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Determination of 1-furan-2-yl-3-pyridin-2-yl-propenone, an anti-inflammatory propenone compound, by high performance liquid chromatography with ultraviolet spectrometry

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Abstract

1-Furan-2-yl-3-pyridin-2-yl-propenone (FPP-3) has recently been synthesized and characterized to have an anti-inflammatory activity. In the present study, pharmacokinetic parameters for FPP-3 and its metabolites were determined at the same time by using high-performance liquid chromatography-ultraviolet spectrometry. Two metabolites were detected in sera when FPP-3 was administered intravenously to male SD rats. The linearity of FPP-3, M1 (1-furan-2-yl-3-pyridin-2-yl-propan-1-one) and M2 (1-furan-2-yl-3-pyridin-2-yl-propan-1-ol) was confirmed in the concentration ranges of 0.5–20, 0.101–4.04 and 1.04–20.4 μg/ml, respectively. The lower limits of quantitation of FPP-3, M1 and M2 were 0.5, 0.1 and 1.0 μg/ml, respectively. The intra- and inter-day precision and accuracy over the concentration range of target compounds were within 13.5 and 14.2%, respectively. The half-lives of FPP-3, M1 and M2 were 16.3, 27.7 and 22.1 min, respectively.
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Keywords: 1-Furan-2-yl-3-pyridin-2-yl-propenone (FPP-3); Rat; Metabolites; Kinetics

1. Introduction

1-Furan-2-yl-3-pyridin-2-yl-propenone (FPP-3) is a chemically synthesized novel propenone compound with anti-inflammatory activities. A recent study demonstrated that FPP-3 could inhibit lipopolysaccharide-stimulated production of nitric oxide and tumor necrosis factor-α in the cultures of RAW 264.7 macrophages [1]. In addition, FPP-3 could not only inhibit cyclooxygenases and 5-lipoxygenase activities but also inhibit cyclooxygenase-2 with 35 times greater selectivity than cyclooxygenase-1 [2]. Moreover, analgesic and anti-inflammatory effects of FPP-3 were as high as those of the conventional non-steroidal anti-inflammatory drug, such as indomethacin, without showing any ulcerogenic effects in rats and mice at the therapeutic doses [3].

In our recent study, when FPP-3 was incubated with rat liver cytosols in the presence of NADPH, two major metabolites were detected by liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) and ^{1}H NMR spectrometry [4]. From the study, two metabolites were believed to be formed by sequential reductions of the propenone (Fig. 1): M1 (1-furan-2-yl-3-pyridin-2-yl-propan-1-one) was the initial metabolite and M2 (1-furan-2-yl-3-pyridin-2-yl-propan-1-ol) was a secondary alcohol believed to be formed from M1 [4].

High-performance liquid chromatography with ultraviolet detection (HPLC-UV) is a simple method for the determination of drugs and metabolites in biological fluids. This paper describes a sensitive method for determination of FPP-3 and its metabolites at the same time in rat serum using HPLC-UV spectrometry.

2. Experimental

2.1. Materials

FPP-3 (purity, >99%) used in this study (Fig. 1) was chemically synthesized in our group [1]. Metabolites were separated by thin layer chromatography (TLC) in our group. Tolbutamide and ammonium formate were obtained from Sigma Chemical

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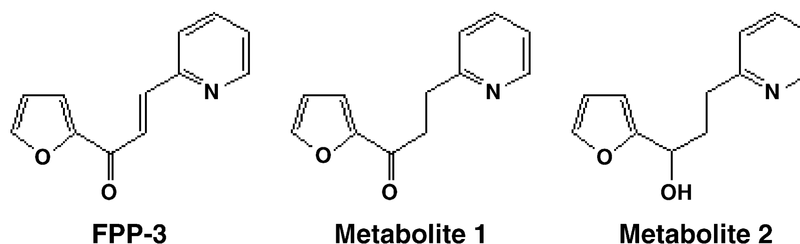

Fig. 1. Chemical structures of FPP-3 and its metabolites.

Co. (St. Louis, MO, USA). TLC alumina sheets (silica gel 60 F_{254}) and acetonitrile (ACN) were HPLC-grades from Merck Ltd. (Poole, UK). All other chemicals were of analytical grade and used as received.

