RECENT RESEARCH ON HPLC METHODS OF ANALYSIS OF LAMIVUDINE AND ZIDOVUDINE: A REVIEW

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ABSTRACT

Highly active antiretroviral therapy (HAART), a combination drug therapy is a topic of current interest in the treatment of HIV and AIDS. Techniques for the analysis and the quality control of antiretroviral drugs, particularly in drug combinations are vital in achieving quality of these drugs and the treatments involved. The HPLC methods available for the analysis of lamivudine and zidovudine, the most widely used drug combination for HIV and AIDS are reviewed in this article.

Keywords: Antiretroviral Drugs, HPLC Methods, Lamivudine, Zidovudine, Combination drug therapy

INTRODUCTION

Antiretroviral Drugs:  
The human immunodeficiency virus (HIV) infects cells of the immune system, destroying these cells as well as the immune system’s ability to fight off the invaders. The aim of antiretroviral therapy (ART) is to keep the amount of HIV in the body at a low level. This stops any weakening of the immune system and allows it to recover from any damage that HIV might have caused already. Antiretroviral drugs have been developed specifically to limit the progression of the retro-virus HIV. The HIV retrovirus causes AIDS, a dysfunction of the immune system associated with a very high mortality rate. Due to the severity of the disease, FDA approval of AIDS-related drugs has been subject to special accelerated processes. There are currently five classes of anti-retroviral drugas follows:

Nucleoside reverse transcriptase inhibitors (NRTIs):  
A Nucleoside reverse transcriptase inhibitors (NRTIs) binds and inhibits the action of reverse transcriptase to prevent the formation of viral RNA from pro viral DNA causing a decrease in the amount of virus in the body and subsequent spread to other healthy cells. The drugs under this category include Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Emtricitabine and Abacavir.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs):  
A Non-nucleoside reverse transcriptase inhibitors (NNRTIs) inhibits the action of HIV reverse transcriptase but a different site on the enzyme than the site targeted by NRTIs block RNA –dependent DNA polymerase activities.

The drugs under this category include Efavirenz, Nevirapine and Delavirdine. The drugs under this category include Efavirenz, Nevirapine and Delavirdine.

Nucleotid reverse transcriptase inhibitors (NtRTIs):  
A Nucleotide reverse transcriptase inhibitors (NtRTIs) inhibits the activity of HIV-1 reverse transcriptase by competing with natural nucleic acid substrates. The NtRTI is then incorporated in viral nucleic acid, causing termination of chain formation. Ex: Tenofovir.

Protease inhibitors:  
A protease inhibitor (PI) inhibits the protease enzyme, which typically cleaves certain HIV protein precursors that are necessary for the replication of new infectious virions. This mechanism results in the production of immature, non infectious virions. These drugs are typically combined with other anti-retroviral drugs and their use has led to marked clinical improvement and prolonged survival among HIV-infected patients. Because PIs are metabolized through cytochrome P-450, drug interactions are common and can be severe. The drugs under this category include Amprenavir, Fosamprenavir, Indinavir, Lopinavir, Nelfinavir, Ritonavir and Atazanavir.

Fusion inhibitors:  
A fusion inhibitors prevents the AIDS virus (HIV) from entering the immune cells. This is a big advance in HIV drugs block replication of the virus only after it has entered the cell. Ex: Enfuvirtide.
Rationale of combination therapy:
Currently, 11 antiretroviral agents are approved by various health authorities worldwide. None of these agents can eradicate the infection but given in combination they can suppress viral replication, improve immunologic status, delay infectious complications and prolong life.

The high viral turnover rate and the error prone nature of the RNA virus replication make it mandatory for potent combination to be used. The combination treatment is known as highly active antiretroviral therapy (HAART). Using a HAART protocol, HIV replication is inhibited, the presence of HIV-RNA in the plasma is greatly prolonged.

