59 (1978).
3) E. Christensen and A.C. Giese, *Arch. Biochem. Biophys.*, **51**, 208 (1954); R. Prat, Cl. Nofre, and A. Cier, *Ann. Inst. Pasteur*, **114**, 595 (1968); D.B.M. Scott, "Aquatic Applications of Ozone," ed. by W.J. Blogoslawski, and R.G. Rice, The International Ozone Institute, Inc., New York, 1975, pp. 1—12.
4) N. Shinriki, K. Ishizaki, A. Ikehata, A. Nomura, and Y. Mizuno, *Nucleic Acids Research, Special Publication*, No. 5, s303 (1978).
5) A.H. Brown, *Arch. Biochem.*, **11**, 269 (1946).
6) M. Daniels, G. Scholes, and J. Weiss, *J. Chem. Soc.*, **1956**, 3771 and references cited therein.
7) "Standard Methods for the Examination of Water and Wastewater," 14th Edition, American Public Health Association, Washington, D.C., 1976, p. 479.
8) P.S. Bailey, "Ozone in Water and Wastewater Treatment," ed. by F.L. Evans, Ann Arbor Science Publishers, Inc., Ann Arbor, 1972, pp. 29—59; K.J. Kolonko, R.H. Shapiro, R.M. Barkley, and R.E. Sievers, *J. Org. Chem.*, **44**, 3769 (1979).

Chem. Pharm. Bull. 29(3) 872—874 (1981)

Determination of Sulthiame in Plasma by High-Performance Liquid Chromatography

Tsuneo Sadanaga,[a] Kozo Hikida,[a] Katsunobu Tameto,[a] Michio Nakanishi,[a] Yoshikazu Matsushima,[b] and Yosuke Ohkura*,[b]

Research Laboratories, Yoshitomi Pharmaceutical Ind. Co., Ltd.,[a] Yoshitomi-cho, Fukuoka, 871, Japan and Faculty of Pharmaceutical Sciences, Kyushu University 62,[b] Maidashi, Higashi-ku, Fukuoka, 812, Japan

(Received September 29, 1980)

A simple high-performance liquid chromatographic method for the determination of sulthiame, N-(4′-sulfamoylphenyl)-1,4-butanesultam, in plasma is described. The method permits the accurate determination of the drug in plasma at concentrations as low as 150 ng/ml and is suitable for monitoring the drug in the therapeutic dose range and for investigation of the bioavailabilities of preparations of the drug.

Keywords——sulthiame; high-performance liquid chromatography; UV detection; plasma; drug monitoring; precise assay

Sulthiame, N-(4′-sulfamoylphenyl)-1,4-butanesultam, is an antiepileptic agent.[1] Several pharmaceutical preparations containing sulthiame have been used clinically. The therapeutic effects of the preparations may be related to its concentration in plasma. Thus, for comparison of the bioavailabilities of the preparations and for drug monitoring during therapy, a rapid and sensitive method of assay was required.

We reported assay methods for psychotropic and other drugs in plasma by means of high-performance liquid chromatography (HPLC).[2,3] HPLC was also found to be effective for the analysis of sulthiame, as described in the present paper. Several methods have been proposed for the determination of sulthiame in biomedical samples, *i.e.* UV spectrometry after separation by thin-layer chromatography,[4] gas-liquid chromatography with[5] or without derivatization,[6] and reversed phase HPLC.[7,8] However, the present method is very sensitive, reliable, and suitable for routine drug assay. Unfailing analytical results have always been obtained, since the method was introduced in our laboratories.

Experimental

Materials——Sulthiame and its preparations were the products of Bayer AG. Aminopyrine was obtained from Ebisu Pharm. Ind. Co. All solvents and chemicals were of reagent grade.

HPLC Instrumentation——A Hitachi model 635 liquid chromatograph equipped with a universal injector and a Hitachi variable wavelength UV effluent monitor operated at 250 nm was used. The column was a Zorbax-SIL (du Pond; particle size, 5 μm; 250×2.2 mm I.D.). The pressure was 50 kg/cm², giving a flow rate of 0.4 ml/min.

