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Determination of Hyoscine N- Butyl Bromide and Paracetamol mixture by zero order and [1]DD first derivative ratio spectrophotometric Method

Nouruddin Ali, Mohammed Gamal *, Mohammed Abdelkawy

Department of analytical chemistry , Faculty of pharmacy, Beni Suef university, Bani Sweif, Bani Sweif Governorate, Egypt

Abstract

In the present work, a simple and sensitive spectrophotometric method was developed for determination of Hyoscine N-Butyl Bromide (HBB) and Paracetamol (PAR) mixture . In this method, PAR could be determined in the presence of HBB by using zero order spectra with at 248 nm over a concentration range of (2-12µg.mL^{-1}) while HBB could be determined in the presence of PAR by using of the first derivative of the ratio spectra (^{1}DD) method at 220.5 nm using the spectrum of PAR 100 µg.mL^{-1} as a divisor over a concentration range of 2 – 45 µg.mL^{-1}. The proposed method was successfully applied for determination of HBB and PAR in pure form as well as in pharmaceutical formulations.

Keywords: Spectrophotometry; Hyoscine N-Butyl Bromide; Paracetamol.

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1.0-Introduction

Hyoscine N-Butyl Bromide (HBB, Figure-1) is a quaternary ammonium salt which exhibits anticholinergic property. It has antispasmodic action on the smooth muscles of the gastrointestinal, biliary, and urinary tracts [1]. Paracetamol (PAR) (Figure-1), 4-acetamidophenol, is an effective analgesic and antipyretic for treatment of minor, non-inflammatory conditions in patients who are prone to gastric symptoms[1] .There are many reports for the determination of HBB and PAR either separately or in combination with other drugs including spectrophotometric method.

ds [2-5], chromatographic methods [6-8], electrochemical methods [9-12], capillary electrophoresis methods [13-14] and titrimetric methods [15-17]. Few methods have been mentioned for analysis of HBB and PAR in binary mixture.

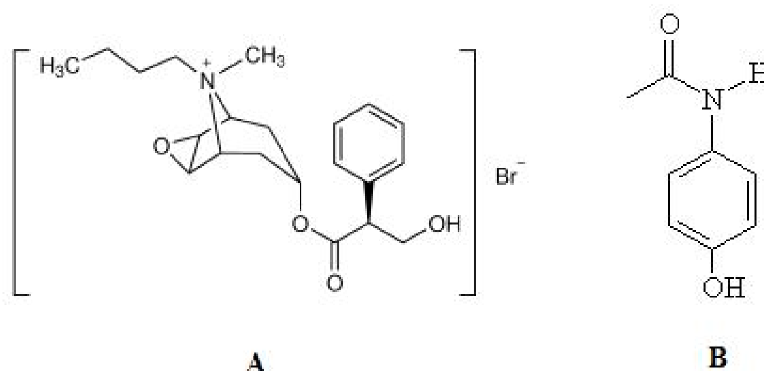

Figure-1: Chemical structure of HBB (A) and PAR (B)

In The first method, Erk [18] analyzed HBB and PAR in their binary mixture by precipitating HBB with ammonium reineckate at pH 6,0 selectively and reading the absorbance of the solution of the precipitate in acetone at 525.0 nm for HBB and by measuring the dA/dλ values at 254.5 nm in the first derivative spectra of the remaining solution for Paracetamol. In the second method [19], solid phase extraction procedure using strong cation exchange cartridges followed by a reversed-phase HPLC assay was applied to the analysis of HBB, PAR and Lidocaine hydrochloride in injection forms. The chromatographic separation was performed on a C-18 column. The mobile phase consisted of a mixture of acetonitrile: ammonium acetate 0.2M, (30:70, v/v) pumped at a flow rate 1.2 mL/min. In the third method [20], The two drugs were separated on RP-18 W/ UV_{254} TLC plates using developing mobile phase consisting of methanol: citrate buffer (pH=1.5): triflouroacetic acid (70:30:0.1, by volume) at room temperature. The obtained bands were scanned simultaneously at 210 nm. In the fourth method [21], A RP-HPLC chromatographic method was developed for the determination of HBB and PAR. where the two drugs were separated using C18 (25 cm × 4.6 mm i.d. 5 µm particle size) column as a stationary phase and water: methanol (50:50, V/V pH adjusted to 3.9 with CF3COOH acid) as a mobile phase, maintaining the flow rate at 1.0 mL min^{-1} with UV detection at 210 nm.

