A Simple HPTLC Method for Simultaneous Estimation of Atenolol and Chlorthalidone in Pharmaceuticals

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Abstract

A new simple, precise, and accurate, high-performance thin-layer chromatography method was developed for the simultaneous determination of Atenolol and Chlorthalidone in tablet dosage forms. In this method, TLC aluminum plates pre-coated with silica gel 60 F254used as the stationary phase. The mobile phase consisting of Methylene Chloride: methanol: Ammonia solution 25%, in the ratio of 8.8:1.3:0.1 v/v is used. The densitometry evaluation was done at wavelength 266 nm using Camag scanner III. The developed method shows a well resolution between two peaks Atenolol and Chlorthalidone with Rf values 0.115 (±0.03) and 0.458(±0.03), respectively. The proposed method was validated as per International Conference on Harmonization (ICH) guidelines for parameters such as specificity, precision, accuracy, linearity, and ruggedness and reported the values. The linear regression analysis shows that the correlation coefficient R2 value for Atenolol is 0.999 and 0.997, respectively. The mean percentage recovery values obtained for Atenolol is 101.4 and for Chlorthalidone is 98.4. The statistical analysis shows that this method is suitable for routine analysis for quantification of Atenolol and Chlorthalidone in single or combined dosage forms of formulations.

Keywords: Atenolol, Chlorthalidone, High-performance thin-layer chromatography (HPTLC), densitometry, Validation.

Introduction

Atenolol and Chlorthalidone tablets are used to treat hypertension to lower the blood pressure and to minimize the risk of stroke of heart attack. Atenolol is beta blocker which affects the heart and maintains the circulation of blood. The chemical name is 4-[2'-hydroxy3'-[(1-methylethyl)amino]propoxy]-benzeneacetamide and molecular structure is $C_{14}H_{22}N_2O_3$ and molecular weight is 266.3 [1-4]. Chlorthalidone is diuretic and causes the body to get rid of extra salt and water which helps to relax the blood vessels. The chemical name is 1-oxo-3-(3-sulfamyl-4-chlorophenyl)-3-hydroxyisoindoline.

The molecular formula is $C_{14}H_{11}ClN_2O_4S$ and molecular weight is 338.7. The Chemical structure for Atenolol and Chlorthalidone are shown in below fig. 1 and fig.2 respectively. [5-13]. Atenolol and Chlorthalidone combination is prescribed as an oral dosage form as tablets and has been determined via well know analytical methods such as HPLC and UV Spectrometry[14-19]. However there is no any HPTLC method for quantification of Atenolol and chlothalidone in combined dosage form. The quantitative estimation of Atenolol and chlothalidone is done by using HPTLC technique.

The mobile phase used is Methylene Chloride: methanol: Ammonia solution 25%, in the ratio of 8.8:1.3:0.1 v/v. The TLC Plate: Merck, HPTLC plates silica gel 60F 254 is used. the Camag Automatic sampler-4 Applicator, TLC Scanner III, Twin trough Chamber TTC, 20cm x 10 cm, Camag, Muttenz, Switzerland, Camag Scanner III operated Server vision CATS-version 2.5.18053.1 used in this study. The developed HPTLC method has been validated as per ICH guidelines. [20]

After Literature review it is observed that there is no HPTLC method available for estimation and quantification of Atenolol and Chlorthalidone in combined dosage forms.

