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A sensitive and selective RP-HPLC method for the determination of lamivudine and stavudine in tablets

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ABSTRACT

A simple, fast, precise and accurate reverse phase high performance liquid chromatographic method developed for the simultaneous determination and validation of lamivudine (3TC) and stavudine (d4T) in tablets. A sunfire $C_{18,}$ 250 × 4.6 mm, 5 µm particle size column in isocratic mode was used with mobile phase comprising of methanol: 0.1 % w/v of ammonium acetate, adjusted to pH 3.8 with glacial acetic acid in the ratio of 15:85, v/v. The flow rate was set at 1.2 ml per minute with UV detection at 266 nm. The retention time of 3TC, d4T was found 5.6 and 8.9 minute respectively. Linearity for lamivudine and stavudine were found in the range of 75-225µg/ml and 20-60µg/ml respectively. Percentage recoveries were obtained in the range of 98.97 % to 99.71 % for lamivudine and 99.22 % to 99.59 % for stavudine. The proposed method is precise, accurate, selective, reproducible, and rapid for the simultaneous estimation of lamivudine and stavudine in tablet dosage forms.

Keywords: Lamivudine, Stavudine, RP-HPLC Method and Simultaneous Estimation.

INTRODUCTION

Multi-drug therapy has become the standard treatment for acquired immunodeficiency syndrome (AIDS)[1]. The situation is imposed by the need to delay the development of resistance by the human immunodeficiency virus (HIV), the causative virus of AIDS, to single anti-HIV drugs and to minimize potential dose dependent side effect[2]. The current typical regimen for treating HIV infection is to use a combination of at least two drugs, a practice known as 'highly active antiretroviral therapy' (HAART)[3].

Lamivudine is chemically known as (2*R*,5*S*)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5yl]-2(1*H*)-pyrimidinone[4] and stavudine is chemically 2',3',didehydro-3'-deoxythymidine[5]. Lamivudine is a nucleoside analog having potent in-vitro and in-vivo inhibitory activity against HIV reverse transcriptase[6]. Lamivudine specifically refers to the (-) enantiomer of the *cis*-racemate.

Literature survey reveals several methods that have been used for the quantitative determination of the two drugs individually or in combination with other drugs in pharmaceutical dosage forms or in human plasma by high performance liquid chromatography[7-10], spectrophotometry[11], LC/MS/MS[12] etc. RP-HPLC method with solid phase extraction procedure has been reported for simultaneous determination of six nucleoside analog reverse transcriptase inhibitors[13] and 13 HIV suppressing drugs[14] of which 3TC and d4T are a part. Besides, simultaneous quantification of several antiretroviral agents including these two drugs has been reported by a solid-liquid extraction procedure using RP-HPLC system[15, 16].

HPLC methods are useful in the determination of drugs in pharmaceutical formulations especially those containing more than one active components. Therefore, the aim of this work was to develop a relatively simple HPLC method for simultaneous quantification of 3TC and d4T in antiretroviral FDCs without the necessity of sample pre-treatment. This paper describes the development and validation of reliable, simple, stable and economic reverse phase HPLC assay, using UV detection for the simultaneous determination of 3TC and d4T in FDC tablets. The method appears to be suitable for quality control in pharmaceutical industry due to its sensitivity, simplicity, selectivity and lack of excipients interference.

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MATERIALS AND METHODS

Chemicals and Reagents:

All chemicals and reagents used were of HPLC grade. Methanol were obtained from E. Merck, Mumbai. Ammonium acetate and glacial acetic acid were obtained from S. D. fine chemicals, mumbai. Reference standard and Stavex- 40 L tablets containing 3TC-150 mg and d4T- 40 mg were provided by Aurbindo Pharmaceuticals Ltd., Hyderabad.

Instruments:

The instrument used for the study was a Shimadzu High Performance Liquid Chromatography equipped with pump (LC- 10 ATVP), inline degasser, UV-Visible detector (SPD- 10 AVP).

Chromatographic Conditions:

Column	:Sunfire (W) C18 (250 × 4.6 mm) x 5 µm particle size
Mobile Phase	:methanol: 0.1 % w/v of ammonium acetate, adjusted to pH 3.8 with glacial acetic acid (15:85, v/v)
Flow rate	:1.2 ml/min.
Detection wavelength	:266 nm
Injection volume	:20 µl
Pump mode	:Isocratic
Run Time	:10 minutes
Retention Time	:5.6 and 8.9 minute respectively

Preparation of Standard Solutions:

About 150 mg of 3TC and 40 mg of d4T was accurately weighed into 100 ml volumetric flask, dissolved in 10 ml of methanol and diluted to volume with water. The solution was further diluted with water to obtain a concentration of 75- 225 µg/ml of 3TC and 20- 60 µg/ml of d4T, respectively.

