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Determination of lovastatin and simvastatin in pharmaceutical dosage forms by MEKC☆

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Abstract

A micellar electrokinetic chromatographic (MEKC) method was developed for the quantification of lovastatin and simvastatin, cholesterol lowering agents in pharmaceutical dosage forms. Lovastatin and simvastatin were separated using an electrolyte system consisting of 12% acetonitrile (v/v) in 25 mM sodium borate buffer pH 9.3 containing 25 mM sodium dodecyl sulphate (SDS) with an extended light path capillary (48.5 cm × 50 μm i.d, 40 cm to detector). The method has been validated and proven to be rugged. Calibration curves were linear over the studied ranges with correlation coefficients greater than 0.996. A limit of detection of 3.2 μg/ml and a limit of quantitation of 10.6 μg/ml were estimated for both the drugs. The proposed method was found to be suitable and accurate for the determination of these drugs in commercial formulations. © 2002 Published by Elsevier Science B.V.

Keywords: Lovastatin; Simvastatin; MEKC; Dosage form; Validation; Peak purity; R.S.D.

1. Introduction

Lovastatin (I), (S)-2-Methyl butyric acid, 8-ester with (4R,6R)-6-[2-[(1S,2S,6R,8S,8aR) -1,2,6,7,8a-hexahydro-8hydroxy-2,6-dimethyl-1-naphthyl]ethyl]tetarhydro-4hydroxy-2H-pyran-2-one and simvastatin (II), 2,2-Dimethyl butyric acid, 8-ester with (4R,6R)-6-[2-[(1S,2S,6R,8S,8aR)-1,2,6,7,8a-hexahydro-8hydroxy-2,6-dimethyl-1-naphthyl]ethyl] tetarhydro-4hydroxy-2H-pyran-2-one are cholesterol lowering agents isolated from a strain of aspergillus terreus [1,2]. In recent years some high performance liquid chromatographic methods were reported for the analysis of lovastatin and simvastatin in bulk drugs, pharmaceutical formulations and in human plasma [3–6]. We focussed our interest on the use of capillary electrophoresis (CE) for the quantification of lovastatin and simvastatin in formulations due to several advantages such as high resolution, efficiency, small sample and buffer volumes [7]. Till date no CE methods were reported for the quantitative determination of these drugs in pharmaceutical formulations. In this paper we report the development of a unique micellar electrokinetic chromatographic (MEKC) method for the deter-

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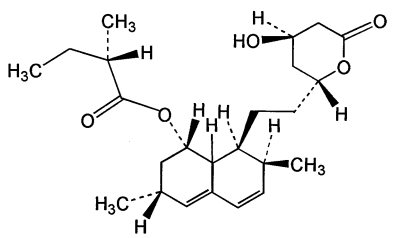
mination of lovastatin and simvastatin in pharmaceutical formulations. Though the developed MEKC method has longer runtime, it is cost effective and more environment friendly when compared with the reported LC methods. Therefore, the developed MEKC method can be used as a versatile alternative over the liquid chromatographic methods for the analysis of lovastatin and simvastain in pharmaceuticals. In the proposed method both the drugs were well resolved with a resolution not less than 2.3. Simvastatin was used as an internal standard for the quantitative determination of lovastatin and vice-versa.

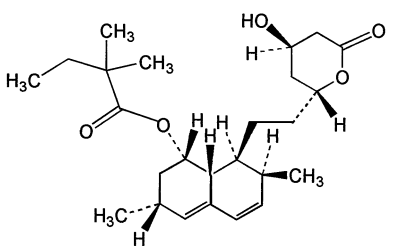
2. Experimental

2.1. Chemicals

Samples of lovastatin (I) and simvastatin (II) were received from Process Research and Technology Development Department of Dr. Reddy's Laboratories, Hyderabad, India. The chemical structures of I and II were given in Fig. 1. Tablets of lovastatin (Lostatin, 20 mg) and simvastatin (Simvotin, 20mg) were purchased from the local market. Lostatin and simvotin contain the following excipients namely cellulose, lactose, magnesium stearate, starch, hydroxypropyl cellulose, hydroxypropyl methylcellulose, talc and titanium dioxide. About 50 mM borate buffer solution pH-9.3 for HPCE was purchased from Hewlett–Packard, Waldbronn, Germany. Ultrapure sodium dodecyl sulphate (SDS electrophoresis) was purchased from Sisco-Research Laboratories Pvt. Ltd., Mumbai, India. HPLC grade acetonitrile was purchased from E. Merck (India) Ltd., Mumbai, India and Milli-Q water was prepared by using Milli Q plus purification system (Millipore, USA).



