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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF ALPHA-BETA ADRENERGIC BLOCKERS: A REVIEW

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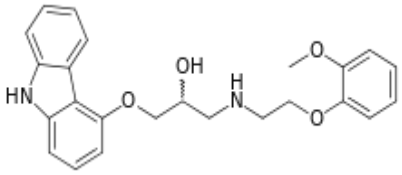
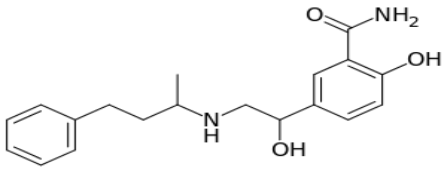
ABSTRACT

Alpha-beta adrenergic blockers belong to a larger class of medicines called adrenergic inhibitors. They combine the effects of two types of medicines Alpha-1 blocking ability (vasodilation) with Beta effects. This review explores the existing analytical methods for the estimation of some commonly used Alpha-beta blockers in pharmaceutical formulations and in biological matrix. There has been significant research on broad range of analytical techniques that could be useful in the estimation Alpha-beta blockers in formulations and in biological matrices. Analytical methods such as Ultraviolet (UV) spectrophotometry, Extractive Spectrometry, High Performance Liquid Chromatography (HPLC), High Pressure Thin Layer Chromatography (HPTLC), Thin Layer Chromatography (TLC), Liquid Chromatography-Mass spectrophotometry (LC-MS), Gas Chromatography-Mass spectrophotometry (GC-MS), Capillary Zone Electrophoresis (CE), Colorimetry, Fluorimetry, Differential voltammetry have been reported for the estimation of Alpha-beta blockers. From the review it could be understand that there are a number of methods developed, but UV Spectroscopy and high performance liquid chromatography with UV detection have been popularly used in the identification and estimation of Alpha-beta adrenergic blockers.

INTRODUCTION: [1,2]

The wide variety of antihypertensive agents now available allows considerable flexibility in the pharmacologic management of hypertension. Among the most important and most widely used drugs are thiazide diuretics, calcium channel blockers, ACE inhibitors, angiotensin II receptor antagonists (ARBs), and beta blockers. The newly available alpha- and beta-adrenergic blocking agents have added considerably to this flexibility. Their safety and efficacy insure that they will find increasing use in the treatment of hypertension. Labetalol and carvedilol, a compound possessing both alpha- and beta-receptor blocking properties, has been found to be effective both as an oral therapy for chronic hypertension and as an intravenous agent in treating hypertensive crisis. Further experience with labetolol and carvedilol will determine its safety and efficacy for the long-term management of hypertension. Of this labetalol is the first adrenergic antagonist capable of blocking both α and β receptors. There are four stereoisomers of labetalol, each of which has a distinct profile of action on sub types of α and β receptors. The commercial preparation has equal parts of each isomer and displays β_1+ β_2+α_1 blocking as well as weak β_2 agonistic activity. Carvedilol is the other Alpha-beta blocker. It is a β_1+ β_2+α_1 adrenoceptor blocker produces vasodilation due to alpha 1 blockade. It has been used in hypertension and is the beta blocker especially employed as cardio protective in CHF.

Table 1: Physico-chemical parameters of different Alpha-beta blockers:

Parameters	Carvedilol	Labetalol
Structure		
IUPAC Name	(±)-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl][2-(2-methoxyphenoxy)ethyl]amine	2-hydroxy-5-{1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl}benzamide
Solubility	Soluble in DMSO, ethanol, methanol, phosphate buffer (pH 4) partly miscible in water, slightly soluble in chloroform, methylene chloride, and ethyl ether. Insoluble in isopropanol	Soluble in ethanol and water
Molecular mass	406.474g/mol	328.406 g/mol
Molecular formula	$C_{24}H_{26}N_2O_4$	$C_{19}H_{24}N_2O_3$
Bioavailability	98%	25%
Protein binding	25–35%	50%

ANALYTICAL METHODS FOR ESTIMATION OF ALPHA-BETA BLOCKERS:

There are several simple, precise, accurate and sensitive methods reported for the estimation of Alpha-beta blockers in dosage form or in biological matrices.

