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Research Article

SPECTROPHOTOMETRIC METHODS FOR THE QUANTIFICATION OF CARISOPRODOL USING FERRIC CHLORIDE, *O*-PHENANTHROLINE, AND *P*-NITROANILINE, SODIUM NITRITE AS ANALYTICAL REAGENTS

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ABSTRACT

Objective: The present study represents the development of two spectrophotometric methods for the determination of carisoprodol (CCP) in pure and formulations using ferric chloride, *o*-phenanthroline, and p-nitroaniline (PNA), sodium nitrate as analytical reagents.

Methods: The proposed spectrophotometric methods were developed based on oxidation of Fe^{3+} by CCP, and then, the resultant product was reacted with *o*-phenanthroline in acidic condition forms an orange-colored complex and diazotization of PNA followed by coupling with CCP in an alkaline medium forms yellow-colored complex.

Results: Under the optimized conditions, the absorbance of CCP concentration obeyed the Beer's law in the ranges of 10–60 µg/mL with good correlation coefficient values of 0.9992 and 0.9990 with the limit of detection values of 1.286 and 2.408 µg/mL, respectively.

Conclusion: The proposed methods were successfully applied for the determination of CCP in pure and in their formulations.

Keywords: Ferric chloride, *o*-Phenanthroline, p-Nitroaniline, Sodium nitrate, Spectrophotometry, Analysis.

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INTRODUCTION

Carisoprodol (CCP) is a skeletal muscle relaxant belonging to monocarboxylic acids and derivatives class of organic compounds [1] that have both sedative and skeletal muscle relaxant effects [2]. The mechanism of CCP is not known exactly. Along with rest and physical therapy, CCP is also used in the treatment of injuries and painful musculoskeletal conditions [3,4]. CCP chemically known as [2-(carbamoyloxymethyl)-2-methylpentyl] N-propan-2-ylcarbamate (Fig. 1). The detailed survey of literature revealed that few methods have been reported for the estimation of CCP by liquid chromatography-tandem mass spectrophotometry [5], gas chromatography [6,7], homogeneous immunoassay [8], high-performance thin-layer chromatography [9], liquid chromatography/mass spectrophotometry [10], and ultraviolet (UV)-high-performance liquid chromatography [11]. The above reported chromatographic methods employed sophisticated and expensive instrumentation. Hence, UV-visible spectrophotometric methods are preferred for the precise, accurate, and cost-effective determination of pharmaceutical substances. There are three extractive spectrophotometric methods that have been reported for the assay of CCP in pure and pharmaceutical formulations [12]. However, the above methods were suffered from one or the other disadvantage such as poor sensitivity, unstable color, and rigid experimental conditions.

By considering into the above disadvantages of the reported methods, the present investigation was aimed to develop and validate some simple, sensitive, precise, accurate, and economical visible spectrophotometric methods.

In the present investigation, two simple and sensitive visible spectrophotometric methods (method-A and method-B) were developed and validated for the analysis of CCP with broad linearity, good precision, and accuracy. These methods could be applied for the quantitative determination of the CCP in their tablet formulations.

METHODS

Instrumentation

An ELICO (Hyderabad, India) double beam model SL 244 digital spectrophotometer with 1 cm matched quartz cell was used for the spectral and absorbance measurements. A Coslab (Ambala Cantt, India) CLE-105 model water bath was used to control the temperature and a Shimadzu (Tokyo, Japan) electronic weighing balance, model BL 220 H, was used for weighing the samples.

Chemicals and reagents

All reagents and chemicals were of analytical reagent grade and used as received. All the solutions were prepared fresh daily using double distilled water. Aqueous solutions of 0.2% (*w/v*) *o*-phenanthroline, 0.54% (*w/v*) ferric chloride, 0.2 M (*v/v*) orthophosphoric acid (Merck Specialties Pvt., Ltd., Mumbai, India), 0.2 M HCl (*v/v*), 0.1% *p*-nitroaniline (PNA), and 0.4% (*w/v*) and 4% (*w/v*) sodium hydroxide (SD-Fine Chemicals Ltd., Mumbai, India) prepared in the usual way.

Stock and working standard solutions

Analytically pure CCP was obtained as a gift sample from the Aurobindo Laboratories Pvt., Ltd., (India) and was used as received. The stock solution of CCP was prepared by dissolving 100 mg of CCP in 20 ml of methanol in a 100 ml volumetric flask and then make up to the mark with distilled water (1.0 mg/ml). The stock solution was diluted stepwise with the same distilled water to obtain working standard solutions of concentration of 200 µg/mL for methods A and B, respectively.

