Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

VALIDATED STABILITY INDICATING HPLC-DAD METHOD FOR THE SIMULTANEOUS DETERMINATION OF AMLODIPINE BESYLATE AND OLMESARTANMEDOXOMIL IN MIXTURE

EKRAM M. HASSAN, MARWA K. EL JAMAL, M. ABDELHADY EL SAYED

Faculty of pharmacy, Beirut Arab University, Lebanon,

Email: Marwa.jamal@hotmail.com, ekram_mahm@yahoo.com

Received: 02 Feb 2014 Revised and Accepted: 17 Feb 2014

ABSTRACT

Objective: The development of a simple and reliable HPLC-DAD procedure was described for the assay of the drug combination containing Amlodipine Besylate (AMB) and OlmesartanMedoxomil (OLM).Such mixture in tablet form is used for the treatment of hypertension.

Methods: Effective chromatographic separation was achieved on licosphere 100 RP-C_8 (250 x 4.6, 5µm) in a relatively short time (3 & 7.6 min for OLM and AMB respectively). The mobile phase was adjusted to be acetonitrile : 0.2MM KH_2PO_4 of pH 6 : triethanolamine ratio (53:42:5).The reliability and performance of the proposed procedure was statistically validated with respect to linearity, ranges, precision, accuracy, selectivity, detection and quantification limit (LOD&LOQ).

Results: Calibration curves were linear in the range of 1-25 and 5-60 µg.mL^{-1} for AMB and OLMrespectively with correlation coefficient (r) > 0.9993. The calculated % relative error (Er%) and % relative standard deviation (%RSD) were less than 2% for both drugs indicating good accuracy and precision.

Conclusion: The validated HPLC-DAD method was successfully applied for the analysis of AMB & OLM in laboratory made tablets with high accuracy exhibiting no interfering peaks from the auxiliary tablet ingredients.The proposed method made use of HPLC –DAD as tool for quality control in compounding AMB and OLM in combination.

Keywords*:* OLM; AMB; HPLC-DAD;combined dosage forms.

INTRODUCTION

Amlodipine Besylate,[3-Ethyl5-methyl(4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzene sulphonate], (AMB) and Olmesartan Medoxomil,[(5-methyl-2-oxo-2H-1,3-dioxol-4-yl)methyl4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazole-5-carboxylate],(OLM) are co-formulated together in Sevikar® tablets.These are indicated as substitution therapy for the treatment of hypertension and for stable coronary heart disease in patients already controlled with separate doses of amlodipine and olmesartan. Combinations of olmesartan and amlodipine are significantly more effective at reducing blood pressure and realizing guideline blood pressure goals in patients with mild to severe hypertension than monotherapy. Combination therapy is well tolerated and associated with a lower incidence of side effects, such as edema, compared to monotherapy with high amlodipine doses[1].

AMB has been determined in presence of OLM using HPLC [2,3] and HPTLC [2].In combined dosage forms, OLM has been determined using HPLC [4-6],HPTLC [6,7], derivative and derivative ratio spectrophotometry [3,8,9].

Few analytical methods have been reported for the simultaneous determination of AMB and OLM in combined dosage forms. These include multi-wavelength spectrophotometry [10], HPLC[11-13].

The aim of the present work was to develop a simple, rapid, sensitive and precise RP-HPLC-DAD for the simultaneous determination of AMBand OLM in combined dosage forms. The optimum conditions for such determination have been investigated.

MATERIALS AND METHODS

Apparatus

pH Measurement: A Mettler Toledo pH meter, Model MP 220 was calibrated with standard buffers at room Temperature.

Balance: Adventurer TM, Ohaus Corp-Pine Brook, NJ USA, sensitivity = 0.1 mg.

Orbital Shaker: Dissolution was done using Wiggen Hauser Shaker OS-150.

