Simultaneous Estimation of Abacavir sulfate and Lamivudine Form Pregnant Rat Plasma By High Performance Liquid Chromatography Technique

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ABSTRACT *Simple, accurate and economic method of HPLC have been described for the simultaneous estimation of Abacavir and Lamivud from Female Pregnant Rat Plasma .Abacavir shows absorption maximum at 284.0nm and Lamivudine shows absorption maximum at 270.0nm in distilled water. Beers law was obeyed in the concentration range of 5-30 µg/ml for Both Abacavir and Lamivudine in Tablet dosage form. The coefficient correlations were found to be 0.9995 for LAM and 0.9992 for ABAC respectively in Tablet dosage form. Plasma, calibration points were prepared by spiking 100µL of biological matrices with 10 µL of each Abacavir- Lamivudine standard solution. The calibration curves of matrices were in the range of 0.05-50 µg/mL.Analytical methods employed for quantitative determination of drugs and their metabolites in biological fluids are the key determinants in generating reproducible and reliable data that in turn are used in the evaluation and interpretation of bioavailability, bioequivalence and pharmacokinetics. absolute recovery of Abacavir ranged from 80 to 96% while Lamivudine recoveries ranged from 63 to 81% in the different biological matrices.*

Keywords: Deep Abacavir , Lamivudine, Rat Plasma,

INTRODUCTION:

Abacavir sulfate is {(1S, 4R)-4-[2-Amino-6-cyclopropyl amino) 9H-Purin-9yl) cyclopent-2-enyl) methanol sulfate. it works by preventing HIV from infecting new cells and taking them over.Abacavir is converted by cellular enzymes to the active metabolite, carbovir triphosphate (CBV-TP), CBV-TP inhibits the activity of HIV-1 reverse transcriptase (RT) by its incorporation into viral DNA[1,2].

LamivudineChemically it is (2R, 5S)-4-Amino1 [2-(Hydroxy methyl)-1, 3-oxathiolan-5yl]-2(1H)-Pyrimidinedione. It is used in HIV infection.Lamivudine is phosphorylated to its active 5'-triphosphate metabolite, lamivudine triphosphate (3TC-TP). The principal mode of action of 3TC-TP inhibition of RT via DNA chain termination after incorporation of the nucleotide analogue[1,2]. Both Abacavir sulfate (ABAC) and Lamivudine (LAM) are official in IP [1]. Both the Drugs are marketed as combined dose tablet formulation and the Ratio is 300:600 mg LAM: ABAC. Literature survey revealed that a number of methods have been reported for estimation of Abacavir sulfate individually or in combination with other drugs and Lamivudine or in combination with other drugs[3-13]. Present work describes two simple, accurate, reproducible, rapid and economical methods for simultaneous estimation of ABAC and LAM in tablet formulation.

A sensitive and accurate HPLC method was developed and validated for the quantification of Abacavir and Lamivudine in rat maternal plasma. The use of acid precipitation and salting out techniques provided an inexpensive and convenient method of sample preparation. This method yielded high recoveries, good linearity, and precision and accuracy in the range of 0.05-50 µg/mL. This method will be useful for pharmacokinetic studies to investigate the fetal and maternal disposition of Abacavir and Lamivudine in pregnant rat.

MATERIAL AND METHOD:

Abacavir and Lamivudine were obtained from Aurobindo pharmaceutical limited Hyderabad, Acetonitrile (HPLC grade,), Methanol (HPLC grade) ,Orthophosphoric acid (AR grade) , and Potassium Dihydrogen orthophosphate (AR grade) were all procured from Qualigens fine chemicals (Mumbai, India).

A] Preparation of stock and standard solutions:

Stock solutions of 1.0mg/ml Abacavir and Lamivudine were individually prepared in deionized water. Standard solutions of ABAC and LAM were prepared by mixing and diluting the appropriate amounts from the individual stock solutions. The final concentrations of the standard solutions were 500,250,50,25,5,2.5 and 0.5 µg/mL. Precision and accuracy standards with concentrations of 400, 50,10, 0.5 µg/mL. Stock

solutions were kept refrigerated when not in use and replaced on a bi-weekly basis. Fresh standard solutions were prepared for each day of analysis or validation.

