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Note

High-performance liquid chromatographic procedure for the determination of rat plasma concentrations of a new antibacterial agent, (±)-9-fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid, for topical use

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The new broad spectrum antibacterial agent (±)-9-fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid (OPC-7251, **I**, Fig. 1A) is structurally related to quinolone carboxylic acid [1]. Compound **I** is presently formulated as a cream product for topical use since it has greater antibacterial activity, especially against *Propionibacterium acnes, Staphylococcus epidermidis, Staphylococcus aureus* and *Pseudomonas aeruginosa* encountered in acne vulgaris and other skin infections [2]. The *P. acnes* and *S. epidermidis* play a central role in the induction

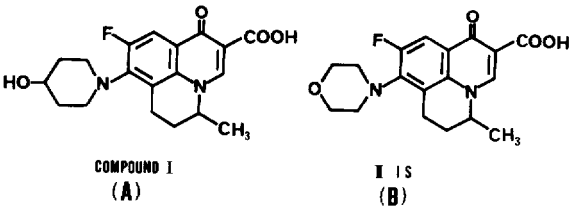

Fig. 1. Molecular structures of compound **I** (A) and the internal standard **II** (B).

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of inflammation in acne vulgaris [3–6]. On the other hand, *S. aureus* and *P. aeruginosa* have been recognized to be potent bacteria causing severe or chronic skin diseases [7, 8].

The pharmacological effect of **I** has been examined in rats with experimental infections of aforementioned bacteria, along with some toxicity studies. Furthermore, **I** is now being evaluated in some clinical studies for the treatment of acne vulgaris and other skin infections.

In this study a simple and accurate high-performance liquid chromatographic (HPLC) method has been established for the determination of **I** in rat plasma, and used to determine the concentration of **I** in rat plasma after a single percutaneous administration of 74 mg/kg to male rats. The method is also suitable for the assay of **I** in human plasma.

EXPERIMENTAL

Chemicals

Compound **I** and the internal standard (OPC-7258, **II**, I.S., Fig. 1B) were supplied by the Second Tokushima Factory, Otsuka Pharmaceutical (Tokushima, Japan). For the animal studies, 10% (w/w) **I** creams were prepared by the Formulation Research Institute. All other reagents and solvents were of analytical grade (Wako, Tokyo, Japan).

Standard solutions with 5, 10, 20, 50, 100 μg/ml concentrations of **I** in methanol were prepared. The standard solutions were added to the rat plasma to prepare 5 to 100 ng/ml concentrations of **I**.

Chromatography

The equipment consisted of a Model 510 solvent-delivery system, a Model 712 WISP sample processor (Waters Japan, Tokyo, Japan) and a Model SPD-6A UV spectrophotometric detector (Shimadzu, Kyoto, Japan). Data analysis was performed on UV peak heights of **I** and I.S. by a Chromatopac Model C-R3A integrator (Shimadzu). A prepacked TSK-gel ODS-80TM reversed-phase column (150 mm×4.6 mm I.D., particle size 5 μm, Toso, Tokyo, Japan) was used, and acetonitrile–tetrahydrofuran–25 m*M* ammonium phosphate buffer (35:3:65, v/v/v, pH 5.5) was used as mobile phase. The flow-rate was 1.0 ml/min, and the eluent was monitored at 295 nm. The separation was performed at room temperature (23°C).

Sample preparation

To 1.0 ml of rat plasma in a glass centrifuge tube, 10 μl of I.S. solution (100 ng/ml in methanol, 1.0 ml of 25 m*M* neutral phosphate buffer (pH 6.86) and 5.0 ml of chloroform were added. The tube was shaken for 10 min and centrifuged at 1800 *g* for 10 min, and the supernatant was discarded. A 3.5-ml aliquot of the chloroform phase was transferred to another centrifuge tube and evaporated to dryness in a water-bath (40°C) under a stream of air. The residue

was redissolved in 120 μl of methanol, and 40 μl of this solution were chromatographed.

Animal study

The animals used were male Sprague-Dawley rats (body weight 200–250 g, Shizuoka Laboratory Animal Center, Shizuoka, Japan). The pharmacokinetic studies of **I** were carried out in these rats after percutaneous administration of 74 mg/kg **I**. Blood samples were collected 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h after the administration. The samples were put into heparinized tubes and centrifuged at 1800 *g* for 10 min. The plasma was stored at −80°C until analysed.

RESULTS AND DISCUSSION

Typical chromatograms of the extracts from a drug-free rat plasma (A), a rat plasma containing 50 ng/ml **I** (B), and a plasma sample taken 2 h after a percutaneous administration of 74 mg/kg **I** (C) are shown in Fig. 2. The peaks of **I** and I.S. are well resolved, with retention times of 6.8 and 11.3 min, respectively. Under these chromatographic conditions, no significant interference from endogenous contaminants or metabolites was observed.

