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Alcohol and metal determination in alcoholic beverages through high-temperature liquid-chromatography coupled to an inductively coupled plasma atomic emission spectrometer

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ABSTRACT

In the present work, an inductively coupled plasma atomic emission spectrometry (ICP-AES) system was used as a high temperature liquid chromatography (HTLC) detector for the determination of alcohols and metals in beverages. For the sake of comparison, a refractive index (RI) detector was also employed for the first time to detect alcohols with HTLC. The organic compounds studied were methanol, ethanol, propan-1-ol and butan-1-ol (in the 10–125 mg/L concentration range) and the elements tested were magnesium, aluminum, copper, manganese and barium at concentrations included between roughly 0.01 and 80 mg/L. Column heating temperatures ranged from 80 to 175 °C and the optimum ones in terms of peak resolution, sensitivity and column lifetime were 125 and 100 °C for the HTLC-RI and HTLC-ICP-AES couplings, respectively. The HTLC-ICP-AES interface design (*i.e.*, spray chamber design and nebulizer type used) was studied and it was found that a single pass spray chamber provided about 2 times higher sensitivities than a cyclonic conventional design. Comparatively speaking, limits of detection for alcohols were of the same order for the two evaluated detection systems (from 5 to 25 mg/L). In contrast, unlike RI, ICP-AES provided information about the content of both organic and inorganic species. Furthermore, temperature programming was applied to shorten the analysis time and it was verified that ICP-AES was less sensitive to temperature changes and modifications in the analyte chemical nature than the RI detector. Both detectors were successfully applied to the determination of short chain alcohols in several beverages such as muscatel, pacharan, punch, vermouth and two different brands of whiskeys (from 10 to 40 g of ethanol/100 g of sample). The results of the inorganic elements studied by HTLC-ICP-AES were compared with those obtained using inductively coupled plasma mass spectrometry (ICP-MS) obtaining good agreement between them. Recoveries found for spiked samples were close to 100% for both, inorganic elements (with both HLTC-ICP-AES and ICP-MS) and alcohols (with both HTLC-ICP-AES and HTLC-RI hyphenations).

1. Introduction

Recent developments in thermally resistant stationary phases have allowed the use of high temperatures in liquid chromatography (HTLC). And so the possibility of increasing the column temperature can dramatically improve the efficiency and speed of a chromatographic separation. The main effects of working with higher temperature than in HPLC are a decrease in the mobile phase viscosity, a higher diffusion of the analytes into and back out of the pores of the stationary phases and higher speed rate in the interactions between the analytes and the stationary phase. In HTLC [1] water can be used under subcritical conditions (*i.e.*, below 374 °C and 22.1 MPa) to achieve a decrease in its polarity thus making it possible to separate low polarity compounds [2]. Additionally, superheated water offers the interest of being compatible with detection modes that are not typically in classical HPLC, like flame ionization detector (FID), nuclear magnetic resonance (NMR) and inductively coupled plasma atomic emission spectrometry (ICP-AES) [3]. Therefore, the development of HTLC-detector hyphenations is one of the areas of interest in chromatography.

Fast determination of volatile compounds like alcohols is of interest in food analysis. So far HTLC has been employed to determine alcohols in association with a flame ionization detector (FID) [4–7]. This detector is aimed at the determination of volatile organic compounds and, hence, water must be used as mobile phase avoiding postcolumn cooling. The use of a FID in HTLC has several features such as low limits of detection, high sensitivity, low background noise and the response tends to be linear across a wide range of concentrations, however it has some important drawbacks such as a tedious optimization of hydrogen and air flow rate and the liq-

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uid flow rate should be decreased so as to avoid flame extinction. This fact requires to switch to capillary columns [8]. And so other detection alternatives can be assayed such as Refractive Index (RI) detector [3]. HTLC is compatible with RI detector at high flow rates or if the mobile phase is well cooled at a constant temperature before entering in the detector. Although no reports on the determination of alcohols have appeared with this hyphenation, it has been demonstrated that HTLC-RI may be used without loss of sensitivity if the column effluent is kept at a rather low temperature (*ca.*, 20–40 °C) before entering the RI detection system. Furthermore, it is able to work with thermal gradient application [9]. The advantages of this detector for HPLC such as the theoretical universality (it is completely non-specific and responsive to almost all compounds) and the possibility to use it for compounds that do not absorb in the UV region, make it to be a good alternative to use with HTLC [10].

On the other hand, inductively coupled plasma atomic emission spectrometry (ICP-AES) is an elemental method in which liquid samples are turned out into aerosols by means of a nebulizer. The generated aerosol is introduced into a spray chamber whose purpose is to select the finest droplets [11]. Only these droplets reach a high temperature argon plasma and the analyte is atomized and excited. A spectrometer and detector are employed to obtain the radiation intensity at a characteristic wavelength. Recently, we developed a HTLC-ICP-AES coupling for the determination of non volatile compounds such as carbohydrates in foods [12]. The quantification of organic compounds was possible through the measurement of the carbon emission signal. The main issues of this coupling were: wider dynamic range and acceptable limits of detection as compared to an Evaporative light Scattering Detector (ELSD). Moreover, the ICP-AES detector provided information about metal content in food samples. This latter characteristic is not found in any of the conventional chromatographic detectors.

As the ICP-AES sensitivity depends directly on the mass of compound reaching the plasma, the introduction of volatile compounds into the ICP system may result advantageous in terms of improved sensitivity as compared to non volatile analytes. Therefore, the aim of the present work was to evaluate the HTLC-ICP-AES and HTLC-RI couplings for the determination of several alcohols (methanol, ethanol, propan-1-ol, butan-1-ol) and their application to the analysis of alcoholic beverages. Additionally, ICP-AES could be used to obtain simultaneous information about the metal and alcohol content. A further goal of the present work was to test the feasibility of column temperature programming to shorten the total analysis time.

