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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF TENOFOVIR BY UV SPECTROPHOTOMETRY

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ABSTRACT

A rapid, specific and economic UV spectrophotometric method has been developed using water as solvent to determine the tenofovir content in bulk and pharmaceutical dosage formulations. At a pre-determined λ max of 260 nm, it was proved linear in the range of 10-60 µg/ml and exhibited good correlation coefficient (0.0206 and 0.9808) and excellent mean recovery (99.00-00.07%). This method was successfully applied to the determination of tenofovir content in marketed brands from India and the results were in good agreement with the label claim. The method was validated statistically and by recovery studies for linearity, precision, repeatability, and reproducibility. The obtained results proved that the method can be employed for the routine analysis of tenofovir in bulks as well as in the commercial formulations.

KEYWORDS

Anti-viral drug- HIV-I infection, Tenofovir, UV spectrophotometric method and Validation.

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INTRODUCTION[1-5]

Tenofovir is chemically {[(2r)-1-(6-amino-9h-purin-9-yl] propan-2-l] oxy} methyl) phosphonic acid. The Structure of tenofovir was shown in Figure No.1. It is an Anti-viral drug- HIV-I infection and also used in the treatment of chronic hepatitis b. Tenofovir is a nucleotide analogue reverse-transcriptase inhibitor. It is analogue of the naturally occurring deoxynucleotides needed to synthesize the viral DNA. It competes with the natural deoxynucleotides for incorporation into the growing viral DNA chain. It inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxydenosine 5'-triphosphate and after incorporation into DNA by DNA chain termination. As the tenofovir lacks a 3'-hydroxyl

group on the deoxyribose moiety which is found in the natural deoxynucleotides substrates. Hence, incorporation of drug cannot form the next 5'-3' phosphodiester bond needed to extend the DNA chain in the virus. Thus, whenever halting of this NtRTI in the viral DNA occurs chain termination. Literature survey reveals that there is no method has been reported for the estimation of tenofovir by U.V. spectroscopy method. The main objective of the research work was to develop a simple, sensitive, rapid UV-Visible spectroscopic method for detection of tenofovir and validate as per ICH guidelines.

MATERIAL AND METHODS

Apparatus

A Shimadzu UV-visible spectrophotometer (UVmini-1700 Pharma sec, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with matched quartz cells.

Materials

All chemicals and reagents were of analytical or HPLC grade. Tenofovir in the form of tenofovir powder and tablets were provided by Arvind remedies Pvt Ltd., Chennai, which was used as the reference standard.

Determination of wavelength of maximum absorption[6-11]

A standard stock solution (LS) of tenofovir (1mg/mL) was prepared using water as diluent and 1mL of LS was then diluted to 10mL with the same diluent to get 100µg/ml. Further the above solution was diluted to obtain 10µg/ml tenofovir reference solution (LR). An UV spectroscopic scanning (190-400 nm) was carried out with the LR and the wavelength with maximum absorbance (λ max) was selected as 260 nm by using water as blank. The spectrum of tenofovir standard was shown in Figure No.2.

METHOD VALIDATION[12-18]

Linearity and range

Tenofovir was found to be linear in the concentration range of 10-60 µg/ml. The absorbance of the solution was noted at the selected wavelength 260 nm. Calibration curve was plotted using concentration Vs absorbance. At a wavelength of 260 nm slope and correlation coefficient values were found to be 0.0206 and 0.9808 respectively. Limit of detection (LOD) and limit of quantification (LOQ) for the assay were also calculated. The results were given in Table No.1 and Figure No.3.

Intra-day precision (repeatability) and inter-day precision study (intermediate precision)

Intraday precision studies was performed by preparing the standard solution and observed at three different times of same concentration 10µg/ml and measuring the absorbance of drug at 260 nm. Inter day Precision studies was performed by preparing the standard solution and observed at three different days of same concentration 10 µg/ml and measuring the absorbance of drugs at 260 nm. The results for the Intraday and interday precision are given in Table No.2 and 3.

Accuracy/recovery study

To study the reliability, suitability and accuracy of the method, recovery studies were carried out. To the formulation equivalent to 10mg of tenofovir at the levels of 80%, 100% and 120% were added. The concentration of drugs present in resulting solution was determined using assay method; percentage recovery and percentage RSD were calculated. The results for the recovery study are given in Table No.8

Assay of content of tenofovir

To determine the purity of the drug assay studies were performed. In assay method both the API of tenofovir and the formulated form (tablet) of tenofovir were taken. Standard solutions of tenofovir in bulk was carried out by accurately weighing 25mg of drug in 25ml (1000µg/ml) of distilled water. Working dilutions 100µg/ml, 10µg/ml were done from the standard solution, absorbance were measured. Standard solutions of tenofovir in formulated form was carried out by accurately weighing the tablet powder equivalent to 50mg of drug in 50ml (1000µg/ml) of distilled water. Working dilutions 100µg/ml, 10µg/ml were done from the stock solution, absorbance were measured and percentage purity was calculated.

