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End-capping of octadecylsilylated silica gels by high-temperature silylation

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Abstract

A novel end-capping method for octadecylsilylated silica gels (ODS) was developed. ODS end-capped by silylation at temperatures above 250°C were less adsorptive for pyridine than those end-capped by silylation in liquid phase. Infrared spectra indicated that the former had a much smaller amount of residual silanols than the latter. The carbon content of ODS prepared from monochlorosilane decreased when end-capping was carried out above 300°C. It is suggested that the decrease was due to the replacement of the octadecylsilyl group on silica gels by end-capping agents. On the other hand, the carbon content of ODS prepared from trichlorosilane did not decrease even when end-capping was carried out at 350°C; therefore, end-capping could be carried out at higher temperatures. Analysis of basic drugs by high-performance liquid chromatography using ODS end-capped at 350°C gave good peak shapes even if anti-tailing agents were not added to the mobile phase.

Keywords: Stationary phases, LC; Endcapping; Silica, octadecylsilylated; Silanol groups; Pyridine; Phenol; Anilines; Basic drugs; Alkaloids

1. Introduction

Octadecylsilylated silica gels (ODS) are used mainly as packings for HPLC. Silanols remain on the surface of silica gel even if they are fully silylated [1] and the residual silanols participate in the retention of solutes [2,3]. It has been reported that the residual silanols show high selectivity because of silanophilic interactions, which leads to adequate separation in some instances [4]. However, the residual silanols cause peak tailing, poor reproducibility and even unexpectedly long retention times in the HPLC of basic compounds. Many studies have been carried out to eliminate the troublesome effects of the residual silanols. The undesirable interactions between basic solutes and the residual silanols can be prevented by two methods, namely addition of modifiers to the mobile phase and end-capping of stationary phases.

The addition of various amines to the mobile phase usually decreases the degree of peak tailing of basic solutes [4–7], and it has been explained that this is due to blocking of the

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residual silanols by the amines [3, 8–10]. Ammonium acetate can also be used as a masking agent for the residual silanols [11]. An ion pair formed by basic solutes and alkylsulfonates added to the mobile phase also prevents the stationary phase from adsorbing basic solutes [3,9,12].

On the other hand, alkylsilylated silica gels are end-capped by trimethylsilylation to reduce the number of residual silanols. Trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) are usually used for end-capping [13,14]. It was shown that HMDS is suitable as an end-capping agent for stationary phases used for the separation of basic solutes whereas TMCS is suitable for the separation of acidic or neutral solutes [15]. End-capping is also effectively carried out using N,O-bis(trimethylsilyl)acetamide [16,17], trimethylsilylphosphine [18] or trimethylimidazole [19]. It has been reported that highly efficient packings could be obtained when trimethylsilylation of about 5% of the total silanols was carried out prior to octadecylsilylation of silica gel [20]. Anti-tailing agents are, however, essential for the HPLC of basic solutes because the end-capping agents cannot block the residual silanols perfectly.

High-temperature silylation (HTS) has been used to deactivate the inner surface of glass or fused-silica capillary columns for gas chromatography before the liquid phase is coated on it [21–23]. We developed a novel method for end-capping of ODS by the application of HTS and found that HTS is a more effective method than conventional liquid-phase silylation for end-capping the residual silanols [24–26]. In this paper, we describe the influence of end-capping conditions on the characteristics of stationary phases and some applications of stationary phases end-capped by HTS for the analysis of basic drugs.

2. Experimental

2.1. Reagents and materials

All silicon chemicals were purchased from Sinetsu Chemical (Tokyo, Japan). The silica gel used was Develosil 100-5 (particle size 5 μm, pore size 100 Å and surface area 300 m^2/g) from Nomura Chemicals (Seto, Japan).

