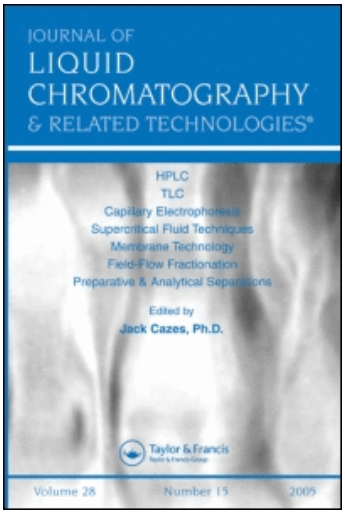


Journal of Liquid Chromatography & Related Technologies

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

Determination of Rebamipide in Human Plasma by HPLC

Min Kyo Jeoung[a]; Chang Soo Kim[a]; Nam Hee Kim[a]; Jin Tae Hong[a]; Youn-Bok Chung[a]; Youmie Park[a]; Kyoung Soon Kim[a]; Dong-Cheul Moon[a]

[a] College of Pharmacy, Chungbuk National University, Heungduk Gu, Cheongju, Korea

Online publication date: 27 May 2004

To cite this Article Jeoung, Min Kyo , Kim, Chang Soo , Kim, Nam Hee , Hong, Jin Tae , Chung, Youn-Bok , Park, Youmie , Kim, Kyoung Soon and Moon, Dong-Cheul(2004) 'Determination of Rebamipide in Human Plasma by HPLC', Journal of Liquid Chromatography & Related Technologies, 27: 12, 1925 — 1935

To link to this Article: DOI: 10.1081/JLC-120038778

URL: http://dx.doi.org/10.1081/JLC-120038778

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 27, No. 12, pp. 1925–1935, 2004

Determination of Rebamipide in Human Plasma by HPLC

Min Kyo Jeoung, Chang Soo Kim, Nam Hee Kim, Jin Tae Hong, Youn-Bok Chung, Youmie Park, Kyoung Soon Kim,* and Dong-Cheul Moon*

College of Pharmacy, Chungbuk National University, Heungduk Gu, Cheongju, Korea

ABSTRACT

A simple determination method of rebamipide in human plasma by using high performance liquid chromatography (HPLC) was developed. The method involves a single liquid–liquid extraction and reversed-phase chromatography with fluorometric detection (excitation, 320 nm; emission, 380 nm). Analytes were extracted from plasma samples that contain an internal standard (ofloxacin) into ethylacetate with a high yield after adjustment to pH 2–3. Separation was accomplished at 60°C on a reversed-phase column using a mobile phase of acetonitrile–water–acetic acid (30 : 70 : 5, v/v, pH 2.4), at a flow-rate of 1.0 mL/min. The linear range of the assay was 2–500 ng/mL of the drug in plasma and

*Correspondence: Kyoung Soon Kim and Dong-Cheul Moon, College of Pharmacy, Chungbuk National University, Gaeshin-Dong San 48, Heungduk Gu, Cheongju 361-763, Korea; E-mail: kyongsoon07@hanmail.net or dcmoon@chungbuk.ac.kr.

DOI: 10.1081/JLC-120038778 1082-6076 (Print); 1520-572X (Online)

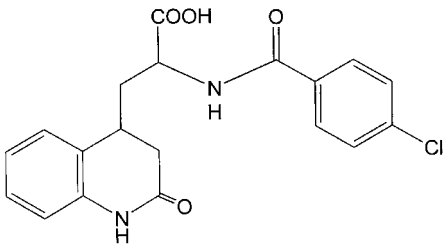
the limit of quantitation was 2.0 ng/mL. The intra- and inter-day relative standard deviation (RSD) were less than 10% and the accuracy of the assay was in the range of 97–104%. Analysis of the drug in human plasma indicates that the procedure can be carried out conveniently and quickly and, therefore, is suitable for obtaining pharmacokinetic profiles in human subjects after oral administration of different types of the drug.

