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Exploration of 1, 2-naphthoquinone-4-sulfonate derivatizing reagent for determination of chlorthalidone in tablets: a spectrophotometric investigation

Suraj R. Chaudhari* and Atul A. Shirkhedkar

Abstract

Background: Chlorthalidone is a diuretic medicinal agent used to treat hypertension. Lowering hypertension results in the prevention of strokes, heart attacks and kidney-related problems.

Results: In this work, the spectrophotometric investigation was employed to examine the reaction between chlorthalidone and 1, 2-naphthoquinone-4-sulfonate. A reaction is performed at the alkaline environment (pH 9.2) and heated at a moderate temperature of 60 ± 1 °C using a water bath. The red colour product was produced, and it displayed a maximum absorbance at 440.50 nm. Further, the advancement of response conditions was explored by UV/visible spectrophotometer. The stoichiometric of the reaction was considered by using Job's plot and the reaction mechanism of the proposed work was drawn. The established approach obeyed the calibration curve in the concentration range of 2–12 μg/mL with a regression coefficient superior to 0.994. The accuracy of the method, assessed by employing the % recovery, was in the range of 99.06–99.60% with the % relative standard deviation being less than 2%. The limit of detection and limit of quantification were 0.58 μg/mL and 1.72 μg/mL, respectively.

Conclusion: A UV/visible spectrophotometric investigation for the analysis of the chlorthalidone in the tablet matrix was successfully established and validated. The established investigation was found to be simple and accurate, and because of its ease of use, the proposed strategy could be applied for quality control investigation of chlorthalidone in pharmaceutical matrices.

Keywords: Chlorthalidone, Job's plot, Spectrophotometry, 1, 2-Naphthoquinone-4-sulfonate

Background

Chlorthalidone (CHL), chemically 2-chloro-5-(1-hydroxy-3-oxo-2*H*-isoindol-1-yl) benzenesulfonamide;N-(2, 6-dichlorophenyl)-4,5-dihydro-1*H*-imidazol-2-amine (Fig. 1), is used in the treatment of hypertension [1]. CHL is a phthalimidine diuretic that shows an extended duration of action up to 72 h after an administration of single-dose, and it shows the antidiuretic effect by inhibiting Na^+ and Cl^- transporters in distal convoluted tubules and increasing Na^+ and Cl^- excretion [2, 3]. Literature survey demonstrated that several analytical methods have been established to investigate CHL in pure form and pharmaceutical preparations alone or combined with other medicinal agents. These reported methods include high-performance liquid chromatography [4–15], high-performance thin-layer chromatography [16–18], spectrophotometry and spectrofluorometry [19–33], capillary electrophoresis [34–36], and potentiometry [37]. Nowadays, 1, 2-naphthoquinone-4-sulfonate is a choice of derivatizing reagent employed for the estimation of drugs containing primary and secondary amino groups using spectrophotometry and spectrofluorometry. These types of drugs are easily

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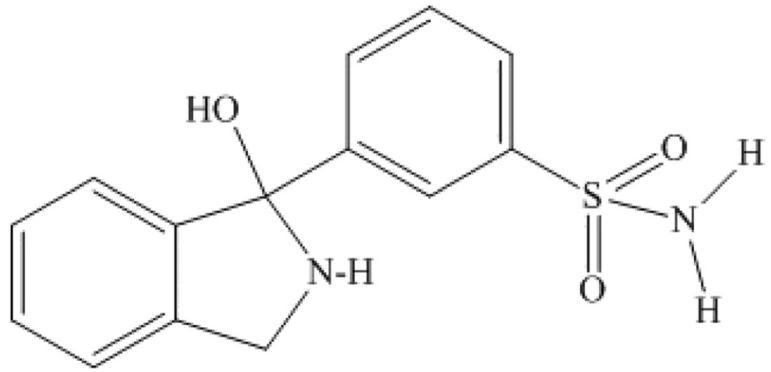

