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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY SYSTEM FOR PACKED CAPILLARY COLUMNS

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SUMMARY

Greatly enhanced mass sensitivity is reported for capillary columns by using 5-mm pathlength flow cells of small volume instead of on-column detection techniques. The variance of the illuminated volume of 1-, 5- and 10-mm pathlength flow cells was measured empirically. The effect of this variance on the observed efficiencies of columns of 0.200, 0.500 and 1.0 mm diameter was calculated. The observed efficiency of 80 000 plates/m and the improved separation of a mixture with a 0.200-mm diameter column demonstrates the suitability of the chromatograph and the 5-mm pathlength flow cell.

INTRODUCTION

For many applications the separation of a complex mixture by high-performance liquid chromatography (HPLC) can only be achieved by employing high-efficiency packed capillary columns[1] that require a well-designed and optimized system. In particular, the performance of the pump and detector is of great importance when packed capillary columns with internal diameters less than 1 mm are employed[2]. Due to the much reduced diameter and corresponding small sample volume, detector flow cells with greatly reduced volumes and long optical pathlengths are required to minimize peak dispersion and maintain maximum sensitivity. The pump should be able to sustain very accurate and pulse-free flow-rates at the very low levels (5 μl/min and less) necessary for packed capillary column chromatography.

Yang[3] and Gluckman *et al.*[4] have reported excellent efficiencies with 0.2-mm packed capillary columns. However, their use of on-column detection seriously impairs the detection limits observed. The modified commercial detectors used in previous work employ optical pathlengths of 0.5 or 0.25 mm. The actual mass sensitivity observed in these systems lags behind the theoretical advantages of these columns discussed by Van der Wal[5].

In this paper, chromatographic performance parameters are reported for a commercially available chromatograph suitable for capillary columns. The chromatograph is comprised of a variable-wavelength absorbance detector with flow cell volumes down to 0.03 μl, and a 50-ml capacity syringe pump with flow-rates down to 0.08 μl/min and a 10 000 p.s.i. pressure limit[6]. Columns are directly connected to the injection valve and flow cell (Fig. 1).

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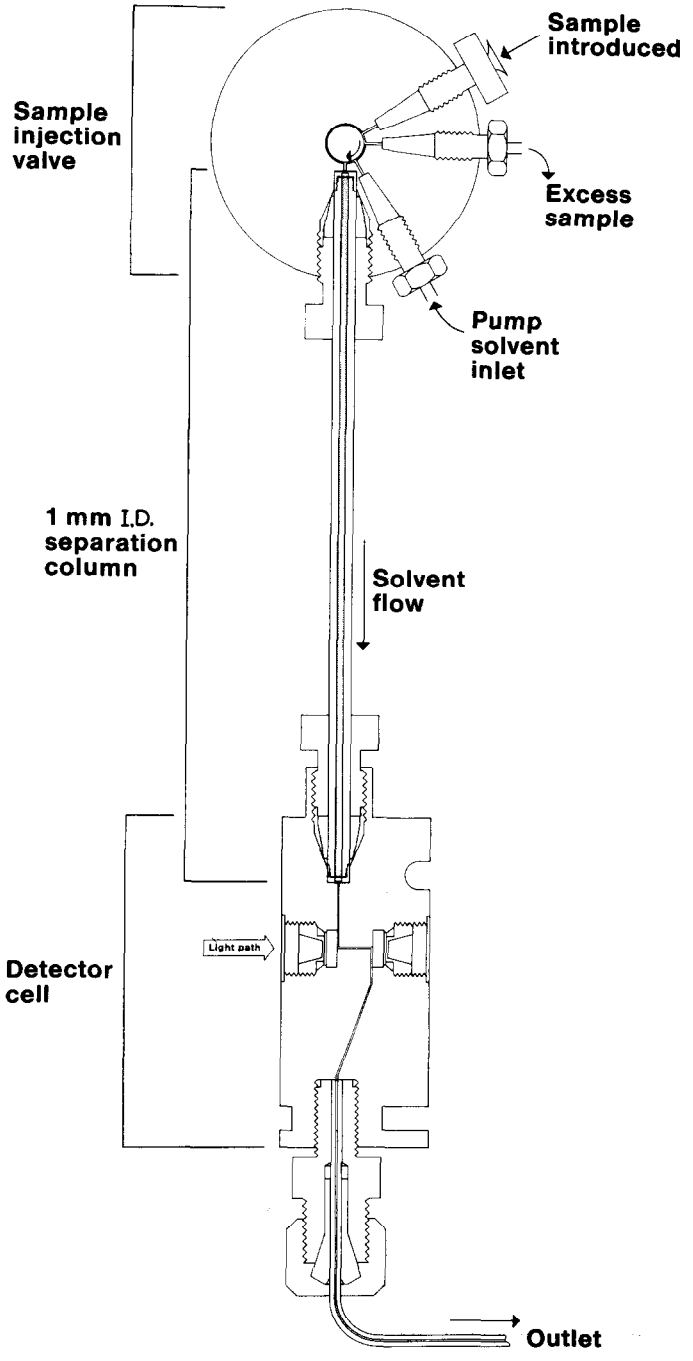

