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# Spectrophotometric Method for the Determination of Furazolidone in Pharmaceutical Formulations and Human Blood Samples with MBTH

**B. Ramachandra, N. Venkatasubba Naidu***

Department of Chemistry, S. V. University, Tirupati - 517502, A.P., India

**Abstract:** ***A simple, sensitive, selective rapid spectrophotometric method has been developed for the determination of synthetic antimicrobial furazolidone in pure form and pharmaceutical formulations based on the oxidative coupling reaction with MBTH reagent, at $P^H$–4.0 which is extractable at 630 nm. Beer's law is obeyed in the concentration ranges 10-60 µg $ml^{-1}$. And 5-30 µg $ml^{-1}$. For blood sample the developed method was applied directly and easily for the analysis of the Pharmaceutical formulations and blood sample. R.S.D was found to be 0.03769% 0.09487and recovery was found between 99.99% 99.38% respectively. The method was completely validated and proven to be rugged. The interferences of the other ingredients and excipients were not observed. The repeatability and the performance of the proved method were established by point and internal hypothesis and through recovery studies.***

**Keywords:** Spectrophotometry, Furazolidone, Blood Sample & MBTH / $FeCl_3$.

## 1. Introduction

Furazolidone is one of the synthetic antimicrobial nitrofurans. It is a stable, yellow, crystalline compound. It is used to treat diarrhoea and enteritis caused by bacteria or protozoan infections in humans. It has been used to treat traveler's diarrhoea, cholera and bacteremic salmonellosis. Use in treating Helicobacter pylori infections has also been reported [1]. Furazolidone is also used for giardiasis (due to Giardia lamblia), though it is not a first time treatment [2]. Furazolidone has been used with some success to treat salmonids cerebral infections as a veterinary medicine. 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 in tissues [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 has also been reported [8].

It is also used for solid phase extraction for the isolation and cleaning up of aderivatised 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]. Tissue-bound metabolites are formed after treatment with the drug, and may be released in an active form on consumption of edible tissues [12]. These are stable even after long storage unlike the parent drug [13] and can be detected in the tissues of animals up to 7 weeks after withdrawal of the drug [14&15]. Journal Nitrofurantoin [nitrofuran; 1-((5-nitro-2-furfurylidene)-1-amino) hydration] is a drug synthesized from nitrofuran that is very useful in the treatment of urinary trac diseases. This drug depending on its concentration at the inflammation may act as a bactericidal agent [16]. 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 nitrofuran. Colorimetric and spectrophotometric methods have been used since 1960 [17, 18&19], and the use of high performance liquid chromatography (HPLC) began in 1970s [20] and [21]. However, according to Jain et al [22], 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 increased cost analysis. These stages also increase the exposure time of the drug to light, which can promote the partial photochemical degradation of the compound. The two initial reports about the use of electro analytical techniques to determine nitrofuran came in sixties and seventies these have been reported Jones et al [23]. Further, Mason and sandman [24] reported the use of the polarographic method and the reductive voltammetric method using a rotating platinum electrode. Recently, three reports have been published that demonstrate the use of square wave cathodic adsorptive stripping voltammetry [25]. A method for the isolation and liquid chromatographic determination of furazolidone in Milk is also reported [26].

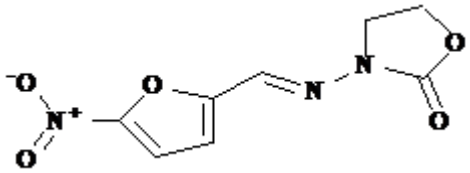


**Figure 1:** Chemical structure of Furazolidone

Depletion of four nitrofuran antibiotics and their tissue-bound metabolites in porcine tissues determination using LC-MS/MS and HPLC-UV have also been reported [27].[3] Determinations of residual furazolidone and Its Metabolite, 3-Amino-2-oxazolidinone (AOZ), in Fish Feeds by HPLC-UV and LC-MS/MS are also reported [28]. However, there is no reported UV- visible spectrophotometric method for the analysis of furazolidone in its technical grade and formulations. This article describes a validated UV- visible spectrophotometric method for the quantitative determination of furazolidone. Functional group used for color development of dasatinib is primary amine group. The results obtained in this method were based on the Oxidative coupling reaction with MBTH/ Ferric chloride. 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.