2. Experimental

2.1. Reagents and solutions

Mobile phase ultrapure water (<18 MΩ) was obtained with a MilliQ water purification system (Millipore, USA). Methanol, ethanol, propan-1-ol and butan-1-ol were purchased from Panreac (Barcelona, Spain). Standards for metal determination were prepared by proper dilution of a 1000 mg/L multielement solution (Merk IV®, Germany).

2.2. Sample preparation

Several alcoholic beverages were analyzed after appropriate dilution in ultrapure water. For muscatel the dilution was 1/500, for vermouth samples it was 1/800, for pacharan and punch 1/1000, whereas the dilution factor for the whiskey samples was 1/2000. Previously to the analysis, all samples were filtered through a nylon filter 45 μm pore id. To avoid alcohol losses through evaporation, dilution was performed in closed vessels with a bore on the top to introduce the pipette. Once the solutions were prepared they were kept at 2 °C.

Metals were determined by means of an external calibration procedure for Mg while standard addition procedure was applied for the elements at low concentrations (Al, Cu, Mn and Ba). Because of the different concentrations, for Mg determination, the sample dilution was 1:100 whereas samples were 1:2 diluted with water for the determination of the remaining elements. The standard addition method was performed by using five solutions of each alcoholic beverage in which a volume of 16 mL of the diluted alcoholic beverage was spiked with increasing volumes of a multielement standard solution prepared from a 1000 mg/L stock solution (Merck ICP IV). Afterward, solutions were made up to a 20 mL final volume by adding Milli-Q water. The response was always taken as the peak area.

2.3. Instrumentation and HTLC-analysis

A Jasco PU 2085 HPLC pump (Tokyo, Japan) equipped with a RI detector Waters 410 (Waters Milford, MA, USA) was used. A 1 m length capillary was directly connected to the detector. This tubing length was taken to cool the effluent and to avoid instabilities in the detection process. The temperature selected for the reference and sample cell was 50 °C. The column temperature was controlled by a GC oven (GC-2014 Shimazdu, Kyoto, Japan) from 80 to 175 °C. A 2 m length 1/16 in. id stainless steel capillary inserted into the oven was employed for mobile phase preheating. The column employed for the separation of alcohols was a Hamilton polymeric reversed phase 5 μm 100 mm × 4.1 mm (PRP-1, Hamilton, Reno, Nevada). A PRP-1 cartridge guard column was also used. For ICP-AES detection, two valves (Mod. 7725 (i), Rheodyne, USA) with 20 μL loop were used (Fig. 1). Valve 1 in Fig. 1 was used to inject the sample (or standards) for separating the organic compounds in the column. Valve 2 in turn was used to inject the sample (or standards) after the column for the determination of metals. In this way, the peaks obtained for the metals present in the sample were registered after the later organic compound left the column. In the case of the RI detector, only valve 1 was used provided that this detector did not give information about the metal content.

An Optima 4300 DV Perkin-Elmer ICP-AES system (Uberlingen, Germany) was employed to simultaneously obtain the intensity of the radiation at the wavelengths studied. Signals were axially taken. The sampling time was set at 1.2 s so as one point was acquired every 1.75 s. This sampling time allowed obtaining from 15 to 20 points per peak. Table 1 summarizes the remaining ICP-AES experimental conditions and wavelengths used. Carbon emission signal was registered for the determination of organic compounds. Addi-

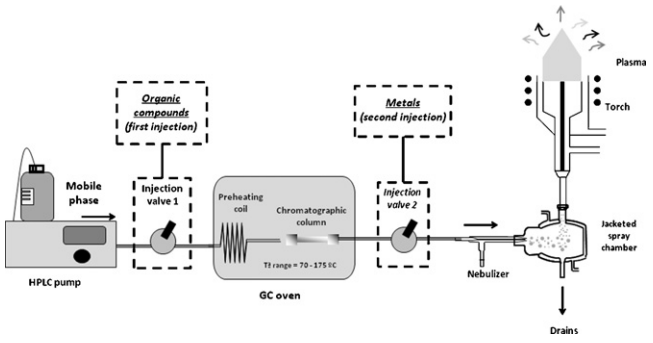

Fig. 1. Experimental setup of HTLC-ICP-AES coupling. In the first injection valve the samples were injected to separate the organic compounds in the column, in the second one the samples were injected after the column for the determination of metals.

Table 1
ICP-AES and ICP-MS instrumental conditions.

ICP-AES system		
RF power (kW)	1.35	
Argon outer gas flow rate (L/min)	15	
Argon intermediate gas flow rate (L/min)	0.2	
Argon central gas flow rate (L/min)	Variable	
Element/wavelength (nm)	C/193.090; Mg/280.271; Al/396.153; Cu/324.752; Mn/257.610; Ba/455.403	
ICP-MS system		
Plasma	RF power (kW)	1.0
	External gas (L/min)	13.5
	Intermediate gas (L/min)	0.82
	Central gas (L/min)	0.75
Acquisition parameters		
	Mode	Peak jump
	Sweeps	70
	Dwell time	20 ms
	Channel per mass	3
	Channel spacing	0.02
	Acquisition time	128 s

Isotopes: ^{24}Mg; ^{27}Al; ^{65}Cu; ^{55}Mn; ^{137}Ba.

tionally five metals were simultaneously determined (Table 1). The results obtained for metals were compared with those measured through a VG PQ ExCell ICP-MS instrument (Thermoelemental, Winsford, Chesire, UK). The acquisition parameters of this spectrometer are also summarized in Table 1.

Two different nebulizers were used to introduce the mobile phase into the ICP-AES system: (1) a glass pneumatic concentric nebulizer (Type TR-30-1A, Meinhard Glass Products, Santa Ana, CA) and, (2) a thermospray, in which the aerosol was generated by thermostating the capillary at the exit of the column at a temperature higher to that used for the separation (*i.e.*, from 150 to 220 °C). To achieve this, a heating tape and a temperature controller (JP Selecta, Barcelona, Spain) fitted to a thermocouple were used.

