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Short communication

Simultaneous determination of arylpropionic acidic non-steroidal anti-inflammatory drugs in pharmaceutical formulations and human plasma by HPLC with UV detection

Yen Sun, Kumiko Takaba, Hideaki Kido, Mihoko N. Nakashima, Kenichiro Nakashima *

Graduate School of Biomedical Sciences, Course of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

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Abstract

A simple and sensitive high-performance liquid chromatography-UV detection method was developed for the simultaneous determination of non-steroidal anti-inflammatory drugs (NSAIDs) having an arylpropionic acid moiety in pharmaceutical formulations and human plasma. Isocratic separation was employed on ODS column (250 × 4.6 mm i.d., 5 μm) at ambient temperature. The mobile phase consisted of acetonitrile, phosphate buffer (pH 3.5; 50 mM), methanol and tetrahydrofuran. The NSAIDs in the eluent were monitored under a wavelength-programme to provide their maximum absorbance. Mefenamic acid was used as an internal standard. Drugs were found to be 96.8–101.9% of their label claim in pharmaceutical formulations. One hundred microliters of human plasma samples were pretreated with a simple liquid-liquid extraction using ethyl acetate. The detection limits of compounds studied at a signal-to-noise ratio of 3 were 11.5–75 ng/ml in human plasma samples. The proposed method is simple, selective and could be applicable for routine analysis of arylpropionic acidic NSAIDs in pharmaceutical as well as in human plasma samples.

Keywords: Non-steroidal anti-inflammatory drugs; Pharmaceutical formulation; Human plasma; HPLC-UV detection

1. Introduction

Arylpropionic acid derivatives belong to a relatively new group of non-steroidal anti-inflammatory drugs (NSAIDs), which have analgesics, antipyretics and anti-inflammatory activities and are widely used in the treatment of acute and chronic pain, osteoarthritis, rheumatoid arthritis and related conditions. Diclofenac is also a relatively new NSAID that is being widely prescribed in Japan. The pharmacological actions of NSAIDs are related to inhibition of cyclooxygenase (COX), a key enzyme of prostaglandin biosynthesis at the site of inflammation. The simultaneous measurement of these NSAID con-

* Corresponding author. Tel.: +81-95-847-1111x2557; fax: +81-95-842-3549
E-mail address: naka-ken@net.nagasaki-u.ac.jp (K. Nakashima).

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centrations in biological samples is required in clinical and toxicological screening, pharmacokinetic studies, as well as in therapeutic monitoring. Furthermore, it is also very important to precisely quantify these NSAIDs in pharmaceutical formulations for quality control Fig. 1.

Until now, a variety of chromatographic methods have been proposed for the determination of commercially available NSAIDs in pharmaceutical formulations and biological fluids, mainly by gas chromatography (GC) [1–4], spectrofluorometry [5], HPLC with fluorescence detection [6], ultraviolet (UV) [7–16], electrochemical detection (ECD) [17], as well as capillary electrophoresis (CE) [18]. GC methods are time-consuming and complex and have been largely replaced by HPLC methods. Among HPLC methods, generally they only concentrated on a single or a few compounds. In other cases, gradient program for separation is needed. Hirai et al. reported a simultaneous analysis of 12 kinds of NSAIDs in human urine [11]. Owen et al. developed a method for determining seven kind of NSAIDs simultaneously in human plasma, yet, under the described condition, ketoprofen and naproxen were not sufficiently separated and also the assay was not sufficiently sensitive to measure the therapeutic concentration of ibuprofen at 254 nm [10]. Generally, although it is almost impossible that one patient would be prescribed for more than one kind of NSAIDs at the same time, simultaneous determination of these widely used NSAIDs still has these advantages: (1) several drugs could be analysed by using the same separation condition which might be very convenient for clinical and pharmaceutical routine analysis; (2) identification of the peak of NSAIDs could be carried out accurately and expeditiously by using a simultaneous analytical system; and (3) for drug-drug interaction study, it might require a simultaneous measurement system to detect more than one drug in biological samples.

