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Analytical Method Development, Validation, and Applications of the HPTLC Densitometric Approach for Simultaneous Quantification of Azelnidipine and Chlorthalidone

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Abstract: For the simultaneous quantification of Azelnidipine and Chlorthalidone in synthetic combination, a high performance thin layer chromatographic method was devised that is quick, accurate, selective, and exact. The mobile phase used in the chromatographic analysis was in the ratio of 5: 4.7: 0.3: 0.1 v/v/v/v, and the stationary phase utilised was silica gel 60 F254 as the precoated stationary phase on aluminium plates. A 10 x 20 cm TLC chamber with a 15-minute saturation period was utilised. Azelnidipine and chlorthalidone were found to have retardation factors (RF) of 0.43 0.03 and 0.30 0.02, respectively. At 242 nm, densitometric analysis was performed. Following the ICH Q2 (R1) standard, a validation study was conducted. The calibration plots' regression data revealed a strong linear association with R2 = 0.999 for the concentration ranges of azelnidipine and chlorthalidone, 400–1200 ng band-1 and 600–1800 ng band-1, respectively. The method's precision, accuracy, and robustness were all validated. For azelnidipine and chlorthalidone, the minimum detectable levels were determined to be 26.71 ng band-1 and 38.39 ng band-1, respectively, and the limits of quantitation were found to be 80.94 ng band-1 and 116.033 ng band-1. Azelnidipine and chlorthalidone can be estimated simultaneously for routine analysis, in drug formulations, and in biological matrices, in short, using the proven analytical approach.

Keywords: Azelnidipine, Chlorthalidone, High-Performance Thin-Layer Chromatographic method, Validation

1. Introduction

Due to the strains of modern life, even younger people are now affected by hypertension, which is the primary cause of mortality among senior patients. Around 25% of people worldwide already have hypertension, and by 2025, this number is predicted to reach 30%. Pre-hypertension, mild hypertension, moderate hypertension, and severe hypertension are all different stages of high blood pressure. To manage hypertension, a variety of antihypertensive drug classes are available. Diuretics, calcium channel blockers, and angiotensin receptor blockers make up the most often prescribed medication class. Due to the various ways in which a combination of medications acts, combination therapy demonstrated superior control of hypertension than monotherapy. Azelnidipine, a calcium channel blocker, and chlorthalidone, a diuretic, are used to lower high blood pressure. This combination has a good impact on lowering the risk of stroke, myocardial infarction, and cardiovascular mortality. Chemically, Azelnidipine (AZEL), 1-(diphenyl methyl) azetidin-3-yl) 5-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate is a calcium channel blocker that is used in the treatment of hypertension [1, 2]. Chlorthalidone (CTD) is a thiazide diuretic used to treat hypertension, nephrogenic diabetes insipidus, and kidney stones. Its chemical name is 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide [3, 4].

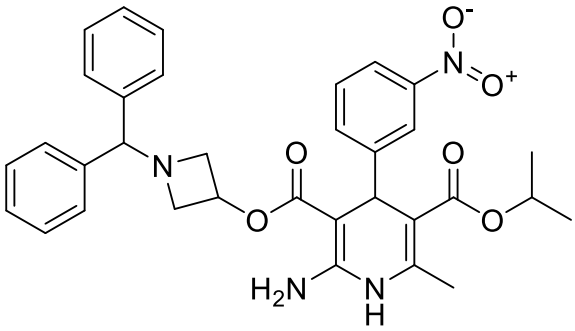

Figure 1. Structure of Azelnidipine.

Azelnidipine is recognized as official in the Indian Pharmacopoeia (IP) and the Japanese Pharmacopoeia (JP) while Chlorthalidone is recognized as official in the Indian Pharmacopoeia (IP) and the British Pharmacopoeia (BP), [1-4] (Figure 1 and 2).

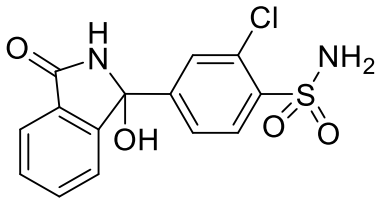

Figure 2. Structure of Chlorthalidone

The estimation of AZEL and CTD alone or in combination with other medications in various pharmaceutical dosage forms and biological matrices has been studied using a variety of UV visible spectrophotometry, liquid chromatographic, and HPTLC methods, according to a thorough literature review. For the proposed combination, no HPTLC method has been published as of yet. The most basic separation method now available to analysts is HPTLC. It can manage multiple samples at once, even ones with distinct natures and compositions. The use of HPTLC over HPLC, which is a flexible, repeatable technology with excellent sensitivity, specificity, and accuracy for the estimation of drug product, has numerous benefits. Since HPTLC methods are being investigated as a crucial tool in routine qualitative and quantitative estimate for medicines, they could be seen as a good alternative. The proposed combination approach was validated in accordance with the ICH guideline Q2 (R1) [5-38] and is described in the current work as being precise, sensitive, accurate, and robust.

