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Fluorous derivatization and fluorous-phase separation for fluorometric determination of naproxen and felbinac in human plasma

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ABSTRACT

Fluorous derivatization followed by fluorous-phase liquid chromatographic (LC) separation exploits the affinity between perfluoroalkyl compounds for highly selective and quantitative isolation of various analytes. However, the applicability of this technique as a simple pretreatment for fluorometric determination in clinical settings has not been fully explored. Here we show the applicability of this technique to the clinical determination of non-steroidal anti-inflammatory drugs (NSAIDs) in human plasma. Naproxen and felbinac, widely used native-fluorescent NSAIDs with a carboxyl group, can have toxic effects at acute doses, and were therefore chosen as representative NSAIDs. Samples were precolumn derivatized with a non-fluorescent fluorous amine, which allowed highly selective retention of only derivatized substances in the fluorous LC column. Thus, subsequently, only the retained fluorous-labeled and fluorescent analytes were detected fluorometrically at appropriate retention times. The detection limits for these two drugs were less than 11 fmol on column. Correlation curves were liner over the range of 0.04–10 and 5–250 nmol/mL plasma for both two drugs ($r > 0.999$) with good repeatability. Thus, this method offers a simple, sensitive, and selective solution for determination of NSAIDs in clinical settings.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of pain, fever, and inflammation [1]. The most widely used NSAIDs are naproxen [(2*S*)-2-(6-methoxynaphthalen-2-yl)propanoic acid, Fig. 1A] and felbinac (biphenyl-4-ylacetic acid, Fig. 1B). Naproxen is mainly administered orally and is approved for over-the-counter (OTC) use in the United States [2], whereas felbinac is mainly administered transdermally [3,4]. However, it is important to note that chronic or acute administration can lead to toxic effects such as gastrointestinal erosion, bleeding, and pathologic changes in the renal cortex, tubules, and papillae [1]. Thus, it is necessary to monitor the concentration of naproxen and felbinac in human plasma.

There are several determination methods for naproxen and felbinac in pharmaceutical preparations and biological samples: methods based on liquid chromatography (LC) with UV detection [5–8], electrochemical detection [9,10], mass spectrometry (MS) method [11,12], and fluorescent detection [13–15]. In general, UV detection is not sensitive and selective, while electrochemical detection tends to lack reproducibility due to hysteretic degradation of the electrode. Although the LC–MS method is sensitive and highly reliable, its apparatus and operating cost are too expensive for routine analyses in many laboratories. Because naproxen and felbinac have native fluorescent, they can be determined with the fluorescent detection [13–15]. However, there are many fluorescent compounds which cause interfering peaks for the chromatogram in biological samples. Therefore, highly sophisticated LC separation conditions [13,15] and/or sample clean-up procedures [14,15] are inevitable to eliminate the interferences from other co-existing compounds.

Recently, we developed a simple LC determination method of native fluorescent compounds based on "fluorous derivatization" [16]. "Fluorous" is that between highly fluorinated compounds and highly fluorinated compounds show the special property to fluorophilic, a branch of organic chemistry is dedicated to research on fluorous chemistry [17–19]. Since 1980s, fluorous reagents and LC columns with fluorous stationary phase have been commercially supplied by some manufacturers [20]. The principle of fluorous derivatization method is described as follows: native fluorescent compounds or analytes with target functional groups are precolumn derivatized with a nonfluorescent fluorous-label. The fluorous derivatized analytes are retained in the fluorous-phase column, whereas underivatized native fluo-

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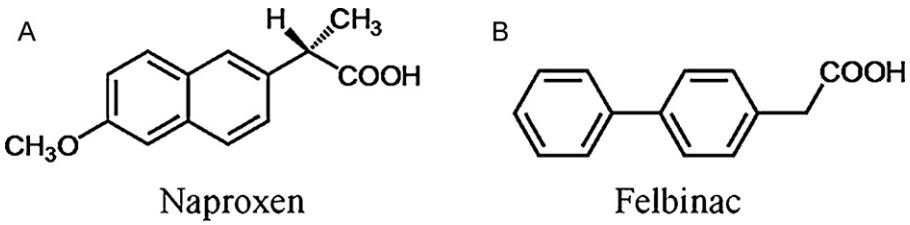

