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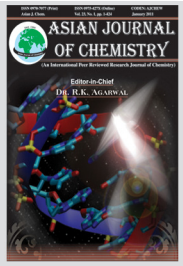

Assay of Efavirenz by Visible Spectrophotometric Methods

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Two simple, rapid and sensitive spectrophotometric methods have been developed to determine the amount of efavirenz in pure form. Method-A is based on the reaction of the drug with 1,2-naphthaquinone-4-sulphonic acid to form N-alkylamono naphthaquinone. In method-B, estimation is based on the reduction of ferric ions to ferrous ions by the drug, which forms green coloured chromogen in the presence of potassium ferricyanide. Beer's law is obeyed in the concentration ranges 2.0-12.0 μg mL^{-1}; 5.0-30.0 μg mL^{-1}, respectively. The molar absorptivity and % RSD are 2.45×10^4; 5.43×10^3 Lt mol^{-1} cm^{-1} and 3.29 and 0.78 for methods A and B respectively.

Key Words: Efavirenz, 1,2-Napthaquinone-4-sulphonic acid, Fe(III), Potassium ferricyanide, Spectrophotometric methods.

INTRODUCTION

Efavirenz (EFZ), an antiretroviral drug used to treat HIV infection in combination with other drugs such as lamivudine/zidovudine (Combivir) or tenofovir/emtricitabine (Truvada). 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, non-nucleoside-RTIs act allosterically by binding to a distinct site away from the active site known as the NNRTI pocket. The chemical name of efavirenz is (4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1*H*-3,1-benzoxazin-2-one with molecular formula $C_{14}H_9NO_2ClF_3$.

Literature survey of efavirenz reveals that there are some HPLC, HPTLC and UV spectrophotometric methods to determine efavirenz in pure form and formulations with different combinations and plasma, but no spectrophotometric method is reported. HPTLC[1] method has been developed and validated for the estimation of efavirenz from bulk drug and capsule formulations. HPLC and reversed-phase HPLC methods[2-13] have been developed for analysis of antiretroviral molecules including efavirenz. The amount of efavirenz in bulk form and formulations is determined by UV-spectrophotometric method at maximum wavelength 247 nm[14]. The purpose of the present investigation is to develop simple, rapid, accurate visible spectrophotometric methods for the determination of efavirenz in pure form.

EXPERIMENTAL

UV-Visible spectrophotometer: An ELICO SL-159 model, 2 nm high resolution, double beam, 1 cm length quartz coated optics, wavelength range190-1100 nm; high stability, linearity, precision instrument was used for all the spectral measurements.

Precision balance: Shimadzu balance of 0.0001 g readability, 200 g capacity, 0.0001 g repeatability, 0.0002 g linearity was used to weigh the required amount of the drug and the reagents.

Preparation of solutions: 100 mg of the efavirenz is accurately weighed and transferred into a 100 mL standard flask and dissolved in doubled distilled water and made up to the mark with constant shaking. 10 mL of this solution is accurately transferred into another 100 mL standard flask by means of a burette and made up to the mark with doubled distilled water. Aqueous solutions of the following reagents are prepared by dissolving required amount of the reagents. All materials and reagents used are of analytical reagent grade. 1,2-Naphthaquinone-4-sulphonic acid (NQS) (Loba, 0.5 %, 500 mg/100 mL), NaOH (E.Merck, 0.02 %, 20 mg/100 mL), PFC (BDH, 0.1 %, 100 mg/100 mL): Fe(III) (Wilson labs, 0.054 %, 54 mg/100 mL), 0.1 N HCl (8.6 mL/100 mL).

Procedure

Method-A: Aliquots of working standard solution of efavirenz (0.5-3.0 mL, 100 μg/mL) are transferred into a series of 25 mL calibrated test tubes. Then 2 mL of NaOH and 0.5 mL of 1,2-naphthaquinone-4-sulphonic acid reagent solutions

are added to each tube and the contents are kept aside for 2 min at room temperature. The solutions are made up to the mark with distilled water The absorbences are measured between 400-500 nm and λ_{max} is found to be 480 nm (Fig. 1) against a reagent blank prepared similarly. The amount of the efavirenz is calculated from its calibration graph (Fig. 3).

Method-B: Aliquots of (0.5-3.0 mL) of standard efavirenz solution are transferred into a series of 10 mL calibrated tubes and then a solution of 1.0 mL of Fe(III) is added. The tubes are stoppered and shaken well for 5 min. Then 0.5 mL of potassium ferricyanide (PFC) solution was added into each tube and is closed with lids. After 5 min 1 mL of 1 N HCl is added and the final volume was made up to 10 mL with distilled water. The absorbance of the solution in each tube is measured immediately between 700-900 nm, giving 720 nm as λ_{max} (Fig. 2) against a similar reagent blank. The amount of the drug is calculated from its calibration graph (Fig. 4).

