https://doi.org/10.48047/AFJBS.6.2.2024.3189-3199

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF ERDOSTEINE IN DOSAGE FORMS

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Article History

Volume 6, Issue 2, Apr-Aug 2024

Received: 5 August 2024

Accepted: 15 August 2024

Published: 15 August 2024

doi:10.48047/AFJBS.6.2.2024.3189-3199

Abstract: Background: A precise, user-friendly, sensitive, and verified spectrophotometric approach has been devised for the determination of erdosteine (ERD) in both pure and dose forms. This approach employed N-bromosuccinimide (NBS) as an eco-friendly oxidizing agent alongside three chosen dyes: amaranth (AM), methylene blue (MB), and indigocarmine (IC). The techniques utilize surplus quantities of NBS in acidic environments to oxidize erdosteine. The unreacted NBS is quantified chemically using fixed amounts of dyes AM, MB, and IC. Absorption was quantified at 520 nm, 663 nm, and 609 nm for AM, MB, and IC dyes, respectively. The analytical technique was executed and validated through a comprehensive examination and optimization of many elements that could potentially interfere with the reaction. Under optimum conditions, substantial linear correlations were identified, with correlation values between 0.9993 and 0.9996. These relationships were valid within concentration ranges of 1.0-16, 1.0-12, and 1.0-14 µg/ml, respectively. The LOD values obtained were 0.29, 0.28, and 0.30 µg/ml, while the LOQ values were established at 0.97, 0.93, and 1.0 µg/ml for the AM, MB, and IC procedures, respectively. The established methods were validated per ICH recommendations and effectively utilized for the analysis of erdosteine in dosage forms, demonstrating high accuracy and precision. The reliability of the approaches was confirmed through recovery studies utilizing the usual addition method. The statistical comparison of findings derived from the proposed methods against those from the published approach, utilizing Student's t-test and variance ratio F-test at a 95% confidence level, demonstrated strong concordance and indicated no significant disparity in accuracy and precision.

Keywords: *Erdosteine; N-bromosuccinimide, Spectrophotometry, Method of validation, Dosage forms*

Introduction

Erdosteine (ERD) is a mucolytic drug employed in the treatment of disorders associated with abnormal mucus production, including respiratory tract infections and chronic obstructive pulmonary disease. Erdosteine is a

mucolytic substance that facilitates the degradation of mucus. It is a thiol derivative utilized for the clinical treatment of chronic obstructive bronchitis, as well as for infectious exacerbations of chronic bronchitis. This medication contains sulfhydryl groups that are liberated following the hepatic first-pass metabolism of erdosteine. Three active metabolites are produced, exhibiting mucolytic properties alongside free radical scavenging activity. Erdosteine regulates mucus formation and its viscosity while enhancing mucociliary transport. It also mitigates the impact of free radicals produced by cigarette smoke [1]. Erdosteine, chemically designated as 2-[2-oxo-2-[(2-oxothiolan-3-yl)amino]ethyl]sulfanylacetic acid (Figure 1), possesses the empirical formula $C_8H_{11}NO_4S_2$ is a thiol derivative formulated for the management of chronic obstructive bronchitis, encompassing acute infective exacerbations of chronic bronchitis. It is not yet officially recognized in any Pharmacopeia.

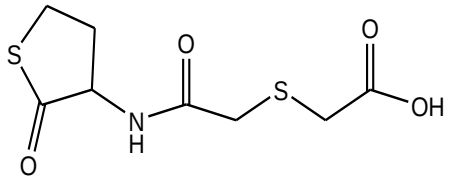


Figure 1. The chemical structure of erdosteine (ERD)

The literature analysis indicates that there are limited published methods for ascertaining ERD in dose forms. These methodologies encompass spectrophotometry [2-11], spectrofluorimetry [12], and chromatography [13-23]. Nevertheless, the previously published approaches were either insufficiently sensitive or demanded considerable effort and depended on costly instruments that are not commonly accessible in many laboratories. Therefore, it was advantageous to establish creative, efficient, and economical spectrophotometric techniques for assessing the concentration of ERD in its pharmaceutical formulations.

N-Bromosuccinimide (NBS) is an environmentally benign reagent employed as a highly effective agent for oxidation and bromination. Spectrophotometric determinations utilizing NBS were performed by directly measuring the chromogenic derivative of the drug or indirectly assessing the residual NBS with colorimetric reagents [24-28].

