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Extractive ion-pair spectrophotometric methods utilizing bromocresol green to estimate chlorpheniramine maleate in pharmaceutical formulations

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Abstract

Two accurate, sensitive, and cost-effective spectrophotometric methods were developed and validated for the quantitative determination of Chlorpheniramine Maleate (CPM). Method A is based on the formation of a yellow ion-pair complex between CPM and bromocresol green (BCG) in an acidic medium. The resulting complex is extracted into dichloromethane, and its absorbance is measured at 414 nm. Method B involves the dissociation of the yellow-colored CPM–BCG ion-pair complex in a basic medium, leading to the development of a blue color that gives a maximum absorbance at 618 nm. Beer's law was found to be valid within the concentration ranges of 1.00–25.0 µg/mL for method A and 0.20–9.00 µg/mL for method B. The molar absorptivities were determined and found to be 1.76×10^4 and 4.47×10^4 L/(mol.cm) for methods A and B, respectively, with Sandell sensitivity values of 0.0222 and 0.0087 µg/cm². Detection and quantification limits were determined and found to be 0.2049 and 0.6210 µg/mL for method A, and 0.0199 and 0.0603 µg/mL for method B, respectively. The developed methods were successfully employed to determine CPM in both its pure form and commercial pharmaceutical formulations. Statistical comparison with the official USP HPLC method showed no significant differences in accuracy or precision, making the developed methods ideal for routine quality control in resource-limited settings.

Keywords Chlorpheniramine maleate, Bromocresol green, Ion-pair complex, Spectrophotometry, Method validation, Pharmaceuticals

1. Introduction

Chlorpheniramine maleate (CPM), also known as chlorphenamine, is chemically defined as (3RS)-(4-chlorophenyl)-N,N-dimethyl-3-(pyridine-2-yl) propan-1-amine hydrogen(Z)-butenedioate (Fig. 1) [1,2].

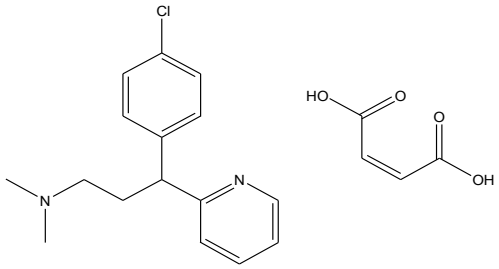


Fig. 1 Structure of the chlorpheniramine maleate

CPM appears as a white to off-white crystalline powder, freely soluble in water and 96% ethanol but slightly soluble in ether and benzene. Its aqueous solutions have a pH of 4–5, and it is stored in amber, airtight containers to maintain stability [1-3]. It is a first-generation alkylamine-class antihistamine and H1-receptor antagonist [4].

CPM is currently listed in major pharmacopoeias, including the United States Pharmacopoeia (USP), British Pharmacopoeia, and European Pharmacopoeia [1,2,5]. Various analytical methods have been reported for the determination of CPM in pharmaceutical formulations, including high-performance liquid chromatography (HPLC) [6,7], ultra-performance liquid chromatography–tandem mass spectrometry [8,9], high-performance thin-layer chromatography [10,11], reversed phase–ultra performance liquid chromatography [12,13], electrochemiluminescence sensors [14,15], Raman spectroscopy [16], chemometrics [17] and spectrophotometry [18-29]. A summary of existing spectrophotometric methods is presented in Table 1, highlighting wide variations in reagents, analytical wavelengths, and performance characteristics. However, many of these methods suffer from limitations such as narrow linear range, incomplete validation data, and reliance on hazardous organic solvents.

While extractive spectrophotometric approaches have been used in the past to estimate CPM, the current study presents two new methods based on the use of a chromogenic reagent i.e., bromocresol green (BCG). The developed methods are suitable for routine quality control

in pharmaceutical laboratories because they have significant benefits in terms of simplicity, sensitivity, affordability, and environmental compatibility.

2. Experimental

2.1.Instrument

Absorbance measurements were conducted using a double-beam ultraviolet–visible spectrophotometer (Specord 200, Analytikjena, Germany), equipped with 1 cm path length quartz cuvettes at room temperature.

2.2.Chemicals and reagents

All chemicals and solvents used were of analytical reagent grade.

- CPM solutions: A stock solution (200 µg/mL) was prepared by dissolving 20 mg of CPM in 100 mL of distilled water. The working solutions of 50 and 20 µg/mL CPM were obtained by appropriate dilution of the stock solution with distilled water and used for method A and method B, respectively.
- BCG solution: A 0.04% (w/v) BCG solution was prepared by dissolving of 0.04 g BCG (BDH, England) in a minimal volume of ethanol, followed by diluting the resulting solution to 100 mL with distilled water.
- Alcoholic potassium hydroxide (KOH) solution: A 0.1 M KOH solution was prepared by dissolving the required amount of KOH (Fisher Scientific, USA) in absolute ethanol.
- Anhydrous sodium sulphate (BDH, England) was used as such as a drying agent.

