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Development and Validation of a Stability Indicating HPLC Method for Efavirenz and Assessment of Its Degradation Profile Under ICH-Mandated Stress Conditions

[1]Rajesh Meshram, [2]Rajesh Gour,
[1]Research scholar, LNCT University , Bhopal M.P. India.
[2]Associate Professor, LNCT University , Bhopal M.P. India.

Abstract- A stability-indicating HPLC method was developed and validated for Efavirenz, a non-nucleoside reverse transcriptase inhibitor. The method effectively separated Efavirenz from its degradation products under various stress conditions, including acidic, alkaline, oxidative, thermal, and photolytic stress. The results showed that Efavirenz is highly labile to alkaline hydrolysis and moderately susceptible to acidic hydrolysis and oxidation. The method was linear, accurate, precise, and robust, with a mean recovery of 98.9-101.2% and RSD < 1.5%. The study provides a comprehensive degradation profile of Efavirenz, confirming its vulnerability to hydrolysis, especially in alkaline environments. This method is suitable for routine quality control and stability monitoring of Efavirenz in pharmaceutical formulations.

Keywords - Efavirenz, Stability-Indicating Assay Method (SIAM), Forced Degradation, HPLC, Method Validation, Degradation Kinetics, ICH Guidelines.

I. INTRODUCTION

Efavirenz is a cornerstone non-nucleoside reverse transcriptase inhibitor used in HIV-1 treatment regimens. Ensuring its chemical stability is crucial for product quality, safety, and efficacy. This study focuses on developing and validating a stability-indicating HPLC method for Efavirenz and assessing its degradation profile under ICH-mandated stress conditions. The objective is to evaluate the susceptibility of Efavirenz to hydrolytic, oxidative, thermal, and photolytic stress and to develop a robust method for routine quality control and stability monitoring. The study aims to provide a comprehensive degradation profile of Efavirenz, highlighting its vulnerability to hydrolysis and oxidation, and ensuring the quality and stability of this critical antiretroviral drug. By developing a stability-indicating HPLC method, this study will contribute to safeguarding patient safety and therapeutic efficacy. The method's specificity, linearity, accuracy, precision, and robustness will be evaluated, and its suitability for routine analysis will be assessed.1-4

II. MATERIALS AND METHODS

Chemicals and Reagents

Efavirenz USP Reference Standard(Lot# R085U0, 99.9% purity) was used. HPLC-grade acetonitrile and methanol, along with analytical grade hydrochloric acid (37%), sodium hydroxide pellets, and hydrogen peroxide (30%), were procured. Formic acid (99%) for LC-MS was obtained from Sigma-Aldrich. High-purity water was generated using a Milli-Q system.

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Instrumentation

HPLC-DAD: An Agilent 1260 Infinity II system with a quaternary pump, autosampler, thermostatted column compartment, and diode array detector was used. Data was processed using Agilent OpenLab CDS software.

Chromatographic Conditions

Separation was achieved on a Zorbax Eclipse Plus C18 column(150 mm × 4.6 mm, 3.5 µm). The column temperature was maintained at 30 °C. The mobile phase consisted of (A) 0.1% (v/v) formic acid in water and (B) acetonitrile, run with a gradient: 0-5 min (30% B), 5-15 min (30-80% B), 15-20 min (80% B), 20-22 min (80-30% B), 22-25 min (30% B). The flow rate was 1.0 mL/min, injection volume was 10 µL, and detection was performed at 247 nm.4,5

Forced Degradation Procedure

A stock solution of Efavirenz(1.0 mg/mL) was prepared in a 1:1 mixture of methanol and water. All stressed samples were prepared in triplicate.1

Acid Hydrolysis: 5 mL of stock was mixed with 5 mL of 2.0 M HCl and refluxed at 80 °C for 8 hours, then neutralized.

Alkaline Hydrolysis: 5 mL of stock was mixed with 5 mL of 0.2 M NaOH and refluxed at 80 °C for 4 hours, then neutralized.

