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Simultaneous determination of triflusal and its major active metabolite, 2-hydroxy-4-trifluoromethyl benzoic acid, in rat and human plasma by high-performance liquid chromatography

Hea-Young Cho, Tae-Jin Jeong, Yong-Bok Lee*

College of Pharmacy and Institute of Bioequivalence and Bridging Study, Chonnam National University, 300 Yongbong-Dong, Buk-Gu, Gwangju 500-757, South Korea

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Abstract

A rapid, selective and sensitive high-performance liquid chromatography (HPLC) method was developed and validated for the simultaneous determination of triflusal and its major active metabolite, 2-hydroxy-4-trifluoromethyl benzoic acid (HTB), in rat and human plasma. HPLC analysis was carried out using a 5-μm particle size, C_{18}-bonded silica column and acetonitrile–methanol–water (25:10:65, v/v/v) as the mobile phase and UV detection at 234 nm. Furosemide was used as the internal standard. The method involved extraction with an acetonitrile–chloroform mixture (60:40, v/v) and evaporation to dryness with nitrogen stream. The chromatograms showed good resolution and sensitivity and no interferences by plasma constituents. The mean absolute recovery for human plasma was 93.5 ± 4.2% for triflusal and 98.5 ± 3.1% for HTB. The lower limits of quantification of triflusal and HTB in human plasma were 20 and 100 ng/ml, respectively. The calibration curves in human plasma were linear over the concentration range 0.02–5.0 μg/ml for triflusal and 0.1–200.0 μg/ml for HTB with correlation coefficients greater than 0.999 and with inter- or intra-day coefficients of variation (CV) not exceeding 10.0%. This assay procedure was applied to the study of metabolite pharmacokinetics of triflusal and HTB in rat and human.

Keywords: Triflusal; 2-Hydroxy-4-trifluoromethyl benzoic acid

1. Introduction

Triflusal (2-acetoxy-4-trifluoromethyl benzoic acid, Fig. 1) is a new molecule related to acetylsalicylic acid which displays a notable activity as a platelet cyclooxygenase inhibitor. After a repeated triflusal administration in healthy subjects and patients, it has been observed that thromboxane B_2 levels descend and platelet aggregation is inhibited. Triflusal is quickly metabolized into its major metabolite, 2-hydroxy-4-trifluoromethyl benzoic acid (HTB, Fig. 1), which has in man an elimination half-life of over 24 h. Recently, it has been observed that HTB also displays antiaggregant activity [1,2].

Previous studies have shown that the determination of triflusal and HTB has different procedures for sample preparation in human plasma [2] and simultaneous determination of triflusal and HTB has low sensitivity in rat, dog and human plasma [3,4]. These methods were inadequate in our hands for metabolite kinetics of triflusal because of its time-consuming procedure, low sensitivity and limited reproducibility.

A simple and routine assay of triflusal and HTB in plasma was required for further investigations of their pharmacokinetics. We have established an high-performance liquid chromatography (HPLC) method available to pharmacokinetics by means of not only simultaneous determination of triflusal and HTB but also improving sensitivity. This type of assay using a reverse-phase HPLC is the subject of this paper.

2. Experimental

2.1. Chemicals

Triflusal and HTB were obtained from J. Uriach & Cia., S.A. (Barcelona, Spain). Furosemide (internal standard, Fig. 1) was purchased from Sigma (St. Louis, MO, USA).

* Corresponding author. Tel.: +82-62-530-2931; fax: +82-62-530-0106.
E-mail address: leeyb@chonnam.chonnam.ac.kr (Y.-B. Lee).

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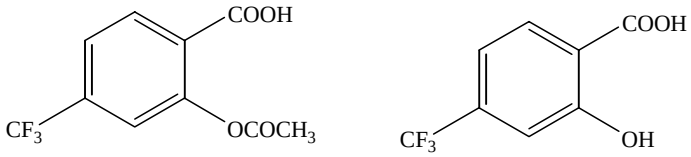
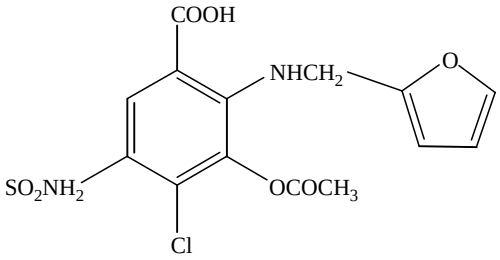


Triflusal

HTB



Furosemide

Fig. 1. Chemical structures of triflusal, 2-hydroxy-4-trifluoromethyl benzoic acid (HTB) and furosemide (IS).