Advantages of combination therapy:
1. Potent inhibition of viral replication
2. Prevention of emergence of resistant strains
3. Sustained clinical improvement
4. Target different cellular reservoirs of HIV
5. Target cells in different stages of activation

Lamivudine and zidovudine combination is most widely used in HAART protocols. Lamivudine is an analogue of cytosine. It is given orally, is well absorbed and excreted unchanged in the urine. The CSF level is 20% of the plasma concentration. Used alone, it could select for HIV mutants that are resistant to both the drug itself as well as other reverse transcriptase inhibitors. Lamivudine is also used in the therapy of hepatitis B infection. Zidovudine is an analogue of thymidine. It can prolong life in HIV-infected individuals and diminish HIV-associated dementia. Given to the parturient mother and then to the new born infant, it can reduce mother-to-baby transmission by more than 20%. It is generally administered orally twice daily but can also be given by intravenous infusion. The bioavailability is 60-80%, and the peak plasma concentration occurs at 30 minutes. Its half life is 1 hour and intracellular half life of the active tri phosphate is 3 hours. The concentration in cerebrospinal fluid (CSF) is 65% of the plasma level. Most of the drug is metabolized to the inactive glucuronide in the liver, only 20% of the active form being excreted in the urine.

Lamivudine and zidovudine are official in IP and USP. Lamivudine and zidovudine combination has significant therapeutic importance. Zidolam tablets (a commercial brand) contain lamivudine (150 mg) and zidovudine (300 mg). Zidolam tablets are used in antiretroviral combination therapy for the treatment of HIV infection. Zidolam tablet reduces the amount of HIV in the body and keeps it at a low level. It also increases CD4 cell counts. CD4 cells are a type of white blood cells that plays an important role in maintaining a healthy immune system to fight against infection.

HPLC Methods of Analysis of Antiretroviral Drugs:
High performance liquid chromatography (HPLC) is a process, which separates mixture containing two or more components under high pressure. In this the stationary phase is packed in a column one end of which is attached to a source of pressurized liquid mobile phase. High performance liquid chromatography is the fastest growing analytical technique for the analysis of drugs. Its simplicity, high specificity and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids. Several HPLC methods were reported for the analysis of antiretroviral drugs in bulk, dosage forms and biological fluids. A summary of research work on HPLC methods reported for the estimation of lamivudine and zidovudine alone and in combination is given in Table 1.

CONCLUSION:
Though several HPLC methods are reported there is a continued need for developing more efficient, sensitive, accurate and precise methods for the analysis of lamivudine and zidovudine alone and in combination in dosage forms and in biological fluids.

Table 1: Summary of Research Work on HPLC Methods for the Estimation of Lamivudine and Zidovudine Alone and in Combination