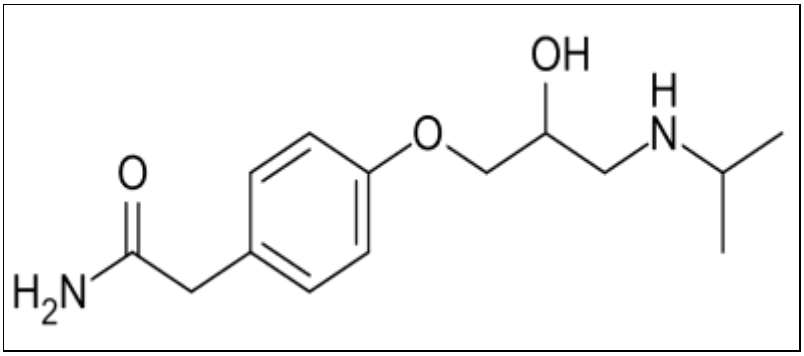

Fig.1. Chemical Structure of Atenolol

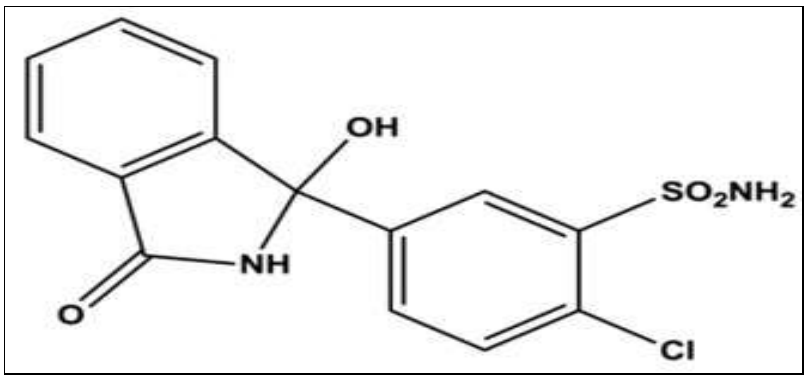

Fig.2. Chemical Structure of Chlorthalidone

Materials and Methods

The solvent and reagents, Methylene Chloride (Analytical Grade), Methanol (HPLC Grade) and Ammonia Solution (25%),that was used to dissolve the Drug/ API (Active Pharmaceutical Ingredient) and in the preparation of mobile phase was purchased from Merck Specialities Ltd. Mumbai, India. The Tenoric-100 (Atenolol 100mg and Chlorthalidone 25mg Tablets) are purchased from market.

Instrumentation

The estimation and quantification of Atenolol and Chlorthalidone tablets was done by using Camag Autosampler-4 Applicator (Muttenz, Switzerland) equipped with twin trough chamber TTC, 20cm x 10 cm is used in the study. The pre-coated silica gel 60 F254 TLC Plates of Merck (Darmstadt, Germany) of 10 cm x 10cm, 0.2mm thickness layer used stationary phase. Camag Scanner III operated Server vision CATS-version 2.5.18053.1 used in this study. Microsyringe (Linomat syringe, Hamilton-Bonaduz Schweiz), UV chamber (Muttenz, Switzerland) were used in this study.

Preparation of mobile phase

The mobile phase used is Methylene Chloride: methanol: Ammonia solution 25%, in the ratio of 8.8:1.3:0.1 v/v/v.

Preparation of Standard Solution (Atenolol)

Prepared standard solution by weighing about 10mg of Atenolol and dissolved in 10mL of methanol and further diluted with methanol to get working concentration of standard solution, 1mg/mL of Atenolol.

Preparation of Standard Solution (Chlorthalidone)

Prepared standard solution by weighing about 25mg of Atenolol and dissolved in 10mL of methanol and further diluted with methanol to get working concentration of standard solution, 0.25mg/mL of Chlorhalidone.

Preparation of Sample Solution

Weighed and crushed 20 tablets into fine powder and transferred sample powder equivalent to 500mg of Atenolol into 100mL volumetric flask, added 60 mL of methanol, sonicated for 20 minutes to get dissolve the material with vigorous intermittent shaking for every 2-3 minutes of interval. Cool to attain the room temperature for 10 minutes. Diluted upto mark with methanol. Filtered through Whatmann filter no.1. Further diluted filtered solution to achieve the concentration of working sample solution having 1mg/mL of Atenolol and 0.25mg/mL of Chlorthalidone.

