Optimization Quantitative Determination of Cimetidine in Pharmaceutical Preparations via Bromothymol Blue Using Central Composite Design

الظروف الفضلى للتقدير الكمي للسميتيدين في المستحضرات الصيدلانية مع البروموثايمول الأزرق باستخدام تصميم التراكم المركزي

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الخلاصة:

تم تحديد الظروف الفضلى لتكوين معقد المزدوج الايوني بين السميتدين و البروموثايمول الازرق باستخدام طريقة تصميم التراكم المركزي (CCD) حيث تم دراسة تأثير ثلاثة عوامل هي الدالة الحامضية و تركيز الكاشف و زمن الرج. وكانت افضل الظروف التي تم الحصول عليها هي pH=5.53 و %BTB=0.05% و زمن الرج = 5.71 دقيقة. وبتطبيق هذه الظروف اظهر منحني المقايسة علاقات خطية لمدى من التراكيز (0.5 – 15) مايكروغرام / مل عند الطول الموجي 427.5 نانومتر وبحد كشف 0.222 مايكروغرام/ مل. لقد اظهرت النتاج التي تم الحصول عليها ان الطريقة المقترحة خالية من تأثير التداخلات المتوقعة من المكونات التي تتواجد عادة في المستحضرات الصيدلانية ، وقد أمكن تطبيق الطريقة بنجاح لتقدير السميتيدين في بعض تلك المستحضرات.

الكلمات المفتاحية: الظروف المثلى, سميتيدين, المزدزج الأيوني, تصميم التراكم المركزي.

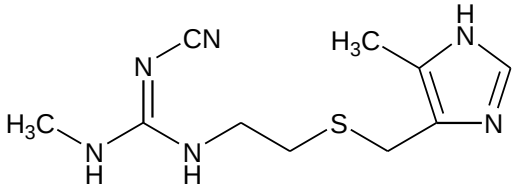
Abstract

Optimum conditions for ion-pair complex formation between cimetidine-bromothymol blue (BTB) are determined using central composite design (CCD) by investigating the effects of three experimental factors including pH, reagent concentration and shaking time. The optimum conditions were found to be pH=5.53, %BTB=0.05% and shaking time=5.71 minutes. The linearity dynamic range for cimetidine are (0.5-15) $\mu g.mL^{-1}$ at 427.5 nm with detection limit of 0.222 $\mu g.mL^{-1}$. The results show the absence of interferences from the excipients on the determination of the above drug. The proposed method has been successfully applied for the determination of cimetidine in pharmaceutical preparations.

Keywords, Optimization, Cimetidine, Ion-pair, Central composite design

Introduction

Histamine H_2-receptors antagonist, competitively inhibit the action of histamine on its receptors of parietal cell (1). Cimetidine was the first histamine H_2- receptor antagonist approved by the Food and Drug Administration USA, for the treatment of duodenal ulcers, Zollinger-Ellison syndrome, and other gastric hypersecretory states (2), the drug was also approved for the treatment of peptic ulcers disorders (3). There are also reports that cimetidine, due to its effects on the immune system and as an H_2-receptor antagonist, can inhibit growth of carcinogen-induced colonic tumors in rats, as well as the in vitro human colon cancer cell (4).It is also prescript for the relief heartburn in peptic, duodenal ulcers and prevents rebleeding in patients which reduce the secretion of gastric acid (5). The Chemical structure of cimetidine is given in (Scheme 1), which shows the imidazole ring (6).



Scheme 1: **The chemical structure of cimetidine**

Several methods have been reported for determination of cimetidine in bulk and pharmaceutical dosage forms, these methods include titrimetry(7), high performance liquid chromatography(8,9) high performance thin layer chromatography(10), ion selective electrode(11,12), and capillary electrophoresis(13), some of these methods are suffer from large solvent consumption and time-consuming, tedious, and/or dedicated to sophisticated and expensive analytical instruments. Spectrophotometry(14-19) are most convenient techniques because of their inherent simplicity, adequate sensitivity, low cost and wide availability in all quality control laboratories.

