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ANALYSIS OF NITROFURATOIN IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORMS BY HPLC METHOD

G.Chandra Sekhar*, B.Satyanarayana, P.Sugunab, B.Narasimhulub
[*] Department of Chemistry,Govt. degree &PG college, Puttur;A.P; India
SGS Arts college,TTD,Tirupati, A.P; India

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ABSTRACT

A simple, economic, selective, precise, and accurate High Performance liquid Chromatographic method for the analysis of Nitrofuratoin in bulk drug and pharmaceutical formulations was developed and validated in the present study. The mobile phase consists of a mixture of Acetonitrile and buffer 88:12. And adjust the pH to 5.0 ± 0.05 with sodium hydroxide solution. This was found to give a sharp peak of Nitrofuratoin at a retention time of 8.423 min. HPLC analysis of Nitrofuratoin was carried out at a wavelength of 254 nm with a flow rate of 1.6 mL/min. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient of 0.999 in the concentration range of 50 to 150 µg ml^{-1}. The linear regression equation was y =53.4120x-168.66. The developed method was employed with a high degree of precision and accuracy for the analysis of Nitrofuratoin. The developed method was validated for accuracy, precision, robustness, detection and quantification limits as per the ICH guidelines. The wide linearity range, accuracy, sensitivity, short retention time and composition of the mobile phase indicated that this method is better for the quantification of Nitrofuratoin.

KEYWORDS: Nitrofuratoin. HPLC. Validation.

INTRODUCTION

Several materials have been tested toward this aim, including solid amalgam electrodes (SAE), carbon paste electrodes (CPE), nanotube carbon paste electrodes (NTCPE), glassy carbon electrodes (GCE), solid composite electrodes (SCE) and boron-doped diamond film electrodes (BDDFE). Several reviews illustrating the use of these materials in the electroanalytical field have been published in recent years [1], [2], [3], [4],[5], [6], [7], [8], [9], [10] and [11].

Among these materials, BDDFE has attracted the attention of electroanalytical researchers as a candidate for the electrochemical determination of pharmaceutical compounds, since BDDFE presents a stable background current, wide potential window for aqueous and non-aqueous electrolytes, high thermal conductivity, high hardness, high electrochemical stability, low organic molecule adsorption and high sensitivity for analytical purposes [5], [8], [9], [12], [13] and [14].

However, little attention has been given in the literature concerning the influence of different boron-doping levels on the electrochemical response of the pharmaceutical compounds. In addition, some reports have been published showing that nitrofurazone, which is in the group of nitrofuran antibacterial agents, has a good electrochemical response on the BDDFE [15] and [16]. This enables the application of this electrode for the electrochemical detection and quantification of compounds belonging to the nitrofuran group. Among them, nitrofurantoin [NFT; 1-((5-nitro-2-furfurylidene)-1-amino)hydantoin] is a drug synthesized from nitrofuran that is very useful in the treatment of urinary tract diseases. Depending on its concentration at the inflammation site, this drug may act as a bacteriostatic or bactericidal agent [17]. As pointed out by Hamman [17], this drug can act against various gram negative bacteria, as well as some gram-positive bacteria, including *Citrobacter*, *Corynebacterium*, *Enterobacter*, *Escherichiacoli*, *Klebsiella*,*Neisseria*, *Salmonella*, *Staph ylococcusaureus* and *Enterococcus faecalis*. It is efficient against these bacterias in the concentration range of 1–32 µg mL^{-1}. However, this drug is partially metabolized, and 24 h after the application of a single oral dose, 30–

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50% of this drug is excreted in its original form and 1% is excreted as aminofurantoin in the urine [17]. Thus, this drug can become a water contaminant, which can be dangerous for human health.