2.2. Preparation of ODS

Three kinds of ODS packing, ODS-Cl_1, ODS-Cl_2 and ODS-Cl_3, were prepared by silylating silica gel using dimethyloctadecylchlorosilane, methyloctadecyldichlorosilane and octadecyltrichlorosilane as the silylating agents, respectively. The following preparation method was employed: a suspension of 100 g of silica gels in 400 ml of concentrated HCl was heated at 100°C for 16 h, then the suspension was cooled to about 25°C, and the silica gels were collected, washed with water until free from acid and dried under vacuum at 140°C for 8 h. The octadecylsilylating agent (0.1 mol) was added to the stirred suspension of 50 g of the dried silica gels in 170 ml of dry toluene under a nitrogen atmosphere, then the calculated amount of dry pyridine equivalent to the amount of octadecylsilylating agent was added. The suspension was refluxed for 16 h, cooled and the silylated silica gels obtained were washed with dry toluene. When methyloctadecyldichlorosilane or octadecyltrichlorosilane was used, residual chloro groups on the bonded silanes were hydrolysed in acetonitrile–water (60:40) with stirring for 3 h after the silica gels had been washed with acetonitrile. The silica gels in every case were washed with methanol and dried under vacuum at 140°C for 4 h.

In the HTS method, the following steps were employed for the end-capping: 3 g of ODS was placed in a 30-ml glass ampoule and dried under vacuum at 140°C for 4 h. Silylating agent (2.9 mmol) was added to the ampoule after it had been cooled to about 25°C. The ampoule was cooled to −60°C and the air in it was replaced with nitrogen. The ampoule was sealed, heated for 24 h, cooled and then opened. The silylated silica gels obtained were washed repeatedly with toluene and then with methanol and dried under vacuum at 140°C for 4 h. In this paper, ODS end-capped with HMDS and hexamethylcyclotrisiloxane (D_3) are denoted ODS-HMDS and ODS-D_3, respectively.

For end-capping ODS by the liquid-phase silylation method, the following steps were employed: HMDS (4 ml) was added to a stirred suspension of 10 g of ODS in 38 ml of dry toluene. The suspension was refluxed for 16 h, cooled, washed sequentially with toluene and methanol and dried under vacuum at 140°C for 4 h.

The packings prepared were packed into stainless-steel tubes (150 × 4.6 mm I.D.) by conventional high-pressure slurry-packing procedures.

2.3. Chromatographic measurements

The HPLC system consisted of a pump (LC-6A; Shimadzu, Kyoto, Japan), a UV detector (SPD-6A; Shimadzu), a data processor (C-R4A; Shimadzu) and an injector (Model 7125; Rheodyne, Cotati, CA, USA).

The columns prepared were tested by separating a mixture of pyridine and phenol using acetonitrile–water (3:7) as the mobile phase. Pyridine has often been used to evaluate the adsorptive activity of stationary phases [27,28]. The relative retention value ($\alpha_{ph/py}$) of phenol, which is inert to silanols, with respect to basic pyridine was used for the evaluation of the effectiveness of end-capping. Asymmetry factors were also calculated using the equation $A_s = b/a$, where a is the distance from the leading edge of the peak to the perpendicular drawn from the peak tip at the 10% peak-height level and b is that from the tailing edge to the perpendicular [29].

To examine the chemical stability of the ODS prepared, the columns were left as they were after pyridine and phenol had been chromatographed on them. Then the columns were washed with 20 ml of methanol and $\alpha_{ph/py}$ values were measured using the same mobile phase again. Repeating this procedure gave stability curves ($\alpha_{ph/py}$ versus standing time) for the prepared ODS.

2.4. Measurement of infrared spectra

Infrared spectra were obtained with a diffuse reflectance infrared Fourier transform (DRIFT) spectrometer (FTS-30; Bio-Rad Labs., Richmond, CA, USA) purged with dry air and equipped with a deuterated triglycine sulfate (DTGS) detector. All spectra were acquired by 100 scans at a nominal resolution of 8 cm^{-1} against pure KBr as a reference. Samples were 20% (w/w) of ODS dispersed in KBr. The samples and KBr were dried under vacuum at 150°C for 8 h and then measured.