Preparation of Sample Solution:

Twenty tablets were weighed and powdered. A quantity equivalent to 150 mg of 3TC was weighed accurately and transferred to a 100 ml volumetric flask, dissolved in 10 ml of methanol and diluted to volume with water. The resultant mixture was filtered through 0.45 µm nylon filter and filtrate was again diluted to have a concentration of 150 µg/

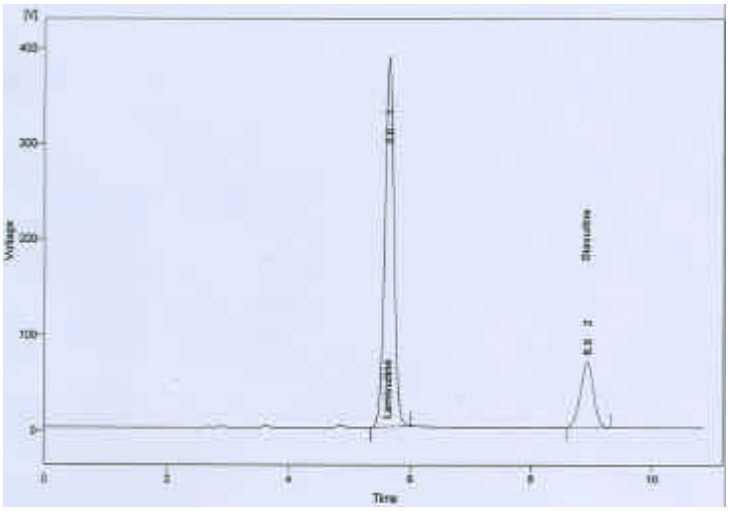
ml of 3TC.

Procedure for Assay:

Standard and sample solutions (20 µl) were separately injected on HPLC system. From the peak area of 3TC and d4T the amount of drugs in the sample were computed.

RESULT AND DISCUSSION

The composition of the mobile phase for development of chromatographic method was optimized by using different solvent mixtures of varying polarity. The best results were obtained using methanol: 0.1 % w/v of ammonium acetate, adjusted to pH 3.8 with glacial acetic acid in the ratio of 15:85, v/v. This mobile phase showed good resolution of 3TC and d4T peak. The wavelength of detection selected was 266 nm, as both the drugs showed optimum absorbance at this wavelength. By our proposed method the retention time of 3TC and d4T was about 5.6 and 8.9 minute, respectively and none of the impurities were interfering in its assay (Fig. 1 & 2).



gure 1: A typical chromatogram of lamivudine and stavudine standard

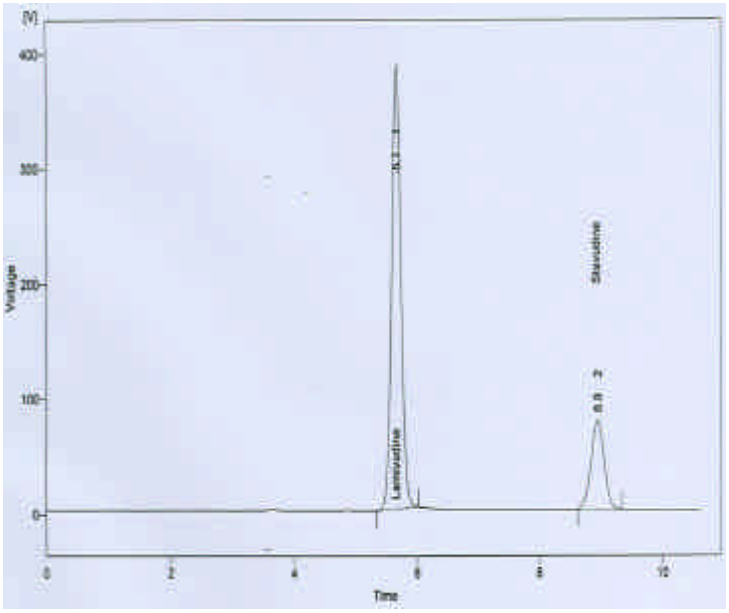


Figure 2: A typical chromatogram of lamivudine and stavudine sample

Validation of the method:

The developed method has been validated for the assay of 3TC and d4T as per ICH guidelines[17] by using following parameters.

Specificity and Selectivity:

Specificity and selectivity were studied for the examination of the presence of interfering components. It was checked by subjecting the drug solution in different stress conditions like Acid, Base, Peroxide and the degradation was noted.

