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Note

Regeneration of reversed-phase high-performance liquid chromatographic columns by flow reversal

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After several months of continuous operation on a high-performance liquid chromatographic (HPLC) column, the maintenance of good chromatographic efficiency usually requires the renewal of the column or its refilling. The most common factors that lead to deterioration of silica-based reversed-phase columns are column head contamination or column inlet void formation. Both problems can sometimes be overcome by repacking the column head with new material identical with that already in the column or, less ideally, with related materials (*e.g.*, pellicular support or glass beads). However, these restorative operations do not last for long, have to be frequently repeated and their use is not advisable when the void or the contaminated region is deeper than 1 cm[1]. The possibility of reversing the flow so that the old column offers an even, well packed and uncontaminated surface is not often considered, because this solution is usually not recommended by the suppliers. However, we have observed that, at least for some chromatographic supports, this is a very good alternative for increasing the lifetime of reversed-phase HPLC columns.

EXPERIMENTAL

The chromatographic analyses were performed on a Waters Assoc. system consisting of a 710 WISP autosampler, a 680 gradient controller, two 6000A pumps, a Model 440 detector fixed at 436 nm and a Data Module integrator/recording unit. The different HPLC columns used are indicated under Results and discussion. A guard column (2.3 × 0.45 cm I.D.) was placed between the injector and the column. This guard column was packed with Corasil/C_{18} pellicular support (Waters Assoc.) when using octadecylsilane columns and with Perisorb/C_2 pellicular support (Merck) when using a trimethylsilane column.

Repacking and void elimination of the column heads was carried out by successive additions of pellicular support (the same pellicular support as used in guard columns) followed by submission of the column to a high pressure (2000–3000 p.s.i.) for 15–30 min between each addition.

Derivatization of amino acids with 4-(dimethylamino)azobenzene-4′-sulphonyl (dabsyl) chloride and their chromatographic separation was accomplished following the method of Chang *et al.*[2].

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RESULTS AND DISCUSSION

Fig. 1 shows the chromatographic behaviour of dabsyl derivatives of eighteen primary amino acids (a) on a C_{18} reversed-phase column with an irreversibly contaminated and channeled head, and (b) on the same column after repacking the column head with Corasil/C_{18} pellicular support and reversing the original flow. It is clear that the reversal of the flow greatly improves the symmetry and resolution of the peaks, giving a chromatogram very similar to those obtained with a new column (not shown). Simple repacking of the former head with Corasil/C_{18} pellicular support also produces a good recovery of column performance, but the resolution is better and lasts for much longer when in addition the flow is reversed. In any event, repacking of the column head has to be carried out before the reversal of the column flow in order to eliminate the generation of voids in the new head. It should be noted that we use Corasil/C_{18} pellicular support (identical with that used in the guard

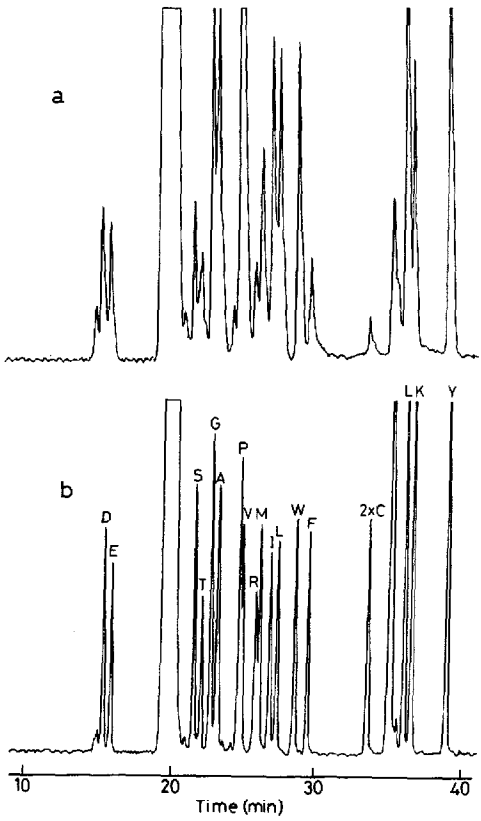

Fig. 1. (a) Chromatographic separation of dabsyl amino acids with a deteriorated reversed-phase C_{18} column and (b) with the same column after repacking the former head with Corasil/C_{18} pellicular chromatographic support and reversal of the flow. Elution was performed by a gradient between 10 m*M* sodium phosphate (pH 6.5) and acetonitrile, both solvents containing 4% of dimethylformamide, according to the method of Chang *et al.*[2]. Column temperature, 50°C. See IUPAC-IUB rules[3] for one-letter notation.

column) instead of the original porous support packed in the column, as the former is cheaper, easier to pack and readily available.

The chromatograms presented in Fig. 1 were obtained with a deteriorated octadecylsilane column with an irregularly shaped support (LiChrosorb RP-18, 5 μm, 25 cm length; Merck), but the same successful operation has also been carried out with a deteriorated octadecylsilane column with a spherical support (Novapak C_{18}, 4 μm, 15 cm length; Waters Assoc.) (not shown). Similar improvements were also obtained in the behaviour of a deteriorated trimethylsilane column with a wide-pore spherical support (Ultrapore RPSC, 5 μm particle size, 300 Å pores, 7.5 cm length; Beckman) with respect to its ability to produce well resolved peptide maps when the above restorative procedures were applied (not shown).

The renegerated columns can be used with good performance over a number of injections similar to those of new columns. Moreover, the regeneration method proposed here can be easily carried out and does not require any special instrumentation (*e.g.*, an HPLC slurry packer) as do other successful regeneration methods recently proposed[4]. These results suggest that the reversal of the flow could be an easy and inexpensive resource for lengthening the life of different kinds of reverse-phase HPLC columns.

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