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TECHNIQUES OF CAPILLARY LIQUID CHROMATOGRAPHY

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SUMMARY

Packed microcapillary columns of the reversed-phase type for liquid chromatography are shown to provide the high efficiencies needed to resolve complex mixtures. Certain demands are placed on the instrumental design for this type of chromatography, with respect to small volumes of sampling and detection. A direct sampling method is demonstrated that compares favorably with the splitting injection. Two step-wise gradient elution techniques have been developed for work with packed microcapillaries. Complex mixtures of polyaromatic compounds are resolved into numerous fractions detectable with small-volume ultraviolet and spectrofluorometric detectors.

INTRODUCTION

Recent interest in capillary liquid chromatography (LC) stems from (a) the potential of achieving greater resolution of mixture components, and (b) drastically reduced flow-rates of the mobile phase. Whereas the separation value of capillary LC columns[1–5] has yet to be carefully assessed against a competitive approach[6,7], typical flow-rates of just several microliters per minute through such columns present some unique and desirable instrumental possibilities.

The capillary LC columns thus far described are of two types: (a) open microtubular columns[2,3,5], and (b) packed microcapillaries[1,4]. Both column types have now been described in adsorption[1,5], partition[3], and bonded-phase[3,4] modes. Various approaches to column technology have yet to be optimized.

Apart from the desirably low solvent consumption, capillary LC columns lend themselves ideally to explorations of certain improved detection techniques (*e.g.*, LC–mass spectrometry and LC–IR combinations, use of concentration-sensitive detectors, and transport-type systems). While some advantages of such columns in this respect are predicted, capillary LC will only be feasible with significant departures from the instrumental design of the conventional HPLC.

The instrument miniaturization efforts by Ishii and co-workers[3,8] have sig-

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nificantly aided the overall development of capillary LC. Such efforts have included both sample introduction and detector cell design.

Whereas relatively simple, low-pressure systems are typical for work with open microtubular columns[3,5], the studies of this laboratory have been mostly associated with (semi-permeable) packed microcapillary columns[1-4]. Since the trend of our work has been toward exploitation of higher pressures, techniques described in this paper are consistent with such a direction. Thus, sampling techniques, operational and detection conditions consistent with packed-microcapillary LC, developed in our laboratory, are described.

In order to overcome problems associated with out earlier used[1] splitter sampling method, direct sample introduction was investigated. The extent of band broadening is compared here for both types of samples techniques. A need for direct sampling is further discussed below.

In order to optimize compound resolution in LC, gradient elution is most frequently applied. While the conventional high-pressure pumping systems are adequate to regulate microcolumn flow-rates in the isocratic operational mode, gradient techniques require a different instrumental design. Gradient elution techniques are needed to optimize resolution of complex mixtures. Since the commercially available pumps are designed for the flow-rates considerably higher than those practiced in capillary LC, development of flow-controlled micropumps will be the ultimate goal. Meanwhile, suggestions are given here to modify typical solvent delivery systems for gradient elution in microcolumn LC, under the conditions of high pressure.

Two stepwise gradient elution methods have been investigated here in the reversed-phase chromatography of polycyclic aromatic hydrocarbons (PAHs). Ultraviolet and spectrofluorometric detectors with cell volumes of less than 0.1 μl are used either individually or in series to detect such compounds.

Finally, resolution of a complex mixture (aromatic fraction of coal tar) is demonstrated. Whereas the earlier components of the mixture could also be analyzed by capillary gas chromatography, volatility problems are experienced with compounds beyond approximately the elution of benzopyrenes. At the same time, conventional HPLC has thus far been unable to provide adequate resolution.

EXPERIMENTAL AND RESULTS

Chromatographic system

Two Varian Model 8500 high-pressure syringe pumps were used to generate the necessary pressures maintaining desired flow-rates through the packed microcapillaries. Both splitter[1] and direct sampling method (to be described in more detail below) were used to introduce samples of different concentrations. The detectors used were the standard Varian UV monitor (operating at a fixed wavelength of 254 nm) and a Schoeffel F.S. 970 LC spectrofluorometric detector. Both detectors were modified with quartz microflow cells (internal volume of less than 0.1 μl) and connected to the column outlet with a piece of small plastic tubing (I.D. 70 μm) according to Tsuda *et al.*[3].

