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CANDESARTAN ANALYSIS METHODS DURING 2000-2020

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ABSTRACT

Angiotensin II type 1 receptor antagonists have been widely used in the treatment of various disorders such as hypertension, heart failure, myocardial infarction, and diabetic nephropathy. Over the years, various analytical methods have been developed in both biological fluids and dosage forms to estimate the desired pharmacological activity. Candesartan becomes very lipophilic with varying oral bioavailability, determination of its level in biological fluids is very important to determine the efficacy of further therapy. Potentiometric titration is an official method in Pharmacopoea. Several UV spectrophotometric, spectrofluorimetric, and electroanalytic techniques have been reported to determine the level of candesartan in formulations and bulk forms. Also, the flow analysis, high- performance liquid chromatographic (HPLC), and high-performance thin-layer chromatographic (HP-TLC) method provide analysis of candesartan with several methods that are systematically optimized using an experimental design. This review provides an overview of various analytical techniques used for candesartan determination both in a single preparation and mixed with other substances.

KEYWORDS: Candesartan cilexetil, spectrophotometric, spectrofluorimetric, electroanalytic, flow injection. HPLC, HP=TLC,

INTRODUCTION

Candesartan is classified as an angiotensin II receptor type 1 antagonist. Angiotensin II receptor type 1 antagonists are widely used in the treatment of diseases like hypertension, heart failure, myocardial infarction, and diabetic nephropathy. Candesartan is an orally active lipophilic drug and possesses rapid oral absorption. It causes a reduction in blood pressure and is used in the treatment of hypertension. It is also used in the treatment of congestive

heart failure and given as prophylaxis to reduce the severity and duration of migraines. Candesartan cilexetil, a prodrug of Candesartan, is available in the market under the trade names- Blopress®, Atacand®, Amias®, and Ratacand®. Candesartan is also available in a combination formulation with a low dose thiazide diuretic, invariably hydrochlorothiazide, to achieve an additive antihypertensive effect.[1]

Over the years, varied analytical methods were developed both in biological fluids as well as dosage forms to estimate their desired pharmacological activity. Candesartan being highly lipophilic with variable oral bioavailability, its estimation in biological fluids is very critical for establishing its subsequent therapeutic efficacy. A few UV spectrophotometric and voltammetric techniques have been reported to estimate candesartan in bulk formulations. Additionally, capillary electrophoresis methods provided a qualitative and quantitative estimation of candesartan with a few methods being systematically optimized using experimental designs. Ultimately, an overview of different HPLC and LC-MS/MS methods explained the importance of the methods while estimating candesartan in various biological fluids (e.g. plasma, urine, etc.) as well as other studies like stability studies.[2]

CLINICAL BACKGROUND

Candesartan binds selectively and non-competitively to the angiotensin II receptor type 1, thus preventing the actions of angiotensin II. Clinical trials have demonstrated its efficacy at a dose range of 2 to 32 mg once daily in hypertension of all grades, heart failure, in reducing urinary albumin excretion in diabetes mellitus and coexisting hypertension and renal failure.[3]

Absorbed candesartan cilexetil is completely metabolized to candesartan. Oral bioavailability is low (about 40%) because of incomplete absorption. Plasma protein binding in humans is more than 99%. The volume of distribution in healthy individuals is 0.13 L/kg. CV-15959 is the inactive metabolite of candesartan.

Candesartan that reaches the systemic circulation is mainly cleared by the kidneys, and to a smaller extent by the biliary or intestinal route. The apparent oral clearance of candesartan is 0.25 L/h/kg after a single dose in healthy individuals. Oral clearance (3.4 to 28.4 L/h) is highly variable among patients. No relevant pharmacokinetic drug-food or drug-drug interactions are known. The terminal elimination half-life remains unclear but appears to be longer than the currently used range of 4 to 9 hours.

Non-compartmental models do not appear to be appropriate for the analysis of candesartan pharmacokinetic data. A 2-compartment analysis revealed a much longer half-life of 29 hours using data from patients with hypertension. However, further in-depth analysis has never been performed. The concentration-effect relationship is unaffected by age. No gender or race differences have been shown in the effect or pharmacokinetics of candesartan.

Renal function affects the pharmacokinetic profile of candesartan. For patients with Creatinine clearances of >60 mL/min · 1.73 m^2, 30 to 60 mL/min·1.73 m^2, and 15 to 30 mL/min·1.73 m^2, the elimination half-life is 7.1, 10.0 and 15.7 hours, respectively, at a dose of 8 mg/day. However, at 12 mg/day an accumulation factor of 1.71 was found. Thus, a maximum daily dose of up to 8 mg appears suitable in patients with severe renal dysfunction. No significant elimination of candesartan occurs with hemodialysis. In patients with mild to moderate hepatic impairment, no relevant pharmacokinetic alterations have been observed. Dosages of up to 12 mg/day do not require precautions in patients with mild to moderate liver disease.

Clinically effective dosages range between 8 and 32 mg/day. The response rate of monotherapy with candesartan in patients with hypertension increases with dosage, but never exceeds 60 % at a daily dosage of 16 mg of candesartan. Dosages up to 32 mg/day do not increase this response rate.[4]

Physicochemical Properties

Candesartan is a tetrazole derivative (five-membered heterocyclic ring with 4 nitrogen atoms). Clinically it is used in the form of an ester prodrug- Candesartan cilexetil. Candesartan cilexetil is chemically (1RS)-1-(Cyclohexyloxycarbonyloxy)ethyl-2-ethoxy-1-{[2?-(1H-tetrazole-5-yl)biphenyl-4-yl]methyl}-1H-benzimidazole-7-carboxylate [145040-37-5] (Figure 1), with chemical formula $C_{33}H_{34}N_6O_6$ and molecular weight 610.66. Candesartan Cilexetil occurs as white crystals or white crystalline powder. It is soluble in acetic acid, sparingly soluble in methanol, slightly soluble in ethanol (99.5 %), and practically insoluble in water. A solution of Candesartan Cilexetil in methanol (1 in 100) shows no optical rotation. Candesartan Cilexetil shows crystal polymorphism.[5]

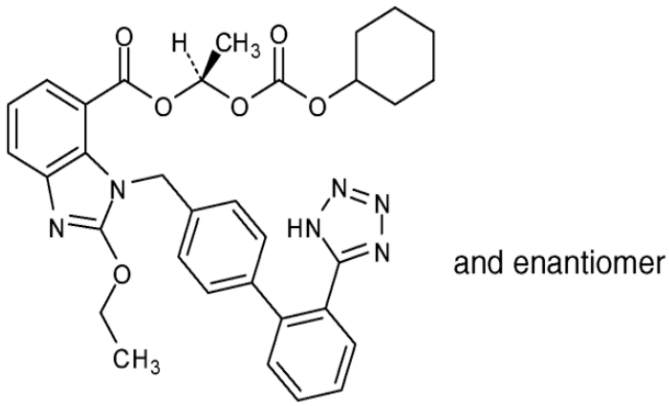

Figure 1: Candesartan Cilexetil.[5]

Candesartan cilexetil (CC) loaded solid lipid nanoparticles (CC-SLNs) were developed to improve oral bioavailability. Components of the SLNs include either of trimyristin/tripalmitin/tristearin and surfactants (Poloxamer 188 and egg lecithin E80). The CC loaded nanoparticles were prepared by hot homogenization followed by the ultrasonication method. The physicochemical properties, morphology of CC-SLNs were characterized. Stable CC-SLNs having a mean particle size of 180–220 nm with entrapment efficiency varying in between 91–96 % were developed. The physical stability of optimized formulation was studied at refrigerated and room temperature for 3 months. Further, freeze-drying was tried for improving the physical stability. DSC and XRD analyses indicated that the drug incorporated into SLN was in amorphous form but not in a crystalline state. The SLN-morphology was found to be nearly spherical by electron microscopic studies.[6]

Data Collection

In compiling this review article, the technique used is to use a literature study by finding sources or literature in the form of primary data in the form of official books and international journals in the last 20 years (2000-2020). Also, in making this review article a data search using online media with keywords was candesartan analysis in pharmaceutical preparations and biological matrices. The search for primary references used in this review article through a trusted web such as ScienceDirect, NCBI, Researchgate, Google Scholar, and other published and trustworthy journals.