Fig. 1. Chemical structures of (A) naproxen and (B) felbinac.

rescent substances are not. Only the retained fluorescent analytes are detected fluorometrically at appropriate retention times, and retained non-fluorescent substrates without fluorophores are not detected. Fluorous derivatization method was carried out for the specific determination of not only native fluorescent carboxylic acids but also sialic acids with MS detection [21]. In these methods, 4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoro-*n*-undecylamine (HFUA) was used as a fluorous derivatization reagent for carboxylic acids with a fluorous structure, and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl) methylmorpholin-4-ium chloride (DMT-MM) was used as an effective reagent for condensation reaction between amino group in HFUA and carboxylic acid structure in the analytes (Fig. 2).

In the present paper, we described a fluorous derivatization method for naproxen and felbinac to exhibit the utility for biomedical field. This method allows a highly sensitive and selective determination of them in human plasma with simple pretreatment. We applied this method for the measurement of drug concentrations in plasma sample from oral administration of naproxen and transdermal administration of felbinac.

2. Experimental

2.1. Reagents and solutions

Unless stated otherwise, all chemicals mentioned below were of the highest purity available and were used as received. Naproxen and DMT-MM were purchased from Wako Pure Chemicals (Osaka, Japan). Felbinac and HFUA were obtained from Alfa Aesar (Ward Hill, MA, USA) and Fluka (Buchs, Switzerland), respectively. All organic solvents were of LC grade. It should be noted that these reagents and solvents are toxic to the eyes, lungs, and skin, and should be used carefully according to guidelines specified in the latest material safety data sheets. Ultrapure water, purified using a Milli-Q gradient system (Millipore, Billerica, MA, USA), was used to produce all aqueous solutions.

Stock solutions (1.0 mM) of analytes were prepared in acetonitrile and stored at room temperature. They were stable for at least one week and diluted further with tetrahydrofuran (THF) to the required concentrations before use. A solution of 20 mM HFUA in THF was usable for at least one week when stored at room temperature. A solution of 10 mM DMT-MM in 90% (v/v) THF was used within one day.

2.2. Pharmaceuticals

NAIXAN® tablet includes 100 mg naproxen was obtained from Mitsubishi Tanabe Pharma (Osaka, Japan). PASSTIME® FX plaster includes 10 mg felbinac per plaster was kindly donated from Yutoku Pharmaceutical Industries Co., Ltd. (Saga, Japan).

2.3. Derivatization procedure

To the sample solution (40 μL) placed in a 1.5-mL vial, 40 μL of 20 mM HFUA and 20 μL of 10 mM DMT-MM were successively added. The vial was tightly sealed and left at room temperature for 5 min. After derivatization, 100 μL of water was added to the vial, and the entire reaction solution was placed in the autosampler of an LC system. A reagent blank was prepared by the same procedure using a 40-μL aliquot of 90% (v/v) THF instead of the sample solution.

