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High-Temperature Liquid Chromatography Inductively Coupled Plasma Atomic Emission Spectrometry hyphenation for the combined organic and inorganic analysis of foodstuffs

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ABSTRACT

The coupling of a High-Temperature Liquid Chromatography system (HTLC) with an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) is reported for the first time. This hyphenation combines the separation efficiency of HTLC with the detection power of a simultaneous ICP-AES system and allows the combined determination of organic compound and metals. The effluents of the column were introduced into the spectrometer and the chromatograms for organic compounds were obtained by plotting the carbon emission signal at a characteristic wavelength versus time. As regards metals, they were determined by injecting a small sample volume between the exit of the column and the spectrometer and taking the emission intensity for each one of the elements simultaneously. Provided that in HTLC the effluents emerged at high temperatures, an aerosol was easily generated at the exit of the column. Therefore, the use of a pneumatic nebulizer as a component of a liquid sample introduction system in the ICP-AES could be avoided, thus reducing the peak dispersion and limits of detection by a factor of two. The fact that a hot liquid stream was nebulized made it necessary to use a thermostated spray chamber so as to avoid the plasma cooling as a cause of the excessive mass of solvent delivered to it. Due to the similarity in sample introduction, an Evaporative Light Scattering Detector (ELSD) was taken as a reference. Comparatively speaking, limits of detection were of the same order for both HTLC–ICP-AES and HTLC–ELSD, although the latter provided better results for some compounds (from 10 to 20 mg L^{-1} and 5–10 mg L^{-1}, respectively). In contrast, the dynamic range for the new hyphenation was about two orders of magnitude wider. More importantly, HTLC–ICP-AES provided information about the content of both organic (glucose, sucrose, maltose and lactose at concentrations from roughly 10 to 400 mg L^{-1}) as well as inorganic (magnesium, calcium, sodium, zinc, potassium and boron at levels included within the 6–3000 mg L^{-1}) species. The new development was applied to the analysis of several food samples such as milk, cream, candy, isotonic beverage and beer. Good correlation was found between the data obtained for the two detectors used (*i.e.*, ICP-AES and ELSD).

1. Introduction

High-Temperature Liquid Chromatography (HTLC) has emerged as a good alternative to High Performance Liquid Chromatography (HPLC) for the separation and determination of organic compounds [1–3]. A common HTLC system requires from a preheating conduction in which the temperature of the mobile phase is accommodated to that of the column in order to avoid temperature gradients during the separation step [4,5]. An additional component is the column heating unit. On this subject there are several approaches such as the use of a Gas Chromatography (GC) oven [6], a heating tape [7] or a heating metallic body [8]. An obvious constraint of this technique is the stability of the stationary phase. Separation degradation caused by a deterioration of the column has been a subject of great concern. The development of new columns containing graphite [9], zirconia [10,11] or polymeric [12,13] stationary phases has renewed the interest on HTLC. As the mobile phase leaves the column at high temperature, a cooling system must be used in order to avoid both positive and negative effects on sensitivity [14].

One of the advantages of working in reversed phase at high temperatures is that pure water can be employed as mobile phase. Thus, from an environmental point of view, HTLC emerges as a "green" separation methodology. Moreover, the preparation is sim-

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plified and the cost of the mobile phase is severely depressed as compared with conventional reversed phase liquid chromatography (RPLC). From an analytical point of view, HTLC shows several important advantages over HPLC [15]: (i) shorter analysis times thus leading to narrower peaks and higher sensitivities in terms of peak height; (ii) improved efficiency and hence, resolution; and, (iii) possibility of using longer columns because of the decrease in the back-pressure. It could be stated that HTLC is especially efficient and suitable in those cases in which a few analytes must be separated.

An additional advantage of HTLC is that the number of detectors that can be adapted becomes larger than in HPLC. On this subject, HTLC has been adapted to detection techniques such as visible–UV spectrophotometry [16,9], fluorimetry [17], refractometry [19], Evaporative Light Scattering Detection (ELSD) [18,19], Flame Ionization Detection [20], Mass Spectrometer [21] and Nuclear Magnetic Resonance [22].

Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) is an elemental analysis technique that has enormously evolved in the last twenty years. This technique is currently used in routine analysis laboratories for the determination of trace metals in samples of different nature. In order to carry out the analysis, the liquid sample should be transformed into an aerosol. Then, a spray chamber is used to introduce only the finest aerosol droplets into the plasma where the different elements are atomized/ionized and further excited. The emission signal obtained at characteristic wavelengths is directly proportional to the analyte concentration in the sample [23]. In ICP-AES the sample introduction system plays a critical role, because it precludes the obtained analytical signal. In general terms, it can be stated that the finer the aerosol generated by the nebulizer the higher the mass of analyte delivered to the plasma and hence the higher the sensitivity. There are some studies that demonstrate the suitability of an ICP-AES as a HPLC detector for the determination of organic compounds [24]. This application implies the measurement of the signal at a carbon characteristic wavelength. The HPLC–ICP-AES hyphenation has been applied to the determination of aminoacids [25–27], saccharides [28,29], carboxylic acids and alcohols [30,31]. Advantages over conventional chromatographic detectors include: (i) better limits of detection for some organic compounds; (ii) wider dynamic ranges; (iii) universality for non-volatile compounds, since the carbon signal only depends on the carbon concentration and is independent of the carbon chemical form; and (iv) cleaner backgrounds. A drawback observed is that retention times are sometimes too long and, hence, the cost of the determination increases severely since the total argon flow under typical ICP-AES operating conditions is about 20 L/min. Therefore, the development of rapid separation methods in crucial to make this detector competitive in terms of analytical cost.

