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SPECTROPHOTOMETRIC DETERMINATION OF AMPRENAVIR IN FORMULATION SAMPLE

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Abstract:

A validate rapid, economical and sensitive visible spectrophotometric method has been developed for quantitative determination of amprenavir in bulk drug and tablet samples.This method is validated for irinotecan with chromogenic reagent namely bromo cresol green (BCG) at λ_{max} 417. The calibration curve was linear over a concentration range of 5-40 µg/ml. The relative standard deviation (R.S.D) is less than 1% and average recovery was above 99.60%. Efficient visible spectrophotometric detection at λ_{max} 417nm enabled determination of the drug with no interference from tablet excipients.

The proposed method is fast, sensitive, precise, accurate and efficient and can be used for analysis in quality control laboratories.

Key Words: *Ultraviolet-Visible Spectrophotometry, amprenavir, bromocresol green(BCG), Phosphate buffer, ion association complex.*

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INTRODUCTION:

Amprenavir is chemically 3S-tetrahydro-3-furyl N-[(1S,2R)-3-(4-amino-N-isobutyl benzene sulfonamido)-1-benzyl-2-hydroxypropyl]carbamate. Amprenavir is a single stereoisomer with the (3S) (1S, 2R) configuration. It has a molecular formula of $C_{25}H_{35}N_3O_6S$ and a molecular weight of 505.64 g/mol (Figure 1). Amprenavir [AGENERASE®] is an inhibitor of the human immunodeficiency virus (HIV) protease [1,2].Amprenavir binds to the active site of HIV-1 protease and thereby prevents the processing of viral gag and gag-pol polyprotein precursors, resulting in the formation of immature non-infectious viral particles.

Amprenavir alone or in combination with other drugs is reported to be estimated by HPLC [3], LC-MS[4-7] and Spectrophotometry[8]. No visible spectrophotometric method for quantitative determination of amprenavir in bulk drug samples and formulations was reported. The present study describes simple, sensitive, accurate, rapid and economical spectrophotometric methods for the estimation of amprenavir in bulk samples & tablet dosage forms.

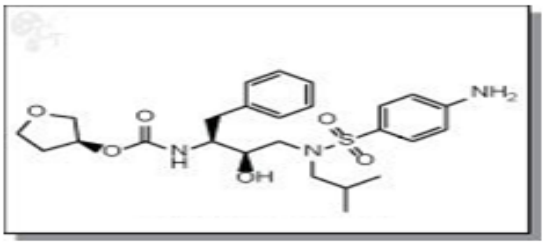


Fig1: Structure of Amprenavir

MATERIAL AND METHODS:

Instrument: Pharmaspec-1700 Ultraviolet-Visible spectrophotometer (double beam) was used for all spectral measurements. Digisun model DI-707 pH meter was used for all the pH measurements.

Materials: Amprenavir is obtained as gift sample from Mylan Laboratories. The reagents bromocresol green (BCG), chloroform, methanol, NaOH, H_3PO_4, NaH_2PO_4, Na_2HPO_4 used were of analytical grade and were used as they are purchased without any further purification.

Preparation of standard drug solution:

About 100 mg of amprenavir was accurately weighed, dissolved in 100 ml of distilled water to obtain a stock solution of 1 mg/ml.

Preparation of sample solution:

A quantity of the powder from capsules equivalent to 50 mg of drug was dissolved in 50 ml of distilled water and analyzed by taking an aliquot and treated as per the procedure for standard.

Methodology for bulk drug sample:

Aliquots of standard drug solution (0.5-4.0 ml) were added to 3 ml of phthalate buffer of pH 2.2[9] contained in a separating funnel followed by 1 ml of BCG [10] (0.1% w/v). The solution was extracted with chloroform and volume made upto 10 ml.

A linear graph was obtained at 417nm after the waiting period of 15 min, against reagent blank prepared simultaneously.

Methodology for formulation sample:

A quantity of the powder from capsules equivalent to 50 mg of drug was dissolved in 50 ml of distilled water. Sample solution was added to 3 ml of phthalate buffer of pH 2.2 contained in a separating funnel followed by 1 ml of BCG solution (0.1% w/v). The compound extracted with chloroform and volume made up to 10 ml.

