

Stability indicating HPLC determination of Erdosteine in bulk drug and pharmaceutical dosage form

Md. M.G. Khan, P.S. Jain*, A.A. Shirkhedkar, R.A. Fursule, N. K. Kale, S. J. Surana

Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dhule (M.S.) 425 405 India

Received on 25th Jul 2013

Accepted on 14th Aug 2013

Available online from 24th Aug 2013

Abstract

A novel stability-indicating high-performance liquid chromatographic assay method was developed and validated for quantitative determination of erdosteine in bulk drugs and in pharmaceutical dosage form. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using an Ace5-C18 (250×4.6 mm, 5 μm) advance chromatography column, and 10 mmol L⁻¹ acetonitrile and Phosphate Buffer (35:65 v/v) as a mobile phase. The detection was carried out at a wavelength of 236 nm. The Erdosteine was subjected to stress conditions of hydrolysis (acid, base), oxidation, photolysis and thermal degradation. Degradation was observed for erdosteine in base, in acid and in 30% H₂O₂. The drug was found to be stable in the other stress conditions attempted. The degradation products were well resolved from the main peak. The percentage recovery of erdosteine was from (99.78 – 101.25 %) in the pharmaceutical dosage form. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, specificity and robustness. The forced degradation studies prove the stability indicating power of the method.

Keywords: Erdosteine, HPLC, validation, stability, degradation.

INTRODUCTION

Erdosteine is chemically (erdotin; (+)-1S-(2- [N-3- (2-oxotetrahydrothienyl)aceta-mido]thioglycolic acid and is official in Martindale – The Extra Pharmacopoeia and Merck Index. It is used as a Mucolytic modulator, anti-oxidant[1,2]. Erdosteine is used in combination with other antiretroviral agents as part of an expanded post exposure prophylaxis regimen to prevent HIV transmission for those exposed to materials associated with a high risk for HIV transmission. It is a white to yellowish white powder, slightly soluble in water, soluble in methanol and chloroform and route of administration is orally.

Various methods as, high performance liquid chromatographic determination of Erdosteine and its optical active metabolites utilizing a fluorescent chiral tagging reagent-(-)-4-(N,N-dimethylaminosulfonyl)-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole using Acetonitrile Water containing 0.1% TFA as a mobile

phase[3]. Quantification of drug in low volume of dog plasma by liquid chromatography tandem mass spectroscopy[4]. Sensitive determination of Erdosteine in human plasma by using solid phase extraction[5]. LC-MS/MS RP-HPLC determination of Erdosteine done in human plasma and its pharmacokinetic studies[6]. Simple spectrophotometric determination was carried out[7]. Sensitive simultaneous determination of cefixime and erdostein by ultra violet spectroscopic technique[8] and high performance thin layer chromatographic method[9] were reported. Till date, no stability-indicating HPLC assay method for the determination of erdosteine is available in the literature. It was felt necessary to develop a stability indicating liquid chromatography (LC) method for the determination of erdosteine in bulk drug and pharmaceutical dosage form and separate the drug from the degradation products in the force degradation studies[10,11]. Therefore, the aim of the present study was to develop and validate a

*Corresponding author E - mail: pritash79@yahoo.com

stability-indicating HPLC assay method for erdosteine as bulk drug and in pharmaceutical dosage form as per ICH guidelines[11].

MATERIALS AND METHODS

Material and reagents: Erdosteine bulk drug (purity 99.8) and tablet erdosteine (100 mg) were obtained from Glenmark Pharmaceuticals (Maharashtra, India). Hydrochloric acid and sodium hydroxide pellets were obtained from Rankem Laboratories India. Acetonitrile, potassium dihydrogen phosphate, o-phosphoric acid was obtained from Merck Specialities Private Ltd. Hydrogen peroxide is obtained from Fischer Scientific, India. All chemicals used are of HPLC grade. Milli-QWater was used throughout the experiment.

