Optimization of the coupling of high-temperature liquid chromatography and flame ionization detection
Application to the separations of alcohols

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Abstract

The feasibility of coupling high-temperature liquid chromatography (HTLC) to flame ionization detection (FID) has been studied. FID parameter values (hydrogen flow-rate, air flow-rate and FID temperature), typically set in gas chromatography are rarely suitable for liquid chromatography. Best values depend obviously on the water flow rate which is defined depending on both column temperature and column internal diameter. The FID parameters were optimized according to the water flow-rate by means of an experimental design. The potential of the method is shown with some alcohol separations and the value of increasing column temperature while reducing the column diameter is highlighted.
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1. Introduction

The interest of pure water as mobile phase has recently grown [1–8] since pure water allows the coupling of liquid chromatography and more sensitive and universal detectors than the traditional UV detector. However, pure water is a very polar mobile phase at ambient temperature. As a result its eluent strength is usually too low in reversed-phase liquid chromatography (RPLC) although in the past some separations were carried out [9–12] at ambient temperature. A few years ago, Hawthorne et al. [13] increased the temperature of water (up to 200 °C) in order to increase its eluent strength. If we consider the dielectric constants, pure water at 150 °C and a methanol–water (50/50, v/v) mixture at ambient temperature are expected to have the same eluent strength [1]. In addition to a decrease in polarity, elevated temperatures also lead to a decrease in eluent viscosity

and to a concomitant increase in the solute diffusion coefficients. As a result high temperatures allow faster separations without loss of efficiency [14]. From a detection point of view, since FID response is not sensitive to water, HTLC–FID coupling is achievable with hot water as mobile phase.

In the past, many attempts were made to couple classical HPLC and FID. Most of them were based on interfaces [15] leading either to eluent vaporization or to the formation of a stream of small droplets at the outlet of the column. Such a system had two major drawbacks, namely the complexity of the design and the very high probability of vaporizing the sample with the solvent. Various technical improvements, all requiring the elimination of aqueous-organic solvent, were introduced to HPLC–FID coupling at ambient temperature. A drop headspace interface conveyed volatile analytes from eluent to the FID [10,11] or an eluent–jet interface generated a jet of droplets by inducing a sharp temperature gradient at the tip of the introduction capillary [15]. The alternative way to achieve HPLC–FID coupling involves superheated

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water. In this case, the eluent has not to be removed before it enters into the detector. The entire effluent [13,16,17] or just a fraction of it (usually 5–10% divided by a post column split) [18] is introduced into the FID. Numerous parameters, including hydrogen and air flow-rates, FID temperature and water flow-rate are known to have a significant effect on both FID sensitivity and FID noise. Optimized values leading to the best performance in terms of signal/noise ratio were found for hydrogen and air flow-rates with various water flow-rates ranging from $20 \, \mu L \, min^{-1}$ to $200 \, \mu L \, min^{-1}$ [13]. This was done by running successive tests at ambient temperature in the flow injection analysis (FIA) mode at a high fixed FID temperature, $400 \, °C$ [13,16], $350 \, °C$ [18] or $300 \, °C$ [15]. In all these different approaches, the restrictor outlet was placed between 3 cm and 5 cm below the tip of the FID flame jet in order to keep the FID lit. These various studies lead to the following conclusions: first, the optimized hydrogen and air flow rates depend on the water flow-rate, second the best FID response is reached with a hydrogen flow-rate as low as possible to keep the flame lit [13] and third the best performance is obtained at a very high detector temperature. For example, for a water flow-rate of $50 \, \mu L \, min^{-1}$, a good performance was obtained [16] with a FID temperature of $400 \, °C$, a hydrogen flow-rate of $100 \, mL \, min^{-1}$ and an air flow rate of $250 \, mL \, min^{-1}$. All sets of optimized conditions are obviously dependent on the used instrument but some common trends are highlighted throughout these different studies.

The aim of this work is firstly to propose a general methodology for simultaneously optimizing the different FID parameters, including the FID temperature and the water flow-rate and secondly to provide helpful suggestions that could im-prove the performance of HTLC–FID coupling regardless of the used instrument.