2. Experimental

2.1 Instrument

The HPTLC device was a CAMAG Linomat 5 sample applicator with a 100-µL applicator syringe (Hamilton). Chromatography was carried out on aluminium HPTLC plates measuring 10 cm ×10 cm that had silica gel G60 F254 pre-coated on them. The generated chromatogram was scanned densitometrically using a CAMAG TLC scanner. On a Shimadzu electronic balance (ATX 224), all the drugs and Chemical substances were weighed.

2.2 Chemicals and reagents

Pure drug samples of AZEL and CTD were acquired from CTX Lifesciences Pvt. Ltd., Surat, Gujarat, and Pure Chem Pvt. Ltd., Ankleshwar Gujarat, respectively. All chemicals and reagents utilized were of AR grade.

2.3 Chromatographic System

Standard and formulation samples of AZEL and CTD were applied to the HPTLC plates as narrow bands measuring 6 mm in length, with 5 mm separating each band application. The bands were placed 15 mm from the plate's edge and 10 mm above the bottom. The samples were applied under a continuous drying stream of nitrogen gas.

2.4 Mobile Phase Development

A mobile phase made up of toluene, ethyl acetate, methanol, and glacial acetic acid in the proportions of 5: 4.7: 0.3: 0.1 v/v/v/v was used to develop the plates. In a twin-trough glass chamber saturated with mobile phase for 30 min, linear ascending development was performed. For each development, 10 mL of the mobile phase 5 mL in the trough containing the plate and 5 mL in the other trough—was utilised and allowed to migrate 80 mm. The HPTLC plates underwent full drying after development.

2.5 Densitometric Analysis

WinCATS planar chromatography programme (CAMAG) controlled densitometric analysis in the absorbance mode. The deuterium lamp served as the radiation source, and the bands were scanned at a wavelength of 242 nm. The slit's dimensions were 5 mm long by 0.45 mm wide, with a 10 mm/s scanning speed. Utilising a linear regression equation, the chemical concentrations were calculated from the intensity of light that was diffusely reflected and analysed as peak areas versus concentrations.

2.6 Preparation of Standard Stock Solution

Accurately weighed amounts of AZEL (10 mg) and CTD (10 mg) were added to 10 ml volumetric flasks and then dissolved in a small amount of methanol. Methanol was used to bring the volumes up to the proper levels, producing a solution with 1000 g/ml of CTD and AZEL, respectively. A working standard of 100 g/ml AZEL and 150 g/ml CTD, respectively, was prepared by taking aliquots from the stock solutions of AZEL and CTD and diluting them with methanol.

2.7 Validation

According to the ICH recommendations, the following parameters are taken into account when validating the method [39].

Linearity

To determine the method's linearity, calibration curves were created for AZEL and CTD at five different concentration levels, spanning a 400–1200 ng/band and 600–1800 ng/band range, respectively.

Accuracy

Recoveries of AZEL and CTD were computed using the method of standard additions, which was used to assess the method's accuracy. At 50, 100, and 150%, pre-analyzed synthetic mixtures were spiked with known concentrations of AZEL and CTD. Utilising

the chromatographic conditions indicated above, the solutions were examined by developing the TLC plate. With the help of a regression equation, the peak area's measurements and the levels of AZEL and CTD were calculated.

2.8 Precision

Repeatability

By spotting AZEL (1000 ng/band) and CTD (1500 ng/band) six times on an HPTLC plate, developing the plate, and noting the peak areas for the spots, the repeatability of the sample was evaluated. By calculating the percent relative standard deviation (%RSD) of the mean peak areas obtained from each spot of the sample, the precision of the procedure was assessed.

Intermediate precision

Inter-day and intra-day precisions were used to evaluate intermediate precision. Analysing sample solutions of AZEL (600, 800, and 1000 ng/band) and CTD (900, 1200, and 1500 ng/band) at three levels encompassing low, medium, and high concentrations of the calibration curve three times on the same day allowed researchers to estimate the intra-day precision. By examining sample solutions of AZEL (600, 800, and 1000 ng/band) and CTD (900, 1200, and 1500 ng/band) at three levels covering low, medium, and high concentrations over the course of three days, inter-day accuracy was ascertained. The peak regions and % RSD values were obtained.