Fig. 1. Schematic drawing of the direct connections of the microbore liquid chromatograph.

Mass sensitivities, determined using a 5-mm pathlength flow cell (0.37 μl volume), are reported for 0.2-, 0.5- and 1-mm columns. Detailed analysis of the variance of the 1-, 5- and 10-mm flow cells is discussed. Change in column efficiency with change in flow cell volume is used to calculate empirical flow cell variances for all three cells. Finally, the effect of the larger flow cell volumes on the efficiency of the columns is calculated for a capacity factor, k', of 2.

EXPERIMENTAL

An Isco microbore LC system consisting of the μLC-500 syringe pump, a Valco injection valve with a 0.1-μl internal loop, and a μLC-10 variable-wavelength absorbance detector was employed without modification for all separations discussed in this paper. The direct connection of injection valve and column reduces broadening enough to allow the use of full injections for 0.2-mm columns as well as 1.0-mm columns. Standard Isco microbore flow cells of 1-, 5- and 10-mm pathlengths with small illuminated volumes were used with packed capillary columns.

Columns of three diameters were packed for comparison in this work. The columns were all packed in this laboratory with Spherisorb® ODS2, 3-μm packing material. Packing slurries were prepared in methanol–carbon tetrachloride (1:13). The 250 × 1 mm column is a commercial Isco C_{18} microbore column.

Two 0.5-mm steel columns were packed by means of an air-driven Haskel pump at 15000 p.s.i. One of these columns was a straight 300-mm long glass-lined rigid stainless-steel tube with standard Isco 1/8-in. end caps and frits 0.5 mm thick and 1 mm diameter. A 840 × 0.5 mm column of polished steel was coiled (diameter 16 cm) and then packed in this configuration. The end pieces of this column were adapted to 1/8 in. which allowed standard Isco end caps to be used.

Porous Teflon® end frits were glued into the fused-silica column using the method described by Shelly *et al.*[7]. The fused-silica columns (0.2 mm I.D. from Scientific Glass Engineering) are more fragile and explode on sudden application of pressure from a compressed-air-actuated Haskel pump. Therefore, the columns were packed with an Isco Model 2600 syringe pump at 6000 p.s.i. This pump delivers fluid at a constant flow-rate, but the maximum pressure setting of the pump produces a more constant pressure than the lower settings of the Haskel pump. Columns were adapted to 1/8-in. fittings with a Vespel® or Kel-F® ferrule and insert. A pin vise with a drill bit was used to adapt the Kel-F inserts to the exact O.D. of the fused-silica tubing. The syringe pump was connected to the packing reservoir with a (Model 02-0121 from Scientific Systems Inc., State College, PA, U.S.A.) three-way valve. The packing reservoir was a 500-μl, 1/8-in. I.D. tube with a small funnel inserted into the column end. A column with the adapter was inserted through a 1/8-in. union directly into the funnel inside the packing reservoir.

