DRUG FORMULATIONS AND CLINICAL METHODS

Stability-Indicating Methods for the Determination of Erdosteine in the Presence of its Acid Degradation Products

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Four accurate, sensitive, and reproducible stability-indicating methods for the determination of erdosteine in the presence of its acid degradation products are presented. The first method involves processing the spectra by using a first-derivative method at 229 nm in a concentration range of 10–70 µg/mL. The mean percentage recovery was 100.43 ± 0.977. The second method is based on ratio-spectra first derivative spectrophotometry at 227.4 and 255 nm over a concentration range of 10–70 µg/mL. The mean percentage recovery was 99.65 ± 1.122% and 100.02 ± 1.306% at 227.4 and 255 nm, respectively. The third method utilizes quantitative densitometric evaluation of the TLC of erdosteine in the presence of its acid degradation products, and uses methanol–chloroform–ammonia (7 + 3 + 0.01, v/v/v) as the mobile phase. TLC chromatograms were scanned at 235 nm. This method analyzes erdosteine in a concentration range of 2.4–5.6 µg/spot, with a mean percentage recovery of 100.03±1.015%. The fourth method is HPLC for the simultaneous determination of erdosteine in the presence of its acid degradation products. The mobile phase consists of water–methanol (65 + 35, v/v). The standard curve of erdosteine showed good linearity over a concentration range of 10–80 µg/mL, with a mean percentage recovery of 99.90±1.207%. These methods were successfully applied to the determination of erdosteine in bulk powder, laboratory-prepared mixtures containing different percentages of the degradation products, and pharmaceutical dosage forms. The validity of results was assessed by applying the standard addition technique. The results obtained agreed statistically with those obtained by a reported method, showing no significant differences with respect to accuracy and precision.

Erdosteine is ±1S-(2-[N3-(2-oxotetrahydro thienyl)] acetamido)-thioglycolic acid (1; Figure 1) and is a known mucolytic (2). It modulates mucus production and viscosity and increases mucociliary transport, thereby improving expectoration (3, 4). It is an expectorant and is used for the treatment of acute exacerbations of chronic bronchitis in adults (i.e., chronic obstructive pulmonary disease) and lower respiratory infections in children above the age of 2 years (5). It also suppresses chemical stimulation-induced cough reflex plasma leakage into the airways. Erdosteine is used for the treatment of acute and chronic bronchitis and has multiple mechanisms of action, which include muco-modulatory, antibacterial, anti-inflammatory, and antioxidant activities (6).

One stability-indicating HPTLC assay procedure was reported for erdosteine (7). Erdosteine and its optically active metabolite have been analyzed by HPLC using a fluorescent chiral tagging reagent (8). The sensitive determination of erdosteine in human plasma has been achieved by utilizing automated 96-well plates with SPE and LC/MS/MS (9). Spectrophotometric methods, measuring absorption at 235 nm, first-order derivative (^{1}D) processing showing a sharp peak at 227.5 nm and calculating the area under the curve in the wavelength range of 230–240 nm (10) have been described. Simultaneous estimation of cefixime trihydrate and erdosteine in pharmaceutical dosage forms by using RP HPLC have also been described (11).

An ideal stability-indicating method quantifies the standard drug alone and resolves its degradation products (12). However, none of these methods is concerned with the identification and elucidation of the structure of the resulting acid degradation products. Thus, the objective of the present study was to develop simple and accurate stability-indicating methods for the selective determination of erdosteine in the presence of its acid degradation products. This information is applicable to pharmaceutical dosage forms and can be further applied to drug quality control.

Experimental

Apparatus

The experiments were performed using a Shimadzu (Kyoto, Japan) UV-1601 PC, dual-beam UV-Vis spectrophotometer, with matched 1 cm quartz cells. The software (v. 3.7), was used to process the absorption and derivative spectra. The spectral band width was 2 nm, with a wavelength-scanning speed of 2800 nm/min. IR spectrophotometer (Shimadzu 435) sampling was also undertaken, and samples were prepared using potassium bromide discs. UPLC MS/MS was performed using a Waters (Milford, MA) Acquity-TQ system.

Densitometric evaluation was performed using precoated TLC plates, (silica gel 60 F_{254}, 20 × 20 cm, 0.25 mm), purchased from E. Merck (Darmstadt, Germany). A 100 µL microsyringe was used to load samples onto the TLC plates. A CAMAG (Muttenz, Switzerland) TLC scanner 3 with winCATS software and equipped with a Linomat 5 autosampler was used for analysis.