The nebulizers were coupled to two different spray chambers: a cyclonic type [13] and a single pass device [14]. Their inner volumes were 42 and 20 cm^3, respectively. Both spray chambers were made at the glass blower services of the University. The main difference between these two designs was related with the aerosol path inside the chambers and the dead volume. The two chambers were refrigerated with water at room temperature in order to lower the mass of vapor solvent reaching the plasma thus preventing the plasma cooling. Obviously, a fraction of the alcohols also condensed and was eliminated.

2.4. Recovery test

Some alcoholic beverages (punch and vermouth) were spiked with a known concentration of propan-1-ol and butan-1-ol (100 mg/L) whereas a given amount of methanol (100 mg/L) was added to the two whiskey samples. The alcoholic beverages were also spiked with a known concentration of multielement solution (Merck IV). The added concentration was 1 mg/L in each element.

Recovery percentage was calculated according to $R(\%) = [C_o \times 100/C_a]$. Where C_o was the analyte concentration found in the sample and C_a was the analyte concentration added to the spiked sample. All recovery assays were performed in triplicate.

2.5. Alcohol plasma transport efficiency

The transport measurements were done to determine the percentage of alcohol reaching the plasma with the HTLC-ICP-AES hyphenation. These experiments were carried out by means of an indirect method in which 20 μL, of a standard containing 1000 mg/L in each alcohol was injected in the chromatographic system. Then the drains were collected from the chamber (Fig. 1) for 2 min. Afterwards, the alcohol content in the drain was determined by HTLC. As the volume of the collected drain was known, the analyte transport efficiency was calculated according to:

$$\%\text{transport efficiency} = \left(\frac{\text{alcohol injected mass} - \text{alcohol mass found in drain}}{\text{alcohol injected mass}}\right)_{\text{alcohol "}i\text{"}} \times 100$$

The transport efficiency was obtained for the three different sample introduction systems and the four alcohols tested. The experiments were done in triplicate the RSD being lower than 10%.

3. Results and discussion

3.1. Optimization of the separation conditions with HTLC-RI hyphenation

Since alcohols cause a change in the solution refraction index, RI is perfectly indicated for their determination in alcoholic beverages. To the best of our knowledge, this is the first time that the HTLC-RI coupling has been applied to the determination of alcohols. The RI detector is sensitive to changes in the temperature of the mobile phase and it has to be very precisely thermostated (±0.001 °C). This is a very critical point that should be taken into account when coupling to HTLC.

The mobile phase flow rate was optimized working at a 100 °C oven temperature. Flow rates included within the 0.6–1.0 mL/min range were assayed. It was found that the signal-to-noise ratio was similar at all the flow rates tested, because, although the peak was higher at lower flow rates, when the flow rate increased, the noise decreased slightly. Consequently, the selected mobile phase flow rate was 1 mL/min because the retention times were shorter. Note that, at 0.6 mL/min the total analysis time was 21.7 min, whereas at 1 mL/min this time was 12.8 min.

As regard the oven temperature, the values of this variable covered the range between 80 and 175 °C because at temperatures below 80 °C the retention time was too long for the last compound detected (butan-1-ol), whereas at temperatures above 175 °C peaks for methanol and ethanol overlapped. Furthermore, the PRP columns can suffer severe damages if they are exposed to temperatures above 200 °C [15]. The van't Hoff plots depicted in Fig. 2 show the experimental linearity with positive slopes. This is because the transfer of the analyte from the mobile phase to the stationary phase was exothermic and the enthalpy and entropy of

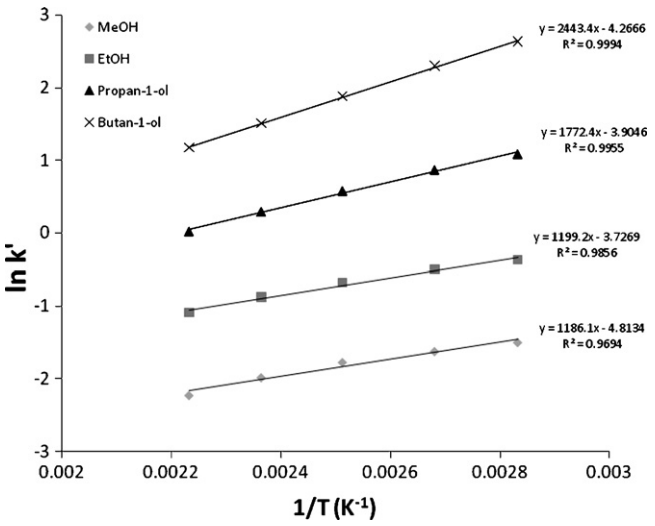

Fig. 2. van't Hoff plots for the studied compounds obtained with the HTLC-RI coupling at five different oven temperatures (80, 100, 125, 150 and 175 °C). Flow rate = 1 mL/min.

transfer were not a function of temperature. Note that the van't Hoff equation can be successfully used to predict retention times [16]. All the compounds showed a decrease in the retention times as the temperature went up, being more noticeable for alcohols with longer retention times. The higher slopes (enthalpies) of the van't Hoff plots for these compounds in Fig. 2, show that there was a stronger interaction between the mobile and stationary phase. At 175 °C column temperature good separations were obtained in 5.5 min. However, if the column temperature was 80 °C the analysis time was 17 min.

The column temperature also affected the sensitivity of the determination. It was found that the peak height increased with oven temperature. This was attributed to a reduction in the compound dispersion along the column. As regard the signal (peak height) to noise ratio, it was found that it increased with temperature up to 125 °C then at 150 °C decreased and at 175 °C this parameter increased again (Fig. 3). This trend was due to the fact that the lowest noise value was found at 125 °C. In all the cases S/N RSDs (three replicates) were lower than 3%.