General assay procedure

Method-A

Delivered aliquots of standard CCP (0.5–3.0 ml, 200 µg/ml) into 10 ml calibrated tubes. To each tube, 1.0 ml of 0.2% O-PHEN was added followed by 1.0 ml of 0.54% $FeCl_3$ solution. The contents of the tubes were mixed well and the resulting solution was heated for 15 min at 100°C, and then,

2.0 ml of 0.2 M orthophosphoric acid was added. The volume in each tube was made up to the mark with distilled water and the absorbance of the colored solution was measured at 495 nm against a reagent blank.

Method-B

To a set of 10 ml calibrated tubes, aliquot volumes (0.5–3.0 ml) of CCP standard solution (200 µg/ml) were quantitatively transferred. To each tube, 0.8 ml of 0.1% PNA followed by 1.0 ml of 0.4% $NaNO_2$ were added and mixed well. The solutions were kept aside for 20 min. Then, 2.0 ml of 4% NaOH was added and again kept aside for 30 min. After the specified time, the tubes were made up to volume with distilled water. The absorbance was measured at 445 nm against a reagent blank.

In both the methods, the calibration graphs were plotted against the absorbance with the final concentration of the CCP (µg/mL).

Procedure for the assay of CCP in pharmaceutical formulations (tablets)

The tablet formulation of CCP, Pain O Soma® (HAB Pharmaceuticals and Research Ltd., Dehradun, India) labeled to contain 250 mg and 350 mg was purchased from a local pharmacist. 10 tablets of each were accurately weighed, finely powdered, and mixed well. A portion of the powder equivalent to 100 mg of CCP was transferred into 100 ml volumetric flasks, about 30 ml methanol was added and the contents of the flask were sonicated for 15 min. The volume was made with distilled water, mixed, and filtered using Whatman No. 1 filter paper. Aliquots covering the working concentration ranges of proposed methods were prepared with distilled water. The procedures described under general analytical procedure were followed. The nominal contents of the CCP in tablets were calculated using the corresponding calibration curve or regression equation.

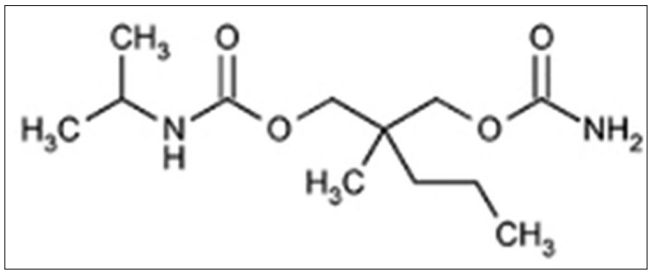


Fig. 1: Structure of carisoprodol

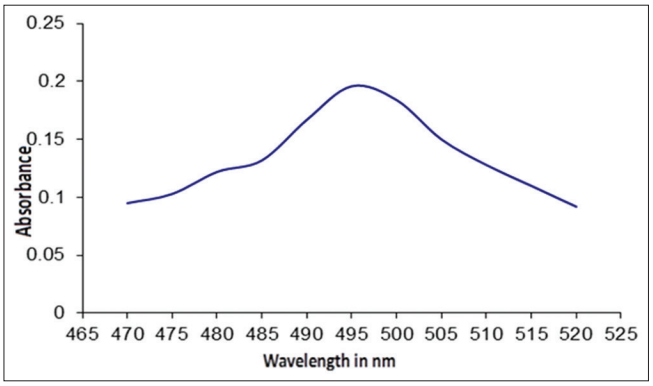


Fig. 2: Absorption spectrum of carisoprodol with $FeCl_3$ and *o*-phenanthroline

RESULTS AND DISCUSSION

Method-A

The reaction of $FeCl_3$ and *o*-phenanthroline with the amino group has been reported for some amino group containing drugs. The results of those reactions demonstrated that the combination of $FeCl_3$ and *o*-phenanthroline is one of the good chromogenic reagents in the development of spectrophotometric methods for the determination of many drugs containing amine groups [13-18]. In this present developed method, CCP was reacted with ferric chloride under suitable experimental conditions which convert ferric chloride into ferrous salt, the amount of conversion correlated to the drug concentration. These ferrous ions react with *o*-phenanthroline to form orange-red-colored chromogen (Scheme. 1) with maximum absorption at 495 nm (Fig. 2) by obeying Beer's law in the concentration range of 10–60 µg/mL.

