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Research Article

Reverse phase HPLC method for the determination of Pravastatin in tablet dosage forms

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ABSTRACT

A simple RP-HPLC method for the determination of pravastatin in tablet dosage form. Numerous HPLC conditions were tested for the determination of pravastatin. The best result was achieved by using Phenomenex® Luna 5µm C18(150x4.6mm) ID column, and a mobile phase consisting of acetonitrile: potassium dihydrogen orthophosphate (0.02M) (30:70) adjusted to pH 3.0 with orthophosphoric acid, a flow rate of 1.5ml/min with ultraviolet detection at 240nm.The correlation coefficient for calibration curves within the detection range of 35.22-65.40µl/ml are 0.9993. The within and between-day precision was determined for both retention time and peak area.

Keywords: Pravastatin; HPLC; Stability

1. INTRODUCTION

Statin drugs are one of the most used pharmaceutical classes of products throughout the world. It has been ranked among the top ten prescription drugs since 1999, with $9.2 billion in sales generated. The widespread use of Pravastatin drug (Fig.1) is of special concern because they can lower the endogenous level of Coenzyme, the naturally occurring form of Ubiquinone in humans. Ubiquinone is widely recognized as an essential component of energy metabolism in the electron transfer system in mitochondrial membrane. (Otter, 1998; Bauer, 2003) reveals that HPLC methods were reported for Pravastatin in urine and plasma. A Differential Pulse Polarographic method (Coskun, 1997) was also adapted for Pravastatin tablets. This paper describes a simple, reliable, precise, stability indicating RP- HPLC method for the determination of Pravastatin in tablets.

2. EXPERIMENTAL

2.1 Chemicals

Pravastatin working standard and pravastatin tablets (Pravator-10 mg) was purchased From Ranbaxy (New Delhi, India). HPLC grade acetonitrile was purchased from Merck (Mumbai, India) and potassium dihydrogen orthophosphate and orthophosphoric acid was obtained from S.D.Fine (Mumbai, India). All chemicals were of analytical grade.

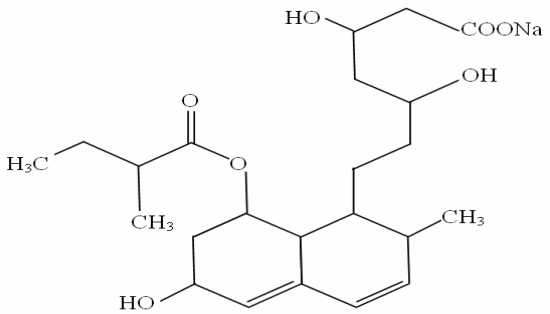

Figure 1: Structure of PRAVASTATIN

2.2 Chromatography

The determination was carried out on waters HPLC model 2690 equipped with 2487 UV visible detector (Dualλ absorbance detector) and waters HPLC model 2690 equipped with 2996 photodiode array detector using data handling system-waters millennium chromatography software. The column used in the development for determination is Phenomenex® LUNA 5µm C18 (150× 4.6mm) I.D column. The detector wavelength was set at 240nm.A flow rate of 1.5ml/min was used for the determination of Pravastatin. The mobile phase composition was acetonitrile: potassium dihydrogen orthophosphate 0.02M (30:70) adjusted to pH

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Table 1: METHOD DEVELOPMENT CONDITIONS

CONDITION	MOBILE PHASE-A	MOBILE PHASE-B	PH OF MOBILE PHASE	RATIO OF A/B	PRAVASTATIN-RETENTION TIME	TAILING FACTOR
1	0.01M KH_2PO_4	Acetonitrile	3.0	70/30	9.01	1
				65/35	4.87	1
				75/25	not eluted	not eluted
2	2.0 Ml of Gl.Acetic Acid	Acetonitrile	3.0	70/30	9.3	1.1
				65/35	5.04	1.1
				75/25	not eluted	not eluted
3	0.02 M NaH_2PO_4	Acetonitrile	4.5	70/30	5.97	0.7
				65/35	3.62	0.7
				75/25	13.68	0.7
4	0.02 M Na_2HPO_4	Acetonitrile	6.0	70/30	2.42	1.5
				65/35	2.18	1.2
				75/25	5.09	1.6
5	2.0 Ml of Gl.Acetic Acid	Methanol	3.0	50/50	not eluted	not eluted
				40/60	7.06	1.0
6	0.02 M NaH_2PO_4	Methanol	4.5	65/35	not eluted	not eluted
				50/50	13.01	0.8
7	0.02 M NaH_2PO_4	Methanol	6.0	65/35	not eluted	not eluted
				50/50	8.95	1.8
8	0.02 M KH_2PO_4	Acetoni-trile/Methanol	4.5	60/20/20	12.13	0.9
9	0.02 M KH_2PO_4	ACN/Meth/THF	4.5	60/20/20	2.63	1.0

- KH2PO4- -Potassium dihydrogen orthophosphate
- Na2HPO4 - Sodium dihydrogen orthophosphate

3 with orthophosphoric acid. The samples and standards were dissolved in the mobile phase and 20 µl samples were injected into the HPLC system at thecolumn and sample temperature of 30° c and 10° c respectively.

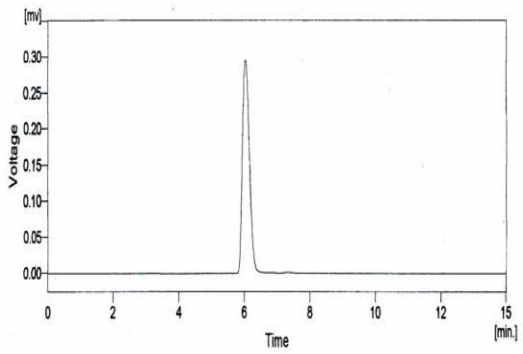

Figure 2: Chromatogram of PRAVASTATIN

2.3 Preparation of solutions

Test and standard solutions of pravastatin sodium and tablets were prepared by dissolving in HPLC grade water in a concentration of 50µg/ml respectively. Samples were filtered through 0.45µm nylon syringe filter (Millipore, England) before they injected into the HPLC system.

