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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF HYDROCHLOROTHIAZIDE AND AMLODIPINE BESYLATE IN BULK AND TABLET DOSAGE FORM BY RP-HPLC TECHNIQUE

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ABSTRACT

A simple, rapid, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous analysis of Hydrochlorothiazide (HTZ) and Amlodipine besylate (AML) in a tablet dosage form has been developed and validated. This method was performed with a thermosil C_{18} (4.6 × 100 mm i.d., 3.7 µm particle column with 35:65 (v/v) 20mM potassium dihydrogen orthophosphate buffer : methanol as mobile phase at a flow rate of 1.0 ml/min. UV detection at 248 nm; HTZ and AML were eluted with retention times of 1.823 and 2.639min, respectively. The method was continued and validated accordance with ICH guidelines. Validation revealed the method is rapid, specific, accurate, precise, reliable, and reproducible. Calibration curve plots were linear over the concentration ranges 6.25-100µg/mL for HTZ and 2.5-40µg/mL for AML. Limits of detection (LOD) were 0.004 and 0.0016µg/ml and limits of quantification (LOQ) were 0.013 and 0.0052µg/mL for HTZ and AML respectively. Statistical analysis was proves the method is suitable for the analysis of HTZ and AML as a bulk, in tablet dosage form without any interference from the excipients. It may be extended for its estimation in plasma and other biological fluids.

Keywords: *Hydrochlorothiazide (HTZ) and Amlodipine besylate (AML), RP-HPLC, Validation.*

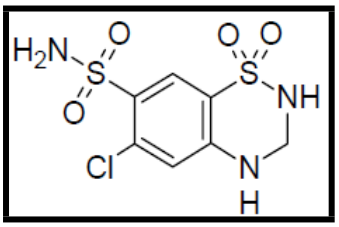
INTRODUCTION

Hydrochlorothiazide (HTZ) chemically 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide (**Fig. 1**). It is a diuretic drug and derivative of the thiazide class [1].

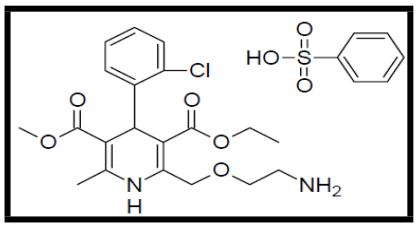
Amlodipine (AML) chemically (RS)-3-ethyl-5-methyle-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (**Fig. 1**). It is a long-acting dihydropyridine-type (DHP) of calcium channel blocker [2]. Amlodipine is also acts as a functional inhibitor of acid sphingomyelinase [3].

In the scientific literature, analysis of HTZ and AML has been reported as individual ingredients and in combination with other compounds. Analytical methods have included estimation of HTZ [4], AML [5] individually. And in two component formulations of HTZ and AML have been analyzed in combination [6, 7]. Simultaneous HPLC analysis of HTZ with other drug [8] and in combinations of HTZ, AML with other drugs analyzed in HPLC [9-15].

No other chromatographic methods are found for simultaneous analysis of HTZ and AML in a combined dosage form. The method described is rapid, economical, precise, and accurate and can be used for routine analysis of tablets. It was validated as per ICH guidelines [16-18].



HTZ



AML

Fig. 1: Chemical structures of Hydrochlorothiazide (HTZ) and Amlodipine besylate (AML)

MATERIALS AND METHODS

1. Experimental:

1.1. Materials and Methods:

Pharmaceutical grade working standards Hydrochlorothiazide (HTZ) and Amlodipine besylate (AML) were obtained from Hetero Labs, Jedcharla, India. All chemicals and reagents were HPLC grade and were purchased from Merck Chemicals, Mumbai, India.

1.2. Instrumentation:

The analysis was performed using Waters-2695 (Modal Alliance) High Performance liquid chromatography, analytical balance (Mettler Toledo), PDA Detector (Standard cell) and data handling system (Empower 2), pH meter (lab India), Sonicator. The column used is Thermosil C_{18} (100×4.6mm, packed with 3.7µm) with the flow rate 1.0ml/min (isocratic).

1.3. Preparation of stock solution:

Accurately weighed 10 mg of HTZ and AML working standard and separately transferred into a 10ml clean dry volumetric flasks, add about 7ml of diluent to each volumetric flask

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and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Calibration standards at five levels were prepared by appropriately mixed and further diluted stock standard solutions in the concentration ranges from 6.25-100µg/ml for HTZ and 2.5-40µg/ml for AML. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the Linearity graphs.

