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Research Article

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Hydrochlorothiazide Used as Diuretic with Antihypertensive Agents in Pharmaceutical Preparations. Estimation by First-Order Derivative and Extractive Spectrophotometry

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Abstract: First derivative UV-spectrophotometric and extractive spectrophotometric methods for the determination of hydrochlorothiazide (HCT) and bisoprolol fumarate (BF) in bulk and combined formulations have been developed. The signals in first derivative (^{1}D) spectrophotometry were measured at 264.5 and 282.5 nm for HCT without interference with BF. The measurement of the values of ^{1}D was used for construction of calibration graph for HCT. Calibration graph was found linear in the range 1.2-59.0 μg mL^{-1}. The extractive spectrophotometry is based on the reaction of BF with bromothymol blue (BTB) dye in solution containing KCl-HCl buffer pH 2.2 to form ion-pair complex (1:1 and 1:2, drug:dye) extractable with chloroform and subsequently measured spectrophotometrically at 412 nm without interference with HCT. Beer's law was obeyed in the range 0.5-40.9 μg mL^{-1} for BF. Both methods are rapid, simple and do not require any preliminary separation or treatment of the samples. Furthermore, the two methods were applied to pharmaceutical dosage form. Interferences of the other ingredients and excipients were not observed.

Keywords: Hydrochlorothiazide, Bisoprolol Fumarate, First Derivative Spectrophotometry, Extractive Spectrophotometry, Bromothymol Blue, Pharmaceutical Preparations.

1. INTRODUCTION

Hydrochlorothiazide, 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide, is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical preparations which decreases active sodium reabsorption and reduces pheripheral vascular resistance [1]. Bisoprolol, 1-[4-[[2-(1-methylethoxy)ethoxyl-methyl]phenoxy]-3-[1-methylethyl)amino]-2-propanol, is a highly selective β1 receptor antagonist. Bisoprolol fumarate is indicated for the management of hypertension. It may be used alone or in combination with other antihypertensive agents [1,2]. The two drugs are employed in antihypertensive pharmaceutical formulations, alone or in association.

Literature survey reveals that both hydrochlorothiazide and bisoprolol fumarate are official in USP [3] and BP [4]. Several methods are available for the determination of the latter compounds by high-

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performance liquid chromatography in different pharmaceutical preparations, either alone [5,6], in combinations of hydrochlorothiazide and bisoprolol fumarate [7,8] or with other active ingredients [9-18]. Many analytical methods were developed for determination of hydrochlorothiazide either alone or jointly with other antihypertensive drugs. These methods include spectrophotometry [18-22], derivative spectrophotometry [12,14,16,18, 23-27], polarography [28,29] and chemiluminescence [30]. Bisoprolol fumarate membrane ion selective electrode [31,32], voltammetric [33] and TLC densitometric [27,34] methods were developed for the determination of bisoprolol fumarate in pharmaceutical formulations.

The aim of this study is to develop two simple, sensitive and validated UV-derivative spectrophotometric and extractive spectrophotometric methods for the determination of hydrochlorothiazide and bisoprolol fumarate and to apply the two methods to the combined commercial pharmaceutical preparations. The results obtained by these two methods were statistically compared.

2. EXPERIMENTAL

2.1. Apparatus

Double beam Spectroscan 80 SD spectrophotometer (England) with 1 cm quartz cells was used for the spectrophotometric measurements under the following operating conditions: scan speed medium (400 nm/min), scan range 200-500 nm and slit width 2 nm. Spectra were automatically obtained by software UV-WIN 5.0. WTW InoLab pH meter calibrated with standard buffers at room temperature and a Thornton sonicator (model T-14) were also used.

2.2. Materials and Reagents

All the chemicals and solvents used were of analytical reagent grade and purchased from E. Merck. Working reference standards of hydrochlorothiazide (HCT) was supplied by Ausun Chemical Co. Ltd., its purity was 99.88 % and bisoprolol fumarate (BF) was supplied by Rarecrown Pharma Chem Co. Ltd. (China), its purity was 99.70 %. Bidistilled water was used in all experiments. Tablets were purchased from Syrian market, containing 10 mg bisoprolol fumarate and 6.25 mg hydrochlorothiazide per tablet.