Calibration curves were constructed by plotting the peak-height ratio of **I** to I.S. versus the concentration of **I**, and showed linearity in the range 5–100 ng/ml. The results are presented in Table I. The equation used for the construction of the calibration curve was $y=0.0141x-0.0146$ with a correlation coefficient of 0.9996 ($n=5$). The accuracy was assessed by analysis of known amounts of **I**. The measured concentrations of **I** ranged from 98% to 107% of the theoret-

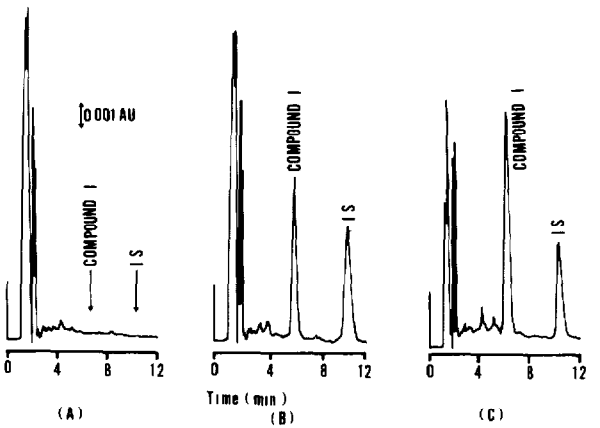

Fig. 2. Chromatograms of (A) drug-free rat plasma; (B) rat plasma containing compound **I** (50 ng/ml) and (C) rat plasma after a single percutaneous dose of 74 mg/kg compound **I**.

TABLE I

LINEARITY AND INTER-ASSAY PRECISION OF HPLC PROCEDURE FOR COMPOUND I IN RAT PLASMA BY INTERNAL STANDARD METHOD

Spiked concentration (ng/ml)	Peak-height ratio (mean ± S.D., $n=5$)	C.V. (%)	Recalculated concentration (ng/ml)	Percentage of theory	Recovery (%)
5	0.061 ± 0.004	6.1	5.4	107	78
10	0.128 ± 0.007	5.3	10 1	101	87
25	0.331 ± 0.003	0.9	24.5	98	91
90	0.688 ± 0.023	3.2	49.8	100	91
100	1.398 ± 0.019	1.4	100 2	100	81

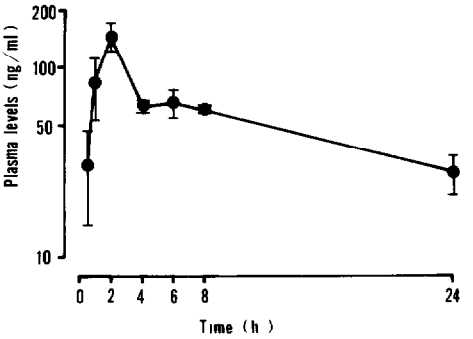

Fig 3. Plasma compound **I** levels after a single percutaneous dose of 74 mg/kg in male rat ($n=3$).

ical concentrations. The inter-day precision of this method was determined by calculating a mean peak-height ratio ± standard deviation (S.D.) for each of five plasma samples containing 20 and 75 ng/ml concentration. The coefficient of variation (C.V.) ranged from 3.3% at 20 ng/ml **I** to 4.1% at 75 ng/ml.

The detection limit of this method was 1 ng/ml for plasma concentration, resulting in a signal-to-noise ratio of 3:1.

The recovery ratio of **I** was determined by the extraction procedure (as described in *Sample preparation*) at the concentrations of 5, 10, 25, 50 and 100 ng/ml **I** in rat plasma. The peak heights of **I** obtained from the plasma were compared with those obtained after the standard solutions at the same concentrations were injected into the chromatograph. The recovery ratios ranged from 78% to 91%.

The present assay method was applied to the pharmacokinetic studies of **I** in rats. Fig. 3 shows the typical plasma concentration versus time profiles after a percutaneous administration of **I** at a dose of 74 mg/kg to male rats ($n=3$). Compound **I** reached a peak plasma concentration of 146 ng/ml at 2 h, and

rapidly declined to 29 ng/ml at 24 h. The procedure was thus shown to be simple, accurate and reproducible for analysis of **I** in rat plasma.

This method was also used successfully for the assay of **I** in human plasma at concentrations greater than 5 ng/ml. The calibration curve for **I** in human plasma was linear over the range 5–100 ng/ml. The coefficient of the calibration curve for human plasma was 0.9991. The C.V. ($n=5$) were 8.9% (10 ng/ml), 3.4% (25 ng/ml) and 1.5% (50 ng/ml).

In conclusion, the present method was found to be useful for analysis of **I** in rat plasma, and to be applicable for analysis of **I** in human plasma.

REFERENCES

1 H. Ishikawa, F. Tabusa, H. Miyamoto, M. Kano, H. Ueda, H. Tamaoka and K. Nakagawa, Chem. Pharm. Bull., 37 (1989) 2103.
2 S. Kawabata, K. Ohguro, F. Mukai, K. Ohmori, H. Miyamoto and H. Tamaoka, Chemotherapy, 37 (1989) 1160.
3 N.H. Shehadesh and A.M. Kligman, Arch. Dermatol., 88 (1963) 829.
4 P.E. Pochi, in D.J. Demis (Editor), Clinical Dermatology, Vol. 2, Harper and Row, New York, 1979, unit 10-2.
5 K.T. Holland, E. Ingham and W.J. Cunliffe, J. Appl. Bacteriol, 51 (1981) 195
6 K. Takisawa, Rinsho Derma (Tokyo), 17 (1981) 515.
7 M. Suwaki, K. Yamamoto and J. Arata, Chemotheraphy, 26 (1978) 324.
8 K. Ishibiki, Y. Yamada, N. Aikawa and S. Yamamoto, Surg. Ther., 41 (1979) 659.