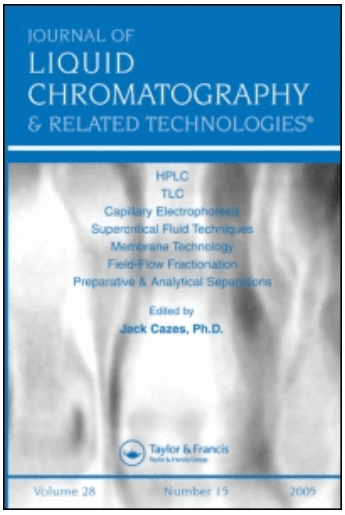

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Development of a Selective Extraction Method for Pravastatin Quantification in Tablets using HPLC with Ultraviolet Detection

María Campos-Lara[a]; José Alberto Mendoza-Espinoza[b]
[a] Unidad de Investigación Médica en Farmacología, Centro Médico Nacional SXXI, Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico [b] Sección Externa de Farmacología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV del IPN), Mexico City, Mexico

To cite this Article Campos-Lara, María and Mendoza-Espinoza, José Alberto(2008) 'Development of a Selective Extraction Method for Pravastatin Quantification in Tablets using HPLC with Ultraviolet Detection', Journal of Liquid Chromatography & Related Technologies, 31: 4, 619 — 623
To link to this Article: DOI: 10.1080/10826070701815288
URL: http://dx.doi.org/10.1080/10826070701815288

Journal of Liquid Chromatography & Related Technologies®, 31: 619–623, 2008

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070701815288

Development of a Selective Extraction Method for Pravastatin Quantification in Tablets using HPLC with Ultraviolet Detection

María Campos-Lara[1] and José Alberto Mendoza-Espinoza[2]

[1]Unidad de Investigación Médica en Farmacología, Centro Médico Nacional SXXI, Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[2]Sección Externa de Farmacología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV del IPN), Mexico City, Mexico

Abstract: A high performance liquid chromatography method for estimation of pravastatin in tablets has been developed. The mobile phase consisted of acetonitrile and phosphates buffer in a volume percentage ratio of 7:3 v/v, pH 2.0 and was delivered at the rate of 1 mL/min and detected at 238 nm, retention times were approximately 7.3 min: this peak was analyzed with mass spectroscopy. The method was fully validated and validation parameters were: linearity range 10–200 ng/mL, correlation coefficient 0.999, mean recovery >99%, limit of quantification 5 ng/mL, and limit of detection 5 ng/mL; this method can be used for a quality control assay.

Keywords: Pravastatin sodium, Tablets extraction, Validation, Mass spectroscopy, Quality control assay

INTRODUCTION

Cholesterol lowering statin drugs (atorvastatin, cerivastatin, fluvastatin, pravastatin and sinvastatin) are the most frequently prescribed substances

Correspondence: José Alberto Mendoza-Espinoza, Sección Externa de Farmacología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV del IPN), Apdo. 4-740, CP 07000, Mexico City, Mexico. E-mail: amendoza@cinvestav.mx

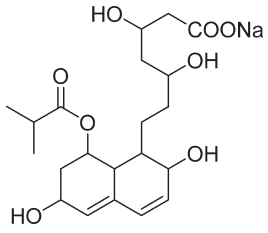

Figure 1. Pravastatin chemical structure.

for reducing the mortality related to coronary heart disease (CHD).[1,2] The elevated plasma cholesterol level and low density lipoprotein cholesterol levels (LDL) have been recognized as the major risk factor for atherosclerotic disease, specifically for CHD.[3] Several methods have been developed for estimation of pravastatin (Figure 1) with ultraviolet (UV) detection.

These methods are very sensitive but they only described the quantification in plasma and urine.[4–6] Only one method has been described for tablets and this method did not describe pravastatin extraction.[5] We, therefore, developed and validated a stable analytical method for pravastatin extraction and quantification in tablets and this method can be used for a quality control assay.

EXPERIMENTAL

Instrumentation

The chromatographic system consisted of a Mod 600 controlled pump and Mod 2487 waters UV absorbance detector. The above system was controlled by a Millennion controller.

Mass Spectroscopy

The Varian Saturn 2000 ion trap (IT) spectrometer (70 ev, ion source temperature 170°C, using column insertion) was used.

Chemical and Reagents

Potassium dihydrogen phosphate, ortho-phosphoric acid, methanol, and acetonitrile were of HPLC or analytical grade, and were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

The pravastatin secondary standard was purchased from Biofade with lot number CR/710/110102, and pravacol tablets were obtained at the pharmacy in Centro Medico Nacional SXXI, IMSS.

Chromatographic Condition

A reversed phase SunFire^{TM} C-18 column (150 mm × 4.6 mm, 5 μm particle size) equipped with a pre-column was employed. The mobile phase was composed of phosphate buffer and acetonitrile (7:3 v/v, pH 2.0) and its flow rate was 1 mL/min. Injection volume was 60 μL, and the experiments were performed at 30°C. Absorption was measured at 238 nm, and retention times for pravastatin were approximately 7.3 min. This peak was collected and analyzed by mass spectroscopy.

