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Note

Potential *in situ* regeneration of octadecyl-silica columns

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The problem of loss of column performance characteristics is continually encountered in high-performance liquid chromatography (HPLC), especially when a single column is used to analyze diverse sample types with widely different matrices and various mobile phases. The common use of buffers and ion-pairing reagents in reversed-phase systems and incomplete daily column rinsing will lead to deterioration in column resolution. The possibility of reclaiming chromatographically lost columns or at least extending their useful lifetime would contribute to cost savings, especially when a large number of columns are routinely used by a laboratory.

Column suppliers often provide information on column maintainance and various authors have given procedures for increasing column longevity[1–3]. Little practical information is available, however, on column regeneration after loss of resolution. While initial preparation of various bonded phases has been recently discussed[3–7] *in situ* preparation or regeneration of bonded phases is not commonly encounterd. Gilpin *et al.*[8] have shown the application of this method to the bonding of octadecyltrichlorosilane to 10-μm LiChrosorb Si 60. They determined that reproducible results were obtained when 30 ml of a 50% octadecyltrichlorosilane solution was used with properly dehydrated columns.

The present paper reports on the application of commercially available silica derivatization and endcapping agents to the regeneration of HPLC column resolution characteristics. The use of octadecyltriethoxysilane and trimethylmethoxysilane will be discussed.

EXPERIMENTAL

Chemicals

Octadecyltriethoxysilane and trimethylmethoxysilane were obtained from Alltech. Water was distilled and filtered (Millipore, 0.45 μm). Methanol used was MCB Omnisolve, chromatographic grade and acetic acid was reagent grade from Corco. Chloroform was Fisher HPLC grade and MCB reagent grade toluene was dried over silica gel.

The mobile phase was deaerated water–methanol–acetic acid (650:350:10). The derivatizing solutions were 5% (v/v) solutions of octadecyltriethoxysilane and trimethylmethoxysilane prepared in dry toluene.

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Standard preparation

A solution was prepared by transferring approximately 325 mg theophylline (Sterling-Winthrop) and 20 mg phenobarbital (Sterling-Winthrop), accurately weighed to a 100-ml volumetric flask. A 20-ml volume of U.S.P. alcohol was added and the solution was diluted to the mark with distilled water. A further dilution of 10.0 to 100.0 ml was then made with distilled water.

Apparatus

Silanizations were done at room temperature using a single-piston mini pump (Milton Roy) at a flow-rate of 1 ml/min.

The chromatographic system consisted of an Altex 110A pump at 1 ml/min, an Altex 153 detector at 254 nm and a sensitivity of 0.01 a.u.f.s. A Fisher 5000 recorder was also utilized.

Columns investigated included a 25-cm Whatman Partisil 10-μm ODS-3, a 25-cm Altex Ultrasphere 5-μm ODS and a 30-cm Waters μBondapak C_{18}. These columns had previously been judged useless after an extended period of normal use.

Derivatization procedure

The procedure suggested by the producer includes equilibrating the column with dry toluene and pumping the derivatizing reagent at a slow rate of 0.3–0.4 ml/min for 5–6 h. Following this 10–20 column volumes of dry methanol are pumped at normal flow-rates[9].

The present method consisted of pumping 50 ml of the following reagents through the column: dry methanol, dry toluene, 5% derivatizing reagent, dry toluene, chloroform and dry methanol.

In order to determine the effect of the derivatizing solvent sequence alone, 50 ml of dry methanol, dry toluene, chloroform and dry methanol were pumped through a 30-cm Waters μBondapak C_{18} column. This was followed by a second and third 50-ml sequence.

Resolution between the theophylline and phenobarbital peaks was measured under initial conditions, after increments of 50-ml treatments with octadecyltriethoxysilane and after endcapping with the same solvent sequence using trimethylmethoxysilane. Resolution factors, R_s, were calculated using the following equation

$$R_s = \frac{2\,(t_2 - t_1)}{w_1 + w_2}$$

where t_1 and t_2 are measured retention times of theophylline and phenobarbital, respectively, and w_1 and w_2 are their base widths measured in the same units.

The number of theoretical plates was measured for phenobarbital using the equation

$$N = 5.54\left(\frac{t_R}{t_{w_{\frac{1}{2}}}}\right)^2$$

where t_R is the retention time of phenobarbital and $t_{w_{\frac{1}{2}}}$ is peak width at half-height for phenobarbital.

TABLE I

RESOLUTION FACTOR MEASURED BETWEEN THEOPHYLLINE AND PHENOBARBITAL

ODS = Octadecyltriethoxysilane.

