Original Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING METHODS FOR DRUGS ACTING ON RESPIRATORY DISORDERS: A REVIEW APPROACH

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Abstract

The current paper is related to the review based on the development and validation of stability indicating methods for drugs acting on respiratory disorders. An extensive literature survey is important for the development of analytical methods for various classes of drugs available in the market. The drugs with few analytical methods were in a need of more simple and accurate analytical methods. The present proposed research attempted to develop and validate new analytical methods. The principle intention of planning this endeavor is to deliver simple, accurate, precise, specific, reproducible, robust, economical, and highly sensitive methods.

Keywords- Drugs, Respiratory Disorders, Analytical Methods, Robust, Economical Methods.

I.INTRODUCTION

Establishing quality and quantity is one of the prime concerns of any pharmaceutical industry for the successful release of drugs into the market. Not only for the release of drugs into the market, had assurance on quality and quantity also stressed for safe human consumption of drugs. These two parameters i.e. quality and quantity are well confirmed in various types of bulk and commercial Pharma products by using different analytical techniques starting from the oldest titrimetric analytical techniques to recently developed hyphenated techniques [1-2]. These analytical techniques are the widest use in the pharmaceutical industry from drug development to the post-marketing stage. Different analytical techniques are also used to get prior knowledge on the selection and design of dosage forms, and stability of drugs during manufacturing, storage, and under different environmental conditions [3]. Analytical techniques are also used to identify the presence of impurities along with their quantity in different pharmaceutical formulations, which is very useful to establish toxicity profiles [4]. Analytical techniques are also used to distinguish and differentiate API from different impurities generated during various types of drug manufacturing processes and stability studies [5]. Analysis of drugs and their metabolites in various body fluids also gives information about the pharmacokinetics behaviors of drugs [6]. All these analytical techniques are based on measuring certain physical properties of substances/compounds under investigation [7]. Various physical properties like volume, weight, refractive index, polarity index, absorbance or transmittance, peak area, and retention time are measured in volumetric analysis, gravimetric analysis,

refractometry, polarimetry, spectroscopy, and chromatography simultaneously [8].

In any pharmaceutical industry, the quality control department is actively engaged from the initial level of sample collection to the ending level of reporting data. The quality control department in the pharmaceutical industry is meant for controlling safety, efficacy, quality& compliance with regulatory authorities like ICH, FDA, MHRA, and CDER [9-10]. Method validation includes the verification of the developed method for its suitability and proposed purpose by studying the following validation parameters with necessary acceptance criteria, Selectivity, Specificity, Linearity, Range, Accuracy, Precision, Limit of detection (LOD), Limit of quantitation (LOQ), Robustness and System suitability [11-12]. The success or efficacy of any formulation is depending upon its quality and quantity, which can be best explored through an important subject intimately known as Pharmaceutical Analysis [13]. As people around the globe are facing several new deadly diseases with different origins, pharmaceutical markets are flooded with a greater number of drugs and formulations. Release of drugs into the market without giving promise on quality, quantity, and stability may cause possible unwanted effects [14]. To establish the above-said parameters viz quality, quantity, and stability during various processes of manufacture and storage in newly developed dosage forms several analytical techniques are well confirmed in the pharmaceutical analysis [15]. Keeping the importance and need of analysis for drugs, we designed our research work with two well-known analytical techniques i.e. HPLC and UV Spectrophotometry [16]. The main premise for engaging in these techniques is because of their adaptability and consistency. Spectrophotometric methods are one of the oldest and less expensive techniques and it does not necessitate much handy knowledge with stringent experimental conditions [17]. As well the criteria for the selection of HPLC are because of its conventionality, wide applicability to nearly 90% of recognizable organic compounds, and knowing its importance by observing its mark in several revolutionary analytical techniques like LC-MS, GC-MS, and UPLC [18].

The usage of less costly solvents, chemicals like purified water, buffers, methanol, acetonitrile, and elution at shorter retention times makes analysis with the developed methods inexpensive, speedy, consistent, and reproducible when compared with the established methods. Further, these developed methods can also be applied to routine quality control of drugs in laboratories and pharmaceutical industries.

II. LITERATURE SURVEY

In this section we have selected some important research papers which are closely related to our research area and they as follows:

Prafulla Kumar Sahu et. al. (2017) [19] "portrayed the various analytical techniques which was enhanced their robust nature with the help of chemometric application for the analytical quality control of drugs. In this review, vast LC methods and hyphenated techniques were analyzed for different drugs. Later they enhanced the description about preliminary screening and optimization of design by various QbD elements. Also had a brief description about Lean Six Sigma concept which act's a quality marker for chromatographical determinations."

