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Review of Determination of Chlortalidone Levels in Pharmaceutical Preparations and Biological Matrix

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Abstract: Chlortalidone is a compound that is often used in the pharmaceutical world as an oral drug to treat hypertension. This review article discusses the summary of methods for determining the concentration of chlorthalidone in pharmaceutical preparations and biological matrices. We took the data collected in this review article was taken through trusted sites such as Google Scholar with the search keywords "chlorthalidone determination," "pharmaceutical preparations," and "biological matrices," with a period of the last twenty years (2001-2021). This review article aims to provide an overview of the assay used to determine the concentration of chlorthalidone either in the form of a single substance or pharmaceutical dosage forms. Overall, the determination of chlorthalidone levels has been carried out using various analytical methods. Including spectrophotometry, high-performance liquid chromatography (HPLC), chemometrics and thin-layer chromatography (TLC) - densitometric, capillary zone electrophoresis (CZE) methods, spectrofluorimetric methods, liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS) methods, ultrafast liquid chromatography (UFLC), supercritical fluid chromatography method and high-performance thin-layer chromatography (HPTLC)-densitometric method. Analysis with the HPLC technique is widely used in research because it can detect samples with the lowest concentrations up to the nanogram level.
Keywords: Chlortalidone, Pharmaceutical preparation, biological matrix, mixtures

1. Introduction

Hypertension is a disease that contributes to an increase in mortality. The death rate due to hypertension is increasing along with an unhealthy lifestyle. One of the efforts to treat hypertension is using diuretic therapy. Diuretic therapy is the primary Lili therapy recommended for people suffering from hypertension [1]. Chlortalidone is a compound that is often used as a diuretic for diuretic therapy. This compound has been shown to have a therapeutic diuretic effect as an antihypertensive [2].

Chlortalidone contains not less than 98.00% and not more than 102.0% $C_{14}H_{11}C_1N_2O_4S$, calculated based on the dried substance. Chlortalidone is practically insoluble in water, ether, and chloroform; soluble in methanol; slightly soluble in ethanol. The chemical name of chlorthalidone is 2-chloro-5(1-hydroxy-3-oxo-1-isoindolinyl)benzenesulfonamide [3].

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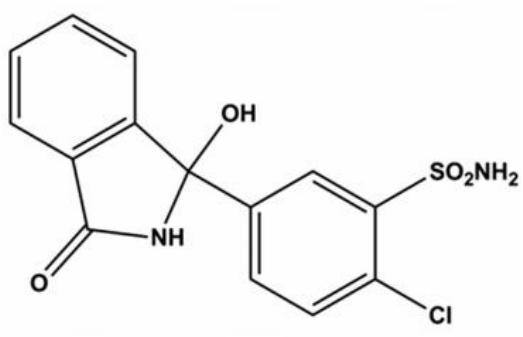

Figure 1: Chemical Structure of Chlortalidone [3]

Several methods have been reported related to the analysis of chlorthalidone in pure compounds and pharmaceutical preparations, and biological fluids. To date, many analytical methods have been developed for the quantitative determination of chlorthalidone levels.

2. Method of collecting data

In preparing this article, the technique used is literature study by searching for sources or literature in official books and international journals in the last 20 years (2001-2021). The keywords used to explore the data were "chlorthalidone assay," "pharmaceutical preparation," and "biological matrix." The primary reference search for this review article is through trusted websites such as Science Direct, NCBI, ResearchGate, Google Scholar, and other published and trustworthy journals.

3. Analysis methods of chlortalidone

3.1 Spectrophotometric Analysis

Spectrophotometric analysis of chlortalidone in various samples is presented in Table 1.

Table 1: Spectrophotometric analysis of chlortalidone in various samples

No.	Samples	Solvents	Methods	Wavelength (nm)	Range of Concentration	Ref.
1.	Chlortalidone tablets	Methanol	zero-, first- and second-order derivative	276 nm	10,0-75,0 µg/mL	[4]
2.	Cilnidipine and Chlortalidone tablets	Methanol	multi-wavelength method	271,83 nm and 278,34 nm 233,83 nm and 250,0 nm	2-10 µg/mL 2,5-12,5 µg/mL	[5]
3.	Chlortalidone and atenolol bulk and compound tablets	Methanol	simultaneous method	284 nm 225 nm	30 µg mL^{-1} - 140 µg mL^{-1} 10 µg mL^{-1} - 60 µg mL^{-1}	[6]

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4	Chlortalidone	Methanol	Visible spectrophotometry with reagent 1,2-naphthoquinone-4-sulfonate	440.50 nm	2–12 µg/mL	[7]
5	Chlortalidone and azilsartan medoxomil	Methanol	simultaneous measurement	257 nm 286 nm	2–20 µg mL^{-1} 8–50 µg mL^{-1}	[8]

Simple, rapid, and accurate zero-, first- and second-order derivative spectrophotometric methods have been developed to determine chlorthalidone (CLT) in commercially available tablets. Normal spectrophotometric scan (zero-order) shows maximum absorbance at 276 nm in methanol solution and good linearity in the range of 10.0–75.0 µg/mL. Linear relations using first (D1) and second (D2) order derivative methods were obtained at 278 and 288 nm for D1 and 286 and 292 nm for D2. The calibration curves were constructed in the range of 1.0–25.0 µg/mL for D1 (R = 0.998) and D2 (R = 0.999). Different analytical validations were determined (accuracy, precision, specificity, recovery, stability, and robustness) to demonstrate their suitability for routine quality control labs. The developed methods were successfully applied to a tablet formulation, and the results were compared statistically with each other and with those obtained by the HPLC reference method [4].

