Original Article

Simultaneous estimation of olmesartan medoxomil and indapamide from bulk and commercial products using a validated reverse phase high performance liquid chromatographic technique

Abstract

Aim: A simple, rapid, accurate, precise and economical reverse phase high performance liquid chromatographicmethod is developed for simultaneous separation and quantification of two anti-hypertensive drugs, viz., olmesartan medoxomil and indapamide. **Materials and Methods:** The separation of both the drugs was achieved on ACE C_{18} AR column (250 × 4.6 mm id, 5 μm particle size) column using a mobile phase of sodium perchlorate and triethylamine buffer solution (at pH 3): Acetonitrile (60:40 v/v). The flow rate was 1 ml/min and detection was done at 280 nm. **Results:** The retention time for indapamide was 5.3 min and for olmesartan medoxomil was 6.8 min. Olmesartan medoxomil and indapamide showed a linear response in the concentration range of 50-300 μg/ml and 3.75-22.5 μg/ml respectively. The correlation co-efficients for olmesartan medoxomil and indapamide were 0.9999 and 0.9998 respectively. The percentage recoveries obtained forolmesartan medoxomil and indapamide ranges from 99.3% to 99.8% and 99.7% to 100.9% respectively. The results of the analysis have been validated as per International conference on Harmonisation (ICH) guidelines. Validation results indicated that method shows satisfactory linearity, accuracy, precision, and ruggedness. **Conclusion:** The extremely low flow rate, simple mobile phase composition makes this method cost effective, rapid, and non-tedious and can also be successfully employed for simultaneous estimation of both drugs in commercial products.

Key words:
High performance liquid chromatography, indapamide, olmesartan medoxomil

Introduction

Olmesartan medoxomil [Figure 1] (a prodrug, which is hydrolyzed in body active olmesartan during absorption from the gastrointestinal tract) is chemically, 2,3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-carboxylate, cyclic-2,3-carbonate. Olmesartan medoxomil is an angiotensin II receptor blocker, which is used as an anti-hypertensive agent.[1,2] The literature survey reveals that olmesartan medoxomil was analyzed by the Liquid chromatography mass spectrometry (LC-MS) and reverse phase high performance liquid chromatography (RP-HPLC) methods[3-8] Indapamide [Figure 2] is an orally administered diuretic and anti-hypertensive drug. Its molecule contains both a polar sulfamoyl chlorobenzamide moiety and a lipid soluble methyl-indoline moiety. It differs chemically from thiazide in a way that it does not possess the thiazide ring system and contains only one sulfonamide group. Indapamide is chemically 3-(aminosulfonyl)-4-chloro-

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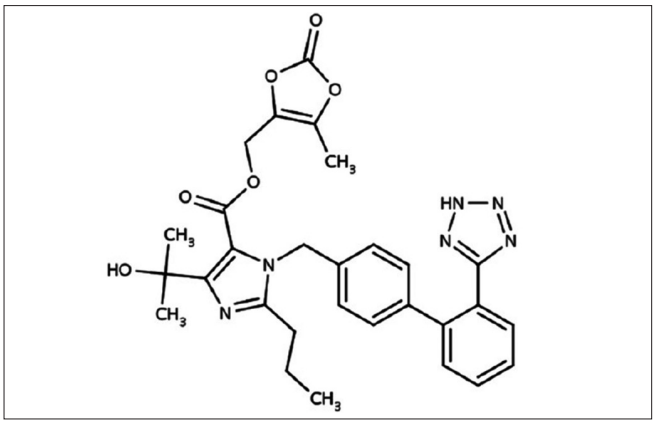

Figure 1: Structure of olmesartan medoxomil

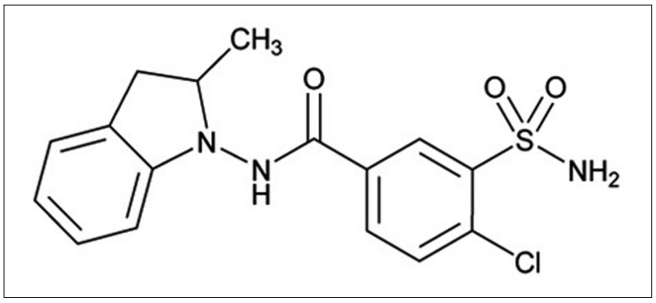

Figure 2: Structure of indapamide

N-(2,3-dihydro-2-methyl-1H-indol-1-yl) benzamide. Literature survey reveals bio-analytical methods by LCMS for detection of indapamide in human serum and blood and few spectrophotometric methods and HPLC methods for the quantitative estimation of indapamide in bulk and pharmaceutical formulations.[9-16] Currently most commonly prescribed medicines for hypertension are angiotensin receptor blockers and diuretics. 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. The present drug combination has promising anti-hypertensive effect. Literature review suggests no analytical methods reported for simultaneous analysis of both the drugs together and henceforth, the following experiment was performed.

