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Spectrophotometric determination of hyoscine butylbromide and famciclovir in pure form and in pharmaceutical formulations

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Abstract

A simple, rapid, and extractive spectrophotometric method was developed for the determination of hyoscine butylbromide (HBB) and famciclovir (FCV) in pure and pharmaceutical formulations. These methods are based on the formation of yellow ion-pair complexes between the basic nitrogen of the drug and four sulphonphthalein acid dyes, namely; bromocresol green (BCG), bromothymol blue (BTB), bromocresol purple (BCP) and bromophenol blue (BPB) in phthalate buffer of pH range (3.0–3.5). The formed complexes were extracted with chloroform and measured at 420, 412, 409 and 415 nm for HBB and at 418, 412, 407 and 414 nm for FCV using BCG, BTB, BCP and BPB, respectively. The analytical parameters and their effects on the reported systems are investigated. Beer's law was obeyed in the range 1.0–20 $\mu g\,mL^{-1}$ with correlation coefficient ($n = 6$) ≥ 0.9997. The molar absorptivity, Sandell sensitivity, detection and quantification limits were also calculated. The composition of the ion pairs was found 1:1 by Job's method in all cases and the conditional stability constant (K_f) of the complexes have been calculated. The free energy changes (ΔG) were determined for all complexes formed. The proposed methods have been applied successfully for the analysis of the studied drugs in pure and pharmaceutical formulations with percentage recoveries ranges from 99.84 to 100.26. The results were in good agreement with those obtained by the official methods.

Keywords: Hyoscine butylbromide; Famciclovir; Spectrophotometry; Ion-pair complex; Acid dyes

1. Introduction

Hyoscine butylbromide (HBB) (1S, 3s, 5R, 7S, 8r)-6,7-epoxy-3-[(*S*)-(3-hydroxy-2-phenylpropiony)oxy]-8-butyl-8-methyl-8-azoniabicyclol [3.2.1] octane bromide is used as an antispasmodic in treating peptic ulcer, gastritis and various disorders of the gastrointestinal tract which are characterized by spam. It has also found employment for the relief of spasmodic conditions of the bile duct and urinary tract and for the treatment of dysmenorrhoea [1]. Famciclovir (FCV) is an antiviral drug and is chemically [2-(acetyloxymethyl)-4-(2-aminopurin-9-yl)-butyl] acetate. Famciclovir, an orally available nucleoside analog with potent in vitro activity against HIV, is being investigated for treatment of chronic hepatitis B. Famciclovir induced rapid, dose-dependent suppression of viral replication and reduction in alanine aminotransferase (ALT), with greatest efficacy in the 500-mg tid treatment group (Scheme 1). Due to the vital importance of HBB determination in pharmaceutical preparations and in biological fluids, many analytical techniques have been reported in the literature.

HBB has been determined in pharmaceutical preparations including titrimetric methods [1], spectrophotometric [2–11], high-performance liquid chromatographic [12–17], capillary electrophoresis [18,19] and electrochemical methods [20,21].

Literature survey revealed many analytical techniques have been reported for determination of FCV including: spectrophotometric [22–25], pharmacological data [26] and high-performance liquid chromatographic determination of antivirals using UV detection and mass spectrometry [27].

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of some drugs [28–36]; therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds.

In the literature no method using acid dyes has been reported. In the present investigation, we report that the development of

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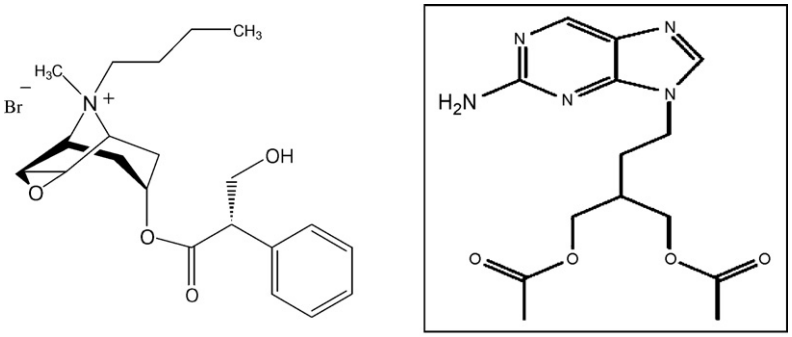

hyoscine butylbromide (HBB) Famciclovir (FCV)

Scheme 1. Chemical structure of the studied drugs.

accurate, reproducible, less time consuming and adequately sensitive four extractive spectrophotometric methods based on the formation of ion-pair complexes between HBB and FCV with anionic dye namely bromocresol green (BCG), bromothymol blue (BTB), bromocresol purple (BCP) and bromophenol blue (BPB) compared with other reported methods. The proposed methods were applied to the determination of HBB and FCV in pharmaceutical formulations. No interference was observed in the assay of HBB and FCV from common excipients in levels found in pharmaceutical formulation. These methods are validated by the statistical data.

