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Extractive Spectrophotometric Method for Determination of Non-Nucleoside Reverse Transcriptase Inhibitorin Bulk and Pharmaceutical Dosage Form

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Abstract: *Two simple, accurate, precise and sensitive spectrophotometric methods have been developed and validated for determination of Rilpivirine in bulk and pharmaceutical dosage form. Method A and B involves the formation of a colored chloroform extractable ion pair complex of drug with bromothymol blue and Bromocresol green absorbing maximally at 425nm and 415nm. Beer's law is obeyed in the concentration range of 4-20µg/ml for methods A and B. Molar absorptivity, Sandell's sensitivity, Association constant, Limit of Quantification and Limit of Detection were calculated. The proposed methods were successfully applied for the determination of Rilpivirine in pharmaceutical formulation.*

Keywords: *Rilpivirine, Spectrophotometry, Bromothymol blue, Bromocresol green, ion-pair complex, Validation.*

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I. Introduction

Rilpivirine Hydrochloride (Figure 1) chemically known as4-{[4-({4-[(E2-cyanovinyl]-2,6-dimethylphenyl}amino)pyrimidin-2yl]amino}benzonitriles. It is a second generation non-nucleoside reverse transcriptase inhibitor (NNRTI) with higher potency and longer half-life. It has activity against NNRTI resistant viral strains due to the flexibility of interactions with the HIV RT. [1,2,3]

Literature review indicates that few UV spectrophotometric method [4-9] HPLC method [10-14], UPLC method [15,16] LC-MS[17] method, HPTLC method [18]. Theabove reportedchromatographic methodsemployedsophisticated and expensive instrumentationthatare generally not availableinmostofthequality controllaboratories ofunderdeveloped anddeveloping countries. As aresult, theapplicationsof thesemethods[4-18]for thequantificationof Rilpivirine inbiologicalsamples, bulkand pharmaceutical formulations are limited.

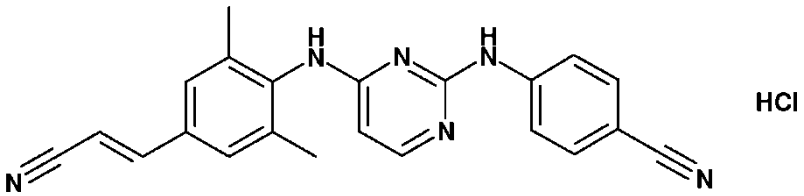

Figure 1: Chemical structure of Rilpivirine

II. Materials and methods

Apparatus

A double beam UV- Visible Spectrophotometer, (LAB INDIA-3000) with UV WIN software and 1cm quartz cell in the wavelength range of 200-400nm was used for spectrophotometric measurements. Drug and the reagents were weighed using Sartorius weighing balance. A calibrated digital pH (Systronics, model-361) was used for pH measurement.

Preparation of reagents and solutions

Standard solution of Rilpivirine

Rilpivirine working standard was procured from HiQ Pharma Labs PvtLtd., Hyderabad, India. Standard stock solution of Rilpivirine was prepared by dissolving accurately weighed 100mg of drug in 100ml volumetric flask and diluted up to the mark with distilled water.

Standard solution of Bromothymol blue

0.1% w/v of Bromothymol blue was prepared by dissolving 0.100g in distilled water in 100ml volumetric flaskand diluted up to the mark with water.

Standard solution of Bromocresol green

0.1% w/v of Bromocresol green was prepared by dissolving 0.100g in distilled water in 100ml volumetric flask by adding 2ml of 0.1M NaOH for better solubility and diluted up to the mark with water.

Preparation of phosphate buffer pH 3.0

1.78g of Sodium dihydrogen phosphate buffer was accurately weighed and dissolved in 1000ml distilled water and pH was adjusted to 3.0.

Standard solution of 0.1N HCl

0.1 N HCl was prepared by dissolving 0.85ml in distilled water in 100ml volumetric flask and diluted up to the mark with water.

General procedure for sample preparation

Method A (BTB)

Aliquots (0.2-1.0ml) of Rilpivirine standard solution were transferred in to 10ml volumetric flask. To each flask 1ml of Bromothymol blue, 0.8ml of 0.1% Hydrochloric acid was added. The volume was adjusted to 5ml with water and then extracted with 5ml chloroform. Absorbance of each solution was measured at 425nm.

