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SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF EFAVIRENZ

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ABSTRACT

Two simple and sensitive spectrophotometric methods have been developed for the estimation of efavirenz. The first method is based on the reaction of the drug with iron (III) and subsequent reaction with 1, 10–phenanthroline to yield an orange red complex with absorption maxima at 497 nm. The second method is based on the oxidation–reduction reaction between efavirenz and Folin–Ciocalteau reagent to form a blue chromogen having absorption maxima at 755 nm. Beer's law limits for both the methods are 5–20 µg/mL. All the variables in the methods were optimized and statistically validated. The proposed methods are selective, simple and economical for the quantitative estimation of efavirenz.

Key word : Efavirenz, Spectrophotometric method.

Efavirenz[1], one of the recent anti–HIV agents, is a non–nucleoside reverse transcriptase inhibitor. Chemically, it is [(S)–6–chloro–4–(cyclopropyl ethynyl)–1, 4–dihydro–4–(trifluoro–methyl)–2H–3,1–benzoxazin–2–one]. A few analytical methods based on HPLC and spectrophotometry have been reported earlier[2–7] for the determination of efavirenz.

Spectrophotometric methods are the instrumental methods of choice in industrial laboratories. Therefore, there is a need to develop faster, low cost and selective methods for routine quality control analysis of pharmaceutical formulations.

The present paper describes two colorimetric methods for determination of efavirenz. In one method, the authors have used ferric chloride to oxidize the drug and the ferrous salt formed consequently was made to react with *ortho*– phenanthroline to form a colored complex. The second method proposed by the authors makes use of phosphomolybdo tungstic acid, the well known Folin–Ciocalteau reagent [FCR].

EXPERIMENTAL

Instrument

A Systronics Model 117 UV–Visible spectrophotometer with 1 cm matched quartz cells was used for all the absorbance measurements.

Chemical and reagents

All the chemicals used in the study were of analytical grade. Aqueous solutions of o–phenanthroline (0.2% w/v, Loba), ferric chloride (0.9% w/v, S.D. Fine chem.), sodium carbonate (5% w/v, Qualigens) and FC reagent (1.0 N, S.D. Fine Chem) were prepared in double distilled water. Freshly prepared solutions were always used.

Preparation of standard solutions

About 100 mg of efavirenz was accurately weighed and dissolved in 5.0 mL of 0.3 M sodium hydroxide and kept aside for 10 hours to undergo hydrolysis This solution was filtered through cotton wool and made upto 100 mL with distilled water in a volumetric flask. From this, 25 mL of solution was taken into a 100 mL volumetric flask and diluted with distilled water further to get a working standard solution of 250 µg/mL for method A, whereas for method B, it was suitably diluted with distilled water to get a working standard solution of 50 µg/mL.

Analysis of pure samples

Method A

Aliquots ranging from 0.2 to 0.8 mL of the working standard solution of efavirenz (250 µg/mL) were transferred into a series of 10 mL graduated test tubes. To each of these tubes, 2.5 mL of ferric chloride and 3.0 mL of 1, 10–phenanthroline were successively added. The tubes were heated on a water bath at 70°C for 40 min. and the final volume was brought to 10.0 mL with distilled water. The absorbances were measured during 30 min at 497 nm against a reagent blank. The amount of the drug in the sample was computed from Beer–Lambert's plot. The colored compound was stable upto 90 min.

Method B

To a series of 10 mL graduated test tubes, aliquots ranging from 1.0–4.0 mL of the working standard solution of efavirenz (50 µg/mL), 5.0 mL of solution of sodium carbonate and 1.5 mL of FC reagent were added simultaneously and kept aside for 5 min at room temperature. Then the solution in each tube was made upto 10.0 mL with distilled water. The absorbance of the blue coloured complex was measured at 755 nm against a reagent blank. The chromophore was stable for about 60 min. The amount of the drug in the sample was computed from Beer–Lambert's plot.

RESULTS AND DISCUSSION

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar extinction coefficient, percent relative standard deviation, (calculated from the eight measurements containing $3/4^{th}$ of the amount of the upper Beer's law limits of efavirenz), % range of error (0.05 to 0.01 confidence limits) were calculated for both the methods and the results are summarized in Table 1.

Table 1. Optical characteristic and precision data

Parameters	Method A	Method B
Beer's law limit (µg/mL)	5–20	5–20
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.0248	0.0286
Molar extinction coefficient (mole^{-1}. cm^{-1})	12.721×10^3	11.017×10^3
% Relative standard deviation	0.528	0.732
% Range of error		
0.05 confidence limits	± 0.4414	± 0.6125
0.01 confidence limits	± 0.6531	± 0.9051
Correlation coefficient	0.9998	0.9999
Regression equation (Y)*		
Slope (a)	0.0395	0.0216
Intercept (b)	9.0×10^{-3}	7.0×10^{-4}

* Y= b + aC, where C is concentration in µg/mL and Y is absorbance unit.

The values obtained for the determination of efavirenz sample by the proposed and reference methods are compared in Table 2. To evaluate the validity and reproducibility of the methods, known amounts of pure drug was added to the previously analyzed samples and the mixtures were analyzed by proposed methods and the percent recoveries are given in Table 2.

Table 2. Estimation of efavirenz in bulk samples

Samples	Samples amount (mg)	Amount obtained (mg)			Percent recovery of the proposed method**	
		Reference Method*	Proposed method			
			A	B	A	B
1	50	48.8	50.2	50.1	100.04	100.04
2	50	47.9	48.2	48.9	99.28	99.56
3	20	19.2	19.8	19.3	99.96	99.86

*Average of five determinations

**UV method developed in our laboratory

The orange red coloured complex formed in method A is due to the coupling of Fe (II) ion [formed by reduction of efavirenz with Fe (III)] with 1, 10–phenanthroline. Three molecules of, 1, 10–phenanthroline attach themselves to the metal ion forming a orange red ferroin complex.

Efavirenz effects a reduction of 1, 2 or 3 oxygen atoms from the tungstate and/or molybdate in FC reagent producing one or more of the possible reduced species, which have a characteristic intense blue colour.

The results indicate that the proposed methods are sensitive, precise and reproducible and can be used for the routine determination of efavirenz in bulk samples.

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