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"STABILITY INDICATING RP-HPLC METHOD FOR DETERMINATION OF ATENOLOL AND CHLORTHALIDONE IN PHARMACEUTICAL DOSAGE FORM."

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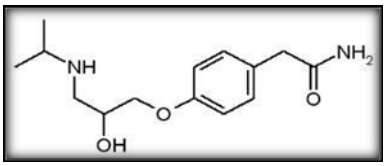
ABSTRACT
A stability-indicating reversed phase liquid chromatographic method for the determination of atenolol and chlorthalidone was developed and validated. The determination was carried out on anusing Sheisedo C_{18} (250* 4.6 mm, 5µm) column and Buffer: Methanol (60:40% v/v) as mobile phase at 1.0 ml/min flow rate. Detection was carried out at 240 nm. R_t was found to be 3.187 min for Atenolol and 5.497min for Chlorthalidone. Atenolol and chlorthalidone showed a linear reshponse in the concentration range of atenolo 12.5-37.5µg/ml And chlorthalidone 3.12-9.37µg/ml. The correlation co-effient for atenolol and chlorthalidone was 0.9999. the developed method was validated with regard to linearity, accuracy, precision, selectivity and robustness and method was found to be precise,accurate,linear and specific.the method was validated as per ICH guidelines.

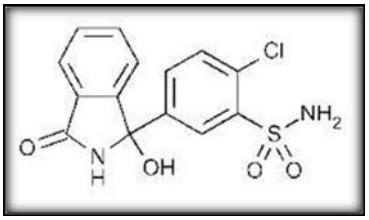
KEYWORDS: atenolol and chlorthalidone, method development, validation, high performance liquid chromatography.

INTRODUCTION
Atenolol Chemically is RS)-2-{4-[2-Hydroxy-3-(propan-2ylamino)propoxy]phenyl} acetamide. It is white to almost white powder used as anti- hypertensive having solubility in methanol and water, sparingly soluble in ethanol. Atenolol is a selective ß1 receptor antagonist, a drug belonging to the group of beta blockers and primarily used in cardiovasculardiseases . Atenolol is one of the most widely used ß-blockers inthe United Kingdom and was once the first-line treatment forhypertension. Hydrochlorothiazide is a diuretic drug of thethiazide class that acts by inhibiting the kidney's ability to retainwater. This reduces the volume of the blood, decreasing bloodreturn to the heart and thus cardiac output and, by othermechanisms, is believed to lower peripheral vascular resistance.



Chemical Structure of Atenolol

chlorthalidone chemically is (RS)-2-chloro-5-(3-Hydroxy-1oxoisoindolin-3-yl) benzenesulphonamide. It is white to yellowish white crystalline and practically odorless. Used as anti-hypertensive having solubility in methanol and insoluble in water, slightly soluble in ethanol. Chlorthalidone is a thiazide-related diuretic, provide longer duration of therapeutic benefit and also control oedema.



Chemical Structure of Chlorthalidone

Experimental and Methods
Materials and reagents
All the chemical used were of Analytical reagent grade, and the solvent were of hplc. Standard sampales of atenolol and chlorthalidone are obtained as gift sample from Umedica and S kant pharmaceuticals Ltd.

Chromatographic conditions
HPLC system Sheisedo-C_{18} (250mm x 4.6mm, 5 µm was used for the study. the HPLC system was equipped with empower software for data processing.isocretic elution was performed using potassium dihydrogen phosphate buffer solution:methanol(60:40v/v,pH 4)with flow rate 1 ml/min.detection was carried out at 240 nm.

Preparation of standard stock solution

Standard Stock I Solution of Atenolol (1000µg/ml): 100mg of Atenolol weighed and transferred to 100mL volumetric flask and dissolved in Methanol and solicited for about 10min. Volume was made up to the mark with Methanol to give a solution containing 1000µg/mL Atenolol solution.