Key Words: Rebamipide; HPLC; Ethyl acetate; Human plasma; Pharmacokinetics; Ofloxacin.

INTRODUCTION

Rebamipide [2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl] propionic acid], a novel quinolinon derivative, is a potent antiulcer agent with its main pharmacological actions being mediated by increasing endogenous prostaglandin synthesis and by scavenging the oxygen-derived free radicals, which play an important role in gastric mucosal cell damage [Fig. 1(a)].[1,2]

(a)



(b)

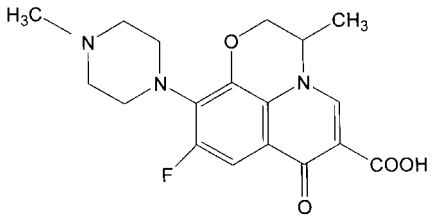


Figure 1. (a) The structure of rebamipide [2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl] propionic acid]. (b) The structure of ofloxacin [9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid].

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

The mechanism of its antiulcer action has been reported to involve increased mucous secretion,[3] enhanced generation of endogenous prostaglandin,[4] suppression of neutrophil function,[5] inhibition of inflammatory cytokines,[6] and scavenging oxygen free radicals.[7] These pharmacological actions have been confirmed by in vitro experimental studies to require concentrations of rebamipide of more than 10^{-6}–10^{-4} M.[3–7]

To date, only a few reports are available for the analysis of rebamipide in human plasma by using reversed-phase high performance liquid chromatography (RP-HPLC).[8–12] However, previous analytical methods have entailed extensive sample preparation and use of an internal standard that is not easily obtainable; in spite of this, they are reported to be sensitive and selective. To overcome these sample preparation constraints, we developed a sample-preparation procedure that provides a more simple and rapid analysis, and requires minimal sample handling, using an internal standard (ofloxacin) that is commercially available [Fig. 1(b)]. Rebamipide was administered in four healthy volunteers at a single dose of one 100 mg tablet of a formulation. Blood samples were drawn off over a 12 hr period, before (time 0) and after each administration, at specific intervals. The plasma concentration of rebamipide was determined by the developed methods in the present study. The non-compartment method was used for pharmacokinetic analysis of rebamipide.

EXPERIMENTAL

Materials

Rebamipide (a standard grade, purity 99.0%) was acquired from the Goo-Ju Pharmaceutical Co., LTD (Seoul, Korea). Ofloxacin was purchased from Sigma (St Louis, MO). Acetonitrile and methanol were HPLC grade (Fisher Scientific Co., Fairlawn, NJ) solvents. Water was passed through Millipore (Bedford, USA), Milli-RO4, and Milli-Q water purification systems. Mobile phase was filtered through 0.45 μm membrane filter (Millipore, CA) and ultrasonically degassed prior to use. All other chemicals and solvents were of analytical-reagent grade, and were used without further purification. Micro-tube (2.0 mL) was purchased from Axygen (CA, USA).

Standard Solutions

Stock solutions of rebamipide (100 μg/mL) and internal standard ofloxacin (10 μg/mL) were freshly prepared in methanol and stored at 4°C. Standard

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

solutions were made by serially diluting the stock solutions in methanol to the required concentrations before use. Calibration standards were prepared with drug-free human plasma spiking with stock solutions, which have the final concentrations of rebamipide, 0 (blank), 2, 10, 50, 100, and 500 ng/mL, respectively, in plasma.

Sample Preparation (Extraction Procedure)

To 0.5 mL of human plasma in a 2 mL micro-tube, 10 μL of internal standard solution and 100 μL of 36% hydrochloric acid were added. Then, the plasma was briefly mixed with a vortex mixer and then 1 mL of ethylacetate was added to the sample mixture. The sample solution was vortex-mixed for 5 min, and centrifuged at 5000*g* for 1 min. The organic layer was transferred to another tube and evaporated to dryness using a speedvac concentrator. The sample residue was re-dissolved with 100 μL of freshly prepared mobile phase, and then 20 μL aliquot was injected onto the HPLC system for analysis.