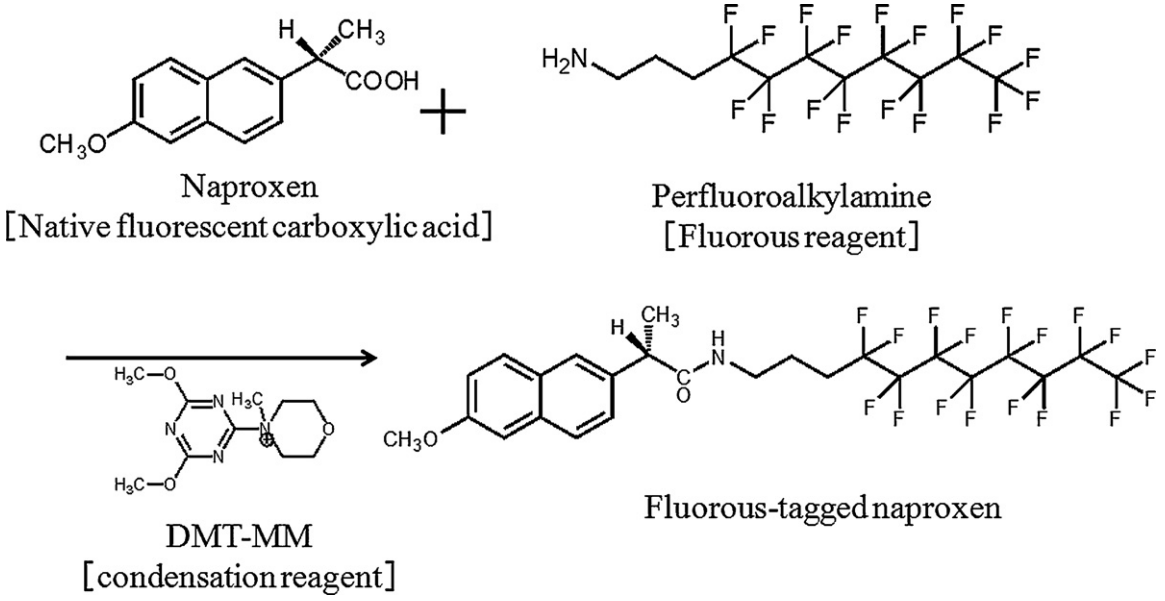

Fig. 2. Fluorous derivatization of naproxen in the presence of DMT-MM.

2.4. Plasma sample and pretreatment

Plasma samples were obtained from the whole blood of healthy volunteers in the laboratory. These volunteer understood the purpose and significance of this experiment and donated blood after signing an agreement. In pharmacokinetic study, blood was collected from one volunteer (male, 23 years old, 57 kg) before and after 0–55 h of oral administration of naproxen or before and after 0–55 h of transdermal administration of felbinac in the lumbar region for 36 h, using a centrifugal blood collection tube (Eiken Chemical, Tokyo, Japan). In order to prevent interference between both the experiments, they were executed at an interval of 10 days. The blood was immediately centrifuged at 2000 × *g* for 5 min at room temperature, and the obtained supernatant was transferred to a screw-capped polypropylene tube and used as plasma sample.

To 10 μL of plasma placed in a 1.5-mL polypropylene tube, 90 μL of THF was added. After vortex mixing for a few seconds, the mixture was immediately centrifuged at 9000 × *g* for 5 min at 4 °C, and the supernatant was passed through a disposable filter (0.20-μm pore size, polytetrafluoroethylene; Advantec Toyo, Tokyo, Japan). Then, a portion (40 μL) of the filtrate was subjected to derivatization.

2.5. LC system and conditions

We used an isocratic LC system consisting of an LC-10AD liquid chromatography pump, a SIL-10A autoinjector, a DGU-12A online degasser, a CTO-10A column oven, a separation column, an RF-10AXL spectrofluorometer equipped with a 12-μL flow cell, and a CBM-20A controller. With the exception of the separation column, all components of the LC system were manufactured by Shimadzu (Kyoto, Japan). Injection of each 20-μL sample into the system was carried out automatically. The flow rate of the mobile phase was set at 1.0 mL/min, and the column temperature was 40 °C. Both the monochromators in the fluorescence detector had a slit width of 15 nm.

A Wakopak Fluofix-II 120E column (150 × 4.6 mm i.d., 5 μm; Wako Pure Chemicals) was used as an analytical column, and a mixture of methanol, water, and acetonitrile (70:20:10, v/v) was used as a mobile phase. The fluorescence detector was operated at excitation/emission wavelengths (nm) of 270/350 (0–22.8 min, detection for naproxen) and 260/316 (>22.8 min, detection for felbinac).