Standard Stock I Solution of Chlorthalidone (1000µg/ml):100mg of Chlorthalidone was accuratelyweighed and transferred to 100mL volumetric flask and dissolved in Methanol and sonicted for about 10min. Volume was made up to the mark with Methanol to give a solution containing 1000µg/mol Chlorthalidone solution.

Standard Stock II Solution of Atenolol(250 µg/ml): 25 mL of Standard stock Solution I was transferred in 100mL volumetric flask and Volume was made up to the mark with Methanol to give a solution containing 250µg/mL Atenolol solution.

Standard Stock II Solution of Chlorthalidone(62.5 µg/ml): 6.25 mL of Standard stock Solution I was transferred in 100mL volumetric flask and volume was made up to the mark with methanol to give a solution containing 62.5µg/mL Chlorthalidone solution.

Selection of Detection Wavelength

- **Working Standard solution of Atenolol (25µg/mL):** 1mL of standard Stock Solution II was transferredin 10mL volumetric flask and Volume was made up to the mark with Methanol to give a solution containing 25µg/mL Atenolol solution.
- **Standard solution Chlorthalidone (6.25µg/mL):** 1mL of Standard Stock Solution II was transferredin 10mL volumetric flask and Volume was made up to the mark with Methanol to give a solution containing 6.25µg/mL Chlorthalidone.

Sample stock solution

20 Tablets were weighed and powdered. Powder equivalent to 25mg of Atenolol and 6.25mg of Chlorthalidone was weighed and transferred to 100mL volumetric flask. And 60mL of diluents was added to it and was sonicated for 10minutes and then volume was made up to the mark with diluent to give a solution containing 250µg/mL Atenolol and 6.25µg/mL Chlorthalidone Solution. Chromatogram is shown.Calibration Curve of both the drugs.

Selectivity

The specificity of the method was checked for the interference of retention time of a blank solution (without any sample) and then a drug solution of 25µg/mL of Atenolol and 6.25µg/mL of Chlorthalidone was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Atenolol and Chlorthalidone.

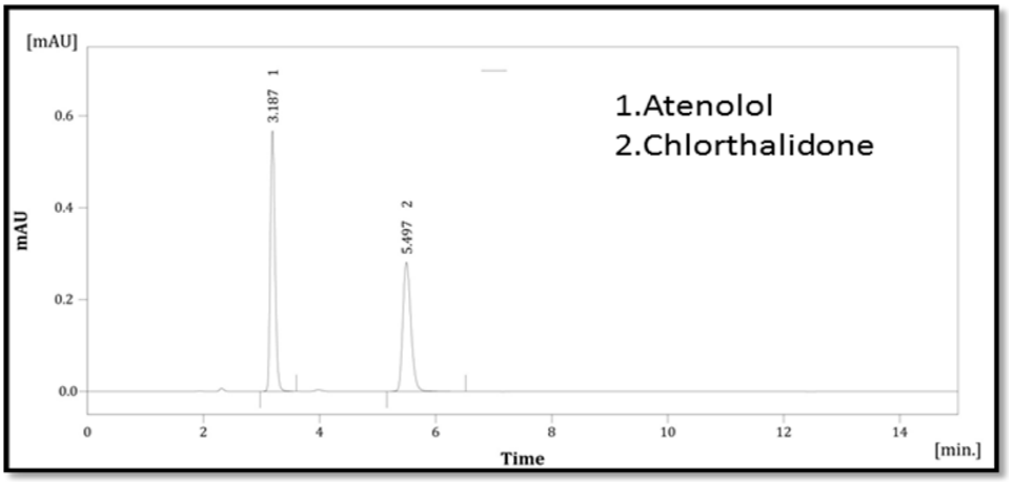


Figure No.1: A chromatogram of the Atenolol and Chlorthalidone.

RESULT AND DUISCUSSION

Method Development

Selection of mobile Phase: According to the solubility of the drugs in different solvents, the mobile phase is selected.

