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An Improved Optimization Study for Determination of Pravastatin in Pharmaceutical Form by Using Reversed Phase Liquid Chromatography Method

Ters Faz Sıvı Kromatografi Yöntemiyle İlaç Formülasyonunda Pravastatin Tayini İçin Geliştirilmiş Optimizasyon Çalışması

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Abstract

Pravastatin belongs to the group of anti-cholestrol agents used in the treatment of hypercholesterolemia. Reversed phase liquid chromatographic (RPLC) method was described in this study for its determination from tablet formulation. This method was improved with a slight change in the pH of the mobile phase. The experiment was carried out a C18 column with a mobile phase of a binary mixture of acetonitrile and water (40:60, v/v) delivered at a flow rate of 1 mL/min and detection was carried out at 239 nm. The linear range was found as 2 - 12 µg/mL. The limits of detection (LOD) and quantitation (LOQ) for pravastatin were found to be 0.260 ug/mL and 0.787 ug/mL, respectively. The optimization results, together with statistical evaluation of the data, demonstrated the reliability of this method.

Keywords: Anti-cholestrol agents, Pharmaceutical dosage form, RPLC, UV detection

Öz

Pravastatin, anti-kolesterol ajanlar grubuna dahil olan ve yüksek kolesterol tedavisinde kullanılan bir ilaçtır. Bu çalışmada pravastatinin tablet formulasyonundan tayini için ters faz sıvı kromatografi (RPLC) metodu tanımlanmıştır. Önerilen metot mobil faz pH'sında küçük değişiklik yapılarak geliştirilmiştir. Kromatografik ayrım C18 kolon kullanılarak, asetonitril-su (40:60, v/v) ikili mobil faz karışımında gerçekleştirilmiştir. Akış hızının 1 mL/dakika olduğu çalışma 239 nm'de yürütülmüştür. Çalışmada lineer aralık 2-12 µg/mL olarak belirlenmiştir. Pravastatin için dedeksiyon limiti (LOD) ve kantitatif yorum limiti (LOQ) değerleri sırasıyla 0.260 µg/mL ve 0.787 µg/mL olarak olarak ölçülmüştür. Optimizasyon sonuçları, istatistiksel verilerle birlikte değerlendirildiğinde metodun güvenilirliğini ortaya koymaktadır.

Anahtar Kelimeler: Anti-kolesterol ajanlar, Farmasötik dozaj form, RPLC, UV dedeksiyon

1. Introduction

Pravastatin is largely used in the treatment of high cholesterol, has genuine pharmacokinetic characteristics among the members of statins. Many *in vivo* and *in vitro* human and animal studies suggest that active transport mechanisms are included in the pharmacokinetics of pravastatin (Hatanaka 2000). Pravastatin varies from other US Food and Drug Administration (FDA)-approved statins because it has major hydrophilicity, as a result of the hydroxyl group joined to its decalin ring (Quion and Jones 1994). Pravastatin has been showed to decrease cholesterol in diseased people with familial and nonfamilial polygenic high cholesterol and patients with diabetes mellitus. Furthermore, it reduces low density lipoproteins (LDL) cholesterol and increases high density lipoproteins (HDL) cholesterol but possibly with fewer adverse effects (Jungnickel et al. 1992).

To date several HPLC-UV methods have been improved for the quantification of pravastatin either lonely or intergration with other drugs in different matrices (Gomes et al. 2009, Chaudhari et al. 2007, Campos-Lara and Mendoza-Espinoza 2008, Önal and Sağırlı 2006, Ashour et al. 2008, Sultana et al. 2008). These methods have generally employed reversed phase liquid chromatography with different detection techniques. A major advantage

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of this technique is the validity of controlling the scope of fractionalization of individual solutes by changing the mobile phase combination. The quality of the separation can conveniently be developed through the careful manipulation of one or more of the diverse experimental parameters for example concentration and type of ion-pairing reagents, pH, the concentration and type of organic modifiers, etc (Smith and Khaledi 1993).

Optimization of liquid chromatography separation methods is a compelling task. For a given chromatographic column, the retention of an analyte depends on diverse mobile phase qualities, namely buffered solution components, pH and solvent composition. The pH of the mobile phase is a basic parameter in the separation of ionisable substances (Rosés et al. 1996). In the HPLC method optimization, the principal target is to ensure the optimal separation conditions. Many approaches have been researched to predict the solute chromatographic behavior as a function of chromatographic conditions. The factors usually selected to optimize the chromatographic separation of ionizable substances are the pH of the mobile phase and ingredient of organic solvent of the eluent. Achievement of good resolution is vital and consistently only possible at specific pH values.

