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Simultaneous Estimation of Stavudine and Lamivudine in Combined Dosage Forms by RP-HPLC Method

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A rapid, selective and precise RP-HPLC method for quantification of stavudine and lamivudine has been developed. The chromatographic resolution was achieved using the mobile phase acetonitrile: methanol:water at 10:1:1 (v/v) ratio. An isocratic HPLC with a single Waters 510 pump, Waters 486 tunable absorbance detector and μ-Bondapack C-18 column was used. The wavelength for detection was 280 nm and the mizolastine was used as an internal standard.

Key Words: Stavudine, Lamivudine, RP-HPLC, Validation.

INTRODUCTION

Stavudine is an analogue of thymidine, chemically it is 2',3'-didehydro-3'-deoxythymidine[1]. Lamivudine is an analogue of cytosine, chemically it is 4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2-pyrimidinone[2]. A review of literature shows a few methods for simultaneous estimation of stavidine and lamivudine using LC-MS[3], HPLC[4-6] and spectroscopic[7] technique, but they are far from being simple. In present study, a simple, validated, rapid and precise method for concurrent estimation of stavudine and lamivudine using RP-HPLC method is developed. The solvent system was optimized to acetonitrile:water:methanol in the ratio of 10:1:1 (v/v) by trial and error method depending on the polarity of the molecules and the internal standard was chosen to give a sharp peak at a suitable R_t difference with the drugs under study. Mizolastine was found to be a suitable internal standard in the solvent system used.

EXPERIMENTAL

An isocratic HPLC (Waters India, USA) with a single Waters 510 pump, Waters 486 tunable absorbance detector and Bondapack C-18 column was used. The HPLC system was equipped with Millennium 32 software. All the solvents used were of HPLC grade (E. Merck India Ltd.), all other reagents used were of AR grade.

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Chromatographic conditions: The mobile phase consisting of acetonitrile:methanol:water (10:1:1 v/v ratio) was prepared and sonicated for 0.5 h for the purpose of degassing before use. The flow rate of the mobile phase was maintained at 1 mL/min. The column temperature was ambient and UV detector at 280 nm detected the drugs. The injection volume was 20 µL. The data were acquired, stored and analyzed with Millennium 32 software.

Preparation of stock solution and working standards

Internal standard: Mizolastine was used as an internal standard for the simultaneous HPLC estimation of stavudine and lamivudine. 10 mg of the drug was weighed accurately and transferred to a 10 mL volumetric flask, dissolved in 5 mL of methanol and the volume was adjusted to the mark with the same to give a stock solution of 1000 ppm. 0.3 mL of this solution was added to the standard drug solutions.

Stavudine: 10 mg of stavudine was weighed accurately and transferred to a 10 mL volumetric flask, dissolved in 5 mL of methanol and the volume was adjusted to the mark with the same to give a stock solution of 1000 ppm.

Lamivudine: 10 mg of lamivudine was weighed accurately and transferred to a 10 mL volumetric flask, dissolved in 5 mL of methanol and the volume was adjusted up to the mark with the same to give a stock solution of 1000 ppm.

Preparation of working standards: From stock solutions of stavudine and lamivudine 0.1 to 2 mL of solutions were transferred to 10 mL volumetric flasks. To these solutions 0.3 mL of mizolastine (internal standard) stock solution were added and the final volume were made up to the mark with methanol to get 10, 20, 30, 40, 50, 100, 200 ppm working standard solutions of both the drugs.

Preparation of the calibration curves of the drug: Each of the working standard solutions were injected 6 times and the mean peak area ratio of each drug to that of internal standard were calculated and plotted against the concentration of the drug. The regression of the concentration of each drug over the mean peak area ratio was obtained and these regression equations were used for the assay of tablets containing these drugs. The reproducibility of the method was suggested by the low coefficient of variation (CV %) in the mean peak area ratio. The precision and accuracy of the method was determined by intra and inter day variation in the mean peak area ratio for a set of working standard solution and by the recovery study, respectively.

RESULTS AND DISCUSSION

The run time for the HPLC method was set at 10 min and the retention time (R_t) of stavudine, lamivudine and mizolastine were 3.070, 3.843 and 5.797 min, respectively. The mean ratio of peak area of stavudine and lamivudine to that of internal standard are shown below in the Table-1. The reproducibility of the method is indicated by low coefficient of variation (less than 2 %).

