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Review Article

Analytical Techniques for Determination of Hydrochlorothiazide and its Combinations: A Review

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Abstract

Hydrochlorothiazide is chemically 6-chloro-1, 1-dioxo-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine-7-sulfonamide. Hydrochlorothiazide is a diuretic drug used for treatment of high blood pressure (hypertension) and accumulation of fluid (edema). It works by blocking salt and fluid reabsorption from the urine in the kidneys, causing increased urine output (dieresis). Hydrochlorothiazide is used to treat excessive fluid accumulation and swelling (edema) of the body caused by heart failure, cirrhosis, chronic kidney failure, corticosteroid medications, and nephrotic syndrome. It can be used alone or in conjunction with other blood pressure lowering medications to treat high blood pressure.

This review focuses on the recent developments in analytical techniques for estimation of Hydrochlorothiazide alone or in combinations with other drugs in various biological media like human plasma and urine. This review will critically examine the (a) sample pretreatment method such as solid phase extraction (SPE), (b) separation methods such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC), high performance thin layer chromatography (HPTLC), liquid chromatography coupled to tandem mass spectrometry (LC-MS) and capillary electrophoresis (CE), (c) other methods such as spectrophotometry, diffuse reflectance near infrared spectroscopy and electrochemical methods.

Keywords: Analytical Techniques, Hydrochlorothiazide, Solid phase extraction, HPLC, Spectrophotometry.

1. Introduction

Hydrochlorothiazide is analogues of 1, 2, 4-benzothiadiazine-1, 1-dioxide. Hydrochlorothiazide diuretics are among the most commonly used antihypertensive and have been available for over 50 years. Thiazides are preferred in hypertensive patients with osteoporosis; Secondary beneficial effect in hypertensive geriatric patients of reducing the risk of osteoporosis secondary to effect on calcium homeostasis and bone mineralization. Thiazide diuretics used in patients who are at an increased risk for developing hyperkalemia [1].

Thiazides achieve their diuretic action via inhibition of the Na^+/Cl^- cotransporter (NCC) in the renal distal convoluted tubule[2-4]. The NCC facilitates the absorption of sodium from the distal tubules back to the interstitium and accounts for approximately 7% of total sodium reabsorption[5]. By decreasing sodium reabsorption, thiazide use acutely results in an increase in fluid loss to urine, which leads to decreased extracellular fluid and plasma volume. This volume loss results in diminished venous return, increased renin release, reduced cardiac output and decreased blood pressure[6]. Within days, the reduction in cardiac output increases total peripheral resistance, which stems mostly from activation of the sympathetic nervous system and renin angiotensin aldosterone system[7,8]. This acute effect is evidenced by the fact that an infusion of dextran, a volume expander, during the acute thiazide treatment phase restores blood pressure to pretreatment levels [9].

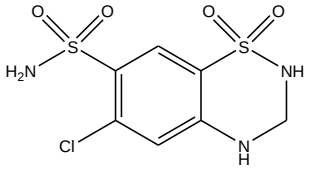
Due to the importance of this class in treatment of hypertension, there is need to review the analytical work reported in the literature for

estimation of Hydrochlorothiazide alone and with its combinations which would be very useful information for an academic and industrial prospective.

2. Physical and Chemical Property

Hydrochlorothiazide is white or almost white, crystalline powder. IUPAC name of Hydrochlorothiazide is 6-Chloro-3, 4-dihydro-2*H*-1, 2, 4-benzothiadiazine-7-sulphonamide 1, 1-dioxide (Fig.1.). Chemical formula of Hydrochlorothiazide is C7H8ClN3O4S2. Molecular weight is 297.7 gm/mol. Very slightly soluble in water, soluble in acetone, sparingly soluble in ethanol (96%). It dissolves in dilute solutions of alkali hydroxides[10-13].

Figure 1: Chemical Structure of Hydrochlorothiazide



3. Analytical Methods

Analytical method development and validation plays an important role in the discovery, development and manufacture of pharmaceuticals. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency and performance of drug products. After therapeutic administration of drug formulation, to check therapeutic concentration of drug in human body, there is a need for analytical methods. Analytical Methods are necessary for therapeutic drug monitoring. High Performance Liquid Chromatography (HPLC) together with various types of detection UV (ultraviolet), FD (fluorescence detection) and MS (mass spectrometry) has become the method of choice for analytical method development. Apart from these methods other analytical methods also used which are High performance thin layer chromatography [50-52], Ultra performance liquid chromatography [59, 60], Thin layer chromatography[62], Voltammetry [16], Capillary electrophoresis[14,15] are also widely used.

3.1 Capillary electrophoresis method

Hillaert *et al*[14] developed a capillary electrophoresis method for the simultaneous determination of Hydrochlorothiazide and several angiotensin-converting enzyme (ACE) inhibitors: enalapril, lisinopril, quinapril, fosinopril, ramipril, and cilazapril. The most critical parameter is the pH of the running buffer. Separation was performed on a fused-silica capillary (52 cm total length ×75 µm I.D.) using a sodium phosphate buffer (pH 7.25; 100 mM). The method was successfully applied to the quantitative determination of these compounds in their corresponding pharmaceutical formulation.

In another study, Alnajjar *et al*[15] developed a capillary electrophoresis method for the separation and quantification of metoprolol (MET) and Hydrochlorothiazide (HCT) in their combined dosage form. MET and HCT were detected at 240 and 214 nm, respectively, using a photodiode array detector. The univariate approach was used for optimizing voltage, injection time and capillary temperature. The optimum conditions were 50 mmol/L phosphate at pH 9.5, injection time 10.0 s, voltage 25 kV and capillary temperature 25C. The method was linear in the range of 2.5–250 mg/mL for both drugs with correlation coefficients above 0.9997. Additionally, acceptable recovery of the contents of MET and HCTZ in their formulations (96.0– 100.3%) with acceptable precision (1.38–2.60 %) were achieved. Moreover, the limits of detection of MET and HCTZ were 0.02 and 0.01 µg/mL, respectively, which were suitable for pharmaceutical analysis.

3.2 Electrochemical method/Voltammetric method

Voltametric method was developed by Omayma[16] for hydrochlorothiazide at glassy carbon electrode was carried out. The drug in Britton–Robinson buffer (pH 3.3) is oxidized at +1040 mV, giving rise to a well-defined peak. The procedure has been applied for the drug determination in human urine with no prior extraction and in commercial tablets. The proposed method has been validated. The limit of detection for the standard solution was 5.0 ng/ml and for the drug in urine was 14.0 ng/ml. The results obtained from the analysis of commercial tablets were compared statistically with those obtained from the USP 26 high-performance liquid chromatographic (HPLC) method.

3.3 Spectrophotometric method

3.3.1 Diffuse reflectance near infrared spectroscopy

Marcus H. Ferreira *et al.* [17] developed and validated a new multivariate diffuse reflectance near infrared method for direct determination of Hydrochlorothiazide in powder pharmaceutical samples. The best partial least squares (PLS) model was obtained in the spectral region from 1640 to 1780 nm, with mean centered data pre-processed by first derivative and Savitzky–Golay smoothing followed by vector normalization. The method was validated according to the appropriate regulations in the range from 21.25 to 29.00 mg of Hydrochlorothiazide per 150 mg of powder (average mass tablet), by the estimate of figures of merit, such as accuracy, precision, linearity, analytical sensitivity, capability of detection, bias and residual prediction deviation (RPD).

