A NOVEL VALIDATED STABILITY INDICATING ANALYTICAL HPTLC METHOD FOR QUANTITATION OF HYDROCHLOROTHIAZIDE AND LISINOPRIL IN TABLET FORMULATION

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ABSTRACT

A new, simple and rapid high-performance thin-layer chromatographic method was developed and validated for quantitative determination of hydrochlorothiazide and lisinopril. Both the drugs were chromatographed on silica gel 60 F_{254} HPTLC plate using chloroform: methanol: ethyl acetate: acetic acid (7:2:1.0:0.2; V/V/V/V) as the mobile phase. Hydrochlorothiazide and lisinopril were quantified by densitometric analysis at 218 nm. The method was found to give compact bands for hydrochlorothiazide and lisinopril at R_f 0.43 and 0.75, respectively. The linear regression analysis data for the calibration plots showed good linear relationship with r2 0.9990 and 0.9992 in the concentration range 200–1200 ng band^{-1} and 250-1500 ng band^{-1}, for hydrochlorothiazide and lisinopril, respectively. The method was validated as per the International Conference on Harmonization guidelines Q2(R1). The minimum detectable amounts were found to be 50.16 ng and 37.93 ng, whereas the limits of quantitation were found to be 150.48 and 113.92 ng for hydrochlorothiazide and lisinopril, respectively. Statistical analysis of the data showed that the method was precise, accurate, reproducible, selective and specific for the analysis of hydrochlorothiazide and lisinopril. The method was successfully employed for the routine quantitative estimation of hydrochlorothiazide and lisinopril in pharmaceutical tablet formulations.

Keywords: Hydrochlorothiazide, lisinopril, HPTLC-densitometric method, quantitative estimation.

INTRODUCTION

Hydrochlorothiazide (HCTZ), chemically 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, is a thiazide diuretic which reduces water in the body by increasing the flow of urine which lowers the blood pressure. Lisinopril (LNP), chemically *(S)*-1-[*N*-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate is an angiotensin converting enzyme inhibitor, by decreasing the tension of blood vessels and blood volume, thus lowering blood pressure. HCTZ and LIS combination is used to treat hypertension. Quantitative estimation of pharmaceutical tablet formulations with such combination is challenging due to their different physicochemical properties. Separation method proposed for quantitation of such binary mixture must be rapid, economical and environment friendly as possible, to enable their application in routine quality control[1-3].

Various spectroscopic and chromatographic methods have been proposed for analysis of HCTZ and LIS in combined dosage forms. El-Gindy et al., described different spectrophotometric and HPTLC-densitometric methods are presented for the simultaneous determination of HCTZ and LIS in pharmaceutical tablets. The spectrophotometric methods include third derivative (^{3}D) ultraviolet spectrophotometry with zero crossing measurement and second derivative of the ratio spectra with measurement. The HPTLC method was based on separation of both drugs followed by densitometric measurements of the spots of HCTZ and LIS[1]. Ivanović et al., reported RP-HPLC method for simultaneous determination of HCTZ, LIS and their impurities in pharmaceuticals[2]. In recent years, a number of RP-HPLC methods have been reported for simultaneous determination of HCTZ and LIS in pharmaceuticals[3-7].

Taken together, this information has led our research group to evaluate the potential of HPTLC-densitometric method as an analytical tool for separation and quantitative estimation of HCTZ and LIS in combined

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tablet dosage forms. The current study elaborates a new and simple HPTLC-densitometric method for quantitative determination of HCTZ and LIS in combined tablet dosage form and the developed HPTLC method as statistically compared with the reported method.

MATERIALS AND METHODS

Materials

Methanol, ethyl acetate, acetic acid, chloroform and ammonia were purchased from Merck (India). All other chemicals and solvents were of analytical reagent grade and used as received without further purification.

Instrumentation

The HPTLC system (CAMAG, Muttenz, Switzerland) consisted of a Linomat 5 autosprayer, a twin-trough chamber for 10 × 10 cm plates, a derivatization chamber, and a plate heater. Precoated silica gel 60F_{254} HPTLC plates (10 × 10 cm, layer thickness 0.2 mm; E. Merck KGaA, Darmstadt, Germany) were used as the stationary phase. Densitometric analysis was carried out using a CAMAG TLC Scanner 3 with winCATS software[8-11].

