STUDY OF QUANTIFICATION OF IMPURITIES AND RELATED SUBSTANCES IN A STATIN USING HPLC TECHNIQUE - METHOD DEVELOPMENT AND VALIDATION FOR THE ANALYSIS

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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. So the present work is to develop and validate a new High Performance Liquid Chromatographic method (HPLC) for such an analysis.

Important study- Bulk drug during its production process, after its scale up, it is necessary to analyse for the presence of any impurities or related substances in it. This is to ensure the impurities and related substances are within their limits as per ICH Guidelines.

The impurities and related substances are defined as; Impurity: Any component present in the drug substance or drug product which is not the desired product, a product-related substance, or excipient including buffer components. It may be either process-related or product-related[1].

Related substances: Those impurities derived from the drug substance. Related substances include degradation products, synthetic impurities of drug substance, and manufacturing process impurities from the drug product[2].

ICH Guideline Q3 A gives the impurities present in a drug substance, ICH Guideline Q3B gives the impurities present in drug product. The Guideline addresses the chemistry and safety aspects of impurities, including the listing of impurities in specifications and defines the threshold for reporting, identification and qualification.

A variety of quantitative techniques are used in the identification and quantification of impurities and related substances, preferably chromatographic methods (HPLC,LC-MS, HPTLC, and GC) are used because it fulfils requirement of various guidelines by effectively separating degraded product from the drug substance. The interest of work emphasizes on HPLC because, in the modern pharmaceutical industry high performance liquid chromatography (HPLC) is the

major and integral analytical tool applied in all stages of drug discovery, development, and production.

HPLC Method development and validation are performed in order to meet the ICH requirements to prepare for regulatory submissions (e.g., NDA). “The object of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose”[3].

Statins or HMG-CoA reductase inhibitors are “a class of drug used to lower cholesterol levels, also called as bad cholesterol by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver”[4]. The drugs coming under statins category includes Pravastatin, Fluvastatin, Atorvastatin, Rosuvastatin, Lovastatin, Simvastatin etc. Increased cholesterol levels have been associated with cardiovascular diseases, and statins are used in the prevention of these diseases, presence of any impurity may lead to severe health hazards and disorders. The present work is to be carried out with a goal of analysing impurities and related substances in statins.

The need of the proposed project is to identify the presence of impurities present in a drug substance and to ensure regulatory complaints as per Harmonized tripartite ICH Guidelines

Chromatographic methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products and compounds in biological fluids. The components monitored include chiral or achiral drug, process impurities, residual solvents, excipients such as preservatives, degradation products, extractable and leachables from container and closure or manufacturing process, pesticide in drug product from plant origin, and metabolites.Methods should be reproducible when used by other analysts, on other equivalent equipment, on other days or locations, and throughout the life of the drug product

Literature survey-patent reading

A literature survey gives an overview of recent trends in development of HPLC methods for determination of impurities in drugs, the review covers the time period from 1995-2001 among the analytical and pharmaceutical chemistry journals, where 450 analytical methods were reported. HPLC with UV detection was found to be choice of many workers; around 200 methods using LC-UV alone were developed[5].

A literature view gives a stability indicating UPLC method development and validation for the simultaneous determination of atorvastatin, fenofibrate and their impurities in tablets. Around six

other known and major unknown impurities were separated. Stability indicating capability was established by forced degradation experiments and separation of known degradation products[6].

According to the literature survey, a simple HPLC method was developed for the analysis of atorvastatin and its impurities in bulk drug and tablets. This method has good resolution for the drug, desfluoro-atorvastatin, diastereomer-atorvastatin and unknown impurities and formulation excipients. A gradient RP-HPLC assay was used with UV detection. Best resolution has been determined using a Luna C18 column with acetonitrile-ammonium acetate buffer pH 4-THF[7].

According to investigators an impurity in Pravastatin was identified by application of Collision-Activated Decomposition Mass Spectra. Mevastatin and 6-epi pravastatin are the main impurities in the fermentation broth as well as in final product. An unknown impurity was detected in analysis of pravastatin sodium sample by reverse phase HPLC-MS. CAD spectrum of the impurity was obtained which was compared with CAD spectra of statins[8].