In experimental chemistry, the optimization of technical system is the process of the adjusting the control variables to find the levels that achieve the best optimization. Usually, many conflicting response must be optimized simultaneously. In lack of systematic approaches the optimization is done by trial and error, or by changing one control variable at a time while holding the rest constant, such methods are requires a lot of experiments to be carried out.

Design of experiment is a structured, organized method that is used to determine the relationship between the different factors affecting a process and the output of that process. This method was first developed by Ronald Fisher in the 1920s and1930, a renowned mathematician and geneticist (20). It could be used in research as well as in industrial settings and also for very different purposes. The primary goal in scientific research is usually to show the statistical significance of an effect that a particular factor exerts on the dependent variable of interest. The (statistical) design of experiments is an efficient procedure for planning experiments so that the data obtained can be analyzed to yield valid and objective conclusions and is widely used in research and development(21,22).

Apparatus:

A Cintra 5 spectrophotometer with 1 cm quartz cells was used for absorbance measurements. pH-meter DW-9421 from Philips instrument, a Sartorius BL 210S balance, and a Pentium 4 computer (DELL) was used for data processing.

Experimental

Material and Reagents

All Chemicals used were of analytical reagent grad unless otherwise is mentioned, Cimetidine standard powder (purity 99.8%) were kindly provided by the State Company for Drug Industries and Medical Appliances, Samara-Iraq (SDI).

Bromothymol blue(BTB) (Aldrich), 0.1% (w/v) solution prepared by dissolving 0.1 g of the dye in 5 mL of methanol and then the solution was diluted to a final volume of 100 mL with distilled water. Working solutions were freshly prepared by subsequent dilutions.

Hydrochloric Acid (Aldrich), ~ 0.1 M, a 0.85 mL of concentrated hydrochloric acid (37% w/w, sp.gr1.18) was added to 50 mL distilled water and diluting to the mark in a 100 mL calibrated flask.

Potassium Hydroxide (fluka), ~ 0.1 M, prepared by dissolving 0.56 g of potassium hydroxide in 25 mL distilled water and diluted to 100 mL in volumetric flask with distilled water.

Phthalate buffer 0.2M solution was prepared by dissolved 4.08 g of potassium hydrogen phthalate (Merck) in 25 mL distilled water and diluted to 100 mL in volumetric flask with distilled water, the pH was adjust to 5.53 by using few drops of 0.1M HCl and\or 0.1M KOH.

Cimetidine Standard solution

Cimetidine stock solution (250 $\mu g.mL^{-1}$), was prepared by dissolving 25 mg of Cimetidine in 5mL methanol and diluting to 100mL in a volumetric flask with distilled water . Working solutions were freshly prepared by subsequent dilutions.

Experimental design and statistical analysis

Designs such as Box-Benhken or Central Composite Face, will support non-linear responses and are generally used for response-surface modeling and optimization applications (22,23). CCD for three factors at three levels was selected to optimize vaned response variable with quadratic model

was employed. Three independent variables namely pH (x_1), reagent concentration (x_2) and shaking time (x_3) were chosen. Each independent variable had 3 levels which were – 1, 0 and +1(23,25). A total 20 different combinations (including six replicates of center point each sighed the coded value 0) were chose in random order according to a CCD configuration for three factors. The coded values of independent variables were found from equations below are given in table1.

$$x_1 = (X1 - 6.5) /1.5$$
$$x_2 = (X1 - 0.04) /0.02$$
$$x_3 = (X1 - 6.0) /4.0$$

Table 1: Un-coded and coded levels of the independent variables

Independent variable	Coded unit		
	-1	0	1
pH	5	6.5	8
Reagent Conc.(%)	0.02	0.04	0.06
Shaking time (min.)	2	6	10

General Recommended Procedure

1 mL aliquots of cimetidine standard solution containing (2.5-75) µg were transferred into a series of 50 mL separating funnels, to each funnel 0.5 mL of phthalate buffer of pH 5.53 and 1 mL of 0.05% BTB reagent solutions were added. The separating funnels were shaken vigorously with 5 mL chloroform for 5.71 mints. The two phases were then allowed for clear separation and the absorbance of the yellow colored organic phase was measured at 427.5 nm against a reagent blank prepared similarly without addition of cimetidine. The calibration graph was constructed by plotting the measured absorbance of the organic phase against the drug concentration.