<table>
<thead>
<tr>
<th>S. No</th>
<th>Drug</th>
<th>Method</th>
<th>Instrument, Mobile Phase, RT</th>
<th>Results of Validation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lamivudine</td>
<td>RP-HPLC</td>
<td>Kromosil C18, 271nm, 0.1% Ortho Phosphoric Acid: Acetonitrile: Methanol</td>
<td>Linearity: 0.1mg/ml, LOD: 20ng</td>
<td>1</td>
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<tr>
<td>2</td>
<td>Lamivudine</td>
<td>HPLC</td>
<td>Octadecylsilanec18, 280 nm, Acetonitrile: Methanol (5-95), RT: 20min</td>
<td>Linearity: 0.2-2mg/ml and 1.6-16µg/ml, Content%= (P&lt;0.05%)</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Zidovudine</td>
<td>RP-HPLC</td>
<td>Nucleosil C18,267nm Methanol, RT: 2.27min</td>
<td>Linearity -400 to 600µg/ml, Percentage Recovery -99.72% (CV&lt; &lt;1%)</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Zidovudine</td>
<td>RP-HPLC</td>
<td>Luna 5u C18, 270mm Acetonitrile: 0.02 M Sodium Dihydrogen Phosphate,(70:30 V/V), RT: 4.5min</td>
<td>Linearity: 10-60µg/ml</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Zidovudine</td>
<td>RP-HPLC</td>
<td>Zodiac C18,270mm Methanol: Acetonitrile (40:60 V/V), RT: 2.51min</td>
<td>Linearity -0.1-0.6µg/ml, LOD: 0.062µg/ml</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Zidovudine</td>
<td>UV</td>
<td>266nm</td>
<td>Linearity=2-20µg/ml, R²= 0.999, Regression Equation -Y=0.0435 C+0.0205, % RSD = 0.630</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Zidovudine</td>
<td>RP-HPLC</td>
<td>Thermohypersilods C18,267nm Methanol: 0.02 M Sodium Dihydrogen Phosphate,(70:30 V/V), RT: 4.5min</td>
<td>Linearity -37.5-225µg/ml (Z) and 75µg to 450µg/ml (L)</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>8</th>
<th>Lamivudine - Zidovudine</th>
<th>RP-HPLC</th>
<th>Symmetry C18, Phosphate Buffer pH 3.6: Methanol (60:40% V/V), RT: 2.338 min (L) and 3.415 min (Z)</th>
<th>Tailing Factor: 1.7, RSD: 0.10 and 0.14 %, Recovery: 98-99%</th>
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</thead>
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<tr>
<td>9</td>
<td>Lamivudine - Zidovudine</td>
<td>RP-HPLC</td>
<td>Pre-Packed Altia 18 5µ, 270nm Ammonium Acetate Buffer: Methanol (80:20)</td>
<td>Linearity: 37.5 - 112.5 mcg/ml (L) and 75 - 225 mcg/ml (Z), Accuracy: 98.50 - 93.30%, Theoretical Plates and Tailing Factor: 3189.33, 1.12 (L) and 7852.83, 1.05 (Z)</td>
</tr>
<tr>
<td>10</td>
<td>Lamivudine - Zidovudine</td>
<td>HPLC,</td>
<td>Hypersil C18, 270nm Methanol; -----</td>
<td>Linearity = 0.1-0.2 µg/ml, Recovery = 100.36 % (L) and 100.46% (Z), LOD = 0.042 mcg/ml (L) and 0.12 mcg/ml (Z), LOQ = 0.039 mcg/ml (L) and 0.12 mcg/ml (Z)</td>
</tr>
<tr>
<td>11</td>
<td>Lamivudine - Zidovudine</td>
<td>UV</td>
<td>279 and 300nm,</td>
<td>Linearity: 10 - 50 mcg/ml Accuracy: 100%, RSD: 0.2 %</td>
</tr>
<tr>
<td>12</td>
<td>Zidovudine - Lamivudine</td>
<td>RP-HPLC</td>
<td>Buffer (0.01m Ammonium Acetate) pH 3.8: Methanol (50:50)</td>
<td>Linearity: 3.75 to 22.5 mcg/ml (L) and 7.5 to 45 mcg/ml (Z), Accuracy: 99.06-99.96% (L), 99.99%-100.25% (Z)</td>
</tr>
<tr>
<td>13</td>
<td>Lamivudine - Efavirenz</td>
<td>RP-HPLC</td>
<td>Luna 5µ C18, 245nm 0.1% Triethylamine (pH 5.11 with 0.1% Orthophosphoric Acid) and Acetonitrile (30:70% V/V), RT: 2.271 min (L), 7.267 min (E)</td>