A simple, accurate, and precise two-wavelength spectrophotometric method was developed for the simultaneous determination of cilnidipine and chlorthalidone in compound dosage forms. The principle of the multi-wavelength method is that the optical density difference between two points in the mixture spectrum is directly proportional to the concentration of the required component. The wavelengths used to determine cilnidipine were 271,83 nm and 278,34 nm, and the wavelengths used to determine chlorthalidone were 233,83 nm and 250,0 nm. Methanol is used as a solvent. Analysis shows that the concentration range of cilnidipine is 210 µg/ml and the concentration range of chlorthalidone is 2512,5 µg/ml. The method has been validated according to various parameters recommended by ICH (such as accuracy, precision, specificity, linearity, reliability, LOD, and LOQ). The linearity of the proposed method was checked in the range of 210 µg/mL cilnidipine (r2 = 0.9990) and 2.5-12.5 µg/ml (r2 = 0,9986) of chlorthalidone. The limits of detection (LOD) were 0.4174 µg/mL and 0.068 µg/mL, and the limits of quantification (LOQ) were 1.264 µg/mL and 0.206 µg/mL, respectively. For cilnidipine and chlorthalidone, each method proposed has been successfully used for drug determination. It is on a commercial tablet [5].

A simple, accurate, accurate, and inexpensive UV spectrophotometric method has been developed for the simultaneous determination of atenolol and chlorthalidone in bulk and compound tablets. The stock solution is prepared with methanol and then diluted with methanol as needed. Atenolol and chlorthalidone are 225 nm and 284 nm, respectively. According to Beer's law, the concentration range of atenolol is 10 µg/mL to 60 µg/mL, and the concentration range of chlorthalidone is 30 µg/ml to 140 µg/mL. The recovery rate research is statistically confirmed and executed. The proposed method can be effectively applied to the simultaneous evaluation of these two drugs in group and combined dosage [6].

In this study, a spectrophotometer probe was used to study the reaction between chlorthalidone and 1,2-naphthoquinone-4-sulfonate, which was carried out in an alkaline medium (pH 9,2). Heat the water bath to a moderate temperature of 60±1°C. The obtained red product showed maximum absorbance at 440,50 nm. Then the evolution of reaction conditions was checked with an ultraviolet/visible spectrophotometer. The reaction's stoichiometry was studied using the work plan and the reaction mechanism of the proposed work. The established approximate value corresponds to the calibration curve within the concentration range of 212 µg/mL, and the regression coefficient is more significant than 0.994. The method's accuracy is estimated from the recovery rate in the range of 99.06-99.60 %, and the relative standard deviation is less than 2 %. The

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detection limit and quantification limits are 0.58 µg/mL and 1.72 µg/mL, respectively. UV/Vis spectrophotometric analysis of chlorthalidone in tablet matrix has been successfully carried out and verified. The research carried out is accurate and straightforward. Because of its ease of use, the proposed strategy can be applied to the quality control research of chlorthalidone in the drug matrix [7].
Azilsartan medoxomil (AZL), the recently approved angiotensin II receptor blocker, has been combined with chlorthalidone (CLT) in its combined dosage form for spectrophotometric and spectrofluidic determination. Ultraviolet spectrophotometry is based on the simultaneous measurement of AZL and CLT spectra obtained for the first time in methanol at 286 and 257 nm, respectively. In the concentration range of 850 µg/ml and 2-20 µg/ml, the linearity, accuracy, and precision of AZL and CLT are satisfactory in spectrophotometry. Determination of test formulations in tablets prepared with medicinal products. The developed process is low-cost and straightforward and can be used for quality control and routine analysis of many marketed drugs and pharmaceutical products [8].

3.2 High-Performance Liquid Chromatography (HPLC)

HPLC analysis of chlortalidone in various samples is presented in Table 2.

