RESEARCH ARTICLE

Forced Degradation Study on Stability Indicating RP-HPLC Method for Estimation of Stavudine and Zidovudine in Combined Dosage Form

Ankit Anchaliya*, Rajesh Nagar, Sudha Vengurlekar, Sachin K Jain

Faculty of Pharmacy, Oriental University, sanwer road Indore 453555, India

Received: 8th Sep,2024; Revised: 21st Oct, 2024; Accepted: 11th Nov, 2024; Available Online: 25th Dec, 2024

ABSTRACT

For the simultaneous measurement of Stavudine and Zidovudine, a novel stability indicating reversed phase high-performance liquid chromatographic technique was created and effectively validated. Chromatographic separation was accomplished using an analytical column, which was kept at 30ºC. At a flow rate of 0.9 mL/min, a buffer (pH 4.6): acetonitrile (80:20) combination was used as the mobile phase. Stavudine and zidovudine were detected at a UV wavelength of 283 nm. Adequate separation of aged and stress-degraded Stavudine and Zidovudine stability samples proved the method's stability-indicating capacity. The International Conference on Harmonization (ICH Q2B) criteria were followed in determining several analytical performance characteristics, including system appropriateness, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness. For every analyte in the target concentration range, the calibration curves' linearity was excellent (r2>0.999).

How to cite this article: Ankit Anchaliya, Rajesh Nagar, Sudha Vengurlekar, Sachin K Jain. Forced Degradation Study on Stability Indicating RP-HPLC Method for Estimation of Stavudine and Zidovudine in Combined Dosage Form. International Journal of Pharmaceutical Quality Assurance. 2024;15(4): 2611-16. doi: 10.25258/ijpqa.15.4.64

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Numerous spectrophotometric and chromatographic techniques, such as UV, colorimetric determination, ratio derivative, and stability indicating HPLC methods, have been reported for the determination of Stavudine and Zidovudine, either alone or in combination with other medications, according to a review of the literature. However, there has been no report of a stability-indicating HPLC technique for the simultaneous quantitative measurement of zidovudine and stavudine in the combination dose form. According to the International Conference on Harmonization's (ICH) current drug stability test guidance Q1A (R2), stress tests should be performed on a drug product to determine its inherent stability characteristics. This will help identify degradation products and support the appropriateness of the suggested analytical procedures. Additionally, it mandates that analytical test methods for stability samples be completely verified and stability indicated. In addition to developing and validating a quick stability-indicating reverse phase assay technique, the current work aimed to establish the inherent stability of Stavudine and Zidovudine via stress experiments conducted under a range of ICH approved test settings.

MATERIALS AND METHODS

Chromatography Conditions

Chromatographic separation was carried out at a wavelength of 283 nm using an HPLC (Alliance with PDA detector). The chromatographic runtime was 10 minutes, the flow rate was 0.7 mL/min, the injection volume was 20 µl, and the mobile phase mixture was acetonitrile: 0.1% OPA in a 55:45% V/V ratio taken in an 80:20 ratio. The column with the reverse phase C18 COSMOSIL (250 x 4.6 mm, 5 microns) was used at 30ºC.

Preparation of Buffer Solution

Precisely measured and transferred 1 milliliter of concentrated orthophosphoric acid into 1000 milliliters of volumetric flaskadd, approximately 900 milliliters of milli-Q water was added, and the volume was finally made up with water. 0.5 milliliters of triethylamine were then added, and the pH was adjusted to 4.6 using a diluted orthophosphoric acid solution.

Stability Studies

The stability studies were carried out by attempting deliberate degradation of the sample with exposure to stress conditions like acidic (2N HCl), alkaline (2N NaOH), 105ºC dry heat, oxidizing agents (H_2O_2), Photo Stability and neutral stability.

Oxidation

One milliliter of 20% hydrogen peroxide (H2O2) was added to one milliliter of the Stavudine and Zidovudine stock solution. The solution was maintained at 600C for 30 minutes. After diluting the resulting solution to achieve concentrations of 150 and 300 µg/ml, 10 µl of the solution was injected into the system, and chromatograms were collected to evaluate the sample's stability for the HPLC investigation.