**Method: Visible Spectrophotometric method by MBTH Reagent[31]**

**Experimental**

Preparation of standard calibration curve of pure drug

**Solvent:** Acetonitrile.

**Preparation of standard stock solution**

Accurately weighed 100 mg of furazolidone was dissolved in 40 ml of acetonitrile in a100 ml volumetric flask and it was made up to the mark with acetonitrile. i.e. 1000 µg $ml^{-1}$ (Stock solution A). From the above stock solution A 10 ml of aliquot was pipetted out into 100 ml volumetric flask and the volume was made up to the mark with acetonitrile to obtain the final concentration of 100 µg $ml^{-1}$ (Stock solution B).

**Preparation of calibration curve**

Fresh aliquots of furazolidone ranging from 1 to 6 ml were transferred into a series of 10ml volumetric flasks to provide final concentration range of 10 to 60 µg $ml^{-1}$. 1ml of (0.5%) MBTH solution was added to each flask followed by 1ml of (0.7%) ferric chloride solution and the resulting solution was heated and finally 1ml (0.5M) hydrochloric acid was added. The solutions were cooled to room temperature and made up to mark with acetonitrile. The absorbance of bluish green colored chromogen was measured at 630 nm against the reagent blank. The color species were stable for 32 hrs. The amount of furazolidone present in the sample solution was computed from its calibration curve.

## 2. Procedure for Formulations

Twenty tablets containing furazolidone were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 100 mg of furazolidone was dissolved in a 100 ml of acetonitrile and mixed for about 5 min and then filtered. The solvent acetonitrile was evaporated. The residue obtained was again diluted with acetonitrile and made up to 100ml to get the stock solution A. 10 ml of aliquots were pipetted out into 100 ml volumetric flasks and the volume was finally made up to the mark with acetonitrile to obtain the final concentration of 100 µg $ml^{-1}$ (Stock solution B). Subsequent dilutions of this solution were made with acetonitrile to get concentrations of 10 to 60 µg $ml^{-1}$.These were analyzed at the selected wavelength, 630 nm and the results were statistically validated.

## 3. Procedure for Blood Sample

Blood samples collected were centrifuged. To isolate furazolidone from plasma sample, acetonitrile was used for protein precipitation. Liquid- Liquid extraction was performed with plasma by alkalinization with 1M sodium hydroxide, followed by extraction with 30% dichloromethane in hexane. The upper organic layer was evaporated to dryness, and reaming dry residue of 100 mg was dissolved in 100 ml of acetonitrile (1000 µg $ml^{-1}$). 10 ml of the above solution was taken into a 100 ml of volumetric flask and made up to the mark with acetonitrile (100 µg $ml^{-1}$). Solutions were in furazolidone was ranging from 0.4-2.4 ml (4-24 µg $ml^{-1}$). These were transferred in to 10 ml volumetric flasks and to each flask 1ml of (0.5%) MBTH solution was added followed by 1ml of (0.2%) ferric chloride solution and were made up to the mark with acetonitrile. Then the resulting solutions were heated and finally 1ml (0.5M) HCL solution was added. The solutions were cooled to room temperature and made up to the mark with acetonitrile. The absorbance of bluish green colored chromogen was measured at 630 nm against the reagent blank. The color species were stable for 32 hrs. The amount of furazolidone present in the sample solution was computed from its calibration curve.

