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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF OLMESARTAN, CLINIDIPINE & CHLORTHALIDONE BY RP-HPLC METHOD

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Olmesartan, Cilinidipine and Chlorthalidone in liquid dosage form. Chromatogram was run through Ascentis C18 150x4.6mm,5µ. Mobile phase containing Buffer and Acetonitrie in the ratio of 43:57 and was pumped through column at a flow rate of 0.8ml/min. Buffer used in this method was 0.01N NH_4. Temperature was maintained at 30°C. Optimized wavelength for Olmesartan. Cilinidipine and Chlorthalidone was 230nm. Retention time of Olmesartan, Cilinidipine and Chlorthalidone. Were found to be 2.253 min, 2.701min and 3.598 min. %RSD of method precision for Olmesartan, Cilinidipine and Chlorthalidone were and found to be 0.5, 0.2 and 0.6 respectively. % recovery was Obtained as 100.15%, 100.09% and 100.01% for Olmesartan, Cilinidipine and Chlorthalidone. respectively. LOD values are obtained from regression equations of Olmesartan, Cilinidipine and Chlorthalidone. were 0.24ppm, 0.01ppm, 0.01ppm, LOQ values are 0.72ppm, 0.02ppm and 0.04 ppm respectively. Regression equation of Olmesartan was y = 23139.x + 15862, Cilinidipinee was y = 6442x +1907 and of Chlorthalidone was y = 8372.x + 3094. Retention times are decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS: Olmesartan, Cilinidipine, Chlorthalidone, RP-HPLC.

INTRODUCTION

Chemically Olmesartan (OLM) is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Olmesartan is selective for AT1 and has a 12,500 times greater affinity for AT1 than the AT2 receptor. Also unlike the well-known ARB losartan, olmesartan does not have an active metabolite or possess uricosuric effects. Structure of the OLM was shown in figure 1 (A).[1]

Chemically Cilnidipine (CLN) is a dihydropyridine calcium-channel blocker. It inhibits cellular influx of calcium, thus causing vasodilatation. It has greater selectivity for vascular smooth muscle. It has little or no action at the SA or AV nodes and -ve inotropic activity is rarely seen at therapeutic doses. Structure of the CLN was shown in figure 1 (B).[2]

Chemically Chlorthalidone (CLR) inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. By increasing the delivery of sodium to the distal renal tubule, Chlorthalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism. Structure of the CLR was shown in figure 1 (C).[3]

Literature survey reveals there are several methods to estimated these drugs in single or in combination of two or three drugs.[5-9] But there is only very few HPLC methods are available for simultaneous estimation of OLM, CLN and CLR, so the scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation processes.

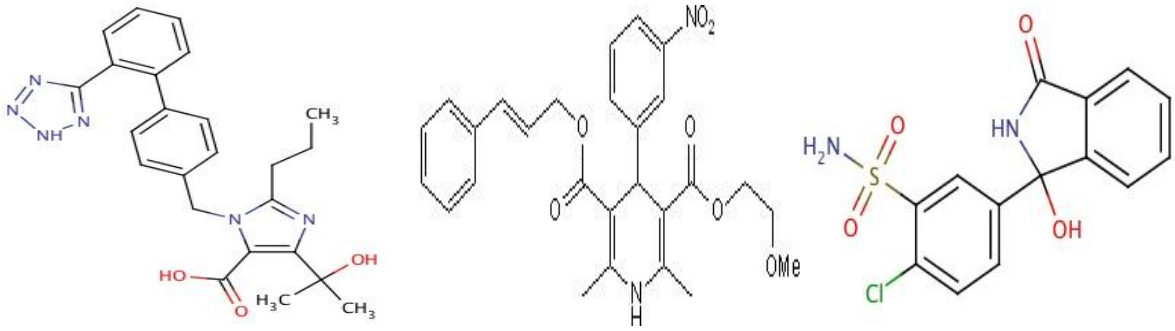

Figure 1: Structure of (A)Olmesartan (B) Clonidipine (C) Chlorthalidone.

