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Preparative high-performance liquid chromatographic columns with an intra-column injection system

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ABSTRACT

Glass and stainless-steel preparative sorbent bed injection columns are described, which can be packed with spherical particles of silica gel and silica-based reversed-phase material, particle size 15 μm, by simple gravity sedimentation. Columns of I.D. 42–44 mm and 250 mm long give symmetrical peaks with loads of 1–2 g; by using sorbent bed injection their lifetime is greatly increased and the use of a compression piston is not needed.

INTRODUCTION

Preparative columns for high-performance liquid chromatography (HPLC) with properties resembling those of analytical columns are attracting increasing attention. With increasing diameter of the columns problems of the maintenance of a sufficiently long lifetime and symmetry of peaks also increase. The usual cause underlying these impaired properties (in addition to inhomogeneities of the sorbent bed and an insufficient distribution and removal of the mobile phase) is the formation of a void volume on the column head, owing to the settling or compression of the sorbent bed during operation. Silica sorbents for HPLC regarded as incompressible dissolve slightly in aqueous mobile phases, so that here also the formation of void volumes should be taken into account.

These difficulties can be forestalled by using a column, the lower part of which consists of a moving piston (Jobin-Yvon, Paris, France), or by radial compression of a column made of elastic plastic in a compression device (Millipore–Waters, Milford, MA, U.S.A.). Further possibilities are offered by systems where the sample is injected directly into the sorbent bed, reported for analytical columns. The injection can be carried out either centrally [1], through the upper end fitting or from the side [2] into the upper part of the column. In such systems difficulties were met when the injection tube had to be introduced into the column packing, and to achieve high efficiencies two pumps or a flow-metering valve system had to be employed in order to ensure suitable ratio of the flows of the mobile phase through the injection branch and the column itself.

Packing of columns of diameter $\geqslant 50$ mm is also a serious problem. A simple geometric increase in systems used in the slurry packing of silica-based analytical

columns at pressures about 50 MPa places great requirements on the pump used. In addition, the strength requirements of the design of the column also increase considerably.

In this paper we describe the design and chromatographic properties of preparative columns with injection into the sorbent bed, the column tubes of which may be made either of glass or of stainless-steel, and can be packed by simple gravity sedimentation of the sorbent suspension.

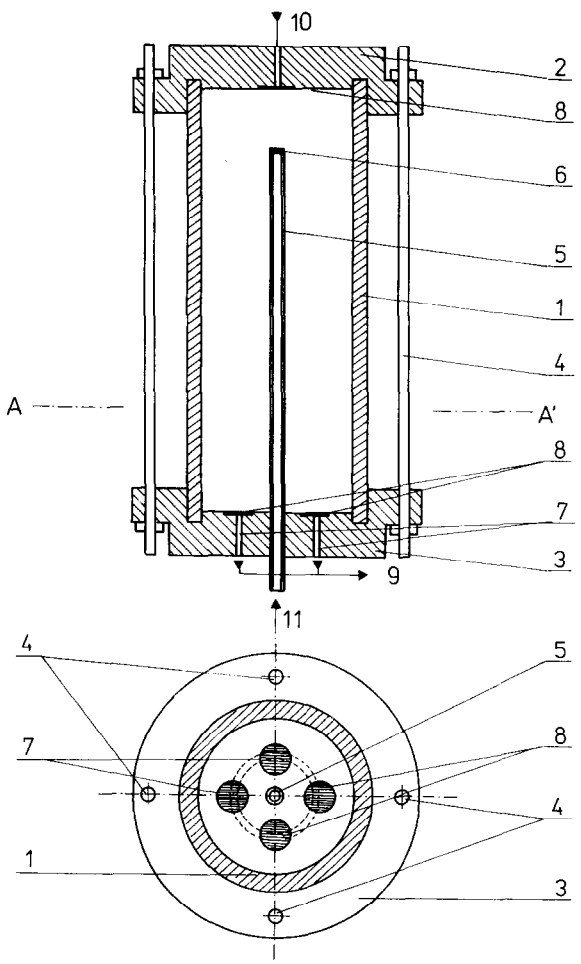

Fig. 1. Schematic diagram of the preparative column with intra-column injection. 1 = Column tube (glass or stainless-steel); 2 = upper end fitting; 3 = lower end fitting; 4 = end fitting fixing clamp; 5 = sample injection tube; 6 = stainless-steel frit; 7 = outlet holes for the mobile phase, placed on a concentric circle with half of the inner diameter of the column tube; 8 = stainless-steel gauzes; 9 = outlet of mobile phase; 10 = inlet of mobile phase; 11 = sample supply.