Specificity

To identify the analyte in the presence of components, which may include contaminants, degradants, and matrix, specificity was examined. Excipients such as microcrystalline cellulose, sodium starch glycolate, PVPK-30, talc, and magnesium stearate were used to prepare the synthetic combination for the experiment. The specificity was assessed using this synthetic mixture, and the interference of excipients was noticed.

Sensitivity

The lowest concentration of analyte that can be detected is the limit of detection (LOD), and the smallest amount of analyte that can be quantified by the method is the limit of quantification (LOQ). We estimated LOD and LOQ using the following equations:

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where σ is the standard deviation of the *y*-intercepts of the regression lines and *S* is the slope of the calibration curve.

Robustness

The effects of small modifications to the mobile phase ratio and scanning wavelength on the outcomes were investigated. A percentage RSD was computed after applying the samples in triplicate.

Analysis of Synthetic Mixture

The excipients microcrystalline cellulose, Sodium starch glycolate, PVPK-30, Talc, and Magnesium Stearate were combined to create a synthetic combination. A precise weighted amount of powder, equal to 10 mg of AZEL and 15 mg of CTD, was placed into a 10 ml volumetric flask. The aforementioned flask received methanol, and it underwent a 15-minute sonication process. Using Whatman filter paper (No. 45), the solution was filtered, and methanol was added to bring the volume up to the required level. To obtain 100 g/ml AZEL and 150 g/ml CTD, the proper volume of the aliquot was transferred to a 10 ml volumetric flask and the volume was topped off as needed with methanol. For the estimate of AZEL and CTD, a 6 µL solution was placed on a TLC plate, yielding concentrations of 1000 ng/band and 1500 ng/band, respectively. The optimised mobile phase was used to create the plate. Following development, the plate was scanned to identify the regions. Keeping these numbers in the regression equation allowed for quantification.

3. Results and Discussion

Based on the solvent's polarity, the mobile phase was chosen to create an HPTLC method for the simultaneous estimate of AZEL and CTD. Different combinations of solvents, including chloroform, toluene, methanol, ethyl acetate, glacial acetic acid, ammonia, and iso-propanol, were explored. The mobile phase was chosen to be a mixture of toluene, ethyl acetate, methanol, and glacial acetic acid (5: 4.7: 0.3: 0.1 v/v/v/v), which produced a distinct, compact band of AZEL and CTD with Rf values of 0.43 and 0.30, respectively (Figure 3). The solvent migration distance was 80 mm, and the optimal mobile phase chamber saturation duration was 30 min.

3.1 Validation

Linearity and Calibration curves

With depicted calibration curves for AZEL and CTD in the 400–1200 ng/band and 600–1800 ng/band ranges, respectively, both medications had linear correlation coefficients (R2) of 0.999 (Figure 4, 5 and 6). Regression data are shown in Table 1 and show a strong linear connection over the specified concentration range, demonstrating the analytical appropriateness of the approach.

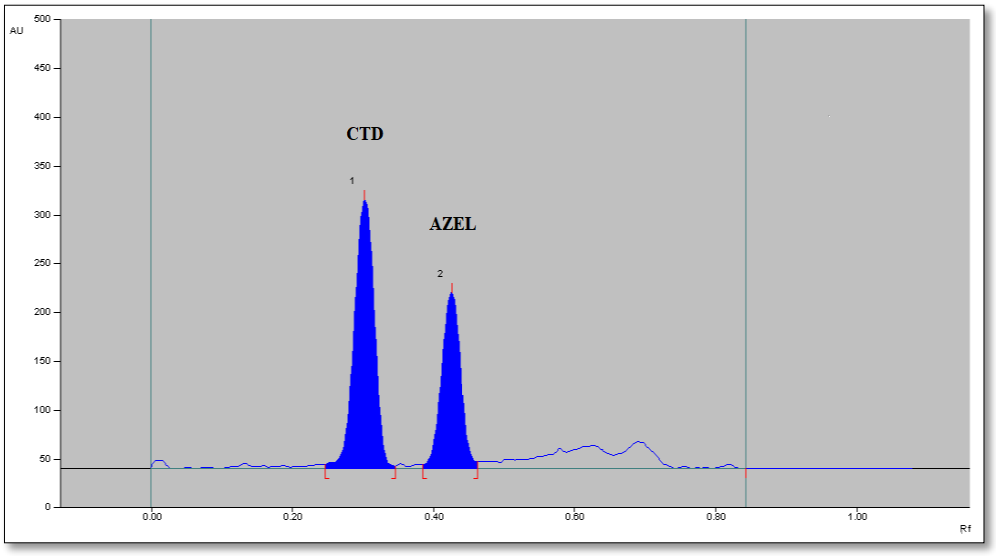

Figure 3. Densitogram of AZEL and CTD in optimized mobile phase

Table 1. Regression analysis of the calibration curve

Parameters	AZEL	CTD
Linearity (ng/band)	400-1200	600-1800
Correlation coefficient (R^2)	0.999	0.999
Slope of regression equation	2.6043	2.3903
Intercept of regression	398.18	1370.7

