A Review on Analytical Methods for Estimation of Azelnidipine and Chlorthalidone in Bulk and Pharmaceutical Dosage Form

Tamilselvi.N[1], Arivukkarasu.R[2], Velmurugan.M[3], Prakash.M[4], Vignesh.S[5]
[1,2,3,4,5]*Department of Pharmaceutical Analysis, KMCH College of Pharmacy, Kovai Estate, Kalapatti Road, Coimbatore – 641048, Tamilnadu, India*

Abstract-Hypertension, other name for high blood pressure, is a common condition characterized by abnormally high blood vessel pressure. A lot of people suffer with hypertension (HT), particularly as they get older. It is a significant risk factor for cardiovascular mortality and morbidity but is not a disease in and of itself. Azelnidipine and chlorthalidone, a more recent combination on the market, are helpful in reducing hypertension activity. This mixture was created to enhance the treatment for Stage II hypertension. This Review focuses on recent development in analytical method development for Azelnidipine and Chlorthalidone, and there was no any method reported for this combination. It provides information about different analytical method development like UV spectrophotometry, HPLC, HPTLC,LC-MS methods reported for Azelnidipine and Chlorthalidone for individual and other drug combination.

Keywords: Azelnidipine, chlorthalidone, Analytical Method, UV Spectrophotmetry, HPLC, HPTLC, LC-MS.

INTRODUCTION

Azelnidipine (AZE) is dihydropyridine derivative and chemically 3-[1-(Benzyldrylazetidin-3-yl] 5-isopropyl- 2- amino6methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5dicarboxylate. Azelnidipine category is Dihydropyridine calcium channel blocker. Azelnidipine calcium channel blocker. Azelnidipine prevents trans-membrane Ca2+ influx through the smooth muscle channels in vascular walls that are voltage-dependent. It is a vasodilator that lowers blood pressure gradually in hypertension. When calcium channels are blocked, the vascular smooth muscle does not contract, resulting in relaxation of vascular smooth muscle walls and decreased blood pressure.[1,2]It is employed in the treatment of hypertension, which lowers blood pressure by blocking calcium channels. and oral dose is 8 – 16 mg once daily. It metabolized in hepatic cytochrome P450 (CYP) 3A4 and has no active metabolite product.[3]

Chlorthalidone(CHL) is a diuretic drug used to treat hypertension, Compared with other medications of the thiazide class, chlorthalidone has the longest duration of action but a similar diuretic effect at maximal therapeutic doses. Chlorthalidone of(RS)-2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene- 1-sulfonamide, represents the class of Chlorthalidone is a diuretic drug used to treat hypertension Chlorthalidone has the longest duration of action but a similar diuretic effect at maximal therapeutic doses., used as an Antihypertensive agent.[4-5] Chlorthalidone inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. Chlorthalidone indirectly increases potassium excretion by boosting sodium supply to the distal renal tubule through the sodium-potassium exchange pathway.

PHYSICAL AND CHEMICAL PROPERTY

Azelnidipine is light yellow to yellow crystalline powder. Its chemically 3-[1- (benzyldrylazetidin-3-yl] 5-isopropyl- 2- amino6methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5dicarboxylate. Molecular formula of azelnidipine is $C_{33}H_{34}N_4O_6$. Molecular weight is 582.646 g/mol. It is insoluble in water, slightly soluble in methanol, soluble in ethyl acetate, freely soluble in acetone and in acetic acid.[6]

Chlorthalidone is white powder. It is a type of thiazide diuretic used to treat hypertension. Its chemically (RS)- 2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol1-yl)benzene-1-sulfonamide. Molecular

formula of chlorthalidone is $C_{14}H_{11}ClN_2O_4S$. Water, methanol, alcohol, DMSO, and methanol were all solvents for chlorthalidone. Pka was 9.57 [7].

