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Detection Modes with High Temperature Liquid Chromatography—A Review

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Detection Modes with High Temperature Liquid Chromatography—A Review

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Abstract: Recent developments of thermally resistant stationary phases based on porous graphitic carbon, zircon, titania, or organic polymers allow the use of high temperatures in liquid chromatography (HTLC). These temperatures can reach 200°C without any degradation of the stationary phase. Temperature becomes an additional parameter to improve the performance of classical HPLC. Indeed, at high temperature, viscosity and mobile phase polarity decrease while solute diffusion coefficients increase. Thus, with the same hydro-organic mobile phase, separations 5 to 20 times faster can be obtained without loss of efficiency and the organic modifier content in the mobile phase can be largely reduced. In some cases, a 100% water mobile phase can be used. This allows the hyphenation of HTLC with some detection modes that could not be used in classical LC. These detection modes are more universal, sensitive, or selective than traditional UV detection. This paper reviews the general use of elevated temperatures (up to 200°C) in liquid chromatography. It focuses on detection modes exposing the drawbacks and advantages of several detection modes used in HTLC: Ultraviolet (UV), Flame Ionization Detection (FID), Mass Spectrometry (MS), Evaporative Light-Scattering Detection (ELSD), Inductively Coupled Plasma (ICP), Nuclear Magnetic Resonance Spectrometry (NMR), and Refractive Index detection (RID).

Keywords: High temperature liquid chromatography, detection modes, temperature effects, FID, mass spectrometry, ELSD, RID

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INTRODUCTION

Most analyses in reversed-phase high-performance liquid chromatography (RP-HPLC) are carried out at room temperature, or around (15–40°C). However, in the development of analysis conditions, chromatographists are more and more faced with three constraints: saving time, environment (ecological mobile phases), and sample (sensitive and universal detection).

The classical approach in HPLC method development consists in optimizing the mobile phase after having chosen the appropriate column (stationary phase). In gas chromatography (GC), temperature is the key parameter acting on solute volatility and retention and on separation selectivity. In liquid chromatography (LC), temperature is often considered as a parameter that has little or no effect on separation quality. LC is generally used in isothermal mode at a constant, often regulated, temperature, generally comprised between room temperature and slightly higher (40–60°C at most). However, temperature acts significantly on liquid physico-chemical parameters, such as viscosity, surface tension, or dielectric constant, all parameters that play a considerable role in LC. The reasons explaining why temperature was not a key factor in LC are simple: (i) 10–20° temperature variation produces small retention factor changes compared to the effect of the organic modifier; (ii) bonded silica stationary phases deteriorate rapidly at temperature higher than 60°C with hydro-organic mobile phases.

Recently, thermally stable new stationary phases appeared on the market along with chromatographic systems able to work with temperature. Temperature becomes thus an interesting parameter to improve the quality of chromatographic separations. At the moment, most high temperature liquid chromatography (HTLC) experiments used UV detection. This review will discuss the possibility to use many other detection modes such as NMR, FID, ELSD, and MS, showing that HTLC can improve the compatibility with these detectors.

INTEREST OF WORKING IN HTLC

Effects of temperature on chromatographic separations were mentioned for the first time, approximately a half-century ago by Strain (1). By comparing adsorption of some organic compounds at 20°C and 95°C, he showed that the compounds adsorption and their elution order depended on the temperature. In 1948, LeRosen and Rivet (2) noted a decrease in the solutes retention in adsorption chromatography between 10 and 70°C. In 1953, the same type of study was reported in a temperature range comprised between −50°C and +200°C (3). The results obtained were rather contradictory. Indeed, according to temperature range and solvent nature, retention factors either increased, or decreased, or did not varied at all. On the other hand, in the 1960s, Giddings noted that significant changes in resolution, linked to

variations of retention and selectivity, could be obtained with temperature changes as small as 20°C (4).

Since these first attempts, many works have tried to explain the kinetic as well as thermodynamic phenomena generated by the rise in temperature. All of them are based on the viscosity and polarity reduction with temperature.

KINETIC ASPECT: EFFICIENCY AND OPTIMAL VELOCITY

Effect of Temperature on u_{opt}, h_{opt}, and Asymmetry

The advantages of working in liquid chromatography at high temperature (>100°C) are clear (5–8): raising the temperature causes a 5- to 10-fold reduction in the mobile phase viscosity generating an increase in solutes diffusion coefficients and a reduction in analysis duration (Figure 1). The plots of H versus u also called Van Deemter curves confirm that the optimum reduced linear velocity (u_{opt}) will always increase proportionally to the solute diffusion coefficient, as shown in Figure 2 (8). Also the optimum mobile phase velocity is much broader. At high temperature, working a little bit above or below the optimum u does not change the optimum H as it does at room temperature (Figure 2). It may be easily concluded that HTLC will dramatically improve productivity. Analyses from 5 to 15 times faster without loss of efficiency can be performed at 200°C compared to room temperature. The time gain depends on the decrease in viscosity. In 1988, Antia and Horvath predicted a 20-fold improvement in analysis duration when a

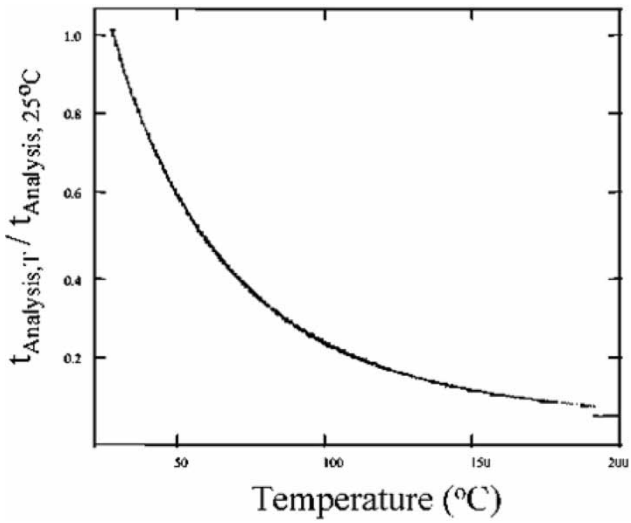

Figure 1. Reduction in analysis retention time due to temperature. Adapted from Ref. 5.

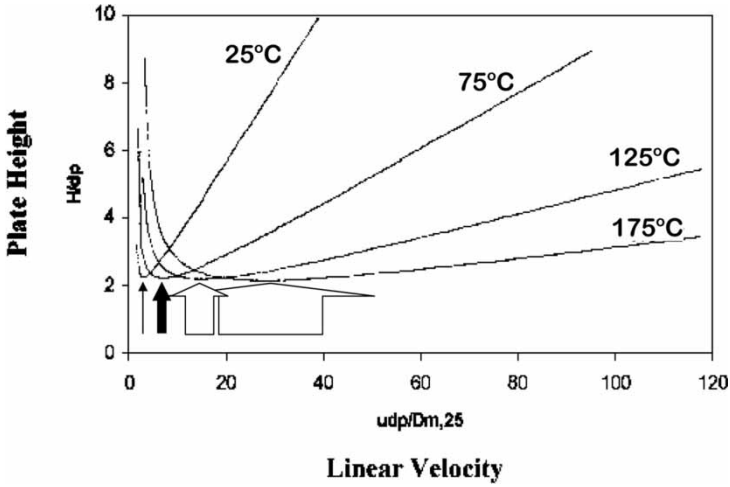

Figure 2. Improvement in the Knox plots with high temperatures. The arrows indicate the optimum linear velocity range much broader at high temperatures. Adapted from Ref. 5.

column is operated at high temperature (150–200°C) rather than at room temperature (5).