I



II

Fig. 1. Chemical structures of lovastatin (I) and simvastatin (II).

2.2. Instrumentation and electrophoretic procedure

Capillary electrophoresis experiments were performed on an Agilent CE system (Agilent Technologies, Waldbronn, Germany) with built in diode array detector (from 190 to 600 nm), an auto injector and a power supply able to deliver up to 30 kV. A CE ChemStation software was used for instrument control, data acquisition, and data analysis. The capillary thermostatting system employed high velocity forced air circulating at 10 m/s. A peltier device was used to control the temperature between 10 °C below ambient and 60 °C, with a precision of 0.1 °C.

An extended light path capillary with a 50 μm internal diameter used was of 48.5 cm length (HP part No. G1600-60232). An alignment interface, containing an optical slit matched to the internal diameter was used and the detection wavelength was set at 238 nm. The background electrolyte (BGE) contains 88 parts of 25 mM borate buffer (pH 9.3) containing 25 mM SDS and 12 parts of acetonitrile. The BGE was filtered through 0.2 μm syringe filter prior to use.

All experiments were carried out in cationic mode. The capillary temperature was established

at 30 °C. A constant voltage of 25 kV, was applied during analysis. Sample injections were achieved using the pressure mode for 5 s ! at 10 mbar.

Before use, the capillary was flushed with water for 10 min, followed by 0.1 M NaOH for 20 min and again with water for 5 min at a system default pressure 900 mbar. To achieve high migration time reproducibility between injections the capillary was equilibrated with the BGE for 10 min.

As electrolysis can change the EOF, which leads to poor quantitative assays in pharmaceutical analysis, the running electrolyte was replaced with a fresh electrolyte after four injections.

2.3. Preparation of background electrolyte (BGE)

A 10.0 ml of 25 mM borate buffer pH 9.3 was prepared by diluting 5.0 ml of 50 mM borate buffer pH 9.3 with 5.0 ml of highly pure water. Then required amounts of SDS was weighed and dissolved in 25 mM borate buffer pH 9.3 to maintain the concentration of 25 mM. Finally BGE was prepared by mixing 88 parts of 25 mM borate buffer pH 9.3 containing 25 mM SDS and 12 parts of acetonitrile.

2.4. Diluting solution

15 mM SDS solution was used as a diluting solution.

2.5. Preparation of sample solutions

Stock solutions of lovastatin and simvastatin were prepared by dissolving each compound in acetonitrile, in order to get a concentration of 2.0 mg/ml. Test solutions of lovastatin and simvastatin were prepared by taking 0.25, 0.375, 0.5, 0.625, and 0.75 ml of corresponding stock solutions in 5.0 ml volumetric flasks. To maintain uniform concentration of internal standard (0.2 mg/ml), 0.5 ml internal standard stock solution was added to each of the flasks and made up to the mark with the diluent. The sample solutions were filtered through 0.2 μm syringe filter prior to use so as to remove particles.

3. Results and discussion

3.1. Method optimization

In order to develop a CE method for the separation of I and II, the following parameters were consecutively optimized.

3.1.1. Influence of buffer pH and organic modifier (acetonitrile)

The first step in the method development process was the selection of optimum pH value and subsequent choice of capillary electrophoresis (CZE, MEKC). The optimization was initiated with a buffer concentration of 25 mM, at a voltage of 30 kV and at a temperature of 30 °C. The p^{Ka} values of I and II were lying between 7.5 and 10.5. Buffer solutions in the pH range 7.0–11.0 were studied in capillary zone electrophoresis mode (CZE) in the absence and in the presence of organic modifier (acetonitrile). None of the attempts gave separation for 1 and II in CZE mode and the peak shape was very broad in the entire studied pH range. MEKC then was applied for the separation of I and II. The introduction of anionic detergent SDS (25 mM) in the run buffer has no effect on the separation of I and II. But the peak shape was improved with the introduction of SDS.

