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AMLODIPINE AND ATENOLOL: AN ANTIHYPERTENSIVE COMBINATION THERAPY WITH VARIOUS ANALYTICAL TECHNIQUES

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ABSTRACT

Among the popularly prescribed agents for hypertension treatment, Atenolol and Amlodipine are frequently used. The estimation of the levels of these compounds is therefore crucial for controlling quality, whether they are used as pharmaceutical preparations, raw materials, or in combinations. The major goal of the current study was to bring together as well as evaluate broadly dispersed findings from published investigations regarding viable, effective, as well as fast analytical techniques for estimating these drugs. The following sections offer information that might be useful to guide as well as facilitate the development of future experiments to enhance analytical procedures in order to detect these compounds in formulations. The study discusses the findings from several analytical techniques assessing Atenolol and Amlodipine separately and in combination that have been reported between 2000 and 2021 incorporating HPLC, U.V-Vis, Electroanalytical, titrimetric, capillary electrophoresis.

KEYWORDS: Atenolol, Amlodipine, hypertension, analytical techniques.

INTRODUCTION

The World Health Organization has classified hypertension as one of the leading causes of illness as well as death in the entire world, accounting for nearly nine million deaths per year.[1,2] In hypertension, the systemic arteries are marked by consistently elevated blood pressure. For calculating an individual's B.P their systolic blood pressure is divided by the diastolic.[3]

The early stages of high blood pressure are often asymptomatic, which is why it's called a "silent killer." Regular blood pressure checks are necessary because of hypertension's asymptomatic nature and the illness load it carries.[4,5,6] Aside from that, uncontrolled hypertension could really negatively impact the brain, heart, blood vessels, kidneys, and other organs. Heart disease, renal failure, blindness, or vision loss, as well as several other serious ailments, may result from harm to these organs.[7] Blood pressure can be controlled by taking medication and adopting changes in lifestyle.[8,9] Either as single-treatment or in combination, they are both effective.[10] When it comes to treating hypertension, for patients with uncomplicated hypertension beta-blockers are used as the first line of treatment because they are safe as well as efficacious.[11] Atenolol is an antagonist of beta-receptor that is cardioselective. Atenolol selectively inhibits beta 1 receptor while for beta-2 receptors there is a reduced affinity. In addition to hypertension, arrhythmias, and angina, Atenolol is being employed for the treatment of heart failure and myocardial infarction.[12,13] There are around 40 million prescriptions written for Atenolol each year in the United States alone, making it one of the top frequently prescribed beta-blockers in the world.[14,15] The initial introduction of Atenolol occurred in 1976 and later on was approved by USFDA as hypertension and CAD (coronary artery disease) treatment since August 1981.[16] Aside from becoming one of the most commonly utilized b-blockers, this has frequently been employed as a reference medication in hypertension randomized controlled trials.[17,18,19]

Atenolol functions through specifically binding with beta-1 adrenergic receptors located in vascular smooth muscle as well as the heart, thereby inhibiting the activities of catecholamines which include isoproterenol, norepinephrine, as well as epinephrine, and preventing sympathetic activation. In addition to lowering heart rate and blood pressure, this activity also reduces myocardial contractility.[20,21] Chemically, Atenolol can be described as follows: 4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzeneacetamide[22,23] 266.34 is the molecular weight of Atenolol ($C_{14}H_{22}N_2O_3$). When it comes to water solubility, at 37 ˚C it has a solubility of 26.5 mg/ml with a 0.23 log partition coefficient value. It comes in the form of powder which is white or almost white in color. In methanol, Atenolol is freely soluble, whereas in acetic acid, DMSO it is soluble, and in 96% ethanol it is sparingly soluble. Atenolol bioavailability ranges from 45% to 55%. About 6-16% of the medication is protein-bound, leading to mainly stable plasma drug levels.

Atenolol has an elimination half-life of 6 to 7 hours and the Kidneys are the main route of excretion.[24, 25, 26] Based on particular indications, the route of administration as well as dosage may differ. Atenolol medication is often initiated using modest doses and increased gradually until the intended therapeutic response is obtained. While discontinuing therapy, dosages must be lowered gradually.[27,28] Side effects[29,30] include confusion, dizziness, diarrhea, dyspnea, headache, vomiting, sleep disorders, constipation, and heart failure.

Amlodipine being a calcium channel blocker of the third generation dihydropyridine (DHP) class that is long-acting, and lipophilic.[31,32] Amlodipine has been a good medication having a wide range of uses, including blood pressure control, anti-atherosclerotic characteristics as well as antianginal effects. Its action is carried out by restricting calcium entering cells of vascular smooth muscle as well as cardiac, thereby minimizing peripheral vascular resistance to lower blood pressure eventually.[33,34] Chemically Amlodipine is 3-~{O}-ethyl 5-~{O}-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5 dicarboxylate.[35,36] The long half-life of Amlodipine makes it suitable for once-daily dosing, that helps facilitate patient compliance.[37] It is recommended that 5 mg of initial dose and 10 mg of highest regular dose be used. 2.5 mg of the initial dose is indicated for the elderly as well as for individuals with hepatic failure. Amlodipine has a 60 to 65% bioavailability upon oral administration as well as peak plasma concentrations are obtained after 6 - 8 hrs of administration. In the liver, it is metabolized predominantly and gradually eliminated having 40 to 50h of elimination half-life. About 98% of the medication is protein bound. There are many reasons to use this medication, including that it is safe, as well as potent. Additionally, it is often used alone or as part of a combination therapy to treat hypertension.[38, 39]

The majority of individuals having high blood pressure need more than one medication for hypertension, especially when co-morbid diseases are present.[40] Fixed-dose combination for hypertension provides various possible advantages, which include faster blood pressure control and better tolerability when compared to monotherapy with a higher dose. Moreover, reducing costs and improving compliance may be some additional advantages[41-44] Amlodipine with Atenolol FDC Treatment has become popular for treating high blood pressure and chronic stable angina.[45] It was discovered by Mettimano M et al. that the hypertensive individuals using Atenolol alone were not able to manage hypertension whereas adding Amlodipine with Atenolol resulted in a significant drop in blood pressure when compared to placebo. The combination of Amlodipine and Atenolol beta-blocker minimized side effects, confirming the combination's usefulness[46]

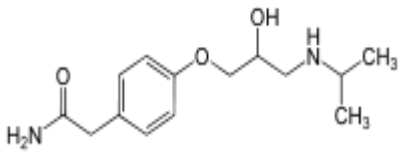

Fig. 1: Structure of Atenolol.

