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Simultaneous Determination of Hyoscine N-Butyl Bromide and Paracetamol by RP-TLC Spectrodensitometric Method

Nouruddin W. Ali[1], Mohammed Gamal[1*] and Mohammed Abdelkawy[2]

[1]Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Alshaheed shehata Ahmed Hegazy St., 62574 Beni-Suef, Egypt.
[2]Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St., 11562, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author NWA designed the study, performed the statistical analysis and wrote the protocol. Author MG managed the analyses of the study and wrote the first draft of the manuscript. Author MA managed the literature searches and wrote the final draft of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: A simple RP-TLC Spectrodensitometric method was developed for determination of Hyoscine N-Butyl Bromide (HBB) and Paracetamol (PAR) either in bulk powder or in their pharmaceutical preparation.
Study Design: Validation study.
Methodology: In this method, HBB and PAR 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. Experimental conditions such as band size, slit width, different developing systems and scanning wavelength were carefully studied and the optimum conditions were selected. The obtained bands were then scanned at 210 nm. The two drugs were satisfactorily resolved with R_F 0.60 ± 0.02 for HBB and 0.81 ± 0.02 for PAR. The validation of spectrodensitometric method was done regarding linearity, accuracy, precision, and specificity.
Results: Linearity of the proposed methods was evaluated and it was found to lie within the concentration range of 2.0-12.0 µg.band^{-1} for HBB and 2.0-14.0 µg.band^{-1} for PAR.

**Corresponding author: Email: mgamalm3000@yahoo.com;*

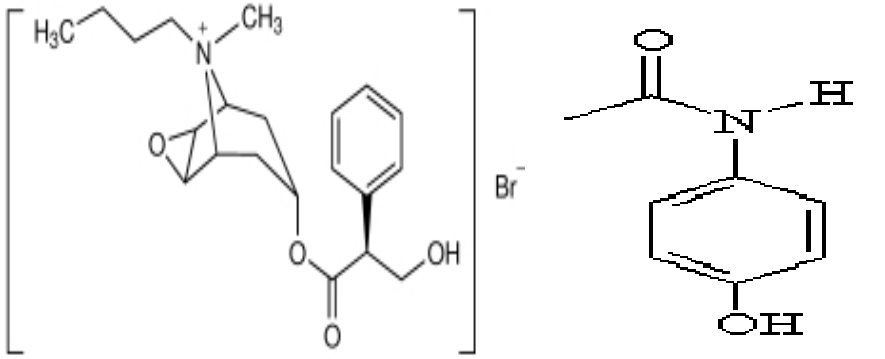
Conclusion: The proposed method was successfully applied for determination of HBB and PAR in pure form and in their different pharmaceutical formulations. The method proved to be specific, accurate and selective.

Keywords: RP-TLC; spectrodensitometry; hyoscine N-butyl bromide; paracetamol.

1. INTRODUCTION

Hyoscine N-ButylBromide is a quaternary ammonium anticholinergic agent. It has been used as antispasmodic due to relaxation effect on the smooth muscles of the gastrointestinal, biliary, and urinary tracts [1].

Paracetamol (PAR), 4-acetamidophenol, is an effective analgesic and antipyretic for treatment of minor, non-inflammatory conditions in patients who are prone to gastric symptoms [1]. The structural formulas of HBB and PAR are shown in Fig. 1.



Molecular formula $C_{21}H_{30}BrNO_4$ *Molar mass 440.4*

Molecular formula $C_8H_9NO_2$ *Molar mass 151.17*

Fig. 1. chemical structure of HBB (A) and PAR (B)

Many reported methods have been mentioned for the determination of HBB and PAR either separately or in combination with other drugs including spectrophotometric methods[2-5], chromatographic methods [6-8], electrochemical methods [9-12], Capillary electrophoresis methods [13,14] and titrimetric method [15-17].

Few methods have been mentioned for analysis of HBB and PAR in binary mixture. In the first method, Erk [18] analysed HBB and PAR 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.

RP-TLC (reversed-phase thin layer chromatography) has been successfully applied for analysis of many drugs as Losartan [20], Oral Antidiabetic drugs [21-22], Ibuprofen [23],

Tocopherol acetate [24], Scopolamine Hydro Bromide [25] and mixture of Dipyrone and Hyoscine N-ButylBromide [26].