Several non-electrochemical techniques have been reported in the literature for the detection of NFT. Colorimetric and spectrophotometric methods have been used since the 1960s [18], [19] and [20], and the use of high performance liquid chromatography (HPLC) began in the 1970s [21] and [22]. However, according to Jain et al. [23], the colorimetric and spectrophotometric techniques do not offer a satisfactory quantification limit for the determination of this drug. In addition, the chromatographic methods require pre-treatment and extraction stages for the sample, which demand a great amount of time and increase the cost of the analysis. These stages also increase the exposure time of the drug to light, which can promote the partial photochemical degradation of the compound.

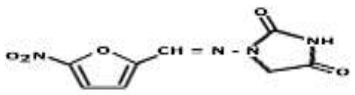
On the other hand, electroanalytical techniques are increasingly used to detect organic compounds. These techniques have several advantages, including that they are quick and reproducible, present low limits of detection and quantification and have relatively low cost compared with the more traditional techniques [8]. The two initial reports about the use of electroanalytical techniques to determine NFT came from the sixties and seventies years, when Jones et al. [24] and Mason and Sandmann [25] reported the use of the polarographic method and the reductive voltammetric method using a rotating platinum electrode, respectively. Recently, three reports have been published that demonstrate the use of square wave cathodic adsorptive stripping voltammetry for the detection on mercury electrodes [17] and [23] and on activated carbon fiber microelectrodes [26]. In these works, this technique was shown to be a very sensitive and selective methodology for NFT analysis [17], [23] and [26].

Resolution of ternary mixtures of nitrofurantoin, furaltadone and furazolidone by partial least-square analysis to the spectrophotometric signals after photo-decomposition
M.C Mahedero· et al2002. Department of Analytical Chemistry, University of Extremadura, 06071 Badajoz, Spain. İ. M. Palabıyık[a] & F. Onur[a*] Liquid Chromatographic and Spectrophotometric Determination of Phenazopyridine Hydrochloride, Ampicilline Trihydrate, and Nitrofurantoine in Pharmaceutical Preparations - 2007. Essam Hammamet al Determination of nitrofurantoin drug in pharmaceutical formulation and biological fluids by square-wave cathodic adsorptive stripping voltammetry (Department of Chemistry, Faculty of Science, Tanta University, 31527 Tanta, Egypt). Received 15 January 2002.

Stability of Nitrofurantoin in Extemporaneously Compounded Suspensions *Mary H.H. Ensomet al* **Differential Effects of Chrysin on Nitrofurantoin Pharmacokinetics Mediated by Intestinal Breast Cancer Resistance Protein in Rats and Mice Atsushi Kawase, etal.** Department of Pharmacy, School of Pharmacy, Kinki University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502,2010.

The empirical formula for Nitrofuratoin is $C_8H_6N_4O_5$ and the molecular weight is238.16 g. It has the following structure.

Figure1.



The HPLC method described here is simple, sensitive, and reproducible for Nitrofuratoin determination in Formulations with low background interference. An attempt has been made to develop and validate to ensure their accuracy, precision and other analytical method validation parameters as mentioned in various gradients. One method reported for the HPLC determination for developed based on the use of a C-18 column, with a suitable mobile phase, without the use of any internal standard. For pharmaceutical formulation the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations.

Instrumentation
HPLC Analytical column ZORBAX Eclipse XDB - C18, 150mm x 4.6mm x 5µ
Table-1.1: Chromatographic conditions of Nitrofuratoin

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Stationary phase	Mobile phase	Flow rate (ml/min)	Run time (min)	Column Temp ($0^{c)}$	Volume of injection loop(µl)	Detection wavelength (nm)	Retention time (min)
ZORBAX Eclipse XDB - C18	Acetonitrile and buffer 88:12	1.6	15	25	20	254	8.244

ANALYTICAL METHODOLOGY

Preparation of Mobile phase

For isocratic system, prepare a mixture of Acetonitrile and buffer in the proportion 88:12 respectively. Mix well, adjust the pH to 7.0 ± 0.05 with sodium hydroxide pellets. Filter through 0.2 µ Nylon membrane filter paper and degas prior to use.

Preparation of Buffer

Weigh accurately and transfer 6.8 g of Potassium dihydrogen orthophosphate to a 1000 ml volumetric flask. Add about 980 ml of water, dissolve and dilute to volume with water.

