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A Review on Reported High-Performance Liquid Chromatography Methods for Determination of Chlorthalidone in Pharmaceutical Dosage Form

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Abstract A persistent medical disease known as hypertension causes high artery blood pressure. The European Society of Cardiology and European Society of Hypertension recommendations a systolic blood pressure (SBP) of at least 140 mmHg and/or a diastolic blood pressure (DBP) of at least 90 mmHg are considered to be signs of hypertension [1]. Combinations of anti-hypertensive medications are used to treat hypertension. Chlorthalidone is a thiazide diuretic, a type of diuretic used to treat hypertension. Na^+ and Cl^- ions are inhibited by chlorthalidone via obstructing the Na^+/Cl^- Symporter, re-absorption in the distal convoluted tubule [2]. Chlorthalidone indirectly increases potassium excretion by boosting sodium supply to the distal renal tubule through the sodium-potassium exchange pathway [3-4]. Although its pharmacological action is similar to that of a sulphonamide, this diuretic differs chemically from thiazides due to the heterocyclic ring structure comparable to those of the thiazides. With the chemical formula $C_{14}H_{11}ClN_2O_4S$, the IUPAC name (RS)- 2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol1-yl) benzene-1-sulfonamide was used.
This review concentrates on the most current advancements in analytical techniques, including high-performance liquid chromatographic techniques for estimating chlorthalidone either by itself or in combination with other medications.

Keywords Chlorthalidone, Analytical method, HPLC, Hypertension

1. Introduction

Chlorthalidone was initially made available to patients in Switzerland in 1959; it is also a generic drug [5]. A 2-chloro-5-(1-hydroxy-3-oxo-2H-isoindol-1-yl) benzene sulfonamide is chlorthalidone (Fig 1) It is a diuretic with a lengthy half-life that is used to treat hypertension and a few kidney disorder [5].

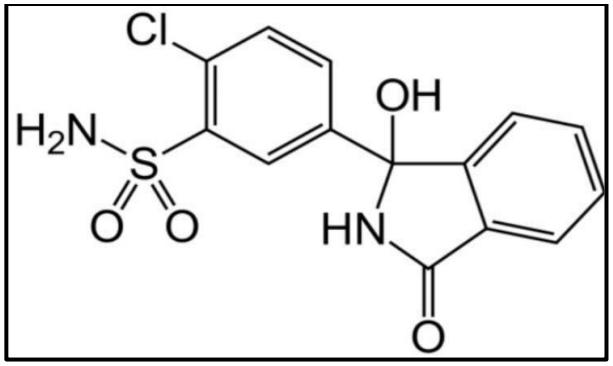

Figure 1: Structure of Chlorthalidone

Molecular Formula: - $C_{14}H_{11}ClN_2O_4S$
Molecular Weight: - 338.8g/mol
Melting point range: - 218 -264℃
It is crystalline, odourless, and white to yellowish-white in colour. It is a substance that is used to treat hypertension and is soluble in methanol but not in water or ethanol [6]. Pka was 9.57 [2].
A diuretic and hypertensive medication, chlorthalidone may be administered either alone or in conjunction with a lowered dose of a hypotensive medication. Although chemically it differs from thiazide diuretics in the structure of the heterocyclic ring, its pharmacological effects are similar to those of thiazide diuretics. It is administered orally once daily in doses ranging from 50 to 200 mg; diuresis begins in 2 hours or less and can persist up to 48 hours. Its biological half-life of 30-80 hours is thought to be the cause of its lengthy duration of activity. Depending on the dose, the medication appears to have a high volume of distribution that results in relatively low peak plasma concentrations of 100–1000 ng /ml [7].
Chlorthalidone is rapidly but incompletely absorbed following oral treatment. There doesn't seem to be much metabolization of it. About 25–40% of a single dose is eliminated in the urine as unaltered. Medication is excreted in the urine in a dose-dependent manner, with around 1% of the drug being removed in the bile. During daily therapy, roughly 25% of the daily dose is removed in the feces and about 50% of the daily dose is excreted unaltered in the urine in 24 hours [8].
At maximum therapeutic doses, chlorthalidone has the longest duration of action but a similar diuretic impact. The Na+/Cl symporter in the apical membrane of the distal convoluted tubule cells of the kidney is largely inhibited, which decreases sodium and chloride reabsorption. Although the precise mechanism of chlorthalidone's anti-hypertensive impact is still being debated, it is generally accepted that enhanced diuresis causes a decrease in the volume of plasma and extracellular fluid, a reduction in cardiac output, and a consequent reduction in blood pressure. Considering the substantial evidence from meta-analyses, chlorthalidone is regarded as a first-line medication for the management of uncomplicated hypertension [5].
Drug development and dosage form formulation heavily relies on the development and validation of analytical methods. The creation of new analytical techniques for assessing the quality of novel, emerging medications is highly necessary. Due to its accuracy and reliable result, HPLC is the primary analytical method for qualitative and quantitative assessment of drugs single or in combination [9].
Uses: It is used to treat oedema and congestive heart failure, liver cirrhosis, and fluid retention brought on by kidney disease. By decreasing the water and electrolyte salts in the body, illness and hypertension can be prevented. It is also used to treat diabetes insipidus and keep calcium kidney stones from forming in persons whose urine has higher calcium levels (hypercalciuria) [10].

