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Research Article

Development and Validation of Visible Spectrophotometric Methods for the Analysis of Etravirine: Application to Tablet Dosage Forms

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Abstract

The present study describes the development and validation of two simple, sensitive, accurate, precise and economical spectrophotometric methods for the estimation of Etravirine (ETR) in bulk and its tablet dosage forms. These methods have developed based on the diazotization of ETR followed by coupling with alkaline β-naphthol (Method-A) and p-nitro aniline in alkaline medium (Method-B). These methods show a linear relation between absorbance and concentration of ETR in the ranges of 5 - 30 µg/mL (M-A) and 2.5 - 15 µg/mL(M-B) respectively. The Molar absorptive for present methods are 3.685 x 10^3 (M-A) and 5.614 x 10^4 (M-B) and LOD, LOQ values are 0.656 µg/mL, 1.990 µg/mL (M-A) and 0.191 µg/mL, 0.578 µg/mL (M-B) respectively. The common excipients in the drug did not interfere in the estimation process and the developed methods are successfully applied to tablet dosage forms of ETR.

Keywords: Etravirine; β-Naphthol; p-Nitro Aniline; Alkaline Medium; Spectrophotometry

Introduction

Etravirine is an antiretroviral agent more specifically classified as a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI). Etravirine (Figure 1) exerts its TEMP effects via direct inhibition of the reverse transcriptase enzyme of human immunodeficiency virus type 1 (HIV-1). It directly binds reverse transcriptase and consequently blocks DNA-dependent and RNA-dependent polymerase activity. Etravirine does not inhibit human DNA polymerase alpha, beta or gamma [1]. Chemically it is 4-({6-amino-5-bromo-2-[(4-cyanophenyl) amino] pyrimidin-4-yl} oxy)-3, 5-dimethylbenzonitrile with M.W. 435.277. The literature survey on ETR revealed that various HPLC [2-6], HPLC-MS [7], HPLC-MS/MS [8], HPTLC [9], LC-MS/MS [10], LC-ESI-MS [11], LC-tandem MS [12,13], UPLC [14], UPLC-MS/MS [15,16] and UV spectrophotometric [17,18] have been reported for estimation of ETR. The reported HPLC, HPLC-MS, HPLC-MS/MS, HPTLC, LC-MS/MS and UPLC methods need sophisticated laboratory, more costly reagents, expensive instrumentation and these take much time to analysis. The reported UV Visible spectrophotometric methods are less selective and the measured absorbance at shorter wave lengths and these methods have developed by using reagents other than β-Naphthol, p-nitro aniline. Hence, it is need to development and validation of simple, selective, precise, cost-effective spectrophotometric methods by using β-naphthol, p-nitro aniline as analytical reagents.

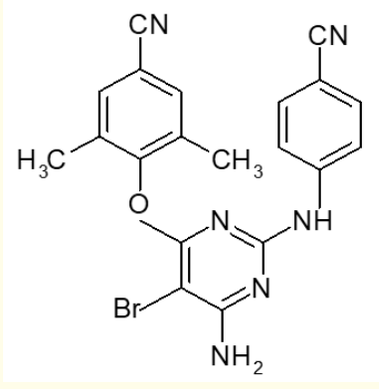

Figure 1: Chemical structure of Etravirine.

Materials and Methods

Materials

An ELICO (Hyderabad, India) double beam model SL 244 digital spectrophotometer was used for spectrophotometric measure-

ments and 1 cm matched quartz cells were used for measurements and Coslab (Ambala Cantt, India) CLE-105 model water bath was used to control the temperature. All chemicals were of analytical reagent grade. Double distilled water was used to prepare all solutions. All 45the solutions were prepared a fresh daily.

Method (M-A)

1. 2N HCl (v/v): Prepared by diluting 17.2 ml of concentrated HCl to 1000 ml with distilled water. This solution was standardized.
2. 0.1% (w/v) sodium nitrite: Prepared by dissolving 100 mg of $NaNO_2$ in 100 ml distilled water.
3. 2% (w/v) sodium hydroxide: Prepared by dissolving 2 gm NaOH in 100 ml distilled water.
4. 0.1% (w/v) alkaline β-naphthol: Prepared by dissolving 100 mg of β-naphthol in 2% NaOH solution.

Method (M-B)

1. 0.2M HCl (v/v): Prepared by diluting 17.2 ml of concentrated HCl to 1000 ml with distilled water.
2. 0.1% p-nitro aniline (PNA): Prepared by dissolving 100 mg of PNA in 100 ml of 0.2M HCl.
3. 0.4% (w/v) sodium nitrite: Prepared by dissolving 400 mg of $NaNO_2$ in 100 ml distilled water.
4. 4% (w/v) sodium hydroxide: Prepared by dissolving 4 gm NaOH in 100 ml distilled water.

