Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 653–660
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Simultaneous determination of HIV-protease inhibitors lamivudine and zidovudine in pharmaceutical formulations by micellar electrokinetic chromatography

R. Sekar*, S. Azhaguvel

Analytical Chemistry Division, Indian Institute of Chemical Technology, Hyderabad 500007, India

Received 17 November 2004; received in revised form 1 May 2005; accepted 1 May 2005
Available online 20 June 2005

Abstract

A micellar electrokinetic chromatographic (MEKC) method for the simultaneous separation and determination of lamivudine (LMV) and zidovudine (ZDV) in pharmaceutical formulation has been developed. Factors that affect the separation, such as buffer pH, surfactant concentration (sodium dodecyl sulfate, SDS), organic solvents and applied voltage were optimized. Buffer consisting of 12.5 mM sodium tetraborate decahydrate and 15 mM boric acid adjusted at pH 10.8, containing 90 mM SDS and 5% (v/v) acetonitrile (ACN) was found to be suitable for the separation of the drugs. *p*-Aminobenzoic acid (PABA) was used as internal standard (I.S.). Detection of analytes and I.S. was performed at a wavelength of 210 nm. It was observed that both the drugs and I.S. were migrated within 20 min at the applied voltage of +10 kV. Validation of the method was performed in terms of linearity, accuracy, precision, limit of detection (LOD) and quantification (LOQ). An excellent linearity was obtained in the concentration range 10–80 μg/ml for LMV and 10–100 μg/ml for ZDV. The detection limits for LMV and ZDV were found to be 2.5 and 2.0 μg/ml, respectively. The optimized method was applied to the simultaneous determination of LMV and ZDV in pharmaceutical formulation and human plasma (spiked) samples. Recovery of both the drugs in tablet dosage form and spiked drugs in plasma were ≥99.72% (relative standard deviation (R.S.D.) ≤ 1.84%) and ≥80.4% (R.S.D. ≤ 5.4%), respectively. In the electropherogram no interfering peaks were observed in the region of analytes and I.S. due to inactive ingredients in the tablets and matrices in plasma.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Zidovudine; Lamivudine; Micellar electrokinetic chromatography; Pharmaceuticals

1. Introduction

The 2′-deoxy-3′-thiacytidine (lamivudine) and 3′-azido-3′-deoxythymidine (zidovudine) are synthetic nucleoside analogues used for the treatment of human immunodeficiency virus (HIV) [1–4]. Intra-cellularly, both of these nucleosides were phosphorylated to their corresponding active 5′-triphosphate metabolites. These metabolites inhibit reverse transcriptase via DNA chain termination after incorporation of the nucleoside analogue [5]. Both the drugs are available in single as well as in combined dosage forms. In recent years, practice of co-administration of multi-drug is increased for the effective treatment of HIV. The combined dosage form (lamivudine (LMV) plus zidovudine (ZDV)) has synergic antiretroviral activity in HIV-1-infected MT4-cells and reduces HIV-1 in seminal [6,7] and blood plasma [8].

Many methods have been reported for the determination of zidovudine [9–14], lamivudine [15–18] and their mixture [19–24] in biological fluids using high-performance liquid chromatography (HPLC). A few HPLC methods also been reported for the determination of ZDV [25] and LMV [26,27] in pharmaceutical formulations when they are present as individual drugs and their mixture [28]. Uslu and Ozkan [22] reported derivative spectrophotometric and HPLC methods for the simultaneous determination of LMV and ZDV in biological fluids as well as pharmaceutical formulations. The above derivative spectrometric method may suscep-

* Corresponding author. Tel.: +91 40 27160123; fax: +91 40 27160387.
E-mail address: seker@rediffmail.com (R. Sekar).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.05.002

tible to interferences due to the degradation product or any UV absorbing species in the formulations. The HPLC method required a costly gradient elution system and more time to equilibrate the column from one analytical run to another. In addition to that the internal standard (Finasteride) used to assay of the drug is not commercially available (Aldrich–Sigma and Merck).

Capillary electrophoresis has been widely adopted for the analysis of drugs and pharmaceuticals [29] due to its simplicity, wide application, high resolution and low running cost with eco-friendly solvents. By using CE technique, individual determinations of LMV and ZDV in biological fluids have been reported in the literature [30–33]. However, no CE method is available for the simultaneous determination of LMV and ZDV in pharmaceutical formulations. Further, the HPLC method [34] described in Indian pharmacopoeia (IP) for the assay of the both drugs is tedious and time consuming.

Hence, a simple, selective, eco-friendly and cost-effective technique is required for their routine analysis in dosage form as well as biological fluids. Therefore, we are reporting first time a CE method for the simultaneous determination of lamivudine and zidovudine in pharmaceutical formulation. Further, the application of this method extended to the analysis of the above drugs (spiked) in the human plasma. The experimental parameters that would influence the peak separation and efficiency, such as buffer pH, surfactant concentration, organic solvents and applied voltage were optimized.

