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ESTIMATION OF NEVIRAPINE BY UV-VIS SPECTROSCOPIC METHOD IN EXTENDED RELEASE(ER) TABLET DOSAGE FORMS AND ITS INVITRO DISSOLUTION ASSESSMENT

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ABSTRACT

A simple, sensitive, rapid and reproducible UV-VIS Spectroscopic Method has been developed and validated for estimation of Nevirapine simultaneously and also the comparative study of invitro data in ER tablet formulation. The solvent used was Ethanol: Water (75:25) %v/v and the λmax or the absorption maxima of the drug was found to be 214nm. A linear response was observed in the range of 4.8-36µg/ml with a regression coefficient of 0.99. The invitro release of various test units was compared for their similarity using the f2 test which limits were found with in the acceptance criteria. All the validation parameters were with in the acceptance range according to ICH norms. The described method was successfully employed for quality control assay of the component simultaneously and dissolution data helpful in generating the further information regarding invivo absorption rate in ER tablet dosage form.

Key words: *Nevirapine; Invitro dissolution study; UV-VIS Spectroscopic*

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INTRODUCTION

Now days, the drugs which exist and those being discovered are of synthetic origin and have limitation of poor water solubility.Low aqueous solubility is the major problem encountered with formulation development of new chemical entites.A number of methodologies can be adapted to improve solubilization of poor water soluable drug and further to improve its bioavailability. The techniques gererally employed for solubilization of drug includes micronization, chemical modification, pH adjustment, solid dispersion,complexation,co-solvency,miceller solubilization,hydrotropy,etc.

Nevirapine is a drug belonging to a class of pharmacological agents known as the non-nucleoside reverse transcriptase inhibitor (NMRTI) of HIV-1.Chemically it is 1 l-cyclopropyl-5,1 l-

dihydro-4-methyl-6H-dipyrido [3,2-b:2',3'-e][l,4] diazepin-6-one

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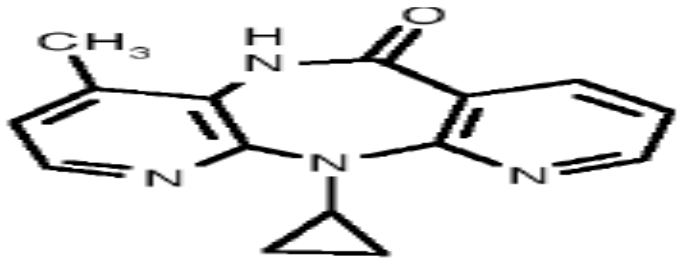


MATERIAL AND METHODS

Chemicals and Materials:

Hetero Labs Ltd supplied Nevirapine respectively. Sodium Lauryl Sulfate (Merck,AR grade), Ethanol (HPLC grade) and Monobasic sodium phosphate monohydrate from Merck AR grade and sodium hydroxide (AR grade) respectively. In-house purified water (USP grade) was used throughout the study.

Dissolution parameters:

Medium	2% SLS in pH 6.8 Phosphate buffer.
Volume	900 mL
Apparatus	USP Type I (Basket 10mesh size)
RPM	100
Temperature	37 ± 0.50C
Time	2,4,8,12 and 18 hours

Instrumentation:

The chromatographic separations were performed using Shimadzu UV 1800 series with UV probe software -VIS Spectroscopic. Electrolab TDT-08L auto sampler dissolution apparatus were used for comparative dissolution study.

Buffer preparation:

Weigh and transfer about 6.9 grams monobasic sodium phosphate monohydrate into a beaker containing 1000 mL of water. Adjust pH of the solution to 6.8 ±0.05 with 10 % w/v sodium hydroxide solution. Add about 20 grams sodium lauryl sulfate and sonicate to dissolve and mix.

Preparation of sodium hydroxide solution (10 % w/v):

Dissolve 10 grams of sodium hydroxide pellets in 100 mL of water and mix.

For Dissolution:

Standard preparation:

Accurately weigh and transfer about 25 mg of Nevirapine working standard into a 500 ml volumetric flask, add about 20 ml of ethanol and sonicate to dissolve. Dilute to volume with dissolution medium. Transfer 5.0 ml of the above solution into a 10 ml volumetric flask. Dilute to volume with dissolution medium and mix.

Sample preparation:

Place 1 tablet each in six different vessels and operate the instrument as mentioned above. Withdraw about 10 mL of the sample solution, filter and dilute 3.0 mL of this to 50ml volumetric flask. Dilute to volume with dissolution medium and mix.