Preparation of stock and working standard solutions

Stock solution of ETR was prepared by dissolving 100 mg of drug in 20 ml of methanol in a 100 ml volumetric flask and then make up to the mark with distilled water (1.0 mg/ml). The stock solution was diluted stepwise with the distilled water to obtain working standard solutions of concentration 100 µg/ml (M-A) and 50 µg/ml (M-B) respectively.

General assay procedure

Method (M-A)

Delivered aliquots (0.5 - 3.0 ml) of standard ETR solution (100 µg/ml) into a series of 10 ml calibrated tubes. To each tube 1.0 ml of 2N HCl and 1.0 ml of 0.1% $NaNO_2$ were added. The contents were subjected to cooling at 0 - 5°C for 10 minutes. After cooling, to each tube 1.0 ml of 0.1% alkaline β-naphthol was added and gently shaken. The tubes are kept aside for 10 minutes. The volume in each tube was made up to the mark and the absorbance of the colored solution was measured at 540 nm against a reagent blank. The amount of drug was deduced from its calibration curve (Figure 2).

Figure 2: Absorption spectrum of ETR-β-naphthol.

Method (M-B)

Delivered aliquots (0.5 - 3.0 ml) of standard ETR solution (50 µg/ml,) into a series of 10 ml calibrated tubes. To each tube 0.8 ml of 0.1% PNA followed by 1.0 ml of 0.4% $NaNO_2$ were added and the tubes were kept aside for 20 minutes. To each tube 2.0 ml of 4% NaOH was added and kept aside for 30 minutes. The volume in each tube was made up to the mark with distilled water and the absorbance was measured at 420 nm against a reagent blank. The amount of drug was computed from its calibration curve (Figure 3).

Figure 3: Absorption spectrum of ETR-PNA.

Assay of ETR in tablet dosage form

Intelence tablets (Janssen Pharmaceuticals, Inc., Titusville NJ) were labeled to contain 100 mg/200 mg ETR per tablet were used in the present investigation. Twenty tablets were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 100 mg of ETR was transferred into a 100 ml calibrated flask and dissolved in about 20 ml of methanol. The contents of the flask were swirled, sonicated for 10 minutes and filtered through Whatman No. 1 filter paper. The filtrate was completed to volume with distilled water. This solution was diluted quantitatively with distilled water to obtain suitable concentrations for the analysis

by the proposed spectrophotometric methods (M-A and M-B). The content of ETR in the tablets was calculated from the corresponding calibration curve or corresponding regression equation.

Results and Discussion

Optimization of experimental conditions

The experimental variables in the present proposed spectrophotometric methods (M-A and M-B), which are found to affect the color intensity and stability of the resulting colored complexes, were optimized to achieve maximum sensitivity and adherence to Beer's law. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in general assay procedures.

Method (M-A)

This method is based on the diazotization of ETR under acidic conditions followed by its coupling with the alkaline β-naphthol. The experimental conditions were established by studying the effect of various parameters like acidity, volume and concentrations of $NaNO_2$, alkaline β-naphthol, diazotization time and diazocoupling time for the maximum and stable color development. The results are incorporated in table 1.

Method (M-B)

In this method diazotization of PNA takes place under acidic conditions followed by its coupling with the ETR. The experimental conditions were established by studying the effect of various parameters like acidity, volume and concentrations of PNA, $NaNO_2$ and NaOH for the stable color development and the time required for diazotization and diazocoupling. The results are incorporated in table 2.

Determination of absorption maxima (λ_{max})

To determine the analytic wavelength, the absorption spectra of the colored chromogens formed in the proposed methods (M-A

Parameter	Investigation conditions	Optimized condition	Remarks
λ_{max} (nm)	500 - 600	540	
Volume of 2 N HCl (ml)	0.5 - 5.0	1.0	Increasing volume of HCl produces an increase in the absorbance upto 1.0 ml. Beyond this volume a gradual decrease in the absorbance is observed.
Volume of 0.1% $NaNO_2$ (ml)	0.5 - 5.0	1.0	1.0 ml of $NaNO_2$ was sufficient to diazotize ETR
Reaction time for diazotization (min)	5 - 25	10	Diazotization goes to almost completion within 10 min. So, 10 min was chosen as optimum time.
Volume of 0.1% alkaline β Napthol (ml)	0.5 - 5.0	1.0	The maximum absorbance was reached with 1.0 ml of β -napthol.
Reaction time for diazocoupling (min)	5 - 20	10	Diazocoupling was completed at 10 min. So, 10 min was chosen as optimum time.