Method-B

PNA is a well-known reagent to react with amines to form the stable diazo complex. In the presence of sodium nitrite and Hcl, PNA) acts as a chromogenic reagent, which has been used for the determination of various drugs containing amino groups [14,18-22]. In the present investigation, PNA undergoes diazotization followed by coupling with CCP in the alkaline medium by the formation of the yellow-colored complex (Scheme. 2) with λ maximum at 445 nm (Fig. 3) by obeying Beer's law in the concentration range of 10–60 µg/mL.

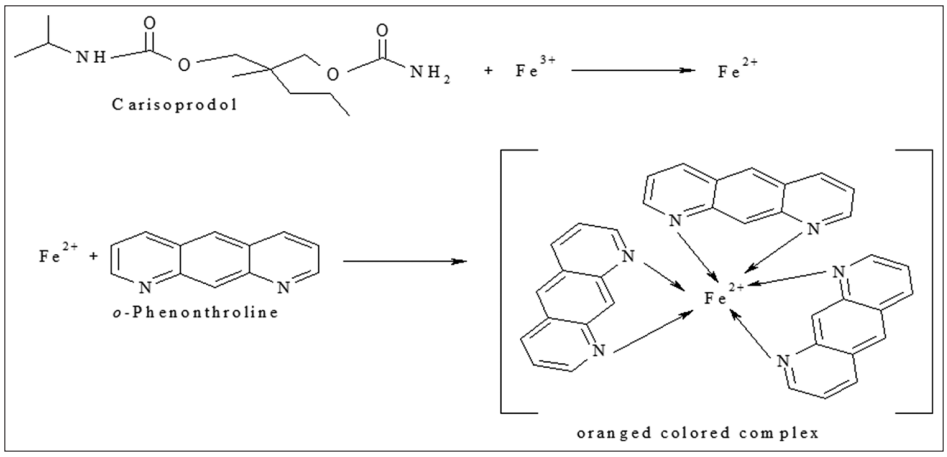
OPTIMIZATION OF THE EXPERIMENTAL CONDITIONS

Method-A

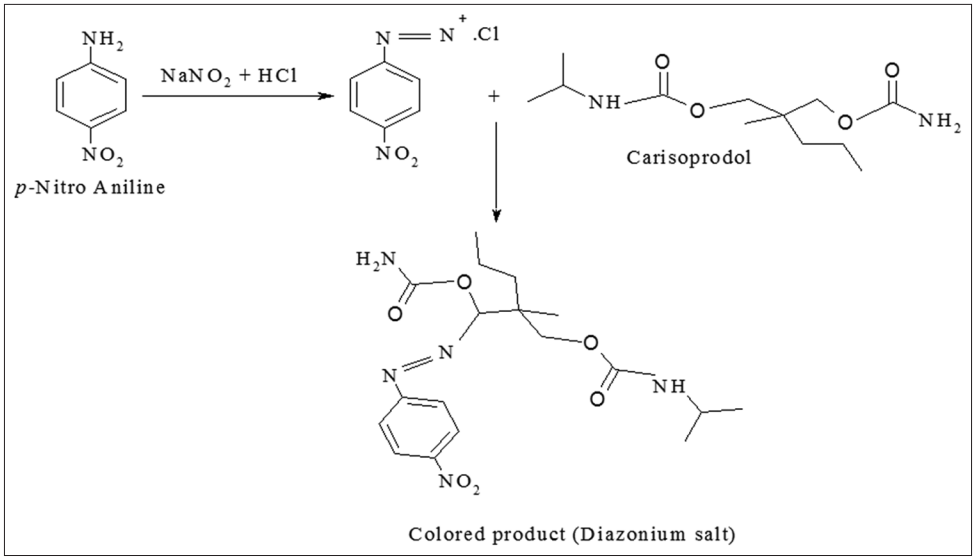
Various factors affect the reaction between the CCP and reagents including $FeCl_3$, *o*-PHEN, and *o*-phosphoric acid concentrations, temperature, and heating time. These were studied to optimize the reaction conditions and to give maximum absorbance. All these optimum values of various factors were maintained throughout the experiment.

