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Short communication

Determination of a new non-narcotic analgesic, DA-5018, in plasma, urine and bile by high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic method was developed for the determination of a new non-narcotic analgesic, DA-5018 (I), in rat plasma, urine and bile samples, using propranolol for plasma samples and protriptyline for urine and bile samples as internal standards. The method involved extraction followed by injection of 100 μl of the aqueous layer onto a C_{18} reversed-phase column. The mobile phases were 5 m*M* methanesulfonic acid with 10 m*M* NaH_2PO_4 (pH 2.5)–acetonitrile, 70:30 (v/v) for plasma samples and 75:25 (v/v) for urine and bile samples. The flow-rates were 1.0 ml/min for plasma samples and 1.2 ml/min for urine and bile samples. The column effluent was monitored by a fluorescence detector with an excitation wavelength of 270 nm and an emission wavelength of 330 nm. The retention time for I was 4.8 min in plasma samples and 10.0 min in urine and bile samples. The detection limits for I in rat plasma, urine and bile were 20, 100 and 100 ng/ml, respectively. There was no interference from endogenous substances.

Keywords: DA-5018; N-[3-(3,4-Dimethylphenyl)propyl]-4-(2-aminoethoxy)-3-methoxyphenylacetamide hydrochloride

1. Introduction

New capsaicin analogs have been synthesized to develop new types of non-steroidal antiinflammatory drugs [1]. KRICT, Korea Research Institute of Chemical Technology (Taejeon, South Korea) has recently developed a capsaicin analog, DA-5018 (I, Fig. 1). Compound I is now being evaluated in phase I clinical trials.

This paper describes a method for the analysis of I in rat plasma, urine and bile using HPLC with fluorescence detection.

2. Experimental

2.1. Chemicals

Compound I [N-[3-(3,4-dimethylphenyl) propyl]-4-(2-aminoethoxy)-3-methoxyphenyl acetamide hydrochloride] was kindly donated by Research Lab-

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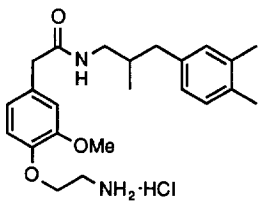
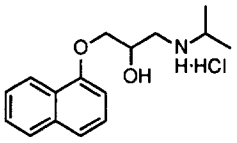
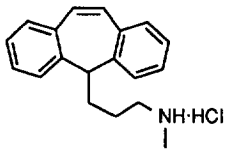


I



Propranolol Hydrochloride



Protriptyline Hydrochloride

Fig. 1. Chemical structures of I and the internal standards, propranolol and protriptyline.

oratories of Dong-A Pharmaceutical. Methanesulfonic acid was purchased from Aldrich (Milwaukee, WI, USA). Propranolol hydrochloride and protriptyline hydrochloride were products of Sigma (St. Louis, MO, USA). The other chemicals were of reagent grade or HPLC grade, and used without further purification.

2.2. Preparation of standard solutions

The stock solution of I was prepared in distilled water. Appropriate dilutions of the stock solution were made with distilled water. Standard solutions of I in water, and rat plasma, urine and bile were prepared by spiking the appropriate volume (less than 10 μl per ml) of diluted stock solution to give final concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 μg/ml. The internal standard solution, propranolol hydrochloride or protriptyline hydrochloride, was prepared by dissolving in distilled water to give a final concentration of 0.05 and 0.2 μg/ml for propranolol and protriptyline, respectively. Only the HPLC assay results on 0.02, 0.05 and 10 μg/ml concentrations for plasma, and 0.1, 0.5 and 10 μg/ml concentrations for urine and bile are listed in Table 1.

2.3. Sample preparation

A 0.1-ml volume of 0.1 *M* NaOH, 0.1 ml of the internal standard (propranolol for plasma samples and protriptyline for urine and bile samples), and 0.6 ml of ethyl ether were added to 0.1 ml of biological samples [2]. The mixture was shaken for 2 min and centrifuged at 3000 *g* for 3 min. The organic layer was transferred to a new tube and 0.15 ml of 0.1 *M* HCl was added. After vortex-mixing and centrifuga-

Table 1
Response factors and relative recoveries of I at various concentrations in rat plasma, urine and bile using the extraction method

Biological sample	Spiked concentration (μg/ml)	Response factor[a]	C.V.[b] ($n=3$) (%)	Relative recovery[c] (%)	Accuracy[d] (%)
Plasma	10	1.32±0.0208	1.57	93.0	93.5
	0.05	1.47±0.0550	3.47	97.5	104
	0.02	1.36±0.0986	7.24	97.9	96.4
Urine	10	0.612±0.00987	1.59	93.8	105
	0.5	0.576±0.0106	1.85	94.6	98.9
	0.1	0.551±0.0101	1.93	108	94.5
Bile	10	0.695±0.0252	3.68	114	106
	0.5	0.626±0.0242	3.79	108	95.4
	0.1	0.652±0.0185	2.79	107	99.5

[a] (Drug peak height divided by its concentration)/(internal standard peak height divided by its concentration); mean±S.D..
[b] Coefficients of variation, (S.D./mean)×100.
[c] Relative recovery compared with water.
[d] (Mean measured concentration/spiked concentration)×100.

tion, 100 μl of the aqueous layer was injected directly onto the HPLC column. After intravenous administration of I to rats, one of its metabolites (the chemical structures are not identified yet) interfered with propranolol assay in urine and bile samples. Therefore, protriptyline (the retention time was longer than that of propranolol) was chosen as an internal standard for bile and urine samples.