This project aims to create revolutionary spectrophotometric methods that are straightforward, extremely sensitive, accurate, and cost-effective for assessing ERD in both pure and dose forms. The suggested methodologies utilize NBS as an eco-friendly agent alongside AM, MB, and IC dyes. The use of commonly used additives at standard concentrations often found in pharmaceutical formulations did not affect the test of ERD. The proposed approaches have been statistically validated for accuracy, precision, sensitivity, selectivity, robustness, and ruggedness in compliance with ICH recommendations [29].

EXERIMENTAL

Equipment

A Shimadzu UV-1601 UV/Vis spectrophotometer (Sweden) fitted with a 10 mm quartz cell was employed for absorbance measurement. It demonstrates remarkable precision in wavelength measuring, with an accuracy of ±0.2 nm. The gadget operates at a scanning speed of 200 nm/min with a bandwidth of 2.0 nm. It encompasses wavelengths from 200 to 900 nm.

Chemicals and reagents

All chemicals, reagents, and solvents utilized in this experiment were of superior analytical reagent purity. Moreover, each answer was reliably produced at regular intervals. The experiment employed double-distilled deionized water.

Pure ERD and dosage forms

A pure sample of the ERD working standard, provided by Global Napi Pharmaceuticals, Cairo, Egypt, had a purity of 100.75 ± 1.35% according to the published method [6]. Mucotec capsules, each containing 300 mg of ERD, were acquired from Global Napi Pharmaceuticals in Cairo, Egypt, whereas Mukemi capsules,

also containing 300 mg of ERD each tablet, were sourced from Mukemi capsules. These tablets were procured from the local market.

Standard solutions preparation

To prepare a standard solution of ERD (100 µg/mL), 10 mg of pure ERD was dissolved in bidistilled water. The solution was subsequently diluted to a final volume of 100 mL with bidistilled water in a 100 mL volumetric flask. The standard solutions maintained stability for a minimum of one week at 4°C. A stock solution of NBS from Sigma-Aldrich, with a concentration of 200 µg/ml, was prepared by dissolving approximately 0.02 g of NBS in the minimal volume of bidistilled water in a 100 ml volumetric flask. Subsequently, the solution was diluted with distilled water to the desired concentration and calibrated [30]. A 1.0% w/v potassium bromide (KBr) solution was prepared by dissolving 1.0 g of KBr in 100 ml of bidistilled water. A 5.0 mol/l HCl solution was prepared by diluting 43 ml of concentrated HCl (Merck, Darmstadt, Germany, Sp. gr. 1.18, 37%) with bidistilled water to a final volume of 100 ml. The solution was subsequently standardized in accordance with the recommended process [31] prior to utilization. All standard solutions were preserved in a refrigerator during periods of inactivity.

A stock solution of AM, MB, or IC (Sigma-Aldrich, 90% dye concentration) was produced by dissolving precisely 112 mg of dye in bidistilled water to achieve a concentration of 1000 µg/ml. The solutions were then diluted to the specified volume using a 100 ml calibrated flask. The solution was then diluted to 200 µg/ml of dye.

Recommended procedures

Various aliquots (0.1-1.6 ml), (0.1-1.2 ml), and (0.1-1.4 ml) of a standard 100 µg/ml ERD solution were dispensed into a series of 10 ml calibrated flasks utilizing AM, MB, and IC techniques, respectively, via a micro burette. To each flask, 2.0 mL of 2.0 mol/l HCl, 1.5 ml of NBS (200 µg/ml), and 1.0 mLl of 1.0% (w/v) KBr were added sequentially. The flasks were sealed, their contents combined, and set aside for 5.0 minutes with intermittent agitation. Subsequently, 1.5 ml of the (AM, MB, or IC) solution was introduced into each flask, well mixed, and the volume was adjusted to the mark with water. The absorbance of each solution was recorded at 520, 663, and 610 nm for the AM, MB, and IC techniques, respectively, after 3.0 minutes against a reagent blank. A standard graph was constructed by graphing absorbance against the amounts of ERD in all methods. The concentration of the unknown analyte was determined from the calibration graph or calculated using the regression equation generated from Beer's law data.