Effect of FeCl3 volume

The effect of 0.54% $FeCl_3$ on the absorbance of the orange-colored complex was studied in the range of 0.5–3.0 mL. The absorbance of the complex increased with the increase in the volume of $FeCl_3$ up to 1.0 mL. Further,



Scheme 1: Reaction of carisoprodol with $FeCl_3$ and *o*-phenanthroline



Scheme. 2: Reaction of carisoprodol with PNA

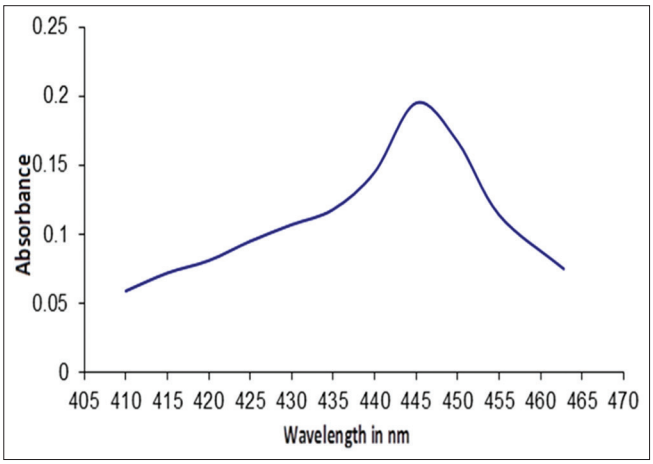


Fig. 3: Absorption spectrum of carisoprodol with p-nitroaniline and $NaNO_2$

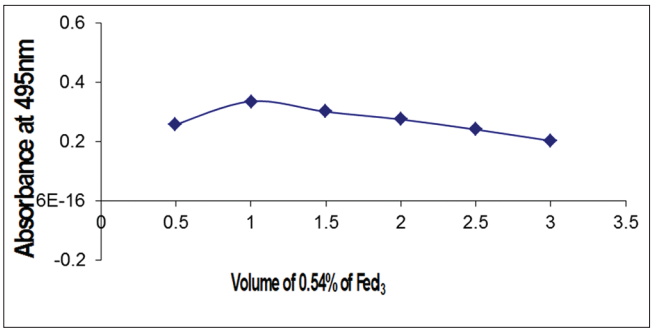


Fig. 4: Effect of the volume of $FeCl_3$

addition of $FeCl_3$ showed a decrease in the absorbance. Therefore, 1.0 mL of 0.54% $FeCl_3$ was considered as an optimum value (Fig. 4).

Effect of o-phenanthroline concentration

The effect of the concentration of *o*-phenanthroline was studied by conducting reaction between 10 µg/mL CCP, 1 mL of $FeCl_3$ and varying volumes (0.5–3.0 ml) of 0.2% *o*-PHEN and 2.0 ml of 0.2 M *o*-phosphoric acid. The reaction indicates that the absorbance of the CCP-$FeCl_3$ and *o*-PHEN complex was increased by increasing the volume of 0.2% *o*-PHEN up to 0.9 ml; later, it became constant at 1.0 mL. Further

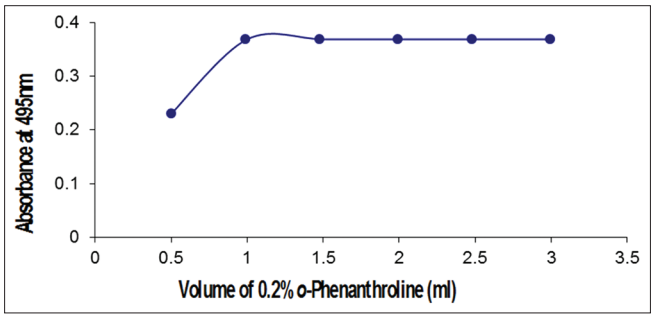


Fig. 5: Effect of the concentration of *o*-phenanthroline

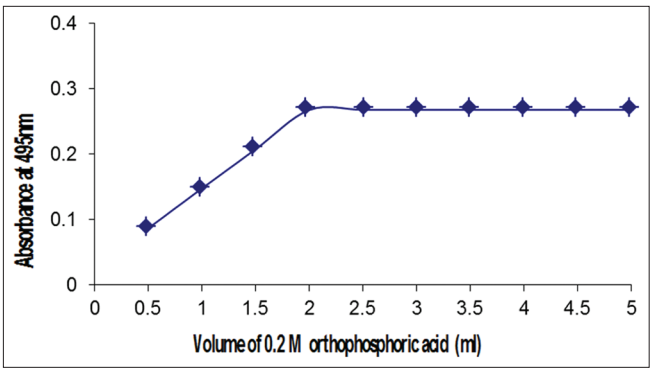


Fig. 6: Effect of the concentration of *o*-phosphoric acid

increase in the volume, there was no change in the absorbance (Fig. 5). Therefore, 1 mL of 0.2% *o*-PHEN solution was chosen as the optimal volume for the development of the colored complex.

Effect of the concentration of orthophosphoric acid

The concentration of 0.2 M orthophosphoric acid effect on the absorbance of the orange-colored complex was studied in the range of 0.5–5.0 ml. The absorbance increased with the increasing the volume of orthophosphoric acid and became constant at 2.0 ml. Further, addition of orthophosphoric acid did not show any change in the absorbance (Fig. 6).