A. Spectrophotometric Methods:

a. Carvedilol:

Y. Nirupa rani et al., [3] developed and validated a spectrophotometric method for the determination of Carvedilol in pure form and in pharmaceutical formulations at the absorption maxima 286 nm in chloroform. The calibration range was studied from 50% -150% and correlation was found to be R2 = 0.998 which was within the limits of ICH guidelines

R. K. Jat et al., [4] has developed a sensitive spectrophotometer method based on the formation of a chloroform soluble ion-pair complex between carvedilol and bromophenol blue in an acidic medium. The complex shows maximum absorbance at 414 nm. Beer's law was obeyed in the concentration range of 5-20 µg /ml.

Dr. C. Theivarasu et al., [5] has developed a ultraviolet spectroscopic methods for the estimation of carvedilol in pure and formulation. The drug solubility and maximum assay sensitivity was found in methanol. The absorbance of carvedilol was measured at 241nm in the wavelength range of 200 - 350 nm. The linear calibration range was found to be 50% - 150%.

K.V. N. Hymavathi et al., [6] developed aspectrophotometric method for the estimation of Carvedilol (CAR) in bulk and in dosage forms using Hydrotropy (Co-solvency) (Method A & B). Method C was done using methanol as a solvent. Absorption maximum is (method A) 265nm, (method B & C) 286nm. Bears law is obeyed in the concentration range of 10-60 (method A), 0.1-0.6 (method B), 0.1-0.5 (method C).

Shinkar Dattatraya Manohar et al., [7] developed a UV spectrophotometric method, drug solubility and maximum assay sensitivity was found in methanol. The absorbance of carvedilol was measured at 284 nm in the wavelength range of 200-350 nm. Beer's law was obeyed in the concentration range of 10-35 µg/ml.

Divya N. Shetty et al., [8] have developed two spectrophotometric methods. Method A is the condensation reaction of carvedilol with p-dimethylaminobenzaldehyde (PDAB), and the reaction mixture exhibits maximum absorbance at 601 nm. Method B is based on the charge transfer complex formation of carvedilol with p-chloraniline; the color developed is measured at

662 nm. The calibration graphs are found to be linear over 50.00–250.00 and 20.00–100.0 μg ml^{-1}.

b. Labetalol:

Manasa O et al., [9] developed and validated a UV spectrophotometric method for the determination of Labetalol in pharmaceutical preparations. The method was developed utilizing 0.5N NaOH. The standard and sample was scanned and the absorbance is scanned at 245.3 Linearity was observed in the concentration range from 20-45 μg/ml with a correlation coefficient (R2) greater than 0.998

Nafisur Rahman et al., [10] determined of labetalol hydrochloride by kinetic spectrophotometry. The method was based on the kinetic investigation of the oxidation of the drug with alkaline potassium permanganate at room temperature (25 ± 1°C). The increase in absorbance of coloured manganate ions was measured at 605 nm. The calibration graphs were linear in the concentration ranges of 2-14 μg mL-1 and 1-10 μg mL-1, using the initial rate and fixed time methods

Chilukuri S. P. Sastry et al.,[11] developed three spectrophotometric methods. The first two are based on the oxidative coupling reaction of labetalol hydrochloride with p-N,N-dimethyl-phenylenediamine dihydrochloride (method A, λ_{max} 685 nm) and 3-methyl-2-benzothiazolinone hydrazone hydrochloride (method B, λ_{max} 545 nm) in the presence of sodium hypochlorite and eerie ammonium sulphate as oxidants, respectively. The third depends on the formation of an ion-association complex of labetalol hydrochloride with suprachen violet 3B at pH 1.3, which is extracted into chloroform (method C, λ_{max} 565 nm).

