ADDENDUM / CORRIGENDUM

1. Indivudual method, not simultaneous

It is not simultaneous method for Lamivudine, Zidovudine and Nevirapine. Aim of the thesis is to develop new method for Lamivudine, Zidovudine and Nevirapine individually in different classification of drugs. Because highly active antiretroviral therapy has been the standard for the treatment of patients infected with the human immunodeficiency virus (HIV). Three classes of drugs, the nucleoside reverse transcriptase inhibitors (NRTIs), the non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) are primarily used for the treatment of patients with HIV infection.Same solvent system (Methanol: Water, 89:11) is used for Lamivudine, Zidovudine (NRTIs), and Nevirapine is (NNRTIs).So the developed method is useful for Therapeutic Drug Monitoring (TDM) of antiretroviral drugs pharmacokinetics in the field of HIV infected patients.

Developed high performance liquid chromatographic method is simple, reliable, reproducible and economical for the analysis of Lamivudine, Zidovudine and Nevirapine in pharmaceutical formulations. All the parameters for LAM, ZID & NVP drugs met the criteria of ICH guidelines for method validation, the developed method with a shorter chromatographic run time, faster preparation steps with a lower requirement of toxic organic solvents and time consuming in contrast to other methods. The RP-HPLC method should useful for monitoring plasma drug concentration and pharmacokinetic studies in HIV patients and reported method can be used successfully for the effective qualitative and quantitative analysis in bulk and pharmaceutical tablet dosage forms and applied to pharmacokinetic *in-vitro* & *in-vivo* studies.

The major advantage of this method as compared to others previously published methods is shorter chromatographic run time in contrast to other methods. Those methods are,

- **Bin Fan and Stewart, 2002,** has determined Zidovudine, Lamivudine and Nevirapine in human plasma by ion pair HPLC using solid phase extraction and developed with run time 12 min, Others long run time of the analysis has been reported in most of the published papers for some anti retroviral drugs.
- **Zheng J J *et al.*, 2001,** developed a method with run time of 17 min.
- **Hoetelmans R M *et al.*,1998,** developed a method with run time of 50min.
- **Zhou X J *et al.*, 1994,** developed a method with run time of 25min.
- **Alnouti Y *et al.*, 2004,** developed a method with run time of 21min.
- **Kenny K B *et al.*, 2000,** developed a method with run time of 30min.
- **Yazen Alnouti *et al.*, 2004,** determined in plasma, amniotic fluid, and rat tissues by liquid chromatography for Lamivudine. The flow rate was 0.2 mL/min and retention time was 7.0 min in the ionized form and 13.0 min in the unionized form
- **Nandini Pai** and **Desai A D, 2007,** developed simultaneous determination of Lamivudine, Zidovudine and Nevirapine by using mobile phase of 50:50 mixtures of water and buffer (0.1 M ammonium acetate in 5% glacial acetic acid) and retention times of Lamivudine, Zidovudine and Nevirapine were found to be 3.2 min,5.2 min,and 8.8 min respectively at flow rate of 1.0 mL/min.
- **Kapoor N *et al.*, 2006,** has developed simultaneous determination of lamivudine and stavudine in antiretroviral fixed dose combinations by first derivative spectrophotometry and high performance liquid chromatography. The mobile phase used was methanol: water (20:80). Retention time was found to be 8.0 for Lamivudine at the flow rate of 0.6 mL/min.

- **Lefebvre I. *et al.*, 2007,** has developed Zidovudine and its monophophate in cell extracts by on- line solid-phase extraction coupled to liquid chromatography by using an aqueous buffer containing an ion pairing reagent as a mobile phase. Under these chromatography conditions, AZTMP was eluted at 11.3 min and AZT at 15.0 min.
- **Hollanders R M F *et al.*, 2000**, developed a method in phosphate buffer and acetonitrile with flow rate 1.5 ml/min and run time was 6.5 min for Nevirapine.
- **Joesph W P *et al.*, 1999** developed method for Nevirapine LC-8 analytical column with mobile phase phosphate buffer, 1-butane sulfonic acid as anion pair reagent, methanol and acetonitrile and peak area was detected with run time of 10 min at a flow rate of 1.0 mL/min.

