CHAPTER - V

STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF QUINAPRIL AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORMS

5.1. PHYSICAL, CHEMICAL AND PHARMACOLOGICAL PROPERTIES OF THE DRUGS

5.1.1. 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	**:**	297.73912 g/mol
Appearance	**:**	White to off-white crystalline powder
Solubility	**:**	Soluble in dilute ammonia, sodium hydroxide, methanol, ethanol, acetone and slightly soluble in water

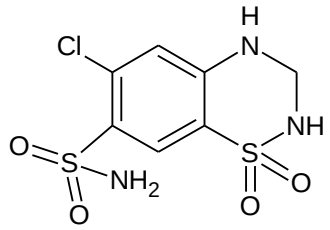

Figure 5.1: Chemical structure of hydrochlorothiazide

Hydrochlorothiazide is a short acting diuretic belonging to the class of compounds known as benzothiadiazines [1,2]. Hydrochlorothiazide is often used to treat hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis and hypoparathyroidism [3-5]. Hydrochlorothiazide decreases the reabsorption of electrolytes from the renal tubules which results in increased excretion of water and electrolytes like chloride, potassium, sodium, and magnesium [6]. The decreased excretion of uric acid and calcium and increased

excretion of iodide occurs in the presence of hydrochlorothiazide [6]. Hydrochlorothiazide may also reduce glomerular filtration rate.

5.1.2. Quinapril

IUPAC name	:	(3S)-2-[(2S)-2-[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino] propanoyl] -3 , 4- dihydro- 1H- isoquinoline-3-carboxylic acid
Molecular formula	:	$C_{25}H_{30}N_2O_5$
Molecular weight	:	438.524 g/mol
Appearance	:	white to off-white amorphous powder
Solubility	:	Soluble in acetone, dimethyl sulfoxide, ethanol, 0.1N HCl, methanol or water

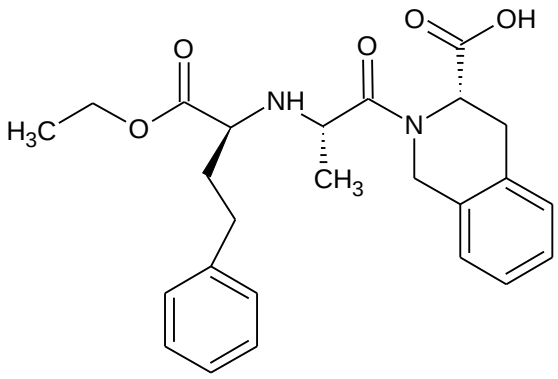

Figure 5.2: Chemical structure of quinapril

Quinapril is a prodrug and an angiotensin converting enzyme inhibitor. The esterases of liver transform quinapril into quinaprilat, an active metabolite. Quinapril is used in the treatment of congestive heart failure and hypertension [7]. Angiotensin converting enzyme catalyses the formation of angiotensin II, a powerful vasoconstrictor and increases blood pressure, from angiotensin I. The inhibition of

angiotensin converting enzyme by quinapril leads to the reduced production of angiotensin II. The result is the reduced plasma concentrations of aldosterone, increased sodium excretion in urine and increased potassium concentration in blood [8,9].

5.2. REVIEW OF LITERATURE

5.2.1. Hydrochlorothiazide

Hydrochlorothiazide is official in the United States Pharmacopoeia, which suggests a liquid chromatographic method for its assay in bulk and tablet formulations [10]. Analytical techniques applicable to hydrochlorothiazide determination in bulk and pharmaceutical preparations (tablets and oral suspension), which include UV spectrophotometry [11], visible spectrophotometry [12], stability-indicating HPLC with UV detection [13,14] and diffuse reflectance spectroscopy [15].

Hydrochlorothiazide in biological samples such as human plasma and serum are estimated using HPLC with electrochemical detection [16], HPLC with UV detection [17] and liquid chromatography–tandem mass spectrometry [18-20]. Different types of voltametric methods such as differential pulse anodic voltammetry [21], anodic stripping voltammetry [22], cyclic voltammetry [22] and adsorptive stripping voltammetry [23] were reported by researchers for the quantification of hydrochlorothiazide in both pharmaceutical dosage forms and biological samples like human urine and plasma.

Biomimetic Sensor for detection of hydrochlorothiazide using amperometric detection and chemometrics was developed for the analysis of hydrochlorothiazide in

spiked urine and pharmaceutical formulations. This method is applied for doping test in sports [24].

5.2.2. Quinapril

Quinapril is official in United States Pharmacopoeia where the liquid chromatographic method was employed for its assay in tablets [25]. Several analytical methods are reported for the assay of quinapril. Three reports using UV spectrophotometry (first order derivative, difference spectrophotometric method, and stability indicating UV spectroscopy) were found [26-28]. First order derivative and difference spectrophotometric method are used for the analysis of quinapril in bulk and pharmaceutical dosage form [25,26]. Stability indicating UV spectroscopy method is applied to study the stability of quinapril in acidic and basic conditions [28].

High performance liquid chromatography with radiochemical detection coupled to liquid scintillation counting spectrometry [29] and high performance liquid chromatography with fluorescent-detection [30] has been suggested for the determination of quinapril in perfusate, perfusate ultrafiltrate, human plasma and human urine. High performance liquid chromatography with UV detection method is employed for the assay of quinapril by in bulk [26,31], tablets [26,31], human plasma [32] and human urine [33].

Sora *et al.*, [34] and Kunal *et al.*, [35] reported liquid chromatographic separation coupled to tandem mass spectrometry detection methods for the determination of quinapril in human plasma and human serum, respectively. Ultra

performance liquid chromatography coupled to tandem mass spectrometry method was developed by Dasandi *et al.,* [36] for the estimation of quinapril and its active metabolite quinaprilat in human plasma.

Square wave voltammetric methods for the electrochemical quantification of quinapril in its pharmaceutical formulations was developed and validated by José *et al.,* [37] and Suslu and Altinoz [38]. Gas chromatography technique with different types of detectors like negative-ion chemical ionization mass spectrometry [39] and electron-capture detection [40] have been described for the assay of quanapril in human plasma and urine. Chi-Yu *et al.,* presented a matrix-assisted laser desorption ionization time-of-flight mass spectrometry with quinolone matrix additives method for determining quinapril in human plasma [41]. Hillaert and Van den [42] and Prieto *et al.,* [43] reported capillary zone electrophoresis for the estimation of quinapril in pharmaceutical formulation and human urine.

5.2.3. Hydrochlorothiazide and quinapril combination

Hydrochlorothiazide is used in the treatment of hypertension either alone or in combination with other antihypertensives like angiotensin converting enzyme inhibitors and beta blockers. The combination of hydrochlorothiazide and quinapril is used to treat high blood pressure [44]. The lowering of high blood pressure helps in prevention of kidney problems, strokes and heart attacks.

Various analytical methods have been reported for quantitative determination of quinapril and hydrochlorothiazide simultaneously in pharmaceutical and biological samples. A second order derivative spectrophotometric method using zero crossing

technique which measures quinapril and hydrochlorothiazide in two component mixtures was reported by and Hopkala [45]. In this method, quinapril was determined at a wavelength of 211.6 nm which is the zero-crossing wavelength point of hydrochlorothiazide. Similarly, hydrochlorothiazide was measured at 270.8 nm which is the zero crossing wavelength point of quinapril.

Simultaneous determination of quinapril and hydrochlorothiazide in tablet dosage forms were accomplished by ratio spectra first order derivative spectrophotometry and chemometric method [46]. In the graphical approach, the absorption spectra of quanapril and its binary mixtures in the range of 210-280 nm were divided by the standard spectrum of hydrochlorothiazide and their absorption spectra were obtained. The ratio spectra of hydrochlorothiazide in the wavelength region of 210-350 nm were obtained by using the standard spectrum of quinapril. The first derivative of the ratio spectra obtained in the above steps was calculated by $\Delta\lambda$=5 nm interval for quinapril and hydrochlorothiazide.

Quantitative determination of quinapril and hydrochlorothiazide in tablets was achieved by Dinç and Baleanu using continuous wavelet transform and chemometric methods [47]. In continuous wavelet transform method, the calibration functions for quinapril at 230.6 nm and for hydrochlorothiazide at 271.1 nm were calculated based on the relationship between the response of continuous wavelet signals and their concentrations. In chemometric approach, partial least squares and principal component regression calibrations were constructed by a training 21 mixtures

containing quinapril and hydrochlorothiazide and their corresponding absorbance data in the wavelength range of 205-290 nm.

Vandana *et al.*, [48] reported dual wavelength spectrophotometry, Fourier transform infrared spectroscopy and stability indicating high performance liquid chromatography methods for the simultaneous estimation of quinapril and hydrochlorothiazide in the combined dosage form. In dual wavelength spectrophotometry method, 220 and 230 nm was selected for estimation of quinapril. At these selected wavelengths quinapril shows a significant difference in absorbance while hydrochlorothiazide shows the same absorbance, hence absorbance difference of hydrochlorothiazide is zero. For estimation of hydrochlorothiazide, 261 nm wavelength was selected where quinapril shows zero absorbance. In fourier transform infrared spectroscopy technique, FT-IR spectrum of the selected drugs was recorded in the range of 4000-400 cm^{-1} and quantitative determination of both drugs were carried out by selecting peak area at 1680- 1615 cm^{-1} (C=O stretch) for quinapril and 1338-1284 cm^{-1} (O=S=O stretch) for hydrochlorothiazide. In stability indicating HPLC method, HiQsil C18 (150 × 4.6 mm, 5 µm) column was used as stationary phase for the analysis of quinapril and hydrochlorothiazide in the presence of stress degradation products. The mobile phase composed of 0.01 M KH_2PO_4 buffer with pH 3.5 and methanol which was run in gradient mode with a flow rate of 1 mL/min. The detection wavelength was 215 nm.