The attentive search of the chromatographic behavior of the studied compound requires information about dissociation constant (pK_a). Information about this value is required to choose the optimum chromatographic conditions for improvement of analytical procedure for determination of studied compound (Talay et al. 2015). Literature survey revealed that reversed phase liquid chromatography (RPLC) procedures have been reported for the determination of pravastatin individualy or several drugs in pharmaceutical formulations (Gomes et al. 2009, Chaudhari et al. 2007, Campos-Lara and Mendoza-Espinoza 2008, Önal and Sağırlı 2006, Ashour et al. 2008, Sultana et al. 2008). In literature there is no procedure that enables optimization study using chromatographic behaviour of pravastatin. Hence, the target of this experiment was to improve and validate more accurate, precise and reliable procedure for the determination of pravastatin in pharmaceutical dosage form.

2. Material and Methods

2.1. Reagents and Solutions

Pharmaceutical grade pravastatin and rosuvastatin (IS) were obtained from Sigma-Aldrich (USA). The pravastatin was Pravachol (Deva, 10 mg), which was purchased from the local pharmacy. Ortho-phosphoric acid (min. 85%) was attained from Riedel-de Haen (Riedel-de Haen Germany); other chemicals (sodium hydroxide, potassium hydrogen phthalate, acetonitrile) employed were of analytical grade Merck (Darmstadt, Germany).

Stock solution of the pravastatin (100 µg/mL) was prepared in the mobile phase. Prior to measurements, stock solution of the pravastatin was diluted with mobile phase so as to prepare the working standard solutions of 12 µg/mL and 2 µg/mL. Fresh working solutions were prepared daily.

2.2. Apparatus

Chromatographic analysis were carried out with a Shimadzu HPLC system (Japan) equipped with a pump (LC-20AD), a 20 µL loop, a diode array dedector (SPD-M20A), a column oven (CTO-10AS VP), and a degasser system (DGU-20A3). The pH measurements of the mobile phase were performed with a Mettler Toledo MA 235 pH/ion analyzer (Switzerland) using M-T glass combined pH electrode model 412. The pH values of the mobile phases were measured versus a 0.05 mol/kg potassium hydrogen phthalate solution as primary standard reference, dissolved in the suitable water acetonitrile binary mixtures in convenience with IUPAC rules (Rondinini et al. 1987).

2.3. Chromatographic Procedure

In the optimized procedure, the separation was performed with a pumped at a flow rate of 1.0 mL/min through a X Terra C18 analytical column (5 um, 250 mm x 4.6 mm I.D.), thermostatized at 25°C. The mobile phases used were binary mixtures at 40% (v/v). At each mobile phase, various pH values were studied, spread over the pH range from 2.5 to 8.0 adjusted by the addition of 1.0 M sodium hydroxide solution containing 25 mM phosphoric acid of the mobile phase.

For studied compounds the retention time values (t_R) were determined from three separate injections for each prepared mobile phase. Retention factors for studied compounds and mobile phase were calculated using the expression $k = (t_R - t_0)/t_0$. The dead time (t_0) was measured by injecting uracil solution (Sigma, USA, 0.1 %, in water), which was determined for each mobile phase and pH studied. The drugs studied had various optimum wavelengths (for pravastatin 239 nm and rosuvastatin (IS) 244 nm).

2.4. Analysis of Tablets

Ten tablets from the sample to be analyzed were correctly weighted and ground until just decreased to a powder. A

correctly weighted amount of the powder equivalent to one tablet was transferred into a 100 mL volumetric flask. About 50 mL of acetonitrile was added and the ingredient of the flask was sonicated for 15 min. The solution in the flask was completed to volume with acetonitrile. Following filtration, suitable solutions were prepared by taking appropirate aliquots of clear filtrate and adding the suitable IS solution, diluting them with mobile phase to achieve the final solution. The quantity of pravastatin was calculated from the corresponding regression equation.

2.5. Recovery Studies

To keep an additional check on the trueness of the method improved, recovery studies were carried out by adding the known quantity of pure drug to preanalyzed tablet samples. Known quantities of the pure drug and a fixed level of an IS were added to preanalyzed tablet solution and the mixtures were analyzed. The percentage of recovery was measured by contrasting the concentration achieved from spiked samples with the real appended concentration. Following three experiments, the mean recovery percent was determined for pravastatin. Hence, the influence of common tablet formulation excipients on chromatograms was researched. Recovery studies also demonstrated the suitability and reliability of the recommended procedure.