3. RESULTS AND DISCUSSION

3.1 Chromatography

Of the conditions tested for method development (Table 1) it was only by using Phenomenex® LUNA 5µm C18 150× 4.6mm I.D column with the mobile phase of acetonitrile: potassium dihydrogen orthophosphate 0.02M (30:70) adjusted to pH 3 with orthophosphoric acid that good for the determination for pravastatin in tablets. The retention time of the compound was found to be 6 mins (Fig.2). The main advantage of this method is that it is simpler to carry out with regard to the preparation of samples and the conditions used and thus it is less time consuming and less costly with the use of Phenomenex® LUNA 5µm C18 column.

3.2 Validation of the analytical method

To validate the RP-HPLC method, a series of tests were made using the most promising conditions.

3.3.1 Linearity

A calibration curve was made and concentrations examined within the detection range of 35.2-65.40 µg/ml for Pravastatin the correlation coefficient was found to be 0.99933.

3.3.2 Precision and accuracy

The within-day precision (expressed as the relative standard deviation (R.S.D)) for area under the curve (AUC) and retention times was determined for Pravastatin for repeated analysis (n=6). Average within-day R.S.D values obtained for retention times were 0.17% and for area under curve were 0.79%. The average R.S.D values for between-day precision obtained for AUC were 0.41 % (Table 2).The assay values obtained by proposed method and the recovery experiment values obtained were performed by adding a fixed amount of drug to preanalyzed formulation summarized in Table.3.

Table 2: PRECISION

COMPOUND	R.S.D (%)	AUC R.S.D (%)	BETWEEN DAY PRECISION R.S.D FOR AUC (%)
Pravastatin	0.17	0.79	0.41

Table 3: ANALYSIS OF PHARMACEUTICAL FORMULATION

PHARMACEUTICAL FORMULATION	LABELLED AMOUNT (mg)	AMOUNT FOUND BY PROPOSED METHOD (mg) [a]	RECOVERY BY PROPOSED METHOD (%) [b]
Pravator tablet	10	9.8±0.4	99.85±0.41

[a] average ±s.d. (n=6)

[b] Recovery of 10mg added to the pharmaceutical preparation. Average of six determinations

3.3.3 Stability

The stability of sample was checked by forced degradation in different conditions and the percentage of degradation was calculated. The peak purity of the analyte was passed in all conditions (purity angle should be less than the threshold value). The following values (Table 4) indicate that the any other impurity is not merging with the main peak. The analyte solution was stable up to 14 hrs.

Table 4: STABILITY

EXPERIMENT	PERCENTAGE DEGRADATION	PURITY ANGLE	PURITY THRESHOLD
Photolytic (2-Days in Sun Light)	3.71	0.120	1.349
Thermal (2-Days in 110°C)	38.23	0.145	1.158
Alkali Deg (2-N NaOH 1ml)	21.48	0.890	2.335
Acid Deg (2-N HCl 1ml)	45.19	0.152	1.180
H_2O_2 50% W/W (1ml)	29.19	0.140	1.136

3.3.4 Robustness

The reliability of the method was determined by made small deliberate variations in method parameters and the RSD values (Table.5) obtained, an indication of its reliability on normal usage.

Table 5: ROBUSTNESS

S.NO	CONDITIONS	% RSD
1	Flow (+10%)	0.20
2	Flow (-10%)	0.09
3	Temperature (35°C)	0.38
4	Organic (+2%Abs)	0.57
5	Organic (-2%Abs)	0.13
6	Wavelength (+5nm)	0.16
7	Wavelength (-5nm)	0.16
8	pH of the buffer(+0.2)	0.32
9	Robustness-pH of the buffer (-0.2)	0.55

4. CONCLUSION

A method was developed for the determination for pravastatin in tablets which is simple, quick, reliable, inexpensive and simple. The results indicate that the described method can be used for quantitative analysis of the compound.

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REFERENCES

Bauer.S, Mwinyi.J, Stoeckle.A, Gerloff.T, Roots. I. J Chromatogr B Analyt Technol Biomed Life Sci. 2005, 818:257.

Coskun. NY, Aycan.S, Sungur.S. Die-Pharmazie.1997, 52:485.

Dumousseaux.C, Muramatsu.S, Takasaki.W, Takahagi.T. J-Pharm-Sci.1994, 83:1630.

Iacona.I, Regazzi.MB, Buggia.I, Villani.P, Fiorito.V, Molinaro.M, Guarnone.E. Ther Drug Monit.1994, 16:191.

Jemal.M, Xia YQ, Whigan DB. Rapid Commun Mass Spectrum.1998, 12:1389.

Kawabata.K, Matsushima.N, Sasahara.K. Biomed Chromatogr.1998, 12: 271.

Mulvana.D, Jemal.M, Pulver. SC. J-Pharm-Biomed-Anal. 2000, 23: 851.

Otter.K, Mignat.C. J Chromatogr B Biomed Sci Appl. 1998, 708:235.

Pasha.MK, Muzeeb.S, Basha.SJ, Shashikumar.D, Mullangi.R, Srinivas.NR. Biomed Chromatogr. 2006, 20: 282.

Siekmeier.R, Gross.W.Marz.W. Int J Clin Pharmacol Ther.2000, 38: 419.

Zhu.Z, Neirinck.L. J Chromatogr B Analyt Technol Biomed Life Sci.2003, 783: 133.