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# Development of a novel octadecyl-bonded silica column and evaluation of its reliability in chromatographic analysis

Hiroyuki Tanaka, Masafumi Kamada, Masahiko Nyudo, Masayoshi Ohira*

*GL Sciences, 237-2 Sayamagahara, Iruma, Saitama 358, Japan*

**Abstract**

The chromatographic characterization of an octadecyl-bonded silica (ODS) column for high-performance liquid chromatography is described. In general, columns of the same type but obtained from various manufacturers give different chromatographic results, due to differences in the purity of column packings, the properties of the silica gel supports and the density of silanols on the surface of the silica gel. In order to solve this problem and to obtain a high performance ODS column, a series of methods with different samples and conditions were evaluated. The result is important for the optimization of conditions for the synthesis of ODS packings and for minimizing the deleterious effects arising from adsorption activity and metal impurity.

*Keywords:* Stationary phases, LC; Octadecylsilica; Silica, bonded

## 1. Introduction

The surface modification of sorbents has advanced greatly and led to the development of high-performance liquid chromatography (HPLC) into a powerful analytical separation technique [1–3]. There is still considerable interest in the evaluation of column performance [4–8]. The chemistry related to a variety of columns has been studied in detail. Extensive work on columns, especially ODS ones, has been accomplished [9,10] and a number of ODS columns for HPLC are now commercially available. However, problems with their practical application still exist; columns obtained from various manufacturers have different properties and lead to obviously different chromatographic results. We investigated a chromatographic method for evaluating/controlling ODS column performance with regard to the adsorption activity. In this paper, representative standard samples were used for chromatographic measurements and a series of investigations were carried out to demonstrate the validity of the evaluation method. This also led to the development of an ODS column with a good performance.

## 2. Experimental

### 2.1. Materials

Octadecylsilica packings were synthesized using four kinds of silica gel (Table 1) and the chromatographic properties of these were compared. Methyloctadecyldichlorosilane and trimethylchlorosilane (TMCS), obtained from Kishida (Osaka, Japan), were used for octadecylsilylation and end-capping, respectively. Pyridine, phenol, oxine-copper, formic acid and acetic acid were purchased from Tokyokasei (Osaka, Japan). Procainamide, chlorpheniramine and barbital were purchased from Sig-

*Corresponding author.


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Table 1
Physical properties of the silica gels synthesized for use in $C_{18}$ columns

| Silica | Particle size[a] (μm) | Surface area[b] ($m^2/g$) | Pore diameter[b] (Å) | Pore volume[b] (ml/g) | Metal impurity level[c] (ppm) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Fe | Na | Al | Ti |
| A | 5 | 350 | 100 | 1.0–1.2 | 22 | 190 | 150 | 160 |
| B | 5 | 320 | 150 | 1.0–1.2 | 2 | 5 | 5 | ND |
| C | 5 | 450 | 80 | 0.7–0.8 | 6 | 5 | 5 | 1 |
| D | 5 | 450 | 100 | 1.1 | 0.5 | 8 | 5 | ND |

[a] By laser scattering method and centrifugal sedimentation.
[b] By nitrogen adsorption method.
[c] By atomic adsorption spectrometry.

ma-Aldrich (Tokyo, Japan). Other reagents and solvents used in the synthetic procedure were of special grade, and obtained from Kishida (Tokyo, Japan). Acetonitrile and methanol, used for the mobile phase, were of HPLC grade and purchased from Kishida. Deionized water was prepared with a Milli-Q system (Nihon Millipore Kogyo, Tokyo, Japan).

Typical physical characteristics of the silicas were measured and are summarized in Table 1.

### 2.2. Preparation of octadecylsilyl-silica gel

Silica gel was dried at 130°C for 3 h and then the methyloctadecyldichlorosilane and solvent were added. Toluene and hexadecane were used as refluxing solvents for comparing octadecylsilyl-silica gels obtained under different refluxing conditions [11]. After refluxing for 6 h, the mixtures were cooled to room temperature. The solvents were filtered off and the octadecylsilyl-silica gels were sequentially rinsed with methylene chloride and acetone. In this study, methyloctadecyldichlorosilane was used to prepare the $C_{18}$-silica gel.