3. Results

The retention of an ionisable analyte can vary exceedingly with the pH of the mobile phase, particularly about the pK_a of the analyte. At this stage, the pH of the mobile phase needs to be firmly controlled. Independent experiments provide the input data: retention factors measured in mobile phases at extreme acidic and basic pH values and at fixed column temperature. In this way, best RPLC condition on the separation for quantitative determination of the pravastatin is determined (Figure 1). Preliminary experiments indicated that the X Terra C18 (5 µm, 250 mm, 4.6mm I.D.) reversed phase column provides efficient and reproducible separation of studied compounds at 25°C. Hence, X Terra C18 column was selected for method optimization and validation. The retention factors were attained over a pH range of 2.5-8.0 for determine chromatographic behaviour of this compound using the RPLC method. In Figure 2, the experimental data achieved at 40% (v/v) acetonitrile-water mixture are plotted for pravastatin, demonstrating the sigmiodal curves. The dissociation constant value of pravastatin (Talay et al. 2015) was calculated by a non linear least squares fit of the data using the programme NLREG (NLREG 2015).

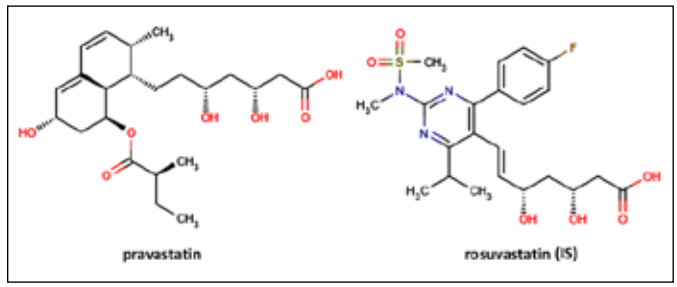

Figure 1. Molecule structures of pravastatin and rosuvastatin (IS).

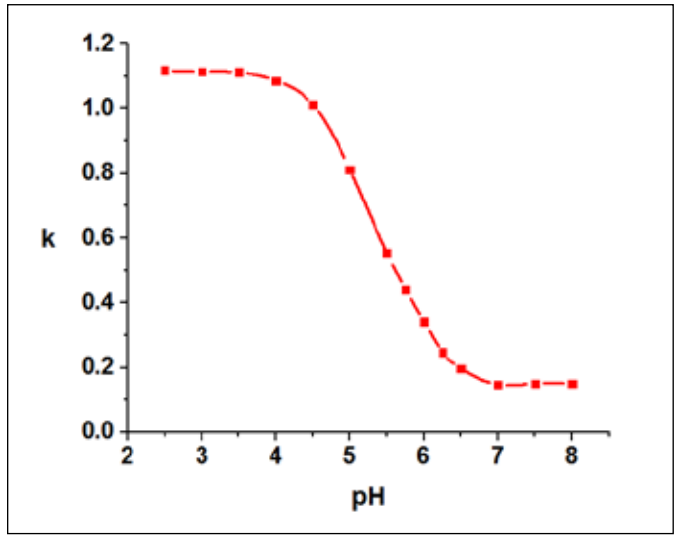

Figure 2. Values of retention factor values vs. pH of the mobile phase for pravastatin on the X Terra C18 column at 25°C using the acetonitrile-water (40:60, v/v) binary mixture. The theoretical results are shown as continuous lines and the plotted points are experimental results.

The most appropriate pH values for separation could be the range of pK_a±1.5. Thus, in the existing procedure pH set to 3.5 using wavelength 239 nm. The peak shape and retention factor were found to be well when the mobile phase containing of the acetonitrile-water pH adjusted to 3.5 with 1 M sodium hydroxide was used in the ratio of 40:60 (%v/v). Present study, the retention, efficiency and selectivity terms were measured in the common way and outlined in Table 1 for optimal separation condition.

An internal standard was used to remove the potential interferences due the excipients of the dosage forms and the change of device response which varies slightly from time to time for reasons that counteract random error in injection volume. Thus, the determination of pravastatin was performed using internal standard technique. Rosuvastatin was selected as the internal standard (IS) because it demonstrated a brief retention time with a well peak

form and a good resolution from the studied drugs peak. According to U.S. Pharmacopoeia 24th, method (McNally 2000) system suitability tests are an integral part of a liquid chromatographic procedure. They are used to validate that the reproducibility and resolution of the chromatographic system are sufficient for the analysis to finished. System suitability tests were performed on prepared standard stock solutions of studied drugs. The parameters involve retention time, theoretical plate number, retention factor, selectivity, tailing factor and RSD% of peak area or height for iterative injections. Tailing factors of 1.161 and 1.084 were achieved for pravastatin and rosuvastatin (IS), respectively. The theoretical plate number values were 6070 for pravastatin and 10671 for rosuvastatin (IS). The selectivity factor was 3.417 for rosuvastatin (IS)/pravastatin. A chromatogram is demonstrated in Figure 3. Pravastatin and rosuvastatin were decent separated in a total duration of 13 min, with well peak resolutions, symmetry and sharpness.