2.6. Method validation

In order to obtain the validation parameters, peak heights were estimated by a Shimadzu software (LC solution, ver. 1.23) and the baseline-to-baseline method was used for the quantification. For the quantitative analysis, calibration standard solutions ($n = 3$ each) with concentration ranges from 4 nM to 1 μM (4 nM, 10 nM, 0.1 μM, 0.5 μM, and 1 μM) for low concentration and from 0.5 μM to 25 μM (0.5 μM, 1 μM, 5 μM, 10 μM, and 25 μM) for high concentration were prepared by diluting the stock solutions. For the plasma sample, calibration spiked solutions ($n = 3$ each) with a concentration range from 0.04 to 10 nmol/mL plasma (0.04, 0.1, 1, 5, and 10 nmol/mL plasma) for low concentration and from 5 to 250 nmol/mL plasma (5, 10, 50, 100, and 250 nmol/mL plasma) for high concentration were prepared by spiking the stock solutions diluted with human plasma. The equations of the calibration curves were determined using least squares linear prediction. Precisions (intraday and interday) pertaining to the present method were estimated during the analytical procedures (sample dilution, pretreatment, derivatization, and LC separation) using the standard solutions (10 nM) and spiked plasma (0.1 nmol/mL plasma). The intraday and interday precisions were assessed by performing an analysis five times on the same day and by conducting the analysis on five different days of a month, respectively. The detection and quantitation limits were determined from signal-to-noise ratio of 3 and 10, respectively. To demonstrate applicability of the real sample, the recovery using plasma samples spiked with standards was evaluated. For this purpose, spiked plasma samples ($n = 3$ each) were prepared standard solutions at low (0.1 nmol/mL plasma), medium (10 nmol/mL plasma), and high (250 nmol/mL plasma) concentration levels were added to plasma sample, and the recovery was calculated on the basis of the results obtained by the proposed method.

3. Results and discussion

3.1. Fluorous LC separation

In the preliminary examination, we tried two fluorous columns whose ligands on stationary phase have different structure in order to obtain the optimum separation. We examined the Fluofix-II 120E column (150 × 4.6 mm i.d., 5 μm) and the Fluophase RP column (150 × 3.0 mm i.d., 5 μm, Thermo Fisher Scientific, San Jose, CA, USA). Then the Fluofix-II 120E column could effectively separate the HFUA derivatives of naproxen and felbinac within 30 min but the Fluophase RP column could not. For the separation, a mixture of methanol, water, and acetonitrile (70:20:10, v/v) was used as the mobile phase. Fig. 3A shows a typical chromatogram obtained with fluorous-labeled naproxen and felbinac. Fig. 3B and C show chromatograms obtained with unlabeled naproxen and felbinac and *n*-undecylamine (the alkylamine that has same carbon number with HFUA)-labeled them. These chromatograms indicate that only fluorous-labeled NSAIDs were retained in the fluorous column but non-fluorous compounds were not. Thus, the fluorous column can selectively retain fluorous compounds including fluorous-labeled analytes on the basis of fluorous separation and not hydrophobicity.

3.2. Derivatization conditions

The optimum conditions for fluorous derivatization reaction were optimized according to the previous method [16]. The concentrations of HFUA and DMT-MM were selected as 20 mM and 10 mM, respectively. The optimal reaction conditions were selected at room temperature for 5 min for the convenient procedure. We guessed that both felbinac and naproxen were derivatized quantitatively under the selected conditions because there are little peaks at the retention time of unlabeled drugs (Fig. 3A) and because it is known that amidation reaction using DMT-MM is faster and milder than that using other condensation reagents [22]. The fluorescence intensities of derivatized naproxen and felbinac were constant even after the mixture was left to stand for at least a week in the dark at room temperature.

3.3. Analysis of standards

The relationship between the amounts of native fluorescent carboxylic acids examined and the peak heights was linear over the concentration range of 4 nM–1 μM (low concentration; 16 fmol–4 pmol on column) and 0.5 μM–25 μM (high concentration; 2–100 pmol on column) in the standard solution. Both the values of the linear correlation coefficients of naproxen and felbinac with both concentration range were 0.999 ($n = 3$). The intraday and interday precisions were estimated during the entire process by repeated determinations ($n = 5$ in each case) using mixtures of standard compounds (10 nM each in a sample solution, 40 fmol each on column); the relative standard deviations of naproxen were 0.3% and 2.1%, respectively, and that of felbinac were 0.8% and 2.3%, respectively. The detection limits (signal-to-noise ratio = 3)