Method Development and Optimization

Initial experiments are conducted on Methylene chloride and methanol in the ratio of 8:2 (v/v), due to this dragging of peaks observed. Hence ammonium solution (25%) was incorporated with different composition and different ratios. The mobile phase consisting methylene chloride: methanol: ammonia solution (25%), 8.8:1.3:0.1 (v/v/v) was used. A suitable volume of standard solution and sample solutions (µl) were spotted on HPTLC plate 10 mm from bottom and 10mm from side edges. Ascending development technique was used in twin trough chambers.

The chamber was saturated with mobile phase for 20 minutes at room temperature at 25°C.The distance covered by solvent front took about 8 cm which took about 15 minutes. The spots are then evaluated by using Camag scanner III at wavelength 266 nm and operated Server vision CATS-version 2.5.18053.1. The quantification and estimation of components done by using intensity of reflected light and peak area responses are used for calculation and evaluation. The TLC procedure was developed and optimized for simultaneous estimation of Atenolol and Chlorthalidone.

The mobile phase methylene chloride: methanol: ammonia solution (25%), 8.8:1.3:0.1 (v/v/v) shows good resolution and sharp and symmetrical peaks of Rf 0.115± 0.02 for Atenolol and 0.458 ± 0.02 for Chlorthalidone. Prewashing of plates are done by using methanol and dried and activated. The plates are saturated for 20 minutes to ensure improved the reproducibility and peak shape for both active components. The developed method was validated and used for determination of Atenolol and Chlorthalidone in formulation.

Method Validation

Specificity

The specificity of the method was produced by analysing blank, sample solution and reference solution. There was good resolution between the two peaks of Atenolol and Chlorthalidone and the identification of bands for both the peaks was confirmed by comparing the R_f values and spectra of bands with those of standards. The typical reference densitograms of Atenolol and Chlorthalidone tablet are shown in Fig.3 and 5. Their values are described in Table 1.