A slurry of 50 mg packing material in 200 μl of packing solvent was thoroughly mixed for 10 min using an ultrasonic bath. The column was filled with packing solvent. The packing reservoir was removed with the column, attached and half-filled with the packing slurry. Packing solvent was layered on top of the slurry until all air was excluded. The system was assembled. About 6000 p.s.i. pressure was applied rapidly by opening the valve of the pressurized syringe pump, filled with methanol. Columns were left unattended at a maximum pressure setting of 6000 p.s.i. overnight.

A minimum pressure setting on the pump was also used a a safety precaution. The valve was then closed, and the column pressure was allowed to equilibrate for 5 h. Columns were then washed overnight with the mobile phase prior to use.

RESULTS AND DISCUSSION

A hydrocarbon standard solution with 12 components was separated to illustrate system performance with respect to both detection limits and resolution for different columns. Figs. 2–6 illustrate these separations. Efficiencies, mass sensitivity, resolution, and analysis time for this standard are presented for four columns in Table I. A 1 m × 0.2-mm fused-silica column with an efficiency of 80 000 plates was packed by the method described herein. The high efficiency of this column allows good comparisons to be made with shorter-length, efficient columns.

Values for the impedance, reduced plate height, and permeability are all in agreement with those reported previously by other workers for these types of columns (see Table I). The only exception is the high impedance of the coiled steel column. This column contains a compacted area even though the efficiency is fairly good.

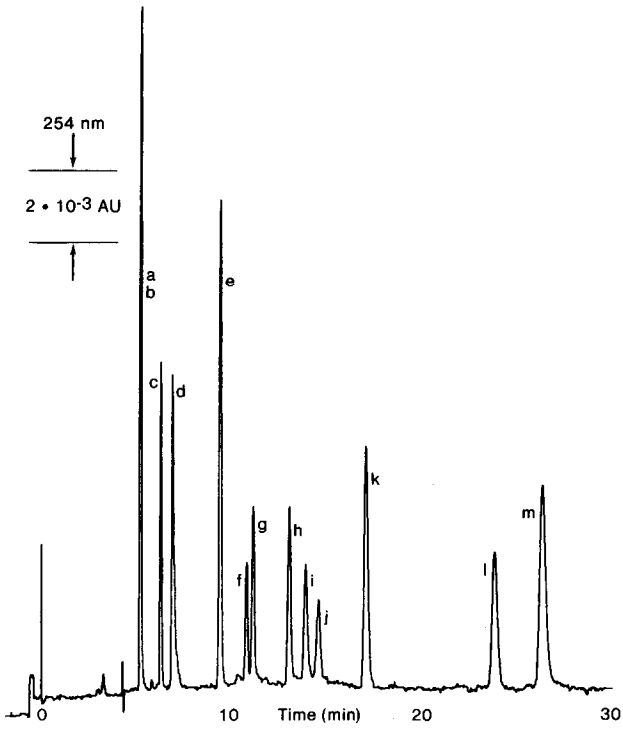

Fig. 2. HPLC of hydrocarbon standard solution with Isco microbore columns, 250 × 1 mm. Conditions: flow-rate, 25 μl/min; packing material, C_{18}, 3-μm particles; flow cell pathlength, 5 mm; flow cell volume, 0.37 μl; mobile phase, methanol–water (80:20); injection volume, 0.1 μl. Peaks: a = nitrophenol, 2.5 mg; b = phenol, 25 mg; c = acetophenone, 0.5 ng; d = nitrobenzene, 2.2 ng; e = benzophenone, 1.0 ng; f = toluene, 50 ng; g = bromobenzene, 150 ng; h = naphthalene, 25 ng; i = impurity; j = xylene, 50 ng; k = biphenyl, 1.0 ng; l = phenanthrene, 0.5 ng; m = anthracene, 1.0 ng.