A survey of literature presents the identification of unknown impurities in simvastatin substance and tablets at a 0.2% level using the liquid chromatography technique with UV (DAD) detector. It is reported that the impurity structures were elucidated by a direct hyphenation of liquid chromatography to high resolution mass spectrometer with electrospray ionisation interface using solutions of formic acid in water and acetonitrile as mobile phase. All three impurities had a modification on lactone ring os statins. Structures here confirmed by LC-UV, LC-MS NMR techniques[9].

And, also the literature survey gives the isolation and structure determination of four oxidative degradation products of atorvastatin. The isolation of oxidative degradation products was carried out on a reversed phase chromatography coupled with a mass spectrometer (LC-MS), high resolution MS(HR-MS), 1D and 2D NMR spectroscopy methods were applied for the structure elucidation. All degradants are due to oxidation of pyrrole ring. Quantitative NMR spectroscopy was employed for the assay determination of isolated oxidative degradation products[10].

According to literature survey, the article has presented the development of HPLC-diode array detection-electrospray ionization tandem mass spectrometry method for analysis and identification of impurities in lovastatin. Lovastatin and impurities were separated on a Diamonsil C18 column, using acetonitrile-water as mobile phase. The elutes were detected with diode array detector and ion trap mass spectrometer in positive mode. The UV spectra of the

impurities are similar with those of lovastatin except for dihydrolovastatin. It indicated that they have the same basic structures[11].

It has been revealed in literature survey regarding the separation of known low level of impurities in lovastatin bulk drug by using high-low chromatography. A column of C-8 (5 µm) Partisil at 30° was used. Gradient elution (1.5 ml/min) was performed with H2O (0.1% H_3PO_4; pH 2.2) - acetonitrile in the ratio 2:3 to 1:4 in 4 min, then to 1:9 (held for 5 min) in 3 min, and detection was at 200 nm, for determination of asterric acid and dihydrolovastatin, and 238 nm for the other five impurities all containing the same diene functional group[12].

The researchers have described about the novel and unique approach used for retention modelling in the separation of simvastatin and six impurities by liquid chromatographic using a micro emulsion as mobile phase. The complexity of the composition of the micro emulsion permits extensive manipulations to be made during method development in order to achieve acceptable resolution of such a complex mixture of substances. Organic solvent, sodium dodecyl sulphate, and n-butanol content were varied within defined experimental domain. Optimal conditions for the separation of simvastatin and its six impurities were obtained using an X Terra 50 x 4.6 mm, 3.5 micron particle size column at 30^0C[13].

A literature review reveals the impurity profiling of pharmaceuticals by thin layer chromatography. Although there is a tendency in current pharmacopoeias for favouring HPLC, thin-layer chromatography (TLC) is still a very popular and frequently used analytical method in the pharmaceutical industry. This article highlights the possibilities of this method in the different areas of pharmaceutical analysis like in-process and intermediate control, illustrated by impurity testing of active ingredients and final products, as well as its application in pharmaceutical research and development[14].

According to the survey, influence of structural and interfacial properties of microemulsion eluent on chromatographic separation of simvastatin and its impurities, with a predictive molecular thermodynamic approach was developed and illustrated. Calculation of predicted radii, area per surfactant and film thickness, as well as its interfacial tension and bending moment enabled better understanding of separation of such a complex mixture. Hydrophobic interactions between solutes and stationary phase, as well as the microstructural characteristics of micro emulsion eluents had a significant influence on chromatographic behaviour of simvastatin and its six impurities[15].

A literature review reveals the physicochemical characterization of major degradation and process-related impurities associated with the synthesis of ezetimibe. This article discusses the structure elucidation of the (R, R, S) stereoisomer as well as ezetimibe degradation product on the bases of NMR, IR and MS data. Other potential impurities of ezetimibe are also described. A selective and stability-indicating HPLC method with dual UV detection was developed for the determination of chemical and stereochemical purity of ezetimibe[16].