Preparation of hydrochlorothiazide and lisinopril standard solution

Accurately weighed 250 mg of HCTZ and 200 mg of LIS working standards were transferred to 100 mL volumetric flask and volume was made up to the mark using methanol (Stock A). Further, 1 mL of stock A was transferred to 10 mL volumetric flask and final volume was made up to the mark using methanol to obtain mixed standard solution containing HCTZ 250 µg mL^{-1} and LIS 200 µg mL^{-1} , respectively.

HPTLC method development

The bands of standard and test solutions of HCTZ and LIS were applied on the HPTLC plate. Linear ascending development technique was used for development of chromatogram in a twin-trough chamber (equilibrated with the mobile phase vapors for 20 min at 25 ± 2 °C, development distance 70 mm). The developed HPTLC plates were dried thoroughly before densitometric evaluation was performed at 218 nm. The calibration curves (n = 6) for HCTZ and LIS were constructed by plotting peak area versus concentration in the range of 250-1500 ng and 200–1200 ng, respectively[8-10].

HPTLC method validation

Specificity, linearity, accuracy, precision, sensitivity, robustness and system suitability of development HPTLC method were evaluated as per the International Conference on Harmonization (ICH) guideline Q2 (R1)[12].

Application of developed TLC-densitometric method

Tablet powder (Listril Forte Tab, Torrent Pharmaceuticals Ltd, Ahmedabad) equivalent to 250 mg HCTZ and 200 mg LIS was weighed accurately and dissolved in 100 mL methanol. The resulted solution was sonicated for 15 min and then passed through Whatman filter paper No. 41 (Florham Park, NJ). The residue was washed thoroughly with 10 mL methanol. The filtrate and washings were combined and diluted up to the mark suitably with methanol. Further, 1 mL of test solution was transferred to 10 mL and dilute up to the mark using methanol to obtain a 250 µg mL^{-1} of HCTZ and 200 µg mL^{-1} of LIS, respectively.

RESULTS AND DISCUSSION

Selection and optimization of chromatographic conditions to develop TLC-densitometric method

Commercially available precoated TLC plates with Silica Gel 60 F_{254} on aluminum backing were selected and used as the stationary phase, which is reasonable and nearly suits all kind of drugs as reported in most of the research papers. Selection of the mobile phase was carried out on the basis of methodology suggested by CAMAG laboratory[13]. As a result, combination of chloroform: methanol: ethyl acetate: acetic acid (7:2:1.0:0.2; V/V/V/V) was found suitable as a mobile phase, which gave good separation of HCTZ and LIS from formulation matrix. Chamber saturation time of less than 15 min and solvent migration distances greater than 70 mm resulted in diffusion of the analyte band. Therefore, combination of chloroform: methanol: ethyl acetate: acetic acid (7:2:1.0:0.2; V/V/V/V) as mobile phase with a chamber saturation time of 20 min at 25 °C and solvent migration distance of 70 mm was used. These chromatographic conditions produced well-defined, compact bands of HCTZ and LIS with optimum migration at R_f = 0.43 ± 0.01 and R_f = 0.75 ± 0.01, respectively at 218 nm (Fig. 1). They also gave a good resolution of the analyte from excipients used in formulations.

HPTLC method validation

Specificity of the method for HCTZ and LIS was proved from the spectral scan (Fig. 2), and peak purity correlation (higher than 0.9999) results for HCTZ and LIS in tablet formulations indicate that there is no co-eluting peak with HCTZ and LIS. These results indicated that

