Research Paper

Development and validation of HPTLC method for simultaneous estimation of Olmesartan medoxomil and Cilnidipine in their combined pharmaceutical dosage forms

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ABSTRACT

An accurate, specific and precise HPTLC method has been developed for the simultaneous estimation of olmesartan medoxomil and cilnidipine in their combined pharmaceutical dosage form. The chromatographic separation was performed using aluminium plate precoated with silica gel 60 F_{254} as stationary phase and toluene: methanol: chloroform (6: 3: 2, v/v/v) as mobile phase. The quantification was carried out at 257 nm. The R_f values were found to be 0.26 ± 0.02 and 0.67 ± 0.02 for olmesartan medoxomil and cilnidipine respectively. The linearity was observed in range of 400-1200 ng/spot for olmesartan medoxomil and 200-600 ng/spot for cilnidipine. The correlation coefficient (R^2) was found to be 0.9928 and 0.9987 for olmesartan medoxomil and cilnidipine respectively. The method was validated for precision, accuracy, LOD and LOQ as per ICH guidelines. The method was applied for simultaneous estimation of olmesartan medoxomil and cilnidipine in their combined pharmaceutical dosage form. The assay results were found to be 99.60% ± 0.15 for olmesartan medoxomil and 98.30% ± 0.16 for cilnidipine of percentage label claim of their combined pharmaceutical dosage form.

Key words: High Performance Thin Layer Chromatography, olmesartan medoxomil, cilnidipine

1. INTRODUCTION

Olmesartan medoxomil is designated chemically as (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]imidazole-4-carboxylate [1]. Olmesartan is angiotensin II type 1 receptor blocker. Chemical structure of olmesartan medoxomil is shown in figure 1(a). Cilnidipine is a calcium channel blocker. Chemically it is designated as 3-O-(2-methoxyethyl) 5-O-[(E)-3-phenylprop-2-enyl] 2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydro pyridine-3, 5-dicarboxylate [1]. Chemical structure of cilnidipine is shown in figure 1(b). The combination of olmesartan medoxomil and cilnidipine is used for the management of hypertension [2, 3].

The literature review described UV-visible spectrophotometric, RP-HPLC and HPTLC method for determination of olmesartan medoxomil individually and in combination with other drugs in their pharmaceutical dosage form [4-11]. The literature review described UV-visible spectrophotometric, RP-HPLC, HPTLC and LC-MS method for determination of cilnidipine individually and in

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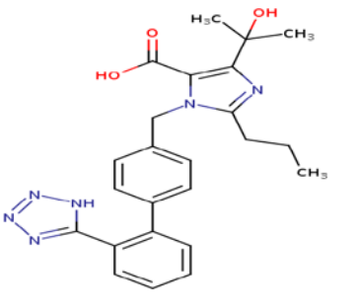
Received – 31/12/2015 Accepted – 13/04/2016

combination with other drugs in their pharmaceutical dosage form [12-16]. The literature review also described UV-visible spectrophotometry and RP-HPLC methods for simultaneous estimation of olmesartan medoxomil and cilnidipine in their combined dosage form [17-20]. The present paper describes HPTLC method for simultaneous estimation of olmesartan medoxomil and cilnidipine in their combined dosage form. The method was validated as per ICH guidelines [21].

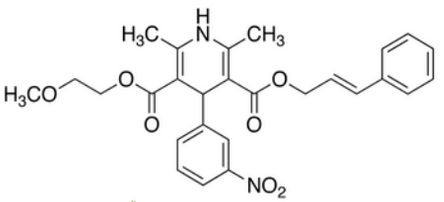
2. MATERIALS AND METHOD:

2.1. Instrumentation:

The HPTLC system (Camag, Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (Camag, Muttenz, Switzerland), twin-trough developing chamber (10 x 10 cm), UV cabinet with dual wavelength UV lamps, winCATS software, syringe (100 µl capacity, Hamilton) were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing purpose.



a



b

Figure 1: Chemical structure of a) Olmesartan medoxomil b) Cilnidipine

2.2. Chemicals and reagents:

Olmesartan medoxomil was supplied as a gift sample by Unichem Laboratires Ltd., Bardez, Goa, India and Cilnidipine was supplied by Tissue Pharma, Surat, Gujarat, India. All chemicals and reagents used were of LR grade and purchased from s.d. Fine-Chem Limited, Mumbai, India. Nexovas-O (Macleods Pharmaceutical Pvt. Ltd.), containing olmesartan medoxomil 20 mg and cilnidipine 10 mg was procured from local pharmacy.