2. Experimental

2.1. Apparatus

All absorption spectra were made using Kontron 930 (UV–visible) spectrophotometer (German) with a scanning speed of 200 nm/min and a band width of 2.0 nm, equipped with 10 mm matched quartz cells. Hanna pH-meter instrument (Portogal) (HI: 9321) was used for checking the pH of phthalate buffer solutions pH ranges from 2.0 to 7.0.

2.2. Materials and reagents

All chemicals and reagents were of analytical grade and water was always bidistilled water.

2.2.1. Materials

- Hyoscine butylbromide (HBB) were kindly supplied by Amriya Pharmaceutical Industries, Alexandria, Egypt. It's purity was found to be 100.28 ± 0.77 ($n = 5$) according to the HPLC procedures [37].
- Famciclovir (FCV) was kindly supplied by Novartis Pharmaceuticals corporation, USA. It's purity was found to be 100.024 ± 0.84 ($n = 5$) [24].

2.2.2. Pharmaceutical formulations

- Nu-Spasm tablets 10 mg HBB per tablet (Egyptian International Pharmaceutical Industries company (EIPICO), Tenth of Ramadan City, Egypt).
- Spasmocin ampoules 20 mg HBB per 1 mL (Memphis Company for Pharmaceutics and Chemistry, Cairo, Egypt).
- Famvir tablet 125 mg FCV per tablet (Novartis Pharma S.A.E., Cairo, Egypt), underlicence from Novartis Pharma AG. Basle, Switzerland.

2.2.3. Standard solution

A stock solution (100 $\mu g\,mL^{-1}$) was prepared by dissolving 10 mg of HBB and FCV in 100 mL of distilled water and further diluted with the same solvent as appropriate.

2.2.4. Reagents

Bromocresol green (BCG), bromocresol purple (BCP) and bromophenol blue (BPB) (BDH Chemicals Ltd., Poole, England) and used without further purification.

A stock solution 0.1% (w/v) and (1.0×10^{-3} M) was prepared by dissolving the appropriate weight of bromocresol green (BCG), bromocresol purple (BCP) bromophenol blue (BPB or bromothymol blue (BTB) in 10 mL 96% ethanol and diluted to 100 mL with bidistilled water.

- These solutions are stable for at least 1 week if kept in the refrigerator.

Series of buffer solutions of KCl–HCl (pH 1.5–4.2), NaOAc–HCl (pH 1.99–4.92), NaOAc–AcOH (pH 3.0–5.6) and potassium hydrogen phthalate–HCl (pH 2.0–7.0) were prepared by following the standard methods [38].

Potassium hydrogen phthalate–HCl (pH 2.0–7.0) was prepared by dissolving 1.28 g potassium hydrogen phthalate in water and completed to 50 mL with water and adjusting pH by addition of 0.2 M hydrochloric acid. Freshly prepared solutions were always employed.

2.3. Construction of calibration curves

2.3.1. Using HBB

Aliquots of (0.2–2.0 mL) the standard drug solutions (100 $\mu g\,mL^{-1}$) were transferred to 10 mL measuring flasks and added 2.0 mL potassium hydrogen phthalate buffers of pH 3.0 and 3.5 using (BCG and BCP) and (BPB or BTB), respectively, then add 2.0 or 1.5 mL of (BCG and BCP) or (BPB and

BTB) reagent solutions 0.1% (w/v), respectively. The mixture was extracted twice with 10 mL chloroform using BCG and BCP or with dichloromethane using BPB and BTB by shaking for 2.0 min, then allowed to stand for clear separation of the two phases and the chloroform layer was passed through anhydrous sodium sulphate. The absorbance of the yellow colored complexes was measured at 420, 412, 409 and 415 nm using BCG, BTB, BCP and BPB, respectively, against corresponding reagent blank similarly prepared. All measurements were made at room temperature (25 ± 2 °C). The procedures were repeated for other analyte aliquots and calibration plots were drawn to calculate the amount of drug in unknown analyte samples.

