Research Article

Method development and validation for the simultaneous determination of amlodipine and benazepril by reverse-phase high-performance liquid chromatography in its bulk and pharmaceutical tablet dosage form using biorelevant dissolution media

Palani Shanmugasundaram*, S. K. Kamarapu

ABSTRACT

Objective: A new, stability-indicating and precise method to develop and validating as per ICH Q2 (R1) guidelines for amlodipine and benazepril in it's pure and marketed tablet formulation in application to biorelevant dissolution (FaSSIF) media using RP-HPLC technique. **Materials and Methods:** The chromatographic separation was done with Inertsil ODS (4.6 × 100 mm, 5 µm) as the stationary phase and mobile phase was a mixture of 0.1% Triethylamine: methanol:acetonitrile (40:30:30) which were monitored at 235 nm, isocratic mode using UV detector. **Results:** By this method, amlodipine and benazepril were eluted with retention times of 2.436 min and 3.332 min. The regression line in calibration graph was linear toward the concentration ranges from 10 to 50 µg/ml for amlodipine and 20–100 µg/ml for benazepril. Limits of detection were 1.05 and 2.78 µg/ml and limits of quantification were 3.18 and 8.45 µg/mL for amlodipine and benazepril, respectively. **Conclusion:** The study of statistical analysis of this method shows that it is relevant for the combination of amlodipine and benazepril in its pure and tablet dosage form in biorelevant dissolution media (FaSSIF) without any intervention from the excipients.

KEY WORDS: 0.1% TEA, Amlodipine and Benazepril, Biorelevant media (FaSSIF), ICH Q2(R1) guidelines, Method validation, RP-HPLC

INTRODUCTION

Amlodipine is a calcium channel blocker.[1,2] It acts as an antihypertensive agent by relaxing the blood vessels. It also controls angina by increasing blood supply to the heart muscle. Hence, it is used as a calcium antagonist[3] and antianginal drugs.[4] The IUPAC name for amlodipine is 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate and it's chemical formula $C_{20}H_{25}ClN_2O_5$. Chemical structure for amlodipine is shown in Figure 1.

Benazepril is an angiotensin-converting enzyme (ACE) inhibitor,[5] used as an antihypertensive agent.[6] It is competitively bound to the active site and inhibits ACE which leads to blocking the conversion of angiotensin I to angiotensin II (vasoconstrictor substance), thereby dilating the blood vessels to dilate and lowers blood pressure.[7] The IUPAC name for benazepril is 2-[(3S)-3-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl] acetic acid and its chemical formula $C_{24}H_{28}N_2O_5$. Chemical structure for benazepril is shown in Figure 2.

From literature review,[8-22] we found that there were no methods available for stability-indicating[23] method development and validation of amlodipine and benazepril in its pure and marketed tablet dosage forms by RP-HPLC using biorelevant dissolution media (FaSSIF)[24-27] and validation was performed according to ICH Q2 (R1) guidelines.[28,29]

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India

***Corresponding author:** Dr. Palani Shanmugasundaram, Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India. E-mail: samsimahi@gmail.com