Concerning the variation of the reduced plate height, h_{opt}, at the optimum reduced velocity (ν_{opt}), contradictory results showed that h_{opt} decreased with temperature (9–12), increased with temperature (13, 14) or was roughly invariant with temperature (5, 15, 16). On one hand, a decrease of column efficiency (increase in h_{opt}) with temperature was often attributed (15) to extra column band broadening, which of course has a higher effect on low solute retentions corresponding to higher temperatures. It was also attributed to the negative impact of temperature due to thermal gradients that may exist inside the column when the eluent temperature at the column inlet is different from the temperature of the column walls. Both problems can easily be overcome: the first one by minimizing the importance of extra-column dispersion (lower extra-column variance value, higher k value), the second one by using an efficient preheating of the mobile phase (see instrumental section later in this review). On the other hand, some authors have noted an increase on column efficiency (decrease in h_{opt}) with temperature (5). This phenomenon was attributed to slow kinetic systems where an increase in temperature may improve the mass transfer resistance and consequently decrease the C term. In the particular case of zirconia, studied by Carr et al. (17), a very significant increase in the efficiency after exposure to temperature was highlighted. Indeed, the plate number of a 100 × 4.6 mm i.d. Zirchrom PBD column always goes from 3000 at 40°C to approximately 9000 at 40°C but after exposure to a temperature of 200°C. An explanation

of this phenomenon could be disappearing of strongly adsorbed compounds at the stationary phase surface (17).

Lastly, many works showed experimentally that the kinetics of some secondary interactions responsible for peak tailings are probably accelerated at elevated temperature. The fast kinetics eliminates the tailing sometimes observed at ambient temperature (5, 17, 18).

Effect of Temperature on Column Backpressure

There is no exception to the decrease in mobile phase viscosity associated to higher temperatures. However, the optimum flow rate at high temperatures is three to four times higher than that at room temperature (Figure 2). Then for a given couple mobile phase/column and under optimum flow rate conditions, the experimental column backpressure will be 1.6 times higher at 200°C (ratio of 473/298) that at 25°C (8). The use of an outlet restrictor (necessary to work with water at high temperature) adds at least 20 bars to the system pressure drop. The working pressure at 200°C is often twice that observed at room temperature (8, 15). Then, contrary to what is sometimes stated in the literature (19, 20), the use of elevated temperatures does not solve the problem of high column backpressure that low diameter particles generate.

THERMODYNAMIC ASPECTS: SELECTIVITY AND RETENTION

Effect of Temperature on Retention Factor

Many studies were done comparing the effect on retention factors of a change in the mobile phase composition and a change in temperature. It was assumed that a given mobile phase was becoming less polar when hot, thus explaining the observed decrease of solute retention factors. When high temperatures were used in reversed-phase and with the alkylbenzene homologous series, Bowermaster and McNair showed that the effect on retention of an increase of 1% in the methanol mobile phase content was about the same that a 4°C increase in temperature (21). Chen and Horvath obtained similar results with the same alkylbenzene series and acetonitrile-water mobile phases (22). These results were also confirmed in another study by Tran et al. (23).

Consequently, as a system (i.e., column and instrument) is optimized for speed, and the eluent temperature is raised, the analyte retention decreases. It may become too low ($k < 1$) to be of practical interest since the resolution may drop dramatically at low ks. In this case and to keep reasonable k values as the temperature is raised during the optimization step, some authors recommend decreasing the volume fraction of the organic solvent (8, 15).

Effect of Temperature on Selectivity

As soon as the 1960s, Giddings described the role of the temperature (4): in his study, significant changes in selectivity could be obtained with an increase in temperature of only 20°C. Recently, Carr et al. published many works on the effect of temperature on selectivity using original zirconia-based columns (9, 24, 25). They showed that observed selectivity changes with temperature depended mainly on the nature of the solutes. They are due to differences in solute-stationary phase (24, 26). When the classical alkylbenzene series is used, the ΔHs are similar and there is little selectivity changes observed. At constant mobile phase composition, raising the temperature tend to decrease selectivity. Conversely, when solutes with varied structures are used, significant selectivity variation is observed. For example, Heinisch et al. (27) demonstrated the important effect of temperature in the isocratic separation of various PAHs on silica-based stationary phases.

In fact, the effects of mobile phase composition and temperature are often complementary and a simultaneous optimization of temperature and composition could become a more convenient means to control retention times and improve selectivity (28, 29). Snyder et al. published many papers showing the utility and the interest to optimize simultaneously temperature and mobile phase composition in gradient and isocratic elution mode (30–44). Then temperature becomes an essential parameter to modulate the selectivity of the most difficult mixtures.

INSTRUMENTAL CONSIDERATIONS—HOW TO WORK CORRECTLY IN HTLC?

High temperatures are not commonly used in liquid chromatography, because there are some stringent requirements and implementation problems. First, the design of a chromatographic system that minimizes thermal mismatch broadening in high-temperature high speed liquid chromatography is necessary. Secondly, the traditional silica-based stationary phases simply are not sufficiently stable at the temperatures needed to achieve the 20-fold improvement in analysis duration. In fact, classical reversed-phase silica columns allow only a relatively small increase (30–40°C) in temperature over ambient.

Preheating

As shown in Figure 3, temperature control of the mobile phase entering in the column should not be neglected since temperature variations of the mobile phase along the column can cancel all possible benefit in efficiency generated by the use of high temperatures (15, 45).

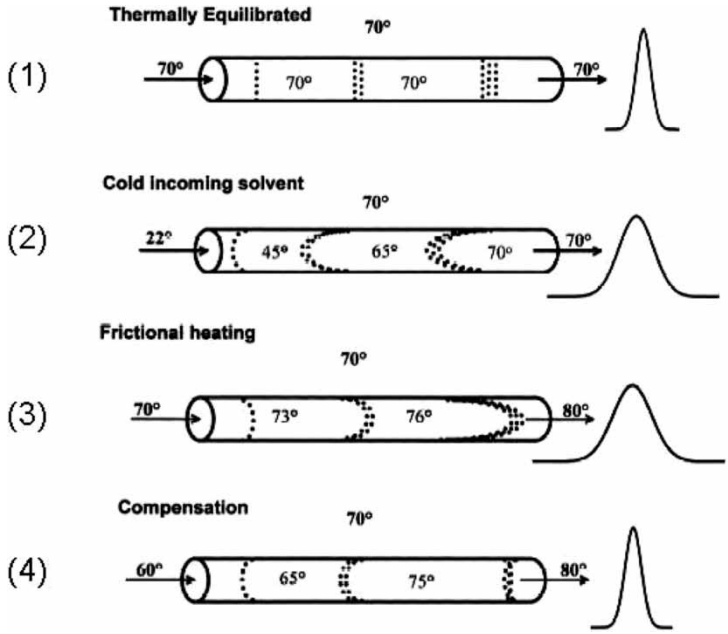

Figure 3. Band broadening due to thermal effects. (1) Ideal case, no thermal effects; (2) effect of incoming mobile phase that is at a much lower temperature than the column; (3) effect of frictional heating; (4) combined effects of cool incoming mobile phase and frictional heating. An oven temperature of 70°C is assumed. Numbers shown inside column suggest plausible solvent temperatures at column center. Adapted from Ref. 46.

In HTLC, many sources of dispersion can come from a bad temperature control throughout the chromatographic system.

- In absence of preheating, the mobile phase entering in the column is not at the oven temperature, involving radial and axial temperature gradients (46, 47).
- The high mobile phase flow rate passing through the column, filled up with stationary phase particles, generates heat. Viscous heat dissipation is clearly a problem if temperature is low and pressure is high. However at high temperatures and high pressures, viscous heat dissipation should be a minor concern (45, 48–50).