The LC consisted of an isocratic pump (Shimadzu LC-10 AD), a UV-Vis wavelength detector (SPD-10A, Shimadzu), and a Rheodyne injector (Model 7725 I; Rohnert Park, CA) equipped with a 20 µL injector loop. The stationary phase was

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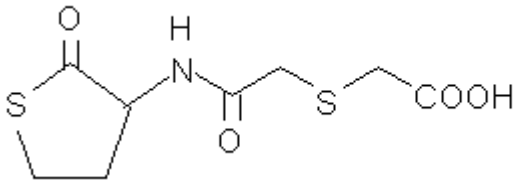

Figure 1. Chemical structure of erdosteine.

a C18 analytical column (250 × 4.6 mm, 5 µm) purchased from Waters. The mobile phase, water–methanol (65+35, v/v), was filtered through a 0.45 µm Millipore (Merck KGaA, Darmstadt, Germany) membrane filter and was degassed for 15 min in an ultrasonic bath prior to use. UV detection was performed at 235 nm. The samples were also filtered through a 0.45 µm Millipore membrane filter, and were injected using a 25 µL Hamilton (Bonaduz, Switzerland) syringe.

Materials and Reagents

Erdosteine was kindly supplied by Global Napi Pharmaceuticals, Cairo, Egypt. Its purity was found to be 100.75 ± 1.352% by a direct spectrophotometric method (10).

Pharmaceutical Dosage Forms

The label from Mucotec® capsules claims 150 or 300 mg erdosteine per tablet (produced by Global Napi Pharmaceuticals, under license of Edmond Pharmaceuticals, Paderno Dugnano, Italy; batch Nos, 920901 and 100537, respectively).

All chemicals and reagents were analytical grade. The chemicals 1 N HCl solution, concentrated ammonia (specific gravity 0.91), methanol, and chloroform were obtained from Adwic, Cairo, Egypt. Deionized water and methanol (E. Merck) were HPLC grade.

Standard Solutions

For the densitometric method, erdosteine standard stock solution (1000 µg/mL) in methanol was prepared by transferring accurately weighed 100 mg of erdosteine into a 100 mL volumetric flask, dissolving it in 20 mL methanol, and diluting to volume with methanol.

For ^{1}D, ratio-spectra first derivative (^{1}DD), and HPLC methods, a working standard solution of erdosteine (100 µg/mL) in methanol was prepared by accurately weighing 10 mg of erdosteine in a 100 mL volumetric flask and dissolving it in methanol.

A standard stock solution (equivalent to 1000 µg/mL erdosteine) was prepared in methanol for the analysis of acid degradation products. Samples for the densitometric method were prepared by accurately weighing 47.25 and 60.44 mg of acid degradates I and II, respectively, into 100 mL volumetric flasks and dissolving them in methanol.

A working standard solution (100 µg/mL) of acid degradation products was prepared in methanol for ^{1}D, ^{1}DD, and HPLC methods by accurately weighing 4.725 mg and 6.044 mg of acid degradates I and II, respectively, into a 100 mL volumetric flask and dissolving them in methanol. These samples were freshly prepared on the day of analysis and stored in a refrigerator, to be used within 24 h.

Preparation of Acid-Degraded Sample

The acid degradation products were prepared by dissolving 200 mg of pure erdosteine in the least amount of methanol and refluxed with 50 mL of 1 N HCl in a 500 mL flask for 4 h at 100°C. This length of time was demonstrated by TLC to be the amount required for complete degradation of the drug. The degraded solution was applied as a band onto several preparative TLC plates. The plates were developed using methanol–chloroform–ammonia (7 + 3 + 0.01, v/v/v) in a chromatographic tank previously saturated for 30 min with the developing solvents, then air-dried. The bands were visualized under UV light at 254 nm. Upon examination of the TLC plate, three different spots were obtained, one for the intact drug (R_f = 0.52), and the other two for two acid degradation products (R_f = 0.35 and R_f = 0.65 for acid degradates I and II, respectively).

The bands were then scraped, and the silica was suspended in the least amount of methanol and filtered. The filtrate was left to dry at room temperature (25°C) to obtain the two degradation products. The purity of the degradation products obtained was tested by dissolving a small portion in methanol, applying it onto TLC plates, and developing them using the previously described solvent system. The structure of the isolated degradation products was elucidated using IR and mass spectroscopy.

^{1}D Method

Two aliquots (approximately 30 µg/mL) of both erdosteine and its degradates as working standard solutions (100 µg/mL each) were separately transferred into two 10 mL volumetric flasks, and the volumes were completed with methanol. The zero-order and first-derivative spectra of the prepared solutions were recorded.

Linearity

A standard curve in the range of 10–70 µg/mL using erdosteine working standard solutions in methanol (100 µg/mL) was prepared. The amplitudes of the ^{1}D peaks were measured at 229 nm with $\Delta\lambda$ = 4 nm and a scaling factor of 100. Calibration graphs were constructed by plotting $\Delta A/\Delta\lambda$ versus concentration. The regression equations were then computed for the studied drug at the specified wavelength and used for determination of unknown samples containing erdosteine.

