Research Article

Development and Validation of pH Independent Spectrophotometric Method for Determination of Pravastatin Sodium in Dosage Forms

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ABSTRACT

A simple, sensitive and pH independent spectrophotometric method has been developed for the determination of pravastatin sodium in pharmaceutical formulations. The method is based on the measurement of absorbance at isosbestic point. Isosbestic point for pravastatin sodium was observed at 249 nm and absorbances were measured at same wavelength. The method is found to be linear in the range of 2.5–30 µg/mL for pravastatin sodium with R^2= 0.9996 (n=5). The proposed method is simple, rapid and gives accurate and precise results. Market formulations of pravastatin sodium were analyzed with the proposed method. Results obtained are in good agreement with the labeled amount of pravastatin sodium (100.62 ± 0.63, n=3) in tablet dosage forms**.** The proposed method can be satisfactorily applied for estimation of Pravastatin sodium in pharmaceutical dosage form. The method can also be applied for estimation of Pravastatin sodium in aqueous medium irrespective of pH of solution.

Keywords: UV spectrophotometry, Isosbestic point, pH independent, Pravastatin sodium

1. INTRODUCTION

Pravastatin sodium (PVS) is a member of the HMG-CoA reductase inhibitors class of lipid lowering drugs. PVS is a selective, competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme that converts 3-hydroxy-3 methylglutaryl-coenzyme A to mevalonate, a precursor of the sterols, including cholesterol. It is mainly used for treatment of hypercholesterolemia and prevention of cardiovascular disease [1,2]. It is chemically 1-Naphthalene-heptanoic acid, 1, 2, 6, 7, 8, 8a-hexahydro-β, δ, 6-trihydroxy-2-methyl-8-(2-methyl-1-oxobutoxy)-, monosodium salt, [1S-[1α(βS*, δS*), 2α, 6α, 8β(R*), 8aα]] [3,4].

PVS is official in Indian Pharmacopoeia 2010 and method for its estimation in bulk drug and formulations is liquid chromatography. Various HPLC, LC-MS, capillary electrophoresis and electrochemical methods have been reported for estimation of PVS in bulk drug, human plasma as well as in dosage forms [5-14]. Various methods for estimation of PVS in combination with other drugs were reported in literature [15-18]. Therefore it was thought of interest to develop simple and effective spectrophotometric method for the estima-

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tion of PVS in aqueous medium irrespective of pH of the solution.

The present study describes development, validation and application of simple and sensitive spectrophotometric method for estimation of PVS in aqueous medium at its isosbestic point. Isosbestic point is the wavelength at which there is no effect of pH on absorbance of the drug. Measurement of drug at isosbestic point allows the analyst to use the method for analysis of drug samples irrespective of the pH of medium. Developed method was successfully utilized to determine PVS in its tablet dosage form.

2. MATERIALS AND METHODS

2.1. Apparatus and Instruments

A Double beam Shimadzu 160A UV-Visible spectrophotometer having two matched quartz cuvettes with 1 cm light path was employed for spectral measurements. Shimadzu-libror AGE-220 balance was used for weighing purpose.

2.2. Reagents and materials

Pravastatin sodium standard was procured as a gift sample from Astron pharmaceutical, Ahmedabad. Sodium hydroxide pellets (Finar chemicals), Citric acid monohydrate (Finar chemicals), Di-sodium hydrogen phosphate (S.d. fine-chem limited), Boric acid (S.d. fine-chem limited), Potassium chloride (Finar chemicals), Hydrochloric Acid (35-38 %, S.d. fine-chem limited) and Distilled water were also used. All the chemicals were of analytical grade. Tablets containing Pravastatin sodium (20 mg) were a commercial product. (PRAVASTIN 20)

2.3. Preparation of solutions

2.3.1 Buffer solutions

Hydrochloric acid buffer solutions (IP 2010) of pH 1.2 and 2.0, citro-phosphate buffer solutions of pH 3.0, 4.0, 5.0, 6.0, 7.0, alkaline borate buffer solutions (IP 2010) of pH 8.0, 9.0, 10.0 and di-sodium hydrogen phosphate & sodium hydroxide buffer solutions of pH 11.0 and 12.0 were prepared [19, 20].

2.3.1. Standard solutions

Accurately weighed Pravastatin sodium (10 mg) was transferred into 10 ml volumetric flask and dissolved in methanol and diluted up to the mark with methanol to give a stock solution having strength of 1 mg/ml. Stock solution was further diluted with distilled water to obtain a standard solution of Pravastatin sodium having concentration 50 µg/ml.

