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Development and Validation of RP-HPLC method for simultaneous estimation of irbesartan and amlodipine besylate: A Review

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ABSTRACT: -To develop and validate a simple, specific, accurate, precise and sensitive reverse phase high performance liquid chromatographic (RP-HPLC) method with forced degradation studies for the simultaneous estimation of amlodipine besylate and irbesartan in the pharmaceutical formulation.

Methods: The chromatographic separation of the two drugs were achieved using Enable C 18G column (250 x4.6 mm; 5 um) in isocratic mode with mobile phase consisting of sodium acetate buffer (pH 4.0) and acetonitrile (30:70, % v/v) with a flow rate of 0.6 ml/min Ultraviolet(UV) detection was carried out at 238 nm. The proposed method was validated for linearity, range, accuracy, precision, robustness, limit of detection (LOD) and limit of quantification (LOQ). The tablet formulation was subjected to stress conditions of degradation including acidic, alkaline, oxidative, thermal and photolysis.

Keywords: Amlodipine Besylate, Irbesartan, RP-HPLC and Forced Degradation

I. INTRODUCTION: -

Amlodipine (as besylate), chemically is 3-Ethyl 5-methyl (4RS)-2- [(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulphonate1 (Figure 1). Amlodipine is a dihydro pyridine derivative with calcium antagonist activity2. It is used in the management of hypertension, angina pectoris and prinzmetal variant angina3. Amlodipine acts by inhibiting the transmembrane influx of calciumions into vascular smooth muscle and cardiac muscle and also acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure. It is official in BP 4. Hydrochlorothiazide, chemically is 6- Chloro-3,4-dihydro-2H-1,2,4- benzothiadiazine-7-sulphonamide 1,1- dioxide (Figure 2), is a diuretic, which inhibits active chloride re absorption at the early distal tubule via the NaCl co- transporter, resulting in an increase in the excretion of sodium, chloride and water. Literature survey reveals few analytical methods for the determination of Amlodipine alone and in combination with other drugs in pharmaceutical preparations and biological fluids, viz. Spectrophotometry5-8, HPTLC9-13 and HPLC14-18. Also there are some analytical methods reported for determination of HCT alone and in combination. No method has been reported for the estimation of Amlodipine (AMLO) and Hydrochlorothiazide (HCT) in combined dosage form with its application to in vitro dissolution. The reported HPLC methods so far in the literature are considered to be uneconomical, time consuming and have poor symmetry. In fact there is a need for the development of a novel, simple, rapid, efficient RP-HPLC analytical method with reproducibility for determination of Amlodipine Besylate and Hydrochlorothiazide in bulk and pharmaceutical dosage forms. The present study illustrates development and validation of a novel, simple, rapid and efficient RP-HPLC analytical method with reproducibilityor determination of Amlodipine Besylate and Hydrochlorothiazide in bulk and pharmaceutical tablet dosage form. The established method was validated with respect to specificity, linearity, precision, accuracy, robustness, LOD and LOQ according to ICH guidelines

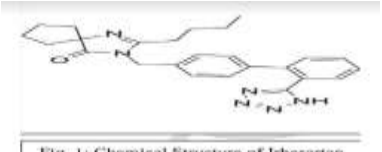
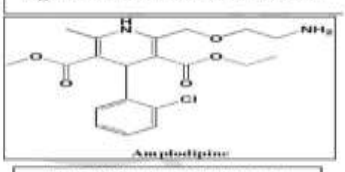