No TLC method has been reported for simultaneous analysis of the two drugs. Therefore, the objective of this work is to develop sensitive and selective RP-TLC method for simultaneous determination of HBB and PAR for routine quality control analysis of these drugs either in bulk powder or in pharmaceutical formulations.

2. EXPERIMENTALS

2.1 Apparatus

1- UV lamp with short wavelength 254 nm (USA).
2- TLC scanner three densitometer (Camage, Muttenz, Switzerland).

The following requirements are taken into consideration:

1- - Slit dimensions: 6.00x0.45, Micrometer-Scanning speed = 20 mm/s
2- Data resolution = 100 µm / step.
3- Sample applicator for thin layer chromatography Linomat IV with 100 µl syringe (Camage, Muttenz, Switzerland).
4- 4-ALUGRAM® RP-18 W/ UV254 TLC plates (10x10 cm) coated with 0.15 mm silica gel RP-18 W with fluorescent indicator UV254 (Macherey-Nagel, Germany).
5- Sonix TV ss-series ultrasonicator (USA).

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.

2.2.2 Market samples

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

2.2.3 Reagents

Analytical grade reagents and chemicals were used without further purification:

1- Sodium Citrate and Hydrochloric acid from (EL – NASR Pharmaceutical Chemicals Co., Abu - Zabaal, Cairo, Egypt).
2- Methanol HPLC grade (Sigma Aldrich, Germany).

3- Trifluoroacetic acid from Spectrochem, India.
1- 4-Deionised Water (SEDICO pharmaceutical Co., 6th October City, Egypt).
2- 5-Citrate buffer pH 1.5 (22 mL 0.1 M sodium citrate and 78 mL 0.1 N HCl are mixed together)

2.3 Preparation OF Standard Solutions

A. A-Paracetamol (PAR) and Hyoscine N-Butyl Bromide (HBB) stock standard solutions (1 mg.mL $^{-1}$). Stock standard solutions of Paracetamol (PAR) and Hyoscine N-Butyl Bromide (HBB) each containing 1 mg.mL $^{-1}$ were prepared in methanol.
B. B- Paracetamol (PAR) and Hyoscine N-Butyl Bromide (HBB) working standard solutions (100 µg.mL^{-1}) working standard solutions (100 µg.mL^{-1}) of these drugs were prepared by appropriate dilution of the stock solution with methanol.

2.4 Procedures

2.4.1 Linearity and construction of calibration curves

Aliquots equivalent to (2.0 – 12.0 µg) of HBB, (2.0-14.0 µg) of PAR were applied accurately from their corresponding stock solutions (l000 µg.mL^{-1}) to RP-TLC plates (10x10cm) as band using the Camage TLC sampler. A space of 1 cm was left between each band and1.5 cm from the bottom edge of the plate. The plate was developed in a previously saturated chromatographic tank for one hour with the developing mobile phase consisting of methanol: citrate buffer (pH=1.5): triflouroacetic acid (70.0:29.9:0.1, by volume) by ascending chromatography at room temperature [25].

The bands were detected under UV - lamp and scanned at 210 nm under the specified experimental conditions. The calibration curves were constructed for each compound by plotting the peak area/ 100 versus the corresponding concentration and then the regression equations were computed.

2.4.2 Analysis of laboratory prepared mixtures

The mixtures containing HBB and PAR in different ratios were prepared and analyzed as mentioned under linearity and construction of calibration curves. The concentrations of the two compounds were calculated from their corresponding regression equations.

2.4.3 Application of TLC-spectrodensitometric method to pharmaceutical formulations

A) **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 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 a concentration of 100.0 and 2.0 µg.mL-1 for PAR and HBB, respectively and a concentration of 5.0 and 0.1 µg.mL-1 PAR and HBB, respectively.

B) For suppositories 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 to coagulate the suppository base 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 get a concentration of 160.0 and 2.0 $\mu g.mL^{-1}$ for PAR and HBB, respectively and a concentration of 8.0 and 0.1 $\mu g.mL^{-1}$ PAR and HBB, respectively.