Received on: 17-05-2019; Revised on: 20-07-2019; Accepted on: 24-08-2019

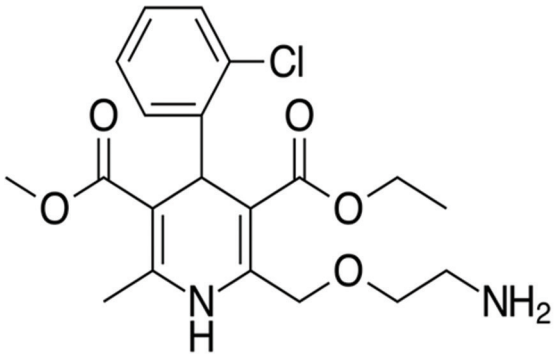

Figure 1: Chemical structure of amlodipine

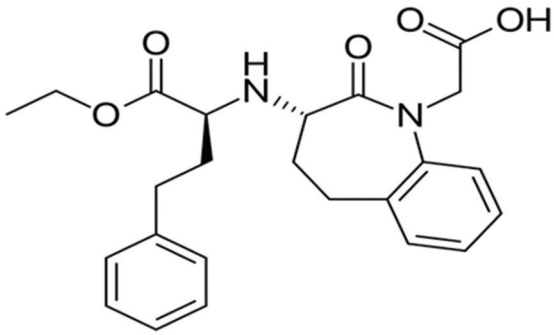

Figure 2: Chemical structure of benazepril

MATERIALS AND METHODS

Reagents and Chemicals

The amlodipine and benazepril pure standards were supplied by Syncorp Clincare Pvt. Ltd., Dilsukhnagar, Hyderabad. The marketed formulation tablets labeled to contain 5 mg of amlodipine and 10 mg of benazepril, manufactured by Systopic Laboratories Pvt. Ltd. (AMACE BP TAB) were obtained from the market. Analytical reagent grade and HPLC grade chemicals were procured from SD Fine-Chem Ltd., Mumbai, India, were used in the research.

Instruments Used

The analysis was performed using HPLC (Waters-2695 series) with UV detector and data handling system is EMPOWER 2 software, UV–visible double beam spectrophotometer (Labindia), analytical balance 0.1 mg sensitivity (Afcoset ER-200A), pH meter (Adwa – AD1020), and ultrasonicator.

Preparation of 0.1% Triethylamine (TEA) Buffer

Take 1 ml of TEA in 1000 ml volumetric flask makeup with HPLC grade water, the final solution was filtered through 0.45 µm filter paper and ultrasonication for 10 min.

Mobile Phase Preparation

Accurately measure and mixed 400 ml of the above buffer, 300 ml of methanol and 300 ml of acetonitrile and degassed through an ultrasonicator water bath for 10 min, thereby filtered using 0.45 µm microfilter paper under vacuum filtration.

Preparation of Blank FaSSIF

Weigh 1.74 g of NaOH, 19.77 g of NaH_2PO_4, and 30.93 g of NaCl dissolve in 5 L of HPLC grade distilled water and adjust the pH 6.5 exactly using 1 N HCl.

Preparation of FaSSIF Diluent

Weigh and dissolve sodium taurocholate 3.3 g in 0.5 L of blank FaSSIF. To this add 11.8 ml of a 100 mg/ml solution contains lecithin in dichloromethane (DCM), it forms an emulsion. The DCM was eliminated under vacuum for 30 min (15 min at 250 mbar and 15 min at 100 mbar) at 40°C to get a clear, micellar and there was no perceptible odor of solution for DCM. Then, the solution was subjected to cool at room temperature, make up the volume 2 L using blank FaSSIF.

Preparation of Standard Stock Solution

Accurately weigh and transfer pure 10 mg of amlodipine and benazepril separately into 10 ml volumetric flasks. Then, add 7 ml of diluent and ultrasonicated for 15 min. Filter the solution using membrane filter paper (0.45 µm) and volume make up to 10 ml using the same diluent. Five levels of linearity concentrations were prepared by mixed appropriately and further diluted to get 10–50 µg/ml of amlodipine and 20–100 µg/ml of benazepril. Inject the series concentrations in triplicate into the column and average peak areas are recorded from chromatograms. Linearity graph was plotted peak area against concentrations.

Preparation of Mixed Working Standard Solution

To prepare separately, 1 mg/ml of amlodipine and benazepril solution using stock solution. From this above solutions pipette out 0.1 ml of amlodipine solution and 0.5 ml of benazepril solution into a 10 ml volumetric flask and make up the volume with a diluent, to get concentrations of 10 µg/ml and 50 µg/ml of amlodipine and benazepril working solutions, respectively.