As sample pretreatment, deproteination, liquid–liquid extraction and solid-phase extraction have been used. Solid-phase extraction was quite time-consuming; deproteination is simple, but since the sample is diluted, the sensitivity is relatively low.

In this study, considering that simple manipulation and widely used UV detector with no special assembly might be convenient and suitable for routine work, an isocratic separation of NSAIDs having an arylpropionic acid moiety and diclofenac by using reversed phase HPLC and UV detection was developed. The proposed method was successfully applied to the determination of

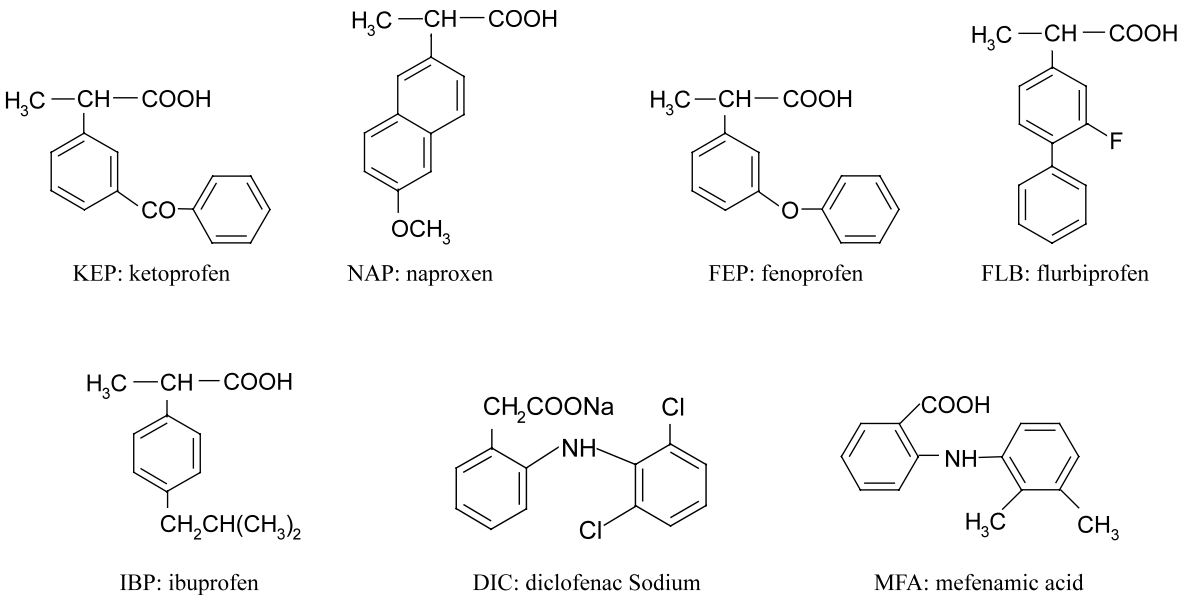

Fig. 1. Chemical structures and abbreviations of NSAIDs studied.