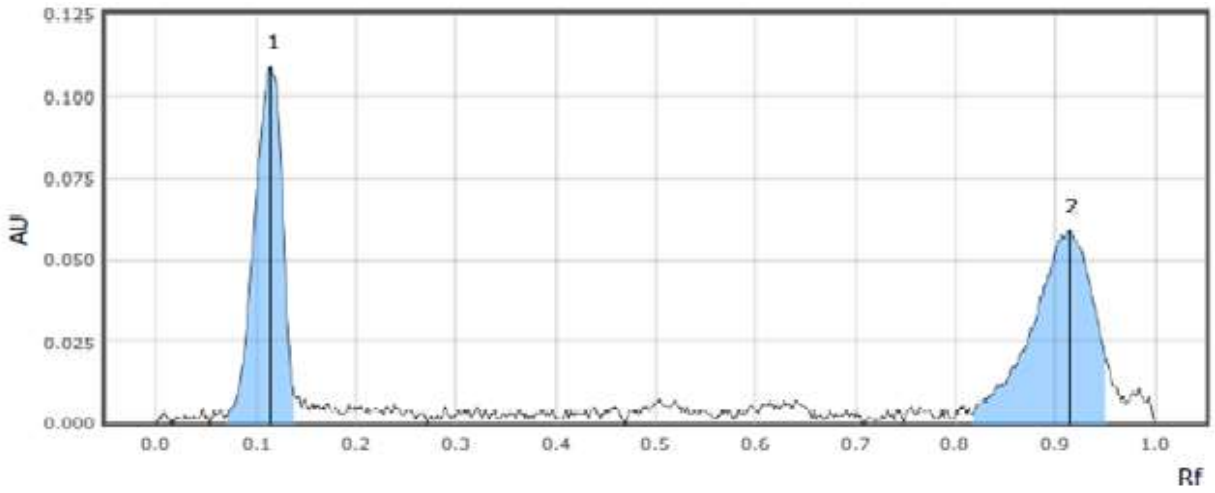

Fig. 3: Reference solution of Atenolol 1mg/mL

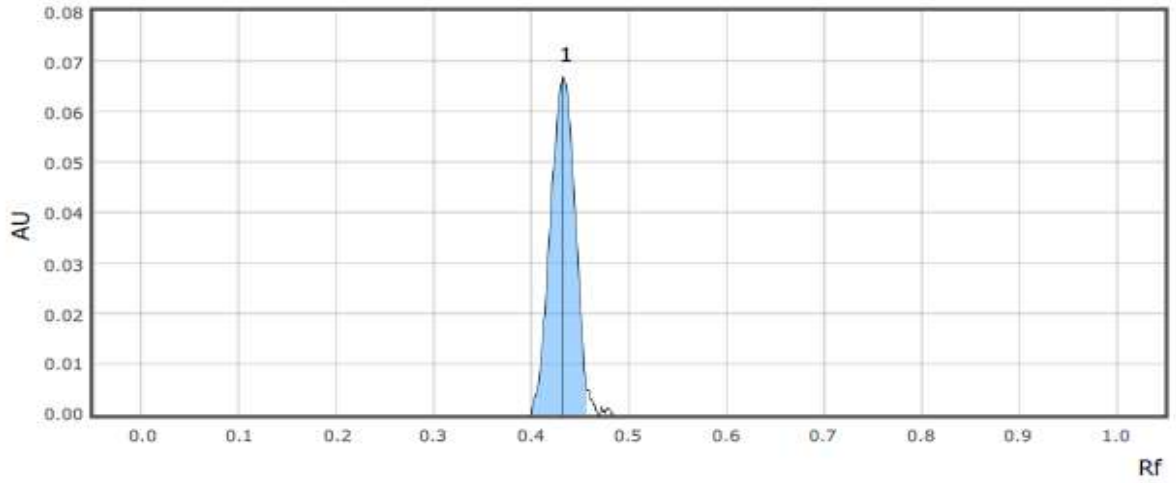

Fig. 4: Reference solution of Chlorthalidone 1mg/mL

Table 1: Specificity data of Atenolol and Chlorthalidone

Name	**Atenolol (Observed Rf)**	**Chlorthalidone (Observed Rf)**
Reference Solution	0.115	0.458
Blank	ND	ND
Mobile Phase	ND	ND

ND=Not Detected

Precision

The Precision of the method was expressed in terms of variation usually Percentage Relative Standard Deviation (%RSD). The intra-day precision and inter day precision was performed. Injection volume (2µl) was spotted of prescribed concentration of Atenolol and Chlorthalidone in the table 2, 3, 4 &5.

Table 2: Intra-day precision for Atenolol

Recovery level	Concentration mg/mL	Area Observed at each spiked level (Area X 1000000)	Mean Std. area observed (Area X 1000000)	% Atenolol	Mean	SD	% RSD
50%	0.50	0.00183132	0.00188559	97.1	96.1	0.88	0.91
50%	0.50	0.00180001		95.5			
50%	0.50	0.00180633		95.8			
100%	1.00	0.00335201	0.00339924	98.6	99.6	0.89	0.89
100%	1.00	0.00339709		99.9			
100%	1.00	0.00340909		100.3			
150%	1.50	0.00500158	0.00515544	97.0	97.8	0.64	0.66
150%	1.50	0.00505944		98.1			
150%	1.50	0.00505840		98.1			

Table 3: Intra-precision for Chlorthalidone

Recovery level	concentration mg/mL	Area Observed at each spiked level (Area X 1000000)	Mean Std. area observed (Area X 1000000)	% Chlorthalidone	Mean	SD	% RSD
50%	0.125	0.00372301	0.00371633	100.2	99.9	0.28	0.28
50%	0.125	0.00370260		99.6			
50%	0.125	0.00370894		99.8			
100%	0.250	0.00709381	0.00718807	98.7	97.3	1.40	1.44
100%	0.250	0.00689192		95.9			
100%	0.250	0.00699191		97.3			
150%	0.375	0.01023653	0.0105803	96.8	96.1	0.59	0.62
150%	0.375	0.01011603		95.6			
150%	0.375	0.01014519		95.9			

