

# Spectrophotometric estimation of D-penicillamine in bulk and dosage forms using 2,6-dichloroquinone-4-chlorimide (DCQ)

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Received 31 March 1999; received in revised form 12 July 1999; accepted 24 July 1999

## Abstract

A simple colorimetric method for the determination of D-penicillamine in pure form and pharmaceutical formulations is described. The method is based on coupling between D-penicillamine and 2,6-dichloroquinone-4-chlorimide (DCQ) in dimethylsulphoxide. The optimum conditions for the reaction were investigated and incorporated into the procedure. The reaction forms a yellow 1:1 complex with maximum absorbance at 431 nm ( $\epsilon = 3700$ ). Regression analysis of Beer's plot showed good correlation ( $r = 0.9998$ ) and the calibration graph was rectilinear over the range 4–20  $\mu\text{g ml}^{-1}$  with a detection limit of 0.15  $\mu\text{g ml}^{-1}$ . The average recovery for the commercial capsules was 101.66% with an RSD of 1.57%. The results obtained were sufficiently accurate, reproducible and in accordance with those given by the official method. The method is specific for the intact drug, and can be adopted in the presence of some interfering substances. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* D-Penicillamine; 2,6-Dichloroquinone-4-chlorimide; Spectrophotometry; Dosage forms

## 1. Introduction

The D-penicillamine, 3-mercapto-D-valine, is the characteristic acid degradation product of  $\beta$ -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 cystiuria, 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 [3–11], fluorimetry [12], chromatography [13–21], flow injection analysis [22–24], electrophoresis [25–27], potentiometry [28,29], voltammetry [30–33] and NMR spectrometry [34,35]. Chiu and Grady [36] and Kucharczyk and Shahinian [37] reviewed other methods of analysis of D-penicillamine before 1981.

Due to the lack of chromophore and/or auxochrome in D-penicillamine molecule, direct spec-

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trophotometry can not be used for its analysis. Most of the reported colorimetric methods are time consuming [7] or lacking selectivity due to the problem of interference with degradation product of coloring agents [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.

2,6-Dichloroquinone-4-chlorimide (DCQ) has numerous applications as an analytical reagent. It has been used for the colorimetric determination of some phenolic [38,39] and thiol-containing drugs [40,41].

In view of the fact that D-penicillamine contains a thiol functional group, it was deemed useful to develop a colorimetric method using DCQ for its determination in bulk and dosage forms.

Therefore, the aim of this work was to develop a new simple spectrophotometric procedure for accurate and rapid analysis of D-penicillamine using DCQ as a coloring reagent. A significant advantage of this study is that it can be applied to the determination of D-penicillamine in the presence of penicillin and its other possible degradation products.

## 2. Experimental

### 2.1. Apparatus

A Shimadzu UV-1601 PC UV-Vis spectrophotometer with 1 cm quartz cuvettes was used.

### 2.2. Materials

A pure sample of D-penicillamine was purchased from Fluka-Chemica (Buchs, Switzerland). Artamine<sup>®</sup> capsules (Batch No. 89498) labeled to contain 50 mg of D-penicillamine were purchased from commercial sources.

### 2.3. Reagents

All chemicals were of analytical or pharmacopoeial grade. 2,6-dichloroquinone-4-chlorimide (DCQ) was obtained from Koch-Light Laborato-

ries (England), a 0.25% w/v of the reagent was freshly prepared in ethanol. Dimethylsulphoxide (DMSO) was obtained from Nentech Ltd (NTL) Brixworth-Northants (UK). Other reagents were either AnalaR or general-purpose reagents.

### 2.4. Sample preparation

A stock solution containing 0.5 mg ml<sup>-1</sup> of D-penicillamine in water was prepared and diluted further to 0.1 mg ml<sup>-1</sup> working solution (A).

### 2.5. Construction of standard curve

Aliquot volumes (1–5 ml) of solution (A) were transferred into 25 ml calibrated flasks. A volume of distilled water was added to each flask to complete the volume to 5 ml, then 15 ml of DMSO followed by 0.5 ml of DCQ solution were added. The volume was completed to mark with water and allowed to cool. The absorbance was measured at 431 nm against a reagent blank. The absorbance was plotted against the final concentration in order to obtain a calibration graph.

### 2.6. Assay procedure for capsules

The contents of 10 Artamine<sup>®</sup> capsules were accurately weighed and the mean value of the weight of one capsule was calculated. An amount of the powder equivalent to about 50 mg of D-penicillamine was accurately weighed and transferred into 100 ml volumetric flask. About 80 ml of distilled water were added and the solution was shaken for 5 min and completed to the volume with water. This solution was further diluted with water to give 0.1 mg ml<sup>-1</sup>, 4 ml of this solution were transferred into a 25 ml standard flask and 1 ml of distilled water was added; followed by 15 ml of DMSO and 0.5 ml of DCQ solution. The solution was completed to volume with water, allowed to cool and the absorbance was measured at 431 nm against a reagent blank.