3.3.2 UV Spectroscopic method

Hany *et al*[18] were developed and validated for the simultaneous estimation of Amlodipine (AML), Valsartan (VAL) and Hydrochlorothiazide (HCTZ) in commercial tablets. The derivative spectrophotometric methods include Derivative Ratio Zero Crossing (DRZC) and Double Divisor Ratio Spectra-Derivative Spectrophotometry (DDRS-DS) methods, while the multivariate calibrations used are Principal Component Regression (PCR) and Partial Least Squares (PLS). The proposed methods were applied successfully in the determination of the drugs in laboratory-prepare mixtures and in commercial pharmaceutical preparations. The validity of the proposed methods was assessed using the standard addition technique. The linearity of the proposed methods is investigated in the range of 2-32, 4-44 and 2-20 µg/mL for AML, VAL and HCTZ, respectively.

A Comparative study between univariate spectrophotometry and multivariate calibration as analytical tools for simultaneous quantitation of Moexipril and Hydrochlorothiazide were carried out. The first method is the new extended ratio subtraction method (EXRSM) coupled to ratio subtraction method (RSM) for determination of both drugs in commercial dosage form. The second and third methods are multivariate calibration which includes Principal Component Regression (PCR) and Partial Least Squares (PLSs). A detailed validation of the methods was performed following the ICH guidelines and the standard curves were found to be linear in the range of 10-60 and 2–30 for MOX and HCTZ in EXRSM method, respectively, with well accepted mean correlation coefficient for each analyte [19].

Two new simple and selective assay methods have been presented for the analysis of eprosartanmesylate (EPR) and Hydrochlorothiazide (HCTZ) in pharmaceutical formulations. The first method is based on first-derivative ultraviolet spectrophotometry with zero- crossing measurements at 246 and 279 nm for EPR and HCTZ, respectively. The assay was linear over the concentration ranges 3.0–14.0 mg/mL for EPR and 1.0–12.0 mg/mL for HCTZ. The quantification limits for EPR and HCTZ were found to be 1.148 and 0.581 mg/mL, respectively, while the detection limits were 0.344 mg/mL for EPR and 0.175 mg/mL for HCTZ. The second method involved isocratic reversed-phase liquid chromatography using a mobile phase composed of acetonitrile –10 mM phosphoric acid (pH 2.5) (40:60, v/v). Olmesartan was used as internal standard and the substances were detected at 272 nm. The linearity ranges were found to be 0.5–30 and 0.3–15.0 mg/mL for EPR and HCT, respectively. The limits of detection were found to be 0.121 mg/mL for EPR and 0.045 mg/mL for HCTZ. The limits of quantification were found to be 0.405 and 0.148 mg/mL for EPR and HCTZ, respectively [20].

A simple, precise, accurate and economic simultaneous UV spectrophotometric method has been developed for the estimation of amlodipine besylate, valsartan and hydrochlorothiazide in combination in bulk mixture and tablet. The estimation was based upon measurement of absorbance at absorbance maxima of 359 nm, 317 nm and 250 nm for amlodipine besylate, Hydrochlorothiazide and valsartan in methanol, respectively in bulk mixture and tablet. The Beer Lambert's law obeyed in the concentration range 5-25 µg/ml, 10-50 µg/ml and 5-25 µg/ml for amlodipine besylate, Hydrochlorothiazide and valsartan, respectively [21].

Bhatia *et al*[22] presented simple spectrophotometric method development for simultaneous estimation of Losartan Potassium (LOS) and Hydrochlorothiazide (HCTZ) in two component tablet formulation. The method employed is a first order derivative spectroscopy. For determination of sampling wavelength, 20 µg/ml of each of LOS and HCTZ were scanned in 200-400 nm range and sampling wavelengths were 257 nm for LOS where HCT showed zero crossing point and 243 nm for HCT where LOS showed zero crossing point in first order derivative spectroscopy, For this method linearity observed in the range of 10-90 µg/ml for LOS and 2.5-22.5 µg/ml for HCTZ.

Naguib *et al*[23] has developed Partial least squares regression (PLSR) and support vector regression (SVR) are two popular chemometric models. The comparison shows their characteristics via applying them to analyze Hydrochlorothiazide (HCTZ) and Benazepril hydrochloride (BZ) in presence of HCTZ impurities; Chlorothiazide (CT) and Salamide (DSA) as a case study. The analysis results prove to be valid for analysis of the two active ingredients in raw materials and pharmaceutical dosage form through handling UV spectral data in range (220–350 nm). An independent test set consisting of 8 mixtures was used to validate the prediction ability of the suggested models.

A new simple spectrophotometric method was developed for the determination of binary mixtures without prior separation. The method is based on the generation of ratio spectra of compound X by using a standard spectrum of compound Y as a divisor. The method was demonstrated by determination of two drug combinations. The first consists of the two antihyperlipidemics: atorvastatin calcium (ATV) and ezetimibe (EZE), and the second

comprises the antihypertensives: candesartan cilexetil (CAN) and Hydrochlorothiazide (HCTZ). For mixture 1, ATV was determined using 10 mg/mL EZE as the divisor to generate the ratio spectra, and the peak to trough amplitudes between 231 and 276 nm were plotted against ATV concentration. Similarly, by using 10 mg/mL ATV as divisor, the peak to trough amplitudes between 231 and 276 nm were found proportional to EZE concentration. Calibration curves were linear in the range 2.5–40 mg/mL for both drugs. For mixture 2, divisor concentration was 7.5 mg/mL for both drugs. CAN was determined using its peak to trough amplitudes at 251 and 277 nm, while HCTZ was estimated using the amplitudes between 251 and 276 nm[24].

Multivariate spectrophotometric calibration for the simultaneous analysis of synthetic samples and commercial tablet preparations containing Hydrochlorothiazide (HCTZ) and amiloride hydrochloride (AMH) were developed by Mo-nica C.F. Ferraro *et al.* [25] The method, validated by the analysis of synthetic mixtures of both drugs, where accuracy over the linear working range as well as inter- and intra-assay precision were determined, was used in the concentration ranges of 21.7-30.4 µg/l for HCTZ and 1.8.3.0 µg/l for AMH.

Different spectrophotometric and HPTLC-densitometric methods are presented for the simultaneous determination of lisinopril and hydrochlorothiazide in pharmaceutical tablets. The spectrophotometric methods include third derivative (3_D) ultraviolet spectrophotometry with zero crossing measurement at 217.4 and 233.4 nm, second derivative of the ratio spectra with measurement at 214.3 and 228.0 nm; both classical least squares and principal component regression were applied to the UV absorption and first derivative spectra of the mixture. The HPTLC method was based on separation of both drugs followed by densitometric measurements of their spots at 210 and 275 nm for lisinopril and Hydrochlorothiazide, respectively[26].

Abdelwahab[27] developed the spectrophotometric methods for simultaneous determination of Carvedilol (CV) and Hydrochlorothiazide (HCTZ) in bulk powder and combined dosage form. Method (I) is based on dual wavelength analysis while Method (II) depends on UV-spectrophotometric determination using Q-analysis (graphical absorbance ratio) method. In Method (I), two wavelengths were selected for each drug in such a way that the difference in absorbance is zero for the second drug. At wavelengths 238 and 248.8 nm HCTZ has equal absorbance values, therefore, these two wavelengths have been used to determine CV, on similar basis 266 and 289.4 nm were selected to determine HCTZ in the combined formulation. Method (II) involves the formation of Q-absorbance equation using the respective absorptivity values at 229.2 nm (isoabsorptive point) and 241 nm (k of CV). The drugs obey Beer's Lambert's law in the concentration range of 1–10 µg/mL for both CV and HCTZ (for Method I) and in the range of 1–10 and 2–10 µg/mL for CV and HCT, respectively (for Method II). The accuracy and precision max were determined and recovery studies confirmed the accuracy of the developed methods that were carried out following the International Conference on Harmonization (ICH) guidelines.