In the present work and with the RI detector, 125 °C and 1 mL/min were selected as the optimum HTLC conditions because they represented a compromise between compound separation, S/N, total analysis time and column lifetime. Under these conditions, the four alcohols evaluated were separated in less than 10 min. Furthermore, it was verified that the mobile phase remained in liquid form. Indeed, the actual temperature of the column effluent was measured with a thermocouple and it was observed that for a nominal 125 °C oven temperature, the actual temperature of the eluent was 79 °C. The analytes, in turn, also remained in liquid form because at nominal oven temperatures of 100 and 125 °C the pressure inside the column was 6.5 and 5.9 MPa, respectively. Under these conditions and according to the phase diagram, the most volatile studied analyte (*i.e.*, methanol) did not evaporate inside the column.

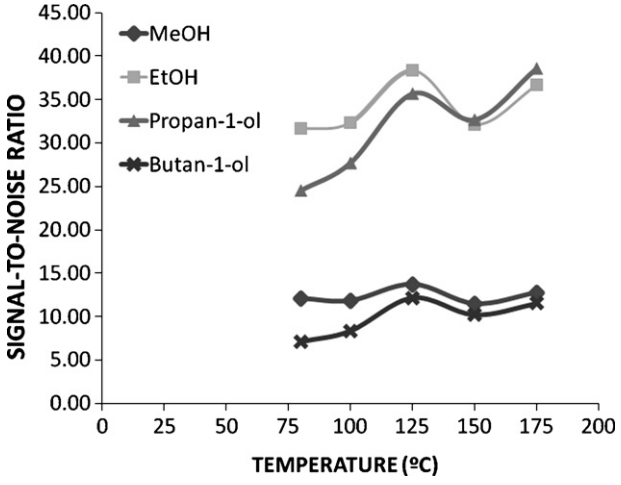

Fig. 3. Influence of column temperature on the signal-to-noise ratio for the studied compounds. Flow rate: 1 mL/min. Detector: RI.

3.2. Development of the HTLC-ICP-AES hyphenation

When coupling HTLC to the ICP-AES detector, the nebulizer and the spray chamber are of capital importance and they should be evaluated separately. First of all, it was observed that, in contrast to HTLC-RI, at temperatures above 100 °C, the methanol and ethanol peaks overlapped. It should be considered that once the solution was nebulized, a fraction of it impacted against the wall of the chamber. The analytes (*i.e.*, alcohols) could be either re-nebulized by the fresh mobile phase or evaporated from the chamber being transported towards the plasma. The net result was that, under a given set of conditions, the peaks became wider in HTLC-ICP-AES than when the RI was employed. Thus for this analysis it was compulsory to work at 100 °C (or below). Moreover, the effect of mobile phase flow rate was studied and 1 mL/min was selected because 0.6 and 0.8 mL/min gave rise to a long analysis time and to the degradation in the separation efficiency (tailing and wider peaks). Thus, the selected working conditions were 100 °C and 1 mL/min.

3.2.1. Pneumatic nebulizer

The nebulizer gas flow rate was optimized and the best value of this variable in terms of carbon sensitivity was 0.6 L/min. The two spray chambers were tested in terms of sensitivity with the same pneumatic nebulizer. The signal-to-noise ratio (S/N) was highest for ethanol, whereas butan-1-ol provided the lowest values of this parameter among the alcohols considered. This was the combination of three effects: the volatility of the solvent, the peak dispersion along the column and the different carbon content. Fig. 4 plots the sensitivity (signal to noise ratio) for the four different alcohols and the two evaluated chambers. It is important to note that due to the fact that ICP-AES is sensitive to the mass of carbon rather

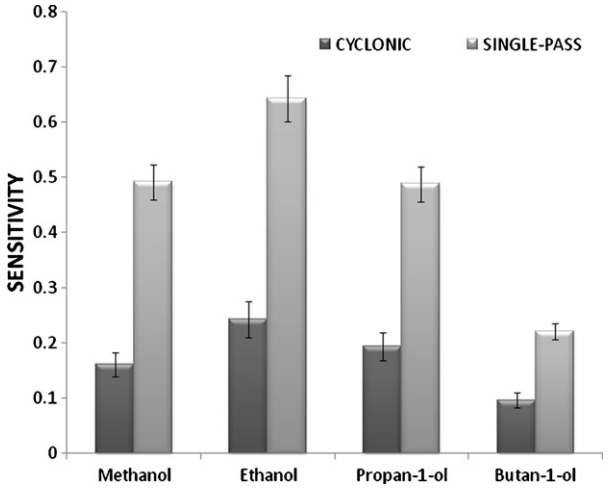

Fig. 4. Effect of the spray chamber design on the signal-to-noise ratio for the studied compounds. Flow rate = 1 mL/min. Oven temperature = 100 °C. Detector: ICP-AES. Pneumatic nebulizer.

than to the mass of analyte reaching the plasma, the signal to noise ratio was divided by the carbon content of each compound (*i.e.*, 100 ppm of ethanol contains 52.09 ppm of carbon (number of carbon atoms × Pa C/Pm) × 100) because the standards were prepared in mg of compound per liter. Another conclusion that can be obtained from the data shown in Fig. 4 is that for a given alcohol, the sensitivities were highly dependent on the spray chamber considered. Indeed the single pass spray chamber improved the results supplied by the cyclonic one. Previous studies had demonstrated that, for other group of compounds such as sugars [14], both cyclonic and single pass spray chambers provide similar ICP-AES sensitivities.

The peak width was also affected by the chamber design. Thus in the case of ethanol this parameter was 43 and 52 s for the single pass and cyclonic chambers, respectively. The lower inner volume in the case of the single pass spray chamber was the reason for these findings. In contrast with these results, the ethanol peak width in HTLC-RI was only 24 s. Therefore, the spray chamber was considered to be the main source of peak widening. In fact it was already observed that for volatile organic compounds, wider peaks were found than for non volatile ones [17]. The reason given was that a fraction of the alcohol could evaporate from the solution on the chamber walls and, thus, be transported to the plasma before the complete chamber rinsing.