HPLC grade acetonitrile and methanol were obtained from Fisher Scientific Company (Fair Lawn, NJ, USA). HPLC grade water was obtained from a Milli-Q water purification system (Millipore Co., Milford, MA, USA) and used throughout the study. The other chemicals and organic solvents were of analytical or HPLC grade and used without further purification. The mobile phase components such as acetonitrile, methanol and deionized water were filtered through a 0.45-μm pore size membrane filter prior to mixing. All plasma samples were immediately stored at −80 °C after collection.

2.2. Instrumentation

The HPLC system was from Shimadzu Corporation (Kyoto, Japan). It consisted of two pumps (model LC-10AD), a degasser (model DGU-12A) and an UV detector (model SPD-10A) set at 234 nm. The detection wavelength, 234 nm, was determined by scanning the maximum absorbance wavelength of triflusal and HTB in the mobile phase with UV spectrophotometer (Uvikon 930, Kontron Instruments, Zürich, Switzerland). Separations were performed on a Brownlee Spheri-10 RP 18 column (5-μm particle size, 100 mm × 4.6 mm i.d.) with NewGuard^{TM} cartridge holder (Alltech Associates Inc., Deerfield, IL, USA). Test samples were injected using a model 7725i injector (Rheodyne, Cotati, CA, USA). Detector output was quantitated on a model class LC-10 integrator (Shimadzu, Kyoto, Japan).

2.3. Calibration standards and quality control samples

Stock solutions of triflusal, HTB and furosemide (1 mg/ml) were prepared in methanol and stored in light-protected glass bottles at 4 °C. All the calibration standards were freshly prepared to give concentrations of 0.02 (only for human plasma), 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 μg/ml for triflusal, 0.1 (only for human plasma), 0.2, 0.5, 1, 5, 10, 20, 50, 100 and 200 μg/ml for HTB in drug-free pooled plasma obtained from nine different sources. In the same manner, quality control (QC) samples at low (0.1 μg/ml for triflusal and 0.5 μg/ml for HTB), medium (0.5 μg/ml for triflusal and 10 μg/ml for HTB) and high (2 μg/ml for triflusal and 100 μg/ml for HTB), respectively, were prepared to evaluate accuracy and precision. An aliquot of 100–200 μl of 0.1 M hydrochloric acid was immediately added to all the calibration standards and QC samples spiked with triflusal in order to prevent the degradation of triflusal due to enzyme in the plasma such as esterase.

2.4. Extraction procedure

The extraction procedures for the simultaneous determination of triflusal and HTB were determined after the evaluation of several extraction solvents. Three extraction solvents were used in order to compare the extraction efficiency of triflusal and HTB from plasma. Solvent A was acetonitrile–chloroform (40:60, v/v) [5], solvent B was diethylether [2] and solvent C was *tert*-butyl methyl ether (TBME) [6]. For a human plasma sample (0.5 ml), an aliquot of 100 μl of the IS (1 μg/ml of furosemide) and 200 μl of 0.1 M hydrochloric acid were immediately added and mixed with vortex-mixer for 30 s. For a rat plasma sample (0.1 ml), an aliquot of 100 μl of the IS (0.5 μg/ml of furosemide) and 100 μl of 0.1 M hydrochloric acid were used and mixed. The sample was then extracted with 1.5–4 ml of the above extraction solvents by vortex-mixing for 1 min. After

centrifugation at 10,000 × *g* for 2 min at 4 °C, the organic layer was separated and evaporated to dryness under a nitrogen stream in a centrifugal evaporator at 4 °C (CVE-200D, Tokyo Rikakikai, Tokyo, Japan). The residue was finally reconstituted in 100 μl of mobile phase by vortex-mixing, of which 50 μl was then injected into the HPLC system.

2.5. Chromatography

Two mobile phases were compared. Mobile phase A consisted of a mixture of 50% methanol and 0.005 M dilution of *n*-tetrabutyl ammonium phosphate (PIC A, Waters, Millipore, Milford, MA, USA) [2–4]. Mobile phase B was consisted of acetonitrile–methanol–water (25:10:65, v/v/v) adjusted to pH 2.5 with 85% (w/v) orthophosphoric acid [5]. All separations were performed isocratically at a flow-rate of 1.0 ml/min. The column was maintained at ambient temperature with an inlet pressure of about 4.7 MPa.