Table 1: Optimization of conditions for the assay of ETR by method M-A.

Parameter	Investigation conditions	Optimized condition	Remarks
λ_{max} (nm)	400 - 500	420	
Volume of 0.1% PNA (ml)	0.2 - 2.0	0.8	It was found that maximum and stable color was formed with 0.8 ml of PNA in final volume of 10 ml.
Volume of 0.4% $NaNO_2$ (ml)	0.5 - 5.0	1.0	1.0 ml of $NaNO_2$ was required to diazotize PNA
Reaction time for diazotization (min)	5 - 30	20	Diazotization goes to almost completion within 20 min. So, 20 min was chosen as optimum time.
Volume of 4% NaOH (ml)	0.5 - 5.0	2.0	2.0 ml of NaOH gave maximum absorbance and therefore 2.0 ml of NaOH was chosen.
Reaction time for diazocoupling (min)	10 - 50	30	Diazocoupling was completed at 30 min. So, 30 min was chosen as optimum time.

Table 2: Optimization of conditions for the assay of ETR by method M-B.

and M-B) were scanned in the wavelength region of 400 - 700 nm against a corresponding reagent blank. The results are graphically presented in the figure 2 and 3.

Method validation

The proposed methods (M-A and M-B) were validated for linearity, sensitivity, selectivity, stability of the colored species, accuracy, precision, robustness and recovery according to the current ICH guidelines.

Linearity and sensitivity

Under the optimum conditions, a linear relation was obtained between absorbance and concentration of ETR in the ranges given in table 3. The calibration graphs (Figure 4 and 5) in each instance is described by the equation: Y = c + mX, (where Y = absorbance, c = intercept, m = slope and X = concentration of ETR in µg/ml). The regression coefficient, intercept and slope for the calibration data are summarized in table 3. The values specify that there is a good correlation between absorbance values and concentration of ETR in the proposed methods.

Parameter Method	Linearity range (µg/ml)	Regression equation (Y=mx+c)*	Regression coefficient (R^2)	Slope (m)	Intercept (c)
M-A	5 - 30	Y = 0.0870x + 0.0051	0.9989	0.0870	0.0051
M-B	2.5 - 15	Y = 0.0289x + 0.0042	0.9993	0.0289	0.0042

Table 3: Linearity and regression parameters of the proposed spectrophotometric methods.

*Y = Absorbance; x = Concentration of drug in µg/ml.

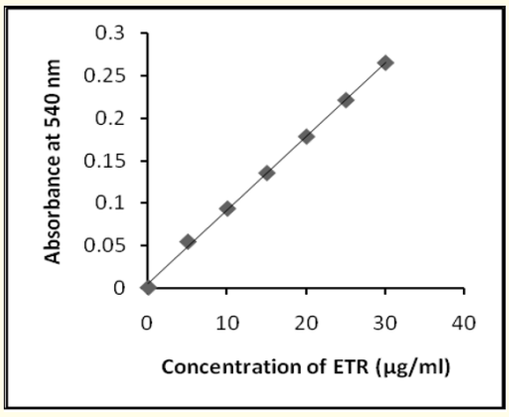

Figure 4: Beer's law plot of ETR-β-naphthol

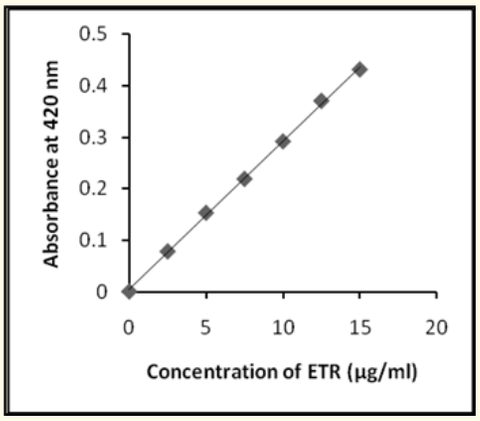

Figure 5: Beer's law plot of ETR-PNA.

Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, the limits of detection (LOD) and quantification (LOQ) are calculated as per the current ICH guidelines and compiled in table 4. The values indicated that the proposed methods (M-A and-M-B) have adequate sensitivity for the analysis of ETR.