Analysis Methods

Many analytical methods have been developed for the quantification and determination of candesartan in biological fluids and pharmaceutical dosage form. Candesartan cilexetil in the form of pharmaceutical raw materials can be determined by using a potentiometry. In most of

the cases, the analysis in the pharmaceutical dosage form was carried using high-performance liquid chromatography (HPLC), spectrophotometry, and voltammetric techniques. However, there are a few reports of biological fluids wherein CC has been determined using methods HPLC or LC-MS/MS.

Potentiometric titration

Candesartan cilexetil in the form of pharmaceutical raw materials can be determined by acid-base titration using a potentiometric indicator. Weigh accurately about 0.5 g of Candesartan cilexetil, dissolve in 60 mL of acetic acid (100 %), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction. Each mL of 0.1 mol/L perchloric acid VS = 61.07 mg of $C_{33}H_{34}N_6O_6$.[5]

Spectrophotometry method

Several spectrophotometry methods have been used for candesartan analysis, either as raw material or in pharmaceutical dosage forms (Table 1).

Table 1: Candesartan cilexetil analysis using spectrophotometry.

No	Sample	Solvent	Mode	Wavelength	Range of Concentration	Reference
1	Tablet	Distilled water	First derivative	270.1 nm	6 - 32 µg/mL	[7]
2	Bulk, tablet	Methanol	Second derivative	291.2 nm	4 - 32 µg/mL	[8]
3	Commercial preparations	methanol: water in the ratio of 9:1	Nonderivative	254 nm	10-90 µg/mL	[9]
4	Tablet	Distilled water	Nonderivative	225 nm 250 nm	1 – 12 µg/mL 2 – 12 µg/mL	[10]
5	Bulk	Distilled water	Kinetic	-	5-30 ug/mL	[11]
6	Bulk	DCQC and NQS in alcoholic media	Visible	550 nm (DCQC) 480 nm (NQS)	5.0-25.0 µg/mL 4.0-20 µg/mL	[12]
7	Bulk, tablet	BCG and BCP in phosphate-buffered solution	Visible	415 nm (BCG), 405 nm (BCP)	Not available	[13]
8	Tablet	Methanol	Nonderivative	258 nm	10 – 50 µg/mL	[14]
9	Tablet	DCQC in water	Visible	467 nm	20 – 100 µg/mL	[15]

This article describes the development and validation of a first derivative UV quantitative analytical method for the determination of candesartan cilexetil in tablet dosage forms. A signal at 270.1 nm of the first derivative spectrum ($ID_{270.1}$) was found adequate for quantification. The limit of quantification was 3.06 µg/mL. The linearity between $ID_{270.1}$ nm and the concentration of candesartan cilexetil in the range of 6.00–32.00 µg/mL presented a correlation coefficient of (r^2) = 0.9990. The mean recovery percentage was 100.97 and 99.23 % for candesartan cilexetil standard solution and candesartan standard cilexetil solution with excipients, respectively. The intraday and interday accuracy of the assay was 98.60 % and 99.10 % respectively. The intraday and interday variability were below 2.0 %. The proposed method is accurate, precise, sensitive, and selective and can be used in quality control laboratories for its intended purpose.[7]

The present work describes a simple stability-indicating second derivative spectrophotometric assay method for the determination of an antihypertensive drug, candesartan cilexetil in the presence of its alkaline degradation product, candesartan. The method was satisfactorily validated concerning linearity, precision, accuracy, selectivity, and sensitivity. The response was linear in the concentration range of 4 - 32 µg/mL (r = 0.99995) at wavelength 291.2 nm, which was the zero-crossing point of candesartan in methanol. The detection and quantitation limits were 0.33 and 1.00 µg/mL, respectively. The suggested method was successfully applied for the analysis of candesartan cilexetil in bulk and commercial tablets. The results were favorably compared statistically to those obtained by a reference method.[8]

A simple, specific, accurate, and stability-indicating UV- Spectrophotometric method was developed for the estimation of candesartan cilexetil, using a Shimadzu, model 1700 spectrophotometer and a solvent composed of methanol: water in the ratio of 9:1 at the wavelength (λ_{max}) 254 nm. Linearity was established for candesartan in the range of 10-90 µg/mL. The percentage recovery was found to be in the range of 99.76 - 100.79 %. The drug was subjected to acid, alkali and neutral hydrolysis, oxidation, dry heat, UV light, and photolytic degradation. Validation experiments performed to demonstrate system suitability, specificity, precision, linearity, accuracy, interday assay, intraday assay, robustness, ruggedness, LOD, and LOQ. While estimating the commercial formulation there was no interference of excipients and other additives. Hence this method can be used for routine determination of candesartan cilexetil in bulk and their pharmaceutical dosage forms. The

proposed method for stability study shows that there was appreciable degradation found in the stress condition of candesartan.[9]

A simple, sensitive, selective, and precise stability-indicating method for the determination of candesartan cilexetil in the presence of its alkaline degradation and tablets was developed and validated. The method is based on the determination of candesartan cilexetil by the bivariate calibration depending on a simple mathematic algorithm that provides simplicity and rapidity. The method showed good linearity in the range of 1- 12 µg/mL at 225 nm and 2-12 µg/mL at 250 nm with mean percentage recovery of 100.29 ± 0.64 %. Candesartan cilexetil can be determined in the presence of up to 80 % of its alkaline degradation, the selectivity of the method was checked using laboratory prepared mixtures. The proposed method has been successfully applied to the analysis of candesartan cilexetil in bulk and commercial tablets without interference from additives or excipients and the results were satisfactory compared with a reference method. Also, the suggested method was successfully applied to the content uniformity testing and degradation kinetic study.[10]

A simple and sensitive kinetic method is described for the determination of the hypotensive drug belonging to the angiotensin II antagonist Candesartan Cilexetil (CAN). This method is based upon a kinetic investigation of drug oxidation with alkaline potassium permanganate. All variables affecting color development have been investigated and the conditions optimized. The kinetic rate was obeying a pseudo-first-order reaction. Among the methods applied were the Initial rate, Rate constant, Fixed-concentration, and Fixed-time methods. Accounting for the applicability, the sensitivity, values of correlation coefficient (r) and intercept (a), the Fixed-time method is selected for this drug assay. The absorbance-concentration plot was rectilinear within the range of 5-30 ug/mL for CAN. The statistical data for the results challenged for the robustness of the fixed-time method.[11]