Procedure for dosage forms

Twenty capsules of ERD were precisely measured and then pulverized into a fine powder. The precise mass of the powdered tablets, corresponding to 10 mg of MXF, was dissolved in bidistilled water within a 100 ml volumetric flask. The solution was stirred for 5.0 minutes and then filtered through Whatman No. 42 filter paper. The filtrate was combined with distilled water in a 100 ml volumetric flask until the appropriate volume was reached, resulting in a stock solution of ERD (100 µg/ml). Spectrophotometric techniques were utilized to examine this solution. Subsequently, a suitable segment was analyzed using the aforementioned approaches. Ascertain the exact quantity of dose forms by utilizing the relevant regression equation.

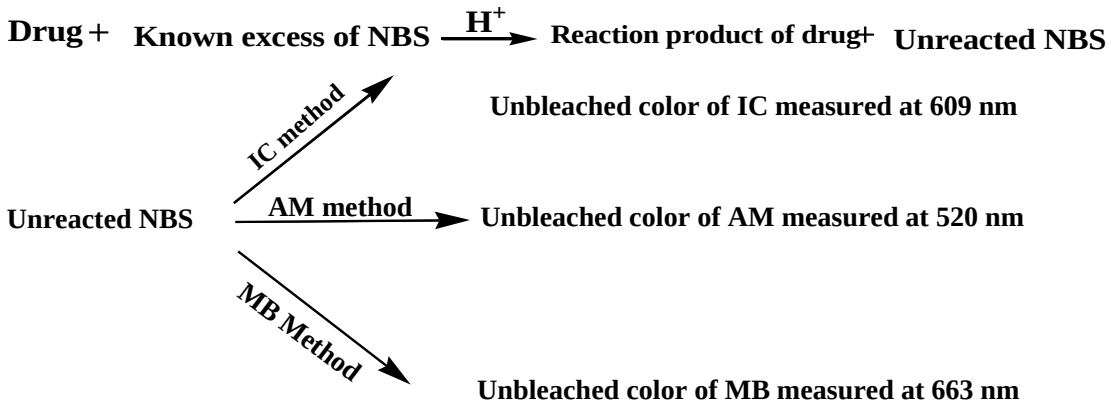
RESULTS AND DISCUSSION

Absorption spectra

In an acidic environment, oxidizing agents induce a permanent transformation of several hues into colorless substances [32]. The described methods rely on the reaction between ERD and a substantial quantity of NBS, followed by the quantification of NBS via its interaction with a specified amount of AM, MB, and IC dye. Subsequently, absorbance is measured at wavelengths of 520, 663, and 609 nm. These methods utilize the bleaching capability of NBS on the dyes, resulting in the loss of color due to oxidative degradation. As the quantity of ERD escalates, the concentration of NBS diminishes due to depletion, leading to a concomitant reduction in its concentration. When a constant amount of dye is introduced to solutions with diminishing concentrations of NBS, there is a corresponding increase in the dye concentration. Thus, a definitive correlation exists between the ERD concentration and the increase in absorbance at the specific λ_{max}.

Chemistry of the reaction

NBS is an exceptionally efficient compound capable of oxidizing or brominating other substances. It is extensively utilized in the analysis of several medicinal agents and is considered the primary organic chemical containing bromine for this application. Furthermore, it is utilized to incorporate bromine atoms into alkenes at the allylic position [33]. The reaction had two successive stages. The initial procedure entailed the bromination of the ERD employing a surplus of NBS in hydrochloric acid solution. During the second stage, the residual NBS was quantified by reacting it with defined quantities of AM, MB, and IC dyes, followed by absorbance measurement at their corresponding λ_{max}. The proposed reaction of the spectrophotometric methods is depicted in Scheme 1. The absorbance exhibited a clear association with the ERD concentration throughout all processes. The previously described methods utilize the whitening characteristics of NBS on dyes, resulting in color fading due to the oxidative degradation of the dye.



Scheme 1. The suggested chemical pathway for the proposed spectrophotometric methods entails the use of NBS and dyes.

Analytical parameters optimization

Identification and quantification of acid type and concentration

The ERD and NBS performed a reaction in various acidic solutions, including HCl, H_2SO_4, HNO_3, and CH_3COOH. Outstanding outcomes were achieved in an HCl environment. A study was performed to assess the impact of HCl content. The HCl concentration was varied between 0.25 and 3.0 ml of HCl (5.0 mol/l), while maintaining constant amounts of NBS and ERD. The results demonstrated that using a volume of 1.0-3.0 ml of hydrochloric acid (HCl) at a concentration of 5.0 mol/l yielded absorbance values that were almost identical in the presence of ERD. When the acid volume was below 2.0 ml, the reaction progressed at a diminished rate and failed to reach completion. Consequently, a constant volume of 2.0 ml of HCl (5.0 mol/l) was utilized, as illustrated in Figure 2.

