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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSES OF SULBENICILLIN AND CARBENICILLIN IN HUMAN URINE

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SUMMARY

A high-performance liquid chromatographic method has been developed for the determination of sulbenicillin (SBPC) and carbenicillin (CBPC) excreted in human urine. The method uses reversed-phase ion-pair chromatography with a bonded hydrophobic stationary phase and methanol–tetra-*n*-butylammonium bromide solution as the mobile phase. Urine is filtered through a micropore membrane and the filtrate introduced directly into a liquid chromatograph. An *in vivo* experiment was conducted by administering intravenously 1 g of SBPC or CBPC to volunteers and analysing their urine. One-compartment model analysis of the time-course data revealed that 73.1% of the amount of SBPC dosed was excreted in the urine with a rate constant of 0.579 h^{-1}, and 91.5% of CBPC with a rate constant of 0.845 h^{-1}.

INTRODUCTION

Sulbenicillin (SBPC) and carbenicillin (CBPC), the semi-synthetic penicillins derived from 6-aminopenicillanic acid, have wide spectra of activity against both Gram-positive and Gram-negative bacteria, the latter being highly resistant to penicillin G. The analytical methods so far employed for the determination of these penicillins include potentiometric titration using Hg^{2+} (ref. 1), infrared[2] and ultraviolet spectroscopies[3,4] ,and microbiological assay[5–10]. The chemical and spectroscopic methods, which utilize successfully the characteristic properties of penicillins, are sensitive but less specific to SBPC and CBPC. Microbiological assay using *Pseudomonas aeruginosa* as a test organism is highly sensitive and specific but time consuming and less accurate. These conventional methods have the common disadvantage of being tedious for the pre-treatment of biological samples. In this work we have attempted to solve such difficulties by utilizing reversed-phase ion-pair high-performance liquid chromatography (HPLC), which is sensitive, specific, accurate and rapid for the determination of the urinary concentration of SBPC and CBPC.

EXPERIMENTAL

Reagents and materials

Distilled water and analytical-reagent grade methanol (Wako, Osaka, Japan) were used after micropore filtration using 0.45-μm pore size triacetylcellulose membrane (Fuji Photo Film, Tokyo, Japan) and degassing. Analytical-reagent grade tetrabutylammonium bromide (Wako) was used after thorough drying. CBPC (Gripenin for injection, GP7640, 1 g as potency) and SBPC (Lilacillin for injection, 0977, 1 g as potency) were gifts from Fujisawa Pharm. Co. (Osaka, Japan) and Takeda Chem. Ind. (Osaka, Japan), respectively.

In vivo experiments and sample preparation

The *in vivo* experiments were conducted by separate administrations of SBPC and CBPC. In each experiment, a 1 g potency of the penicillin was dosed intravenously to each of four healthy male volunteers who had been fasting for 12 h and drug-free at least 1 week before administration. The urine samples were taken just before and at 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h after administration. After measurement of urine volume, a 1–2-ml portion was filtered through a 0.45-μm pore size membrane filter and the filtrate was injected directly into a liquid chromatograph. In the calibration procedure, known amounts of the penicillin (the same batch as that used for administration) were dissolved in normal urine and the solution was treated in the above manner.

Chromatography

An ALC/GPC 204 liquid chromatograph (Waters Assoc., Milford, Mass., U.S.A.) equipped with a UV detector (254 nm, Waters Assoc. Model 440) was used in a reversed-phase ion-pair system with μBondapak C_{18} as the stationary phase (30.4 cm $\times$ 4.0 mm I.D.; Waters Assoc.) and a 4:7 (v/v) mixture of methanol and 0.01 *M* tetrabutylammonium bromide solution as the mobile phase. The flow rate of the mobile phase was maintained at 3.0 ml/min (inlet pressure 3500 p.s.i.) for CBPC and 2.0 ml/min (3000 p.s.i.) for SBPC. The volume of the urine sample injected was 10 μl for CBPC and 20 μl for SBPC.

