

Research Article

Development and Validation of Novel Analytical Method for the Determination of Caroverine in Pharmaceutical Formulations

Asad Raza* and Tariq Mahmood Ansari

Division of Analytical Chemistry, Institute of Chemical Sciences Bahauddin Zakariya University, Multan, Pakistan

***Corresponding author:** Asad Raza, Division of Analytical Chemistry, Institute of Chemical Sciences Bahauddin Zakariya University, Multan, Pakistan**Received:** December 05, 2014; **Accepted:** February 03, 2015; **Published:** February 05, 2015**Abstract**

Caroverine is a spasmolytic drug but no monographs are available in official compendia for the analysis of this substance in raw and pharmaceutical dosage forms. The objective of this work was to develop a simple, fast and validated spectrophotometric method for routine analysis of caroverine in tablets. The newly proposed method depends on the charge-transfer complex formation between caroverine as n-electron donor and chloranil as π -acceptor to give a colored complex that absorbs maximally at 560 nm. Beer's law is obeyed in the concentration ranges 2-30 $\mu\text{g/ml}$ with molar absorptivity of $2.8 \times 10^4 \text{ L mole}^{-1} \text{ cm}^{-1}$. The results of analysis of commercial formulations and the recovery study (standard addition method) of caroverine suggested that there is no interference from any excipients, which are present in pharmaceutical formulations. The proposed method is found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of caroverine bulk and commercially dosage form.

Keywords: Spectrophotometric; Caroverine; Pharmaceutical formulation; Charge transfer reactions

Introduction

A counterfeit medication or a counterfeit drug is a medication or pharmaceutical product which is produced and sold with the intent to deceptively represent its origin, authenticity or effectiveness. A counterfeit drug may contain inappropriate quantities of active ingredients, or none, may be improperly processed within the body (e.g., absorption by the body), may contain ingredients that are not on the label [1]. 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 parameters for the quantitative analysis of drugs in pharmaceutical raw and dosage forms.

Caroverine 1-(2-diethylaminoethyl)-3-(p-methoxybenzyl)-1,2-dihydro-2-quinoxalin-2-on-hydrochloride is chemically derived from isoquinoline, the basic structure of papaverin. 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 a drug used as a spasmolytic and otoneuroprotective (inner ear protective) agent in some countries. It acts as an N-type calcium channel blocker, competitive AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) receptor antagonist, and

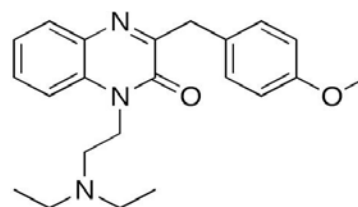


Figure 1: Chemical structure of caroverine.

non-competitive NMDA (N-methyl-D-aspartate receptor) receptor antagonist [2]. It also has potent antioxidant effects [3]. In Pakistan, caroverine is marketed as Saprina Tablets 20 mg (Biopharma, Multan, Pakistan) for oral smooth muscle spasms (Figure 1).

Detailed survey of literature for caroverine revealed that not a single analytical method is available for the quantitative determination of caroverine in pharmaceutical raw and dosage forms. The assay of Caroverine in pure and dosage forms, as far as we know, is not official in any pharmacopoeia, and therefore, requires much more investigation. The molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge transfer complexes, which absorb radiation in the visible region [4]. A variety of electron donating compounds have been reported to yield charge-transfer complexes with various π -acceptors [5-9].

The objective of this study was to develop a simple and validated spectrophotometric method for routine analysis of caroverine tablets in pharmaceutical laboratories.

Experimental

Equipment and reagents

All spectrophotometric measurements were made using a UV/V is spectrophotometer (model U-1100 Hitachi, Japan) with a quartz cuvette (optical path of 1.0 cm). All reagents and solvents were of Analytical Reagent Grade and used as such without further purification. Saprina tablets (20 mg caroverine per tablet) and pure caroverine reference standard were provided by Bio Fine Pharmaceuticals (Pvt.) Ltd. Multan, Pakistan. Its potency was 99.34 % according to certificate of analysis. Chloranil, dimethyl sulfoxide DMSO and 1,4-dioxane (Sigma-Aldrich, Germany) were purchased from local supplier. All laboratory reagents were freshly prepared before use on daily basis.

