RP-HPLC Method Development and Validation of Abacavir Sulphate in Bulk and Tablet Dosage Form

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Abstract

RP-HPLC method was developed for the estimation of abacavir sulphate in bulk and pharmaceutical dosage form (tablets) by using INERTSIL ODS 3V column, C18 (250x4.6 ID) mobile phase consisting of a mixture of 10mM phosphate buffer: ACN (60:40 v/v %) P^H: 4.0 with detection of 287 nm. The retention time was found to be 2.430min and linearity was observed in the range 60-140µg /ml. Still now there were a number of analytical methods were developed for the estimation of abacavir in pharmaceutical dosage form and also in biological samples like spectroscopic methods, chromatographic methods, etc. But the present method was met the validation parameters according ICH guidelines like accuracy, precision, linearity, range, robustness, ruggedness, limit of detection and limit of quantitation, etc. with a short around time. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2.

Key words: Abacavir, UV detection, RP-HPLC, etc.

Introduction

The chemical name of the abacavir is (1S, cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol sulfate (salt) (2:1). The IUPAC name of the abacavir is [(1S, 4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl] cyclopent-2-en-1-yl] methanol. The chemical structure is as follows

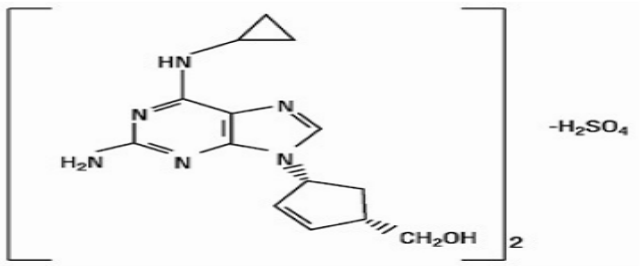


Fig No: 1 Chemical structure of abacavir sulphate

The molecular formula is $(C_{14}H_{18}N_6O)_2 \cdot H_2SO_4$and is soluble in water, methanol and buffers, fine crystal form with white in color. The mechanism of action by inhibiting the activity of HIV-1 reverse transcriptase (RT) [1, 2, 3]. The present study is to develop a RP-HPLC method for the estimation of abacavir sulphate in bulk and pharmaceutical dosage form (tablet) and method is proposed to validation.

Materials and methods

Table No: 1 List of materials

UV-Visible Spectrophotometer	Nicolet evolution 100
UV-Visible Spectrophotometer software	Vision Pro
HPLC software	Spin chrome (LC SOLUTIONS)
HPLC	Shimadzu(LC 20 AT VP)
HPLC Column	Inertsil ODS 3V(250x4.6mm) 5µm
Water	HPLC Grade
Methanol	HPLC Grade
Potassium Phosphate	AR Grade
Acetonitrile	HPLC Grade
Abacavir drugs	Gift Samples obtained from Chandra labs, Hyd.
Abavir (300 mg) tablet (contains Abacavir-300 mg label claim) Mfg. by : Genx (Hetero Healthcare Ltd)	Obtained from local pharmacy.

Method Development

Determination Of Working Wavelength (λmax)

Preparation of standard stock solution of abacavir

25 mg of abacavir was weighed and transferred in to 250ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drug in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no: 2and the absorption curve shows characteristic absorption maxima at 287 nm for abacavir.

Table No: 2 Optimized chromatographic conditions

Mobile phase	10mM KH2PO4: CAN (40:60)
P^H	4.0
Column	Inertsil ODS, 250×4.6mm ID, 5µm Particle size
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	287nm
Injection volume	20 µl
Run time	5 min
Retention time	2.430 min for abacavir
Detector	UV detector

Assay

Preparation of samples for Assay

Standard sample

Weigh accurately 100 mg of abacavir in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 100 µg/ml of abacavir is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

20 tablets (each tablet contains 100 mg of abacavir) were weighed and taken into a mortar uniformly mixed. Test stock solutions of abacavir (100µg/ml) and was prepared by dissolving weight equivalent to 100 mg of abacavir and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100 µg/ml of abacavir was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of abacavir present in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{\text{LC}} \times 100$$

Where,

AS: Average peak area due to standard preparation

AT: Peak area due to assay preparation

WS: Weight of ABACAVIR in mg

WT: Weight of sample in assay preparation

DT: Dilution of assay preparation

And the results were shown in table No 3.

Method Validation

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated and the results were shown in table No 4.