B]Chromatographic system:

Chromatographic separation was performed on Jasco High Performance Liquid Chromatograph with a PU-2080 HPLC pump equipped with a 20-µL loop and a Jasco UV-2075 variable wavelength detector. A double beam spectrophotometer, UV-630V made by Jasco, with the scanning range of 190 to 1100 nm, was used for scanning and selecting the detection wavelength. Chromatogram and data recorded on Borwin chromatography version 1.2. A reversed-phase HiQSil C_8 column (250 mm × 4.6 mm, 5µm) was used for analysis.

C]Chromatographic conditions:

The mobile phase comprising of mixture of 0.01 mol/L potassium Dihydrogen orthophosphate buffer, acetonitrile and methanol (60:25:15), and PH adjusted to 4.5 with Orthophosphoric acid, at a flow rate 1 mL/min. The injection volume was 20µL and detection wavelength was set at 275nm. Under the chromatographic conditions described, Abacavir and Lamivudine eluted at 5.608 and 3.458 min. respectively.

D]Calibration curves:

Plasma, calibration points were prepared by spiking 100µL of biological matrices with 10 µL of each Abacavir- Lamivudine standard solution. The calibration curves of matrices were in the range of 0.05-50 µg/mL.

E]Precision and accuracy:

This method was validated using four QC points for each calibration curve. Five replicates of each QC points were analysed every day to determine the intra-day accuracy and precision. This process was repeated three times over 3 days in order to determine the inter-day accuracy and precision. The concentrations of the QC points for all four matrices were 0.05, 0.1, 5, 40 µg/mL.

F]Sample preparation:

Amniotic fluid samples were prepared with protein precipitation. After spiking, samples were vortexed briefly and 10µL of 2M perchloric acid was added. Samples were vortexed and centrifuged for 10 min. at 10,000 rpm. The supernatant was removed and the pellet was discarded. Plasma and placental and fetal tissues were extracted using salting out technique. Three hundred microlitres of saturated ammonium sulfate solution and 400µL acetonitrile were added to 100 µL of the biological matrices containing the analytes. The samples were vortexed and centrifuged at 10,000rpm for 10 min. the upper organic layer was then evaporated to dryness in a vacuum centrifuge and then residues reconstituted in 100µL deionized water. An injection volume of 20 µL was used for all samples.

G]Sample collection:

The use of animal in this study was approved by the animal use and care committee. The rats were housed one animal per cage in the college of pharmacy animal facility (AALAC accredited). The environment was controlled with daily feeding of standard chow pellets and water ad libitum.

Timed pregnant female Sprague-Dawley rats weighing an average of 172gm were used. On day of 19 of gestation rats were anaesthetized using ketamine (50mg/kg) injected intramuscularly. A cannula was placed in the right jugular vein and a laparotoy was performed to allow concurrent serial sampling of blood and fetal sac, each containing a fetus, placenta and amniotic fluid. The rats were administered an i.v. bolus dose of Abacavir (12.5mg/kg) and Lamivudine (12.5mg/kg) dissolved in 0.1N NaOH in physiological saline (pH 7.4) via the jugular cannula. Individual blood and fetal sac samples were collected at 5,10,15,30,45,60,90,120,180,240 after dosing and stored on ice until processed. Blood samples were collected in heparinized tubes and centrifuged at 10,000 rpm for 10 min .to enable plasma collection. Placental and fetal tissues samples were homogenized in two volumes of deionized water. All samples were stored at – 20^0C until analysis.

RESULTS AND DISCUSSION:

A Reverse phase High Performance Liquid Chromatography (HPLC) method was developed and validated for the simultaneous determination of Abacavir (ABAC) and Lamivudine (LAM), in rat plasma, amniotic fluid, fetal, and placental tissues. Extraction of ABAC and LAM in amniotic fluid was carried out by protein

precipitation. Extraction from plasma, fetal and placental homogenate was achieved by using salting out technique. Chromatographic separation was performed using a HiQSil C_8 column (250 mm × 4.6 mm, 5µm) , with a mobile phase comprising of mixture of 0.01 mol/L potassium Dihydrogen orthophosphate buffer, acetonitrile and methanol (60:25:15) , and PH adjusted to 4.5 with Orthophosphoric acid, at a flow rate 1 ml/min. The method was validated over the range from 0.05-50µg/ml for both Abacavir and Lamivudine in the four biological matrices. The absolute recovery of Abacavir ranged from 80 to 96% while Lamivudine recoveries ranged from 63 to 81% in the different biological matrices.

