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Der Pharmacia Lettre, 2015, 7 (6):220-224
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Method development and validation of amlodipine besylate and hydrochlorothiazide in their bulk and combined dosage form

Ghodke Deepa. Vyankatrao*, Bhusnure Omprakash[1] and Kulkarni Aditi Anil[2]

**Department of Quality Assurance in Maharashtra College of M.Pharmacy Nilanga*
[1]Department of Medicinal chemistry in Maharashtra College of Pharmacy Nilanga
[2]Maharashtra College of Pharmacy Nilanga, Dist-Latur

ABSTRACT

A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the determination of Amlodipine Besylate and Hydrochlorothiazide in bulk and combined dosage form by RP- HPLC using Hypersil BDS C18 column (Dimention : 250mmx 4.6mm, 5µ). The sample was analysed using Acetonitrile:Water 50:50% v/v mobile phase at a flow rate of 1.0ml/min and detection at 254nm. The retention time for Amlodipine Besylate and Hydrochlorothiazide was found to be 1.9 and 3.2 min respectively, and recoveries from combined dosage form were between 100.2% and 99.17%. The method can be used for routine analysis of Amlodipine Besylate and Hydrochlorothiazide in bulk and combined dosage form.

Keywords: Amlodipine besylate, Hydrochlorothiazide, RP-HPLC.

INTRODUCTION

Amlodipine is chemically as (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate. Amlodipine Besylate is used with or without other medications to treat high blood pressure. Lowering high blood pressure helps prevent strokes, heart attacks, and kidney problems. Amlodipine belongs to a class of drugs known as calcium channel blockers. It works by relaxing blood vessels so blood can flow more easily.

Hydrochlorothiazide is chemically designated as 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide, 1-dioxide.It is a first-line diuretic drug. Diuretic drug of the thiazide class that acts by inhibiting the kidney's ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance.

Hydrochlorothiazide is sold both as a generic drug and under a large number of brand names, including Apo- Hydro, Aquazide H, Dichlotride, Hydrodiuril, HydroSaluric, Microzide, Esidrex, and Oretic.

Literature survey reveals the availability of several methods for determination of both Amlodipine besylate and Hydrochlorothiazide includes UV, HPLC as alone or in combination with other drugs. No method has been reported for the estimation of Amlodipine and Hydrochlorothiazide in combined dosage form. Present work emphasizes on

the determination of Amlodipine Besylate and Hydrochlorothiazide in their bulk and combined dosage form by RP-HPLC.

MATERIALS AND METHODS

Pure samples of Amlodipine Besylate - were obtained from Cipla Pharmaceutical Limited, Mumbai and HCTZ from Biocon Limited, Banglore Commercial tablet of amlodipine besylate (5mg) and Hydrochlorthiazide (12.5mg) were procured from the local drug market respectively for the determination Amlodipine besylate and Hydrochlorothiazide. HPLC grade Water, Acetonitrile and Methanol were procured from Qualigens fine chemicals.

Experimental

A High Performance Liquid Chromatograph system, with LC solutions data handling system (WATERS ALLIANCE 510 Separation module).The data was recorded using AUTOCHROM software. The purity determination performed on a stainless steel column 250mm long, 4.6mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5µm diameter (Hypersil BDS C18, 5µ, 250mm x 4.6mm). Optimized chromatographic conditions are listed in Table 1.

Preparation of Standard stock solution: 25 mg of each drug Amlodipine Besylate and Hydrochlorothiazide were weighed separately and dissolved in 10 ml of HPLC grade methanol and then volume was made up to 25 ml so as to get the concentration 1 mg/ml. From standard stock solution of each drug, appropriate dilution was done using the methanol to get mixed standard solutions containing two drugs in the ratio of 1: 2.5. The final concentration of the solution was 100 µg/ml of Amlodipine Besylate and 250 µg/ml of Hydrochlorothiazide Sonicated for 15min and cooled to room temperature. Mixed well and filtered through Whatman No.1 filter paper. Discarded first few ml of the filtrate. Injected separately 20µl of the standard preparation in to the equilibrated HPLC system in 5 replicate and measured the response of the major peak due to Amlodipine besylate and Hydrochlorothiazide.

