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Spectrophotometric Determination of Etravirine in Bulk and Pharmaceutical Formulations

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ABSTRACT

Two simple, rapid, sensitive, accurate, precise and economical Visible Spectrophotometric methods have been developed for the determination of Etravirine in pure and pharmaceutical formulations. These methods (A and B) were based on nucleophilic substitution and oxidative coupling reactions of Etravirine by 1,2-naphtha quinone-4-sulphonate (NQS) in alkaline medium and 3-methyl-2-benzothiazolinone hydrazone (MBTH) in acidic medium with the maximum absorbance at 414 nm and 635 nm respectively. Linearity was obtained in the concentration range of 5 - 30 µg/ml and 2 - 10 µg/ml which was corroborated by the correlation coefficient (r) values of 0.9995 and 0.9996 respectively. The methods developed were validated with respect to linearity, accuracy (recovery), precision, Sandell's sensitivity, molar extinction coefficient and specificity. The proposed methods are successfully applied for the determination of Etravirine in bulk and pharmaceutical formulations and results were validated statistically by recovery studies.

KEYWORDS

Etravirine; 1,2-Napthaquinone 4-Sulphonate; 3-Methyl-2-Benzothiazolinone Hydrazone

1. Introduction

Etravirine (ETR) is a new non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immune deficiency virus type 1 (HIV-1) [1-4] chemically 4-[6-Amino-5-bromo-2-[(4-cyanophenyl) amino] pyrimidin-4-yl] oxy-3,5-dimethylbenzonitrile (**Figure 1**). Etravirine is a highly potent inhibitor of HIV-1 replication, with activity in the nanomolar range comparable to that of the commonly prescribed NNRTI Efavirenz [2].

A detailed literature survey for Etravirine revealed that various methods based on varied techniques namely LC-MS/MS [5-8], HPLC-MS [9,10], HPLC [11,12], and a direct UV-Visible spectrophotometric method for validation of Etravirine at 310 nm performed with equal ratio of acetonitrile and water as solvent, were reported [13]. The experimental results were applied for statistical analysis and found satisfactory precision and reproducibility. The objective of the present study was to develop simple, precise, accurate and economic analytical methods with better detection range for the estimation of Etravirine in bulk and pharmaceutical formulations. These two methods were based on nucleophilic substitution and oxidative coupling of ETR with reagents such as 1,2-naphtha quinone-4-sulphonate (NQS) and 3-methyl-2-benzothiazolinone hydrazone (MBTH) producing colored products.

2. Experimental

2.1. Instrumentation

An ELICO-244, Double Beam UV-Visible Spectrophotometer with 1cm matched quartz cells was used for all spectral and absorbance measurements. An ELICO LI-120 digital pH meter was used for all pH measurements.

2.2. Chemicals and Reagents

Etravirine was obtained as a gift sample from Arabindo Laboratory, Pvt, Ltd., Hyderabad, India. The analytical

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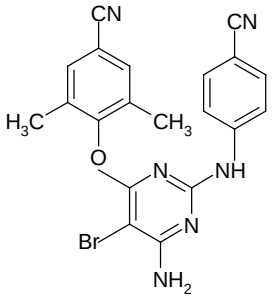


Figure 1. Structure of Etravirine.

reagents namely NQS and MBTH were supplied by Merk Specialties Pvt Ltd; Mumbai and were used without any further purification. Chemicals like NaOH, $NaIO_4$, glacial Acetic Acid (AR grade) supplied by Sd Fine Chemicals Ltd; Mumbai were used throughout the work.

3. Standard and Test Solutions

3.1. Preparation of Standard Drug Solution

An accurately weighed quantity of Etravirine (100 mg) was transferred to 100 ml volumetric flask and dissolved in methanol to obtain standard stock solution having concentration of ETR as 1 mg/1ml. This stock solution was further diluted with the same solvent to get 100 µg/ml of working standard for both (Method A & Method B) respectively.

3.2. Preparation of Test Solution

An accurately weighed tablets content equivalent to about 300 mg of ETR was added to 100 ml methanol and sonicated it for 30 minutes to dissolve and dilute to 100 ml with water. 10 ml of the above solution was transferred into a 100 ml volumetric flask and diluted with water and mixed. The solution was then filtered through 0.5 µ HVLP nylon filter. The filtrate was appropriately diluted for the preparation of formulation solutions for different methods as given under standard solution preparations to fit into the calibration graph.