2.6. Method validation

2.6.1. Specificity

The interference of endogenous compounds was assessed by analyzing standard triflusal, HTB and IS, drug-free plasma samples, plasma spiked with triflusal, HTB and IS, and plasma samples obtained from subjects given triflusal. All the peaks presenting the retention time of triflusal, HTB and IS were investigated using a diode array detector (2017 Daiode Array, Shiseido, Tokyo, Japan).

2.6.2. Sensitivity

The lower limit of quantitation (LLOQ) was defined as the lowest concentration measured with an imprecision of less than 20% (coefficients of variation, CV) and accuracy of 80–120%. It was determined at 0.02 μg/ml for triflusal and 0.1 μg/ml for HTB in five replicate samples.

2.6.3. Linearity

Quantitation was based on electronically integrated peak-height ratios of triflusal and HTB to furosemide using calibration standards covering the expected concentration ranges. The straight-line regression equations were treated statistically and calibration curves were obtained by weighted linear regression (weight factor: $1/[\text{concentration}]^2$) [7]. The unknown concentrations of triflusal and HTB were calculated by inverse prediction from the calibration equations.

2.6.4. Precision and accuracy

In order to assess the intra- and inter-day precision and accuracy of the assay, QC samples were prepared as described above. The intra-day precision of the assay was assessed by calculating the coefficients of variation for the analysis of QC samples in five replicates, and inter-day precision was determined through the analysis of QC samples on five consecutive days. Accuracy was determined by comparing the calculated concentration using calibration curves to known concentration.

2.6.5. Recovery

The absolute recovery from plasma was assessed by comparison of the peak-height from extracted QC samples to the height of standard corresponding to respective concentrations followed by application of a correction factor. The relative recovery from plasma was assessed by comparison of the peak-height from extracted QC samples to those from extracted samples containing water instead of plasma. The mean recovery was determined at low, medium and high concentrations in five replicates.

2.6.6. Stability

The storage stability of triflusal in plasma was assessed because of a previous report that triflusal was readily hydrolyzed to HTB [3]. To test the short- and long-term stability of triflusal and HTB, two QC samples, containing low (0.1 μg/ml for triflusal and 1 μg/ml for HTB) and high (5 μg/ml for triflusal and 100 μg/ml for HTB) concentration with or without the addition of 0.1 M hydrochloric acid, were stored under different conditions: at −80 °C up to 4 weeks; at 4 °C up to 24 h; at 37 °C up to 24 h. Three freezing–thawing cycles (−80 °C/ice-water bath) were applied to the above QC samples on three consecutive days. To test the stability of stock solutions stored in the light-protected glass bottles at 4 °C, they were diluted with mobile phase and injected into the HPLC system at predetermined time intervals up to 4 weeks. Besides, the stability of triflusal and HTB in the mobile phase was determined at room temperature up to 6 h.

On the other hand, in order to evaluate the effect of 0.1 M hydrochloric acid on the hydrolysis of triflusal in plasma, the degradation of triflusal to HTB in blank plasma prior and posterior to adding an aliquot of 100–200 μl of 0.1 M hydrochloric acid was determined at the plasma triflusal samples (5 μg/ml). After spiking of triflusal, they were immediately stored at 4 °C up to 24 h and at 37 °C up to 24 h, respectively, and its degradation to HTB was monitored at predetermined time intervals.

The compounds were considered stable if the variation of assay ($n = 5$) was less than 10% of initial time response.

2.7. Pharmacokinetic studies of triflusal

Nine normal healthy male Korean volunteers (21–26 years, 53.0–78.2 kg) participated in the pharmacokinetic study of oral triflusal after giving written informed consent. All subjects fasted at least 10 h before drug administration and continued to fast up to 4 h thereafter. They abstained from consumption of alcohol or xanthine-containing foods and beverages during the study. Each volunteer received a single oral dose of 300-mg triflusal capsule (Disgren®, Myungin Pharmaceutical Co. Ltd., Seoul, Korea) with 240 ml of spring water.

