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VISIBLE SPECTROPHOTOMETRIC ESTIMATION OF AMPRENAVIR IN PHARMACEUTICAL FORMULATIONS

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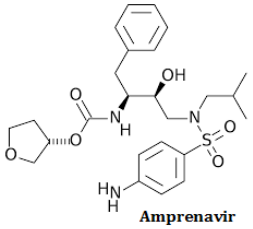
ABSTRACT

Two simple, accurate, rapid and sensitive methods (A and B) have been developed for the estimation of Amprenavir in its pharmaceutical dosage form. The Method A is based on the formation of bluish-green colored chromogen, due to reaction of Amprenavir with Folin–Ciocalteu reagent (FCR) reagent in the presence of alkaline medium, which exhibits λ_{max} at 715 nm. Method B is based on the reaction of Amprenavir with PDAC in the presence of Sulfuric acid to form a red colored chromogen, which shows maximum absorbance at 505 nm. The absorbance-concentration plot is linear over the range of 50-500 µg/mL for Method A, 10-100 µg/mL for Method B. Results of analysis for all the methods were validated statistically and by recovery studies. Literature survey reveals only HPLC methods deals with biological fluids and in fixed pharmaceutical dosage forms. The proposed methods are economical and sensitive for the estimation of Amprenavir in bulk drug and in its formulations.

KEYWORDS: UV-Visible Spectrophotometry, Amprenavir, Folin–Ciocalteu reagent (FCR), Sodium bicarbonate, p-dimethylaminocinnamaldehyde (PDAC), Sulfuric acid

INTRODUCTION

Amprenavir is an inhibitor of is an inhibitor of the human immunodeficiency virus (HIV) protease.[1-2] The chemical name of amprenavir is (3*S*)-tetrahydro-3-furyl *N*-[(1*S*,2*R*)-3-(4-amino-*N*-isobutylbenzenesulfonamido)-1-benzyl-2-hydroxypropyl]carbamate.[3,5] Amprenavir is a single stereoisomer with the (3*S*)(1*S*,2*R*) configuration. It has a molecular formula of $C_{25}H_{35}N_3O_6S$ and a molecular weight of 505.64. Amprenavir is a white to cream-colored solid with a solubility of approximately 0.04 mg/mL in water at 25°C. Amprenavir has the following structural formula:



Amprenavir

Figure 1: Chemical Structure of amprenavir.

Amprenavir drug substance is a white to almost white powder. Amprenavir is sparingly soluble in water over a wide pH range. It is practically insoluble in propylene glycol, very slightly soluble in ethanol, and slightly soluble in acetone. It is soluble in dichloromethane and freely soluble in some organic solvents (e.g.,

tetrahydrofuran and N, N-dimethyl formamide). AGENERASE Capsules (amprenavir capsules) are available for oral administration. Each 50mg capsule contains the inactive ingredients d-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS), polyethylene glycol 400 (PEG 400) 246.7 mg, and propylene glycol 19mg. The capsule shell contains the inactive ingredients d-sorbitol and sorbitans solution, gelatin, glycerin, and titanium dioxide. The soft gelatin capsules are printed with edible red ink. Each 50- mg AGENERASE Capsule contains 36.3 IU vitamin E in the form of TPGS. The total amount of vitamin E in the recommended daily adult dose of AGENERASE is 1,744 IU. Survey of literature reveals that the drug is determined by using HPLC in simultaneous estimation in biological fluids [6,7] and in pharmaceutical dosage form[8,9] The present study describes simple, sensitive, accurate, rapid and economical Spectrophotometric Methods A and B for the estimation of Amprenavir in its Tablet formulations.

EXPERIMENTAL: Instrument: Elico Ultraviolet-Visible double beam spectrophotometer SL-164 with 1 cm matched quartz cells was used for all spectral measurements.

REAGENTS: All the chemicals used were of analytical reagent grade.

1. Para dimethyl amino cinnamaldehyde (PDAC)
2. Folin–Ciocalteu reagent (FCR) reagent - 2%w/v
3. Sodium Bicarbonate
4. Sulfuric acid concentrated
5. Dilute HCl (I N).

PROCEDURE: Standard stock solution was prepared by dissolving 10 mg of Amprenavir in 1.5 mL of 10% Hydrochloric acid .The volume was made up to 10 mL with distilled water to get the primary working standard solution at the concentration of 1000 µg/mL. This was further diluted to get the working standard solution. 1 mL of the above solution is further diluted with distilled water up to 20 mL in volumetric flask to working standard solution.

ASSAY PROCEDURE

METHOD A: Aliquots of standard drug solution of Amprenavir 1 – 10 mL (50 µg/mL) were taken and transferred into series of 10 mL graduated test tubes. To each test tube 1mL of Folin–Ciocalteu reagent (FCR) reagent - 2%w/v and 0.5mL of Sodium biocarbonate (2N) were added. After thoroughly shaking, the test tubes were set aside for 10 minutes, for the reaction to complete. The volumes in each test tube were adjusted to 20 mL with methanol. The absorbance's of the solutions were measured at 715 nm against reagent blank, and the calibration curve was plotted. Similarly the absorbance of the sample solution was measured, and the amount of Amprenavir was by referring to the calibration curve.