2.3.2. Using FCV

Aliquots of (0.1–1.6 mL) the standard drug solutions (100 μg mL^{-1}) were transferred to 10 mL measuring flasks and added 2.0 mL potassium hydrogen phthalate buffers of pH 3.0 and 3.3 using (BCG and BPB) and (BCP or BTB), respectively, then add 2.0 ml of reagent solution 0.1% (w/v), respectively. The mixture was extracted twice with 10 mL chloroform by shaking for 2.0 min, then allowed to stand for clear separation of the two phases and the chloroform layer was passed through anhydrous sodium sulphate. The absorbance of the yellow colored complexes was measured at 418, 412, 407 and 414 nm using BCG, BTB, BCP and BPB, respectively, against corresponding reagent blank similarly prepared. All measurements were made at room temperature (25 ± 2 °C). The procedures were repeated for other analyte aliquots and calibration plots were drawn to calculate the amount of drug in unknown analyte samples.

2.3.2.1. Procedures for pharmaceutical formulations. Procedures for tablets: The contents of ten tablets (Nu-Spasm, 10 mg HBB per tablet or Famvir tablet, 125 mg FCV per tablet) were crushed, powdered, weight out and the average weight of on tablets was determined. An accurate weight equivalent to 10 mg HBB was dissolved in 20 mL of bidistilled water with shaking for 5.0 min and filtered. The filtrate was diluted to 100 mL with bidistilled water in a 100 mL measuring flask to give 100 μg mL^{-1} stock solution. An aliquot of the diluted drug solution was treated as described under Sections 2.3.1 and 2.3.2.

Procedures for ampoules: The contents of 5.0 ampoules of HBB (Spasmocin, 20 mg HBB per 1 mL) were mixed and the average volume of one ampoule was determined. An accurate volume equivalent to 20 mg mL^{-1} of HBB was transferred to a 100 mL measuring flask and completed to the mark with bidistilled water. This solution was further diluted stepwise to the requisite concentration 100 μg mL^{-1} of HBB with bidistilled water and analyzed as described under the general procedure described under Section 2.3.1. For further conformation, the standard addition technique was applied to test the reliability and recovery of the proposed procedures, in which variable amount of HBB were added to the previously analyzed portion of pharmaceutical preparations.

3. Results and discussion

Absorption spectra: The nitrogenous drugs are present in positively charged protonated forms and anionic dyes of sulphonpthalein group present mainly in anionic form at pH ≥ 3. So when treated with an acid dye at pH range (3.0–3.5) of potassium hydrogen phthalate buffer, a yellow ion-pair complex which is extracted with chloroform or dichloromethane is formed. The absorption spectra of the ion-pair complexes, which were formed between HBB or FCV and each of BCG, BTB, BCP and BPB were measured in the range 350–550 nm against the blank solution and shown in (Fig. 1). The ion-pair complexes show maximum absorbance at 420, 412, 409 and 415 nm for HBB and at 418, 412, 407 and 414 nm for FCV using BCG, BTB, BCP and BPB methods, respectively. The optimum reaction conditions for determination of the ion-pair complexes were established. Then linearity, accuracy, precision, sensitivity, and stability of proposed methods were described and these developed methods applied to pharmaceutical preparations and obtained results evaluated statistically.

3.1. Optimum reaction conditions for complex formation

The optimization of the methods was carefully studied to achieve complete reaction formation, highest sensitivity and maximum absorbance. Reaction conditions of the ion-pair complex were found by studying with preliminary experiments such as pH of buffer, type of organic solvent, volumes of the dye, and shaking time for the extraction of ion-pair complexes.

3.1.1. Selecting of the extracting solvents

The effect of several organic solvents *viz.*, chloroform, carbon tetrachloride, ethyl acetate, xylene, diethylether, butyl acetate, toluene, dichloromethane and chlorobenzene were tried for effective extraction of the colored species from aqueous phase. Chloroform and dichloromethane were found to be the most suitable solvent for extraction of colored complex for (BCG or

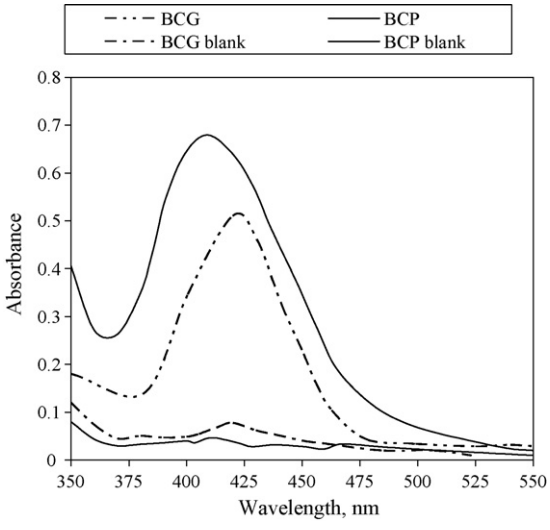

Fig. 1. Absorption spectrum of ion-associate complexes of HBB–BCG and HBB–BCP [HBB] (10 μg mL^{-1}) against reagent blank.

