World Journal of Pharmaceutical Science and Research

www.wjpsronline.com

Research Article

ISSN: 2583-6579
SJIF Impact Factor: 6.916
Year - 2026
Volume: 5; Issue: 9
Page: 340-364

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE SIMULTANEOUS DETERMINATION OF ENALAPRIL MALEATE, HYDROCHLOROTHIAZIDE AND PARACETAMOL IN BULK DRUGS AND PHARMACEUTICAL TABLETS USING UV SPECTROPHOTOMETRY AND RP-HPLC

Dr. Prashanthi Evangelin, D. Simhadri*, K. Vidya Sagar, A. Suresh Kumar Reddy

Department of Analysis, Sims College of Pharmacy, Guntur, 522001, India.

Article Received: 13 July 2026 | | ***Article Revised: 03 August 2026*** | | ***Article Accepted: 23 August 2026***

***Corresponding Author: D. Simhadri**
Department of Analysis, Sims College of Pharmacy, Guntur, 522001, India.
DOI: https://doi.org/10.5281/zenodo.22159902

How to cite this Article: Dr. Prashanthi Evangelin, D. Simhadri, K. Vidya Sagar, A. Suresh Kumar Reddy (2026) DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE SIMULTANEOUS DETERMINATION OF ENALAPRIL MALEATE, HYDROCHLOROTHIAZIDE AND PARACETAMOL IN BULK DRUGS AND PHARMACEUTICAL TABLETS USING UV SPECTROPHOTOMETRY AND RP-HPLC. World Journal of Pharmaceutical Science and Research, 5(9), 340-364.

ABSTRACT

The objective of the present investigation was to establish and validate a convenient, sensitive, precise, and reproducible RP-HPLC procedure for the simultaneous determination of Enalapril Maleate, Hydrochlorothiazide, and Paracetamol in bulk materials and pharmaceutical tablet preparations. Chromatographic conditions were systematically optimized by evaluating the column, mobile-phase composition, flow rate, detection wavelength, and column temperature. The optimized separation was achieved using a Thermo Hypersil-BDS C8 column (250 × 4.6 mm, 5 µm) with a 75:25 (v/v) mixture of phosphate buffer, prepared from potassium dihydrogen phosphate and dipotassium hydrogen phosphate, and acetonitrile as the mobile phase. An isocratic elution mode was employed at a flow rate of 1.0 mL/min, with detection performed at 210 nm and the column maintained at 30°C. A 20 µL injection volume was used, and the mobile phase served as the diluent. During method development, different chromatographic conditions were investigated to obtain satisfactory separation, peak symmetry, resolution, and column efficiency. The optimized procedure provided distinct and adequately resolved responses for all three analytes with suitable chromatographic characteristics. The analytical procedure was validated according to ICH recommendations by assessing parameters such as specificity, linearity, accuracy, precision, robustness, ruggedness, and system suitability. The validation results indicated that the method possesses suitable performance characteristics for simultaneous quantitative analysis of Enalapril Maleate, Hydrochlorothiazide, and Paracetamol. The applicability of the proposed method was demonstrated through the analysis of pharmaceutical tablet formulations containing the selected drug combination. The procedure requires relatively simple chromatographic conditions and provides reproducible analytical results, making it appropriate for routine quality-control testing of bulk drugs and finished pharmaceutical dosage forms. Thus, the developed RP-HPLC method provides a practical analytical approach for the simultaneous determination of Enalapril Maleate, Hydrochlorothiazide, and Paracetamol.

KEYWORDS: Enalapril Maleate; Hydrochlorothiazide; Paracetamol.

INTRODUCTION

Pharmaceutical analysis is an essential part of drug development and quality control because the identity, purity, strength, and consistency of pharmaceutical products must be established before they are used for therapeutic purposes. Analytical procedures provide the scientific basis for determining the amount of active pharmaceutical ingredients present in bulk materials and finished dosage forms. When more than one active ingredient is present in a formulation, the analytical procedure must be capable of distinguishing and accurately measuring each component without interference from the other ingredients or formulation excipients. Therefore, the development of suitable, reliable, and validated analytical methods is an important requirement in pharmaceutical research.

Chromatographic techniques are among the most useful approaches for pharmaceutical analysis because they permit separation of individual components from mixtures before their identification or quantification. Chromatography operates through the differential distribution of substances between a stationary phase and a mobile phase. Differences in the physicochemical properties of analytes determine the extent to which they interact with each phase and consequently influence their movement through the chromatographic system. This principle makes chromatography particularly useful when several compounds are present simultaneously in a sample.

High-performance liquid chromatography (HPLC) is a widely utilized chromatographic technique for the analysis of pharmaceutical substances. In HPLC, the mobile phase is passed through a column packed with a suitable stationary material under pressure. The use of small stationary-phase particles provides efficient interaction between the analytes and the chromatographic system, resulting in good separation within a relatively short period. HPLC is therefore applicable to the assay of active ingredients, determination of impurities, evaluation of pharmaceutical formulations, and other quantitative analytical investigations.

Reversed-Phase high-performance liquid chromatography

Several modes of HPLC are available, and the selection of a particular mode depends mainly on the chemical characteristics of the compounds being analyzed. Normal-phase, reversed-phase, ion-exchange, size-**exclusion, chiral,** and ion-pair chromatographic approaches are among the commonly used modes. Reversed-phase chromatography is particularly important in pharmaceutical analysis because it can accommodate a broad range of organic compounds and is suitable for many polar and moderately polar drug substances.

In reversed-phase HPLC (RP-HPLC), the stationary phase is comparatively hydrophobic and the mobile phase is more polar. Columns containing chemically bonded alkyl groups, particularly C8 and C18 phases, are frequently used. The separation of analytes is largely associated with their different interactions with the non-polar stationary phase. Compounds that interact weakly with the stationary phase generally pass through the column earlier, whereas compounds having stronger hydrophobic interactions require a longer time for elution.

The composition of the mobile phase is an important factor in achieving satisfactory chromatographic separation. Aqueous buffers may be **combined** with organic solvents such as methanol or acetonitrile, and changes in solvent composition, pH, and flow rate can significantly influence retention and resolution. Consequently, appropriate optimization of these variables is required when developing an RP-HPLC procedure for simultaneous estimation of multiple pharmaceutical ingredients.

HPLC method development

The development of an HPLC method involves a systematic evaluation of chromatographic conditions until adequate separation and reliable quantitative performance are achieved. Information regarding the analytes, including their chemical structure, solubility, polarity, ionization characteristics, stability, and ultraviolet absorption properties, can assist in selecting the initial experimental conditions. Sample preparation, column selection, mobile-phase composition, flow rate, temperature, injection volume, and detection wavelength are among the important variables considered during method development.

Column selection is particularly important because the stationary phase determines the nature and extent of analyte retention. Parameters such as column dimensions, packing material, particle size, pore characteristics, and bonded phase can affect separation efficiency and peak characteristics. In reversed-phase analysis, C8 and C18 columns are commonly considered for pharmaceutical compounds. A suitable column should provide satisfactory retention, good peak symmetry, adequate resolution, and reproducible chromatographic performance.

Optimization of the mobile phase is another critical stage of method development. The proportion and nature of organic solvent, aqueous buffer, buffer strength, and pH can alter analyte retention and selectivity. The selected conditions should provide adequate separation of all target compounds while maintaining reasonable analysis time and acceptable peak shape. Flow rate also influences retention time, column back pressure, resolution, and peak symmetry and must therefore be appropriately adjusted.

The present project describes a series of trials performed to obtain suitable chromatographic conditions for the simultaneous determination of Enalapril Maleate, Hydrochlorothiazide, and Paracetamol. Different mobile-phase compositions and chromatographic conditions were evaluated before selecting the optimized system. The final conditions reported in the project employed a mixed phosphate buffer and acetonitrile in a 75:25 ratio, an isocratic flow rate of 1.0 mL/min, a C8 column, and UV detection at 210 nm. These conditions produced satisfactory separation, plate count, peak shape, and retention characteristics.

Analytical method validation

Method development is followed by validation to demonstrate that the established analytical procedure is appropriate for its intended application. Validation provides documented evidence that an analytical method can generate consistent and dependable results when used under specified conditions. It is particularly important for pharmaceutical analysis because analytical results are directly associated with the quality and conformity of drug substances and finished dosage forms.

Important validation characteristics include specificity, linearity, accuracy, precision, detection capability, quantification capability, range, robustness, and system suitability. Specificity refers to the ability of an analytical procedure to determine the analyte in the presence of other substances that may be present in the sample. Linearity establishes the relationship between the analytical response and concentration over a defined working range. Accuracy indicates how closely the measured value corresponds to the accepted or reference value, whereas precision reflects the closeness of agreement between repeated measurements.

The limit of detection and limit of quantification describe the analytical capability at low analyte concentrations. Robustness evaluates whether small deliberate changes in experimental conditions significantly influence the analytical result. System suitability testing is used to verify that the complete chromatographic system is functioning appropriately before analytical measurements are performed. Parameters such as retention time, theoretical plate count, resolution, peak symmetry, tailing factor, and repeatability can be considered when evaluating chromatographic performance.

Present study

The present investigation is directed toward the development and validation of an RP-HPLC method for the simultaneous estimation of Enalapril Maleate, Hydrochlorothiazide, and Paracetamol in bulk drug substances and pharmaceutical dosage forms. The project identifies the need for an analytical procedure capable of determining these three active ingredients together and describes the optimization of chromatographic conditions for this purpose.

Enalapril Maleate is an antihypertensive drug, Hydrochlorothiazide is an antihypertensive and diuretic agent, and Paracetamol is an analgesic and antipyretic drug. The simultaneous presence of these active ingredients requires an analytical procedure capable of providing adequate separation and reliable quantification of each component. The study therefore involves selection of suitable chromatographic conditions, optimization of the mobile phase and flow rate, determination of an appropriate detection wavelength, and evaluation of the developed method through validation studies.

