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Analytical Method Development and Validation for Simultaneous Estimation of Cilnidipine, Olmesartan and Chlorthalisone in Synthetic Mixture by RP-HPLC Method

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ABSTRACT:

To developed and validate a simple and rapid isocratic reversed-phase high-performance liquid chromatography method (RP-HPLC) for the simultaneous estimation of Cilnidipine, Olmesartan and Chlorthalidone in synthetic mixture. The chromatographic separation was achieved by using mobile phase acetonitrile, methanol and water (40:20:20 %v/v/v) with 5ml of 0.5% ortho phosphoric acid (pH 3.8), sheisedo C18 (4.6mm*250mm) 5µm. The mobile phase was pumped at a flow rate of 1.0ml/min and the eluents were monitored at 226nm. Retention times were 5.366 min, 2.693 min and 3.760 min for Cilnidipine, Olmesartan and Chlorthalidone respectively. Linearity was observed in the concentration range of 50-300 µg/ml, 100-600 µg/ml and 60-360 µg/ml for Cilnidipine, Olmesartan and Chlorthalidone respectively. The percentage recoveries found to be as for Cilnidipine 98.9-101.52%, Olmesartan 99.21-100.88% and Chlorthalidone 99.03-100.81% respectively. All the parameters are validated as per ICH guidelines for the method validation and found to be suitable for routine quantitative analysis in pharmaceutical dosage forms.

KEY WORDS: Validation, Development, Cilnidipine, Olmesartan, Chlorthalidone, RP-HPLC.

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1. INTRODUCTION[1-9]

Cilnidipine is dihydropyridine calcium channel blocker and is used as an anti-hypertensive, Which is chemically describe as O3-(2-ethoxymethyl) O5-[(E)-3-pheny-prop-2-enyl] 2,6-dimethyl-4(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.It is a dihydropyridine calcium channel blocker. It inhibits cellular influx of calcium, thus causing vasodilation. It has greater selectivity for vascular smooth muscle.[4]

Olmesartan is Angiotensin II Type 1 Receptor Blockers and is used as an anti-hypertensive, which is chemically described as (5-methyl-2-oxo-1,3-dioxol-4-yl) methyl ester of 4-(1-hydroxy-1methyl ethyl)-2-propyl-1{[2-(1H-tetrazole-5-yl)(1,1´-biphenyl]-4-yl] methyl}-1H-imidazole-5-carboxylic acid.It is an ARB it selectively inhibits the binding of angiotensive II to AT1 that is found in many tissues for example vascular smooth muscle and the adrenal glands. It is effectively inhibits AT1- mediated vasoconstriction and aldosterone secreting special effects of angiotensin II and its results to reduction vascular resistance and blood pressure. It is selective for AT1 also that 12,500 times greater affinity for AT1 than the AT2 receptor.[5, 6]

Chlorthalidone is Sodium chloride symporter inhibitors and used as an anti-hypertensive agent and diuretic, Which is chemically describe as (RS)-2-

chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide.It prevents sodium ion transport across the renal tubular epithelium in cortical diluting segment of ascending limbs of henle. As increasing the delivery of sodium to distal renal tubule that ultimately increasedpotassium excretions via sodium- potassium exchange mechanism.[7, 8, 9]

Literature review indicated that many spectrophotometric and HPLC method were reported for individual drug and in combination with other drugs.[10-14]

There are number of tablets used in anti-hypertensive in the market. It is well known that variation in quality is observed for different brands of dosage forms as well as in a same brand between the batches.

So, the aim of present study was to develop a simple, rapid, precise, accurate and selective chromatographic method for the estimation of Cilnidipine, Olmesartan and Chlorthalidone in synthetic mixture.