HPLC-DAD: The HPLC-DAD system (Jasco, Japan) consisted of quaternary pump, PU-20 89/ i plus, vacuum degasser and a quaternary gradient pump. Diode array and multiple wavelength detector MD 2018 plus. The LC system is equipped with a manual injection which uses a Rheodyne port sample injection valve and fitted with 20 µl sample loop. All are Jasco PU-2089 series.LC separations are performed on Lichrosphere 100 RP C_8 analytical column (250mm x 4.6 mm, 5 µm) at ambient temperature. Mobile phase used was degassed and filtered by passing through 0.5 µm pore size membrane filter (Zefluor™ 47 mm PALL Corporation) prior to use. The samples were also filtered using PTFE 0.2 µmMinisart SRP 15 (Sartorius Stedim) disposable filters.

Materials and Reagents

AMB and OLM (supplied by PHARCO Pharmaceuticals,Alexandria), Egypt, Acetonitrile (SIGMA-ALDRICH CHROMASOLV®FOR HPLC≥99.9%),KH_2PO_4 (Merck KGaA),Na_2HPO_4 (BDH), triethanolamine (AnalaR NORMAPUR).

General procedure

The mobile phase was prepared by mixing 0.2 M phosphate buffer, acetonitrile and triethanolamine 10 % v/v aqueous solution in a ratio (42:53:5) by volume. The pH was adjusted to 6.0 with phosphoric acid. The flow rate was 1 mL.min^{-1} and the injection volume 20 µL using Column C_8. The eluent was monitored by diode array detector from 190 to 400 nm and chromatograms were recorded at 250 nm.All determinations were performed at 25°C.AMB stock solution (500µg.mL^{-1}) and OLM stock solution (1000µg.mL^{-1}) were prepared in HPLC-grade acetonitrile.The working solutions were prepared by dilution 10 times of stock solution with the same solvent.

Calibration graphs

Into series of 10-mL measured flasks 1.0-5.0 mL from working standard AMB or 2.0-6.0 mL OLM were transferred and diluted to volume with acetonitrile to give the final concentrations stated in table 2. The above solutions were filtered using 0.2 µm disposable filters. 20 µL portions of the working standard solutions of AMB and OLM were injected in triplicates and chromatographed under the chromatographic conditions mentioned above. The peak area values of each drug were plotted against the corresponding concentrations to obtain the calibration graph for each drug. The concentrations of AMB and OLM from the corresponding calibration graphs were computed.

Analysis of synthetic mixtures

Accurate volumes of working standard solutions of AMB and OLM were transferred into a four separate 10-ml calibrated flasks and diluted to the mark with the mobile phase to give synthetic mixtures containing AMB and OLM in the ratios stated in table 3. The solutions were filtered using 0.2 µm disposable filters.Volumes of 20 µL portions of the mixture solutions were injected in triplicates and chromatographed under the chromatographic conditions mentioned above. The peak areas for each drug were measured and the corresponding concentrations in the mixtures were derivedreferring to calibration graph.

Tablet Assay

Twenty tablets were weighed and finely powdered (laboratory made tablets containing 5 mg AMB and 20 mg OLM per tablet in addition to lactose, starch, talc and magnesium stearate as tablet fillers). Accurate weights, of the finely powdered tablets equivalent to 10 mg AMB and 40 mg OLM were transferredinto 100 mL calibrated flask and extracted using acetonitrile by shaking for 15 minutes in ultrasonic bath. The volumes were dilutedwith the same solvent. A volume of 0.5 mL aliquot of the filtrate was filtered, transferred into 10-ml calibrated flask and diluted to the mark with acetonitrile to prepare tablet solution containing 5 µg.mL^{-1} AMB and 20 µg.mL^{-1} OLM. The procedure was completed as under preparation of calibration graphs starting from "The above solutions were filtered ……."

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The present study aimed at developing a chromatographic system capable of eluting and resolving AMB and OLM from one another. The preliminary investigations were directed towards the effect of various variables on the system suitability to maximize the resolution and sensitivity of the analytical procedure. The parameters included the detection wavelength, the type and quantity of organic modifier and the pH of the mobile phase.