Table 1 Summary of existing visible spectrophotometric methods for the determination of CPM

No.	Reference Number	λ_{max} (nm)	Molar absorptivity, L/(mol.cm)	Linear range, µg/mL	LOD/LOQ, µg/mL	Used reagent/s	Method description	Method remarks
1	18	530	3.0×10^3	200–240	Not reported	Chloranilic acid	A charge-transfer complex is formed in organic solvents	Incomplete validation data, narrow linear range, less sensitivity, and insufficient reproducibility details
2	19	628	Not reported	25–125	8 / 15	Cobalt-thiocyanate	CPM reacts with cobalt-thiocyanate and extracts the formed blue complex into chloroform	Narrow linear range, molar absorptivity was not stated; relies on chloroform
3	20	482	Not reported	50–175	Not reported	Aniline and cyanogen bromide	CPM reacts with aniline in the presence of cyanogen bromide to yield a yellow derivative	Molar absorptivity, LOD, and LOQ were not reported, narrow linear range, and color stability for 2 min only
4	21	p-Cl: 540 p-I: 524 C2B: 536 C2R: 524	p-Cl: 4.67×10^3 p-I: 6.22×10^3 C2B: 7.86×10^3 C2R: 8.57×10^3	p-Cl: 0 - 3.10×10^3 p-I: 0 - 2.36×10^3 C2B: 0 - 1.90×10^3 C2R: 0 - 1.70×10^3	Not reported	p-Cl, p-I, C2B, and C2R	CPM forms an ion-pair complex with each reagent, followed by the extraction of the formed complex into the methylene chloride solvent	LOD and LOQ are not reported. The lower concentration that can be determined is unclear
5	22	ARS: 425 BCP: 395 ECR: 503 CR: 408	ARS: 3.2×10^3 BCP: 1.4×10^4 ECR: 3.2×10^3 CR: 4.6×10^4	ARS: 1–10 BCP: 1-30 ECR: 2–120 CR: 4–120	ARS: 0.66/ 2.20 BCP: 0.71/ 2.40 ECR: 0.69/ 2.30 CR: 1.5/ 5.00	ARS, BCP, ECR and CR	Formation of ion-pair complexes between CPM and dyes, followed by extraction of these complexes into an organic solvent	Use of chloroform, ARS, and ECR methods is less sensitive
6	23	Method A: 550 Method B: 610	Not reported	Method A: 2.5-20 Method B: 2.5-20	Method A: 0.47/1.14 Method B: 0.83/0.64	Method A: acidic $KMnO_4$ Method B: alkaline $KMnO_4$	CPM reacts with $KMnO_4$ in an acidic or basic medium. The resulting-colored product is measured at the corresponding λ_{max}	Low selectivity and limited product stability
7	24	575	3.84×10^4 for CPM	Not reported	Not reported	TBPE	The assay is based on the reaction of CPM with TBPE to form a red ion-associate that can be extracted into 1,2-dichloroethane	The procedure is "somewhat tedious" due to temperature control and dual-wavelength measurements at two temperatures
8	25	423	2.26×10^3	39–273	Not reported	Methyl orange	The method involves the formation of an ion-pair complex between CPM and methyl orange	Less sensitive, and a narrow linear range
9	26	580	6.5×10^4	0 – 4.5	Not reported	Qn. Ph., Ti (IV), F^- , and SDS	The method involves the formation of a complex between CPM and Qn. Ph., Ti (IV), F^- , and SDS	The method requires careful buffering because the pH range is narrow. Interference from other metal ions is possible
10	27	550	2.69×10^4	4–18	0.0299/0.0997	Rose Bengal dye	Formation of an ion-pair complex between CPM and rose Bengal dye in a weak acidic medium	The method requires careful buffering because the optimal pH is critical
11	28	420	Not reported	5–15	Not reported	Methyl orange	The method uses the formation of complex between CPM and methyl orange in chloroform	Narrow linear range, requires 20 min standing before measurement and 20 mL of chloroform which is toxic
12	29	520	852	30-250	1.65/4.97	p-Chloranilic acid	The method involves formation of charge transfer complex between CPM and p-Chloranilic acid	Less sensitive, and color stability is limited

ARS: Alizarin Red S, BCP: Bromocresol Purple, C2B: 2-(p-nitrophenylazo)-chromotropic acid, C2R: 2-(phenylazo)-chromotropic acid, CR: Cresol Red, ECR: Eriochrome Cyanine R, F^-: Fluoride, LOD: Limit of detection, LOQ: Limit of quantitation, p-Cl: p-chlorophenylazo chromotropic acid, p-I: p-iodophenylazo chromotropic acid, SDS: Sodium dodecyl sulfate, TBPE: Tetrabromophenolphthalein ethyl ester, Ti (IV): Titanium (IV), ε: Molar absorptivity, Qn.Ph.: O-hydroxyhydroquinonephthalein.

2.3. Recommended procedures

2.3.1. Method A

Different aliquots (0.1 – 2.5 mL) of the standard 50 µg/mL CPM solution were accurately transferred separately into a series of 100 mL separating funnels. Then, 5.5 mL of water and 1.0 mL of 0.04% (w/v) BCG solution were added. The contents were mixed thoroughly and allowed to stand for 5 minutes. Then, 5 mL of dichloromethane (DCM) was added to each funnel using a micro-burette, and the mixture was shaken vigorously for 1.5 minutes at room temperature. After complete separation of the immiscible solvents, the DCM layer was passed through anhydrous sodium sulfate to eliminate any remaining moisture. The absorbance of the formed yellow-colored ion-pair complex was measured at 414 nm versus the reagent blank.

2.3.2. Method B

A series of 5 mL volumetric flasks were filled with different volumes (0.05–2.25 mL) of the CPM–BCG ion-pair complex solution (equal to 20 µg/mL CPM, prepared in accordance with Method A). The DCM was used to bring the contents of each flask to 2.5 mL, then 1.0 mL of a 0.1 M alcoholic KOH solution was added. The mixtures were thoroughly shaken and left to stand for 5 minutes. Ethanol was then added to each flask to bring its final volume up to the mark. The resulting blue color's absorbance was measured at 618 nm in versus the corresponding reagent blank.