2.3. Chromatographic conditions

Chromatographic separation was performed on 10 × 10 cm aluminium plates pre-coated with 250 µm layer of silica gel 60 F_{254} (E. Merk, Darmstadt, Germany). The TLC plate was pre-washed with methanol and activated at 60 °C for 5 min prior to spotting. The samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semiautomatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 100 nl/s. The TLC plate was developed in twin through chamber using toluene: methanol: chloroform (6: 3: 2, v/v/v) as mobile phase at temperature, 27 ± 2 ˚C; relative humidity, 35 ± 5 %; chamber saturation time, 30 min; migration distance, 75 mm. The TLC plate was dried, scanned and analysed by TLC Scanner IV and WinCATS software using following parameters: slit dimension, 4 × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 257 nm.

2.4. Preparation of solutions:

2.4.1. Preparation of stock solution of olmesartan medoxomil:

Accurately weighed 10 mg of standard olmesartan medoxomil was transferred to 10 ml volumetric flask, dissolved and diluted to mark with methanol to get standard stock solution having strength of 1000 µg/ml of olmesartan medoxomil.

2.4.2. Preparation of stock solution of Cilnidipine:

Accurately weighed 10 mg standard cilnidipine was transferred to 10 ml volumetric flask, dissolved and diluted to mark with methanol to get standard stock solution having strength of 1000 µg/ml of cilnidipine.

Table 1: Summary of validation parameters

Sr. No	Parameters	Results	
		OLM	CIL
1	Linearity Range (ng/spot)	400-1200	200-600
2	Correlation coefficient (R^2)	0.9928	0.9987
3	Precision (%RSD)		
	Repeatability of measurement of peak area (n=7)	0.70	0.54
	Repeatability of sample application (n=7)	1.20	0.81
	Intraday (n=3)	0.93 – 1.55	0.55 – 1.22
	Interday (n=3)	1.02 – 1.63	1.39 – 1.50
4	Accuracy (% Recovery)	99.37 – 100.08	99.28 – 100.11
5	Limit of Detection (ng/spot)	34.71	13.31
6	Limit of Quantitation (ng/spot)	105.20	40.36
7	Specificity	Specific	Specific

2.4.3. Preparation of combined working standard solution:

The mixed working standard solution was prepared by mixing of 1 ml of olmesartan medoxomil standard stock solution and 0.5 ml of cilnidipine standard stock solution in to 10 ml volumetric flask and diluted to mark with methanol to get a solution having strength of 100 µg/ml of olmesartan medoxomil and 50 µg/ml of cilnidipine.

2.4.4. Procedure for calibration curve:

From combined working standard solution, 4, 6, 8, 10 and 12 µl were spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. Calibration curve was obtained by plotting peak area against respective concentration of both drugs.

2.5. Method Validation:

2.5.1. Specificity:

From combined working standard solution of olmesartan medoxomil and marketed formulation, 8 µl were spotted on same TLC plate. The TLC plate was developed and dried as described under chromatographic conditions [Section 2.3]. The spot of olmesartan medoxomil and cilnidipine from standard and marketed formulation were scanned in range of 200 nm to 700 nm to obtain in situ UV spectrum of all spots. The spot of olmesartan medoxomil and cilnidipine from marketed formulation were confirmed by comparing its R_f values and reflectance-absorbance spectrum with that of standard olmesartan medoxomil and cilnidipine. The peak purity of olmesartan medoxomil and cilnidipine was ascertained by correlating the spectra of olmesartan medoxomil and cilnidipine scanned at peak start, peak apex and peak end position of the spot.

2.5.2. Linearity:

From combined working standard solution 4, 6, 8, 10 and 12 µl were spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. Linearity of proposed method was evaluated by repeating same procedure for five times. Calibration curve for olmesartan medoxomil and cilnidipine was obtained by plotting graph of mean peak area of five determinations vs. respective concentration of both the drugs. The correlation coefficient and regression line equation was calculated.

2.5.3. Precision:

2.5.3.1. Repeatability of sample application:

From combined working standard solution, 8 µl was spotted seven times on a same TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. The peak area of seven spots was measured and %RSD of peak area was calculated for both the drugs.

2.5.3.2. Repeatability of measurement of peak area:

From combined working standard solution, 8 µl was spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. The spots were scanned for seven times without chang-

ing plate position and %RSD for measurement of peak area was calculated for both the drugs.