3. RESULTS AND DISCUSSION

3.1 Method Development and Optimization

The aim of this work is to develop an applicable method that can be used successfully for separation and quantification of the studied drugs. Studying of the optimum parameters for maximum separation was carried out by investigating the effect of different variables. Different developing systems with different compositions and ratios were tried, but complete separation of HBB and PAR was achieved by using the reported developing mobile phase [25] consisting of methanol: citrate buffer (pH=1.5): triflouroacetic acid (70.0: 29.9: 0.1, by volume) .Also different scanning wavelengths (210, 230, 254 nm) were tested, but the best sensitivity obtained when 210 nm was used as scanning wavelength.

Different band dimensions (4, 6, 8, 10 mm) were tested to obtain sharp and symmetrical peaks. The optimum band length chosen was 6 mm and the inter space between bands was 1 cm. The slit dimensions of the scanning light beam should ensure complete coverage of band dimensions on the scanned track without interference of adjacent bands. Different slight dimensions were tried where 6 mm x 0.45 mm provided the highest sensitivity. The method is based on the difference in R_f values of HBB (R_f = 0.60) and PAR (R_f = 0.81) as shown in Fig. 2.

3.2 Method Validation

Method validation was performed according to ICH guidelines [27]. Linearity of the TLC-spectrodensitometric method was evaluated and it was found to lie within the concentration range of 2.0-12.0 $\mu g.band^{-1}$ for HBB and 2.0-14.0 $\mu g.band^{-1}$ for PAR, Figs. (3-4). Good linearity was evident by the high value of the correlation coefficient and the low intercept value, (Table 4). The method can detect low concentrations of the two drugs, the sensitivity of the method is relatively similar to that of spectrophotometric method.

The regression equations were calculated and found to be:

$Y_1 = 0.155\ C_1 + 0.333$ $\quad r_1 = 0.9998$
$Y_2 = 0.274\ C_2 + 0.169$ $\quad r_2 = 0.9996$

Where Y_1 and Y_2 are the peak area /100, C_1 and C_2 are HBB and PAR concentrations in $\mu g.band^{-1}$ respectively and r_1 and r_2 are the correlation coefficients.