CONCLUSION:

A sensitive and accurate HPLC method was developed and validated for the quantification of Abacavir and Lamivudine in rat maternal plasma, amniotic fluid, placental and fetal tissues. The use of acid precipitation and salting out techniques provided an inexpensive and convenient method of sample preparation. This method yielded high recoveries, good linearity, and precision and accuracy in the range of 0.05-50 µg/mL. This method will be useful for pharmacokinetic studies to investigate the fetal and maternal disposition of Abacavir and Lamivudine in pregnant rat.

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LIST OF TABLES:

Table 1:Absolute Recoveries of ABAC and LAM from plasma (n=15)

Analyte	Concentration	Plasma
ABAC	40	80 ± 3.5
	5	85 ± 3.1
	0.1	87 ± 5.9
	0.05	89.08± 4.6
LAM	40	79 ± 2.9
	5	76 ± 2.8
	0.1	78 ± 3.5
	0.05	80 ± 2.3

Table 2 :Intra-day (n=5) and inter-day (n=15) precision (% RSD) and accuracy (% error) measured for QC points for Abacavir from plasma

T.C	Day1			Day 2			Day 3			Inter-day		
	E.C	R.S.D	Error	E.C	R.S.D	Error	E.C	R.S.D	Error	E.C	RSD	Error
Plasma												
40	43.2	8.32	8.23	42.3	5.26	4.02	40.6	2.9	1.7	39.8	1.6	0.75
5	4.95	3.68	2.9	4.7	4.0	2.7	5.2	1.82	0.3	4.72	6.0	2.5
0.1	0.09	4.86	5.89	0.07	2.16	5.6	0.1	3.95	2.35	0.08	5.6	3.6
0.05	0.051	6.59	4.3	0.05	4.23	0.6	0.05	3.86	1.96	0.05	6.5	2.3

T.C. denotes theoretical concentration and E.C denotes experimental concentration.

LIST OF FIGURES:

Fig. 1: pregnant female Sprague-Dawley rat weighing an average of 172gm

Fig. 2: Pregnant female Sprague-Dawley rat

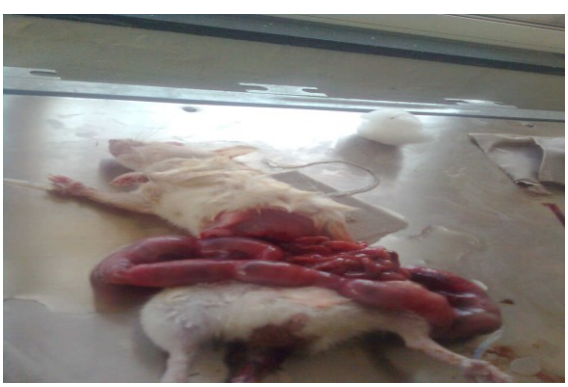

Fig. 3: Blood sample collected via. Jugular cannula

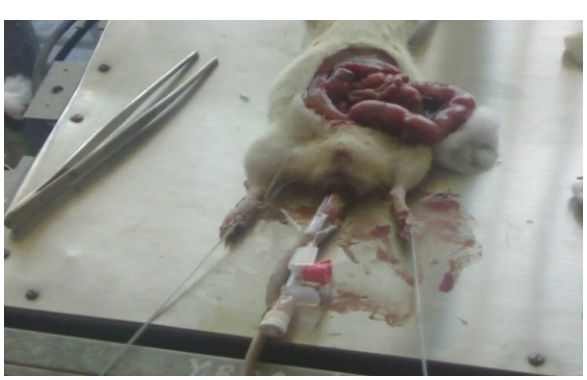

Figure 4: Peaks of abacavir sulfate and Lamivudine