Acid Degradation Studies

1 ml of 2NHydrochloric acid was added to 1 ml of stock solution of Stavudine and Zidovudine, and the mixture was refluxed for 30 minutes at 600C. After diluting the resulting solution to achieve concentrations of 150 and 300 µg/ml, 10 µl of the solution was injected into the system, and

**Author for Correspondence: prachu5316@gmail.com*

Table 1: Results of percent degradation for Stavudine and Zidovudine

Parameter	% Degradation (Stavudine)	% Degradation (Zidovudine)
Acid Degradation Study		
In 1 N HCl for 12 Hour	No degradation	No degradation
In 1 N HCl for 24 Hours	No degradation	No degradation
In 1 N HCl for 48 Hours	No degradation	No degradation
Alkali Degradation Study		
In 1 N NaOH for 12 Hour	No degradation	No degradation
In 1 N NaOH for 24 Hours	No degradation	No degradation
In 1 N NaOH for 48 Hours	No degradation	No degradation
Oxidative Degradation Study		
In 10% H_2O_2 for 12 Hour	No degradation	No degradation
In 10% H_2O_2 for 24 Hours	No degradation	No degradation
In 10% H_2O_2 for 48 Hours	No degradation	No degradation
Thermolytic Degradation Study		
At 70°C for 12 Hour	No degradation	No degradation
At 70°C for 24 Hours	No degradation	No degradation
At 70°C for 48 Hours	No degradation	No degradation
Photolytic Degradation Study		
At Short and Long wavelength for 12 Hour	No degradation	No degradation
At Short and Long wavelength for 24 Hours	No degradation	No degradation
At Short and Long wavelength for 48 Hours	No degradation	No degradation

chromatograms were obtained to evaluate the sample's stability.

Alkali Degradation Studies

1 ml of 2N sodium hydroxide was added to 1 ml of Stavudine and Zidovudine stock solution, and the mixture was refluxed for 30 minutes at 600C. After diluting the resulting solution to achieve concentrations of 150 and 300 µg/ml, 10 µl of the solution was injected into the system, and chromatograms were obtained to evaluate the sample's stability.

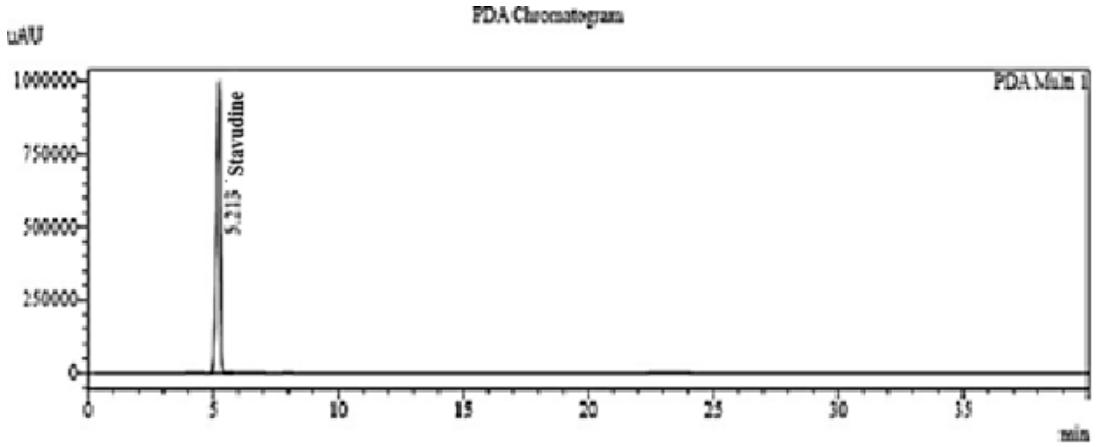

Figure 1: Chromatogram of Stavudine (in 1 N HCl) (after 48 hours)

