

A New Spectrophotometric Method for Determination of Penicillamine in Pharmaceutical Formulation Using 1, 2-naphthoquinone-4-sulfonate (NQS)

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Abstract

A rapid, simple and sensitive method for the determination of D-Penicillamine using sodium 1,2-naphthoquinone-4-sulfonate (NQS) has been developed. The method is based on the fact that a brown product can be formed by the reaction between D-Penicillamine and sodium NQS in a buffer solution of a pH 12.0. Beer's law is obeyed in the range 10-30 $\mu\text{g/mL}$ of D-Penicillamine at maximum wavelength of 425 nm. The linear regression equation of the calibration curve is $A=0.1684+0.01624C$ ($\mu\text{g/mL}$), with a linear regression correlation coefficient of 0.997. The detection limit is 3.12 $\mu\text{g/mL}$ and the recovery rate is in the range of 99.21-108.2%. The method has been successfully applied to the determination of D-Penicillamine in pharmaceutical formulation.

Keywords: Spectrophotometric; D-Penicillamine; Pharmaceutical formulation; Sodium 1,2-naphthoquinone-4-sulfonic (NQS)

Introduction

The D-Penicillamine, 3-mercapto-D-valine, is the characteristic acid degradation product of β -lactam antibiotics. It is a chelating agent, which is used to aid the elimination of copper in the treatment of hepatolenticular degeneration (Wilson's disease) [1]. It has been also used in cystinuria, in heavy metal poisoning and for the treatment of rheumatoid arthritis [2].

Several methods have been reported for the analysis of D-Penicillamine in both pharmaceutical preparations and biological samples. These methods include colorimetry [3-10], fluorimetry [11], chromatography [12-19], flow injection analysis [20-22], electrophoresis [23-25], potentiometry [26], voltammetry [27-29] and NMR spectrometry [30]. Due to the lack of chromophore and or auxochrome in D-penicillamine molecule, direct spectrophotometry cannot be used for its analysis.

Most of the reported colorimetric methods are time consuming or lacking selectivity due to the problem of interference with degradation product of coloring agents [7,9]. Therefore the need for a fast, sensitive, simple and selective method is obvious, especially for routine quality control analysis of pharmaceutical products containing D-penicillamine.

NQS has been used as a color-developing reagent in the spectrophotometric determination of many pharmaceutical amines [31-39]. The applications of NQS for determination of pharmaceutical bearing amine group have recently been reviewed by Elbashir et al. [39]. The reaction between NQS and D-penicillamine has not investigated yet. Therefore, the present study was devoted to investigate the reaction between NQS and D-penicillamine, and use this color reaction in the development of simple rapid spectrophotometric method for determination of D-penicillamine in its dosage form.

Experimental

Apparatus

The spectral measurements were carried out by using a spectrophotometer model Shimadzu 1800, with quartz cells of 1cm optical path length. pH meter was used for pH measurements.

Material and reagents

D-penicillamine, sodium-1,2-naphthoquinone-4-sulfonate (NQS) solution of 0.2% (w/v) was prepared by dissolving 0.2 g in distilled water, transferred into a 100 mL volumetric flask and diluted to the mark with distilled water and mixed well. The solution was freshly prepared and protected from light during use. Buffer solution of pH 12.0 was prepared by mixing 100 mL of 0.2 M aqueous solution of sodium dihydrogen phosphate with 50 mL of 0.2 M aqueous solution of sodium hydroxide in 100 mL volumetric flask, and adjusted by pH meter.

Preparation of standard and sample solutions

Stock standard solution of D-penicillamine (1000 $\mu\text{g/mL}$): An accurately 0.1 g of D-penicillamine standard was dissolved in distilled water, transferred into a 100 mL volumetric flask and diluted to the mark with water and mixed well. This stock solution was further diluted with water to obtain working solutions in the ranges of 5-30 $\mu\text{g/mL}$.

