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Original Article

NOVEL HPLC-UV METHOD USING VOLATILE BUFFER FOR SIMULTANEOUS DETERMINATION OF AMLODIPINE BESYLATE AND ATORVASTATIN CALCIUM

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ABSTRACT

Objective: The purpose of this work was to develop and validate a novel HPLC-UV method using triethylamine (TEA) as a volatile buffer for simultaneous determination of amlodipine besylate (AML) and atorvastatin calcium (ATV).

Methods: System suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), selectivity, accuracy, and precision was validated using Hitachi L-2000 system with detector: DAD L-2455 at a detected wavelength of 245 nm. Stationary phase: Phenomenex Luna RP-C18 (250 mm x 4.6 mm, 5 µm) and mobile phase: acetonitrile-methanol-TEA pH 4.0 (ratio 52:18:30 v/v/v) were used. Samples' volume of 20 µl was run at room temperature with the flow rate at 1 ml/min.

Results: The linearity demonstrated good correlation in the concentration range at 2-40 ppm and 4-80 ppm for AML and ATV, respectively. The method was repeatable with relative standard deviation (RSD) of the intermediate precision test less than 1%. The recovery rate was 100.03% and 99.58% for AML and ATV, respectively. The method was also validated for dissolution studies with excellent compatibility.

Conclusion: A new, simple and easy HPLC-UV method was successfully developed and validated for the determination of AML and ATV in both quantification test and dissolution test.

Keywords: Amlodipine, Atorvastatin, Simultaneous, Dissolution, HPLC, Quantification, Volatile buffer

INTRODUCTION

Recently, cardiovascular diseases (CVDs) have been the number one cause of death in the world, especially in low-and-middle-income countries. In 2012, approximately 17.5 million people died from these illnesses, attributing 31% of deaths globally [1]. Among many risk factors, namely, unhealthy diet, tobacco smoke, physical inactivity, and alcohol abuse, that can contribute to the onset of CVDs, hypertension and hyperlipidemia are considered the most frequent and important ones [1].

AML (fig. 1) is the besylate conjugated form of amlodipine, a dihydropyridine-type calcium channel blocker that can widen the blood vessel, hence, lower the blood pressure. On the other hand, ATV (fig. 2) is the calcium salt of a lipid lowering agent which works by inhibiting 3-hydroxy-3-methyl-glutaryl-CoA reductase, an enzyme that plays a key role in the cholesterol synthesis pathway. The combination of AML and ATV have been proved a significant synergistic effect on reducing the overall CVDs risks [2-4].

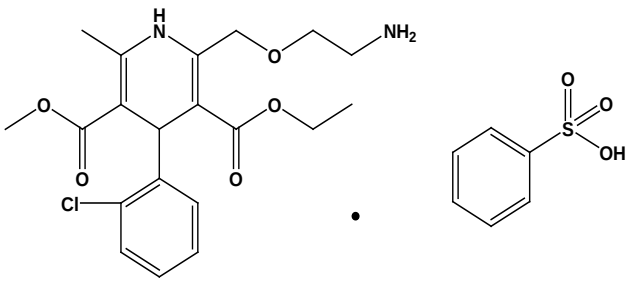

Fig. 1: Chemical structure of amlodipine besylate, (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate besylate

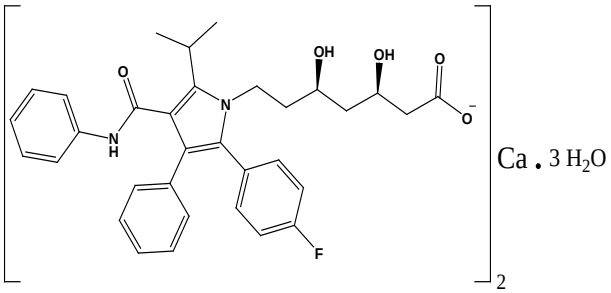

Fig. 2: Chemical structure of atorvastatin calcium, (3R,5R)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoate calcium

Some simultaneous determination methods for this combination have been published, utilizing the UV-Vis spectrophotometry [5, 6], the HPLC-UV [7-9], the HPLC-MS/MS [10, 11], or the capillary electrophoresis [12]. No outstanding methods have been recognized so far; since UV-Vis spectrophotometry method has low sensitivity and selectivity; HPLC-MS/MS method is the most expensive one, and therefore, used only in determining drug concentration in human plasma. The suitable method for measuring both AML and ATV in pharmaceutical dosage forms is HPLC-UV.

However, to the best of our knowledge, the mobile phases of previously published methods contained nonvolatile buffers, mainly phosphate salts (table 1). These salts can block the capillaries and the column, hence, increase the system pressure, reduce the method's robustness, and consume longer column cleaning time [13]. Also, no methods reported so far have been applied for the simultaneous determination of AML and ATV in tablet dissolution profiles.