MATERIALS AND METHODS

Reagents and Chemicals: Olmesartan, Clinidipine & Chlorthalidone pure drugs (API), Combination Olmesartan, Clinidipine & Chlorthalidone, Distilled water, Acetonitrile, Tri ethyl amine, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instrumentation: HPLC (waters 2695) system with Empower-2 software and 2996 module photo diode array detector equipped with a quaternary solvent delivery pump, automatic sampler unit, Discovery C18 150x4.6mm, 5m. As part of experimentation, additional equipment such as sonicator (ultrasonic cleaner power sonic 420), pH meter, vacuum oven (wadegati), water bath and other glassware were used for the present investigation.

Chromatographic conditions: The Discovery C18 150x4.6mm, 5m column was used for analytical separation. Potassium dihydrogen ortho phosphate and one drop of triethyl amine in every 100ml of buffer solution (pH3.0) and Acetonitrile was taken in the ratio of (57:43%v/v) mobile phase for the investigation with a flow rate of a 0.8ml/min. The temperature was maintained at 30^0C. The injection volume was 10µl and the UV detection was achieved at 230nm.

Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0): Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.45 with dil. Orthophosphoric acid solution.

Preparation of mobile phase

Mixture of 600 ml of 0.01N KH_2PO_4 buffer (pH-3.5) and 400 ml of Acetonitrile in the ratio of 57:43 v/v 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.

Preparation of mixture Standard stock solution: Accurately weighed 5mg of Olmesartan, 2.5 mg of Cilinidipine and 3.125mg of Chlorthalidone and transferred to three 25ml volumetric flasks separately. 10ml of Diluent was added to flasks and sonicated for 20mins. Flasks were made up with water and methanol (50:50) and labeled as Standard stock solution 1, 2 and 3.

Preparation of Sample (Tablet) stock solutions: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 25mL of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered.

Optimized chromatographic conditions

Column Used :Ascentis C18 150 x 4.6 mm, 2.7µ.
Mobile phase : buffer: Acetonitrile (57:43v/v)
Flow rate : 0.8ml/min
Wavelength : 230.0 nm
Temperature : 30°C
Injection Volume : 10.0µl

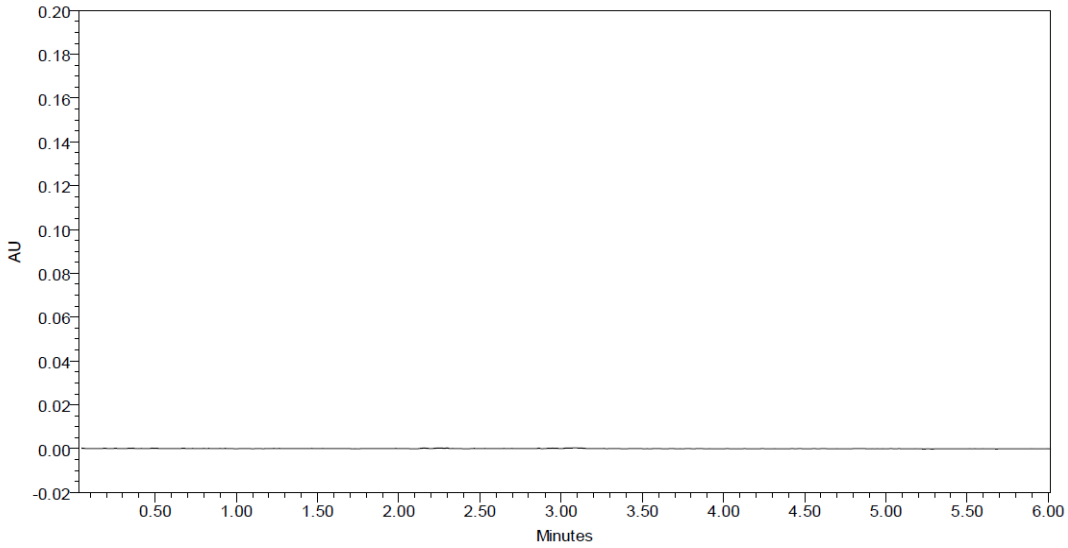

Figure 2: Blank chromatogram.