4. Methods

4.1. Preparation of Calibration Curve

Calibration curves were constructed in accordance with the optimum conditions.

4.1.1. Method-A

Aliquots of standard Etravirine solution, 0.5 to 3.0 ml (100 µg/ml) were transferred into a series of calibrated tubes containing 0.2 ml of 0.02 N NaOH and 0.2 ml of 0.5% NQS reagent was added in each tube then the contents were heated at 50˚C for 5 minutes and cooled. This operation was performed in the dark. Then 0.5 ml of conc. H_2SO_4 was added slowly, mixed and absorbance (**Figure 2**) was measured after 5 minutes at λ_{max} 414 nm against a reagent blank prepared similarly.

4.1.2. Method-B

Aliquots of standard Etravirine solution, 0.5 to 2.5 ml (100 µg/ml) were transferred into a series of calibrated tubes 1.0 ml $NaIO_4$ and 1.0 ml 20% glacial acetic acid were added. The volume was adjusted to 10 ml with distilled water and kept on a water bath for 40 minutes. The solutions were cooled then 1.0 ml of MBTH solution was added and kept aside for 15 minutes. Then it was diluted to 25 ml with distilled water and measured the absorbance (**Figure 3**) at 635 nm against similar reagent blank.

The calibration graphs (**Figures 4** and **5**) were linear over the permissible concentration ranges. The optical characteristics and statistical data for the regression equation of the proposed methods are presented in (**Table 1**). The amount of ETR was calculated from the Beer-Lambert's plot.

4.2. Optimization of Reaction Conditions

The reactions were investigated on the effect of the reaction time, temperature and volume of chemicals and reagents used. Controls were carried out by measuring absorbance at 414 and 635 nm for a series of solutions varying one and fixing the other parameters for Method A and B, respectively.

5. Result and Discussion

Etravirine involved in nucleophilic substitution reaction in basic medium with NQS as well as oxidative coupling reaction in acidic medium with MBTH. The absorbance spectra of both methods (Method A & B) were drawn by plotting absorbance against wavelengths (**Figures 2** and **3**). From the respective absorption spectra the absorbance maximum were found to be at 414 and 635 nm respectively.

5.1. Optical Characteristics

In order to test the colored products formed in these methods adhere to Beer's law, the absorbance at maximum wavelength of a series of different concentrations were plotted against concentration of the drug in µg/ml. Beer's law limits, Molar absorptivity, Sandell's sensitivity and optimum photometric range were calculated and presented in **Table 1**.

A linear relationship was found between the absorbance and concentration of the drug in the ranges 5.0 - 30.0 µg/ml and 2.0 - 10.0 µg/ml for Methods A and B respectively (**Figures 4** and **5**). Regression analysis of

