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Stability Indicating Analytical Method Development and Validation of Efavirenz Quantification by High Performance Liquid Chromatographic Technique

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Abstract: A stability-indicating high performance liquid chromatography (HPLC) method is developed for the quantification of efavirenz drug substance. Selected mobile phase is a combination of 50 volume of acetonitrile and 50 volume of 0.86% w/v solution of ammonium dihydrogen phosphate, pH adjusted to 3.0 with orthophosphoric acid. Optimized column is a stainless steel column packed with base deactivated octa decylsilyl silica gel and at 252 nm wavelength. Linearity of the method is found 40 µg/mL to 160 µg/mL with regression coefficient of 0.9995. The method is validated according to ICH guidelines.

Keywords: Efavirenz, Liquid chromatography, Antiretroviral, Validation

Introduction

The aim of the current study is to establish stability indicating HPLC quantification method for efavirenz drug substance as per international conference for harmonization of technical requirement for pharmaceutical in human use[1]. The study has been carried out as part of bigger project. The revised parent drug stability test guideline issued by international conference for harmonization (ICH) requires the stress testing on the drug substance have been taken in to account to establish its stability characteristics and for supporting the stability of proposed analytical procedures. ICH suggested that stress testing should include the effect of temperature, humidity, light and oxidizing agent across a wide range of pH values[2]. ICH also recommended that analysis of stability samples should be done with the use of validated stability indicating methods[3-4].

Several analytical methods reported for the determination of efavirenz drug substance but not any method accounted for the products formed under condition of hydrolysis, oxidization and thermal stress which are required to be considered according to ICH. Efavirenz is an antiretroviral drug. Efavirenz falls in the NNRTI class of antiretrovirals. Both nucleoside and non-nucleoside RTIs inhibit the same target, the reverse transcriptase enzyme, an essential viral enzyme which transcribes viral RNA into DNA. Unlike nucleoside RTIs, which bind at the enzyme's active site, NNRTIs act allosterically by binding to a distinct site away from the active site known as the NNRTI pocket. Efavirenz is not effective against HIV-2, as the pocket of the HIV-2 reverse transcriptase has a different structure, which confers intrinsic resistance to the NNRTI class[5] as most NNRTIs bind within the same pocket, viral strains which are resistant to Efavirenz are usually also resistant to the other NNRTIs, nevirapine and delavirdine. The most common mutation observed after efavirenz treatment is K103N, which is also observed with other NNRTIs[6]. Psychiatric symptoms, including insomnia, confusion, memory loss, and depression, are common[7] and more serious symptoms such as psychosis may occur in patients with compromised liver or kidney function[8-9]. Rash, nausea, dizziness and headache may occur efavirenz can cause birth defects and should not be used in women who might become pregnant[10]. Safety in children has not been established Use of efavirenz can produce a false positive result in some urine tests for marijuana[11-12].

Experimental:

Efavirenz drug substance was supplied by Hetro labs Ltd (Hyderabad) and used without further purification. Ammonium dihydragen orthophosphate (AR grade) and orthophosphoric acid was purchased from Renkem India Ltd. India. Acetonotire, methanol (HPLC grade) was purchased from Merck India Ltd. Sodium luryl sulphate, hydrogen peroxide was also purchased from Merck India Ltd. Sodium hydroxide and hydrochloric acid was purchased from s.d fine Chemicals Ltd. India. Milli Q water was obtained from millipore water purifier.

Instrumentation

The HPLC system consisting of an alliance system (Waters Milford, USA) with UV/VIS detector (Waters 2487). Data acquisition and processing was done by using Empower software. Another HPLC used with photo diode array detector (Waters 996) to check the peak purity. Stress studies under neutral, acidic and alkaline condition were performed by using high precision water bath with digital temperature controller.

Chromatographic condition

The chromatographic separation was carried out on Zorbax RX C18 (4.6x250) mm, packed with base deactivated octadecylsilyl silica gel HPLC column (5 µm) and mobile phase was filtered and degassed mixture of 50 volume of acetonitrile and 50 volume of 0.86% w/w solution of ammonium dihydrogen phosphate, pH of which was adjusted 3.0 with diluted orthophosphoric acid. Mobile phase was filtered with 0.45 µm filter (Millipore membrane) and degassed. The flow rate of mobile phase was 1.5 mL/min and the injection volume was 20 µL. Quantification of efavirenz drug substance was carried out at 252 nm.

Sample preparation

Efavirenz drug substance sample was prepared in mobile phase at the concentration of 100 µg/mL.

Degradation studies

Drug at a concentration of 100 µg/mL was used in all degradation studies condition employed for performing stress studies were as follows. Acid decomposition studies were performed by heating of drag in 5 mL 0.1 N HCl at 80 ^{0}C for 4 h. The studies in alkaline condition were performed by heating drug in 0.1 N NaOH (sodium hydroxide) at 80 ^{0}C for 4 h. For the study in neutral condition, the drug dissolved in water and the solution was heated at 80 ^{0}C for 4 h. In oxidative studies the drug dissolved in 3% hydrogen peroxide and keeps it 4 h at room temperature.

