ISSN:0975-1459
Nasiruddin Ahmad Farooqui et al, /J. Pharm. Sci. & Res. Vol.2 (7), 2010, 412-416
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Analytical Method Development and Validation of Secnidazole Tablets by RP-HPLC

Nasiruddin Ahmad Farooqui[1], A.Anton Smith*[1], H.K.Sharma[2] and R.Manavalan[1]

[1]Department of Pharmacy, Annamalai University, Annamalainagar – 608002, Tamilnadu.
[2]Unichem Laboratories Ltd., Ghaziabad – 201001, Uttar Pradesh.

Abstract:
A simple, rapid, sensitive specific, accurate, RP-HPLC method was developed and validated for the determination of secnidazole in formulation. The method utilized HPLC (Alliance 2695 with 2487PDA) model and a column (Inertsil, ODS- 3V, 250× 4.6mm, 5µ). The mobile phases were comprised of buffer: 0.01M KH_2PO_4 : ACN (85:15). Validation experiments were performed to demonstrate system suitability, specificity, precision, linearity and range, accuracy, ruggedness and robustness. The developed method was linear over the concentration range of 30-70µg/ml^{-1}. The method showed good recoveries (98.0 – 102.0%) and the relative standard deviations of intra and inter-day assay were 0.008 and 0.12% respectively. The proposed method is precise, accurate, selective and rapid for the determination of secnidazole for the quality control test.
Key words: *Secnidazole, RP- HPLC, Quality control, Determination*

Introduction:

Secnidazole is 1-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol. Antiprotozoal and antiamoebic activity [1], of secnidazole is by nitro group of nitroimidazole is chemically reduced by ferrodoxin which is ferrodoxin-Linked Metabolic process. After entering into the microorganism by diffusion, its nitro group is reduced to intermediate compound which cause cytotoxicity, by damaging DNA. Its selectivity high activity against anaerobic organism has suggested interference with electron transport from NADPH or other reduced substrate [2], secnidazole is completely absorbed after oral administration, secnidazole is having abdominal pain, dizziness, Neurological disturbance and head ache as Adverse effects. Detailed survey of literature for Secnidazole revealed several methods based on techniques viz. HPLC [3, 4] and Spectrophotometric [5-7] for its determination in pharmaceutical dosage form and in human plasma. This paper presents simple, rapid, reproducible and economical method for RP- HPLC simultaneous estimation of secnidazole in tablet dosage form.

Experimental Method:

Apparatus

The analysis was performed by using HPLC was Alliance 2695 with 2487 dual wavelength detector. Column used in HPLC was of Inertsil ODS-3V, 250cm ×4.6 mm 5µ.

Reagents and solutions

Pure sample of secnidazole and other ingredients were kindly supplied by Unichem Laboratories Ltd, Uttar Pradesh. Acetonitrile and water used were of HPLC and Milli-Q grade respectively and are obtained from Unichem Laboratories Ltd, Uttar Pradesh. All other chemicals like mobile phase consists of Buffer: 0.01M KH_2PO_4: ACN (85:15) PH 6.0, 0.1N hydrochloric acid, orthophosphoric acid and sodium hydroxide used were of AR grade. Optimized chromatographic conditions are listed in Table 1.

Standard Preparation

Secnidazole 50mg of working standard was weighed accurately in a 100 mL of volumetric flask, dissolved and diluted up to the mark with mobile phase. 5ml of the above solution was diluted to 50 ml in a volumetric flask with mobile phase and mixed well.

Sample preparation:

Powdered sample of 20 tablets of secnidazole 50mg was weighed accurately in 100ml volumetric flask, about 50ml of mobile phase was added, sonicated, and diluted up to mark. The solution was filtered through 0.45µ membrane filter and from the

Table 1: Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Alliance 2695 with 2487 PDA)
Column	Inertsil ODS- 3, 250cm×4.6mm 5u
Mobile Phase	Buffer: 0.01M KH_2PO_4: ACN (85:15).
Flow rate	1.0 ml/min
Detection	UV at 228 nm
Injection volume	20µl
Column temperature	30°C

above solution 5ml was diluted to 50 ml in a 50 ml volumetric flask with mobile phase.