2. Experimental

2.1. Chemicals and reagents

All chemicals used in the analysis were of analytical-reagent grade. Sodium tetraborate decahydrate, sodium dodecyl sulfate (SDS), acetonitrile (ACN), *p*-aminobenzoic acid (PABA), methanol and boric acid were purchased from S.D. Fine Chem. Ltd. (Mumbai, India). Drug standards were obtained from the pharmacology division of our institute. Different brands of LMV and ZDV tablets were purchased from local pharmacies (Hyderabad, India). Deionized water was obtained by using a Milli-Q water purification system (Millipore, Molsheim, France). Plasma samples were obtained from healthy volunteers after obtaining their written consent.

2.2. Apparatus

CE experiments were performed using a Prince CE system (Prince Technologies, Model No. 460, The Netherlands) equipped with a Lambda 1010 UV–vis detector, an auto sampler. An uncoated fused-silica capillary of 75 μm i.d. (Polymicro, Phoenix, AZ, USA) with a total length of 72 cm (60.5 cm effective length) was used for the separation. The capillary was thermostated at 25 °C. Samples were kept in the auto sampler and injected by applying a pressure of 50 mbar for 6 s. A constant voltage of +10 kV was applied throughout the analysis. Detections were performed at a UV wavelength of 210 nm. Data acquisition and analysis were carried out with the DAx software supplied by the Prince Technologies.

A new capillary was conditioned by rinsing with 1.0 M sodium hydroxide for 15 min, water for 5 min and finally, with the buffer solution for 10 min. Between each run, the capillary was rinsed with water for 2 min, 0.1 M sodium hydroxide for 2 min, water for 1 min and the buffer solution for 3 min, successively.

2.3. Preparation of buffer solution and standards

The running buffer consisted of 12.5 mM sodium tetraborate decahydrate, 15 mM boric acid and 90 mM SDS was prepared in deionized water. Prior to the analysis, the pH of the buffer solution was adjusted to 10.8 with 1 M NaOH and filtered through 0.45 μm syringe filter and added 5% (v/v) of ACN.

Lamivudine (50 mg) and zidovudine (50 mg) were accurately weighed separately in a 50-ml volumetric flask; it was dissolved in methanol and finally made up to 50 ml with the same solvent. Working standards containing LMV, ZDV and internal standard (I.S.) were prepared by diluting stock standards (1 mg/ml) to get a final concentration of 25, 50 and 50 μg/ml, respectively, with deionized water.

2.4. Preparation of sample

2.4.1. Tablets

Twenty tablets were crushed into fine powder in a mortar and homogenized. A portion of the powder that equivalent to 25 mg of LMV and 50 mg of ZDV was weighed accurately and transferred into a 100 ml conical flask. The powder was mixed uniformly in methanol using a mechanical stirrer. The solution was filtered and made up to 50.0 ml with methanol. A portion of filtrate solution was diluted to get 25 μg/ml of LMV, 50 μg/ml of ZDV and spiked with I.S. (50 μg/ml) at the time of analysis. Sample and standard solutions were stored in a refrigerator at 4 °C, when it is not used. They were stable for more than a month under these storage conditions. Buffer solution and samples were filtered through 0.45 μm membrane prior to use.

2.4.2. Plasma sample

Blood samples (at least 72 h prior to blood sample collection, no drug was administrated) were collected from healthy volunteers after obtaining their consent. The plasma was separated immediately after collection by centrifuging at 5000 rpm for 10 min. To a known volume (0.5 ml) of plasma sample, different concentrations of drugs were spiked to get the final concentration 10–60 μg/ml of LMV and ZDV and constant amount 20 μg/ml of I.S. The solution was treated with 2 ml of methanol in order to precipitate proteins. The sample mixture tubes were vortexed for 2 min and centrifuged for 15 min at 5000 rpm. The entire volume of the clear supernatant was transferred carefully into a tube and evaporated

under a gentle nitrogen flow at room temperature. The evaporated residue was reconstituted in 0.5 ml of deionized water and used for CE analysis.

3. Results and discussion

3.1. Method development

The structures of LMV, ZDV and PABA (I.S.) and their pK_a values are shown in Fig. 1. The simultaneous separation and determination of LMV and ZDV is not possible over a buffer pH range 3.5–7.0 by capillary zone electrophoretic (CZE) method, because at this pH range, ZDV expected to be neutral, while LMV in cationic form. And above pH 7, a poor peak shape of ZDV was observed, however, a sharp and symmetric peak was observed while using SDS surfactant [32]. Hence, for simultaneous separation and determination of both the drugs, a micellar electrokinetic chromatographic (MEKC) system was adopted. In MEKC, analytes are getting separated based on their interaction with micelle and solvent phase in addition to the electrophoretic behavior.