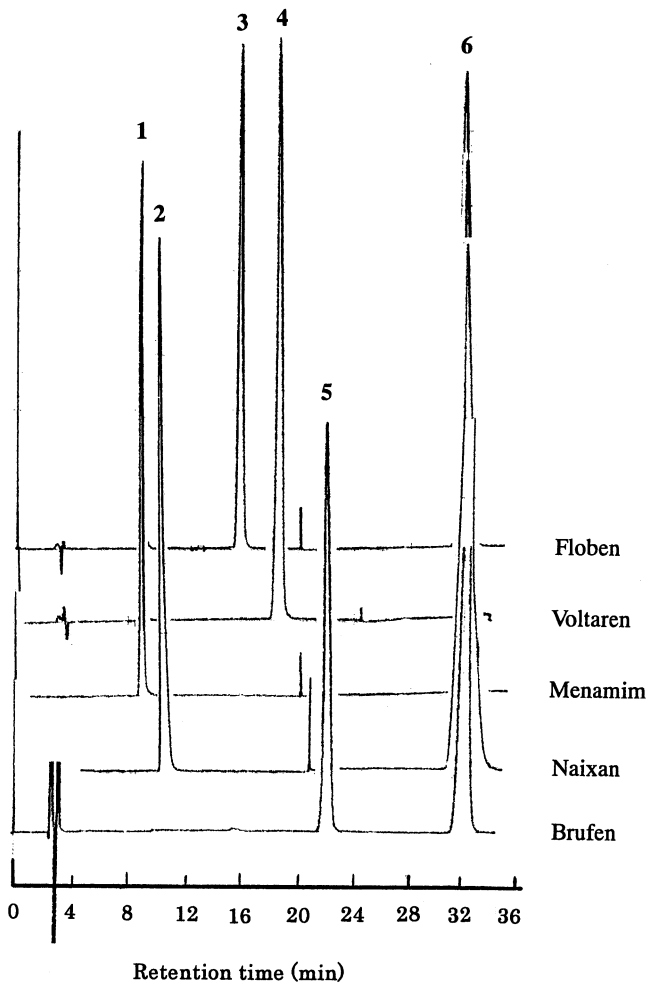

Fig. 2. Chromatograms of NSAIDs in pharmaceutical formulations. 1: KEP in Menamim; 2: NAP in Naixan; 3: FLB in Floben; 4: DCL in Voltaren; 5: IBP in Brufen; and 6: MFA (I.S.).

each compound in human plasma sample, as well as the commercially available pharmaceutical formulations in Japan.

2. Experimental

2.1. Materials and reagents

Ketoprofen (KEP), flurbiprofen (FLB), ibuprofen (IBP), diclofenac sodium (DIC), mefenamic acid (MFA) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Naproxen (NAP) was obtained from ICN Biomedical Co. (OH). Fenoprofen (FEP) was purchased from Sigma Chemical Co. (Steinheim, Germany). Sodium hydrogenphosphate, sodium acetate, acetic acid, phosphoric acid, methanol, acetonitrile and ethyl acetate were of analytical reagent grade (Wako). Water was passed through a pure line WL21P (Yamato Sciences, Tokyo, Japan).

2.2. Apparatus

The HPLC system consisted of a Jasco 880-pu pump (Tokyo, Japan), a Jasco 875-UV multi-

wavelength UV/VIS detector, a Rheodyne 7125 sample injector with a 20-μl loop (Cotati, CA) and a Rikadenki R-01 recorder (Tokyo, Japan). Chromatographic separations were carried out on a Daisopak ODS column (SP-120-5-ODS-BP, 250 × 4.6 mm i.d., Daiso, Osaka, Japan).

2.3. Preparation of standard solutions

Standard stock solutions of KEP, NAP, FLB, IBU, DIC (10 mg/ml), FEP (1.0 mg/ml) and MFA (2.0 μg/ml) as an internal standard (I.S.) were prepared by dissolving appropriate amounts of them in methanol. These stock solutions were subsequently used in the preparation of working standards by further dilution with methanol. All stock solutions were kept in refrigerator at 4 °C, which were stable for at least 3 months. NSAIDs spiked plasma samples were prepared as follows: after evaporating a proper volume of each working standards, 100 μl of drug-free plasma were added to the residue to obtain the final concentration range of 0.1–100 μg/ml.

2.4. Chromatographic conditions

The NSAIDs were isocratically separated with acetonitrile-acetate buffer (pH 3.5; 0.1 M)-methanol (35:40:25, v/v/v) as an eluent at a flow rate of 1.0 ml/min. The effluent was monitored at 255 nm for 0–11.6 min, 240 nm for 11.6–16.8 min and 220 nm for 16.8–35 min. The chart speed was set at 2.5 mm/min.

