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Stability indicating RP-HPLC method for determination of nevirapine in pure and tablet form

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ABSTRACT

In the present study, determination of Nevirapine in pure and tablet form by stability indicating RP-HPLC method was developed. The drug was resolved using a mobile phase of buffer (pH 2.5) and methanol in the ratios of 50:50 on a Thermo BDS Hypersil C_{18} column in isocratic mode. The retention time of nevirapine was 8.583 min. The % RSD was less than 2 in intraday, inter-day precision and in each parameter of robustness. The Lower limit of Detection and the limit of quantitation were found to be 0.2563µg/ml and 0.7767µg/ml respectively which indicates that the method is sensitive. The percentage of average recoveries was obtained in the range of 98 to 102%. Further forced degradation studies of Nevirapine were carried out under acidic, alkaline and hydrolytic conditions as per SIAM (Stability Indicating Assay Methods). The drug is more stable in the acidic and basic medium than the hydrogen peroxide medium. The developed method is simple, new, accurate, precise, robust, sensitive and reproducible and can be utilized for routine laboratory analysis of pure and tablet dosage form.

Keywords: Nevirapine, RP-HPLC, Stability indicating assay method, Validation

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INTRODUCTION

Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Nevirapine is structurally a member of the dipyridodiazepinone chemical class of compounds. The chemical name of nevirapine (Fig. 1) is 11-Cyclopropyl-4-methyl-5, 11-dihydro-6H-dipyrido [3, 2-b: 2′, 3′-e] [1, 4] diazepin-6-one; used to treat HIV-1infection and AIDS. A single dose of Nevirapine given to both mother and child reduced the rate of HIV transmission by almost 50%. Mechanism of action is that, NNRTIs exhibit a classical noncompetitive inhibition pattern with the enzyme. Nevirapine is readily absorbed after oral administration with a peak plasma concentration at 4 hr. C_{max} for Nevirapine is 1-2 µg/ml. The concentration of the drug in the CNS is 45% of that in plasma. It crosses the placenta and has been detected in breast milk. Nevirapine undergoes extensive metabolism in the liver mainly by the cytochrome P450 isoenzymes of the CYP3A family. It is excreted via urine as the glucuronide conjugates of the hydroxylated metabolites. The drug is widely distributed in body tissues and the CNS [1-5].

A literature survey revealed that the few analytical methods available for estimation of nevirapine from pharmaceutical formulations [6-10] and from human plasma [11-16]. The reported method for the estimation of

nevirapine from pharmaceutical formulations includes HPLC, Spectrophotometry and HPTLC method of analysis. Also the above methods are not validated for its performance under stress conditions thus rendering them unsuitable for stability studies. Thus an attempt was made to develop a new, simple, accurate and validated method for determination of Nevirapine by reverse phase high-performance liquid chromatographic method along with its stability studies. The method was validated as per the procedures and acceptance criteria of ICH guidelines [17].

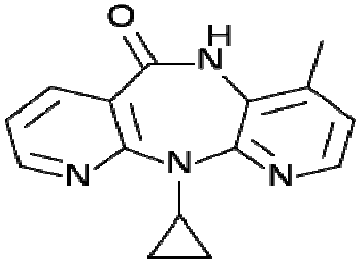

Fig. 1: Chemical Structure of Nevirapine

MATERIALS AND METHODS

Materials:
Nevirapine (NAV) generous gift samples from Cipla Ltd. (Mumbai, India). A commercial NEVIMUNE (Cipla) and NEVIPAN (Crosland's) tablets containing 200 mg of NAV were purchased from a local market and used within their shelf-life period. The HPLC grade acetonitrile, methanol and water were purchased from Rankem (New Delhi, India). All other chemicals used were of pharmaceutical or analytical grade from Rankem (New Delhi, India).

Instrumentation:
The HPLC system consisted of a Shimadzu model LC 2010 CHT series equipped with quaternary constant flow pump, auto injector, SPDM 10 A VP Shimadzu Photodiode Array Detector and LC Solution Version 1.22 SP1 Software, Thermo BDS Hypersil C_{18} column (250 mm × 4.6 mm 5µ) forms the stationary phase.

