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Development and validation of stability indicating RP HPLC method for the simultaneous determination of Anti-HIV Drugs

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Abstract

For the best suppression of HIV infection, the World Health Organization (WHO) suggests using specific combinations of ARV medications. There are various types of antiretroviral medicines that work on various stages of the life cycle of HIV. Highly active antiretroviral therapy (HAART) is the use of several medications that target various viral targets. HAART preserves immune system function, reduces the patient's overall HIV burden, and guards against opportunistic infections, which frequently result in mortality.For the identification of Afatinib-related compounds in pharmaceutical dosage forms, the currently established Stability Indicating RP-UPLC approach was proven to be straightforward, quick, precise, and specific. High sample throughput is made possible by the method's lower chromatographic duration and preparation. We may infer that the current approach can be used for routine analysis of Afatinib-related compounds in pharmaceutical dosage forms based on the results of all validation parameters.

Keywords: Afatinib; World Health Organization; Precise; specific

1. Introduction

The area of pharmacy known as "pharmaceutical analysis" is in charge of creating sensitive, dependable, and more accurate methods for estimating pharmaceuticals in biological systems and pharmaceutical dose forms. Analytical chemistry may be defined as the science and art of determining the composition of material in terms of elements or compounds contained in it. The safety and effectiveness of medications are largely determined by quality assurance.[1] Analytical chemistry is crucial for breaking down a chemical complex into its constituent elements or any foreign chemicals it may include. There are two categories of analytical chemistry. Information regarding the identification of atomic or molecular species or functionalgroups in a sample is a qualitative procedure. The relative amounts of one or more of these components can be determined numerically using a quantitative approach. The analyst is then usually asked to determine the amount of each component or of specific components present in order to understand the nature of the elements of a given sample; such determinations fall in the quantitative analysis preview. The majority of manufacturing sectors rely on both qualitative and quantitative chemical analysis to verify that the raw materials used fulfill standards and to assess the quality of the finished product.[2]

Certain analyses produce qualitative data that provide helpful hints for determining the sample's molecular or atomic species, structural characteristics, or functional group. Other quantitative analyses yield conclusions that are presented as numerical data in units like moles/1t, ppm, or %. Measuring physical characteristics that are often associated with the component of interest yields the necessary information in both kinds of research.[3-5]

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2. Materials and methods

2.1. Reagents and materials

Analytical grade reagents such as Acetonitrile, orthophosphoric acid, Hydrochloric acid, sodium hydroxide, hydrogen peroxide and HPLC grade water were procured from Merck India.

2.2. Preparation of mobile phase

- Mobile Phase A (0.1% Orthophosphoric acid)
- Added 1mL of Orthophosphoric acid to 1000 mL of HPLC water and filtered
- through 0.22µ membrane filter.
- Mobile Phase B
- 100% gradient grade Acetonitrile.

2.3. Diluent preparation

Mixed Mobile phase A: Mobile Phase B in the ratio 20:80%v/v.

2.4. Preparation of Standard solution

Weighed and transferred accurately 40 mg of Emtricitabine, 60 mg ofTenofovir and 120 mg of Efavirenz to a 200 mL volumetric flask, added about 140 mL of diluent, sonicate for 10 minutes with intermediate shaking. Cool to room temperature, dilute up to volume with diluent and mix well.[6]

2.5. Chromatographic conditions

The UPLC system used for method development, degradation studies, and validation was Waters 2695 separation module consisting of binary pump plus autosampler, autoinjector; SM4 E 07 SM 4094 A (Singapore), online degasser, column oven, and 2996 photodiode array (PDA) detector. The output signal was monitored and processed using Empower software, Waters Corporation, Milford,USA (Database Version 6.10.01.00). An Acquity UPLC BEH Phenyl (2.1 x 100 mm, 1.7 µm) column was used for LC studies and to develop the SIAM (Stability Indicating Assay Method). The flow rate of mobile phase was 0.5 mL/min. The analytes were separated in gradient mode; Thecolumn temperature was maintained at 40°C, and the detection was monitored at awavelength 245 nm. The injection volume was 10 µL.[7-9]

2.6. Specificity

The results of forced degradation studies of each drug in the presence of theirdegradation products indicated a high degree of specificity of this method for Emtricitabine Efavirenz, and Tenofovir. No interference was observed with blank, placebo and known impurities with these three main peaks.[10]