Accuracy and precision

To determine the accuracy and precision of the proposed methods, pure ETR solution at three different concentration levels (M-A - 5,15 and 30 µg/ml; M-B - 2.5,7.5 and 15 µg/ml) were prepared and analyzed in five replicates during the same day (intra-day precision) and on three consecutive days (inter-day precision) by the proposed methods (M-A-M-B). The results are presented in table 5 and 6. The reported values in tables give the information that the accuracy of the proposed methods is satisfactory and the low values indicating repeatability of the proposed methods in the routine analysis of ETR.

Recovery studies

The accuracy and validity of the proposed methods were further demonstrated by performing recovery studies. Pre analyzed tablet powder was spiked with pure ETR at three concentration levels (50, 100 and 150%) and the total concentration was once again analyzed by the proposed methods (M-A and M-B). The results of this study are presented in table 7. The percent recovery of the ETR is in the range of 99.88-99.94%, and 99.04-99.92% for the methods M-A and M-B, respectively. The good percentage of recovery values indicate that the excipients present in the tablets did not interfere

Parameter / Method	Molar Absorptivity (L/mole/cm)	Sandell's sensitivity (µg cm^{-2})	LOD (µg/ml)	LOQ (µg/ml)
M-A	3.685 x 10^3	1.1310	0.656	1.990
M-B	5.614 x 10^4	0.3424	0.191	0.578

Table 4: Sensitivity and stability parameters of the proposed spectrophotometric methods.

Method	Concentration of ETR (µg/ml)		SD	RSD (%)	Recovery (%)	Confidence limit	
	Taken	Found*				0.05	0.01
M-A	5	4.988	0.02216	0.444	99.76	0.01852	0.02741
	15	14.995	0.03108	0.207	99.96	0.02598	0.03844
	30	30.001	0.02985	0.099	100.00	0.02495	0.03692
M-B	2.5	2.503	0.02727	0.108	100.12	0.00228	0.00337
	7.5	7.506	0.02349	0.312	100.08	0.00196	0.00290
	15	14.996	0.03124	0.208	99.97	0.00261	0.00386

Table 5: Evaluation of intra-day precision and accuracy of the proposed spectrophotometric methods.

*: Average of five determinations.

Method	Concentration of ETR (µg/ml)		SD	RSD(%)	Recovery(%)	Confidence limit	
	Taken	Found*				0.05	0.01
M-A	5	4.970	0.02576	0.518	99.40	0.02153	0.03186
	15	14.986	0.03286	0.219	99.90	0.02747	0.04065
	30	29.996	0.02097	0.069	99.98	0.01753	0.02594
M-B	2.5	2.476	0.03155	1.274	99.04	0.00263	0.00390
	7.5	7.50	0.01979	0.263	100.00	0.01654	0.02448
	15	14.988	0.02126	0.141	99.92	0.01777	0.02630

Table 6: Evaluation of inter-day precision and accuracy of the proposed spectrophotometric methods.

*: Average of five determinations.

Method	Spiked level	Concentration of ETR (µg/ml)			SD	RSD (%)	Recovery (%)
		Taken	Spiked	Found*			
M-A	50	5	2.5	7.491	0.00907	0.121	99.88
	100	5	5	9.994	0.01608	0.160	99.94
	150	5	7.5	12.493	0.01410	0.112	99.94
M-B	50	2.5	1.25	3.742	0.03155	1.274	99.04
	100	2.5	2.5	4.997	0.03215	0.859	99.78
	150	2.5	5	7.494	0.01401	0.186	99.92

Table 7: Results of recovery studies of the proposed spectrophotometric methods.

*: Average of three determinations.

in the assay. The non-interference of excipients also demonstrates the selectivity of the proposed methods.

Robustness

To evaluate the robustness of the proposed spectrophotometric methods, the experimental variables were altered deliberately and the effect of these change on the performance of the proposed methods (M-A and M-B) were studied. The robustness of the methods was studied at two different concentration levels (M-A - 5 and 30 µg/ml; and M-B - 2.5 and 15 µg/ml). The results of this study are presented in table 8. From the %RSD values presented in table 8

