A RP-HPLC method for the simultaneous assay of amlodipine and hydrochlorthiazide in combined dosage form

M.Prasada Rao[1,*], M. Srikanth[2], K. Umamaheswari[3]

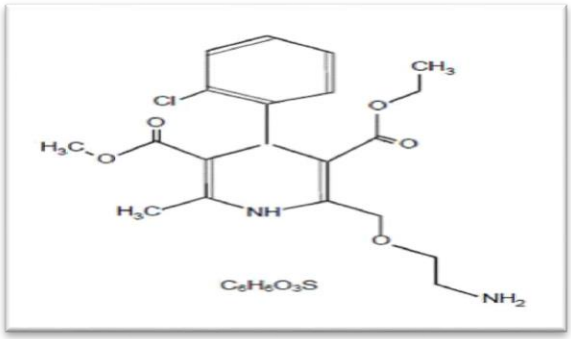
[1]Professor, [2,3]Assistant Professor, Department of Pharmaceutical Analysis, M.A.M College of Pharmacy, Kesanupalli, Andhra Pradesh, India

***Corresponding Author:**
Email: prins2mam@gmail.com

Abstract
This is the present work which is a new method development and validation was carried out for the estimation of Amlodipine besylate and hydrochlorthiazide by RP-HPLC technique. The wavelength selection was made at 245 nm in various literature were having a maximum absorbance at around 240 to 250 nm. Hence the wavelength was selected at 245 nm for the detection of the compound. In this method development several trials had been carried out and reported. Leading this to the optimized chromatographic conditions for the estimation of Amlodipine besylate and hydro chlorthiazide pharmaceutical dosage form. Preliminary studies involved trying C_{18}, columns and several mobile phase compositions for the effective separation of these drugs by using symmetry C_{18}Hypersil-BDS column eluted with the mobile phase Degassed water and acetonitile with a flow rate of 1.0ml/ min and a detection wavelength of 245 nm with injection volume of 20 ml at ambient (25°C) temperature yielded the good results.

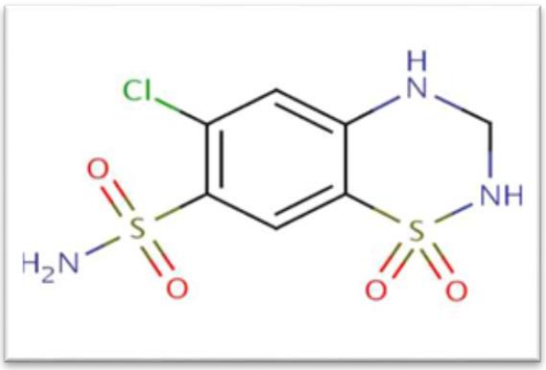
Keywords: Hydrochlorthiazide, RP-HPLC technique, C_{18}, Amlodipine besylate.

Introduction
Amlodipine is 1, 4-dihydropyridine calcium channel blocker which is a long-acting. Its primary activity is on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. Main mechanism is to inhibit the influx of the calcium into the smooth muscle cells and also prevents the calcium-dependent myocyte contraction and the vasoconstriction. The other mechanism for the drug's vasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition of the smooth muscle carbonic anhydrase. Evidential proof has been obtained that the amlodipine also exerts inhibitory effects on voltage-gated N-type calcium channels. These are the channels which are located in the central nervous system which may be involved in nociceptive signaling and pain sensation. Amlodipine is used in treating the hypertension and chronic stable angina.



Amlodipine

This is a thiazide diuretic which is often considered the prototypical member of this class. It helps in reducing the reabsorption of the electrolytes from the renal tubules. As a result of this there is an increased excretion of water and electrolytes, including sodium, potassium, chloride, and magnesium. It treats several disorders including edema, hypertension, diabetes insipidus, and hypoparathyroidism.