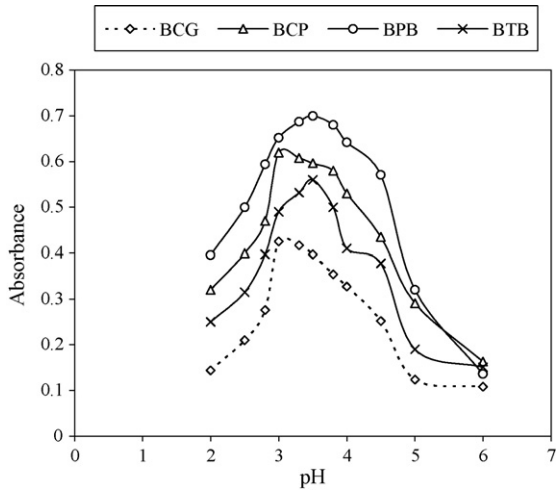

Fig. 2. Effect of pH of potassium hydrogen phthalate buffer solution on the HBB–dye ion-pair complexes.

BCP) and (BPB or BTB), respectively, for HBB and chloroform was found to be the most suitable solvent for extraction of colored complex for all reagents in case for FCV. Double extraction with total volume 10 mL, yielding maximum absorbance intensity and considerably lower extraction ability for the reagent blank and the shortest time to reach the equilibrium between both phases.

3.1.2. Effect of time and temperature

The optimum reaction time was investigated from 0.5 to 4.0 min by following the color development at ambient temperature ($25 \pm 2\,^{\circ}C$). Complete color intensity was attained after 2.0 min of mixing for all complexes. Raising the temperature up to 30 °C has no effect on the absorbance of the formed complexes, whereas above 30 °C, the absorbance start to decay. The absorbance remains stable for at least 48 h.

3.1.3. Effects of pH on the ion-pair formation

The effect of pH was studied by extracting the colored complexes in the presence of various buffers such as KCl–HCl (pH 1.5–4.2), NaOAc–HCl (pH 1.99–4.92), NaOAc–AcOH (pH 3.0–5.6) and potassium hydrogen phthalate–HCl (pH 2.0–7.0). It was noticed that the maximum color intensity and highest absorbance value were observed in potassium hydrogen phthalate–HCl buffer of pH 3.0 and 3.5 of potassium hydrogen phthalate buffer using (BCG and BCP) and (BPB or BTB), respectively, in case of HBB, whereas for FCV pH 3.0 and 3.3 are the optimum pH values of buffer using (BCG and BPB) and (BCP or BTB), respectively. In addition to the stability of the color without affecting the absorbance at the optimum pH values (Fig. 2). Further, 2.0 mL potassium hydrogen phthalate buffers gave maximum absorbances and reproducible results.

3.1.4. Effects of reagent concentration

The effects of the reagents were studied by measuring the absorbances of solutions containing a fixed concentration of HBB or FCV ($10\ \mu g\ mL^{-1}$) and varied amounts of the respective

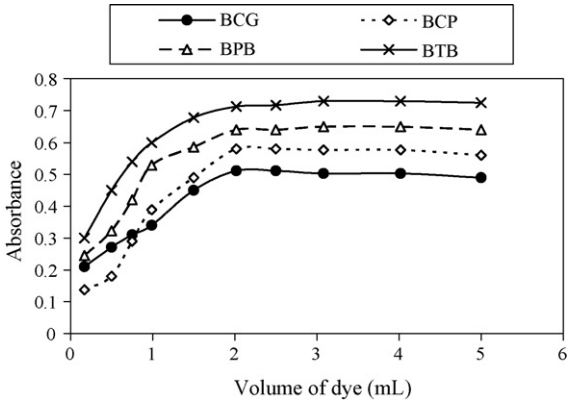

Fig. 3. Effect of reagent concentration on the reaction of ($10\ \mu g\ mL^{-1}$) FCV with BCG, BCP, BPB and BTB (0.1, w/v).

reagent. Maximum color intensity of the complex was achieved with 2.0 or 1.5 mL of 0.1% (w/v) (BCG and BCP) or (BPB and BTB) reagent solutions, respectively, for HBB and with 2.0 ml of 0.1% (w/v) of each reagent solutions for FCV. Although a larger volume of the reagent had no pronounced effect on the absorbances of the formed ion-pair complex (Fig. 3).

