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Research Article

Spectrophotometric Determination of Amprenavir by Complex Formation in Bulk Drug and Formulation Samples

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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 formulation samples. This method is validated for irinotecan with chromogenic reagent 3-methyl benzothiazolinone hydrazine (MBTH). The calibration curve was linear over Beer's concentration range of 25-350 µg/ml. The relative standard deviation is less than 1% and average recovery is above 99.80%. Efficient visible spectrophotometric detection at the respective absorption maxima enabled determination 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.

Keywords: Ultraviolet-VisibleSpectrophotometry,amprenavir,3-methyl benzothiazolinone hydrazine (MBTH).

INTRODUCTION

Amprenavir is chemically 3S-tetrahydro-3-furyl N-[(1S, 2R)-3-(4-amino-N-isobutyl benzene sulfonamido)-1-benzyl-2-hydroxy propyl] 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.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.

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 3-methyl benzothiazolinone hydrazine (MBTH) and ferric chloride 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 (0.25-3.5 ml) were transferred into series of 10ml graduated test tubes, 2ml of ferric chloride (0.3 % w/v) and ml of MBTH[9](0.2% w/v) were added to each test tube and made upto 10ml using distilled water.

The absorbance of resulting solution was measured at 569nm against reagent blank prepared simultaneously and a linear graph was obtained.

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.

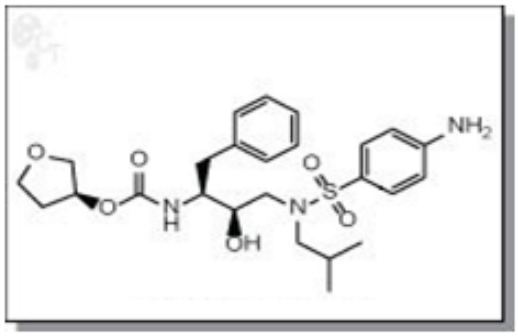

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Table 1: Result of recovery studies (n=6).

Sample[a]	Labelled Amount (mg)	Amount obtained (mg)[b]		Percentage Recovery[b,c]
		Proposed method	Reference method	
T_1	50	99.88±0.33	99.88±0.25	99.98±0.23
T_2	50	99.96±0.60	99.77±0.47	99.86±0.22

a - T1 and T2 are the tablets from different batches (Celonib, Celon)
b – Mean ± SD of 6 determinations
c – 50 mg of pure drug was added and recovered

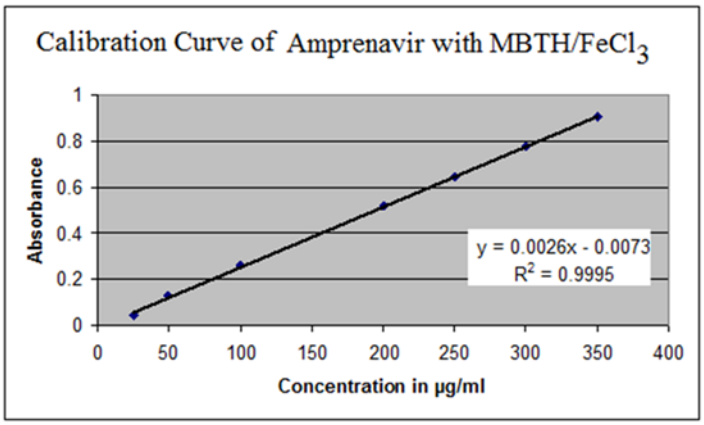

Figure 1: Calibration Curve of amprenavir.

Table 2: Values of optical and regression of amprenavir.

Parameter	Values
λ_{max} (nm)	569
Beer's law range (µg/ml)	25-350
Molar extinction coefficient ($L.mole^{-1} cm^{-1}$)	0.44×10^4
Sandell's sensitivity(µg/cm^2/0.001)	0.38
Regression equation (y = mx + b)	
Slope (m)	0.0026
Intercept (b)	-0.0073
Correlation coefficient (r)	0.9997
Precision (%Relative Standard Deviation)	0.09%

Table 3: Interday precision studies.

Concentration	Absorbance	Stastical analysis	
350	0.905		
350	0.904	MEAN	0.904
350	0.904	SD	0.000816
350	0.906	% RSD	0.09%
350	0.905		
350	0.904		

Sample solution was transferred into 10 ml graduated test tube, 2 ml of ferric chloride and 1 ml of MBTH were added to the test tube and made upto 10 ml with distilled water. The absorbance of resulting solution was measured at 569nm against reagent blank prepared simultaneously.

Accuracy

Commercially available tablets of amprenavir were analyzed by the proposed method and as additional check on the accuracy recovery experiments were also conducted. 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 metho 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 method 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.

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 for this method is shown in Figures 1. The spectral analysis showed the λ_{max} of amprenavir is 569 nm. The calibration curve was obtained for a series of Beer's concentration in the range of 25-350 µg/ml.