1.4. Sample Preparation:

For the analysis of a tablet dosage form, 20 tablets were weighed individually and their average mass was determined. Then, the tablets were crushed to a fine powder. The powder equivalent to 12.5mg of HTZ and 5mg of AML were transferred to a 10 mL volumetric flask and dissolved in 10mL of diluent, sonication was done for 15 min with swirling. After sonication, the solution was filtered through a membrane filter paper (#0.45µ). From the above stock solution 0.2mL was transferred in to 10mL volumetric flask and made volume upto the mark with diluent, the final concentrations were 25µg/ml and 10µg/ml of HTZ and AML respectively, then injected into the chromatographic system, and analyzed quantitatively. The analysis was repeated six times and the possibility of excipient interference with the analysis was examined.

1.5. Optimization of HPLC Method:

The HPLC method was optimized and developed with a simultaneous assay method for HTZ and AML respectively. The mixed standard stock solution (25µg/mL of HTZ and 10µg/mL of AML) injected in HPLC. Different ratios of methanol and potassium dihydrogen orthophosphate buffer at different pH and molarities were tried.

1.6. Method validation:

The method validation was done according to the ICH guidelines. The following validation characteristic parameters are accuracy, precision, linearity, and specificity, LOD, LOQ and robustness.

1.6.1. Linearity and range:

Linearity of the method was studied by the injecting the mixed standard solutions with the concentration ranges from 6.25-100µg/ml for HTZ and 2.5-40µg/ml for AML levels of target concentrations were prepared and injected six times into the HPLC system keeping the constant injection volume. The peak areas were plotted against the concentrations to obtain the linearity graphs.

1.6.2. Precision:

The precision of the optimized method was evaluated by carrying out six independent assays of test sample. %RSD of six assay values was calculated. Intermediate precision was carried out the samples by using another instrument and with different analyst.

1.6.3. Limit of Detection and Quantification:

The LOD and LOQ procedures were performed on samples contain very lower concentrations of analytes under the ICH guidelines. By applying the visual evaluation method, LOD was expressed by establishing the lowest concentration at which the analyte can be detected. LOQ was considered as the lowest concentration of analytes that can be detected and quantified, with acceptable accuracy and precision.

1.6.4. Robustness:

Robustness was studied by evaluating the effect of small variations in the chromatographic conditions. The conditions studied were flow rate altered by ±0.1ml/min, mobile phase composition with methanol ±5ml. These chromatographic variations are evaluated for resolution between HTZ and AML.

1.6.5. System suitability:

The system suitability parameters with respect of tailing factor, theoretical plates, repeatability and resolution between HTZ and AML peaks were defined.

1.6.6. Specificity:

The specificity of the analytical method is the ability of the method to estimate the analyte response in the presence of additional components such as impurities, degradation products and matrix [19]. The peak purity of HTZ and AML were assessed by comparing the Retention time of standard HTZ and AML good correlation was obtained between the Retention time of standard and sample of HTZ and AML.

The specificity method was also evaluated to ensure that there were no interference products resulting from forced degradation studies.

1.6.6.1. Forced degradation study:

Forced degradation or Stress testing of a drug substance will help to identify the degradation products, which can help to establish the intrinsic stability of the molecule.

All stress decomposition studies were performed at an initial drug concentration 25µg/mL of HTZ and 10µg/mL of AML. The degradation conditions are selected on the basis of literature survey [20-24].

The Stability indicating study of HTZ and AML were undergoes acid, alkali and oxidation degradation, photolysis and heat condition.

Placebo Interference: The placebo (in the present of excipients in tablet) sample were prepared as per the test method and analyzed in the HPLC. It expressed there is no additional peaks at the retention time of HTZ and AML in the chromatograph it indicates that there is no placebo interference.

Acid Degradation: Sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume upto the mark with diluent and analyzed using HPLC.

Alkali Degradation: Sample was treated with 3ml of 1N sodium hydroxide and kept for 10hr. After 10hr the solution was neutralized with 3ml of 1N hydrochloric acid, made the volume upto the mark with diluent and analyzed using HPLC.

Oxidative Degradation: HTZ and AML solutions of 25 and 10µg/ml were mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume upto the mark with diluent and analyzed using HPLC.