In the early 1980s, Poppe et al. highlighted the necessity to preheat the mobile phase before it enters the chromatographic column (51, 52). The role of the preheating tube, placed between the injection valve outlet and the column inlet, is to limit temperature variations inside the column, especially with macrobore columns.

One of the major problems in HTLC lies in the control of the temperature throughout the chromatographic column and especially at the column inlet. Many concepts were proposed to carry out separations in isothermal mode; they are listed in a recent review (53). They consist either of using a relatively long tubing length placed inside an oven between the injector and the column inlet (15, 46), or a short tubing length in contact with a powerful heating block (47, 54).

In 1993, Schneider patented a countercurrent heat exchanger in which heat is transferred from the mobile phase leaving the column, to the mobile phase entering it (55). This heat exchanger was successfully used by Carr and coll (17, 56). A similar configuration was also proposed by Plant and Dumas (57).

The optimal length of preheating tube to use according to the flow rate and temperature conditions was the subject of intense work (8, 15, 45, 46, 58). Indeed, a short tube length does not produce band broadening but may not be able to raise the mobile phase temperature correctly. A long tubing length will heat the mobile phase correctly but it dilutes the injected sample. In a study realized by Wolcott et al. (45) various temperature-related problems which could result in a failure of method transfer for nonambient RP-LC methods were examined. A thermocouple was introduced in the chromatographic system to evaluate the mobile phase temperature all along the column, to check the preheating quality and to ensure method transferability. In the case where low internal diameters columns (500 μm i.d. or less) were used, preheating was not a problem since ten centimetres of tubing were enough (8). This is the usual length needed to link the injector valve to the column inlet. In this case, there will be no problem of band broadening or back pressure due to the preheating tube. The use of a miniaturized LC system with small bore or capillary column is then the easiest way to work in HTLC.

Heating Mode

The column temperature must be controlled correctly because small variations have a large effect on retention and/or selectivity. Early on, Locke and Martire insisted on this need (59). In the 1970s the opinions diverged on the precision of the temperature control. According to Kirkland, a very precise control of the temperature was not necessary since the changes involved on retention factors by a 1°C variation were less than 1% (60). On the other hand, Maggs showed that it was necessary to control the temperature at ± 0.2°C to obtain a good repeatability on retention factors ($<1\%$) (61). Kikta and Stange implemented in 1977 a heating system allowing to thermostat columns up to 85°C with a precision of ± 0.1°C (62). This low cost system consists in covering the chromatographic column with an insulating sheath of Tygon and placing inside the resistors and thermocouple probe to control temperature changes. This equipment was used to separate

4 alkylphenones in less than 30 min using a temperature gradient between 40 and 66°C (62).

In 1996, half of HPLC systems did not have any way to thermostat the column (63). Today most commercial systems propose systems to regulate the column temperature up to 60°C and sometimes 90°C. It remains still difficult to find systems allowing to reach very high temperatures. To work at 150 or 200°C, it is necessary to use "homemade systems" either modifying a traditional GC oven (21) or with an oil bath (15).

In 2003, a patent was filled for the first complete equipment allowing the use of temperatures higher than 100°C (64). This apparatus is composed of a heat exchanger allowing heating the mobile phase, of an oven allowing maintaining the column in a completely adiabatic environment and finally a second heat exchanger whose role is to cool the mobile phase before it enters the detector. In this system, the heating unit is powerful enough to heat at 200 or 250°C the mobile phase flowing at $5\,mL \cdot min^{-1}$. Temperature gradients were carried out using this new equipment on carbon, zirconia and polymer-based columns (65).

Stable Stationary Phases

During last ten years, chromatographers were interested in acting on mobile phase parameters such as composition, pH, or ionic strength to improve separations. But temperature received less attention. This was due to the thermal instability of some solutes and stationary phases. Thermal instability of the solutes may not be an insuperable problem since the solutes are exposed to high temperature only for a very short period of time in ultra fast separations and/or may not have enough time to degrade (57). However, the solute stability problem must be looked at very critically since the pharmaceutical industry is still highly septic about the fact that solutes do not degrade at high temperatures. When working with real pharmaceutical samples often minor impurities have to be looked at and possible degradation of analytes cannot be ignored. The problem, for the moment, is that not enough data is available linking the rate of solute degradation to eluent temperature and the time spent by the solute in the stationary phase.

For stationary phases, thermal stability is however essential. The stationary phase is indeed continuously exposed to high temperatures (66). For several years, in the commercial literature, temperature was just another parameter used to promote the sales of new thermally stable stationary phases. Columns containing zirconia (9, 15, 17, 24, 67), titania, carbon (68), or organic polymers (69, 70) were developed. These columns are stable at high temperatures. For example, in 2000, Wilson used a Hypercarb column at 225°C, polymeric stationary phases at 210°C and zirconia-based columns at 190°C (69). Something more surprising in this article is the use of silica-based columns at a temperature of 160°C, although it is specified that these

columns are not stable at this temperature. Other examples of silica-based phases used at high temperature are given in the literature: a Nucleosil C_{18} column allowed the separation of anilines, phenols and alkylbenzenes at 200°C (71). A Spherisorb ODS2 column was also used at 140°C for the separation of phenolics compounds (72). In this last case, the prolonged use of this phase at high temperatures involved a reduction in retentions, suggesting a stability problem with this type of material.

Stability of the bonded phase and the support are important, but the column housing and its materials may also be a limiting factor. The presence of polyetheretherketone (PEEK) components within the column body, for example, will limit the usable temperature to approximately 110°C, as its pressure resistance drops dramatically above this point.

Silica-Based Stationary Phases

It is known that silica dissolves quickly in heated water containing mobile phases. Separations on silica-based stationary phases were always done at room temperature or slightly above. The surface silanols are responsible for silica dissolution either in high pH or in hot solution. Then, screening the surface silanols is the solution to increase the thermal as well as basic stability of silica. Embedded or hybrid silica-based stationary phases that were developed to work at elevated pHs are thus generally also able to work at temperatures up to 100–120°C.

The thermal stability of silica-based of stationary phases was not tested in depth. In a recent series of papers, a "high pH" stationary phase was tested at room temperature passing 1100 dead volume of alkaline mobile phase at pH 10 (73, 74). The efficiency obtained on these poly (methyl-octyl-siloxane) and poly (methyl-octadecyl-siloxane) columns remained constant. However, the efficiency obtained with the same columns deteriorated rapidly after only 450 volumes of a pH 8.4 mobile phase was passed at 60°C (74). Taking into account the reproducibility of retention factors and number of plates, He and Yang (75) studied the thermal stability of three types of silica-based columns with a 100% water mobile phase. They found that the Zorbax RX-C_8 and Nucleosil C_{18} columns could maintain their properties at 100°C after the passage of 6000 volumes and 8000 volumes of water, respectively. Most silica-based materials very rapidly degrade at temperatures above 120°C and only few datas are available concerning the long-term stability of these materials. Nevertheless, new modifications of silica stationary phases (e.g., endcapped phases etc.) will improve long-term stability.

Carbon-Based Stationary Phases

Graphitic carbon is much more hydrophobic than the other existing stationary phases. For many solutes, graphite stationary phases are highly retentive.

Consequently, a high percentage of organic modifier is required in the mobile phase to elute solutes in a reasonable amount of time. This percentage is 20 to 40% higher than what was needed on silica-based stationary phases.

In addition to strong retention, the kinetics of solute-stationary phase interactions on porous graphite varies widely, producing poor peak shape and/or efficiency for some solutes. However graphite stationary phases are thermally very stable. They can work up to 225°C (69). Porous graphite thermal stability was studied by Yamaki (76). His work describes a peptide and amino acid separation at 160°C with a carbon based stationary phase. The column was stable during the time needed to pass more than 3000 column volumes at this temperature.