3.1.1. Effect of buffer pH

In general, in MEKC system, the pH of the buffer below the neutral value is not preferred, because at lower pH a slow electro-osmotic flow (EOF) that leads a longer time of analysis. To optimize buffer pH, buffer solution consisting of 12.5 mM sodium tetraborate decahydrate, 15 mM boric acid and 30 mM SDS, was adjusted to pH 9–11 with 1.0 M NaOH. Base line separation of analytes and I.S. was observed at pH 10.8. However, LMV migrates closer to the EOF peak and this find difficult to measure the peak area accurately.

3.1.2. Effect of SDS

Influence of SDS concentration on the separation of the analytes in borate buffer of pH 10.8 was investigated. Fig. 2 shows the separation of analytes as a function of SDS (15–90 mM) concentration. As can be seen from Fig. 2, while increasing concentration of SDS, the migration velocity of LMV is markedly increased, whereas ZDV was slightly influenced. As the interior cores of SDS micelles are highly hydrophobic, the selectivity of solutes is mainly governed by

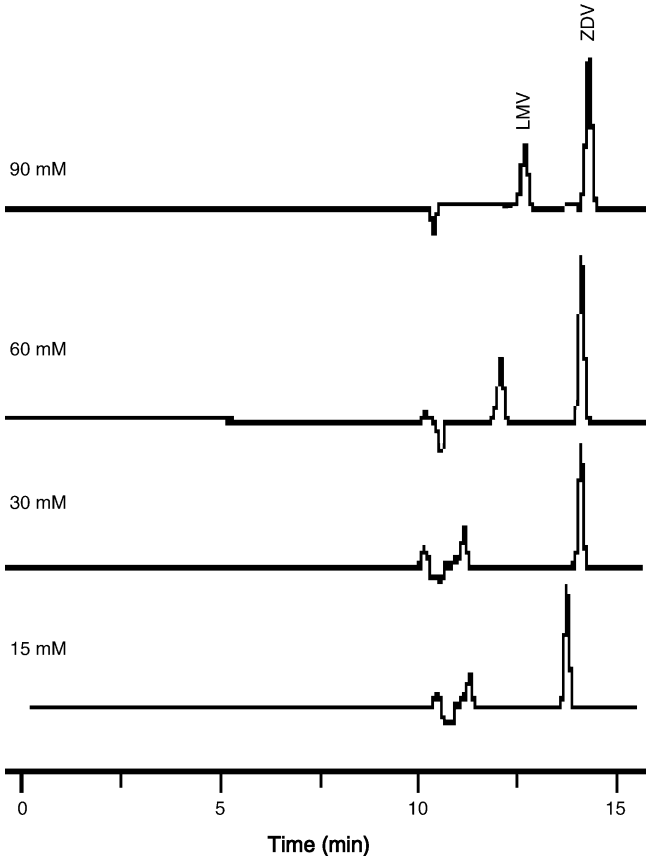

Fig. 2. Effect of SDS concentrations on migration of LMV and ZDV. Electrophoretic conditions: 12.5 mM sodium tetraborate decahydrate, 15 mM boric acid (pH 10.8) and SDS 15–90 mM; capillary length: 72 cm (effective length: 60.5 cm) × 75 μm i.d.; applied voltage: 10 kV; hydrodynamic injection: 50 mbar for 6 s; UV detection: 210 nm.

the hydrophobic interaction. At 90 mM SDS concentration, LMV peak migrated away from the EOF peak, the reason is due to the interaction of neutral LMV with SDS micelles via solubilization and the extent of interaction is depend on the hydrophobicity of the molecule [35]. In MEKC method, neutral solutes migrate only by the chromatographic process, whereas ionic solutes migration depends upon the degree of micellar solubilization and electrophoretic mobility of the molecules in the ionized state [36]. The reason for the increasing migration time LMV is due to the negatively charged

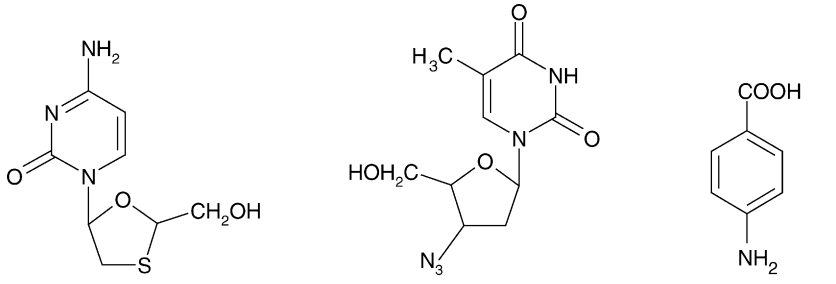

Fig. 1. Chemical structures of LMV, ZDV, I.S. and their pK_a values.

micelle, which was electrophoretically attracted towards anode and this, would subsequently lead to increased migration time of neutral molecules [37]. Even though the better separation of analytes appears at 60 mM SDS concentration, 90 mM SDS concentration was optimized because the LMV migrated away from EOF peak, and hence, it can be integrated the peak area accurately. Further at this concentration the both analytes gave maximum peak efficiency ($N \geq 31{,}000$ for LMV and 50,000 for ZDV, where N are theoretical plates calculated by the half width method).