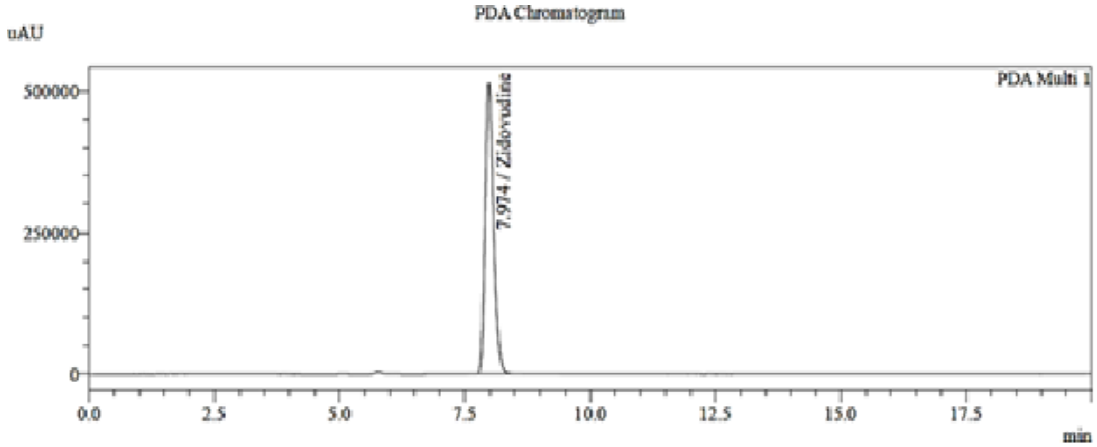

Figure 2: Chromatogram of Zidovudine (in 1 N HCl) (after 48 hours)

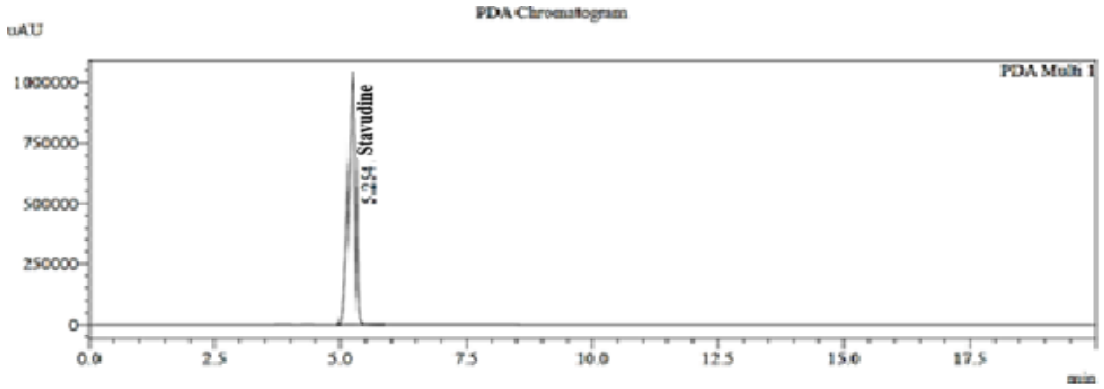

Figure 3: Chromatogram of Stavudine (in 1 N NaOH) (after 48 hours)

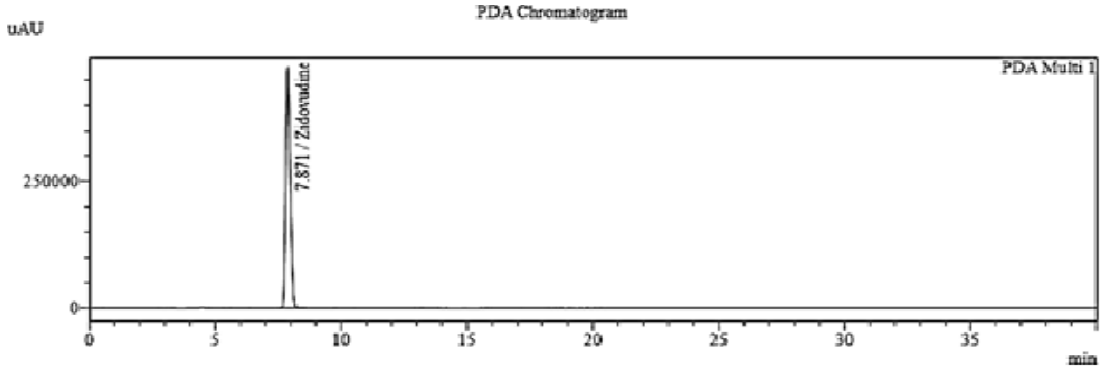

Figure 4: Chromatogram of Zidovudine (in 1 N NaOH) (after 48 hours)

