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SIMULTANEOUS ESTIMATION OF PERINDOPRIL AND AMLODIPINE IN COMBINED DOSAGE FORM BY RP-HPLC METHOD

V. BHASKARA RAJU[a] and A. LAKSHMANA RAO[*]

V. V. Institute of Pharmaceutical Sciences, GUDLAVALLERU – 521356 (A.P.) INDIA
[a]Sri Vasavi Institute of Pharmaceutical Sciences, TADEPALLIGUDEM – 534101 (A.P.) INDIA

ABSTRACT

An accurate and precise HPLC method was developed for the simultaneous determination of perindopril and amlodipine. Separation of the drugs was achieved on a reverse phase C_{18} column using mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 65 : 35 v/v. The flow rate was 0.6 mL/min and the detection wavelength was 237 nm. The linearity was observed in the range of 10-50 µg/mL for amlodipine and 200-1000 µg/mL for perindopril. The proposed method was validated for its linearity, accuracy, precision and robustness. The proposed method can be employed to estimate the drug contents in marketed formulations.

Key words: Perindopril, Amlodipine, RP-HPLC, Validation.

INTRODUCTION

Perindopril erbumine is a nonsulfhydryl prodrug that belongs to the angiotensin-converting enzyme (ACE) inhibitor class of medications. Chemically, it is (2S, 3aS, 7aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxopentan-2-l]amino]propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic acid[1,2] (Fig. 1). Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Amlodipine besylate is chemically described as 3-ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate[1,2] (Fig. 2). A few spectroscopic[3-6], GC[7], HPLC[8-13], LC-MS[14] and TLC[15] methods were reported earlier for the individual determination of perindopril and amlodipine in pharmaceutical dosage forms. Till now no HPLC method has been developed for the estimation of these drugs simultaneously. In this communication, We proposed a rapid, sensitive, accurate and precise HPLC method

[*]Author for correspondence; Ph.: (M) +098660 99916; E-mail: dralrao@gmail.com

for the simultaneous estimation of perindopril and amlodipine in bulk samples and in tablet dosage forms.

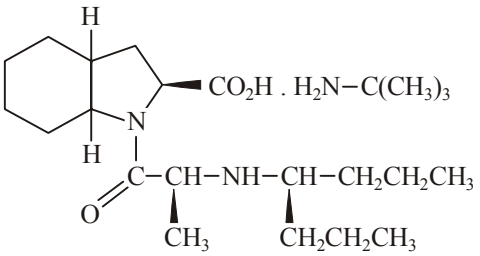

Fig. 1: Chemical structure of perindopril

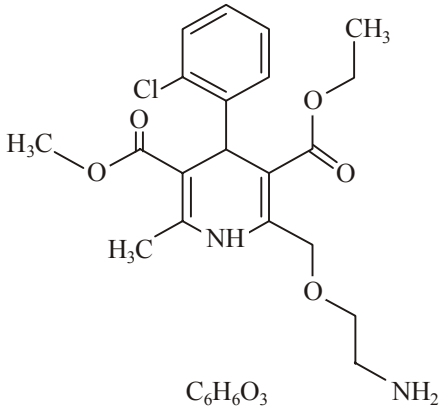

Fig. 2: Chemical structure of amlodipine

EXPERIMENTAL

Chromatographic conditions

The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C_{18} column (100 mm x 4.6 mm; 5 μm), a 2695 binary pump, a 20 μL injection loop and a 2487 dual absorbance detector and running on Waters Empower software. The UV spectrum of the drugs was taken using a Elico SL-159 UV-Visible spectrophotometer.

Chemicals and solvents

The reference sample of perindopril and amlodipine were supplied by Sun Pharmaceutical Industries Ltd., Baroda. HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric

acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Preparation of phosphate buffer (pH 3.0)

Seven grams of KH_2PO_4 was weighed into a 1000 mL beaker, dissolved and diluted to 1000 mL with HPLC water and pH was adjusted to 3.0 with orthophosporic acid.

Preparation of mobile phase and diluents

650 mL of the phosphate buffer was mixed with 350 mL of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µ filter under vacuum.

Procedure

A mixture of buffer and acetonitrile in the ratio of 65 : 35 v/v was found to be the most suitable mobile phase for ideal separation of perindopril and amlodipine. The solvent mixture was filtered through a 0.45 µ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 0.6 mL/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 237 nm. The run time was set at 11 min. Under these optimized chromatographic conditions, the retention time obtained for the drugs perindopril and amlodipine was 5.28 min and 8.50 min. A typical chromatogram showing the separation of the drug is given in Fig. 3.