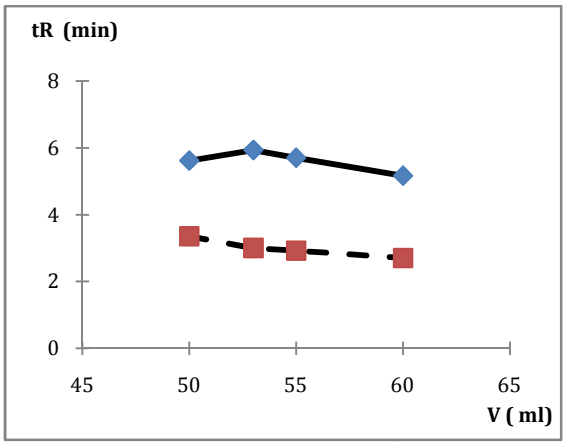

Fig. 1: It shows the effect of volume of acetonitrile on the retention time of 5µg.mL^{-1} AMB (- - -) and 20 µg.mL^{-1} OLM (—)

Methanol and acetonitrile were investigated as organic modifiers,that reflected on retention time. The use of acetonitrile in the mobile phase allowed the elution of both AMB and OLM at reasonable retention time. The mixture of standards was injected with mobile phases containing different proportions of acetonitrile. Fig. 1 shows the retention times obtained for the two drugs as a function of acetonitrile content in mobile phase. Acetonitrile 53 parts was chosen to provide optimum separation with the most symmetric and well defined peaks. At lower acetonitrile concentrations, separation occurred but with excess tailing. Increasing acetonitrile concentrations caused the decrease in the retention time of amlodipine with decreased base line separation.

Optimum pH of the aqueous phase was investigated using phosphate buffer(0.2 M potassium dihydrogen phosphate of different pH values adjustable with orthophosphoric acid or sodium hydroxide) together with acetonitrile (Fig. 2).

Therefore, the final mobile phase composition was adjusted to be acetonitrile: 0.2 M aqueouspotassium dihydrogen phosphate (pH 6.0):aqueoustriethanolamine 10% v/v (53: 42:5).The use of triethanolamine was necessary to give chromatographic peaks with acceptable symmetry factors.

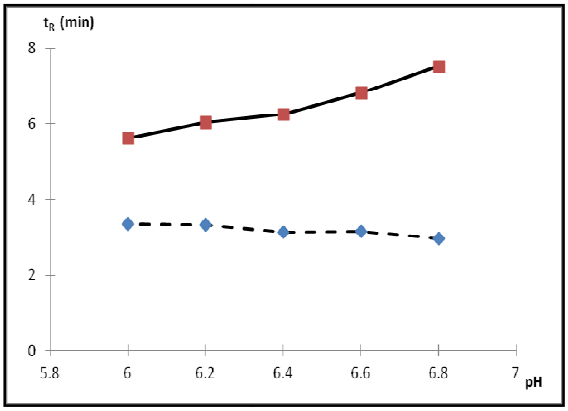

Fig. 2: It shows the effect of pH on the retention time of 5µg.mL^{-1} AMB (- - - -) and 20 µg.mL^{-1} OLM (—)

Wavelength Detection

The multiple wavelength detector offers the advantage of measuring each analyte at itsλ_{max}, thus improving sensitivity. In addition, diode array detection enhances the power of HPLC as an elegant option for assessing method specificity by comparison of recorded spectra during peak elution. Quantification was achieved and based on peak area measurement.

The standard solutions of AMB (5 µg.mL^{-1}) and OLM (20 µg.mL^{-1}) were scanned separately in the wavelength range 200 - 400 nm. AMB absorption spectrum exhibited two absorbance maxima at 239 and 360 nm where as OLM showed its absorbance maximum at 256 nm. The optimum chromatographic conditions mentioned previously were applied for all measurements. Fig. 3, shows the separation of OLM at 3 min and AMB at 6.55 min at the optimized chromatographic conditions.