The reversed-phase packed microcapillary columns were prepared according to a recently described procedure[4] and tested with the series of polycyclic aromatic

hydrocarbons. Whenever needed, the microcapillary columns were placed in a constant-temperature bath during chromatographic experiments.

Sample introduction

While splitter sampling was used exclusively in the previous work[1], a need for direct sampling techniques has been realized. Obvious drawbacks of the splitter mode are its incompatibility with trace analysis work as well as difficulties with sampling concentrated (and, often viscous) solutions. Consequently a high-pressure version of the technique similar to the "micro-feeder" method[3] was investigated; sample solution is introduced by suction into a short stainless-steel capillary (5 × 0.15 mm I.D.) fixed in a PTFE adapter. During the stop-flow sampling, the adapter with the sample capillary is inserted into the analytical system. The regenerated mobile-phase flow through the sample capillary introduces the injected material into a capillary column.

In order to evaluate the extent of band-broadening due to this injection method and to compare it with the previously used splitting mode[1], a 5.0 m × 70 μm I.D. microcolumn was used under the same flow-rate and sample size conditions. The result is demonstrated in Fig. 1, indicating that the direct sampling is only slightly worse than the use of the splitter. However, if further reduction of the column diameter is desirable in the future, better sampling techniques must be developed.

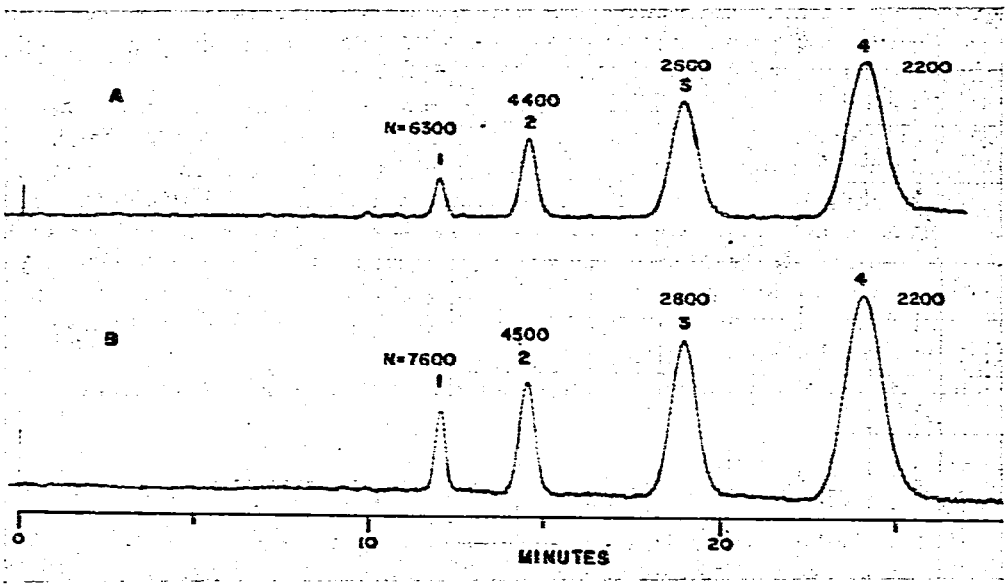

Fig. 1. Comparison of the direct injection (A) and the splitter injection (B) techniques; the numbers of theoretical plates are indicated in the figure. Chromatographic conditions: 5.0 m × 70 μm I.D. column, packed with 30-μm basic alumina–octadecylsilane; mobile phase, acetonitrile–water (1:1) at 2.2 μl/min. Sample components: (1) benzene, (2) naphthalene, (3) fluorene, (4) anthracene. Column temperature, ambient.

