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A NEW RP-HPLC ASSAY METHOD FOR DETERMINATION AND QUANTITATION OF NITROFURANTOIN API

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ABSTRACT

A simple, specific and precise RP-HPLC method was developed and validated for determination of Assay for Nitrofurantoin Active Pharmaceutical ingredient (API). The method validation was performed as per ICH Q2 (R1) and FDA guidelines. The final chromatographic condition comprises of HPLC column i.e. Ascentis Express C18 (dimension 7.5cm x 4.6mm and particle size 2.7µm) and mobile phase containing a mixture of 0.1% Triethylamine pH-3.0 and Acetonitrile in the ratio of 80:20 v/v. The flow rate and column temperature were constantly maintained at 1.0 ml/min and at 30ºC respectively. The detection was performed at wavelength 254 nm using PDA detector. The retention time obtained for Nitrofurantoin peak was about 2.0 minutes. There was no interference due to the mobile phase or diluent was observed at retention time of Nitrofurantoin peak. Also, the data of linear regression analysis shows a linear relationship of Nitrofurantoin over the concentration range of 50-150µg/mL and the correlation coefficient value obtained was 0.9999. The Analytical method validation data shows that the method was Specific, Linear, Precise and Robust for the Assay determination of Nitrofurantoin API.

Keywords – *Nitrofurantoin, API, ICH guideline, Acetonitrile, RP-HPLC*

1. INTRODUCTION

Nitrofurantoin belongs to the class of organic compounds known as hydantoins [1,2]. Chemical name of Nitrofurantoin is 1-[(Z)-[(5-nitrofuran-2-ethylidene] amino] imidazolidine-2,4-dione. Molecular formula is $C_8H_6N_4O_5$ and molecular weight is 238.15 Da. These are heterocyclic compounds containing an imidazolidine substituted by ketone group at positions 2 and 4. Nitrofurantoin exhibits bacteriostatic or bactericidal effects by inhibiting the synthesis of DNA, RNA, protein and cell wall synthesis.

Nitrofurantoin is active against some gram-positive organisms such as *S. aureus, S. epidermidis, S. saprophyticus, Enterococcus faecalis*, *S. agalactiae*, group *D streptococci*, viridians streptococci and Corynebacterium. Its spectrum of activity against gram negative organisms includes *E. coli, Enterobacter, Neisseria, Salmonella* and *Shigella.* It may be used as an alternative to trimethoprim/sulfamethoxazole for treating urinary tract infections though it may be less effective at eradicating vaginal bacteria. May also be used in females as prophylaxis against recurrent cystitis related to coitus. Nitrofurantoin is highly stable to the development of bacterial resistance, a property thought to be due to its multiplicity of mechanisms of action.

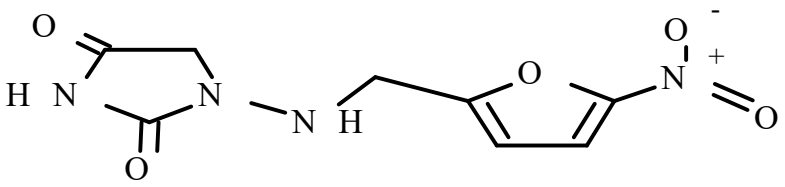


Fig.1: Chemical Structure[3]

The HPLC method for assay determination of Nitrofurantoin is official in USP, BP, IP and also several methods have been reported in literatures which includes a variety of analytical techniques such as Derivative spectrophotometry[3-12], absorbance measures to diverse wavelengths and combination of the measured values, or multivariate analysis, HPLC, have been reported to characterize the Nitrofurantoin[13-24].

These stability indicating analytical methods (SIAM) are validated for assay of Nitrofurantoin, and not for analyzing the drug in the presence of its known impurities. Since there are no methods available with short run time for Assay determination, an attempt was made to develop a simple, rapid, RP-HPLC method for the estimation of Nitrofurantoin.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Working standard and Nitrofurantoin sample were received as gift from KP Labs Hyderabad, HPLC Grade water was obtained from Mill-Q Water system (Millipore), Acetonitrile HPLC grade from (Ranbaxy), Triethylamine (S.D fine), Ortho Phosphoric acid (S.D. fine), Nylon membrane filters (0.45 µm)

2.2 Equipments

Chromatographic separation was achieved by using HPLC System (Agilent 1100 series) having PDA detector. The software used was Chemstation Software® for capturing and processing chromatographic/Analytical data.

2.3 Selection of UV Wavelength

Structure determination of Active pharmaceutical ingredient confirmed the presence of multiple number of chromophores e.g. unsaturated double bonds as part of Heterocyclic ring as well as presence of Carbonyl group. Also, as reported in literature the UV max for nitrofurantoin is 254nm which was employed for quantification by using HPLC with UV detector.

