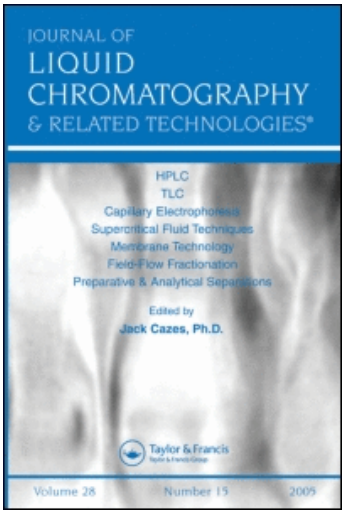

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Optimization of a Pravastatin Quantification Method Using HPLC with Ultraviolet Detection in Human Serum for Monitoring Dyslipidemic Patients

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Abstract: A high performance liquid chromatography (HPLC) method for the estimation of pravastatin in human serum samples has been developed to monitor dyslipidemic patients. The method was fully validated and validation parameters were in the linearity range 10–200 ng/mL, correlation coefficient 0.99, mean recovery >0.73, quantification limit 10 ng/mL, and limit of detection, 5 ng/mL; this method was applied for pravastatin determination in human serum from Mexican dyslipidemic patients. Pravastatin values found for three studied patients were 73, 57, and <10 ng/mL, indicating the importance of monitoring, due to the metabolic variability of these chronic patients.

Keywords: Pravastatin, Serum levels, Dyslipidemic patients

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INTRODUCTION

Cholesterol lowering statin drugs (atorvastatin, cerivastatin, fluvastatin, pravastatin, and sinvastatin) are the most frequently prescribed substances for reducing coronary heart disease (CHD) mortality rates.[1,2] The elevated plasma cholesterol level and low density lipoprotein cholesterol levels (LDL) have been recognized as a major risk factor for atherosclerotic disease, specifically for CHD.[3] Several methods have been developed for estimation of pravastatin (hexahydro-6-hydroxy-2-methyl-8-(2-methylbutyloxy)-1-naphthyl-3,5-dihydroxyheptanoate, (Figure 1), with ultraviolet (UV) detection.

These methods are very sensitive but only describe quantification in plasma and urine and not in serum of healthy volunteers.[4–6] The evaluation in the serum of dyslipidemic patients would be useful because they have different metabolisms than healthy volunteers. Thus, we developed and validated a stable analytical method for pravastatin quantification in the serum of dyslipidemic patients, linking this with the cholesterol related parameters. Our objective was to evaluate the quantity of pravastatin in serum to maintain the therapeutic window, in order to control skeletal muscle abnormality related side effects.

EXPERIMENTAL

Instrumentation

The chromatographic system consisted of a Waters mod 600 controlled pump, a Waters 2487 UV absorbance detector, and a Waters 717 plus autosampler. This system was controlled by a Millennium controller.

Chemical and Reagents

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

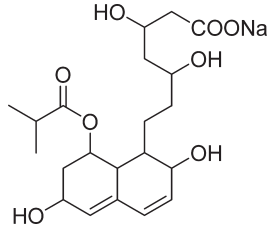

Figure 1. Pravastatin chemical structure.

Chromatographic Condition

A reversed phase SunFire™ C-18 column (150 × 4.6 mm, 5-μm particle size) equipped with a pre-column was employed. The mobile phase was composed of phosphate buffer (sodium dodecyl sulphate 1 mM + monobasic sodium phosphate 20 mM) and acetronitrile (7:3 v/v, pH 2.0) with a 1 mL/min flow rate. Injection volume was 60 μL, and experiments were performed at 30°C. Absorption was measured at 238 nm, and retention times for pravastatin were approximately 7.3 min.

Standard Solution and Calibration Curve

The stock solution of pravastatin was prepared by dissolving 10 mg in 100 mL water. 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, 25, and 5, and quality control 150, 40, and 10 ng/mL by serial dilution with water. For the calibration standard, the working solution was prepared with 100 μL of standard solution and 200 μL human serum adjusted to pH 3.0 with HCl, 1 N, and was pre-purified as follows: After conditioning with 1 mL each of methanol and distilled water, the working solution was introduced into the 1 cc oasis column; the samples were washed with 1 mL of 5% methanol in water, and eluted with 1 mL of methanol and afterwards were evaporated to dryness at 60–65°C under a nitrogen stream. The dried extracts were redissolved in 100 μL of distilled water. The complete reconstituted extract was subjected to high performance liquid chromatography (HPLC) analysis.

