Research Article
Volume 13:5, 2022

Chemical Sciences Journal

ISSN: 2150-3494 Open Access

Development and Validation of RP-HPLC Chromatographic Method for the Simultaneous Estimation of Perindopril Erbumine and Amlodipine Besylate in Formulation

Sachin Babar*, S. L. Padwal and P. V. Raut

Department of Chemistry, K B P Mahavidyalaya Pandharpur, Maharashtra, India

Abstract

The present paper reports the simple, rapid, accurate and precise RP-HPLC method for the simultaneous estimation of Perindopril erbumine and Amlodipine besylate in bulk and formulated drug substance. The reverse phase liquid chromatographic analysis has been performed on a Kromasil C8 (4.6 mm × 250 mm, 5 µ particle size) column with mobile phase Buffer (6.8 g Potassium dihydrogen orthophosphate) and Acetonitrile in the ratio 59:41 with adjusted pH 2.6 with orthophosphoric acid and column oven temperature 40°C. The flow rate of mobile phase was adjusted 1.0 ml/min. and the injection volume 10 µl. Detection was performed at 210 nm. The retention time of Perindopril erbumine and Amlodipine Besylate were found to be 4.483 min. and 6.767 min, the linearity was observed in the concentration range from 20% to 160% of nominal concentration of Perindopril erbumine and Amlodipine Besylate correlation coefficient was 0.999 for both drugs. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 99.4% for perindopril erbumine and 99.6% for Amlodipine besylate. The %RSD below 2.0 shows high precision of proposed method.

Keywords: RP-HPLC • UV detector • Amlodipine besylate • Perindopril erbumine • Tablet dosage form

Introduction

Perindopril erbumine1 is chemically 2-Methylpropan-2-amine (2S, 3aS, 7aS)-1-[(2S)-2-[[(1S)-1-(ethoxy carbonyl) butyl] amino Propanoyl] octahydro ethyl-1H-indole-2-carboxylate. It is angiotension -converting enzyme inhibitor. It is used in patients with hypertension and heart failure. Having molecular formula of dicarboxylate calcium $C_{23}H_{43}N_3O_5$ and molecular weight 441.613 g/mol. Its pressure solubility freely soluble in water and in ethanol (96 per cent), soluble or sparingly soluble in methylene chloride. Amlodipine Besylate1 is chemically 3-Ethyl 5-methyl- 2-[(2-aminoethoxy) methyl]-4-(2-chloro-phenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzensulfate. Belongs to the class of calcium channel blocker that widens blood vessels. Having molecular formula $C_{26}H_{31}ClN_2O_8S$ and the molecular weight was 567.05 g/mol. Its solubility slightly soluble in water, freely soluble in methanol, sparingly soluble in anhydrous ethanol, slightly soluble in 2-propanol [1].

The perindopril erbumine and Amlodipine besylate tablets are a combination of two active ingredients, perindopril and Amoldipine. Amlodipine is a calcium channel blocker that dilates (widens) blood vessels and perindopril is an angiotensin converting enzyme inhibitor. Together they work to widen and relax the blood vessels, which results in a reduction of blood pressure. So, blood can flow through the body very easily. From detailed literature survey, it was found that individual and combination these drugs has been analyzed by many spectroscopic methods. The Amlodipine besylate API is official in British pharmacopoeia and Indian pharmacopoeia. And the Perindopril erbumine is official in the British. The combination of perindopril erbumine and Amlodipine besylate is not included in any pharmacopoeias. So, objective of this work describes simple, rapid, economical, selective, precise and reproducible HPLC method for pharmaceutical importance. The method was validated as per ICH guidelines [2].