Preparation of stock solution

1 mg ml⁻¹ stock solution of pure drug was prepared in analytical grade dimethyl sulfoxide. Working standard solutions were prepared by further dilution of these solutions with same solvent. A 0.2 mg/ml chloranil solution was prepared in 1,4-Dioxane.

Calibration curve

Serial volumes of stock solutions ranging from 2-30 µg/ ml were transferred to 10 ml volumetric flasks. To each flask 0.5 ml chloranil solution was added and the volume was brought to mark by adding DMSO. The color developed instantaneously. The absorbance was measured within the stability period of 2 hours after dilution at 560 nm against a reagent blank. Calibration graph was prepared by plotting absorbance vs. concentration.

Pharmaceuticals formulations

Twenty tablets were separately weighed and finely powdered. Accurately weighed portion of powder tablets equivalent to 100 mg of pure drug was dissolved in dimethyl sulfoxide (DMSO) solvent and shaken well for proper mixing. These solutions were allowed to stand for five minutes and then sonicated for complete solubilization of drug. Then the contents were filtered on Whatman filter paper No.42 to separate the insoluble excipients and volume was made up to 100 ml with the same solvent. The measurements were carried out according to the procedure described under the preparation of calibration curve.

Determination of the molar ratio

The Job's method of continuous variation [10] was employed. Master equimolar solutions of the drug and chloranil were prepared. The concentration of the drug solution was 10 µg/ml. A series of 10

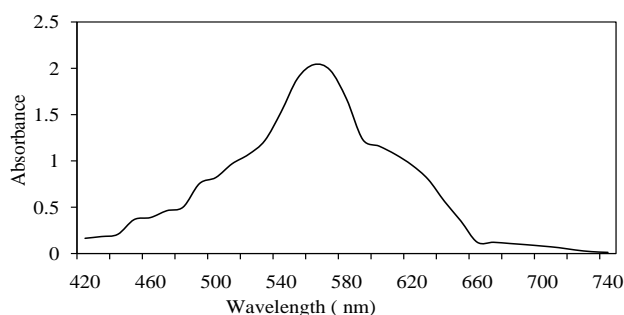
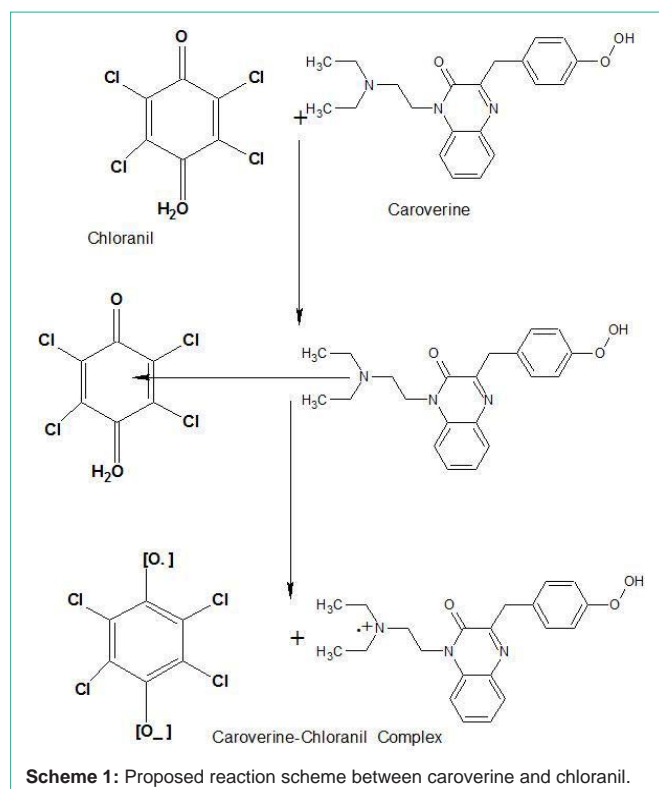


Figure 2: Absorption spectra of caroverine and chloranil complex.



Scheme 1: Proposed reaction scheme between caroverine and chloranil.

ml portions of the master solutions of the drug with chloranil reagent were made up comprising different complementary proportions (0 : 10, 1 : 9, ..., 9 : 1, 10 : 1) in 10 ml volumetric flasks. After the reaction was allowed to proceed at room temperature (25 ± 5°C), the absorbance of the solutions was measured at 560 nm against the reagent blank.