Chromatographic conditions

Separation was performed on ZORBAX Eclipse XDB - C18, 150mm x 4.6mm x 5µ Column. Methanol used as a Diluent and Mobile phase consists of mixture of Buffer, **Buffer** and Acetonitrile in the proportion 88:12. Injection volume of 20 µl was used. Mobile phase was filtered before use through 0.5 µm Nylon membrane filter paper and degassed with helium purge for 15 min. The components of the mobile phase were pumped from solvent reservoir to the column at flow rate 1.6 ml/min and wavelength was set to 254 nm. The column temperature was set at 25ºC.

Preparation of Nitrofuratoin Standard Solution: (pure sample)

Weighed accurately about 100 mg of **Nitrofuratoin**working standard and transferred to a 100 ml volumetric flack. Add 10 ml of diluents and sonicated to dissolve. Dilute to volume with diluents and volume was made up to the mark with diluents. i.e. 1000 µg/ml (Stock solution A)

From the above stock solution A 10 ml of solution was pipette out into 100 ml volumetric flask and the volume was made up to the mark with methanol to obtained the final concentration of 100 µg/ml (Stock solution B) From the stock solution B ranging from 5-15 ml were transferred into a series of 10 ml volumetric flasks to provide final concentration range of 50-150 µg/ml, and each flask made up to the mark with diluents.

Preparation of Test Solution :(Formulation)

Twenty tablets containing **Nitrofuratoin** were weighed and finely powered. An accurately weighed portion of the powder equivalent to 100 mg of **Nitrofuratoin** was transferred into a 100 ml volumetric flask. Added about 10 ml of diluents and shaken for 20 minutes by manually and further sonicate for 30 minutes. Diluted up to the mark with diluents. Centrifuge this solution at 8000 rpm for 10 minutes. Decanted the supernatant solution into another test tube (. i.e. 1000 µg/ml) and transferred 10 ml of supernatant solution into another 100 ml volumetric flask and make up the volume with diluents (100 µg/ml). Further transfer 5-15 ml of solution into another 10 ml volumetric flask and make up the volume with diluents. Filtered the solution through 0.45 µm Nylon membrane filter paper. (50-150 µg/ml)

Assay procedure:

The column was equilibrated for at least 30 minutes with mobile phase flowing through the system with a flow rate of 1.0 ml/min. Detector was set at a wavelength of 254 nm. Five sets of the Drug solutions were prepared in diluents containing **Nitrofuratoin** at a concentration range of 50 - 150 µg/ml. Then 20 µl of each standard and sample solution were injected for Five times separately. The retention time for **Nitrofuratoin** was found to be 8.243 min (Fig -6.15). The peak areas of the Drug concentrations were calculated. The Regression of the Drug concentration over the amount of Drug in formulation

System Suitability Solution:

Use Nitrofuratoin standard working solution as system suitability solution.