2.4. Isosbestic point determination

Isosbestic point of Pravastatin sodium was determined by zero order spectrophotometric method and difference spectrophotometric method.

2.4.1. Using zero order spectrophotometric method

Solutions of same concentration (15 µg/mL) of Pravastatin sodium solution were prepared in 0.1 N HCL, citro-phosphate buffer solution of pH 4.0, alkaline-borate buffer solution of pH 9.0, buffer solution of pH 12.0, distilled water and 0.1N NaOH. The overlay zero order UV spectra of above solutions were recorded using respective solution as blank. (Figure 1)

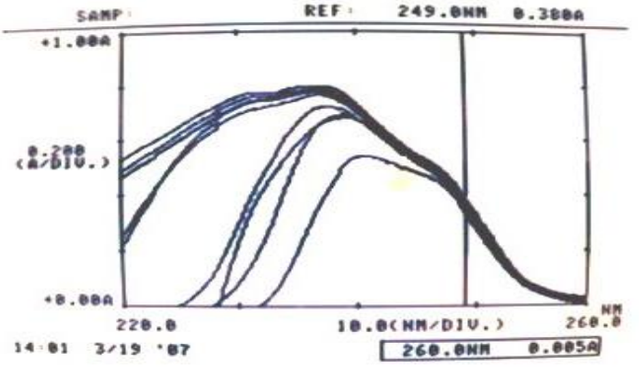

Figure 1: Isosbestic point of pravastatin sodium (15µg/ml) by zero order spectroscopic method.

2.4.2. Using difference spectrophotometric method

The overlay difference spectra of equimolar (20 µg/mL) solutions of Pravastatin sodium (in pH 3.0

buffer solution and in pH 11.0 buffer solution) were recorded. (Figure 2)

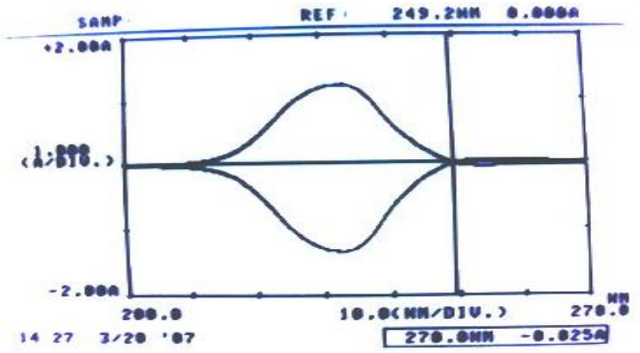

Figure 2: Isosbestic point of pravastatin sodium (15µg/ml) using difference spectrophotometric method.

2.5. Calibration curve preparation

Standard solutions of Pravastatin sodium in the range of 2.5-30 µg/mL were prepared by diluting working standard solution with 0.1 N HCl, buffer solutions of pH 1.2 -12.0 and 0.1 N NaOH. Absorbance of each solution was measured at 249 nm using respective buffer solution as blank. The plot of absorbance at 249 nm versus respective concentration was plotted. (Figure 3)

2.6. Validation of proposed method

The proposed method was validated in terms of linearity, range, accuracy, precision, limit of detection and limit of quantification.

2.6.1. Linearity and range

Experiments were carried out to find out the range of concentration in which Beer's law is obeyed. The linearity of calibration curve for standard drug was determined in terms of correlation co-efficient following the procedure described under calibration curve preparation (n=6).

2.6.2. Accuracy

To a fixed amount of pre-analyzed sample of PVS increasing amount of PVS standard solution was added in all the levels of calibration curve (table 1). The absorbance of each solution was measured at 249 nm using respective solution as blank. The amount of Pravastatin Sodium was calculated at each level and % recovery was calculated.

2.6.3. Precision

Precision of method was determined by performing repeatability, intraday variation and interday variation studies. Precision of repeatability was studied by measuring the absorbance of same solution of PVS seven times at selected wavelength and relative standard deviation (RSD) was calculated. Intraday precision was determined by analysing working standard solution of PVS over entire calibration range five times on the same day and interday precision was determined by performing same experiment daily for five days. Results were reported in terms of RSD.

2.6.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The values of LOD and LOQ were calculated using equations, LOD = 3.3 × (α/b) and LOQ = 10 × (α/b), where α = Standard deviation of the Y- intercepts of the 5 calibration curves and b = mean slope of the 5 calibration curves.

2.7. Analysis of pharmaceutical formulations

Twenty tablets were weighed accurately and finely powdered. The quantity of the powder equivalent to 10 mg pravastatin sodium was accurately weighed and transferred into 10 ml volumetric flask, methanol (5 mL) was added and sonicated for 10 minutes to dissolve the drug. The solution was filtered through Whatman filter paper No. 42, washed with methanol and volume was adjusted up to mark with methanol.

