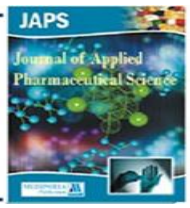
Journal of Applied Pharmaceutical Science
Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 24-12-2011
Revised on: 09:01:2012
Accepted on: 18-01-2012

Analytical Method Development and Validation of Anti-HIV Drug Abacavir Sulphate

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ABSTRACT

Analytical method development and validation was useful for estimation of drugs in bulk and biological fluids. They help to improve the reliability, consistency and accuracy of analytical data. Present investigation involves development and validation of UV spectroscopic method for abacavir sulphate as per ICH guidelines. The present work describes sensitive and robust method development of abacavir sulphate by UV spectroscopic method for estimation of abacavir sulphate in bulk and pharmaceutical dosage forms using UV-1700 pharma spec (Shimadzu). The method was validated for accuracy, precision, linearity, ruggedness and robustness to check its consistency. The wavelength scan of UV spectroscopic method showed absorption maxima 249 nm obeying beers law with linearity range of 0-40µg/ml and correlation coefficient of 0.99939. Molar absorptivity and sandell's sensitivity are found to be 1.2404472 $\times 10^{-4}$ and 0.0208 respectively. The accuracy was found to be 99.94%-100.2% with recovery 99.76%, 100.06%, 100.07% for 50%, 100%, 150% solutions. The obtained results indicated that the developed analytical method was sensitive, accurate, with low standard deviation values for all validation parameters and could be used in day to day regular analysis of abacavir sulphate in bulk and pharmaceutical formulations.

Keywords: Abacavir sulphate, UV Spectroscopic method, Validation, Linearity, Accuracy.

INTRODUCTION

Analytical method development involves initial study of system suitability and specificity parameters study for standard and sample analytes. Abacavir sulphate (ABC) is a nucleoside reverse transcriptase inhibitor with half life 1.54 ± 0.63 h used to treat HIV and AIDS with molecular formula $C_{14}H_{18}N_6O$ H_2SO_4 and is soluble in methanol, Acetonitrile and water. In previous literature studied the UV spectroscopic method development and validation parameters for the present work revealed that (Pant et al., 2009) developed colorimetric method for determination of acyclovir at 400 nm. (Devyani et al., 2009) developed a spectrophotometric method for simultaneous estimation of lamivudine and silymarin using 270.9 nm and 326.4 nm as two analytical wavelengths. Both the drugs obey Beer's law in the concentration ranges employed for this method. (Gnana babu et al., 2009) developed two simple and sensitive spectrophotometric methods for the estimation of lamivudine in both pure and tablet dosage form based on the condensation reaction to form yellow chromogen at 476 nm and 474 nm respectively. Beer's law is valid over 2-10 µg/ml. This method was validated for precision, accuracy, ruggedness and robustness.

(Preethi et al., 2006) developed a simple, sensitive, rapid, accurate and precise spectrophotometric method for estimation of acyclovir in bulk and pharmaceutical dosage forms. Acyclovir shows maximum absorbance at 253nm with beer's concentration range of 2-20 µg/ml. The literature revealed that there is very few or merely no method reported for abacavir estimation by UV method directly.Hence with this rationale the present study undertaken for development and validation of spectroscopic method of abacavir sulphate for system suitability parameters, specificity followed by validation for accuracy, linearity, precision, ruggedness and robustness for the proposed drug candidate estimation in bulk and dosage forms.

MATERIALS AND METHODS

Abacavir sulphate API and tablets were procured from Hetero labs Pvt Ltd., Hyderabad, A.P. India. Methanol and o-phosphoric acid were obtained from Merck, Bangalore. All other Polymers, chemicals and solvent ethanol used in the investigation were of AR grade. UV – 1700 of Shimadzu spectrophotometer model was used for analytical studies.

UV spectroscopic method development of abacavir sulphate

(Shalini S et al., 2009, Basavaiah K et al., 2007, Verweijvan WC et al., 2005).

Preparation of Abacavir sulphate standard stock solution (1000ppm)

Weigh accurately about 100 mg of Abacavir sulphate working standard and transfer to a 100 ml volumetric flask. Add 75 ml of methanol, shake for 5 minutes to dissolve and make up the volume with 25 ml 1% ortho phosphoric acid.

Preparation of Abacavir sulphate sample stock solution (1000ppm)

10 tablets of Abacavir sulphate (300 mg) are taken and average weight is considered, tablets are powdered and weight equivalent to 100 mg of Abacavir sulphate i.e 266 mg Abacavir sulphate tablet powder was accurately weighed and transferred into a 100 ml volumetric flask. Add 75 ml of methanol, shake for 5 minutes to dissolve and make up the volume with 25 ml 1% ortho phosphoric acid.

