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Estimation of Furazolidone In Bulk Drug and Pharmaceutical Dosage Forms by HPLC Method

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ABSTRACT

A simple, economic, selective, precise, and accurate High Performance liquid Chromatographic method for the analysis of Furazolidone in bulk drug and pharmaceutical formulations was developed and validated in the present study. The mobile phase consists of a mixture of potassium dihydrogen phosphate and acetonitrile 80:20. And Adjust pH 6.50±0.1 with dilute potassium hydroxide solution. This was found to give a sharp peak of Furazolidone at a retention time of 4.245 min. HPLC analysis of Furazolidone was carried out at a wavelength of 354 nm with a flow rate of 1.0 mL/min. The linear regression analysis data for the calibration curve showed a good linear relationship with a regression coefficient (r^2) of 1 in the concentration range of 25 to 150 µg ml^{-1}**.** The linear regression equation was $y = 46002x+40407$. The developed method was employed with a high degree of precision and accuracy for the analysis of Furazolidone. 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 Furazolidone.

Keywords: Furazolidone, HPLC, Validation.

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INTRODUCTION

Furazolidone is one of the synthetic antimicrobial nitrofurans. It is a stable, yellow, crystalline compound 3-(5-nitrofurfurylideneamino)-2-oxazolidinone (figure 1). In humans it has used to treat diarrhoea and enteritis caused by bacteria or protozoan infections. It has been used to treat traveler's diarrhoea, cholera and bacteremic salmonellosis. Use in treating Helicobacter pylori infections has also been proposed[1]. Furazolidone is also used for giardiasis (due to Giardia lamblia), though it is not a first line treatment[2]. As a veterinary medicine, furazolidone has been used with some success to treat salmonids for Myxobolus cerebralis infections. It has also been used in aquaculture[3]. Furazolidone is rapidly and completely degraded in liver, kidney and muscle tissues of calves[4]. Biotransformation of furazolidone results in formation of protein bound metabolite, 3-amino-2-oxazolidinone (AOZ) which has been shown to have a long residence time intissues[5]. Furazolidone, (N-(5-nitro-2-furfurylidodene)-3-2-oxazolidone) is an example of an anticoccidial drug that has been used for years for treatment of bacterial and protozoal infections[6&7]. Determination of Furazolidone in animal feeds using LC-MS detection[8]. It is also used for solid phase extraction for the isolation and clean-up of a derivatized furazolidone metabolite from animal tissues[9]. The methods for detecting residues of furazolidone by measuring the parent drug are inappropriate as it is rapidly metabolized and does not persist in edible tissues[10&11]. Monitoring for use of furazolidone is possible, however, by analyzing for tissue bound metabolites. Tissue-bound metabolites are formed after treatment with the drug, and may be released in an active formon consumption of edible tissues[12]. Unlike the parent drug, they are stable even after long storage[13] and can be detected in the tissues of animals for up to 7 weeks after withdrawal of thedrug[14&15]. Method for the Isolation and Liquid Chromatographic Determination of Furazolidone in Milk[35].[min]

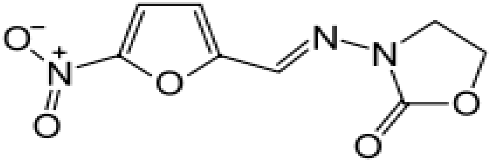


Figure 1.Structure of Furazolidone

Depletion of four nitrofuran antibiotics and their tissue-bound metabolites in porcine tissues and determination using LC-MS/MS and HPLC-UV[36].[3.]. Determinations of Residual Furazolidone and Its Metabolite, 3-Amino-2-oxazolidinone (AOZ), in Fish Feeds by HPLC-UV and LC-MS/MS, Respectively[3] Among these materials, BDDFE has attracted the attention of electro analytical 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[16,17,18,19,20&21] . 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[22&23]. 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) hydration] 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[24]. As pointed out by Hamman[24], this drug can act against various gram-negative bacteria, as well as some gram-positive bacteria, including Citrobacter, Corynebacterium, Enterobacter, Escherichiacoli, Klebsiella, Neisseria, Salmonella, Staphylococcus aureus 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, 30–50% of this drug is excreted in its original form and 1% is excreted as aminofurantoin in the urine[24]. 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[25, 26&27], and the use of high performance liquid chromatography (HPLC) began in the 1970s[28] and[29]. However, according to Jain et al[30], 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, electro analytical 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[17]. The two initial reports about the use of electro analytical techniques to determine NFT came from the sixties and seventies years, when Jones et al[31]. And Mason and Sandmann[32] 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[24&30] and on activated carbon fiber microelectrodes[33]. In these works, this technique was shown to be a very sensitive and selective methodology for NFT analysis[24, 30&33].