2.2. Standard solution

To collect the standard compounds, FPP-3 at 3 mM final concentration was incubated with 4 mg/ml of cytosolic protein in 0.1 M potassium phosphate buffer, pH 7.4, at 37 °C in a final volume of 50 ml. Cytosolic fraction was prepared from male Sprague–Dawley rats as described previously [4]. The reaction was initiated by the addition of an NADPH-generating system containing 0.8 mM NADPH, 10 mM glucose 6-phosphate and 1 U of glucose 6-phosphate dehydrogenase into the reaction mixture. The reaction was stopped by the addition of 100 ml ethyl acetate (EtOAc). Following mixing and centrifugation, 95 ml of the organic layer was separated and concentrated to 2 ml.

The concentrated EtOAc extracts were loaded on a TLC alumina sheet. The plates were eluted with hexane:EtOAc (2:8). Each spot separated from the plates was extracted by EtOAc. The fractions were identified as FPP-3, M1 and M2 by LC/ESI-MS and ^{1}H NMR spectra [4].

2.3. Sample preparation

After an intravenous penile vein injection of male Sprague–Dawley rats with FPP-3 at 4 mg/kg, approximately 500 μl of blood was obtained from the subclavian vein immediately before and 1, 3, 5, 10, 20, 40 and 60 min after the dose. A 200 μl serum sample, prepared by a centrifugation of the blood at 3000 × *g* for 15 min at 4 °C, was extracted with 800 μl of EtOAc following an addition of 25 μl internal standard solution, 12.5 μg/ml of tolbutamide. After mixing and centrifugation, 600 μl of the organic layer was separated. The organic layer was dried under a stream of nitrogen gas. A residue was reconstituted in 100 μl ACN and a 20 μl aliquot was injected into an HPLC column.

2.4. Chromatographic conditions

The samples (20 μl) were injected into the chromatographic system (GL Sciences Inc., Tokyo, Japan). Separation was conducted using a linear gradient system from 5:95 to 90:10 for 13 min (100% ACN:20 mM ammounium formate buffer, pH 4.0). The analyses were performed at room temperature (25 °C) at a flow rate of 1.0 ml/min. The eluate was monitored at 265 nm. The data were collected with a dsCHROM$^{99(PI)}$ chromatograph data system (Donam Inc., Sungnam, Korea). The data were acquired as the ratio of the AUC under the peak of samples with the AUC of internal standard.

2.5. Instrumentation

A GL science Intertsil® ODS-3 column (5 μm, 4.6 mm × 150 mm) and a guard column C18 (4 mm, 3.0 mm i.d., Phenomenex, USA) were used for the HPLC separation. FPP-3 and its metabolites in the extracted samples were determined with a PU610 pump and a UV620 detector (GL Sciences Inc.).

2.6. Validation test

2.6.1. Linearity and calibration curve

To prepare calibration standards, 10 μl of each stock solution in ACN was added to 190 μl drug-free serum. The spiked concentrations of the calibration standards were 0.5, 1.0, 5, 10 and 20 μg/ml of FPP-3, 0.101, 0.202, 1.01, 2.02 and 4.04 μg/ml of M1 and 1.04, 5.01, 10.2, 15.3 and 20.4 μg/ml of M2. The samples were then processed as described in Section 2.3. The calibration curves were constructed by plotting the peak-area ratio of FPP-3, M1 and M2 to the internal standard, respectively.

2.6.2. Accuracy and precision

Spiked samples of three concentrations (0.5, 5.0 and 20 μg/ml, $n = 5$) were prepared and assayed to determine the intra-day accuracy expressed as the relative error, and the precision as the relative standard deviation (R.S.D.). The same method was used over 5 days for the inter-day assay.

2.7. Pharmacokinetic parameters

The pharmacokinetic parameters were determined using the standard non-compartmental method. Serum AUC was calculated using WinNonlin (version 1.1, Scientific Consulting, KY, USA) with a log linear trapezoidal method.

3. Results and discussion

3.1. Sample preparation and analysis

Representative UV chromatograms of blank serum and spiked serum sample are shown in Fig. 2. No endogenous sources of interference were observed at the retention time of the analyte. FPP-3, M1 and M2 were observed in HPLC-UV with a

