Available online at www.icjpir.com

ISSN: 2349-5448

INTERCONTINENTAL JOURNAL OF PHARMACEUTICAL INVESTIGATIONS AND RESEARCH

ICJPIR |Volume 2 | Issue 2 | April-June - 2015

Research article

Simultaneous analytical method development and validation of amlodipine besylate and hydrochlorthiazide using reverse phase HPLC method in pharmaceutical dosage form

Mohamed H. Assaleh*, Fathi H. Assaleh

Department of Chemistry, Faculty of Science, University of Zawia, Az-Zawiyah, Libya.

Email: mohammed_assaleh@icloud.com

Abstract

The aim of the present work is to develop a precise, accurate, simple and reliable, less time consuming, RP-HPLC method for the simultaneous estimation of Amlodipine besylate and Hydrochlorothiazide in pharmaceutical formulation. First of all, maximum absorbance was found to be at 245 nm and the peaks purity was excellent. Injection volume was selected to be 20 µl which gave a good peak area. The column used for study was Hypersil C_{18}, BDS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0 mL/min because of good peak area and satisfactory retention time. Different ratios of mobile phase were studied, mobile phase with ratio of 50:50 Water: Acetonitrile was fixed due to good symmetrical peaks and for good resolution. The present recovery was found to be 98.0-101.5 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.77 for Amlodipine and 0.35 for Hydrochlorthiazide. The analytical method was found linearity over the range of 20-80 ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

Key Words: RP HPLC method, Amlodipine, Hydrochlorthiazide, Linearity.

Introduction

The modern form of column chromatography has been called high performance, high pressure, and high-resolution and high-speed liquid chromatography[1-6]. High-Performance Liquid Chromatography (HPLC) is a special branch of column chromatography in which the mobile phase is forced through the column at high speed[7-12]. Amlodipine Besylate chemically is [3-ethyl-5-methyl (4RS)-2-[2-aminoethoxy) methyl]-4-(2- chloro phenyl)-methyl-1-dihydropyridine-3,5-dicarboxylate benzene sulphonate is long acting calcium channel blocker used as antihypertensive agents. Hydrochlorothiazide is a first-line calcium sparing thiazide diuretic drug that acts by inhibiting the kidneys' ability to retain water. This reduces cardiac output and hence causes antihypertensive action[13-34]. The aim of the present work is to develop a precise, accurate, simple and reliable, less time consuming, RP-HPLC method for the simultaneous estimation of Amlodipine besylate and Hydrochlorothiazide in pharmaceutical formulation.

EXPERIMENTAL

Materials

Hydrochlorothiazide, API – IPCA Laboratories Gujarat, Amlodipine Besylate API – Cadila Healthcare Ltd., Ankleshwar.

Apparatus and Equipment

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

Chromatographic conditions

Column: Hypersil -BDS C_{18} (250 x 4.6 mm, 5 µ); Detector: 245 nm; Injection Volume: 5 µl; Flow Rate: 1.0 mL min^{-1}; Temperature: 30ºC; Run Time: 5 min; Mobile Phase: Water: Acetonitrile (70:30); Drug RT (min): 1.9 min for Amlodipine, 3.2 min for Hydrochlorthiazide.

Mobile Phase

Degassed Water and Acetonitrile in the ratio of 70:30 V/V.

Preparation of stock solution

Weighed about 10 mg of Amlodipine besylate and Hydrochlorothiazide RS drugs and dissolved in 10 mL of Mobile phase taken in two 10 mL of volumetric flasks separately and sonicated for 20 min to get 1000 ppm and 1 mL was taken from each solution and diluted to 10 mL with mobile phase.

Preparation of working standard solution:

Reference solution (a): The solution was prepared by dissolving 10.0 mg of accurately weighed Amlodipine Besylate RS and 25.0 mg Hydrochlorothiazide RS in methanol, in a 100.0 mL volumetric flask.

Reference solution (b): The solution was prepared by diluting 10.0 mL of reference solution (a) with methanol into a 50.0 mL volumetric flask.

Preparation of sample drug solution for pharmaceutical formulations

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 10 mg Amlodipine and 25 mg Hydrochlorthiazide was weighed and dissolved in the 70 mL methanol with the aid of ultrasonication for 10 min. The content was diluted to 100 mL with methanol to furnish a stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 10.0 mL of the filtrate was diluted into a 50.0 mL volumetric flask to give a test solution containing 20 µg/mL Amlodipine and 50 µg/mL Hydrochlorthiazide.

Validation of Data

System suitability testing

The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Amlodipine besylate and Hydrochlorothiazide combination, retention times and peak areas. The %RSD for retention times and peak areas were found to be within the limit. The results are summarized in Tables 1 and 2.