RESULTS AND DISCUSSION

As CBPC and SBPC are synthesized by coupling 6-aminopenicillanic acid with the optically active moiety including one asymmetric carbon atom, these penicillins comprise D(−)- and L(+)-diastereomers. The mixing ratio can be determined by nuclear magnetic resonance (NMR) spectrococpy. The NMR spectra of SBPC and CBPC used in this study are shown in Figs. 1 and 2, respectively, where the peaks marked A,B,C,. . ., are due to the protons indicated in the structure[11]. Nomura *et al.*[12] reported that proton B of D(−)-SBPC gives a signal at a lower δ-value than that of the L(+)-isomer. Fig. 1 shows two distinct peaks at 4.27 and 4.31 ppm, indicating that the SBPC is a mixture of diastereomers. The percentage of the D(−)-isomer estimated on the basis of the peak-height ratio of proton B signals is about 77%. The same method was also applied to CBPC and the percentage of the D(−)-isomer is about 55%.

Fig. 3 shows the chromatogram of CBPC dissolved in normal human urine.

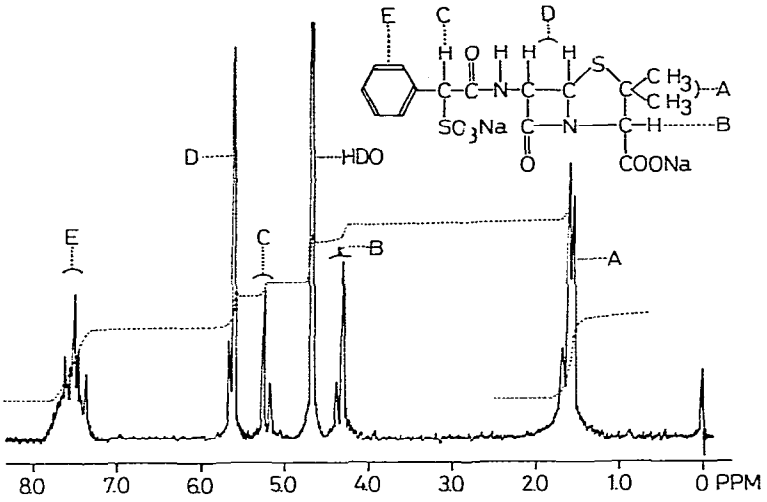

Fig. 1. Nuclear magnetic resonance spectrum of sulbenicillin in D_2O (60 MHz, TMS as internal standard).

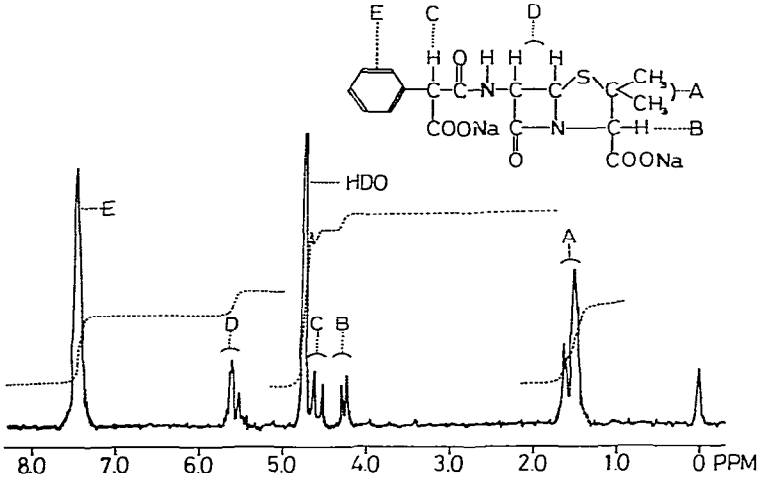

Fig. 2. Nuclear magnetic resonance spectrum of carbanicillin in D_2O (60 MHz, TMS as internal standard).

CBPC gave two incompletely resolved peaks, probably due to the diastereomers, and these peaks were well separated from background peaks. The usual reversed-phase system with 0.05% acetic acid solution–methanol as the mobile phase instead of the ion-pair elution system gave poor resolution of the isomers and unsatisfactory separation from normal urinary components. In the present study, therefore, CBPC was quantitated based on the combined peak area measured by gravimetry, *i.e.*, by cutting the peaks from the recording paper and weighing them. The calibration graph thus obtained for CBPC in the range 0.162–12.6 mg/ml showed good linearity and passed through the origin, with a correlation coefficient 0.999. SBPC, unlike CBPC, did not

