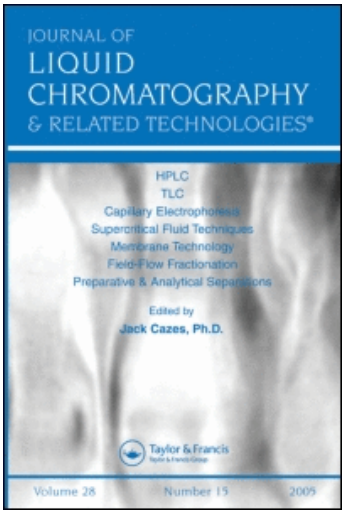

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High-Performance Liquid Chromatographic Determination of Ketoprofen in Pharmaceutical Dosage forms and Plasma

Chi-yin Wong[a]; Ming-kung Yeh[b]; Da-peng Wang[b]
[a] Kaohsiung Veterans General Hospital Kaohsiung, Taiwan, Republic of China [b] Department of Clinical Pharmacy Tri-Service General Hospital School of Pharmacy, National Defense Medical Center Taipei, Taiwan, Republic of China

To cite this Article Wong, Chi-yin , Yeh, Ming-kung and Wang, Da-peng(1992) 'High-Performance Liquid Chromatographic Determination of Ketoprofen in Pharmaceutical Dosage forms and Plasma', Journal of Liquid Chromatography & Related Technologies, 15: 8, 1215 — 1225
To link to this Article: DOI: 10.1080/10826079208018283
URL: http://dx.doi.org/10.1080/10826079208018283

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(8), 1215-1225 (1992)

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF KETOPROFEN IN PHARMACEUTICAL DOSAGE FORMS AND PLASMA

CHI-YIN WONG[1], MING-KUNG YEH[2], AND DA-PENG WANG[2]
[1]*Kaohsiung Veterans General Hospital*
Kaohsiung, Taiwan, Republic of China
[2]*Department of Clinical Pharmacy*
Tri-Service General Hospital
School of Pharmacy, National Defense Medical Center
Taipei, Taiwan, Republic of China

ABSTRACT

A high-performance liquid chromatographic (HPLC) method for determining ketoprofen in dosage forms and plasma is developed. The drug was chromatographed in a C18 column, using a mobile phase consisting of acetonitrile and 1 % acetic acid aqueous solution with ratio 40 : 60, and detected at 254 nm. The linear detector response to the concentration was confirmed. Recoveries from standard addition to tablets and nonaqueous solution were 100.43 and 100.15, for humans and for rabbit plasmas 82.4 and 82.7,respectively . The procedure showed excellent reproduction inside of two days.

It is suggested that this HPLC method could be used for routine assay of ketoprofen in pharmaceutic dosage forms and plasma.

INTRODUCTION

Ketoprofen [2-(3-benzoylphenyl) propionic acid] is widely used in rheumatologic treatment because of its analgesic and anti-inflammatory properties. There are many reports (1-11) of applications to analyze the drug content in dosage forms and pharmacokinetics studies of ketoprofen such as colorimetric, polarographic, TLC, HPLC and GLC methodology. However, these analytical methods generally require large quantities of test samples and are also time consuming.

In this study, a high-performance liquid chromatographic (HPLC) assay eliminates these drawbacks and enables the rapid determination of plasma and dosage form.

EXPERIMENTAL

Apparatus

The following apparatus was used :

A model 440 high-performance liquid chromatograph with 745 data module, a 254 nm UV detector from WATERS ASSOCIATES, and a uBONDAPARK C18 column.

Conditions

The HPLC condition, used to a uBondapak C_{18} column (4.6mm*15cm), fixed with a 254 nm detector, was used at a pressure of 2000 psig. The column ran a mobile phase (acetonitrile and 1 % acetic acid, 40 : 60) with a flow rate of 2.0 mL/min at room temperature. The injection volume was 20 uL, the chart speed was set at 0.5 cm/min.

Materials

Ketoprofen was ordered from CHENG HSING TANG Chemical CO.,LTD R.O.C. Other chemicals were analytical grade. Mill-Q water and HPLC grade methanol were used in the mobile phase preparation. Placebo tablet6s were prepared by a lot of

ketoprofen, lactose, corn starch, and PEG 400 purchased from Stokes' single punch machine (Model 519-2). Nonaqueous solution was prepared to dissolve ketoprofen in propylene glycol. Pharmaceutical dosage forms were purchased from the PARIS Compound, France.

Internal standard

Two different concentrations of isopropylphenazone aqueous solution with 0.002 mg/mL and 0.1 mg/mL were prepared as the internal standard.