The content of D-penicillamine was calculated either from the calibration graph or from the corresponding regression equation.

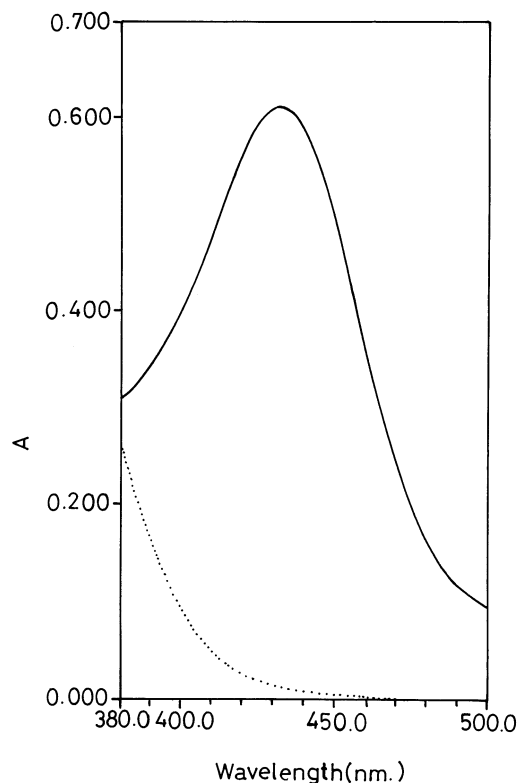


Fig. 1. Absorption spectra of D-penicillamine ( $30 \text{ mg ml}^{-1}$ ) DCQ complex (—) and reagent blank (...).

Table 1  
Effect of pH on formation, intensity and stability of the reaction product (D-penicillamine =  $30 \mu\text{g ml}^{-1}$ )

Buffer (pH)	$\lambda_{\text{max}}$ (nm)	Absorbance <sup>a</sup>	Stability for 20 min
Acetate buffer (5)	405	0.112	Stable
Citrophosphate (7)	429	0.404	Stable
0.1 M $\text{NaHCO}_3$ (8)	433	0.475	Stable
Borate buffer (9)	441	0.507	Not stable
Ammonia (10)	431 and 365	0.550	Not stable

<sup>a</sup> Average of 3 separate determinations.

## 2.7. Interferences study

Into a 100 ml volumetric flask, an amount of the powder equivalent to 10 mg of D-penicillamine was accurately weighed and mixed with each of possible interfering materials as shown in Table 4. The solution was completed to volume and the procedure was carried out as described under standard curve.

## 3. Results and discussion

D-Penicillamine contains a thiol group and is therefore expected to react with DCQ to give a colored product. Fig. 1 shows the absorption spectrum of the reaction product.

The different experimental parameters affecting the color development were extensively studied to determine the optimal conditions for the assay procedure. All conditions studied were optimized at room temperature ( $25 \pm 1^\circ\text{C}$ ). The reaction was conducted at different pH values to investigate the reaction time, color intensity, and stability. In the pH range used (from pH 5 to 9), the color was developed immediately. Maximum color intensity was obtained using either borate buffer at pH 9 or ammonia buffer at pH 10. However, the developed color in these media was unstable. The color developed with other buffers, namely, acetate (pH 5), citrophosphate (pH 7) and sodium bicarbonate (pH 8) was stable but have low absorbance values (Table 1).

The effect of different solvents, namely, acetonitrile, water, acetone, methanol, ethanol, dimethylformamide (DMF) and DMSO on the color development was studied in the presence and absence of the studied buffers. In the presence of the buffers, turbid colored solutions were obtained with these solvents. The study was then carried out in the absence of these buffers. Immediate, intense and stable color was obtained with DMF and DMSO solvents compared to the other solvents studied (Table 2). The observed heat released upon addition of DMF or DMSO to the aqueous solution of the drug (exothermic reaction) and the change of the viscosity of the solution seems to give the optimal conditions for the

Table 2

Effect of some solvents on the color formation and intensity of reaction product (D-penicillamine = 30  $\mu\text{g ml}^{-1}$ )

Solvent	Color formation	Absorbance	$\lambda\text{Max}$ (nm)	D.E. <sup>a</sup>	Density
Acetonitrile	Very slow	0.054	424	37.5	0.78
Water	Slow	0.112	419	80	1.0
Acetone	Slow	0.178	425	20.7	0.79
Methanol	Slow	0.244	427	32.7	0.79
Ethanol	Slow	0.371	428	24.6	0.80
Dimethylformamide	Immediate	0.599	431	36.7	0.95
Dimethylsulphoxide	Immediate	0.631	431	46.7	1.1

<sup>a</sup> Dielectric constants.

reaction of the drug with the reagent. This is confirmed by the finding that the sequence of addition of the reagents is essential for the maximal color intensity. Solutions were therefore allowed to cool before measuring the absorption. The least color intensity observed with acetonitrile (showing an endothermic reaction) could further confirm this finding as it has the lowest density.

