A novel spectrophotometric determination of caroverine in pharmaceutical formulations via derivatization with Folin-ciocalteu Phenol reagent

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Abstract: In this study we have reported a new, fast and extraction free spectrophotometric procedure for the assessment of caroverine in pharmaceutical raw and tablet dosage forms. In the reported visible spectrophotometric procedure tungstate in Folin-Ciocalteu phenol reagent is reduced in alkaline medium and produces the blue colored chromogen that shows λ_{max} at 740nm with the calibration range of 2-28µg/ml. The LOD and LOQ values are 1.15 and 3.81µg/ml respectively. The newly developed analytical procedure is used to determine caroverine in raw material of and commercial tablets dosage forms. The spectrophotometric method represented in this study is simple, rapid and extraction free. It may easily be utilized for the determination of caroverine in pharmaceutical laboratories for quality control and stability studies purpose.

Keywords: Caroverine, Folin-Ciocalteu phenol reagent, spectrophotometric method, formulation.

INTRODUCTION

A counterfeit medication is the product which is presented and marketed with less quantities of active ingredients, or may contain ingredients that are not on the label (Davison*et al*., 2011). The concern about the quality of drugs marketed increases every year not only in commercial terms, but also legal and ethical aspects, since the health of patients depends on the quality and effectiveness of these drugs. For this purpose different regulatory authorities around the world are demanding specific and validated analytical methods for the registration of new drugs to ensure their quality. So there is a great interest in developing rapid and efficient analytical methods that provide precise and accurate quantitative results of drugs in pharmaceutical raw and dosage forms.

Caroverine is chemically derivative of isoquinoline that is a basic structure of papaverin (fig. 1). It is clinically available in some countries as a spasmolytic drug based on its unspecific Ca^{2+} channel blocking activity for more than 40 years. Caroverine is used for relieve spasm of smooth muscle in some countries. It performs as N-type calcium channel blocker and competitive AMPA receptor antagonist (Udilova *et al*. 2003). It also has potent antioxidant effects (Noh*et al*., 2003). In Pakistan, caroverine is marketed as Saprina tablets 20mg (BioPharma, Multan, Pakistan) for smooth muscle spasms.

Detailed survey of literature for caroverine revealed that not a single analytical method is present for evaluation of drug in raw and tablets. The assay procedure of caroverine in raw material and dosage forms is not official in any pharmacopoeia up to our knowledge.

Recently our medicine mart is contaminated with bogus and inferior medical products. Our aim of study is to establish a new, delicate and cheap visible spectrophotometric procedure that provide accuracy comparable to costly and complicated technique like HPLC.

MATERIALS AND METHODS

Equipment

All absorption measurements were made on spectrophotometer (U 1100 Hitachi, Japan) equipped with 1 cm alike quartz cells.

Chemicals

Analytical Reagent Grade chemicals used were in all study. Double distilled water was used throughout the research work. Folin-Ciocalteu reagent (Fluka, Germany), sodium hydroxide (Merck, Germany). Dimethyl sulfoxide DMSO (Sigma-Aldrich, Germany) was used for dilution. A pure caroverine (pharmaceutical grade) sample and Sparina tablets were provided by BioFine Pharmaceuticals (Pvt.) Ltd., Multan, Pakistan.

Drug and reagent solutions

100 mg of pure caroverine was dissolved in 100ml DMSO in volumetric flask to make the concentration of 1.0mg/ml. Further working concentration of caroverine was made by dilution above solution with same solvent. Folin ciocalteu phenol reagent solution (2.0 N) was used as such. 4.0gm of NaOH was dissolved in 100mL distilled water to produce 1.0 N solution.

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Determination of absorption spectra

Caroverine solution equivalent to 10µg/ml was mingled with 2.5mL of NaOH solution and 2mL of folin-Ciocalteu reagent in a 10-mL volumetric flask. After 10 min, the volume was diluted up to the volume with water and the content was quaked completely. Similarly the reagent blank was prepared in the same way without caroverine. This solution was scanned on visible spectrophotometer from 400-800 nm against the regent blank. The observed λ_{max} was 740 nm that was used for further study.