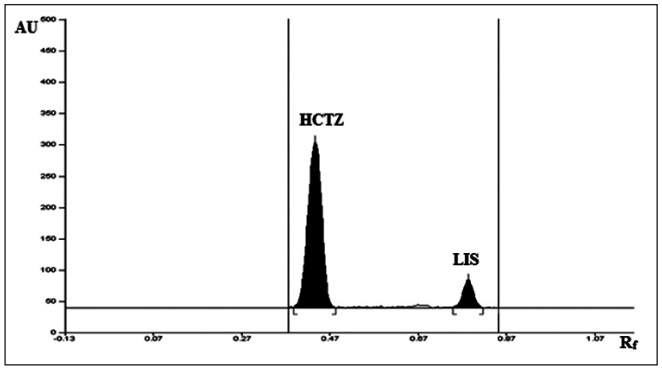


Fig. 1: Chromatogram of standard solution containing hydrochlorothiazide (1000 ng band^{-1}) and lisinopril (800 ng band^{-1}) using proposed TLC-densitometric method

the proposed method is specific, and can be applied for stability studies and QC analysis of HCTZ and LIS in pharmaceutical products.

Under the experimental conditions used, the minimum detectable amounts were found to be 50.16 ng and 37.93 ng, whereas the limit of quantitation was found to be 150.48 ng and 113.92 ng for HCTZ and LIS, respectively (Table I).

Table I: Regression analysis of calibration graphs for hydrochlorothiazide and lisinopril by proposed TLC-densitometric method

Parameter	HCTZ	LIS
Concentration range	250-1500 ng	200-1200 ng
Slope	4.146	0.214
Residual SD	67.83	2.55
intercept	1980.68	554.39
SD of the intercept	63.15	2.37
Correlation coefficient	0.9990	0.9992
LOD	50.16 ng	37.93 ng
LOQ	150.48 ng	113.92 ng

The linearity results demonstrated the acceptable linearity for HCTZ and LIS over the range of 80 to 120 % of the target concentration. Linear correlation was obtained between peak areas and concentrations of HCTZ and LIS in the range of 250–1500 ng and 200–1200 ng, respectively. The following regression equation was found by plotting the peak area (y) versus the HCTZ and LIS concentrations (x) expressed in ng: y = 4.146 x + 1980.68; y = 0.214 x + 554.39, respectively. The coefficient of determination (r^2: 0.9990 and 0.9992 for HCTZ and LIS, respectively) obtained for the regression line demonstrated the excellent relationship between peak area and concentration of HCTZ and LIS. Data

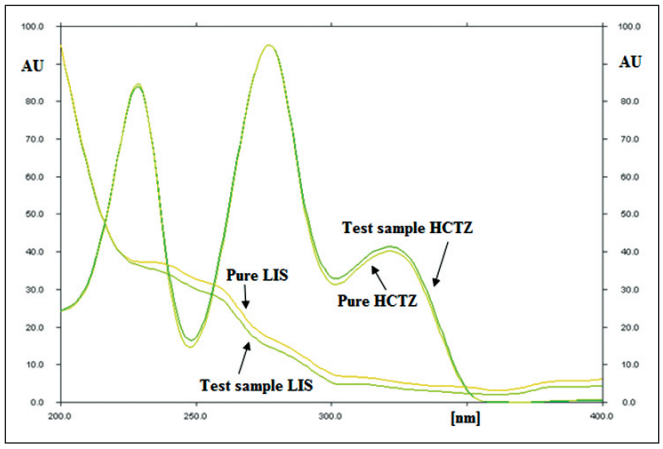
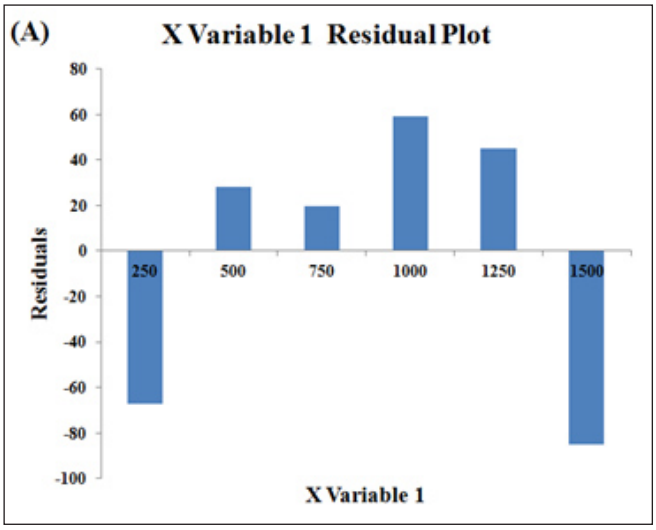


