A STUDY OF ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CHLORTHALIDONE, OLMESARTAN, AND CLINIDIPINE IN BULK AND TABLET DOSAGE FORM BY RP-HPLC METHOD

Kalaiselvi ponnusamy*, ArunkumarVinayagam* and Senthil Kumar Natesan

*Department of Pharmaceutical Analysis, JKKMRF-Annai J.K.K SampooraniAmmal College of Pharmacy, Komarapalayam, Namakkal, Tamil Nadu, India. (Affliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India).

ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Chlorthalidone, Olmesartan and Clinidipine in bulk and tablet dosage form. Chromatogram was run through Waters XBridge C18 250x4.6mm, 5µ. Mobile phase containing 0.05 m dibasic sodium phosphate Buffer (pH 2.5) and Acetonitrie in the ratio of 40:60 and was pumped through column at a flow rate of 0.8ml/min. Buffer used in this method was 0.05 M Na2HPo4. Temperature was maintained at 30°C. Optimized wavelength for Chlorthalidone, Olmesartan and Clinidipine was 240nm. Retention time of Chlorthalidone, Olmesartan and Clinidipine. Were found to be 5.026 min, 9.161 min and 16.379 min. %RSD of method precision for Chlorthalidone, Olmesartan and Clinidipine were and found to be 0.1, 0.1 and 0.0 respectively. % recovery was obtained as 100.47%, 99.92% and 101.58% for Chlorthalidone, Olmesartan and Clinidipine. Respectively. LOD values are obtained from regression equations of Chlorthalidone, Olmesartan and Clinidipine. Were1.28ppm, 1.88ppm, 0.29ppm, LOQ values are 3.89ppm, 5.71ppm and 0.81 ppm respectively. Regression equation of Chlorthalidone was y = 88195x-4000000, Olmesartan was y = 184043x-2000000 and of Clinidipine was y = 99672x-3000000. Retention times are decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS: Chlorthalidone, Olmesartan and Clinidipine, RP-HPLC.

1. INTRODUCTION

Chlorthalidone, identified by its chemical structure as 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl)benzenesulfonamide, has the chemical formula C14H11ClN2O4S and a molecular weight of approximately 338.77 g/mol. This compound is classified as a thiazide-like diuretic, primarily utilized in the treatment of hypertension. It functions by targeting the distal convoluted tubule of the nephron, where it inhibits the Na^+/Cl^- cotransporter, resulting in increased excretion of sodium and chloride, and subsequently water. This mechanism effectively reduces blood volume and lowers blood pressure. Chlorthalidone is recognized as a first-line therapy for uncomplicated hypertension, supported by robust evidence from meta-analyses indicating that thiazide diuretics, including chlorthalidone, significantly decrease the risk of stroke, myocardial infarction, heart failure, and overall cardiovascular mortality in hypertensive patients.(1)

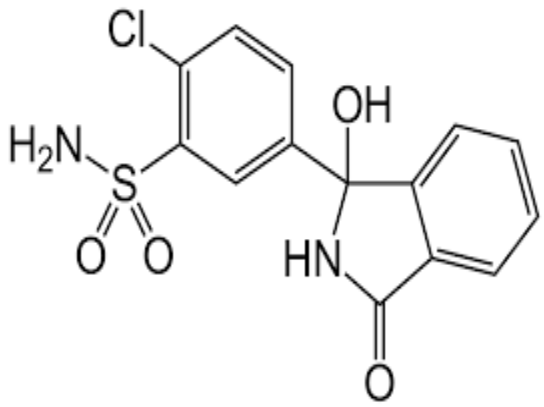


Figure no: 1. Structure of Chlorthalidone

Olmesartan is an anti-hypertensive agent and an angiotensin receptor antagonist. It identified by the chemical structure 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] imidazole -4- carboxylic acidwhich belongs to thechemical formula$C_{29}H_{30}N_6O_6$ and a molecular weight is 558.595 g/mol. Olmesartan selectively blocks the binding of angiotensin II to the AT_1 receptors found in vascular smooth muscle and the adrenal gland. Angiotensin II normally causes vasoconstriction and stimulates the release of aldosterone, leading to sodium and water retention, which increases blood pressure. By inhibiting these effects, Olmesartan causes vasodilation**,**decreasesaldosterone secretion**,** and reducessodium and water reabsorption**,**therebylowering blood pressure**.** [2]