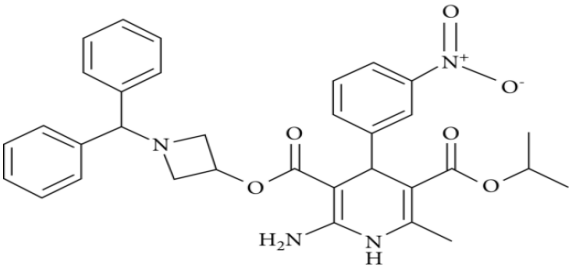

Figure 1.a) chemical structure of azelnidipine

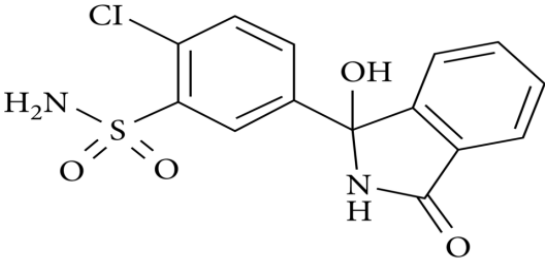

Figure.2 b) chemical structuire of chlorthalidone

ANALYTICAL METHOD DEVELOPMENT

Development and validation of analytical methods play a crucial role in pharmaceutical product assembly as well as medication discovery and advancement. It comprises determining a drug substance's toxicity and purity. Development of analytical methods is the process of choosing an exact assay method to ascertain a formulation's composition. It involves demonstrating that an analytical technique can be used in a lab to determine the concentration of future samples. The procedures and acceptance criteria outlined in the ICH guidelines Q2 must be applied when developing analytical techniques in GMP and GLP environments (R1).

In the discovery, development, and production of pharmaceuticals, analytical method development and validation are crucial processes. The following literature review reveals that no single approach has been described for the combination of azelnidipine and chlorthalidone. But for the quantification of CTL and AZE with other analytes in the formulations, UVspectrophotometric methods [8–14], spectrofluorometric methods [8, 13], HPLC [14–16], and HPTLC [17] methods were used. For the quantification of CTL and AZE separately, stability-indicating RP-HPLC methods [18-22] were also published in the literature. Additionally, CTL and AZE concentrations in biological samples were determined using LCMS techniques [23, 24] both alone and in combination with other medicines. For the simultaneous determination of CTL and AZE in bulk and formulation, no analytical technique has been created.

Table 1: Reported methods for assessment of azelnidipine.

S.NO	TITLE/METHOD	DISCRIPTION	RF.NO
1.	UV spectrophotometric method development and validation for determination of azelnidipine in pharmaceutical dosage form.	Model: Shimadzu 1800 UV Visible spectrophotometer Solvent: Methanol Wavelength (nm): 255nm Linearity: 2 - 14 µg/ ml	[25]
2.	Simultaneous determination of azelnidipine and olmesartanmedoxomil by first derivative spectrophotometric method.	Model: Shimadzu – 1800 UV Visible Spectrophotometer Solvent:Methanol Method: 1.First Derivative Spectrophotometric method Wavelength (nm):AZL - 217nm OLM- 239.4 nm Linearity : 4 - 32 µg/ ml	[26]
3.	Spectrophotometric estimation of azelnidipinein bulk and pharmaceutical dosage form by second order derivative methods	Model: Shimadzu 1800 UVVisible Spectrophotometer Solvent:Methanol Method: 1. Second Derivative Spectrophotometric method Wavelength: 233.8 nm Linearity: 1 - 20 µg / ml	[27]
4.	Method development and validation of azelnidipine by RP-HPLC	Column: C_{18} column (250 mm x 4.5 mm, 5 µm) Mobile Phase: Methanol: Water (75:25) v/v,0.1%glacial aceticacid.	