For the construction of a calibration curve, six standard solutions with pravastatin at concentrations ranging from 2.0 to 12 µg/mL were prepared and determined. In each sample 1 µg/mL of rosuvastatin (internal standard) was added. The peak area ratio of pravastatin to that of the internal standard was plotted versus the corresponding concentration to attain a calibration graph. Lower limit of quantitation (LOQ) and limit of detection (LOD) were calculated for studied compound. Results analyzed can be seen in Table 2. Intra-day and inter-day variations of the procedure were determined using five separate injections of two concentrations and analyzed on the same day and three diverse days. Accuracy, reproducibility and precision results summarized in Table 3 were assessed by performing repetitive analysis of the standard solutions in mobile phase.

Method precision and accuracy were tested using tablet sample of pravastatin including 10 mg of active substance. Three samples of tablets were prepared for each experiment. Precision was denoted as relative standard error (RSD%) of three determinations. Accuracy was determined by spiking of pravastatin tablets with a known quantity of pravastatin standard and was denoted as % of recovery (Table 4). The applicability of this method was tested by the determination of pravastatin in tablet dosage forms. The results achieved are satisfactorily precise and accurate as shown by the great recovery% and SD < 2 (Table 4). Tablets common excipients, for example lactose, gelatin, talc, starch, or magnesium stearate did not interfere with the assay. No undesirable peaks were found in the chromatogram (Figure

Table 1. Separation, retention and selectivity factors pravastatin and rosuvastatin (IS), at 40% acetonitrile at pH 3.5.

Factor	Rosuvastatin (IS)/Pravastatin
k_2	3.785
α	3.417
$k_2/1+ k_2$	0.791
$(\alpha-1)/ \alpha$	0.707
(¼)√N	25.825
R_s	14.45

Table 2. Calibration data of pravastatin.

Calibration parameters	Pravastatin
Linearity range (µg/mL)	2-12
Intercept	0.120
Slope	1.287
SD of slope	0.012
SD of intercept	0.094
Detection limit (µg/mL)	0.260
Quantitation limit (µg/mL)	0.787
Correlation coefficient	0.999

Table 3. Intra-day and inter-day precision of pravastatin.

Precision Results	Pravastatin
Theoretical concentration (µg/mL)	4.000
	10.000
Intraday measured concentration mean (µg/mL)	4.043
	10.037
RSD (%)	0.259
	0.176
Interday measured concentration mean (µg/mL)	4.662
	10.423
RSD (%)	0.642
	0.640

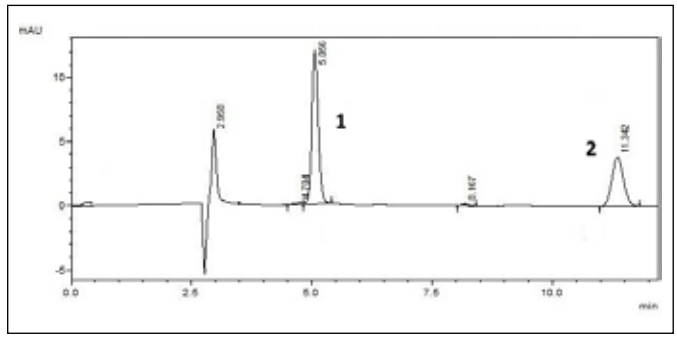

Figure 3. The representative chromatogram obtained analysis of standard mixture. 1) Pravastatin 2) Rosuvastatin (IS).

4). Moreover, when a known quantity of the drug solution was added to a powdered sample of the tablet dosage form and subjected to a prediction of the drug by the existing method, there was a high recovery of pravastatin.

4. Discussion

In existing study, the chromatographic behavior of pravastatin was researched using various mobile phase pH conditions on a reversed phase column, for research the combined effect of pH and acetonitrile percentage on the retention in RPLC. In order the find the optimum chromatographic conditions for RPLC determination of pravastatin, the affect of pH on the mobile phase were analysed. The pH effect demonstrated that optimized conditions are reached when the pH value is 3.5, producing decent peak shape and retention value. Under the optimum condition, the desirable retention time, selectivity ($\alpha \geq 1.15$), retention factor ($1<k<10$) and resolution ($Rs \geq 1.5$) were achieved. The results indicate that the proposed chromatographic system achieves preferred selectivity and reasonable retention for pravastatin and rosuvastatin (IS). The chromatographic conditions described ensured sufficient resolution and retention for all analytes. The results achieved from the system suitability tests satisfy the USP requirements. Using the explained analytical method, an optimum resolution of the analytes was obtained.