The determination of organic compounds by ICP-AES requires the use of mobile phases free of organic solvents. This later point fits perfectly with some applications and work done in HTLC. So far ICP-AES has not been used in conjunction with HTLC to determine organic compounds. Besides this, none of the detectors mentioned previously provides elemental information for inorganic species. The main goal of the present work was thus to develop a new HTLC–ICP-AES coupling for the simultaneous determination of carbohydrates and metals in food samples. Both groups of species are very important to control the quality and safety of foodstuffs [32]. In order to accomplish the proposed aim the interface between both techniques was studied and optimized so as to obtain the best separation efficiencies and analytical figures of merit. The results were compared against those provided by a conventional ELSD that, as in the case of ICP-AES, requires the sample to be transformed into an aerosol. The developed coupling was applied to the analysis of several food samples.

2. Experimental

2.1. Apparatus

Fig. 1 shows a schematic layout of the HTLC–ICP-AES system employed in the present work. A HPLC pump Model PU-2085 (Jasco Inc., Tokyo, Japan) was used throughout. Two valves (Mod. 7725(i), Rheodyne, USA) with 20 μL loops were adapted. 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 before the first organic compound left the column. The heating system consisted of a CG oven (GC-2014 Shimazdu, Kyoto, Japan). In the present work temperatures ranged from 70 to 175 °C. A 2 m length 1/16″ id stainless steel capillary inserted into the oven was employed for mobile phase preheating. A 100 mm length 4 mm id column with 5 μm graphitic carbon porous spherical particles was employed for the first time for the separation of carbohydrates (Hypercarb, Thermo electron corporation, UK) [33]. This column has been previously used for the determination of monosaccahides at temperatures below 60 °C using water as mobile phase [34].

An Optima 4300 DV Perkin-Elmer (Uberlingen, Germany) ICP-AES system was used to simultaneously obtain the intensity at the wavelengths studied. Signals were axially taken, because of the increased sensitivity. This system was equipped with a 40.68 MHz free-running generator and a polychromator with an échelle grating. Two Segmented-array Charge-coupled Device (SCD) detectors allowed the simultaneous measurement of several lines in the UV and visible electromagnetic spectrum zones. The sampling time was set at 1.2 s so as a point was acquired every 1.75 s. This sampling time allowed to obtain from 15 to 20 points per peak. Under these conditions peak area RSDs were always lower than 5%. Table 1 summarizes the ICP-AES experimental conditions used. Carbon emission signal was registered for the determination of organic compounds. Additionally six metals were simultaneously determined (Table 1).

A glass pneumatic concentric nebulizer (Type TR-30-1A, Meinhard Glass Products, Santa Ana, CA) was employed to introduce the mobile phase into the ICP-AES system (Fig. 1). In a different approach, the aerosol was generated by thermostating the capillary at the exit of the column at a temperature close 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 (Fig. 1B). These nebulizers were coupled to three different spray chambers. Two of them were of Cyclonic type [35] and the third one was a single pass device [36]. Their inner volumes were 44, 42 and 20 cm^3, respectively. Cyclonic #1 and single pass spray chambers were home-made whereas Cyclonic #2 was purchased from Glass Expansion (Australia). The main difference among designs was related with the aerosol path inside the chambers and the dead volume. All three 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.

Table 1
Instrumental conditions for the ICP-AES spectrometer used.

RF power (kW)	1.35
Argon outer gas flow rate (L/min)	15
Argon intermediate gas flow rate (L/min)	0.2
Element/wavelength (nm)	C/193.090; Na/589.592; Ca/317.933; Zn/206.200; Mg/280.271; K/766.490; B/249.677

