THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

P.V.V. Satyanarayana **et al, The *Experiment, AUGUST.2012 Vol.1 (2), 48-54***

NEW SPECTROPHOTOMETRIC METHODS FOR THE QUANTITATIVE ESTIMATION OF TIPRANAVIR IN FORMULATIONS

ABSTRACT

Five simple and sensitive visible spectrophotometeric methods have been developed for the quantitative estimation of Tipranavir in bulk drug and pharmaceutical preparations including the un-interruption of the excipients and impurities. All these method have different linearity ranges. All these method are simple, Accurate, precise and very effective even at low concentrations and used for the quantitive estimation of Tipranavir in commercial formulations.

KEYWORDS: Tipranavir, spectrophotometeric methods, Sodium nitroprusside, Citric acid – Acetic anhydride, Cobalt Thiocyanate Method, Iso Nicotanic hydrazide, TPOOO.

INTRODUCTION

Tipranavir, or tipranavir disodium, is a nonpeptidic protease inhibitor. It is administered with ritonavir in combination therapy to treat HIV infection. Tipranavir has the ability to inhibit the replication of viruses that are resistant to other protease inhibitors and it recommended for patients who are resistant to other treatments. Resistance to tipranavir itself seems to require multiple mutations.[1] It is very potent and is effective in salvage therapy for patients with some drug resistance. However, side effects of tipranavir can be more severe than other anti-retrovirals. Some side effects include intracranial hemorraging, hepatitis[3], and diabetes mellitus. The drug has also been shown to cause increases in total cholesterol and triglycerides

EXPERIMENTAL PROCEDURE

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. Double beam UV-Visible Spectrophotometer is used for measuring the absorbance's of the color formed during the analysis.

Preparation of reagents:

1. Sodium nitroprusside Solution: Weigh accurately 5g of sodium nitroprusside and is dissolved in 100ml of distill water.
2. Hydroxil amine hydrochloride solution: 500mg of Hydroxil amine hydrochloride is dissolved in 100ml of distill water.
3. Sodium carbonate solution: 10mg of sodium carbonate is dissolved in 100ml, of distill water.
4. Citric acid – Acetic anhydride solution: Prepared by dissolving 1.2g of citric acid in 5ml methanol and made up to 1ml with acetic anhydride.
5. Cobalt Thiocyanate solution: The solution was prepared by dissolving 7.25g of cobalt nitrate and 3.8g of ammonium thiocyanate in 100ml of distill water.
6. P^H 2 Buffer solution: the solution was prepared by mixing 306ml of 0.1M tris sodium citrate with 694ml of 0.1M HCL and the P^H adjusted to 2.
7. Iso Nicotanic hydrazide solution: Weigh accurately 800 mg of Iso Nicotanic hydrazide and is dissolved in 100 mL of MeOH containing 1% of conc. HCl.
8. Tropaeolin-ooo solution: weigh 200 mg of Tropaeolin-ooo (Tpooo) and is dissolved in 100ml of distill water.
9. HCL Solution: dissolve 8.6 ml of concentrated hydrochloric acid in 1000ml of distill water

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

Preparation of working standard drug solution:

The standard Tipranavir (100 mg) was weighed accurately and transferred to volumetric flask (100 ml). It was dissolved properly and diluted up to the mark with methanol to obtain final concentration of 1000 µg /ml (stock solution I). 20 ml of stock solution I was diluted to 100 ml with Methanol (Stock solution II, 200 µg/ml) and the resulting solution was used as working standard solution.

METHODS

Sodium nitroprusside Method: (M1)

Aliquot of standard drug solution was transferred in to a series of 25ml calibrated volumetric flasks. Then 1ml of Sodium nitroprusside Solution and 1ml of Hydroxil amine hydrochloride solution were added and kept aside for 5min. then 1ml of Sodium carbonate solution solution was added and shaken for 15min. the volume w3as made up to the mark with distill water. The absorbance was measured after 10min at 580nm against a reagent blank solution prepared similarly without drug.

Citric acid – Acetic anhydride Method: (M2)

From the standard stock solution II of Tipranavir, appropriate concentration (20 to140 ppm) is pipetted out in to a 25 ml volumetric calibrated tube, and gently evaporated on a boiling water bath to dryness. To this 10 ml of Citric acid – Acetic anhydride reagent was added, and flask was immersed in a boiling water bath for 30 min. the tubes were cooled to room temperature and made up to mark with acetic anhydride. The absorbance of the formed color was measured at 580 nm against a reagent blank.