Photolytic Degradation: The **s**amples were kept under UV light for different time intervals (15mins – 7days) and made the volume upto the mark with diluent and analyzed using HPLC.

Thermal Degradation: Samples were heated at 80^0 C for 15mins - 60mins and 220^0 C for 2-5mins and analyzed.

1.6.7. Accuracy:

Accuracy was carried out by applying the method to drug sample (HTZ and AML combination of tablets) to which known amounts of HTZ and AML standard powder corresponding to 50, 100 and 150% of label claim was added, mixed and the powder was extracted and determined by the system in optimized mobile phase. The experiment was performed in triplicate and percentage recovery, % RSD was calculated.

1.6.8. Analysis of marketed formulation:

The marketed formulation was assayed by above description. The peak areas were monitored at 248nm, and determination of sample concentrations were using by multilevel calibration developed on the same HPLC system under the same conditions using linear regression analyzed for HTZ and AML in the same way as described above.

RESULTS

The simultaneous estimation of HTZ and AML was done by RP-HPLC and in the optimized method the mobile phase consists of buffer (350 volumes of phosphate buffer and 650 volumes of Methanol and the pH was adjusted to be 2.8. Then finally filtered using 0.45µ membrane filter paper and degassed in sonicator for 15 minutes. The detection is carried out using PDA detector at 248nm. The solutions are following at the constant flow rate of 1.0 ml/min.

The retention time for HTZ and AML was 1.823 and 2.639minutes respectively. Linearity ranges for HTZ and AML were 6.25-100µg/mL and 2.5-40µg/mL respectively and the results were found for in the acceptable as (R^2) = 0.999 for HTZ and AML also. LOD were 0.004 and 0.0016µg/ml and LOQ were 0.013 and 0.0052µg/mL for HTZ and AML respectively. The all parameters value of RSD is less than 2.0% indicating the accuracy and precision

of the method. The percentage recoveries were found 99.93-100.52% and 99.6-100.8% for HTZ and AML respectively.

DISCUSSION

1. Method Development and Optimization:

The HPLC procedure was optimized with a view to develop a suitable LC method for the analysis of HTZ and AML in fixed dose for bulk and combined dosage form. It was found that 35:65 v/v (20mM) potassium dihydrogen orthophosphate buffer: methanol gave acceptable retention time (1.823min for HTZ and 2.639min for AML), plates, and good resolution for HTZ and AML at the flow rate of 1.0ml/min (**Table. 1**; **Fig. 2 & 3**).

Table No. 1: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (column)	Thermosil C_{18} (100×4.6mm, packed with 3.7µm)
Mobile Phase	40:60v/v, (0.02M Phosphate Buffer : Methanol)
pH	2.8 ± 0.02
Flow rate (ml/min)	1.0
Run time (minutes)	8.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	15
Detection wavelength (nm)	248
Drugs RT (min)	1.823 & 2.639