The absorbance of resulting solution was measured at 417nm after the waiting period of 15 min, against reagent blank prepared simultaneously.

Accuracy and Recovery Studies

Commercially available tablets of amprenavir were analyzed by the proposed method and as additional check on the accuracy, recovery experiments were also conducted by spiking known amounts of pure drug in pre analyzed formulation. The percentage recovery was calculated in each of the case using the regression line equation developed under the linearity experiment. Assay results of the proposed methods were compared with that of reference method and statistically evaluated using one-way ANOVA with post-test followed by Dunnett multiple comparison test. The results showed that $P > 0.05$ and the means of the proposed methods are not significantly different from that of reference method. The assay and accuracy results were presented in Table 1. The interference studies indicated the common additives and excipients present in formulations did not interfere with the proposed method.

Table 1: Result of recovery studies (n=6)

Sample[a]	LabelledAmount(mg)	Amount obtained (mg)[b]		Percentage Recovery[b,c]
		Proposed method	Reference method	
T_1	50	99.95±0.68	99.88±0.25	99.75±0.31
T_2	50	99.96±0.45	99.77±0.47	99.65±0.15

a - T_1 and T_2 are the tablets from different batches.
b – Mean ± SD of 6 determinations.
c – 50 mg of pure drug was added and recovered.
For the sample T1 and T2 One-way ANOVA with post-test followed by Dunnett multiple comparison test was performed. The results showed that P > 0.05 and the means of the proposed methods are not significantly different from that of reference method.

Linearity

By using the method of least squares regression analysis was performed to evaluate the slope (m), intercept (b) and correlation coefficient (r) was computed from various concentrations .The graph showed negligible intercept as described by the regression equation y = mx + b where y is the absorbance and x is the concentration in µg/ml. Calibration curve is shown in Figures 2.The spectral analysis showed that λ_{max} of amprenavir is 417 nm. The calibration curve was obtained for a series of Beer's concentration 5-40µg/ml(Table 2).

Table 2: Values of optical and regression of amprenavir

Parameter	Values
λ_{max} (nm)	417
Beer's law range (µg/ml)	5-40
Molar extinction coefficient ($L.mole^{-1} cm^{-1}$)	4.34×10^4
Sandell's sensitivity(µg/cm^2/0.001)	0.039
Regression equation (y = mx + b) * Slope (m) Intercept (b)	0.0249 0.0035
Correlation coefficient (r)	0.9998
Precision (%Relative Standard Deviation)	0.06%

- Molar extinction coefficient(a) = A/(b*c)

A - Absorbance
b - Pathlength
c – Concentration

- Sandell's sensitivity calculated as per ICH guideline
- y=mx+c

Where, y is absorbance of of standard solution, x is concentration, m and c are slope and intercept of line respectively.

- Correlation coefficient (r)

$$r = \frac{\Sigma(x-\bar{x})(y-\bar{y})}{\sqrt{[\Sigma(x-\bar{x})^2(y-\bar{y})^2]}}$$

y - y-coordinate
$\bar{y}$ – mean of y values
x - x coordinate
$\bar{x}$ – mean of x values

- % Relative Standard Deviation - S*100/ $\bar{x}$

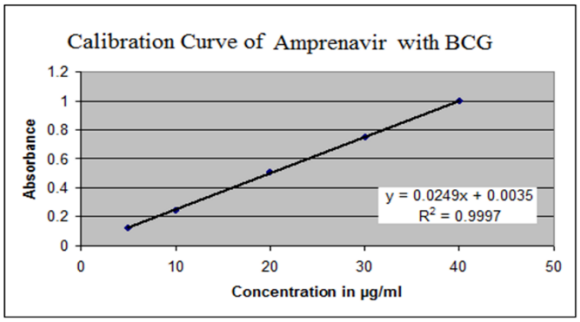


Fig 2: Calibration Curve of Amprenavir

Precision

The reproducibility of this method was evaluated by analysing the amprenavir sample of concentration 40µg/ml in six replicates.Intraday and interday precession studies were carried out and results tabulates in Table 3 and Table 4. The % RSD was proved satisfactory.