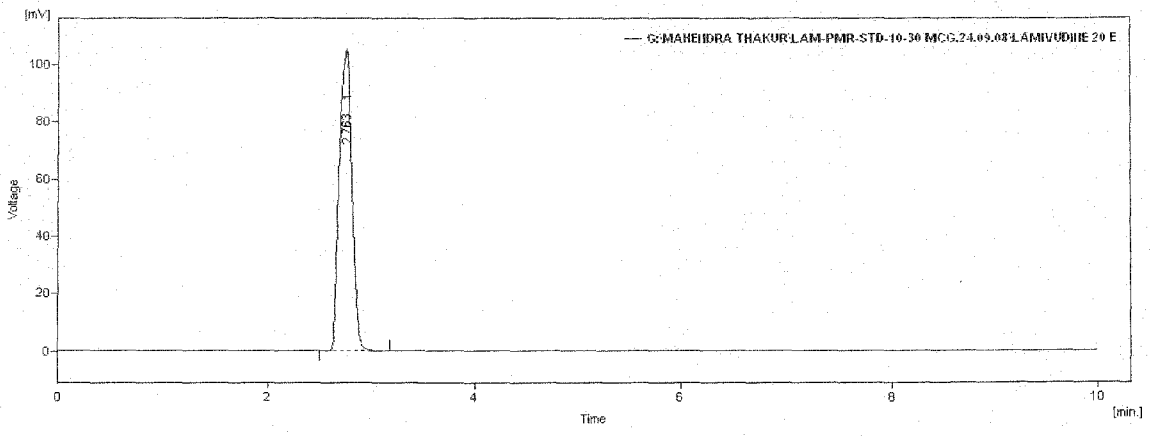
2. Impurity Studies

Yes, the developed method is suitable for profiling of impurities, because Specificity studies has also been done in the thesis for validation. Once a chromatography method is developed, it has to be validated. Method validation is carried out to ensure that an analytical method is accurate, precise, specific, sensitive, reproducible and robust over the specified range that an analyte will be analyzed. So Specificity is the ability of an analytical method to assess unequivocally the analyte in the presence of all potential sample components like Titanium dioxide, Red oxide, Yellow oxide, Talc and Methyl cellulose. In the developed method chromatograms shows that the method is specific. A chromatogram of blank methanol injection without the drugs, chromatogram of drugs blended with excipients shows that the excipients did not interfere with the drugs peak in the thesis Figures. 71-73.

Fig 71

Typical Chromatogram Specificity of Lamivudine

Column Mode	C_{18} RP-HPLC
Detector	UV - Visible
Type of Analysis	Peak Area & Peak height
Detection Limit	272 nm
Flow Rate	1.0 mL/min
Run Time	10 min
Injection volume	20 µL