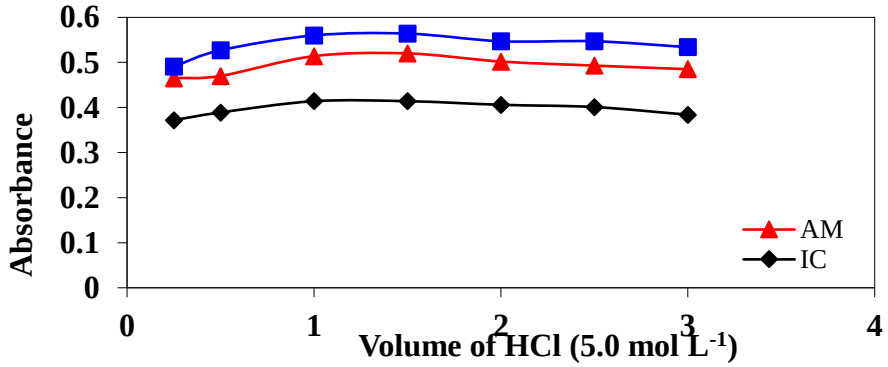


Figure 2. Effect of volume of HCl (2.0 mol/L) of the oxidation product of ERD (12 µg/mL) with NBS and dyes (200 µg/mL).

Effect of NBS

To determine the ideal concentration of NBS, several amounts of NBS (200 µg/ml) ranging from 0.25 to 3.0 ml were combined with a fixed amount of dye in HCl solution. The analysis determined that the highest absorbance value was attained with the application of 1.5 ml of NBS (200 µg/ml) (Figure 3).

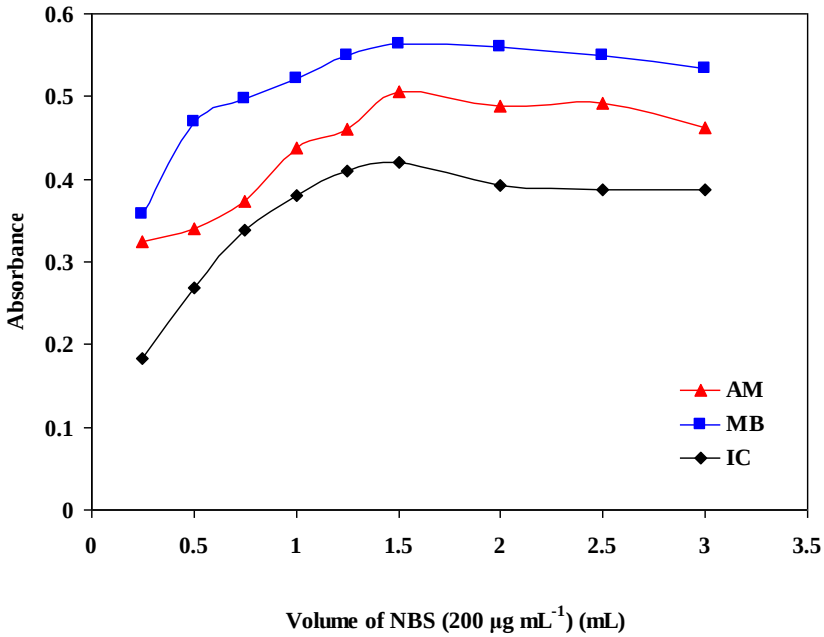


Figure 3. Effect of volume of NBS volume (200 µg/ml) on the oxidation product of ERD (12 µg/ml) in presence of dyes in HCl medium.

Effect of KBr

The influence of KBr concentration on the oxidation process was examined by altering the volume of KBr (1.0%, w/v) between 0.5 and 2.5 ml, while maintaining constant concentrations of ERD (12 µg/ml), acid (HCl, 5.0 mol/L), oxidant (NBS, 200 µg/ml), and dye (200 µg/ml). A volume of 1.0 mL of KBr (1.0%, w/v) was selected as the optimal quantity to enhance the oxidation process.

