Research Article

Development and validation of HPTLC method for simultaneous estimation of Lercanidipine Hydrochloride and Enalapril Maleate in their synthetic mixture

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ABSTRACT

A precise and accurate HPTLC method has been developed for simultaneous estimation of Lercanidipine Hydrochloride and Enalapril Maleate in its synthetic mixture. The chromatographic separation was performed on aluminum plates pre-coated with silica gel 60F254 using toluene:n-butanol:formic acid in proportion of 6:4:1 v/v/v as mobile phase and densitometrically scanned at 224 nm wavelength. The Rf values were found to be 0.34 ± 0.02 and 0.55 ± 0.02 for Enalapril Maleate and Lercanidipine Hydrochloride respectively. The linearity range for both Lercanidipine Hydrochloride and Enalapril Maleate were found to be 400 – 800 ng/band with correlation coefficients of 0.9979 and 0.9961 respectively. The method was validated for linearity, precision, accuracy as per ICH guidelines. The method was applied for simultaneous estimation of Lercanidipine Hydrochloride and Enalapril Maleate in its synthetic mixture. The assay results were found to be 99.48 ± 0.25% and 99.34 ± 0.15% for Lercanidipine Hydrochloride and Enalapril Maleate.

Keywords: Lercanidipine, Enalapril, HPTLC, Simultaneous

1. INTRODUCTION [1-14]

Antihypertensive therapy seeks to prevent the complications of high blood pressure, such as-stroke and myocardial infarction. Among the most important and most widely used drugs are thiazide diuretics, calcium channel blockers, ACE inhibitors, angiotensin II receptor antagonists (ARBs), and beta blockers. Lercanidipine hydrochloride (LER) is a calcium channel blocker of dihydropyridine class and Enalapril maleate (ENM) is an ACE inhibitor. Chemical structures are depicted in (Figure 1).

The therapeutic importance of ENM initiated several reports viz: spectrophotometry[2]' polarography, HPLC [3], fluorimetry[4], flow injection chemiluminescence method[5], stripping voltammetry[6],and capillary electrophoresisfor its determination both in formulations and in biological fluids.

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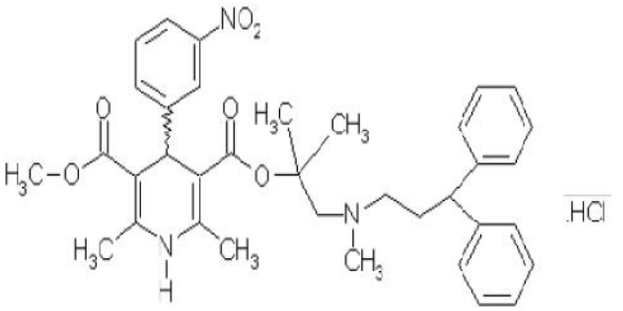
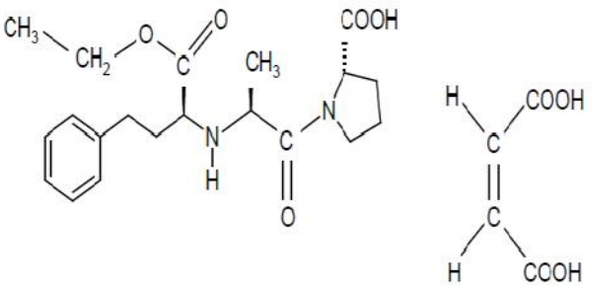
There are several reports viz: voltammetry[7] spectrophotometry[8,9], HPLC [10] and capillary electrophoresis for determination of LER. To the best of our knowledge, no HPTLC method has yet been described for simultaneous estimation of ENM and LER in their synthetic mixture or combination formulation.



(a)



(b)

Figure 1. Chemical Structure of a) Lercanidipine HCl b) Enalapril Maleate

2. MATERIALS ANDMETHODS

2.1. Instruments

The HPTLC system (CAMAG Switzerland)consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (CAMAG Muttenz, Switzerland), twin-trough developing chamber (10x 10cm), UV cabinet with dual wavelength lamps, winCATS software, syringe (100 µl capacity, Hamilton) were used forchromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all theweighing purpose.

2.2. Chemicals and Materials

Methanol, Chloroform, Toluene, n-butanol, n-hexane, formic acid and ethyl acetate (AR grade)were obtained from S. D. Fine Chemicals Limited, Mumbai, India.Lercanidipine Hydrochloride and Enalapril Maleate were obtained as gift samples from Alembic Pharmaceuticals, Vadodara and Cadila Healthcare, Ahmedabad, India respectively.