Simultaneous determination of Losartan potassium (LSP) and Hydrochlorothiazide (HCTZ) in a binary mixture form has developed by Rao *et al*[28]. The present work was carried out on Shimadzu electron UV1800 double beam UV-Visible spectrophotometer. The absorption spectra of reference and test solutions were carried out in 1 cm matched quartz cell over the range of 200-400 nm. The first method is the application of simultaneous equation. Where the linearity ranges for LSP and HCTZ were 5–25 µg/ml and 1–20 µg/ml, respectively. The second method is the determination of ratio of absorbance at 272 nm, the maximum absorption of HCTZ and isobestic wavelength 266.5nm, the linearity ranges for LSP and HCTZ were 5-80µg/ml and 1-25µg/ml respectively. The third method is the first order derivative method, where the linearity ranges for LSP and HCTZ were 1-30 µg/ml and 1-40 µg/ml respectively[28].

3.4 Chromatographic method

3.4.1 High performance liquid chromatography

A simple and precise high performance liquid chromatographic method has been developed and validated for the simultaneous determination of bisoprolol fumarate (BF) and hydrochlorothiazide (HCTZ) in a tablet formulation. Chromatography was carried out at 25C on a 4.6 mm×250 mm, 5 µm cyano column with the isocratic mobile phase of 0.1 M aqueous phosphate buffer, acetonitrile and tetrahydrofuran (85:10:5, v/v/v) at a flow rate of 1.0 ml/min. The UV detection was carried out at 225 nm. HCTZ and BF were separated in less than 10 min with good resolution and minimal tailing, without interference of excipients. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all cases. The method was linear in the range of 50–150 µg/ml for BF and 125–375 µg/ml for HCTZ[29].

Kevin b. Alton *et al.* [30] developed high-performance liquid chromatographic assay was developed for the quantitative determination of

Hydrochlorothiazide (HCTZ) in human urine. Reversed-phase separation of HCT and the internal standard, trlchloromethiazide (TCMT), was accomplished on a 300 x 3.9 mm N Bondapak Phenyl column has been routinely employed in bioavailability studies evaluating a variety of formulations as well as characterizing the pharmacokinetics of this drug from urinary excretion data.

Simultaneous high performance liquid chromatographic analysis of serum samples and commercial tablet formulation containing hydrochlorothiazide, olmesartan medoxomil and irbesartan were developed by Sultana Najma *et al.* [31]. Good chromatographic separation was achieved using N -Bondapak, C column (15 cm 4.6 mm, 5 m), and a mobile phase consisting of acetonitrile -0.2% acetic acid aqueous solution (5050, v/v) at a flow rate of 1.0 mL/min. The ultraviolet detector was set at a wavelength of 260 nm. hydrochlorothiazide, olmesartan medoxomil, and irbesartan were eluted at 1.2, 3.8, and 4.4 min, respectively. The linear ranges for Hydrochlorothiazide, Olmesartan medoxomil, and Irbesartan were 6.25–18.75, 20–60, and 75–225 ng/mL, respectively. The recoveries of hydrochlorothiazide, olmesartan medoxomil, and irbesartan in spiked samples were all greater than 98%, and their relative standard deviations were less than 2.0%.

A simple, rapid, and precise method was developed for the quantitative simultaneous determination of telmisartan and hydrochlorothiazide in combined pharmaceutical dosage form. Chromatographic separation of two drugs was achieved on an ACE 5 C_{18}25-cm analytical column using buffer–acetonitrile (60:40, v/v) of pH 5.5, adjusted with acetic acid. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantification, and robustness. The calibration curve shows excellent linearity over the concentration range for telmisartan and hydrochlorothiazide were 10–150 and 5–75 µg/mL, respectively. The correlation coefficient for telmisartan and hydrochlorothiazide were 0.9999[32].

Suchara *et al*[33]described the quantitation of target analytes in complex matrices such as biological samples require special calibration approaches to compensate for additional capacity or activity in the matrix samples. The performance of the analytical method was evaluated. Relative standard deviation, limit of detection, and limit of quantification are respectively 0.5–0.6%, 0.169–0.75 µg/mL, and 0.565–2.5 µg/mL. Linear range falls within the range of 0.3 to 63.8 µg/mL for both compounds. Accuracy ranged between 94% and 101%.

Hegazy[34] developed and validated and stability indicating chromatographic methods for Hydrochlorothiazide (HCTZ) and Spironolactone (SPR) determination in their mixtures and in presence of their impurities and degradation products. The first method was based on thin layer chromatographic (TLC) combined with densitometric determination of the separated spots. The separation was achieved using silica gel 60 F TLC plates and ethyl acetate–chloroform– formic acid–triethyl amine (7:3:0.1:0.1, by volume) as a developing system. The second method was based on the high-performance liquid chromatography with ultraviolet detection, by which the proposed components were separated on a reversed phase C_{18}analytical column using gradient elution system with deionized water–acetonitrile (97:3, v/v) for 8 min. The flow rate was maintained at 2 mL/min and the detection wavelength was at 230 nm. Linear regressions were obtained in the range of 4.0–50 µg/mL and 5.0–50 µg/mL for both HCTZ and SPR, respectively.

A Liquid chromatographic method for the determination of hydrochlorothiazide in human plasma was presented by R. Brent Miller el al. [35]. The reported method herein for the determination of HCTZ is linear over the range of 2.0-1000 $ngml^{-1}$ in human plasma.

3.4.2 High performance liquid chromatography with UV spectroscopy

The development of a reversed phase liquid chromatographic method for the simultaneous determination of seven angiotensin converting enzyme (ACE) inhibitors were developed by Elsebaei *et al*[36]. The method utilizes a simple gradient procedure for the separation in a 11 min run time using acetonitrile aqueous ammonia buffer (pH 9) solution and an Extend RP-C_{18} (25 µm particle size, 4.6 mm×250 mm, Agilent) HPLC column. The effluent was monitored on a UV detector at 215 nm. The limits of detection (LOD) and limits of quantification (LOQ) from standard drug solutions lie in the range of 17–64 and 56–212 $ngml^{-1}$, respectively. Correlation coefficient values (r) higher than 0.997 were obtained for all the studied drugs in spiked human plasma and urine samples.

High performance liquid chromatography (HPLC) was developed for the simultaneous determination of Telmisartan (TELM) and Hydrochlorothiazide (HCTZ) in human plasma using indapamide as internal standard. The method utilizes proteins precipitation with acetonitrile as only sample preparation prior to RP-HPLC. The analytes were chromatographed on shim-pack cyanopropyl column

in isocratic elution with methanol: 10 mM ammonium acetate solution (pH 6.0) (35:65 v/v) as mobile phase at a flow rate of 1 ml/min and the wavelength of detection was 270 nm. The method was validated over the concentration range of 1–10 µg/ml for TELM and 0.31– 3.12 µg/ml for HCTZ in human plasma. Inter- and intra-run precision of TELM and HCTZ were less than 3.60% and the accuracy was less than 1.868% [37].