2.4 HPLC instrumentation and Analytical Conditions

The proposed method involves use of HPLC column Ascentis Express C18 having dimension 7.5cm x 4.6mm and particle size 2.7µ. The mobile phase consists mixture of 0.1%Triethylamine pH-3.0 (Previously adjusted with orthophosphoric acid) and Acetonitrile in the ratio of 80:20 v/v. The flow rate and column temperature were constantly maintained at 1.0 ml/min and 30ºC respectively throughout the experiment. The detection was performed at wavelength 254 nm using PDA detector. Injection volume was kept 5 µL.

2.5 Preparation of solutions

Standard and sample solution were prepared in mobile phase and injected on the HPLC system.

2.5.1 Standard solution: Weighed accurately about 20 mg of Nitrofurantoin standard into a 20 ml volumetric flask, added about 10 mL of mobile phase and sonicated to dissolve. Allowed the solution to attain the room temperature and finally diluted up to mark with mobile phase. (1000 ppm)

Further 1 ml of above solution was diluted to 10 ml with mobile phase. (100 ppm)

2.5.2 Sample solution: Weighed accurately about 20 mg of Nitrofurantoin sample into a 20 ml volumetric flask, added about 10 mL of mobile phase and sonicated to dissolve, Allowed the solution to attain the room temperature and finally diluted up to mark with mobile phase. (1000 ppm)

Further 1 ml of above solution was diluted to 10 ml with mobile phase. (100 ppm)

2.5.3 Calculation:

The percentage of Nitrofurantoin in the portion of sample taken is calculated by using below formula,

$$\% \text{ Assay} = \frac{\text{Sample Area} \times \text{Standard dilution factor} \times \text{Purity of standard} \times 100}{\text{Average standard Area} \times \text{Sample dilution factor} \times 100}$$

3. RESULTS AND DISCUSION

3.1 Method development

The initial development was started with simple mobile phase comprising of water and Methanol in the ratio of 80:20 v/v and Inerstil C18, 25cm x 4.6mm, 5 µm was selected. The flow rate was set at 1.5ml/ min and 10 µL of 50 ppm Nitrofurantoin standard (prepared in Acetonitrile) and injected on the chromatographic system. The broad peak of nitrofurantoin was observed at about 18 minutes. So various trails were taken to improve the peak shape and to reduce the retention time for Nitrofurantoin like a) Changing the column to Hypersil C18 ,25cm x 4.6mm, 5µ b) by modifying the diluent to Acetonitrile: Water 25:75 v/v, c) Varying the flow rate of mobile phase, d) Changing the column temperature etc.

Further mobile phase was modified to 0.1%TEA whose pH was adjusted to 3.0 with Ortho Phosphoric acid to improve the peak shape. A broad peak shape was observed with high retention time (About 14 minutes). To further optimize the peak broadening, a mixture of Acetonitrile and buffer (0.1% TEA pH 3.0) was employed in the mobile phase, comparatively a sharp peak was observed at about 8 minutes. Since attempt was taken for assay method development method for shorter run time the conventional column was replaced with new generation column having fused core technique. So Ascentis Express C18 column with dimension of 7.5cm x4.6 mm, 2.7 µ was used. The peak shape obtained after the usage of new column shows considerable improvement in the peak shape and in retention time.

3.2 Method validation [11]

The developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines, Validation of Analytical Procedures: Q2(R1)[7], for the parameters like system suitability, Specificity, linearity and range, precision (repeatability) , Intermediate precision (ruggedness) and robustness.

3.3 System suitability

The system suitability test performed according to USP39. The standard solution was injected six times and results were recorded to find the adequate peak shape, percentage relative standard deviation for area and retention time, peak asymmetry and theoretical plates. The results obtained were compiled in Table-1.

