DETERMINATION OF ABACAVIR IN HUMAN PLASMA FOR PHARMACOKINETIC STUDIES

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2.1 INTRODUCTION

Abacavir belongs to the class nucleoside reverse transcriptase inhibitors. The agent is anabolized by a unique intracellular mechanism form carbovir triphosphate, which potently and selectively inhibits human immunodeficiency virus (HIV) reverse transcriptase\(^1\). The nucleoside reverse transcriptase inhibitors (NRTIs) form the backbone of current antiretroviral treatment for HIV. The first drug approved for use, zidovudine (ZDV), is a member of this class of compounds. Lamivudine (3TC) and abacavir (ABC) are also NRTIs and are frequently given in combination with zidovudine in a formulation marketed as TRIZIVIR\(^\circledR\). All members of the NRTI class of antiretroviral agents, while differing in pharmacokinetics, toxicity and efficacy, require conversion to the triphosphate form in order to inhibit viral replication.

Efficacy of this class of compounds depends on many factors including parent drug pharmacokinetics (absorbance, clearance, etc.), intracellular metabolism factors such as uptake/transport into the cell, multi-step phosphorylation and de-phosphorylation or other enzymatic processes and for the activation status of the cells\(^{1-3}\). Since the triphosphate form is the active form of the NRTI class of compounds\(^{4-6}\), it is essential to measure the triphosphate levels in the target cells, primarily peripheral blood mononuclear cells (PBMC). Previous studies have shown that intracellular concentrations of zidovudine triphosphate (ZDV-TP) correlate with antiviral activity and immunological response to therapy\(^{2,7-8}\).

Abacavir is a choice of drug which could be administered along with zidovudine to prevent transmission from mother to child and its estimation in human matrices poses challenges in method development by various scientists\(^{10-16}\). To date, only few HPLC\(^{17-22}\), LC-MS/MS\(^{23-25}\) methods have been developed and reported for abacavir analysis in combination with other co-drugs. However the reported methods had certain limitations viz., less sensitivity, long run time, facing difficulty in processing large samples and requires more plasma volume\(^{17-26}\).

Prompted by the importance, in this experiment, we made an attempt to have unique method for reducing several hurdles faced by scientific community by using high through put LC/MS/MS instrument for quantification of abacavir in human plasma. This method will also pave way for processing large number of samples.
2.2.1 Study Objective
Purpose of the present study was to develop and validate a LC-MS/MS method for
determination of abacavir in human plasma for pharmacokinetic studies.

2.2 EXPERIMENTAL

2.2.2 Reference Compound: Abacavir Sulphate

*IUPAC Name:* (1S,cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-
cyclopentene-1-methanol sulfate (salt) (2:1).

*Molecular Formula:* \((C_{14}H_{18}N_{6}O)_{2} \cdot H_{2}SO_{4}\)

*Molecular Weight:* 670.76

*Purity:* 99.8%

*Supplier:* Vardada biotech Pvt.Ltd, (Bombay, India).

![Structure of Abacavir](image)

**Fig.2.1. Structure of Abacavir**

*Reference Compound:* Tenofovir Disoproxil Fumarate

*IUPAC Name:* \((\{(2R)-1-(6-amino-9H-purin-9-yl) pro yl\}oxy\)methyl)

phosphonic acid

*Molecular Formula:* \(C_{9}H_{14}N_{5}O_{4}P\)

*Molecular weight:* 287.213

*Purity:* 98.9%

*Supplier:* Vardada biotech Pvt.Ltd, (Bombay, India)

2.2.3 Chemicals, Reagents and Materials
2.2.3.1 Chemicals

All the solvent were of high purity and before analysis, all the glass ware was carefully cleaned and rinsed with Milli Q water (Type-1 grade). The reagent were obtained as stated:-

- Acetonitril from Labscan (Mumbai, India)
- Methanol from Labscan (Mumbai, India)
- Ethyl acetate from Labscan (Mumbai, India)
- Formic acid from Merck (Darmstadt, Germany)
- Water deionised and purified by a Milli-Q water purification system from Millipore (Bedford, MA, USA)
- Blank K$_2$EDTA plasma was obtained from Innovative Research, (USA)

2.2.3.2 Reagents

- Mobile Phase Buffer: Accurately weighed 0.77g of ammonium acetate was transferred to a reagent bottle; to this 1000mL of milli Q water was added and sonicated to dissolve. pH was then adjusted to 5.0 with acetic acid (20%).
- Mobile Phase: In a 1000mL volumetric flask, 800mL of acetonitrile was taken, to this, 200mL of mobile phase buffer solution was added, mixed and filtered through 0.2µm filter.