Alexander H Schmidt et al. (2013) [20] "reported a UPLC integrated with Quality by design for ebastine to develop a stable method. Here screening for various stationary phases act as primary task and then the next stage i.e., optimization done with Drylab 4 programming software".

SushantBhimraoJadhav et al. (2016) [21] "worked on Omeprazole to identify the impurities which are not in pharmacopoeia with the help of 2 level factorial design using RP UPLC tandem with TOF/MS. They utilized Acquaity BEH RP 18 column as a stationary phase. With the help of design-expert the degraded impurities are identified and further validated."

V SreeJanardhaman et al. (2016) [22] "utilized Phenomenex C18 column and methanol, acetonitrile KH2PO4 buffer for the estimation of 4 drugs as Rosuvastatin, Telmisartan, ezetimibe and Atorvastatin. In this paper they clearly explained about how screening has been done and optimization with the employment of Factorial design. Various models for the representation of design space have been depicted they are the perturbation graphs to show the interactions, 3D figures and desirability of individual parameters. The most robust method was

described for the cardio vascular regimen."

Christine Bousses et al. (2015) [23] "proposed a method for Dextromethorphan by an inventive consolidation of QbD with analytical green chemistry methods utilizing UHPLC. In spite of the fact that the utilization of QBD is emphatically suggested by regulatory rules, this methodology isn't yet generally connected in analytical method developments. The final goal of this work was to make a more robust quality methodology in regulatory bodies."

Aneesh T. P. et al. (2012) [24] "described the importance of forced degradation studies for in depth understanding of stability of bulk and drug product (DP). In those situations where there is little information about probable degradants these studies are highly useful as they afford information on degradation pathways and degradation products which are likely to form during storage. These assist in areas which require knowledge of chemical behavior such as formulation development, manufacturing and packaging for fulfilling of regulatory needs."

Blessy M. et al. (2014) [25] "discussed about recent advances for performance of forced degradation studies. Also stressed on importance of conducting forced degradation studies and make available of strategies for conducting stress studies. A deep insight into degradation mechanism and degradation products of the drug substance assists in elucidation of the structure of the degradation products. During the development of formulation, the chemical behavior of the molecule plays an escalatory role so forced degradation studies may furnish this information."

Bhattacharyya Indrani et al. (2015) [26] "carried a forced degradation study on Atenolol and Hydrochlorthiazide, a beta1 (β1) receptor blocker and diuretic drug respectively under specified stressed conditions in acidic and basic environment in order to deconvolute the possible degradation product. In this study they found that under acidic conditions atenolol and hydrochlorothiazide were spliced into various degradants. Structural identification and characterization was done by FT-IR and Proton NMR. This study assisted on the degradation behavior of drugs and furnished information on structural elucidation of degradants."

EsenBellurAtici et al. (2015) [27] "identified, synthesized and characterized process related impurities in Benidipine hydrochloride, a drug used in the treatment of hypertension. Their mechanisms of formation were discussed. Aultra performance liquid 26 chromatographic method has been developed and stress-testing were done under various conditions as specified by ICH guidelines. A process related impurity of benedipine was detected using the developed method. This helps for the identification, synthesis of process related impurities."

Tapas Kumar Laha et al. (2017) [28] "developed novel and simple stability indicating RP-HPLC method for a drug namely leflunomide an anti-rheumatoid drug. The method has been designed for detection the drug even in the presence of degradants formed during stress studies. Further degradation products formed under different stress conditions were separated. The developed method can be employed for analysis of the samples of stability study. This provides data on how to perform stress studies and conditions need to be employed."

R. Nageswara Rao et al. (2013) [29] "developed RP-HPLC for assay of doxofylline which can separate its related substances and degradants which were formed under an acidic, basic, thermal, photo, and peroxide conditions. These were further characterized by ESI-MS/MS,1H, and 13C spectroscopy. This method has been effectively applied for the assay of doxofylline in bulk drugs but also to quantify related substances and degradation products. This also aids in the characterization of degradants by various spectrophotometric techniques. Gulshan Bansal et al. (2015) [30] carried out and concluded that doxorubicin was sensitive to alkaline hydrolysis even at RT, acid hydrolysis at 80°C, and oxidation at RT. For their characterization, a six-stage mass fragmentation (MS6) pattern of doxorubicin was outlined through mass spectral studies in the positive mode of electrospray ionization (ESI). The probable mechanisms of degradant formation were outlined and discussed."