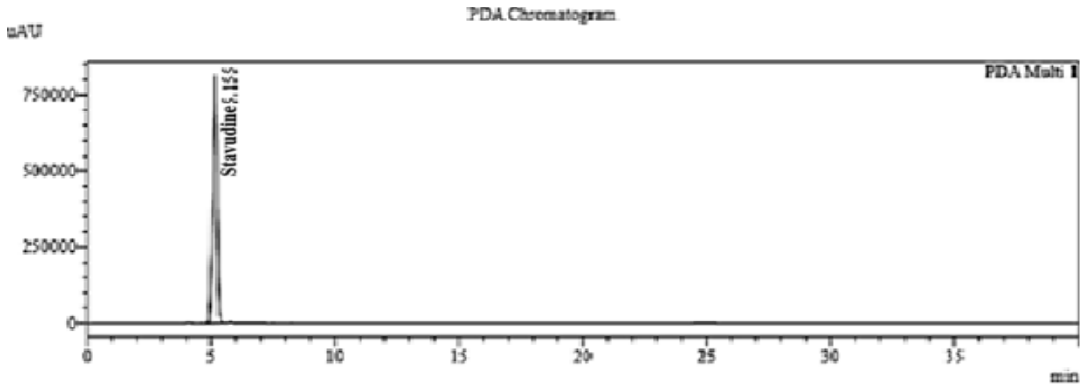

Figure 5: Chromatogram of Stavudine (in 3% H2O2) (after 48 Hours)

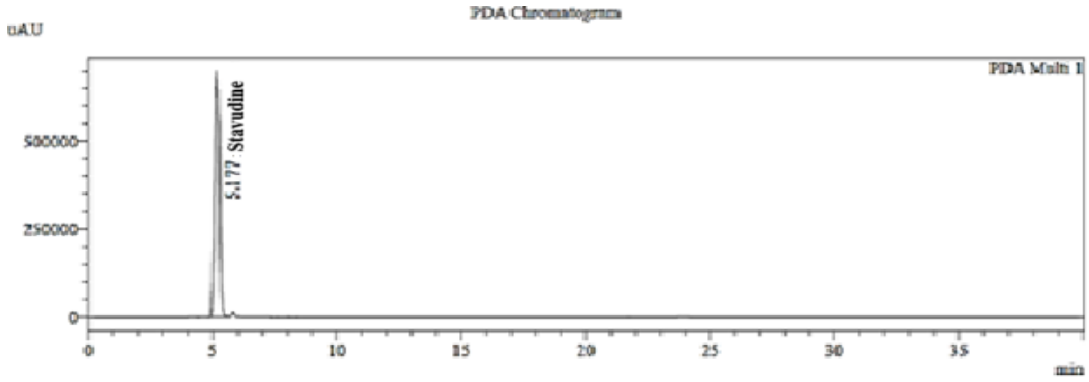

Figure 6: Chromatogram of Stavudine (in 10% H2O2) (after 48 Hours)

Photo Stability Studies

By placing the beaker in a UV chamber for six hours and subjecting the standard drug solution to UV light, the photochemical stability of the medication was also investigated. After diluting the resulting solution to achieve concentrations of 150 and 300 µg/ml, 10 µl of the solution

was injected into the system, and chromatograms were collected to evaluate the sample's stability for the HPLC investigation.

RESULTS AND DISCUSSION

Method Development

To increase the chromatographic system's efficiency, several chromatographic settings were tested. The composition of the mobile phase, the wavelength of detection, the column, the column temperature, and the buffer's pH were among the parameters that were tuned.

Stability Studies

Acid Degradation Study

The chromatograms for 12, 24 and 48 were recorded (1N HCl for 48 Hours).