3.4.3 Reverse phase high performance liquid chromatography

Reversed-phase column liquid chromatographic method has been developed and validated for the simultaneous determination of Zofenopril (ZOF) and Hydrochlorothiazide (HCTZ) in pharmaceutical preparations. Analyses were carried out on a C_{18} column using methanol-water (pH 2.5 with $H3PO_4$) as the mobile phase, delivered in a gradient mode. Detection was performed using an UV–vis detector monitored at 270 nm. Quantitation was achieved using an external calibration curve. The linearity for ZOF concentrations ranging between 1.0–70.0 µg/mL, and between 2.5–35.0 µg/mL for HCTZ (r > 0.999) was established. The recovery (R %) of the active ingredients from the samples ranged between 99.42% and 100.67%. Intra- and inter-day precisions were less than 1.5%. The limits of detection (LOD) and quantitation (LOQ) were 0.129 and 0.292 µg/mL for ZOF, and 0.183 and 0.556 µg/mL for HCTZ, respectively [38].

Sheth *et al*[39] Describe simultaneous estimation of rosuvastatin calcium and hydrochlorothiazide from bulk and commercial products using a validated reverse phase high performance liquid chromatographic technique. The separation of both the drugs was achieved on ACE C_{18} AR (AR - Aromatic) column (250 × 4.6 mm, 5 µm) using a mobile phase of sodium perchlorate buffer solution (at pH 3.0): Acetonitrile (60:40 v/v). The flow rate was 1 ml/min and detection was done at 280 nm. The retention time for Hydrochlorothiazide was 3.9 min and for Rosuvastatin calcium was 10.3 min. Rosuvastatin calcium and Hydrochlorothiazide showed a linear response in the concentration range of 5-30 µg/ml and 6.25-37.5 µg/ml respectively. The correlation co-efficients for Rosuvastatin calcium and Hydrochlorothiazide were 0.9998 and 0.9999 respectively. The percentage recoveries obtained for Rosuvastatin calcium and Hydrochlorothiazide range from 99.3% to 100.4% and 99.2% to 100.4% respectively. The results of analysis have been validated as per the International conference on Harmonisation (ICH) guidelines.

A simple, sensitive and specific liquid chromatographic method with UV detection (230 nm) was developed for the simultaneous estimation of Hydrochlorothiazide, amlodipine and losartan in tablet dosage form and Telmisartan as an internal standard. Separation was achieved with a phenomenexluna 5µ CN100R, 250 × 4.60 mm5 micron size column, ambient temperature with a low pressure gradient mode with mobile phase containing acetonitrile, water and 0.4% of potassium dihydrogen phosphate buffer pH 2.7 adjusted with orthophosphoric acid (45:35:20). The flow rate was 1 mL min^{-1} and eluent was monitored at 230 nm. The linearity range of hydrochlorothiazide, amlodipine and losartan found in the range of 12.5-62.5 µg ml^{-1}, 2.5-12.5 µg ml^{-1}methodand 20-250 µg ml^{-1}respectively[40].

Reversed phase high performance liquid chromatography (RP-HPLC) method was developed for determination of related substances of Telmisartan and Hydrochlorothiazide in tablet dosage form presented by Mukhopadhyay *et al.* [41]. Simultaneous determination of related substances was performed on Kromasil C_{18} analytical column (250 × 4.6 mm; 5µm particle size) column at 40°C employing a gradient elution. Mobile phase consisting of solvent A (solution containing 2.0 g of potassium dihydrogen phosphate anhydrous and 1.04 g of Sodium 1- Hexane sulphonic acid monohydrate per liter of water, adjusted to pH 3.0 with orthophosphoric acid) and solvent B (mixture of Acetonitrile: Methanol in the ratio 80:20 v/v) was used at a flow rate of 1.0 ml/min. UV detection was performed at 270 nm.

Joshi *et al*[42] Developed HPLC method for the simultaneous determination of Bisoprolol fumarate and Hydrochlorothiazide in pharmaceutical dosage form. The separation was achieved on an Inertsil ODS 3V (25 cm×4.6 mm) 5 µm column with isocratic flow. The mobile phase at a flow rate of 1.0 mL min^{-1} consisted of 0.1 M potassium dihydrogen phosphate buffer and acetonitrile(70:30, v/v). The UV detection was carried out at 228 nm. A linear response was observed over the concentration range 2.5–50 µg $ml^{-1}$of Bisoprolol fumarate and the concentration range 6.25–125 µg $ml^{-1}$of hydrochlorothiazide. Limit of detection and limit of quantitation for bisoprolol fumarate were 0.01 and 0.03 µg/mL, respectively and for hydrochlorothiazide were 0.01 and 0.05µg ml^{-1}, respectively.

High pressure liquid chromatographic method has been developed for the simultaneous estimation of Nebivolol and Hydrochlorothiazide from pharmaceutical formulation. Phenomenex Gemini C_{18}(25 cm×4.6 mm i.d, 5 µ) column with a mobile

phase consisting of acetonitrile: 50mMammonium acetate (adjusted to pH 3.5 using orthophosphoric acid) (70:30 v/v) at a flow rate of 1.0 ml/min was used. Detection was carried out at 254 nm. Probenecid was used as an internal standard. The retention times of probenecid, Nebivolol and Hydrochlorothiazide were 13.05, 3.32 and 4.25 min, respectively[43].

Rawool *et al*[44]. Presented RP-HPLC method of two different drug components hydrochlorothiazide and metoprolol tartrate present in a tablet formulation. It is a simple, fast, precise and accurate high performance liquid chromatographic method. It is performed using phosphate buffer along with methanol as mobile phase, in the proportion of 60:40. The separation is done on a C18column and it is estimated at 226 nm with a flow of 1 ml/min. The detection limits range from a 0.013 to 0.075 mg/ml for Hydrochlorothiazide and 0.10 to 0.60 mg/ml for metoprolol tartrate, respectively.

3.4.4 Stability indicating chromatographic method

A simple and selective HPLC-DAD stability indicating method was developed for the simultaneous determination of the three antihypertensive drugs amlodipine besylate (AML), valsartan (VAL) and Hydrochlorothiazide (HCTZ) in their combined formulation describe by Shaalan *et al*[45]. Effective chromatographic separation was achieved using Zorbax SB-C8 column (4.6 × 250 mm, 5 µm ps) with gradient elution of the mobile phase composed of 0.025 M phosphoric acid and acetonitrile at a flow rate of 1 mL/min. The multiple wavelength detector was set at 238 nm for measurement of AML and 225 nm for both VAL and HCTZ. Quantification was based on measuring the peak areas. The three compounds were resolved with retention times of 4.9, 6.4 and 8.3 min for HCTZ, AML and VAL respectively.

P.S. Jain *et al.* [46] A simple, specific, accurate and precise stability-indicating reversed-phase high-performance liquid chromatographic method was developed for simultaneous estimation of olmesartan medoxomile (OLME), amlodipine besylate (AMLO) and hydrochlorothiazide (HCTZ) in tablet dosage form. The method was developed using an RP C_{18} base deactivated silica column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of triethylamine(pH 3.0) adjusted with orthophosphoric acid (A) and acetonitrile (B), with a timed gradient program of T %B: 0/30, 7/70, 8/30, 10/30 with a flow rate of 1.4 mL/min. Ultraviolet detection was used at 236 nm. The retention times for OLME, AMLO and HCTZ were found to be 6.72, 4.28 and 2.30 respectively.

Stability study of losartan / Hydrochlorothiazide tablets is presented by Lusina *et al*[47]. Losartan (angiotensin II receptor antagonist) and Hydrochlorothiazide (diuretic) are successfully used in association in the treatment of hypertension. Stability study of losartan/Hydrochlorothiazide tablets consisted of three steps: stress test (forced degradation study), preliminary testing (selection of packaging) and formal stability testing. Based on the first 12 months of the formal stability study, a shelf life of 24 months was proposed.