Solvent delivery

The first version of a "stepwise gradient technique" is explained by Fig. 2. Both pumps employed were Varian 8500 type. While pump 1 contains the starting

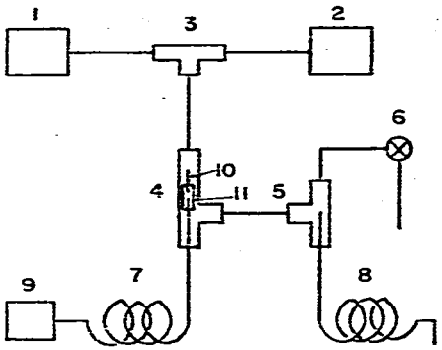

Fig. 2. Schematic diagram of the chromatographic system: 1 and 2, high-pressure syringe pumps; 3, mixing tee; 4, sampling port, 5, auxillary tee; 6, high-pressure shut-off valve; 7, analytical column; 8, flow-controlling column; 9, detector; 10, sample capillary; 11, PTFE attachment.

solvent, pump 2 contains the final solvent mixture. Prior to sample introduction, the pressure inside pump 2 was kept at the operational level by closing another valve situated between this syringe pump (pump 2) and the mixing tee, 3. Following the sample introduction, pump 1 was driven quickly to the necessary pressure and the flow-rate set at several milliliters per hour. While most solvent is bled off through the flow-controlling column, 8, pressure on the analytical column 7, can be kept constant. When solvent change is necessary, the flow-rate of each pump is adjusted manually. Obviously, the system could be easily automated.

A typical course of solvent change in the stepwise gradient elution (as monitored by the UV detector) is shown in Fig. 3, where pump 1 contained pure methanol and pump 2 a 0.1% benzene solution in methanol. The total flow-rate was 8 ml/h. The flow-rate of each pump was changed by 2 ml in each step, while the total flow was maintained. If two mixing solvents have similar viscosity, the operational pressure remains constant. However, as shown in Fig. 4, pressure may change due to viscosity. Obviously, this procedure provides a quick solvent change needed in a gradient elution. Fig. 4 also demonstrates a superior resolution of the model mixture of PAHs, quite typical of this chromatographic method.

Another stepwise gradient technique, similar to that described by Ishii *et al.*[8],

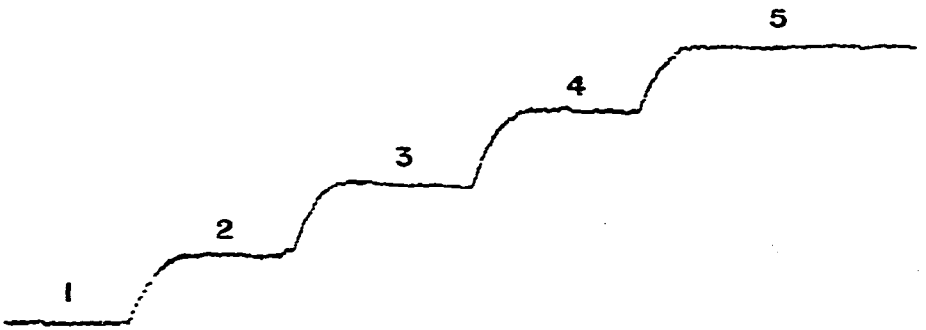

Fig. 3. Typical solvent change in the stepwise gradient elution technique. Time intervals, 10 min; pump 1, pure methanol; pump 2, 0.1% benzene in methanol; total flow-rate, 8 ml/h, with solvent changes of: (1) 8/0, (2) 6/2, (3) 4/4, (4) 2/6, and (5) 0/8.