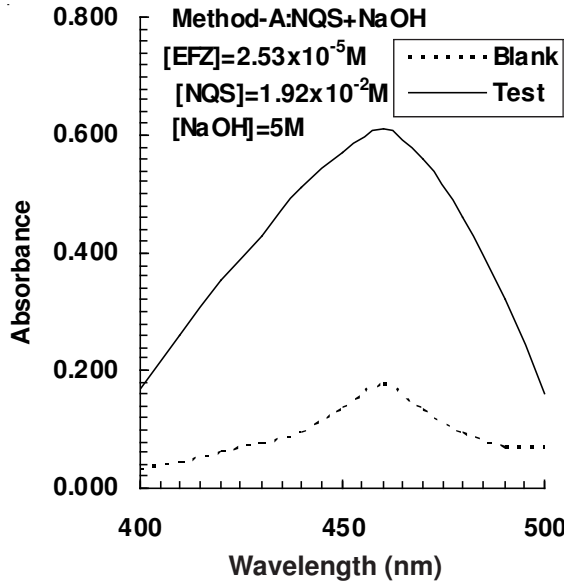

Fig. 1. Absorption spectrum of efavirenz with 1,2-naphthaquinone-4-sulphonic acid

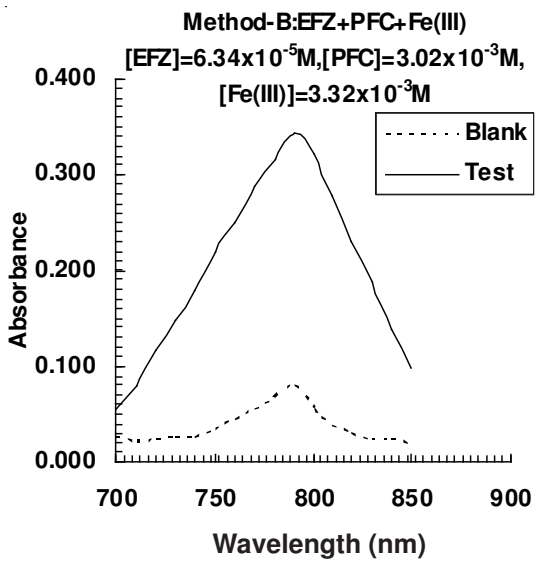

Fig. 2. Absorption spectrum of efavirenz with Fe(III), $[Fe(CN)_6]^{-3}$

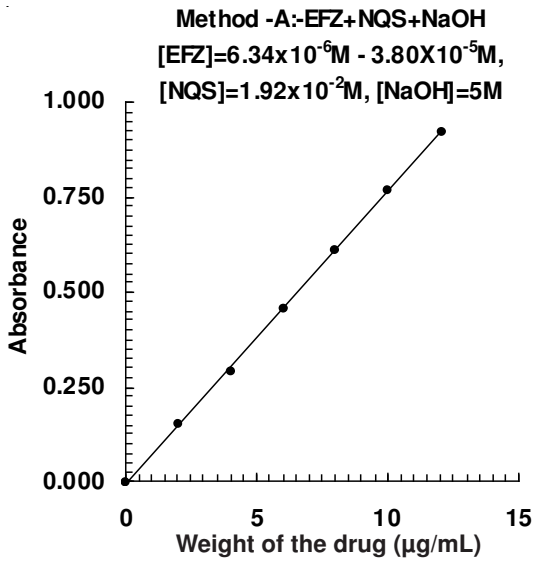

Fig. 3. Beer's law plot of efavirenz with 1,2-naphthaquinone-4-sulphonic acid

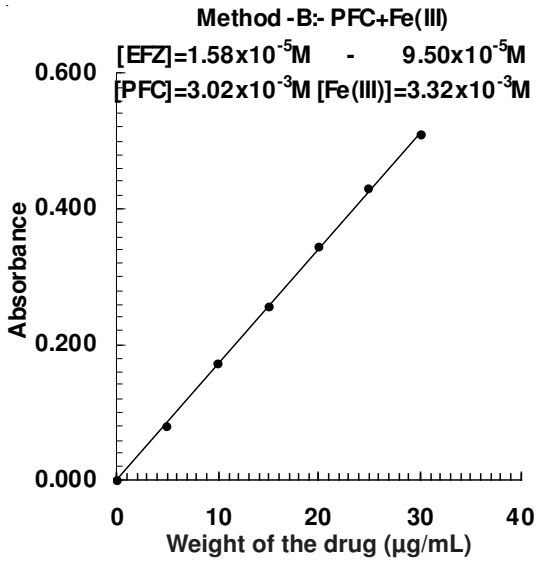

Fig. 4. Beer's law plot of efavirenz with Fe(III), $[Fe(CN)_6]^{-3}$

Optimum conditions established in method-A and method-B: The optimum conditions in these methods are fixed based on the study of the effects of various parameters such as volumes of the reagents. Concentration of the drug, type of acid, temperature and time of heating, changing one parameter keeping other constant for complete colour development, the stability and intensity of the coloured species after final dilution are established by measuring absorbance's at 460 and 740 nm for methods A and B, respectively.