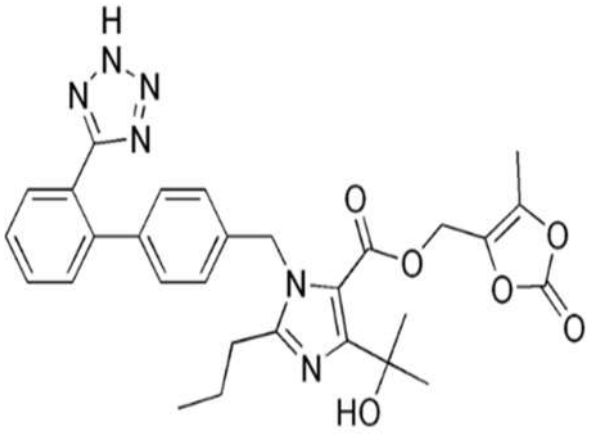


Fig. no: 2. Structure of Olmesartan

Clinidipine is a dihydropyridine calcium channel blockerwithadual L-type and N-type calcium channel blocking action. It having the chemical structure of 3-(2-methoxyethyl)-5-methyl-2-(3-nitrophenyl)-4-(3-phenylpropyl)-1,4-dihydropyridine-6-carboxylic acid 2-methoxyethyl ester, molecular formula is $C_{27}H_{33}N_3O_7$ and a molecular weight about 527.57 g/mol. Clinidipine exerts its effects primarily through L-type calcium channel blockade in vascular smooth muscle and cardiac muscle.By inhibiting calcium influx via these channels, Clinidipine promotes relaxation of vascular smooth muscle, leading to vasodilation of arterioles.This process effectively reduces peripheral vascular resistance and lowers blood

pressure without triggering reflex tachycardia, making it a valuable therapeutic option for managing hypertension. Additionally, clinidipine acts on N-type calcium channels located in sympathetic nerve endings. By blocking calcium entry into these neurons, clinidipine diminishes the release of norepinephrine, which in turn decreases sympathetic overactivity.This results in a reduced increase in heart rate and a lower cardiac workload, while also enhancing renal hemodynamics and offering protection against proteinuria.[3]

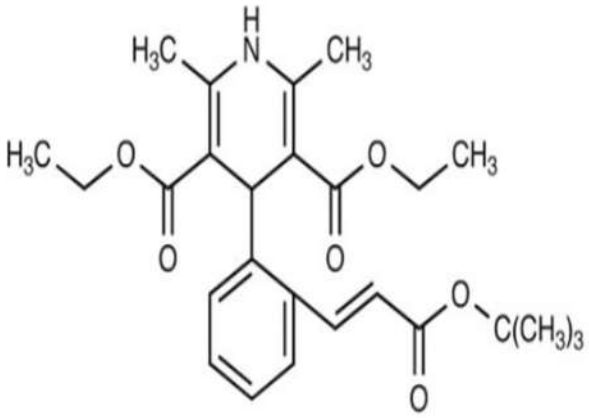


Fig. no: 3. Structure of Clinidipine

2. MATERIALS AND METHODS

2.1. Reagents and Chemicals:

The study utilized several reagents and chemicals, including pure drugs such as Chlorthalidone, Olmesartan, and Clinidipine, as well as their combination. Additionally, water, acetonitrile, dibasic sodium phosphate buffer, and ortho-phosphoric acid were employed in the experiments. All chemicals and solvents were sourced from Qualigens, ensuring high-quality materials for the research.

2.2.Instrumentation:

SHIMADZU HPLC system with Lab solution software and photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, Discovery Waters X Bridge C18 250x4.6mm, 5µ. As part of experimentation, additional equipment such as sonicator (Sonica ultrasonic cleaner power-model 2200 MH), ELICO pH meter (Model L1-120), Centrifuge apparatus and other glassware were used for the present investigation.

2.3. Development of Optimum MobilePhase:

A good mobile phase should give dense compact spots and a desirable extent of separation of drugs from each other, such mobile phase is selected for the study. For this purpose different mobile phases were tried, the results of which are given below.