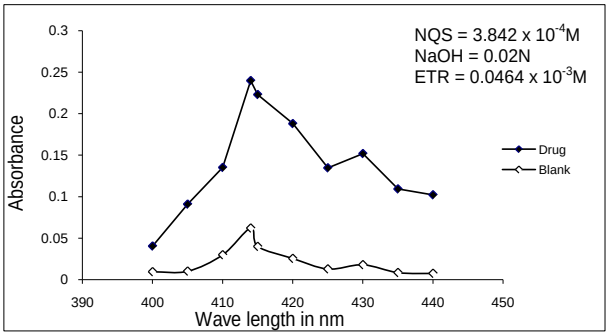


Figure 2. **Absorbance spectrum of ETR with NQS.**

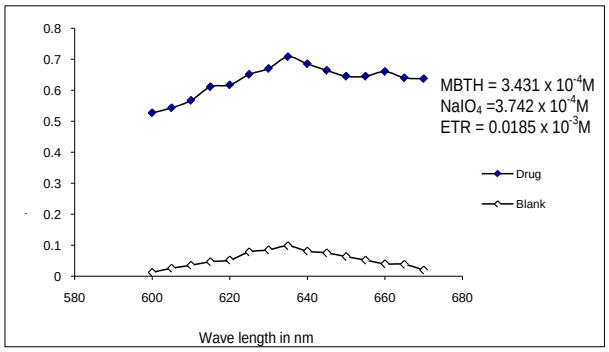


Figure 3. **Absorbance spectrum of ETR with MBTH.**

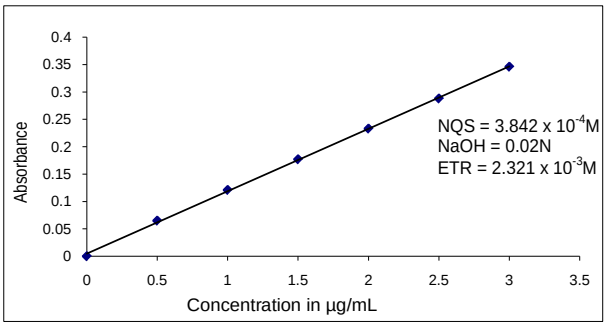


Figure 4. **Beer's Lambert plot of ETR with NQS.**

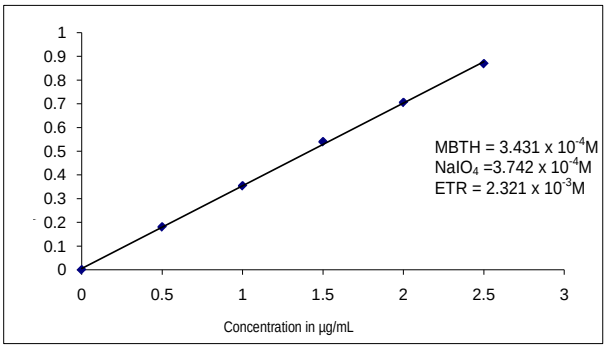


Figure 5. **Beer's Lambert plot of ETR with MBTH.**

the Beer's law plots at λ_{max} reveals a good correlation. The graphs shows negligible intercept and were described by the regression equation, Y = bC + a (where Y is the absorbance of 1 cm layer, b is the slope, a is the intercept and C is the concentration of the measured solution in µg/ml). The molar absorptivities of the resulting colored complexes indicate the high sensitivity of the methods. Linear regression parameters are also given in **Table 1**.

Table 1. **Optical characteristics and linear least square regression analysis of proposed methods for Etravirine.**

Parameters	METHOD-A	METHOD-B
Maximum wave length λ_{max}	414	635
Beer's law Limits (µg/ml)	5.0 - 30.0	2.0 - 10.0
Optimum photometric Range µg/ml	12.7 - 26.02	3.6 - 7.5
Sandell's sensitivity	0.0826	0.0111
Molar absorptivity lt/mole/cm	5.86×10^4	8.35×10^4
Slope (b)	0.114	0.348
Intercept (a)	0.0047	0.0055
Standard deviation on slope (S_b)	0.150	0.241
Standard deviation on Intercept (S_a)	0.0306	0.0302
Correlation coefficient (r)	0.9995	0.9996
Limit of detection (LOD) µg/ml	0.0561	0.0826
Limit of quantification (LOQ) µg/ml	0.1709	0.2504

5.2. Precision and Accuracy

Precision of the developed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of the test solution. The percent of relative standard deviation and percent range of errors were calculated and presented in **Table 2** for the developed methods. To determine the accuracy of these methods, three different amounts of bulk samples within the Beer's law limits were prepared and analyzed by the developed methods. The results are presented in **Table 3**. Percent of relative standard deviation (% RSD) were found to be less than 2, which indicate that the developed methods were precise. The percentage recoveries of the drug by these methods were found to be within the range of 100.03 - 100.24 and 100.09 - 100.32 for method A and method B, respectively, indicating that the developed methods are accurate. Studies also revealed that the common excipients and other additives usually present in tablets did not interfere in the proposed methods.

6. Application to the Pharmaceutical Dosage Forms

It is evident from the above mentioned results that the proposed methods gave satisfactory results with Etravirine in bulk. Thus its pharmaceutical dosage forms (Intelence) were subjected to analysis of their Etravirine con-

tents by proposed and reference methods. An ICH validated spectrophotometric method with UV-Visible detection for determination of Etravirine in tablets was carried out. The label claims of Etravirine in tablet dosage forms are 100 mg and 200 mg whose percentages were 100.38, 100.35 and 199.95, 199.92 for Method A and Method B respectively. The statistical data was presented in **Table 4**. This result was compared with the reference method obtained statistical analysis with respect to the accuracy (by t-test) and precision (by F-test). No significant differences were found between the calculated and theoretical values of t-test and F-tests at 0.05% confidence level proving similar accuracy and precision in the determination of Etravirine by both methods.