Preparation of Sample solution:

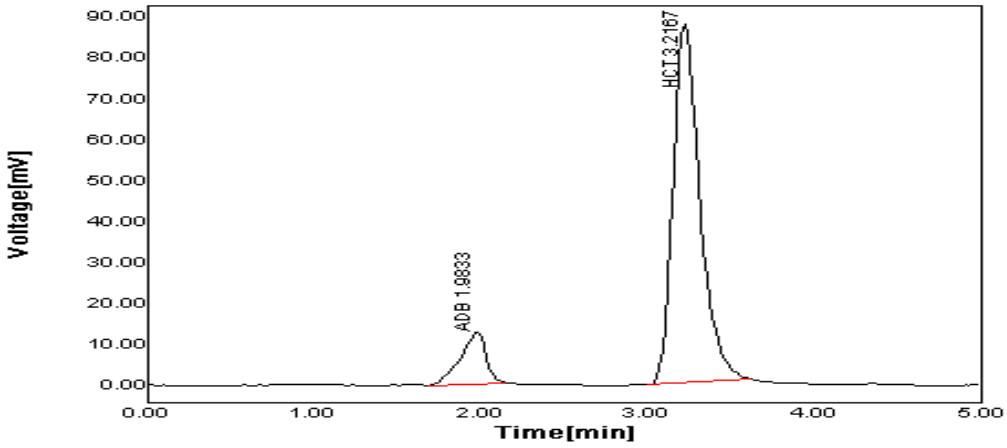
Weighed and finely powdered not less than 20tablets. Transferred an accurately weighed portion of the powder equivalent to about 10mg of each drug Amlodipine Besylate and Hydrochlorothiazide were weighed separately and dissolved in 10 ml mobile phase and then volume was made up to 10 ml so as to get the concentration 1 mg/ml. From standard stock solution of each drug, appropriate dilution was done using the mobile phase to get mixed standard solutions containing two drugs in the ratio of 1: 2.5. The final concentration of the solution was 10 µg/ml of Amlodipine Besylate and 25 µg/ml of Hydrochlorothiazide. Sonicated for 15min and cooled to room temperature. Mixed well and filtered through Whatman No.1 filter paper. Discarded first few ml of the filtrate. Then injected separately 20µl of the sample preparation into duplicate and measured the response of the major peak due to Amlodipine besylate and Hydrochlorothiazide. And calculated the content of Amlodipine besylate and Hydrochlorothiazide.

Validation of the Method

Table 1: Optimized Chromatographic conditions

Parameter	Optimized condition
Instrument	HPLC WATERS Alliance Series 510.Separation module
Mode	Isocratic
Column	Hypersil BDS C18 Dimension: 250mm X 4.6mm, 5 µ
Mobile phase	Acetonitrile :Water(50:50)
Flow rate	1ml/min
Detection	254nm
Injection volume	20µl
Temperature	Ambient

Optimized Chromatogram



Sr. No	Name	Retention Time	Area	Plate Count	Tailing Factor
1	**AMD**	1.9833	132.6957	4356	0.9348
2	**HCTZ**	3.2167	985.3930	2967	0.7694

RESULTS AND DISCUSSION

The method was validated in terms of linearity, accuracy, precision and specificity of the sample applications. The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulation at 50%, 100% and 150% levels. The recovery studies were carried out and the percentage recovery and mean of the percentage recovery were calculated and given in Table 3and 4. From the data obtained, it was observed that the recoveries of standard drugs were found to be accurate and within the specified limits.

The precision of the method was determined by studying repeatability and reproducibility. The area of drug peaks and relative standard deviation were calculated. The results revealed that the developed method was found to be reproducible in nature.

