INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND HYDROCHLORTHIAZIDE IN TABLET DOSAGE FORM BY RP-HPLC

M.V.Kumudhavalli*, K.Anand babu, B.Jayakar
Department of pharmaceutical analysis, Vinayaka mission college of pharmacy, salem-636008, Tamilnadu, India

Article Received on: 18/04/2011 Revised on: 22/05/2011 Approved for publication: 18/06/2011

*E-mail:Kumudhuphd@rediffmail.com

ABSTRACT
A simple, precise, rapid and accurate RP-HPLC method has been developed for the simultaneous estimation of Amlodipine besylate and Hydrochlorthiazide in tablet formulations. The chromatographic separation was achieved on a Shimadzu Symmetry C18 column (250 mm x 4.6mm, 5.0 µ particle size) using Methanol: Acetonitrile: 50mM Na_2HPO_4 pH7.0 (60:20:20v/v/v) with 1% triethylamine. Flow rate was 1ml/min and column was maintained at ambient temperature. Quantification and linearity was achieved at 254 nm over the concentration range of 1 to 8 µg /ml for Amlodipine besylate and 2.5 to 20 µg /ml Hydrochlorthiazide. The method was validated for specificity, linearity, accuracy, and precision, LOD, LOQ and Robustness.
Key words: Amlodipine besylate, Hydrochlorthiazide**,** RP HPLC, Validation.

INTRODUCTION

Amlodipine (as besylate, mesylate or maleate), chemically is 3-Ethyl-5-methyl (±) -2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydr o-6-methyl-3,5-pyridinedicarboxylate benzenesulfonate[1]. Amlodipine is a dihydropyridine derivative with calcium antagonist activity[2]. It is used in the management of hypertension, chronic stable angina pectoris and prinzmetal variant angina[3]. Amlodipine acts by inhibiting the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle and also acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure[4]. Hydrochlorothiazide is a 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine- 7-Sulphonamide 1, 1-dioxide, is a diuretic, which inhibits active chloride reabsorption at the early distal tubule via the Na-Cl co-transporter, resulting in an increase in the excretion of sodium, chloride and water[5].

Literature survey reveals few analytical methods for the determination of Amlodipine alone and in combination with other drugs in pharmaceutical preparations and biological fluids, viz. spectrophotometry[6-9], HPLC[10]and HPTLC[11-13]. Also there are some analytical methods reported for determination of HCT alone and in combination [14-18].

No method has been reported for the estimation of Amlodipine besylate and Hydrochlorothiazide in combined dosage form. Present work emphasizes on the quantitative estimation of Amlodipine besylate and Hydrochlorothiazide in their combined dosage form by UV Spectroscopic methods.

MATERIALS AND METHODS

Materials and instruments

Reference standards of Amlodipine besylate and Hydrochlorthiazide were obtained as gift samples from Micro labs. Market formulation of this combination Amlong-H was procured from the local market. HPLC grade acetonitrile and methanol were obtained from Merck (India). Analytical grade disodium hydrogen phosphate buffer were purchased from SD Fine chemicals, India. Water obtained from Millipore with milli Q system, filtered through 0.45 µ nylon-66 membrane was used for the HPLC work. The LC system consisted of isocratic pump, auto sampler and UV detector. The output signal was monitored and integrated using LC solutions chromatography Manager Software (Prominence HPLC, Shimadzu, Japan).

Chromatographic conditions

Instrument - High performance liquid Chromatography equipped with Auto sampler and UV detector
Column - C_{18}, 250x4.6mm, 5µ, Phenomenox Luna Column
Column oven Temperature - Ambient
Wave length - 254 nm
Flow rate - 1 ml per min
Injection volume - 20 µl
Runtime - 15 min
Mobile phase - Methanol: Acetonitrile: 50mM Na_2HPO_4 (60:20:20) with 1% triethylamine

Preparation of buffer solution

Weighed 7.42gm of Na_2HPO_4 and dissolved in 1000ml of distilled water then added 0.1% Triethylamine, adjusted the pH 7.0 with Orthophosphoric acid.