Even though there are many methods available for estimation of BAT and PAT, all these methods suffer from one or other disadvantages. Hence in the present work, an attempt has been made to develop sensitive and simple spectrophotometric method for determination of HBB and PAR for routine quality control analysis of these drugs in bulk powder and in pharmaceutical formulations.

2.0 Materials and methods

2.1. Apparatus

1-Double beam UV-Visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with a quartz cell of 1 cm path length, connected to IBM compatible computer was used during the present work. The software was UVPC personal spectroscopy software version 3.7. The spectral bandwidth was 2 nm and wavelength-scanning speed was 2800nm/min.

2.2. Materials

2.2.1. Pure samples

Paracetamol (PAR) and Hyoscine N-Butyl Bromide (HBB) were kindly supplied by CID Co. Chemical Industries Development, Giza, Egypt. Their purity was found to be 99.94±1.537 and 99.21±1.012, respectively, according to the company certificate of analysis (COA).

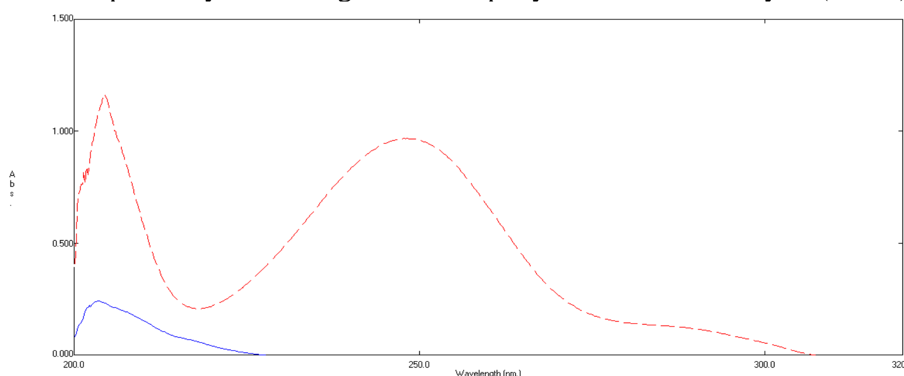

Figure-2: Zero order spectra of HBB (──) and PAR (-----) each of 10 µg.mL^{-1} in methanol

2.2.2. Market samples

1- Buscopan plus® tablets (Batch No 116738T) labeled to contain 500 mg of (PAR) and 10 mg of (HBB), CID Co. Chemical Industries Development, Giza, Egypt. 2- Buscamol.F.C® tablets (Batch No 12001025) labeled to contain 500 mg of (PAR) and 10 mg of (HBB), DELTA PHARMA, Egypt.3- Buscopan plus® Suppositories (Batch No 105) labeled to contain 800 mg of (PAR) and 10 mg of (HBB), CID Co. Chemical Industries Development, Giza, Egypt.

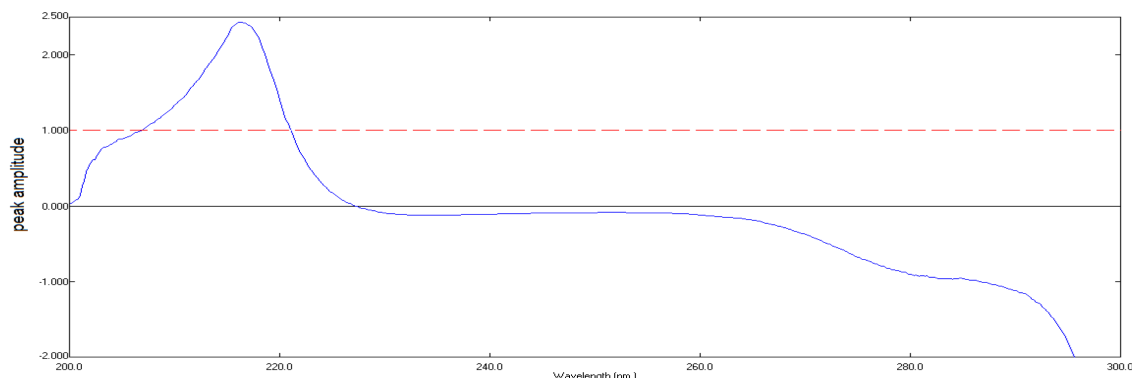

Figure-3: Ratio spectra of of HBB 10 µg.mL^{-1} (──) and PAR 2 µg.mL^{-1} (-----) in methanol using 100 µg.mL^{-1} of PAR as a divisor

2.3. Reagents

All reagents and chemicals used were of analytical grade and were used without further purification.Methanol (EL - Nasr Pharmaceutical Chemicals Co., Abu -Zabaal, Cairo, Egypt).