EXPERIMENTAL

Materials and apparatus

The silica-based sorbents used (mean particle size d_p = 15 μm), Separon SGX and Separon SGX C_{18}, were obtained from Tessek (Prague, Czechoslovakia).

All chemicals were of analytical-reagent grade. Methanol and isopropanol were supplied by Lachema (Brno, Czechoslovakia) and *n*-heptane by Merck (Darmstadt, F.R.G.). All test substances were supplied by Lachema.

The preparative chromatograph consisted of a membrane pump (Orlita type) constructed in the laboratory, a UVD 254 UV detector (Development Workshop, Czechoslovak Academy of Sciences, Prague, Czechoslovakia) and a TZ 4221 recorder (Laboratory Instruments, Prague, Czechoslovakia).

Columns and injection procedure

A schematic diagram and cross-section of the column [3] are shown in Fig. 1. The sample is injected into the sorbent through tube 5, provided with a frit 6 and terminated

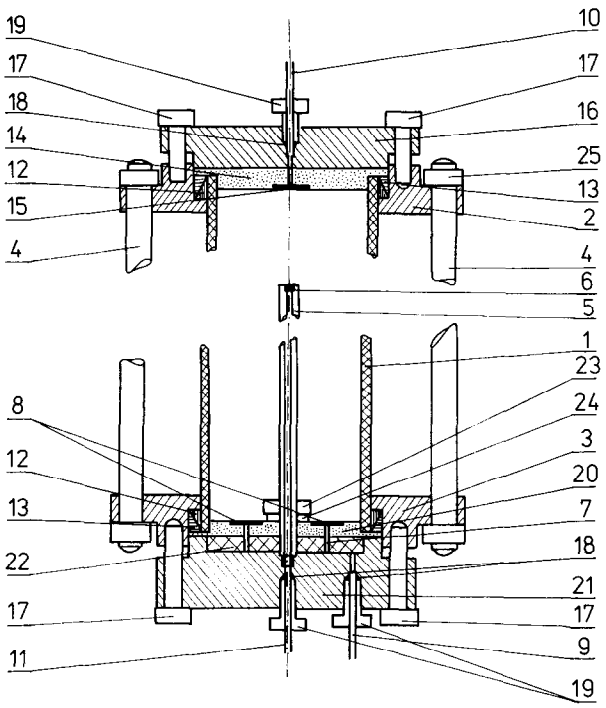

Fig. 2. Design of glass preparative column. 1 = Glass tube (borosilicate glass, 250 mm × 42 mm I.D., wall thickness 3.5 mm); 2 = upper end fitting; 3 = lower end fitting; 4 = fixing clamp (stainless-steel, diameter 6 mm); 5 = injection tube (200 mm × 2 mm I.D.); 6 = stainless-steel frit (porosity 7 μm); 7 = flow-off opening; 8 = stainless-steel gauze (porosity 7 μm); 9 = outlet capillary; 10 = inlet capillary for mobile phase; 11 = sample inlet capillary; 12 = PTFE sealing ring; 13 = stainless-steel sealing ring; 14 = PTFE sealing piece; 15 = stainless-steel gauze (porosity 7 μm); 16 = upper part of inlet end fitting; 17 = screw; 18 = sealing cone; 19 = bored screw; 20 = PTFE sealing piece; 21 = upper part of lower end fitting; 22 = plug of lower end fitting; 23 = injection tube fixing nut; 24 = support; 25 = clamp nut.

at four fifths of the column length in the stop-flow regime. The flow of the mobile phase by-passes the column by means of the three-way valve; at the same time, the inlet into the column is closed. At the same moment, the closing injection valve is opened, the sample is injected manually or by using an auxilliary pump, the injection valve is closed and the mobile phase flow through the column is renewed by switching over the three-way valve. Functioning of the two valves can be joined and substituted using a standard six-way valve.

