

NEW SPECTROPHOTOMETRIC ESTIMATION OF PRULIFLOXACIN USING 2,4-DINITROPHENYL HYDRAZINE REAGENT

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ABSTRACT

Objective: A new, simple, and reproducible spectrophotometric analytical method was developed for the estimation of Prulifloxacin, from its tablet dosage form.

Methods: The method is based on the oxidation of (2,4-DNP) 2,4- dinitrophenyl hydrazine and coupling of the oxidized product with drug to give intensely colored chromogen. Condensed product of Prulifloxacin at its λ_{max} 480 nm shows linearity in the concentration range of 1-7 $\mu\text{g/ml}$. The results of analysis for the method have been validated statistically and by recovery studies.

Result: Linear relationship with good correlation coefficients (0.994) was found between absorbances and the corresponding concentrations of the drug. The percentage recovery ranged from 98.8% and 99.58% for this method.

Conclusion: The developed spectrophotometric method is precise, accurate, selective and sensitive.

Keywords: Prulifloxacin, 2, 4- Dinitrophenyl hydrazine (2,4-DNP), Validation, Spectrophotometric.

INTRODUCTION

Prulifloxacin¹⁻² is an older synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class. It is a prodrug which is metabolized in the body to the active compound ulifloxacin. It has been approved for the treatment of uncomplicated and complicated urinary tract infections, community-acquired respiratory tract infections and gastroenteritis, including infectious diarrhoeas. The chemical name of Prulifloxacin is (RS)-6-Fluoro-1-methyl-7-[4-(5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl-1-piperazinyl]-4-oxo-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylic acid, as shown in chemical structure of Prulifloxacin in figure 1.

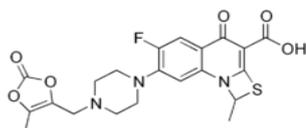


Fig. 1: Structure of Prulifloxacin.

Literature surveys have revealed a number of methods for the estimation of Prulifloxacin depending on different analytical techniques, LC-MS/MS³, RP-HPLC⁴, HPLC⁵⁻⁶, UV Spectrophotometric methods⁷⁻⁹. The present investigation is based on the oxidation of 2,4- dinitrophenyl hydrazine (2,4-DNP) and coupling of the oxidized product with drug to give intensely colored chromogen. Condensation reaction is one in which two molecules joins together with the loss of a water molecule in the process (as shown in figure 2). In terms of mechanism, this is a nucleophilic addition-elimination reaction. The 2,4 DNP first adds across the carbonyl group to give an intermediate compound which then loses a molecule of water¹⁰⁻¹¹.

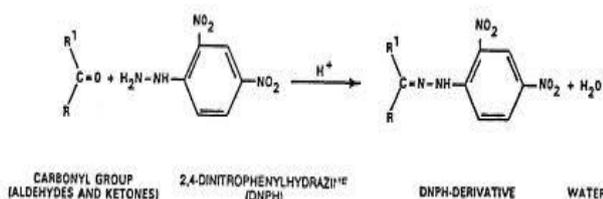


Fig. 2: General reaction of drug containing carbonyl group with 2, 4-Dinitrophenyl hydrazine.

MATERIALS AND METHODS

Instrumentation

A double beam UV/visible spectrophotometer, lab India 3000 make with 1cm quartz cells was used for the absorbance measurements. Instrument used were calibrated.

Chemicals

All the reagents used for the analysis were of analytical grade. Distilled water was used throughout the study. Standard Prulifloxacin was obtained as a gift sample from Micro labs, Bangalore. The Tablet Percin of strength 600 mg were produced from the market manufactured by Lupin Pharma.

Reagents

2, 4-Dinitrophenyl hydrazine (2, 4-DNP) 0.08 % (w/v): A 0.08% w/v of the reagent solution were freshly prepared by dissolving 0.08 g of 2, 4- DNP in 2 ml of concentrated H₂SO₄ and diluting to 100 ml with water.

10N Sodium hydroxide (NaOH) solution: 40 g of sodium hydroxide dissolve in 100 ml of distilled water.

Potassium iodate (KIO₃) 4 % (w/v): 4% w/v potassium iodate solution was prepared by dissolving 4 g in 100 ml of distilled water.