Ramisetti Nageswara Rao et al. (2013) [31] "developed a new RP-HPLC method and further performed forced degradation studies by exposing the drug carisbamate to acid, base, peroxide oxidation, exposure to light and heat and isolated process related to 27 impurities in bulk drugs. Drug was found to be more sensitive to acid/base, the formed degradants were further isolated and characterized by ESIMS, 1H and 13C NMR. The

possible isomerization of carbimasate were discovered by MS/MS and 2D-NMR (COSY and HSQC)."

Li Ding et al. (2016) [32] "developed novel and simple high-performance liquid chromatographic method and performed stress testing of posaconazole injection under various stress conditions to estimate the intrinsic stability. Four degradation products formed in oxidative and thermal were detected. Among these three are unknown degradants which are formed under oxidative stress condition. Isolation of the degradants was done by preparative HPLC and explicitly elucidated by LC-TOF MS, Proton Nuclear magnetic resonance spectroscopy, 13C NMR spectroscopy and 2D NMR. Probable mechanisms for the formation of the degradants were proposed."

Mohamed R. Elghobashy et al. (2012) [33] "reported four HPLC methods for estimation of bupropion hydrochloride in existence with alkaline degradants and related substances. Among the two methods the former method is isocratic RP-high performance liquid chromatographic method achieved excellent separation of bupropion hydrochloride from its alkaline degradants and related impurities. Method B is the first derivative (D1) measurement of the drug at 259 nm, method C is first derivative ratio spectra (DD1) and method D is based on the determination of drug and related substances by Q value method. The developed method was found suitable for the analysis of bupropion hydrochloride API and pharmaceutical formulation and produced data on various techniques for analysis of drugs in the presence of impurities."

Nageswara Rao Ramisetti et al. (2014) [34] "proposed the probable degradation of lacosamide (LAC) in the presence of ICH prescribed stress conditions. Seven degradants formed were resolute and their structures were elucidated employing hyphenated techniques like ESI-Q-TOF-MS/MS technique 28 e. This study demonstrates a comprehensive approach of LAC degradation studies during its development phase and use of hyphenated techniques for structure elucidation of degradants."

Qingmei Ye et al. (2016) [35] "implied about implementation of novel RP-HPLC methods for determination of impurities and degradation products in API of Aztreonam. Three unknown degradants were separated by employing preparative HPLC and characterized by mass,1D and 2D NMR spectroscopy. Quantity of the impurity was found out by quantitative NMR and use of small amount of sample abolished the necessity for the synthesis of bulk quantities of standard reducing cost and time."

Saranjit Singh et al. (2004) [36] "described novel RP-HPLC method for determination of prazosin, terazosin and doxazosin in the presence of degradation products generated from forced decomposition studies. These three drugs belong to the class of alpha-adrenergic blockers used in the treatment of cardiovascular diseases. This provides the basis for the resolution of ternary mixture in the presence of degradants."

Mahesh Kumar Mone et al. (2013) [37] "established the degradation pathway of sitagliptin in bulk and tablet during stress study and isolation of formed major degradation products in pure form. Structure elucidation of the new impurities was done by mass and NMR spectroscopy. RP-HPLC method was developed on Poroshell 120 ECC18 (3×150mm, 2.7µ) column using mobile phase comprised of 5mM ammonium acetate and acetonitrile in the presence of spiked degradation products and impurities."

Mastanamma SK et al. (2018) [38] "developed a simple, specific, accurate and economic reverse phase liquid chromatographic method for the simultaneous estimation of Sofosbuvir and Ledipasvir in bulk and tablet dosage form. The method has shown adequate separation of Sofosbuvir and Ledipasvir from their degradation products. Separation was achieved on a Luna C18, 250 mm x 4.6 mm, 5 mm Column at wavelength of 227 nm, using a mobile phase acetonitrile: Triethylamine Buffer (pH-2.5) (50:50) in an isocratic elution mode at a flow rate of 1.0 mL/min. The retention time for Sofosbuvir and Ledipasvir was found to be 4.905 and 2.751 min correspondingly. The above drug combination was subjected to acidic, base, neutral hydrolysis, thermal and photolytic stress environment. Thus, stressed samples were analyzed by the proposed analytical method. Quantitation was achieved with UV detection at 227 nm based on peak area with linear calibration curve at concentration range 1-15 mg/mL for Sofosbuvir and 0.25-3.75 mg/mL for Ledipasvir. The LOD's were 0.25 and 0.0625 for Sofosbuvir and Ledipasvir respectively. The LOQ's were found to be 0.5 for Sofosbuvir and 0.505 for Ledipasvir. The proposed method was established to be precise and Forced Degradation as no interfering peaks of degrades and excipient was observed. The proposed method was therefore suitable for purpose in