3. Results and discussion

3.1. Effect of end-capping temperature

Table 1 shows the effect of end-capping temperature in the case of end-capping ODS-Cl_1 with HMDS. ODS-Cl_1-HMDS end-capped by HTS gave faster elution of pyridine, larger $\alpha_{ph/py}$ values and less tailing and a better shape of the pyridine peak than ODS-Cl_1 end-capped by liquid-phase silylation in toluene. The results indicate that end-capping by HTS effectively blocked the residual silanols on the surface of ODS-Cl_1. An increase in the end-capping temperature led to a slight decrease in the $\alpha_{ph/py}$ value and a slight improvement in the peak shape of pyridine, although the carbon contents of ODS-Cl_1-HMDS decreased markedly. The results suggest that the siloxane bond between octadecylsilyl groups and silanol groups on silica gels was not cleaved but octadecylsilyl groups were replaced with the trimethylsilyl groups from the end-capping agent. As shown in Table 1, the carbon content of ODS-Cl_1 decreased slightly when it was heated without silylating agent by the same procedure as HTS. This supports the suggestion that the marked decrease in carbon content with increasing end-capping temperature is mainly due to the replacement of octadecylsilyl groups by the trimethylsilyl groups. Slight differences in $\alpha_{ph/py}$ values were observed with increasing end-capping temperature; these may be due to their carbon content rather than the silanol activity for basic solutes.

Fig. 1 shows the chemical stability of the end-capping group on ODS-Cl_1-HMDS end-capped at 250, 300 and 350°C. In all instances the $\alpha_{ph/py}$

Table 1
Effect of end-capping temperature on characteristics of ODS-Cl_1-HMDS

Packing No.	End-capping temperature (°C)	k'		$\alpha_{py/ph}$	A_s [a]	Change in carbon content[b] (%)	Nitrogen content (%)
		Pyridine	Phenol				
1	250	0.83	2.89	3.48	2.47	+2.0	0.07
2	300	0.86	2.93	3.41	2.47	−8.4	0.10
3	350	0.93	2.91	3.13	2.09	−23.7	0.25
4	Reflux in toluene	1.40	2.97	2.12	–	+1.9	0.08
5	350[c]	2.21	2.94	1.33	2.70	−4.3	0.01

End-capping agent, HMDS; reaction time, 24 h; carbon content of ODS-Cl_1, 17.58%.
[a] A_s = asymmetry factor of pyridine peak.
[b] Expressed by $[(C_1 - C_0)/C_0] \cdot 100$, where C_0 and C_1 are the carbon contents before and after end-capping, respectively.
[c] ODS-Cl_1 was heated without silylating agent.

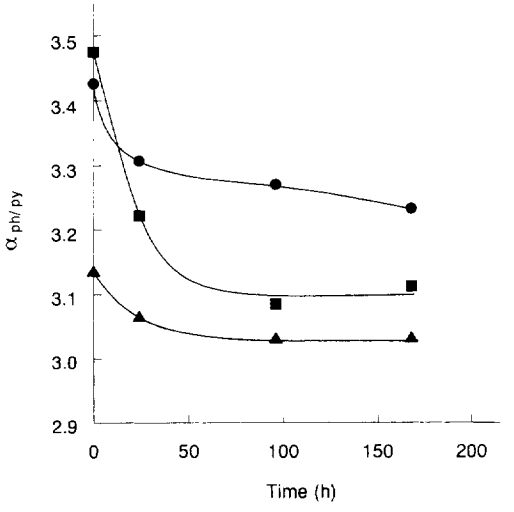

Fig. 1. Stability of ODS-Cl_1-HMDS end-capped at (■) 250, (●) 300 and (▲) 350°C when exposed to acetonitrile–water (3:7).

values decreased when the packings were repeatedly exposed to acetonitrile–water (3:7); pyridine began to interact with the silanols formed by hydrolysis of the trimethylsilyl groups. The decrease was smaller when the end-capping temperature was higher; ODS-Cl_1-HMDS end-capped at 350°C exhibited the smallest decrease in $\alpha_{ph/py}$ value and the highest stability compared with ODS-Cl_1-HMDS end-capped at lower temperatures. Hydrolysis of ODS-Cl_1-HMDS end-capped at higher temperature became more difficult. This may suggest that end-capping proceeded more effectively with increasing end-capping temperature.