Method Validation:

Linearity

Several aliquots of standard solutions of secnidazole were taken in different 10ml volumetric flasks and diluted up to the mark with mobile phase, such that the final concentration of secnidazole lies between 30 – 70 µg/ml. Linearity study solution was measured at 228 nm, peak area was recorded for all the peaks. A model chromatogram was presented in fig 1. The slope and intercept value for calibration curve was Y = 19356 X (R^2 = 1) for secnidazole. The results showed that an excellent correlation exists between peak area and concentration of drug within the concentration range indicated above. The regression curve was presented in fig 2.

Assay

20 µl of standard and sample solutions were injected into the chromatograph, from the peak area of secnidazole amount of drug present in the samples were computed. The values are given in Table 2.

Limit of Detection and Limit of Quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for secnidazole was found to be 0.00038. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was found to be 0.0012 and the data were presented in the table 3.

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like waters HPLC and Shimadzu HPLC by different operators using different columns of similar type like Inertsil, Luna C18. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was found to be that there were no marked changes in the chromatograms, which demonstrated that the HPLC method developed, are rugged and robust.

Recovery Studies

To study the accuracy and reproducibility of the proposed method recovery experiments were carried out. A fixed amount of pre-analyzed sample was taken and standard drug was added at 50%, 60%, and 70% levels. Each level was repeated three times. The contents of secnidazole per tablet was found by the proposed method are shown in table 4. The lower values of RSD of assay indicate the method is accurate. The mean recovery of secnidazole was in the range of 98% to 102%, which shows that there

