International Journal of Advances in Pharmaceutics

Journal home page:http://ijap.ssjournals.com

Research Article

Devlopment and validation of RP-HPLC Method for estimation of Metronidazole and Norfloxacin in suspension form

Kuldip D Trivedi*, Ankit B Chaudhary and Sellapan Mohan

Saraswati Institute of Pharmaceutical Sciences, Dhanap, Gandhinagar, Gujarat, India.

***Correspondence Info:**

Kuldip D Trivedi
Saraswati Institute of Pharmaceutical Sciences, Dhanap, Gandhinagar, Gujarat, India
Email: trivedi.kuldeep007@gmail.com

Keywords:
Metronidazole,
Norfloxacin,
RP-HPLC,
Validation

Abstract

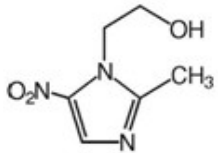
A simple reversed- phase high-performance liquid chromatographic method (RP-HPLC) has been developed and validated for estimation of Development & Validation of Analytical method for Estimation of Metronidazole and Norfloxacin in pharmaceutical dosage form**.** Chromatographic separation was carried out on YL 9100 equipped with PDA detector using C_{18} (250mm × 4.6mm, 5µ) as stationary phase and mobile phase Triethylamine: 0.02M Potassium Dihydrogen phosphate (pH 3.5): Methanol (0.01:70:30) at flow rate of 1ml/min. Wavelength for UV detection was 292nm. The retention time for Metronidazole and Norfloxacin was found to be 6.10 and 3.50 min. The method was validated as per ICH guideline and and can be applied for estimation of Metronidazole and Norfloxacin in suspension. The linearity was found over concentration range of 12.5-37.5µg/ml for Metronidazole and Norfloxacin.

1. Introduction

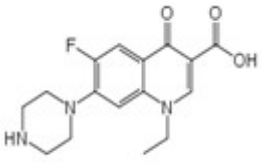
Metronidazole is a prodrug. Chemically is a 2-(2-methyl-5-nitroimidazol-1-yl), ethanol used as Antibiotic, Amebicide[1]. Norfloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. Chemically is a 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid used in treatment of urinary tract of infection[2].

Literature survey reveals that, UV, HPLC, Spectrophotometric method are reported for Metronidazole alone and combination with other drugs[3-10]. UV, HPLC, Spectrophotometric method and simple electrolytical method are reported for Norfloxacin alone and combination with other drugs[11-17]. The chemical structure of Metronidazole and Norfloxacin are given below.

Figure 1: Chemical Structure of (a) Metronidazole and (b) Norfloxacin



a)



(b)

2. Materials and Methods

Metronidazole (METRO) and Norfloxacin (NOR) were gifted by Molecule Lab Pvt. Ltd. (Ahmedabad, Gujarat). All chemical and reagents used were of HPLC grade for HPLC analysis and analytical grade for spectroscopic study. Commercial pharmaceutical suspension preparation was claimed to contain 100mg/5ml of Metronidazole and 100mg/5ml of Norfloxacin.

2.1 Instrument and Reagents

High performance liquid chromatography YL 9100 with PDA detector was used.Mobile phase comprising of was Triethylamine: 0.02 M Potassium Dihydrogen phosphates (pH 3.5): Methanol (0.01:70:30) at flow rate of 1ml/min was performed on C18 column (250mm × 4.6mm, 5µ).The effluent was detected at 292nm.The retension times of Metronidazole and norfloxacin were 6.10 and 3.50 min. The volume of injection was 20µl.

