REPORT

Novel UV-spectrophotometric method for quantitative estimation of furazolidone using mixed hydrotropic agent

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Abstract: A novel, eco friendly, accurate, sensitive, economic and safe spectrophotometric method was developed by application of mixed hydrotropy using 2 M sodium acetate, 8 M urea, 2 M niacinamide and 2 M sodium benzoate solution (25:25:25:25% V/V) as hydrotropic agent, for the solubalizing of poorly water-soluble Furazolidone (FZ) (solubility:- 3.64e-01 mg/mL in water). There were more than 32 times enhancements in the solubility of FZ were found in mixed hydrotropic solution as compared to solubilities in distilled water. FZ shows maximum absorbance at 360 nm where sodium acetate, urea, niacinamide, sodium benzoate and other tablets excipients did not show any absorbance above 300 nm, and thus no interference in the estimation was seen. FZ was obeyed Beer's law in the concentration range of 10 to 50 µg/ml (r^2=0.9992) in mixed hydrotropic solvent with mean recovery ranging from 97.32% to 98.9%. Proposed method is new, simple, economic, safe, rapid, accurate and reproducible and was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values.

Keywords: Furazolidone; mixed hydrotropic; sodium acetate; urea; sodium benzoate; niacinamide; spectrophotometry.

INTRODUCTION

Furazolidone (O'neil *et al*., 2001), chemically, 3-(5-nitrofurfurylideneamino)-2-oxazolidinone, a synthetic antimicrobial nitrofurans, which act as antibacterial spectrum covering the majority of gastrointestinal tract pathogens including E.coli, Staphylococci, Salmonella, Shigella, Proteus, Aerobacter aerogenes, Vibrio cholerae and Giardia lamblia. Its bactericidal activity is based upon its interference with several bacterial enzyme systems; this antimicrobial action minimizes the development of resistant organisms. The chemical structure of FZ is shown in fig. 1.

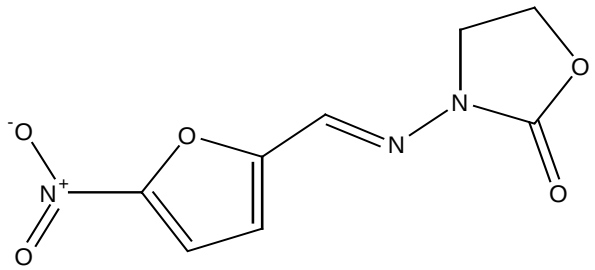

Fig. 1: Chemical structure of furazolidone

FZ is official in IP (IP, 1996), BP (BP 2009) and USP (USP, 2005). FZ is the water insoluble drug so hydrotropic agents utilized to increase the water solubility. Urea, sodium benzoate, niacinamide, Sodium salicylate, sodium citrate and sodium acetate are the most common examples of hydrotropic agent. The solubility of various poorly water-soluble drugs were increased by hydrotropic solubilization phenomenon (Jain *et al.,* 2010[a,b]; Maheshwari *et al.,* 2006). Hydrotropic solution may be a proper abundance as a solvent to exclude the use of organic solvents. Literature survey reveals that various analytical methods has been developed such as HPLC (Cieri, 1979; Shirke *et al.,* 1994), UV-Visible spectrophotometry (Ravisankar *et al.,* 1998), Liquid Chromatography with Electrochemical Detection (Germain *et al.,* 1990), Turbidimetric method (Gang and Shaikh, 2006) for estimation of FZ in biological fluids and in Pharmaceutical formulations.

To the best of our knowledge, there is no work in the literature reported about the spectrophotometric method for the analysis of FZ using hydrotropic agent. In the preliminary solubility studies there were more than 32 fold enhancements in the solubility of FZ in mixed hydrotropic solution. Therefore, it was thought worthwhile to employ this hydrotropic solution to extract out the drug from fine powder of tablets to carry out spectrophotometric estimation. This prompted us to disclose our results, consisting of new and accurate method for the determination of drug in pharmaceutical formulations.

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MATERIALS AND METHODS

Materials

Instruments

1. UV-visible spectrophotometer (1700 Shimadzu).
2. Sonicator.
3. Micropipette, Variable volume 20-200µL (Bio-system classic).

Reagents

1. 2 M sodium acetate, 8 M urea, 2 M niacinamide and 2 M sodium benzoate solution (25:25:25:25% V/V) used as hydrotropic agent.
2. Furazolidone was a generous gift from GSK Ltd. Mumbai.
3. Double Reverse Osmosis (R.O.) water.

Procedure

Preparation of standard stock solution and selection of wavelength

The standard stock solution of 100 µg/ml for FZ was prepared in mixed hydrotropic solution comprised of 2 M sodium acetate, 8 M urea, 2 M niacinamide and 2 M sodium benzoate solution (25:25:25:25% V/V) 25±1°. From this stock solution, appropriate dilution was made and scanned in the UV range 400-200 nm. The absorbance of FZ was recorded at 360 nm. The solubility of FZ was increased more than 32 times in mixed hydrotropic solution, as compare with distilled water. This increased solubility of FZ is due to the hydrotropic solubilization phenomenon.