Ruggedness

Ruggedness is reproducibility under normal but variable laboratory conditions. It is done by 2

methods. In one method, three working standard dilutions of 10µg/ml by 2 different analysts were prepared and tested their absorbances at fixed wavelength in the same equipment and in another method, three working standard dilutions of 10µg/ml were prepared by the same analyst and the measurement of absorbance was done at 2 different systems. The results for the ruggedness are given in Table No.4-6

Robustness

Robustness is ability to remain unaffected by small changes in parameters. It was carried out by calculating absorbances with the same concentration (10µg/ml) of solution at different wave lengths (258nm, 260nm, 262nm). The results for robustness are given in Table No.7

RESULTS AND DISCUSSION[19-20]

A simple, accurate and precise UV spectroscopic method was developed for the estimation of tenofovir in Pharmaceutical dosage forms. Analytical method development was done based on the detection of wave length. Selection of the solvent was done based on the solubility of the tenofovir. Validation of the developed method was done as per the ICH guidelines Q_2 (R1).

The validated parameters such as precision, assay, accuracy, linearity, ruggedness, robustness, limit of detection, limit of quantification were evaluated. The precision studies were carried out in intraday and inter day method and the mean, standard deviation (SD) and percentage (%RSD) were calculated and found to be within the limit that is less than 2%. Accuracy is reported as % nominal of the analyzed concentration. The results indicate that the recovery of tenofovir was consistent at all levels and the percentage nominal of the tenofovir was in between 80% to 120% respectively. Linearity and range of the methods were analyzed by preparing calibration curves using different concentrations range of standard tenofovir (10 to 60µg/ml) solutions. The calibration curve was plotted using absorbance and concentration of the standard solutions. The results revealed that linear regression equation for tenofovir was y =0.0206x with correlation coefficient (R^2) value 0.980 respectively. The limit of detection and limit of quantification of tenofovir was found to be 0.1µg/ml and 0.3µg/ml respectively. The method was found to be rugged and robust since there was no change in the results. The assay of tenofovir was performed and the percentage purity found to be 101.31% w/v was within range.

Table No.1: Linearity

S.No	Concentration (µg/ml)	Absorbance
1	10	0.2034
2	20	0.4154
3	30	0.5705
4	40	0.7910
5	50	1.1303
6	60	1.1964

Table No.2: Intraday Precision

S.No	Hours	Concentration(µg/ml)	Absorbance	AVG	S.D	% RSD
1	10 AM	10	0.2358 0.2354 0.2359	0.2357	0.000265	0.112251
2	1 PM	10	0.2350 0.2353 0.2353	0.2352	0.000173	0.073642
3	4 PM	10	0.247 0.249 0.243	0.24633	0.003055	1.2401

Table No.3: Inter day Precision

S.No	Day	Concentration(µg/ml)	Absorbance	AVG	S.D	% RSD
1	1	10	0.239 0.242 0.237	0.2393	0.002517	1.051509
2	2	10	0.2350 0.2353 0.2353	0.2352	0.000173	0.073642
3	3	10	0.2315 0.2352 0.2306	0.2324	0.00243	1.0488

Table No.4: Ruggedness (Analyst-I)

S.No	Absorbance	AVG	S.D	% RSD
1	0.229	0.22503	0.00189	0.840021
2	0.2265			
3	0.2257			

Table No.5: Ruggedness (Analyst-II)

S.No	Absorbance	AVG	S.D	% RSD
1	0.2108	0.207133	0.003807	1.837952
2	0.2074			
3	0.2032			

Table No.6: Ruggedness (system to system variation)

S.No	Single Beam	Double Beam
1	0.2479	0.248
2	0.2466	0.246
3	0.2465	0.0248
AVG	0.247	0.248
S.D	0.000781	0.02
% RSD	0.316204	0.806452

Table No.7: Robustness

S.No	At 258 nm	At 260 nm	At 262 nm
1	0.2218	0.2461	0.2193
2	0.2217	0.2473	0.2190
3	0.222	0.2472	0.2191
AVG	0.221833	0.2468	0.219133
S.D	0.000153	0.000666	0.000153
% RSD	0.06885	0.269714	0.069708

