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Short communication

Simultaneous quantification of stavudine, lamivudine and nevirapine by UV spectroscopy, reverse phase HPLC and HPTLC in tablets

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Abstract

In the present study, simultaneous quantification of stavudine (SV), lamivudine (LV) and nevirapine (NV) in tablets by UV spectroscopy, reverse phase HPLC (RP-HPLC) and HPTLC methods were developed. In the UV multi-component spectral method, SV, LV and NV was quantified at 266, 271 and 315 nm, respectively. In the RP-HPLC method, the drugs were resolved using a mobile phase of 20 mM sodium phosphate buffer (containing 8 mM 1-octanesulphonicacid sodium salt):acetonitrile (4:1, v/v) with pH adjusted to 3.5 using phosphoric acid on a C_{18}-ODS-Hypersil (5 μm, 250 mm × 4.6 mm) column in isocratic mode. The retention time of SV, LV and NV was 2.85, 4.33 and 8.39 min, respectively. In the HPTLC method, the chromatograms were developed using a mobile phase of chloroform:methanol (9:1, v/v) on precoated plate of silica gel 60 F_{254} and quantified by densitometric absorbance mode at 265 nm. The R_f of SV, LV and NV were 0.21–0.27, 0.62–0.72 and 0.82–0.93, respectively. Recovery values of 99.16–101.89%, percentage relative standard deviation of <0.7 and correlation coefficient (linear dynamic range) of 0.9843–0.9999 shows that the developed methods were accurate and precise. These methods can be employed for the routine analysis of tablets containing SV, LV and NV.

Keywords: Stavudine; Lamivudine; Nevirapine; HPLC; HPTLC

1. Introduction

Stavudine (SV), lamivudine (LV) and nevirapine (NV) are anti-HIV drugs (reverse transcriptase inhibitors). As HIV develops resistance rapidly [1], HAART (highly active antiretroviral therapy) is a combination of ≥3 drugs with ≥1 drug penetrating the blood–brain barrier is essential to avoid resistance. Such a combination dosage form will be adhering to effective therapy and enhancing better patient compliance. None of these drugs are official in any pharmacopoeia. SV [2–6], LV [7–17] and NV [18–29] have been reported to be quantified individually or in combination with other drugs in biological fluids by various techniques. LV along with zidovudine [30] has been reported to be estimated in tablets by reverse phase HPLC (RP-HPLC). LV and SV [31] have been reported to be simultaneously quantified in tablets by visible spectroscopy. To our knowledge, there is no method reported for the simultaneous quantification of SV, LV and NV in tablets. In the present study, simultaneous quantification of SV, LV and NV in tablets by RP-HPLC and HPTLC methods were developed.

2. Experimental

2.1. Instrumentation

UV spectral measurements were recorded in Shimadzu (Japan) 1601 UV-Visible spectrophotometer. RP-HPLC was

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performed in Shimadzu (Japan) HPLC VP Series, Pump LC 10 AT & UV Detector model SPD–10 A with Rheodyne injector and 20 μl loop. The column used was C_{18}-ODS-Hypersil column (5 μm, 250 mm × 4.6 mm). Win chrome (Mumbai, India) computer based data station was used. HPTLC was performed in Camag HPTLC (Camag, Muttenz, Switzerland) system (precoated plate of silica gel 60 F_{254}, E. Merck, Mumbai, India) equipped with linomat IV sampler applicator, twin trough plate development chamber, TLC scanner II with Cats software V4.0.

2.2. Chemicals and reagents

Hetero Drugs Ltd., India, generously gifted pure SV, LV and NV. Commercial tablets (two different brands) containing SV (30 mg), LV (150 mg) and NV (200 mg) were used for the study. Water, acetonitrile, chloroform and methanol used were of HPLC grade (E. Merck, Mumbai, India). All the other chemicals used were of analytical grade (E. Merck, India).

2.3. UV multi-component method

Three stock solutions were prepared by dissolving 0.25, 1.25 and 1.666 mg of SV, LV and NV in 100 ml of 0.01 M hydrochloric acid, respectively. Seven mixed standard solutions were prepared from the stock solutions with different concentration ranging from 1.6–6.4, 8–32 and 10.66–42.64 μg/ml of SV, LV and NV, respectively. All the mixed standard solutions were scanned over the range of 200–400 nm in the multi-component mode using three sampling points, 266, 271 and 315 nm, that is the λ_{max} of SV, LV and NV, respectively. These solutions were used to calculate the linear dynamic range and for the relative quantification of the tablets.

About 20 tablets were weighed and powdered. A powder equivalent of 25 mg of SV was weighed accurately and transferred to a 100 ml volumetric flask. The tablet powder was dissolved in 0.01 M hydrochloric acid and filtered through a Whatman filter paper. The solution was further diluted and UV measurements were recorded. The analytical data are presented in Table 1.