7. Scheme of the Colored Products

7.1. Method-A

The results obtained in Method A were based on the nucleophilic substitution reaction of ETR with NQS in the presence of NaOH at 50˚C, yields a cherry colored product having maximum absorption at a wavelength of 414 nm against the corresponding reagent blank. The colored species is represented as given in the **Scheme 1** [14].

7.2. Method-B

The results obtained in Method B were based on the oxidative coupling reaction of ETR with MBTH in the presence of $NaIO_4$ in acidic medium, yields a green colored product having maximum absorption at a wavelength of 635 nm against the corresponding reagent blank. Actually, this is sodium metaperiodate catalyzed oxidative coupling reaction of MBTH with the primary amino group of the drug. Under the reaction conditions, on oxidation, MBTH loses two electrons and accept one proton forming an electrophilic intermediate, which is the active coupling species. This intermediate undergoes electrophilic substitution with the ETR to form the colored product. The proposed reaction mechanism is presented in **Scheme 2** [15].

Table 2. Precision of the test method.

Statistical Parameter Value	METHOD-A	METHOD-B
Concentration (µg/ml)	15.0	8.0
Mean (µg/ml)*	15.02	7.98
Standard deviation (s)	0.0019	0.0087
% relative standard deviation (% RSD)	1.624	1.617
0.05 level confidence limit µg/ml	0.00162	0.00165

*Mean of six determinations.

Table 3. Accuracy of the proposed methods.

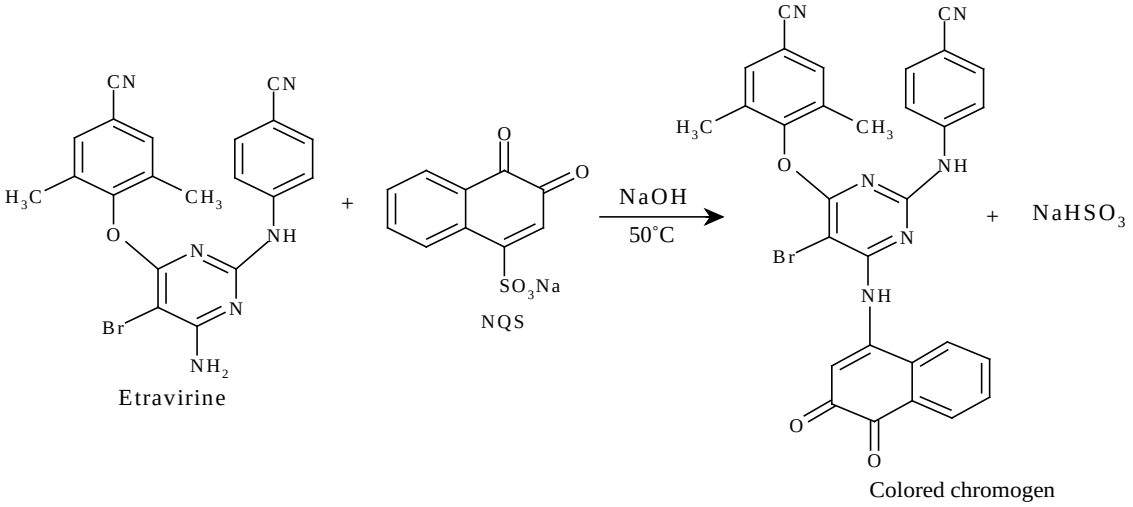
Method Number	METHOD-A		METHOD-B	
Amount Taken (µg/ml)	1.50	15.0	2.0	8.0
Amount Found (µg/ml)*	1.51	15.06	2.01	8.02
SD	0.0079	0.1123	0.0224	0.0169
% Recovery	99.33	99.60	99.64	99.87
% RSD	0.527	0.745	0.688	0.211

*Mean of five determinations.

