ORIGINAL ARTICLE

Novel UV spectrophotometer methods for quantitative estimation of metronidazole and furazolidone using mixed hydrotropy solubilization

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Ruchi Jain [a],*, **Nilesh Jain** [b], **Deepak Kumar Jain** [c], **Vijay Kumar Patel** [c], **Harish Rajak** [c], **Surendra Kumar Jain** [b]

[a] *Suresh Gyan Vihar University, Jaipur, Rajasthan, India*
[b] *Sagar Institute of Research & Technology-Pharmacy, Ayodhya Bypass Road, Bhopal 462 041, MP, India*
[c] *Medicinal Chemistry Research Laboratory, SLT Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur 495 009, CG, India*

Abstract Two simple, accurate, novel, safe and precise methods were developed for the simultaneous estimation of poorly water-soluble drugs Metronidazole (MTR) and Furazolidone (FZ) in a tablet dosage form using 2 M sodium acetate and 8 M urea solution (50:50%V/V) as a mixed hydrotropic solution. MTR and FZ show maximum absorbances at 319 and 364 nm, respectively. Sodium acetate and urea solution did not show any absorbance above 240 nm and thus no interference in the estimation of drugs was seen. MTR and FZ follow Beer's law in the concentration range of 10–50 µg/ml and 5–25 µg/ml ($r^2 = 0.9992$ and 0.9996). Method-A employs the simultaneous equation method using 319 and 364 nm as two analytical wavelengths, method-B employs the absorption ratio method, which uses 339.2 and 364 nm as two analytical wavelengths for estimation of MTR and FZ. The mean percent label claims of tablet dosage were found to be 98.715 ± 1.012 and 98.74 ± 0.912 in method A, 98.99 ± 0.872 and 97.89 ± 0.903 in method B for MTR and FZ, respectively. The developed methods were 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 therefore both methods can be used for routine monitoring of MTR and FZ in industry in the assay of bulk drug and tablets.

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* Corresponding author. Mobile: +91 9425184145.
  E-mail address: jainruchi02@gmail.com (R. Jain).
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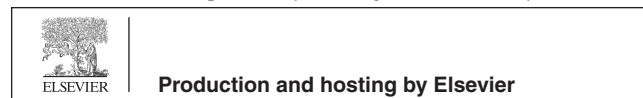
1. Introduction

Metronidazole (MTR) chemically 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol (Fig. 1), is a nitroimidazole used to treat amebiasis, vaginitis, trichomonas infections, giardiasis, anaer-
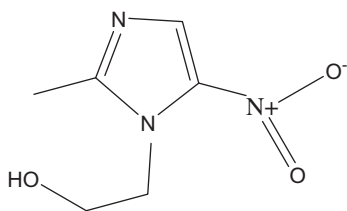
Figure 1   Chemical Structure of Metronidazole.

obic bacteria and treponemal infections. It is also used to treat Crohn's disease (Sweetman, 1999; Oneil et al., 2001). Furazolidone (FZ) chemically 3-(5-nitrofurfurylideneamino)-2-oxazolidinone (Fig. 2), is synthetic antimicrobial nitrofurans, which acts in a broad antibacterial spectrum covering the majority of gastrointestinal tract pathogens including *Escherichia coli*, staphylococci, *Salmonella*, *Shigella*, *Proteus*, *Aerobacter aerogenes*, *Vibrio cholerae* and *Giardia lamblia* (Sweetman, 1999; Oneil et al., 2001).

Metronidazole is official in IP (Indian Pharmacopoeia, 2010), BP (British Pharmacopoeia, 2004), USP (The United States Pharmacopoeia and The National Formulary, 2000). The literature survey reveals that several analytical methods viz. the UV method (Rehman et al., 2005; Adegoke and Umoh, 2009), HPLC (Mustapha et al., 2006; Cox et al., 2009) and voltammetry (Bartlett et al., 2005) method have been reported for quantitative estimation of MTR. Quantitation of metronidazole and spiramycin by LC–MS/MS (Sagan et al., 2005), HPLC with UV detection method (Maher et al., 2008) and potentiometric (Khattab et al., 2011) method has been reported.