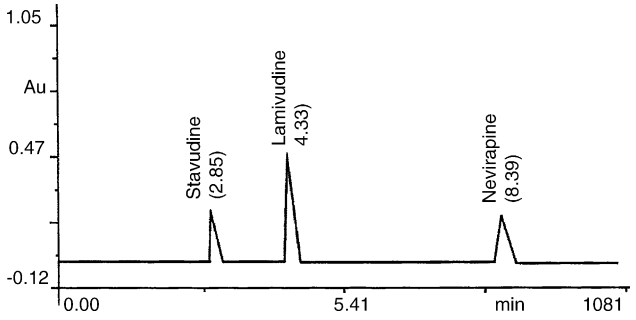

Fig. 1. RP-HPLC chromatogram of the tablet sample (SV, LV and NV).

2.4. RP-HPLC method

The phosphate buffer was prepared by dissolving 3.12 g of sodiumdihydrogen phosphate and 1.87 g of 1-octanesulphonic acid sodium salt in distilled water and made up to the volume 1000 ml. The drugs were resolved using a mobile phase of 20 mM sodium phosphate buffer (containing 8 mM 1-octanesulphonic acid sodium salt):acetonitrile (4:1, v/v) with pH adjusted to 3.5 using phosphoric acid, filtered using membrane filter and degased. The flow rate was 1.5 ml/min and the effluents were monitored at 265 nm (Fig. 1).

A stock solution was prepared by dissolving 30, 150 and 200 mg of SV, LV and NV in 100 ml of mobile phase. The stock solution was further diluted with the mobile phase to obtain various concentration of SV (10–50 μg/ml), LV (50–250 μg/ml) and NV (66.6–333.2 μg/ml), respectively. These solutions were used to calculate the linear dynamic range and for the relative quantification of the tablets.

About 20 tablets were weighed and powdered. A powder equivalent of 25 mg of SV was weighed accurately and transferred to a 100 ml volumetric flask. The tablet powder was dissolved in the mobile phase and filtered through a membrane filter (0.45 μm). The sample solution was suitably diluted and used for the analysis. Twenty microlitres of standard and sample solutions were injected, respectively, under the specified conditions and scans were recorded. Each

Table 1
Quantification parameters of stavudine, lamivudine and nevirapine

Sample	Label claim (mg)	UV method		RP-HPLC method		HPTLC method	
		Assay[a] (mean (%) ± S.E.M.)	%R.S.D.	Assay[a] (mean (%) ± S.E.M.)	%R.S.D.	Assay[a] (mean (%) ± S.E.M.)	%R.S.D.
Tablet-A							
Stavudine	30	99.95 ± 0.274	0.477	100.55 ± 0.265	0.4565	99.92 ± 0.199	0.3449
Lamivudine	150	100.01 ± 0.246	0.4261	99.47 ± 0.16	0.2786	100.15 ± 0.159	0.2749
Nevirapine	200	99.91 ± 0.209	0.3623	99.75 ± 0.311	0.5401	100.31 ± 0.255	0.4403
Tablet-B							
Stavudine	30	100.34 ± 0.262	0.4523	100.37 ± 0.426	0.7352	100.64 ± 0.243	0.4182
Lamivudine	150	99.88 ± 0.224	0.3885	100.52 ± 0.154	0.2653	101.18 ± 0.299	0.5117
Nevirapine	200	99.89 ± 0.206	0.3572	101.22 ± 0.115	0.1968	101.51 ± 0.32	0.5461

[a] Each value is a mean of six observations.

Table 2
System suitability parameters (RP-HPLC)

Parameters	Stavudine	Lamivudine	Nevirapine
Retention time (min)	2.85	4.33	8.39
Theoretical plates	9661	10766	15561
Tailing factor	1.29	1.21	1.36
Calibration range (μg/ml)	10–50	50–250	66.6–333.2

solution was run thrice at an interval of 20 min to ensure the elution of earlier injection. The amount of SV, LV and NV present per tablet was calculated by comparing the peak area of sample with that of standard. The stability [32] of the sample in the mobile phase was analyzed after 24 h; it was found that there was no change in the analytical parameters, which was indicative of the stability of all of these drugs in the solvents employed for the analysis. The quantification data and system suitability data are presented in Tables 1 and 2, respectively.

2.5. HPTLC method

The drugs were resolved using a mobile phase of chloroform:methanol (9:1, v/v). A stock solution was prepared by dissolving 30, 150 and 200 mg of SV, LV and NV in 100 ml of mobile phase. The stock solution was further diluted with the mobile phase to obtain various concentration of SV (10–60 μg/ml), LV (50–300 μg/ml) and NV (60–400 μg/ml), respectively. These solutions were used to calculate the linear dynamic range and for the relative quantification of the tablets (Fig. 2).

