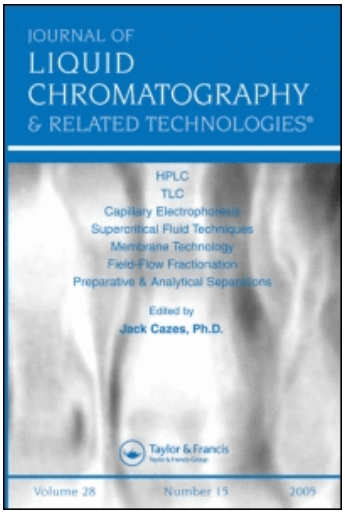

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COMPARISON OF A MULTIPORE COLUMN WITH A MIXED-BED COLUMN FOR SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

A new type of column packed with polystyrene gels which have a broad pore size distribution in a particle (henceforth referred to as the multipore column) was developed, and its performance was compared with the so-called linear column (henceforth referred to as the mixed-bed column). The chromatogram of the epoxy resin (Epikote 1009) on the multipore column showed a monomodal distribution, but the chromatogram on the mixed-bed column showed the inflection on the chromatogram at the molecular mass of about 10,000 as polystyrene equivalent. There was a big difference in the pore size distributions of gels packed in the two types of columns; the

pore size distribution of the multipore column showed the trapezoid, while that of the mixed-bed column had sharp maxima for pores with a diameter of 0.08 μm. This difference in the pore size distribution may be the main reason for the inflection phenomenon, though the calibration curves of these two types of columns were almost the same. The multipore column also had the advantage of the mixed-bed column in the linearity of the calibration curve.

INTRODUCTION

Size-exclusion chromatography (SEC) has been routinely and widely used for determining the molecular mass distributions and molecular mass averages of polymers, because of its rapidity, ease of operation, and good reproducibility.[1,2] The SEC columns are the most important part in the SEC system, and column technology in SEC has made great progress in the areas of high resolution and a linearity of a calibration curve during the past decade.

One of the possible procedures to obtain a linear calibration curve of the SEC columns is to mix polystyrene (PS) gels of several different pore sizes, followed by packing them.[3] This type of SEC column is called a linear column, which can separate polymers with a wide range of molecular masses in one column. This type of SEC column is referred to as a mixed-bed column in this paper.

However, when commercial epoxy resins were separated by SEC using the mixed-bed columns, a bimodal distribution (the inflection of the chromatogram) was often observed in our laboratory. The epoxy resins should have a monomodal distribution (a smooth chromatogram) according to the reaction mechanism. This observation may be attributed to the inadequate pore size distribution of PS gels in the column, or to the imperfect mixing of different pore sizes of the PS gels in the column, though the calibration curve was almost linear from 1,000 up to several million of PS molecular masses.

To overcome these problems, new PS gels which have a broad pore size distribution in a PS gel particle were developed. Details of the PS gels will be reported in the near future. Normally, the PS gels in a mixed-bed column are mixtures of particles having pores of the sizes with narrow distribution. On the contrary, new PS gels have pores of several different sizes with continuous distribution in every particle. In this paper, the column packed with this new type of PS gels is referred to as a multipore column.

Also, in this paper, chromatograms of epoxy resin having a broad molecular mass distribution were measured using both columns; the mixed-bed and the multipore columns. The problem on the inflection on the chromatograms was discussed, and then the precision of calibration curves for PS standards obtained with these two types of columns was discussed.

EXPERIMENTAL

Chromatographic System

Size-exclusion chromatography was carried out using the TOSOH (Tokyo, Japan) system, consisting of a CCPM pump, an SD-8012 on-line degasser, an AS-8000 auto sampler equipped with a 100-mL loop, a CO-8011 column oven, a UV-8010 ultraviolet (UV) detector at 254 nm, an LS-8000 LALLS photometer, and an RI-8012 refractive index (RI) detector controlled at 40°C. The data acquisition and analysis of the UV detector were performed by an SC-8020 data processor, and a C-7000 data processor was used to collect and calculate the LALLS and the RI data. Elution was delivered at a flow rate of the pump dial adjusted to 1.0 mL/min, and the pressure of the pump head was 4 MPa. The temperature of the columns was controlled at 40°C.