Separation studies on stress samples

In all HPLC run the mobile phase was filtered with 0.45 µm filter (Millipore HVLP type) and degassed. The flow rate of mobile phase was 1.5 mL/min and the injection volume was 20 µL. The detection wavelength was 252 nm.

Validation of method

Validation of the optimized HPLC method was done with following parameters. Specificity of the method towards the drag was studies by determination of purity for analyte peak in stress samples using photodiode array detector. The study of resolution factor of the drug peak from the degradation product peak was also done. Precision of the method was carried out by repeatability studies. Precision of the assay method was assessed by making six determinations sample solution at concentration of 100 µg/mL of the drug. The relative standard deviation was calculated to determine by precision. The accuracy of the method was studied at three concentration of the drug in triplicate. The amount of drug was calculated and compared with the amount of drug added. Linearity of the method was studied by injecting six concentration of the drug. A stock solution of the drug (100 µg/mL) was prepared. This stock solution was diluted to prepare a solution containing 40-160 µg/mL (*i.e.* 40% to 160%) of drug with mobile phase. The injections were injected in the triplicate in HPLC system keeping the injection volume constant (20 µL).

Filter standardization

The Filter standardization was evaluated with the standard and test preparation filtered using HVLP (0.45 µm) filter and disc filter in terms of mean area in % variation.

Stability of solution

The stability of solution for efavirenz was studied after keeping the standard and sample solution for 24 h at room temperature.

Results and Discussion

Degradation behavior of efavirenz

HPLC analysis of samples obtained on stress testing of efavirenz under different conditions. It was observed that around 1.8 to 2% drug degraded on heating it in 0.1 N HCl for 4 h at 80 ^{0}C but there was no corresponding formation of degradation product as compared to the standard solution of drug. Peak purity for efavirenz peak was within acceptance criteria *i.e.* purity angle should be less than purity threshold.

The result obtained efavirenz in alkaline condition was found that around 62 to 63% of drug degradation on heating it with 0.1 N NaOH for 4 hrs at 80 ^{0}C. The major product was seen around 2 min and 8 min respectively during HPLC analysis of alkali degraded sample. Peak purity of efavirenz peak was within an acceptance criteria. In neutral condition only

1.6 to 2% degradation of the drug was found on heating the drug at 80 ^{0}C for 4 h. Peak purity of efavirenz peak was within acceptance criteria. The drug was found to be stable in 3% H_2O_2 and there was no corresponding formation of degradation products. The result is presented in Table 1.

Table 1. Forced degradation study for efavirenz

Wt of efavirenz Std. taken = 99.42 mg Mean area of efavirenz peak =3454236			
Stress condition	Assay degraded/ Unstressed sample, %	Peak purity test for efavirenz peak	
		Peak Purity Angle	Peak purity Threshold
Unstressed	99.16	0.150	0.260
Acidic:5 mL 0.1 N HCl / @ 80 ^{0}C for 4 h	97.36	0.142	0.257
Alkaline:-5 mL of 0.1 N NaOH @ 80 ^{0}C for 4 h.	36.05	0.194	0.416
Oxidative:5 mL of 3%H_2O_2 Room temp for 4 h.	99.26	0.139	0.257
Neutral 5 mL water heat @ 80 ^{0}C for 4 h	97.48	0.135	0.260
Acceptance Criteria	Purity angle should be less than purity threshold.		

It was observed that there is no interference of the sample diluents at the retention time of the efavirenz peak. More ever the method was also selective to degradation products as their peak were pure, which was proved through PDA purity studies. Data obtained from precision experiments are given in below table. The relative standard deviation in precision study is less than 2% which confirm that the method is sufficiently precise. The results of precision are given in Table 2.

Table 2. Precision data for developed method

Wt of efavirenz Std. taken = 100.71 mg Mean area of efavirenz peak =3536110						
Wt. of test sample taken, mg	Test-1	Test-2	Test-3	Test-4	Test-5	Test-6
	103.99	104.01	104.02	103.98	103.97	104.00
Mean area	3716107	3712904	3714252	3724452	3730791	3727138
Assay %	101.25	101.15	101.18	101.50	101.68	101.54
Mean	101.38					
% RSD	0.2					
Acceptance criteria	RSD should be not more than 2%					

Accuracy of the method was studies at three levels (65%, 104% and 130%) of assay concentration. The amount of efavirenz was calculated and compared with amount of efavirenz added. The result of accuracy studies are given in Table 3. Linearity of the method was studied from 40%, 60%, 80%, 100%, 120%, 140% and 160% of the test concentration. The injections were injected in triplicate. The results obtained are given in Table 4.