B. Chromatographic methods:

a. Carvedilol:

Bhavna A. Patel et al., [12]developed and validated a RP-HPLC –Fluorescence method. The separation was achieved by a Brownlee analytical C18 column (250mm X 4.6mm, 5μm) in isocratic mode, with mobile phase comprises of Acetonitrile : Methanol : Buffer in proportion of 70:20:10v/v/v, buffer was 5mM Potassium Di-hydrogen Phosphate (pH 3.5 adjusted with Ortho Phosphoric Acid). The flow rate of mobile phase was 1.0ml/min and employing fluorescence detection with 280nm excitation and 340nm emission wavelengths. The retention time of Carvedilol Phosphate was 2.20 min.The calibration curve was found to be linear within the concentration range of 10ng/ml to 60ng/ml.

B. A. Patel et al., [13]developed and validated a RP-HPTLC method using TLC silica gel 60 GF_{254} aluminum plates with a solvent system comprised of n-hexane: ethyl acetate: methanol (3:5:2 v/v/v) and the R_f value was found to be 0.36 cm. the linearity range was 100ng/ spot to 200ng/spot.

Pattana Sripalakit et al., [14] developed and validated a RP-HPLC –Fluorescence method. Chromatographic separation was achieved on a Alltima® C18 (250 mmX4.6 mm) column using a mobile phase containing 0.01 M Na_2HPO_4 in water and acetonitrile (30:70 v/v) adjusted to pH 3.0 by orthophosphoric acid at a flow rate of 1.0 ml/min and employing fluorescence detection with 300- nm excitation and 343-nm emission wavelengths.

Basaveswara rao M.V et al., [15] developed and validated RP–HPLC method. Isocratic elution at a flow rate of 1.0ml/min was employed on symmetry C18 (250 mm x 4.6 mm, 5µm) Column at ambient temperature. The mobile phase consisted of Methanol: Acetonitrile: 1% OPA in the ratio of 80:18:2 v/v/v. The UV detection wavelength was 240nm and 20µl sample was injected. The retention time for Carvedilol was 2.1 min

Jelena stojanovi et al., [16] developed a stability-indicating high-performance liquid chromatographic method for the analysis of carvedilol. The chromatographic separation was achieved on a Chromolit RP8e, 100X 4.6 mm analytical column. The mobile phase consisted of a mixture of acetonitrile and water (45:55, V/V) (pH 2.5), pH adjusted with formic acid. The absorbance was monitored with a UV detector at 280 nm and the temperature of the analyses was 40 °C. The flow rate was 0.5 mL/min. The recovery was found to be 99.71–101.58.

P. K. Manna et al., [17] developed and validated a reverse phase High performance liquid chromatography (RP-HPLC) method. The chromatographic system consist of a steel plated C18 column, an isocratic mobile phase composed of phosphate buffer pH 3.0, acetonitrile and water (75:625:300) and UV detection at 240.0 nm. Carvedilol was eluted at 2.8 minutes. The linearity was observed over the range from 1 to 50 µg/mL (R2 =0.9999) and validated statistically according to ICH guidelines.

E.Bernabeu et al., [18] has tested three paediatric oral liquid formulations using high performance liquid chromatography. The liquid chromatographic system consisted of an isocratic solvent delivery pump (ShimadzuLC-20AT) that pumped a mixture of (A: 35; B:65) (A: acetonitrile and B:phosphatebuffer, pH: 2.0)througha15-cm_4.6-mm reverse-phaseC-85 mm column (RP-

8Microsorb-MV100-5)at1.0ml/min. The column was maintained at55^0 C. The column effluent was monitored with a variable wavelength ultra- violet detector (Waters486) at 285nm. To establish the stability-indicating nature of the method, carvedilol (1mg/ml) was subjected to forced acidic degradation (1N HCl), basic degradation (1N NaOH) and oxidation (10% H2O2) at a temperature of 100^{0}C for 1hr. The analytical method validation was carried out according to the specifications inUSP31, chapter1225. Aliquots were collected from each container on days 0, 3, 7, 14, 28 and 56.These were diluted with HPLC mobile phase and immediately analysed. And it is linear in the 20–64 mg/ml range of concentrations with an accuracy of 98.157±2.98%.