The developed RP-HPLC procedure is intended to provide a practical analytical approach for routine examination of the selected drug combination. According to the project, the optimized chromatographic system provided satisfactory separation of the three analytes and was subsequently subjected to analytical validation. The study therefore demonstrates the application of RP-HPLC as a suitable technique for simultaneous pharmaceutical analysis of Enalapril Maleate, Hydrochlorothiazide, and Paracetamol.

MATERIALS AND METHODS

Chemicals, Reference Standards and Reagents

Enalapril maleate, hydrochlorothiazide and paracetamol working standards were used for method development and validation. The pharmaceutical formulation containing enalapril maleate, hydrochlorothiazide and paracetamol was also analyzed. Potassium dihydrogen orthophosphate, dipotassium hydrogen phosphate, orthophosphoric acid, HPLC-grade methanol, HPLC-grade acetonitrile, triethylamine and double-distilled water were used during the analytical procedure. According to the project records, the chemicals and reagents were obtained from Fisher, Merck and other listed suppliers, while the drug standards were obtained from the respective pharmaceutical manufacturers. The source document identifies enalapril maleate with Taro Pharmaceuticals, hydrochlorothiazide with Novartis and paracetamol with Ranbaxy.

Instruments and Equipment

Chromatographic analysis was performed using a Waters Alliance 2695 HPLC system equipped with a UV detector and operated using Empower 2 software. UV spectrophotometric measurements were performed using a Shimadzu 1800 UV-visible spectrophotometer operated with UV Win software. An ER200A analytical balance, SE60US sonicator and

AD102U pH meter were also used. The corresponding manufacturers recorded in the project were Waters, Labindia, Ascoset, Enertech and Adma, respectively.

Method Development

The RP-HPLC method was developed for the simultaneous determination of enalapril maleate, hydrochlorothiazide and paracetamol in bulk drug substances and pharmaceutical tablets. Initial chromatographic conditions were selected on the basis of the literature survey and the physicochemical characteristics of the analytes. Reversed-phase chromatography was selected because it is suitable for the separation of polar to moderately polar pharmaceutical compounds.

Selection of Detection Wavelength

Individual standard solutions of enalapril maleate, hydrochlorothiazide and paracetamol were prepared at a concentration of 10 µg/mL and scanned in the UV-visible region from 400 to 200 nm. The overlaid spectra were examined, and 210 nm was selected as the detection wavelength for the RP-HPLC method.

Optimization of Chromatographic Conditions

Several chromatographic trials were performed by varying the stationary phase, mobile-phase composition and flow rate. The initial trial employed acetonitrile and water in a 50:50 ratio on a C18 Nucleosil column (125 × 4.6 mm, 5 µm), at a flow rate of 1.0 mL/min and detection at 210 nm. The chromatographic separation was performed isocratically at 30 °C with a 20 µL injection volume. The observed USP plate count was low.

In the second trial, 0.01 M phosphate buffer and acetonitrile were used at a ratio of 60:40 with the same C18 Nucleosil column and chromatographic conditions. The plate count remained unsatisfactory and the resolution was inadequate.

In subsequent trials, different buffer systems and stationary phases were evaluated. A 0.01 M sodium dihydrogen phosphate buffer–acetonitrile system and C18 columns were examined. Further optimization using a C18 Zodiac column and an ammonium acetate buffer–acetonitrile system was also carried out. These trials showed inadequate resolution and/or additional peaks, with unsatisfactory peak symmetry for enalapril.

Optimized RP-HPLC Conditions

The final chromatographic conditions were selected after optimization because they provided satisfactory separation and resolution, a USP plate count greater than 2000, acceptable peak symmetry and relatively short retention times. The optimized method employed a **Thermo Hypersil-BDS C8 column (250 × 4.6 mm, 5 µm)**. The mobile phase consisted of mixed phosphate buffer and acetonitrile in a ratio of 75:25 (v/v). The flow rate was maintained at 1.0 mL/min, the detection wavelength was 210 nm and the column temperature was maintained at 30 °C. The injection volume was 20 µL and the method was operated under isocratic conditions. The mobile phase was also used as the diluent, while water (90:10, v/v) was used as the needle-wash solution.

The optimized procedure represents a modification of the chromatographic conditions investigated during the initial development trials, particularly with respect to the stationary phase and mobile-phase composition. The project literature review reports previously published RP-HPLC procedures for simultaneous determination of enalapril maleate and hydrochlorothiazide and related combinations; these publications provided the basis for the present method-development work.

Preparation of Phosphate Buffer

For preparation of the mixed phosphate buffer, 2.95 g of potassium dihydrogen orthophosphate and 0.55 g of dipotassium hydrogen phosphate were accurately weighed and transferred into a 1000 mL volumetric flask. Distilled water was added and the solution was sonicated until the salts were completely dissolved. The prepared buffer was subsequently used for preparation of the mobile phase.

Preparation of Mobile Phase

The phosphate buffer and acetonitrile were mixed in the ratio of 75:25 (v/v). The resulting mobile phase was sonicated and subsequently degassed by vacuum filtration through a 0.4 µm membrane filter before use. The degassed mobile phase was used as the chromatographic mobile phase and as the diluent for preparation of standard and sample solutions.

Preparation of Standard Stock Solution

Accurately weighed quantities of 10 mg enalapril maleate, 325 mg paracetamol and 25 mg hydrochlorothiazide working standards were transferred into a 100 mL volumetric flask. Approximately 50 mL of diluent was added and the solution was sonicated to ensure complete dissolution. The solution was then diluted to volume with the same diluent and mixed thoroughly.

Preparation of Standard Solution

Ten millilitres of the prepared standard stock solution were transferred into a 100 mL volumetric flask and diluted to volume with the diluent. The resulting solution was used as the working standard solution for chromatographic analysis.

Preparation of Tablet Sample Solution

Twenty tablets were accurately weighed and finely powdered. An amount of the powdered tablet sample equivalent to 10 mg of enalapril maleate, 325 mg of paracetamol and 25 mg of hydrochlorothiazide was accurately transferred into a 100 mL volumetric flask. Approximately 50 mL of diluent was added, and the mixture was sonicated for 10 min to facilitate complete extraction and dissolution of the active ingredients. The solution was then diluted to volume with the same diluent and mixed thoroughly.

The resulting solution was filtered through filter paper to remove insoluble excipients. Ten millilitres of the filtrate were subsequently transferred into a 100 mL volumetric flask and diluted to volume with mobile phase. The final solution was used for chromatographic determination.

Chromatographic Procedure

Blank, placebo, standard and sample solutions were prepared as described above. A 20 µL volume of each solution was injected into the HPLC system under the optimized chromatographic conditions. Blank and placebo chromatograms were examined to identify any interfering peaks. Standard and sample chromatograms were subsequently recorded and the peak areas corresponding to enalapril maleate, hydrochlorothiazide and paracetamol were used for quantitative determination.

Assay Calculation

The amount of each active pharmaceutical ingredient present per tablet was calculated by comparing the average peak area obtained from the sample solution with that obtained from the standard solution, while taking into consideration the dilution factors, standard purity, molecular factor and average tablet weight. The percentage of labeled amount was calculated by comparing the calculated amount per tablet with the respective label claim.

Analytical Method Validation

The optimized RP-HPLC method was validated using the analytical performance characteristics described in the project and based on ICH validation principles. The validation characteristics included accuracy, precision, linearity, ruggedness, robustness and specificity. The project also describes system suitability evaluation as an important component of chromatographic method validation.

Accuracy

Accuracy was evaluated by the standard-addition approach at 80%, 100% and 120% of the target assay concentration. Appropriate quantities of enalapril maleate, hydrochlorothiazide and paracetamol working standards were accurately weighed and transferred into volumetric flasks, dissolved with diluent using sonication and diluted to volume.

For the 80% level, 80 mg of the combined working standards were initially dissolved and diluted to volume. Appropriate aliquots corresponding to 32.5 mL paracetamol, 2.5 mL hydrochlorothiazide and 1 mL enalapril were transferred into a 100 mL volumetric flask and diluted to volume with diluent.

The 100% and 120% accuracy solutions were prepared similarly using 100 mg and 120 mg quantities, respectively. The prepared solutions were injected into the HPLC system and the amount found, percentage recovery and mean recovery were calculated for each analyte. The acceptance criterion specified in the project was 98.0–102.0% recovery.

Precision

Precision was assessed by replicate analysis of the standard solution under the optimized chromatographic conditions. A standard stock solution was prepared by dissolving 10 mg of enalapril maleate, 325 mg of paracetamol and 25 mg of hydrochlorothiazide in 100 mL of diluent. Ten millilitres of this solution were diluted to 100 mL with diluent to obtain the standard preparation.

The standard solution was injected repeatedly and the peak areas were recorded. Precision was expressed as percentage relative standard deviation (%RSD), calculated from the standard deviation and mean response. The project specifies an acceptance criterion of not more than 2% RSD for replicate standard injections.

Linearity

Linearity was evaluated by preparing standard solutions at different concentration levels covering the working range of the analytical method. The project reports concentrations ranging from 2.5–15.0 µg/mL for enalapril maleate, 6.25–37.50 µg/mL for hydrochlorothiazide and 81.25–487.50 µg/mL for paracetamol. The reported correlation coefficient for each analyte was 0.999.

Specificity

Specificity was evaluated by injecting blank, placebo, individual standards, mixed standard and sample solutions. The chromatograms were examined for interference from the diluent, formulation excipients and other components at the retention positions of the analytes. The project reports that no significant interference from blank or placebo was observed at the principal analyte peaks.

Robustness

Robustness was assessed by deliberately varying selected chromatographic parameters. The project reports evaluation by changing the flow rate by ±10% and the column temperature by ±5 °C. The system suitability parameters remained acceptable under the investigated conditions, with no meaningful change in retention time or peak area.