2.4. Chromatographic conditions:

The Discovery Waters X Bridge C18 250x4.6mm, 5µ column was used for analytical separation. Dibasic sodium phosphate buffer solution (pH2.5) and Acetonitrile was taken in the ratio of (40:60%v/v) mobile phase for the investigation with a flow rate of a 0.8ml/min.

The temperature was maintained at 30°C. The injection volume was 20µl and the UV detection was achieved at 240nm.

2.5. Preparation of mobile phase:

Mixture of 400 ml of 0.05m K2HPO4 buffer (pH-2.5) and 600 ml of Acetonitrile were mixed and degassed in ultrasonic water bath for 15 minutes and filtered through 0.45 µ filter paper. Mobile phase was used as a diluent.

2.6. Preparation of standard stock solution:

Weighed accurately about 12.5 mg of, Chlorthalidone, 40 mg of Olmesartan and 10 mg of Clinidipine were transferred into a 100 ml volumetric flask separately and dissolved with minimum quantity of mobile phase and the volume was made up to the mark with mobile phase.The concentration of the solution contains 125µg/ml for Chlorthalidone, 400 µg/ml for Olmesartan and 100µg/ml for Clinidipine.

2.7. Preparation of standardsolution:

From above standard stock solution 5ml pipetted into 10ml volumetric flask, diluted to volume with diluent and mixed well.The concentration of the solution contains 62.5µg/ml for Chlorthalidone, 200 µg/ml for Olmesartan and 50µg/ml for Clinidipine.

2.8. Preparation of sample stock solution:

Twenty tablets of formulations (Olvan Trio) were weighed accurately. The average weight of the tablet was calculated and powdered. The tablet powder equivalent to one tablet of Olvan Trio (12.5 mg of, Chlorthalidone, 40 mg of Olmesartan and 10 mg of Clinidipine) was weighed and transferred into 100 ml volumetric flask. About 15ml of mobile phase was added to dissolve the substance. Then the solution was sonicated for 15 mins. The volume was made up to 100ml with the same solvent and centrifuge at 3000 rpm. Then the solution was filtered through whatmann filter paper No:41 to get 125µg/ml for Chlorthalidone, 400 µg/ml for Olmesartan and 100 µg/ml for Clinidipine.

2.9. Preparation of sample solution: From the clear solution, pipetted 5.0ml of above stock solution into 10ml volumetric flask, diluted to volume with mobile phase.The concentration of the solution contains 62.5µg/ml for Chlorthalidone, 200 µg/ml for Olmesartan and 50µg/ml for Clinidipine. A steady base line was recorded withoptimized chromatographic conditions. After the stabilization of base line for 30 minutes, the test solutions were injected and recorded the chromatogram. The concentration of each test solutions was determined by using slope and intercept values from the calibration graph.

2.10. Optimized chromatographic condition

Mode of separation - Gradient
Stationary phase - C18 column (250 mm×4.6 mm i.d 5µ)
Mobile phase - Acetonitrile: 0.05 m dibasic sodium phosphate buffer (pH 2.5)
Proportion of mobile phase- 60:40 %v/v
Detection wavelength - 240 nm
Temperature - 30℃
Sample Load - 20 µl
Flow rate -0.8ml/min

3. Result and Discussion:

3.1. REVERSEPHASEHPLCMETHOD:

Three factors—acetonitrile concentration (A), phosphate buffer pH (B), and flow rate (C)—were optimized within specified ranges using a design of experiments approach. The responses studied were the capacity factor of Chlorthalidone (K1), the retention time of the second eluted drug (Rt2), and the resolution between Olmesartan and Clinidipine peaks (Rs2,3). Experiments were randomized, and central points were replicated to estimate experimental error. A second-order polynomial model incorporating linear, quadratic, and interaction terms was used, and insignificant terms ($p > 0.05$) were removed by backward elimination. The models showed good fit with adjusted $R^2 \geq 0.80$, significant p-values (< 0.05), adequate precision ratios ($6.25–7.16 > 4$), and acceptable reproducibility ($C.V < 10\%$). The strongest interaction observed was between buffer pH and flow rate (BC, +0.0024) in the K1 model, which was highly significant ($p < 0.0001$). Increasing buffer pH led to a rapid decrease in Chlorthalidone flow rate at both low and high acetonitrile concentrations.**(Table no: 1)**