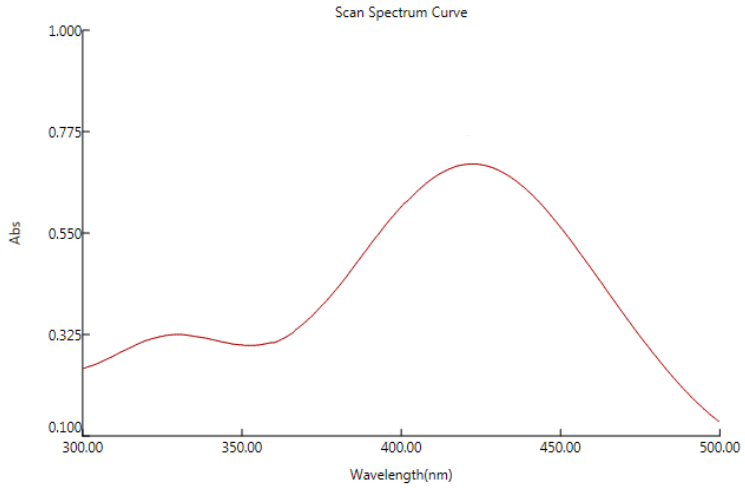

Figure 2: Absorption spectra of Rilpivirine – BTB complex showing absorption maxima at 425nm

Method B (BCG)

Aliquots (0.2-1.0ml) of Rilpivirine standard solution were transferred in to 10ml volumetric flask. To each flask 2ml of Bromocresol green, 2ml of buffer solution was added. The mixture was extracted with 10ml chloroform. The organic phase was extracted and dehydrated by passingoveranhydrous sodium sulphate and volume was made up to the mark with chloroform. Absorbance of each solution was measured at 415nm against a reagent blank.

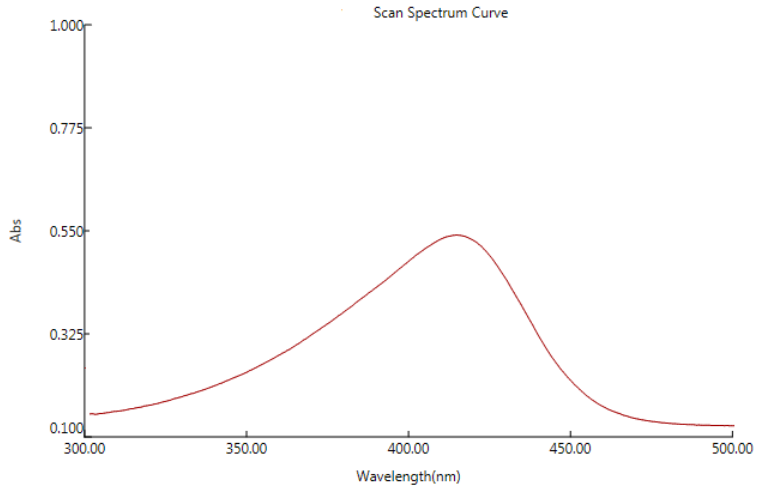

Figure 3: Absorption spectra of Rilpivirine – BCG complex showing absorption maxima at 415nm

Procedure for the assay of dosage forms

The tablet formulation of Rilpivirine labeled to contain 25mg was purchased. Twenty tablets were accurately weighed and finely powdered in a mortar. A portion of tablet powder equivalent to 25mg was weighed and transferred into 100ml volumetric flask and the mixture was sonicated for 15mins. The mixture was filtered through Whatman No. 1 filter paper. The solution was made up to the mark with distilled and contents were analyzed by the proposed methods.

III. Results and discussion

Method development

Rilpivirine forms ion-pair complexes with bromothymol blue and Bromocresol green. This property of drug was followed for development of sensitive colorimetric methods for analysis of drug. The complex of Rilpivirine with BTB and BCG showed maximum absorbance at 425nm and 415nm respectively. [19-26]