Standard curve in dosage form

Five different standards were prepared as following, transferred

1.0, 3.0, 5.0, 7.0 and 9.0 mL of 1 mg/mL ketoprofen standard solution to 25 mL volumetric flasks separately, each was also added 1.0 mL internal standard solution (0.1 mg/ml) and then made volume with the mobile phase. The standards were prepared daily.

Standard curve in plasma

0.5 mL of rabbit plasma and human plasma containing varied amounts of ketoprofen with a range of 0.4 to 2.0 ug/mL and an internal standard (0.002 mg/mL) were prepared in 20 * 15 mm centrifuge tubes, then 1 mL of acetonitrile was added, the mixture rotated for 5 minutes, and centrifuged at 3000 rpm for 15 minutes. Following centrifugation the upper layer was filtrated by 0.45 um millpore and transferred to a clear tube , then 20 ul of this solution was injected into the HPLC in triplicate.

Procedure

A. Dosage forms preparation

Solution : Accurately transfer the solution equal to 20.0 mg of ketoprofen into a 100 mL volumetric flask. 1.0 mL

of internal standard solution (0.1 mg/mL) was added. The volume was brought up to 100 mL with the mobile phase. The final mixture was analyzed by HPLC.

Tablet : Twenty tablets were taken and ground into a fine powder. A portion of the power equivalent to 20 mg of ketoprofen was dissolved with the 50 mL mobile phase, mixed well and filtered. The 1.0 mL of internal standard solution (0.1 mg/ml) was added and the final solution was diluted to 100 mL in 100 ml volumetric flask with the mobile phase, and then 20 uL of the solution was injected into the HPLC under the described conditions.

B. Human or rabbit plasma preparation

Pharmacokinetics Study : Male NewZealand white rabbits in the weight range of 2.5 to 3.0 kg were used in this study. An initial loading dose of 4.0 mg/kg of ketoprofen (10 mg/ml in nonaqueous solution) was intramuscularly injected (I.M) at the vatus intermedias of the right leg in the rabbits. The animals were conscious and individually housed in cages during the experiment. At designated times after I.M injection, each rabbit was awakened and blood samples were collected in heparinized tubes by withdrawing directly from the left abdominal aorta. Blood samples were drawn from the left abdominal aorta without anticoagulation prior to drug administration at 0,5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 225, 270, 330, 390, 480 and 600 min. Plasma was separated by centrifugation immediately after collection and the samples were frozen at -20°C until analysis.

Extraction process

A 2.0 ug/ml of 1 ml isopropylphenazone stock solution (internal standard) and 1 ml acetonitrile were added to 0.5

ml plasma by mechanical mixing for 5 min, then centrifuged at 3500 rpm for 15 min. Following centrifugation the upper layer was filtered by 0.45 um millpore and transferred to a clear tube , then 20 ul of this solution was injected into the HPLC.

Sample analysis

The ketoprofen plasma concentration was determined by HPLC method. A standard curve for each rabbit plasma sample was generated.

Reproducibility of the analytical method was evaluated by triplicate analysing of the same dosage form over a period of two days.

Calculations

Preliminary investigations showed that the ratio of peak areas (ketoprofen/isopropylphenazone) were directly related to concentrations of ketoprofen. The percentage recovery of ketoprofen may be obtained either by a calibration graph method or by calculations using the equation (1). (the mass of sample taken is assumed to be the same as the mass of standard ketoprofen in the standard solution).

$$\text{Drug Content \%} = \frac{D/IS\ (\text{sample})}{D/IS\ (\text{standard})} * 100\ \% \quad \text{-----(1)}$$

where D is the peak area of ketoprofen and IS is the peak area of the internal standard.

Results and Discussion

The HPLC profiles for the quantitative determination of ketoprofen in dosage form and plasma are shown in Fig 1. Isopropylphenazone was an ideal internal standard because it was stable under the assay conditions more than two days and