Fatma M Abdel-Gawad et al., [19] developed RP-HPLC and TLC densitometric methods, analysis was performed on Agilent C18 column using a mobile phase consisting of 0.05 M potassium di hydrogen phosphate (pH 2.5±0.1) and acetonitrile (60:40, v/v) with a flow rate of 2.0 mL/min and UV detection at 245nm. The second method uses thin-layer liquid chromatography (TLC) separation was carried out on silica gel 60 F254 using acetone-toluene-ethanol-ammonia solution 33% (45:45:10:1, v/v/v/v) as mobile phase. The methods were linear in the range of 10-200 µg mL-1 and 2.0-37.4 µg/spot for HPLC and TLC. The methods were validated according to USP and ICH guidelines

Dey Suddhasattya et al., [20] developed and validated a RP-HPLC method. The mobile phase used was 1.77g of potassium dihydrogen phosphate dissolved in distilled water and diluted to 650 ml with the same solvent; pH was adjusted to 2.0 with phosphoric acid. 350 ml of acetonitrile was added and mixed. Then it was filtered through 0.2Î¼ membrane filter. The specification of the chromatographic system, column 4.6mm X 25cm X 5 micron (c8), wave length 240nm, flow rate 1ml/min and the oven temperature 55°c. A linear response was observed between the concentration ranges of 806-1202Î¼g/ml with a regression coefficient of 0.994. The method was then validated for different parameters as per the ICH (International Conference for Harmonization) guidelines.

R. Srinivas et al., [21] have developed an isocratic liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI–MS) for the separation and identification of stress degradation products (DPs) in carvedilol. Here carvedilol was exposed to hydrolytic (acidic, alkaline and neutral), oxidative, photolytic and thermal stress conditions as per ICH guidelines Q1A (R2). The drug degraded under acidic, basic and photolytic stress conditions and studies were carried

using Agilent XDB C-18 column (4.6 × 150 mm, 5 µm) using a mobile phase comprising of 20 mM ammonium acetate (pH adjusted to 6) (solvent A) and acetonitrile (solvent B) in an isocratic elution method. The structures of the degradation products were elucidated by using LC–ESI–MS/MS combined with accurate mass measurements.

LJ Patel et al., [22] developed RP-HPLC and HPTLC methods for estimation of carvedilol in bulk drug and pharmaceutical formulations. Analysis was performed on Lichrospher 100 C-18, 5 µm column consisting of 200×4.6 mm i.d. in isocratic mode, with mobile phase containing 50 mM KH_2PO_4 buffer (pH 3.0±0.1): acetonitrile: methanol (60:50:10 v/v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 242 nm. The retention time was 4.56±0.03 min. For the high performance thin layer chromatography method a Camag high performance thin layer chromatography system comprising of Linnomat V automatic sample applicator, Hamilton Syringe, Camag TLC Scanner-3, Camag Win CAT software with stationary phase precoated silica gel 60F 254 and mobile phase consisting of ethyl acetate: toluene: methanol (1:4:3.5 v/v/v) were used. The detection of spot was carried out at 242 nm. The Rf value was 0.65±0.02. The linearity curves were found to be linear over 1-35 µg/ml for high performance liquid chromatography and 50-300 ng/spot for high performance thin layer chromatography.

b. Labetalol:

USP [23]: In USP it is RP-HPLC method. The mobile phase used was 0.1M monobasic sodium potassium and methanol (65:35). 2000 mg equivalent tablet powder was weighed and dissolved in distilled water and diluted to 500 ml with the same solvent; filter and transfer 10 ml and makeup the volume to 100ml with mobile phase. The specification of the chromatographic system are column 4.6mm X 25cm X 5 micron (c8), wave length 302nm, flow rate 1.5ml/min and the oven temperature 60±1°c.