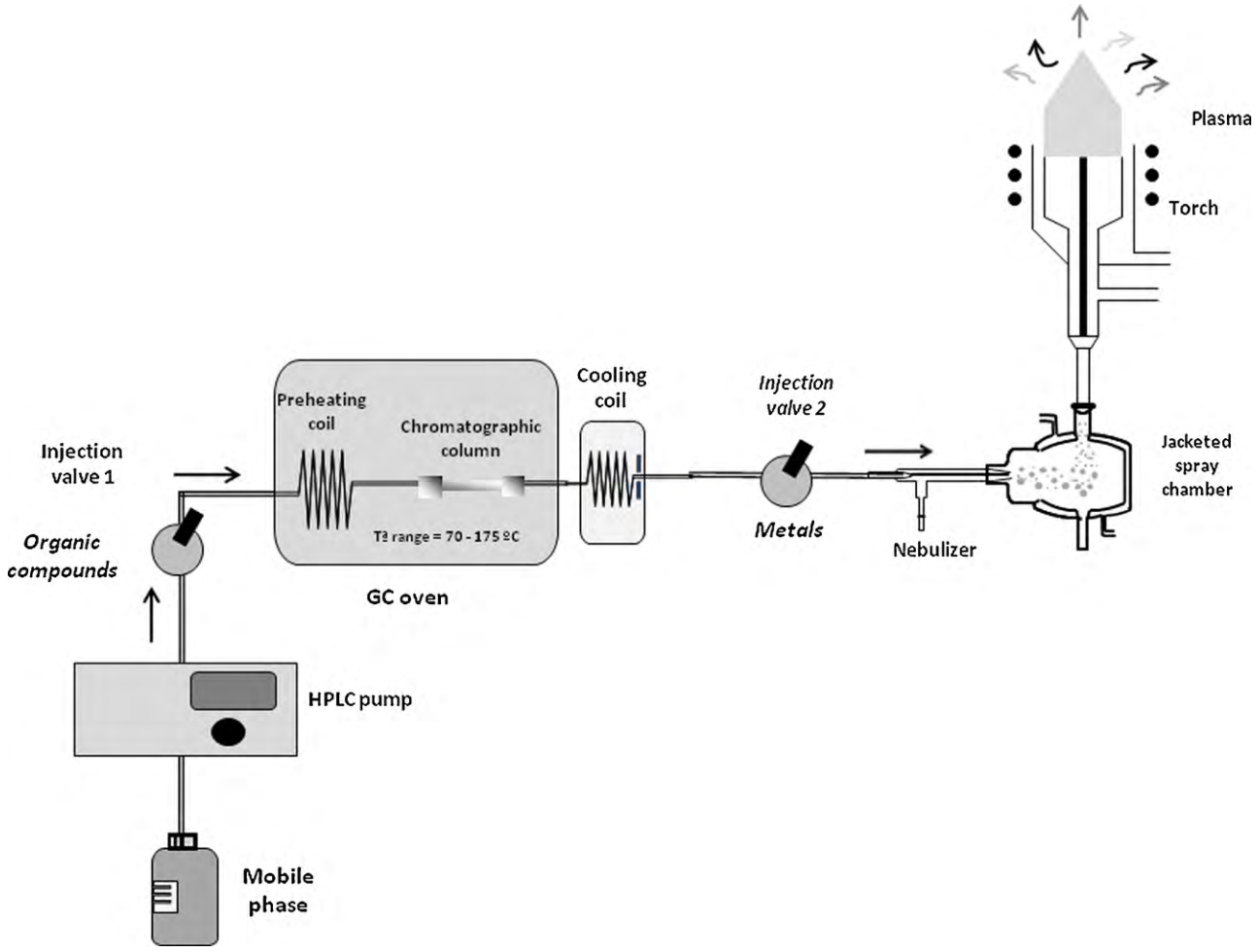

Fig. 1. Experimental setup of the HTLC–ICP-AES system with pneumatic nebulization.

Alternatively, an ELSD system (SoftA Corporation, Tokyo, Japan) was employed as a reference detector. In this detector, the column effluent was transformed into an aerosol by means of a plastic pneumatic nebulizer. Argon was used as the nebulization gas. Then, the aerosol solvent was removed through a cooling and heating cycle produced inside two tubes called spray chamber and drift tube, respectively. The selected conditions were 10 °C for the spray chamber and 90 °C for the drift tube, being the argon gas pressure 4.5 bars (1.2 l L/min). The analyte particles intercept a laser beam and the dispersed light is correlated with the analyte concentration. When the ELSD was used, the second valve shown in Fig. 1 was removed provided that this detector was not able to detect simultaneously both organic compounds and metals. In this case, a 1 m length capillary is directly connected to the detector. This tubing length has been selected to cool the effluent and avoid instabilities in the detection process.

Volume drop size distribution was measured for the aerosols generated by the nebulizers (*i.e.*, primary aerosols). This was done by means of a laser Fraunhofer diffraction system (model 2600c, Malvern Instruments, Malvern Wolcestershire, UK). The sizer was equipped with a 63 mm lens focal length what enabled the system to measure droplets with diameters included within the 1.2–118 μm range. The nebulizer tip was placed at 30 mm from the lens and at 2 mm from the laser beam centre.

2.2. Reagents and solutions

All reagents were of analytical grade. D(+)-Glucose, D(+)-sucrose, D(+)-maltose and D(+)-lactose were purchased from Flucka (Buchs, Switzerland). Standards for metal determination were prepared by proper dilution of a 1000 mg/L multielement solution (Merk, Germany). Mobile phase consisted of milli-Q water (<18 MΩ).

