

Development and Validation of RP-UPLC Method for Simultaneous Estimation of Abacavir Sulphate and Lamivudine in Combined Tablet Dosage Form

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Abstract: A novel stability-indicating Ultra high-performance liquid chromatography (UPLC) method has been developed and validated for the simultaneous estimation of abacavir sulphate and lamivudine in the capsule dosage form. Chromatographic separations were carried using Acquity BEH C8 (100 mm x 2.1 mm) 1.7 μm column) with a mobile phase composition of triethylamine phosphate buffer (pH 2.5) and methanol in the ratio 50:50% (V/V) have been delivered at a flow rate of 0.5 mL min^{-1} and the detection was carried out using UV detector at wavelength 230 nm. The retention time for abacavir sulphate and lamivudine were 0.83 and 1.62 minute respectively. The correlation coefficient values in linearity were found to be 0.9999 for both at concentration range 2.509-50.190 $\mu\text{g mL}^{-1}$ and 20.093 - 401.860 $\mu\text{g mL}^{-1}$ respectively. The recovery results were found in the range from 99.47- 101.08%. The results of study showed that the proposed RP-UPLC method is a simple, accurate, precise, rugged, specific, robust, ultra fast and reproducible, which may be useful for the routine estimation of abacavir sulphate and lamivudine in pharmaceutical dosage form.

Keywords: Abacavir sulphate, lamivudine, RP-UPLC, Simultaneous estimation.

INTRODUCTION

Abacavir is chemically [(1R)-4-[2-amino-6-(cyclopropylamino) purin-9-yl]-1-cyclopent-2-enyl] methanol (Fig. 1). It is a white crystalline powder used as antiretroviral agents, for the treatment of HIV infection. It has an empirical formula of $\text{C}_{14}\text{H}_{18}\text{N}_6\text{O}$ and molecular weight of 286.3323. Abacavir belongs to a class of antiretroviral drugs known as nucleoside reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1)¹. Literature survey reveals that very few analytical

methods has been established for the determination of abacavir viz. abacavir, lamivudine and zidovudine in Pharmaceutical Tablets, Human Serum and in Drug Dissolution Studies by HPLC², Hypersensitivity reaction to abacavir is strongly associated with the presence of the HLA-B 5701 allele³, Simple and Reliable HPLC Method of Abacavir Determination in Pharmaceuticals, Human Serum and Drug Dissolution Studies from Tablets⁴, Spectrophotometric determination of abacavir sulphate⁵, HPTLC method for simultaneous determination of Lamivudine and Abacavir Sulphate in tablet dosage form⁶ were reported.

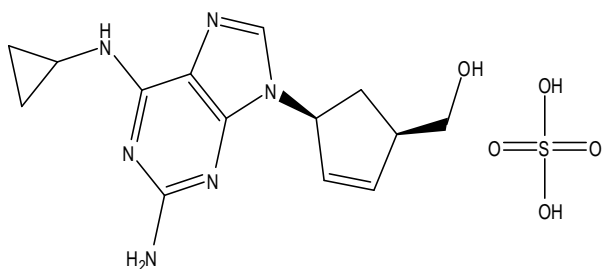


Fig. 1: Chemical structure of Abacavir Sulphate

Lamivudine is a synthetic nucleoside analogue with activity against HIV-1 and HBV^{7, 8}. The chemical name of lamivudine is (2R, cis)-4- amino-1-(2-hydroxymethyl-1, 3-oxathiolan-5-yl) - (1 H)-pyrimidin-2- one. Lamivudine is the (-) enantiomer of a dideoxy analogue of cytidine. Lamivudine has, also been referred to as (-) 2', 3'- dideoxy, 3'-thiacytidine. It has a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight of 229.3. It has the structural formula (Figure 2). Lamivudine is a white to off-white crystalline solid with a solubility of approximately 70 mg/ml in water at 20°C⁹. The drug is officially listed in Martindale, the Extra Pharmacopoeia¹⁰. Several analytical methods that have been reported for the estimation of Lamivudine in biological fluids or pharmaceutical formulations include high performance liquid chromatography, titrimetry and UV-visible spectrophotometer¹¹⁻²¹.

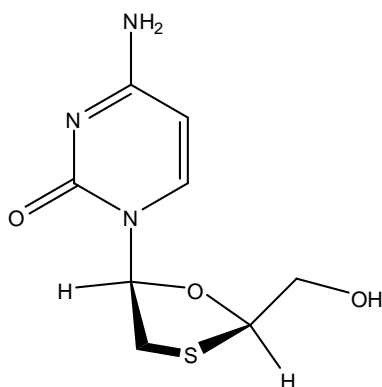


Fig. 2: Chemical structure of Lamivudine

MATERIALS AND METHODS

Instrumentation

An Ultra High performance liquid chromatography (UPLC) system consisted of Waters Acquity with PDA detector and data-handling system Empower Pro and all pH measurements were performed on a pH meter (Metrohm, model 654 Herisau).

