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Simple UV Spectrophotometric Method for the Determination of Fluvastatin Sodium in Bulk and Pharmaceutical Formulations

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Abstract: A simple and cost effective spectrophotometric method is described for the determination of fluvastatin sodium in pure form and in pharmaceutical formulations. When the drug reacts with sodium hydroxide shows absorption maximum at 304 nm and obeys beer's law in the concentration range 5-25 μg mL^{-1}. The absorbance was found to increase linearly with increasing concentration of FVS, which is corroborated by the calculated correlation coefficient value of 0.9999 (n=5). The apparent molar absorptivity and sandell sensitivity were 1.1905×10^4 and 0.0368844 μg cm^{-2} cm respectively. The slope and intercept of the equation of the regression line are 0.027112 and 0.003539 respectively. The limit of detection and limit of quantification was found to be 0.0811 μg mL^{-1} & 0.2460 μg mL^{-1}. The validity of the described procedure was assessed. Statistical analysis of the result has been carried out revealing high accuracy and good precision. The proposed method was successfully applied to the determination of FVS in pharmaceutical formulations without any interference from common excipients. The relative standard deviations were ≤ 0.937%, with recoveries of 98.60% -101.70%.

Keywords: Fluvastatin sodium (FVS), UV spectrophotometry, Validation, Pharmaceuticals.

Introduction

Fluvastatin sodium (FVS)[1] is [*R**, *S**-(*E*)]-(±)-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1*H*-indol-2-yl]-3, 5-dihydroxy-6-heptenoic acid, monosodium salt. Figure 1 shows the structure of fluvastatin sodium. Literature survey reveals that, few chromatographic[2-4], capillary electrophoresis (CE)[5] and electrochemical methods such as differential pulse voltammetry (DPV)[6] methods have been reported for the estimation fluvastatin sodium. To the best of our

knowledge, there is no UV method for the analysis of FVS in pharmaceutical formulations has been reported in literature The aim of this study is to develop a fast, simple, reliable, selective, sensitive and inexpensive UV spectrophotometric method for the determination of FVS in bulk drug and commercial pharmaceutical formulations as tablet.

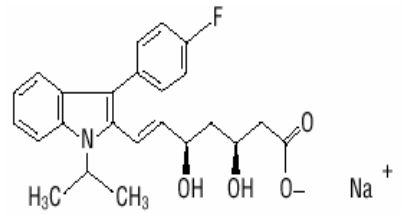

Figure 1. Chemical structure of FVS.

Fluvastatin sodium is a new drug used in lipid lowering agent, which has not been launched in local market. Fluvastatin sodium is an official drug in USP[7] 2007 available in international market (England, Qatar, Switzerland). There were no UV methods reported for fluvastatin sodium. The proposed method was developed and validated according to the evaluation of the validation parameters[8-9]. The developed method was applied to the determination of FVS in pharmaceutical formulations.

Experimental

The spectrophotometric measurements were carried out using a shimadzu model 1700 UV-VIS spectrophotometer with a diode array detector (DAD) (190-1100 nm). UV spectra of standard and sample solutions were recorded in 1cm quartz cells at the wavelength ranges of 190-350 nm. The statistical analysis was performed with SPSS software (Version 10.7).

Chemicals and reagents

FVS was kindly provided from Orchid chemicals and Pharmaceuticals Ltd (Chennai, India) and it was used without further purification. Melting point, UV and IR spectra of FVS were evaluated to check purity and no impurities were found. Lescol tablets (80 mg FVS per tablet) were procured from Granada Medical, Doha, Qatar. QualigensIndia® water was used for the preparation of solutions. All solvents and other chemicals were analytical reagent grade. Acetonitrile was purchased from Merck.

Standard solutions

Standard stock solutions of FVS (250 μg mL^{-1}) was prepared in sodium hydroxide and kept in the dark and at +4 °C maximum for 2 months. Working standard solutions were daily prepared by diluting stock solutions at the concentrations of 5-25 μg mL^{-1} in sodium hydroxide. Then the absorbance of these solutions was measured. Sodium hydroxide was used as a blank solution.

