ACTA CHROMATOGRAPHICA, NO. 19, 2007

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHIC METHOD FOR DETERMINATION OF ERDOSTEINE IN PHARMACEUTICAL DOSAGE FORMS

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SUMMARY

A sensitive, selective, precise, and stability-indicating method for quantitative analysis of erdosteine, in the presence of its degradation products, both as the bulk drug and in a formulation has been established and validated. High-performance thin layer chromatography (HPTLC) on aluminium-backed silica gel 60 F_{254} plates with toluene–methanol–acetone–ammonia 3.5:3.5:2.5:0.05 (*v*/*v*) as mobile phase was followed by densitometric measurement at 254 nm. This system was found to give compact bands for erdosteine (R_F 0.45 ± 0.02). The method was validated in accordance with ICH guidelines There was no chromatographic interference from capsule excipients. Erdosteine was subjected to acid and alkaline hydrolysis, oxidation, dry heat, wet heat, and UV degradation. The drug is degraded by acid and alkaline hydrolysis, oxidation, and UV irradiation. The drug was found to be stable under wet and dry heat conditions. Because the method could effectively separate the drug from its degradation products it can be regarded as stability-indicating.

INTRODUCTION

Erdosteine (erdotin; (+)-1*S*-(2-[*N*-3-(2-oxotetrahydrothienyl)acetamido)thioglycolic acid; Fig. 1 [1]) is a thiol derivative developed for treatment of chronic obstructive bronchitis, including acute infective exacerbation of chronic bronchitis.

Erdosteine contains two blocked sulfhydryl groups which are released after first-pass metabolism. The three active metabolites have mucolytic and free radical-scavenging activity. Erdosteine modulates mucus produc-

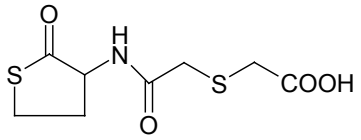

Fig. 1

The chemical structure of erdosteine

tion and viscosity and increases mucociliary transport, thereby improving expectoration [2]. It is registered as expectorating and for the treatment of acute exacerbations of chronic bronchitis in adults, and for the treatment of lower respiratory infections in children above the age of 2 years in 7 to 10 days [3]. It also suppresses the chemical stimulation-induced cough reflex and plasma leakage into the airways. Erdosteine is used for treatment of acute and chronic bronchitis on the basis of its unique mechanism of action – muco-modulatory activity, antibacterial activity, anti-inflammatory activity, and antioxidant activity [4].

Erdosteine and its optical active metabolite has been analysed by high-performance liquid chromatography using a fluorescent chiral tagging reagent [5]. HPLC with on-line mass spectrometric detection has been used in a preliminary study to elucidate the metabolism of erdosteine [6]. Sensitive determination of erdosteine in human plasma has been achieved by automated 96-well solid-phase extraction and LC–MS–MS [7]. As far as we are aware no HPTLC methods for stability-indicating chromatographic determination of erdosteine in pharmaceutical dosage forms have been reported in literature.

The International Conference on Harmonization (ICH) guideline entitled “Stability Testing of New Drug Substances and Products” requires stress testing to be conducted to elucidate the inherent stability characteristics of the active substance [7]. Susceptibility to oxidation is one of the tests required, as are hydrolytic and photolytic stability. An ideal stability-indicating method is one that quantifies the standard drug alone and also resolves its degradation products [8].

The objective of this work was to develop stability-indicating HPTLC method for determination of erdosteine in presence of its degradation products for assessment of the purity of the bulk drug and the stability of its dosage forms. The proposed method is simple, accurate, specific, repeatable, and stability-indicating, reduces the duration of analysis, and is suitable for routine determination of erdosteine. The method was validated in accordance with ICH guidelines [9,10] and their recent updates [11,12].