Reagents and chemicals

Abacavir sulphate and Lamivudine were obtained as pure standards, samples (Capsules containing

Abacavir sulphate and Lamivudine in the ratio of 25mg; 200 mg respectively) from wockhardt Research Center, Aurangabad, India. HPLC grade solvents, Methanol, Triethylamine, Orthophosphoric acid, trifluoroacetic acid was from Merck-Specialties private Ltd., India. Water was deionised and further purified by means of a Milli-Q Plus water purification system, Millipore Ltd (U.S.A).

Chromatographic conditions and measurement procedure

The mobile phase consisted of a mixed and degassed solution of triethylamine phosphate buffer and methanol in the ratio of 50:50 (v/v). The buffer solution was prepared by adding 1 mL Triethyl amine in 1000 mL of water mixed and adjusted to pH 2.5 with orthophosphoric acid, filtered through 0.45µm or finer porosity membrane filter. The peak separations were achieved on Acquity BEH C8 (100 mm x 2.1 mm) 1.7 µm column. Maintained column oven temperature at 400C and detection with PDA detector at 230 nm, the injection volume was 1.0µl. flow rate of mobile phase was 0.5 mL min⁻¹.

Preparation of diluent1

2 ml of Trifluoroacetic acid was added in 1000 ml of Water and mixed well.

Preparation of diluent2

The suitable Buffer pH 2.5 and Methanol was mixed in the ratio 50: 50 (v/v).

Preparation of standard solution

Accurately weighed 25 mg of Abacavir sulphate working standard and 200 mg of Lamivudine working standard was added into 100 ml volumetric flask then about 70 ml of diluent-1 added and sonicated to dissolve and diluted to volume with diluent-1 and mixed well. Transferred 5 ml of standard stock solution into 50 ml volumetric flask and diluted to volume with diluent-2 and mixed well. (To get a final concentration of 25 µg mL⁻¹ and 200 µg mL⁻¹ of Abacavir sulphate and Lamivudine respectively) filtered through 0.45 µm membrane filter.

Preparation of sample

Average net content of 10 capsules was determined. Accurately transferred quantitatively the whole content of 10 sample capsules carefully (Equivalent to 250 mg of Abacavir sulphate and 2000 mg of Lamivudine) in 250 ml volumetric flask and added about 200 ml of diluent-1 and sonicated for about 30 minutes with intermittent shaking volume made with

diluent-1 and mixed well. Further diluted 5ml of this solution into 50 ml volumetric flask and diluted the volume with diluent-2 and mixed well. Further diluted 5 ml of this solution into 20 ml with diluent-2 and mixed well. Filtered through 0.45 μm nylon membrane filter by discarding first few mL filtrates.

RESULTS AND DISCUSSIONS

Selection of wavelength maximum

Abacavir sulphate showed two absorbance maxima at 226.3nm (-1) and 275.3nm (-2) where as Lamivudine showed two absorbance maxima at 231.2nm (-1) and 283.9nm (-2). Simultaneous estimation of both Abacavir sulphate and Lamivudine a common absorption point was selected as wavelength maxima at 230 nm.

Method development

During method development and optimization of chromatographic separation of major three component peaks were critical, two active ingredients Abacavir sulphate and lamivudine and salicylic acid a main degradation impurity of Abacavir sulphate. The pH of buffer was tried as acetate buffer 4.4 to phosphate buffer 2.5 in various combinations of methanol. Each trial mixture of

known components were injected and observed resolution and tailing factor of peaks. Addition of triethyl amine in buffer showed improved peak symmetry and resolution. Abacavir sulphate degraded rapidly in water: methanol and 2% aqueous acetic acid: methanol solutions. Both Abacavir sulphate and lamivudine were found to be soluble and stable in a mixture of 2% aqueous trifluoroacetic solution and methanol. Lamivudine is highly sensitive to mobile phase composition. Finally mobile phase composition was optimized to 50:50 v/v as it was found that both peaks were well resolved, Resolution 12.60, USP tailing 1.30 and 1.54, K prime value 1.23 and 1.72 for Abacavir sulphate and lamivudine respectively. The RT of Abacavir sulphate was found to be 0.83 min. and for Lamivudine 1.62 min. chromatogram shown in Fig. 3.