C. Bio analytical methods:

a. Carvedilol:

Abolghasem jouyban et al., [24] developed and validated a isocratic HPLC-UV method for simultaneous quantification of 5 drugs(carvedilol, losartan, diltiazem, furosemide and propranolol). Analysis was performed using MZ-analytical column (15 mm × 4.6 mm, 5 µm) and mobile phase is acetonitrile/2-propanol/15 mM phosphate buffer (pH = 2) (32.5/2.5/65 v/v/v. The sample preparation consisted of a protein precipitation procedure using a mixture of

acetonitrile and zinc sulphate solution prior to injection of sample to the chromatographic system. The maximum wavelength for the all detections were 225 nm. Method shows acceptable precision, accuracy and linearity [carvedilol (0.025-0.800 µg/mL), losartan (0.050-0.800 µg/mL), diltiazem (0.050-0.800 µg/mL), furosemide (0.025-0.800 µg/mL) and propranolol (0.025-0.800 µg/mL). Hye Jung Lee et al., [25] determined carvedilol in human plasma using a high-performance liquid chromatography with tandem mass spectrometer (HPLC-MS/MS). Plasma samples were deproteinized using acetonitrile and the supernatant was directly injected. Chromatography was performed on a reversed-phase (C18) column with isocratic mobile phase for 2 min. The calibration curve was linear over the range of 2 to100 ng/ml ($R2 > 0.9998$).

Bilal Yilmaz et al., [26] determined carvedilol in human plasma by GCMS using atenolol as internal standard. Carvedilol and atenolol (internal standard) were extracted from human plasma with a mixture of diethylether and ethylacetate at basic pH with liquid–liquid extraction. The extracts were derivatized with n- Methyl-n-(trimethylsilyl) tri fluoro acetamide and analyzed by gas chromatography-mass spectrometry (GC–MS). Calibration curves were linear over the concentration range 15–500 ng/mL.

Mogallapalli et al., [27] developed and validated a RP-HPLC using UV detector. Analytes were extracted from serum samples that were previously mixed with sodium hydroxide solution into an n- hexane, dichloromethane (7:3) solvent system. The mobile phase consisted of acetonitrile: orthophosphoric acid (37:63). The filtered mobile phase components were pumped from the respective reservoirs at a flow rate of 1.0 mL/min. Celecoxib was used as internal standard. Serum samples containing the carvedilol and internal standard, celecoxib were eluted through a C18 column. Retention times of carvedilol and celecoxib are 9.12 and 11.49 min.

Yamsani VV et al., [28] determined carvedilol in pig serum. Carvedilol and internal standard (IS) were extracted into n-hexane-dichloromethane solvent system and separated using an isocratic mobile phase on a Phenomenex C(18) column. The eluent was monitored by spectroflourimetric detector at a flow rate of 1.0 mL/min. The linearity range of proposed method was 1-1000 ng/mL and mean recovery was more than 89.95 and 94.27 for carvedilol and IS.

Yamsani Madhusudan Rao et al., [29] developed and validated a RP-HPLC method for Determination of Carvedilol in Human Serum Analytes were extracted from serum samples, that were previously mixed with 300 µL of 0.1 N sodium hydroxide solution into an n-hexane,

dichloromethane (7:3) solvent system. The mobile phase was made of acetonitrile, 15 mM orthophosphoric acid (37:63), and 0.25% v/v of triethylamine, with a flow rate of 1 mL/min. Serum samples containing the carvedilol and internal standard, amitriptyline were eluted through a C8, Kromasil KR 100 5C8 column. Retention times of carvedilol and amitriptyline were 6.10 min and 8.44 min and linear over a concentration range of 5–500 ng/mL. The extraction recovery of carvedilol is more than 75%.