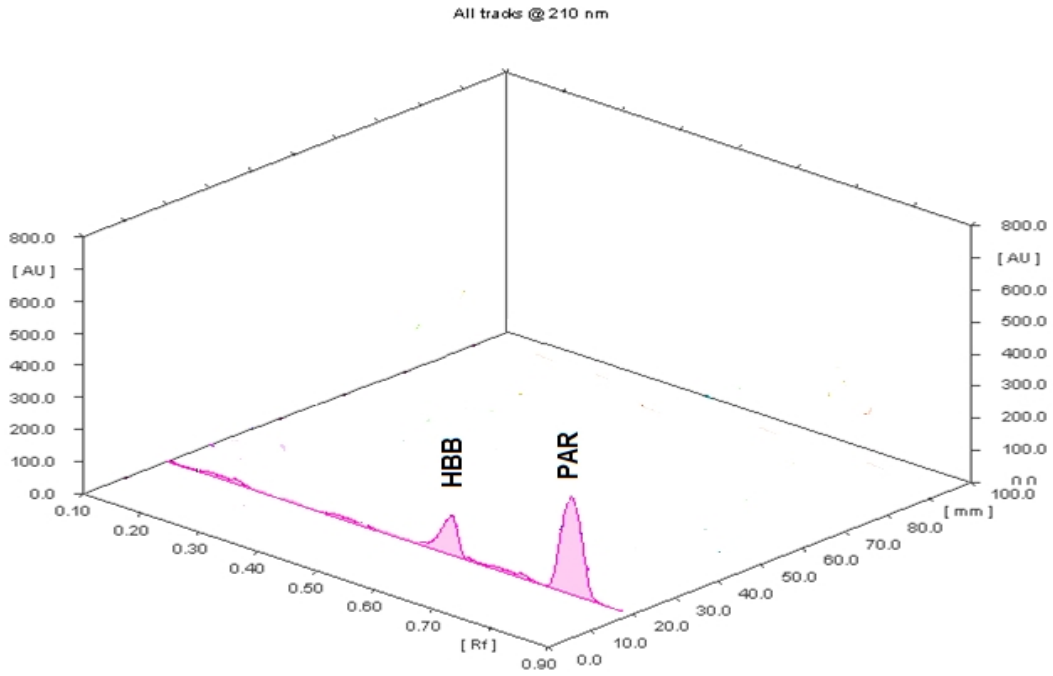


Fig. 2. A 3D diagram of a TLC chromatogram showing an example of separated mixture of HBB (5 µg band^{-1}) and PAR (10 µg band^{-1}) using methanol: citrate buffer (pH=1.5): triflouroacetic acid (70.0:29.9:0.1, by volume) as a mobile phase and scanning at 210 nm

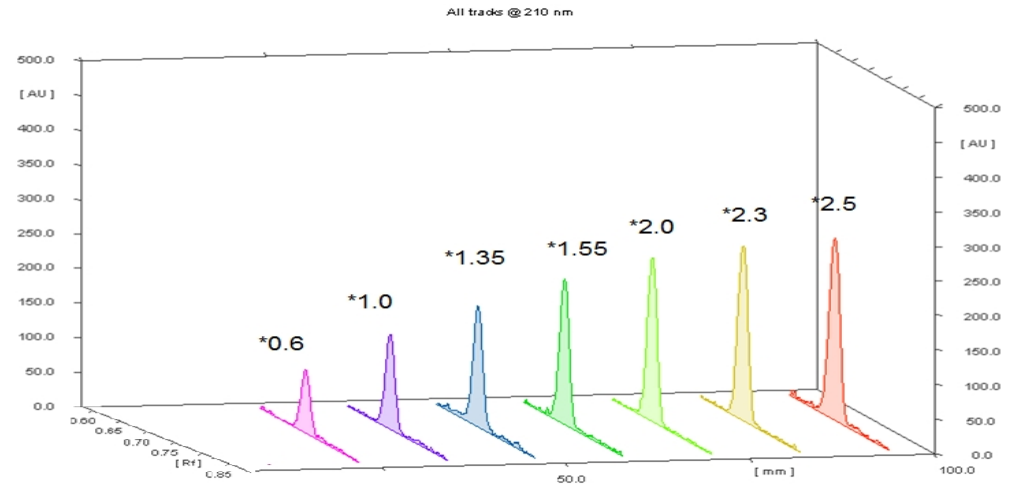


Fig. 3. A 3D diagram showing separation of HBB (R_f =0.60) over a concentration range 2.0 – 12.0 µg band^{-1} at 210 nm. * AUP/100

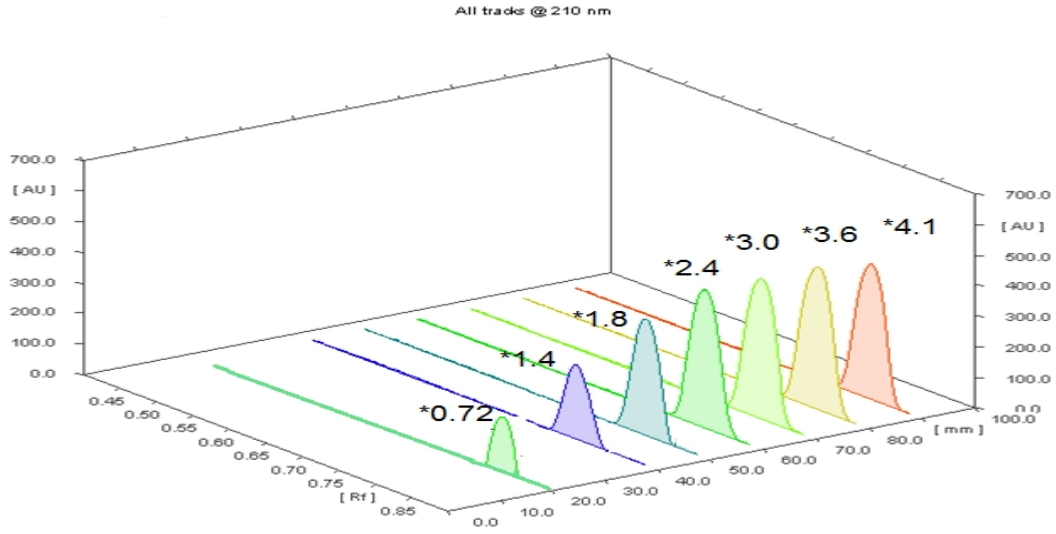


Fig. 4. A 3D diagram showing separation of PAR (R_f =0.81) over a concentration range 2.0 – 14.0 µg band^{-1} at 210 nm. * AUP/100

