Original Article

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Spectrophotometric Determination of Erdosteine at Capsule Dosage Forms

Erdostein'in Kapsül Dozaj Formlarında Spektrofotometrik Olarak Analizi

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ABSTRACT

Objective: Two simple, rapid and sensitive methods were developed for determination of Erdosteine (ERD) in pure form as well as in their pharmaceutical formulations.

Methods: The methods were based on formation of colored charge transfer complexes with ERD with chloranil (CA) and 7,7,8,8-tetracyanoquinodimethane (TCNQ). The obtained charge-transfer complexes were measured at 454 and 843 nm for CA and TCNQ methods, respectively. Optimization of different experimental conditions were investigated.

Results: Beer's plots were obeyed in a general concentration range of 10-500 μg mL^{-1} and 20-600 μg mL^{-1} for CA and TCNQ methods, respectively. The validity of methods in terms of specificity, linearity, accuracy, precision, limit of detection and limit of quantitation were evaluated.

Conclusion: The methods were applied successfully in the determination of ERD in capsule dosage forms. Developed new spectrophotometric methods have been found to be very practical and practical. The lack of complex sample preparation increases the applicability of the method.

Keywords: Erdosteine, charge transfer complex, TCNQ, CA, spectrophotometry, validation, pharmaceutical formulation

ÖZ

Amaç: Erdostein'in (ERD) farmasötik formülasyonlarında tayini için iki basit, hızlı ve hassas yöntem geliştirilmiştir.

Yöntemler: Metotların esası, kloranil (CA) ve 7,7,8,8-tetrasiyanokinodimetan (TCNQ) ile ERD arasında renkli yük transfer komplekslerinin oluşturulmasına dayanmaktadır. Maksimum dalga boyları CA ve TCNQ yöntemleri için sırasıyla 454 ve 843 nm'de ölçülmüştür. Deney koşullarının optimizasyonu araştırılmıştır.

Bulgular: Beer kuralı, CA ve TCNQ metotları için sırasıyla 10-500 μg mL^{-1} ve 20-600 μg mL^{-1} konsantrasyon aralığında bulundu. Yöntemler özgünlük, doğrusallık, doğruluk, kesinlik, gözlenebilme sınırı, tayin sınırı gibi validasyon parametreleri açısından değerlendirildi.

Sonuç: Metodlar ayrıca ilaç içeren kapsüllerde ERD'nin tayininde de başarıyla uygulandı. Geliştirilen yeni spektrofotometrik metotların oldukça pratik ve uygulanabilir olduğu belirlenmiştir. Örnek hazırlama aşamasında karmaşık numune hazırlama işlemlerine gereksinim duyulmaması yöntemin uygulanabilirliğini artırmaktadır.

Anahtar Sözcükler: Erdostein, yük transfer kompleksi, TCNQ, CA, spektrofotometri, validasyon, farmasötik formülasyon

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Introduction

Erdosteine (ERD), a potent mucolytic agent, shows pharmacological activity by lowering the viscosity of mucus in the respiratory system and thus reducing the ability of the bacteria to adhere to the cellular membrane. ERD has anti-inflammatory properties in the bronchial airways and scavenges free radical compounds from the airways. Chemical structure of ERD consists of thiolactone and carboxyl group. Its chemical formula is 2- [2-oxo-2 - [(2-oxothiolan-3-yl) amino] ethyl] sulfanylacetic acid (Figure 1) (1-3).

Various analytical methods such as UV spectrophotometric method (4-8) and high performance liquid chromatography (9-14) have been found in the literature for the determination of ERD from pharmaceutical preparations and biological fluids. In this study, two spectrophotometric methods were proposed to determine the amount of ERD in pharmaceutical preparations using easily accessible materials and equipment. In the proposed methods, CA and tetracyanoquinodimethane (TCNQ) reagents were reacted with ERD to form charge transfer complexes (CT complexes). For quantitative analysis of pharmaceutical compounds in pharmaceutical dosage forms, the use of CA and TCNQ reagents to obtain CT complexes is preferred because they do not require a buffer, they are fast, precise and cost-effective (15-18). Therefore, in this study, we considered it appropriate to develop two methods for the analysis of ERD using these two markers, and these developed methods were successfully applied in the analysis of ERD in pharmaceutical formulations.

