A Validated Method Development for Quantification of Pravastatin Sodium using Diffuse Reflectance Fourier Transform Spectroscopy

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ABSTRACT

Objective: The plan of the proposed work is development and validation of a new, simple and cost- effective method using Diffuse Reflectance Fourier Transform Infrared Spectroscopy as a method of choice for analysis of Pravastatin in the solid dosage forms. **Method:** Spectrum using Fourier transform infrared (DRS 8000) was analyzed after preparing solid state sample through diluting in dry potassium bromide. The method was validated according to the International conference on Harmonization guidelines including linearity, accuracy, precision, robustness and selectivity. **Results:** A linear relationship was found in the selected wave number 1740 -1705 cm-1denoting the hydroxyl peak in the concentration range of 5-30% w/w admitting a good correlation coefficient of 0.9978. The three marketed tablet dosage form selected for the determination of % recovery of Pravastatin was in the range of 97.38-98.14% w/w. The % relative standard deviation for day 1 precision studies of five samples was found in the acceptable range of 0.68–1.83 similarly for day 3 precision studies the range was 0.727–1.379. All the results found for the validation parameters were excellent in the proposed method. **Conclusion:** The present research work depicts that Diffuse Reflectance Infrared Fourier Transforms has a tremendous potential which can be selected as a method of choice for determination of drug content in Pravastatin.

Key words: Pravastatin Sodium, Validation, Diffuse Reflectance Infrared Fourier Transform Spectroscopy, Fourier Transform Infra Red Spectroscopy, International Conference on Harmonization Guidelines, Method Development.

INTRODUCTION

Pravastatin sodium, called chemically as 1-Naphthalene heptanoic acid,1,2,6,7,8,8a-hexahydro-β,δ,6-trihydroxy-2-methyl-8-(2-methyl-1-oxobutoxy)-,monosodium salt Figure 1. Pravastatin sodium that has an inhibitory action for 3 – hydroxyl 3 methyl glutaryl coenzyme A which is also known as HMG-CoA Reductase, is an anti-hypercholesterolemia agent. It is the rate-determining enzyme during the cholesterol synthesis and for the translation of 3-Hydroxy 3-Methyl Glutaryl – Coenzyme A to mevalonate.[1,2] Pravastatin is pigeonholed as one of the most excellent because the hydroxyl group is attached to the decalin ring that raises the hydrophilicity parameter when compared to 3-Hydroxy 3-Methyl Glutaryl – Coenzyme A reductase inhibitors.

Fourier Transform Infrared Spectroscopy is has been since a long time and has been a widely explored technique for the analysis of versatile sample groups in pharmaceutical and chemical industries for identifying compounds, impurities and functional groups in qualitative analysis. Theoretically, it can be stated that traditionally available dispersive instruments do not carry some vital advantages as with interferometer that is a

Multiplex advantage also known as Fellgett advantage, Jacquinot advantage. The Fellgett advantage is gained by taking multiple analog or digital signals instead of direct or successive measurement with lead to improvement of signal to noise ratio. Throughput advantage also designated as Jacquinot advantage varies as wave number and depends on resolution as slit width changes. Fourier Transform Infrared Spectroscopy also has negligible stray light because each source wavelength is modulated by the interferometer.

It is also been revealed in pieces of literatures that some pharmaceuticals have been quantified using Ultra-Violet Visible spectroscopy and Fourier Transform Infra Red spectroscopy moreover by analyzing the transmittance or absorbance of the solid state pharmaceutical in potassium bromide or chloroform.[3–8] As found in many compendia Fourier Transform Infrared Spectroscopy is getting more thought of the analytical scientists in the investigation and utilizing Fourier Transform Infrared Spectroscopy technique. Adding hereby that Diffuse Reflectance Infrared Fourier Transform that was proposed as a comparatively a profound techniques for quantitatively analyzing solid-state samples.