System suitability

Various parameters including capacity factors (k′), selectivity (α), resolution (R_s), asymmetry factor (A_f) and number of theoretical plates (N) are listed in table 1. All these parameters were satisfactory and indicative of the good efficiency and selectivity of the method for separation of AMB and OLM binary mixtures.

Analytical performance of the proposed method

After establishing the optimum experimental conditions, validation of the proposed method was conducted involving determination of

the required analytical performance characteristics. These included linearity, limits of detection and quantification, accuracy, precision and specificity.

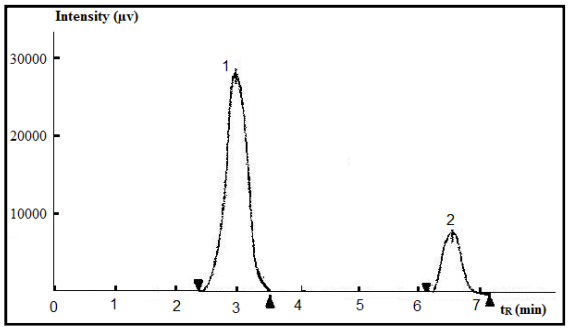

Fig. 3: It shows the HPLC chromatogram of a 20µL injection of a standard mixture of 5µg.mL^{-1} AMB and 20 µg.mL^{-1} OLM using the optimized chromatographic conditions.

Linearity and concentration ranges

Under the optimized experimental conditions, the graph obtained by plotting the peak areas versus the concentrations of AMB or OLM, was found to be linear over the concentration ranges given in table 2. The slopes, intercepts and correlation coefficients obtained by the linear least squares [14,15]treatments of the results are also given in table 2. The correlation coefficients (r) obtained were higher than 0.999 for both drugs with high values of F (low significant F) which confirmed the linearity of the calibration curves. An important statistical parameter for indicating the random error in the estimated values of y is the standard deviation of the residuals $S_{y/x}$. Also, the importance of $S_{y/x}$ originates from being used to calculate S_a and S_b, the standard deviation of the intercept (a) and the slope (b). These values showed the good linearity of the calibration graphs and the compliance to Beer's law.

Limit of detection (LOD) and limit of qualification (LOQ)

The concentration of the analyte showing signal-to-noise ratios 3:1 and 10:1 were considered as LOD and LOQ, respectively [16]. LOD and LOQ for AMB and OLM using the proposed HPLC methods are presented in table 2.

Accuracy and precision

In order to test for accuracy and precision of the proposed methods, five replicate determinations of laboratory prepared mixtures of the two drugs were carried out. The concentrations of the two drugs in the prepared synthetic mixtures were within the linearity range of each drug. The assay was repeated five times.The calculated % relative error (Er %) and RSD % were found to be less than 2 % for AMB and OLM indicating the good accuracy and precision for the proposed method (table 3).

Table 1: It shows the HPLC system suitability parameters for the determination of AML and OLM using the proposed method

Analyte	Retention time (t_R)	Capacity factor (k′)	N^o of theoretical plates (N)	Asymmetry factor (A_F)	Selectivity (α)	Resolution (R_s)
OLM	3.00	1.14	303	1.09	3.23	5.6
AMB	6.55	3.68	1709	1.34		

Table 2: It shows the assay parameters for the determination of AMB and OLM using the proposed HPLC method

Parameters	AMB	OLM
Conc range (µg.mL^{-1})	1-25	5-60
λ_{nm}	250	250
Intercept (a)	-3737	-3167
Slope (b)	21605	43520
Correlation coefficient (r)	0.9993	0.9999
S_a	6942	8380
S_b	457.58	232.10
$S_{y/x}$	2.56 x10^8	6.79 x10^8
$(S_b)^2$	209380	53871
% S_b	45758	76199
F	2229	3321
Sig. F	2.09 x10^{-5}	3.01 x 10^{-4}
LOD (µg.ml^{-1})	0.964	0.578
LOQ (µg.ml^{-1})	3.21	1.93

Table 3: It shows the accuracy and precision for the simultaneous determination of AMB and OLM in laboratory-made mixtures using the proposed HPLC method.