2.3.3. Procedure for the formulations

2.3.3.1. The tablets assay procedure

To ensure uniformity, ten pills containing 4 mg of CPM each were precisely weighed, finely powdered, and well combined. After carefully weighing a portion of the powder equal to

5.0 mg of CPM, it was put into a 100 mL volumetric flask. To ensure full CPM extraction, about 60 mL of distilled water was added, and the mixture was shaken vigorously for 15 minutes. After that, the solution was diluted to the mark with distilled water, the mixture was well mixed and filtered using a Whatman No. 42 filter paper. The resultant filtrate was used directly for Method A and contained 50 µg/mL CPM. The same tablet solution used for Method A was utilized to prepare the appropriate CPM–BCG ion-pair complex solution (20 µg/mL CPM) for use in Method B. From these solutions, appropriate aliquots were then analyzed in accordance with the recommended procedures.

2.3.3.2. The syrup assay procedure

For Method A, the syrup formulations were treated to produce a working solution of 50 µg/mL CPM. This was achieved by precisely filling two different 25 mL volumetric flasks with a 2.5 mL aliquot of Histat syrup (2.5 mg/5 mL) or a 3.125 mL aliquot of Afeel solution (2 mg/5 mL). About 10 mL of distilled water and 5 mL of phosphate buffer (pH 5.7) were added to guarantee appropriate dispersion and pH stability. After 15 minutes of shaking, the mixtures were diluted to volume using distilled water. The resultant 50 µg/mL CPM solutions were utilized for method A. The same syrup solution used for Method A was utilized to prepare the appropriate CPM–BCG ion-pair complex solution (20 µg/mL CPM) for use in Method B. From these solutions, appropriate aliquots were then analyzed in accordance with the recommended procedures.

2.3.3.3. The injection assay procedure

After withdrawing and carefully mixing the contents of five injection ampoules, a precisely measured 1 mL portion of the mixture, equivalent to 10 mg of CPM, was put into a 100 mL volumetric flask. A 100 µg/mL CPM solution was obtained by adding around 60 mL of

water to the previous flask, shaking the mixture thoroughly, and then adding water up to the mark. The resulting solution was then diluted with water to obtain a 50 µg/mL CPM solution for use in method A. The same injection solution used for Method A was utilized to prepare the appropriate CPM–BCG ion-pair complex solution (20 µg/mL CPM) for use in Method B. From these solutions, appropriate volumes were then analyzed according to the recommended procedures.

3. Results and discussion

3.1. Absorption spectra

There are two different steps in the developed methods. The first step involved the reaction of CPM with BCG and formation of a yellow ion-pair complex due to the electrostatic interaction between the positively charged CPM and the negatively charged BCG dye. DCM effectively extracts this ion-pair complex from the aqueous phase. The extracted complex shows a distinct absorption maximum at 414 nm, which is the wavelength at which method A's measurements were made (Fig. 2).

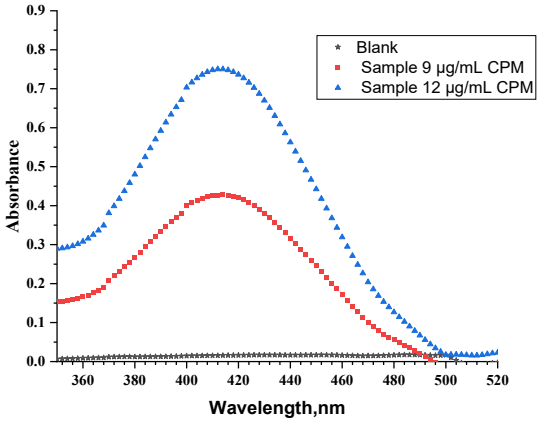


Fig. 2 Absorption spectra for the formation of CPM–BCG ion-pair complex versus the reagent blank (method A)

In the next step, the extracted CPM–BCG ion-pair complex is then treated with an alcoholic potassium hydroxide solution, which causes the ion-pair complex to dissociate and transforms BCG into its dianionic form. This process produces a more intensely colored chromogen and induces a pronounced bathochromic shift, with a new absorption maximum appearing at 618 nm (Fig. 3). This shift corresponds to the formation of the dianionic form of the dye and forms the basis of measurements in method B.