Sample solution: Five capsules (D-penicillamine 250 mg/capsule) were weighed, and finely powdered. A portion of the power equivalent to 10 mg of the drug was weighed and dissolved in distilled water then transferred into 100 mL volumetric flask to give a solution of 100 $\mu\text{g/mL}$. The solution was completed to the mark with distilled water, shaken well, filtered and then analyzed by the following procedure.

Procedure

A 1.0 mL of 200 $\mu\text{g/mL}$ of D-penicillamine was transferred into 10 mL volumetric flask, 1.0 mL of 0.2% sodium-1,2-naphthoquinone-4-sulfonate was added and followed by 1.0 mL pH 12.0 $\text{NaH}_2\text{PO}_4^-$

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NaOH buffer solution. The reaction was completed to volume with distilled water, and the resulting solution was measured at 452 nm against reagent blank treated similarly.

Job's method

The Job's method of continuous variation [40] was employed. Master equimolar (1×10^{-3} M) aqueous solution of D-penicillamine and NQS were prepared. Series of 10 mL portions of the master solution of D-penicillamine and NQS were made up comprising different complementary proportions (1:9, ... 9:1, inclusive) in 10 mL volumetric flask containing 1 ml of buffer solution (pH=12.0). The solution was further manipulated as described under the general recommended procedures.

Results and Discussion

Absorption spectra

The absorption spectrum of D-penicillamine was recorded against water (Figure 1), it was found that D-penicillamine exhibits a maximum absorption peak (λ_{max}) at 198 nm. Because of highly blue shifted λ_{max} of D-penicillamine, its determination in the dosage form based on the direct measurement of its absorption for ultraviolet is susceptible to potential interferences from the common excipients. Therefore, derivatization of D-penicillamine red-shifted light-absorbing derivative was necessary. The reaction between D-penicillamine and NQS was performed, and the absorption spectrum of the product was recorded against reagent blank (Figure 1). It was found that the product is brown colored exhibiting λ_{max} at 452 nm, and the λ_{max} of NQS was 362 nm. The λ_{max} of D-penicillamine-NQS derivative was red-shifted, eliminating any potential interference. Therefore, the measurements were carried out at 452 nm.

Optimization of variables

Effect of pH: The effect of pH on the reaction between D-penicillamine and NQS was examined by varying pH from 6.0 to 13.5. As shown in figure 2, the absorbance of the product is low at pH 6.0, indicate that D-penicillamine has difficulty to react with (NQS) in acidic media. This was possibly due to the existence of the amino group of D-penicillamine in the form of hydrochloride salt, thus it loses its nucleophilic substitution capability.

As the pH increased, the readings increased rapidly, as the amino

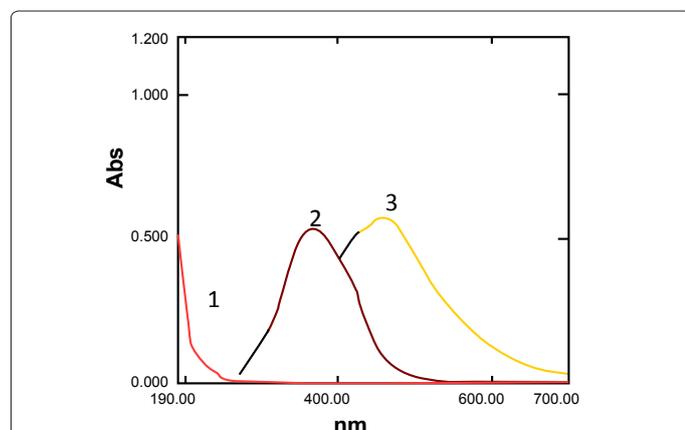


Figure 1: Absorption spectra of D-penicillamine (20 µg/mL) against water line (1), NQS (0.2%, w/v) against water line (2), and the reaction product of D-penicillamine (20 µg/mL) with NQS against reagent blank line (3).