Procedure for formulations

Twenty tablets of lescol were accurately weighed and finely powdered and mixed. A portion of the powder equivalent to the average weight of one tablet was transferred into a 100 mL volumetric flask and 30 mL of sodium hydroxide was added. The content of the flask was sonicated for 15 min and diluted to volume with sodium hydroxide. This solution was centrifuged for 15 min at 5000 rpm to separate out the insoluble excipients. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant and diluting them with sodium hydroxide to give final concentration (15 μg mL^{-1}). Then the absorbance of these solutions was measured. The amount of FVS per tablet was calculated using the calibration curve.

Results and Discussion

FVS is very poorly soluble in acidic media. So, basic solution was used to prevent its possible precipitation. Sodium hydroxide was investigated and the UV spectrum of FVS was measured. In sodium hydroxide solutions, well defined peak was obtained in sodium hydroxide solution shows maximum absorbance at wavelength of 304 nm (Figure 2). At the end of these studies, sodium hydroxide was chosen for the working solution. The spectrum shows a well defined peak at 304 nm in the measuring wavelength range 190-350 nm. This wavelength was used for the determination of FVS.

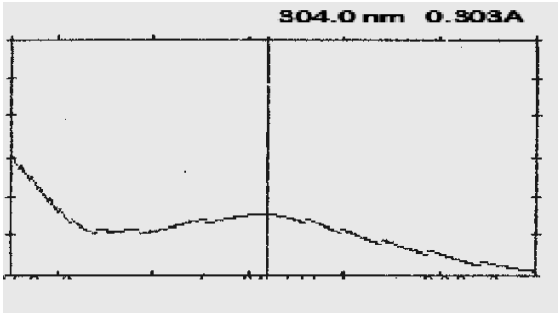

Figure 2. UV spectrum of FVS in 0.1 M sodium hydroxide.

Validation

Validation is one of the most important steps in method development for analytical determinations. The main validation parameters[8-9] such as stability, linearity, sensitivity, precision, accuracy, recovery, specificity, robustness and ruggedness were evaluated in developed method[8].

Stability

The standard stock solutions of FVS were stored +4 ^{0}C for 2 months. During this period, the solutions were analyzed with UV spectrophotometric method, the spectrum was compared with the spectrum of daily prepared standard solution and no difference was obtained between them as peak shape and maximum absorbance of FVS. Therefore, FVS is highly stable in the mentioned conditions.

Linearity range

Under the experimental conditions, the calibration graphs of the absorbance *versus* concentration were found to be linear over the range of 5-25 µg mL for proposed method. The calibration graphs Figure 3 were constructed after analysis of 5 different concentrations with each concentration was measured six times. Each point of the calibration graph corresponded to the mean value obtained from 5 independent measurements. The regression equations (with standard error of intercept and slope) and correlation coefficients of the mean of 5 consecutive calibration curves are given in Table 1. The regression equation was y = 0.027112x+0.003539 where y is the absorbance and x is the concentration in µg mL^{-1} (r = 0.9999)

Sensitivity

The limit of quantification (LOQ) is the lowest concentration of FVS on the calibration curve that can be quantified with acceptable precision and accuracy[8,9]. The LOQ was found as 0.246 µg mL^{-1} (RSD = 1.51%) (n=5) for proposed method.