Table 2: HPLC analysis of chlortalidone in various samples

No	Sample	Column	Mobile Phase	Detector	Chromatographic Condition	Ref.
1	Chlortalidone tablets	reverse phase C-18 column (Lichrospher, Merck®) (250 × 4 mm, particle size 5 µm)	50 mM disodium hydrogen phosphate:methanol: acetonitrile in a 70:30:05 ratio (pH adjusted to 3.5 with orthophosphoric acid) as mobile phase	UV (220 nm)	Flow rate: 1.0 mL/min	[9]
2	Chlortalidone tablets	HiQ Sil C8 column (4.6 mm x 250 mm x 5 µm)	mobile phase 20 mM potassium dihydrogen orthophosphate buffer pH 4.0: methanol (30:70 % v/v)	UV (230 nm)	Flow rate: 1.0 mL/min	[10]
3	Chlortalidone in bulk and formulation	column C8 (250 × 4.6 mm; particle size 5 µm)	Mobile phase A consisted of a buffer solution (diammonium hydrogen orthophosphate (10 mM, pH 5.5)) and methanol with a ratio of 65: 35 (v/v), and mobile phase B consists of a buffer solution and methanol in a ratio of 50: 50 (v/v)	UV (220 nm)	Flow rate : 1.4 mL/min	[11]
4	Two ternary mixtures containing amiloride	Shim-pack cyanopropyl column 5 µm (250 × 4,6 mm)	The mobile phase consists of 10 mM Buffer KH_2PO_4 (pH 4.5) and methanol with a ratio	UV (275 nm)	Flow rate: 1.0 mL/min	[12]

ISSN: 2519-9889
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	hydrochloride, atenolol, hydrochlorothiazide, and chlorthalidone		of (75 + 25% v/v)			
5	Combination of chlorthalidone and losartan	C18 Phenomenex column (250 mm × 4.6 mm, 5 µm)	the mobile phase contains a mixture of acetonitrile and water with a ratio of 80:20 percentage v/v	UV (284 nm)	Flow rate: 1.0 mL/min	[13]
6	A mixture of chlorthalidone and benidipine in tablet dosage form	Chromacil C18 column (5 µm particles, 250 mm × 4.6 mm)	the mobile phase consisting of methanol-0.1M potassium dihydrogen phosphate buffer (40:60, v/v)	UV (260 nm)	Flow rate: 1.0 mL/min	[14]
7	Chlortalidone and atenolol in human plasma	Shim-pack cyanopropyl column 5 µm (250 × 4.6 mm)	10 mM KH_2PO_4 (pH 6,0) – methanol (70:30, v/v)	UV (225 nm)	Flow rate: 1.0 mL/min	[15]
8	Chlortalidone and azilsartan medoxomil	column C 18G (250 × 4.6 mm, 0,5 µm)	the mobile phase consisting of acetonitrile and 0.1% trifluoroacetic acid in water in a ratio of 40:60%v/v	UV (240 nm)	Flow rate: 0.8 mL/min	[16]
9	Chlortalidone and atenolol formulation	Xterra RP8 column (150 x 4.6 mm, 5 µm)	potassium dihydrogen phosphate buffer solution: methanol (50:50v/v, pH 3.6)	UV (240 nm)	Flow rate: 0.5 mL/min	[17]
10	Chlortalidone and azilsartan medoxomil	Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm)	methanol/potassium hydrogen phosphate buffer (pH 8, 0.05 M) (40:60, v/v) in isocratic mode	UV (210 nm)	Flow rate: 0.8 mL/min	[18]
11	Chlortalidone bulk and tablets	Phenomenex HyperClone C 18 column (250 × 4.6 mm, 5 µm)	methanol : acetonitrile : phosphate buffer (20 mM) (pH 3.0 adjusted for o-phosphoric acid): 30 : 10 : 60% v/v	UV (241 nm)	Flow rate: 1.0 mL/min	[19]
12	Chlortalidone and eprosartan mesilate	Phenomenex, Gemini C18 column (250 × 4.6 mm, 5 µm)	mobile phase 55:45 water: acetonitrile with pH adjusted to 3.4 with orthophosphoric acid	UV (250 nm)	Flow rate: 1.0 mL/min	[20]
13	Chlortalidone and Olmisartan	BDS C18 column (250 x 4.6 mm, 5 µm)	10 mM buffer of orthophosphoric acid and acetonitrile (45:55v/v)	UV (212 nm)	Flow rate: 1.0 mL/min	[21]
14	Chlortalidone dan Olmesartan medoxomil	Phenomenex, Gemini C18 column (250 x 4.6 mm, 5 µm)	55:45 water: acetonitrile with pH adjusted to 3.0 with orthophosphoric acid	UV (250 nm)	Flow rate: 1.0 mL/min	[22]

ISSN: 2519-9889
Impact Factor: 3.426

15	Chlortalidone and Olmesartan medoxomil	STD Hypersil C18 column (150 x 4.6mm, particle size 5µm)	Phosphate buffer (KH_2PO_4) adjusted to pH 5.0 with dilute orthophosphoric acid and methanol in a ratio of 40:60 % v/v	UV (240 nm)	Flow rate: 1.2 mL/min	[23]

A simple, specific, sensitive, and precise, high-performance liquid chromatography method was established to determine chlorthalidone in tablet form. The technique was carried out on a C18 reversed-phase column (Lichrospher, Merck®) (particle size 250 × 4 mm, 5 µm), using 50 mM sodium hydrogen phosphate: methanol: acetonitrile in a ratio of 70:30:05. With 3,5-orthophosphoric acid was used as the mobile phase; hydrochlorothiazide was used as internal standard; UV detector was used for detection, wavelength 220 nm, column temperature 30±20℃; the calibration curve was found to be linear in the range of 0.1 to 3.2 µg/mL. The intra-day percentage changes were 3.3085 and 0.3702, respectively [9].

