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RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF PRAVASTATIN AND COENZYME Q10 IN THEIR COMBINED FORMULATED DOSAGE FORM

Mr. HIREN K. KADIKAR, Dr. RAGIN SHAH

Department of Pharmacy, JJT University, Jhunjhunu, Rajasthan.

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Corresponding Author

Mr. Hiren K. Kadikar

Department of Pharmacy, JJT University, Jhunjhunu, Rajasthan.

hirenkadikar@gmail.com

Abstract

A Simple, precise, accurate and rapid RP-HPLC method developed and validated for the simultaneous estimation of Pravastatin and Coenzyme Q10 in pure and formulated dosage form. The quantification was carried out using symmetry C18 column, 250 x 4 mm, i.d, 5µm particle size in isocratic mode, with mobile phase compressing of Acetonitrile and Tetrahydrofuran (80:20). The flow rate was 1 ml/min and the detection was carried out by UV detector 254nm. The retention times were 6.47 and 3.64 min for Coenzyme Q10 and Pravastatin, respectively. Percentage recovery for SIM was 98.72-101.45%, while for COQ10, it was found to be in range of 98.00-101.27%. The method was validated as per ICH guideline.

INTRODUCTION

Pravastatin is (1S,3R,7S,8S,8aR)-8-{2-[(4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate. Pravastatin is a selective and competitive inhibitor of 3-hydroxy-3- methylglutaryl -coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to Mevalonate, a precursor of cholesterol[1]. Pravastatin is a member of the class of Statins, used to treat hypercholesterolemia and related conditions and to prevent cardiovascular disease. It increases the number of hepatic LDL (Low Density Lipoprotein) receptors on the cell surface to enhance uptake and catabolism of LDL. Secondly, Pravastatin inhibits hepatic synthesis of VLDL (Very Low Density Lipoprotein), which reduces the total number of VLDL and LDL particles[2]. Coenzyme Q10 is a 2-[(2*E*,6*E*,10*E*,14*E*,18*E*,22*E*,26*E*,30*E*,34*E*)-3,7,11,15,19,23,27,31,35,39-Decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaen-1-yl]-5,6-dimethoxy-3-methyl-2,5cyclohexadiene-1,4-dione.

Coenzyme Q10 is nutrient or dietary supplement with Pravastatin. It's mainly used in cardiovascular disease, Neurodegenerative disease, Diabetics, Cancer, Asthma Migraine, Immune disorders, HIV/AIDS, Male fertility. A daily dosage up to 3600mg was found to be tolerated by healthy as well as unhealthy person.

Pravastatin has a side effect of myopathy and Rhabdomyolosis. Number of clinical trial supports this and also it is supported by the official USFDA Site. This side effect is due to Pravastatin block HMGCO A reductase because in synthetic pathway of cholesterol one side chain from fernesyl PP which is converted in to decaprinyl PP which combines with the 4-OH benzoate forms endogenous Coenzyme Q10. So Pravastatin block not only Cholesterol synthesis but also coenzyme Q10 so side effect of Pravastatin due to Coenzyme Q10 block so it has been prove that giving Coenzyme Q10 as a adjuvant with the

Pravastatin decrease the Myopathy and rhabdomylosis[3-6].

A detailed survey of analytical literature for Pravastatin revealed several methods based on varied techniques, viz, HPLC, LCMS Spectrophotometry and High-Performance Thin- Layer Chromatography (HPTLC) and similarly, a survey of the analytical literature for COQ10 revealed several methods based on HPLC, spectroflorymetry and Ampherometry for determination in pharmaceuticals in combination with other drugs were reported.

The combination of this both drug are still under clinical trials and hence till date we have not come across any reported method for simultaneous estimation of the Pravastatin and coenzyme Q10 during our continues reference work.

MATERIALS & METHODS

Chemicals and Reagents

Acetonitrile for HPLC (RFCL LMT, Rankem)

Tetrahydrofuran for HPLC (RFCL LMT, Rankem).

Apparatus and Instrumentation

HPLC: Thermo fisher

UV-Visible Detector: UV 2000, ss420

Column :- Thermo Hypersil-Keystone, 250mm x 4mm, 5 µm

UV 1800 Spectrophotometer - Shimadzu

Shimadzu – AUX 220 balance Uni Bloc - Shimadzu

Sonicator-Soltec-Sonica Ultrasonic Cleaner (Spincotech Pvt. Ltd.)

Durasil-Pipettes of 2 and 5 ml capacity were used.

Glassware - Borosil-Volumetric flasks of 10 and 25ml capacity were used.

Chromatographic conditions

Stationary phase: Thermo Hypersil-Keystone, 250mm x 4mm, 5 µm

Mobile phase: Acetonitrile:tetrahydrofuran (80:20v/v)

Flow rate: 1.0 ml/min

Wavelength: 254 nm

Run time: 8min

EXPERIMENTAL

All chemicals and reagents used were of AR/HPLC grade.

Preparation of mobile phase

A degassed mixture of THF and Acetonitrile in the ratio of 20:80 (v/v) was prepared and the mixture was filtered through 0.45 µ membrane filters and it was degassed.

