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Application Note

Identification of Atenolol and Chlorthalidone in Tablets by TLC acc. to USP and Quantification Using the TLC Explorer

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Abstract

This study presents a method for the identification of atenolol and chlorthalidone in tablet formulations using Thin Layer Chromatography (TLC) as per the USP monograph. The TLC Explorer system was employed for enhanced digital documentation and automated evaluation, facilitating also quantitative analysis and by that extended the use of TLC for this application. Results indicate that the test samples exhibited R_f values comparable to standard solutions, confirming identity. Quantitative assessments yielded concentrations within 97.6% to 102.6% of nominal values, indicating the method's applicability. This application underscores the TLC Explorer's efficacy in pharmaceutical TLC analysis and documentation.

Introduction

Atenolol and chlorthalidone combination tablets are used to treat high blood pressure (hypertension). Atenolol (2-[p-[2-hydroxy-3-(isopropylamino) propoxy]phenyl]acetamide) is a cardio-selective beta blocker that works by affecting the response to some nerve impulses in the heart. As a result, the heart beats slower and decreases the blood pressure. Chlorthalidone (2-chloro-5-(1-hydroxy-3-oxoisoindolin-1-yl)benzene sulfonamide) is a diuretic that reduces the amount of water in the body by increasing the flow of urine, which helps to lower blood pressure.[1]

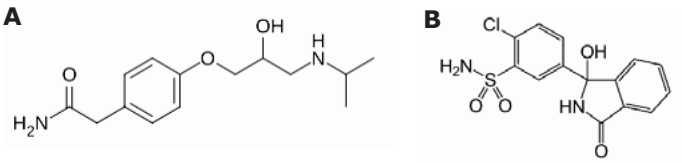

Figure 1. Chemical structures of (**A**) atenolol and (**B**) chlorthalidone.

The United States Pharmacopeia (USP) monograph for atenolol and chlorthalidone tablets lists thin layer chromatography (TLC) as one of the methods for the identification test.[2] In many pharmacopeial methods, TLC is a frequently cited technique for identity testing. HPTLC, a high-performance version of TLC and often used with automation, is a robust, reliable, rapid, and inexpensive tool used in qualitative and quantitative analysis of pharmaceutical compounds. This technique delivers chromatographic separations/fingerprints that can be visualized for identification/quantification and saved as electronic images for documentation.[3-4]

In this application note, the USP monograph specified identification test of atenolol and chlorthalidone in tablets by TLC is performed on the new TLC Explorer documentation system (**Figure 2**) that is also used to extend this method to a quantitative evaluation by video densitometry.

Figure 2. TLC Explorer System

The TLC Explorer documentation system enables the digital and automated evaluation of TLC plates, enhancing the efficiency and accuracy of thin layer chromatography analyses. The device offers three illumination modes using LED light sources – white light (VIS), UV-A (366 nm), and UV-C (254 nm) – for the detection and fast measurement of the compounds of interest. The software offers special features like automated track recognition, simultaneous measurement of multiple plates and background signal correction. Overall, the TLC Explorer offers accurate TLC imaging for reliable densitometric measurements, enabling quantitative analysis and reliable data interpretation.
(read more at **SigmaAldrich.com/tlc-explorer**)

Experimental

Reagent Preparation

Mobile phase: Dissolve 6.80 mL of ammonia solution 25% in 100 mL water to obtain 1 N ammonium hydroxide. Mix 40 mL of this solution with 200 mL n-butyl alcohol to obtain a mixture of n-butyl alcohol and 1 N ammonium hydroxide (5:1, v:v).

Standard Preparation

Atenolol

- *Standard stock solution 1 (50 mg/mL of atenolol):* Weigh and dissolve 125 mg of atenolol in 2.5 mL of methanol.
- *Standard solution 1 (20 mg/mL atenolol):* Dilute 400 µL of standard stock solution 1 to 1000 µL with methanol.