2.2.3.3 Materials

- API 3200 triple Quadra pole Mass Spectrometer (MDS - SCIEX, Canada)
- Shimadzu HPLC system with C18 Gemini column (150mm x 4.6mm, 5µ)
- Micropipettes from Eppendorf, Hamburg, Germany
- Repetitive pipette and tips Handy Step Electronic of Brand (Germany)
- Solvent filter (0.2µm), Millipore, Bangalore, India
- Vortex mixer, Spinix, Tarson, India
- Analytical balance AB265-S from Mettler Toledo (Germany)
- Micro balance MX-5 of Mettler from Toledo (Germany)
- Filtration Setup Millipore (Millipore, Bangalore, India)
- Sonicator Bandelin Sonorex (Zymark, Germany)
- Class A calibrated glass ware from different supplier
2.2.4 Liquid Chromatographic Conditions

A Shimadzu HPLC system with C18 Thermo column (50mm x 4.6mm, 5µ) that contains packing of octadecylsilane chemically bonded to porous silica was used for chromatographic separation. The mobile phase was prepared by addition of ammonium acetate buffer (pH adjusted to 5.0 with acetic acid) in acetonitrile (20:80 v/v). The flow rate of 0.1mL/min was used to carry out separation. The column temperature was set at 40°C, the auto-sampler was conditioned at 5°C and the injection volume was 5µL with a run time around 3min.

2.2.5 Mass Spectrometric Conditions and Data Processing

The Mass Spectrometry was operated in positive ion detection mode. Nitrogen was used as a nebulizing turboionspry. The temperature of vaporizer was set at 500°C and the ESI needle voltage was 5000V. The declustering potential was set at 30 volts for abacavir and 90 volts for Internal Standard (IS). Collision energy was set at 27 volts for abacavir and 40 for IS. The mass spectrometer was operated at unit mass resolution with a dwell time of 200ms per transition. The collision associated dissociation (CAD) was set at 6 volts for abacavir and IS. Quantification was performed using multiple reaction monitoring (MRM) of the transition $m/z$ 287.10 (parent ion) $\rightarrow m/z$ 191.20 (product ion); $m/z$ 288.10 (parent ion) $\rightarrow m/z$ 176.20 (product ion) for abacavir and IS respectively.

2.2.6 Standard Solutions (Calibration Standards and Quality Control Samples)

Stock solution (1mg/mL) was prepared by dissolving 10mg of abacavir in 10mL of methanol. Spiking solution of abacavir was prepared from stock solution by serial dilution method in methanol: water (1:1, v/v). Eight levels of calibration curve standards were prepared by adding the spiking solution in plasma and achieved the concentration levels of 20.065, 40.129, 150.30, 500.99, 1802.1, 4505.3, 8002.4, 10003ng/mL. Four levels of quality control samples were prepared by adding the spiking solutions in plasma the concentration levels of 20.163, 50.406, 3500.4, and 7000.9ng/mL were achieved.

2.2.7 Extraction Procedure

All the calibration standards (50µL) or QC samples (50µL) were taken in polypropylene tubes, 50µL of internal standard (10µg/mL of tenofovir) was added and vortexed for 10s.
Fifty micro liter of 10% formic acid was added to the above samples and vortexed for 20s. Ethyl acetate (3ml) was added to the above samples and vortexed for 10min. The two phases were separated by centrifugation at 4000rpm (rotations per minutes) for 3minutes. The upper organic layer was transferred in to another glass tube and completely evaporated to dryness at 40°C and 5PSI (pounds per square inch). The dry residue was reconstituted with 3mL of mobile phase 20:80, vortexed for about 30 seconds and transferred to auto injector vial. 5µL was chromatographed and analyzed by LC-MS/MS.