2.4. HPLC apparatus

The HPLC system consisted of a Model 7125 injector (Rheodyne, Cotati, CA, USA), a Model 9600 ternary gradient pump (Eldex, San Carlos, CA, USA), a reversed-phase column (Waters Assoc. Milford, MA, USA, Symmetry C_{18}, 15 cm×3.9 mm I.D., 5 μm), a fluorescence detector (Linear fluor LC304, Linear, Reno, NV, USA) set at excitation wavelength of 270 nm and emission wavelength of 330 nm, and an integrator (D-2500, Hitachi, Japan) [3]. The mobile phases were 5 m*M* methanesulfonic acid with 10 m*M* NaH_2PO_4 (pH 2.5)–acetonitrile, 70:30 (v/v) for plasma samples, and 75:25 (v/v) for urine and bile samples. The flow-rates were 1.0 ml/min for plasma samples and 1.2 ml/min for urine and bile samples. The composition and flow-rate of the mobile phase for urine and bile samples were different from those for plasma samples because of interferences in the urine and bile samples.

3. Results and discussion

Fig. 2 shows typical chromatograms of drug-free rat plasma (A), drug standards in plasma (B), and plasma collected at 15 min after intravenous administration of 5 mg/kg of I to a rat (C), using the extraction method; the corresponding chromatograms for rat urine and bile are shown in Figs. 3 and 4, respectively. No interference from endogenous substances was observed in any of the chromatograms of the rat plasma, urine, or bile samples. The peaks of I and the internal standard, propranolol or protriptyline, were symmetrical. The retention times for I and propranolol in plasma samples were 4.8 and 2.5 min, respectively, and for I and protriptyline in urine and bile samples were 10.0 and 12.1 min, respectively.

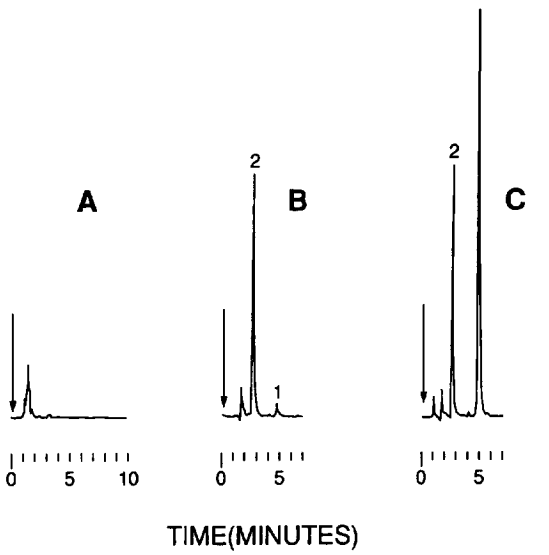


Fig. 2. Chromatograms of extracts from blank rat plasma (A), the rat plasma spiked with 0.05 μg/ml of I and 0.05 μg/ml of an internal standard, propranolol hydrochloride (B) and plasma obtained at 15 min after intravenous administration of I (5 mg/kg) to a rat (C). Peaks: 1=I (4.8 min); 2=propranolol hydrochloride (2.5 min). The arrows mark the points of injection. The detector sensitivity was set at 50 FUFS (fluorescence unit full-scale) and recorder sensitivity was set at 64 mV.

The detection limits for I in rat plasma, urine and bile were 20, 100 and 100 ng/ml, respectively (Table 1), based on a signal-to-noise ratio of 3.0 using the extraction method. The mean within-day coefficients of variation (C.V.s) for I in rat plasma, urine and bile using extraction method were lower than 7.99, 5.28 and 4.97%, respectively (Table 1). The between-day C.V.s for the analysis of the same samples on three consecutive days for I in rat plasma, urine and bile using extraction method were lower than 7.80%. The analytical relative recoveries compared with water from rat plasma, urine and bile samples spiked with I using extraction method were 93.0–110, 93.0–108 and 107–114%, respectively (Table 1). The mean accuracy (measured concentration/spiked concentration) from rat plasma, urine and bile samples spiked with I using extraction method were 93.5–104, 94.5–105 and 95.4–106%, respectively (Table 1).