3.2. Optimized chromatogram:

The assay conditions were optimized using a mobile phase composed of acetonitrile and sodium monophosphate buffer at pH 2.2, with a flow rate set at 0.8 ml/min and UV detection at 240 nm. The predicted response values for the variable D were determined to be K1 = 1.513, Rt_2 = 9.161 min, and $Rs_{2,3}$= 14.984. A comparison between the predicted and experimental responses revealed a strong correlation, with discrepancies remaining within 5.0%. The percentage of prediction error was calculated using a specific equation, and the results are presented in **Table 2**. Additionally, the optimized chromatogram is illustrated in **Figure no. 3.**

3.3. METHOD VALIDATION (ICH Q2A, Q2B Guidelines) (62,63)

A). SYSTEM SUITABILITY

The system suitability test serves as a critical validation tool, ensuring that a method yields accurate and precise results during a specific analysis. Each test's outcomes are evaluated against predetermined acceptance criteria; if the results meet these standards, the method is considered acceptable for that instance. The established criteria include an asymmetry factor not exceeding 2.0, a minimum of 2000 theoretical plates, and a peak area % RSD of no more than 2.0. All parameters assessed in this study fell within the specified acceptance limits. The findings from the system suitability evaluation of the developed method are presented in **Table no: 03.**

B). LINEARITY:

The linearity of an analytical method refers to its capacity to produce test results that are directly proportional to the concentration of the analyte within a specified range. To assess this, working stock solutions were created by diluting the stock solution with the mobile phase, achieving concentrations of 50-75 µg/ml for Chlorthalidone, 160-240 µg/ml for Olmesartan, and 40-60 µg/ml for Clinidipine. Following the injection of these solutions, chromatograms were recorded at a wavelength of 220 nm. The analysis confirmed that the specified concentration ranges exhibited linearity, with correlation coefficients exceeding 0.999 for all three drugs, as detailed in the accompanying **Table no: 4.**Thecalibration curve was shown in **Figure no: 04-06**.

C). METHOD PRECISION:

The tablet formulation (Olvan Trio tablet formulation containing 12.5 mg of Chlorthalidone, 40 mg of Olmesartan and 10 mg of Clinidipine) was selected for analysis. The percentage label claim present in tablet formulation was found to be 100.26, 99.36 and 99.60 % for Chlorthalidone, Olmesartan and Clinidipine respectively. The % RSD values were found to be 0.6195, 1.2816 and0.9036 for Chlorthalidone, Olmesartan and Clinidipine respectively. The reports were shown in **Table no:5.**

D). SYSTEM PRECISION:

The precision of the method was confirmed by the analysis of formulation was repeated in six times. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.0065, 0.0063and0.0048 for Chlorthalidone, Olmesartan andClinidipine respectively**.** The results of the analysis are shown in**Table no: 6.**

E). ACCURACY:

Recovery studies confirmed the accuracy of the method. In the pre-analyzed formulation, a known quantity of analytes raw materials was added at different concentration levels. The absorbance of the solutions was measured and the percentage recovery was calculated. The percentage recovery was found to be in the range of 100.24-100.64%for Chlorthalidone, 99.46-100.20%for Olmesartan and 100.37 – 102.46 % for Clinidipine. The low % RSD value for analytes indicated that this percentage recovery revealed no interference due to the excipients used in the formulation. Therefore, the developed method was found to be accurate. The results were shown in the **Table no: 7.**

F). RUGGEDNESS:

Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. The percentage RSD value for analyst I found to be 1.4273, 0.6135 and 1.4230% for Chlorthalidone, Olmesartan and Clinidipine respectively. The percentage RSD values for analyst II were 0.6000, 1.2884 and 0.5447 % for Chlorthalidone, Olmesartan and Clinidipine respectively.The results were shown in the **Table no: 8.**

G). ROBUSTNESS:

The robustness study indicated that the factors selected remained unaffected by small variation of flow rate and the mobile phase composition. The system suitability results were within the limit. Hence the method was robust. The results were shown in **Table no: 9.**

