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Novel Analytical Method Development and Validation for Estimation of Clinical Important Simvastatin in Bulk and Pharmaceutical Dosage Form by UV Spectrometric Method Using Phosphate Buffer Solubility

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ABSTRACT: According to World Health Organization statistics, more than 16 million people die of cardiovascular disease each year, and 7.2 million deaths in 2001 were caused by heart disease. By the year 2020, approximately 25 million deaths annually worldwide are expected from cardiovascular disease, and almost half of those deaths (11.1 million) will be from coronary heart disease. Simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, is administered in the form of lactone prodrug. Simvastatin lowers plasma cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase and found most effective for the treatment of cardiovascular disease. The present study was undertaken to develop and validate a simple, accurate, precise, reproducible and cost effective UV-Visible spectrophotometric method for the estimation of simvastatin in bulk and pharmaceutical formulation. The solvent used throughout the experiment was methanol and water. Absorption maximum of the drug was found to be 238nm in phosphate buffer pH 7.4. Beer's law was obeyed in the range of 02-200µg/ml. The method was shown linear in the mentioned concentrations having line equation y = 0.021x + 0.063 with correlation coefficient R2 of 0.977. The recovery values for simvastatin ranged from 99.95%-100.21%.The percent relative standard deviation (%RSD) of interday and intraday precision range was found below 2%. The percent relative standard deviation of robustness and ruggedness of the method was found within the prescribed limit as per recommended guideline of ICH. Hence, proposed method was precise, accurate and cost effective. This method could be applicable for quantitative determination of the bulk drug as well as dosage formulation.

Keywords: UV Spectroscopy; Method Development; Validation; Simvastatin and Regulatory Requirements for Drug Development.

INTRODUCTION: Hyperlipidemia is commonly diagnosed each year. There is a strong correlation between hypercholesterolemia and coronary heart disease.[1-4] Statin is a group of drugs that used primarily in lowering blood cholesterol. Statins were discovered in 1976 when Endo et al. found that a product of mould Penicillium citricum was able to inhibit the activity of one of the enzymes in the cascade of cholesterol synthesis, 3-hydroxy-3 methylglutaryl coenzyme A reductase (HMG CoA reductase). This substance was used for further name statins (vastatins). Two decades of research after discovery were sufficient for the conclusion that statins were considered a very important group of drugs (professor Roberts stated that statins were for atherosclerosis the same as was penicillin for infectious diseases).[6-9] Statins have become one of the basic pillars in the secondary and primary prevention of atherosclerosis. Their role is documented in preventing the progression of atherosclerosis, and they are even able to cause regression of the disease. The decrease of cardiovascular endpoints, but also total mortality found in clinical studies could not be explained only by the decrease of atherogenic lipids.[10-16] It was greater than benefit from lipid lowering. Speculation about so-called pleiotropic effects (non-lipid-modifiable) appeared and was confirmed by further research activity. Statin is generally capable of lowering cholesterol by 20 to 60 percent. The discovery of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme-A which is act as inhibitors called statin that was a breakthrough in the prevention of hypercholesterolemia and related diseases such as cardio-

vascular diseases related to high levels of cholesterol are among the main causes of death in our societies, there is a high incentive for developing processes for the production of statins, an FDA approved drug.[17-18]

All natural statins have a common molecular structure, a hexahydro-naphthalene system and a -hydroxy-lactone, but they differ from each other due to side chains and a methyl group around the ring. Statins also are fungal secondary metabolites and were the first enzyme in cholesterol biosynthesis. Statins are available either in Tablet or capsule form; statins are usually taken with dinner or bedtime.[19] The results are typically evident after a period of four to six weeks of use. Medications in this group are usually easy to tolerate and cause few side effects. The mechanism that involved in controlling the production of plasma cholesterol 14 levels is the reversible inhibition of HMG-CoA reductase by the statins that are related to the structural. The statins differ with respect to their ring structure and substituents. These differences in structure affect the pharmacological properties of the statins.[20-21] Sometimes, statins have been grouped into two groups of statins according to their structure. Statins that belong to type-1 are pravastatin and simvastatin. Statins that are fully synthetic and have larger groups linked to the HMG-like moiety is often referred to as type 2 statins. Statins that belong to this group are atorvastatin and rosuvastatin. Simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, is administered in the form of lactone prodrug. Simvastatin lowers plasma cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-CoAreductase.[22-23]