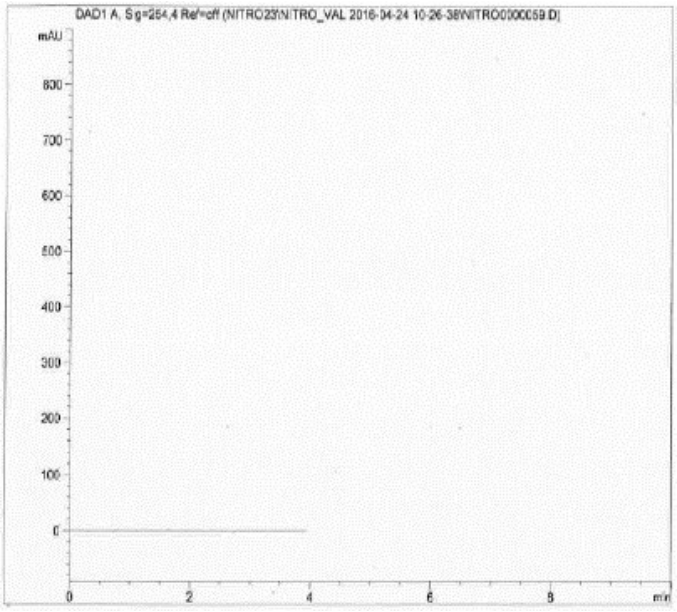
Table 1: System suitability results

Parameter	Observed value	Acceptance Limit
% RSD for Area for replicate injection of standard solution	0.2	**NMT 2.0%**
Theoretical plates	3883	**NLT 2000**
Tailing factor	1.2	**Should be between 0.8 to 2.0**

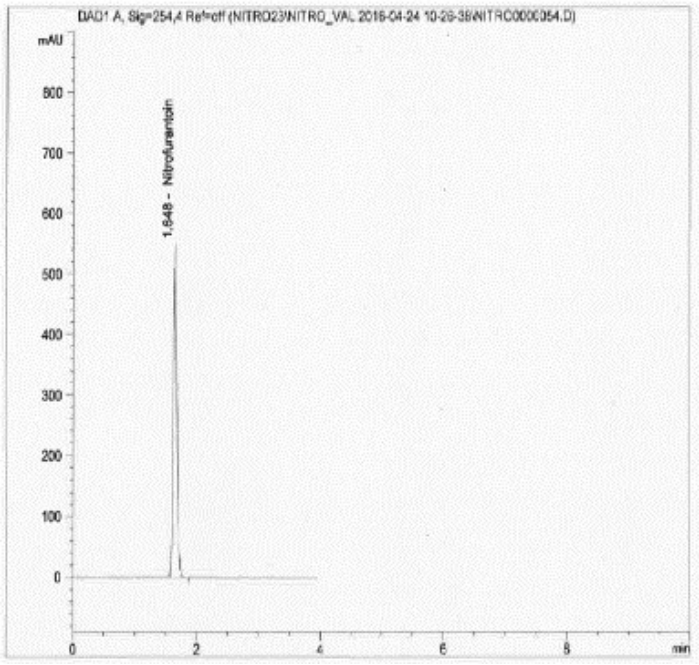
The percentage Relative standard deviation (% RSD) for Area, Tailing factor and Theoretical plates for analyte peaks were within the acceptance limit which shows that the method has good system suitability.

3.4 Specificity

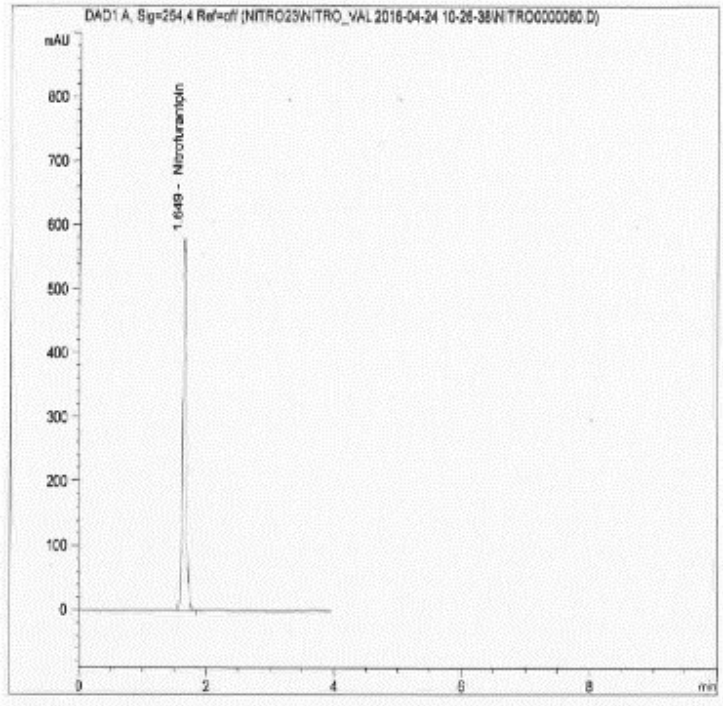
Specificity was performed to detect the presence of interference peak at the retention time of the analyte peak. The specificity of the method was checked by comparison of chromatograms obtained from test sample solution and the blank solution. The representative chromatogram shown in figure-2. No interference at retention time of analyte peak due to blank was observed.



