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RP-HPLC Method for the Simultaneous Estimation of Calcium Channel Blocker with Diuretic and Antihypertensive in Bulk Drugs and Marketed Formulation

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ABSTRACT

Simultaneous estimation of Amlodipine and Indapamide using RP-HPLC and UV methods in bulk and marketed formulation. The HPLC system used was LC SHIMADZU UFLC-2000 Prominance LC-20AD Binary Gradient System, SPDM 20A detector with Rheodyne injector and Enable C18 G column 250x 4.6mm, 5µm. Injection volume of 20µL was injected and eluted with the mobile phase of Phosphate buffer, Mixed pH 4.0 (adjusted with glacial acetic acid): Acetonitrile in the ratio of 40:60v/v at the flow rate of 1.0mL/min. The peaks of Amlodipine and Indapamide were found well separated with retention time 3.54min and 4.87min respectively. The % RSD for method, system, interday, intraday precision was found to be NMT 2%. The recovery studies from tablet are indicative of accuracy of method and are found in between 99.87-101.43% at three different levels of standard additions. Precision studies showed satisfactory results. The proposed method was found to be specific, linear, accurate, precise, robust and rugged. Hence the RP- HPLC method developed and validated can be used routinely for the simultaneous estimation of Amlodipine and Indapamide in bulk drugs and marketed formulation.

INTRODUCTION:

Stability plays an important role in the drug development process. It explains several factors that affect the expiration dating of drug products, including the chemical and physical stability during the pre-clinical formulation stages, process development, packaging development, and post-marketing life. The evaluation of the physicochemical stability of a given product requires an understanding of the physical and chemical properties of the drug substance[1].

Pharmaceutical stability may be applied in several ways; therefore, the performance of a drug will be evaluated depending on whether it assesses a drug substance, a formulation, a drug product, or a packaged product[2]. The safety and efficacy of a drug product are established during the development process via preclinical animal and human clinical studies. The quality attributes such as identity, concentration, and purity are defined, and testing is developed. Should drug properties change beyond the accepted criteria during a stability study, then the established safety and efficacy data may no longer be applicable. Changes in drug stability could risk patient safety, since the dosage amount to patient may be lower than expected. Instability may also lead to formation of toxic degradants.

If instability of a drug product leads to these unwelcome effects on patients, it could also lead to expensive costs to manufacturers as they attempt to discover the reasons for instability and methods of minimizing them. An unstable product would highlight an uncontrolled process, and could require a substantial product and process investigation with possible product recalls. FDA has authority to issue cGMP violations with follow-up warning letters and possible consent decrees and criminal prosecutions. Stability testing therefore allows the establishment of recommended storage conditions, retest periods, and ultimately product shelf-life and expiry dating.

Stability considerations will dictate the environment for drug substance preparation and storage, choice of packaging, and allowable shelf-life of the final drug product. Should a drug substance be sensitive to environmental factors such as temperature, humidity, pH, light and oxygen exposure, these must be considered and controlled when designing processing, storage, and final packaging of the drug product.

Stability studies Provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as

Temperature, Humidity, and Light. Lack of drug substance or drug product stability may affect the purity, potency, and safety of the drug product.

How each one of these factors has the capability to catalyze, accelerate or mediate one or more of the various degradation reactions like hydrolysis, oxidation, photolysis or some other unwanted conversion of the drug substance or product and Understanding the degradation mechanism. To provide information on drug sub product characteristics. Identification of potential degradants. Development of stability indicating assays. *(SIAMs).*

To establish a *re-test period* for the drug substance or a shelf life for the drug product and recommended *storage conditions*, Process development, Design and optimization of manufacturing process. Design of formulation (including selection of excipient for formulation.), Packaging development, and Stability studies are used to provide data to support clinical trials, registration submission, or commercialization.

Types of stability studies

Stability studies are used to provide data to support clinical trials, registration submission, or commercialization. There are different types of stability studies during the drug development processes[3].

Each phase of drug development requires addressing the time period that the drug product continues to maintain its specifications. This period is called *expiration dating* period of a drug product. Current GMP indicates that the purpose of stability testing of the final packaged drug product is to assure that a drug product meets applicable standards of identity, strength, quality, and purity at the time of use.

Stability of Active Pharmaceutical Ingredient (API)

Before any formulation work is developed, it is necessary to determine the nature of the API. Its purity profile must be established and specifications set for the allowed levels of impurities. The change of impurities with storage time must be established by subjecting the API to various accelerated and stress storage conditions to establish conditions which minimize the formation of degradants. These early stability studies may determine that the API should be stored under non-ambient conditions such as low temperature, low humidity, and non-oxidizing and low-light environments. These stability studies should be continued to determine the optimum storage conditions for holding the bulk API before actual processing.