Optimized chromatographic conditions: RP-HPLC analysis was performed by isocratic elution with flow rate of 1 ml/min. The mobile phase consisting of 0.02 M ammonium dihydrogen phosphate buffer having pH 2.5 was adjusted with formic acid (98%) and methanol were used in the ratio of (50:50) to obtain well-resolved peaks of Nevirapine as shown in the Fig. 3. Injection volumes of 20 µl each of standard solutions were injected into the column. The detection wavelength and chromatographic run time were selected at 264 nm and 25 min, respectively.

Preparation of stock and standard solutions with calibration curve: An accurately weighed quantity of 25mg was transferred to 100 ml volumetric flask, which was then dissolved and made up to volume with mobile phase in order to get 250µg/ml. Suitable aliquots 1, 2, 4, 6 and 8ml of standard stock solutions were taken in five different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 25, 50, 100, 150 and 200 µg/ml of Nevirapine respectively. The solutions were injected using a 20µl fixed loop system and chromatograms were recorded [17-23]. Calibration curve was drawn by plotting average peak area versus concentrations as shown in the Fig. 2. The linearity table of Nevirapine is shown in Table 1.

Table 1: Linearity data of Nevirapine

Concentration (µg/ml)	Area
0	0
25	1085098
50	2023103
100	3902112
150	5680122
200	7458131

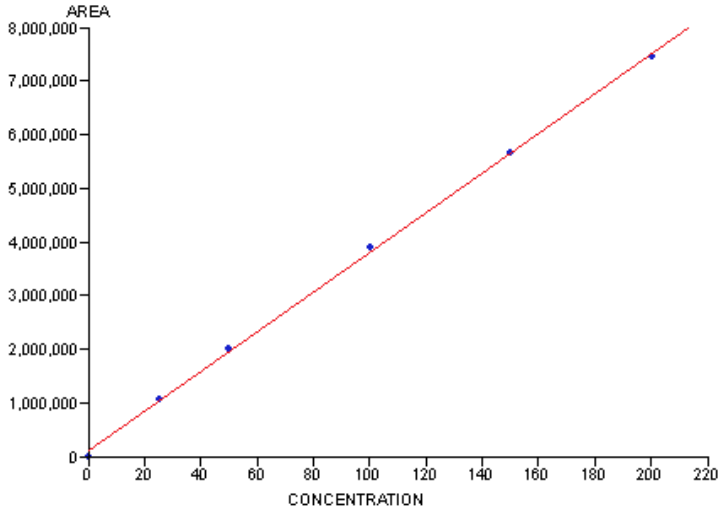

Fig. 2: Calibration curve of Nevirapine

Estimation of nevirapine from tablets: Twenty tablets were weighed accurately and crushed to form fine powder. The powder equivalent to 200 mg of Nevirapine was dissolved in 100 ml of volumetric flask with the mobile phase. The flask was sonicated for 20 min and then the solution was filtered using Whatmann filter paper no. 40. Appropriate volumes of the aliquots (0.5ml) were transferred into five different 20 ml volumetric flasks and the volume was made up to the mark with mobile phase to obtain approximately 50 µg/ml of Nevirapine. The experiments were performed under the optimized chromatographic conditions as described above and peak areas were measured [17-23]. The concentration in the sample was determined by using linear regression equation and the results are shown in the Table 2. The chromatogram of tablet sample Nevirapine is given in Fig. 4.

Table 2: Analysis of Pharmaceutical formulation (Nevimune [R])

Formulation concentration (µg/ml)	Label claim (mg/tab)	Found (mg/tab)	C.I.	%RSD	SE	t
50	200	201.06	100.530 ±2.756	1.722	0.865	0.612

SD: Standard deviation, % SE: Percent standard error, C.I.: Confidence Interval within which true value may be found at 95% confidence level = R ± ts/√n, R: Mean percent result of analysis (n = 4). Theoretical 't' values at 95% confidence level for n - 1 degrees of freedom t (0.05, 3) = 3.1827

Method Validation: The proposed method was validated according to ICH guidelines Q_2B [16]. System suitability test was carried out on freshly prepared standard stock solution of Nevirapine (100µg/ml) and the results of parameters were obtained by five replicate injections. The system suitability results are given in the Table 3. All parameters were satisfactory with good specificity for the stability assessment of nevirapine.