The standard drug solutions in varying concentrations ranging from 20 to 60 % of the targeted level of the assay concentration were examined by the assay procedure. Amlodipine and Hydrochlorothiazide were found to be linear in the range of 20 to 60 mg/ml. The slope, intercept and correlation coefficient values were also calculated that given in Table 6. The correlation coefficient of Amlodipine and Hydrochlorothiazide were found to be 0.9999 and 0.9999 respectively. The calibration curves were plotted as peak area Vs concentration of the standard solutions. The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. These data demonstrates that the methods have adequate sensitivity to the concentration of the analytes.
The Range demonstrates that the method is linear outside the limits of expected use. The additional peaks were observed in the chromatogram of the formulation, which may be due to excipients present in the formulation. These peaks do not interfere with the standard peaks, which clearly confirm the assay method was found to be highly specific.

The system suitability studies were performed for the standard solutions and were presented in Table 2. The values obtained demonstrated the suitability of the system for the analysis of the above drug combination. From the above experimental data results and parameters it was concluded that the developed RP-HPLC method has the following advantages.

As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase ,altering flow rate were made to check the method's capacity to remain unaffected. The change was made in the flow rate study was performed at flow rate 0.9, 1.0 and1.1ml/min. The % RSD was found to be within the limit given in

Table 5.Therefore, the HPLC method for the determination of Amlodipine besylate and hydrochlorothiazide is robust.
Stability study was performed by keeping the sample under various stressed conditions as at 85^0C and at 85^0C by adding 1ml of 0.1N HCl, 0.1N NaOH, 3% H2O2, exposing with UV light at 254nm. Acid and base degraded sample showed degradation product peaks which is well resolved from drug peak, Dry heat degradation and peroxide degradation study revealed that there is no degradation peak for Amlodipine besylate and Hydrochlorothiazide. Amlodipine besylate and Hydrochlorothiazide was found to be stable to peroxide and dry heat at room temperature and at 70 °C . The drug was found to be stable

From the above experimental data results and parameters it was concluded that the developed RP-HPLC method has the following advantages.

- The standard and sample preparation requires less time.
- No tedious extraction procedure was involved in the analytical process.
- Suitable for the analysis of raw materials.
- Run time required for recording chromatograms were less than 15 times.

Hence, the chromatographic method developed for Amlodipine and Hydrochlorothiazide were found to be simple, precise, accurate and cost effective and it can be effectively applied for routine analysis in laboratories, quality control department in industries, approved testing laboratories.

Table 2: System Suitability Parameters

Parameter	Amlodipine Besylate	Hydrochlorothiazide
Area	138.6721	991.8805
Retention Time	1.9700	3.21
Resolution	9.2	
Theorotical Plates	4320.4	2961
Tailing Factor	0.96702	0.7509

Table-3: Data of Accuracy for Amlodipine Besylate

Concentration % of spiked level	Area	Amount added µg/ml	Amount found µg/ml	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	97.8610	19.83	20	99.15%	**MEAN**	100.98%
50% Sample 2	101.9170	20.56	20	101.95%		
50% Sample 3	99.3353	20.36	20	101.84%	**%RSD**	1.21
100 % Sample 1 1111 1111 1 1	130.0623	39.68	40	99.2%	**MEAN**	99.17%
100 %Sample 2	130.3973	40.35	40	100.8%		
100%Sample 3	131.4166	39.01	40	97.52%	**%RSD**	1.65
150%Sample 1	140.8396	59.87	60	99.78%	**MEAN**	100.09%
150%Sample 2	150.4400	59.99	60	99.98%		
150%Sample 3	141.3896	60.31	60	100.5%	**%RSD**	1.25

Table-4: Data of Accuracy for Hydroclorothiazide

Concentration % of spiked level	Area	Amount added µg/ml	Amount found µg/ml	% Recovery	Statistical Analysis of % Recovery	
50% Sample 1	298.1478	19.63	20	98.15	**MEAN**	99.48
50% Sample 2	306.2543	19.82	20	99.1		
50% Sample 3	302.1368	20.24	20	101.2	**%RSD**	1.5
100 % Sample 1	989.0457	39.62	40	99.05	**MEAN**	99.41
100 %Sample 2	999.5291	39.56	40	98.9		
100%Sample 3	1011.8907	40.12	40	100.3	**%RSD**	0.7
150%Sample 1	1325.4521	59.63	60	99.39	**MEAN**	99.58
150%Sample 2	1356.1472	59.84	60	99.74		
150%Sample 3	1329.2463	59.77	60	99.61	**%RSD**	1.1