Furazolidone is official in IP (Indian Pharmacopoeia, 2010) and USP (The United States Pharmacopoeia and The National Formulary, 2000). The literature survey reveals that various analytical methods have been developed such as HPLC (Cieri, 1979), HPTLC (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, 1972) for the estimation of FZ in biological fluids and in pharmaceutical formulations. Some reports are available for the estimation of MTR and FZ in a tablet dosage form by UV methods (Lopez-de-Alba et al., 1997; Basu and Mahalanabis, 1991; Kale et al., 2012; Chemate et al., 2012).

Hydrotropic solubilization is the phenomenon by which aqueous solubility of poorly water soluble drugs and insoluble drugs increases. Various techniques have been employed to enhance the aqueous solubility and hydrotropy is one of them. Sodium salicylate, sodium benzoate, urea, nicotinamide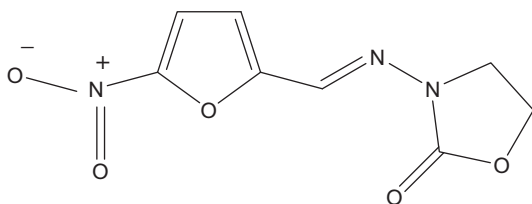, sodium citrate and sodium acetate are the most common examples of hydrotropic agents utilized to increase the water solubility of the drug. Maheshwari (2005, 2006) and (Jain et al. (2010a,b,c,d) have analyzed various poorly water-soluble drugs using hydrotropic solubilization phenomenon viz. ketoprofen, salicylic acid, frusemide, torsemide, hydrochlorothiazide, pramipexole and amlodipine besylate. Various organic solvents such as methanol, chloroform, dimethyl formamide and acetonitrile have been employed for solubilization of poorly water-soluble drugs to carry out spectrophotometric analysis. Drawbacks of organic solvents include their higher cost, toxicity and pollution. Hydrotropic solution may be a proper choice to preclude the use of organic solvents.

Therefore, it was thought worthwhile to employ this mixed hydrotropic solution to extract out the drug from fine powder of tablets to carry out spectrophotometric estimation. There are no reports yet for the determination of this combination by proposed methods. The present work emphasizes on the quantitative estimation of MTR and FZ in their combined dosage form by UV Spectroscopic methods.

2. Experimental procedure

2.1. Materials and methods

Pure sample of MTR and FZ was obtained as a gift sample from Swan pharmaceutical, Indore and GSK Ltd. Mumbai, respectively. Sodium acetate and urea were obtained from Merck Chemical Division, Mumbai. Reverse osmosis water was used throughout the study. A Shimadzu UV/VIS 1700 spectrophotometer with 1 cm matched quartz cells was used for the estimation.

2.2. Preliminary solubility studies of drugs

Solubility of both drugs was determined at $25 \pm 1\ °C$. An excess amount of drug was added to two screw capped 25 ml of volumetric flasks containing different aqueous systems viz. distilled water and different combination of hydrotropic agent. The volumetric flasks were shaken mechanically for 12 h at $25 \pm 1\ °C$ in a mechanical shaker. These solutions were allowed to equilibrate for next 24 h. and then centrifuged for 5 min at 2000 rpm. The supernatant liquid was taken for appropriate dilution after filtering through Whatman filter paper #41 and analyzed spectrophotometrically against water as blank. After analysis, it was found that the enhancement in the solubility of MTR and FZ was found to be more than 36 and 28 folds, respectively in a mixture of 2 M sodium acetate and 8 M urea solution (1:1) as compared to solubility studies in other solvents.

2.3. Selection of hydrotropic agent

MTR and FZ were scanned in hydrotropic agent in the spectrum mode over the UV range 200–400 and a mixture of 2 M sodium acetate and 8 M urea (50:50% V/V) solution was found to be most appropriate because:

- MTR and FZ are soluble in it (36 and 28 fold enhancement of solubility)
- MTR and FZ are stable in hydrotropic agent.

Figure 2   Chemical Structure of Furazolidone.