Chromatographic conditions

The HPLC system used was a Shimadzu 2010CHT system equipped with a photodiode array detector. A chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with octadecyl silane Ace5-C18 (Advance Chromatography Technology, USA) stationary phase with particle size 5 μ m were used. The instrumental setting was at a flow rate of 1 mL min⁻¹. The injection volume was 20 μ L. The detection wavelength was 232 nm.

Mobile phase

The mobile phase consisted of acetonitrile: phosphate buffer in the ratio (35:65 v/v). The pH 3.2 of mobile phase is adjusted with o-phosphoric acid in double distilled water. The mobile phase was premixed and filtered through a 0.45 μ m membrane nylon filter and degassed.

Preparation of standard stock solutions

All solutions were prepared on a weight basis and solution concentrations were also measured on weight basis to avoid the use of an internal standard. Standard solution of erdosteine was prepared by dissolving the drugs in the diluents and diluting them to the desired

concentration. Diluent A was composed of acetonitrile and diluent B was composed of phosphate buffer in the ratios of (35:65 v/v). Approximately 5 mg of erdosteine was accurately weighed, transferred in a 50 mL volumetric flask, dissolved and diluted to 50 mL with the diluent A. From these stock solutions 2 mL of erdosteine standard solution were transferred in a 10 mL volumetric flask and diluted with diluent B. This final solution contained 20 μ g mL⁻¹ of erdosteine.

Sample solution (tablets)

Ten tablets of erdosteine (100 mg) (Erdozet 100) were finely ground using agate mortar and pestle. The ground material, which was equivalent to 10 mg of the active pharmaceutical ingredient, was extracted into diluent A in a 25 mL volumetric flask by vortex mixing followed by ultra sonication. The Take 2 mL of it taken and diluted to 40 mL with diluent B. The solution was filtered through a 0.45 μ m membrane nylon filter and an appropriate concentration of sample (20 μ g mL⁻¹ assay concentration) was prepared at the time of analysis.

Procedure for forced degradation study

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light and enables recommendation of storage conditions, retest periods, and shelf life to be established. The two main aspects of drug product that play an important role in shelf-life determination are assay of the active drug and the degradation products generated during stability studies.

Preparation of sample solution

Acidic degradation: The 5 mg drug was dissolved in the 5 mL of the diluent A. 50 mL of the 3 mol L⁻¹ hydrochloric acid was added to it. The solution was kept for 1 h. 10 mL of solution was taken from it and neutralized with 3 mol L⁻¹ sodium hydroxide. Then the solution was diluted

with diluent B to prepare working solution of 20 $\mu\text{g mL}^{-1}$ (pH of solution was 2.3).

Alkaline degradation: The 5 mg drug was dissolved in the 5 mL of the diluent A. 50 mL of the 0.5 mol L⁻¹ sodium hydroxide was added to it. The solution was kept for 1 h. 10 mL of solution was taken from it and neutralized with 0.5 mol L⁻¹ hydrochloric acid. Then the solution was diluted with diluent B to prepare working solution of 20 $\mu\text{g mL}^{-1}$ (pH of solution was 14).

Oxidative degradation: The 5 mg drug was dissolved in 5 mL of diluent A and 50 mL of 30% H₂O₂ was added. The solution was kept for 4 h. Then the solution was diluted with diluent B to prepare working solution of 20 $\mu\text{g mL}^{-1}$.

Thermal degradation: 10 mg drug was kept in the hot air oven for 48 h at 100°C temperature. Then the working solution was prepared using diluent A and diluent B.

Photo degradation: 10 mg of drug is exposed to the short wavelength (254 nm) and long wavelength (366 nm) UV light for 48 h. Then the working solution was prepared using diluent A and diluent B.

Validation of method: The proposed methods were validated as per ICH guidelines[11].