2. MATERIALS AND METHODS

Chemicals and Reagents:

Cilnidipine from Unique Pharmaceutical Laboratories, Olmesartanmedoxomil from Vapi care pvt ltd. and Chlorthalidone from Vapi care pvt ltd. HPLC grade acetonitrile, methanol and water was purchased from Lichrosoly- E. Merck (India) Ltd. Mumbai

Instrumentation:

High Performance Liquid Chromatography:
Model: Shimadzu
Software: LC solution
Column: Sheisedo C_{18} (4.6mm*250mm) 5µm
Pump: Isocratic
Wavelength range: 200-800 nm
Detector: UV-VIS detector

Chromatographic conditions

- Stationary Phase:Sheisedo C_{18} (4.6mm*250mm), 5µm was used at ambient temperature
- Mobile phase: acetronitrile: methanol: water (40:40:20 %v/v/v) with 5ml of 0.5% ortho phosphoric acid (pH 3.8)
- Flow rate: 1.0ml/min
- Injection volume: 10 µL
- Detection: At 226nm with UV visible detector

Standard solution and calibration graphs for chromatographic measurement

Accurately weighed 50 mg Cilnidipine, 50 mg Olmesartan and 50 mg of Chlorthalidone were transferred to 50 ml volumetric flask. It was dissolved with sufficient methanol and diluted up to mark with methanol to give concentration of 1000 µg/ml of Cilnidipine, 1000 µg/ml of Olmesartan and 1000 µg/ml of Chlorthalidone. 0.5 ml of this solution was further diluted to 10 ml with methanol to get 50 µg/ml of Cilnidipine, 1.0 ml of Olmesartan dilute up to 10 ml with methanol to get 100 µg/ml of Olmesartan and 0.6ml of Chlorthalidone solution was further diluted to 10 ml with methanol to get 60 µg/ml of Chlorthalidone. Above solution was diluted further to get the concentration range of 50-300 µg/ml for Cilnidipine, 100-600 µg/ml for Olmesartanand 60-360 µg/ml for Chlorthalidone. Peak areas were plotted against the corresponding concentrations to obtain calibration graphs.

Assay of synthetic mixture formulation

Twenty synthetic mixture tablets were weighed and finely powdered. A quantity equivalent to 10 mg Cilnidipine, 20 mg Olmesartanmedoxomil and 12.5 mg Chlorthalidone were transferred in to 50 ml volumetric flask and dissolved on about 25 ml methanol. The solution was ultrasonicated for 5 minutes and filtered through 0.45µ whatmann filter paper no. 41. and the volume was made up to mark with same solvent. Above solution was taken to prepare a dilution of 10 µg/ml of Cilnidipine, 20 µg/ml of Olmesartanmedoxomil and 12.5 µg/ml of Chlorthalidone. The amount of drug was determined and three replicates were done. The result of assay was shown in table no. 1

3. RESULT AND DISCUSSION

Method development and optimization

To develop a suitable method for the estimation of Cilnidipine, Olmesartan and chlorthalidone by using different mobile phases were employed to achieve the best separation and resolution. The development was intiated with using a mobile phase of acetronitrile and methanol, methanol and water and finally mobile phase consisting of acetronitrile, methanol and water (40:20:20 %v/v/v) with 5ml of 0.5% ortho phosphoric acid (pH 3.8) mixture was found to be appropriate allowing good separation and resolution of compound at a flow rate

1.0ml/min using Sheisedo C_{18} (4.6mm*250mm) 5µm column. In order to obtain a satisfactory and full detection for this new method, UV spectra of standard Cilnidipine, Olmesartan and Chlorthalidone solution were obtained. Based on the highest UV absorbance for Cilnidipine, Olmesartan and Chlorthalidone at 226 nm was chosen. Retention times were 5.366 min, 2.693 min and 3.760 min for Cilnidipine, Olmesartan and Chlorthalidone respectively.