Existing study is the first study advance with the assignment of optimum chromatographic condition of pravastatin using chromatographic behaviour at fixed proportion of acetonitrile-water binary mixture and various pH values of mobile phase. The LOQ, short chromatographic time and small sample volume of this procedure makes it advantageous for adaptation to ordinary assay requirements

Table 4. Results of the assay and the recovery analysis of pravastatin in tablet dosage form.

Sample No	Amount of Pravastatin in tablets (µg/mL)	Recovery%
1	9.926	99.260
2	10.143	101.426
3	10.096	100.962
Mean	10.055	100.549
SD	0.114	1.141
RSD%	1.134	1.135

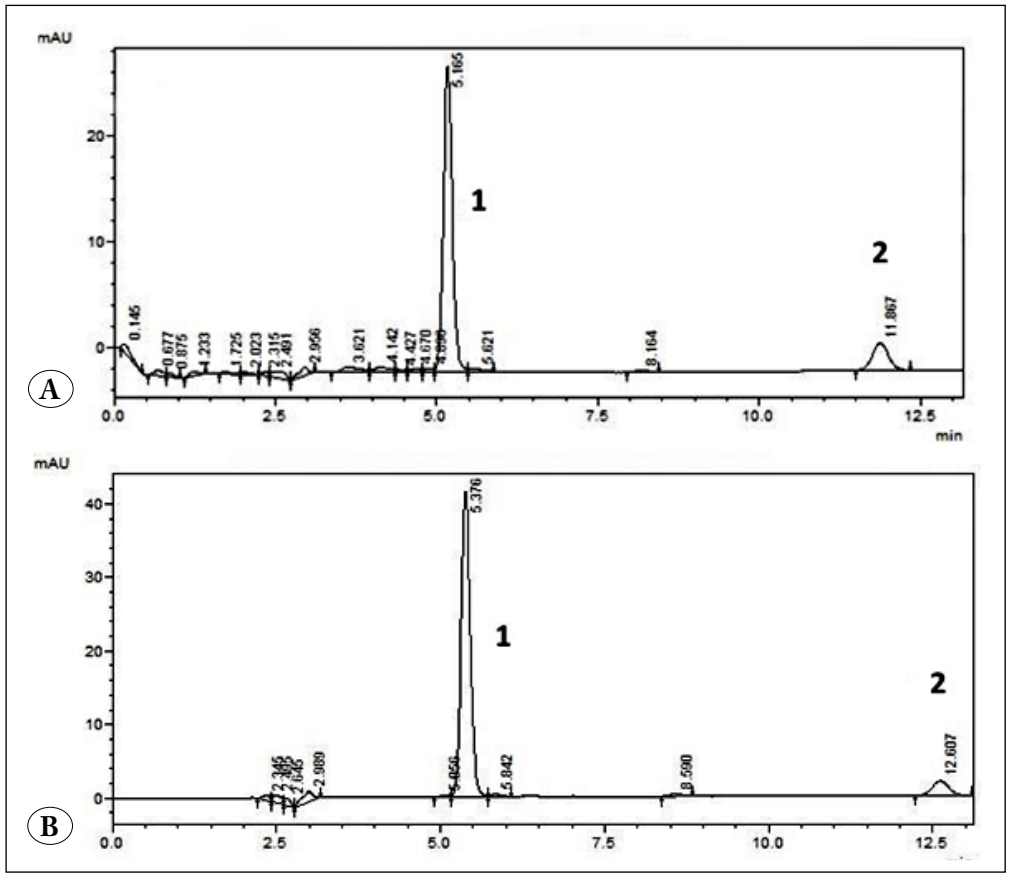

Figure 4. **(A)** Chromatograms of pravastatin and rosuvastatin (IS) in pharmaceutical dosage form. 1) Pravastatin (4 µg/mL), 2) Rosuvastatin (IS) (1 µg/mL), **(B)** Pharmaceutical dosage form spiked with each drug at 1) Pravastatin (8 µg/mL), 2) Rosuvastatin (IS) (1 µg/mL).

and enables same time determination of pravastatin and rosuvastatin because of well resolution and separation of the chromatographic peaks. The achieved results are in decent aggreement with the declared content of tablet dosage form. Method validation produced great results for accuracy, linearity, precision, limit of detection and limit of quantitation. The statistical results shown that the values were within the desirable range and the method was adequately precise and accurate.

5. Acknowledgment

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