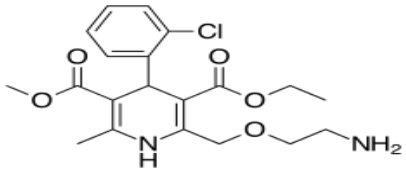

Fig. 2: Structure of Amlodipine.

ANALYTICAL METHODOLOGIES FOR ATENOLOL

Ultaviolet (UV) and Visible spectrometric methods

Aboud KM. *et al.*[47] developed analytical method which is sensitive, simple, and validated with the goal of assaying Atenolol raw materials as well as estimating actual Atenolol concentration in pharmaceutical tablets utilizing spectrophotometer equipment. As a first step, the study examined the absorption spectrum of Atenolol in (0.1N) hydrochloric acid against a blank solution in the UV range. For linearity, the method has been tested by making preparations over a range of different concentrations then measuring their absorptions, as well as calculating the correlation coefficient, Sandell's sensitivity as well as molar absorptivity. Recovery experiments were used to check the method's accuracy. The RSD was used to express the method's precision. It was found that a 0.1N hydrochloric acid solution of Atenolol showed maximum absorption at the wavelength of 225 nanometers (nm). The concentration-absorbance relationship appeared linear, having 0.9979 correlation value with molar absorptivity and Sandell's sensitivity value 7.163103Lmol^{-1}cm^{-1}, and 0.034g/cm^2 respectively. Concentrations between 5 to 40µg/ml is suitable for this technique. Validation results on this method revealed that it was statistically validated. When used to quantify Atenolol in its pharmaceutical formulations, the proposed approach proved to be accuracy, precise, and specific.

Lalitha G. *et al.*[48] developed a new U.V spectrophotometric method for determining Atenolol in tablet formulation as no published analytical procedure utilized solvent ammonium acetate solution ph 6 enabling Atenolol estimation in single or in a combination dosage form. The maximum absorbance of Atenolol in ammonium acetate solution was 273.2nm. As long as the concentration was between 2 and 30 micrograms per milliliter, it followed Beer's law. The obtained correlation coefficient value of 0.9976 confirms that the absorbance increases linearly when Atenolol concentration increases. The Atenolol's slope estimated was 0.0348. The detection and quantification limits were determined to be 0.05µg/mL and 0.17 µg/mL,

respectively. In addition, reproducibility values ranged from 98.75 percent to 100.16 percent, with RSD of ≤0.055 percent. Since the method uses solvent ammonium acetate solution for the analysis, this novel, simple, accurate, and reproducible analytical method could be routinely applied to the Atenolol analysis in tablet formulations.

Singh S. *et al.*[49] developed a simple spectroscopic method estimating in bulk Atenolol that was efficient, economical, as well as accurate. A UV-visible spectrophotometer (double beam) was used to accomplish the spectroscopic procedure (1700, Pharmaspace, Shimadzu, Kyoto, Japan). With varied pH solvents (2.5, 4.5, 6.5), various dilution concentrations were made to determine the absorbance of the solution. For performing validation, measured each buffer's maximum absorbance (max) and found that it was 224.30 for pH 2.5, 224.50 for pH 4.5, and 225.00 for pH 6.5. Studies were conducted on accuracy, (interday and intraday) precision, recovery, and linearity. In addition to the limit of detection, the limit of quantification is also included. Atenolol in pharmaceutical bulk, as well as dose form, can be estimated and validated using this method.

Syed S. *et al.*[50] developed and validated in accordance with ICH requirements a UV spectrophotometric approach which was sensitive and rapid for assessing Atenolol routinely in tablets. At 226.6 nm, the UV spectroscopy was conducted, and for preparation of samples phosphate buffer at p^H 6.8 was used. As a result of the linearity, a correlation coefficient value 0.9988 was obtained. Also, measured several validation parameters including LOD, LOQ, precision, accuracy, recovery study as well as range. In addition, the presented technique was easy to use and could be utilized to analyze Atenolol on a routine basis.

Zhuk YN. *et al.*[51] quantified Atenolol using a visible spectrophotometric approach relying upon the colored product absorbance by bromthymol blue and Atenolol reaction at a wavelength of 402 nm using acetone medium. For estimating Atenolol quantitatively in the pharmaceutical medications the ideal conditions were determined. Atenolol and bromthymol blue stoichiometric relationship coefficients are calculated. It appears that the technique is reproducible and adequate for routine quality control, based on the results of the experiments. In accordance with Ukrainian Pharmacopeia's validation requirements, the presented method was found to be valid.

Prashanth KN. *et al.*[52] proposed 3 spectrophotometric approaches that are sensitive and simple to estimate Atenolol in tablets as well as bulk. The method involves brominating Atenolol with bromine produced in situ as a result of acid action on the bromate–bromide mixture after which unreacted bromide is determined through reaction either with MCP (metacresol Purple) at 540nm i.e. method A, at 445nm i.e. method B or with erioglaucine (EGC) at 630nm i.e. method C. According to Beer's law, methods A, B and C is applicable within 1.0–20.0, 2.0–40.0, and 1.0–8.0 µg/mL of concentrations Furthermore, correlation coefficients and Sandell's sensitivity value as well as the limits of quantification and detection were included. T and f student tests were applied comparing recovery results to a reference method statically. The presented work is unique in that it uses MCP to show two separate colors. At 540 nm in acid, medium MCP shows the red-pink color and at 445nm brominated MCP shows yellowish-orange color.

Madhurai P *et al.*[53] developed a visible spectrophotometric method to determine Atenolol in the pharmaceutical dosage form. Colored chromogen (-napthol) was used as the basis for the method. Both crude and commercial samples of Atenolol had a λmax of 549nm and beer's law was used for the analysis. When the concentration was between 2 -10µg/ml, Beer's law was followed. For regular Atenolol determination, the proposed techniques were deemed to be the original type since they were definite, clear, consistent, and absolute. The approach has been statistically validated. For visible spectra, 5.88µg/ml and 17.83µg/ml were determined as the LOD and LOQ respectively. 0.999 was the correlation coefficient value. As a result, the purity was 99.5 percent.