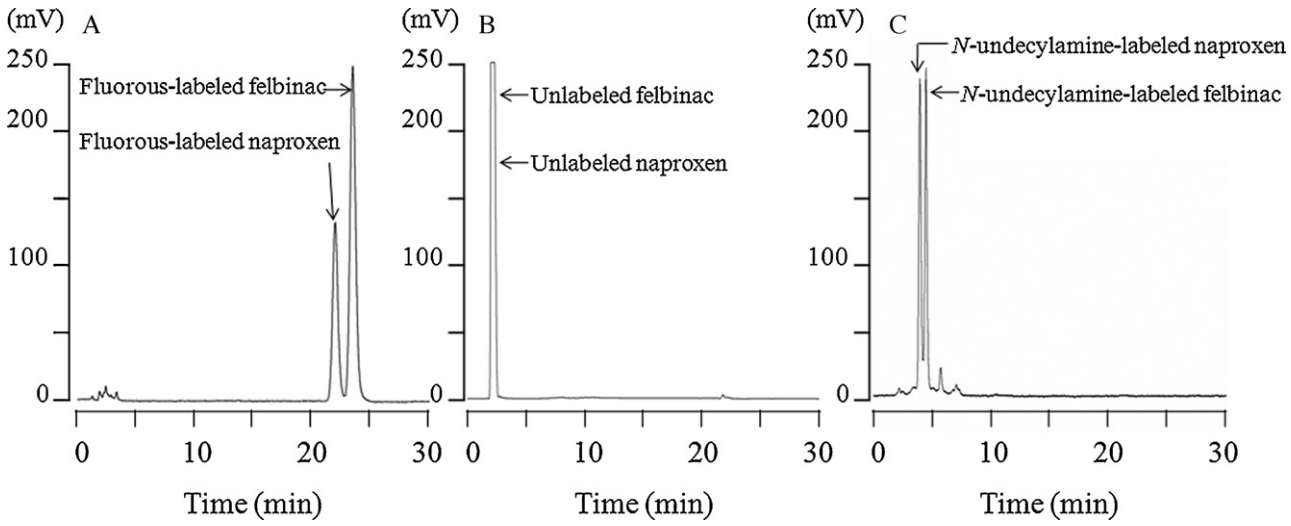

Fig. 3. Chromatograms obtained with (A) fluorous-labeled naproxen and felbinac, (B) unlabeled naproxen and felbinac, and (C) *n*-undecylamine-labeled naproxen and felbinac. Amounts: 4 pmol each on the fluorous column.

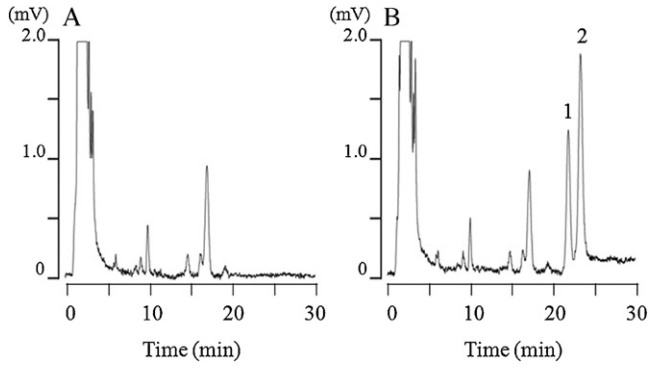

Fig. 4. Chromatograms obtained with plasma samples by fluorous derivatization. Samples: (A) drug-free plasma; (B) plasma spiked with naproxen and felbinac (0.1 nmol/mL plasma). Peaks: 1, fluorous-labeled naproxen; 2, fluorous-labeled felbinac; others, endogenous compounds.

that were dependent on the fluorescence intensities of naproxen and felbinac were 11 and 6 fmol, respectively, per 20-μL injection volume.