2.3. Chromatographic conditions

2.3.1.1. Optimization of Mobile Phase

The mobile phase was optimized to get desired Rf value range [0.2 - 0.8] with a resolution of > 1.5. It was achieved by trying different mobile phases containing solvents of different polarities in different ratios like toluene, n-hexane, n-butanol, methanol, and ethyl acetate. Modifier was added to reduce tailing effect and get sharp spot.

2.3.1.2. Optimized chromatographic conditions

Chromatographic separation was performed on 20 × 20 cm aluminium plates pre- coated with 250 µm layer of silica gel 60 F_{254} (E. Merk, Darmstadt, Germany). The TLC plate was pre-washed with methanol and activated at 60 °C for 5 min prior to spotting. The samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semiautomatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 0.1 µl/s. TLC plate was developed in previously saturated twin trough chamber (20 cm x 10 cm) using toluene : n-butanol : formic acid in proportion of 6 : 4 : 1, v/v/v as mobile phase with filter paper at temperature, 25 ± 2 ˚C (air conditioning system), relative humidity, 35-40 %; chamber saturation time, 30 min and migration distance, 75 mm. The TLC plate was dried, scanned and analysed by TLC Scanner IV and WinCATS software using following parameters: slit dimension, 4 mm × 0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 224 nm.

2.4. Preparation of Solutions

2.4.1. Preparation of stock solutions of Lercanidipine hydrochloride and Enalapril maleate

Accurately weighed 10 mg LER and 10 mg of ENM were transferred to 10 ml volumetric flasks, dissolved in methanol and diluted up to mark with methanol to get the final concentrations of 1000 µg/ml each. Further dilution was carried out to make final concentration of 100 µg/ml.

2.4.2. Preparation of combined working standard solution:

The combined working standard solution was prepared by mixing 1 ml each of LER and ENM stock solution in a 10 ml volumetric flask and diluted up to mark with methanol. Aliquot of 1 ml of this mixture solution was transferred to 10 ml volumetric flask and diluted up to mark with methanol to get the final concentration of 10 µg/ml for LER and ENM.

2.4.3. Preparation of Synthetic Mixture[15]

Synthetic Mixture containing ENM 10 mg and LER 10 mg in combination was prepared in laboratory by using suitable excipients (prepared by mixing 10.0 mg ENM, 10.0mg LER, 5.0 mg talc powder, 20.0 mg maize starch and lactose, q.s., to 200 mg.

2.4.4. Solution Stability

Freshly prepared working solutions of LER (10 µg/ml) and ENM (10 µg/ml) were stored at Room temperature (25±2°C). The solutions were analyzed after 12 hours and compared with initial area.

2.5. Procedure for Calibration Curve

From combined working standard solution, five tracks of 4, 5, 6, 7 and 8 µl were spotted on a TLC plate. The TLC plate was developed, dried and analysed as describedunder chromatographic conditions [section 2.3]. Calibration curves were obtained by plotting peak area of both standard drugs against respective concentration of both drugs.

2.6. Validation of proposed method

2.6.1. Specificity

The specificity of the method was ascertained by analyzing drugs from standard and synthetic mixture. The bands of LER and ENM from the sample were confirmed by comparing the Rf and UV spectra of the respective band with those obtained from standard. The peak purity was assessed by comparing UV spectra acquired at three different positions of the band, i.e. peak start (s), peak apex (m), and peak end (e).

2.6.2. Linearity

Linearity response was determined by repeating procedure for calibration curve five times.Linearity curves for LER and ENM were obtained by plotting graph of mean peak area of five determinations vs. respective concentration of both drugs. Correlation coefficient and regression line were computed.

2.6.3. Precision

2.6.3.1. Repeatability of measurement of peak area

From combined working standard solution, 6 µl was spotted on a TLC plate. The plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. The band was scanned seven times without changing plate position and RSD for measurement of peak area was calculated for both drugs.

2.6.3.2. Repeatability of Sample application

From combined working standard solution, seven tracks of 6 µl were spotted on a TLC plate. The TLC plate was developed, dried and analysed as described under chromatographic conditions [Section 2.3]. The peak area of seven bands for each drug was measured and RSD of peak area was calculated for both drugs.

