ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Suppl 2, 2014

ISSN - 0974-2441

Research Article

VALIDATION METHOD FOR MEASURING SIMVASTATIN IN HUMAN PLASMA BY HPLC-UV AND ITS APPLICATION IN STUDY SIMVASTATIN STABILITY IN PLASMA AND WORKING SOLUTION

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Received: 23 April 2014, Revised and Accepted: 2 May 2014

ABSTRACT

Objective: The aim of this study by HPLC-UV method for determination of simvastatin in human plasma has been developed, after extraction by ethyl acetate and hexane (90/10%, v/v) using lovastatin as internal standard.

Methodology: Solutes are separated on a C_{18} column with mobile phase consist a mixture of acetonitrile-water (51/49%, v/v) mL/min and UV detection at 238 nm. The calibration curve was linear from 20 to 1000 ng/mL (r = 0.9996).

Result: The intra-day coefficients of variation were less than 6.00% and the accuracies were between 97.52 and 104.80% for human control plasma containing 50, 200 and 500 ng/mL of simvastatin, respectively. The inter-day coefficients of variation were less than 9.00% and the accuracies were between 101.65 and 105.16% for human control plasma containing 50, 200 and 500 ng/mL of simvastatin, respectively. The entire run time for analysis was only 11 min. The limit of quantitation of 20 ng/mL was achieved.

Conclusion: The stability studies of simvastatin in human plasma for two months at -85^{0}C, in acetonitrile and water for one month at 4^{0}C, did not show any significant degradation.

Keywords: HPLC-UV, simvastatin and human plasma

INTRODUCTION

Simvastatin, an analogue of lovastatin, is the lactone form as shown in Fig. 1. Simvastatin is one of the major statin drugs inhibits 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase that catalyzes the conversion of HMG-CoA to mevolanate, which is an early rate-limiting step in cholesterol biosynthesis in body [1]. This agent is highly effective in reducing total cholesterol and the low density lipoprotein level. It is a highly effective cholesterol lowering agent, which is widely used in the treatment of hypercholesterolemia [2]. The absorption, excretion and tissue distribution of simvastatin have been reported [3-5]. It is important that to study simvastatin stability under storage in human plasma and in working solution. However, stability of simvastatin under various storage conditions such as -750C -700C, -200C and 40C have been previously reported [6-8]. In this paper HPLC-UV was validated to study simvastatin stability in human plasma under storage condition for two months at -850C and the stability studies of simvastatin in acetonitrile and water for one month at 40C. Therefore, this paper describes a specific, accurate, precise, simple and reproducible HPLC-UV method using a simple and rapid liquid-liquid extraction procedure for sample preparation prior to analysis of simvastatin in human plasma.

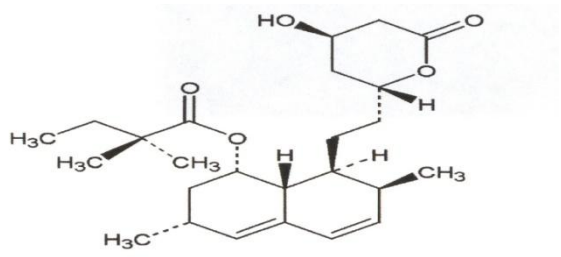

Figure 1. Chemical structure of simvastatin

EXPERIMENTAL

Chemicals

Simvastatin and its internal standard, lovastatin were supplied by Ranbaxy Company. Acetonitrile (HPLC grade) and hexane (analytical grade) were obtained from Merck Darmstadt, Germany. Ethyl acetate (analytical grade) and formic acid (analysis grade) were obtained from Fisher Scientific International Company, United Kingdom. Water was purified and deionized using a Milli-Q ion exchange filtration system. Water was filtered through WCN 0.45 µm filters Whatman Ltd, UK. The analytical HPLC column used was Symmetry C18 column, 5 µm (3.9 mm, i.d x 50 mm) from Waters.