3.1.5. Stoichiometric relationship

Job's method of continuous variation [39] of equimolar solutions was employed: a 1.0×10^{-3} M standard solution of drugs base and 1.0×10^{-3} M solution of BCG, BTB, BCP, and BPB, respectively, were used. A series solutions was prepared in which the total volume of drug and reagent was kept at 10 mL for BCG, BTB, BCP, and BPB, respectively. The absorbance was measured at the optimum wavelength. The molar ratio of the reagents (drug:dye) in the ion-pair complexes was determined by the method continuous variations (Job's method) (Fig. 4). The results indicate that 1:1 (drug:dye) ion-pairs are formed through the electrostatic attraction between positive protonated HBB^{+} or FCV^{+} and negative BCG^{-}, BTB^{-}, BCP^{-} and BPB^{-}. The extraction equilibrium can be represented as follows:

$$HBB_{(aq)}{}^{+} + D_{(aq)}{}^{-} \leftrightarrow HBB^{+}D_{(aq)}{}^{-} \leftrightarrow HBB^{+}D_{(org)}{}^{-}$$

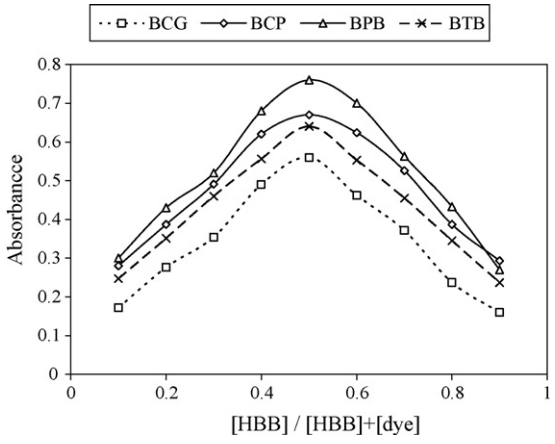

Fig. 4. Job's method of continuous variation graph for the reaction of HBB with acid dyes BCG, BCP, BPB and BTB, [drug] = [dye] = 1×10^{-3} M.

where HBB^+ and D^- represent the protonated hyoscine hydrobromide and the anion of the dye, respectively, and the subscripts (aq) and (org) refer to the aqueous and organic phases, respectively.

3.1.6. Conditional stability constants (K_f) of the ion-pair complexes

The stability of the ion-pair complexes was evaluated. The formation of the ion-pairs was rapid and the yellow color extracts were stable 48 h for drug–dye without any change in color intensity and the maximum absorbance at room temperature.

The conditional stability constants (K_f) of the ion-pair complexes for the studied drug were calculated from the continuous variation data using the following equation [40]:

$$K_f = \frac{A/A_m}{[1 - A/A_m]^{n+2} C_M (n)^n}$$

where A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_M the mole concentration of drug at the maximum absorbance and n is the stoichiometry with which dye ion associates with drugs. The $\log K_f$ values for drug–dye ion-pair associates were 4.96 ± 0.13, 5.32 ± 0.07, 4.72 ± 0.16 and 4.95 ± 0.09 for HBB and 5.13 ± 0.14, 5.22 ± 0.06, 4.68 ± 0.21 and 5.08 ± 0.07 for FCV using BCG, BTB, BCP and BPB, respectively.

The standard free energy changes of complexation (ΔG°) were calculated from the association constants (Table 1) by the following equation [41]:

$$\Delta G^\circ = -2.303 RT \log K_f$$

where (ΔG°) is the free energy change of the complex ($kJ\,mol^{-1}$), R the gas constant ($1.987\,cal\,mol^{-1}\,degree^{-1}$), T the temperature in Kelvin (273+°C), and K_f is the association constant of drug–reagent ion-pair complexes ($l\,mol^{-1}$).

