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R E S E A R C H A R T I C L E

Development and Validation of a HPLC Method for Nelfinavir and Tenofovir in Pharmaceutical Dosage Forms

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A B S T R A C T

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Nelfinavir and Tenofovir in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. From literature review and solubility analysis initial chromatographic conditions Mobile phase ACN: ortho Phosphate buffer pH 4.0 (70: 30 % v/v) were set (Buffer P^H 2.45 adjusted with Triethylamine), Agilent C18 column (4.6×150mm) 5µ Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 230 nm. As the methanol content was increased Nelfinavir and Tenofovir got eluted with good peak symmetric properties. The retention times for Nelfinavir and Tenofovir was found to be 2.462 & 3.737 min respectively. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 50% to150 % levels, R^2 value was found to be as 0.999 by using above method assay of marketed formulation was carried out, 100.7% was present. Full length method was not performed; if it is done this method can be used for routine analysis of Nelfinavir and Tenofovir.

Keywords: HPLC, Nelfinavir, Tenofovir, Ortho Phosphoric Acid Buffer: ACN, Agilent C18.

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Nelfinavir is a potent HIV-1 protease inhibitor. It is used in combination with other antiviral drugs in the treatment of HIV in both adults and children. Nelfinavir inhibits the HIV viral proteinase enzyme which prevents cleavage of the gag-pol polyprotein, resulting in noninfectious, immature viral particles.

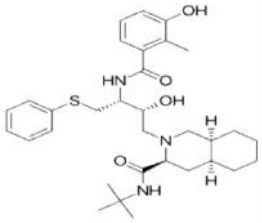

Figure 1 Nelfinavir

IUPAC Name: (3S,4aS,8aS)-N-tert-butyl-2-[(2R, 3R)-2-hydroxy-3-[(3-hydroxy-2-methylphenyl)formamido]-4-(phenylsulfanyl)butyl]-decahydroisoquinoline-3-carboxamide
Chemical formula : $C_{32}H_{45}N_3O_4S$
Molecular weight : 567.782

Tenofovir inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination

Figure 2 Tenofovir

IUPAC Name: 4-(1-hydroxy-2-{[6-(4-phenylbutoxy) hexyl] amino} ethyl)-2-(hydroxymethyl)phenol
Chemical formula: $C_{25}H_{37}NO_4$
Molecular weight: 415.5656 g/mol
pKa: 2.86

2. Methodology

Table.1 List of chemicals and standards used

S.No	Chemicals	Manufacturer Name	Grade
1.	Water	Merck	HPLC grade
2.	Methanol	Merck	HPLC grade
3.	Acetonitrile	Merck	HPLC grade
4.	Ortho phosphoric acid	Merck	G.R
5.	KH_2PO_4	Merck	G.R
6.	K_2HPO_4	Merck	G.R
7.	0. 22µ Nylon filter	Advanced lab	HPLC grade
8.	0.45µ filter paper	Millipore	HPLC grade
9.	Nelfinavir and Tenofovir	In – House	In- House

Trial-1
Chromatographic conditions
Column : Symmetry C18 4.6x150mm, 5µm
Mobile phase ratio: ACN: H2O (70:30%v/v)
Detection wavelength : 240nm
Flow rate: 1ml/min
Injection volume : 10µl
Run time : 5min
Retention time: 1.250 min&1.496 min

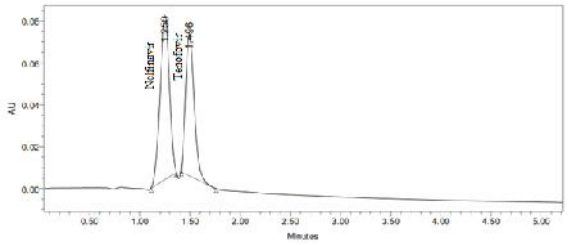

Figure 3. Chromatogram showing trial 1 injection

Observation: The trial shows no proper separation of peaks in the chromatogram, so more trials were required for obtaining peaks.

Trial - 2
Chromatographic conditions
Column : Inertsil C18 4.6x150mm 5µm
Mobile phase ratio: Methanol: H2O (70:30%v/v)
Detection wavelength: 230 nm
Flow rate: 1ml/min
Injection volume : 10µl
Column temperature: Ambient
Auto sampler temperature: Ambient
Run time: 10.0 min
Retention time: 1.536 min & 3.584 mins

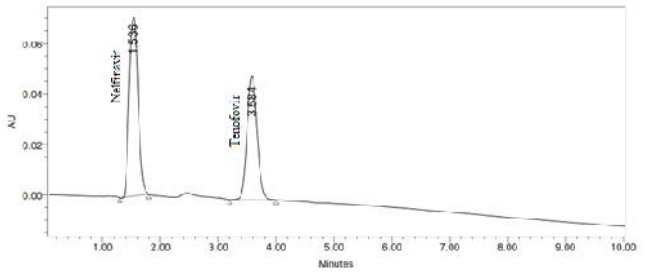

Figure 4. Chromatogram showing trial-2 injection

Observation
In this trial both peaks were eluted well, but there was no proper baseline. Need some more trails.