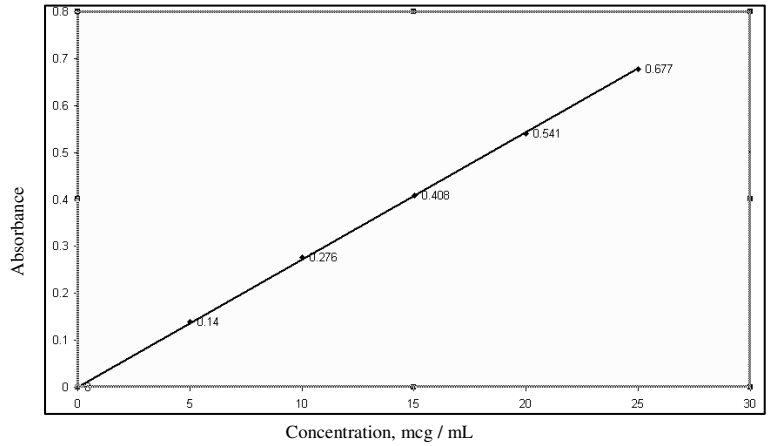

Figure 3. Calibration curve of FVS in 0.1 M sodium hydroxide.

Table 1. Analytical characteristics of FVS by proposed UV spectrophotometric method (n=5).

Parameters	UV Spectrophotometric method
λ_{max}, nm	304
Beers law limit, µg/mL	5-25
Molar absorptivity, L mol^{-1} cm^{-1}	1.1905 X 10^4
Regression equation (y)a	y = 0.027112x +0.003539
Linearity range, µg mL	5-25
Sandell's sensitivity, µg/cm^2/0.001A.U	0.0368844
Slope	0.027112
Intercept	0.003539
Correlation coefficient (r)	0.9999
Limit of Detection, LOD, µg/mL	0.0811
Limit of quantification, LOQ, µg/mL	0.2460
Number of data points	5

y = bx + a where x is the concentration in µg mL, y is amplitude for UV spectrophotometry

Precision

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Three different concentrations of FVS in the linear range (3, 6 and 9 µg mL^{-1}) were analyzed in 6 independent series in the same day (intra-day precision) and 3 consecutive days (inter-day precision) from three measurements of every sample in each series. The precision of the analysis was determined by calculating the relative standard deviation (RSD %). The RSD values of intra-day and inter-day studies varied from 0.96 to 1.90% showed that the intermediate precision of the method was satisfactory (Table 2).

Accuracy and recovery

The accuracy of the method was determined by calculating the percentage relative error (Bias %) between the measured mean concentrations and added concentrations at the same concentration of FVS. Table 2 shows the results obtained for intra and inter-day accuracy. The results obtained for intra and inter-day accuracy were between 0.96-1.80%. Observed concentration values are in good agreement with the expected ones. Recovery[10] studies for the

accuracy of the method were performed by spiking synthetic mixture with known amount of FVS tablet. The amount of 15 mg of FVS was found to be 14.99±0.08 with RSD of 1.10%. The mean percentage recoveries were found as 99.94±0.42 with RSD of 1.10% Table 3.

Table 2. Precision and accuracy data of the developed spectrophotometric method for the analysis of FVS (n=5).

Inter-day				Intra-day		
Added, Mg mL^{1}	Found[a], µg mL^{-1}	Precision RSD %	Accuracy[b] Bias	Found[a], µg mL^{-1}	Precision RSD %	Accuracy[b] Bias
3.0	3.05±0.02	1.80	1.66	3.02±0.02	1.54	0.86
6.0	6.02±0.02	1.69	0.33	5.95±0.06	1.21	-0.83
9.0	8.98±0.04	1.14	-0.22	8.92±0.04	0.96	-0.88

Found[a] $\overline{x}$ *= mean ± standard error, RSD % = Relative standard deviation, Accuracy[b] = [(Found - Added) / Added] x 100*

Table 3. The results of percentage recovery value in synthetic mixture of FVS for proposed method (Added FVS for Tablet; 15 mg) (n=7).