		Flow rate: 1 mL/min Wavelength: 254nm Linearity: 10 - 50 µg/ml Retention Time: 6.13min	[28]
5	RP-HPLC Method development and validation of azelnidipine	Column: C_{18} column (250 mm x 4.5 mm, 5 µm) Mobile Phase: Methanol: Water (80:20) v/v,Orthophosphoric acid (pH-3) Flow rate:1 mL/min. Wavelength:257 nm Linearity:20-100µg/ml Retention Time: 6.5 min.	[29]
6.	Simultaneous determination of Azelnidipine and two metabolites in Human Plasma using Liquid chromatography-tandem mass spectrometry.	Column: Intersil ODS-3 C_{18} (2.1 mm × 150 mm,5µm) Mobile Phase:Methanol: Water: Acetic Acid (800:200:0.2)v/v Flow rate: 0.2ml/min. Wavelength: 256nm Linearity: 0.5-40 mg/ml Retention Time: AZL–3.6min. M-1(Aeromatizedform)-10.2min. M-2(Hydroxylated Form)-6.8min.	[30]
7.	Simultaneous determination of azelnidipine and olmesartanmedoxomil in pharmaceutical dosage forms by UFLC method.	Column : ODS (250mm x 4.6mm, 5µm) Mobile Phase: Methanol : Water (85:15) v/v Flow Rate: 1.5ml/min. Wavelength: 255nm Linearity: 2-16 mg/ml Retention Time: AZL - 6.80 min. OLM -1.72 min.	[31]
8.	Stability indicating analytical method development and validation for estimation of azelnidipine.	UV Spectrophotometric method: Solvent : Methanol: Water (80:20) v/v Methods: Method 1- Zero order Spectrophotometric method Method 2 - First order Derivative Spectrophotometric method Wavelength: Method 1 -257 nm Method 2- 242.6 nm Linearity: 2-10 µg/ml Method 3 – RP HPLC Method Column: ODS C_{18} (250mm×4.6mm.,5µm) Mobile phase: Sodium diabasic Phosphate Buffer: Acetonitrile: Methanol (10:50:40)v/v/v, orthophosphoric acid (pH - 4.5) Flow rate: 1mL/min Wavelength: Method 3 -256nm Linearity: 2-12 µg/ml Retention Time : 6.1 min.	[32]
9.	Validated stability-indicating RP-HPLC method for the simultaneous determination of azelnidipineand olmesartanin their combined dosage form.	Column: Hypersil GOLD C_{18} (150 mm × 4.6mm, 5 µm) Mobile Phase: Methanol :Acetonitrile :Water(40:40:20)v/v/v Flow rate :0.5mL/min Wavelength :260 nm Linearity : AZL – 2 - 48 µg/ml OLM- 2.5 - 60 µg/ml Retention Time: AZL -8.56min. OLM - 3.04 min	[33]
10.	Validation and forced stability-indicating HPTLC method for determination of azelnidipine.	Stationary Phase: Silica gel 60 F_{254} (20cm × 10cm , 0.2mm) Mobile Phase: Chloroform: Ethyl acetate: methanol 6.5:3.5: 0.1 (v/v/v) Wavelength : 255nm Linearity:300-800ng/band Rf Value :0.59,0.60	[34]
11.	Sensitive analysis of azelnidipine and related derivative in human plasma by Ultra-Performance Liquid Chromatography-tandam mass spectrometry.	Column : C_{18} (50 mm × 2.1 mm.,1.7 µm) Mobile Phase: A (20 mM Ammonium acetate aqueous solution) B (0.1 % formic acid in Acetonitrile) Flow Rate: 0.5 mL/min Linearity: 0.01-10 mg/ml Retention Time: AZL -1.38 min. IS -1.26 min.	[35]

Table 2: Reported methods for assessment of chlorthalidone.