Antakli S. *et al.*[54] has developed a spectrophotometric method for rapid as well as simple Atenolol determination both in tablet as well as bulk formulation. The basis of the method involves the use of 1,2-dichloroethane medium for the creation of yellow ion-pair complex by Bromocresol green and Atenolol. 414nm was the complex's maximum absorption wavelength determined. The effect of solvents, time, reagent concentration, correlation ratio, and other various reaction-related parameters were all optimized. At maximal absorption, the generated complex was spectrophotometrically measured. It was found that 2.66 - 26.63µg/mL was the Linearity range. 0.9999 was found to be the correlation coefficient value in regression analysis. 0.22µg/mL and 0.66µg/mL were to be the detection and quantification limits, respectively. For Atenolol, the average recovery percent was 97.23–101.53%. The approach has been effectively employed in six Syrian pharmaceutical trademarks to estimate Atenolol in pharmaceutical tablets. It is a sensitive, direct, simple, and no extraction process required method that is easily adapted to routine regular analysis and quality control.

High performance liquid chromatography (HPLC) methods

Jadhav S. *et al.*[55] performed Atenolol analysis in bulk, developed and validated Reverse Phase -HPLC method that was easy, accurate, sensitive, and precise. At 25°C HPLC separation was performed using a HiQSil C18HS column with a ph 5 Ammonium Acetate Buffer (20mM):

Acetonitrile (80:20) mobile phase flowing at 0.75 ml/min. At a wavelength of 273 nm, UV detection has been performed. A retention time of 4.606 min was found. ICH guidelines were followed for range and linearity, system appropriateness and selectivity as well as precision and accuracy as well as detection and quantitation limits. Calibration range 5 – 40 µg/ml resulted in a linear calibration curve having an r2 value = 1. It was found that the method was precise having 0.3508 and 1.2135 as repeatability and intermediate precision values respectively. Using the standard deviation method there were 0.075µg/ml and 0.23µg/ml as detection and quantitation limits for Atenolol, respectively.

Mohammed NS. *et al.*[56] determined Atenolol using An RP-HPLC method in bulk as well as pharmaceutical formulations and validated it. To optimize the method, the following chromatographic conditions were used: Inertsil column with the volume of injection 20 µL at 30 °C temperature keeping 1 mL/min flow rate, acetonitrile: water 60: 40 with 276nm detection wavelength. The method results were not significantly different when employing two HPLC systems, one paired with a PDA detector and the other with a UV detector. As a result of the method's validation, both systems showed good precision with less than 1 RSD %, 0.5 and 1.5 µg/mL are detections and quantification limits respectively and R2 value more than 0.998 showing acceptable linearity. A high recovery of 97.7% was achieved after application on the tablet dosage form. Therefore the method may be used for routine analysis being rapid, economic, and simple.

Bhaskara BL. *et al.*[57] has developed and validated a new method using RP-HPLC for Atenolol quantification in pharmaceutical formulations. For performing HPLC determination Atlantis dC18, a reversed-phase column was used with mobile phase flowing at 1.0 mL/min and 225 nm set for ultraviolet detection. From the 1-100 lig mL-1 range, the relationship of concentration and mean peak area of Atenolol was found to be rectilinear, exhibiting 1.0 lig/ml and 0.4 lig/ml as quantitation and detection limit. Following the ICH guidelines, validation parameters were developed including specificity, precision, linearity and range, ruggedness, and robustness. Student's F and t-test were used to compare the results to that of the literature or reference methods. Accuracy was 98.3-102.5 percent when evaluated using the spike recovery method. A retention time of 3.39 minutes characterized the method. In addition to being accurate and precise, the method has shown to be a good fit for routine application in pharmaceutical quality control.

Eaga CM. *et al.*[58] determined in pharmaceutical formulation, Atenolol enantiomers by developing a method based on RP-HPLC that rapid, sensitive, precise for development of approach column a Chiralcel AGP was used, a buffer of sodium phosphate of 10 mM having pH-7 to methanol in ratio 95:5 v/v used as the mobile phase along with this the detection wavelength used was 225nm keeping 0.9 ml/min flow rate. From 10 to 100 µg/ml, the linearity ranged. 7.05 - 9.43 and 2.69 - 3.97 were the assay method's inter and intraday coefficient of variation respectively. RP-HPLC can be used to analyze Atenolol enantiomers in a variety of pharmaceutical dosage forms.

Hussein AA. *et al.*[59] for Atenolol determination in human plasma author developed an HPLC method which is new, sensitive, specific, and precise that can be employed for pharmacokinetics study. (75:25) dichloromethane: 2-propanol was used to extract the drug-using technique of liquid-liquid extraction from plasma. For an internal standard, bamethan sulfate was employed. For achieving adequate resolution, the ODS-3 C18 Intertsil column was employed for analyzing samples along with using mobile phase in isocratic mode composed of 0.5 % triethylamine: methanol in 90:10v/v ratio and 3.5 as final pH and 1.3 ml/min was the flow rate. Into the HPLC system, a 60µl sample was injected using Spectra auto sample and performed separations at room temperature while monitoring the excitation and emission wavelengths at 228 and 298 nanometers, respectively. Atenolol and bamethan showed a peak area with retention times of 6.4 and 10.4 minutes, respectively. At 30, 400 and 700 ng /ml the inter and intraday precision were determined to be 3.229%, 1.471%, 3.246% and 1.909%, 1.571%, 1.358% respectively. Atenolol's relative recovery percent determined were 100.733, 99.948, and 98.599 at 30, 400, and 700ng/ml. For the Atenolol quantification in human plasma, the method proved to be accurate, as well as sensitive. This method has been effectively applied in pharmacokinetics studies.

Yilmaz B. *et al.*[60] presents a method for Atenolol determination in human plasma using high-performance liquid chromatography. Method liquid-liquid extraction was used to extract metoprolol and Atenolol from plasma. A reverse-phase Ace C18 column was used for the development of the method, along with (50:50, v/v) methanol-water as a mobile phase comprising trifluoroacetic acid in 0.1%. A linear calibration curve was obtained throughout 5–150 ng/mL of concentration range. For Atenolol the precision values were below 6.1 for both Intra-day and inter-day and for plasma 98.4% was Atenolol's mean recovery. 1.5 and 5 ng/mL were detection and quantification limits respectively.