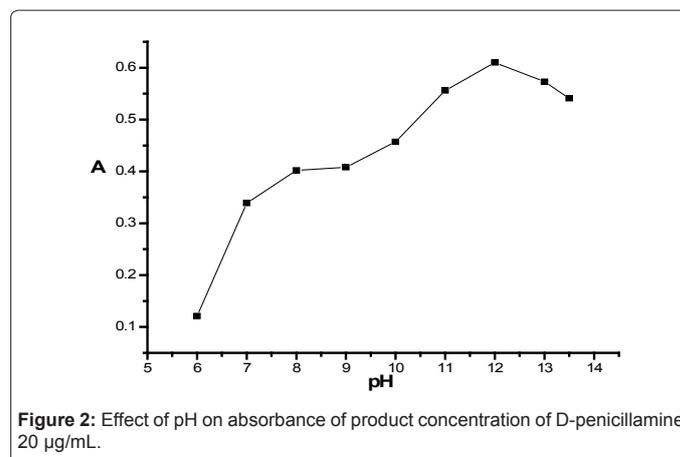


Figure 2: Effect of pH on absorbance of product concentration of D-penicillamine 20 µg/mL.

group of D-penicillamine turns into the free amino group, thus facilitating the nucleophilic substitution. The maximum readings were attained at pH values of 12.0. At pH values more than 12.0 decrease in the readings occurred. This was attributed probably to the increase in the amount of hydroxide ion that holds back the reaction of D-penicillamine with NQS.

Effect of temperature and standing time: The absorbance of product I was determined at different time (Figure 3). Keeping other conditions unchanged, the absorbance of the product I was measured after standing for different time periods at 25°C. The results show that D-penicillamine react with NQS at 25°C and the absorbance begins to increase instantly and becomes constant after 20 min. Furthermore, it is also observed that the absorbance remain constant for 30 min.

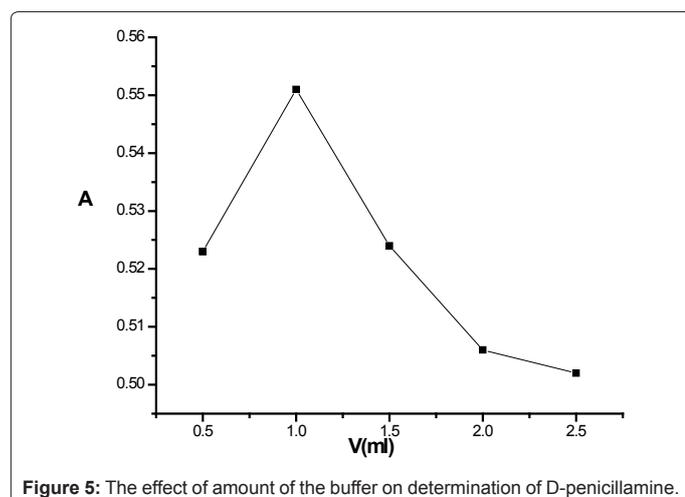
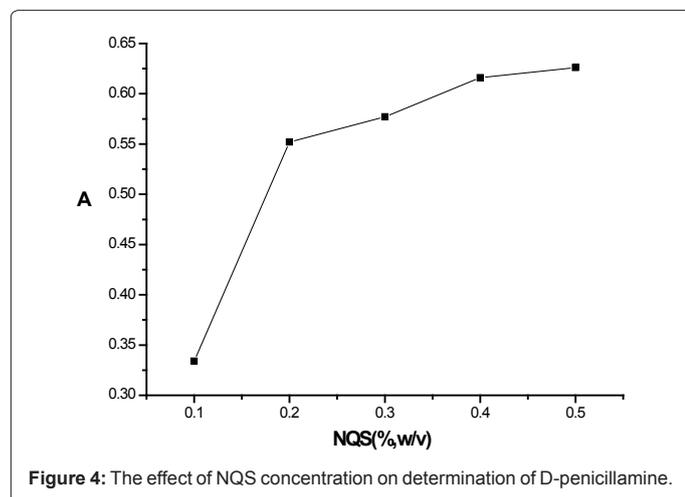
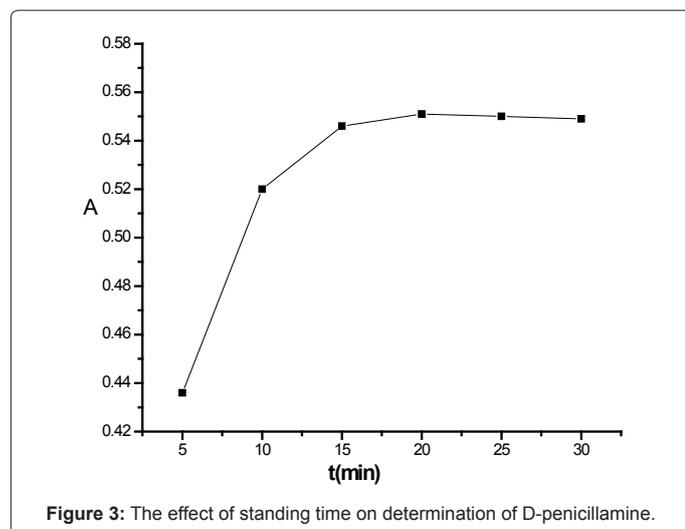
Effect of NQS concentration: The studying of NQS concentrations revealed that the reaction was dependent on NQS reagent. The highest absorption intensity was attained at NQS concentration of 0.4% (w/v), and higher concentration of NQS (0.5%, w/v) had no effect on the absorption values, as shown in figure 4.