Fig. 1 Chemical structure of chlorthalidone

reacting with NQS under the alkaline medium and at moderate temperatures to yield a derivative product which can be efficiently detected through spectrofluorometric and spectrophotometric methods [38]. So far in the literature, no research work was accounted for being related to the utilization of UV/vis-spectrophotometric technique for assessment of CHL in tablet dosage form using 1, 2-naphthoquinone-4-sulfonate. Although several UV/vis-spectrophotometric methods have been reported for estimation of CHL, to our knowledge, in all approaches, different wavelengths were used for the analysis of CHL. Therefore, there is a vital need for the development of a specific UV/vis-spectrophotometric method which uses a fixed wavelength for analysis of CHL. Till date, there is no single report available on literature for the analysis of CHL using NQS. Hence, it was decided to explore 1, 2-naphthoquinone-4-sulfonate derivatizing agent for the establishment of sensitive and precise UV/vis-spectrophotometric investigation for CHL analysis in the tablet matrix. Moreover, the stoichiometric ratio of reaction between CHL and 1, 2-naphthoquinone-4-sulfonate was successfully established using Job's method.

Methods

Chemicals

Chemicals and materials used in proposed work include working standard CHL which was obtained as a gift sample from the J. B. Chemicals and Pharmaceuticals Ltd. Ankleshwar, India. 1, 2-Naphthoquinone-4-sulfonate sodium salt and HPLC grade methanol were purchased from Sigma-Aldrich Mumbai, India.

Instrumentation

A spectrophotometric optimization was performed on a double-beam UV/visible spectrophotometer (Shimadzu, Japan), with a deuterium lamp, electronic balance (Model Shimadzu AUX 120), pH meter (model EQ 621, Equiptronics, India), and thermostatically monitored water bath.

Preparation of stock standard solution

The stock standard solution was prepared by accurately dissolving 10 mg of CHL in 10 mL of methanol to the obtained concentration of 1 mg/mL. From it, appropriate volume was further diluted with the same solvent to achieve a concentration of 0.1 mg/mL.

Preparation of reagent

1, 2-Naphthoquinone-4-sulfonate (0.5% w/v)

An accurate weight of 50 mg of 1, 2-naphthoquinone-4-sulfonate was transferred into a 10-mL amber colour volumetric flask, dissolved in 5 mL of water, and sonicated for 10 min, and further volume was adjusted to mark with the same solvent to an obtained concentration of 0.5% *w/v*.

Preparation of buffer solution (pH 9.2)

The buffer solution of pH 9.2 was prepared by dissolving 7.645 g of sodium bicarbonate and 0.954 g of sodium carbonate in 800 mL of water, and finally, the volume was adjusted up to 1000 mL using the water [39].

The general protocol for optimization of chlorthalidone-1, 2-naphthoquinone-4-sulfonate complex

A 1 mL of a solution of CHL was transferred into 10 mL of the calibrated flask; subsequently, 1 mL of sodium carbonate buffer (pH 9.2) and 1 mL of 0.5% 1, 2-naphthoquinone-4-sulfonate solution were added into the calibrated flask. The resulting solution was kept on a water bath at 60 ± 1 °C for 20 min. After heating, the reaction solution was cooled at room temperature and the volume of the calibrated flask was adjusted up to the mark up with methanol. The CHL-1, 2-naphthoquinone-4-sulfonate complex depicted maximum absorbance at a wavelength of 440.50 nm with methanol as the blank solvent. The UV/visible spectrum of CHL-1, 2-naphthoquinone-4-sulfonate complex was shown in Fig. 2.

Measurement of the stoichiometric ratio of reaction between chlorthalidone-1, 2-naphthoquinone-4-sulfonate

Job's method for continuous variation was successfully applied to determine the stoichiometric of a reaction within CHL-1, 2-naphthoquinone-4-sulfonate under operative condition [40, 41]. An equimolar methanolic solution of CHL and aqueous solutions of 1, 2-naphthoquinone-4-sulfonate of 0.002 M (2×10^{-3} M) were prepared separately in the calibrated flask. Soon after, a set of 10 mL of portions of the equimolar solutions of CHL and 1, 2-naphthoquinone-4-sulfonate were mixed in calibrated flasks in different proportions (9:1, 8:.2, 7:3....1:9, 2:8, 3:7) containing 1 mL of buffer solution of (pH 9.2). The subsequent arrangements were additionally drawn closer for a general convention for estimation of the stoichiometric proportion of response.