3.1.3. Effect of organic solvents

To optimize the organic solvents several aspects to be considered: resolution, speed of analysis time, efficiency and reproducibility. The influence of organic solvents on peak efficiency and resolution of LMV and ZDV were tested by adding methanol, 2-propanol and acetonitrile to the buffer solution (12.5 mM sodium tetraborate decahydrate, 15 mM boric acid, 90 mM SDS and pH 10.8). On increasing concentration of organic solvents in buffer, migration of analytes and I.S. were increased. The organic solvents not only changes the pH, dielectric constant and viscosity, but also affects the zeta potential and in results decrease in EOF [38–41] and thereof, the migration velocity of analytes changes. However, the migration time on addition of ACN has not significantly increased compared to alcoholic solvents (methanol and 2-propanol) and the reason for this is ACN has a tendency to form solvent clusters rather than hydrogen bonding in aqueous buffer and has a different effect on EOF compared to alcoholic solvents. Hence, with an increase in the percentage (v/v) of ACN in the buffer electrolyte, the magnitude of the EOF changed only to a small extent compared to alcoholic solvents [42]. Fig. 3 shows the peak efficiencies (theoretical plate numbers) and peak-to-peak resolution of LMV and ZDV at 5% (v/v) content of methanol, 2-propanol and acetonitrile in running buffer. From figure, it can be seen that on

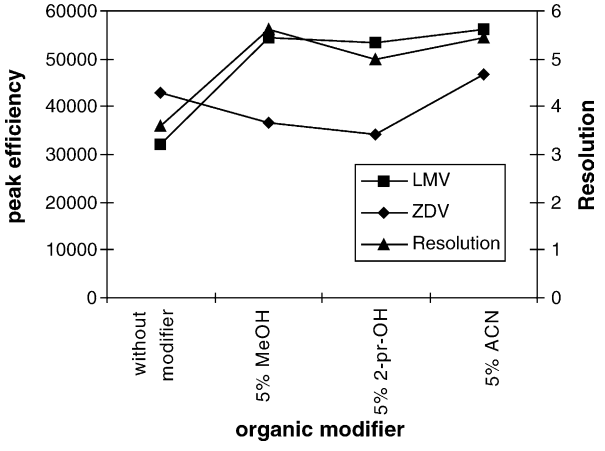

Fig. 3. Effect of organic modifiers on peak efficiencies and resolution. Electrophoretic conditions: 12.5 mM sodium tetraborate decahydrate, 15 mM boric acid (pH 10.8) and 90 mM SDS; capillary length: 72 cm (effective length: 60.5 cm) × 75 μm i.d.; applied voltage: 10 kV; hydrodynamic injection: 50 mbar for 6 s; UV detection: 210 nm.

an addition 5% ACN (v/v) provides greater peak efficiency (≥544,30) and resolution (≥6.39) compared to the absence of ACN with reasonable analysis time. Therefore, ACN was chosen as an organic modifier for the separation of LMV and ZDV.

3.1.4. Effect of applied voltage and injection (pressure/time)

Under the optimized condition, the effect of applied voltage on peak efficiency of the analytes was studied over the range of 5–20 kV. As expected, on increasing the applied voltage leads to shorter migration time and sharper peak. However, higher applied voltage exhibits higher current and increased joule heating. Further, poorer reproducibility of the analytes and I.S. was observed. Hence, a voltage of 10 kV (≈53.2 μA) was chosen for the reasonable analysis time with best peak efficiency. Under this condition the migration time of LMV, ZDV and I.S. were 12.5, 15.0 and 18.6 min, respectively.

In order to obtain the highest sensitivity without affecting the peak shape and resolution of the analytes, the injection pressure (25 and 50 mbar, hydrodynamic) and the duration of injection (3, 6 and 12 s) were varied by keeping one parameter is constant and varying the other at a time. While increasing pressure and injection time, peak area is increased correspondingly, however, peak broadening is observed. Injection time longer than 6 s (at 50 mbar pressure) caused a loss of efficiency greater than 8%. Therefore, 50 mbar injection pressure for a period of 6 s was optimized to get better sensitivity without peak broadening.

3.1.5. Selection of internal standard

In CE, internal standards were used in the same way as in chromatography. The use of an internal standard was crucial in order to obtain a good reproducibility and to compensate the injection errors and loss of solvents due to evaporation [43]. To select a suitable internal standard, the following compounds were screened: deoxycytidine, imidazole, nicotinic acid, indole, paracetamol and *p*-aminobenzoic acid. Among them, *p*-aminobenzoic acid was found to be suitable in terms migration time and UV response, whereas others were either overlapped with analyte peaks or migrated after a long time.