Method validation[15]

The method was validated according to the ICH guidelines. The following validation characteristics were addressed: linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

System suitability testing (SST)[16]

It is a vital part of chromatographic technique. These tests are used to prove that the resolution and reproducibility of the system are suitable for the analysis to be accomplished. It provides assurance that method will deliver accurate and precise data for its intended use. It is based on the conception that the electronic, equipment, samples constitute and analytical operations that an important system that can be evaluated as a whole.It involved test parameters as resolution, retention time, tailing factor and number of theoretical plate noted. The Result of system suitability testing was shown in table no. 2

Linearity

The linearity is expressed in term of correlation co-efficient of linear regression analysis. The linearity of response for Cilnidipine, Olmesartan and Chlorthalidonewas assessed by analysis of five independent levels of calibration curve in range of 50-300 µg/mlforCilnidipine, 100-600 µg/mlforOlmesartan and 60-360 µg/mlfor Chlorthalidone.The result of linearity was shown in table no. 3

Accuracy

The % recovery experiment was performed by standard addition method. In these method target concentration for Cilnidipine 100 µg/ml, Olmesartan 200 µg/ml and Chlorthalidone120 µg/ml in theses spiked concentration for Cilnidipine(0, 80, 100, 120 µg/ml), Olmesartan(0, 160, 200, 240 µg/ml) and Chlorthalidone(0, 96, 120, 144 µg/ml) was added at 0%, 80%, 100% & 120%. Area of peak obtained with each solution was measured for Cilnidipine, Olmesartan and Chlorthalidone. The result of accuracy was shown in table no. 4, 5, 6

Presicion

Result should be expressed as relative standard deviation.

A. Repeatability

In these for mixture solution of 50, 150 and 250µg/ml of Cilnidipine, 100, 300 and 500µg/ml of Olmesartan and 60, 180 and 300 µg/ml of Chlorthalidoneconcentration take that three times analyzed.The peak area was obtained with each solution was measured and % R.S.D was calculated. The result of repeatability was shown in table no.7

B. Intraday presicion

Mixed solutions containing 50, 150, 250µg/ml of Cinidipine, 100, 300,500 µg/ml of Olmesaratnand 60,180, 300 µg/ml of Chlorthalidonewere analyzed on the same day and % R.S.D was calculated. The result of intraday precision was shown in table no. 8

C.Interday precision

Mixed solutions containing 50, 150, 250µg/ml of Cilnidipine, 100, 300,500 µg/ml of Olmesartanand 60,180, 300 µg/ml of Chlorthalidonewere analyzed on different days and % R.S.D was calculated. The result of interday precision was shown in table no. 8

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) of the method refers to that minimum concentration of the active component that can be effectively estimated based on visual evaluation and the limit of quantitation (LOQ) is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. The result of LOD and LOQ was shown in table no. 9

The LOD was estimated from the 5 calibration curves. The LOD may be calculated as

LOD = 3.3 × (S.D. / Slope)

Where, S.D. = Standard deviation of the Y- intercepts of the 5 calibration curves

The LOQ was estimated from the calibration curves used to determine method linearity. The LOQ may be calculated as

LOQ = 10 × (S.D./Slope)
Where, S.D. = Standard deviation of the Y- intercepts of the 5 calibration curves

Robustness

The solution containing concentration 100 µg/ml of Cilnidipine, 200 µg/ml of Olmesartan and 120 µg/ml of Chlorthalidone was analyzed in different pH, mobile phase and flow rate. The peak area obtained with each solution was measured and % R.S.D was calculated. The result of robustness was shown in table no.10,11,12

Discussion

Validation of an analytical method is the process by which it is established by laboratory studies and the performance characteristics of the method meet the requirements for the intended analytical application. Validation is require for any new or amended method to ensure that it is capable of giving reproducible and reliable results when used by different operators employing the same or different laboratories.

4. CONCLUSION

All the parameters are validated as per ICH guidelines for the method validation and found to be suitable for routine quantitative analysis in pharmaceutical dosage forms. The result of linearity, accuracy, precision proved to be within limits with lower limits of detection and quantification. Robustness of method was confirmed as no significant were observed on analysis by subjecting the method to slight change in the method condition. Assay results obtained by proposed method are in fair agreement.