2.3. Solutions

Standard stock solutions of HCT and BF (1.0 mg mL^{-1} of both) were prepared by direct weighing of standard substance with subsequent dissolution in 0.1 N NaOH. Working standard solutions were then prepared by suitable dilution of the stock solutions with 0.1 N NaOH. A stock standard solution of BF 10^{-3} M was prepared by dissolving the appropriate weight in bidistilled water. Working standard solutions were then prepared by suitable dilution of the stock solution with water. Bromothymol blue (BTB) 1×10^{-3} M solution in water was prepared. Buffer solutions (pH range from 1.4 to 4.6) were prepared by mixing specific volumes of KCl (0.2 M) and HCl (0.2 M).

2.4. Procedure for the Assay of Bulk Samples

2.4.1. Calibration for Derivative Spectrophotometry

HCT and BF stock solutions in 0.1 N NaOH were freshly prepared. Standard solutions were obtained by diluting the stock solutions for the preparation of calibration curve in the concentration range of 1.2-59.0 μg mL^{-1} HCT in the presence of BF with the same concentration in 0.1 N NaOH for derivative spectrophotometric method. HCT and BF mixtures were initially scanned to determine the zero-crossing absorption bands. Calibration graph was constructed in the concentration range of 1.2-59.0 μg mL^{-1} of HCT in the presence of BF with the same concentration against 0.1 N NaOH as blank. The values of ^{1}D amplitudes at 282.5 and 264.5 nm were measured for the determination of HCT in presence of BF.

2.4.2. Calibration for Extractive Spectrophotometry

Into a series of 50mL separating funnels, 3 mL of buffer solution of pH 2.2 and 2.0 mL of BTB were placed. An appropriate volume of 10^{-3} M standard drug solution (0.022–0.84 mL) was added to each

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funnel and mixed well. The funnels were shaken vigorously with 10mL chloroform for 2 min, and then allowed to stand for clear separation of the two phases. The separated organic phase was transferred to a 50 mL beaker, dried over anhydrous sodium sulfate, and transferred to a 10 mL volumetric flask. Then the combined extract was made up to the mark with the solvent and mixed. The absorbance of the organic phase was measured at 412 nm for BF-BTB complex against a reagent blank similarly prepared. The standard calibration plot was prepared to calculate the amount of the analyst drug in unknown samples. The cooler is stable for at least 24 hrs up to 30 °C.

2.5. Assay Validation

Synthetic mixtures prepared by adding known amounts of HCT and BF were analyzed by both developed methods using the procedure outlined above. The mean percentage recoveries and relative standard deviations (RSD) were calculated.

2.6. Stoichiometric Ratio

The molar ratio and continuous variation methods were applied to study the stoichiometric ratio of the ion pair formed. 1×10^{-3} M solutions of BF and BTB were used. In the former method a constant volume of 1×10^{-3} M BF solution was employed and BTB concentration was changed to obtain different ratios for the ion pair complex, while in the latter method, a series of solutions was kept at 2.0 mL. BTB was mixed in various proportions and then diluted to volume in a 10 mL calibrated flask with chloroform. The absorbance of the resulting solutions were measured at λ_{max} against reagent blanks treated similarly.

2.7. Analysis of pharmaceutical dosage form samples

2.7.1. Procedure for Derivative Spectrophotometry

Twenty tablets containing HCT and BF were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 25 mg HCT was dissolved in a 25 mL of methanol and sonicated for 5 minutes and then filtered. The combined filtrate was evaporated to about the dryness. The remaining portion of solution was diluted in a 25 mL volumetric flask to the volume with 0.1 N NaOH. The general procedure was then followed in the concentration range mentioned above.