Rane *et al*[48]presented stability-indicating liquid chromatography Method is developed and validated for the quantitative simultaneous estimation of Irbesartan (IRB) and Hydrochlorothiazide (HCTZ) in combined pharmaceutical dosage form. A chromatographic separation of the two drugs was achieved with an Ace5 C_{18}25-cm analytical column using buffer–acetonitrile (70:30 v/v). The buffer used in mobile phase contains 50 mM ammonium acetate pH adjusted 5.5 with acetic acid. The instrumental settings are flow rate of 1.5 mL/min, column temperature at 30°C, and detector wavelength of 235 nm using a photodiode array detector. Peak homogeneity data of IRB and HCTZ is obtained using photodiode array detector. The described method shows excellent linearity over a range of 10–200 µg/mL for IRB and 5–100 µg/mL for HCTZ. Methyl paraben was used as internal standard. The correlation coefficient for IRB and HCTZ are 0.998 and 0.999. The mean recovery values for IRB and HCTZ ranged from 100.45% to 101.25%. The limit of detection for IRB and HCTZ were 0.019 and 0.023 µg/mL, respectively, and the limit of quantification was 0.053 and 0.070 µg/mL respectively.

The ultimate goal of this work was to develop and validate a single high-performance liquid chromatography method selective for the eight main components of tablets HYZAAR.A single method was developed to afford simultaneous quantitation of actives and degradates for each of the two existing formulations. Each method is presented herein and demonstrated to be suitable for quantitation to 0.1% levels of all relevant degradates, as well as 100% levels of respective drug substances[49].

3.4.5 High performance thin layer chromatographic method

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of Irbesartan and Hydrochlorothiazide in combined dosage forms[50]. The stationary phase used was precoated silica gel $60F_{254}$. The mobile phase used was a mixture of acetonitrile: chloroform:

glacial acetic acid (7:3:0.1 v/v/v). The detection of spots was carried out at 260 nm. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 100 to 700 ng/spot for irbesartan and 100 to 350 ng/spot for hydrochlorothiazide. The limit of detection and the limit of quantification for the Irbesartan were found to be 30 and 100 ng/spot respectively and for hydrochlorothiazide 25 and 100 ng/spot respectively.

Different spectrophotometric and HPTLC-densitometric methods are presented by Gindy *et al* [51] for the simultaneous determination of lisinopril and hydrochlorothiazide in pharmaceutical tablets. The spectrophotometric methods include third derivative (D) ultraviolet spectrophotometry with zero crossing measurement at 217.4 and 233.4 nm, second derivative of the ratio spectra with measurement at 214.3 and 228.0 nm; The HPTLC method was based on separation of both drugs followed by densitometric measurements of their spots at 210 and 275 nm for lisinopril and hydrochlorothiazide, respectively. The separation was carried out on Merck HPTLC aluminum plates of silica gel 60 F_{254}, using chloroform–ethylacetate–acetic acid (10:3:2 by vol.) as mobile phase.

Khodke *et al* [52**]** presented development and validation of a stability indicating HPTLC method for simultaneous estimation of Irbesartan and Hydrochlorothiazide using TLC plates precoated with Silica gel 60F254 and the mobile phase comprising Acetonitrile: Chloroform in the ratio of 5:6 v/v. Irbesartan and Hydrochlorothiazide were well resolved with Rf 0.27 ± 0.03 and 0.45 ± 0.03, respectively. Wavelength selected for the quantization was 270 nm. Inherent stability of these drugs was studied by exposing both drugs to various stress conditions as per ICH guidelines viz. Dry heat, oxidative, photolysis (UV and cool white fluorescent light) and hydrolytic conditions under different pH values.

3.4.6 High performance liquid chromatography coupled with mass spectrometry

A sensitive, specific and selective method has been developed for the simultaneous determination of Bisoprolol and Hydrochlorothiazide in human plasma presented by Tutunji *et al*[53]. The method employed a state of the art LC–MS/MS operated in the positive and negative ionization switching modes. A simple sample preparation step involving protein precipitation with acetonitrile has been optimized; the analytes and the internal standard moxifloxacin were separated on a Purosphere®STAR C_8column (125 mm×4 mm, 5 µm). The mobile phase was an ammonium acetate solution (1 mM) with formic acid (0.2%): methanol and acetonitrile (65:17.5:17.5, v/v/v (%)), the flow rate was set at 0.65 mL/min. Linearity was demonstrated over the concentration range 0.10–30.0 (ng/mL) for Bisoprolol and 1.00–80.00 ng/mL for hydrochlorothiazide. The limits of detection were 0.100 (ng/mL) for bisoprolol and 1.00 (ng/mL) for hydrochlorothiazide. The validated method was successfully applied to a pharmacokinetic study of 5 mg bisoprolol fumarate with 12.5 mg hydrochlorothiazide tablet in healthy volunteers.

Parekh *et al*[54] described a rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been developed and validated for the simultaneous estimation of hydrochlorothiazide, quinapril and its metabolite quinaprilat in human plasma. After solid phase extraction (SPE), the analytes and IS were chromatographed on ahypurity C8 (100 mm×2.1 mm i.d, 5 µm particle size) column using 2 µL injection volume with a run time of 2.8 min. An isocratic mobile phase consisting of 0.5% (v/v) formic acid: acetonitrile (25:75, v/v) was used to separate all these drugs. The precursor and product ions of these drugs were monitored on a triple quadrupole mass spectrometer, operating in the multiple reaction monitoring mode (MRM) without polarity switch. The proposed method was validated over the range of 5–500 ng/mL for hydrochlorothiazide method and 5–1500 ng/mL for quinapril and quinaprilat.

High performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) was developed for the simultaneous determination of olmesartan (OLM) and hydrochlorothiazide (HCTZ) in human plasma and urine. Solid-phase extraction (SPE) was used to isolate the analytes from biological matrices followed by injection of the extracts onto a C_{18} column with isocratic elution. Detection was carried out on a triple quadrupole tandem mass spectrometer in multiple reactions monitoring (MRM) mode using negative electrospray ionization (ESI). The method was validated over the concentration range of 1.00–1000 ng/mL and 5.00–5000 ng/mL for OLM in human plasma and urine as well as 0.500–200 ng/mL and 25.0–25,000 ng/mL for HCTZ in human plasma and urine, respectively [55].

A rapid and sensitive method for the simultaneous quantitation of hydrochlorothiazide (HCTZ) and metoprolol (MET) in human plasma based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been developed and validated by Gao *et al*[56]. Sample preparation by liquid–liquid extraction with diethyl ether–dichloromethane (60:40, v/v) was followed by

chromatography on a Venusil MP-C_{18} column using methanol–ammonium acetate (10 mM)–formic acid (pH 3.4) (50:50:0.05, v/v/v) at a flow rate of 0.8 mL/min. The method was linear in the concentration range 3–1000 ng/mL for both HCT and MET using 100 µL human plasma. Intra- and inter-day precisions (as relative standard deviation) for HCT were 2.9–3.9% and 3.9–4.7%, respectively and for MET were 2.4–4.1% and 4.7–6.2%, respectively. Accuracies were ±3.8% and ±2.6% for HCT and MET, respectively.

Gadepalli *et al*[57] developed novel and accurate liquid chromatography tandem mass spectrometry method using electrospray ionization mode has been developed and validated for the simultaneous determination of amlodipine (AMD), valsartan (VAL) using losartan (LOS) as an internal standard (IS), and Hydrochlorothiazide (HCTZ) using furosemide (FSD) as an IS. The separation was carried on Aquasil C_{18}(50 mm × 2.1 mm, 5 µm) reversed phase column using acetonitrile and water containing 0.1% formic acid (50:50, v/v) as the mobile phase. The method was validated in terms of linearity, accuracy and precision over the concentration range of 1–1000 ng/mL.