Ruggedness

Ruggedness was evaluated by examining the performance of the analytical method under changed analytical circumstances. The reported ruggedness values were 0.360% RSD for enalapril maleate, 0.319% RSD for hydrochlorothiazide and 0.412% RSD for paracetamol, all within the stated acceptance criterion of not more than 2% RSD.

System Suitability

Before routine analysis, chromatographic system suitability was evaluated using replicate injections of the standard solution. Parameters such as theoretical plate count, tailing factor, resolution and percentage RSD were considered. The project specifies desirable system suitability criteria including a capacity factor greater than 2.0, theoretical plates greater than 2000, resolution greater than 2 between the analyte and the nearest potential interferent, and a tailing factor below 2.

Reference to Previously Published Procedures

The method-development strategy was based on the literature cited in the project, including previously reported RP-HPLC methods for enalapril maleate and hydrochlorothiazide and related pharmaceutical combinations. The project specifically cites the work of Pawar et al. describing an RP-HPLC method for simultaneous estimation of enalapril maleate, hydrochlorothiazide and paracetamol in bulk and tablet dosage forms. Other cited reports include methods developed by Uslu et al., Nikolova Maslarska et al., Dinç et al. and Carlucci et al.

The present procedure was optimized experimentally from these literature-guided starting conditions. The major modification was the selection of a Thermo Hypersil-BDS C8 column together with a mixed phosphate buffer–acetonitrile mobile phase at 75:25 (v/v), a flow rate of 1.0 mL/min and UV detection at 210 nm. These optimized conditions produced satisfactory chromatographic separation, resolution, plate count and peak shape according to the project results.

Note on Manufacturer Addresses

The supplied project records the manufacturers/suppliers of the principal instruments and materials but **does not provide complete postal addresses for those manufacturers**. Therefore, addresses have not been invented or added here. The manuscript should insert the verified manufacturer address for each trade-name instrument, column and

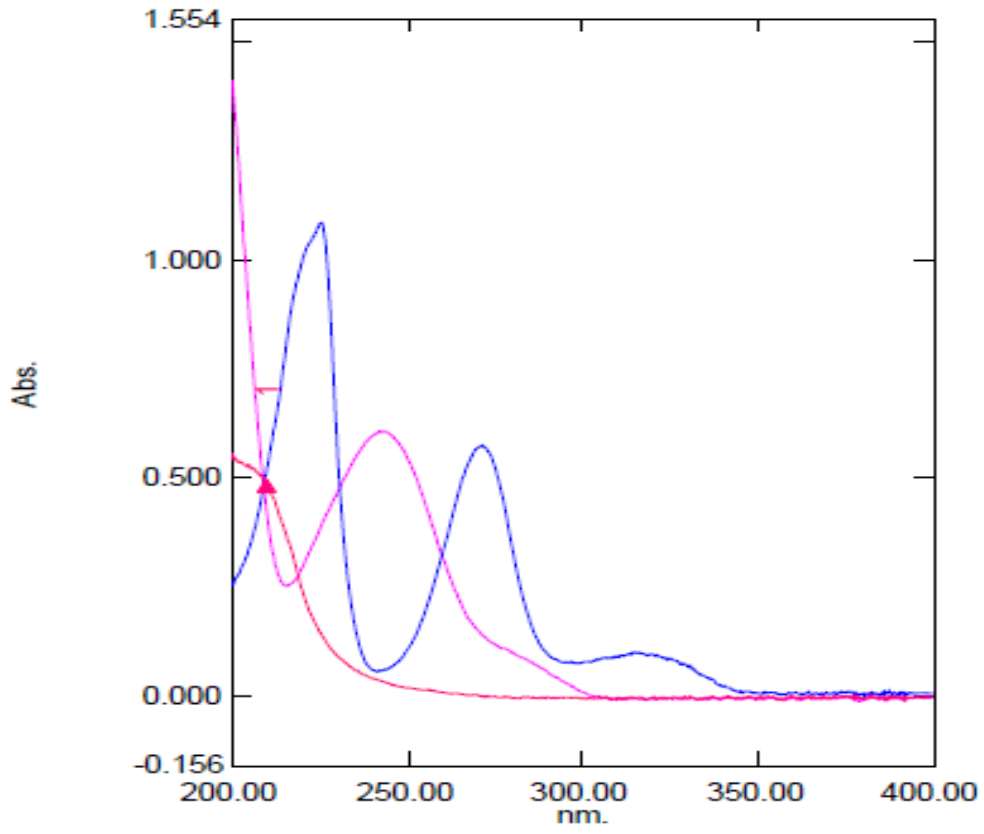
relevant reagent before journal submission. This preserves the source information without introducing unsupported details.

RESULTS AND DISCUSSION

1. Optimization of RP-HPLC Method

The RP-HPLC method was optimized by evaluating different stationary phases and mobile-phase compositions. Initial chromatographic trials produced inadequate plate counts, poor resolution, additional peaks and, in some cases, unsatisfactory peak symmetry. The optimized conditions consisted of a mixed phosphate buffer containing potassium dihydrogen phosphate and dipotassium hydrogen phosphate with acetonitrile in the ratio of 75:25 (v/v), using a Thermo Hypersil-BDS C8 column (250 × 4.6 mm, 5 µm), a flow rate of 1.0 mL/min and UV detection at 210 nm.

The optimized method provided satisfactory chromatographic separation, acceptable peak symmetry and a theoretical plate count greater than 2000. The mean retention times reported for enalapril maleate, paracetamol and hydrochlorothiazide were approximately 2.925, 3.825 and 4.583 min, respectively.



No.	Wavelength	Absorbance	Description
1	210.20	0.484	HCZ_Para_E
2			

Figure 1: UV Overlay Spectrum.

UV spectrum was recorded for Enalapril, Hydrochlorothiazide and Paracetamol individually. The isobestic point of Enalapril, Hydrochlorothiazide and Paracetamol was found to be at 210nm. UV spectrum is shown in figure 1.

ASSAY

CHROMATOGRAMS

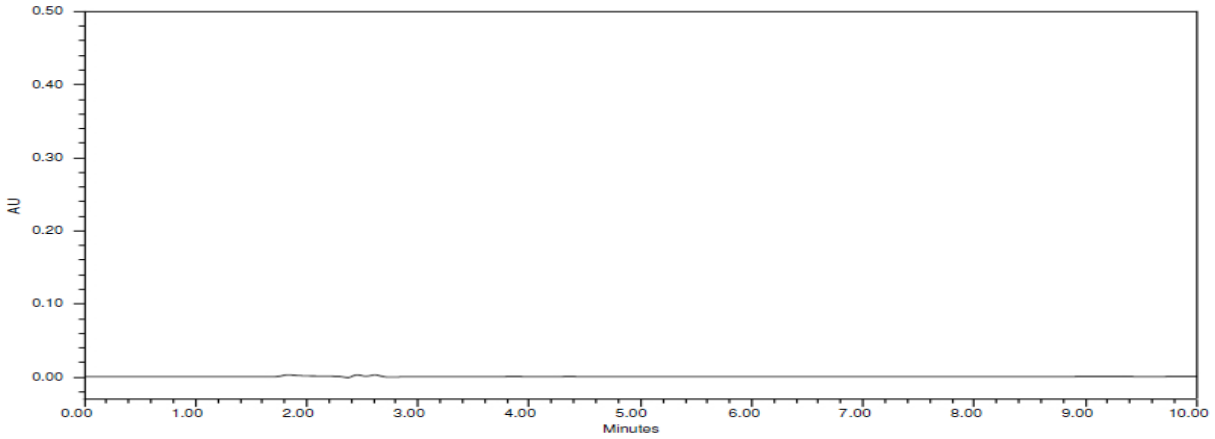


Figure 2: Blank chromatogram.

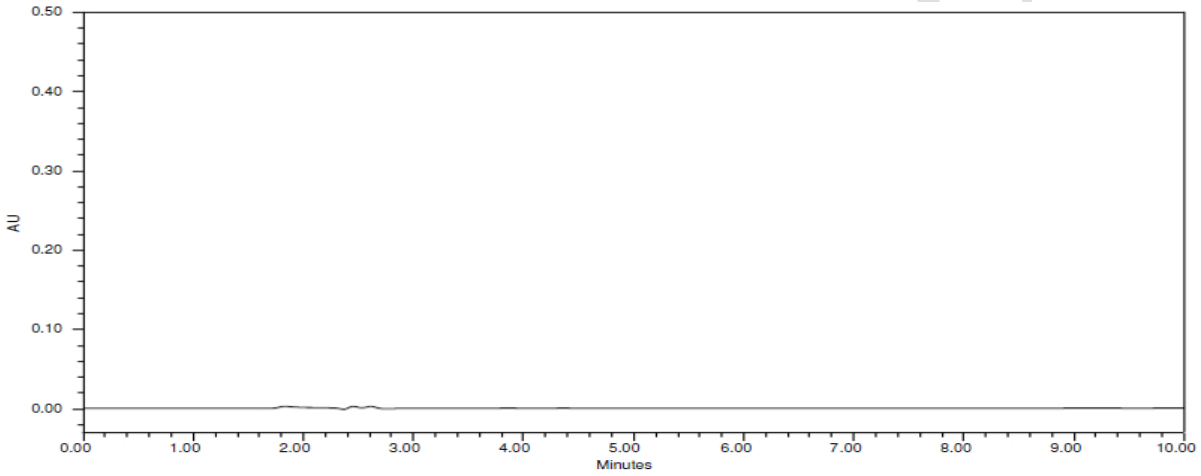


Figure 3: Placebo chromatogram

% Assay:

$$\frac{A_T}{A_S} \times \frac{WS}{D_S} \times \frac{D_T}{WT} \times \frac{P}{100} \times \frac{AW}{Label\ Claim} \times 100$$

Where:

A_T = Average peak area of respective active in chromatogram of sample solution

A_S = Average peak area of respective active in chromatogram of standard solution

WS = Weight of working standard taken in mg.

D_S = Dilution factor for the standard solution

D_T = Dilution factor for the sample solution

P = Percentage purity of working standard used (as is basis), in mg

AW = Average weight of tablets in mg

L = Label claim of respective active in mg per tablet

Table 1: Optimized chromatographic conditions.

