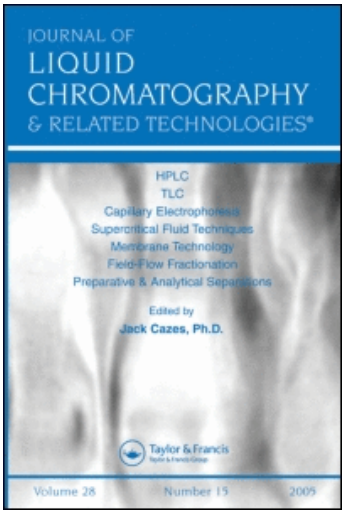

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SIMPLE DETERMINATION OF ERDOSTEINE IN HUMAN PLASMA USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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SIMPLE DETERMINATION OF ERDOSTEINE IN HUMAN PLASMA USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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□ *A simple and rapid high-performance liquid chromatography (HPLC) method with UV detection (220 nm) was developed for the determination of erdosteine in human plasma. The plasma was prepared by deproteination based on treatment with 6.25% trichloroacetic acid (TCA). After the mixture was centrifuged, the supernatant was separated on CAPCELL PACK C_{18}(4.6 × 250 mm) column. The mobile phase consisted of acetonitrile in phosphate-heptane sulfonate buffer at the volume ration of 5:95 (pH 2.0). Citiolone was used as an internal standard. The calibration curve was linear in concentrations of 0.5–8 μg/mL with correlation coefficient of 0.999. The limit of quantitation (LOQ) was 0.5 μg/mL. This method was sensitive with reproducibility and specificity and successfully applied to the bioequivalence study of erdosteine (900 mg) in healthy subjects.*

Keywords bioequivalence, erdosteine, HPLC, UV

INTRODUCTION

Erdosteine, ±-{[2-oxo-2[(tetrahydro-2-oxo-3-thienyl)amino]ethyl]thio}-acetic acid, is a potent mucolytic and mucoregulator agent which contains

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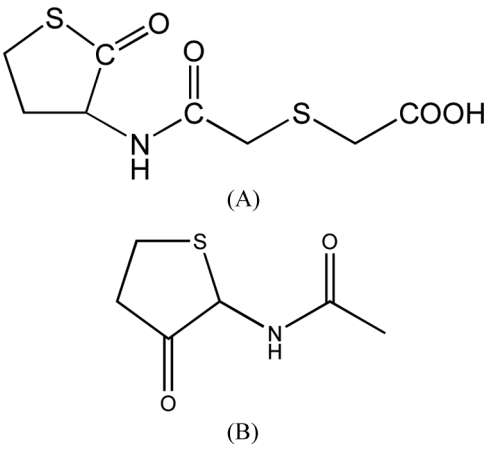

FIGURE 1 Chemical structures of erdosteine (A) and citiolone (B).

both a carboxyl group and a thiolactone ring in the structure (Figure 1).[1–3] It is important as free radical scavenger and antioxidant because two sulfhydryl groups of the drug have reducing potential.[4,5] The mucokinetic-mucolytic properties as well as the antitussive effect of erdosteine have been reported.[6] Also, this has a therapeutic application of erdosteine in preventing oxidative lung disease caused by the elastase inhibitory capacity of a 1-antitrypsin induced by cigarette smoke.[7,8]

In a recent study, erdosteine, which appeared to be the most active drug[9,10] and its optical active metabolites were analyzed by the liquid chromatography-mass spectrometry (LC-MS).[11] LC-MS with selective ion monitoring is not easily adapted to pharmacokinetic studies, because the instrument is quite expensive and it requires a specific technique to operate. Also, reproducibility of LC-MS is lower than HPLC, whose instrument, column, and method development are compensating for weak points such as lower sensitivity and higher sample requirement. Also, LC-MS/MS is too expensive to use for routine measurements in the preclinical laboratory.[12–14]

Accordingly, it is harder to adapt to this study as it has to be reproducible and treat the bioequivalence study. Therefore, to be effective, a simple analysis method with routine equipment is needed.

The aim of this study was to develop a simple, rapid, and robust analytic procedure for determination of erdosteine using a HPLC system. The signals were monitored with UV detection at 220 nm. This method was applied to perform a bioequivalence study of two capsule formulations of erdosteine in 24 healthy volunteers.