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TABLES AND FIGURES

Table No: 1. Assay Result of Synthetic Mixture Formulation

Amount Taken (mg)			Obtained Amount (mg)			% Assay Mean ± S.D. (n = 3)		
CIL	**OLM**	**CHLOR**	**CIL**	**OLM**	**CHLOR**	**CIL**	**OLM**	**CHLOR**
10	20	12.5	9.89	19.72	12.38	100.23 ±	99.68 ±	100.18 ±
10	20	12.5	10.06	19.91	12.57	1.19	1.15	1.01
10	20	12.5	10.12	20.18	12.62	30	57	29

Table No: 2. Result of System Suitability testing

Parameters	Data Obtained		
	CIL	**OLM**	**CHLOR**
Retention time	5.366	2.693	3.760
Theoretical plate	8776	4356	4462
Tailing factor	1.200	1.379	1.148
Resolution	-	0.318	0.512

Table No: 3. Linearity data for Cilnidipine, Olmesartan and Chlorthalidone

Conc. (µg/ml) CIL	Mean Area ± S.D.(n= 5) CIL	Conc. (µg/ml) OLM	Mean Area ± S.D. (n= 5) OLM	Conc. (µg/ml) CHLOR	Mean Area ± S.D. (n= 5) CHLOR
50	1675.06 ± 9.8902	**100**	1978.44 ± 8.5837	**60**	2182.31 ±5.1147
100	3054.87 ± 7.4335	**200**	3464.33 ± 7.0919	**120**	3746.42 ± 8.1372
150	4378.08 ± 8.0832	**300**	4938.34± 8.5176	**180**	5330.24 ± 5.6758
200	5723.49 ± 9.1163	**400**	6459.42 ± 7.4817	**240**	6869.18 ± 7.2115
250	6967.55 ± 8.7685	**500**	7868.04 ± 6.7272	**300**	8280.84 ± 7.7586
300	8284.84 ± 8.4752	**600**	9266.66 ± 9.1722	**360**	9856.06 ± 5.4566

Table No: 4. Accuracy Data for Cilnidipine

% Recovery	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Final Conc. (µg/ml)	Obtain Conc. (µg/ml)	% Assay	S.D.	Mean	% R.S.D
0%	100	0	100	98.9	98.9 %	1.4157	99.9	1.4171
	100	0	100	99.28	99.28%			
	100	0	100	101.52	101.52%			
80%	100	80	180	178.86	99.36%	0.8491	100.15	0.8478
	100	80	180	180.12	100.06%			
	100	80	180	181.89	101.05%			
100%	100	100	200	198.23	99.11%	0.9457	100.11	0.9446
	100	100	200	200.46	100.23%			
	100	100	200	201.98	100.99%			
120%	100	120	220	218.64	99.38%	0.7351	100.12	0.6074
	100	120	220	220.32	100.14%			
	100	120	220	221.87	100.85%			

Table No: 5. Accuracy Data for Olmesartan

% Recovery	Target Conc. (µg/ml)	Spiked Conc. (µg/ml)	Final Conc. (µg/ml)	Obtain Conc. (µg/ml)	% Assay	S.D.	Mean	% R.S.D
0%	200	0	200	198.43	99.21%	0.8361	100.07	0.8355
	200	0	200	200.25	100.12%			
	200	0	200	201.76	100.88%			
80%	200	160	360	357.42	99.28%	0.6559	100.01	0.6559
	200	160	360	360.72	100.2%			
	200	160	360	361.98	100.55%			
100%	200	200	400	397.86	99.46%	0.5434	100.05	0.5431
	200	200	400	400.64	100.16%			
	200	200	400	402.12	100.53%			
120	200	240	440	437.	99.4	0.5	100	0.5

%				63	6%	963	.12	956
	200	240	440	441.25	100.28%			
	200	240	440	442.75	100.62%			

Table No: 6. Accuracy Data for Chlorthalidone

% Recovery	Target Conc. (µg/ml)	Spiked Con. (µg/ml)	Final Conc. (µg/ml)	Obtain Conc. (µg/ml)	% Assay	S.D.	Mean	% R.S.D
0%	120	0	120	119.06	99.21%	0.8411	100.16	0.8397
	120	0	120	120.56	100.46%			
	120	0	120	120.98	100.81%			
80%	120	96	216	214.62	99.36%	0.5146	99.83	0.5155
	120	96	216	215.46	99.75%			
	120	96	216	216.84	100.38%			
100%	120	120	240	238.23	99.26%	0.7202	99.97	0.7204
	120	120	240	239.89	99.95%			
	120	120	240	241.68	100.7%			
120%	120	144	264	261.46	99.03%	0.6051	99.62	0.6074
	120	144	264	262.98	99.61%			
	120	144	264	264.64	100.24%			