The design of the glass laboratory version of the column is shown in Fig. 2. To seal the column tube 1 in stainless-steel end fittings 2 and 3, an earlier arrangement was used [4] which prevents axial pressures on the sealed tube. The channels joining flow-off openings 7 are formed in the upper part 21 of the lower end fitting 3 under the plug 22. The injection tube 5 with the frit 6 is screwed into the upper part of the end fitting 21.

The columns were packed by the simple gravity sedimentation of a 10–20% suspension of the respective sorbent in a suitable solvent. For silica gel, methanol, dioxane or a mixture thereof can be used, whereas ethyl acetate, acetone or heptane is suitable for use in the reversed-phase mode. Packed columns were washed with ethyl acetate (silica) or methanol (reversed-phase mode) and equilibrated with the corresponding mobile phase (*ca.* ten column volumes).

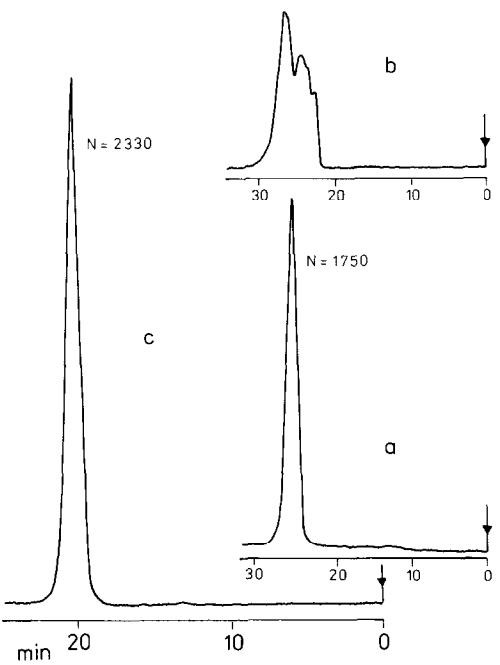

Fig. 3. Comparison between conventional loop injection and intra-column injection. (a) Freshly packed column; (b) column after 14 days of operation; in both instances 1-ml loop injection at the column head; (c) intra-column injection. Column, 250 mm × 44 mm I.D., Separon SGX C_{18}, 15 μm; mobile phase, methanol–water (3:1); injection, 15 mg of benzene in 1 ml of mobile phase; flow-rate, 23 ml/min. N = Plate number.

RESULTS AND DISCUSSION

The importance of the use of intra-column injection for the lifetime of columns is illustrated in Fig. 3. With classical loop injection through a six-way valve in a freshly packed column, a peak completely acceptable with regard to efficiency and asymmetry is obtained (Fig. 3a). After 14 days of operation, however, the same column with classical loop injection appears to be completely unsuitable for use (Fig. 3b). With intra-column injection (Fig. 3c), the column gave a symmetrical peak also after daily operation for 2 months, and its efficiency was also higher, in spite of the fact that with such an injection procedure its effective length is shorter than that obtained with the classical loop.

In view of the fact that the aim of preparative HPLC is to isolate the largest possible amount of maximally pure product, the paramount requirement to be met by preparative columns is symmetry of peaks at a high mass load. The requirement of a high efficiency with small injections, which is the key requirement with analytical