EXPERIMENTAL

Chemicals and Reagents

Pharmaceutical grade erdosteine was kindly supplied as a gift by Glenmark Pharmaceuticals, Nasik, India; it was certified to contain 99.85 % (w/w) on dry basis and was used without further purification. All other chemicals and reagents used were analytical grade and were purchased from Merck Chemicals, India.

A stock solution (1000 μg mL^{-1}) of erdosteine was prepared by dissolving 100 mg in 100 mL methanol. Standard solutions for calibration were prepared by dilution of the stock solution with methanol; the concentrations were such that amounts of erdosteine between 30 and 1000 ng were applied to the plates.

Chromatography

HPTLC was performed on 20 cm × 10 cm aluminium plates coated with 250-μm layers of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany; supplied by Anchrom Technologists, Mumbai). Before chromatography the plates were pre-washed by development with methanol and activated at 60°C for 5 min. Samples were applied to the plates as bands 6 mm wide and 6 mm apart by use of a Camag (Muttenz, Switzerland) Linomat IV applicator fitted with a 100 microlitre syringe (Hamilton, Switzerland). The rate of sample application was constant at 0.1 μL s^{-1}.

Linear ascending development with toluene–methanol–acetone–ammonia 3.5:3.5:2.5:0.05 (*v*/*v*) as mobile phase was performed, in the dark, in a Camag twin-trough glass chamber previously saturated with mobile phase vapour for 10 min at room temperature (25°C) and relative humidity of 60 ± 5% by lining the two largest sides with filter paper that had been soaked thoroughly in the mobile phase. The development distance was 9 cm and the development time approximately 30 min. The volume of mobile phase used for chromatography was 10 mL.

After development the plates were dried in a current of air by means of an air dryer in wooden chamber with adequate ventilation. The flow of air in the laboratory was maintained unidirectional (laminar flow, towards exhaust). Densitometric scanning at 254 nm in reflectance–absorbance mode was then performed with a Camag TLC Scanner III operated by CATS software (V 3.15; Camag). The slit dimensions were 5 mm × 0.45 mm and the scanning speed was 10 mm s^{-1}. The monochromator bandwidth was 20 nm. Each track was scanned three times and baseline correction was used.

The source of radiation was a deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm.

Calibration of the Method

Each calibration solution was applied to the plate six times and the plate was developed as described above. Peak areas were plotted against the corresponding concentrations to obtain the calibration graph.

Method Validation

Precision

The precision of the method was determined with the product, samples of which were accurately weighed and assayed. System repeatability was determined by performing six replicate analyses of each of three different amounts (300, 500, and 900 ng band^{-1}), measurement of peak area for the active compound, and calculation of relative standard deviation (*RSD*, %) and standard error (*SE*). Method repeatability was measured as the *RSD* obtained by repeating each assay six times on the same day (intra-day precision). Intermediate precision was assessed by sixfold assay of the three samples on each of two different days (inter-day precision).

Robustness

To measure robustness the experimental conditions were deliberately changed by small amounts and the effects on the results were examined. Chromatograms were obtained with mobile phases of composition toluene–methanol–acetone–ammonia 3.5:2.5:3.5:0.05, 3.5:3.0:2.5:0.05, and 3.5:3.5:2.5:0.05 (*v*/*v*). The amount of mobile phase, the temperature, and the relative humidity were also varied by ±5%. After prewashing of the plates with methanol they were activated at 60 ± 5°C for 2, 5, or 7 min before chromatography. The time from application to chromatography and from chromatography to scanning were also varied (0, 20, 40 and 60 min). The robustness of the method was measured for three different amounts of erdosteine – 300, 500, and 900 ng band^{-1}.

Limits of Detection and Quantitation

To estimate the limit of detection (*LOD*) and the lower limit of quantitation (*LLOQ*), blank methanol was applied six times and the band was chromatographed by following the same method. The signal to noise ratio (*S*/*N*) was determined. *LOD* was considered as 3 × *S*/*N* and *LLOQ* as 10 × *S*/*N*.