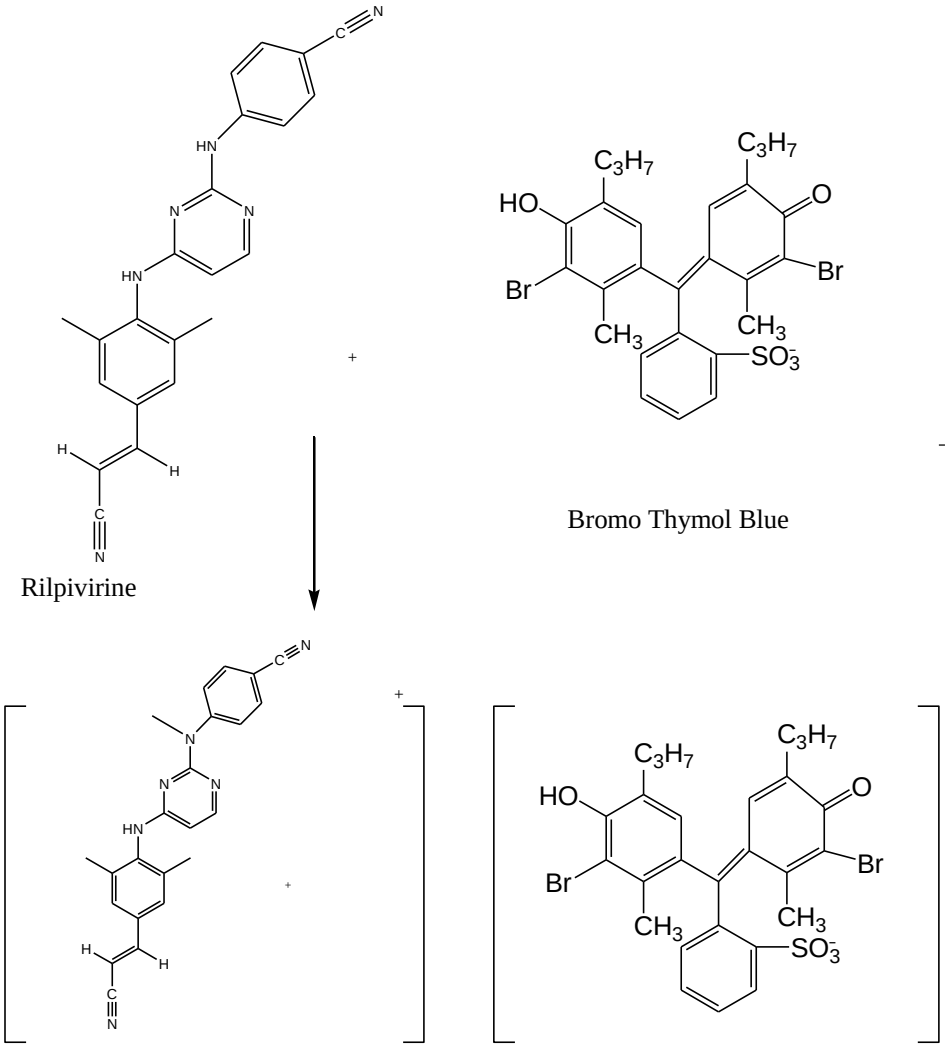

Figure 3: Reaction Pathway between Bromothymol blue and Rilpivirine

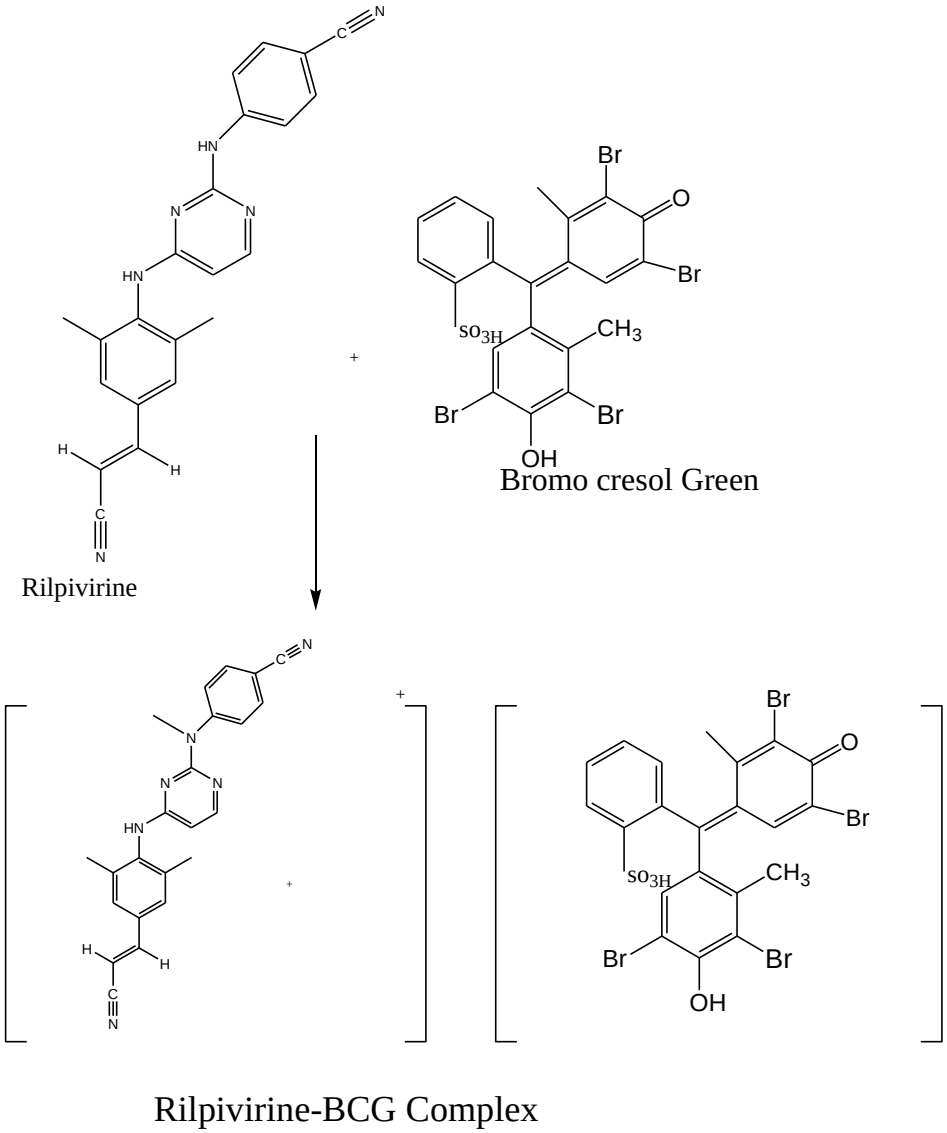

Figure 4: Reaction Pathway between Bromocresol green and Rilpivirine

Effect of BTB

The effect of the volume of 0.1% w/v Bromothymol blue on the absorbance of the yellow colored complex was studied in the range of 0.2-2.0ml. The absorbance increases with the increase in the volume of Bromothymol blue up to 1ml. Further addition of Bromothymol blue showed decrease in the absorbance. Therefore, 1ml of 0.1% w/v Bromothymol blue was chosen as an optimum value (Figure 5).