Blood samples were withdrawn from the forearm vein before oral administration and at 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 6, 12, 24, 48 and 72 h after the oral administration, transferred to Vacutainer® (K3E, 13 mm × 75 mm, Becton Dickinson, Plymouth, UK) tubes, immediately kept in icebox, and centrifuged. Following centrifugation (10,000 × *g*, 2 min, 4 °C), plasma samples were transferred to polyethylene tubes containing an aliquot of 200 μl of 0.1 M hydrochloric acid, immediately vortex-mixed for 10 s, and stored at −80 °C until analysis.

Male Sprague–Dawley rats, weighing 200–250 g and aged 8–10 weeks, were fasted overnight before drug administration. The bolus injection of triflusal (10 mg/kg in 2 ml of 7.2 mM sodium bicarbonate solution, pH 7.4) was made intravenously to each rat ($n = 9$). Blood samples were collected immediately before dosing and up to 72 h postdose, using EDTA sodium salt (0.1% final solution) as the anticoagulant. Plasma was separated and stored as described above.

The plasma concentration–time data of triflusal and HTB were evaluated by means of a nonlinear regression analysis using WinNonlin software (Pharsight Corporation, CA, USA) [8]. The relevant pharmacokinetic parameters were calculated according to the procedures described in Ref. [9].

3. Results and discussion

3.1. Chromatographic analysis

Triflusal is a trifluoromethyl derivative of acetylsalicylic acid (aspirin). HTB corresponds to 2-hydroxy benzoic acid, the major circulating metabolite of aspirin. Thus, the analysis of triflusal and HTB is mainly based on the analysis of aspirin and salicylic acid [6,10–12]. There are two problems associated with the HPLC analysis of triflusal and HTB in biological fluids like aspirin and salicylic acid. Firstly, triflusal hydrolyses to HTB in protic solvents such as water or methanol, and also in plasma as aspirin does to salicylic acid [4,13]. Secondly, part of HTB like salicylic acid may be lost by sublimation during sample treatment when evaporation steps are included [11]. In this study, we circumvented these analytical problems as follows.

The chromatograms of triflusal and HTB in mobile phase B showed more stable base lines and better resolutions than those in mobile phase A using *n*-tetrabutyl ammonium as the ion-pairing counter ion. The UV absorption spectrum of triflusal in this mobile phase B indicated the optimal absorption maximum was at 234 nm. Fig. 2 shows chromatograms using mobile phase B of (A) blank human plasma; (B) human plasma sample containing triflusal (1.0 μg/ml), HTB (10.0 μg/ml) and furosemide, the IS (0.2 μg/ml); (C) human plasma sample taken at 30 min after single 300-mg oral dose administration of triflusal and spiked with furosemide. Triflusal, HTB and furosemide were eluted as peaks after 8.0, 14.6 and 5.1 min, respectively, in areas of the chromatogram free from endogenous peaks. Also, the same results could be obtained from the chromatograms using rat plasma.

In order to select the proper IS, the following acidic drugs were dissolved in the mobile phase B and injected into the HPLC system: acetylsalicylic acid, antipyrine, benzoic acid, furosemide, isopropyl antipyrine, methyl benzoic acid, naproxene, phenylbutazone and salicylic acid. The retention time of above drugs in turn was 2.8, 10.5, 3.3, 5.1, 11.5, 4.1, 24.2, 15.3 and 4.3 min, respectively. Furosemide and naproxene were selected as the candidates for the IS. The absolute recovery of furosemide and naproxene was 92.5 ± 3.1 and 75.3 ± 4.1%, respectively. Furosemide was selected as the