2.4. Preparation of standard solutions

Paracetamol (PAR) and Hyoscine N-Butyl Bromide (HBB) stock standard solutions (1 mg.mL $^{-1}$).

Accurately weighed (0.1 gram of each drug) was taken into two separate 100-mL volumetric flask, 50 mL methanol was added with vigorous shaking, and then the volume was made to the mark with methanol.

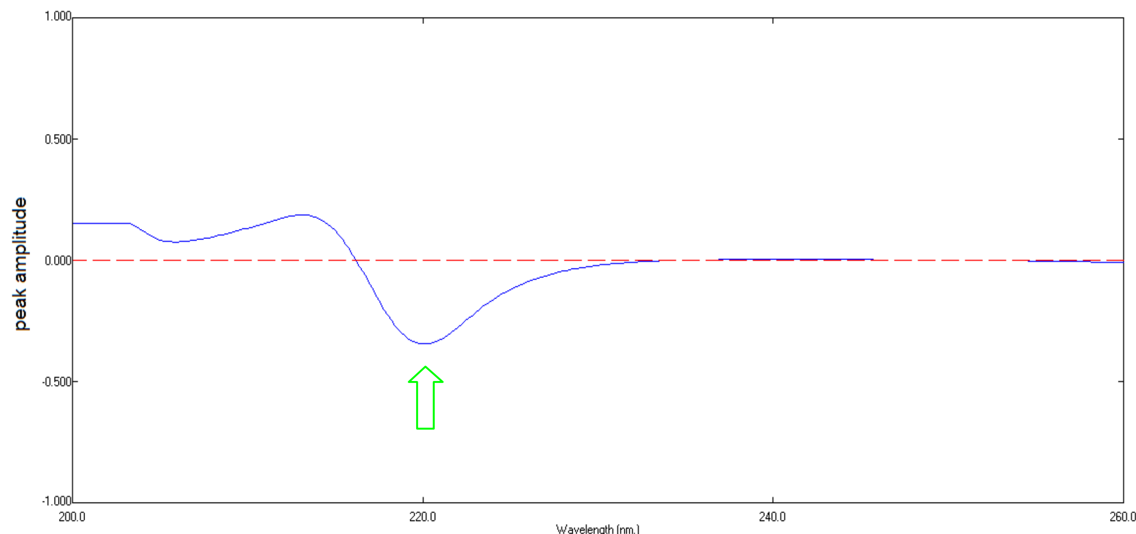

Figure-4: First derivative of ratio spectra of of HBB 10 µg.mL^{-1} (──) and PAR 2 µg.mL^{-1} (-----) in methanol using 100 µg.mL^{-1} of PAR as a divisor

Paracetamol (PAR) and Hyoscine N-Butyl Bromide (HBB) working standard solutions (100 µg.mL^{-1})

10 mL of the above stock solutions were transferred to two separate 100-mL volumetric flasks and volume was made upto 100 ml with methanol to get 100 µg.mL^{-1} working solution for each drug.

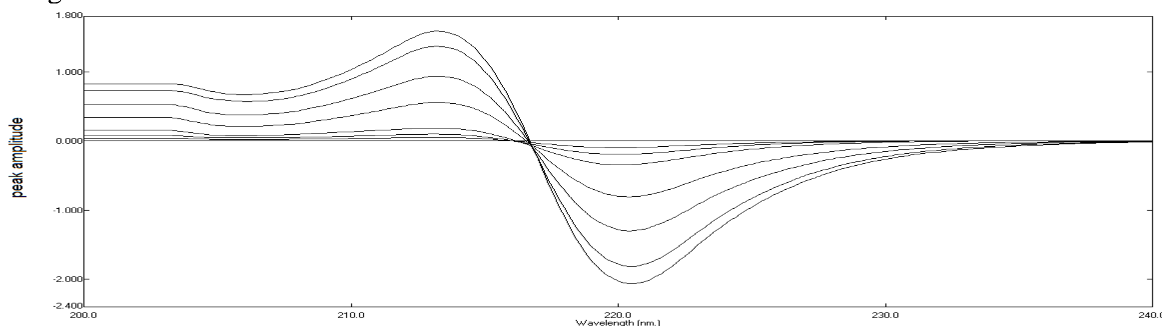

Figure-5: Linearity of first derivative of ratio spectra of HBB in methanol using 100 µg.mL^{-1} of PAR as a divisor