Solvent composition

Methanol and 1% ortho phosphoric acid in ratio 75:25.

Determination of absorption maxima of Abacavir sulphate in selected solvent blend

From the standard stock solution, different aliquots were taken and diluted to 10 ml mark with solvent to obtain series of concentrations. The solutions were scanned on spectrophotometer in the UV range of 200-380 nm and find out the absorption maxima.

Selection of optimum pH for maximum absorbance

Absorbance's of different concentrations of standard solutions were recorded at different pH ranges and at pH were maximum absorbance is observed is selected for method.

Validation of proposed UV method of abacavir sulphate

(Devmurari VP et al., 2010, Rolf WS et al., 2001, Veldkampa AI et al., 1999).

Determination of linearity range and calibration curve for Abacavir sulphate

Standard solutions of Abacavir sulphate in the concentration range of 1-100 µg / ml were prepared and absorbance was measured at 249 nm taking solvent blend as a blank to determine the linearity range.

Appropriate aliquots from standard Abacavir sulphate stock solutions were transferred to series of 10 ml volumetric flasks. The volume was adjusted to the mark with solvent blend pH 5.2 to obtain concentrations of 5, 10, 15, 20, 25, 30, 35 and 40µg/ml. Absorbance spectra of each solution against respective solvent blend as blank were measured at 249 nm and concentration vs absorbance was plotted and interpreted statistically.

Accuracy

The accuracy was evaluated applying the proposed method to the analysis formulations with known amounts of drug. The accuracy was calculated as the percentage of the drug recovered from the formulations.

Preparation of standard Abacavir sulphate solution

Transfer accurately weighed about 50 mg of Abacavir sulphate working standard to a 50 ml volumetric flask, add about 40 ml of solvent blend to dissolve, dilute up to the mark with solvent blend and mix. Aliquots of stock solution were further diluted with solvent blend to get desired concentrations.

Preparation of Abacavir sulphate sample solution

Weigh accurately 5 tablets and grind in a mortar and transfer equivalent to 50 mg of Abacavir sulphate into a 50 ml volumetric flask, add 40 ml of solvent blend and shake to dissolve. Dilute to volume with solvent blend mix the contents and filter through 0.45 µm membrane filter.

Transfer aliquots of the filtrate to 25ml volumetric flask and dilute to volume with the solvent blend to get desired concentration.

Procedure

Recovery studies were carried out by adding known amount of standard drug (10µg/ml as 50%, 20µg/ml as 100% and 30µg/ml as 150%) to the solvent blend measure the absorbance and calculate the amount from the calibration curve. The % recovery was calculated in terms of % RSD and it should be less than 2%.

Precision

The precision was determined by repeatability (intra-day) and intermediate precision (inter day). Repeatability was evaluated assaying 3 determinations at the same concentration (20 µg/ml), during the same day, under the same experimental conditions. Intermediate precision was analyzed comparing the assays in 3 determinations at the same concentration (20 µg/ml) during 3 different days. Precision (repeatability and intermediate precision) was expressed as % relative standard deviation (RSD).

Preparation of Abacavir sulphate sample solution

Weigh accurately 5 tablets and grind in a mortar and transfer equivalent to 50 mg of Abacavir sulphate into a 50 ml volumetric flask, add 40 ml of solvent blend and shake to dissolve. Dilute to volume with solvent blend mix the contents and filter through 0.45 µm membrane filter. Transfer aliquots of the filtrate to 25ml volumetric flask and dilute to volume with the solvent blend to get desired concentration.

Procedure

Intraday precision was determined by analyzing abacavir sulphate for three times in the same day (morning, afternoon, evening) at respective absorption maxima using respective solvent blends. Inter day precision was determined by analyzing daily once (morning) for three days at respective absorption maxima using respective solvent blends. The % RSD values were calculated and it should be less than 2%.

Robustness

Robustness of the proposed methods was determined by the analysis of samples and standard solutions (20µg/ml) at different wavelength (251nm), at different ratios of solvent blend. Appropriate concentrations of Abacavir sulphate from bulk and formulations were prepared in respective solvent blends. Analysis was carried out at a different wavelength (251nm). Amount found was calculated at three different wavelengths in terms of % RSD and values should be less than 2%.

Ruggedness

Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from analyst to analyst and instrument to instrument. Appropriate concentrations of Abacavir sulphate from bulk and formulations were prepared in respective solvent blend. Analysis was carried out through two different instruments.

Solution stability

Check the stability of drug substance in solution form at different time intervals.

Measure the absorbance initially at 0 min and after specified time intervals i.e. every15 min time interval and up to 120 min.

