# REVIEW

# HPTLC method for estimation of Olmesartan medoxomil in tablet formulation with stability studies

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**Abstract**: A rapid resolution high performance thin layer chromatography (HPTLC) method has been developed and validated for estimation of Olmesartan medoxomil in tablet formulations. This paper describes accurate, precise, specific and reproducible method and its degradation products, related impurities for assessment of purity of bulk drug and stability of its tablet formulations. The method involve silica gel 60 $F_{254}$ high performance thin layer chromatography and densitometric detection at 264 nm using toluene - acetonitrile- methanol - ethyl acetate - acetic acid (5:3.5:0.3:1:0.3 v/v/v/v). Calibration curve ranges between 300-800 ng/spot-1 Olmesartan medoxomil. Experimental design was involved forced degradation of drug, optimization of mobile phase, detection made and other chromatographic phase and study of linearity range. The total time for chromatographic separation was 6 min with a total analysis time 15 min. The proposed method was validated for its linearity, precision, recovery studies and robustness.

**Keywords**: Olmesartan medoxomil, HPTLC, validation, experimental design.

## INTRODUCTION

Olmesartan medoxomil is one of the most widely used angiotensin II type I (ATI) receptor antagonist in the treatment of hypertension. Chemically Olmesartan medoxomil is nonpeptide benzimidazole derivatives. The drug is not official in any pharmacopoeia usual dose is 10 mg once daily. The drug is practically insoluble in water, sparingly soluble in methanol, acetone and freely soluble in acetonitrile. The literature survey revealed that few analytical methods are reported for estimation pf Olmesartan medoxomil in biological fluids and in combination with other drugs in tablet dosages form. The literature search has shown several analytical method for analysis of antihypertensive drugs, including Olmesartan medoxomil in biological fluids (Yang *et al., 2006*), olmesartan in human plasma by HPLC-MS with solid phase extraction (Xiaoli *et al., 2006*), simultaneous estimation of Olmesartan medoxomil and hydrochlorthiazide by HPTLC (Shah *et al., 2007*), comparative analysis of the efficacy of Olmesartan medoxomil, hydrochlorthiazide, valsartan, Irbesartan and telmisartan combination (Venketa *et al., 2004*). Determination of Olmesartan medoxomil and hydrochlorthiazide in the tablet by capillary zone electrophoresis (Celebier and Altinoz, 2007), there are no reference in the literature concerning for estimation of Olmesartan medoxomil in bulk and tablet formulations (Abbas e*t al.,* 2006, Bakshi and Singh, 2000, 2002, 2004, Kaul *et al.,* 2003, 2*004*, 2005, Vedera *et al., 2006*, Jain *et al.,* 2007, Venkatachalam *et al.,* 2007, Bari and Rote, 2009). The present study therefore aimed to provide search an economically viable HPTLC method.

System suitability tests were also carried out to verify reproducibility and results are summarized in table 1. For quantitative applications linear calibration graphs were obtained with correlation coefficients of 0.9998 and 0.9999 for OLM. Limits of detection (LOD) were 0.143 $mL^{-1}$ and limits of quantitation (LOQ) 0.43 $mL^{-1}$ for OML which showed good precision for the proposed HPTLC method (ICH, 1996).

## MATERIALS AND METHODS

### *Chemicals and reagents*

Methanol, Toluene (Merck, Germany), water (HPLC grade) were used to prepare the mobile phase, methanol (analytical grade, Merck, Germany) was used as solvent. Merck pre-coated aluminum sheet with silica gel 60 $F_{254}$ were used for this study. Olmesartan medoxomil was obtained as gift sample from Blessings pharmaceuticals, India; Nagpur and marketed formulations were purchased by local market, Rohtak, India.

Validation studies were performed as per ICH guidelines (ICH: Q1A 2003, ICH 2005).

### *Preparation of standard and test solutions*

**Standard solution A**

Accurately weighed 5 mg of OLM was dissolved in methanol and the volume was made upto 50 mL to obtain

