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# High Performance Thin Layer Chromatographic determination of Enalapril maleate Hydrochlorothiazide in Pharmaceutical dosage form

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**Abstract:** A simple and precise High-performance thin-layer chromatographic (HPTLC) method for determination of Enalapril Maleate and Hydrochlorothiazide (HTZ) in their bulk and tablet dosage forms has been developed and validated. This employs a precoated silica gel $60F_{254}$ on aluminium sheets and mobile phase was Ethanol: Toluene in the ratio of 7:3 v/v. having chamber saturation for 20 minutes at room temperature. The TLC plate was run up to 8 cm. Detection was performed at 211 nm. The RF values were 0.52 for Enalapril Maleate and 0.83 for Hydrochlorothiazide.

The developed method validated for its accuracy and precision. The LOD and LOQ were found to be 0.015 *µg/ml and* 0.047 *µg/ml* 0.018 *µg/ml and* 0.056 *µg/ml,* for Enalapril Maleate and Hydrochlorothiazide. The recovery was carried out by standard addition method. The Average recovery was found to be 99.22% and 100.73% for Enalapril Maleate and Hydrochlorothiazide respectively.

**Keywords:** High Performance Thin layer chromatography, Method validation, Enalapril Maleate and Hydrochlorothiazide.

## INTRODUCTION:[1,2,3]

Enalapril maleate Angiotensin converting enzyme inhibitor after oral administration Enalapril maleate is hydrolysed by enzyme esterase in liver to produce parent dicarboxylic acid as enalaprilate, which is a highly potent inhibitor of ACE. Chemically it is (2*S*)-1-[(2*S*)-2-[[(1*S*)-1-(Ethoxycarbonyl)-3-phenylpropyl] amino] propanoyl]pyrrolidine-2-carboxylic acid (*Z*)-butenedioate.

Hydrochlorothiazide is a Thiazide diuretic. Inhibits water reabsorption in the nephron by inhibiting the sodium-chloride symporter in the distal convoluted tubule, which is responsible for 5% of total sodium reabsorption. Chemically it is 6-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide1,1-dioxide.

Literature survey reveals that availability of several methods for estimation of Enalapril Maleate and Hydrochlorothiazide includes UV spectrophotometry, RP-HPLC and HPTLC alone or in combination with other drugs. No method has been reported for the estimation of Enalapril Maleate and Hydrochlorothiazide in combined dosage form. Present work emphasizes on the quantitative estimation of by high performance thin layer chromatography.[3,4]

## MATERIALS AND METHODS:

### EXPERIMENTAL:

**Table 1: Instrument specification**

| Instrument | Description |
|---|---|
| HPTLC system | Camag HPTLC system |
| Sample applicator | camag linomat V automatic TLC sample applicator |
| Scanner | CAMAG TLC Scanner |
| Software | Camag Wincats software (version 1.4.5) |
| Saturation chamber | Camag Twin-trough chamber (10×10cm) / (20×10 cm) |
| HPTLC plates | Merck HPTLC plates coated with silica gel 60 $F_{254}$ (0.2 mm thickness) on aluminium sheets |
| Syringe | Hamilton syringe (100µl) |

### REAGENTS AND STANDARDS :

Enalapril Maleate and Hydrochlorothiazide standards were obtained from Cipla Ltd, Mumbai, India. Analytical grade methanol, ethanol, toluene, were purchased from Merck Chemicals, India and formulation containing Enalapril Maleate and Hydrochlorothiazide (Enace-D) were used.

### PREPARATION OF WORKING STANDARD SOLUTIONS:

Standard stock solutions of Enalapril Maleate and Hydrochlorothiazide were prepared by dissolving 10 mg of Enalapril Maleate and 10 mg of Hydrochlorothiazide bulk drug in 10 ml of methanol to get a concentration of 1000µg/ml for Enalapril Maleate and 1000µg/ml for Hydrochlorothiazide separately. The standard working solutions were prepared by dilution of the stock standard solution with methanol to reach a concentration of 100µg/ml Enalapril Maleate for and 250µg/ml for Hydrochlorothiazide respectively.

### PREPARATION OF SAMPLE SOLUTIONS:

Twenty tablets were weighed accurately, finely powdered and powder equivalent to 5 mg of Enalapril Maleate was weighed accurately and dissolved in 10ml of methanol, solution was sonicated for 20 min, allowed the solution to cool to room temperature and then volume made up to the mark with methanol, the resulting solution was used for further dilutions. Required dilutions were made to get 100 µg/ml for Enalapril Maleate and 250 µg/ml for Hydrochloro - thiazide respectively.