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Concentration (ppm)	Mean peak area ratio of stavudine (n = 6)	CV (%)	Mean peak area ratio of lamivudine (n = 6)	CV (%)
10	0.201119837	0.53	0.242663685	0.58
20	0.389168695	0.76	0.465008128	0.82
30	0.566256780	1.02	0.681797773	1.09
40	0.736887365	0.61	0.910668829	0.67
50	0.920855951	0.19	1.176053241	0.26
100	1.824452618	0.97	2.313864958	1.02
200	3.637133178	0.43	4.645093792	0.57

The regression of concentration of stavudine and lamivudine over their peak area ratio were found to be y = 0.0181x + 0.0159 and y = 0.0232x – 0.0015, respectively. These regression equations were used to estimate the drugs in their formulation and in validation study.

The developed HPLC method was validated for intra- and inter-day variation. When the solution containing 20 and 40 ppm of both the drugs along with 30 ppm of mizolastine (internal standard) were injected repeatedly on the same day and on the next day the mean peak area ratio of the drugs found are given in the Table-2. The low coefficient of variation in the study shows the precision and reproducibility of the HPLC method developed.

TABLE-2
PRECISION OF THE PROPOSED METHOD

Drug	Conc. (ppm)	Intra-day			Inter-day		
		Mean peak area ratio (n = 6)	Mean amount (n = 6)	CV (%)	Mean peak area ratio (n = 6)	Mean amount (n = 6)	CV (%)
Stavudine	20	0.38916869	20.01	0.27	0.3327253	19.92	0.25
	40	0.73688736	40.03	0.77	0.6932555	40.05	1.07
Lamivudine	20	0.46500812	19.97	0.57	0.5112732	20.08	0.50
	40	0.91066882	40.05	0.65	0.9000235	40.07	0.81

The accuracy of the method was established by high recovery of the drugs, when a known amount of the drugs (20 and 40 mg) were added to a known concentration of drug solution (20 ppm) there was a high recovery of both the drugs indicating that the developed method is highly accurate. The results are shown in the Table-3.

TABLE-3
RECOVERY STUDY

Drug	Amount added (μg)	Mean amount recovered (μg)	Amount recovered on mean value (%)
Stavudine	20	20.04 ± 0.09	100.200
	40	39.87 ± 1.02	99.675
Lamivudine	20	20.06 ± 0.31	100.360
	40	40.07 ± 0.07	100.350

The lower limit of detection (LOD) and the lower limit of quantitation (LOQ) were calculated as per the ICH guidelines using the formula 3.3 × SD/slope and 10 × SD/slope, respectively. The lower limit of detection and lower limit of quantitation were found to be 0.01 and 0.04 ppm, respectively for stavudine and for lamivudine limit of detection (LOD) and limit of quantitation (LOQ) were 0.01 and 0.04, respectively.

Assay of formulations: Two brands of tablet of the combination of stavudine and lamivudine were assayed by the developed HPLC method and the results obtained are given in Table-4. 20 Tablets of each brand were triturated and powder equivalent to 20 mg of stavudine and 10 mg of lamivudine was weighed and transferred to a 10 mL volumetric flask containing 5 mL of methanol. The solution was then kept for 0.5 h with intermittent sonication to assure the complete solubility of the drug. The solution is then filtered and the volume was adjusted to 10 mL with methanol. From this solution 0.1 mL was taken in another 10 mL volumetric flask containing 0.3 mL of mizolastine stock solution and the volume was adjusted up to the mark with methanol to get a solution containing 20 ppm of stavudine, 10 ppm of lamivudine and 30 ppm of mizolastine (internal standard).

TABLE-4
ASSAY OF STAVUDINE AND LAMIVUDINE IN DOSAGE FORMS

Brand name	Stavudine			Lamivudine		
	Label claim (mg)	Mean amount (±SD) found (n = 6) (mg)	Purity on mean value (%)	Label claim (mg)	Mean amount (±SD) found (n = 6) (mg)	Purity on mean value (%)
Livin-S	30	30.03 ± 0.05	100.100	150	150.07 ± 0.10	100.466
Lamostad	30	29.98 ± 0.07	99.993	150	149.71 ± 0.06	99.806

These solutions are subjected to quantitative analysis by the developed HPLC method. The HPLC method developed can be used in the routine analysis of the drugs in the combinations as the method is simple, sensitive and validated and uses the most common column (RP-C18) and UV detector.

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