- MTR and FZ, both exhibit good spectral characteristics in it.
- Sodium acetate and urea solution have no interference with the λ_{max} of MTR and FZ, 319 and 364 nm, respectively (Fig. 3).

2.4. Establishment of stability profile

Stability of MTR and FZ was observed by dissolving in a mixture of 2 M sodium acetate and 8 M urea (50:50% V/V) solution used as hydrotropic agent. Solution of MTR and FZ was scanned under time scan for 30 min. Spectra of the drug under time scan shows that drug is stable in hydrotropic solution.

2.5. Linearity range and calibration graph

2.5.1. Preparation of standard stock solutions of MTR and FZ
Standard stock solutions of 1000 µg/ml were prepared by dissolving separately 100 mg of each drug in mixed hydrotropic solution and the flask was sonicated for about 10 min to solubilize the drug (Stock-A).

2.5.2. Preparation of working standard solution for calibration curve
The standard solution (1000 µg/ml) was further diluted in different dilutions and prepared ranging from 10–50 µg/ml for MTR and 5–25 µg/ml for FZ. The calibration curve was plotted between concentrations and absorbances. Linearity data and result of their optical characteristics are shown in Table 1).

2.6. Study of overlay spectra of drugs and selection of method

The spectra exhibit major absorbance maxima at 319 nm and 364 nm for MTR and FZ, respectively and isosbestic point at 339.2 nm Fig. 3. Due to difference in absorbance maxima and having no interference with each other both drugs can be simultaneously estimated by the simultaneous equation method (Method A) and the Q-analysis method (Method B).

2.6.1. Vierordt's simultaneous equation method (Method A)
The wavelength 319 nm (λ_{max} of MTR) and 364 nm (λ_{max} of FZ) was selected. The absorbencies of MTR and FZ were measured at 319 nm and 364 nm. This method of analysis is based on the absorption of drugs X and Y at the wavelength maxima of the other. The quantification analysis of MTR and FZ in a binary mixture was performed by using Eqs. (1) and (2). Where C_X and C_Y are the concentrations of MTR and FZ, respec-
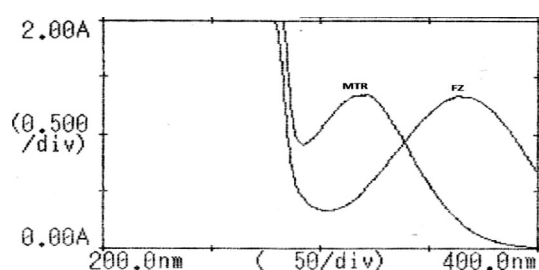
Figure 3 Overlay Spectra of MTR 50 µg/ml and FZ 25 µg/ml.

Table 1 Optical characteristics and linearity data of MTR and FZ.

Sr. No.	Parameters	MTR	FZ
1	Working λ	319 nm	364 nm
2	Beer's law limit (µg/ml)	10–50	5–25
3	Correlation coefficient (r^2)[*]	0.9992	0.9996
4	Slope (m)[*]	0.0277	0.0600
5	Intercept (c)[*]	−0.0229	0.0115
6	LOD (µg/ml)	0.323	0.443
7	LOQ (µg/ml)	0.082	0.743

[*] Average of five determinations.

tively in the diluted sample, ax_1 and ax_2 are absorptivities of MTR at λ_1 and λ_2, ay_1 and ay_2 are absorptivities of FZ at λ_1 and λ_2, respectively (Table 2)). A_1 and A_2 are the absorbances of samples at the 319 and 364 nm, respectively (Beckett et al., 2002).