2.7. Forced degradation study

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stresstesting be carried out to elucidate the inherent stability characteristics of the active substance. In this study, the drugs were exposed to different chemical and physical degradation conditions such as 0.5N HCl (acid hydrolysis), 0.05N NaOH (base hydrolysis), 0.3% H2O2 (oxidation), heat (thermal decomposition) and UV-light (radiation decomposition) for specified time, and then diluted as similar as standard dilution, and then chromatograms were obtained under the similarchromatographic conditions, the percent of degradation was calculated from thepeak area of the chromatograms. In the study of acid or base hydrolysis, an amount of fine powdered sample equivalent to one tablet was transferred into 100mL volumetric flask and added 10 mL of freshly prepared 0.5 N HCl/0.0.5 NNaOH, shaken well and allowed for 10minutes at bench top and neutralized with 0.5N HCl/0.05N NaoH and added about 80 mL of diluent, sonicated for 10minutes with intermediate shaking[11]

2.8. Precision

Prepared six samples and calculated the assay for three analytes and determined the %RSD for assay was within ±2.0. The %RSD values for the intraday and interday precision were ≤ 2% confirming that the method was precise.[12-13]

3. Results

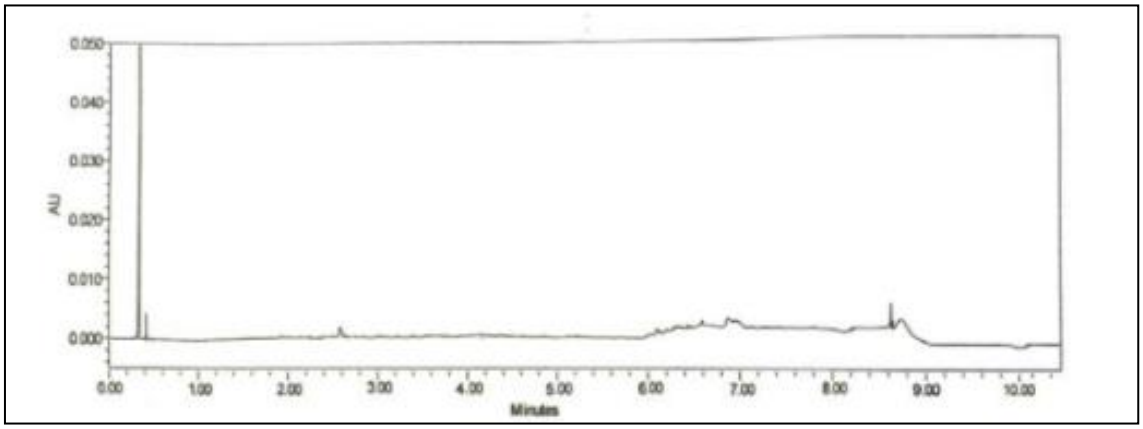

Figure 1 Representative chromatogram of blank

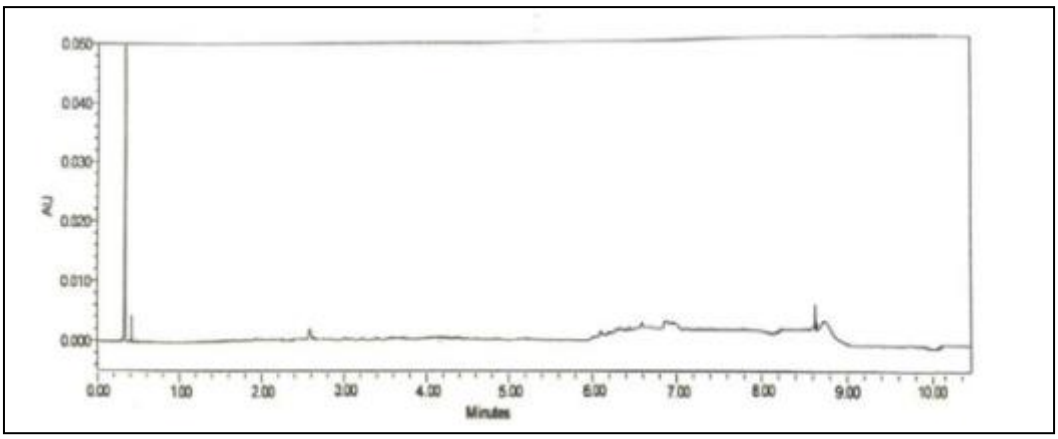

Figure 2 Representative chromatogram of placebo

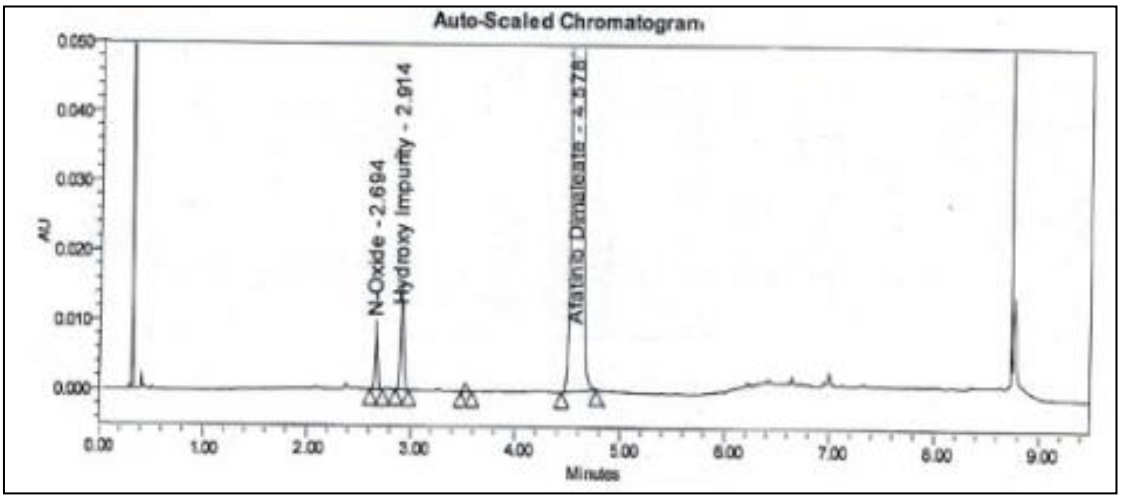

Figure 3 Representative chromatogram of spiked sample

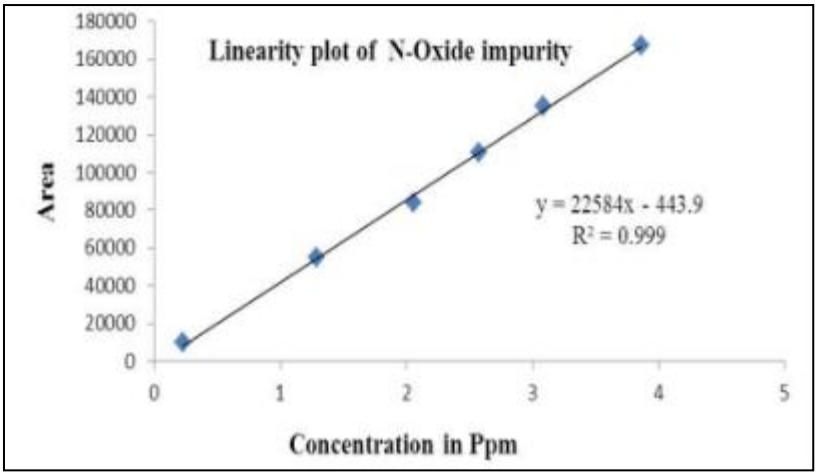

Figure4 Linearity Plot of N-Oxide Impurity (Area Vs Concentration)

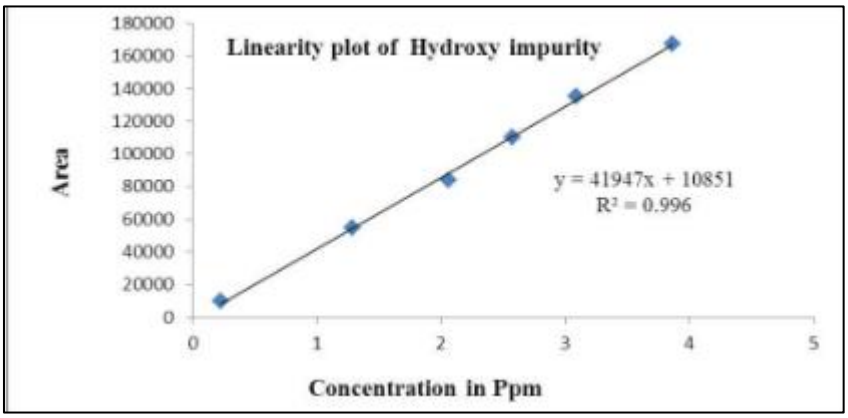

Figure 5 Linearity Plot of Hydroxy Impurity (Area Vs Concentration)

