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Determination of pravastatin in tablets by capillary electrophoresis

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Abstract

Pravastatin (PRA) is an inhibitor of HMG-CoA reductase enzyme, which is clinically used as a hypolipidemic agent to reduce cholesterol level. A capillary electrophoretic method for the determination of PRA in pharmaceutical tablet formulations is described. PRA and lansoprazole as an internal standard (IS) were well migrated in the background electrolyte of 10 mM borate buffer (pH 8.5) and 10% acetonitrile using a fused silica capillary. The separation was achieved by applying 27.5 kV, detecting at 200 nm and injecting the sample for 0.5 s and with an average migration time (t_m) for PRA and IS of 4.7 and 3.9 min, respectively, at ambient temperature. The results were precise and repeatable for areas of the peaks and peak normalization ratio (PN_{PRA}/PN_{IS}). Linearity was found in the concentration range of 1.56–7.78 × 10^{-5} M. Intra-day and Inter-day assays were performed and reliable results were obtained. Limit of detection and limit of quantitation were 8 × 10^{-6} and 2.4 × 10^{-5} M, respectively. The proposed method was successfully applied for the analysis of PRA in the pharmaceutical tablet formulation. The method proved simple, precise and fast since the analysis can be performed in less than 5 min.

1. Introduction

Pravastatin (PRA, Fig. 1), is an inhibitor of HMG-CoA reductase enzyme which catalyses 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) into mevalonate. It is one of the statins, which decreases cholesterol level in the human body [1–3].

Several methods have been reported for the determination of PRA. These include: high performance liquid chromatography (HPLC) [4–6], with laser-induced fluorescence (LIF) detector [7], liquid chromatography/tandem mass spectrometry (LC/MS/MS) [8,9], capillary ion electrophoresis (CIE) [10]. In addition to these methods, a liquid chromatographic using atmospheric pressure chemical ionization mass spectrometry (LC/APCIMS) [11] and gas chromatographic employing chemical ionization mass spectrometry (GC/CIMS) [12] methods have been used for the analysis of PRA. Ertürk et al. recently published an updated review on the analytical methods for determination of HMG-CoA inhibitors in biological fluids [13].

No capillary electrophoretic (CE) method has not been used for the determinations of PRA. However validated analysis for fluvastatin, one of the statins members has been recently studied in tablets and serum samples [14].

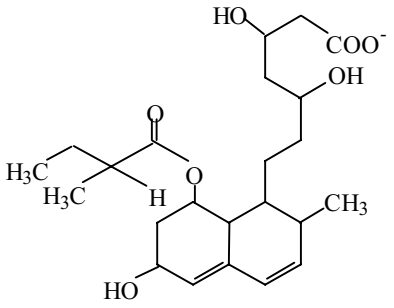

Fig. 1. Chemical structure of PRA.

The aim of this study is to report a validated CE method for the determination of PRA in tablets pharmaceutical formulations.

2. Experimental

2.1. Chemicals

PRA standard material was supplied by Bristol-Myers Squibb (Istanbul, Turkey). All the other chemicals used in the

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experiments were the products of Merck (Darmstadt, Germany) and they were all of analytical grade. Double distilled water and ethanol was used for the preparation of the solutions. The pharmaceutical tablet formulation of PRA, Pracachol® 10-mg tablet, was purchased from a local market.

2.2. Apparatus

The CE experiments were conducted using a Spectrophoresis 100 system equipped with Modular Injector, a model Spectra FOCUS ultraviolet and visible scanning detector (Thermo Separation Products, CA, USA) cabled to a Model Etacomp 486 DX4-100 computer. The data processing was done by using a PC 1000 (Version 2.6) working under OS/2 Warp program (Version 3.0). The capillary was a 86 cm × 75 μm ID (50 cm to detector) fused silica capillary tube (Phenomenex, CA, USA). All the solutions used during the experiments were filtered from a model Phenex microfilter (25 mm, 0.45 μm) (Phenomenex, CA, USA) and degassed using a model B-220 ultrasonic bath (Branson, Shelton, CT, USA). The pH of the solutions was measured employing a model P 114 pH meter (Consort, Belgium). Spectrophotometric studies were conducted using a model UV-2401PC spectrophotometer (Shimadzu, Japan).