S.NO	TITLE/METHOD	DISCRIPTION	RF.NO
1.	Method development and validation for the simultaneous estimation of azilsartan and chlorthalidone by RP-HPLC in pharmaceutical dosage form	Column : ODS (250mm: 4.6mm, 5µ) Mobile phase : 0.1% Ortho phosphoric acid buffer and acetonitrile in the ratio of (30:70) v/v Flow rate : 1ml/min Wavelength : 230nm Linearity : y = 20261x + 2072 and y = 13573x + 1593 Retention time :Chlorthalidone and Azilsartan were eluted at 2.266min and 4.568min	[36]
2.	Method development and validation of irbesartanchlorthalidone and cilnidipine in their combined tablet dosage form by high performance liquid chromatography	Column :C18 column (250 x 4.6mm, 5Î¼m) Mobile phase : Buffer, Acetonitrile and TEA in a proportion of 80:20:0.1 %v/v/v Flow rate : 1.0 ml/min Wavelength : 222 nm Linearity : 30-90 Î¼g/ml, 1.25-3.75 Î¼g/ml and 1-3 Î¼g/ml for IrbesartanChlorthalidone and cilnidipine Retention time :IrbesartanChlorthalidone and cilnidipine were 3.807 min, 4.667 min, and 6.887 min	[37]
3.	Rp-hplc method for simultataneous determination of irbesartan, losartan, hydrochlorothiazide and chlorthalidone–application to commercially available drug products	Column :Hypersil BDS (Length 250 mm × Diameter 4.6 mm Particle size 5 µm) Mobile phase :0.05 M sodium dihydrogen phosphate buffer and acetonitrile (Gradient ratio) Flow rate : 1.0 mL min−1 Wavelength : 220 nm. Linearity : 10 –150 µg mL−1 for all Hydrochlorothiazide, Chlorthalidone, Irbesartan and Losartan	[38]
4.	Development and validation of an UV spectrophotometric method for simultaneous determination of cilnidipine and chlorthalidone	LOD : 0.4174 µg/mL and 0.068 µg/ml LOQ : 1.264 µg/ml and 0.206 µg/ml Wavelength selection : Cilnidipine are 271.83 nm and 278.34 nm and Chlorthalidone are 233.83 nm and 250.0 nm Lineariry: f 2-10mg/mL (r2=0.9990) for Cilnidipine and 2.5 - 12.5mg/mL (r2 = 0.9986) for Chlorthalidone Solvent : methanol	[39]
5.	Method development and validation for simultaneous estimation of telmisartan and chlorthalidone by RP-HPLC in pharmaceutical dosage form	Column: CAPCELL C18 (250mm x 4.6mm id ,5 µm) Mobile phase : potassium di hydrogen ortho phosphate buffer: acetonitrile : methanol (35: 45: 20) % v/v/v Flowh rate : 0.8ml/min Lineariry : 20-100 µg/ml and 6.25-31.25 µg/ml for telmisartan and chlorthalidone Retention time : 3.640min and 4.937min for chlorthalidone and telmisartan	[40]
6.	Stability-indicating RP-HPLC method for the simultaneous estimation of azilsartanmedoxomil and chlorthalidone in solid dosage forms	Column: BDS C18 column (100 x 4.6 mm, 5µ, Hypersil) Mobile phase : Phosphate Buffer and Acetonitrile (90:10)% v/v Flowh rate : 0.9 ml/min Wavelength : 260nm Lineariry:AzilsartanMedoxomil and Chlorthalidone was in the range of 10.0 to 60.0µg/ml and 6.25 to 37.5µg/ml Retention time :AzilsartanMedoxomil and Chlorthalidone was 2.36±0.1 mins and 5.54±0.5 mins	[41]
7.	RP-HPLC method development and validation for simultaneous estimation of cilnidipine, atenolol and chlorthalidone	Column:Hypersil-keystone C18(4.6 x 250mm, 5µm) Mobile phase : methanol and triple distilled water (80/20, v/v) having pH 7 Flowh rate : 1.0 mL/min Wavelength : 225 nm Lineariry :10-50 µg/ml for CDP, 10-50 µg/ml, for ATL and 6-36 µg/ml for CTD Retention time : CDP (Rt: 3.25 min), ATL (Rt: 5.366 min) and CTD (Rt: 9.025 min)	[42]