In a silylation procedure, so-called end-capping, TMCS was used in place of methyloctadecyldichlorosilane as described above. Finally, sixteen packings that were prepared under different conditions (four silica gels, two refluxing solvents, end-capping and no end-capping) were obtained (Table 2). These packing materials were packed into a stainless-steel column (150×4.6 mm I.D.).

### 2.3. Chromatographic test

Pyridine–phenol (0.05 ml of pyridine and 11 mg of phenol in 1 ml of acetonitrile–water, 50:50, v/v), oxine-copper (0.1 mg in 1 ml of acetonitrile) and formic acid–acetic acid (50 ml of formic acid and 5.0 ml of acetic acid in 1 ml of water) were used as the samples to evaluate the surface activity of ODS packing materials. To compare the properties of the columns, several commercial ODS columns (Table 3) were also evaluated using formic acid and acetic acid, the physical characteristics of which are summarized in Table 3.

An ODS column that gave good results during the deactivating test was Inertsil ODS-3V. Furthermore, the column was evaluated using pharmaceutical samples such as procainamide, barbital and chlorpheniramine by determining the reproducibility of peak area and height. The mobile phases used in the pharmaceutical analyses are shown in Table 4.

### 2.4. Instrumentation

The HPLC system consisted of a PU-610 pump, a UV-620 variable-wavelength UV–Vis detector equipped with a flow cell (8.0-μl inner volume), a CO-630 column oven with a built-in valve injector and an AS-640 auto-sampler. This system is designed to give minimal dead volume. AVStation data analyzing processor was used to analyze the chromatographic data. All instruments were from GL Sciences (Tokyo, Japan) unless otherwise specified.

Table 2
List of synthesized ODS columns

| ODS column | Silica gel | Octadecylsilylation conditions[a] | Carbon loading (C, %, w/w) | End-capping[b] |
|---|---|---|---|---|
| ODS-A1 | A | I | 12 | × |
| ODS-A2 | A | I | 12 | ○ |
| ODS-A3 | A | II | 16 | × |
| ODS-A4 | A | II | 16 | ○ |
| ODS-B1 | B | I | 11 | × |
| ODS-B2 | B | I | 11 | ○ |
| ODS-B3 | B | II | 12 | × |
| ODS-B4 | B | II | 12 | ○ |
| ODS-C1 | C | I | 12 | × |
| ODS-C2 | C | I | 12 | ○ |
| ODS-C3 | C | II | 14 | × |
| ODS-C4 | C | II | 14 | ○ |
| ODS-D1 | D | I | 13 | × |
| ODS-D2 | D | I | 13 | ○ |
| ODS-D3 | D | II | 18 | × |
| ODS-D4 | D | II | 18 | ○ |

[a] I=Refluxing with toluene; II=refluxing with hexadecane.
[b] ×=No end-capping; ○=end-capping.

Table 3
Properties of commercial columns

| Column | Particle size (μm) | Pore size (Å) | Surface area ($m^2/g$) | Carbon loading (%) |
|---|---|---|---|---|
| Column A | 5 | – | – | – |
| Column B | 5 | 120 | 300 | 16 |
| Column C | 5 | 100 | – | 16 |
| Column D | 4 | 80 | – | 9 |
| Column E | 4 | 80 | – | 14 |
| Column F | 4 | 80 | – | 22 |
| Column G | 5 | 100 | 340 | 19 |

Table 4
Mobile phase in pharmaceutical analysis

| |
|---|
| *Procainamide* |
| Methanol–0.01 *M* phosphate, pH 2.5 (10:90, v/v) |
| Methanol–0.01 *M* phosphate, pH 4.9 (20:80, v/v) |
| Methanol–0.01 *M* phosphate, pH 7.5 (10:90, v/v) |
| *Chlorpheniramine* |
| Methanol–0.01 *M* phosphate, pH 2.5 (20:80, v/v) |
| Methanol–0.01 *M* phosphate, pH 4.9 (50:50, v/v) |
| Methanol–0.01 *M* phosphate, pH 7.5 (50:50, v/v) |
| *Barbital* |
| Methanol–0.01 *M* phosphate, pH 2.5 (20:80, v/v) |
| Methanol–0.01 *M* phosphate, pH 4.9 (50:50, v/v) |
| Methanol–0.01 *M* phosphate, pH 7.5 (20:80, v/v) |
| Methanol–water (15:85, v/v) |