This research involves developing and validating a simple, accurate, and rapid high-performance liquid chromatography (HPLC) method to analyze chlorthalidone in tablet formulations. A method for qualitative and quantitative evaluation of chlorthalidone tablets has been developed. Separation was achieved on the HiQ Sil C8 column (4.6 mm x 250 mm x 5 µm) using 20 mM potassium dihydrogen phosphate (buffer pH 4.0): methanol (30: 70% v/v) as mobile phase. The mobile phase was pumped at a rate of 1.0 mL/min, the eluent was monitored at 230 nm, and the retention time was 3,334±0,042 min. Linearity was observed in the concentration range of 530 µg/mL, and the correlation coefficient (R2) 0, 9915. Approved according to ICH guidelines and found to be suitable for routine testing of dosage forms [10].

Low-dose thiazides and thiazide diuretics are often used as first-line treatments for hypertension. Chlorthalidone is a diuretic commonly used to treat hypertension and congestive heart failure. In this study, an improved reversed-phase HPLC method was developed to simultaneously identify and quantify the decomposition of chlorthalidone in pharmacopeia and process contaminants, as well as drugs and bulk products. Chromate was separated on a C8 column (250 × 4,6 mm; particle size 5 µm) at a flow rate of 1.4 mL/min, and the detection wavelength was 220 nm. Mobile phase A consists of buffer solution (diammonium hydrogen phosphate) (10 mM, pH 5,5)) and methanol with a ratio of 65:35 (v/v), mobile phase B consists of a buffer solution (diammonium hydrogen phosphate) (10 mM, pH 5,5)) with a ratio of 50:50 (v/v) Buffer solution and methanol composition. (The active ingredients and preparations are exposed to acidic, alkaline, oxidative, thermal, and photolysis conditions and other pressure conditions. Validation studies are carried out on impurities in the internal process to determine specificity, the limit of detection (LOD), the limit of quantification (LOQ), linearity, accuracy, precision, and reliability. Our improved RP-HPLC method is designed to separate all known and unknown impurities with acceptable resolution and tailing ratio [11].

They have developed high-performance liquid chromatography to simultaneously determine two ternary mixtures containing amiloride hydrochloride, atenolol, hydrochlorothiazide, and chlorthalidone to treat hypertension. The use of the cyanopropyl column allows the two mixtures to be separated satisfactorily. The mobile phase consists of 10 mM KH_2PO_4 buffer (pH 4,5) and methanol (75 + 25% v/v), and the flow rate is 1 mL/min. The UV detector operates at 275 nm. For amiloride hydrochloride, atenolol, hydrochlorothiazide, or chlorthalidone, the linear concentration ranges are 2 to 10, 20 to 200, 10 to 100, and 5 to 50 µg/mL. The daily and daily accuracy values (relative standard deviation) of mixture I (amiloride hydrochloride, atenolol, chlorthalidone) are 1.13, and mixture II (amiloride hydrochloride, atenolol, hydrochlorothiazide)) Has a daily accuracy value of 0,93. They have two combinations of laboratory and commercial drug mixtures successfully determined with high precision and accuracy. Statistical comparisons with published methods showed very consistent results, indicating no significant difference between them [12].

A simple, precise, and accurate HPLC method was developed for simultaneous quantification of the antihypertensive drug losartan and chlorthalidone compound preparation. The separation is performed on a Phenomenex C18 column by a simple isocratic process—a mixture of acetonitrile and water in a volume ratio

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of 80:20 v/v. The flow rate is 1.0 mL/min, and the column is kept at room temperature. The detector wavelength is 284 nm, the retention times of losartan and chlorthalidone are 1.72 minutes and 2.64 minutes, respectively, and the total running time is 10 minutes. The described method has been verified for system applicability, specificity, linearity, accuracy, and precision. A low percentage of RSD indicates that the procedure is accurate and precise and can quantify the drug in drug forms and biological fluids [13].