Cobalt Thiocyanate Method: (M3)

In to a series of 125ml separating funnels, aliquots of standard drug solution were taken. Then add 2ml of buffer solution and 8ml of Cobalt Thiocyanate solution. The volume of each aqueous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel, 10ml of Nitrobenzene was added and the contents were shaken for 2min. the two phases were separated and organic layer was collected. The absorbance of the organic layer was measured at 680nm against a similar reagent blank.

Iso Nicotanic hydrazide Method: (M4)

Aliquot of standard drug solution (5-30ppm) was delivered into a series of 10 ml of calibrated tubes. Then 2.0 mL of Iso Nicotanic hydrazide solution was added to each tube and heated for 10 min at 60 ^{0}C. The solution in each tube was cooled and made up to 10 mL with methanol. The absorbance was measured at 480 nm against the reagent blank.

TPOOO Method: (M5)

In a series of 125 ml separating funnels containing aliquots of standard drug solution was taken. To this 6ml of HCl solution and 2ml of Tpooo solutions were added successively. The total volume of the aqous phase in each separating funnel was adjusted to 15ml with distill water. To each separating funnel 10ml of Chloroform was added and the contents were shaken for 2 min. the two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 480nm against a similar reagent blank.

Assay Procedure for Formulations:

An amount of finely ground tablet powder equivalent to 100 mg of Tipranavir (Jakafi - 10mg) was accurately weighed into a 100 ml calibrated flask, 60 ml of water added and shaken for 20 min. Then, the volume was made up to the mark with water, mixed well, and

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

filtered using a Whatman No 42 filter paper. First 10 ml portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (1000 µg mL-1 Tipranavir) was diluted appropriately to get suitable concentrations for analysis by proposed methods.

Method Validation:

Selection of analytical concentration ranges: (linearity test)

Linearity test was evaluated by measuring the absorbance values of standard solutions. The standard stock solution of Tipranavir, appropriate aliquots were pipetted out in to a six or seven series of volumetric flasks and add the solutions required in required for each individual method. After color formation absorbance of each concentration was measured at wavelength found for the proposed method. Results were shown in Table: 1 and Standard graphs of linearity for proposed methods were shown below.