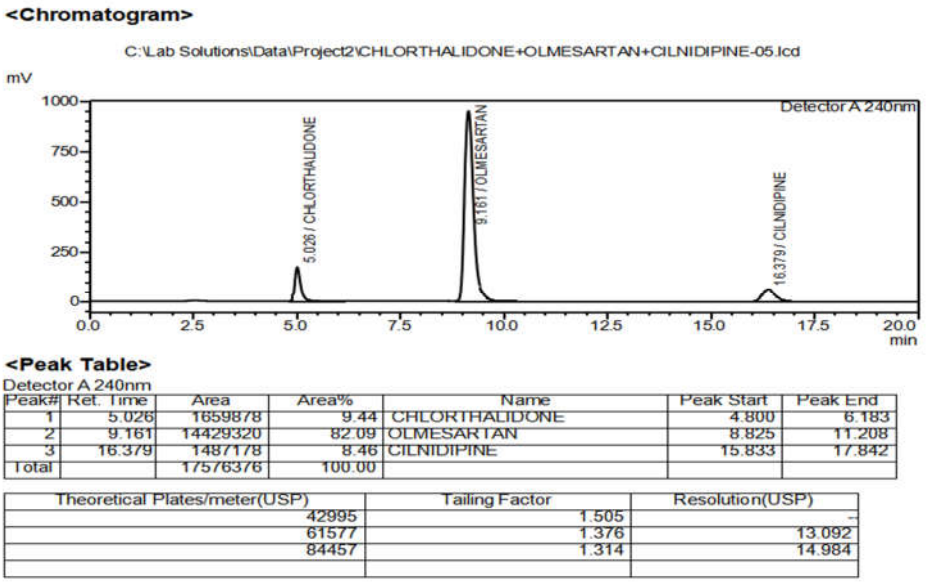
Table no: 1.Central Composite arrangement and Responses

Run	Space type	CCN Con (%v/v)	Na mono PO4 buffer pH	Flow rate (mL /min)	Capacity factor (K1)	Retention time (Rt2)	Resolution (Rs2,3)
7	Factorial	55	2.2	0.8	1.511	9.162	14.980
10	Factorial	65	2.2	0.8	1.516	9.163	14.984
12	Factorial	55	2.8	0.8	1.501	9.159	14.983
17	Factorial	65	2.8	0.8	1.51	9.160	14.982
18	Factorial	55	2.2	1.2	1.512	9.161	14.980

19	Factorial	65	2.2	1.2	1.508	9.160	14.981
2	Factorial	55	2.8	1.2	1.509	9.162	14.982
4	Factorial	65	2.8	1.2	1.514	9.16	14.982
13	Axial	51.591	2.5	1	1.515	9.163	14.984
15	Axial	68.409	2.5	1	1.507	9.162	14.982
16	Axial	60	1.994	1	1.513	9.159	14.980
20	Axial	60	3.004	1	1.502	9.162	14.983
1	Axial	60	2.5	0.663	1.504	9.159	14.982
3	Axial	60	2.5	1.336	1.507	9.158	14.982
5	Center	60	2.5	1	1.505	9.161	14.984
6	Center	60	2.5	1	1.505	9.161	14.984
8	Center	60	2.5	1	1.505	9.161	14.984
3	Center	60	2.5	1	1.505	9.161	14.984
2	Center	60	2.5	1	1.505	9.161	14.984
1	Center	60	2.5	1	1.505	9.161	14.984

Table no: 2. Comparison of Experimental and Predictive values of different functions under optimal conditions

Optimum conditions	ACN (%v/v)	NamonoPO$_4$ buffer pH	Flow rate (ml/min)	K_1	Rt_2	$Rs_{2,3}$
Predictive	55.00	2.2	0.8	1.513	9.155	14.981
Experimental	55.00	2.2	0.8	1.505	9.161	14.984
Average error				0.528	0.0654	0.0200
Desirability value(D)=0.747						



<Peak Table>
Detector A 240nm

Peak#	Ret. Time	Area	Area%	Name	Peak Start	Peak End
1	5.026	1659878	9.44	CHLORTHALIDONE	4.800	6.183
2	9.161	14429320	82.09	OLMESARTAN	8.825	11.208
3	16.379	1487178	8.46	CILNIDIPINE	15.833	17.842
Total		17576376	100.00			