Ratio AML : OLM	Nominal Value µg.mL^{-1}		Mean Recovery ± SD^a RSD%^{b} Er%^{c}	
	AMB	OLM	AMB	OLM
1:2	5	10	98.52 ± 0.98 0.99 -1.48	100.09 ± 1.25 1.25 0.09
1:4	5	20	98.2 ± 1.52 1.55 -1.8	99.82 ± 0.88 0.88 -0.18
2:3	10	15	99.61 ± 1.34 1.35 -0.39	98.82 ± 0.92 0.93 -1.18
4:1	20	5	99.6 ± 1.09 1.09 -0.40	98.34 ± 1.42 1.44 -1.66

[a]Mean ± SD for the five determinations, [b]% Relative standard deviation, [c]% Relative error

Selectivity

Method selectivity was examined by analyzing several laboratory-made mixtures of AMB and OLM at various concentrations levels within the linearity ranges mentioned in table 2. The good % recoveries indicated the capability of the method to resolve and quantify the analytes in different ratios (table 3). Also, the selectivity of the proposed HPLC-DAD method can be assessed by the system suitability parameters like retention time (t_R) and resolution (R_s). The FDA guidance indicates that well separated peaks, with resolution, R_s>2 between the peak of interest and the closest eluted peak are essential for reliable quantification [17]. The separated chromatographic peaks met this specification where the resolution factor was 5.6 (table 1).

Tablet assay

Due to the unavailability of the commercial tablets in the Lebanese market, laboratory-made tablets were prepared and analyzed by the proposed HPLC-DAD method. The active ingredients were extracted with acetonitrile and dilution was made to reach concentration levels within the specified ranges with acetonitrile. The active ingredients were eluted at their specific retention times.

The diode- array detection enabled peak purity verification where no signs of co-elution from any inactive components were detected. The recoveries were calculated using the external standard method. The assay results revealed satisfactory accuracy and precision as indicated from % recovery, SD and RSD % (table 4).

Table 4: It shows the assay results for the determination of AMB and OLM in laboratory-made tablets using the proposed HPLC-DAD method

Drug	Nominal Value µg.mL^{-1}	Mean Recovery ± SD[a]	RSD %[b]	Er %[c]
AMB	5 ug.mL^{-1}	98.50 ± 0.98	0.99	-1.50
OLM	20 ug.mL^{-1}	99.45 ± 1.07	1.08	-0.55

[a]Mean ± SD for five determinations,[b]% Relative standard deviation,[c]% Relative error

CONCLUSION

In this study, a validated simple and reliable HPLC-DAD procedure was described for the assay of the drug combination containing AMB and OLM, indicated for hypertension treatment. The analytes were separated at retention times 3.0, 6.55 min for OLM and AMB, respectively on a RP-C_8 column in a relatively short run time (less than 7 min). The method used the diode-array detector (DAD) as a tool for peak purity confirmation and multiple wavelength detection. Meanwhile, the method could be adapted to conventional HPLC with UV detection. Reliability was guaranteed by testing various validation parameters of the method and successful application to laboratory made tablets. The developed method was found to be accurate and precise. Therefore, it can be recommended for the routine analysis of the studied drugs either in bulk form or in combined tablet formulations.