Table 1: Summary of HPLC-UV methods for simultaneous determination of AML and ATV. MeOH: Methanol; ACN: Acetonitrile

S. No.	Sample solvent	Stationary phase	Mobile phase	Ref
1	MeOH, water, mobile phase	PerfectSil Target ODS-3 (250 mm x 4.6 mm, 5 µm)	ACN-NaH_2PO_4 pH 4.5 (55:45)	[7]
2	Mobile phase	Grace Smart RP C-18 (250 mm x 4.6 mm, 5 µm)	ACN-MeOH-H_3PO_4 (43:4:53)	[8]
3	Mobile phase	RP C-18 Kromasil (250 mm x 4.6 mm, 5 µm)	ACN-$NH_4H_2PO_4$ (40:60)	[9]

Therefore, in this study, we aimed to develop a new HPLC-UV method, using a volatile buffer, for simultaneous determination of AML and ATV in both tablet quantification and dissolution profiles.

MATERIALS AND METHODS

Chemicals and reagents

AML and ATV references were purchased at Institute of Drug Quality Control, Ho Chi Minh City, Vietnam, with purity of 100.34% and 94.25%, respectively. AML and ATV ingredients, USP grade, were acquired from India. Amdepin Duo® film coated tablets (AML 5 mg, ATV 10 mg) of Cadila, India, register number VN-4367-07, batch number ADE2004, were obtained from Kim Loi drugstore, Can Tho, Vietnam. Test tablets were manufactured in Department of Pharmaceutical Technology, Can Tho University of Medicine and Pharmacy, Vietnam with pharmaceutical grade ingredients (i.e. starch, povidone K30, talc, magnesium stearate, avicel PH-102, $CaCO_3$, ethanol 50%) imported from China. Acetonitrile (ACN), methanol (MeOH), glacial acetic acid, formic acid, orthophosphoric acid, TEA, NaOH, KH_2PO_4 were purchased from Merck, Germany. All reagents used were qualified as analytical grade, except for ACN, MeOH and double distilled water with HPLC grade.

HPLC instrumentation and conditions

The HPLC system used was Hitachi L-2000, detector: DAD L-2455, stationary phase was Phenomenex Luna RP-C18 (250 mm x 4.6 mm, 5 µm). Samples were run at the volume of 20 µl with optimized mobile phase consisted of ACN-MeOH-TEA buffer pH 4.0 (ratio 52:18:30 v/v/v) at room temperature with the flow rate at 1 ml/min. The eluent was detected at the wavelength of 245 nm.

Method validation

Method development and validation were based on International Council for Harmonisation (ICH) guideline [14, 15] with the parameters, namely system suitability, linearity, LOD, LOQ, selectivity, accuracy, and precision for both quantification study and dissolution study.

Tablet formulation

Three new tablet formulas contained both AML and ATV were manufactured by wet granulation method [16]. Briefly, active ingredients, starch, and avicel PH-102 were mixed for 15 min. Approximately 140 ml ethanol 50% solution of povidone K30 was added to the mixture and granulated with Erweka AR-402 (Germany) machine. The granules were mixed with talc and magnesium stearate for 15 min before tableting with Rimek Mini Press-I (India), die diameter of 7 mm, to get 150 mg tablet with a hardness of 50-60 N.

Dissolution study

Amdepin Duo® tablet and three formulations were tested dissolution profiles with Pharmatest PT-DT8S Tester (Germany) using paddle apparatus, 75 rpm/min, in 900 ml of phosphate buffer pH 6.8, at 37 ^oC. 10 ml of each sample was withdrawn at each time interval of 5, 10, 15, and 30 min, filtered, and determined the drug concentration based on validated method. All tests were done in triplicate.

Standard solution preparation for quantification

10 mg AML and 20 mg ATV references were accurately weighed and dissolved in 100 ml MeOH. After 10 minute sonication, the solution was diluted to 200 ml with water. This solution was further diluted ten times to get the final concentration of AML and ATV at 5 ppm and 10 ppm, respectively.

Sample solution preparation for quantification

Twenty Amdepin Duo® tablets or test formulations were weighed, crushed and mixed. An accuracy amount of powder equal to the weight of one tablet was dissolved in 50 ml MeOH and sonicated for 10 min. The solution was diluted with 50 ml of water and filtered. This solution was then further diluted ten times with water and filtered through a Millipore membrane (0.45 µm) (Merck, Germany) to get the concentration of AML and ATV at approximately 5 ppm and 10 ppm, respectively.