Preparation of standard stock:

Standard stock solution was prepared by accurately weighing 10mg of Prulifloxacin which was solubilised by 50 ml of Methanol and final volume was achieved with same in 100 ml of volumetric flask.

Procedure for calibration graph:

Standard solutions of Prulifloxacin in methanol, having final concentrations in the range of 1-7 $\mu\text{g/ml}$ were transferred into a series of 10 ml volumetric flasks, to these solutions 1.5 ml of 2,4-DNP (0.08%), 1.5 ml of KIO₃ and 1ml of NaOH (10 N) were added. The yellowish green colour hence developed was further diluted with water.

The absorbance of each solution was measured at 480 nm (as shown in figure 3) against the reagent blank prepared in the same manner, without the analyte.

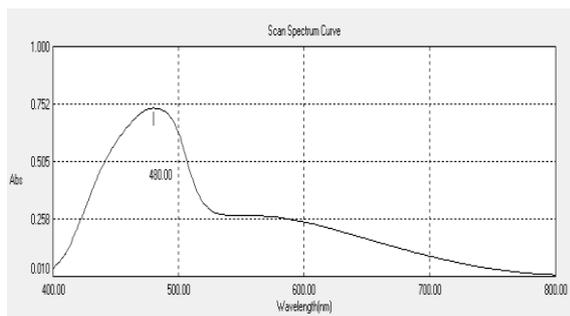


Fig. 3: Spectra of Prulifloxacin at 480 nm.

Assay of Prulifloxacin in tablet dosage form

Twenty tablets were weighed, crushed and their contents are mixed thoroughly. An accurately weighed portion of powder equivalent to the 10 mg of Prulifloxacin was weighed into a 100 ml volumetric flask containing about 50 ml of Methanol. It was shaken thoroughly for about 5-10 min, and final volume was made with the same. Filter thoroughly with whatman filter paper No. 41 to remove any insoluble matter. To the aliquot of stock solution 1.5 ml of 2, 4- DNP, 1.5 ml of KIO₃ and 1 ml of NaOH were added. The mixture was then gently shaken until the appearance of yellowish green colour. The contents were diluted up to 10 ml with distilled water.

Table 1: Optical parameters

| Parameters | Values |
|---|---------------------------|
| λ _{max} | 480 nm |
| Beers law limits | 1-7 µg/ml |
| Equation of the line | y=0.100x-0.058 |
| Correlation coefficient (R ²) | 0.994 |
| Slope (b) | 0.100 |
| Intercept (c) | -0.058 |
| Sandell's sensitivity(µg/ cm ²) | 0.0152 µg/cm ² |

y = bx + c, where x is the concentration of drug in µg/ml.

Quantification

The limits of the Beer's law and the Sandell's sensitivity values were evaluated. The Regression analysis of the Beer's law plots at λ_{max} 480 nm revealed a good correlation, and are described by the regression equation, y = bx + c (where y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and x is the concentration of the drug in µg/ml) obtained by the least-squares method. The results are summarized in Table 1.

Table 2: Result of accuracy by Recovery studies

| Conc. of sample added. (µg/ml) | Level of addition (%) | Conc. of standard added (µg/ml) | Total Conc. (µg/ml) | Absorbance at 480 nm * | Conc. obtained from graph. (µg/ml) | % recovery (%) |
|--------------------------------|-----------------------|---------------------------------|---------------------|------------------------|------------------------------------|----------------|
| 3 | 80 | 2.4 | 5.4 | 0.481 | 5.39 | 99.58 |
| | 100 | 3 | 6 | 0.539 | 5.97 | 99 |
| | 120 | 3.6 | 6.6 | 0.598 | 6.56 | 98.8 |
| | | | | | Mean | 99.12 |
| | | | | | SD | 0.330 |
| | | | | | %RSD | 0.33 |

*Average of three absorbances.