2.5.3.3. Intraday precision:

From combined working standard solution, 6, 8, and 10 µl were spotted on TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. Same procedure was carried out three times on same day and % RSD of peak area was calculated for both the drugs.

2.5.3.4. Interday Precision:

From combined working standard solution, 6, 8, and 10 µl were spotted on TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. Same procedure was carried out on three consecutive days and % RSD of peak area was calculated for both the drugs.

2.5.4. Accuracy:

The accuracy was determined by standard addition method. The proposed method was applied for estimation of olmesartan medoxomil and cilnidipine in their combined dosage forms. The recovery experiment was carried out in triplicate by spiking previously analysed sample i.e. 500 ng/spot of olmesartan medoxomil and 250 ng/spot of cilnidipine with different concentration of both standard drugs at 80%, 100% and 120%. The percentage recovery of olmesartan medoxomil and cilnidipine were calculated at each level.

2.5.5. Limit of detection and limit of quantification:

LOD and the LOQ of the method were calculated using the following equations as per ICH guidelines Q2 (R1).

$$LOD = 3.3\ N/S$$
$$LOQ = 10\ N/S$$

Where,
N = Standard deviation of intercepts of five calibration curves
S = Mean slope of five calibration curves

2.6. Procedure for assay of marketed formulation:

Twenty tablets were weighed accurately, finely powdered and mixed. Powder equivalent to 20 mg of olmesartan medoxomil or 10 mg of cilnidipine was accurately weighed and transferred to 10 ml volumetric flask and mixed with 5 ml of methanol. The solution was sonicated for 10 min and diluted to mark with methanol. The solution was filtered through Whatman filter paper (No. 41). Further, 0.5 ml aliquot from the filtrate was diluted to 10 ml with methanol. From resulting solution, 8 µl was spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. Amount of olmesartan medoxomil and cilnidipine from marketed formulation was calculated using calibration curve data.

3. RESULTS AND DISCUSSION:

3.1. Selection of wavelength:

The overlein UV spectra of olmesartan medoxomil and cilnidipine showed reasonable absorbance at 257 nm wavelength (Figure 2). Therefore, 257 nm wavelength was selected for simultaneous estimation of olmesartan medoxomil and cilnidipine.