S. No	Parameter	Optimized condition
1.	Column	Thermo Hypersil-BDS C8
2.	Column dimensions	250 × 4.6 mm, 5 µm
3.	Mobile phase	Mixed phosphate buffer: acetonitrile
4.	Mobile-phase ratio	75:25 (v/v)
5.	Elution	Isocratic
6.	Flow rate	1.0 mL/min
7.	Detection wavelength	210 nm
8.	Column temperature	30 °C
9.	Injection volume	20 µL
10.	Diluent	Mobile phase
11.	Needle wash	Water: acetonitrile (90:10)

ASSAY CALCULATION

	Enalapril Maleate		Paracetamol		Hydrochlorothiazide	
	RT	Area	RT	Area	RT	Area
Standard-1	2.956	412215	3.856	4147231	4.592	538659
Standard-2	2.953	410250	3.852	4136677	4.589	538838
Average	**2.955**	**411233**	**3.854**	**4141954**	**4.5905**	**538749**
Sample-1	2.961	410326	3.860	4123652	4.594	538659
Sample-2	2.963	410411	3.865	4139451	4.597	537841
Average	**2.962**	**410369**	**3.863**	**4131552**	**4.5955**	**538250**

Assay Calculation for Enalapril Maleate

410369	10.02	10	100	100	99.88	450	**mg/tab**	**% of Assay**
411233	100	100	450.0	10	100		**9.99**	**99.87**

Assay Calculation for Paracetamol

4131552	325.1	10	100	100	99.82	450	**mg/tab**	**% of Assay**
4141954	100	100	450.0	10	100		**323.70**	**99.60**

Assay Calculation for Hydrochlorothiazide

538250	25.12	10	100	100	99.91	450	**mg/tab**	**% of Assay**
538748	100	100	450.0	10	100		**25.07**	**100.30**

Acceptance Criteria: The % of amount should be between 98.0 to 102.0%.

Discussion

The chromatograms for blank, standard and sample of Enalapril Maleate, Hydrochlorothiazide and Paracetamol were shown in figure 2-7. The assay limits for ENP, HCZ and PCM were between 98-102%. The results obtained for ENP, HCZ and PCM were found to be 99.87%, 100.30%, 99.60% respectively and results were found to be within the limits. The results of ENP, HCZ and PCM were shown in Table No.2

The optimized chromatographic system was therefore considered suitable for simultaneous estimation of the three analytes.

2. Assay of Enalapril Maleate, Hydrochlorothiazide and Paracetamol

The developed method was applied to the analysis of the marketed tablet formulation. Representative blank, placebo, standard and sample chromatograms were obtained. No significant interference was observed from the blank or placebo at the retention positions of the analytes.

The average retention times obtained for enalapril maleate, paracetamol and hydrochlorothiazide were approximately 2.962, 3.864 and 4.596 min, respectively, in the sample chromatograms. The calculated assay values were within the specified acceptance range of 98–102%.

Table 2: Assay results obtained by RP-HPLC.

S. No	Drug	Mean retention time (min)	Mean sample peak area	Assay (%)	Acceptance criteria
1.	Enalapril maleate	2.962	410369	99.87	98–102%
2.	Paracetamol	3.863	4131552	99.60	98–102%
3.	Hydrochlorothiazide	4.596	538250	100.30	98–102%

The assay results demonstrated that the amounts of all three active ingredients were within the specified limits. Enalapril maleate showed an assay of 99.87%, paracetamol 99.60% and hydrochlorothiazide 100.30%. These findings indicate satisfactory quantitative performance of the proposed RP-HPLC method.

Assay of the three active ingredients

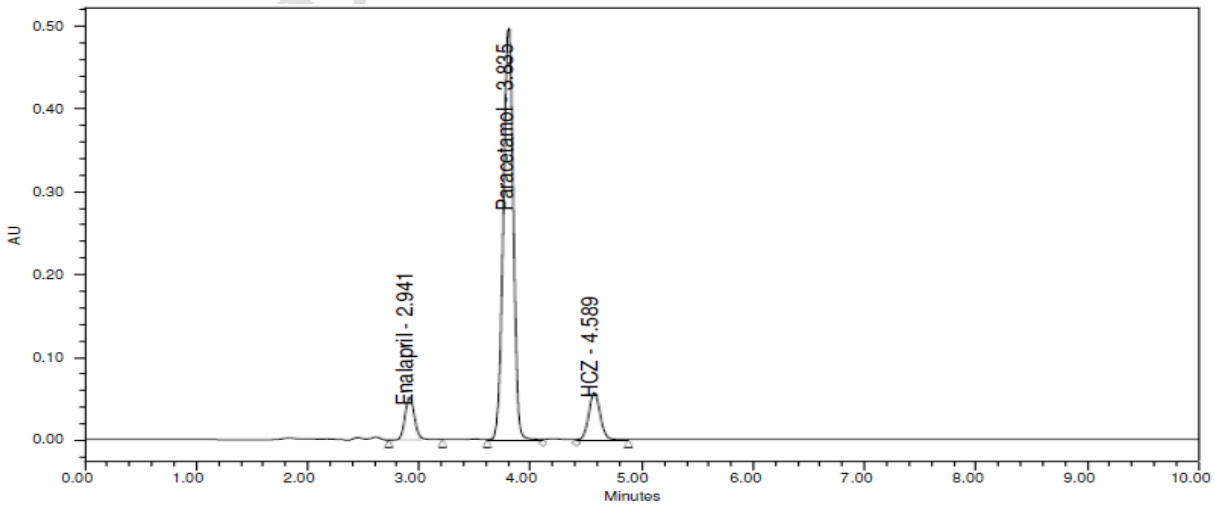
Percentage assay obtained for enalapril maleate, hydrochlorothiazide and paracetamol by the developed RP-HPLC method. 98% 99% 100% 101% 102% Enalapril maleate Hydrochlorothiazide Paracetamol

Acceptance range reported in the project: 98–102%.

3. Accuracy

Accuracy was evaluated at 80%, 100% and 120% concentration levels. The percentage recoveries obtained for all three analytes were within the acceptance range of 98–102%. For enalapril maleate, recoveries of 99.05%, 99.68% and 99.05% were obtained at 80%, 100% and 120%, respectively. Hydrochlorothiazide showed recoveries of 99.53%, 99.81% and 99.65%, whereas paracetamol showed recoveries of 99.29%, 99.74% and 99.66%, respectively. Refer Figure 4 to 6.

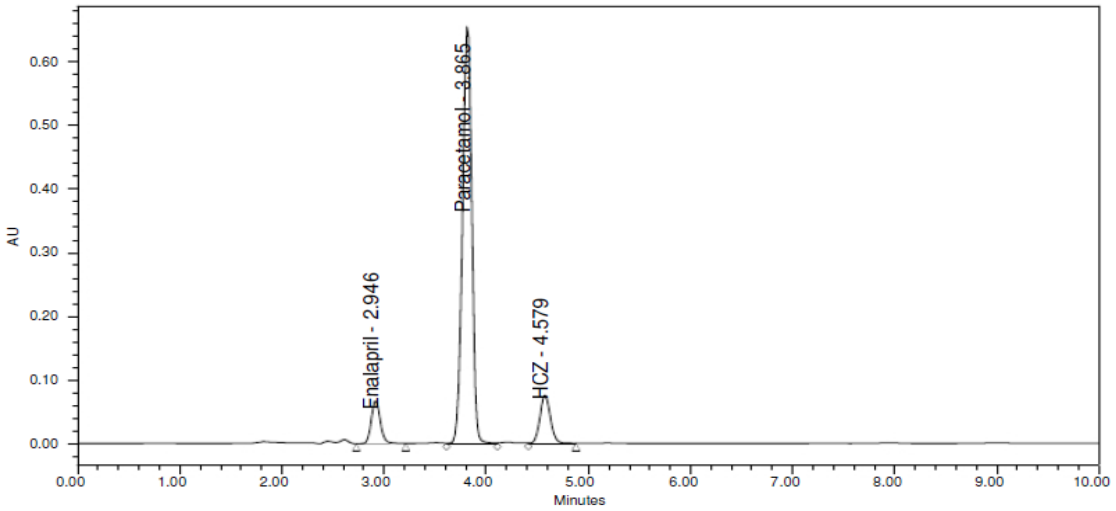
ACCURACY 80%



	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor	Resolution
1	Enalapril	2.941	322548	52156	8.06	5659	1.09	
2	Paracetamol	3.835	3265954	513569	81.44	8378	0.97	5.75
3	HCZ	4.589	428566	58654	10.50	9635	1.08	4.84

Figure 4: Accuracy 80% chromatogram – 1.

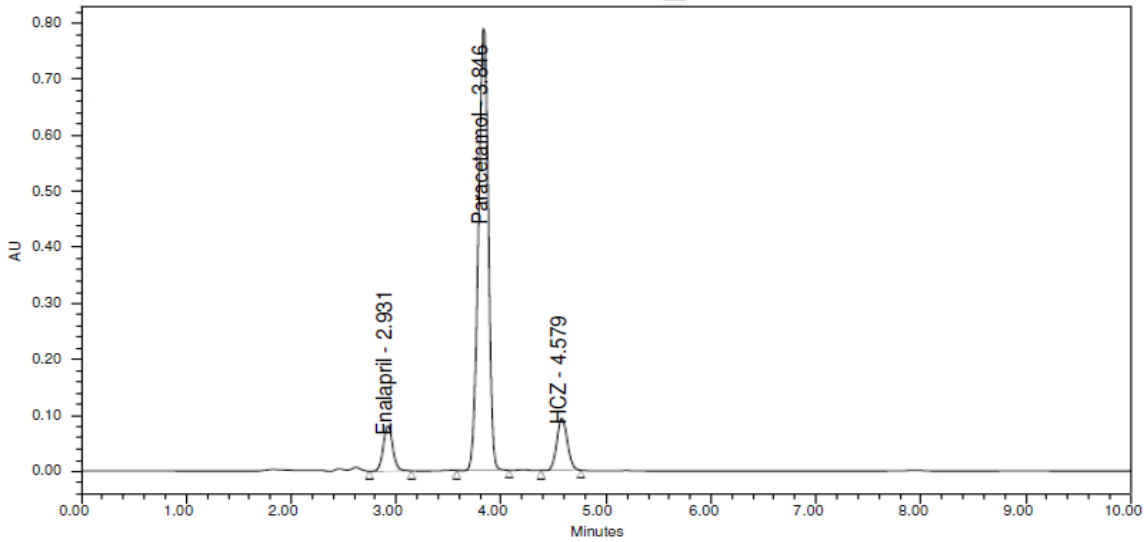
ACCURACY 100%



	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor	Resolution
1	Enalapril	2.946	407526	66784	8.04	5875	1.06	
2	Paracetamol	3.865	4123564	653255	81.45	8896	0.97	5.75
3	HCZ	4.579	535266	75481	10.51	9986	1.07	4.82

Figure 5: Accuracy 100% chromatogram – 3.