Results and Discussion

The proposed method was designed to develop charge transfer complexation reaction between caroverine donor and chloranil as π -acceptor. The absorbance of formed charge transfer complex was measured on a UV/Visible spectrophotometer. The mechanism of reaction is based on the transfer of electron from electron rich donor having lone pair of electron to electron deficient π -acceptor, which further dissociates due to high ionizing power of the polar solvent, and leads to the formation of radical ions. The newly formed complex shows absorption maxima at 560 nm respectively against reagent blank prepared under the same conditions. Figure 2 represents the electronic absorption spectra of complex and the proposed reaction is illustrated in Scheme 1.

Optimization of experimental conditions

In order to establish the optimum reaction conditions suitable for the complexation, various analytical parameters were studied. The effect of each parameter was observed by altering one parameter at a time while keeping others constant. Various analytical solvents were checked like methanol, water, acetonitrile and Dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO). But the DMSO was found to be a suitable solvent due to high sensitivity and maximum absorbance. To study the optimum reaction time the absorbance of complex was measured 0, 2, 5 and 10 minutes. It was noticed that complete color

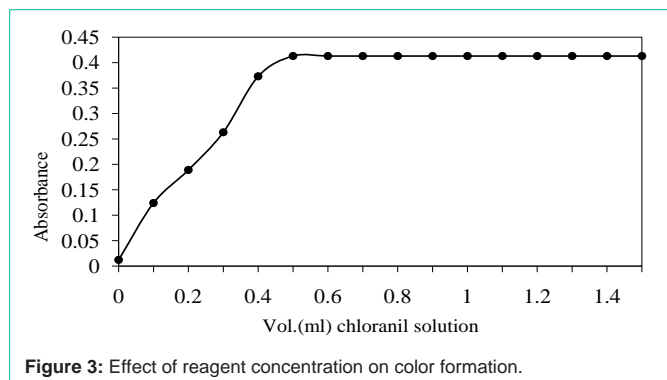


Figure 3: Effect of reagent concentration on color formation.

development was achieved instantaneously at ambient temperature ($25 \pm 2^\circ\text{C}$) and there was no effect on absorbance at different time interval. The formed color complex was found to be stable for 24 h. To determine the effect of chloranil concentration, the concentration of caroverine was kept constant and the concentration of chloranil was changed by varying the ml of stock solution.

Reagent concentration

We found that 0.5 ml is the optimum volume of chloranil for carrying the assay of caroverine stock solution. It can be concluded that the elevated concentrations of the reagent have no marked effect on the color development (Figure 3).

Reaction time

Following the absorbance of the developed color at different time intervals at ambient temperature ($25 \pm 5^\circ\text{C}$) for the reagent was determined. Complete color development was attained instantaneously. But for more accuracy and safety we placed the reaction mixture for five minutes at room temperature before taking the absorbance measurement.

Stoichiometric ratio of complex

The stoichiometric ratio of caroverine and chloranil was established by applying Job's method of continuous variation using equimolar solutions by taking absorbance of complex solutions of different ratios (0:10, 1:9,10:0) (donor: acceptor). The graph was plotted between the mole fractions of drug vs. absorbance. Figure 4 indicates that caroverine interacted with chloranil in stoichiometric ratio of 1:1.

Association constants and standard free energy changes

The association constant was determined for the interaction of caroverine with chloranil complex using Benesi Hildebrand equation [11].

$$\frac{C_a}{A} = \frac{1}{\epsilon} + \frac{1}{Kc \times \epsilon} \times \frac{1}{C_b}$$

Where C_a and C_b are the concentrations of the acceptor and donor respectively, A is the absorbance of the complex, ϵ is the molar absorptivity of the complex and Kc is the association constant of the complex. The standard free energy changes of complexation (ΔG°) were calculated from the association constants by the following equation [12].

$$\Delta G^\circ = -2.303RT \log Kc$$

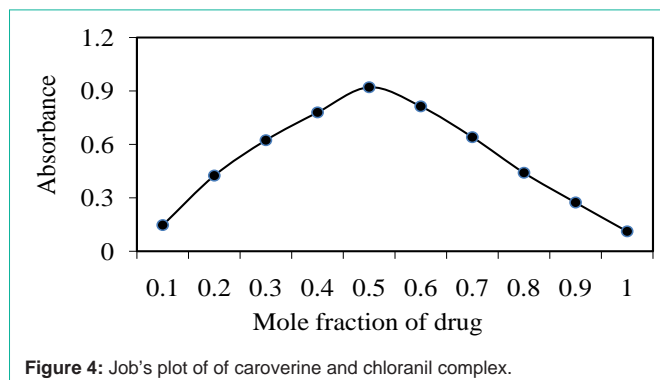


Figure 4: Job's plot of caroverine and chloranil complex.