Method	Parameter	Concentration of ETR (µg/ml)		SD	Recovery (%)	RSD (%)
		Taken	Found*			
M-A	Volume of 0.2N HCl (1.0 ± 0.1 ml)	5	4.99	0.0122	99.80	0.245
		30	29.98	0.0316	99.93	0.105
	Volume of 0.1% $NaNO_2$ (1.0 ± 0.1 ml)	5	5.03	0.0644	100.60	1.280
		30	30.03	0.0561	100.10	0.186
	Volume of 0.1% β Naphthol (1.0 ± 0.1 ml)	5	4.98	0.0367	99.60	0.737
		30	30.01	0.0357	100.03	0.118
	Diazotization time (10 ± 2 min)	5	5.01	0.0406	100.20	0.810
		30	30.01	0.0433	100.03	0.144
	Diazo coupling time (10 ± 2 min)	5	4.98	0.0269	99.60	0.540
		30	29.98	0.0367	99.93	0.122
M-B	Volume of 0.1% PNA (0.8 ± 0.1 ml)	2.5	2.49	0.0254	99.60	1.023
		15	15.01	0.0295	100.06	0.197
	Volume of 0.4% $NaNO_2$ (1.0 ± 0.1 ml)	2.5	2.51	0.0229	100.40	0.912
		15	15.01	0.0285	100.06	0.197
	Volume of 4% NaOH (1.0 ± 0.2 ml)	2.5	2.49	0.0187	99.60	0.751
		15	14.98	0.0300	99.86	0.200
	Diazotization time (20 ± 2 min)	2.5	2.51	0.0357	100.40	1.422
		15	15.02	0.0374	100.13	0.249
	Diazo coupling time (30 ± 2 min)	2.5	2.51	0.0229	100.40	0.912
		15	15.01	0.0295	100.06	0.197

Table 8: Robustness of the proposed spectrophotometric methods.

*Average of three determinations.

(0.105 - 1.280%, 0.197 - 1.422% for methods M-A and M-B, respectively) one can conclude that the proposed methods are robust.

Application to the tablet dosage forms

The proposed methods (M-A and M-B) were successfully applied to the estimation of ETR in commercially available tablets (Intelence tablets, Janssen Pharmaceuticals, Inc., Titusville NJ, labeled to contain 100 mg/200 mg ETR per tablet). The results are compiled in table 9. The good percent recovery value with low relative standard deviation (< 1%) value confirms the suitability of the proposed methods (M-A and M-B) for the routine analysis of ETR in tablet dosage forms.

The results obtained were statistically compared with the labeled claim values by applying the Student's t-test for accuracy and F-test for precision at 95% confidence level. As can be seen from table 9, the calculated t- and F- values at 0.05 confidence level did not exceed the tabulated values of 1.833 and 5.19 respectively. This indicates that the proposed methods possess sufficient accuracy and precision.

Chemistry of the colored products

Method M-A

The results obtained in method M-A were based on the diazotization of the ETR under acidic conditions, followed by its coupling with β-naphthol in alkaline medium to form a colored complex [19]. In method M4, initially ETR was converted to its diazonium salt with $NaNO_2$ in the presence of HCl. The reaction is usually carried out in an ice bath. The diazonium salt form of ETR couples with the β-naphthol in the presence of NaOH resulting in the formation of the colored azo-dye having maximum absorption at 540 nm against the corresponding reagent blank.

Method M-B

In this method, PNA was converted to the diazonium salts by treatment with $NaNO_2$ in acidic medium. The reaction is usually carried out in an ice bath. The diazonium salt form of PNA couples with the ETR at alkaline pH resulting in the formation of the colored azo-dye having maximum absorption at 420 nm against the corresponding reagent blank [20].

Method	Labeled claim (mg)	Found*	SD	% RSD	% Recovery	*t*-Value**	F-Value***
	200	199.99	0.02959	0.1477	99.99	0.1879	3.723
M-A	100	100.12	0.02413	0.2406	100.33	0.2425	1.212
	200	200.04	0.02577	0.1286	100.16	0.2265	2.085
M-B	100	99.49	0.02590	0.2603	99.49	0.1548	2.6897
	200	199.50	0.02723	0.1364	99.75	0.3061	3.0614

Table 9: Assay of ETR in tablets by the proposed spectrophotometric methods.

*: Average of six determinations. *: Average of six determinations. **: Tabulated t value - 1.833. ***: Tabulated F value - 5.19.

Figure A

Conclusion

In the present investigation, the developed methods were validated by testing their linearity, sensitivity, accuracy, precision, selectivity and robustness and the statistical data from the validation studies demonstrated the methods to be accurate, precise, selective, sensitive, linear and robust. The procedures which have followed in the present investigation meet all the criteria of the demands of analytical chemists namely simplicity, accuracy, reliability and cost-effective analysis. Application of the proposed methods (M-A and M-B) was checked by analyzing with its formulations that revealed good recovery with low relative standard deviation values ensuring the suitability for routine quality control and drug analysis of ETR in tablet dosage forms.

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Figure B

Conflict of Interest

Authors do not have any conflict of interest for the present investigation.

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