The chromatographic separation was performed in a reverse phase system, using isocratic elution. The mobile phase consisted of 0.1 M dipotassium hydrogen phosphate buffer: methanol (40:60, v/v) at a flow rate of 1 mL/min. They used a PDA detector set at 260 nm was used to detect and measure benidipine and chlorthalidone. Benidipine and chlorthalidone tablet samples decompose under acidic, neutral, alkaline, heat, light, and oxidative conditions. The proposed method is effectively applicable to the measurement of benidipine and chlorthalidone as part of a combined tablet. The elution time of benidipine and chlorthalidone is approximately 4573 minutes and 6422 minutes, respectively. This method was tested in the concentration range of benidipine 26 µg/ml (R2 = 0.9997) and Chlorthalidone 6.25–18.75 µg/mL (R2 = 0.9998). In terms of accuracy (RSD% of benidipine = 0.106% and RSD% of chlorthalidone = 0.031%) and accuracy (average recovery of benidipine is 99.95-100.25% and extraction rate is 99.60-99.63%), a good result was obtained. The average value of chlorthalidone). They also found the stability to be acceptable. During the degradation study, the study drug analysis did not change. The study shows that this method can determine drug combinations in daily practice [14].

A simple, sensitive, and fast chromatographic method has been established and tested for the simultaneous quantitative determination of atenolol and chlorthalidone in human plasma using rotthiazide hydrochloride as an internal standard (IS). In this method, the precipitation of protein and acetonitrile is used as the only method for sample preparation before reduction—liquid chromatography phase. Use 10 mM KH_2PO_4 (pH 6,0)-methanol (70:30, v/v) at room temperature with a flow rate of 1 ml/min and 225 nm UV detection isocratically eluted chromatographic analytes on a Shimpack cyanopropyl column. The mixing processing time is less than 10 minutes. The calibration curve is linear in the range of 0,110 µg/ml. This method's accuracy, precision, recovery, freezing stability, table stability, and re-injection repeatability have been verified. The accuracy is 15% within the limit within a few days. The analyte remained stable after three freeze-thaw cycles (deviation< 15%). The proposed method is dedicated to the simultaneous determination of atenolol and chlorthalidone in human plasma without interference from the human body's biological substances [15].

After a forced degradation study of the drug, a simple, specific, and accurate RP-HPLC method was developed and validated for the simultaneous evaluation of azilsartan medoxomil and chlorthalidone. This method was developed using an Enable C 18G chromatographic column (250 x 4,6 mm, 0.5 µm) and a mobile phase. The mobile phase was composed of acetonitrile and 0.1% trifluoroacetic acid in water with a volume ratio of 40:60 v/v. The flow rate is 0.8 mL/min; UV detection is performed at 240 nm. For Azilsartan, Metozolamide, and Chlorthalidone, 6.982 and 7.748 minutes, respectively. The proposed method uses various parameters for verification, such as linearity, range, accuracy, precision, reliability, LOD, and LOQ. Linearity was observed between 580 µg/ml azilsartan medoxomil (r2 = 0.9995) and 2.525 µg/ml chlorthalidone (r2 = 0.9973). The %RSD for daily and daily accuracy is defined as 0.41, 0.41 for azilsartan medoxomil, 0.32, and 0.47 for chlorthalidone, LOD, and LOQ 0.0189 µg/mL and 0.0305 µg/ml and chlorthalidone is 0.1109 and 0.3522 µg/mL, respectively [16].

Reversed-phase high-performance liquid chromatography (RP HPLC) has been developed and validated for the simultaneous evaluation of atenolol and chlorthalidone in commercial formulations. The mobile phase was measured on an Xterra RP8 column (150 x 4,6 mm, 5 µm). Potassium dihydrogen phosphate buffer solution: methanol (50:50 v/v, pH 3,6) with a flow rate of 0,5 ml/min (UV detection at 240 nm). The retention time of atenolol is 3,2 minutes, and the retention time of chlorthalidone is 5,0 minutes. Chlorthalidone showed a linear response in the concentration range of 50-150 µg/ml. The correlation coefficient ("r" value) between atenolol and chlorthalidone is 0.9996. The developed method has been verified for linearity, accuracy, precision, selectivity, and reliability and has proven accurate, precise, linear, and specific. This method has been validated according to ICH guidelines. The RSD for intraday and intraday accuracy is less than 2%. The benefits

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obtained by atenolol and chlorthalidone are 100.54 x 103.32% and 98.03 x 102.77%, respectively, which is the amount stated on the label of the pharmaceutical preparation [17].

The RPLC method was developed and validated for the simultaneous determination of the active substances Azilsartan Medoxomil (AZL) and Chlorthalidone (CLT) based on the prescription of the new antihypertensive combination. Chromatographic separation was achieved on the Eclipse XDBC18 column (4.6 x 150 mm), 5 µm). Use a mobile phase composed of buffer methanol/potassium hydrogen phosphate (pH 8, 0.05 M) (40:60, v/v) in isocratic mode. This method has been validated according to ICH guidelines. For AZL and CLT, linearity, accuracy, and precision are static (r2 = 0.9999) within the concentration range of 5.0–50.0 and 2.5–25.0 µg/mL. The LODs of AZL and CLT are 0.90 and 0.32 µg/mL, respectively, while the LOQs are 2.72 and 0.98 µg/mL, respectively. Both drugs have been subjected to forced degradation studies under hydrolytic conditions (neutral, acidic, and alkaline), oxidation, and extensive folic acid stress. The proposed method shows stability, which is demonstrated by the dissolution of the investigated drug and its breakdown products. In addition, the acid degradation kinetics of AZL and the basic degradation kinetics of CLT were also studied. The Arrhenius diagram was created to calculate the first-order rate constant, half-life, service life, and activation energy of the decomposition process; this method has been successfully applied to the simultaneous determination of test drugs in compound tablets. The developed method is specific and shows the stability of the quality control and formal analysis of the specified drugs in its pharmaceutical preparations [18].