System suitability

System suitability parameters such as no. theoretical plates, peak tailing and K prime value were determined. The results obtained are shown in Table-1.

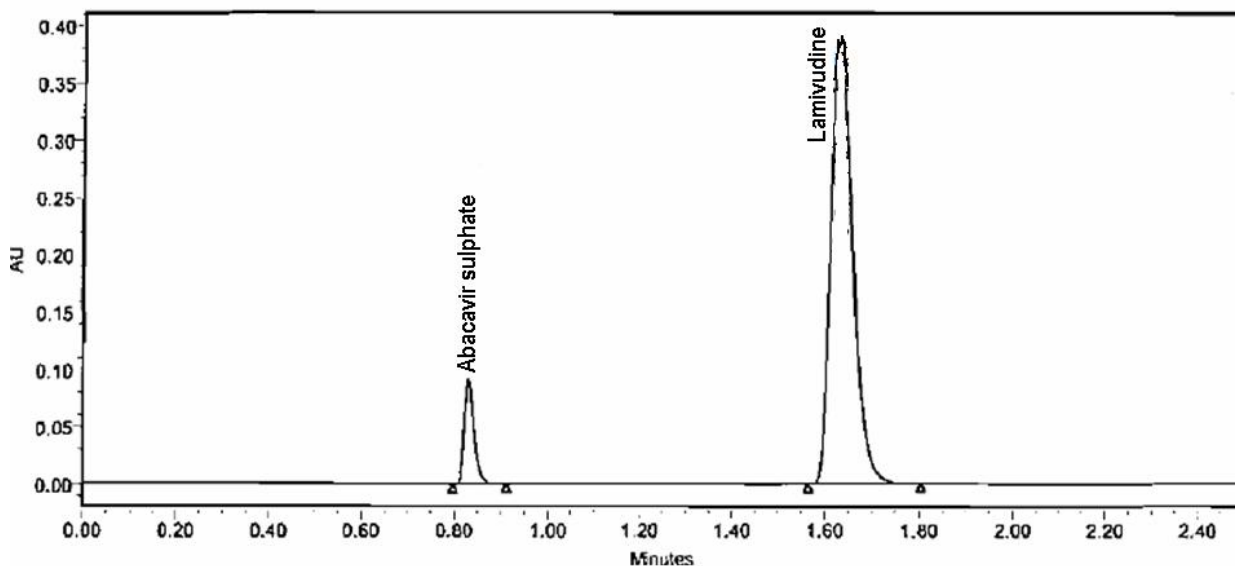


Fig. 3: Representative chromatogram of Abacavir sulphate and lamivudine

Table 1: Results of system suitability

Serial No	Parameters	Abacavir sulphate	Lamivudine
1	No. of theoretical plates	7340	5994
2	Tailing factor	1.30	1.54
3	K prime	1.23	1.72

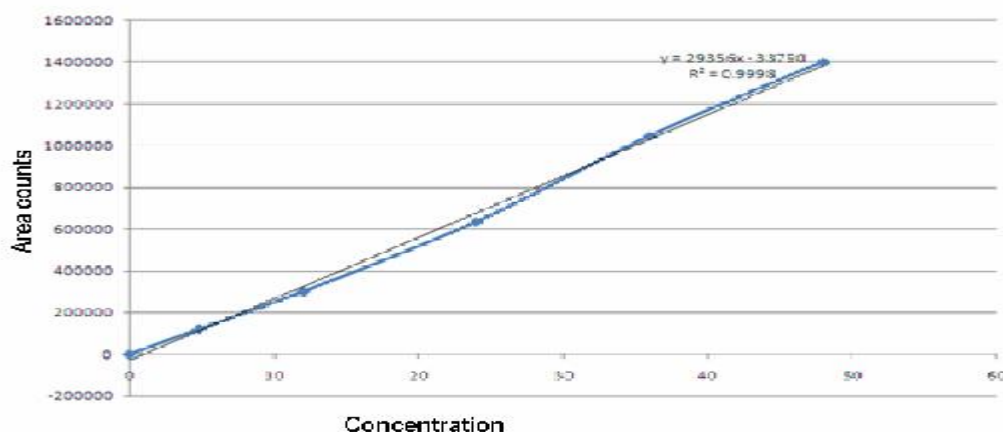
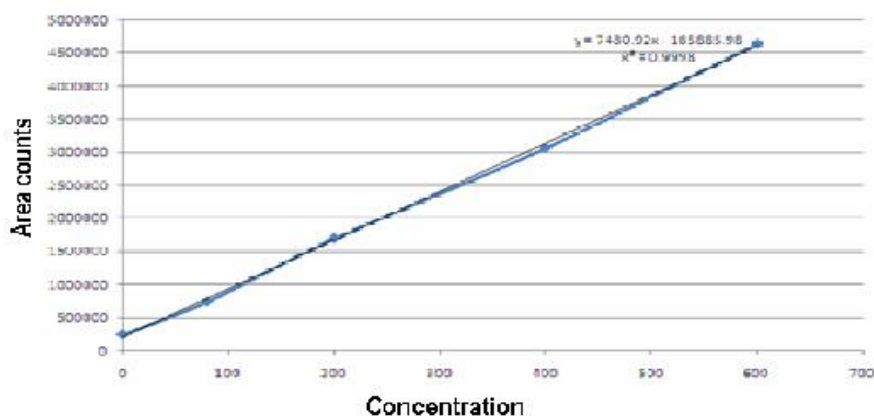
Method validation**Specificity**