Table 3: System suitability

Parameter	Results of Nevirapine	I.P. Limits
Asymmetry factor	1.46	NMT 1.5
Retention Time (mins)	8.583	------
Theoretical plates	7934.693	NLT 7500
Repeatability (%RSD)	0.898	NMT 2

Linearity: The linearity range was found in between 25-200 µg/ml. The linear regression equation of Nevirapine is Y = 36,416.5302 x +205,977.5244 and Co-relation coefficient r^2 = 0.9998.

Specificity: The peak purity of Nevirapine was assessed by comparing the retention time (R_t) of standard Nevirapine Good correlation was also found between the retention time of standard and sample of Nevirapine.

Precision: Precision study was performed to find out intraday and inter-day (within three days) variations in the estimation of Nevirapine of different concentrations with the proposed method. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. Results are shown in Table 4.

Table 4: Precision data of Nevirapine

Inter day			Intra day		
Concentration (µg/ml)	Area	%RSD	Concentration (µg/ml)	Area	%RSD
50	2024053	0.040	50	2029053	0.205
100	3905363	0.117	100	3920363	0.430
150	5689522	0.143	150	5767022	1.752

Accuracy: It was found out by recovery study using standard addition method. Known amounts of standard Nevirapine was added to pre-analyzed samples at a level from 80%, 100% and 120% and then subjected to the proposed HPLC method. Results of recovery studies are shown in Table 5.

Table 5: Results of recovery study of Nevirapine

% Level of recovery	Formulation (µg/ml)	Amount of standard drug added (µg/ml)	Amount of standard drug found (µg/ml)	C.I.	%RSD	SE	t
80	50	40	40.007	100.018±1.1386	0.871	0.435	0.043
100	50	50	50.027	100.055±1.762	1.1069	0.553	0.099
120	50	60	60.282	100.470±1.137	0.7116	0.357	1.317

SD: Standard deviation, % SE: Percent standard error, C.I.: Confidence Interval within which true value may be found at 95% confidence level = R ± ts/√n, R: Mean percent result of analysis of Recovery study (n = 4). Theoretical 't' values at 95% confidence level for n - 1 degrees of freedom t (0.05, 3) = 3.1827

Robustness: It was done by making small changes in the chromatographic conditions and found to be unaffected by small changes like flow rate ± 1%, pH changes ± 0.1, temperature ± 2^{o} C, wavelength ±2 nm and ± 2% change in organic solvent in the mobile phase. The results are shown in the Table 6.

Table 6: Results of robustness study of Nevirapine at different conditions

Analyte	% of Drug*	RSD	% of Drug*	RSD
Nevirapine	Flow rate (+1%)		Flow rate (-1%)	
	99.976	0.689	100.463	0.547
	pH (+0.1 Units)		pH (-0.1 Units)	
	100.466	0.922	99.523	0.7907
	Temperature (+2°C)		Temperature (-2°C)	
	99.7533	0.6246	100.533	0.6408
	Wavelength (+2 nm)		Wavelength (-2nm)	
	99.8166	0.84085	100.35	0.4825
	Organic solvent in Mobile phase (+2%)		Organic solvent in Mobile phase (-2%)	
	98.65	0.554	100.173	0.710

Sensitivity: The sensitivity of the method was determined with respect to LOD and LOQ. The LOD and LOQ were separately determined based on the standard calibration curve. LOD = (3.3 x S.D /S and LOQ = 10 x S.D/S, where, S.D is the standard deviation of the y- intercepts of regression line and S is the average slope of the calibration curve. The Lower limit of detection and limit of quantitation were found to be 0.2563µg/ml and 0.7767µg/ml respectively.