The samples withdrawn above were analyzed on UV-VIS Spectroscopic about λmax 214nm using 0.2 cm^2 cell.

Applied method to compare dissolution profiles:

The description of the in vitro dissolution profiles was calculated by using model-independent method. In this study, as model-independent approaches, two fit factors were applied to the dissolution data that compare the dissolution profiles of a pair of drug product. These fit factors directly compare the difference between the percent drug dissolved per unit time for a test and reference product. The fit factors are f2 (similarity factor).

The specification of dissolution method is set by considering the solubility, permeability, dissolution and pharmacokinetics of the drug substance. A model-independent method was used for the comparison of in vitro dissolution profiles. In this study f2 (similarity factor) was calculated. The use of these factors was also recommended for dissolution profile comparison in the FDA's guides for industry.

For Assay:

Diluent: Prepared a mixture of (Ethanol: Water) 75:25%v/v

Standard preparation:

Accurately weigh and transfer about 25 mg of Nevirapine working standard into a 100 ml volumetric flask, add about 75 ml of ethanol and

sonicate to dissolve. Dilute to volume with water. Transfer 5.0 ml of the above solution into a 50 ml volumetric flask. Dilute to volume with diluent and mix.

Sample preparation:

Weigh accurately tablets powdered equivalent to about 200 mg of Nevirapine in to 200-mL volumetric flask. Add about 150-mL ethanol and sonicate it for 30 minute to dissolve. Dilute to volume with water. Transfer 5.0 ml of the above solution into a 200 ml volumetric flask. Dilute to volume with diluent and mix. Filtered it through 0.45 µ HVLP nylon filter.

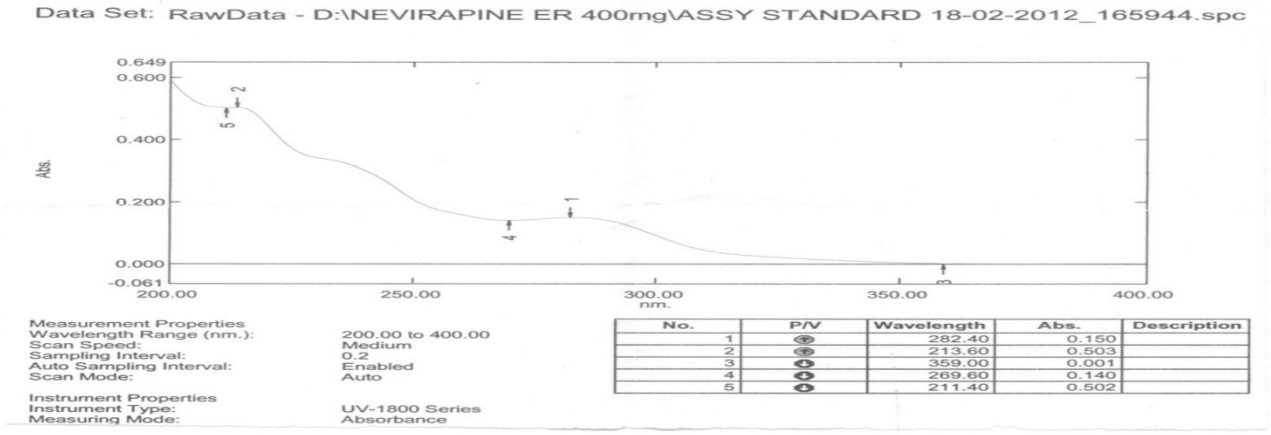
RESULTS

The UV-VIS Spectroscopic wavelength of 214 nm was chosen in order to achieve a good sensitivity for quantitative determination of Nevirapine in ER tablet dosage.

Table 1. Method Precision

Compound	Concentration (µg/mL) (n=6)		% Assay Mean (n=6)	%RSD of Assay
Nevirapine	25		99.85	1.0

Standard Solution(fig.1)



Test Solution(fig.2)