Optimized method:

Chromatographic conditions

Column : Agilent column (4.6×150mm)5µ

Mobile phase ratio: ACN: Phosphate buffer pH 4.0 (70: 30 % v/v)

Detection wavelength: 230 nm

Flow rate: 1.0ml/min

Injection volume : 10µl

Column temperature: Ambient

Auto sampler temperature : Ambient

Run time: 10min

Retention time: 2.462 & 3.737mins

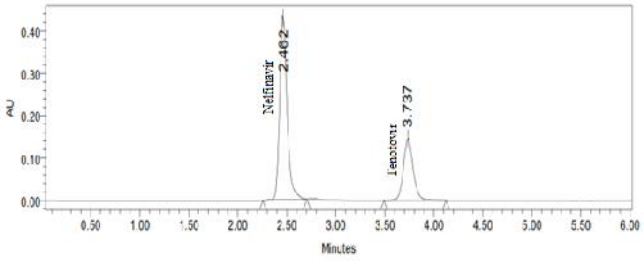

Figure 5. Optimized Chromatogram

Observation

The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Preparation of phosphate buffer

2.95 grams of $KH_2PO_4$and 5.45 grams of K_2HPO_4 was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 300 ml (30%) and 700 ml of methanol (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation

Mobile phase was used as the diluent.

Preparation of the Nelfinavir and Tenofovir standard and sample solution

Sample solution preparation:

An equivalent tablet power such that 5 mg of Nelfinavir and 2 mg Tenofovir tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 1ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent. (Concentration is 50 ppm for Nelfinavir and 20 ppm for Tenofovir)

Standard solution preparation

5 mg Nelfinavir and 2 mg Tenofovir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. (Concentration is 50 ppm for Nelfinavir and 20 ppm for Tenofovir)

System suitability

5 mg of Nelfinavir and 2mg of Tenofovir working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of Nelfinavir and Tenofovir from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. (Concentration is 50 ppm for Nelfinavir and 20 ppm for Tenofovir)

3. Results and Discussion

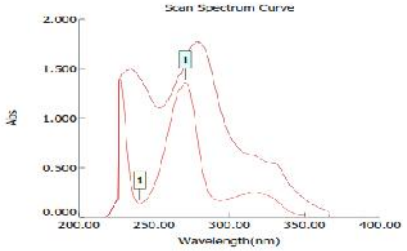

Figure 6. Spectrum showing overlapping spectrum of Nelfinavir and Tenofovir

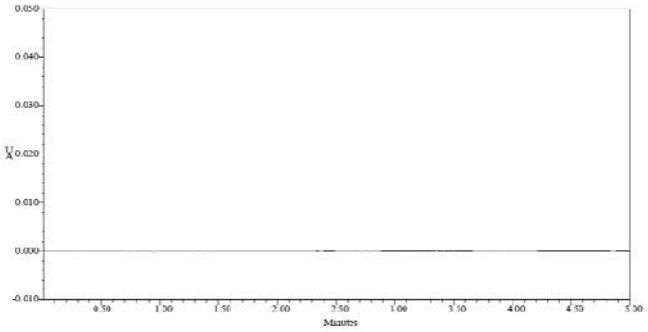

Figure 7. Chromatogram showing blank preparation (mobile phase)

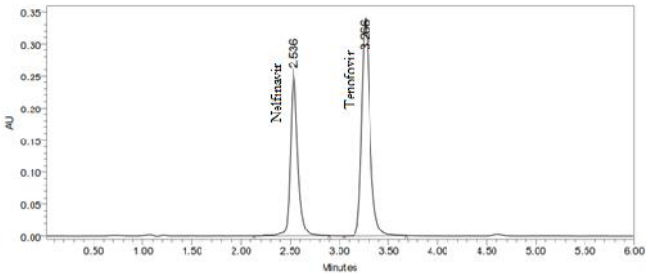

Figure 8. Assay calculation for Nelfinavir and Tenofovir

	Name	Rt	Area
1	Nelfinavir	2.536	2497318
2	Nelfinavir	2.536	2494274
Mean			2495796
Std.dev			1152.9
%RSD			0.06

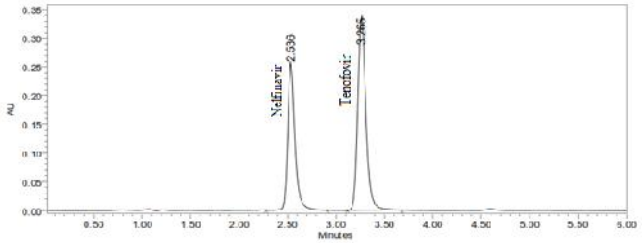

Figure 9. Assay calculation for Nelfinavir and Tenofovir

	Name	Rt	Area
1	Tenofovir	3.265	958190
2	Tenofovir	3.265	965083
Mean			961637
Std.dev			38733
%RSD			0.48