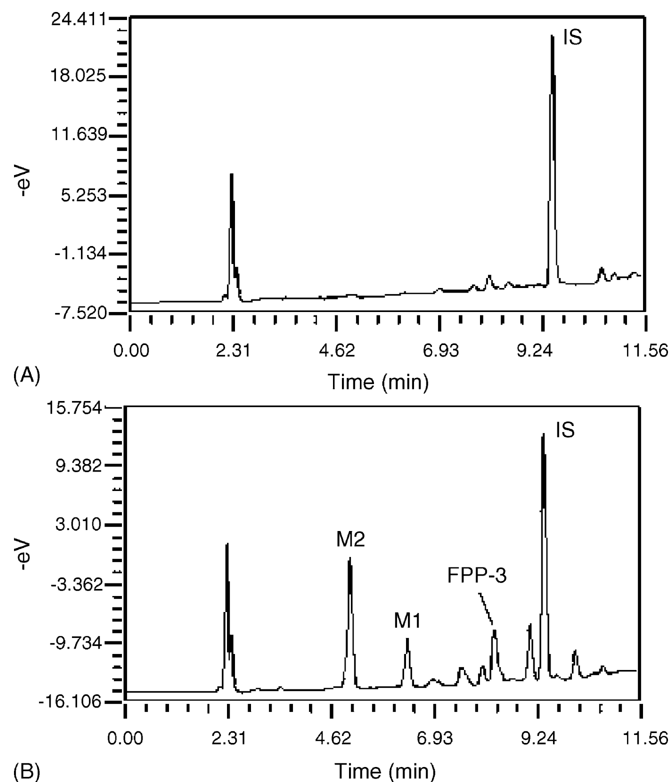

Fig. 2. Chromatograms of the extract of rat serum blank (A) and rat serum intravenously treated with FPP-3 at 4 mg/kg (B). The concentration of the internal standard, tolbutamide, was approximately 12.5 μg/ml.

retention time of 8.3, 6.3 and 5.1 min, respectively. Following the reduction of propenone structure of FPP-3, the UV absorption bands of metabolites were shifted to a shorter wavelength (Fig. 3). The extinction co-efficient of FPP-3, M1 and M2 were determined with 6.88, 16.12 and 7.09 $mM^{-1}\,cm^{-1}$ in the each 0.1 mM standard methanol solution at 265 nm, respectively.

FPP-3 seemed to be relatively stable because FPP-3 was decreased 8%, when FPP-3 was heated to evaluate the stability for 30 min at 100 °C. In addition, M1 and M2 were not detected by the heating, indicating that the production of M1 and M2 might not be due to a spontaneous degradation of FPP-3. All stock solutions were stable for at least 6 months when stored at 4 °C (data not shown).

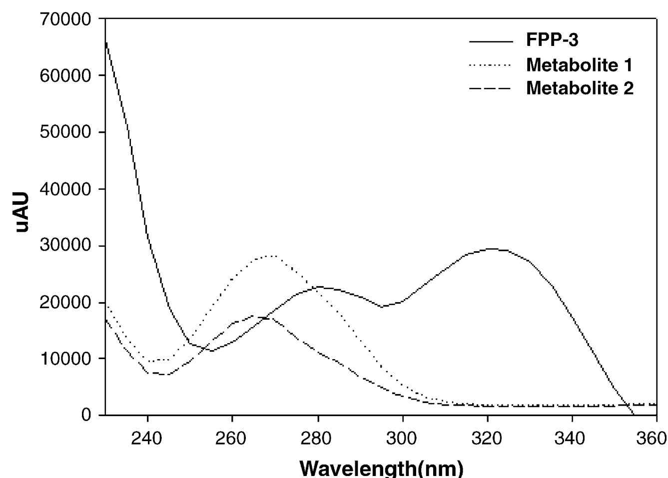

Fig. 3. UV spectra of FPP-3 and its metabolites.

3.2. Linearity and limit of quantification

Acceptable linearity was observed over the concentration range 0.5–20 μg/ml of FPP-3 (r = 0.9924), 0.1–4.0 μg/ml of M1 (r = 0.9985) and 1.0–20 μg/ml of M2 (r = 0.9982), respectively. The lower limit of quantitation (LLOQ) of FPP-3, M1 and M2 were 0.5, 0.1 and 1.0 μg/ml, respectively. These were the lowest concentration of the analytes that can be measured with a coefficient of variation and accuracy both less than 15%.

3.3. Intra- and inter-day precision and accuracy

The intra- and inter-day precision and accuracy are shown in Table 1. The inter-day accuracy of FPP-3, M1 and M2 were less than 107.8, 113.4 and 114.2%, respectively, and intra-day accuracy of FPP-3, M1 and M2 were less than 103.4, 105.6 and 114.8%, respectively.