Summary of precession results showing repeatability

Table 3: Interday precession studies:

Concentration (µg/mL)	Absorbance
40	0.995
40	0.994
40	0.994
40	0.993
40	0.994
40	0.994
MEAN	0.994
SD	0.00063
% RSD	0.0636%

mean ($\bar{x}$) - $\frac{\text{Sum of six observations (X)}}{6\ (N)}$

Standard deviation (S) - $\sqrt{(\Sigma(x-\bar{x})^2 / N-1)}$ where x is absorbance

% RSD - S*100/ $\bar{x}$

Table 4: Intraday precession studies:

	Day 1	Day 2	Day 3	Avg. RSD
% RSD	0.0757%	0.0519%	0.0519%	0.0598%

Mean ($\bar{x}$) - $\frac{\text{Sum of six observations (X)}}{6\ (N)}$

Standard deviation (S) - $\sqrt{(\Sigma(x-\bar{x})^2 / N-1)}$ where x is absorbance

% RSD - S*100/ $\bar{x}$

Average %RSD – %RSD (Day 1)+%RSD(Day 2)+%RSD(Day 3)

3

RESULTS AND DISCUSSION:

In the present work a method have been developed for the estimation of amprenavir from tablet formulation, based on formation of colored complex with BCG. The drug showed absorption at λ_{max} 417nm over concentration range of 5-40µg/ml (Table 2). Regression equations of calibration curves are y=0.0249X+0.0035(R^2=0.9997). Interday and intraday precession studies were carried out and % RSD in found within limit (Table 3 and Table 4).

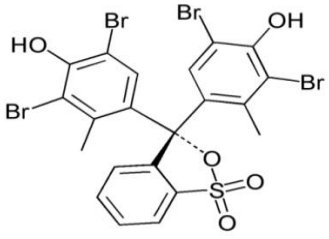
Optimization of parameters for proposed method:

The optimum conditions were established by changing one parameter while fixing the other parameters and noting the effect on absorbance of chromogen. This method has been developed for the estimation of amprenavir in tablet formulation, based on formation of colored complex with bromocresol green.

1ml of BCG (0.1% w/v) dye was added to the sample with manual stirring. Addition of less than 1ml of dye solution resulted in low absorbance particularly with high concentrations of Beer's law limits. Addition of more than 1 ml of the dye resulted in high blank value. The optimum pH required for complexation and efficiency of the solvent to extract the ion pair is 2.2,which is maintained by addition of 3ml phthalate buffer .The time taken for formation of complex is 15 minutes at a temperature of 29^{0}C.The stability of coloured complex is >40 minutes.

Mechanism of formation of colored species:

The amino group of amprenavir forms the ion-pair complexes with acidic bromocresol green dye. As amprenavir possesses secondary amine group that involves in ion association complex formation with acid dye BCG which is extractable into chloroform from the aqueous phase. The protonated nitrogen moiety (positive charge) of amprenavir is expected to attract the oppositely charged part (negative charge) of dye and behave as a single unit being held together by electrostatic attraction.



+ Amprenavir →

[Ion-Association complex]

Absorption Maximum

Absorption spectra of amprenavir by proposed method was shown in figure 3. The λ_{max} is 417 nm.

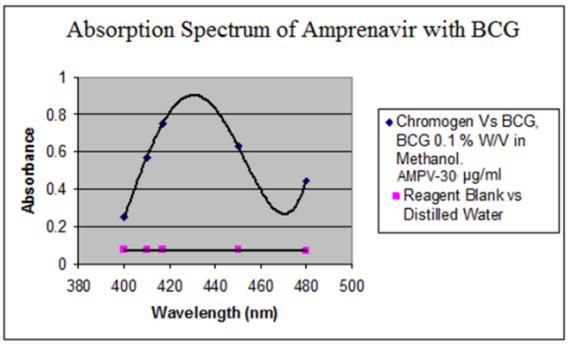


Fig 3: Absorption Spectrum of amprenavir with BCG

CONCLUSION:

The proposed visible spectrophotometric methods enable quantitative determination of amprenavir in bulk drug samples and formulations.. The calibration curves were linear over a concentration range of 5-40 µg/ml. The relative standard deviations (R.S.D) were less than 1% and average recovery was above 99.60%. Efficient visible spectrophotometric detection of the drug at 417nm absorption maxima enabled determination with no interference from tablet excipients.

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