2.5. Procedure

2.5.1 Spectral characteristics

Into two separate 10-mL volumetric flasks transfer accurate aliquots equivalent to 100 µg of each drug from their working standard solutions, complete the volume with methanol. Record the zero order, first derivative, second derivative and third derivative spectra for each solution using methanol as a blank. For first derivative of ratio spectra (^{1}DD) of HBB divide the stored

spectra of HBB by the spectrum of 100 µg.mL^{-1} of PAR then obtains the first derivative of ratio spectra (1DD) using Δλ= 4 nm and scaling factor = 100.

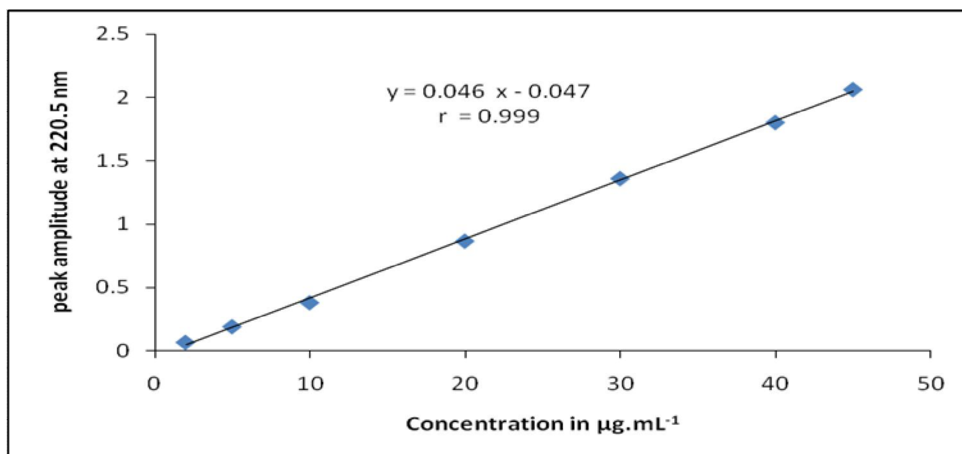

Figure-6: Calibration of first derivative of ratio spectra of HBB in methanol using 100 µg.mL^{-1} of PAR as a divisor, relating its peak amplitude to its corresponding concentration at 220.5 nm.

Taken (µg mL^{-1})		Found* (µg mL^{-1})		Recovery %	
HBB	**PAR**	**HBB**	**PAR**	**HBB**	**PAR**
4.00	3.00	4.02	2.97	100.50	99.00
8.00	4.00	7.88	4.04	98.50	101.00
10.00	6.00	10.05	5.97	100.50	99.50
20.00	8.00	19.60	8.02	98.00	100.25
30.00	10.00	30.33	9.95	101.10	99.50
40.00	11.00	39.40	11.04	98.50	100.36
Mean ±SD				99.52± 1.327	99.94± 0.731

*** Average of three determinations**

Table-1: Results of accuracy for determination of pure authentic of HBB and PAR by the proposed spectrophotometric method

2.5.2 Linearity and construction of calibration curves

For PAR, transfer aliquots equivalent to (20 – 120 µg) of PAR from its working solution (l00 µg.mL^{-1}) into a series of 10-mL volumetric flasks complete to volume with methanol to give a final concentration range of (2 – 12 µg.mL^{-1}) of PAR. Record the absorption spectra of PAR in the range of (2 - 12 µg.mL^{-1}) using methanol as a blank and store them in the computer. Record the zero order absorbencies of PAR at λ_{max} 248 nm. Construct the calibration curve representing the relation between the absorbencies at 248 nm and the corresponding concentration then compute the regression equation. 2 - For HBB transfer aliquots equivalent to (20 – 450 µg.mL^{-1}) of HBB from its working solution (100 µg.mL^{-1}) into a series of 10-mL volumetric flasks complete to volume with methanol to give a final concentration range of (2 – 45 µg.mL^{-1}).

