



RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESYLATE AND OLMESARTAN MEDOXOMIL FROM TABLET

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ABSTRACT

The present work describes RP-HPLC method for simultaneous estimation of Amlodipine Besylate (AML) and Olmesartan Medoxomil (OLM) from tablet. Best resolution of two drugs was achieved with the mobile phase having composition of acetonitrile and water in the ratio 60:40. The linearity response of the HPLC system for both OLM and AML was obtained over the range of 5-35 $\mu\text{g ml}^{-1}$. Optimum retention time with greater resolution of the two drugs and internal standard eluting within six minutes was achieved with a flow rate of 1 ml min^{-1} . After recording the spectra of the two drugs and internal standard, 248 nm was selected as suitable wavelength for estimation. The result of analysis showed excellent recoveries for both the drugs ranging from 99.75 % to 100.62 % for OLM and 98.91 % to 102.05 % for AML. Which suggested the good accuracy of the method. The results of analysis of tablet indicated that no interference due to common tablet excipients was observed with the developed method.

Keywords: Amlodipine besylate, Olmesartan medoxomil, HPLC

INTRODUCTION

Combinations of two or more drugs in the pharmaceutical dosage forms are very much useful in multiple therapies. Market survey revealed that, day-by-day new drugs and their combination with another drugs are being introduced in market as they have more patient compliance than a single drug¹. The analytical chemistry hence has challenge in developing the methods for their analysis with the help of number of analytical techniques, which are available for the estimation of the drugs and their combination². Analytical monitoring of pharmaceutical product or of specific ingredients within the product is necessary to ensure the safety and efficacy throughout the shelf life, including storage, distribution and use³.

Amlodipine Besylate and Olmesartan Medoxomil are recently introduced in the market as combined tablet dosage form, which is widely used in the treatment of hypertension. There is various methods as UV⁴, HPTLC⁵, Mass⁶, LC-MS-MS⁷, CZE⁸, HPLC^{9,10} for OLM alone or in combination with other dosage form and also for AML alone or in combination with other dosage form such as HPLC¹¹⁻²⁶, HPTLC²⁷, UV²⁸⁻³² for simultaneous estimation. But there is no method reported for simultaneous estimation of OLM and AML from dosage forms by RP-HPLC. In the analysis of formulations containing two or more drugs, one drug can interfere in the estimation of another drug. To avoid this, separation of components of mixture by extraction is usually carried out which make the procedure time consuming and complicated and often lacks accuracy.

The present work was undertaken to develop such method of analysis, which can estimate both the drugs in combination without prior separation which is a precise, accurate, simple, reliable and less time consuming method for estimation of drugs in tablet.

MATERIALS AND METHODS

Apparatus

The instrument used for the present study was PC based Jasco V-530 UV-Visible double beam Spectrophotometer with 1 cm matched pair quartz cell and spectral bandwidth of 2 nm. and HPLC with HIQ SII C₁₈ column-10 (4.5 mm x 250 mm).

Reagents and materials

Olmesartan Medoxomil and Amlodipine Besylate were obtained as a gift sample from Cipla, Vapi, India. Acetonitrile were purchased from Loba fine, India. Double distilled water was used throughout the experiment. Olsar-A in a tablet dosage form containing OLM and AML were purchased from local commercial sources.

Determination of wavelength for detection of OLM and AML:

The suitable wavelength for detection of OLM and AML was selected from overlain spectrum of OLM and AML, Wavelength selected was 248 nm.

Selection of mobile phase

Based on sample solubility, stability and suitability various mobile phases and compositions were tried to get a good resolution and sharp peak.

The standard solution containing mixture of OLM and AML as well as individual drugs were run in different mobile phases.

The following mobile phases were tried:

1. ACN: Methanol [70:30 % v/v].
2. ACN: Methanol [50:50 % v/v].
3. ACN: Methanol: Water [50:30:20 % v/v/v].
4. Water: ACN [50:50 % v/v].
5. Water: ACN [60:40 % v/v].

Each mobile phase was filtered through 0.22 μ membrane filter and degassed by sonication for 20 minutes. From the various mobile phases tried, mobile phase containing ACN and Water in 60:40 % v/v proportion was selected, since it gave sharp completely resolved peaks with symmetry within limits and significant retention times for both the drugs

Seperation conditions

Chromatographic mode	Chromatographic conditions
Standard Solution	100- $\mu\text{g ml}^{-1}$ of Olmesartan medoxomil and 100- $\mu\text{g ml}^{-1}$ of Amlodipine Besylate.
Stationary Phase	HIQ SII C ₁₈ column-10 (4.5 mm x 250 mm).
Mobile phase	ACN: Water (60:40).
Detection Wavelength	248 nm.
Flow Rate	1 ml min^{-1} .
Sample Size	20 μl .