Fig. 2: Spectral comparison of standard and test of hydrochlorothiazide and lisinopril using proposed TLC-densitometric method

of regression analysis are summarized in Table I. The calibration can be accepted as linear, if it is statistically proved. However, declaring method linearity on the basis of value of coefficient of determination as a sole proof of linearity is inappropriate. Different approaches for checking linearity objectively are reported and should be used. Residual plot is the simplest method of linearity test for HPTLC method. In this study, linearity was confirmed (Fig. 3), since residuals are randomly distributed around the regression line (no lack of fit). Further, CAMAG has described parameter such as 'sdv' (residual standard deviation of the standard point), for fit of calibration curve for TLC-densitometric method by using WinCATS software. The lower the value of sdv means, the closer the points to the curve. The acceptance limit of sdv is not more than 5.0 %[9-13]. In the present study, sdv value was found to be 2.17 % and 2.88 % for HCTZ and LIS, respectively, which is lower than specified limit. Three-dimensional overlay of calibration band and spectral comparison of various aliquots of HCTZ and LIS during linearity studies are presented in Fig. 4.

Results of the accuracy studies from excipient matrix are shown in Table II. The HCTZ and LIS recoveries were obtained in a range of 99.96 % to 100.02 % and 99.98 % to 100.23 %, respectively for tablet formulations, using the proposed HPTLC method. Recovery values demonstrated the accuracy of the method in the desired range.

The precision results obtained are shown in Table II. In all instances, RSD values were less than 5 %, confirming the precision of the method. Aliquots of 10 µL of samples containing of HCTZ (500, 750, and 1000 ng) and LIS (400, 600, and 800 ng), respectively, were analyzed by the proposed HPTLC method. In order to control scanner parameters, i.e., repeatability of measurement of peak area,



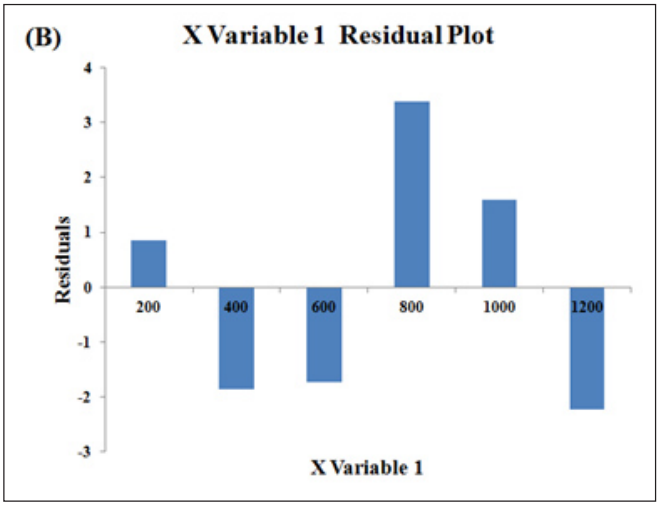


Fig. 3: Residual plot for (A) hydrochlorothiazide and (B) lisinopril using proposed TLC-densitometric method

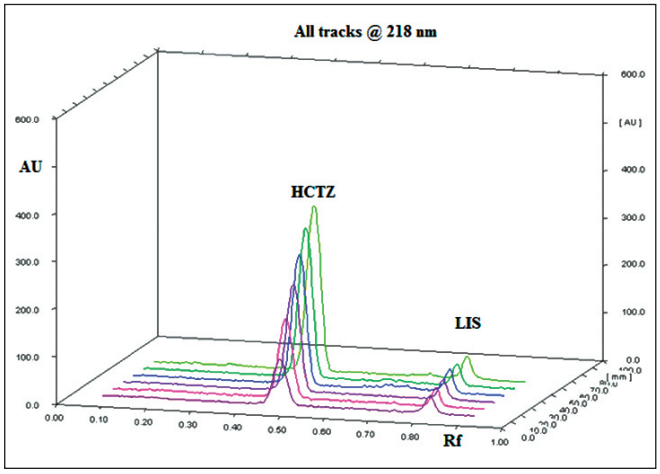


Fig. 4: Three-dimensional overlay of densitograms of calibration bands of hydrochlorothiazide and lisinopril using proposed TLC-densitometric method

one band was analyzed without changing the position of the plate ($n = 7$). By applying and analyzing the same amount seven times, precision of the automatic spotting device was evaluated. RSD was consistently less than 5% (Table II), which was well below the instrumental specifications.