3.4. Determination of naproxen and felbinac in human plasma

To investigate the applicability of the fluorous derivatization method to biomedical analysis, it was used for the determination of native fluorescent NSAIDs (naproxen and felbinac) in human plasma. Plasma was diluted 10-fold with THF to deproteinize and optimize the reaction solvent. In this deproteinization method total (free and bond) drugs were determined [16]. Fig. 4 shows typical chromatograms obtained with derivatized plasma samples. No peaks of the plasma components were observed at the retention times around and after the objective peaks, because the native fluorescent compounds without carboxyl groups in the plasma were not derivatized and not retained in the column. Furthermore, non-fluorescent carboxylic acids were not detected, even though they were retained. The components of peaks 1 and 2 in Fig. 4 were identified as HFUA-labeled naproxen and felbinac, respectively, by comparing their retention times with those in Fig. 3 and by co-chromatography using various mobile phases. When HFUA solution was replaced with only a solvent, it was found that all compounds were eluted in the solvent front. Thus, there are few compounds retained in the fluorous column in human plasma and, the biogenic fluorescent compounds did not affect the determination of naproxen and felbinac. The peak components observed at retention times of 15–20 min might be attributed to the HFUA derivatives of endogenous native fluorescent carboxylic acids.

Validation data of spiked-plasma samples are presented in Table 1. The calibration graphs of naproxen and felbinac in plasma were linear ($r = 0.999$) in the concentration range corresponding to 0.04–10 nmol/mL plasma and 5–250 nmol/mL plasma. No significant changes in the slopes of the graphs were observed with the plasma used. Recoveries of naproxen and felbinac using plasma samples spiked with naproxen and felbinac were evaluated. For this purpose, spiked plasma samples were prepared at low (0.1 nmol/mL plasma), medium (10 nmol/mL plasma), and high (250 nmol/mL plasma) concentration levels. The recoveries were calculated from the ratio of the peak heights of spiked plasma samples and those of standards ($n = 3$ each); 95.1–99.6% NSAIDs were recovered from the plasma (Table 1). The intraday and interday precisions were established by repeated determination ($n = 5$) of naproxen and felbinac in plasma (0.1 nmol/mL plasma); the relative standard deviations were within 1.4% (intraday) and 4.6% (interday), respectively. The quantitation limits (signal-to-noise ratio = 10) that were dependent on the fluorescence intensities of

Table 1
Validation data of plasma samples.

	r[a]		Recovery[b] (%, mean ± SD, $n = 3$)			Repeatability[c] (RSD, $n = 5$)	
	Low	High	Low	Medium	High	Intraday (%)	Interday (%)
Naproxen	0.999	0.999	95.1 ± 1.6	99.6 ± 1.5	98.8 ± 1.3	0.9	3.8
Felbinac	0.999	0.999	97.9 ± 1.2	98.0 ± 1.5	97.4 ± 1.4	1.4	4.6

[a] Correlation curve in the concentration range of 0.04–10 nmol/mL plasma (low) and 5–250 nmol/mL plasma (high).
[b] The ratio of the peak height of spiked plasma (low, 0.1 nmol/mL; medium, 10 nmol/mL; high, 250 nmol/mL) and that of the standard.
[c] Relative standard deviation of peak height for 0.1 nmol/mL plasma.

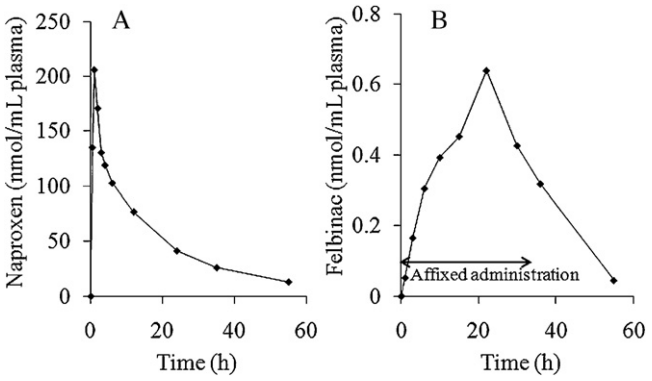

Fig. 5. The plasma concentration–time curves of (A) naproxen and (B) felbinac.

naproxen and felbinac were 0.073 and 0.038 nmol/mL, respectively, per 20-μL injection volume. This method was the same or more sensitive than MS method [12] and other fluorescence detection methods [13–15] with simple pretreatment.