Organic Polymer Stationary Phases

To date, most of the studies done in HTLC were carried out with polystyrene–divinyl benzene based stationary phases. Indeed, this type of highly reticulated polymer remains stable at high temperature even with a 100% water mobile phase. The thermal stability of a PRP-1 stationary phase was studied in 2 steps (75): the column was initially tested at 100°C during 11,000 column volumes and it remained stable, even after being heated at 150°C during 9000 more column volumes. The stability was evaluated in term of retention and efficiency. A PRP-1 column was also used successfully with pressured water at 225°C (77). The problem with this kind of material is similar to that mentioned for porous graphite: the kinetics is often slow producing a low efficiency, this largely depending on the nature of the solutes.

Zirconia-Based Stationary Phases

Zirconia, ZrO_2, is much less water soluble than silica. Alkyl-bonded zirconia stationary phases have great thermal stability. The manufacturer (ZirChrom) recommends that most columns be used at a maximum temperature of only 150°C. Most thermal stability studies of zirconia based stationary phases were done by Carr et al. A preliminary study showed that this type of column remained stable after 1300 column volumes passed at 200°C (17). It was surprisingly noted than the column efficiency often improved after an exposure to high temperatures (150–200°C). Indeed, they noted a 300% increase in plate count after elution of 1300 column volumes at 200°C. Recently, it was shown that the zirconia phases did not lose their properties after 5000 column volumes were passed at 195°C. Although these columns are generally used between 100 and 200°C, they were tested in water as hot as at 370°C (78). One problem to mention with this kind of material is that some bleeding was observed when temperature gradients were performed. This bleeding generated repeatability problems, particularly in quantitative

measurements (65). However, more data needs to be generated to evaluate bleeding effects fully.

The thermal stability of zirconia-based stationary phases is very satisfying when considering their chromatographic properties and robustness and comparing with silica-based stationary phases. Zirconia-based stationary phases are on the way to become the most used stationary phases when working at very high temperatures.

TEMPERATURE GRADIENTS AND MICROBORE COLUMNS

Temperature Gradient Rather Than Isothermal Separations

Complex mixtures are commonly separated at room temperature using gradients of mobile phase composition. A temperature programming can also be interesting. Temperature gradients consist in gradually increasing temperature of the column. It is the usual way to work in GC where the solute volatility increases and then, the solute retention decreases with the temperature. In LC, up to recent time, temperature gradients were ignored because solute retention and selectivity are less sensitive to changes of temperature. However, for a number of applications, a temperature programming can bring improvements in terms of methods simplification, changes of selectivity or compatibility with some detectors.

Studying theoretically all possible gradients: mobile phase composition, stationary phase composition (connecting in series columns with different selectivities), temperature, and flow rate variation, Snyder showed in 1970 that the first parameter (mobile phase composition) was the best one to vary to obtain a maximum resolution per unit of time (79). The worse resolution per unit of time is obtained in isocratic elution mode. Some applications in normal phase mode confirmed these theoretical predictions. Many other authors have also been interested, in temperature gradients. They modeled solute retentions and peaks capacity according to the gradient duration, its slope and other conditions (46, 76, 80, 81).

The Need of Reduced Column Diameters

The use of temperature gradients started 40 years ago and the need to reduce column diameters was immediately seen.

- The first attempt in temperature gradient was described in the middle of the sixties (82). A small bore 12 mm internal diameter column was used between 15 and 70°C, in a normal phase mode. A separation of 4 compounds was done between 20 and 90°C in 10 h with a temperature change of 7°C per h. In 1970, a temperature gradient, also using the normal phase mode was carried out with a slope of 15°C per hour and a

final temperature of 100°C (79). In the early 1980s a 20–80°C temperature programming was used to separate several acid disulphonic anthraquinone geometrical isomers on a 4.6 mm i.d. column (83). It was even tried to use inverse temperature gradients: cooling from 25°C to −60°C with a slope of $-5°C \cdot min^{-1}$ allowed some improvements in the separation of a steroid sample (84). Analysis duration was the driving interest in the use of a temperature gradient until 100°C in the separation of oligonucleotides on a 2 mm i.d. column (85).

- In the mid-1980s, association between temperature gradients and microbore columns became common. Hirata and Sumiya proposed fatty acids separations with temperature gradients and 0.3 mm i.d silica or glass capillary columns (86). The gradient slope was 1°C/min between 20°C and 200°C. Mc Nair and Bowermaster evaluated the potential of the temperature programming (until 100°C) on 1 mm i.d. C_{18} microbore columns and studied the chromatographic behavior of ten alkylbenzene homologues in the broad temperature range of −60°C to 180°C (21, 87, 88). Jinno et al. (89) and Takeuchi et al. (90) also used C_{18} microcolumns with i.d. <1 mm to separate PAHs as well as alkylbenzene homologues using temperature programming. They concluded that the advantages of the use of temperature gradients were comparable in LC and GC. The advantages were: reduction in analysis time and increase in sensitivity and peak capacity. Trivially, low column diameters offer a lower resistance to thermal transfers compared to the classical column diameters. A 4.6 mm i.d. column contains 20 times more mobile phase than a 1 mm i.d. microbore column, making its thermal equilibration much longer with likely radial temperature gradients.
- Some recent applications were obtained on filled capillary columns. The filling limits radial temperature gradients and the associated losses of efficiency. Temperature gradients up to 80°C were realized on a 180 μm i.d. column filled with silica and purely aqueous mobile phases (22) and up to 150°C on a 320 μm i.d. column with hydro-organic mobile phases (91).

Macrobore columns were found not compatible with good temperature programming (65). In a recent paper, Greibrokk et al. tested various columns with diameters comprised between 0.32 and 4.6 mm and temperature gradients with slopes ranging from 1 to $20°C \cdot min^{-1}$ (92). They showed that the column diameter was not a limiting factor in temperature gradient between 30 and 90°C. However, the most benefits of high temperature appear at 150°C and above. There, only low diameter columns could be used. They also demonstrated significant advantages of temperature programming over composition gradient through many separations on capillary columns (93–100). Most separations were carried out on C_{18} silica-based stationary phases up to 120°C with high molecular mass solutes. Some surprising results were found: inverse temperature gradients are better in the separation of polyethylene glycols (93, 94). It seems that large molecules have a retention increasing

with temperature. The best way to reduce analysis time maintaining a high selectivity was to start at a high temperature and work with a negative gradient: for example, a gradient from 80°C to 25°C with a slope of $-1.5°C/min$ (94).

Houdiere et al. (101) evaluated the effects of simultaneous temperature and flow rate programming, on chromatographic performances. This approach is logical since the optimal flow rate increases with temperature. It becomes thus possible to decrease the retention of most retained compounds keeping the efficiency high. This type of double gradient was carried out on capillary and microbore columns. It allowed the separation of a mixture of 10 aromatic solutes with a slope of $6°C \cdot min^{-1}$, the maximum flow and temperature being $3\,mL \cdot min^{-1}$ and 130°C. These two gradients made simultaneously allowed a reduction of 30% over the analysis time compared to the only temperature gradient.

HTLC SEPARATIONS SORTED BY DETECTION MODES

UV detection is the dominant detection mode in HPLC due to its versatility and cost. Most early attempts to study the effect of temperature in liquid chromatography were done detecting with a UV detector. In the progress of the knowledge of the use of high temperatures in LC, it was found that low organic solvent mobile phases and even 100% water mobile phases could be used in some cases. This opened the way for the use of the FID detector and other detection modes.