Preparation of Test Solution

According to I. P method, take 20 tablets and weighed. Then, the tablets are triturated in a mortar to get a smooth powder. The amount of drug presents in a powder which is equivalence to the standard drug of 5 mg of amlodipine. The powder was transfer to a 100 ml of volumetric flask and add approximately 70 ml of diluent, the resulted solution was subjected to sonication for 15 min using ultrasonicator. Then, the solution was filtered using membrane filter paper (0.45 µm) and volume make up to 100 ml using the same diluent. From this, take 1 ml and transfer to six 10 ml volumetric flasks, then

the volume was make up mark with the diluent. These solutions are injected 3 times each sample solution into the column and the results are mentioned as a function of the mean of all replicas.

Method Development

Selection of wavelength

Working standard solutions were scanned an entire range of UV in a 1 cm path length cell against blank using UV spectrophotometer. The absorption maxima of amlodipine and benazepril were selected from spectral data and isosbestic wavelength was selected from overlain spectra of UV spectrophotometer. An isosbestic point was found to be at 235 nm. The UV spectrum of for amlodipine and benazepril is shown in Figure 3.

Optimized Chromatographic Conditions

The selected and optimized mobile phase was 0.1% TEA: methanol:acetonitrile (40:30:30) Inertsil ODS (4.6 × 100 mm, 5 µm) as stationary phase and conditions optimized were as follows: flow rate (1.0 ml/min), wavelength (235 nm, UV detector), run time was 10 min, and injection volume was 20 µl.

Method Validation

This method was validated according to ICH Q2 (R1) guidelines. The validation parameters performed such as system suitability, linearity range, accuracy data, precision (intra and inter), limits of detection (LOD), limit of quantification (LOQ), and robustness.

Forced Degradation Studies

Active pharmaceutical ingredients of amlodipine and benazepril were subjected to keep in degradation ways and find out the extent of degradation of a product by this method. The parameters that were carried out for forced degradation studies are acid, base, peroxide, thermal, and photodegradation.

RESULTS AND DISCUSSION

Method Development and Optimized Method

This method was accurate, specific, linear, precise, and suitable for analysis of amlodipine and benazepril by RP-HPLC method. The HPLC instrument comprised a Waters HPLC with an autosampler and UV detector. The Inertsil ODS-3 (4.6 × 100 mm, 5 µm) column is used. The ratio of mobile phase used is 0.1% TEA buffer: methanol:acetonitrile (40:30:30 v/v). Mode of separation is isocratic; its temperature of the column is ambient. The optimized HPLC conditions and chromatogram are presented in Tables 1 and 2 and Figure 4.

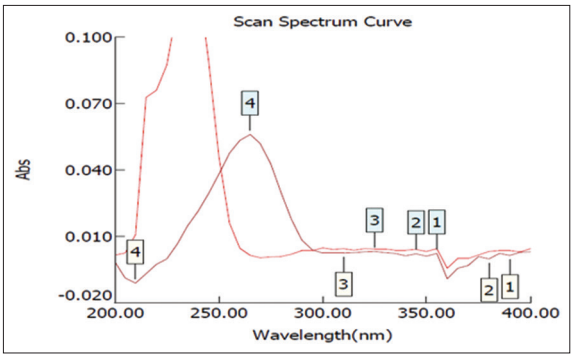

Figure 3: Overlay spectrum for isosbestic point

Method Validation

This method was validated according to ICH Q2(R1) guidelines for various parameters.

System Suitability

The mixed working standard solution was injected 6 times into the chromatographic column. The mean of each system suitability parameter was calculated from the obtained chromatogram. The results are tabulated in Table 3.

Linearity and Range

The linearity study was performed for the series concentrations of 10–50 µg/ml and 20–100 µg/ml of amlodipine and benazepril, respectively. The obtained values are tabulated in Tables 4 and 5. The graph for both the drugs is shown in Figures 5 and 6 and overlay chromatogram in Figure 7.