Table No: 7. Repeatability Data for Cilnidipine, Olmesartan and Chlorthalidone

Drug	Conc. (µg/ml)	Mean Area ± S.D. (n=3)	% R.S.D
Cilnidipine	**50**	1692.799 ± 4.9414	0.2919
	150	4382.48 ± 6.5431	0.1493
	250	6954.88 ± 6.5123	0.0936
Olmesartan	**100**	1988.90 ± 3.5222	0.1770
	300	4935.34 ± 6.0130	0.1218
	500	7869.13 ± 3.5137	0.0446
Chlorthalidone	**60**	2187.19 ± 6.8050	0.3111
	180	5327.98 ± 7.7837	0.1460
	300	8282.98 ± 6.5208	0.0787

Table No: 8. Intraday &Interday Data for Cilnidipine, Olmesartan and Chlorthalidone

Drug	Conc. (µg/ml)	Intraday Mean Area ± S.D. (n=3)	% R.S.D	Interday Mean Area ± S.D. (n=3)	% R.S.D
Cilnidipine	**50**	1686.13 ± 8.5150	0.5050	1687.12 ± 8.7453	0.5183
	150	4375.46 ± 7.0125	0.1602	4375.48 ± 8.9950	0.2055
	250	6963.57 ± 9.0050	0.1293	6956.86 ± 9.6694	0.1389
Olmesartan	**100**	1991.24 ± 6.013	0.3019	1985.25 ± 6.9950	0.3523
	300	4936.69 ± 9.0833	0.1839	4935.99 ± 9.0220	0.1827
	500	7868.44 ± 5.5931	0.0710	7864.79 ± 9.8719	0.1255
Chlorthalidone	**60**	2187.20 ± 7.7909	0.3562	2185.86 ± 8.1331	0.3720
	180	5334.31 ± 8.0643	0.1511	5327.67 ± 9.1423	0.1716
	300	8283.64 ± 8.5358	0.1030	8284.65 ± 9.1917	0.1109

Table No: 9. LOD & LOQ Data for Cilnidipine, Olmesartan and Chlorthalidone

Parameter	CIL	OLM	CHLOR
Intercept	136.01	182.89	231.71
Slope	29.802	19.113	41.711
LOD (µg/ml)	15.06	31.57	18.33
LOQ (µg/ml)	45.63	95.69	55.55

Table No: 10. Robustness Data for Cilnidipine

Conc. (µg/ml)	pH (+0.2units)	pH (-0.2units)	Flow rate (+0.2units)	Flow rate (-0.2units)	Mobile phase (+2%unit)	Mobile phase (-2%units)
100	2964.53	3058.46	2944.52	3062.45	2956.52	3071.46
100	2971.46	3047.43	2954.53	3056.47	2962.57	3068.45
100	2958.62	3051.44	2959.54	3069.46	2971.56	3059.47
Mean	2964.87	3052.44	2952.86	3062.79	2963.55	3066.46
S.D.	6.4288	5.5849	7.6473	6.5054	7.5685	6.2380
%R.S.D	0.2168	0.1829	0.2589	0.2124	0.2553	0.2034

Table No: 11. Robustness Data for Olmesartan

Conc. (µg/ml)	pH (+0.2units)	pH (-0.2units)	Flow rate (+0.2units)	Flow rate (-0.2units)	Mobile phase (+2%unit)	Mobile phase (-2%units)
200	3362.32	3446.35	3373.35			
200	3356.35	3452.34	3365.36			
200	3368.34	3461.35	3359.34			
Mean	3362.33	3453.35	3366.02			
S.D.	5.9945	7.5518	7.0254			
% R.S.D	0.1912	0.2186	0.2087	0.2838	0.1793	0.2178