## 3. Results and discussion

### 3.1. Surface activity and metal impurity

The elution behavior of pyridine–phenol is influenced by the residual silanol group on the packing's surface [12]; therefore, a pyridine–phenol solution is used as the sample, to evaluate ODS packings according to their elution order and peak shape as well as the separation factor. The chromatograms shown in Fig. 1 were obtained when the pyridine–phenol mixture was analyzed with sixteen kinds of ODS column. Although a well-deactivated

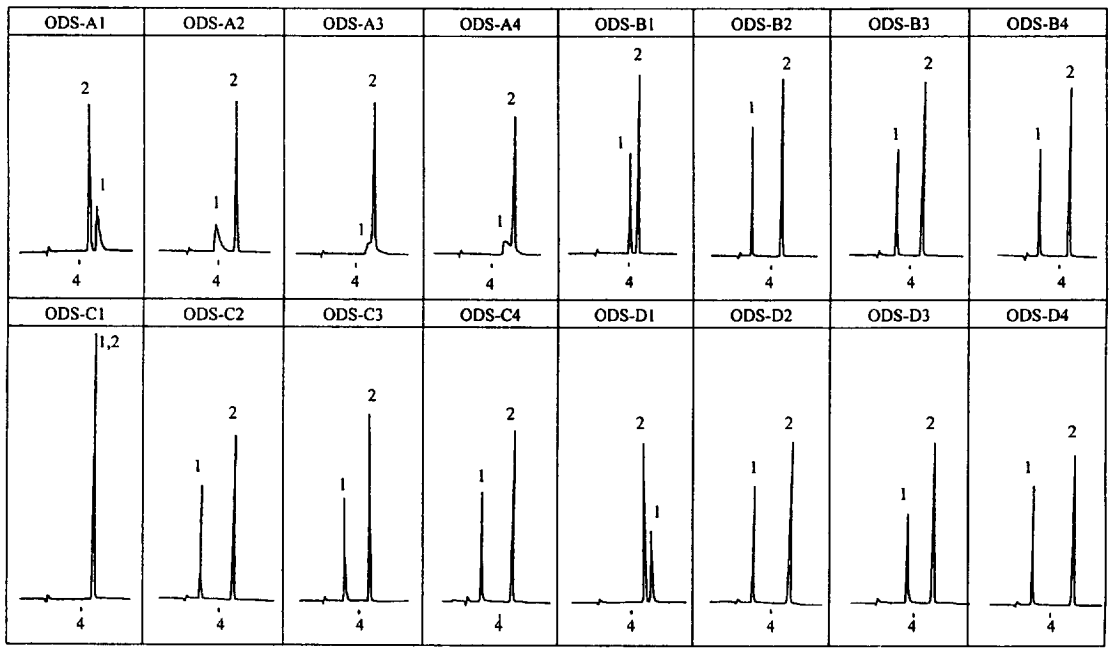


Fig. 1. Chromatograms of pyridine–phenol, with a mobile phase of acetonitrile–water (30:70, v/v); flow-rate, 1.0 ml/min; UV detection at 254 nm; column temperature, 40°C; sample volume, 5 μl; Peaks: 1=pyridine (0.05%, v/v), 2=phenol (0.1%, v/v).

column is expected to elute in the order of pyridine< phenol, some of the columns synthesized under differing conditions showed the opposite elution order and the ODS-A columns (1–4) synthesized from silica gel A led to the formation of broad peaks. On the other hand, the Inertsil ODS-3V column was promising compared with ODS-A columns. The reason for this is that the silica gel A contained a high level of metal impurity, which resulted in an undesirable interaction between pyridine and metal. Moreover, an interaction between the residual silanol group and pyridine is apparent (e.g., ODS-D1 of Fig. 1), because the peak shape of the pyridine and the elution order of the pyridine–phenol were improved by end-capping (ODS-D2 of Fig. 1). The results confirmed that the use of these samples for evaluating/controlling column performance is very valid, as the elution of the pyridine–phenol is strongly influenced by the characteristics of the silica gel and the conditions of octadecylsilylation and end-capping.