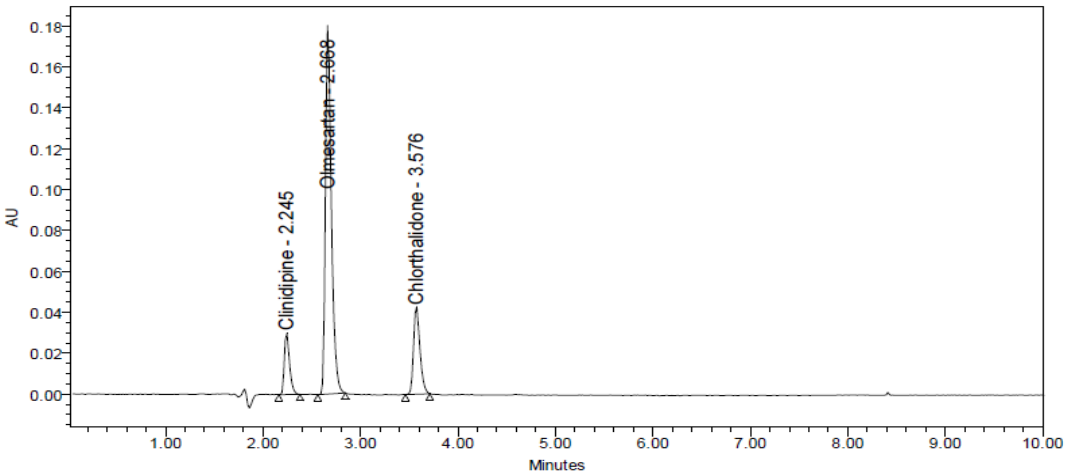

Figure 3: Chromatogram of standard mixture of OLM, CLN & CLR.

	Peak Name	RT	Area	USP Tailing	USP Resolution	USP Plate Count
1	Olmesartan	2.668	2303655	1.57±0.02	5	5447±792
2	Clinidipine	2.245	320565	1.48±0.03	4.9	5691±258.66
3	Chlorthalidone	3.578	538932	1.41±0,03	4.6	9797±813

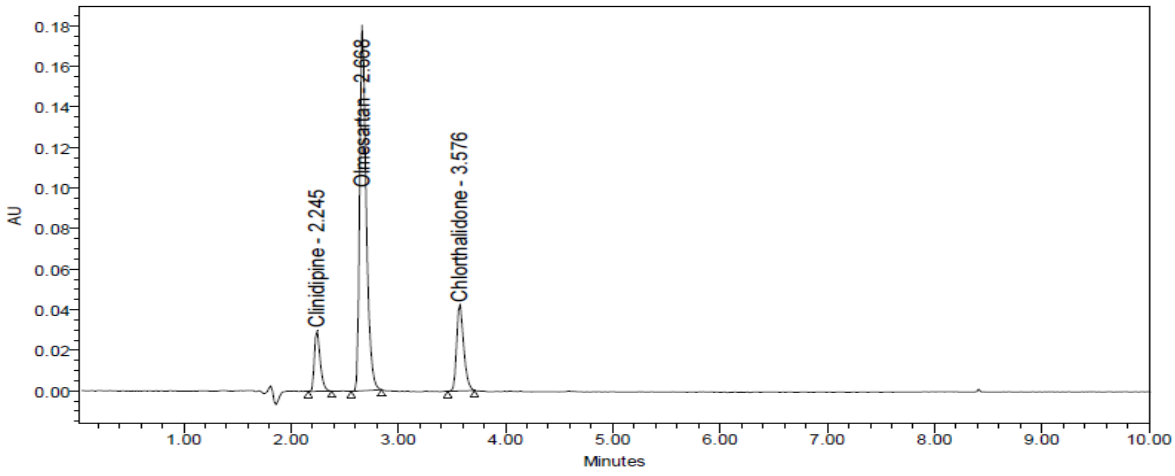

Figure 4: Chromatogram of sample mixture of OLM, CLN & CLR.