ExperimentalCondition –1 N HCl (STV)(48 Hours)

Experimental Condition – 1 N HCl (ZDV) (48 Hours)

Alkali Degradation Study

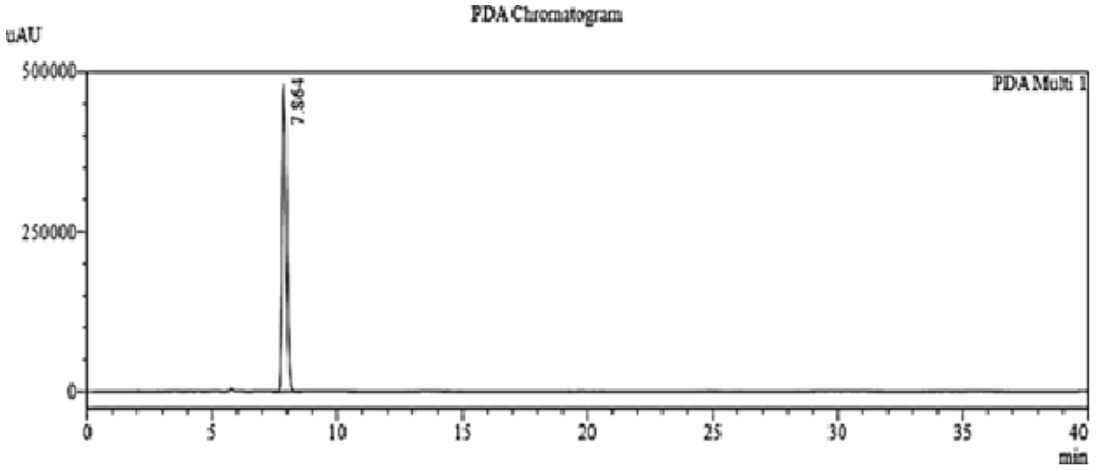

Figure 7: Chromatogram of Zidovudine (in 3% H2O2) (after 48 Hours)

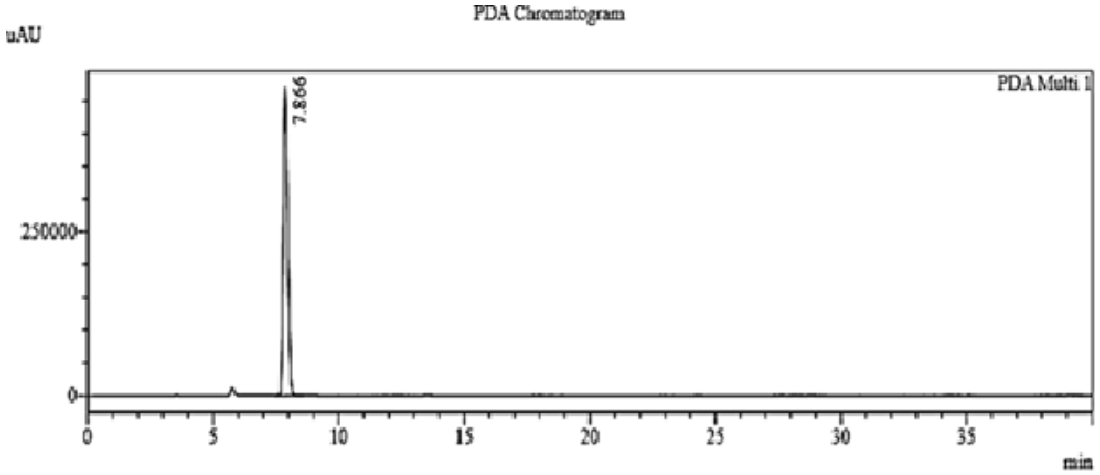

Figure 8: Chromatogram of Zidovudine (in 10% H2O2) (after 48 Hours)