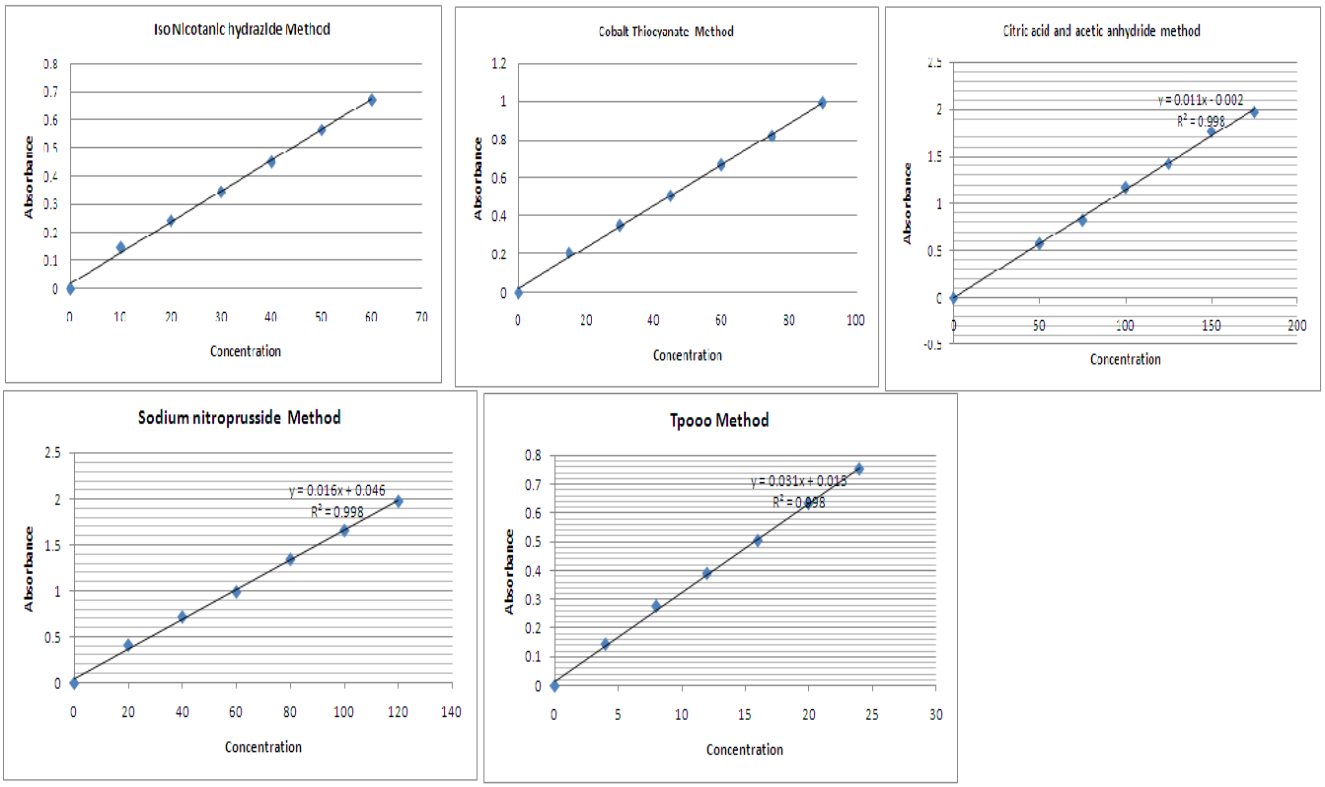

Figure 1: Calibration curves for the proposed methods.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

S.NO	Parameter	M1	M2	M3	M4	M5
1	Wavelength Max	580nm	580nm	680nm	480nm	490nm
2	Concentration Range	20-120 ppm	50-175 ppm	15-90 ppm	10-60 ppm	4-24 ppm
3	Correlation coefficient	0.9991	0.999	0.9992	0.999	0.9994
4	Slope	0.016	0.011	0.01	0.01	0.031
5	Intercept	0.045	-0.003	0.021	0.024	0.014
6	RSD of Precision	0.28	0.37	0.65	0.78	0.87
7	Average recovery	99.69	99.93	99.44	100.57	99.33
8	Stability period	150min	210min	140min	150min	180min
9	LOD	0.85ppm	1.5ppm	2 ppm	1 ppm	0.06ppm
10	LOQ	2.8 ppm	5 ppm	10 ppm	3.3 ppm	0.2 ppm
8	% Assay of Formulation	98.66	99.02	99.4	99.20	99.00

Table 1: Summery results of the proposed methods

PRECISION

To evaluate the accuracy and precision of the methods, pure drug solution (Within the working limits) was analyzed and being repeated six times. The relative error (%) and relative standard deviation (%) were less than 2.0 and indicate the high accuracy and precision for the proposed methods (Table 2).

SNO	M1	M2	M3	M4	M5
Concentration	80PPM	100PPM	45ppm	30PPM	12PPM
1	1.347	1.171	0.505	0.346	0.391
2	1.341	1.172	0.509	0.344	0.395
3	1.344	1.169	0.507	0.349	0.399
4	1.342	1.165	0.502	0.341	0.394
5	1.349	1.177	0.5	0.345	0.39
6	1.35	1.175	0.506	0.347	0.391
RSD	0.28	0.37	0.506	0.78	0.87

Table 2: Precision results of the proposed methods

RECOVERY STUDIES

To ensure the accuracy and reproducibility of the results obtained, known amounts of pure drug was added to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The Percentage recoveries thus obtained were given in Table 3.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

Method	Recovery	Concentration In ppm	Amount found in ppm	% of recovery	Average Recovery
M1	50%	40	39.36	98.4	99.69
	100%	80	80.63	100.79	
	150%	120	119.86	99.88	
M2	50%	50	49.21	98.42	99.33
	100%	100	100.52	100.52	
	150%	150	151.28	100.85	
M3	50%	30	29.61	98.7	99.44
	100%	60	59.29	98.82	
	150%	90	90.73	100.81	
M4	50%	10	9.915	99.15	100.57
	100%	20	20.33	101.65	
	150%	30	30.27	100.9	
M5	50%	6	5.89	98.17	99.33
	100%	12	11.88	99	
	150%	18	18.15	100.83	

Table3: Recovery results of the proposed methods

Application to Analysis of Commercial Sample:

In order to check the validity of the proposed methods, Tipranavir was determined in commercial formulation. From the results of the determination it is clear that there is close agreement between the results obtained by the proposed methods and the label claim. These results indicating that there was no significant difference between the proposed methods and the reference methods in respect to accuracy and precision.

S.NO	Method	Formulation	Amount prepared	Amount found	% Assay
1	M1	Aptivus(250mg)	80ppm	78.93	98.66
2	M2	Aptivus(250mg)	100ppm	99.02	99.02
3	M3	Aptivus(250mg)	45ppm	44.73	99.4
4	M4	Aptivus(250mg)	30ppm	29.76	99.2
5	M5	Aptivus(250mg)	12ppm	11.88	99.00

DISCUSSION

In method M1 Aconitic acid formation takes place from Acetic acid and Acetic anhydride through dehydration. Aconitic acid forms an internal salt with the 3^0 amine group of the drug. Results form a violet color show absorbance at 580nm.