Table 1: HPLC single method reported for determination of Chlorthalidone in pharmaceutical dosage form

No	Method	Analytical condition	References
1	Quantitative determination of chlorthalidone in pharmaceutical dosage forms by high-pressure liquid chromatography.	Stationary phase: Stainless steel tube (1m X 2.2 mm.) polyamide-coated stationary phase. Mobile phase: 2-propanol-acetic acid-water-n hexane ((30:1.5:0.5:68% v/v) λmax: 254nm Flow rate:2ml/min	[11]
2	A validated RP-HPLC stability method for the estimation of chlorthalidone and its process-related impurities in an API and tablet formulation.	Stationary phase:C8 column (250 ×4.6 mm 5 µ) Mobile phase: Mobile phase A consists of buffer solution (diammonium hydrogen orthophosphate (10 mM, pH 5.5)) and methanol (65: 35 % v/v), and mobile phase B consists of buffer solution and methanol (50: 50 %v/v). λmax:220nm Retention time: 6.729min Flow rate: 1.4ml/min Linearity: 1.0- 2.8 µg/ml	[12]
3	Development and validation of stabilityindicating method for estimation of chlorthalidone in bulk and tablets with the use of experimental design in forced degradation experiments	Stationary phase: Phenomenex Hyperclone C 18 column (250 × 4.6 mm, 5 μ) Mobile phase : Methanol: acetonitrile: phosphate buffer (20 mM) pH 3. (30 : 10: 60% v/v.) λmax:241nm Flow rate :1 ml/min Linearity: 2-12µg/ml	[13]
4	Stability-indicating assay for chlorthalidone formulation: evaluation of the USP analysis and a high-performance liquid chromatographic analysis.	Stationary phase: ODS columns Mobile phase: Acetonitrile-2% acetic acid (30:70%v/v) λmax:280nm Flow rate:1.5ml/min	[14]
5	Determination of chlorthalidone in human plasma by reversed-phase micellar liquid chromatography.	Stationary phase: C 8 column reversed-phase column (220 x 4.6 mm 10µ) Mobile phase: 0.05 molar sodium dodecyl sulphate – propanol (95+5) λmax:235nm Retention time:9.4min Flow rate:1.3ml/min Linearity:50 -800ng/ml	[7]
6	Rapid and sensitive determination of chlorthalidone in blood, plasma and urine of man using high-performance liquid chromatography.	Stationary phase: Stainless-steel C18 column (15 cm X 4.6 mm. 5 µ) Mobile phase: of 0.01 M sodium acetate in water and acetonitrile (400:100 v/v) λmax:226nm	[15]

		Flow rate:1.6ml/min	
7	Analysis of chlorthalidone in biological fluids by high performance liquid chromatography using a rapid column cleanup procedure.	Stationary phase: C18 column (RCM100) Mobile phase:0.001M Aqueous sodium acetate mixed with acetonitrile (80:20% v/v) λmax:210nm Retention time:8.6min Flow rate:2ml/min	[16]
8	Simple, sensitive and selective highperformance liquid chromatographic method for analysis of chlorthalidone in whole blood.	Mobile phase: (77% 0.01 M sodium acetate in acetonitrile) λmax:214nm Retention time:7.5min Flow rate:1.5 ml/min Linearity:2- 0.0625 µg/ml	[17]
9	On-line solid-phase extraction and highperformance liquid chromatographic determination of chlorthalidone in urine.	Stationary phase: column Cl8 (250x4.6 mm.,5 µ) Mobile phase: Acetonitrile-0.01 M phosphate buffer pH 7 (20:80 %v/v) λmax:214nm Flow rate:2ml/min Linearity:0.1-200 µg/ml	[18]
10	An approach to select linear regression model in bioanalytical method validation.	Stationary phase: C18 column (250 × 4.6 mm, 5 µ). Mobile phase: Methanol: water (60:40%, v/v) λmax:276nm Retention time:6.825min Flow rate:1ml/min Linearity:100 -320ng/ml	[19]
11	Stability indicating RP-HPLC method development and validation for the quantitative estimation Chlorthalidone in API and tablet dosage form.	Stationary phase: Develosil ODS HG-5 RP C18, (15cmx4.6mm 5µ) column Mobile phase:0.1% OPA: Acetonitrile: Methanol (12:18:70 %v/v/v) λmax:245nm Retention time:3.444min Flow rate:1ml/min Linearity :0-14µg/ml	[20]
12	Method validation and development of chlorthalidone by RP-HPLC.	Stationary phase: Phenomenex Luna C18, 100A, 5µm, (250mmx4.6mm) Mobile phase: Phosphate dihydrogen phosphate buffer: Methanol (55:45%v/v) pH=3.4 λmax:244nm Retention time:3.91 min Flow rate:1ml/min Linearity:6 -14µg/ml	[21]