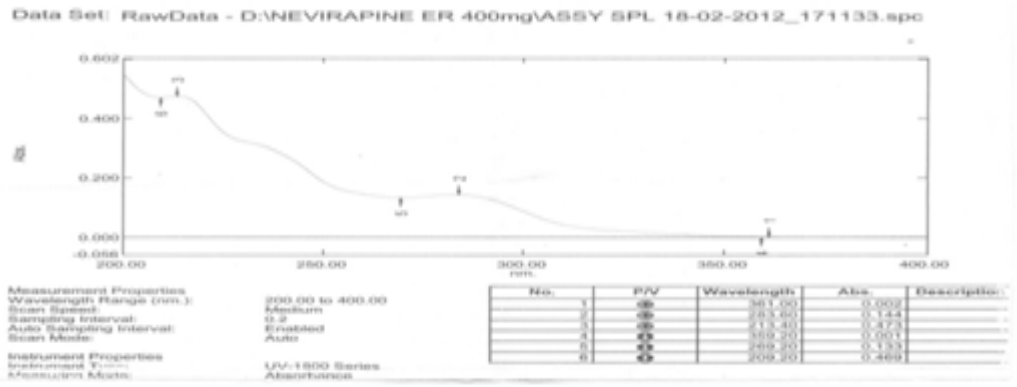
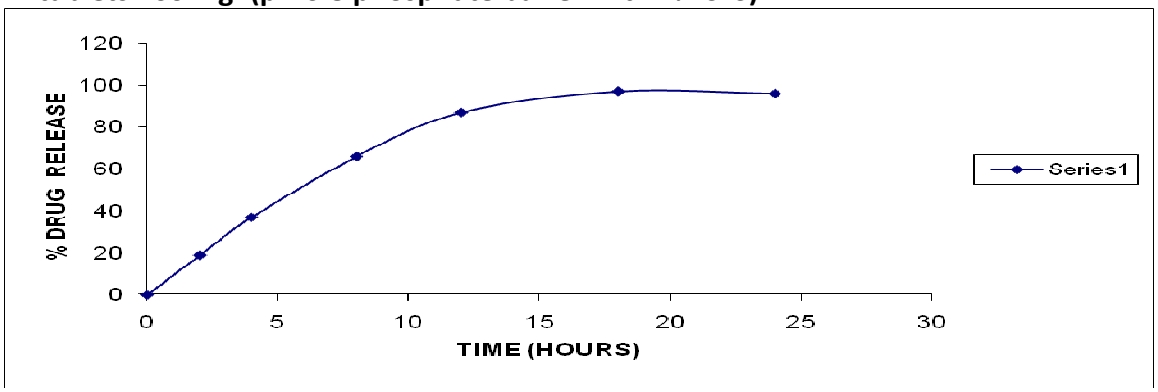


Table 2. Method Accuracy

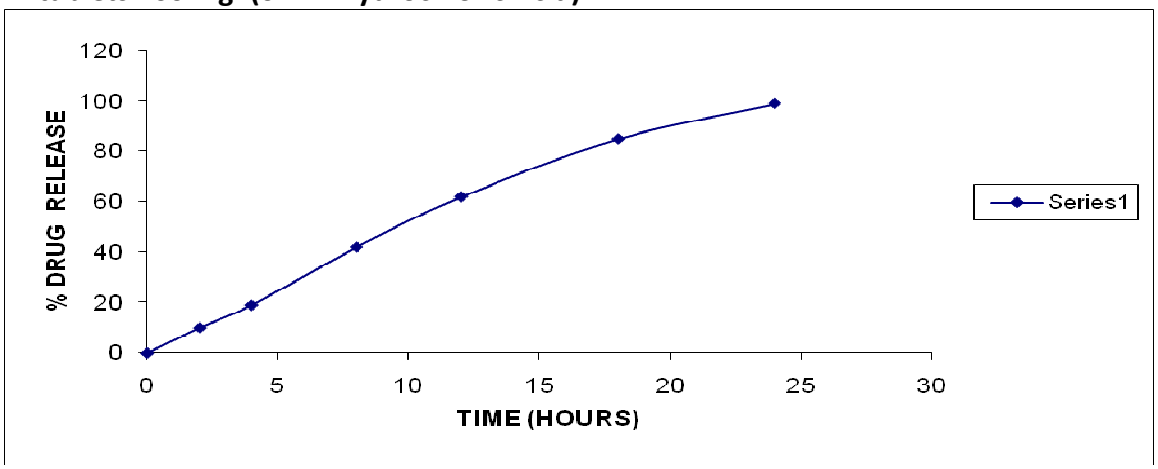
Drug	Level	Drug Added	Drug recovered	%Assay	% Assay(n=3)
Nevirapine	50%	12.62	12.89	102.14	0.5
	100%	25.56	25.10	98.20	0.7
	150%	38.23	38.92	101.80	0.5

Comparative Dissolution Data:

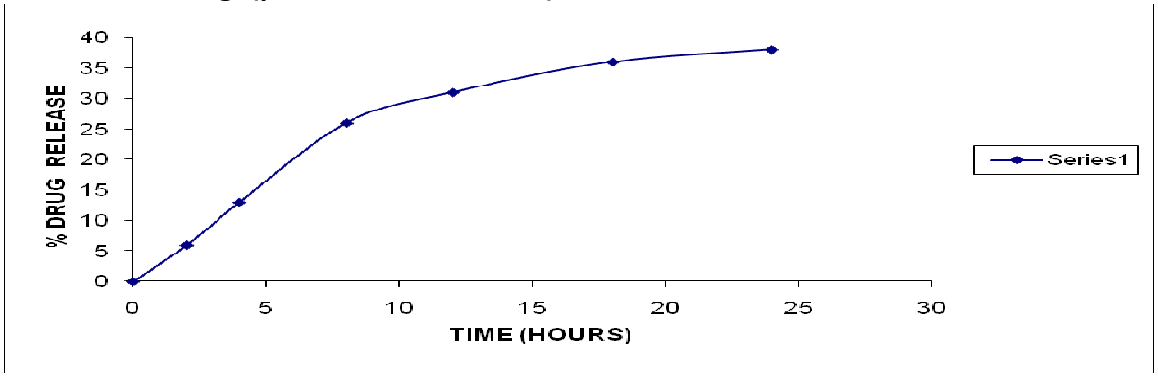
Viramune XR tablets 400 mg: (pH 6.8 phosphate buffer with 2% SLS)



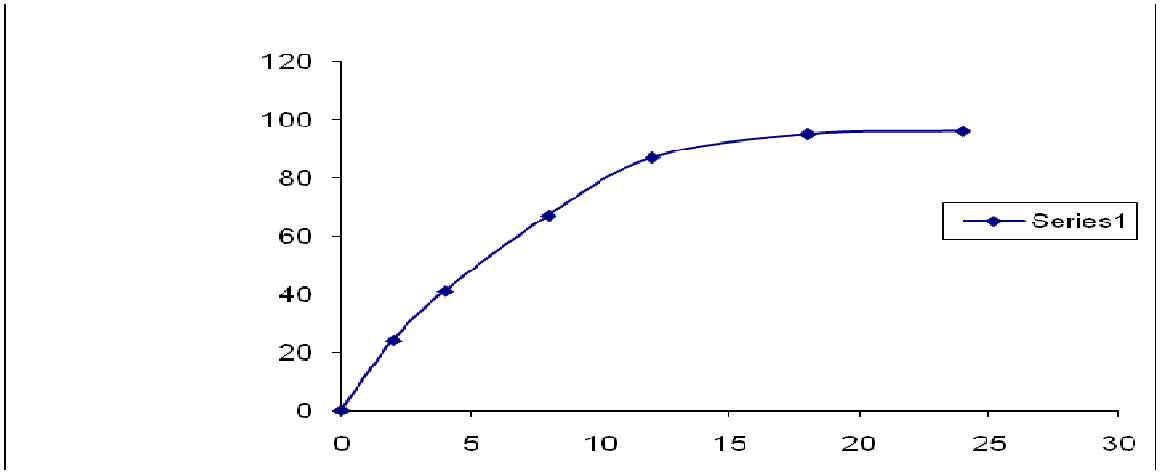
Viramune XR tablets 400 mg: (0.1 N Hydrochloric Acid)