Linearity and range

Preparation of standard stock solution

Weigh accurately 100 mg of abacavir in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase and further dilutions were prepared. The linearity graph was shown in fig No 4 and results were shown in table No 5.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80%, 100%, 120%. The results were shown in table No 6.

Precision

Method precision

Prepared sample preparations of abacavir as per test method and injected 6 times in to the column. The results were shown in table No 7.

Limit of Detection

$$\text{LOD} = \frac{3.3\sigma}{\text{S}}$$

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. Limit of Quantification

$$\text{LOQ} = \frac{10\sigma}{\text{S}}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision. The chromatograms of abacavir for robustness were shown in fig No 5-8.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts and the results were shown in table No 8.

Results and discussion

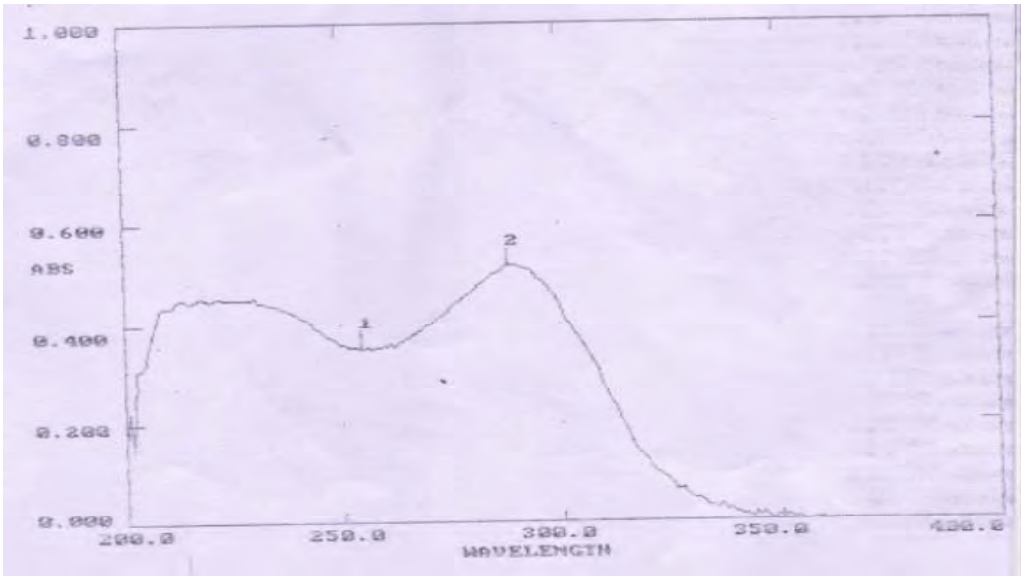


Fig No: 2 UV-VIS spectrum of abacavir

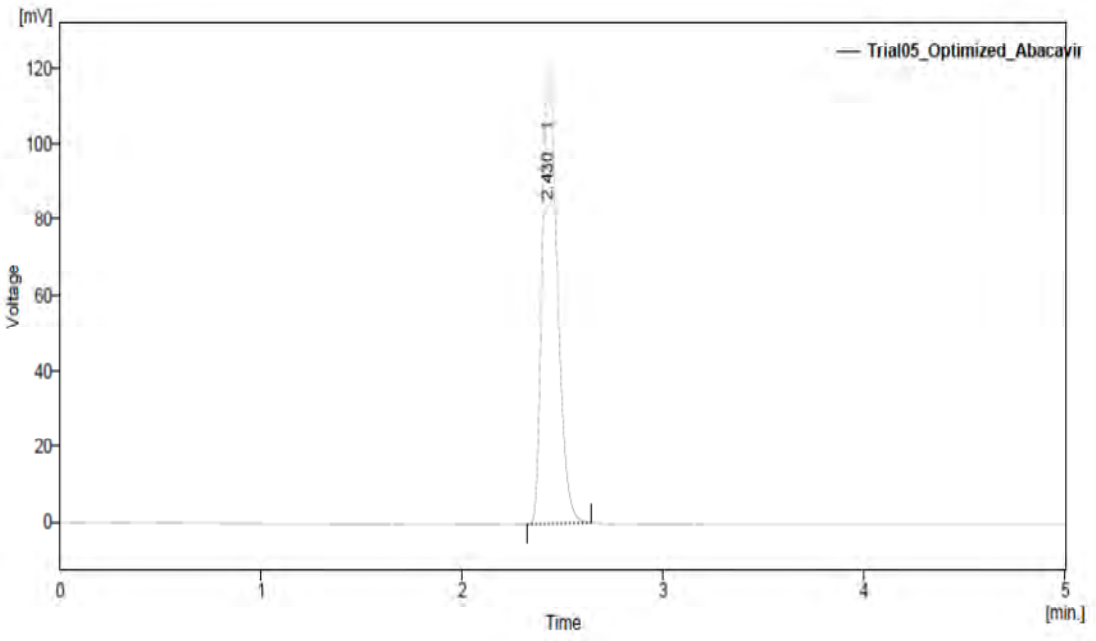


Fig No: 3 Chromatogram of abacavir using mobile phase

Table No: 3 Assay results of abacavir

ABACAVIR		
	Standard Area	Sample Area
Injection-1	639.416	642.878
Injection-2	641.607	641.488
Injection-3	641.868	642.967
Injection-4	640.550	639.797
Injection-5	641.503	639.655
Average Area	640.9637	641.357