From the literature brought to the researcher's interest that Diffuse Reflectance Infrared Fourier Transform Spectroscopy techniques have been already used for the quantitative estimation of mixtures of sulfamethoxazole polymorphs,[9] simultaneous quantization of ethenzamide, isopropyl antipyrine, caffeine, and allyl isopropyl acetyl urea in tablet dosage forms.[10] Hereby, Diffuse Reflectance Infrared Fourier Transform Spectroscopy can be seen as the perspective to provide a competent method for quantification of solid-state pharmaceuticals.

Copious methods have been reported for development and validation for the estimation of Pravastatin in plasma counting high-performance liquid chromatography with Ultra-Violet detection,[11-13] liquid chromatography/ tandem mass spectrophotometry and hyphenated techniques (LC/MS/MS).[14-20] A Simple Ultra-Violet spectroscopic method, also first order derivative spectroscopic method as well as Area under Curve method in addition to absorption ratio methods are reported for determination of the Pravastatin in different marketed formulations. Several chromatographic methods already exist for the determination of the Pravastatin only or in combination with other drugs and in biological fluids like human plasma and urine. Many Chromatographic methods like High performance liquid chromatography and Reverse phase High-Performance Liquid Chromatography, High-performance thin layer chromatography (High-Performance Thin Layer Chromatography) with Ultra-Violet detection have been reported already.

The novelty of the present work is based on above literature survey that revealed that an analytical method for the Validation and quantification of Pravastatin in bulk and tablet dosage form (solvent free method) using Fourier Transform Infra Red Spectrophotometry is yet not available. Considering these facts and Figures the goal of the present work was to develop and validate a sensitive, specific and reproducible Fourier Transform Infra Red method for quantification of Pravastatin in solid-state and to assess the practicability of Diffuse Reflectance Infrared Fourier Transform Spectroscopy application for pharmaceuticals. The proposed Diffuse Reflectance Infrared Fourier Transform Spectroscopy method in the present study was performed on standard Pravastatin and three marketed tablet dosage forms, namely, (Pravachol, Lipostat, Pravalip; Label Claim 40mg Pravastatin).The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Typical validation characteristics considered in the presented compendia are: [21,22]

Linearity and Range The linearity is ability of the assay to return the values that are directly proportional to the concentration of the target analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Precision Precision is variability of data from the replicate determination of the same homogeneous sample under the normal assay conditions.

Accuracy Accuracy is the acceptance between the values found and accepted true value.

MATERIALS AND METHODS

Chemicals and Reagents. A standard sample of Pravastatin sodium, (B. No. AR No. 0301036909) obtained as a gift sample from Zydus Cadila and Potassium bromide UVSOL (AR) from the Merck, Germany.

Fourier Transform Infra Red Instrumentation. Shimadzu IR Affinity – 1 spectrophotometer having diffuse reflectance sampling interface DRS 8000 (wave number range between 4000 and 650 cm−1has been used for recording Fourier Transform Infra-Red spectra, an average 45 scans for each sample with a small resolution of 4 cm−1 and Potassium bromide as background spectrum. The software used for collection, analyzis and interpretation of data was IR Solutions

Calibration Curve. Five different concentrations of Pravastatin sodium in the concentrations range of 5-30%w/w were used for the preparation of calibra-

tion curve. By using a suitable quantity of potassium bromide, Pravastatin sodium was diluted to get around 1000 mg and triturated to certify proper homogeneity in the sample. Apiece calibration standard was analzsed for six replicates. The Area under curve (AUC) analogous to the hydroxy peak in the region of 1740–1705 cm−1 used in the quantification and an average six measurements was used to obtain a calibration curve. For all the statistical calculations and calibration curve plotting IRSolutions software for Windows 7 were used.

Method Validation. The following parameters were validated for the developed Diffuse Reflectance Infrared Fourier Transform Spectroscopy method.