Girija *et al.*, [49] determined quinapril and hydrochlorothiazide simultaneously by HPTLC in pharmaceutical formulations. The quinapril and hydrochlorothiazide were separated on silica gel 60 F254 plates using ethyl acetate:

acetone: acetic acid (6.5: 3: 0.5 *v/v/v*) as the mobile phase. The plates were dried and scanned at 208 nm using Camag TLC Scanner.

Liquid chromatography-tandem mass spectrometry method has been developed and validated by Parekh *et al.,* [50] for the simultaneous estimation of hydrochlorothiazide and quinapril in human plasma. The analytes and internal standard were chromatographed on a hypurity C8 (100 mm x 2.1 mm i.d., 5 µm particle size) column. An isocratic mobile phase consisting of 0.5 % formic acid and acetonitrile in the ratio of 25:75 (*v/v*) was used to separate all the drugs.

In an RP-HPLC method described by Harini *et al.,* [51] quinapril and hydrochlorothiazide were separated on Zorbax Eclipse XDB C18 column (150 x 4.6 mm, 5 µm) maintained at 30 °C using acetonitrile and phosphate buffer (pH 4.5) in the ratio of 35:65 *v/v* as a mobile phase at flow rate of 0.9 mL/min and detected at 210 nm. The isocratic RP-HPLC method was developed, optimized and validated by Rani *et al.,* [52] for the estimation of quinapril and hydrochlorothiazide in tablets. The selected drugs were estimated using Hypersil BDS C18 (150 mm x 4.6 mm id 5 µm particle size) column. A mobile phase composed of triethylamine buffer, acetonitrile in the proportion of 60:40 (*v/v*) at a flow rate of 1.0 mL/min was used for the separation. Detection was performed at 220 nm.

A HPLC method has been reported Srikanth *et al*., [53]. The separation of quinapril and hydrochlorothiazide was carried out on Inertsil ODS column (250 mm × 4.6mm, 5 µm) with containing phosphate buffer (pH 4.0), acetonitrile and methanol in the ratio of 50:40:10 (*v/v/v*) as the mobile phase. The flow rate was 1.2 mL/min.

The column effluents were monitored with UV detector set at 210 nm. This method was applied to the quantification of quinapril and hydrochlorothiazide in pharmaceutical dosage forms. Serkan *et al.*, [54] reported an RP-HPLC method for the simultaneous determination of quinapril and hydrochlorothiazide in pharmaceutical dosage forms. The separation and analysis are achieved on Hichrom C18 (250 × 4.6 mm, 10 μm) column. The mobile phase used was a mixture of acetonitrile: 0.067 M potassium dihydrogen phosphate (pH 2.5) (40:60 *v/v*). Ultra violet detection was done at 211 nm. A RP-HPLC method (Govinda *et al.*,) [55] for determination of quinapril and hydrochlorothiazide in dosage form has been reported and was carried out on a ACE C18 column (150 mm × 4.6 mm, 5 μm) with phosphate buffer and acetonitrile in distilled water (90:10 *v/v*) as the mobile phase. The flow rate was 1 mL/min and column effluents were monitored at 225 nm.

An ion-pair HPLC method for the determination of quinapril and hydrochlorothiazide in tablet was performed on an RP -C18 Gemini and (150 × 4.5 mm, 5 μm) column was proposed by Gandhimathi and Ravi [56]. The optimized chromatographic conditions were 0.1 % triethylamine (pH 3.5), containing 1 mM of hexane sulphonic acid: acetonitrile (30:70 *v/v*) as mobile phase with a flow rate of 1 mL/min and detection with photo diode array detector set at 220 nm.

Stability indicating HPLC method was reported by Shabeen and Kumar in the literature for quinapril and hydrochlorothiazide quantification in bulk and tablet dosage form [57]. The separation was carried out on an Inertsil ODS C18 (150 mm × 4.6 mm, 5 μm particle size) column. A simple combination (phosphate buffer and

acetonitrile in the ratio of 26:74 *v/v*) delivered at a flow rate of 1.2 mL/min is used as mobile phase. The effluents were detected using photodiode array detector set at 210 nm.

A gradient stability indicating liquid chromatographic method was developed by Marta de *et al.*, [58]. The method was applied for the assay of quinapril and hydrochlorothiazide quantification in pharmaceutical samples. The separation was achieved on Purospher RP-8 column (124 mm x 4.0 mm, 5 µm). The method employs acetonitrile and phosphate buffer (pH 4.6) as mobile phase in a gradient mode. The flow rate was 1 mL/min and the detection was done at 216 nm.

Most of the above reported methods [45-47, 49-56] were not stability indicating. Disadvantages of the reported stability indicating methods (48,57,58) are narrow range of linearity, more flow rate (> 1 mL/min), long runtime (> 6 min), large sample volume (> 10 µL) and were not fully validated. In the present investigation, we report a rapid, reliable and fully validated RP-HPLC method with photodiode array detector for the precise and accurate determination of hydrochlorothiazide and quinapril concentrations in bulk and combined tablet dosage form.

5.3. MATERIALS AND METHODS

5.3.1. Reference drugs and tablet dosage forms

Both quinapril and hydrochlorothiazide reference standards were kindly provided by Lara Drugs Private Limited (Telangana, India). Accuretic tablets (labeled to contain quinapril 20 mg, hydrochlorothiazide 12.5 mg) from Pfizer were purchased from the local pharmacy store.

5.3.2. Chemicals

Methanol of HPLC grade was from Merck India Ltd., Mumbai, India. Potassium dihydrogen orthophosphate, hydrogen peroxide, hydrochloric acid and sodium hydroxide were of analytical reagent grade and obtained from Sd. Fine Chemicals Ltd., Mumbai, India. Milli-Q water (Millipore, USA) was used right through the experiments.

5.3.3. HPLC instrumentation

The chromatographic separation and analysis of quinapril and hydrochlorothiazide was performed on an Alliance Waters HPLC system equipped with Alliances 2695 series Quaternary pump, Waters 2998 photodiode array detector and an auto sampler. Data collection and processing was done using Waters Empower 2.0 software. Degassing of mobile phase was done with Spectra lab DGA 20 A3 ultrasonic bath sonicator. The chemicals and drugs were weighed using Electronic balance ELB 300. Digisun pH meter was used for all pH measurements.

5.3.4. Chromatographic conditions

Mobile Phase: 0.1 M KH_2PO_4 and methanol in the ratio of 65:35 (*v/v*).

pH was adjusted to 4.5 with dilute orthophosphoric acid.

Analytical column: Agilent C18, 250 mm × 4.6 mm, 5 µm

Mobile phase flow rate: 1.0 mL/min

Run time: 6 min

Column temperature: 25±2 ˚C

Injection volume: 10 µL

Detection wavelength: 210 nm

5.3.5. Stock and working standard solutions

A stock standard solution of quinapril (200 μg/mL) and hydrochlorothiazide (125 μg/mL) was prepared by dissolving 20 mg of quinapril and 12.5 mg hydrochlorothiazide reference drugs in 40 mL of mobile phase in a 100 mL volumetric flask. The final volume was made up to mark using the same solvent. The stock standard solution was further diluted with mobile phase to obtain working standard solutions in a range of 10-30 μg/mL quinapril and 6.25-18.75 μg/mL hydrochlorothiazide.

5.3.6. Calibration curve

Working standards at concentrations of 10, 15, 20, 25, 30 μg/mL quinapril and 6.25, 9.375, 12.5, 15.625, 18.75 μg/mL hydrochlorothiazide were prepared and analyzed thrice. Calibration curves (peak area response of the analyte *versus* the nominal concentration of analyte) were fitted by least squares linear regression.

5.3.7. Assay of quinapril and hydrochlorothiazide in tablets

Twenty Accuretic tablets were weighed. The average weight was calculated. Tablets were crushed to a fine powder. Amounts equivalent to 20 mg of quinapril and 12.5 mg of hydrochlorothiazide were transferred to a 100 mL volumetric flask and 30 mL of mobile phase was added. The flask was shaken sonically for 20 min and the solution was then diluted to the volume with the same solvent. The solution was filtered through 0.45 μm pore size membrane filter. From this solution 1 mL aliquot was transferred to 10 mL volumetric flask and made up to the volume with mobile phase to give a final concentration of 20 μg/mL quinapril and 12.5 μg/mL

hydrochlorothiazide. 10 µl of this solution was injected into the HPLC system thrice and analyzed by the proposed RP-HPLC method.

5.3.8. Stability indicating nature of the proposed method

The stability indicating nature of the proposed RP-HPLC method was assessed through forced degradation of quinapril and hydrochlorothiazide tablet sample solution in acidic condition using 0.1 N HCl (sonication for 30 minutes at room temperature), basic condition using 0.1 N NaOH (sonication for 30 min at room temperature), oxidative condition using 30 % H_2O_2 (sonication for 30 min at room temperature), photolytic (direct exposure of tablet powder to sun light for up to 24 h) and thermal (exposure of tablet powder to 105 ^oC for 30 min in oven) [59]. Stressed samples were analyzed at a concentration of 20 µg/mL quinapril and 12.5 µg/mL hydrochlorothiazide by the proposed RP-HPLC. The peaks of quinapril and hydrochlorothiazide were observed for the retention times, peaks interference and spectra purity.