Selection of chromatographic conditions

The selection of HPLC method depends upon the nature of the

sample, its molecular weight and solubility. RP-HPLC method was selected for the initial separations because of its simplicity and suitability.

The chromatographic variables such as mobile phase flow rate and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as selectivity, asymmetric factor, and peak resolution were calculated. The condition that gave the best resolution, symmetry and selectivity was selected for estimation.

Optimization of chromatographic parameters

Optimisation in HPLC is the process of finding a set of conditions that adequately analyze the quantification of the analyte with acceptable accuracy, precision, sensitivity, specificity, cost, ease, and speed of analysis.

Optimization of mobile phase strength

For selection of mobile phase, various mobile phase compositions containing phosphate buffer and acetonitrile in different ratios was tried but the resolution was not found to be satisfactory. Finally, mobile phase containing acetonitrile and water in 60:40 proportions was found to give best resolution for both the drugs.

Optimization of detection wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. For good response, optimization of wavelength was done at different wavelengths by UV detector. In the present study, drug solutions of $10\text{-}\mu\text{g ml}^{-1}$ of OLM and $10\text{-}\mu\text{g ml}^{-1}$ of AML were prepared in acetonitrile. After observing UV spectra of both the drugs, wavelength of 248 nm was selected for further study is shown in Fig. 1.

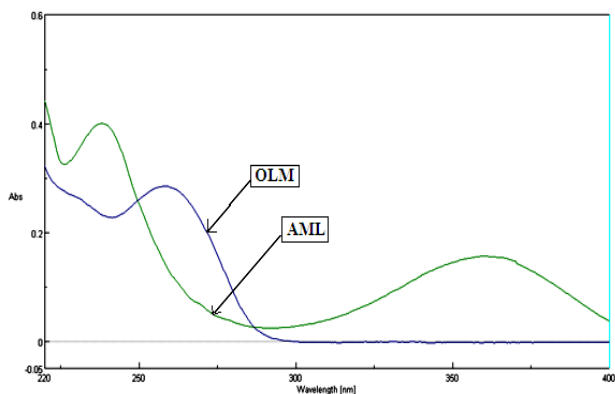


Fig. 1: It shows overlain spectrum of OLM and AML

Selection of internal standard

Hydrochlorothiazide (HCTZ) was selected as an internal standard after observing retention behavior of several drugs.

Preparation of standard drug solution

Standard stock solution containing OLM and AML was prepared by dissolving 10 mg and 10 mg of OLM and AML respectively in 100 ml of acetonitrile. It was then sonicated for 20 minutes and then final volume of the solution was made up to 100 ml with acetonitrile to get stock solution containing $100\text{-}\mu\text{g ml}^{-1}$ OLM and $100\text{-}\mu\text{g ml}^{-1}$ AML in 100 ml volumetric flask

Preparation of internal standard solution

Standard stock solution containing $100\text{-}\mu\text{g ml}^{-1}$ of HCTZ was prepared by dissolving 10 mg of HCTZ in 50 ml of acetonitrile and then sonicated for 20 minutes. The final volume of solution was made up to 100 ml with acetonitrile to get $100\text{-}\mu\text{g ml}^{-1}$ of HCTZ in

100 ml volumetric flask.

Linearity study of drug at selected wavelength

In a series of 10 ml volumetric flasks, 0.5 to 3 ml of OLM solution ($100\text{-}\mu\text{g ml}^{-1}$) and AML solution ($100\text{-}\mu\text{g ml}^{-1}$) respectively were pipetted and to each flask 2 ml of ($100\text{-}\mu\text{g/ml}$) HCTZ was added and then final volume of the solutions was made up to 10 ml with acetonitrile. A $20\text{-}\mu\text{l}$ of sample solution was injected into the injection port of chromatographic system having fixed volume loop injector.

Chromatograms were noted and response factor was plotted against concentration to get calibration curve. The data for calibration curve is given in Table 1. and Table 2 for OLM and AML respectively,

Table 1: Linearity of OLM 248 nm

Sr. No.	Concentration ($\mu\text{g ml}^{-1}$)	Response Factor
1.	5	1.7237
2.	10	2.04353
3.	15	2.35564
4.	20	2.78003
5.	25	3.09330
6.	30	3.45805
7.	35	3.75928

Table 2: Linearity of AML 248 nm

Sr. No.	Concentration ($\mu\text{g ml}^{-1}$)	Response Factor
1.	20	1.7237
2.	40	2.04353
3.	60	2.35564
4.	80	2.78003
5.	100	3.09330
6.	120	3.45805
7.	140	3.75928

The chromatogram of physical mixture is shown in Fig. 2. While overlain chromatogram of physical mixture is shown in Fig. 3.