^{1}DD Spectrophotometric Method

Standard serial concentrations in the range of 10–70 µg/mL of erdosteine working standard solution (100 µg/mL) were prepared in methanol and separately transferred to a series of 10 mL volumetric flasks. Aliquots equivalent to 30 µg/mL of the degradate working standard solutions (100 µg/mL, to be used as a divisor) were transferred into the 10 mL volumetric flasks, and the volume was completed with methanol. The spectra of the prepared standard solutions were then scanned from 200 to 400 nm. The stored spectra of erdosteine were divided (amplitude at each wavelength) by the spectrum of 30 µg/mL of the degradation product. The ^{1}DD with $\Delta\lambda$ = 4 nm and a scaling factor of 10 was obtained. The amplitudes of the ^{1}D peaks of erdosteine were measured at both 227.4 and 255 nm. Calibration graphs were constructed relating the peak amplitudes of ^{1}DD to the corresponding concentrations. The regression equations

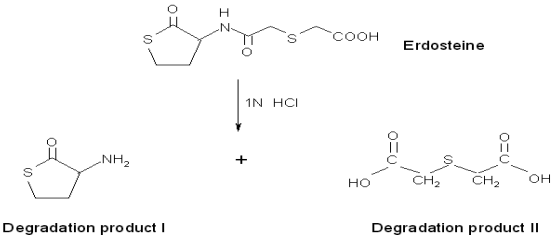

Figure 2. Acid degradation of erdosteine.

were then computed for the studied drug at the two specified wavelengths and used for determination of unknown samples containing erdosteine.

Assay of Laboratory-Prepared Mixtures

Aliquots equivalent to 10–60 µg/mL were accurately transferred from erdosteine working standard solution (100 µg/mL) into a series of 10 mL volumetric flasks. Aliquots equivalent to 10–60 µg/mL of acid degradation products (100 µg/mL) were added to the previous solutions. The volumes were completed with methanol and mixed thoroughly. The ^{1}DD values were recorded at both 227.4 and 255 nm. The concentration of erdosteine was calculated from its regression equation. Each concentration was calculated from four experiments.

Spectrodensitometric Method

Aliquots equivalent to the 2.4–5.6 µL range of erdosteine standard stock solution (1000 µg/mL) were spotted using a CAMAG Linomat autosampler with a 100 µL microsyringe. Spots were spaced 1.5 cm apart from each other and 2 cm from the bottom edge of the plate. The plate was developed in a chromatographic tank previously saturated for at least 1 h with the developing mobile phase, methanol–chloroform–ammonia (7 + 3 + 0.01, v/v/v), in the ascending mode. The plate was removed and dried in air, and the spots were visualized under a UV lamp at 254 nm and scanned at 235 nm. The calibration curve was plotted between the recorded area under the peak and the corresponding concentration, from which the regression equation was calculated. The calibration curve was made from the average of three experiments.

Assay of Laboratory-Prepared Mixtures

Aliquots equivalent to 2.4–5.6 µg/mL were accurately transferred from erdosteine stock standard solution (1000 µg/mL) into a series of 10 mL volumetric flasks. Aliquots equivalent to 2.4–5.6 µg/mL of acid degradation products standard stock solution (1000 µg/mL) were added to the previous solutions. The volumes were completed with methanol and mixed thoroughly. The procedures were the same as those described above under *Linearity*, and concentrations were calculated from the corresponding regression equation.

HPLC Method

Accurately measured volumes of erdosteine working standard solution (100 µg/mL) were transferred into 10 mL volumetric

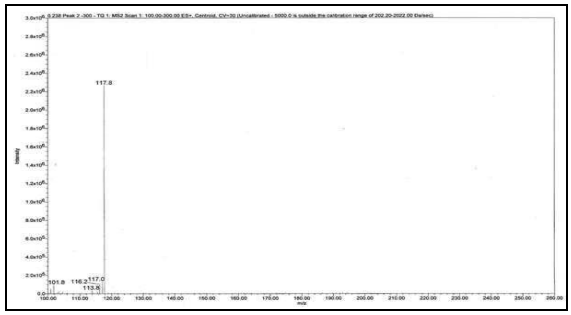

Figure 3A. Mass spectrum of erdosteine acid degradation product I.

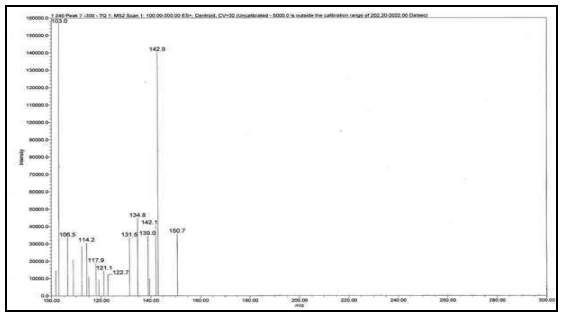

Figure 3B. Mass spectrum of erdosteine acid degradation product II.

flasks and diluted to volume with methanol to obtain the final concentration range of 10–80 µg/mL. The samples were then chromatographed using a C18 analytical column (250 × 4.6 mm id, 5 µm; Waters). The mobile phase, which consisted of water–methanol (65 + 35, v/v), was filtered through a 0.45 µm Millipore membrane filter and was degassed for about 15 min in an ultrasonic bath prior to use; flow rate was 1 mL/min at approximately 25°C, with UV detection at 235 nm. The samples were also filtered through a 0.45 µm membrane filter and injected using a 25 µL Hamilton analytical syringe. The relative peak area ratios to that of external standard (40 µg/mL) were then plotted versus the corresponding concentrations of erdosteine to obtain the calibration graph and compute the corresponding regression equation. Concentrations of unknown samples of erdosteine were determined using the obtained regression equation.