The effect of the DMSO volume on color intensity is shown in Fig. 3, where the absorbance versus volume of DMSO are reported at constant penicillamine concentration (30  $\mu\text{g ml}^{-1}$ ). The volume required of DMSO was 15 ml in the 25 ml volumetric flask (60% v/v). DMF can also be used in the same % range with slight decrease in color intensity. The optimal volume and concentration for the DCQ reagent required was found to be 0.5 ml of 0.25% w/v in ethanol or 0.5 ml of 0.5% w/v in isopropanol (Fig. 4); both giving the same intensity for a 30  $\mu\text{g ml}^{-1}$  drug solution using 15 ml DMSO.

The validity of the method was tested by analyzing synthetic samples of D-penicillamine. Under the experimental conditions employed, Beer's law was found to be valid over the concentration range 4–20  $\mu\text{g ml}^{-1}$ . The molar absorbance ( $\epsilon$ ) was found to be  $3.7 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ . This value, being relatively high, confirms the sensitivity of the method. The relationship between absorbance,  $A$ , at 431 nm and concentration,  $C$ , in  $\mu\text{g ml}^{-1}$  was expressed by the following equation:  $A = 0.0069 + 0.025C$ , with a correlation coefficient of 0.9998 at 95% confidence limits indicates excellent linearity. The detection limit of the stud-

ied drug was 0.15  $\mu\text{g ml}^{-1}$ ; which represents the minimum absorbance value that can be measured. The color was stable for more than 5 h (Fig. 2)

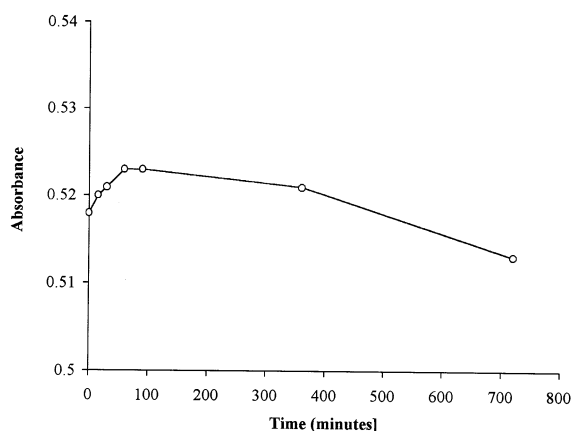


Fig. 2. The effect of time on the formation and stability of the reaction product (D-penicillamine 20  $\mu\text{g ml}^{-1}$ ).

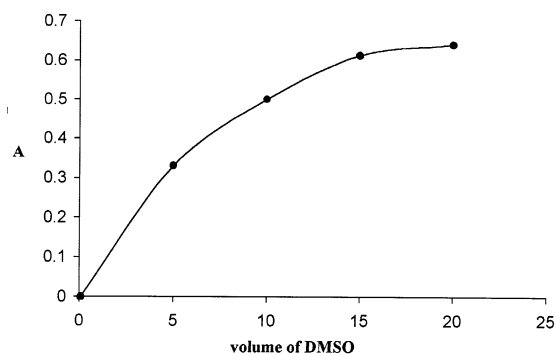


Fig. 3. Effect of the volume of DMSO on the absorbance of the reaction product (D-penicillamine 30  $\mu\text{g ml}^{-1}$ ).

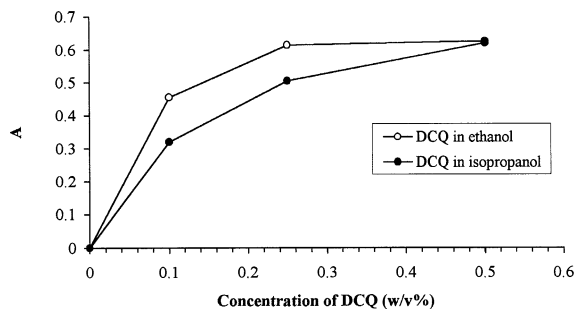
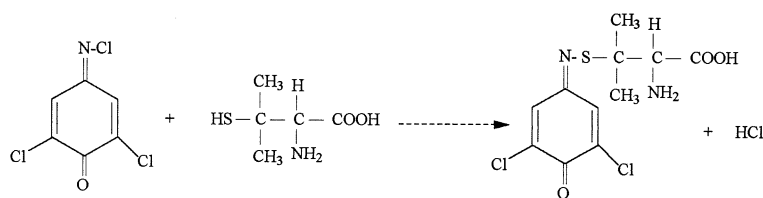


Fig. 4. Effect of the concentration of DCQ on the absorbance of the reaction product (D-penicillamine  $30 \mu\text{g ml}^{-1}$ ).