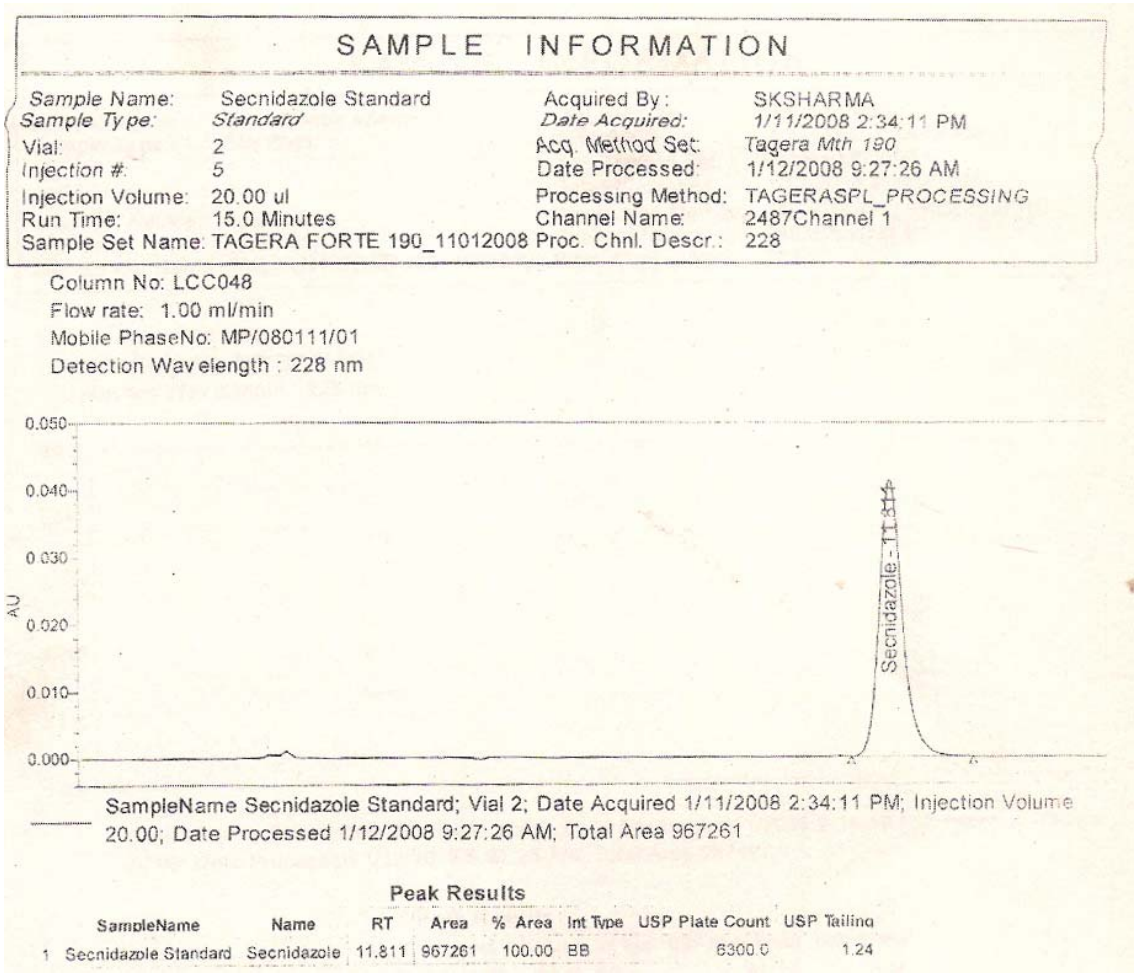

Fig 1: Chromatogram of secnidazole

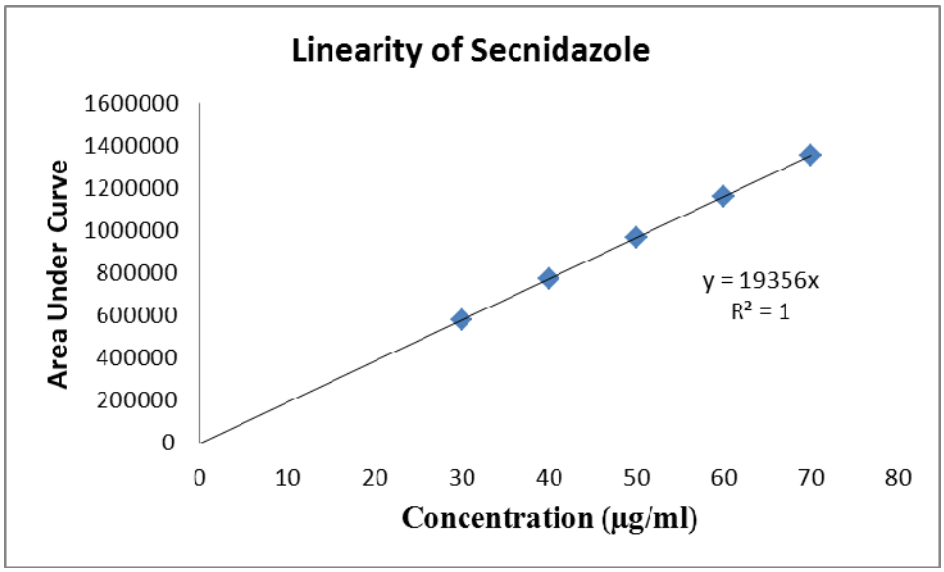

Fig 2: Linearity of Secnidazole

Table 2: Analysis of formulation and recovery studies for Secnidazole

Label claim (mg/ml)	Estimation		Recovery	
	Mg/tablet	% label claim	Amount added µg/ml	% Recovery
50	50	98.34	50	98.82
50	50	98.34	60	101.97
50	50	98.34	70	99.76

was no interference with the excipients.

Results and Discussion:

Satisfactory resolution was obtained using the mobile phase system of Buffer: 0.01M KH_2PO_4: ACN (85:15) at a flow rate of 1.0 ml min^{-1}. The UV-spectrum of the drug shows that the absorption maxima band at 228nm. Under the experimental conditions, the chromatogram of (Fig. 1) showed a single peak of the drug around 15 min.