Preparation of SIM stock solution

Accurately weighed Pravastatin (25 mg) was transferred to 25 ml volumetric flask and dissolved in mobile phase and diluted up to the mark with mobile phase to give a stock solution having strength 1 mg/ml (1000 µg/ml).

Working standard solution of SIM

100 µg/ml of Pravastatin working standard solution was prepared by diluting 2.5 ml of stock solution with methanol to 25 ml with mobile phase.

Preparation of COQ10 stock solution

Accurately weighed COQ10 (25 mg) was transferred into 25 ml volumetric flask and dissolved in mobile phase and diluted up to the mark with mobile phase to give a stock solution having strength 1 mg/ml (1000 µg/ml).

Working standard solution of COQ10

100 µg/ml of COQ10 working standard solution was prepared by diluting 2.5ml of stock solution to 25 ml with mobile phase.

Calibration curve

Calibration curves were prepared by taking appropriate aliquots of 0.5, 1.0, 1,5, 2.0, 2.5, 3.0ml of above mixed preparation and diluted with the mobile phase to get 5,10,15,20,25,30 µg/ml of both drugs. Representative Chromatogram of calibration curve for Pravastatin (5-30 µg/ml) and Coenzyme Q10 (5-30 µg/ml) is shown in Fig. 2.

Procedure for analysis of tablet formulation

Total 20 tablets were accurately weighted and triturated with glass mortar and pestle. The powder equivalent to 10 mg of Pravastatin and 20 mg of Coenzyme Q10 was taken in 100 ml volumetric flask; mobile phase was added and the flask was kept in an ultrasonic bath for 10 min. The volume was made up to mark and the solution was filtered through 0.2 micron nylon membrane filter. 1ml of above filter solution was further diluted to 10 ml with mobile phase. The above solution was

analyzed under optimized chromatographic conditions.

VALIDATION OF RP-HPLC METHOD

Linearity

Aliquots of standard solutions of Pravastatin and coenzyme Q10 in range of 5-30 µg/ml, was prepared from working standard solution and injected to system with stated chromatographic conditions and analyzed. The graph of peak area obtained versus respective concentration was plotted. The mean area with its standard deviation and % relative standard deviation of peak were calculated.

PRECISION

1. Repeatability

Three different standard solutions of Pravastatin and Coenzyme Q10 (10, 15, 20µg/ml) were prepared from working standard solution and injected three times to system with stated chromatographic conditions and analyzed.

2. Intraday precision

Standard solutions Pravastatin and coenzyme Q10 (5-30 µg/ml) were prepared from working standard solution and injected in to system with stated chromatographic conditions and analyzed, three times in a day.

3. Interday precision

Standard solutions Pravastatin and coenzyme Q10 (5-30 µg/ml) were prepared from working standard solution and injected in to system with stated chromatographic conditions and analyzed, three days.

ACCURACY

Accuracy may often be expressed as percentage recovery. The accuracy was determined by standard addition method.

To a fixed amount of pre-analyzed sample (1.0 ml) mixture of Pravastatin (50µg/ml) and Coenzyme Q10 (100 µg/ml) increasing amount of its working standard solution (2.5,5.0,7.5ml of 10µg/ml of Pravastatin and 20 µg/ml coenzyme Q10) were added in three different 10 ml volumetric flask and made up to mark with methanol. Samples were injected to system and analyzed. The mean % recovery from of peak areas calculated (Table 1).

LIMIT OF DETECTION (L.O.D.)

The L.O.D. was estimated from the set of 5 calibration curves is shown in eq. 1.

LOD = 3.3 × (S.D./Slope) ----------1

Where,

S.D. = Standard deviation of the Y-intercepts of the 5 calibration curves.

Slope= Mean slope of the 5 calibration curves.

LIMIT OF QUANTIFICATION (L.O.Q.)

The L.O.Q. was estimated from the set of 5 calibration curves is shown in eq. 2.

LOQ = 10 × (S.D./Slope) ----------**2**

Where,

S.D.= Standard deviation of the Y-intercepts of the 5 calibration curves

Slope = Mean slope of the 5 calibration curves.

RESULTS AND DISCUSSION

For RP-HPLC method different mobile phases were tried and the mobile phase containing Acetonotrile and Tetrahydrofuran (80:20, v/v) was found to be optimal for obtaining well defined and resolved peaks with mean retention times 3.64 min and 6.47 min for SIM and COQ10 respectively. The representative chromatogram of the standard solution of mixture is shown in Figure 1.

Results were found to be linear in the concentration range of 5-30 mg/mL for both drugs. The correlation coefficients for the plots were 0.999 for Pravastatin and 0.999 for COQ10. The proposed method was also evaluated by the assay of formulated tablets containing SIM and COQ10. The % assay was found to be 99.79 ± 0.542 for Pravastatin and 101.69 ± 0.709 for COQ10 (mean ± S.D., n = 6). The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2. The summary of validation parameters of proposed HPLC method is given in Table 2.