1) Stationary phase concist of Stainless steel tube (1m X 2.2mm.) 2-propanol, acetic acid, water, and n-hexane (30:1.5:0.5:68% v/v/v)) make up the mobile phase, wavelength measurement at 254 nm. With 1 ml/min of flow.

2) The C8 column (250 ×4.6 mm5µ) makes up the stationary phase. Mobile phase B was composed of buffer solution and methanol at a (50:50%v/v) flow rate of 1.4 ml/min, whereas mobile phase A was composed of buffer solution and methanol at a (65:35% v/v) of diammonium hydrogen orthophosphate (10 mM, pH 5.5) in the mobile phase.
3) Using a Phenomenex Hyperclone C18 column (250 × 4.6mm, 5µ), the mobile phase was composed of 30:10:60% v/v of methanol, acetonitrile, and phosphate buffer (20 mM) with a pH 3.0 adjustment made with OPA. Eluent was detected at 241 nm while the flow rate was held constant at 1 ml/min. In research involving calibration curves, linearity was discovered to be between 2 and 12 µg/ml.
4) The mobile phase was acetonitrile-:2% acetic acid (30:70%v/v) the wavelength detected at 280 nm flow rate was 1.5 ml/min for microparticulate octadecylsilane columns.
5) The reconstituted residues are then examined on a C8 reversed-phase column using a mobile phase of 0.05 M sodium dodecyl sulphate - propanol(95 + 5) after the organic phase has evaporated. Utilizing UV absorption at 235 nm, the medication and internal standard are found. Chlorthalidone's retention time is 9.4 minutes. 1.3 ml/min flow rate with linearity between 50 and 800 ng/ml
6) A stainless steel column (15 cm X 4.6 mm 5µ) filled with LiChrosorb RP C18, was used for high-performance liquid chromatography. Maximum absorbance is 226 nm in a solution of 0.01 M sodium acetate in water and acetonitrile (400:100 v/v). Chlorthalidone flows at a rate of 1.6 ml/min.
7) Enabling a minimum concentration of 1 mg/ml to be determined Chromatogram fitted with a C18 column of 10 m radial compression (RCM100) at 0.001M Acetonitrile with sodium acetate in water (80:20% v/v) detection of 210 nm wavelength Chlorthalidone has a retention duration of 8.6 minutes and a flow rate of 2 ml/min.
8) At 214 nm, sodium acetate at a concentration of 0.01 M in acetonitrile is detected by the mobile phase (77%). Chlorthalidone has a retention time of 7.5 minutes. Chlorthalidone flows at a rate of 1.5 ml/min.
9) The method was performed by reversed-phase chromatography and UV detection at 214 nm using an acetonitrile-0.01 M phosphate buffer pH 7 (20:80 %v/v) eluent. The precolumn is renewed and prepared for the subsequent sample after the run while the LC separation is being carried out. detection of the 214 nm wavelength. The linearity of the chlorthalidone is between 0.1 and 200 µg/ml. flow rate is 2 ml/min.
10) In the concentration range of 100-3200 ng/ml, the calibration curve standards were investigated. Methanol: water (60:40%, v/v) was used as the mobile phase during the chromatography, which was carried out on a C18 column (250 ×4.6 mm, 5µ) in an isocratic mode at a flow rate of 1 ml/min. The retention time of chlorthalidone was measured at 276 nm and is.6.825 minutes.
11) The Develosil ODS HG-5 RP C18 (5µm, 15 cm x 4.6 mm). a column with UV detection at 245 nm and 0.1% OPA: Acetonitrile: Methanol (12:18:70 v/v/v) ratio at a flow rate of 1.0 ml/min was used to standardize the chromatographic technique. The procedure was linear between 0 and 14 µg/ml. It was discovered that chlorthalidone had a retention time of 3.44 minutes.
12) The Phenomenex Luna C18, 100A, (5 µm, 250 mm x 4.6 mm) column was used for the stationary phase of the chromatography, and the mobile phase was made with a solution of phosphate dihydrogen phosphate buffer: methanol (55:45 %v/v)(pH 3.4) flowed at 1.0 ml/min with an injection volume of 20 µl, at a detection wavelength of 244 nm, and run Chlorthalidone can be estimated using the analytical approach over a range of 6-14µg/ml.