A sensitive, specific and selective liquid chromatography/tandem mass spectrometric method has been developed and validated for the simultaneous determination of irbesartan and hydrochlorothiazide in human plasma. Plasma samples were prepared using protein precipitation with acetonitrile, the two analytes and the internal standard losartan were separated on a reverse phase C_{18}column (50 mm×4 mm, 3 µm) using water with 2.5% formic acid, methanol and acetonitrile (40:45:15, v/v/v (%)) as a mobile phase (flow rate of 0.70 mL/min[58].

Singh *et al* [59] Validated ultra-performance liquid chromatography mass spectrometric method (UPLC–MS/MS) was used for the simultaneous quantitation of candesartan (CN) and hydrochlorothiazide (HCTZ) in human plasma. The analytes were extracted using a liquid–liquid extraction (LLE) method by using 0.1 mL of plasma volume. The lower limit of quantitation for CN and HCT was 1.00 ng/mL whereas the upper limit of quantitation was 499.15 ng/mL and 601.61 ng/mL for CN and HCTZ respectively. The chromatography was achieved within 2.0 min run time using a C18 Phenomenex, Gemini NX (100 mm ×4.6 mm, 5 mm) column with organic mixture: buffer solution (80:20, v/v) at a flow rate of 0.800 mL/min.

An ultra-high performance liquid chromatography tandem mass spectrometry (U-HPLC–MS/MS) method was developed and validated to determine irbesartan (IRB) and hydrochlorothiazide (HCTZ) in human plasma simultaneously presented by Qiu *et at*[60]. Plasma samples were prepared using protein precipitation with acetonitrile, the two analytes and the internal standard losartan were separated on an Acquity U-HPLC BEH C_{18} column and mass spectrometric analysis was performed using a QTrap5500 mass spectrometer coupled with electro-spray ionization (ESI) source in the negative ion mode. The MRM transitions of m/z 427.2 → 206.9 and m/z 296.1 → 204.9 were used to quantify for IRB and HCTZ, respectively. The linearity of this method was found to be within the concentration range of 5–3000 ng/mL for IRB, and 0.5–300 ng/mL for HCT in human plasma, respectively. The lower limit of quantification (LLOQ) was 5 ng/mL and 0.5 ng/mL for IRB and HCTZ in human plasma, respectively[60].

3.4.7 HPLC with on-line solid-phase extraction

Shang *et al*[61] described a HPLC method with on-line solid phase extraction (SPE) and DAD detection was developed for the simultaneous determination of nitrendipine and hydrochlorothiazide in spontaneously hypertensive rat (SHR) plasma. Plasma samples (100 µL) were injected directly onto a CAPCELL MF C_8 SPE column. High-abundance proteins and most matrixes in plasma were removed by on-line SPE technology, while nitrendipine and hydrochlorothiazide trapped on the SPE column were effectively separated on a C_{18}analyticalcolumn. The column temperature was maintained at 20°C. The optimal detection wavelength was 237 nm for NTDP and 271 nm for HCTZ. The total analytical run time was 34 min. The proposed method was linear over the range 5–500 ng/mL for nitrendipine and 10–1000 ng/mL for hydrochlorothiazide. The lower limit of detection (LLOD) was 0.5 and 0.6 ng/mL for nitrendipine and hydrochlorothiazide, respectively.

3.4.8 Thin layer chromatography

TLC simultaneous determination of valsartan (VAL) and hydrochlorothiazide (HCTZ) in pure form and in tablets using new butyl-modified Aleppo bentonite (BAC4) with mobile phase of acetonitrile: water: acetic acid (49.35:49.35:1.3, v/v), at pH 3.2 and at wavelength λ= 260 nm was developed. The surface properties of butyl-modified bentonite were studied by nitrogen adsorption at 77K. The retardation factors (Rf) of valsartan and hydrochlorothiazide were 0.49 and 0.78, respectively. Linearity for determination of VAL and HCTZ was in the range 2.00-20.00 and 1.00-10.00 µg/spot, respectively. The minimum determined concentration was 2.0 µg/spot for VAL and 1.0 µg/spot for HCTZ

with percent relative standard deviation (RSD %) does not exceed 3.1% and 2.0%, respectively. The limits of quantification (LOQ) were 0.61 and 0.20 µg/spot, and the limits of detection (LOD) were 0.20 and 0.066 µg/spot for determination of VAL and HCTZ, respectively. The proposed method was novel, simple, accurate and successfully applied to simultaneous determination of VAL and HCTZ in pharmaceuticals with average recovery of 97.9 to 102.4%, the results obtained agree well with the contents stated on the labels [62].

Table 1: Development Parameters for Chromatographic Methods

Drugs	Method	Year	Mobile phase (v/v)	Column	Flow rate (mL/min)	Wave length(nm)	Ref
Bisoprolol fumarate and Hydrochlorothiazide	HPLC	2008	0.1 M aqueous phosphate buffer: acetonitrile: tetrahydrofuran (80:10:5)	5 µm cyano column	1	225	29
Hydrochlorothiazide	HPLC	1986	0.1 M potassium di-hydrogen phosphate: acetonitrile: tetrahydrofuran (85:10:5)	Bondapack phenyl column	2	280	30
Olmesartan medoxomil and Irbesartan and Hydrochlorothiazide	HPLC	2008	acetonitrile : 0.2% acetic acid (50:50)	Bondapak, C_{18} column	1	260	31
Telmisartan and Hydrochlorothiazide	HPLC	2008	Buffer : acetonitrile (60:40)	ACE 5 C_{18}	1	260	32
Acetaminophen and Hydrochlorothiazide	HPLC	2008	water : methanol (85 : 15)	MICROSORB-MV 100 C_{18}	1.2	254	33
Hydrochlorothiazide and Spironolactone	HPLC	2011	ethyl acetate : chloroform : formic acid : triethyl amine (7:3:0.1:0.1)	silica gel 60 F_{254} TLC	2	230	34
Hydrochlorothiazide	LC	1992	Sodium phosphate monobasic: acetonitrile: methanol (90:6:4)	Nucleosile C_8 column	1.2	273	35
Angiotensin Converting Enzyme Inhibitors together with Hydrochlorothiazide	HPLC-UV	2011	acetonitrile : ammonia buffer	Extend RP- C_{18}	1	215	36
Telmisartan and Hydrochlorothiazide	HPLC – UV	2011	methanol: ammonium acetate (35: 65)	cyanopropyl column	1	270	37
Zofenopril and Hydrochlorothiazide	RP – LC	2011	Methanol : water (30: 70)	C_{18} column	1	270	38
Rosuvastatin calcium and Hydrochlorothiazide	RP – HPLC	2012	sodium perchlorate buffer solution (at pH 3.0): Acetonitrile (60:40)	ACE C_{18} AR	1	280	39
Hydrochlorothiazide, Amlodipine, and Losartan	RP – HPLC	2013	acetonitrile : water : potassium dihydrogen phosphate buffer (45:35:20)	phenomenexluna 5µ CN	1	230	40
Telmisartan and Hydrochlorothiazide	RP- HPLC	2011	Acetonitrile: Methanol (80:20)	Kromasil C_{18} column	1	270	41
Bisoprolol fumarate and Hydrochlorothiazide	RP- HPLC	2010	potassium dihydrogen phosphate buffer and acetonitrile (70:30)	Inertsil ODS 3V column	1	228	42
Nebivolol and Hydrochlorothiazide	RP- HPLC	2008	acetonitrile: ammonium acetate (70: 30)	Phenomenex Gemini C_{18}	1	254	43
Hydrochlorothiazide and Metoprolol Tartrate	RP- HPLC	2011	phosphate buffer: methanol (60:40)	C_{18} Column	1	226	44
Amlodipine besylate, Valsartan and Hydrochlorothiazide	Stability indicating HPLC-DAD	2013	phosphoric acid : acetonitrile	Zorbax SB-C_8 column	1	225	45
Olmesartan Medoxomile, Amlodipine Besylate and Hydrochlorothiazide	Stability indicating RP – HPLC	2012	Triethylamine : acetonitrile	RP C_{18}	1.4	236	46
Losartan/ Hydrochlorothiazide	Stability indicating HPLC	2005	acetonitrile : water : orthophosphoric acid : triethylamine (400:600:1:1)	Hypersil ODS (C18) column	1	220	47
Irbesartan and Hydrochlorothiazide	Stability indicating LC	2010	ammonium acetate : acetonitrile.(70 :30)	Ace5 C_{18}	1.5	235	48
Losartan potassium,	Stability	2002	phosphate buffer:	C_8 columns	1.0	250	49