Capillary zone electrophoresis (CZE) methods

Hasanzadeh M. *et al.*[61] employed capillary electrophoresis (CE) in combination with a UV-diode array detector for Atenolol photodegradation product characterization. for this assay, at 298 K and in less than 7 minutes optimum separation was achieved using fused-silica capillary column along with running buffer of pH 5.3 of acetate buffer (60 mM) mixed with methanol/ethanol mixture in 20:80% v/v ratio. 16.0 kV

of voltage was used to inject the samples. The capillary electrophoresis approach has been shown to be effective in Atenolol photodegradation products detection. The proposed approach was employed to determine the studied medication in human serum, as well as the findings, had been compared to other methods that are previously reported.

Azzama KMA. *et al.*[62] developed a method for the determination of Atenolol, chlorthalidone, and amiloride, simultaneously in pharmaceutical formulations using capillary zone electrophoresis. Examined various factors having influence including (buffer pH, concentration, applied voltage, capillary temperature, and injection time). As internal standard phenobarbital was used and within 4 min. Separation of all analytes occurred. Normal polarity mode was used for performing separation with 25 kV as well as employing (10 s) hydrodynamic injection at 25°C. A fused-silica capillary was used for the separation with 25 mm H3PO4 background electrolyte adjusted using 1 m NaOH solution having pH 9.0. At 198 nm detection was performed. Validation was performed in terms of linearity, the limit of detection and quantification, precision, accuracy, and selectivity. For both chlorthalidone as well as Atenolol linear calibration curves were obtained in the 1–250 μg/mL range whereas for amiloride it was in the 2.5–250 μg/mL range. amiloride, Atenolol, and chlorthalidone were simultaneously determined using the approach with good accuracy as well as precision in a variety of pharmaceutical tablet formulations.

Arias R. *et al.*[63] developed method in which Atenolol a beta-blocker was determined in plasma using capillary zone electrophoresis. 58.5 cm (with 50 cm effective length) uncoated silica capillary, which had a 375 mm I.D., was employed for carrying out separation, and 194nm was selected for detection. Studies were conducted on the effects of injection time, buffer, plasma clean-up technique, and applied voltage. At 50 mbar, Hydrodynamic injection of 4 seconds together with +25 kV applied voltage was utilized for Atenolol determination within 3 minutes employing in 50:50, v/v ratio electrolyte of H3BO3 (50 mM) –Na2B4O7 (50 mM), pH 9. Nine patients with hypertension (aged 39 to 73) were employed for this method and Atenolol was determined in their plasma. The concentrations of Atenolol discovered range from 30 to 585 ng/ml.

Titrimetric Methods

Basavaiah K. *et al.*[64] developed titrimetric method regarding Atenolol determination in pharmaceutical bulk form as well as in tablet form based on (CAT)chloramine-T. The procedure entails Iodometric back-titration to determine the remaining amounts of oxidants after the drug has been oxidized to an excessive amount of chloramine-T assessed in an acid media. The applied range of titrimetry is 3-20 mg. There is a discussion of the ideal experimental settings selection for obtaining the best possible accuracy precision, and sensitivity precision, and accuracy. No interference is caused by excipients and other ingredients contained in tablet forms. In tablets, the method described has been used successfully to determine medication. 96.43-103.74 percent was the recovery range.

Prashanth KN. *et al.*[65] have developed two non-aqueous titrimetric approaches that are cost-effective, reliable, simple, and accurate regarding Atenolol determination in pharmaceutical bulk and formulation. To determine the endpoint, either visually employing crystal violet for the indicator purpose or potentiometrically employing electrode-SCE system, drug titration in CH_3 COOH with acetous perchloric acid is used. For the 1.5-15 mg Atenolol range, the approaches might be applied, while the calculation relies upon 1:1 reaction proportions (stoichiometry). When in dosage form Atenolol was determined using these methods results obtained by these methods and the reference method were in good agreement. The results of precision were good as the values of RSD were below 2.35 percent. In addition, the accuracy results were also good. According to the standard addition approach, utilized for the recovery experiment, the proposed techniques have no interference from excipients utilized in drug formulations showing a recovery percentage of 99.56 -101.2 percent and ≤ 0.31 percent of S.D.

Electrochemical Method

Cervini P. *et al.*[66] the authors in pharmaceutical preparations determined Atenolol by employing a substitute electrode graphite–polyurethane composite. It was found that for 4–100 mol/L an analytical curve was linear when utilizing the DPV technique. 3.16 mol/L was the LOD obtained value and around 95.5 -108 percent were the recoveries. From other drugs that were antihypertensive Interference was identified although not with normal tablet components. There was about 95 percent agreement between the proposed method's results and those of HPLC.

Goyal RN. *et al.*[67] described that at physiological pH, the voltammetric assessment of Atenolol utilizing carbon electrode C60 was discovered as having good electrocatalytic activity. As the overpotential related to the oxidation of Atenolol decreases, it suggests the presence of an electrocatalytic electrode. Using modified electrode at 7.2 pH Atenolol assessments was performed. When Atenolol concentrations were 0.25–1.5mM, high co-linearity was shown by the calibration plot together with 0.997 value of correlation coefficient. The method was being determined to have a sensitivity of 8.58 μA mM^{-1}. A 0.16 mM detection limit has been reported. The devised approach can be used effectively, in pharmaceutical formulations as well as urine samples regarding the assessment of Atenolol. The surface coverage of the modified electrode with C60 was great.

Goyal RN. *et al.*[68] Differential pulse voltammetry was used to study the methylprednisolone (MP) electrochemical response at carbon electrode modified fullerene-C60. The findings of the experiments indicate that methylprednisolone oxidation has an electrocatalytic impact by the modified electrode leading to peak current response significant improvement. MP concentration was shown to be linearly related to the oxidation peak current in the 5.0 nM–1.0μM range and $0.0107\mu A\mu M^{-1}$ value were for sensitivity. According to estimates, 5.6 nM was the detection limit. This method has also been effectively used for the MP determination in urine samples, and also in human serum together with pharmaceutical preparations as before the analysis there was no need for treatment of sample and even extraction steps. The findings of MP estimation in biological fluids were cross-validated using the GC–MS method, the obtained findings demonstrated a variation of about 2% achieved from the method proposed.

GC/MS Methods

Yilmaz B. *et al.*[69] developed a procedure in order to estimate Atenolol in human plasma and validated it. At basic pH Utilizing butanol and chloroform mixture the extraction of Atenolol as well as internal standard metoprolol from plasma was performed. GC–MS was used to evaluate the extracts after they were derivatized. For 15–250 ng mL^{-1} range concentration linear Calibration curves were reported. In human plasma, it was discovered that precision values remained below 7.4 for Atenolol. 90.46 percent on average of Atenolol was recovered from human plasma. It was found that 5 and 15 ng/mL were Atenolol's detection and quantitation limits respectively. The approach had been effectively used on six hypertensive patients who were provided Atenolol 50 mg oral tablet.