2.3 RESULTS AND DISCUSSION

2.3.1 Method Development

During the method development mass spectrometric conditions, extraction procedure and chromatographic conditions were optimized. The ideal condition of MS/MS detection was expected to be advantageous in developing a selective and sensitive method. Optimum mass acquisition parameters were obtained by direct infusion of 500ng/mL solution of both analyte and internal standard at a flow rate of 10µL/min. The mass spectrometer was operated in the MRM condition under positive ion mode. The transition of ions were monitored m/z 287.1 (parent ion) -191.20 (product ion) for abacavir and m/z 288.1 (parent ion) - 176.20 (product ion) for internal standard (tenofovir). An earlier report had suggested the ionspray voltage of 1500V for the linearity range of 1 - 500ng/mL. However in the present study ionspray voltage of 5000V was optimized along with other tuning parameters for the enhanced sensitivity and wider linearity range.

Thomas Le Saux et al, Agnes I. Veldkmp et al and Rolf W. Sparidans et al have reported a protein precipitation method to separate abacavir a polar drug from human plasma. Thus, the extraction was carried out initially via protein precipitation with common solvents like acetonitrile, methanol, acetone, but the sensitivity and reproducibility were very poor and lot of matrix interference were observed by this method, which resulted high back pressure in the column due to endogenous interference.

Liquid-liquid extraction technique was performed to isolate abacavir from plasma using diethyl ether, dichloromethane, methyl tert butyl ether, isopropyl alcohol and ethyl
acetate in acidic and basic conditions. Ethyl acetate extraction in acidic condition has produced very good sensitivity, accuracy and reproducibility, when compared with other solvent conditions.

Based on the earlier reports\textsuperscript{17-19,25-26}, solid phase extraction using Waters Oasis HLB, Water Oasis MCX, Waters Oasis WAX, Orochem and Phenomenex Strata cartridges was carried out. None of the method produced good extraction efficiency and reproducibility. Extraction with ethyl acetate in acidic condition was found to be cost effective and efficient compared to the earlier reported method\textsuperscript{23}, which used liquid-liquid extraction followed by protein precipitation for the extraction of abacavir.

It was essential to have a chromatographic separation of abacavir and IS to minimize any interference during quantitation. Chromatographic analysis of abacavir and IS was initiated under isocratic conditions to obtain adequate response, sharp peak shape and a shorter run time. As the pKa value of abacavir is 5.01, mobile phase has been planned to proceed in the acidic condition with pH equivalent to pKa±1. Thus, separation was attempted using various combinations of methanol/acetonitrile, acidic buffers and additives like formic acid on different reversed-phase columns [ACE C18 (50×4.6mm, 5µm), Gemini C18 (50mm×4.6mm, 5µm), Thermo C18 (50mm×4.6mm, 5µm), Kromasil C18 (50mm×4.6mm, 5µm), and Chromolith RP18 (100mm×4.6mm, 5µm)]. Best results in terms of reproducibility, complete separation and peak shape without any interference were obtained with Thermo C18 (50mm×4.6mm, 5µm) column compared to others and hence was selected for further study. Earlier reports\textsuperscript{17, 19} used the ion pairing agents for the mobile phase, which shows slow equilibration of the column and fluctuation of retention times. In our method, mobile phase buffer consisted of 0.077% ammonium acetate in water, pH adjusted to 5.0 with acetic acid (20%). Mobile phase buffer and acetonitrile (20:80v/v) without any ion pairing agent was found most suitable for eluting abacavir and internal standard at around 1.4min. A flow-rate of 0.1mL/min produced good peak shapes and permitted a run time of 3.0 min per analysis. Earlier publications have reported longer run times (7min to 30min) when compared with this chromatographic condition\textsuperscript{17-19, 23-24, 26}. By virtue of its similarity in therapeutic group, chromatographic behaviour and ionization pattern, tenofovir was selected as the internal standard (IS).
2.3.2 Method Validation

2.3.2.1 Selectivity

Possible interferences at the retention times of abacavir and IS from endogenous compounds were checked during the validation by testing six different batches of K₂EDTA human plasma, one lipemic blank plasma and one lot of haemolysed blank in order to check the absence of signals for the retention times of each compound. Selectivity was carried out by analyzing the six blank plasma samples spiked with abacavir (LLOQ level) and IS. Representative chromatograms of extracted blank plasma (Fig. 2.2), blank plasma fortified with IS (Fig. 2.3) are shown no interference in the blank plasma at the retention time of analyte and the IS.