Two new rapid, simple, sensitive reproducible and economical spectrophotometric methods are described for the determination of Candesartan cilexetil (CDC) in bulk form. Both methods are based on the formation of colored complexes due to the action of 2, 6-Dichloroquinone chlorimide (DCQC) and 1, 2 Napthaquinone, 4-Sulphonic acid (NQS) on CDC in the alcoholic medium. Under optimized conditions, they show an absorption maxima at 550 nm (DCQC) and 480 nm (NQS), with molar absorptivities of 1.237 x 104 and 3.366 x 104 $mole^{-1}$ cm^{-1} and Sandell's sensitivities of 0.06329 and 0.1578 per 0.001 absorbance unit for DCQC and NQS, respectively. The color is stable for 5 min after extraction. Beers law is

obeyed between 5.0-25.0 µg/mL for DCQC and 4.0-20 µg/mL for NQS. The proposed methods were successfully extended to bulk and Pharmaceutical dosage forms.[12]

Two simple, sensitive, accurate, rapid spectrophotometric and conductometric methods were developed for the determination of candesartan (CAND) in raw material and its pharmaceutical preparation. The proposed methods depend upon the reaction of bromocresol green (BCG) or bromocresol purple (BCP) with candesartan in phosphate-buffered solution to form a stable colored ion-pair complex, which was extracted in chloroform. The yellow-colored complexes were determined at λmax 415, 405 nm with BCG, BCP, respectively. Using conductometric titration, candesartan could be evaluated in acetone. The optimizations of various experimental conditions were described. The results obtained showed a good recovery of 100.14 % (n=6) with a relative standard deviation of 0.62 % (n=6). Applications of the proposed methods to representative pharmaceutical formulations are successfully presented compared with official methods.[13]

In the present work simple, precise, specific, accurate, and cost-effective UV spectrophotometric method has been developed for the estimation of Candesartan Cilexetil in pharmaceutical formulation. The wavelength of the Candesartan Cilexetil detection was 258 nm in methanol and obeys Beer's law in the concentration range of 10 – 50 µg/mL with a correlation coefficient of 0.9999. The results of the analysis were validated by recovery studies. The percentage recovery of the drug after standard addition was found to be 99.45 - 99.85 %. The relative standard deviation was found to be < 2.0 % in all cases. The result of the analysis was found to be 99.83 ± 0.226 %. The Proposed spectrophotometric method was validated as per the ICH Q2 (R1) guidelines. The proposed method was found to be suitable for the quantitative determination of Candesartan Cilexetil in bulk form and pharmaceutical formulations.[14]

The spectrophotometric method was developed and applied for the determination of candesartan cilexetil (CDC) in tablets formulations. This new method was applied for determination (CDC) in several Syrian trademark drugs. The proposed method is based on the formation of a colored complex between 2, 6-Dichloroquinone chlorimide (DCQC) with CDC. Under optimized conditions, it shows maximum absorption at 467 nm. The analytical parameters and their effects are investigated. Beer's law was obeyed in the range of 20 – 100 µg/mL, with a correlation coefficient R2, = 0.9988. The average recovery of the CDC was between 98.09 and 102.86 %. The limit of detection (LOD) and limit of qualification (LOQ)

were 2.49 and 7.55 µg/mL, respectively. The proposed method has been successfully applied to the analysis of the studied drug in pure form and pharmaceutical formulations.[15]

Spectrofluorimetry method

The acid-base equilibrium constants of a new family of antihypertensive drugs, the angiotensin II receptor antagonists (ARA II), Losartan, Irbesartan, Valsartan, Candesartan cilexetil, its metabolite Candesartan M1 and Telmisartan were determined by spectrofluorimetry. Relative fluorescent intensity (*I*)–pH data were treated by graphical (derivatives and curve-fitting) and numerical methods (LETAGROP SPEFO). The resultant p*K* values at an ionic strength of 0.5 M were (3.15±0.07) for Losartan, (4.70±0.06) for Irbesartan, (4.90±0.09) for Valsartan, (6.0±0.1) for Candesartan cilexetil, (3.9±0.1) for Candesartan M1, and (4.45±0.09) for Telmisartan.[16]

A Simple, accurate, and highly sensitive spectrofluorimetric method is presented for the determination of candesartan cilexetil in bulk and tablets. The proposed method is based on the direct measurement of candesartan cilexetil fluorescence intensity. The excitation and emission wavelengths were 260 nm and 381 nm, respectively. The method was linear over the concentration range of 3.00 – 288.60 ng/mL. The limit of detection was 0.25 ng/mL, and the limit of quantitation was 0.77 ng/mL. To validate the method the results were compared with those obtained by a high-performance liquid chromatography method. The proposed method was successfully applied to the analysis of candesartan cilexetil in pure form and tablets with good precision and accuracy compared to the reported method as revealed by *t*- and *F*- tests. The method was shown to be highly specific in the presence of hydrochlorothiazide which is commonly combined with candesartan cilexetil in tablets.[17]

A simple, rapid, accurate and highly sensitive spectrofluorimetric method has been developed for the determination of some angiotensin II receptor antagonists (AIIRA's), namely Losartan potassium (Los-K), Irbesartan (Irb), Valsartan (Val) and Candesartan cilexetil (Cand) in pure forms as well as in their pharmaceutical dosage forms. All the variables affecting the relative fluorescence intensity (RFI) were studied and optimized. Under the optimum conditions, linear relationships with good correlation coefficients (0.9982–0.9991) were obtained over the concentration range from 0.006 µg/mL to 1.7 µg/mL. Good accuracy and precision were successfully obtained for the analysis of tablets containing each drug alone or combined with hydrochlorothiazide (HCTZ) without interferences from the co-formulated HCTZ or the additives commonly present in tablets.[18]

High-performance liquid chromatography method

Candesartan cilexetil in tablet preparations can be determined by high-performance liquid chromatography as follows.[5] Weigh accurately the mass of not less than 20 Candesartan Cilexetil Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 6 mg of candesartan cilexetil, add exactly 15 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) to make 150 mL, shake vigorously for 10 minutes, and allow to stand. Filter the supernatant liquid through a membrane filter with a pore size not exceeding 0.45 mm. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of candesartan cilexetil for assay (separately determine the water in the same manner as Candesartan Cilexetil), dissolve in acetonitrile to make exactly 50 mL. Pipet 4 mL of this solution, add exactly 10 mL of the internal standard solution, then add a mixture of acetonitrile and water (3:2) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 mL each of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios, QT and QS, of the peak area of candesartan cilexetil to that of the internal standard.

An isocratic reversed-phase liquid chromatographic method has been developed for the quantitative determination of candesartan cilexetil, used to treat hypertension, in the bulkdrug and pharmaceutical dosage forms. The method is also applicable to the analysis of related substances. Chromatographic separation was achieved on a 250 mm × 4.6 mm, 5 µm particle, CN column with a 50:50 (*v/v*) mixture of phosphate buffer, pH 3.0, and acetonitrile as mobile phase. The flow rate was 1.0 mL min and the detection wavelength was 210 nm. The resolution of candesartan cilexetil and six potential impurities was greater than 2.0 for all pairs of compounds. The drug was subjected to hydrolytic, oxidative, photolytic, and thermal stress and substantial degradation occurred in alkaline and acidic media and under oxidative and hydrolytic stress conditions. The major product obtained as a result of basic hydrolysis was different from that produced by acid hydrolysis and aqueous hydrolysis. The stress samples were assayed against a reference standard and the mass balance was found to be close to 99.6%. The method was validated for linearity, accuracy, precision, and robustness.[19]

This paper describes the analytical method suitable for the validation of candesartan cilexetil (CDN) by reversed-phase liquid chromatography (RP-LC) method. The method utilized RP-