ANALYTICAL TECHNIQUES FOR AMLODIPINE

Ultaviolet (UV) and Visible spectrometric methods

Bhargavi P. *et al.*[70] within pharmaceutical preparations, regarding the assessment of Amlodipine Besylate a fast as well as efficient visible spectrophotometry approach has been devised. The basis of the approach has been green complex production employing H2SO4 50percent as well as 0.1N Potassium dichromate. This produced complex exhibits 600 nm as maximum absorption and in 0.7-7μg/ml range complies with Beer's rule. Considering applications in the Q.C laboratory, the approach established was validated statistically with 1.7% and 1.8 % intra and interday precision values respectively. 99.98-100% was the recovery range with 0.00666 $\mu g/cm^3$ Sandell's sensitivity.

Naveed S. *et al.*[71] employed spectrophotometric approach to perform Amlodipine assay. 238nm was selected as absorbance maxima with water as solvent forming the basis of the approach. An Amlodipine-containing solution and other dilutions were prepared by dissolving the drug sample with water. The test has been centered upon the measurement of Amlodipine dilution absorption at 238 nm of wavelength. The % assay of 4 distinct dilutions - 6.25, 12.5, 25,and 50ppm was estimated.

Bernard S. *et al.*[72] developed UV approach enabling Amlodipine besylate estimation employing urea solution (2 M) in formulations as well as in pharmaceutical bulk. To perform the spectrophotometric study, several solvents including acetonitrile, dimethyl formamide, chloroform, and methanol were used to solubilize weakly water-soluble compounds. 243 nm was the absorption maxima of Amlodipine. Beyond 225 nm there was a lack of absorbance by urea indicating no interference in drug determination. Throughout the 5-25μg/mL concentration level, beer law was proven to have complied with. Under the present procedure, no diluents, as well as common additives, are interfered with. 0.99863 was the Amlodipine correlation co-efficient value. Amlodipine had a percentage recovery of 99.94 to 99.96. The procedure was exact, accurate as well as cost-effective. The analysis findings were validated according to ICH criteria.

Patil VP. *et al.*[73] proposed spectrophotometric technique regarding Amlodipine Besylate analysis as well as performed validation in bulk drugs. Within Sodium Bicarbonate availability Amlodipine Besylate having primary amine reacts with ninhydrin forms the basis of the approach. The reaction takes 15 minutes to complete at about 97°C and at 566 nm generated product of purple color shows maximum absorbance. 50-250μg/ml was the range of concentrations in which Beer-Lambert's law holds true having 0.999 as the regression coefficient value. In order to optimize the overall technique, examined the important variables impacts including color stability, heating duration, temperature, and color reagent concentration. 0.2729 and 0.09 μg/ml were the LOQ and LOD values respectively for Amlodipine. The presented technique exhibits great accuracy, sensitivity, stability, and precision according to the validated findings. The process is environmentally clean as well as economical since there are no organic solvents employed.

Jaina N *et al.*[74] measured the Amlodipine besylate quantitatively in tablet dosage form with, an accurate, safe, and novel spectrophotometric approach which has been devised utilizing sodium acetate solution (2 mol/L). When compared to pure water, Amlodipine besylate solubility in sodium acetate solution was 75 times higher. At 365nm maximum absorbance was shown by Amlodipine besylate. Over 240nm there was no absorbance by Sodium acetate so there was zero interference with drug evaluation. Within 50 to 250 $\mu g.mL^{-1}$ concentration Beer's Law was complied by the drug sample having 97.84 to 100.16% of the recovery. In compliance with ICH criteria the method created has been validated, and it was determined being in good agreement with specified results.

Varma BHR. *et al.*[75] have established UV procedure for Amlodipine besylate estimation quantitatively in tablet and pure form. To perform the assay 366nm was the obtained λ max. It was discovered that 5-25 µg/ml concentration was linear. 0.999 was the obtained r2 value. The accuracy, Linearity, robustness, and precision of the approach were all validated. Detection, as well as quantification limits for the drug, were determined to be 0.136, 0.400 respectively. 99.80% was the determined recovery of the drug.

Jampana PK. *et al.*[76] has developed and validated specific, precise, and fast Visible method for Amlodipine besylate measurement in tablet and pure form. To perform the assay 456nm was the obtained λmax. It was discovered that 3-30 µg/ml concentration was linear. 0.999 was the obtained r2 value. The accuracy, Linearity, ruggedness, and precision of the approach were all validated. Detection, as well as quantification limits for the drug, were determined to be 0.104, 0.322 respectively. 99.90% was the determined recovery of the drug.

Naveed S. *et al.*[77] conducted investigations on the degradation studies of Amlodipine besylate several brands. In compliance with the recommendations of ICH varied stress conditions were applied to the drug. To analyze the medicine during the availability of the degrading components, a UV approach was created. As a solvent, distilled water has been employed. 238 nm was selected for absorbance estimating the degraded drug's quantity. UV light contact deteriorated brands first, second, and fifth, while following heat exposure first and the second Brand was degraded. Basic ph degradation was indicated by brand first, second, fourth, and fifth. A considerable deterioration is shown by the first, second, fourth, and fifth brands with a 1N HCL introduction. It has been established that the procedure is easy as well as economical.

High performance liquid chromatography (HPLC) methods

Jat S. *et al.*[78] Developed and validated method of analysis to estimate Amlodipine besylate in pharmaceutical preparation. The C18 column with 1ml/min. The flow was applied to the system together with 10 min of run time. For the analysis of sample 44:46:10 ratio of acetonitrile, potassium dihydrogen orthophosphate buffer, methanol was utilized. With help of OPA, pH 3 was obtained and at 239 nm PDA detector was used for scanning eluents. 2.39 min was the Amlodipine retention time.

Kumar S. *et al.*[79] established HPLC method regarding Amlodipine besylate assessment in pharmaceutical formulations. On the HPLC of Shimadzu having integrated with C18Column and UV-Vis detection system separation was achieved. 50 V: 35 V: 15 V of Buffer (PH 3.0): methanol: acetonitrile was used as mobile phase with 1ml/min flow and 237 nm selected for examining the eluents. Amlodipine besylate has been shown to have a 12.3 minute retention time. 0.41 and 0.58 were the RSD percentage of system and method precision respectively. Within 35-105 µg/ml concentration the technique has been determined as linear. From 99.50 to 99.91 percent the method's accuracy ranged.