Analysis of cimetidine in pharmaceutical preparations

i. In tablets:

The content of 10 tablets were mixed well and a certain amount of fine powder was accurately weighted to give an equivalent to 200 mg was dissolve in 5 mL of methanol and diluted to 100mL in a volumetric flask with distilled water. The solution was filtered by using Whatman filter paper No.41 to avoid any suspended or un dissolved material before use. Working solutions were freshly prepared by subsequent dilutions with distilled water and analyzed by the recommended procedure.

ii. In Ampoules

The volume of 10 ampoules were quantitatively transferred into 250 mL volumetric flask and diluted to the mark with distilled water. An accurately measured volume (2.5mL) was transferred into 100 mL volumetric flask and diluted to the mark with distilled water. Working solutions were freshly prepared by subsequent dilutions with distilled water and analyzed by the recommended procedure.

Results and Discussion

Extractive spectrophtometric procedures are popular for their sensitivity in the assay of drugs and hence, ion pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds (26-29).

cimetidine react with BTB in acidic buffer to give yellow color chloroform soluble ion-pair complex, which exhibit absorption maxima at 427.5 nm against their reagent blanks, (Figure1). Under the experimental conditions the reagent blank showed negligible absorbance thereby permit good analytical conditions for quantitative determination of cimetidine in pharmaceutical dosage forms.

Absorption spectra

Preliminary investigations reveled that cimetidine react immediately with BTB in acidic buffered medium to produce an intense yellow colored chloroform-soluble ion-pair complex, which exhibit an absorption maximum at 427.5 nm against blank (Figure. 1). Under the experimental conditions the reagent blank showed a negligible absorbance thereby permits good analytical conditions for quantitative determination of cimetidine in pharmaceutical dosage forms.

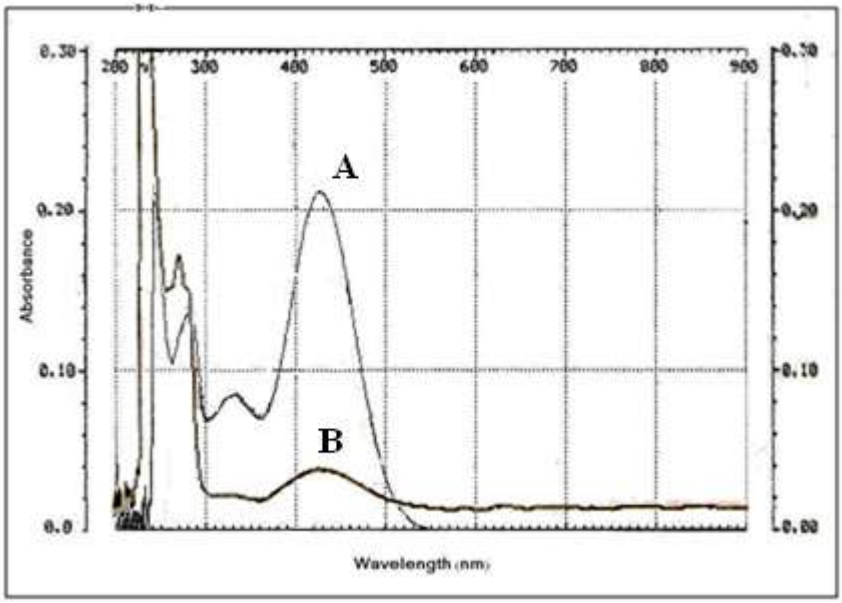


Figure 1: Absorption spectra of : (A) 4 µg.mL^{-1} cimetidine-BTB ion-pair complex against reagent blank, (B) blank solution against chloroform under the recommended procedure.