RESULTS AND DISCUSSION

Hypertension and hyperlipidemia are the most popular and dangerous risk factors of CVDs [1]. AML and ATV combination (i.e. Amdepin Duo®) can reduce these risks significantly. However, to the best of our knowledge, no perfect method to determine both AML and ATV in both quantification test and dissolution profiles. Hence, our newly developed method may be considered for these purposes.

Method optimization

Optimal conditions for a suitable method can be characterized as follows: short retention time, sharp peak, asymmetric factor in the range of 0.8-1.2. Since the pK_a of AML and ATV are 8.6 and 4.5, respectively, the suitable buffer pH should be from 3 to 6. The UV spectrum of AML showed absorption peaks at 238 and 361 nm. On the other hand, ATV has maximum absorption at the wavelength of 245 nm. Hence, the wavelength of 245 nm was used to simultaneous determine both substances because not only it can yield the best chromatography results, but also reduces the noise from HPLC mobile phase namely ACN, MeOH [13]. In addition, as mentioned earlier in the introduction part, the use of nonvolatile buffers is not preferable [13]. The volatile buffer TEA can eradicate this problem. After several preliminary studies, we chose the optimal chromate-graphic conditions as demonstrated in the materials and methods section.

Method validation

System suitability

Six replicative standard and test samples were injected into the system. Results are shown in table 2. All values had RSD<2%, the resolution between peaks were larger than 1.5, asymmetric factors were in the range of 0.8-1.2, and theoretical plates were higher than 1500. Hence, the method had system suitability.

Specificity

Five samples included standard sample, test sample, blank sample, standard and mixture test sample, and mobile phase sample, were measured and the chromatograms were overlaid (fig. 3). Specificity was validated with no interference from other substances, and test sample demonstrated similar retention time with the references.

Linearity

Five different concentrations of standard AML and ATV, ranging from 2 to 40 ppm and from 4 to 80 ppm, respectively, was used to determine the calibration curves. Each concentration was measured three times, and average values were obtained. The result is illustrated in fig. 4. Calculated based on ICH guideline [14, 15], the LOD and LOQ of AML is 0.3169 and 1.0563; of ATV is 0.3572 and 1.1907, respectively. The linearity was confirmed, the calibration curves of both AML and ATV had a coefficient of determination (R^2) of an acceptable value of 0.9998.

Table 2: System suitability of method on standard and test samples. sAML: standard AML; sATV: standard ATV; $\overline{X}$: mean; RSD%: relative standard deviation; t_R: retention time; S: peak area; Rs: resolution; N: theoretical plates; As: asymmetric factor; K': retention factor

		T_r	S	Rs	N	As	K'
sAML	$\overline{X}$	3.72	691387.20	1.55	3873.67	1.07	0.69
	RSD%	0.23	0.74	0.49	1.61	1.27	0.59
sATV	$\overline{X}$	5.76	1772524.83	8.18	7824.00	1.11	1.62
	RSD%	0.10	0.71	0.30	0.60	0.93	0.34
AML	$\overline{X}$	3.74	851418.67	1.71	4055.33	1.06	0.70
	RSD%	0.28	1.19	0.57	1.61	1.39	0.00
ATV	$\overline{X}$	5.75	1748552.33	8.14	7908.83	1.10	1.61
	RSD%	0.09	0.77	0.59	0.87	0.94	0.32

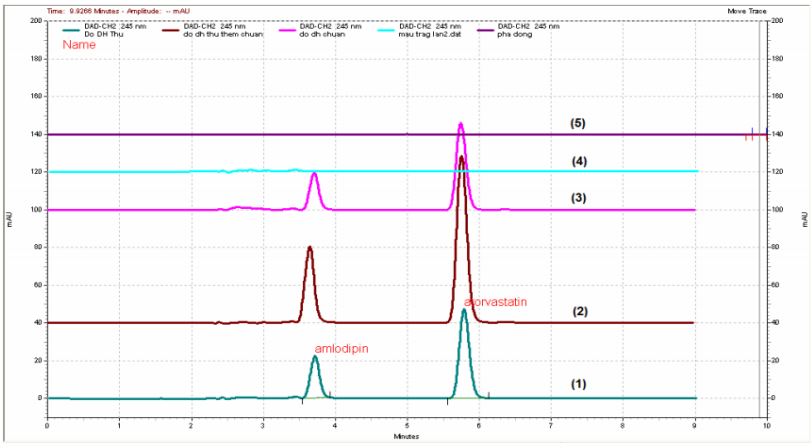

Fig. 3: Overlaid chromatogram of specificity validated samples. (1): test sample, (2): standard and test mixture sample, (3): standard sample, (4): blank sample, (5): mobile phase sample