2.7.2. Procedure for Extractive Spectrophotometry

Twenty tablets containing HCT and BF were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 25 mg of BF was dissolved in a 25 mL of methanol and sonicated for 5 minutes and then filtered. The combined filtrate was evaporated to about the dryness. The remaining portion of solution was diluted in a 25 mL volumetric flask to the volume with double distilled water, and the resulting solution was used for analysis by the recommended procedure in the concentration range mentioned above.

3. RESULTS AND DISCUSSION

3.1. Derivative UV-spectrophotometry

The absorption spectra of HCT, BF and mixture of them in 0.1 M NaOH medium are clearly shown in Figure 1A. The spectra are strongly overlapped and as a result, simultaneous determination of two drugs cannot be possible for reliable direct measurements. In contrast, the first derivative (^{1}D) spectrum (Figure 1B) of each pure drug was found to show zero-crossing points and was sufficiently enough to demonstrate the resolving power of the proposed derivative spectrum method for determination of HCT. Among the number of points where the derivative spectra cross the zero line, the most interesting were 264.5 and 282.5 nm, by applying the ^{1}D; at these wavelengths, the $dA/d\lambda$ value of BF is equal zero, allowing to analyze HCT in the presence of BF.

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3.1.1. Linearity of the Derivative Procedure

Under the optimum experimental conditions described above. The linearity of the proposed method was evaluated for HCT by analyzing different concentrations of drug, within the concentration range in Table 1 in the absence and presence of certain concentrations of the other component in the mixture. The corresponding ^{1}D for HCT drug was measured at the specific wavelength, and plotted against its concentration (Table 1). A straight line was obtained in each case and linear regression equations (intercepts and slopes) for pure HCT and mixtures of HCT and BF were established. The calibration graphs were constructed from ^{1}D signal by measuring the signal at 282.5 nm for HCT (Figure 2). All the validation parameters, such as concentration range, detection limit, quantitation limit and correlation coefficient are summarized in Table 1. The high values of the correlation coefficients and the values of *Y*-intercepts close to zero indicate the good linearity of the calibrations, so the proposed method is free from error in the procedure. The lowest detection limit calculated indicating the highest sensitivity.