quality-control laboratories for quantitative analysis of both the drugs individually and in combined dosage form, as it is simple and rapid with tremendous precision and accuracy."

Rote AP et al. (2017) [39] "determined a specific, accurate, simple, selective and stability-indicating RP-HPLC method for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form. RP-HPLC method was performed on the systronics isocratic HPLC System equipped with SP930 D HPLC pump and dual wavelength UV-VIS detector and C18 column (250 mm × 4.6 mm, 5µm), using the mobile phase (Methanol: Water 83:17 V/V) pH 3.0 with 0.05% acidic acid at a flow rate of 1.0 mL/min, injection volume 20µL and UV detection at 245 nm. This method was validated according to BP, USPand ICH requirements for new methods, which include accuracy, precision, robustness, ruggedness, LOD, LOQ, linearity and range. Linear relationships were obtained in the ranges of 10-50µg/mL and 40-200µg/mL with correlation coefficients of 0.9991 and 0.9994 at Rt value of 7.45 min and 3.50 min for sofosbuvir and ledipasvir respectively. The Forced Degradation studies as acidity, alkalinity, oxidation and hydrolytic degradation were performed according to ICH guidelines."

Sunder B. S. et al. (2018) [40] "developed a novel, sensitive and accurate high-performance liquid chromatography with ultraviolet/visible light detection (HPLC-UV/VIS) method for the quantification of ledipasvir and Sofosbuvir in plasma was developed and validated. The analytes were extracted by liquid extraction method and chromatograph using a mobile phase consisting of acetonitrile and buffer solution, Methanol and Acetonitrile in the ratio of 200:600:200 (V/V) using Oyster BDS RP-C18 column. The flow rate 1.0 mL/min and UV detection at 238 nm were employed. The retention time for Ledipasvir and Sofosbuvir was 4.61 and 9.09 min respectively. Linearity for ledipasvir and Sofosbuvir was found to be in the range of 250-2000 ng/mL for both drugs respectively. Intra-and inter-day precision was less than 2% coefficient of variation. The method was validated as per the USFDA guidelines and the results were within the acceptance criteria for selectivity, sensitivity, linearity, precision, accuracy, recovery stability of the solution, the stability of solution in plasma and dilution integrity."

Ganapaty S et al. (2018) [41] "developed stability indicating reversed-phase high performance liquid chromatography method and validated for simultaneous quantification of sofosbuvir and ledipasvir in tablets. The chromatographic separation was done in an isocratic mode using the Discovery C18 (250×4.6 mm, 5 µ particle size) column. The mobile phase 0.1 % ortho phosphoric acid and acetonitrile 45:55 (%V/V) at a flow rate of 1.0 mL/min and at ambient temperature was used. The wavelength used for detection was 270 nm. The retention time for sofosbuvir was found to 2.08 min and that of ledipasvir was 3.06 min. Sofosbuvir and ledipasvir were linear in the concentration ranges of 100 to 600 µg/mL and 22.5 to 135 µg/mL, respectively. The developed method was validated and found to be accurate, specific and robust. Both the drugs were subjected to the stressed conditions like acidic, basic, oxidative, photolytic and thermal conditions. The degradation results were found satisfactory. This method could be applied for the simultaneous estimation of sofosbuvir and ledipasvir in tablets."