Precision of the proposed TLC-spectrodensitometric method was evident as shown in Table 4 where the intra-day and inter-day relative standard deviations of the average of concentrations (4.0 ,6.0 and 10.0 µg band^{-1} for each drug were calculated and found to be 1.014 and 1.182 for HBB , 1.122 and 0.814 for PAR.

Accuracy of the TLC-spectrodensitometric method was checked by applying the method for determination of different samples of pure HBB and PAR. The concentrations of HBB and PAR were calculated from the corresponding regression equations. The results obtained were shown in Table 1. The accuracy for HBB was found to be 100.22±0.733 while it was found to be 99.82±1.048 for PAR.

Accuracy of the TLC-spectrodensitometric method was further assessed by applying the standard addition technique on Buscopan plus® tablets, Buscamol® tablets and Buscopan plus® suppositories where good recoveries were obtained as shown in Table 3 revealing good accuracy of the proposed method.

Specificity of the described method is evident from the TLC-spectrodensitometric chromatogram as shown in Fig. 2 where each drug of the mixture appears at certain R_f value (0.61 for HBB and 0.81 for PAR. Specificity of the proposed method is also evident from Table 2 where the accuracy for HBB was found to be 100.25±1.084 while it was found to be 100.53±0.704 for PAR.

Robustness of the TLC-spectrodensitometric method was evaluated in the development phase by making small changes in the composition of mobile phase and detection wavelength (209, 211 nm). The low values of %RSD show that the method is robust and that deliberate small changes in the studied factors did not lead to a significant change in R_f values, area or symmetry of the peaks.

System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as whole. System suitability is used to ensure system performance before or during the analysis of the drugs. System suitability was checked by calculating the capacity factor(K'), symmetry factor, the selectivity factor(γ) and resolution(Rs), where the system was found to be suitable as shown in Table 5.

Table 1. Results of accuracy for determination of pure authentic of HBB and PAR by the proposed TLC-spectrodensitometric method

Taken(µg.band^{-1})		Found* (µg .band^{-1})		Recovery %	
HBB	**PAR**	**HBB**	**PAR**	**HBB**	**PAR**
2.00	2.00	2.03	1.99	101.50	99.50
4.00	4.00	4.02	3.95	100.50	98.75
6.00	6.00	5.96	5.91	99.33	98.50
8.00	8.00	7.99	8.04	99.88	100.50
10.00	10.00	10.00	10.08	100.00	100.80
12.00	14.00	12.01	14.12	100.08	100.86
				100.22±0.733	99.82±1.048

Average of three determinations

Table 2. Determination of HBB and PAR in laboratory prepared mixtures by the proposed TLC-spectrodensitometric method

Mix. No.	Ratio	HBB			PAR		
	HBB:PAR	Taken (µg. band^{-1})	Found* (µg. band^{-1})	Recovery %	Taken (µg. band^{-1})	Found* (µg. band^{-1})	Recovery %
1	1:1	2.00	1.98	99.00	2.00	2.02	101.00
2	1: 2	2.00	2.00	100.00	4.00	3.98	99.50
3	1: 5	2.00	2.04	102.00	10.00	10.09	100.90
4	1 :10	2.00	2.00	100.00	20.00	-------	-------
		1.00	------	--------	10.00	10.03	100.30
5	1: 50**	2.00	2.02	101.00	100.00	------	-------
		0.20	--------	--------	10.00	10.09	100.90
6	1:80***	2.00	1.99	99.50	160.00	------	-------
		0.10	--------	--------	8.00	7.96	99.50
Mean ±SD				100.25 ±1.084			100.35 ±0.704

