ANALYSIS ON ESTIMATION OF STAVUDINEDRUG BY SIMPLE UV AND COLORMETRIC METHOD

[1]B.Rajesh, [2]P.Navitha, [3]K Raghotham Reddy
[1,2]Assistant Professor, [3]Director, Department of H & S, Brilliant Institute of Engineering and Technology, Hyderabad, India

Abstract
For the measurement of stavudine in pharmaceutical dosage forms and bulk, a new simple, sensitive, and exact approach has been developed with the use of the MBTH reagent. The objective of this analytical validation technique is to demonstrate that the approach satisfies the requirements for use in a laboratory by validating it through laboratory testing. Stavudine's secondary amine group reacts with 3-methyl-2-bezothiazoline hydrazone in the presence of the oxidant ferric chloride. The resulting compound was apple green in hue and had a maximum absorbance at 626 nm. The results of the analysis have statistical backing.
Keywords: Colorimetric analysis, MBTH, stavudine, UV-Visible

Introduction

Pharmaceuticals go through a number of stages before they are ready for consumer markets. The formulation, lab preparation, and quality inspection are all involved [1]. The substance, quality, and yield of the drugs are examined using a variety of both conventional and modern analytical techniques. Several analytical techniques, including titration, chromatography (TLC, GC, HPLC), spectroscopy (FT-IR, UV), electrophoresis, and electrochemical, were employed for the examination of the purity and chemical composition of the various drugs [3]. Analytical testing of drugs both before and after they are put on the market is crucial to their success.

In the field of chemistry and its related sciences, spectroscopy is a flexible technology with many potential applications. A study of the interaction of radiation with matter at a specific wavelength is called spectroscopy. Among various spectroscopy techniques, UV-Visible spectroscopy is used widely to analysis samples [3]. In UV-Visible the transitions are generally occurs between a bonding or lone pair orbitals and an untitled non-bonding orantibonding orbital concerned.

Stavudine, a drug belongs to nucleoside reverse transcriptase inhibitors (NRTI) even though Stavudine is not a cure for HIV infection. Use of Stavudine with other HIV medicines helps to decrease the amount of HIV in your body so your immune system can work better. This improves your quality of life by lowering your chance of getting HIV[5]. This medicine can be takenby mouth with or without food, usually every 12 hours or as per doctor direction**.** Based on the detailed literature survey it was observed that there are few analytical methods reported for Stavudine either individually or in combination with other drugs by UV, HPLC and HPTLC method[6]. There is no analytical method reported for the estimation of Stavudine in Pharmaceutical dosage form by Colorimetric method. This led to the development of colorimetric method for the estimation of stavudine in pharmaceutical dosage form. Thepresent work is aimed for the development of Colorimetric method using MBTH(3-methyl-2- bezothiazolinehydrazone) reagent and its validation using ICH guideline (validation parameter)[12].

Materials and Methods

The materials used were procured commercially and used without further purification, Stavudine (SISCON), 3-methyl-2-benzothiazonium hydrazones hydrochloride (Sigma Aldrich), Hydrochloric acid (SRL), Ferric chloride (SRL).

Methodology

Experiment

The carboxyl group of the drug Stavudine was reacting with MBTH reagent (99.7%) in the presence

Hydrochloric acid (0.1M) and Oxidizing reagent of $FeCl_3$ to give a green colored product. Under the acidic conditions, on oxidation, MBTH looses two electrons and one proton forming an electrophilic intermediate which is that the active coupling species. This intermediate undergoes electrophilic substitution with the drug to make the coloured product.

Aliquots of the standard solution of the drug 1ml - 6 mlwere transferred into 10 ml calibrated flasks. To each, aqueous solution of $FeCl_3$ (1 ml), and 1 ml of HCl (0.1M) and aqueous solution of MBTH (1ml, 0.2%) were added. The solutions were swirled and allowed to stand for 5mins at room temperature and made up to the mark with distilled water. The absorbance was measured at 626nm against the corresponding reagent blank. Colorimetric method wasused to study the optical characteristics of the prepared colored compound.