Optimization of experimental variables

Three major parameters (pH, reagent concentration, and shaking time) were optimized by the CCD procedure, while the other minor parameters were obtained by the univariate method (30). The values of these parameters were selected within specified boundaries for each at which they affected the measured absorption signal of the colored products as in table 1.

The study was carried out according to the central composite design and the experimental points used according to the design are shown in Table 2.

Table 2: The Central composite design with three independent variables (un-coded variables) and their experimental absorption values of 10 µg.mL^{-1} cimetidine ion-pair complex.

Exp. No.	pH	Reagent Conc. (%)	Mixing Time (min)	Abs.
1	5	0.02	2	0.300
2	5	0.02	10	0.291
3	5	0.06	2	0.429
4	5	0.06	10	0.417
5	8	0.02	2	0.237
6	8	0.02	10	0.230
7	8	0.06	2	0.340
8	8	0.06	10	0.330
9	5	0.04	6	0.470
10	8	0.04	6	0.372
11	6.5	0.02	6	0.322
12	6.5	0.06	6	0.460
13	6.5	0.04	2	0.420
14	6.5	0.04	10	0.408
15	6.5	0.04	6	0.460
16	6.5	0.04	6	0.460
17	6.5	0.04	6	0.460
18	6.5	0.04	6	0.460
19	6.5	0.04	6	0.460
20	6.5	0.04	6	0.460

A second-order polynomial equation(24,25) was used to express the absorbance as a function of independent variables (i.e. pH, reagent concentration and shaking time).

$$\textbf{Abs.} = \beta_0 + \beta_{1*}(pH) + \beta_{2*}(\textit{Reg. conc.}) + \beta_{3*}(\textit{Mix. time}) + \beta 4_*(pH * \textit{Reg. conc.}) + \beta_{5*}(pH * \textit{Mix. time}) + \beta_{6*}(\textit{Reg. conc.} * \textit{Mix. time}) + \beta_{7*}(pH)^2 + \beta_{8*}(\textit{Reg. conc.})^2 + \beta_{9*}(\textit{Mix. time})^2$$

The coefficients of the response surface equation were determined by STATISTICA 8.0 software (StatSoft. Inc, release 2007), Table 3.

Table 3: Regression coefficients, p or probability for absorption of cimetidine ion-pair complex.

Variable	Regression coefficient	S.D	t-value	P
Constant	0.457709	0.002534	180.6409	0.000000
pH	-0.039800	0.002331	-17.0760	0.000000
(pH)^2	-0.033273	0.004445	-7.4861	0.000021
Reg. conc.	0.059600	0.002331	25.5710	0.000000
(Reg. conc.)^2	-0.063273	0.004445	-14.2359	0.000000
Mix. time	-0.005000	0.002331	-2.1452	0.057526
(Mix. Time)^2	-0.040273	0.004445	-9.0611	0.000004
*pH * Reg. conc.*	-0.006500	0.002606	-2.4944	0.031752
*pH * Mix. time*	0.000500	0.002606	0.1919	0.851681
*Reg. conc. * Mix. time*	-0.000750	0.002606	-0.2878	0.779368

The statistical analysis indicates that the model was adequate, possessing no significant lack of fit and with very satisfactory of the R^2 (0.9955). The closer the value of R^2 to the unity, the better the empirical model fits the actual data.

The variable values that maximize the response obtained can be calculated mathematically. Results are 5.53 for the pH, 0.05 % for the BTB concentration and 5.71 min. for the shaking time. These values were considered as conditions the determination of cimetidine by ion-pair complex formation with BTB. The response surface predicted by the model can be visualized (Figure 2-4) if one of the three variables is maintained constant.

