after installing the modified metallic piece in the system, the sample was analyzed in each case by transferring all the fractions pre-separated in the LC step to the GC in consecutive runs. The LC flow rate during the transfer step was reduced to 0.1 ml/min, and a helium flow rate of 1000 ml/min was applied for solvent elimination on the basis of previous studies with the same compounds, in which the most satisfactory results were reached at a similarly high helium flow rate during transfer [41]. It should be noted that such studies were not carried out using the TOTAD interface but another PTV-based interface (which did not allow the automation of the overall analysis). However, with regard to the helium flow rate to be applied for solvent removal, the results can be extrapolated.

Under these conditions, the best results were obtained with an initial LC flow rate of 1 ml/min and transferring the fraction between 1.3 and 1.6 min (Fig. 2A), which implies sending a volume of 0.3 ml to the GC. In this way, seven ethyl esters were satisfactorily identified (Fig. 2B). However, ethyl butyrate was not detected, probably due to its co-evaporation with the eluent. Indeed, the most volatile esters were also partially lost during the LC–GC analysis.

Bearing these results in mind, the test mixture was injected again, decreasing the helium flow rate for solvent removal from 1000 to 200 and 100 ml/min. Fig. 3A corresponds to the GC profile obtained with a flow rate of 100 ml/min. However, these experimental values did not improve the results obtained with the above mentioned conditions because the chromatograms showed that ethyl pentanoate was not detected and rest of the peaks were smaller than those previously achieved. This effect can be explained because the volume of eluent removed was lower than in Fig. 2, so that partial or total losses due to co-evaporation with the solvent seem to be easier in this case. On the other hand, when the LC flow applied during the transfer step was increased from

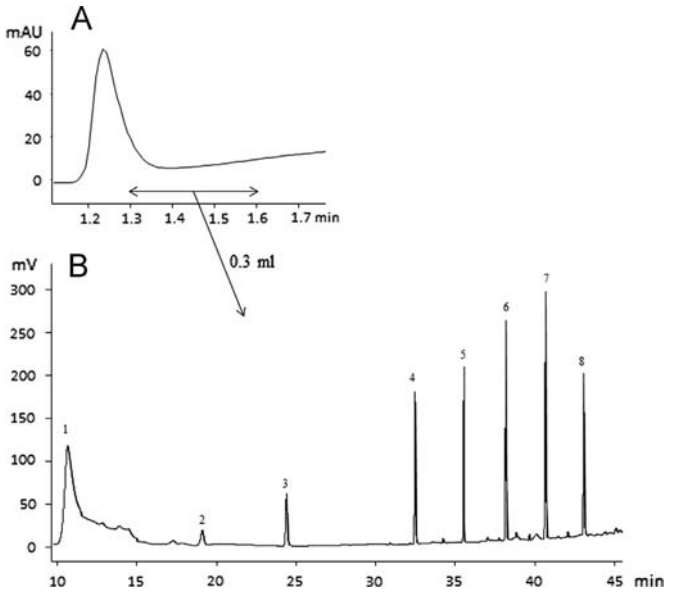

Fig. 2. Liquid chromatogram obtained after injecting the test mixture (100 mg/l) (A) and gas chromatogram resulting from the transfer, from LC into GC, of a 0.3-ml fraction when performing the RPLC–GC analysis of the sample tested (10 mg/l) (B). The helium flow rate applied for eluent removal was 1000 ml/min, and the LC flow during transfer step was 0.1 ml/min. Identification peak numbers: 1, solvent peak; 2, ethyl pentanoate; 3, ethyl hexanoate; 4, ethyl octanoate; 5, ethyl nonanoate; 6, ethyl decanoate; 7, ethyl undecanoate; 8, ethyl dodecanoate.

0.1 to 1 ml/min, very small peaks were recorded (Fig. 3B), probably because the transfer speed was too high for a transferred volume of 0.3 ml and the analytes could not be retained on the adsorbent material.

At this point of the study, we decided to validate the reliability of the modification introduced by enlarging the range of the experimental variables, while maintaining the initial PTV-temperature at 50 °C. With this goal, both the LC flow rate applied during the transfer step (0.4, 1.0, 1.5 or 2.0 ml/min) and the transferred volume from LC to GC (0.45, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0 or 5.0 ml) were considered. The initial LC flow rate for each situation was also adapted.