2.3. Procedure

Several food samples were analyzed. Condensed, liquid milk and coffee cream samples were diluted with ultrapure water. The dilution factor depended on the detector employed. For the ELSD it was 1/3000, 1/250 and 1/400, respectively, whereas these values were 1/25, 1/5 and 1/10 in the case of the ICP-AES. This was necessary because the former detector was saturated at relatively low sugar concentrations. Then the samples were prepared as follows: 8 mL of a 2:1 chloroform:methanol mixture were added to 2 g of diluted sample in order to separate sugars from proteins and fatty compounds. The mixture was shaken for 1 min into a mechanic agitator (Promax 1020, Heidolph Instruments, Kelheim, Germany). Then the solutions were centrifuged for 30 min at 3500 rpm. Three immiscible phases were obtained and the uppermost phase was taken, because it contained the sugars dissolved into methanol. The methanolic solution was poured into a flask and evaporated to dryness by means of a rotavapor (Mod. R-II, Buchi, Flawil, Switzerland). In order to accomplish this, the solution was heated at 60 °C and the vacuum pressure was set at 300 MPa. Once this was completed, the dry residue was dissolved with 10 mL of ultrapure water and forced to pass through a nylon 45 μm pore id filter.

A lemon – honey candy sample was also analyzed. In this case the sample was dissolved in water, the dilution factor being 1:600.

In order to determine sucrose by means of the ELSD, this solution was further diluted (1:3). Glucose and maltose determinations did not require from this second dilution. To analyze beer and isotonic beverages, the samples were degasified by sonication and 1:50 diluted with water. Only for the latter sample, a further 1:4 dilution was required so as to avoid ELSD saturation.

3. Results and discussion

3.1. Optimization of the separation conditions

In the present work, the separation was optimized by coupling the column to the ELSD system. The oven temperature ranged from 75 to 175 °C because at temperatures below 75 °C the retention times were too long for disaccharides, whereas at temperatures above 175 °C the resolution was degraded. It was observed that the higher the oven temperature, the shorter the retention time. Furthermore, the peaks became narrower and higher as the temperature raised. This behavior was more pronounced for the three disaccharides studied (sucrose, maltose and lactose) than for glucose. At 175 °C column temperature good separations were obtained and the four compounds considered eluted in 2 min. This corresponded to a significant shortening in the analysis time. If the column temperature was 75 °C the analysis time was about 12 min. Meanwhile, glucose left the column at retention times close to 1 min. Note that, according to previous results in HPLC [37], this sugar left the column 10 min after its injection. In the present work, the column temperature selected was 150 °C because of the improved sensitivity. At this temperature, the time of analysis was still lower than 3 min. This time was almost the same as that required to run one solution for the analysis of metals through ICP-AES. Therefore, the use of HTLC–ICP-AES allowed to obtain a larger amount of information about the sample without increasing the time and price of analysis at all.

The column temperature also affected the sensitivity of the determination and it was found that the signal (peak height) to noise ratio peaked at 150 °C. The increase in peak height with oven temperature was attributed to the decrease in the analyte dispersion in the column. This had as a main consequence a reduction of the peak width and a concomitant increase in the height. Additionally, the nebulization and/or aerosol transport processes could be enhanced as the temperature increased thus leading to an increase in the aerosol mass reaching the ELSD detector [38]. The latter phenomenon can be clearly observed as the peak widths above 125 °C did not change appreciably with the oven temperature. Therefore, a compromise existed between separation efficiency, analysis time and sensitivity. At 175 °C the sensitivity was about 10–50% lower than at 150 °C depending on the compound considered. As regards analysis time, the respective values of this parameter were 2 and 2.5 min. According to these results 150 °C was selected as the optimum separation temperature.

The mobile phase flow rate was also optimized. Flow rates included within the 0.5–1.2 mL/min range were used. It was found that 1 mL/min represented again a good compromise between separation efficiency, sensitivity and analysis time.

3.2. Development of the HTLC–ICP-AES hyphenation

The characteristics and operating conditions required in HTLC made this technique to be highly compatible with an ICP-AES spectrometer. This was because: (i) hot liquid samples are tolerated by the detector although attention should be paid to the solvent plasma load, since if it is too high the plasma can be altered what causes a degradation in its performance and likely its eventual extinction; and, (ii) the optimum liquid flow rate is in fully agreement with the rates normally used to carry out routine determinations. Furthermore, as ICP-AES is a technique used for elemental analysis, it is possible to develop a single method to provide information about the content of organic as well as inorganic species in foodstuffs.

The interface of the developed coupling was a critical point. In ICP-AES the sample is normally introduced as an aerosol (as in the case of the ELSD). This aerosol is often produced by means of a pneumatic nebulizer. In this device the aerosol is generated as a result of the interaction between the mobile phase and a high velocity gas stream. Following this step a spray chamber is employed so as to select the aerosol finest droplets. Both components are of capital importance and should be evaluated [39]. In the present study the three spray chambers previously described were employed together with a pneumatic concentric nebulizer. For this sort of nebulizers, the liquid and gas flow rates precluded the sensitivity and, hence, they were optimized. A 0.6 L/min gas flow rate was selected as it provided the best sensitivity for carbon for the three spray chambers tested. Note that special attention was paid on carbon provided that it presented higher limits of detection than the metals. In order to optimize the liquid flow rate, a thorough study was performed for the three chambers considered. The results are shown in Fig. 2. It can be observed that the trends were highly dependent on the spray chamber considered. Thus, in the case of the Cyclonic #1 spray chamber (Fig. 2a), the higher the liquid flow rate, the higher the signal to noise ratio (being the signal the peak height and the noise an average value of 20 measurements of the difference between the maximum and minimum signals obtained for the base line within a time range equal to the peak width). This result was a combined consequence of the increase in the peak height and the reduction in the signal noise as the liquid flow rate increased up to 1 mL/min. The latter value was then considered as the optimum one. Nonetheless, for the Cyclonic #2 spray chamber a signal to noise ratio maximum was observed at 0.5 mL/min. Meanwhile, the single pass spray chamber provided good results at 0.75 mL/min. These results showed that modifications in the spray chamber geometry caused significant changes in the instrument performance thus giving a proof of the critical role played by this component of the sample introduction system.