Table No: 12. Robustness Data for Chlorthalidone

Conc. (µg/ml)	pH (+0.2units)	pH (-0.2units)	Flow Rate (+0.2units)	(
120	3648.61	3752.63	3657.63	
120	3641.62	3764.65	3667.65	
120	3653.64	3766.66	3653.67	
Mean	3647.95	3761.32	3659.65	
S.D.	6.0345	7.5882	7.2072	8.1696 7.3815 8.4913
% R.S.D	0.1654	0.2017	0.1969	0.2171 0.2018 0.2256

Figure No: 1. Structure of Cilnidipine

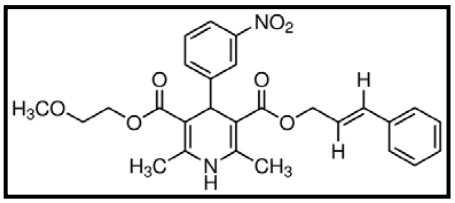

Figure No: 2. Structure of Olmesartanmedoxomil

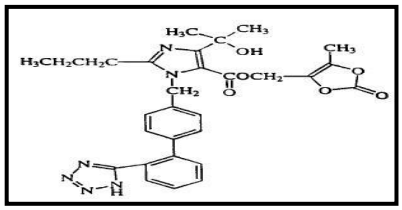

Figure No: 3. Structure of Chlorthalidone

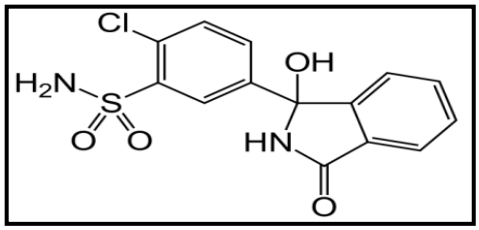

Figure No: 4. Chromatogram of different concentration of ternary mixture of Cilnidipine, Olmesartan and Chlorthalidone

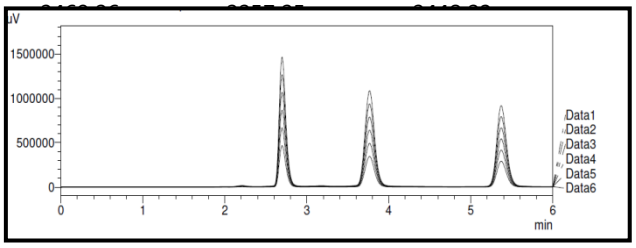

Figure No: 5. Calibration Curve of Cilnidipine

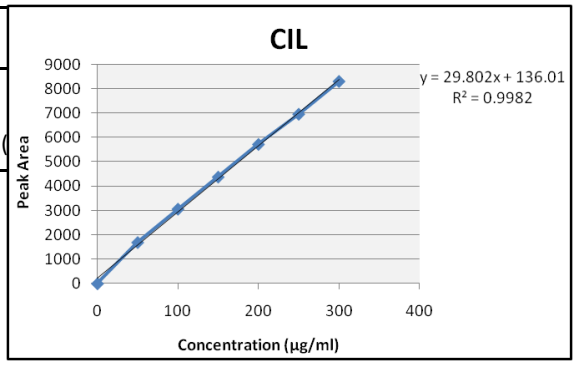

Figure No: 6. Calibration Curve of Olmesartan

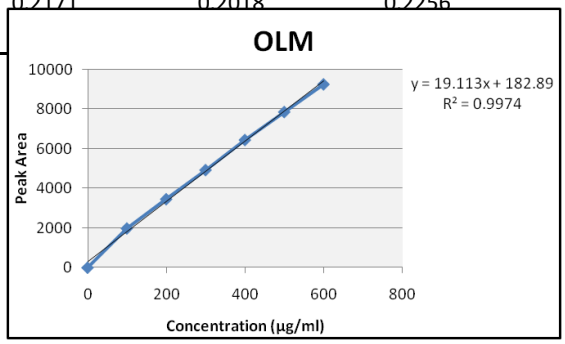

Figure No: 7. Calibration Curve of Chlorthalidone