Fig. 1: Chemical Structure of Irbesartan



Amplodipine

Fig. 2: Chemical Structure of Amlodipine Besylate

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II. MATERIALS AND METHODS

Chemicals and Reagents The reference samples of Amlodipine besylate and Hydrochlorothiazide standards were kindly supplied as gift samples by Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. All the chemicals were analytical grade. Potassium dihydrogen orthophosphate and phosphoric acid from Rankem Ltd., Mumbai, India, while acetonitrile (HPLC grade) and triethylamine (HPLC grade) from Merck Pharmaceuticals Private Ltd., Mumbai, India. Ortho phosphoric acid used was of HPLC grade and purchased from Merck Specialties Private Ltd., Mumbai, India. Commercial tablets AMLONG-H were procured from local market. Instruments Quantitative HPLC was performed on a isocratic high performance liquid chromatograph (Shimadzu LC-20AT Prominence Liquid Chromatograph) with a LC-20AT VP pump, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength Shimadzu SPD-20A Prominence UV-Vis detector and Welchrom C18 Column (4.6 X 250 mm, 5 µm particle size). The HPLC system was equipped with "Spinchrome" software. In addition an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40), UV-Visible Spectrophotometer (Systronicsmodel-2203) were used in this.

Chromatographic Conditions Amlodipine Besylate and Hydrochlorothiazide were analyzed by various reversed phase columns like C8 and C18 columns. Among C8 and C18 columns, C18 (250 mm X 4.6 mm, 5 µm) column was selected. Various combinations of acetonitrile, phosphate buffer and methanol with triethylamine as column modifier were tested. The mixture of 10 mM Phosphate buffer (pH adjusted to 3.0 using triethylamine) and Acetonitrile in ratio of 50 : 50 v/v was selected as mobile phase and UV detection wavelength was 230 nm with a flow rate of 1 mL.min-1. Injection volume was 20 µL, with ambient temperature, run time was 6 minutes. Preparation of Mobile Phase A 10 mM Phosphate buffer was prepared by dissolving 6.056 g of potassium dihydrogen orthophosphate in 445 mL of HPLC grade water. To this 55 mL of 0.1 M phosphoric acid was added and pH was adjusted to 3.0 with triethylamine. The above prepared buffer and acetonitrile were mixed in the proportion of 50 : 50 v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication. Preparation of Standard Solution About 100 mg of pure Amlodipine besylate and Hydrochlorothiazide were accurately weighed and separately dissolved in 100 mL of mobile phase to get 1 mg.mL-1 stock solution. Working standard solutions were prepared with mobile phase. The final volume was made with the mobile phase. The standard solutions were filtered through 0.45 µm nylon membrane filter and degassed by sonication. Preparation of Sample Solution The content of 20 tablets of Amlong-H were accurately weighed and transferred into a mortar and ground to a fine powder. From this, tablet powder which is equivalent to one tablet was taken and the drug was extracted in 10 mL of mobile phase. The resulting solution was filtered using Whatman Grade No.1 filter paper and degassed by sonication. This solution was further suitably diluted for chromatography. Calibration Curve for Amlodipine Besylate and Hydrochlorothiazide Replicates of each calibration standard solutions (1-5 µg / mL for Amlodipine and 3-15 µg / ml for Hydrochlorothiazide) were injected using a 20 µL fixed loop system and the chromatograms were recorded. Calibration curves were constructed by plotting concentration on X-axis and peak areas on Y-axis and regression equations were computed. The calibration data is presented in Table 2. In vitro Dissolution Studies In vitro dissolution of six tablets containing AML and HCT was performed using 900 mL volume distilled water as the dissolution media at 50 rpm using an USP Apparatus II. The dissolution study was carried out in a 900 mL volume of distilled water as the dissolution media at 37°C (± 0.5) using the paddle method. 5 mL sample aliquots were withdrawn at 10, 20, 30, 45, 60 and 75 minutes using micropipettes and immediately replaced with equal volumes of fresh medium at the same temperature to maintain constant total volume during the test. All samples were filtered through 0.45 µm membrane filters. The concentrations of AML and HCT in samples were determined by the proposed HPLC method. Validation of the Proposed Method The developed method of analysis was validated as per the ICH guidelines for the parameters like system suitability, specificity, linearity, precision, accuracy, robustness and system suitability, limit of detection (LOD) and limit of quantitation (LOQ). System Suitability System suitability tests are an integral part of chromatographic method which was used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 10 µg