Precision. Precision study was carried out by analyzing six samples of five different concentrations (5-30% w/w) of Pravastatin sodium six times on the same day (Day 1). Likewise, the intermediate precision of the method was examined by repeating studies on day 3 (Intraday Precision).

Accuracy. The accuracy of the method was performed by standard addition method by recovering the pure drug from the excipient at three different concentrations (80, 100, and 120%w/w). A known amount of Pravastatin sodium standard powder was added to preanalyzed powdered drug Pravacol equivalent to 80, 100, and 120% of label claim. A through mixing was ensured for making an appropriate dilution of 1%w/w with Potassium bromide in a set of six.

Linearity. Linear regression method was used for assessing the linearity in the assay method by analyzing the six samples of five different concentrations (ppm) (5-30% w/w) of Pravastatin in a set of six.

Analysing Marketed Tablet Formulations. For the drug content determination three diverse brands of Pravastatin sodium namely Pravachol, Lipostat, Pravalip; Label Claim 40 mg Pravastatin were selected. Accurately weighed ten tablets was used for determining their average weight and finely powdered. 1% w/w of Pravastatin sodium was achieved by appropriately diluting each of the tablet powder with potassium bromide .Triturating was done to ensure a thorough mixing. The analysis was made using six samples in a set of six.

RESULT AND DISCUSSION

Diffuse reflectance infrared spectroscopy has tremendous potentials in sampling powdered or crystalline materials in the mid-IR and near-IR spectral ranges results in a relatively long path lengths which increase the interaction of infrared light with the sample. Diffuse Reflectance Infrared Fourier Transform Spectroscopy also has an advantage that it can be used in the analysis

of intractable solid samples. Concentrated samples can cause higher noise for which the samples are diluted with nonabsorbing material such as potassium bromide before sampling. Diffuse reflectance has an excellent benefit that it is free from the time-consuming process of pressing pellets for transmission measurement.

The range of 3244.27, 3064.89, 2964.59, 2873.94, 1726.29, 1705.07, 1560.41, 1398.39, 1328.95, 1186.22, 1157.29 and 1039.63 cm−1 showed the Fourier Transform Infra Red spectrum for pure of Pravastatin. Potassium bromide was used as diluents as the low-intensity bands were slight affected. The transmittance band analogous to hydroxyl group was found in the range of 1740–1705 cm−1 for diluted sample of Pravastatin in Potassium bromide within 2.0 transmittance unit. The transmittance spectra of Pravastatin are shown in Figure 2 (overlay) and Figure 3. The calibration curve was prepared by using the area under curve (AUC) centred in the range of 1740-1705 cm−1as shown in Figure 4. Initially, the samples in the concentration range of 5-50% w/w were analyzed to determine the linearity. The calibration curve showed a good linearity range in 5-30%w/w Pravastatin in potassium bromide. The consequent linear regression equation was $y = 38.58x + 114.5$ and the R^2 value for calibration curve was 0.9978 Figure 4 and Table 1. The precision was articulated by a coefficient of variation
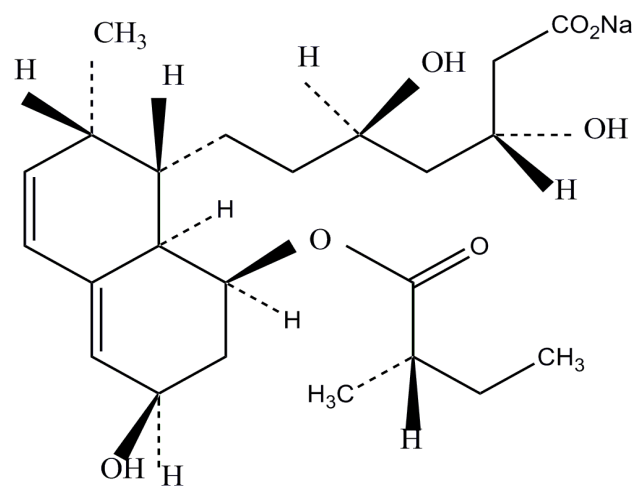

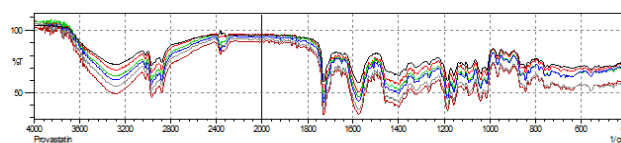**Figure 1: Chemical Structure of Pravastatin Sodium.**

Figure 2: Transmittance spectra of various diluted samples of Pravastatin (Overlay Graph).