Chromatography

The HPLC system consisted of a Jasco PU-980 pump, model FP-2020 plus fluorescence detector (λ_{ex} 320 nm, λ_{em} 380 nm) equipped with L-7200 autosampler (Hitachi, Tokyo, Japan), a column heater (CH-150, Eldex Lab. Inc., CA, USA), and Ds-CHROM chromato-integrator (Donam instruments Inc., Seoul, Korea). The chromatographic separation was performed using a Luna C18 analytical column (250 mm × 4.6 mm I.D., 5 μm, Phenomenex; Torrance, CA), preceded by a guard column (4 mm × 3 mm I.D.) packed with the same material (Phenomenex; Torrance, CA). Both the analytical and guard columns were kept at 60°C within a column heater. The mobile phase consisted of acetonitrile–water–acetic acid (30 : 70 : 5, v/v, pH 2.4) and was delivered at a flow-rate of 1.0 mL/min.

Extraction Recoveries

Extraction recoveries of rebamipide (0.1 μg/mL) from plasma samples were determined according to the pH changes of the plasma sample controlled together with extracting solvents. Each 0.5 mL of rebamipide solution (0.1 μg/mL) was added to the blank plasma samples (each, 0.2 mL) at five different pH (pH 2, 3, 4, 5, and 7). Five hundred micro-liters, each, of three

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

different solvents, ethylether, *n*-butanol, and ethylacetate were used to extract the analytes from test solutions. Extraction was performed by vortex-mixing for 5 min and centrifugation at 5000*g* for 1 min. The organic layer was transferred to another tube and 20 μL aliquots were injected directly into the HPLC. The extraction recoveries were calculated by comparing the peak area obtained from extracts of spiked plasma samples and the peak area from direct injection of known amounts (20 μL) of standard solutions of rebamipide (0.1 μg/mL).

Method Validation

Batches, consisting of four calibration standards at each concentration, were analyzed on four different days to complete the method validation. In each batch, QC samples at 10, 50, 100, and 400 ng/mL were assayed in sets of four replicates to evaluate the intra- and inter-day precision and accuracy.

Pharmacokinetic Study

The study was carried out on four healthy male volunteers, aged from 20 to 25 years. The protocol was previously approved by the Korean Food and Drug Administration (KFDA), and the volunteers signed a formal written consent to participate in the study. A physical and biological examination was carried out before and after completion of the study.

Rebamipide tablets (100 mg) were given after an overnight fasting with 200 mL of tap water. Food was allowed 4 hr (lunch) and 12 hr (dinner) after drug intake. After each administration, blood samples were drawn into venoject heparin-containing tubes, just prior to and up to 12 hr after administration. After centrifugation at 1700*g* for 10 min at 4°C, the plasma was deep frozen at −70°C until assayed.

A non-compartmental pharmacokinetic analysis was performed on plasma concentrations using PCNONLIN software.[13] Levels lower than the limit of quantitation were taken as zero. Maximum plasma concentration (C_{max}) and corresponding time to C_{max} (T_{max}) were obtained through direct observation of plasma concentration–time curves. The area under the plasma concentration–time curves, from time zero to time of the last quantifiable concentration (AUC_{0-last}), was calculated by the trapezoidal rule, whereas $AUC_{0-\infty}$ was calculated according to $AUC_{0-\infty} = AUC_{0-last} + C_{last}/\beta$, where β is the slope of the terminal phase of the plasma concentration curve using log-transformed concentrations, and C_{last} is the last concentration

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

higher than the limit of quantitation. Plasma half-life ($t_{1/2}$) was calculated using the formula $t_{1/2} = 0.693/k$.