ACCURACY 120%



	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor	Resolution
1	Enalapril	2.931	481251	80054	8.04	5574	1.05	
2	Paracetamol	3.846	4936986	786596	81.58	7658	0.98	5.47
3	HCZ	4.579	641859	92154	10.38	9687	1.07	4.58

Figure 6: Accuracy 120% chromatogram – 1.

$$\text{Amt.Recovered} = \frac{\text{Avg.area of Enp} - \text{Placebo Area}}{\text{Avg.area of std}}$$

$$\%\text{Recovered} = \frac{\text{Amt Recovered}}{\text{Amt Taken}} \times 100$$

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Discussion

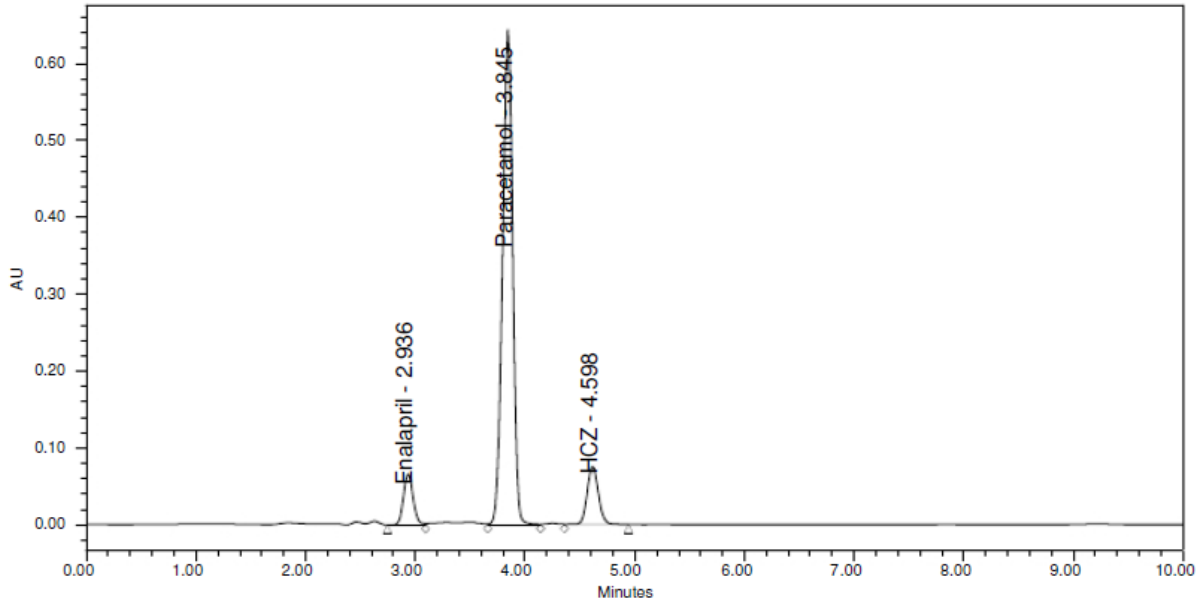
The accuracy studies were calculated as % recovery. At 80% for ENP it was (99.05), HCZ (99.53) and PCM(99.29). At 100% for ENP it was (99.68), HCZ (99.81) and PCM (99.74). At 120% for ENP it was (99.05), HCZ (99.65) and PCM (99.66). The limits of % recovery of drugs were 98-102% and above results which indicates that the method was accurate and also revealed that the commonly used excepients present in the Pharmaceutical formulation do not interfere in the proposed method. The chromatograms of accuracy shown in Figure 8-19. The results were shown in Table No.3.

Table 3: Accuracy results of the proposed RP-HPLC method.

S. No	Level	Enalapril maleate (% recovery)	Hydrochlorothiazide (% recovery)	Paracetamol (% recovery)
1.	80%	99.05	99.53	99.29
2.	100%	99.68	99.81	99.74
3.	120%	99.05	99.65	99.66
4.	Acceptance range	98–102%	98–102%	98–102%

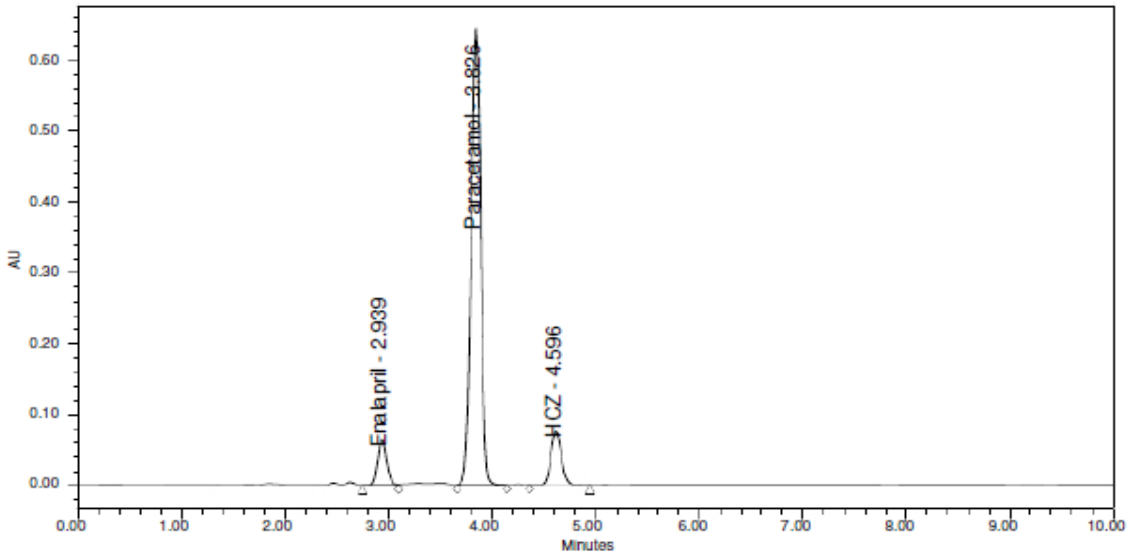
The close agreement between the added and recovered amounts confirms the accuracy of the proposed method. The results also indicate that commonly used formulation excipients did not produce a significant effect on the estimation of the three drugs.

5. Precision



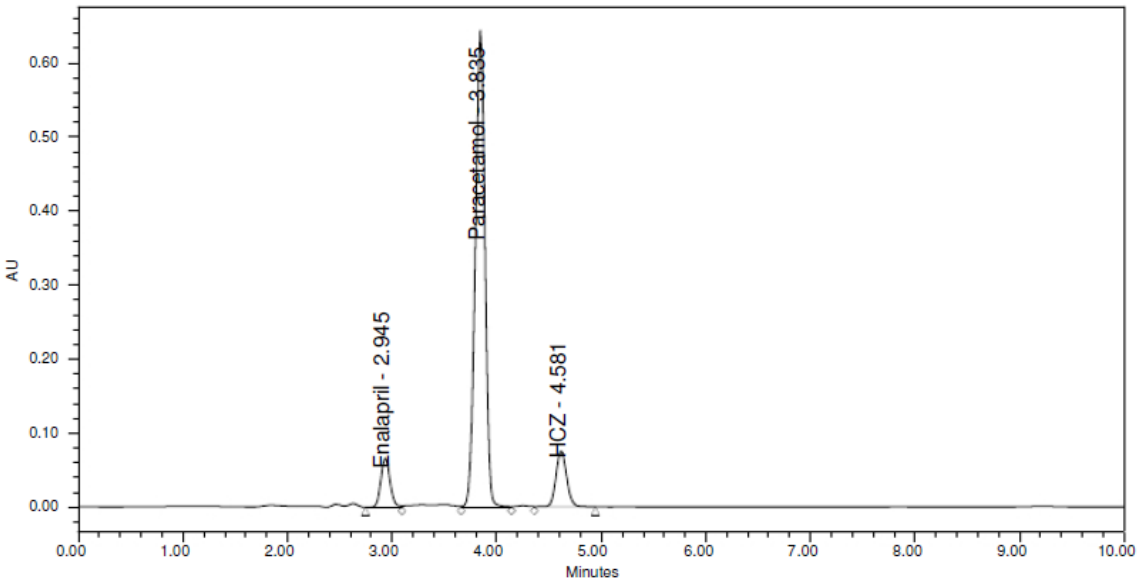
	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor	Resolution
1	Enalapril	2.936	409659	66265	8.03	5326	1.09	
2	Paracetamol	3.845	4125696	639568	81.39	8174	0.98	5.51
3	HCZ	4.598	536998	75154	10.58	9775	1.08	4.36

Figure 7: System precision chromatogram-1.



	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor	Resolution
1	Enalapril	2.939	407865	66132	8.05	5387	1.08	
2	Paracetamol	3.826	4156598	639796	81.37	8168	0.98	5.65
3	HCZ	4.596	539598	75359	10.58	9768	1.07	4.75

Figure 8: System precision chromatogram-2.