Effect of dye

A study was conducted to determine the optimal volumes of AM, MB, and IC dyes at a concentration of 200 µg/ml that would produce the highest color intensity. A study was conducted to examine the effect of dye volume, varying from 0.25 to 3.0 ml, for each dye. The experiment revealed that the oxidation products had the highest color intensity with the application of 1.5 ml of dye solution (Figure 4).

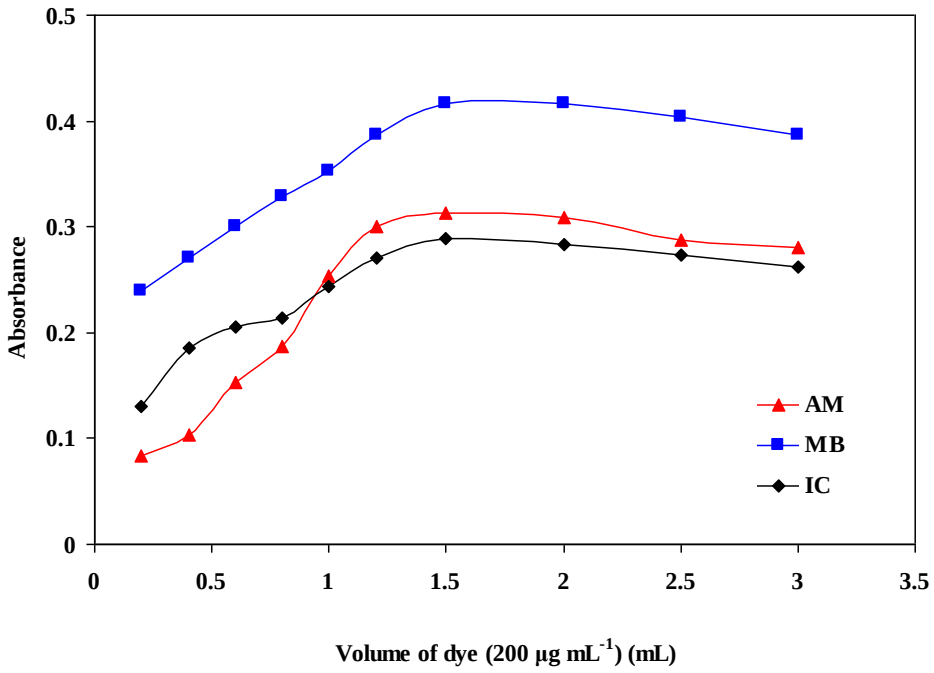


Figure 4. Effect of volume of dyes (200 µg/ml) of the oxidation product of ERD (12 µg/ml) with NBS in HCl medium.

Effects of temperature and duration of mixing

A study was conducted to examine the effect of temperature on various sample and blank solutions. The solutions experienced temperature fluctuations between 25 and 60°C by immersion in a water bath. The highest level of color intensity was achieved at 25±2°C. Increasing the temperature yields inconsistent outcomes. Thus, the effect of time on the oxidation process completion was examined across a duration of 2.0 to 20 minutes. The experiment revealed that sustaining contact for 5.0 minutes at a temperature of 25±2°C reliably yielded strong and reproducible absorbance values. A standing duration of 3.0 minutes is necessary to fully eliminate the dye pigment utilizing the residual NBS. The dye absorbance that did not react remained stable for at least 12 hours thereafter.

Impact of the sequence of addition

The ideal order of addition was: ERD, HCl, NBS, KBr, and finally, the dye. Under identical testing settings, the alternate sequences exhibited reduced absorbance values.

Method validation

Linearity and sensitivity

A clear association was seen between the absorbance at the peak wavelength and the ERD concentration. This relationship was established under optimal conditions. The ERD concentration ranges were 1.0-16, 1.0-12, and 1.0-14 µg/ml for the AM, MB, and IC techniques, respectively. The Ringbom concentration range [34] was determined to provide precise results. The limits of detection (LOD = 3s / k) and quantitation (LOQ = 10s / k) for the proposed methodologies were established. The variable "s" is the standard deviation of ten repeated measurements of the reagent blank, whereas "k" signifies the sensitivity, defined as the slope of the calibration curve. The resultant LOD values were 0.29, 0.28, and 0.30 µg/ml, while the LOQ values were established at 0.97, 0.93, and 1.0 µg/ml for the AM, MB, and IC procedures, respectively. The proposed methodologies were evaluated for validity by statistical analysis [35], juxtaposing the outcomes obtained from these methodologies with the established method [6]. The results of the Student's t-test and variance ratio F-test (Table 1) indicate

no statistically significant difference in accuracy and precision between the proposed strategy and the one outlined in reference [6].