Study Protocol of Dyslipidemic Patients

After a single oral dose of 4 × 10 mg Pravacol® tablets, the samples of three dyslipidemic patients were analyzed and compared with some clinical data after 1.5 h.

Analysis of Samples from Dyslipidemic Patients

Samples were centrifuged for 10 min at 3,500 rpm at 10°C. The clear supernatant was processed in the same way as the calibration curves described previously, and simultaneously a quality control standard was injected.

RESULTS

Separation and Specificity

Pravastatin retention times were 7.3 min; no important interferences were observed in serum samples. Figure 2 shows the chromatogram of serum samples as follows: (a) a sample at the lowest limit of quantification (LOQ); and (b) a quality control standard with a pravastatin concentration 40 ng/mL. Figure 3 shows serum samples from three dyslipidemic patients 1.5 h (C-max in healthy volunteers) after administration of 4×10 mg of Pravacol® tablets.

Limit of Detection (LOD) and Limit of Quantification

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 sanalyzed in serum that could be quantified with an inter-assay coefficient of variation (CV) of $<20\%$ and accuracy between 80 and 120% (Table 1).

Recovery and Linearity

Recovery of pravastatin after the process was approximately 76% (Table 2). Mean slopes, intercepts, and R^2 values with standard deviation (SD) and ranges are reported in Table 3.

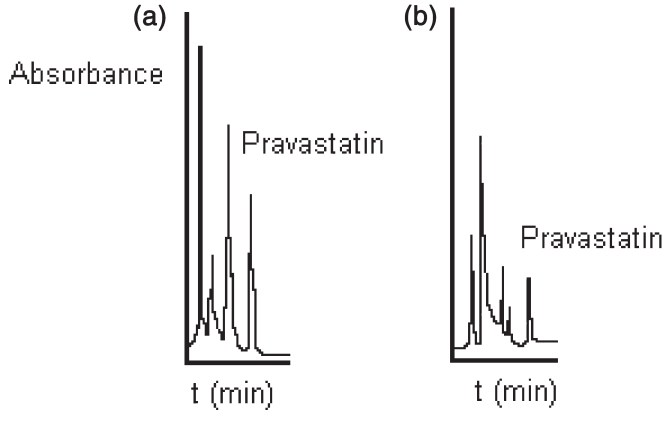

Figure 2. a) Lowest LOQ (10 ng/mL), b) Quality control standard (40 ng/mL).

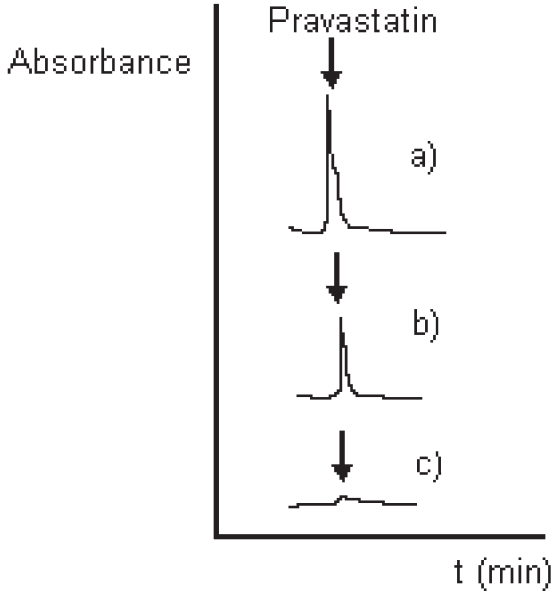

Figure 3. Pravastatin quantification in three different dyslipidemic chronic patients: a) 73 ng/mL; b) 57 ng/mL; c) <10ng/mL.

Intra- and Inter-Assay Variation

Intra-assay %CV for pravastatin in serum ranged from 1.01–2.74%, and the inter-assay %CV, from 1.54–2.32% (Table 4). These values were below the allowed range of 10–20%.