Effect of temperature

The effect of the temperature on the orange-colored complex was studied between the temperatures 30–110°C. The maximum absorbance

was attained when the temperature reached 100°C. Further increasing the temperature >100°C, the intensity of the color and the absorbance started to decrease (Fig. 7).

Effect of time

Time also one of the important factors which affect chemical reactions. In the present investigation, the effect of heating time on the formation of the orange-colored complex was also studied and optimized. At room temperature, the intensity of the color increased by the increasing the time, maximum absorbance was obtained at 15 min and remained constant for 30 min. After 30 min of the time interval, the increase in the heating time did not cause any change in intensity of color (Fig. 8).

Method-B

In this method, PNA carried out diazotization reaction with $NaNO_2$ under acidic conditions followed by coupling with the CCP. The experimental conditions were optimized by studying the effect of various parameters such as volume and concentrations of PNA, $NaNO_2$, and NaOH for the maximum color development and the time required for diazotization and diazo coupling.

Effect of the concentration of 0.1% PNA

To the study of the effect of the concentration of 0.1% *p*-nitroaniline for yellow-colored development, different volumes (0.2–2.0 ml) of 0.1% PNA were mixed with different volumes of CCP (10 µg/mL) and followed by 1.0 ml of 0.4% $NaNO_2$, and then, 2.0 ml of 4% NaOH was added. The study indicates that the addition of 0.8 ml of 0.1% PNA gave the maximum absorbance, which remained constant up to 2.0 ml. Therefore, 0.8 ml of the 0.1% PNA was chosen for the determination of the CCP for the present experiment (Fig. 9).

Effect of the volume of $NaNO_2$

The effect of the volume of 0.4% $NaNO_2$ was studied during the formation of yellow color. This study carried out by addition of an aliquot of CCP containing 10 µg/ml to different volumes (0.5–3.0 ml) of 0.4% $NaNO_2$ and 0.8 ml of 0.1% PNA and then 2.0 ml of 0.4% NaOH. The results were shown that the maximum absorbance was attained with 1.0 ml of 0.4% $NaNO_2$. Further, addition of $NaNO_2$ showed decrease in the absorbance. Therefore, 1.0 ml of 0.4% $NaNO_2$ was used throughout the experiment (Fig. 10).

Effect of time for diazotization

To study the effect of time for diazotization, 1 mL of CCP (10 µg/mL) and 0.8 ml of 0.1% PNA followed by 1.0 ml of 0.4% $NaNO_2$ were added and mixed well. Later, the solutions were kept aside for 20 min. Color intensity was obtained maximum at 20 min. Further, increase in time no effect on diazotization. Hence, 20 min was chosen as the optimum time for diazotization (Fig. 11).

Effect of the volume of NaOH

The effect of the volume of 4% sodium hydroxide on yellow color formation was studied by addition of different volumes (0.5–4.0 ml) of 4% sodium hydroxide to an aliquot of CCP (10 µg/ml) and mixed well then added 0.8 ml of 0.1% PNA followed by 1.0 ml of 0.4% $NaNO_2$ to that mixture. The solutions were kept aside for 20 min. The results are presented in Fig. 12, which indicate that the addition of 2.0 ml of 4% NaOH gave the maximum absorbance, which remained constant up to