5.4. RESULTS AND DISCUSSION

5.4.1. Method development

Different stationary phases and several mobile phase compositions for the effective separation and analysis of quinapril and hydrochlorothiazide were tried during the preliminary investigation. C8 and C18 stationary phases were tested. Good separation of quinapril, hydrochlorothiazide, and the degradation products was obtained with C18 (250 mm × 4.0 mm, 5 µm) stationary phase. So the same stationary phase was used. Regarding the mobile phase, a mixture of 0.1 M potassium dihydrogen phosphate and methanol in different ratios was tested in isocratic elution

mode. The separation of the two drugs and the degradation products was good when 0.1 M potassium dihydrogen phosphate and methanol was used in the ratio of 65:35 (*v/v*). Regarding the pH of the mobile phase, different pH values were tested and found that pH 4.5 was the best as it gave a better separation. Different flow rates of 0.8, 1.0 and 1.2 mL/min were tested and found that 1.0 mL/min was the best one. Room temperature was good for this separation and so the entire analysis was carried at room temperature. Detection at 210 nm was used, as it gave high peak area response for quinapril and hydrochlorothiazide. Using the above optimized chromatographic conditions, good separation of quinapril and hydrochlorothiazide was obtained (Figure 5.3). The complete details of the optimized HPLC conditions are shown in Table 5.1.

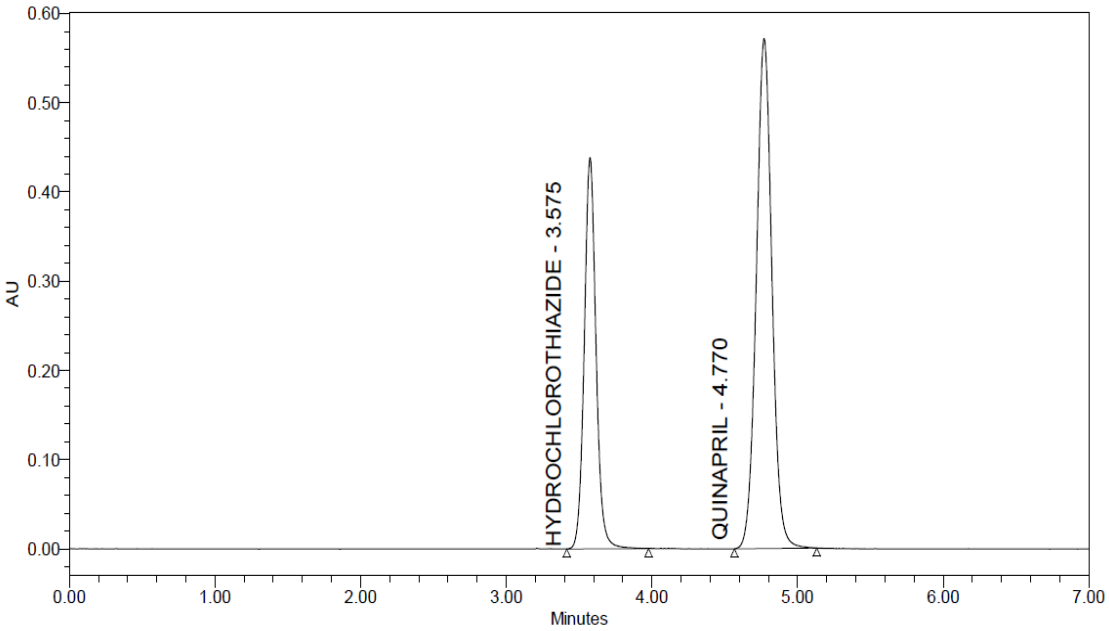

Figure 5.3: Chromatogram of well separated peaks of quinapril and hydrochlorothiazide with optimized chromatographic conditions

Table 5.1: Optimized chromatographic conditions

S.No	Parameter	Value
1	Column	Agilent C18 (250 mm x 4.6 mm, 5 µm particle size)
2	Mobile phase	0.1 M KH_2PO_4 and methanol in the ratio of 65:35 (*v/v*). pH 4.5
3	Flow rate	1.0 mL/min
4	Diluent	Mobile phase
5	Column temperature	25 ± 1 ^oC
6	Runtime	6 min
7	Retention time	Hydrochlorothiazide – 3.575 min and Quinapril – 4.770 min
8	Volume of injection	10 µL
9	Detection wavelength	210 nm

5.4.2. Method validation

The method was validated according to ICH guidelines for system suitability, selectivity, specificity, linearity range, accuracy, precision, limit of detection (LOD)/limit of quantification (LOQ) and robustness [60].

5.4.2.1. System suitability study

To evaluate the system suitability of the method, the peak area, plate count, tailing factor resolution and retention time of five replicate injections of a standard solution of concentration 20 µg/mL quinapril and 12.5 µg/mL hydrochlorothiazide were used. The determined values were compared with recommended limits and the %RSD values were calculated in each case. The results of the system suitability

parameters are shown in Table 5.2. The obtained values indicate the good performance of the system.

Table 5.2: System suitability parameters

Parameters	Quinapril		Hydrochlorothiazide		Recommended limit
	Value*	RSD (%)	Value*	RSD (%)	
Retention time	4.770	0.071	3.576	0.063	RSD ≤2
Peak area	4255934	0.343	2442465	0.223	RSD ≤2
USP resolution	6.862	0.417			> 1.5
USP plate count	9572	0.927	9806	0.263	> 2000
USP tailing factor	1.026	0.534	1.072	0.417	≤ 2

**Average of five values*

5.4.2.2. Linearity and range

A linear relationship was found between the peak area response and the concentration of analytes in the range of 10 to 30 µg/mL for quinapril and 6.25 to 18.75 µg/mL for hydrochlorothiazide. The representative linear equation, calculated by the least squares method, was

$y = 12210x + 1608$ ($R^2 = 0.9999$) - Quinapril

$y = 34048x - 114.8$ ($R^2 = 0.9999$) - Hydrochlorothiazide

The regression coefficient values indicating good linearity ($R^2 > 0.999$).

Table 5.3 presents the linear regression equations, concentration ranges, regression coefficients, intercept and slope. Calibration curves of quinapril and hydrochlorothiazide are given in Figures 5.4 and 5.5, respectively.

Table 5.3: Linearity studies for quinapril and hydrochlorothiazide

Linearity of quinapril		Linearity of hydrochlorothiazide	
Concentration (µg/mL)	**Peak area**	**Concentration (µg/mL)**	**Peak area**
10	1224617	6.25	2121720
15	1832061	9.375	3199057
20	2443146	12.5	4259430
25	3059829	15.625	5315658
30	3660022	18.75	6383717
Regression equation: y = 12210 x + 1608 (R^2= 0.9999)		Regression equation: y = 34048 x - 114.8 (R^2 = 0.9999)	