3.2. Comparison of end-capping effects among ODS-Cl_1-HMDS, ODS-Cl_2-HMDS and ODS-Cl_3-HMDS

Table 2 shows the characteristics of ODS-Cl_1-HMDS, ODS-Cl_2-HMDS and ODS-Cl_3-HMDS end-capped at 350°C for 24 h. ODS-Cl_1-HMDS exhibited the largest decrease in carbon content among the three. Since the more electronegative substituents on the Si atoms of the siloxane more strongly attract the unshared electron pairs of the siloxane oxygen, reactions involving electrophilic attack on these electrons become more difficult [30]. Hence the reactivity of the octadecylsiloxane bond of ODSs is inferred as follows. Electrophilic attack on the oxygen of the octadecylsiloxane bond ($C_{18}H_{37}Si–O–Si$) of ODS-Cl_3 is the most difficult because the two substituents on the Si atom of the octadecylsiloxy group ($C_{18}H_{37}Si–O–$) of ODS-Cl_3 are oxy groups which are electronegative, whereas electrophilic attack on the oxygen of the octadecylsiloxane bond of ODS-Cl_1 is the easiest because the two substituents on the Si atom of the octadecylsiloxy group of ODS-Cl_1 are methyl groups which are electron donors. The degree of

Table 2
Characteristics of ODS-Cl_1-HMDS, ODS-Cl_2-HMDS and ODS-Cl_3-HMDS

Packing No.	Starting packing	k'		$\alpha_{py/ph}$	A_s	Carbon content of starting packing (%)	Change in carbon content (%)	Nitrogen content (%)
		Pyridine	Phenol					
3	ODS-Cl_1	0.93	2.91	3.13	2.09	17.58	−23.7	0.25
6	ODS-Cl_2	0.87	2.82	3.24	2.05	17.79	−11.1	0.20
7	ODS-Cl_3	1.05	2.79	2.66	1.93	17.47	+1.6	0.14

End-capping reagent, HMDS; end-capping temperature, 350°C; reaction time, 24 h.

decrease in the carbon content reflects well the reactivity of the octadecylsiloxane bond. This supports the idea that the decrease in carbon content is due to a substitution reaction.

The $\alpha_{ph/py}$ value of ODS-Cl_3-HMDS was the lowest among the three. Since ODS-Cl_3 has the largest number of silanol groups [31], the amount of ammonia generated by HTS with HMDS is estimated to be the largest. Therefore, the inhibition of HTS by ammonia might have led to insufficient end-capping.

3.3. End-capping with D_3

Table 3 shows the effect of end-capping by HTS with D_3. The packings treated at the same temperature showed similar $\alpha_{ph/py}$ values, although they gave different amounts of the end-capping by-product (water) since they have different numbers of residual silanols. The result suggests that, in this instance, the by-product (water) does not inhibit HTS, unlike the case of ammonia from HMDS. The decrease in carbon content with increasing temperature exhibits a trend similar to that of HTS with HMDS.

In contrast to HMDS, in the case of D_3, the $\alpha_{ph/py}$ values increased with increasing silylation temperature. When a higher temperature is required for end-capping because of an improvement in $\alpha_{ph/py}$, the decrease in carbon content during HTS can be prevented by use of ODS-Cl_3 as the starting material.

The $\alpha_{ph/py}$ value of ODS-HMDS was higher

Table 3
Characteristics of ODS-Cl_1-D_3, ODS-Cl_2-D_3 and ODS-Cl_3-D_3

Packing No.	Starting packing	End-capping Temperature (°C)	k'		$\alpha_{py/ph}$	A_s	Change in carbon content (%)	Nitrogen content (%)
			Pyridine	Phenol				
8	ODS-Cl_1	250	1.63	2.85	1.75	2.51	+1.0	0.01
9	ODS-Cl_1	300	1.47	2.74	1.86	4.00	−0.85	0.02
10	ODS-Cl_1	350	1.16	2.81	2.42	1.91	−24.9	0.02
11	ODS-Cl_1	370	1.04	2.74	2.63	1.90	−36.2	0.02
12	ODS-Cl_2	250	1.50	2.81	1.87	2.83	+1.3	0.02
13	ODS-Cl_2	300	1.25	2.53	2.02	1.56	+4.3	0.03
14	ODS-Cl_2	350	1.13	2.65	2.35	1.62	−1.1	0.03
15	ODS-Cl_2	370	1.07	2.84	2.65	1.93	−10.0	0.03
16	ODS-Cl_3	250	1.75	2.79	1.59	4.53	+4.4	0.03
17	ODS-Cl_3	300	1.33	2.51	1.89	1.45	+6.9	0.02
18	ODS-Cl_3	350	1.15	2.83	2.46	1.28	+1.0	0.04
19	ODS-Cl_3	370	1.06	2.86	2.70	1.74	−5.9	0.03