Specificity

The specificity of the method was ascertained by analysing drug standard and sample. The identity of the band for erdosteine in the sample was confirmed by comparison of the R_F and spectrum of the sample band with those from the standard. The peak purity of erdosteine was assessed by comparing spectra acquired at the peak start (S), peak apex (A), and peak end (E) positions of the band by use of the densitometer.

Recovery

Recovery was determined by applying the method to drug sample to which known amounts of erdosteine corresponding to 50, 100, and 150% of the label claim had been added (standard-addition method). Six analyses were performed for each amount added and the results obtained were compared with those expected.

Analysis of the Marketed Formulation

To determine the erdosteine content in conventional capsules (label claim 300 mg per capsule) the capsule contents were weighed. Powder equivalent to 300 mg erdosteine was extracted with methanol, the sample being sonicated for 30 min to ensure complete extraction of the drug. The volume of the extract was diluted to 100 mL and 1 μL of the resulting solution was diluted to 10 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the supernatant was analysed for drug content by applying 3 μL of the solution to a plate (600 ng band^{-1}) and analysis as described above. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

Accelerated Degradation of Erdosteine [13,14]

A stock solution containing 100 mg erdosteine in 100 mL methanol was prepared. This solution was used for forced degradation to provide an indication of the stability-indicating nature and specificity of the method. In all degradation studies the average peak area of erdosteine after application (500 ng band^{-1}) for HPTLC of seven replicates was obtained. To study the degradation of erdosteine by use of the HPTLC method most of the study was conducted by single development of the plate to prevent the movement of non-polar degradation products to the extreme end of the plate.

Acid and Base-Induced Degradation

HCl (0.1 M, 10 mL) or NaOH (0.1 M, 10 mL) were added separate-

ly to 10 mL methanolic stock solution and the mixtures were heated under reflux for 4.0 h at 50°C in the dark (to exclude the possible degradative effect of light). The resulting solutions (10 µL; 500 ng band^{-1}) were then analysed as described above.

Hydrogen Peroxide-Induced Oxidation

Hydrogen peroxide (10.0% or 30% (*w/v*), 10 mL) was added to 10 mL methanolic stock solution and the mixtures were heated in a boiling water bath for 10 min to completely remove excess hydrogen peroxide. Then the same mixtures were heated under reflux for 4.0 h at 50°C. The resulting solutions (10 µL; 500 ng band^{-1}) were then analysed as described above.

Dry and Wet Heat Treatment

The drug was stored in oven at 100°C for 5.0 h to study dry thermal degradation and the stock solution was heated under reflux for 5.0 h on a boiling water bath to study wet thermal degradation.

UV Degradation

The UV stability of the drug was studied by exposing the stock solution to direct outdoor sunlight for 15 days. After suitable dilution the resulting solution (10 µL; 500 ng band^{-1}) was analysed as described above.

Neutral Hydrolysis

Double-distilled water (10 mL) was added to 10 mL methanolic stock solution and the mixture was heated under reflux for 5.0 h at 50°C to study the degradation occurring under neutral conditions.

RESULTS AND DISCUSSION

Optimization of the Procedure

The TLC procedure was optimized with the objective of developing a stability-indicating method. Both pure drug and the degraded products were applied to the TLC plates and chromatographed with different mobile phases. Initially toluene–methanol–carbon tetrachloride–acetone 2.0:3.0:3.0:2 (*v/v*), 3.0:3.0:3.5 (*v/v*), and 3.5:4.5:2.5: (*v/v*) were tried. Addition of 0.05 mL ammonia to these mobile phases improved the characteristics of the bands. Finally, the mobile phase toluene–methanol–acetone–ammonia

3.5:3.5:2.5:0.05 (*v*/*v*) was found to enable good resolution with a sharp and symmetrical peak of R_F 0.45. Well defined bands were obtained when the chamber was saturated with mobile phase for 30 min at room temperature (Fig. 2).