Chlorthalidone is affected by various forced degradation conditions. Chlorthalidone decomposes significantly under acidic, alkaline, and oxidizing conditions. Then the full factorial design of the experiment was applied to the acidic and alkaline conditions of forced degradation, in which acid-base power, temperature, and heating effects. Time is regarded as the independent variable (factor), and % degradation is regarded as the dependent variable (response). Use Yates analysis and Pareto chart to statistically evaluate the factors that lead to acid and alkali decomposition. Optimizing digestion 10%. All chromatographic separations were performed on Phenomenex HyperClone C 18 columns (250 x 4,6 mm, 5 µ), and the mobile phase consisted of methanol: acetonitrile: phosphate buffer (20 mM) (pH 3,0 acid correction) : 30:10: 60% v/v. The flow rate is kept constant at 1 ml/min, and the eluent is measured at 24 ± 1 nm. In the experiment using the calibration curve, the linear range was 212 µg/mL. Experimental validation proves good method accuracy and precision [19].

A simple, fast, accurate, and precise RP-HPLC method has been developed and validated to determine eprosartan mesylate (EPM) and chlorthalidone (CHL) in pharmaceutical products. Chromatographic separation was performed on Phenomenex, Gemini C18 column (250 × 4,6 mm, 5 µm) in thermal isocratic mode, mobile phase water: acetonitrile 55:45, adjusted to the lowest pH value of 3,4 with orthophosphoric acid at a flow rate of 1 ml/ min. The peak intensities of the two formulations were monitored at 250 nm using UV detection. Time (RT) EPM and CHL are 2,14 and 3,80 minutes, respectively. The linear range of EPM and CHL is 10-400 µg/ml for EPM and 0,5-12,5 µg/ml. It has been verified for accuracy, precision, linearity, detection limit, and quantification limit. In addition, no interference from other pharmacopoeial carriers in the tablets was observed, indicating that they can be used for routine quality control analysis of EPM and CHL in drugs [20].

To quantify olmesartan and chlorthalidone, the two antihypertensive drugs simultaneously, a simple, fast, accurate, precise, and inexpensive high-performance reversed-phase liquid chromatography method was developed. The separation of the two preparations was achieved in BDS C18 (250 mm x 4.6 mm, 5 µm) using a mobile phase buffer of 10 mM phosphoric acid and acetonitrile (45:55 v/v) at a flow rate of 1.0 mL/min performed at 212 nm using a photodiode array detector (PDA). According to the various stress conditions of ICH, including hydrolysis (neutral, acidic, and alkaline), oxidation, photolysis, and thermal decomposition. The proposed method has been validated in terms of specificity, linearity, accuracy, precision, the limit of detection (LOD), and the limit of quantification (LOQ), and stability and reliability comply with ICH recommendations. The proposed analysis method can effectively separate the drug from its decomposition products, proving stability [21].

Chromatographic separation was performed on Phenomenex, Gemini C18 column (250 x 4,6 mm, 5 µm) in thermal isocratic mode, mobile phase water: acetonitrile 55:45, pH 3,0, phosphoric acid, flow rate 1 ml/ min. The peak intensities of the two formulations were monitored at 250 nm using UV detection. The retention

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times (RT) of OLM and CHL are 2,95 and 3,91 minutes, respectively. The linear ranges of OLM and CHL are 10-60 µg/ml (OLM) and 5-30 µg/ml (CHL). The detection and quantification limits of OLM are 15 µg/ml and 70 µg/ml, and CHL is 20 µg/ml and 60 µg/ml. The proposed method has been validated in accuracy, precision, linearity, detection, and quantification limits. In addition, no interference from other pharmacopoeial carriers in tablets was observed, indicating that they can be used for routine quality control analysis of OLM and CHL in pharmaceutical preparations [22].