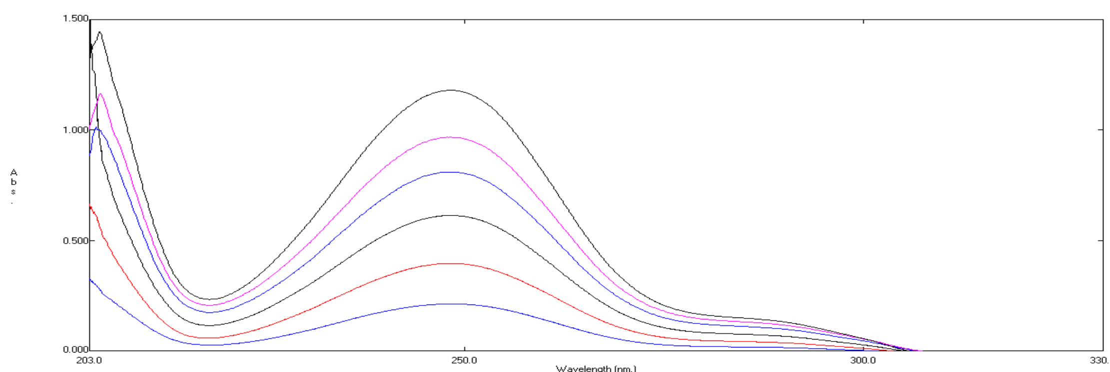

Figure -7: Linearity of zero order spectra of PAR in methanol .

Record the absorption spectra of HBB using methanol as a blank and store them in the computer.Divide the absorption spectra of HBB in the range of (2 – 45 µg.mL^{-1}) by absorption spectrum of 100 µg.mL^{-1} PAR, differentiate the obtained ratio spectra with respect to wavelength to obtain the first derivative curves then record the peak amplitude of HBB at λ_{max} 220.5 nm.Construct the calibration curve representing the relation between the peak amplitudes at 220.5 nm and the corresponding concentrations and compute the regression equation.

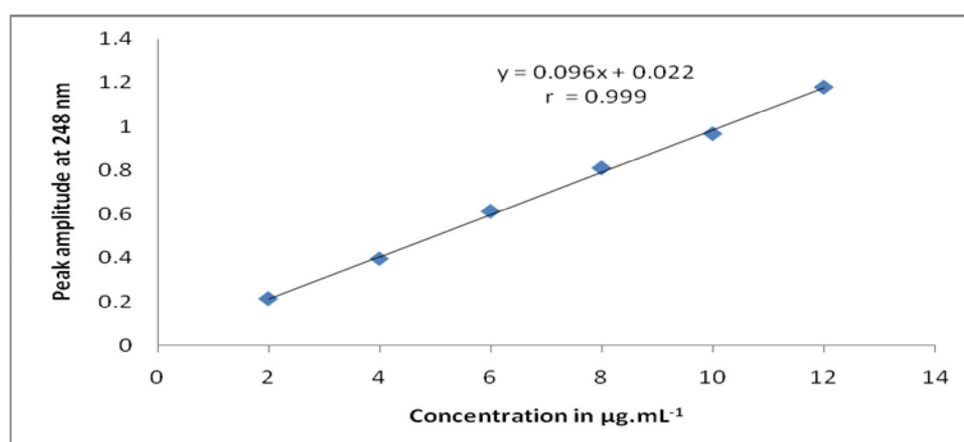

Figure-8: Linearity of zero order spectra of PAR in methanol, relating its peak amplitude to its corresponding concentration at 248.0 nm.

2.5.3 Analysis of laboratory prepared mixtures

Prepare mixtures containing PAR and HBB in different ratios and proceed as detailed under linearity. Calculate the concentrations of PAR and HBB from their corresponding regression equations.

2.5.4 Application of the proposed method to pharmaceutical formulations.

For tablet dosage form

The contents of ten tablets of Buscopan plus® (also for Buscamol®) were thoroughly powdered and mixed then an amount of the powder equivalent to 500 mg of PAR and 10 mg of HBB was weighed accurately in a 250-mL beaker, 70 mL of methanol was added, stirred for about 30 min then filtered through filter paper into a 100-mL volumetric flask, the beaker and the funnel were washed and the volume was completed with methanol to get a concentration of 5.0 and 0.10

mg.mL^{-1} for PAR and HBB, respectively. Appropriate dilutions were made to get the concentration of 125.0 and 2.5 µg.mL^{-1} for PAR and HBB, respectively and a concentration of 5.0 and 0.1 µg.mL^{-1} PAR and HBB, respectively.