Vikas PM et al. (2016) [42] "developed a validated chromatographic method which is simple, precise, accurate, reproducible and specific RP-HPLC method for estimation of Sofosbuvir in bulk. Separation of SFS was successfully achieved on a Hisil C18 (4.6 x 250mm, 5 µm) Waters or equivalent in an isocratic mode utilizing Phosphate Buffer (4.0 pH): Methanol (50:50% V/V) at a flow rate of 0.8 mL /min and eluate was monitored at 262 nm, with a retention time of 1.01 minutes. The method was validated and the response was found to be linear in the drug concentration range of 5 µg/mL to 30µg/mL. The values of the slope, intercept and the correlation coefficient were found to be 0.07, 0.4 and 1.000 respectively. The RSD values for system precision and method precision were found to be 0.19% (Intra-day), 0.21% (Inter-day) and 0.20% (Intra-day), 0.23% (Inter-day) respectively."

Vejendla R. et al. (2016) [43] "developed a validated chromatographic method which is simple, sensitive, precise, and accurate isocratic reverse phase high pressure liquid chromatographic method for sofosbuvir in bulk and tablet dosage form. To optimize, a column Phenomenex prodigy ODS-3V (150 mm x 4.6 mm, 5 µm), mobile phase mixture of methanol and (0.1%) trifluro acetic acid as buffer having pH of 3.2 in the ratio of (30:70 V/V) found to be an efficient system for elution of drug with good peak shape as well as retention time 2.990 min., flow rate 1.0 mL/min at UV wavelength of 260nm. Quantitative linearity was obeyed in the

concentration range of 100 to 600 µg/mL, with regression coefficient R^2 = 0.996. The number of theoretical plates obtained was 2604.352 which indicate the efficient performance of the column. The limit of detection was 0.01 µg/mL and limit of quantification was 0.03 µg/mL, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by the proposed RP-HPLC method."

Guguloth R et al. (2016) [44] "developed a validated chromatographic method of Sofosbuvir by reversed Phase High Performance Liquid Chromatography (RP-HPLC) method. The method utilized RP-HPLC (Water 2695 with PDA detector) model and a column Agilent C18 4.5×100 mm 3.0 µm. The mobile phases were comprised with 60:40 of Methanol: Water at a flow rate of 1.0 mL/min. UV detection at 235 nm Sofosbuvir was eluted with retention times of 2.351min. The method was continued and validated accordance with ICH guidelines. Validation revealed the method is rapid, specific, accurate, precise, reliable, and reproducible. Calibration curve plots were linear over the concentration ranges 320-480µg/mL (R2 = 0.9993). Limit of detection (LOD) was 1.5µg/mL and limit of quantification (LOQ) was 4.7µg/mL. The method showed good recoveries (99.1 -99.9%). Statistical analysis proves the method is suitable for the analysis of Sofosbuvir as a bulk, in tablet dosage form without any interference from the excipients."

Rezk MR et al. (2016) [45] "developed a novel and sensitive LC-MS/MS method was developed and validated for determination of sofosbuvir (SF) using eplerenone as an internal standard. The Xevo TQD LC-MS/MS was operated under the multiple-reaction monitoring mode using electrospray ionization. Extraction with tert-butyl methyl ether was used in sample preparation. The prepared samples were chromatographed on Acquit UPLC BEH C18 (50 × 2.1 mm, 1.7 µm) column by pumping 0.1% formic acid and acetonitrile in an isocratic mode at a flow rate of 0.35 mL/min. Method validation was performed as per the US Food and Drug Administration guidelines and the standard curves were found to be linear in the range of 0.25-3500 ng/mL for SF. The intra- and inter-day precision and accuracy results were within the acceptable limits. A very short run time of 1 min made it possible to analyze more than 500 human plasma samples per day. A very low quantification limit of SF allowed the applicability of the developed method for determination of SF in a bioequivalence study in human volunteers."

Bhatt D et al. (2018) [46] "developed a new isocratic simple and rapid reverse phase high performance liquid chromatographic method was developed and successively validated for the estimation of Sofosbuvir. In this newly developed method, chromatographic separation of Sofosbuvir was achieved on a Phenomenex C18-column (250 × 4.6) mm within a short runtime of 6 min using mobile phase containing 0.1% Formic acid in water (pH at 2.3) and Acetonitrile in the ratio of 50:50% V/V. Sofosbuvir was estimated with UV detection at 262 nm and it was found to be eluted at 2.983 min. The above-mentioned method was validated as per International Conference on Harmonization (ICH) guidelines with respect to accuracy, precision, linearity, limit of detection (LOD) and limit of quantitation (LOQ) and robustness. The method was found specific for Sofosbuvir and linear (r2 =0.9994) over concentrations ranging from 20 to 100 µg/mL. The method was found statically accurate (mean recovery = 99.46%), precise with both intra-day and inter-day relative standard deviation (RSD) values < 1.0%, and robust. The obtained results concluded that the proposed RP-HPLC method was convenient, reliable, and useful in routine analysis for the estimation of Sofosbuvir in its bulk form and dosage form."