Standard weight	100	
Sample weight	125.5	
Average Wt.	125.5	
Label claim	100	
Standard Purity	98.8	
Assay in mg	98.86	
%Assay	98.86	

Table No: 4 System suitability results of abacavir

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.460	647.790	4828	1.556
2	2.453	647.230	4439	1.611
3	2.453	645.963	4439	1.611
4	2.447	645.534	4415	1.611
5	2.450	641.381	4427	1.474
6	2.450	641.381	4427	1.474
Mean	2.4522	644.880	-	-
SD	0.0044	2.831	-	-
%RSD	0.18	0.44	-	-

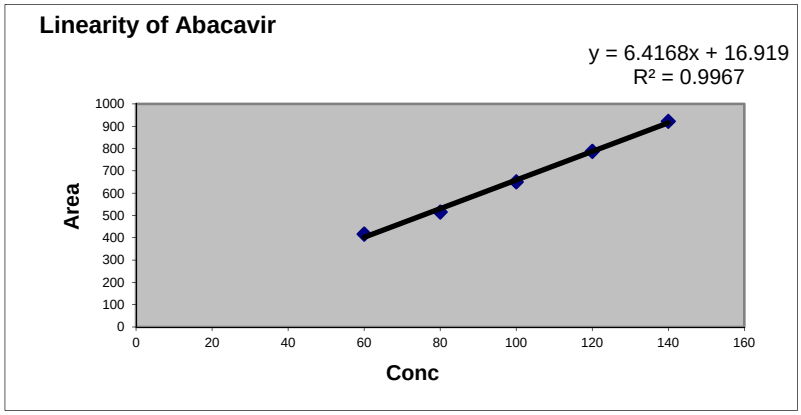


Fig No: 4 Linearity of abacavir

Table No 5: Linearity of abacavir

S. No.	Conc.(µg/ml)	Area
1	60	416.656
2	80	515.508
3	100	650.922
4	120	787.647
5	140	922.268

Table No: 6 Accuracy results of abacavir

Recovery level	Accuracy ABACAVIR					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	100	639.813	638.870	99.14	99.14	99.92%
	100	634.816				
	100	641.982				
100%	120	787.277	786.922	120.89	100.74	
	120	786.212				
	120	787.277				
120%	140	914.510	917.962	139.85	99.90	
	140	919.688				
	140	919.688				

Table No: 7 precision results of abacavir

ABACAVIR		
S. No.	Rt	Area
1	2.460	647.790
2	2.453	647.230
3	2.453	645.963
4	2.447	645.534
5	2.450	641.381
6	2.450	641.381
Average	2.4522	644.880
Standard Deviation	0.0044	2.831
%RSD	0.18	0.44

Limit of detection

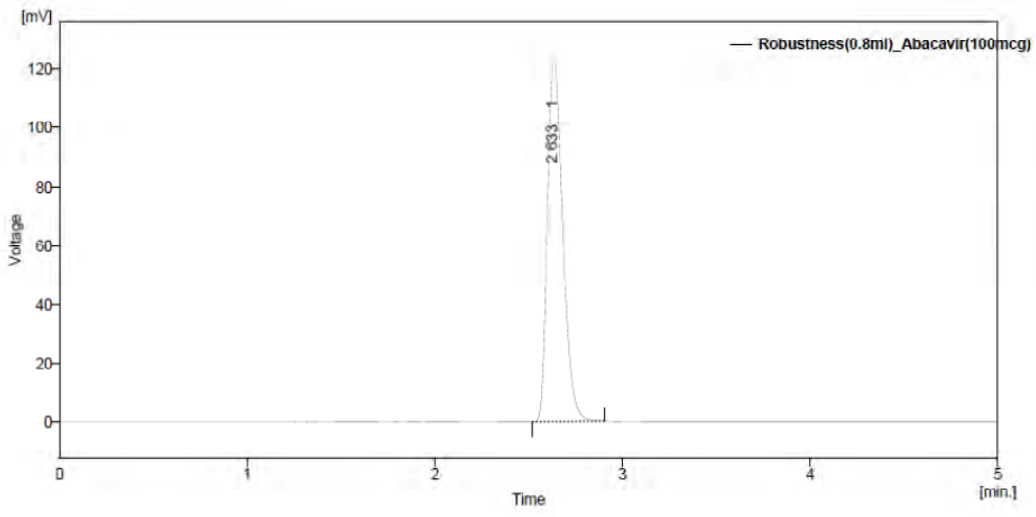
The limit of detection for abacavir was found to be 16.28 µg/ml & area 104.64.