2. Experimental

The HTLC–FID system as shown in Fig. 1 consisted of a Shimadzu LC 10 AD vp pump, a Perkin-Elmer Auto System GC used as oven and FID system and either a rheodyne 7520 injection valve (500 nL internal sample loop) or a Cheminert C4 injection valve (50 nL sample internal loop). Chromatograms were processed with AZUR acquisition software (Datalys, France). The acquisition frequency was set at 20 Hz.

A capillary tube with 50 cm length was placed between the column outlet and the FID system, in order to provide an adequate back-pressure for keeping the mobile phase in a liquid state all along the column: a $50 \, \mu m$ I.D. restrictor for columns with internal diameters higher than 0.5 mm, a $20 \, \mu m$ I.D. restrictor for columns with smaller internal diameters.

The mobile phase preheating was achieved by using a tube placed into the oven between the injector and the column inlet. Its required length, determined into a previous work [14] depends on both the oven temperature and the water flow-rate.

2.1. Analytical columns

Three different columns were used: a $100 \, mm \times 1 \, mm$ I.D. ($5 \, \mu m$ Hypercarb, Thermo France) for the optimization of FID parameters, a $100 \, mm \times 0.32 \, mm$ I.D. ($5 \, \mu m$ Hypercarb, Thermo France) and a $100 \, mm \times 0.5 \, mm$ I.D. ($3 \, \mu m$
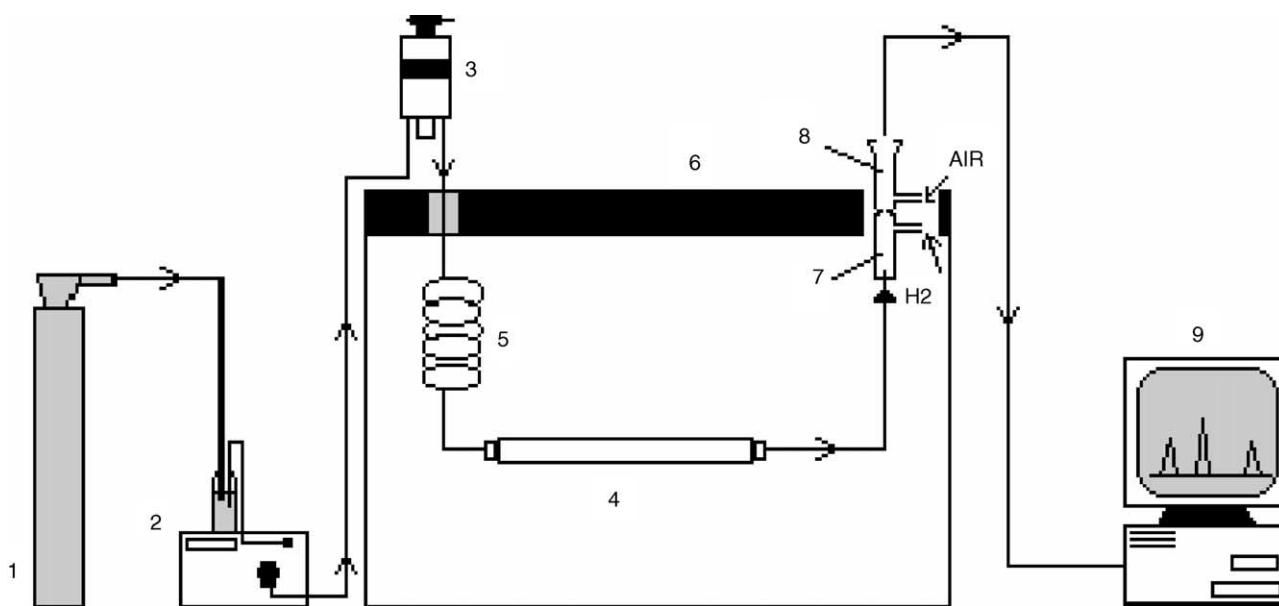

Fig. 1. Scheme of the HTLC–FID system: 1, nitrogen for purging the mobile phase; 2, pump; 3, injection valve; 4, column; 5, preheating coil; 6, GC oven; 7, FID jet; 8, ion collector; 9, computerized data acquisition system.