Singh K et al. (2019) [47] "developed a rapid, selective, precise, and accurate method for the estimation of % drug release of Sofosbuvir in a pharmaceutical formulation. Stability indicating HPLC method was developed using Zorbax eclipse plus C18 (100 × 4.6 mm), 3.5 µ as an analytical column, and a combination of ammonium acetate buffer pH 5.3 and methanol in the ratio (45:55 V/V) was used as mobile phase in isocratic mode. UV detection was carried out at 260 nm, column temperature was maintained at 25 °C, and the flow rate was 1.5 mL/min. The method was validated as per internationally accepted ICH guideline and found to be specific for blank and placebo solution, precise, robust, accurate and linear in range 9.2 to 69.0 µg/mL of Sofosbuvir. This method can be used for routine analysis of pharmaceutical formulation in any quality control laboratory leading to delivery of good quality healthcare solution."

Eldin AS et al. (2017) [48] "developed a simple, rapid and reproducible reversed phase high performance liquid chromatography (RP-HPLC) and ultraviolet derivative spectrophotometric (UVDS) methods for the simultaneous determination of daclatasvir (DAC) and sofosbuvir (SOF) in pure and in pharmaceutical dosage forms have been developed and validated. The chromatographic separation of DAC and SOF was achieved using Agilent Zorbax SB C18 (4.6 x 250 mm, 5 µm) column at temperature of 40 ° C. The mobile phase used was 9 mM dipotassium hydrogen orthophosphate buffer (pH 4±0.1): acetonitrile (60:40, V/V). The flow rate was maintained at 1 mL/min with UV detection at 265 nm. The calculated resolution was 4.56 (> 2), which ensures complete separation. The tailing factor was 1.13 and 1.40 (≤ 2) for DAC and SOF, respectively. Intermediate precision value was ≤ 2% indicates acceptable ruggedness. The two methods were validated according to the International Conference on Harmonization (ICH) guidelines in terms of linearity, precision, accuracy, selectivity, specificity, detection limit, quantification limit, robustness and ruggedness."

A LR et al. (2019) [49] "developed a simple, accurate and precise stability indicating RP-HPLC method for the simultaneous estimation of the Sofosbuvir and Velpatasvir in tablet dosage form. Chromatogram was run through m) column. Mobile phase containing buffer Discovery C18 (250 x 4.6 mm, 5 0.1% OPA: acetonitrile taken in the ratio 50:50 V/V was pumped through column at a flow rate of 1 mL/min. Temperature was maintained at 30°C. Optimized wavelength selected was 240 nm. The method was linear over the concentration range for Sofosbuvir is 100-600µg/mL and for Velpatasvir is 25- 150µg/mL. The retention times of Sofosbuvir and Velpatasvir were found to 2.473 min and 3.316 min respectively. %RSD of the Sofosbuvir and Velpatasvir were found to be 0.2 and 0.3 for system precision, 0.4 and 0.5 for repeatability and 0.2 and 0.3 for intermediate precision respectively. %Recovery was obtained as 99.32% and 100.43% for Sofosbuvir and Velpatasvir respectively. LOD and LOQ values obtained from regression equations of Sofosbuvir and Velpatasvir were 0.44, 1.32 and 0.33, 1.01 respectively. The method was validated and was successfully employed for the routine quantitative analysis of pharmaceutical formulations containing Sofosbuvir and Velpatasvir in combined tablet dosage form."

Pal N et al. (2016) [50] "developed a validated chromatographic method for Ledipasvir by using reverse phase High performance liquid chromatography (HPLC). The procedure involved use of isocratic elution where the stationary phase was a BDS column (250 mm, 4.6 mm, 5 µm), mobile phase 0.05% triflouroacetic acid in methanol and 0.05% triflouroacetic acid in acetonitrile (55:45). pH of the chromatographic system was maintained at 3.0, flow rate 1 mL/minute, eluent was monitored by PDA detector wavelength at 270 nm. Retention time was found to be 2.749 minutes, regression analysis shown the value of correlation coefficient 0.999. Value for limit of detection (LOD) was 1.064µgm/ml and limit of quantification (LOQ) was 3.224 µgm/mL. Linearity range was designed 15µg/mL to 300µg/mL for Ledipasvir. Accuracy study revealed percentage recovery 99.81%-100.10% and precision result in terms of standard deviation was 1986.515 and percentage relative standard deviation was 0.08511%. Test for intermediate results also were well within the limit for Ledipasvir. Robustness study results proved the suitability of the method under different chromatographic condition. The developed method was validated as per ICH guideline and was found to be an ideal one for regular analysis in the laboratory."