3.1.6. Selection of suitable detection wavelength

The reported HPLC method [22] for the detection of LMV and ZDV performed at UV wavelength of 265 nm. In the present study, UV detections were initially carried out for both the drugs at 265 nm as well as at 210 nm. The corrected peak areas (arbitratory units) for a concentration of LMV (25 μg/ml) and ZDV (50 μg/ml) at 265 nm were 2.28×10^{-5} and 3.68×10^{-5}, and at 210 nm, 3.53×10^{-5} and 6.34×10^{-5}, respectively. Detector response for the both drugs was almost 1.5 times higher at 210 nm than that of 265 nm for the same concentration. Therefore, 210 nm was selected for the detection of both the drugs.

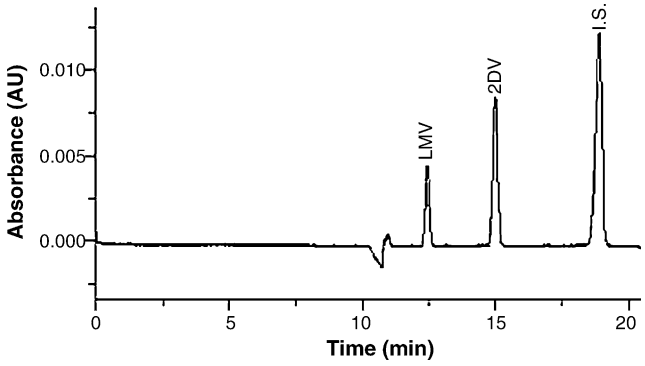

Fig. 4. Typical electropherogram of standard mixture of LMV (25 μg/ml), ZDV (50 μg/ml) and I.S. (50 μg/ml). Electrophoretic conditions: 12.5 mM sodium tetraborate decahydrate, 15 mM boric acid (pH 10.8) and 90 mM SDS + 5% (v/v) ACN; capillary length: 72 cm (effective length: 60.5 cm) × 75 μm i.d.; applied voltage: 10 kV; hydrodynamic injection: 50 mbar for 6 s; UV detection: 210 nm.

3.1.7. Optimized conditions

As shown in Fig. 4, baseline separation of the LMV, ZDV and I.S. was achieved in less than 20 min using buffer composition of 12.5 mM sodium tetraborate decahydrate and 15 mM boric acid (pH 10.8), containing 90 mM SDS and 5% (v/v) ACN. This optimized separation was performed at 25 °C, when applying a 10 kV voltage.

3.2. Method validation

3.2.1. Linearity range

Under the optimized conditions, calibration curves were obtained for LMV and ZDV. Five standard mixtures of different concentrations were prepared. The corrected peak areas were used for constructing the calibration graph. Linear range, regression equation, correlation coefficient, slope, intercept, standard error of slope, standard error of intercept, limit of detection and quantification of the two drugs were listed in Table 1.

Table 1
Statistical data for the calibration graphs of LMV and ZDV

Statistical parameters[a]	ZDV	LMV
Linearity range (μg/ml)	10–100	10–80
Regression equation	$y = 0.01203x + 0.00848$	$y = 0.00125x + 0.00242$
Correlation coefficient (r^2)	0.9998	0.9997
Slope	0.01203	0.00125
Intercept	0.00848	0.000242
S.D. for slope	0.0013	0.00002
S.D. for intercept	0.00844	0.00118
LOD (μg/ml)	2.0	2.5
LOQ (μg/ml)	5.8	7.6
% R.S.D. (LOQ)	1.92	1.86

[a] $n = 5$.

Table 2
Accuracy

Drug	Added amount (μg)	Recovered amount (μg)[a]	Recovery (%)	R.S.D. (%) ($n = 3$)
ZDV	15	14.97	99.82	1.88
	20	19.72	98.60	1.06
	40	39.92	99.80	1.52
LMV	15	15.02	100.15	1.21
	30	30.42	101.34	1.79
	50	49.84	99.68	1.65

[a] Mean of three injections.

3.2.2. Accuracy

In order to examine the accuracy of the method and to check the interference from excipients used in tablet dosage formulation, the recovery studies were carried out by standard addition method. In this method, three different amounts of LMV and ZDV were added to a constant known concentration of the composite tablet solution. Each solution was injected in triplicates and the amounts determined were compared to theoretical amounts. The results of the recovery were summarized in Table 2. The recoveries ranged from 98.60 to 99.82% for ZDV and from 99.68 to 101.34% for LMV. The relative standard deviation (R.S.D.) of ≤1.88% was observed for the both drugs, indicates that the method gives sufficient accuracy.

3.2.3. Precision

Precision of the method was tested in terms of peak area and migration time repeatability. Intra- and inter-day (over 5 days) precisions were evaluated performing five replicated injections covering lower and higher concentration of LMV and ZDV. The R.S.D. values obtained for peak area and migration time for intra- and inter-day were ≤1.98, 1.65 and ≤3.21, 1.97%, respectively (Table 3). It can be seen from Table 3, a good precision of migration time and peak area was obtained for both the drugs.