2.5. Determination of NSAIDs in pharmaceutical formulations

The contents of ten capsules of KEP and ten tablets of NAP, FLB, DIC and IBP were finely ground. An accurately weighed powdered sample containing the labeled amount of each drug was transferred to a 100-ml volumetric flask. The volume was adjusted with methanol and the resultant solution was sonicated for 5 min. A portion of the solution was then filtered through a 0.45 μm filter (Kanto Chemical Co., Tokyo, Japan). To each 100 μl of filtrate, 100 μl of the I.S. solution (0.5 mg/ml of MFA) were added and the mobile phase was added to make the volume up to 1.0 ml. An aliquot (20 μl) of each solution was injected onto the column.

2.6. Determination of NSAIDs in human plasma

Human blood samples were collected from healthy volunteers in our laboratory. The blood was drawn into the test tube including EDTA. After centrifugation at 1000 *g* for 10 min, the plasma was taken and kept in a freezer (−20 °C) until analysis.

For drug analysis, 100 μl of human plasma were transferred to a test tube and 200 μl of phosphate buffer (pH 2.5; 50 mM) was added and mixed well. To the mixture, 700 μl of ethyl acetate was added and vortex-mixed for 1 min. After centrifugation at 1000 × *g* for 10 min, the organic layer (600 μl) was transferred to a test tube. The extraction procedure was repeated once and the collected ethyl acetate layer was evaporated to dryness. Thereafter, 200 μl of mobile phase were added to the residue, centrifuged at 1000 *g* for 10 min and 20 μl of the supernatant was injected onto the HPLC system.

3. Results and discussion

3.1. Chromatographic separations

In this study, an isocratic separation of NSAIDs in standard solution was carried out by using a mixture of acetonitrile-acetate buffer (pH 3.5; 0.1 M)-methanol (35:40:25, v/v/v). The capacity factor (k') value of each compound remarkably decreased in the pH value of mobile phase above 4.5, whereas there were little changes up to pH 4.0; the pH 3.5 was chosen as the optimal condition. The proposed separation condition was applied to the analysis of NSAIDs in pharmaceutical formulations (Fig. 2). However, FEP and DIC were interfered with the endogenous peak from plasma by using acetate buffer in mobile phase. After changing to a phosphate buffer (pH 3.5; 50 mM) and the slight modification of acetonitrile and methanol composition in mobile phase, a complete separation of all compounds in plasma