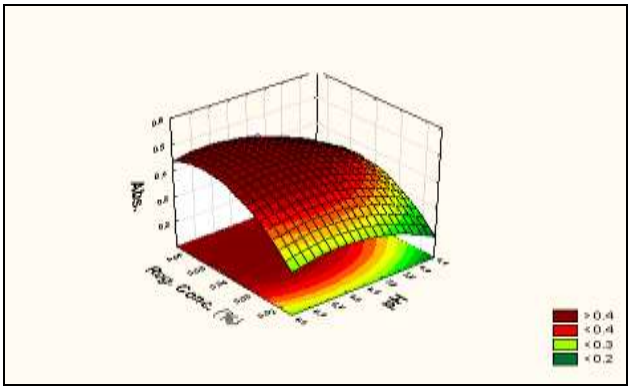


Figure 2: The response surface for absorbance of cimetidine ion-pair complex as a function of pH and reagent concentration (at constant at optimum value of mixing time, 5.71 min).

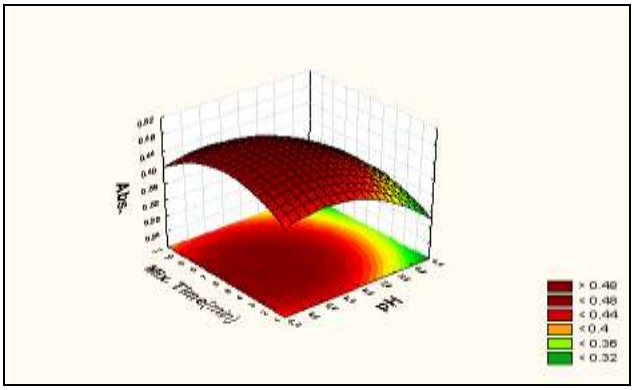


Figure 3: The response surface for absorbance of cimetidine ion-pair complex as a function of pH and mixing time (at constant at optimum value of reagent concentration, 0.05 %).

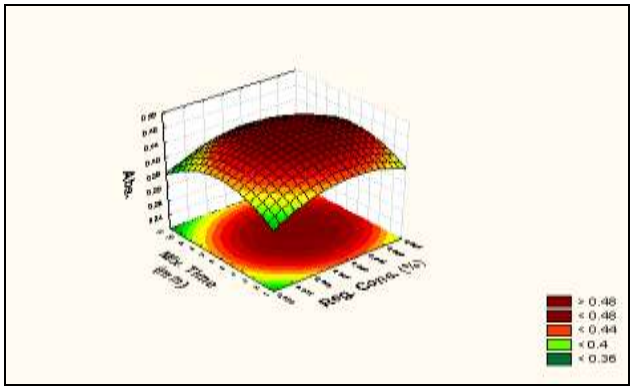


Figure 4: The response surface for absorbance of cimetidine ion-pair complex as a function of reagent concentration and mixing time (at constant at optimum value of pH , 5.53).

From Figure 2 the graph shows the highest value of absorbance is at the pH 5.53 and reagent concentration 0.05 % (when fixed the mixing time at 5.71 min.). Figures 3 and 4 show the same behavior for the other variables.

Calibration graph

Employing the experimental conditions (under univariable method), linear calibration graph for cimetidine was obtained (Figure 5), which shows that Beer's law is obeyed in the concentration range of (0.5 – 15.0 µg.mL^{-1}). The correlation coefficient, intercept and slope for calibration data are calculated.

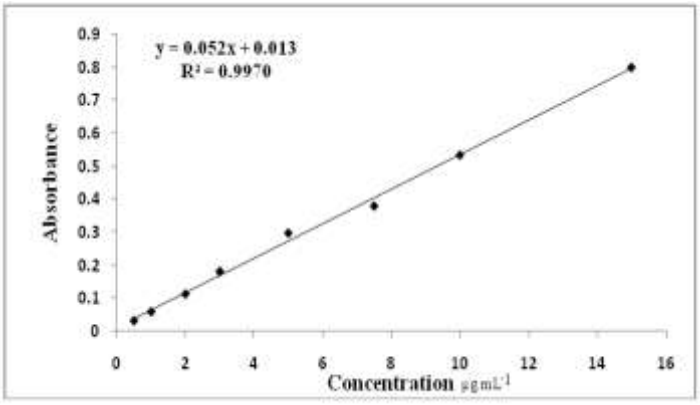


Figure5: Calibration graph for the determination of cimetidine under optimum experimental conditions.