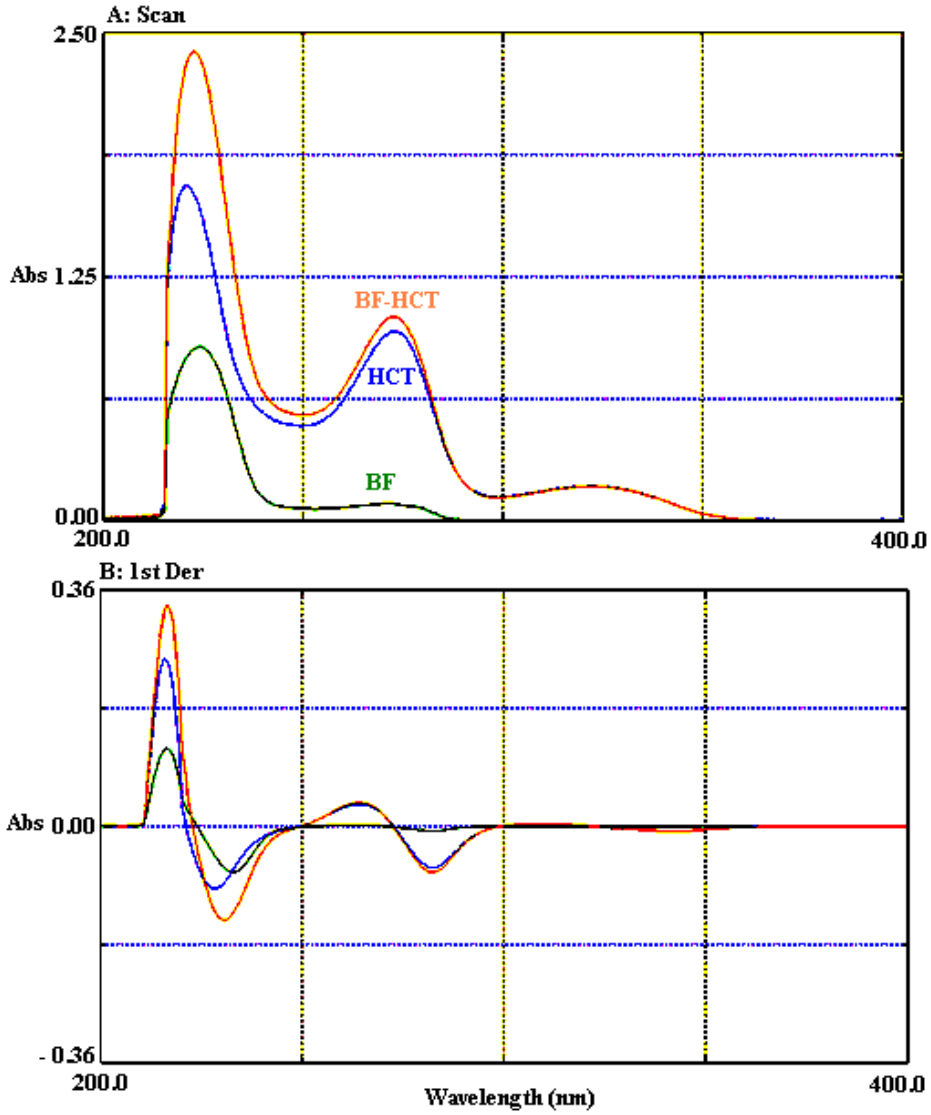

Figure 1. A: Zero order spectra of BF 25 μg mL^{-1}, HCT 15.6 μg mL^{-1} and mixture of HCT and BF have the same concentration in 0.1 N NaOH. **B**: First derivative spectra of BF 25 μg mL^{-1}, HCT 15.6 μg mL^{-1} and mixture of them have the same concentration in 0.1 N NaOH.

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Table 1. Characteristic parameters for the regression equations of ^{1}D and extractive spectrophotometric methods for determination of HCT and BF.

Parameter	$^1D_{282.5}$ spectrophotometry		Extractive spectrophotometry
Compound	HCT	Mixture HCT+BF	BF
Linearity range (μg mL^{-1})	1.2 – 59.0		0.5 – 40.9
Detection limit (μg mL^{-1})	0.26	0.27	0.14
Quantitation limit (μg mL^{-1})	0.85	0.89	0.45
Regression equation (A)[a]: slope (m)	0.0035	0.0037	0.0472
SD of slope (S_m)	4.1×10^{-5}	3.2×10^{-5}	1.31×10^{-3}
Intercept (b)	0.0003	0.0090	0.0754
SD of intercept (S_b)	3.0×10^{-4}	3.3×10^{-4}	2.15×10^{-3}
Correlation coefficient (r^2)	0.9998	0.9997	0.9999
Reproducibility	99.8 ± 0.14	100.2 ± 0.04	100.68 ± 0.40

[a] *A=mC+b, where A is the absorbance and C is the concentration of drug in μg mL^{-1}.*