$$C_X = A_2ay_1 - A_1ay_2/ax_2ay_1 - ax_1ay_2 \tag{1}$$

$$C_Y = A_1ax_2 - A_2ax_1/ax_2ay_1 - ax_1ay_2 \tag{2}$$

2.6.2. Q-analysis method (Method B)
In this method absorbances of both the drugs were calculated at two selected wavelengths; among which λ_1 is the wavelength of isoabsorptive point of both drugs and λ_2 is the λ_{max} of either drug among both drugs. From the overlain spectra wavelength 339.2 nm (isoabsorption point) and 364 (λ_{max} of FZ) were selected for the study. The absorbencies at 339.2 nm and 364 nm for MTR were obtained and similarly for FZ absorbencies are measured at 339.2 nm and 364 nm. The concentrations of the individual components were calculated by using the following equations;

$$C_X = Q_m - Q_y/Q_x - Q_y \times A_1/ax_1 \tag{3}$$

$$C_Y = Q_m - Q_y/Q_y - Q_x \times A_1/ax_1 \tag{4}$$

where $Q_m = A_2/A_1$, A_1 is absorbance of sample at isoabsorptive point, A_2 is absorbance of sample at λ_{max} of one of the two components. ax_1 and ax_2 represent absorptivities of MTR at λ_1 and λ_2 and ay_1 and ay_2 denote absorptivities of FZ at λ_1 and λ_2, respectively (Table 2); C_X and C_Y are the concentrations of MTR and FZ, respectively (Beckett et al., 2002; Pernarowski et al., 1960)

3. Analysis of tablet formulation

Twenty marketed tablets of MTR and FZ, Metrofur (Western Remedies) were weighed and ground to a fine powder; amount equal to 200 mg of MTR was taken in a 10 ml volumetric flask. The FZ present in this amount of tablet powder was 100 mg. Then 80 ml of sodium acetate and urea solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with RO water to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected

Table 2 Absorptivities of MTR (x) and FZ (y) at λ_1 and λ_2.

Drug	Method-I				Method-II			
	319 nm (λ_1)		364 nm (λ_2)		339.2 nm (λ_1)		364 nm (λ_2)	
MTR	ax_1	0.0249	ax_2	0.0054	ax_1	0.0180	ax_2	0.0054
FZ	ay_1	0.0239	ay_2	0.0614	ay_1	0.0435	ay_2	0.0614
					Q_X	0.3000	Q_Y	1.4115
				$N = 5$				

wavelengths and the concentrations were obtained from the simultaneous equation method and the absorbance ratio method. The result of statistical evaluation of tablet analysis is reported in Table 3.

4. Validation of method

The developed methods for simultaneous estimation of EPS and HCZ were validated as per ICH guidelines (Linearity, Accuracy, Precision and Robustness) (ICH, 2005).

4.1. Linearity

Linearity of MTR and FZ was established by response ratios of drugs. Response ratio of both drugs was calculated by

dividing the absorbance with respective concentration and then a graph was plotted between concentration and response ratio.

4.2. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of MTR and FZ were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines. The limit of detection (LOD) and limit of quantification (LOQ) for MTR and FZ were found to be 0.323 µg/ml, 0.082 µg/ml and 0.443 µg/ml, 0.743 µg/ml, respectively (Table 1) indicating that the proposed UV method is highly sensitive.

Table 3 Results and statistical parameters for tablet analysis: Metrofur (MTR-200/FZ-100).

S. No.	Drug	Label claim	Amount found	Recovery%[*]	S.D.[*]	%COV[*]	STD. error[*]
Method A	MTR	200	197.43	98.715	1.012	1.025	0.187
	FZ	100	98.74	98.74	0.912	0.924	0.169
Method B	MTR	200	197.98	98.99	0.872	0.881	0.161
	FZ	100	97.89	97.89	0.903	0.922	0.169

[*] Average of five determinations.

Table 4 Results of Recovery Studies on Marketed Formulations.

Recovery level%	% Recovery (Mean ± SD)[*]			
	Method A		Method B	
	MTR	FZ	MTR	FZ
80	98.39 ± 1.023	98.36 ± 0.868	98.44 ± 0.869	98.67 ± 0.721
100	97.45 ± 0.903	98.42 ± 0.475	97.93 ± 0.722	98.40 ± 0.172
120	98.64 ± 0.172	98.54 ± 0.673	97.85 ± 0.343	97.74 ± 0.451
Mean	98.16 ± 0.699	98.44 ± 0.672	98.07 ± 0.644	98.27 ± 0.448

[*] Average of five determination.