LC (Shimadzu LC-10AT with UV detector) model and Hypersil ODS C-18 (250 x 4.6 mm, packed with 5 microns) columns were used for the separation. The mobile phase was consist of acetonitrile: 0.05 M KH_2PO_4 buffer in the ratio of 65: 35 at a flow rate of 1.5 mL/min. Validation experiments were performed to demonstrate system suitability, specificity, precision, linearity, accuracy study. The method was linear over the concentration range of 0.5-400 µg/mL. The method showed good recoveries (99.54-100.41 %) and the recovery studies were carried out by adding different amounts (80 %, 100 % & 120 %) of bulk samples of candesartan cilexetil along with internal standard were 100.04%, 99.98 % & 99.64 % respectively. The proposed method is precise, accurate, reproducible, and rapid for the determination of candesartan cilexetil in bulk and their pharmaceutical dosage forms.[20]

A simple, sensitive and inexpensive isocratic reverse-phase liquid chromatographic method has been developed for the quantitative determination of candesartan cilexetil, as a bulk drug, and in pharmaceutical dosage forms. Chromatographic separation was achieved on an octadecyl silyl column (C-18, 250 mm x 4.6 mm, 5 µm particle) with a 20: 80 (v/v) mixture of phosphate buffer, pH 2.5, and acetonitrile as mobile phase. The flow rate was 1.0 mL/min and the detection wavelength was 215 nm. The resolution of candesartan cilexetil was greater than 2.0. The drug was subjected to forced degradation such as photochemical oxidation, chemical oxidation, acid hydrolysis, base hydrolysis, different pH range, aqueous and nonaqueous solution, and thermal stress. The substantial degradation occurred in alkaline and acidic media and under oxidative and hydrolytic stress conditions and also in aqueous and non-aqueous hydrolysis. The method was validated for accuracy, precision, specificity, the limit of detection, the limit of quantification, and linearity range.[21]

A simple, sensitive gradient rapid resolution liquid chromatographic assay method has been developed for the quantitative determination of candesartan cilexetil in bulk active pharmaceutical ingredient, used for the treatment of hypertension. The developed method is also applicable toto the process-related impurities determination. Efficient chromatographic separation was achieved on a C18 stationary phase with a simple mobile phase combination delivered in a gradient mode and quantification was by ultraviolet detection at 210 nm at a flow rate of 0.4 mL/min. In the developed UPLC method the resolution between candesartan cilexetil and its two potential impurities was found to be greater than 2.0. Regression analysis showed an *r* value (correlation coefficient) greater than 0.99 for candesartan cilexetil and its two impurities. This method was capable to detect two impurities of Candesartan Cilexetil at

a level of 0.003 % concerning test concentration of 1.0 mg/mL-1 for a 2 µL injection volume. The bulk active pharmaceutical ingredient was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur in oxidative stress conditions. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.5%. The developed RP-LC method was validated with respect to linearity, accuracy, precision and robustness.[22]

A selective, specific, and sensitive Ultra High-Pressure Liquid Chromatography (UPLC) method was developed for determination of candesartan cilexetil impurities as well asits degradent in tablet formulation. The chromatographic separation was performed on Waters Acquity UPLC system and BEH Shield RP18 column using gradient elution of mobile phase A and B. 0.01 M phosphate buffer adjusted pH 3.0 with orthophosphoric acid was used as mobile phase A and 95 % acetonitrile with 5 % Milli Q Water was used as mobile phase B. Ultraviolet (UV) detection was performed at 254 nm and 210 nm, where (CDS-6), (CDS-5), (CDS-7), (Ethyl Candesartan), (Desethyl CCX), (N-Ethyl), (CCX-1), (1 N Ethyl Oxo CCX), (2 N Ethyl Oxo CCX), (2 N Ethyl) and any unknown impurity were monitored at 254 nm wavelength, and two process-related impurities, trityl alcohol and MTE impurity, were estimated at 210 nm. Candesartan cilexetil and impurities were chromatographed with a total run time of 20 min. Calibration showed that the response of impurity was a linear function of concentration over the range limit of quantification to 2 µg/mL ($r^2 \geq 0.999$) and the method was validated over this range for precision, intermediate precision, accuracy, linearity, and specificity. For the precision study, percentage relative standard deviation of each impurity was < 15 % (n= 6). Conclusion: The method was found to be precise, accurate, linear, and specific. The proposed method was successfully employed for estimation of candesartan cilexetil impurities in pharmaceutical preparations.[23]

Candesartan was degraded together under different stress test conditions prescribed by International Conference on Harmonization. The samples so generated were used to develop a stability-indicating high-performance liquid chromatographic (HPLC) method for the candesartan. The drug was well separated from degradation products using a reversed-phase (C-18) column and a mobile phase comprising of acetonitrile: buffer (80:20 v/v) at pH- 3. Other HPLC parameters were: flow rate, 1 mL/min; detection wavelength, 225 nm; and injection volume 20 µl. The method was validated for linearity, precision, accuracy etc. Results obtained after validation study, indicating that the proposed single method allowed

analysis of candesartan, in the presence of their degradation products formed under a variety of stress conditions. The developed procedure was also applicable to the determination of stability of the drug in commercial pharmaceutical dosage form.[24]

Two stability-indicating methods were developed for the determination of candesartan cilexetil in the presence of its degradation products. The first method uses isocratic RP-HPLC with an Agilent C18 column. The mobile phase was phosphate buffer (pH = 2.8 ± 0.1)–acetonitrile (60 + 40, v/v). The flow rate was 2.0 mL/min, and the UV detection was at 254 nm. The second method depends on TLC-densitometric measurements of drug spots at 254 nm. The separation was carried out on silica gel 60 F plates using ethyl acetate–methanol–toluene–ammonia 33% (40 + 25 + 20 + 2, v/v/v/v) mobile phase. The methods were validated according to U.S. Pharmacopeia guidelines, and the acceptance criteria for accuracy, precision, linearity, specificity, robustness, LOD, LOQ, and system suitability were met in all cases. Linear ranges of the methods were 10.0–200.0 μg/mL and 1.0–9.0 μg/spot for HPLC and TLC, respectively. The proposed methods were successfully applied to the drug in bulk powder, in laboratory-prepared mixtures with its degradation products, and in commercially available tablets. The results were compared statistically at the 95 % confidence level with each other. There were no significant differences between the mean recovery and precision of the two methods.[25]

A simple, rapid, selective and reproducible reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the estimation of release of candesartan cilexetil (CC) in tablets. Analysis was performed on an Agilent, Zorbax C8 column (150 mm · 4.6 mm, 5 lm) with the mobile phase consisting of phosphate buffer (pH 2.5) acetonitrile (15:85, v/v) at a flow rate of 1.0 mL/min. UV detection was performed at 215 nm and the retention time for CC was 2.2. The calibration curve was linear (correlation coefficient = 1.000) in the selected range of analyte. The optimized dissolution conditions include the USP apparatus 2 at a paddle rotation rate of 50 rpm and 900 mL of phosphate buffer (pH 7.2) with 0.03 % of polysorbate 80 as dissolution medium, at 37.0 ± 0.5 C. The method was validated for precision, linearity, specificity, accuracy, limit of quantitation and ruggedness. The system suitability parameters, such as theoretical plate, tailing factor and relative standard deviation (RSD) between six standard replicates were well within the limits. The stability result shows that the drug is stable in the prescribed dissolution medium. Three different batches (A, B and C) of the formulation containing 8 mg

of candesartan cilexetil was performed with the developed method and the results showed no significant differences among the batches.[26]