Materials and Methods

Reagents and chemicals

Acetonitrile (HPLC Grade), Orthophosphoric acid (AR Grade), Potassium dihydrogen orthophosphate (Merck, AR Grade), water (HPLC Grade), The standard drug samples of perindopril erbumine

***Address to Correspondence:** Sachin Babar, Department of Chemistry, K. B. P. Mahavidyalaya Pandharpur, Maharashtra, India, Tel: 8600855040; E-mail: sachin.babar86@gmail.com

Received: 14 October, 2019, Manuscript No. CSJ-19-3538; **Editor assigned:** 17 October, 2019, PreQC No. P-3538; **Reviewed:** 31 October, 2019, QC No. Q-3538; **Revised:** 28 June, 2022, QI No. Q-3538; Manuscript No. R-3538; **Published:** 26 July, 2022, DOI: 10.37421/ 2150-3494.2022.13.5.290

and amlodipine besylate, as well as tablet available in the ratio of 1:1 containing perindopril erbumine 5 mg and Amlodipine 5 mg, perindopril erbumine 10 mg and Amlodipine 10 mg gifted from Generic Healthcare Pvt. Ltd., Pune. Analysis was performed on chromatographic system consisted of Shimadzu, series LC 2010 A (pump Quaternary system). Separation was carried out with Kromasil C8 (4.6 mm × 250 mm, 5 µ particle size) column at 40°C temperature, flow rate 1.00 mL per minute with an isocratic mobile phase constituting Buffer and Acetonitrile in the ratio 59:41 pH adjusted to 2.6 with orthophosphoric acid. perindopril and Amlodipine was determined by UV detection at 210 nm. The injection volume of 10 µL and the run time was 10 min.

Preparation of buffer solution for mobile phase

About 6.8 gm of potassium dihydrogen orthophosphate were accurately weighed and transfer in to 500 mL of HPLC grade water, shake and sonicate to dissolved completely and finally make the solution 1000 mL with HPLC grade water [3].

Preparation of standard solution

50 mg of Perindopril erbumine and 69 mg of Amlodipine Besylate were weighed accurately and transferred to 100 mL volumetric flask and dissolved in 70 mL of mobile phase and then volume was made up to the mark with mobile phase to get 500 µg/mL of perindopril erbumine and 690 µg of Amlodipine besylate stock solution respectively. The final solution was prepared by 5 mL of this solution in to 100 mL volumetric flask then volume was made up to the mark with mobile phase to get 50 µg/mL of perindopril erbumine and 69 µg/mL of Amlodipine besylate respectively. Figure 1 represents the typical chromatogram of standard Perindopril and Amlodipine respectively [4].

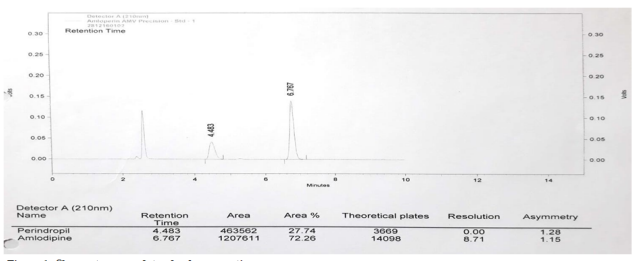

Figure 1. Chromatogram of standard preparation.

Preparation of sample solution

For assay, twenty tablets labeled as containing 5 mg of perindopril erbumine and 5 mg of Amlodipine besylate together with excipients, was accurately weighed, and finely powdered. The weight of powder equivalent to 5 mg of Perindopril erbumine and 5 mg of Amlodipine was weighed accurately and transferred in to 100 mL volumetric flask and added 50 mL mobile phase. The contents were sonicated for 10 mins. Then cooled and volume made up to the mark with mobile phase. Filtered sufficient amount of this solution through 0.45 µm membrane syringe filter. The final solution was prepared by 5 mL of this filtered solution in to 100 mL volumetric flask then volume was made up to the mark with mobile phase to get 50 µg/mL of perindopril erbumine and 69 µg/mL of Amlodipine besylate respectively. Figure 2 represents the typical chromatogram of sample Perindopril and Amlodipine respectively [5].