y – peak area of the drug: x - concentration of drug in µg/mL

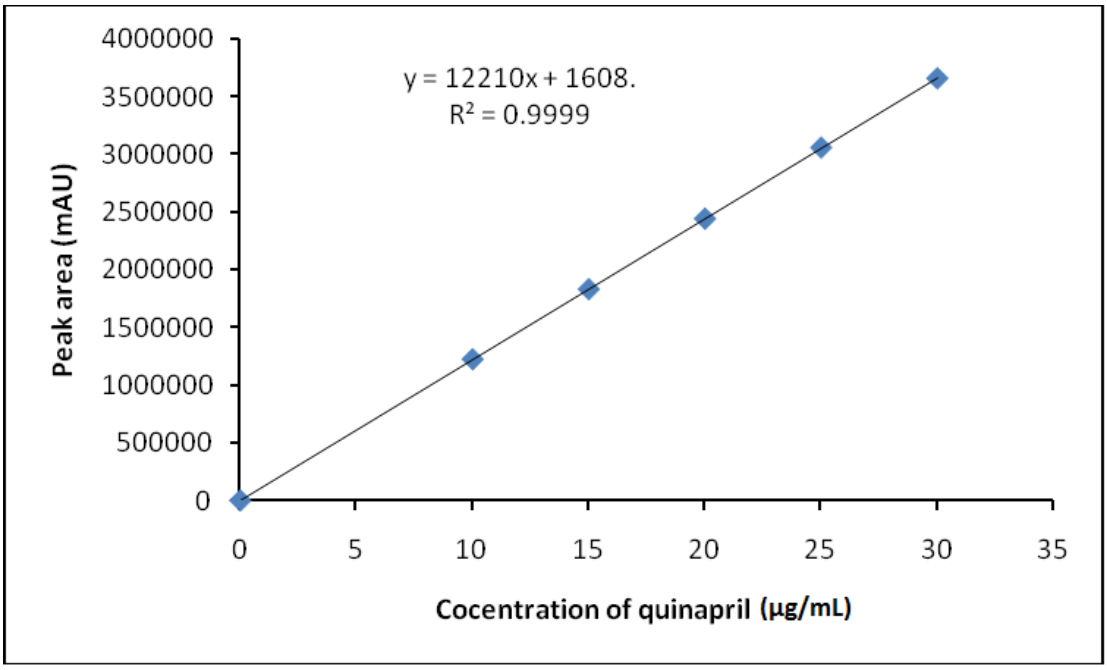

Figure 5.4: Linearity curve of quinapril

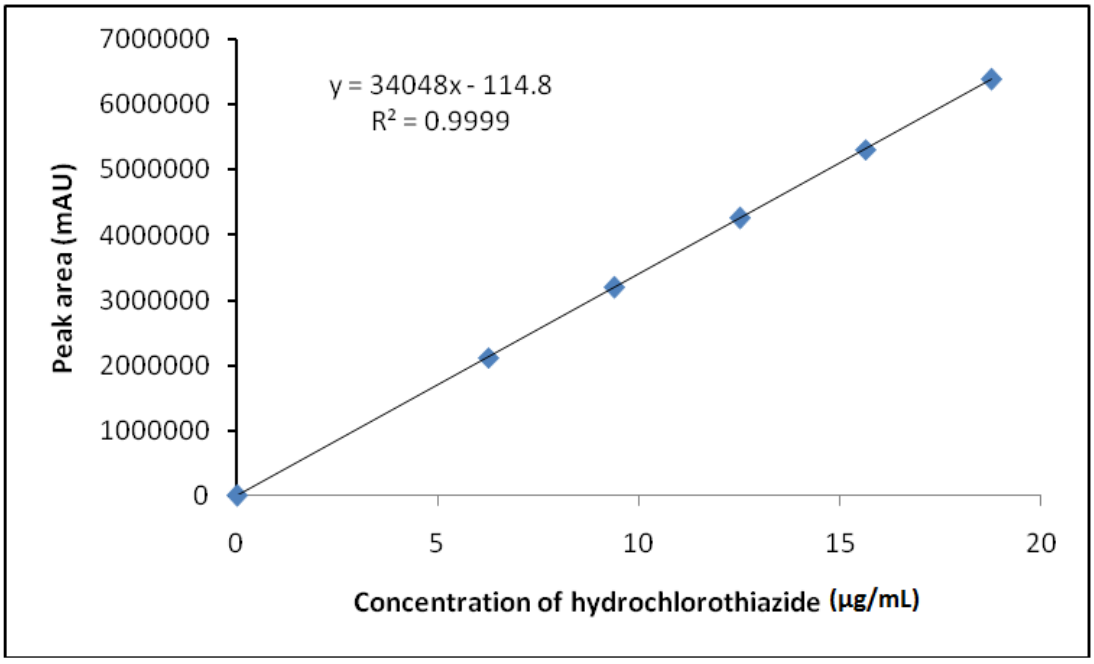

Figure 5.5: Linearity curve of hydrochlorothiazide

5.4.2.3. Sensitivity

The LOD and LOQ of the developed method were calculated based on the standard deviation of the peak area response and slope approach as given in ICH guidelines [60]. The detection limit and quantification limit for quinapril are 0.045 µg/mL and 0.149 µg/mL and for hydrochlorothiazide are 0.021 µg/mL and 0.071 µg/mL, respectively. The low values of LOD and LOQ indicates the adequate sensitivity of the method for the assay of quinapril and hydrochlorothiazide simultaneously. The LOD and LOQ chromatograms of quinapril and hydrochlorothiazide are shown in Figures 5.6 and 5.7.

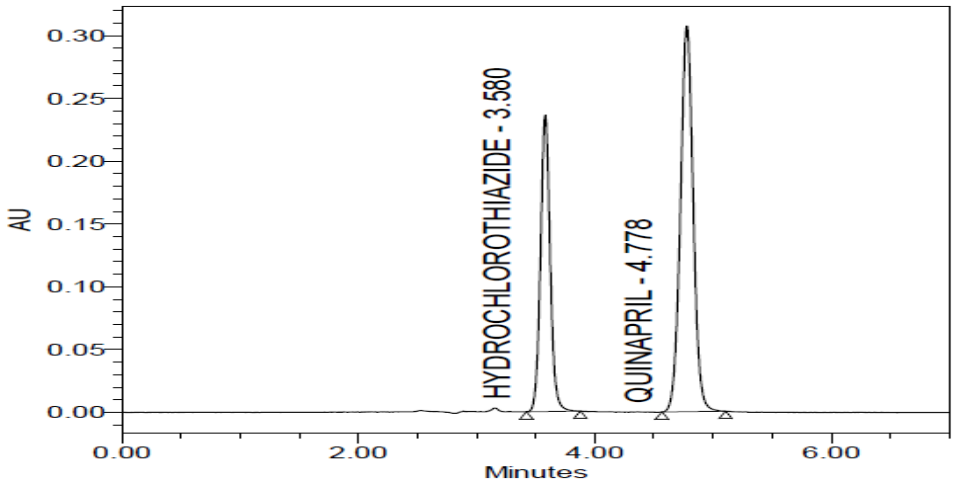

Figure 5.6: Chromatogram of quinapril and hydrochlorothiazide at LOD level

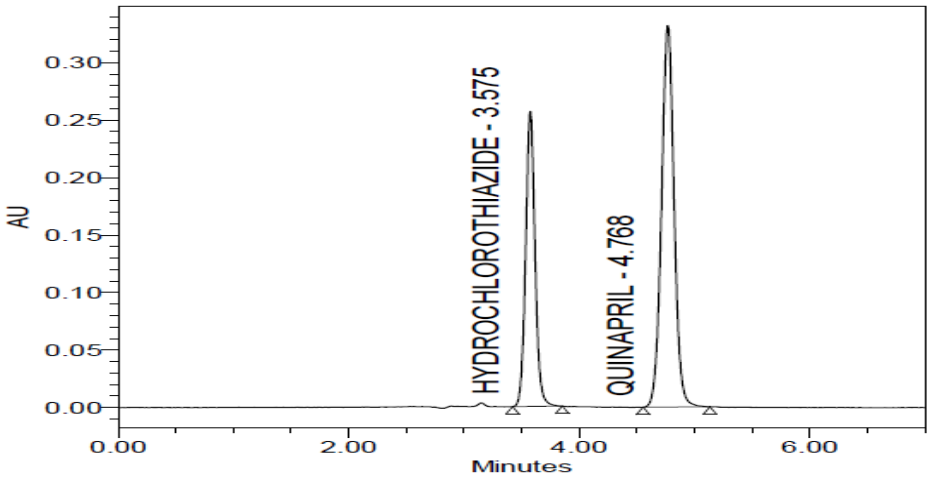

Figure 5.7: Chromatogram of quinapril and hydrochlorothiazide at LOQ level

5.4.2.4. Selectivity

The selectivity of the method was confirmed by comparison of the chromatograms of placebo (Figure 5.8), mobile phase blank (Figure 5.9), tablet sample (Figure 5.10) and working standard (Figure 5.11) solutions. The results showed that the common excipients of the placebo, excipient used in the preparation of tablets and components of mobile phase do not interfere with the analysis of quinapril and hydrochlorothiazide.