Hydrochlorthiazide

HPLC: This is the modern form of column chromatography which has been called as high performance, high pressure, and high-resolution and high-speed liquid chromatography. High-Performance Liquid Chromatography (HPLC) is a branch of column chromatography where the mobile phase is being forced through the column at high speed. In the year 1960's chromatographers started enhancing the polar nature of silanol group by chemically reacting silica with the organic silanes. The main aim was to make less polar or non-polar so that the polar solvents can be used to

separate the water-soluble polar compounds. As the ionic nature of the chemically modified silica is now reversed i.e. it is non-polar or the nature of the phase is reversed. The chromatographic segregation carried out with such silica is referred to as reversed- phase chromatography

Materials and Methods

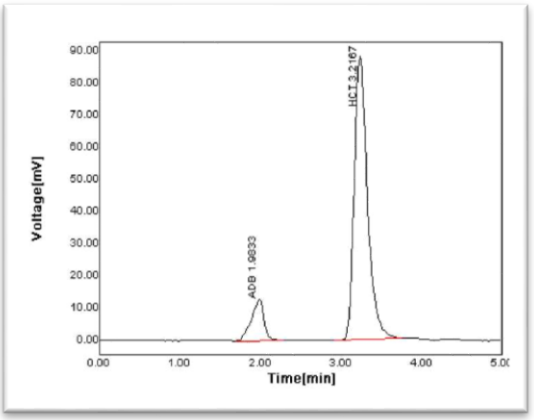
Instruments: HPLC - Electronic balance (SARTORIOUS), WATERS Model NO.2690/5 series,Digital pH meter(POLOMAN), Compact System Consisting of Hypersil-C18 BDS column, Sonicator (FAST CLEAN).

Chemicals: Acetonitrile HPLC Grade, Purified water HPLC Grade**.**

Raw Material: Hydrochlorothiazide Working Standardsand Amlodipine besylate.

Preparation of Standard Solution: Weigh down 10mg's of Hydrochlorothiazide RSand Amlodipine besylate drugs and dissolved in 10ml of Mobile phase which is taken in two 10ml of volumetric flasks seperatelyand those aresonicated for a period 20 minutes to get 1000ppms and 1 ml was taken from each solution and diluted to 10 ml with the mobile phase.

Chromatogram of Standard



Graph 1

Inference: Obtained the chromatogram at RT's of 1.9min to Amlodipine besylate and 3.2min to Hydrochlorothiazide for standard.

Table 1

S.No.	Name of the peak	Retention time (min)
1	Amlodipine Besylate	1.9
2	Hydrochlorothiazide	3.2

Method Validation

System Suitability: Right after completing the method development, the validation of the current method has been performed in accordance to the USP requirements for assay determination (Category-I: analytical methods for quantitation of active ingredients in finished pharmaceutical products) which include the accuracy, precision, selectivity, linearity and range, robustness and ruggedness.

Table 2: Data of System Suitability for Amlodipine besylate

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	1.9665	143	9462	0.9577
2	1.9834	142	9264	0.9786
3	1.9667	141	9765	0.9355
4	1.9663	141	9314	0.9888
5	1.9667	142	9546	0.9745
Mean	1.9655	142.4721	9470.2	0.9670
SD	0.0075	0.8365		
% RSD	0.38	0.59		

Table 3: Data of System Suitability for Hydrochlorothiazide

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.2334	1002	12456	0.7533
2	3.2000	999	12765	0.7299
3	3.2009	998	12764	0.7625
4	3.2167	995	12348	0.7555
5	3.2000	997	12543	0.7533
Mean	3.2102	998.6805	12575.2	0.7509'
SD	0.0147	2.6214		
% RSD	0.46	0.26		

Acceptance criteria: %RSD values are within the limit

Precision: This is the analytical method which was determined by assaying the sufficient number of sample and relative standard deviation was calculated.