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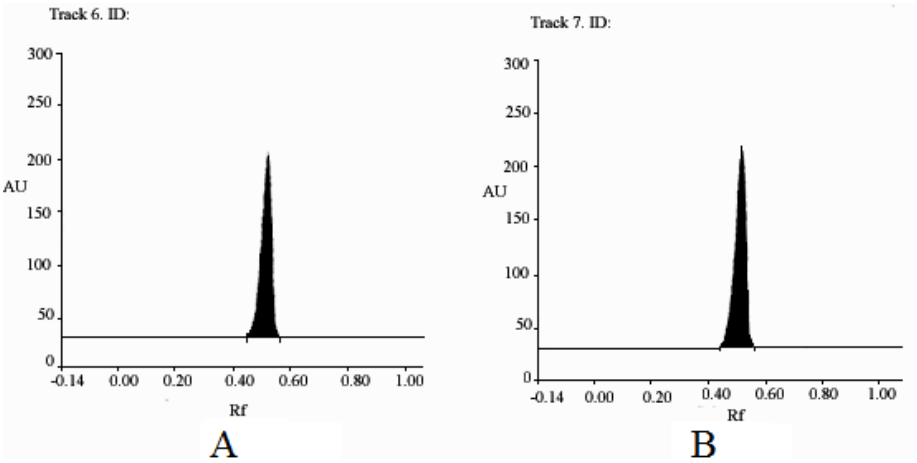


**Fig. 1**: Densitogram of Olmesartan medoxomil (a) Standard and (b) Tablet

conc 100 µg/mL. From this 5 mL of solution was pipette out and diluted to 10 mL with acetonitrile to obtain conc 50 µg/mL.

**Degradation of *Olmesartan medoxomil***

***Preparation of acid degradation products***

Accurately weighed 25 mg of OLM was dissolved in 10 mL methanol and 5 mL of 0.1N HCl was added to it. This mixture was kept at room temperature for 6 h.

***Preparation of base degradation products***

Accurately weighed 25 mg of OLM was dissolved in 10 mL methanol and 10 mL of 0.01 N NaOH was added to it. This mixture was kept at room temperature for 6 h.

***Preparation of hydrogen peroxide induced degradation products***

Accurately weighed 25 mg of OLM was dissolved in 10 mL methanol and 10 mL of 1% hydrogen peroxide was added to it. This mixture was kept at room temperature for 4.5 h.

***Dry and wet heat degradation products***

The powdered drug was stored in oven at 130˚C for 4 h to study heat degradation and 25 mg of OLM was separately dissolved in 25 mL methanol and refluxed for 3 h on boiling water bath for wet heat degradation.

***Photo-degradation products***

The photo stability of the drug was also studied by exposing the 25 mg of OLM to direct UV radiation for 56 h in UV chamber. All above degraded sample solution were made up to 25 mL with methanol to obtain solution of 1000 µg/mL. For this solution 10 mL was pipette out and to make up the volume 100 µg/mL, which was used for further study.

***Selection of mobile phase***

Solvent system toluene - acetonitrile - methanol – ethyl acetate - acetic acid in the ratio 5:3.5:0.3:1:0.3 v/v/v/v.

***Selection of wavelength for densitometric evaluation***

The wavelength selected for densitometric determination was λmax 264 nm.

***Chromatographic condition***

The chromatographic conditions were optimized to obtain reproducible results.

Separation were performed using mere pre-coated aluminum sheet with silica gel 60 $F_{254}$ TLC plate as stationary phase plate size and band width was 10X 10 cm 4 mm 1.6 µm particle size at 25˚c. Injection volume was 10 µL. UV detection was done at 264 nm. All mobile phase were filtered through a 0.2 µm milipore filter.

***Study of linearity of response and apparatus***

Standard solution was applied on TLC plate by microliter syringe with the help of Linomat IV. Sample applicator in the range of 6-16 µL (OLM : 300 to 800 mg) (ICH, 2000). The plate was then developed in a twin through glass chamber. After development, the plate was scanned at 264 nm with the help of Camag TLC scanner 3, which was attached to a Wincat's software made by Anchrom.

***Estimation of Olmesartan medoxomil in tablet by propose method***

***Standard solution***

It was prepared as described in standard solution A.

***Sample solution***

Twenty tablets were weighed and finely powdered. An accurately weighed tablet powered equivalent to 5 mg of OLM was transferred into 50 mL volumetric flask and 25 mL methanol was added. The flask content was sonicated for 25 min and the volume was adjusted to 50 mL with methanol. This solution was filtered through grade 1 filter paper and 5 mL filtrate was diluted upto 10 mL with acetonitrile to get final concentration of 50 µg/ mL.