EXPERIMENTAL

Chemicals and Reagents

Standard erdosteine (Figure 1A) was obtained from Hanmi Pharmaceutical Co. Ltd. (Seoul, Korea). Citiolone (Figure 1B), as an internal standard, was obtained from Sam-il Pharm. Co. Ltd. (Seoul, Korea). Eldos® capsules (300 mg), as a reference, were obtained from Daewoong Pharmaceutical Co. Ltd. (Seoul, Korea). As a test drug, erdosteine capsules (300 mg) made by Hanmi Pharmaceutical Co. Ltd. were used. Potassium phosphate monobasic, 1-heptanesulfonic acid, and 6.25% trichloroacetic acid were purchased from Sigma (St. Louis, MO, USA). HPLC grade phosphoric acid (85%) and acetonitrile were purchased from J. T. Baker (Phillipsburg, NJ, USA) and all other chemicals were analytic grade and used without further purification.

Preparation of Standard Solution

Stock solutions (1 mg/mL) of erdosteine and citiolone as an internal standard were prepared by dissolving them in methanol at −70°C. Standard solutions of erdosteine in human plasma were prepared from stock solutions by spiking the suitable volume (>10 μL/mL) of various diluted stock solutions for daily calibration.

Sample Preparation

Each 4 μL of internal standard solution, citiolone (500 μg/mL), was spiked to 0.4 mL of plasma in a glass tube, then each tube was stirred and 120 μL of trichloroacetic acid solution (6.25%) was added for deproteination. Each tube was shaken for 1 min then placed at room temperature. After 10 min, the mixtures were centrifuged at 10,000 rpm for 10 min and then filtered. The supernatant was transferred to the autosampler vial. Then, 30 μL of supernatant was injected into the chromatographic system.

Instrument and Chromatography

Analyses were performed using following HPLC system equipped with a LC-10AS pump (Shimadzu, Japan), SPD-10A UV/VIS detector (Shimadzu), and Waters™ 717 plus Autosampler (Waters, Milford, MA, USA).

The CAPCELL PAK C_{18} MG (4 × 250 mm) was employed. The plasma samples were separated by isocratic elution of the mobile phase consisted of acetonitrile (ACN) and buffer mixed potassium phosphate (0.68 g/L) and 1-heptane sulfonic acid (1.01 g/L), pH 2.0. ACN, and buffer described

previously at a volume ration of 5:95. The flow rate was 0.8 mL/min at room temperature. The eluents were monitored with UV detector at 220 nm. The HPLC system was controlled with dsChrom software (Donam Int., Seoul, Korea).

Calibration Curve and Assay Validation

The stock solution of erdosteine (1 mg/mL) was diluted with drug free plasma for the calibration standards at concentration of 0.5, 1, 2, 4, and 8 μg/mL.

The specificity and sensitivity of this assay was determined on the basis of different plasma sample. The interference of endogenous compounds was assessed by analysis of their given plasma samples. The lower limit of quantitation (LOQ) was the lowest non-zero concentration level which could be accurately and reproducibly quantitated.

The linearity was determined by a calibration curve in the concentration ranges of 0.5–8 μg/mL. The calibration curve was plotted versus the ratio of erdosteine concentration to that of citiolone as an internal standard. Peak area ratio of erdosteine to citiolone was detected and the calibration curve was obtained from the least square linear regression presented with the correlation coefficient. This regression line was applied to calculate the respective concentrations of erdosteine in the 24 healthy volunteers.

The precision, defined as the coefficient of variation, and the accuracy (%), defined as the bias between added and calculated concentration, of method were evaluated for five times. Inter- and intra-day precision were evaluated for 5 concentrations described previously, corresponding to quality samples of the method. Accuracy was determined by comparing the calculated concentration using calibration curves to known concentrations.

Bioequivalence Study

After overnight fasting, 24 healthy adult males taking no concurrent medications or alcohol were orally administered erdosteine capsules (900 mg); half of the volunteers received the referenced-erdosteine and the second half of the volunteers received the tested-erdosteine in the first week, then vice versa in the second week. Volunteers were divided into two groups according to a randomization table. Investigations were carried out using a double blind test in a cross manner with the lapse of a week. The blood samples were obtained after dosing at twelve time-intervals: 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, and 7 h. The plasma samples were stored at −70°C until assayed and prepared with the method describe previously.

