Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2014, 5(1):23-28

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Development and validation of liquid chromatographic method for metronidazole

Nandini R. Pai* and Deeptaunshu Atul Pusalkar

Department of Chemistry, D. G. Ruparel College, Mahim, Mumbai, Maharashtra, India

---

ABSTRACT

A simple isocratic, rapid and sensitive high performance liquid chromatographic method has been developed for quantitative determination of Metronidazole and its process related impurity. The method has been validated for determination of related substance in metronidazole using C8 ODS (250X4.6mm) column by keeping the flow rate of 1ml/min and having sensitivity of 4.The elution is carried out by using mobile phase of 300ml acetonitrile,200ml methanol, 100ml THF and 400 ml water(1.56gms/litre K_2HPO_4). pH is adjusted to 11 by TEA. The detection is carried out at 254nm with injection volume of 10 microliter. Specificity, system suitability, linearity, precision, ruggedness, robustness has been carried out for metronidazole. Limit of quantification and limit of detection has been carried out for the impurities of metronidazole.

Keywords: HPLC, metronidazole, related impurity, validation

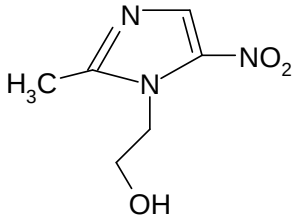
INTRODUCTION

Metronidazole is an antibiotic. It fights bacteria in your body. Metronidazole is an antibiotic, amebicide, and antiprotozoal. It's a off white crystalline powder with IUPAC name as 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol. Several liquid chromatographic methods are reported for estimation of metronidazole in various matrix systems.

For the development and validation for liquid chromatographic method of metronidazole following parameters were evaluated: Specificity, system suitability, accuracy, linearity, precision, ruggedness, robustness, limit of Quantification and Detection according to USP and ICH guidelines.

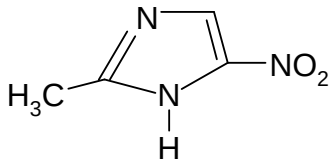
Structure of metronidazole and its impurity is as follows:

Metronidazole



2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol

Impurity



2-Nitro-5-Methyl imidazole

MATERIALS AND METHODS

Reagents and Chemicals

Metronidazole drug substance is synthesized from the impurity and taken as a reference standard for validation study. Analytical reagent grade K_2HPO_4 was purchased from merck Chemicals and HPLC grade methanol, Acetonitrile, from S.D Fine chemicals.

Preparation of Solutions, Chromatographic Conditions and System Suitability Parameter

Chromatographic Conditions

The elution is carried out by using mobile phase of 300ml acetonitrile,200ml methanol, 100ml THF and 400 ml water(1.56gms/litre K_2HPO_4). pH is adjusted to 11 by TEA. The detection is carried out at 254nm with injection volume of 10 microliter.

Standard solution Preparation

About 10 mg of Metronidazole Reference standard, accurately weighed was transferred in 50 mL volumetric flask, dissolved in sufficient mobile phase and diluted to the mark. This solution was further diluted with mobile phase to obtain required ppm solutions.

The proposed method for estimation of related substances of metronidazole is validated as per the guideline of United States Pharmacopoeia and ICH guidelines.

Method Validation

The proposed method for estimation of related substances of Metronidazole is validated as per the guideline of United States Pharmacopoeia and ICH guidelines.

(1) Specificity

Diluent, Metronidazole and its related impurity of 100 ppm are injected individually and in combination into the chromatograph.

Retention times of all the components are given in table no 1. From retention time, it can be seen that, all the components have different retention time. Diluent, Metronidazole and its impurities show different retention times. The entire components are well separated indicating the specificity of analytical method.

(2) Linearity

About 10 mg of accurately weighed sample is taken in 50ml volumetric flask and dissolved in sufficient amount of mobile phase and diluted up to the mark. This is taken as stock solution. From stock solution serial dilutions are made of different concentration level and injected for Metronidazole and its process related impurity which are given in table no 2.

The graph of concentration (on X-axis) Vs Area (on Y-Axis) is linear in nature passing through origin.

(3) Precision-

To ensure analytical system is working satisfactorily and giving precise results, 100ppm solution (from stock solution) of Metronidazole and its impurity were injected 5 times. RSD for retention time and area are calculated and tabulated below. Limit RSD: +/-2.0% [98.0% to 102.0%]. The individual area is found to be within 98.0 to 102.0% indicates that analytical system is well précised. The areas of metronidazole and its process related impurity are given in table no 3.

(4) Ruggedness

Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. Its a degree of exactness of a measurement to its true value.

Ruggedness of Metronidazole and its impurity are given in table no 4 .The individual area is found to be within 98.0 to 102.0% indicates that analytical system is well precise.

(5) Accuracy

Accuracy of Metronidazole is given below in table no 5. From stock solution of 200 ppm further dilutions are made for analysis.