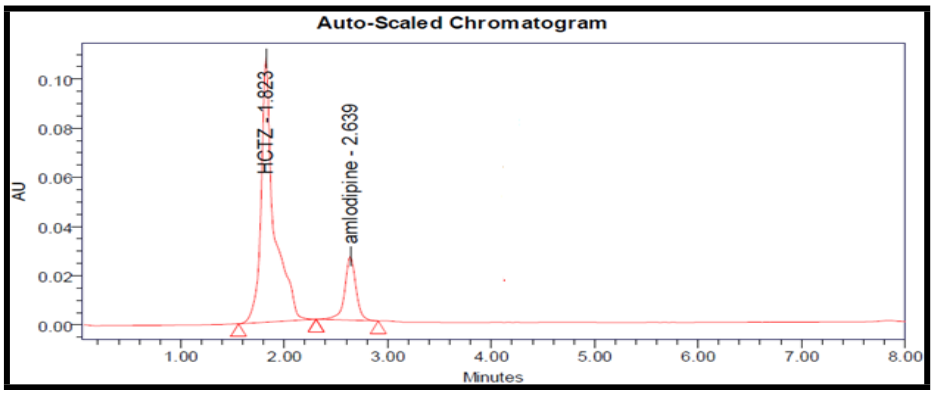

Fig. 2: Chromatogram of HTZ and AML at 248nm from bulk drug

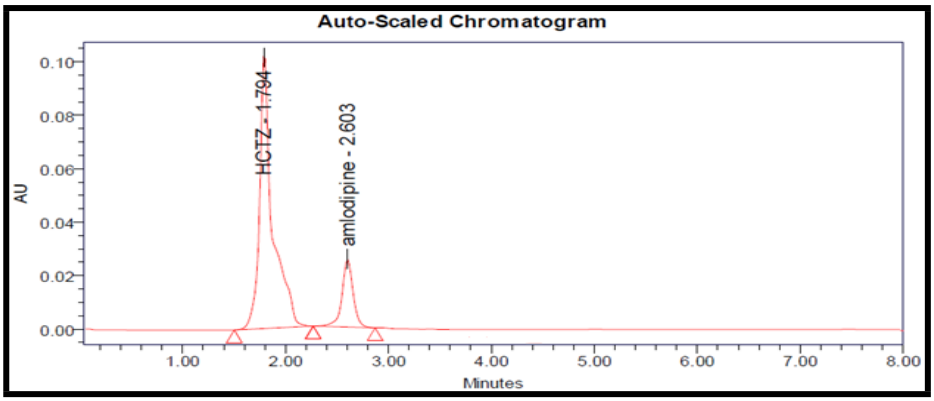

Fig. 3: Chromatogram of HTZ, and AML at 248nm from pharmaceutical formulation

2. Validation of Developed method:

2.1. Linearity:

Linearity was evaluated by analysis of working standard solutions of HTZ and AML of five different concentrations. The range of linearity ranges from 6.25-100µg/ml for HTZ and 2.5-40µg/ml for AML for TLM (**Table. 2**). The result of correlation coefficients of HTZ and AML (R^2) = 0.9993 and 0.9991 respectively (**Fig. 4-6**). There was an excellent correlation between peak areas and concentrations of each drug.

Table 2: Linearity Data for HTZ and AML

Analyte	Concentration range (µg/mL)	Correlation Coefficient (R^2)	Slope	Intercept
HTZ	6.25-100	0.9993	37791x	59447
AML	2.5-40	0.9991	13125x	76149

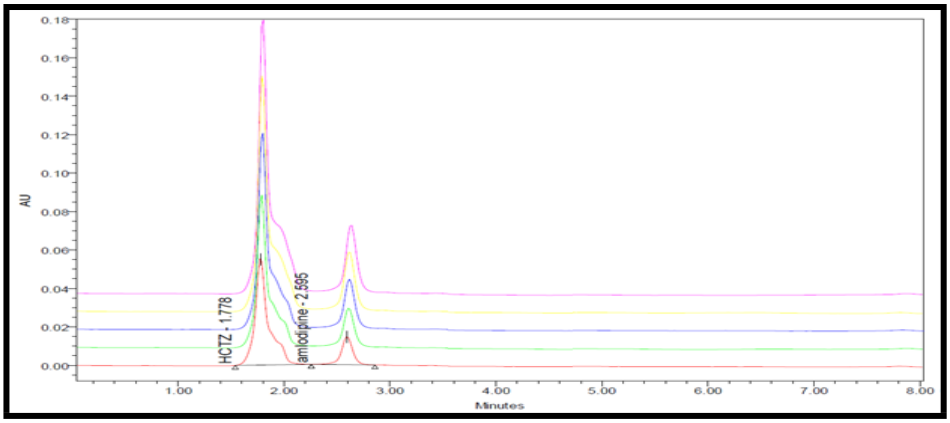

Fig. 4: Overlay linearity Chromatogram for HTZ and AML

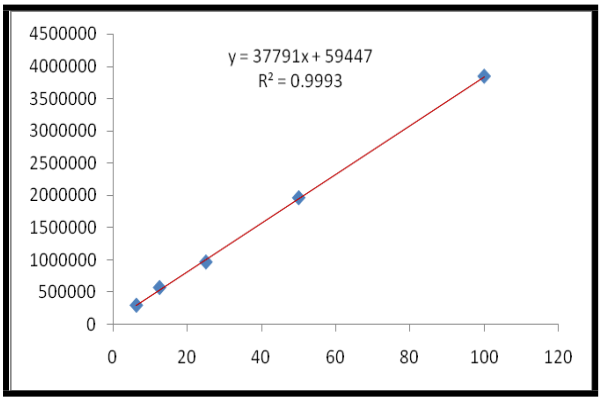

Fig. 5: Linearity Curve of Standard Hydrochlorothiazide (HTZ)