Forced degradation studies: Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method. Peroxide degradation was carried out by exposing 25.01 mg of Nevirapine in a 25ml volumetric flask containing 3% H_2O_2 for four hours. Then aliquot 1ml was transferred into another 10 ml volumetric flask and made up to the mark with mobile phase to obtain the below mentioned concentration of Nevirapine. Acidic hydrolysis was carried out by exposing 25.05 mg of Nevirapine in a 25ml volumetric flask containing acid (0.1 N HCL, 40^{0}C) for four hours. Then aliquot 1ml was transferred into another 10 ml volumetric

flasks and made up to the mark with mobile phase to obtain the below mentioned concentration of Nevirapine. Basic (0.1 M NaOH) hydrolysis degradation was carried out by exposing 25.16 mg of Nevirapine in a 25ml volumetric flask containing basic (0.1 N NaOH, 40^0C) solvent for four hours. Then aliquot 1ml was transferred into another 10 ml volumetric flask and made up to the mark with mobile phase to obtain the below mentioned concentration of Nevirapine. The results of forced degradation study of Nevirapine are shown in Table 7. Acidic, basic and oxidative degradation chromatograms of Nevirapine are shown in Fig. 5, 6 and 7 respectively.

Table 7: Results of forced degradation study of Nevirapine

Sl. No.	Agent	Drug	Initial concentration (µg/ml)	Conc. found (µg/ml)	% Degradation
1	3% H_2O_2	NEV	100.10	69.66	30.40
2	0.1 M HCL	NEV	100.20	85.43	14.74
3	0.1M NaOH	NEV	100.64	82.82	17.70

% degradation = [(initial concentration - stressed concentration) / initial concentration] x 100

RESULTS AND DISCUSSION

The modalities adopted in experimentation were successfully validated as per analytical procedures laid down in routine. The results of specificity studies indicated no interference from excipients, impurities and degraded products due to various stress conditions and assured that the peak response was due to a single component only. The chromatogram of samples degraded with hydrogen peroxide showed one small peak having retention time 3.083 mins and well resolved from the drug peak, the chromatogram is shown in Fig. 7. There were no extra peaks were observed in the acidic and basic degradation chromatogram of Nevirapine. The drug is more stable in the acidic and basic medium than the hydrogen peroxide medium. The drugs are degraded less than < 30 in the case of acidic and basic medium. All the system suitability parameters found in the developed method are complying with the Indian Pharmacopoeia 2007. The % RSD was less than 2 in intraday, inter-day precision and in each parameter of robustness. So the proposed method is more precise and robust. The Lower limit of Detection and the limit of quantitation were found to be 0.2563µg/ml and 0.7767µg/ml respectively which indicates that the method is sensitive. The proposed method was validated by preliminary analysis of standard sample and by recovery studies. The percentage of average recoveries was obtained in the range of 98 to 102%. The results of analysis of average recoveries obtained in each instance were compared with the theoretical value of 100 percent by means of Student's't' test. As the calculated't' values are less than theoretical' values (Table 5), it is concluded that the results of recoveries obtained in agreement with 100 percent for each analyte are accurate. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets.

CHROMATOGRAMS:

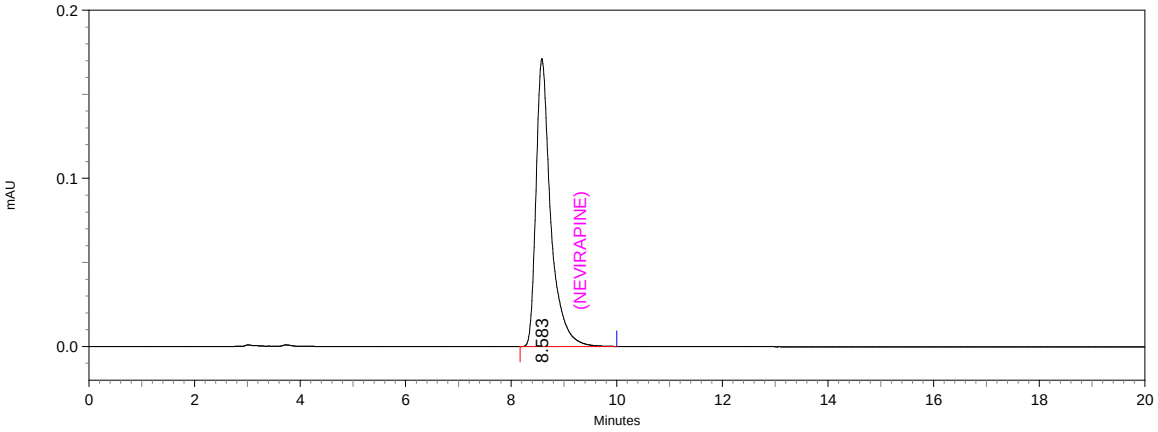

Fig 3: Representative chromatogram of Standard Nevirapine

Pk #	Retention Time	Area	Area %	Detector A (264nm) Asymmetry	Theoretical plates	Height	Resolution	Width	Capacity factor
1	**8.583**	**3317088**	**100.000**	**1.58**	**5416.51**	**171219**	**0.00**	**1.83**	**857.33**

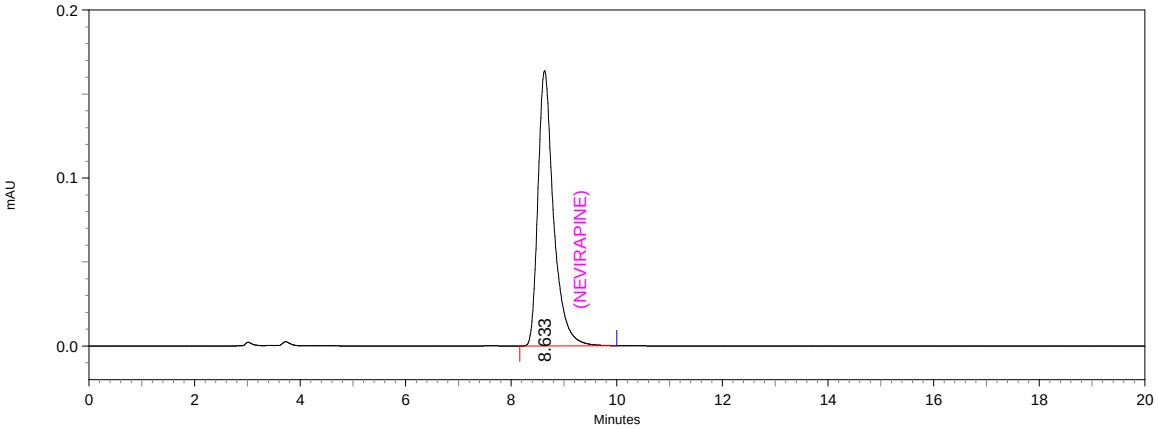

Fig 4: Representative chromatogram of tablet sample of Nevirapine

Detector A (264nm)									
Pk #	**Retention Time**	**Area**	**Area %**	**Asymmetry**	**Theoretical plates**	**Height**	**Resolution**	**Width**	**Capacity factor**
1	**8.633**	**3366235**	**100.00**	**1.43**	**4611.03**	**163853**	**0.00**	**1.84**	**862.33**