The author has developed RP-HPLC method based on the use of symmetry column, without use of any internal standard. An attempt has been made to develop and validate all methods to ensure their accuracy, precision, repeatability, reproducibility and other analytical method validation parameters as mentioned in the various guidelines. For pharmaceutical formulation the proposed method is suitable for their analysis with virtually no interference of the usual additives presented in pharmaceutical formulations.

MATERIAL AND METHODS

Chromatographic Conditions

Waters e 2695 separation module with high pressure liquid chromatographic instrument provided with a Inertsil C_{18} column (150 mm x 4.6 mm ; 5 μ) and 2489 UV-Visible detector, auto injector, auto sampler with Empower 2 software from Waters corporation, Milford USA was employed in the study. HPLC grade acetonitrile was purchased from Ranbaxy, India, and Potassium dihydrogen phosphate, potassium hydroxide was purchased from SD Fine Chem Mumbai, India was used in the study.

Drug Samples

The reference samples were obtained from M/s. Richer Pharmaceuticals, Hyderabad, India, and the formulation samples were purchased from local market.

Mobile phase

Accurately weigh 1.36gms of potassium dihydrogen phosphate in 1000ml of water adjust pH 6.5±0.1 with dilute potassium hydroxide and acetonitrile in the ratio 80:20 v/v was filtered through 0.4µ membrane filter and was degassed. Mobile phase was used as diluent for preparing the working solution of the drug. The mobile phase was filtered and sonicated by using Bio-Technics India, Mumbai before use. The flow rate of the mobile phase was maintained at 1ml/min. The column temperature was maintained at 35ºC and the detection of the drug was carried out at 354nm

Preparation of Furazolidone Standard stock solution and Standard Solution

Weighed accurately 100 mg of Furazolidone working standard and transferred to a 100 ml volumetric flask. Added 30 ml of mobile phase and sonicate to dissolve dilute to volume gives 1000 µg/ml (Stock solution A). From the above stock solution A, dilute 10 ml to 100ml with mobile phase gives 100 µg/ml used as a Standard solution.

Preparation of Test Solution

Weighed accurately previously weighed and crushed 20 tablets powder equivalent 100 mg of Furazolidone transferred to 100ml volumetric flask make up to the mark with mobile phase sonicated and filtered through what man filter paper. Further dilute 10 ml to 100 ml with mobile phase.

Linearity and Construction of Calibration Curve

Linearity of the peak area response was determined by taking measurement at six concentration points working standard dilution of Furazolidone in the range of 25-150µg/ml. 20µl quantity of the dilution was injected each time in to the column. The drug elutes was monitored at 354 nm at a column temperature of 35ºC and the corresponding chromatograms were obtained. The linearity of the calibration curve was plotted between the mean peak areas versus respective concentration (figure 2).

Assay

Transfer accurately previously weighed and crushed 20 tablets powder equivalent 100 mg of Furazolidone into 100ml volumetric flask make up to the mark with mobile phase sonicate and filtered through what man filter paper. Further dilute 10 ml to 100 ml with mobile phase. The diluted preparation was injected in duplicate and the results were calculated and presented in Table 7. The chromatogram of sample preparation shown in figure 3.

METHOD VALIDATION

The proposed method was validated as per ICH guidelines as follows

System Suitability

System suitability of the proposed method is determined the parameters like tailing factor, theoretical plates and the standard deviation. The optical and system suitability parameters are tabulated in Table 1.

Linearity

The Linearity of the proposed method was performed over a concentration range 25 to 150 mcg/ml. The regression concentration and areas are given in table 2. And the regression characters are given in figure 4.

Accuracy

The accuracy of the proposed method was determined by using recovery method. The accuracy studies carried out by adding sample solution in three different levels. The percentage recovery and standard deviation were calculated. The drug content in tablets was quantified using the proposed analytical method are given in table 3.

Robustness

The deliberate changes in the method have not much affected the peak tailing, Theoretical plates and the percent assay. This indicated the robustness of the method. The robustness study results are presented in table 4**.**

Precision

The system precision was established by six replicate injections of the standard solution containing analyte of interest. The values of relative standard deviation were found within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analyte six times using the proposed method. The relative standard deviation was found within the limit, indicating the injection repeatability of the method. The precision results were presented in Table 5&6.