The HMG-CoA reductase inhibitors, or statins, have a beneficial effect on the primary and secondary prevention of cardiovascular morbidity and mortality, primarily by lowering the concentration of circulating LDL. Statins exert their effect by inhibition of HMG-CoA reductase (HMGCR), the rate-limiting enzyme in the cholesterol biosynthesis pathway. Therefore, the HMGCR gene is a good candidate for studies on genetic variation influencing the cholesterol-lowering effect of statin therapy. Studies have shown that polymorphisms in the HMGCR gene are associated with a lower reduction in levels of total and LDL-cholesterol, within different populations and settings. Another important candidate gene is the LDL receptor (LDLR) gene, since statins increase LDLR expression. Studies on genetic variation in this gene showed a decreased response to statin therapy. Furthermore, cytochrome P450 3A4 (CYP3A4) metabolizes simvastatin, atorvastatin, and lovastatin, and in a recent study, the CYP3A4 intron 6 C>T SNP was associated with an increased total and LDL-cholesterol lowering response to simvastatin therapy. Nevertheless, there is a considerable variability between individuals in response to statins, in terms of both cholesterol lowering and clinical outcomes, of which the origins are poorly understood. Simvastatin belongs to another class of cholesterol-lowering agents, the statins. Statins work by inhibiting the first step involved in cholesterol synthesis.[24-25] They competitively block HMG-CoA reductase and lower the amount of cholesterol that is synthesized by the body. This is the rate-limiting enzyme in the liver necessary for cholesterol production.[26-28]

Pharmaceutical analyses is one of the most challenging field of analytical chemistry.[29-34] Pharmaceutical analysts carry out the qualitative and quantitative control of APIs and drug products and also develop and validate appropriate methods. These methods are routinely used by manufacturing companies in-process testing and by authorities for the quality control of drug products. In the vast majority of pharmaceutical analyses, instrumental analytical methods are applied. It is the subject of science, which deals with the interaction of radiation and matter.[35-46] All atoms and molecules are capable of absorbing energy in accordance with certain restrictions, these limitations depending upon the structure of the substance.[47-49] Spectroscopic analytical methods are based on measuring the amount of radiation produced and absorbed by molecular or atomic species. The kind and amount of radiation absorbed depends upon the number of molecules interacting with the radiation. The study of these dependencies is called absorption spectroscopy.[50-54] Absorption spectroscopy is one of the most valuable analytical techniques; its advantages include simplicity, speed, specificity and sensitivity. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formula for the calculation, etc.[55-59] A suitable and validated method has to be available for the analysis of drug in the bulk, in drug

delivery systems, from release dissolution studies and in biological samples. If a suitable method, for specific need, is not available then it becomes essential to develop a simple, sensitive, accurate, precise, reproducible method for the estimation of drug samples. The efficient analytical method development and its validation are critical elements in the development of pharmaceuticals. An analytical method is selected on the basis of criteria such as accuracy, precision, sensitivity, selectivity, robustness, ruggedness, and the amount of available sample, the amount of analyte in the sample, time, cost, and availability of equipment.[60-72] Thus, present study was undertaken to develop and validate a simple sensitive, accurate, precise and reproducible UV method for simvastatin.

MATERIAL AND METHODS:

Instruments: The analysis was performed by using the analytical balance (Rodwag Wagi Electronics Switzerland model AS22201xs), pH meter (Cyber scan), UV spectrophotometer (UV-1800 Shimadzu,Japan. equipped with variable wavelength detector and data integration software).

Reagents and solutions: Simvastatin IP grade and reagents for phosphate buffer were used as analytical grade.

Preparation of solvent system:

Phosphate buffer of pH 7.4: 50ml of 0.2M potassium dihydrogen phosphate was placed in 200 ml volumetric flask, 39.1 ml of 0.2M sodium hydroxide was added, water was added to volume and pH adjusted to 7.4 accordingly.

Standard stock solution of simvastatin: 100 mg of simvastatin weighted accurately and transferred into a 100 ml volumetric flask containing phosphate buffer. Solution sonicated to dissolve simvastatin and cooled at room temperature and mix well. Pipette out 10ml solution and transferred into 100 ml volumetric flask resulting solution contained 100µg/ml solution (Stock solution A). Pipette out 10, 20, 30, 40, 50ml solution respectively of standard stock solution A and transferred into a 100 ml volumetric flask, mixed well and diluted up to volume with buffer solution. The resulting solution contains 10 to 50µg/ml of simvastatin.