Solvent and Samples

Stabilized tetrahydrofuran (THF) as the eluent was analytical grade and was purchased from Wako (Osaka, Japan) without any treatment.

An epoxy resin sample was obtained from Yuka Shell Epoxy (Tokyo, Japan). Twenty PS standards with narrow-molecular-mass distribution were obtained from TOSOH (Tokyo, Japan). The weight-average molecular mass measured by low-angle laser light scattering and the groups used to evaluate the accuracy of the approximated calibration curve are shown in Table 1.

Separation Columns

Two column systems were used: (a) two multipore columns, TOSOH TSKgel Multipore H_{XL}-M (7.8 mm I.D. × 300 mm) packed with the new PS gel particles of about 5-μm diameter having an exclusion limit of Mw 2×10^6 and (b) two mixed-bed columns, TOSOH TSKgel GMH_{HR}-M (7.8 mm I.D. × 300 mm) packed with a mixture of polystyrene gel particles with different pore sizes

Table 1

The Weight-Average Molecular Mass of PS Standards and the Groups Used to Evaluate the Accuracy of the Approximated Calibration Curve

	Groups of PS Standards					
Mw_{LS}[a] $\times 10^{-5}$	1	2	3	4	5	6
0.103	*[b]	*	*	*	*	*
0.184	*	*	*	*	*	
0.194	*					
0.433	*	*	*	*	*	*
0.467	*					
0.698	*	*	*	*		
0.826	*					
1.00	*	*	*			
1.02	*					
1.07	*					
1.15	*	*	*	*	*	*
1.77	*	*	*	*	*	
1.83	*					
2.35	*	*	*	*		
3.62	*	*	*	*	*	*
4.08	*	*				
4.10	*					
4.76	*	*	*			
6.71	*	*	*	*	*	*
7.91	*	*				
Number of PS	20	13	11	9	7	5

[a] The weight-average molecular mass measured by low-angle laser light scattering.

[b] *: Selected PS standard.

of 5-μm diameter having an exclusion limit of Mw 4×10^6. The number of theoretical plates of the multipore column and the mixed-bed column measured with toluene was 47,000 plates/m and 42,000 plates/m. The values of the resolution of the multipore column and the mixed-bed column measured with two PS standards (Mw : 4.39×10^4 and 3.55×10^5) were 4.53 and 4.55, respectively.

The pore size distributions of PS gels packed into the two types of columns were determined using an AUTOSCAN-60 (Yuasa Ionics, Japan) mercury porosimeter.

Preparation of Sample Solutions

The epoxy resin was dissolved in THF in a concentration of 0.1, 0.05, 0.025, and 0.0125 mg/mL, respectively. Polystyrene standards were dissolved in a concentration of 1.0 mg/mL. Polystyrene standards were left 12 - 24 hrs prior to use with occasional gentle swirling. Toluene was added in a concentration of 0.04 vol% as a flow marker to all sample solutions. An analysis of each sample was repeated at least three times.

Calculation of Molecular Mass of Standard Polystyrenes

To determine molecular mass averages of the polystyrene standards used for calibration, low-angle laser light scattering (LALLS) was used to measure the weight-average molecular masses (Mw) of twenty polystyrene standards. The Mw thus obtained was adopted in the calibration curve.

Using the polystyrene standard with Mw 3.55×10^5 (vendor value), the instrumental constant (K') of LALLS was calculated by

$$K' = S_{LS} / S_{RI}Mw; \quad Mw = 335{,}000 \qquad (1)$$

where S_{LS} and S_{RI} are the peak area of the LALLS data and the peak area of the RI data of the polystyrene standard, respectively.

RESULTS AND DISCUSSION

Separation of an Epoxy Resin

Figure 1 shows chromatograms of epoxy resin (Epikote 1009) which have a broad molecular mass distribution measured with the multipore column and the mixed-bed column, respectively. The chromatogram obtained with the multipore column (Figure 1,a) shows a monomodal distribution; however, that obtained with the mixed-bed column (Figure 1,b) shows the inflection on the chromatogram at a molecular mass of about 10,000 as PS equivalent (marked by an arrow).