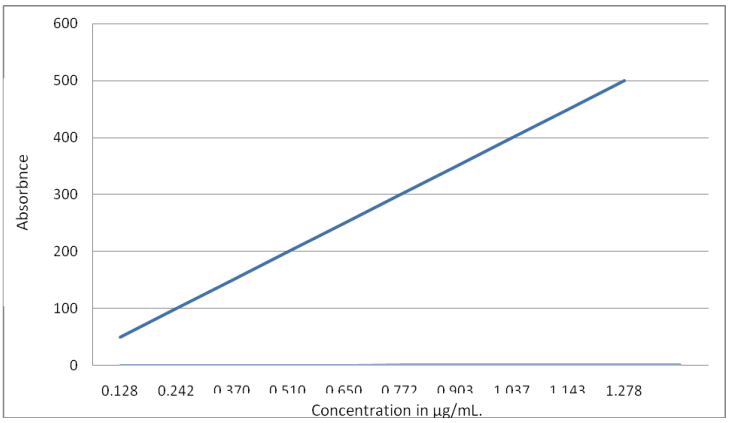


Figure 2: Calibration Curve of Amprenavir with FC-reagent and Sodium Bicarbonate.

METHOD B: Aliquots of Amprenavir solution (100µg/mL) ranging from 0.2 to 2.0 mL were transferred into a series of 10 mL volumetric flasks. To each flask 1.0 mL of p-Dimethylamino- cinnamaldehyde (PDAC) solution and 0.1 mL of sulphuric acid solution were added. After five minutes the volume was brought up to 10 mL with methanol to get the orange red coloured species. The absorbance's of the solutions were measured at optimized absorbance maxiama (505 nm) against reagent blank, and the calibration curve was plotted. Similarly the absorbance of the sample solution was measured, and the amount of Amprenavir was by referring to the calibration curve was measured at 505 nm against reagent blank. The amount of Amprenavir was computed from calibration curve.

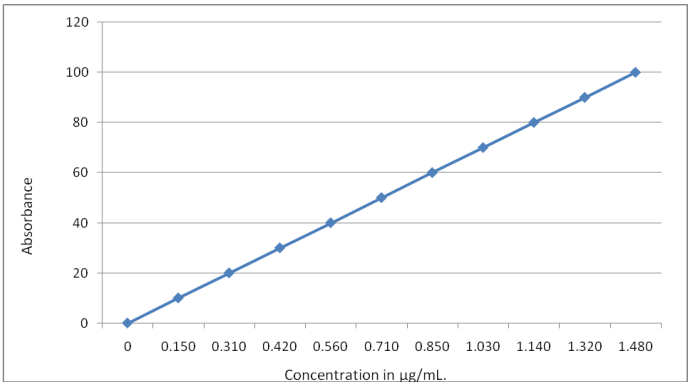


Figure 3: Calibration Curve of Amprenavir with PDAC-Sulfuric acid.

The methods were extended for the determination of Amprenavir from capsule formulations, (Agerenase® 50 mg capsules Glaxo smikline Inc). The total contents of 20 capsules were empied, weighed, blended uniformLy and powder equivalent to 10 mg was dissolved in 10 mL of distilled water in a volumetric flask to get the primary working sample solution. The resulting solution was further diluted by taking 1mL of primary standard up to 10 mL to get the 100 µg of working standard solution. The above solution was further diluted and analyzed as described, in the above mentioned methods. The analysis procedure was repeated three times with Tablet formulations and the results of analysis are shown in Table II.

Recovery Studies

To ensure the accuracy and reproducibility of the results obtained, adding known amounts of pure drug 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 II**.

RESULTS AND DISCUSSIONS

In the present study, the Method A involves quantitative reaction of the drug with FC-reagent in alkaline medium. The reaction is based on the condensation of Amprenavir with Folin–Ciocalteau reagent, in alkaline media thereby producing greenish blue colored chromogen with maximum absorbance of 715 nm. The Folin–Ciocalteu reagent which is a mixture of tungstates and molybdates works on the mechanism of oxidation–reduction reaction. The method strongly relies on the reduction of the mixture heteropolyphosphotungsates–molybdates by the phenolic compound which results in the formation of blue coloured chromogen. The phenolic compounds react with Folin–Ciocalteu reagent only under basic conditions adjusted by sodium carbonate solution. Under Basic conditions it has been observed that the phenolic compound undergoes dissociation to form a phenolate anion which reduces the Folin–Ciocalteu reagent i.e. the mixture of tungstates and molybdates rendering a blue coloured solution. The colour intensity of the formed blue chromogen can be measured by the absorbance readings using a spectrophotometer Stability study of the developed chromogen was carried out by measuring the absorbance values at time intervals 15 min for 3 hrs, and it was found to be stable for more than 3 hrs at room temperature. The linearity was found to be in the concentration range of 50-500 µg/mL. The most probable reaction mechanism involving the formation of colored species has shown in fig-2.