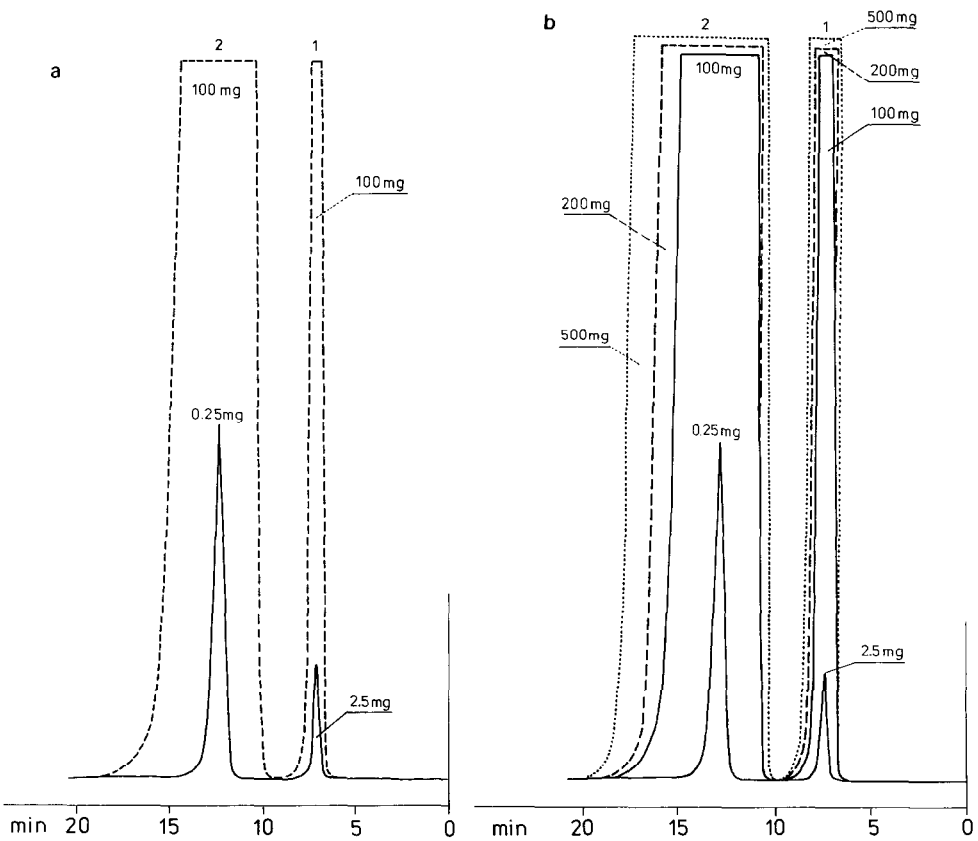

Fig. 4. Loadability test of (a) glass and (b) stainless-steel columns with intra-column injection in the normal-phase mode. Columns: 250 mm × 42 mm I.D., Separon SGX, 15 μm, mobile phase, *n*-heptane–0.3% isopropanol; flow-rate, 29 ml/min. Sample: 1 = toluene; 2 = nitrobenzene.

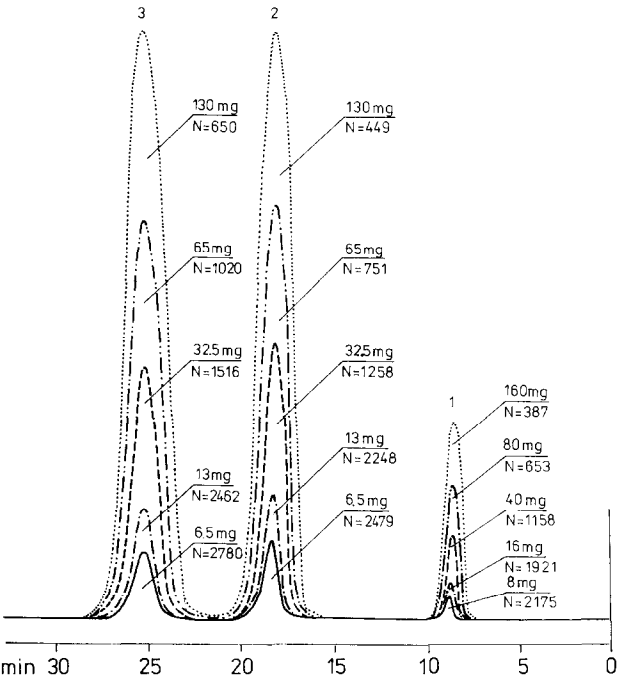

Fig. 5. Effect of the amount of substance injected into the sorbent bed on efficiency. Column: 250 mm × 42 mm I.D., stainless-steel, Separon SGX C_{18}, 15 μm; mobile phase, methanol–water (3:1); flow-rate, 24 ml/min. Sample: 1 = acetone; 2 = benzene; 3 = toluene.

columns, is of secondary importance. This is why our greatest attention was concentrated on the shape of the peaks and on the loadability of the columns to be observed with baseline separation.

The loadability of the normal-phase system and the shape of the peaks in the glass column are illustrated in Fig. 4a. Superposition of the chromatograms shown in Fig. 4b, obtained with a stainless-steel variant of the column, shows that baseline separation could be achieved with loads higher than the maximum value shown (1 g).