Theoretical Plates/meter(USP)	Tailing Factor	Resolution(USP)
42995	1.505	-
61577	1.376	13.092
84457	1.314	14.984

Figure no: 4. Optimized chromatogram

Table no: 3. System suitability parameter of Chlorthalidone, Olmesartan and Clinidipine

Parameters	Chlorthalidone	Olmesartan	Clinidipine
Retention time(min)	5.026	9.161	16.379
Tailing factor	1.505	1.376	1.314
Peak Area	1659878	14429320	1487178
Theoretical plates (USP)	42995	61577	84457
Resolution (min)	0.00	13.092	14.984

Table no: 4. Linearity of Chlorthalidone, Olmesartan and Clinidipine

Parameters	Chlorthalidone	Olmesartan	Clinidipine
Beers Law limit (µg/ml)	50-75	160-240	40-60
Correlation coefficient(r^2)	0.9997	0.9993	0.9998
Regression equation	y=88195X-4000000	y=18043X-2000000	y=99672X-3000000
Slope (m)	88195	18043	99672
Intercept (c)	4000000	2000000	3000000
LOD (µg/ml)	1.28	1.88	0.29
LOQ (µg/ml)	3.89	5.71	0.89

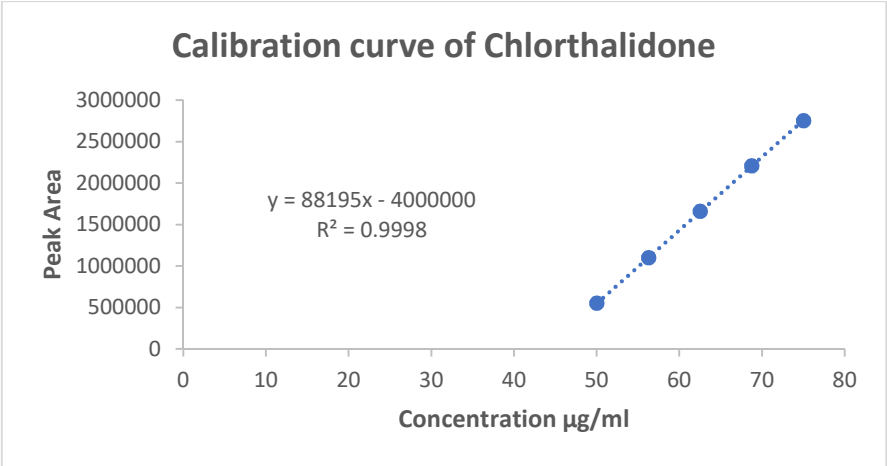


Figure no: 4.Calibration Curve of Chlorthalidone

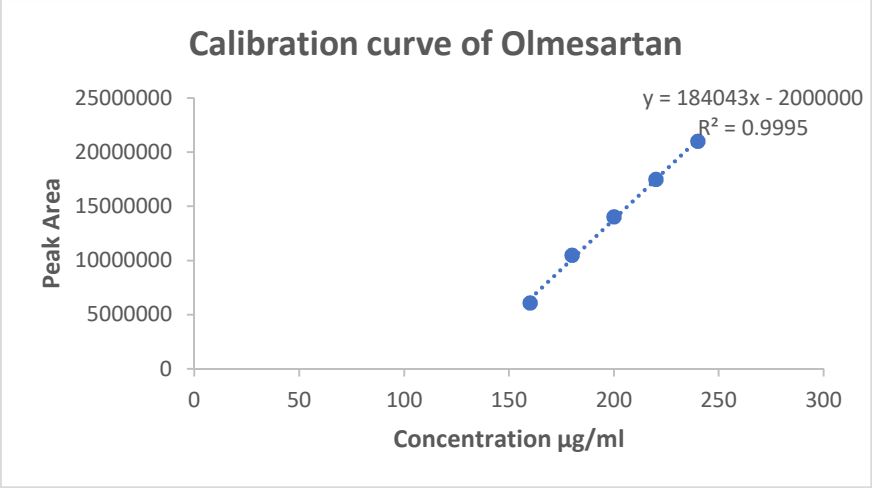


Figure no: 5. Calibration Curve of Olmesartan

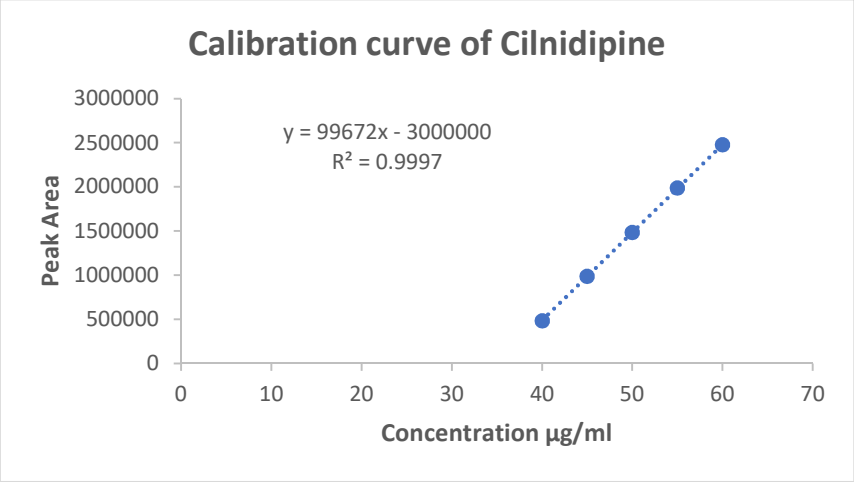


Figure no: 6.Calibration Curve for Clinidipine

Table no: 5. Quantification of formulation data (Olvan trio tablet MP)

Drug	Sample No	Labeled Amount (mg/tab)	Amount Found (mg/tab)	Percentage Obtained	Average (%)	SD	% RSD
CHLOR	1	12.5	12.56	100.48	100.29	0.6213	0.6195
	2	12.5	12.60	100.80			
	3	12.5	12.45	99.60			
OLME	1	40	41.45	101.12	99.36	1.2735	1.2816