System precision:

Table 4: Data of Repeatability (System precision) for Amlodipine besylate

	Injection	Peak Areas of Amlodipine besylate	%Assay
	1	142	99.92
Concentration	2	140	98.58
40ppm	3	142	99.44
	4	141	99.33
	5	141	98.98
Statistical	**Mean**	141.3386	99.25
Analysis	**SD**	1.0961	0.5033
	% RSD	0.77	0.51

Table 5: Data of Repeatability (System precision) for Hydrochlorothiazide

	Injection	Peak Areas of Hydrochlorothiazide	%Assay
Concentration 40ppm	1	998	100.06
	2	999	100.17
	3	997	99.96
	4	999	100.21
	5	999	100.20
Statistical Analysis	**Mean**	999.0045	100.12
	SD	1.075	0.1075
	% RSD	0.11	0.11

Repeatability

Table 6: Data of Repeatability (Method precision) for Hydrochlorothiazide

	Injection	Peak Areas of Hydrochlorothiazide	%Assay
	1	998	100.04
Concentration	2	998	100.02
40ppm	3	997	99.96
	4	999	100.20
	5	997	99.96
	6	999	100.13
Statistical	**Mean**	998.3124	100.05
Analysis	**SD**	0.951	0.096
	% RSD	0.095	0.096

Accuracy (Recovery)

The accuracy is the measurement of an analytical method to the closeness of that results obtained by that method to the true value. This accuracy may often be expressed as percent recovery by the assay of known added amount of analytic.

Table 7: Data of Accuracy for Amlodipine besylate

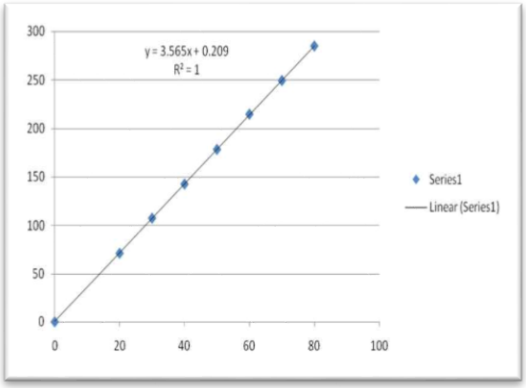
Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.96	99.80	**MEAN**	99.95
50% Injection 2	20	19.83	99.15		
50% Injection 3	20	20.18	100.90	**%RSD**	0.89
100 % Injection 1	40	39.76	99.40	**MEAN**	99.84
100 % Injection 2	40	39.95	99.87		
100% Injection 3	40	40.10	100.25	**%RSD**	0.43
150% Injection 1	60	59.65	99.42	**MEAN**	99.64

Table 8: Data of Accuracy for Hydrochlorothiazide

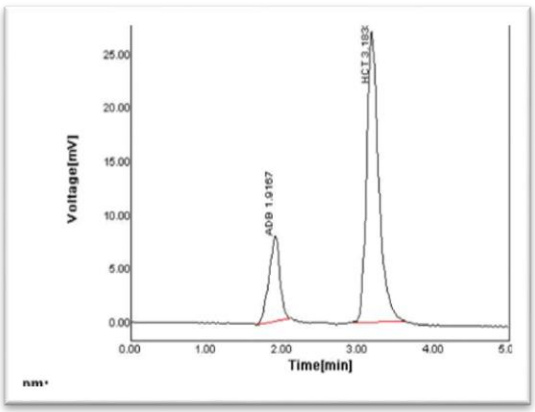
Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.97	99.85	**MEAN**	99.88
50% Injection 2	20	19.96	99.80	**%RSD**	0.10
50% Injection 3	20	20.00	100.00		
100 % Injection 1	40	40.03	100.07	**MEAN**	100.07
100 % Injection 2	40	39.99	99.97		
100% Injection 3	40	40.05	100.17	**%RSD**	0.10
150% Injection 1	60	60.08	100.13	**MEAN**	100.07
Injection 2	60	60.03	100.05		
150% Injection 3	60	60.02	100.03	**%RSD**	0.053

Linearity: Linearity of the analytical method for assay is by injecting the various concentrations of standard preparations prepared. The linearity between the peak-area and the concentration was examined.