To study the stoichiometry of the reaction, the molar ratio of DCQ to D-penicillamine in the reaction mixture was determined by the molar ratio method [41] using  $4 \times 10^{-3}$  M solutions for both DCQ and D-penicillamine. The molar ratio was found to be 1:1, indicating that one molecule of D-penicillamine reacts with one molecule of DCQ. According to Karmer and Gamson [40], DCQ reacts via the chlorine atom of the chlorimide group with thiols to give a yellow to brown color of dichloroquinone sulfenimide as proposed in Scheme 1.

The appreciable value of molar absorbance ( $3.7 \times 10^3 \text{ l mol}^{-1} \text{ cm}^{-1}$ ) and the stability of the product permit the successful application of the proposed method to the determination of D-penicillamine either in pure form or in commercial



Scheme 1. Proposed reaction pathway between DCQ and D-penicillamine.

Table 3

The analysis of D-penicillamine capsules (Artamine<sup>®</sup>) by the recommended procedure and the official USP method

Recovery of proposed method	Recovery of official method	<i>t</i> -Value	<i>F</i> -Value
$101.66 \pm 1.6$ ( $n = 6$ )	$100.75 \pm 0.69$ ( $n = 5$ )	1.43 cal <sup>a</sup> (2.26 tab <sup>b</sup> )	5.38 cal <sup>a</sup> (6.26 tab <sup>b</sup> )

<sup>a</sup> Calculated *t* or *F*-value.

<sup>b</sup> Tabulated *t* or *F*-value.

Table 4

The percentage recovery of D-penicillamine in the presence of possible interfering substances

Interference	Amount added (mg) to 0.01% (w/v) D-penicillamine	% Recovery of D-penicillamine
Benzylpenicillin	20	99.5
Clavulanic acid	10	104.6
Benzathine penicillin	10	102.0
EDTA	10	77.3
Ascorbic acid	25	0.0
Sodium ampicillin	10	99.4

formulations. The results were statistically compared with those obtained by the official titrimetric method (USPXXII) [42]. Variance ratio (*F*) and *t*-tests indicate a non-significant difference with regard to precision and accuracy (Table 3). When the proposed method was applied to capsules labeled to contain 150 mg D-penicillamine, the mean percentage recovery found was  $101.66 \pm 1.6$ . The official titrimetric method gave  $100.75 \pm 0.69$  for the same capsule.

An amount of authentic D-penicillamine ( $6 \mu\text{g ml}^{-1}$ ) was added to the capsules ( $10 \mu\text{g ml}^{-1}$ ) and subsequently assayed by the proposed method. The mean percentage recovery of the added quantity was found to be  $101.13 \pm 1.13$ .

This indicates that the proposed method gives accurate results. To test for the reproducibility for the described colorimetric method, six replicate analyses were carried out for a concentration of  $16 \mu\text{g ml}^{-1}$  of D-penicillamine. The relative standard deviation (RSD) was 1.36%. This shows that the method is satisfactorily reproducible.

D-Penicillamine capsule was analyzed by HPLC on a ASI column of ODS with 1mM Na-hexanesulphonate in 50mM phosphate buffer at pH 3 [19]. The coefficient of variation was 0.16%, recovery was 100.2% and the detection limit was 40 ng. The proposed method, in comparison with HPLC method, is equally accurate and precise, less sensitive, but simpler. Although HPLC method offers high degree of specificity, yet, sample clean-up and the instrument limitations preclude their use in routine clinical studies.

Possible interferences from penicillamine capsules excipients and other structurally related degradation products were investigated under the conditions of the assay. Some substances apparently interfere with the proposed method while others do not. Table 4 shows the assay of the sample by the described method in presence of the previously mentioned ingredients. As can be seen from the table, only Vitamin C interferes with the assay. In the case of Ethylenediaminetetracetic acid (EDTA), the results indicate that with the amount of EDTA added to the preparation sample (10 mg to 0.01% w/v D-penicillamine), an error in the determination of D-penicillamine of about 33% can be expected.

In conclusion, DCQ is a suitable chromogenic reagent for the determination of D-penicillamine in pure form or in its dosage forms. The suggested method is simple, time saving (the analysis of one-sample takes about 5 min), sensitive, and reproducible. Therefore the proposed method can be used advantageously as a routine method for the determination of penicillamine in quality control and industry.

### Acknowledgements

The author gratefully acknowledges Mr. El-Rasheed A. Gad Kariem for his technical assistance.

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