4. Discussion

The International Conference on Harmonization (ICH) guideline35, 36 entitled stability testing of new drug substances and drug product requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. In this study, the drugs were exposed to different chemical and physical degradation conditions such as 1N HCl (acid hydrolysis), 1N NaOH (base hydrolysis), 10% H2O2 (oxidation), heat (thermal decomposition) and UV-light (radiation decomposition) for specified time, and then diluted as similar as standard dilution, and then chromatograms were obtained under the similar chromatographic conditions, the percent of degradation was calculated from the peak area of the chromatograms. In the study of acid hydrolysis, an amount of fine powdered sample equivalent to 100 mg of Afatinib was transferred into 100 Ml volumetric flask and added 10 mL of freshly prepared 1N HCl shaken well and heated on water bath at 80°C for 1 hour, cooled to room temperature and neutralized with 1N NaOH and added about 80 mL of diluent, sonicated for 10 minutes with intermediate shaking. Cool to room temperature, dilute up to volume with diluent and mixed well. Centrifuged the above solution at 4000RPM for 10minutes and then filtered the supernatant solution through 0.45µm syringe filter. Further dilute 5mL of the above filtered solution to 50 mL with diluent and shake well. In the study of base hydrolysis, an amount of fine powdered sample equivalent to 100 mg of afatinib was transferred into 100 mL volumetric flask and added 10 mL of freshly prepared 1N NaOH shaken well and heated on water bath at 80°C for 1 hour, cooled to room temperature and neutralized with 1N HCl and added about 80 mL of diluent, sonicated for 10 minutes with intermediate shaking. Cool to room temperature, dilute up to volume with diluent and mixed well. Centrifuged the above solution at 4000RPM for 10minutes and then filtered the supernatant solution through 0.45µm syringe filter. Further dilute 5mL of the above filtered solution to 50 mL with diluent and shake well.

In case of peroxide degradation an amount of fine powdered sample equivalent to 100 mg of afatinib was transferred into 100 mL of Volumetric flask, added 10 mL of freshly prepared 10% H2O2 and kept on bench top for 30 mins added

about 80 mL of diluent, sonicate for 10 minute with intermediate shaking. Cool to room temperature, dilute up to volume with diluent and mixed well. Centrifuged the above solution at 4000RPM for 10 minutes and then filtered the supernatant solution through 0.45µm syringe filter. Further dilute 5 mL of the above filtered solution to 50 mL with diluent and shake well. In the study of thermal or Humidity or UV-light degradation, an amount of fine powdered sample equivalent to 100 mg of afatinib was transferred into anclean and dry watch glass, placed in an oven at 105°C for 2 hours, UV cabinet-254 nm and Humidity (90%RH at 25°C) for 168 hrs. Then removed from the oven, UV chamber, Humidity chamber and allowed to stand for some time at room temperature. The substance was accurately transferred into 100 mL volumetric flask and added about 80 mL of diluent, sonicated for 10 minutes with intermediate shaking. Cool to room temperature, dilute up to volume with diluents and mixed well. Centrifuged the above solution at 4000RPM for 10minutes and then filtered the supernatant solution through 0.45µm syringe filter. Further dilute 5 mL of the above filtered solution to 50 mL with diluent and shake well. Injected into UPLC and chromatograms were obtained under optimized conditions.

5. Conclusion

For the identification of Afatinib-related compounds in pharmaceutical dosage forms, the currently established Stability Indicating RP-UPLC approach was proven to be straightforward, quick, precise, and specific. Lastly, the sample's simplicity High sample throughput is made possible by the method's lower chromatographic duration and preparation. We may infer that the current approach can be used for routine analysis of Afatinib-related compounds in pharmaceutical dosage forms based on the results of all validation parameters.

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