Table 3. Accuracy data for developed method

Wt of efavirenz Std. taken =100.71 mg Mean area of efavirenz peak -3536110									
Conc.	65%			104%			130%		
Efavirenz added, mg	65.16	65.16	65.16	104.29	104.29	104.29	130.26	130.26	130.26
Mean area of Efavirenz peak	2258862	2249506	2251068	3615549	3605293	3627739	4574199	4631767	4489092
Efavirenz found	64.45	64.18	64.22	103.19	102.90	103.53	130.45	132.09	128.03
% Recovery	98.91	98.50	98.57	98.95	98.67	99.28	100.15	101.41	98.29
Mean Recovery %		98.66			98.97			99.95	
Overall mean %					99.19				
Range %					98.29-101.41				
Acceptance criteria					% recovery should be between 98 to 102.0				

Table 4. Linearity data for developed method

Concentration	Linearity @ 40%	Linearity @ 60%	Linearity @ 80%	Linearity @ 100%	Linearity @ 120%	Linearity @ 140%	Linearity @160%
Area	391596	2092975	2737806	3467470	4190796	4939134	5690720
Regression equation	35534x-77544						
Correlation coefficient	$R^2 = 0.9995$						
Acceptance criteria	Correlation coefficient should be more than 0.99						

Filter Standardization

Science the percent variation in terms of assay (in %) by comparison of unfiltered against HVLP filter is within +. 2%. Hence HVLP filter is suitable. The % variation in terms of assay (in %) by comparison of unfiltered against Disc is more than 2% of test solution. Hence disc filter is not suitable. The results are given in Table 5.

Table 5. Filter standardization data for developed method

Sample	Standard		Test	
	Mean area observed	% Variation	Mean area observed	% Variation
Unfiltered sample	3516042	N.A	3871336	N.A.
Filtered with HVCP filter	3482521	-0.96%	3823306	-1.81%
Filtered with Disc filter	3485822	-0.37%	3588641	-7.88%

Stability of solution

The results of stability of solution are given in Table 6.

Table 6. Stability of solution data for developed method

Wt. of efavirenz std. taken = 100.38mg Mean area of efavirenz peak = 3465705									
	Initial 0 h	After 3 h	After 6 h	After 9 h	After 12 h	After 15 h	After 18 h	After 21 h	After 24 h
Standard area	3465705	3488552	3474117	3502432	3507478	3513254	3514627	3524467	3531319
%RSD	-	0.5	0.4	0.6	0.6	0.7	0.7	0.8	0.9
Acceptance criteria	The % RSD of the area of Efavirenz peak should not be more than 20%								

Conclusion

In this study, stability of efavirenz was established through employment of ICH recommended stress study[13-14]. The drag was found of degradation extensively in alkaline condition and mild degradation was also found in acidic and neutral condition. The HPCL method was developed, which is simple, accurate, precise, specific, selective and can be used to analyze the drug in stability sample.

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References

1. ICH, Stability Testing of New Drug Substances and Products, International Conference on Harmonization, IFPMA, Geneva, 1993.
2. ICH, Stability testing: photo stability testing of new drug substances and products, in: Proceedings of the International Conference on Harmonization, IFPMA, Geneva, 1996.
3. Bakshi M and Singh S, *J Pharm Biomed Anal*., 2002, **28**, 1011-1040.
4. Bakshi M, Singh B, Singh A and Singh S, *J Pharm Biomed Anal*., 2001, **26(5-6)**, 891-897.
5. Ren J, Bird L E and Chamberlain P P, Stewart-Jones G B, Stuart D I and Stammers D K**,** *Proc Natl Acad Sci.,* USA 2002, **99(22)**, 14410.
6. Sustiva (efavirenz) capsules and tablets, Product information*,* April 2005.
7. Cespedes M S and Aberg J A, *Drug Safety,* 2006, **29(10)**, 865-874.
8. Hasse B, Günthard H F, Bleiber G and Krause M, *Clinical Infectious Diseases*, 2005, **40(3),** e22. PMID 15668854
9. Lowenhaupt E A, Matson K, Qureishi B, Saitoh A and Pugatch D, *Clinical Infectious Diseases*, 2007, **45(10)**, 128-130.
10. DHHS Panel, Guidelines for the use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents*,* October 10, 2006.
11. Rossi S, Yaksh T, Bentley H, van den Brande G, Grant I and Ellis R, *Clin Chem.*, 2006, **52(5)**, 896-897.
12. Röder C S, Heinrich T, Gehrig A K and Mikus G, *AIDS*, 2007, **21,** 1390-1391.
13. Mahalaxmi D, Samarth M M, Shiravadekar H S and Sanghavi N M, Drug Dev *Ind Pharma*., 1996, **22**, 1037-1039.
14. Brightman K, Finlay G, Jarvis I, Knowlton T and Manktelow C T, *J Pharm Biomed Anal.,* 1999, **20(3)**, 439-447.