2.6.3.3. Intra-day and inter-day precision

Intra-day precision (RSD) was determined by analysing combined working standard solution over

the entire calibration range three times on same day. The inter-day precision (RSD) was determined by analysing combined working standard solution over the entire calibration range for three days.

2.6.4. Accuracy

The accuracy of the method was determined by calculating recovery of LER and ENR using the standard addition method. Accurately weighed known quantity of LER and ENR was spiked at 80, 100 and 120 % level to pre-analysed sample of LER and ENR.

2.6.5. Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ of the method were estimated from the set of five calibration curves using following equations.

LOD = 3.3 N/S and LOQ = 10 N/S, where N = Standard deviation of intercepts of five calibration curves, S = Mean slope of five calibration curves

2.6.6. Procedure for assay of synthetic mixture

The powder equivalent to 10 mg of LER & 10 mg of ENM was accurately weighed and transferred to 100 ml volumetric flask, 70 ml of methanol was added and the solution was sonicated for 10 min and diluted up to 100 ml with methanol and filtered through whatman filter paper No. 41. From the above solution 1 ml was transferred in to 10 ml volumetric flask and diluted up to mark with methanol. Two separate bands were spotted by applying 6 µl of each resulting solution for estimation of LER and ENM respectively. Plate was developed and analyzedas described under chromatographic conditions [Section 2.3]. Amount of LER and ENM was calculated using calibration curves of respective drugs.

3. RESULTS

3.1. Characterization of Drug Substance

Drug substances were characterized by melting point determination and studying the IR spectra.

3.2. Melting Point Determination

Melting points were found to be 116-120°C for Lercanidipine Hydrochloride and 140-144˚C for Enalapril Maleate . reported values of both drug 118-120 and 142-144°C Lercanidipine Hydhrochloride and Enalapril Maleate respectively.

3.3. Selection of wavelength

Working standard solutions of both drugs were scanned from 200 nm to 700 nm using UV spectrophotometer and the spectra were overlain. For simultaneous estimation 224 nm was selected as wavelength for detection (Figure 2).