Method

A series of concentrations ranging from 10-50µg/ml were prepare by taking different aliquots from the stock solution and diluted with the Double R.O. water. The absorbance of these solutions was measured at 360 nm and a calibration curve was plotted between concentration and absorbance at selected wavelength; the spectral characteristics and linearity data is shown in table 1. The spectrum of FZ is shown in fig. 2.

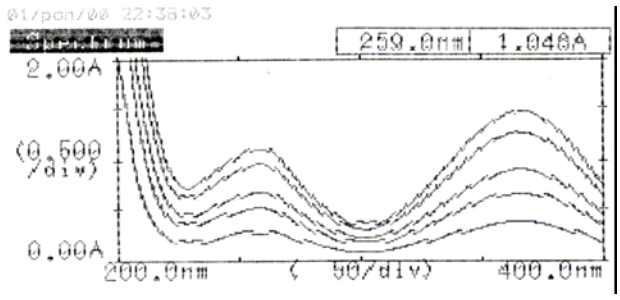

Fig. 2: UV absorbance spectra of Furazolidone in mixture of 2 M sodium acetate, 8 M urea, 2 M niacinamide and 2 M sodium benzoate solution (25:25:25:25% V/V).

Table 1: Optical characteristics and linearity data

Parameters	FZ
Absorption maximum (nm)	360
Beer's law limit (µg/ml)	10 – 50
Correlation coefficient	0.9992
Regression equation (Y=m X+C)	Y=0.80887 X+ (-0.0004)
Intercept (C)	-0.0004
Slope (m)	0.80887

Table 2: Analysis of tablet formulation

Brand	(* % Amount found ± SD)
Furoxon (100 mg)	96.95±0.69

*Mean of nine determinations (3 replicates at 3 concentration level)

Table 3: Results from Recovery Studies

Furazolidone	Quantity of Drug Added	(* % Amount found ± SD)
10	10	97.4±1.50
20	20	97.36±1.90
30	30	98.58±1.18
40	40	98.9±0.84
50	50	97.32±1.37

*Mean of fifteen determinations (3 replicates at 5 concentration level)

Table 4: Results from precision

Validation Parameters	* % Mean ± S.D*. (n=6)	* %RSD
Repeatability	97.21±0.19	0.72
Intermediate precision		
Day to Day	96.52±0.29	0.86
Analyst to Analyst	96.98±0.12	0.42
LOD	0.135 µg/ml	
LOQ	0.466 µg/ml	
Robustness	96.03±1.11	1.14

*****Mean of fifteen determinations (3 replicates at 5 concentration level)

Preparation and analysis of tablet formulations

Twenty tablets were accurately weighed, average weight determined and ground to fine powder. An accurately weighed quantity of powder equivalent to 100 mg of FZ was transferred into 100 ml volumetric flask containing 80 ml of hydrotropic solution. The flask was sonicated for about 20 min to solublize the drug, volume was adjusted to mark with Double R.O. water and filtered through Whatmann filter paper no. 41. The resulting solution was further diluted to get concentration of 10, 20, 30, 40 and 50 µg/ml of FZ. Absorbances of sample solution were

analyzed on UV spectrophotometer at 360 nm against Double R.O. water as blank. Drug content of tablet formulation were calculated using prepared calibration curve. The results of analysis of tablet formulation were presented in table 2.

Validation of Method (ICH guidelines, 2005)
The method was validated with reference to accuracy, precision, LOD, LOQ, and robustness.

Accuracy
The accuracy of the proposed method was assessing by recovery studies. The recovery studies were conceded by adding known amount of standard solution of FZ to preanalyzed tablet solutions. The resulting solutions were then re-analyzed by proposed methods; the results were shown in table 3.

Precision
Precision of the methods was established at three levels; as repeatability, intermediate precision and reproducibility. Study was performed by analyzing, the five different concentration of drug for three times in the same day (Inter-day precision), three days in a week (Intra-day precision) and with different analyst (Analyst to Analyst pricion). Reproducibility was performed by analyzing same concentration of drugs for five times in different lab. The results are shown in table 4.

LOD and LOQ
LOD and LOQ of the proposed method were calculated by using the standard deviation method

RESULT

Stability studies (to confirm the stability of drug in the mixed hydrotropic solvent, drug was kept with mixed hydrotropic solvent for 6 hr. and analyzed them by 1 hr. interval by spectrophotometricaly) shows that drug is stable in 2 M sodium acetate, 8 M urea, 2 M niacinamide and 2 M sodium benzoate solution (25:25:25:25% V/V). One wavelengths 360 nm (λ_{max} for FZ) was selected for analysis of FZ. Linearity was observed in the range 10-50 µg/ml with correlation coefficient (r^2)=0.9992 for FZ. The amount of drug estimated by the proposed method was in resembled with the label claim. The proposed method was validated as per the ICH guideline. The accuracy of the method was assessed by recovery studies and it was found to be 97.32 to 98.9%. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. This method was found to be precise as shown by the Repeatability (97.21±0.19), Day to Day analysis (96.52±0.29), Analyst to Analyst analysis (96.98±0.12), LOD (0.135 µg/ml) and LOQ (0.466 µg/ml) showing %RSD less than 2. The results of precision are shown in table 4.

DISCUSSION

All statistical data proves validity of the method and can be used for routine analysis of pharmaceutical formulations containing this drug by using hydrotropic agent. Use of hydrotropic agent in the analysis of drugs makes method eco-friendly and economic.

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