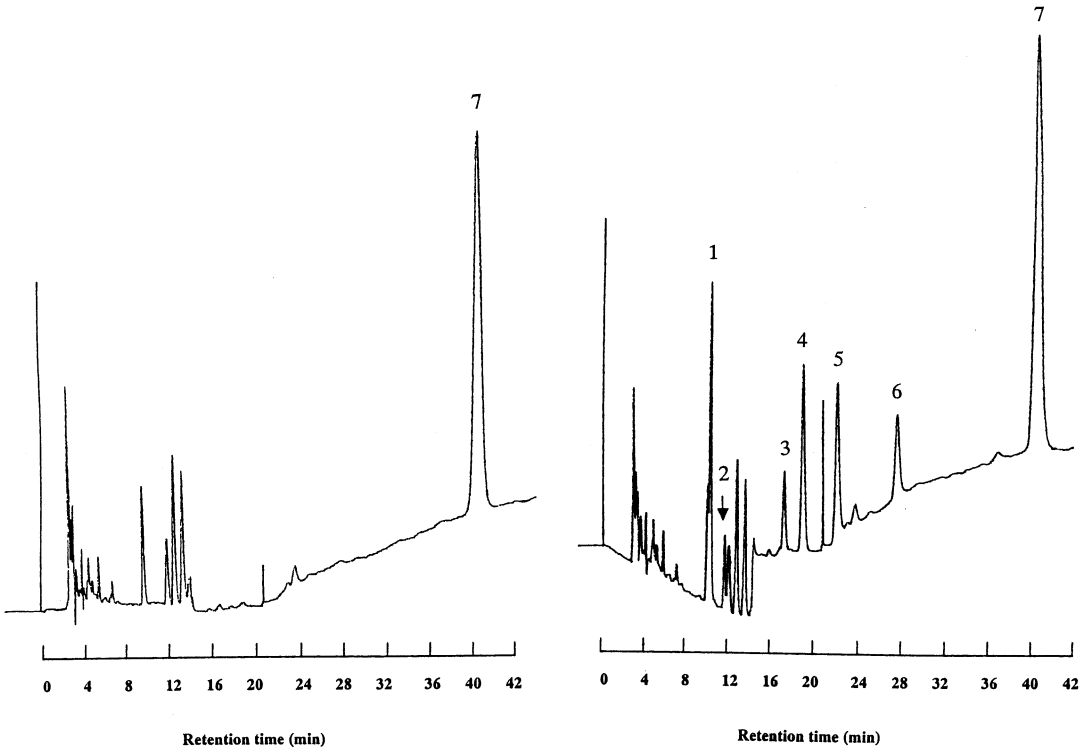

Fig. 3. Chromatograms of NSAIDs in human plasma. (A) Drug-free human plasma; (B) human plasma spiked with each drug at 1.0 μg/ml. 1: KEP; 2: NAP; 3: FEP; 4: FLB; 5: DCL; 6: IBP; and 7: MFA (I.S.).

sample could be achieved at acetonitrile-phosphate buffer (pH 3.5; 50 mM)-methanol-tetrahydrofuran (25:40:35:1.5, v/v/v) (Fig. 3).

Table 1
Maximum UV absorption of NSAIDs

Compound	λmax (nm)[a]
KEP	256
NAP	232
FEP	210
FLB	246
DIC	274
IBP	226
MFA	222

[a] UV absorption of each compound was measured in mobile phase [acetonitrile–acetate buffer (pH 3.5; 0.1 M)–methanol = 35:40:25, v/v/v].

3.2. Selection of detection wavelength

As shown in Table 1, each compound exhibited different maximum UV absorbance. In order to detect NSAIDs sensitively, a wavelength-programme was performed in this study. For human plasma analysis, the program was set as follows: 255 nm (0–13.6 min), 240 nm (13.6–20.6 min) and 220 nm (20.6–42.0 min). For pharmaceutical formulation analysis, each drug was monitored at its maximum wavelength. Under these conditions, standard calibration curves showed good linearity ($r > 0.999$), ranging from 0.05 to 100 μg/ml for KEP, NAP, FEP; 0.1–100 μg/ml for FLB, IBP, DIC.

3.3. Determination of NSAIDs in pharmaceutical formulations

The accuracy of the proposed method was evaluated by recovery assays. In this study, the

recovery assessment was made on the formulation samples instead of preparing placebos. Thus, known amounts of each compound were spiked into their corresponding formulation at two levels and each spiked matrices were prepared in four replicates. The recovery was calculated as follows:

$$\text{Recovery (\%)} = [(\text{measured conc.} - \text{original conc.})/\text{spiked conc.}] \times 100 \quad (1)$$

The precision of the proposed method was performed by spiking each compound to the selected formulation on different days. The precision (% R.S.D.) for all the studied components in different dosage forms were <6.7 and 4.9% for intra-day and inter-day assay, respectively (Table 2).

The method was applied to seven different pharmaceutical formulations (capsules and tablets) for determining the content of NSAIDs. As shown in Table 3, the value ranged of 96.8–101.9% as the percentage of the label claim.

3.4. Determination of NSAIDs in human plasma

3.4.1. Linearity

The calibration curves were prepared over the concentration range of 0.1–100 μg/ml in human plasma for each compound by assaying in duplicates at eight different concentrations. A linear relationship was obtained between the peak height ratio and concentration for each drug in spiked human plasma samples. As shown in Table 4, the calibration curves were linear over the spiked range for each compound with the good correlation coefficient ($r > 0.999$). Limit of detections (LODs) of each compound were 11.5–75 ng/ml at a signal-to-noise ratio of 3. Compared with those of other publications [10,11], the proposed method was three to 30 times more sensitive.