Table 1: Optimized HPLC conditions

Optimization parameters	Method conditions
Stationary phase	Inertsil ODS-3 (4.6×150 mm, 5 m)
Mobile phase	0.1% TEA buffer: methanol: acetonitrile (40:30:30 v/v)
pH	4.8±0.02
Flow rate	1.0 ml/min
Analysis time each injection	10.0 min
Temperature of column	Ambient °C
Fixed injection loop volume	20 µl
Detection wavelength	235 nm
Drugs retention time	2.436 and 3.332 min

Table 2: Chromatogram data for mixed standard amlodipine and benazepril solution

Drug	RT	Peak area	USP tailing	USP plate count
Amlodipine	2.436	504911	1.59	3466.29
Benazepril	3.332	476545	1.41	5169.00

Table 3: System suitability data for amlodipine and benazepril

Parameter	Amlodipine	Benazepril
Retention time (min)	2.447	3.325
Peak area	505,395.8	475,133.8
Resolution (Rs>2)	3.14	3.09
USP plate count	3482.23	5206.15
USP tailing	1.54	1.40

Note: Mean of six determinations

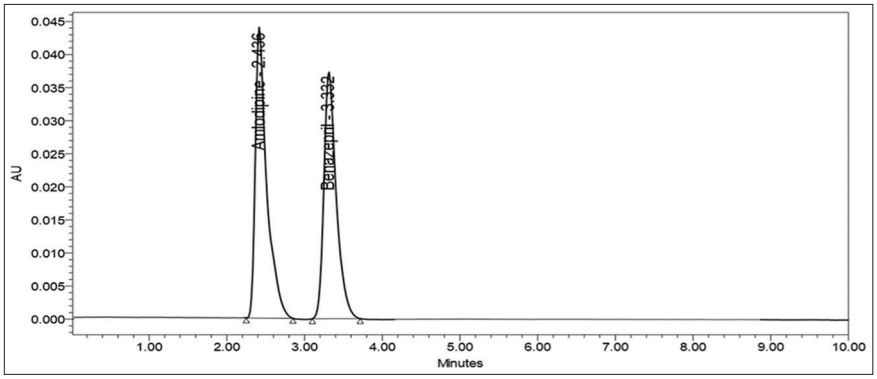

Figure 4: Chromatogram for mixed standard amlodipine and benazepril solution at 235 nm

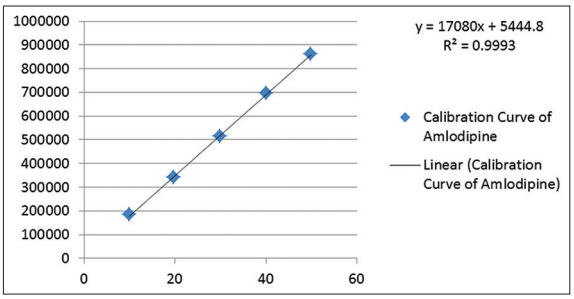

Figure 5: Calibration curve of amlodipine

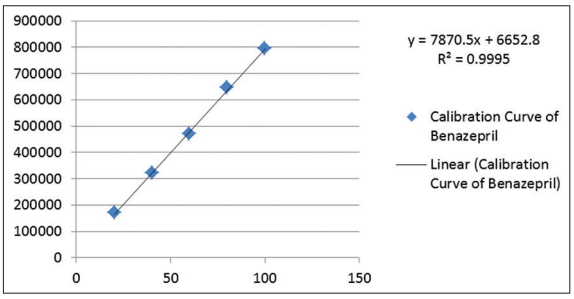

Figure 6: Calibration curve of benazepril

Accuracy

Accuracy was achieved by applying the method to drug sample (AMACE BP TAB) to which known amounts of amlodipine and benazepril standard drug corresponding to three levels of percentage of mentioned label claim on marketed formulation were added and estimated by the system in optimized chromatographic conditions where three levels are injected in triplicate injections into a chromatographic column. The peak area of all levels is used for the calculation of percentage recovery of drug. The results are tabulated in Table 6.

Precision

The study of precision in this method was based on intraday and interday variations. The mixed working standard solution of amlodipine and benazepril has injected six replicates and measured the peak area of each replicate injection in HPLC. The mean and %RSD are tabulated in Tables **7** and 8.