VALIDATION

The above optimized chromatographic method has been validated for the assay of OLM, CLN & CLR using the following parameters [International Conference on Harmonization (ICH) 1995]. Linearity was studied to find out the relationship of concentration with Peak area. Six different concentrations of Olmesartan, Clinidipine and Chlorthalidone (OLM, CLN & CLR)drug mixtures respectively. Each concentration of solution was injected into the HPLC and chromatogram was recorded. The calibration graph was constructed by plotting the peak versus the final concentration of the each drug (µg/ml) and the corresponding regression equation derived. Precision was studied to find out variations in the test methods of mixtures of Olmesartan(5mg)+Clonidipine (2.5mg)+chlorthalidone(3.125mg) respectively. The precision of each method was ascertained separately from the peak area by actual determination of five replicates of a fixed amount of Olmesartan(5mg)+ Clonidipine(2.5mg)+ chlorthalidone (3.125mg) respectively. The %RSD (percentage relative standard deviation) was calculated for precision and ruggedness. The accuracy of the method was shown by analyzing the model mixtures containing 80,100 and 120% of Olmesartan, Clinidipine & Chlorthalidone. After the measurement, the Amount found and individual recoveries were calculated. Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated based on the linearity data using the formulae LOD = 3.3×standard deviation /slope; LOQ = 10×standard deviation /slope. Robustness was performed by following the same method with different flow rate.

RESULTS AND DISCUSSION

The regression equation for OLM was found to be y = 23138x+1562 (slope, intercept and correlation coefficient were found to be 23138, 1562 and 0.999 respectively) and linear over beer's range of 25-150 µg/ml. The regression equation for CLN was found to be y = 6442x + 1907 (slope, intercept and correlation coefficient were found to be 6442, 1907 and 0.999 respectively) and linear over beer's range of 12.5-75 µg/ml. The regression equation for CLR was found to be y = 8372x + 3094 (slope, intercept and correlation coefficient were found to be 8372, 3094 and 0.999 respectively) and linear over beer's range of 15.625-93.75 µg/ml. Linearity graph of OLM, CLN & CLR were shown in Figure 5, 6 & 7 respectively. Linearity data was shown in table 1. The precision and ruggedness were determined using the % RSD of the peak area for six replicate preparations of the drug. %RSD of system precision for Olmesartan, Clonidipine and Chlorthalidone were and found to be 1.0, 1.0 and 1.0 respectively. %RSD of method precision for Olmesartan, Clonidipine and Chlorthalidone were and found to be 0.5, 0.2 and 0.6 respectively. % recovery was obtained as 100.15%, 100.09% and 100.01% for Olmesartan, Clonidipine and Chlorthalidone respectively. The calculated RSD values were less than 2. Precision and ruggedness data are presented in Table 2. In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures contained 50%, 100% and 150% of standard solution of drug OLM, CLN & CLR and along with 5 µg/mL of placebo solution within the linearity ranges. The mean percentage recoveries were found to be 100.15%, 100.09% and 100.01% w/w for 50%, 100% and 150% respectively. The results of accuracy were shown that the developed method have a good percentage recovery at different concentrations of drugs. LOD for OLM, CLN and CLR was found to be 0.24 µg/ml, 0.01 µg/ml and 0.01 µg/ml respectively. LOQ for OLM, CLN and CLR was found to be 0.72 µg/ml, 0.02 µg/ml and 0.04 µg/ml respectively. Summary of all the validation parameter shown in table 6.

Degradation

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Conclusion

A simple, accurate, precise method was developed for the simultaneous estimation of the Albuterol and Ipratropium in Tablet dosage form was developed and the proposed method as suitable for routine analysis of OLM, CLN & CLR.

Table 1: Linearity table for OLM, CLN and CLR.

Olmesartan		Clinidipine		Chlorthalidone	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0	0	0
25	606592	12.5	80401	15.625	134136
50	1153196	25	164434	31.25	269631
75	1733061	37.5	247706	46.875	394529
100	2395300	50	325353	62.5	528587
125	2932749	62.5	405236	78.125	653492
150	3437805	75	481471	93.75	788368

Table 2: System precision table of OLM, CLN & CLR.