3.2.2. Thermal nebulization

Another liquid sample introduction mode evaluated in the HTLC-ICP-AES hyphenation was the thermal nebulization [18], this approach is known as thermospray. Because the mobile phase left the column at high temperature, a heating tape was used thus giving rise to an aerosol at the exit of the stainless steel capillary. Therefore it was possible to introduce the mobile phase directly into the spray chamber without using a nebulizer taking advantage of the energy supplied to the sample in the oven. As an argon stream was not used to generate the aerosol, it was necessary to supply an additional argon flow in order to drive the aerosol through the spray chamber towards the plasma. With this nebulization system the selected chamber was the single pass spray chamber because the cyclonic one does not provide good results. An optimization of this aerosol carrier gas flow rate was performed and the optimum value was higher than that for the pneumatic concentric nebulizer (*i.e.*, 1 L/min).

An important variable precluding the characteristics of the generated aerosol and, hence, the sensitivity was the capillary temperature. The studied temperatures were 180, 200 and 220 °C. Temperatures below 180 °C and above 220 °C caused plasma degradation. For ethanol, the S/N values (RSD < 4%) were 41, 27 and 36 at 180, 200 and 220 °C, respectively. By comparison between thermospray and pneumatic nebulizer, it was observed that the signal-to-noise ratio was slightly higher for the former nebulization method. Thus the S/N values for the thermospray divided by those for the pneumatic nebulizer were 1.4, 1.5, 1.7 and 1.7 for methanol, ethanol, propan-1-ol and butan-1-ol, respectively. In the case of non volatile compounds, it was found that the former nebulization approach improved the sensitivities by a factor of up to two [12].

3.2.3. Transport measurements

It is widely known that in ICP-AES an important mass fraction of the nebulized solution (*i.e.*, typically 98–99%) does not reach the plasma and is lost via de spray chamber drains [11]. The experiments carried out in the frame of the present study proved that, also for alcohols, a fairly low fraction of the mass being injected reached the plasma. For instance, efficiencies for methanol with the single pass spray chamber were 17.2% with thermal nebulization and 15.9% with the pneumatic one and with the cyclonic spray chamber with the pneumatic nebulizer the efficiencies were around 8%. With these experiments it was observed that the highest transport efficiency was obtained with the thermal nebulization introduction system. Meanwhile, worst results were obtained with the cyclonic spray chamber. As regard the compound nature, the higher the alcohol volatility the higher the analyte transport efficiency.

3.3. Temperature gradients

Temperature programming in HTLC is equivalent to gradient elution in HPLC. Causon et al. demonstrated the benefits of using temperature-programmed elution for the determination of alcohols [5].

In the present work, preliminary studies about the possibility of using temperature gradients with the evaluated couplings were carried out. For the alcohol determination with HTLC-ICP-AES, the initial temperature (100 °C) was held for 4 min to separate compounds with short retention times. Note that higher alcohols require higher temperatures, whereas lower alcohols need lower temperatures to elute [6]. Then the temperature was increased at a 150 °C/min rate up to 150 °C. The use of temperature gradient gave rise to an important change in the baseline (Fig. 5a). In fact, the ICP-AES background carbon emission signal increased by a factor of 1.5 from 4 to 10 min retention time. This fact was likely due to the release of the carbon dioxide dissolved in the mobile phase as the temperature increased. This problem could be solved by subtracting the baseline background to the obtained chromatogram. Fig. 5b shows the chromatogram obtained after baseline subtraction. Another solution was to fit an additional 1 m stainless steel capillary at the exit of the oven (as in the HTLC-RI setup employed in the present work). In this way the mobile phase temperature decreased what prevented the release of carbon dioxide. In fact, it was verified that with this capillary the chromatograms obtained

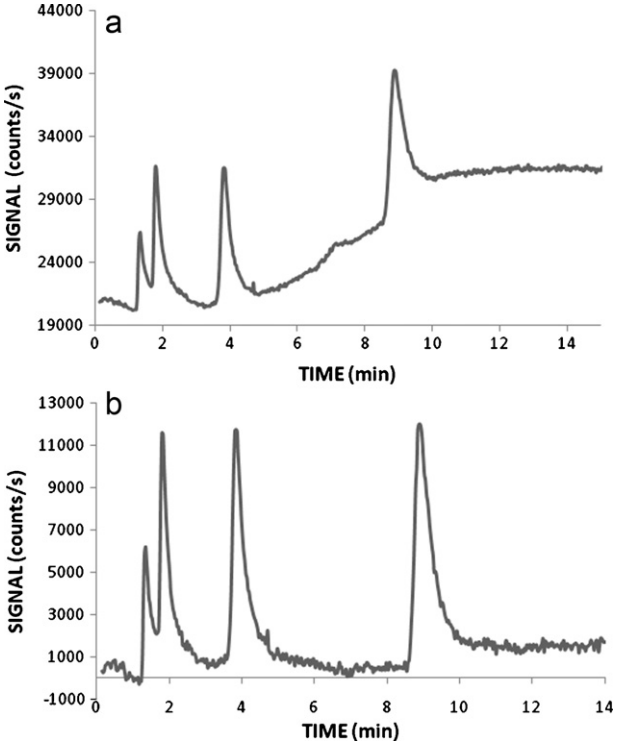

Fig. 5. (a) Chromatogram obtained under temperature programming for the studied compounds. (b) Chromatogram obtained under temperature programming for the studied compounds in which the background has been subtracted. Temperature gradient: 100 °C for 4 min and 150 °C/min up to 150 °C. Compounds from left to right: methanol, ethanol, propan-1-ol and butan-1-ol. Flow rate = 1 mL/min. Detector: ICP-AES.