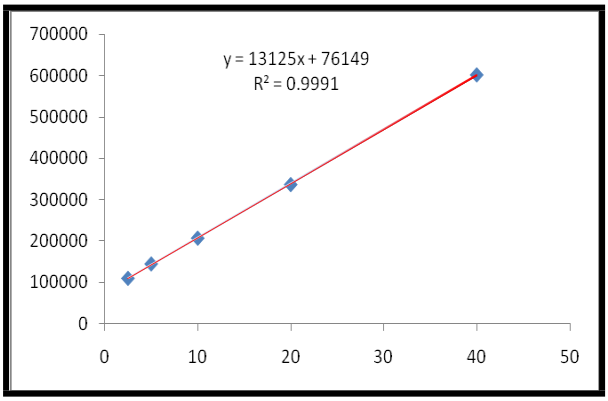

Fig. 6: Linearity Curve of Standard Amlodipine besylate (AML)

2.2. Precision:

The results of precision method were evaluated by carrying out six independent test samples of HTZ and AML. The percentage of RSD of six sample peak area values was calculated. Different analyst from the same laboratory conditions analyzed the intermediate precision for the optimized method. The RSD values of intra-day and inter-day studies for HTZ and AML confirming good precision of the optimized method (**Table. 3**).

Table No. 3: Intra-day and inter-day Precision results of HTZ and AML from tablets

No. of Preparation	HTZ		AML	
	Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision
Pre-1	901825	897825	184724	189714
Pre-2	906134	895724	185617	187193
Pre-3	907721	890162	182816	188167
Pre-4	902835	891826	186272	189782
Pre-5	910026	889017	184726	186825
Mean	905708.2	892910.8	184831	188336.2
St. dev.	3398.9184	3740.2482	1301.6312	1379.1238
% RSD	0.3752	0.4188	0.7042	0.7322

2.3. LOD and LOQ:

The LOD and LOQ values were found to be 0.004 and 0.013µg/mL for HTZ and 0.0016 and 0.0052µg/mL for AML (**Table. 4**).

2.4. Specificity

Injected the extracted solutions commonly used excipients were performed to demonstrate for the absence of interaction with the drugs. These results are expressed that there was no interference from the other excipients in the tablet formulation; therefore, confirm the method was specific.

2.5. System suitability

System suitability parameters such as the theoretical plates count, resolution, % RSD and peak tailing factors are determined (**Table. 4**).

Table No. 4: System suitability parameters for HTZ and AML

System suitability parameters	HTZ	AML
Retention time (min)	1.823	2.639
Repeatability of retention time; %R.S.D (n=5)	0.163793	0.293152
Repeatability of peak area; %R.S.D= (S.D./Mean)×100	0.57259161	0.482787475
Resolution (Rs)	-	4.53
Tailing factor (asymmetric factor)	1.45	0.96
USP plate count	7130	9456
LOD (µg/mL)	0.004	0.0016
LOQ (µg/mL)	0.013	0.0052

2.6. Robustness:

To ensure the insensitivity of the optimized RP-HPLC method to small alteration in the experimental conditions. The conditions studied were flow rate altered by ±0.1ml/min, mobile phase composition with methanol ±5ml. These chromatographic variations are evaluated for resolution between HTZ and AML (**Table. 5**).

Table No. 5: Robustness study for analytical method validation of HTZ and AML tablets

Parameters		Adjusted to	Mean Area [a]	Mean RT	SD	% RSD
HTZ	Flow Rate ±0.1ml/min	0.9 ml/min	1016052.2	2.03	1746.39	0.17
		1.1ml/min	798422.00	1.63	1139.00	0.14
	Mobile Phase (40:60) (±5ml)	35:65	898142.67	1.63	1309.34	0.15
		45:55	986892.83	1.80	1927.57	0.20
AML	Flow Rate ±0.1ml/min	0.9 ml/min	216963.17	2.91	964.66	0.44
		1.1ml/min	168430.5	2.32	1277.65	0.76
	Mobile Phase (40:60) (±5ml)	35:65	187967.83	2.32	1328.81	0.71
		45:55	198902.67	3.11	619.49	0.31

[a] = 5 *Replicates*

2.7. Solution stability studies:

Three different concentrations of HTZ (25µg/mL) and AML (10µg/mL) were prepared from the sample solution and stored at room temperature for 24 hrs. Then injected into the HPLC system and the additional peaks were not found in the chromatograms so, it was indicating the stability of HTZ and AML tablet in the solution (**Table. 6**).