The sample was prepared as that of HPLC method and filtered through a Whatman filter paper. The sample solution was suitably diluted and used for the analysis. Two microlitres of standard and sample solutions were applied as bands 4 mm long at 4-mm intervals under a stream of nitrogen. The developed chromatograms were evaluated by scanning in densitometric mode at 265 nm. The amount of SV, LV and NV present per tablet was calculated by comparing the peak area of sample with that of standard. The analytical data are presented in Table 1.

2.6. Recovery studies

Recovery studies were carried out by adding known quantities of standards at different levels to the pre-analyzed sample to study the linearity, accuracy and precision of the proposed methods. The recovery studies also reveals whether there is a positive or negative influence on the quantification parameters by the additives usually present in the dosage forms. The recovery study data are presented in Table 3.

3. Results and discussion

In the UV multi-component spectral method, the linearity of SV, LV and NV was 0.8–6.4 μg/ml ($r = 0.996$), 4–32 μg/ml ($r = 0.9962$) and 5.33–42.64 μg/ml ($r = 0.9843$), respectively. The recovery values were 99.88–100.34% with percentage relative standard deviation (%R.S.D.) of <0.48.

In the RP-HPLC method, system suitability (Table 2) was applied to a representative chromatograph to check various parameters such as efficiency, resolution and peak tailing which was found to comply with the BP requirements [33]. The retention time of SV, LV and NV was 2.85, 4.33 and 8.39 min with a linear dynamic range of 10–50 μg/ml ($r = 0.9968$), 50–250 μg/ml ($r = 0.9999$) and 66.6–333.2 μg/ml ($r = 0.9996$), respectively. The recovery values were 99.47–100.55% with %R.S.D. of <0.74.

In the HPTLC method, the R_f of SV, LV and NV was 0.21–0.27, 0.62–0.72 and 0.82–0.93 with a linear dynamic range of 10–50 μg/ml ($r = 0.997$), 50–250 μg/ml ($r = 0.998$) and 66.6–333.2 μg/ml ($r = 0.9993$), respectively. The recovery values were 99.42–101.64% with %R.S.D. of <0.55.

The proposed methods for the quantification of SV, LV and NV in different brands of tablets were simple, precise, accurate, rapid and selective. The methods are linear in the concentration range reported. The developed method are free from interference due to the excipients present in various brands of tablets and can be used for routine simultaneous quantitative estimation of SV, LV and NV in tablets. In

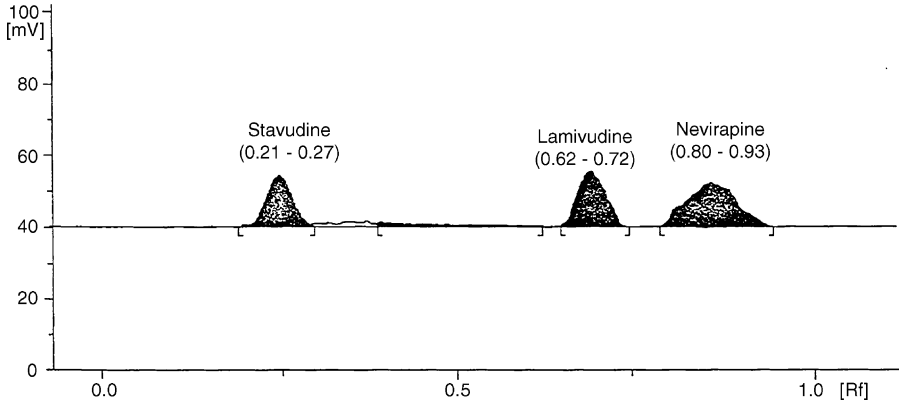

Fig. 2. HPTLC chromatograms of the tablet sample (SV, LV and NV).

Table 3
Recovery studies of stavudine, lamivudine and nevirapine in tablets

Sample	Label claim (mg)	Fortified amount (mg)	% Recovery[a]		
			UV	RP-HPLC	HPTLC
Tablet-A					
Stavudine	30	10	98.88	101.1	98.2
		20	98.33	102.9	99.46
Lamivudine	150	50	99.88	99.69	98.40
		100	98.16	100.59	98.35
Nevirapine	200	75	99.87	98.89	99.89
		150	99.51	100.30	100.92
Tablet-B					
Stavudine	30	10	99.86	100.86	99.96
		20	98.51	101.89	100.86
Lamivudine	150	50	99.87	99.76	99.2
		100	98.89	100.64	100.78
Nevirapine	200	75	99.86	99.33	100.68
		150	98.43	100.46	100.79

[a] Each value is a mean of six observations.

conclusion, the results have shown that HPLC method is best for a simultaneous quantification of SV, LV and NV in tablets.

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