Results and Discussion

Linearity and range

Different concentrations starting from 10µg/ml - 60µg/ml were prepared, scanned and therefore the absorbance was measured at 626nm and are given in table 1

Table 1: Absorbance data of different concentrations of Stavudine

S. No.	Concentration of Stavudine in µg/ml	Absorbance at 626nm
1.	10	0.149
2.	20	0.213
3.	30	0.28
4.	40	0.349
5.	50	0.415
6.	60	0.478

.

Figure 1: Color development of Stavudine standard

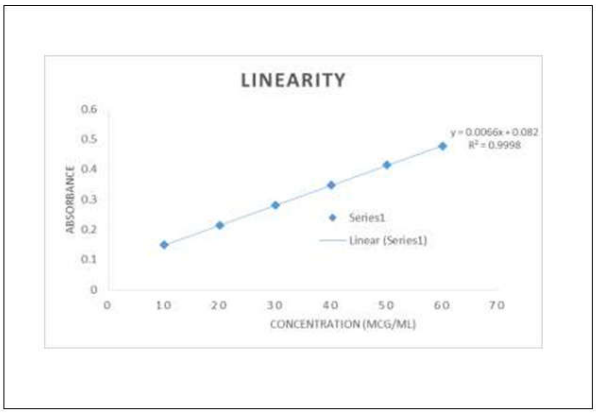

Figure 2: Calibration graph of Stavudine

Table 2: Correlation Coefficient, slope and intercept values of both the methods

Drug	Slope	Intercept	Correlation Coefficient
Stavudine	0.0066	0.082	0.9998

The correlation coefficient value was found to be within limit (0.996). The slope andintercept values are used for forming the regression equation y = mx + c
Where, m- Slope
x – Concentration in µg/ml c – Intercepty – Absorbance

Assay

To check the accuracy of the method the prepared marketed formulation was subjected toanalysis.

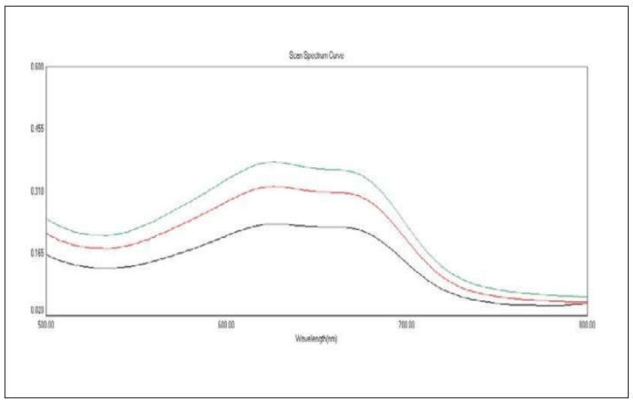

Figure 3: Assay of Stavudine

Assay of Stavudine

Table 3: Assay results showing accuracy of the methods

S.No	Drug	Amount Present Concentration (µg/ml)	Amount found (µg/ml)	% Purity
1	Stavudine	20	19.92	99.6
		30	29.97	99.9
		40	40.01	100.02
				99.84*

The estimated percentage purity was found to be close to 100% which provesthe accuracy of themethod.The results obtained by repeating the estimation procedure six times were observed to have good statistical parameters.

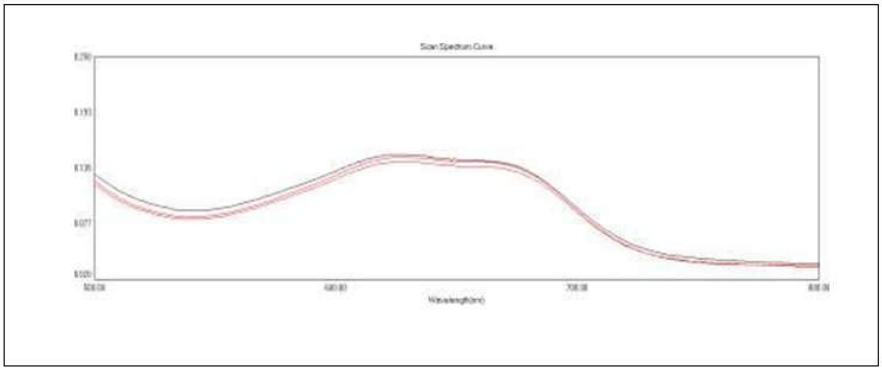

Figure 4: Precision of Stavudine

Table 4: Precision of Stavudine

METHOD	Drug	Amount found (µg/ml)	S.D	%RSD	Standard error	
MBTH	Stavudine	30.35	0.7133	0.7203	2.575 ± 0.40[a]	2.575 ± 0.29[b]

a) 95%confidential limit
b) 99%confidential limit

The Standard deviation, Relative Standard deviation, standard error were found to be low andhence proves the precision of the method.