(%relative standard deviation). The % relative standard deviation for day 1 precision studies of five samples was found in the acceptable range of 0.68–1.83 similarly for day 3 precision studies the range was 0.727–1.379 Table 2. The proposed validated method was applied for the quantification of Pravastatin in tablet dosage form. The Fourier transform infra-red spectra for the representative samples of tablet dosage forms diluted with potassium bromide which indicates that there is no interference of excipients used in the formulation of tablet dosage form. Three different brands of Pravastatin tablets were analyzed using the developed method and the results of analysis are shown in Table 4. The average recoveries of Pravastatin in all the three formulations were in the range of 97.38–98.14%w/w of label claim and the % relative standard deviation values were in the range of 0.67-1.39. The % RSD of recovery studies was within acceptable limits (%RSD < 2%) Table 3.
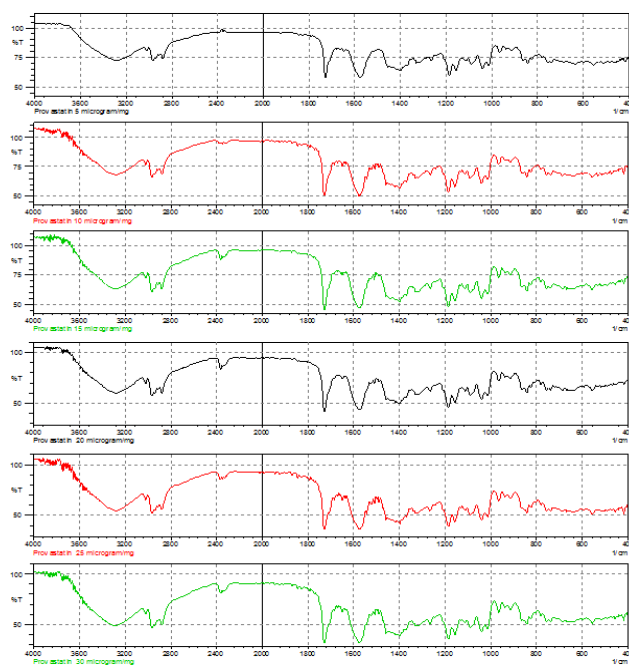

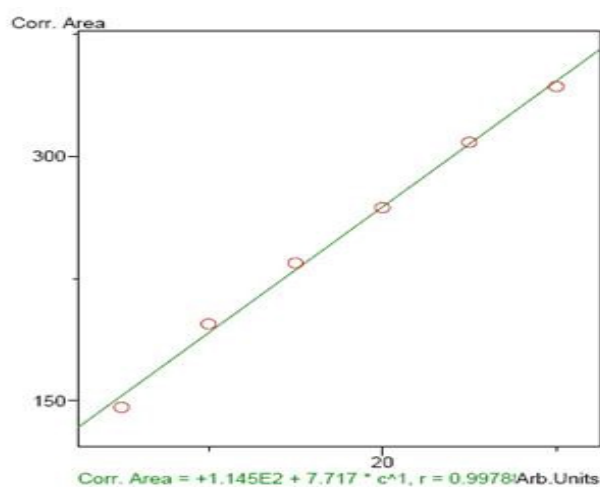Figure 3: Transmittance spectra of various diluted samples of Pravastatin.

Corr. Area = +1.145E2 + 7.717 * c^1, r = 0.9978 Arb.Units

Figure 4: Calibration curve for Pravastatin (5 -30 % w/w).