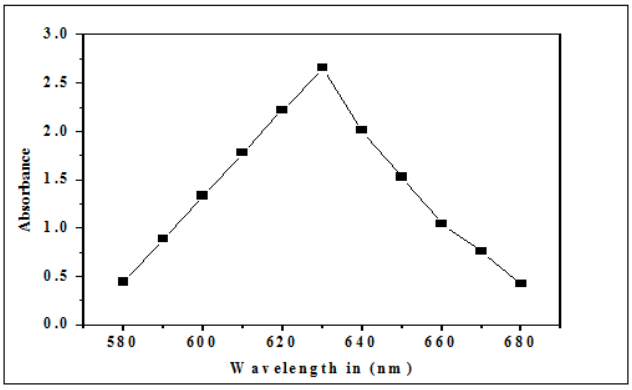



**Figure 2:** Absorption spectrum of Furazolidone with MBTH /$FeCl_3$

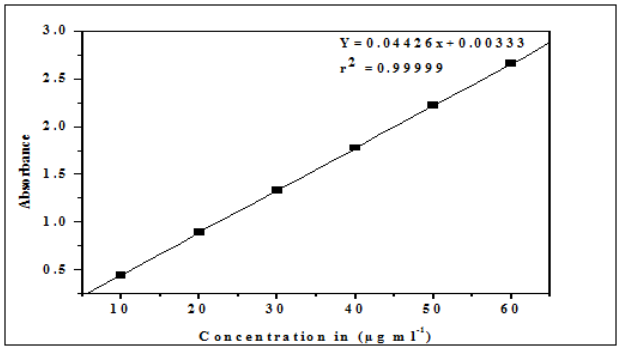



**Figure 3:** Beer's law plot of Furazolidone with MBTH/$FeCl_3$

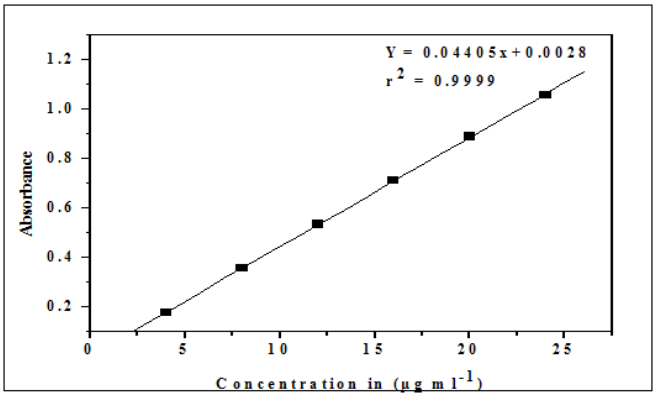



**Figure 4:** Beer's law plot for MBTH in Blood sample

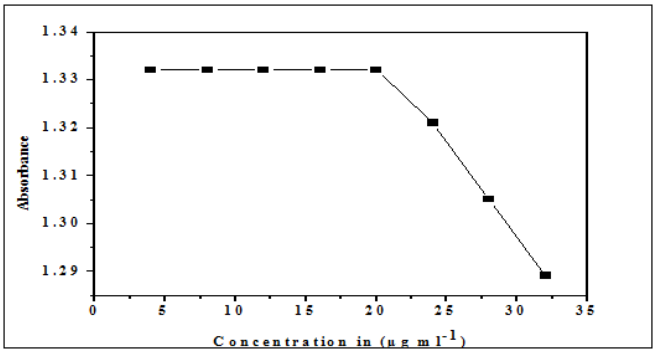
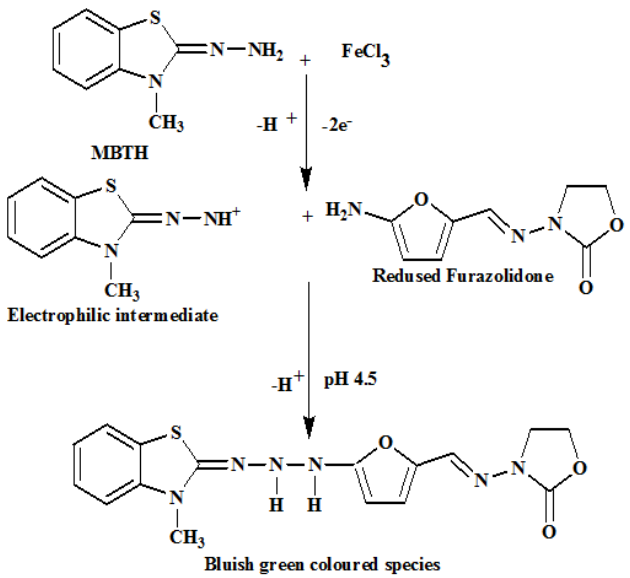