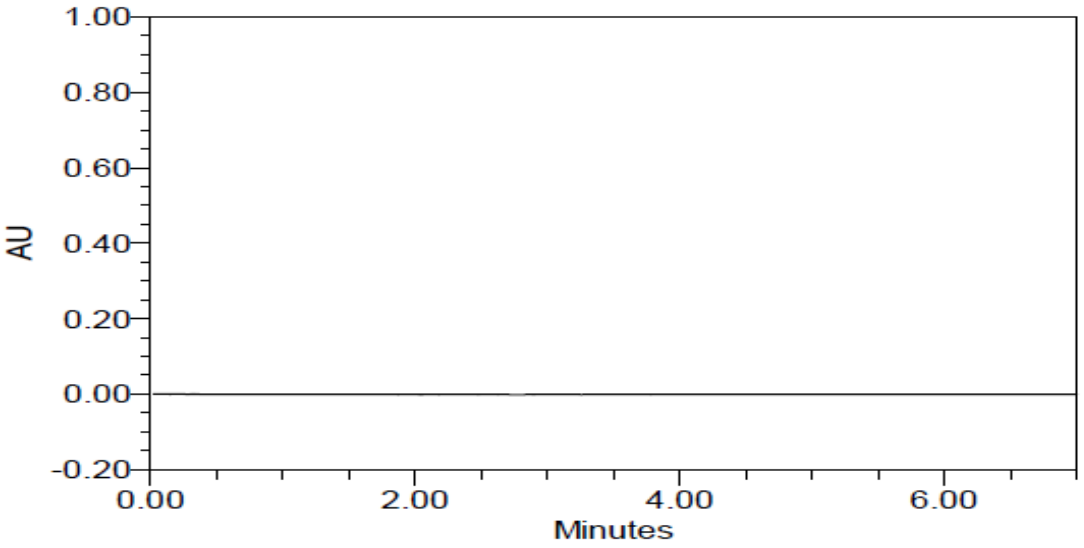

Figure 5.8: Chromatogram of placebo blank

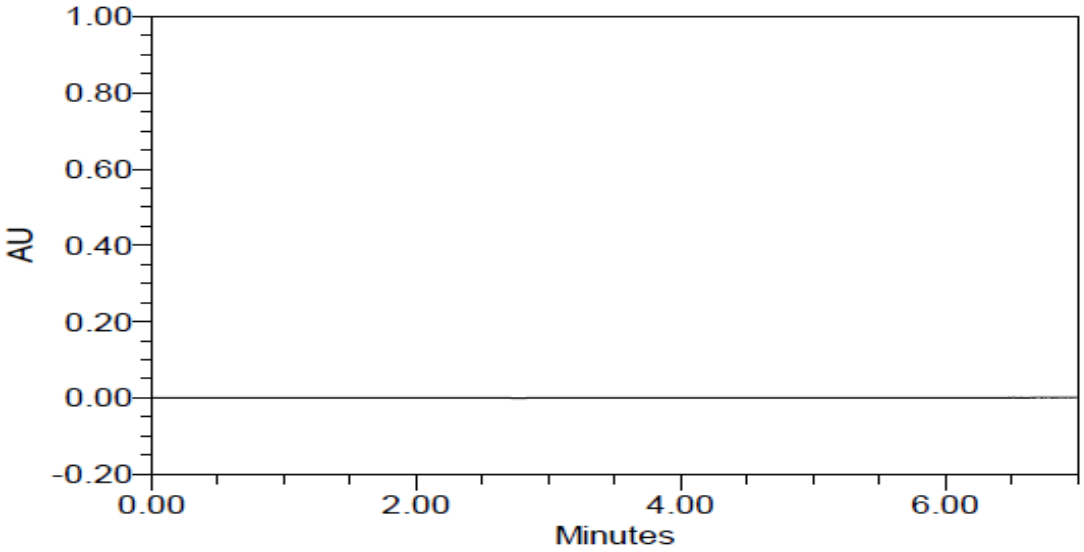

Figure 5.9: Chromatogram of mobile phase blank

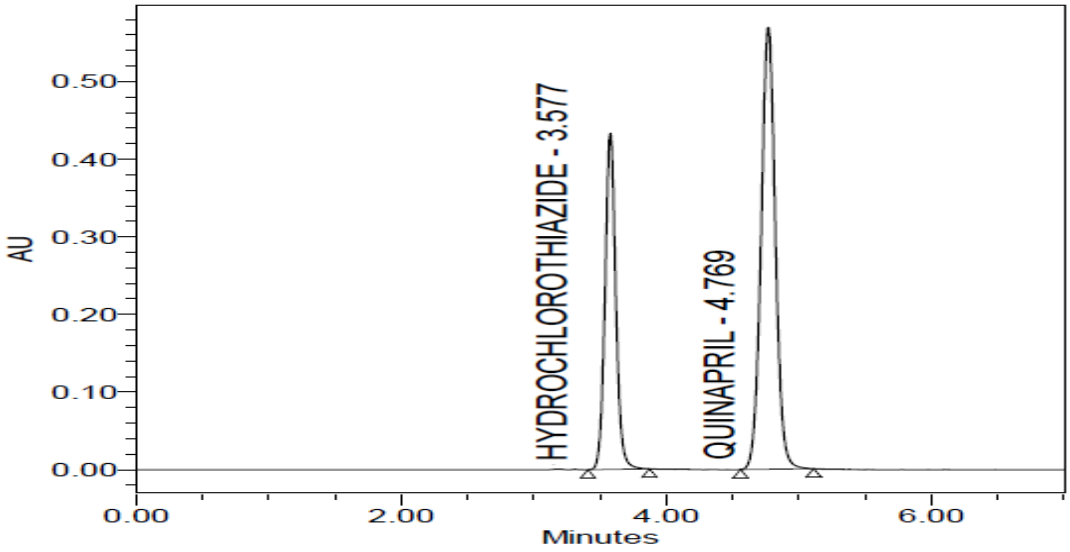

Figure 5.10: Chromatogram of tablet sample (20 µg/mL quinapril and 12.5 µg/mL hydrochlorothiazide)

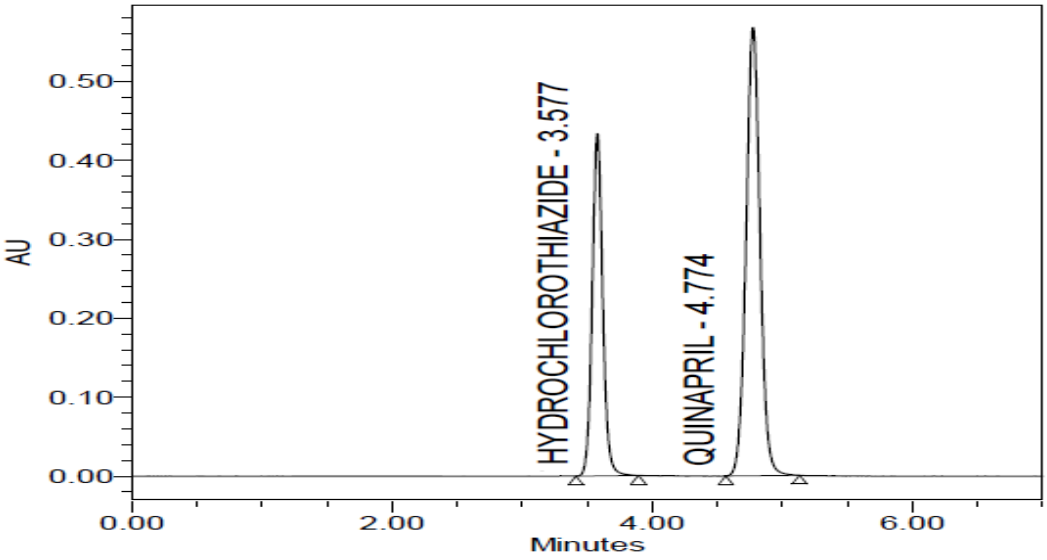

Figure 5.11: Chromatogram of working standard (20 µg/mL quinapril and 12.5 µg/mL hydrochlorothiazide)

5.4.2.5. Specificity

Specificity and stability indicating nature of the proposed RP-HPLC method was demonstrated by the forced degradation of quinapril and hydrochlorothiazide tablet sample solution using various ICH prescribed stress conditions such as acidic, basic, oxidative, thermal and photolytic [59]. The chromatograms under various degradation conditions are shown in Figures 5.12, 5.13, 5.14, 5.15, and 5.16. The results of degradation studies are given in Table 5.4.

All the degradation conditions applied were adequate to degrade quinapril and hydrochlorothiazide. The percent degradation values showed that hydrochlorothiazide is stable as compared to quinapril. Under acidic conditions, hydrochlorothiazide was degraded up to 4.62 % and quinapril was degraded up to 6.82 %. Under basic stress, hydrochlorothiazide was degraded up to 5.74 % and quinapril was degraded upto 5.94 % under basic stress. Under oxidative stress, hydrochlorothiazide was degraded up to 4.55 % and quinapril 5.24 %. Under thermal stress, quinapril was degraded up to 7.38 % and hydrochlorothiazide was degraded up to 5.13 %. Under photolytic stress hydrochlorothiazide and quinapril were degraded up to 6.10 % and 7.90 %, respectively. From these degradation studies, it was concluded that hydrochlorothiazide and quinapril are not stable in basic, acidic, oxidative, thermal and photolytic degradation conditions. One degradation product was produced under acidic (Figure 5.12) and oxidative (Figure 5.14) degradation conditions. Two degradation products were produced in basic (Figure 5.13), photolytic (Figure 5.15) and thermal (Figure 5.14) degradation conditions. The developed RP-HPLC method well separated the degradation products from hydrochlorothiazide and quinapril peaks

(Figures 5.12, 5.13, 5.14, 5.15, and 5.16). The results indicated that this method is specific for its intended use.

Using PDA detector, the homogeneity and purity of hydrochlorothiazide and quinapril peaks were assessed. The peak purity test results (Table 5.4) confirmed that hydrochlorothiazide and quinapril peaks were pure and homogeneous in all the analyzed degradation conditions. Thus confirms the stability-indicating power of the method.

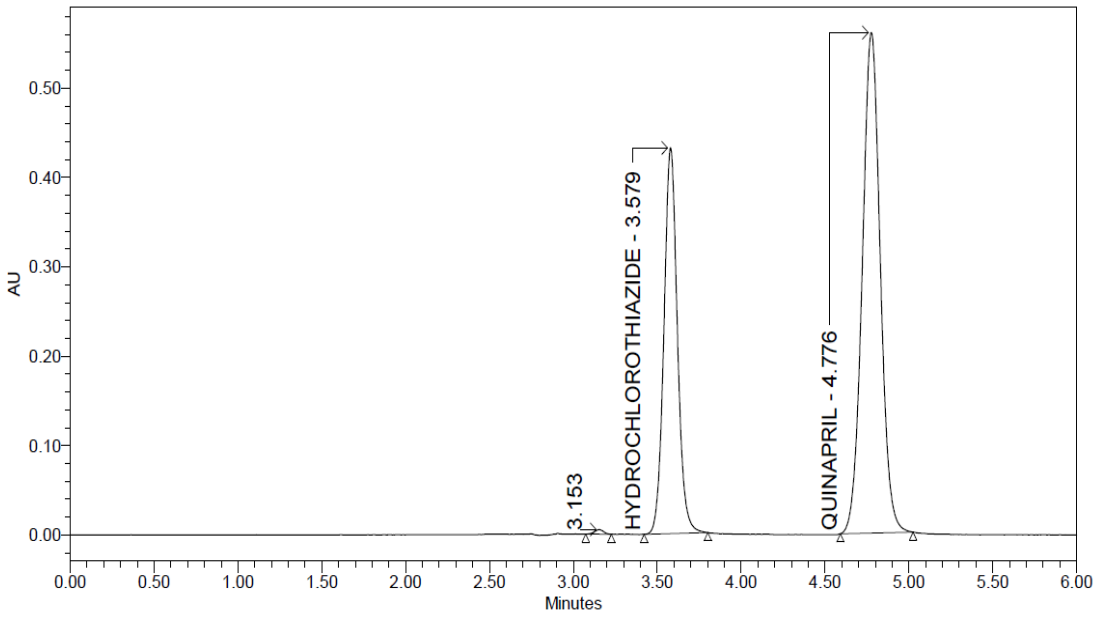

Figure 5.12: Chromatogram of hydrochlorothiazide and quinapril under acidic degradation