End-capping reagent, D_3; reaction time, 24 h.

than that of ODS-D_3 at all end-capping temperatures investigated. However, it has been reported that compounds containing nitrogen remain on the surface of silica gels when silanols are silylated with HMDS [13,15,32,33]. Tables 1–3 show that ODS-HMDS had a higher nitrogen content than ODS-D_3 at all end-capping temperatures investigated. If the compounds containing nitrogen block the effect of silanol, which leads to faster elution of pyridine, it cannot be concluded that the residual silanols were more effectively end-capped with HMDS than with D_3. On the other hand, as shown in Tables 2 and 3, the asymmetry factor of ODS-D_3 end-capped at 350°C was closer than that of ODS-HMDS at 350°C. Hence D_3 may provide fewer residual silanols on the ODS and a more inert surface for basic compounds than HMDS at 350°C. Since smaller tailing of peaks leads to higher resolution, end-capping with D_3 is more useful than that with HMDS.

Fig. 2 shows the chemical stability of the end-capping group on ODS-Cl_1-HMDS, ODS-Cl_1-D_3, ODS-Cl_3-HMDS and ODS-Cl_3-D_3 end-capped at 350°C. The $\alpha_{ph/py}$ values of ODS-Cl_1-HMDS and ODS-Cl_3-HMDS began to decrease earlier than those of ODS-Cl_1-D_3 and ODS-Cl_3-D_3. This result indicates that end-capping trimethylsilyl groups were hydrolysed to form silanols on the surface of silica gel. On the other hand, the $\alpha_{ph/py}$ values of ODS-Cl_1-D_3 and ODS-Cl_3-D_3 decreased negligibly within the given stability test period. A possible explanation of these results is the following. Three electron-donating groups (methyl groups) are bonded to the Si atoms of trimethylsilyl groups, which are the general end-capping moieties, whereas two electron-donating groups are on the Si atoms of D_3 bonded to silica gels. Therefore, the electrophilic attack on the siloxane oxygen located between D_3 and silica gels is more difficult than that on the siloxane oxygen located between trimethylsilyl groups and silica gels. Since packings with fewer residual silanols are more stable, the result of the chemical stability test also suggests that end-capping with D_3 is more efficient than with HMDS.

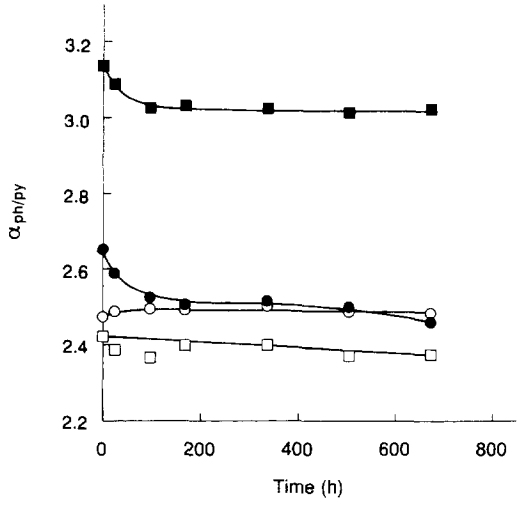

Fig. 2. Stability of (■) ODS-Cl_1-HMDS, (□) ODS-Cl_1-D3, (●) ODS-Cl_3-HMDS and (○) ODS-Cl_3-D3 when exposed to acetonitrile–water (3:7).

Fig. 3 shows the DRIFT spectra of ODS-Cl_3 before end-capping, ODS-Cl_3 end-capped with HMDS in the liquid phase and ODS-Cl_3-D_3 (350°C). The silanol groups are observed between 3200 and 3700 cm^{-1} in addition to alkyl groups near 2900 cm^{-1}. The DRIFT spectra also indicate that the ODS end-capped by HTS had much smaller amounts of residual silanols than that end-capped in the liquid phase.