2.2 Selection of mobile phase

Sr. No.	Mobile Phase	Inference
1	Methanol : Water (50 : 50)	1st Peak showed fronting
2	Acetonitrile : Water (50 : 50)	1st Peak showed fronting
3	0.02M Potassium Dihydrogen phosphate (pH 4.5) : Methanol (50 : 50)	Less resolution with tailing
4	0.02M Potassium Dihydrogen phosphate (pH 4.5) : Methanol (60:40)	Both peak resolve but with tailing
5	0.02M Potassium Dihydrogen phosphate (pH 3.5) : Methanol (60:40)	Both peak resolve but 1st peak come at solvent level
6	Triethylamine : 0.02M Potassium Dihydrogen phosphate (pH 3.5) : Methanol(0.01:70:30)	Well resolved peaks with good shape

2.3 Preparation of Mobile phase

2.72gm of Potassium Dihydrogen Phosphate was weighed and dissolved it in to 500ml of HPLC water. Volume was made upto 1000ml with water. The final pH 3.5was adjusted using O-Phosphoric Acid. Finally composition of mobile phase was Triethylamine: 0.02 M Potassium Dihydrogen phosphates (pH 3.5): Methanol (0.01:70:30). The prepared mobile phase was filter through 0.45µm membrane filter paper.

2.4 Standard Stock Solution

An accurately weighed quantity of standard MET (50 mg) and NOR (50 mg) powder were transferred to 50 ml volumetric flasks. Both drugs were dissolved in 30 ml of mobile phase. The flasks were shaken and sonicated for 15min and volumes were made up to mark with mobile phase. 25 ml of resulting solution was transferred in 100 ml volumetric flask and made up to mark with mobile phase to get 250 µg/ml MET and NOR solution. Linearity was determined in the range of 12.5-37.5µg/ml for MET and NOR.

2.5 Assay in formulation

Accurately measured 1.25 ml of Metronidazole and Norfloxacin suspension was transferred to 50 ml volumetric flask and dissolved in 30 ml of mobile phase. The flask was shaken and sonicated for 5 min and volumes were made up to mark with mobile phase to get 100 µg/ml. The sample solution was further diluted with mobile phase to obtain the final concentration of 25µg/ml for each.

3. Result and Discussion

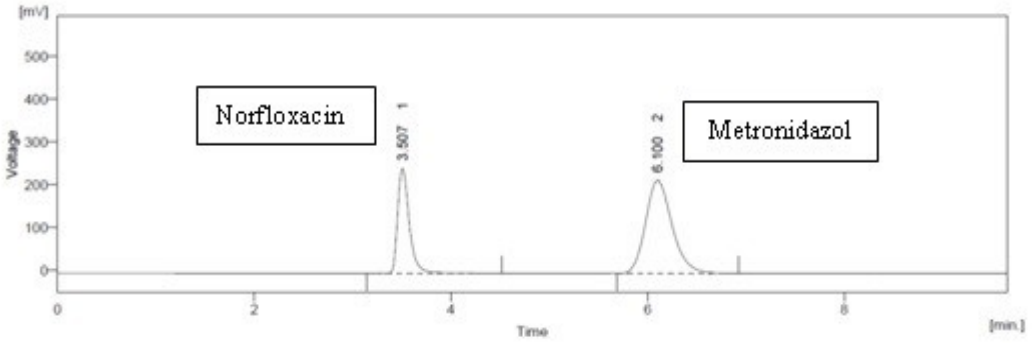
To optimize the RP-HPLC parameters, several mobile phase combination were tried. A satisfactory separation and good peak symmetry was found in a mixture of Triethylamine: 0.02 M Potassium Dihydrogen phosphates (pH 3.5): Methanol (0.01:70:30) at flow rate 1ml/min proved to be better than the other mixture in terms of resolution and peak shape. The optimum wavelength for detection was set at 292nm at which much better detector responses for both drug were

obtained. As show in Fig. 2, the retention times were 6.10 min for Metronidazole and for 3.50 min for Norfloxacin. The optical regression characteristics and validation parameters are showed in table 1.