3.2. Method validation

3.2.1. Linearity

At described experimental conditions for HBB and FCV determination, standard calibration curves for HBB and FCV with BCG, BTB, BCP and BPB calibrations were constructed by plotting absorbances versus concentrations. The statistical parameters were given in the regression equation calculated from the calibration graphs, along with the standard deviations of the slope (S_b) and the intercept (S_a) on the ordinate and the standard deviation residuals ($S_{y/x}$).

The linearity of calibration graphs was proved by the high values of the correlation coefficient (r) and the small values of the y-intercepts of the regression equations. The apparent molar absorptivities of the resulting colored ion-pair complexes and relative standard deviation of response factors for each proposed spectrophotometric method were also calculated and recorded in Table 1. The molar absorptivity of BPB > BCG > BTB > BCP ion-pair complexes for HBB, while for FCV the molar absorptivity of BPB > BCG > BCP > BTB ion-pair complexes.

3.2.2. Sensitivity

The detection limit (LOD) for the proposed methods were calculated using the following equation [42]:

$$\mathrm{LOD} = \frac{3s}{k}$$

where s is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and k is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits were found to be 0.16, 0.21, 0.26 and 0.165 $\mu g\,mL^{-1}$ for BCG, BTB, BCP and BPB methods, respectively. Whereas for FCV, the detection limits were found to be 0.15, 0.16, 0.23 and 0.14 $\mu g\,mL^{-1}$ for BCG, BTB, BCP and BPB methods, respectively.

The limits of quantitation, LOQ, defined as [42]:

$$\mathrm{LOQ} = \frac{10s}{k}$$

According to this equation, the limits of quantitation were found to be 0.53, 0.70, 0.87 and 0.55 $\mu g\,mL^{-1}$ for BCG, BTB, BCP and BPB methods, respectively. Whereas for FCV, the detection limits were found to be 0.50, 0.53, 0.77 and 0.47 $\mu g\,mL^{-1}$ for BCG, BTB, BCP and BPB methods, respectively.

3.2.3. Specificity, precision, and accuracy

Specificity of ion-pair reaction and selective determination of HBB and FCV which was the basic nitrogenous compounds with sulphonphthalein dyes could be possible. Percentage relative standard deviation (R.S.D.%) as precision and percentage relative error (Er %) as accuracy of the suggested method were calculated. Precision was carried out by six determinations at four different concentrations in these spectrophotometric methods. The percentage relative error calculated using the following equation:

$$\mathrm{Er}\,\% = \left[\frac{\text{founded} - \text{added}}{\text{added}}\right] \times 100$$

The inter-day and intra-day precision and accuracy results are shown in (Table 2). These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility.

3.2.4. Robustness and ruggedness

For the evaluation of the method robustness, some parameters were interchanged; pH, dye concentration, wavelength range, and shaking time. The capacity remains unaffected by small deliberate variations. Method ruggedness was expressed as R.S.D.% of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical differences between different analysts and instruments suggesting that the developed methods were robust and rugged.

Table 1
Statistical analysis of calibration graphs and analytical data in the determination of the studied drugs using the proposed methods ($n = 6$)