Alaama M. *et al.*[80] developed and validated method utilizing RP-HPLC system contained with UV detector. For the preparation of standards as well as samples the drug was dissolved in mobile phase followed by 5 min sonication. A quaternary pump fitted RP-HPLC was used for sample analysis. 60:40 v/v ratio of buffer and acetonitrile was utilized as mobile phase and C18 column for separation. 1 ml/min flow rate with 248 nm UV detection was employed for measuring Amlodipine. The approach has been validated in accordance with the ICH. Over 6 minutes of run time Amlodipine has 3.44 ± 0.41 of retention time. 0.5 - 40 µg/ml range showed the linear method with 0.999 R2 value. The approach demonstrated excellent precision as well as reproducibility, with a recovery rate ranging from 98.09 to 100.19 percent. Every validated parameter met ICH criteria.

Pawar HA. *et al.*[81] developed and validated an approach employing reversed-phase HPLC for Amlodipine besylate estimation in the dissolution study. The Amlodipine besylate was eluted isocratically using a C18 analytical column with a 40:60 v/v ratio of buffer and methanol. At 239 nm with 1ml/min flow rate samples have been examined. 20-150 µg/ml concentration method was linear. The recovery rates were good, varying between 98.06% to 99.22%. Having a percent RSD of below 2, the approach demonstrated high precision.

Klinkenberg R. *et al.*[82] developed and validated the HPLC method for residues of Amlodipine estimation in swab samples. From stainless steel to produce a sufficient Amlodipine recovery process of swabbing has been optimized. As 2 methanol-soaked swabs were utilized, the average recovery was about 90%. Using RP-18 stationary phase at 25 ˚C the Amlodipine residues have been examined with 15:35:50 v/v/v ratio of acetonitrile, methanol, and triethylamine pH 3.0 solution as mobile phase. 237 nm was used for performing UV detection. The approach has been demonstrated both specific as well as linear in 0.39 to 1.56 µg/ml concentration range. 0.08µg/ml and 0.02 µg/ml have been determined as detection and quantification limits.

Electrochemical methods

Altiokkaa G. *et al.*[83] dihydropyridine group oxidation on electrode surface during both rotating as well as stationary settings have been used to develop a differential-pulse voltammetric approach in order to measure Amlodipine. For the evaluation of starting potential, the investigations have been performed under

supporting electrolyte comprising: methanol, KCl 0.2M, and phosphate buffer 0.1M. In rotational as well as stationary settings there was zero adsorption impact when 0mv of potential together with 5.5 pH electrolyte solution was used. Detection and quantification limits of stationary, as well as rotating approaches, have been reported as 0.0072 and 0.004 mg/ml. with 0.022 and 0.012 mg/ml as LOQ respectively. In the case of Amlodipine tablets, the presented approach yielded acceptable results at 95%. Level of probability.

Djaalab E. *et al.*[84] produced novel biosensor detecting Amlodipine besylate medication with biodegradable content relying upon lipase by utilizing a gelatin and iron oxide fusion. For the synthesis of Polyaniline polymerization technique has been used in an oxidant-containing solution of ammonium persulfate then analyzed using UV, SEM, and FTIR. In order to immobilize the lipase, cross-linking agent glutaraldehyde was used to entrap the enzyme in the biocomposite matrix film. The biosensor's concept relies upon the Amlodipine besylate electrochemical characteristics that have been evaluated employing the voltammetric technique for the 1st time.

Jadon N. *et al.*[85] developed voltammetric technique by employing pencil graphite electrode (PGE) in order to estimate the Amlodipine. Through adopting voltammetry of differential pulse as well as square-wave, various test factors including pH, frequency, amplitude, concentration, and deposition potential have been adjusted. At 700 mV, the peak potential was observed. 0.8 nM–51.2 nM range the calibration curve was linear. With about 99.9 percent recovery, the presented technique has been applied to pharmaceutical brands for estimating drug content. In serum also Amlodipine was estimated using the method with about 99% recovery together with 0.21 pM LOD.

Titrimetric methods

Haque SKM. *et al.*[86] developed and validated titrimetric approach in order to estimate Amlodipine Besylate containing content of acetic acid. The acid-base reaction forms the basis of titration which produces salt and water. Strong base NaOH is titrated against CH3COOH which is a weak acid. 0.75 to 30.25 µg/ml range having 0.9999 as r2 value the approach proved linear. The detection limit was 0.61 µg/ml whereas the quantification limit was 1.85 µg/ml. 98.20–99.97% was the obtained recovery percentage with below 2% RSD. The findings for ruggedness as well as robustness seemed great. Excipients have no effect on the method's accuracy and precision.

Basavaiah K. *et al.*[87] developed titrimetric approach using which Amlodipine besylate is estimated. The method involves directly titrating Amlodipine besylate acidified solution with a mixture of bromate-bromide while utilizing indicator methyl orange. From the method drug of 1 to 10 mg can be estimated. 0.9984 value of correlation coefficient demonstrates the linearity among endpoint and amount of drug.

Capillary Electrophoresis (CE) methods

Cârje AG. *et al.*[88] developed a method for Amlodipine enantiomers separation utilizing cyclodextrines through Capillary Electrophoresis and also estimated methods performance. Amlodipine CE assessment has been conducted employing fused-silica capillaries which were uncoated plus cyclodextrins of several kinds as chiral selectors. The amlodipine enantiomers had migration times of 8.15 and 8.45 min for S-AML and R-AML respectively. P^H 3.0 buffer solution consisting of H3PO4 25 mM as well as chiral selector α-CD 20 mM with 15°C of the maintained temperature of capillary, has been found to provide the ideal CE requirements.

Fakhari AR. *et al.*[89] developed a method for Amlodipine enantiomers assay confirming stability and separation utilizing CD in tablet formulations. It was determined that the optimum findings were achieved when using phosphate buffer that was composed of hydroxypropyl- α -CD 5mM with fused-silica capillary. This approach successfully separated enantiomers of Amlodipine and degradation components. In order to impart stress factors, this medication has been treated with photolysis, hydrolysis, heat, and oxidation. Each of the enantiomers had a quantification range of 5–150 µg/mL. Each enantiomers has 5 µg/ mL quantification limit and 0.5 µg/mL detection limit. Stress-induced degradation components have zero interference with enantiomer detection.