### CHROMATOGRAPHIC CONDITIONS:[5]

The experiment was performed on a silica gel 60 $F_{254}$ (0.2 mm thickness) HPTLC plates (20×10cm) without prewashing. Samples were applied to the plates as 8 mm bands, 8 mm apart and 10 mm from the edges of the plate, with a Camag Linomat automatic sample applicator; the speed of sample application was 10 mm s–1. The plates were developed by the ascending technique, to a distance of 8.0 cm, at 25 ± 5°C, relative humidity 50–60%, in a Camag twin-trough glass chamber with a stainless steel lid, using a mobile phase Ethanol: Toluene in the ratio of 7:3v/v. and the chamber saturation time 20 min. After development, plates were dried with a hot-hair dryer, viewed in a Camag UV cabinet, and then scanned with a Camag TLC Scanner, using win CATS software (version 1.4.5) , in absorbance mode, with slit dimensions 6.00 x 0.45 mm, Micro. The detection wavelength 211 nm, was selected by overlain spectra of the drugs acquired in-situ from the plate. The *R*f values of 0.52 and 0.83 for Enalapril Maleate and Hydrochlorothiazide, respectively.

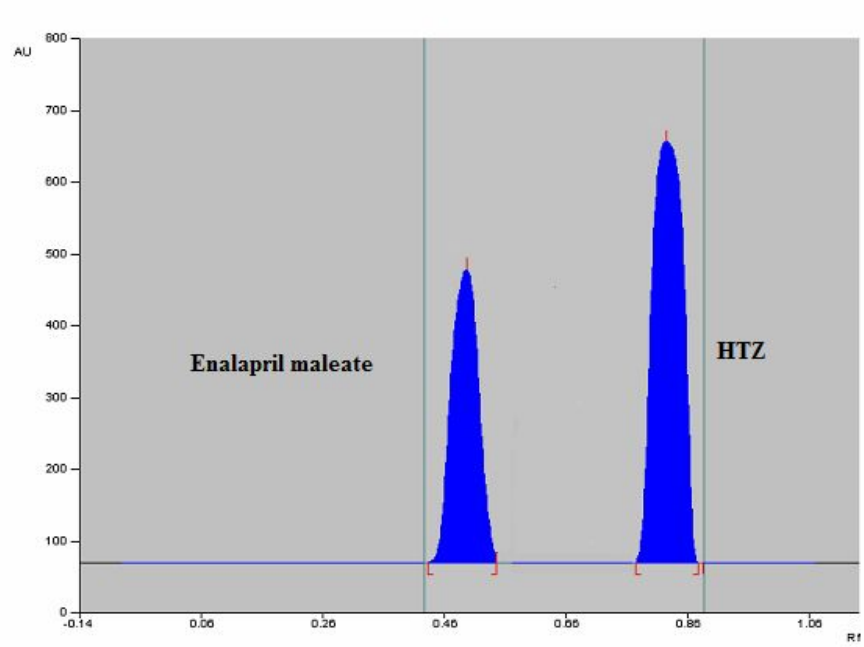


**Figure 1. Typical chromatograms obtained in analysis for Enalapril Maleate and Hydrochlorothiazide by HPTLC.**

**METHOD VALIDATION:**[6]

**LINIARITY:**

The linearity of the method was evaluated by triplicate analysis of standard solutions containing 100 ng to 500 ng of (figure: 2) and 250 ng to 1250 ng Hydrochlorothiazide. (figure: 3) Peak area was recorded for each concentration and a calibration plot was obtained by plotting peak area against concentration. Correlation coefficients $R^2$ were 0.999 for Enalapril Maleate and 0.998 for Hydrochlorothiazide. The average linear equations were for Enalapril Maleate y = 3031.5x + 397 and y = 2639.9x + 532 for Hydrochlorothiazide.