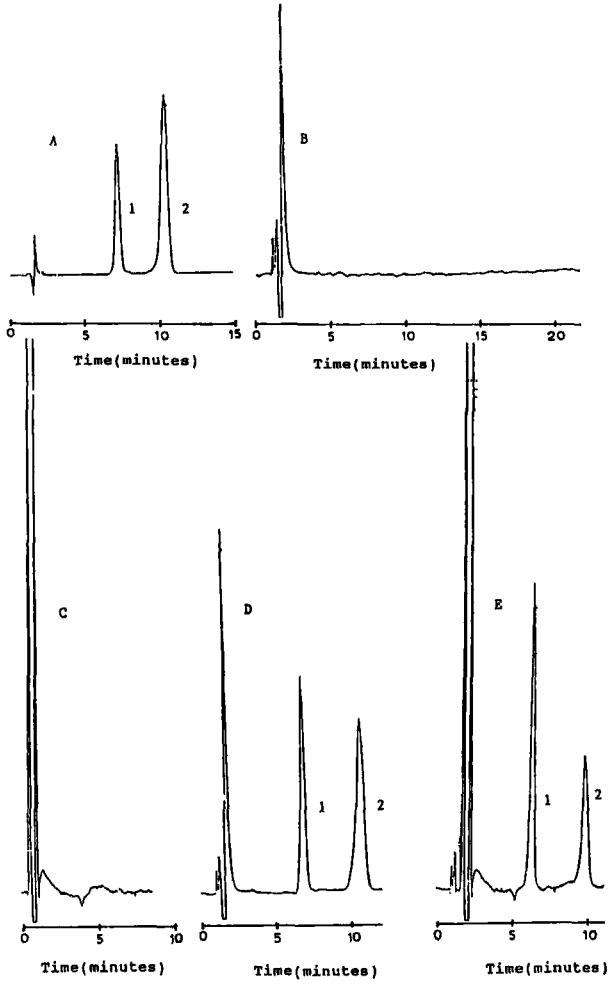

Fig. 1. HPLC profiles of ketoprofen (A) solution, (B) human plasma without ketoprofen, (C) rabbit plasma without ketoprofen, (D) ketoprofen (0.4 ug/mL) in human plasma, (E) ketoprofen (0.4 ug/mL) in rabbit plasma.

Table 1. Calibration Curve for Plotting Peak Area Ratios Versus Varying Concentration of Ketoprofen in Plasma and Solution.

Sample	Concentration	Area Ratio*		Slope	Intercept	r
		Mean	C.V. %			
	0.04 mg/ml	0.19	0.32			
	0.12 mg/ml	0.57	0.37			
Solution	0.20 mg/ml	0.95	0.44	4.5310	0.0084	0.9998
	0.28 mg/ml	1.33	0.31			
	0.36 mg/ml	1.70	0.21			
	0.4 ug/ml	1.09	3.7			
	0.8 ug/ml	2.18	4.2			
Human	1.2 ug/ml	3.27	3.8	2.7104	0.0074	0.9994
plasma	1.6 ug/ml	4.37	4.7			
	2.0 ug/ml	5.46	3.6			
	0.4 ug/ml	1.02	4.8			
	0.8 ug/ml	2.04	3.6			
Rabbit	1.2 ug/ml	3.05	2.9	2.5204	0.0094	0.9996
plasma	1.6 ug/ml	4.07	3.7			
	2.0 ug/ml	5.09	3.5			

*.results of five replicate determinations; C.V. coefficient of variation
r : regression coefficient.

separated well from the ketoprofen. The relative retention times for ketoprofen and isopropylphenazone were 6.82 and 10.66 minutes, respectively. Table 1 shows the calibration curve for plotting peak area ratios versus varying concentrations of ketoprofen (0.4-2.0 ug/mL) in the sample of human and rabbit plasma and (0.04-0.36 mg/mL) in dosage

Table 2. The Recovery of Placebo Samples and Plasmas

Sample	Labeled Amount	n	% Recovery*		
			Mean	S.D.	C.V. %
Tablet#	100.0 mg/tab	5	100.43	0.43	0.42
Nonaqueous#	10.0 mg/mL	5	100.15	0.37	0.37
Human plasma	1.0 mg/mL	5	82.4	3.7	4.49
Rabbit plasma	1.0 mg/mL	5	82.7	4.1	4.96

*.S.D. standard deviation; C.V. coefficient of variation.

#.synthetic dosage forms were prepared in our pilot pharmaceutical factory.

Table 3. The Reproducible of Solution and Plasma in the Inter and Intraday

Sample	Concentration (mg/mL)	% Recovery*					
		Mean	S.D.(1)	Mean	S.D.(2)	Mean	S.D.(3)
Solution	10	10.2	10.43	10.24	0.37	10.18	0.45
Human plasma	1.0	0.824	3.7	0.821	3.8	0.815	4.1
Rabbit plasma	1.0	0.827	4.1	0.813	4.3	0.811	4.4

*. results of five replicate determinations; S.D. standard deviation.

(1) the first analysis time at AM. 8:00.

(2) the second analysis time at PM 8:00.

(3) the third analysis time at PM 8:00 the next day.