Found, 15 mg	Recovery, %
14.72	98.60
15.18	100.40
14.96	99.80
14.84	99.20
15.34	101.70
14.82	99.10
15.06	100.30

$\overline{x}$ *: 14.99±0.08, 99.87%, SD: 0.22, RSD %: 1.10,* $\overline{x}$ *: Mean ± standard error, SD: Standard deviation. RSD %: Relative standard deviation*

Specificity

The spectra obtained from tablet and synthetic tablet solution was identical with that obtained spectrum from standard solution containing an equivalent concentration of FVS. Tablet solutions showed that the wavelength of maximum absorbance of FVS did not change. It was concluded that the excipients did not interfere with quantification of FVS in this method and the proposed method could be considered specific. These data showed that there was no spectral interaction in the analysis of FVS in pharmaceutical formulations by the proposed method. Therefore, the calibration curve method, which is easier and quicker than the standard addition method, was used in quantitative analysis of FVS. These values showed that no significant excipients interference, thus the procedures was able to determination of FVS in the presence of excipients. In the proposed method, there was no need for pre-separation and only centrifugation was applied to make the solution clear.

Robustness

The robustness of the proposed method was examined by evaluating the influence of small variations of some of the most important procedure variables such wavelength (302 nm and 306 nm). Each deliberate small change was analyzed 7 independent series containing 15 µg mL^{-1} only one parameter was changed in the experiments at a time. The statistically comparison was done with Friedman analysis and no difference was found between results

($p = 0.062 > p = 0.05$) (Table 4). The results obtained from the various conditions were not different compared to the optimum conditions and none of these variables significantly affected the assay of FVS and the proposed method could be considered robust.

Table 4. The robustness data of developed method (n=7).

Solution	Found, μg mL^{-1}	RSD %
Standard, 10.00 μg mL^{-1}	10.25±0.05	1.41
Sodium hydroxide, 0.1 M	10.25±0.07	1.71
Wavelength, 302 nm	10.32±0.06	1.42
Wavelength, 306 nm	9.96±0.05	1.42
Friedman analysis: $p = 0.062 > p = 0.05$		

$\overline{x}$ *: Mean ± standard error.* $\overline{x}$ *RSD %: Relative standard deviation*

Ruggedness

The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 15 μg mL^{-1} of FVS using the same instrument by two different analysts under the same optimized conditions at different days. The obtained results were found to reproducible, since there was no significant difference between analysts ($p = 0.075 > p = 0.05$) (Table 5). Thus, the proposed methods could be considered rugged.

Table 5. The ruggedness of proposed method (Added of FVS amount of 10.00 μg mL^{-1}) (n=7).

1. Analyst Found, μg mL^{-1}	2. Analyst Found, μg mL^{-1}
$\overline{x}$: 9.96±0.02	$\overline{x}$: 10.06±0.05
SD: 0.04	SD: 0.12
RSD %: 0.40	RSD %: 1.19

Wilcoxon Paired Test: p=0.075>p=0.05, $\overline{x}$ *: Mean±standard error, RSD %: Relative standard deviation.*

Analysis of pharmaceutical formulations

The optimized spectrophotometric method was applied to the direct determination of FVS in tablet using calibration curve method without any sample extraction or filtration. The average amount present was determined by taking average of six replicate analysis and the amount present were found to be 79.918±0.570 mg/tab. The percentage RSD was found to be 0.937 for lescol respectively. The results show that the proposed method was successfully applied for the assay of FVS in its pharmaceutical formulations (Table 6).

Table 6. Assay of pharmaceutical formulations containing FVS analyzed by proposed method (n=6).

Solution	Amount found	% found	% RSD
Fluvastatin sodium 80 mg	79.3862	99.23	0.937
	80.4805	100.06	
	78.8013	98.50	
	80.5330	100.66	
	79.6703	99.58	
	80.6402	100.80	
	79.918	99.89	

Conclusion

In this study a simple, fast and reliable UV spectrophotometric method was developed and validated for the determination of FVS in pharmaceutical formulations. This method was applied directly to the analysis of pharmaceutical dosage forms without the need for separation such as extraction steps prior to the drug analysis. As this proposed method has the lowest LOD value and wider linear range is more sensitive method. From the results obtained, we concluded that the suggested method showed high sensitivity, accuracy, reproducibility and specificity. Moreover, this method is simple and inexpensive and it can be employed for the routine quality control of FVS in pharmaceutical formulations

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