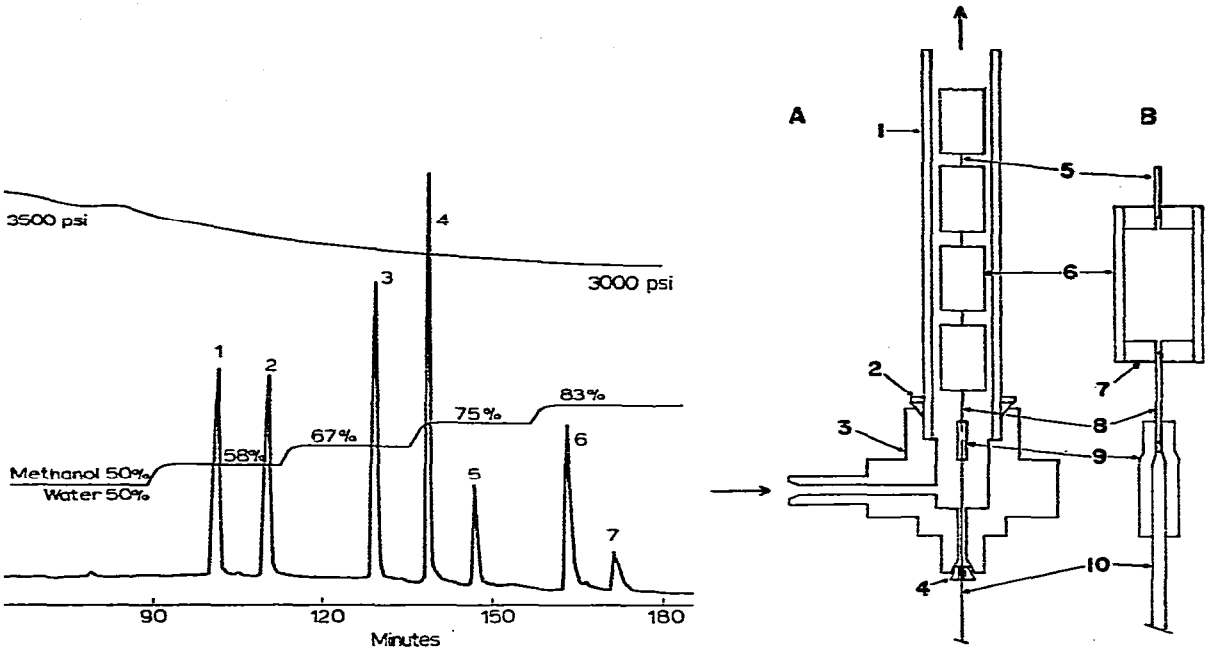

Fig. 4. Chromatogram of standard polycyclic aromatic hydrocarbons under the conditions of stepwise gradient. Column: 27 m × 70 μm I.D., acidic alumina (25 μm)–octadecylsilane; total flow-rate through the system, 6 ml/h (pump 1, methanol–water (1:1); pump 2, methanol), flow-rate, 1 μl/min. Components: (1) toluene, (2) naphthalene, (3) fluorene, (4) anthracene, (5) pyrene, (6) chrysene, and (7) benzo[*e*]pyrene.

Fig. 5. Modification of the sampling port shown in Fig. 2, for the stepwise gradient elution technique using one pump. (A) whole sampling port; (B) magnification of a sample capillary and a solvent reservoir. 1, large stainless steel tube; 2, metal ferrule; 3, injector body; 4, polyimide ferrule sealing the analytical column; 5, stainless steel capillary; 6, PTFE micro-reservoir tube; 7, PTFE capillary; 8, stainless steel capillary; 9, PTFE capillary; 10, analytical capillary column.

was investigated in this work. In this procedure, as shown in Fig. 5, a series of miniature reservoirs (with different solvent composition) is used while employing only one high-pressure pump. While the essential elements of this gradient technique are detailed in Fig. 5, the arrows show the direction of solvent delivery during the initial pressurizing and the removal of air bubbles from the system.

Detection methods

As stressed in the previous communications[3,4,8], a detector for the microcolumn and capillary LC work must have minimal volume to avoid an extra-column band broadening. The miniaturization of the optical detection cells[8] was also performed in this work, together with carefully designed small dead-volume connections between the column and detectors.

While UV detection has been used in much of our work, a spectrofluorometric detector was added in series to the UV monitor for the detection of PAH mixtures. Fig. 6 shows a simultaneous recording of a standard PAH mixture with both detectors. Only a minor peak broadening (about 13% efficiency reduction) is observed