System Suitability

Twenty microliters of the solvent mixture and the working standard solutions were injected. The system suitability parameters, retention time, tailing factor, number of theoretical plates (*N*), height equivalent to a theoretical plate, separation between the erdosteine peak and its degradation products peak (resolution), and column capacity were calculated.

Assay of Laboratory Prepared Mixtures

Aliquots equivalent to 10–70 µg/mL were accurately transferred from the erdosteine working standard solution (100 µg/mL) into a series of 10 mL volumetric flasks. Aliquots equivalent to 10–70 µg/mL of acid degradation products (100 µg/mL) were added to the previous solutions. The volumes were completed with methanol and mixed thoroughly. Procedures were as

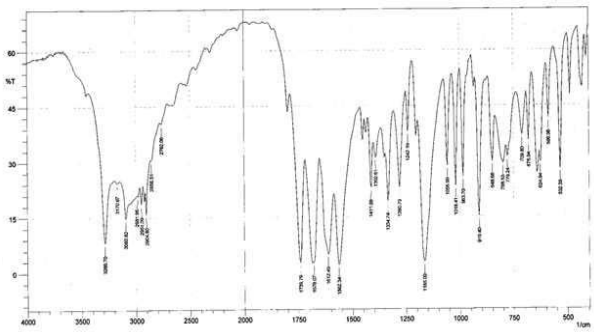

Figure 4A. IR spectrum of intact erdosteine.

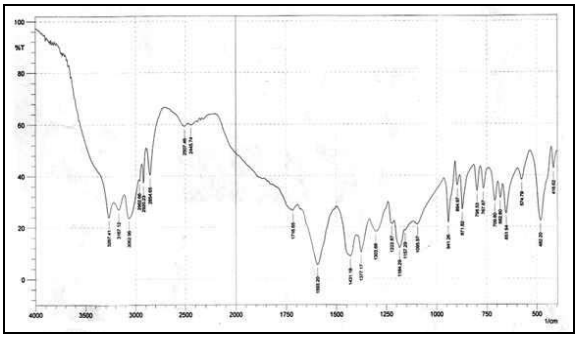

Figure 4B. IR spectrum of erdosteine degradation product I.

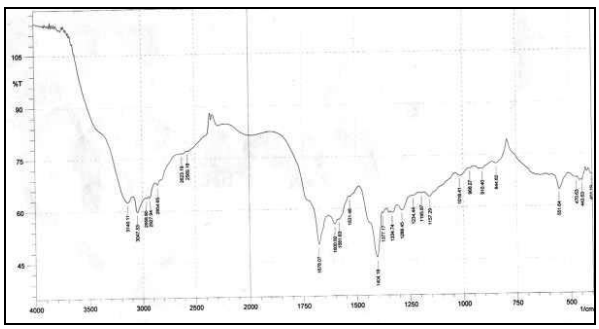

Figure 4C. IR spectrum of erdosteine degradation product II.

described above, and concentrations were calculated from the corresponding regression equation.

Assay of Pharmaceutical Formulations (Mucotec Capsules)

Twenty Mucotec capsules were emptied. A portion of the powder equivalent to 100 mg erdosteine was accurately weighed into a 100 mL beaker and dissolved in methanol. For the densitometric method (4×20 mL), this solution was then filtered into a 100 mL volumetric flask. The volume was completed with the same solvent (1000 µg/mL). A 10 mL amount of this tablet stock solution (1000 µg/mL) was transferred into a 100 mL volumetric flask and diluted with methanol to get a final concentration of 100 µg/mL for the ^{1}D , ^{1}DD, and HPLC methods. Each concentration was repeated four times.

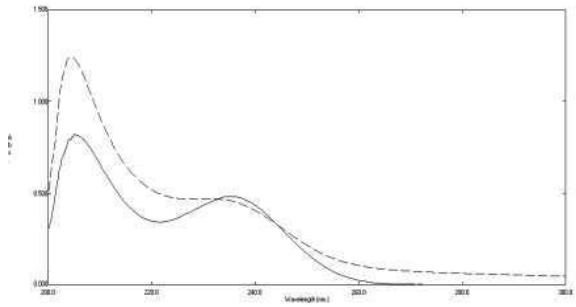

Figure 5. Absorption spectra of 30 µg/mL erdosteine (—) and 30 µg/mL of its acid degradation products (⋯).