Specificity and Selectivity

The specificity of the proposed method was determined by the complete separation of analyte and other components in the sample. When it was subjected to forced degradation as per ICH guidelines which was carried out with 0.1N HCL, 0.1N NaOH and Heat degradation. The method does not permit detection of degradation product for Furazolidone

LOD and LOQ

The proposed method shows good sensitivity, the LOD and LOQ values found to be 1.725mcg/ml and 5.228 mcg/ml respectively.

RESULTS AND DISCUSSION

The present study was aimed at developing a simple economical precise and accurate HPLC method for the analysis of Furazolidone in bulk drug and in pharmaceutical dosage form. In order to achieve optimum separation of the component peaks, mixture of acetonitrile with water in different combinations were tested as mobile phase on a $C_{18 \text{ stationary}}$ phase. A mixture of Phosphate buffer pH 6.5: acetonitrile in a proportion of 80:20 v/v was selected as the chromatographic peaks were well defined and resolved with no tailing. The retention time obtained for Furazolidone was 4.245 min. A good linear relationship $r^2 = 1$ was observed indicates the proposed method is linear over the range 25 to 150mcg/ml.

High recovery values between 99.83 to 100.06% obtained from the pharmaceutical dosage form by the proposed method indicates the method is accurate. The deliberate changes in the method have not much affected the peak tailing, Theoretical plates and the percent assay. This indicated the robustness of the method. When test solutions were analyzed by the proposed method for finding out system precision and method precision low co-efficient of variation <1.0 was observed. The absence of additional peaks indicated non-interference of common excipients used in the tablets. The specificity of the HPLC method was determined by the complete separation with Furazolidone. When it was subjected to forced degradation as per ICH guidelines which was carried out with 0.1N HCL, 0.1N NaOH and Heat degradation. The method does not permit detection of degradation product for Furazolidone. The lowest value of LOD and LOQ as obtained by the proposed method by calculated using 3.3xstdev/slope for LOD and 10xstdev/slope for LOQ. The standard solution of the drug was stable up to 24 hrs as the difference in percent assay during the above period is within limit.

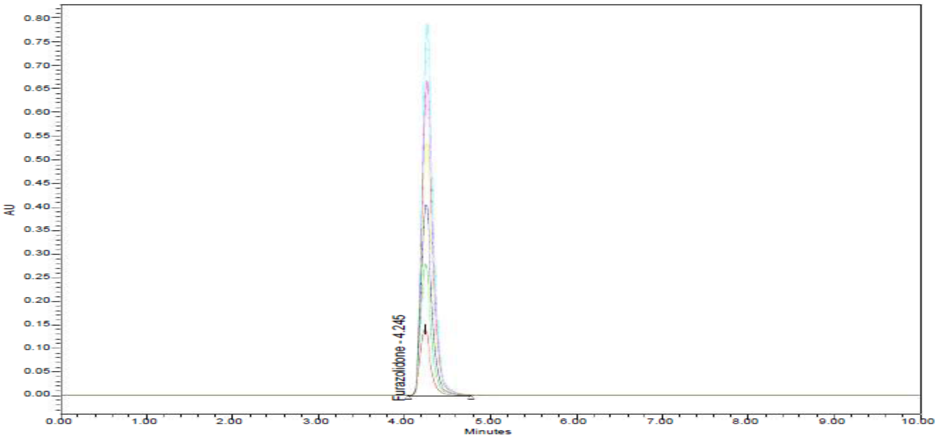
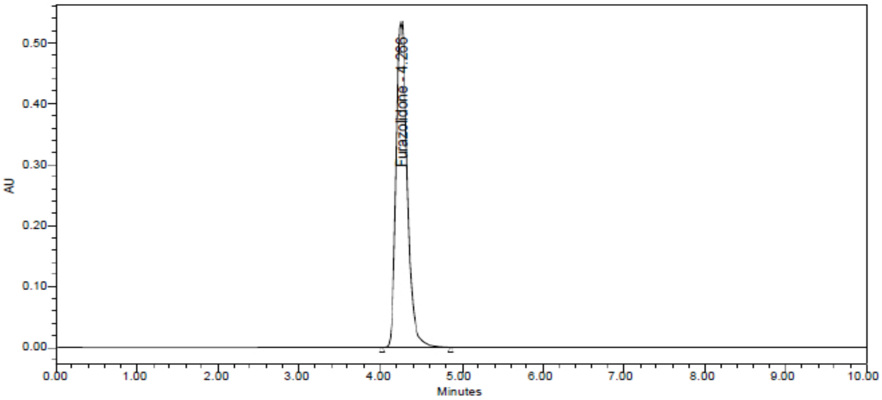


Figure-2: Overlaid Linearity Chromatogram of Furazolidone



	Peak Name	RT	Area	Height	% Area	USP Plate Count	USP Tailing
1	Furazolidone	4.266	4620562	533634	100.00	5832	1.26