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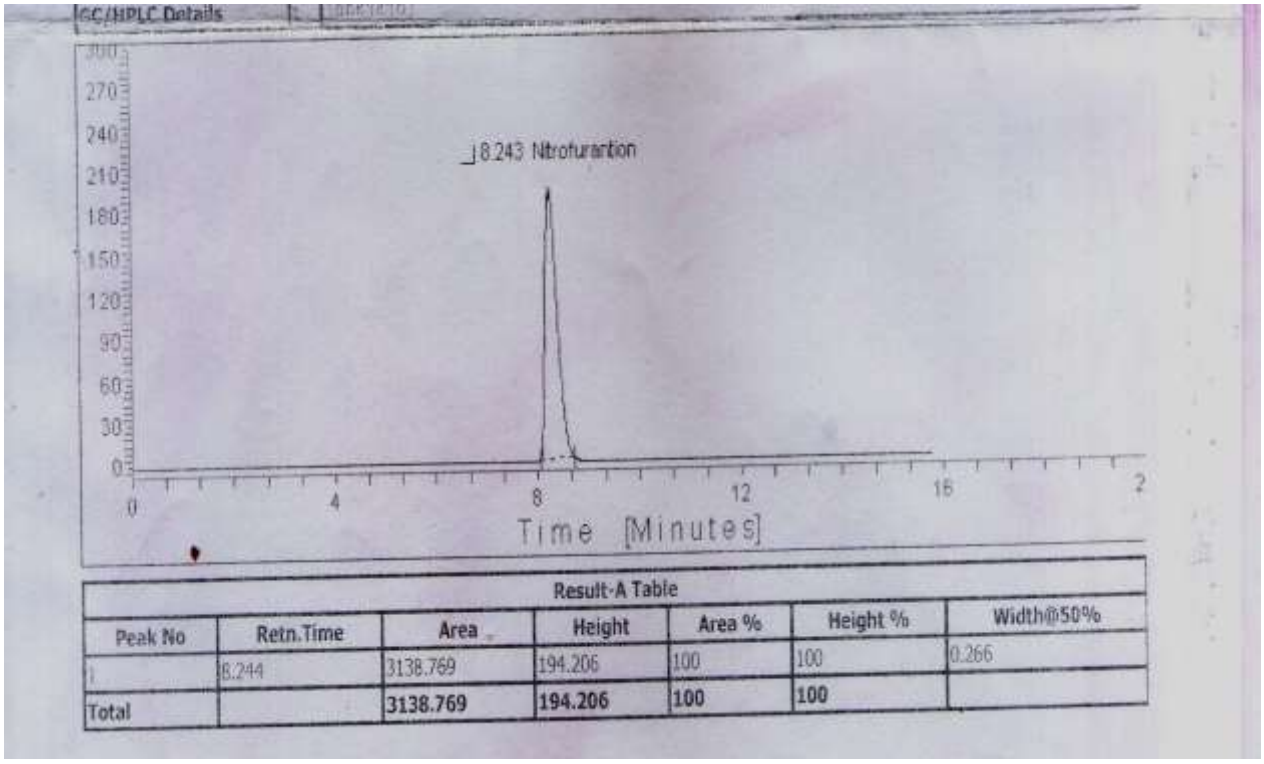
Procedure:

Separately inject equal volumes of blank, five replicate injections of system suitability solution (Nitrofuratoin standard working solution). Then inject two injections of test solution and record the chromatograms. Disregard any peak due to blank in the test solution. Calculate % RSD of five replicate injections of system suitability solution (Nitrofuratoin standard working solution). Check tailing factor and theoretical plates of the peak in the chromatogram obtained with 5th injection of system suitability solution (Nitrofuratoin standard working solution).



Result-A Table

Peak No	Retn.Time	Area	Height	Area %	Height %	Width@50%
1	8.244	3138.769	194.206	100	100	0.266
Total		3138.769	194.206	100	100	

