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Research article

SPECTROPHOTOMETRIC DETERMINATION OF RITONAVIR BY CONDENSATION METHOD USING NINHYDRIN AND ASCORBIC ACID

M.L.N.Acharyulu[*1], Ch.V.R.Murthy[2], T.S.Reddy[3], S.V.Maruti Prasad[4] and S.Ashok[5]

[1] Department of AS&H, VITAM College of Engineering, Mindivanipalem, Anandapuram Mandal,Visakhapatnam, A.P,India.531173.

[2]Department of Chemical Engineering, AU College of Engineering (A), Andhra University, Visakhapatnam-530003 Andhra Pradesh (India),

[3] DLR College (Degree & P.G Courses), Gollalamamidada, E.G.Dtt, Pin,533344, A.P

[4]Department of BS&H, AdityaInstitute of Tecnology&Management,Tekkali,Srikakulam Dist, A.P

[5]Department of BS&H,G.V.P.College of Engineering for Women, Madhurawada,Visakhapatnam

ABSTRACT: A simple and sensitive extractive visible spectrophotometric method for the assay of Ritonavir in pure and pharmaceutical Formulations based on the reaction between peptide group in RIT and Ninhydrin in the presence of ascorbic acid affords a blue violet coloured product (λ_{max} 560nm). Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges (20-60) µg/ml. The percent recoveries are obtained as 99.64 ± 0.47 to 100.40 ± 0.45 by proposed method and 99.51 ± 0.25 to 99.92 ± 0.20 by reference method for the formulations respectively. The method can be applied successfully for the estimation of the Ritonavir in the presence of other ingredients that are usually present in formulations. The method offers the advantage of rapidity, simplicity and sensitivity and low cost without the need for expensive instrumentation and reagents.

Keywords: Statistical analysis, Ritonavir(RIT),Ascorbic Acid, Ninhydrin,Peptide group.

INTRODUCTION

Ritonavir(United States Pharmacopeia,2007;European Pharmacopeia;1997;CIMS-97) is chemically known as 1,3-thiazol-5-yl methyl [3-hydroxy-5-[3-methyl-2-[methyl-[(2-propan-2-yl-1,3-thiazol-4-yl) methyl] carbamoyl] amino-butanoyl] amino-1,6-diphenyl-hexan-2-yl] amino formate(Fig.1). It is an antiretroviral drug from the protease inhibitor class used to treat HIV infection and AIDS and is exceptional as the only anti retroviral drug that inhibits a liver enzyme that normally metabolizes away protease inhibitors. It can cause a large number of side effects on its own. It is now rarely used for its own antiviral activity but remains widely used as a booster to other protease inhibitors. The analytically useful fuctional groups are Thiazole, Sulphur, Nitrogen, Peptide,secondary hydroxyl respectively.

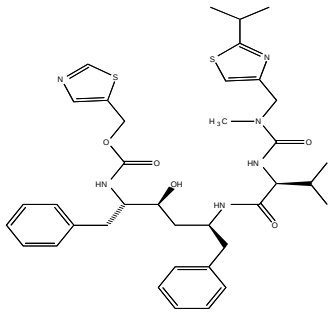

Fig.1.Chemical Structure of Ritonavir

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A few methods were observed in the literature UV(Chutima Mataytsuk Phechkrajang *et al.*,2012),LC(Dias,CL,*et al.*,2009),HPLC(CMarzolini,*et al.*,2000;VirginieProust*etal.*,2000;Wagner Wollinger *et al.*,2012; K.Anand Babu,*et al.*,2011; R.W.Sparidans *et al.*,2001;A.Anton Smith *et al.*,2012; K.Chiranjeevi,*et al.*,2011),RPLC(Antonio Checa *et al.*,2008 VeeraVenkata Satyanarayana Peruri *et al.*,2011),Spectrophotometeric (Carolina LupiDias*et al.*,Erk,N,2004;AninditaBehera *et al.*,2011),Voltammetric(Nevin rk,2004),Electrochemical reduction(Burçin Bozal *et al.*,2011).

Upon thorough study of literature, It is found that no attempt has been made to exploit the functional group peptide present in RIT, to develop a spectrophotometric method to determine the assay of it using condensation methods. Hence, the author has made use of it and made it to condense with Ninhydrin to hydrindantin, the bimolecular Hemi Acetal. The blue colour(Leong,1977;Zhang,Z,*et al.*,1984;Sastry,C.S.P,*et al.*,1988) developed is based on strecker degradation in weakly acidic solution to the anion formed by the reaction of liberated ammonia with Ninhydrin and its reduction product hydrindantin. The stability of the blue colored product is enchanced by making use of reducing agents Ascorbic acid. In the present investigation, the drug RIT which possesses peptide group when heated with Ninhydrin in presence of ascorbic acid afforded a blue violet colour product.

MATERIALS AND METHODS

Preparation of standard drug solution: A 1mg/ml stock solution of RIT was prepared by dissolving 100 mg of the drug in aldehyde free 100 ml Methanol. This stock solution was further diluted with appropriate solvent to get the working standard solutions (50-250 µg/ml).
Pharmaceutical formulation solution: Tablets were mixed thoroughly and 20 tablets were selected at random and grinded to a fine powder. A portion of the mixed powder, equivalent to 100 mg of RIT was dissolved in methanol (2 ml×15ml) and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in 100 ml methanol to achieve a concentration of 1 mg/ml. This solution was further processed as required for analysis**.**

Recommended procedure: Aliquots of standard RIT Solution (0.5-2.5ml,400µg) was transferred into a series of calibrated tubes containing 4.0ml of buffer (pH 5.0),1.0ml Ninhydrin (5.605×10^{-5}M) solution and 0.5ml of Ascorbic acid (5.678×10^{-3}M) solution. The volume in each tube was adjusted to 8.0ml with distilled water and was kept in boiling water bath. After 15min tubes were removed and chilled in ice water. The solution in each tube was made up to 10.0ml with distilled water. The absorbances were measured at 560nm (Fig.2) after 10min gainst a reagent blank prepared similarly. The amount RIT was calculated from its Beer"s plot (Fig.3).