Average of three determinations

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

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

Table 3. Application of standard addition technique to analysis of HBB and PAR in dosage forms by the TLC-spectrodensitometric method

Dosage form	Drug	Taken ($\mu g.mL^{-1}$)	Found* ($\mu g.mL^{-1}$)	Found %	Pure added ($\mu g.mL^{-1}$)	Pure Found* ($\mu g.mL^{-1}$)	Recovery %	Mean ±SD
Buscopan plus® tablets Batch No 116738T	HBB	2.00	1.97	98.50	2.00	2.00	100.00	100.38± 0.333
					4.00	4.02	100.50	
					8.00	8.05	100.63	
	PAR	5.00	5.02	100.40	1.00	1.01	101.00	101.09± 0.471
					3.00	3.02	100.67	
					5.00	5.08	101.60	
Buscamol.F.C® tablets Batch No 12001025	HBB	2.00	1.98	99.00	2.00	2.01	100.50	100.33± 0.289
					4.00	4.02	100.50	
					8.00	8.00	100.00	
	PAR	5.00	5.04	100.80	1.00	0.99	99.00	100.22± 1.072
					3.00	3.02	100.67	
					5.00	5.05	101.00	
Buscopan plus® suppositories Batch No 105	HBB	2.00	2.02	101.00	2.00	2.04	102.00	100.46± 1.369
					4.00	4.00	100.00	
					8.00	7.95	99.38	
	PAR	8.00	8.08	101.00	1.00	0.99	99.00	100.27± 1.102
					3.00	3.03	101.00	
					5.00	5.04	100.80	

** Average of six determinations*
*** Average of three determinations*

Table 4. Results of assay validation parameters of the proposed TLC - spectrodensitometric method for the determination of HBB and PAR in binary mixture

Parameters	HBB	PAR
Range (µg band^{-1})	2.0-12.0 (µg.band^{-1})	2.0-14.0 (µg.band^{-1})
Slope	0.155	0.274
Intercept	0.333	0.169
Correlation coefficient (r)	0.9991	0.9996
Accuracy(mean ± SD)	100.22±0.733	99.82±1.048
Precision Repeatability (RSD%)*	1.014	1.122
Intermediate precision*	1.182	0.814
Limit of detection (LOD) 3.3xSD/Slope	0.606	0.779
Limit of quantization (LOQ) 10xSD/Slope	1.837	2.361

**the intra-day and inter-day relative standard deviations of the average of concentrations (4.0 , 6.0 and 10.0 µg band^{-1} for each drug) .*

Table 5. Statistical analysis of parameters required for system suitability testing of the proposed TLC-spectrodensitometric method

Parameters	For TLC-densitometric method		
	Obtained value		Reference value
	HBB	PAR	
Resolution (R_s)	2.52		›1.5
Capacity factor(K')	0.67	0.23	0- 10 acceptable
Symmetry factor	1.00	1.22	≈ 1
Selectivity factor(γ)	2.91		› 1

Table 6. Statistical analysis of the results obtained by proposed method and reference method for the determination of HBB and PAR

Parameter	Spectrodensitometric method		Reference method[a]	
	HBB	PAR	HBB	PAR
Mean %	100.22	99.82	99.21	99.94
SD	0.733	1.048	1.012	1.537
n	6	6	6	6
Student 's t-test (2.23)[b]	0.079	0.878		
F-value (5.05)[b]	1.906	2.151		

a manufactured HPLC method via personal communications .
b the values between parenthesis are the theoretical values for t and F at P=0.05

4. CONCLUSION

The proposed method is efficient for providing sensitive and accurate quantitative analysis for simultaneous determination of HBB and PAR in bulk powder and in pharmaceutical formulations. TLC- spectrodensitometric method has the advantages of that several samples can be run simultaneously using a small quantity of mobile phase and provides high sensitivity and selectivity. The separation power of chromatographic methods allows determination of mixture of drugs by any ratio in pharmaceutical formulations.

Statistical analysis was determined by comparing the results of the TLC-spectrodensitometric method with those of manufacturer HPLC method. No significant difference was estimated regarding accuracy and precision, as shown in Table 6.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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