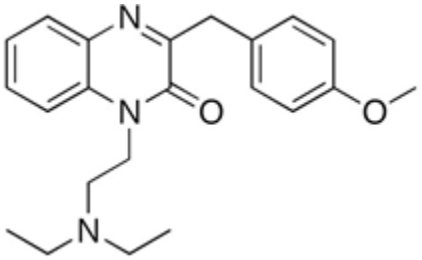

Fig. 1: Chemical structure of Caroverine

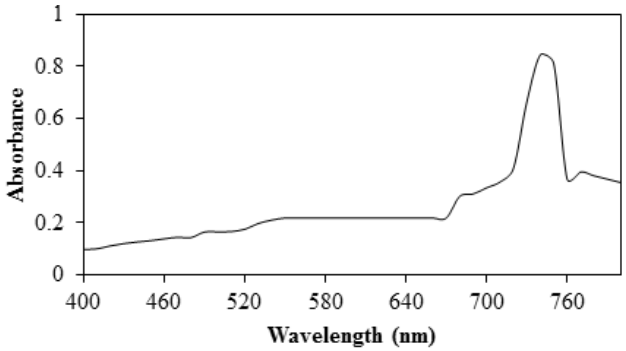

Fig. 2: Absorption maximum of caroverine and FCR Complex

General analytical procedure

Various samples of the working standard of caroverine solution (10µg mL^{-1}), ranging from 2-28µg mL^{-1} were delivered into a chain of 10-mL volumetric flasks and the final volume was brought to 10mL with water. To each flask, 2.5mL of sodium hydroxide solution and 2.0mL of folin-Ciocalteu reagent solution were added by micro burette. The flasks were plugged; contents were mingled completely and kept at room temperature for 10 min. The volume was completed with DMSO and the absorbance of each solution was recorded at 740 nm.

Assay procedure for tablets

Average weight of ten tablets was calculated and grinded into fine powder. An approximate amount to 100 mg of caroverine was carefully weighed and transferred to a 100-mL volumetric flask. About 50mL of DMSO was added to the flask and agitated for 2.0 minutes. Finally the volume was made up to the mark with same solvent. Then we kept this solution at room temperature for 5 minutes and filtered through Whatman No. 42. Then a suitable portion was used for the assay as described under "General analytical procedure".

RESULTS

The involved reaction and absorption spectra

The current study depends on the oxidation of the free phenolic group in caroverine with Folin-Ciocalteu reagent. The newly formed blue colored was deliberated at 740 nm. Fig. 2 shows the absorption spectrum of the reaction product.