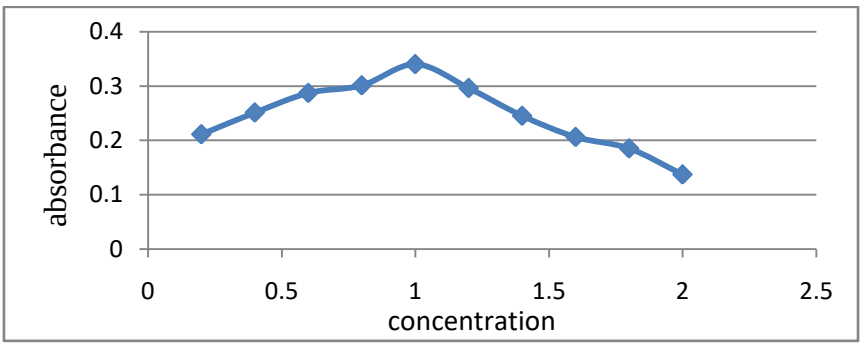

Figure 5: Effect of volume of Bromothymol blue

Effect of BCG

The effect of the volume of 0.1% w/v Bromocresol green on the absorbance of the yellow colored complex was studied in the range of 0.4-4.0ml. The absorbance increases with the increase in the volume of Bromocresol green up to 2ml. Further addition of Bromothymol blue showed decrease in the absorbance. Therefore, 2ml of 0.1% w/v Bromocresol green was chosen as an optimum value (Figure 6).