Precision

The reproductivity of this method was evaluated by analysing the amprenavir sample of concentration 350 µg/ml in six replicates. The % RSD calculated and proved satisfactory. Intraday precession studies were carried out by preparing drug solution of same concentration and analysing it at three different times of same day. The same procedure was followed for 3 different days to determine interday precession.

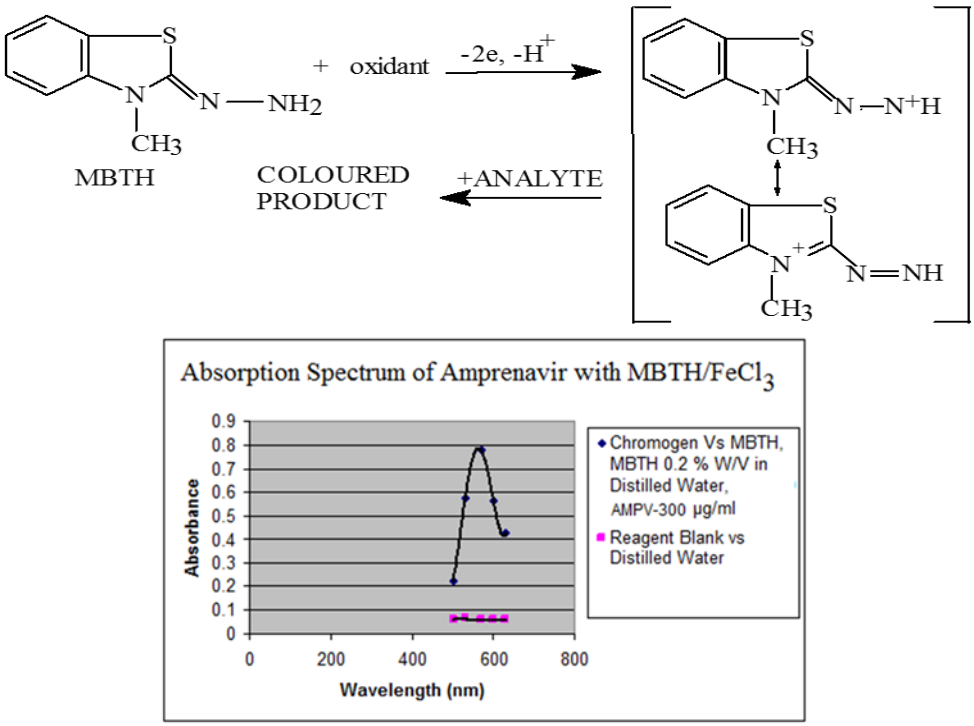

Figure 2: Absorption spectrum of amprenavir.

Table 4: Intraday precision studies.

Concentration	Day 1	Day 2	Day 3	Average RSD
350	0.905	0.905	0.905	
350	0.904	0.906	0.905	
350	0.905	0.905	0.904	
350	0.906	0.904	0.905	0.082%
350	0.906	0.904	0.905	
350	0.904	0.904	0.904	
% RDS	0.098%	0.09%	0.057%	

RESULTS AND DISCUSSION

Optimization of parameters

The optimum conditions were established by changing one parameter while fixing the other parameters and noting the effect on absorbance of chromogen. In the present work a simple method have been developed for the estimation of amprenavir from tablet formulation, based on formation of colored complexes with MBTH - $FeCl_3$.The conditions required for the formation of colored complexes were optimized.

2ml of $FeCl_3$ (0.3% w/v) is added to the sample followed by 1ml of MTBH (0.2 % w/v) and stirred manually. Addition of less than 2ml of $FeCl_3$ results in low absorbance particularly with high concentrations of Beer's law limits. The time taken for formation of colored complex is 5 minutes at temperature of 29^0C.The stability of coloured complex is >40 minutes.

Mechanism of formation of colored species

The amino group of amprenavir undergo oxidative coupling with MBTH in ferric chloride solution. Under the reaction conditions, MBTH loses two electrons and one proton in presence of $FeCl_3$ forming the electrophilic intermediate which has been postulated to be the active coupling species. The intermediate reacts with amine by electrophilic attack on the aromatic ring of the amine and the resulting intermediate is spontaneously oxidized with an oxidant to form the colored species via oxidative coupling mechanism.

Absorption Maxima

Absorption spectra of amprenavir is shown in Figure 2. The spectral analysis showed the λ_{max} of amprenavir.

CONCLUSION

The proposed visible spectrophotometric methods enable quantitative determination of amprenavir in bulk drug samples and formulations. Efficient visible spectrophotometric detection at the respective absorption maxima enabled determination with no interference from tablet excipients. The calibration curve is linear over a concentration range from 25-350 µg/ml. The relative standard deviation (R.S.D.) is less than 1% and average recovery was above 99.80%. The proposed method is fast, sensitive, precise, accurate and efficient and can be used for analysis in quality control laboratories.

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