Spectral characteristics of the proposed methods

According to the optimum experimental conditions of the proposed methods, the regression plots showed linear dependence of absorbance signals on the concentrations of the studied drugs in the studied range of cimetidine concentration. The regression equation, correlation coefficient, molar absorptivity, detection limit and Sandell's sensitivity in addition to other parameters are given in Table 4.

Table 4: Spectral characteristics and statistical data of the regression equations for determination of cimetidine using ion–pair formation.

Parameter	Value
λ_{max} (nm)	427.5
Color	Yellow
Linearity range (µg.mL^{-1})	0.5 – 15.0
Molar absorptivity (L.mol^{-1}.cm^{-1})	13172
Regression equation	A = 0.052 [Cim. µg.mL^{-1}] + 0.013
Calibration Sensitivity	0.052
Sandell's Sensitivity (µg.cm^{-2})	19.157
Correlation of Linearity (R^2)	0.9970
Correlation coefficient (R)	0.9984
Detection limit (µg.mL^{-1})	0.222

2-3-5 Stoichiometry of the complex:

To establish molar ratio between cimetidine with BTB in their ion-pair complex, mole-ratio method and Job's method of continuous variation have been used (Figures 6 and 7). The results showed that 1:1 complexes were formed with BTB through the electrostatic attraction between the positive prorogated cimetidine with the anion of BTB. Accordingly, the proposed path way for the formation of the ion-pair complex can be represented in (Scheme 2).

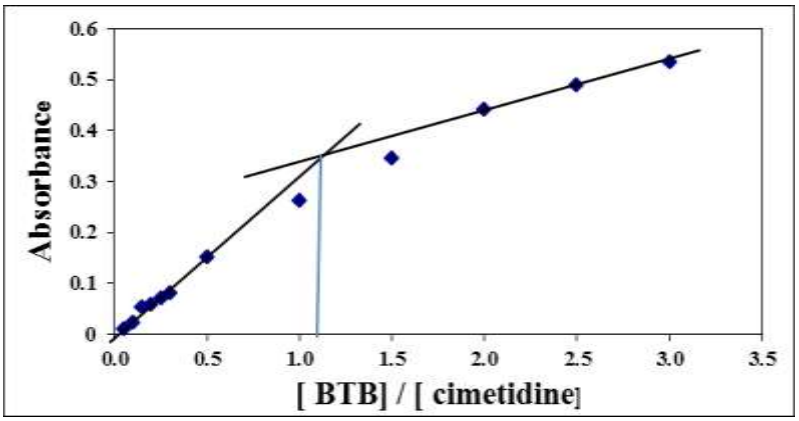


Figure 6: Mole ratio method: for 3.962 x 10^{-5} M cimetidine with variable concentrations of bromothymol blue.

