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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 drugsas 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 underthis 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.

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

Nucleotide 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 andAtazanavir

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 a entered the cell.
Ex: Enfuvirtide.

Rationale of combination therapy:

Currently, 11 antiretroviral agents are approved by various health authorities world wide. 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 reduced to undetectable levels and patient survival 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

S. No	Drug	Method	Instrument, Mobile Phase, RT	Results of Validation	Reference
1	Lamivudine	RP-HPLC	Kromosil C18, 271nm, 0.1% Ortho Phosphoric Acid: Acetonitrile: Methanol	Linearity:0.1mg/ml, LOD: 20 ng	1
2	Lamivudine,	HPLC,	Octadecylsilanec18, 280 nm, Acetonitrile: Methanol (5:95), RT : 20min	Linearity: 0.2-2mg/ml and 1.6-16µg/ml, Content= (P<0.05%)	2
3	Zidovudine	RP-HPLC	Nucleosil C18,267nm Methanol, RT: 2.37min	Linearity -400 to1600µg/ml, Percentage Recovery -99.72% (CV= <1%)	3
4	Zidovudine	RP-HPLC	Luna 5µ C18, 270nm Acetonitrile: 0.02 M Sodium Dihydrogen Phosphate,(70:30 V/V), RT: 4.5min	Linearity : 10-60µg/ml	4
5	Zidovudine	RP-HPLC	Zodiac C18,270nm Methanol: Acetonitrile (40:60 V/V) RT :2.51min	Linearity -0.1-0.6µgml-1, LOD-0.062µg/ml	5
6	Zidovudine	UV	266nm	Linearity-2-20µg/ml, R^2= 0.999, Regression Equation -Y=0.0435 C+ 0.0205, % RSD = 0.630	6
7	Zidovudine - Lamivudine	RP-HPLC	Thermohypersilbds C18,267nm Methanol: Acetonitrile (70:5:25)	Linearity = 37.5-225µg/ml (Z) and 75µg to 450µg/ml (L)	7