CONCLUSION

The proposed method is simple, sensitive and reproducible and hence can be used in routine for determination of Pravastatin and Coenzyme Q10 in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The developed method can be used for routine

quantitative estimation of Pravastatin and Coenzyme Q10 in pharmaceutical preparation. The mobile phase CAN and THF (80:20) was found to be ideal for estimation of Pravastatin and Coenzyme Q10. The elution was as followed (For Pravastatin RT-3.64 and COQ10 RT-6.47).The mean recovery was (For Arte 99.00% and Amo 98.83%).

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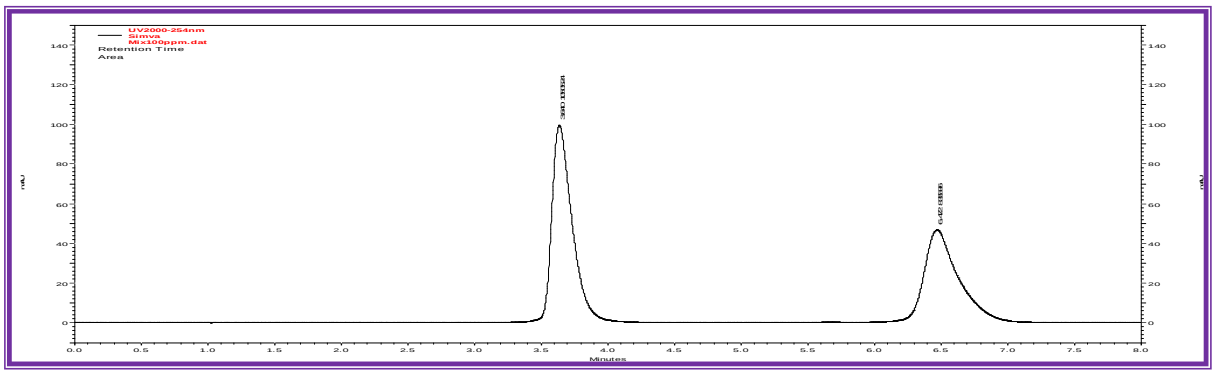

Figure 1: Representative chromatogram obtained for standard mixture containing Pravastatin (50 mg/ml, 3.64 min) and COQ10 (50 mg/ml, 6.47 min)

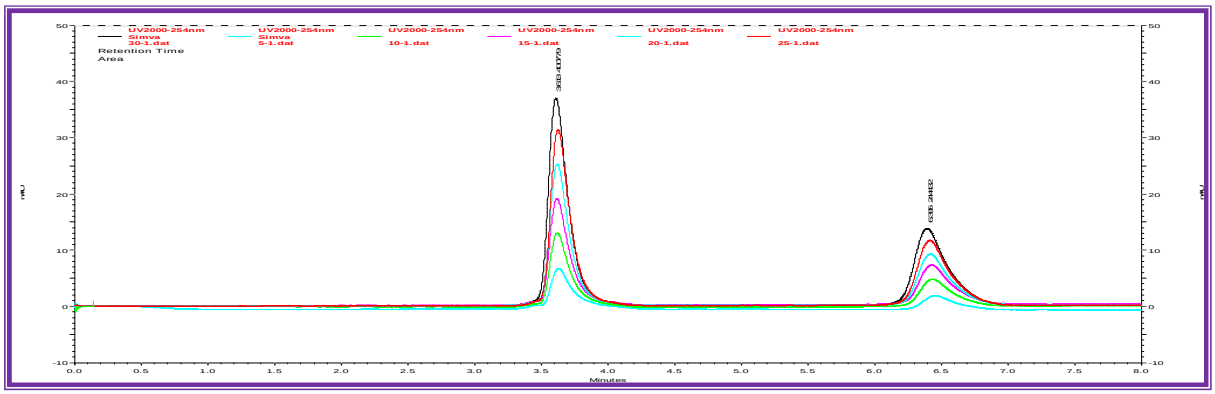

Figure 2: Representative Chromatogram of calibration curve for Pravastatin and Coenzyme Q10 (5-30 µg/ml)

Table 1.

Accuracy for Pravastatin and Coenzyme Q10

Prava statin	COQ10	STD added		Total		Amount recovered		% recovery	
		Prava statin	COQ10	Prava statin	COQ10	Prava statin	COQ10	Prava statin	COQ10
5	10	2.5	5	7.5	15	2.53	5.06	101.45	101.27
5	10	5	10	10	20	5.1	9.93	101.80	99.37
5	10	7.5	15	12.5	25	7.6	15.12	101.44	100.84

Table 2

Summary of Validation parameters for Pravastatin and Amodiaquine

Sr. no.	Parameters	Results	
		Pravastatin	**COQ10**
1	Linearity Range (µg/ml)	5-30	5-30
2	Correlation coefficient	0.999	0.999
3	Precision (%C.V.)		
	1. Repeatability	0.27-1.78	0.4-1.78
	2. Intraday precision	0.11-0.96	0.057-1.41
	3. Interday precision	0.68-1.96	0.38-1.61
4	Accuracy (%recovery)	98.72-99.07	98.00-99.15
5	Limit of Detection (L.O.D.) (µg/ml)	0.27	0.31
6	Limit of Quantitation (L.O.Q.) (µg/ml)	0.82	0.95

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