Aliquots of the filtrate (0.5, 1.5 and 2.5 ml) were transferred into series of 100 ml volumetric flasks and diluted up to mark with distilled water. The absorbance of above solutions was measured at 249 nm using distilled water as blank. The content of pravastatin sodium was calculated using the equation obtained in the calibration curve of pravastatin sodium.

Table 1: Recovery study of pravastatin sodium from sample solution

Volume of preanalysed sample solution of PVS (50 µg/mL)	Volume of standard PVS solution (50 µg/mL) spiked	Diluted up to	Concentration of PVS in final solution (µg/mL)	Concentration of PVS found (µg/mL)	Recovery (%)
2.0 mL	0.0 mL	10 mL	10	10.02	---
2.0 mL	1.6 mL	10 mL	18	18.04	100.23
2.0 mL	2.0 mL	10 mL	20	20.42	102.11
2.0 mL	2.4 mL	10 mL	22	22.22	101.00

Table 2: Summary of validation parameters for pravastatin sodium

Parameters	Results
Linearity Range	2.5-30 µg/ml
Correlation Co-efficient	0.9996
Precision (RSD)	
Repeatability(n=7)	0.1659
Intra day precision(n=5)	0.43 - 2.11
Inter day precision(n=5)	0.28 - 2.32
% Recovery	100.23 - 102.11
Limit of Detection (LOD)	0.25 µg/ml
Limit of Quantification (LOQ)	0.76 µg/ml

3. RESULTS AND DISCUSSION

Overlay zero order UV spectra (Figure 1) and overlay difference spectra (Figure 2) clearly indicated that, at 249 nm wavelength, absorbance values at different pH conditions were found to be identical. Therefore 249 nm was considered as isosbestic point and selected for measurement of absorbance. Good linearity was observed at different pH conditions at this wavelength. The method was validated according to ICH Q2B guidelines in order to determine linearity, range, accuracy, precision, limit of detection and limit of quantification. Calibration curve data were found to be linear in concentration range of 2.5 to 30 µg/mL with correlation coefficient of 0.9996 (Figure 3). The recovery of PVS was found to be in range of 100.23-102.11 % as presented in Table 1. Results of validation parameters of developed method are summarized in Table 2. Assay results obtained for pravastatin sodium tablets are in good agreement with the labeled amount of pravastatin sodium (100.6256 ± 0.630, n=3), which are shown in Table 3.

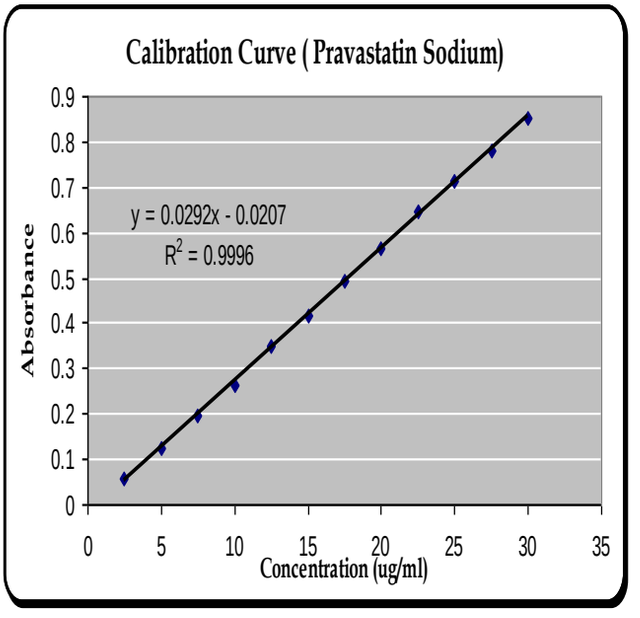

Figure 3: Calibration curve for Pravastatin Sodium at 249 nm

Table 3: Assay results of Pravastatin Sodium in market formulations

Formulations	Pravastatin Sodium labelled value (mg)	% Pravastatin Sodium found Mean ± SD (n=3)
PRAVASTIN 20	20	100.62 ± 0.63

4. CONCLUSIONS

The proposed spectrophotometric method is simple, sensitive and suitable for determination of PVS in pharmaceutical dosage forms. The proposed method offers the advantage of accuracy, time saving as well as pH independency. This study suggests that this spectrophotometric method can be employed for routine analysis of PVS in dosage forms and aqueous medium without interference of pH.

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