Preparation of mobile phase

The mixture of Methanol: Acetonitrile: 50mM Na_2HPO_4 in the ratio of 60:20:20 (v/v/v) was prepared. Filtered and degassed the mobile phase.

Preparation of Amlodipine Besylate and Hydrochlorothiazide Standard Solution

Weighed accurately 50mg of Amlodipine Besylate as working standard and 50mg of Hydrochlorothiazide as working standard and transferred into 50ml volumetric flasks, and dissolved separately in methanol and made up the volume with Methanol. Pipette out 1ml of both solutions into a 10ml volumetric flasks and made up the volume with mobile phase. And again pipette out 1ml of this solution into a 10ml volumetric flask and made up the volume with mobile phase.

Preparation of test solution

Twenty tablets were weighed accurately and powdered. Powder equivalent to 10mg of Hydrochlorothiazide was weighed and transferred to 50ml volumetric flask and dissolved in methanol by shaken the flask for 15minutes. Filtered the first 20ml of the filtrate through 0.25 µ filter. Pipette out 5ml of the solution into a 10ml volumetric flask and made up the volume with mobile phase. And again pipette out 5ml of this solution into a volumetric flask and made up the volume with mobile phase. All the determinations were conducted six times to ensure repeatability of the method. The mean peak area of the each drug was calculated.

RESULTS AND DISCUSSION

The purpose of the present study was to develop a rapid and sensitive RP-HPLC method for the simultaneous estimation of Amlodipine besylate and Hydrochlorthiazide in combined dosage form using Phenomenox C18 analytical column with UV detection

System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing were determined. The results obtained are shown in Table-1. The number of theoretical plates for Amlodipine besylate and Hydrochlorthiazide were 7979 and 3303 respectively.

Linearity

Under the experimental conditions described above, linear calibration curves for both Amlodipine besylate and Hydrochlorthiazide were obtained with five concentration level each. Peak area (A) and concentration (C) of each drug substance was subjected to regression analysis to calculate the regression equation and the correlation coefficients. The regression equation obtained for Amlodipine besylate and Hydrochlorthiazide were (r=0.99995, n=5) and (r=0.99996, n=5). The linearity range of Amlodipine besylate was 1-8µg/ml and 2.5 to 20µg/ml for Hydrochlorthiazide.

Accuracy

The accuracy of an analytical method is the closeness of test results obtained by method to the assay value. Accuracy should be established across the specified range of the analytical procedure. The accuracy was then calculated as the percentage of analytes recovered by the assay. Mean recoveries (mean±S.D.) for Amlodipine besylate and Hydrochlorthiazdie from the combination formulation are shown in Table 2&3 indicating good accuracy of the method.

Precision

Method precision was investigated by the analysis of six separately prepared samples of the same batch of tablets. From this six separate sample solutions was injected and the peak areas obtained used to calculate mean and percentage R.S.D. values. The results obtained are shown in Table 4. In all instances the accepted criteria of % R.S.D. of less than 2% was met. Precision of the system was evaluated by injecting a freshly prepared standard solution six times. The %R.S.D. results obtained 0.857 and 0.671 for Amlodipine besylate and Hydrochlorthiazide, respectively, all well below the accepted maximum of 1%.

Limit of detection and limit of quantitation

The LOD was calculated to be 0.314 µg/mL for Amlodipine besylate and 0.635 µg/mL for Hydrochlorthiazide. And the LOQ of Amlodipine besylate and Hydrochlorthiazide were found to be 0.95 µg/mL and 1.92 µg/mL, respectively.

Robustness

The robustness was determined by carrying out the assay during which the flow rate was altered slightly. When the flow rate was altered to 0.8ml/min, percent RSD was found to be 0.549% for Amlodipine besylate and 1.134% for Hydrochlorthiazide. And for 1.2ml/min percent RSD was found to be 0.667% for Amlodipine besylate and 1.02% for Hydrochlorthiazide which indicated that the method is robust, also indicating lack of influence on the test results by operational variable for the proposed method. The results obtained are shown in Table 5.