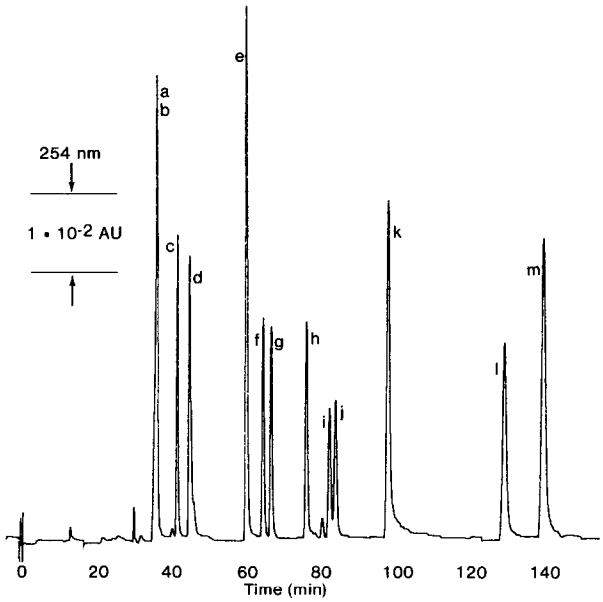

Fig. 3. HPLC of hydrocarbon standard solution with packed capillary column, 1000 × 0.2 mm. Conditions as in Fig. 2, except flow-rate, 1.2 μl/min. Peaks as in Fig. 2.

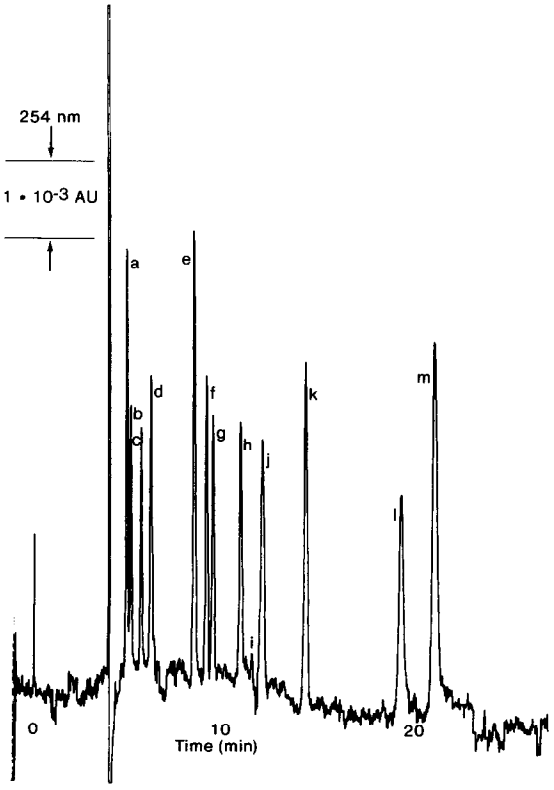

Fig. 4. HPLC of hydrocarbon standard solution with 1-mm pathlength flow cell. Conditions as in Fig. 2, except column, 300 mm × 0.5 mm glass-lined; flow-rate, 10 μl/min. Peaks as in Fig. 2.

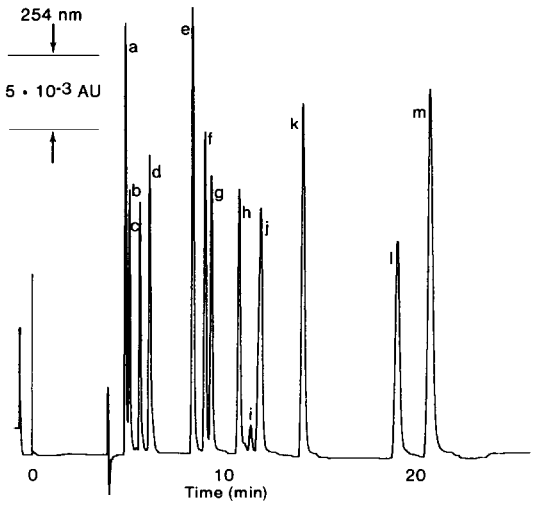

Fig. 5. HPLC of hydrocarbon standard solution with 5-mm pathlength flow cell. Conditions as in Fig. 4. Peaks as in Fig. 2.