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mL-1 for Amlodipine besylate and Hydrochlorothiazide to check the reproducibility of the system. At first the HPLC system was stabilized for 40 minutes. One blank followed by six replicates of a single calibration standard solutions of Amlodipine besylate and Hydrochlorothiazide were injected to check the system suitability. To ascertain the systems suitability for the proposed method, the parameters such as theoretical plates, peak asymmetry, retention time and parameters were taken and results were presented in Table 1. Specificity The effect of wide range of excipients and other additives usually present in the formulation of Amlodipine Besylate and Hydrochlorothiazide in the determinations under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms. The common excipients such as lactose anhydrous, microcrystalline cellulose and magnesium stearate have been added to the placebo solution and injected and tested. The representative chromatogram of placebo was shown in Figure 3. The specificity results were presented in Table 4. Linearity The linearity graphs for the proposed assay methods were obtained over the concentration range of 1-5 µg / mL for Amlodipine besylate and 3-15 µg/ ml for Hydrochlorothiazide. A calibration curve was plotted between concentration and area response and statistical analysis of the calibration curve is shown in Figure 6 and 7. Precision Intraday and interday precision study of Amlodipine besylate and Hydrochlorothiazide was carried out by estimating corresponding responses 3 times on the same day and on 3 different days for the concentration of 10 µg. The percent relative standard deviation (% RSD) was calculated which is within the acceptable criteria of not more than 2.0. The results for intraday and interday precision were presented in Table 5. Accuracy (Recovery Studies) The accuracy of the method was determined by calculating recovery of Amlodipine besylate and Hydrochlorothiazide by the method of addition. Known amount of Amlodipine besylate and Hydrochlorothiazide at 80 %, 100 % and 120 Limito f Detection and Limit of Quantitation were % was added to a pre quantificd sample solution. The calculated using following formula recovery studies were carried out in the tablet in triplicate cach in the presence of placebo. The mean percentage

LOD=3.3(SD)/S and LOQ= 10 (SDY'S, recovery of Amlodipine besylate and Hydrochlorothiazide Where SD- standard deviation of risponse (cak area) and 2 each I level was not less than 99 % and not more than 101
s=slope of the calibration curve.
%. The results were presented in Table 6 The LOD and LOQ values are presented in Table 8.

Robustness

The Robustness vas evaluated by the analysis of Amlodipine besylate and Hydrochlorothiazide uander different experimental conditions such as making small changes in flow rate (# 0.2 ml/ min), detection wavelength H 5 nm), Mobile phase composition 5 %). The results were presented in Table

In vitro Dissolution Studies

The average percentage drugs released within 75 minutes as detected bv the proposed HPLC method after in vitro dissolution of tablets containing combination drug product are depicted in Figure 14. The dissolution pattern complies with the FDA standards, indicating suitability of the proposed method for the dissolution study of the two drugs According to the FDA Guidance (Qui, Xu 2007) no less han 85 % of the active ingredients of the labeled claim should be dissolved within 30 minutes. Dissolution values are presented in Table 9. The representative chromatograms of dissolution are shown in Figure 8 to Figure 13.

LOD and LOQ

Limit of Detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation SY the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy

APPARATUS AND EQUIPMENT

HPLC (Shimadzu-LC 20AT) C18 column (250 mm × 4.6 mm i.d., particle size 5 µm)

Camag Linomat V (Semiautomatic Spotting device) Camag Twin Tough Chamber (10 × 10 cm2) Camag TLC Scanner-3 Camag win CATS v.1.3.4 Software Hamilton Syringe (100 µl) Digital weighing balance– Denver SI234, Germany Volumetric flask – 10 ,25 and 100 ml Pipettes – 1, 2, 5 and 10 ml

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III. CONCLUSION

The simultaneous determination of combination anti-hypersensitive amlodipine besylate and irbesartan using the suggested RP-HPLC method was found to be sensitive, accurate, precise, easy to use, and quick. The majority of the work should go into method development and optimisation when creating an HPLC method because doing so will enhance the performance of the finished method. It was discovered that specific chromatographic conditions may distinguish between irbesartan (Rt = 5.133) and amlodipine besylate (Rt = 3.170) with a resolution of 7.778. The methods were validated for linearity, accuracy, precision, limit of detection, limit of quantification, and sensitivity in accordance with ICH criteria. For routine examination of the raw ingredients in combinational dose formulations combining amlodipine besylate and irbesartan, the present RP-HPLC method can therefore be utilised.