Chlorthalidone

- *Standard stock solution 2 (25 mg/mL chlorthalidone):* Weigh and dissolve 62.5 mg of chlorthalidone in 2.5 mL of methanol.
- *Standard solution 2 (10 mg/mL chlorthalidone):* Dilute 400 µL of standard stock solution 2 to 1000 µL with methanol.

Standard mix solutions I-VI: Prepare a total of six standard solutions (nos. I-VI) by pipetting 40 µL, 100 µL, 200 µL, 300 µL, 400 µL and 440 µL each of standard stock solution 1 and standard stock solution 2 into six separate 2 mL vials. Add methanol to get to final volume of 1 mL. The resulting solutions contain 2.0, 5.0, 10.0, 15.0, 20.0, and 22.0 mg/mL of atenolol and 1.0, 2.5, 5.0, 7.5, 10.0, and 11.0 mg/mL of chlorthalidone, respectively.

Sample Preparation

As sample, tablets were used with a atenolol to chlorthalidone content ratio of 1:2.

Test solution I (for identification): Weigh and shake a quantity of powdered combination tablets, equivalent to 50 mg of atenolol (corresponding to 25 mg chlorthalidone), with 2.5 mL of methanol for 15 minutes and filter through 0.45 µm PVDF membrane. Nominal resulting concentration of 20 mg/mL atenolol and 10 mg/mL chlorthalidone.

Test solution II (for quantification): Weigh and shake a quantity of powdered combination tablets, equivalent to 25 mg of atenolol (corresponding to 12.5 mg chlorthalidone), with 2.5 mL of methanol for 15 minutes and filter through 0.45 µm PVDF membrane. Nominal resulting concentration of 10 mg/mL atenolol and 5 mg/mL chlorthalidone.

Recovery test solutions I-III: Prepare three samples by shaking a quantity of powdered tablets, equivalent to 25 mg of atenolol (corresponding to 12.5 mg chlorthalidone), spiked with 125 µL, 250 µL and 500 µL each of standard stock solution 1 and standard stock solution 2 for 15 minutes. Add methanol to get to final volume of 2.5 mL and filter the solutions.

Instrument Parameters

Table 1. TLC conditions

TLC parameters	
Plate:	Silica Gel 60G F_{254}, 20 x 20 cm (**1.00390**)
Sample application:	10 µL of each solution, manual application
Mobile phase:	n-Butyl alcohol:1 N ammonium hydroxide (5:1, v:v)
Chamber conditions:	Twin trough chamber with chamber saturation
Migration distance:	17 cm
Drying:	Air-drying
Detection:	UV, 254 nm and 366 nm

Results

The identification of atenolol and chlorthalidone in tablets performed according to USP monograph on the TLC Explorer under UV 254 nm and UV 366 nm is demonstrated in **Figure 3**. It additionally shows the calibration and recovery experiments performed on the TLC Explorer. **Table 2** summarizes the obtained chromatographic results (R_f values). As required by the USP monograph for the identification, the principal spots obtained from the test solution (track 3) correspond to the R_f value size and intensity of the respective standards solutions (tracks 1 & 2).

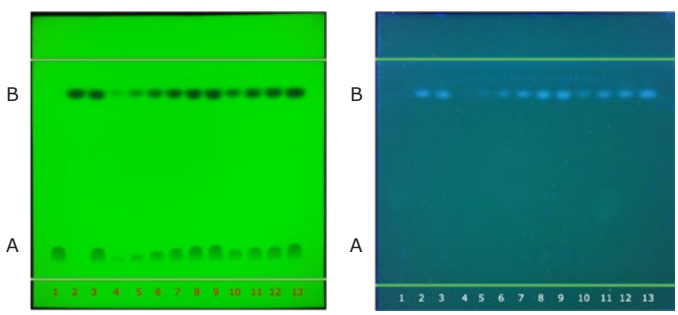

Figure 3. TLC chromatogram demonstrating the identification studies (track 1-3), calibration studies (tracks 4-9), test sample (10) and recovery studies (tracks 11-13) of atenolol (A) and chlorthalidone (B) in tablets under UV 254 nm (left) and UV 366 nm (right) by the TLC Explorer.