Ruggedness

The ruggedness of the method was determined by performing the same assay by different analysts and performing the assay on different days to check the

reproducibility. The test results were found to provide percentage content of 100.85% for Amlodipine besylate(day to day), 100.4%(analyst to analyst) and 100.17% (day to day) and 99.84% (analyst to analyst)for Hydrochlorthiazide.

CONCLUSION

A simple, rapid, accurate and precise HPLC method was developed for the determination of Amlodipine besylate and Hydrochlorthiazide in pure form and in tablets. The analytical conditions and solvent system developed provided a good separation for Amlodipine besylate and Hydrochlorthiazde within a short analysis time. The method was validated and demonstrated a wide linear dynamic range, a good precision and accuracy. Thus, the method can be proposed for routine analysis laboratories and for quality control.

REFERENCES

1. The Merck Index. Whitehouse Station, 23rd ed. New Jersey; 2008.p.516, 6235.
2. Martindale, Sweetman SC., The complete drug reference. 32nd ed. Pharmaceutical Press; 1999.p.822, 907.
3.Oneil MJ, Smith A, Heckelman PE, Budawari S.The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals. 13rd ed.,Merck and Co Inc., White House Station, New jersey; 2001.p.488, 865.
4. www.wikipedia.org/wiki/Amlodipidine.
5. Goodman and Gilman's, in: Gilman A G, Rall T W, Nies A S, Taylor P. The Pharmacological Basis of Therapeutics. (Eds.) Pergamon Press Oxford; 1996.p.743.
6. Devi Ramesh, S Ramakrishna. New spectrophotometric methods for simultaneous determination of amlodipine besylate and atorvastatin calcium in tablet dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences. 2010; Vol 2, Issue 4:215-219.
7. Priyanka R Patil, Sachin U Rakesh, PN Dhabale, KB Burade. Simultaneous UV Spectrophotometric Method for Estimation of Losartan Potssium and Amlodipine Besylate in Tablet Dosage Form. Asian J. Research Chem.2009; 2(1): 183-187.
8. Chandan Kumar Giri, M.S. Kondawar and D.D Chougule. Simultaneous estimation of Nebivolol hydrochloride and Amlodipine besylate in combined tablet dosage form by Qanalysis method. International Journal of Pharma. Research and Development.2010;2(5):1-8.
9.Bhatia N. M., Mohite A. S., Bhatia M. S. Simultaneous spectrophotometric estimation of atenolol and amlodipine besylate by multi-wavelength method. The Indian Pharmacist. 2007; vol. 6: 59-62.
10. Safeer K, Anbarasi B, N.Senthil Kumar. Analytical Method Development and Validation of Amlodipine and Hydrochlorothiazide in combined dosage form by RP-HPLC. International Journal of ChemTech Research.2010; Vol.2, No.1: 21-25.
11.Chandrashekhar T G, Rao P S N , Smrita K. Analysis of amlodipine besylate by HPTLC with fluorimetric detection: a sensitive method for assay of tablets. J.Planar. Chromatogr. Mod. TLC. 1994; 7: 458.
12. Pandya K K, Satia M, Gandhi T P. Detection and determination of total amlodipine by high performance thin layer chromatography: A useful technique for pharmacokinetic studies. J. Chromatogr. B; Biomed. Appl. 1995; 667: 315.
13. Ilango K, Kumar P B, Prasad V RV. Simple and rapid high performance thin layer chromatographic determination of amlodipine in pharmaceutical dosage forms. Indian J. Pharm. Sci. 1997; 59: 336.
14. Carlucci.G. and Palumbo. G. Simultaneous determination of losartan and hydrochlorothiazide in tablets by high performance liquid chromatography. J. Pharm. Biomed. Anal., 2000; 23(1):185-90.
15. Alaa. E.G. and Ahmed. A. Application of LC and HPTLC Densitometry for the Simultaneous determination of Benazepril Hydrochloride and Hydrochlorothiazide. J. Pharma.Biomed.Anal., 2001; 25(2):171-179.
16. Kartal. M. and Erk. N. Simultaneous determination of Hydrochlorothiazide and Amiloride Hydrochloride by Ratio Spectra Derivative Spectrophotometry and High-Performance Liquid Chromatography. J. Pharma. Biomed. Anal., 1999; 19(3):477-485.
17. Palled. M. S. and Rajesh. P. M .N. RP-HPLC Determination of Telmisartan in Tablet Dosage Forms, Indian J. Pharm.Sci., 2005; 67: 108-110.
18. N.J.Shah et al, Development and validation of HPTLC method for Simultaneous estimation of Telmisartan and Hydrochlorothiazide in tablet dosage form. Indian Journal of Pharmaceutical Sciences. 2007; 203 .