Parameters	HBB				FCV			
	BCG	BTB	BCP	BPB	BCG	BTB	BCP	BPB
Wavelengths	420	412	409	415	418	412	407	414
λ_{max} (nm) pH	3.0	3.5	3.0	3.5	3.0	3.3	3.3	3.0
Beer's law limits ($\mu g\,mL^{-1}$)	2.0–14	2–16	2–20	2–14	1.0–12	2.0–14	2.0–18	2.0–12
Molar absorptivity, ε ($L/mol^{-1}\,cm^{-1}$)	2.521×10^4	2.310×10^4	2.082×10^4	2.886×10^4	1.320×10^4	1.087×10^4	1.131×10^4	1.683×10^4
Sandell's sensitivity ($ng\,cm^{-2}$)	17.47	19.07	21.15	15.26	24.35	29.55	28.42	19.09
$\log K_f$	4.96 ± 0.13	5.32 ± 0.07	4.72 ± 0.16	4.95 ± 0.09	5.13 ± 0.14	5.22 ± 0.06	4.68 ± 0.21	5.08 ± 0.07
Free energy change (ΔG°) ($kJ\,mol^{-1}$)	6.764	7.255	6.437	6.750	6.996	7.118	6.382	6.927
Regression equation (y)[a]								
$S_{y/x}$	0.2737	0.3118	0.361	0.3384	0.191	0.1668	0.233	0.2263
Intercept (a)	0.0003	0.0051	−0.0019	−0.0137	−0.0048	−0.0037	0.0088	−0.0086
S.D. of intercept (S_a)	0.234	0.2647	0.2976	0.32	0.1526	0.1438	0.2091	0.2107
$\pm tS_a$	0.651	0.7359	0.8273	0.8896	0.423	0.3997	0.5813	0.5857
Slope (b)	0.0571	0.0497	0.0463	0.0678	0.0425	0.0345	0.0338	0.0541
S.D. of slope (S_b)	0.0261	0.0249	0.0224	0.0339	0.0213	0.0173	0.0169	0.027
$\pm tS_b$	0.0726	0.0692	0.0612	0.094	0.059	0.0481	0.047	0.0751
Correlation coefficient (r)	0.9999	0.9999	0.9998	0.9997	0.9999	0.9998	0.9997	0.9999
LOD ($\mu g\,mL^{-1}$)	0.16	0.21	0.26	0.165	0.15	0.16	0.23	0.14
LOQ ($\mu g\,mL^{-1}$)	0.53	0.70	0.87	0.55	0.50	0.53	0.77	0.47
R.S.D.%	0.7973	0.771	0.9615	1.084	0.70	1.14	0.8698	0.6551
Mean ± S.D.	99.84 ± 0.796	100.24 ± 0.772	100.26 ± 0.964	99.97 ± 1.084	100.16 ± 0.701	100.26 ± 1.143	99.93 ± 0.8692	100.06 ± 0.6555
RE%	0.8369	0.793	1.01	1.138	0.736	1.196	0.9128	0.6875
t-Test[b]	0.9293	0.3956	0.0325	0.5375	0.283	0.394	0.166	0.078
F-Test[b]	1.069	1.134	1.567	1.982	1.44	1.85	1.07	1.64

LOD, limit of detection; LOQ, limit of quantification; ε, molar absorptivity.

[a] $y = a + bC$, where C is the concentration in $\mu g\,mL^{-1}$ and y is the absorbance units.

[b] The theoretical values of t and F at $P = 0.05$ are 2.31 and 6.39, respectively.

Table 2
The intra-day precision and accuracy data for the studied drugs obtained by the proposed methods

Method	HBB					FCV			
	Added ($\mu g\,mL^{-1}$)	Recovery (%)	Precision R.S.D.%[a]	Accuracy Er %	Confidence limit[b]	Recovery (%)	Precision R.S.D.%[a]	Accuracy Er %	Confidence limit[b]
BTB	4.0	99.72	0.99	−0.275	3.989 ± 0.041	99.20	0.76	−0.80	3.968 ± 0.032
	8.0	99.15	0.85	−0.85	7.932 ± 0.071	100.2	0.57	0.2	8.016 ± 0.048
	12	98.94	1.12	−1.06	11.873 ± 0.140	99.90	0.66	−0.1	11.988 ± 0.083
	16	99.45	0.88	−0.55	15.912 ± 0.147	100.31	0.39	0.313	16.05 ± 0.0657
BCP	4.0	100.1	0.84	0.1	4.004 ± 0.0353	100.75	0.46	0.75	4.03 ± 0.0193
	8.0	99.57	0.92	−0.425	7.966 ± 0.077	100.625	0.83	0.625	8.05 ± 0.070
	12	99.95	0.77	−0.05	11.994 ± 0.097	99.17	0.64	−0.833	11.90 ± 0.080
	16	100.2	1.08	0.2	16.032 ± 0.182	99.38	0.98	−0.625	15.90 ± 0.164
BCG	3.0	100.12	0.76	0.133	3.004 ± 0.024	99.70	1.08	−0.3	2.991 ± 0.0339
	6.0	99.85	0.79	−0.15	5.991 ± 0.050	98.92	0.54	−1.083	5.935 ± 0.0336
	9	99.92	0.81	−0.078	8.993 ± 0.076	99.30	0.45	−0.70	8.937 ± 0.0422
	12	100.15	1.23	0.15	12.018 ± 0.155	100.10	0.61	0.10	12.012 ± 0.0769
BPB	3.0	99.67	0.64	−0.33	2.99 ± 0.020	100.67	0.57	0.67	3.02 ± 0.0181
	6.0	101.17	0.58	1.17	6.07 ± 0.037	100.50	0.72	0.50	6.03 ± 0.0456
	9	100.11	0.73	0.11	9.01 ± 0.069	99.78	0.69	−0.22	8.98 ± 0.0650
	12	99.83	0.88	−0.17	11.98 ± 0.111	100.08	0.94	0.083	12.01 ± 0.1185

n, number of determination, R.S.D.%, percentage relative standard deviation; Er %, percentage relative error.
[a] Mean of five determination.
[b] Confidence limit at 95% confidence level and five degrees of freedom ($t = 2.571$).