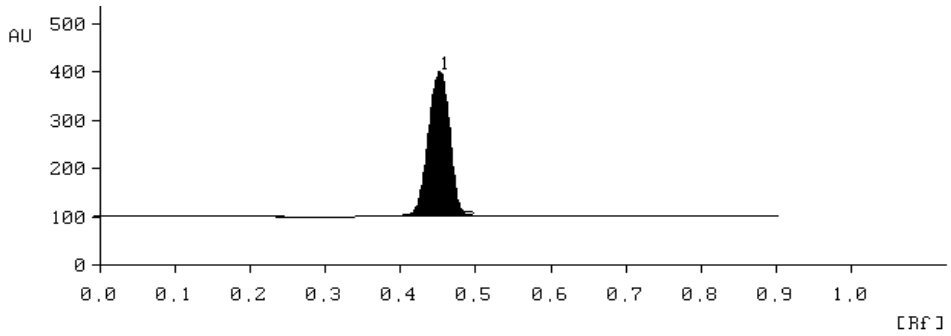

Fig. 2

Densitogram obtained from erdosteine standard (peak 1, R_F 0.45 ± 0.02, 500 ng band^{-1})

Linearity

The calibration graph was linear, i.e. the system adhered to Beer's law, over the range 30–1000 ng band^{-1} ($r^2 \pm SD = 0.998 \pm 0.002$). Linearity was evaluated by duplicate analysis of six standard working solutions equivalent to 30–1000 ng band^{-1} erdosteine. The relationship between peak area and concentration was subjected to least-squares linear-regression analysis to calculate the calibration equation and correlation coefficients. The regression data showed linearity was good over the concentration range investigated; this was apparent from the high value of the correlation coefficient

Table I

Linear regression data for the calibration plots[a]

Regression data	Value
Linear range	300–1000 ng band^{-1}
$r^2 \pm SD$	0.998 ± 0.002
Slope ± *SD*	0.073 ± 0.004
Intercept ± *SD*	36.78 ± 1.50
Confidence limit[b], slope	0.059–0.066
Confidence limit[b], intercept	38.74–40.48

[a] $n = 6$
[b] 95% confidence limit

and the *SD* for the intercept of <2%. There was no significant difference between the slopes plots from 30–100 ng band^{-1} or from 300–1000 ng band^{-1} (ANOVA; $P < 0.05$). Typical linearity data are given in Table I.

Validation

Precision

Repeatability of sample application and measurement of peak area, as *RSD* (%), was 1.12 and 1.21, respectively. *RSD* for inter-day and intra-day analysis was <2%. The values are shown in Table II.

Table II

Results of measurement of intra and inter day precision[a]

Intra-day precision			Inter-day precision		
SD of area	*RSD* (%)	*SE*	*SD* of area	*RSD* (%)	*SE*
1.42	1.12	0.51	1.86	1.21	0.76

[a] $n = 6$
Results are averages from analysis of three concentrations – 300, 500, and 900 ng band^{-1}

Robustness

The standard deviation of peak area was calculated for each change of conditions parameter and *RSD* was found to be <2% (Table III).

Table III

Results from robustness testing[a]

Condition changed	*SD* of peak area	*RSD* (%)
Mobile phase composition	1.74	1.50
Amount of mobile phase	1.48	1.28
Temperature	1.23	1.12
Relative humidity	1.18	0.98
Plate pretreatment	1.02	0.86
Time from application to chromatography	0.82	0.59
Time from chromatography to scanning	0.78	0.37

[a](n = 6)
Results are averages from analysis of three concentrations – 300, 500, and 900 ng band^{-1}

LOD and LOQ

The *LOD* and *LLOQ*, for signal-to-noise ratios were 3:1 and 10:1, respectively, were 5 and 10 ng band^{-1}.

Specificity

When the peak purity of erdosteine standard was assessed by comparing spectra acquired at the peak-start, peak-apex, and peak-end positions of a band, $r_{(\text{start, middle})} = 0.996$ and $r_{(\text{middle, end})} = 0.9994$. Good correlation ($r = 0.9998$) was also obtained between spectra of erdosteine obtained from standards and samples.