Also, the literature suggests the information regarding the comparison of UV and charged aerosol detection approach in pharmaceutical analysis of statins. CAD (charged aerosol detector) has recently become a new alternative detection system in HPLC. This detection approach was applied in a new HPLC method for the determination of three of the major statins - simvastatin, lovastatin and atorvastatin. The method was optimized and the influence of individual parameters on CAD response and sensitivity was carefully studied. The limits of quantitation (0.1µg/ml) were found to be two times lower than those of UV detection[17].

The researchers gave a stability Indicating RP-HPLC Method for Simultaneous Determination of Simvastatin and Ezetimibe from Tablet Dosage Form. The method uses C18 ODS Hypersil column and isocratic elution. The mobile phase composed of acetonitrile:phosphate buffer. UV detector was programmed at 232 nm for first 10 min and at 238 nm for 10 to 20 min. The development method was also applied suitably for determining the degradation products of ezetimibe and simvastatin[18].

Literature review revealed the rapid detection and identification of the impurities of simvastatin using high resolution sub 2 micron particle LC coupled to hybrid quadruple time of flight MS operating with alternating high-low collision energy.LC coupled to electrospray MS is a powerful tool that has been employed for the identification of impurities, natural products, drug metabolites, and proteins. In this study, they show how sub 2 micron porous particle LC has been coupled to hybrid quadruple orthogonal TOF mass spectrometer to profile and identify the impurities of the common cholesterol lowering drug simvastatin. Using this process it was possible to identify all of the common impurities of simvastatin in a single 10 min run. During the analysis a new impurity of simvastatin was detected and identified as the saturated ring form of simvastatin[19]

Rosuvastatin calcium is a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is used for the treatment of hyperchole-sterolemia. HMG-CoA reductase is the rate-limiting enzyme in de novo cholesterol synthesis. HMG-CoA reductase inhibitors reduce the production of mevalonic acid from HMG-CoA, resulting in a reduction in hepatic

cholesterol synthesis. This in turn results in a compensatory increase in the expression of high affinity low-density lipoprotein (LDL) receptors on hepatocyte membranes and stimulation of LDL catabolism. It is in this manner that produces the lowering of plasma total and LDL cholesterol levels observed in patients with hypercholesterolemia. Reductions in the hepatic pool of cholesterol have also been associated with a decrease in the rate of production of very-low-density lipoprotein (VLDL) and/or LDL by the liver.

Rosuvastatin calcium is a reversible competitive inhibitor of HMG-CoA reductase, which is the most important rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, a precursor for cholesterol [8, 9]. It is more potent than other statins such as atorvastatin, simvastatin and is 8-fold more potent than the hydrophilic comparator, pravastatin [9, 10, 11]. Literature survey reveals that an HPLC method was developed for quantitative determination of rosuvastain in the presence of its degradation products in raw material (Hasumati A. Raj *et.al*.), HPLC method for the estimation of rosuvastatin (RST) in rat plasma (Thammera ranjith kumar *et.al)*, liquid chromatography/tandem mass spectrometry method for the quantification of rosuvastatin in human plasma (Ke Lan *et.al)*, microbore high-performance liquid chromatography (HPLC) in combination with tandem mass spectrometry (MS/MS) for the sensitive detection of rosuvastatin (Crestor™) in human plasma (Kathalijne A. Oudhoff *et.al*.) and an HPLC method for the simultaneous quantitation of five 3-hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase inhibitors, viz. atorvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin, in pharmaceutical formulations and extended the application to in vitro metabolism studies of these statins (Pasha, Md Khalid *et.al)*. This paper describes a simple, sensitive, validated and economic method for the determination of Rosuvastatin Calcium in bulk and pharmaceutical dosage form

Ammonium Acetate (AR Grade), Glacial acetic acid (AR Grade), Triethylamine (AR Grade), Acetonitrile (HPLC Grade), Rosuvastatin calcium (IP), Lactose DCL-15 (BP), Tri basic calcium phosphate(BP), Microcrystalline Cellulose (Sancel PH -102, BP), Cross Povidone (BP), Magnesium stearate (BP)

Preparation of standard solution: Accurately weigh 50 mg of Rosuvastatin WS equivalent to 52.085 mg Rosuvastatin calcium & transfer to a 100 ml volumetric flask. Add 20 ml of methanol & sonicate for 5.0 minutes, make up the volume with methanol. From the above solution take 1.0 ml & transfer to a 25 ml volumetric flask, make up the volume with dissolution media (Concentration 20.0 mcg / ml).