(a) Blank Chromatogram



(b) Standard Chromatogram



(c)Sample Chromatogram

Fig. 2: A typical chromatogram of Blank, Standard and Sample solution for Assay determination

3.5 Precision

3.5.1 Repeatability and Ruggedness (Intermediate Precision)

Method precision was evaluated by carrying out six different test sample solution preparation. Intermediate precision was performed on different day in the same laboratory by carrying out six different test sample solution preparation. The assay of these samples was determined. Precision and intermediate precision of the method was evaluated by calculating the %RSD. The values were given in Table 2.

Table 2: Precision and Intermediate Precision results

Parameter	Precision (Day -1)	Intermediate Precision (Day-2)	Average for Precision (Day-1 and Day-2)	% RSD for Precision (Day-1 and Day-2)	Acceptance Criteria
Average % Assay	100.1	100.0	100.05	0.65	%RSD should not be more than 2.0% for day-1 and day-2.
% RSD	0.59	0.71			

The percentage Relative standard deviation (%RSD) obtained was found to be less than 2 .0 % for day-1 and day-2.

3.6 Linearity and Range

The linearity of detector response was determined by preparing a series of solution of the working standards over the range of 50 to 150 % of specification level targeted concentration. These solutions were injected onto the chromatographic system and response area were recorded. Calibration curve was constructed by plotting area against concentration and regression equation was computed. The

linearity plots with values were shown in Figure 3. The results obtained for correlation coefficient and % y-intercept were within acceptance limit.

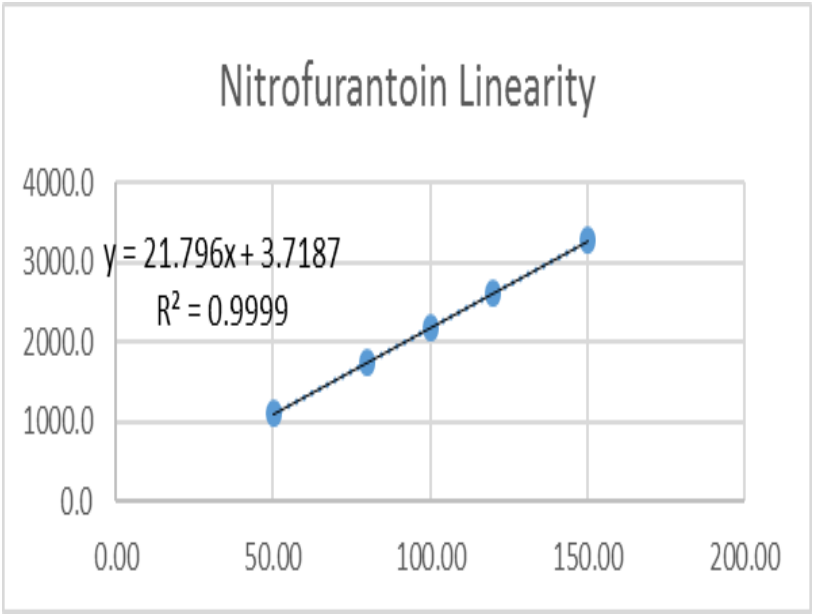


Fig. 3: Calibration curve for Nitrofurantoin showing the Linearity

Table 3: Linearity study results

Parameter	Observed value	Acceptance Limit
Correlation coefficient	0.9999	0.999
% y-Intercept	0.2	Should be between ± 2 %.

3.7 Robustness: Effect of variation in Column temperature and pH of Mobile Phase Buffer

To study robustness of the test method, small, deliberate changes were made to the chromatographic condition. A study was performed by changing the column temperature and pH of Mobile Phase Buffer. Standard solution prepared as per the test method and was injected into the HPLC system at 25°C and 35°C temperature. The pH of mobile phase buffer was altered to 2.5 and 3.5. The system suitability parameters were evaluated on the modified conditions.

By performing deliberate changes in pH and column temperature, no significant impact on the system suitability test parameter was observed which demonstrates robustness of the method.

4. CONCLUSION

The RP-HPLC method for Assay determination of Nitrofurantoin is simple, rapid, specific, linear, precise and robust. The developed method has short run time which results in less solvents consumption, low amount of waste generated, maximum utilization of HPLC instrument and low cost per analysis. The shorter run time will also help in the analysis of multiple batches in short duration thus increasing the output of batch analysis.

The HPLC method was validated as per ICH Q2 (R1) guidelines, shows satisfactory data. Hence, the proposed method can be used for assessing the Assay determination of Nitrofurantoin for Routine and R& D analysis.

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