The Method B is based on the coupling reaction between amine group and PDAC in sulfuric acid. Certain amines condense with various aldehydes in strongly acidic medium able to give color. Among many, the following aldehydes are widely used. They are dimethylaminonzaldehyde, vanillin, formaldehyde, benzaldehyde, salicyaldehyde, paraldehyde, aminobenzaldehyde, m- and p-nitrobenzaldehyde, etc. The reaction with aromatic amines produces Schiff bases. The mechanism of aldehydes which condenses with the aromat condensation of the aldehydes to release the oxygen molecule and then it combines with the amine group to form the yellow Schiff's base in the presence of acidic medium such as sulfuric acid which forms a red colored complex with maximum absorbance of 505 nm. The linearity was found to be in the concentration range of 10-100 µg/mL. The colored species was stable for 3 hrs. The reaction mechanism has been shown in fig—3

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sand ell's sensitivity are presented in **Table I.** The regression analysis using the method of least squares was made for slope (m), intercept (b) and correlation obtained from different concentrations and the results are summarized in **Table I.**

The reproducibility and precision of the methods are very good as shown by the standrard values of %RSD. The Mean percentage recovery value of 98.4% for Method A, 98.4% for Method B, indicates non-interferences from the excipients present in the tablets. All the validated parameters are summarized in Table II.

In conclusion, the proposed methods are simple, sensitive, accurate and economical for the routine analysis of Amprenavir in bulk and in its formulations (Tablets).

Chemistry of the Colored Species

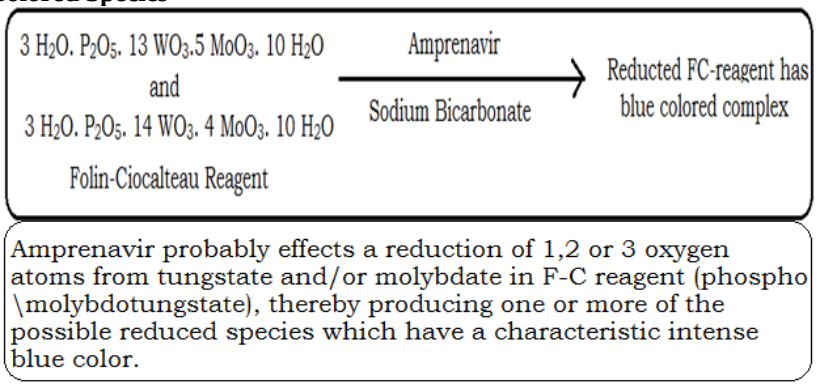


Fig. 4: Probable reaction mechanism involved in Method A:

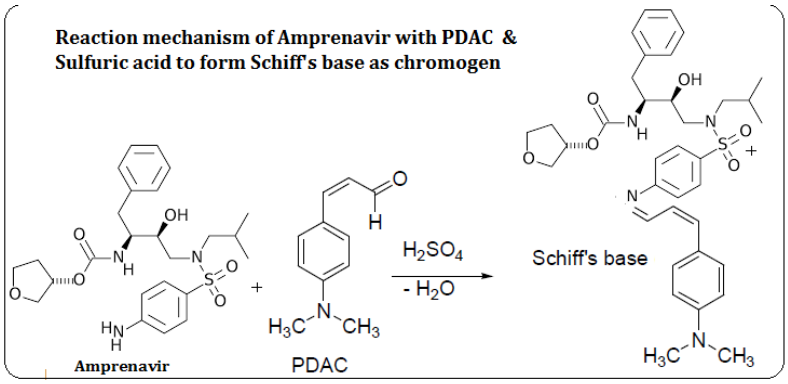


Fig. 5: Probable reaction mechanism involved in Method-B:

Table I: OPTICAL CHARACTERISTICS AND PRECISION DATA:

Parameters	Method A	Method B
λ_{max} (nm)	715	505
Beer's law limits	50-500	10-100
Molar absorptivity (l/mol.cm)	1.3 X 10^3	7.1 X 10^3
Sand ell's sensitivity (micrograms/cm^2/0.001 absorbance unit)	7.6	1.4
Regression Equation* (Y=mx+C) Slope (m) Intercept (c)	 0.002 2.6	 0.73 0.01
Correlation Coefficient(r)	1.000	0.999
Precision (%Relative Standard Deviation)	0.32	0.55

*Y=mx+c, where X is the concentration in micrograms/mL and Y is absorbance unit.

Table II: ASSAY OF AMPRENAVIR IN TABLET FORMULATIONS.

***Average of three determinations. ** After spiking the sampl**

S.No.	Label Claim(mg)	*Amount Obtained (in mg) by the proposed method.	**% Recovery by the proposed method.

		Method-A	Method-B	Method-A	Method-B
1	50	48.2	49.2	98.28	98.27
2	50	49.6	49.4	98.76	98.94
3	50	48.8	49.8	98.16	99.05

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