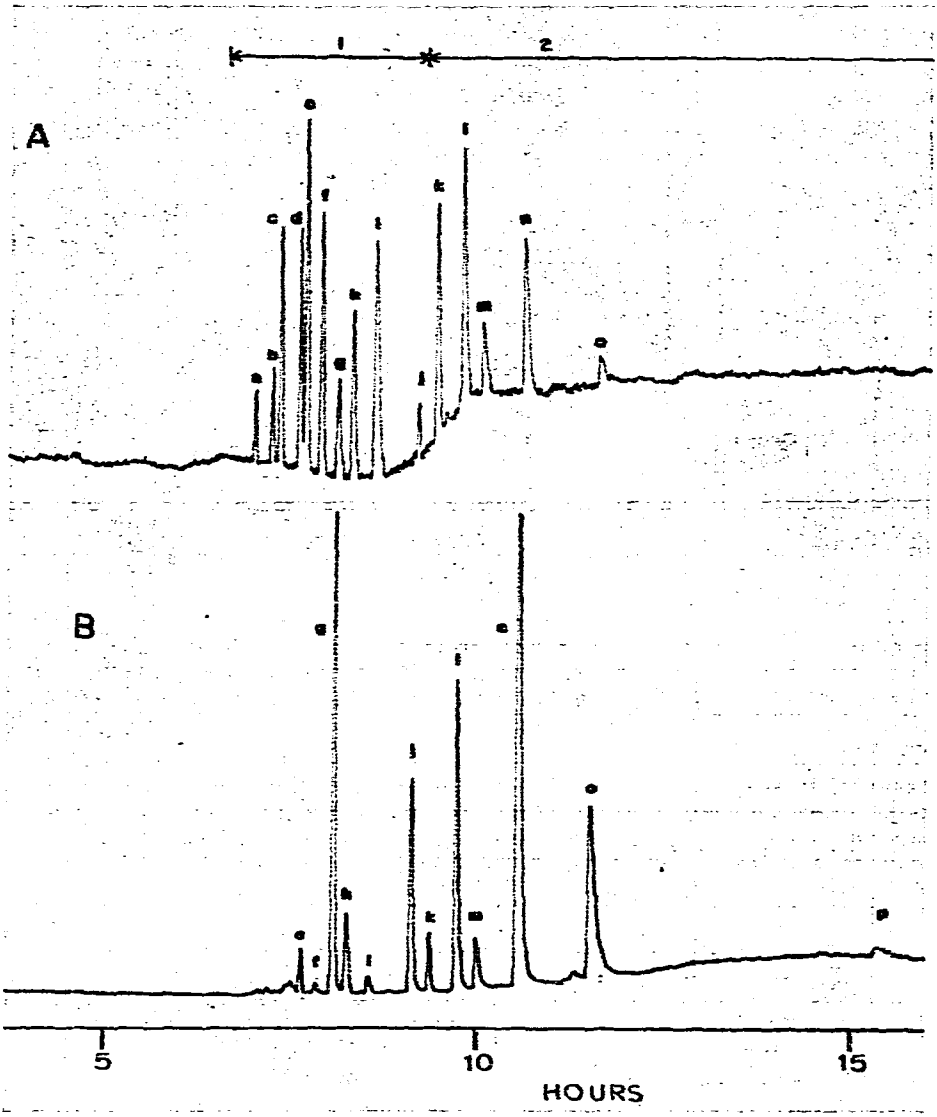

Fig. 6. Chromatogram of a standard mixture of polycyclic aromatic hydrocarbons under the conditions of a two-step gradient, detected with two different detectors in series. (A) UV detection at 254 nm; (B) spectrofluorometric detection with λ_{exc} = 290 nm and 370 nm cut-off filter. Column: 55 m × 70 μm I.D., basic alumina (30 μm)–octadecylsilane; column temperature, 30°; inlet pressure, 100 atm; mobile phase (stepwise gradient): (1) methanol–water (90:10), (2) pure methanol. Components: (a) benzene, (b) naphthalene, (c) biphenyl, (d) fluorene, (e) phenanthrene, (f) anthracene, (g) fluoranthene, (h) pyrene, (i) triphenylene, (j) benz[*a*]anthracene, (k) chrysene, (l) benzo[*e*]pyrene, (m) perylene, (n) benzo[*a*]pyrene, (o) dibenzo[*ghi*]perylene, (p) coronene. Except for a and b, sample quantities are less than 50 ng.

with the spectrofluorometric detector in spite of the fact that interconnecting plastic tube had to be used between the two detectors. Whereas the UV absorption chromatogram shows a significant baseline drift due to a change in solvent composition together with a significant noise, none of these problems are observed with the fluorometric response. The latter detector was shown to exhibit an adequate sensitivity.

A chromatogram of a complex mixture of aromatic compounds (originating from coal tar) is shown in Fig. 7. Here, the spectrofluorometric detector alone was used. While 23 h were needed to accomplish this separation, good resolution is observed over a wide range of molecular weights. The sample (an aromatic fraction of coal tar) was prepared following the liquid–liquid partition scheme used previously in this laboratory[9].