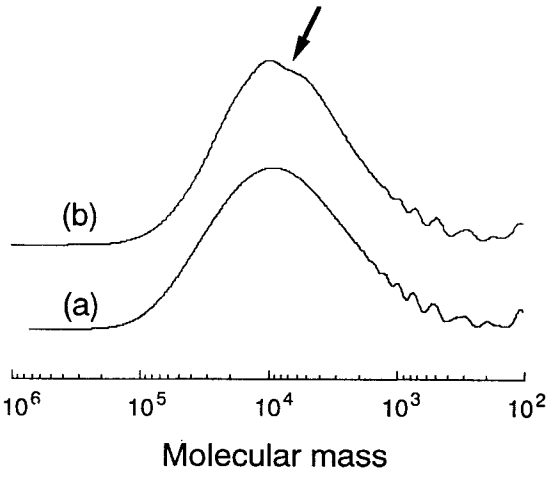

Figure 1. Chromatograms of the epoxy resin (Epikote 1009). (a) multipore column; (b) mixed-bed column.

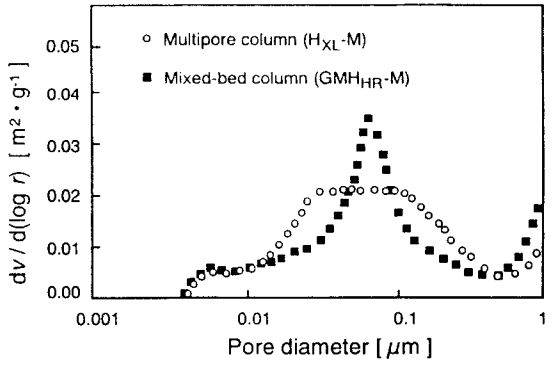

Figure 2. Pore size distribution of multipore column and mixed-bed column, measured by means of mercury porosimetry; $dv/d(\log r)$ is the derivative of the pore volume (v) with respect to the logarithm of the pore radius (r).

From these experimental results, it may be difficult to decide which chromatogram showed the correct molecular mass distribution. Taking into account the polymerization mechanism of epoxy resin, the molecular mass distribution may appear to be a monomodal distribution. Therefore, it may be concluded that Figure 1,a showed a correct molecular mass distribution. A

similar observation was reported by F.P.Warner et al.[4] using several individual columns inadequately connected. Accordingly, the chromatogram obtained with the multipore column can be assumed as normal.

There is no difference in the number of theoretical plates and the resolution between the two kinds of columns. The inflection on the chromatogram remained unchanged, even though the concentration of the epoxy resin was decreased from 0.1 to 0.0125 mg/mL. Therefore, the other factors except the number of theoretical plates, the resolution and capacity of the mixed-bed column, will cause the inflection on the chromatogram of the epoxy resin.

PS gels of five different pore sizes were mixed and packed in the mixed-bed column (GMH_{HR}-M). The separation range (10^2 - 4×10^6) of the molecular mass of the mixed-bed column is covered with those of five individual gels, and the calibration curve of the mixed-bed column is indistinguishable from that of the multipore column (Figure 3). The reason for the inflection on the chromatogram obtained with the mixed-bed column is neither the lack of PS gels which are adequate for the separation of the epoxy resin, nor the imperfect mixing of the gels of different pore sizes.

The pore size distributions of gels packed in the multipore column and also in the mixed-bed column are shown in Figure 2. The distributions measured by means of mercury porosimetry are good indexes for the pore size distribution of the gels in the column,[5] though the pore size distribution is determined in the dry state. In the mixed-bed column, the pore size distribution has sharp maxima for pore with a diameter of 0.08 μm, though the overall pore size distribution is ranging from 0.006 to 0.6 μm in pore diameter. On the other hand, the pore size distribution of the multipore column shows a trapezoid shape ranged from 0.02 to 0.1 μm in pore diameter. The pore volume of the gels in the multipore column around 0.01 to 0.05 μm and around 0.1 and 0.6 μm in pore diameter is greater than in the mixed-bed column. This difference in the pore size distribution may be the main reason for the inflection phenomenon.

Comparison of Calibration Curves

The mixed-bed column is called a "linear" column. However, the calibration curves are fitted to a cubic equation, in general. We were interested in making sure whether the calibration curve could be adequately approximated by a linear equation or not.