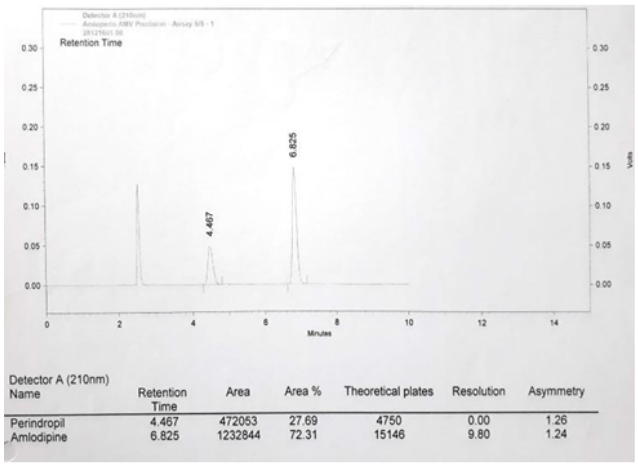

Figure 2. Chromatogram of sample preparation (assay).

For content uniformity, one tablet was placed in to each of ten 100 mL volumetric flask. Approximately 70 mL of mobile phase was added to each volumetric flask and sonicate until the tablets were dispersed in the solution. The resultant solutions were cooled and volume was made up to the mark with the mobile phase. The solution was shaken well for uniform distribution. Filtered a portion of solution using 0.45 µm membrane syringe filter and filtrate was injected for analysis. Figure 3 represents the typical sample chromatogram of Perindopril and Amlodipine respectively.

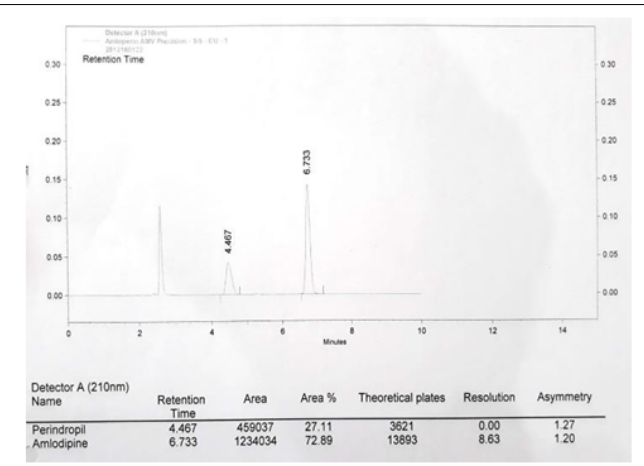

Figure 3. Chromatogram of sample preparation (CU).

Results and Discussion

Specificity and system suitability

Specificity test determines the effect of excipients on the assay result. To determine the specificity of method, filtered as well as unfiltered solutions of blank, placebo, diluent and standard of Perindopril erbumine and Amlodipine Besylate injected. System suitability study of the method was carried out by five replicate analysis of solution containing 100% target concentration of perindopril erbumine and Amlodipine besylate. Various chromatographic parameter such as retention time, peak area, column efficiency, tailing factor and resolution betweenion the peaks were determined and the method was evaluated by analyzing these parameters [6].

Linearity and range

Linearity of the method was determined by constructing calibration curves. Standard solution of perindopril erbumine and Amlodipine besylate of different concentration level (20%, 40%, 60%, 100%, 120%, 140% and 160%) were used for this purpose.

Each measurement was carried out and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients [7]. Tables 1 and 2 represents the results were directly proportional to the concentration of analyte in the sample.