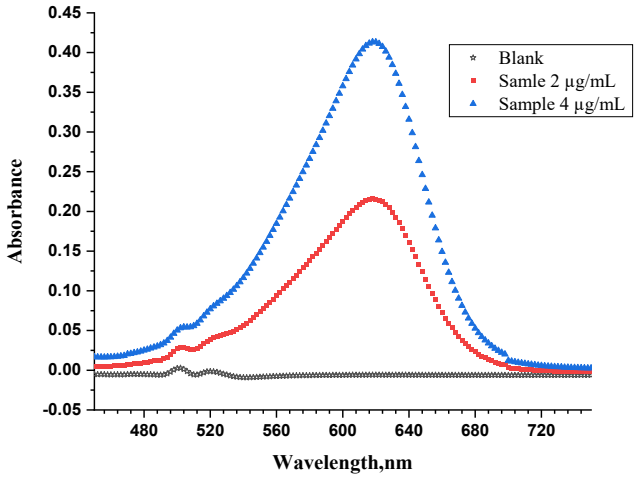
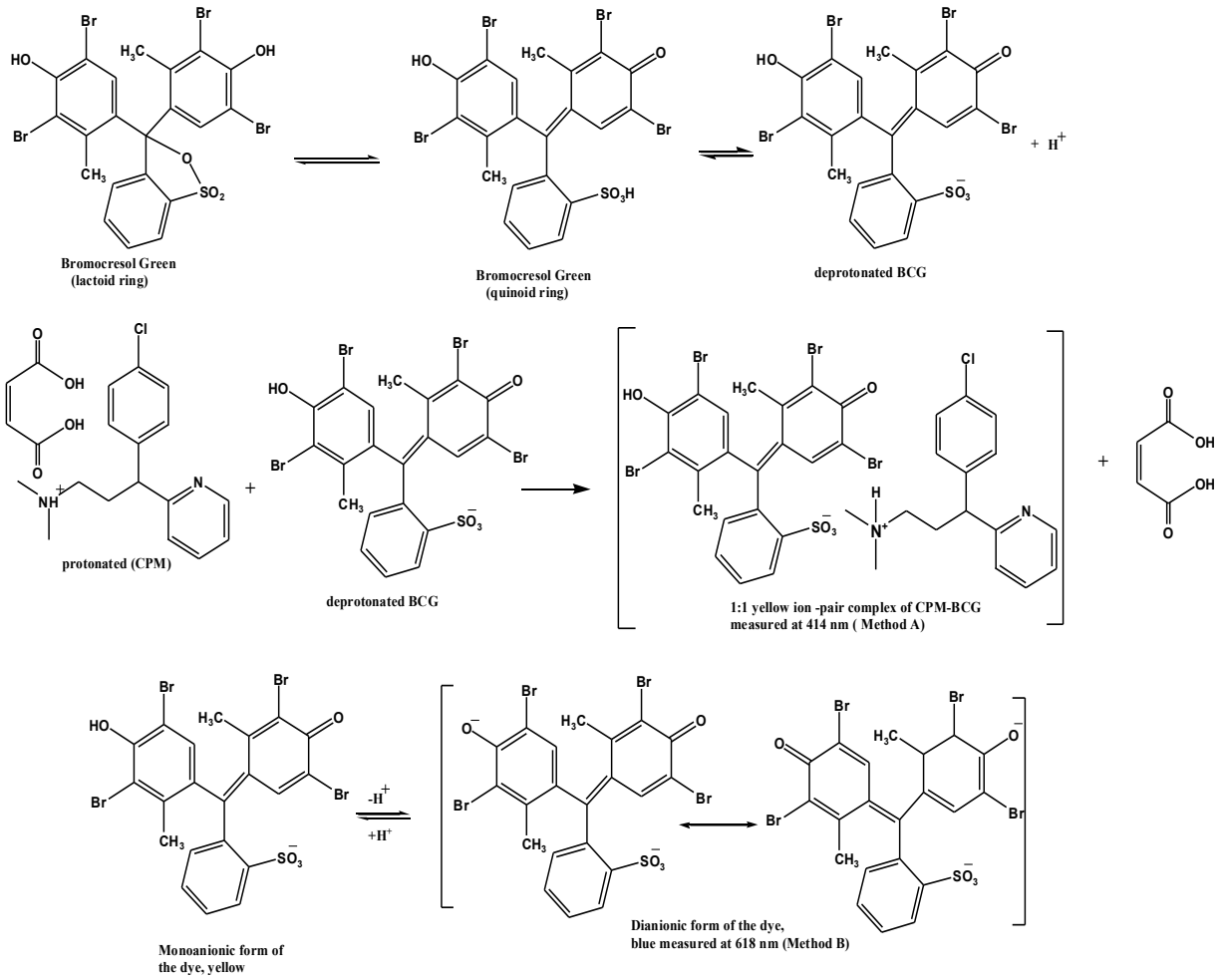


Fig. 3 Absorption spectra of the dianionic form of BCG dye versus the reagent blank (method B)

3.2. Reaction mechanism

The structure of chlorpheniramine has two nitrogen atoms: an aliphatic tertiary amine nitrogen and an aromatic heterocyclic nitrogen in the pyridine ring. The first is significantly more basic than the second because the aliphatic nitrogen is sp^3 hybridized, whereas the aromatic nitrogen is sp^2 hybridized. When the drug reacts with BCG in aqueous medium, the aliphatic nitrogen atom becomes protonated and is attracted via electrostatic interactions to the negatively charged sulfonate group of BCG, forming a CPM-BCG ion-pair complex (method A). This stable ion-

pair complex is extracted into DCM and exhibits a characteristic yellow color, distinct from the spectra of the individual components [30,31]. In the subsequent step, the ion-pair complex is treated with alcoholic KOH. The base disrupts the electrostatic interaction, causing dissociation of the complex. Consequently, BCG is converted into its fully deprotonated dianionic form, producing a bathochromic shift with an absorption maximum at 618 nm due to the formation of a blue-green color (method B). This transformation demonstrates the dynamic nature of the ion-pair system and highlights how complex stability depends on the protonation states of CPM's aliphatic nitrogen atom and on the pH of the medium [32,33]. The proposed mechanisms for both methods are illustrated in Scheme 1.



Scheme 1 Proposed reaction mechanisms for the formation and dissociation of the CPM–BCG ion-pair complex.

3.3. Optimization of reaction conditions

3.3.1. Selection of the extracting solvent

Several organic solvents were evaluated, including chloroform, DCM, hexane, and benzene. DCM was ultimately chosen due to its quantitative extraction efficiency, high stability of the complex (for over 24 hours), low reagent blank absorbance, and rapid phase equilibrium. The effect of different solvents on the absorbance of the formed CPM–BCG complex is shown in Figure 4.