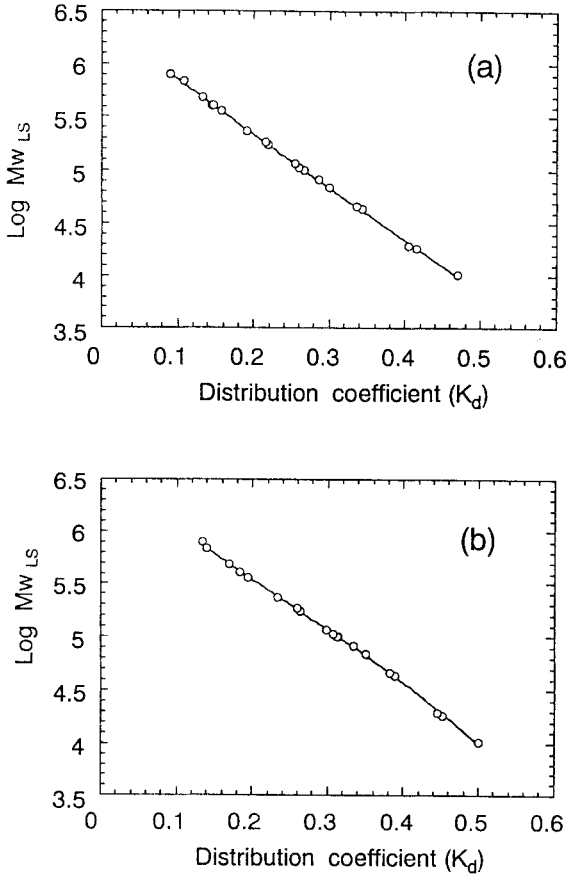

Figure 3. Polystyrene calibration curves fitted to a cubic equation. (a) multipore column; (b) mixed-bed column.

Using both the multipore and the mixed-bed columns, the precision of the calibration curves fitted to a cubic equation was compared with those fitted to a linear equation. Distribution coefficient K_d was given by

$$K_d = (V_e - V_0) / (V_t - V_0) \quad (2)$$

$$V_t = V_0 + V_i \quad (3)$$

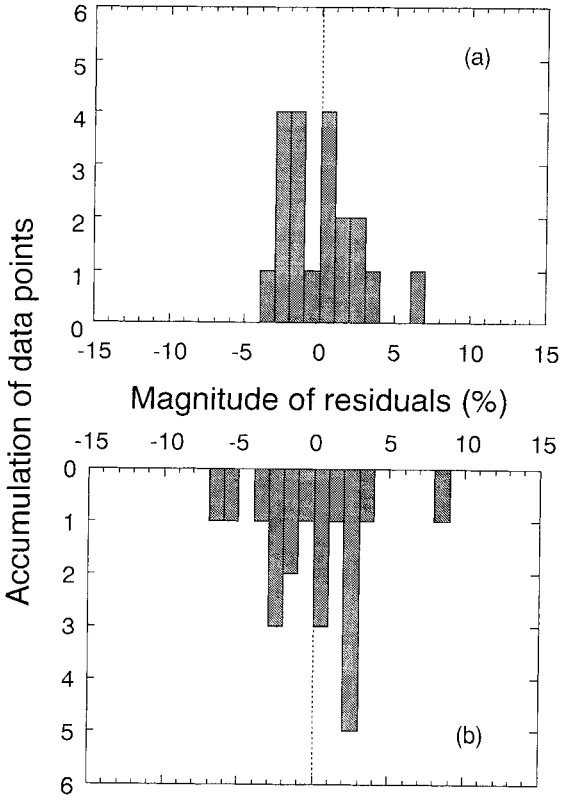

Figure 4. Plot of accumulation of the data points versus the magnitude of residuals fitted to a cubic equation. (a) multipore column; (b) mixed-bed column.

where V_e, V_0, V_t, and V_i refer to the retention volume of the solute, the void volume, the total liquid volume, and the internal pore volume of the gels in the column, respectively. The PS standards with Mw 8.42×10^6, 1.41×10^7 and 2.06×10^7 (vendor values) and the toluene were used as probes to estimate the accurate void volume and the total liquid volume, respectively. The void volume and the total liquid volume of the multipore column were 9.03 mL and 22.59 mL. Those of the mixed-bed column were 9.72 mL and 21.34 mL.