2.3. Procedures

2.3.1. Preparation of solutions

Standard stock solution containing PRA was prepared by dissolving 12.9 mg in 10 ml ethanol and completed to 50 ml with bidistilled water. Lansoprazole (LNS) was used as an internal standard (IS) and the solution was prepared by dissolving 5.5 mg and adding 1 drop of 0.1 N NaOH, it was completed to 50 ml of water. The final LNS concentration is 5.96×10^{-5} M. Background electrolyte is consisting of 10 mM borate buffer (pH 8.5) and 10% acetonitrile. The 10 mM borate buffer was prepared by diluting from 100 mM borate stock solution.

2.3.2. Capillary zone electrophoretic procedure

Fused silica capillary was conditioned by washing with 0.1 M NaOH and distilled water for 2 min and then with background buffer for 2 min before each run. After the washing step, the instrument was set on 27.5 kV of applied voltage and the detection at 200 nm. The samples were introduced by using 0.5 s of vacuum injection corresponding to 25 nl. Samples at a concentration of 5.96×10^{-5} M LNS were used for the optimization of CZE parameters. The experiments were carried out at ambient temperature.

2.3.3. Application of methods to PRA tablet

For the determination of PRA in tablets, 10 PRA tablets were accurately weighed. The tablets were grounded and a sufficient amount of tablet powder equivalent to 5 mg of PRA was accurately weighed, transferred to a 50-ml flask and made up to volume by distilled water. It was magnetically stirred for 10 min and made up to the final volume with related solvent. The solution was centrifuged at 5600 *g* for 10 min. The supernatant and fix amount of IS solutions were diluted with background electrolyte and was injected to the CE.

3. Results and discussion

During the method development, there are some related parameters that they should be optimized to provide a sufficient analysis time to be efficient. It can be expressed into two groups; analytical parameters or procedures such as the content of background electrolyte, concentration of the components, pH, ionic strength and instrumental parameters such as injection time, wavelength and applied voltage.

Since PRA carries a carboxylic group, a basic buffer system was preferred to have a suitable migration time (t_m). It is known that when the concentration of the borate buffer is low, the electroosmotic flow (EOF) mobility of the molecule is high and it has an early apparent appearance in the electropherogram. A model background electrolyte (BGE) consisting of 10-mM borate and 10% acetonitrile at pH 8.5 was used. Using the BGE and injecting the solutions 0.5 s, applying voltage of 27.5 kV, and detecting at 200 nm the PRA appeared at 4.7 min. This is considered a reasonable time for analysis.

Another issue was to find a suitable IS. Certain compounds were tested and the most convenient IS is LNS. It was appeared at 3.9 min. The electropherogram of PRA and IS is shown in Fig. 2.

The validation of the method was discussed in the analytical and instrumental conditions. A concentration of 6.20 ×

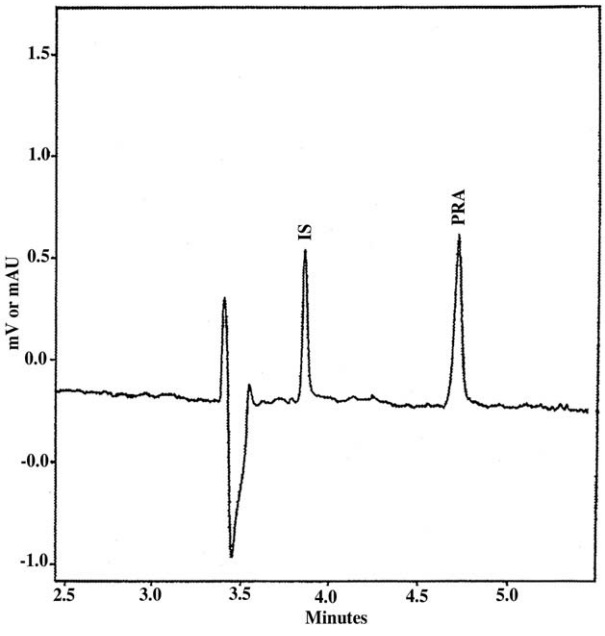

Fig. 2. The electropherogram of standard PRA and IS under the optimum experimental conditions described.

Table 1
The precision values of PRA for retention time and peak area ratios

	Area of PRA	t_m of PRA	Area of IS	t_m of IS	PN_{PRA}	PN_{IS}	PN_{PRA}/PN_{IS}
(x)	2163.4	4.75	1410	3.9	455.6	361.4	1.3
S.D.	118.8	0.1	71.8	0.1	31.5	22.2	0.05
% RSD	5.5	1.9	5.1	2.2	6.9	6.2	4.1

10^{-5} M PRA and 5.96×10^{-5} M IS was prepared and the sample was injected six times. The integration outputs of the results as t_m, and area was evaluated and the results were presented in Table 1.