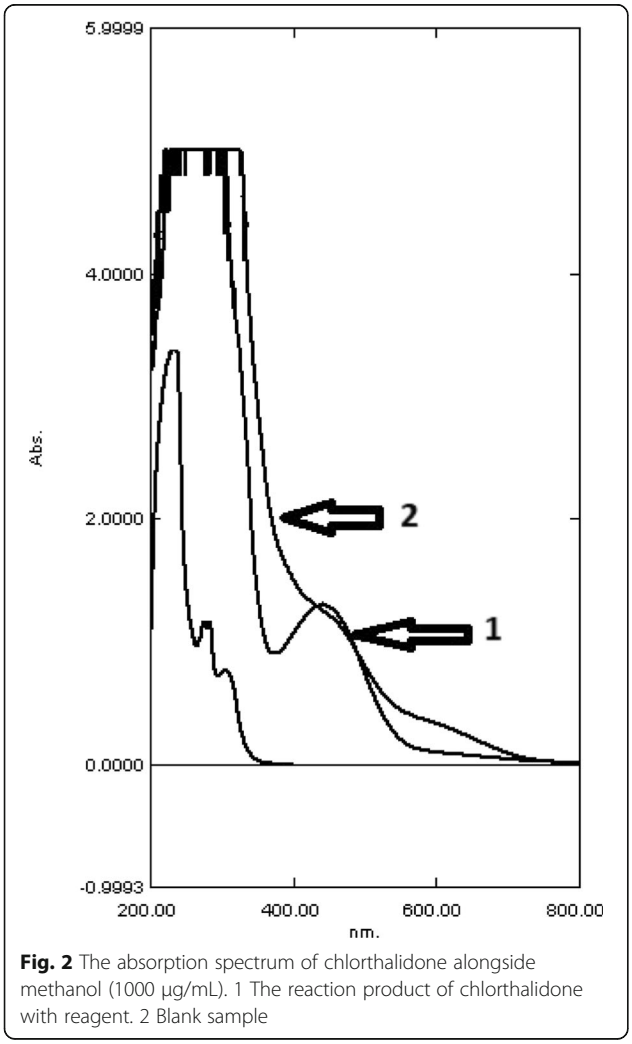

Fig. 2 The absorption spectrum of chlorthalidone alongside methanol (1000 µg/mL). 1 The reaction product of chlorthalidone with reagent. 2 Blank sample

Results

Quantification of wavelength

Initially, the UV/vis absorption spectrum of CHL was measured in methanol as a reference solvent using a concentration of a 10 µg/mL. From there, it was observed that the CHL show maximum absorbance (0.9847) at 214.80 nm. The determination of CHL in tablets was quite difficult using this wavelength (214.80 nm) because of potential interference from common excipients. Hence, an attempt to shift the lambda max of CHL at higher wavelength was needed for its determination. Therefore, we carried out the reaction of CHL with 1, 2-naphthoquinone-4-sulfonate using an alkaline medium at a temperature of 60 ± 1 °C for 20 min. After the reaction, red-orange colour solution (product) was observed and the UV/vis spectrum of the product was determined using methanol as a blank. It was observed that the lambda max of CHL has shifted to longer wavelength, i.e. 440.50 nm. Similarly, a blank determination was performed to study the effect of reagent; from these, it was noticed that the blank solution shows lambda max at 360 nm for 1, 2-naphthoquinone-4-sulphonic acid. To estimate the CHL in tablets and to avoid interference from excipients, 440.50 nm was selected as the wavelength for rest of the investigation. The stoichiometric reaction between drug and reagent was examined by Job's method, under the established optimized conditions. The symmetrical bell-shaped curve (Job plot) was