3.5. Pharmacokinetic study

The method has been successfully applied to the pharmacokinetic study. A healthy volunteer was administered 300-mg naproxen by a single oral dose and blood was collected before and 0.1, 0.5, 1, 2, 3, 4, 6, 12, 24, 35, and 55 h after administration. The plasma concentration–time curve of naproxen is shown in Fig. 5A. The main pharmacokinetic parameters of naproxen were calculated. After administration of naproxen, T_{max} and C_{max} values were found to be 1 h and 206 nmol/mL, respectively. The AUC_{0-55} value was 2820 nmol h/mL. These results are similar to the reported results [23].

Furthermore, the method has been applied to the pharmacokinetic study of felbinac as well. A healthy volunteer was transdermally administered 40-mg felbinac in the lumber region for 36 h and blood was collected before and 0.1, 1, 3, 6, 10, 15, 22, 30, 36, and 55 h after administration. The plasma concentration–time curve of felbinac is shown in Fig. 5B. After administration of felbinac, T_{max} and C_{max} values were found to be 22 h and 0.64 nmol/mL, respectively. The AUC_{0-55} values were 18.2 nmol h/mL. These results are similar to the reported result [4].

4. Conclusion

This paper reports the development and application of the determination of NSAIDs by using fluorous derivatization and fluorous-phase separation. NSAIDs could be analyzed by simple pretreatment and rapid derivatization, and detected with high selectively and sensitivity without interference of contaminants in the biological sample. Furthermore this method was successfully used to monitor the concentration of orally administered naproxen and transdermally absorbed felbinac. Examination concerning simultaneous determination of NSAIDs with some metabolites [24,25] are on progress.