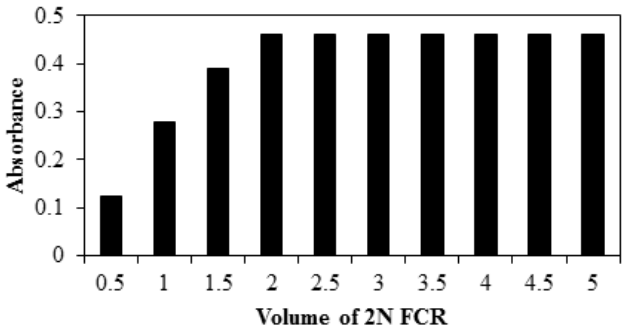

Fig. 3: Effect of volume of reagent on color development

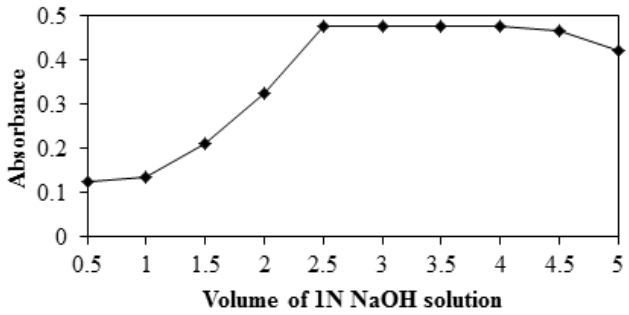

Fig. 4: Effect of volume of base on color development

Optimization of the reaction conditions

The ideal conditions were obtained by changing one circumstance at a time while keeping other constant. Then we examined their effect at λ_{max}740 nm. Effect of color producing reagent on the formation of color complex was studied. Different volume of 2N folin-ciocalteu reagent was used without further dilution. It was observed that 2 ml volume of folin-ciocalteu reagent is sufficient for the maximum color development as shown in fig. 3. To discover a suitable medium for the reaction different aqueous bases were investigated. Favorite results were achieved by the use of 1N NaOH. It was detected that high and consistent absorbance was obtained in the concentration range of 0.5 to 5ml of 1N sodium hydroxide thus 2.5mL of 1N NaOH was fixed as optimal as shown in fig. 4. The reaction time was observed by quantifying the absorbance of the blue chromogen after commixing the reactants over a period from 1 to 30 minutes. Maximum color developed with 10 minutes and was stable for at least 60 minutes. Quantifications were therefore made only after 10min throughout the investigation.

Table 1: Percent recovery of caroverine (10µg/ml) in the presence of excipients/additives

Excipients/ additives	Amount taken (µg/ml)	Recovery (% ± S.D.)
Microcrystalline cellulose	300	99.78 ± 0.35
Lactose monohydrous	400	100.67 ± 0.46
Magnesium stearate	200	100.71 ± 0.43
Starch	450	100.04 ± 0.76
Titanium dioxide	100	100.13 ± 0.48

Table 2: Consequences of recovery and precision

Ingredients	Amount of drug (mg) in formulation	Amount Added (mg)	Amount Recovered (mg)	% Recovery	Precision (Intra Day)*	Precision (Inter Day)*
Caroverine	20	05	24.75	99.00	1.03	1.31
	20	10	29.69	98.96	1.16	1.19
	20	15	34.32	98.05	1.01	1.24

*Percentage RSD of three Samples

Table 3: Statistical and analytical data for the complex of caroverine-Folin-Ciocalteu reagent

Parameters	Values
λ_{max} (nm)	740
Beer's law range (µg/ml)	2-28
Molar absorptivity (L mole^{-1}cm^{-1})	2.15×10^4
Sandell's sensitivity	1.7×10^{-2}
Limit of Detection LOD (µg/ml)	1.15
Limit of Quantification LOQ (µg/ml)	3.81
Slope	0.0580
Intercept	-0.00343
Correlation coefficient	0.9998

Table 3: Assay of tablet dosage forms

Parameter	Labeled claim	Amount obtained	% assay
Sparina tablets	20 mg	19.86	99.31

Table 4: Consequence of content uniformity testing of Sparina tablets using developed procedure

Parameter	% age of the label claim
Tablet 1	99.78
Tablet 2	100.05
Tablet 3	98.02
Tablet 4	99.67
Tablet 5	99.45
Tablet 6	100.34
Tablet 7	98.37
Tablet 8	98.23
Tablet 9	99.2
Tablet 10	99.47
Mean (X)	99.258
S.D.	0.7552
% RSD	0.7609
% Error	0.0026
Acceptance value (AV)	1.8125
Max.Approved value (L1)	15

Interference effect

Up to 100% recovery of caroverine was achieved in the presence of various excipients and additives. The very most common additives used in tablet formation are microcrystalline cellulose, lactose monohydrous, magnesium stearate, starch and titanium dioxide. About 10µg/ml of pure drug was used with various concentrations of excipients. The usually used excipients and additives concentrations presented in table 1 do not show any conflict with the recovery results.