New analytical methods have been developed and validated on high performance liquid chromatography (HPLC) to assess the assay, content uniformity and dissolution of immediate release candesartan cilexetil 32 mg tablets. Method development studies were performed on cyano column. Mobile phase of assay and content uniformity test consisted of mixture of 0.05 M phosphate buffer, pH 4.5 and methanol (40 : 60, v/v) adjusted to pH 4.0 with trifluoroacetic acid, whereas mobile phase of dissolution test consisted of mixture of 1 mM phosphate buffer and acetonitrile (50 : 50, v/v) adjusted to pH 2.0 with trifluoroacetic acid. Mobile phases were pumped at flow rate of 1.0 mL/min, ultraviolet-visible (UV) detector was operated at 254 nm, injection volume was set at 20 µL, column temperature was held at 25 ^{o}C. Dissolution medium was 0.05 M phosphate buffer, pH 6.5 including 0.70 % (w/v) polysorbate 20. Validation studies met acceptance criteria of system suitability, specificity, linearity and range, accuracy, precision, detection limit (LOD), quantitation limit (LOQ) and robustness parameters.[27]

Electroanalytic method

A sensitive, simple and rapid square-wave adsorptive stripping voltammetric method was developed and validated for the determination of candesartan cilexetil in pharmaceutical formulations. The proposed method was based on electrochemical reduction of candesartan cilexetil at a hanging mercury drop electrode in phosphate buffer at pH 5.0. A well-defined reduction peak was observed at -1340 mV with 30 s of accumulation time and -1100 mV of accumulation potential. Under these optimized conditions, the square-wave adsorptive stripping voltammetric peak current showed a linear correlation on drug concentration over the range of 0.25–1.34 µg/mL with a correlation coefficient of 0.9986 for the proposed method. The detection and quantitation limits for this method were 1×10^{-2} and 2.5×10^{-1} µg/mL, respectively. The results obtained for intra-day and inter-day precision (as RSD %) were between 1.10 and 3.90 %. This method was applied successfully for the determination of candesartan cilexetil in its tablet dosage forms with mean recoveries of 101.13 ± 0.78 % with RSD of 2.06 % for 8 mg tablet and 99.84 ± 0.89 % with RSD of 2.36 % for 16 mg tablet. The results obtained from the developed square-wave adsorptive stripping voltammetric method were compared with those obtained by the analytical method reported in the literature.[28]

The aim of this study was to develop a new, simple, rapid, and sensitive square-wave adsorptive stripping voltammetric method for the determination of candesartan cilexetil in bulk form and pharmaceutical formulations by complex formation with Cu(II). The experimental and instrumental parameters affecting the response of the candesartan cilexetil-Cu(II) complex were investigated and optimized. This method was based on electrochemical reduction of candesartan cilexetil-Cu(II) complex at a hanging mercury drop electrode in phosphate buffer at pH 3.0 containing $CuCl_2.2H_2O$. A well-defined reduction peak was observed at 325 mV with 30 s accumulation time and 150 mV accumulation potential versus an Ag/AgCl reference electrode in the proposed method. The developed voltammetric method was validated in terms of linearity, sensitivity, precision, accuracy, recovery, selectivity, robustness, and ruggedness. The linear calibration range was 0.50-177 g/mL (r = 0.9972). The LOD and LOQ of this method were 0.10 and 0.50 g/mL (n 7), respectively. The developed method was fully validated and applied to the pharmaceutical formulations, including candesartan cilexetil.[29]

A new electrochemical method is introduced for the determination of candesartan cilexetil using a new sensor and Continuous Coulometric Fast Fourier transformation cyclic voltammetry (CCFFTCV). The new sensor was designed based on silicon carbide nanoparticles and graphene nanosheets hybrid mixed with ionic liquid (1-Butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF6]) on a glassy carbon electrode. The sensor response calculated in form of coulomb by integrating the charge changes under the peak current in a selected potential range. The experimental conditions, for the electrochemical measurement were optimized. The linear concentrations range of candesartan cilexetil was from 0.5 to 120×10^{-8} M with a detection limit of 5.2×10^{-9} M. Moreover, the proposed sensor exhibits good accuracy, the response time less than 6s, high sensitivity with repeatability (RSD value of 3.5 %) and long term stability, 55 days with a decrease of 2.3 % in the response.[30]

Two simple, sensitive, accurate, rapid spectrophotometric and conductometric methods were developed for the determination of candesartan (CAND) in raw material and in its pharmaceutical preparation. The proposed methods depend upon the reaction of bromocresol green (BCG) or bromocresol purple (BCP) with candesartan in phosphate buffered solution to form stable colored ion-pair complex, which was extracted in chloroform. The yellow colored complexes were determined at λ_{max} 415, 405 nm with BCG, BCP, respectively. Using

conductometric titration, candesartan could be evaluated in acetone. The optimizations of various experimental conditions were described. The results obtained showed good recover of 100.14 (n=6) with relative standard deviation of 0.62 (n=6). Applications of the proposed methods to representative pharmaceutical formulations are successfully presented compared with official methods.[13]

Flow injection method

This article describes the development of flow injection methods for the determination of candesartan cilexetil using spectrophotometery and spectrofluorimetry in pharmaceutical formulations. The first method is based on the UV absorption at 270 nm in phosphate buffer containing cetyl trimethylammonium bromide (CTAB) and methanol at pH 6.5. The second method based on the fluorescence excitation and emission at 260 and 384 nm respectively, in phosphate buffer containing sodium dodecyl sulphate (SDS) and methanol at pH 4.0. Calibration graphs were linear over the concentration range of 0.1 to 4.0 µg/mL for UV-method and 0.03 to 2.0 µg/mL for fluorescence method. The limits of detection (3 s) of 0.01 µg/mL for both methods with sample throughput of 80 and 100/h were obtained respectively. The relative standard deviations of 1.2 to 3.2 % (n = 4) for UV-method and 0.5 to 1.8 % for fluorescence method were achieved in the concentration range studied. The developed methods were applied to pharmaceuticals and the results obtained were compared with HPLC reference method and no significant difference between these methods was observed at 95 % confidence level.[31]

Analysis of the candesartan cilexetil-pitavastatin calcium mixture

A comparative study of smart spectrophotometric chemometric assisted techniques and RP-HPLC for the determination of candesartan cilexetil (CAN)-pitavastatin calcium (PIT) and clopidogrel bisulfate (CLO)-rosuvastatin calcium (ROS), binary co-administered drugs were developed and validated. The spectrophotometric chemometric assisted methods included two simple techniques, namely Fourier transform convolution (FF) and ratio spectra of Fourier transform convolution (FFR) methods. FFR is considered as a hybrid divisor ratio spectra method where Fourier functions are applied to divisor ratio signals. The RP-HPLC method involves a rapid separation on a C18 column using a mobile phase consisting of acetonitrile: sodium dihydrogen phosphate (adjusted to pH 2.6 using orthophosphoric acid) in the ratio of 70: 30 v/v at a flow rate of 1 mL/min in isocratic mode. CLO and ROS were monitored at 220 nm however CAN and PIT were monitored at 238 nm. The

spectrophotometric chemometric assisted methods proved their ability to quantify each of the studied drugs in their binary mixtures, where excellent percentage recoveries were obtained. FF and FFR method proved to be linear over the concentration range of 10-50 µg/ml for CLO, 4 - 20 µg/mL for ROS, 8 - 20 µg/mL for CAN and 2 - 10 µg/mL for PIT. The RP-HPLC method was able to separate the drugs in the study; retention times were found to be 3.9 min and 14.4 min for ROS-CLO, 4.2 min and 14.5 min for PIT-CAN respectively. The RP-HPLC method was found to be linear in the concentration range of 0.1 - 0.5 µg/mL for CLO, 0.04 - 0.2 µg/mL for ROS, 0.5 - 1 µg/mL for CAN and 0.05-0.1 µg/ml for PIT. System suitability parameters proved that peaks were well resolved from each other. The spectrophotometric and chromatographic methods were validated according to ICH guidelines. Recovery was found to be in the range of 95.9 % - 100.5 % in synthetic laboratory mixtures. The suggested spectrophotometric methods have the advantage over other methods that they do not require a preliminary separation. Statistical analysis between the suggested spectrophotometric chemometric assisted and RP-HPLC methods, using student's t-and F-test revealed that there is no difference between the applied methods.[32]