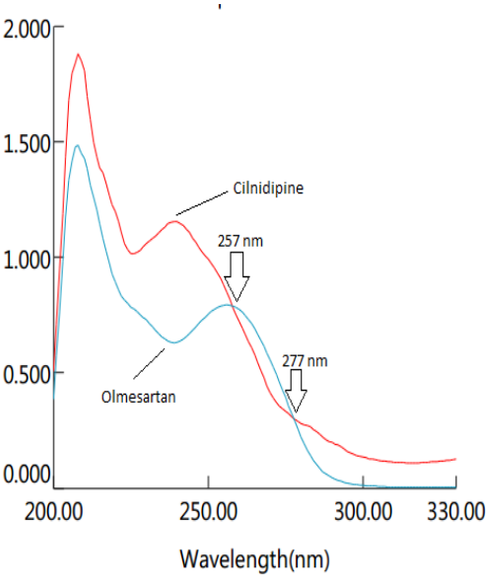


Figure 2: Overlain UV spectra of Olmesartan medoxomil and Cilnidipine

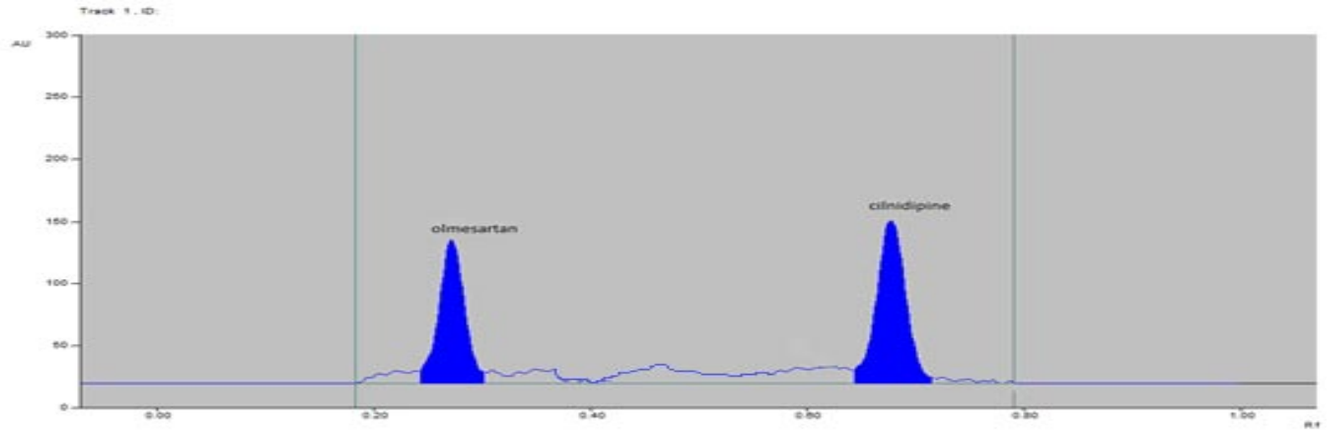


Figure 3: Chromatogram of standard olmesartan medoxomil (800 ng/spot); peak 1 (R_f: 0.26 ± 0.02) and cilnidipine (400 ng/spot); peak 2 (R_f: 0.67 ± 0.02), measured at 257 nm

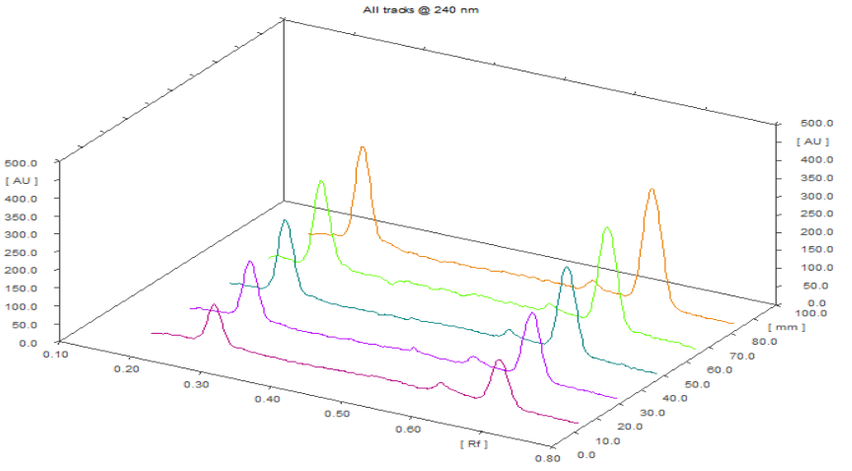


Figure 4: 3D chromatogram of olmesartan medoxomil (400-1200 ng/spot; R_f: 0.26 ± 0.02) and cilnidipine (200-600 ng/spot; R_f: 0.67 ± 0.02) at 257 nm

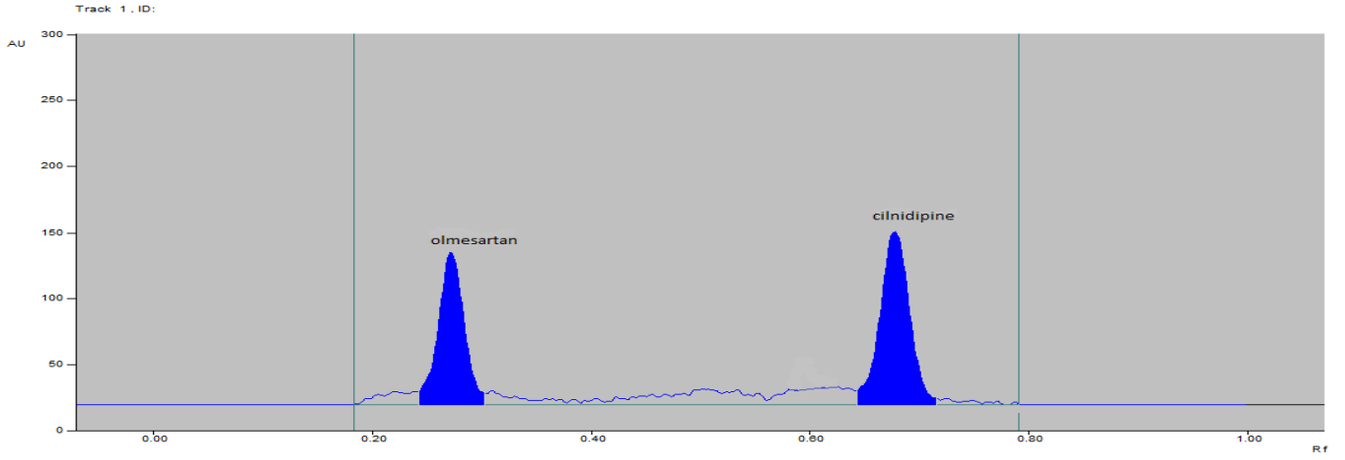


Figure 5: Chromatogram of olmesartan medoxomil; peak 1 (R_f: 0.26 ± 0.02) and cilnidipine; peak 2 (R_f: 0.67 ± 0.02) from marketed formulation, measured at 257 nm.