3.2.4. LOD and LOQ

LOD is established at a signal-to-noise ratio (S/N) of 3, whereas LOQ is at a signal-to-noise ratio of 10. The LOD

Table 3
Intra- and inter-day precision

Analyte	Concentration (μg/ml)	R.S.D.[a,b] (%)			
		Intra-day		Inter-day	
		Peak area	Migration time	Peak area	Migration time
ZDV	25	1.18	0.96	2.12	1.32
	50	1.34	0.98	2.38	1.38
	75	1.98	1.03	2.62	1.82
LMV	20	1.96	1.12	2.21	1.26
	40	1.32	1.23	2.64	1.48
	60	1.56	1.65	3.21	1.97

[a] $n = 5$ times.
[b] $n = 5$ days.

and LOQ were experimentally verified by injecting various concentrations of sample ranging from 1.0 to 15.0 μg/ml. The signal-to-noise ratio is calculated by using software supplied by Prince Technologies. The LOD and LOQ of 2.5 and 7.6 μg/ml for LMV and 2.0 and 5.8 μg/ml for ZDV were observed, respectively.

3.2.5. Specificity

The effect of inactive ingredients (placebo) on the determination of both the drugs in tablet formulation was studied. Each tablet contains 150 mg of LMV and 300 mg of ZDV along with some inactive ingredients, such as colloidal silicon dioxide, hypromellose, magnesium stearate, polysorbate 80, sodium starch glycolate and titanium oxide. In the electropherogram, no interfering peaks were observed in the region of analytes and I.S. due to inactive ingredients in the tablets (figure not shown). Therefore, a simple filtration is sufficient to remove the undissolved matrices. Further, thymine, one of the related substances of ZDV [44] did not interfere in the determination, as evident from its migration time 17.0 min and which is different from those of LMV and ZDV.

3.2.6. Robustness

Robustness of the proposed method was tested by small but deliberate variations of pH (10.5–11.0), SDS (85–95 mM), applied voltage (9–10 kV) and capillary length. The variations for corrected peak area and peak-to-peak resolution between LMV and ZDV were ±5 and ±8%, respectively, from the optimized condition values.

3.3. Application to the pharmaceutical formulation

The validated method was employed to quantify LMV and ZDV in commercially available composite tablets. Typical electropherogram of the Duovir tablet (LMV 150 mg plus ZDV 300 mg) is shown in Fig. 5. The analysis results (Table 4) have shown good agreement with the labelled content and the recovery of LMV and ZDV in tablets were ≥99.72% with R.S.D. ≤ 1.84%. Thus, the method was suitable for simultaneous determination of LMV and ZDV in routine quality control laboratories.

Table 4
Results of LMV and ZDV analysis in composite tablets

	LMV		ZDV	
	Brand 1	Brand 2	Brand 1	Brand 2
Labelled amount (mg/tablet)	150	150	300	300
Amount found (mg/tablet)[a]	149.58	150.58	300.26	299.89
Recovery (%)	99.72	100.38	100.08	99.96
R.S.D. (%)	1.08	1.48	1.46	1.84

[a] Mean of the six determinations.

3.4. Application to the plasma sample

The applicability of the proposed method was tested for the determination of both drugs simultaneously in biological matrices (human plasma). Both LMV and ZDV were spiked to the human plasma and used as a test sample. As described in Section 2, the only pre-treatment, we performed was to deproteinize the plasma with the addition of methanol, followed by centrifugation. The precision of the method was assessed by determining four concentrations of each drug within the range of 10–60 μg/ml. Even though CE is known to be less sensitive than HPLC, the present method was able to detect up to 10 μg/ml of ZDV comparable to the reported HPLC method by Uslu and Ozkan [22]. The recovery percentage and precision at four different concentrations in plasma are summarized in Table 5. Extraction recoveries of both drugs were higher than 80.4%. Fig. 6A shows the electropherogram of LMV and ZDV along with I.S. extracted from plasma as given in Section 2.4.2. Plasma blank (Fig. 6B) electropherogram indicate that no interfering peaks due to endogenous

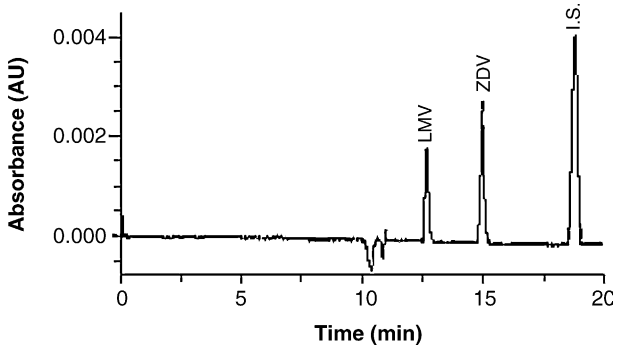

Fig. 5. Typical electropherogram of Duovir tablet formulation with I.S. (PABA). Electrophoretic conditions as given in Fig. 4.