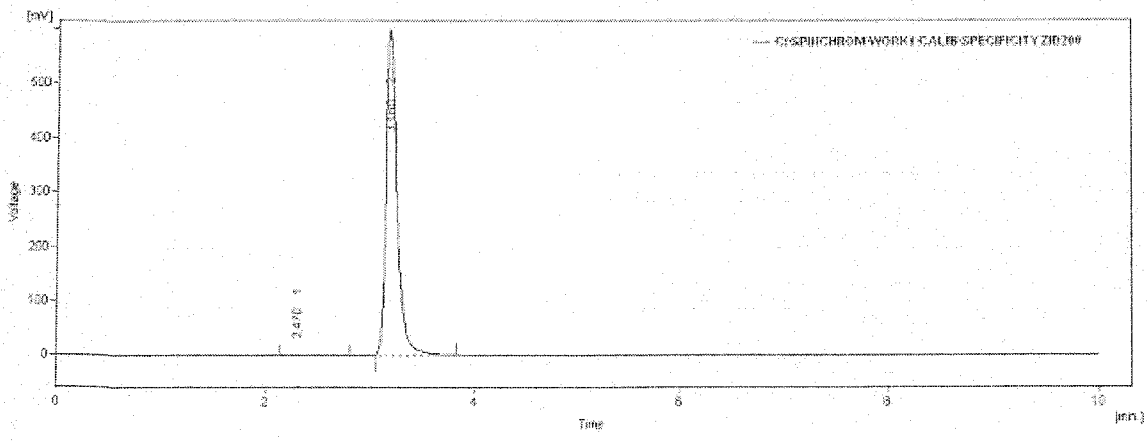


Retention time *(min)*	Peak area *(mV.s)*	Peak height *(mV)*	Area %
2.763	505.536	64.224	100.0
Total	505.536	64.224	100.00

Fig 72

Typical Chromatogram Specificity of Zidovudine

Column Mode	C_{18} RP-HPLC
Detector	UV - Visible
Type of Analysis	Peak Area & Peak height
Detection Limit	272 nm
Flow Rate	1.0 mL/min
Run Time	10 min
Injection volume	20 µL

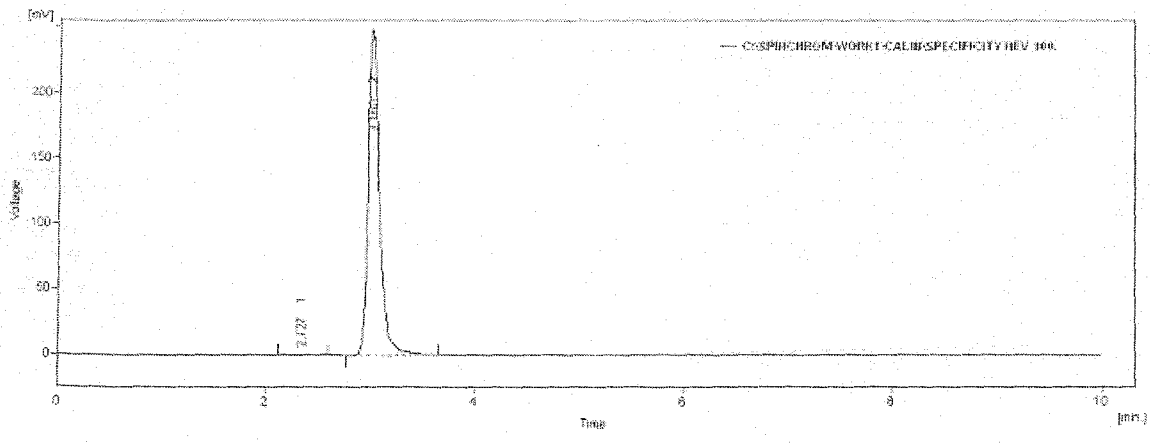


Retention time *(min)*	Peak area *(mV.s)*	Peak height *(mV)*	Area %
2.470	1.917	0.375	4.127
3.160	4431.081	583.011	95.873
Total	4332.998	583.386	100.00

Fig 73

Typical Chromatogram Specificity of Nevirapine

Column Mode	C_{18} RP-HPLC
Detector	UV - Visible
Type of Analysis	Peak Area & Peak height
Detection Limit	272 nm
Flow Rate	1.0 mL/min
Run Time	10 min
Injection volume	20 µL



Retention time *(min)*	Peak area *(mV.s)*	Peak height *(mV)*	Area %
2.721	3.577	0.681	0.1
3.160	3193.281	494.118	99.9
Total	3196.858	494.799	100.00

3. Brief Summary on Stability Studies

Subjecting the API (Active Pharmaceutical Ingredients) or drug product to common stress conditions provides insight into the stability of the analyte under different conditions. The common stress conditions include acidic, basic, different temperature, oxidation and photo degradation. These studies help to determine the significant related substances to be used in method development and to determine the sample solvent that gives the best sample solution stability. Physical (e.g., solubility) and chemical (e.g., UV activity, stability, pH effect) properties of the sample matrix will help to design sample preparation scheme.