Linearity level	Level 1 (20%)	Level 2 (40%)	Level 3 (60%)	Level 4 (100%)	Level 5 (120%)	Level 6 (140%)	Level 7 (160%)	Regression coefficient	% Y intercept
Conc. of perindopril w.r.t. working level conc. (i.e. 0.5 mg/ml)	0.01	0.02	0.03	0.05	0.06	0.07	0.08		
Peak area injection 1	98762	190813	288351	480470	576022	672996	765324		
Peak area injection 2	98817	190906	288256	479660	576169	672772	765810		
Peak area injection 3	98903	190716	288507	480000	576429	673135	768005	0.99999	0.3
Peak area injection 4	98907	-	-	479778	-	-	766996		
Peak area injection 5	98975	-	-	479484	-	-	766358		
Average peak area	98873	190812	288371	479878	576207	672968	766499		
Response factor	9887300	9540600	9612367	9597560	9603450	9613829	9581238		
Relative response factor	1.0302	0.9941	1.0015	1	1.0006	1.0017	0.9983		
Average	1.0038								
%RSD	1.19								

Table 1. Linearity and range (for perindopril peak).

Linearity level	Level 1 (20%)	Level 2 (40%)	Level 3 (60%)	Level 4 (100%)	Level 5 (120%)	Level 6 (140%)	Level 7 (160%)	Regression coefficient	% Y intercept
Conc. of perindopril w.r.t. working level conc. (i.e. 0.5 mg/ml)	0.01	0.02	0.03	0.05	0.06	0.07	0.08		
Peak area injection 1	252727	485684	733158	1219653	1460105	1704408	1936794		
Peak area injection 2	252303	485804	732671	1216435	1459867	1702293	1935237		
Peak area injection 3	252403	485529	733670	1218202	1459349	1703700	1941934	0.99999	0.6
Peak area injection 4	252280	-	-	1216642	-	-	1938703		
Peak area injection 5	252394	-	-	1216177	-	-	1937040		

Average peak area	252421	485672	732833	1217422	1459774	1703467	1937942
Response factor	25242100	24283600	24427767	24348440	24329567	24335243	24224275
Relative response factor	2.6301	2.5302	2.5452	2.5369	2.5350	2.5356	2.5240
Average	2.5481						
%RSD	1.44						

Table 2. Linearity and range (for amlodipine peak).

Precision

System precision and method precision: Method precision performed by carrying out standard replicate and six independent sample preparations of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. The percentage relative standard deviation for both analyte was found less than 2.0%.

Intermediate precision: Intermediate precision performed by carrying out standard replicate and six independent sample by two different analyst using different chromatographic system on different days. The chromatographic sample results are summerised in below Tables 3 and 4, the percentage relative standard deviation for both analytes was found less than 3.0% [8].

Sr. No	Method precision			Intermediate precision (Analyst 2, day 2)		
	Content in %			Content in %		
	Perindopril	Amlodipine	Perindopril (MP)	Perindopril (IP)	Amlodipine (MP)	Amlodipine (IP)
1	99.7	100	99.7	98.5	100	97
2	99.9	100.2	99.9	98.3	100.2	96.8
3	100.3	100.6	100.3	98	100.6	96.5
4	100.1	100.4	100.1	98.3	100.4	96.8
5	100.2	100.5	100.2	97.9	100.5	96.4
6	99.6	99.9	99.6	98.8	99.9	97.4
Mean	100	100.3	99.1		98.5	
%RSD	0.3	0.3	0.9		0.9	

Table 3. Precision study for assay.

Accuracy (Trueness): The accuracy of the test method was determined by preparing recovery samples in triplicate by standard addition method at 50%, 100% and 150% known amount of standard Perindopril erbumine and Amlodipine besylate and calculated the % recovery as % label claim and amount recovered [9]. It obtained between 98.0% to 102.0% of labeled amount. The values were given in Table 5.

Sr. No	Method precision			Intermediate precision (Analyst 2, day 2)		
	Content in %			Content in %		
	Perindopril	Amlodipine	Perindopril (MP)	Perindopril (IP)	Amlodipine (MP)	Amlodipine (IP)
1	97.7	100.9	97.7	101.8	100.9	99.4
2	98.1	101.4	98.1	97.1	101.4	100.8
3	97.9	101.2	97.9	102.1	101.2	99.7
4	102.1	103.9	102.1	97.2	103.9	100.9

5	102.3	104.1	102.3	97.4	104.1	101.1
6	97.8	100.8	97.8	102.2	100.8	99.7
7	98.2	101.4	98.2	97.4	101.4	101.2
8	97.9	101	97.9	102.4	101	99.8
9	102.1	103.8	102.1	97.4	103.8	101.1
10	102.5	104.1	102.5	97.3	104.1	101
Mean	99.7	102.3	99.4		101.4	
% RSD			2.3		1.4	

Table 4. Precision study for CU.