Table 1: System suitability parameters of amlodipine besylate

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	1.9663	141	9314	0.9888
2	1.9665	143	9462	0.9577
3	1.9834	142	9264	0.9786
4	1.9667	141	9765	0.9355
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 2: System suitability parameters of hydrochlorthiazide

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

Specificity

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system. The chromatograms of Standard and Sample were same identical with same retention time.

Precision

1. System precision: Standard solution prepared as per test method and injected five times.
2. Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

Test results are showing that the test method is precise. Refer tables 2 and 3 for system precision and for method precision.

Intermediate precision (analyst to analyst variability):

Individual %assays and %RSD of Assay are within limit and passes the intermediate precision. The results are shown in Tables 3 and 4.

Table 3: Precision data of hydrochlorthizide

Concentration 40 ppm	Injection	Peak Areas of Hydrochlorothiazide	% Assay
	1	998	100.11
	2	997	100.00
	3	999	100.17
	4	998	100.10
	5	997	99.96
	6	999	100.18
Statistical Analysis	**Mean**	998.5998	100.09
	SD	0.889	0.0894
	% RSD	0.089	0.089

Table 4: Precision data of Amlodipine besylate

Concentration 40ppm	Injection	Peak Areas of Amlodipine besylate	% Assay
	1	140	98.63
	2	142	100.02
	3	143	100.68

	4	142	100.08
	5	142	99.44
	6	141	99.00
Statistical Analysis	**Mean**	142.2995	99.64
	SD	1.0824	0.7602
	% RSD	0.76	0.76

Accuracy (recovery)

Drug Assay was performed in triplicate as per test method with equivalent amount of Amlodipine besylate and Hydrochlorothiazide into each volumetric flask for each spike level to get the concentration of Amlodipine besylate and Hydrochlorothiazide equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The recovery results indicating that the test method has an acceptable level of accuracy (Table 5 and 6).

Table 5: Recovery studies of 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
150% Injection 2	60	59.78	99.63		
150% Injection 3	60	59.93	99.88	**%RSD**	0.23

Table 6: Recovery studies of hydrochlorthizide

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		
50% Injection 3	20	20.00	100.00	**%RSD**	0.10

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
150% Injection 2	60	60.03	100.05		
150% Injection 3	60	60.02	100.03	**%RSD**	0.053

Linearity of test method

A Series of solutions are prepared using Amlodipine besylate and Hydrochlorothiazide working standards at concentration levels from 25ppm to 150 ppm of target concentration. Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

The linear fit of the system was illustrated graphically. The results are presented in Figure 1 and 2.

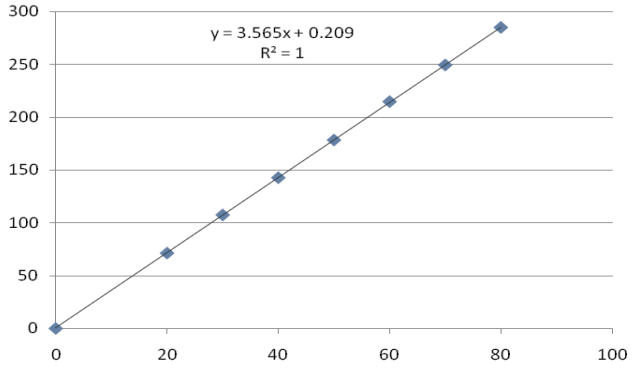

Figure 1: Linearity plot of amlodipine besylate

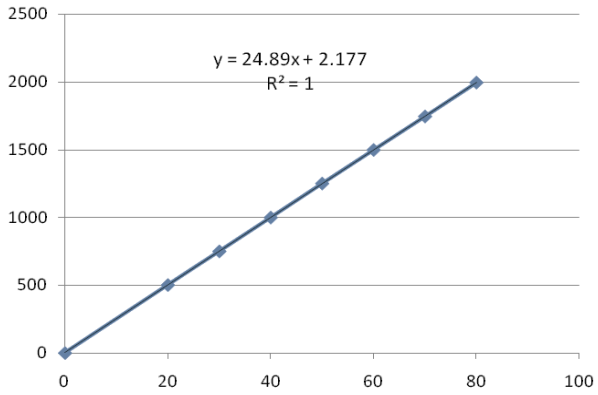

Figure 2: Linearity plot of hydrochlorthiazide

Ruggedness of test method

System to system variability

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability. The % RSD was found within the limit (Table 7 and 8).

Column to column variability

Column to column variability study was conducted by using different columns. Six samples were prepared and each was analysed as per test method

The results obtained by comparing with both two types were within limit. Refer tables: 3 and 4

Table 7: Assay of amlodipine besylate

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 8: Assay of hydrochlorthizide

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

Robustness

a) Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0 mL/min and 1.2 mL/min. The system suitability parameters were evaluated and found to be within the limits for 1.0 mL/min and 1.2 mL/min flow.