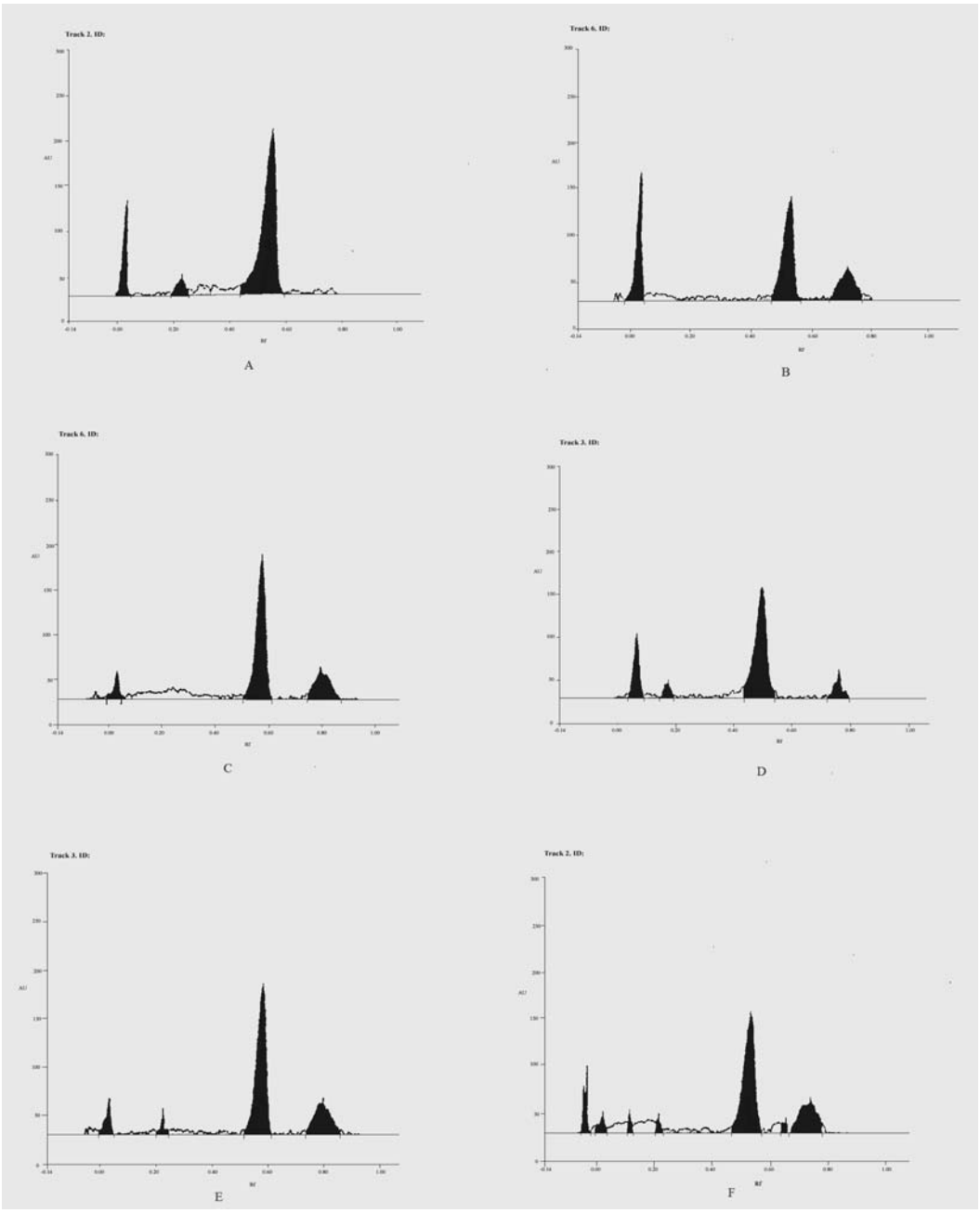


**Fig. 2**: Densitogram of Olmesartan medoxomil and its degradation products (A) Acid treated (B) Base treated (C) Oxide treated (D) UV treated (E) Dry heat treated (F) Wet heat treated.

***Procedure***

Two band of standard solution and six bands of sample of equal volume (10 µL) were applied on TLC plate and the plate was developed and scanned as per optimized chromatographic conditions.

**Validation procedure**

***Accuracy***

Accuracy of proposed method was ascertained on the basis of recovery studies by standard addition method at different level of labeled claim (60 to 140% of labeled claim) (ICH Q1A (R2) 2003, ICH Q2 (R1), 2005).

***Standard solution***

Standard solution was prepared as described in standard solution A.

***Standard solution***

An accurately weighed quantity of preanalyzed tablet powder equivalent to 2.5 mg of OLM was taken into 50 mL volumetric flask and known quantities of pure OLM and 25 mL methanol was added. The flask content was sonicated for 15 min and the volume was adjusted to 50 mL with methanol, 5 mL from resultant solution was

pipette out and diluted upto 10 mL with acetonitrile to give 50 µg/mL.