In the most severe conditions tested, up to 5 ml of the test mixture could be transferred from LC to GC at a flow rate of 2 ml/min, with excellent solvent elimination (unsaturated solvent peak was obtained). The helium flow rate for eluent elimination was 1000 ml/min. The solvent saturation was neither observed when this flow rate decreased to 100 ml/min, eluent elimination being almost complete before the sample reached the GC column even at this low helium flow rate.

This is an important point which deserves to be emphasized, since during the on-line coupling RPLC–GC the slow evaporation rate of polar eluents limits the LC flow rate along the transfer step [42]. In this respect, it is interesting to consider that during experimental work it was possible to transfer a volume of 5 ml at high speed (2 ml/min). However, as expected, adverse effects on the quality of the chromatograms obtained with regard to the number and concentration of the peaks recorded were observed, because both the elevated transferred volume and the excessively high transfer speed (which interfere with the retention of the compounds on the adsorbent material) were inadequate for these compounds.

Actually, the sealing system used so far in the TOTAD interface has already allowed volume fractions as high as 5 ml to be transferred from LC to GC. Thus, the results reported in the present work show that the proposed new sealing system maintain the previously described capabilities of the TOTAD interface while also

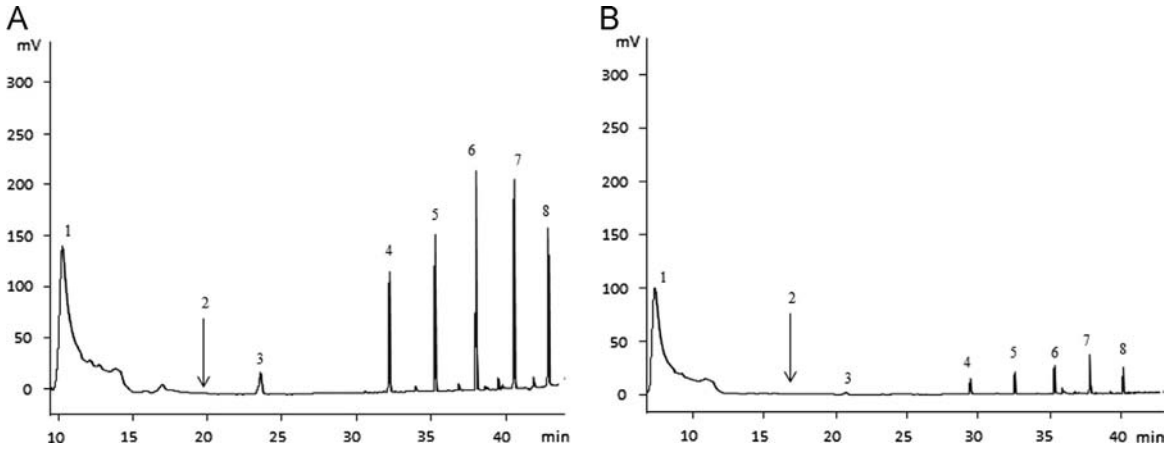

Fig. 3. Gas chromatograms obtained by transferring 0.3 ml of the standard mixture (10 mg/l) from LC into GC at a helium flow rate for solvent elimination of 100 ml/min, and an LC flow rate during transfer step of 0.1 ml/min (A), and by increasing the LC flow rate during the transfer step (up to 1 ml/min) (B). Identification peak numbers as in Fig. 2.

Table 1
LC–GC analysis of the test mixture: Relative Standard Deviation (RSD) from absolute peak areas and retention times ($n=4$), detection limits (LODs), recoveries, coefficients of determination (R^2) and regression equations for the linear calibration (ranging from 1 to 100 mg/l).

Compound	RSD (%)		LOD (mg/l)	Recovery (%)	Linearity	
	Area	t_R			R^2 (%)	Regression equation
Ethyl pentanoate	2.05	1.38	0.02	28.39	98.77	$y=30.25+7.84x$
Ethyl hexanoate	3.89	0.57	0.01	54.60	99.78	$y=38.26+18.50x$
Ethyl octanoate	5.78	0.09	0.01	75.59	99.01	$y=40.51+33.81x$
Ethyl nonanoate	9.37	0.07	0.01	83.47	99.16	$y=17.65+38.08x$
Ethyl decanoate	10.35	0.08	0.01	80.54	99.71	$y=4.43+44.39x$
Ethyl undecanoate	12.65	0.07	0.01	85.64	98.63	$y=8.42+51.47x$
Ethyl dodecanoate	8.89	0.07	0.02	68.69	96.90	$y=-18.79+34.52x$

improving its performance as far as it provides a more simple maintenance of the injector.