RESULTS

Chromatography

Figure 2 shows typical chromatograms of a human plasma sample spiked with rebamipide (10 ng/mL) and internal standard (1.0 μg/mL) (B), and a drug-free human plasma sample (A). The retention times of rebamipide and internal standard were 8.2 and 12.9 min, respectively, and the total run time was within 15 min. Rebamipide was identified by comparing its retention time, spectral data, and quantified from the calibration curve based on peak height ratio of analyte to internal standard. The isocratic elution with the

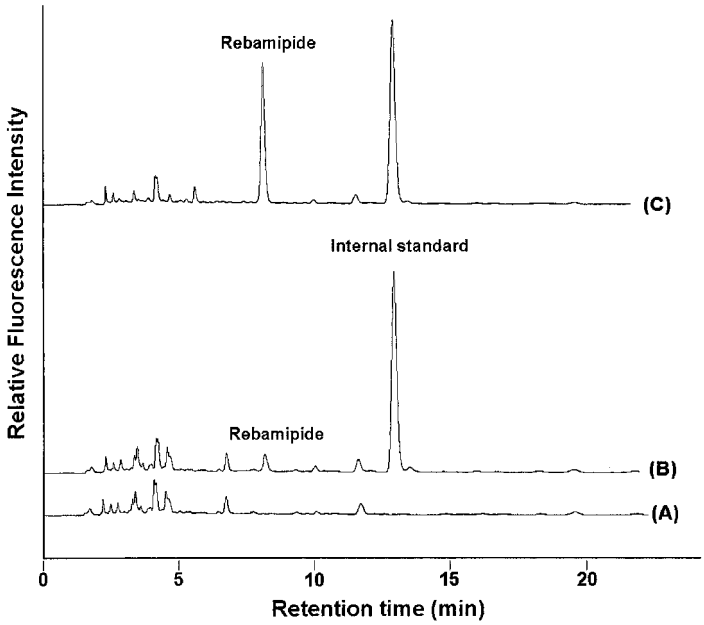


Figure 2. Chromatograms of human plasma containing rebamipide and internal standard (ofloxacin): (A) 0.5 mL of drug-free human plasma (blank plasma); (B) 0.5 mL of plasma containing 0.01 μg/mL of rebamipide and 1.0 μg/mL of ofloxacin; (C) 0.5 mL human plasma 8 hr after oral administration of one tablet with 100 mg rebamipide (104.77 ng/mL).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

mobile phase afforded good separation of rebamipide and internal standard from endogenous constituents in human plasma.

Extraction Recovery (Efficiency)

Two factors affecting the recovery of the drug, extracting solvents and the controlled pH of plasma sample, were investigated and optimized for the sample clean-up procedure. Rebamipide was totally extracted (above 99%) into butanol from solution, over the pH range 1–7, whereas in ethyl acetate 90% is extracted over the pH range 2–3, and very little into ethyl ether. Butanol caused not only emulsion formation during the extraction step, but was too difficult to remove from the extracts. Accordingly, ethyl acetate was selected as the best solvent for the practical purpose of extracting the drug that is stable, even at the lower pH from the acidified sample solution (pH 2–3).

Table 1 showed that the extraction recoveries ranged from 87.95% to 95.93% with their CV values of 5.72–12.12%, in four different concentrations between 10 and 500 ng/mL of rebamipide.

Linearity

The calibration curve constructed for the determination of rebamipide exhibited good linearity by plotting the peak-height ratio of the analyte to internal standard against the concentration, 2.0–500 ng/mL in plasma ($y = 0.0191x - 0.0032$, $r = 0.9999$).

Sensitivity

The limit of detection (LOD) was determined to be 2 ng/mL, as defined by the concentration of the analyte giving a signal to noise ratio of 3 : 1. The

Table 1. Extraction recovery of the rebamipide in human plasma.

Rebamipide concentration added (ng/mL)	Recovery (%) ($n = 6$)	CV (%) ($n = 6$)
10	95.93	12.12
50	93.63	5.72
100	91.46	8.58
500	87.95	9.72

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

limit of quantification (LOQ) was 10 ng/mL as defined by the lowest concentration in linear range that can be detected with variation within 6.7%. The relative standard deviation (RSD) of seven replicate determinations was in the range, 1.86–7.43%.