Lamprecht, Guenther et al., [30] developed and validated a HPLC column-switching method for the enantioselective determination of (R)- and (S)-carvedilol in human plasma. Sample preparation was performed either off-line, by extraction with trichloromethane and back-extraction into 0.01. M aqueous citric acid which was injected on to a LiChrosorb RP 8 column, or on-line, by injecting diluted (0.1 M formic acid) plasma on to a LiChrosorb ADS column. In both instances separation was performed by gradient elution and on-line transfer of the fraction containing the carvedilol on to an enantioselective Teicoplanin column. The enantiomers of carvedilol were separated isocratically by use of methanol-acetonitrile-triethylammonium acetate, 70:30:0.05 (v/ v/w), as mobile phase. With fluorescence detection the limits of quantitation were 0.30 ng mL-1 for (R)-carvedilol and 0.26 ng mL-1 for (S)-carvedilol

B. Labetalol:

Radi et al., [31] determined labetalol in pharmaceuticals and Spiked Human Urine using differential voltammetry. For analytical purposes, a well-defined adsorption-controlled anodic peak was obtained in Britton—Robinson buffer at pH 2.0. By anodic adsorptive linear sweep and differential pulse voltammetry, linear calibration plots were obtained in the ranges of 2.5 × 10−6—1.0 × 10−5 mol dm−3 and 2.5×10−8—1.0 × 10−5 mol dm−3 for both techniques.

DavidR. Luke et al., [32] assayed labetalol in plasma by high-performance liquid chromatography, with 5-{2-[4-(4-chlorophenyI) ethyl]} salicylamide hemihydrate as the internal standard. Plasma samples are extracted with acetonitrile, evaporated under nitrogen, reconstituted in the mobile phase, and injected onto a PRP-1 (Hamilton) column packed with particles of poly(styrene-divinylbenzene) copolymer. Fluorescence, enhanced by post-column introduction of NH_4OH, was measured in the effluent (excitation wavelength 340 nm, emission wavelength 418 nm). Rt for labetalol and the internal standard were 1.99 and 3.32 mm, respectively. The assay standard curve is linear from 1 to 250 /1g/L. Some commonly co administered drugs were tested and did

not interfere. Sk Manirul Haque et al., [33] developed spectrophotometric method for the analysis of labetalol hydrochloride in pharmaceutical, urine and blood samples. The method is based on the oxidation of the drug with ferric ammonium sulphate that yields a green colored product. The increase in absorbance of colored product is measured at 535 nm. Beer's law is obeyed over the concentration range 10 - 200 µg/mL with molar absorptivity of 2.13 ×103 L mol-1 cm-1.

M. Ganesan et al., [34] developed a liquid chromatography–tandem mass spectrometry method for estimation of Labetalol in human plasma. The analyte and internal standard (Metoprolol) were extracted by liquid/liquid extraction with ethyl acetate. The chromatographic separation was performed on reverse phase Phenomenax Luna column (C18, 5µm, 100 x 4.6 mm) with a mobile phase of 2 mM ammonium formate (pH 5.0) / methanol (20:80 v/v), which was pumped at a flow rate of 0.5 mL / min with split ratio of 20:80. The protonated analyte was quantitated in positive ionization by multiple reactions monitoring with a mass spectrometer. The mass transitions m/z 329.01→161.95 and 267.99→115.86 were used to measure Labetalol and Metoprolol, respectively. The lower limit of quantitation was 3.1800 ng/mL with a relative standard deviation of less than 15%. Acceptable precision and accuracy were obtained for concentrations over the calibration curve ranges (3.1800 to 700.8760 ng/ml). Sample analysis time of 2.5 min for each sample made it possible to analyze a throughput of more than 400 human plasma samples per day.