Table 3: System suitability parameters

Parameter	Secnidazole
Calibration range (µg/ml)	30-70
Theoretical plates	6314
Tailing factor	1.24
LOD (µg/ml)	0.00038
LOQ (µg/ml)	0.0012

The calibration curve was prepared by plotting the peak area of secnidazole against drug concentration and was linear in the range of 30-70 µg/ml. Peak area and concentration were subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficients. The regression equation was found as Y = 19356 X (r =1, *n = 5*) *(Y = aX + b,* where *Y* is the peak area of secnidazole, *a* is the slope, *b* is the intercept and *X* is the concentration of the measured solution in µg ml^{-1}).

Limit of detection (LOD) value was found as 0.00038µg ml^{-1} which is the concentration that yields a signal-to-noise ratio of 3:1. Limit of quantization (LOQ) value under the described condition was 0.0012µg ml^{-1}, which is the signal-to-noise ratio of 10:1. The repeatability of sample application and measurement of peak area were expressed in terms of % RSD which revealed intra-day *(n* = 6) and inter-day *(n* = 6, at 5 different day) The RSD values were found to be 0.008 – 0.004 and 0.08 - 0.12%, for intra- and inter-day variation, respectively, indicating good precision. To examine the accuracy of the method, recovery studies were carried out by standard addition method.

Table 4: Validation Parameters

Parameters	Secnidazole
Linearity range	30 – 70 µg/ml
Correlation coefficient	1
Slope	403458
Y Intercept	50
Recovery(n=3)	100.18 %
Intra-day(n=6)(% RSD)	0.008
Inter-day(n=6) (% RSD)	0.12%

The average percent recoveries obtained as 98 - 102% indicate that good accuracy of the method. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small variations in method conditions. The composition of HPLC mobile phase, flow rates at 1 ± 0.05 ml min^{-1}, the effect of pH (varying ± 0.2 pH units) and two analytical columns were studied to evaluate for the robustness of the method with % RSD values 0.08 - 1.8 for pH 3 and 4 respectively.

Conclusion:

The developed HPLC method for the qualification of secnidazole was performed in mobile phase pH 3 of Buffer: 0.01M KH_2PO_4: ACN (85:15). The results obtained on the validation parameters by this developed method meet the ICH requirements. It is thus inferred that this newly developed method is found to be specific, simple, precise, accurate, reproducible, reliable, Linear and proportional it follows Lambert- Bears Law. The method was found to be rapid. Hence it can be inferred that the developed method is useful in routine laboratory analysis with a high degree of accuracy and precision.

References:

[1] Laurence L. Brunton, Louis Sanford Goodman, John S. Lazo., Goodman and Gilman , The Pharmacological Basis of Terapeutics, McGraw-Hill Companies, Inc.,11th edition, 2006.

[2] Gillis JC and Wiseman LR., Secnidazole A review of its antimicrobial activity, pharmacokinetic properties and therapeutic use in the management of protozoal infections and bacterial vaginosis**.,** Drugs, 51(4), 621-38, 1996.

[3] Sekhar K, Ravi, M. U. R. Naidu, E. C. Sekhar, T. R. K. Rao, J. C. Shobha, P. U. Rani and Kumar J. Surya., Biomedical Sciences and Applications, J. Chrom., 691(1), 208-211, 1997.

[4] Xiaoyu Li, Jianguo Sun, Guangji Wang *, Yuanting Zheng, Bei Yan, Haitang Xie, Yi Gu, Hongchan Ren, Biomed. Chrom., 21(3), 304-309, 2007.

[5] Saffaj M, Charrouf M, Abourriche A, Abboud Y, Bennamara A and Berrada M, Farmaco., 59 (10), 843-846, 2004.

[6] Afaf Abul khier, Magda M, Elhenawee and Manal S, Elmasry., Eur.J..Chem , 5 (S2), 1087-1097, 2008.

[7] Ravichandran V, Sankar V, Sivanand V, Velrajan G and Raghuraman S., Ind. J. Pharm. Sci., 64 (4), 396-398, 2002.