Materials and Methods

Reference standards of olmesartan medoxomil and indapamide were received from Glenmark pharmaceuticals Ltd., Ankleshwar, Gujarat. Marketed preparation Olmy-I tablets was obtained from Zydus Cadila Healthcare Ltd., Moraiya Ahmedabad. Dose ofolmesartan medoxomil was 20 mg and indapamide was 1.5 mg in Olmy I tablets. Acetonitrile (HPLC Grade) used was purchased from Merck (India) Ltd., Mumbai. Orthophosphoric acid, sodiumperchlorate, and triethylamine were purchased from spectrochem. India. RP-HPLC was performed using Shimadzu HPLC system (LC 2010CHT, Shimadzu corporation, Japan) equipped with quaternary pump, auto injector, column oven and Photo diode array (PDA) detector.

Chromatographic condition

Column: ACE C_{18} AR Column (250 × 4.6 mm), 5 µ
Detector: 280 nm
Injection volume: 10 µl
Flow rate: 1.0mL/minTemperature : 30ºC
Run time: 9 min
Mobile phase: Sodium perchlorate and triethylamine buffer (at pH 3):Acetonitrile (60:40)
Diluent: Water: Acetonitrile (50:50)
Retention time: Indapamide (5.3 min) and olmesartan medoxomil (6.8 min)

Experimental work

Buffer preparation

Accurately weighed 4.3 g sodium perchlorate was dissolved in to 1000 ml Milli-Q water and 1 ml Triethylamine was added to this buffer solution, than pH was adjusted to 3.0 with ortho-phosphoric acid.

Preparation of standard solution

The standard stock solution olmesartan medoxomil (1000 ppm) and indapamide (75 ppm) were prepared by weighing olmesartan medoxomil 200 mg and indapamide 15 mg in 200 ml volumetric flask respectively and making volume up to mark with diluent. Then 10 ml of standard stock solution was diluted to 50 ml with diluent to make final standard concentration of olmesartan medoxomil (200 ppm) and indapamide (15 ppm) respectively.

Preparation of test solution

Accurately, 20 intact tablets were weighed and average weight of tablet was calculated. Then tablets were finely crushed, powdered and sample powder about 3500 mg (Tablet powder equivalent to 200 mg olmesartan medoxomil and 15 mg indapamide. i.e., 10 tablets' powder was added in the flask for assay of tablets.) was transferred into 200 ml volumetric flask. Then add about 100.0 ml diluent was added and sonicate for 40 min with intermittent shaking. Then volume was made up to mark with diluent. Then 10 ml of standard stock solution was diluted to 50 ml with diluent to make final standard concentration of olmesartan medoxomil (200 ppm) and indapamide (15 ppm) respectively. The test solution was filtered through 0.45 µ (PVDF Millipore filter) and analyzed by using HPLC system. The isocratic program was adopted to analyze both components in a single run by HPLC as shown in Figure 3.

Method validation

Validation was carried out with respect to various parameters, as required under ICH guideline Q2 (R1). The developed method validated with respect to parameters such as linearity, precision, accuracy, specificity, ruggedness, robustness, and solution stability.[17]

System suitability and system precision

The results of system suitability and system precisionare presented in Table 1.

Linearity

To achieve linearity and range, stock solution containing olmesartan medoxomil (1000 ppm) and indapamide (75 ppm) were prepared. Olmesartan medoxomil and indapamidestock solutions were diluted to yield solutions in the concentration range of 50-300 µg/mL and 3.75-22.5 µg/mLrespectively. The solutions were analyzed by using HPLC. Calibration curve for both the drugs are shown in Figures 4 and 5. The results of linearity are presented in Tables 2 and 3.