Nafisur Rahman et al., [35] developed two spectrofluorimetric methods. In method A, fluorescence was measured at 432 nm after excitation at 312 nm. The second method (method B) is based on the formation of a ternary complex between zinc (II), eosin and LBT. The fluorescence intensity of the ternary complex was measured at 452 nm after excitation at 317nm. Optimum conditions for the determination were also investigated. The linear range and detection limit for method A and B were found to be 1.25-30 µg/ml; 0.24 µg/ml and 0.5-4 µg/ml; 0.08 µg/ml. El-Enany et al., [36] developed Micellar enhanced spectrofluorometric method for the determination of labetalol through complexation with Aluminium(III) .LBT was reacted with Al^{3+} , both in acetate buffer of pH 4.5 (Procedure I) and borate buffer of pH 8.0 (Procedure II), to produce highly fluorescent stable complexes. The fluorescence intensity could be enhanced by the addition of sodium dodecyl sulfate, resulting in 3.5- and 2.7-fold increases in the fluorescence intensity for Procedures I and II. In both procedures, the fluorescence intensity was

measured at 408 nm after excitation at 320 nm. The fluorescence intensity-concentration plots were rectilinear over the range of 0.020.1 and 0.010.05 g/mL with a detection limit of 0.003 and 0.001 g/mL for Procedures I and II. Furthermore, the method was applied for the determination of LBT in spiked human plasma, and the recovery (n = 4) was 93.30 ±2.62%.

Woodman, Tracy F. et al., [37] developed a HPLC method for estimation of labetalol in serum. The applicable range of the assay is 10 to 400 ng/ml using 1 ml of plasma or serum. Analysis uses the mobile phase of acetonitrile/pH 3.0 phosphate, and ultraviolet detection at 207 nm. The percentage recovery of labetalol and internal standard (propericiazine) (3-cyano-10-[3-4-hydroxypiperidino) propyl] phenothiazine from serum was 95.6% and 75.5. An approximate fourfold variation in plasma labetalol concentration, with plasma levels ranging from 20 to 93 ng/ml, was seen 10 to 11 hours after a dose in a group of patients taking 200 mg twice daily.

C Ceniceros et al., [38] developed a high-performance liquid chromatographic (HPLC) method with amperometric detection for the quantitation of labetalol in urine. The chromatography was performed at 30°C using a reversed-phase column with a base deactivated silica stationary support and an alkylamide bonded phase (Supelcosil ABZ+Plus). A 5 m***M*** acetate buffer (pH 4.5)–acetonitrile (70:30, v/v) mixture was employed as the mobile phase, pumped at a flow-rate of 1 ml/min. Sample preparation was carried out using a simple solid-phase extraction (SPE) procedure, and recoveries were found to be higher than 85%.

Other methods:

Abolghasem Jouyban et al., [39] developed a capillary electrophoresis method for assay of some degradation products of carvedilol using running buffer 80 mm acetate dissolved in methanol/ethanol mixture (65:35% v/v), applied voltage of 19 kv, temperature is 20 ºc and the wavelength range of 200-350 nm.

T. Mahajan et al., [40] determined the traces of amorphous carvidilol content in carvedilol drug substance and drug product using modulated differential scanning colorimetry and Powder X-Ray diffraction (PXRD). The characteristic glass transition (Tg) of amorphous carvedilol is exhibited at about 35°C.

CONCLUSION

This review represents the reported spectrophotometric, chromatographic and bioanalytical methods developed and validated for the determination of carvedilol and labetalol in different dosage forms. Here this review shows simple, accurate, precise methods development of the different drug formulations. The method development take place for determination of alpha-beta adrenergic blockers using UV spectrophotometry, colorimetry, HPLC, capillary electrophoresis, powder X-ray diffraction, liquid chromatography–electrospray ionization-mass spectrometry, differential voltammetry, fluorimetry, HPLC-MS, GC-MS. But commonly used methods are UV spectrophotometric method and RP-HPLC method.

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