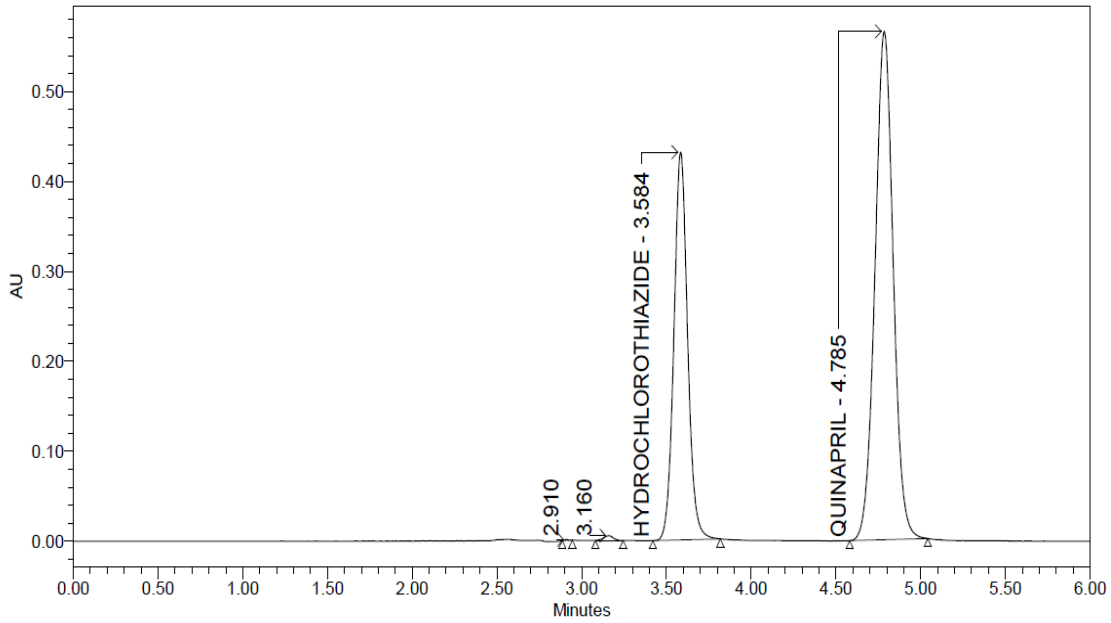

Figure 5.13: Chromatogram of hydrochlorothiazide and quinapril under basic degradation

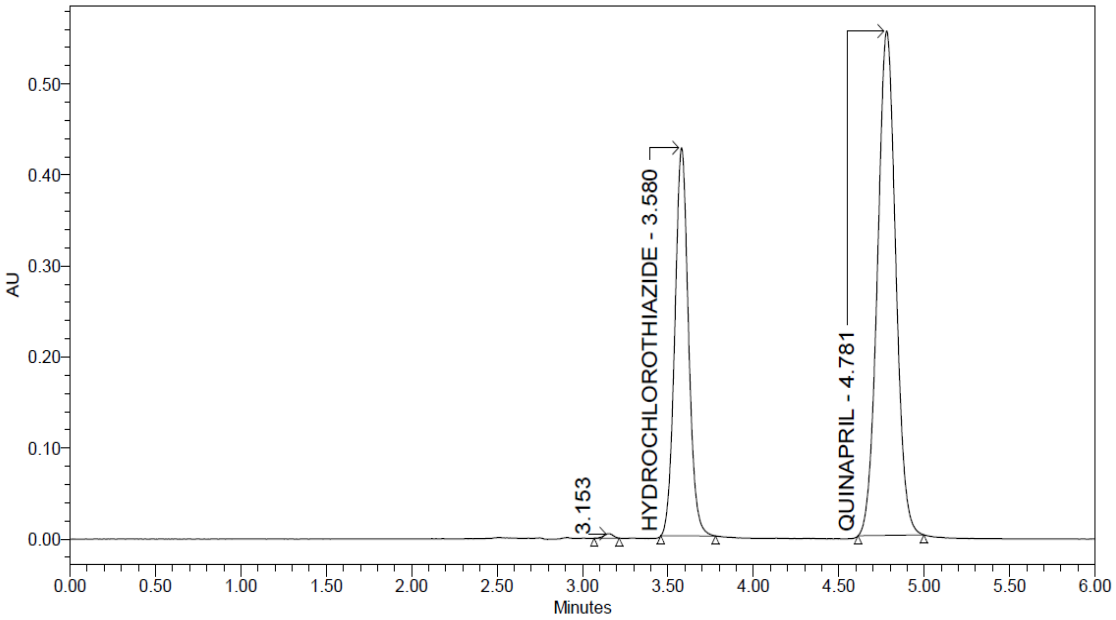

Figure 5.14: Chromatogram of hydrochlorothiazide and quinapril under oxidative degradation

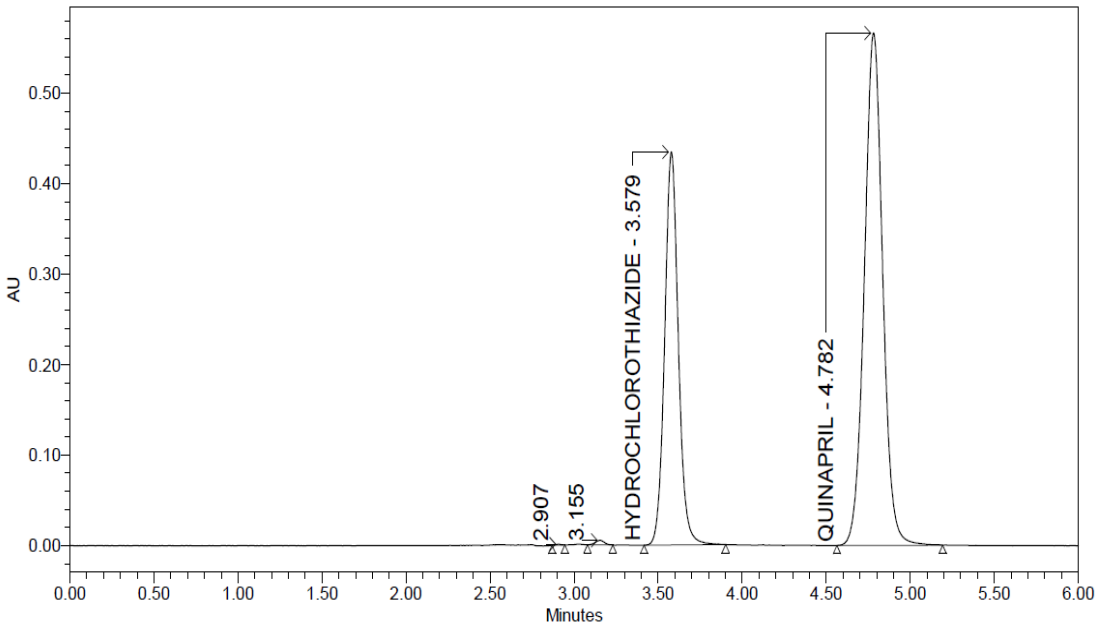

Figure 5.15: Chromatogram of hydrochlorothiazide and quinapril under thermal degradation

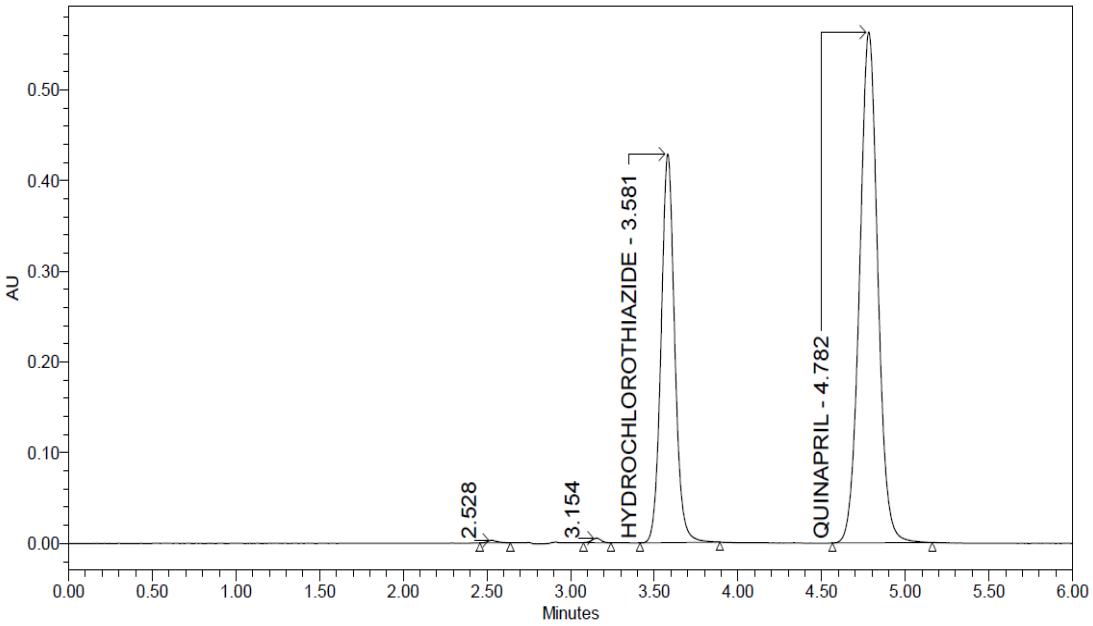

Figure 5.16: Chromatogram of hydrochlorothiazide and quinapril under photo degradation