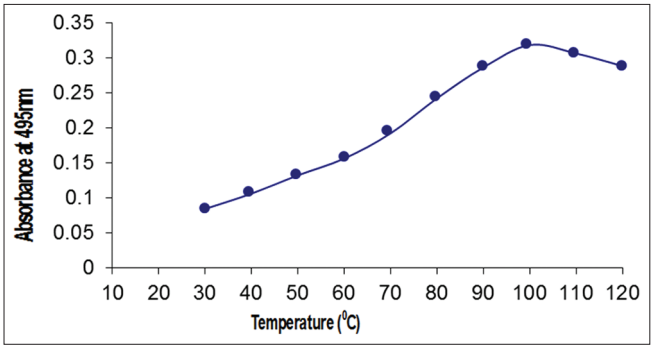


Fig. 7: Effect of temperature

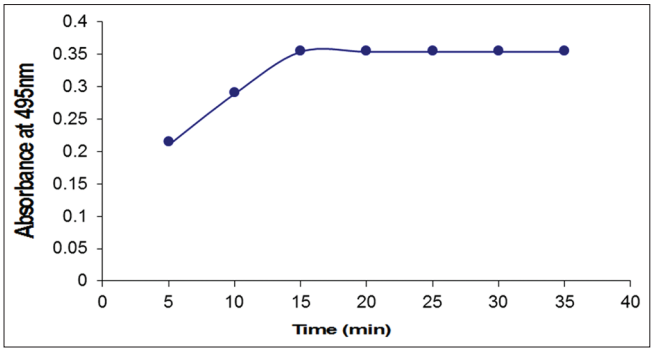


Fig. 8: Effect of time

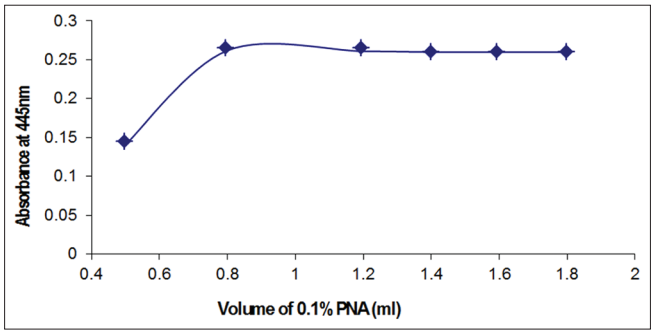


Fig. 9: Effect of the concentration of p-nitroaniline

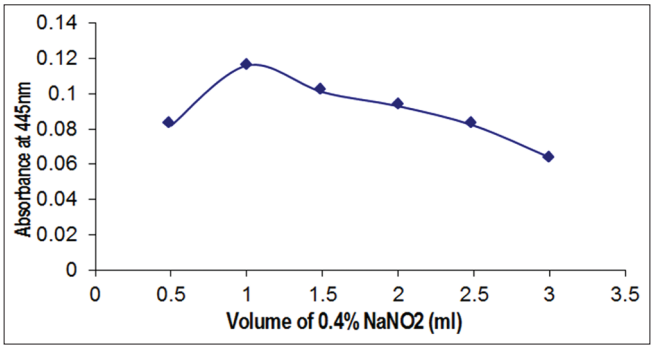


Fig. 10: Effect of the volume of $NaNO_2$

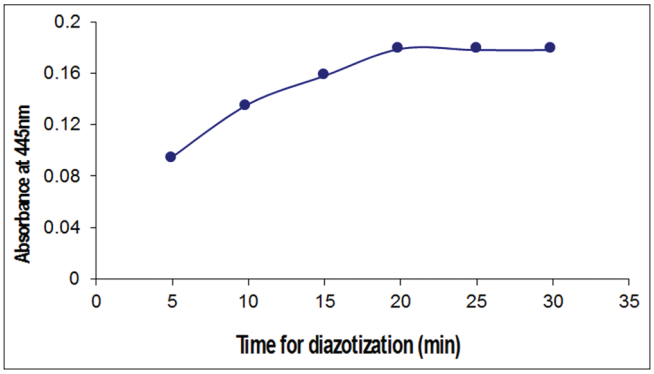


Fig. 11: Effect of time for diazotization

3.0 ml later started to decrease. Therefore, 2.0 ml of the 4% NaOH was chosen for the quantification of the CCP throughout the experiment.