Recovery

Table 5: Result of recovery studies.

S.No	Amount present (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	%Recovery
1	10	0	9.85	98.50
2	10	10	20.05	100.25
3	10	20	29.65	98.83
4	10	30	39.84	99.6
5	10	40	49.52	99.04
Meanvalues				99.24

The pre analyzed synthetic mixture was subjected to further analysis to check thereproducibility of the method. The results are shown below.

The estimated percentage recovery was found to be within the normal range (97%–103%), this proves the reproducibility of the method

Table 6: Optical Characteristics of Stavudine by Colorimetric Method

Parameter	Colorimetric method
Λ max (nm)	626
Beer's law limit (µg/ml) Correlation	10-60
coefficient (r) Regression equation (y	0.9998
=mx+c)* Slope (m)	
Intercept (c)	0.0066
Limit of detection(LOD) µg/ml Limit of	0.0082
quantization (LOQ) µg/ml Relative	0.9798
standard deviation Standarderror	2.9692
95% Confidencelimit	0.7203 %
99% Confidencelimit	
	1.96±0.2923
	2.575±0.2923

Conclusion

The goal was to create and validate an analytical method using the MBTH reagent for stavudine. The employed concentration range of 0.5 mg per ml is shown to have linear response by the linearity calibration curve. The repeatability of the assay procedure was shown to be sufficient by the precision data, and the correctness of the method was confirmed by the recovery studies. The method that was developed and validated was successfully applied to medication formulation estimation. The results were proven to be significantly within the permissible range of 97-103%. Since the described methods were proved to be simple, quick, and economical, they can be used for routine analysis of stavudine in bulk and in pharmaceutical formulations.

Reference

1. S. Anbazhagan, N. Indumathy, P. Shanmugapandiyan, and S. K. Sridhar, "Simultaneous quantification of stavudine, lamivudine and nevirapine by UV spectroscopy, reverse phase HPLC and HPTLC in tablets," J. Pharm. Biomed. Anal., vol. 39, no. 3–4, pp. 801–804,2005
2. Poizot-Martin, M. P. Milon, P. Enel, J. A. Gastaut, and J. Vion-Dury, "Discrepancy between blood and cerebral Didanosine effects in HIV patients: A magnetic resonance spectroscopy study.," Eur. Neurol., vol. 53, no. 4, pp. 223– 225, 2005
3. M. Rangapriya and N. N. Rajendran, "Validation of UV spectroscopy for simultaneous estimation of stavudine, lamivudine and nevirapine in tablet formulations.," Int. J. Pharma Sci. Res., vol. 5, no. 10, pp. 656–664,2014.
4. D. Anantha Kumar, G. SrinivasaRao, and J. V. L. N. SeshagiriRao, "Simultaneous determination of lamivudine, zidovudine and abacavir in tablet dosage forms by RP HPLC method.," E-Journal Chem., vol. 7, no. 1, pp. 180– 184, 2010.
5. S. Anbazhagan, N. Indumathy, P. Shanmugapandiyan, and S. K. Sridhar, "Simultaneous quantification of stavudine, lamivudine and nevirapine by UV spectroscopy, reverse phase HPLC and HPTLC in tablets," J. Pharm. Biomed. Anal., vol. 39, no. 3–4, pp. 801–804,2005.
6. J. Anbu, C. Roosewelt, A. Anjana, G. S. Rao, and R. Sathish, "Simultaneous estimation of Lamivudine and Stavudine in tablet dosage form by RP-HPLC.," Int. J. Life Sci. Pharma Res., vol. 2,no11
7. R. Zhao, Y. Du, and Z. Hong, "Investigation of vudine pharmaceutical compounds by terahertz spectroscopy and density functional theory.," YaowuFenxiZazhi, vol. 34, no. 1, pp. 108–114,2014.
8. D. Anantha Kumar, G. SrinivasaRao, and J. V. L. N. SeshagiriRao, "Simultaneous determination of lamivudine, zidovudine and abacavir in tablet dosage forms by RP HPLC method.," E-Journal Chem., vol. 7, no. 1, pp. 180– 184, 2010.