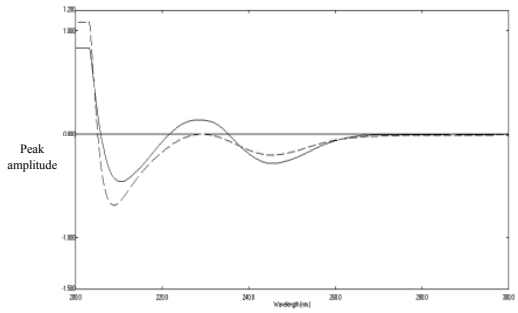

Figure 6. ^{1}D absorption spectra of erdosteine 30 µg/mL (—) and its acid degradates 30 µg/mL (----) using methanol as a solvent.

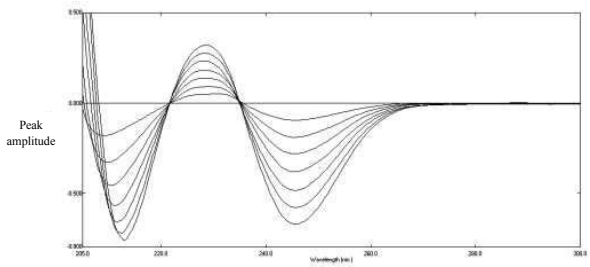

Figure 7. ^{1}D absorption spectra of 10–70 µg/mL of erdosteine.

Results and Discussion

Degradation of Erdosteine

Many pharmaceutical compounds undergo degradation during storage or even during the different processes of their manufacture. Several chemical or physical factors can lead to the degradation of drugs (13). Hydrolysis and oxidation are the most common chemical degradation routes for drugs (14, 15). The main functional groups found in drugs that are subject to degradation are esters, amides, and lactams. Erdosteine has an amide linkage, so trials were conducted for its degradation in either an acidic or a basic medium. It was found that the drug was liable to degradation upon refluxing in a strong acid medium to give two degradates, as demonstrated in Figure 2.

In this work, acid-hydrolyzed erdosteine degradation products were prepared and separated, and their structures were identified by MS. Erdosteine acid degradate I showed a molecular ion peak at 117.8 m/z (Figure 3A) whereas its acid degradate II molecular

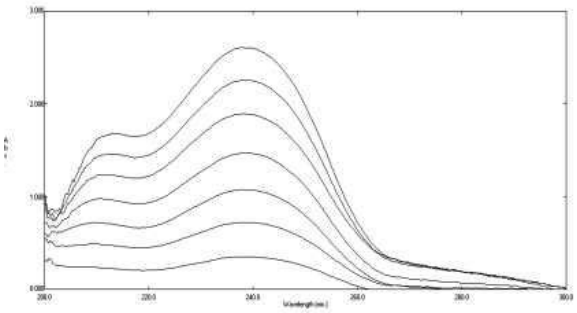

Figure 8. Zero-order of ratio spectra of erdosteine (10–70 µg/mL) using the spectrum of 30 µg/mL of acid degradates as a divisor.

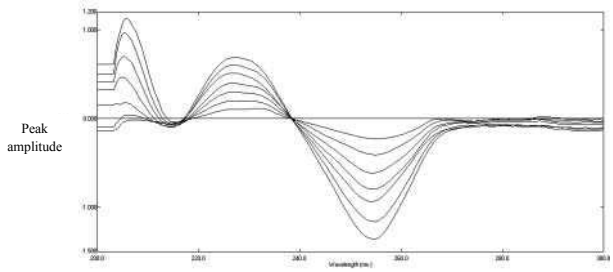

Figure 9. ^{1}D ratio spectra of erdosteine (10–70 µg/mL) using 30 µg/mL of its acid degradates as a divisor.

ion peak appeared at 150.7 m/z (Figure 3B); these peaks are equivalent to their respective molecular weights. This indicates that the amide group suffered cleavage by 1 N HCl, and this was further confirmed by IR spectroscopy. The IR spectrum of intact erdosteine is characterized by the absorption frequency of two stretching bands of the carbonyl group at 1680 and 1740 cm^{-1} (Figure 4A). The IR spectrum of erdosteine acid degradate I showed a stretching band of the carbonyl group at 1716 cm^{-1}, and a forked peak of the primary amine at 3167 and 3267 cm^{-1} (Figure 4B). The IR spectrum of erdosteine acid degradate II showed stretching bands from the carbonyl and hydroxyl groups of the carboxylic acid appearing at 1678 and 3140 cm^{-1}, respectively (Figure 4C). Also, by applying the HPLC method, the retention times for erdosteine and its acid degradate I were 2.26 and 3.46 min, respectively, and 9.95 min for acid degradate II. The present work was conducted for the selective determination of erdosteine in the presence of its acid-hydrolyzed degradation products, with application to pharmaceutical dosage forms.