**Figure 5:** Color stability data for MBTH Method




**Scheme 1:** Schematic reaction Mechanism of Furazolidone with MBTH

**Table 1:** Optical characteristics and precision by MBTH

| *Parameter* | *Visible method* |
|---|---|
| Color | Bluish Green |
| Absorption maxima (nm) | 630 |
| Beer's law limits (µg $ml^{-1}$) | 10-60 |
| Molar absorptivity (l $mol^{-1}cm^{-1}$) | $0.04423x10^4$ |
| Sandell's Sensitivity (µg $cm^{-2}$) | 0.37678 |
| Regression equation (Y*) | Y=mx+c |
| Slope (b) | 0.04426 |
| Intercept(a) | 0.00333 |
| Standard deviation(SD) | 0.00345 |
| Correlation coefficient ($r^2$) | 0.99999 |
| %RSD (Relative Standard deviation) | 0.03769 |
| Limits of detection (µg $ml^{-1}$) | 0.23384 |
| Limits of quantification (µg $ml^{-1}$) | 0.77948 |

RSD of 6 independent determinations

**Table 2:** Assay results of Furazolidone in formulations by visible method

| *Name of the Formulation* | *Formulation (mg )* | *Amount found by the proposed method (mg )* | *Amount found by the reference method[29,30] (mg)* | *% Recovery* |
|---|---|---|---|---|
| FUROXONE | 250 | 249.99<br>T=0.002968<br>F=1.8320 | 249.21 | 99.21 |
| DEPENDAL-M | 250 | 249.97<br>T=0.002967<br>F=1.83082 | 248.01 | 98.01 |

- T and F- values refer to comparison of the proposed method with reference method.
- Theoretical values at 95% confidence limits T= 0.0029 and F= 1.7043.

**Table 3:** Determination of accuracy of Furazolidone

| *Amount of FRZ in formulation (mg)* | *Amount of Standard FRZ added (mg)* | *Total amount found (mg)* | *% Recovery* |
|---|---|---|---|
| 249.98 | 200 | 449.96 | 99.96 |
| 249.84 | 200 | 449.71 | 99.71 |
| 249.70 | 200 | 449.46 | 99.46 |
| 249.97 | 250 | 499.94 | 99.94 |
| 249.86 | 250 | 499.72 | 99.72 |
| 249.74 | 250 | 499.48 | 99.48 |
| 249.96 | 300 | 549.91 | 99.91 |
| 249.82 | 300 | 549.60 | 99.60 |
| 249.72 | 300 | 549.38 | 99.38 |

**Table 4:** Statistical data for accuracy determination

| *Total amount found (mean)* | *Standard deviation* | *% RSD* |
|---|---|---|
| 449.71 | 0.25 | 0.05559 |
| 499.71 | 0.23007 | 0.04604 |
| 549.63 | 0.26627 | 0.048445 |