The mean arterial plasma concentration–time pro-

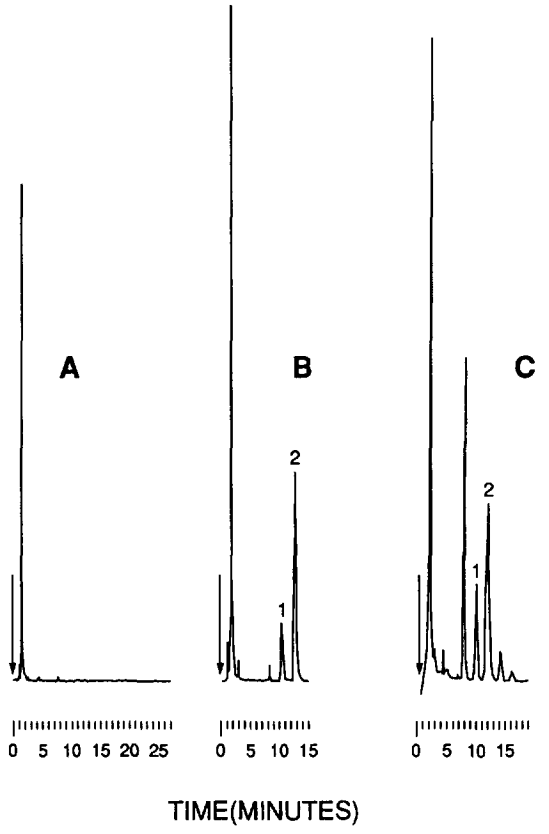


Fig. 3. Chromatograms of extracts from blank rat urine (A), the rat urine spiked with 0.5 μg/ml of I and 0.2 μg/ml of an internal standard, protriptyline hydrochloride (B) and urine collected between 0–24 h after intravenous administration of I (5 mg/kg) to a rat (C). Peaks: 1=I (10.0 min); 2=protriptyline hydrochloride (12.1 min). The arrows mark the points of injection. The detector sensitivity was set at 50 FUFS and recorder sensitivity was set at 64 mV.

files of I after 1-min intravenous administration of 5 mg/kg of I to 4 rats are shown in Fig. 5. The mean values of terminal half-life, mean residence time, total body clearance and apparent volume of distribution at steady state of I were 1.15 h, 0.702 h, 2720 ml/min/kg and 1870 ml/kg, respectively. The percentages of intravenous dose of I excreted in 24-h urine as unchanged I were 0.853% and in 24-h bile were 3.06% when the I, 5 mg/kg, was administered intravenously to 4 rats.

Stability of I in plasma, blood, 4% human serum albumin (HSA), and phosphate buffer of pH 7.4, blood partition of I between plasma and blood cells,

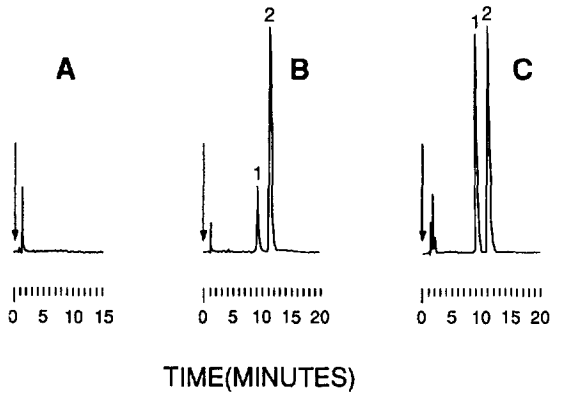


Fig. 4. Chromatograms of extracts from blank rat bile (A), the rat bile spiked with 0.5 μg/ml of I and 0.2 μg/ml of an internal standard, protriptyline hydrochloride (B) and rat bile collected between 2–4 h after intravenous administration of I (5 mg/kg) to a rat (C). Peaks: 1=I (10.0 min); 2=protriptyline hydrochloride (12.1 min). The arrows mark the points of injection. The detector sensitivity was set at 50 FUFS and recorder sensitivity was set at 64 mV.

and protein binding of I to 4% HSA were reported [4] using the present HPLC method. Compound I was stable in both rat blood and plasma, 4% HSA, and the buffer up to 4–24 h standing at 25°C, the mean value of rat blood cells to plasma concentration ratio of I was 1.31, and the binding of I to 4% HSA

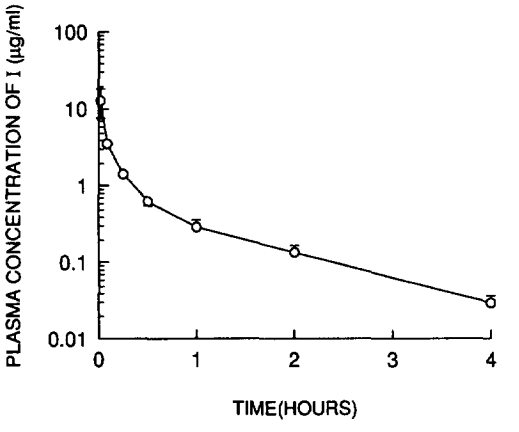


Fig. 5. Mean arterial plasma concentration–time profile of I after 1-min intravenous administration of I (5 mg/kg) to 4 rats. Bars represent standard deviation.

was dependent on concentrations of I; from 87.3% (at 0.1 μg/ml) to 52.5% (at 50 μg/ml) [4].

Acknowledgments

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