RESULTS AND DISCUSSION

Simple, rapid and reproducible UV spectroscopic method was developed and validated as per ICH guideline for an antiretroviral drug viz., Abacavir sulphate. The developed method was further validated for accuracy, precision, specificity, robustness, and ruggedness with statistical data. The λ_{max} with characteristic peak for Abacavir sulphate in solvent phase methanol: 1%ortho phosphoric acid (75:25) at 249 nm was observed and was showed in figure 2.

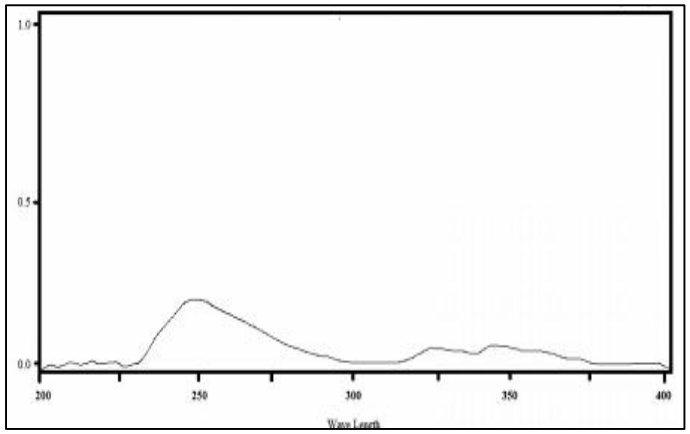


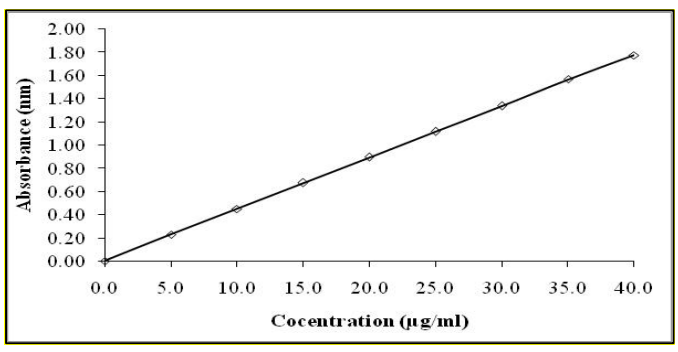
Fig 2: UV Absorption maxima of Abacavir sulphate.

The linearity range for Abacavir sulphate was studied at respective absorption maxima in the concentration range of 0-40µg/ml. The beer`s range was found to be 0-40 µg/ml with correlation coefficient of 0.99939. The linearity range data and graph was showed in table 1 and figure 3.

Table 01: UV Linearity studies of Abacavir sulphate:

Conc. (µg/ml)	Absorbance
0	0
5	0.229
10	0.449
15	0.673
20	0.893
25	1.114
30	1.333
35	1.564
40	1.772

Figure 03: Calibration curve for Abacavir sulphate:



The calibration curve for Abacavir sulphate in the concentration range of 0-40 µg/ml and are shown with high correlation coefficient in the concentration range of 0-40 µg/ml; Molar absorptivity was found to be 1.2404472 $\times 10^{-4}$; Sandell's sensitivity was found to be 0.0208; Best fit value slope was found to be 0.04434 ± 0.0001153. The P value is < 0.0001 indicate proposed method is statistically significant data was showed in table 2.

Table 2: UV Statistical data of calibration curve for Abacavir sulphate.

λ_{max}(nm)	249
Beer's law limits (µg / ml)	0-40
Molar Absorptivity ($mol^{-1}cm^{-1}$)	1.2404472 $\times 10^{-4}$
Sandell's sensitivity	0.0208
Best-fit values	
Slope	0.04434 ± 0.0001153
Y-intercept when X=0.0	-0.001400 to 0.01158
X-intercept when Y=0.0	-0.2625 to 0.03141
1/slope	22.55
95% Confidence Intervals	
Slope	0.04407 to 0.04461
Y-intercept when X=0.0	-0.001400 to 0.01158
X-intercept when Y=0.0	-0.2625 to 0.03141
Goodness of Fit	
R square	0.999976
P value	< 0.0001

The accuracy was found to be 99.94 % -100.2% in proposed method for the estimation of Abacavir sulphate in bulk. The % recovery of Abacavir sulphate in formulation was found to be 99.76%, 100.06%, 100.07% respectively for 50%, 100%, 150% solutions, where as 20 µg/ml was taken as 100% and 10 µg/ml taken as 50% and 30 µg/ml as 150%. The results suggest that proposed methods were accurate in estimation and data was showed in tables 03. The % RSD values of intraday and inter day precision for Abacavir sulphate were found to be 1.4% and 0.23% which were within the acceptance limits.