Amlodipine besylate and Hydrochlorothiazide was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0 mL/min. The tailing factor for Amlodipine besylate and

Hydrochlorothiazide was found to be within the limits as shown in Table 9.

b) Effect of variation of temperature:

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºC. The tailing factor for Amlodipine besylate and Hydro chlorothiazide is found to be within the limits.

Table 9: Tailing factors of proposed method

Flow 0.8 mL	Std Area	Tailing factor	Flow 1.0 mL	Std Area	Tailing factor	Flow 1.2 mL	Std Area	Tailing 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

Limit of detection and quantitation (LOD and LOQ):

From the linearity data calculate the limit of detection and quantitation, using the following formula.

LOD= 3.3 σ/S

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

LOQ = 10 σ/S

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

RESULT AND DISCUSSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 245nm and the peaks purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Hypersil C_{18}, BDS chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0 mL/min because of good peak area and satisfactory retention time. Different ratios of mobile phase were studied, mobile phase with ratio of 50:50 Water:Acetonitrile was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed 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 min because analyze gave peak around min and also to reduce the total run time. The present 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 within range. Detection limit was found to be 0.77 for Amlodipine and 0.35 for Hydrochlorthiazide. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 20-80ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

CONCLUSION

Thus proposed HPLC method was found to be simple, accurate, precise selective and economical for simultaneous routine analysis of Hydrochlorothiazide and Amlodipine besylate in tablet dosage form.

REFERENCES

1. R. W. Yost, L.S. Ettre and R. B. Conlon, Practical Liquid Chromatography;1980.
2. Snyder L.R, Gilajch J.L. Practical HPLC method development, ED-2nd, John Wiley Sons, 1998:21-50.
3. Kaur.H, Pragathi Prakashan, Meerut. High Performance Liquid Chromatography, Instrumental methods of chemical analysis, ED-3rd, 2006:798 -809.
4. Sethi P.D. HPLC Quantitative Analysis of Pharmaceutical Formulations, CBS Publishers and distributors, New Delhi, 2001:58-67.
5. The International Pharmacopoeia, WHO, Vol.1-5, Ed-3rd,2003-2004:667.
6. Verzele M, Dewaele C and M. D, Micro-LC: what are its chances? LC-GC,1988:6, 966,
7. International Conference on Harmonization, Draft Guideline on Validation of Analytical Procedure, Federal Register, (1995), 11260-11262.
8. Johnson J. D. and Van Buskirk G. E., Analytical Method Validation. J. Validation Technol 1998: 88-89.
9. International Conference on Harmonization, Validation of Analytical Procedure: Methodology, Federal Register,1996:1.
10. Loyd R. Synder, Joseph j. Kirkland, Joseph L. Glajch, Practical HPLC Method Development ED-2nd, 1997.
11. Sethi P.D., HPTLC Quantitative Analysis of Pharmaceutical Formulations, ED-1st., CBS Publishers and distributors, New Delhi, 1996:22.
12. Connors K.A., A Textbook of Pharmaceutical Analysis, ED-3rd, A Wiley- Interscience Publication, John Wiley and Sons, 1982:173-201.
13. The Merck Index, Maryadele J.O., ED-13th., Published by Merck research lab, Division of Merck and co, White house station, NJ, USA, 2001:148.
14. The United State Pharmacopoeia USP 28/NF 23, U S Pharmacopoeial Convention, Inc., Rockville, 2174. 2005.
15. D. N. Vora and A. A. Kadav. Development and Validation of a Simultaneous HPLC Method for Estimation of Bisoprolol Fumarate and Amlodipine Besylate from Tablets. Indian J Pharm Sci. 2008 Jul-Aug; 70(4): 542–546. doi: 10.4103/0250-474X.44616.
16. P. S. Jain, M. K. Patel, S. B. Bari, and S. J. Surana. Development and Validation of HPTLC Method for Simultaneous Determination of Amlodipine Besylate and Metoprolol Succinate in Bulk and Tablets. Indian J Pharm Sci. 2012 Mar-Apr; 74(2): 152–156.doi: 10.4103/0250-474X.103849.
17. K. Safeer, B. Anbarasi, N. Senthil Kumar. Analytical Method Development and Validation of Amlodipine and Hydrochlorothiazide in combined dosage form by RP-HPLC. International Journal of Chem.Tech Research. CODEN(USA): IJCRGG ISSN: 0974-4290. Vol.2, No.1, pp 21-25, Jan-Mar 2010
18. Bokya, G. Tsvetkova, Lily P. Peikova. Development and validation of RP-HPLC method for simultaneous determination of Amlodipine Besylate and Hydrochlorothiazide in pharmaceutical dosage form. Journal of Chemical and Pharmaceutical Research, 2013, 5(1):271-275
19. Gaurav Patel, Sanjay Patel, Dhamesh Prajapiti, Rajendra Mehta. RP-HPLC Method for Simultaneous Estimation of Amlodipine Besylate and Hydrochlorothiazide in Combined Dosage Forms. Stanford journal of pharmaceutical sciences. Vol 3, no 1 (2010): 49-53
20. N. Delhiraj, S. Anbazhagan, Delhiraj. validated chromatographical methods for the simultaneous estimation of antihypertensive drugs in pharmaceutical dosage forms. Int J Pharm 2013; 3(1):103-107 ISSN 2249-1848
21. Nilesh Jain, Ruchijain, Jitendrabanweer, Deepakkumarjain. Development and validation of a rapid RP-HPLC method for the determination of Amlodipine besylate and olmesartan medoxomil in their combined tablet formulation. 08 Jan 2010.
22. R.S.CH. Phani, K.R.S. Prasad, Useni Reddy Mallu. High resolution RP-HPLC method for the determination of hypertensive drug products. Int J Pharm Bio Sci 2013 Jan; 4(1): (P) 440 – 454