Linearity and range: The linearity of the response of the drug was verified at seven concentration levels, ranging from 10 to 150% of the targeted level (20 $\mu\text{g mL}^{-1}$). Concentration standard solutions containing 2-30 $\mu\text{g mL}^{-1}$ of erdosteine in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area versus the concentration data and were treated by least-squares linear regression analysis.

Sensitivity: The limit of detection (LOD) and limit of quantification (LOQ) were determined by calibration curve method.¹¹ Specific calibration curve was constructed using samples containing the analytes in the range of LOD and LOQ. LOD and LOQ were calculated by using the following equations. $\text{LOD} = 3.3S_a/b$, $\text{LOQ} =$

$10S_a/b$. where S_a is the standard deviation of the calibration curve and b is the slope of the calibration curve.

Precision: Assay of method precision (intra-day precision) was evaluated by carrying out six independent assays of erdosteine test samples against reference standard, the percentage of RSD of six assay values obtained was calculated. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, different HPLC systems and different days in the same laboratory (Table 1).

Table 1: Result of precision of test method

Sample number	Assay of erdosteine as % of labeled amount	
	Analyst 1 (intra-day precision)	Analyst 2 (inter-day precision)
1	99.97	99.75
2	99.87	99.40
3	99.98	99.23
4	99.56	99.31
5	99.45	99.43
6	99.68	99.13
Mean	99.75	99.45
RSD	0.12	0.101

Accuracy: Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80, 100 and 120% of the label claim of the tablet (100 mg of erdosteine). The recovery samples were prepared in the aforementioned procedure, and then 5 mL of erdosteine solution were transferred into a 50 mL volumetric flask and diluted to volume with diluent B. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve.

Specificity: Specificity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradation products,

matrix, etc.[11]. The specificity of the developed HPLC method for erdosteine was carried out in the presence of its degradation products. Stress studies were performed for erdosteine bulk drug to provide an indication of the stability indicating property and specificity of the proposed method.

RESULTS AND DISCUSSION

The primary target in developing this stability indicating HPLC method is to achieve the resolution between erdosteine and its degradation products. To achieve the separation of degradation products we used a stationary phase C-18 and combination of mobile phase 10 mmol L⁻¹ acetonitrile with phosphate buffer. The separation of the degradation product and erdosteine was achieved on Ace5 octadecyl silane C-18 stationary phase and 10 mmol L⁻¹ acetonitrile and phosphate buffer (35:65 v/v) as a mobile phase. The tailing factor obtained was less than two and retention time was about 3.3 min for the main peak and less than 4 min for the degradation products, which would reduce the total run time and ultimately increase the productivity thus reducing the cost of analysis per sample. Forced degradation study showed the method is highly specific and entire degradation products were well resolved from the main peak. The developed method was found to be specific and method was validated as per international guidelines.

Degradation was not observed for erdosteine samples during stress conditions like heat, UV and light, except in base, acid and oxidation. Erdosteine was degraded into acid (Figure 2), base (Figure 3) and oxidation (Figure 4) and forms polar impurities. In the acidic condition 5.65%, in the basic condition 8.56% after 1 h and in the oxidative condition 59.60% after 4 h, degradation was observed for erdosteine. Peak purity results greater than 990 indicate that the erdosteine peak is homogeneous in all stress conditions tested. The unaffected drug assay of erdosteine in the tablets confirms the stability indicating power of the method (Table 2).

TABLE 2: Summary of forced degradation results

Stress conditions	Time (Hrs)	Assay of active Substance	Degradation (%)	Peak purity ^a
Acid hydrolysis (3 mol L ⁻¹ HCl)	1	87.69	5.65	999
Base hydrolysis (0.5 mol L ⁻¹ NaOH)	1	89.89	8.56	999
Oxidation (30% H ₂ O ₂)	4	39.08	59.60	999
Thermal (100°C)	48	99.90	No degradation	999
Photo	48	99.55	No degradation	999

The contents of drug in tablets were determined by the proposed method using the calibration curve and % assay determined was 99.44% – 101.68%. The precision of the method was determined by calculating % RSD value and for both intra-day and inter-day; the value is within the acceptance limit (Table 1).