In any case, none of these analyses (*i.e.*, LC flow rate applied during the transfer step ranging from 0.4 to 2 ml/min and transferred volume ranging from 0.45 to 5 ml) improved the GC chromatogram achieved in Fig. 2B. So, as a result of this study, the optimal conditions for the RPLC–GC analysis of the esters tested were as follows: initial LC flow rate of 1 ml/min, LC flow rate during transfer step of 0.1 ml/min, transfer volume of 0.3 ml and helium flow rate for solvent removal of 1000 ml/min.

3.3. Method validation

To confirm that the adaptation of the lower end of the tubular metallic piece to house the sealing system improves the performance of the TOTAD interface, repeatability, detection limits, recoveries and linearity were evaluated (Table 1). All validation parameters were determined from four replicates using the optimal conditions described for the compounds tested.

The repeatability was evaluated by injecting 20 μl of the test mixture (10 mg/l) into the LC–GC system. The Relative Standard Deviation (RSD) from the absolute peak areas was lower than 10% for most compounds, whereas for retention times RSD values ranged from 0.07% to 1.38%. Detection limits (LODs) were calculated as the amount of product giving a signal-to-noise ratio of 3, and these varied from 0.01 to 0.02 mg/l. Recoveries were estimated by comparing the splitless mode injection of the test mixture (1 μl injected, 10 mg/l of each ester) with the LC–GC analysis of the same sample (20 μl injected, 10 mg/l of each ester). Values around 80% were found for this parameter in most cases, although lower recoveries were obtained for the most volatile compounds (mainly for ethyl pentanoate), likely due to losses in the solvent removal step. Recovery of ethyl dodecanoate decreases comparatively, probably because this compound may not be completely desorbed from the retaining material due to its high boiling point.

The coefficient of determination (R^2) and the regression equation for the linear calibration were also studied. For this purpose, 20 μl of the standard solution containing 1, 10, 25, 50, 75 and 100 mg/l of each ester were consecutively injected and the absolute peak areas were considered. Generally speaking, good linearity was found for all esters, with coefficients of determination ranging from 96.90% to 99.78%.

3.4. Analysis of real samples

The proposed method was applied to the analysis of two different flavorings, namely white grape and champagne grape pomace. Two GC chromatograms were obtained for the white grape aroma (Fig. 4A), corresponding to 8 μl of white grape flavoring diluted in 4 ml of methanol (Fig. 4A.1) and the same flavoring solution enriched with 1 mg/l of each ester tested (Fig. 4A.2). As can be observed, six ethyl esters were identified in the white grape sample, beginning with ethyl hexanoate. Ethyl pentanoate was not detected in this flavoring, in which the most abundant esters were ethyl decanoate and ethyl dodecanoate.

Fig. 4B shows the GC chromatograms recorded for champagne grape pomace (8 μl of flavoring in 4 ml of methanol). In this case, a lower number of esters was expected since the pomace is the solid remains of grapes after pressing for juice during winemaking. Only four esters were identified in the sample (Fig. 4B.1), as can be seen