<td>Validated for linearity, accuracy, precision, LOD, LOQ as per ICH guidelines. No results were given</td>
</tr>
<tr>
<td>14</td>
<td>Lamivudine - Stavudine</td>
<td>RP-HPLC</td>
<td>C18, 257nm Water: Methanol (90:10 V/V), RT: 5.621 min (S) and 4.176 min (L)</td>
<td>Precession: RSD &lt; 2 % Accuracy: 99.2%-101.5% for both drugs. Other parameters are in accordance with ICH guidelines</td>
</tr>
<tr>
<td>15</td>
<td>Lamivudine - Stavudine</td>
<td>RP-HPLC</td>
<td>C18, 254nm Methanol: Acetonitrile and 0.05m Phosphate Buffer of pH 4.5, (60:20:20 V/V) RT: 2.50 min (L), 4.25 min (S)</td>
<td>Linearity: 10 - 602 µg/ml (L), 10-690 µg/ml (S)</td>
</tr>
<tr>
<td>16</td>
<td>Lamivudine - Stavudine</td>
<td>RP-HPLC</td>
<td>Kronosil C18, 265nm 0.2 M Ammonium Acetate Buffer (pH 4.5), Acetonitrile, (1:1) RT: 13.66 min (L), 16.51 min (S)</td>
<td>Linearity: 2.5-50 µg/ml (L) and 0.5-10 µg/ml (S) LOD: 0.82 µg/ml (L) and 0.33 µg/ml (S)</td>
</tr>
<tr>
<td>17</td>
<td>Lamivudine - Stavudine</td>
<td>RP-HPLC</td>
<td>Reverse Phase C18, 266nm Methanol: Water (80:20 V/V)</td>
<td>Accuracy: 97 and 103%, Precession: RSD = 1% for both the drugs</td>
</tr>
<tr>
<td>18</td>
<td>Efavirenz - Lamivudine - Zidovudine</td>
<td>RP-HPLC</td>
<td>Enable C18, 275nm Acetonitrile: 0.02m Sodium Dihydrogen Orthophosphate, pH 3.2 (30:70 V/V), RT: 2.01 min (E), 2.90 min (L), 7.5 min (Z)</td>
<td>Linearity: 75-450 mcg/ml (E), 185-112.5 mcg/ml (L), 37.5-225 µg/ml (Z), Precision: RSD: 0.15% (E), 0.24% (L), 0.37% (Z), LOD: 20 ng/ml (E), 1.0 ng/ml (L), 2.0 ng/ml (Z) LOQ: 50 ng/ml (E), 2.5 ng/ml (L), 5 ng/ml (Z)</td>
</tr>
<tr>
<td>19</td>
<td>Lamivudine - Zidovudine - Abacavir</td>
<td>RP-HPLC</td>
<td>Intersil Ods, 270nm, Ammonium Hydrogen Phosphate: and Diammonium Hydrogen Phosphate buffer of pH 3.9 and Methanol in gradient mode</td>
<td>Validated for specificity, LOD, LOQ, linearity, accuracy and precision as per ICH guidelines. No results were given</td>
</tr>
<tr>
<td>20</td>
<td>Lamivudine - Zidovudine - Nevirapine</td>
<td>RP-HPLC</td>
<td>Nucleodur C18 Buffer: Methanol (60:40 V/V)</td>
<td>Linearity: 24 - 36 mcg/ml (L) and 32 - 48 mcg/ml (N), Precision: RSD &lt; 2 % Recovery (%): -100.313 (L), 100.79 (Z), 99.96</td>
</tr>
<tr>
<td>21</td>
<td>Lamivudine - Zidovudine - Abacavir</td>
<td>UPLC</td>
<td>Symmetry C18, 280nm Buffer: Methanol (60:40), RT: 1.276 min (A), 1.010 min (L), 1.641 min (Z)</td>
<td>Validated for specificity, LOD, LOQ, linearity, accuracy and precision as per ICH guidelines. No results were given</td>
</tr>
<tr>
<td>22</td>
<td>Lamivudine - Zidovudine - Nevirapine</td>
<td>RP-HPLC</td>
<td>Quatsilisib C18 Acetonitrile: Water, pH Adjusted with Ortho Phosphoric Acid To 5.0 (70:30), RT: 3.1 min (L), 4.41 min (Z), 7.0 min (N)</td>
<td>Linearity: 1-1.15 mcg/ml (L), 3.24 mcg/ml (Z), 2.5 - 20 mcg/ml (N)</td>
</tr>
<tr>
<td>23</td>
<td>Lamivudine - Didanosine - Efavirenz</td>
<td>RP-HPLC</td>
<td>Oyster Bds C18, 245nm Water: Acetonitrile: Tetrahydrofuran (45.83: 20.83: 33.34 % V/V), RT: 2.01 min (L), 3.01 min (D), 8.61 min (E)</td>
<td>Linearity: 0.080 mcg/ml - 0.120 mg/ml for all drugs, Accuracy (Assay)-99.98% (L), 99.96% (Z) and 100.14 (E) Recovery (%): 100.7 (L), 100.28 (Z) and 100.45 (E) Precision: RSD &lt; 2.0 %</td>
</tr>
</tbody>
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REFERENCES