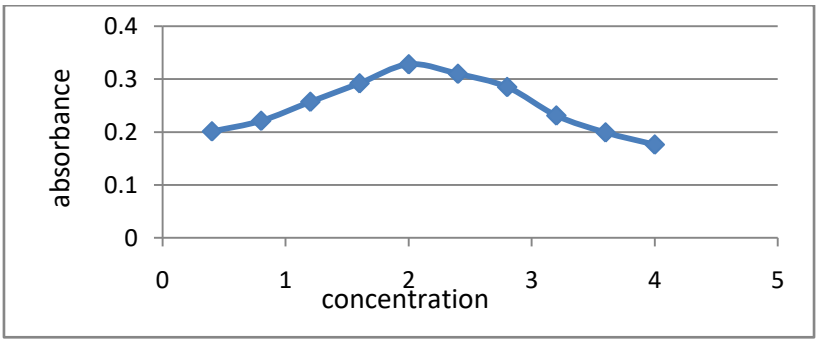

Figure 6: Effect of volume of Bromocresol green

Effect of volume of 0.1N HCl

The effect of volume of 0.1N HCl on the absorbance of yellow colored complex was studied in the range 0.2-2.0ml. The absorbance increases with increase in the volume of Sodium carbonate and becomes constant at 0.8ml. Further addition of HCl showed decrease in the absorbance. Hence 0.8ml of 0.1N HCl was selected as an optimum value (Figure 7).

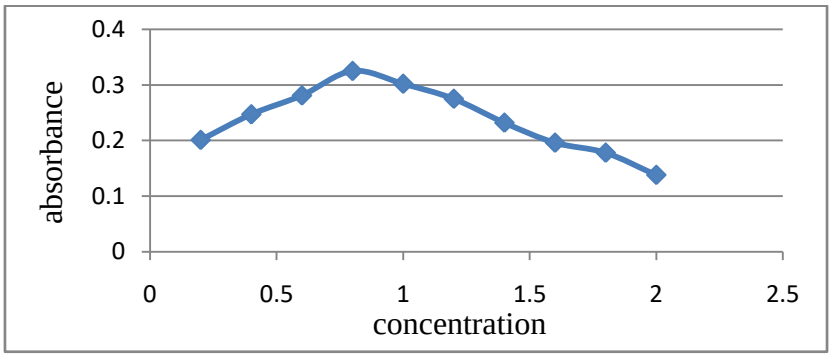

Figure 7: Effect of volume of 0.1N HCl

Effect of pH

The influence on pH on the ion-pair formation between Rilpivirine and BCG was studied using sodium dihydrogen phosphate buffer in the range of 2-5. The maximum absorbance value was obtained at pH 3. It was also observed that addition of 2ml of buffer showed maximum absorbance (figure 8).

Figure 8: Effect of Ph

Effect of extracting solvents

Different solvents (methanol, dichloromethane, chloroform, ethyl acetate and 1,2-dichloromethane) were tested. Maximum absorbance and higher selective extraction of the ion-pair complex were achieved using chloroform as an extracting solvent.

Associationconstantandthe freeenergy changesof thecomplexes

Theassociationconstantofcomplex was determinedby employing theBenesi–Hildebrand method [27],besides theassociation constant was calculated byusingthe following equation:

$[Ao]/A\lambda=1/\varepsilon+(1/Kc.\varepsilon).1/[Do]$

Where,

[Do] = Concentration of the drug,

[Ao]= Concentration of thereagent,

$A\lambda$= Absorbanceofthe complexat445nm,

ε= Molarabsorptivityofthe complex at445nm,

Kc=Association constant of the complex.

The$\Delta G°$ (thestandardfreeenergyof complexation) and the association constant Kc arerelatedbythefollowingequation [20]

$$\Delta G°=-2.303RT\log Kc$$

Where,

$\Delta G°$=Freeenergychangeofthe complex,

R = Gas constant ($1.987 calmol^{-1} degree^{-1}$),

T = Temperature in Kelvin,

K= Association constant ($Lmol^{-1}$) of the drug-reagent complex.

The results are summarized in the (Table 1).

The negative values of the standard free energy indicated that the complexes are stable and started to form spontaneously.

Method validation

Sensitivity:

According to the ICH guidelines [28], the sensitivity parameters like molar absorptivity, Sandell's sensitivity, Limit of Detection and Limit of Quantification were calculated and summarized in (Table 1).