23. Lakshmi Narasimham Y.S, Barhate V.D. 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. 4:1 (2010) 1-20
24. Vidhya K. Bhusari, Sunil R. Dhaneshwar. Validated HPTLC Method for Simultaneous Estimation of Atenolol, Hydrochlorothiazide and Amlodipine Besylate in Bulk Drug and Formulation. International Journal of Analytical and Bioanalytical Chemistry.05 October 2011
25. CVN. Prasad, Ch. Santhosh Kumari and Sree Ramulu j. simultaneous determination of telmisartan, Amlodipine besylate and hydrochlorothiazide in a combined poly pill dosage form by stability-indicating high performance liquid chromatography. international journal of research in pharmacy and chemistry 2011, 1(3) ISSN: 2231□2781
26. S. T. Kumbhar, G. K. Chougule, V. S. Tegeli, G. B. Gajeli, Y. S. Thorat, U. S. Shivsharan. A Validated HPTLC Method for Simultaneous Quantification of Nebivolol and Hydrochlorothiazide in Bulk and Tablet Formulation. International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(1): 62-66
27. M. M. Deshpande, M P. Mahajan and S. D. Sawant. Simultaneous estimation of valsartan and hydrochlorothiazide in fixed dose combination in UV spectrophotometry. IJPSR, 2012; Vol. 3(1): 236-240
28. RB Kakde, VH Kotak, AG Barsagade, NK Chaudhary and DL Kale. Spectrophotometric Method for Simultaneous Estimation of Amlodipine Besylate and Bisoprolol Fumarate in Pharmaceutical Preparations. Research J. Pharm. and Tech. 1(4): Oct.-Dec. 2008,
29. Nilesh Jain, Ruchi Jain, Jitendra Banweer, Deepak Kumar Jain. Development and validation of a rapid RP HPLC method for the determination of Amlodipine besylate and olmesartan medoxomil in their combined tablet formulation. International Journal of Current Pharmaceutical Research Vol 2, Issue 2, 2010
30. Mohammad Younus, T. Karnaker Reddy, Y. Ravindra Reddy, Md. Fasiuddin Arif. RP-HPLC Method Development and Validation for Simultaneous Estimation of Amlodipine besylate, Valsartan and Hydrochlorothiazide in Tablet Dosage Form. Journal of Pharmacy Research 2010, 3(11),2647-2650
31. Delhiraj N and Anbazhagan S. Validated chromatographical methods for the simultaneous estimation of antihypertensive drugs in pharmaceutical dosage forms. Int J Pharm 2013; 3(1):103-107
32. Ravisankar Panchumarthy, Devala Rao Garikapati. An Improved Rapid HPLC Method for the Separation of Five Anti-Hypertensive Agents Using C18 Column: Application to Hydrochlorothiazide Determination in Bulk and Tablet Dosage form. IOSR Journal Of Pharmacy. Volume 3, Issue 5 (June 2013), pp. 07-19
33. Naman doshi, Avani sheth, C N Patel. Validated reverse phase high performance liquid chromatographic method for simultaneous estimation of olmesartan medoxomil, hydrochlorothiazide and Amlodipine besylate in newly designed pharmaceutical dosage form. International Research Journal of Pharmacy. 2012, 3(11).
34. Bheru S.Malviya, Prince Shivhare, Pramod Hinnariya and Vidhi Jain. Development and Validation of UPLC Method for Simultaneous Determination of Irbesartan, Hydrochlorothiazide, Amlodipine Besylate and Amlodipine besylate. International journal of drug discovery and herbal research (ijddhr) 2(4): Oct.-Dec.: (2012), 481-486.