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A New RP-HPLC Method for Simultaneous Determination of Olmesartan Medoxomil and Indapamide in Tablet Dosage Forms

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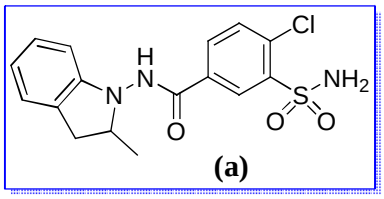
A rapid, accurate, and precise RP-HPLC method has been developed for simultaneous separation and quantification of olmesartanmedoxomil and indapamide in tablet dosage forms. The separation of both the drugs was achieved on an Enable C_{18}column (250 x 4.5 mm; 5µm) using a mixture of methanol and acetonitrile (95 : 5 v/v) as the mobile phase. The flow rate was 0.8mL/min and detection of the drugs was done at 245 nm. The retention times obtained for olmesartan and indapamide were 2.1 and 3.5 min respectively. The linearity range found for both olmesartan and indapamide was 5-25 µg/mL. The average percentage recoveries obtained by the proposed method for olmesartanmedoxomil and indapamide were 99.45% and 99.42% respectively. The results of the analysis were validated as per ICH guidelines. The proposed method can also be successfully employed for simultaneous determination of both the drugs in tablet dosage forms.

INTRODUCTION

Olmesartanmedoxomil is an angiotensin II receptor antagonist, which has been used for the treatment of high blood pressure.[1] Olmesartanmedoxomil is chemically (5-methyl-2-oxo-2H-1,3-dioxol-4-yl) methyl-4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)-phenyl]phenyl}methyl)-1H-imidazol-5-carboxylate. It is a pro-drug containing an ester moiety, which is rapidly cleaved to release the active form of olmesartan after oral administration.[2] This drug has a potent and long-lasting action, which is effective when given in a once-daily dose regimen. It works by blocking the binding of angiotensin II to the AT_1receptors in vascular muscle. As a result of this blockage, olmesartan reduces vasoconstriction and the secretion of aldosterone. This lowers blood pressure by producing vasodilation and decreasing peripheral resistance.[3]



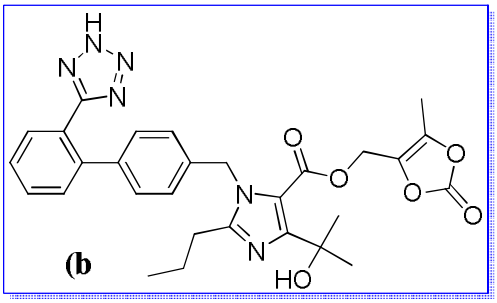


Figure 1. a) Structure of Indapamide b) Structure of Olmesartanmedoxomil.

Indapamide is a sulphonamide derivative pharmacologically related to thiazide group of diuretic drugs, generally used in the treatment of hypertension, and decompensated heart failure. Chemically, it is 4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)-3-sulfamoyl-benzamide. Currently, most commonly prescribed medicines for hypertension are angiotensin receptor blockers and diuretics.[4] Monotherapy with oral anti-hypertensive agents is not sufficient to achieve target blood pressure levels and henceforth, a combination tablet formulation is beneficial in terms of its convenience and patient compliance.[5] The present drug combination of olmesartan with indapanide has promising anti-hypertensive effect.[6] A literature survey reveals very few methods are available for simultaneous analysis of both the drugs in formulations and there is need for asensitive and selective stability indicating analytical method.[7,8] Therefore the current method was developed and validated.

MATERIALS AND METHODS

HPLC System

The study was carried out on a Shimadzu UFLC system (LC-20AD/Prominance) with an SPD-M20A Prominance diode array detector. The instrument was equipped with a quaternary pump, manual injector, column oven, and a 20 µL Hamilton syringe.

Drugs and Solvents

Reference standard samples of olmesartanmedoxomil and indapamide were procured from Piramal Healthcare Pvt. Ltd., Hyderabad. Commercial tablets of OLMESAT ID 20 mg (Biocon Limited, Bangalore) were obtained from the local market in Hyderabad. Acetonitrile and methanol (HPLC Grade) used were purchased from Merck (India) Ltd., Mumbai.

Mobile Phase

Based on a literature survey we have conducted several trials for a suitable mobile phase for ideal separation of olmesartanmedoxomil and inapamide. A mobile phase consisting of methanol : acetonitrile in the ratio of 95:5 (%v/v), was found to separate these two drugs with sharp peaks and good retention times. The retention times obtained for olmesartanmedoxomil and indapamide were 2.1 and 3.5 min respectively.

Stock and Working Solutions of Drugs

The standard stock solutions (1000 µg/mL) of olmesartanmedoxomil and indapamide were prepared individually by accurately weighing 10 mg of the appropriate drug in 10 mL volumetric flasks and making up the volume with the methanol. From these standard stock solutions, the combined working standard solution was prepared by taking 2 mL of olmesartan solution and 0.15 mL of indapamide solution in 10 mL volumetric flask and making up the volume with methanol. The combined working standard solution thus contains 200 µg/mL of olmesartan and 15 µg/mL of indapamide.