Table 2: HPLC combination method reported for determination of Chlorthalidone in the pharmaceutical dosage form.

No	Method	Analytical condition	Reference
[1]	RP-HPLC method for simultaneous estimation of enalapril maleate and chlorthalidone in a synthetic mixture.	Stationary phase: Hypersil BDS C18 (250 x 4.6mm, 5 µ) column Mobile phase: Phosphate buffer: Acetonitrile: Methanol	[22]

		(65:25:10% v/v/v) λmax:210nm Retention time:4.247 min Flow rate:1 ml/min Linearity:12.5-37.5 µg/ml	
[2]	RP-HPLC method development and validation for simultaneous estimation of benidipine hydrochloride and chlorthalidone in pharmaceutical dosage form.	Stationary phase:C18Agilent Zorbax Bonus – RP (250 x 4.6 mm, -5µ) column Mobile phase: Methanol and 0.1% OPA (45:55%v/v) λmax:238nm Retention time:3.52min Flow rate:1ml/min Linearity:100-150 µg/ml	[5]
[3]	Bioanalytical method development and validation for simultaneous determination of chlorthalidone and cilnidipine drugs in human plasma by RP-HPLC.	Stationary phase: Inertsil C18 (150×4.6 mm; 5µm) column Mobile phase: Acetonitrile and 0.1% OPA buffer (35:65% v/v) λmax:248nm Retention time:3.516min Flow rate:1ml/min Linearity:0.05-5.00 µg/ml	[23]
[4]	Determination of azilsartan medoximil and chlorthalidone in tablets exposed to forced degradation by using RP-HPLC.	Stationary phase: C18G (250 × 4.6 mm, 0.5 µm) column Mobile phase: Acetonitrile and 0.1% trifluoroacetic acid (40:60%v/v) λmax:240nm Retention time:7.748min Flow rate:0.8ml/min Linearity:2.5-25 µg/ml	[24]
[5]	Determination of chlorthalidone and clonidine hydrochloride in tablets by HPLC.	Stationary phase: ODS bonded, 5 to 6-m, spherical silica Mobile phase: 65% methanol in pH 7.9 phosphate buffer λmax:254nm Flow rate:1 ml/min	[25]
[6]	Simultaneous determination of atenolol and chlorthalidone in plasma by high-performance liquid chromatography Application to pharmacokinetic studies in man.	Stationary phase: ODS column Mobile phase:0.05 M sodium dodecyl sulphate in phosphate buffer (pH 5.8)-n-propanol (95:5,%v/v) λmax:225nm Flow rate:1.3ml/min Linearity:10-1000 ng/ml	[26]
[7]	HPLC method for the simultaneous determination of atenolol and chlorthalidone in human breast milk.	Stationary phase: Cyanide column. Mobile phase: ACN/water (35:65 %v/v) and buffered at pH 4.0 λmax:225nm	[27]

		Retention time:5min Flow rate:1ml/min Linearity:0.25 -5µg/ml	
[8]	Simple and rapid HPLC method for simultaneous determination of atenolol and chlorthalidone in spiked human plasma.	Stationary phase: cyanopropyl column Mobile phase:10 mM KH2PO4 (pH 6.0) – methanol (70:30 % v/v) λmax:225nm Flow rate:1ml/min Linearity:0.1 -10µg/ml	[8]
[9]	Simultaneous estimation of metoprolol succinate and chlorthalidone in pharmaceutical solid dosage form by using a developed and validated reverse phase highperformance liquid chromatographic technique.	Stationary phase: Inertsil ODS 3 column (100 × 4.6 mm, 5 µ) Mobile phase: Diammonium hydrogen phosphate buffer solution (pH 5.5): Methanol (70:30 %v/v). λmax:254nm Retention time:9.94min Flow rate:1ml/min Linearity:12.5-75µg/ml	[28]
[10]	Development of validated RPHPLC method for the simultaneous estimation of atenolol and chlorthalidone in combine tablet dosage form.	Stationary phase: Comosil RP-C18 (4.6 x 250mm, 5µ) column Mobile phase: Methanol: Water (pH 3)(60:40%v/v) λmax:226nm Retention time:3.36min Flow rate:1ml/min Linearity:10- 50 µg/mL	[6]
[11]	Validated method development for simultaneous estimation of losartan potassium and chlorthalidone in tablet dosage form by RP-HPLC method.	Stationary phase: Agilent XDB C18 (150 x 4.6 mm, 5µ) Mobile phase: Mixture of 0.02M Potassium dihydrogen orthophosphate (KH2PO4) buffer: acetonitrile (70:30 % v/v pH 3.5) λmax:254nm Retention time:2.718min Flow rate:1ml/min Linearity:1.55 – 9.35 µg/ml	[29]
[12]	RP-HPLC-PDA method for the simultaneous estimation of metoprolol succinate and chlorthalidone in bulk and pharmaceutical dosage forms.	Stationary phase: Inertsil ODS column Mobile phase: Mixture of 10mM ammonium acetate: acetonitrile in the ratio of (70:30%v/v) λmax:220nm Retention time:7.5min	[30]