Figure-2: Chromatogram of Nitrofuratoin

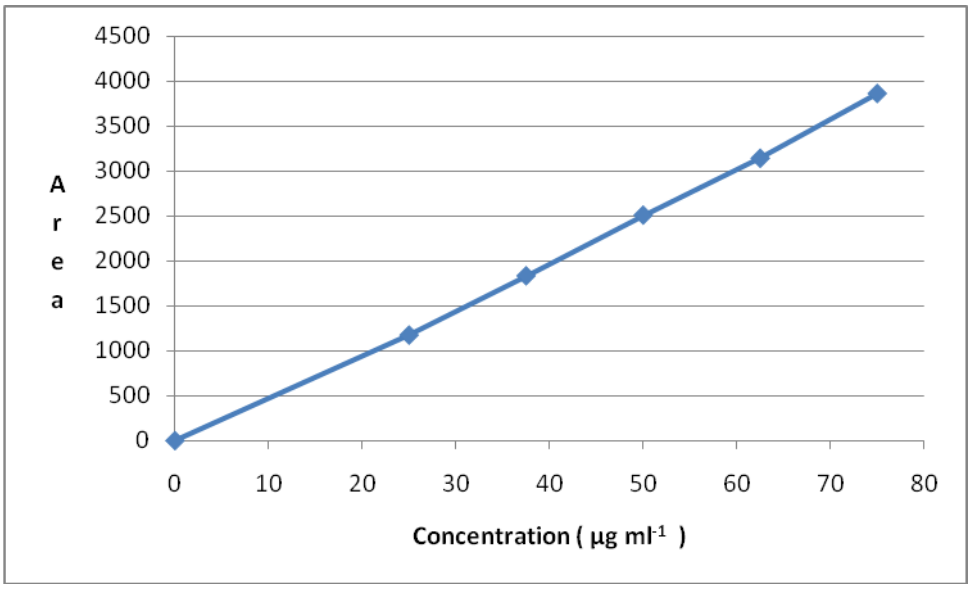


Figure -3: Linearity of Nitrofuratoin standard

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Table -1.2: Performance calculations, detection characteristics precision and accuracy of the proposed method for Nitrofuratoin

Parameter	HPLC Method
Wavelength (nm)	**254**
Retention time (t) min	**8.243**
Linearity range (µg ml^{-1})	**25-75**
LOD (µg ml^{-1})	**0.82655**
LOQ (µg ml^{-1})	**2.7552**
Regression equation (y=bc+a)	
Slope (b)	**53.4120**
Intercept (a)	**168.66**
Standard deviation (SD)	**14.716**
Correlation coefficient(r^2)	**0.9998**
Relative Standard deviation (%RSD)	**0.5881**
Intermediate Precision (%RSD)	**0.28**
Range of errors	
Confidence limits with 0.05 level	**12.8989**
Confidence limits with 0.01 level	**16.952**

RSD of 5 independent determinations
RSD of 15 independent determinations (5 independent samples per day for 3 days)

Table – 1.3: System suitability – Selectivity

Sr. No.	Area of Nitrofurantoin	Tailing factor	Theoretical plates
1	2456.92	1.04	4211
2	2463.09		
Mean	**2460.01**		
Standard Deviation (±)	**4.36**		
(%) Relative Standard Deviation	**0.18**		

Table -1.4: System suitability - Linearity of standard

Sr. No.	Area of Nitrofurantoin	Tailing factor	Theoretical plates
1	2500.30	1.13	4120
2	2580.52		
3	2561.30		
4	2498.42		
5	2526.65		
Mean	**2533.44**		
Standard Deviation (±)	**36.62**		
(%) Relative Standard Deviation	**1.45**		

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Table -1.5: Results of linearity of standard

Linearity Level	Sample Concentration (in µg ml $^{-1}$)	Average Area (n = 2)	Correlation Coefficient
Level – 1	25	1173.19	
Level – 2	37.5	1832.46	
Level – 3	50	2506.97	**0.999**
Level – 4	62.5	3138.77	
Level – 5	75	3858.29	

Table 1.6: Results of linearity of sample

Linearity Level	Sample Concentration (in µg ml $^{-1}$)	Average Area (n = 2)	Correlation Coefficient
Level – 1	25	1168.41	
Level – 2	37.5	1822.55	
Level – 3	50	2512.51	**0.999**
Level – 4	62.5	3168.56	
Level – 5	75	3920.27	

Table -1.7: Results of linearity of standard in presence of placebo

Linearity Level	standard Concentration (in µg ml $^{-1}$)	Placebo added to the standard solution	Average Area (n = 2)	Correlation Coefficient
Level – 1	25	52.4mg	1153.78	
Level – 2	37.5	52.4mg	1815.98	
Level – 3	50	52.4mg	2501.00	**0.999**
Level – 4	62.5	52.4mg	3206.30	
Level – 5	75	52.4mg	3928.80	

Table -1.8: System precision

Sr. No.	Area of Nitrofurantoin	Tailing factor	Theoretical plates
1	2463.86		
2	2463.22		
3	2466.71		
4	2467.04	1.12	4056
5	2486.91		
6	2493.46		
7	2502.42		