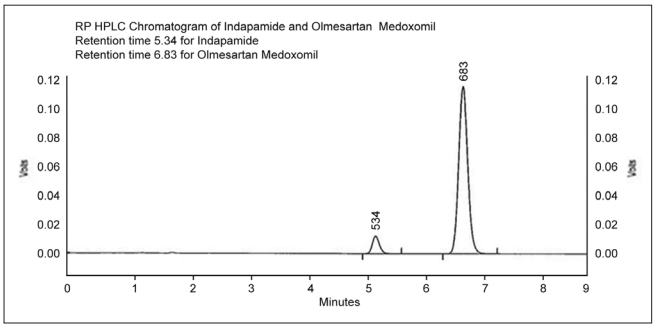

Figure 3: High performance liquid chromatography chromatogram of olmesartan medoxomil and indapamide

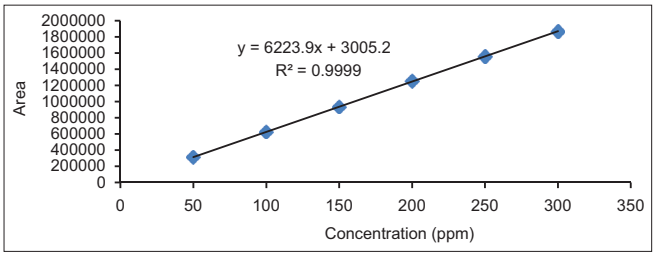

Figure 4: Calibration curve of olmesartan medoxomil

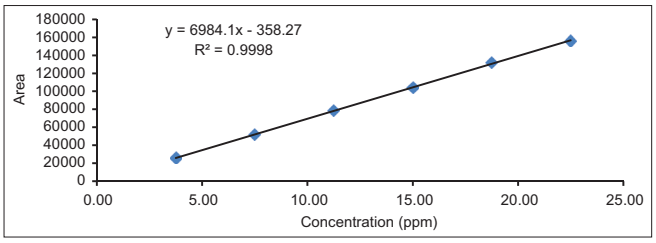

Figure 5: Calibration curve of indapamide

Precision

The method precision was done by preparing six different sample preparations by one analyst. The results are presented in Table 4. The results obtained were within 2% Relative Standard Deviation (RSD).

Ruggedness

Ruggedness test was determined between different analyst, instrument and column. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results are presented in Table 4.

Accuracy

The difference between theoretical added sample amount

Table 1: General parameters found after reverse phase high performance liquid chromatography analysis

Compound	Retention time	Theoretical plates	Asymmetry
Indapamide	5.8±0.0022	8361	1.09
Olmesartan medoxomil	6.83±0.0031	8919	1.12

Table 2: Linearity data of olmesartan medoxomil

Linearity range (%)	Stock solution taken in ml	Diluted to volume ml	Final Conc (ppm)	Area
25	2.50	50	50	309360
50	5.00	50	100	628320
75	7.50	50	150	937246
100	10.0	50	200	1254142
125	12.5	50	250	1556739
150	15.0	50	300	1867282

Table 3: Linearity data for indapamide

Linearity range (%)	Stock solution taken in ml	Diluted to volume ml	Final conc (ppm)	Area
25	2.50	50	3.75	25853
50	5.00	50	7.50	51919
75	7.50	50	11.25	78245
100	10.0	50	15.00	103944
125	12.5	50	18.75	131723
150	15.0	50	22.50	156163

Table 4: Results of method precision and ruggedness

Parameters	Olmesartan medoxomil		Indapamide	
	% assaymean (*n*=6)	% RSD	% assaymean (*n*=6)	% RSD
Method precision	99.9	0.2	101.2	0.3
Ruggedness	100.5	1	100.5	1.1

RSD – Relative standard deviation

to the placebo and practically achieved sample amount from placebo (after HPLC analysis) is called accuracy of analytical method. Accuracy was determined at three different level 50%, 100%, and 150% of the target concentration in triplicate. The results are presented in Tables 5 and 6.

Solution stability

The standard and sample solutions were found stable up to 24 hr at room temperature. After 8, 12, 24 hr the solutions were analyzed and results related to solution stability are summarized in Tables 7 and 8.

Robustness

Robustness of the method was carried out by deliberately made small changes in the flow rate, pH, organic phase ratio, and column oven temperature. Results are presented in Tables 9 and 10.

Limit of detection and limit of quantitation

In order to estimate the limit of detection LOD and limit of quantitation LOQ) values, the blank sample was injected six times and the peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level while 10 times the noise value gave the LOQ. The results of LOD and LOQ are mentioned in Table 11.