Table 1: Optical regression characteristics and Validation parameters

Parameters	MET	NOR
Calibration range (µg/ml)	12.5-37.5	12.5-37.5
Regression Equation (y = mx + c)	y = 129.3x + 31.83	y = 78.84x + 14.53
Quantitation limit (µg/ml)	3.980	3.245
Detection limit (µg/ml)	1.313	1.071
Slope(m)	129.3	78.84
Intercept(c)	31.83	14.53
Correlation coefficient(r)	0.998	0.999
Intraday RSD* %	0.53-1.27	0.49-1.28
Interday RSD*%	0.76-1.59	0.97-1.54

Figure 2: Chromatogram for Metronidazole (25 µg /ml) and Norfloxacin (25 µg/ml)



3.1 Method validation

3.1.1 Linearity

The linearity of the response for MET and NOR was determined by preparing and injecting standard solutions of 12.5-37.5µg/ml MET and 12.5-37.5µg/ml NOR. The calibration curves of MET and NOR (5 replications) showed in Figure 4.9 and 4.10 respectively indicate that the response was linear over the concentration range. Correlation coefficient (r) was found 0.998 for MET and 0.999 for NOR as shown in Figure 3 and 4 respectively. The calibration curve was obtained by plotting absorbance(y axis) → concentrations(x axis).

Figure 3: Calibration curve of Metronidazole

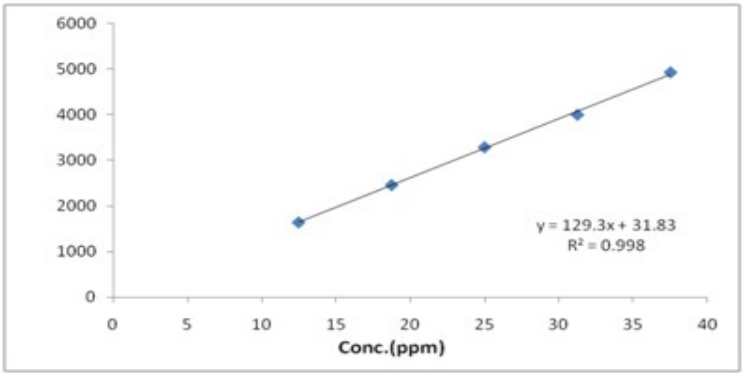
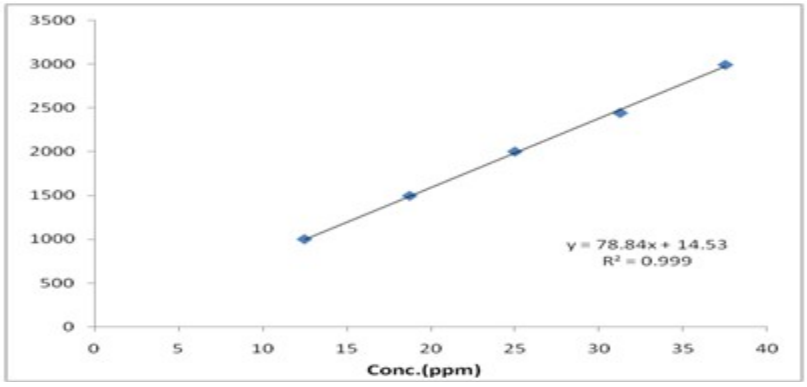


Figure 4: Calibration curve of Norfloxacin



3.1.2 Accuracy

Accuracy was performed at 50%, 100% and 150% levels by Standard addition method. Each concentration was analyzed 3 times and average recoveries were measured as shown in Table 2.

Table 2: Recovery studies of Metronidazole and Norfloxacin

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Total Amount (µg/ml)	Amount found* (µg/ml)	% Recovery ± S.D. (n=3)
MET	50%	10	5	15	14.99	99.93±0.048
	100%	10	10	20	19.98	99.9±0.23
	150%	10	15	25	25.034	100.13±0.23
NOR	50%	10	5	15	14.89	99.26±0.050
	100%	10	10	20	19.87	99.35±0.22
	150%	10	15	25	24.95	99.8±0.31

3.1.3 Limit of Detection and Quantification

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The detection limit(LOD) and quantification limit (LOQ) may be expressed as:

$LOD = 3.3 \times N/S$

$LOQ = 10 \times N/S$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of Detection for MET and NOR was found to be 1.313µg/ml and 1.071µg/ml respectively.