Limit of Quantification

The LOQ for this method was found to be 49.33 µg/ml & area 317.09 for abacavir.

Robustness

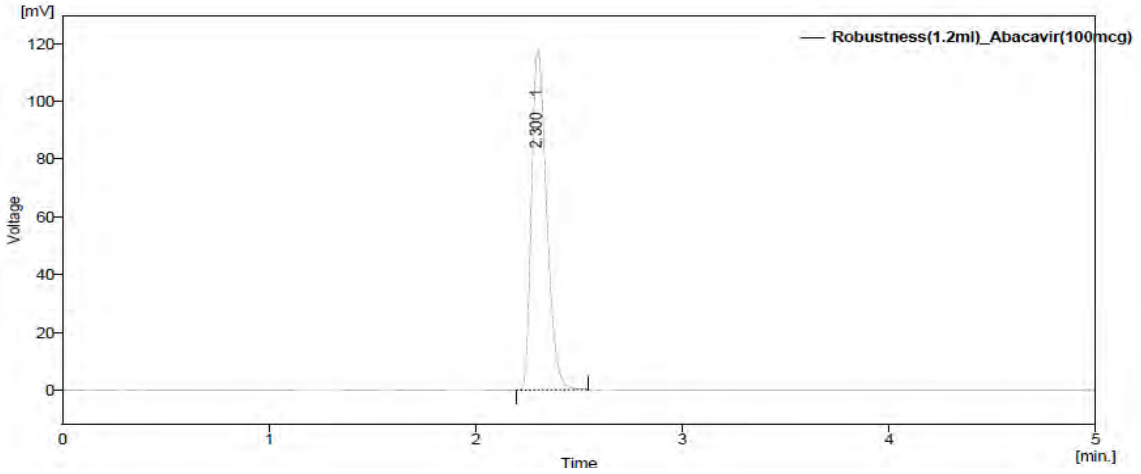
Fig No: 5 Chromatogram of abacavir Robustness (Flow: 0.8mL/min)



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.633	698.387	123.536	100.0	100.0	0.09
	Total	698.387	123.536	100.0	100.0	

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.633	0.090	1.632	0.00	4743	47428	-

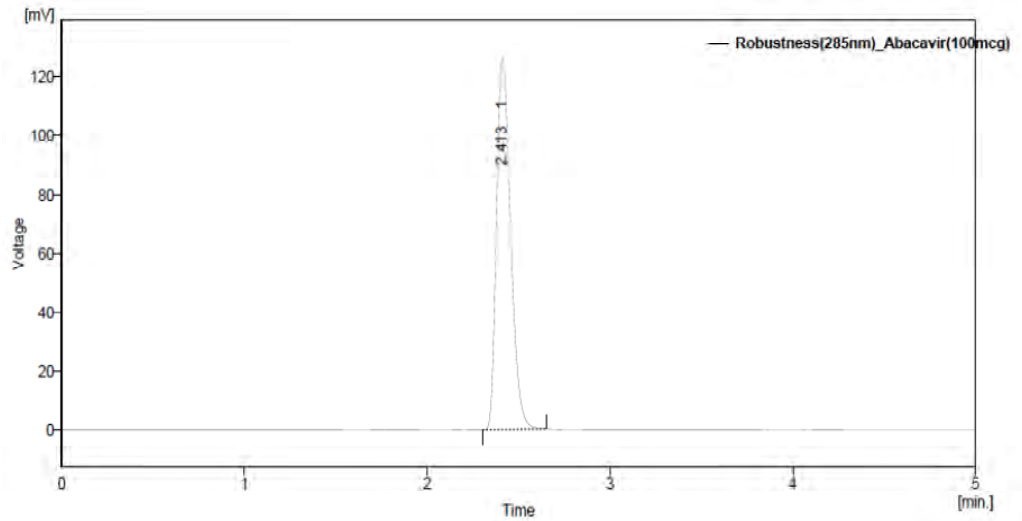
Fig No: 6 Chromatogram of abacavir for Robustness (Flow: 1.2mL/min)