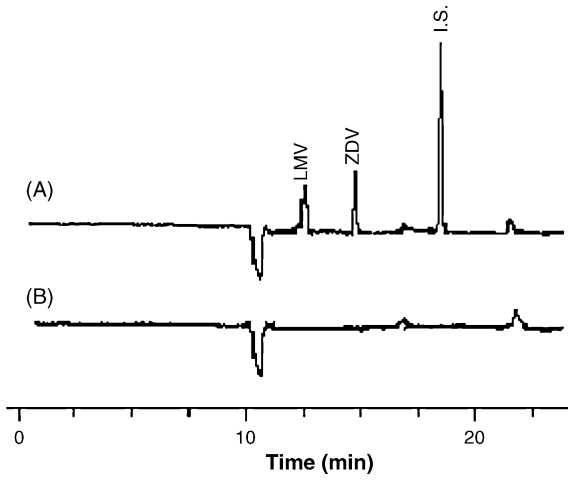

Fig. 6. Typical electropherogram of: (A) human plasma spiked with mixture of LMV (10 μg/ml), ZDV (20 μg/ml) and I.S. (20 μg/ml) and (B) blank human plasma. Electrophoretic conditions as given in Fig. 4.

Table 5
Recovery and precision of LMV and ZDV in human plasma samples

Drug	Amount added (μg)	Amount found (μg)	Recovery (%)[a]	R.S.D.
LMV	10.0	8.42	84.2	3.4
	20.0	18.08	90.4	2.8
	30.0	28.62	95.4	1.7
	60.0	58.56	97.6	1.2
ZDV	10.0	8.04	80.4	5.4
	20.0	17.94	89.7	4.2
	30.0	28.26	94.2	3.4
	60.0	57.78	96.3	2.5

[a] Mean recovery of four determination.

substances were observed in the region of drugs and I.S. The limit of detection of LMV and ZDV in Plasma samples was 3.5 and 2.8 μg/ml, respectively.

4. Conclusion

The present MEKC method is simple and precise for the simultaneous determination of ZDV and LMV in pharmaceutical formulations. A good separation of analytes was achieved using 12.5 mM sodium tetraborate decahydrate and 15 mM boric acid buffer solution (pH 10.8), containing 90 mM SDS and 5% (v/v) ACN. The recovery of active ingredients in drug formulations was ≥99.72% with ≤1.84% R.S.D., which indicates that the method is suitable for quantify LMV and ZDV in pharmaceutical formulation without any interference of other inactive ingredients. As compared with the reported derivative spectrophotometric and HPLC method [22], the present method is simple and fast; the above method requires long time to equilibrate the column and gradient elution for separations of drugs.

Acknowledgments

Authors are grateful to Dr. J.S. Yadav, Director and Dr. M. Vairamani, Head, Analytical Chemistry Division, of this Institute for their constant encouragement to pursue this research work.