Oxine-copper, which is used as a pesticide because it has a bactericidal action, is also a good sample for use in the evaluation of column performance [12]. The chromatograms shown in Fig. 2 were obtained when the oxine-copper was analyzed with sixteen kinds of ODS column. The oxine-copper has a very strong coordination linkage, the elution behavior of which is dependent on metal impurity in the silica gel. Under the analytical conditions used, the oxine-copper could not be eluted from ODS columns that were made from silica gel A, which had a high level of metal impurity, as shown in Table 1. There are different peak shapes in the same measurement, although silica gels B, C and D contained the same level of metal impurity. This suggests that the elution behavior of the samples is not only influenced by the level of metal impurity on the silica gel support, but also by the residual silanol groups on the silica gel structure.

In general, the pyridine and the oxine-copper are used as a basic compound and a coordination

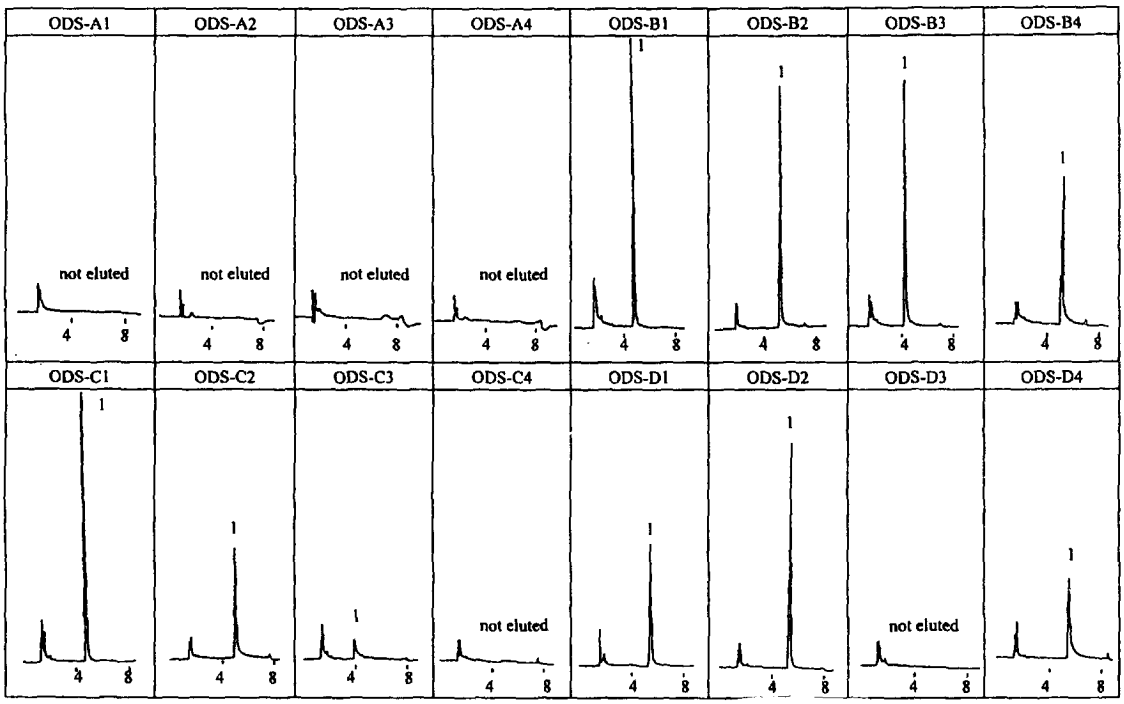


Fig. 2. Chromatograms of oxine-copper, with a mobile phase of acetonitrile–20 m*M* $H_3PO_4$ (5:95, v/v); flow-rate, 1.0 ml/min; UV detection at 240 nm; column temperature, 40°C; sample volume, 20 μl; Peak: 1=oxine-copper (0.1 mg/ml).