	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor	Resolution
1	Enalapril	2.945	408889	66054	8.03	5397	1.05	
2	Paracetamol	3.835	4141452	639547	81.36	8215	0.98	5.75
3	HCZ	4.581	538959	75278	10.61	9659	1.06	4.87

Figure 9: System precision chromatogram-6.

Precision was evaluated using replicate injections of the standard and sample preparations. The percentage RSD values obtained for the three analytes were low and remained below the specified acceptance criterion of 2%. The reported system and method precision values were 0.314% for enalapril maleate, 0.344% for hydrochlorothiazide and 0.446% for paracetamol. Refer figure 6 to 9.

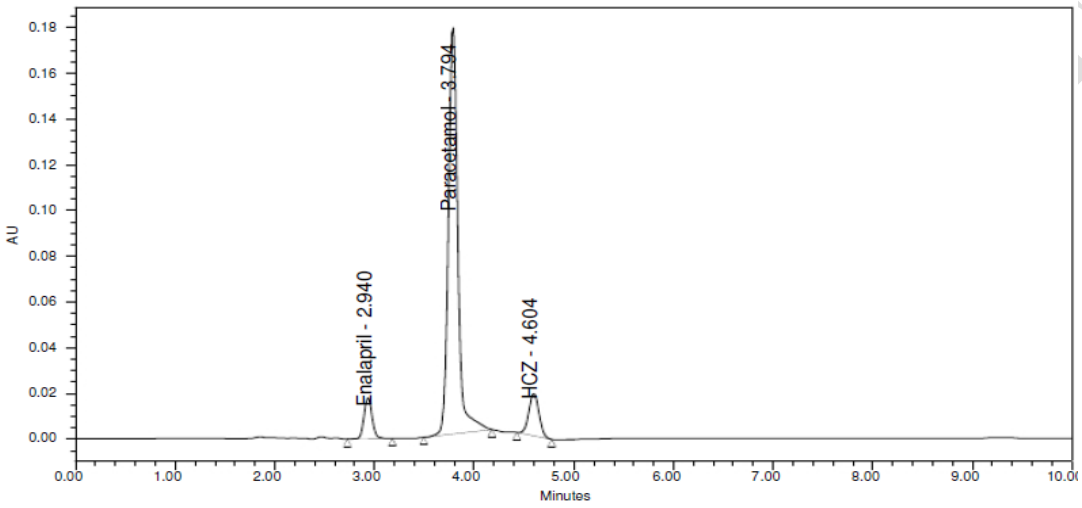
Table 4: Precision results.

S. No	Drug	Precision(% RSD)	Acceptance criterion
1.	Enalapril maleate	0.314	NMT 2%
2.	Hydrochlorothiazide	0.344	NMT 2%
3.	Paracetamol	0.446	NMT 2%

The low %RSD values demonstrate good repeatability and precision of the developed chromatographic procedure.

4. Linearity

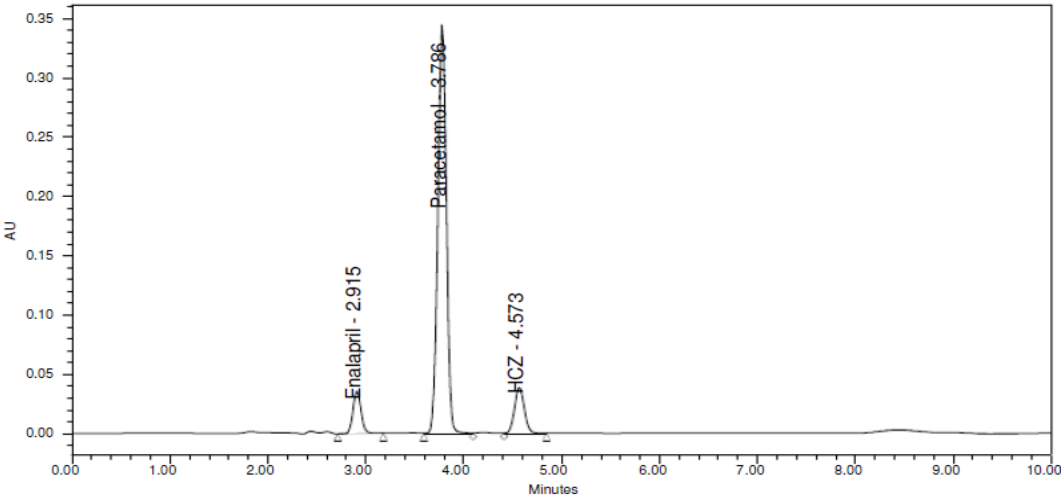
Level-I



	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor	Resolution
1	Enalapril	2.940	101874	17954	7.25	6332	1.06	
2	Paracetamol	3.794	1171198	178365	83.39	8474	1.06	5.48
3	HCZ	4.604	131390	18463	9.36	9431	1.12	4.61

Figure 10: Linearity chromatogram-1.

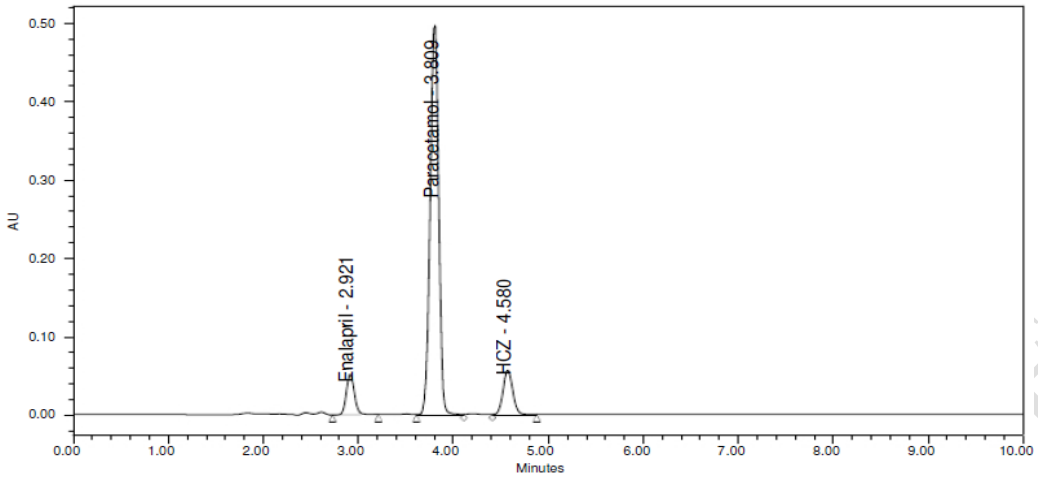
Level –II



	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor	Resolution
1	Enalapril	2.915	205496	35436	8.05	5868	1.06	
2	Paracetamol	3.786	2081061	342460	81.49	8823	0.98	5.61
3	HCZ	4.573	267278	38639	10.47	9943	1.08	4.60

Figure 11: Linearity chromatogram-2.

Level -III



	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor	Resolution
1	Enalapril	2.921	307252	51628	8.07	5575	1.10	
2	Paracetamol	3.809	3102043	500418	81.43	8303	0.96	5.56
3	HCZ	4.580	400142	57028	10.50	9698	1.07	4.41

Figure 12: Linearity chromatogram-3.

The calibration curves for enalapril maleate, hydrochlorothiazide and paracetamol demonstrated linear responses over the investigated concentration ranges. The project reports correlation coefficients of 0.999 for all three analytes. Refer level figure I to III

Table 5: Linearity range of the proposed RP-HPLC method.

S. No	Drug	Linearity range (µg/mL)	Correlation coefficient
1.	Enalapril maleate	2.5–15.0	0.999
2.	Hydrochlorothiazide	6.25–37.50	0.999
3.	Paracetamol	81.25–487.50	0.999

The correlation coefficients demonstrate a strong linear relationship between analyte concentration and detector response within the investigated ranges. The results satisfy the criterion reported in the project and confirm the suitability of the method for quantitative analysis.

5. SPECIFICITY CHROMATOGRAMS

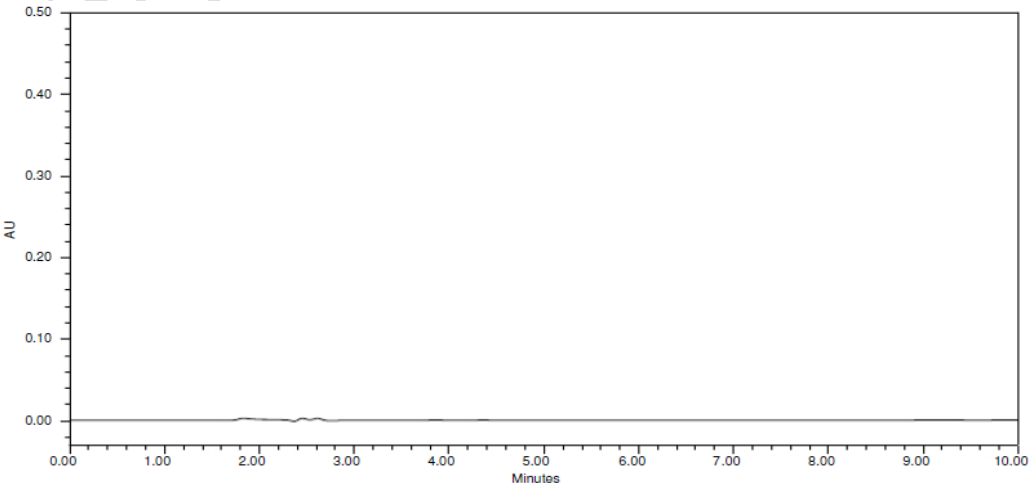
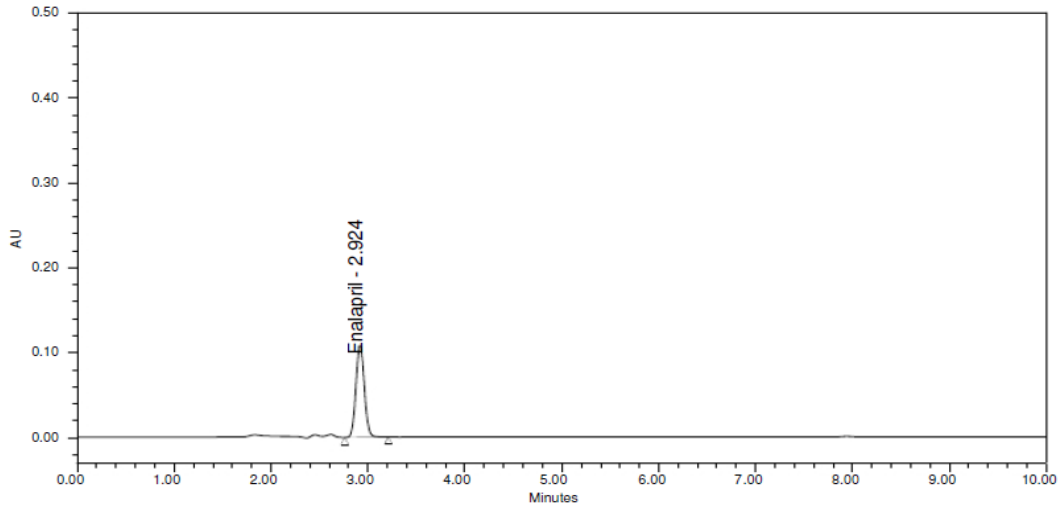