Specificity of analytical assay method carried out by analyzing blank, placebo and sample solution spiked with known impurities at 1 % level in triplicate with two injections of each. The % assay difference is < 2.

Linearity

The linearity of this method for assay determination was carried out by analyzing in the range from about

50 % to 150 % of test concentration. Peak responses of the components on Y-axis and the corresponding concentrations on X- axis were drawn and the correlation coefficient (r) estimated. The linearity study showed that the calibration curve for Abacavir sulphate and Lamivudine was found to be linear with correlation coefficient (r^2) values 0.99998 and 0.99998 respectively. Linearity plot of Abacavir sulphate and Lamivudine were shown in Fig. 4 and Fig. 5 respectively.

**Fig. 4 Linearity of Abacavir sulphate****Fig. 5 Linearity of Lamivudine**

Accuracy

The accuracy study of assay was performed with known amount of drug substance (API) was spiked in placebo at about 50 %, 100 % & 150 % of test concentration in triplicate at each level and was injected each level in duplicate. Amount of drug recovered was quantified and % recovery was calculated from amount found and actual amount added. The accuracy study of this method for estimation of percent assay of Abacavir sulphate and Lamivudine in capsule dosage was found to be in the range of 99.47 % - 101.08 % as shown in Table-2.

Precision

The method precision study showed that the results of percent assay in six different samples preparations of same sample were within limits (%RSD < 2) as shown in Table-3. The Intermediate Precision study was performed within laboratory variation by different analysts, on different days, different instruments, and different column by using different standard and sample solution of the same sample as specified in method precision and the results were

compared with method precision. The ruggedness study showed that it passes the limits (%RSD < 2).

Repeatability

Injecting six replicate injections of standard solution from the same vial performed the system precision study; the results found were within limits (%RSD < 2).

Robustness

As per ICH guidelines small but deliberate changes have been made in parameters. The Robustness study for proposed analytical method for the determination of assay was performed and checked by preparing sample solutions in triplicate as per test method and was injected in duplicate by varying the organic phase/least component composition by ± 2 % (absolute) or 10 % relative which is lower, pH of the mobile phase by ± 0.1 unit, column oven temperature by ± 5 %, flow rate by ± 10 % and wavelength of the detector by ± 5 nm, the results showed that the percent assay of Abacavir sulphate and Lamivudine was not more than 2% as compared to method precision results, hence the developed method was found to be robust.

Table 2: Results of accuracy (recovery)

	Spiked level %, n=3)	Amount added (mg)	Amount recovered (mg)	Amount recovered (mg)
Abacavir Sulphate	50	12.5	12.4	99.47
	100	25	24.9	99.60
	150	37.5	37.8	100.80
Lamivudine	50	100	99.9	100
	100	200	199.5	99.75
	150	300	303.2	101.08

Table 3. Precision results determined during method validation

Parameter	Spiked level	%RSD	
		Abacavir sulphate	Lamivudine
Repeatability	50%	0.9	0.2
	100%	0.7	0.1
	150%	0.5	0.2
Intermediate Precision	50%	1.2	1.3
	100%	0.5	0.4
	150%	0.3	0.3

Stability of sample solution

The sample solution was stable up to 37 hr. 48 min. at 50C temperature and did not show any appreciable change in sample area.

CONCLUSION

This intended study can be concluded as: the proposed method is economical, simple, ultra fast, sensitive and reliable and is found to be more accurate, precise, specific, stability indicating, rugged and robust hence it can be employed for routine estimation of capsules containing Abacavir

sulphate and Lamivudine. Conventional reported HPLC methods may be replaced by the proposed UPLC method because of its superiority in cost effectiveness, Savings of analysis time per sample and better detection. For faster samples testing routinely in QC lab the validated method may be used.

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