[13]	Development and validation of RP-HPLC method for the Simultaneous Estimation of Eprosartan mesylate and chlorthalidone in Tablet Dosage Form.	Flow rate:1ml/min Linearity:2-6 µg/ml Stationary phase: Phenomenox, Gemini C18 (250×4.6 mm, 5µ) column Mobile phase: Mobile phase (55:45 %v/v)water: acetonitrile with pH adjusted to 3.4 with OPA λmax:250nm Retention time:3.80min Flow rate:1ml/min Linearity:0.5-12.5 µg/ml	[31]
[14]	Development and validation of RP-HPLC method for the simultaneous estimation of olmesartan medoxomil and chlorthalidone in tablet dosage form.	Stationary phase: Phenomenox, Gemini C18 (250×4.6 mm, 5 µm) column Mobile phase: water: acetonitrile (pH3.0) (55:45 %v/v) λmax:250nm Retention time:3.91min Flow rate:1ml/min Linearity: 5-30 µg/ml	[32]
[15]	Method development and validation of stability indicating RP-HPLC method for simultaneous Estimation of azilsartan and chlorthalidone in pure and pharmaceutical dosage form.	Mobile phase: Buffer: acetonitrile (45:55%v/v) λmax:270nm Retention time:3.652min Flow rate:1ml/min Linearity:31.25 -187.5 µg/ml	[2]
[16]	Novel and validated stabilityindicating HPLC method for simultaneous estimation of olmesartan and chlorthalidone in oral solid form.	Stationary phase: BDS C18 (250mm x 4.6 mm, 5µ) column Mobile phase: 10 mM OPA buffer and acetonitrile (45:55%v/v) λmax:212nm Retention time:2.113 min Flow rate:1ml/min Linearity:6.24 -31.25 µg/ml	[33]
[17]	Spectrophotometric and high performance liquid chromatographic determination of chlorthalidone and losartan potassium in combined dosage form.	Stationary phase: C18 column (250 mm × 4.6 mm, 5 µ) Mobile phase: Acetonitrile: Water (50: 50 % v/v) λmax:220nm Retention time:1.857 min Flow rate:1ml/min Linearity:10-30 µg/ml	[34]

[18]	Development and validation of stability indicating gradient RP-HPLC method for simultaneous estimation of telmisartan and chlorthalidone in bulk API and fixed-dose combination.	Stationary phase: Agilent Extend C18 (150 mm× 4.6 mm, 5 µ) column Mobile phase: Disodium Hydrogen Phosphate Buffer of pH-6.5: Acetonitrile (75:25% v/v) λmax:235nm Retention time:3.82min Flow rate:1ml/min Linearity:6-18 µg/ml	[35]
[19]	RP-HPLC method for simultaneous estimation of cilnidipine and chlorthalidone.	Mobile phase: Methanol: water (80:20% v/v) λmax:231.6nm Flow rate:1ml/min Linearity:10-70µg/ml	[36]
[20]	Validated stability-indicating RP-HPLC method for simultaneous estimation of cilnidipine and chlorthalidone in tablet dosage form.	Stationary phase: Inertsil ODS column (250 mm x 4.6 mm, 5 µ) column Mobile phase: Methanol: 0.025 M Potassium dihydrogen phosphate Buffer pH 5.5 (50:50 %v/v) (Solution A) and Acetonitrile, 0.025 M Potassium dihydrogen phosphate Buffer pH 5.5 (75:25% v/v) (Solution B), λmax:225nm Retention time:3.580 min Flow rate:1ml/min Linearity:6.25-37.5 µg/ml	[37]
[21]	Strategies for stabilizing formulation and QbD assisted development of robust stability indicating method of azilsartan medoxomil/chlorthalidone	Stationary phase: Inertsil C8 column (150 x 4.6 mm, 5 µ), column Mobile phase:0.025 M phosphate buffer pH 2.7: acetonitrile (52.5: 47.5%v/v) λmax:225nm Flow rate:1.5ml/min Linearity:1.5 – 25 µg/ml	[38]
[22]	Development and validation of novel RP- HPLC method for related substances in chlorthalidone and fimasartan formulations.	Stationary phase: Zodiac C18 (250 ×4.6mm,5µ)column Mobile phase: Buffer: 100% methanol pH 3 (50:50%v/v) λmax:230nm Retention time:12.342 min Flow rate:1.5ml/min Linearity:0.5-1.5µg/ml	[39]
[23]	Stability indicating method to analyze benidipine and chlorthalidone using HPLC technique: establishment, validation and application to tablets.	Stationary phase:C18 Kromasil (250 mm × 4.6 mm 5µ) column Mobile phase: Methanol-0.1M	[40]