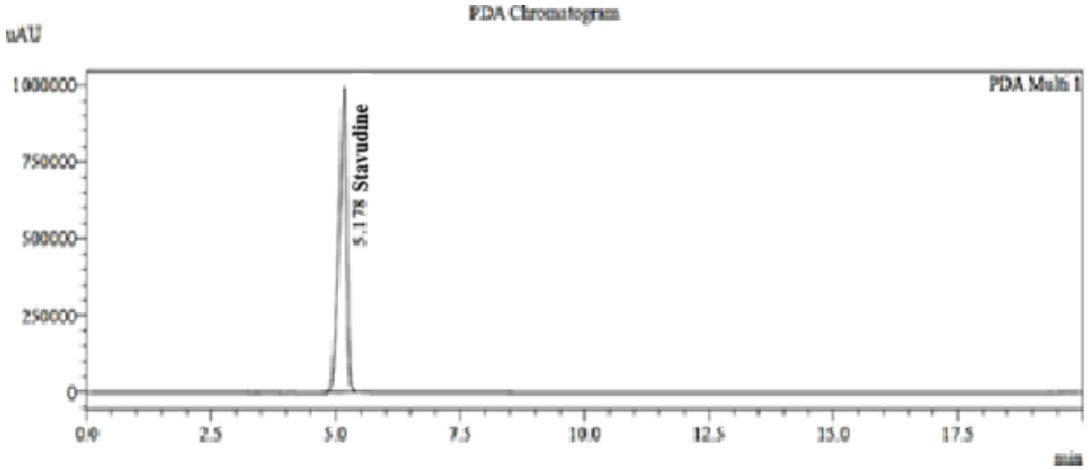

Figure 9: Chromatogram of Stavudine (Temperature of 70°C) (after 48 Hours)

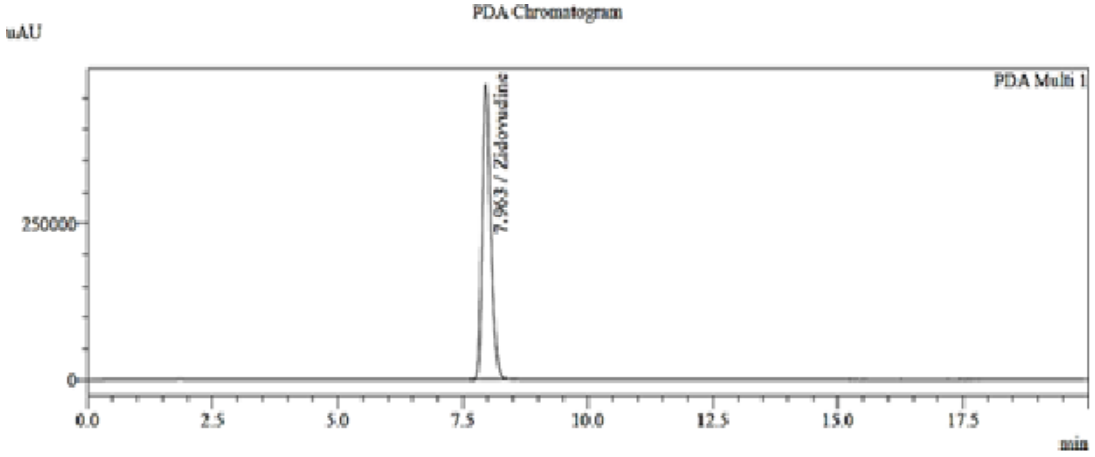

Figure 10: Chromatogram of Zidovudine (Temperature of 70°C) (after 48 Hours)

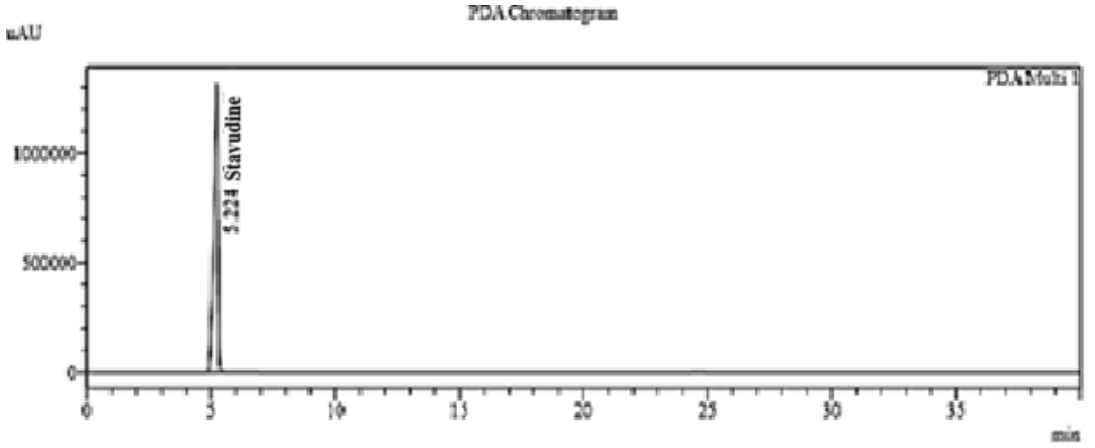

Figure 11: Chromatogram of Stavudine (Short and Long wavelength) (after 48 Hours)