Preparation of Working Standard Solution

1mL of Atenolol Standard Stock Solution II and 1mL of Chlorthalidone Standard Stock Solution II was transferred in 10mL volumetric flask. Volume was made up to the mark with Mobile Phase used for trials to give a solution containing 25µg/mL Atenolol and6.25µg/mL of Chlorthalidone solution.

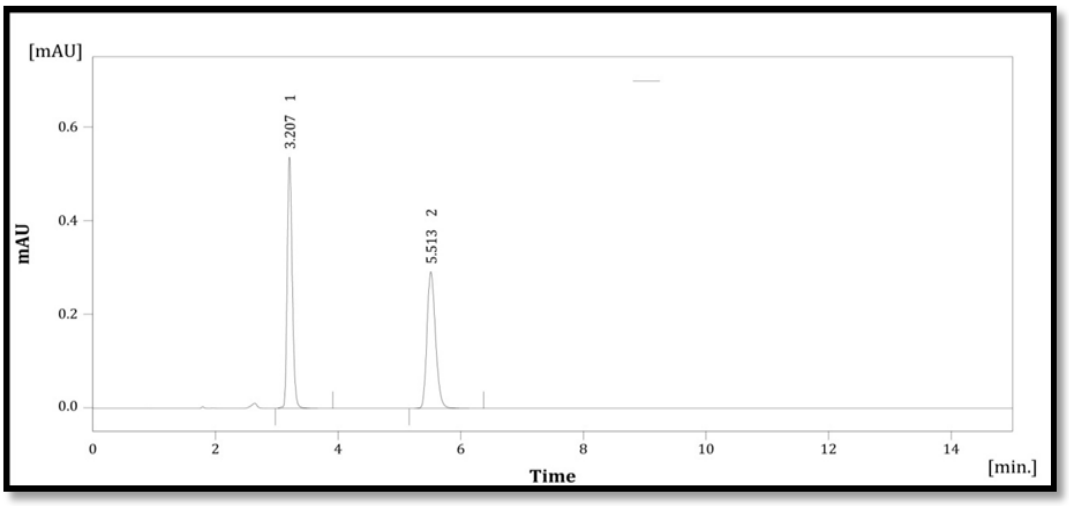


Figure No.2: Chromatogram of Standard Sample.

System Suitability Parameters

Table No.1: System Suitability Parameters Atenolol and Chlorthalidone.

Factor	ATENOLOL	CHLORTHALIDONE
Conc (µg/ml)	25 µg/ml	6.25 µg/ml
R_t (min)	3.346	5.589
Resolution	11.15	
Theoratical plate number	6870	7206
Tailing factor	1.370	1.381

Method Validation

Forced Degradation Studies

Acid Degradation Blank

2mL of 0.1N HCl was transferred to 10mL volumetric flask and then 2mL of 0.1N NaOH was added for neutralization and diluted up to the mark with Mobile Phase. Chromatogram of blank acid Hydrolysis is shown.

Atenolol, Chlorthalidone and Formulation Acid Degradation

1mL of Standard Stock Solution II of Atenolol, Chlorthalidoneand Sample Stock Solution were transferred in three different 10mL volumetric flask; to it 2mL of 0.1NHCl was added and kept for 4hrs and then 2mL of 0.1N NaOH was added for neutralization and diluted up to the mark with Mobile Phase. Chromatogram of Atenolol, Chlorthalidoneand Formulation under Acid Hydrolysis is shown.

Alkaline Hydrolysis

Alkaline Degradation Blank

2mL of 0.1N NaOH was transferred to 10mL volumetric flask and then 2mL of 0.1N HCl was added for neutralization and diluted up to the mark with Mobile Phase. Chromatogram of blank alkaline Hydrolysis is shown.

Atenolol, Chlorthalidone and Formulation Acid Degradation

1mL of Standard Stock Solution II of Atenolol, Chlorthalidoneand Sample Stock Solution were transferred in three different 10mL volumetric flask; to it 2mL of 0.1N NaOH was added and kept for 4hrs and then 2mL of 0.1N HCl was added for neutralization and diluted up to the mark with Mobile Phase. Chromatogram of Atenolol, Chlorthalidoneand Formulation under Alkaline Hydrolysis.