	2	40	39.53	98.82			
	3	40	39.26	98.15			
CILNI	1	10	10.05	100.50	99.60	0.90	0.9036
	2	10	9.87	98.70			
	3	10	9.96	99.60			

Table no: 6. Precision study data of Chlorthalidone, Olmesartan and Clinidipine

S.No	Peak Area		
	Chlorthalidone	Olmesartan	Clinidipine
1	11568436	3044256	1959215
2	11569615	3044654	1959316
3	11568843	3044515	1959235
4	11569212	3044316	1959432
5	11568919	3044455	1959347
6	11567216	3044764	1959436
Mean	11568706.83	3044493.33	1959330.16
SD	757.127	194.312	94.207
%RSD	0.0065	0.0063	0.0048

Table no:7. Recovery Analysis of formulation data

Drug	Percentage	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount recovered (µg/ml)	% Recovery	SD	% RSD
CHLOR	50	62.5	50.0	112.5	50.12	100.24		
	100	62.5	62.5	125.0	62.90	100.64	0.2071	0.2061
	150	62.5	75.0	137.50	75.40	100.53		
					Mean	100.47		
	50	200.0	160.0	360.0	159.15	99.46		
OLME	100	200.0	200.0	400.0	200.40	100.20	0.3983	0.3986
	150	200.0	240.0	440.0	240.26	100.10		
					Mean	99.92		
CILN	50	50.0	40.0	90.0	40.15	100.37	1.0816	1.0647
	100	50.0	50.0	100.0	51.23	102.46		
	150	50.0	60.0	110.0	61.15	101.91		
					Mean	101.58		

Table no: 8. Ruggedness study data- Different Analyst

S.no	Drug	Condition	Mean %	±SD	%RSD
1	Chlorthalidone	Analyst 1	100.65	1.4250	1.4157
2		Analyst 2	100.51	1.6640	1.6555
3	Olmesartan	Analyst 1	99.64	0.9439	0.9473
4		Analyst 2	99.63	1.0554	1.0593
5	Clinidipine	Analyst 1	100.56	1.3930	1.3852
6		Analyst 2	100.38	0.9118	0.9083