A simple and accurate RP-HPLC method has been developed and validated, proving the drug's stability and the pure dosage form of the simultaneous determination of olmesartan medoxomil and chlorthalidone. Chromatography was performed on a Hypersil C18 STD analytical column (150 × 4,6 mm, particle size 5 µm), the mobile phase was phosphate buffer (KH_2PO_4), and the ratio of dilute phosphoric acid and methanol was adjusted to pH 5,0 at a ratio of 40:60 v/v. The flow rate is 1,2 mL/min. The analyte was monitored at 240 nm with a KPC detector. The retention times of olmesartan, medoxamide, and chlorthalidone were found to be 2240 minutes and 3042 minutes, respectively. In the concentration range of 60-180 µg/mL and 18.75-56.25 µg/mL, the linearity of olmesartan, methazolamide, and chlorthalidone was observed and the correlation coefficient was 0,999. The average recoveries of olmesartan, methazolamide, and chlorthalidone were 100,02% and 99,97%, respectively—exercise tests, including acid and alkaline hydrolysis. Hydrolysis, peroxide, photolysis, and thermal decomposition were performed to test the specificity of the proposed method and achieve decomposition. According to ICH recommendations, the developed method has been statistically verified and, given the value, proved to be simple, precise, and precise. Therefore, the proposed RP-HPLC method was successfully used to determine stability, demonstrating the simultaneous evaluation of standard quality control assays for bulk and commercially available olmesartan, methazolamide, and chlorthalidone [23].

3.3 Analysis of chemometric methods and TLC-Densitometry

In this study, the combined use of two known antihypertensive drugs showed a better therapeutic effect than either one alone, which was determined selectively in the presence of degradation products. To be selective, determine atenolol (ATE) and chlorthalidone (CLT), and hydrolytic degradation products. Principal component regression (PCR) and partial least squares (PLS) chemometric models have been developed. The model has been updated to predict ATE and CLT for other dosage forms, including amiloride hydrochloride (AMH). The updated model can predict the concentration of the three new dose components with good accuracy and precision without rebuilding the calibration set. The development of the TLC optical density method depends on the use of ATE, CLT, atenolol (ATE Deg) decomposition products (ATE Deg) and chlorthalidone decomposition products (CLT Deg) thin layer chromatogram at 227 nm and chloroform quantitative optical density separation on silica gel plate: methanol: ethyl acetate: 28: 2: 1,6, by volume) as the development system. The linearity results obtained using the chemometric method are 32-40 µg/ml for atenolol, 2-10 µg/ml for chlorthalidone, 3-5 µg/ml for atenolol, and 0,5-2,5 µg/ml for chlorthalidone. The TLC optical density method includes 2-10 µg $band^{-1}$ for atenolol and 0,22 µg $band^{-1}$ for chlorthalidone, 2-10 µg $band^{-1}$ for degradation of atenolol and 0,2-1,8 µg $band^{-1}$ for chlorthalidone decreasing [24].

3.4 Capillary Zone Electrophoresis (CZE) Method

A capillary zone electrophoresis (CZE) method for the simultaneous determination of the b-blocker drugs atenolol and chlorthalidone in pharmaceutical formulations has been developed. The CZE separation was performed under the following conditions: capillary temperature, 25 C; applied voltage, 25 kV; 20 mM H_3PO_4–NaOH running buffer (pH 9.0); and detection wavelength, 198 nm. Phenobarbital was used as an internal standard. The method was validated and showed not only good precision and accuracy but also good robustness. The technique has been successfully applied to the simultaneous determination of both atenolol and chlorthalidone in pharmaceutical tablets [25].

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3.5 Spectrofluorometric method

In this study, a method for simultaneous determination of chlorthalidone (CLT) and telmisartan (TEL) in human plasma samples and the recently released tablet (TelmikindCT 40®) is groundbreaking and optimized. Based on the fluorescence intensity measurement in the synchronous fluorescence mode combined with the signal processing based on microbeads, 0,5% cetyltrimethylammonium bromide is used as a cationic surfactant increase the fluorescence signal intensity and increase the sensitivity of the method. The synchronized spectra obtained from CLT and TEL are well separated by two zero-crossing points, making it possible to measure CLT and TEL at 362 nm and 351 nm, respectively. Various experimental parameters were carefully checked and optimized, and CLT and TEL calibration curves were created within the concentration ranges of 20-1200 µg/mL and 5-800 µg/mL, respectively. The developed method is fast and straightforward. The analysis parameters are confirmed according to ICH recommendations, and high sensitivity is achieved. The limits of detection (LOD) of CLT and TEL are 4.69 and 1.58 µg/mL, respectively [26].

3.6 Liquid chromatography-tandem mass spectrometry method

A simple, sensitive, selective, and rapid liquid chromatography-tandem mass spectrometry method was developed and validated for the simultaneous separation and quantitation of atenolol and chlorthalidone in human plasma metoprolol and hydrochlorothiazide as an internal standard. Following solid-phase extraction, the analytes were separated by an isocratic mobile phase on a reversed-phase C_{18} column and analyzed by MS in the multiple reaction monitoring modes (atenolol in positive and chlorthalidone in the negative ion mode). The limit of quantitation for this method was 10 and 15 ng mL^{-1}, and the linear dynamic range was generally 10–2.050 ng mL^{-1} and 15–3.035 ng mL^{-1} for atenolol and chlorthalidone, respectively [27].