Solution Preparation

Standard Solution and Calibration Curve

Stoke solution of pravastatin was prepared by dissolving 10 mg of pravastatin secondary standard in 100 mL waters (100 μg/mL). This solution was utilized for the preparation of calibration standards and quality control samples; these solutions were stored at 4°C. The highest calibration standard with a concentration of 200 ng/mL pravastatin was prepared with 1 mL of base solution in 100 mL of water, which was then used to generate standard samples with final pravastatin concentrations of 200, 100, 75, 50, 25, 10, and 5 and quality control 150, 40, and 10 ng/mL by serial dilution with HPLC water.

Extraction Study

Pravastatin from the pravacol tablets (10 mg) was extracted using acetyl-acetate (50 mL), the organic layer was washed with water HPLC (20 mL) and taken to dryness in an N_2 flow; the solid residue (in theory 10 mg) was utilized to prepare the solution with final theoretical concentrations of 200, 100, 75, 50, 25, 10, and 5 ng/mL, which was compared with the pravastatin secondary standards curve.

RESULTS AND DISCUSSION

Separation and Specificity

Pravastatin retention times were 7.3 min. This time was validated for mass analysis. The pravastatin obtained from the tablets and the pravastatin secondary standard, show the same spectrum.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD determined as the amount of drug corresponding to a signal to noise ratio of 3:1 was 5 ng/mL, while the LOQ was determined as the lowest concentration analyzed, at 5 ng/mL.

Table 1. Assay linearity for the quantification of pravastatin

System	Mean	Std. error	T	P
Tablets extraction n = 7				
Slope	0.00747	1.28 e^{-04}	58.404	<0.001
Constant	−5.112	1.906	−2.682	0.044
R	0.999			
R^2	0.999			
Secondary standard n = 7				
Slope	0.00725	8.20 e^{-05}	88.392	<0.001
Constant	−6.039	1.268	−4.762	0.005
R	1.000			
R^2	0.999			

Recovery and Linearity

The recovery of pravastatin after the process was approximately 99% indicating good extraction. Mean slopes, intercepts, and r^2 values for the secondary standard and for the pravastatin extraction are reported in Table 1. These statistic parameters were obtained with the sigma stat program *version* 2.0.

Intra-Assay and Inter-Assay Variation

The intra-assay and inter-assay for pravastatin were obtained in the range of 1–3% (Table 2).

Table 2. Intra-assay and inter-assay accuracy of the pravastatin extraction

	Variability (C.V., %)	
Concentration (ng/mL)	Intra-assay (n = 6)	Inter-assay (n = 6)
150	1.01	1.54
75	2.74	2.32
25	1.31	1.63

Table 3. Accuracy of three concentrations using secondary standard

Concentration (ng/mL)	C.V. (%)	N
150	1.61	6
75	1.65	6
25	1.89	6

Accuracy

The accuracy of the measurements was determined using the calibration standards and three quality control samples for pravastatin in at least six runs (Table 3).

CONCLUSION

We introduced one method to quantify pravastatin in tablets using high performance liquid chromatography with ultraviolet light detection. The method was fully validated and validation parameters were: linearity range 10–200 ng/mL; correlation coefficient 0.999; mean recovery >999%; limit of quantification 5 ng/mL, and limit of detection 5 ng/mL. This method can be used for the quality control assay of pravastatin in tablets.

REFERENCES

1. Harman, J.G.; Limbird, L.E.; Goodman, G.E. *Las bases farmacológicas de la terapéutica*; McGraw Hill: México, 2003; 996.
2. Alberts, A.W.; Chen, J.; Kuron, G.; Hunt, V.; Huff, J.; Hoffman, C.; Rothrock, J.; López, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Albers-Schonberg, G.; Hensens, O.; Hirshfield, J.; Hoogsteen, K.; Liesch, J.; Springer, J.; Mevinolin. A highly potent competitive inhibitor of hyrdoxymethyl-glutaryl-coenzyme, a reductase and cholesterol-lowering agent. In *Proc Natl. Acad. Sci.* USA, **1980**; 3957.
3. Bauer, S.; Mwinyi, J.; Stoecke, T.G.; Roots, T. Quantification of pravastatin in human plasma and urine after solid phase extraction using high performance liquid chromatography with ultraviolet detection. J. Chromatogr. B **2005**, *818*, 257–262.
4. Kocijan, A.; Grahch, R.; Bastarda, A.; Kralj, L.Z. Fast analysis of pravastatin in production media. J. Cromatogr. B **2005**, *822*, 311–315.
5. Önal, A.; Sagirli, O. Development of a selective LC method for the determination of pravastatin sodium. Cromatography **2006**, *64*, 157–162.
6. Bischoff, H.; Angerbauer, R.; Bender, J.; Bischoff, E.; Faggioto, A.; Petinna, D.; Pfitzner, J.; Porter, M.C.; Schmidt, D.; Thomas, G. Cerivastatin; pharmacology of a novel synthetic and highly active HMG-CoA reductase inhibitor. Atherosclerosis **1997**, *135*, 119–130.

Received September 9, 2007
Accepted October 3, 2007
Manuscript 6204