The most significant advantage of the smaller packed columns is in improved mass sensitivity. The mass sensitivity of the 0.2-mm column is 13.2 times better than that of the 1-mm column. This improvement is due to the small void volume, 15 μl, of the 0.2-mm column. This results in less dilution of the sample and, consequently,

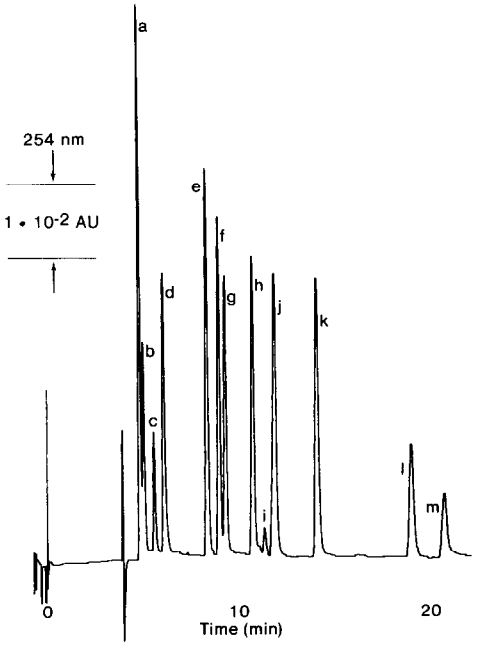

Fig. 6. HPLC of hydrocarbon standard solution with 10-mm pathlength flow cell. Conditions as in Fig. 4. Peaks as in Fig. 2.

TABLE I

COLUMN PARAMETERS

N = efficiency (plate number) of biphenyl ($k' = 2.6$); h = reduced plate height; minimum detection limit: biphenyl measured at a signal-to-noise ratio of 2 (5-mm flow cell); R_s = resolution of bromobenzene and toluene; E = impedance[9] = $t_0 \Delta P / N^2 \eta$, where η = viscosity = $8.1 \cdot 10^{-4}$ Ns/m^2, t_0 = void time and P = pressure; K_0 = specific permeability = H^2/E; H = plate height.

Column dimensions (mm)	N ($\times 10^4$)	h	*Minimum detection limit (pg)*	R_s	*Analysis time (min)*	E ($\times 10^3$)	K_0 ($\times 10^2$ cm^2)
250 × 1	3.4	2.4	111	1.25	30	2.7	1.9
300 × 0.5	3.3	3.0	48	1.33	23	4.5	1.8
840 × 0.5 (coiled)	5.6	4.9	63	1.9	140	11.1	1.9
1000 × 0.2	8.0	4.1	8.4	2.0	140	4.0	3.8

improves detection limits. By using a 5-mm pathlength flow cell instead of on-column detection[3,4] with a 0.2-mm pathlength, mass sensitivity is greatly enhanced. The narrow bores and small volume of the flow cell result in minimal broadening of the peaks, as illustrated by the excellent efficiencies reported with a 5-mm flow cell.

Flow cell geometry and volume becomes more important for smaller columns. To test the suitability of the Isco flow cells, three identical separations, by means of the 300 × 0.5 mm glass-lined column, were monitored with three different flow cells: 1-, 5- and 10-mm pathlength (Figs. 4–6). The data are presented in Table II. The relative intensity of the biphenyl signal between the 5- and 1-mm flow cells was 5 (0.97), which is close to the expected ratio. Because of the excellent response and lack of distortion from the 5-mm flow cell, this flow cell was used as the standard for comparison of mass sensitivity, efficiencies and other column parameters listed in Table I. Further calculations were performed to describe the performance of these flow cells for small-diameter columns.