Table 4: Inter-day precision for Atenolol

Recovery level	Concentration mg/mL	Area Observed at each spiked level (Area x 10^6)	Mean Std. area observed (Area x 10^6)	% Atenolol	Mean	SD	% RSD
50%	0.500	0.001678256		97.6			
50%	0.500	0.00166095	0.00171952	96.6	97.3	0.67	0.68
50%	0.500	0.001682594		97.9			
100%	1.000	0.00338971		97.1			
100%	1.000	0.00339541	0.00349124	97.3	97.4	0.44	0.45
100%	1.000	0.00341852		97.9			
150%	1.500	0.004863063		98.7			
150%	1.500	0.004761900	0.00492820	96.6	98.0	1.20	1.22
150%	1.500	0.004865013		98.7			

Table 5: Inter-day precision for Chlorthalidone

Recovery level	Concentration mg/mL	Area Observed at each spiked level (Area x 10^6)	Mean Std. area observed (Area x 10^6)	% Chlorthalidone	Mean	SD	% RSD
50%	0.125	0.00349139		95.6			
50%	0.125	0.00350660	0.00365353	96.0	96.3	0.94	0.98
50%	0.125	0.00355703		97.4			
100%	0.250	0.00659521		96.8			
100%	0.250	0.00657241	0.00681256	96.5	97.2	0.93	0.96
100%	0.250	0.00669191		98.2			
150%	0.375	0.00961153		97.8			
150%	0.375	0.00949103	0.00983030	96.5	97.5	0.82	0.85
150%	0.375	0.00964519		98.1			

System Precision

The system precision was performed by analysing the Standard solution of Atenolol and Chlorthalidone and the reproducibility was confirmed by calculating the mean area response, standard deviation and percentage relative standard deviation (% RSD). The %RSD obtained for six replication of Atenolol and Chlorthalidone are 1.84 & 1.38 respectively.

Recovery

The accuracy of the developed method was performed by spiking known amounts of standards into placebo at 80%, 100% and 120% levels in triplicate. The amount of drug was calculated based on the amount of drug added and amount of drug found. The percentage recovery obtained for both the active was in between 95.0 to 105.0. The recovery results for Atenolol and Chlorthalidone are as shown below in table 6 and 7 respectively.

Table 6: Recovery for Atenolol

Recovery for Atenolol					
Recovery Level	**Concentration mg/mL**	**Area Observed (Area x 10^6)**	**Mean Area Observed (Area x 10^6)**	**Mean Std. Area Observed (Area x 10^6)**	**% Recovery**
80%	0.80	0.00291517	0.00286664	0.002832694	101.2
80%	0.80	0.00283043			
80%	0.80	0.00285432			
100%	1.00	0.00339334	0.003469173	0.003449723	100.6
100%	1.00	0.00350709			
100%	1.00	0.00350709			
120%	1.20	0.00410986	0.00417101	0.004073838	102.4
120%	1.20	0.00434656			
120%	1.20	0.00405661			
				Mean	**101.4**

Table 7: Recovery for Chlorthalidone

Recovery for Chlorthalidone					
Recovery level	**Concentration mg/mL**	**Area Observed (Area x 10^6)**	**Mean Area Observed (Area x 10^6)**	**Mean Std. Area Observed (Area x 10^6)**	**% Recovery**
80%	0.20	0.00603675	0.006015073	0.005896492	102.0
80%	0.20	0.00600416			
80%	0.20	0.00600431			
100%	0.25	0.00709373	0.007052403	0.007190783	98.1
100%	0.25	0.0070115			
100%	0.25	0.00705198			
120%	0.30	0.00792926	0.008062887	0.008464155	95.3
120%	0.30	0.00815237			
120%	0.30	0.00810703			
				Mean	**98.4**