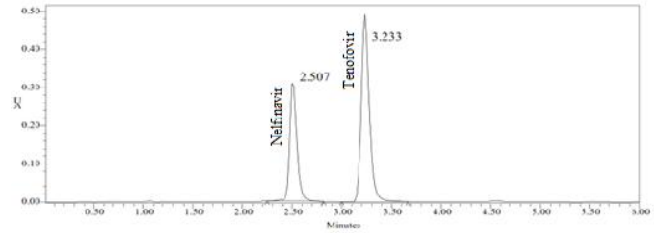

Figure 10. Chromatogram showing standard injection

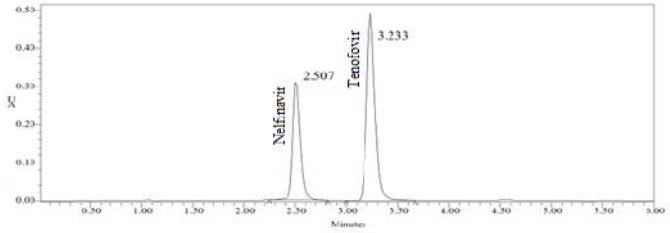

Figure 11. Chromatogram showing sample injection

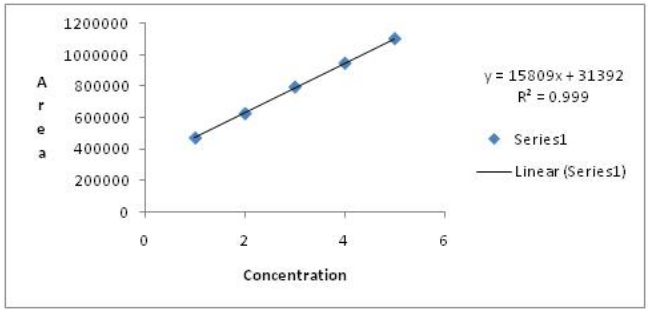

Figure 12. Calibration curve of Nelfinavir

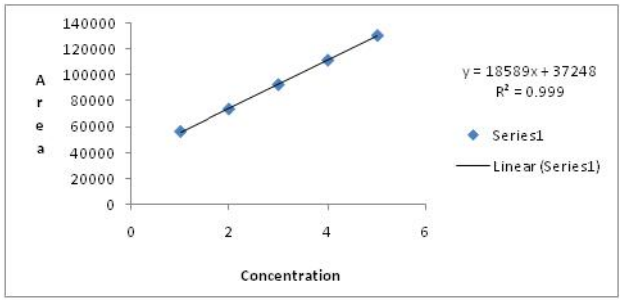

Figure 13. Calibration curve of Tenofovir

Table 2. Showing %RSD results for Tenofovir

	Peak name	RT	Area
1	Tenofovir	3.230	925541
2	Tenofovir	3.239	923214
3	Tenofovir	3.246	923881
4	Tenofovir	3.257	920840
5	Tenofovir	3.271	926447
Mean			923984.6
Std.dev			1948.274
%RSD			0.210856

Table 3. Showing %RSD results for Nelfinavir

	Peak name	RT	Area
1	Nelfinavir	2.506	2367917
2	Nelfinavir	2.516	2324161
3	Nelfinavir	2.519	2390163
4	Nelfinavir	2.531	2323428
5	Nelfinavir	2.544	2329454
Mean			2347025
Std.dev			27150.26
%RSD			1.156795

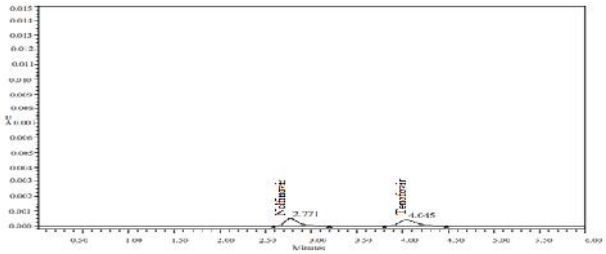

Figure 14. Showing results LOD

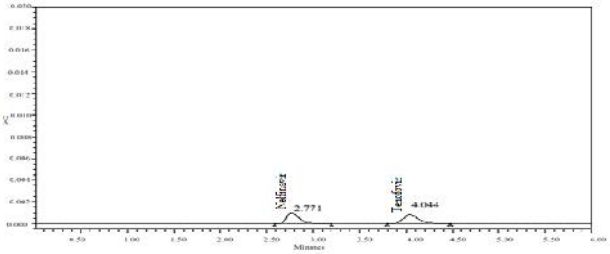

Figure 15. Showing results LOQ

4. Conclusion

A new method was established for simultaneous estimation of Nelfinavir and Tenofovir by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Nelfinavir and Tenofovir by using Agilent column (4.6×150mm)5µ, flow rate was 1.0 ml/min, mobile phase ratio was (70:30 v/v) ACN: phosphate buffer($KH_2PO_4$and K_2HPO_4) phosphate pH 4.0 (pH was adjusted with orthophosphoricacid),detection wavelength was 230 nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.462 & 3.737mins. The % purity of Nelfinavir and Tenofovir was found to be 99.87% and

100.27% respectively. The system suitability parameters for Nelfinavir and Tenofovir such as theoretical plates and tailing factor were found to be 2733, 1.6 and 3500 and 1.4, the resolution was found to be 4.6. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)).

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