Table 1: Percent recovery of the caroverine in the presence of possible excipients used in tablet formulation.

Excipients	Amount taken ($\mu\text{m/ml}$)	% Recovery \pm RSD (n=5)
Microcrystalline cellulose	200	99.58 \pm 0.41
Magnesium stearate	80	99.1 \pm 0.41
Titanium dioxide	100	99.48 \pm 0.33
Lactose	300	99.15 \pm 0.72

Table 2: Spectral and Benesi-Hildebrand data for the reaction of caroverine and chloranil complex.

Parameters	Values
λ max (nm)	560
Beer's law limits ($\mu\text{g/ml}$)	2-30
Molar absorptivity ($\text{L mole}^{-1} \text{cm}^{-1}$)	2.8×10^4
Detection limit ($\mu\text{g/ml}$)	0.95
Quantification limit ($\mu\text{g/ml}$)	3.13
Sandle sensitivity ($\mu\text{g cm}^{-2}$)	1.3×10^{-2}
Slope	7.2×10^{-2}
Intercept	-1.7×10^{-2}
Correlation coefficient	0.9998
Association Constant (Kc)	3.7×10^5
Standard free energy change (ΔG°)	-7.6889

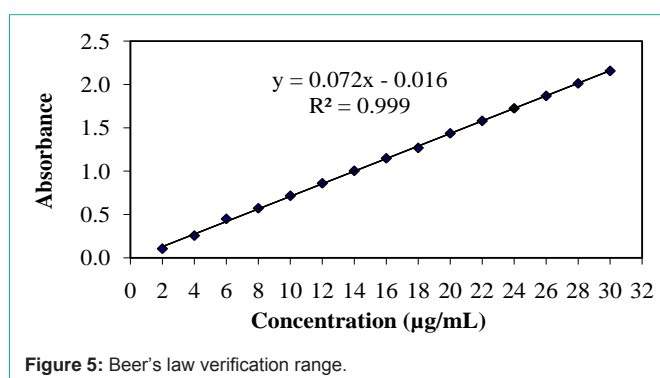


Figure 5: Beer's law verification range.

Where ΔG° is the free energy change of the complex (kJ mol^{-1}), R the gas constant ($0.001987 \text{ K cal mol}^{-1} \text{ deg}^{-1}$), T the temperature in Kelvin ($273 + ^\circ\text{C}$) and Kc is the association constant of drug- acceptor complex (1 mol^{-1}).

Table 3: Results of recovery study by standard addition method and precision.

Tablets (API)	Amount of drug (mg) in formulation	Amount Added (mg)	Amount Recovered (mg)	% Recovery	RSD (%)	Precision (Intra Day)*	Precision (Inter Day)*
Sparina	20	5	24.75	99.0	0.56	1.04	0.99
Tab	20	10	30.04	100.13	0.47	0.97	1.15
Caroverine	20	15	34.89	99.68	0.34	0.73	1.02

*Percentage of three determination sake.

Interference of excipients

More than 99 % recovery of caroverine was obtained in the presence of possible excipients and other additives in tablet formulations such as microcrystalline cellulose, magnesium stearate, titanium dioxide and lactose. Under the experimental conditions employed, to a known amount of drug (caroverine 10 µg/ml), excipients in different concentrations are added and analyzed. The results of the recovery analysis are presented in Table 1. Excipients up to the concentrations shown in the Table 1 do not interfere with the assay. In addition recoveries in most cases are 100 %.

Validation of method and applications

The method was validated as per the ICH [13] in terms of linearity, accuracy and specificity, intra-day and inter-day precision, repeatability of measurement of absorbance. Optical characteristics, statistical data for the regression equation and Benesi Hildebrand equation of the proposed method are given in Table 2. The proposed method was found to give linear calibration curve over the concentration ranges of 2- 30 µg/ml with a regression coefficient (r) of 0.9998, indicating good linearity (Figure 5). Assay were performed in triplicate at different levels. This was repeated with a second instrument, standard and sample preparation on different days. These results of accuracy and precision show that the proposed method has good repeatability and reproducibility. Also the assay results are unaffected by the presence of excipients, thus establish specificity of the method. The proposed method was applied for the determination of the caroverine in commercial preparations. Three replicate determinations were made. Moreover, to check the validity of the proposed method, the standard addition method was applied by adding pure caroverine to the previously analyzed tablets. The recovery of the drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of analysis of commercial dosage forms and the recovery study (standard addition method) of the cited drug (Table 3) suggested that there is no interference from any excipients, which are present in tablets.