The introduction of organic modifier (acetonitrile) in the presence of SDS in the run buffer has played a key role on the separation of I and II. The organic modifier alters the retention mechanism by changing the electrolyte viscosity and the zeta potential [8]. I and II were co eluted up to 5% acetonitrile concentration (v/v). On the other hand, the separation between I and II was getting increased with the increase in further levels of acetonitrile. Acetonitrile 12% concentration (v/v) was selected as a compromise between resolution and analysis time.

The retention of I and II was decreased with the increase in pH. However, borate buffer pH at 9.3 was selected for the further experiments as a compromise between resolution, peak shape and analysis time.

3.1.2. Influence of borate buffer concentration

The effect of borate buffer concentration on the separation was studied by varying it from 15 to 30 mM. The migration of I and II was decreased with the increase in buffer concentration. I and II were well separated in the entire studied concentration range. However, about 25 mM borate buffer was considered as optimum for its good resolution, peak shape and analysis time.

3.1.3. Influence of SDS concentration

The effect of SDS concentration on the separation was studied by varying it from 15 to 35 mM. The resolution between I and II was increased with the increase in SDS concentration. About 25 mM SDS was selected for the experiments because it gave sharp peaks with an acceptable current (45 μA) and analysis time.

3.1.4. Influence of capillary temperature and applied voltage

The effect of temperature on the separation between 20 and 40 °C was investigated. The resolution between I and II was decreased with the increase in capillary temperature. A temperature of 30 °C was selected as a compromise between run time, resolution and current generated in the capillary.

The effect of applied voltage on the separation was studied by varying it from 15 to 30 kV. The resolution between I and II was getting decreased with increase in applied voltage. A potential of 25 kV yielded the best compromise in terms of resolution and current generated in the capillary.

3.1.5. Optimized conditions

The optimized conditions consisted of electrolyte containing of 12% acetonitrile (v/v) in 25 mM borate buffer pH 9.3 containing 25 mM sodium dodecyl sulphate (SDS). The quantification was carried out at 30 °C and 25 kV. Under these conditions, lovastatin and simvastatin were separated to the baseline below 15 min (Fig. 2). The system suitability results of the developed method are given in Table 1.

During MEKC the electroosmotic flow of the system was measured by injecting methanol as marker substance. The electroosmotic mobility was found to be 1.1×10^{-5} cm^2/volt.sec.

3.2. Performance evaluation

3.2.1. Precision

Method precision was determined by measuring repeatability (intra-day precision) and inter-day precision of migration times and peak area ratios of I and II.

In order to determine the repeatability of the method, replicate injections ($n = 6$) of a solution containing I and II each at a concentration of 0.2 mg/ml were carried out in the optimized conditions. R.S.D. < 1.3 and 1.2% for intra-day migration times and peak area ratios were obtained for both the drugs. The inter-day precision was also evaluated over 3 days by performing six successive injections on each day. R.S.D. < 1.9 and 2.0% for inter-day migration times and peak area ratios were obtained for both the drugs. This demonstrates the good reproducibility of the method.

3.2.2. Linearity

The target analyte concentration of both the drugs was fixed as 0.2 mg/ml. Linear calibration plots were obtained over the calibration ranges tested, i.e. 0.1–0.3 mg/ml for I and II; the corresponding linear regression equations, with correlation coefficients ≥ 0.997, were, respectively, $y = 5.66 \times -0.02$ and $y = 5.63 \times -0.03$. Linearity was checked for three consecutive days for the same concentration range from the same stock solutions. The %R.S.D. values of the slope and intercept of the calibration curves for I and II were 1.5, 15 and 1.9, 28 respectively.