Method

Devices

Spectrophotometric measurements were performed using a Hitachi spectrometer Model U-2900 equipped with a xenon lamp and 1 cm quartz cells.

Reagents and Solutions

ERD was obtained from EnzyChem Lifesciences (Korea), TCNQ Fluka (Neu-Ulm, Germany) and CA Merck (Darmstadt, Germany). The pharmaceutical preparation (ERDOSTIN® 300 mg) was obtained from the pharmacy. All chemicals and reagents were used for analytical purity.

Stock Solutions

Stock solutions of ERD were prepared in methanol to make up 1 mg/mL. Solutions of 0.2% (w/v) for TCNQ and 0.2% (w/v) for CA were prepared in acetonitrile. The solutions were determined to be stable for 1 week at 4 °C.

General Analysis Method

ERD stock solution in volumes of 0.050-2.5 mL and 0.100-3.0 mL was added to 5 mL calibrated flasks for CA and TCNQ methods, respectively. The volume of the stock solutions in each flask was brought to 2.5 mL with acetonitrile for the CA method and 3.0 mL for the TCNQ method, and 0.75 mL CA and 1 mL TCNQ reagents were added to them. The reaction mixture was heated at 80 °C for 5 min for the TCNQ method and then stood at room temperature for 5 min for CA. After the cooling process, it was diluted to 5 mL with methanol and its absorbance was measured against the blank test at 454 and 843 nm for CA and TCNQ methods, respectively. Calibration charts were prepared by measuring the absorbance against the ERD concentration.

Analysis Method for Capsules

The amount equivalent to 300 mg ERD was weighed and dissolved in 125 mL of methanol. Then it was extracted in a mechanical mixer for 20 minutes and in an ultrasonic bath for 20 minutes. The volume was made up to 250 mL and then filtered through filter paper. The filtrate was diluted with methanol and studied as in the preparation of the calibration curve. The amount of substance in the capsule was measured using the calibration graph and the corresponding regression equation.

Results

The maximum absorption of the CT complexes obtained as a result of the reaction formed by ERD with CA and TCNQ reagents was observed at 454 and 843 nm, respectively (Figure 2).

Optimum conditions such as reaction time, temperature, type of solvent, amount of reagents used and reaction stoichiometry were also investigated for the reaction, which were explained in detail below.

Choosing the Most Suitable Solvent

Various solvents commonly used in analytical procedures including acetonitrile, chloroform, methanol, acetone, ethanol, 1,4-dioxane and methylene chloride were used to determine

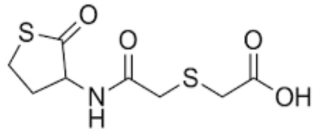

Figure 1. Chemical structure of Erdosteine

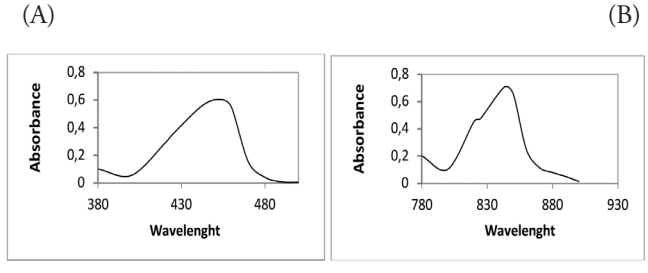

Figure 2. (A) Absorption spectrum of ERD-CA complex against blank solution (400 µg mL-1) (B) Absorption spectrum of ERD-TCNQ complex against blank solution (600 µg mL-1)

CA: Chloranil, TCNQ: Tetracyanoquinodimethane, ERD: Erdosteine

the most suitable solvent. It was observed that the most suitable solvent was obtained by using methanol.