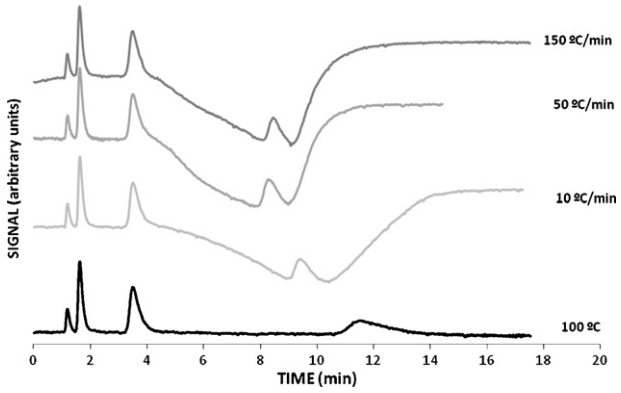

Fig. 6. Chromatogram obtained under three different temperature programming and an isothermal (100 °C) for the studied compounds. Temperature gradient: 100 °C for 4 min and (1) 150 °C/min, (2) 50 °C/min and (3) 10 °C/min up to 150 °C. Compounds from left to right: methanol, ethanol, propan-1-ol and butan-1-ol. Flow rate = 1 mL/min. Detector: RI.

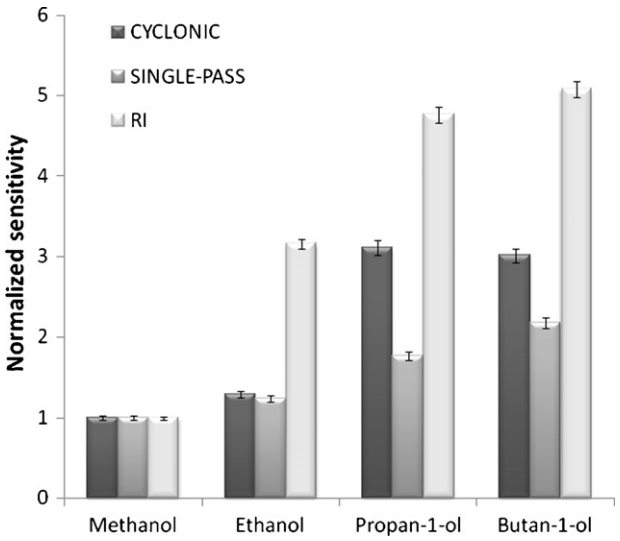

Fig. 7. Sensitivities normalized with respect to those for methanol. Flow rate = 1 mL/min. Alcohol concentration 100 mg/L each one. In the case of HTLC-ICP-AES the sensitivity has been calculated as the ratio between the peak area and the carbon concentration.

were virtually identical to the included in Fig. 5b with only a 10% increase in the background signal as the temperature went from 100 to 150 °C. Under the operating conditions employed in the present work, it was possible to carry out the four alcohol separations with a 3 min saving in the total analysis time.

Similar studies were carried out with HTLC-RI coupling. In this case three different temperature gradients were tested (Fig. 6). The initial temperature (100 °C) was held for 4 min to separate methanol, ethanol and propan-1-ol, and then the temperature was increased at 150 °C/min, 50 °C/min and 10 °C/min rates up to 150 °C in all the cases. The total analysis time was reduced in 3, 3 and 2 min, respectively, but the baseline experimented an important drift. For the three studied heating rates, the variation of the background with time was nearly the same. This was due to a change in the refraction index of the mobile phase when the temperature raised because the RI detector is highly sensitive to changes in temperature. Comparatively speaking the change in the background was much more severe for the RI than in the case of the ICP-AES detector. Thus, for the RI detector the baseline change was more than three times the butan-1-ol peak height, whereas in the case of ICP-AES the change was lower.

3.4. Comparison between detectors

The two hyphenations were compared in terms of analytical figures of merit. As regard LODs, both couplings provided similar values of this magnitude for all the studied compounds. In HTLC-ICP-AES the LODs were lower with the thermal nebulization than with the pneumatic one. Table 2 shows the obtained results. In both cases the limits of detection were below the maximum tolerable [19] and allowed levels for all the compounds. For example, according to the Wine International Organization [20], the maximum methanol allowable level is 400 mg/L. This value is far above the LODs included in Table 2.

The peak height obtained with the RI detector depended strongly on the type of alcohol being determined. The ICP-AES is a detector whose response is directly related with the carbon concentration. As the compound concentration instead of the carbon one was kept constant the peak area was divided by the actual carbon concentration for every alcohol, thus giving rise to a sensitivity parameter. Fig. 7 shows the sensitivities obtained normalized to that for methanol. The corresponding data for the RI detector are also summarized but, in this case, the area was not divided by the solution carbon concentration because the refraction index depended on the compound concentration (not on the carbon one). If the nature of the compound does not affect the performance of the detector the normalized sensitivity must be equal to the unity. As it may be seen, the results for the HTLC-ICP-AES coupling were closer to the unity than for the HTLC-RI association.

An interesting observation emerged when comparing the results obtained for the two chambers considered. Thus it was found that for the single pass spray chamber, the relative sensitivities were closer to 1 than for the cyclonic type chamber (Fig. 7). Also from this point of view, the single pass chamber proved to be more suitable than the cyclonic one to carry out the determination of volatile compounds.

Table 2
Limits of detection (mg of compound/L) obtained for the different alcohols at the optimum temperatures of each detector: RI. Oven temperature = 125 °C, ICP-AES: oven temperature = 100 °C. Flow rate = 1 mL/min.

	Methanol	Ethanol	Propan-1-ol	Butan-1-ol
RI detector	9.8	4.9	6.8	25.1
ICP-AES pneumatic nebulization	9.8	8.4	11.3	20.5
ICP-AES thermal nebulization	7.1	5.5	6.6	11.5

3.5. Analysis of real samples

HTLC-ICP-AES and HTLC-RI were used to determine volatile compounds in several alcoholic beverages. Table 3 gathers the concentrations obtained for ethanol. Student's t test revealed that the ethanol concentration obtained with both methods was not significantly different for the samples considered (*i.e.*, $t_{calculated} < t_{tabulated}$).