Oxidative Degradation

Oxidative Degradation Blank

2mL 3% $H_2O_2$was transferred to 10mL volumetric flask and then diluted up to the mark with Mobile Phase. Chromatogram of blank oxidative degradation.

Atenolol, Chlorthalidone and Formulation Oxidative Degradation: 1mL of Standard Stock Solution II of Atenolol, Chlorthalidoneand Sample Solution were transferred in three different 10mL volumetric flask; to it 2mL of 3% $H_2O_2$was was added and kept for 3hrs and then diluted up to the mark with Mobile Phase. Chromatogram of Atenolol, Chlorthalidoneand Formulation under Oxidative degradation.

Thermal degradation

Thermal Degradation Blank

2mL of Mobile Phase was transferred in 10mLvolumetric flask and was heated at 80ºC in oven for 1hrsthen diluted up to the mark with Mobile Phase. Chromatogram of Blank Thermal Degradation is shown.

Atenolol, Chlorthalidone and Formulation Thermal Degradation

1mL of Standard Stock Solution II of Atenolol, Chlorthalidoneand Sample Solution were transferred in

three different 10mL volumetric flask was heated at 80ºC in oven kept for 5hrs and then diluted up to the mark with Mobile Phase. Chromatogram of Atenolol, Chlorthalidoneand Formulation under Oxidative degradation.

Table No. 2: Summary of Stability indicating RP – HPLC Method.

Stress Condition	% Degradation of API		% Degradation of pharmaceutical dosage form	
	ATNO	CHLO	ATNO	CHLO
Acid Hydrolysis	8.14	7.85	8.13	7.78
Alkaline Hydrolysis	7.80	6.74	6.50	6.92
Oxidative	9.95	9.98	10.23	10.15
Thermal	6.89	6.24	6.92	6.75

RESULT AND DISCUSSION

System Suitability

System suitability tests are an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. System Suitability was performed on standard solution and system suitability parameters were calculated at the start of study for each parameter. The test includes Parameters like Number of Theoretical Plates, Resolution, Retention time and tailing factor.

Linearity and Range

The linearity was determined at three levels over the range of 12.5-37.5 µg/ml Atenolol and 3.12-9.37 µg/ml Chlorthalidone. Peak area of above linearity solution preparations were taken at each concentration three times. Mean Peak Area at each concentration was calculated and Graph of Mean Peak Area (yaxis) versus Concentration (x-axis) was plotted.

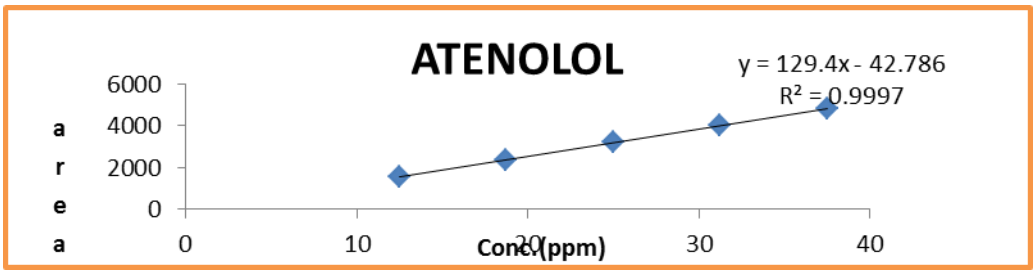


Figure No.3: Linearity Curve of Atenolol.