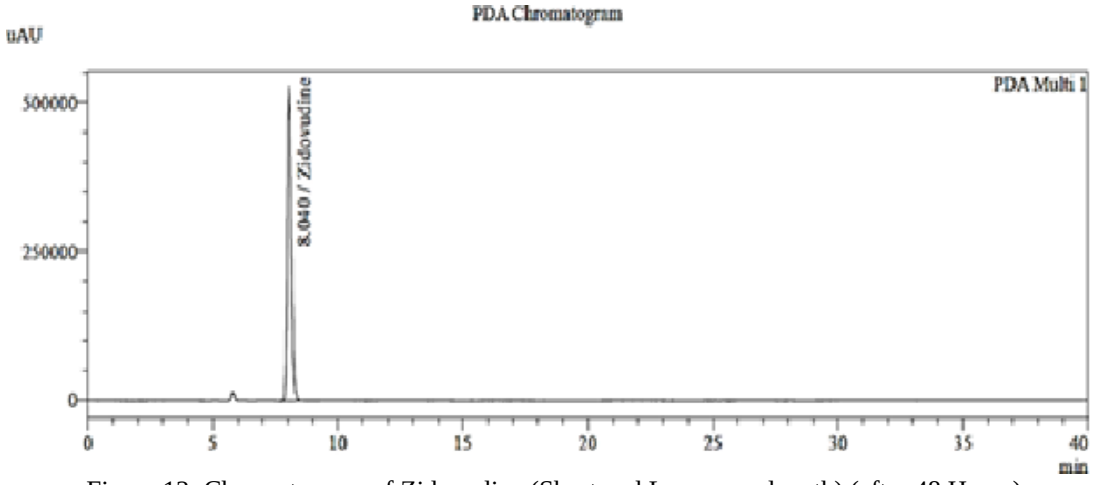

Figure 12: Chromatogram of Zidovudine (Short and Long wavelength) (after 48 Hours)

The chromatograms for 12, 24 and 48 hours were recorded (1N NaOH for 48 hours).

Experimental Condition – 1 N NaOH (STV) (48 Hours)

Experimental Condition – 1 N NaOH (ZDV) (48 Hours)

Oxidative Degradation Study

As shown in Figures 5-7 (3% H2O2 for 48 hours) and 6 (10% H2O2 for 48 hours), the chromatograms were taken after 12, 24, and 48 hours to assess the sample's stability.

Experimental Condition – 3% H2O2 (STV) (48 Hours)

Experimental Condition – 10% H2O2 (STV) (48 Hours)

Experimental Condition – 3% H2O2 (ZDV) (48 Hours)

Experimental Condition – 10% H2O2 (ZDV) (48 Hours)

Thermolytic Degradation Study

The chromatograms were recorded after 12, 24 and 48 Hours to access the stability of sample as shown in Figure 5.17 (70°C for 48 hours).

Experimental Condition – Temperature of 70°C (STV) (48 Hours)

Experimental Condition – Temperature of 70°C (ZDV) (48 Hours)

Photolytic Degradation Study

The chromatograms were recorded after 12, 24 and 48 Hours to access the stability of sample as shown in Figure 5.18 (Short and Long wavelength for 48 hours).

Experimental Condition – Short and Long wavelength (STV) (48 Hours)

Experimental Condition – Short and Long wavelength (ZID) (48 Hours)

CONCLUSION

The RP-HPLC technique that was developed turned out to be quick, easy, sensitive, accurate, exact, and specific. Two medications were isolated throughout the eight-minute chromatographic duration. All of the method validation parameters evaluated showed good results once the technique was verified. Stavudine and zidovudine may be quantitatively determined simultaneously in the presence of degradation products using the developed stability-indicating approach. Compared to existing HPLC techniques, the new approach was able to provide quicker elution while retaining better separation. For regular analysis in the pharmaceutical industry, the short retention durations make it more cost-effective since they enable the analysis of many samples in a short amount of time. It is appropriate for both single drug analysis and quick and precise quality control of zidovudine and stavudine in combination dose forms.