2.3.2.2 Linearity

Linearity of the method was evaluated using bulk spiked plasma samples in the concentration range as mentioned in section 2.2.6 using the method of least squares. Three such linearity curves were analyzed. Each calibration curve consisted of a blank sample, a zero sample (blank + IS) and eight concentrations. The standard curves were linear over the concentration range of 20 to 10000ng/mL. The mean correlation coefficient was 0.9980. Samples were quantified using the ratio of peak area of analyte to that of IS. A weighting factor linear regression (1/concentration) was performed with the nominal concentrations of calibration levels. Peak area ratios were plotted against plasma concentrations, the limit of quantitation was 20ng/mL.

2.3.2.3 Recovery

The recovery of drug and IS was evaluated at three concentration levels namely low, medium and high quality control. Recovery was calculated by comparing its response in replicate samples with that of neat standard solution responses. Analyte recovery from a sample matrix (extraction efficiency) is a comparison of analytical response from an amount of analyte added to that determined from sample matrix. Because of basic properties of abacavir, extraction was carried out using ethyl acetate as organic solvent. Experiments with spiked compounds resulted in recoveries of analyte 62.86 - 63.62 % and for IS 60.26 - 62.49 % as summarized in Table 2.1
Fig. 2.2. Representative Chromatograms of Extracted Blank Plasma

**Extracted Blank - IS**

**Extracted Blank - Analyte**
Fig. 2.3. Representative Chromatograms of Blank Plasma and IS
2.3.2.4 Precision and Accuracy

Intra-day accuracy and precision were evaluated from replicate analyses (n = 6) of quality-control samples containing abacavir at different concentrations on the same day. Inter-day accuracy and precision were also assessed from the analysis of the same QC samples on separate occasions in replicate (n = 6). QC samples were analyzed against calibration standards.

All calibration curves were found to be linear over the range of 20 to 10000ng/mL. The precision for the six plasma samples spiked at LOQ concentration was 4.3% with a mean accuracy of 90.3% (Table 2.2). The inter-batch assay accuracy ranged between 99.0 - 103.7%, whereas intra-batch accuracy ranged between 93.9-103.8%. The inter-batch precision ranged between 4.3-16.9%. Intra batch precision ranged between 2.5 - 4.4%. The results are presented in Table 2.3. All the results were found within the acceptable limit of precision, not more than 15.0% and accuracy 85.0 - 115.0%, except LLOQ for which precision was not more than 20% and accuracy was between 80.0 - 120.0% was noted.

2.3.2.5 Matrix Factor

The matrix of plasma constituents over the ionization of analyte was determined by comparing the response of post-extracted plasma standard QC samples (n = 6) with the response of analyte from neat samples at equivalent concentrations\(^{27-29}\). The matrix effect intended method was assessed by using chromatographically screened human plasma. Precision (%CV) is 5.0% and 2.6% for abacavir and IS respectively (Table 2.4).

<table>
<thead>
<tr>
<th>Nominal Concentrations (ng/mL)</th>
<th>% Recovery</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abacavir</td>
<td>IS</td>
</tr>
<tr>
<td>50.406</td>
<td>63.62</td>
<td>62.49</td>
</tr>
<tr>
<td>3500.4</td>
<td>63.53</td>
<td>60.26</td>
</tr>
<tr>
<td>7000.9</td>
<td>62.86</td>
<td>60.71</td>
</tr>
</tbody>
</table>

---

Table 2.1. The Percentage Recovery of Abacavir and IS
Table 2.2. Precision and Accuracy Data of Back-Calculated Concentrations