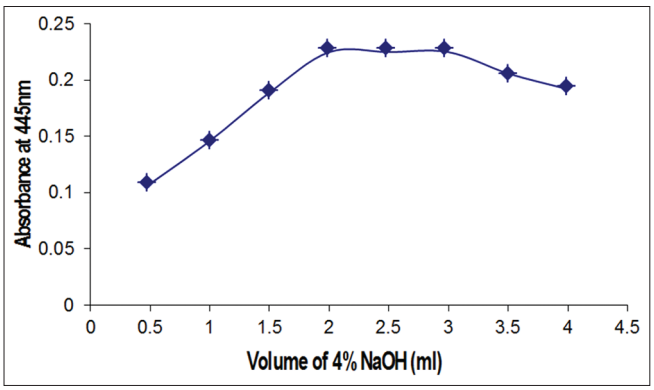


Fig. 12: Effect of the volume of NaOH

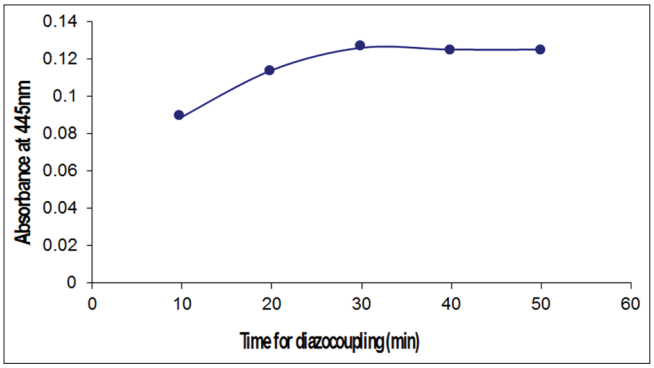


Fig. 13: Effect of time for diazo coupling

Table 1: Optical and regression characteristics of the proposed methods

Parameters	Method-1	Method-2
λ max	495	445
Beer's limit (µg/mL)	10–60	10–60
Molar absorptivity (L mole-1 cm^{-1})	2.874×10^3	7.078×10^3
Sandell's sensitivity (µg cm^{-2}/0.001 Absorbance unit)	0.2032	0.1960
Stability of colored products (mins)	50	70
Regression equation (Y=mx+c)**		
Slope (m)	0.005	0.005
Intercept (c)	−0.0016	0.0030
Regression coefficient (r^2)	0.9992	0.9990
LOD (µg/mL)	1.286	2.408
LOQ (µg/mL)	3.898	7.298

**Y=mx+c, Where y is the absorbance and x is the concentration of drug in µg/mL. LOD: Limit of detection, LOQ: Limit of quantification

Effect of time for diazo coupling

To study the effect of time for diazo coupling, 1 mL of CCP (10 µg/mL) and 0.8 ml of 0.1% PNA followed by 1.0 ml of 0.4% $NaNO_2$ were added and mixed well. The solutions were kept aside for 20 min. Then, 2.0 ml of 4% NaOH was added and again kept aside for 30 min. Diazo coupling was completed at 30 min. Hence, 30 min was chosen as optimum time (Fig. 13).

VALIDATION OF THE DEVELOPED METHODS

The developed methods were validated by following the ICH guidelines [23]. Various parameters including linearity, sensitivity, precision, accuracy, and robustness were studied.

Linearity

Linearity was studied in the concentration range from 10 to 60 µg/mL for methods - A and B, respectively. The drug showed good linearity in the tested range. The regression coefficient values for methods - A and B were found to be >0.9989. The obtained results have a good and dynamic linearity range of the developed methods.

Sensitivity

The sensitivity of the proposed methods was estimated in terms of Sandell's sensitivity, limit of quantitation, limit of detection, and molar absorptivity. The results are presented in Table 1, the results showed the high sensitivity of the developed methods.

Accuracy and precision

Precision and accuracy were investigated by analyzing different concentrations of CCP (10, 30, and 60 µg/ml) - for both methods (methods - A and B) in five independent replicates on the same day (intraday precision and accuracy) and on 3 consecutive days (interday precision and accuracy). The data are represented as the relative standard deviation (%RSD) and percent recovery for precision and accuracy, respectively. The results have shown in Tables 2 and 3. Low RSD (<1.0) values and good recovery values for intra- and inter-day analysis show good precision and accuracy data of the proposed methods (methods - A and B), respectively.

RECOVERY STUDIES

The accuracy of the proposed methods was further determined by the standard addition method. A known amount of CCP at three different levels (5, 100, and 150%) was added to the pre-analyzed sample solution and the amount of CCP was estimated by the reported methods. The results were reported as the RSD and percent recovery are shown in Table 4. The recovery studies showed that there was no interference from excipients in the determination of the CCP by the proposed methods.

Robustness

The robustness of the proposed methods was studied by making small changes in the experimental parameters at two different concentration levels (10 and 60 µg/mL). The results are presented in Table 5. The results showed that the slight changes did not adversely influence the absorbance intensity.

Table 2: Intraday precision and accuracy

Method	Amount of CCP (µg/mL)		RSD (%)	Recovery (%)	Error (%)
	Taken	Found*±SD			
1	10	10.035±0.047	0.473	100.35	0.35
	30	30.030±0.062	0.209	100.10	0.10
	60	60.020±0.057	0.095	100.03	0.03
2	10	10.003±0.025	0.259	100.03	0.03
	30	30.015±0.040	0.135	100.05	0.05
	60	60.016±0.066	0.110	100.02	0.02

*Average five determinations. CCP: Carisoprodol, SD: Standard deviation, RSD: Relative standard deviation

APPLICATION OF THE PROPOSED METHODS TO ANALYSIS OF CCP IN TABLET FORMULATIONS

From the above-mentioned results, the proposed methods gave satisfactory results with CCP in bulk. Therefore, the proposed methods were successfully applied for the determination of CCP in their formulations. The results are shown in Table 6. The percent recovery and %RSD clearly showed no interference of any excipients of formulation, thus proving accuracy and precision in the quantification of CCP by both methods.