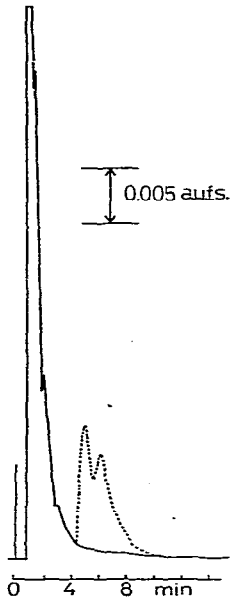

Fig. 3. HPLC separation of carbenicillin (— — —) from ordinary excretions (———) in human urine. Concentration of carbenicillin, 16 mg/ml; volume injected, 10 μl.

resolve the diastereomers, yielding a single peak with slight tailing, completely separated from the background peaks (Fig. 4). The quantitation of SBPC was achieved on the basis of the tailing peak, the area being measured by the triangulation method.

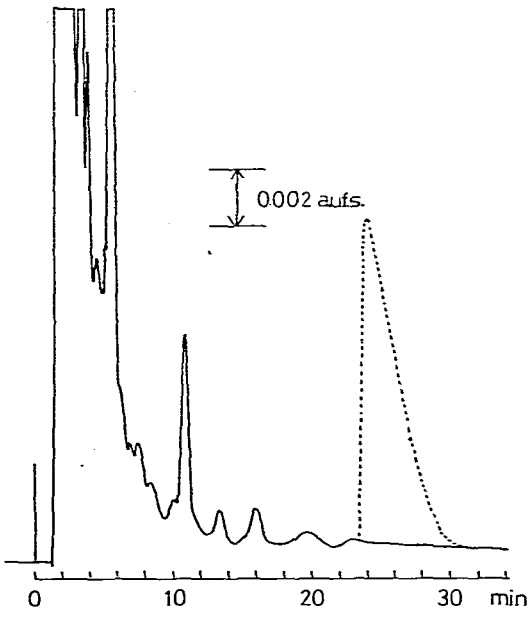

Fig. 4. HPLC separation of sulbenicillin (— — —) from ordinary excretions (———) in human urine. Concentration of sulbenicillin, 4.46 mg/ml; volume injected, 20 μl.

The calibration graph showed good linearity over a urinary concentration range of 0.223–8.92 mg/ml, with a correlation coefficient 0.999.

Prior to the urinary excretion experiments, the stability of CBPC was examined. The effluents corresponding to the two CBPC peaks were collected and each fraction was immediately re-chromatographed under the same LC conditions. No extra peaks appeared on the chromatogram. This result confirms that the mixing ratio of D(—)- and L(+)-CBPC remained constant during the chromatographic procedure. Penicillin G, which may be present in CBPC products, was not detected on the chromatogram within the full-scale redorder response of the CBPC peak. The elution profile of a urine sample taken from a volunteer administered CBPC was coincident with that shown in Fig. 3, indicating that diastereomers of CBPC underwent the same biological effects in the human body with the absence of metabolism. The same results were obtained with SBPC.

Tables I and II give the results for the amounts of CBPC and SBPC, respectively, excreted by each of four volunteers. The excretion rate–time curves are illustrated

TABLE I

URINARY EXCRETION OF CARBENICILLIN FOLLOWING A SINGLE INTRAVENOUS ADMINISTRATION OF 1 g TO HEALTHY VOLUNTEERS

Subject	K.Y.	M.M.	J.H.	S.N.
Age (years)	28	28	23	23
Body weight (kg)	68	68	62	59
Time after administration (h)	*Amount excreted (mg)*			
0 –0.5	270	411	283	282
0.5–1.0	254	223	183	265
1.0–1.5	143	142	133	127
1.5–2.0	64	93	90	59
2.0–3.0	108	87	80	71
3.0–4.0	12	25	27	61
4.0–5.0	—	16	9	46
5.0–6.0	—	—	—	34
Total	851	997	805	945

TABLE II

URINARY EXCRETION OF SULBENICILLIN FOLLOWING A SINGLE INTRAVENOUS ADMINISTRATION OF 1 g TO HEALTHY VOLUNTEERS

Subject	K.Y.	M.M.	J.H.	N.H.
Age (years)	29	28	24	27
Body weight (kg)	68	68	62	57
Time after administration (h)	*Amount excreted (mg)*			
0 –0.5	164	328	276	195
0.5–1.0	150	116	136	143
1.0–1.5	87	91	96	137
1.5–2.0	56	64	47	76
2.0–3.0	89	107	76	116
3.0–4.0	64	53	50	27
4.0–5.0	33	29	37	35
Total	668	808	749	739