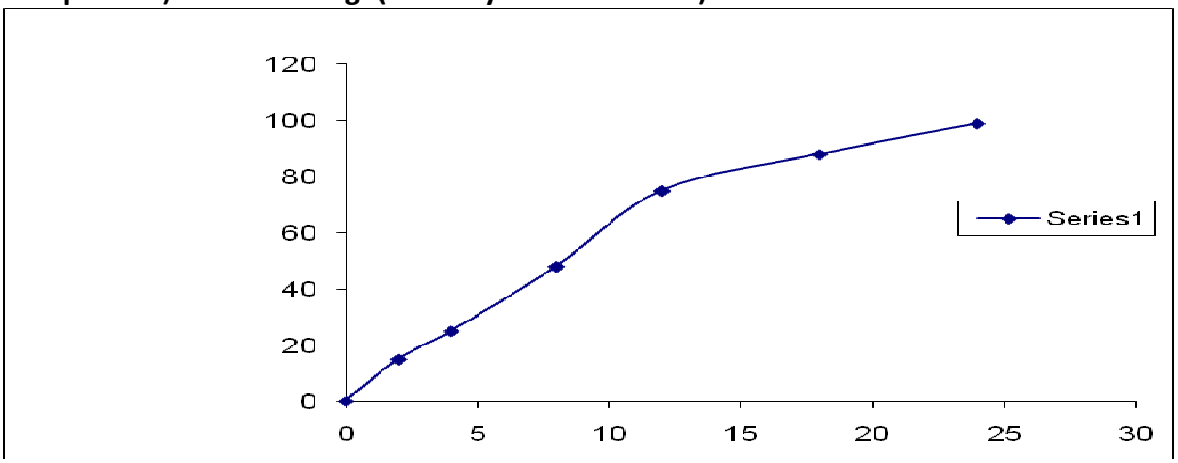
Viramune XR tablets 400 mg: (pH 4.5 Acetate buffer)



Hetero (Nevirapine ER) Tablet 400mg: (pH 6.8 phosphate buffer with 2% SLS)



Hetero (Nevirapine ER) Tablet 400mg: (0.1 N Hydrochloric Acid)



Hetero (Nevirapine ER) Tablet 400mg: (pH 4.5 Acetate buffer)

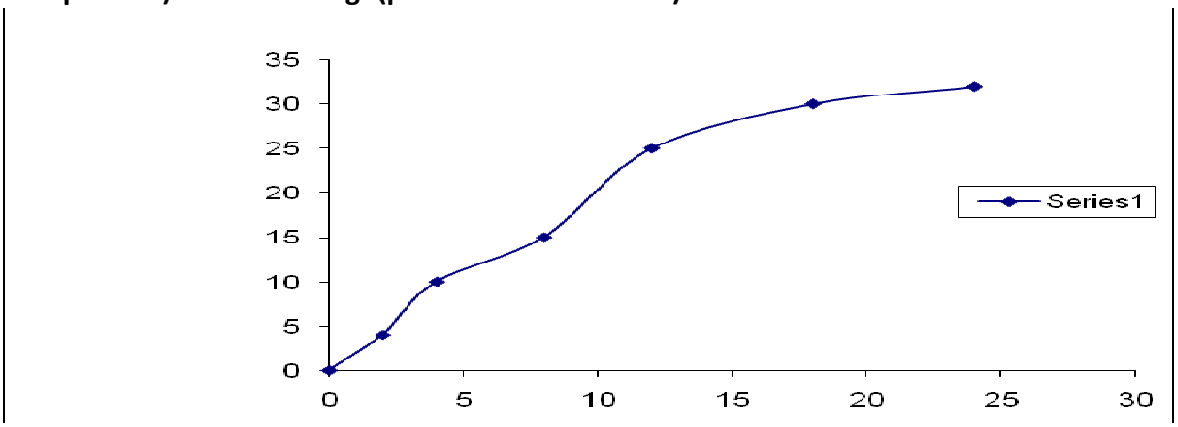


Table 3: Method Ruggedness

Day	Compound	% Assay Mean	%RSD
Day 1	Nevirapine	99.56	0.6
Day 2	Nevirapine	98.45	0.5