Required brief study

- The primary objective of the study is to develop HPLC method and validate it for the detection and quantification of impurities and related substances in the manufacturing batch of statins and to ensure regulatory compliance as per ICH Guidelines.

Method development is performed with an adaptation of ICH Q2 B Guidelines. The method development process of HPLC method involves the following scheme:

1) **Analysis of impurities and related substances by one of**
a) Area percent method.
b) High-low concentration.
c) External standard method.

2**)** **Suitability of impurities and related substances analysis**.
a) Linear range is a critical factor.
b) Low end of a linearity curve is about 50% of the ICH reporting limit.
c) High end of a linearity curve is the nominal concentration, 100%.

3) **Preparation before method validation**

a) Critical related substances.

b) Lower and upper concentration range for method validation.

c) Method procedure.

d) Critical experimental parameters for robustness.

e) System suitability tests.

- The secondary objective is to evaluate certain parameters for HPLC method to ensure compliance as per ICH Q2 (R1) Guidelines such as:

Specificity

- Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present[20].
- Most related substance methods will be used in a stability study, and therefore they have to be stability indicating.
- Stability indicating means that the method has sufficient specificity to resolve all related substances and the drug substance from each other.

Quantitation limit and Detection limit

- The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be determined quantitatively with a suitable precision and accuracy.
- The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not quantitated as an exact value.
- Quantitation limit can be found out using any of the following methods:

1. Signal to noise approach.
2. Visual examination.
3. Standard deviation.

Linearity

- The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample.solution[20].
- If the linearity does not support such a wide range of concentration, linearity is determined from 50% of the ICH reporting level to 150% of the proposed shelf life specifications of the related substance (for the high–low and external standard methods) as a minimum.

Accuracy

- The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or as an accepted reference value and the value found[20].
- Accuracy for the area percent method should be established from 50% of the ICH reporting limit to the nominal concentration of drug substance in the sample solution.
- For the high–low and external standard methods, determine accuracy from 50% of the ICH reporting level to 150% of the proposed shelf life specification of the related substances.

Overall accuracy:

- Matrix effect.
- Sample preparation.
- Calculation error.

Precision

Precision can be determined by detecting any of the following:

Repeatability:

- Repeatability expresses the precision under the same operating conditions over a short interval of time.
- Repeatability is also termed intra-assay precision.
- Repeatability of a method can be determined by multiple replicate preparations of the same sample.
- This can be done either by multiple sample preparations (n = 6) in the same experiment or by preparing three replicates at three different concentrations.

Intermediate Precision:

Intermediate precision expresses, within laboratories variations, different days, different analysts, different equipment, and so on.

Reproducibility:

Reproducibility expresses the precision between laboratories (collaborative studies are generally used, for standardization of methodology).

Range:

- The range of an analytical procedure is the interval between the upper and lower concentrations of analytes in the sample.
- Typically, linearity and accuracy determination covers a wide concentration range (e.g., 50% of the ICH reporting limit to 150% of specification).

Robustness:

- The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal use.
- This is to verify that the method performance is not affected by typical changes in normal experiments. Therefore, the variation in method conditions for robustness should be small and reflect typical day-today variation.

MATERIALS AND METHODS

1 Source of Data:

Data will be collected from various related websites (ScienceDirect, Scirus, Pub Med), literature surveys, abstracts, journals and related articles from libraries of Krupanidhi College of Pharmacy, ICH Guidelines, FDA Guidelines, USP etc.

2 Method of collection of data:

Data will be collected from the following step wise experimental procedures proposed in the study:

Two conditions are taken into account:

1) HPLC conditions:

a) HPLC column (lot, age, brand).

b) Mobile-phase composition (pH ± 0.05 unit).

c) HPLC instrument (dwell volume, detection wavelength ± 2 nm, column.temperature ± 5^{o}C, flow rate).

2) Sample preparation:

a) Sample solvent (pH ± 0.05 unit).

b) Sample preparation procedure (shaking time, different membrane filters).

c) HPLC solution stability.