Zirchrom-PBD, Zirchrom Separations, Anoka, USA) for the applications.

2.2. Materials and reagents

Water was deionised and distilled. Nitrogen was used to purge oxygen dissolved in water. All the solutes were of high purity from Sigma–Aldrich (France). The auxiliary gas, hydrogen and air were of analytical purity. The hydrogen and air flow-rates were adjusted using a digital GC flowmeter (VICI, Switzerland).

3. Results and discussion

3.1. Selection of the parameter space

The optimization of the FID parameters was performed using an experimental design in HTLC conditions with a Hypercarb column (1 mm I.D.) known to be thermally resistant and with methanol as test solute. Numerous parameters may have a significant effect on the performance of the FID system, namely the hydrogen flow-rate, the air flow-rate, the FID temperature, the oven temperature, the length of the restrictor capillary entering into the FID jet and the water flow-rate. This latter parameter is obviously dependent on chromatographic considerations such as the required column linear velocity and the column internal diameter [14]. It was shown by different authors [13,18] that the FID system was not stable in the splitless mode at water flow-rates greater than $150\,\mu\text{L min}^{-1}$. Therefore, we limited our study to water flow rates, F_{water}, ranging from $20\,\mu\text{L min}^{-1}$ to $100\,\mu\text{L min}^{-1}$; this range is totally suitable for column internal diameters ranging from 0.3 mm to 1 mm [14] as shown by Table 1. After several trials, it appeared that whatever the other conditions were, the FID response was not significantly affected by the restrictor position provided of course that the flame could be ignited. Thus, we placed our restrictor 3 cm below the tip of the FID flame jet. This observation is consistent with the results of other authors [18,15,13]. From the results of various experiments performed at different oven temperatures, it was obvious that the oven temperature was not critical. Therefore, it was fixed at 100 °C. Thus, the search for optimum conditions was restricted to the four following parameters: the water flow-rate, F_{water}, the FID temperature, T_{FID}, the hydrogen flow rate, F_{H_2}, and the air flow-rate, F_{air}. F_{air} was studied within a range from $250\,\text{mL min}^{-1}$ to $500\,\text{mL min}^{-1}$, T_{FID} within a range from 180 °C to 340 °C and F_{H_2} within a range varying from $40\,\text{mL min}^{-1}$ to $100\,\text{mL min}^{-1}$. The studied experimental factors and their limits are listed in Table 2. Within this parameter space, conditions exist that either prevent the flame to be ignited or are detrimental to the FID stability. Working zones for these four parameters have been defined by performing numerous trial-and-error experiments within the parameter space. The air flow-rate appeared to have no effect on FID stability inside the studied range. The suitable zones (in dark on the diagrams of Fig. 2) represent the resulting parameter space for the experimental design. The four diagrams are given as a function of both water and hydrogen flow-rates, each corresponding to a given range of FID temperatures.

3.2. Selection of the response function

Since the high-frequency noise is dependent on detector electronics, we first verified that it was also actually dependent on the operating conditions and not the result of a random process. Thirty different sets of conditions were tested for 1 min throughout the parameter space defined above. The high-frequency noise represented the mean value of the 60 differences (one per second) between maximum and minimum amplitudes. An analysis of variance by the Fisher test among the 60 data obtained with the same operational conditions and the 30 data points obtained at the same time established a method effect with a confidence level higher than 99.999%.

In our opinion, the most relevant response function for evaluating the performances of the four mentioned FID parameters, including the water flow-rate, is the peak area to noise ratio, peak area/N, which is directly related to sensitivity/N, N being the high-frequency noise. It is given by

$$\frac{\text{peak area}}{\text{N}} = \frac{\text{sensitivity}}{\text{N}} \times Q_{\text{injected}} \qquad (1)$$

where Q_{injected} is the injected amount of solute.

The S/N ratio was often chosen by other authors. However, the peak height, i.e. the signal, varies with F_{water}, according to

$$\text{peak height} = \frac{\text{sensitivity} \times F_{\text{water}} \times Q_{\text{injected}}}{\sigma_{\text{v}}\sqrt{2\pi}} \qquad (2)$$

where σ_{v} is the peak standard deviation in volume units which also varies with F_{water} since the peak dispersion is affected if the HPLC column is removed (FIA mode) or not. Therefore the S/N is not a very suitable response function if the water flow-rate is taken into account.