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8	2500.88		
9	2499.23		
10	2508.34		
Mean	**2485.21**		
Standard Deviation (±)	**18.12**		
(%) Relative Standard Deviation	**0.73**		

Table – 1.9: Results of method precision,

Test Solution	% Assay of Nitrofurantoin
1	100.68
2	100.40
3	100.75
4	100.97
5	100.63
6	100.48
Mean	**100.65**
Standard Deviation (±)	**0.20**
(%) Relative Standard Deviation	**0.20**

Table – 1.10: Results of intermediate precision

	% Assay of Nitrofurantoin
1	100.31
2	100.20
3	99.87
4	99.57
5	100.25
6	99.92
Mean	**100.02**
Standard Deviation (±)	**0.28**
(%) Relative Standard Deviation	**0.28**

Table – 1.11: Results of twelve test solutions of Nitrofurantoin in NIFTAS 50 (Six of method precision & six of intermediate precision)

Analysis performed during method precision study By Analyst 1 on system 1 and on column 1 on day 1	
Same column	**% Assay of Nitrofurantoin**
1	100.68
2	100.40
3	100.75

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4	100.97
5	100.63
6	100.48
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Column sr. no.	**015337030136 01**
Test Solution	**% Assay of Nitrofurantoin**
7	100.31
8	100.20
9	99.87
10	99.57
11	100.25
12	99.92
Mean of twelve samples	**100.34**
Standard Deviation (±)	**0.41**
(%) Relative Standard Deviation	**0.40**

Table-1.12(A): Determination of accuracy of Nitrofuratoin

Level of % Recovery	Amount of MET in formulation (mg)	Amount of Standard MET added (mg)	Total amount found (mg)	% Recovery
50%	99.95 99.27 97.80	100 100 100	199.9 196.54 195.60	99.94 98.23 97.75
100%	99.87 99.32 99.89	150 150 150	249.67 248.3 249.72	99.86 99.31 99.88
150%	99.98 99.84 99.13	200 200 200	299.94 299.52 297.4	99.97 99.83 99.12

Table-1.12(B): Statistical data for accuracy determination

Level of % Recovery	Total amount found (mean)	Standard deviation	% RSD
50%	197.34	2.2606	1.1455
100%	249.23	0.8057	0.3232
150%	298.95	1.3615	0.4554

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Table – 1.13: Results for Change in Column Lot

Flow rate →	**Same column**	**Diff column**
Sample	**% Assay**	
Test solution	100.12	99.25
Average assay result from method precision	100.65	100.65
Mean	**100.39**	**99.95**
Standard Deviation (±)	**0.37**	**0.99**
(%) Relative Standard Deviation	**0.37**	**0.99**

Table – 1.14: Results for change in flow rate

Flow rate →	**1.4mL/minute**	**1.8 mL/minute**
Sample	**% Assay**	
Test solution	100.48	99.78
Average assay result from method precision	100.65	100.65
Mean	**100.57**	**100.22**
Standard Deviation (±)	**0.12**	**0.62**
(%) Relative Standard Deviation	**0.12**	**0.61**

Table – 1.15: Results for change in wavelength

Wavelength →	**252 nm**	**256 nm**
Sample	**% Assay**	
Test solution	99.31	100.30
Average assay result from method precision	100.65	100.65
Mean	**99.98**	**100.48**
Standard Deviation (±)	**0.95**	**0.25**
(%) Relative Standard Deviation	**0.95**	**0.25**

Table – 1.16: Results for change in pH of mobile phase

pH	**6.8**	**7.2**
Sample	**% Assay**	
Test solution	100.43	100.82
Average assay result from method precision	100.65	100.65
Mean	**100.54**	**100.74**
Standard Deviation (±)	**0.16**	**0.12**
(%) Relative Standard Deviation	**0.15**	**0.12**

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Table – 1.17: Results for solution stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	**% Assay of Nitrofurantoin**
0^{th} hr	100.09
12^{th} hr	99.97
24 hr	99.77
36 hr	100.06
48 hr	99.81
Mean	99.94
Standard Deviation (±)	0.14
(%) Relative Standard Deviation	0.14

RESULTS AND DISCUSSION

The appropriate wavelength in UV region has been selected for the measuring of active ingredient in the proposed method. This method was validated by linear fit curve and all the parameters were calculated.