References

[1] H. Mitsuya, K.J. Weinhold, P.A. Furman, M.H.St. Clair, S. Nusinoff Lehrman, R.C. Gallo, D. Bolognesi, D.W. Barry, S. Broder, Proc. Natl. Acad. Sci. U.S.A. 82 (1985) 7096–7100.
[2] P.A. Furman, J.A. Fyfe, M.H.St. Clair, K. Weinhold, J.L. Rideout, G.A. Freeman, S. Nusinoff Lehrman, D.P. Bolognesi, S. Broder, H. Mitsuya, D.W. Barry, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 8333–8337.
[3] H. Nakashima, T. Matsui, S. Harada, N. Kobayashi, A. Matsuda, T. Uede, N. Yamamoto, Antimicrob. Agents Chemother. 30 (1986) 933–937.
[4] J.A.V. Coates, N. Cammack, H.J. Jenkinson, A.J. Jowerr, M.J. Jowelt, B.A. Pearson, C.R. Penn, P.L. Rouse, K.C. Viner, J.M. Cameron, Antimicrob. Agents Chemother. 36 (1992) 733–739.
[5] R. Yarchoan, H. Mitsuya, C.E. Myers, S. Broder, N. Engl. J. Med. 321 (1989) 726–738.
[6] B.L. Gilliam, J.R. Dyer, S.A. Fiscus, C. Marcus, S. Zhou, L. Wathen, W.W. Freimuth, M.S. Cohen, J.J. Eron, J. Acquir. Immun. Defic. Syndr. Hum. Retrovirol. 15 (1997) 54–60.
[7] P.L. Vernazza, B.L. Gilliam, M. Flepp, J.R. Dyer, A.C. Frank, S.A. Fiscuss, M.S. Cohen, J.J. Eron, AIDS 11 (1997) 1249–1254.
[8] S. Staszewski, C. Loveday, J.J. Picazo, P. Dellarnomica, P. Skinhoj, M.A. Johnson, S.A. Danner, P.R. Harrigan, A.M. Hill, L. Verity, H. McDade, JAMA 276 (1996) 111–117.
[9] J.D. Unadkat, S.S. Crosby, J.P. Wang, C.C. Hertel, J. Chromatogr. 430 (1988) 420–423.
[10] M.A. Hedaya, R.J. Sawchuk, Clin. Chem. 34 (1988) 1565–1568.
[11] S.S. Good, D.J. Reynolds, P. De Mirande, J. Chromatogr. 431 (1988) 123–133.
[12] T. Nadal, J. Ortuno, J.A. Pascual, J. Chromatogr. A 721 (1996) 127–137.
[13] R.M. Ruprecht, A.H. Sharpe, R. Jaenish, D. Trites, J. Chromatogr. 528 (1990) 371–383.
[14] E. Font, O. Rosario, J. Satana, H. Garcia, J.P. Sommadossi, J.F. Rodriguez, Antimicrob. Agents Chemother. 43 (1999) 2964–2968.
[15] A.J. Harker, G.L. Evans, A.E. Hawky, D.M. Morris, J. Chromatogr. B 657 (1994) 227–232.
[16] D.M. Morris, K. Selinger, J. Pharm. Biomed. Anal. 12 (1994) 255–264.
[17] X.J. Zhou, J.P. Sommadosi, J. Chromatogr. B 691 (1997) 417–424.
[18] R.M. Hoetelmans, M. Profijit, P.L. Mennhornst, J.W. Mulder, J.H. Beijnen, J. Chromatogr. B 713 (1998) 387–394.
[19] K.B. Kenney, S.A. Wring, R.M. Carr, G.N. Wells, J.A. Dunn, J. Pharm. Biomed. Anal. 22 (2000) 967–983.
[20] A.S. Pereira, K.B. Kenney, M.S. Cohen, J.E. Hall, J.J. Eron, R.R. Tidwell, J.A. Dunn, J. Chromatogr. B 742 (2000) 173–183.
[21] J.F. Rodriguez, J.L. Rodriguez, J. Santana, H. Garcia, O. Rosario, Antimicrob. Agents Chemother. 44 (2000) 3097–3100.
[22] B. Uslu, S.A. Ozkan, Anal. Chim. Acta 466 (2002) 175–185.
[23] Y. Alnouti, C.A. White, M.G. Bartlett, Biomed. Chromatogr. 18 (2004) 641–647.
[24] B. Fan, J.T. Stewart, J. Pharm. Biomed. Anal. 28 (2002) 903–908.
[25] W.V. Caufield, J.T. Stewart, Chromatographia 54 (2001) 561–568.
[26] H.Y. Aboul-Enein, M.M. Hefnawy, Anal. Lett. 36 (2003) 2527–2538.
[27] S.A. Ozkan, B. Uslu, J. Liq. Chromatogr. Rel. Technol. 25 (2002) 1447–1456.
[28] P. Djurdjevic, A. Laban, S. Markovic, M. Jelikic-Stankov, Anal. Lett. 37 (2004) 2649–2667.
[29] K.D. Altria, M.A. Kelly, B.J. Clark, Trends Anal. Chem. 17 (1998) 204–214 (and references therein cited).
[30] B. Fan, J.T. Stewart, J. Cap. Electrophor. 7 (2002) 103–106.
[31] N. Mesplet, P. Morin, C. Francois, L.A. Agrofoglio, J. Chromatogr. A 927 (2001) 161–168.
[32] B. Fan, J.T. Stewart, J. Pharm. Biomed. Anal. 30 (2002) 955–960.
[33] L.A. Agrofogolio, X. Cahours, T.T. Tan, H. Dessans, C. Kieda, P. Morin, Nucleosides Nucleotides Nucleic Acids 20 (2001) 375–381.
[34] Indian Pharmacopoeia, third ed., Controller of Publication, Government of India, New Delhi, Addendum 2002, pp. 914–916.
[35] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111–113.
[36] S. Orlandini, S. Fanali, S. Furlanetto, A.M. Marras, S. Pinzauti, J. Chromatogr. A 1032 (2004) 253–263.
[37] C.P. Ong, C.L. Ng, H.K. Lee, S.F.Y. Li, J. Chromatogr. A 686 (1994) 319–324.

[38] S. Hillaert, Y. Vander Heyden, W. Van den Bossche, J. Chromatogr. A 978 (2002) 231–242.
[39] L. Barthe, J.P. Ribet, M. Pelissou, M.J. Degude, J. Fahy, A. Duflos, J. Chromatogr. A 968 (2002) 241–250.
[40] R. Loos, R. Niessner, J. Chromatogr. A 835 (1999) 217–229.
[41] J. Cai, Z. El Rassi, J. Chromatogr. 608 (1992) 31–45.
[42] S.S. Yang, I. Smetena, A.I. Goldsmith, J. Chromatogr. A 746 (1996) 131–136.
[43] A. Kunkel, S. Gunter, C. Dette, H. Watzig, J. Chromatogr. A 781 (1997) 445–455.
[44] European Pharmacopoeia, fifth ed., Council of Europe, Strasbourg, France, 2005, pp. 3043–3044.