Table 4: Linearity results (for amlodipine)

S. No.	Linearity concentration (µg/ml)	Peak area
1	10	184,810
2	20	339,375
3	30	510,488
4	40	692,083
5	50	862,450
Correlation coefficient		0.9993

Table 5: Linearity results (for benazepril)

S. No.	Linearity concentration (µg/ml)	Peak area
1	20	168,693
2	40	318,093
3	60	471,391
4	80	642,911
5	100	793,338
Correlation coefficient		0.9995

LOD and LOQ

The LOD and LOQ for amlodipine and benazepril were separately determined by based on calculating the signal-to-noise ratio. Detection limit = 3.3 σ/s; quantification limit = 10 σ/s; where, σ stands for the SD of the Y-intercept of the regression line and "s" stands for the slope of the calibration curve. The results are tabulated in Table 9.

Robustness

It is an assessment for the method as stable and unaffected by small changes were made in method development. The robustness data conducted for variations in flow rate and percentage of composition in the mobile phase was performed. The obtained values are presented in Tables 10 and 11.

Assay of Marketed Tablet

The assay study was performed for the amlodipine and benazepril in the marketed tablet dosage form. For each determination, 3 times inject the solution into the column. The assay results are mentioned in Table 12 and chromatogram is shown in Figure 8.

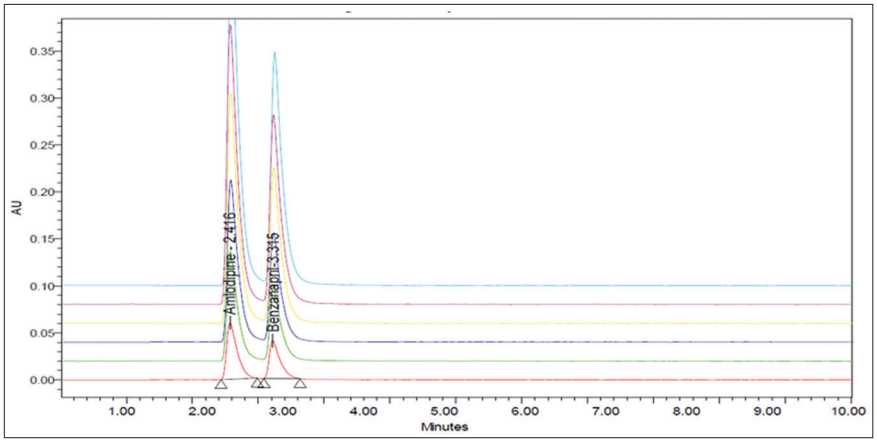

Figure 7: Overlay chromatogram for linearity

Table 6: Accuracy data for amlodipine and benazepril

Brand name	Drug	Recovery levels (%)	Actual amount (mg)	Amount added (mg)	Theoretical amount (mg)	Amount found (mg)	% recovery	Mean
AMACE BP TAB	Amlodipine	50	5	2.5	7.5	7.45	99.33	99.78
		100	5	5	10	10.09	100.9	
		150	5	7.5	12.5	12.39	99.12	
	Benazepril	50	10	5	15	14.96	99.73	99.97
		100	10	10	20	19.99	99.95	
		150	10	15	25	25.06	100.24	

Acceptance limits: The percentage recovery for three levels is 98.0–102.0%

Table 7: Intraday precision data

S. No.	For amlodipine				For benazepril			
	RT	Peak area	USP tailing	USP plate count	RT	Peak area	USP tailing	USP plate count
1	2.424	505,591	1.50	3466.58	3.311	470,021	1.41	5215.36
2	2.436	503,459	1.55	3518.39	3.316	473,537	1.39	5205.88
3	2.439	504,693	1.55	3497.12	3.324	477,253	1.41	5183.78
4	2.443	506,230	1.57	3463.83	3.327	476,197	1.41	5189.79
5	2.455	504,599	1.54	3461.70	3.335	479,198	1.41	5197.78
6	2.457	507,803	1.55	3485.21	3.339	474,597	1.41	5244.34
Mean	2.442	505,395.8	1.54	3482.13	3.325	475,133.8	1.40	5206.15
SD	0.012	1510.5	0.02	22.55	0.010	3197.4	0.01	21.82
%RSD	0.5	0.3	1.5	0.6	0.3	0.7	0.5	0.4