***Precision***

Precision of any analytical method in expressed as SD and % RSD of series of measurement.

***Specificity***

Specificity study was performed by analyzing standard of drug and sample. The spot for OLM in sample was confirmed by comparing the Rf value and spectra of the spot with that of standard. The peak purity of OLM was assayed by comparing the spectra at three different level i.e. peak start apex and peak end positions of the spot.

***Limit of detection (LOD) and Limit of Quantitation (LOQ)***

Several approaches for determining the LOD and LOQ are possible; the approach used is based on visual evaluation (ICH Draft guidelines on validation of analytical procedures, 2000).

***Linearity***

Linearity is reported as the correlation coefficient and the scope of the regression line.

***Robustness***

Robustness testing was performed in accordance with central composite circum scribed (CCC) design.

## RESULTS

Peak height and peak area were recorded and concentration vs response curves were plotted concentration response curve are depicted in table 1.

The instrument directly gives the weight of constituents in volume of sample solution applied by comparison with concentration of standard. These values subsequently converted to percent of labeled claim using fallowing equation:

% estimation = winCATs Value X DF X Avg Wt / Vol X LC X Wt taken X 10 eq (1)

Where DF – Dilution Factor, LC – Labelled Claim (mg/tab) of the respective tablet and Vol – Volume applied (5 µL)

**Table 1**: Values of Lineraity study

| Drug | Linearity range ng/spot | | Coefficient of slope | | | |
|---|---|---|---|---|---|---|
| | By height | By area | By height | By area | By height | By area |
| OLM | 300-800 | 300-800 | 0.9998 | 0.9999 | 360.01 | 11367 |

**Table 2**: Values of estimation of Olmesartan medoxomil in tablets, marketed tablet (Avg wt 152.99 mg for 20 mg of OLM)

| Sr. No. | Wt of tablet powder taken (mg) | Amount of OLM estimated in applied 10µL vol (mg) | | % drug estimation | |
|---|---|---|---|---|---|
| | | By height | By area | By height* | By area* |
| 1 | 39.12 | 5.032 | 5.031 | 99.41 | 99.89 |
| 2 | 39.10 | 4.972 | 5.006 | 99.64 | 99.89 |
| 3 | 38.78 | 4.989 | 4.998 | 99.68 | 99.30 |
| 4 | 38.62 | 5.033 | 5.026 | 101.87 | 101.27 |
| 5 | 37.24 | 5.112 | 5.013 | 100.08 | 100.18 |
| | | | Mean | 100.08 | 100.24 |
| | | | ± S.D. | 0.8316 | 1.5274 |
| | | | % RSD | 0.8332 | 1.5236 |

*Each value is mean of five observations

**Table 3**: Values of degradation study of Olmesartan medoxomil

| Sr. No. | Treatment | Time | % of OLM* |
|---|---|---|---|
| 1 | 5ml of 0.1 N HCl at R.T | 5 | 87.16 |
| 2 | 5ml of 0.1 N NaOH at R.T | 0.5 | 79.99 |
| 3 | 5ml of 1% $H_2O_2$ at R.T | 3.6 | 85.76 |
| 4 | Wet refluxed on boiling water bath | 1.5 | 87.13 |
| 5 | Dry heat 120˚C | 2.5 | 84.12 |
| 6 | In UV chamber | 56 | 84.11 |

*Each value is mean of five observations

**Table 4**: Value of recovery study, Avg wt of tablet 152.99 mg for 20 mg of OLM

| Sr. No. | Wt of tablet powder + pure drug (mg) | Amount of OLM estimated in applied 10µL Val (mg) | | % recovery | |
|---|---|---|---|---|---|
| | | By height | By area | By height* | By area* |
| 1 | 19.35±1.5 | 4.012 | 3.998 | 100.07 | 99.94 |
| 2 | 18.34±2.5 | 5.027 | 5.019 | 101.10 | 100.09 |
| 3 | 19.98±3.5 | 6.026 | 6.045 | 100.76 | 101.21 |
| | | | Mean | 100.64 | 100.41 |
| | | | ±S.D | 0.5290 | 0.7570 |
| | | | % RSD | 0.5255 | 0.7536 |

*Each value is mean of five observations

**Table 5**: Values of precision studies

| Sr. No. | Different analyst | % drug estimation | | | |
|---|---|---|---|---|---|
| | | Marketed formulation (Tablet) | | Marketed formulation (Tablet) | |
| | | By height* | By area* | By height* | By area* |
| 1 | I | 99.64 | 99.59 | 99.13 | 99.19 |
| 2 | II | 100.19 | 101.10 | 99.95 | 98.92 |
| 3 | III | 100.54 | 99.45 | 98.62 | 99.25 |
| | Mean | 100.07 | 100.01 | 99.26 | 99.78 |
| | ±SD | 0.4508 | 0.9564 | 0.6659 | 0.2676 |
| | RSD | 0.4507 | 0.9469 | 0.6709 | 0.2700 |

*Each value is mean of five observations.