The flow path of the flow cell consists of two parts: the first, effectively a narrow tube-shaped path between the column and illuminated light path, and the second, the illuminated volume. The variance of the narrow tube, σ_t^2 is calculated

TABLE II

FLOW CELL VARIANCE

Column: 300 × 0.5 mm C_{18}, 3 μm. N_{eff} = Number of effective plates; $\sigma^2_{flowcell}$ = variance from the flow cell.

Optical pathlength (mm)	*Illuminated volume (µl)*	$1/N_{eff}$ ($\times 10^{-5}$)			$\sigma^2_{flowcell}$ ($\times 10^{-2}$ µl^2)
		$k' = 1.1$	$k' = 2.6$	$k' = 3.8$	
10	0.50	4.93	3.39	2.89	13.6
5	0.25	3.79	3.00	2.94	4.2
1	0.03	3.63	2.80	2.71	1.1

using the second term of the Taylor–Golay equation, as discussed by Guiochon and Colin[8]:

$$\sigma_t^2 = \frac{\pi r^4 l F}{24 D_m} = (1.1 \times 10^{-3}\ \mu\text{l/min})\ (F)$$

where r = radius of the tube (0.051 mm), l = length (10 mm), F = flow-rate, and D_m (solvent diffusion coefficient) = $1.3 \cdot 10^{-4}$ mm^2/s which is a low estimate[8], appropriate for calculating the upper limit of σ_t^2.

The illuminated volume of the flow cell, V_{iv}, is a hollow cylinder, parallel to the light beam, which maximizes the pathlength for optimum mass sensitivity. This cylinder acts as a mixing chamber, and the variance of this illuminated volume, σ_{iv}^2, is determined by the following equation:

$$\sigma_{iv}^2 = \frac{V_{iv}^2}{K}$$

where K is a constant.

The effective efficiency, N_{eff}, is defined as $N_{eff} = V_R^2/\sigma^2$, where V_R is the retention volume, and $\sigma^2 = \sigma_{iv}^2 + \sigma_{system}^2$ (σ_{system}^2 = variance of column and system, except for illuminated volume.) Rearranging and substituting for σ^2, the following relationship can be derived:

$$\frac{1}{N_{eff}} = \frac{V_{iv}^2}{K V_R^2} + \frac{\sigma_{system}^2}{V_R^2}$$

A plot of $1/N_{eff}$ *versus* V_{iv}^2 was made for several components separated by the 300 × 0.5 mm column. The data are presented in Table II. The value of K was found to be 2 from the slope and the constant value, V_R^2, of the benzophenone data, k' = 1.1. Later-eluted peaks, k' = 3.8, displayed no measurable variance when similar plots were used. The total variance from the flow cell is

$$\sigma_{flowcell}^2 = \sigma_{iv}^2 + \sigma_t^2 = \frac{V_{iv}^2}{K} + (1.1 \cdot 10^{-3}\ \mu\text{l/min})\ (F)$$

The variances of three flow cells have been reported at 10 μl/min for comparison.

The effect of flow cell variance on the efficiency of the columns used in this work is calculated by:

$$\frac{N_{eff}}{N} = \frac{1}{1 + \dfrac{\sigma_{flowcell}^2}{\sigma_{column}^2}}$$

where σ_{column}^2 is column variance.

An optimum k' for separation, k' = 2, was used for the calculation of column variance. Results of these calculations are reported in Table III. The 1-mm flow cell exhibits excellent efficiency with all columns and would be the flow cell of choice to

TABLE III

EFFECT OF FLOW CELL VARIANCE ON OBSERVED EFFICIENCY

All data are calculated for $k' = 2$. $\sigma^2_{column} = V_R^2/N = V_0^2 (k' + 1)^2/N = 9 V_0^2/N$, where V_0 is the void volume.