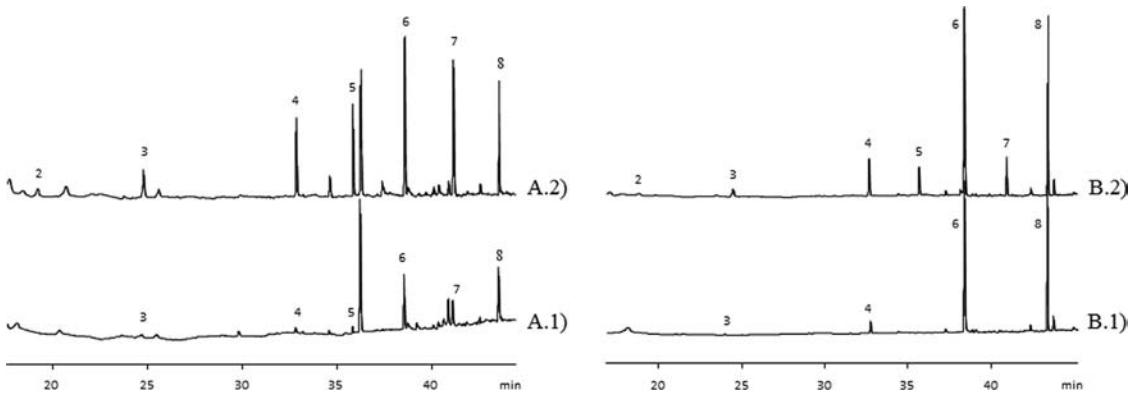

Fig. 4. White grape flavoring (8 μl) diluted in 4 ml of methanol (A.1), and the same flavoring doped with 1 mg/l of each ester tested (A.2); champagne grape pomace (8 μl) in 4 ml of methanol (B.1), and the same flavoring doped with 1 mg/l of each standard (B.2). Identification peak numbers as in Fig. 2.

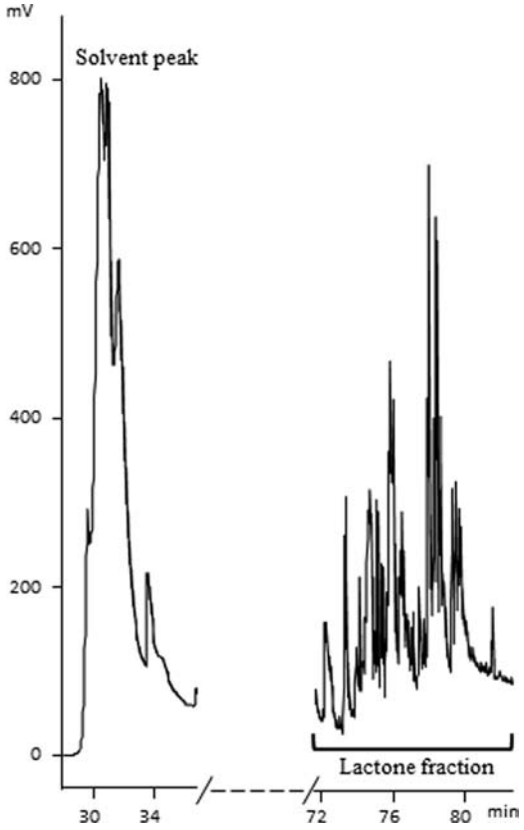

Fig. 5. Gas chromatogram obtained by transferring 1.05 ml of white wine from LC into GC at a helium flow rate for solvent elimination of 200 ml/min, and an LC flow rate during the transfer step of 0.1 ml/min.

by comparing it with the same flavoring fortified with 1 mg/l of the standard solution (Fig. 4B.2).

In both cases, reliable analysis could be eventually performed as relatively simple and clear chromatograms were obtained due to the fact that exclusively a specific fraction of the real sample was transferred from LC to GC.

Additionally, the analysis of a more complex sample was performed, specifically white wine, which requires increasing the transfer volume. In this case, a 1.05-ml volume fraction (corresponding to wine lactones) was transferred from LC into GC (Fig. 5). As lactones are compounds extracted into wine during barrel ageing, thereby providing distinctive properties (color, flavor, texture), their analysis is often required. Wine was injected previously filtered directly into the LC, using a 250-μl loop to increase the sensitivity of the overall analysis because lactones are at low concentrations. The results showed an excellent performance of the interface under these experimental conditions, although to ensure good retention of the analytes of interest on the adsorbent material it seemed convenient to reduce, from 1 to 0.1 ml/min, the flow rate applied during the transfer step. In any case, it is interesting to emphasize that flow rates during transfer as high as 2.0 ml/min can be applied when required.

4. Conclusion

The proposed modification for the TOTAD interface, as well as its original design, enables the automatic analysis of compounds with a wide range of volatilities. From the data obtained in this work it can be seen that up to 5 ml were transferred to the TOTAD interface at an LC flow rate of 2 ml/min during the transfer, solvent elimination being almost complete before starting the GC analysis. Thus, as regards the capacity/effectiveness of the interface to eliminate the eluent coming from the LC, the proposed modification allows the transfer of high volume fractions at high speed while maintaining the toric joint in place and, therefore, the experimental work is simplified. In this way, efficient solvent removal was observed all throughout the approximately 250 analyses which were performed to obtain the reported data.

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