Table 03: Accuracy and recovery studies of Abacavir sulphate.

	Sample concn (PPM)	at % recovery	Absorbance				% of recovery
			1	2	3	Avg	
Standard	10	50	0.412	0.411	0.414	0.412	99.94
	20	100	0.892	0.891	0.892	0.891	100.22
	30	150	1.358	1.357	1.355	1.356	100.02
Sample	10	50	0.420	0.421	0.420	0.420	99.76
	20	100	0.893	0.893	0.895	0.8936	100.06
	30	150	1.356	1.354	1.355	1.355	100.07

The results suggested that the proposed method was precise and reproducible for the estimation. The data was showed in table 04.

Change in the λ_{max} from the actual in robustness analysis the % recovery of Abacavir sulphate was found to be 99.06. At 249 nm the % RSD is 0.23% and at 251 nm %RSD is 0.13%, similarly with the change in pH 5.2 to 5.4 the % RSD was found to be 0.17% and with change in solvent phase ratio % RSD was found to be 0.23%.Results significantly indicate proposed method is robust and the data was showed in table 05.

The proposed method also showed ruggedness as the %RSD was found to be 0.13% and 0.18% respectively, when the method was performed on two different systems and the data was showed in table 06.

There was no significant change during solution stability studies in absorbance up to 120 min at room temperature, indicating that the solvent blends used in the analysis were stable. Absorbences were recorded for both standard and sample solutions up to 120 min. The data was showed in table 07.

The above results indicated that the developed and validated UV spectroscopic method was found to be economic, accurate, precise, reproducible and could be used for analysis of abacavir sulphate in bulk and pharmaceutical dosage forms.

Table 04: Intraday and Inter day Precision studies of abacavir.

	Absorbance (n=6)						Mean	SD	%RSD
Intra day	0.893	0.894	0.861	0.892	0.893	0.895	0.888	0.0132	1.40%
Inter day	0.886	0.885	0.884	0.881	0.887	0.885	0.8846	0.0021	0.23%

Table 05: Robustness studies of Abacavir sulphate.

	Condition	S.no	Parameter	Absorbance	Mean	Std dev	%RSD
Standard	Change in wave length	1	251	0.883	0.88233	0.00115	0.13%
		2	251	0.881			
		3	251	0.883			
	Change in pH	1	5.4	0.863	0.86433	0.00153	0.17%
		2	5.4	0.864			
		3	5.4	0.866			
	Change in solvent ratio	1	Methanol:1% OPA 80:20	0.863	0.863	0.002	0.23%
		2		0.864			
		3		0.866			
Sample	Change in wave length	1	251	0.885	0.88467	0.00058	0.06%
		2	251	0.885			
		3	251	0.884			
	Change in pH	1	5.4	0.883	0.88267	0.00058	0.06%
		2	5.4	0.882			
		3	5.4	0.883			
	Change in solvent ratio	1	Methanol:1% OPA 80:20	0.879	0.87833	0.00115	0.13%

Table 06: Ruggedness studies of Abacavir sulphate.

	Trials	Absorbance	Mean	SD	% RSD
System-1	1	0.892	0.8915	0.00122	0.13%
	2	0.890			
	3	0.890			
	4	0.893			
	5	0.892			
	6	0.892			
System-2	1	0.890	0.89033	0.00163	0.18%
	2	0.889			
	3	0.888			
	4	0.892			
	5	0.891			
	6	0.892			

Table 07: Abacavir sulphate standard and sample solution stability studies.

	Time(Min)	Absorbance	Mean	SD	%RSD
Standard	15	0.892	0.8865	0.00648	0.73%
	30	0.891			
	45	0.891			
	60	0.889			
	75	0.887			
	90	0.886			
	120	0.884			
	145	0.872			
Sample	15	0.892	0.88088	0.01537	1.74%
	30	0.892			
	45	0.891			
	60	0.891			
	75	0.890			
	90	0.873			
	120	0.866			
	145	0.852			

CONCLUSION

The results obtained in this study demonstrate that the UV method described in the protocol was valid for the determination and assay of Abacavir sulphate. Therefore the method is suitable for its intended use. The present study described a highly sensitive, accurate and reproducible UV method for determination of Abacavir sulphate. This method involves simple, rapid and inexpensive sample preparation method. The very low quantification limit obtained with a UV detector helped to avoid use of fluorimetric detection, which demands more expensive equipments.

UV detectors give more reproducible and stable responses than fluorimetric detectors.Hence it could be concluded that proposed method was successfully applied for the analysis of marketed tablets and also used for the routine analysis of abacavir sulphate in bulk and formulation using UV method.

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