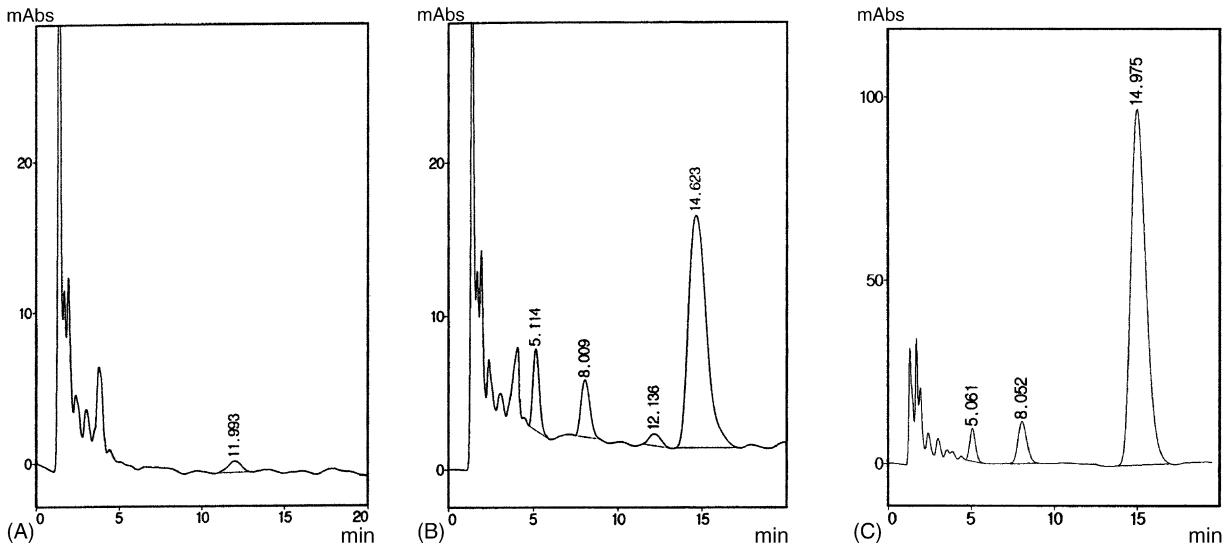


Fig. 2. High-performance liquid chromatograms of (A) blank human plasma; (B) human plasma sample containing triflusal (1.0 μg/ml), HTB (10.0 μg/ml) and furosemide, the IS (0.2 μg/ml); (C) human plasma sample taken at 30 min after single 300-mg oral dose administration of triflusal and spiked with furosemide. Peaks: furosemide, the IS (5.1 min), triflusal (8.0 min), HTB (14.6 min).

most proper IS in this study. And, the chromatographic run time selected was 20 min.

The chromatograms with extraction solvent A showed no interference of plasma, and represented better resolution and more stable baseline than those with the other two extraction solvents. The absolute recovery of triflusal (0.5 μg/ml) for human plasma extracted by extraction solvent A, B and C was 92.5 ± 4.3, 75.3 ± 4.7 and 38.7 ± 3.9%, respectively. That of HTB (10 μg/ml) was 95.9 ± 3.2, 96.8 ± 4.1 and 28.5 ± 3.1%, respectively. It was found that the mean absolute recoveries had no significant differences ($P < 0.05$) between rat and human plasma. Therefore, the extraction solvent A, acetonitrile–chloroform mixture (40:60, v/v), was selected.

3.2. Specificity

There were no peaks of interferences with triflusal, HTB and IS at their retention times in the blank rat and human plasma. We could confirm the each identical peak spectrum with the diode array detector. The extraction with an acetonitrile–chloroform mixture (40:60, v/v) and the chromatographic analysis using mobile phase B were essential to minimize interfering peaks.

3.3. Sensitivity

The lower limit of quantitation (LLOQ) was defined as those quantities producing a background noise of approximately 10 times that could be estimated with an intra- and inter-day imprecision less than 20% (CV), and inaccuracy between ±20% (bias). The LLOQ for human plasma was estimated to be 0.02 μg/ml for triflusal and 0.1 μg/ml for HTB, and that for rat plasma was 0.05 and 0.2 μg/ml, respectively. The mean percent accuracy values of triflusal and HTB for human plasma were 83.0 and 116.0% with CV of 18.4 and 16.9% at the LLOQ, respectively. Particularly, the LLOQ values of triflusal and HTB for human plasma were 50- and 10-fold lower than those reported by Ramis et al. [2], respectively.

3.4. Linearity

The mean (±S.D.) regression equations from nine replicate calibration curves on different days for human plasma: $y = (0.6301 \pm 0.0573)x + (0.0333 \pm 0.0035)$ for 0.02–5 μg/ml triflusal and $y = (0.3394 \pm 0.0288)x + (0.1294 \pm 0.0110)$ for 0.1–200 μg/ml HTB (where y is the peak-height ratio and x is the concentration), showed significant linearity ($r = 0.9993 \pm 0.0002$ for triflusal, 0.9991 ± 0.0002 for HTB), with statistically insignificant ($P > 0.01$) nonlinear elements in the residual sum of squares, as determined by analysis of variance. The mean regression equations for rat plasma: $y = (0.2677 \pm 0.0186)x + (0.0146 \pm 0.0015)$ for 0.05–5 μg/ml triflusal and $y = (0.1864 \pm 0.0144)x + (0.0994 \pm 0.0087)$ for 0.2–200 μg/ml HTB, showed the same linearity.

