Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF NIMORAZOLE IN BULK AND DOSAGE FORM BY RP-HPLC

H. S. Ghorpade*[1] and P. S. Dabhade[1]

[1]*Department of Quality Assurance Technique, SND College of Pharmacy, Savitribai Phule University, Babhulgaon, Yeola, India.

ABSTRACT

A new simple, precise, rapid and accurate reverse phase high performance liquid chromatographic method had been developed for estimation of nimorazole in pure and its pharmaceutical dosage form[1,2]. The chromatographic separation was achieved on inertil ODS 3V 150mmx4.6mm, 5µm particle size column was used with UV detector by using mobile phase containing mixture of phosphate buffer pH 4: methanol (70:30) was used. The flow rate was 1ml/min and effluents were monitored at 300 nm[3,4]. Chromatogram showed peak correspondence to nimorazole at retention time 4.9 min. the method was linear over the concentration range 5-35 µg/ml. The developed method was validated in according to ICH guidelines.

KEYWORDS

Nimorazole, RP-HPLC, Validation and ICH.

Author for Correspondence:

Ghorpade H S,
Department of Quality Assurance Technique,
SND College of Pharmacy,
Savitribai Phule University,
Babhulgaon, Yeola, India.

Email: hemantghorpade0@gmail.com

INTRODUCTION[1-4]

Nimorazole is an morpholine derivative. Chemically it is 4-(2-(2-nitro-1H-pyrrol-1-yl)ethyl) morpholine in Figure No.1. It is mainly used as an antimicrobial with activity against anaerobic bacteria and protozoa[5,6].

Literature survey stated that, few analytical method such HPLC, RP-HPLC and UV were reported estimation of nimorazole either individually or combined with other drugs[7]. However no method is reported in the literature for the analytical method development and validation for estimation of nimorazole.

EXPERIMENTAL[8-19]

Sample

Working standard of nimorazole was supplied by AN therapeutics,(Pondicherry, India), and HPLC grade water, arranged from Milli-Q-Academic system, Millipore, Bangalore,, India, were used throughout the experiment. The pharmaceutical formulation was used in this study was Nimorazole tablet (Lupin Ltd, Mumbai, India) procured from the local market and labelled to contain 200mg per tablet.

Instrument and chromatographic condition

A shimadzu HPLC system consist of LC-10AT-vp solvent delivery system (pump), UV detector, Rheodyne injector with 20µL loop volume, LC solution assisted foe data collection and processing. The mobile phase consisted of phosphate buffer pH 4: methanol (70:30) was delivered at flow rate of 1ml/min. Separation was achieved using inertil ODS 3V 150mmx4.6mm, 5µm particle size column was kept at an ambient temperature. The column effluent was monitored at 300 nm. The mobile phase filtered through 0.45µ filter before using Summarized in Table No.1.

Preparation of phosphate buffer solution

8.954 gm. of disodium hydrogen phosphate and 3.4023 gm. of potassium hydrogen phosphate was dissolved in sufficient water (HPLC grade) with aid of sonicatore and volume was made up to 1000ml with water.

Standard stock solution

Accurately weighed 10 mg of Nimorazole and transferred to 100 ml volumetric flask containing a mixture of Phosphate buffer pH 4: Methanol (70:30) as mobile phase. The volume was made up to the mark using same mixture of mobile phase. The resulting stock solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min.

Sample solution

Twenty tablets (Label claim 500 mg of Nimorazole, Lupin) were weighted and average weight was determined and powdered. Powder equivalent to 10 mg (20.72mg) was transferred to 100 ml of mobile phase. The resulting solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10 min. From the stock 0.8, 1.0, 1.2 ml solutions were pipette out and diluted up to 10ml using mobile phase to obtain resultant solution of 8, 10 and 12 µg/ml representing 80, 100 and 120 % of API in formulation. The above three standard solutions of API were spiked in these three levels and injected in given HPLC system to determine mean are of three replicate injections. The results were subjected to statistical analysis. Finally, the percent recovery was determined from mean measured area and area obtained from injections of nominal concentration of drug (10µg/ml).

RESULTS AND DISCUSSION

Method validation

The developed method was validated as per the ICH guidelines with respect to system suitability, linearity, precision, accuracy, robustness and % recovery.

System suitability

To ensure the resolution and reproducibility of the HPLC system was adequate for the analysis, a system suitability test was established. Data from six injection of standard solution of 5µg/ml were used for the evaluation of system suitability parameter like theoretical plate, area and tailing factor. The system suitability result obtained for nimorazole summarized in Table No.2.

