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RP-HPLC method development and validation for the simultaneous estimation of hydrochlorothiazide and amlodipine in tablet dosage form

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Abstract

A simple, selective, rapid, precise and economical reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed for simultaneous estimation of Hydrochlorothiazide (Hctz) and Amlodipine (Amlo) in tablet dosage forms. The method is carried out on C18 (25 cm x 4.6 mm i.d., 5µm) column with a mobile phase consisting of methanol (MeOH): Phosphate buffer 50 mmol (KH₂PO₄, 0.05M) (pH adjusted to 5.5 using *o*-phosphoric acid) in the ratio of 75:25 (v/v). The retention time of Hctz and Amlo is 3.2 and 5.1 min, respectively, with the flow rate of 1 mL/min with diode array detection at 236 nm. The linear regression analysis data from the linearity plot showed good linear relationship with a correlation coefficient (R²) value for Hctz and Amlo of 0.9969 and 0.9958 in the concentration range for Hctz is 10-35µg ml⁻¹ and Amlo is 4-14 µg ml⁻¹, the assay report of Hctz and Amlo is 98.4 and 99.6%. The relative standard deviation for intraday precision has been found to be <2.0%. The method is validated according to the ICH guidelines. The developed method is validated in terms limit of detection, limit of quantitation, robustness and solution stability. The proposed method can be used for simultaneous estimation of these drugs in marketed dosage forms.

Keywords: RP-HPLC, hydrochlorothiazide, amlodipine, and column C18 (25 cm x 4.6mm i.d., 5µm), methanol: phosphate buffer 50 mmol (75:25 (v/v))

Introduction

The present research aims to develop and validate a RP-HPLC method for the simultaneous estimation of hydrochlorothiazide and amlodipine in tablet dosage form. Hydrochlorothiazide belongs to thiazide class of diuretics and used to treat hypertension and congestive heart failure. Amlodipine is a calcium channel inhibitor. The various brands available in Combination of Hydrochlorothiazide and Amlodipine are Amlokind-H, Amlong-H, Amosafe-H. Extensive literature survey revealed that there are various methods for estimation of hydrochlorothiazide & amlodipine in individual or in combination with other drugs [1-12] are present but there is no method available for simultaneous analysis of Hydrochlorothiazide & Amlodipine by HPLC.

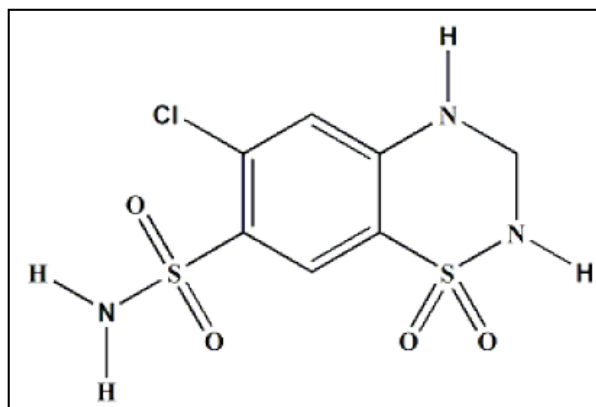


Fig 1: Chemical Structure of hydrochlorothiazide

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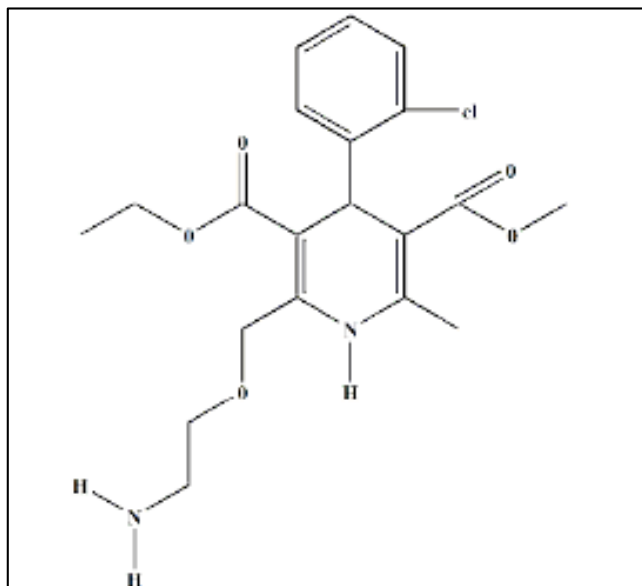