Table 4: Linearity

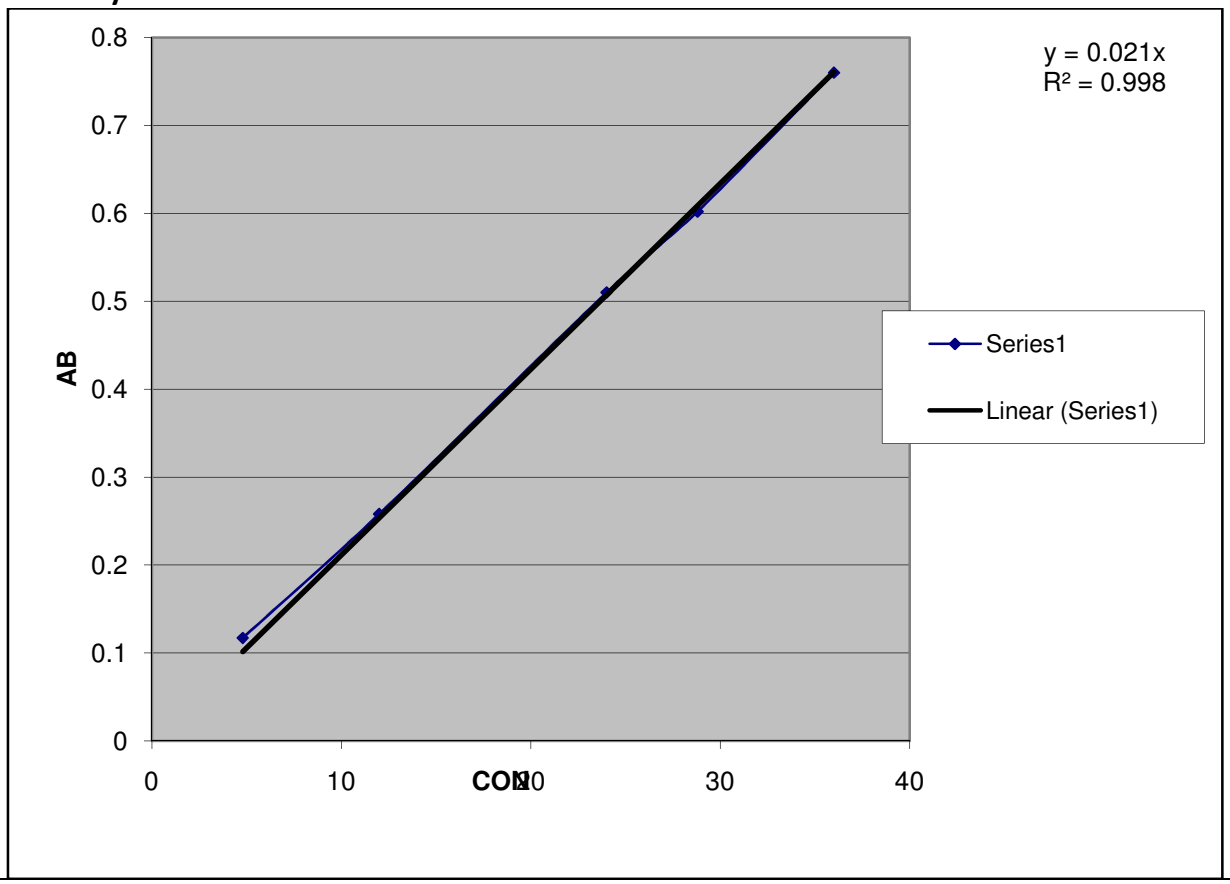


Table 5. Comparative Dissolution Profile for Nevirapine XR tablets		
	Reference	Test
Manufactured by	Boehringer Ingelheim, USA	Hetero
Apparatus	USP-I Basket 10 mesh size	
RPM	100	
Dissolution Media	900 ml, 2%SLS in pH 6.8 phosphate buffer	
% of Drug release for Nevirapine		
Time in hours	Reference	test
2	19	24
4	37	41
8	66	67
12	87	87
18	97	95
24	98	96
F2(Dissimilarity factor)	73.97	
Dissolution Media	900 ml, 0.1N HCl	
% of Drug release for Nevirapine		
Time in hours	Reference	test
2	10	15
4	19	25
8	42	48

12	62	75
18	85	88
24	99	99
F2(Dissimilarity factor)	56.30	
Dissolution Media	900 ml, pH 4.5 Acetate buffer	
% of Drug release for Nevirapine		
Time in hours	Reference	test
2	6	4
4	13	10
8	26	15
12	31	25
18	36	30
24	38	32
F2(Dissimilarity factor)	59.59	

Standard and sample solution stability:

Standard and sample solution stability was evaluated at room temperature for 48 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 24 h at room temperature.

Specificity:

There was no interference from Standard, sample, placebo and The values of f2 were calculated for the dissolution in three different medias. As can be seen in Table 5 data obtained for f2 were found to be with in the acceptable criteria. In three different media phosphate buffer shows the better result and water was not found to be suitable as dissolution media.

Method precision:

The relative standard deviation for six replicate injections was less than 1.0 %, which met the acceptance criteria established for the method. The results obtained were presented in Table 1.

Accuracy/recovery:

The data presented in Table 2 show excellent recoveries at all levels. The average recoveries for triplicate determinations at 50,100, and 150% levels were with in the acceptable criteria. Excellent recovery and low relative standard deviation value showed that the method is suitably accurate for potency assay of Nevirapine simultaneously in the drug substances.