Linearity

The Linear regression data for the calibration plots revealed good linear relationships between the responses and the injection volume for Atenolol and Chlorthalidone. The correlation coefficient obtained for Atenolol and Chlorthalidone are 0.999 and 0.9968 respectively. The linearity plots are shown in below fig.4 and fig.5. The values are described in below table 8 and table 9.

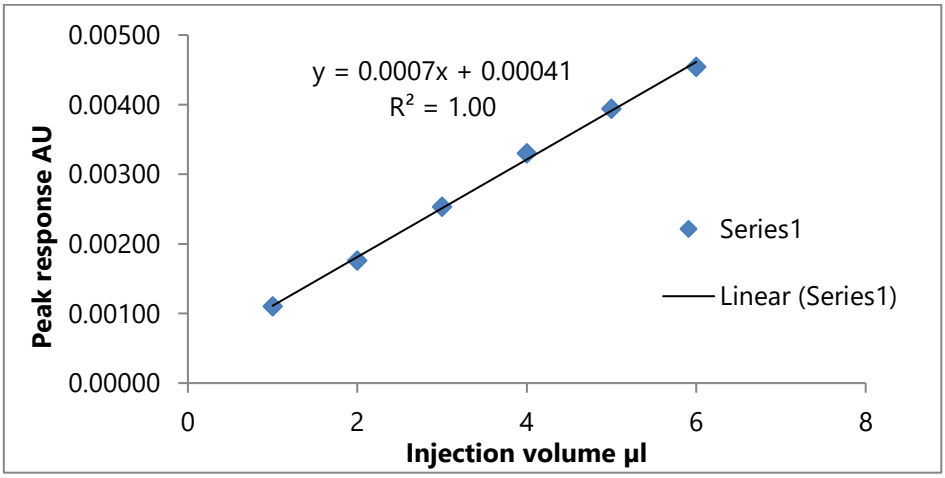

Fig. 5: Linearity of Atenolol

Table 8: Linearity of Atenolol

Linearity Level (%)	Injection Volume (µL)	Area observed
1	1	0.00110
2	2	0.00176
3	3	0.00253
4	4	0.00330
5	5	0.00394
6	6	0.00454
Intercept		0.000410667
Slope		0.000700
Correlation Coefficient		0.9990

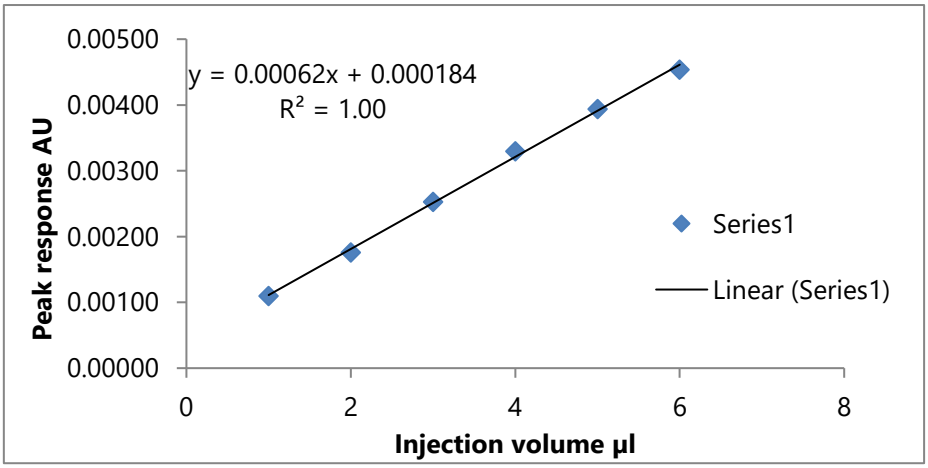

Fig. 6: Linearity of Chlorthalidone

Table 9: Linearity of Chlorthalidone

Linearity Level (%)	Injection Volume (µL)	Area observed
1	1	0.00089
2	2	0.00131
3	3	0.00211
4	4	0.00263
5	5	0.00319
6	6	0.00400
Intercept		0.000184
Slope		0.00062
Correlation Coefficient		0.9968