Spectral study: The final stock solution scanned in UV spectrophotometer over the range 200-400nm (Figure 1).

Validation of the Method:

Linearity and Range: A series of dilutions were prepared in the concentration range of 10-50mg/ml. Separate calibration curve was plotted between concentration Vs response and slope, intercept and correlation coefficient value (r^2) was determined.

Accuracy: To test accuracy, recovery studies were performed. To a preanalyzed sample solution, a definite quantity of known concentration of standard drug solution was added and then its recovery was studied. Different concentration of pure drug was added to preanalyzed sample, and then the solution was analyzed to determined recovery study.

Precision: A standard stock solution of drug was prepared in same manner. The tests were repeated thrice for all concentration. The intermediate precision was performed by doing repeatability, day-to-day variation, and analyst-to-analyst variation.

RESULTS AND DISCUSSION: The methods discuss in the present work provide a convenient, precise and accurate way for simvastatin pharmaceutical dosage form. An absorption maximum of simvastatin was selected at 238nm for the analysis. Regression analysis shows linearity over the concentration range of 02-200µg/ml for correlation coefficients of 0.977 (Figure 2). The % RSD for repeatability (n=6) precision was found to be less than 2% indicating the precision of method. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as percentage recovery. Percentage recovery for simvastatin was found within the range of 99.15 % and 101.21%. The assay for simvastatin was found to be 101.00 ± 0.22. The % RSD value for simvastatin was found to be less than 2%. In this study the simvastatin was carried out by UV Spectroscopy method satisfactorily. The result of developed method and validation was given in table 1.

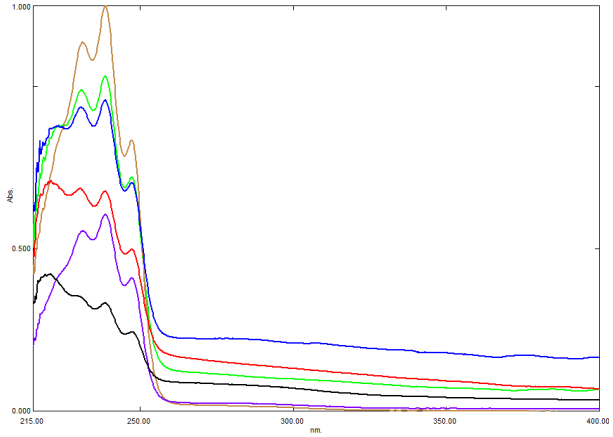

Figure 1: UV Spectra of Simvastatin.

Table 1: Result of method development and validation.

Method	Parameter	Observation	SD*	RSD*
Linearity	Slope and Y-Intercept	0.021x + 0.063	-	-
	R^2	0.977		
Accuracy	% Recovered	99.15 to 101.21	0.3628	0.891
Precision	Repeatability	Method was found repeatable (Validated three times as per ICH guideline)	0.4872	0.597
	Analyst to analyst	Method was found precise when performed by different analyst (Validated three times as per ICH guideline)	0.7580	0.766
	Day to day	Method was found precise when performed three consecutive days (Validated three times as per ICH guideline)	0.1894	0.957
Ruggedness & Robustness		Compiled (Validated three times as per ICH guideline)	0.2254	1.735

**Each value is mean of three replicates*

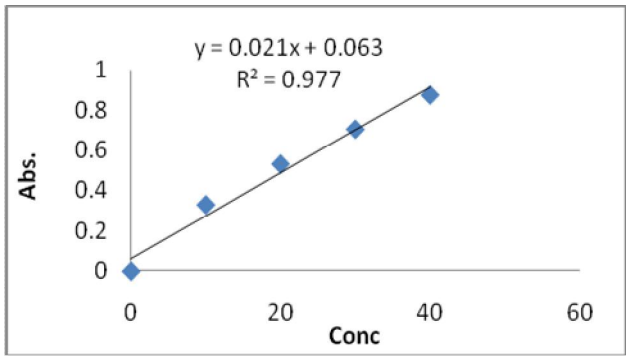

Figure 2: Calibration curve of Simvastatin.

CONCLUSION: The analytical method for determination of simvastatin has been validated according to validation protocol of ICH guidelines. All parameters mentioned in the protocol were tested and they fulfilled the requirement of ICH analytical method validation for the drug. The results obtained are well within the set limit; indicates that the described analytical method is suitable for determination of simvastatin in bulk as well as tablet formulation.

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