Effect of amount of the buffer: Keeping pH at 12.0, the effect of amount of buffer solution on the absorbance of product I was also studied. It shows that the absorbance of product I enhances rapidly with the rise of amount of buffer solution, and becomes maximal when the amount of buffer solution is 1.0 mL. Therefore, the amount of 1.0 mL buffer solution was selected to ensure the highest absorbance of product I, as shown in figure 5.

Validation of the method

Calibration curve: Calibration curve for the determination of D-penicillamine by its reaction with NQS was constructed by plotting the absorbance's as a function of the corresponding concentrations. The regression equation for the results was $A=0.1684+0.01624C$ ($r=0.997$), where A is the absorbance at 452 nm, C is the concentration of D-penicillamine in µg/mL in the range of 10-30 µg/mL, and r is correlation coefficient. The limit of detection (LOD) and limit of quantification (LOQ) were determined according to the following formula $LOD=3.3 \times S_{Da}/b$, and $LOQ=10 \times S_{Da}/b$, S_{Da} is the standard deviation of intercept, b is the slope. The LOD and LOQ were found to be 3.12 and 9.44 µg/mL, respectively, (Table 1).

Robustness: Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others



were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were recorded in table 2. This indicated the reliability of the proposed method to routine application for the analysis of D-penicillamine.

Parameter	Value
Measurement wavelength(nm)	452
Linear range (µg/mL)	10-30
Intercept	0.1682
Standard deviation of the intercept	0.0153
Slope	0.0162
Standard deviation of the slope	0.0007
Correlation coefficient (r)	0.9970
Limit of detection, LOD (µg/mL)	3.12
Limit of quantification, LOQ (µg/mL)	9.44

Table 1: Parameters for the performance of the proposed method.

Parameters	Recovery (% ± SD) ^a
Recommended conditions	104.6 ± 0.01
NQS concentration (% w/v)	
0.15	93.3 ± 0.013
0.25	96.7 ± 0.01
Buffer solution (pH)	
11.8	93.8 ± 0.005
12.2	104.8 ± 0.049
Reaction time (min)	
15	106.3 ± 0.013
25	105.7 ± 0.002

^aValues are mean of three determination.

Table 2: Influence of small variation in the assay condition on the analytical performance of the proposed spectrophotometric method for determination of D-penicillamine using NQS reagent.

Taken µg (drug) D-penicillamine	Added µg (standard)	Found µg	Recovery (% ± SD) ^a
10	2	12.46	103.83 ± 0.01
10	8	19.48	108.2 ± 0.006
10	18	27.78	99.21 ± 0.005

^aValues are mean of three determination

Table 3: Determination of D-penicillamine in pharmaceutical preparation, applying standard addition method.