Sample Analysis

The market samples of Atenolol and Chlorthalidone Tablets were analysed by using the proposed method and calculated via the assay value for both Atenolol and Chlorthalidone. The results obtained are as summarized below in Table 10.

Table 10: Assay of market Sample

Atenolol						
Sample	**Label claim in mg/tablet**	**concentration mg/mL**	**Area Observed (x 1000000)**	**Mean Std. area observed (x 1000000)**	**% Assay**	**% Mean**
Sample-1	100	1.00	0.00296334	0.003000783	98.8	98.0
Sample-2	100	1.00	0.00291709		97.2	
Chlorthalidone						
Sample	**Label claim in mg/tablet**	**concentration mg/mL**	**Area Observed (x 1000000)**	**Mean Std. area observed (x 1000000)**	**% Assay**	**% Mean**
Sample-1	25	0.25	0.00670373	0.00690783	97.0	97.8
Sample-2	25	0.25	0.00681115		98.6	

Forced degradation

Forced degradation study was performed under acid hydrolysis, base hydrolysis and peroxide degradation conditions. The degradation sample solutions of Atenolol and Chlorthalidone were prepared. For acid hydrolysis samples are treated using 5 mL of 1N HCl, heated on water bath at 70°C for 30 minutes, cooled and neutralized the solution with 1N NaOH. For base hydrolysis samples are treated using 5 mL of 1N NaOH, heated on water bath at 70°C for 30 minutes, cooled and neutralized the solution with 1N HCl.

In peroxide degradation of sample, added 30% H_2O_2 solution and heated on water bath at 70°C for 30 minutes. It was observed that there is no any secondary peak generated for Atenolol and Chlorthalidone. The peroxide degradation shows little degradation. The results are summarized in below table 11.

Table 11: Forced Degradation Study Data

Atenolol					
Sample	**Condition**	**Sample Wt. (mg)**	**Concentration mg/mL**	**Area Observed (x 1000000)**	**% Assay**
Control	as such	107.20	1.00	0.00304251	97.1
Acid degradation	5 mL 1N HCl @ 70°C/30 min.	105.40	1.00	0.00298256	96.8
Base degradation	5 mL 1N NaOH @ 70°C/30 min.	105.20	1.00	0.00294567	95.7
Peroxide degradation	2 mL 30% H_2O_2 @ 70°C/30 min.	106.80	1.00	0.00302512	96.9
Chlorthalidone					
Sample	**Condition**	**Sample Wt. (mg)**	**Concentration mg/mL**	**Area Observed (x 1000000)**	**% Assay**
Control	as such	107.20	0.25	0.00690824	98.6
Acid degradation	5 mL 1N HCl @ 70°C/30 min.	105.40	0.25	0.00671245	97.4
Base degradation	5 mL 1N NaOH @ 70°C/30 min.	105.20	0.25	0.00669421	97.3
Peroxide degradation	2 mL 30% H_2O_2 @ 70°C/30 min.	106.80	0.25	0.00675651	96.8

Conclusion

The proposed HPTLC method was validated as per ICH guidelines. The analysis for combined dosage form was observed highly reliable and reproducible. As there were no prior HPTLC methods available for determination of combination of drug products containing Atenolol and Chlorthalidone; the proposed method can be adapted for routine analysis of Atenolol and Chlorthalidone in pharmaceutical dosage forms.

Acknowledgement

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