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.300	599.569	117.706	100.0	100.0	0.08
	Total	599.569	117.706	100.0	100.0	

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.300	0.080	1.647	0.00	4579	45792	-

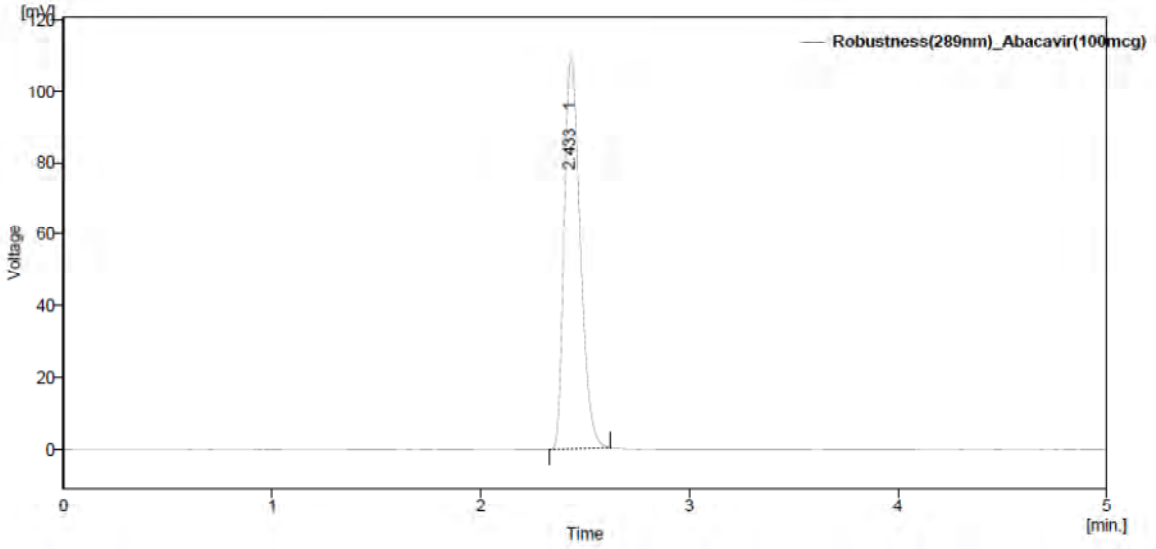
Fig No: 7 Chromatogram of abacavir for Robustness (285nm)



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.413	675.111	126.428	100.0	100.0	0.09
	Total	675.111	126.428	100.0	100.0	

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.413	0.087	1.474	0.00	4296	42958	-

Fig No: 8 Chromatogram of abacavir for Robustness (289nm)



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.433	583.521	109.487	100.0	100.0	0.08
	Total	583.521	109.487	100.0	100.0	

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.433	0.083	1.611	0.00	4724	47236	-

Ruggedness

Table No: 8 Results for Ruggedness

ABACAVIR	%Assay
Analyst 01	98.84
Analyst 02	99.10
%RSD	0.18

Discussion

A simple and selective LC method is described for the quantitative estimation of Abacavir in tablet dosage form. Chromatographic separation was achieved on a c_{18} column using INERTSIL ODS 3V column, C18 (250x4.6 ID) mobile phase consisting of a mixture of 10mM phosphate buffer: ACN (60:40 v/v %) P^H: 4.0 with detection of 287 nm. Linearity was observed in the range 60-140µg /ml for Abacavir (r^2 =0.9967) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Conclusion

RP-HPLC method development and validation of abacavir in bulk and pharmaceutical dosage form (tablets) with the facilities and the results are incorporated in this thesis.

In conclusion a validated RP-HPLC method has been developed for determination of Abacavir in tablets. The results show that the method was found to be specific, simple, accurate, precise and sensitive. The

method was successfully applied for the determination of both drugs in combined tablet dosage form. In the future, this method may be applied for routine analysis of both the drugs in API and in tablet formulation.

Several analytical procedures have been proposed for the quantitative estimation of Abacavir separately and in combination with other drugs. So attempt was taken to develop and validate a reversed-phase high performance liquid chromatographic method for the quality control of Abacavir in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time.

References

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