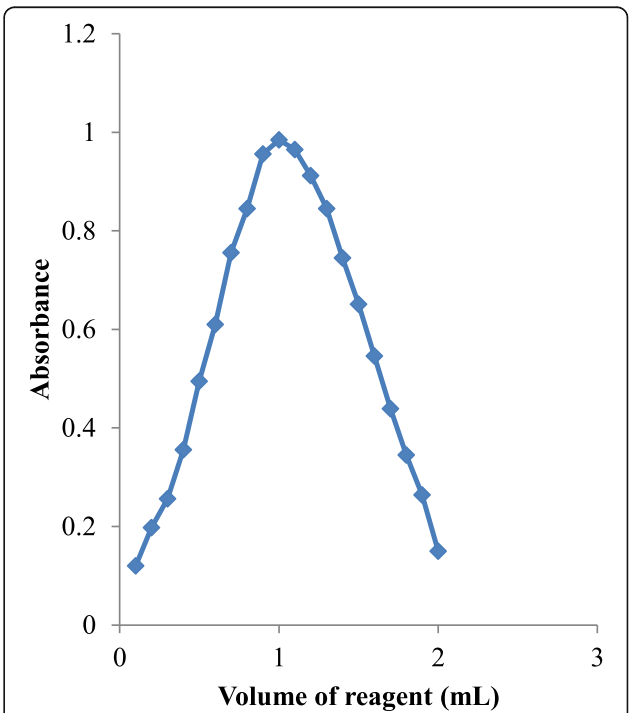

Fig. 3 Job's plot for the determination of stoichiometric reaction between drug and reagent (1:1 ratio)

plotted (Fig. 3), and from these, it is clear that the stoichiometric ratio is 1:1.

Optimization of reaction condition for chlorthalidone-1, 2-naphthoquinone-4-sulfonate complex

Effect of temperature

The impact of temperature on the reaction among CHL and 1, 2-naphthoquinone-4-sulfonate was estimated by performing the reaction at distinct temperatures (40–80 °C). The absorbance of reaction increased with the increase in temperature up to a certain level, and then the absorbance declined. The decrease in the absorbance of a complex was due to the instability of the reaction product at a higher temperature (85–95 °C). The higher absorbance of a reaction product was achieved at 60 ± 1 °C, and absorbance was remained steady up to 80 °C.

Effect of time

The impact of time on the formation of the complex was examined by permitting the reaction for a changing time. The most favourable heating time for the development of complex was 20 min as depicted in Fig. 4.

Effect of pH

The effect of pH on the development of complex was investigated in different pH ranges (9.2–10.6) with carbonate-bicarbonate buffer. The pH of the reaction is the most stringent factor because the absorbance intensity of the complex was directly depending on the pH of the reaction medium. The maximum absorption of the complex was achieved using the pH 9.2, and the absorption of the complex was remained steady for the pH 10.6. Therefore, the pH 9.2 buffer solution was used for the rest of the analysis as illustrated in Fig. 5.

Effect of 1, 2-naphthoquinone-4-sulfonate concentration (% w/v)

While measuring the impact of concentration of 1, 2-naphthoquinone-4-sulfonate on the complex formation reaction, it was noticed that the complex of reaction depended on the concentration of 1, 2-naphthoquinone-4-sulphonic acid. The concentration of 1, 2-naphthoquinone-4-sulfonate increased as the absorbance intensity of the complex formation reaction was also increased. Excellent results were obtained using 0.5% *w/v* concentration of 1, 2-naphthoquinone-4-sulphonic acid. Therefore, the 0.5% *w/v* concentration of 1, 2-naphthoquinone-4-sulfonate was selected for the rest of the analysis as shown in Fig. 6.

Stability of complex

The stability of the reaction product was checked by measuring the absorbance intensity of the complex at a different time at room temperature. From there, it was revealed that the complex was stable for 1 h at room temperature.

Influence of diluting solvent

Influence of diluting solvent was studied using water and methanol. In diluting the reaction solution with water, the colour intensity of the reaction solution was changed because of the instability of the reaction product in water. Diluting the reaction solution with methanol gave the maximum intensity of the reaction product, and hence, the methanol was used as the diluting solvent for the rest of the analysis. The reaction scheme for CHL with 1, 2-naphthoquinone-4-sulfonate was depicted in Fig. 7.