Table 1: Result of system suitability

S.No	Parameters	Amlodipine besylate	Hydrochlorthiazide
1.	No.of Theoretical plates	7979	3303
2.	Tailing factor	1.416	1.326

Table 2: Results of the HPLC analysis for tablets

S.NO	Parameters	Amlodipine besylate	Hydrochlorthiazide
1.	% Mean*	99.09	98.97
2.	S.D.	0.84	0.47
3.	%RSD	0.85	0.47

* Mean of fifteen determinations (3 replicates at 5 concentration level)

Table 3: Accuracy(recovery) study results

Analyte (n=6)	Amount percent(mean)		%RSD of assay	
Percentage of target concentration	Amlodipine besylate(% recovery)	Hydrochlorthiazide(% recovery)	Amlodipine besylate	Hydrochlorthiazide
50%	99.60	99.67	1.1709	0.8867
100%	100.04	99.72	0.6385	0.5168
150%	100.84	99.43	1.0094	0.6246

Table 4: Results of precision

S.No.	Validation Parameter	% Mean*		S.D.		% R.S.D	
		AML	HCZ	AML	HCZ	AML	HCZ
1.	Repetability	99.95	99.96	0.857	0.671	0.857	0.671
2.	Intermediate precision(day to day)	100.85	100.17	0.354	0.467	0.350	0.465
3.	Intermediate precision (analyst to analyst)	100.4	99.84	1.697	0.516	1.690	0.517

*Mean of fifteen determinations (3 replicates at 5 concentration level)

Table 5 : Results of robustness

S.No	Validation parameter	% Mean*		S.D.		%RSD	
	Robustness (flow rate)	AML	HCZ	AML	HCZ	AML	HCZ
1.	0.8ml/min	**98.80**	**99.23**	**0.543**	**1.126**	**0.549**	**1.134**
2.	1.2ml/min	**98.87**	**98.18**	**0.659**	**1.010**	**0.667**	**1.020**

* Mean of six determinations

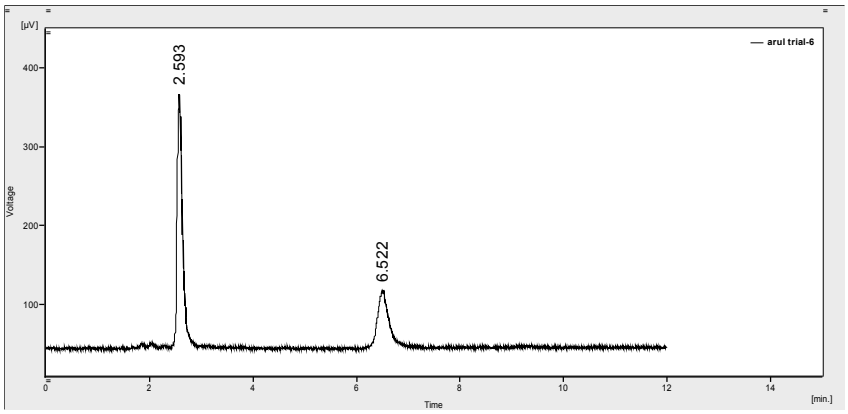

Fig.1: Chromatogram of Amlodipine besylate and Hydrochlorthiazide.

Source of support: Nil, Conflict of interest: None Declared