Table II: Summary of validated parameters of the proposed TLC-densitometric method for hydrochlorothiazide and lisinopril

Parameter	HCTZ	LIS
Accuracy, (%, n=3)	99.96 to 100.02	99.98 to 100.23
Repeatability (RSD, %, n=6)	0.30	0.24
Precision (RSD, %)		
Interday (n=3)	0.66-0.75	0.29-0.62
Intraday (n=3)	0.14-0.31	0.15-0.23
Specificity	Specific	Specific
Robustness	Robust	Robust

Table III: System suitability test parameters for hydrochlorothiazide and lisinopril by proposed TLC-densitometric method

Parameter	HCTZ (n=6)	LIS (n=6)
Retardation factor (R_f)	0.43±0.01	0.75 ± 0.01
Peak Area	5118.23±3.11	681.10±0.36
Resolution	-	3.76±0.12
Tailing factor	0.90±0.01	1.00±0.01
Number of theoretical plates	2317.47±9.21	5485.71±13.65
Peak purity	r (S, M)-0.9999 and r(M, E) 0.9999	r (S, M)-0.9999 and r(M, E) 0.9999
Repeatability (%RSD)	0.29	0.25

The results and the experimental range of the selected variables (chamber saturation time, development distance, mobile phase composition, size of the plates and development chamber) evaluated in the robustness test suggested that there were no significant changes in the chromatographic pattern when the deliberate modifications were made in the experimental conditions, thus showing the method to be robust.

System suitability parameters are shown in Table III; the results indicated that the system was suitable for the analysis intended.

The quantitative estimation of HCTZ and LIS in its pharmaceutical formulation was successfully performed using an optimized and validated HPTLC method in triplicate (n=3). The assay results for two different batches were found to be 98.85 ± 0.47 and 99.65 ± 0.78 % w/w for HCTZ and 99.96 ± 0.59 and 100.25 ± 0.17 for LIS % w/w, respectively (Table IV).

Table IV: Assay results for hydrochlorothiazide and lisinopril in different pharmaceutical tablet dosage forms using the proposed TLC-densitometric method

Sample	HCTZ ± SD (n = 5), %	LIS ± SD (n = 5), %
Tablet formulation Batch 1	99.85±0.47	99.96 ± 0.59
Tablet formulation Batch 2	99.65±0.78	100.25±0.17

Comparison of methods

Results obtained by the proposed HPTLC-densitometric method for determinations of HCTZ and LIS in its pharmaceutical formulations were statistically compared to those obtained by applying the reported HPTCL method (1). The calculated *t*- value were found to be 4.2903 ($P = 0.1458$) and 0.0.5254 ($P = 0.6920$) for HCTZ and LIS, respectively, at 95 % confidence level ($P = 0.05$). The obtained values were found to be less than the theoretical ones; this indicates no significant difference in the determined content of HCTZ and LIS, confirming accuracy and precision at 95% confidence level.

CONCLUSION

A new HPTLC-densitometric method has been developed for quantification of HCTZ and LIS. Low cost, fast speed, satisfactory precision (RSD less than 5.0 %), and accuracy (99.96 % to 100.02 %, HCTZ and 99.98 % to 100.23 %, LIS) are the main features of this method. The method was successfully validated in accordance with ICH guideline, and statistical analysis proved that the method is sensitive (LOD 50.16 ng and 37.93 ng, whereas LOQ 150.48 ng and 113.92 ng HCTZ and LIS, respectively), specific, and repeatable (RSD less than 5.0 %). The results were statistically compared to those obtained by the reported HPTLC methods, where no significant difference was found; indicating the ability of proposed HPTLC-densitometric method to be used conveniently for analysis HCTZ and LIS as a bulk drug and in tablet formulations without any interference from excipients.

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