Fig 2: Chemical Structure of Amlodipine

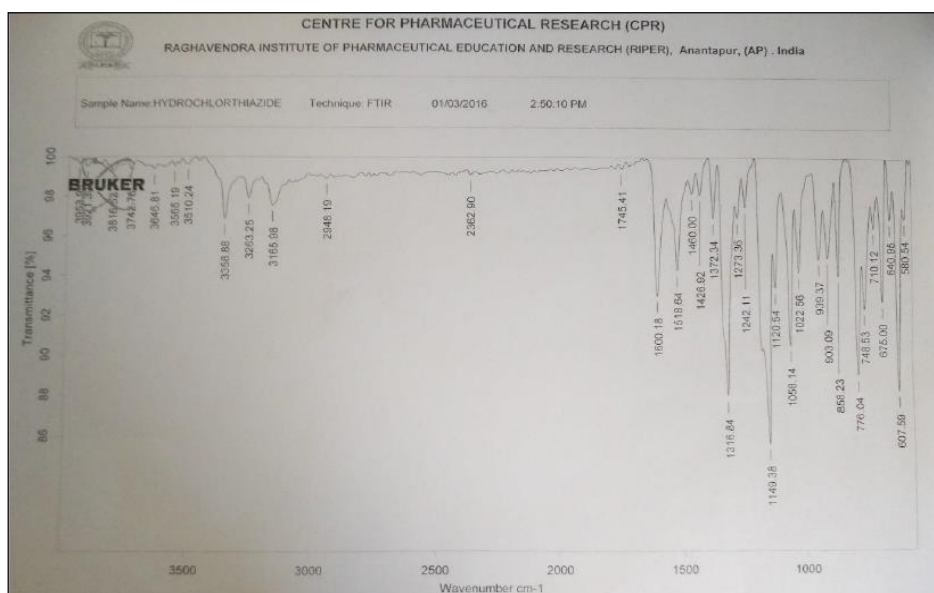


Fig 3: IR Spectrum of hydrochlorothiazide

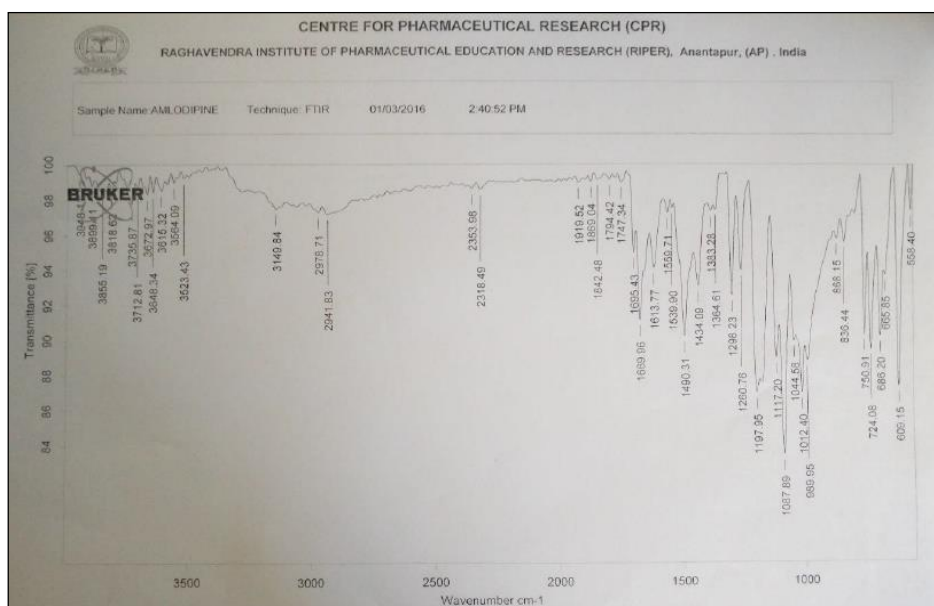


Fig 4: IR spectrum of Amlodipine

Materials & Methods

Reference standards of both APIs (Hctz and Amlo) were respectively procured from Micro labs (Bangalore India) and Hetero drugs (Hyderabad, India) with a purity of 99.91% and 99.95%.

Chemicals Used

Table 1: Chemicals Used

S. No	Name	Grade	Manufacturer
1	Methanol	HPLC	Merck
2	Acetonitrile	HPLC	Merck
3	Potassium hydrogen orthophosphate	AR	LobaChemie
4	Distilled water	HPLC	Merck

Instrument used

Agilent LC-1200 system with C18 column (250 mm x 4.6 mm, 5 micron), photo diode array (PDA) detector, Rheodyne (loop) injector (20 microliters) and binary pumps was used in this study.

Preparation of standard drug solutions

Accurately about 10 mg of the Hctz and Amlo pure drugs were weighed and transferred into 10ml clean, dried standard

volumetric flasks separately. The volume was filled with mobile phase up to the mark. 150 µl and 60 µl of the above solutions were transferred into 10ml volumetric flasks and the volume was filled with mobile phase up to the mark to obtain 15 µg/ml and 6 µg/ml concentration of Hctz and Amlo this is standard. From the first stock solution, different concentrations of Hctz were prepared in the concentration range of 10-35 µg/mL and Amlo were prepared in the concentration range 4-14 µg/ml. The solutions were injected under above mentioned chromatographic conditions and peak areas were measured. However for initial trails drug concentrations of 20 µg/mL of both Hctz and Amlo was used in the initial trails.