Levels	Perindopril	Amlodipine
1 (50%)	99.4	99
2 (100%)	99.7	100.8
3 (150%)	99	98.9
Average	99.4	99.6
% RSD	0.4	1.1

Table 5. 3% recovery.

Robustness: To evaluate the robustness of the developed RP-HPLC method, small deliberate variation in optimised method parameters were done. The effect of change in flow rate, column oven temperature and mobile phase composition. The method was found to be unaffected by small changes ± 0.1 mL changes in flow rate, ± 2°C changes in column oven temperature and ± 5% changes in mobile phase composition [10].

Effect of variation in flow rate: The study was performed to determine the effect of variation in flow rate. The standard and test solution was prepared and injected in to the HPLC system by keeping flow rate 0.9 mL/min, 1.0 mL/min and 1.1 mL/min and evaluated system suitability parameter. The values were given in Table 6.

Flow rate 0.9 ml/Min			Flow rate 1.1 ml/Min		
Sr. No.	**Perindopril in %**	**Amlodipine in %**	**Sr. no.**	**Perindopril in %**	**Amlodipine in %**
Sample-A	97.5	100.3	Sample-A	97.6	100.1
Sample-B	98	100.9	Sample-B	98.1	100.5
Average	97.8	100.6	Average	97.9	100.3

Table 6. Flow rate (Robustness).

Column oven temperature 38°C			Column oven temperature 42°C		
Sr. No.	**Perindopril in %**	**Amlodipine in %**	**Sr. No.**	**Perindopril in %**	**Amlodipine in %**
Sample-A	98.1	100.9	Sample-A	97.6	100.7
Sample-B	97.9	100.7	Sample-B	97.6	100.6
Average	98	100.8	Average	97.6	100.7

Table 7. Column oven temperature (Robustness).

Effect of variation in column oven temperature: The study was performed to determine the effect of variation in temperature. The standard and test solution was prepared and injected in to the HPLC system by keeping temperature 28°C, 30°C and 32°C and evaluated system suitability parameter. The values were given in Table 7.

Effect of variation in mobile phase: The study was performed to determine the effect of variation in mobile phase composition. The standard and test solution was prepared and injected in to the HPLC system by changing ± 5% mobile composition and evaluated system suitability parameter. The values were given in Table 8.

	Mobile phase (-5%)			Mobile phase (+5%)	
Sr. No.	**Perindopril in %**	**Amlodipine in %**	**Sr. No.**	**Perindopril in %**	**Amlodipine in %**
Sample-A	99.3	99.1	Sample-A	99.2	98.9
Sample-B	99.1	98.9	Sample-B	98.6	98.5
Average	99.2	99	Average	98.9	98.7

Table 8. Mobile phase (Robustness).

Solution stability

To demonstrate the stability of standard solution during analysis, solution was analysed over a period of 24th at room temperature. The results showed that for all the solutions, the retention times and peak areas of Perindopril and Amlodipine remained almost unchanged (RSD%) indicating that no significant degradation occurred within this period. i.e. solutions were stable for at least 24th hour [11]. Which was sufficient to complete the whole analytical process. The results were displayed in Table 9.

Sr. No.	Time interval in hour	Content of perindopril in %	% Relative difference with time interval	Content of Amlodipine in %	% Relative difference with time interval
1	2 hour	96.4	0	97.2	0.1
2	4 hour	96.4	0	97.2	0.1
3	8 hour	96.3	0.1	97	0.31
4	12 hour	96.4	0	97.1	0.21
5	16 hour	96.3	0.1	96.9	0.41
6	20 hour	96.3	0.1	97	0.31
7	24 hour	96.3	0.1	96.9	0.41

Table 9. Solution stability.