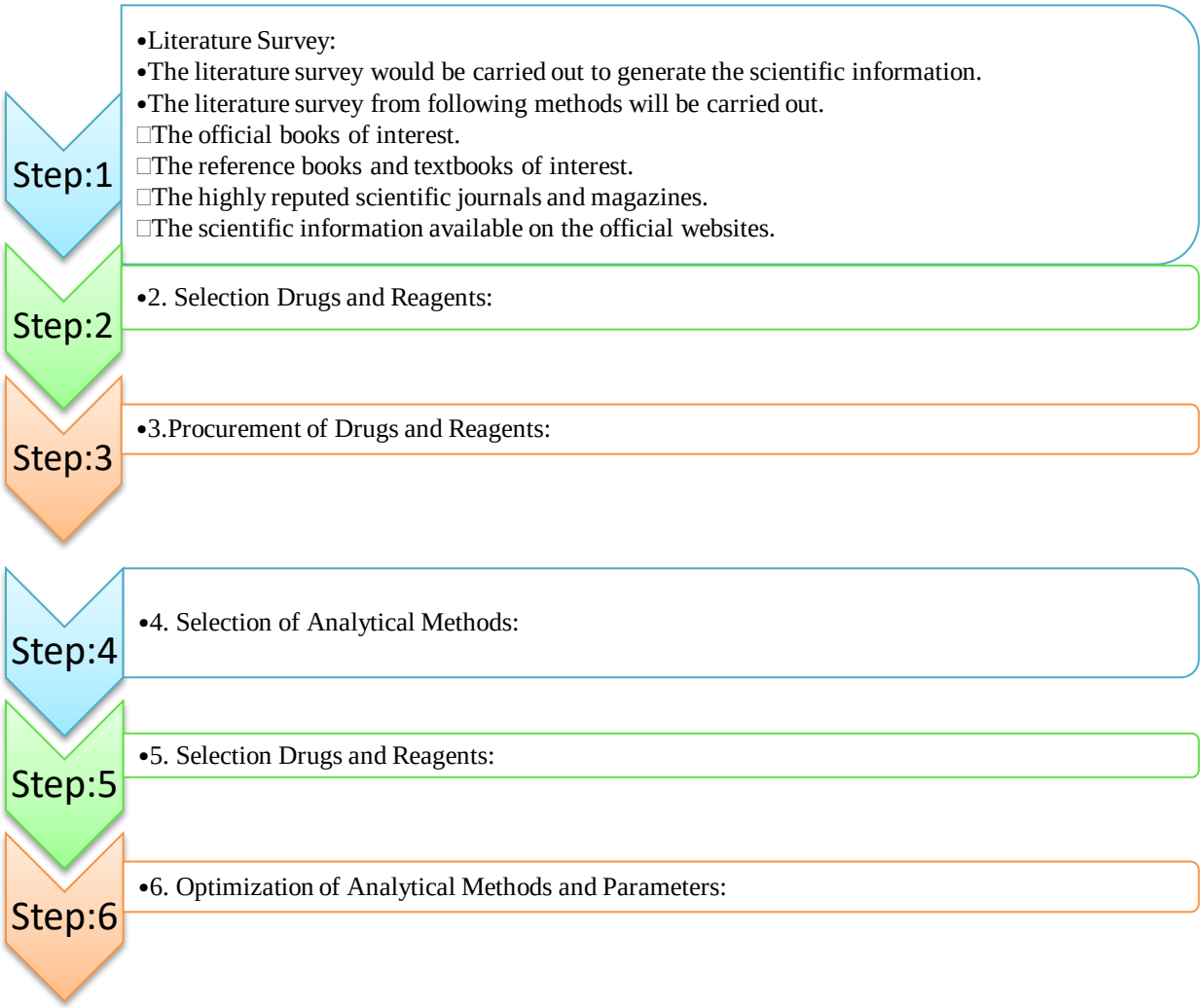
M SK et al. (2014) [51] "developed a new stability indicating reversed phase high performance liquid chromatographic method was developed and successfully validated for the simultaneous estimation of Lamivudine and Zidovudine. A Hypersil BDS C18 (250 x 4.6 mm, 5µ.) analytical Column was used for chromatographic separation and column temperature was maintained at 30ºC.Mobile phase used was a mixture of Buffer (pH 4.6): Acetonitrile (80:20) at a flow rate of 0.9 mL/min. The UV wavelength used for detection was 272 nm for Lamivudine and Zidovudine. The stability-indicating capability of the method was demonstrated through adequate separation of aged and stress degraded Lamivudine and Zidovudine stability samples. Different analytical performance parameters such as system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ) and robustness were determined according to International Conference on Harmonization (ICH Q2B) guidelines. The linearity of the calibration curves for each analyte in the desired concentration range was good (r2 =0.999). The recovery of the method was 100.32% and 100.51 % for Lamivudine and Zidovudine respectively. Hence the proposed suitability-indicating method was rapid, simple, highly sensitive, precise and accurate and it can be successfully applied to determine the amount of Lamivudine and Zidovudine in the formulations."