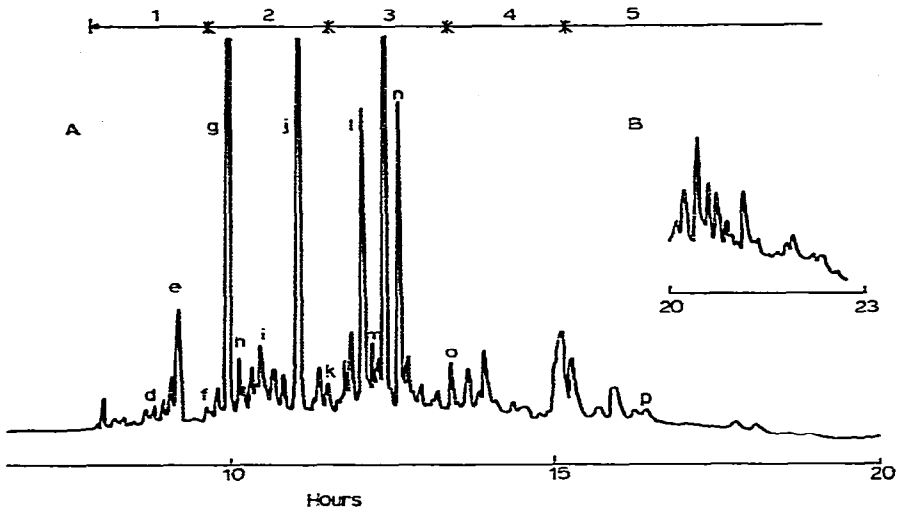

Fig. 7. Chromatogram of the aromatic fraction of coal tar. Mobile phase, stepwise gradient: (1) methanol–water (80:20), (2) methanol–water (90:10), (3) methanol, (4) 1% methylene chloride in methanol, (5) 3% methylene chloride in methanol. Column, other operational conditions (A) and component designation as in Fig. 6. After 20 h, the conditions were changed (B) as follows: inlet pressure, 200 atm; temperature, 60°; detector sensitivity setting was increased by a factor of 5.

DISCUSSION

There has been an increasing awareness that substantially higher column efficiencies are needed in order to address the resolution problems of very complex mixtures. It is expressed appropriately by Scott[7] in a recent article that "... today, such efficiences are novel and are required only in a minority of circumstances. As the technique develops and expands into the field of biological materials, very high efficiency columns will no longer be a luxury but a necessity." The current approaches to higher efficiencies in HPLC all involve columns of reduced internal diameters (but not necessarily the "small-particle technology"): (a) the tightly packed long columns, with 1 mm I.D., of Scott and Kucera[6,7]; (b) packed microcapillary columns, such as discussed in this work and elsewhere[1,4]; and, (c) open microtubular columns[2,3,5]. While the potential and limitations of all three approaches remain to be assessed carefully, there are certain unique features to all of them.

Although packed microcapillary columns are exclusively dealt with in the present communication, the techniques described here may be of benefit to any future work with small-bore columns. While the column performance studies are reported elsewhere[4], the primary purpose of this article has been to describe the technique aspects.

Development of new preconcentration/sampling techniques will be extremely important for future investigations in capillary LC and its applications. The present injection techniques, including those described here, are less than ideal. While the direct sampling used in this work permits semiquantitative analyses with columns of internal diameter above approximately 60 μm, further volume reduction of the injector will be needed for smaller columns. For example, design of a high-pressure loop injector with approximately 50 nl volume would be highly desirable.

While instrumental requirements for capillary LC are presently quite high, the previous reports of Ishii *et al.*[3,8] demonstrate the feasibility of a miniature design in both detector and column interfacing technology. As shown in this report, connection of two different detectors in series is possible with no substantial reduction of column efficiency. Since a simultaneous use of different detectors is desirable in chromatographic analysis of complex mixtures, size reduction of other detectors is eminently worthwhile.

The chromatogram of the aromatic coal tar fraction (Fig. 7) attests to the complexity expected of such mixtures. With further improvements in column efficiency, an increasing number of peaks will be demonstrated, placing additional demands on ancillary structural methods. Even though the time of analysis is currently long, resolution is considerably more important in a number of cases, including many important biochemical and environmental problems. Just as it is now a common practice in capillary gas chromatography, column optimization studies with respect to a suitable compromise between resolution and time of analysis, are needed in capillary LC.

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