Table no: 9. Robustness study data

Peak Name	Parameter	Conditions	Theoretical plate	Tailing factor
Chlorthalidone	Mobile phase (Acetonitrile concentration)	53 ml	42604	1.58
		55 ml	42613	1.54
		57 ml	42606	1.59
	Flow rate	0.6 ml/min	42619	1.53
		0.8 ml/min	42613	1.54
		1.0 ml/min	42609	1.57
Olmesartan	Mobile phase (Acetonitrile concentration)	53 ml	61789	1.40
		55 ml	61577	1.38
		57 ml	61478	1.35
	Flow rate	0.6ml/min	61318	1.33
		0.8ml/min	61577	1.38
		1.0ml/min	61423	1.35
Clinidipine	Mobile phase (Acetonitrile concentration)	53ml	84489	1.34
		55ml	84494	1.33
		57 ml	84448	1.36
	Flow rate	0.6 ml/min	84477	1.31
		0.8 ml/min	84494	1.33
		1.0 ml/min	84447	1.35

4. Conclusion:

The developed RP-HPLC method for the simultaneous estimation of Chlorthalidone, Olmesartan, and Clinidipine was found to be simple, precise, accurate, and highly sensitive. The method demonstrated excellent linearity with correlation coefficients above 0.999, along with satisfactory recovery and low %RSD values, confirming its accuracy and precision. The use of chemometric optimization ensured high resolution, reduced analysis time, and superior peak quality. Therefore, the proposed RP-HPLC method is reliable and suitable for routine quality control and quantitative analysis of Chlorthalidone, Olmesartan, and Clinidipine in bulk and combined pharmaceutical formulations.

5. CONTRIBUTIONS:

1. KALAISELVI. P[1] – Contributed for the conceptual work in schemes of research work.

2. ARUNKUMAR. V[*1]- Contributed for the laboratory works in research and literature works.

3. SENTHIL KUMAR. N- Contributed for the literature works and a moral support.

6. Reference:

1. https://go.drugbank.com/drugs/DB00310.
2. https://go.drugbank.com/drugs/DB00275.
3. https://go.drugbank.com/drugs/DB09232.
4. Lloyd.R.Slyder, Joseph.J.Kirkland and Joseph.L.Glajch. Practical HPLC Method Development. John Wiley and Sons, Inc, USA; 2nd edn; 1997: 1-14.
5. Beckett, A.H and Stenlake, J.B. Practical Pharmaceutical Chemistry, Part-2, CBS Publishers and Distributers: New Delhi; 4th edn; 2003: 85, 86& 92.
6. Sethi. P.D. Quantitative Analysis of Drugs in Pharmaceutical Formulations. CBS Publishers and Distributors; New Delhi; 3rd edn; 1997: 6-9.
7. Douglas.A.Skoog, Donald.M.West, James.F.Holler and Stanley.R.Croch. Fundamentals of Analytical Chemistry. Thomson Asia Pvt. Ltd; Singapore; 8th edn; 2007: 4, 921, 975.
8. Horbart.H.Willard, Lynne.L.Merritt, John.A.Dean and Frank.A.Settle. Instrumental Methods of Analysis. CBS Publishers and Distributors; New Delhi; 7th edn; 1986: 1, 592, 622-628.
9. http://www.rpi.edu.htm
10. David.G.Watson. Pharmaceutical Analysis, A text for pharmacy students and pharmaceutical chemists. Churchill Livingston: Philadelphia; 2005: 237-273,277-286.
11. http;//pharmtech.com.
12. Fifield.F.W. and Kealey.D. Principles and Practise of Analytical Chemistry, Blackwell Sciences Itd USA; 5th edn; 2000: 80-95.
13. Sharma.B.K. High Performance Liquid Chromatography, Instrumental Methods of Chemical Analysis, GOEL publishing house, Meerut, 6th edn; 2007: C 290-291.
14. Sandy Lindsay. HPLC by Open Learning, John Wiley and Sons: New York; 1998: 30-45.
15. George Lunn. HPLC methods of recently approved pharmaceuticals drugs development; 2005: 123-128.
16. www.artclejpj.com
17. Haleem.J. Issaq. A century of separation science, Marcel Dekker, inc. USA, New York; 2002: 231-252.
18. David Harvey. Modern Analytical Chemistry. Mc-Graw Hill companies, New York; 2000: 35-52, 549- 559.