AMLODIPINE AND ATENOLOL COMBINATION METHODS

Ultraviolet methods

Avula S. *et al.*[90] developed UV method based on dual-wavelength for Amlodipine besylate and Atenolol estimation simultaneously in the combined formulation. For Atenolol 230nm, as well as 242nm, were selected wavelengths. And also for Amlodipine besylate 263nm, as well as 277nm, were selected wavelengths. The solvents employed included distilled water and methanol. Beer's law held true for Amlodipine besylate and Atenolol at a concentration between 1-6 µg/mL and 5–30 µg/mL respectively. In the dual-wavelength approach, Amlodipine besylate and Atenolol had the Correlation coefficient values of 0.9987 and 0.9983 respectively. Within 98.0-102.0 percent the approach was accurate. There was a good level of precision in the method with %CV below 2. Detection and quantification limits were 0.0825 µg and 0.25 µg for Amlodipine besylate whereas 0.162 µg and 0.492 µg for Atenolol respectively.

Mohammad MY. *et al.*[91] demonstrated using spectrophotometry second derivative to assess Amlodipine and Atenolol in both pure as well as marketed preparations. It was found that the approach was accurate, economic, precise, and easy. Both medications within combined preparation were evaluated

using the zero-crossing point approach. At 251 nm, 5.0–50.0 µg/ml was the range for Atenolol whereas, at 264 nm, 5.0–45.0 µg/ml was the range for Amlodipine in which the technique has been determined to be linear. The presented technique has been effectively used to assess Amlodipine and Atenolol in both combination and individual dosages. Moreover acquired findings proved consistent with the standard procedure.

Girdhari J. *et al.*[92] established Spectrophotometric approach in order to determine Amlodipine besylate and Atenolol concurrently utilizing HCL 0.1N in tablet formulation. Vieordt's approach or Simultaneous equation is used in this procedure. Amlodipine besylate had λmax 239.6 nm whereas Atenolol had λmax 224.6 nm. As long as the concentration is in between 4-32 µg/ml, exhibiting 0.9932 correlation coefficient for Amlodipine and 4-28 µg/ml, exhibiting 0.9991 correlation coefficient for Atenolol, Beer's law is obeyed. This devised technique might be utilized to estimate the Amlodipine besylate as well as Atenolol in various preparations.

Godge RK. *et al.*[93] established spectroscopic approach in order to determine Amlodipine besylate as well as Atenolol concurrently both in combination and bulk form. The absorption maxima obtained were 238.2 nm and 224nm for Amlodipine and Atenolol respectively. 2- 34 µg/ml concentration of Amlodipine and 2- 24 µg/ml concentration of Atenolol followed Beer's law. At 224 nm and 232.2 nm, the absorptivity and isoabsorptive point respectively were evaluated using the Q analysis approach. The approach's accuracy has been proved by the recovery investigations ranging from 99.05- 101.16 percent. The outcomes of precision testing were satisfactory.

Pawar PY. *et al.*[94] determined Amlodipine besylate and Atenolol by establishing a UV-Spectrophotometric approach that was precise and simple. A double beam Spectrophotometer has been employed to conduct the spectroscopic experiments. Amlodipine Besylate and Atenolol combined preparation Primol-AT 10 TAB was utilized together with a solvent of 0.1N HCL. At 228nm and 239nm, the drugs Atenolol and Amlodipine indicated maximum absorbance. On 233nm wavelength, an isoabsorptive point has also been discovered which was chosen to measure Amlodipine and Atenolol simultaneously. Linear calibration graphs have been obtained having 0.993 and 0.996 correlation coefficient values of Atenolol and Amlodipine respectively. Regarding both the drugs, the approach has proven relevant throughout 4-24ug/ml range. Using this approach, bulk, as well as tablets samples, maybe quickly & regularly analyzed.

Suresh P. *et al.*[95] established UV approach to evaluate Amlodipine besylate and Atenolol individually as well as concurrently in binary tablet preparations. This technique relies upon the absorbance method to determine one medication even in another medication's presence. The technique involved the selection of 2 wavelengths for each drug, 265 and 278 nm for Amlodipine whereas 209 and 244.5 nm for Atenolol, and the difference in absorbance across these wavelengths appeared practically nil. The concentration of drug Amlodipine and Atenolol is in proportionate with the difference in their absorbance i.e. within 265 and 278 nm for Atenolol and 209 and 244.5 nm for Amlodipine besylate.

High performance liquid chromatograpgy (Hplc) methods

Manisha D. *et al.*[96] established an approach for tablet preparation, in order to measure Amlodipine besylate and Atenolol simultaneously utilizing RP-HPLC. The separation has been accomplished by HPLC Water's 717 Plus having C18 column which is octadecyl silane bonded and ambient temperature was chosen for the operation. The mobile phase consisted of Acetonitrile, Methanol, buffer in 45, 35, and 20 volumes respectively. Tetrabutylammonium hydroxide has been used to make the buffer, and then Ortho-Phosphoric Acid was employed for managing the pH 3.0. 1.5ml/min was the maintained flow with 10 min of run time and at 225nm complete analysis got carried out with 20 µl of load volume.

Blessen P. *et al.*[97] devised RP-HPLC technology with the aim of Amlodipine Besylate and Atenolol quantification simultaneously in each of two tablets and bulk form. The separation has been accomplished by HPLC of Agilent technologies having a C18 Inertsil column. The mobile phase consisted of 4:3.5:2.5 volume/volume Buffer: Acetonitrile: Methanol. 1ml/min was the maintained flow with 10 min of run time and at 225nm complete analysis got carried out with 10µl of load volume. Ambient temperature was chosen for the operation. Triethylamine has been used to make the buffer, and then Ortho-Phosphoric Acid was employed for managing the pH 3. Atenolol had a retention time of 2.23 minutes with 100.1% mean recoveries whereas Amlodipine besylate had a retention time of 5.97 minutes with 100.4% mean recoveries. 5-15 µg/ml and 50-150 µg/ml concentration of Amlodipine besylate and Atenolol demonstrated linear response. 0.9998 and 0.9992 were Amlodipine besylate and Atenolol's correlation coefficient. The approach's accuracy has been proved by the recovery investigations, also the outcomes have been validated in compliance with ICH Criteria.