compound, respectively, in order to evaluate and control the column's performance. However, no acidic compounds have been recommended especially at present. Therefore, formic acid ($pK_a$=3.7) and acetic acid ($pK_a$=4.7) were selected for use in the evaluation, because these compounds have small molecular masses and comparatively strong acidities, and are easy to obtain. Also, the small-sized molecules that are allowed to pass through the attached organic layer to reach the silica gel surface are influenced by the surface form of ODS packings. As shown in Fig. 3, good results were obtained when the formic acid and acetic acid were analyzed with sixteen kinds of ODS column. In addition, similar analyses were carried out using several commercial columns (A–G of Fig. 4); however, a good peak shape in not obtained on elution of formic acid from these columns. One possible reason is that the residual catalyst (i.e., used in synthetic procedures) on the surface of the packing material or on any products derived during the octadecylsilylation and end-capping process led to undesirable results. In particular, basic residues from the bonding process may cause total adsorption of these acids.

### 3.2. Peak reproducibility

The peak shapes obtained with pyridine–phenol, oxine-copper and formic acid–acetic acid are representative. The Inertsil ODS-3V column, which gave better results than other columns when these samples were applied (Fig. 5), also gave satisfactory peak reproducibility in pharmaceutical analysis, in comparison with the ODS-A4 column.

For the analysis of the basic compounds, a buffer solution with a pH of 2.5 is generally chosen as the mobile phase. At low pH, protonation of the basic compound is promoted and the dissociation of residual silanol groups is suppressed. Thus, the adsorption of the sample to the surface of the

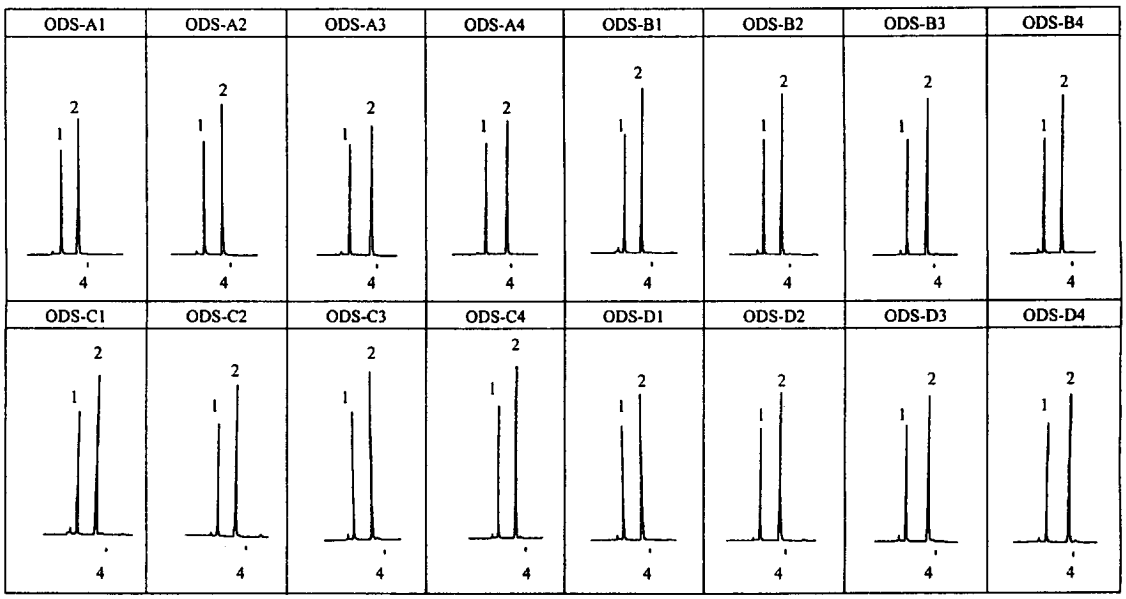


Fig. 3. Chromatograms of formic acid–acetic acid, with a mobile phase of 0.1% (v/v) $H_3PO_4$; flow-rate, 1.0 ml/min; UV detection at 210 nm; column temperature, 40°C; sample volume, 20 μl; Peaks: 1=formic acid (0.05%, v/v), 2=acetic acid (0.05%, v/v).