TLC Fractionation

TLC monitoring of drug degradation was done on thin layer plates of silica gel F_{254} using methanol–chloroform–ammonia (7 + 3 + 0.01, v/v/v) as the developing solvent. The developed plates were visualized under a short UV lamp. The degradates (R_f = 0.35 and 0.65) for acid degradates I and II, respectively, showed that they could be separated from the intact drug (R_f value = 0.52).

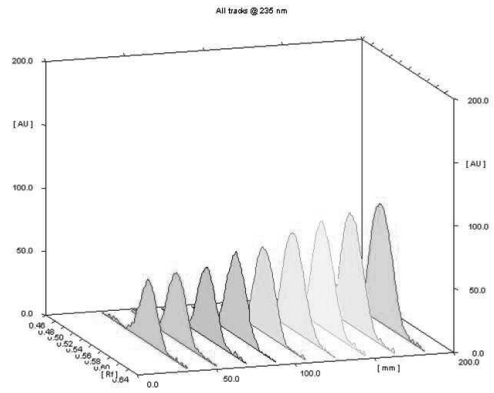

Figure 10. Scanning profile of the TLC chromatogram of erdosteine (2.4–5.6 µg/spot) at 235 nm.

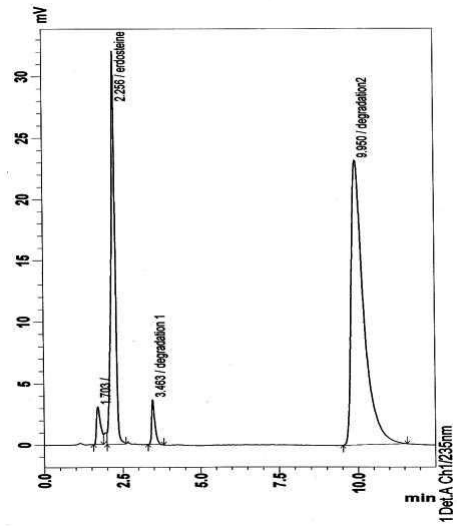

Figure 11. HPLC chromatogram of erdosteine (20 µg/mL, R_t 2.26 min.), its acid degradate I (20 µg/mL, R_t 3.46 min), and its acid degradate II (20 µg/mL, R_t 9.95 min.)

Spectrophotometric Methods

^{1}D Method

Derivative spectrophotometry is a powerful tool in the quantification of a mixture of drugs. It can also be used as a stability-indicating method for the analysis of drugs in the presence of their degradation products, as it can solve the problem of overlapping absorption bands.

A simple, rapid, and selective spectrophotometric technique was proposed and applied for the determination of erdosteine in the presence of its degradation products, either as a raw material or in pharmaceutical formulations. This was done by applying the ^{1}D treatment of the UV spectra. This method can solve the problem of overlapping spectral bands between erdosteine and its degradates without the need for sample pretreatment or time-consuming separation steps.

The zero-order absorption spectra of erdosteine and its degradation products showed severe overlap over the entire spectrum of the intact drug (Figure 5). Therefore, the use of direct absorbance measurements for assaying erdosteine in the presence of its degradation products was not possible.

Table 1. Determination of erdosteine in Mucotec capsules by the proposed methods

	Methods				
		^{1}DD method			
Preparation, mean ± SD	^{1}D method at 229 nm	At 227.4 nm	At 255 nm	TLC densitometric method	HPLC method
Mucotec capsules, 150mg, Batch No. 920901	100.26 ± 0.870	99.69 ± 0.815	99.66 ± 1.064	99.30 ± 0.544	99.71 ± 0.684
Mucotec capsules, 300mg, Batch No. 100537	100.65 ± 0.826	99.88 ± 0.675	100.57 ± 0.957	100.01 ± 0.941	100.38 ± 0.922

When the ^{1}D spectra (Figure 6) were examined, it was found that erdosteine can be determined at 229 nm, whereas its degradates have no contribution (zero crossing). The clear zero crossing of the degradates allows accurate determination of erdosteine in the presence of any level of its degradates up to 85%. A linear relationship was obtained in the range of 10–70 μg/mL for erdosteine at 229 nm (Figure 7). The corresponding regression equation was computed to be:

$$^1D = 0.0046C - 0.0006 \quad (r = 0.9998) \qquad (1)$$

where ^{1}D is the peak amplitude of the first-derivative curve ($\Delta A/\Delta\lambda$) at the corresponding wavelength, C is the concentration of erdosteine (μg/mL), and r is the correlation coefficient.

The precision of the proposed method was confirmed by the analysis of different concentrations of authentic marketed samples ($n = 3$). The mean percentage recovery was 100.05 ± 0.634%.

^{1}DD Method

^{1}DD is an analytical technique of good utility that offers background correction and better selectivity than normal spectrophotometry for resolving binary mixtures and some ternary mixtures (16).