Stability studies of the API will provide data to establish a retest time for the raw materials used in the process. Stability indicating methods must be developed to monitor the purity of the API as well as identification and quantitation of impurities. If impurities are shown to be process related, then they may be monitored at release but do not need to be monitored during long-term stability. However, if any of these impurities are shown to increase during storage, or if new impurities are developed, these are referred to as "degradants" or "degradation products", and analytical methods must be developed to monitor these degradants during stability studies. Quality specifications and limits must also be set for the degradants as required by ICH.

Stability Studies to Support Formulation Development

Excipients or non-active constituents may be added to an API to develop a formulation which meets the intended performance criteria of the drug product. These excipients may be necessary for purposes of adding color, or controlling pH, moisture, or oxygen content. Interaction of the excipients with one another or with the API will be determined, as well as the rates of these reactions, through stability studies. Data of these studies, so-called *excipient compatibility*, will be used to determine the appropriate formulation for the drug product. If interactions occur, then the products of these interactions (degradants) must be evaluated for safety, and analytical procedures for identification and quantitation must be developed. Krummen gave an overview of some issues which can arise in stability testing during preparation development. He indicated that stability testing is a continuous process as information on the drug substance and the first provisional dosage forms is synergistic and builds the basis for the development of the dosage form which will be marketed[4]. Many companies also manufacture small batches at the extreme of the manufacturing process capabilities. These batches are then placed on stability stations to determine the stability profiles of the drug product, to better understand the process capabilities.

Stability Studies to Support Production and Use of Pre-clinical and Clinical Supplies

During the formulation development studies, batches are made to support clinical studies. Pre-clinical stage formulations are usually used for testing in animals. Stability studies are performed to show that pre-clinical samples maintain their specifications over the entire time span of the animal study. The formulation being tested must be stable to assure that all animals receive the nominal dose and purity from start to finish of the study. As the drug product enters subsequent clinical phases, materials are needed to support these clinical

evaluations. Stability studies are necessary to support these materials. In most cases such studies would only require long-term storage; however, most companies conduct additional accelerated or stress studies on the clinical materials to gain more understanding of the drug product. This data set is also used to set expiry of clinical supplies.

Stability Studies to Support Drug Registration

Final packaged product must be shown to be stable up to at least the expiry date. These stability data are obtained by actual testing through the expiry date and beyond. Early term stability data may be submitted to FDA or other regulatory bodies to support preliminary expiry dating. These data as well as data obtained under accelerated storage conditions may be utilized to predict ultimate stability and to establish rates and kinetics of degradation. ICH requires at least 12-month long-term stability data of three batches of drug products as necessary for drug registration. In addition, accelerated and stress studies are also conducted to establish a tentative expiration date.

Stability Studies to Support Marketed Products

Expiry dating of a drug product must be determined on the actual packaged drug product over the period of time indicated by the expiry date. Although extrapolated stability data may be used to support product registration, real time data must be established to support actual product dating. In addition, sampling of newly manufactured production lots of product must be monitored on a continuing basis, at least to the projected expiration date or beyond, and data submitted to FDA. After approval is received for the drug product, stability studies are continued to support commercialization of the drug product. Representative lots are put on stability station for annual product monitoring. In addition, post approval studies would also be necessary if there is any change to the processing or packaging of the drug product.

The components of the mixture are separated by HPLC method by the principle of partition and then their contents are determined individually.

Table No. 1: Chromatographic Condition

Instrument	SHIMADZU UFLC-2000 Prominance LC-20AD Binary Gradient System SPDM 20 A Detector
Injector	Rheodyne
Column	Enable C18 G Column 250×4.6mm,5µm
Detector	PDA
Injection volume	20 µL
Flow Rate	1 mL/min
Wavelength	247 nm
Mobile phase	Phosphate buffer, Mixed pH 4.0:Acetonitrile (40:60) v/v

Reagents and chemicals

1. Disodium hydrogen phosphate (Thermo Fisher Scientific India Pvt. Ltd)

2. Potassium dihydrogen ortho phosphate (Thermo Fisher Scientific India Pvt. Ltd)

3. Glacial acetic acid (Thermo Fisher Scientific India Pvt. Ltd)

4. Acetonitrile, HPLC grade (SD Fine- Chem Limited)

5. Milliporewater

6. Amlodipine Besylate (Glochem Industries Limited, Hyd)

7. Indapamide (Supra Chemicals, Thane)

A method was developed for simultaneous determination of Amlodipine Besylate and Indapamide on HPLC by selecting the appropriate λ max, optimum mobile phase and flow rate which gives peaks with sharp and good resolution.