Precision and Accuracy

The intra-day assay variations were determined by analyzing plasma samples spiked with 2, 10, 50, 100, 500 ng/mL of rebamipide. The inter-day assay variations were determined by analyzing plasma samples spiked with the same amount, in duplicates, on four separate days. In both cases, accuracy was within the range 97–123% over the concentrations investigated, and the RSD was less than 10% at any concentrations studied (Table 2).

Pharmacokinetic Study

The mean plasma profiles of rebamipide are shown in Fig. 3. The following mean pharmacokinetic parameters were found for healthy human. The mean terminal half-life, maximum plasma concentration (C_{max}), T_{max}, area under the plasma concentration–time curves from time zero to time of the last quantifiable concentration (AUC_{0-24}), and $AUC_{0-\infty}$ were 1.93 hr, 215 μg/mL, 1.5 hr, 1346 μg/mL/hr, and 1375 μg/mL/hr, respectively.

Table 2. Precision (CV) and accuracy (relative error) for determination of rebamipide in spiked plasma ($n = 7$).

Rebamipide added (ng/mL)	Concentration (ng/mL)	Found RSD (%)	Accuracy (%) [mean ± SD]
Intra-day assay ($n = 4$)			
10	9.94	6.70	99.48 ± 6.65
50	50.33	3.43	100.66 ± 3.46
100	97.77	4.02	97.77 ± 3.93
500	500.12	1.86	100.02 ± 1.86
Inter-day assay ($n = 4$)			
10	10.26	6.73	102.69 ± 6.91
50	51.14	3.45	102.28 ± 3.53
100	104.12	4.52	104.12 ± 4.70
500	500.78	7.43	100.15 ± 7.44

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

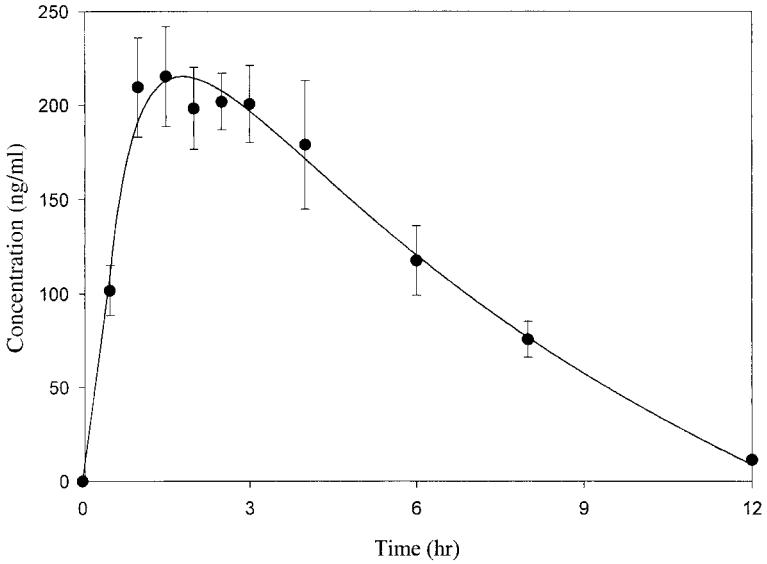


Figure 3. Rebamipide concentration in human plasma after oral administration of one tablet (100 mg). Each point represents the mean (SD) of four volunteers.

DISCUSSION

To date, there is no simple procedure for the determination of rebamipide in human plasma that can be applied to the pharmacokinetic studies of rebamipide, because sample pretreatment procedure is usually quite complicated and time-consuming. Therefore, the purpose of present study is to develop a simple and rapid HPLC method to determine rebamipide in human plasma. Sample pre-treatment steps were reduced and, thus, extraction recovery increased greater than 90% in the present method. Also, the detection limit with fluorescence detection was 2 ng/mL, which was 1000 times more sensitive than the previous report with UV detection.[10] With the advantages of simple extraction procedure and short analysis time, the present method would be applicable to pharmacokinetic studies of rebamipide after oral administration. We have described a simple, accurate, and precise HPLC method for the determination of rebamipide, within the plasma concentration range observed in the human plasma after its oral administration. It has been currently used in the laboratory for investigating the pharmacokinetics of rebamipide in humans. The present method has been applied successfully in a study of the pharmacokinetics of rebamipide, and these results will be presented elsewhere.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ACKNOWLEDGMENT

This study was supported in part by a National Research Laboratory (NRL) program (#MI-0302-00-0069) of the Ministry of Science and Technology, Republic of Korea.