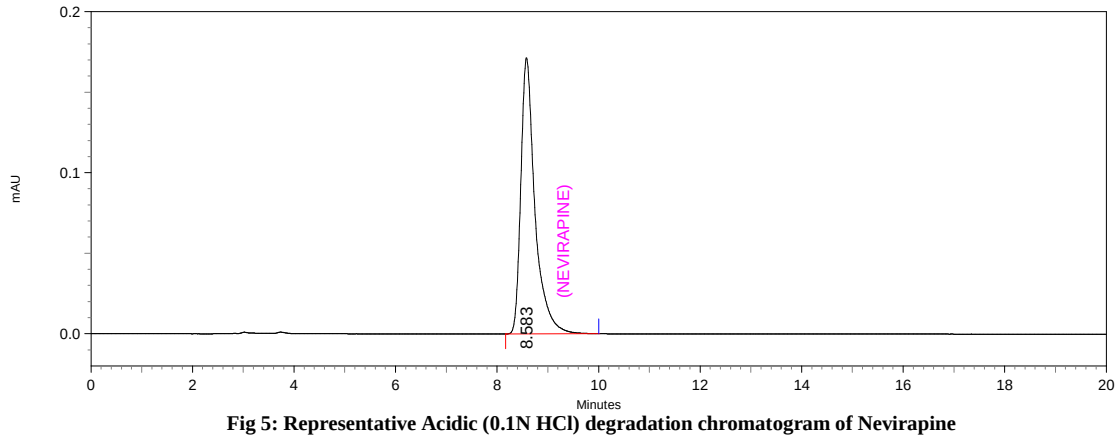

Fig 5: Representative Acidic (0.1N HCl) degradation chromatogram of Nevirapine

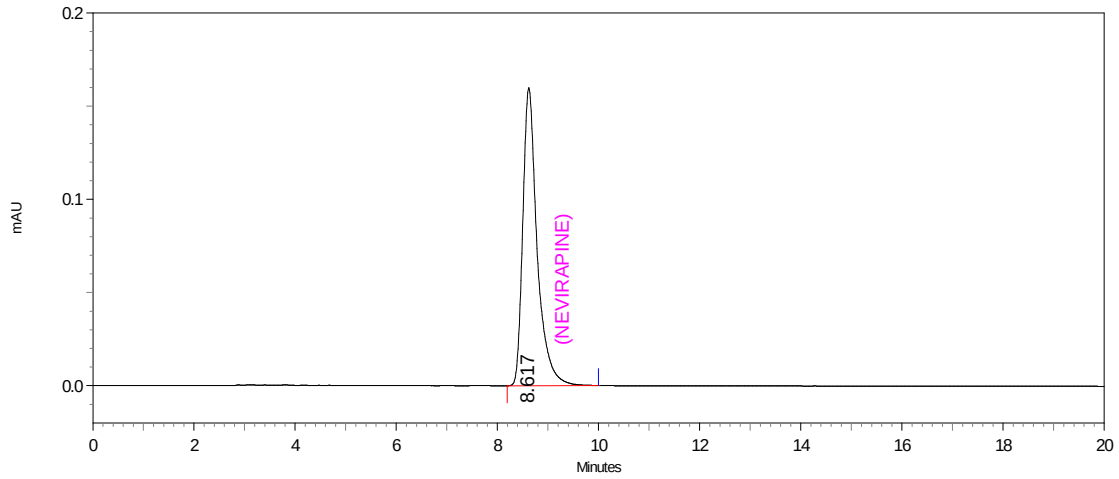

Fig 6: Representative Basic (0.1N NaOH) degradation chromatogram of Nevirapine

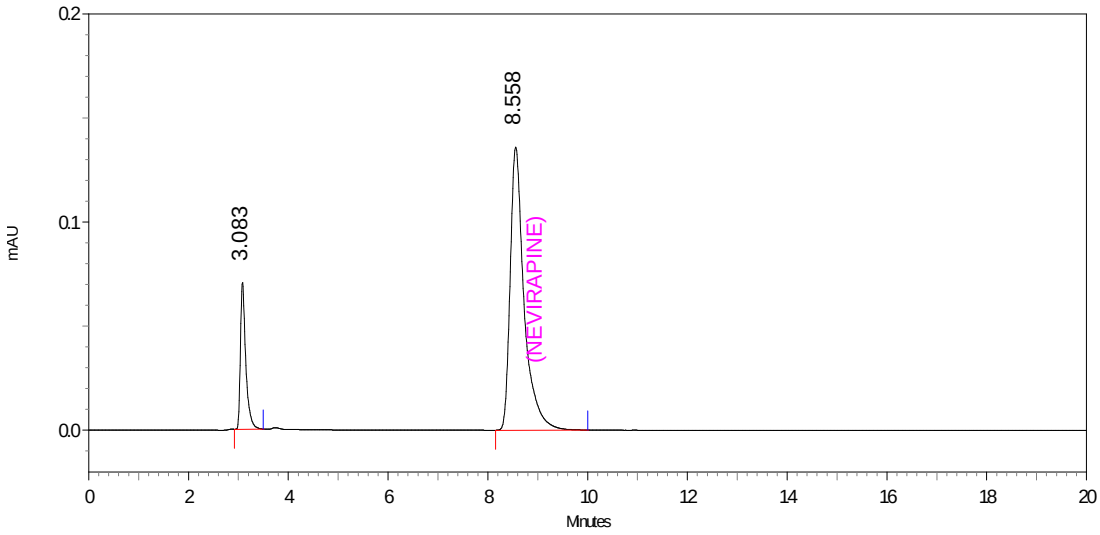

Fig 7: Representative Oxidative chromatogram of Nevirapine

CONCLUSION

The advantages of the proposed method in comparison to the compendia method are shorter analysis time, less toxic organic solvent used and no sample extraction required. So, the proposed method is simple, economy, accurate, precise, reliable and suitable for the routine quality control and stability indicating studies of nevirapine for bulk drug and tablets.

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REFERENCES

[1] *Indian Pharmacopoeia, Volume II*, 5th ed. Ghaziabad: Indian Pharmacopoeia Commission; **2007**: 1433-1435.
[2] *British Pharmacopoeia, Volume I*, 3rd ed. London: British Pharmacopoeia Commission; **2001** supplement:1519.
[3] S.C. Sweetman. *Martindale: The complete drug Reference*, 33rd ed. London: Pharmaceutical Press; **2002**: 635, 645, 637.
[4] http://en.wikipedia.org/wiki/Nevirapine.
[5] A. Renuka Devi and R. Shyamala. *Der Pharmacia Lettre*, **2012**, 4: pp. 1835-1836.