Validation of the commenced method

The established method was verified for linearity, accuracy, precision, sensitivity, and robustness as per the recommendations of an International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline [42].

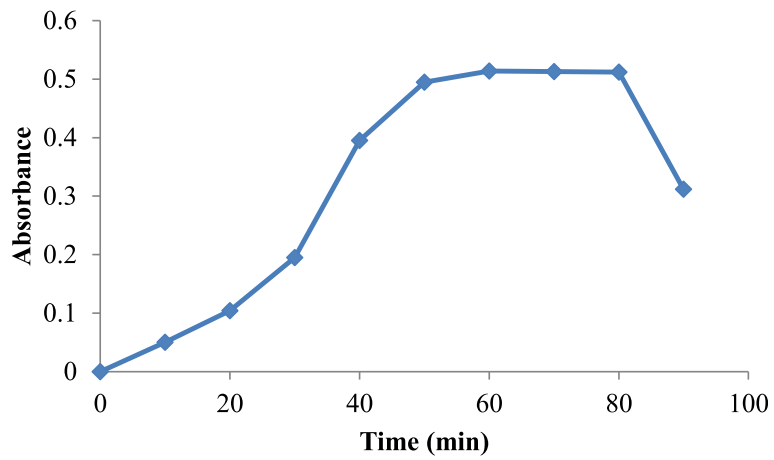

Fig. 4 Effect of time on the reaction product

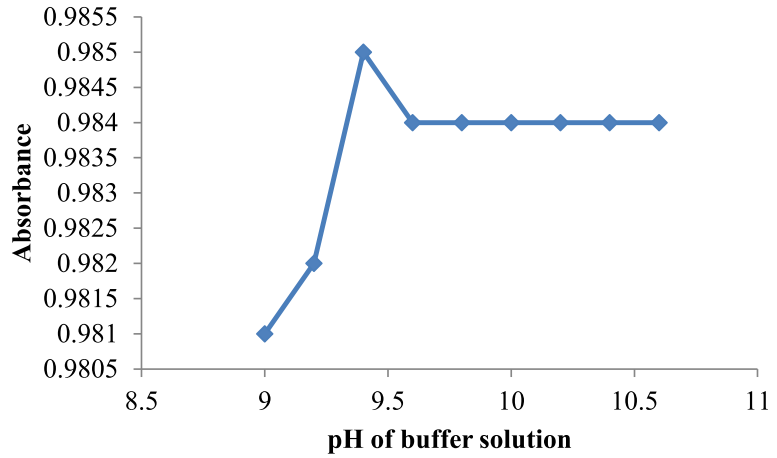

Fig. 5 Effect of pH of buffer solution on the reaction product

Linearity study

To establish the calibration curve of the method, accurate volumes in the range of 2.0–12 mL were transferred from a secondary stock solution into a series of 10-mL calibrated flask and performed as per general protocol to obtain concentrations in the range of 2–12 μg/mL. All measurements were repeated five times for each concentration, and calibration plot was constructed by the plotting concentration of CHL-1, 2-naphthoquinone-4-sulfonate (μg/mL) complex against absorbance. A linear regression equation $y = 8.77x + 7.51$ was found after plotting calibration curve with coefficient correlation ($r^2 = 0.994$).

Accuracy

The accuracy of the proposed analysis was executed using the standard addition method, where a known amount of the drug standard was employed in three distinct levels 80, 100, and 120% to tablet matrix. Accuracy of this study was measured in terms of % recovery.

Precision

The precision of the established method is demonstrated in terms of intra-day precision and inter-day precision. The repeatability of the established method for CHL-1, 2-naphthoquinone-4-sulfonate complex was assessed by analyzing the concentration of 10 μg/mL, and (%) RSD of absorption spectra was calculated for CHL-1, 2-naphthoquinone-4-sulfonate complex. Intra-day variation was measured by three replicates of three different concentrations of 4, 5, and 6 μg/mL which were analyzed on the same day and three successive days for inter-day precision.