Table 1. Analytical and regression parameters of the proposed approaches for determining ERD.

Parameters	**AM**	**MB**	**IC**
Beer's law limits, µg/ml	1.0-16	1.0-12	1.0-14
Ringboom limits, µg/ml	3.0-13	3.0-10	3.0-12
Molar absorptivity, x 10^4 L/mol.cm	0.4316	1.1135	0.7285
Sandell sensitivity, ng/cm^2	57.76	22.39	34.22
Regression equation[a]			
Intercept (a)	0.0034	0.0107	0.0056
Standard deviation of intercept (S_a)	0.014	0.008	0.004
Slope (b)	0.0312	0.0451	0.0269
Standard deviation of slope (S_b)	0.016	0.011	0.003
Correlation coefficient, (r)	0.9994	0.9995	0.9995
Mean ± SD	100.04±1.12	100.36±1.40	99.83±1.34
RSD%	1.12	1.40	1.34
RE%	1.18	1.47	1.41
Limit of detection, µg/ml	0.29	0.28	0.30
Limit of quantification, µg/ml	0.97	0.93	1.0
Calculated *t*-value [b]	0.47	0.72	0.38
Calculated *F*-value [b]	1.14	1.78	1.63

[a] $A = a + bC$, where C is the concentration in µg mL^{-1}, A is the absorbance units, a is the intercept, b is the slope.

[b] The theoretical values of t and F are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

Accuracy and precision

To assess the accuracy and precision of the proposed approaches, we produced and examined solutions with three distinct ERD concentrations. A study was conducted on six comparable samples. The findings from this study are succinctly presented in Table 2. To evaluate the accuracy and precision of the proposed methods, one may examine reduced values of the percentage relative error (RE%) and relative standard deviation (RSD%), respectively. To assess the accuracy and precision of the provided approaches, one can analyze lower values of the relative standard deviation (RSD%) and percentage relative error (RE%). The findings from this study are succinctly presented in Table 2. The inter-day and intra-day accuracy and precision of the processes were assessed, revealing that the proposed techniques exhibit outstanding levels of accuracy and precision, indicating high repeatability and reproducibility.

Table 2. Intra-day and inter-day accuracy and precision of the proposed approaches.

Method	Taken (µg/ml)	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
	Intra-day				
AM	5.0	99.40	0.63	-0.60	4.97 ± 0.033
	10	99.20	1.20	-0.80	9.92 ± 0.125
	15	99.60	1.53	-0.40	14.94 ± 0.24
MB	3.0	99.60	0.80	-0.40	2.988 ± 0.025
	6.0	100.50	1.10	0.50	6.03 ± 0.07
	9.0	100.30	1.80	0.30	9.027 ± 0.171
IC	4.0	99.60	0.88	-0.40	3.984 ± 0.037
	8.0	100.30	1.06	0.30	8.024 ± 0.089
	12	99.20	1.50	-0.80	11.904 ± 0.187
	Inter-day				
AM	5.0	99.30	1.04	-0.70	4.965 ± 0.054
	10	100.80	1.60	0.80	10.08± 0.169
	15	100.70	2.05	0.70	15.105 ± 0.325
MB	3.0	99.50	0.90	-0.50	2.985 ± 0.028
	6.0	100.40	1.30	0.40	6.024 ± 0.082
	9.0	99.70	1.25	-0.30	8.973 ± 0.118
IC	4.0	100.40	0.63	0.40	4.016 ± 0.027
	8.0	99.60	0.92	-0.40	7.968 ± 0.077
	12	99.20	1.70	-0.80	11.904 ± 0.212

[a] RSD%, percentage relative standard deviation; RE%, percentage relative error.

[b] Mean ± standard error, confidence limit at 95% and five degrees of freedom (t = 2.571).

Robustness and ruggedness

The robustness was assessed by analyzing the impact of minor variations in technique variables, such as the concentration of analytical reagents and reaction time, on the efficacy of the suggested methods. The analysis was conducted using modified parameters, utilizing three distinct concentrations of ERD. The employed methodologies remained unchanged, as evidenced by the RSD% ranging from 0.50% to 2.50%. The robustness of the methodologies was assessed by calculating the RSD% of the procedure performed by three different analyzers using three independent instruments. The intra-analyst RSD% ranged from 0.80% to 2.30%, while the inter-instrument RSD% ranged from 0.70% to 2.25%, demonstrating the reliability and consistency of the proposed techniques. The data is displayed in Table 3.