8.	Novel NP and RP-HPTLC in praxis for simultaneous estimation of chlorthalidone and cilnidipine in bulk and pharmaceutical formulation	Stationary phase: silica gel F254 TLC plate Mobile phase : NP-HPTLC -toluene: ethyl acetate: methanol 3.2:1.3:0.5 (v/v/v), RP-HPTLC - methanol: water 3.2:1.8 (v/v) Wavelength :275 nm Lineariry : 250-1500 ng/band for CHL and 200-1200 ng/band for CIL both methods Retention factor :CHL and CIL at retention factor (Rf) of 0.34 ± 0.02 and 0.79 ± 0.02 for NP-HPTLC and 0.24 ± 0.02 and 0.81 ± 0.02 RP-HPTLC	[43]
9.	HPTLC-densitometric method for simultaneous estimation of olmesartanmedoxomil and chlorthalidone in tablet dosage form	Stationary phase : silica gel 60 F254 Mobile phase : Toluene: Ethyl acetate: Methanol: Glacial acetic acid (5:4.7:0.3:0.1 % v/v/v/v) Wavelength : 238 nm Linearity : 100-1200 ng/band for Olmesartanmedoxomil and 62.5-750 ng/band for Chlorthalidone	[44]
10.	Estimation of telmisartan, amlodipine and chlorthalidone in bulk and fixed dose combination using stability indicating high performance thin layer chromatography	Stationary phase : silica gel 60 F254 of size (20 cm × 10 cm) Mobile phase : Chloroform: Toluene: Methanol: Glacial Acetic Acid (6:2:2:0.1 % V/V/V/V) Wavelength : 254 nm Linearity : 400-4800 ng/band for TEL, 50-600 ng/band for AML and 125-1500 ng/band for CHL Retention factor : 0.64 ± 0.008, 0.25 ± 0.008 and 0.48 ± 0.01 for TEL, AML and CHL	[45]

CONCLUSION

This article gives an idea about improved activity of azelnidipine and chlorthalidone from other drugs. The presented review provides information about the various methods available in the literature for the determination of azelndipine and chlorthalidone. The different analytical methods are reported for the individual and other combination like UV Spectrophotometry, HPLC, LC-MS, HPTLC. This article also present with Pharmacological action, chemical structure, solubility of azelnidipine and chlorthalidone. The given literature review focus that there is not a single method reported for azelnidipine and chlorthlidone combination. This article also suggest that reported methods for azelnidipine and chlorthalidone for individual and other combinations. This review will help in future to develop the analytical method for this new combination and also gives the knowledge about its characteristics of both drugs.

REFERENCE

[1] Goodman and Gilman's. The pharmacological basis of therapeutics; 10th Edn; Medical publishing division, 2001;1804.

[2] Tripathi KD. Essentials of medical pharmacology; 6th ed., New Delhi, Jaypee Brothers Medical Publishers Ltd.,271-275.

[3] Drug Profile, "Azelnidipine", December 2020.http://www.drugbank.ca/drugs/DB09230

[4] "Drug profile for Chlorthalidone", Sep2015 ,http://www.drugbank.ca/drugs/DB00310

[5] "DrugprofileforChlorthalidone",Sep2015,http://en.wikipedia.org/wiki/Chlorthalidone.

[6] The Indian Pharmacopoeia, Government of India, Ministry of Health and Family welfare; 7th ed., The Indian pharmacopeia commission, Ghaziabad, 2018; II: 1304-1305, 3319-3320.

[7] Niraimathi V et al. "Uv Spectrophotometric methods for the estimation of chlorthalidone in bulk and oral dosage form"., Indo American J Pharm Res., (2013); 3(9): 7160-7167.

[8] Ebeid W. M et al. "Spectrophotometric and spectrofluorimetric studies on azilsartanmedoxomil and chlorthalidone to be utilized in their determination in pharmaceuticals.," Analytical Chemistry Insights., 2014; 7(9): 33–40.

[9] Abdullah N. S et al."Spectrophotometric determination of chlorthalidone in pharmaceutical formulations using different order derivative methods.," Arabian Journal of Chemistry., 2017; 10(10): 426–433.

[10] Darwish H. W et al."Full spectrum and genetic algorithm-selected spectrum-based chemometric methods for simultaneous determination of azilsartanmedoxomil, chlorthalidone, and

azilsartan: development, validation, and application on commercial dosage form.," Open Chemistry., 2021; 19(1): 205–213.

[11] Yuvasri S et al."First-order derivative and UV-spectrophotometric methods for simultaneous determination of telmisartan and azelnidipine in bulk and tablet dosage form.,"European Journal of Biomedical and Pharmaceutical Sciences., 2021; 8(5): 290–294.