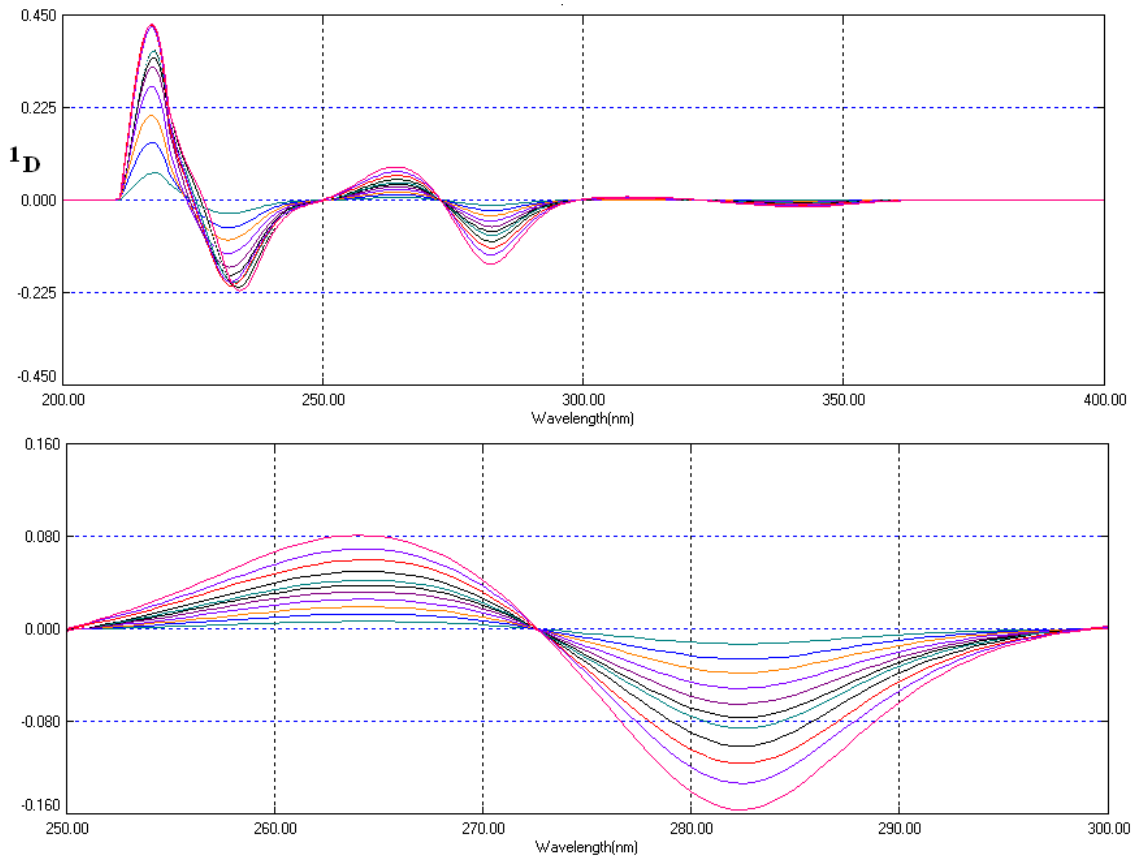

Figure 2. ^{1}D spectra of HCT 1.2-59.0 μg/mL in the presence of BF with the same concentration in 0.1 N NaOH.

3.1.2. Accuracy and Precision of the Derivative Procedure

Accuracy and precision were determined by carrying out six replicate determinations of HCT in synthetic mixtures of HCT and BF (Table 2). Percentage relative standard deviation (RSD%) as precision, recovery and percentage relative error (Er%) as accuracy of the suggested method were calculated. The

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recovery ranged from 99.17 to 101.87% for HCT. The RSD% of standards were less than 5% over the selected range indicating that the method is sufficiently accurate and precise. These results of accuracy and precision show that the proposed method had good repeatability and reproducibility.

Table 2. Determination of HCT and BF in pure form by the proposed methods.

Method	μg mL^{-1}		Relative error (%)	RSD (%)	Recovery (%)
	Taken	Found*			
HCT	1.20	1.19	-0.84	2.89	99.17
$^1D_{282.5}$ spectrophotometry	10.00	10.11	1.08	1.57	101.10
	20.00	20.14	0.69	0.94	100.70
	30.00	30.48	1.57	0.76	101.60
	40.00	40.75	1.84	0.58	101.87
	50.00	50.79	1.55	0.41	101.58
	59.00	59.96	1.60	0.39	101.63
BF	0.50	0.51	1.96	3.29	102.00
Extractive spectrophotometry	1.00	1.01	0.99	2.97	101.00
	4.00	4.06	1.47	2.01	101.50
	10.00	10.05	0.49	1.16	100.50
	20.00	20.40	1.96	1.05	102.00
	40.00	40.12	0.30	0.48	100.30