Linearity

Linearity should be established across the range of the analytical procedure. Linearity is generally reported as the correlation coefficients, the slope of regression line i.e, $R^2 \geq 0.999$. The linearity of a test procedure was performed across the range of 5-35µg/ml and the results so obtained were as shown in Table No.3. From the results obtained, it can be suggested that the Nimorazole showed linear relationship between concentration and the corresponding peak area across the range 5-35µg/ml. Therefore, it can be said that the method was found to be linear between 5-35µg/ml standard concentration and this was supported by the correlation coefficient of 0.999 as shown in Figure No.2.

Precision

Precision of the method was established by intra-day and inter day measurements of QC standards (7, 22, 32 µg/ml) selected at three levels across the calibration range. Six replicates for each QC standard were performed with given instrument setting. The results were recorded for area, retention time, theoretical plates, and USP symmetry factor and found to be in agreement with each other. The area for each QC standard was statistically evaluated for standard deviation and percent RSD as illustrated in Table No.4 and Table No.5. The percent RSD obtained was in conventionality with the ICH principle. As a consequence, it was accomplished that the method was precise for the specified range.

% Accuracy

Accuracy of analytical procedure should be established across the specified range of analyte. Accuracy was calculated from data obtained from precision study by an equation of calibration curve i.e. Y = mX + c.

The results obtained for percent accuracy were as cited in Table No.6. From the results obtained it was established that the method was accurate at three levels of QC standards across range and it passed for the test of accuracy as per ICH guideline Q2R1.

Robustness

This is the measure of capacity of method to give consistency of result during schedule usage. Purposeful minute transforms in analytical method were made to study the steadiness of the method even after these small variations during custom analysis of Nimorazole. Given method was subjected to intentional changes as per Table No.7 and Table No.8. The method stayed impervious by these small but premeditated dissimilarity and same was confirmed by percent assay standards within limit.

Percent recovery

Percent recovery is determination of percent purity of given analyte finished product. The accuracy of the methods was determined by calculating recoveries of Nimorazole by the standard addition method. Known amount of standard solutions of Nimorazole (8, 10 and 12 µg/ml) were added to a pre-analyzed sample solution of Nimorazole (10µg/ml). Each solution was injected in triplicate and the percentage recovery was calculated by measuring the peak areas and fitting these values into the regression equations of the calibration curves. Data acquired was represented in Table No.9.

Table No.1: Optimize chromatographic conditions

S.No	Column :	Inertil ODS 3V (150mm×4.6mm),5µm, C18
1	Flow Rate	1 ml/min
2	Wavelength	300 nm
3	Injection volume	10µl
4	Column oven temperature	Ambient (25^{o} C)
5	Run Time	8 minutes
6	Mobile phase	Phosphate buffer pH4: Methanol (70:30)

Table No.2: Results of system suitability testing

S.No	Parameter	Mean	Limit
1	Area (AU)	96216	%RSD (<2%)
2	Retention time (min)	4.829	< 10-5
3	Theoretical plates	5894	>2000
4	Tailing factor	1.01	<2

Table No.3: Results obtained for linearity of Nimorazole

S.No	Concentration (µg/ml)	Mean Area
1	5	97340
2	10	191607
3	15	295390
4	20	394379
5	25	488361
6	30	588038
7	35	689700

Table No.4: Intra-day precision

S.No	Concentration (µg/ml)	Mean Area*± SD	%RSD
1	7	135182 ± 418.7	0.31
2	22	430344.33 ± 2948.34	0.68
3	32	629728.67 ± 2990.2	0.48

Table No.5: Inter-day Precision

S.No	Concentration (µg/ml)	Mean Area*± SD	%RSD
1	7	133779 ± 473.85	0.35
2	22	431504.22 ± 2261.89	0.52
3	32	627295.89 ± 475.91	0.07

Table No.6: % Accuracy

S.No	Concentration(µg/ml)	Mean area	Amount found (µg/ml	%Assay (w/w)
1	7	135182	6.98	99.73
2	22	430344.33	21.94	99.71
3	32	629728.67	32.04	100.13

Table No.7: Robustness study for Flow Rate variation

S.No	Flow rate (ml/min)	Conc. (µg/ml)	Mean RT (min)	Mean Area	% Assay (w/w)
1	1.0 (std)	20	4.84	392380	98.76
2	1.1 (+0.1ml)	20	4.62	393527	99.05
3	0.9 (-0.1ml)	20	5.13	394322	99.25