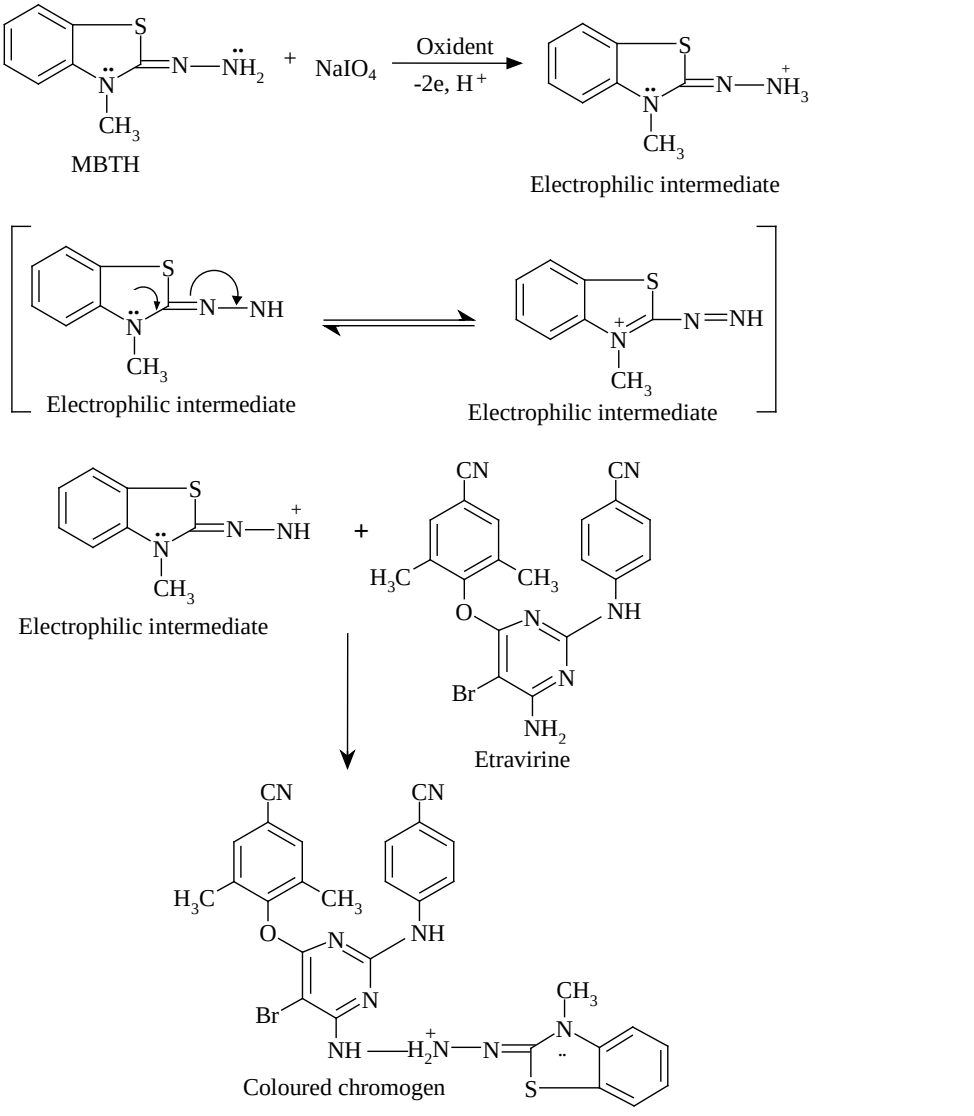
Table 4. Assay of formulations of Etravirine.

Compound	Label claim (mg)	Amount of compound found by proposed methods (mg)*		% REC		% RSD		SD		F-test		t- test	
		M-A	M-B	M-A	M-B	M-A	M-B	M-A	M-B	M-A	M-B	M-A	M-B
Intelence	100	100.38	100.35	100.38	100.02	0.612	0.764	±0.614	±0.766	1.16	1.33	0.478	0.479
Intelence	200	199.95	199.92	99.94	99.92	0.283	0.284	±0.565	±0.284	1.30	1.31	0.173	0.215

*Mean of six determinations. Theoretical values at 0.05 level of confidence limit F = 5.19, t = 1.833. 100 mg/tablet and 200 mg/tablet formulations are analyzed (average of six determinations). M-A: Method-A, M-B: Method-B.



Scheme 1. **Nucleophilic substitution reaction of Etravirine with NQS.**



Scheme 2. **Oxidative coupling reaction of Etravirine with MBTH.**

8. Conclusion

The proposed methods were simple, selective, and reproducible and can be used in the routine analysis of Etravirine in bulk drug and pharmaceutical formulations with reasonable accuracy and precision.

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