3.4.2. Extraction recovery

Recovery of the extraction procedure was determined for each compound at 1.0 μg/ml. The recovery of I.S. was determined at 2.0 μg/ml as its working concentration. Recoveries were calculated by comparing the peak heights of each compound spiked in plasma, to those of standards in the

Table 2
Recoveries and precision of the proposed method in pharmaceutical formulations

Compound	Spiked level (mg/ml)	Conc. Found (mg/ml)	Recovery (Accuracy, %) ($n=4$)	Precision	
				Intra-day ($n=4$) (R.S.D.%)	Inter-day ($n=4$) (R.S.D.%)
KEP	0	0.16±0.023	–	1.1	0.5
	0.07	0.23±0.028	100.2	4.9	4.9
	0.14	0.30±0.023	99.9	1.2	2.8
NAP	0	0.08±0.002	–	2.4	3.6
	0.04	0.12±0.003	99.9	6.7	3.6
	0.16	0.24±0.001	100.1	0.3	2.8
FLB	0	0.048±0.002	–	1.4	1.5
	0.025	0.071±0.001	95.2	4.8	3.8
	0.075	0.123±0.001	100.5	0.2	2.8
IBP	0	0.09±0.005	–	0.6	3.6
	0.045	0.13±0.005	98.1	1.4	4.8
	0.09	0.18±0.004	100.5	0.4	3.7
DIC	0	0.018±0.0001	–	1.3	2.0
	0.01	0.028±0.0005	89.4	1.8	2.9
	0.04	0.057±0.0002	101.2	0.5	1.2

Table 3
Contents of NSAIDs in pharmaceutical formulations and OTC drugs

Drug	Dosage form	Claimed (mg)	Found[a] (mg)	Content[b] (%)
Pharmaceutical formulations				
Menamim (KEP)	Capsule	150	151.5±4.55	101.0
Naixan (NAP)	Tablet	100	101.2±1.09	101.2
Froben (FLB)	Tablet	40	38.7±1.17	96.8
Voltaren (DIC)	Tablet	25	24.9±1.16	99.6
Brufen (IBP)	Tablet	100	101.9±2.75	101.9
OTC drugs				
EVE A (IBP)	Tablet	75	73.4±0.24	97.8
NARON ACE (IBP)	Tablet	72	69.7±1.90	96.8

[a] Mean ± S.D. of four replications.
[b] Content (%) = Found/Claimed × 100.

mobile phase. Several different solvents (dichloromethane, hexane, chloroform, ethyl acetate) were studied for liquid-liquid extraction and ethyl acetate was chosen as adequate. Recoveries of each compound were also tested at different pH by acidified with 50 mM phosphate buffer (pH 1.5–5.0). The maximum recovery of each drug was obtained at pH 2.5. As shown in Table 5, recoveries for NSAIDs were 97.1–114.6% at studied concentrations, which were relatively higher than those of 73.1–95.1% in other published method [11].

3.4.3. Assay accuracy and precision

Assessment of the intra-day accuracy and precision of the method were performed in several different lots of drug-free plasma samples spiked with each drug at low (0.1 μg/ml), middle (1.0 μg/ml) and high (10 μg/ml) concentrations. Six replicate samples at each concentration were analyzed for the intra-assay assessment. For the inter-day precision, analysis of the spiked plasma at the same concentrations of each drug was performed on 5 different days. Precision and accuracy are shown as R.S.D.% and percent accuracy [(mean observed conc./spiked conc.) × 100], respectively. The results are given in Table 5. The intra- and inter-day precision of the assay were observed to be < 8.4 and 10.1%, respectively. The assay accuracy was found to be 90.0–111.5% for all compounds.