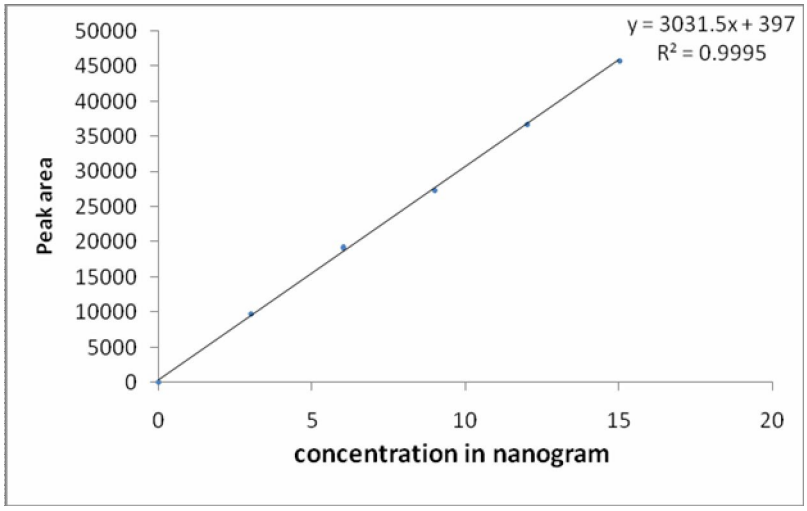


**Figure 2: linearity of Enalapril Maleate.**

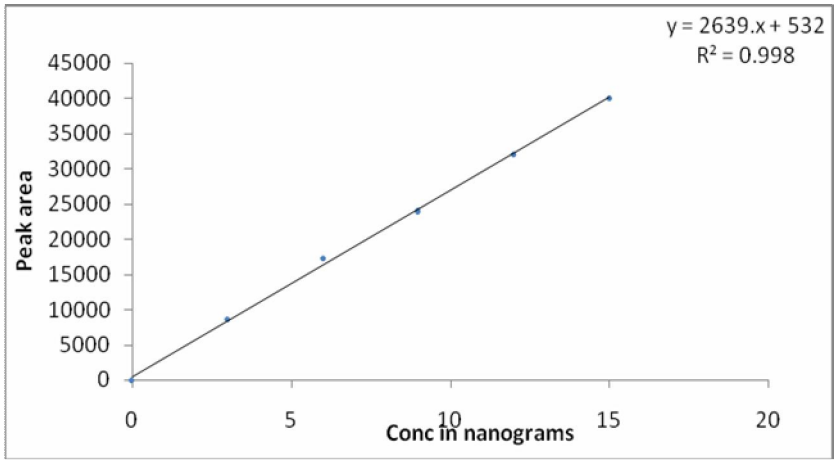


**Figure 3: linearity of Hydrochlorothiazide.**

**LIMIT OF DETECTION AND LIMIT OF QUANTITAON:**[6]

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the slope (s) of the calibration plot and the standard deviation of the response (SD). The values were found to be0.015 *µg/ml and* 0.045 *µg/ml* 0.018 *µg/ml and* 0.054 *µg/ml,* respectively for Enalapril Maleate and Hydrochlorothiazide.

**PHARMACUTICAL PREPARATION ASSAY, ACCURACY, AND PRECISION EVALUATION:**[6]

The amounts of Enalapril Maleate and Hydrochlorothiazide were found by the number of replicates of both pharmaceutical preparations (n=3) performed by the inter-day assays. The statistical parameter and results are reported in Table 2. These results were in close agreement to the label claim of the pharmaceutical preparations, and the standard deviation observed for both the drugs were very low.

**RECOVERY:**[6]

To confirm the accuracy of the proposed method, recovery experiments were carried out by standard addition technique by adding a known amount of standard to the sample. Each level was repeated three times ($n$ = 3). Results and statistical parameters are reported in Table 3. from the amount of drug found, the percentage recovery was calculated.

**Table 2. Evaluation of Enalapril Maleate and Hydrochlorothiazide amounts in pharmaceutical formulations (*n = 3*)**

| Brand | Sr. No. | Amount of Enalapril Maleate found (label claim: 5 mg per tablet) | Amount of Hydrochlorothiazide found (label claim: 12.5mg per tablet) |
|---|---|---|---|
| Enace D 5 | 1 | 4.96 | 12.47 |
| | 2 | 4.97 | 12.48 |
| | 3 | 4.94 | 12.47 |
| Mean assay | | 4.957 | 12.47 |
| Mean assay (%) | | 99.14 | 99.76 |
| Standard deviation | | 0.0152 | 0.0115 |

**Table 3. Recovery studies (*n = 3*)**

| Drug | Recovery level (%) | Initial amount (nanogram) | Amount added (nanogram) | Recovery (%) | Mean | Standard deviation |
|---|---|---|---|---|---|---|
| Enalapril Maleate | 80 | 500 | 400 | 99.91 | 99.86 | 0.17018 |
| | 100 | 500 | 500 | 99.77 | | |
| | 120 | 500 | 600 | 99.92 | | |
| Hydrochlorothiazide | 80 | 1250 | 1000 | 99.57 | 99.44 | 0.12602 |
| | 100 | 1250 | 1250 | 99.47 | | |
| | 120 | 1250 | 1500 | 99.28 | | |

## DISCUSSION

The mobile phase Ethanol: Toluene: in the ratio of 7:3 v/v enables good resolution of the drugs on silica gel 60 $F_{254}$ TLC plates The *R*f values were 0.52 for a Enalapril Maleate and 0.83for Hydrochlorothiazide (Figure 1). The conditions used for chromatography were optimized on the basis of experimentation.

Plots of concentration against peak area were linear over the ranges 100-500 ng $mL^{-1}$ for Enalapril Maleate and 250-1250 ng $mL^{-1}$ for Hydrochlorothiazide; the correlation coefficients (r) were 0.999 for Enalapril Maleate and 0.998 for Hydrochlorothiazide. Recovery of the drugs from the sample, evaluated on the basis of standard additions, was % and % for Enalapril Maleate and Hydrochlorothiazide respectively, indicating the accuracy of the method and non-interference from the excipients.

## CONCLUSION:

First time, a HPTLC method was developed for analysis of Enalapril Maleate and Hydrochlorothiazide in pharmaceutical dosage form. Use of HPTLC enables analysis of several samples at the same time. The method is very simple, rapid and provides accurate and precise results. The whole procedure may be useful for screening of pharmaceutical preparations.

## ACKNOWLEDGEMENT

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