The results are the mean of five readings at each level of recovered

**Table 5:** Repeatability data for FRZ at 630 nm

| **Conc. (µg $ml^{-1}$)** | **Abs 1** | **Abs2** | **Abs3** | **Mean** | **Std. deviation** | **(%)RSD** |
|---|---|---|---|---|---|---|
| 10 | 0.444 | 0.443 | 0.442 | 0.443 | 0.001 | 0.22573 |
| 20 | 0.888 | 0.884 | 0.886 | 0.886 | 0.002 | 0.22573 |
| 30 | 1.332 | 1.330 | 1.329 | 1.3303 | 0.00153 | 0.11501 |
| 40 | 1.776 | 1.771 | 1.773 | 1.7733 | 0.00252 | 0.14210 |
| 50 | 2.220 | 2.218 | 2.217 | 2.2183 | 0.00153 | 0.06897 |
| 60 | 2.654 | 2.652 | 2.653 | 2.653 | 0.001 | 0.03769 |

Average of five determinations

**Table 6:** Color stability data for MBTH Method

| *Conc. in µg $ml^{-1}$* | *Time in Hours* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 |
| 30 | 1.332 | 1.332 | 1.332 | 1.332 | 1.331 | 1.321 | 1.305 | 1.289 |

**Table 7:** Assay results of Furazolidone in Blood sample

| *Name of the Formulation* | *Formulation in (mg)* | *Amount found by the proposed method in (mg)* | *Amount found by the reference method[29,30] (mg)* | *% of Recovery* |
|---|---|---|---|---|
| FUROXONE | 2 | 1.99 T=0.002969 F=1.79002 | 1.87 | 99.99 |
| DEPENDAL-M | 2 | 1.98 T=0.002968 F=1.78288 | 1.89 | 99.98 |

- T and F values refer to comparison of the proposed method with reference method.
- Theoretical values at 95% confidence limits T=0.00196 and F= 1.5274.

**Table 8:** Determination of accuracy of Furazolidone

| *Name of the Formulation in (mg)* | *Amount of Drug in Blood sample (mg)* | *Amount of Standard Drug added in (mg)* | *Total amount found (mg)* | *% Recovery* |
|---|---|---|---|---|
| FUROXONE (2mg) | 1.99 | 2 | 3.98 | 99.98 |
| DEPENDAL-M (2mg) | 1.98 | 2 | 3.96 | 99.96 |

The results are the mean of five readings at each level of recovered

**Table 9:** Repeatability data for Furazolidone at 630nm

| *Concentration in (µg $ml^{-1}$)* | *Abs 1* | *Abs 2* | *Abs 3* | *Mean* | *Std. Deviation* | *(%) RSD* |
|---|---|---|---|---|---|---|
| 4 | 0.177 | 0.176 | 0.175 | 0.176 | 0.001 | 0.56818 |
| 8 | 0.355 | 0.354 | 0.353 | 0.354 | 0.001 | 0.28248 |
| 12 | 0.532 | 0.531 | 0.532 | 0.53167 | 0.00058 | .0.10909 |
| 16 | 0.710 | 0.709 | 0.708 | 0.709 | 0.001 | 0.14104 |
| 20 | 0.888 | 0.887 | 0.886 | 0.887 | 0.001 | 0.11273 |
| 24 | 1.055 | 1.053 | 1.054 | 1.054 | 0.001 | 0.09487 |

Average of five determinations

## 4. Results and Discussion

### Optical Parameters

In order to ascertain the optimum wavelength of maximum absorption ($\lambda_{max}$) formed in UV spectrophotometric method the colored species formed in this visible spectrophotometric method, specified amount of furazolidone in final solutions which contained 10 µg $ml^{-1}$ were taken and the color was developed following the procedures described earlier. The absorption spectra were scanned on spectrophotometer in the wavelength region of 200-800 nm against corresponding reagent blanks. The regent blank absorption spectrum of each method was also recorded against distilled acetonitrile. The results are graphically represented in figure 2.

### Parameters fixation

A systematic study of the effects of various relevant parameters encountered in the methods were under taken by verifying one parameter at a time and controlling all other parameters to get the maximum color developed with MBTH method. Reproducibility and reasonable period of stability of colored species formed were also studied.