Table 3. Robustness and ruggedness results of the developed methods. (n=3).

Methods	**Nominal amount concentration (µg/ml)**	**RSD%**			
		Robustness		**Ruggedness**	
		Variable alerted [a]			
		Acid volume	Reaction time	Different analysts	Different instruments
AM	5.0	1.25	0.90	1.20	0.80
	10	1.30	1.90	1.80	1.60
	15	2.0	2.10	2.30	1.90
MB	3.0	1.30	0.50	0.80	0.70
	6.0	1.80	1.10	1.20	1.20
	9.0	2.10	1.70	2.30	2.20
IC	4.0	1.0	0.60	0.80	0.90
	8.0	1.70	1.80	1.60	1.80
	12	2.30	2.50	1.80	2.25

[a] Volume of (5.0 mol/ml) HCl is (2.0±0.2 mL) and reaction time is (5.0±2.0 min) (after adding NBS) were used.

Recovery studies and application

A recovery experiment was done using the usual addition approach to evaluate the accuracy, reliability, and validity of the proposed methodologies. The experiment involved administering three distinct MXF concentrations to a predetermined amount of ERD in tablets that had previously been analyzed. The final concentration was then determined according to the prescribed protocols. The determination was conducted three times at each level, and the recovery was computed using the following equation:

$$\textbf{\% Recovery} = \frac{[C_F - C_T]}{C_p} \times 100$$

The variable C_F denotes the total concentration of ERD that was identified. C_T denotes the concentration of ERD present in the pill. C_P denotes the unadulterated ERD concentration that was introduced into the pill. The results of this study, as presented in Table 4, demonstrate that the accuracy of the proposed procedures was unaffected by the various compounds incorporated into the formulations. Table 4 presents the statistical variance of the data obtained from evaluating ERD using the proposed approaches and the previously described method [6], by calculating Student's t-test and F-value at a 95% confidence level with 5 degrees of freedom [35]. The results corroborate the specified label claim. Therefore, there is no significant difference between the proposed methods and the established ones.

Table 4. Application of the developed methods for the determining ERD in dosage forms.

Sample	**Taken (µg/ml)**	**Added (µg/ml)**	**Recovery [a] (%)**			**Reported method [6]**
			AM	**MB**	**IC**	
Mucotec capsules	4.0	2.0	99.20	99.60	99.0	
		4.0	99.30	99.70	99.20	
		6.0	98.90	99.10	100.50	
Mean ± SD[a]			99.13±0.21	99.47±0.32	99.57±0.814	99.40±0.46
RSD% [a]			0.21	0.32	0.814	0.463
Variance			0.043	0.103	0.663	0.21
t-value [b]			1.19	0.28	0.41	
F-value [b]			4.88	2.04	3.16	
Mukemi capsules	4.0	2.0	99.60	99.30	100.30	
		4.0	99.80	99.10	99.0	
		6.0	100.90	99.80	99.50	
Mean ± SD[a]			100.10±0.70	99.40±0.36	99.60±0.66	99.70±0.58
RSD% [a]			0.70	0.36	0.66	0.58
Variance			0.49	0.13	0.43	0.336
t-value [b]			0.98	0.98	0.25	
F-value [b]			1.46	2.58	1.28	

[a] Average of six determinations; SD: standard deviation; RSD%: percentage relative standard deviation.

[b] Theoretical values of t and F are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

CONCLUSION

The suggested spectrophotometric approaches provide good sensitivity, selectivity, cost-effectiveness, accuracy, and precision for the quantification of ERD in dosage forms. The procedures are not affected by color instability due to the involvement of dye bleaching. The duration necessary for the complete analysis is about 10 to 15 minutes. They also obviate the necessity for critical experimental elements, perilous extraction methods, and the use of toxic solvents, thereby conserving time. These methods accurately quantified the quantity of ERD in both pure and dose forms. The methods used employ NBS as a sustainable brominating agent and have been rigorously validated according to International Council for Harmonisation (ICH) standards. Consequently, the suggested methodologies are advisable for the standard analysis of ERD in quality control laboratories.

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