Conditions	*Whatman ODS-3 10 μm*	*Waters μBondapak C_{18} 10 μm*	*Altex ultrasphere ODS 5 μm*
Initial	1.89	0.0	5.33
50 ml ODS	3.05	1.43	5.51
100 ml ODS	3.79	1.32	5.76
100 ml ODS plus 50 ml endcapping	4.14	–	5.52
150 ml ODS	–	2.24	–
200 ml ODS	–	2.10	–
250 ml ODS	–	2.68	–

RESULTS AND DISCUSSION

The results obtained in column regeneration will depend on many factors. Primary among these is the initial state of the column. A completely degraded column would be difficult to regenerate by the procedure outlined above. Other factors affecting the outcome include reagent concentration, flow-rate, column pressure, temperature, the presence of catalysts, total volume of reagent used and size and type of silica particles comprising the packing. Here columns with irregular 10-μm silica and 5-μm spherical particles were subjected to the same treatment. Initially, before treatment the 5-μm column gave a better separation of theophylline and phenobarbital as would be expected from normally operating columns. Table I shows the improvement in resolution for the Partisil 10-μm irregular silica ODS-3 column as the treatment progressed. An initial value of 1.89 was modified to a final 4.14 after endcapping with

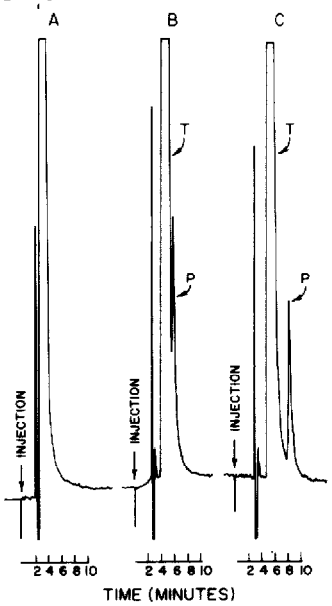

Fig. 1. Separation of theophylline (T) and phenobarbital (P) on 10-μm Waters μBondapak C_{18} column. A, Original untreated conditions; B, following treatment with 100 ml octadecyltriethoxysilane; C, following treatment with 250 ml octadecyltriethoxysilane. Mobile phase: water–methanol–acetic acid (650:350:10); flow-rate 1.0 ml/min.

TABLE II

NUMBER OF THEORETICAL PLATES FOUND FOR PHENOBARBITAL
ODS = Octadecyltriethoxysilane.

Conditions	*Whatman ODS-3 10 μm*	*Waters bondapak C_{18} 10 μm*	*Altex ultrasphere ODS, 5 μm*
Initial	2021	–	1725
100 ml ODS	1825	1622	4114
250 ml ODS	–	2038	–

trimethylsilyl groups. Similarly the resolution factor for the μBondapak 10-μm column increased irregularly from an initial value of 0.0 to 2.68. This was obtained after five increments of 50 ml of the octadecyltriethoxysilane reagent. Table II also shows this improvement with the number of theoretical plates for phenobarbital increasing to 2038. Fig. 1 shows results for this column when the sample was run under the same conditions initially, after 10 ml and after 250 ml of derivatizing treatment.

The resolution on the 5-μm Ultrasphere column was not improved to such a degree giving an increase from 5.33 to 5.76 in resolution factor at maximum. The final endcapping appears to have caused a reversal of part of the improvement made with the octadecyltriethoxysilane. Peak shape was improved, however, in the case of this column by the full treatment indicating that more uniformly coated particles resulted, giving a better partitioning behavior.

An adverse effect observed in the case of the 5-μm column following siloxane treatment was the buildup of column back pressure at each stage giving a final value of 4500 p.s.i. This high value for 1 ml/min could have resulted from a polymerization effect of the silanol groups caused by the trifunctional derivatization agent[3,7]. Accordingly even though an increase in resolution and number of theoretical plates were observed, the regeneration in this case was unsuccessful. Such a pressure increase was not seen for the 10-μm columns with final pressures of 1200 p.s.i. for the ODS-3 and 1900 p.s.i. for the μBondapak C_{18} treated.

The possibility that the solvent sequence alone without silylation reagent could result in an increased column efficiency was examined. The resolution between the theophylline and phenobarbital peaks showed little improvement from an initial value of 2.93 to values of 2.80, 2.93 and 2.80 after a first, second and third sequence of 50 ml of each solvent.

The potential for regaining lost resolution on columns which would normally have been discarded makes this a useful procedure. While a return to like-new conditions is not claimed, the increases in theoretical plate number and resolution factors would allow for an extended column life. It is interesting that such good results are obtained under the very mild conditions employed for the reaction.

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