SD: Standard deviation

Table 8: Interday precision data

S. No.	For amlodipine				For benazepril			
	RT	Peak area	USP tailing	USP plate count	RT	Peak area	USP tailing	USP plate count
1	2.436	505,591	1.50	3466.58	3.324	470,021	1.41	5215.36
2	2.443	503,459	1.55	3518.39	3.326	473,537	1.39	5205.88
3	2.446	507,867	1.52	3517.70	3.327	476,185	1.41	5241.05
4	2.451	503,326	1.53	3491.57	3.329	470,009	1.39	5161.94
5	2.451	504,599	1.54	3461.70	3.332	475,679	1.41	5186.86
6	2.458	505,716	1.56	3485.24	3.341	479,198	1.41	5197.78
Mean	2.447	505,093	1.53	3490.19	3.329	474,104.8	1.40	5201.47
SD	0.007	1695.1	0.02	24.27	0.006	3647.5	0.01	26.71
%RSD	0.3	0.3	1.4	0.6	0.2	0.8	0.7	0.5

Acceptance limits: The results of % RSD values are not more than 2%. SD: Standard deviation

Forced Degradation Studies

According to ICH guidelines, the stability testing of new drug substances and product requires stress testing to be carried out to evaluate the stability indicative of the pharmaceutical drug substance. The aim of the study was to perform the stress degradation studies on amlodipine and benazepril by applying the proposed method. The data obtained in forced degradation studies reveal that the developed method is more stable in some stress conditions. The obtained values are tabulated in Table 13.

Table 9: LOD and LOQ for amlodipine and benazepril

Parameter	Amlodipine	Benazepril
LOD	1.05	2.78
LOQ	3.18	8.45

Acceptance limits: Signal-to-noise ratio value should be 3 for LOD solution and 10 for LOQ solution

CONCLUSION

The obtained results for this method validation all are within acceptance criteria. This method was more economical and stable. This method could selectively quantify amlodipine and benazepril in a pharmaceutical

Table 10: Robustness data for variation in flow rate

Drug	Flow rate (ml/min)	System suitability			
		RT	Peak area	USP tailing	USP plate count
Amlodipine	0.9	2.771	509,234	1.52	3374.87
	1*	2.436	504,911	1.59	3466.29
	1.1	2.192	501,551	1.57	3430.34
Benazepril	0.9	3.767	470,253	1.44	5100.55
	1*	3.332	476,545	1.41	5169.0
	1.1	2.978	477,886	1.37	5150.94

*The values for actual flow rate (1.0 ml/min) have been considered from the standard assay

Table 11: Robustness data for variation in percentage of composition in the mobile phase

Drug	Variation in the mobile phase	System suitability			
		RT	Peak area	USP tailing	USP plate count
Amlodipine	10% less	2.769	506,252	1.52	3422.22
	*Actual	2.436	504,911	1.59	3466.29
	10% more	2.185	501,522	1.57	3242.86
Benazepril	10% less	3.761	471,426	1.44	5659.98
	*Actual	3.332	476,545	1.41	5169.0
	10% more	2.964	475,363	1.37	5365.28

*The values for actual composition have been considered from the standard assay

Table 12: Assay data for marketed tablets

Tablet (AMACE BP TAB)	Label claim	Amount estimated*	Amount estimated	Acceptance range
Amlodipine	5 mg	4.96 mg	99.33%	98–102%
Benazepril	10 mg	9.97 mg	99.72%	

*Mean of three determinations

Table 13: Data for forced degradation

Drug	For amlodipine				For benazepril			
Degradation parameter	RT	Peak area	USP tailing	USP plate count	RT	Peak area	USP tailing	USP plate count
Standard	2.436	504,911	1.59	3466.29	3.332	476,545	1.41	5169.00
Acid	2.443	487,362	1.55	3518.39	3.324	456,373	1.39	5205.88
Base	2.472	478,637	1.54	3461.7	3.360	446,563	1.41	5197.78
Peroxide	2.424	481,627	1.50	3466.58	3.306	451,837	1.41	5215.36
Thermal	2.475	472,882	1.56	3485.24	3.359	449,377	1.41	5186.86
Photo	2.464	479,898	1.53	3485.24	3.334	441,345	1.41	5197.78