3.5. Precision and accuracy

Table 1 shows a summary of intra- and inter-day precision and accuracy for triflusal and HTB in human plasma. The intra-day accuracy of triflusal and HTB for human plasma samples was 87.0–107.8 and 93.8–104.6% at QC samples with the imprecision (CV) less than 9.6 and 8.6%, respectively. The inter-day accuracy of triflusal and HTB for human plasma samples ranged from 92.0 to 108.6% and from 90.2 to 114.4% at QC samples with the imprecision (CV) less than 10.0 and 7.7%, respectively. The imprecision and inaccuracy of both drugs for rat plasma were found to be within 13.5% and were apparently not significantly different ($P < 0.05$) from the corresponding values in human plasma. So, it is expected that the present method will be applicable to the metabolite kinetics of triflusal for human and rat.

3.6. Recovery

The extraction recovery of triflusal and HTB was determined at its QC samples in five replicates (Table 2). The mean absolute recovery of triflusal and HTB for human plasma was found to be 93.5 ± 4.2% for triflusal and 98.5 ± 3.1% for HTB. That for rat plasma was determined

Table 1
Imprecision and accuracy of HPLC analysis of triflusal and HTB in human plasma

Triflusal				HTB			
Added (μg/ml)	Measured (mean ± S.D.)	Imprecision (CV, %)	Accuracy[a] (%)	Added (μg/ml)	Measured (mean ± S.D.)	Imprecision (CV, %)	Accuracy[a] (%)
Intra-day ($n = 5$)							
0.1	0.087 ± 0.005	6.3	−13.0	0.5	0.523 ± 0.045	8.6	4.6
0.5	0.539 ± 0.052	9.6	7.8	10.0	9.781 ± 0.308	3.2	−2.2
2.0	1.780 ± 0.102	5.7	−11.0	100.0	93.770 ± 1.875	2.0	−6.2
Inter-day ($n = 5$)							
0.1	0.092 ± 0.007	7.7	−8.0	0.5	0.520 ± 0.031	6.0	4.0
0.5	0.543 ± 0.043	7.9	8.6	10.0	11.440 ± 0.709	6.2	14.4
2.0	1.975 ± 0.198	10.0	−1.3	100.0	90.205 ± 6.946	7.7	−9.8

[a] Defined as: [(measured concentration − added concentration)/added concentration] × 100%.

Table 2
Absolute and relative recovery of triflusal and HTB for human plasma

Concentration (μg/ml)	Triflusal recovery (mean ± S.D., $n = 5$) (%)		Concentration (μg/ml)	HTB recovery (mean ± S.D., $n = 5$) (%)	
	Absolute	Relative		Absolute	Relative
0.1	90.8 ± 4.9	95.8 ± 5.9	0.5	98.8 ± 3.9	97.9 ± 4.9
0.5	93.5 ± 3.9	96.3 ± 4.6	10.0	97.5 ± 2.9	98.8 ± 5.8
2.0	95.9 ± 4.1	94.9 ± 5.1	100.0	98.9 ± 2.4	99.1 ± 4.0

to be 91.2 ± 4.6 and 96.7 ± 4.2%, respectively. The mean relative recovery of triflusal and HTB for human plasma was determined to be 95.6 ± 5.1% for triflusal and 98.7 ± 4.9% for HTB. That for rat plasma was found to be 92.8 ± 5.5 and 97.8 ± 6.6%, respectively. There was no significant difference ($P < 0.05$) in the recovery between rat and human plasma. This high, reproducible recovery of triflusal and HTB was able to increase their assay sensitivity and the simple liquid–liquid extraction with acetonitrile–chloroform (40:60, v/v) has been successfully applied to the extraction of triflusal and HTB from human and rat plasma.

3.7. Stability

HTB QC samples were stable at −80 °C up to 4 weeks, at 4 °C up to 24 h, and at 37 °C up to 24 h with quantitation variation less than 7.4% irrespective of adding 0.1 M hydrochloric acid to plasma. Triflusal QC samples with 0.1 M hydrochloric acid were also stable at −80 °C for at least 4 weeks, at 4 °C for at least 24 h and at 37 °C for at least 24 h with quantitation variation less than 10.0%. However, triflusal degraded to HTB at 4 and 37 °C in plasma without 0.1 M hydrochloric acid (Fig. 3). The faster degradation at 37 °C than at 4 °C indicated a temperature-dependent hydrolysis and 0.1 M hydrochloric acid could obstruct this hydrolysis. But, triflusal in plasma could be stored at −80 °C for at least 4 weeks without the addition of 0.1 M hydrochloric acid (CV was less than 9.8%).