Chromatographic conditions

The chromatographic system (Shimadzu LC 6A), was connected to UV spectrophotometer SPD-6A with a D2500 chromato-integrator and a Rheodyne (model 7125) sample injector equipped with a 20 µL loop. The separation was performed on an analytical column C18, 5 µm (3.9 mm, i.d x 50 mm). An isocratic mobile phase consisted of acetonitrile and formic acid 3 mM (51:49%, v/v) was delivered at a flow rate of 1 mL/min. The column was maintained at room temperature (250C) and the compounds thus eluted were recorded by detector at a constant wavelength of 238 nm.

Preparation of stock solution and calibration standard

Stock solution of simvastatin was prepared in acetonitrile (1 mg/mL) and was diluted with acetonitrile and water 80:20%. Stock solution of lovastatin was prepared in acetonitrile and water 80:20% (1 mg/mL) and was diluted with acetonitrile and water 80:20% to obtain the desired concentrations. The stock solutions were kept at -20°C. The calibration standard curves in human plasma were prepared by adding known amounts of simvastatin to blank plasma (200 µL) in order to prepare final concentrations of 20, 50, 100, 200, 500 and 1000 ng/mL. The calibration curves for simvastatin were generated by measuring peak height ratio of the analyte to that of the respective internal standard.

Assay validation

The intra-day coefficient of variation (C.V.) and accuracy of the quantification were assessed for the determination of simvastatin by analysis of plasma samples (n = 7) spiked with the analyte at three different concentrations 50, 200 and 500 ng/mL. The C.V. and accuracy for inter-day assay were evaluated using plasma samples (n = 5) at the same concentrations for five days.

Extraction procedure

The human plasma sample (0.2 mL) was dispensed into glass test tube containing 50 µL of internal standard (500 ng/mL). An aliquot of 3 mL of a mixture of ethyl acetate and hexane (90:10%, v/v) was added to all the tubes and vortex-mixed for 30 seconds. This step was performed in a fume cupboard. Following centrifugation at 4000 rpm for 10 min, the upper organic phase was transferred into another set of clean tubes and evaporated to dryness under a stream of nitrogen at 40°C. The dry residues were dissolved with 200 µL of solution acetonitrile and water (80/20%, v/v), then vortex for 15 second in which 20 µL was injected into the chromatography in HPLC-UV.

Extraction efficiency

The extraction efficiencies of simvastatin and lovastatin were determined at concentrations of 50, 200, and 500 ng/mL, by comparing peak heights of solutions of extracts versus aqueous standards.

Stability Studies

The stability studies of analytes in human plasma were carried out for two months at -850C and the stability studies of simvastatin in acetonitrile and water (working solution) for one month at 40C. The stability of simvastatin was determined by using plastic tubes as container system. Aliquots of simvastatin with different concentrations at 50, 200 and 500 ng/mL (Low, medium and high) of plasma and acetonitrile/water were prepared. The spiked plasma samples were then stored at -850C whereas simvastatin solutions in acetonitrile/water were stored at 40C. A series of standard samples were prepared from freshly made stock solutions in the same solvent used for the assay with a respective low, medium and high concentrations of the quality controls were used. Three aliquots of each concentration were processed and quantified immediately in order to provide the reference (initial) values and other three aliquots of each concentration were processed at different time intervals. The samples were analyzed by injecting into the HPLC-UV.

RESULTS AND DISCUSSION

Detector Linearity, Calibration curves, coefficient of variations and accuracy.

The linearity was improved by setting the detector at the maximum absorbance of simvastatin at 238 nm Fig. 2 shows wavelengths for simvastatin. The results indicated that the detector response towards simvastatin was linear over the range of 4 - 200 ng/mL.

