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ANALYSIS OF ANTIPYRETICS BY SEMIMICRO LIQUID CHROMATOGRAPHY

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SUMMARY

Using semimicro columns (150 × 1 mm I.D.) of octadecyl silica and styrene–divinylbenzene porous polymer, antipyretic drugs were analyzed by means of high-performance liquid chromatography. The antipyretics studied were sulpyrin, caffeine, guaiacol glycerol ether, acetaminophen, 3-hydroxy-*p*-butyrophenetidine, methyl *p*-hydroxybenzoate, phenacetin, mefenamic acid, aspirin, salicylamide, salicylic acid, *o*-ethoxybenzamide, theobromine, theophylline and their preparations. The semimicro columns are economical in solvents in chromatographic analysis.

INTRODUCTION

High-performance liquid chromatography (HPLC) on micro or semimicro columns has aroused interest, since it enables the analysis time and amounts of samples and solvents to be reduced[1–5]. In addition, HPLC with semimicro columns requires no or only minor changes to the conventional chromatographic system. The present paper is concerned with the application of semimicro columns (150 × 1 mm I.D.) to the analysis of antipyretics and their pharmaceutical preparations. The packing materials used were octadecyl silica gel and styrene–divinylbenzene porous polymer.

EXPERIMENTAL

Column preparation

The octadecyl silica gel used was Hitachi-Gel 3057 (particle size 3 μm, Hitachi Co.). ODS Silica (200 mg) was dispersed in 1.0 ml of 2-propanol, containing 0.1 ml of dioxane. The slurry was packed into a 150 × 1 mm I.D. stainless-steel column at a pressure of 400 kg/cm^2 for 30 min with methanol.

The porous polymer used was Hitachi-Gel 3011. A 200-mg amount was dispersed in 1.7 ml of methanol by sonication and packed into a semimicro column at a pressure of 200 kg/cm^2 for 1 h with methanol.

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Chemicals

The chemicals and solvents used were of reagent grade and obtained from commercial sources. Antipyretics and their preparations were pharmaceuticals standardized according to the 10th edition of the *Japanese Pharmacopoeia.* Water used as solvent was freshly distilled and deionized.

Apparatus

A Hitachi Model 655-15 high-performance liquid chromatograph, equipped with a Rheodyne valve for semimicro columns and a Hitachi Model 655-0510 UV monitor operated at 254 nm, was used. The pharmaceuticals were dissolved in 80% aqueous acetonitrile for the ODS silica column and in methanol for the porous polymer column. A volume of 1.0 μl was injected into the chromatograph with a Hamilton syringe. The system was opoerated at room temperature.

RESULTS AND DISCUSSION

Seven antipyretic drugs, sulpyrin, caffeine, guaiacol glycerol ether, acetaminophen, 3-hydroxy-*p*-butyrophenetidine, methyl *p*-hydroxybenzoate and phenacetin, were chromatographed on the ODS silica column at a flow-rate of 30 μl/min. The mobile phases were mixtures of acetonitrile and water, degassed by sonication. The results are summarized in Fig. 1. With mobile phases containing more than 40% acetonitrile, the retention times of the seven drugs were similar, and they were not separated.

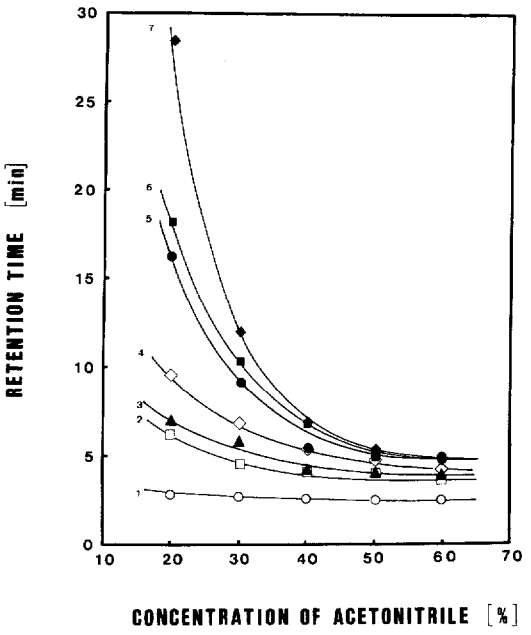

Fig. 1. Effect of acetonitrile concentration in the mobile phase on the retention times of sulpyrin (1), caffeine (2), guaiacol glycerol ether (3), acetaminophen (4), 3-hydroxy-*p*-butyrophenetidine (5), methyl *p*-hydroxybenzoate (6) and phenacetin (7).