To determine whether the method was stability indicating, pure samples were stressed under different conditions to promote degradation according to ICH.

Duration of Stability indicating studies carried for the Lamivudine, Zidovudine and Nevirapine are given in the Table .

Duration of Stability Indicating Studies Carried Out

During Stress studies

STABILITY STUDIES	LAMIVUDINE(HRS)	ZIDOVUDINE(HRS)	NEVIRAPINE(HRS)
Alkali (0.1N NaOH)	1-120	1-72	1-72
Acidicmetry (0.1N HCl)	1-120	1-72	1-72
Oxidation (H_2O_2)	1-48	1-48	1-24
Thermal decomposition	40 ° C-70°C	40 ° C-70°C	40 ° C-70°C
Photolysis (UV light)	1-4	1-7	1-6

Stability indicating studies were performed for Lamivudine 10 µg/mL, Zidovudine 25 µg/mL and Nevirapine 25 µg/mL from pure samples under the different stability indicating studies. The different conditions are Alkali (0.1N NaOH) study, Acidimetric (0.1N HCl) stress study, Oxidation stress study with Hydrogen Peroxide (5%),Thermal decomposition study and Photolysis study with UV Light. The degradation products were completely distinguishable from the parent compound with Retention time 2.710, 2.920 and 3.167. Peak areas were calculated as % RSD and found to be less than 2%. Average % RSD values are given in the Table,

Average %RSD values of Lamivudine, Zidovudine and Nevirapine

Name of the Drug	Stress Studies	Avg % RSD
Lamivudine	Alkali (0.1N NaOH)	0.671
	Acidimetric (0.1N HCl)	1.012
	Oxidation (H_2O_2)	0.83
	Thermal decomposition	0.933
	Photolysis (UV light)	1.236
Zidovidine	Alkali (0.1N NaOH)	0.395
	Acidicmetry (0.1N HCl)	0.602
	Oxidation (H_2O_2))	0.471
	Thermal decomposition	0.134
	Photolysis (UV light)	0.263
Nevirapine	Alkali (0.1N NaOH)	0.14
	Acidicmetry (0.1N HCl)	0.986
	Oxidation (H_2O_2)	0.629
	Thermal decomposition	0.827
	Photolysis (UV light)	0.295

Kinetic Studies

The purpose of carrying kinetic studies is to examine the developed method is able to quantify and separate the free drug from the biological samples. As the evaluator mentioned the work was additional to prove our method can be used in biological samples. And the grapes were drawn on the basis of the data available. Hence we carried out the kinetic studies to study the sensitivity of the method in biological samples which our method is able to detect and quantify the drugs.In given table below explain about drug release kinetic models.

Table 97 Pharmacokinetic Assessment of Release Data of Lamivudine at Different Group

Kinetics	Parameters	Group A	Group B	Group C	Group D
Zero order	Kr^0	0.319	0.997	0.834	0.818
	r^2	0.206	0.331	0.231	0.240
First order	Kr^1	0.061	0.060	0.061	0.061
	r^2	0.156	0.153	0.157	0.157
Higuchi	Kr^2	2.684	2.947	2.497	2.447
	r^2	0.331	0.351	0.250	0.260
Korsmeyer & Peppas	Kr^3	0.382	0.407	0.386	0.385
	r^2	0.533	0.577	0.542	0.562

r^2 is the coefficient of correlation;

Kr^0, Kr^1, Kr^2 and Kr^3 are the release rate constants for Zero order, First order, Higuchi models and Korsmeyer & Peppas, respectively.

Signature of the Candidate

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30/01/2011

Signature of the Supervisor

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