The stock solutions of triflusal, HTB and the IS stored in the light-protected glass bottles at 4 °C were stable for at least 4 weeks with quantitation variation less than 10.0%. And also, the mobile phase used did not alter the stability

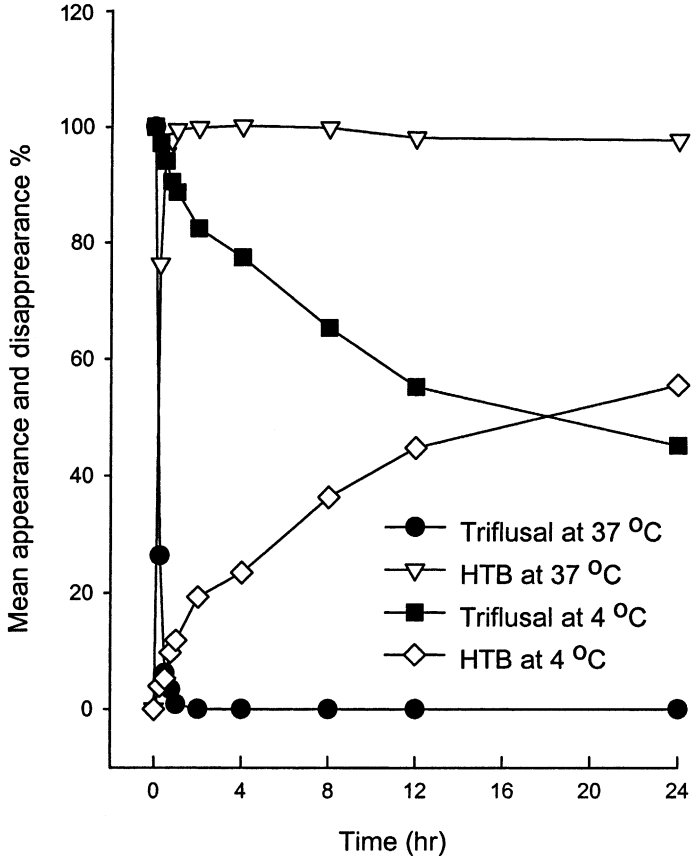


Fig. 3. Percent mean metabolic disappearance of triflusal (5 μg/ml) and appearance of HTB in human plasma incubated at 4 and 37 °C over 24 h without 0.1 M hydrochloric acid ($n = 5$).

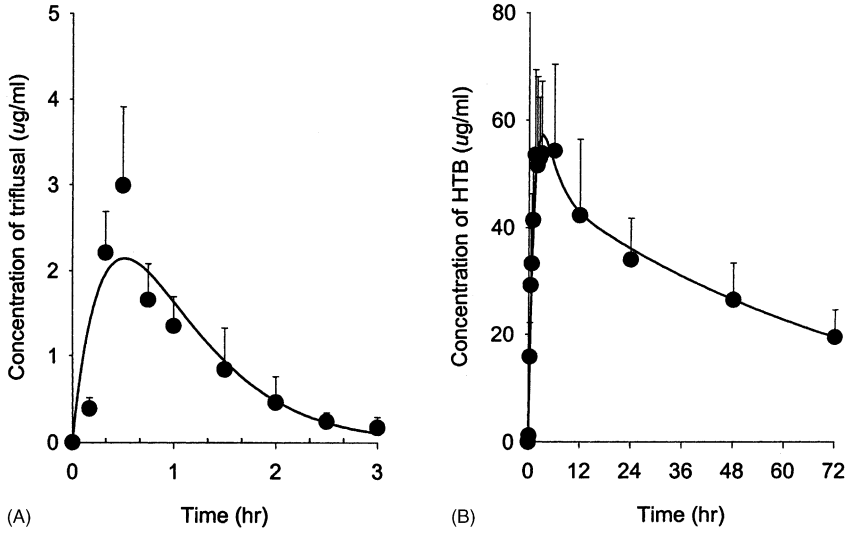


Fig. 4. Mean venous plasma concentration–time profiles of (A) triflusal and (B) HTB after oral administration of single 300-mg triflusal capsule to humans ($n = 9$). Each solid line was calculated by WinNonlin program. Vertical bar represents the standard deviation of the mean.

of triflusal and HTB at room temperature up to 6 h (CV was less than 2.6%).