9. S. Anbazhagan, N. Indumathy, P. Shanmugapandiyan, and S. K. Sridhar, "Simultaneous quantification of stavudine, lamivudine and nevirapine by UV spectroscopy, reverse phase HPLC and HPTLC in tablets," J. Pharm. Biomed. Anal., vol. 39, no. 3–4, pp. 801–804,2005.
10. J. Anbu, C. Roosewelt, A. Anjana, G. S. Rao, and R. Sathish, "Simultaneous estimation of Lamivudine and Stavudine in tablet dosage form by RP-HPLC.," Int. J. Life Sci. Pharma Res., vol. 2,no
11. S. Choudhury, R. K. Patnaik, T. K. Laha, and S. Sen, "Simultaneous determination of stavudine and lamivudine in combined dosage forms by RP- HPLC method.," Asian J. Chem., vol. 20, no. 7, pp. 5254–5258,2008
12. R. A. Dayaramani and P. U. Patel, "Simple, rapid and cost effective method for routine analysis of Stavudine and Lamivudine in tabletdosage P. HariPrasad, P. M. Patel, D. Vijaysree, and K. VamshiSharathnath, "Simultaneous estimation oflamivudine and stavudine by using RP-HPLC and method development as per ICH guidelines.," Int. J. Pharma Sci. Res., vol. 3,no. 7, pp. 416–420,
13. S. Jayaseelan, S. Ganesh, M. Rajasekar, V. Sekar, and P. Perumal, "A new analytical method development and validation for the simultaneous estimation of Lamivudine and Stavudine in tablet dosage form by RP-HPLC method.," Int. J. PharmTech Res., vol. 2, no. 2, pp. 1539–1542,2010.
14. S. Jayaseelan, S. Suresh, G. Sathishkumar, V. Sekar, and P. Perumal, "Bioanalytical method development and validation of lamivudine by RP-HPLC method.," Int. J. ChemTech Res., vol. 2, no. 1, pp. 163–167,2010.
15. C. Jothimanivannan, S. AnandaThangadurai, M.Ramya Krishna, C. Arjun, andV. M. K. Gowtham, "Simultaneous determination of Stavudine and Lamivudine in pharmaceutical dosage forms by RP-HPLC.," Asian J. Pharm. Clin. Res., vol. 6, no. 2, pp. 26–29, 2013.
16. E. K. Kano, C. H. dos Reis Serra, E. E. M. Koono, S. S. Andrade, and V. Porta, "Determination of lamivudine in human plasma by HPLC and its use in bioequivalence studies.," Int. J. Pharm., vol. 297, no. 1–2, pp. 73–79,2005.
17. S. Kaul, K. A. Dandekar, and K. A. Pittman, "Analytical method for the quantification of 2',3'-didehydro-3'-deoxythymidine, a new anti-human immunodeficiency virus (HIV) agent, by high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection in rat and monkey plasma.," Pharm. Res., vol. 6, no. 10, pp. 895– 899,1989.
18. G. Kumaraswamy, J. M. Rajendra Kumar, J. V. L. N. SheshagiriRao, D. Vinay Kumar, D. Prabakar, and U. Ashok Kumar, "HPLC method development and validation for simultaneous estimation of lamivudine and stavudine in bulk and combinedtabletdosageform.,"Int.J.Pharm.Pharm.Sci.,vol.3,no.3,pp.142– 146, 2011.
19. Z.Li,H.Li,Y.Liu,A.Zhao,andX.Ren,"Methodofdeterminationofmethadone hydrochlorideinplasmabyHPLCinpatientsofanti- virustreatment.,"ZhongguoYiyuanYaoxueZazhi, vol. 28, no. 17, pp. 1446–1447,2008.
20. S. Jayaseelan, S. Ganesh, M. Rajasekar, V. Sekar, and P. Perumal, "A new analytical method development and validation for the simultaneous estimation of Lamivudine and Stavudine in tablet dosage form by RP-HPLC method.," Int. J. PharmTech Res., vol. 2, no. 2, pp. 1539– 1542,2010.