Fig. 5 demonstrates the superposition of chromatograms in the reversed-phase mode, showing the dependence of the column efficiency on the increasing mass loading. The loadability test carried out with a glass (Fig. 6a) and a stainless-steel (Fig. 6b) column shows that a small change in the composition of the mobile phase can increase the loadability from 780 mg up to as much as 2 g, extending the separation time only slightly while maintaining the symmetry of peaks and separation virtually to the baseline. The separation of the phenol–*p*-cresol pair as an example of a mixture of more polar solutes in the reversed-phase mode is shown in Fig. 7. It can be seen that a 720-mg injection again gives separation to the baseline and symmetrical peaks.

Results of tests of the peak shapes obtained using an optimized variant of the stainless-steel column in the reversed-phase mode are summarized in Fig. 8. It can be seen that also with injection of 60 mg of benzene as a single solute (Fig. 8a) and 120 mg of benzene–toluene mixture (Fig. 8b) the peak asymmetry factor is unity. The same is

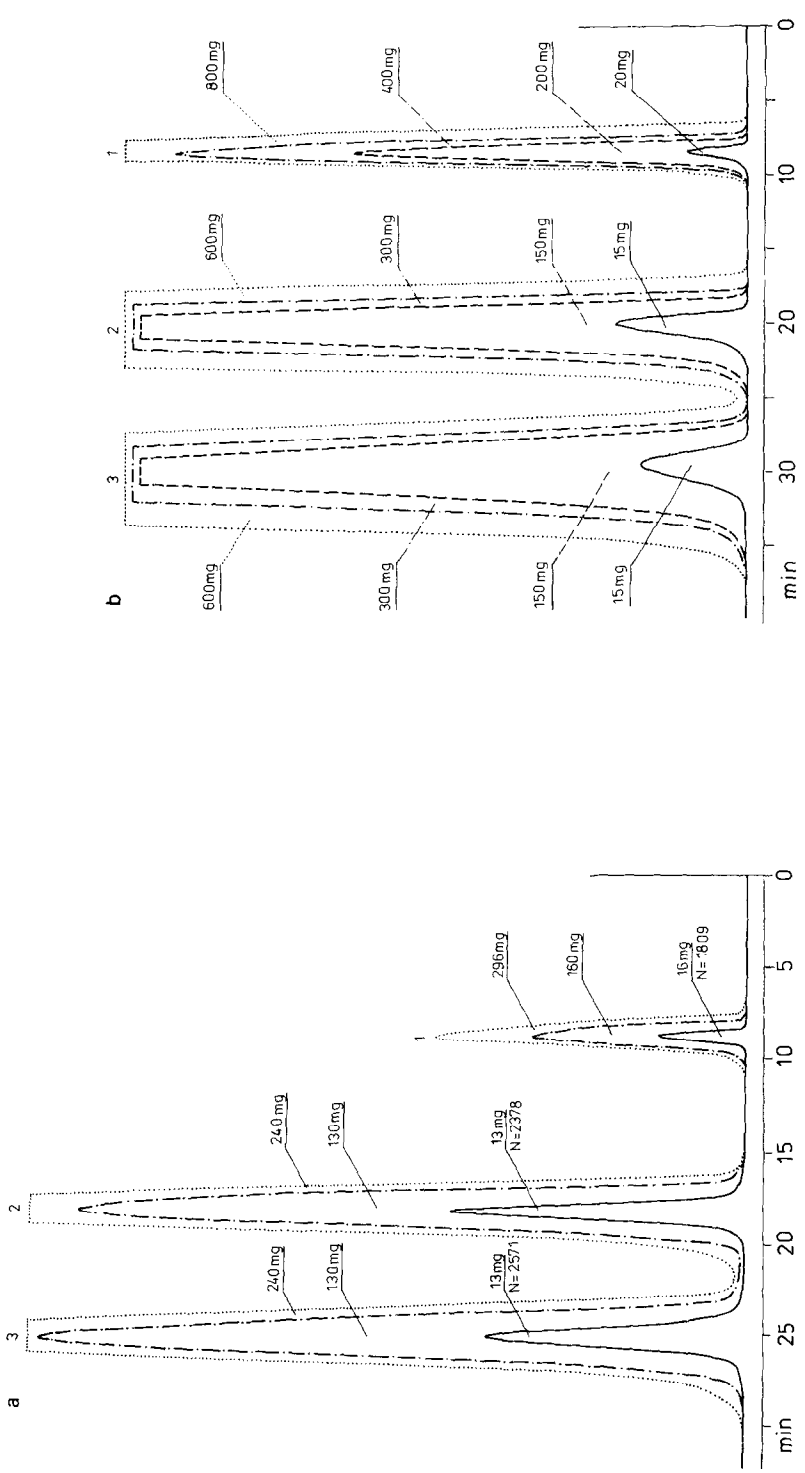

Fig. 6. Effect of change in composition of the mobile phase on loadability of column with sorbent bed injection. (a) Mobile phase, methanol–water (3:1); glass column, 250 mm × 42 mm I.D. (b) Mobile phase, methanol–water (7:3); stainless-steel column, 250 mm × 42 mm I.D. Packing, Separon SGX C_{18}, 15 μm; flow-rate, 24 ml/min. Sample: 1 = acetone; 2 = benzene; 3 = toluene.

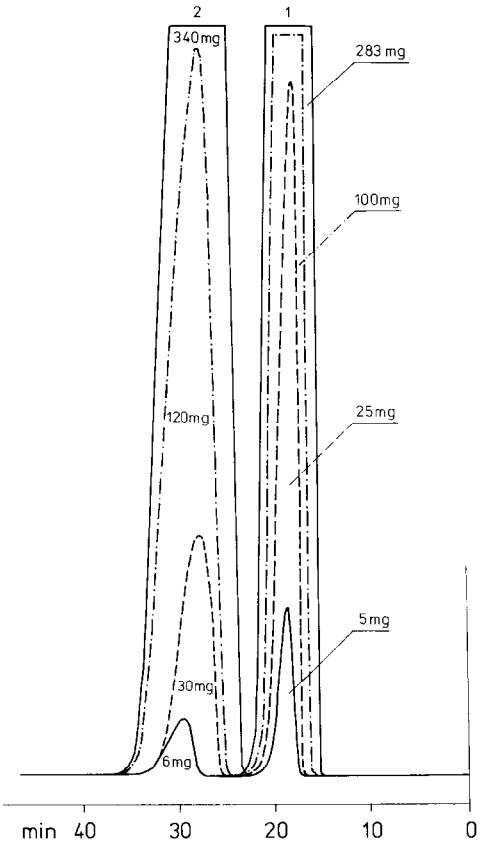

Fig. 7. Loadability of column with sorbent bed injection in the separation of the pair phenol (1) and *p*-cresol (2). Stainless-steel column (250 mm × 42 mm I.D.), Separon SGX C_{18}, 15 μm; mobile phase, methanol–water (49:51); flow-rate, 25 ml/min.

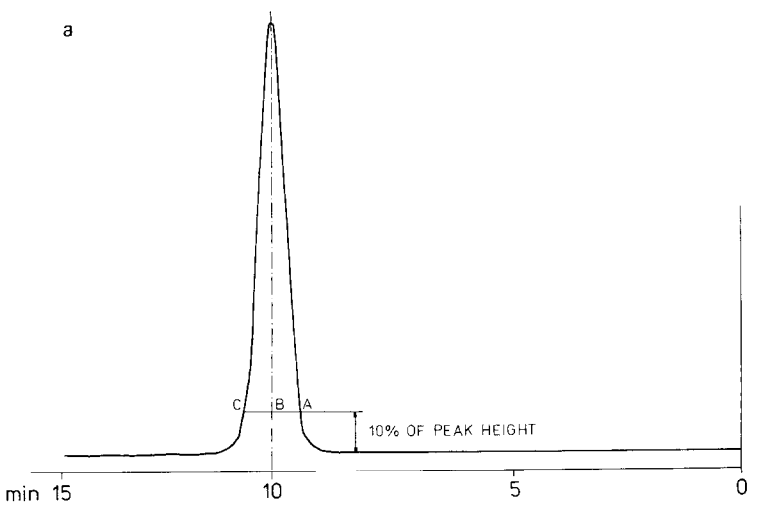

Fig. 8.