Linearity:

The plot of peak area responses against concentration. It can be seen that plot is linear over the concentration range of 4.8 to 36 µg/mL of Nevirapine respectively with a correlation coefficient (r2) 0.998. The results of linearity, limit of detection and limit of quantification were presented in Table 4.

Method Ruggedness:

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in Table 3

DISCUSSION

Considering the efficiency of UV-VIS Spectroscopic, attempt has been made to develop simple, accurate, precise, rapid and economic method for simultaneous estimation of Nevirapine in a ER tablet dosage form. Thus method described enables to the quantification of Nevirapine. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. Dissolution testing is very important invitro test to evaluate drug product. This data form the part of the pharmaceutical development report, but can also be included in the bioequivalance study report. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, this UV-VIS

Spectroscopic method can be used for analysis of commercial formulation and dissolution data provides useful information for invivo studies.

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REFERENCES

1. Rey D. et al, Tolerance of a Short Course of Nevirapine, Associated with 2 Nucleoside Analogues, in Postexposure Prophylaxis of HIV. J. Acquir. Immune. Defic. Syndr., 2004; 37: 1454-6.
2. USP-NF, The Official Compendia of Standards, published by The United States Pharmacopoeia Convention, City Press, Baltimore, US, 2009, Vol III, pp 3072-3.
3. Mohanraj P, Deb Kumar S, Choudhury T and Gauthaman K. A Simple and Rapid RP-HPLC Method for the Estimation of Nevirapine in Bulk and Pharmaceutical Dosage Forms. E-J. Chem., 2008; 5 (S2): 1081-6.
4. Prasada Rao CH, Channabasavaraj KP, Lakshmi Aswini G. Development and validation of RP-HPLC method for the estimation of nevirapine in bulk and tablets. J. Pharm. Sci., 2009: 1(2): 78-82.
5. Ananth Kumar D, Naveen Babu MV, Seshagiri Rao JVNL and Jayathirtha Rao V. Simultaneous determination of Lamivudine, Zidovudine and Evirapine in Tablet Dosage Forms by RP-HPLC Method. Rasayana J. Chem., 2010; 3 (1): 94-9.
6. Vaishali PN and Kishor PB. A validated UV spectrophotometric method for the simultaneous estimation of Lamivudine, Nevirapine and zidovudine in combined tablet dosage form. J. Pharm. Res., 2009; 2 (4): 666-9.
7. Venkatesh P and Rajesh Kumar R. Sepectrophotometric determination of nevirapine in pharmaceuticals after derivatization with 2,4-dinitro phenlyhydrazine. Asian J. Pharm. Health Sci., 2011; 1 (1): 33-4.
8. Purnima DH, Mitesh DP and Nitul S. Quantitative estimation of nevirapine by high performance thin layer chromatography. J. Pharm. Res. Health Care. 2009; 1 (2): 197-216.
9. Venkata Kumar Ch, Ananth Kumar D and Seshagiri Rao JVNL. A new validated RP-HPLC method for the determination of nevirapine in human plasma. E-J. Chem., 2010; 7 (3): 821-6.
10. Pav JW, Rowland LS and Korpalski DJ. HPLC-UV method for the quantitation of nevirapine in biological matrices following solid phase extraction. J. Pharm. Biomed. Anal., 1999; 20 (1-2): 91-8.
11. Heeswijk RPG, Hoetelmans RMW, Meenhorst PL, Mulder JW and Beijnen JH. Rapid determination of nevirapine in human plasma by ion-pair reversed-phase high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. B, 1998; 713 (2): 395-9.
12. S. Staszewski, J. Morales-Ramirez, K. T. Tashima, A. Rachlis, D. Skiest, J. Stanford, R. Stryker, P. Johnson, D. F. Labriola, D. Farina, D. J. Manion, N. M. Ruiz, N. Engl. J. Med. 341 (1999) 1865.
13. J. S. G. Montaner, P. Reiss, D. Cooper, S. Vella, M. Harris, B. Conway, M. A. Wainberg, D. Smith, P. Robinson, D. Hall, M. Myers, J. M. A. Lange, JAMA, 279 (1998) 930.
14. D. Podzamczer, E. Ferrer, E. Consiglio, J. M. Gatell, P. Perez, J. L. Perez, E. Luna, A. Gonzalez, E. Pedrol, L. Lozano,Ocana, J. M. Llibre, A. Casiro, M. Aranda, P. Barrufet, J. Martinez-Lacasa, J. M. Miro, X. Badia, A. Casado, S. Lupo, P. Cahn, M. Manos, J. Estela, Antivir Ther. 7 (2002) 81.
15. L. I. Malaty, J. J. Kupper, Drug Sar. 20 (1999)147.
16. D. Back, S. Gibbons, S. Khoo, J. Acquir. Immune Defic. Syndr. 34 (2003) S8–14.
17. R. Panchagnula, S. Agrawal, Y. Ashokraj, M.V.S. Varma, K. Sateesh, V. Bhardwaj, S. Bedi, I. Gulati, J. Parmar, C. Kaul, B. Blomberg, B. Fourie, G. Roscigno, R. Wire, R. Laing , P. Evans, T. Moore, Methods Find. Exp. Clin. Pharmacol. 26 (2004) 703.