Result and Discussions

The mobile phase was optimized after several trials with methanol, acetonitrile, and buffer solutions in various proportions and at different pH values. The ion pair reagent triethylamine used in method helps to improve peak shape and enhances retention time of olmesartan medoxomil. The values of relative standard deviation are satisfactorily low and recovery was close to 100% which indicated accuracy and reproducibility of methods. Literature review reveals only individual methods for estimation of olmesartan medoxomil and indapamide individually, but no methods were reported for simultaneous estimation of olmesartan medoxomil and indapamide was reported till date. So, method much superior to previously published methods of individual estimation of all drugs was developed. The detection wavelength of 280 nm was chosen in order to achieve a good sensitivity for quantitative determination of olmesartan medoxomil and indapamide in solid dosage form.

Conclusion

Thus proposed method was found to be simple, rapid, accurate, selective, and economical for simultaneous routine analysis of olmesartan medoxomil and indapamide in bulk and commercial dosage form. This method can also be used for determination of content uniformity and dissolution profiling of this product.

Table 5: Results of accuracy of olmesartan medoxomil

Level %	Amount of drug added (mg)	Amount of drug recovered (mg)	Recovery (%)	Mean %	% RSD
50	100.10	100.09	99.9	99.6	0.3
	100.20	99.95	99.7		
	100.60	100.06	99.4		
100	200.10	199.92	99.9	99.8	0.1
	200.30	199.97	99.8		
	200.60	200.03	99.7		
150	300.30	297.77	99.3	99.3	0.1
	300.10	298.11	99.4		
	300.50	297.72	99.2		

RSD – Relative standard deviation

Table 6: Results of accuracy of indapamide

Level %	Amount of drug added (mg)	Amount of drug recovered (mg)	Recovery (%)	Mean %	% RSD
50	7.20	7.11	98.7	99.9	1.1
	7.10	7.14	100.5		
	7.00	7.04	100.6		
100	15.50	15.50	100.0	99.7	0.4
	15.70	15.70	100.0		
	15.20	15.30	99.3		
150	22.0	22.32	101.4	100.9	0.8
	22.10	22.41	101.4		
	22.50	22.50	100		

RSD – Relative standard deviation

Table 7: Results of standard solution stability olmesartan medoxomil and indapamide

Time (hours)	Area		Difference (% RSD)	
	Olmesartan	Indapamide	Olmesartan	Indapamide
0	1254187	102536	–	–
8	1258362	102563	0.17	0.07
24	1299652	102942	1.50	0.17
% mean RSD			0.44	0.09

RSD – Relative standard deviation

Table 8: Results of sample solution stability olmesartan medoxomil and indapamide

Time (h)	Area		% Difference	
	Olmesartan	Indapamide	Olmesartan	Indapamide
0	1248253	102489	–	–
8	1251321	102599	0.12	0.05
24	1292544	102234	1.48	0.13
% mean RSD			0.54	0.08

RSD – Relative standard deviation

Table 9: Olmesartan medoxomil robustness study

	Sys. Suit.	Temp. −5°C	Temp. +5°C	Flow −10%	Flow +10%	Org. −2%	Org. +2%	pH=3.2	pH=2.8
% RSD *N*=5	0.1	0.2	0.4	0.6	0.5	0.7	0.3	0.9	0.3
Mean % RSD	0.4								

RSD – Relative standard deviation

Table 10: Indapamide robustness study

	Sys. Suit.	Temp. −5°C	Temp. +5°C	Flow −10%	Flow +10%	Org. −2%	Org. +2%	pH=3.2	pH=2.8
% RSD	0.2	0.6	0.1	0.1	0.3	0.1	0.8	0.1	0.6
Mean % RSD	0.3								

RSD – Relative standard deviation

Table 11: Summary of validation parameters of reverse phase high performance liquid chromatographic method for simultaneous estimation of olmesartan medoxomil and indapamide

Parameter	Acceptance criteria	Olmesartan medoxomil	Indapamide
Range of linearity	Follows beer lambert's law	50-300 μg/ml	3.75-22.5 μg/ml
Correlation coefficient	Correlation coefficient r^2>0.999 or 0.995	0.9999	0.9998
LOD	S/N>2 or 3	5 μg/ml	0.375 μg/ml
LOQ	S/N>10	15 μg/ml	1.125 μg/ml
Precision %	RSD<2	0.2	0.3
Ruggedness %	RSD<2	1.0	1.1
Accuracy %	Recovery 98-102	99.3-99.8	99.7-100.9
Specificity	No intereference of placebo	Complies	Complies
Solution stability	>12 h	Stable for 24 h % RSD=0.7	Stable for 24 h % RSD=0.9
Robustness	RSD NMT 2% in modified condition	Complies	Complies

RSD – Relative standard deviation; NMT – Not more than; LOD – Limit of detection; LOQ – Limit of quantitation

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