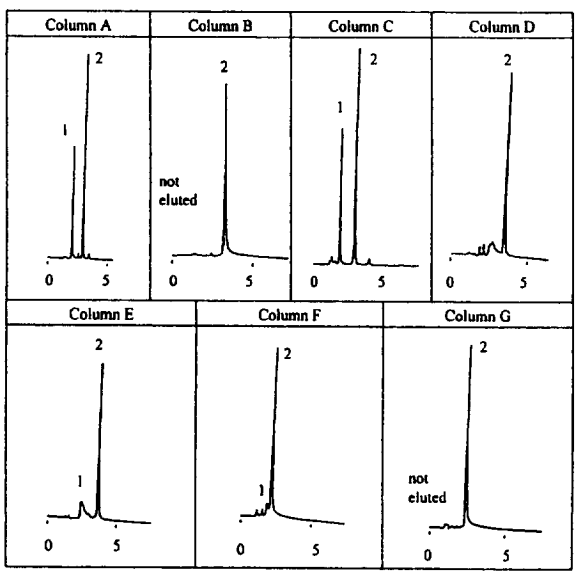


Fig. 4. Chromatograms of formic acid–acetic acid obtained using commercial ODS columns. Mobile phase, 0.1% (v/v) $H_3PO_4$; flow-rate, 1.0 ml/min; UV detection at 210 nm; column temperature, 40°C; sample volume, 20 μl; Peaks: 1=formic acid (0.05%, v/v), 2=acetic acid (0.05%, v/v).

packing material is suppressed and the peak shape becomes symmetrical. On the other hand, when using a neutral buffer solution, the hydrophobicity of the basic compound increases, as does the dissocia-

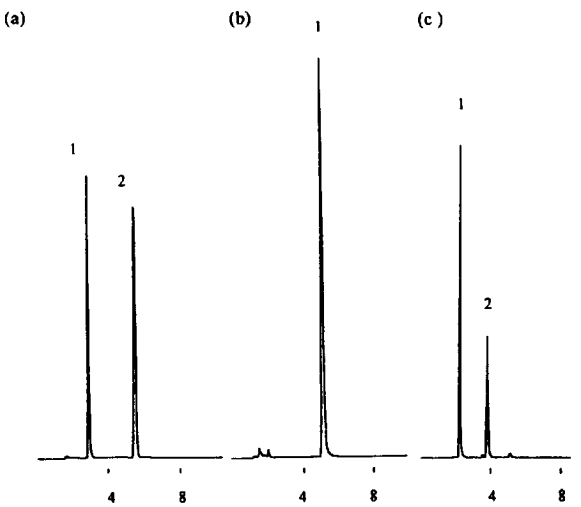


Fig. 5. Chromatograms of (a) pyridine–phenol, (b) oxine-copper and (c) formic acid–acetic acid obtained using an Inertsil ODS-3V column. Chromatographic conditions of (a) are as in Fig. 1, of (b) are as in Fig. 2 and of (c) are as in Fig. 3.

Table 5
Peak reproducibility obtained using Inertsil ODS-3V

| Sample | Buffer pH | R.S.D. (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Peak area | | | | Peak height | | | |
| | | 1.0 (mg/ml) | 10 | 50 | 100 | 1.0 (mg/ml) | 10 | 50 | 100 |
| Procainamide | 2.5 | 3.11 | 0.11 | 0.18 | 0.27 | 2.71 | 0.23 | 0.10 | 0.21 |
| | 4.9 | 2.15 | 0.55 | 0.41 | 0.36 | 2.15 | 0.55 | 0.41 | 0.36 |
| | 7.5 | 2.88 | 1.45 | 0.04 | 1.07 | 2.88 | 1.45 | 0.04 | 1.07 |
| Chlorpheniramine | 2.5 | 10.5 | 2.30 | 0.92 | 0.85 | 3.98 | 1.88 | 0.68 | 0.50 |
| | 4.9 | – | 20.9 | 1.90 | 1.39 | – | 20.9 | 1.97 | 1.39 |
| | 7.5 | – | 5.70 | 2.0 | 5.97 | – | 3.62 | 1.54 | 1.90 |
| Barbital | 2.5 | 9.31 | 0.86 | 0.41 | 0.19 | 8.60 | 0.64 | 0.34 | 0.33 |
| | 4.9 | 2.07 | 0.57 | 0.25 | 0.99 | 6.13 | 0.61 | 0.18 | 0.51 |
| | 7.5 | 16.5 | 2.71 | 0.98 | 0.20 | 13.7 | 1.26 | 0.36 | 0.10 |
| | MeOH–$H_2O$ | 5.28 | 4.22 | 0.98 | 0.44 | 13.8 | 1.26 | 0.36 | 0.10 |