3.4. HPLC of basic compounds using ODS-Cl_3 end-capped with D_3 by HTS

Figs. 4–6 show the chromatograms of basic compounds on ODS-Cl_3 end-capped by (A) liquid-phase silylation and (B) HTS with D_3 at 350°C. The solutes in Fig. 5 are the standard test mixture proposed by Engelhardt et al. [34]. Figs. 4 and 5 indicate that pyridine is most sensitive to the residual silanols among these basic solutes. Fig. 6 shows the chromatograms of tropane alkaloids. HPLC analysis of tropane alkaloids is generally performed using ODS. However, the strong interaction between the residual silanols and the basic drugs results in peak tailing and poor reproducibility, especially in trace analysis. In order to suppress the interaction, masking agents for silanols have been added to the mobile phase [35] and/or an acidic mobile phase has

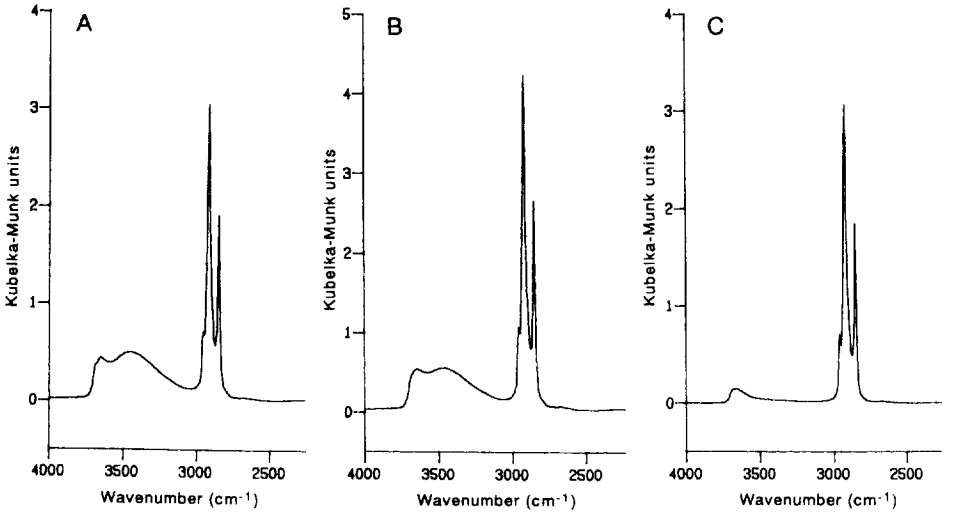

Fig. 3. DRIFT spectra of (A) ODS-Cl_3 before end-capping, (B) ODS-Cl_3 end-capped with HMDS in the liquid phase and (C) ODS-Cl_3-D_3 at 350°C.

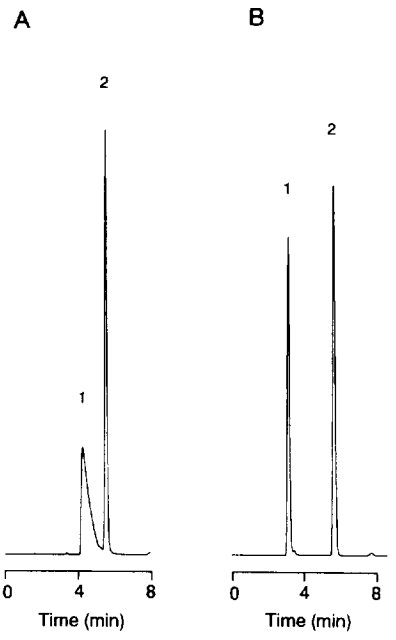

Fig. 4. Chromatograms of pyridine and phenol on ODS-Cl_3 end-capped by (A) liquid-phase silylation and (B) HTS with D_3 at 350°C. Chromatographic conditions: mobile phase, acetontrile–water (30:70); flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1 = pyridine; 2 = phenol.