**Table 6**: Values of LOD and LOQ

| Parameter | OLM | |
|---|---|---|
| | Height | Area |
| LOD(µg/mL) | 0.0144 | 0.0098 |
| LOQ(µg/mL) | 0.0433 | 0.0297 |

The chromatogram of the acid degraded sample for OLM showed additional peak at Rf 0.05 and 0.25 indicating that OLM undergoes degradation under acidic condition (fig. 2a).

The chromatogram of the base degraded sample for OLM showed additional peak at Rf 0.05 and 0.071 indicating that OLM undergoes degradation under alkaline condition (fig. 2b).

The chromatogram of the hydrogen peroxide degraded sample for OLM showed addition peak at Rf 0.07 and 0.74 indicating that OLM undergoes degradation in the presence of hydrogen peroxide (fig. 2c).

The chromatogram of the UV degradation sample for OLM showed additional peak at Rf 0.05 and 0.72 indicating that OLM undergoes degradation in UV radiation (fig. 2d).

The chromatogram of the dry heat degraded sample for OLM showed additional peak at Rf 0.05, 0.26, 0.68 and 0.78 indicating that OLM undergoes degradation under dry heat condition (fig. 2e).

The chromatography of the wet heat degraded sample for OLM showed additional peak at Rf 0.05, 0.15, 0.25, 0.68 and 0.78 indicating that OLM undergoes degradation in wet-heat condition (fig. 2f).

For the validation procedure, the total amount of each drug was calculated and the percent recovery was calculated by using following equation:

$$\% \text{ Recovery} = A/ B+C \times 100 \quad (2)$$

Where A-Total drug estimated (mg), B-Cut (mg) of drug contributed by tablet powder, C-Amount of pure drug added (mg)

Precision of this method is expressed as SD and % RSD of series of measurement by the different analyst and by interday and intraday.

Specificity was performed by analyzing standard drug and sample. The spot for OLM in sample was confirmed by comparing the Rf value and spectra of the spot with that of standard. Densitogram of OLM standard and tablet shown in fig. 3.

**Table 7**: Values of Linearity

| Drug | Particulars | Methods | Coefficient of correction | Slope |
|---|---|---|---|---|
| OLM | Linearity 300-800mg /spot | By height | 0.9998 | 358.09 |
| | | By area | 0.9999 | 11365 |

**Table 8**: Values of Robustness studies

| Sr. No. | Change in wavelength ± 2 | % drug estimation Tablet formulation | |
|---|---|---|---|
| | | By height* | By area* |
| 1 | 261 | 99.81 | 99.71 |
| 2 | 263 | 100.55 | 100.47 |
| 3 | 264 | 99.89 | 100.12 |
| | Mean | 100.06 | 100.12 |
| | ±SD | 0.4076 | 0.3698 |
| | RSD | 0.4075 | 0.3691 |

*Each value is mean of five observations

LOD and LOQ i.e. the quantitation limit is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analytes can be quantified with acceptable accuracy and precision.

Linearity is reported as the correlation coefficient, the slope of the regression line results are shown in table 7.

The proposed stability indicating its HPTLC method developed for the estimation of OLM in the bulk and marketed formulations, due to good separation and resolution of the chromatographic peaks and robustness toward reasonable changes in chromatographic parameters. Several individual solvents and blends of solvent were used to develop mobile phase for estimation of OLM by HPTLC method. The mobile phase comprising toluene - acetonitrile - methanol - ethyl acetate - acetic acid in the ratio 5:3.5:0.3:1:0.3 v/v/v/v was found to be most suitable for HPTLC as it resolved degradation products from pure drug.

## DISCUSSION

The developed HPTLC method are very sensitive and stability indicating statistical analysis proved that these methods are reproducible, selective for the analysis of OLM in bulk drug and in tablet formulations and can be used to determine the purity of the drug even in the presence of excipients and related impurities. As HPTLC methods separate the drug from its degradation products, it can be employed to isolate degradation products. This study can be extended to study the degradation kinetics of OLM and for estimation in plasma and other biological fluids.

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