18. D. Rey, M. Partisani, H. -K. Georgette, V. Krantz, M. Priester, C. Christine, B. -H. Claudine, E. de Mautort, L. Decroix, J.-M. Lang, J. Acquir. Immune Defic. Syndr. 37 (2004) 1454.
19. C. C. J. Carpenter, M. A. Fischl, S. M. Hammer, M. S. Hirsch, D. M. Jacobsen, D. A. Katzenstein, J. S. G. Montaner, D. D. Richman, M. S. Saag, R. T. Schooley, M. A. Thompson, S. Vella, P. G. Yeni, P. A. Volberding, J. Am. Med. Assoc. 77 (1997) 1962.
20. R. M. Gulick, J. M. Mellors, D. Havlir, J. J. Eron, C. Gonzalez, D. McMahon, D. D. Richman, F. T. Valentine, L. Jonas, A. Meibohm, E. A. Emini, J. A. Chodekewitz, N. Engl. J. Med. 337 (1997) 734.
21. W. Cavert, D. W. Notermans, K. Staskus, W. W.Stephen, Z. Mary, G. Kristin, H. Keith, Z. –Q. Zhang, R. Mills, H. McDade, J. Goudsmit, S. A. Danner & T. H. Ashley, Science 276 (1997) 960.
22. A. Carr, D. A. Cooper, Adv. Exp. Med. Biol. 394 (1996) 299.
23. S. M. Hammer, J. Infect. Dis. 192 (2005) 1.
24. M. Hartmann, S. Witte, J. Brust, D. Schuster, F. Mosthaf, M. Procaccianti, J. A. Rump, H. Klinker, D. Petzoldt, Int. J.STD. AIDS 16 (2005) 404.
25. P. Barreiro, V. Soriano, F. Blanco, C. Casimiro, J. J. de la Cruz, J. Gonzalez-Lahoz, AIDS 14 (2000) 807.
26. N. L. Rezk, R. R. Tidwell, A. D. M. Kashuba, J. Chromatogr. B (2004) 805, 241.
27. C. F. Silverthorn, T. L. Parsons, Biomed. Chromatogr. 20 (2006) 23.
28. G. Ramachandran, A. K. Hemanthkumar, V. Kumaraswami, S. Swaminathan, J. Chromatogr. B 843 (2006) 339.
29. B. H. Chi, A. Lee, E. P. Acosta, L. E. Westerman, M. Sinkala, J. S. A. Stringer, HIV Clin. Trials 7 (2006) 263.
30. R. ter Heine, H. Rosing, E. C. M. van Gorp, J. W. Mulder, W. A. van der Steeg, J. H. Beijnen, A. D. R. Huitema, J. Chromatogr. B 867 (2008) 205.
31. R. Sekar, S. Azhaguvel, Chromatographia 67 (2008) 389.
32. S. Notari, C. Mancone, T. Alonzi, M. Tripodi, P. Narciso, P. Ascenzi, J. Chromatogr. B 863 (2008) 249.
33. G. Ramachandran, A. K. Hemanthkumar, V. Kumaraswami, S. Swaminathan, J. Chromatogr. B 843 (2006) 339.
34. G. R. Da Silva, G. P. Lages, G.A. Pianetti, E.D.A. Nunan, C. D. V. Soares, L. M. M. De Campos, Quimica Nova 29 (2006) 1159.
35. C. F. Silverthorn, T. L. Parsons, Biomed. Chromatogr. 20 (2006) 23.
36. P. Lemmer, S. Schneider, M. Schuman, C. Omes, V. Arendt, J.-C. Tayari, L. Fundira, R. Wennig, Therapeutic Drug Monitoring 27 (2005) 521.
37. U. S. Pharmacopeia 29 NF 24 (2006) 29 15-19-15200

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