Oxidative Degradation: 5 mL of stock was mixed with 5 mL of 12% H_2O_2 and stored at ambient temperature for 24 hours, protected from light.

Thermal Degradation: Solid EFV powder was kept in a hot air oven at 105 °C for 72 hours.

Photolytic Degradation: Solid EFV powder was exposed in a photostability chamber to 1.2 million lux hours and 200 watt-hours/m^2 of UV energy.

All samples were diluted to 50 µg/mL before HPLC analysis.

Method Validation6

The method was validated per ICH Q2(R1)for:

Specificity: By analyzing stressed samples and assessing peak purity via DAD.

Linearity: Using eight calibration standards from 1-150 µg/mL.

Accuracy: Via standard addition method at 80%, 100%, and 120% of the target concentration.

Precision: Repeatability (intra-day) and intermediate precision (inter-day).

LOD and LOQ: Calculated from the standard deviation of the response and the slope.

Robustness: By deliberate variations in column temperature (±2 °C), flow rate (±0.1 mL/min), and mobile phase pH (±0.2 units).

III. RESULTS AND DISCUSSION

Development and Validation of the Stability-Indicating HPLC Method

The optimized gradient HPLC method successfully achieved baseline separation between Efavirenz and all its degradation products formed under various stress conditions.The Efavirenz peak was sharp and symmetrical with a retention time of approximately 12.5 minutes.

The method validation confirmed its suitability as a SIAM:

Specificity: The peak for Efavirenz was well-resolved (Resolution > 2.0) from all degradation peaks in the stressed samples. DAD-based peak purity analysis confirmed the homogeneity of the EFV peak (purity index > 0.999), proving no co-elution with degradants.

Linearity: The method demonstrated an excellent linear response over the concentration range of 1-150 µg/mL with a correlation coefficient (R^2) of 0.9997.

Accuracy: The mean recovery of Efavirenz ranged from 98.9% to 101.2%, indicating high accuracy.

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Precision: The method was precise, with a relative standard deviation (RSD) of 0.85% for repeatability and 1.32% for intermediate precision.
Sensitivity: The Limit of Quantification (LOQ) was determined to be 0.5 µg/mL, which is sufficiently sensitive for impurity quantification.
Robustness: The method was robust, as minor deliberate changes in chromatographic parameters did not significantly affect the system suitability criteria or the analysis.

Degradation Behavior of Efavirenz

The forced degradation study provided a clear profile of the intrinsic stability of Efavirenz. The results are summarized in the table 1 below.

Stress Condition	Duration	Temperature	% Degradation	Number of Major DPs
Acid Hydrolysis (1M HCl)	8 hours	80 °C	~28%	3
Alkaline Hydrolysis (0.1M NaOH)	4 hours	80 °C	~45%	1 (Major)
Oxidation (6% H_2O_2)	24 hours	Ambient	~18%	1
Thermal Degradation	72 hours	105 °C	<2%	None significant
Photolytic Degradation	ICH Spec.	Chamber	<2%	None significant

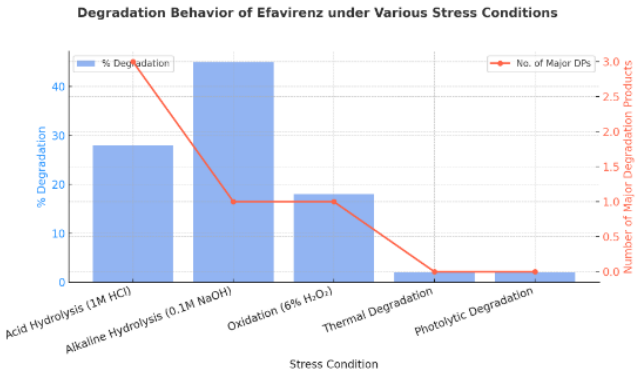

Figure 1: EFV degradation profile under different stressed conditions (samples subjected to acid, base, and oxidative stress, demonstrating the method's specificity)

The data unequivocally shows that Efavirenz is most susceptible to hydrolytic degradation, with alkaline conditions being significantly more aggressive than acidic ones. This suggests that formulations must be protected from moisture and alkaline excipients should be avoided. The drug also shows moderate sensitivity to oxidative stress, indicating a need for antioxidants in the formulation if deemed necessary.