Acid Stress (0.1 M HCl)

Table 1: Specificity testing (Acid stress)

Concentration (µg/ml)		Time (hrs)	Retention time (min)		RT of degraded product
3TC	d4T		3TC	d4T	
150	40	0	5.60	8.90	-
		8	5.95	8.88	-
		24	5.98	8.89	-

Base stress (0.1M NaOH)

Table 2: Specificity testing (Base stress)

Concentration (µg/ml)		Time (hrs)	Retention time (min)		RT of degraded product
3TC	d4T		3TC	d4T	
150	40	0	5.99	8.90	-
		8	5.94	8.89	-
		24	5.98	8.91	-

Peroxide stress (5% H_2O_2)

Table 3: Specificity testing (Peroxide stress)

Concentration (µg/ml)		Time (hrs)	Retention time (min)		RT of degraded product
3TC	d4T		3TC	d4T	
150	40	0	5.61	8.91	_
		8	5.99	8.89	_
		24	5.98	8.90	_

Linearity:

Linearity was studied by preparing standard solutions of 3TC and d4T at different concentration levels (Fig. 3 & 4). The responses were found linear in the range of 75-225 µg/ml and 20-60 µg/ml for 3TC and d4T, respectively.

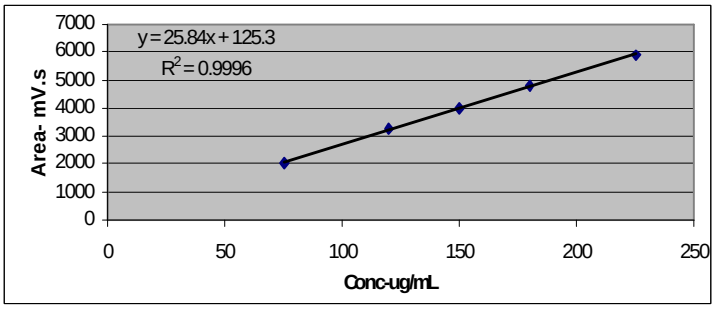


Figure 3 : Linearity curve of standard Lamivudine

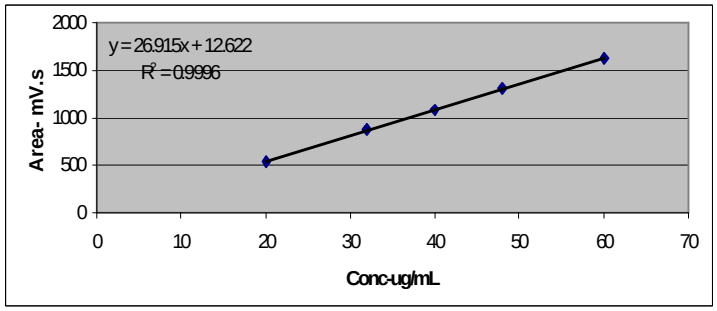


Figure 4 : Linearity curve of standard Stavudine

Accuracy:

Accuracy was determined by assay and recovery studies of 3TC and d4T. A known amount of standard of 3TC and d4T were added into pre-analysed sample and were subjected to the proposed HPLC method. Results of recovery studies are shown in Table 4. The study was carried out at three different concentration levels.

Table 4: Results of Analysis of Formulation and Recovery Studies

Drug	Amount (mg/tab) Labelled	Found*	% Label Claim*	Recovery Studies Amount Added (mg)	Amount Recovered (mg)	% Recovery*
Lamivudine	150	148.8	99.20	120	119.65	99.71
Stavudine	40	39.5	98.75	150	148.46	98.97
				180	178.92	99.40
				32	31.87	99.59
				40	39.69	99.23
				48	47.73	99.44

Precision:

Precision was studied to find out intra and interday variations in the test methods of 3TC and d4T in the concentration range of 75-225 µg/ml and 20-60 µg/ml respectively for three times on the same day and interday. Precision was determined by analysing corresponding standard daily for a period of three days. The inter-day and intra-day precision expressed as % RSD (< 2) as shown in Table 5. indicates that the proposed method is quite precise and reproducible.

Table 5: Results from determination of precision of analysis of 3TC and d4T

Concentration [µg/ml]		Intraday precision RSD [%]		Interday precision RSD [%]	
3TC	d4T	3TC	d4T	3TC	d4T
75	20	1.31	0.87	1.36	0.92
150	40	1.41	0.82	1.46	0.92
225	60	1.48	0.88	1.51	0.83

Robustness and Ruggedness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of 3TC and d4T was noted. The factors selected were flow rate and % methanol in the mobile phase. The results remained unaffected by small variations in these parameters. Ruggedness of the method was checked by using different analysts and instruments. The relative standard deviation of the results obtained from different analysts and instruments was < 1.0 %.