3.7 Ultra-fast liquid chromatography (UFLC) method

A chiral ultra-fast liquid chromatographic method was developed and validated for the separation of chlorthalidone enantiomers both in bulk and pharmaceutical formulations. The resolution was obtained in a normal phase mode on a Kromosil TBB chiral stationary phase (150 x 4.6 mm, 5 µm) with a mobile phase composed of n-hexane, 2-propanol, acetic acid, and triethylamine (92:8:0.3:0.01 v/v), at a flow rate of 1.2 mL/min. The enantiomers and internal standard (2-nitro aniline) were detected at 260 nm wavelength. The internal standard and both the enantiomers were detected at 3.3 min, 5.2 min and 6.2 min, respectively. The correlation coefficient for linear regression curves of enantiomer 1 and enantiomer 2 was ≥ 0.999. The inter-day precision and intra-day precision, expressed as %RSD, was less than 2. The accuracy determined by the average recovery of enantiomer 1 and enantiomer 2 was within the acceptable limits. Low levels of the limit of detection (<0.1µg/mL) and limit of quantitation (< 0.3 µg/mL) for both enantiomers, makes the method sensitive. The method was useful for determining the ratio of the two enantiomers in the bulk drug and the final formulated tablets [28].

Develop and validate new, reliable, and straightforward enantioselective reverse-phase ultra-fast liquid chromatography (RP-UFLC) method to quantify chlorthalidone in the bulk and pharmaceutical dosage form. In the present study, the isocratic RP-UFLC method was developed on Phenomenex® Lux cellulose 4 columns (250 × 4.6 mm, 5 µm) and disodium hydrogen phosphate buffer (pH 3.6): methanol (40:60 v/v) as mobile phase. Elute was monitored at 240 nm with a flow rate of 1 mL/min. The described method provided linear correlation (R^2=0.999) between the range of 2-10 µg/mL. Chlorthalidone enantiomers showed good resolution with a retention time (t_R) of 5.75 min and 7.46 min, respectively. The precision of the method revealed that relative standard deviation is within the acceptable limit. The percentage recovery of each chlorthalidone enantiomers was found to be 99.98% and 100.09%, respectively. ICH validated the method harmonized tripartite guidelines, validating analytical procedures: text and methodology Q2 (R1). An economical, accurate, sensitive, and precise RP-UFLC method was developed and fully validated for quality control analysis of chlorthalidone in pharmaceutical dosage form [29].

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3.8 Supercritical fluid chromatography method

Supercritical liquid chromatography with a chiral stationary phase is a popular separation technique for enantioselective separation. The main advantages of supercritical liquid chromatography are short analysis time, low consumption of organic modifiers, low cost, and environmental friendliness. According to reports, chlorthalidone is a widely used diuretic, and its separation demonstrates the benefits of supercritical liquid chromatography. The effects of the amount and type of organic modifier, temperature, and back pressure on enantioselectivity and enantiomeric separation were evaluated. An optimized system consists of Chiralpak AD column, 50/50 (v/v) CO_2 / MeOH mobile phase, 40 °C temperature, 4,0 ml/min flow rate, and 120 bar back pressure less 2,5 minutes. In addition, the enantiomers of chlorthalidone have been identified in two commercially available drugs. It can be easily transferred to the semi-finished product scale [30].

3.9 High-performance thin-layer chromatography (HPTLC)

The principle motive behind this work is the densitometric high-performance thin-layer chromatography (HPTLC) method has been developed and validated for the simultaneous estimation of Olmesartan medoxomil and Chlorthalidone in three different brands of the tablet dosage form. The technique involves silica gel 60 F_{254} high-performance thin-layer chromatography and densitometric detection at 238 nm using Toluene: Ethyl acetate: Methanol: Glacial acetic acid (5:4.7:0.3:0.1 % v/v/v/v) as a mobile phase. The calibration curve was linear over a range of 100-1200 ng/band for Olmesartan medoxomil and 62.5-750 ng/ band for Chlorthalidone. Precision provided relative standard deviations (RSD) lower than 2 % of both analytes. Percent recoveries were performed and found to be 99.53-99.90 % for Olmesartan medoxomil and 98.18-99.49 % for Chlorthalidone. Validation of the method was carried out as per International Conference on Harmonization (ICH) guideline Q2 (R1). The HPTLC densitometric-driven way is highly suitable for analyzing Olmesartan medoxomil and Chlorthalidone in dosage form without interference [31].

4 Conclusion

Overall, various analytical methods have been used to determine chlorthalidone levels from 2001 to 2021. Chlorthalidone levels in raw materials, mixtures, and pharmaceutical preparations have been carried out using various analytical methods. It is including spectrophotometry and high-performance liquid chromatography (HPLC), chemometrics and TLC-Densitometric, capillary zone electrophoresis (CZE) method, spectrofluorometry method, LC-MS-MS method, ultra-fast liquid chromatography (UFLC) method, supercritical fluid chromatography method, and HPTLC-densitometric method. Analysis using the HPLC technique is more often used in research because it can detect samples with low concentrations up to the nanogram level.

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A Brief Author Biography

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