Hydrochlorothiazide	indicating HPLC		acetonitrile(93: 7)				
Irbesartan and Hydrochlorothiazide	HPTLC	2007	Acetonitrile : chloroform : glacial acetic acid (7:3:0.1)	-	-	260	50
Lisinopril and Hydrochlorothiazide	spectrophotometric and HPTLC	2001	Chloroform : ethylacetate : acetic acid (10:3:2)	-	-	275	51
Irbesartan and Hydrochlorothiazide	HPTLC	2010	Acetonitrile: Chloroform(5:6)	Silica gel 60F254	-	270	52
Bisoprolol and Hydrochlorothiazide	HPLC-MS	2009	formic acid (0.2%): methanol : acetonitrile (65:17.5:17.)	Purosphere ® STAR C_8 Column	0.65	-	53
Hydrochlorothiazide, Quinapril and Quinaprilat	LC–MS	2008	formic acid : acetonitrile (25: 75)	hypurity C_8	0.2	-	54
Olmesartan and Hydrochlorothiazide	LC-MS	2010	Acetonitrile : formic acid :methanol (60 : 36 :4)	XTerra MS C_{18}	0.2min during 0-1.2 min and was change into 0.35 ml/min during 1.2 – 2 min	-	55
Hydrochlorothiazide and Metoprolol	LC-MS	2010	methanol :ammonium acetate : formic acid (50:50:0.05)	Venusil MP-C_{18}	0.8	-	56
Amlodipine, Valsartan and Hydrochlorothiazide	LC ESI-MS/MS	2003	acetonitrile : water containing 0.1 % formic acid (50:50)	Aquasil C_{18}	0.2	-	57
Irbesartan and Hydrochlorothiazide	HPLC -MS	2010	2.5% formic : methanol : acetonitrile (40:40:15)	C_{18} column	0.70	-	58
Candesartan and Hydrochlorothiazide	UPLC-MS/MS	2014	organic mixture : buffer solution (80:20)	C_{18} phenomenex, Gemini NX	0.800	-	59
Irbesartan and Hydrochlorothiazide	UPLC -MS	2014	0.1% formic Acid in water : acetonitrile	BEH C_{18} column	0.45	-	60
Nitrendipine and hydrochlorothiazide	HPLC with on-line solid-phase extraction	2011	Acetonitrile : aqueous formic acid	CAPCELL MF C8 SPE column	-	237 for NTDP and 271 for HCTZ	61
Valsartan and hydrochlorothiazide	TLC	2013	acetonitrile: water: acetic acid (49.35:49.35:1.3)	-	-	260	62

Table 2: Validation Parameters for Chromatographic Methods

Drugs	Method	Year	Linearity range	LOD	LOQ	Ref.
Bisoprolol fumarate and Hydrochlorothiazide	HPLC	2008	50–150 µg/ml (BF). 125–375 µg/ml (HCTZ).	-	-	29
Olmesartan medoxomil and Irbesartan and Hydrochlorothiazide	HPLC	2008	6.25 –18 ng/ml (HCTZ). 20–60 ng/ml (OLME). 75-225 ng/ml (IRBE).	1ng/ml (HCTZ). 2 ng/ml (OLME). 3 ng/ml (IRB).	3 ng/ml (for all drugs).	31
Telmisartan and Hydrochlorothiazide	HPLC	2008	10–150 µg/ml (TELM). 5–75 µg/mL (HCTZ).	0.018 µg/ml (TELM).0.022 µg/m (HCTZ).	0.052 µg/ml (TELM). 0.068 µg/ml (HCTZ).	32
Acetaminophen and Hydrochlorothiazide	HPLC	2008	0.3 to 63.8 µg/mL (both drugs)	0.169 µg/ml (AC). 0.75 µg/ml (HCTZ).	0.565 µg/ml (AC). 2.5 µg/ml (HCTZ).	33
Hydrochlorothiazide and Spironolactone	HPLC	2011	4.0–50 µg/ML (HCTZ). 5.0–50 µg/mL (SPR).	-	-	34
Hydrochlorothiazide	LC	1992	2.0-1000 ng /ml	-	2 ng/ml	35
Angiotensin Converting Enzyme Inhibitors together with Hydrochlorothiazide	HPLC - UV	2011	-	17–64 ng/ml	56–212 ng /mL	36
Telmisartan and Hydrochlorothiazide	HPLC – UV	2011	1–10 µg/ml (TELM). 0.31– 3.12 µg/ml(HCTZ).	0.018 µg/ml (TELM). 0.022 µg/ml (HCTZ).	0.052 µg/ml (TELM). 0.068 µg/ml (HCTZ).	37
Zofenopril and Hydrochlorothiazide	RP – LC	2011	1.0–70.0 µg/mL (ZOF). 2.5–35.0 µg/mL (HCTZ).	0.129 µg/ml (ZOF). 0.183 µg/ml (HCTZ).	0.292 µg/ml (ZOF). 0.556 µg/ml (HCTZ).	38
Rosuvastatin calcium and Hydrochlorothiazide	RP – HPLC	2012	5-30 µg/ml (ROSU). 6.25-37.5 µg/ml (HCTZ).	0.16 µg/ml (ROSU). 0.20 µg/ml (HCTZ).	0.48 µg/ml (ROSU). 0.60 µg/ml (HCTZ).	39
Hydrochlorothiazide, Amlodipine, and Losartan	RP – HPLC	2013	12.5-62.5 mg/ml (HCTZ). 2.5-12.5 mg/ml (AMLO). 50-250 mg/ml (LSP).	0.03 µg/ml (HCTZ).0.03 µg/ml (AMLO). 0.108 µg/ml (LSP).	0.1 µg/ml (HCTZ).0.1 µg/ml (AMLO). 0.228 µg/ml (LOP).	40
Telmisartan and Hydrochlorothiazide	RP-HPLC	2011	-	0.020 µg/ml (HCTZ). 0.010 µg/ml (TELM).	0.060 µg/ml (HCTZ). 0.031 µg/ml (TELM).	41
Bisoprolol fumarate and Hydrochlorothiazide	RP-HPLC	2010	2.5–50 µg/mL (BF). 6.25–125 µg/mL (HCTZ).	0.01 µg/ml (BF). 0.01 µg/ml (HCTZ).	0.03 µg/ml (BF). 0.05 µg/ml (HCTZ).	42