Figure 13: Blank chromatogram.



	Peak Name	RT	Area	Height	% Area	USP Plate Count	Symmetry Factor
1	Enalapril	2.924	657463	108643	100.00	5230	1.07

Figure 14: Enalapril chromatogram.

Specificity was assessed by comparing chromatograms obtained from blank, individual standards, mixed standard, placebo and sample solutions. The project reports that the blank and placebo did not interfere with the principal peaks of enalapril maleate, hydrochlorothiazide and paracetamol.

Table 6: Specificity results.

S. No	Parameter	Standard RT (min)	Sample RT (min)	Observation
1.	Enalapril maleate	2.925	2.929	Comparable
2.	Hydrochlorothiazide	4.583	4.582	Comparable
3.	Paracetamol	3.825	3.823	Comparable

No significant interference from the diluent or placebo was observed. The close agreement between standard and sample retention times confirms the specificity of the method.

7. Robustness

Robustness was investigated by deliberate variation of chromatographic parameters, particularly flow rate and column temperature. The flow rate was varied by ±10% and column temperature by ±5 °C. The system suitability parameters remained acceptable under the investigated conditions and the reported %RSD values remained below 2%.

Table 7: Robustness assessment.

S. No	Parameter varied	Variation studied	Result
1.	Flow rate	±10%	System suitability passed
2.	Column temperature	±5 °C	System suitability passed

These findings indicate that small deliberate changes in the selected chromatographic parameters did not significantly affect the performance of the method.

8. Ruggedness

Ruggedness/intermediate precision was assessed under changed analytical conditions. The reported %RSD values were 0.360% for enalapril maleate, 0.319% for hydrochlorothiazide and 0.412% for paracetamol. All values were below the specified limit of 2%.

Table 8: Ruggedness results.

S. No	Drug	% RSD	Acceptance criterion
1.	Enalapril maleate	0.360	NMT 2%
2.	Hydrochlorothiazide	0.319	NMT 2%
3.	Paracetamol	0.412	NMT 2%

The low variation observed under the investigated conditions demonstrates satisfactory ruggedness of the analytical procedure.

DRUG PROFILE

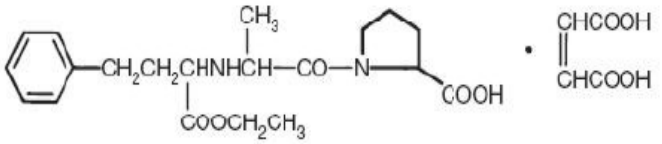
2.1 DRUG NAME: ENALAPRIL MALEATE

IUPAC NAME: (s)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-Lalanyl]proline,butenedioate salt (1:1).

Molecular Formula: $C_{20}H_{28}N_20_5$

Molecular Weight: 376.44

Molecular Structure



Category: Anti-hypertensive agent

Physical Properties:

- **Appearance:** White powder
- **Melting Point:** 143-144.5°c
- **Solubility:** Freely soluble in methanol and ethanol, slightly soluble in water.
- **Dosage Form:** Tablet, solution, IV forms

PK_A Value: 2.97

Polarisability: 40.41

Refractivity: 99.57

Mechanism of Action: The main mechanism of action of Enalapril is inhibition of ACE, that leads to block the hypertension. If not causes

ACE

Angiotensin1 ⟶ Angiotensin2

↓

VASOCONSTRICTION

↓

Blood Pressure Rises (hypertension)

Adverse Effects: Fatigue, asthenia, diarrhea, nausea, headache, dizziness.

Brand Names: Vasotec, Gadopril, Kinfil.

Manufacturer: Taro pharmaceuticals, Teva pharmaceuticals

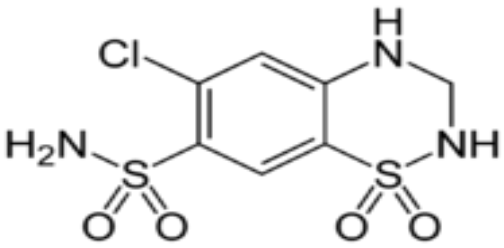
2.2 DRUG NAME: HYDROCHLOROTHIAZIDE

IUPAC NAME: 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide

Molecular Formula: $C_7H_8ClN_3O_4S_2$

Molecular Weight: 297.74

Molecular Structure



Category: Anti-hypertensive agent, Diuretic

Physical Properties:

- **Appearance:** White powder
- **Melting Point:** 274^0c
- **Solubility:** Freely soluble in methanol and ethanol, slightly soluble in water.
- **Dosage Form:** Tablet, capsule, elixir.

PK_A Value: 7.9

Polarisability: 25.35

Refractivity: 63.11

Mechanism Of Action: Hydrochlorothiazide belongs to thiazide class of diuretics. It reduces blood volume by acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electroneutral Na+-Cl- co-transporter by competing for the chloride site on the transporter. By blocking the sodium-chloride symporter, hydrochlorothiazide effectively reduces the osmotic gradient and water reabsorption throughout the nephron.

Adverse Effects: Hypokalemia, Hypomagnesemia, Hyponatremia, Headache, Nausea and Vomiting.

Brand Names: Apresazide, Aquafills, Caplaril

Manufacturer: Novartis, Hetero.

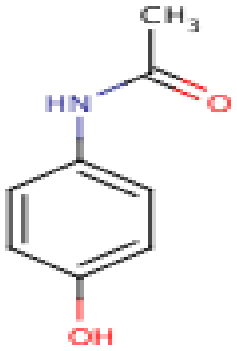
2.3 DRUG NAME: PARACETAMOL

IUPAC NAME: *N*-(4-hydroxyphenyl)ethanamide/*N*-(4-hydroxyphenyl)acetamide.

Molecular Formula: $C_8H_9NO_2$

Molecular Weight:151.16.

Molecular Structure



Category: Analgesic, Antipyretic.

Physical Properties:

- **Appearance:** White powder
- **Melting Point:** 170^0c
- **Solubility:** Freely soluble in methanol and ethanol, slightly soluble in water.
- **Dosage Form:** Tablet, Suspension, solution, capsule.

PK_A Value: 9.38

Polarisability: 15.52

Refractivity: 42.9

Mechanism of Action: The main mechanism of paracetamol is inhibition of cox enzyme. Acetaminophen is thought to act primarily in the CNS, increasing the pain threshold by inhibiting both isoforms of cyclooxygenase, COX-1, COX-2, and COX3 enzymes involved in prostaglandin (PG) synthesis. Unlike NSAIDs, acetaminophen does not inhibit cyclooxygenase in peripheral tissues and, thus, has no peripheral anti-inflammatory affects.

Adverse Effects: Hypokalemia, hypomagnesemia, Hyponatremia, Headache, Nausea and Vomiting.

Brand Names: Acetagesic, Abenol, Acetalgin, Crocin, Calpol

Manufacturer: Ranbaxy, Roxane, Polymedia

9. Summary of Validation Results

Table 9: Summary of validation characteristics of the developed RP-HPLC method.

S. No.	Validation parameter	Acceptance criterion	Result
1	Accuracy	98–102% recovery	ENP: 99.05–99.68%; HCT: 99.53–99.81%; PCM: 99.29–99.74%
2	Precision	NMT 2% RSD	ENP: 0.314%; HCT: 0.344%; PCM: 0.446%
3	Linearity	Correlation coefficient ≥0.999	r all three drugs
4	Ruggedness	NMT 2% RSD	ENP: 0.360%; HCT: 0.319%; PCM: 0.412%
5	Specificity	No interference	No significant blank/placebo interference
6	Robustness	System suitability should pass	Passed under investigated conditions

The validation results demonstrate that the proposed RP-HPLC method possesses satisfactory accuracy, precision, linearity, specificity, ruggedness and robustness.

10. Significance of the Results

The developed RP-HPLC method provided satisfactory simultaneous separation and quantification of enalapril maleate, hydrochlorothiazide and paracetamol in the investigated pharmaceutical formulation. The analytes were adequately resolved within a relatively short chromatographic run, with retention times of approximately 2.9–4.6 min. The assay results were within the specified 98–102% range, while the accuracy and precision studies demonstrated low variability. The linearity results showed correlation coefficients of 0.999 for all three analytes. Furthermore, the method remained satisfactory following deliberate changes in selected chromatographic conditions.

Therefore, the results support the suitability of the developed RP-HPLC procedure for routine simultaneous estimation of enalapril maleate, hydrochlorothiazide and paracetamol in the studied pharmaceutical formulation.