The three chambers were compared in terms of sensitivity and peak width. At 1 mL/min, the Cyclonic #1 afforded the highest sensitivities. This device improved the sensitivities by a factor included within the 2–3 range as compared to the Cyclonic #2. As regards the peak dispersion it was found that despite their very different inner volumes all three chambers provided similar peaks widths. Thus for example, at 1 mL/min the sucrose peaks were only 5% narrower for the single pass spray chamber than for the cyclonic ones. Obviously the differences were intensified as the mobile phase flow rate decreased. Note that at 0.5 mL/min this difference increased up to 25%. Therefore, it was concluded that the Cyclonic #1 spray chamber exhibited the best performance for the determination of carbohydrates through ICP-AES. Fig. 3 shows the obtained chromatogram for the four carbohydrates considered. These compounds were eluted in less than 3 min. This was an important shortening of the analysis time as compared with previous HPLC–ICP-AES work [37]. The Cyclonic #1 spray chamber appeared to be more appropriate to cope with HTLC, because it provided the best performance at the optimum mobile phase flow rate in terms of separation efficiency.

At the temperatures employed in the separation step an aerosol was produced at the exit of the stainless steel capillary, therefore it was possible to remove the nebulizer (Fig. 4). Therefore, by thermostating this capillary it was possible to introduce the solution directly into the spray chamber thus reducing the peak broadening caused by the dead volume of the pneumatic nebulizer. This system

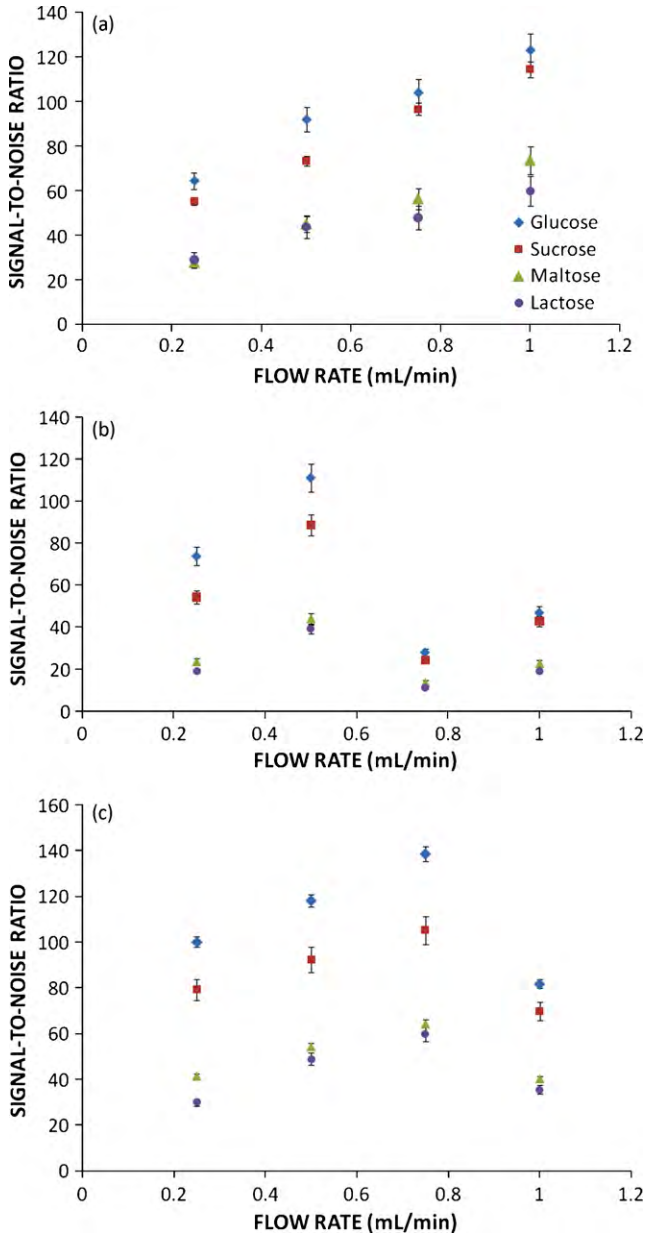

Fig. 2. Effect of the liquid flow rate on the signal to noise ratio for (a) cyclonic spray chamber 1; (b) cyclonic spray chamber 2; and (c) single pass spray chamber with neumatic nebulizer. Oven temperature: 150 °C.

is similar to the so-called Thermospray employed in HPLC–MS and ICP techniques [40]. This approach took advantage of the energy supplied to the sample in the oven and a similar idea has been described for HTLC–MS coupling employing an electrospray as nebulization system [41]. In these approaches the capillary also acted as a linear restrictor that avoided phase transition of the mobile phase in the column.