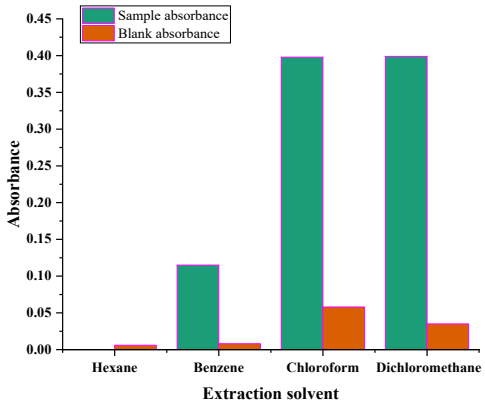


Fig. 4 The effect of different solvents on the absorbance of the formed CPM–BCG complex (10 µg/mL CPM)

3.3.2. Assessment of the stability of the measured species

The resultant yellow ion-pair complex (method A) and the blue dianionic form of the dye (method B) both exhibited constant absorbance for at least 24 hours at room temperature. This confirms the reproducibility and robustness of both methods for routine quantitative analysis.

3.3.3. Reagent concentration's effects on the absorbed species

In method A, the ion-pair complex displayed the greatest color intensity when 1 mL of 0.04% (w/v) BCG solution was employed. The absorbance of the formed complex did not significantly

alter when the dye amount was increased over 1 mL (Fig. 5). As a result, method A consistently used 1 mL of 0.04% (w/v) BCG solution.

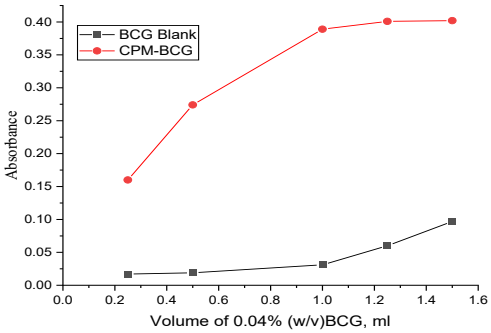


Fig. 5 Effect of BCG concentration on the absorbance of CPM–BCG complex (10 µg/mL CPM)

In method B, the impact of 0.1 M alcoholic potassium hydroxide on the ion-pair complex dissociation and the creation of the dye's dianionic form was examined. The absorbance at 618 nm was determined after testing different volumes of the base (Fig. 6). It was observed that 1 mL of 0.1 M alcoholic KOH solution produced maximum absorbance, while higher volumes had no significant effect.

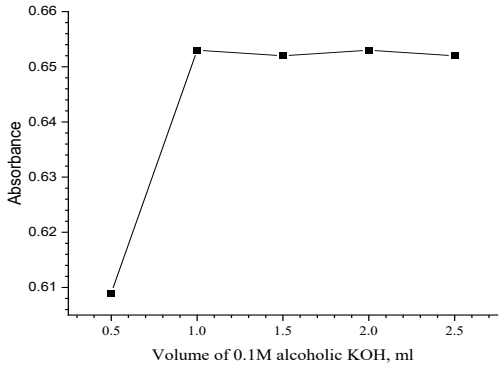


Fig. 6 Effect of 0.1 M alcoholic KOH on the absorbance of the dianionic form of the dye (6 µg/mL CPM)

3.3.4. Effect of reaction time

The effect of contact time between CPM and BCG was examined over a period of 0–30 minutes prior to extraction, and it was determined that 5 minutes was sufficient to achieve maximum absorbance at 414 nm (Fig. 7). In method A, shaking times ranging from 0.5 to 3 minutes yielded constant absorbance; consequently, a shaking time of 1.5 minutes was adopted throughout. In method B, the duration required for complete dissociation of the complex after the addition of 0.1 M alcoholic KOH solution was investigated, and 5 minutes was found sufficient for complete dissociation (Fig. 8).

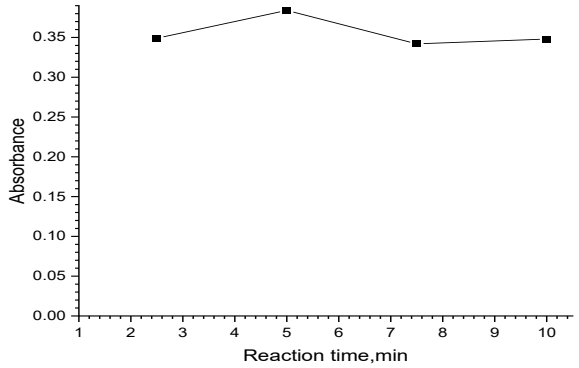


Fig. 7 Effect of reaction time on the absorbance of CPM–BCG complex (10 µg/mL CPM)

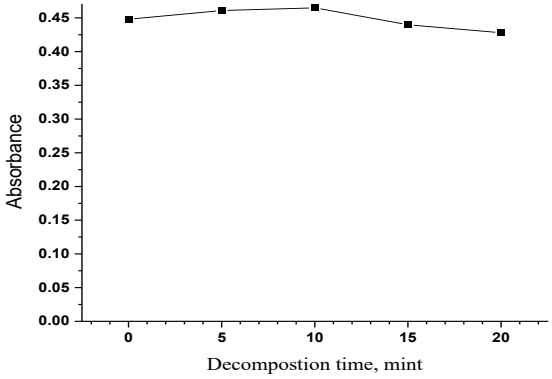


Fig. 8 Effect of time on the dissociation of CPM–BCG complex (4 µg/mL CPM)

3.3.5. Determination of the stoichiometry of ion-pair complex

Using Job's method of continuous variations, the interaction between equimolar amounts of CPM and BCG was investigated, confirming the formation of an ion-pair complex. The absorbance of each solution was recorded and plotted against the mole fraction of CPM ($V_{CPM}/(V_{CPM} + V_{BCG})$), as shown in Figure 9. Maximum absorbance was observed precisely at a mole fraction of 0.5. This result clearly indicates the formation of a 1:1 (CPM: BCG) ion-pair complex. Furthermore, the conditional stability constant (K_f) of the resulting complex was calculated and found to be 7.53×10^7, a value which reflects the high stability of the formed ion-pair complex.