		dipotassium hydrogen phosphate buffer (40:60% v/v), λmax:260nm Retention time:6.422 min Flow rate:1ml/min Linearity:6.25 - 18.75 µg/ml	
[24]	Method development and validation for the simultaneous estimation of anti-hypertensive drugs atenolol and chlorthalidone in solid dosage forms by RP-HPLC.	Stationary phase: Develosil ODS HG-5 RP C18, (15cmx4.6mm) column Mobile phase: Methanol: Acetonitrile (85:15% v/v) λmax:258nm Retention time:5.861min Flow rate:1ml/min Linearity:0-28µg/ml	[41]
[25]	Experimental design optimization of simultaneous enantiomeric separation of atenolol and chlorthalidone binary mixture by highperformance liquid chromatography using polysaccharide-based stationary phases.	Stationary phase: Chiral stationary phase Mobile phase: Hexane: ethanol: DEA: TFA (60:40:0.2:0.1%, v/v/v/v) λmax:230nm Flow rate:1ml/min Linearity:12.5–150 µg/ml	[42]
[26]	Analytical method development and validation for assay of fimasartan potassium trihydrate and chlorthalidone in tablet dosage form by using RP- HPLC.	Stationary phase: Prontosil C18 (250 mm ×4.6 mm,5µ) column Mobile phase: Potassium Phosphate Buffer (pH 3): ACN λmax:230nm Retention time:2.6min Flow rate:1.5ml/min Linearity:5 - 10 µg/ml	[9]
[27]	Method development and validation for the simultaneous estimation of azilsartan and chlorthalidone by RP-HPLC in pharmaceutical dosage form.	Stationary phase: ODS (250mm: 4.6mm,5µ) column Mobile phase:0.1% OPA buffer: acetonitrile (30:70%v/v) λmax:230nm Retention time:2.266min Flow rate:1ml/min Linearity:31.25-187.5 µg/ml	[2]
[28]	Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Azelnidipine and Chlorthalidone in Tablet Dosage Form	Stationary phase: Kromasil C18 (150 x 4.6 x 5 µm) column Mobile phase: 20 mM diammonium hydrogen phosphate: Methanol (65:35% v/v) pH 5.5 λmax:235nm Retention time: 4.616 min. Flow rate: 1.2 ml/min. Linearity: 31.16-93.47 µg/ml	[43]
[29]	Development and Validation of a RP - HPLC Method	Stationary phase: Inertsil C18 (4.6mm	[44]

for the Simultaneous Determination of Azelnidipine and Chlorthalidone in Pure and Pharmaceutical Dosage Form	×250mm, 5µm) column. Mobile phase: Methanol: Phosphate buffer (55:45%v/v) pH 4.8. λmax: 282nm Retention times: 3.282min Flow rate: 1ml/min. Linearity: 30-70 µg/ml