The recovery values for erdosteine ranged from 99.78 to 101.25%. The average recoveries of three levels for erdosteine were 100.02% (Table 3).

Table 3: Results of recovery tests of erdosteine

Level of addition (%)	Amount added (µg)	Recovery (%)	Average recovery (%)
80	4.8	98.78	
100	6	99.99	100.34
120	7.2	101.25	

The equation of the calibration curve for erdosteine obtained $y = 28074 X + 4287$, the calibration graphs were

found to be linear in the aforementioned concentrations. The coefficient of determination is 0.9995.

The LOD and LOQ for erdosteine in LC were 0.44 and 0.95 $\mu\text{g mL}^{-1}$, respectively. Precision at limit of quantification and limit of detection was checked by analyzing six test solutions prepared at LOQ and LOD levels and calculating the percentage RSD of area.

CONCLUSION

The developed method is stability indicating and can be used for assessing the stability of erdosteine bulk drugs and pharmaceutical dosage form. The developed method is specific, selective, robust, rugged and precise. This method can be conveniently used for assessing stability assay of selected substances and dissolution of tablets containing erdosteine in quality control laboratory. The study showed that the drug is stable for the thermal and photo degradation conditions where as moderately degraded in acid (5.65%) and base (8.56%) conditions but highly degraded in the oxidative (59.60%) conditions.

ACKNOWLEDGEMENTS

The authors thank the Principal, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur for providing the facilities to carry out the research work.

REFERENCES

1. Sweetman SC, Martindale-The Complete drug reference, 29th Edn., the pharmaceutical Press: Council of Royal Pharmaceutical Society of Great Britain; 2006, 920.
2. Budavari S, The Merck Index, 12th Edn, Merck and Co., White House station: USA; 1996, 624.
3. Norikazu M, Toshimasa TO, Kazuo Y. Satoshi, K. High-performance liquid chromatographic determination of erdosteine and its optical active metabolite utilizing a fluorescent chiral tagging reagent, *R*-($-$)-4-(*N,N*-dimethylaminosulfonyl)-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole. *J chromatogr B* 1998; 719: 177-189.
4. Liu D, Wang Y, Tang Y, Chen X, Zhong D. Quantification of Erdosteine in low volume of Dog Plasma by Liquid Chromatography tandem Mass spectroscopy *Chem Res Chin* 2007; 23: 736-741.
5. Hohyun K, Kyu YC, Hee JL, Sang BH, Kyung RL. Sensitive determination of Erdosteine in human plasma by use of automated 96-well Solid Phase extraction and LC-MS/MS. *J Pharm Biomed Anal* 2004; 34: 661-69.
6. Liu H, Wang BJ, Yaun GY. RP-HPLC determination of erdosteine in human plasma and its pharmacokinetic studies. *J Pharm Anal* 2007; 27: 1540-43.
7. Nanda RK, Gaikwad J, Prakash A. Spectrophotometric estimation of erdostein in pharmaceutical dosage form. *Int J Pharm Tech Res* 2009, 3, 492.
8. Dhoka MV, Gawande VT, Joshi PP, Gandhi SV, Patil NG. Simultaneous estimation of Cefixime and Erdostein in bulk and pharmaceutical dosage form by spectrophotometric method. *Hind Antibiot Bull* 2009; 51: 29-32.
9. Mhaske DV, Dhaneshwar SR. High-performance Thin-Layer Chromatographic method for determination of erdostein in pharmaceutical dosage forms. *Acta Chromatogr* 2007; 19: 170-184.
10. Singh S, Bakshi M. Development and validated stability-indicating assay method- A critical review. *J Pharm Biomed Anal* 2002; 28: 1011-1040.
11. <http://private.ich.org/cache/compo/363-272-1.html>, accessed in September 15, 2009.