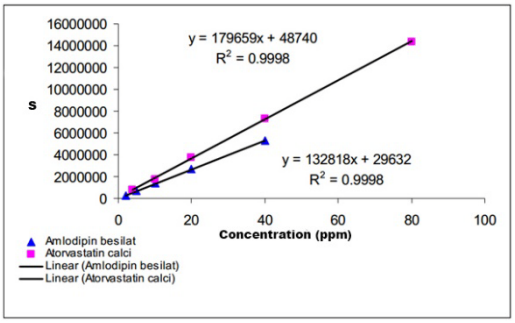

Fig. 4: Calibration curves in linearity study of AML and ATV, in a concentration range of 2-40 ppm and 4-80 ppm, respectively. S: peak area

Precision

The intermediate precision test was performed. Twelve test samples were run consecutively. Six samples were injected into HPLC Hitachi L-2000, detector DAD L-2455, column Phenomenex Luna RP-C18 (250 mm x 4.6 mm, 5 µm) at Can Tho University of Medicine and Pharmacy, Vietnam (condition 1). Another six were injected into HPLC Hitachi L-2000, detector DAD L-2455, column Restek Pinnacle II C18 (250 mm x 4.6 mm, 5µm) at the Can Tho Institute of Drug Quality Control, Vietnam (condition 2). Data are shown in table 3. Intermediate precision in two different locations at two different times shown RSD<1%, indicating good repeatability.

Accuracy

Standard solution of AML and ATV with a concentration of 80%, 100%, and 120% compared to the estimated amount of AML and ATV was added to the test samples. The accuracy was determined by the average recovery rate of the samples. Results are demonstrated in table 4. The satisfied recovery rate at 100.03% and 99.58% of AML and ATV, respectively, demonstrate excellent accuracy. Overall, our method was validated successfully with acceptable parameters according to ICH.

Dissolution test

The dissolution tests were done with three formulations (A, B, C) along with the reference drug Amdepin Duo® after re-validating the method with buffer pH 6.8 as a sample solvent. The profiles are illustrated in fig. 5.

Table 3: Intermediate precision test at two different places and times

Sample	Intermediate precision			
	AML (%)		ATV (%)	
	Condition 1	Condition 2	Condition 1	Condition 2
1	100.8	99.2	99.8	98.1
2	102.0	99.0	100.5	97.2
3	99.4	99.6	98.8	97.7
4	100.4	100.0	98.8	97.6
5	100.8	98.8	99.8	97.3
6	99.4	99.0	98.5	97.1
Mean	100.4	99.2	99.4	97.5
RSD%	1.04	0.47	0.78	0.39
Intermediate precision	99.8		98.5	
RSD%	0.75		0.58	

Table 4: Experimental results for the accuracy test for AML and ATV

Substance	Added amount		Found amount (ppm)	Recovery (%)	Average recovery (%)
	%	ppm			
AML	80	4.09	4.05	99.02	100.03
	100	5.12	5.20	101.56	
	120	6.14	6.11	99.51	
ATV	80	7.58	7.47	98.55	99.58
	100	9.47	9.52	100.53	
	120	11.37	11.33	99.65	

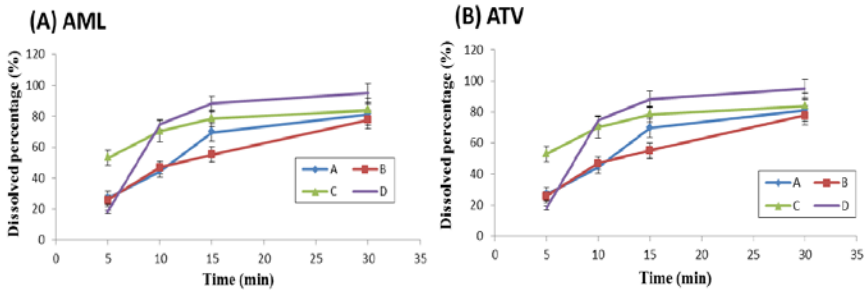

Fig. 5: Dissolution profiles of AML (A) and ATV (B) in 4 formulations, namely A, B, C, and Amdepin Duo® (D)

The method has a broad range of concentration value from 40% to 800% of quantification value (i.e. 2-40 ppm for AML and 4-80 ppm for ATV). Therefore, it is suitable for determining the dissolution profiles of pharmaceutical dosage forms. To accomplish this, we validated the method again in sample solvent at pH 6.8 (data not shown). Hence, the method can be used for dissolution purpose.

CONCLUSION

In this study, we developed and validated a novel, simple, and flexible HPLC-UV method using volatile buffer triethylamine for simultaneous determination of amlodipine besylate and atorvastatin calcium. Furthermore, this approach can be applied for both quantification and dissolution profiles.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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