Pharmacokinetic Application and Statistical Analysis

The pharmacokinetic parameters were assessed for 7 hr; the area under the plasma concentration-time curves of erdosteine measured (AUC_{0-7h}), the maximum plasma concentration (C_{max}), and time to reach the C_{max} (T_{max}) were calculated directly from the plasma concentration-time plot by visual expectation.

For the analysis of the bioequivalence study, a two-way ANOVA performed with K-BE Test 2002 program was used.[15] Bioequivalence of test and reference treatment was assessed on the basis of the confidence intervals for the test/reference mean ratios of these raw variables in the relation to the bioequivalence range of 80 to 120% for raw data.

RESULTS AND DISCUSSION

Specificity and Sensitivity

After the deproteination step, the chromatograms of erdosteine in human plasma were shown in Figure 2. The retention time for citiolone as an internal standard (IS) and erdosteine were about 9.9 and 19 min, respectively. There were no peak-interferences between erdosteine and IS at their retention times in the blank and sample prepared, and the resolution between erdosteine and surrounding peak was sufficient for good qualitative and quantitative analysis. The limit of quantitation (LOQ) was 0.5 μg/mL.

Linearity

The calibration curve has linearity in the studied range. The equation of the calibration curve consisting of 5 points was $y = 0.1901(\pm 0.004)x - 0.0532(\pm 0.023)$ with correlation coefficient $R^2 = 0.999$, where x and y represent the concentration of erdosteine and the peak-ratio of erdosteine, respectively.

Precision and Accuracy

The precision and accuracy of the assay are listed in Table 1. The accuracy deviated from 92.99% to 105.97%, with a theoretical value between 80 and 120%. This assay was precise because the precision value was less than 20%, and the ratio of signal to noise at low concentration was better than 10. This assay was successfully applied to the bioequivalence experiment. Moreover, the results showed that this simple, rapid, and robust method is sufficiently sensitive to accurately follow human plasma concentrations of erdosteine.

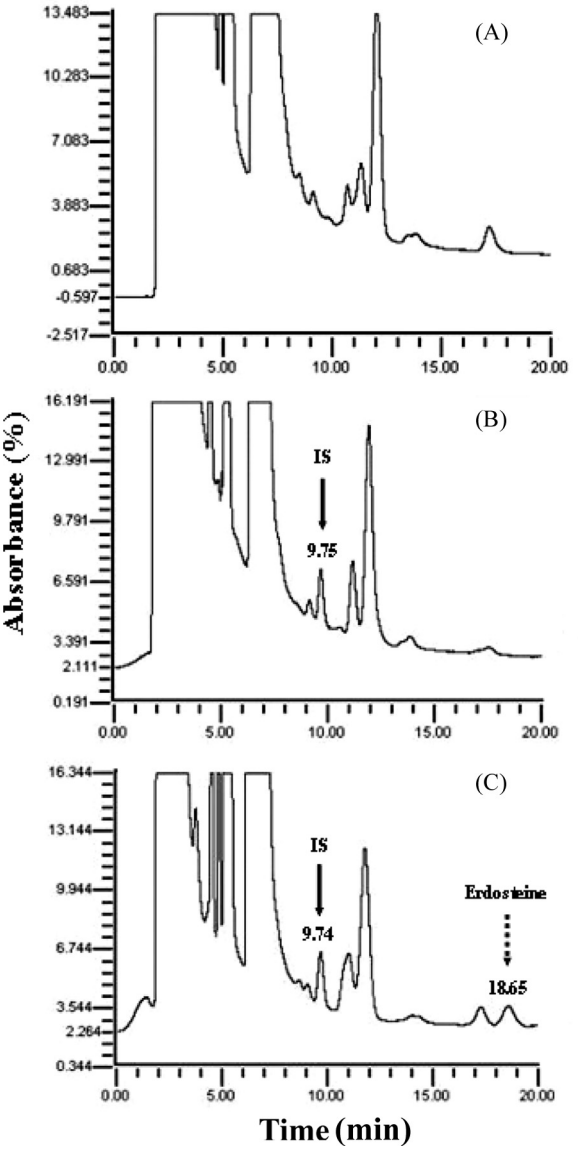

FIGURE 2 HPLC chromatogram of erdosteine in human plasma. (A) Blank human plasma, (B) blank plasma spiked with internal standard (100 μg/mL) and (C) both blank plasma with erdosteine (4 μg/mL) and internal standard were represented.