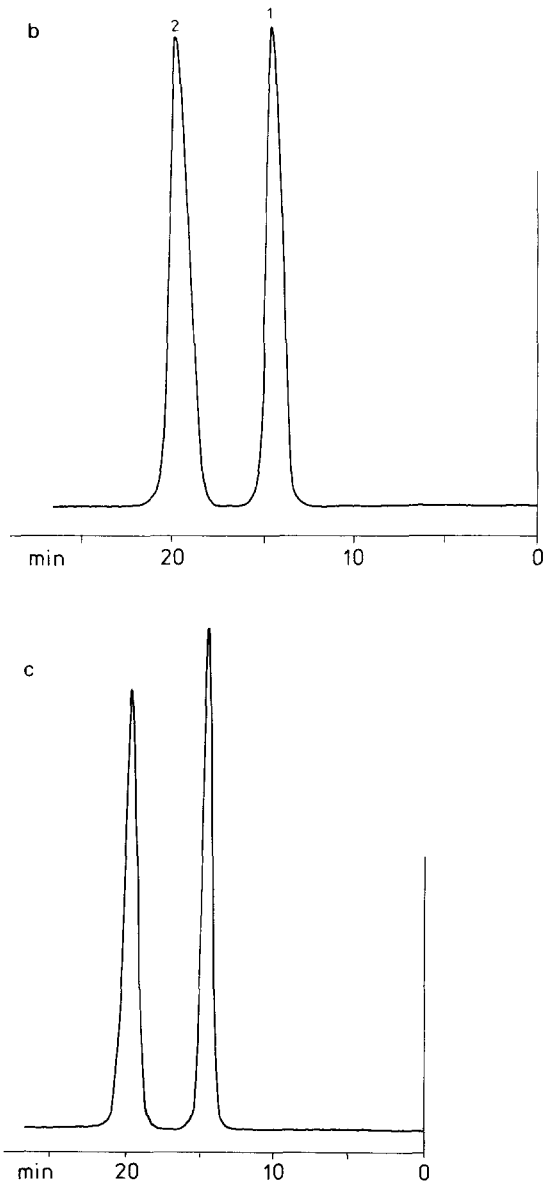

Fig. 8. Test of the peak shape of sorbent bed injection column: (a) 60 mg of benzene in 5 ml of mobile phase, $N = 1282$, peak asymmetry factor = AB/BC = 1.0; (b) mixture of 60 mg of benzene (1) and 60 mg of toluene (2) in 4 ml of mobile phase, $N = 1162$ and 1644; (c) mixture of 15 mg of benzene (1) and 15 mg of toluene (2) in 1 ml of mobile phase, $N = 2501$ and 2739. Stainless-steel column (250 mm × 42 mm I.D.), Separon SGX C_{18}, 15 μm; mobile phase, methanol–water (3:1), flow-rate, (a) 43.6 and (b and c) 31.2 ml/min.

true for the injection of 30 mg of a benzene–toluene mixture (Fig. 8c), where the efficiency with the given particle size increases to values comparable to that of columns of analytical size.