Stock and Working Solutions of Formulation

10 tablets were weighed accurately and the average weight of a tablet was calculated. Then the tablets were finely powdered and from this,a quantity of powder equivalent to 1 tablet (20 mg of olmesartanmedoxomil and 1.5 mg indapamide) was added intoa 100 mL volumetric flask. Then to this about 50 mL methanol was added and sonicate for 40 min with intermittent shaking. Then volume was made up to mark with methanol. The test solution was filtered through a PVDF Millipore filter (0.45 µ) and the solvent analyzed by using proposed method.

Table 1. Optimized chromatographic conditions.

Stationary phase	ENABLE C_{18} column (250 mmx4.6 mm; 5 µ)
Mobile phase	Methanol : Acetonitrile (95 : 5 %v/v)
Flow rate	0.8 mL/min.
Column temperature	30°C
Injection volume	20 µL
Detection wavelength	245 nm
Run time	10 min.

RESULTS AND DISCUSSION

The mobile phase was optimized after several trials with methanol, acetonitrile, and buffer solutions in various proportions and at different pH values. The isobestic point of olmesartanmedoxomil and indapamide was found to be 245nm by scanning in UV region. The chromatographic conditions were optimised with mobile phase consisting of Methanol : Acetonitrile (95 : 5) and Enable C_{18} column (250 mm x 4.5 mm; 5 µ) (Table 1). All the validation parameters were determined at a wavelength of 245nm. Accuracy was determined by calculating the recovery (Table 5 and 6) and the results were in acceptable limits (98-102%). The method was successfully used to determine simultaneously the amount of olmesartanmedoxomil and indapamide present in the tablet. The results obtained were in good agreement with the corresponding labelled amount. The method was linear in the concentration range of 5 to $25 \mu g/mL$ for both the drugs. Precision was calculated as repeatability and intra- and inter-day variations (% RSD) for the drug. Robustness and ruggedness results were in acceptable range (Table 7 and Table 8).

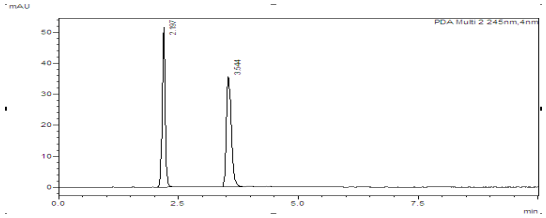


Figure 2. Optimised chromatogram of olmesartanmedoxomil and indapamide.

A summary of the results of validation parameters for the method is given in Table 10. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence, the

method can be employed for the routine analysis of olmesartanmedoxomil and indapamide in tablet dosage forms.

Method validation

Validation of the method was carried out as per ICH guideline Q2 (R1) with respect to parameters such as linearity, precision, accuracy, ruggedness, robustness, specificity, limit of quantification (LOQ) and limit of detection (LOD).

Table 2. Calibration data of olmesartanmedoxomil.

S. No.	µg/mL	Area
1	5	149393
2	10	235611
3	15	324395
4	20	414242
5	25	503792

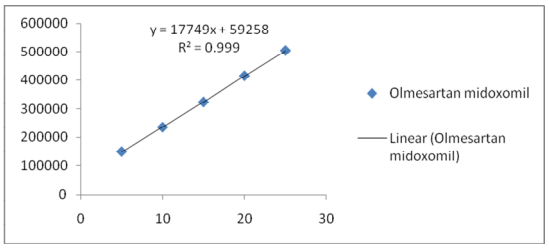


Figure 3. Calibration curve of olmesartanmedoxomil.

Linearity

To study the linearity range, the stock solutions of olmesartanmedoxomil (1000 µg/mL) and indapamide (1000 µg/mL) were prepared separately. Then, the individual stock solutions were diluted to yield solutions in the concentration range of 5- 25 µg/mL. The solutions were analysed on the HPLC system. Each dilution was injected tries and the average area was computed. The calibration curves for both the drugs are shown in the Fig. 3 & 4. The results of linearity are presented in the Table 2 & 3.

Table 3. Calibration data of indapamide.

S. No.	µg/mL	Area
1	5	172435
2	10	289040
3	15	410526
4	20	533100
5	25	662066

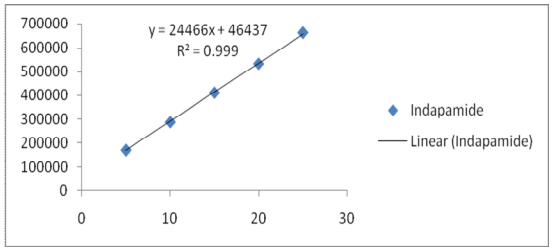


Figure 4. Calibration curve of indapamide.

Precision

The method precision was done by analyzing six replicate samples of the combined working standard solution prepared by only one analyst. The results of the precision are depicted in Table 4.

Limits: The results obtained were within 2% Relative Standard Deviation (RSD).

Table 4. Method precision (repeatability).

