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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF ANTIPYRETICS ON CHEMICALLY MODIFIED POROUS GLASS

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SUMMARY

An octadecylsilyl porous glass was prepared and used as the packing for reversed-phase high-performance liquid chromatography. Five antipyretic drugs (aspirin, caffeine, guaiacol glycerol ether, 3-hydroxy-*p*-butyrophenetidine, and phenacetin) were separated in 2 min with a mobile phase of 20% acetonitrile at a flow-rate of 3.0 ml/min. A pharmaceutical preparation, containing aspirin, phenacetin, caffeine and chlorpheniramine maleate was analysed in 2 min with a mobile phase of acetonitrile–water–acetic acid (20:79:1). The packing seems promising for the rapid analysis of pharmaceuticals and biomedical compounds.

INTRODUCTION

There is an urgent need for new packing materials in order to achieve rapid chromatographic separation of pharmaceuticals and biomedical materials. To meet this dimand, we prepared chemical modifications of porous glass as packing materials[1]. Porous glass has been used in the chromatographic purification of proteins[2–5]. Antipyretics were successfully separated by the use of an octadecylsilyl porous glass (ODS glass) as the packing for reversed-phase high-performance liquid chromatography (HPLC). HPLC on ODS glass has not been described so far in the literature. The present paper describes results that suggest that this packing material saves much time in the analysis compared with conventional materials.

EXPERIMENTAL

Preparation of ODS glass

Porous Vycor glass with a pore size of 350 Å was treated with concentrated nitric acid, rinsed, and allowed to react with octadecylchlorosilane in boiling toluene for 3 h. The results of elemental analysis showed that the modified glass contained

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6.46% carbon. By treatment with trimethylchlorosilane, unchanged silanol groups in the modified glass were inactivated.

The electronograph and the BET method indicated that the distribution of pore size was fairly small.

ODS glass with a particle size distribution of 10–15 μm was packed into a 150 × 4 mm I.D. stainless-steel column by a high-pressure slurry technique.

Chemicals

Acetonitrile and acetic acid were purchased from Wako (Tokyo, Japan). Antipyretics and their preparations used in the study were pharmaceuticals standardized according to the 10th edition of the *Japanese Pharmacopoeia*. Water used as a solvent was freshly distilled and deionized. Other chemicals were of reagent grade. Vycor glass was obtained from Fuji Photofilm (Tokyo, Japan).

Apparatus

A Hitachi Model 655 high-performance liquid chromatograph, equipped with a Rheodyne Model 7125 valve and a Hitachi Model 655A UV monitor, operated at 254 nm, was used. Pharmaceuticals were dissolved in 80% acetonitrile–water and injected into the chromatograph with a Hamilton syringe. The system was operated at room temperature.

RESULTS AND DISCUSSION

Five antipyretic drugs, aspirin, caffeine, guaiacol glycerol ether, 3-hydroxy-*p*-

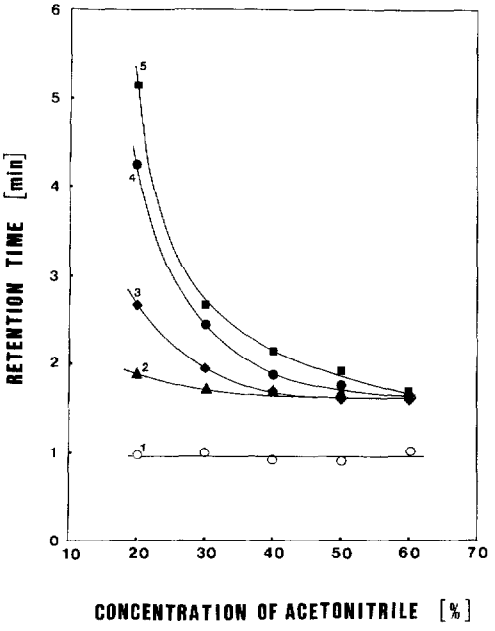
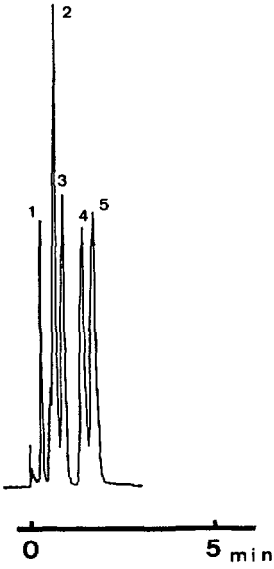