Comparison with Previously Reported Methods

The literature reviewed in the project demonstrates that several analytical procedures have previously been developed for enalapril maleate and hydrochlorothiazide. Pawar et al. reported an RP-HPLC procedure specifically involving enalapril maleate, hydrochlorothiazide and paracetamol in pure and tablet dosage forms. Uslu et al. developed a gradient RP-HPLC procedure for enalapril maleate and hydrochlorothiazide using a Waters µ-Bondapak C18 column. Nikolova Maslarska et al. reported a rapid RP-HPLC procedure using a Lichrosorb C18 column and an acetonitrile–phosphate buffer mobile phase with UV detection at 215 nm.

Other reported approaches included HPLC–PDA chemometric determination by Dinç et al. and HPLC/derivative spectrophotometric procedures reported by Carlucci et al. These studies collectively demonstrate the feasibility of chromatographic and spectrophotometric determination of enalapril-containing combinations.

In comparison, the present investigation used a **C8 stationary phase rather than the C18 phases commonly reported in the cited literature**, together with a phosphate-buffer/acetonitrile mobile phase at 75:25 (v/v). The method provided retention times of less than 5 min for all three analytes and satisfactory assay, accuracy, precision and linearity results. The experimental trials in the present work indicate that this combination of stationary phase and mobile phase was preferable to the initial C18-based conditions investigated.

Therefore, the principal significance of the present method is not simply that it confirms the feasibility of RP-HPLC analysis, which has already been established in the literature, but that it provides an experimentally optimized chromatographic system for the **simultaneous determination of enalapril maleate, hydrochlorothiazide and paracetamol** with satisfactory validation characteristics.

DISCUSSION

Taken together, the results demonstrate that the developed RP-HPLC method provides satisfactory chromatographic separation and reliable quantitative determination of enalapril maleate, hydrochlorothiazide and paracetamol. The assay results were close to the respective label claims, recoveries were within 98–102%, precision was associated with low %RSD values, and the calibration responses were linear with correlation coefficients of 0.999.

The results are broadly consistent with the previously published analytical procedures cited in the project, particularly the RP-HPLC methods reported by Pawar et al. and Dubey et al. for simultaneous estimation of these drugs. At the

same time, the present work demonstrates that changing the stationary phase and optimizing the mobile-phase composition can improve the chromatographic behavior of this three-component formulation.

The successful specificity and robustness studies further indicate that the method can tolerate the investigated minor variations in analytical conditions without compromising quantitative performance. Consequently, the developed RP-HPLC procedure appears suitable for routine quality-control analysis of pharmaceutical formulations containing enalapril maleate, hydrochlorothiazide and paracetamol.

CONCLUSION

The present research successfully developed and evaluated analytical methods for the **simultaneous estimation of enalapril maleate, hydrochlorothiazide and paracetamol in bulk drugs and pharmaceutical dosage forms** using RP-HPLC and UV spectrophotometric techniques. The principal objective of the study was achieved through systematic optimization of analytical conditions followed by validation of the developed procedures.

RP-HPLC Method

The RP-HPLC method was successfully optimized after evaluating different chromatographic conditions. The optimized method employed a **Thermo Hypersil-BDS C8 column (250 × 4.6 mm, 5 µm)** with mixed phosphate buffer and acetonitrile in the ratio of **75:25 (v/v)** as the mobile phase. An isocratic flow rate of **1.0 mL/min** and a detection wavelength of **210 nm** were selected. Under these conditions, the three analytes exhibited satisfactory chromatographic separation, with average retention times of approximately **2.925 min for enalapril maleate, 3.825 min for paracetamol and 4.583 min for hydrochlorothiazide**.

The developed RP-HPLC method demonstrated satisfactory analytical performance. The assay values obtained for the marketed formulation were **99.87% for enalapril maleate, 99.60% for paracetamol and 100.30% for hydrochlorothiazide**, indicating that the drug contents were within the specified acceptance range.

The validation results further demonstrated the reliability of the method. Accuracy studies produced recoveries within the specified **98–102%** range. Precision studies showed low %RSD values, while the calibration curves demonstrated a correlation coefficient of approximately **0.999** for the investigated analytes. Specificity, robustness and ruggedness studies also produced satisfactory results.

UV-Spectrophotometric Method

A simultaneous UV spectrophotometric method was also developed for estimation of the three active ingredients. The method was optimized with respect to solvent, wavelength and concentration and subsequently validated. The reported assay values were **99.67% for enalapril maleate, 99.92% for hydrochlorothiazide and 100.12% for paracetamol**, all of which were within the specified acceptance range.

The UV method demonstrated satisfactory accuracy, precision, linearity and ruggedness. The project reports recoveries close to 100%, low %RSD values and a correlation coefficient of **0.999**, supporting its suitability for simultaneous estimation of the three drugs.

Significance and Relevance

The significance of the present investigation lies in the development of analytical procedures capable of determining **three active pharmaceutical ingredients simultaneously**, thereby avoiding separate analytical determinations for each drug. The RP-HPLC procedure provides chromatographic separation and quantitative estimation, whereas the UV method offers a comparatively straightforward approach for routine analysis.

The findings are relevant to pharmaceutical quality-control laboratories because the developed methods demonstrated satisfactory accuracy, precision and reproducibility when applied to the investigated formulation. The results are also consistent with the literature cited in the project, including previously reported RP-HPLC methods for simultaneous determination of enalapril maleate with hydrochlorothiazide and related combinations.

Table 10: Summary of Major Findings.

S. No	Parameter	RP-HPLC
1.	Analytes	Enalapril maleate, hydrochlorothiazide, paracetamol
2.	Principal detection condition	210 nm
3.	Assay of enalapril maleate	99.87%
4.	Assay of hydrochlorothiazide	100.30%
5.	Assay of paracetamol	99.60%
6.	Linearity	$r \approx 0.999$
7.	Accuracy	Within 98–102%
8.	Precision	%RSD < 2%
9.	Overall outcome	Validated and suitable for analysis

In conclusion, the study established that the developed RP-HPLC method is a reliable and suitable procedure for simultaneous estimation of enalapril maleate, hydrochlorothiazide and paracetamol in the investigated pharmaceutical formulation. The UV spectrophotometric method also demonstrated satisfactory analytical performance and provides an alternative approach for routine estimation. Together, these methods provide useful analytical tools for pharmaceutical quality control and contribute to the reliable assessment of the identity, strength and consistency of formulations containing these three active ingredients.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support and guidance received during the course of this research work. The authors express their sincere thanks to the management and faculty members of the concerned institution for providing the necessary laboratory facilities, instrumentation and academic support required to carry out the analytical investigations.

The authors also acknowledge the assistance provided during the development and validation of the RP-HPLC and UV spectrophotometric methods for the simultaneous estimation of enalapril maleate, hydrochlorothiazide and paracetamol. The authors are thankful to the suppliers and manufacturers who provided the drug substances, chemicals and analytical materials used during the study.

The authors further express their gratitude to all individuals who contributed directly or indirectly to the successful completion of this research work.

REFERENCES

1. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva: ICH; 2005.
2. Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review. J Pharm Biomed Anal, 2002; 1011–1040.
3. Sayali SK, Gandhi SV, Ranjane PN, Ranher SS. HPTLC analysis of olmesartan medoxomil and hydrochlorothiazide in combination tablet dosage forms. J Planar Chromatogr, 2009; 425–428.
4. Shethi PD. HPTLC: High Performance Thin Layer Chromatography. New Delhi: CBS Publishers, 1996:1–68.
5. Wall PE. Thin-Layer Chromatography: A Modern Practical Approach. Cambridge, UK: Royal Society of Chemistry, 2005:6–5.
6. Martindale. The Complete Drug Reference. 36th ed. London, UK: Pharmaceutical Press, 2009:1276.
7. Tripathi KD. Essentials of Medical Pharmacology. 5th ed. New Delhi: Jaypee Brothers Medical Publishers, 2003: 506.
8. Anonymous. Indian Pharmacopoeia. 5th ed. Delhi, India: Controller of Publication, Government of India, 2007: 460.
9. Carlucci G, Palumbo G, Mazzeo P, Quaglia MG. J Pharm Biomed Anal, 2000; 23: 185.
10. Walily AF, Belal S, Heaba E, Kresh A. J Pharm Biomed Anal, 1995; 13: 851.
11. Franeta JT, Agbaba D, Eric S, Pavkov S, Aleksi M, Vladimirov S. Il Farmaco, 2002; 57: 709.
12. Erk N, Ozkan Y, Banoglu E, Ozkan SA, Senturk Z. J Pharm Biomed Anal, 2001; 21: 469.
13. Dinç E, Kokdil G, Onur F. J Pharm Biomed Anal, 2001; 26: 769.
14. Lou H, Yuan H, Raun Z, Jiang B. J Chromatogr B., 2010; 878: 682.
15. Erk N. J Pharm Biomed Anal, 2002; 27: 901.
16. Erk N. J Pharm Biomed Anal, 2001; 24: 603.
17. Pawar PY, Joshi, et al. A simple, reproducible and efficient reverse-phase high-performance liquid chromatographic method for estimation of enalapril maleate, hydrochlorothiazide and paracetamol in pure and tablet dosage form. Der Pharmacia Sinica, 2011; 2(5): 121.
18. Prasad CVN, et al. Simultaneous determination of amlodipine-enalapril maleate and amlodipine-lisinopril in combined tablet preparations by derivative spectrophotometry, 1999; 5(6): 383–388.
19. Shah KV, et al. Simultaneous estimation of nebivolol hydrochloride and hydrochlorothiazide in bulk and tablet dosage form by multicomponent and simultaneous estimation method. Int J Pharm Sci, 2011; 2(1): 27–35.
20. Foda, et al. A sensitive and specific HPLC method for simultaneous determination of enalapril and hydrochlorothiazide in human plasma using a C18 reversed-phase column. J Pharm Sci Res., 2010; 2(11): 786.