REFERENCES

[1]. Bhardwaj SK, Dwivedi K, Agarwal DD. A review: HPLC method development and validation. International Journal of Analytical and Bioanalytical Chemistry. 2015;5(4):76-81.

[2]. Waghmare AN, Muddukrishna BS, Vasantharaju SG. Analytical method development and validation of simultaneous estimation of amlodipine and atorvastatin by RP-UPLC. Mintage Journal of Pharmaceutical & Medical Sciences. 2014;3(2):22-5.

[3]. Patwekar SL, Sakhare RS, Nilesh NN. HPLC Method Development and Validation–A General Concept. International Journal of Chemical and Pharmaceutical Sciences. 2015 Mar;6(1):8-9.

[4]. Shankar CH, Suthakaran R, Kumar PB. A New RP-HPLC Method Development and Validation for the Simultaneous Estimation of Febuxostat And Ketorolac in Bulk and Tablet Dosage Form. International Journal of Biomedical Investigation. 2018; 1: 113.doi: 10.31531/2581.;4745(2):4- 6.

[5]. Svec F, Fréchet JM. Continuous rods of macroporous polymer as high-performance liquid chromatography separation media. Analytical Chemistry. 1992 Apr 1;64(7):820-2.

[6]. Kazakevich YV, Lobrutto R. HPLC for pharmaceutical scientists. John Wiley & Sons; 2007 Feb 16.

[7]. Tamaoka J, Komagata K. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS microbiology letters. 1984 Nov 1;25(1):125-8.

[8]. Striegel A, Yau WW, Kirkland JJ, Bly DD. Modern size-exclusion liquid chromatography: practice of gel permeation and gel filtration chromatography. John Wiley & Sons; 2009 Jul 31.

[9]. Kopaciewicz W, Rounds MA, Fausnaugh J, Regnier FE. Retention model for high-performance ionexchange chromatography. Journal of Chromatography A. 1983 Aug 26;266:3-21.

[10]. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. John Wiley & Sons; 2012 Dec 3.

[11]. Van Leeuwen JA, Buydens LM, Vandeginste BG, Kateman G, Schoenmakers PJ, Mulholland M. RES, an expert system for the set-up and interpretation of a ruggedness test in HPLC method validation: Part 1: The ruggedness test in HPLC method validation. Chemometrics and Intelligent Laboratory Systems. 1991 Apr 1;10(3):337-47.

[12]. McCalley DV. Selection of suitable stationary phases and optimum conditions for their application in the separation of basic compounds by reversed-phase HPLC. Journal of separation science. 2003 Mar 1;26(34):187-200.

[13]. Swartz M. HPLC detectors: a brief review. Journal of Liquid Chromatography & Related Technologies. 2010 Jul 13;33(9-12):1130-50.

[14]. Subirats X, Roses M, Bosch E. On the Effect of Organic Solvent Composition on the pH of Buffered HPLC Mobile Phases and the p K a of Analytes—A Review. Separation & Purification Reviews. 2007 Aug 1;36(3):231-55.

[15]. Ahuja S, Rasmussen H, editors. HPLC method development for pharmaceuticals. Elsevier; 2011 Sep 21.

[16]. Muñiz-Valencia R, Ceballos-Magaña SG, Rosales-Martinez D, Gonzalo-Lumbreras R, Santos-Montes A, Cubedo-Fernandez-

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Trapiella A, Izquierdo- Hornillos RC. Method development and validation for melamine and its derivatives in rice concentrates by liquid chromatography. Application to animal feed samples. Analytical and bioanalytical chemistry. 2008 Oct 1;392(3):523-31.

[17]. Pham-Tuan H, Kaskavelis L, Daykin CA, Janssen HG. Method development in high-performance liquid chromatography for high-throughput profiling and metabonomic studies of biofluid samples. Journal of Chromatography B. 2003 Jun 15;789(2):283-301.