Table 2. Chromatographic data observed for standard solutions and test solutions under UV 254 nm by the TLC Explorer

Track No.	Solution name	Concentration of Atenolol (µg/spot)	Concentration of Atenolol in applied solution (mg/mL)	R_f Atenolol	Concentration of Chlorthalidone (µg/spot)	Concentration of Chlorthalidone in applied solution (mg/mL)	R_f Chlorthalidone
1	Standard solution 1	200	20.0	0.122	n.a.*	n.a.*	n.a.*
2	Standard solution 2	n.a.*	n.a.*	n.a.*	100	10.0	0.845
3	Test solution I	200#	20.0	0.124	100#	10.0	0.841
4	Standard mix solution I	20	2.0	0.092	10	1.0	0.848
5	Standard mix solution II	50	5.0	0.104	25	2.5	0.851
6	Standard mix solution III	100	10.0	0.114	50	5.0	0.847
7	Standard mix solution IV	150	15.0	0.121	75	7.5	0.847
8	Standard mix solution V	200	20.0	0.129	100	10.0	0.844
9	Standard mix solution VI	220	22.0	0.133	110	11.0	0.845
10	Test solution II	100#	10.0	0.122	50#	5.0	0.848
11	Recovery test solution I	125	12.5	0.124	62.5	6.25	0.849
12	Recovery test solution II	150	15.0	0.127	75	7.5	0.852
13	Recovery test solution III	200	20.0	0.139	100	10.0	0.848

nominal values; *n.a.: not applicable

Calibration

The results of the calibration experiments utilizing standard mix solutions I-VI and the calibration function of the TLC Explorer unit are displayed in **Figures 4 & 5** and **Table 3** (for atenolol), and **Figures 6 & 7** and **Table 4** (for chlorthalidone).

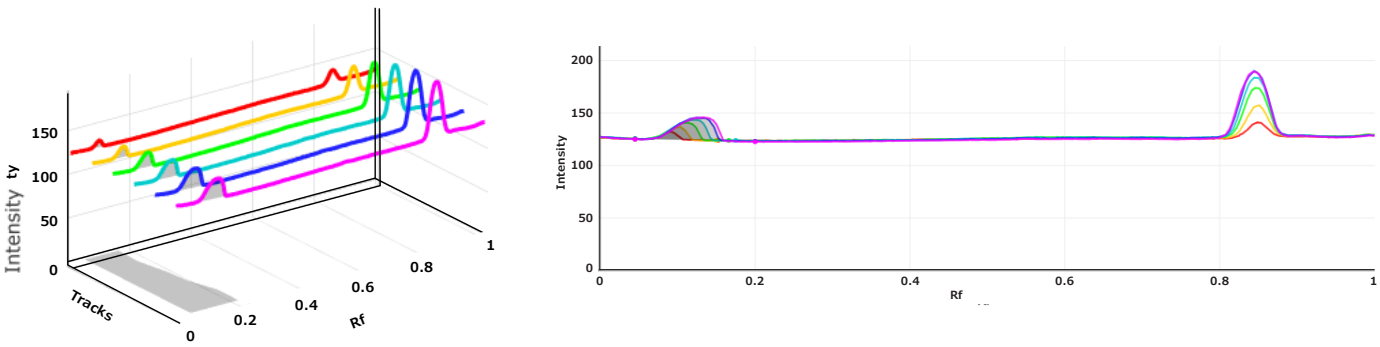

Figure 4. 3D densitogram (left, tracks 4 to 9) and 2D densitogram (right) demonstrating the calibration studies for atenolol utilizing standard mix solutions I-VI.