Table 1

Optimum linear velocities, u_{opt}, and optimum water flow rates depending on both the column internal diameter and the column temperature (calculated with particle diameter of 5 µm, column porosity of 0.7 and reduced linear velocity of 3) [14]

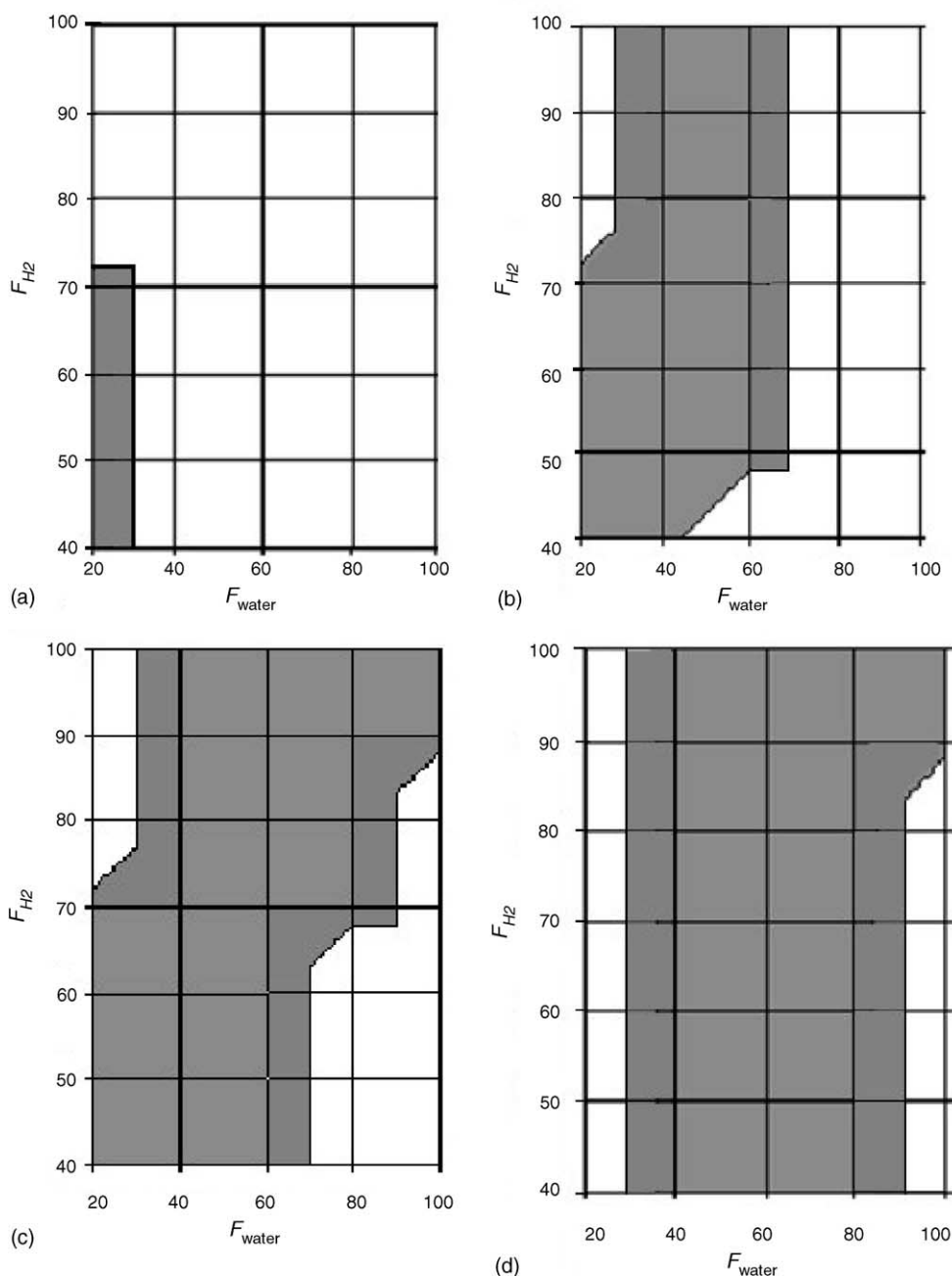|	Temperature		
	30 °C	100 °C	200 °C
Water viscosity (cP)[a]	1	0.3	0.15
u_{opt} (mm s^{-1})	1	4	10
Optimum water flow rates (µL min^{-1})			
4.6 mm I.D.	700	2900	7300
2.1 mm I.D.	145	600	1500
1 mm I.D.	33	130	340
0.5 mm I.D.	8	35	90
0.32 mm I.D.	3	14	35