REFERENCES

1. Roussos, A., Stavudine treatment for acute severe hepatitis B: report of a case and review of the literature. Acta gastro-enterologica belgica 71.1 (2008): page: 30-32.
2. Cload, PA .A review of the pharmacokinetics of zidovudine in man. Journal of Infection 18 (1989): page:15-21.
3. Harker AJ, Evans GL, Hawley AE, Morris DM. High-performance liquid chromatographic assay for 2'-deoxy-3'- thiacytidine in human serum. J Chromatogr B Biomed Appl. 1994 Jul 1; 657(1):227-32.
4. Zhou XJ, Sommadossi JP. Rapid quantitation of (-)-2'-deoxy-3'-thiacytidine in human serum by high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Sci Appl. 1997 Apr 11; 691(2):417-24.
5. Hoetelmans RM; Profijt M; Mennhorst PL; Mulder JW; Beijnen JH Quantitative determination of (-)-2'-deoxy-3'- thiacytidine (Stavudine) in human plasma, saliva and cerebrospinal fluid by high-performance liquid chromatography with ultraviolet detection. January30, 1999 J Chromatogr B Biomed Sci Appl. 1998 Aug 25; 713(2):387-94.
6. Zheng JJ, Wu ST, Emm TA. High-performance liquid chromatographic assay for the determination of 2'-deoxy-3'- thiacytidine (Stavudine) in human plasma. J Chromatogr B Biomed Sci Appl. 2001 Sep 25;761 (2):195-201.
7. AlnoutiY, White CA, Bartlett MG. Simultaneous determination of Zidovudine and Stavudine from rat plasma, amniotic fluid and tissues by HPLC. Biomed Chromatogr. 2004 Nov; 18(9):641-7.
8. Good SS, Reynolds DJ, de Miranda P. Simultaneous quantification of Zidovudine and its glucuronide in serum by high-performance liquid chromatography. J Chromatogr. 1988 Sep 23; 431 (1):123-33.
9. Ruprecht RM, Sharpe AH, Jaenisch R, Trites D. Analysis of 3'-azido-3'-deoxythymidine levels in tissues and milk by isocratic high-performance liquid chromatography. J Chromatogr. 1990 Jun 29; 528 (2):371-83.
10. Burger DM, Rosing H, Koopman FJ, Mennhorst PL, Mulder JW, Bult A, Beijnen JH. Determination of 3'-amino-3'- deoxythymidine, a cytotoxic metabolite of 3'-azido-3'-deoxythymidine, in human plasma by ion-pair high- performance liquid chromatography. J Chromatogr. 1993 Dec 22; 622 (2):235-42.
11. TanX, Boudinot FD. Simultaneous determination of zidovudine and its monophosphate in mouse plasma and peripheral red blood cells by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl. 2000 Apr 14;740 (2):281-7.
12. Aymard G, Legrand M, Trichereau N, Diquet B. Determination of twelve antiretroviral agents in human plasma sample using reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl. 2000 Jul 21; 744(2):227-40.
13. Marchei E,Valvo L, Pacifici R, Pellegrini M, Tossini G, Zuccaro P. Simultaneous determination of Zidovudine and nevirapine in human plasma by RP-LC. J Pharm Biomed Anal. 2002 Aug 1; 29(6):1081-8.
14. Verweij-vanWissen CP, Aarnoutse RE, Burger DM. Simultaneous determination of the HIV nucleoside analogue reverse transcriptase inhibitors Stavudine, didanosine, stavudine, Zidovudine and abacavir in human plasma by reversed phase high performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2005 Feb 25; 816(1-2):121-9.
15. Stability testing of new drug substances and productsQ1 A (R2).International conference on harmonization, IFPMA, 2003, Geneva.
16. SinghS, Bakshi M. Guidance on Conduct of Stress tests to determine inherent stability of drugs. Pharm Technol Online.2000; 1–24.
17. International Conference on Harmonization. Photostability testing of new drug substance and products Q1 B. International Conference on Harmonization, IFPMA, 1996, Geneva.
18. Bakshi M, Singh S. J Pharm Biomed Anal. Development of validated stability-indicating assay methods—critical review.2002; 28: 1011–1040.