The calibration data in the molecular mass range of 10^4 - 10^6 for both the multipore column and the mixed-bed column plotted as log Mw measured by LALLS versus K_d of twenty PS standards, group 1 in Table1, are shown in Figure 3. The calibration curve can be usually fitted to a cubic equation. The

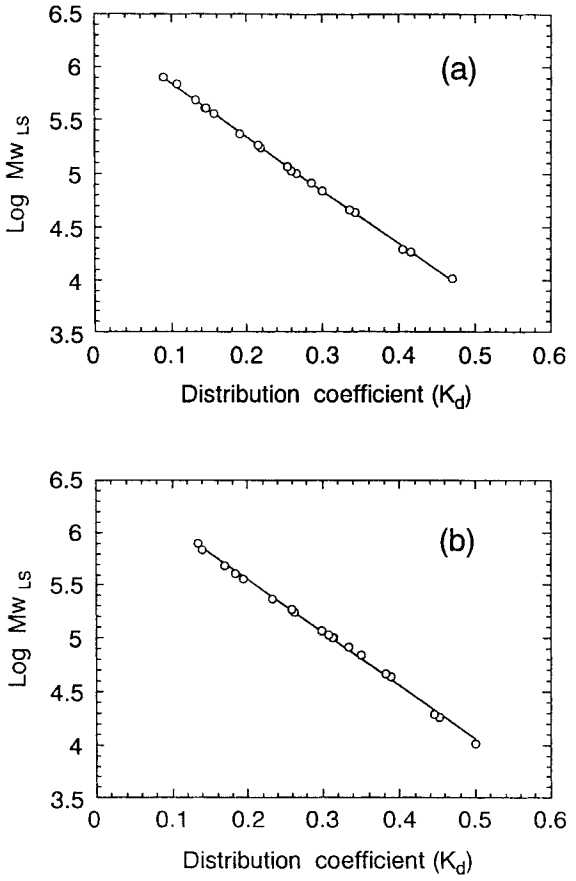

Figure 5. Polystyrene calibration curves fitted to a linear equation. (a) multipore column; (b) mixed-bed column.

fit of a cubic equation is displayed in Figure 3. To evaluate the validity of the cubic equations, the difference between the PS molecular mass values calculated by using the K_d values of the PS standards and the experimental values by LALLS was calculated as follows:

The definition of the residual[6] is defined as

$$\text{Residual (\% Error)} = 100(Mw_{fit} - Mw_{LS}) / Mw_{LS} \quad (4)$$

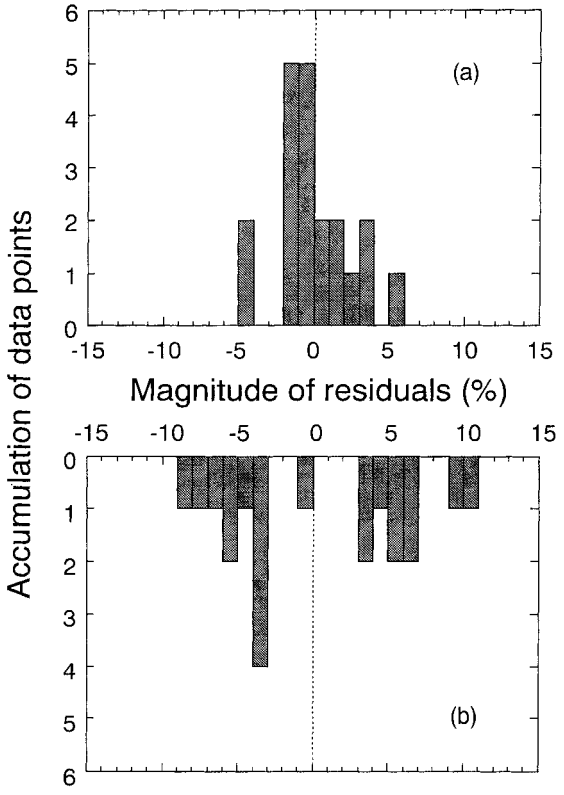

Figure 6. Plot of accumulation of the data points versus the magnitude of the residuals fitted to a linear equation. (a) multipore column; (b) mixed-bed column.

where Mw_{fit} is the molecular mass calculated by the fitted equation, and Mw_{LS} is the molecular mass measured by LALLS. The accumulation number of the residuals with the same %Error was plotted against the magnitude of the residuals in Figure 4. The residuals of the multipore column and the mixed-bed column are spread over the range of about ± 7%, except one data. The calibration data and the fit of a linear equation for the multipore column and also for the mixed-bed column are shown in Figure 5. Accumulations of the data points were plotted against the magnitude of the residuals in Figure 6 as the same manner as in Figure 4. The residuals of the multipore column are distributed over the range of -5 to 6%, while those of the mixed-bed column are spread over the range of about ± 10% and there are few data around 0%.