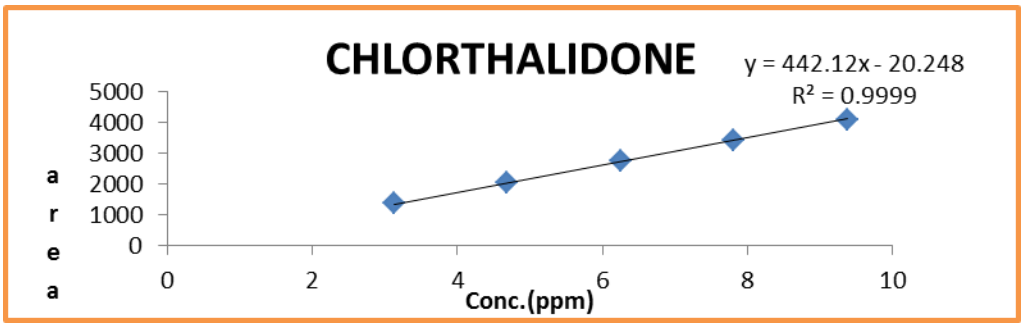


Figure No.4: Linearity Curve of Chlorthalidone.

Table No.3: Linearity for Atenolol

ATENOLOL (n=5)		
Conc. (µg/ml)	**Mean. Area±S.D**	**% RSD**
12.5	1591.735±0.7342	0.046128
18.75	2384.063±2.3465	0.098428
25	3204.18±0.5718	0.01784
31.25	4014.615±0.5481	0.01365
37.5	4802.111±0.544	0.011329

Table No.4:Linearity for Chlorthalidone.

CHLORTHALIDONE(n=5)		
Conc. (µg/ml)	**Mean. Area±S.D**	**% RSD**
3.125	1363.0403±0.5501	0.021886
4.6875	2043.801±0.4473	0.021886
6.25	2745.644±0.54875	0.019986
7.8125	3447.223±0.71631	0.020779
9.375	4115.435±0.8572	0.02083

Accuracy

Recovery studies was carried out by addition of standard drug solution to the sample solution at 3 different concentration levels (80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and results are calculated.

Table No.5: Accuracy of Atenolol.

% ADDED	TARGET CONC. (µg/ml)	SPIKED CONC. (µg/ml)	FINAL CONC. (µg/ml)	CONC. OBTAINED	% RECOVERY	SD	%RSD
80%	12.5	10	22.5	10.086	100.861	0.627	0.617
	12.5	10	22.5	10.185	101.854		
	12.5	10	22.5	10.202	102.020		
100%	12.5	12.5	25	12.584	100.668	0.706	0.702
	12.5	12.5	25	12.656	101.274		
	12.5	12.5	25	12.480	99.842		
120%	12.5	15	27.5	14.984	99.891	0.816	0.819
	12.5	15	27.5	14.822	98.816		
	12.5	15	27.5	15.063	100.418		

Table No.6: Accuracy of Chlorthalidone.

% ADDED	TARGET CONC. (µg/ml)	SPIKED CONC. (µg/ml)	FINAL CONC. (µg/ml)	CONC. OBTAINED	% RECOVERY	SD	%RSD
80%	3.125	2.5	5.62	2.524	100.955	1.165	1.153
	3.125	2.5	5.62	2.496	99.839		
	3.125	2.5	5.62	2.554	102.168		
100%	3.125	3.125	6.25	3.148	100.733	0.518	0.514
	3.125	3.125	6.25	3.167	101.342		
	3.125	3.125	6.25	3.135	100.310		
120%	3.125	3.75	6.87	3.748	99.940	1.527	1.537
	3.125	3.75	6.87	3.660	97.612		
	3.125	3.75	6.87	3.768	100.486		

Precision

Repeatability Study

Standard solutions of 25 µg/ml Atenolol and 6.25 µg/ml Chlorthalidone were prepared and chromatograms were recorded. Area was measured of the same concentration solution six times and %RSD was calculated.

Intra-day precision

Mixed solutions containing 50,100,150 µg/ml Atenolol and 50,100,150 µg/ml Chlorthalidone were analysed three times on the same day and % RSD was calculated.

Inter-day precision

Mixed solutions containing 50,100,150µg/ml Atenolol and 50,100,150µg/ml Chlorthalidone were analysed on three different days and % RSD was calculated.

Table No.7: Repeatability Study of Atenolol & Chlorthalidone.