S. No	Area of Olmesartan	Area of Cliinidipine	Area of Chlorthalidone
1.	2277811	315129	542991
2.	2288486	318395	535162
3.	2292417	321020	531939
4.	2302398	323239	537041
5.	2338012	322942	540812
6.	2322803	322664	545644
Mean	2303655	320565	538932
S.D	22684.5	3214.7	5133.3
%RSD	1.0	1.0	1.0

Table 3: degradation data of OLM.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.92	0.864	0.333
2	Alkali	4.07	0.170	0.337
3	Oxidation	4.43	0.170	0.337
4	Thermal	1.85	0.136	0.354
5	UV	0.48	0.119	0.336
6	Water	0.40	0.145	0.339

Table 4: degradation data of CLN.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	6.08	5.560	0.335
2	Alkali	3.91	0.566	0.332
3	Oxidation	2.69	0.566	0.332
4	Thermal	0.89	0.775	0.355
5	UV	1.86	0.142	0.343
6	Water	0.40	0.918	0.374

Table 5: degradation data of CLR.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	3.25	0.250	0.356
2	Alkali	2.32	0.132	0.325
3	Oxidation	1.80	0.132	0.325
4	Thermal	1.00	0.147	0.316
5	UV	0.52	0.135	0.349
6	Water	0.35	0.146	0.347

Table 6: summary of validation data of OLM, CLN & CLR.

Parameters	Olmesartan	Cliinidipine	Chlorthalidone	LIMIT
Linearity Range (µg/ml)	25-150µg/ml	12.5-75 µg/ml	15.625-93.75µg/ml	R< 1
Regressioncoefficient	0.999	0.999	0.999	
Slope(m)	23139	6442	8372	
Intercept(c)	1562	1907	3094	
Regression equation (Y=mx+c)	y = 23138.x + 1562	y = 6442.x +1907	y = 8372.x + 3094	
Assay (% mean assay)	100.37%	100.23%	100.17%	90-110%
Specificity	Specific	Specific	Specific	No interference of any peak
System precision %RSD	1.0	1.0	1.0	NMT 2.0%
Method precision %RSD	0.5	0.2	0.6	NMT 2.0%
Accuracy % recovery	100.15%	100.09%	100.01%	98-102%

LOD		0.24µg/ml	0.01 µg/ml	0.01 µg/ml	NMT 3 µg/ml
LOQ		0.72 µg/ml	0.02 µg/ml	0.04 µg/ml	NMT 10µg/ml
Robustness	**FM**	0.8	1.2	0.9	%RSD NMT 2.0
	FP0	0.6	1.0	1.1	
	MM	1.1	1.3	0.4	
	MP	0.8	0.2	0.7	
	TM	0.9	0.7	0.6	
	TP	0.2	1.1	1.3	

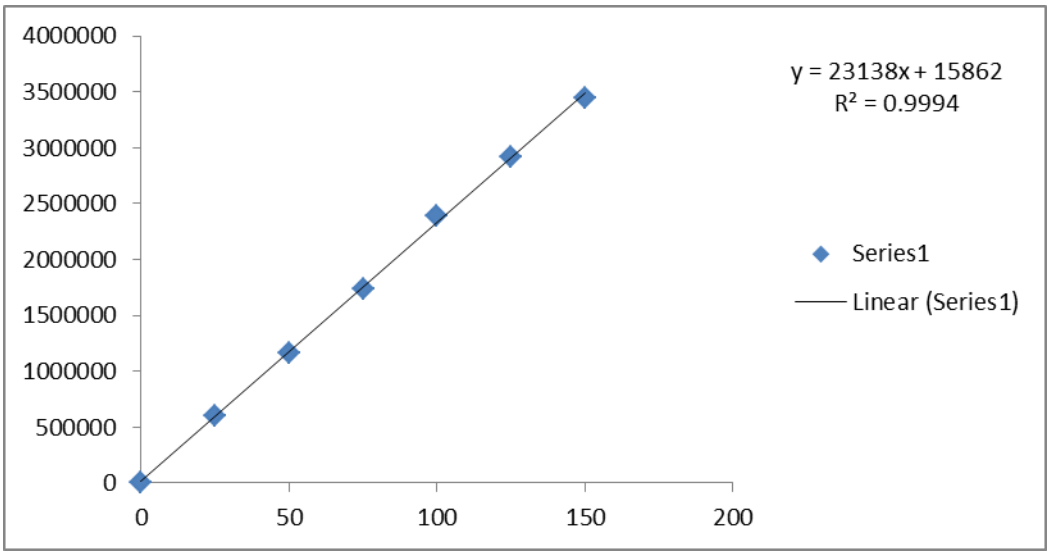

Fig No 7: Linearity curve of Olmesartan.