Conclusion

From the results obtained by all validation parameters, it is concluded that developed RP-HPLC method is sensitive, accurate, linear, Robust, precise and can be adopted for routine analysis for simultaneous estimation of Perindopril erbumine and Amlodipine Besylate.

References

1. Guideline, ICH Harmonised Tripartite. "Validation of Analytical Procedures: Text and Methodology." *Q2 (R1)* 1 (2005): 5.
2. Duraisamy, Kalaiyarasi, and Jaganathan KS. "Method Development and Validation of HPLC Tandem/Mass Spectrometry for Quantification of Perindopril Arginine and Amlodipine Besylate Combination in Bulk and Pharmaceutical Formulations." *Res Pharm Sci* 12 (2017): 307.
3. Patel, Deval B, Mehta Falgun A, and Bhatt Kashyap K. "Simultaneous Estimation of Amlodipine Besylate and Indapamide in a Pharmaceutical Formulation by a High Performance Liquid Chromatographic (RP-HPLC) Method." *Sci Pharm* 80 (2012): 581-590.
4. Zaazaa, Hala E, Abbas Samah S, Essam Hebat Allah M, and El-Bardicy Mohammed G. "Validated Chromatographic Methods for Determination of Perindopril and Amlodipine in Pharmaceutical Formulation in the Presence of their Degradation Products." *J Chromatogr Sci* 51 (2013): 533-543.
5. Erk, Nevin. "Comparison of Spectrophotometric and an LC Method for the Determination Perindopril and Indapamide in Pharmaceutical Formulations." *J Pharm Biomed Anal* 26 (2001): 43-52.
6. Gumieniczek, Anna, Maczka Paulina, Inglot Tadeusz, and Pietras Rafal, et al. "New HPLC Method for *in vitro* Dissolution Study of Antihypertensive Mixture Amlodipine and Perindopril Using an Experimental Design." *Central Eur J Chem* 11 (2013): 717-724.
7. Gumustas, Mehmet, and Ozkan Sibel A. "A Validated Stability-Indicating RP-LC Method for the Simultaneous Determination of Amlodipine and Perindopril in Tablet Dosage Form and their Stress Degradation Behavior under ICH-Recommended Stress Conditions." *J AOAC Int* 96 (2013): 751-757.
8. Ali, Nouruddin W, Abdelwahab Nada S, Zaki Marco M, and Abdelkawy M. "Validated Chromatographic Methods for Simultaneous Determination of Amlodipine Besylate and Perindopril Arginine in Binary Mixtures and in Pharmaceutical Dosage Form." *J Chromatogr Sep Tech* 3 (2012): 134-139.
9. Szabo, Zoltan-Istvan, Reti Zenko-Zsuzsanna, Gagyi Laszlo, and Kis Erika Lilla, et al. "Simultaneous Quantification of Related Substances of Perindopril Tert-Butylamine Using a Novel Stability Indicating Liquid Chromatographic Method." *J Chromatogr Sci* 53 (2015): 424-430.
10. Georgakakou, Sofia, Kazanis Michael, and Panderi Irene. "Hydrophilic Interaction Liquid Chromatography/Positive Ion Electrospray Ionization Mass Spectrometry Method for the Quantification of Perindopril and its Main Metabolite in Human Plasma." *Anal Bioanal Chem* 397 (2010): 2161-2170.
11. Bhadani, S, and Sellappan M. Development and Validation of RP-HPLC for Simultaneous Estimation of Perindopril Erbumine and Indapamide in Combined Dosage Form. *Am J Pharm Tech Res* 3 (2013): 703-711.

How to cite this article: Babar, Sachin, SL Padwal, and PV Raut . " Development and Validation of RP-HPLC Chromatographic Method for the Simultaneous Estimation of Perindopril Erbumine and Amlodipine Besylate in Formulation." *Chem Sci J* 13 (2022): 290.