Standard Extraction Procedure——To 1.0 ml of plasma placed in a 50-ml glass-stoppered centrifuge tube, 8.0 ml of H_2O and 1.0 ml of 5% HCl were added. The sample was extracted once with 15 ml of $CHCl_3$ with vigorous shaking for 5 min. The phases were subsequently separated by centrifugation (1000 **g**, 10 min). After removal of the aqueous layer by aspiration, 8 ml of the $CHCl_3$ layer was transferred to a 20-ml glass-stoppered conical flask and evaporated to dryness *in vacuo* in a water bath at 45°. After cooling, the residue was dissolved in 0.25 ml of $CHCl_3$ containing aminopyrine (5 μg/ml) as a reference standard. An 8 μl volume of the $CHCl_3$ solution was injected into the liquid chromatograph.

For the preparation of the calibration curve, a series of standard sulthiame solutions was prepared by successive dilution of a stock solution of sulthiame. To 1.0 ml of drug-free plasma, 6.0 ml of H_2O, 1.0 ml of 5% HCl, and 2.0 ml of the standard sulthiame solution (0.5—10.0 μg/ml) were added, and the mixture was extracted with 15 ml of $CHCl_3$. These standards were carried through the procedure described above.

Results and Discussion

A chromatogram of a plasma sample containing sulthiame is shown in Fig. 1. The mobile phase used was dichloromethane containing 1.0% methanol and 0.1% aqueous ammonia. The retention times were 6.0 min for sulthiame and 10.5 min for aminopyrine. No interfering peak arose from endogenous plasma components.

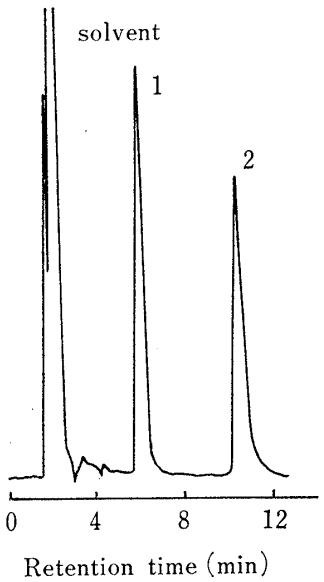

Fig. 1. A Chromatogram of Dog Plasma Containing Sulthiame

(1) sulthiame, (2) aminopyrine (reference standard).

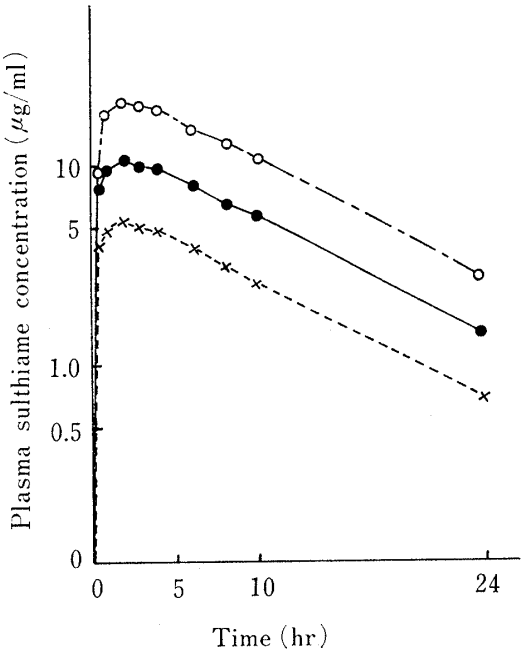

Fig. 2. Plasma Concentration of Sulthiame Following Its Oral Administration to Dogs

Doses of sulthiame were 400 mg (—○—), 200 mg, (—●—), and 100 mg (—×—) per animal. Analytical data presented are the averages of three animals.

The ratio of the peak height of sulthiame to that of aminopyrine was plotted against the amount of sulthiame in the standard. The calibration curve thus obtained was linear up to 20.0 μg/ml and passed through the origin. In the determination of sulthiame in plasma containing 10.0 μg/ml, the standard deviation was 0.15 μg (n=15). The present method permits the accurate determination of sulthiame in plasma at concentrations as low as 0.15 μg/ml. The method is suitable for monitoring the drug in plasma in the therapeutic dose range (200—600 mg/day/adult).