In order to control the capillary temperature a heating tape was carefully wound around it (Fig. 4). For the Thermospray, the aerosol is also generated through a liquid and gas interaction step. In this case it is necessary to use an additional argon stream in order to drive the aerosol through the spray chamber towards the plasma. An optimization of this aerosol carrier gas flow rate was performed. The optimum value was higher than that for the pneumatic concentric nebulizer (*i.e.*, 1 L/min).

A new variable was then optimized, namely the capillary temperature. Fig. 5 shows the chromatograms obtained for the

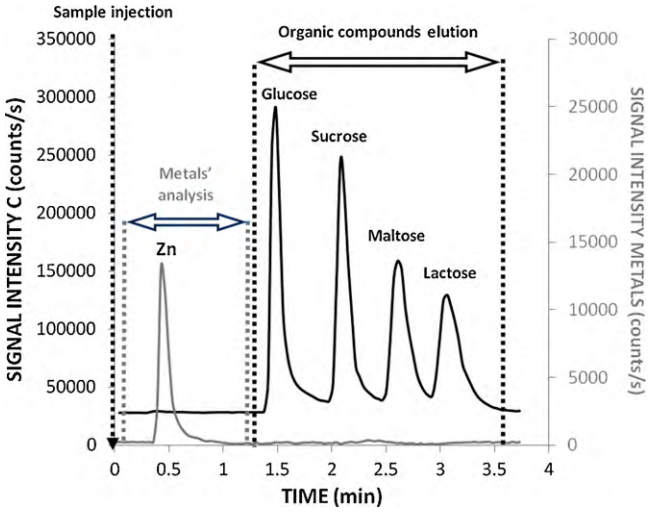

Fig. 3. Chromatograms for the HTLC–ICP-AES coupling using pneumatic nebulizer. Flow rate: 1 mL/min; Oven temperature: 150 °C.

Thermospray at three different capillary temperatures. It was found that the peak height became higher as the temperature went up. In the case of glucose, for instance, the peak height at 220 °C was about three times higher than that at 170 °C. At temperatures above 220 °C, precision was severely degraded whereas at temperatures below 170 °C no aerosol was generated. In order to understand the reasons of the signal enhancement with temperature, aerosol drop size distributions were measured. The aerosol surface mean diameter, also known as Sauter mean diameter, $D_{3,2}$, gave an indication of the aerosol fineness in global terms. This parameter took values of 11.1 and 7.6 μm at 170 and 220 °C, respectively. Therefore, finer aerosols were produced at higher temperatures. As a result, a higher mass fraction of aerosol was transported towards the plasma, hence increasing the sensitivity. Additionally, the increase in the mass of solvent delivered to the plasma could cause a degradation in its thermal characteristics. In contrast, the 420.069 nm argon emission increased with the capillary temperature, what meant that the plasma excitation conditions were enhanced at high values of this variable. The fact that a chilled chamber was used also contributed to prevent plasma degradation.

Fig. 5 also shows that the background increased with the capillary temperature. This could be explained by taking into account that the carbon dioxide dissolved in the mobile phase left the solution more quickly as the solution temperature raised. Therefore the mass of carbon delivered to the plasma increased with this variable.

As with the pneumatic nebulizer, the Cyclonic #1 spray chamber supplied the best results in terms of sensitivity at the optimum separation liquid flow rate (*i.e.*, 1 mL/min). Nevertheless, the sensitivity improvement with respect the other two chambers was lower than for the pneumatic nebulizer. For glucose, Cyclonic #1 yielded 20% higher sensitivities than Cyclonic #2. Single pass spray chamber afforded the lowest sensitivities when the Thermospray was used.

Comparatively speaking, the use of the Thermospray was advantageous with respect to the pneumatic concentric nebulizer. In fact the former system provided sensitivities up to two times higher (maltose) than the latter. Table 2 summarizes the limits of detection obtained for the different compounds and sample introduction systems employed in the present work. It can be observed that, although for all the studied situations the LODs were of the same order, the Cyclonic #1 spray chamber together with the Thermospray gave rise to the lowest LODs among the systems considered. Another advantage was that the peak width was lower for the Ther-