Table 1: Linear regression data for Pravastatin	
Statistical parameters Values	
Concentration range	05-30% w/w
Regression equation	y = 38.58x +114.5
Correlation coefficient	0.9978

Table 2: Intraday and Interday precision and accuracy for analysis data of Pravastatin (n = 6).						
Nominal concentration (% w/w)	Day 1			Day 3		
	AUC	**SD**	**RSD (%)**	**AUC**	**SD**	**RSD (%)**
5	146.065	2.6809	1.83	149.065	1.7940	1.2175
10	197.206	1.3514	0.68	195.456	1.5256	0.7794
15	234.492	2.0973	0.887	235.482	1.7154	0.7276
20	268.438	2.3588	0.885	269.585	3.0427	1.1388
25	308.467	2.1338	0.696	310.467	2.8010	0.9122
30	342.624	3.8976	1.14	343.525	4.6997	1.3796

Table 3: Recovery data of Pravastatin in tablet (n = 6).							
Tablet brand name	Label claim (mg)	Amount of drug added (mg)	Total	Amount recovered (mg)	% Recovery	SD	RSD (%)
	40	32	72	73.152	101.6	1.179	1.599
Pravachol	40	40	80	78.586	98.23	0.858	1.098
	40	48	88	87.547	99.48	0.903	1.025

Table 4: Assay results of tablets (n = 6).					
Tablet brand names	Label claim (mg)	Amount recovered (mg)	% Recovery	SD	RSD (%)
Pravachol	40	39.256	98.14	0.547	1.39
Lipostat	40	38.954	97.38	0.259	0.67
Pravalip	40	39.145	97.86	0.509	1.29

The accuracy of the Fourier Transform Infra Red method was calculated by %relative standard deviation method and it was observed within the acceptable limits (as stated in USP not less than 90.0%and not more than 110%of stated amount of Pravastatin)

CONCLUSION

The proposed Diffuse Reflectance Infrared Fourier Transform Spectroscopy method for the quantification of solid state pharmaceutical Pravastatin and its application in pharmaceutical sciences is accurate, precise and eco-friendly. Conventionally, Fourier Transform Infrared Spectroscopy was used in the qualitative analysis of pharmaceuticals; but with advent of the sampling techniques, Diffuse Reflectance Infrared Fourier Transform spectroscopy may serve as a valuable technique for qualitative and quantitative analysis of pharmaceuticals in solid-state. In the present paper, we account that the % relative standard deviation for all the validation parameters was establish to be less than two, that revealed the validation of the new method and its assay results obtained by the current method are fairly agreeable with a pro that it is cost effective, solvent-free which needs simple sample preparations. When compared to other presented method the proposed method is simple, inexpensive and not using any polluting agents. Therefore it can be concluded that the developed Diffuse Reflectance Infrared Spectroscopic method can be employed successfully for the routine quality control analysis of Pravastatin as an alternate for Ultra Violet, High-Performance Liquid Chromatography and High-Performance Thin Layer Chromatography methods.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