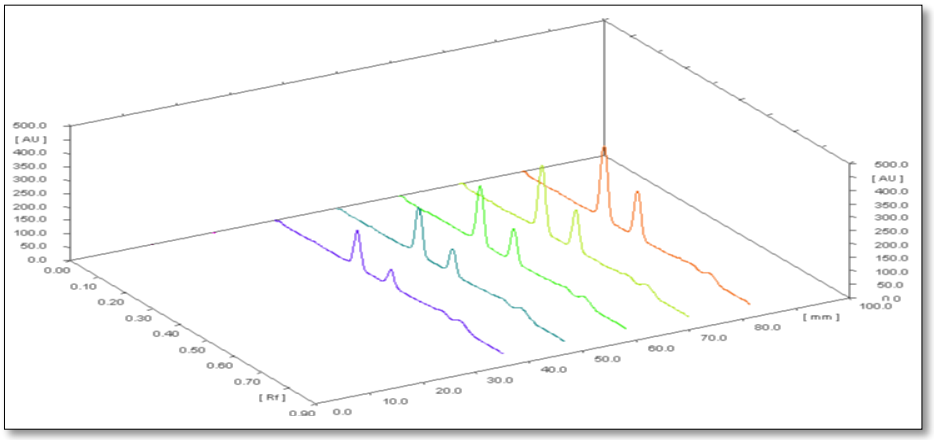

Figure 4. Chromatogram of Linearity for AZEL and CTD

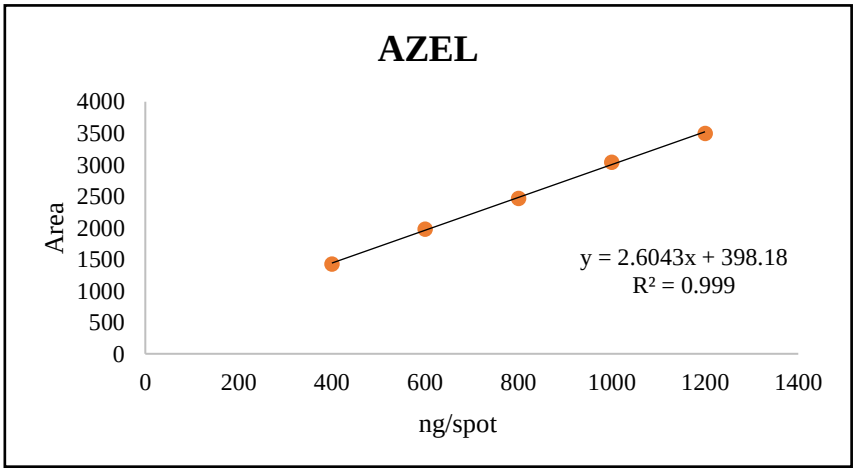

Figure 5. Linearity curve for AZEL

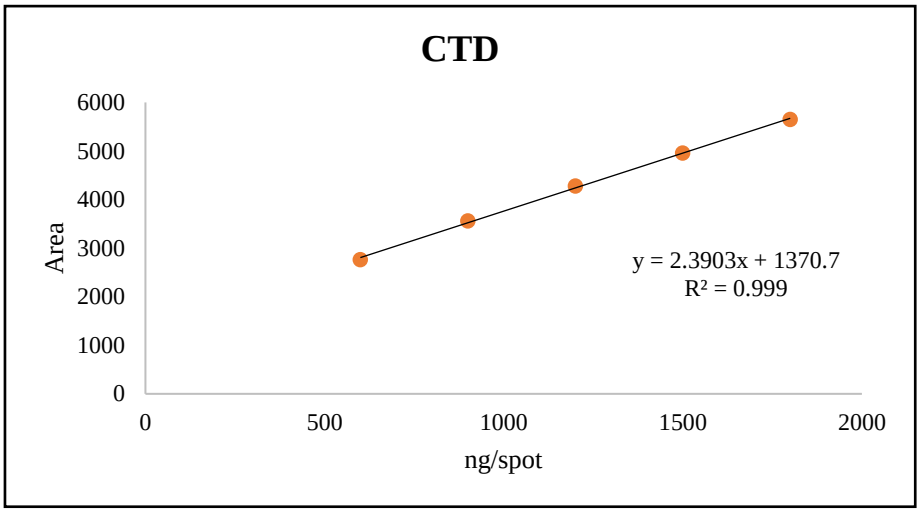

Figure 6. Linearity curve for CTD

Table 2. Summary of Validation Parameters

Parameters		AZEL	CTD
Linearity (ng/band)		400-1200	600-1800
Limit of Detection (ng/band)		26.71	38.39
Limit of Quantitation (ng/band)		80.94	116.33
Accuracy (%)		99.82-100.86	100.06-100.81
Precision (%RSD)	Repeatability (n=6)	0.22	0.23
	Intraday (n=3)	0.17-0.39	0.17-0.44
	Interday (n=3)	0.26-0.41	0.16-0.21
Robustness (%RSD)		0.24-0.69	0.18-0.75

Table 3. Analysis of the Synthetic mixture

Synthetic Mixture (n=3)		
	AZEL	**CTD**
Amount of drug taken (ng/band)	1000	1500
Amount of drug found (ng/band)	1009.8	1503.1
%Amount of drug ± SD	100.98 ± 0.45	100.20 ± 0.35

Accuracy

The amount of drug recovered from the synthetic mixture was accurately disclosed at three degrees of standard addition. The percentage recoveries for AZEL and CTD were determined to be between 99.82 - 100.86% and 100.06 -100.81%, respectively. The fact that the results were close to the true value (100%) shows that the procedure is accurate (Table 2).

3.2 Precision

Repeatability

Applying and analysing AZEL (1000 ng/band) and CTD (1500 ng/band) six times was done as part of a repeatability study. Both drugs' % RSD readings were found to be under 2%.

Intermediate precision

Interday precision was checked by measuring three concentrations on three different days, and%RSD were found to be 0.26-0.41% for AZEL and

0.16-0.21 for CTD. Intraday precision was checked by measuring three concentrations three times a day, and%RSD of AZEL and CTD were found to be 0.17-0.39% and 0.17-0.44%, respectively.

Limit od Detection (LOD) and Limit of Quantitation (LOQ):

The LODs for AZEL and CTD were discovered to be 26.71 and 38.39 ng/band and the LOQs for AZEL and CTD to be 80.94 and 116.33 ng/band. This suggests that the approach is sensitive.

Robustness