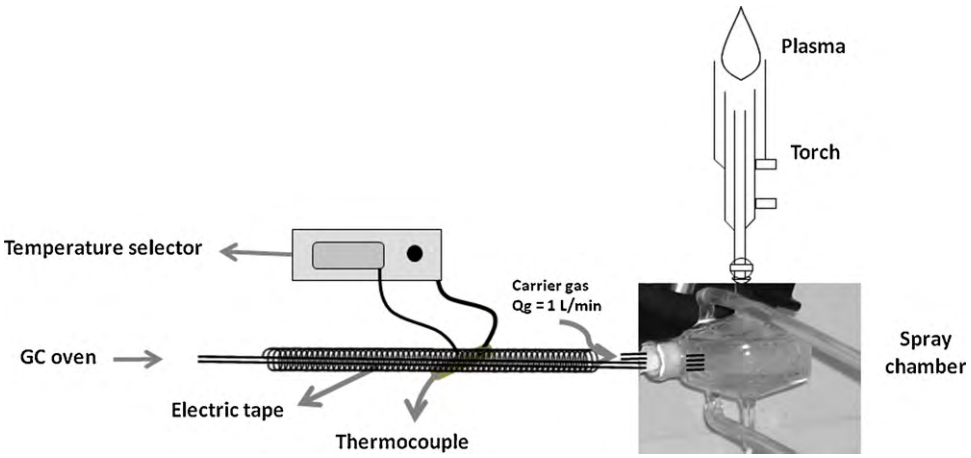

Fig. 4. Scheme of the Thermospray.

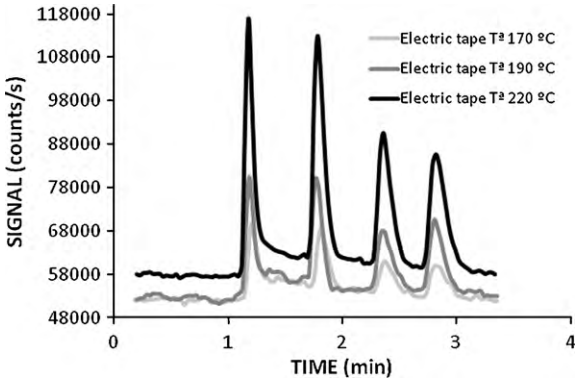

Fig. 5. Chromatograms obtained with HTLC–ICP-AES with the thermospray (without the pneumatic nebulizer) at three different capillary temperatures. Q_l = 1 mL/min; Oven temperature: 150 °C. Compounds: glucose, sucrose, maltose, lactose.

mospray than when the pneumatic concentric nebulizer was used. In fact by comparing Figs. 3 and 5 it was observed that the peak width for sucrose was about 30 and 15 s when using the pneumatic nebulizer and the Thermospray, respectively.

3.3. Comparison between detectors

A comparison between HTLC–ELSD and HTLC–ICP-AES was established in terms of analytical figures of merit. As regards LODs, it was observed that for sucrose, both couplings provided similar values of this magnitude. In the case of the remaining compounds the ELSD provided LODs about two times lower than ICP-AES because of the extremely stable background registered for the former. Anyhow these LODs were low enough to determine the evaluated carbohydrates in food samples.

Table 2
Limits of detection (mg of compound/L) obtained in HTLC–ICP-AES for the different carbohydrates and liquid sample introduction systems.

Spray chamber	Nebulizer	Glucose	Sucrose	Maltose	Lactose
Cyclonic #1	Pneumatic	24.3	26.1	40.5	49.7
	Thermospray	14.8	16.7	29.6	36.3
Cyclonic #2	Pneumatic	59.9	70.3	129.3	144.3
	Thermospray	19.4	24.7	39.6	50.9
Single pass	Pneumatic	37.6	42.9	74.1	84.1
	Thermospray	29.3	35.1	64.8	80.9

The dynamic range (range of concentrations along which there is a linear relationship between the signal and concentration) was also evaluated. In this case, it was found that a curve was obtained when the ELSD was used for quantification. Therefore the analysis was complicated because on the one hand it was necessary to prepare a relatively high number of standards and, on the other, samples had to be diluted, since the detector was quickly saturated. In addition, polynomial regression curves were required. In contrast, there was a good linear relationship between compound concentration and sensitivity in the case of the HTLC–ICP-AES coupling. When using this detector, dynamic range was about two orders of magnitude wider than for the ELSD. This means that it is possible to carry out analysis of samples with a very different content in carbohydrates (*i.e.*, from 50 to about 5000 mg/L).

An obvious advantage of the HTLC–ICP-AES coupling lies in the fact that elements others than carbon can be determined in the same chromatographic run. In the present work, the time elapsed until the first carbon peak was used to obtain the peaks corresponding to the metals. To accomplish this, a second valve was used (Fig. 1). The Zn peak shown in Fig. 3 illustrates this possibility. Note that, because a simultaneous ICP-AES system was used, the peaks for several elements were obtained from a single injection through valve 2.

3.4. Analysis of real samples

To illustrate the applicability of the HTLC–ICP-AES coupling, several food samples were analyzed and the results were compared against those provided by the ELSD system. Table 3 gathers the concentrations obtained for the four carbohydrates determined. By taking into account the confidence intervals, it can be indicated that in general terms the results obtained with both methods were not significantly different for all the compounds and samples considered.

Metals were also determined with ICP-AES. The calibration method employed was the internal standardization to compensate for matrix effects. Therefore, every sample was spiked with 1 mg Y/L. In the case of the determination of carbohydrates this was unnecessary because the matrix introduced into the plasma was always water corresponding to the mobile phase. Table 4 summarizes the data obtained for 5 different elements. In this case it was possible to detect them in the same injection because a simultaneous spectrometer was used that recorded the emission wavelengths characteristic of each element. The results obtained were in fully agreement with the contents declared in the products labels.