Injection	Olmesartan medoxomil		Indapamide	
	RT	Area	RT	Area
1	2.166	235611	3.535	227478
2	2.180	231448	3.543	269256
3	2.193	226955	3.550	262924
4	2.197	226765	3.544	255570
5	2.195	229337	3.534	258657
6	2.196	231814	3.542	263515
AVG	13.127	230321.7	3.541	264233
STD	0.01238	3359.364	0.005989	3309.891
%RSD	0.094	1.458553	0.169114	1.25264

RT - Retention Time

Accuracy

Accuracy was determined at three different concentration levels i.e. 50, 100 and 150% of the target concentration in triplicate. The results indicate that the recoveries are well within the acceptance range. Therefore, method is accurate and it can be used for the estimation of olmesartanmedoxomil and indapamide. The results obtained are given in Table 5 and 6.

Table 5. Accuracy results of olmesartanmedoxomil.

% level	AT (µg /mL)	Area	Avg. area	AR (µg /mL)	% of Recovery
50	50	4499791			
	50	4435301	4449844	49.473	98.946
	50	4414441			
100	100	8928191			
	100	8834371	8905091	99.679	99.679
	100	8952710			
150	150	13305322			
	150	12880574	34362090	149.611	99.74
	150	13822734			

AT - Amount Taken; AR - Amount Recovered

Table 6. Accuracy results of indapamide.

% level	AT (µg /mL)	Area	Avg. area	AR (µg /mL)	% of Recovery
50	50	647861			
	50	648487	648555.3	49.22	98.44
	50	649318			
100	100	1247049			
	100	1246713	12794500	99.853	99.853
	100	1310073			
150	150	1844926			
	150	15995	1880897	149.959	99.97
	150	1881769			

AT - Amount Taken; AR - Amount Recovered

Robustness

Robustness is the measure of a method remain unaffected by small and deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here, the detection wavelength varied by ±2nm and the flow rate by ±0.2 mL/min and temperature varied by ±5. The results were shown in Table 7.

Table 7. Results of Robustness Study.

Chromatographic Conditions		Number of Theoretical Plates		Peak Asymmetry	
		Olme	Inda	Olme	Inda
Flow Rate (mL/min)	0.6	13544	9827	0.992	1.012
	0.8	13584	9868	0.998	1.001
	1.0	13538	9852	0.997	1.024
Temperature (°C)	25	13548	9819	0.993	1.011
	30	13550	9835	0.997	1.000
	35	13511	9845	0.992	1.014
Wavelength (nm)	240	13524	9854	0.996	1.025
	245	13585	9851	0.998	1.002
	250	13511	9611	0.995	1.010

Ruggedness

Ruggedness test was determined between different analysts, instrument and column. The results of the ruggedness are presented in Table 8.

Limits: The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method.

The detection and quantification limits of olmesartanmedoxomil and indapamide are given in Table 9.

Specificity of the Method

Under optimization condition**,** the retention times of the standard olmesartanmedoxomil and indapamidewere found to be 2.1 and 3.5 min. respectively. A processed sample solution of the pharmaceutical dosage form (tablet) was then injected and the chromatogram was obtained. The retention times of the drugs in the dosage form were also found to be 2.1 and 3.5 min. respectively. There is no specific change in the chromatogram of the formulation. This indicates that there is no interference from of the excipients. This confirms that the developed method is a suitable for the simultaneous estimation of olmesartanmedoxomil and indapamide in dosage forms.

Table 8. Results of Ruggedness Study.

Analyst No. & Drug		Standard Area	Sample Area	No. of Theoretical Plates	Peak Asymmetry	%Assay	%RSD
Analyst-1	Olmesartan medoxomil	226955	229337	13589	0.999	99.87	1.42%
Analyst-2		2267436	229897	13558	0.998	99.69	
Analyst-3	Indapamide	227436	229146	9865	1.001	98.54	1.28%
Analyst-4		235611	231814	9870	1.002	98.64	

Table 9. Validation results of the method.

S. No.	Parameter	Value obtained of olmesartanmedoxomil	Value obtained of indapamide
1	Linearity range (µg/mL),	5-25 µg/mL	5-25 µg/mL
	Correlation coefficient (R^2)	0.9999	0.9999
2	LOD	0.01µg/mL	0.01 µg/mL
3	LOQ	0.03µg/mL	0.03 µg/mL
4	Method precision (%RSD, n=6)	0.094	0.169
5	Robustness (Peak asymmetry)		
	a) Flow rate	0.996	1.001
	b) Temperature	0.998	1.000
	c) Wavelength	0.997	0.999
6	Ruggedness (%RSD)	1.42	1.28

Conclusions

The proposed HPLC method was found to be sensitive, accurate, precise and rugged for simultaneous determination of olmesartanmedoxomil and indapamide in tablet dosage forms. The common excipients and other additives usually present in the tablet dosage form do not interfere in the analysis of olmesartanmedoxomil and indapamide and hence, it can be conveniently adopted for routine quality control analysis of these drugs in pharmaceutical formulations. This method can also be used for determination of content uniformity and dissolution profiling of this product.

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Notes and References

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