[6] J.W. Pav, L.S. Rowland, D.J. Korpalski. *J. Pharm. Biomed. Anal*. **1999**, 20: pp. 91-98.
[7] C. Marzolini, A. Béguin, A. Telenti, A. Schreyer, T. Buclin, J Biollaz, L.A. *J. Chromatogr. B.* **2002**, 2: pp. 127-140.
[8] M. Sarkar, S. Khandavilli, R. Panchagnula. *J. Chromatogr. B.* **2006**, 830: pp. 349-354.
[9] S. Anbazhagan, N. Indumathy, P. Shanmugapandiyan, S.K. Sridhar. J. *Pharm. Biomed. Anal*. **2005**, 39: pp. 801-804.
[10] A. K. H. Kumar, V. Sudha, S. Swaminathan, G. Ramachandran. *Ind. J. Med. Res*. **2010**, 132: pp. 390-394.
[11] R.P.G.V. Heeswijk, R.M.W. Hoetelmans, P.L. Meenhorst, J.W. Mulder, J.H. Beijnen. *J. Chromatogr. B.* **1998**, 713: pp. 395-399.
[12] G. Ramachandran, A.K. Hemanthkumar, V.K. Swami, S. Swaminathan. *J. Chromatogr*. B. **2006**, 843: pp. 339-344.
[13] M. Lopez, L. Pou, M.R. Gomez, I. Ruiz, J. Monterde. *J. Chromatogr. B.* **2001**, 751: pp. 371-376.
[14] H.N. Mistri, A.G. Jangid, A. Pudage, N. Gomes, M. Sanyal, P. Shrivastav. *J. Chromatogr.* B. **2007**, 853: pp. 320-332.

[15] E. Marchei, L. Valvo, R. Pacifici, M. Pellegrini, G. Tossini, P. Zuccaro. *J. Pharm. Biomed. Anal.* **2002**, 29: pp. 1081-1088.
[16] N.L. Rezk, R.R. Tidwell, A.D.M. Kashuba. J. *Chromatogr. B.* **2002**, 774: pp. 79-88.
[17] ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2B (R1), Nov. **2005**.