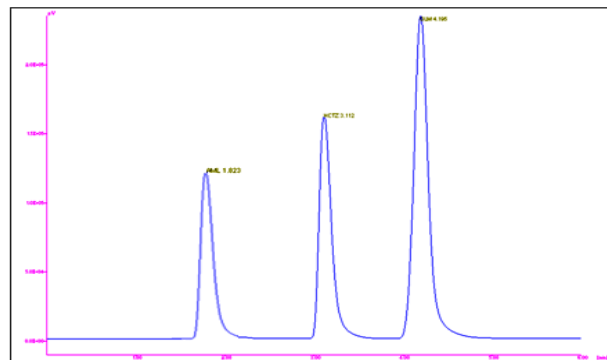


Fig. 2: It shows chromatogram of physical mixture

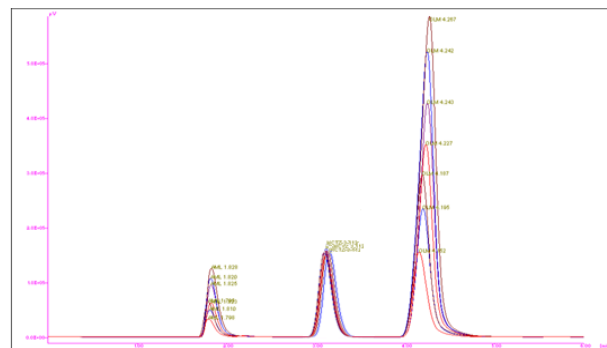


Fig. 3: It shows Overlain Chromatogram of Physical Mixture

Analysis of tablet formulation

From the triturate of 20 tablets, an amount equivalent to 20 mg of OLM and 5 mg of AML was weighed and dissolved in 50 ml of acetonitrile by sonicating for 20 minutes. The solution was filtered through 0.22 μ membrane filter and then final volume of the solution was made up to 100 ml with acetonitrile to get stock solution containing 200 $\mu\text{g ml}^{-1}$ of OLM and AML 000 $\mu\text{g ml}^{-1}$ in 100 ml volumetric flask. After appropriate dilutions, the solutions were run on HPLC system and the concentration of each analyte was determined with the equations generated. The statistical data obtained after replicate determinations (n = 6) is shown in Table 3. and chromatogram is shown in Fig 4.

Table 3: Results of tablet analysis

Analyte	Label claim (mg/tab)	% Label claim estimated* (Mean \pm S. D.)	R.S.D.
OLM	20	99.83 \pm 1.1424	1.1443
AML	5	99.98 \pm 0.8267	0.8268

*Average of six determinations; S.D.: Standard Deviation; R.S.D. Relative Standard Deviation

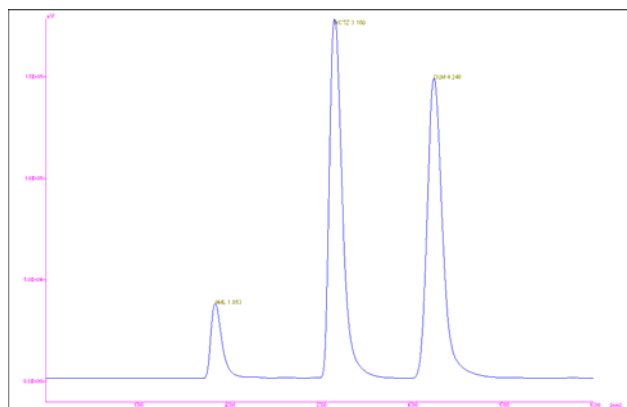


Fig. 4: It shows Chromatogram of Tablet Solution

Recovery studies

Accuracy of analysis was determined by performing recovery studies by spiking different concentrations of pure drug in the preanalyzed tablet sample. Results of recovery studies indicated that the method is rapid, accurate and reproducible and is shown in Table 4 and chromatogram is shown in Fig 5.

Table 4: Results of recovery study:

Analyte	Label claim (mg/tab)	% Recovery estimated* (Mean \pm S. D.)	R.S.D.
OLM	20	100.41 \pm 0.4386	0.4368
AML	5	100.76 \pm 1.01597	1.0082

*Average of six determinations; S.D.: Standard Deviation; R.S.D.; Relative Standard Deviation.