Accuracy and Precision

Accuracy and precision were investigated by analyzing caroverine tablets (i.e. 20 mg tablet) in three independent replicates on the same day (Intra-day accuracy and precision) and on three consecutive days (Inter-day accuracy and precision). Intra-day and Inter-day relative standard deviation (RSD) values and also the low RSD values obtained from the analysis of the pharmaceutical tablet formulation (Table 3) indicated good intermediate precision of method.

Conclusion

The proposed spectrophotometric method is novel because none of the analytical method is available in the literature for the quantitative determination of caroverine in pharmaceutical raw and

dosage forms. The method is rapid, simple, precise and accurate. The proposed procedure is free from tedious steps like extraction or heating and involves the least number of experimental variables, which is reflected in high precision. An additional advantage of this method is its specificity. Since basic nitrogen is the reaction site, the method is specific to caroverine since none of the excipients normally used in dosage forms contains basic nitrogen. Furthermore, all the analytical reagents are inexpensive, have excellent shelf life, and are easily available in any analytical laboratory. The proposed method can be applied in quality control laboratories for the routine analysis of the caroverine in raw materials and pharmaceutical formulations.

Acknowledgement

The work reported in this paper is a part of PhD thesis of Mr. A. Raza. He is thankful to Bio-Fine Pharmaceuticals Ltd, Multan for providing instrumental facilities to carry out this work. We are grateful to the Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan for providing support and laboratory facilities to accomplish this task.

References

- Davison M. Pharmaceutical Anti-Counterfeiting: Combating the Real Danger from Fake Drugs: Wiley; 2011.
- Udilova N, Kozlov AV, Bieberschulte W, Frei K, Ehrenberger K, Nohl H. The antioxidant activity of caroverine. *Biochem Pharmacol.* 2003; 65: 59-65.
- Nohl H, Bieberschulte W, Dietrich B, Udilova N, Kozlov AV. Caroverine, a multifunctional drug with antioxidant functions. *Biofactors.* 2003; 19: 79-85.
- Foster R, Organic Charge-Transfer Complexes. Vol. 51. London Academic Press; 1969.
- Raza A, Utility of Certain p-Acceptors for the Spectrophotometric Determination of Tranexamic Acid in Commercial Dosage Forms. *Analytical Letters.* 2006; 39: 2217-2226.
- Raza A. Development and application of spectrophotometric methods for the determination of citalopram hydrobromide in dosage forms. *Chem Pharm Bull (Tokyo).* 2006; 54: 432-434.
- Raza A, Ansari TM, Niazi SB, Rehman A. Spectrophotometric determination of citalopram in pharmaceutical formulations. *J.Chem.Soc.Pak.* 2008; 30: 241- 245.
- Raza A, Ansari TM, Niazi SB. A Novel Spectrophotometric Method for the Determination of Zolmitriptan in Pharmaceutical Formulations. *J. Chin. Chem. Soc.* 2007; 54:1413-1417.
- Raza A, Ijaz AS, Rehman A, Rasheed U. Spectrophotometric determination of on dansetron hydrochloride in pharmaceutical bulk and dosage forms. *J.Chin.Chem.Soc.* 2007; 54: 223-227.
- Job P: Advanced Physicochemical Experiments. 2nd Ed. Edinburgh, Oliner and Boyd; 1964.
- Bensei HA, Hidelbrand J. The Benesi-Hildebrand Method for Determination of Kf for DA association and ε Values for DA CT absorption. *J. Am. Chem. Soc.* 1949; 71: 2703.

12. Martin AN, Swarbrick J, Cammarata A. Physical Pharmacy 3rd Ed. Philadelphia, Lee & Febiger; 1969.
13. ICH Guidance on Analytical Method Validation. In: Proc. Int. Convention on Quality for the Pharmaceutical Industry, Toronto, Canada; 2002.