REFERENCES

1. Arakawa, T.; Watanabe, T.; Fukuda, T.; Yamasaki, K.; Kobayashi, K. Rebamipide, a novel prostaglandin-inducer, accelerates healing and reduces relapse of acetic acid-induced rat gastric ulcer comparison with cimetidine. Dig. Dis. Sci. **1995**, *40*, 2469–2472.
2. Takemoto, T. Clinical evaluation of proamipide (OPC-12759) in gastric ulcer: a multicenter double-blind comparison study with cetraxate. J. Adult. Dis. **1989**, *19*, 127–153.
3. Ishihara, K.; Komura, Y.; Nishiyama, N.; Yamasaki, K.; Hotta, K. Effect of rebamipide on mucus secretion by endogenous prostaglandin-independent mechanism in rat gastric mucosa. Arzneim-Forsch/Drug. Res. **1992**, *42*, 1462–1466.
4. Yamasaki, K.; Kanbe, T.; Chijiwa, T.; Ishiyama, H.; Morita, S. Gastric mucosal protection by OPC-12759, a novel antiulcer agent, in the rat. Eur. J. Pharmacol. **1987**, *142*, 23–29.
5. Murakami, K.; Okajima, K.; Uchiba, M.; Harada, N.; Johno, M.; Okabe, H.; Takatsuki, K. Rebamipide attenuates indomethacin induced gastric mucosal lesion formation by inhibiting activation of leukocytes in rats. Dig. Dis. Sci. **1997**, *42*, 319–325.
6. Aihara, M.; Azuma, A.; Takizawa, H.; Tsuchimoto, D.; Funakoshi, Y.; Shindo, Y.; Ohmoto, Y.; Imagawa, K.; Kikuchi, M.; Mukaida, N.; Matsushita, K. Molecular analysis of suppression of interleukin-8 production by rebamipide in *Helicobater pylori* stimulated gastric cancer cell lines. Dig. Dis. Sci. **1998**, *43*, 174S–180S.
7. Yoshikawa, T.; Naito, Y.; Kondo, M. Free radical scavenging activity of the novel anti-ulcer agent rebamipide studied by electron spin resonance. Arzneim-Forsch/Drug. Res. **1993**, *43*, 363–366.
8. Yoshihide, S.; Takefumi, S. High performance liquid chromatographic procedure for the determination of a new anti-gastric ulcer agent, 2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propionic acid, in human plasma and urine. J. Chromatogr. **1988**, *434*, 283–287.
9. Zhao, R.; Duan, J.; Yan, B. Determination of rebamipide in plasma by HPLC and its pharmacokinetic study. Zhongguo Yaoxue Zazhi. **1999**, *34*, 369–399.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

10. Zu, L.; Li, L.; Yu, D. Determination of rebamipide in plasma by high performance liquid chromatography. Zhongguo Yiyuan Yaoxue Zazhi. **2002**, *22*, 353–355.
11. Xu, H.; Zhou, Y.; Fu, Y. RP-HPLC determination of rebamipide in tablets. Yaowu Fenxi Zazhi. **2001**, *21*, 362–363.
12. Cho, H.Y.; Jeong, H.C.; Oh, I.; Moon, J.D.; Lee, Y.B. Bioequivalence of rebamipide tablet to mucosta tablet (rebamipide 100 mg). J. Kor. Pharm. Sci. **2001**, *31* (4), 281–287.
13. Metzler, C.M.; Weiner, D.L. PCNONLIN, SCI Software, Lexington, 1992.

Received January 24, 2004
Accepted February 29, 2004
Manuscript 6330

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!