Table 3: Interday precision and accuracy

Method	Amount of CCP (μg/mL)		RSD (%)	Recovery (%)	Error (%)
	Taken	Found*±SD			
1	10	10.035±0.067	0.675	100.35	0.35
	30	30.036±0.059	0.199	100.12	0.12
	60	59.991±0.015	0.026	99.98	-0.02
2	10	10.006±0.086	0.864	100.06	0.06
	30	30.013±0.050	0.168	100.04	0.04
	60	59.998±0.012	0.020	99.99	-0.01

*Average five determinations. CCP: Carisoprodol, SD: Standard deviation, RSD: Relative standard deviation

Table 4: Results of recovery studies by standard addition technique

Method	Amount of CCP mg		Found*	RSD (%)	Recovery (%)
	Tablet	Spiked			
1	10	5	14.992	0.619	99.94
	10	10	20.005	0.055	100.02
	10	15	25.006	0.046	100.03
2	10	5	15.018	0.131	100.08
	10	10	20.022	0.116	100.11
	10	15	25.007	0.032	100.29

*Average of three determinations, CCP: Carisoprodol, RSD: Relative standard deviation

Table 5: Results of robustness of the proposed methods

Method	Parameter	Concentration of CCP (μg/ml)		SD	Recovery (%)	RSD (%)
		Taken	Found*			
1	Volume of 0.2%	10	10.01	0.032	100.10	0.323
	O-PHEN (1.0±0.1 ml)	60	59.99	0.025	99.98	0.042
	Volume of 0.54% $FeCl_3$ (1.0±0.1 ml)	10	9.99	0.007	99.90	0.070
		60	60.01	0.029	100.01	0.049
	Volume of 0.2 M H_3PO_4 (2.0±0.2 ml)	10	10.02	0.041	100.20	0.414
		60	59.99	0.021	99.98	0.036
	Temperature	10	10.03	0.051	100.30	0.513
	(100±5°C)	60	60.01	0.025	100.01	0.042
	Boiling time	10	10.02	0.037	100.20	0.373
	(15±2 min)	60	60.01	0.033	100.01	0.056
2	Volume of 0.1% PNA (0.8±0.1 ml)	10	9.96	0.033	99.60	0.336
		60	60.01	0.036	100.01	0.060
	Volume of 0.4% $NaNO_2$ (1.0±0.1 ml)	10	10.02	0.039	100.20	0.392
		60	60.03	0.053	100.05	0.089
	Volume of 4% NaOH (2.0±0.2 ml)	10	9.98	0.020	99.80	0.200
		60	60.01	0.012	100.01	0.020
	Diazotization time	10	10.06	0.041	100.60	0.415
	(20±2 min)	60	60.11	0.069	100.18	0.115
	Diazo coupling time	10	10.06	0.042	100.60	0.424
	(30±2 min)	60	60.13	0.077	100.21	0.129

CCP: Carisoprodol, SD: Standard deviation, RSD: Relative standard deviation

Table 6: Results of the analysis of CCP in tablet formulations

Method	Labeled claim (mg)	Found*	SD	% RSD	% recovery	*t*-value[$]	F-value[$$]
1	250	250.03	0.040	0.016	100.012	0.097	3.195
	350	350.06	0.052	0.015	100.017	0.116	4.616
2	250	249.973	0.023	0.094	99.989	0.465	2.353
	350	350.015	0.032	0.092	100.004	0.988	3.082
3[@]	250	250.09	0.025	0.071	100.036	----	----
	350	249.91	0.034	0.067	99.964	----	----

*Average of three determinations. [$]tabulated t value - 2.306. [$$]tabulated F value - 6.39. [@]Reference method. CCP: Carisoprodol, SD: Standard deviation, RSD: Relative standard deviation

CONCLUSION

In the present investigation, there are two simple, rapid, cost-effective, accurate, precise, and robust spectrophotometric methods developed for the estimation of CCP, using $FeCl_3$, *o*-phenanthroline, and PNA, $NaNO_2$ as analytical reagents in bulk drug and tablet forms. The developed methods have the advantages over the reported spectrophotometric methods in being more sensitive, cost-effective, robust, precise, and accurate. Furthermore, the developed methods are inexpensive and do not require sophisticated instrumentation and elaborate treatments allied with chromatographic methods. Therefore, the proposed methods are applied for the routine analysis of CCP in quality control laboratories.

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AUTHORS' CONTRIBUTION

D Murali has developed and validated. G Purna Chandra Rao has computed and given suggestions to investigate the findings of this work. Both the authors discussed and contributed to the final manuscript.

CONFLICTS OF INTEREST

Authors have no conflicts of interest.

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