[12] NeupaneN. P et al. "Analytical method development and validation of azelnidipine by UV-visible spectroscopy," World Journal of Pharmaceutical Research, 2021; 10(6): 858–872.

[13] Elsonbaty M. A et al. "Synchronous spectrofluorimetry coupled with third-order derivative signal processing for the simultaneous quantitation of telmisartan and chlorthalidone drug combination in human plasma," Journal of Fluorescence, 2021; 31(1): 97–106.

[14] . Hinge M. A et al. "Spectrophotometric and high-performance liquid chromatographic determination of chlorthalidone and losartan potassium in combined dosage form," Analytical Chemistry Letters, 2016; 6(4): 408–420.

[15] . Kharat C et al. "A validated RP-HPLC stability method for the estimation of chlorthalidone and its process-related impurities in an API and tablet formulation," International Journal of Analytical Chemistry, 2020; 20(8): 11.

[16] Joglekar P., "Method development and validation for the estimation of telmisartan and chlorthalidone in bulk and pharmaceutical dosage form by HPTLC method," Research Journal of Pharmacy and Technology, 2015; 8(4): 376–381.

[17] ChaudharyR. and. Dave J. B, "Estimation of telmisartan, amlodipine and chlorthalidone in bulk and fixed dose combination using stability indicating high performance thin layer chromatography," Indo Global Journal of Pharmaceutical Sciences, 2020; 10(3): 06–20.

[18] Sonawane S et al. "Development and validation of stability-indicating method for estimation of chlorthalidone in bulk and tablets with the use of experimental design in forced degradation experiments," Scientific, 2016; 16(8): 9.

[19] Sonawane S. S, Bankar P. C, and Kshirsagar S. J., "Stability-indicating LC method for quantification of azelnidipine: synthesis and characterization of oxidative degradation product," Turkish Journal of Pharmaceutical Science, 2021; 18(5): 550–556.

[20] Patel J. K. and. Patel N. K, "Validated stability-indicating RP-HPLC method for the simultaneous determination of azelnidipine and olmesartan in their combined dosage form," Scientia Pharmaceutica, 2014; 82(3): 541–554.

[21] Kumar M. U., Garg C. A., and Gupta P., "A stability indicating RP-HPLC method validation for simultaneous estimation of azelnidipine and telmisartan in a fixed-dose combination," International Journal of Pharmaceutical Sciences and Drug Research, 2021; 13(3): 288–294.

[22] Peddi P., Tulasi S. L., Usha Rani N., and. Rajeswari T. R, "A validated stability indicating Rp-Hplc method for determination of azelnidipine and its impurities in pharmaceutical formulation," Indian Drugs, 2020; 57(8): 70–76.

[23] Vekariya,.Pandya S, Pethani T., and Vadia N., "A novel liquid chromatography-tandem mass spectrometry method for simultaneous quantification of telmisartan and chlorthalidone in rat plasma and its application to a pharmacokinetic study," Analytical Chemistry Letters, 2021;11(5): 741–755.

[24] UrasakiY et al., "Enantioselective determination of azelnidipine in human plasma using liquid chromatography-tandem mass spectrometry," Journal of Chromatography B, 2007; 852(2): 389–397.

[25] Raskapur KD., PatelM., Captain AD, "UV-Spectrophotometric method development and Validation for Determination of Azelnidipine in Pharmaceutical Dosage Form.," Int. J. Pharm. Pharm. Sci., 2012; 4(1): 238-240.

[26] Patel N., Patel J., et al., ":Simultaneous Determination of Azelnidipine and Olmesartanmedoxomil by First Derivative Spectrophotometric Method.," Der Pharm. Lettre., 2012; 4(4):1080-1084.

[27] Rele RV. et al. "Spectrophotometric estimation of Azelnidipine bulk and pharmaceutical dosage form by second order derivative methods.," J. of Chem. and Pharma. Res., 2014; 6(8):198-202.

[28] Prabhakar D., Sreekanth J., JayaveeraKN., et al. "Method Development and Validation of Azelnidipineby RP-HPLC.," Int. J. of ChemTech. Research, 2017; 10(10):418-423.