ETHICAL APPROVAL

This article does not contain any study with human participants or animals performed by any of the authors.
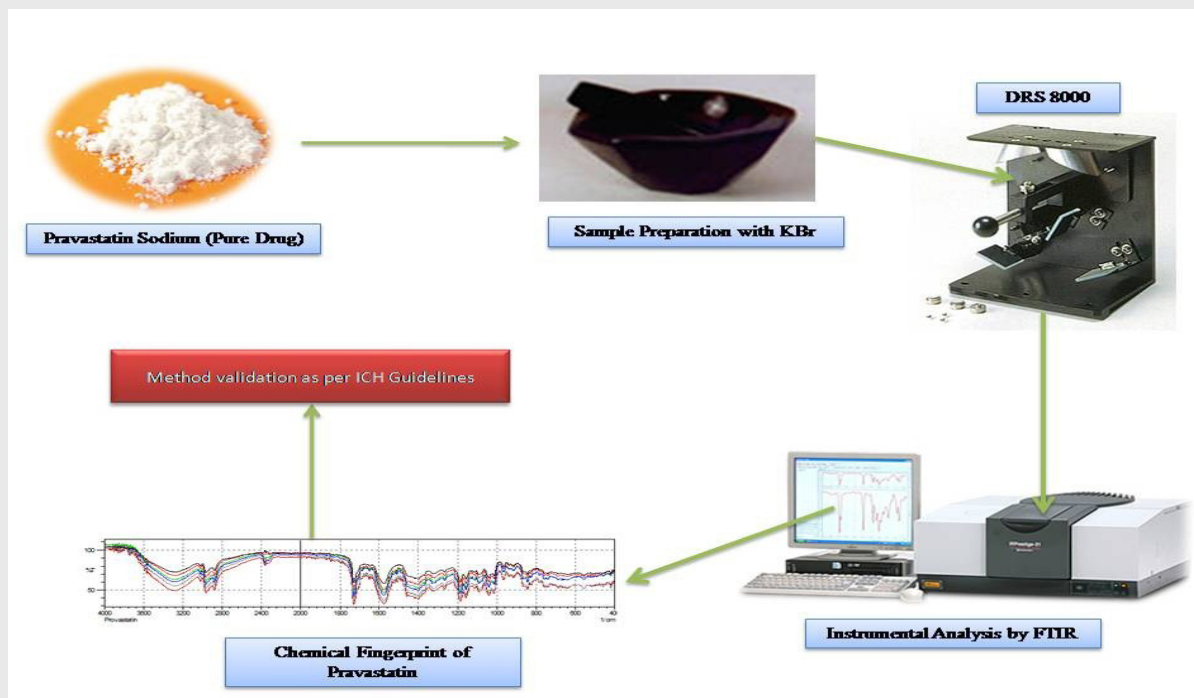
ABBREVIATION USED

FTIR: Fourier Transform Infra red Spectroscopy; **xDRIFTS:** Diffuse Reflectance Infra red Fourier Transform Spectroscopy; **ICH:** International Conference for Harmonization.

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PICTORIAL ABSTRACT

Pravastatin Sodium (Pure Drug)

Sample Preparation with KBr

DRS 8000

Method validation as per ICH Guidelines

Chemical Fingerprint of Pravastatin

Instrumental Analysis by FTIR

SUMMARY

- The present paper accounts a Diffuse Reflectance Infrared Fourier Transform Spectroscopy method for the quantification of solid state pharmaceutical Pravastatin and its application in pharmaceutical sciences is accurate, precise and eco-friendly where that the % relative standard deviation for all the validation parameters was establish to be less than two, that revealed the validation of the new method and its assay results obtained by the current method are fairly agreeable with a pro that it is cost effective, solvent-free which needs simple sample preparations. When compared to other presented method the proposed method is simple, inexpensive and not using any polluting agents. Spectrum using Fourier transform infrared (DRS 8000) was analyzed after preparing solid state sample through diluting in dry potassium bromide. The method was validated according to the International conference on Harmonization guidelines including linearity, accuracy, precision, robustness and selectivity. A linear relationship was found in the selected wave number 1740 -1705 cm − 1denoting the hydroxyl peak in the concentration range of 5-30% w/w admitting a good correlation coefficient of 0.9978. The three marketed tablet dosage form selected for the determination of % recovery of Pravastatin was in the range of 97.38-98.14% w/w. The % relative standard deviation for day 1 precision studies of five samples was found in the acceptable range of 0.68−1.83 similarly for day 3 precision studies the range was 0.727−1.379. All the results found for the validation parameters were excellent in the proposed method. The present research work assures that Diffuse Reflectance Infrared Fourier Transforms budding technology which can be selected as a method of choice for determination of drug content in Pravastatin.

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