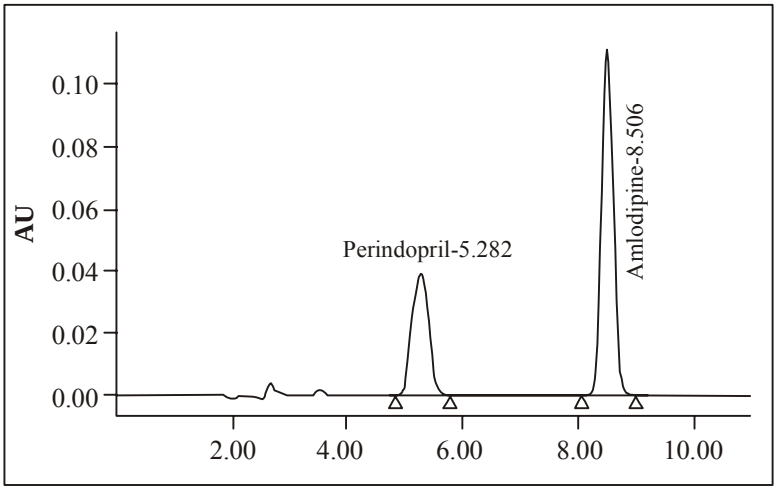

Fig. 3: Typical chromatogram of perindopril and amlodipine

Calibration plot

About 100 mg of perindopril and 100 mg of amlodipine was weighed accurately, transferred into a 100 mL volumetric flask and dissolved in 25 mL of a 65 : 35 v/v mixture of phosphate buffer and acetonitrile. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 1000 µg/mL solution. From this, a working standard solution of the drugs (20 µg/mL for amlodipine and 400 µg/mL for perindopril) was prepared by diluting the above solution to 10 mL in a volumetric flask. Further dilutions ranging from 10-50 µg/mL for amlodipine and 200-1000 µg/mL for perindopril were prepared from the solution in 10 mL volumetric flasks using the above diluent. 20 µL of each dilution was injected six times into the column at a flow rate of 0.6 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 10-50 µg/mL for amlodipine and 200-1000 µg/mL for perindopril. The relevant data are furnished in Tables 1 and 2. The regression equations of this curve was computed. This regression equation was later used to estimate the amount of perindopril and amlodipine in tablets dosage forms.

Table 1: Calibration data of the method for amlodipine

Concentration (µg/mL)	Mean peak area (n = 5)
10	821061
20	1528220
30	2194775
40	2874126
50	3613261

Table 2: Calibration data of the method for perindopril

Concentration (µg/mL)	Mean peak area (n = 5)
200	420293
400	793838
600	1181098
800	1522084
1000	1919281

Validation of the proposed method

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of perindopril and amlodipine. Solution containing 20 µg/mL for amlodipine and 400 µg/mL for perindopril was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Tables 3 and 4. The accuracy of the HPLC method was assessed by analyzing solutions of perindopril and amlodipine at 50, 100 and 150% concentrated levels by the proposed method. The results are furnished in Tables 5 and 6. The system suitability parameters are given in Table 7.

Table 3: Precision of the proposed HPLC method for amlodipine

Concentration of amlodipine (20 µg/mL)	Peak area	
	Intra-day	**Inter-day**
Injection-1	1515057	1533531
Injection-2	1506206	1537321
Injection-3	1516059	1543169
Injection-4	1512946	1542100
Injection-5	1516721	1540454
Average	1513398	1539315
Standard deviation	4266.3	3915.9
% RSD	0.28	0.25

Table 4: Precision of the proposed HPLC method for perindopril

Concentration of perindopril (400 µg/mL)	Peak area	
	Intra-day	**Inter-day**
Injection-1	790956	798173
Injection-2	785055	800725
Injection-3	788935	802996
Injection-4	788753	802947

Cont…

Concentration of perindopril (400 μg/mL)	Peak area	
	Intra-day	Inter-day
Injection-5	789934	803014
Average	788727	801571
Standard deviation	2233.5	2137.0
%RSD	0.28	0.27

Table 5: Accuracy studies for amlodipine

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	10	10.04	100.8%	
100%	20.1	20.06	99.6%	99.6%
150%	30.6	30.4	98.3%	

Table 6: Accuracy studies for perindopril

Concentration	Amount added (mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	105	106.6	101.5%	
100%	200.2	201.2	100.5%	100.7%
150%	299	299.2	100.1%	