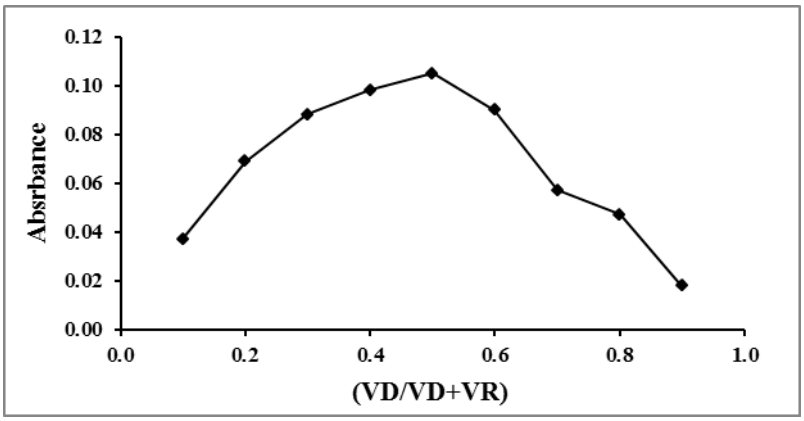
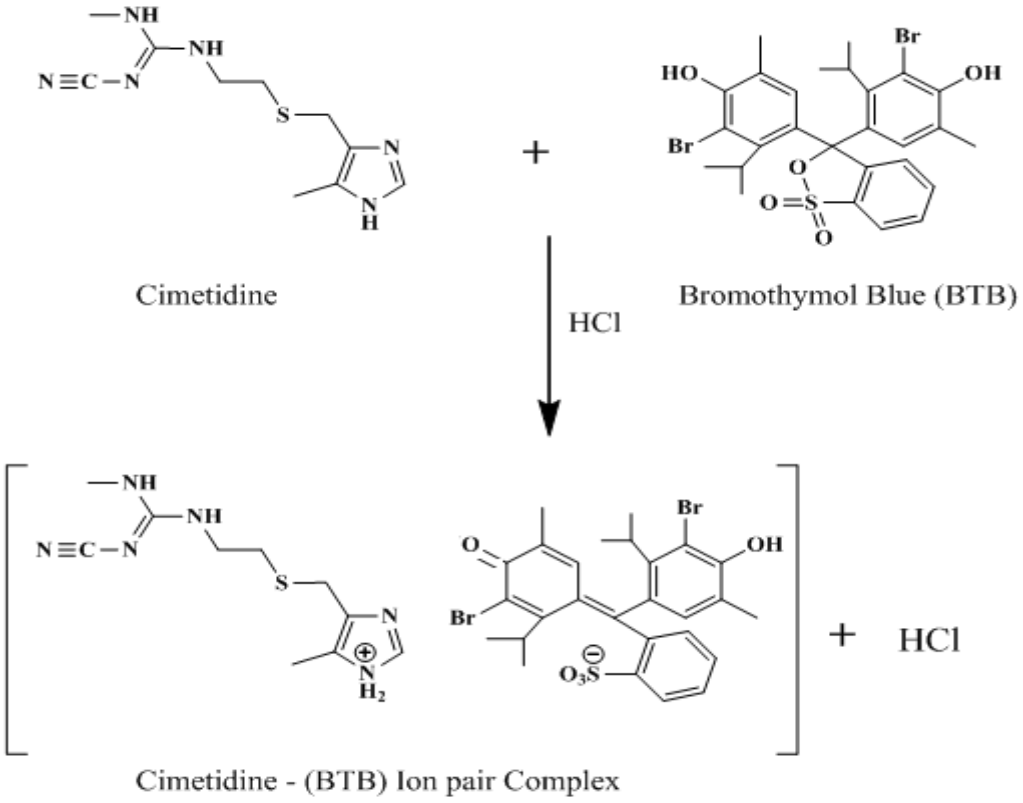


Figure 7: Continuous variation of 9.907x10^{-5} M cimetidine, 9.907x10^{-5} M BTB.



Scheme 2: The proposed reaction pathway between cimetidine and BTB.

Accuracy and precision:

The accuracy of the proposed method was confirmed by performing three replicate analyses for three different amounts of cimetidine (selected within the obtained calibration rang) by calculating the relative error percentage (Table 5). The results indicated good accuracy of the method at each concentration level. The precision was determined by calculating the percentage relative standard deviation (RSD %) for three determinations at each of the studied concentration level and were found to be in the range of 1.158-2.003%.

The proposed method was compared statistically with other methods found in the literature and the results are shown in (Table 6).

Table 5: Evaluation of accuracy and precision of the proposed method.

Cimetidine Conc. (μg.mL^{-1})		Relative Error %	R.S.D.* %
Taken	Found*		
2	1.971	-0.145	1.538
4	3.972	-0.700	1.158
10	9.936	-0.640	2.003

*Average of three determinations.

Table 6: Analytical Parameters for the analysis of cimetidine by the proposed and others methods.