Parameter	BTB	BCG
Beer's law limit (µg/mL)	4-20	4-20
Regression Equation(y=mx+c)	Y = 0.0368x + 0.0027	Y = 0.0332x + 0.0027
Slope (m)	0.0368	0.0332
Intercept (x)	0.0056	0.0057
Molar Absorptivity	1.36×10^4	1.22×10^4
Regression coefficient (r^2)	0.9994	0.9998
Sandell's sensitivity	0.027322	0.030326
LOD (µg/mL)	0.7	0.03
LOQ (µg/mL)	1.17	0.12
Association constant ($L\ mole^{-1}$)	1.66×10^4	2.1×10^4
Free energychange	-5.04×10^4	-4.87×10^4

Table1: Thermodynamic studies,linearity and Sensitivity of the proposed methods

Linearity:

The relation between the absorbance and final concentration ofRilpivirine was found to be linear over the concentration range of 4-20µg/ml for methods A and B. Results are shown in (Figure 9, 10) respectively.

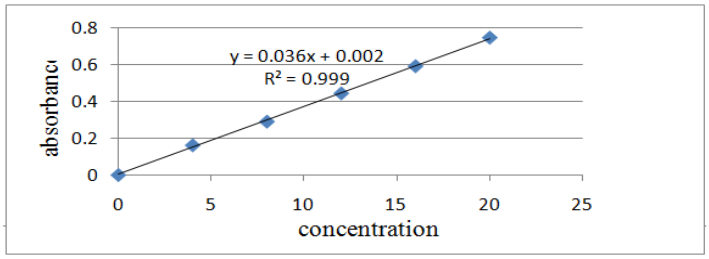

Figure 9: linearity graph of Rilpivirine (Method A)

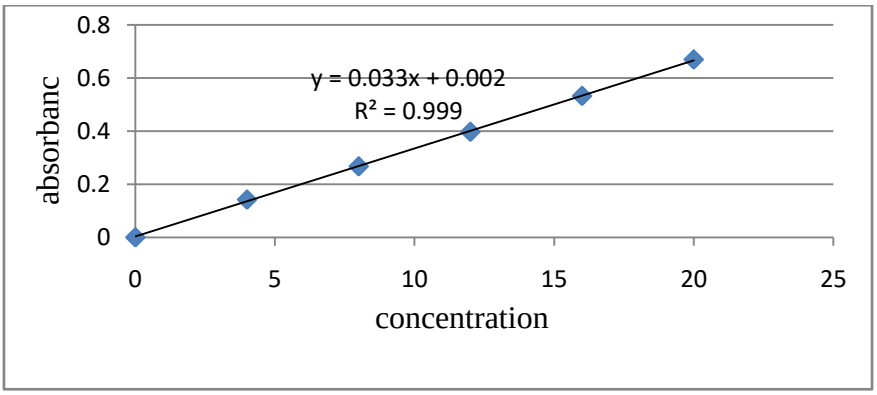

Figure 10: linearity graph of Rilpivirine (Method B)

Precision:

The repeatability (intra-day precision) of the proposed method was determined by replicate analysis (n=5) of standard solutions at three concentration levels (4µg/ml, 12µg/ml and 20µg/ml). The intermediate precision (inter-day precision) was conducted by repeating the analysis over a period of three consecutive days.

The precision of the methods was expressed as standard deviation (SD) and percentage relative standard deviation (%RSD). The results are summarized in (Table 2).The SD and % RSD obtained by both methods are found to be in the acceptable range. Therefore, it can be considered to be satisfactory.

Methods	Type of Assay	Concentration(µg/mL) Taken	Found	SD	%RSD	%Recovery	%Error
A (BTB)	Inter day	4	4.008	0.016	0.396	100.2	-0.2
		12	11.989	0.031	0.258	99.9	0.1
		20	20.02	0.03	0.15	100.08	-0.08
	Intra day	4	3.981	0.029	0.726	99.5	0.5
		12	11.33	0.083	0.455	99.3	0.7
		20	20.0	0.143	0.714	100	0.0
B (BCG)	Inter day	4	3.991	0.016	0.402	99.8	0.2
		12	12.010	0.017	0.153	100.1	-0.1
		20	20.080	0.017	0.086	100.4	-0.4
	Intra day	4	4.011	0.032	0.799	100.3	-0.3
		12	12.002	0.052	0.434	100.05	-0.1
		20	19.99	0.03	0.13	99.97	0.0

Table 2: Accuracy and Precision of the proposed methods

Accuracy

The accuracy of the proposed method was established by performing intra-day and inter-day assays by determining at different levels of drug concentrations [lower concentration (50%), intermediate concentration (100%) and higher concentration (150%)] within 1 day and 3 consecutive days, respectively. The accuracy of the methods is expressed as percentage recoveries and percentage error. The results obtained by both the methods are found to be in the acceptable range. Therefore, we can say it can be considered as satisfactory.