Dosage form	Drug	Taken (µg.mL^{-1})	Found* (µg.mL-1)	Found %	Pure added (µg.mL^{-1})	Pure Found** (µg.mL^{-1})	Recovery %	Mean ±SD
Buscopan plus® tablets Batch No 116738T	HBB	2.50	2.48	99.20	8.00 10.00 20.00	7.92 10.05 20.20	99.00 100.50 101.00	100.17± 1.041
	PAR	5.00	5.04	100.80	2.50 3.00 5.00	2.50 3.03 5.08	100.00 101.00 101.60	100.87± 0.808
Buscamol.F.C® tablets Batch No 12001025	HBB	2.50	2.47	98.80	8.00 10.00 20.00	8.04 10.06 19.89	100.50 100.60 99.45	100.18± 0.637
	PAR	5.00	5.08	101.60	2.50 3.00 5.00	2.45 2.97 4.82	98.00 99.00 96.40	97.80± 1.311
Buscopan plus® suppositories Batch No 105	HBB	2.50	2.53	101.20	8.00 10.00 20.00	8.08 9.96 19.65	101.00 99.60 98.25	99.62± 1.375
	PAR	5.00	5.05	101.00	2.50 3.00 5.00	2.55 2.97 5.02	102.00 99.00 100.40	100.47± 1.501

* Average of six determinations ** Average of three determinations

Table-2: Application of standard addition technique to analysis of HBB and PAR in dosage forms by the spectrophotometric method

The PAR concentrations were calculated from zero order calibration spectrophotometric method while the proposed derivative ratio spectrophotometric calibration method was applied for the analysis and calculation of HBB concentrations.

For suppository dosage form

The contents of five suppositories of Buscopan plus® were thoroughly cut to small fragments then an amount of the fragments equivalent to 800 mg of PAR and 10 mg of HBB was weighed accurately in 250-mL beaker, 70 mL of methanol was added, stirred for about 30 min, leave to cool then filtered through filter paper into a 100-mL volumetric flask, the beaker and the funnel were washed and the volume was completed with methanol to get a concentration of 8.0 and 0.10 mg.mL^{-1} for PAR and HBB, respectively. Appropriate dilutions were made to bring up a concentration of 200.0 and 2.5 µg.mL^{-1} for PAR and HBB, respectively and a concentration of 5.0 and 0.06 µg.mL^{-1} PAR and HBB, respectively. The proposed spectrophotometric method was applied for the analysis and calculation of HBB and PAR concentrations.

3.0 Results and discussion

3.1. Method development and optimization

The aim of this work is to develop a method that can be applied successfully for selective quantification of the studied drugs.The zero order spectra of PAR and HBB show overlapping with HBB at its maximum absorbance wavelength at 205.0 nm while PAR can be measured directly at 248.0 nm in the presence of HBB that don't interfere as shown in Figure -2.

Parameters	HBB	PAR
Range (μg.mL^{-1})	2-45	2- 12
Slope	0.046	0.096
Intercept	-0.047	0.022
Correlation coefficient (r)	0.9999	0.9998
Accuracy (mean ± SD)	99.52± 1.327	99.94± 0.731
Precision (RSD%) Repeatability*	0.673	1.301
Intermediate precision*	0.842	1.324

* The intra-day and inter-day relative standard deviations of the average of concentrations (4, 6 and 8 μg mL^{-1} for each).

Table-3: Results of assay validation parameters of spectrophotometric method for the determination of HBB and PAR in binary mixture

The first derivative order, the second derivative order and the third derivative order fail to give suitable peaks that can be used for selective determination of HBB in the presence of PAR. The problem of overlapped spectra of PAR with HBB was solved using ^{1}DD spectrophotometric technique.

Mixture No	Ratio of HBB:PAR (μg. mL^{-1})	HBB Taken (μg. mL-1)	HBB Found* (μg. mL-1)	HBB Recovery %	PAR Taken (μg. mL-1)	PAR Found* (μg. mL-1)	PAR Recovery %
1	1:1	10.00	9.84	98.40	10.00	9.90	99.00
2	4:3	8.00	8.02	100.25	6.00	5.99	99.83
3	4:5	8.00	7.99	99.88	10.00	10.30	103.00
4	2:1	10.00	9.88	99.80	5.00	4.92	98.40
5	1:50**	2.50	2.43	97.20	125.00	---	---
		0.20	---	---	10.00	9.98	99.80
6	1:80 ***	2.50	2.45	98.00	200.00	---	---
		0.125	---	---	10.00	10.20	102.00
Mean ± SD				98.76± 1.150			100.34± 1.786

*** Average of three determinations**

**** The ratio present in Buscamol® tablets and Buscopan plus® tablets**

***** The ratio present in Buscopan plus® suppositories**

Table-4: Determination of HBB and PAR in laboratory prepared mixtures by the proposed spectrophotometric method.