Dosage form	Recovery (% ± SD) ^a
D-penicillamine capsule	104.6 ± 0.01

^aValues are mean of five determination.

Table 4: Analysis of D-penicillamine containing dosage form by the proposed method.

Recovery of D-penicillamine: To check the validity of the proposed method, the standard addition method was applied by adding D-penicillamine to the previously analyzed D-penicillamine capsule solution. The recovery of each was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of analysis of pharmaceutical dosage form and the recovery study (Table 3) suggest that there is no interference from any excipients, which are present in capsules (Table 4).

Reaction mechanism

It has been reported that NQS could react with amino group of primary amine derivative. Similarly, amino group of D-penicillamine, taking on nucleophilicity due lone electron pair of nitrogen atom, trend to attack on the electron-deficient center in NQS, namely no.4 carbon atom (3,4-C=C carbon bond conjugate with 2-C=O, as a result 4-C of NQS becomes electron lacking center). At the same time, it has been proved that the composition of product I is 1:1 of D-penicillamine and NQS (Figure 6). So it is concluded that amino group of D-penicillamine react with 4-sodium sulphonate of NQS molecule respectively, to form

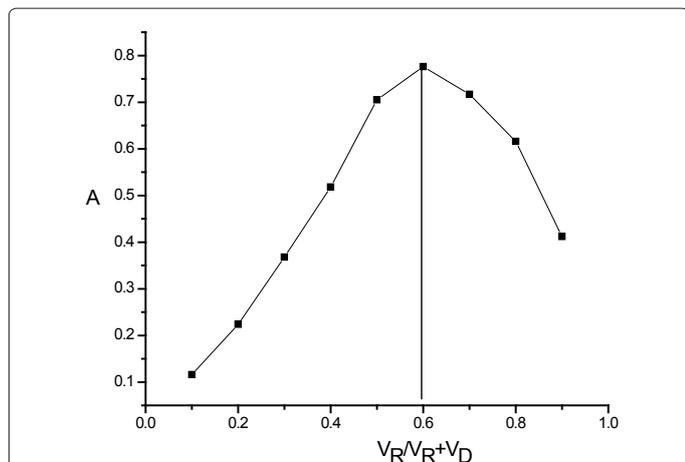
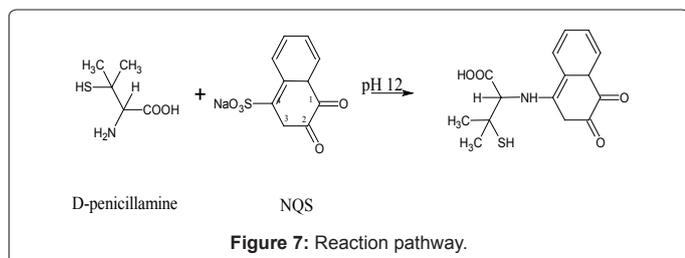


Figure 6: Determination of product I formation by continuous variation method. V_R : NQS (1×10^{-3} M); V_D : D-penicillamine (1×10^{-3} M); $V_R + V_D = 10$ mL; total volume of reaction solution 11 mL.



brown N-alkyl-amino-naphthoquinone. The reaction equation is shown in figure 7.

Application of the proposed method to analysis of D-Penicillamine in dosage form

The pharmaceutical dosage forms (D-penicillamine capsule) were subjected to the analysis of their D-penicillamine content by the proposed method. The label claim percentage was 104.6%, (Table 3).

Conclusion

The procedure presented here does not need necessitate any expensive apparatus and is simple, sensitive and rapid to be carried out. These merits make it applicable for common laboratories. Moreover, this method can be used successfully for the determination of D-D-penicillamine in pharmaceutical preparations with satisfactory results. Finally, it should be noted that, our method may be applied to the determination of other primary amine derivatives as well.

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