Table 4. Assay of Ketoprofen in Pharmaceutical Dosage Forms

Preparation	Labelled Amount	% Recovery*		
		Mean	S.D.	C.V.%
Ketoprofen	100.0 mg/vial	100.04	0.43	0.43

*.results of five replicate determinations; S.D.: standard deviation; C.V.: coefficient of variation.

#. PARIS FRANCE Compound, lot no. 1772-1

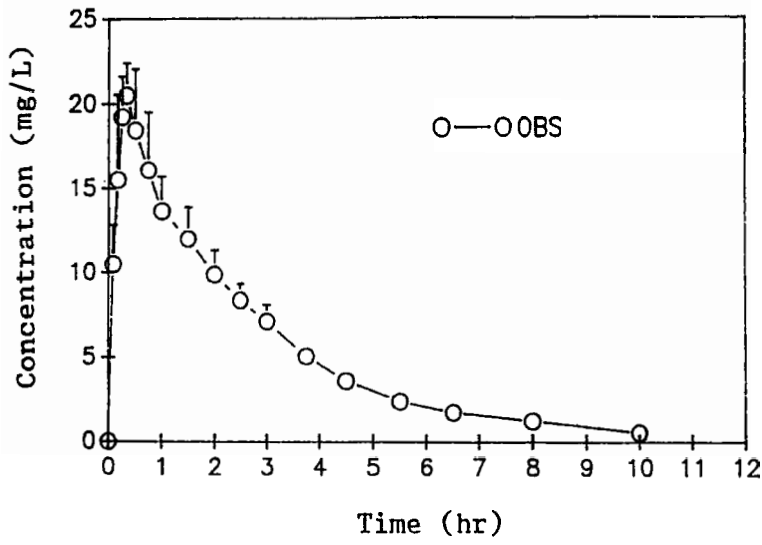

Fig. 2. The time course of ketoprofen concentration in plasma for I.M. administration of 4 mg/kg of ketoprofen injection in six rabbits.

forms. The equations were Y = 2.5204 X - 0.0094 with a correlate coefficient of 0.9996 for animal plasma, Y = 2.7104 X - 0.0074 with a correlation coefficient of 0.9994 for human plasma and Y = 4.531 X - 0.0084 with a correlation coefficient of 0.9998 for dosage forms. These results all demonstrated a good linear relationship.

Recovery studies for plasma, placebo tablet, and nonaquous solution were done by adding specific amounts of ketoprofen to plasma samples (1.0 mg/ml), tablet (100 mg/tab), and nonaqueous solution (10 mg/mL) and an analysis of the actual quantity of ketoprofen obtained using the extraction procedure when compared with the actual drug added. Table 2 is the recovery of the placebo samples and plasmas.

Reproducible data showed that assay precision were determined by the ketoprofen concentration of 1.0 mg/mL in plasma, and 10 mg/mL in the nonaqueous solution, a relatively standard deviation and coefficient of variation (Table 3).

This method was checked to assay commercially available products, the coefficients of variation of the described method are sufficiently low to be acceptable (Table 4). Animal pharmacokinetics studies showed the mean plasma-concentration-time curve for ketoprofen after a I.M. adminstration of 4 mg/kg to the remaining 6 rabbits (Fig 2). The above results indicated that this HPLC method is simple, rapid and has a high degree of precision.

REFERENCES

1. R. Ballerini, A. Cambi, P. D. Soldato, F. Melani, and A. Meli, J. Pharm. Sci., 68, 366(1979)

2. R. Farinotti, and G. Mahuzier, J. Pharm. Sci., 68, 484 (1979)

3. W. O. Thomas, and R. T. Parfitt, J. Chromatogr. Biomed. Appl., 162, 122(1979)

4. A. Bannier, K. L. Brazier, and C. Quiny, J. Pharm. Sci., 69, 763(1980)

5. B. C. Sallustio, A. Abas, P. J. Hayball, Y. J. Purdie, and P. J. Meffin, J. Chromatogr. Biomed. Appl., 374, 329 (1986)

6. S. Bjiorkman, J. Chromatogr. Biomed. Appl., 414, 465 (1987)

7. M. Lempiainen, and A. L. Makela, Int. J. Clin. Pharmacol. Res., 7, 265(1987)

8. R. T. Foster, F. Jamali, Drug Metab Dispos Jul-Aug., 16, 623(1988)

9. B. Floubat, A. Roux, Arzneimittelfotschung Jul., 39, 812 (1988)

10. B. W. Berry, F. Jamali, Pharm Res Feb., 5(2), 123(1988)

11. R. Mehvar, F. Jamali, Pharm Res Jan., 5, 53(1988)

Received: September 24, 1991
Accepted: October 16, 1991