Preparation of Sample drug solutions

Appropriate tablet powder was weighed, dissolved and the final dilution of 15µg/ml of Hctz and 6µg/ml of Amlo used for assay. The sample injection was performed in replicate and amount was calculated from regression equation of calibration plot.

Results & Discussion

Specificity

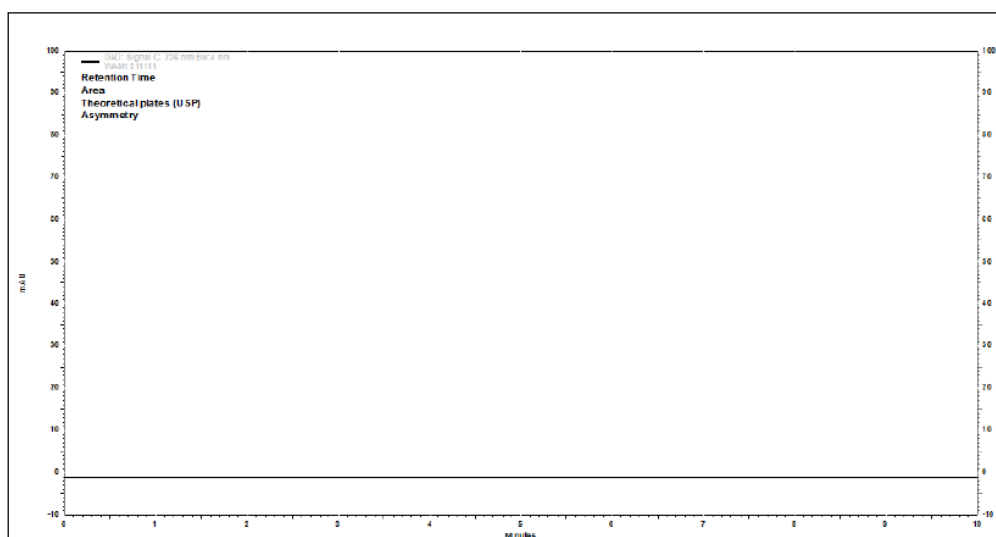


Fig 5: Blank Chromatogram for specificity

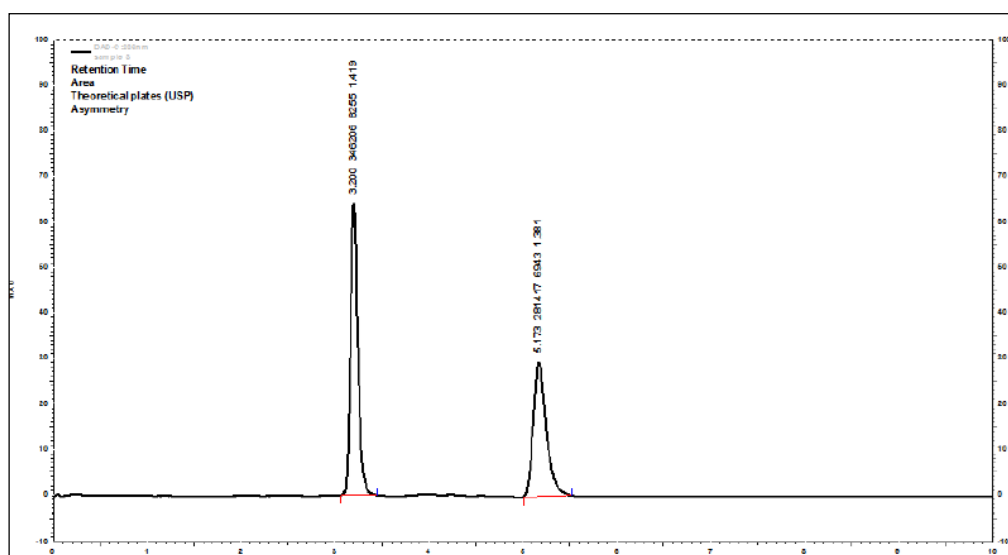


Fig 6: Optimized chromatogram of Bromofenac Sodium

Linearity

Table 2: Results for Linearity and range of Hydrochlorothiazide

S. No	Conc. of Hctz (µg/mL)	Peak area mean
1	10	221717
2	15	349192
3	20	466856
4	25	627683
5	30	739449
6	35	856852

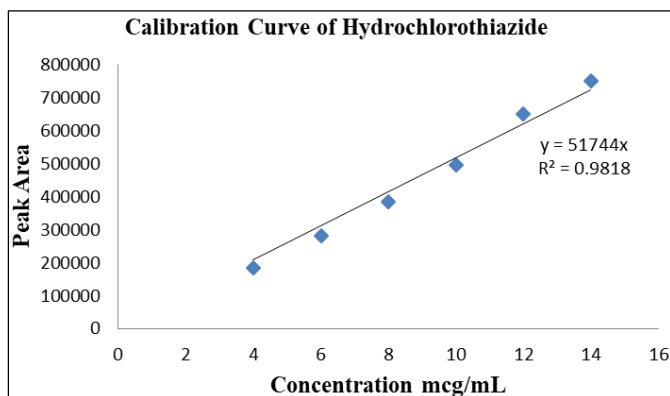


Fig 7: Calibration Curve of Hydrochlorothiazide

Table 3: Results for Linearity and range of amlodipine

S. No	Conc. of Hctz (µg/mL)	Peak area mean
1	4	185241
2	6	280514
3	8	384521
4	10	495241
5	12	650010
6	14	751201

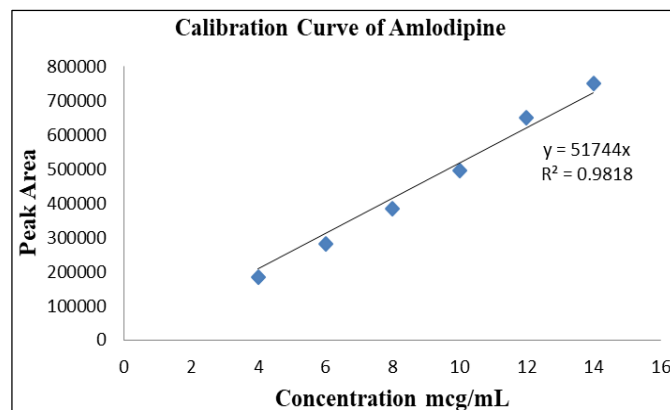


Fig 8: Calibration Curve of Amlodipine

Accuracy

Table 4: Recovery Report of Hydrochlorothiazide and Amlodipine

Drug name	Pre-analyzed concentration taken (µg/ml)	Recovery level	Amount of drug added (µg/ml)	Amount of drug found (µg/ml) Mean± SD	% Recovery
Hydrochloro Thiazide	15	80	12	26.7	101.8
		100	15	30.1	99.08
		120	18	33.1	101.9
Amlodipine	6	80	4.8	10.1	98.6
		100	6	12.1	100.8
		120	7.2	13.8	100.9

Precision

Table 5: Repeatability Data

S. No	Conc. (µg/mL)		Peak area (n=8)	
	Hctz	Amlo	Hctz	Amlo
1	15	6	346259	286434
2	15	6	356612	284678
3	15	6	341648	278956