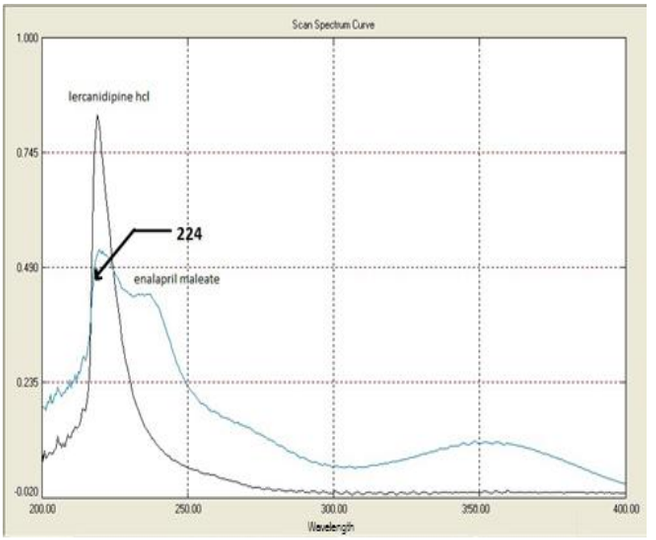


Figure 2. Overlain UV spectra of LER and ENM

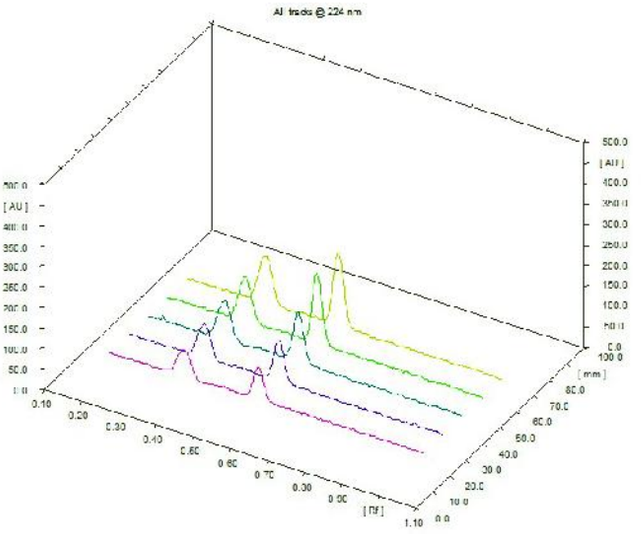


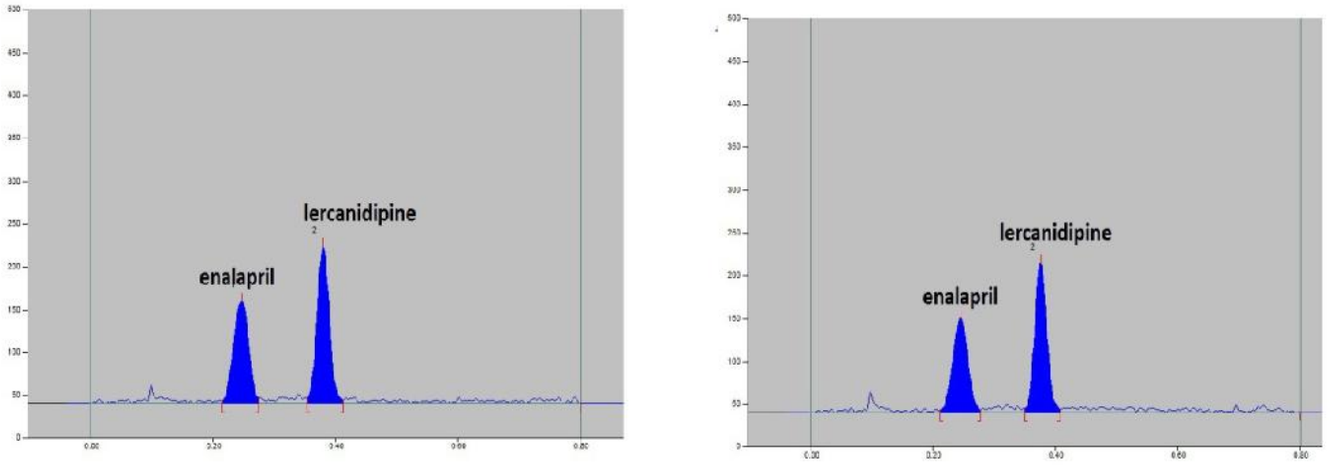
Figure 3. 3D Chromatogram of LER and ENM

3.4. Mobile phase optimization

Different solvent systems have been tried for separation of LER and ENM, but good separation was found to be in toluene: n-butanol: formic acid (6: 4: 1, v/v/v). The R_f values were found to be 0.34 ± 0.02 and 0.55 ± 0.02 for LER and ENM, respectively. TLC plates were observed in UV chamber using short UV (254 nm), long UV (366 nm) and white light.

3.5. Solution Stability Study

The peak area at initial time were found to be 2513.60 ± 30.62 and 2122.68 ± 31.32 for LER and ENM and after 12 hr 2492.04 ± 40.45 and 2067.44 ± 40.01 respectively .



(a) (b)

Figure 4. Comparison of Chromatogram of a) Standard and b) Sample

Table 1. Summary of Validation Parameters

Sr. No	Parameters	LER	ENM
1.	Linearity range (ng/band)	400 – 800	400 – 800
2.	Correlation Coefficient	0.9979	0.9961
3.	Precision (RSD)-		
	Repeatability of Sample Application	0.68	0.89
	Repeatability of Measurement of Peak Area	0.24	0.44
	Intra-Day	0.50-0.88	0.56-0.98
	Inter-Day	1.04-2.30	1.93-2.87
4.	Limit of Detection (LOD)	41.11	91.26
5.	Limit of Quantification (LOQ)	124.57	275.02
6.	Accuracy (%Recovery)	97.39 - 98.22	95.21 – 96.50
7.	Specificity	Specific	Specific

Table 2 . Data of Recovery Study for LER and ENM

Drug	Amount of drug in pre-analysed Sample (ng)	Amount of Standard Spiked (ng)	Total amount (ng/band)	Recovered amount (ng) ï SD (n=3)	Mean % Recovery
LER					
	300	240	540	235.74±1.05	98.22%
	300	300	600	293.78±2.86	97.68%
	300	360	660	350.63±1.10	97.39%
ENM					
	300	240	540	231.6±2.88	96.50%
	300	300	600	285.62 ±3.50	95.21%
	300	360	660	347.31 ±4.14	96.47%