1) Phosphate buffer: ACN : methanol was employed as the mobile phase (65:25:10 %v/v/v) drug was detected at 210 nm using a flow rate of 1.0 ml/min. With the help of Hypersil BDS C18 (250 x 4.6mm, 5µ), the separation was accomplished. Enalapril Maleate and Chlorthalidone both showed linearity in the 5–15 µg/ml and 12.5–37.5 µg/ml ranges, respectively. Chlorthalidone has a retention time of 4.247 minutes.
2) This method made use of C18 Agilent Zorbax Bonus - RP (250 x 4.6 mm 5µ). With a flow rate of 1 ml/min, the mobile phase is made up of methanol and 0.1% OPA (45:55%v/v) (Photodiode array Detector). Chlorthalidone had a retention time of 3.52 min and the detection concentration was linear over the ranges of and 100-150 µg/ml.
3) By using an acetonitrile and 0.1% orthophosphoric acid (OPA) buffer mixture with a flow rate of 1 ml/ml and an injection volume of 10 ml as the mobile phase, the content of the medicines was evaluated. The Inertsil C18, (150 ×4.6 mm 5µ) analytical column was used for chromatographic separation, and the effluents were observed using a photodiode array (PDA) detector at 248 nm. Chlorthalidone had retention times of 3.516 min, and a total run duration of 8 min. For Chlorthalidone, linearity was established at concentrations between 0.05 and 5.00 µg/ml.
4) The Enable C18 G column (250 ×4.6 mm, 0.5 µ) and a mobile phase consisting of acetonitrile and 0.1% trifluoroacetic acid in water at a ratio of (40:60%v/v) with a flow rate of 0.8 ml/min have
5) been used to develop the method. At 240 nm, UV detection was conducted. and chlorthalidone to have retention times of 7.748 min. For chlorthalidone, linearity is seen around 2.5–25 µg/ml.
6) Individual tablets or composite samples were sonicated in water, diluted with methanol, and filtered before chromatographing. Chlorthalidone, formulated at 15mg/tablet, was chromatographed on ODS bonded, spherical silica with 50% methanol in a water mobile phase. drug was determined with a spectrophotometric detector at 254 nm.
7) A 0.05 M sodium dodecyl sulphate in phosphate buffer (pH 5.8)-n-propanol (95:5% v/v) solution, given at a flow rate of 1.3 ml/min, was used to separate the medicines on an ODS column at room temperature. Linearity was discovered in the 10-1000 ng/ml range.
8) The samples were put into a cyanide column using a mobile phase made of ACN/water (35:65% v/v) that was buffered at pH 4.0 and flowed at a rate of 1.0 ml/min. UV detection at 225 nm was used to quantify the data. Chlorthalidone has a linearity of 0.25 to 5.0 µg/ml. The chlorthalidone retention time was 5 minutes.
9) The analytes were chromatographed on a Shim-pack cyanopropyl column at room temperature with an isocratic elution of 10 mM KH2PO4 (pH 6.0) - methanol (70:30 %v/v) and UV detection at 225 nm. For the mixture, the chromatographic run time was under 10 minutes. Over the concentration range of 0.1–10 lg/ml, the calibration curves were linear.
10) On an Inertsil ODS 3 column (100 ×4.6 mm, 5 µ), the two medicines were separated using a mobile phase of diammonium hydrogen phosphate buffer solution (pH 5.5): methanol (70:30 %v/v). Detection was carried out at 254 nm at a flow rate of 1.0 ml/min. while chlorthalidone had a retention time of 9.94 min. Chlorthalidone responded linearly when the concentrations were between 12.5-75 µg/ml. chlorthalidone had correlation values of 0.9998.
11) Utilizing Comosil RP-C18 (4.6 x 250mm, 5 µm) in a gradient mode and a mobile phase made up of Methanol: Water, an RP-HPLC technique for the quantification of ATN (atenolol) and CTN

(chlorthalidone) in combination dose form was created (pH 3 using OPA) The effluent was measured at 226.0 nm and the flow rate was 1 ml/min. The retention time was determined to be 3.36 minutes.

12) On an Agilent XDB C18 (150 x 4.6 mm, 5µ) particle size column with a PDA detector, chromatographic separation was accomplished using a mobile phase comprising a mixture of 0.02 M potassium dihydrogen orthophosphate (KH2PO4) buffer and acetonitrile (70:30% v/v pH 3.5). 1 ml /min of flow was detected at a wavelength of 254 nm. Chlorthalidone was shown to have retention times of 2.718 minutes. The procedure was linear for the concentration ranges of 1.55 - 9.35 µg/ml for chlorthalidone and 12.5 - 75 µg/ml for losartan potassium, respectively
13) The procedure was run on an Inertsil ODS column with a mobile phase consisting of a combination of 10mM ammonium acetate and acetonitrile in a 70:30%v/v ratio. PDA detection at 220nm was used with a flow rate of 1ml/min. 7.5 minutes was the CT retention time and CT demonstrated good linearity at a concentration range of 2-6 µg/ml.
14) Chromatographic separation was carried out in the thermo isocratic mode on a Phenomenox, Gemini C18 (250 x 4.6 mm, 5 µm) column using a mobile phase of 55:45% water: acetonitrile with a pH adjustment of OPA at a flow rate of 1 ml/min. Both medicines' peak intensities were tracked at 250 nm using a UV detector. Chlorthalidone was found to have retention times (RT) of 3.80 minutes and the linearity was determined to be between 0.5-12.5 µg/ml, respectively.
15) Chromatographic separation was carried out in the thermo isocratic mode on a Phenomenox, Gemini C18 (250× 4.6 mm, 5µ) column using a mobile phase of 55:45 water: acetonitrile with a pH adjustment of OPA at a flow rate of 1 ml/min. Both medicines' peak intensities were tracked at 250 nm using UV detection. Results chlorthalidone was found to have retention times (RT) of 3.91 min and linearity was determined to be between 5 and 30 µg/ml.
16) Chlorthalidone had respective retention durations of 3.652 minutes. Acetonitrile and buffer make up the mobile phase (45:55%v/v) Both medicines' peak intensities were measured at 270 nm.There is a 1 ml/min flow. Chlorthalidone has linearity between 31.25 µg/ml -187.5 µg/ml
17) Using a mobile phase of 10 mM orthophosphoric acid buffer and acetonitrile (45:55%v/v) at a flow rate of 1.0 ml min-1, separation of both pharmaceuticals was accomplished on BDS C18 (250mm x 4.6mm, 5µ), and detection was carried out at 212 nm using a photodiode array (PDA) detector. Chlorthalidone retention was discovered at 2.113 min with a linearity of 6.24 -31.25 µg/ml.
18) Acetonitrile: Water (50:50 % v/v) mobile phase was employed on a C18 (250 mm ×4.6 mm, 5µ) column at a flow rate of 1.0 ml/min. Based on the peak height ratios, quantification was accomplished using UV detection at 220 nm. For chlorthalidone, calibration curves were linear in the concentration range of 10–30 µg/ml.
19) In the RP-HPLC procedure, the separation was carried out on an Agilent Extend C18 (150 mm× 4.6 mm, 5 µm) column using a gradient run with a starting ratio of (75:25% v/v)of acetonitrile as the mobile phase and a detection wavelength of 235 nm. Chlorthalidone had retention times of 3.82 min. The linearity was discovered to be between 6mcg/ml and 18mcg/ml respectively.
20) Method was developed using a flow rate of 1 ml/ min. The mobile phase consist of methanol:water (80:20% v/v) with UV detection at 231.6 nm. In the concentration ranges of 10-70 µg/ml for chlorthalidone demonstrated linearity.
21) On an Inertsil ODS column (250 mm x 4.6 mm, 5 µ), the separation was accomplished in gradient mode. Methanol, 0.025 M Potassium Dihydrogen Phosphate Buffer pH 5.5, adjusted by 10% v/v OPA (50:50 % v/v), and Acetonitrile, 0.025 M Potassium Dihydrogen Phosphate Buffer pH 5.5, adjusted by 10% v/v OPA (75:25% v/v), made up the mobile phase. The response was detected at 225 nm after the gradient for chlorthalidone, the retention times were determined to be 3.580 minutes.
22) Inertsil C8 column (150 x 4.6 mm, 5µ), linearity in the concentration range of 1.5 -25 µg/ml, detection wavelength 225 nm at 33 °C, mobile phase of 0.025 M phosphate buffer pH 2.7 and acetonitrile (52.5: 47.5%).