Validation parameter

The method was validated by using the following parameters as shown in Table 6.

Table 6 : Validation parameter of HPLC method for Lamivudine and Stavudine

Validation Parameter	Lamivudine (3TC)	Stavudine (d4T)
Linearity Range (µg/ml)	75-225	20-60
Regression equation	y=25.84x+ 125.3	y=26.915x+ 12.622
Correlation Coefficient (r^2)	0.9996	0.9996
Accuracy	98.97-99.71	99.23-99.59
Precision		
Inter-day Precision (RSD %)	1.36-1.51	0.83-0.92
Intra-day Precision (RSD %)	1.31-1.48	0.82-0.88

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of less amount of solvents and change of set of conditions requires a short time. Many samples can be simultaneously and suitably analysed for the routine quality control analysis of 3TC and d4T in bulk and its tablet dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

REFERENCES

1. Clercq, E. D. New developments in anti-HIV chemotherapy. Biochimica Biophysica Acta, 2002, 1587, 258-275.
2. Beach, J. W. Chemotherapeutic agents for human immunodeficiency virus infection: mechanism of action, pharmacokinetics, metabolism and adverse reactions. Clinical Therapeutics, 1998, 20, 2-25.
3. Gallant, J. E. Initial therapy of HIV infection. Journal of Clinical Virology, 2002, 25, 317-333.
4. Anonymous, Indian Pharmacopoeia 2007, fifth edition, Vol. II, The Indian Pharmacopoeia Commission, Ghaziabad. 2007; Page no: 1276.
5. Anonymous, Indian Pharmacopoeia 2007, fifth edition, Vol. III, The Indian Pharmacopoeia Commission, Ghaziabad. 2007; Page no: 1745-1746.
6. Gilman, A. G. Antiretroviral agents, in: J.G. Hardman, L. E. Limbird (Eds), The Pharmacological Basis of Therapeutics, McGraw Medical Publishing Division, New York, 2001, Page no : 1349.
7. Tarinas, A.; Tapanes, R. D.; Ferrer, G.; Perez, J. Validation of high-performance liquid chromatography methods for determination of zidovudine, stavudine, lamivudine and indinavir in human plasma. Farmacia Hospitalaria, 2007, 31(4), 243-247.
8. Kapoor, N.; Khandavilli, S.; Panchagnula, R. Simultaneous determination of lamivudine and stavudine in antiretroviral fixed dose combinations by first derivative spectrophotometry and high performance liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis, 2006, 41(3), 761-765.
9. Kapoor, N.; Khandavilli, S.; Panchagnula, R. Simultaneous determination of lamivudine, stavudine and nevirapine in antiretroviral fixed dose combinations by high performance liquid chromatography. Analytica Chimica Acta, 2006, 570(1), 41-45.
10. Verweij-van Wissen C. P. W. G. M.; Aarnoutse, R. E.; Burger, D. M. Simultaneous determination of the HIV nucleoside analogue reverse transcriptase inhibitors lamivudine, didanosine, stavudine, zidovudine and abacavir in human plasma by reversed phase high performance liquid chromatography. Journal of Chromatography B., 2005, 816(1-2), 121-129.
11. Sankar, D. G.; Reddy, M. V. V. N.; Rajendrakumar, J. M.; Murthy, T. K. Indian Journal of Pharmaceutical Sciences, 2002, 64, 504-506.
12. Kenney, K. B.; Wring, S. A.; Carr, R. M.; Wells, G. N.; Dunn, J. A. Journal of Pharm Biomed Anal, 2000, 22, 967.
13. Rezk, N.; Tidwell, R.; Kashuba, A. Journal of Chromatography B, 2003, 791, 127-147.
14. Simon, V.; Thiam, M.; Lipford, L. Journal of Chromatography A, 2001, 913, 447-453.
15. Aymard, G.; Legrand, M.; Trichereau, N.; Diquet, B. Journal of Chromatography B, 2000, 744, 227-240.
16. Notari, S.; Bocedi, A.; Ippolito, G.; Narciso, P.; Pucillo, L. P.; Tossini, G.; Donnorso, R. P.; Gasparrini, F.; Ascenzi, P. Journal of Chromatography B, 2006, 831, 258-266.
17. Anonymous, ICH Guidelines: Validation of Analytical Procedures: Methodology Q2(B), 2003.

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