Table No.8: Accuracy

S.No	Level	80%	100%	120%
1	% Recovery	80.04	100.07	120.38

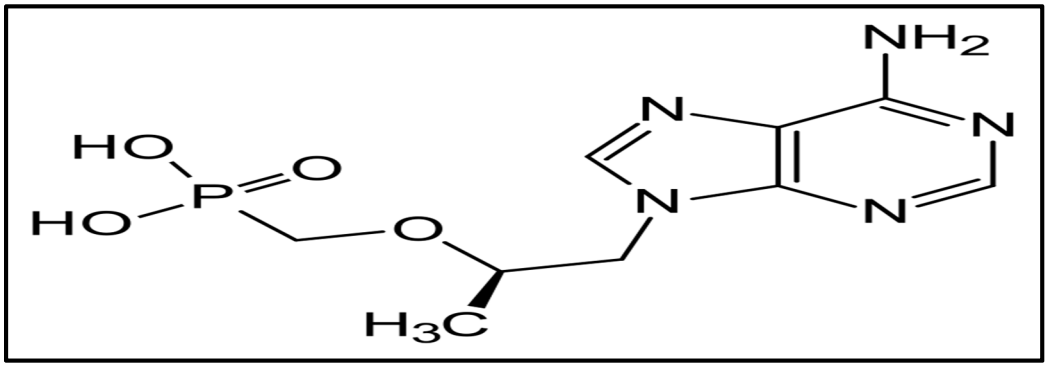

Figure No.1: Structure of tenofovir

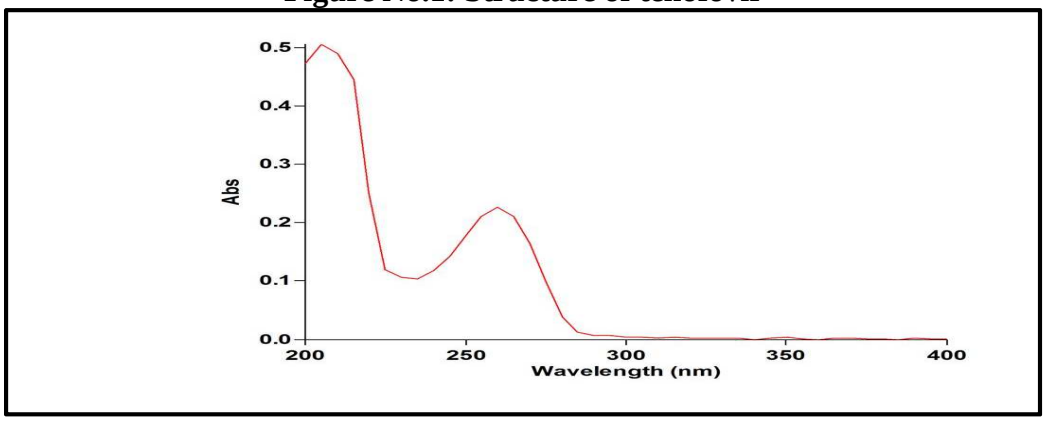

Figure No.2: UV Spectrum of Tenofovir standard

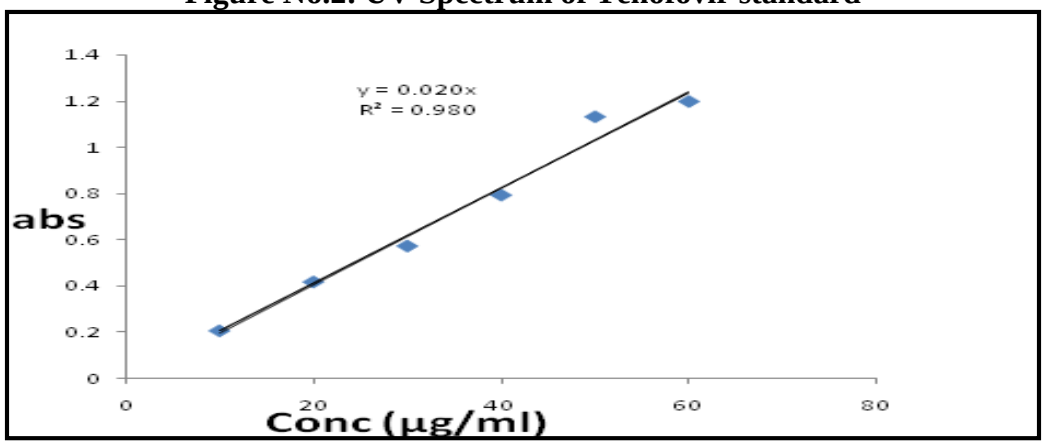

Figure No.3: Linearity curve of tenofovir

CONCLUSION

Literature survey revealed that there was no method has been reported for the estimation of tenofovir by U.V. spectroscopy method. The developed UV spectroscopic method for estimation of tenofovir was found to be simple, precise, accurate and sensitive. The method was validated as per ICH guidelines. Hence, the developed method was found to be satisfactory and can be used for routine analysis of tenofovir in bulk and tablet dosage form.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Beckett A H and Stenlake J B. "Practical pharmaceutical chemistry", *CBS publications, New delhi*, 4th Edition, Part- 2, 2007.
2. Sharma Y R. "Text elementary organic spectroscopy principles and chemical applications", *Chand publications*, Multi colour edition, Revised Edition, 1980, 376.
3. Ravi Shankar S. "Text book of pharmaceutical analysis", *Rx publications, Tirunelveli*, 3rd Edition, 2006, 440.
4. Sharma B K. "Text book of instrumental of chemical analysis", *Geiol publishing house*, 27th Edition, 2000, 308.
5. Gurdeep Chatwal R. Text book of instrumental methods of chemical analysis, *Himalaya publishing house*, 5th Edition, 2012, 424.
6. Arun Ramaswamy, Anton Smith Arul Gnana Dhas. "Development and validation of analytical method for quantitation of Emtricitabine, Tenofovir, Efavirenz based on HPLC", *Arabian Journal of Chemistry*, 2(4), 2014, 1-7.
7. Syed Sajjad Hussen, Premnath Shenoy, Udu pa, Muddu Krishna. "Development and validation of Stability Indicating RP-HPLC Method for Tenofovir Nanoparticle Formulation", *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(2), 2010, 45-55.
8. Akula Srinath, Sneha, Akhila Alladi, Rayees Ahmed and Kulkarni. "Method Development and Validation for Simultaneous Estimation of Lamivudine, Tenofovir and Efavirenz in Combined Tablet Dosage Form by RP-HPLC and Uv-Spectroscopic Method", *IJPSR*, 5(12), 2014, 37-45.
9. Sivaram Manavarthi and Gurmeet Singh Chhabra. "Stability Indicating RP-HPLC Method Development and Validation of Tenofovir in Bulk and Pharmaceutical Formulation", *Der Pharma Chemical*, 6(2), 2014, 401-409.