Table 5.4: Stress testing results of hydrochlorothiazide and quinapril

Degradation condition	Peak area	Drug remained (%)	Purity threshold	Purity angle
Quinapril				
Acidic	2334370	93.18	0.642	0.581
Basic	2355867	94.04	0.643	0.589
Oxidative	2373927	94.76	0.697	0.603
Thermal	2320264	92.62	0.643	0.582
Photo	2307206	92.10	0.643	0.583
Hydrochlorothiazide				
Acidic	4079864	95.38	0.594	0.442
Basic	4031679	94.26	0.596	0.466
Oxidative	4082767	95.45	0.598	0.466
Thermal	4057847	94.87	0.594	0.45
Photo	4016492	93.90	0.595	0.473

5.4.2.6. Precision

Precision was evaluated by measuring peak area response of six different samples at the same concentration (20 µg/mL quinapril and 12.5 µg/mL hydrochlorothiazide) under the same experimental conditions. The method proved to be precise presenting relative standard deviation values for quinapril (0.120 %) and hydrochlorothiazide (0.080 %) lower than 1 %. The results are shown in Table 5.5.

5.4.2.7. Accuracy

Accuracy was estimated by determining percent recovery of six different samples at the same concentration (20 µg/mL quinapril and 12.5 µg/mL hydrochlorothiazide) under the same experimental conditions. The method proved to

be accurate presenting good percent recovery values for quinapril (99.804 %) and hydrochlorothiazide (99.498 %). The results are shown in Table 5.5.

The accuracy was evaluated further by assaying, in triplicate, tablet samples of known concentration with the addition of three different concentrations (50 %, 100 %, and 150 %) of quinapril and hydrochlorothiazide reference standards. The percent recovery of the pure drugs added was calculated. The concentration of quinapril and hydrochlorothiazide in the assay of accuracy was between 99.70 % to 100.21 % and 100.51 % to 100.52 % and 58.3 % (Tables 5.6 and 5.7). Thus, the method can be considered accurate. The tablet excipients did not interfere with the assay of quinapril and hydrochlorothiazide. The chromatograms of quinapril and hydrochlorothiazide at three different concentration levels are shown in Figures 5.17, 5.18 and 5.19

Table 5.5: Values determined for the parameter precision and accuracy of quinapril and hydrochlorothiazide working standard

Sample No.	Quinapril		Hydrochlorothiazide	
	Peak area	**Recovery (%)**	**Peak area**	**Recovery (%)**
1	2449497	99.99	4251921	99.41
2	2447469	99.90	4258602	99.56
3	2447132	99.89	4258583	99.56
4	2440264	99.61	4257847	99.54
5	2445867	99.84	4252767	99.43
6	2444370	99.78	4251679	99.4
Mean*	**2445020.4**	**99.804**	**4255895.6**	**99.498**
RSD	**0.120**	**0.119**	**0.080**	**0.077**

**Average of six values*

Table 5.6: Values of recovery test for quinapril

Spiked Level	Quinarpril			
	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Mean* (%)
50 %	10.00	10.02	100.20	100.21
	10.00	10.03	100.26	
	10.00	10.02	100.17	
100 %	20.00	19.93	99.63	99.80
	20.00	19.97	99.85	
	20.00	19.98	99.92	
150 %	30.00	29.92	99.73	99.70
	30.00	29.89	99.62	
	30.00	29.93	99.76	

**Average of three values*

Table 5.7: Values of recovery test for hydrochlorothiazide

Spiked Level	Hydrochlorothiazide			
	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Mean* (%)
50 %	6.19	6.21	100.41	100.51
	6.19	6.22	100.54	
	6.19	6.22	100.57	
100 %	12.38	12.45	100.58	100.52
	12.38	12.43	100.47	
	12.38	12.44	100.52	
150 %	18.56	18.66	100.51	100.52
	18.56	18.67	100.58	
	18.56	18.65	100.46	

**Average of three values*

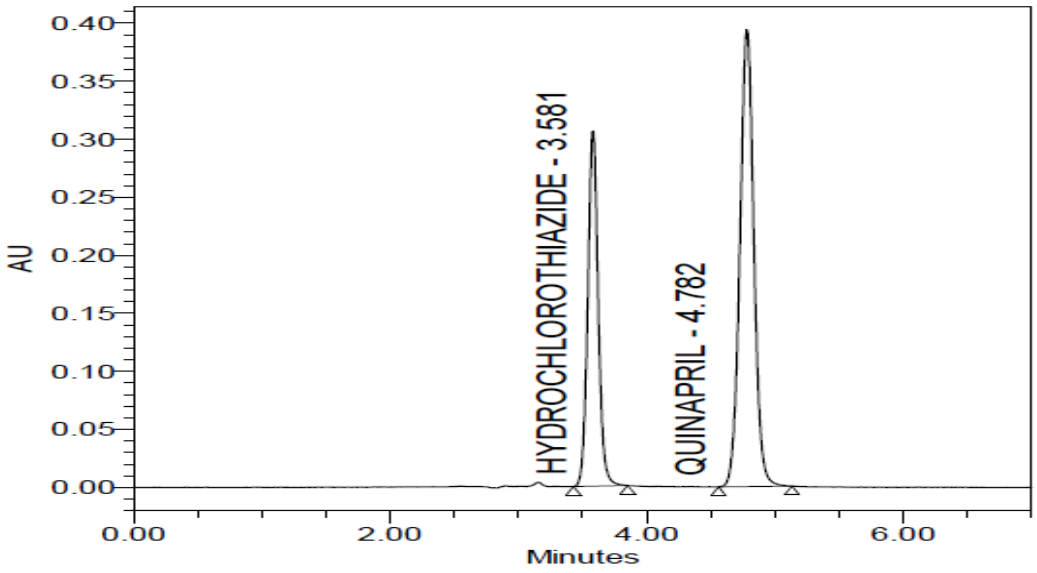

Figure 5.17: Chromatogram of quinapril and hydrochlorothiazide at 50 % accuracy level

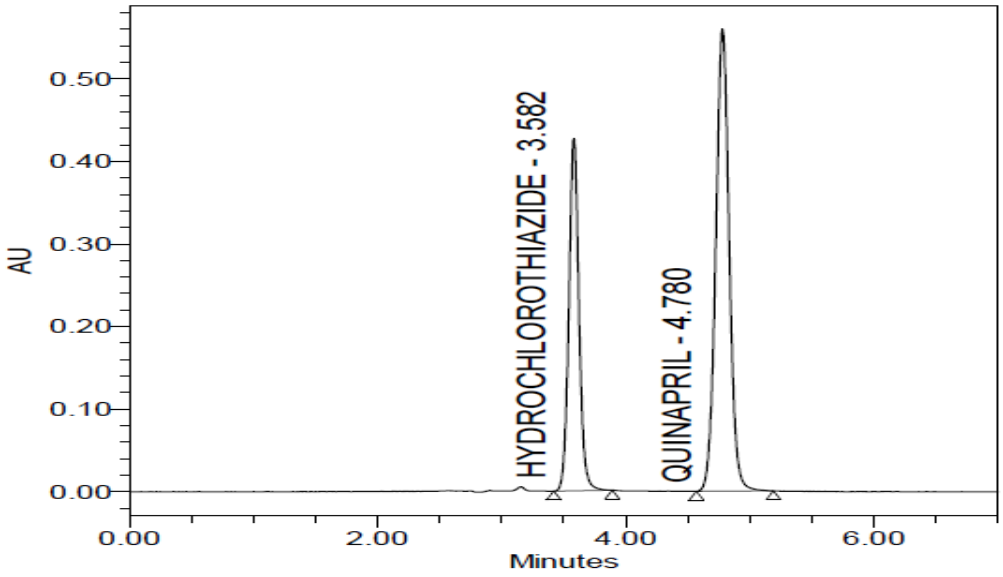

Figure 5.18: Chromatogram of quinapril and hydrochlorothiazide at 100 % accuracy level

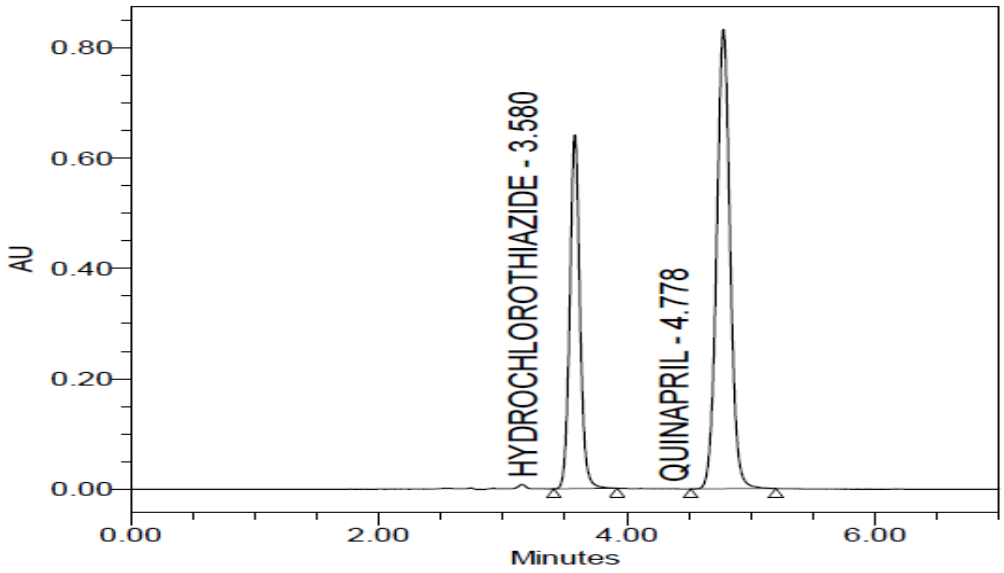

Figure 5.19: Chromatogram of quinapril and hydrochlorothiazide at 150 % accuracy level

5.4.2.8. Robustness

Robustness was determined at a concentration of 20 µg/mL quinapril and 12.5 µg/mL hydrochlorothiazide. By introducing small changes in the mobile phase flow rate (± 0.1 mL) and column temperature (± 5 ^{o}C), the effects on the system suitability parameters were examined. The system suitability parameters are within the recommended limits, indicated the robustness of the method (Tables 5.8).