[29] Gore M., Dabhade PS. RP-HPLC Method Development and Validation of Azelnidipine. Int. J. of Pharma. Sci. and Research, 2016; 7(12): 5111-5114.
[30] Kawabata K., Urasaki Y. "Simultaneous determination of Azelnidipine and two metabolites in human plasma using liquid chromatography-tandem mass spectrometry.,"J. Chromatogr. B., 2006; 4(1):45-52.
[31] Amin A., Saad M., Amin et al." Simultaneous Determination of Azelnidipine and OlmesartanMedoxomil in Pharmaceutical Dosage Forms by UFLC Method.," J. of PharmaSciTech., 2016; 6(2): 69-74.
[32] Modi J., Patel SK., ParikhN.,ShahS., PradhanP., Upadhyay U., "et al.Stability Indicating Analytical Method Development and Validation For Estimation Of Azelnidipine.,"World J. of Pharma. Res., 2016; 5(2):831-847.
[33] Patel J., Patel N. "Validated Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Azelnidipine and Olmesartan in Their Combined Dosage Form.," Sci. Pharm., 2014; 4(1):541–554.
[34] Rane A., Mahajan S., et al. "Validation and Forced Stability-Indicating HPTLC Method For Determination of Azelnidipine.," World J. of Pharma. Res., 2016; 5(9):1053-1062.
[35] Suneethaet al. "Sensitive Analysis of Azelnidipine and Related Derivative in Human Plasma by Ultra-Performance Liquid Chromatography-Tandam Mass Spectrometry.," Asian J. of Chemistry, 2013; 15(18):10319-10321.
[36] Sravani P et al."Method Development and Validation for the Simultaneous Estimation of Azilsartan and Chlorthalidone by RP-HPLC in Pharmaceutical Dosage Form.," International Journal of Pharma Sciences., 2014; 4(5): 725-729.
[37] Rishabh K Dagariya "method development and validation of irbesartanchlorthalidone and cilnidipine in their combined tablet dosage form by high performance liquid chromatography.," Journal of Drug Delivery and Therapeutics. 2017; 7(4): 2250-1177
[38] Mhaske R. A et al. "RP-HPLC method for simultataneous determination of irbesartan, losartan, hydrochlorothiazide and chlorthalidone–application to commercially available drug products.," international journal of pharmaceutical sciences and research.2012; Vol. 3(4): 1116-1123.
[39] SnehalN. Patelet al." Development and validation of an UV spectrophotometric method for simultaneous determination of cilnidipine and chlorthalidone" Journal of Pharmacy Research. 2015,9(1),41-45
[40] Vinaja et al." method development and validation for simultaneous estimation of telmisartan and chlorthalidone by RP-HPLC in pharmaceutical dosage form" asian journal of pharmaceutical analysis, 2015; 5(4): 171-177.
[41] Naazneen1 S., Sridevi A." stability-indicating RP-HPLC method for the simultaneous estimation of azilsartanmedoxomil and chlorthalidone in solid dosage forms" International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(6): 6-11
[42] Vishal Singh Solanki et al." RP-HPLC method development and validation for simultaneous estimation of Cilnidipine, Atenolol and Chlorthalidone" Journal of Drug Delivery and Therapeutics. 2018; 8(6):78-82
[43] Ravsaheb H. Rathod et al." Novel NP and RP-HPTLC in Praxis for Simultaneous Estimation of Chlorthalidone and Cilnidipine in Bulk and Pharmaceutical Formulation" analytical chemistry letters.2018; 8(6) : 862-871
[44] Amitkumar J." HPTLC-Densitometric Method for Simultaneous Estimation of Olmesartanmedoxomil and Chlorthalidone in Tablet Dosage Form" analytical chemistry letters.2020; 10(4): 498-506
[45] Bhamini R. Chaudhary ,Jayant. B. Dave ." Estimation of Telmisartan, Amlodipine and Chlorthalidone in Bulk and Fixed Dose Combination Using Stability Indicating High Performance Thin Layer Chromatography" Indo Global Journal of Pharmaceutical Sciences, 2020; 10(3): 6-20