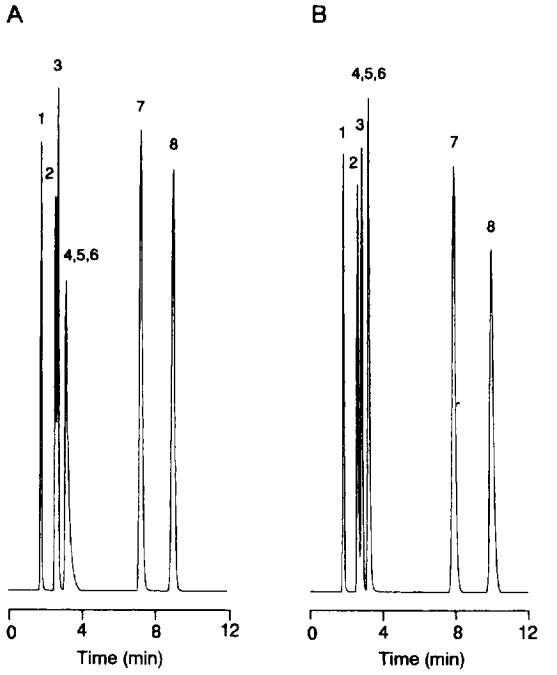

Fig. 5. Chromatograms of the standard test mixture proposed by Engelhardt et al. [34] on ODS-Cl_3 end-capped by (A) liquid-phase silylation and (B) HTS with D_3 at 350°C. Chromatographic conditions: mobile phase, methanol–water (65:35); flow-rate, 1 ml/min; detection, UV at 254 nm. Peaks: 1 = thiourea; 2 = aniline; 3 = phenol; 4–6 = *o*-, *m*- and *p*-toluidine; 7 = N,N-dimethylaniline; 8 = toluene.

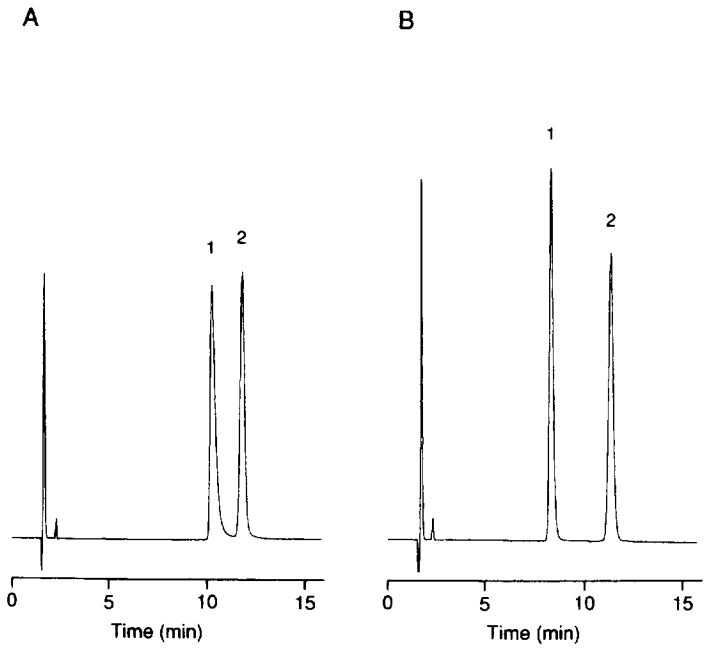

Fig. 6. Chromatograms of tropane alkaloids on ODS-Cl_3 end-capped by (A) liquid-phase silylation and (B) HTS with D_3 at 350°C. Chromatographic conditions: mobile phase, acetonitrile–20 mmol/l phosphate buffer (pH 6.8) (15:85); flow-rate, 1 ml/min; detection, UV at 210 nm; injection volume, 20 μl. Peaks: 1 = atropine (50 mg/l); 2 = scopolamine (50 mg/l).

been used [36]. However, the addition of modifiers causes various problems: (1) the background becomes higher with low-wavelength UV detection, (2) a long time is required to equilibrate and to wash a column, (3) it sometimes leads to inconvenience for LC–MS analysis and (4) there is difficulty in separating the modifier from the fractions obtained from HPLC. On the other hand, when ODS-Cl_3-D_3 end-capped by HTS is used, peaks without tailing can be easily obtained even when a neutral mobile phase free of anti-tailing agents is used. End-capping by HTS allowed a choice of an appropriate mobile phase for the analysis.

4. Conclusions

ODS were successfully deactivated by end-capping using HTS. The most inert and stable ODS were obtained by end-capping of the octadecyltrichlorosilanized silica gels (ODS-Cl_3) with D_3 at temperatures above 350°C. The inert packing material gave sharp peaks of basic drugs even if masking agents for silanols were not added to the mobile phase.

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