Limit of Quantitation for MET and NOR was found to be 3.980µg/ml and 3.245µg/ml respectively.

3.1.4 Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Intraday precision was determined by analyzing MET and NOR three times in the same day. Inter day precision was determined by analyzing both the drugs three successive days.

Table 3: Precision study of Metronidazole

Conc. (µg/ml)	Intraday Mean ± S.D (n=3)	%RSD	Interday Mean ± S.D (n=3)	%RSD
12.5	1656.69±19.23	1.16	1645.043±24.21	1.47
25	3272.42±41.83	1.27	3253.49±24.88	0.76
37.5	4808.87±25.87	0.53	4894.75±77.99	1.59

Table 4: Precision study of Norfloxacin

Conc. (µg/ml)	Intraday Mean ± S.D (n=3)	%RSD	Interday Mean ± S.D (n=3)	%RSD
12.5	1008.81±5.01	0.49	1004.40±9.75	0.97
25	1990.50±25.52	1.28	2002.19±24.82	1.23
37.5	1990.68±30.37	1.01	2977.72±46.07	1.54

3.1.5 Robustness

Robustness was carried by varying experimental parameters of proposed method. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, and flow rate. No significant change was observed.

Table 5: Robustness parameter of Metronidazole and Norfloxacin.

Factor	Value	AREA	
		MET	NOR
Ph	3.3	3252.26	2020.37
	3.5	3308.81	2012.494
	3.7	3312.06	1992.90
	Mean±S.D.	3291.033± 33.61	2008.59± 14.14
	%R.S.D.	1.021	0.704
Mobile phase	Triethylamine: 0.02M Potassium Dihydrogen phosphate (pH 3.5) : Methanol (0.01:68:32)	3341.905	2042.219
	Triethylamine: 0.02M Potassium Dihydrogen phosphate (pH 3.5) : Methanol (0.01:70:30)	3308.81	2012.494
	Triethylamine: 0.02M Potassium Dihydrogen phosphate (pH 3.5) : Methanol (0.01:72:28)	3289.293	1997.092
	Mean±S.D.	3313.33± 26.59	2017.26±22.93
	%R.S.D.	0.802	1.13
Flow rate	0.9	3367.71	2049.67
	1	3308.81	2012.494
	1.1	3279.78	1988.28
	Mean±S.D.	3318.76± 44.80	2016.81±30.92
	%R.S.D.	1.34	1.53

3.2 Assay of Pharmaceutical dosage form

One brands of MET and NOR combination was estimated and results are shown in Table 6.

Table 6: Analysis of marketed formulation

Formulation	MET			NOR		
	Amount Labelled (mg)	Amount Found* (mg)	%Amount Found ± S.D (n=3)	Amount labeled (mg)	Amount Found* (mg)	% Amount Found ± S.D (n=3)
Brand I	100	99.95	99.95±0.93	100	101.73	101.73±0.75

3.3 System suitability testing

System suitability test parameters like Resolution, Retention Time, Theoretical plate and Tailing factor are shown in Table 7.

Table 7: System suitability parameters of RP-HPLC method

Parameters	MET	NOR
Retention Time	6.10	3.50
Theoretical plate	2568	4246
Tailing factor	1.352	1.667
Resolution	7.444	

4. Conclusion

All these factors lead to conclude that the proposed method is accurate, precise, rapid and can be applied successfully for the estimation of MET and NOR in suspension without interference and with good sensitivity.