Table 5 :Data of Robustness

Sr No	Validation Parameter	Avg Peak Area		S.D.		% R.S.D.	
		AMD	HCTZ	AMD	HCTZ	AMD	HCTZ
1	Change in Flow +1	121.9930	831.4928	1.1257	7.7256	0.9	0.9
2	Change in Flow -1	201.281	1339.0785	3.4737	0.01911	1.6	1.8

Table 6: Data of Linearity for Amlodipine Besylate and Hydrochlorothiazide

Sr.No	Conc µg/ml	Area of AMD	Area of HCTZ	Statistical Analysis of AMD		Statistical Analysis of HCTZ	
1	20	58.2869	542.793	**Slope**	2.853	**Slope**	25.94
2	30	86.1396	794.563	**Intercept**	0	**Intercept**	o
3	40	114.9927	1041.208	**Correlation Coefficient**	0.999	**Correlation Coefficient**	0.999
4	50	143.0799	1303.420				
5	60	169.6245	1532.931				

REFERENCES

[1] D.A. Skoog, F.J. Holler, S.R. Crouch, Principle Of Instrumental Analysis, 6th ed., Thomson Publications, India, **2007**, pg. 1-3, 145-147, 180.
[2] FDA Guidance for Industry-Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER), August **2000**
[3] G.R.Chatwal, S.K.Anand, Instrumental Methods of Chemical Analysis, 5th Edition,Himalaya Publishing House, **2002**,pg.149.
[4] P.D. Sethi, 'High Performance Liquid Chromatography', Quantitative Analysis of Pharmaceutical Formulations, 1st ed., CBS Publishers and Distributors, New Delhi, **2001**, pg. 3-11, 116-120.
[5] Remington. The Science & Practice of a Pharmacy Vol. I, 20th Edition. pg .587-613.
[6] Monika Bakshi, Saranjit Singh. Development of validated stability-indicating assay methods—critical review. Journal of Pharmaceutical and Biomedical Analysis 28 (**2002**) pg.1011–1040.
[7] Mallikarjuna Rao, Gowri Sankar.D, *Journal of Pharmacy and Pharmaceutical Science Research* April **2011**, 1 (1) pg.1-5.
[8] Sharma Hemandra Kumar, Vinod Sahu, Rahul Sahu, Neha Sengar, Sneha Kulkerni *International journal Research and Pharmaceutical Science*, **2011**,1(3),pg. 66-74.
[9] M.V. Kumudhani, K. Anand Babu, B. Jayakar, *International Journal Research and Pharmacy* 2 (6) **2011**, pg.144-147.
[10] Safeer K. Anbarasi B.N.,Senthil Kumar *International Journal of ChemTech Research* , Jan-Mar **2010** , Vol.2, (1), pg.21-25.
[11] Mohammad Younus, Karnaker Reddy, Ravindra Reddy, Fasiuddin Arif, *Journal of Pharmacy Research*, **2010**, 3(11), pg.2647-2650.
[12] Jabir Aboobacke, Venkatachalam T, Senthilkumar N, Vijayamiruthraj R, Kalaiselvi P *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **2012** Volume 3 (3) pg.509.
[13] Gaurang P. Pandya and Hitendra S. Joshi Development and validation of stability indicating HPLC assay method for simultaneous determination of amlodipine besylate, olmesartan medoxomil and hydrochlorothiazide in tablet. *Pelagia Research Library*, **2013**, 4(2), pg 145-152.
[14] P.S.Jain**,** M. K. Patel, A.P. Gorle, A.J. Chaudhari and S.J. Surana *Journal of Chromatographic Science* **2012**, pg 1–8.
[15] Richa Sah and Saahil Arora, *Journal of Advanced Pharmacy Education & Research*, 2 (3) 93-100 (**2012**) pg.2249-3379
[16] Alagar Raja M and Selvakumar D, *International Journal of Research and Pharmaceutical science*,1(3), **2010**, pg.369-371