The plasmas of dogs administered sulthiame orally were analyzed by the present method. The results are shown in Fig. 2. The plasma sulthiame level increased immediately after the administration, reached a maximum in 2 hr, and then decreased in a first order manner. The biological half-life was about 7.3 hr.

The bioequivalence of sulthiame-containing tablets (Ospolot Tablets) of two formulas, which were the same in content of sulthiame but different in the kind and contents of inactive ingredients, was demonstrated in dogs. Details will be reported elsewhere.

References and Notes

1) P. Lerman and E. Nussbaum, *Acta Neurol. Scand.*, Suppl. **60**, 7 (1975).
2) T. Sadanaga, K. Hikida, K. Tameto, M. Nakanishi, Y. Matsushima, and Y. Ohkura, *J. Chromatogr.*, **183**, 246 (1980); *idem*, *Japan. J. Clin. Chem.*, **9**, 351 (1980).
3) T. Sadanaga, K. Hikida, K. Tameto, K. Sugimoto, M. Nakanishi, Y. Matsushima, and Y. Ohkura, *J. Chromatogr.*, **233**, 243 (1981).
4) O.V. Olesen, *Acta Pharmacol. Toxicol.*, **26**, 22 (1968).
5) K.J. Simmons and R.H. Levy, *J. Pharm. Sci.*, **61**, 1252 (1972).
6) P. Friel, J.R. Green, and H.J. Kupferberg, *Epilepsia*, **13**, 273 (1972); L.P. Hackett and L.J. Dusci, *Clin. Chim. Acta*, **66**, 443 (1976); P.N. Patsalos, V.D. Goldberg, and P.T. Lascelles, *Proc. Anal. Div. Chem. Soc.*, **12**, 270 (1975).
7) L.P. Hackett and L.J. Dusci, *Clin. Toxicol.*, **13**, 551 (1978).
8) D.J. Berry, L.A. Clarke, and G.E. Vallins, *J. Chromatogr.*, **171**, 363 (1979).

[Chem. Pharm. Bull. 29(3) 874—878 (1981)]

Effect of Extract from Paeoniae Radix on Urea-nitrogen Concentration in Rat Serum. I[1)]

Shinya Shibutani,[a] Tetsuro Nagasawa,[a] Hikokichi Oura,*,[a] Gen-ichiro Nonaka,[b] and Itsuo Nishioka[b]

Department of Biochemistry, Research Institute for WAKAN-YAKU, Toyama Medical and Pharmaceutical University,[a] 2630, Sugitani, Toyama, 930-01, Japan and Faculty of Pharmaceutical Science, Kyushu University,[b] 3-1-1, Maidashi, Higashi-ku, Fukuoka, 812, Japan

(Received July 29, 1980)

Intraperitoneal administration of the extract from Paeoniae Radix decreased the urea-nitrogen (BUN) concentration in rat serum. An attempt was made to extract and purify the active components, monitoring the BUN-decreasing activity. The acetone fraction from Paeoniae Radix decreased BUN concentration. After partition between ethyl acetate (EtOAc) and water, BUN-decreasing activity was detected in the EtOAc fraction. Further purification was carried out by Sephadex LH-20 column chromatography, and 1,2,3,4,6-penta-O-galloyl glucose, (+)-catechin, and gallic acid were purified from the EtOAc fraction. (+)-Catechin and gallic acid showed no activity. It became clear that one of the BUN-decreasing components from Paeoniae Radix was 1,2,3,4,6-penta-O-galloyl glucose, which is a typical tannin of the gallotannins.

Keywords——paeoniae radix; blood urea nitrogen; gallotannin; 1,2,3,4,6-penta-O-galloyl glucose; (+)-catechin; gallic acid

It was previously reported that intraperitoneal administration of each of 6 Kanpo prescriptions from among 65 varieties resulted in decreases of urea-nitrogen (BUN) concentrations in rat serum.[2)] Furthermore, administration of each of 7 crude drugs (Rhei Rhizoma, Coptidis