10. Mohan Goud V, Srinivasa Rao, Ranjith Kumar. "Method Development and Validation for Simultaneous Determination of Lamivudine and Tenofovir in Tablet Dosage Form by Rp- HPLC", *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(3), 2013, 10-18.
11. Bojja Soumya, Thimmaraju Manish Kumar and Nerella Raghunandhan. "Simultaneous Determination of Tenofovir disoproxil fumarate and Lamivudine by UV Spectro photometric method", *International Journal of Pharmacy and Pharmaceutical Science Research*, 2(1), 2012, 9-15.
12. International conference on harmonization, "Validation of analytical procedures Methodology", 14, *Federal Register*, 62(96), Nov.1996, 1-8.
13. Ashok Gorja, Jahnavi Bandla. "Method Development and Validation for the Simultaneous Estimation of Lamivudine and Tenofovir Disoproxil Fumarate in Pharmaceutical Dosage Forms by Rp-Hplc", *IJARPB*, 2(1), 2013, 22-32.
14. Mangoankar K and Desai A (2008). "Simultaneous estimation of Emtricitabine, Tenofovir disoproxil fumarate and Efavirenz from tablets by Reverse Phase High Performance Liquid Chromatography method", *Indian Drugs*, 45(3), 2015, 188-92.
15. Naresh Chandra Joshi, Pradeep Kumar, Rakesh Kumar Jat. "Development and Validation of Rp-Hplc Method for the Estimation of Antiretroviral Drugs and Their Pharmaceutical Formulations", *International Journal of Drug Research and Technology*, 6(3), 2016, 228-238.
16. Honey Diana B, Bibi Khader Shaik, Sita Kumari K. "New validated RP-HPLC method for simultaneous estimation of Lamivudine and tenofovir disoproxil fumerate in tablets", *International journal of advances in pharmaceutical analysis*, 5(1), 2015, 10-15.

17. Anil Dubbaka, Sireesha and Vasudha Bakshi. "Analytical Method Development and Validation for the Simultaneous Estimation of Lamivudine and Tenofovir Disoproxil Fumarate by RP-HPLC Method, *MOJ Proteomics and Bioinformatics,* 4(5), 2016, 126-134.
18. Rajesh Sharma and Pooja Gupta. "A Validated RP - HPLC Method for Simulataneous Estimation of Emtricitabine and Tenofovir Disoproxil Fumarate in a Tablet Dosage Form", *Eurasian J. Anal. Chem*, 4(3), 2009, 276-284.
19. Shirkhedkar Atul, Bhirud Charushila, Suran a. "Application of UV spectrophotometric Methods for Estimation of Tenofovir Disoproxil Fumarate in Tablets", *Pak. J. Pharm. Sci*, 22(1), 2009, 27-29.
20. Sagar S P, Pratik D, and Annapurna M M. "Developed a stability indicating ion-pair RP- HPLC method for estimation of tenofovir disoproxil", *International journal of analytical sciences,* 1(3), 2013, 478-81.

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