3.2.3. Assay of lovastatin and simvastatin in commercial formulations

Ten tablets of Lostatin (equivalent to 20 mg of lovastatin in each tablet) and Simvotin (equivalent to 20 mg of simvastatin in each tablet) were ground, extracted into acetonitrile and then diluted to 0.16, 0.2 and 0.24 mg/ml solutions with the diluting solution. The assay results from lovastatin formulation were given in Table 2 and the assay results from simvastatin formulation were given in Table 3. The percentage recoveries were ranged from 98.6 to 102.1.

In the formulation samples of I and II it was noticed that excipients did not interfere with the

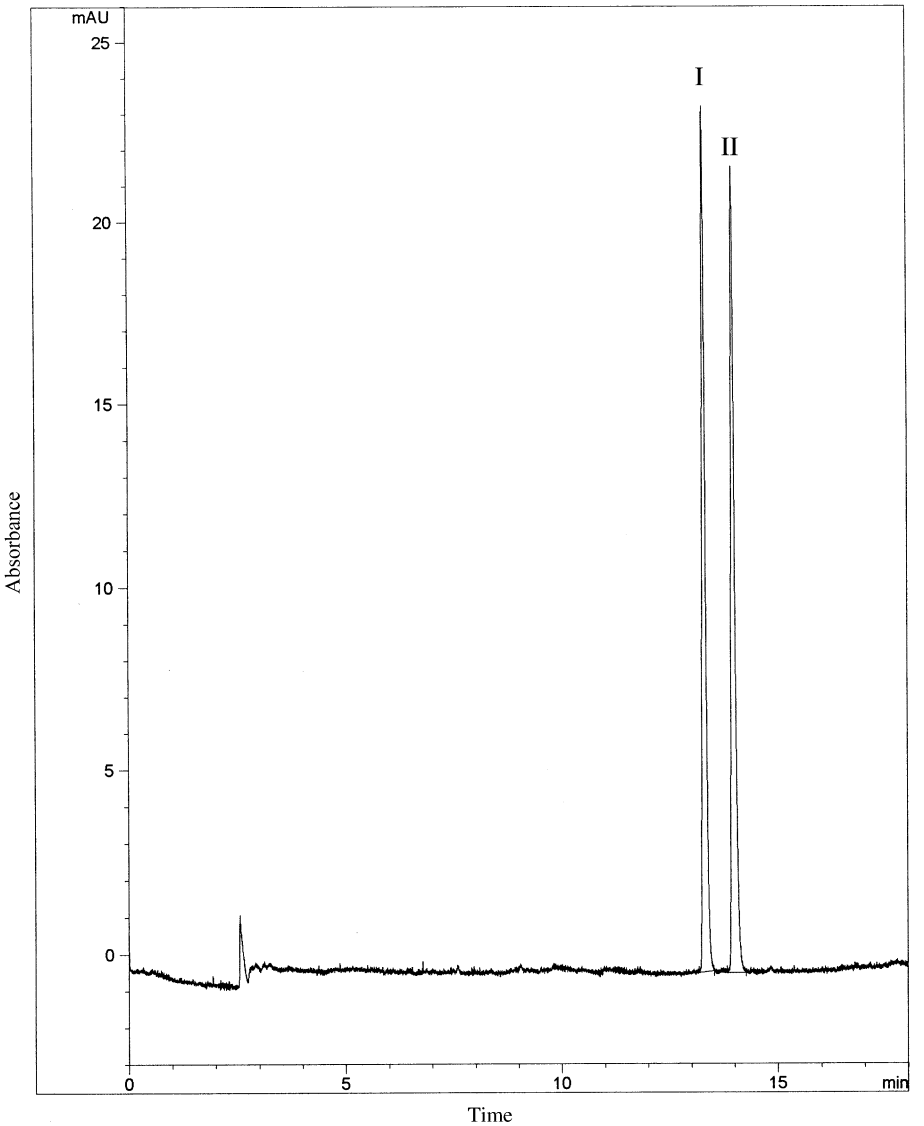


Fig. 2. Micellar electrokinetic chromatogram of lovastatin (I) and simvastatin (II). Operating conditions: uncoated extended light path fused silica capillary L 48.5 cm, l 40 cm, I.D. 50 μm; 30 °C; 25 kV; 238 nm; BGE − 12% acetonitrile (v/v) in 25 mM sodium borate buffer pH 9.3 containing 25 mM sodium dodecyl sulphate and concentrations of I and II are 0.2 mg/ml.

peaks of interest. Hence the method is specific and applicable for the quantitative determination of I and II in pharmaceutical dosage forms.