Bioequivalence of Erdosteine

The proposed method was applied to a determination of two erdosteine capsules (reference and test treatment) in human plasma samples. Plasma samples of 24 healthy volunteers were periodically collected for 7 hr after

TABLE 1 Reproducibility of Erdosteine in Human Plasma (n = 5)

Concentration (μg/mL)	Precision (%)		Accuracy (%)
	Intraday	Interday	
0.5	19.9	12.8	106.0
1	6.8	12.3	103.0
2	11.7	4.7	93.0
4	5.8	2.6	102.9
8	1.7	3.0	99.7

TABLE 2 Pharmacokinetic Parameters of Erdosteine in Plasma of 24 Healthy Human Subjects After Oral Administration of 900 mg Erdosteine Capsules

Parameter	Erdosteine	
	Reference	Test
[a]AUC_{0-7h} (μg · hr/mL)	5.01 ± 2.08	4.68 ± 2.31
[b]C_{max} (μg/mL)	3.10 ± 1.42	2.69 ± 1.01
[c]T_{max} (hr)	1.18 ± 0.35	1.20 ± 0.36

[a]AUC: area under the plasma concentration versus time curve.
[b]C_{max}: peak plasma concentration.
[c]T_{max}: time to reach C_{max}.

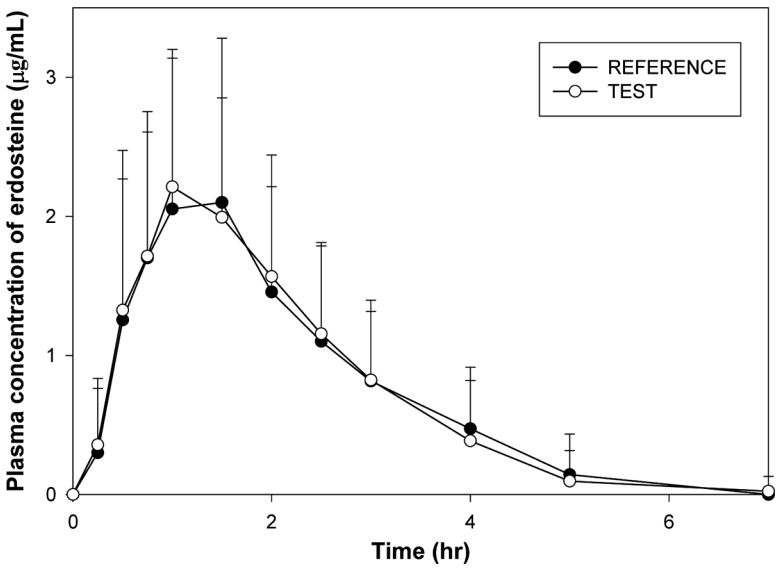

FIGURE 3 Mean plasma concentrations versus time plot of erdosteine in human subjects after oral administration of 900 mg erdosteine. Each point with vertical bar represents the mean and standard deviation of 24 healthy volunteers.

oral administration of erdosteine capsules (900 mg dose) to each volunteer. As a result, pharmacokinetic data were obtained as seen in Table 2. The T_{max} of reference and test were reached at 1.14 ± 0.40 and 1.20 ± 0.36 h. The C_{max} were at 2.82 ± 1.29 and 2.75 ± 1.08 μg/mL. The AUC_{0-7h} was at 4.72 ± 1.84 μg · h/mL and 4.79 ± 1.97 μg · h/mL, respectively.

The mean plasma concentration versus time plot of erdosteine following an oral administration of erdosteine (900 mg) to human subjects was shown in Figure 3. The profiles both referenced and tested erdosteine and were increased until T_{max} after oral administration, thereafter the plasma level declined. In this profile, the degree of decline might be related to a relatively short half-life.

CONCLUSION

Simple and rapid HPLC method with UV detection was established for treatment of a large amount of human plasma samples. This method was validated in aspect of specificity, sensitivity, linearity, and reproducibility as well as suitable for the bioequivalence study of erdosteine in human plasma.

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