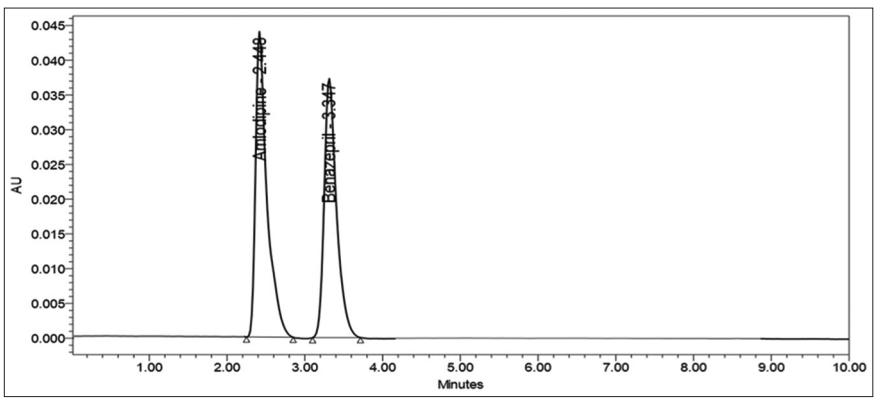

Figure 8: Chromatogram for amlodipine and benazepril at 235 nm from pharmaceutical dosage form (AMACE BP TAB)

tablet dosage form. From the obtained experimental data, the developed method is more accurate, precise, and selective so this method was suitable for routine analysis successfully for this combination in its bulk and marketed formulations by RP-HPLC using biorelevant dissolution media (FaSSIF).