Column dimensions (mm)	σ^2 *column* (μl^2)	N_{eff}/N		
		Flow cell pathlength		
		1	*5 mm*	*10 mm*
250 × 1	1.62	0.98	0.96	0.91
300 × 0.5	0.45	0.98	0.92	0.77
840 × 0.5	1.71	0.97	0.98	0.92
1000 × 0.2	0.03	0.93	0.48	0.19

improve resolution. The 5-mm flow cell improves mass sensitivity but with a small loss of efficiency and resolution. Consequently, this flow cell may be the flow cell of choice for improved mass sensitivity.

Although the Isco flow cells with longer illuminated path exhibit some broadening, when used with 0.2-mm columns in peaks with short retention times ($k' < 1$), the improved mass sensitivity outweighs this effect for many applications. Theoretically, the 5-mm flow cell should improve the sensitivity of a 0.2-mm column by a factor of at least 25 over on-column detection. These flow cells have the advantage of commercial availability.

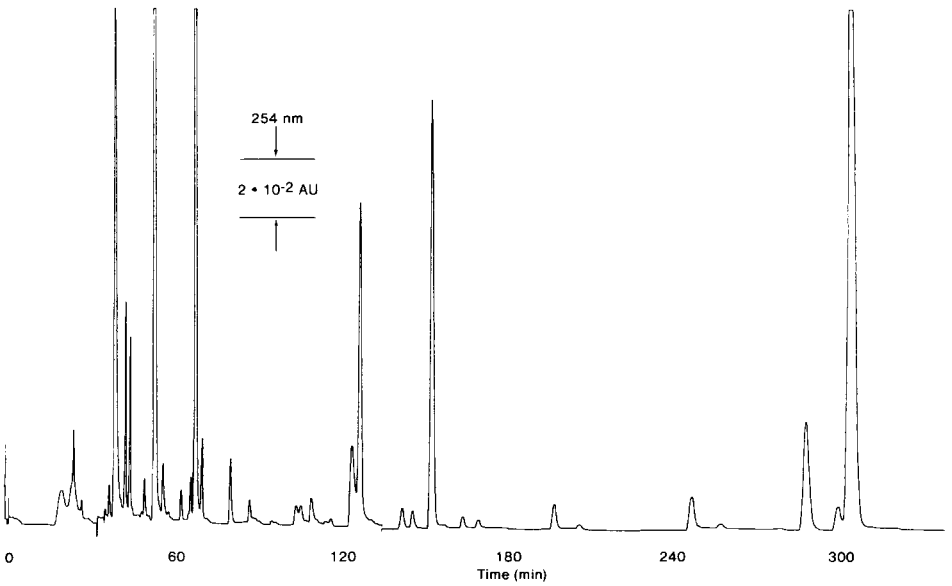

Fig. 7. HPLC of Halston cologne with packed capillary column. Conditions as in Fig. 3. Sample: Halston cologne diluted 1/10 with mobile phase.

Resolution was improved with the extended column lengths and high efficiencies reported; however, the time required for analyses with these longer columns may diminish this advantage. Two analyses with shorter columns and different mobile phases may be faster than one analysis with a 1-m column. However, where samples are limited and valuable, utilizing a column of 80 000 plates is still an important option. Fig. 7 illustrates a complex mixture, Halston cologne, separated on a 0.2-mm column with 80 000 plates. Several components, which were not resolved on a 1-mm column with 33 000 plates, are resolved in this separation by the increased plate number of the longer column.

CONCLUSIONS

The chromatograph described is suitable for monitoring separations of 0.200-mm columns with 5-mm pathlength flow cells. By packing these 0.200-mm fused-silica columns with a syringe pump, highly efficient columns with improved mass sensitivity are produced. Mass sensitivity is improved by a factor of 13 over that observed for 1-mm columns with direct connections. Work with shorter 0.200-mm columns will reduce analyses times greatly and improve the mass sensitivity.

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