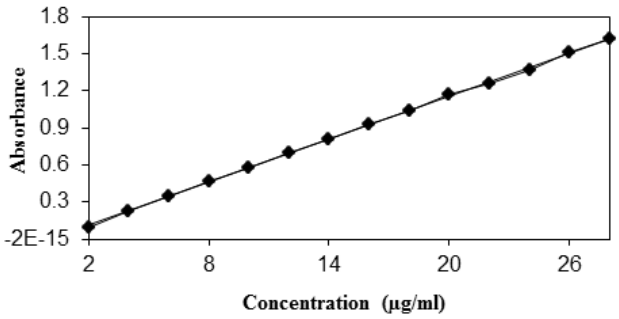

Fig. 5: Beer's law verification range

Validation of the suggested method

The validation of suggested spectrophotometric method was observed with reference to Beer's law range, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ).

Beer's law range

The samples of drug solution range from 2-28µg/ml were scanned at absorbance maximum value of 740 nm. The absorbance range was observed 0.096-1.624 with regression of 0.9998 as shown in fig. 5.

Accuracy and precision

Accuracy and precision of the suggested method was calculated by testing caroverine tablets (i.e. 20 mg tablet) in three replicates on the same day and on three consecutive days. The calculated data is presented in table 2. The RSD values of same or intra-day and three consecutive days or inter-day values indicated good precision.

The standard addition technique was used by mixing excipients at level of 25% (5mg), 50% (10mg) and 75% (15mg), respectively in sample solution for validation purpose. 100% recoveries of the three concentrations show high accuracy of the method.

Limit of detection and limit of quantification

LOD (k=3.3) and LOQ (k=10) of the method were calculated in the light of ICH guidelines. The calculated values of LOD and LOQ of proposed method are reported in table 3. In this study, LOD and LOQ were based on the standard deviation of the blank and the slope of calibration curve. Suggested formula is

LOD = 3.3 S/M; LOQ=10 S/M

Where S is the standard deviation of the absorbance of the blank and M is the slope of the calibration curve. Statistical and analytical data is shown in table 3.

DISCUSSION

The structure of caroverine permits the use of Folin-Ciocalteu reagent for its quantitative determination. In the proposed method blue color is produced when caroverine is reacts with the Folin-Ciocalteu reagent in alkaline medium. Peterson explained this blue color as follows (Peterson 1979). Following chemicals species are produced due to mixed acids in the Folin-Ciocalteu reagent.

$3H_2O{\cdot}P_2O_5{\cdot}13WO_3{\cdot}5MoO_3{\cdot}10H_2O$ and
$3H_2O{\cdot}P_2O_5{\cdot}14WO_3{\cdot}4MoO_3{\cdot}10H_2O$

Perhaps caroverine reduce the molybdate in the Folin-Ciocalteu reagent. The formed blue color is may be due to this reason.

It is also reported that Folin-Ciocalteu reagent can react with phenols and non-phenolic reducing substances to form color products that can be measured by spectrophometry (Sivakumar *et al*., 2010; Muruganathan *et al*., 2008). Caroverine contain free phenolic group which may react with the Folin-Ciocalteu reagent in alkaline medium and give blue color. This blue color is due to oxidation of the caroverine and the reduction of Folin-Ciocalteu reagent. The colored chromogen was found to be stable for almost one hour at room temperature and have λ_{max} of 740 nm.

Applications

I - Dosage form analysis

Sparina tablets containing 20 mg of caroverine were tested by the proposed methods. Results are shown in table-3.

II - Content uniformity test

Contents uniformity test was also applied by using the proposed method. The acceptance value (AV) was determined that was found to be lesser than the maximum approved acceptance value. The results are presented in table 4.

CONCLUSION

Based on the results obtained, it is concluded that the newly proposed visible spectrophotometric method for the determination of caroverine is rapid, accurate, extraction free and economical. It may be used as alternative to HPLC due to its simplicity, sensitivity and selectivity. It may be used with confidence as a routine laboratory method due its short time of operation and ease of handling. However, this method can be used in quality

control laboratories of pharmaceutical industry where modern instruments are not present.

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