Table 3: Assay Data for Synthetic Mixture

No	Amount of drug in synthetic mixture (mg)		Amount found (mg) (n=3)		Assay (%)	
	LER	ENM	LER	ENM	LER	ENM
1	10	10	9.75	9.44	99.50	99.51
2	10	10	9.61	9.40	99.22	99.20
3	10	10	9.86	9.42	99.73	99.32
Mean					99.48	99.34
Standard Deviation (n=3)					0.255	0.154
% RSD					0.25	0.15

3.6. Method Validation

3.6.1. Linearity and range

Representative calibration curves of LER and ENM were obtained by plotting mean peak area of LER and ENM against concentration over range of 400-800 ng/band (n=5) (Figure 3). They were found to be linear in above mentioned range with correlation coefficient of 0.9979 and 0.9961 for LER and ENM, respectively. RSD for LER and ENM were found to be in range of 1.29-1.79 % and 1.46-1.94 % respectively. Average linear regressed equation for curves were y=4.7136x + 401.82 and y=2.8506x + 428.48 for LER and ENM respectively.

3.6.2. Specificity

Comparison of chromatograms for LER and ENM from standard and that from synthetic mixture showed identical R_f values for both drugs, i.e. 0.34 ± 0.02 for LER and 0.55 ± 0.02 for ENM (Figure 4). Excipients and other components present in synthetic mixture did not interfere in separation and resolution of LER and ENM. Apart from R_f values, UV spectra of individual bands from sample were also correlated with spectrum of standard drugs.

3.6.3. Precision

Precision of instrument was checked by repeated scan of same band of respective drugs seven times without changing plate position and the RSD for measurement of peak area was found to be 0.24 % and 0.44 % for LER and ENM, respectively. Repeatability of sample application (RSD) for area of LER and ENM was found to be 0.68 % and 0.89 %, respectively which ensures the precision of spotting device. The intraday precision (RSD) was found to be in range of 0.50-0.88 % for LER and 0.56-0.98 % for ENM. RSD for inter-day precision was found to be in range of 2.12-2.94 % for LER and 2.14-2.87 % for ENM.(Table 1)

3.6.4. Accuracy

Accuracy was determined by standard addition method. The proposed method was applied for estimation of LER and ENM in their synthetic mixture after spiking with known quantity of standard drugs. The percentage recovery was found to be 97.39 % - 98.22 % for LER and 95.21 % - 96.50 % for ENM (Table 2)

3.6.5. LOD and LOQ

The LOD was found to be 41.11 ng/band for LER and 91.26 ng/band for ENM. The LOQ was found to be 124.57 ng/band for LER and 275.02 ng/band for ENM.

3.6.6. Assay of synthetic mixture

The bands at R_f0.34 for LER and 0.55 for ENM were observed in the chromatogram of the drug sample from synthetic mixture. The drug content was found to be 99.48 % ± 0.25 and 99.34% ± 0.15 for LER and ENM respectively (Table 3).

4. DISCUSSION

Purity of both drugs were confirmed by melting point and IR spectroscopy.Melting point for both the drugs matched with reported values of LER and ENM and the recorded IR spectra of the drugs were in concordance with reported spectra. Overlain spectra showed considerable absorbance for both the drugs at 224 nm and hence it was selected for estimation. In the selected mobiles phase, both drugs showed compact, sharp and well resolved bands. LER and ENM showed linear relationship

in the range of 400-800 ng/band respectively. Comparison of spectra scanned at peak start (s), peak apex (m) and peak end (e) positions of individual bands of sample LER and ENM showed high degree of correlation [i.e., r(s,m) = 0.9989 and r(m,e) = 0.9988], [r(s,m) = 0.9992 and r(m,e) = 0.9991] respectively confirmed purity of corresponding band. The percentage RSD for all the precision studies was found less than 3%, that indicates the developed method is precise. The percentage recovery for both drugs was found in the range of 95-105 %, which indicates method is accurate. LOD and LOQ was in nanogram level, hence method shows sensitivity.There was no additional peak observed in the chromatogram of synthetic mixture except LER and ENM that indicate no interference of excipients in estimation of LER and ENM in their synthetic mixture.

5. CONCLUSION

Novel HPTLC method was developed for estimation of LER and ENM in synthetic mixture. The method was validated as per ICH (Q2 R1) guidelines. The proposed method was found to be specific, accurate, precise, sensitive and cost effective. The developed method was successfully applied for quantitative analysis of LER and ENM in their synthetic mixture. The proposed method can be applied for routine analysis of LER and ENM.

6. ACKNOWLEDGEMENT

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