[a] From [20].

Fig. 2. Working zone (feasibility study): F_{H_2} vs. F_{water} for different zones of FID temperatures: 180–230 °C (a); 230–260 °C (b); 260–320 °C (c); 320–340 °C (d). Working zones are represented in dark.

Peak areas were measured for a 50 ng of methanol injected. The high-frequency noise was measured during 1 min at the beginning of each chromatogram, its value corresponding to the mean difference observed between maximum and minimum amplitudes throughout 1 s period of time.

3.3. Structure of the experimental design

The experimental design is not traditional: All possible combinations of values for the four factors are listed in Table 2, each factor being coded on 5, 6 or 7 levels. Using

Table 2
Studied experimental factors, their limits and levels for the experimental design

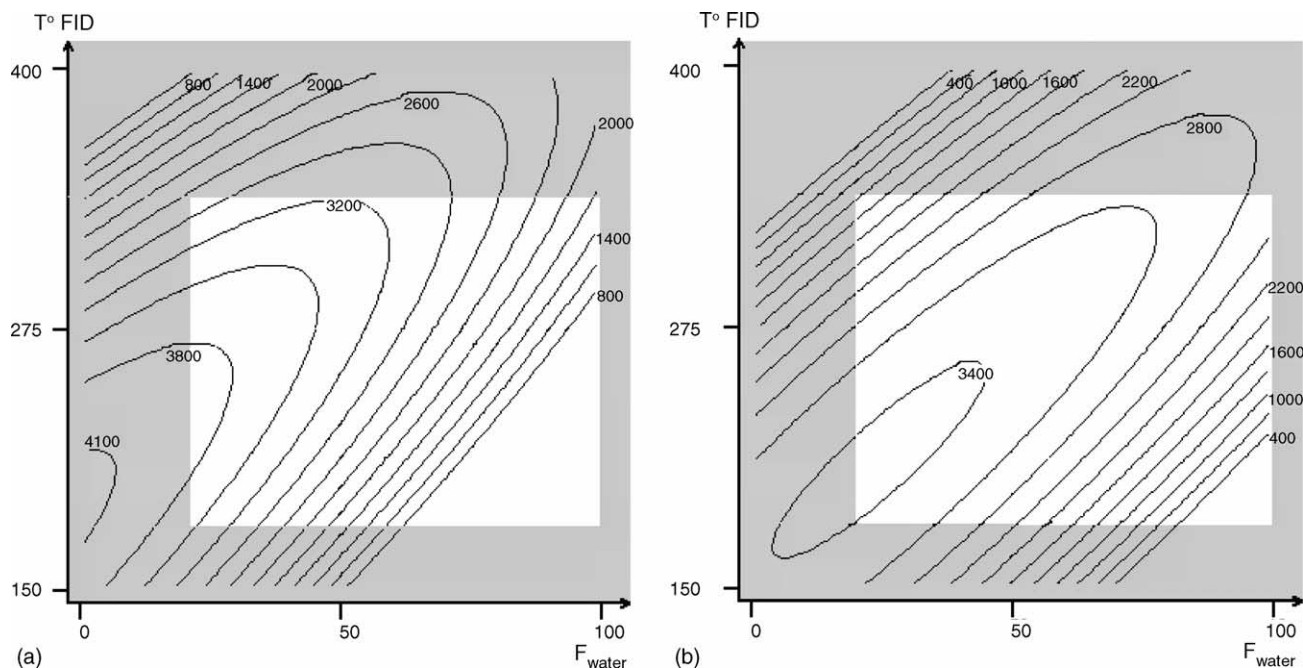| Experimental factors	Unit	Number of levels	Levels
F_{water}	$\mu L\,min^{-1}$	5	20, 40, 60, 80, 100
F_{H_2}	$mL\,min^{-1}$	7	40, 50, 60, 70, 80, 90, 100
F_{air}	$mL\,min^{-1}$	6	250, 300, 350, 400, 450, 500
T_{FID}	°C	7	180, 200, 230, 260, 290, 320, 340