Nebivolol and Hydrochlorothiazide	RP-HPLC	2008	-	10 ng/ml (NEB). 5 ng/ml (HCTZ).	30 ng/ml (NEB). 15 ng/ml (HCTZ).	43
Amlodipine besylate, Valsartan and Hydrochlorothiazide	Stability indicating HPLC-DAD	2013	5–200 µg/mL (AMLO). 5–200 µg/mL (VAL). 10–200 µg/mL (HCTZ).	0.26 µg/ml (AMLO). 0.24 µg/ml (VAL). 0.12 µg/ml (HCTZ).	0.85 µg/ml (AMLO). 0.80 µg/ml (VAL). 0.40 µg/ml (HCTZ).	45
Olmesartan Medoxomile, Amlodipine Besylate and Hydrochlorothiazide	Stability indicating RP – HPLC	2012	50–150 mg/ml (OLME). 12.5–37.5 mg/ml (AMLO). 31–93 mg/mL (HCTZ).	0.19 µg/ml (OLME). 0.16 µg/ml (AMLO). 0.22 µg/ml (HCTZ).	0.57 µg/ml (OLME).0.49 µg/ml (AMLO).0.66 µg/ml (HCTZ).	46
Irbesartan and Hydrochlorothiazide	Stability indicating LC	2010	10–200 µg/mL (IRB). 5–100 µg/mL (HCTZ).	0.019 µg/ml (IRB). 0.023 µg/ml (HCTZ).	0.053 µg/ml (IRB). 0.070 µg/ml (HCTZ).	48
Irbesartan and Hydrochlorothiazide	HPTLC	2007	100 to 700 ng/spot (IRB). 100 to 350 ng/spot (HCTZ).	30 ng/spot (IRB). 25 ng/spot (HCTZ).	100 ng/spot (IRB). 100 ng/spot (HCTZ).	50
Irbesartan and Hydrochlorothiazide	HPTLC	2010	200-1000 ng/band (IRB). 200-600 ng/band (HCTZ).	30 ng/band (IEB). 66 ng/band (HCTZ).	100 ng/band (IRB). 200 ng/band (HCTZ).	52
Bisoprolol and Hydrochlorothiazide	HPLC – MS	2009	0.10–30.0 ng/mL (BF). 1.00–80.00 ng/mL (HCTZ).	0.100 ng/ml (BF). 1 ng/ml (HCTZ).	0.10 ng/ml (BF). 1 ng/ml (HCTZ).	53
Hydrochlorothiazide, Quinapril and Quinaprilat	LC – MS	2008	5–500 ng/mL (HCTZ). 5–1500 ng/mL (QUI).	-	LLOQ 5 ng/ml	54
Hydrochlorothiazide and Metoprolol	LC – MS	2010	3–1000 ng/mL for both HCTZ and MET	-	LLOQ for Both drug 3 ng/ml	56
Irbesartan and Hydrochlorothiazide	HPLC - MS	2010	0.06–6.00 µg/mL (IRB). 1.00–112.00 ng/mL (HCTZ.)	0.01 µg/ml (IRB). 0.51 µg/ml (HCTZ).	0.06 µg/ml (IRB). 0.1 ng/ml (HCTZ).	58
Candesartan and Hydrochlorothiazide	UPLC-MS	2014	-	-	1 ng/ml (CN). 1 ng/ml (HCTZ)	59
Irbesartan and Hydrochlorothiazide	HPLC - MS	2014	5–3000 ng/mL (IRB). 0.5–300 ng/mL (HCTZ).	-	5 ng/ml (IRB). LLOQ for 0.5 ng/ml (HCTZ).	60
Nitrendipine and hydrochlorothiazide	HPLC with on-line solid-phase extraction	2011	5–500 ng/mL (NITR). And 10–1000 ng/mL (HCTZ).	0.5 ng/mL (NITR).0.6 ng/mL (HCTZ).	-	61
Valsartan and hydrochlorothiazide	TLC	2013	2-20. µg/spot (VAL). 1-10 µg/spot (HCTZ)	0.61µg/spot (VAL). 0.20 µg/spot (HCTZ)	0.20 µg/spot (VAL). 0.066 µg/spot (HCTZ).	62

4. Method Benefits and Limitations

General considerations for method selection are discussed, focusing on important analytical parameters as selectivity, efficiency, sensitivity and robustness. The sensitivity is the most important method characteristic in the evaluation of the samples with small amount of analyte. The limit of detection of the most of analytical methods described in table 3. The principal advantages of voltammetric method are its rapidity and simplicity. Each voltammetric run takes few seconds. It involves no clean up procedures. Capillary electrophoresis method gives more specificity, and accuracy. Spectrophotometry has always provided analytical techniques characterized by instrumental simplicity, moderate cost and portability. In these methods, fluorescence detection offers superior sensitivity and selectivity compared to that provided by UV detection. On the other hand, the HPLC shows advantages over spectrophotometric methods lies in its separation capability. Through chromatographic separations, the analytes of interest can be detected and quantified in the presence of degradation products and excipients. Additionally, capillary electrophoresis can be an excellent alternative separation technique to HPLC, however its limit of detection is usually about 50 times poorer than that of HPLC. At present, HPLC is the most widely used technique for the analysis of bulk drugs and their formulations. However, HPLC methods show limitations as cost of columns, solvents and a lack of long-term reproducibility due to the proprietary nature of column packings. The choice of proper detection mode in HPLC analysis is crucial to ensure that all the components are detected. With UV detection, the use of this problem could be overcome by using a multiple wavelength scanning program which is capable of monitoring several wavelengths simultaneously. In general, fluorescence detection offers superior sensitivity and selectivity compared to that provided by UV detection. Liquid chromatography combined with mass spectrometry (LC-MS) is considered as one of the most important techniques of the last decade of 20th century. It became the method of choice for analytical support in many stages of quality control and assurance within the pharmaceutical industry .The main advantage of mass spectrometric is its capability for analyzing both chromophoric and non-chromophoric drug compounds with superior selectivity and sensitivity. As the other techniques, this method shows some

disadvantages as high cost, few well-defined analytical procedures fit in the current GMP requirements and variability associated with unstable ionization source and significant interference from the formulation excipients.

Table 3: Comparison of sensitivity of various analytical methods

Method	Detection	Sample	Limit of detection	Ref.
Capillary electrophoresis	UV	Dosage form	0.12 mg/mL.	15
Voltammetric method		Urine and tablet	14.0 ng ml−1(urine) 5.0 ng ml−1 (std.)	16
Spectrophotometric method	IR	Pharmaceutical sample	-	20
	UV	Bulk mixture and tablet	0.91 µg/ml	21
	UV	Tablet	1.0137 µg/ml	22
	UV	Binary mixture	0.32 µg/ml	24
	UV	Dosage form	o.33 µg/ml	27
HPLC	UV	Tablet	0.12 µg/ml	45
	UV	Tablet	0.22 µg/ml	46
	UV	Pharmaceutical preparation	0.023 µg/ml	48
	UV	Degradation product	-	49
	MS	Human plasma	1 ng/ml	53
	MS	Human plasma	-	54
	MS	Human plasma and urine	-	55

5. Conclusions

The presented review provides information about the various methods available in the literature for the determination of Hydrochlorothiazide alone and with combination. The analysis of the published data revealed that the HPLC was exclusively used for determination of Hydrochlorothiazide. Determination of Hydrochlorothiazide in formulation and biological samples, were commend HPLC-MS/MS method, since this method combines the HPLC Separation ability with MS Sensitivity and selectivity, allowing the unambiguous identification of Hydrochlorothiazide. Sometime, HPLC with UV Detection is applicable because this method provides accurate results and low cost compared to more advanced detection techniques. This review carried out an overview of the current state-of-art analytical methods for determination of Hydrochlorothiazide.

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