Figure -3: Sample Chromatogram of Furazolidone

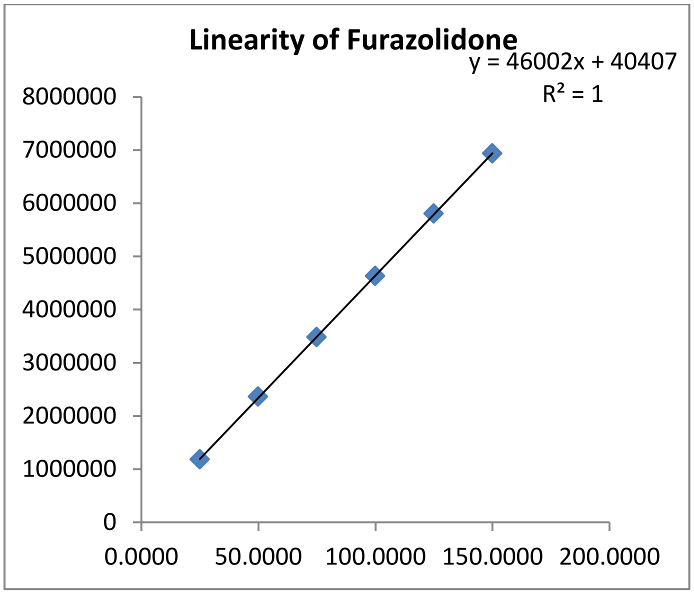


Figure 4 . Linearity of Furazolidone

Table 1 : System suitability parameters

Parameter	HPLC Method
Wavelength (nm)	354
Retention time (t) min	4.245
Linearity range (µg ml^{-1})	25-150
LOD (µg ml^{-1})	1.725
LOQ (µg ml^{-1})	5.228
Slope (b)	46002
Intercept (a)	40407
Correlation coefficient(r^2)	1
Tailing Factor	1.25
Theoretical Plates	5856

Table 2: Calibration of the proposed method

Conc(mcg)	Area
25.0000	1183480
50.0000	2359900
75.0000	3478830
100.0000	4630379
125.0000	5803425
150.0000	6937370

Table 3: Accuracy Data (Triplicate values at 50,100 &150 percent levels)

S.No	Spike level	Peak area	Amount Added (µg/ml)	Amount Recovered (µg/ml)	%Recovery	Avg	% RSD
1	50%	2325635	50	50.23	100.46	100.06	0.41
		2316859	50	50.04	100.08		
		2306582	50	49.82	99.64		
2	100%	4625326	100	99.90	99.90	99.84	0.06

		4622302	100	99.83	99.83		
		4620214	100	99.79	99.79		
3	150%	6936325	150	149.81	99.88	99.83	0.05
		6933025	150	149.74	99.83		
		6929536	150	149.67	99.78		

Table 4 : Robustness Study

Drug Name	Parameter	Chromatographic Conditions			
		Retention time	Area	Theoretical Plates	Asymmetry
	Flow Change ±0.1ml				
	1.1ml/min	3.798	4172309	5272	1.27
	1.0ml/min	4.258	4610245	5856	1.25
	0.9ml/min	4.853	5354654	6303	1.28
Furazolidone	Temp Change±5˚C				
	30˚C	4.341	4672580	5996	1.25
	35˚C	4.258	4610245	5856	1.25
	40˚C	4.166	4704055	5631	1.27

Table 5: Precision Study

Sample	RT	Area
Injection-1	4.258	4610245
Injection-2	4.258	4605986
Injection-3	4.259	4618659
Injection-4	4.263	4621523
Injection-5	4.265	4625245
Injection-6	4.265	4622485
Avg	4.261	4617357
Std Dev	0.003	7582.2
% RSD	0.079	0.16

Table 6: Method Precision study

Sample	RT	Area
Sample-1	4.257	4598659
Sample-2	4.259	4599068
Sample-3	4.261	4623025
Sample-4	4.265	4625235
Sample-5	4.266	4622301
Sample-6	4.268	4626365
Avg	4.263	4615776
Std Dev	0.004	13182.4
% RSD	0.101	0.29

Table 7: Assay results

Drug	Amount present/tablet	Amount found	% of Assay
Furazolidone	100 mg	99.72 mg	99.72

CONCLUSION

Hence it can be concluded that the proposed HPLC method was simple, precise, accurate, sensitive, robust and rugged for estimation of furazolidone in pharmaceutical formulations, this method can be used for the analysis of furazolidone in bulk and tablet formulations in regular quality control analysis.

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