(6) Robustness

Robustness can be described as the ability to reproduce the (analytical) method in different laboratories or under different circumstances without the occurrence of unexpected differences in the obtained results. It was carried out by change in flow rate, change in mobile phase composition, change in wavelength and change in pH. It is observed that method is unaffected by small changes in experimental conditions complies the robustness. Results are mentioned in table no7 to 14.

(7) Limit of quantification

Limit of quantification is lowest amount of analyte present in sample that can be determined with acceptable precision and accuracy under stated experimental conditions. Limit of quantification is calculated from signal to noise ratio.

(8) Limit of detection

The detection limit is characteristic of limit test. It is lowest amount of analyte present in sample that can be detected but not necessarily quantities, under stated condition. Limit of detection is calculated from signal to noise ratio. Results are given in table no 15.

Table 1: Specificity of Metronidazole

Concentration (ppm)	Component	Retention time (mins)
	Diluent	-
100	Metronidazole	3.14
100	Imp A	2.76

Table 2: Linearity of Metronidazole and its process related impurity

Sr No.	Concentration (ppm)	Metronidazole	Impurity
1	60	456.365	1655.419
2	70	537.637	1898.305
3	80	612.261	2146.805
4	90	692.267	2385.953
5	100	771.862	2672.124

Table 3: Precision of Metronidazole and its process related impurity

Sr No.	Concentration (ppm)	Metronidazole	Impurity
1	100	771.862	2672.124
2	100	776.815	2641.268
3	100	778.174	2639.204
4	100	782.416	2646.269
5	100	785.179	2646.335
	MEAN	778.8892	2649.04

Table 4: Ruggedness for Metronidazole and process related impurity

Sr No.	Concentration (ppm)	Metronidazole	Impurity
1	100	771.862	2672.124
2	100	776.815	2641.268
3	100	778.174	2639.204
4	100	782.416	2646.269
5	100	785.179	2646.335
	MEAN	778.8892	2649.04

Accuracy for metronidazole and process related impurities

Table 5: Metronidazole

Level	Concentration (ppm)	Area	Amount recovered	% Recovery
80%	80%	612.261	78.60	98.25
100%	100%	778.8892		
120%	120%	939.262	120.58	100.49

Table 6: Impurity

Level	Concentration (ppm)	Area	Amount recovered	% Recovery
80%	80%	2146.805	80.34	100.425
100%	100%	2672.124		
120%	120%	3235.125	120.69	100.575

Change in flow

Table 7: Metronidazole (100ppm)

Flow	R.T (min)	Area
1ml/min	3.29	1074.372
	3.13	1069.246
	3.33	1085.223
0.8ml/min	3.98	1175.716
	3.92	1183.863
	3.92	1198.836
1.2ml/min	2.62	899.351
	2.65	910.556
	2.63	918.554

Table 8: Imp A (100ppm)

Flow	R.T (min)	Area
1ml/min	2.86	2801.788
	2.88	2807.670
	2.89	2809.772
0.8ml/min	3.40	3329.323
	3.42	3321.033
	3.40	3324.331
1.2ml/min	2.32	2300.379
	2.33	2292.747
	2.31	2303.513

Change in wavelength

Table 9: Metronidazole (100ppm)

Wavelength (nm)	R.T (min)	Area
252	3.14	975.073
	3.13	995.036
	3.14	1002.538
254	3.29	1074.372
	3.31	1069.246
	3.33	1085.223
256	3.08	909.803
	3.14	915.139
	3.16	912.607

Table 10: Impurity (100ppm)

Wavelength (nm)	R.T (min)	Area
254	2.75	2672.124
	2.76	2641.268
	2.74	2629.204
256	2.79	1116.871
	2.77	1122.204
	2.77	1127.008
252	2.76	1212.070
	2.77	1209.467
	2.79	1225.280

Change in pH

Table 11: Metronidazole (100ppm)

Wavelength (nm)	R.T (min)	Area
10.80	3.03	1263.710
	3.05	1304.640
	3.07	1404.751
11.00	3.29	1074.372
	3.31	1069.246
	3.33	1085.223
11.20	3.03	1104.749
	3.03	1135.971
	3.05	1177.099

Table 12: Imp A (100ppm)

Wavelength (nm)	R.T (min)	Area
10.80	2.96	1180.920
	2.88	1192.941
	2.94	1213.368
11.00	2.86	2801.788
	2.88	2807.670
	2.89	2809.772
11.20	2.85	1309.714
	2.88	1356.815
	2.85	1398.587

Change in mobile phase composition

Mobile phase1— 330ml acetonitrile +200ml methanol+100 ml THF+70 ml water (1.56gm/litre K_2HPO_4)

Table : 13

Compound	R.T(min)	Area
Metronidazole	2.85	789.665
	2.84	792.892
	2.86	794.289
Impurity	2.48	874.769
	2.48	890.037
	2.49	887.642

Mobile phase2— 260 ml acetonitrile+ 100 ml methanol+ 100 ml THF +440 ml water (1.56 gm/ litre K_2HPO_4)