REFERENCES

1. Niemeijer MG, Cleophas TJ, Combination Therapy with Olmesartan and Amlodipine in the Treatment of Hypertension. Pharmaceuticals22009; 125-133.
2. Kamble AY,Mahadik MV, Khatal L, Dhaneshwar SR, Validated HPLC and HPTLC method for simultaneous quantitation of amlodipine besylate and olmesartanmedoxomil in bulk drug and formulation,Anal. Lett.2010; 43 (1-3):251-258.
3. Kardile DP, Kalyane NV, Thakkar TH,Patel MR, Moradiya RK, Simultaneous estimation of amlodipine besylate and olmesartanmedoxomil drug formulations by HPLC and UV-spectrophotometric methods,J. Pharm. sci. & Res., 2010; 2 (9): 599-514.
4. Sagirli O,O''nal A,Toker SE, Ensoy DS, Simultaneous HPLC Analysis of Olmesartan and Hydrochlorothiazide in Combined Tablets and in vitro Dissolution Studies,Chromatographia, 2007; 66: 213-218.
5. Ashok kumar J, Sathya A,Senthilkumar K,Patilsagar N,Prathap B,LokeshSB, Gopal V,Simultaneous estimation of Olmesartanmedoxomil and Hydrochlorothiazide by RP-HPLC Method from Combined Dosage Forms,Int.J.Res.Pharm.Sci. 2010; 1 (1): 24-27.
6. Bari PD, Rote AR,RP-LC and HPTLC methods for the determination of olmesartanmedoxomil and hydrochlorothiazide in combined tablet dosage forms,Chromatographia,2009; 69 (11-12): 1469- 1472.
7. B. Moussa, M. Mohamed, N.Youssri,simultaneous densitometric TLC analysis of olmesartanmedoxomil and hydrochlorothiazide in the tablet dosage form,J. Planar Chromatogr-Mod. *TLC,* 24 (1) (2011) 35-39.
8. M. Farouk, O.Abd EL-Aziz, A. Hamdan, M.Shehata,Simple Novel Spectrophotometric and Spectrofluorimetric Methods for Determination of Some Anti-hypertensive Drugs,J.American Sci.,7(1) (2011) 300-312.
9. Hemke AT, Bhure MV, Chouhan KS, Gupta KR,Wadodkar SG, UV Spectrophotometric Determination of Hydrochlorothiazide and OlmesartanMedoxomil in Pharmaceutical Formulation,E-J.Chem., 2010; 7 (4):1156-1161.
10. P.S.Patil, N.D.Chivate, S.S.Shinde, H.N. More, S.A.Pishwikar,RP-HPLC method for simultaneous estimation of amlodipine besylate and olmesartanmedoxomil from tablet,Int. J.Chem. Tech. Research,3(1) (2011) 267-273.
11. S.S.Qutab,S.N.Razzaq, M.Ashfaq, I.U. Khan, A. M.Mumtaz, simultaneous quantitation of olmesartanmedoxomil and amlodipine besylate in combined tablets using hplc, J. chil. Chem. Soc., 54 (3) (2009) 234-237.
12. S. Shah, A.Asnani, D.Kawade, S.Dangres, S. Arora, S.Yende,simultaneous quantitative analysis of olmesartanmedoxomil and amlodipine besylate in plasma by high-performance liquid chromatography technique,J. Young pharm., 4(2) (2012) 88-94.
13. P.S.Patil, H.N. More, S.A.Pishwikar,RP-HPLC method for simultaneous estimation of amlodipine besylate and olmesartanmedoxomil from tablet, Int. J. pharm. Pharm. Sci., 3 (3) (2011) 146-149.
14. P. Armitage, G.Berry,Statistical Methods in Medical research, 3rd Edition, Black well, Oxford, UK, (1994) PP 283-285,
15. J.N. Miller, J.C. Miller, Statistics and Chemometrics for Analytical Chemistry, 5thEdition,Pearson Prentice Hall, London (2005).
16. The United States Pharmacopeia (2009), Second edition, The National Formulary, Twent- seventh edition, United States Pharmacopeial Convention, Inc.
17. FDA Center for Drug Evaluation Research (CDER), Reviewer Guidance: Validation of Chromatographic Methods, Washington, USA (1994).