4	15	6	342615	287453
5	15	6	348617	291456
6	15	6	346386	284621
7	15	6	348679	278648
8	15	6	348845	284795
Mean			347457.6	284630.1
%RSD			1.32	1.49

Table 6: Intermediate Precision Data for Hydrochlorothiazide and Amlodipine

S. No	Analyte	Conc (mcg/mL)	Intraday Peak area mean	Interday Peak area mean
1	Hctz	15	364271	374102
2	Amlo	6	263431	287958

LOD & LOQ

The LOD&LOQ for Hctz is 0.256 mcg/mL & 0.776 mcg/mL and 0.123 mcg/mL & 0.327 mcg/mL for Amlo respectively.

Robustness

Table 7: Robustness studies of Hydrochlorothiazide and Amlodipine

S. No	Parameter	Modification	Retention time		% RSD	
			Hctz	Amlo	Hctz	Amlo
1	Flow rate	0.9	3.25	5.31	0.8	0.66
		1.1	2.85	4.88	0.5	0.77
2	Mobile phase: MeOH: KH ₂ PO ₄ 0.05M	77:23	2.85	4.7	0.03	0.31
		73:27	3.2	5.3	0.56	0.09
3	Wavelength	234	3.1	5.1	0.47	0.71
		238	3.11	5.11	0.56	0.48

Assay

Table 8: Assay studies of Hydrochlorothiazide and Amlodipine

S. No	Drug name	Peak area	Amount found (mg)	Assay	% RSD
1	Hctz	341817	14.72	98.4%	0.23
2	Amlo	270428	5.953	99.6%	0.66

Conclusion

The present study represents an accurate, precise and specific HPLC method for routine analysis of Hydrochlorothiazide and Amlodipine in Bulk form. The use of C18 column in the

present work has shown better elution of analytes with good resolution, improved plate count, capacity factor and reduced tailing. So the C18 column can be used to achieve high specificity in shorter time of analysis of Hydrochlorothiazide and Amlodipine as per ICH Q2 (R2) guidelines.

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