The ^{1}DD method was suggested to solve this problem. The theory of derivative ratio spectrophotometry, which is based on the use of first (or second) derivatives of the ratio spectra of the mixture and divided (amplitudes at each wavelength) by the absorption spectrum of a standard solution of one of the components, has been applied extensively to simultaneous determination of substances with overlapping spectra as an economic alternative to HPLC methods (17), and to solve the problem of the overlapping absorption spectra of erdosteine and its acid degradation products. In the present investigation, the careful choice of the divisor and working wavelength were of great importance as it affected both sensitivity and selectivity. Accordingly, different concentrations of the degradation products (10, 20, 30, and 60 μg/mL) were tried as divisors. It was found that 30 μg/mL was the best, as it produced minimum noise and gave better results in agreement with selectivity.

The zero-order of the ratio spectra of erdosteine and the first-order of the derivative ratio spectra are presented in Figures 8 and 9, respectively. The concentration of the divisor was studied. It was found that dividing by 30 μg/mL (of the degradation products) led to the best results in terms of sensitivity, repeatability, and S/N.

Linear calibration graphs were obtained for erdosteine in the concentration range of 10–70 μg/mL by recording the peak amplitudes at 227.4 and 255 nm using the absorption spectra of 30 μg/mL from the acid degradation products as a divisor. The regression equations were computed and found to be:

$$(^1DD_{227.4nm}) = 0.0099C + 0.0074 \quad (r = 0.9996) \qquad (2)$$

$$(^1DD_{255nm}) = -0.0187C - 0.04 \quad (r = 0.9997) \qquad (3)$$

where ^{1}DD is the peak amplitude of the first-derivative curve for erdosteine/its degradates, C is the concentration of erdosteine in μg/mL, and r is the correlation coefficient.

The precision of the proposed method was checked by the analysis of different concentrations of authentic samples in triplicate. The mean percentage recoveries were 99.91±0.950% and 100.72±0.862% at 227.4 and 255 nm, respectively.

Table 2. System suitability parameters of the HPLC method for the analysis of erdosteine in the presence of its acid degradation products

	Obtained value			
Parameter	Erdosteine	Degradate I	Degradate II	Reference value (19)
Resolution (*R*)	5.11	6.89		*R* > 0.8
T (tailing factor)	1.05	1.02	1.5	*T* = 1 for a typical symmetric peak
α (selectivity factor)	3.12[a]	4.71[b]		>1
K (column capacity)	0.33	1.03	4.85	Till–10 acceptable
N (column efficiency)	971.72	5911.90	546.22	Increases with efficiency of the separation
HETP (height equivalent to theoretical plates)	0.026	0.0043	0.046	The smaller the value, the higher the column efficiency

[a] Relative to degradation product I.

[b] Relative to degradation product II.

Table 3. Validation of the results obtained by applying the suggested methods for the determination of erdosteine

Parameters	^{1}D method, 229 nm	^{1}DD method		TLC densitometric method	HPLC method
		227.4 nm	255 nm		
Range	10–70, µg/mL	10–70, µg/mL	10–70, µg/mL	2.4–5.6, µg/spot	10–80, µg/mL
Slope	0.0046	0.0099	–0.0187	0.844	0.0251
SE of the slope	3.96×10^{-5}	0.000116	0.000185	0.003021	0.000198
Intercept	–0.0006	0.0074	–0.04	–0.212	0.0124
SE of the intercept	0.001771	0.005198	0.008258	0.006647	0.009976
Accuracy	100.43 ± 0.977	99.65 ± 1.122	100.02 ± 1.306	100.03 ± 1.015	99.90 ± 1.207
Specificity[a]	100.26 ± 0.859	99.99 ± 1.091	99.67 ± 0.945	99.62 ± 0.649	100.23 ± 1.118
Correlation coefficient (r)	0.9998	0.9996	0.9997	0.9993	0.9998
Repeatability[b]	0.634	0.951	0.856	0.918	0.731
Intermediate precision[c]	0.946	1.121	0.876	0.763	0.760

[a] Specificity was calculated from the analysis of laboratory-prepared mixtures.

[b] The intraday RSD % of samples of erdosteine (20, 40, 60 µg/mL) for the ^{1}D, ^{1}DD, and HPLC methods, (2.80, 3.60, 4.00 µg/spot) for the TLC densitometric method.

[c] Interday RSD % of samples of erdosteine (20, 40, 60 µg/mL) for ^{1}D, ^{1}DD and for HPLC method, (2.80, 3.60, 4.00 µg/ spot) for TLC densitometric method.

Spectrodensitometric Method

TLC densitometry overcomes the problem of overlapping absorption spectra of a mixture of drugs by separating these components on TLC plates and determining each ingredient by scanning the corresponding chromatogram. The TLC-UV method has the advantage of simultaneously determining the active ingredients in multicomponent dosage forms (18).