Moreover, a recovery study was done to evaluate the accuracy of the method by spiking some samples with propan-1-ol, butan-1-ol and methanol (100 mg/L in each alcohol) (Table 4). Recoveries of around 100% for all of the analytes were obtained independently of the employed detector.

One of the problems found with some of the analyzed samples was that compounds such as sugars coexisted with alcohols. As a result some peak overlapping occurred with the employed column. In fact, in the present work it was observed that for some beverages (*e.g.*, punch) an interfering peak emerged at a retention time very close to that for methanol. Therefore, severe difficulties appeared if the aim of the analysis was to determine methanol in these sam-

Table 3
Ethanol concentration (g/100 g of sample) found in the alcoholic beverages studied.[a]

	Muscatel	Pacharan	Punch	Vermouth	Whiskey 1	Whiskey 2
ICP-AES	10.4 ± 0.9	23.7 ± 2	19 ± 1	13.7 ± 0.7	40 ± 2	35 ± 1
RI	11.03 ± 0.2	19.2 ± 0.3	18.4 ± 0.2	12.2 ± 0.2	38.6 ± 0.2	34.2 ± 0.6
t[b]	2.7	7.9	0.4	7.1	3	4.4

[a] The confidence interval is defined as $\pm(ts/\sqrt{n})$, where s is the standard deviation ($n = 3$) t is obtained for a 99% confidence level and n is the number of replicates.
[b] These Student's t tests were calculated taking into account that the standard deviations between methods were different. Tabulated t for a 99% confidence level and two degrees of freedom: 9.9.

Table 4
Percentages of recovery ($n = 3$) in spiked samples of alcoholic beverages with methanol, propan-1-ol, butan-1-ol.[a]

Alcohol	Punch	Vermouth	Whiskey 1	Whiskey 2
Methanol			91 ± 6	91 ± 11
			105 ± 8	99 ± 6
Propan-1-ol	100 ± 1	110 ± 5		
	100 ± 2	100 ± 5		
Butan-1-ol	98 ± 6	100 ± 7		
	108 ± 3	108 ± 4		

[a] First row: data obtained with HTLC-ICP-AES.
Second row: data obtained with HTLC-RI.
The confidence interval is defined as $\pm(ts/\sqrt{n})$, where s is the standard deviation ($n = 3$) t is obtained for a 95% confidence level and n is the number of replicates.
The spike concentration was 100 mg/L in every analyte in the injected sample.

ples. To solve this problem the sample preparation method had to be modified. In the present work, a punch sample was spiked with methanol. The sample was evaporated to almost dryness in a heating plate at 75 °C during 20 min and then it was reconstituted with water. Additionally, an independent non spiked and non evaporated punch sample was prepared. Both samples were injected in HTLC-RI. The results demonstrated that initially methanol was not present in the sample, because the interfering peak had the same area in both the spiked and evaporated and the non spiked samples. All these studies were done by triplicate.

An obvious advantage of the HTLC-ICP-AES coupling over HPLC-RI was that elements other than carbon could be determined in the same chromatographic run as alcohols. Metals can be present in beverages through several sources including raw materials (from soil, water, fertilization and pesticide use in phytosanitary treatments), process type and equipment, bottling, storage (wood casks), and adulteration. Metals have positive as well as negative effects on the final quality of the beverage. For example, the turbidity increases with the metal content and color changes can be attributed to the formation of metal complexes with anthocyanins, tannins and polyphenols. In contrast, some metals enhance the flavor of these beverages. Therefore, the control of these inorganic elements is very important to protect the public health against the dietary exposure and in quality analysis and authentication. Chemometrics and pattern recognition methods are used for the distinction of beverages according to their origin, quality, variety, type and other features [21–23].

In the present work, the time elapsed after the last carbon peak was used to obtain the peaks corresponding to the metals. To accomplish this, a second valve was used (Fig. 1). 20 μL of the sample were injected and the peaks for several metals were simultaneously obtained. Note that, because a simultaneous ICP-AES system was used, the peaks for several elements were obtained from a single injection through the valve located after the GC oven. This is a clear advantage of the present hyphenation over the remaining existing methods for the determination of the concentration of alcohols. Metals were also determined with ICP-AES in the alcoholic beverages. It is widely known that organic solvents cause severe interferences in ICP-AES [24]. This kind of matrices may modify the aerosol generation and transport processes as well as the plasma thermal characteristics [25]. The final result is a modification in the sensitivity with the concomitant degradation of the accuracy. For this reason, the calibration was done by means of the standard addition method. Table 5 summarizes the data obtained for five different elements. For the sake of comparison the results obtained through ICP-MS are included in Table 5. It may be observed that by taking into account the confidence intervals, the two elemental methods provided similar metal concentrations. In Table 5 it can be observed a high concentration of Mg for some studied beverages, it can be explained because one of the main sources of this metal can be the dilution water added after distillation, the bottling water, the equipment and because magnesium is a natural component found in the fruit used to make these beverages. The content of Mn and Cu in the studied samples could come from the pesticides, fungicides and fertilizers and from the equipment used to elaborate the alcoholic beverage, moreover Mn is an oligoelement and can be a natural component depending on the origin of the raw fruit used to produce the beverage. However, Cu was not found in whiskey, possibly due to the formation of complexes that bind to wood during storage and aging; therefore Cu is thus removed from the liquor. A large number of factors can increase the presence of Al in alcoholic beverages, such as the use of bentonite during the process, the use of Al tanks for storage, levels of this metals in the soil or the dis-

Table 5
Concentration of five elements in the studied alcoholic beverage samples.[a]