In TPooo method drug being a base form an ion association complex with acid dye Tpooo. The formed complex is extractable in to Chloroform from the aqueous phase. The protonated nitrogen positive charge of the drug molecule in acid medium is expected to attack the positive charge of the dye. Hence form a colored complex which is extracted with Chloroform.

In method M4, the keto group of the drug reacts with Iso Nicotinic hydrazode to gives a colored Hydarzone. The formed color chromogen shows absorbance at 480nm.

Cobalt thiocyanate is a valuable reagent for the detection and determination of Amino compound. A coordinate complex is formed when the secondary amine group of the drug is treated with Cobalt thiocyanate. The formed complex shows color. The colored complex is extractable with the Nitrobenzene from the aqueous solution. The obtained color shows absorbance at 680nm.

In Sodium nitroprusside method, Aromatic 3^0 amine group in the drug functions as an electron donor. Sodium nitroprusside in the presence of hydroxyl amine and alkali exists as ferrocyanide. The color obtained in this reaction may be due to the formation of inner complex with 3^0 nitrogen in drug. As it exhibit ligandary property.

The linearity ranges of Tipranavir are found to be 20-120ppm, 50-175 ppm, 15-90ppm, 10-60ppm, 4-24ppm for M1 to M5 respectively. A linear correlation was found between absorbance and concentration of Tipranavir. The graphs showed negligible intercept and are described by the equation: Y = a + bX (where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in ug mL-1 max) .Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient(r) for each system according to ICH guide

The accuracy of the proposed methods was further ascertained by performing Accuracy studies. The Relative standard deviations of results for the proposed were very low and the values are within the range below 2. It indicates that the high accuracy and precision for the proposed methods. The Recovery results were very close to the actual range and it revealed that co-formulated substances did not interfere in the determination.

CONCLUSIONS

Five useful micro methods for the determination of Tipranavir have been developed and validated. The methods are simple and rapid taking not more than 20-25 min for the assay. These spectrophotometric methods are more sensitive than the existing UV and HPLC methods, and are free from such experimental variables as heating or extraction step. The methods rely on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

REFERENCES

1. doyon l, tremblay s, bourgon l, wardrop e, cordingley m (2005). "selection and characterization of hiv-1 showing reduced susceptibility to the non-peptidic protease inhibitor tipranavir.". *antiviral res* 68 (1): 27–35.
2. "new aptivus (tipranavir) oral solution approved for treatment-experienced pediatric and adolescent hiv patients" boehringer ingelheim. 2008-06-24.
3. "aptivus (tipranavir) capsules, 250 mg, prescribing information", boehringer ingelheim, 2007. revised february 5, 2007.
4. doyon l, tremblay s, bourgon l, wardrop e, cordingley m (2005). "selection and characterization of hiv-1 showing reduced susceptibility to the non-peptidic protease inhibitor tipranavir.".*antiviral res* 68 (1): 27–35.
5. "new aptivus (tipranavir) oral solution approved for treatment-experienced pediatric and adolescent hiv patients" (press release). boehringer ingelheim. 2008-06-24. retrieved 2008-09-02.
6. aptivus (tipranavir) capsules, 250 mg, prescribing information", boehringer ingelheim, 2007. revised february 5, 2007.
7. walmsley s, leith j, katlama c, et al. pharmacokinetics and safety of tipranavir/ritonavir (tpv/r) alone and in combination with saquinavir (sqv), amprenavir (apv), or lopinavir (lpv): interim analysis of bi1182.51. in: program and abstracts of the xv international aids conference; july 11-16, 2004; bangkok, thailand. abstract weorb1236.

8. damodar katasani, srinu bhogineni, bala ramanjaneyulu, new spectrophotometric methods for the quantitative estimation of aztreonamin formulations, *j.atoms and molecules*, 2(1); 2012 /109–116.
9. hicks c, et al. resist-1: a phase 3 randomized, controlled, open-label multicenter trial comparing tipranavir/ritonavir (tpv/r) to an optimized comparator protease inhibitor/r (cpi/r) regimen in antiretroviral (arv) experienced patients: 24-week data. in: program and abstracts of the 44th interscience conference on antimicrobial agents and chemotherapy; october 31-november 2, 2004; washington. abstract 1137a.
10. keil k, difrancesco r, morse gd, determination of tipranavir in human plasma by reverse phase liquid chromatography with uv detection using photodiode array, ther drug monit. 2006 aug;28(4):512-6.

P.V.V. Satyanarayana*, Alavala Siva Madhavi

Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India

Contact Mail id: sivamadhavi@rocketmail.com