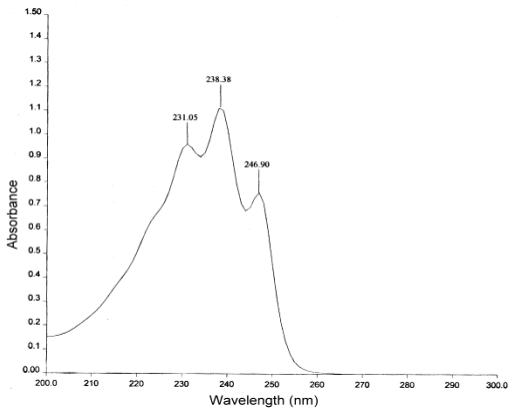

Figure 2. The absorbance of simvastatin at 10 µg/mL

Simvastatin has three maximum wavelengths (231, 238, and 246 nm). However, simvastatin can not be accurately at the entire three wavelengths quantified due to interferences. Simvastatin extracted using liquid-liquid, showed changes in the peak area at the baseline while, peak area of simvastatin with aqueous solution did not change, this may be due to extraction of the simvastatin from plasma by using liquid-liquid, so there is different results between the peak area and the peak height ratio. At the low concentration of simvastatin extracted using liquid-liquid there is no different while at the high concentration there is clear different in peak area. Therefore, peak height measurements are more accurate than peak area, because peak height is less subject to interference by adjacent, overlapping peaks. However, the choice of peak height or peak area measurements requires an understanding of chromatographic parameters on the precision of each approach. As well as, peak height analysis can in some cases yield non-linear calibration; in this case, peak area analysis is usually preferred. Causes of such non-linearity for peak height response include tailing bands, column overload and detectors with large response times. Therefore, the quantification of simvastatin in this method was achieved by measurement of peak height at 238 nm. Figura 3 shows the different between peak area at baseline for both standard solution and spiked plasma sample of simvastatin at 1000 ng/mL.

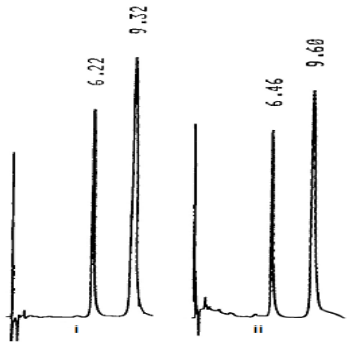

Fig.3: Chromatograms of (i) 1000 ng/mL simvastatin in acetonitrile/water, and (ii) 1000 ng/mL simvastatin spiked in human plasma after extraction from plasma with ethyl acetate/hexane

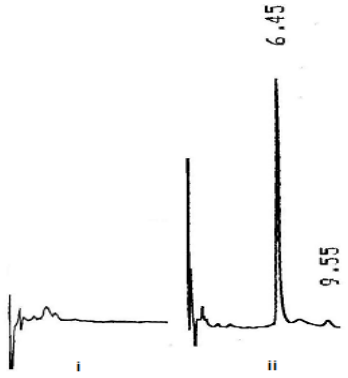

Fig.4: Chromatograms of (i) Blank plasma. (ii) LOQ of simvastatin at 20 ng/mL with internal standard

The calibration curves exhibited linearity over the range of 20-1000 ng/mL with correlation co-efficient greater than 0.999. The mean calibration curve for simvastatin was given by the equation, y = 0.001x - 0.01066, (R = 0.9996) where y indicates the peak height ratio, x represents the concentration of respective analyte in ng/mL. The calibration curves were drawn on each analysis day. Fig. 4 shows the representative chromatograms of the lactone fraction obtained from drug free plasma, internal standard, lovastatin (500 ng/mL) and plasma spiked with 20 ng/mL simvastatin. The results of intra-day and inter-day are summarized in Table 1 and Table 2,

respectively. The intra-day coefficients of variation did not exceed 6.00% and the intra-day accuracies were between 97.52 and 104.80% for human control plasma containing 50, 200 and 500 ng/mL analyte. The inter-day coefficients of variation did not exceed 9.00% and the inter-day accuracies were between 101.65 and 105.16% for human control plasma containing 50, 200 and 500 ng/mL simvastatin. The limit of quantification for analyte was 20 ng/mL, which is the lowest concentration of the analyte that can be measured with a coefficient of variation and accuracy from theory of less than 20%. The limit of detection is considered to be about 3 times the signal-to-noise (S/N) ratio is 15 ng/mL. The retention time in this method for simvastatin and its internal standard lovastatin were 9.3-9.6 min and 6.2-6.4 min respectively. The total run time was 11 min.