Reference

1. "Metronidazole Introduction", September 2012 http://www.drugbank.ca/drugs/DB00916
2. "Norfloxacin Introduction", September 2012 http://www.drugbank.ca/drugs/DB01059
3. Mishra AK, Yadava R, Mishra A, Verma A, Chattopadhyay P, "Development and validation of U.V. spectrophotometric method for the determination of Metronidazole in tablet formulation" *Int. J. Pharma Res. Dev.* 2010, 2(6), 1-5.
4. Thulasamma P and Venkateswarlu P, "Spectrophotometric method for the determination of Metronidazole in pharmaceutical pure and dosage forms" *Rasayan J. Chem*. 2009, 2(4), 865-869.
5. Farhadi K and Bahar S, "Kinetic-Spectrophotometric determination of Metronidazole Benzoate in surfactant medium" *J. Indian Chem. Soc.* 2007; 54, 1521-1527.
6. Hassan S, Hussain S, Ansari MT, "Quantitation of Metronidazole in pharmaceutical suspension using High Performance Liquid Chromatographic method" *Pakistan J. Zool.*, 2011; 43(5), 909-914.
7. Patel S, "Development and Validation of HPLC method for simultaneous estimation of Ofloxacin and Metronidazole from pharmaceutical formulation" *Int. J. Pharmaceut. Fro. Res*., 2011; 1(2), 68-74.
8. Mishal A and Sober D, "Stability indicating reversed-phase liquid chromatographic determination of Metronidazole Benzoate and Diloxanide Furoate as bulk drug and in suspension dosage form" *J. Pharm. Biomed. Anal.* 2005, 39(4), 819–823.
9. Vega E, Dabbene V, Nassetta M, Sola N, "Validation of a reversed-phase LC method for quantitative analysis of intravenous admixtures of Ciprofloxacin and Metronidazole" *J. Pharm. Biomed. Anal.* 1999, 21(5), 1003-1009.

10. Gattavecchia E, Tonelli D, Breccia A, "Determination of Metronidazole, Misonidazole and its metabolite in serum and urine on rp-18 high-performance thin-layer chromatographic plates" *J. Chrom. B*, 1981; 224(3), 465-471.

11. Singh S,Inamullah, Sharma S, Yadav AK, Gautam H, "Estimation of Norfloxacin in tablet dosage form by using UV-visible spectrophotometer" *Der Pharmacia Lettre*, 2012; 4(6), 1837-1842,.

12. Boonme P, Phadoongsombut N, Ingkatawornwong S, Faroongsarng D, "The Formulation Development and Stability Study of Norfloxacin Suspension" *Thammasat Int. J. Sc. Tech.*, 2002; 7(1), 1-4.

13. Sher M, Hussain MA, Mehmood MH, Hassan MN, Bashir S, "Bioequivalence of Norfloxacin by HPLC-UV method" *J. Chil. Chem. Soc.* 2010, 55(2), 203-205.

14. Rege PV, Sathe PA, Salvi VS, "A simple electroanlytical method for estimation of Norfloxacin and Tinidazole individually from pharmaceutical formulation" *Res. J. Pharm. Bio. Che. Sci*. 2011, 2(2), 495-505.

15. Kassab NM, Singh AK, Kedor-Hackmam ERM, Santoro MIRM, "Quantitative determination of Ciprofloxacin and Norfloxacin in Pharmaceutical preparations by high performance liquid chromatography" *Braz. J. Pharm. Sci.* 2005, 41(4), 507-513.

16. Morton SJ, Shull VH, Dick JD, "Determination of Norfloxacin and Ciprofloxacin concentrations in serum and urine by high-pressure liquid chromatography" Antimicrob. *Agents and Chemother.*, 1986 30(2), 325-327.

17. Sebaiy MM, El-Shanawany AA, Adl SM, Abdel-Aziz LM, Hashem HA, "Rapid RP-HPLC method for simultaneous estimation of Norfloxacin and Tinidazole in tablet dosage form" *AJP. Ana*. 2011, 1(4), 79-84.