HTLC-UV (Ultraviolet)

Selected Applications with UV Detection in Isothermal Mode

Recently, Yan et al. (15) described the new concept of UFHTLC for "Ultra Fast High Temperature Liquid Chromatography." The authors used an experimental setup allowing working with flow rate and temperatures as high as $15\,mL \cdot min^{-1}$ and 150°C. As shown in Figure 4, a separation of 5 alkylphenones was carried out in 20 s instead of 20 min at ambient temperature. It should be noted that, at $15\,mL \cdot min^{-1}$ and 150°C, the pressure drop was $350\,kg \cdot cm^{-2}$ (5000 p.s.i.) with a conventional 50×4.6 mm column with 2.5 μm small particles. Mao and Carr invented the concept of "thermally tuned tandem chromatography" (T^3C) (102, 103). The principle of T^3C is to connect, in series, two different columns as shown in Figure 5. Each column is placed in an oven that can be set at a different temperature. A complex separation of 10 triazines was thus carried out in 10 min at $3\,mL \cdot min^{-1}$ by coupling an ODS silica column set at 30°C with a zirconia-based column set at 125°C (103). A separation of 12 carbamates could also be done in 8 min. The ODS column was set at 39°C while the zirconia

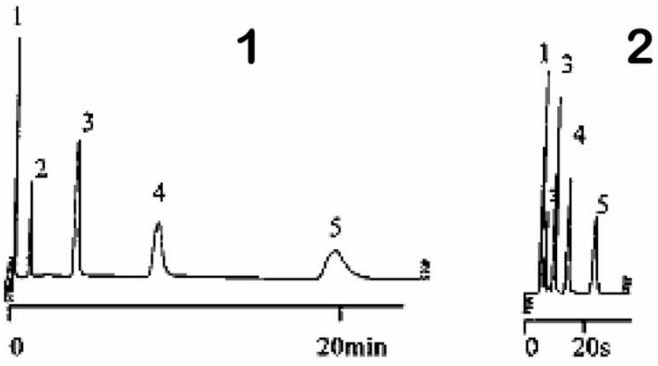

Figure 4. Chromatograms showing the effect of temperature on the separation of alkylphenones. Experimental conditions: mobile phase 1, 30% ACN (v/v) and flow rate is 4 mL/min at 25°C; mobile phase 2, 25% ACN (v/v) and flow rate is 15 mL/min at 150°C. Peaks: 1, acetophenone; 2, octanophenone; 3, decanophenone; 4, dodecanophenone; 5, tetradecanophenone. Adapted from Ref. 15.

column was set at 89°C (103). In this method, flow rate may be a problem: it is not possible to adjust independently the flow rate on both columns, and the optimal flow rate is not the same at 30°C and 125°C. Also the pressure drop on the system is rapidly elevated since there is addition of the back pressures on the two columns serially connected.

UV Absorbing Basic Compounds

As shown by different authors, the pKa of basic compounds decreases when the temperature increases and so does the compound positive ionization. This phenomenon allows an easier elution of basic compounds in their molecular form without the need to work at extreme pHs (104–106).

Low UV Wavelength with 100% Water Mobile Phase

Another particularly interesting application of HTLC is the use of pure water as a mobile phase, much less expensive and polluting that the traditional organic solvents. Many works (58, 71, 72, 75, 107–110) using 100% water as mobile phase showed the feasibility and the interests of such a technique, when, of course, the stationary phases and the solutes resist to very hot aggressive water. Smith and Burgess used since 1996, pure water at 200°C to separate phenols, amides, esters, or barbiturates on a polystyrene divinylbenzene

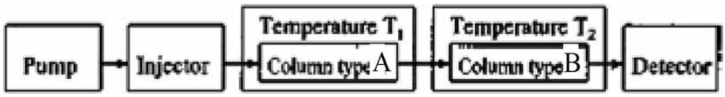

Figure 5. Block diagram of the T^3C system. Adapted from Ref. 103.

phase (PS-DVB) (72, 107, 109). Fields et al. studied the retention of testosterone with a purely aqueous mobile phase on a zirconia-based column coated with polybutadiene (58). The high UV transparency of water allows working with low 200 nm wavelengths, where most of the compounds have a maximum absorbance (109). It is thus possible to improve sensitivity by working at wavelength under 200 nm with clean samples. In the case of real samples, the interest of working below 200 nm is limited since most matrix components will also highly absorb the very short UV wavelength hiding the analyte.

HTLC-FID (Flame Ionization Detection)

The FID operation principle was described for the first time in 1958 (111, 112). This detector was quickly optimized and it is now the most common, sensitive and reliable GC detector. Since FID detects all carbon containing molecules, it is almost universal. However, in both normal and reversed phase modes the LC mobile phases contain organic molecules that will blind the FID. Many attempts were done to couple it with LC. All were based on mobile phase nebulization and evaporation of the small droplets. In the 1980s, LC-FID prototypes were using a moving belt (113), wire (114), chain (115), or rotating disc (116), with the column effluent being applied on the moving substrate and evaporating the mobile phase. The analytes should be nonvolatile to be seen by the FID. Tsuda et al. (117) and Veening et al. (118) combined μLC and FID via a moving-wire interface. All these systems had a complex design and the loss of volatile analytes during solvent evaporation did not make them very useful. Krejci and coworkers (119) described the first direct on-line coupling of capillary LC columns (5–15 μm i.d.) with modified FID using 100% water at a $60\,pL \cdot min^{-1}$ flow rate. These last years, many technical improvements were proposed for LC-FID coupling. But they all eliminate the organic solvent before detection limiting the usefulness (120–122).

Use of Pure Water as a Mobile Phase

The LC-FID coupling implies to work with 100% water mobile phases (5, 9, 15, 69, 72, 107, 109). A major problem is that water is a very weak mobile phase in RPLC at room temperature. However, for selected compounds and with appropriate stationary phases, separations could be realized with pure water at ambient temperature (110, 111, 123, 124). The stationary phases were nonporous and large diameter silica particles involving a reduction in the volume phase ratio compared to classical columns. This type of nonporous phases generates weaker retention factors for a given mobile phase composition (120, 124). Other stationary phases

used contained trifluoropropylsiloxane (124) or cyano propyl groups (121). They are more polar than a C_{18} stationary phase, producing lower retention factors with 100% water mobile phase.

To solve the problem of eluent strength, Hawthorne and colleagues used the fact that water polarity decreases as temperature increases and they worked up to 200°C (6, 125, 126). As an example, the polarity of water at 150°C, in term of permitivity, is equivalent to that of a mixture of water–methanol 50 : 50% v/v at ambient temperature ($\varepsilon = 44$) (107). These two mobile phases, at their respective temperatures, have nearly identical eluent strength and consequently lead to similar retention factors. Since 100% water does not contain any organic molecule, the direct coupling with FID seems possible (77, 125, 127). It was sometime needed to split high flow rates, sending only 5 to 10% in the FID (128).

Even if it is well known that bacterial growth can be an issue when working with 100% water mobile phase over a long period, we do not observe such problems, and it does not appear necessary in our case to clean or regenerate chromatographic column. This behavior was attributed to the fact that pure water was used at temperatures higher than 150°C. At these temperatures, sterilization occurred killing all living material that could be present at ambient temperature.

Optimization of FID Conditions

The flame in the FID is obtained by burning air + hydrogen. The column outlet is added in the flame. It should not blow it off like a strong water steam jet could do. The parameters to adjust in the LC-FID coupling include: hydrogen and air flow rates, FID temperature, water mobile phase flow rate and capillary length entering into the FID. Some authors made trial-and-error adjustments (122, 126). Miller et al. set arbitrarily the FID temperature at 400°C; they tried to find out suitable conditions for hydrogen and air flow rates by successive tests according to the water eluent flow rate (126). They found out that the hydrogen flow rate was the key parameter. Hooijschuur et al. choose to optimize the FID temperature between 150°C and 300°C (122). The best response was obtained at the maximum 300°C, for hydrogen and air flow rates fixed and equal respectively to $55\,\text{mL}\cdot\text{min}^{-1}$ and $1000\,\text{mL}\cdot\text{min}^{-1}$.