Recovery

When the method was used for extraction and subsequent analysis of erdosteine in pharmaceutical dosage form spiked with 50, 100, and 150 % extra drug, recovery was 98–102% of erdosteine as bulk and in dosage form results are listed in Table IV.

Table IV

Results from recovery studies[a]

Amount of standard added to matrix[b] (mg)	Amount recovered (mg)	Recovery (%)	*RSD* (%)	*SE*
0	198.8	99.44	0.82	0.25
100	300.15	100.05	0.47	0.12
200	403.24	100.81	0.58	0.29
300	505.2	101.41	1.71	0.85

[a] $n = 6$
[b] The matrix contained 200 mg drug

Stability in Sample Solution

Solutions of erdosteine of two different concentrations, equivalent to 400 and 800 ng band^{-1}, were prepared and stored at room temperature for 3 days. The solutions were then chromatographed on the same TLC plate and the densitograms obtained were evaluated for additional bands, if any. There was no instability in the sample solution. The results obtained are listed in Table V.

Table V

Stability of erdosteine in sample solutions (n = 6)

Amount (ng band^{-1})	Mean area	Area range	*RSD* (%)	*SE*
400	7724.56	7720.12–7730.58	0.58	0.36
800	15449.12	1544.36–15457.75	1.58	0.72

Band Stability

If the sample is not stable on the layer, the time the sample is left on the plate before chromatographic development can affect the results from analysis of the separated bands. This must be investigated during validation [15]. Two dimensional chromatography using same mobile phase was used to discover decomposition occurring during development. If decomposition occurs during development, peak(s) of decomposition product(s) should be obtained from the analyte in both the first and second developments. No decomposition was observed after application or during development.

Analysis of the Marketed Formulation

A single band at R_F 0.45 was observed in the densitogram of drug samples extracted from capsules. There was no interference from excipients commonly present in the capsules. The drug content was found to be 100.28%, *RSD* 0.57%. Statistical evaluation of the accuracy and precision of the results was performed by use of Student's *t*-test and the *F*-ratio at 95% confidence level. It was found that degradation of erdosteine had not occurred in the marketed formulations analysed. The low *RSD* value indi-

Table VI

Applicability of the proposed method for analysis of commercial capsules[a]

Property	Value
Label claim (mg)	300
Drug content (%) ± *SD*	100.28 ± 0.88
RSD (%)	0.57
SE	0.36
t-value[b]	2.44
F-value[b]	9.27

[a]n = 6
[b]Theoretical values for t and F (P = 0.05)

cated the suitability of this method for routine analysis of erdosteine in pharmaceutical dosage forms (Table VI).

Stability-Indicating Property of the Method (Accelerated Degradation)

Acid and Base-Induced Degradation

The densitogram obtained from the acid-degraded sample of erdosteine contained two peaks of degradation products at R_F 0.10 and 0.65 (Fig. 3). The densitogram obtained from the base-degraded sample for erdosteine contained two peaks of degradation products at R_F 0.28 and 0.55

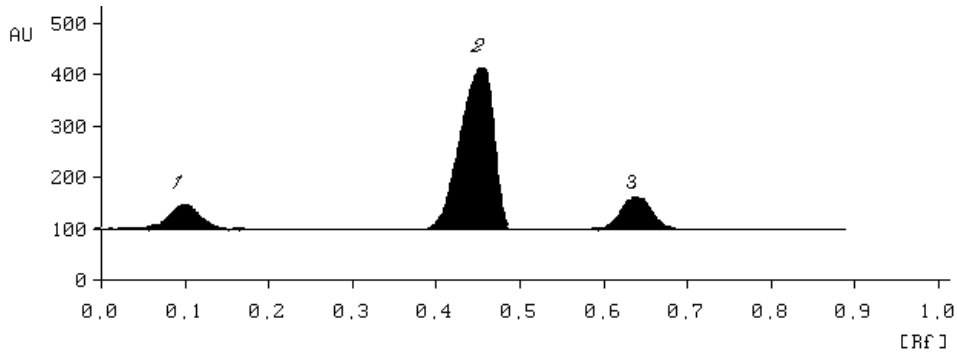

Fig. 3

Densitogram obtained from acid-degraded erdosteine. Peaks 1 and 3 (R_F 0.10 and 0.65, respectively) are degradation products