Selection of Mobile Phase:

Several solvent systems were tried to get optimum resolutions of Amlodipine and Indapamide in the proposed method. The observations obtained with various mobile phases in different ratios and the final mobile phase ratio is phosphate buffer, Mixed (pH 4.0) and Acetonitrile (40:60) v/v.

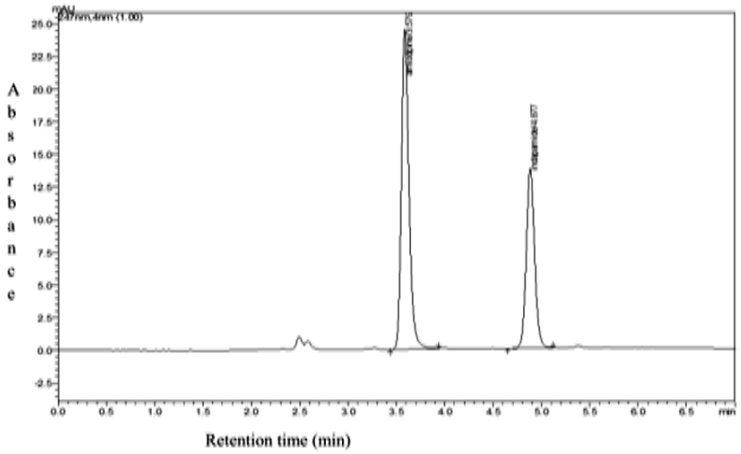

Fig. No. 1: Chromatogram showing peak separation Peaks of Amlodipine and Indapamide were well resolved with the solvent system of phosphate buffer, Mixed (pH 4.0) and Acetonitrile (40:60) v/v

DISCUSSION:

Simultaneous estimation of drug combinations in single dosage form plays a very important role in the field of pharmacy. One such combination is Amlodipine Besylate and Indapamide which is widely used for treatment of hypertension. Amlodipine Besylate and Indapamide combination dosage forms are marketed as Natrilam amongst others.

From literature survey it was found that many methods had been reported for determination of Amlodipine Besylate and Indapamide individually and in combination with other drugs. It this project work, it was proposed to develop and validate a RP-HPLC method for simultaneous determination of Amlodipine Besylate and Indapamide in bulk and marketed dosage formulations.

Standard drug of Amlodipine Besylate and Indapamide were obtained from Glochem Industries Limited, Hyderabad and Supra Chemicals, Mumbai. Natrilam tablet formulation was procured from market, containing 5 mg of Amlodipine Besylate and 1.5 mg of Indapamide. The objective of the proposed project was to develop and validate a RP-HPLC method for simultaneous estimation of Amlodipine Besylate and Indapamide in bulk and marketed dosage forms.

Various combinations of solvent system in different ratios of mobile phase were tried and the mobile phase of Phosphate buffer, Mixed (pH: 4.0) and Acetonitrile in ratio of 40:60 v/v was

selected and standardized as it evoked satisfactory resolution. The peaks with good resolution were observed for Amlodipine Besylate and Indapamide at the optimum flow rate of 1.0 mL/min which was used for our further study.

A RP-HPLC method was developed for the simultaneous estimation of Amlodipine Besylate and Indapamide using a C18 column (Enable C18 G, 250 mm x 4.6 mm, 5 µm) mobile phase consisting of Phosphate Buffer, Mixed pH 4.0: Acetonitrile in the ratio of 40:60 v/v, with flow rate of 1.0 mL/ min, The retention time of Amlodipine Besylate and Indapamide was observed at 3.54 and 4.87 min respectively. The developed method was then validated by using various parameters like accuracy, precision, linearity, specificity, ruggedness and robustness etc., as per ICH guidelines.

Specificity: The specificity of the proposed method was determined by studying the effect of impurities etc at the retention time of Amlodipine Besylate and Indapamide. Hence there was no interference from impurities with the peaks of Amlodipine Besylate and Indapamide, indicating a high degree of specificity for the proposed method.

Linearity: The linearity for the drugs by the proposed method was determined to study its ability to elicit test results which are directly proportional to concentration of the analyte in the sample. Standard solutions in the concentration range of **1 to 10 µg/mL** for Amlodipine Besylate and **0.3-3 µg/mL** for Indapamide in the mobile phase of Phosphate Buffer and Acetonitrile in the ratio of 40:60 v/v, flow rate of 1.0 mL/min and UV detection at 247 nm were injected in to the chromatograph. From the peak areas obtained the standard calibration curve was prepared. The proposed method is found to be linear at the concentration range of **1 to 10 µg/mL** for Amlodipine Besylate and **0.3-3µg/mL** for Indapamide. The percentage curve fittings were found to be **99.93%** for Amlodipine Besylate and **99.9%** for Indapamide.