Table 14

Compound	R.T(min)	Area
Metronidazole	2.89	796.380
	2.90	793.175
	2.89	791.550
Impurity	2.43	933.347
	2.24	926.010
	2.46	923.084

Table 15: LOQ and LOD for impurities of Metronidazole

Compound	LOQ (ppm)	LOD (ppm)
Impurity	0.3	0.1

CONCLUSION

A. Analytical method is found to be specific as proved by injecting known amount of component into the chromatogram.
B. Limit of quantification and limit of detection for process related Impurity of metronidazole has been established and it is found to be within the range.
C. Analytical method is found to be linear over a specific range.
D. Analytical method is found to be précised and accurate.
E. Analytical method is found to be robust.
F. Sample prepared in analytical solution is found to be stable for at least 24 hrs.

The above mentioned isocratic method for the analysis of metronidazole and its related impurity is found to be Simple, rapid and sensitive.

The method is completely evaluated for its specificity linearity, precision, accuracy, robustness, ruggedness, limit of quantification and detection

REFERENCES

[1] Wubayehu Kahaliw and Ayenew Ashenef, *International journal of pharmaceutical science and research* **2013** Vol. 4(4): 1384-1391.
[2] Mbah C. C., Emosairue C. O., Builders P. F., Isimi C. Y., and Kunle O. O. *African journal of pharmacy and pharmacology* June **2012,** Vol 6(24), pp 1710-1725
[3] Elena Gabriela Oltean, A. Nica *Medicamentul Veterinar / Veterinary* Drug December **2011** vol 5(2)
[4] Kuldip Dilipkumar Trivedi, Ankit B Chaudhary, Sellapan Mohan *International journal of advances in pharmaceutics*.**2013** vol 2 No 1.
[5] Metronidazole Introduction", September **2012** http://www.drugbank.ca/drugs/DB00916
[6]"Norfloxacin Introduction", September **2011** http://www.drugbank.ca/drugs/DB01059
[7] Mishra AK, Yadava R, Mishra A, Verma A, Chattopadhyay P, *Int. J. Pharma Res. Dev***. 2010**, 2(6), 1-5.
[8] Thulasamma P and Venkateswarlu P, *Rasayan J. Chem.* **2009**, 2(4), 865-869.
[9] Farhadi K and Bahar S, *J. Indian Chem. Soc***. 2007**; 54, 1521-1527.
[10] Hassan S, Hussain S, Ansari MT, *Pakistan J. Zool*., **2011**; 43(5), 909-914.
[11] Patel S, *Int. J. Pharmaceut. Fro. Res.*, **2011**; 1(2), 68-74.
[12] Mishal A and Sober D, *J. Pharm. Biomed. Anal*. **2005**, 39(4), 819–823.
[13] Vega E, Dabbene V, Nassetta M, Sola N, *J. Pharm. Biomed. Anal*. **1999**, 21(5), 1003-1009.
[14] Gattavecchia E, Tonelli D, Breccia A, *J. Chrom. B*, **1981**; 224(3), 465-471.
[16] Singh S,Inamullah, Sharma S, Yadav AK, Gautam H, *Der Pharmacia Lettre***, 2012**; 4(6), 1837-1842,.
[17] Boonme P, Phadoongsombut N, Ingkatawornwong S, Faroongsarng D, *Thammasat Int. J. Sc. Tech*., **2002**; 7(1), 1-4.
[18] Sher M, Hussain MA, Mehmood MH, Hassan MN, Bashir S, *J. Chil. Chem. Soc*. **2010**, 55(2), 203-205.
[19] Rege PV, Sathe PA, Salvi VS, *Res. J. Pharm. Bio. Che. Sci*. **2011**, 2(2), 495-505.
[20] Kassab NM, Singh AK, Kedor-Hackmam ERM, Santoro MIRM, *Braz. J. Pharm. Sci*. **2005**, 41(4), 507-513.
[21] Morton SJ, Shull VH, Dick JD, *Antimicrob. Agents and Chemother*., **1986** 30(2), 325-327.
[22] Sebaiy MM, El-Shanawany AA, Adl SM, Abdel-Aziz LM, Hashem HA, *AJP. Ana*. **2011**, 1(4), 79-84.
[23] Marina Silva, Simone Schramm, Eunice Kano, Eunice Koono, Valentina Porta, and Cristina Serra *Journal of Chromatographic Science*, October **2009** Vol. 47
[24] Danao K.R., Hiradeve S.M., Moon R.S., Kasture A.V., Yeole P.G. *Internal journal of pharmacy and life science* June **2010**, 1(2),82-85
[25] Hesham Salem, Safaa M. Riad, Mamdouh R. Rezk and Kholoud Ahmed, *Pharmaceutica Analytica acta* **2013** Vol 3 issue 10
[26] Essam Ezzeldin and Toqa M. El-Nahhas *Tropical Journal of Pharmaceutical Research* October **2012**; 11 (5): 799-805