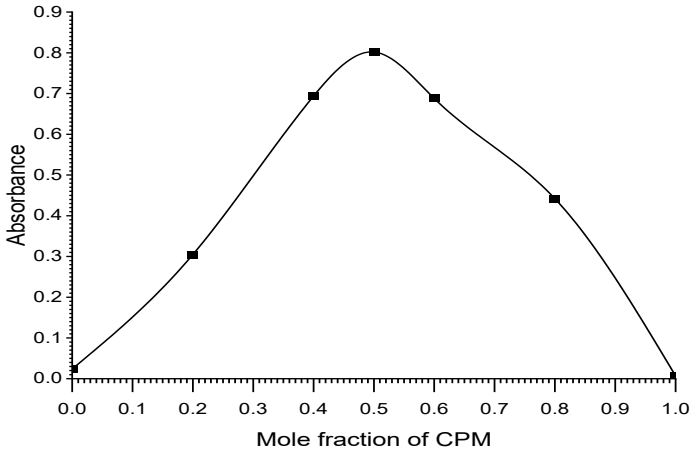


Fig. 9 Job's continuous variation plot for the formed CPM–BCG complex using equimolar solutions of CPM and BCG (1.279×10^{-4} M each)

3.4. Method validation

The proposed methods were validated following the current International Council for Harmonisation (ICH) guidelines [34].

3.4.1. Analytical parameters

Under the specified experimental conditions for CPM determination, the absorbance versus concentration plots were linear over the concentration ranges presented in Figures 10 and 11. Statistical parameters, including the standard deviations of the slope (S_b) and the intercept (S_a), were derived from the regression analysis of the calibration graphs. The linearity of the calibration curves was confirmed by high correlation coefficients (r) and y-intercepts close to zero. Additionally, the apparent molar absorptivity and Sandell sensitivity values for methods A and B were calculated and are summarized in Table 2.

Table 2 Analytical variables and regression

Parameter	Method A	Method B
λ_{max}, nm	414	618
Beer's law limits, µg/mL	1.00–25.0	0.2-9.0
Color stability, h	>24	>24
Molar absorptivity, L/(mol.cm)	1.76×10^4	4.47×10^4
Sandell sensitivity, µg/cm²	0.0222	0.0087
Limit of detection, µg/mL	0.2049	0.0199
Limit of quantification, µg/mL	0.6210	0.0603
Regression equation (y = mx + c)		
Intercept (a)	- 0.0178	- 0.0059
Slope (b)	0.0454	0.1144
Correlation coefficient (r)	0.9995	0.9997
Coefficient of determination (R^2)	0.9990	0.9994
Standard deviation of intercept (S_a)	0.0088	0.0073
Standard deviation of slope (S_b)	0.0006	0.0013

* Y is the absorbance, and X is the concentration in µg/mL.

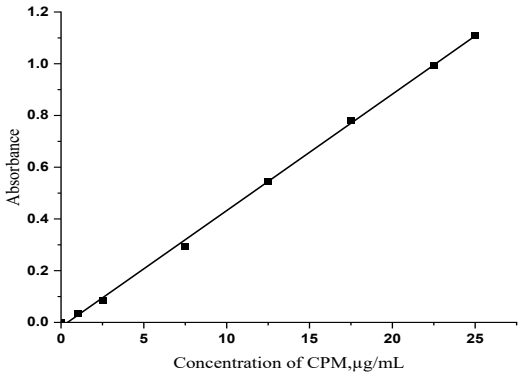


Fig. 10 Linear calibration curve for the CPM–BCG complex

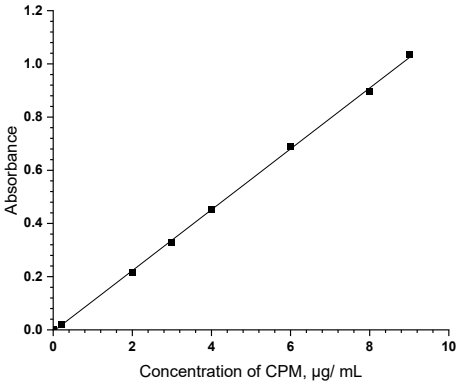


Fig. 11 Calibration curve for the formation of the dianionic form of the dye

3.4.2. Precision and accuracy

To assess the precision and accuracy of the developed methods, three different concentrations of CPM were prepared and analyzed in five replicates. Precision was evaluated by calculating the percent relative standard deviation (%RSD) across all concentrations, which ranged from 0.24% to 1.85% (Table 3), indicating high precision for both methods. Repeatability (intra-day precision) was assessed by analyzing the samples five times within the same day, while intermediate precision (inter-day precision) was evaluated over five consecutive days. The consistent, low %RSD values under both intra- and inter-day conditions demonstrate the reproducibility of the analytical methods. Accuracy was assessed using the percent relative error (%RE), which generally remained below 2.1% (e.g., ranging from -1.38% to 2.08%). These low %RSD and %RE values collectively indicate that the methods yield precise and accurate results, closely approximating the true concentrations of CPM, thereby confirming their reliability and suitability for routine quantitative analysis.