LOD & LOQ:

The LOD and LOQ were determined by visualization method. The LOD was determined to find out the lowest amount of Amlodipine Besylate and Indapamide that can be detected and it was found to be **0.0002 µg/mL**and **0.00017 µg/mL** respectively. The LOQ was determined to find out the lowest amount of Amlodipine Besylate and Indapamide that can be quantified and it was found to be **0.00047 µg/mL** and **0.00021 µg/mL** for Amlodipine Besylate and Indapamide respectively, indicating that the small concentration in micrograms level can be determined with acceptable accuracy and precision.

Precision:

The precision of method and system was determined to study the concordance of data between the series of measurements. In system precision the % RSD value of peak area was found to be **1.06%** for Amlodipine Besylate and **0.74%** for Indapamide. In method precision the % RSD value of peak area was found to be **1.09%** for Amlodipine Besylate and **0.79%** for Indapamide. The intermediate precision of the method was determined by performing the assay at two different days (inter day and intraday) to check the reproducibility. On intraday % RSD value of peak area was found to be **0.77%** for Amlodipine Besylate and **0.27%** for Indapamide. On interday % RSD value of peak area was found to be **0.87%** for Amlodipine Besylate and **1.31%** for Indapamide. All the values of % RSD for precision study obtained were well within the acceptance criteria of NMT 2%. Thus, the proposed method was found to be providing high degree of precision and reproducibility.

Robustness:

The robustness of the method was determined by carrying out the assay after performing slight changes in the flow rate and mobile phase ratio. The flow rate was slightly altered from 1.0mL/min to **1.1mL/min, 0.9 mL/min** the % assay for Amlodipine Besylate was found to be **101.9 and 102.19%** and for Indapamide was found to be **100.2 and 100.4%** respectively. The composition of mobile phase was slightly altered from 40:60 v/v to **50:50 v/v** and **30:70 v/v,** the % assay of Amlodipine Besylate was found to be **99.1%** and **100.9%** and for Indapamide was found to be **98.4%** and **101.2%** respectively.

All the robustness results indicated that the new method developed was robust and did not show significant variations on slight changes in the flow rate and mobile phase ratio indicating lack of influence on the test results by operational variables for the proposed method.

Accuracy:

The accuracy was determined through recovery study of the drugs by spiking the standard drug of Amlodipine Besylate and Indapamide at three different levels 50%, 100% and 150% with the previously assayed samples of known fixed concentration. The percentage recovery was found to be **97.5% to 102.3%** for Amlodipine Besylate and **98.5% to 104.7%** for Indapamide indicating no interference of Amlodipine Besylate and Indapamide, the percentage recovery was in total agreement with acceptance criteria of **90- 110%.**

System suitability:

The system suitability parameters were calculated to ascertain the suitability of the proposed method in mobile phase Phosphate Buffer, pH 4.0: Acetonitrile from 40:60 v/v with flow rate of 1.0 mL/ min on Enable G C18 column (250 mm x 4.6 mm, 5µm particle size), The number of plates was found to be **28719** for Amlodipine Besylate and **52125** for Indapamide. The HETP was found to be **0.0087 mm** and **0.0047mm** for Amlodipine Besylate and Indapamide respectively, indicating the system suitability of the method. The asymmetry factor was calculated statistically and found to be **1.0** for both Amlodipine Besylate and Indapamide. The resolution of the method was good as found from the value of **1.33** indicating good and complete separation of the two components from each other with a well-defined baseline.

The developed HPLC method was then applied for the simultaneous estimation of Amlodipine Besylate and Indapamide in marketed formulations (Natrilam Tablets). The % assay of Amlodipine Besylate and Indapamide by proposed method was found to be in the range of **97.07-101.59%** and **98.74%-102.11%** respectively which was well within the acceptance criteria limit of 90% -110% indicating that the method can be applied for simultaneous determination of Amlodipine Besylate and Indapamide in formulations.

CONCLUSION:

A HPLC method was developed for the simultaneous estimation of Amlodipine Besylate and Indapamide in bulk drugs and marketed formulation. The HPLC system used was SHIMADZU UFLC-2000 Prominance LC-20AD Binary Gradient System. SPDM 20A detector with Rheodyne injector and Enable C18 G column 250x 4.6mm, 5µm. Injection volume of 20µL was injected and eluted with the mobile phase of Phosphate buffer, Mixed (pH4.0):Acetonitrile in the ratio of 40:60v/v at the flow rate of 1.0mL/min and UV detection at 247nm. The peaks of Amlodipine Besylate and Indapamide were found well separated with retention time 3.54min and 4.87min respectively. Hence the stability indicating HPLC method developed and validated can be used routinely for the simultaneous estimation of Amlodipine Besylate and Indapamide in bulk drugs and marketed formulation.

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