Fig. 3. Isoresponse curves as a function of T_{FID} and F_{water}; $F_{air} = 450\,mL\,min^{-1}$; $F_{H_2} = 40\,mL\,min^{-1}$ (a); $F_{H_2} = 100\,mL\,min^{-1}$ (b).

a full factorial design, the number of required experiments would reach the very high value of 1470 ($5 \times 7 \times 6 \times 7$). It was necessary to drastically reduce this number. The parameter space was first reduced by studying the FID ignition as previously mentioned. The large number of experiments left (678 experiments) was then reduced to 30 using the Nemrod W [19] algorithm of D-optimal construction design. In order to take into account the long-term variability and to obtain reliable results, six identical experimental designs (one per day) were performed. The mean response value was retained. A second order polynomial was used to model the response function. A statistical study of the coefficient values proved that the response was well fitted with a determination coefficient R^2 equal to 0.94.

3.4. FID optimization

This study first showed that F_{air} had no significant effect on the FID response provided it was high enough (350–500 mL min^{-1}). Isoresponse curves as a function of T_{FID} and F_{water} are shown in Fig. 3 for two F_{H_2} values, 40 mL min^{-1} (Fig. 3a) and 100 mL min^{-1} (Fig. 3b), with 450 mL min^{-1} air flow-rate. White zones indicate response values calculated inside the parameter space and dark zones reflect extrapolated values. These results give rise to two relevant comments concerning the best FID working. First, it appears that the optimum FID temperature is highly dependent on the water flow rate. Whereas high FID temperatures (350–400 °C) are appropriate at high water flow rates, lower temperatures (250 °C or less) are required when water flow rates are low (less than 50 μL min^{-1}). For example, with a

water flow-rate of 20 μL min^{-1} the response increases four-fold (Fig. 3a) or six-fold (Fig. 3b) when T_{FID} is changed from 375 °C to 200 °C. Secondly, it appears that response values are higher at low water flow-rates. Indeed, a loss of about 50% is seen when the water flow-rate is increased from 20 μL min^{-1} to 70 μL min^{-1} regardless of the hydrogen flow rate, 40 mL min^{-1} (Fig. 3a) or 100 mL min^{-1} (Fig. 3b). Best values for higher water flow rates are located on the edge of the studied parameter space but the response values undoubtedly tend to decrease when the water flow rate increases.