**Average of six determinations.*

3.2. Extractive Spectrophotometry

Containing cationic nitrogen, the cited drug (BF) reacts with BTB in the presence of HCT to form a yellow ion-pair complex between the basic nitrogen of BF in KCl-HCl buffer and BTB. The drug–dye complex, with two oppositely charged ions, behaves as a single unit held together by an electrostatic force of attraction. The complex is quantitatively extracted into chloroform. Absorption spectrum of the yellow BF–BTB ion-pair complex extracted into chloroform with its λ_{max} at 412 nm is shown in Figure 3. The colorless blank has practically negligible absorbance.

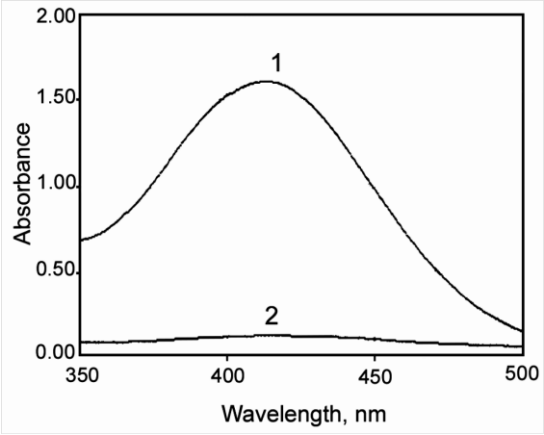

Figure 3. Absorption spectrum of BF-BTB complex extracted into 10 mL chloroform: (1) 0.25 mL of 10^{-3} M BF + 2 mL of 10^{-3} M BTB + 3 mL of pH 2.2 buffer, against its respective blank (2) vs. distilled water.

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3.2.1. Optimization of Variables

Optimum conditions necessary for rapid and quantitative formation of colored ion-pair complex with maximum stability and sensitivity were established by a number of preliminary experiments. KCl–HCl buffer (Clark&Lubs buffer) was found to be suitable for BTB method. Chloroform was preferred to other solvents (carbon tetrachloride, dichloromethane, and ether) for method for its selective and quantitative extraction. Optimum conditions were fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance at 412 nm for BF-BTB. The effect of pH and volume buffer was studied by extracting the colored complex species at different pH value and volume buffer. Maximal absorbance was observed at the pH 2.2 using 3 mL of buffer (Figure 4).

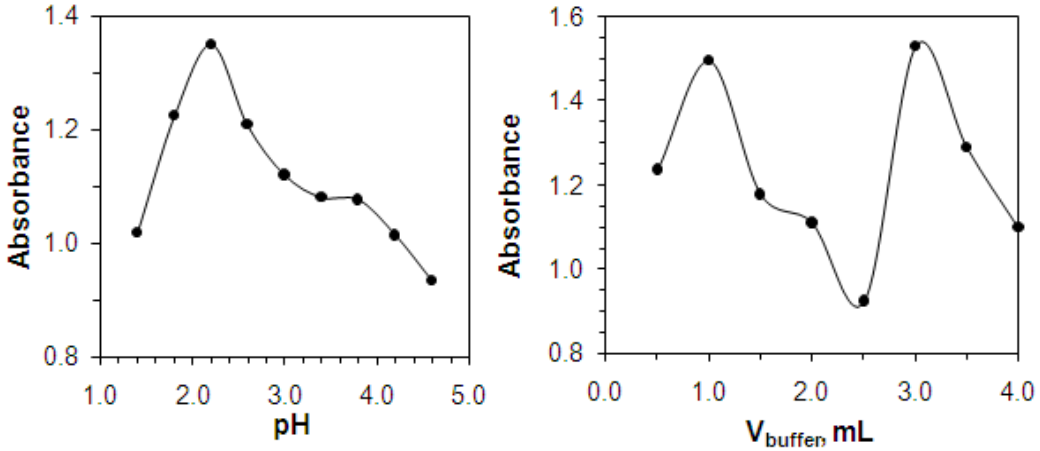

Figure 4. Effect of pH and volume buffer pH 2.2 on the absorbance at 412 nm of BF (28 μg mL^{-1})-BTB complex.