Table 2

Influence of the Number of the PS Standards for Calibration

No. of PS	Multipore Column (Linear Equation) Mw	Multipore Column (Cubic Equation) Mw	Mixed-Bed Column (Cubic Equation) Mw
13	4.30×10^4	4.43×10^4	4.10×10^4
11	4.31×10^4	4.25×10^4	4.06×10^4
9	4.32×10^4	4.41×10^4	4.24×10^4
7	4.33×10^4	4.29×10^4	4.25×10^4
5	4.35×10^4	4.29×10^4	4.16×10^4
RSD(%)	0.45	1.86	2.01
13	3.53×10^5	3.52×10^5	3.60×10^5
11	3.51×10^5	-[a]	3.61×10^5
9	3.51×10^5	3.67×10^5	3.68×10^5
7	3.54×10^5	3.60×10^5	3.67×10^5
5	3.54×10^5	3.58×10^5	3.63×10^5
RSD(%)	0.43	1.72	0.96

[a] The equation was not calculated by the SC-8020 data processor.

As can be seen in the above experiments, a more accurate calibration curve is obtained by the fit of a cubic equation for the conventional mixed-bed column, while even if by the fit of a linear equation, a calibration curve for the multipore column can be obtained accurately as done by a cubic equation. The approximation of a calibration curve to a linear equation is important for the use of SEC for many purposes in quality control sections and analytical sections in factories. The approximation of a calibration curve to a linear equation can decrease the number of PS standards used for constructing the calibration curve without the sacrifice of accuracy and also can save the time for calibrating experiment.

The number of the PS standards for calibration was decreased from 13 every 2 down to 5, from group 2 to 6 in Table 1. One was selected among PS standards having similar molecular mass. The PS standards were culled out to keep equal intervals between log Mw_{LS}. The calibration curve for the multipore column was fitted to a linear equation and a cubic equation, and that for the mixed-bed column was fitted to a cubic equation. Two polystyrene standards (Mw_{LS} : 4.33×10^4 and 3.62×10^5) were used to calculate Mw using these

calibration curves, and the results are listed in Table 2. In the case of the fit to a linear equation for the multipore column, the weight-average molecular mass (Mw) was almost the same value regardless of the number of PS standards.

On the other hand, in the case of the fit to a cubic equation for both the multipore column and the mixed-bed column, the value of Mw varied with the number of PS standards.

CONCLUSIONS

The inflection on the chromatogram of the epoxy resin was caused by the inadequate pore size distribution of PS gels packed in the mixed-bed column. The trapezoid pore size distribution may be required for determination of adequate molecular mass distribution of the polymers with broad molecular mass distributions.

The best fit of the calibration curve for the mixed-bed column was a cubic equation. The calibration curve for the multipore column was fitted adequately to both a cubic equation and a linear equations.

The multipore column has the merit of using fewer PS standards for constructing the calibration curve without the sacrifice of accuracy.

REFERENCES

1. H. G. Barth, B. E. Boyes, C. Jackson, Anal. Chem., **66**, 595R (1994).

2. T. Provder, H. G. Barth, M. W. Urban (Editors), **Chromatographic Characterization of Polymer**, Advances in Chemistry Series 247, ACS, Washington, DC, 1995.

3. Y. Kato, S. Kido, T. Hashimoto, J. Polym. Sci., Polym. Phys. Ed., **11**, 2329 (1973).

4. F. P. Warner, Z. Dryzek, L. L. Lloyd, Soc. Plast. Eng. Annu. Tech. Conf., **44**, 457 (1986).

5. A. Schmid, L-I. Kulin, P. Flodin, Macromol. Chem., **193**, 1579 (1992).

6. T. H. Mourey, S. M. Miller, S. T. Balke, J. Liq. Chromatogr., **13**, 435 (1990).

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