**Method**

The results obtained in this method were based on oxidation followed by coupling reaction of furazolidone with MBTH, ferric chloride and HCL. This results in the formation a bluish green colored chromogen that exhibited maximum absorption at 630 nm against the corresponding reagent blank. The functional group used for the color development in this method is primary amine group. A schematic reaction mechanism of furazolidone with MBTH reagent was shown in (schem-1). The effect of various parameters such as concentration and volume of MBTH and strength of acid was studied by means of control experiments varying one parameter at a time.

**Optical characteristics**

The reference method follows Beer's law. The absorbance at appropriate wave length of a set of solutions that contained different amounts of furazolidone and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blank. In order to test whether the colored species formed in the method adhere to Beer's law, the absorbance at appropriate wavelength of a set of solutions contained different amounts of furazolidone and specified amount of reagents (as described in the recommended procedure) were noted against appropriate reagent blanks. The Beers law plots of the system are illustrated graphically (fig: 3&4). Least square regression analysis was carried out for the slope; intercept and correlation coefficient, Beer's law limits, molar absorptivity, sandells sensitivity for furazolidone with each of mentioned reagents were calculated. The optical characteristics are presented in the table – 1.

**Precision**

The precision of each one among the three proposed spectrophotometric methods was ascertained separately from the absorbance values obtained by actual determination of a fixed amount of furazolidone 10 µg $ml^{-1}$ in a final solution. The percent relative standard deviations were calculated for the proposed method and are presented in table -1.

**Analysis of Formulations**

Commercial formulations of furazolidone were successfully analyzed by the proposed methods. The values obtained from the proposed and reference methods were compared statistically by the T and F tests and the comparison showed that those proposed method do not differ significantly from the reported method and they are presented in table - 1. The proposed method also applied for biological samples (Blood) and good recoveries were observed which are recorded in table-2 &7.

**Accuracy** Recovery studies were carried by applying the standard method to drugs samples present in formulations to which known amount of furazolidone. By applying the same method to biological sample (Blood) to which known amount of furazolidone correspond to 2 mg formulations taken by the patient. By following of standard addition method, 2 mg of label claim was added. And the contents were transferred to 100 ml volumetric flash and dissolved in acetonitrile. Finally the volume was made up to the mark with solvent. The solution was filtered through Whitman No. 41filter paper. The mixed sample solutions were analyzed and their absorbance values were determined. At each level of recovery five determinations were performed and these are presented in Table-3&8. The results obtained were compared with expected results and were statistically validated in Table-4.

**Linearity and Range**

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyze in a sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyze that have been demonstrated within a suitable level of precision, accuracy and linearity.

**Specificity and Selectivity**

Specificity is a procedure to detect quantitatively an analyze in the presence of components that may be expected to the present in the sample matrix. While selectivity is a procedure to detect the analyze qualitatively in presence of components that may be expected to present in the sample matrix. The results of the present study show that they follow rules of Specificity and Selectivity.

**Repeatability**

Standard solutions of furazolidone were prepared and absorbance was measured against the solvent as the blank. The observance of the same concentration solution was measure five times and standard deviation was calculated and the results are presented in tables -5&9.

**Solution stability**

The stability of the solutions under study was established by keeping the solution at room temperature for 32 hours. The results indicate that no significant change occurs in assay values indicating stability of drug in the solvent used during analysis. The results are given in Table-6.

**Interferences Studies**

The effect of wide range of inactive, ingredients usually present in the formulations for the assay of Furazolidone under optimum conditions was investigated. None of them interfered in the proposed methods even when they were present in excess.

## 5. Conclusion

The proposed method was found to be simple, economical and sensitive. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the method. Analysis of blood samples and formulation containing Furazolidone showed no interference from common excipients. Hence this method could be

considered for the determination of dasatinib in quality control laboratories.

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