Analytical conditions: 150×4.6 mm I.D. column; flow-rate, 1.0 ml/min; column temperature, 40°C; UV detection at 254 nm; sample volume, 1 μl; relative standard deviation (R.S.D.) calculated with $n=3$.

tion of residual silanol groups; therefore, it is difficult to elute the sample. Three mobile phases with different pH values (2.5, 4.9 and 7.5) were used in the experiment. As the results obtained with Inertsil ODS-3V (Table 5) show, over the range of pH values studied, high reproducibility can be achieved with very low concentrations of procainamide (for a 1-μl injection, the accuracy of the AS-640 autosampler is within ±0.5%). However, the ODS-A4 column gave an irreproducible peak at pH 7.5 (Table 6). In measuring traces of chlorpheniramine, the use of ODS-A4 not only led to an irreproducible result in the designated pH range, but the elution of the sample was impossible at pH 7.5.

Under the pH conditions used, no difference in peak reproducibility was found between Inertsil ODS-3V and ODS-A4 columns when barbital was applied. These data were obtained using formic acid–acetic acid as the sample, with no tailing of the peak shape with either column. In addition, when a

Table 6
Peak reproducibility obtained using an ODS-A4 column

| Sample | Buffer pH | R.S.D. (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Peak area | | | | Peak height | | | |
| | | 1.0 (mg/ml) | 10 | 50 | 100 | 1.0 (mg/ml) | 10 | 50 | 100 |
| Procainamide | 2.5 | 10.0 | 2.83 | 0.65 | 0.26 | 10.0 | 2.83 | 0.65 | 0.26 |
| | 4.9 | 8.69 | 4.16 | 0.27 | 0.54 | 9.49 | 0.62 | 0.62 | 0.15 |
| | 7.5 | 40.1 | 18.6 | 8.79 | 2.73 | 12.7 | 9.92 | 3.53 | 0.57 |
| Chlorpheniramine | 2.5 | 52.9 | 9.74 | 4.20 | 2.56 | 49.8 | 4.32 | 0.94 | 0.94 |
| | 4.9 | – | – | 13.3 | 6.20 | – | – | 7.44 | 2.52 |
| | 7.5 | – | – | – | – | – | – | – | – |
| Barbital | 2.5 | 16.1 | 0.41 | 0.63 | 0.64 | 14.6 | 1.30 | 0.35 | 0.23 |
| | 4.9 | 23.3 | 1.18 | 1.33 | 0.17 | 6.51 | 0.80 | 0.99 | 0.23 |
| | 7.5 | 25.0 | 3.10 | 0.63 | 0.45 | 26.1 | 1.85 | 0.24 | 0.64 |

Analytical conditions: 150×4.6 mm I.D.; flow-rate, 1.0 ml/min; column temperature, 40°C; UV detection at 254 nm; sample volume, 1 μl; R.S.D. calculated with $n=3$.

mobile phase without buffer was used, reproducible results could not be obtained using ODS-A4, due to the influence of residual silanol groups and/or metal impurities in the silica gel.

## 4. Conclusion

The analytical results obtained using pyridine–phenol, oxine-copper and formic acid–acetic acid are strongly controlled by the kind of silica gel used and by the degree of octadecylsilylation and end-capping. For the evaluation/controlling of column performance, the analytical method using the samples (i.e., pyridine–phenol, oxine-copper and formic acid–acetic acid) is very effective. The results found with these samples correspond well with those obtained on analysis of pharmaceutical compounds. These data are important in the optimization of conditions for the synthesis of ODS packing materials.

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