Sensitivity

The sensitivity of the developed method was determined by calculating the limit of detection (LOD) and limit of

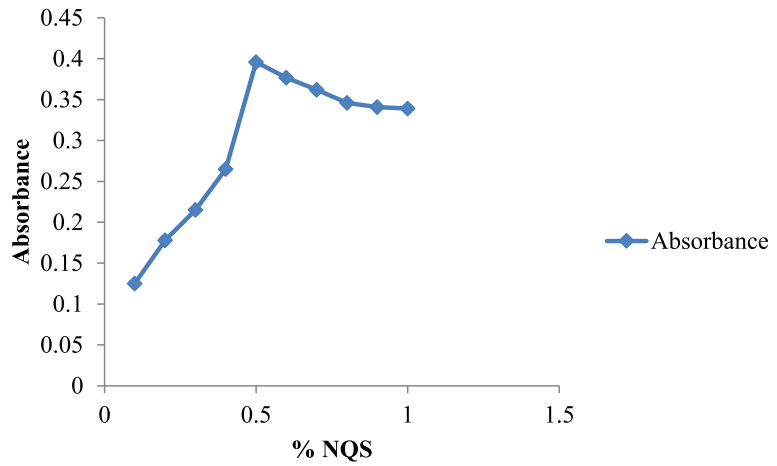

Fig. 6 Effect of concentration of 1, 2-naphthoquinone-4-sulfonate on the reaction product