Table 3 Assessment of precision and accuracy

Method	CPM taken µg/mL	Precision and accuracy during the day Intra-day (n= 5)			Day-to-day precision and accuracy Inter-day (n =5)		
		CPM found[a] µg/mL	%RSD[b]	%RE[c]	CPM found[a] µg/mL	%RSD[b]	%RE[c]
A	5.00	5.01	1.85	0.18	5.05	1.71	0.98
	10.0	9.86	0.89	-1.38	10.01	1.51	0.13
	15.0	14.92	1.26	-0.53	14.84	1.36	-1.07
B	2.00	2.03	1.18	1.70	2.04	1.41	1.97
	4.00	4.06	0.50	1.55	4.08	0.71	2.08
	6.00	6.10	0.24	1.65	6.10	0.24	1.70

[a] An average of five measurements, [b] Percent relative standard deviation, [c] Percent relative error.

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291 The performance of the developed methods was statistically compared with the official
292 HPLC method described in the USP [35]. The official USP method involves the determination
293 of CPM by HPLC using a C18 column. The mobile phase consists of a mixture of water and
294 methanol (40:60, v/v) containing monobasic potassium phosphate, triethylamine hydrochloride,
295 sodium lauryl sulfate, and phosphoric acid. The analysis was performed at a flow rate of 2
296 mL/min using UV detection at 214 nm. As shown in Table 4, the calculated t- and F-values did
297 not exceed the theoretical tabulated values at the 95% confidence level. This confirms that there
298 is no significant difference between the proposed methods and the standard pharmacopoeia
299 procedure in terms of accuracy and precision.

Table 4 Results of the analysis of CPM formulations using the proposed and official methods, and their statistical evaluation

Brand name	Nominal amount	Measured values expressed as % of the nominal amount* ± SD		
		Official USP method	Proposed methods	
			Method A	Method B
Anallerge tablets	4 mg	98.87 ± 0.94	98.22 ± 0.72 t =1.24 F =1.70	99.80 ± 1.90 t = 1.03 F =4.09
Afeel tablets	4 mg	99.15 ± 0.85	98.82 ± 0.97 t =0.57 F =1.30	100.1 ± 0.60 t =2.07 F =2.01
Chlorpheniramine Maleate injection	(10 mg/ 1 mL)	98.43 ± 1.39	99.44 ± 0.75 t =1.49 F =3.43	99.80 ± 0.89 t =1.90 F =2.44
Afeel syrup	2 mg / 5 mL	99.54 ± 0.86	98.00 ± 1.30 t =2.25 F =2.29	100.4±0.91 t =1.54 F =1.12
Histat syrup	2.5 mg / 5 mL	98.37 ± 1.02	98.72 ± 1.01 t =0.54 F =1.02	98.69 ± 1.56 t =0.39 F =2.34

*An average value of five measurements, SD: standard deviation.
The tabulated *t* value at the 95% confidence level is 2.78.
The tabulated *F* value at the 95% confidence level is 6.39.

300

301 **3.4.3. Robustness**

302 The robustness of the two methods was assessed by analyzing the impact of small, deliberate
303 changes in two crucial parameters- the volume of the reagent (BCG dye) and the reaction time.
304 As seen by the consistently low %RSD values—none of which exceeded 1.86% across all tested
305 concentrations and variations—the results, which are summarized in Table 5, demonstrate that
306 both developed methods exhibit excellent robustness.

307 The amount of BCG dye (Method A) or KOH (Method B) was purposefully changed for
308 reagent volume (0.9, 1.0, and 1.1 mL), and the resulting %RSD values stayed low, ranging from
309 0.32% to 1.85%. Similarly, the corresponding %RSD values, which ranged from 0.25% to
310 1.86%, confirmed the stability of the developed methods when the reaction time was changed
311 (4, 5, and 6 minutes). These findings show that the methods are robust and can withstand slight
312 changes in the experimental conditions without compromising analytical performance.

313

Table 5 Results of methods' robustness

Method	CPM taken, µg/mL	%RSD	
		Volume of the reagent [a]	Reaction time [b]
Method A	5.00	1.85	1.86
	10.0	1.64	1.60
	15.0	0.32	1.15
Method B	2.00	0.66	1.34
	4.00	0.43	0.63
	6.00	0.67	0.25

[a] The amount of reagent used in both methods was 0.9, 1.0, and 1.1 mL
[b] In both methods, the reaction time was taken as 4, 5, and 6 minutes

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315

3.4.4. Selectivity

The absence of background interference was confirmed by repeated tests of the placebo blank, which produced absorbance values similar to those of the reagent blank. The proposed methods yielded recovery percentages of the pure CPM that ranged from 98.22±1.03 to 101.4 ± 0.28% when analyzing a synthetic mixture at three concentration levels. These findings support the developed methods' selectivity by showing that the excipients in the formulations do not interfere with the determination of CPM.