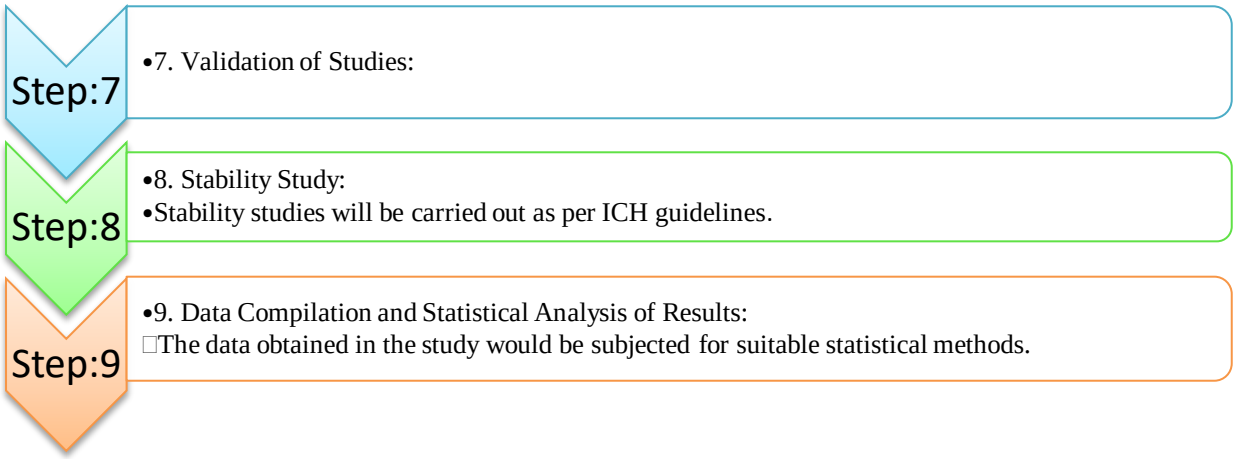
Extensive literature survey is important for the development of analytical methods for various classes of drugs available in the market. The drugs with few analytical methods were in a need of more simple and accurate analytical methods. The present research was attempted so as to develop and validate new analytical methods. The principle intention of planning this endeavor is to deliver simple, accurate, precise, specific, reproducible, robust, economical and highly sensitive methods.

III. PROPOSED METHODOLOGY

The following steps are followed as methodology for doing research.

Figure 1. Methodology for Project Implementation





VI. OBECTIVES OF PROPOSED WORK

The objectives of proposed work are:

- To develop and validate MS compatible HPLC methods that can be applied for the analysis of the selected drug.
- To develop and validate Forced degraded stability indicating methods for selected drugs
- To deliver simple, accurate, precise, specific, reproducible, robust, economical and highly sensitive methods.
- To calibrate the developed methods quantitatively.
- To validate the developed methods as per the course of action framed by USP and ICH.

VII. CONCLUSION

It is concluded that extensive literature survey is important for the development of analytical methods for various classes of drugs available in the market. The drugs with few analytical methods were in a need of more simple and accurate analytical methods. The present research was attempted so as to develop and validate new analytical methods. The principle intention of planning this endeavor is to deliver simple, accurate, precise, specific, reproducible, robust, economical and highly sensitive methods.

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