The proposed procedure is based on the difference in R_f values of erdosteine (R_f = 0.52) and its acid degradation products (R_f = 0.35 and 0.65 for acid degradates I and II, respectively). Various developing systems were tried, but complete separation was achieved using methanol–chloroform–ammonia (7 + 3 + 0.01, v/v/v).

The linearity was confirmed by plotting the measured peak area versus the corresponding concentrations at 235 nm over a range of 2.4–5.6 µg/spot, where a linear response was obtained. The scanning profile of different concentrations of erdosteine at 235 nm is shown in Figure 10. The linear regression equation was:

$$A = 0.844C - 0.212 \quad (r = 0.9993) \qquad (4)$$

where A is the integrated area under the peak × 10^{-4}, C is the concentration in µg/spot, and r is the correlation coefficient.

The precision of the proposed method was confirmed by the analysis of different concentrations of authentic marketed samples in triplicate. The mean percentage recovery was 100.34 ± 0.921%.

HPLC Method

A simple HPLC method was adopted for the simultaneous determination of erdosteine in the presence of its acid degradation products without previous separation. Different mobile systems were tried, such as water–methanol–acetonitrile

Table 4. Statistical analysis of the results obtained by applying the proposed methods and a reported spectrophotometric method for the analysis of pure erdosteine

Values	^{1}D method, 229 nm	^{1}DD method		TLC densitometric method	HPLC method	Reported method (Ref. 10)[a]
		227.4 nm	255 nm			
Mean	100.43	99.65	100.02	100.03	99.90	100.75
±SD	0.977	1.122	1.306	1.015	1.207	1.352
n	7	7	7	9	8	6
Variance	0.955	1.259	1.706	1.030	1.457	1.828
t	0.538	1.789	1.139	0.052	1.398	—
t theoretical	(2.201)[b]	(2.201)[b]	(2.201)[b]	(2.160)[b]	(2.179)[b]	
F	1.914	1.452	1.072	1.803	1.255	—
F theoretical	(4.39)[b]	(4.39)[b]	(4.39)[b]	(4.21)[b]	(3.97)[b]	

[a] Direct UV spectrophotometric method at 235 nm.

[b] The values in parenthesis are the corresponding tabulated *t* and *F* values at *P* = 0.05.

(40 + 20 + 40, v/v/v) and acetonitrile– phosphate buffer (at different pH values), for the chromatographic separation of the drug from its acid degradation products. The best resolution was achieved with a mobile phase consisting of water–methanol (65+35, v/v) and UV detection at 235 nm to obtain a retention time 2.26 min for erdosteine, 3.46 for acid degradate I, and 9.95 for acid degradate II (Figure 11).

A linear relation was obtained between peak area and the concentration of erdosteine in the range of 10–80 µg/mL. The linear regression equation was:

$$A = 0.0251C + 0.0124 \quad (r = 0.9998) \qquad (5)$$

where A is the relative peak area, C is the concentration in µg/mL, and r is the correlation coefficient.

Stability-Indicating Method

To assess the stability-indicating efficiency of the proposed methods, the degradation products of erdosteine were mixed with its intact sample in different ratios and analyzed by the proposed methods.

The suggested methods were successfully applied for the determination of erdosteine in its pharmaceutical formulation, showing good percentage recoveries. The validity of the suggested methods was further assessed by applying the standard addition technique (Table 1). System suitability tests, which are used to ensure system performance before or during the analysis of drugs, were performed. The obtained values of erdosteine and its acid degradation products agreed with the stated reference values (19; Table 2).

Table 3 illustrates good selectivity in the determination of erdosteine in the presence of up to 85% (w/w) of its degradates in the ^{1}D and the ^{1}DD methods, up to 70% (w/w) by the densitometric method, and up to 87% (w/w) by the HPLC method. Assay validation was done by repeating the procedures three times on three different days (interday) and three times at different time intervals within the same day (intraday), for the analysis of different concentrations of erdosteine (Table 3). The results show that the analysis methods were accurate, precise, and specific.

Results of the suggested methods for the determination of erdosteine were statistically compared with those obtained by applying a reported spectrophotometric method (10). The calculated Student's *t*-test and *F*-values were less than the corresponding theoretical ones, confirming good accuracy and excellent precision (Table 4).

Conclusions

Four methods—^{1}D, ^{1}DD, TLC, and HPLC—were developed for the determination of erdosteine in the presence of its acid degradation products. The methods provide simple, accurate, rapid, and reproducible quantitative analysis of erdosteine in bulk powder, laboratory-prepared mixtures, and a pharmaceutical dosage form.

The ^{1}D and ^{1}DD methods have the advantages of being more economical, rapid, and environmentally secure than the other methods. The TLC method was more sensitive than the ^{1}D or ^{1}DD methods. The HPLC method gave a good resolution between erdosteine and its acid degradation products within a short time period and across a dynamic range. These methods can be used as stability-indicating procedures in quality control laboratories where cost control and time management are essential.

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