2.8. Recovery studies:

Good recoveries of the HTZ and AML were obtained at different added concentrations for the tablets (**Table. 7**).

Table No. 6: Solution stability study for analytical method validation of HTZ and AML tablets

Name	*Replicate (n = 5)*	*Initial*	*After 3 hrs*	*After 6 hrs*	*After 12 hrs*	*After 24 hrs*
HTZ	Mean	896022.8	894439.4	893842	892867.6	889681.6
	SD	5069.394	3945.409	3430.281	3568.615	3792.128
	% RSD	0.565766	0.441104	0.383768	0.39968	0.426234
AML	Mean	188451.6	188439.4	188234.2	188052.8	187667.2
	SD	457.8638	422.4941	351.0131	399.2207	407.4907
	% RSD	0.242961	0.224207	0.186477	0.212292	0.217135

Tablet No. 7: Accuracy Results of HTZ and AML from tablets

Analyte	*Levels (%)*	*Actual Conc. (µg/mL)*	*Added Conc. (µg/mL)*	*Theoretical Conc. (µg/mL)*	*Found Conc. (µg/mL)*	*% Recovery*	*% RSD*	*% Error [a]*
HTZ	50 %	12.5	6.25	18.75	18.78	100.16	0.183	0.16
	100 %	12.5	12.5	25	25.13	100.52	0.048	0.52
	150 %	12.5	18.75	31.25	31.23	99.93	0.047	-0.06
AML	50 %	5	2.5	7.5	7.56	100.8	0.693	0.80
	100 %	5	5	10	10.06	100.06	0.546	0.60
	150 %	5	7.5	12.5	12.45	99.6	0.314	-0.40

[a] *[found conc. – theoretical conc./theoretical conc.] x 100.*

2.9. Analysis of a commercial formulation:

Experimentally the results for the amount of HTZ and AML in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interaction from the excipients which are commonly present in formulation of tablets.

CONCLUSION

A new RP-HPLC method described in this manuscript provides a simple, convenient and reproducible approach for the simultaneous estimation and quantification of Hydrochlorothiazide and Amlodipine Besylate in routine quality control analysis.

REFERENCE:

1. Duarte JD, Cooper-DeHoff RM. "Mechanisms for blood pressure lowering and metabolic effects of thiazide and thiazide-like diuretics". Expert Rev. Cardiovasc. Ther., **2010**; 8(6):793-802. doi:10.1586/erc.10.27.
2. WHO Model List of EssentialMedicines. World Health Organization. October **2013**. Retrieved 22 April 2014.
3. Kornhuber J, Muehlbacher M, Trapp S, Pechmann S, Friedl A, Reichel M, Mühle C, Terfloth L, Groemer TW, Spitzer GM, Liedl KR, Gulbins E, Tripal P. **2011**.
4. V. Vijayasree, C. Pallavan and J.V.L.N. Seshagiri Rao. Development and Validation of an RP-HPLC Method for the Estimation of Hydrochlorothiazide in Tablet dosage forms, IJPSR, **2013**; 4(3): 1052-1055.
5. Richa Sah and Saahil Arora. Development and validation of a HPLC analytical assay method for Amlodipine besylate tablets: A Potent Ca+2 channel blocker. Journal of Advanced Pharmacy Education & Research, **2012**; 2(3): 93-100.
6. Boyka G. Tsvetkova and Lily P. Peikova. Development and validation of RP-HPLC method for simultaneous determination of Amlodipine Besylate and Hydrochlorothiazide in pharmaceutical dosage form, J. Chem. Pharm. Res., **2013**; 5(1): 271-275.
7. Safeer K, Anbarasi B, N. Senthil Kumar. Analytical Method Development and Validation of Amlodipine and Hydrochlorothiazide in combined dosage form by RP-HPLC, Int. J. ChemTech. Res., **2010**; 2(1): 21-25.
8. Subhashini. E, B. Syama Sundhar. New Analytical Method Development And Validation For The Simultaneous Estimation Of Telmisartan And Hydrochlorothiazide In Bulk And Tablet Dosage Form Using RP-HPLC, Carib. J. SciTech., **2014**; 2: 519-529.
9. Madhukar A, N. Kannappan and Mahendra Kumar CB. Analytical Method Development and Validation for the Determination of Hydrochlorothiazide, Amlodipine besylate and Telmisartan hydrochloride in Multicomponent Tablet dosage form and in Biorelevant media (FASSIF) by RP-HPLC Techniques, Int. J. Pharm. Pharm. Sci., **2015**; 7(1): 218-225.
10. Ridhdhi S Sinojiya et al., Development and Validation of Rp-HPLC Method for the Simultaneous Determination of Telmisartan, Amlodipine Besylate & Hydrochlorothiazide in a Tablet Dosage Form, Journal of Pharmacy Research, **2012**; 5(8): 4154-4157.