REFERENCES

1. Murdoch D, Heel RC. Amlodipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in cardiovascular disease. Drugs 1991;41: 478-505.
2. Brown MJ, McInnes GT, Papst CC, Zhang J, MacDonald TM. Aliskiren and the calcium channel blocker amlodipine combination as an initial treatment strategy for hypertension control (ACCELERATE): A randomised, parallel-group trial. Lancet 2011;377:312-20.
3. Reid JL, Meredith PA, Donnelly R, Elliott HL. Pharmacokinetics of calcium antagonists. J Cardiovasc Pharmacol 1988;12 Suppl 7:S22-6.
4. Taylor SH. Amlodipine in post-infarction angina. Cardiology 1992;80 Suppl 1:26-30.
5. Juillerat L, Nussberger J, Ménard J, Mooser V, Christen Y, Waeber B, *et al.* Determinants of angiotensin II generation during converting enzyme inhibition. Hypertension 1990;16:564-72.
6. Jamerson K, Weber MA, Bakris GL, Dahlöf B, Pitt B, Shi V, *et al.* Benazepril plus amlodipine or hydrochlorothiazide for hypertension in high-risk patients. N Engl J Med 2008;359:2417-28.
7. Li J, Hirose N, Kawamura M, Arai Y. Antiatherogenic effect of angiotensin converting enzyme inhibitor (benazepril) and angiotensin II receptor antagonist (valsartan) in the cholesterol-fed rabbits. Atherosclerosis 1999;143:315-26.
8. Jagadeeswaran M, Gopal N, Sivakumar T. Simultaneous estimation and validation of amlodipine besylate and nebivolol hydrochloride in tablet formulation by RP-HPLC method. Asian J Res Chem 2010;3:640-2.
9. Mirza AZ, Arayne MS, Sultana N. Method development and validation of amlodipine, gliquidone and pioglitazone: Application in the analysis of human serum. Anal Chem Lett 2014;4:302-12.
10. Prajapati J, Patel A, Patel MB, Prajapati N, Prajapati R. Analytical method development and validation of amlodipine besylate and perindopril erbumine in combine dosage form by RP-HPLC. Int J PharmTech Res 2011;3:801-8.
11. Çelebier M, Kaynak MS, Altinoz S, Sahin S. Validated HPLC method development: The simultaneous analysis of amlodipine and valsartan in samples for liver perfusion studies. Hacet Univ J Fac Pharm 2008;28:15-30.
12. Chaudhari BG, Patel NM, Shah PB. Stability indicating RP-HPLC method for simultaneous determination of atorvastatin and amlodipine from their combination drug products. Chem Pharm Bull (Tokyo) 2007;55:241-6.
13. Shanmugasundaram P, Kamarapu SK. RP-HPLC method for the simultaneous estimation and validation of amlodipine besylate and atenolol in bulk and tablet dosage form in biorelevant dissolution medium (Fassif). Res J Pharm Technol 2017;10:3379.
14. Mohammadi A, Rezanour N, Dogaheh MA, Bidkorbeh FG, Hashem M, Walker RB. A stability-indicating high performance liquid chromatographic (HPLC) assay for the simultaneous determination of atorvastatin and amlodipine in commercial tablets. J Chromatogr B 2007;846:215-21.
15. Rajeswari KR, Sankar GG, Rao AL, Seshagirirao J. RP-HPLC method for the simultaneous determination of atorvastatin and amlodipine in the tablet dosage form. Indian J Pharm Sci 2006;68:275-7.
16. Zarghi A, Foroutan SM, Shafaati A, Khoddam A. Validated HPLC method for determination of amlodipine in human plasma and its application to pharmacokinetic studies. Farmaco 2005;60:789-92.
17. Radhakrishna T, Rao DS, Vyas K, Reddy GO. A validated method for the determination and purity evaluation of benazepril hydrochloride in bulk and in pharmaceutical dosage forms by liquid chromatography. J Pharm Biomed Anal 2000;22:641-50.
18. Naidu KR, Kale UN, Shingare MS. Stability indicating RP-HPLC method for simultaneous determination of amlodipine and benazepril hydrochloride from their combination drug product. J Pharm Biomed Anal 2005;39:147-55.
19. Chitlange S, Imran M, Sakarkar D. RP-HPLC method for simultaneous estimation of amlodipine and metoprolol in tablet formulation. Asian J Pharm 2008;2:232.
20. Dai SY, Qiu ST, Wu W, Fu CM. Development and validation of an RP-HPLC method for simultaneous determination of ramipril and amlodipine in tablets. J Pharm Anal 2013;3:440-6.
21. Shah DA, Patel DV, Mehta FA, Chhalotiya UK, Bhatt KK. High-performance thin-layer chromatography method for estimating the stability of a combination of irbesartan and amlodipine besylate. J Taibah Univ Sci 2015;9:177-86.
22. Tengli AR, Gurupadayya BM, Soni N. Simultaneous estimation of hydrochlorothiazide, amlodipine, and losartan in tablet dosage form by RP-HPLC. Int J Chem Anal Sci 2013;4:33-8.
23. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs-a review. J Pharm Anal 2014;4:159-65.
24. Marques M. Dissolution media simulating fasted and the fed states. Dissolution Technol 2004;11:16.
25. Manish S. Bio-relevant dissolution media development. J Bioequivalence Bioavailab 2017;9:1-2.
26. Shanmugasundaram P, Sudheerkumar K. Prospectus review of biorelevant dissolution medium. Pharm Res Lib 2016;4:132-37.
27. Bhagat NB. A review on development of biorelevant dissolution medium. J Drug Deliv Ther 2014;4:140-8.
28. ICH. Validation of Analytical Procedures: Text and Methodology Q2 (R1). International Conference on Harmonization; 2005. Available from: http://www.gmp-compliance.org/guidemgr/files/Q2(R1).PDF. [Last cited on 2019 Mar 02].
29. Abraham J. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. In: Brouder A, Tietje C, editors. Handbook of Transnational Economic Governance Regimes. Brill; 2009. Available from: http://nijhoffonline.nl/book?id=nij9789004163300_nij9789004163300_i-1081. [Last cited on 2019 Mar 02].

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