3.3. Effects of interference

To assess the usefulness of the method, the effect of diluents, excipients and additives which often accompany HBB and FCV in its dosage forms (starch, lactose, glucose, sugar, talc, sodium chloride, titanium dioxide and magnesium stearate) was studied. The results indicated that there is no interference from the degradation, indicating a high selectivity for determining the studied HBB and FCV in its dosage forms.

3.4. Analysis of pharmaceutical preparations

The proposed methods have been successfully applied to the determination of HBB in commercial tablet. The results obtained are shown in (Table 3). The four suggested methods were applied successfully to the determination of HBB and FCV in commercial tablet. Six replicate determinations were made. (Table 4) shows that satisfactory recovery data were obtained and the assay results were in a good agreement with the label claims. Moreover, to check the validity of the proposed methods, dosage forms were tested for possible interference with standard addition method. There was no significant difference between slopes of calibration curves and standard addition methods at four methods. Therefore, it is concluded that the excipients in pharmaceutical dosage forms of HBB and FCV such as starch, lactose, talc, stearic acid, titan dioxide, yellow iron oxide were not found any interference in the analysis of HBB and FCV. At 95% confidence level the calculated *F*-value did not exceed the theoretical *F*-value indicating no significant difference between the four proposed methods and the reference method (Table 4).

Table 3
Determination of the studied drugs in pharmaceutical dosage forms

Sample	Official methods ($n = 5$)	Proposed methods ($n = 6$)		Recovery[a] ± S.D.%	
		BCG	BCP	BPB	BTB
Nu-Spasm tablets (10 mg HBB/tablet)	99.70 ± 0.16 0.00512	99.86 ± 0.19 $t^* = 1.51$ $F^* = 1.41$	99.55 ± 0.18 $t = 1.46$ $F = 1.27$	99.80 ± 0.22 $t = 0.87$ $F = 1.89$	99.91 ± 0.20 $t = 1.93$ $F = 1.56$
Spasmocin ampoules (20 mg HBB/mL)	99.89 ± 0.61	100.02 ± 0.72 $t = 0.324$ $F = 1.39$	99.94 ± 0.68 $t = 0.13$ $F = 1.24$	99.63 ± 0.81 $t = 0.606$ $F = 1.76$	100.06 ± 0.52 $t = 0.492$ $F = 1.38$
Famvir tablet (125 mg FCV/tablet)	99.95 ± 0.37 0.02738	100.09 ± 0.43 $t = 0.583$ $F = 1.35$	99.90 ± 0.50 $t = 0.19$ $F = 1.83$	100.11 ± 0.29 $t = 0.79$ $F = 1.63$	99.82 ± 0.48 $t = 0.507$ $F = 1.68$

[a] Average of six determinations.
[*] The theoretical values of *t* and *F* at $P = 0.05$ are 2.31 and 6.39, respectively.

Table 4
Determination of the studied drugs in their pharmaceutical dosage forms applying the standard addition technique

Sample	Taken ($\mu g\,mL^{-1}$)	Added ($\mu g\,mL^{-1}$)	Proposed methods		Recovery[a] (%)	
			BCG	BCP	BPB	BTB
Nu-Spasm tablets (10 mg HBB/tablet)	4.0	2.0	99.92	100.01	99.89	99.95
		6.0	99.85	99.98	99.56	100.06
		8.0	100.03	100.08	99.90	99.91
Spasmocin ampoules (20 mg HBB/mL)	4.0	2.0	100.05	100.02	99.87	99.93
		6.0	99.94	99.85	99.90	100.03
		8.0	99.90	99.96	99.95	99.92
Famvir tablets (125 mg FCV/tablet)	4.0	2.0	99.94	99.97	99.65	99.86
		6.0	100.05	100.03	99.73	99.90
		8.0	100.09	99.88	99.95	99.93

[a] Average of six determinations.

4. Conclusion

Unlike GC and HPLC procedures, the spectrophotometer is simple and is not of high cost. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component in complex dosage formulations. The reagents utilized in the proposed methods are cheaper, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as pH and reagent concentration. Moreover, the methods are accurate, reproducible, adequately sensitive and free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control is well established by the assay of HBB and FCV in pure form and in pharmaceutical preparations.

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