3.5. Interest of column miniaturization

According to Table 1, miniaturization of the column geometry will provide the best performance for coupling HPLC and FID. Furthermore, since the FID response is mass flow dependent, the signal is proportional to the column linear velocity and as a result is greatly improved at high column temperatures since high column temperatures require higher flow-rates [14]. This is illustrated by Fig. 4: under optimum FID conditions, a S/N ratio of 20:1 is observed for the separation of four alcohols (1 ng injected) at 150 °C and 50 μL min^{-1} with a 0.32 mm I.D. Hypercarb column. Under these conditions and despite the low injected quantity, the detection limit is still greatly exceeded. The value of high column temperature is also underlined by Fig. 5 that shows two separations of five linear alcohols on a Zirchrom PBD column. These separations were carried out under optimum FID conditions with two different sets of chromatographic conditions. The first chromatogram (Fig. 5a) was obtained at ambient temperature
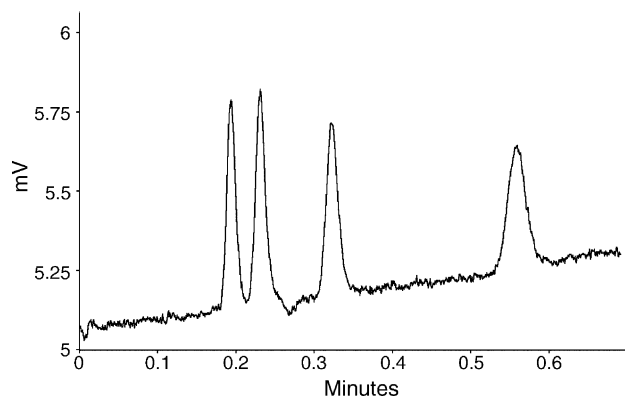
Fig. 4. Separation of 1-propanol, 1-butanol, 1-pentanol and 1-hexanol (1 ng injected) on a Hypercarb column (100 mm × 0.32 mm) at 150 °C and 50 μL min^{-1} water flow rate under optimized FID conditions (F_{H_2} = 100 mL min^{-1}, F_{air} = 450 mL min^{-1} and T_{FID}° = 260 °C).
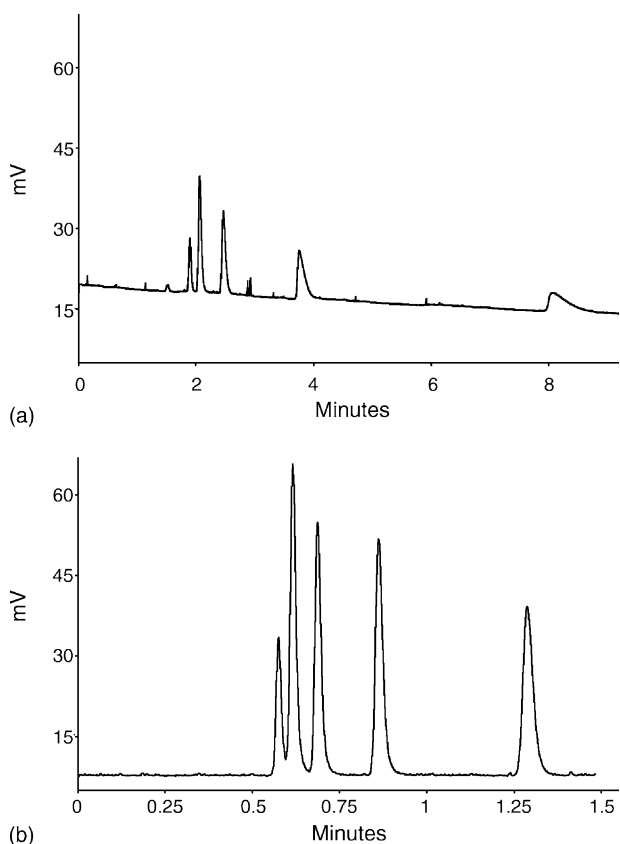

Fig. 5. Separations of a mixture of five linear alcohols (150 ng injected) on a Zirchrom PBD column (100 mm × 0.5 mm) at 30 °C and 10 μL min^{-1} water flow-rate (a); at 120 °C and 30 μL min^{-1} water flow-rate (b); elution order: methanol, 1-propanol, 1-butanol, 1-pentanol and 1-hexanol.

while the second one (Fig. 5b) was obtained at 120 °C. In both cases, the water flow rate was close to the optimum value. As shown elsewhere [14], elevated temperatures have a significant effect on the peak shapes. In addition, the separation of

the five alcohols is obtained in less than 80 s. Indeed, the analysis time is reduced six-fold while the S/N ratio is radically improved.

4. Conclusion

The use of pure water as mobile phase allows the coupling of liquid chromatography to FID. A methodology has been developed that requires a limited number of experiments to optimize the four main FID parameters. This study has shown that the optimized FID parameter values, the FID temperature in particular, are greatly dependent on the water flow rate. Moreover, the FID response is to a large extent improved by decreasing the water flow-rate through decreasing the column internal diameter. Additional improvements in FID response are also expected when the column temperature is increased. As regards analytical performance, a separation of alcohols (1 ng injected) has been obtained at 150 °C with a 20:1 signal-to-noise ratio. Detection limits are then expected to be in the 0.1–1 ng range, comparable to those obtained by GC.

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