A volume of 2 mL of 1×10^{-3} M BTB was found to be optimal for complete complexation between the cited drug and dye, since the absorbance at maximum wavelength was found to be maxima at the mentioned volume. The effect of the reagent's concentration on the absorbance is shown in Figure 5.

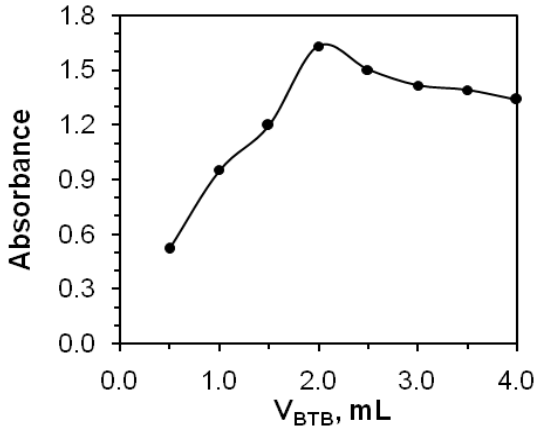

Figure 5. Effect of reagent concentration on the formation of the yellow colored ion-pair complex (C_{BF} =27 μg mL^{-1}).

3.2.2. Stoichiometric Relationship

The stoichiometric ratio of the drug to dye in the colored complex was determined using the molar ratio and continuous variation methods. It is apparent from the data that ion-pair complex with drug to dye ratio 1:1 and 1:2 is formed (Figure 6).

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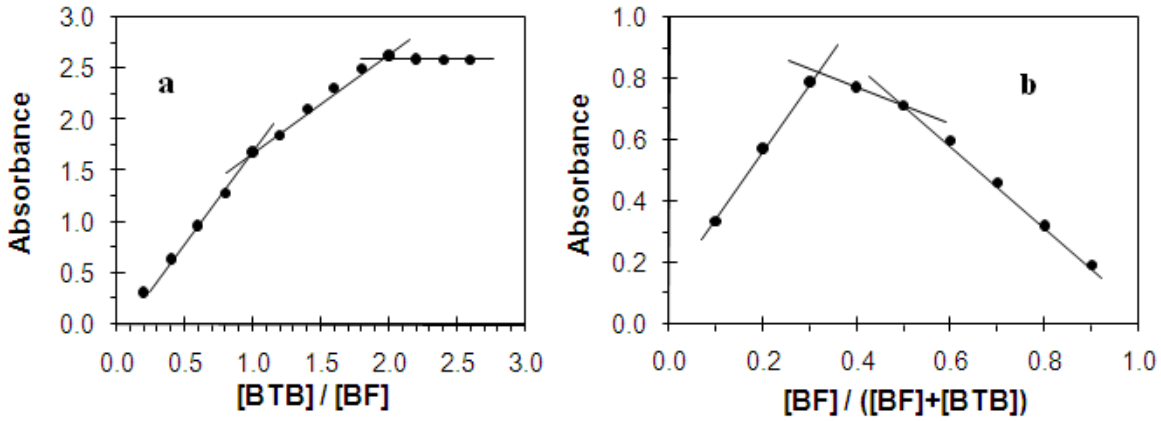

Figure 6. (a) Mole-ratio method of BF-BTB complex (C_{BF} = 1×10^{-4} M) and (b) Job's method of continuous variations of BF-BTB system, $C_{BF} + C_{BTB} = 2\times10^{-4}$ M.

The logarithmic stability constants of the formed complex is calculated from the Harvey and Manning method [35] using the data of the molar ratio and continuous variation method and found to be 6.85.