11. Jabir Aboobacker O et al., Method Development and Validation of Hydrochlorothiazide, Amlodipine Besylate and Telmisartan in Tablet Dosage Form by RP-HPLC Method, Research Journal of Pharmaceutical, Biological and Chemical Sciences, **2012**; 3(3): 509-517.
12. S. Nalwade *et al.* Rapid Simultaneous Determination of Telmisartan, Amlodipine Besylate and Hydrochlorothiazide in a Combined Poly Pill Dosage Form by Stability-Indicating Ultra Performance Liquid Chromatography, Sci. Pharm., **2011**; 79: 69-84.
13. Samya M. El-Gizawy et al., Development and Validation of HPLC Method for Simultaneous Determination of Amlodipine, Valsartan, Hydrochlorothiazide in Dosage Form and Spiked Human Plasma, American Journal of Analytical Chemistry, **2012**; 3: 422-430.
14. Prathyusha W et al., Development and Validation of a Stability Indicating RP-HPLC Method for Simultaneous Estimation of Aliskiren Hemifumarate, Amlodipine Besylate and Hydrochlorothiazide in Bulk and Pharmaceutical Dosage Forms, IOSR Journal of Pharmacy and Biological Sciences, **2014**; 9(1 IV): 114-123.
15. Vijay Kumar Rekulapally, Vinay U Rao. Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Aliskiren, Amlodipine and Hydrochlorthiazide in Tablet dosage form, Int. J. Pharm. Pharm. Sci., **2014**; 6(1): 724-730.
16. Code Q2(R1)-Text on Validation of Analytical Procedure Step-3 Consensus Guideline. ICH Harmonised Tripartite Guideline, **2005**.
17. Code Q2A-Text on Validation of Analytical Procedure Step-3 Consensus Guideline. ICH Harmonised Tripartite Guideline, **1994**.
18. Code Q2B-Validation of Analytical Procedure Methodology Step-4 Consensus Guideline. ICH Harmonised Tripartite Guideline, **1994**.
19. International Conference on Harmonization. Photo stability testing of new drug substance and products Q1B. International Conference on Harmonization, IFPMA, Geneva, **1996**.
20. Potale LV, Damle MC, Khodke AS, Bothara KG. A validated stability indicating HPTLC method for simultaneous estimation of Ramipril and Telmisartan, Int. J. Pharm. Sci. Rev. Res., **2010**; 2: 7.
21. Dongre VG, Shah SB, Karmuse PP, Phadke M, Jadhav VK. Simultaneous determination of metoprolol succinate and amlodipine besylate in pharmaceutical dosage form by HPLC, J. Pharm. Biomed. Anal., **2008**; 46(3): 583-586. doi:10.1016/j.jpba.2007.11.006.
22. Sudhakar P, Nirmala M, Moses Babu J, Vyas K, Madhusudan Reddy G, Vijaya Bhaskar B, Pratap Reddy P, Mukkanti K. Identification and characterization of potential impurities of amlodipine maleate, J. Pharm. Biomed. Anal., **2006**; 40: 605-613. doi:10.1016/j.jpba.2005.10.029.
23. Lakshmi Narasimham YS, Barhate VD. Development and validation of stability indicating UPLC method for the simultaneous determination of beta-blockers and diuretic drugs in pharmaceutical dosage forms, J. Chem. Metrol., **2010**; 4: 1-20.
24. Naidu KR, Kale UN, Shingare MS. Stability indicating RP-HPLC method for simultaneous determination of amlodipine and benazepril hydrochloride from their combination drug product, J. Pharm. Biomed. Anal., **2005**; 39: 147-155. doi:10.1016/j.jpba.2005.04.001.

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