Reviewing the literature concerning repeatability some studies have reported employing the values of t_m and peak areas [15–17] or PN values [16–19]. The % relative standard deviation (RSD) values, which correspond to repeatability, are tabulated in Table 1.

The results in Table 1 indicates reliable result by using the ratio of peak normalization of PRA to peak normalization of IS. Therefore, this parameter was employed for the quantitative procedures for the rest of the study.

The velocity of the electroosmosis is calculated to be 0.245 cm s^{-1}. The net electrophoretic velocity of IS and PRA is found 0.031 and 0.07 cm s^{-1} by using the formula of $V_{net} = V_{eo} - V_{ep}$, respectively, where V_{eo} is electroosmotic velocity and V_{ep} is the electrophoretic velocity.

3.1. Linearity

Three groups and five dilutions in each group were prepared. Each dilution contained 5.96×10^{-5} M LNS and PRA concentrations were in the range of $1.56–7.78 \times 10^{-5}$ M. Intra-day and inter-day assays were determined and the results were through PN_{PRA}/PN_{IS} values and the results are summarized in Table 2.

Accurate and precise results are obtained for intra-day and inter-day calibrations studies. The slope values are highly close each other.

3.2. Application of the method

The applicability of the proposed method was applied for the analysis of PRA in the pharmaceutical tablet formulation and the results obtained were compared to the pharmacopeial method. The samples were prepared as explained in the experimental section and injected after washing and conditioning the capillary as discussed earlier. Electropherogram of PRA recovered from tablets is shown in Fig. 3. There were no interference for the peaks of PRA and IS and the tablet excipients which indicate the selectivity of the proposed method.

The results of 10-mg PRA tablets analysis by the proposed CE method were statistically shown in Table 3.

As shown in Table 3, the mean value of the tablet analysis was 107.6% (3.55% RSD). Furthermore, this study achieved such results within a short time period (≤5 min) and can be applied for the routine quality control of pharmaceutical formulations of PRA.

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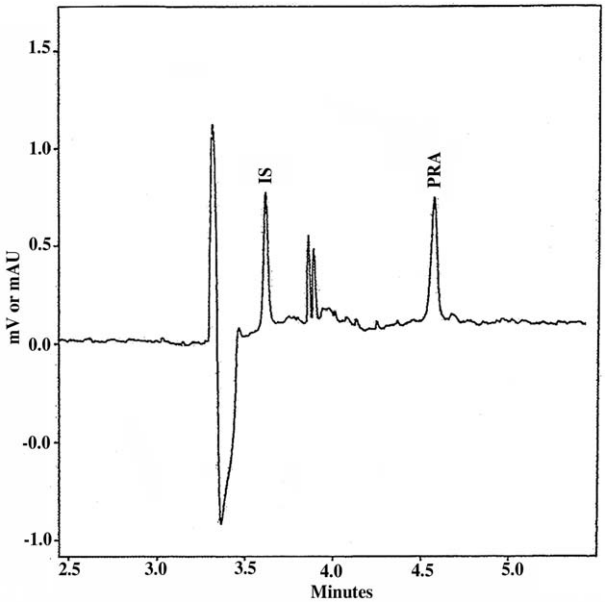

Fig. 3. The electropherogram of PRA recovered from tablets and spiked IS in the optimum experimental conditions.

Table 2
The intra-day and inter-day data for analysis of PRA

Parameters	Intra-day, $n = 5$	Inter-day, $n = 15$
Slope ± S.D.	20745.2 ± 182.4	21155.8 ± 997.3
Intercept	0.007 ± 0.0003	–0.029 ± 0.001
Correlation coefficient (R)	0.9999	0.9989

n: Number of determinations.

Table 3
Statistical results of PRA recovered from 10-mg tablets using the proposed CE method

% mean (in 10-mg PRA tablets)	107.6
S.D.	3.82
% RSD	3.55
Confidence limits (CL) $P = 0.05$	±4.01

References

[1] H. Lennernäs, G. Fager, Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors, Clin. Pharmacokinet 32 (1997) 403–425.