3.2. Mobile phase optimization:

Different solvent systems were tried for separation of olmesartan medoxomil and cilnidipine but better separation was found to be in toluene: methanol: chloroform (6: 3: 2, v/v/v). The R_f values were found to be 0.26 ± 0.02 and 0.67 ± 0.02 for olmesartan medoxomil and cilnidipine respectively (Figure 3).

3.3. Method validation:

3.3.1. Calibration curve and Linearity:

A linear relationship over the concentration range 400 to 1200 ng/spot for olmesartan medoxomil and concentration range 200 to 600 ng/spot for cilnidipine was observed. The correlation of coefficient was found to be 0.9928 for olmesartan medoxomil and 0.9987 for cilnidipine. The regression line equation for olmesartan medoxomil and cilnidipine was found to be y = 4.646x + 424.9 and y = 11.94x + 512.5 respectively. The 3D chromatogram of calibration curve for olmesartan medoxomil and cilnidipine is shown in figure 4.

3.3.2. Specificity:

Both the track, one of standard drugs and second of marketed formulation, showed only two spots having same R_f values 0.26 ± 0.02 and 0.67 ± 0.02 for olmesartan medoxomil and cilnidipine respectively. The in situ UV spectra of both tracks were recorded. Peak purity check of both drug from marketed formulation showed high degree of correlation between spectra scanned at peak start, peak apex and peak end position. The good correlation between absorbance reflectance spectrum of both standard drugs and sample drugs from combined marketed formulation confirms the identity of both drugs.

3.3.3. Precision:

The %RSD for repeatability of sample application was found to be 1.20 and 0.81 for olmesartan medoxomil and cilnidipine respectively. The %RSD for repeatability of measurement of peak area was found to be 0.70 and 0.54 for olmesartan medoxomil and cilnidipine respectively. The %RSD for intraday precision was found to be 0.93 – 1.55 for olmesartan medoxomil and 0.55 - 1.22 for cilnidipine. The %RSD for interday precision was found to be 1.02 – 1.63 for olmesartan medoxomil and 1.39 - 1.50 for cilnidipine.

3.3.4. Accuracy:

The percentage recovery was found to be 99.37% - 100.08% for olmesartan medoxomil and 99.28% - 100.11% for cilnidipine.

3.3.5. LOD and LOQ:

The LOD was found to be 34.71 ng/spot for olmesartan medoxomil and 13.31 ng/spot for cilnidipine. The LOQ was found to be 105.20 ng/spot for olmesartan medoxomil and 40.36 ng/spot for cilnidipine. The summary of validation parameters is shown in Table 1.

3.4. Assay of marketed formulation:

The spots at R_f 0.26 (for olmesartan medoxomil) and 0.67 (for cilnidipine) were observed in the chromatogram of the drug sample from marketed formulation (Figure 5). The drug content was found to be 99.60% ± 0.15 and 98.30% ± 0.16 for olmesartan medoxomil and cilnidipine of label claim of their combined dosage form respectively (Table 2). There was no additional peak observed in the chromatogram of marketed formulation indicates that there is no interference of excipients and additives of tablets in estimation of both the drugs in their pharmaceutical dosage form.

Table 2: Assay data for marketed formulation (Nexovas-O tablet)

Sr. No.	Drug	Label Claim (mg)	Amount found (mg) (n=3)	Assay (% Label Claim)
1	Olmesartan medoxomil	20	19.92	99.60 ± 0.15
2	Cilnidipine	10	9.83	98.30 ± 0.16

4. CONCLUSION:

The specific, accurate and precise HPTLC method was developed for simultaneous estimation of olmesartan medoxomil and cilnidipine in tablet dosage form. The developed method was applied

for assay of tablet formulation and results were found to be in agreement with the label claim. The proposed method can be applied for routine analysis of tablet formulation.

5. ACKNOWLEDGEMENT:

The authors are thankful to Unichem Laboratires Ltd., Bardez, Goa, India for providing the gift sample of olmesartan medoxomil and Tissue Pharma, Surat, Gujarat, India for providing gift sample of cilnidipine.

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