Analysis of candesartan cilexetil-amlodipine mixture

A new, simple, accurate and sensitive UV-spectrophotometric absorption correction method has been developed for simultaneous determination of candesartan cilexetil and amlodipine besylate in bulk utilizing concept of standard addition. The method is based upon determination of amlodipine besylate at 237 nm and candesartan cilexetil at 254 nm in methanol as a solvent. Overlay spectra of both drugs shows absorbance at 244 nm. Linearity was observed in range of 10 - 60 µg/mL and 2 -10 µg/mL for Candesartan Cilexetil and Amlodipin Besylate respectively. The correlation coefficient value was found to near to 1. All methods were statistically validated as per ICH guidelines.[33]

The aim of this paper consisted in the development and validation of a high-performance liquid chromatography (HPLC) method for the determination of three antihypertensive substances from a mixture and pharmaceutical formulations. The three selected antihypertensive substances were candesartan, amlodipine and hydrochlorothiazide. The extraction and quantification of the constituents by HPLC was carried out using an analytical column, C18, and a mobile phase consisting in a mixture of buffer solution pH = 3.5 and methanol (15:85 v/v), at a flow rate of 1 mL/min. The UV detection was performed at 240 nm for candesartan and amlodipine and at 270 nm for hydrochlorothiazide. The three

compounds mentioned above were separated with good resolution, reproducibility and sensitivity under these conditions. The proposed HPLC method was applied to the analysis of the in-house dosage forms.[34]

Analysis of candesartan cilexetil - hydrochlorothiazide mixture

Two-component mixtures of candesartan cilexetil (CAN) and hydrochlorothiazide (HYD) were assayed by first derivative and ratio derivative spectrophotometry. The first method depends on zero-crossing and peak to base measurement. The first derivative amplitudes at 270.1 and 255.5 nm were selected for the assay of (CAN) and (HYD), respectively. The second method depends on first derivative of the ratio spectra by division of the absorption spectrum of the binary mixture by a normalized spectrum of one of the components and then calculating the first derivative of the ratio spectrum. The first derivative of the ratio amplitudes at 236, 250, 232, 267 and 280 nm were selected for the determination of (CAN) and (HYD), respectively. Calibration curves were established for 6.0 - 38.0 µg/mL for (CAN) and 4.0 - 28.0 µg/mL for (HYD) in binary mixtures. Good linearity, precision and selectivity were found, and the two methods were successfully applied to the pharmaceutical dosage form containing the above-mentioned drug combination without any interference by the excipients.[35]

A method for simultaneous estimation of candesartan cilexetil and hydrochlorothiazide in tablet dosage form has been described. The method is based on UV-Spectrophotometric determination using Q-absorbance method. It involves, formation of Q-absorbance equation at 258.14 nm (isoabsorptive point) and 271 nm λ_{max} of hydrochlorothiazide in methanol. Linearity was obtained in the range 2 – 24 µg/mL for candesartan and 2 - 24 µg/mL for hydrochlorothiazide. The method allows rapid analysis of binary pharmaceutical formulation with accuracy. The % recovery lies in the range of 101.2 – 102.1 for CAN and 99.2 – 99.7 for HCTZ. Result were validated statistically and were found satisfactory.[36]

Candesartan cilexetil (CND), and hydrochlorothiazide (HCT) are co-formulated in a single-dose combination for the treatment of hypertensive patients whose blood pressure is not adequately controlled on either component monotherapy. In this work, two multivariate calibration methods were applied for simultaneous spectrophotometric determination of CND and HCT in their combined pharmaceutical tablets. The multivariate methods are principal component regression (PCR) and partial least squares (PLS). Both methods are useful in spectral analysis because of the simultaneous inclusion of many spectral wavelengths instead

of the single wavelength used in derivative spectrophotometry. The optimum assay conditions were established and the proposed methods were successfully applied for the assay of the two drugs in an independent validation set and combined pharmaceutical tablets with excellent recoveries. No interference was observed from common pharmaceutical additives. The results were favorably compared with those obtained by a reference HPLC method.[37]

A new simple derivative spectrophotometric method has been developed for the simultaneous determination of candesartan cilexetil and hydrochlorothiazide in tablet dosage forms. The first derivative method is based on the measurement of absorbance of one drug at the zero crossing point of another drug. Candesartan cilexetil and hydrochlorothiazide were determined at two different wavelengths 222.69 nm (zero crossing point of hydrochlorothiazide) and 254.63 nm (zero crossing point of candesartan cilexetil) from the derivative spectra respectively. The methods hows linearity over the concentration range 0.5 - 50 and 0.1 - 50 µg/mL for candesartan cilexetil and hydrochlorothiazide respectively in phosphate buffer. The proposed method was validated and can be used for routine analysis of combined tablet dosage forms containing candesartan cilexetil and hydrochlorothiazide.[38]

The simultaneous determination of candesartan cilexetil and hydrochlorothiazide mixtures by using spectrophotometric method is a difficult task in analytical chemistry, due to spectral interferences. Ultra violet-spectra of the studied drugs were collected from 200 nm to 350 nm in ethanol solutions. In this range the studied drugs showed a considerable degree of spectral overlapping (91.82 %). Initial studies of the UV-spectra of the individual compounds showed that satisfactory linear regression calibration models could be constructed in the concentration range of 5 - 50 µg/mL and 2 - 20 µg/mL for candesartan and hydrochlorothiazide respectively. Resolution of binary mixtures of the drugs has been accomplished by using 1D (first derivative), 1D ratio (first derivative ratio). The multivariate methods, classical least squares (CLS) and principal components regression (PCR) has also been used for determination the studied drugs. Thus, a simple, rapid and inexpensive method for the simultaneous determination of these drugs (either in their pure powder forms, laboratory prepared samples or in their pharmaceutical formulation) was researched and developed with the aid of chemometrics assisted spectrophotometric methods.[39]

A simple, rapid and precise high-performance thin-layer chromatographic (HPTLC) method for analysis of candesartan cilexetil and hydrochlorothiazide in their tablet dosage forms has been developed and validated. Chromatography was performed on silica gel 60 GF_{254} plates

with acetone-chloroform-ethyl acetate-methanol 3:3:3:0.5 (*v/v*) as mobile phase. Detection was performed at 280 nm. The R_F values were 0.27 for candesartan cilexetil and 0.45 for hydrochlorothiazide. Regression plots revealed good linear relationships in the concentration ranges 50.6 - 253.0 µg for hydrochlorothiazide and 65.0 - 325.0 µg for candesartan cilexetil. When accuracy was checked by conducting recovery studies, average recovery was 99.78% and 98.09% for candesartan cilexetil and hydrochlorothiazide, respectively. The amounts of the drugs in the marketed formulation was 100.26 %. and 99.28 % for candesartan cilexetil and hydrochlorothiazide, respectively.[40]