Triflusal in plasma without 0.1 M hydrochloric acid was not stable under the condition of one freezing–thawing cycle (−80 °C/ice-water bath). However, triflusal and HTB in plasma with 0.1 M hydrochloric acid, and HTB in plasma without 0.1 M hydrochloric acid resulted stable even if submitted to three freezing–thawing cycles.

3.8. Application

The mean plasma profiles of triflusal and HTB after single oral administration of 300-mg triflusal capsule to nine normal healthy male Korean volunteers and intravenous bolus administration of triflusal (10 mg/kg) to nine rats, are shown in Figs. 4 and 5, respectively. The plasma concentration–time profiles of triflusal and HTB followed the mono- and bi-exponential pattern, respectively, as pointed out in Refs. [3] and [4]. The mean (±S.D.) terminal half-life and area under the plasma concentration–time curve (AUC) of triflusal in human were 38.4 (±5.0) min and 3.0 (±0.5) μg h/ml, respectively. The mean (±S.D.) terminal half-life of HTB and ratio of AUC of HTB formed from triflusal to AUC of triflusal in human were 59.7 (±11.3) h and 1321 (±304), respectively. On the other hand, the mean (±S.D.) terminal half-life, AUC, total body clearance and mean

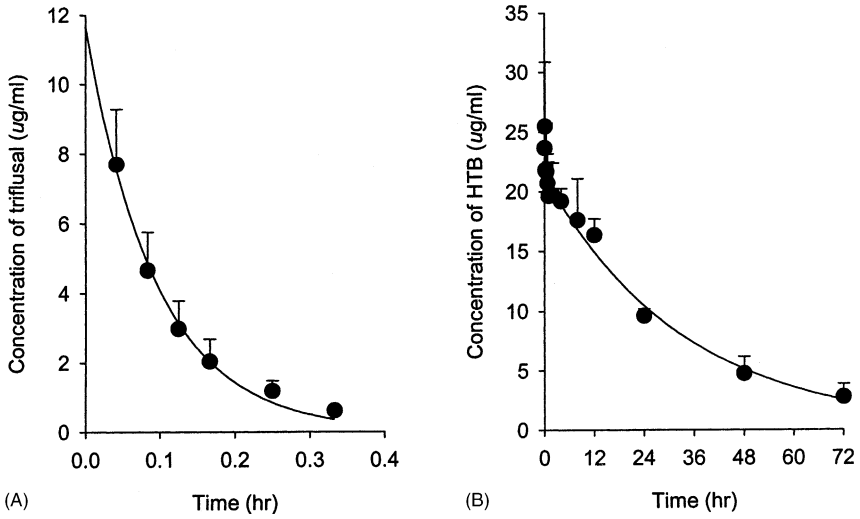


Fig. 5. Mean arterial plasma concentration–time profiles of (A) triflusal and (B) HTB after femoral vein administration of triflusal (10 mg/kg) to rats ($n = 9$). Each solid line was calculated by WinNonlin program. Vertical bar represents the standard deviation of the mean.

residence time (MRT) of triflusal in rats were 5.7 (±1.0) min, 74.4 (±10.9) μg min/ml, 132.1 (±18.6) ml/min/kg and 6.8 (±0.5) min, respectively. The mean (±S.D.) terminal half-life of HTB and AUC ratio of HTB to triflusal in rats were 31.7 (±4.6) h and 756.2 (±90.7), respectively. The parameter values of triflusal in human and rat were similar to those of other investigators [3,4]. It is of interest to note that the terminal half-lives of triflusal and HTB in human were much longer than those in rat, and that AUC ratio of HTB to triflusal was dependent on the administration route and species. Besides, it is very interesting that there are similarities in the chemical stability, chromatographic behavior, metabolism and pharmacokinetics of triflusal and aspirin [14].

4. Conclusion

We have described a simple, robust, accurate, precise and validated HPLC method for the simultaneous determination of triflusal and its major metabolite, HTB, within the plasma concentration range observed in rat and human. It is currently used in this laboratory for investigating the metabolite kinetics of triflusal following intravenous and chronic oral triflusal therapy in rat and human. The present method was applied to a study of the metabolite kinetics of triflusal and these results will be presented elsewhere.

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