With a mobile phase of acetonitrile–water (25:75), a mixture of sulpyrin, caffeine, guaiacol glycerol ether, methyl *p*-hydroxybenzoate and phenacetin was eluted at various flow-rates, ranging from 10 to 50 μl/min. The five peaks were well separated at all flow-rates in this range. The time required for the elution was 55 min with a flow-rate of 10 μl/min, 20 min with 30 μl/min and 10 min with 50 μl/min. The amount of mobile phase required was *ca.* 550 μl. Fig. 2 shows a chromatogram obtained at a flow-rate of 30 μl/min.

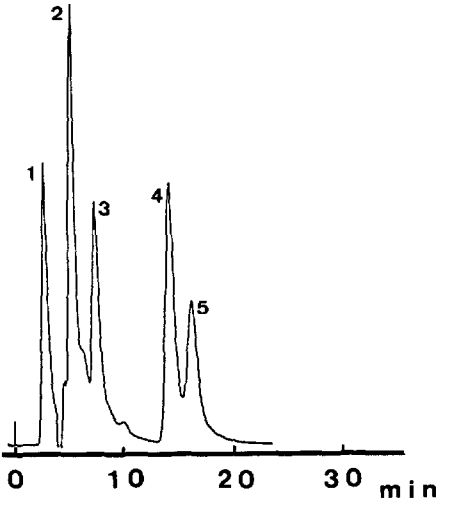

Fig. 2. A chromatogram of a mixture of sulpyrin (1), caffeine (2), guaiacol glycerol ether (3), methyl *p*-hydroxybenzoate (4) and phenacetin (5).

With the porous polymer semimicro column, the antipyretic drugs were chromatographed with three mobile phases at a flow-rate of 30 μl/min. The mobile phases were methanol, methanol containing 1% acetic acid and methanol containing 1% aqueous ammonia. The retention times are listed in Table I.

Pulvis aspirini, phenacetini et coffeini (aspirin, phenacetin and caffeine powder) is a pharmaceutical preparation standardized according to the 10th edition of the *Japanese Pharmacopoeia*. The three ingredients of the preparation were well separated with methanol containing 1% aqueous ammonia as the mobile phase at a flow-rate of 30 μl/min. The chromatogram is shown in Fig. 3.

A preparation containing acetaminophen, caffeine and *o*-ethoxybenzamide (ethenzamide) is a popular antipyretic pharmaceutical. The three drugs were well separated with any of the three mobile phases.

Methanol containing 1% aqueous ammonia was most suitable for the separation of a mixture of aniline antipyretics, acetaminophen, phenacetin, 3-hydroxy-*p*-butyrophenetidine and mefenamic acid. The peak of mefenamic acid was smaller or not detectable with other mobile phases. A mixture of xanthine drugs, caffeine, theobromine and theophylline, was also separated with a mobile phase containing ammonia.

TABLE I

RETENTION TIMES (min) OF ANTIPYRETICS

	Mobile phase		
	Methanol (100%)	*Methanol–1% acetic acid*	*Methanol–1% ammonia*
Aniline antipyretics			
Acetoaminophen	4.48	4.54	3.26
Phenacetin	5.84	5.68	5.70
3-Hydroxy-*p*-butyrophenetidine	5.18	5.20	5.24
Mefenamic acid	–	14.40	3.16
Salicylic acid antipyretics			
Aspirin	8.04	5.06	3.52
Salicylamide	4.98	5.00	3.16
Salicylic acid	–	7.10	3.56
o-Ethoxybenzamide	5.84	5.86	5.86
Pyrazolones			
Sulpyrin	3.42	8.58	3.62
Xanthines			
Caffeine	8.16	8.08	8.06
Theobromine	5.56	5.62	5.18
Theophylline	6.24	5.94	3.00

The results described indicate that semimicro columns can be used for the analysis of antipyretic agents. Such columns are economical in chromatographic solvents and seem promising for analysis of pharmaceuticals and biomedical compounds.

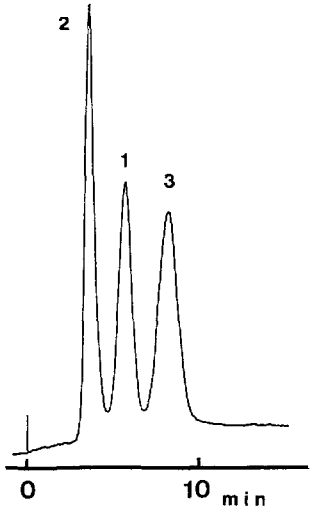

Fig. 3. A chromatogram of a preparation containing aspirin (1), phenacetin (2) and caffeine (3).

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