Reagent Concentration

The optimum reagent concentration was investigated by changing the concentrations of TCNQ and CA reagents and keeping the ERD concentration constant. As shown in the figure, the optimum reagent amount was 0.75 mL CA [0.2% (w/v)] and 1.0 mL TCNQ [0.2% (w/v)] (Figure 3).

Reaction Time

The time required to complete the reaction between ERD and CA and TCNQ was studied spectrophotometrically at room temperature and 60-80 °C, respectively. A reproducible color development was achieved in 5 minutes for CA and TCNQ, respectively, at room temperature and 80 °C. The color reaction resulting from the CT complexes was observed stably for 12 hours (Figure 4).

Reaction Stoichiometry

Job's continuous change method was used for the reaction stoichiometry (19). According to the results, the equivalent molarity of ERD and reagents was defined as the 1:1 ratio (compound/reagent).

Method Validation

The proposed analytical methods were validated according to the ICH guideline Q2 (R1) (20). Calibration curves were generated for all methods under the above conditions. Regression equation, correlation coefficients, Beer's law limits, limit of observability (LOD) and determination limit (LOQ) data for each method are given in Table 1.

According to the results obtained, a linear correlation between 10-500 μg mL^{-1} and 20-600 μg mL^{-1} was observed for CA and TCNQ methods, respectively.

The formula of LOD/LOQ = κSDa/b was used to calculate LOD or LOQ. Here the value of is 3 for LOD and 10 for LOQ. SDa indicates the standard deviation of the scale curve intersept and b is the slope. The results are shown in Table 1.

Sensitivity values of intra-day and inter-day were examined at 50, 100 and 500 μg/mL for the TCNQ and CA method (n=5 for each) for 5 consecutive days. The % RSD values for the inter-day precision % and the inter-day precision results for all

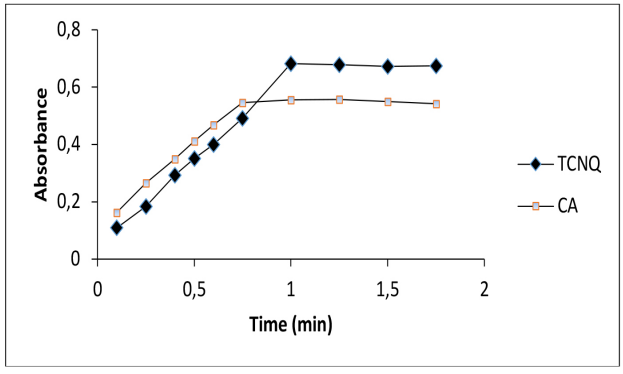

Figure 3. Effect of volumes of CA (0.2%, w/v) and TCNQ (0.2%, w/v) reagents on the formation of the reaction product of ERD with CA and TCNQ

CA: Chloranil, TCNQ: Tetracyanoquinodimethane, ERD: Erdosteine

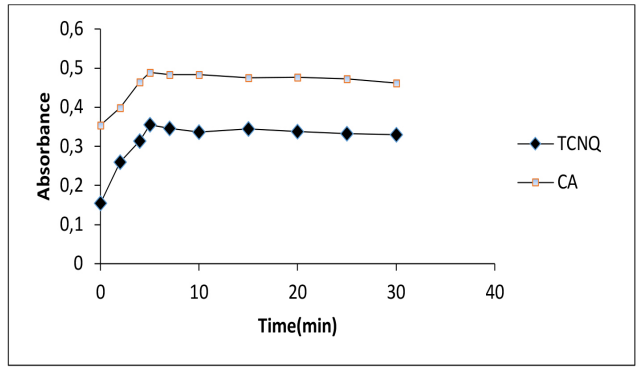

Figure 4. Effect of temperature and heating time on reaction of TCNQ (at 80 °C) and CA (at room temperature) reagents with ERD

CA: Chloranil, TCNQ: Tetracyanoquinodimethane, ERD: Erdosteine

Table 1. Validation parameters

	CA reagent	TCNQ reagent
Linear range[a] (μg mL^{-1})	10-500	20-600
Regression equation[b]		
Slope ± SD	0.0016±0.000008	0.0013±0.000015
Intersept ± SD	0.0711±0.00032	0.0612±0.00014
Correlation coefficient, r	0.9997	0.9995
LOD (μg mL^{-1})	0.00034	0.00074
LOQ (μg mL^{-1})	0.0011	0.0025
Precision		