Guillarme et al. selected a chemometric approach (129). Working with four parameters: hydrogen and air flow-rates, detector temperature and length of the capillary tube entering into the FID jet, several experiments were done following an experimental design. The isoresponse curves are presented by Figure 6. It appeared that the FID sensitivity was quite independent on the position of the capillary tube provided that it allowed the flame to be ignited, thus confirming the results obtained by Yang et al. (128). It was confirmed that air flow rate had no significant effect on the FID response provided it was high enough. The optimum FID temperature is highly depending on the water flow rate. Whereas high FID

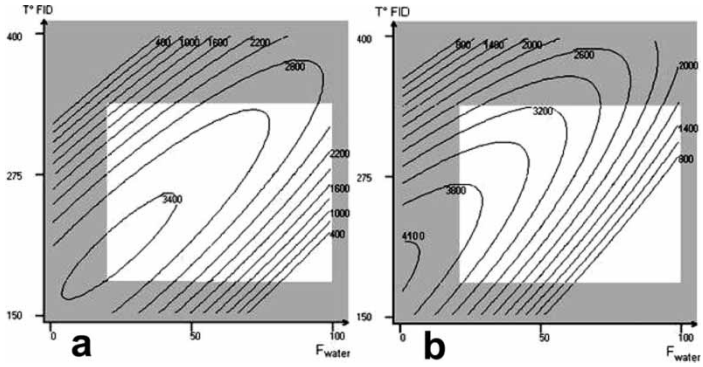

Figure 6. Isoresponse curves as a function of T_{FID} and F_{water}; $F_{air} = 450\,mL \cdot min^{-1}$; (a) $F_{H2} = 40\,mL \cdot min^{-1}$; (b) $F_{H2} = 100\,mL \cdot min^{-1}$. Adapted from Ref. 131.

temperatures (350 to 400°C) are appropriate to high water flow rates, lower temperatures (250°C or less) are required to obtain maximum sensitivity when water flow rates are low (less than $50\,\mu L \cdot min^{-1}$) (129). The limits of detection (LOD) obtained after optimization were compared to that of other works. Nakajima et al. had LODs of 26 to 57 ng for alcohols (130). Then 10 times lower LODs (few ng) were obtained under optimized conditions (Figure 7).

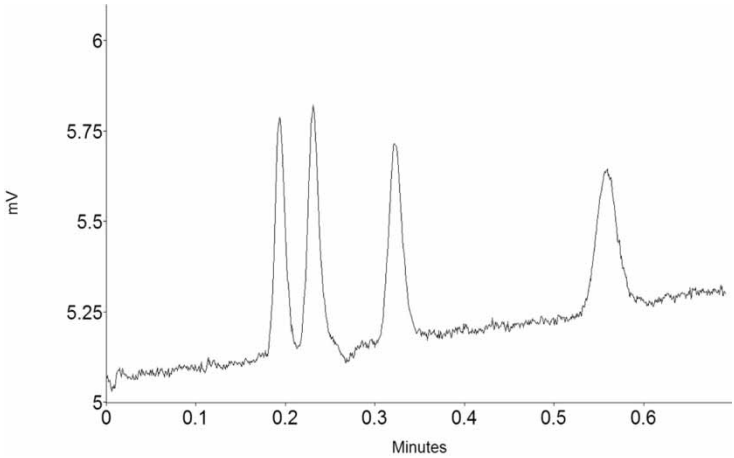

Figure 7. Separation of 1-propanol, 1-butanol, 1-pentanol and 1-hexanol (1 ng injected) on a Hypercarb column (100 mm × 0.32 mm i.d.) at 150°C and $50\,\mu L \cdot min^{-1}$ water flow rate under optimized FID conditions ($F_{H2} = 100\,mL \cdot min^{-1}$, $F_{air} = 450\,mL \cdot min^{-1}$ and $T^{\circ}_{FID} = 260°C$). Adapted from Ref. 131.

Applications

Most of the applications carried out in HTLC-FID are separations of linear and ramified non-UV-absorbing alcohols between C_1 and C_6 (77, 122, 125, 129, 131) generally performed on carbon-based stationary phases, polymeric ones or more recently on zirconia ones (129). The isothermal elution mode (77, 122, 125, 129) is generally chosen for this family of solute, although some separations were done in temperature programming (77, 125). A particularly interesting application of the alcohols separation is the measurement of ethanol in alcoholic beverages such as wine, whisky or sake by Nakajima et al. (130). Results obtained in HTLC-FID were compared with those obtained in GC-FID or GC-MS and excellent correlation was found between both methods.

Many non-UV absorbing compounds were tested in HTLC-FID. Carbohydrates were separated on porous graphitic carbon retentive column such as the hypercarb one (77). Carboxylic acids were separated in isothermal mode (31) or temperature gradient; and finally amino acids were separated on PRP-1 column (125, 128) or hypercarb with FID. Peak shapes were always good for these separations.

In order to further extend the range of application of the HPLC-FID system, it is necessary to increase the elution strength and the selectivity of water. Formic acid gives a very weak FID signal. Formic acid additions to water were examined (77, 122). There are no clear conclusions on the benefit of formic acid additions. Ingelse et al. mentioned FID instabilities and possible capillary clogging (77). Hooijschuur et al. (122) with 1% formic acid additions in the separation of alcohols, amino acids, and carboxylic acids got improved results in term of peak shapes.

Last, the "low temperature enrichment" method was proposed (77). It can be said that solid phase extraction is done at room temperature at the column head injecting a large volume of extremely diluted sample. When the temperature is raised, the compound is eluted and detected by FID. LODs as low as 5 to 10 pg were obtained (77).

HTLC-ELSD (Evaporative Light Scattering Detection)

Evaporative Light Scattering Detection was introduced in 1966 by Ford and Kennard (131). The principle is based on the nebulization of the eluent from a liquid chromatographic column with a gas; drying the droplets in a hot chamber and detection of any nonvolatile residue by light-scattering and photomultiplier tube amplification. Compared to mass spectrometry, this detection mode is a relatively inexpensive alternative method for the detection of heavy compounds that do not absorb UV light (132). UV detection depends on chromophores; ELSD will detect all compounds that are solid or less volatile than the mobile phase. The detector response is almost independent of the solute structure. Most of the ELSD applications

were developed for lipids, amino acids, sugars and carbohydrates or non-UV-absorbing surfactants and polymers separated in size exclusion chromatography. The weak points of ELSD are a limited sensitivity, nonlinearity between mass and signal intensity, and a response depending somewhat on the mobile phase composition (133–135).

These drawbacks may explain why there are few studies on the coupling between HTLC and ELSD. It could be thought that the fact of working at high temperature could facilitate the evaporation step. Greibrokk et al. worked intensively on HTLC-ELSD coupling (93, 95, 99, 100, 134, 136). They modified an ELSD in order to adapt it to the use of capillary columns in HTLC (136). A classical ELSD was improved for use with flow rates as low as $5\,\mu L \cdot min^{-1}$. This modified system was evaluated in term of detection limits, linearity, and response to temperature programming. The main problem, noted first in a 1998 article (99), was a rising baseline with temperature ramps higher than $6^{\circ}C \cdot min$. There was no clear explanation for this problem. Analyses could however be performed with temperature ramps steeper than $6^{\circ}C/min$, but then the range from start to the end temperature had to be minimized (99). The baseline problem was mostly solved by working with an external nebulizer gas heating device (136). The chromatograms given in Figure 8 were obtained with the modified ELSD device and prove that the baseline signal shows little or no sensitivity towards temperature ramps as high as $50^{\circ}C/min$. In the case of isocratic mode, very good LODs as low as 1 ng were obtained with an acceptable dynamic range of 1 to 500 ng (136).