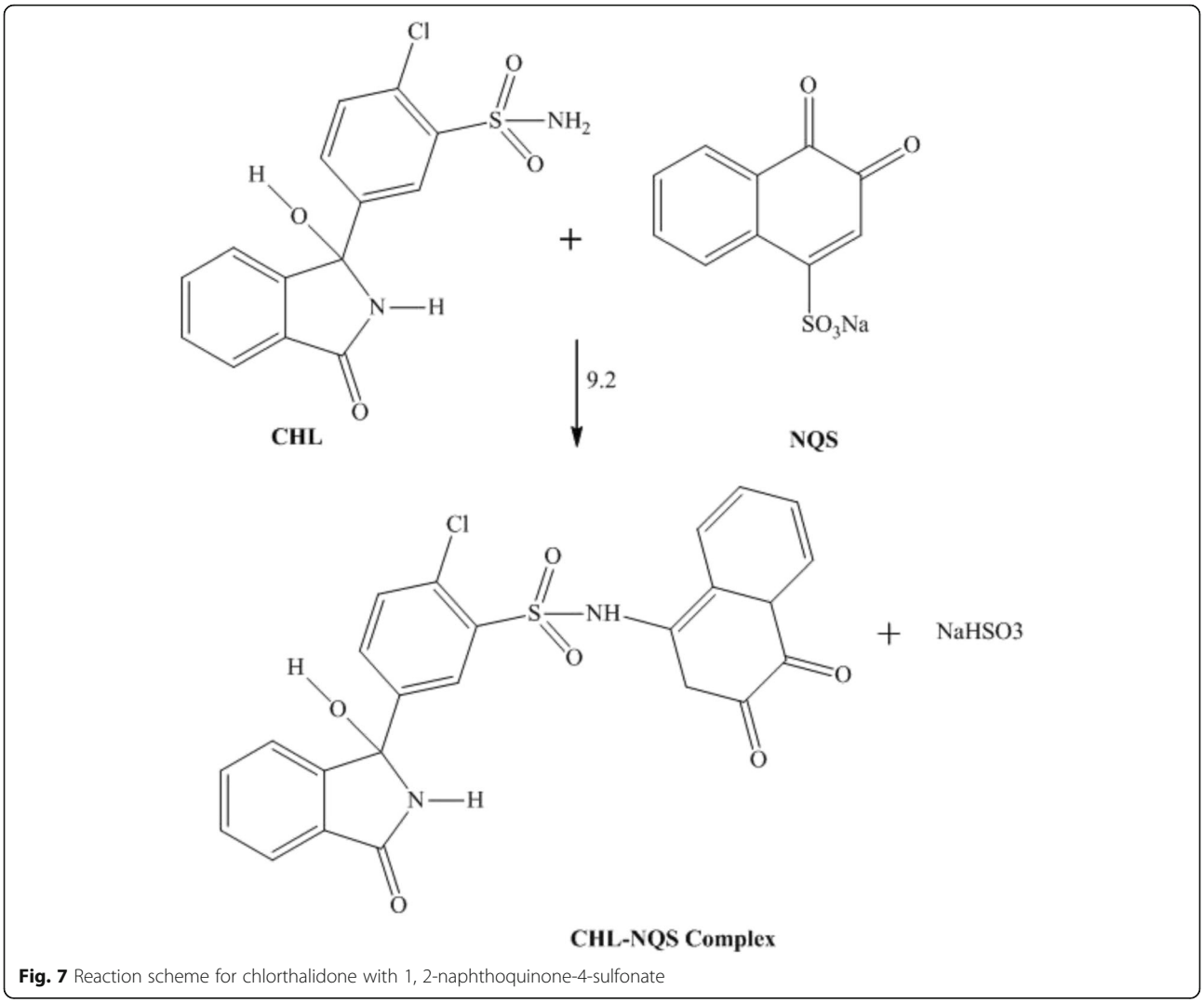

Fig. 7 Reaction scheme for chlorthalidone with 1, 2-naphthoquinone-4-sulfonate

quantification (LOQ). For determination of LOD and LOQ, different concentrations in the range of 2–4.5 μg/mL were prepared and investigated by using the general protocol. The limit of detection and limit of quantification were estimated using the formula LOD = 3.3 (SD)/S and LOQ = 10 (SD)/S, where SD = standard deviation of response and S = the slope of the calibration plot. The LOD and LOQ of the proposed method were found to be 0.58 μg/mL and 1.72 μg/mL for CHL-1, 2-naphthoquinone-4-sulfonate complex.

Robustness

Robustness of the method was studied by determining the impact of small but deliberate variation in independent variables and investigating its effect on the performance of the method. For determination of the robustness of the method, three different independent variables were selected as the volume of 1, 2-naphthoquinone-4-sulfonate solution (0.8–1.2 mL), pH of buffer solution (9.0–9.4), and water bath temperature (58–62 °C). The results of the robustness study were shown in Table 1, and a robustness study was performed using a sample solution of 10 μg/mL.

Application of the proposed method for analysis of chlorthalidone in a pharmaceutical preparation

For the preparation of the sample solution, ten tablets were taken (Thalidon-6.25 tablets, 6.25 mg/tablet), weighed, and finally powdered with mortar and pestle. An accurate weight powder drug equivalent to one tablet (6.25 mg) was taken into a 100-mL volumetric flask, dissolved in 50 mL of methanol and sonicated for 10 min. Further, the volume of the flask was made up to mark with the same solvent. The resulting solution was filtered through a 0.45-μm filter (Millifilter, Milford, MA, USA). An accurate volume was further diluted with the methanol to obtain a concentration of 6 μg/mL. A sample solution of 6 μg/mL was prepared and subjected to

Table 1 Robustness investigation

Independent variables	Variation level	% Recovery ± % RSD
Volume of 1, 2-naphthoquinone-4-sulfonate solution (mL)	0.8	98.45 ± 1.26
	1.0	99.95 ± 0.45
	1.2	98.69 ± 0.37
pH of buffer solution	9.0	100.10 ± 1.14
	9.2	98.85 ± 1.56
	9.4	98.87 ± 1.85
Water bath temperature (℃)	58	98.56 ± 1.32
	60	100.05 ± 1.24
	62	97.45 ± 1.65

estimation of CHL in the tablet matrix. The result of the determination of CHL in a pharmaceutical formulation was shown in Table 2.