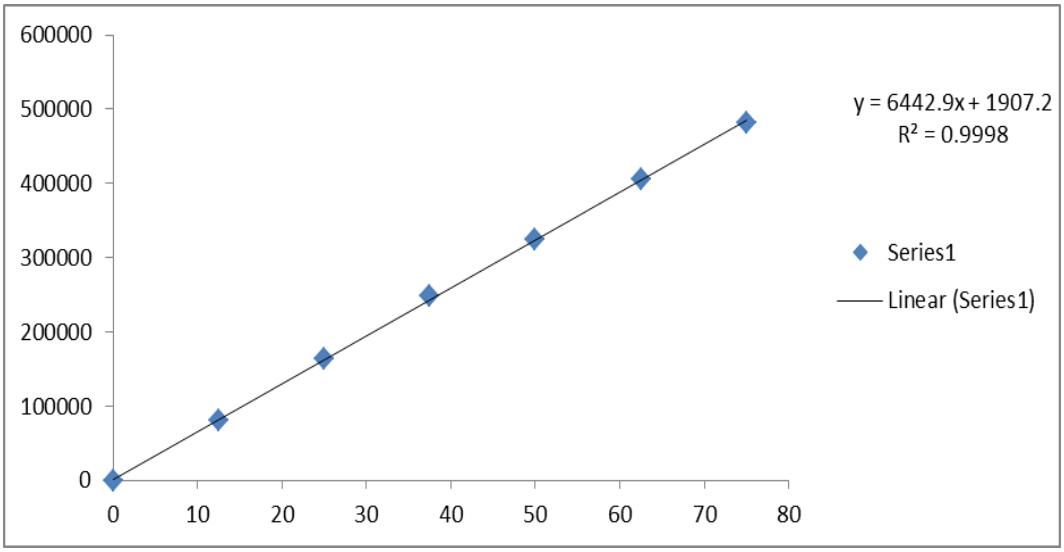

Fig No 8: Linearity curve of Clinidipine.

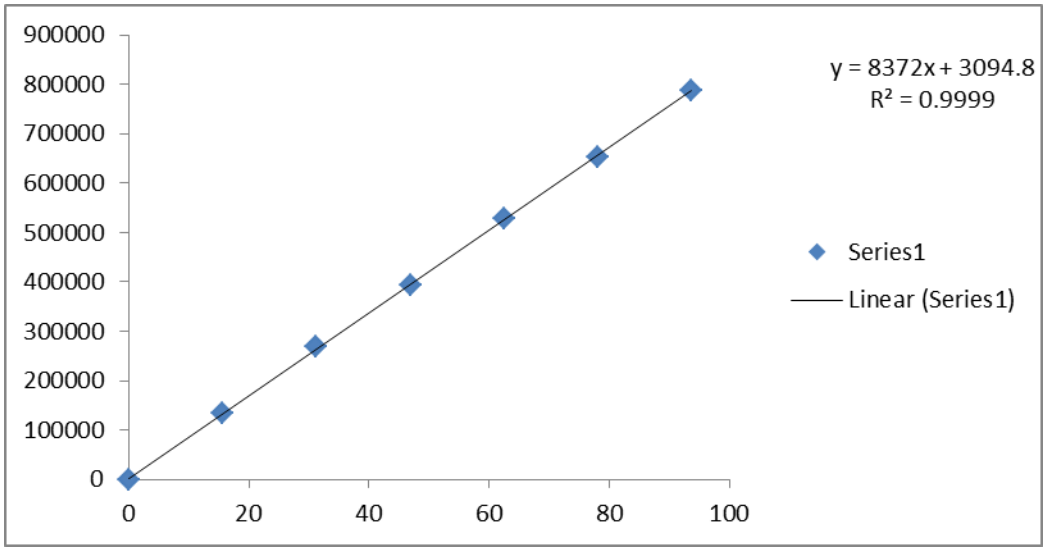

Fig No 9: Linearity curve of Chlorthalidone.

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