RESULTS AND DISCUSSION

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar absorptivity and regression characteristics like slope, intercept, correlation coefficient, standard deviation of slope (S_b), standard deviation of intercept (S_a), % range of error and detection limits are calculated for the efavirenz. The values obtained by the proposed methods are presented in Table-1. Beer's law is obeyed in the concentration ranges 2.0-12.0 and 5.0-30.0 µg/mL are shown in Figs. 3 and 4

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS

Name of the parameter	Method-A	Method-B
Maximum wavelength λ_{max} (nm)	460	790
Beer's law limits (µg/mL)	2.0-12.0	5.0-30.0
Optimum photometric range (µg/mL)	4.0-12.0	10.0-25.0
Sandell's sensitivity (µg/cm^2/0.001 absorbance)	1.32E-02	6.25E-02
Molar absorptivity L/mol/cm	2.45E+04	5.43E+03
Slope (b)*	7.75E-02	1.72E-02
Intercept (a)*	-9.40E-03	-2.73E-03
Standard deviation on slope (S_b)	6.86E-04	1.38E-04
Standard deviation on intercept (S_a)	5.34E-03	2.68E-03
Correlation coefficient (r)	0.9999	0.9998
Standard deviation (S)	0.263	0.155
% Relative standard deviation**	1.201	0.784
0.05 Level confidence limit (µg/mL)	0.433	0.257
Limit of detection (LOD) (µg/mL)	0.215	0.473
Limit of quantification (LOQ) (µg/mL)	0.691	1.564

*Regression equation Y = a + bC
where Y stands for absorbance and C is concentration in µg/mL.
**% Relative standard deviation is calculated for six determinations.

and the optimum photometric ranges are 4.0-12.0 and 10.0-25.0 µg/mL for methods A and B, respectively. These methods are simple, rapid, accurate and do not involve any critical reaction conditions, or tedious sample preparation.

Chemistry of coloured products

Method-A: The reaction of 1,2-naphthaquinone-4-sulphonic acid (NQS) with secondary aromatic amine is the replacement of sulphonate group of the naphthaquinone sulphonic acid by an amino group gives N-alkyl amino naphthaquinone. In the case of secondary amine the quinone structure can be taken into account. It may be concluded that, under the conventional analytical conditions, equilibrium between two forms (I and II) may intervene for the derivatives obtained from primary amines, but the quinone structure is mostly favoured. The scheme of the reaction is shown in **Scheme-A**.

Method-B: This method is based on the oxidation of efavirenz by excess ferric salt to give products of oxidation inclusive of Fe(II) (reduced form of oxidant). The reduced form of oxidant subsequently reacts with ferricyanide to give ferro ferricyanide. The reaction is presented in **Scheme-B**.

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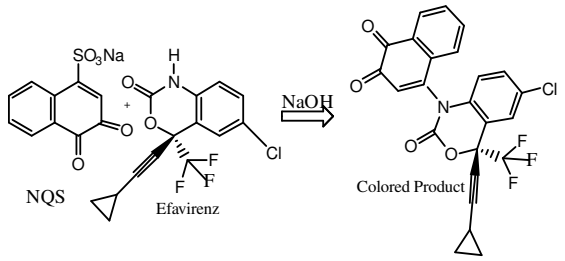
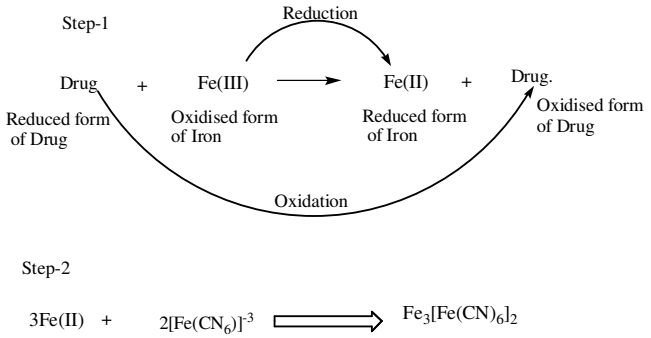
Scheme-A



Scheme-B



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