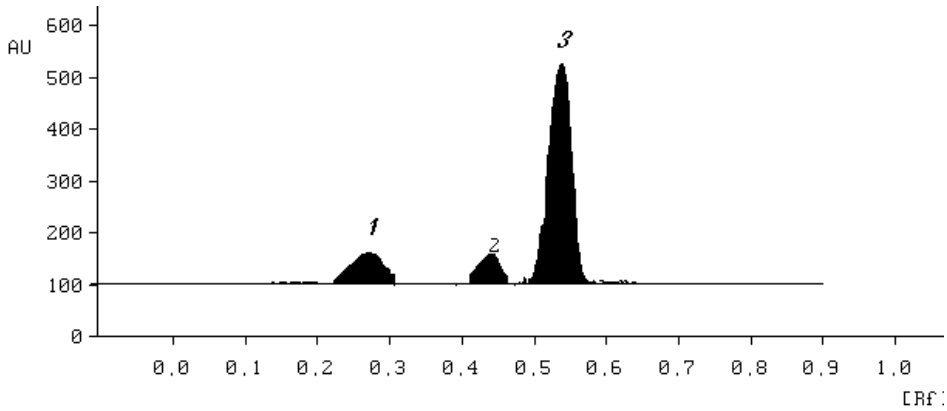

Fig. 4

Densitogram obtained from base-degraded erdosteine. Peaks 1 and 3 (R_F 0.28 and 0.55, respectively) are degradation products

(Fig. 4). The concentration of the drug was found to have changed from the initial concentration indicating that erdosteine undergoes degradation under acidic and basic conditions.

Hydrogen Peroxide-Induced Degradation

The densitogram obtained from the sample degraded with 10% hydrogen peroxide contained two peaks of degradation products at R_F 0.16 and 0.55 (Fig. 5).

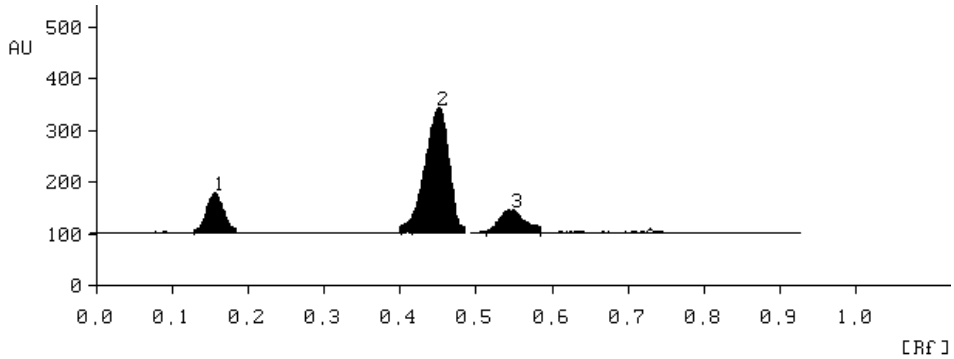

Fig. 5

Densitogram obtained from hydrogen peroxide-degraded erdosteine. Peaks 1 and 3 (R_F 0.16 and 0.55, respectively) are degradation products

Dry and Wet Heat Degradation

No peaks of degradation products were observed in densitograms obtained from samples subjected to wet heat and dry heat conditions (Fig. 6).