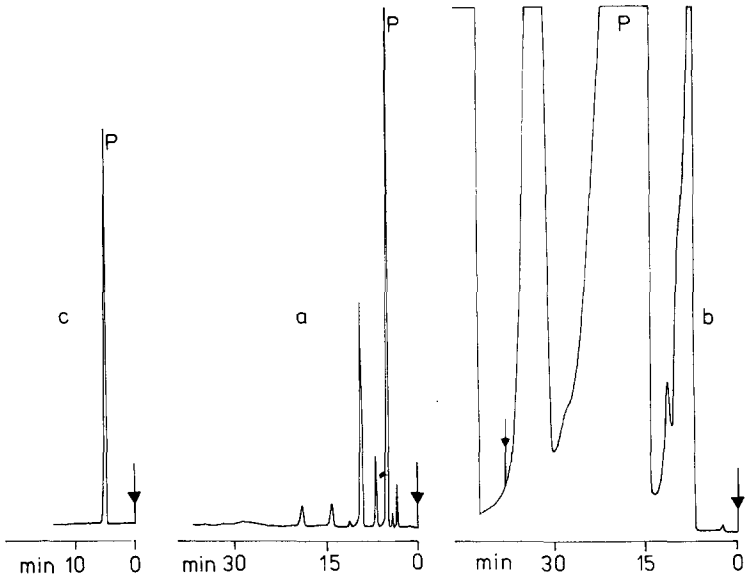

Fig. 9. Analytical and model part of the separation of anilinomonochlorohydrin from the aniline–epichlorohydrin reaction mixture: (a) starting reaction mixture and (c) final product (P). Column, CGC (150 mm × 3.3 mm I.D.), Separon C_{18}, 7 μm; mobile phase, methanol–water (6:4); flow-rate, 0.4 ml/min; injection, starting mixture 5 μl, product 0.5 μl. (b) Model separation. Column, 200 mm × 6 mm I.D., Separon SGX C_{18}, 15 μm; mobile phase, methanol–water (45:55), 0–35 min, and washing with methanol; flow-rate, 1.4 ml/min; injection, 10 mg of reaction mixture in 400 μl of mobile phase.

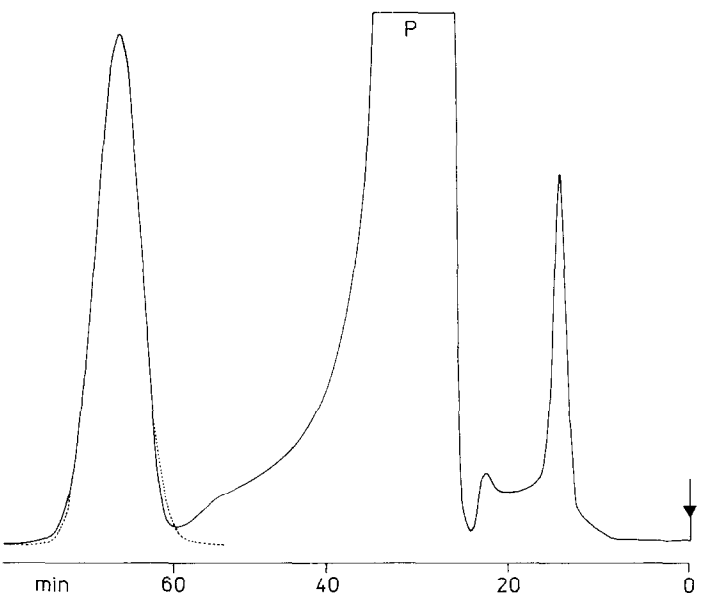

Fig. 10. Preparative separation of anilinomonochlorohydrin (P). Column, 250 mm × 44 mm I.D., Separon SGX C_{18}, 15 μm; mobile phase, methanol–water (45:55); flow-rate, 36.5 ml/min; injection, 0.5 g of reaction mixture in 20 ml of mobile phase.

A practical example is the isolation of anilinomonochlorohydrin from the reaction mixture after aniline in excess has reacted with epichlorohydrin and the main part of unreacted aniline has been removed by distillation; an analytical chromatogram of the mixture is shown in Fig. 9a. Fig. 9b shows a model preparative separation (200 mm × 60 mm I.D. column), with the same packing as in the preparative column, and Fig. 9c illustrates the purity of the product (P). Isolation of the product from the injection of 0.5 g of the mixture in 20 ml of the mobile phase is illustrated in Fig. 10. The shape of the peak next to the product illustrates the symmetry obtained; the gaussian shape is represented by the dotted line. It can be seen that in this instance a column of the given dimensions allows a few grams of pure product to be obtained for further synthetic work. The total time required for a single preparative experiment, including washing with methanol after trapping the product and equilibration of the column in the initial mobile phase composition, did not exceed 80 min; the yield in one injection was about 350 mg.

The column described above offers several advantages. It can be easily packed with sorbent by simple gravity sedimentation. Owing to the counter-current flow arrangement of the injection tube from below, complicated introduction of the injection system into the sorbent bed is avoided, and the injected sample is adequately distributed along the cross-section of the column. The sorbent layer above the injection point "replaces" the compression piston, serves as the saturation column in those instances where the sorbent dissolves slightly in the mobile phase and may also trap some impurities from the mobile phase. With glass column tubes coloured impurities can be observed visually, and the sorbent layer above the injection point can easily be supplemented or exchanged without reducing the column efficiency.

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