In addition, accuracy and validity of the proposed methods were determined by standard addition technique. The pre analyzed samples were spiked with additional 50,100 and 150% were once again analyzed by the proposed methods. The accuracy of the methods was evaluated by percentage recovery of the Rilpivirine. The average recovery and percentage standard deviation values (Table 3) of the methods lying in the acceptable range show that the methods are accurate.

Method	Tablet Concentration(mg)	Spiked	Found	SD	%RSD	%Recovery
A (BTB)	25	10	9.990	0.041	0.408	99.9
	25	20	19.84	0.08	0.39	99.19
	25	30	29.66	0.22	0.75	98.87
B (BCG)	25	10	9.953	0.040	0.406	99.5
	25	20	20.02	0.03	0.150	100.08
	25	30	29.985	0.03	0.09	99.95

Table 3: Results of standard addition technique of proposed method

Robustness:

The robustnessoftheproposedmethodswas checkedforeach operationalparameterand investigated. The operational parameters were:

Volume of 0.1% Bromothymol blue: 1.0 ± 0.1 mL

Volume of 0.1N HCl: 0.8± 0.1 mL

Volume of 0.1% w/v Bromocresol green: 2.0 ± 0.1 ml

The robustnessof themethodswas assessed byanalyzing the Rilpivirine attwo different concentrationlevels (4 and20µg/mL).The percent recovery and % RSD of the method (Table4)was foundtobe satisfactory, indicatingthat the method is robust.

S.No	Parameter	vol	(4µg/ml) Absorbance	% Recovery	% RSD	(20µg/ml) Absorbance	% Recovery	% RSD
1	Bromothymol blue	0.9	0.205	99.52	1.29	0.881	99.55	1.09
		1.0	0.202	97.58	0.75	0.887	100.23	0.45
		1.1	0.207	100	0.39	0.884	99.89	0.79
2	Hydrochloric acid	0.7	0.206	99.52	0.37	0.883	99.77	1.21
		0.8	0.203	98.07	0.40	0.885	100	0.51
		0.9	0.205	99.03	0.71	0.884	99.89	0.62
3	Bromocresol green	1.9	0.143	100	0.40	0.667	99.9	0.15
		2.0	0.142	99.3	0.51	0.668	100	0.10
		2.1	0.143	100.0	0.40	0.668	100	0.10

Table 4: Robustness of proposed method

Application of the methods

The developed spectrophotometric methods were applied to the pharmaceutical formulation containing Rilpivirine through complex formation by BTB and BCG in chloroform. Thepercentrecoveryand %RSD(Table 5)clearly showednointerferenceofany excipientsofformulation,thus provingaccuracy &precisioninthequantification of Rilpivirine

Method	Formulation	Labeled claim(mg)	Found ± SD (n=5)	RSD (%)	Recovery (%)
A (BTB)	EDURANT	25	24.86 ± 0.094	0.953	99.52
B (BCG)	EDURANT	25	24.79 ± 0.091	0.948	99.49

Table 5: Results of analysis in tablet formulation

IV. Conclusion

A sensitive visible spectrophotometric method for the determination of Rilpivirine have been developed and validated. The present methods demonstrate that Bromothymol blue and Bromocresol green can be used for the quantitative determination of Rilpivirine in bulk and in pharmaceutical dosage forms. The reagents used in the developed method are cheap and readily available. From the values of molar absorptivity, Sandell's sensitivity, LOD and LOQ, it was observed that the new methods are more sensitive than the reported methods. The proposed method is fully validated and found to be sensitive, accurate, reproducible, selective, robust and rugged. These advantages support the application of the proposed methods in routine quality control analysis of Rilpivirine.

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