23) The separation was carried out on Zodiac C18 (250 ×4.6mm,5µ) columns with a flow rate of
24) 1.5 ml/min and a run time of 50 min. The method was developed using a Shimadzu LC Prominence-i 2030 model with chameleon software. The mobile phase contained buffer pH 3.0 and 100% methanol in a 50:50 ratio, and 230 nm was utilized as the detecting wavelength. The injection volume was 20 µl. For chlorthalidone, the retention times were determined to be 12.342 min, and linearity in the concentration range of 0.5 - 1.5 µg/ml.
25) A methanol-0.1M dipotassium hydrogen phosphate buffer mobile phase with a flow rate of 1 ml/min. Benidipine and chlorthalidone were detected and measured using the photodiode array (PDA) detector set at 260 nm. chlorthalidone took around 6.422 minutes to elute. Chlorthalidone concentrations between 6.25 and 18.75 µg/ml (R2 = 0.9998) were used to validate the procedure.
26) Isocratic mode with mobile phase containing Methanol: Acetonitrile in a ratio of (85:15% v/v) column was made of Develosil ODS HG-5 RP C18, (15cmx4.6mm). The effluent was seen at 258 nm and the flow rate was 1.0 ml/min. Chlorthalidone was reported to have linearity ranges of 0 to 28 and retention times of 5.861 minutes.
27) Atenolol and chlorthalidone were separated simultaneously into their enantiomers using highperformance liquid chromatography and stationary phases made of polysaccharides. According to the optimization method, a mobile phase made up of hexane, ethanol, DEA, and TFA (60:40:0.2:0.1%, v/v/v/v) was used to separate and quantify the drug combination chirally at 230 nm, where the linearity range of chlorthalidone was discovered to be 12.5-150 µg/ml.
28) The injection volume was 20µl, and the mobile phase was composed of Potassium Phosphate Buffer (pH 3) and ACN in gradient mode. In an 8-minute run, the detection wavelength was 230 nm. Chlorthalidone had respective retention times of 5.0. It was discovered that linearity was 5–10 µg/ml.
29) For the simultaneous measurement of azilsartan and chlorthalidone in pharmaceutical dose form by RP-HPLC methodology, a straightforward, exact, and accurate method has been devised. A mobile phase consisting of 0.1% OPA buffer and acetonitrile was passed down an ODS (250mm× 4.6mm, 5µ) column at a flow rate of 1ml/min. The column oven was kept at a temperature of 30°C. The optimized wavelength is 230 nm. Water and acetonitrile were used as diluents in a 50:50 ratio to create the stock and working solutions. The run time was set at 9 minutes. Chlorthalidone was eluted at 2.266min. Linearity was discovered between 31.25 µg/ml - 187.5 µg/ml.

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