Table 3
Concentration of carbohydrates for different food samples[a].

Compound	Milk	Coffee cream	Condensed milk	Candy	Isotonic beverage	Beer
Lactose	44 ± 2	41 ± 3	201 ± 11			
	49 ± 4	47 ± 3	231 ± 79			
Sucrose			363 ± 30	366 ± 14	33 ± 2	
			352 ± 42	370 ± 40	39 ± 6	
Glucose				47 ± 9		
				64 ± 2		
Maltose				312 ± 59		6 ± 0.2
				316 ± 60		6 ± 0.2

The confidence interval is defined as $\pm \frac{ts}{\sqrt{n}}$, where s is the standard deviation ($n = 3$) t is obtained for a 95% confidence level and n is the number of replicates. First row corresponds to the concentrations obtained with the HTLC–ICP-AES hyphenation, whereas second row shows the data for HTLC–ELSD.

[a] The concentration is given in g compound/Kg sample.

Table 4
Metal concentration for different food samples[a].

Element and wavelength (nm)	Milk	Coffee cream	Condensed milk	Candy	Isotonic beverage	Beer
Mg 280.271	117 ± 13	108 ± 10	272 ± 12		1.7 ± 0.2	105 ± 5
Ca 317.933	1254 ± 88	1299 ± 64	2980 ± 156	318 ± 37	74 ± 19	87 ± 13
Na 589.592	366 ± 38	347 ± 18	992 ± 75		204 ± 8	56 ± 1
Zn 206.200			9.5 ± 1.2			
K 766.490	1723 ± 37	1595 ± 162	3905 ± 163	86.9 ± 0.5	30 ± 6	690 ± 37
B 249.677		6 ± 1	7.002 ± 0.625			

The confidence interval is defined as $\pm \frac{ts}{\sqrt{n}}$, where s is the standard deviation ($n = 3$) t is obtained for a 95% confidence level and n is the number of replicates.

[a] The concentration is given in mg element/Kg sample.

4. Conclusions

Nowadays a current trend in HTLC is its association to additional detection systems. The present study explores and demonstrates the feasibility of the HTLC–ICP-AES coupling. Advantages of this hyphenation includes: (i) information of both inorganic and organic components of the same sample is obtained. This is accomplished by correlating carbon emission signal with organic compound concentration and because ICP-AES is a technique implemented for the determination of metals, (ii) shorter analysis time when compared to conventional HPLC–ICP-AES hyphenation due to the use of high temperatures in the separation step. This is especially relevant because of the high cost of operation of a plasma spectrometer. Note that the total argon gas flow rate is close to 20 L/min. With the HTLC–ICP-AES coupling it is possible to determine the organic compounds and metals in approximately the same time as that required for the determination of metals using conventional ICP-AES methodologies. This is a significant improvement with regard to HPLC–ICP-AES provided that the cost of analysis is not increased, (iii) in addition, due to the high temperatures used in the separation step, at the exit of the heating system an aerosol is generated. This fact makes it possible to suppress the nebulizer of the spectrometer and, thus, to improve the shape of chromatographic peaks, (iv) the wide dynamic range exhibited by an ICP-AES system simplifies significantly the sample preparation step when compared to other detection systems such as ELSD.

Nevertheless, the present study has showed that the HTLC–ICP-AES hyphanation has several drawbacks that need to be addressed and overcome namely: (i) high carbon background signals, (ii) problems related to the high temperature reached inside the capillary that could lead to the precipitation and hence retention of metals inside the capillary. This can cause a depression in the analytical signal, on the one hand and a partial capillary blocking, on the other. This problem can be prevented by carefully selecting and optimizing the capillary heating temperature.

The application of the information drawn from this kind of hyphenation to the food industry ranges from quality and fraud control to toxicological issues. Additional fields can be explored such as pharmaceutical or environmental in which changes in the sample composition can be quickly monitored.

Finally, it has been verified that, unlike HTLC–ELSD, the HTLC–ICP-AES coupling could also be applied to the analysis of volatile compounds such as alcohols. The ELSD detector in turn did not provide any signal at all even for high concentrations in compounds such as methanol or ethanol. These studies are being currently performed in our laboratories and will be the subject of an additional contribution.

A common practice in HTLC is to place a long capillary inside the oven *prior* to the column. Obviously, this point compromises the separation efficiency and additional improvements could be studied. One of those would be to use microbore and nanobore columns. With this kind of columns the separation efficiency could increase because of two reasons: (i) the reduced sample dispersion; and, (ii) the improved efficiency of the heating of the mobile phase, what would decrease the temperature gradients in the column. Furthermore, these columns would allow to work at low mobile phase flow rates hence reducing the impact of the solvent on the plasma thermal properties. However, this latter approach could also degrade the sensitivity of the determination because of the reduced analyte mass delivered to the plasma per unit of time. All these approaches are being now evaluated in our laboratory and could be the subject of a future contribution.

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