Atenolol			Chlothalidone		
Conc. (µg/ml)	**Area Mean ± S.D. (n=6)**	**% RSD**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=6)**	**% RSD**
25	3256.061667±29.8327	0.916221	6.25	2789.485±27.81213	0.997035

Intra-Day Precision

Table No.8: Intra-day precision of Atenolol & Chlorthalidone.

Atenolol			Chlothalidone		
Conc. (µg/ml)	**Area Mean ± S.D. (n=3)**	**%RSD**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**%RSD**
50	1611.9743±9.63500	0.5977	50	1378.3403±6.1681	0.4475
100	3197.4453±9.74115	0.3046	100	2736.058±3.0491	0.1114
150	4772.1583±9.9702	0.2090	150	4084.029±15.9358	0.3901

Inter-Day Precision

Table No.9:Inter-day Precision of Atenolol & Chlorthalidone.

Atenolol			Chlothalidone		
Conc. (µg/ml)	**Area Mean ± S.D. (n=3)**	**%RSD**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**%RSD**
50	1586.522±7.13417	0.4496	50	1362.019±4.69807	0.3449
100	3194.071±14.8960	0.4663	100	2732.585±17.838	0.6528
150	4783.032±17.4833	0.3655	150	4102.735±14.574	0.3552

Limit of detection (LOD) and limit of Quantitation (LOQ)

Limit of Detection (LOD)

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The limit of detection (LOD) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline: LOD = 3.3× Intercept / Slope.

Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline.

LOQ = 10 × Intercept / Slope.

Table No.10: LOD and LOQ of Atenolol and Chlorthalidone.

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Atenolol	0.02421	0.07338
Chlothalidone	0.00465	0.01411

Robustness

The robustness of the method was established by making deliberate minorvariations in the following method parameters

a) pH of mobile phase:±0.2

b) Flow rate: ±0.2 ml/min

c) Change in the ratio of component in the mobile phase: ± 2%.

Table No.11: Robustness of Atenolol.

Sr. no.	Atenolol (50 µg/ml)					
	pH		Flow rate		Mobile phase	
	+ 0.2 units	**-0.2 units**	**+0.2 units**	**-0.2 units**	**+2.0 %**	**-2.0 %**
1	3090.801	3264.984	3125.226	3249.347	3153.824	3217.571
2	3109.38	3284.602	3118.955	3262.399	3157.468	3240.139
3	3093.793	3278.011	3140.691	3272.25	3163.273	3259.627
Mean	3097.991	3275.866	3128.291	3261.332	3158.188	3239.112
S.D	9.975686	9.983402	11.18738	11.48872	4.765507	21.04679
% R.S.D	0.322005	0.304756	0.35762	0.352271	0.150894	0.64977

Table No.12: Robustness of Chlorthalidone.

Sr. no.	Chlorthalidone (12.5 µg/ml)					
	pH		Flow rate		Mobile phase	
	+ 0.2 units	**-0.2 units**	**+0.2 units**	**-0.2 units**	**+2.0 %**	**-2.0 %**
1	2648.52	2797.713	2678.018	2784.462	2702.595	2757.152
2	2643.22	2794.795	2672.578	2774.305	2718.89	2776.511
3	2651.05	2808.844	2670.73	2803.915	2710.618	2784.962
Mean	2647.597	2800.451	2673.775	2787.561	2710.701	2772.875
S.D	3.995827	7.413819	3.78866	15.04624	8.147817	14.25708
% R.S.D	0.150923	0.267437	0.141697	0.539764	0.30058	0.514162

CONCLUSION

- A simple, accurate and precise RP-HPLC method of Atenolol and Chlorthalidone in Pharmaceutical dosage form has been developed and validated.Separation of drugs was carried out using Buffer(ph 4) : Methanol mobile phase at 15 min run time and 240 nm.
- It is concluded that the developed method is specific. The test parameters were also performed and were found to be within acceptable criteria. The method can be successfully employed for the stability determination of Atenolol and Chlorthalidone in pharmaceutical formulation.

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