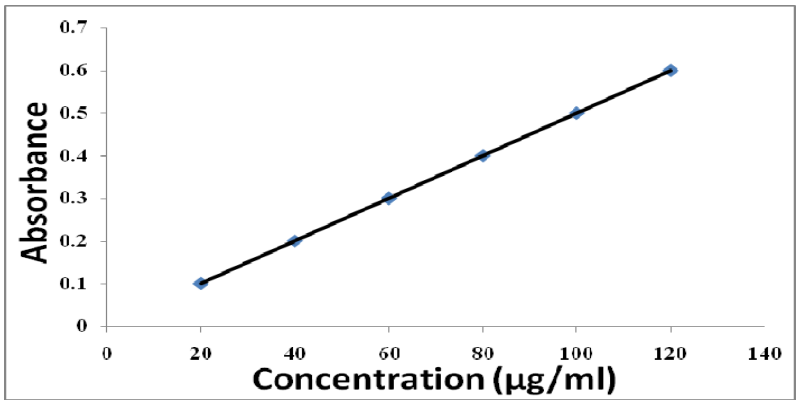

Fig.3 Beer"s plot of RIT With NH/ASA System

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RESULTS AND DISCUSSION

Optimum operating conditions used in the procedure were established adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of reagents, Vol. of Ninhydrin) in acetone required ,Volume and pH of the buffer, Volume of AA solution, order of addition, time and temperature for maximum color development, stability of the coloured species after final dilution, and solvent for final dilution of the colored species were studied.The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements, regression characteristics like standard deviation of slope(Sb), standard deviation of intercept (Sa), standard error of estimation (Se) and % range of error(0.05 and 0.01 confidence limits) were calculated and the results are summarized in Table-1.Commercial formulations containing RIT were successfully analyzed by the proposed method. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-and F-test and found not to differ significantly. These results are summarized in Table-2.

Table 1: Optical Characteristics, Precision, Accuracy of the Methods Proposed in the Determination of Rit

S.No	Optical Characteristics	NH/ASA
1	λmax(nm)	560
2	Beer's Law limits(µg/ml)	20-60
3	Molarabsorptivity(l mol-1cm-1)	1.54x103
4	Correlation coefficient (r)	0.9997
5	Sandell's sensitivity (µg/cm2/0.001absorbance unit)	0.3661
6	Regression equation(y=a+bc) (i)slope (b)	3.76896x10-3
	(ii) Standard deviation on intercept(Sb)	0.00249
	(iii)intercept (a)	7.5x10-4
	(iv) standard deviation (Sa)	0.1139
	(v)Standard error of estimation(Se)	0.209
7	Optimum photometric range (µg/ml)	25.12-120
8	Relative Standard deviation *	0.479
9	Detection limit	0.196
10	% of range of error(confidence limit) (i)0.05 level	0.5028
	(ii)0.01 level	0.8277

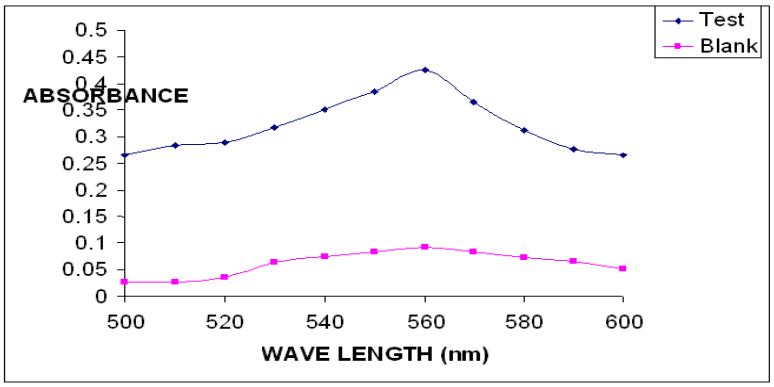

Fig. 2 Absorption Spectrum of RIT with NH/ASA System

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Table.2 Determination of Rit in Pharmaceutical Formulations

Pharmaceutical Formulations	LABELLED AMOUNT	Percentage Recovery	
		Proposed methods	Reference method
Tablets – T1	200mg	99.64 ± 0.47 t = 0.52 F = 1.42	99.51 ± 0.25
Tablets – T2	200mg	100.33 ± 0.53 t = 1.08 F = 2.94	99.92 ± 0.20
Tablets – T3	200mg	100.40 ± 0.45 t = 1.32 F = 2.66	99.56 ± 0.45
Tablets – T4	200mg	99.76 ± 0.57 t = 1.40 F = 2.76	99.83 ± 0.50

*Tablets from four different pharmaceutical companies.

**Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method.

CONCLUSION

A significant advantage of an extraction spectrophotometric determination is that it can be applied to the determination of individual compounds in a multi component mixture. This aspect of spectrophotometric analysis is of major interest in analytical chemistry, since, it offers distinct possibilities in assay of a particular component in a complex dosage formulation. In the present study, RIT was determined successfully as pure compound as well as a single component in representative dosage formulations. The proposed method applicable for the assay of drug and the advantage of wider range under Beer's law limits.

The proposed visible spectrophotometric method is validated as per ICH guide lines and possess reasonable precision, accuracy, simple, sensitive and the proposed method report a new for the determination of RIT in pharmaceuticals. The method can be extended for the routine assay of RIT in formulations.

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