The absorption spectra PAR and HBB were divided by the spectrum of 100 μg.mL^{-1} of PAR where the obtained ratio spectra, Figure-3, were differentiated with respect to wavelength, Figure-4, for determination of HBB. To optimize the ^{1}DD spectrophotometric method, it was necessary to test the effect of different variables

The divisor and its concentration

Different concentrations of PAR were tested (2, 5, 10,100 μg.mL^{-1}), the concentration of 100 μg.mL^{-1} gave the best results regarding selectivity.

Instrumental parameters:-

Different smoothing factor (Δλ) values given by the program (2,4 ,8 and 16) were tried; smoothing factor = 4 showed a suitable signal to noise ratio , and the spectra showed good resolution. Different scaling factor values (1, 10, 100, and 1000) were tried, scaling factor = 1 was suitable for good resolution of the signal of PAR and HBB to facilitate measurement and to diminish the error in reading the signal.

Parameter	Spectrophotometric method		Reference method	
	HBB	PAR	HBB	PAR
Mean %	99.52	99.94	99.21	99.94
SD	1.327	0.731	1.012	1.537
n	6	6	6	6
Student 's t-test (2.23)[b]	0.660	1.000	---	---
F-value (5.05)[b]	1.719	4.421	---	---

a-manufactured method personal communications .

b-the values between parenthesis are the theoretical values for t and F at P=0.05.

Table-5: Statistical analysis of the results obtained by proposed method and reference method for the determination of HBB and PAR

Method validation

Method validation was performed according to ICH guidelines [22].

Linearity of the proposed method was evaluated where the absorption spectra of HBB in a concentration rang of 2- 45 $\mu g.mL^{-1}$, were divided by the spectrum of 100 $\mu g.mL^{-1}$ of PAR where the obtained ratio spectra were differentiated with respect to wavelength. ^{1}DD values showed good linearity and accuracy at 220.5 nm for HBB, figure-5 and figure-6. The absorption spectra of PAR in a concentration rang of 2- 12 $\mu g.mL^{-1}$ were measured directly at 248 nm in zero order spectra, Figure-7and 8.

The regression equations were computed and found to be:

$$Y_1 = 0.046\, C_1 - 0.047 \qquad r_1 = 0.9999 \qquad \text{for HBB at } \lambda = 220.5 \text{ nm}$$

$$Y_2 = 0.096\, C_2 + 0.022 \qquad r_2 = 0.9998 \qquad \text{for PAR at } \lambda = 248.0 \text{ nm}$$

Where Y_1 is the peak amplitude at 220.5 nm for HBB and Y_2 is the peak amplitude at 248.0 nm for PAR, C_1 and C_2 is the concentration of HBB and PAR, respectively, in $\mu g.mL^{-1}$ and r_1 and r_2 is the correlation coefficient of HBB and PAR , respectively.

Good linearity was evident by the high value of the correlation coefficient and the low intercept value. The accuracy of the results was checked by applying the proposed method for determination of different blind samples of pure HBB and PAR. The concentrations were calculated from the corresponding regression equations. The results obtained are shown in Table-1.Accuracy of the proposed method was further assessed by the use of the standard addition technique. The results obtained are shown in Table-2. The precision of the proposed Spectrophotometric method was evident as shown in Table-3. Specificity of the method was achieved by the analysis of different laboratory prepared mixtures of the two drugs within the linearity range. Satisfactory results were shown in Table-4.Statistical analysis was performed by comparing the results of the proposed method with those of manufacturer method. No significant difference was observed regarding accuracy and precision, as shown in Table-5.

4.0 Conclusion

The proposed method is efficient for providing sensitive and accurate quantitative analysis for determination of HBB and PAR in bulk powder and pharmaceutical formulations, without any interference from excipients. Spectrophotometric method has the advantages of simplicity and the availability of the device in every quality control unit so it is suitable for routine analysis.

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