Parameters Fixation

In developing methods, systematic study of the effects of various parameters was undertaken by varying one parameter at a time controlling all other parameters. The following studies were conducted for this purpose.

Mobile phase characteristics

In order to get sharp peaks and baseline separation of the components, carried out number of experiments by varying different components like percentage of organic phase in the mobile phase, total pH of the selected mobile phase and flow rate by changing one at a time and keeping all other parameters constant. The optimum conditions obtained were included in the procedure proposed.

Detection Characteristics

To test whether Nitrofuratoin had been linearly eluted from the column, different amounts of Nitrofuratoin were taken and analyzed by the above mentioned procedures. The peak area ratios of component areas were calculated and the values are graphically represented in Fig – 2, the linear fit of the system was illustrated graphically. Least square regression analysis for the method was carried out for the slope, Intercepts and correlation coefficient. The results are presented in Table -1.2.

Performance Calculations

To ascertain the system suitability for the proposed method, a number of statistical values have been calculated with the observed readings and the results are recorded in Table-1.2.

Method validations

The UV absorption maximum for Nitrofuratoin was fixed at 254 nm respectively. As the final detection was made by the UV absorption spectrum, each method was validated by linear fit curve.

Precision

The precision of the method was ascertained separately from the peak area ratios obtained by actual determination of a fixed amount of Drug. The percent of Relative Standard deviation calculated for Nitrofuratoin and are presented in Table-1.8, 1.9. 1.10 & 1.11. The precision of the assays was also determined in terms of intra and inter-day variation in the peak areas for a set of Drug solution was calculated in terms of coefficient of variation (CV)

Accuracy

To determine the accuracy of the proposed methods, different of technical grade samples of Nitrofuratoin within the linearity limits were taken and analyzed by the proposed methods. The results (percent error) are recorded n Table-1.12.

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Interference Studies
The effect of wide range of excipients and other additives usually present in the formulations of Nitrofuratoin in the determinations under optimum conditions were investigated. The common excipients such as colloidal Silicon dioxide, ethyl cellulose, hydroxyl propyl methyl cellulose, magnesium state, microcrystalline cellulose provide have been added to the sample solutions and injected. They have not disturbed the elution or quantification of Drug. In fact many have no absorption at this UV maximum.

Analysis of Formulation
To find out the stability of the proposed methods for the assay of formulations containing Nitrofuratoin was analyzed by the proposed and reference methods. The proposed method does not differ significantly in precision and accuracy from reference method. The results are recorded in Table-1.12.

Ruggedness and Robustness
Ruggedness of the proposed method was determined by carrying out the analysis by two different analysts using similar operational i.e. Robustness with Change in Column Lot, Change in Flow rate, Change in wavelength and Change in pH of the Mobile phase . The results were indicated by % CV in Table -1.13, 1.14, 1.15 & 1.16.

Robustness of the method was determined by carrying out the analysis at two different wavelengths i.e. at 252 nm and 256 nm and the results were indicated by % CV in Table -1.15.

Recovery Studies
Recovery studies were conducted by analyzing each formulation in the first instance for the active ingredient by the proposed methods known amounts of pure Drug was then added to each of the previously analyzed formulations and the total amount of the Drug was once again determined by the proposed methods after bringing the active ingredient concentration within the linearity limits. The results are recorded in Table -1.12.

Solution Stability
The stability of the solutions under study was established by keeping the solution at room temperature for 48 Hours. The results indicate no significant change in assay values indicating stability of Drug in the solvent used during analysis. The results are recorded in Table -1.17.

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