System Suitability Parameters

System suitability parameters were analyzed on freshly prepared standard stock solutions of OLM and AML. Both the drugs were injected into the chromatographic system under the optimized chromatographic conditions. Parameters were validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte³³. The suitability of the system were

- Number of theoretical plates.
- Tailing/ asymmetric factor.

c) Resolution.

d) Retention time.

e) Calibration Curve.

f) Limit of Detection and Limit of quantitation. The results are shown in Table No. 5.

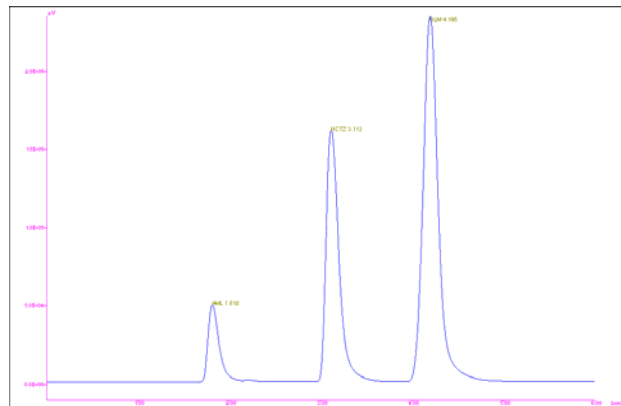


Fig. 5: It shows Chromatogram of Recovery Solution

Table 5: System Suitability Parameters:

Sr. No.	Parameters	OLM	AML
1.	Theoretical Plates	2137.91	2521.93
2.	Tailing Factor	1.35	1.52
3.	Resolution	4.26	00
4.	Retention Time in minutes	4.24	1.82
5.	Selectivity	1.35	0.00
5.	Calibration Range ($\mu\text{g ml}^{-1}$)	20-140	5-35
6.	Limit of Detection ($\mu\text{g ml}^{-1}$)	0.0735	0.0343
7.	Limit of Quantitation ($\mu\text{g ml}^{-1}$)	0.2227	0.1039

RESULTS AND DISCUSSION

The proposed method describes a RP-HPLC procedure employing a C₁₈ column and a mobile phase containing acetonitrile and water in 60:40 proportions. To develop the method with good resolution the change in proportion of organic solvents were studied. Acetonitrile, methanol, phosphate buffer and water in various ratios were tested to get an appropriate mobile phase composition. The mixtures of acetonitrile, methanol and water at various ratios were examined, which resulted in very close retention times of the two drugs. Also in case of phosphate buffer results in broadening of peaks were observed. Best resolution of two drugs was achieved with the mobile phase having composition of acetonitrile and water in the ratio 60:40. The linearity response of the HPLC system for both OLM and AML was obtained over the range of 5-35 $\mu\text{g ml}^{-1}$. Various drugs were checked for use as an internal standard to obtain well-resolved peaks along with the two drugs in the formulations. Retention time of both the drugs was studied with flow rate of mobile phase at 0.5, 1, 1.2 ml min⁻¹. Optimum retention time with greater resolution of the two drugs and internal standard eluting within six minutes was achieved with a flow rate of 1 ml min⁻¹. The two drug solutions having concentration of 10 $\mu\text{g ml}^{-1}$ were scanned in the UV Range of 200 nm to 400 nm on a UV-Visible spectrophotometer for selection of sampling wavelength. After recording the spectra of the two drugs and internal standard, 248 nm was selected as suitable wavelength for estimation.

Accuracy of the method was checked by adding known amounts of pure drug to each known concentration of the tablet formulations. The resulting mixtures were run on HPLC. The result of analysis showed excellent recoveries for both the drugs ranging from 99.75 % to 100.62 % for OLM and 98.91 % to 102.05 % for AML. The

recovery study data by the standard addition method suggested the good accuracy of the proposed method. The results of analysis of tablet indicated that no interference due to common tablet excipients was observed with the developed method.

CONCLUSION

The developed method is the first report for simultaneous estimation of OLM and AML. The developed HPLC method was found to be more accurate, precise and reproducible. The analysis of tablets containing two drugs gave the satisfactory results. The statistical parameter of this method showed good results. The recovery studies revealed excellent accuracy and high precision of the method. The statistical parameters and recovery studies of HPLC method were compared with the developed spectrophotometric method of analysis of the same dosage form. The HPLC method was found to give better results. Therefore the proposed method could be applied for routine analysis in quality control laboratories.

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