Table 1
Intra- and inter-day R.S.D. and accuracy for the determination of FPP-3 in rat serum

Theoretical concentration (μg/ml)	Intra-day			Inter-day		
	Concentration measured (μg/ml)	% R.S.D.	Accuracy (%)	Concentration measured (μg/ml)	% R.S.D.	Accuracy (%)
FPP-3						
0.5	0.45 ± 0.03	13.4	90.0	0.44 ± 0.02	8.2	87.5
5	4.64 ± 0.16	7.6	92.8	4.29 ± 0.10	5.3	85.8
20	20.7 ± 0.85	9.2	103.4	21.6 ± 0.68	7.1	107.8
M1						
0.1	0.11 ± 0.01	8.9	105.6	0.10 ± 0.01	8.0	94.5
1	1.06 ± 0.06	13.5	105.4	1.15 ± 0.06	10.9	113.4
4	4.22 ± 0.10	5.5	104.5	3.64 ± 0.16	9.8	90.1
M2						
1	0.90 ± 0.05	11.8	87.9	0.97 ± 0.03	6.9	94.6
10	11.7 ± 0.20	3.8	114.8	10.9 ± 0.53	10.9	106.5
20	21.4 ± 1.21	12.6	104.9	23.3 ± 0.76	7.3	114.2

Each value represents mean ± S.E. of five determinations.

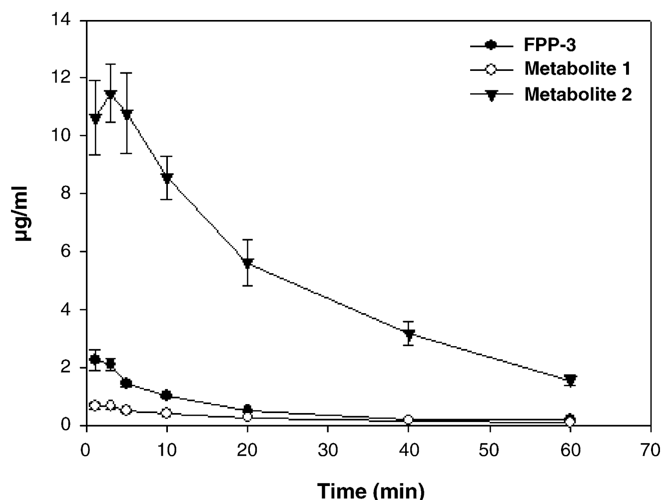

Fig. 4. Mean serum concentrations of FPP-3 and its metabolites in male SD rats following an intravenous penile vein injection with FPP-3 at 4 mg/kg. Blood was obtained from the subclavian vein immediately before and 1, 3, 5, 10, 20, 40 and 60 min after the dose. All the detailed procedures for the analyses were described in the Experimental. Each value represents the mean ± S.E. of five animals.

3.4. Pharmacokinetic investigation in SD male rats

The validated method was applied to determine FPP-3 concentration in rat serum after single i.v. treatment of 4 mg/kg. Blood samples were collected at 1, 3, 5, 10, 20, 40 and 60 min post dose. Fig. 4 shows the mean serum concentration–time curve of FPP-3, M1 and M2. The pharmacokinetic parameters for FPP-3, M1 and M2 are shown in Table 2.

The C_{max} of M2 was 4.8-fold higher than the parameter for FPP-3, and the AUC of M1 and M2 was 0.45- and 7.95-fold higher than FPP-3, respectively. Moreover, FPP-3 was rapidly converted to M1 and M2 within 2 min in biological fluids possibly by dehydrogenases or reductases present in liver and blood [5–8].

Table 2
Phamacokinetic parameters after i.v. treatment of male SD rats with FPP-3

Parameters	FPP-3	M1	M2
C_{max} (μg/ml)	2.4 ± 0.3	0.69 ± 0.05	11.6 ± 0.9
T_{max} (min)	1.8 ± 0.5	1.8 ± 0.5	3.4 ± 0.4
AUC (μg min/ml)	46.1 ± 8.6	20.7 ± 1.1	366.4 ± 32.5
Half-life (min)	16.3 ± 2.4	27.7 ± 1.6	22.1 ± 1.2

Each value represents mean ± S.E. of five animals.

In our previous report, M2 was only produced from M1, indicating that metabolism of FPP-3 to M1 and subsequently to M2 is sequentially occur [4]. With regard to the identity of other peaks in the chromatogram (Fig. 2), we do not have an answer on this at the present time. To identify the other peaks whether they are possible metabolites produced in vivo, studies are currently under way. Nevertheless, no other metabolites except two reduced metabolites confirmed in the present study were observed in the in vitro incubation of FPP-3 with rat liver cytosols in the presence of NADPH (data not shown).

Because FPP-3 was metabolized very rapidly in vivo, M1 and M2 should be simultaneously determined with FPP-3 for pharmacokinetic and bioavailability studies. In this regard, the method described in this report affords the sensitivity, accuracy and precision necessary for quantitative measurements in pharmacokinetic studies and therapeutic monitoring of FPP-3 and its metabolites.

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