Haque MA. *et al.*[98] developed and validated RP-HPLC technique assessing Amlodipine and Atenolol in tablets. The employed detector and pump were UV-Vis SPD-20 A and LC-20 AT respectively. For attaining separation C18 column had been applied accompanied by 55:10:35 v/v ratio of methanol-acetonitrile- buffer to make up the mobile phase. Ambient temperature was kept throughout the operation with 1ml/min flow. 1.67 and 5 min were the obtained retention times of Atenolol and Amlodipine.

Amlodipine, as well as Atenolol peaks, were found fairly separated. Within 80 to 120 percent concentration, linear results of the calibration curve were obtained in addition to r2 of each compound 0.999. Recovery of 100.72% and 99.44% of Atenolol and Amlodipine are representative of the accurate method. The percent RSD of Amlodipine and Atenolol had 0.067-1.518 and 0.53-0.152 values as intraday variation whereas the percent RSD of Amlodipine and Atenolol had 0.034-1.518 and 0.024-1.518 as an inter-day variation. This approach was then utilized for evaluating marketed brands, whose potency was 97.4% to 100.4% and 99.02% to 100.02% for Amlodipine and Atenolol respectively.

Naikini P. *et al*.[99] has developed and validated a new method using RP-HPLC for Atenolol as well as Amlodipine quantification in pharmaceutical formulations and bulk. For performing HPLC determination BDS C18, a reversed-phase column was used. In 40: 30: 30 volume /volume ratio Phosphate buffer mixture pH 5, methanol, and acetonitrile formed the mobile phase. At a wavelength of 213 nm, UV-Vis detection has been performed using isocratic mode. This optimized approach resulted in Amlodipine as well as Atenolol retention durations of 2.540 and 5.950, respectively, also within the ICH standards asymmetry and theoretical plates were found. From 60-140µg/ml and 6-14µg/ml, Atenolol and Amlodipine linearity ranged having 0.9983 r2 value of each compound. Amlodipine & Atenolol had percent assays values of 99.47 and 99.22 percent, respectively. This developed approach was measured to be robust, precise, specific, stable, and accurate having 99.97% and 99.20% recoveries of Amlodipine and Atenolol.

Ubale M. *et al*.[100] devised reverse-phase technique quantifying in tablets Amlodipine besylate and Atenolol. Complete assessments were conducted on a stainless steel reverse phase C18 ODS column. In 50:50 ratio Acetonitrile and Potassium buffer of 0.03 M comprised the mobile phase. OPA was exploited to modify to 3.4 pH. Prior to application, the solution was degassed. 1.0ml/minute was the maintained flow with 237 nm wavelength of detection. Amlodipine Besylate had 8 to 12 µg/ml whereas Atenolol had 16-22 µg/ml range in which method was linear. Considering the Amlodipine and Atenolol assessment, the technique presented proved as being simple and efficient.

Shanmugasundaram P. *et al*.[101] developed and validated reliable, fast and efficient procedure estimating Atenolol as well as Amlodipine besylate simultaneously by making use of RP-HPLC in both Biorelevant media as well as tablet form. By making use of C18 kromasil column separation got accomplished. 15:30:55 was the ratio in which the mobile phase was prepared consisting of Acetonitrile, pH 3.0 Potassium dihydrogen phosphate solution (0.01M), and methanol. For analyzing the eluents PDA detector at 254 nm was applied. Using this approach drugs got eluted, Atenolol had 3.711 while Amlodipine Besylate had 2.589 min of retention times. In compliance with ICH criteria, this approach was then validated which demonstrated the approach being reliable, specific, rapid, reproducible, and accurate. Amlodipine Besylate had 25- 125 whereas Atenolol had 5-25µg/mL range in which the approach was linear. There were detection limits of 0.005, and 0.01µg/ml and quantification limits of 0.015, and 0.004µg/ml for Atenolol and Amlodipine besylate, respectively.

Srivani A. *et al*.[102] developed for pharmaceutical dosage form an approach analyzing Amlodipine and Atenolol by adopting RP-HPLC which was reliable, efficient, and repeatable. 62:38v/v ratio of Potassium dehydrogenate phosphate in 0.02M and acetonitrile comprised the mobile phase (OPA was used to modify to 3.56 pH) for conducting the separation by making use of column Hypersil, BDS, and 238nm for UV detection together with 0.8ml/min flow. Atenolol had 1.998 and Amlodipine had 6.093 min. of retention time respectively. Each of the compounds had exhibited high linearity in the 25-75 and 250-750 µg/ml range. In compliance with ICH recommendations, provided approach was validated. This approach exhibited high precision having below 2% percentage RSD. Atenolol had 99.06-100.94% whereas Amlodipine had 99.12-100.95% recovery suggesting the accuracy of the approach.

Electrochemical Methods

Moraes JT. *et al*.[103] explained CP-BDDE based Atenolol and Amlodipine besylate voltammetric estimation concurrently. Through cyclic voltammetry, at CP-BDDE Atenolol as well as Amlodipine had peak potentials (anodic) of 1.32 and 0.727 V respectively utilizing pH 7.0 phosphate buffer. By comparing the CP-BDDE against alternative carbon-based electrodes under similar conditions, made it evident that it operates better enabling Atenolol and Amlodipine assessment simultaneously. Within range of 9.8-190 µmol L^{-1} and 2.9-33 µmol L^{-1} analytical curves of Atenolol and Amlodipine appeared linear having 0.22 and 0.17 µmol L^{-1} LOD. For pharmaceutical products, an application of described technique measuring Atenolol and Amlodipine concurrently was successful.

Silva AA. *et al*.[104] demonstrated batch injection analysis in order to quantify Atenolol and Amlodipine accompanied by pulsed amperometric detection. The operating electrode was a boron-doped diamond. Detection for Atenolol and Amlodipine had been performed at +1.65 and +1.00 V respectively. The approach described has proved being easy, precise as having below 3.2 percent RSD, robust, as well as having a great frequency of more than 70 injections per hour, together with less waste production and low manipulation of the sample. Based on comparisons with HPLC results, the presented approach produced equivalent outcomes with a level of confidence of 95%.

CONCLUSION

We can clearly say considering the findings of this study that, while a wide variety of methodologies exist that analyze Amlodipine and Atenolol, researchers have been continuously researching towards inventing the latest approaches as well as optimizing them for not only saving time but also resources, confirming the effectiveness of the proposed technique. After examining overall validation information of published approaches, the HPLC methods have been found to be suitable for the drug analysis both separately and simultaneously, being more rapid as well as sensitive, while being less expensive than alternatives.

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