Furthermore, the assay of lovastatin and simvastatin in commercial formulations (i.e. Lostatin and Simvotin) was also carried out using USP methods in the same concentration range as mentioned above and the assay results obtained from the developed MEKC method were compared with these reference methods using *F*-test [5,6]. The calculated value of *F*-test of variance ratio, close to 1.0, implies that the developed MEKC method and USP reference methods have equal precision.

Table 1
System-suitability report

Compound ($n = 3$)	Migration time (min)	USP Resolution (R)	USP Tailing factor (t)	No. of theoretical plates (N) Tangent method
I	13.2	–	1.1	90 000
II	14.0	2.5	1.2	100 000

n = number of determinations.

3.2.4. Limit of detection and limit of quantitation

The limit of detection (LOD) represents the concentration of analyte that would yield a signal-to- noise ratio of 3 [9]. The limit of quantitation (LOQ) represents the concentration of analyte that would yield a signal-to-noise ratio of 10 [9]. LOD and LOQ for both the drugs were 3.2 and 10.6 μg/ml respectively.

3.2.5. Peak purity

Peak purity was obtained for both I and II by overlay of the spectra captured at the apex, upslope and downslope using photo diode array detector and no interference was noted for I and II. Hence the developed method is a stability indicating method.

3.2.6. Ruggedness

To determine ruggedness, the developed MEKC method was performed by different analysts with different capillary lots, different lots of reagents on different days. The change of migration times of both the drugs was within a ± 1.2 min with all these variations. This confirms the ruggedness of the method.

3.2.7. Robustness

The electrophoretic resolution of the I and II peaks, were used to evaluate the method under modified conditions. Sufficient resolution for lovastatin and simvastatin was obtained under all separation conditions tested (Table 4), demonstrating sufficient robustness.

3.2.8. Stability

Solution stability of I and II was carried out by leaving the solutions (0.2 mg/ml) in tightly capped volumetric flasks at room temperature on a laboratory bench. They were tested for 1 day in 6 h interval. The solutions were found to be stable for the study period.

4. Conclusions

A MEKC method was developed for the separation and quantitative determination of lovastatin and simvastatin in pharmaceutical formulations. The introduction of acetonitrile in the BGE has played a key role on the separation. The developed MEKC method, as an alternative to existing LC methods, is suitable for routine use and offers advantage of simplicity of operation, flexibility and low cost (requiring only a few milliliters of electrolyte and inexpensive capillaries). The method was validated showing satisfactory data for selectivity, linearity, ruggedness and robustness. The developed method is stability indicating and results obtained from commercial pharmaceutical formulations attest the precision and accuracy of the method.

Table 2
Assay results from lovastatin formulation

S.No.	Taken	Recovery	%Recovery	%R.S.D.
I	0.1611	0.1615	100.2	
		0.1645	102.1	0.9
		0.1632	101.3	
II	0.2006	0.1995	99.4	
		0.2015	100.4	0.7
		0.2022	100.8	
III	0.2412	0.2425	100.5	
		0.2413	100.0	0.5
		0.2436	101.0	

Table 3
Assay results from simvastatin formulation

S.No.	Taken	Recovery	% Recovery	% R.S.D.
I	0.1621	0.1598	98.6	
		0.1632	100.7	1.5
		0.1647	101.6	
II	0.2016	0.2022	100.3	
		0.2039	101.1	0.5
		0.2041	101.2	
III	0.2425	0.2436	100.4	
		0.2393	98.7	1.5
		0.2464	101.6	

Table 4
Robustness of the method

Parameter	Resolution
Buffer concentration (*mM*)	
20	2.0
25	2.4
30	2.7
Temperature (°C)	
26	2.6
30	2.3
35	2.1
Applied voltage (*kV*)	
20	2.6
25	2.4
30	2.1

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