Ref. No.	Methods	Linear range $\mu g.mL^{-1}$	ε $L.mol^{-1}.cm^{-1}$	Correlation Coefficient. (R)	Recovery	RSD%
8	**H.P.L.C**	50 - 3000	-	-	71 - 81	less than 6%
9	**H.P.L.C**	0.25 - 83.0		0.998	99.2 - 100.8	
10	**H.P.TL.C.**	5 - 50	-	-	100.39 ±1.33	
18	**Spectrophotometric 1st derivative**	25.0-150.0	-	-	100.27 ± 0.68	-
	Spectrophotometric Complex formation	10.0 - 60.0	-	-	99.84 ± 0.858	-
19	**Spectrophotometric**	8.0 - 30.0	6710		99.8 - 100.2	0.810- 0.840
31	**Spectrophotometric**	2.0 - 16.0	13660	0.9989	99.8 - 100.7	0.740- 0.920
-	**Proposed method**	0.5 - 15.0	13172	0.9984	98.55 - 99.36	1.158- 2.003

Interferences Study

Under optimum experimental conditions, the effect of various foreign species which may be present in pharmaceutical products and affecting the reaction between cimetidine and BTB were examined. The results showed that no interferences were found even in the presence of 1000 µg of the studied excipients (lactose, sucrose, starch, glucose, magnesium stearate and sodium citrate) in the determination of cimetidine (Table 7).

Table 7: Percent recovery for 10 $\mu g.mL^{-1}$of cimetidine in the presence of 1000$\mu g.mL^{-1}$of Excipients.

Excipients	Conc. Fond $\mu g.mL^{-1}$	% Recovery
Lactose	9.928	99.280
Sucrose	9.934	99.340
Starch	9.932	99.320
Glucose	9.929	99.290
Magnesium Stearate	9.935	99.350
Sodium Citrate	9.933	99.330

*Average of three determinations.

Analysis of dosage forms

It is evident from the aforementioned results that the proposed method gave satisfactory results with the investigated drugs. Thus, their pharmaceutical dosage forms were subjected to analysis of their contents of the active ingredient by the proposed method (ion-pair formation). The results given in Table 8 were satisfactory.

The recommended method was statistically compared with official, standard and other methods, no significant differences were found between the calculated and theoretical values of t- test at 95% and F- test at 99.5%, 99.9% and 95% confidence limit respectively, (Table 9).

Table 8: Spectrophotometric determination of cimetidine in pharmaceutical Preparations using Ion –Pair Formation .

Sample	Labeled amount	Found amount	Conc. taken	Conc.* found	Rec. %	S.D*	R.S.D* %
Tagadine (Cimetidine) 200mg/ tablet SDI/Iraq	200	204.840	5	5.121	102.42	0.048	0.937
		200.760	10	10.038	100.38	0.051	0.508
Cimedine^{R} (Cimetidine) 200mg/ tablet DAD	200	180.320	5	4.508	90.16	0.029	0.615
		179.820	10	8.991	89.91	0.029	0.316
Histale (Cimetidine Hydrochloride) 200mg/Ampoule	200	199.000	5	4.975	99.5	0.029	0.582
		198.840	10	9.942	99.42	0.157	1.579

*Average of three determinatios.

Table 9: t- and F- values for analysis of cimetidine in pharmaceutical compounds (S.D.I).

Proposed Method, N=3 μ=10.038	t- values[a]	F-values[b]	Other Methods (N=5)	μ	S. D.	Ref. No.
	0.387	136.667	Official	9.89	0.640	19
	0.159	288.34	Other	9.95	0.930	31
	0.177	9.334	Standard	10.020	0.167	32

a- Theoretical value for t-test, for N=6, at 95% confidence limit is (2.447),
b- Theoretical values for F-test, for N= (4, 2), at 99.5% is (199.25), 99.9% is (999.25) and at 95% is (19.274) respectively.
c- Theoretical value for t-test for N=2, at 95% confidence limit is 4.304.

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