Robustness tests were carried out by purposefully making minor adjustments to technique parameters like the proportion of mobile phase to detection wavelength. Toluene: Ethyl acetate: Methanol: Glacial acetic acid (3: 6.7: 0.3: 0.1 v/v/v/v) and Toluene: Ethyl acetate: Methanol: Glacial acetic acid (7: 2.7: 0.3: 0.1 v/v/v/v) were substituted for the mobile phase ratio (5: 4.7: 0.3: 0.1 v/v/v/v). The 242 nm scanning wavelength was modified to 240 nm and then 244 nm. None of these modifications had an impact on how well the created HPTLC approach worked. For AZEL, the %RSD values were determined to be 0.24–0.69% and for CTD, 0.18–0.75%. The Rf value did not fluctuate significantly, demonstrating the robustness of the technique.

Analysis of Synthetic Mixture

The proposed approach was used to analyse the synthetic combination, and the results showed %Recoveries of 100.98 ± 0.45% w/w for AZEL and 100.20 ± 0.35% w/w for CTD. Table 3 displays the findings of the study of the synthetic mixture.

4. Conclusion

For the quantification of AZEL and CTD, a high-performance thin-layer chromatographic technique has been devised that is accurate and precise. The HPTLC approach is both time and money efficient because just 10 ml of mobile phase are needed for a single analysis. In terms of accuracy and precision, the results are comparable to those of the liquid chromatographic approach. The mobile phase of toluene, ethyl acetate, methanol, and glacial acetic acid (5: 4.7: 0.3: 0.1 v/v/v/v) was used to develop the method. For AZEL and CTD, the Rf values were found to be 0.43 and 0.30, respectively. The method was found to be linear for AZEL and CTD between 400 and 1200 ng/band and 600 and 1800 ng/band, respectively. The technique was approved in accordance with ICH Q2 (R1) principles. The technique was used to estimate the presence of both medications in the synthetic combination, and it resulted in a percentage recovery for both pharmaceuticals of more than 98%. The technique is applicable to the routine analysis of in-process quality control of samples and dosage forms.

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Conflict of interest

The Authors have no conflicts of interest to declare that they are relevant to the content of this article.

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