3.2.3. Linearity and Range

A linear relationship was found between the absorbance at λ_{max} and the concentration of BF in the range 0.5-40.9 μg mL^{-1}, in the presence of certain concentrations of the other component (HCT) in the mixture, for the proposed method in the final measured volume of 10 mL. Regression analysis of the Beer's law plots at λ_{max} reveals a good correlation as given in Table 1. The graph shows negligible intercept and is described by the regression equation, $A=mC+b$ (where A is the absorbance of 1cm layer, m is the slope, b is the intercept and C is the concentration of the measured solution in μg mL^{-1}) obtained by the least-squares method [36]. The high molar absorptivity of the resulting colored complex (4.11×10^4 L mol^{-1} cm^{-1}) indicates the high sensitivity of the method.

3.2.4. Validation of the method

The validity of the method for the analysis of BF in its pure state and in its formulations was examined by analyzing the samples of BF in synthetic mixtures of HCT and BF using the proposed procedure. The results obtained for BF drug are given in Table 2. The precision and accuracy of the method were tested by analyzing six replicates of the drug. The low values of relative standard deviation (RSD%) indicate good precision and reproducibility of the method. The results of analysis of dosage forms are given in Table 3. The results were reproducible with low RSD% values. The average percent recoveries obtained were quantitative (100.50–102.00%), indicating good accuracy of the method.

3.3. Application to the Pharmaceutical Dosage Forms

The proposed techniques were applied to the tablets. The ingredients in the tablets did not interfere in the experiments. The applicability of the proposed methods for the assay of HCT and BF in formulations was examined by analyzing various formulations and the results are tabulated in Table 3 were compared to the official non-aqueous titration method for BF and HPLC method for HCT [4] by means of *t*- and *F*-values at 95% confidence level. In all cases, the average results obtained by proposed methods and official methods were statistically identical, as the difference between the average values had no significance at 95% confidence level. The proposed methods are simple, sensitive and reproducible and can be used for routine analysis of HCT and BF in pure form and in formulations. The commonly used additives such as starch, lactose, titanium dioxide and magnesium stearate do not interfere with the assay procedure.

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Table 3. Determination of HCT and BF in tablets by the proposed and official methods.

Sample	%Recovery[a] ± SD			
	Hydrochlorothiazide		Bisoprolol fumarate	
	^{1}D method	Official method [4]	Extractive method	Official method [4]
Pure	100.16±0.14	99.88±0.12	100.68±0.40	99.70±0.36
t-value[b]	1.28	1.12	1.89	2.02
F-value[b]	1.36		1.23	
Bisoprol HCT (6.5 mg HCT and 10 mg BF/tablet)				
Mean±SD[a]	101.46±0.52	100.85±0.48	103.90±0.86	102.84±0.79
t-value[b]	1.75	1.63	1.66	1.82
F-value[b]	1.17		1.18	
Bizocor Plus (6.5 mg HCT and 10 mg BF/tablet)				
Mean±SD[a]	100.86±0.68	99.87±0.59	102.22±0.94	101.91±0.88
t-value[b]	1.82	2.04	1.16	1.36
F-value[b]	1.33		1.14	

[a] Five independent analyses.[b] Theoretical values for t and F-values at five degree of freedom and 95% confidence limit are t =2.776 and F=6.26.

4. CONCLUSION

In conclusion the proposed first derivative and extractive spectrophotometric methods provide simple (as there is no need for expensive solvents), rapid (as it only requires measurements of $^1D_{282.5}$ and absorbance values at suitable wavelength), direct (as it estimates each drug independently of the other) and sensitive methods suitable for the quality control analysis of HCT and BF in dosage forms. These methods have some advantages in comparison to similar studies such as wide range of linearity that makes it suitable for all in vitro studies of the tablets formulation. The described first derivative spectrophotometric method could be considered for the analysis of HCT either alone or in the presence of BF and the extractive spectrophotometric method could be considered for the analysis of BF either alone or in the presence of HCT in the quality control laboratories.

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The authors declare no conflict of interest

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