3.5. Applications to pharmaceutical formulation analysis

The quantitative estimation of CPM in a variety of commercially available pharmaceutical formulations, such as Afeel and Anallerge tablets, Afeel and Histat syrups, and CPM injection formulations, was successfully applied using the developed methods. The findings, which are compiled in Table 6, show the approaches' accuracy, selectivity, and practical utility for regular quality control. Both methods produced consistent CPM content across all tested formulations, suggesting that the common excipients and formulation matrices—such as binders in tablets or flavoring ingredients in syrups—did not interfere significantly with the spectrophotometric readings.

A recovery study was carried out utilizing the standard addition method in order to further confirm the accuracy of the developed methods. Pre-analyzed pill, syrup, and injection samples were spiked with pure CPM at three distinct concentrations. High recovery percentages, ranging from 98.14% to 100.8%, were consistently produced by both methods, with minimal standard deviations (usually less than 2.40%).

In particular, recovery percentages for tablet formulations (Afeel and Anallerge) ranged from 98.14% to 100.4% whereas liquid formulations (syrups and injections) showed recoveries between 99.04% and 100.8%. The suggested methods' repeatability is confirmed by these high

recovery percentages and minimal variability across replicates, demonstrating their appropriateness for accurate CPM quantification in a variety of pharmaceutical formulations.

Table 6 Recovery results via the standard addition method

Method	Formulation	CPM in tablet, µg/mL	Pure CPM added, µg/mL	Total CPM found, µg/mL	Pure CPM recovered*, (% ±SD)
Method A	Afeel Tablet (4 mg)	7.91	4.00	11.89	99.66 ± 1.70
		7.91	8.00	15.84	99.18 ±1.69
		7.91	12.00	19.96	100.4 ± 0.77
	Anallerge Tablet (4 mg)	7.86	4.00	11.86	100.1 ± 2.22
		7.86	8.00	15.85	99.87 ±1.40
		7.86	12.00	19.84	99.65 ±1.91
	Chlorpheniramine Maleate injection (10 mg/ 1 mL)	7.96	4.00	11.92	99.04±1.80
		7.96	8.00	15.94	99.39±1.27
		7.96	12.00	19.91	99.59±1.52
	Afeel Syrup (2 mg/ 5 mL)	7.84	4.00	11.81	99.33± 1.50
		7.84	8.00	16.00	100.7 ± 2.33
		7.84	12.00	19.76	99.32 ± 2.39
	Histat Syrup (2.5 mg/ 5 mL)	7.90	4.00	11.89	99.85 ±2.10
		7.90	8.00	15.86	99.56 ± 1.11
		7.90	12.00	19.99	100.8 ± 2.17
Method B	Afeel Tablet (4 mg)	3.00	1.50	4.50	99.69±1.21
		3.00	3.00	6.00	99.96±0.61
		3.00	4.50	7.51	100.1±1.27
	Anallerge Tablet (4mg)	2.99	1.50	4.47	98.14± 1.17
		2.99	3.00	5.99	99.86± 1.18
		2.99	4.50	7.49	99.96 ± 2.23
	Chlorpheniramine Maleate injection (10 mg/ 1 mL)	2.99	1.50	4.49	99.69± 0.89
		2.99	3.00	5.97	99.18± 0.58
		2.99	4.50	7.52	100.6± 0.98
	Afeel Syrup (2 mg/ 5 mL)	3.01	1.50	4.52	100.4 ± 1.17
		3.01	3.00	6.02	100.2 ± 0.45
		3.01	4.50	7.47	99.11± 2.05
	Histat Syrup (2.5 mg/ 5 mL)	2.96	1.50	4.47	100.35 ± 1.54
		2.96	3.00	5.95	99.81±1.47
		2.96	4.50	7.49	100.73 ± 0.92

***** Mean value of three determinations

4. Conclusion

This work successfully illustrated the use of BCG as a flexible chromogenic reagent for the estimation of CPM using two different spectrophotometric methods. Although both methods demonstrated robust and reliable performance, method B showed greater sensitivity made it especially appropriate for low-dose formulations. The results of the validation, which was

carried out in accordance with ICH guidelines, verified that the developed methods have excellent precision, accuracy, and solution stability. These methods' applicability is demonstrated by their effective application to a variety of pharmaceutical formulations, such as tablets, syrups, and injections, without the need for complicated pre-treatment. For routine pharmaceutical analysis and quality assurance, these methods are a good substitute for sophisticated chromatographic techniques due to their affordability and avoidance of highly toxic solvents like chloroform.

List of abbreviations

C2R	2-(phenylazo)-chromotropic acid
C2B	2-(p-nitrophenylazo)-chromotropic acid
ARS	Alizarin Red S
BCG	Bromocresol green
BCP	Bromocresol Purple
CPM	Chlorpheniramine Maleate
CR	Cresol Red
DCM	Dichloromethane
ECR	Eriochrome Cyanine R
HPLC	High-performance liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantitation
Qn.Ph	O-hydroxyhydroquinonephthalein
p-Cl	p-chlorophenylazo chromotropic acid
%RE	Percent relative error
%RSD	Percent relative standard deviation
p-I	p-iodophenylazo chromotropic acid
SDS	Sodium dodecyl sulfate
SD	Standard deviation
TBPE	Tetrabromophenolphthalein ethyl ester
USP	United States Pharmacopoeia

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Author contributions

FAM conceptualized, collected relevant information from the literature review, discussed the findings, edited the manuscript, and supervised the work. SHAA assisted in obtaining the CPM drug's raw material and formulations, designed the research methodology, carried out the experiments, and wrote the original draft of the manuscript. SAMA provided the work plan, assisted in developing the methods, discussed the results, revised the manuscript. The final manuscript was reviewed and approved by all authors.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study did not require ethical approval.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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