Table 3: Result of method precision

| Conc. of solution (µg/ml) | Absorbance | | | Average | %RSD (%) | Conc. from graph (µg/ml) |
|---------------------------|------------|-------|-------|---------|----------|--------------------------|
| | Day 1 | Day 2 | Day 3 | | | |
| 2 | 0.131 | 0.135 | 0.136 | 0.134 | 1.6 | 1.92 |
| 6 | 0.545 | 0.536 | 0.549 | 0.543 | 1 | 6.01 |
| 7 | 0.633 | 0.636 | 0.638 | 0.635 | 0.34 | 6.93 |

VALIDATION ¹¹

Linearity and range

Linearity plot is shown in figure 4. The response for Prulifloxacin was linear in the concentration range of 1- 7 µg/ml. The regression equation calculated by least square method was y = 0.100x - 0.058 with coefficient of correlation R² = 0.994.

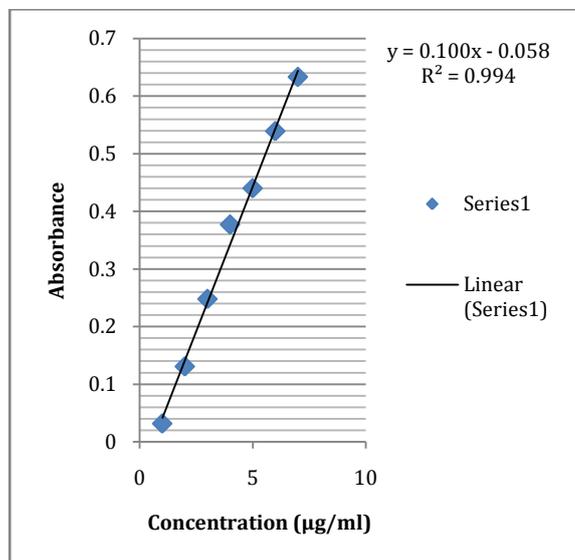


Fig. 4: Calibration curve of Prulifloxacin.

Accuracy

Accuracy studies were done as percent recovery; it was performed by adding constant amount of the standard drug to the sample at levels of 80%, 100% and 120% of the test concentration. The results are tabulated in Table no 2.

The % recovery of the added pure drug was calculated as

$$\% \text{ recovery} = [(C_t - C_s) / C_a] \times 100,$$

Where

C_t is the total drug concentration measured after standard addition;

C_s is drug concentration in the formulation sample;

C_a is drug concentration added to formulation.

Precision

1. Method precision: Data obtain from precision experiments are given in Table no 3. Precision was calculated for Interday.

The data obtained shows that method is sufficiently precise. Precision is calculated as % Relative Standard Deviation.

2. Repeatability: The repeatability of the proposed methods was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method. The results are given in Table no 4.

RESULTS AND DISCUSSION

The absorption spectra of the reaction product of oxidized 2,4-DNP with drug show maximum absorption (λ_{max}) at 480 nm. The blank solution was slightly yellowish in colour. Thus formed colour was stable for around two hours. The 2, 4- DNP is oxidized by KIO_3 to

give diazonium cation which reacts with drug by a nucleophilic addition- elimination reaction to give coloured chromogen. Beer's law is obeyed in the range of 1-7 $\mu\text{g/ml}$ for Prulifloxacin.

Optimization of parameter

The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance. By varying one and keeping other experimental parameters and the amount of drug constant, the effect of 2, 4-DNP, oxidizing agents and sodium hydroxide were studied. Different concentrations and different volumes were tried for the reagents, by varying one parameter at a time.

For the method it was found that optimum concentration of KIO_3 was 4% w/v and optimum concentration of 2, 4-DNP was 0.08% w/v and optimum concentration of NaOH was 10N. The optimum volume was found to be 1.5 ml for KIO_3 and that of 2, 4-DNP was 1.5 ml and NaOH was 1 ml.

Table 4: Result of Repeatability.

| Conc ($\mu\text{g/ml}$) | Absorbance at 480 nm | Conc from graph ($\mu\text{g/ml}$) |
|---------------------------|----------------------|--------------------------------------|
| 3 $\mu\text{g/ml}$ | 0.246 | 3.02 $\mu\text{g/ml}$ |
| | 0.246 | |
| | 0.248 | |
| | 0.245 | |
| | 0.238 | |
| | Mean: 0.244 | |
| | SD: 0.00349 | |
| | RSD:1.43% | |

CONCLUSION

The reagents utilized in the proposed method are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the method is free from interference by common additives and excipients. The wide applicability of the new procedure for routine quality control was well established by the assay of Prulifloxacin in pure form and in pharmaceutical preparations.

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