Fig. 1. Effect of the acetonitrile concentration in the mobile phase on the retention times of aspirin (1), caffeine (2), guaiacol glycerol ether (3), 3-hydroxy-*p*-butyrophenetidine (4) and phenacetin (5).



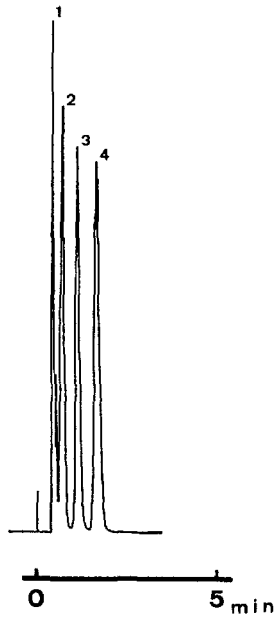


Fig. 2. Chromatogram of a mixture of aspirin (1), caffeine (2), guaiacol glycerol ether (3), 3-hydroxy-*p*-butyrophenetidine (4) and phenacetin (5).

Fig. 3. Chromatogram of the preparation containing chlorpheniramine maleate (1), caffeine (2), aspirin (3), and phenacetin (4).

butyrophenetidine and phenacetin, were chromatographed at room temperature at a flow-rate of 1.0 ml/min. The mobile phases were mixtures of acetonitrile and water, degassed by sonication. The results are shown in Fig. 1. With mobile phases containing more than 40% acetonitrile, the retention times of the five drugs were close together, and they were not separated.

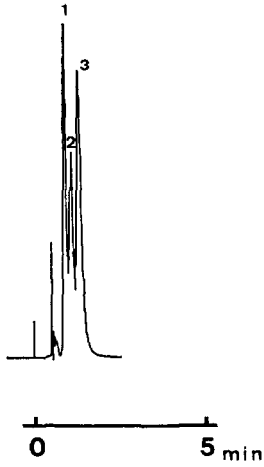


Fig. 4. Chromatogram of a mixture of salicylamide (1), aspirin (2) and salicylic acid (3).

With a mobile phase of 20% acetonitrile, the variation of retention times at different flow-rates was studied. The five peaks were well separated with flow-rates in the range 1.0–3.0 ml/min. Fig. 2 is a chromatogram obtained at a flow-rate of 3.0 ml/min, indicating that mixtures of the five drugs can be analysed in 2 min.

Pulvis aspirini, phenacetini et coffeini compositus (Compound Aspirin, Phenacetin and Caffeine Powder) is a pharmaceutical preparation standardized by the 10th *Japanese Pharmacopoeia*. It contains aspirin, phenacetin, caffeine and chlorpheniramine maleate. Determination of these four ingredients was required for the analysis of this preparation. The peaks of aspirin and chlorpheniramine maleate partially overlapped with acetonitrile–water (20:80) as the mobile phase. To facilitate complete separation, 1% of acetic acid was added to the mobile phase: this addition increased the retention time of aspirin. With acetonitrile–water–acetic acid (20:79:1), the ingredients of the preparation can be separated, as shown in Fig. 3. The separation of aspirin and its derivatives with this mobile phase was also possible. Fig. 4 is a chromatogram of a mixture of aspirin, salicylamide and salicylic acid.

The results shown in Figs. 2–4 indicate that the antipyretic agents can be analysed in 2 min. ODS glass seems to be a promising packing material for the rapid analysis of pharmaceuticals and biomedical compounds. Further studies are in progress in our laboratories.

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