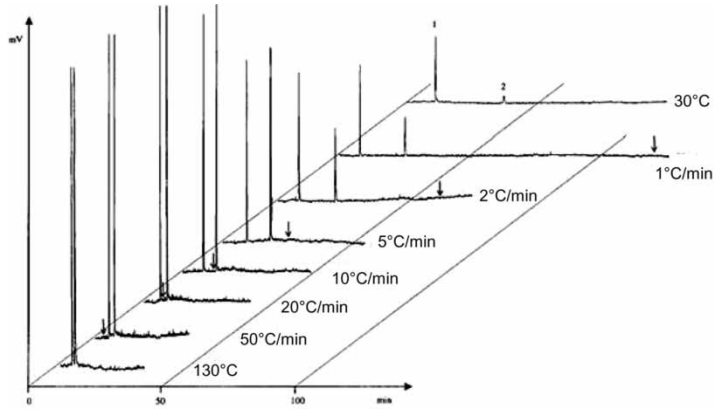

Figure 8. The modified ELSD's response to isothermal temperatures (30°C and 130°C) and different temperature ramps from 1–50°C/min, starting at 30°C and ending at 130°C. The arrows indicate the end of each temperature program. Compounds: Irganox 3114, Irganox 1076. The column was 0.32 × 280 mm, 3 μm dp Hypersil ODS. Flow rate was 5 μL/min and acetonitrile was used as mobile phase. Drift tube temperature 85°C and N_2 gas flow was 2.2 L/min. Adapted from Ref. 140.

The HTLC-ELSD coupling is found in several other works (93, 95, 100, 134). However, in these studies, ELSD is used as a first detection mode before mass spectrometry. The separation of high-molecular-mass-hindered amine light stabilizers was optimized with ELSD. Next, an ESI-TOF-MS detection was used for full identification (95, 134). The same approach was used for the separation and identification of mixtures of polyglycerol fatty acid esters (93, 100).

HTLC-MS (Mass Spectrometry)

Mass spectrometry is the only detection mode that not only says: "there is a product" but also "and this product is ...." Analysts were eager to couple such a powerful detection mode to any separation method. If the GC-MS coupling was soon realized, the LC-MS coupling was much more difficult due to the liquid mobile phase. Solutions were found and the early methods, including thermospray (137–140), particle beam interface (141) or pneumatic nebulizer interface, were difficult to use and lacked robustness. The introduction of atmospheric pressure chemical ionization (APCI) (142–144), electrospray ionization (ESI) (145–147) and atmospheric pressure photo-ionization (APPI) (148) led to the development of reliable and easy to use interfaces (149). APCI and ESI techniques are now the method of choice for many LC-MS applications.

HTLC must also be coupled with MS detection. The compatibility between HTLC and MS with electron-impact ionization was shown more than a decade ago (11). A temperature programmed microcolumn liquid chromatography/mass spectrometry (μLC-MS) system has been developed as a practical alternative to conventional solvent-programmed LC-MS. This method enabled the complete resolution of fatty acids in fish oil using 200 μm i.d. capillary columns and a temperature programming with a slope of $0.35^{\circ}C \cdot min^{-1}$ between 40 and 80°C. Temperature programming was proposed in this case, as a way to minimize problems associated with changes in the mobile phase viscosity and surface tension as a result of alterations in the composition of the mobile phase (11).

Today, as in classical LC-MS, APCI (150) and ESI (100, 134, 151) are the dominating ionization techniques used in HTLC-MS. The efficiency of these MS interfaces that are based on nebulisation, vaporization and desolvatation processes, can normally be expected to vary with mobile phase properties (surface tension, viscosity, and polarity). Very few applications could be found using an APCI-MS detector in HTLC. This is due to the lack of sensitivity induced by the low flow rate and the mass-dependence of this kind of ionization mode. A separation was performed in open-tubular liquid chromatography at 150°C and a flow rate of about 1 μL/min (150).

Interesting examples were given in ESI-MS detection by Greibrokk et al. (100, 134) and Molander et al. (151), both using temperature programming.

These applications concerned phosphatidylserine species, hindered amine stabilizer and fatty acids. The sensitivity obtained using a temperature programming was superior to that obtained with mobile phase composition gradients. Hot mobile phases were easily vaporized allowing a large decrease in noise on chromatogram.

HTLC-NMR (Nuclear Magnetic Resonance)

NMR spectroscopy is well established as a powerful analytical approach to molecular structure determination and the study of a variety of dynamic processes, including diffusion, binding, and macromolecular folding. The field of NMR has developed a sophisticated array of experimental capabilities and hardware but NMR is still orders of magnitude less sensitive that most other analytical methods. NMR has, however, a significant advantage: it is nondestructive.

Most of the practical problems associated with coupling LC and NMR have been resolved in recent years. The strong background signal for protons in nondeuterated mobile phases is the major problem. NMR-quality deuterated solvents with minimal interfering proton signals, such as deuterated methanol or acetonitrile, can be used, but these are expensive. For HPLC-NMR the use of superheated deuterated water as the eluent is advantageous compared to conventionally HPLC organic–aqueous eluents because deuterated water of great purity is affordable. The NMR spectra obtained with heavy water have much less interference from the mobile phase than with conventional HPLC-RMN eluents (152). Furthermore, the combination of superheated heavy water with NMR-MS chromatography enables both NMR and MS spectra to be obtained simultaneously for the same sample; one or two dimensional spectra could then be obtained easily (152).

HTLC-NMR-MS separations of barbiturates was reported with on-flow and stop-flow detection modes (153). HTLC-NMR-MS of model drugs (152), sulfonamides (154) and a ginger extract (155) were also reported. A PS-DVB column was often used (152, 154–158), but silica-based stationary phases are not excluded (155). Separations were performed in isothermal mode with hydro-organic mobile phase and temperature programming with 100% water mobile phases. Direct on-flow NMR analyses produced acceptable identification but stop-flow analyses provided spectra with better signal to noise ratio and enabled structural identification of diluted compounds.

Figure 9 shows that some more complex combinations of spectrometers could also be done. HTLC-IR-UV(DAD)-NMR-MS with superheated D_2O was used to analyze a model drug mixture (156) and ecdysteroids in plants extracts (158) in on- and off-line mode. As can be expected with such highly hyphenated systems, the NMR and IR instruments were the least sensitive requiring injections of more than 100 μg for each compound in the column.

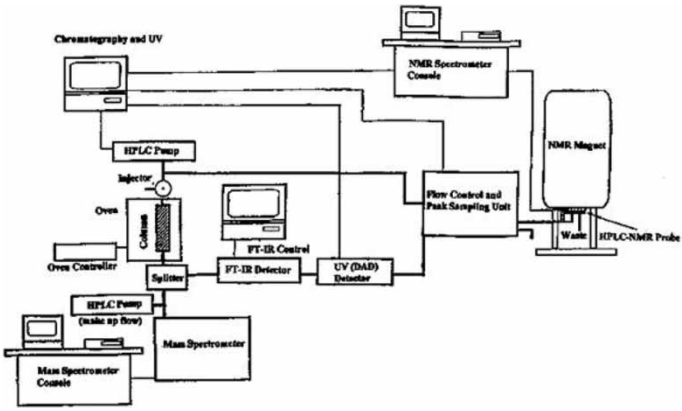

Figure 9. Experimental layout of the various spectrometers used in the superheated water HPLC-MS-IR-UV-NMR system. Adapted from Ref. 164.

ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) or ICP-MS (Inductively Coupled Plasma Mass Spectrometry)

Combining the HTLC high resolving power with ICP-MS element specific detection, is a promising combination for the determination of organometallic compounds at low concentrations. Furthermore, imposing elevated temperatures on the packed capillary column, and thereby heating the mobile phase before the nebulization process, was assumed to have a positive effect on the production of ions in the plasma, because less energy would be consumed from the plasma for evaporation of the mobile phase.

In 1989 Biggs et al. were the first to investigate "thermal gradient liquid chromatography" coupled with ICP-AES (159). They concluded that a temperature gradient had a potential for replacing a mobile phase gradient that had a negative influence on plasma stability. Their experiments were conducted with 2 mm or 4.6 mm i.d. columns coupled to a standard spray chamber as the nebulizer. Greibrokk et al. used a laboratory-made microconcentric nebulizer as an interface for the coupling of μ-HTLC with ICP-MS (160). They concluded that elevated column temperature did not affect positively or negatively the signal response. In this case, the repeatability of peak height and area at the investigated column temperatures (75–150°C) was quite good. The tin LOD was 9 pg for tetraethyltin, whereas others authors had an LOD of 51 pg tetraethyltin using conventional HPLC coupled to ICP-MS (161). The ICP-MS response was found stable even when high temperature gradients, up to $32^{\circ}C \cdot min^{-1}$ were used.

An application of HTLC-ICP-MS was the detection of carbon-containing compounds with entirely aqueous eluents at high temperature, in order to

eliminate completely the organic modifier (162). Separations obtained using 100% water were far more sensitive than those done with methanol in the mobile phase.

Other Detection Modes

The other detection modes include refractive index, amperometric detector, conductivity detector, and Fourier transform infrared (FTIR) detections. The principal problem with all these detection modes, working perfectly in isocratic conditions, is the elution gradient. An interesting alternative solution to the composition gradient could be temperature programming.

Generally, baseline instability is a drawback of temperature gradient. Baseline drift is more serious with some detection modes, like refractometer and conductimeter (26). In these cases, it is due to variations in mobile phase temperature entering the detector, which induce modifications of the mobile phase physicochemical properties (viscosity, refractive index, and conductivity). The drift can be corrected by commercially available software (163).

Refractive Index Detection

Refractive index based detectors are theoretically universal. However they are not extremely sensitive and are traditionally plagued by baseline drift and noise level much higher than other detectors. A refractive index based detector able to work with thermal gradient applications in liquid chromatography has been implemented about 10 years ago (164). The conclusions of the study were that liquid chromatography at high temperature with the refractive index detector may be used without loss of sensitivity for analytes if high flow rates are used, or if the eluent is well cooled at a constant temperature before entering the refractive index detection system. This suggests that the RI detector can be used with care in high-temperature liquid chromatography without substantial temperature-based sensitivity losses.

Amperometric Detector

A miniaturized amperometric detector compatible with temperature programmed packed capillary liquid chromatography has been constructed and evaluated (26). Temperature programming could successfully be used, for electrochemically active compounds, as a tool for retention control. The temperature changes did not influence the detector performance. A simple mathematical approach eliminated the baseline drift during temperature programming, and synchronizing the data sampling against the in-house electric facility in combination with proper grounding significantly lowered the noise level (26).

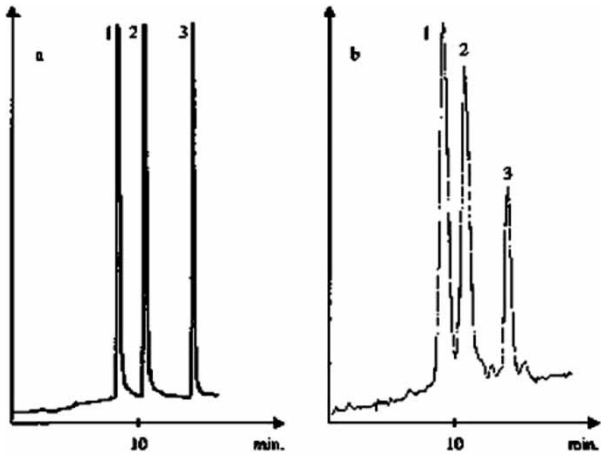

Figure 10. Temperature-programmed packed capillary LC separation of Irganox 3114 (1), Irganox 1010 (2), and Irganox 1076 (3) dissolved in DMF (300, 300, and 180 ng of each, respectively) with UV detection (a) and off-line FTIR detection (b). Temperature program: 50°C for 2 min, then $4°C \cdot min^{-1}$ to 120°C. Adapted from Ref. 171.

FTIR (Fourier Transform Infrared Spectroscopy)

In LC-MS hyphenation, MS lacks the ability to discriminate between isomers. Infrared spectroscopy (IR), however, is suitable for the characterization of functional groups in addition to differentiating between structural isomers. Hence, the coupling of LC and FTIR is a technique complementary to LC-MS. Here again, a nebulization process is needed for the hyphenation and temperature programming may be superior to gradient elution.

The coupling between HTLC and FTIR was tested by Greibrokk et al. (165). Their study of the separation of complex antioxidants demonstrates that temperature gradients did not influence the nebulizer spray performance or the solute deposition, making temperature programming preferable to solvent gradient elution. An example of separation obtained is given Fig. 10 that shows that UV detection, when possible, has great power.

CONCLUSION

The advantages and drawbacks of high temperature liquid chromatography were noted briefly. The major disadvantage comes from the traditional silica stationary phases, which are quickly degraded as temperatures pass 100°C. This problem was solved by replacing silica-based stationary phases by thermally stable new stationary phases with comparable performances. The advantages of working at high temperature are real. It is possible to

obtain ultrafast separations with improved efficiency and peak shapes. It is possible to reduce the organic modifier content. Selectivity can also be improved by temperature changes. Last, it is possible to work with universal and/or sensitive detectors such as FID, ELSD, MS, NMR and/or more specific detectors, such as IRFT, RID, and ICP-AES.

The coupling possibility of HTLC is due to the fact that it is possible to work with 100% water as the mobile phase. FID becomes compatible with LC since pure water does not give any background response in FID. In the case of NMR detection, deuterated water of great purity is much more affordable than other deuterated organic solvents. It makes the HTLC-NMR coupling attractive. The possibility of using temperature gradients instead of composition gradients renders HTLC compatible with refractometer, ELSD, conductimeter, FTIR, ICP, MS, or NMR, and improves the baseline stability. Last, hot mobile phases improve the nebulization process, which is a preliminary step of many detection modes such as ICP-AES, ICP-MS, MS, FTIR, and ELSD. HTLC makes possible to improve significantly the sensitivity of these detection modes. Another interesting point would be to have some results concerning the compatibility and the use in HTLC of the new, dubbed universal, detector "Charged Aerosol Detector" (CAD) from ESA (166). This promising detection mode has a principle based upon APCI ionization source (corona discharge) and ELSD (mobile phase evaporation before detection). It has several advantages upon the universal ELSD, but it has not yet completely been established experimentally (higher sensitivity and reproducibility, linear response ...).

In the future, with the development of new thermally ultrastable metal oxide-based stationary phases for HPLC (zirconia-, alumina-, or titania-based) (167, 168), and the marketing of new commercial HTLC system like Selerity polaratherm 9000 (169) or ZirChrom Metalox 200-C (170), it is expected that liquid chromatography at high temperature will expand even more.

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