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References

[1] C.S. Boynton, C.F. Dick, G.H. Mayor, NSAIDs: an overview, J. Clin. Pharmacol. 28 (1988) 512–517.
[2] D.W. Hallesy, L.D. Shott, R. Hill, Comparative toxicology of naproxen, Scand. J. Rheumatol. 2 (1973) 20–28.
[3] T. Shiba, Y. Shikata, N. Takagawa, M. Sasaki, M. Yanaka, Y. Sugiyama, E. Murai, K. Nishiki, N. Inomata, T. Hama, M. Nawada, S. Hisaichi, A. Hasui, K. Hattori, K. Shirakawa, R. Konishi, Antiinflammatory and analgesic activities of felbinac adhesive preparation, Jpn. Pharmacol. Ther. 20 (1992) 81–94.
[4] N. Shinkai, K. Korenaga, H. Takizawa, H. Mizu, H. Yamauchi, Percutaneous penetration of felbinac after application of transdermal patches: relationship with pharmacological effects in rats, J. Pharm. Pharmacol. 60 (2008) 71–76.
[5] P.Z. Milani, M.B. Jalali, H. Tajerzadeh, Y. Azarmi, H. Valizadeh, Simultaneous determination of naproxen, ketoprofen and phenol red in samples from rat intestinal permeability studies: HPLC method development and validation, J. Pharm. Biomed. Anal. 39 (2005) 624–630.
[6] E.M. Costi, I. Goryacheva, M.D. Sicilia, S. Rubio, D.P. Bendito, Supramolecular solid-phase extraction of ibuprofen and naproxen from sewage based on the formation of mixed supramolecular aggregates prior to their liquid chromatographic/photometric determination, J. Chromatogr. A 1210 (2008) 1–7.
[7] G. Carlucci, P. Mazzeo, G. Palumbo, Simultaneous determination of rufloxacin, fenbufen and felbinac in human plasma using high-performance liquid chromatography, J. Chromatogr. B 682 (1996) 315–319.
[8] T. Hirai, S. Matsumoto, I. Kishi, Simultaneous analysis of several non-steroidal anti-inflammatory drugs in human urine by high-performance liquid chromatography with normal solid-phase extraction, J. Chromatogr. B 692 (1997) 375–388.
[9] Y. Sun, Z. Zhang, Z. Xi, Z. Shi, Determination of naproxen in human urine by high-performance liquid chromatography with direct electrogenerated chemiluminescence detection, Talanta 79 (2009) 676–680.
[10] N. Adhoum, L. Monser, M. Toumi, K. Boujlel, Determination of naproxen in pharmaceuticals by differential pulse voltammetry at a platinum electrode, Anal. Chim. Acta 495 (2003) 69–75.
[11] A. Aresta, T. Carbonara, F. Palmisano, C.G. Zambonin, Profiling urinary metabolites of naproxen by liquid chromatography–electrospray mass spectrometry, J. Pharm. Biomed. Anal. 41 (2006) 1312–1316.
[12] P. Gallo, S. Fabbrocino, F. Vinci, M. Fiori, V. Danese, L. Serpe, Confirmatory identification of sixteen non-steroidal anti-inflammatory drug residues in raw milk by liquid chromatography coupled with ion trap mass spectrometry, Rapid Commun. Mass Spectrom. 22 (2008) 841–854.
[13] E. Mikami, T. Goto, T. Ohno, H. Matsumoto, M. Nishida, Simultaneous analysis of naproxen, nabumetone and its major metabolite 6-methoxy-2-naphthylacetic acid in pharmaceuticals and human urine by high-performance liquid chromatography, J. Pharm. Biomed. Anal. 23 (2000) 917–925.
[14] H. Ibrahim, A. Boyer, J. Bouajila, F. Couderc, F. Nepveu, Determination of non-steroidal anti-inflammatory drugs in pharmaceuticals and human serum by dual-mode gradient HPLC and fluorescence detection, J. Chromatogr. B 857 (2007) 59–66.
[15] M. Siluveru, J.T. Stewart, Determination of fenbufen and its metabolites in serum by reversed-phase high-performance liquid chromatography using solid-phase extraction and on-line post-column ultraviolet irradiation and fluorescence detection, J. Chromatogr. B 682 (1996) 89–94.
[16] Y. Sakaguchi, H. Yoshida, K. Todoroki, H. Nohta, M. Yamaguchi, Separation-oriented derivatization of native fluorescent compounds through fluorous labeling followed by liquid chromatography with fluorous-phase, Anal. Chem. 81 (2009) 5039–5045.
[17] J.A. Gladysz, D.P. Curran, I.T. Horvth, Handbook of Fluorous Chemistry, Weinheim, Wiley-VCH, 2004.
[18] K. Uneyama, Organofluorine Chemistry, Oxford, Blackwell Publishing, 2006.
[19] Z. Luo, Q. Zhang, Y. Oderaotoahi, D.P. Curran, Fluorous mixture synthesis: a fluorous-tagging strategy for the synthesis and separation of mixtures of organic compounds, Science 291 (2001) 1766–1769.
[20] W. Zhang, Fluorocarbon stationary phases for liquid chromatography applications, J. Fluorine Chem. 129 (2008) 910–919.
[21] T. Hayama, Y. Sakaguchi, H. Yoshida, M. Itoyama, K. Todoroki, M. Yamaguchi, H. Nohta, Fluorous determination combined with liquid chromatography/tandem mass spectrometry: a method for the selective and sensitive determination of sialic acids in biological samples, Rapid Commun. Mass Spectrom. 24 (2010) 2868–2874.
[22] M. Kunishima, C. Kawachi, K. Hiroki, K. Terao, S. Tani, Formation of carboxamides by direct condensation of carboxylic acids and amines in alcohols using a new soluble condensing agent: DMT-MM, Tetrahedron 57 (2001) 1551–1558.
[23] N.M. Davies, K.E. Anderson, Clincal pharmacokinetics of naproxen, Clin. Pharmacokinet. 32 (1997) 268–293.
[24] Y. Sugawara, M. Fujihara, Y. Miura, K. Hayashida, T. Takahashi, Studies on the fate of naproxen. II. Metabolic fate in various animals and man, Chem. Pharm. Bull. 26 (1978) 3312–3321.
[25] A. Ohnishi, H. Fujiwara, M. Kitamura, T. Sasaki, Study of pharmacokinetics and safety of felbinac patch, Jpn. Pharmacol. Ther. 20 (1992) 115–138.