8	Lamivudine - Zidovudine	RP-HPLC	Symmetry C18, Phosphate Buffer pH-3.6: Methanol (60:40%V/V), RT : 2.338 (L) and 3.415min (Z)	Tailing Factor-1.7, RSD-0.10 and 0.14 %, Recovery-98-99%	8
9	Lamivudine-Zidovudine	RP-HPLC	Pre-Packed Altiac 18 5µ, 270nm Ammonium Acetate Buffer: Methanol (80:20)	Linearity:37.5 -112.5 mcg/ml (L) and 75- 225 mcg/ml (Z), Accuracy: 98.50 - 98.30%, Theoretical Plates and Tailing Factor: 3189.33, 1.12 (L) and 7852.83,1.05 (Z)	9
10	Lamivudine - Zidovudine	HPLC,	Hypersilss C18, 270nm Methanol; -----	Linearity = 0.1-0.2µg/ml, Recovery = 100.36 % (L) and 100.46% (Z), LOD= 0.042 mcg/ml (L) and 0.12 mcg /ml (Z), LOQ- 0.039mcg/ml (L) and 0.12 mcg/ml (Z)	10
11	Lamivudine - Zidovudine	UV	279 and 300nm,	Linearity : 10-50 mcg/ml Accuracy : 100% RSD: 0.2 %	11
12	Zidovudine-Lamivudine	RP-HPLC	Luna 5µ C18, 270nm Buffer (0.01m Ammonium Acetate) pH-3.8: Methanol (50:50)	Linearity: 3.75 to22.5 mcg/ml (L) and 7.5to45mcg/ml (Z), Accuracy: 99.06-99.96% (L), 99.99%-100.25% (Z)	12
13	Lamivudine-Efavirenz	RP-HPLC	Luna 5µ C18,245nm 0.1%Triethylamine (pH-5.11 with 0.1% Ortho Phosphoric Acid) and Acetonirile (30:70%V/V), RT : 2.271 min (L), 7.267min (E)	Validated for linearity, accuracy, precession, LOD, LOQ as per ICH guidelines. No results were given	13
14	Lamivudine - Stavudine	RP-HPLC	C18, 257nm Water: Methanol (90:10 V/V) RT : 5.621 min (S) and 4.176 min (L)	Precession : RSD< 2 % Accuracy: 99.2%-101.5% for both drugs. Other parameters are in accordance with ICH guidelines	14
15	Lamivudine-Stavudine	RP-HPLC	C18, 254nm Methanol: Acetonitrile and 0.05m Phosphate Buffer of pH- 4.5, (60:20:20 V/V) RT : 2.50 min (L), 4.25min (S)	Linearity: 10 - 602 2 µg/ml (L), 10-60µg/ml (S)	15
16	Lamivudine - Stavudine	RP-HPLC	Kromosil C18, 265nm 0.02 M Ammonium Acetate Buffer (pH-4.5), Acetonitrile, (1:1) RT :13.66 min (L),16.51min (S)	Linearity 2.5-50µg/ml(L) and 0.5-10µg/ml (S) LOD:0.82µg/ml (L) and 0.33µg/ml (S)	16
17	Lamivudine-Stavudine	RP-HPLC	Reverse Phase C18, 266nm Methanol: Water (80:20 V/V)	Accuracy : 97and103%, Precession : RSD<1% for both the drugs	17
18	Efavirenz - Lamivudine-Zidovudine	RP-HPLC	Enable C18,275nm Acetonitrile: 0.02m Potassium Dihydrogen Orthophosphate, pH-3.2 (30:70 V/V) RT : 2.01 min (E),2.90 min (L), 7.5min (Z)	Linearity-75-450 mcg/ml (E), 18.75-112.5 mcg /ml (L), 37.5-225µg/ml (Z), Precision : RSD-0.15% (E):0.24% (L):0.37% (Z), LOD-20ng/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)	18
19	Lamivudine-Zidovudine-Abacavir	RP-HPLC	IntersilOds, 270nm, Ammonium Dihydrogen Phosphate: and Diammonium hydrogen Phosphate buffer of pH-3.9 and Methanol in gradient mode	Validated for specificity, LOD, LOQ, linearity, accuracy and precision as per ICH guidelines. No results were given	19
20	Lamivudine-Zidovudine-Nevirapine	RP-HPLC	Nucleodur C18 Buffer: Methanol (60:40 V/V)	Linearity : 24 -36 mcg/ml (L), 48-72 mcg/ml (Z) and 32 – 48 mcg/ml (N) Precision : RSD< 2 % Recovery (%): -100.313 (L),100.79 (Z), 99.96	20
21	Lamivudine-Zidovudine-Abacavir	UPLC	Symmetry C18, 280nm Buffer pH-3: Methanol(60:40), RT : 1.276 min (A),1.010 min (L),1.64min (Z)	Validated for specificity, LOD, LOQ, linearity, accuracy and precision as per ICH guidelines. No results were given	21
22	Lamivudine-Zidovudine-Nevirapine	RP-HPLC	Qualisilbds C18 Acetonitrile: Water, pH Adjusted with Ortho Phosphoric Acid To 5.0 (70:30) RT : 3.1 min (L),4.4min (Z), 7.0 min (N)	Linearity-1-15 mcg/ml (L), 3-24 mcg/ml (Z), 2.5 -20 mcg/ml (N)	22
23	Lamivudine-Didanosine-Efavirenz	RP-HPLC	Oyster Bds C18, 245nm Water: Acetonitrile: Tetrahydrofuran (45.83: 20.83: 33.34 % V/V) RT : 2.01min (L), 3.01 min (D), 8.61min (E)	Linearity :15-90 mcg/ml (L),12.5-75 mcg/ml (D), 30-180µg/ml (E): Recovery : 99.85,99.78,99.94%, LOD : 0.61 mcg/ml (L), 0.43 mcg/ml (D), 0.65 mcg/ml (E) LOQ; 1.85µg/ml (L), 1.31µg/ml (D), 1.97µg/ml (E)	23
24	Lamivudine-Zidovudine-Efavirenz	RP-HPLC	Symmentry C18,250nm Methonal: Water (65:35 V/V) RT :2.51 min (L), 3.01(Z), 24.10(E) min	Linearity: 0.080 mg/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< 2.0 %	24

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