Table No.8: Robustness study for mobile phase ratio variation

S.No	Mobile phase ratio	Conc. (µg/ml)	Mean of RT	Mean of area	% Assay (w/w)
1	70:30 (std)	20	4.81	393678	99.09
2	72:28(High)	20	4.62	391349	98.50
3	68:32(Low)	20	4.95	392180	98.71

Table No.9: Percent recovery study using Tablet dosage form

S.No	Recovery Level (%)	Standard conc. (µg/ml)	Amount of sample spiked (µg/ml)	Mean area	Amount recovered (µg/ml)	Mean % recovery (98-102)
1	80	10	8	345974	17.66	98.12
2	100	10	10	388129	19.80	98.99
3	120	10	12	429563	21.89	99.53

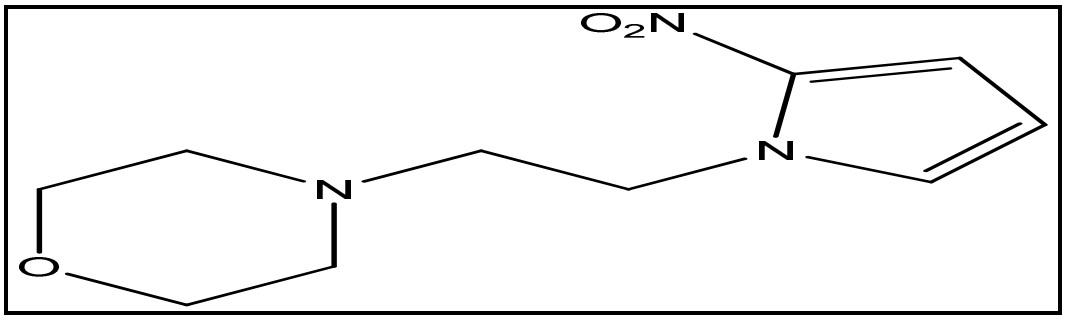

Figure No.1: Structure of Nimorazole

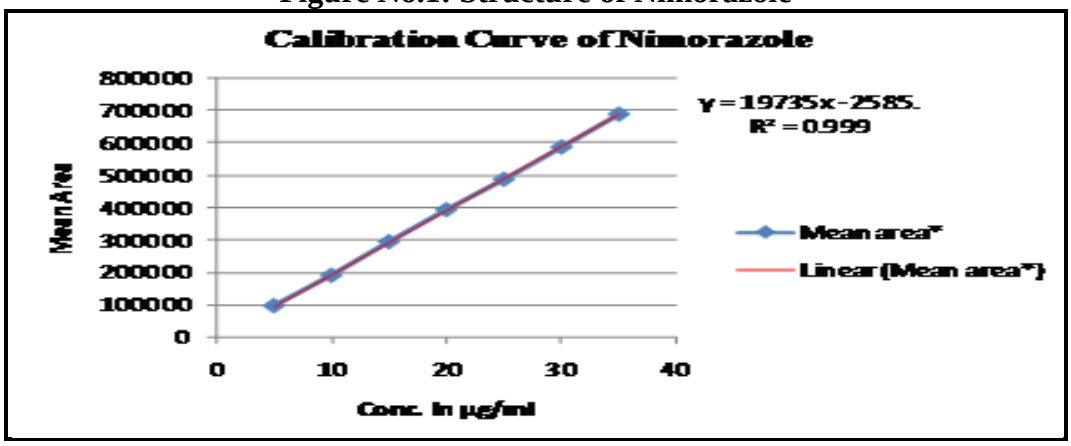

Figure No.2: Linearity graph of Nimorazole

CONCLUSION

This developed method is considered as the first method for the determination of nimorazole using RP-HPLC. The various validation characteristics were applied and determined, to assure the suitability of the method. This investigation also proved that, the chromatographic technique provide a complete profile of separation process, making this technique a powerful analytical tool. Therefore, this validated RP-HPLC method can be readily adapted for the determination of nimorazole in bulk and pharmaceutical dosage form as a routine quality control analysis.