3.4.4. Stability

The freeze-thaw stability of NSAIDs in plasma was assessed over two freeze-thaw cycles. Each compound was spiked in plasma at concentration of 1.0 μg/ml, which was frozen at −20 °C and thawed at room temperature for two consecutive

Table 4
Calibration curves and limit of detections (LODs) of NSAIDs in human plasma

Compound	Concentration range (μg/ml)	Equation	r	LOD (S/N = 3) (ng/ml)
KEP	0.1–100	$Y = 1.86X - 0.25$	0.999	11.5
NAP	0.1–100	$Y = 0.46X - 0.14$	0.999	75.0
FEP	0.1–100	$Y = 0.49X - 0.046$	0.999	75.0
FLB	0.1–100	$Y = 1.08X - 0.13$	0.999	13.6
DIC	0.1–100	$Y = 0.95X - 0.22$	0.999	30.0
IBP	0.1–100	$Y = 0.51X - 0.0034$	0.999	42.8

Table 5
Intra-, inter-day accuracy, precision and recoveries of NSAIDs in human plasma

Compound	Nominal (μg/ml)	Found conc. (μg/ml)	Accuracy (%)	R.S.D. (%)		Recovery (%)
				Intra-day ($n=6$)	Inter-day ($n=5$)	
KEP	0.1	0.11±0.01	111.5	5.0	8.6	97.1
	1.0	1.05±0.06	105.4	5.8	4.3	
	10.0	10.4±0.45	104.4	4.3	3.6	
NAP	0.1	0.90±0.01	90.0	5.6	8.8	98.6
	1.0	1.02±0.05	102.3	5.0	7.9	
	10.0	10.7±0.50	107.1	4.7	3.2	
FEP	0.1	0.09±0.01	90.1	5.6	8.8	110.0
	1.0	0.90±0.02	90.0	2.7	2.6	
	10.0	10.7±0.59	106.6	5.5	1.6	
FLB	0.1	0.10±0.01	103.0	6.7	6.7	97.1
	1.0	1.00±0.04	99.8	4.3	4.8	
	10.0	10.8±0.50	108.8	4.6	3.2	
DIC	0.1	0.11±0.01	109.4	8.4	4.7	108.8
	1.0	0.99±0.02	99.4	2.5	2.2	
	10.0	10.3±0.42	103.1	4.1	0.7	
IBP	0.1	0.11±0.003	110.7	5.4	10.1	114.6
	1.0	1.04±0.03	104.0	3.2	1.9	
	10.0	10.3±0.46	102.5	4.4	2.3	
MFA (I.S.)	2.0	–	–	–	–	100.0

Table 6
Stability of NSAIDs in human plasma

Compound	Conc. spiked (μg/ml)	Conc. measured (μg/ml)				
		Initial	Free-thaw stability		Stored at room temperature	
			Cycle 1	Cycle 2	24 h	48 h
KEP	1.0	0.97	0.96	0.99	0.96	1.03
NAP	1.0	1.01	0.96	0.99	0.99	1.07
FEP	1.0	0.98	0.95	1.04	0.96	1.01
FLB	1.0	0.97	0.96	1.01	1.00	1.05
DIC	1.0	1.02	1.00	0.99	0.97	1.03
IBP	1.0	1.00	0.98	1.02	0.99	1.08

times. As shown in Table 6, freeze-thaw stabilities of NSAIDs were evaluated to be 0.99–1.04 μg/ml, room temperature stabilities until 48 h were within 0.96–1.08 μg/ml, with spiked concentration of 1.0 μg/ml. Overall results indicate that NSAIDs studied are stable in plasma samples following two freeze-thaw cycles and room temperature storage.

4. Conclusions

A simple and sensitive HPLC-UV detection method for the simultaneous determination of NSAIDs having arylpropionic acid moiety, as well as diclofenac was developed. The proposed method was successfully applied to the determina-

tion of these NSAIDs in pharmaceutical formulations, as well as in the human plasma samples. The LODs of each compound in human plasma were as low as 11.5–75 ng/ml at signal-to-noise ratio of 3, which was enough for monitoring the plasma concentrations of each compound. In addition, the proposed method might be applicable for screening and determination of other NSAIDs having carboxylated acid group (e.g. loxoprofen, penicillamine, indomethacine, etc.) by slight modification of the analytical conditions.

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