Table 3. Calibration data obtained for atenolol (20.0-220.0 µg/Spot), 6 calibrants

Conc. (µg/spot)	Area Response
20	291.8
50	624.3
100	1064.9
150	1524.3
200	1997.7
220	2164.2
Slope	9.2898
STEYX	20.3671
LOD (µg/mL)	7.23
LOQ (µg/mL)	21.92

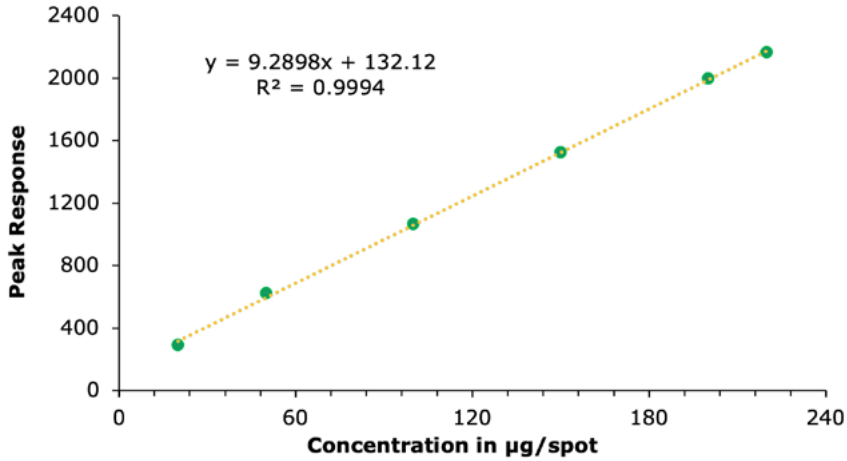

Figure 5. Calibration curve obtained for atenolol using standard mix solutions I-VI (c = 20.0, 50.0, 100.0, 150.0, 200.0 and 220.0 µg/spot).

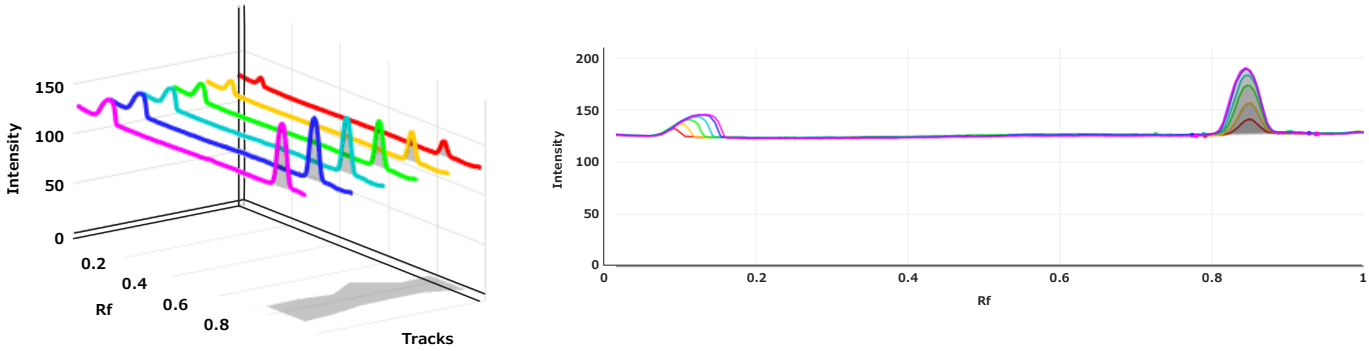

Figure 6. 3D densitogram (left, tracks 9 to 4) and 2D densitogram (right) demonstrating the calibration studies of chlorthalidone utilizing standard mix solutions I-VI.

Table 4. Calibration data obtained for chlorthalidone (20.0-220.0 µg/Spot), 6 calibrants

Conc. (µg/spot)	Area Response
10	959.6
25	1631.6
50	2694.5
75	3683.5
100	4607.1
110	4932.4
Slope	39.7473
STEYX	71.8758
LOD (µg/mL)	5.97
LOQ (µg/mL)	18.08