Two methods are described for the simultaneous determination of candesartan cilexetil (CAN) and hydrochlorothiazide (HYD) in binary mixture. The first method was based on HPTLC separation of the two drugs followed by densitometric measurements of their spots at 270 nm. The separation was carried out on Merck HPTLC aluminium sheets of silica gel 60 F_{254} using chloroform: methanol (8:2, v/v) as mobile phase. Linear regression analysis data used for the regression line were in the range of 0.05 - 0.70 and 0.05 - 0.50 µg/band for CAN and HYD, respectively. The second method was based on difference and derivative-difference spectrophotometry with a zero-crossing measurement technique. Linear calibration graphs of absorbance difference values (ΔA) at 292 nm and 338 nm were obtained versus concentration in the range 20 - 100 mg/L for CAN and HYD. Also, linear regression equations of second derivative difference values (ΔD2) at 296 nm for CAN and first derivative difference values (ΔD1) at 299 nm for HYD versus concentration in the ranges 10 - 100 and 5 - 70 mg/L for CAN and HYD, respectively, were obtained. The two methods were validated according to ICH guidelines and applied on bulk powder and pharmaceutical formulation.[41]

The present study deals with development and validation of stability indicating HPTLC method for quantitative determination of candesartan cilexetil (CDT) and hydrochlorothiazide (HCT) in pharmaceutical dosage form.. Chromatographic separation was performed on aluminum plate precoated with Silica Gel 60 F_{254} using toluene: chloroform: ethanol: glacial acetic acid (2:7:1:0.1 v/v) as mobile phase. The wavelength selected for densitometry scanning was 270 nm. Regression plots revealed linear relationships in the concentration range of 500 - 5000 and 400 - 2000 ng/band for CDT and HCT respectively. The correlation coefficient of calibration curves was found to be more than 0.99 for both analytes. The chromatographic conditions gave compact spots at Rf value (± SD) 0.12 (±

0.01) and 0.70 (± 0.02) for HCT and CDT respectively. The method was validated as per International Conference on Harmonization (ICH) guidelines, demonstrating to be accurate and precise within the corresponding linearity range of titled analytes. Inherent stability of these drugs was studied by exposing drug substances to various stress conditions as per ICH guidelines namely oxidative, photolysis and hydrolytic conditions under different pH. Relevant degradation was found to take place under these conditions. A new simple, accurate, precise, sensitive and economic stability-indicating HPTLC method has been developed and validated for the simultaneous determination of CDT and HCT in pharmaceutical dosage form. The proposed method can be used for the routine estimation of CDT and HCT in bulk and formulation and can be employed for stability-indicating analysis.[42]

No methods were reported for the simultaneous determination of HCZ and CAN in the presence of CHL and DSA; accordingly, the aim of the presented research work is to provide highly selective, accurate and simple HPTLC method for the determination of the proposed mixture in pure forms and dosage form. TLC Scanner 3 Densitometer (Camag, Muttenz, Switzerland) was used for separation of HCZ, CAN, CHL and DSA mixtures and for quantification of HCZ and CAN on HPTLC silica gel 60 F using ethyl acetate: methanol: glacial acetic acid (95:5:0.05 by volume), followed by densitometric measurement of bands at 270 nm. HPTLC method was successfully applied for separation of HCZ, CAN, CHL and DSA mixtures and for quantification of HCZ and CAN. Good resolution is indicated by the difference in the retention factor (R_f) values of (HCZ = 0.73, CAN = 0.36, CHL =0.53, DSA = 0.86). The proposed method was validated according to International Conference on Harmonization (ICH) guidelines. The proposed HPTLC method provides sensitive, accurate, and selective means for determination of mixtures of HCZ and CAN in the presence of impurities of HCZ; (CHL and DSA) using HPTLC method; which gives an advantage over previously reported methods for the analysis of HCZ and CAN mixtures that do not consider interference of impurities. The proposed method could be applied for the determination of pure HCZ and CAN in the presence of (CHL and DSA) both in bulk powder and in pharmaceutical formulations.[43]

An HPLC-UV method for simultaneous determination of candesartan cilexetil and hydrochlorothiazide in compound candesartan cilexetil tablets was established. A Phenomenex C18 column (250 mm × 4.6 mm 5 μm) was used with the mobile phase of acetonitrile - 0.1 mol/L sodium dihydrogen phosphate solution (added 0.1 % triethylamine,

adjusted pH 5.0 with phosphoric acid) (65:35) at the detection wavelength of 262 nm. The flow rate was 1.0 mL/min and the column temperature was 35 ^{o}C. The calibration curves of candesartan cilexetil and hydrochlorothiazide were linear in the ranges of 1.6 - 32.0 µg/mL (r = 0.9998) and 1.2 - 23.8 µg/mL (r = 0.9999), respectively. The average recoveries (n = 9) were 102.1 % and 100.7 %, respectively. This method is accurate,quick and suitable for content determination of compound candesartan cilexetil tablets.[44]

A simple, sensitive, and inexpensive high-performance liquid-chromatographic method has been developed for simultaneous determination of hydrochlorothiazide and candesartan cilexetil in pharmaceutical formulations. Chromatographic separation was achieved on a Phenyl-2 column with a 25:75:0.2 mixture of 0.02 M potassium dihydrogen phosphate, methanol, and triethylamine, final pH 6.0 ± 0.1, as mobile phase. Detection was at 271 nm. Response was a linear function of concentration in the range 5 - 45 µg/mL for hydrochlorothiazide and 12 - 56 µg/mL for candesartan cilexetil; the correlation coefficients were 0.9993 and 0.9991, respectively. Total elution time for the two components was less than 5 min.[45]

A high-performance liquid chromatographic method was developed for simultaneous determination of candesartan cilexetil and hydrochlorothiazide in binary mixtures and in pharmaceutical dosage forms. Active substances were separated by gradient system in the mobile phase, consisting of acetonitrile and 0.02 M sodium acetate. The flow rate of the mobile phase was 1 mL/min. Separation was achieved on Kromasil 100 C18 column (4.6 mm i.d. × 25 cm length, 5 µm). Detection was carried out using UV detector set at 265 nm. The detector response was linear in the range of 16 - 200 ng/injection (10 µL) for candesartan cilexetil and 12.5 - 1250 ng/injection (10 µL) for hydrochlorothiazide. The relative standard deviation of the recovered amounts of candesartan and hydrochlorothiazide were 0.27 and 0.60 %, respectively. The limit of detections were 4 ± 0.05 and 5 ± 0.06 ng/injection for hydrochlorothiazide and, candesartan, respectively. The method was applied for the determination of candesartan and hydrochlorothiazide in pharmaceutical tablets.[46]