Discussion

CHL is effectively being used to treat hypertension and fluid retention developed due to different conditions like heart diseases. In commenced research work, a complex of 1, 2-naphthoquinone-4-sulfonate and CHL was effectively established. A red colour product was obtained in an alkaline environment (pH 9.2) and at a moderate temperature of 60 ± 1 °C for 20 min which obeyed maximum absorbance at wavelength 440.50 nm. After establishing the complex, the reaction conditions for CHL-1, 2-naphthoquinone-4-sulfonate complex were optimized for effect of temperature, effect of time, effect of pH, effect of 1, 2-naphthoquinone-4-sulfonate concentration (% *w/v*), the stability of complex, and influence of diluting solvent. Then, the stoichiometric reaction between drug and reagent was studied by using Job's method under investigated conditions. After that, the symmetrical bell-shaped curve (Job plot) was constructed, and from these, it is concluded that the stoichiometric ratio is 1:1 [43].

CHL-1, 2-naphthoquinone-4-sulfonate complex obeyed good determination coefficient more than (r^2= 0.99) in the given concentration range of 2–12 μg/mL. Accuracy was appraised as a % recovery study at three distinct levels, i.e. 80, 100, and 120%. % Recovery study was performed using standard addition method demonstrating excellent % recovery at three distinct levels in the range of 99.06–99.60%, and their percentage RSD was less than 2 satisfying the acceptance criteria for % recovery study. The precision of the present investigation was evaluated for intra-day, inter-day, and repeatability and was acknowledge as RSD (%). The intra-day and inter-day precision were studied using the 4, 5, and 6 μg/mL concentrations, and repeatability was at 10 μg/mL and demonstrated RSD (%) less than 2% suggesting precision of the investigation. LOD and LOQ were found to be 0.58 μg and 1.72 μg, respectively. The small values of LOD and LOQ show the adequate sensitivity of the investigation. Robustness experiment for the proposed investigation has appraised the impact of independent variables on the CHL-1, 2-naphthoquinone-4-sulfonate complex. The independent variables selected for this experiment are volume of 1, 2-naphthoquinone-4-sulfonate solution, pH of buffer solution, and water bath temperature. From this, it was observed that a small variation in the independent variables did not show any impact on response indicating the robustness of investigation [44].

The tablet matrix (Thalidon-6.25 tablets) containing CHL (6.25 mg/tablet) when studying using the proposed investigation displayed excellent recovery. The amount of CHL in the tablet matrix was found to be 99.17 ± 0.40%. Form this, it was observed that none of the matrix ingredients interferes with CHL-1, 2-naphthoquinone-4-sulfonate complex. Therefore, the proposed method could be applied for quality control analysis of CHL in a pharmaceutical formulation.

The summary of validation parameters for design investigation is summarized in Table 3.

Conclusion

A simple, specific, and accurate spectrophotometric method has been developed and validated for the determination of CHL in tablet matrix. In the present study, 1, 2-naphthoquinone-4-sulfonate was successfully

Table 2 Analysis of tablet matrix containing Chlorthalidone (6.25 mg/tablet)

Complex	Amount found (μg/mL)	% Amount found ± SD	% RSD
CHL-1, 2-naphthoquinone-4-sulfonate	5.95	99.17 ± 0.40	0.51

Table 3 Summary of validation parameters

Parameters	Outcomes of parameters
Linearity range (μg/mL)	2–12
Accuracy	
Mean recovery % (% RSD)	99.32 ± 0.70 0.71
Precision (n = 3)	
Intra-day precision (% RSD)	0.36–0.82
Inter-day precision (% RSD)	0.56–1.20
LOD (μg/mL)	0.58
LOQ (μg/mL)	1.72
Robustness	Robust
% Assay of drug	99.17 ± 0.40

% RSD percent relative standard deviation

employed for the determination of CHL and the proposed method is the first method for determination of CHL in tablet matrix using 1, 2-naphthoquinone-4-sulphonic acid; the established method was efficiently applied for the determination of CHL in the tablet matrix without hindrance from excipients.

Abbreviations

CHL: ChlorthalidoneLODLimit of detection and limitLOQLimit of quantitation

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Authors' contributions

SRC carried out the proposed research work and organized the preliminary draft of the manuscript. AAS gave technical contribution for completing the research work. The authors read and approved the final manuscript.

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Ethics approval and consent to participate

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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