Table 5.8: Robustness study of quinapril and hydrochlorothiazide

Parameter	Quinapril			Hydrochlorothiazide		
	USP Tailing	USP plate count	USP resolution	USP Tailing	USP plate count	USP resolution
Temperature 25 + 5 ^{o}C	1.01	10495	7.14	1.05	10805	-
Temperature 25 - 5 ^{o}C	1.04	8448	6.48	1.07	8418	-
Flow rate 1.0 + 0.1 mL/min	1.00	10223	7.07	1.06	10594	-
Flow rate 1.0 – 0.1 mL/min	1.03	8271	6.46	1.06	8575	-

5.5. COMPARISON STUDIES

A variety of analytical methods have been reported for the assay of hydrochlorothiazide [section 5.2.1. references: 10-24] and quinapril [section 5.2.2. references: 25-43] alone in pharmaceutical formulations and/or biological samples. They include UV spectrophotometry, visible spectrophotometry, stability-indicating HPLC with UV detection, diffuse reflectance spectroscopy, HPLC with electrochemical detection, HPLC with radiochemical detection coupled to liquid scintillation counting spectrometry, HPLC with fluorescent detection, HPLC with UV detection, liquid chromatography–tandem mass spectrometry, UPLC with tandem mass spectrometry, differential pulse anodic voltammetry, anodic stripping voltammetry, matrix-assisted laser desorption ionization time-of-flight mass spectrometry, cyclic voltammetry, adsorptive stripping voltammetry, biomimetic sensor using amperometric detection and chemometrics and capillary zone electrophoresis. All the reported methods are applied only for the individual quantification of hydrochlorothiazide or quinapril. They are not applicable for simultaneous estimation of hydrochlorothiazide and quinapril

Few methods are existing for the simultaneous analysis of quinapril and hydrochlorothiazide combination. These methods include UV spectrophotometry [45-48], Fourier transform infrared spectroscopy [48], high performance thin layer chromatography [49], liquid chromatography-tandem mass spectrometry [50], high performance liquid chromatography [51-55], ion-pair high performance liquid chromatography[56] and stability indicating high performance liquid chromatography [48, 57,58].

Though the spectrophotometric methods [45-48] are simple, they are low in accuracy, precision, and selectivity when compared with the chromatographic methods. High performance thin layer chromatography [49] and liquid chromatography-tandem mass spectrometry [50] methods are sensitive but they are time consuming, costly and require expertise. Furthermore, liquid chromatography-tandem mass spectrometry [50] method was applied for the analysis of quinapril and hydrochlorothiazide in human plasma samples. The spectrophotometry [45-48], high performance thin layer chromatography [49] and liquid chromatography-tandem mass spectrometry [50] methods are not stability indicating methods.

RP-HPLC is considered as the most specific, sensitive and first choice of quantitation for drugs in bulk, pharmaceutical dosage forms, and biological samples. Few RP-HPLC methods are found in the literature [48, 51-58]. The details of the reported and proposed RP-HPLC methods are summarized in Table 5.9. From the values of percent RSD and percent recovery, it was observed that the proposed stability indicating RP-HPLC method was more precise and accurate than the reported RP-HPLC methods [48, 51-58]. The proposed method has quite lower detection limit as compared to several reported methods [48, 51-53, 54-58]. The shorter run time makes the proposed method more rapid than all reported RP-HPLC method [48, 51-55, 57,58], except ion-pair HPLC method [56]. The shorter run time decreases the utilization of solvents and therefore cost of analysis per single analysis. Except the methods of Shabeen and Rubesh kumar [57], de Diego *et al.*, [58] and Vandana *et al.*, [48] the remaining all other methods are not stability indicating.

Though the methods of Shabeen and Rubesh kumar [57], de Diego *et al.*, [58] and Vandana *et al.*, [48] are stability indicating, peak purity analysis was not reported and the methods were not fully validated. As compared to the reported stability indicating RP-HPLC methods, the proposed method has advantages, including small volume of the sample [58,59], less flow rate [58] and isocratic mode of elution [48,59]. The less flow rate of mobile phase and isocratic mode of elution decrease the utilization of solvents and make the method economical.

Table 5.9: Performance of reported and proposed high performance liquid chromatographic methods

Method	Drug	Detection wavelength (nm)	Run Time (min)	Linearity (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	RSD (%)	Recovery (%)	Reference
RP-HPLC	Qui	210	10	50-300	3.1	9.1	0.12-0.21	100.82-101.11	Harini *et al.*, [51]
	Hyd			31.25-187.5	3	9.8	0.05-0.26	99.72-100.82	
RP-HPLC	Qui	220	7	50-150	0.0592	0.1793	1.284	99.70-101.50	Rani *et al.*, [52]
	Hyd			50-150	0.0509	0.1543	0.067	98.80-100.40	
RP-HPLC	Qui	210	10	25-150	0.44	2.3	0.24-0.46	99.91-100.09	Srikanth *et al.*, [53]
	Hyd			31.25-187.5	0.15	0.76	0.23-0.56	99.72-100.11	
RP-HPLC	Qui	211	8	2-30	0.0195	0.0639	0.506-0.652	98.40-101.00	Serkan *et al.*, [54]
	Hyd			1.25-18.75	0.0030	0.0098	1.304-1.611	100.23-102.50	
RP-HPLC	Qui	225	15	5-30	0.547	1.659	0.5	99.98	Govinda *et al.*, [55]
	Hyd			6-37	0.578	1.751	0.4	99.24	
Ion-pair HPLC	Qui	220	6	30-150	0.05	0.4	1.373	102.20-102.50	Gandhimathi and Ravi, [56]
	Hyd			30-150	0.02	0.1	0.776	99.66-99.67	

Table 5.9 (continued): Performance of reported and proposed high performance liquid chromatographic methods

Method	Drug	Detection wavelength (nm)	Run Time (min)	Linearity (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	RSD (%)	Recovery (%)	Reference
Stability indicating RP-HPLC	Qui	210	8	25-150	0.3978	1.2055	0.4	99.66-100.42	Shabeen and Rubesh kumar, [57]
	Hyd			31.25-187.5	0.9245	3.552	0.6	99.78-100.20	
Stability indicating RP-HPLC	Qui	216	12	40-200	0.35	1.06	1.03-3.14	99.52-100.14	de Diego *et al.*, [58]
	Hyd			25-125	0.61	1.85	0.75-1.77	100.37-102.78	
Stability indicating RP-HPLC	Qui	215	8	2-10	0.60	1.83	0.91-1.99	100.71	Vandana *et al.*, [48]
	Hyd			2.5-12.5	0.54	1.65	0.80-1.89	99.96	
Stability indicating RP-HPLC	Qui	210	6	10-30	0.045	0.149	0.120	99.70-100.21	Proposed method
	Hyd			6.25-18.75	0.0214	0.0715	0.080	100.51-100.52	

Qui – Quinapril; Hyd - Hydrochlorothiazide

5.6. SUMMARY AND CONCLUSION

The overall results obtained for the proposed method validation were tabulated in Table 5.10.

Table 5.10: Summary of the proposed method

Parameter	Quinapril	Hydrochlorothiazide
Linearity (µg/mL)	10-30	6.25-18.75
Regression equation	y = 12210 x + 1608	y = 34048 x - 114.8
Regression coefficient (R^2)	0.9999	0.9999
LOD (µg/mL)	0.045	0.021
LOQ (µg/mL)	0.149	0.071
Precision (%RSD)	0.120	0.080
Accuracy (%Recovery)	99.70-100.21 %	100.51-100.52 %

A stability indicating RP-HPLC method with photodiode array detection has been developed and validated for the quantification of quinapril and hydrochlorothiazide combination. The newly developed RP-HPLC method is more superior to reported RP-HPLC methods due to its better sensitivity, more rapid, economy, precise, accurate and lower detection limits. Application of the method for the estimation of quinapril and hydrochlorothiazide in tablets with good recovery demonstrated its suitability for analysis of quinapril and hydrochlorothiazide simultaneously. The less runtime (5 min) in the proposed RP-HPLC method enabled the determination of a number of samples in a limited time without any hindrance from the excipients of tablets or degradation products produced during degradation conditions. The proposed RP-HPLC method could be useful for quality control laboratories.

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