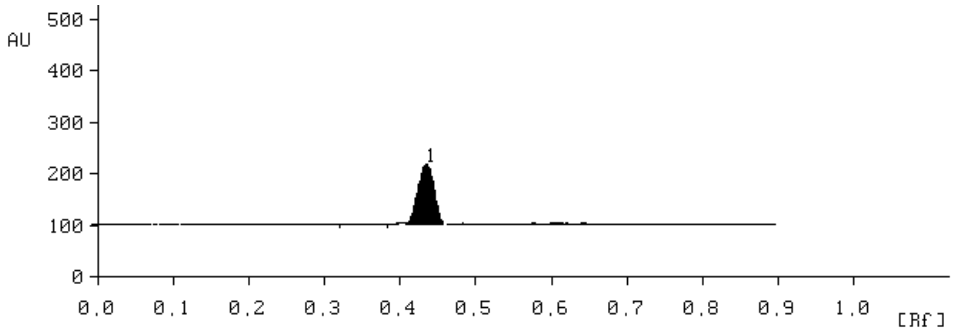

Fig. 6

Densitogram obtained from sample subjected to wet heat

UV Degradation

The densitogram obtained from the sample for 15 days subjected to UV irradiation contained a degradation product peak at R_F 0.51 (Fig. 7).

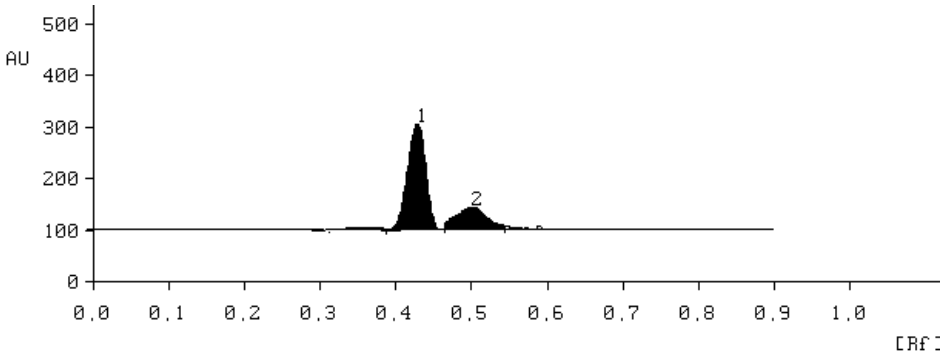

Fig. 7

Densitogram obtained from sample subjected to UV irradiation. Peak 2 (R_F 0.51) is that of a degradation product

Neutral Degradation

No peaks of degradation products were observed in densitograms obtained from samples degraded under neutral conditions.

Results obtained from study of erdosteine degradation are summarized in Table VII.

Table VII

Results from study of erdosteine degradation

Condition	Recovery (%)	R_F values of degradation products
Acid, 0.1 M HCl, reflux, 4.0 h, 50°C	66.13	0.10, 0.65
Base, 0.1 M NaOH, reflux, 4.0 h, 50°C	82.65	0.28, 0.55
H_2O_2 10% (*w*/*v*), reflux, 4.0 h, 50°C	87.20	0.16, 0.55
Neutral hydrolysis, 50°C, 5 h	98.02	–
Dry heat, 100°C, 5 h	98.00	–
Wet heat, 100°C, 5 h	99.05	–
UV, sunlight, 15 days	72.01	0.51

CONCLUSION

This HPTLC method for quantitative analysis of erdosteine in pharmaceutical formulations is precise, specific, accurate, reproducible, and stability-indicating, without interference from the excipients or from degradation products resulting from treatment with acid, alkali, oxidizing agent, or from UV irradiation. The method was validated in accordance with ICH guidelines. The method reduces analysis time compared with other methods and seems to be suitable for routine analysis of pharmaceutical formulations in quality-control laboratories, where economy and speed are essential. This drug is separated from its degradation products, and hence can be regarded as stability-indicating.

ACKNOWLEDGEMENT

The authors thank Glenmark Pharmaceuticals, Nasik, India, for providing a sample of erdosteine as a gift.

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