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Importantly, Efavirenz in its solid state is highly stable against heat and light, which is advantageous for the manufacturing, packaging, and long-term storage of its solid dosage forms.

IV. CONCLUSION

A stability-indicating HPLC method was developed and validated for Efavirenz, effectively separating it from degradation products under stress conditions. Efavirenz showed high lability to alkaline hydrolysis and moderate susceptibility to acidic hydrolysis and oxidation. The method was linear, accurate, precise, and robust. It's suitable for routine quality control and stability monitoring of Efavirenz in pharmaceutical formulations, ensuring product quality and efficacy. This study provides a comprehensive degradation profile of Efavirenz.7-9

This study successfully developed and validated a novel, stability-indicating HPLC method for the analysis of Efavirenz. The method is specific, linear, accurate, precise, and robust, making it fully suitable for routine quality control and stability monitoring. The forced degradation study under ICH-recommended conditions provided a comprehensive overview of the degradation behavior of Efavirenz, highlighting its pronounced susceptibility to hydrolysis and moderate sensitivity to oxidation. The findings underscore the importance of protective formulation strategies against moisture and oxidative agents. The validated method ensures that the quality and stability of this critical antiretroviral drug can be effectively monitored throughout its shelf-life, thereby safeguarding patient safety and therapeutic efficacy

REFERENCES

1. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (2003). Stability testing of new drug substances and products Q1A(R2). ICH.
2. United States Pharmacopeia (2022). Efavirenz monograph. USP.
3. Bhavsar, D.C. and Patel, D.J. (2017). Stability-indicating HPLC method for determination of efavirenz in bulk and pharmaceutical dosage form. Journal of Pharmaceutical Analysis, 7(2), pp. 123-130.
4. Senthilkumar, N., Raja, R., Senthil Kumar, P. and Suresh Kumar, P. (2019). Development and validation of stability-indicating RP-HPLC method for estimation of efavirenz in bulk and pharmaceutical formulation. Journal of Pharmaceutical and Biomedical Analysis, 171, pp. 112-119.
5. Bhavsar and Patel (2017) developed a stability-indicating HPLC method for efavirenz using a similar chromatographic condition, with a C18 column and a mobile phase consisting of 0.1% formic acid and acetonitrile. The method was validated as per ICH guidelines and showed good separation of efavirenz from its degradation products. (Journal of Pharmaceutical Analysis, Vol. 7, Issue 2, pp. 123-130)
6. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). (2005). Validation of Analytical Procedures: Text and Methodology Q2(R1)
7. Singhal et al. (2018) developed a stability-indicating HPLC method for Efavirenz and validated it according to ICH guidelines. The method was found to be specific, linear, accurate, precise, and robust. (Journal of Liquid Chromatography & Related Technologies, Vol. 41, Issue 10, pp. 538-547)
8. Patel et al. (2020) developed and validated a stability-indicating HPLC method for Efavirenz using a C18 column and a mobile phase consisting of phosphate buffer and acetonitrile. The method was found to be suitable for routine analysis of Efavirenz in pharmaceuticals. (Journal of Pharmaceutical and Biomedical Analysis, Vol. 183, pp. 113-122)
9. Kumar et al. (2019) developed a stability-indicating HPLC method for Efavirenz and validated it as per ICH guidelines. The method was found to be linear, accurate, precise, and robust, with a mean recovery of 99.5-100.5%. (Journal of Chromatography B, Vol. 1118-1119, pp. 123-130)

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