ACKNOWLEDGEMENT

The authors are thankful to the SND College of Pharmacy, Savitribai Phule University, Babhulgaon, Yeola, India for providing help in carrying the research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Fossheim R, Joslyn A, Solo A J, Luchowski E, Rutledge A and Triggle D J. **"**Crystal structures and pharmacologic activities of 1,4-dihydropyridine calcium channel antagonists of the isobutylmethyl2, 6-dimethyl-4-(substituted phenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate (nisoldipine) series", *Med Chem*, 31(2), 1988, 300-305.
2. Shetty S K, Surendranath K V, Jogul J and Tripathi U M. "Determination of Zaleplon in the presence of its degradation products by a stability indicating UPLC method", *Analytical Chemistry*, 8(3), 2009, 360-367.
3. Gupta A, Gaud Ram S, and Ganga S. "Liquid chromatographic method for determination of Nisoldipine from pharmaceutical samples", *E Journal of chemistry*, 7(3), 2010, 751-756.
4. Pathare D V, Jadhav A S, Shingare M S. "A validated stability indicating LC method for Nateglinide, *Drug development and Industrial pharmacy*, 33(5), 2007, 551-557.

5. Baokar S B, Erande R S, and Shaikh S G. "Analytical Method Development and validation for estimation of Ezetimibe from Tablet Dosage Form Using RP–HPLC", *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2(2), 2011, 833-841.
6. Pathare D V, Jadhav A S, Shingare M S. "A validated stability indicating RPLC method for Tazarotene", C*hromatographia*, 66(3-4), 2007, 247-250.
7. Ninfa A J, Ballou D P, Benore M. "Fundamental Laboratory Approaches for Biochemistry and Biotechnology", *Wiley*, 2nd Edition, 2009, 133.
8. Guideline, ICH, Validation of analytical procedures: text and methodology, *Q2 (R1)*, 1, 2005.
9. ICH Q2R1 guideline, validation of analytical procedures: text and methodology, Current Step 4 version Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005), 1 to 13) ICH Q2B guideline, Validation of Analytical Procedures: Methodology, *Guidance for Industry*, 1-10.
10. Kashid A M, Anup, A, Dhange A I, Mulani D A G and Shashikant C D. "RP-HPLC Method development and validation for Nimorazole", *American Journal of pharm tech research*, 2(6), 2012, 818-823.
11. Arayne M S, Sultana N, and Zuberi M H. "Development and validation of RP-HPLC method for the analysis of Metformin", *Pak J Pharm Sci*, 19(3), 2006, 231-235.
12. Fontana M C, Bastos M O, and Beck R C R. "Development and validation of a fast RP-HPLC method for the determination of Clobetasol propionate in topical nanocapsule suspensions", *Journal of chromatographic science*, 48(8), 2010, 637-640.
13. Rao A L, Kumar B N V and G Sankar G. Development of RP-HPLC method for the estimation of Rabeprazole in pure and tablet dosage form", *Journal of Chemistry*, 5(S2), 2008, 1149-1153.
14. Jyothirmai B, Tnvss S, Santosh T, and Sundar B S. "Development and validation of an Rp-HPLC method for the determinaton of Olmesartan in human plasma", *IJRPC*, 4(2), 2014, 457-466.
15. Laxmi M, Ghosh S, and Ravikumar B V V. "Analytical method development and validation of Artemether in bulk drug by RP-HPLC method as per ICH guidelines, *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(2), 2014, 498-501.
16. Reddy Y. "RP-HPLC method for the estimation of Nitroxazepine hydrochloride in pharmaceutical dosage forms, *INBPT*, 1(3), 2010, 1181-1187.
17. Ghari T, Kobarfard, F, and Mortazavi S A. "Development of a Simple RP-HPLC-UV Method for Determination of Azithromycin in Bulk and Pharmaceutical Dosage forms as an Alternative to the USP Method", *Iranian journal of pharmaceutical research: IJPR*, 12, 2013, 57-63.
18. Chua Y A, Abdullah W Z, and Gan S H. "Development of a high-performance liquid chromatography method for Warfarin detection in human plasma", *Turkish Journal of Medical Sciences*, 42(5), 2012, 930-941.
19. Nataraj K S, Duza M B, Pragallapati K, and Kumar D K. "Development and validation of RP-HPLC method for the estimation of Omeprazole in bulk and capsule dosage forms", *International Current Pharmaceutical Journal*, 1(11), 2012, 366-369.

Please cite this article in press as: Ghorpade H S and Dabhade P S. Analytical method development and validation for determination of nimorazole in bulk and dosage form by RP-HPLC, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 5(2), 2017, 73-78.