	Muscatel	Pacharan	Punch	Vermouth	Whiskey 1	Whiskey 2
Mg	75 ± 1	20 ± 0.2		73.6 ± 0.6		
	72 ± 4	22 ± 1		79 ± 2		
Al			0.45 ± 0.06	2.96 ± 0.07	0.8 ± 0.01	0.53 ± 0.04
			0.45 ± 0.05	3.15 ± 0.06	0.8 ± 0.07	0.44 ± 0.05
Cu	0.27 ± 0.01			0.26 ± 0.02		
	0.26 ± 0.03			0.23 ± 0.01		
Mn	0.76 ± 0.05	0.24 ± 0.01		1.25 ± 0.03	0.012 ± 0.003	0.025 ± 0.005
	0.73 ± 0.03	0.20 ± 0.01		1.32 ± 0.04	0.011 ± 0.006	0.025 ± 0.001
Ba					0.62 ± 0.03	0.63 ± 0.08
					0.7 ± 0.06	0.7 ± 0.06

[a] The concentration is given in mg of element/L. The confidence interval is defined as $\pm(ts/\sqrt{n})$, where s is the standard deviation ($n = 3$), t is obtained for a 99% confidence level and n is the number of replicates.
First row: ICP-AES; second row: ICP-MS.

criminate use of pesticides, moreover Al can combine with tartaric acid and organic acids thus increasing its bioavailability [21,26].

Finally, a recovery study was carried out by both ICP-AES and ICP-MS with the aim to check the validity of these methods. Results around 100% for every element were found (*e.g.*, for muscatel, the recovery of manganese was 101.9 ± 3.8 by ICP-AES and 101 ± 2.2 in the case of ICP-MS).

4. Conclusions

The present work shows two alternatives to the existing methods for the determination of volatile compounds such as alcohols in alcoholic beverages. The HTLC-ICP-AES coupling shows several advantages as compared with HPLC-ICP-AES determinations. The retention times for alcohols are shortened what is extremely important for an expensive detector such as the ICP-AES. Furthermore, the interface HTLC-ICP-AES can be simplified by removing the nebulizer and taking advantage of the mobile phase heating to produce the aerosol. Finally, a single pass spray chamber can be used in order to obtain narrower peaks than those found with conventional cyclonic spray chambers.

The HTLC-RI coupling is a good choice because this detector is very common in routine laboratories and it shows satisfactory results in combination with HTLC if some considerations are taking into account. The ICP-AES detector in turn appears to be a useful tool for the determination of volatile and non volatile organic compounds as well as metals. HTLC-ICP-AES hyphenation shows as an alternative to obtain both organic and inorganic information in the same chromatographic run. Moreover, the use of temperature programming is advisable when working with HTLC-ICP-AES. In this case, the changes in the baseline caused by a rise in the mobile phase temperature can be reduced to acceptable levels by merely placing an additional stainless steel capillary between the oven and the spectrometer. Therefore, this detector appears to be advantageous against the RI to work under temperature gradient conditions.

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References

[1] L.A. Cole, J.G. Dorsey, K.A. Dill, Anal. Chem. 64 (1992) 1324.
[2] K. Hartonen, M.L. Riekkola, Trends Anal. Chem. 27 (2008) 1.
[3] D. Guillarme, S. Heinisch, Sep. Purif. Rev. 34 (2005) 181.
[4] D. Guillarme, S. Heinisch, J.Y. Gauvrit, P. Lanteri, J.L. Rocca, J. Chromatogr. A 1078 (2005) 22.
[5] T.J. Causon, R.A. Shellie, E.F. Hilder, Analyst 134 (2009) 440.
[6] B.A. Ingelse, H.-G. Janssen, C.A. Cramers, J. High Resolut. Chromatogr. 21 (1998) 613.
[7] D.J. Miller, S.B. Hawthorne, Anal. Chem. 69 (1997) 623.
[8] M.H. Chen, C. Horváth, J. Chromatogr. A 788 (1997) 51.
[9] L.R. Lima, R.E. Synovec, Anal. Chem. 65 (1993) 128.
[10] J.-M. Mermet, M. Otto, M. Valcárcel, R.A. Kellner, H.M. Widmer, Analytical Chemistry. A Modern Approach to Analytical Science, second ed., Wiley-VCH, 2004.
[11] J.L. Todolí, J.M. Mermet, Liquid Sample Introduction in ICP Spectrometry. A Practical Guide, Elsevier, Amsterdam, 2008.
[12] A. Terol, E. Paredes, S.E. Maestre, S. Prats, J.L. Todolí, J. Chromatogr. A 1217 (2010) 6195.
[13] S.E. Maestre, J. Mora, J.L. Todolí, A. Canals, J. Anal. At. Spectrom. 14 (1999) 61.
[14] J.L. Todolí, J.M. Mermet, J. Anal. At. Spectrom. 17 (2002) 211.
[15] M.O. Fogwill, K.B. Thurbide, J. Chromatogr. A 1139 (2007) 199.
[16] T. Teutenberg, High-Temperature Liquid Chromatography. A User's Guide for Method Development, RSC Publishing, Cambridge, 2010.
[17] E. Paredes, S.E. Maestre, S. Prats, J.L. Todolí, Anal. Chem. 78 (2006) 6774.
[18] J.A. Koropchak, M. Veber, Crit. Rev. Anal. Chem. 23 (1992) 113.
[19] A. Paine, A.D. Davan, Hum. Exp. Toxicol. 20 (2001) 563.
[20] Wine International Organisation (OIV). Resolution OENO 19/2004.
[21] J.G. Ibanez, A. Carreon-Alvarez, M. Barcena-Soto, N. Casillas, J. Food Compos. Anal. 21 (2008) 672.
[22] J. Baciela, M. Vilar, S. García-Martín, R.M. Peña, C. Herrero, Anal. Chim. Acta 628 (2008) 33.
[23] L. Elçi, Z. Arslan, J.F. Tyson, J. Hazard. Mater. 162 (2009) 880.
[24] R.I. Botto, J.J. Zhu, J. Anal. At. Spectrom. 11 (1996) 675.
[25] D.G.J. Weir, M.W. Blades, Spectrochim. Acta 49B (1994) 1231.
[26] F.F. Lopez, C. Cabrera, M.L. Lorenzo, M.C. Carmen, Sci. Total Environ. 220 (1998) 1.