<table>
<thead>
<tr>
<th>Concentrations added (ng/mL)</th>
<th>Concentrations determined (ng/mL) (mean ± S.D)</th>
<th>Precision (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.065</td>
<td>18.125 ± 0.69878</td>
<td>4.3</td>
<td>90.3</td>
</tr>
<tr>
<td>40.129</td>
<td>41.895 ± 0.98162</td>
<td>2.2</td>
<td>104.4</td>
</tr>
<tr>
<td>150.3</td>
<td>157.46 ± 4.1938</td>
<td>2.5</td>
<td>104.8</td>
</tr>
<tr>
<td>500.99</td>
<td>516.52 ± 11.174</td>
<td>2.1</td>
<td>103.1</td>
</tr>
<tr>
<td>1802.1</td>
<td>1784.7 ± 38.374</td>
<td>2.2</td>
<td>99.0</td>
</tr>
<tr>
<td>4505.3</td>
<td>4455.7 ± 170.31</td>
<td>3.9</td>
<td>98.9</td>
</tr>
<tr>
<td>8002.4</td>
<td>7671.6 ± 185.45</td>
<td>2.5</td>
<td>95.8</td>
</tr>
<tr>
<td>10003</td>
<td>10426 ± 293.12</td>
<td>2.7</td>
<td>104.2</td>
</tr>
</tbody>
</table>

Table 2.3. Precision and Accuracy of Method for Determining Abacavir Concentrations in Plasma Samples

<table>
<thead>
<tr>
<th>Conc. added (ng/mL)</th>
<th>Within-batch precision (n=6)</th>
<th>Between-batch precision (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. determined (ng/mL)</td>
<td>Precision (%)</td>
</tr>
<tr>
<td></td>
<td>(mean ± S.D)</td>
<td></td>
</tr>
<tr>
<td>20.163</td>
<td>18.933 ±0.77821</td>
<td>4.4</td>
</tr>
<tr>
<td>50.406</td>
<td>52.321 ± 1.3462</td>
<td>2.5</td>
</tr>
<tr>
<td>3500.4</td>
<td>3577.4 ± 105.87</td>
<td>2.9</td>
</tr>
<tr>
<td>7000.9</td>
<td>6846.8 ± 192.27</td>
<td>2.9</td>
</tr>
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</table>
Table 2.4. Matrix Effect for Abacavir

<table>
<thead>
<tr>
<th>LLOQ QC</th>
<th>Internal Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processed (A)</td>
<td>Aqueous (B)</td>
</tr>
<tr>
<td>7245</td>
<td>11885</td>
</tr>
<tr>
<td>7564</td>
<td>11858</td>
</tr>
<tr>
<td>6950</td>
<td>11659</td>
</tr>
<tr>
<td>6963</td>
<td>11961</td>
</tr>
<tr>
<td>6659</td>
<td>11885</td>
</tr>
<tr>
<td>6676</td>
<td>12251</td>
</tr>
<tr>
<td>Mean</td>
<td>11916.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.029</td>
</tr>
<tr>
<td>% CV</td>
<td>5.0</td>
</tr>
</tbody>
</table>

2.3.2.6 Dilution Integrity

During the course of study, the probability of encountering samples with concentrations above the upper limit of quantitation (ULOQ) could not be ruled out and therefore dilution with drug-free plasma is necessary to bring them within the calibration range. To establish the effect of dilution on the integrity of samples, six aliquots of 14000ng/mL of abacavir were prepared. The samples were subjected to two fold dilution (n = 6) and five fold dilution (n = 6) with drug-free human plasma to bring them within the calibration range. The samples were processed, analyzed and the concentrations obtained were compared with theoretical values.

The precision for dilution integrity standards at 1:2 and 1:5 for abacavir were 2.0 and 1.8%. The mean accuracy for dilution integrity of 1:2 and 1:5 for abacavir were 101.0 and 101.1%. These are within the acceptance limits of 85.00 - 115.00% (Table 2.5).
2.3.2.7 Stability Study

Evaluation of the stability of samples was based on the comparison of various samples against freshly prepared sample of the same concentration. Percentage difference between the back calculated concentrations obtained for the sample under investigation and freshly prepared sample was evaluated. Four aliquots, each of LQC and HQC concentrations were used for stability study.