Built-in Robustness in Method procedure:

- Weighing error.
- Dilution error.
- Sonication.
- Mobile phase as sample solvent.
- Buffer.

3 Does the study require any investigations of interventions to be conducted on patients or other human or animals? If so please describe briefly?

No.

4 Has ethical clearance been obtained from your institute in case of as above?

Not applicable.

LIST OF REFERENCES

1) http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html

2) Analytical method validation and instrument performance Verification. New Jersey: A John Wiley & Sons, Inc., Publication;2004. Chan C, Lam H, Lee Y and Zhang XM

3) Method Validation in Pharmaceutical Analysis Weinheim: WILEY-VCH Verlag GmbH & Co. KGaA;2005. Ermer J and Miller J

4) http://en.wikipedia.org/wiki/Statin

5) J Pharm Biomed Anal 2003 Oct;33(3):335-377. An overview of the recent trends in development of HPLC methods for determination of impurities in drugs Rao R and Raju N

6) Kadav AA and Vora DN. Stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets. J Pharm Biomed Anal 2008 Sep;48(1):120-126.

7) Ertürk S, Akta E, Ersoya L and Ficiciolub S. An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablet. J Pharm Biomed Anal 2003 Dec; 33(5):1017-1023.

8) . Kocijan A, Grahek R and Kralj L. Identification of an Impurity in Pravastatin by Application of Collision-Activated Decomposition Mass Spectra. ActaChimSlov 2006;53: 464-468.

9) . Vuletic M, Cindric M and Koružnja J. Identification of unknown impurities in simvastatin substance and tablets by liquid chromatography/tandem mass spectrometry. J Pharm Biomed Anal 2005 Apr; 37(4):715-721.

10) Kracuna M, Kocijana A, Bastarda A, Graheka R, Plavecb J and Kocjanb D. Isolation and structure determination of oxidative degradation products of atorvastatin. J Pharm Biomed Anal 2009 Dec;50(5):729-736.

11) . Wu YJ, Zhu W, Shao Q and Cheng YY.Identification of impurities in lovastatin by high performance liquid chromatography-diode array detection-mass spectrometry.FenxiHuaxue 2006;34(1):115-118.

12) . Houck A, Thomas S and Ellison D. Liquid-chromatographic determination of the known low level impurities in lovastatin bulk drug: an application of high-low chromatography. Talanta 1993;40(4):491-494.

13) . Malenovic A, Ivanovic D, Medenica M and Markovic S. Retention modelling in liquid chromatographic separation of simvastatin and six impurities using a microemulsion as eluent. J Sep Sci 2004 Sep;27(13):1087-92.

14) Fodora K, Végha Z, and Rengerb B. Impurity profiling of pharmaceuticals by thin-layer chromatography. J Chromatogr A 2011 May;1218(19):2722-2732.

15) Anđelijaa M, Darkoa I, Mirjanab M, Biljanaa J and Slavkoc M. Influence of structural and interfacial properties of microemulsion eluent on chromatographic separation of simvastatin and its impurities. J Chromatogr A 2006 Oct;1131(1-2):67-73.

16) . Filipa K, Bańkowskia K, Sidoryka K, Zagrodzkaa J, Łaszcza M, Trzcińskaa K , et al. Physicochemical characterization of ezetimibe and its impurities. J MolStru 2011 Apr; 991(1-3):162-170.

17) Novakovaa L, Lopezb SA, Solichovac D, Šatinskya D, Kulichovad B and Hornad A. Comparison of UV and charged aerosol detection approach in pharmaceutical analysis of statins. Talanta 2009 May;78(3):834-839.

18) Dixit RP, Barhate CR, Padhye ISG and Viswanathan CL. Stability Indicating RP-HPLC Method for Simultaneous Determination of Simvastatin and Ezetimibe from Tablet Dosage Form.Ind J Pharm Sci 2010 Mar–Apr;72(2):204–210.

19) . Plumb RS, Jones MD, Rainville P and Castro-Perez JM. The rapid detection and identification of the impurities of simvastatin using high resolution sub 2 microm particle LC coupled to hybrid quadrupole time of flight MS operating with alternating high-low collision energy. J Sep Sci 2007 Nov;30(16):2666-75.