Table 1. Intra-day coefficient of variation and accuracy of measurement of simvastatin in human plasma of individual samples (n=7)

Concentrations		Coefficient of variation (%)	Accuracy (%)
Spike (ng/mL)	Determined (ng/mL)		
50	48.76 ± 2.12	4.30	97.52
200	209.58 ± 11.73	5.60	104.80
500	493.30 ± 13.51	2.70	98.66

The value of concentration determined represents mean ±S.D.

Table 2. Inter-day coefficient of variation and accuracy of measurement of simvastatin in human plasma of individual samples (n = 5)

Concentrations		Coefficient of variation (%)	Accuracy (%)
Spike (ng/mL)	Determined (ng/mL)		
50	52.58 ± 4.60	8.80	105.16
200	203.29 ± 10.50	5.20	101.65
500	511.34 ± 39.90	7.80	102.27

The value of concentration determined represents mean ±S.D.

Table 3. Extraction efficiency of simvastatin (n=3)

Sample concentration (ng/mL)	50	200	500
Extracted Concentration Mean (CV%)	48.60 (3.90)	209.00 (6.70)	493.00 (1.40)
Non-extracted Concentration Mean (CV%)	53.00 (2.00)	222.00 (3.20)	544.00 (1.50)
Recovery %	91.69	94.10	90.60

Table 4. Stability study of simvastatin working solution at 4°C (n=3). Results presented as mean (CV %)

	Concentrations (ng/mL)		
Reference	Initial	After 15 days	After 30 days
50	48.83 (7.3)	48.23 (4.3)	50.54 (8.7)
200	197.00 (3.2)	202.30 (2.9)	198.27 (4.6)
500	491.20 (2.8)	498.50 (3.6)	498.80 (3.9)

Table 5. Stability of simvastatin in human plasma at -85°C (n=3). Results presented as mean (CV %)

	Concentrations (ng/mL)	
Reference	Initial	After 60 days
50	45.26 (2.2)	46.30 (5.47)
200	203.53 (2.77)	213.30 (1.2)
500	471.40 (10.8)	511.33 (4.9)

Recovery

The extraction efficiency of this method was assessed by recovery experiments. The recoveries for simvastatin in human plasma are presented in Table 3.3. The mean recoveries for 50, 200 and 500 ng/mL in plasma were 91.70, 94.10 and 90.60%, respectively. The major advantage of this method is short time and simple preliminary treatment of the sample, while other methods the operation and clean up procedure prior to analysis seems to be complicated. In this method the precision of recovery is indicating a good reproducibility < 7%.

Stability

Tests performed and visual inspection of the working solutions does not present any significant degree of degradation after 15 and 30 days. Therefore, working solutions do not need to be freshly prepared everyday, during the duration of study. The stability of working solutions in this method is similar to methods previously reported for simvastatin stability at 40C by Jemal et al (2000). Results indicated that, the simvastatin at low, medium and high concentrations in human plasma can be stored at - 850C for at least two months without any degradation, this method is similar to methods previously reported by Jemal et al (2000) for studying the simvastatin stability in plasma for 2 months at -700C, also Barrett et al (2006) for studying the simvastatin stability in plasma for 3 months -750C. Additionally, this method is similar to method previously reported by Carlucci et al (1992) which studies simvastatin stability in plasma for 1 week at -200C. The results of stability studies are summarized in Tables 4 and 5

CONCLUSION

The specific, accurate, precise, simple and reproducible HPLC-UV method for the analysis of simvastatin has been developed and validated based on liquid-liquid extraction. The inter-day and intra-day precisions are well within the acceptable values (<10%). The stability studies of simvastatin in working solutions and human plasma did not show any perceivable degradation at the temperatures and time periods used.

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