Table 7: System suitability parameters

Parameter	Result (amlodipine)	Result (perindopril)
Linearity (μg/mL)	10-50	200-1000
Correlation coefficient	0.9998	0.9998
Theoretical plates (N)	5234	2270
Tailing factor	1.05	1.02
LOD (μg/mL)	0.03	1.76
LOQ (μg/mL)	0.10	5.9

Estimation of perindopril and amlodipine in tablet dosage forms

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate perindopril and amlodipine in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 100 mg of perindopril and 100 mg of amlodipine was transferred into a 100 mL volumetric flask and dissolved in 25 mL of a 65 : 35 v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 25 mL of the diluent was added. The flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution was further diluted to get the required concentrations. This solution was injected into the column six times. The average peak area of the drugs was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Tables 8 and 9.

Table 8: Assay and recovery studies for amlodipine

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand 1	20	20.02	99.90
Brand 2	20	20.03	99.85

Table 9: Assay and recovery studies for perindopril

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand 1	200	199.45	100.27
Brand 2	200	200.65	99.67

RESULTS AND DISCUSSION

In the proposed method, the retention time of perindopril and amlodipine was found to be 5.28 min and 8.50 min. Quantification was linear in the concentration range of 10-50 µg/mL for amlodipine and 200-1000 µg/mL for perindopril. The regression equation of the linearity plot of concentration of perindopril and amlodipine over its peak area was found to be $Y = 127196 + 69303X$ ($r^2 = 0.9998$) for amlodipine and $Y = 49452 + 1863X$ ($r^2 = 0.9998$) for perindopril, where X is the concentration of perindopril and amlodipine (µg/mL) and Y is the corresponding peak area. The number of theoretical plates calculated was 5234 for

amlodipine and 2270 for perindopril, which indicates efficient performance of the column. The limit of detection and limit of quantification for amlodipine were found to be 0.03 µg/mL and 0.10 µg/mL and for perindopril were found to be 1.76 µg/mL and 5.9 µg/mL respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 65 : 35 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of perindopril and amlodipine and can be reliably adopted for routine quality control analysis of perindopril and amlodipine in its tablet dosage forms.

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REFERENCES

1. The Merck Index, 13th Edition, Code 7247 (Perindopril), 491 (Amlodipine), **86** (2001) p. 1286.
2. Martindale, 33rd Edition, Code 729-S (Perindopril), 10499-b (Amlodipine), 838 (2002) p. 953.
3. E. Nevin, J. Pharm. Biomed. Anal., **26**, 43 (2001).
4. E. A. Hisham, J. Pharm. Biomed. Anal., **17**, 1267 (1998).
5. E. A. Hisham, M. A. Magda and A. T. Elham, J. Pharm. Biomed. Anal., **18**, 1021 (1999).
6. S. B. Wankhede, K. C. Raka, S. B. Wadkar and S. S. Chitlange, Int. J. Pharm. Sci., **72**, 136 (2010).
7. S. J. Lin, H. L. Wu, S. H. Chen and Y. H. Wen, Anal Lett., **29**, 1751 (1996).
8. H. Van Den Berg, G. Resplandy, A. De Bie, W. Floor, M. Bertrandt and C. J. M. Arts, J. Pharm. Biomed. Anal., **9**, 517 (1991).

9. M. Medenica, D. Ivanovic, M. Maskovic, B. Jancic and A. Malenovic, J. Pharm. Biomed. Anal., **44**, 1087 (2007).

10. D. A. Shah, K. K. Bhatt, M. B. Shankar, R. S. Mehta, T. R. Gandhi and S. L. Baldania, Ind. J. Pharm. Sci., **68**, 796 (2006).

11. A. Zarghi, S. M. Foroutan, A. Shafaati and A. Khoddam, Farmaco, **60**, 789 (2005).

12. G. D. Vaijanath, B. S. Sweta, P. K. Pravin, P. V. Manisha and K. Jadhav, J. Pharm. Biomed. Anal., **46**, 583 (2008).

13. S. C. Sohan, I. Mohammed and M. S. Dinesh, Asian J. Pharm., **2**, 232 (2008).

14. S. J. Deepak, G. Subbaiah, M. Sanyal, C. P. Umesh and S. Pranav, J. Chromatogr. B, **837**, 92 (2006).

15. K. R. Nesrin, M. M. Heba and A. M. Azza, Anal Lett., **43**, 570 (2010).

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