[a]Average of 6 studies
A=mC+b [C: Concentration (μg mL^{-1}) and A: Absorption at λ_{max})
SD: Standard deviation, CA: Chloranil, TCNQ: Tetracyanoquinodimethane, LOD: Limit of obsercability detection, LOQ: Limit of quantification

proposed methods provided good reproducibility. Results are given in Table 2. The accuracy of the developed methods was examined using the standard addition technique. Pure analyte was mixed with standard solutions at 3 different concentration levels on the sample solution and analyzed. The results obtained are presented in Table 3. It was observed that the average recovery percentages calculated were 100.31% for CA and 100.82% for TCNQ, proving the method to be of high accuracy (Table 2). The methods developed were been successfully applied in the analysis of the drug substance in pharmaceutical preparations, and according to these results, no interference from additives and excipients was observed. The results are given in Table 4. Small changes were made to the method developed to test the robustness of the method and the effect of these changes on the method was examined. For this, changes in TCNQ and CA reagent concentrations (%, w/v ±0.05) and reaction times (optimum time ±0.5 min) were made, and when the results were examined in terms of recovery and RSD values, it was observed that there was no significant difference.

Table 2. Precision results

Precision results	Added concentration (μg mL^{-1})	Concentration found (μg mL^{-1}) (mean ± SD[c])	RSD (%)
intra-day			
CA reagent	50	50.01±0.28	0.56
	100	100.28±0.87	0.87
	500	501.64±1.43	1.43
TCNQ reagent	50	50.04±0.37	0.74
	100	99.97±0.92	0.92
	500	503.36±1.65	0.33
inter-day			
CA reagent	50	50.16±0.56	1.12
	100	101.97±1.34	1.31
	500	502.64±1.83	0.35
TCNQ reagent	50	50.29±0.47	0.93
	100	101.31±1.34	1.32
	500	503.37±2.2	0.44

SD: Standard deviation, CA: Chloranil, TCNQ: Tetracyanoquinodimethane, RSD: Relative standard deviation

Table 3. Recovery results

Method developed	Concentration taken[a] (μg mL^{-1})	Added concentration (μg mL^{-1})	Concentration found[b] (μg mL^{-1}) (mean ± SD[c])	Recovery (%)	RSD (%)
CA reagent	100	10	109.36±1.12	99.42	1.02
		200	301.28±2.74	100.43	0.91
		400	505.39±3.86	101.08	0.76
TCNQ reagent	100	20	120.68±1.24	100.57	1.03
		200	303.87±3.01	101.29	0.99
		500	603.54±4.27	100.59	0.71

[a]ERDOSTIN® 300 mg, [b]n=5, [c]Standard deviation, SD: Standard deviation, RSD: Relative standard deviation, TCNQ: Tetracyanoquinodimethane

Table 4. Analysis of capsules containing 300 mg erdosteine (n=5)

	When Using CA reagent (mean ± SD[c])	When Using TCNQ reagent (mean ± SD[c])
Mean ± SD	302.08±3.28	300.94±2.64
Recovery (%)	100.69	100.31
RSD (%)	1.09	0.88

[a]ERDOSTIN® 300 mg
[b]n=5
[c]Standard deviation, SD: Standard deviation, CA: Chloranil, TCNQ: Tetracyanoquinodimethane

Conclusion

As a result, the new spectrophotometric methods developed are very practical and applicable. Not requiring complicated sample preparation processes before hand increases the applicability of the method. The developed methods enable the analysis of ERD in pharmaceutical preparations with high accuracy and precision. This method can be used in routine analysis of the drug.

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: C.Ö., Design: C.Ö., Data Collection or Processing: C.Ö., D.D., Analysis or Interpretation: C.Ö., D.D., Literature Search: D.D., Writing: C.Ö., D.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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