[2] T. Hatanaka, Clinical pharmacokinetics of pravastatin: mechanisms of pharmacokinetic events, Clin. Pharmacokinet 39 (2000) 397–412.

[3] M. Haria, D. McTavish, Pravastatin. A reappraisal of its pharmacological properties and clinical effectiveness in the management of coronary heart disease, Drugs 53 (1997) 299–336.

[4] K. Otter, C. Mignat, Determination of pravastatin in human plasma by high-performance liquid chromatography with ultraviolet detection, J. Chromatogr. B 708 (1998) 235–241.

[5] R. Siekmeier, W. Gross, W. Marz, Determination of pravastatin by high-performance liquid chromatography, Int. J. Clin. Pharmacol. Therap. 38 (2000) 419–425.

[6] D.B. Whigan, E. Ivashkiv, A.I. Cohen, Determination of pravastatin sodium and its isomeric metabolite in human urine by HPLC with UV detection, J. Pharm. Biomed. Anal. 7 (1989) 907–912.

[7] C. Dumousseaux, S. Muramatsu, W. Takasaki, H. Takahagi, Highly sensitive and specific determination of pravastatin sodium in plasma by high-performance liquid chromatography with laser-induced fluorescence detection after immobilized antibody extraction, J. Pharm. Sci. 83 (1994) 1630–1636.

[8] D. Mulvana, M. Jemal, S.C. Pulver, Quantitative determination of pravastatin and its biotransformation products in human serum by turbo ion spray LC/MS/MS, J. Pharmaceut. Bio. Anal. 23 (2000) 851–866.

[9] Z. Zhu, L. Neirinek, High performance liquid chromatography coupled with negative ion tandem mass spectrometry for determination of pravastatin in human plasma, J. Chromatogr. B 783 (2003) 133–140.

[10] J.B. Nair, C.G. Izzo, Anion screening for drugs and intermediates by capillary electrophoresis, J. Chromatogr. 640 (1993) 445–461.

[11] K. Kawabata, N. Matsushima, K. Sasahara, An automated method for the simultaneous determination of pravastatin and its main metabolite in human plasma by high-performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry, Biomed. Chromatogr. 12 (1998) 271–275.

[12] M.J. Morris, J.D. Gilbert, J.Y.K. Hsieh, B.K. Matuszewski, H.G. Ramjit, W.F. Bayne, Determination of the HMG-CoA reductase inhibitors simvastatin, lovastatin and pravastatin in plasma by gas chromatography/chemical ionization mass spectrometry, Bio. Mass Spectrom. 22 (1993) 1–8.

[13] S. Ertürk, A. Önal, S.M. Çetin, Analytical methods for the quantitative determination of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in biological samples, J. Chromatogr. B 793 (2003) 193–205.

[14] D. Dogrukol-Ak, K. Kircali, M. Tuncel, H.Y. Aboul-Enein, Validated analysis of fluvastatin in a pharmaceutical capsule formulation and serum by capillary electrophoresis, Biomed. Chromatogr. 15 (2001) 389–392.

[15] F. Tagliaro, G. Manetto, S. Bellini, D. Scarcella, F.P. Smith, M. Marigo, Simultaneous chiral separation of 3,4-methylenedioxymethamphelamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy ethyl amphetamine (MDE), ephedrine, amphetamine and methamphetamine by capillary electrophoresis in uncoated and coated capillaries with native β-cyclodextrin as the chiral selector: preliminary application to the analysis of urine and hair, Electrophoresis 19 (1998) 42–50.

[16] Y. Daali, S. Cherkaoui, P. Christen, J.L. Veuthey, Experimental design for enantioselective separation of celiprolol by capillary electrophoresis using sulfated β-cyclodextrin, Electrophoresis 20 (1999) 3424–3431.

[17] S. Chinaka, S. Tanak, N. Takayama, N. Tsuji, S. Takou, K. Ueda, High sensitivity analysis of cyanide by capillary electrophoresis with fluorescence detection, Anal. Sci. 17 (2001) 649–652.

[18] L. Mateus, S. Cherkaoui, P. Christen, J.L. Veuthey, Enantioseparation of atropine by capillary electrophoresis using sulfated β-cyclodextrin: application to a plant extract, J. Chromatogr. A 868 (2000) 285–294.

[19] The United States Pharmacopoeia XXIV, Marck Printing, Co., Easton, Philadelphia, USA, 2000, pp. 992–993 pp. 1521–1522.