Graph 2: Linearity Plot (Concentration Vs Response) of Amlodipine



Graph 3

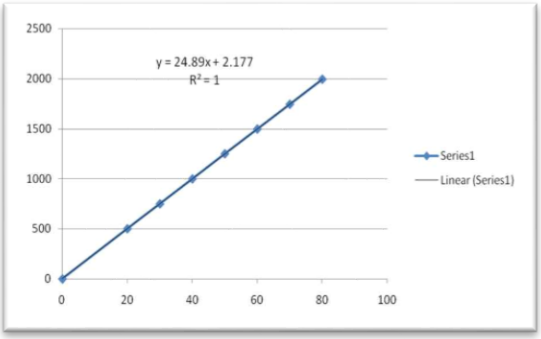


Acceptance Criteria:

Correlation Coefficient should be not less than

0.9990. % of y- Intercept should be ±2.0. % of RSD for level 1 and Level 6 should be not more than 2.0%.

Graph 4: Linearity Plot (Concentration Vs Response) of Hydrochlorothiazide



Ruggedness:

Table 9

S.No	Peak area	Assay % of Amlodipine besylate
1	142	99.92
2	140	98.63
3	142	99.97
4	144	101.42
5	143	100.68
6	142	100.08
Mean	142.9770	100.12
%RSD	0.924	0.92

Table 10

S. No.	Peak area	Assay % of Hydrochlorothiazide
1	998	100.06
2	997	100.01
3	999	100.13
4	997	100.00
5	999	100.17
6	998	100.10
Mean	998.5715	100.08
%RSD	0.068	0.067

Table 11: Data for Effect of variation in flow rate (Amlodipine besylate)

Flow	Std Area	Tailing	Flow	Std Area	Tailing	Flow	Std Area	Tailing
0.8ml		factor	1.0ml		factor	1.2ml		factor
	201.2458	1.0654		142.9203	0.9745		119.5373	0.8677
	202.2735	1.0666		141.7788	0.9656		121.6676	0.8656
	198.7938	1.0659		143.6957	0.9824		121.8238	0.8555
	203.1705	1.0432		139.4545	0.9752		120.6342	0.8746
	202.9369	1.0236		143.7711	0.9751		119.5126	0.8656
Avg	201.6841	1.0529	Avg	142.3241	0.9746	Avg	120.6351	0.8658
SD	1.78	0.019	SD	1.793	0.006	SD	1.112	0.007
%RSD	0.88	1.80	%RSD	1.26	0.61	%RSD	0.92	0.81

Table 12: Data for Effect of variation in flow rate (Hydrochlorothiazide)

Flow	Std Area	Tailing	Flow	Std Area	Tailing	Flow	Std Area	Tailing
0.8 ml		factor	1.0 ml		factor	1.2 ml		factor
	1324.5469	0.8295		998.7905	0.7612		831.9437	0.6555
	1322.4299	0.8133		999.5291	0.7522		830.7733	0.6598
	1323.6787	0.8345		998.3930	0.7632		829.4346	0.6494
	1323.6045	0.8267		997.4566	0.7756		830.4743	0.6654
	1322.1327	0.8266		999.0457	0.7662		830.8384	0.6498
Avg	1323.2785	0.8261	Avg	998.6430	0.7637	Avg	830.6929	0.6560
SD	0.988	0.0078	SD	0.781	0.0084	SD	0.898	0.0068
%RSD	0.074	0.944	%RSD	0.078	1.099	%RSD	0.108	1.036

Summary and Conclusion

This is analytical method which was developed by studying various parameters. First of all, maximum absorbance was found to be at 245nm and the peaks purity was good. Injection volume was selected to be 20ml which gave a good peak area. The Hypersil Ci8 is the column used for study. Optimum temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at l ml/min because of good peak area and retention time. The various ratios of mobile phase were being studied, mobile phase with ratio of 50:50, Water: Acetonitrile was fixed due to good resolution and the symmetrical peaks. This is the reason for selection of this mobile phase for the present study. Acetonitrile was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be minimum because analyze gave peak around minimum and also to reduce the total run time.

The recovery was found to be 98.0-101.50 was linear and precise over the same range. Both system and method precision was found to be accurate and well vary within the range. Detection limit was found to be 0.77 for Amlodipine and 0.35 for HCTZ. This analytical method was found out to be linear over the range of 20-80ppm of the target concentration for both of the drugs. The analytical method passed both robustness and ruggedness tests. On both cases, relative standard deviation were satisfyingly good enough.

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