Table 5 Results of validation (Mean ± SD).

Parameter	Method – A				Method – B			
Precision (Mean ± SD)[*]	MTR	% RSD	FZ	% RSD	MTR	% RSD	FZ	% RSD
Repeatability	98.12 ± 1.09	1.111	99.55 ± 0.93	0.934	98.09 ± 0.32	0.326	98.62 ± 0.94	0.953
Day to day	98.35 ± 0.85	0.864	99.46 ± 0.65	0.654	98.86 ± 0.47	0.475	98.43 ± 0.88	0.894
Analyst to analyst	98.24 ± 0.83	0.845	98.73 ± 0.93	0.942	98.74 ± 0.61	0.618	98.41 ± 0.39	0.396
Reproducibility	97.69 ± 0.73	0.747	98.24 ± 1.12	1.140	98.66 ± 0.46	0.466	98.34 ± 0.22	0.224
Robustness[*] (Ratio)	98.38 ± 0.25	0.254	98.19 ± 1.02	1.039	98.84 ± 1.04	1.052	99.51 ± 0.81	0.814
(Temperature)	97.68 ± 0.93	0.952	98.55 ± 0.28	0.284	98.85 ± 0.61	0.617	97.86 ± 0.76	0.776
(Concentration)	98.78 ± 0.27	0.273	98.69 ± 0.85	0.861	98.78 ± 0.57	0.577	98.79 ± 0.46	0.465

[*] Average of five determinations.

4.3. Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e., 80%, 100% and 120%. The recovery studies were carried out by adding a known amount of standard solution of MTR and FZ to preanalyzed tablet solutions. The resulting solutions were then re-analyzed by proposed methods. Total amount of drug found and percentage recovery were calculated. Results of recovery studies are reported in Table 4.

4.4. Precision

Precision of the methods was studied at three levels as repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility (Table 5).

4.5. Robustness

For the robustness of the analytical method we changed the temperature 25 ± 5 °C, centrifugation time 10 min and the ratio of hydrotropic solution. Instead the 50:50 ratios of sodium acetate and urea and 60:40 sodium acetate and urea were used as solvent (Table 5).

5. Results and discussions

Based on the solubility and stability and spectral characteristics of the drugs, 2 M sodium acetate and 8 M urea solution (50:50% W/V) were used as a mixed hydrotropic solution. It was found that solubility enhancement of MTR and FZ was more than 36 and 28-fold, respectively in mixed hydrotropic solution as compared with distilled water. MTR and FZ show maximum absorbances at 319 and 364 nm, respectively. Sodium acetate and urea solution did not show any absorbance above 240 nm and thus no interference in the estimation of drugs was seen. MTR and FZ follow Beer's law in the concentration range of 10–50 μg/ml and 5–25 μg/ml ($r^2 = 0.9992$ and 0.9996). Method-A employs the simultaneous equation method using 319 and 364 nm as two analytical wavelengths, method-B employs the absorption ratio method, which uses 339.2 and 364 nm as two analytical wavelengths for estimation of MTR and FZ. The optimized methods showed good reproducibility and mean recovery with 98.16 ± 0.699 and 98.44 ± 0.672 in method A and 98.07 ± 0.644 and 98.27 ± 0.448 in method B for MTR and FZ, respectively. The mean percent label claims of tablet dosage were found to be 98.715 ± 1.012 and 98.74 ± 0.912 in method A, 98.99 ± 0.872 and 97.89 ± 0.903 in method B for MTR and FZ, respectively. The standard deviation, coefficient of variance and standard error were obtained for MTR and FZ were satisfactorily low. Result of precision at different levels was found to be within acceptable limits (RSD < 2).

6. Conclusion

There was no interference of 2 M sodium acetate and 8 M urea solution (50:50% W/V) in the estimation and hence the two UV spectrophotometric methods were found to be simple, accurate, economic and rapid for simultaneous estimation of MTR and FZ in bulk and tablet dosage forms. The proposed method can be successfully employed for the routine analysis of MTR and FZ containing dosage forms.

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