Table 1. High and lowest LOQ determination

Concentration (ng/mL)	C.V. (%)	n
200	5.35	6
10	3.56	6

Table 2. Percentage of recovery = [(Water area–Serum area)/Water area] × 100

Concentration (ng/mL)	Water	Serum	Recovery (%)
150	21231	15871	75
75	10838	8608	79
10	2205	1866	85

Table 3. Assay linearity for the quantification of pravastatin

System	Mean	SD	t	p
Serum human n = 7				
Slope	$9.5864185e^{-03}$	$1.49\ e^{-03}$	64.354	<0.001
Intercept	−6.5591005	1.748	−3.752	0.013
R	0.9993968			
R^2	0.9987939			
Water n = 7				
Slope	$7.2636741e^{-03}$	$8.20\ e^{-03}$	88.392	<0.001
Intercept	−6.5738065	1.268	−4.762	0.005
R	0.9996801			
R^2	0.9993603			

Accuracy

Accuracy of the measurements was determined using the calibration standards and the three quality control samples for pravastatin in at least three runs (Table 5).

Table 4. Intra-assay and inter-assay accuracy of the pravastatin quantification in human serum

	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 5. Accuracy of three concentrations using secondary standard

Concentration (ng/mL)	C.V. (%)	n
150	0.61	6
75	0.65	6
25	0.89	6

Study of Dyslipidemic Patients

Dyslipidemic patient results after injecting four quality control standards were 73, 57, and <10 ng/mL. The cholesterol data, before and after pravastatin treatment, were obtained from the Hospital of Cardiology, National Medical Center SXXI (Table 6).

DISCUSSION

This is a new method to quantify pravastatin using HPLC with UV detection in human serum to monitor dyslipidemic patients. The method was fully validated and validation parameters were in the linearity range 10–200 ng/mL, correlation coefficient 0.99, mean recovery >0.73, limit of quantification 10 ng/mL, and limit of detection, 5 ng/mL. The method was applied for pravastatin determination in the human serum of dyslipidemic patients after single oral doses of 4×10 mg Pravacol® tablets. The analyses obtained from dyslipidemic patient samples indicated the importance of monitoring serum levels; for example, the first patient studied presented anemia (hemoglobin levels, 12.9 g/dL) and the pravastatin value in human serum was high (73 ng/mL, Table 6) compared with that of healthy volunteers reported in the literature.[3,4] This could be due to liver failure.[1] This result indicates the importance of normal recuperation parameters; thus, we are required to repeat pravastatin quantification in serum with the purpose of maintaining the therapeutic window. The second patient studied showed pravastatin levels of 57 ng/mL, which is within the normal range. There are no cholesterol levels before treatment for this patient, because pravastatin was prescribed preventively before our evaluation (Table 6). Pravastatin

Table 6. Clinical data of dyslipidemic patients

Data	Total cholesterol	LDL	HDL	Triglycerides
Patient 1[a]				
Pre-treatment	260	150	37	210
Post-treatment	180–380	120–160	30–40	180–204
Patient 2[b]				
Pre-treatment	NA	NA	NA	NA
Post-treatment	175–190	115–132	35–43	135–152
Patient 3[c]				
Pre-treatment	235–272	141–174	19–30	258–400
Post-treatment	200–369	104–188	38–45	215–1264

Pravastatin quantification in serum: [a] 73 ng/mL, [b] 57 ng/mL, [c] <10 ng/mL.
NA: not available.

quantification in the third patient studied was not possible because it fell under the LOD (<10 ng/mL); however, the LDL value was normal, but not that of the triglycerides, this indicating a different absorption and suggesting a complete pharmacokinetic study of the patient (Table 6). These studies indicate the importance of pravastatin monitoring due to differences in the statin metabolism observed in these patients, and the difficulty of maintaining the therapeutic window and of controlling the drugs side effects.

REFERENCES

1. Harman, J.G.; Limbird, L.E.; Goodman, G.E. *Las Bases Farmacológicas de la Terapéutica*; Mc.Graw 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. 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 analisis of pravastatin in production media. J. Cromatogr. B **2005**, *822*, 311–315.
5. Bischoff, H.; Angerbauner, 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.
6. Campos-Lara, M.; Mendoza-Espinoza, J.A. Development of a selective extraction method for pravastatin quantification in tablets, using high performance liquid chromatography with ultraviolet detection. J. Liq. Chromatogr. R. T. **2008**, *31*, 619–623.

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