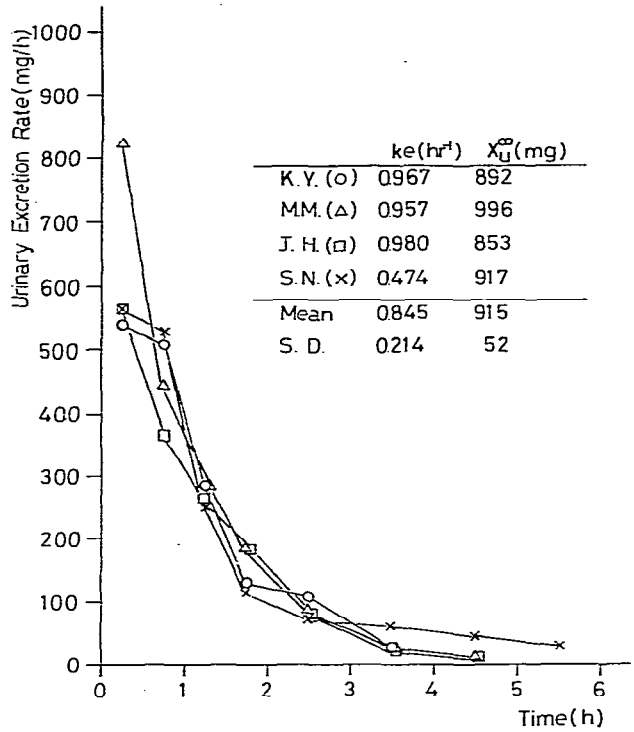

Fig. 5. Urinary excretion rate of carbenicillin following intravenous administration of 1 g of CBPC.

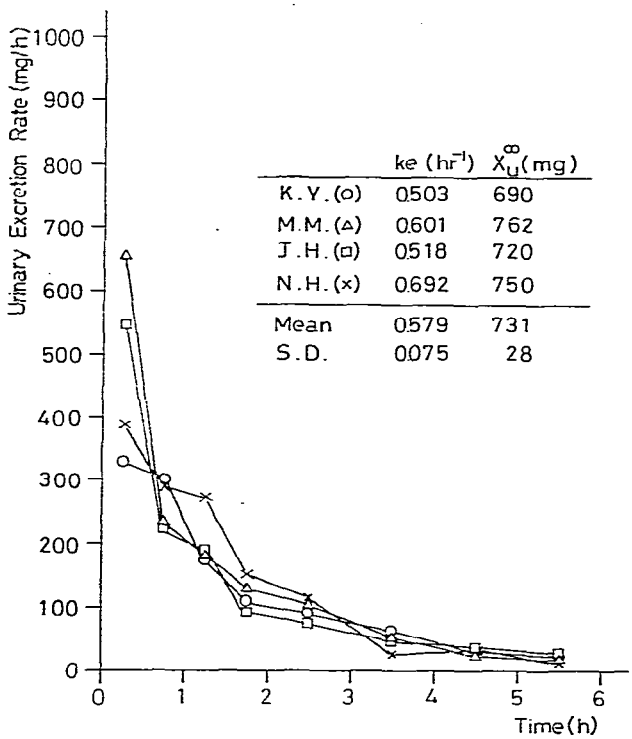

Fig. 6. Urinary excretion rate of sulbenicillin following intravenous administration of 1 g of SBPC.

n Figs. 5 and 6. One-compartment open model analysis of these data predicted that an average 91.5% of the dosed amount (1 g) of CBPC was finally excreted in urine as the intact form with a rate constant of 0.845 h^{-1}, and the corresponding values for SBPC were 73.1% and 0.579 h^{-1}. These results for CBPC agree with those reported by Acred *et al.*[13] and Smith and Finland[14], who, however, used microbiological assay as a quantitation method. Shimura *et al.*[15] found that bile excretion is a significant path for elimination of SBPC from the human body, while CBPC is excreted mostly in urine. This would explain the differences in the cumulative excretion ratios (X_e^∞) and excretion rate constants (k_e) between CBPC and SBPC.

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