A simple, sensitive and reproducible reverse phase high performance liquid chromatographic method has been developed for simultaneous estimation of candesartan cilexetil and hydrochlorothiazide in combined tablet dosage form. Chromatography was performed on a 250 mm x 4.6 mm, 5-µm particle size, C8 Hypersil BDS column with a 60:40 (v/v) mixture of acetonitrile and Triethylamine (0.02 %) as a mobile phase and the pH was adjusted to 5.5

with dilute o-phosphoric acid. The detection of the combined dosage form was carried out at 262 nm and a flow rate employed was 1.0 mL/min. The retention times were 2.449 and 4.895 min for candesartan and hydrochlorothiazide respectively. Linearity was obtained in the concentration range 50 to 150 µg/mL for candesartan cilexetil and 75 - 225 µg/mL for hydrochlorothiazide, with a correlation coefficient of 0.9999 and 0.9999. The results of the analysis were validated statistically and recovery studies confirmed the accuracy and precision of the proposed method.[47]

A simple and sensitive RP-HPLC method was developed and validated for the determination of candesartan cilexetil and hydrochlorothiazide in pharmaceutical dosage forms. The separation of components was achieved on a SHIMADZU Hypersil ODS-C18 column (250 × 4.6 mm, 5 µm) with UV detection at 270 nm. Isocratic elution with a mobile phase consisting of 10 mM (pH 3.37) tetra butyl ammonium hydrogen sulphate: methanol (15:85, V/V), at a flow rate 1.0 mL/min was employed. Linearity was observed in the concentration range 0.625 - 62.5 µg/mL for hydrochlorothiazide and 0.8 - 80 µg/mL for candesartan cilexetil respectively. The linear regression equation was found to be Y = 64002X - 1412.6 for hydrochlorthiazide and Y = 24649X - 6701.8 for candesartan cilexetil respectively with correlation coefficients greater than 0.999. The LOD was found to be 0.1385 and 0.1892 µg/mL for hydrochlorothiazide and candesartan cilexetil respectively, whereas the LOQ was found to be 0.4394 and 0.6187 µg/mL for hydrochlorthiazide and candesartan cilexetil respectively. The mean analytical recovery in determination of candesartan cilexetil and hydrochlorothiazide tablets was 99.31 - 100.08 % hydrochlorothiazide and 99.58 - 100.39 % for candesartan cilexetil respectively. Thus, the proposed method is applicable for routine determination of candesartan cilexetil and hydrochlorothiazide in pharmaceutical formulations.[48]

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of candesartan cilexetil and hydrochlorothiazide in pharmaceutical dosage form. The column used was Hypersil BDS C18 (150 x 4.6mm, 5µm) in isocratic mode, with mobile phase containing phosphate buffer-acetonitrile (55:45) adjusted to pH 4.6 using ortho phosphoric acid was used and injection volume of 20 µL, with a flow rate of 1.0 mL/min. and effluents were monitored at 244 nm. The retention times of candesartan cilexetil and hydrochlorothiazide were 2.6 min and 3.6 min, respectively. The linearity for candesartan cilexetil and hydrochlorothiazide were in the

range of 38.4 - .6 mcg/mL and 30 - 70 mg/mL respectively with correlation coefficient of r^2 = 0.999 for both. The assay of the proposed method was found to be 99.38 % and 99.26 %. The recoveries of candesartan cilexetil and hydrochlorothiazide were found to be 101.3 % and 99.86 %, respectively. The % RSD from reproducibility was found to be < 2 %. The proposed method was statistically evaluated and can be applied for routine quality control analysis of candesartan cilexetil and hydrochlorothiazide in bulk and in pharmaceutical dosage form.[49]

A simple, rapid Reverse phase – Ultra Performance liquid chromatography (RP-UPLC) method was developed and validated for the simultaneous determination of thirteen known potential impurities present in candesartan cilexetil and hydrochlorothiazide fixed dose combination drug product. Chromatographic separation attained using 0.1 % perchloric acid in water and acetonitrile as Mobile phase-A and B respectively. The components were efficiently separated in Acquity UPLC HSS T3, 100 mm x 2.1 mm with 1.8 µm particle size column. Flow gradient elution mode with initial flow rate of 0.5 mL.min-1 followed by 0.6 mL/min was used. The impurities were quantified at a working wavelength of 220 nm. The developed method was validated as per International Conference on Harmonization (ICH) recommendations for specificity, linearity, precision, ruggedness, accuracy, sensitivity (limit of detection & limit of quantitation) and robustness. The present stability indicating method is having shorter run time which is helpful for fast analysis of samples during quality control testing with reduced solvent consumption in a cost and time effective approach.[50]

An isocratic HPLC method was developed for rapid simultaneous separation and determination of carvedilol, candesartan cilexetil and hydrochlorothiazide in pure and tablet dosage form. Separation was carried out on a Hypersil gold C18 (15 µm, 100 x 4.6 mm) column. Mobile phase composed of methanol, acetonitrile and 0.1% ortho-phosphoric acid in the ratio of (35:50:15, v/v/v). The flow rate was 0.8 mL/min and UV detection was measured at 215 nm. Linearity range was observed from 1 - 90 µg/mL for the three drugs. The proposed method was validated in terms of linearity, accuracy, precision and limits of detection and quantitation according to ICH. The obtained results were compared with reference methods.[51]

Development, validation and comparison of two stability-indicating LC methods, one with photodiode array detector (DAD) and the other with evaporative light scattering detector

(ELSD), were performed for simultaneous determination of candesartan cilexetil (CANC) and hydrochlorothiazide (HCTZ), in pharmaceutical samples. A RP-18 column (125 mm × 4 mm, 5 μm) was used for separation of CANC, HCTZ and its major degradation products, using acetonitrile and phosphate buffer (pH 6.0) for DAD method and acetonitrile and water with acetic acid and triethylamine (pH 4.1) for ELSD method, as mobile phase in a gradient mode. The response with ELSD was fitted to a power function and the DAD response by a linear model over a range of 32 - 160 μg/mL for CANC and 25 - 125 μg/mL for HCTZ. The precision and accuracy of the methods were similar, with RSD below 3.0 % and recovery between 98.1 % and 103.9 %. The drugs were subjected to stress conditions of hydrolysis, oxidation, photolysis, humidity and temperature. The degradation products were satisfactory separated from the main peaks and from each other. Both drugs mainly degrade by hydrolysis, showing the formation of one degradation product for HCTZ and two for CANC; its identification was conducted by LC/MS/MS. The methods were successfully applied to the analysis of CANC and HCTZ in combined commercial tablets. The performance of DAD and ELSD methods are comparable, therefore both methods are suitable for stability study and determination of CANC and HCTZ in pharmaceutical samples.[52]

The study describes method development and subsequent validation of RP-HPLC method for simultaneous estimation of candesartan and hydrochlorothiazide in combined tablet dosage forms. Chromatographic separation was achieved on a Hypersil BDS C8 column (250 mm x 4.6 mm, 5 μm) using a mobile phase consisting of (40: 60 v/v) acetonitrile: triethylamine (0.02 %) at a flow rate of 1 mL/min. The detection wavelength is 262 nm. The retention times of candesartan and hydrochlorothiazide were found to be 2.449 min and 4.895 min respectively. The developed method was validated as per ICH guidelines. The developed and validated method was successfully used for the quantitative analysis of candesartan and hydrochlorothiazide in tablet dosage forms.[53]

CONCLUSION

Overall, various analytical methods have been used to determine candesartan cilexetil levels. Spectrophotometry, spectrofluorimetry, electroanalytic, flow injection, high-performance liquid chromatography, and thin-layer chromatography methods are simple and easy to apply. However, the HPLC and HP-TLC analysis methods are often used in research because it can detect samples with low concentrations. The HPLC and HP-TLC methods can be applied in mixture of candesartan cilexetil with other drug.

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