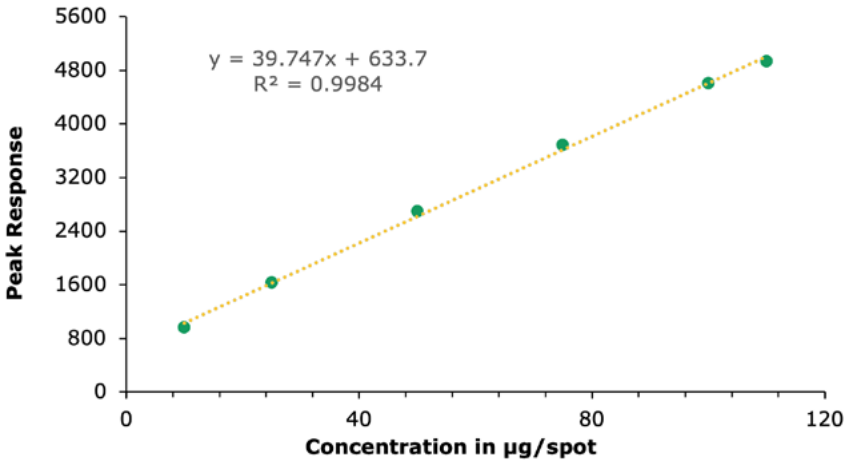

Figure 7. Calibration curve obtained for chlorthalidone using standard mix solutions I-VI (c = 10.0, 25.0, 50.0, 75.0, 100.0 and 110.0 µg/spot)

The determined sensitivities LODs and LOQs were 7.23 and 21.92 µg/spot for atenolol and 5.97 and 18.08 µg/spot for chlorthalidone respectively. These represent concentrations in the applied sample solutions (10 µL) of 0.72 and 2.19 mg/mL for atenolol and of 0.59 and 1.80 µg/mL chlorthalidone.

Recovery

The results of the spike recovery experiments utilizing recovery test solution I-III are displayed in **Table 5** and **Table 6** and were found to be in the range of 90.03 to 98.6%.

Table 5. % Recovery determined for atenolol using recovery test solution I-III

Sample	Spike concentration (µg/spot)	Determined spike concentration (µg/spot)	% Recovery
Recovery test solution I	25	24.67	98.68
Recovery test solution II	50	46.04	92.08
Recovery test solution III	100	96.85	96.85

Table 6. % Recovery determined for chlorthalidone using recovery test solution I-III

Sample	Spike concentration (µg/spot)	Determined spike concentration (µg/spot)	% Recovery
Recovery test solution I	12.5	11.25	90.03
Recovery test solution II	25	24.21	96.83
Recovery test solution III	50	48.13	96.26

Results Test Sample

Quantification of the test sample II by applying the established calibration curve led to resulting concentrations of 97.64 µg/spot for atenolol and 51.32 µg/spot for chlorthalidone (**Table 7**), representing 97.64 % and 102.64 % of the respective nominal concentrations in the applied solutions (10 µL) of 10 mg/mL atenolol and 5 mg/mL chlorthalidone.

Table 7. % Assay determined for atenolol and chlorthalidone in test solution II

Sample	Area response	Determined concentration (µg/spot)	Nominal concentration (µg/spot)	% Assay
Atenolol	1039.2	97.64	100	97.64
Chlorthalidone	2673.5	51.32	50	102.64

Conclusion

A method was applied following the USP monograph specified identification test of atenolol and chlorthalidone in tablets using TLC. The assessment and the documentation of the chromatographic results were done with the TLC Explorer documentation system. The principal spots in the chromatogram obtained from the test solution were similar in R_f value, size, and intensity to the principal spot in the chromatogram obtained with the standard solution as required by the monograph. Additionally, beyond the USP monographs TLC use for identification, the method was extended to a quantitative assessment for the two analytes using the quantification option of the TLC Explorer. The quantification of the test sample resulted in 97.6 -102.6% agreement with the nominal concentrations.

This application demonstrates that the TLC Explorer documentation system is an efficient and practical tool for data collection and documentation, track identification, and R_f calculation as well as quantitation by video densitometry.

References

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Chlorthalidone, European Pharmacopoeia (EP) Reference Standard	**C1950000**
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