The bench top stability (at room temperature) was determined for 10h by comparing the ratio of means of the concentrations for the low and high QCs which were found to be 105.2 and 97.8% respectively. The freeze-thaw stability was determined by measuring the assay precision and accuracy of the LQC and HQC samples, which underwent four freeze thaw cycles. In each freeze thaw cycle, the frozen plasma samples were thawed at

### Table 2.5. Dilution Integrity for Abacavir

<table>
<thead>
<tr>
<th>Description</th>
<th>Concentration Obtained for (\frac{1}{2}) Dilutions (ng/mL)</th>
<th>Concentration Obtained for (\frac{1}{5}) Dilutions (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample conc.</td>
<td>With dilution factor</td>
</tr>
<tr>
<td>Aliquot 1</td>
<td>7160.25</td>
<td>14320.5</td>
</tr>
<tr>
<td>Aliquot 2</td>
<td>7211.20</td>
<td>14422.4</td>
</tr>
<tr>
<td>Aliquot 3</td>
<td>6977.15</td>
<td>13954.3</td>
</tr>
<tr>
<td>Aliquot 4</td>
<td>6912.60</td>
<td>13825.2</td>
</tr>
<tr>
<td>Aliquot 5</td>
<td>7236.35</td>
<td>14472.7</td>
</tr>
<tr>
<td>Aliquot 6</td>
<td>7228.55</td>
<td>14457.1</td>
</tr>
<tr>
<td>Mean</td>
<td>7121.02</td>
<td>14242.033</td>
</tr>
<tr>
<td>SD</td>
<td>140.480</td>
<td>280.959</td>
</tr>
<tr>
<td>% CV</td>
<td>2.0</td>
<td>1.8</td>
</tr>
<tr>
<td>% Mean Accuracy</td>
<td>101.0</td>
<td>101.1</td>
</tr>
</tbody>
</table>
room temperature for 2-3 h and refrozen for 12-24h. After completion of each cycle the samples were analyzed and the results were compared with that of zero cycle. The results showed that the analyte was stable in human plasma through freeze thaw cycles. The ratio of means of concentrations for the low and high QC was 98.1 and 106.9%. This was within the acceptable range of 85-115%. The results demonstrated that human plasma samples could be thawed and refrozen without compromising integrity of the samples. Auto sampler stability of the plasma samples were over 9h 10min. All the stability results (Table 2.6), were found within the acceptable limit.

**Table 2.6. Stability Results for Abacavir**

<table>
<thead>
<tr>
<th>Sample concentrations (ng/mL) (n=6)</th>
<th>Concentrations determined (ng/mL) (mean, n=6)</th>
<th>Precision (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench top stability (10h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50.406</td>
<td>53.035</td>
<td>2.9</td>
<td>105.2</td>
</tr>
<tr>
<td>7000.9</td>
<td>6843.4</td>
<td>2.9</td>
<td>97.8</td>
</tr>
<tr>
<td>Freeze thaw stability (after 4 cycles)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50.406</td>
<td>49.440</td>
<td>14.8</td>
<td>98.1</td>
</tr>
<tr>
<td>7000.9</td>
<td>7481.6</td>
<td>4.9</td>
<td>106.9</td>
</tr>
<tr>
<td>Auto sampler stability (9h at 10°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50.406</td>
<td>45.349</td>
<td>1.2</td>
<td>90.0</td>
</tr>
<tr>
<td>7000.9</td>
<td>6575.0</td>
<td>7.6</td>
<td>93.9</td>
</tr>
</tbody>
</table>
2.4 CONCLUSION

A sensitive and specific method for determination of abacavir in human plasma has been developed. The developed method was validated according to FDA guidelines. The assay method was found to be precise (inter batch 4.3-16.9% ; intra batch 2.5-4.4%) and accurate (inter batch 99.0-103.7%; intra batch 93.9-103.8%) over a wide concentration range (20.065-10003ng/mL), with no interference by endogenous compounds. This simple, rapid, and robust assay will enable the complete processing of large number of samples for pharmacokinetic studies in human plasma.
2.5 REFERENCES


13. Ibrahim S.S. and Boudinot F.D., Pharmacokinetics of 2,3- dideoxy cytidine in rats: application to interspecies scale-up J Pharm Pharmacol 1989; 41, 12, 829-834.


