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Stability Indicating Reverse Phase High Performance Liquid Chromatographic Method with Photodiode Array Detection for the Simultaneous Quantification of Quinapril and Hydrochlorthiazide in Bulk and Tablet Dosage Forms

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The combination of hydrochlorothiazide and quinapril is used to treat high blood pressure. In the current study, a stability-indicating RP-HPLC method with photodiode array detection was developed and validated for the simultaneous determination of quinapril and hydrochlorthiazide in bulk and tablet dosage form. Separation and analysis of selected drug combination in the presence of degradation products was achieved on Agilent C_{18} (250 mm × 4.6 mm, 5 µ) column. A mixture of 0.1 M KH_2PO_4 and methanol (65:35, v/v) was used as mobile phase. The developed method was linear over the concentration range of 10-30 µg/mL (quinapril) and 6.25-18.75 µg/mL (hydrochlorothiazide) with acceptable precision and accuracy. The degradation studies showed that quinapril and hydrochlorthiazide are relatively unstable under acidic, basic, oxidative, thermal and photo degradation conditions applied. No interference peaks from common tablet excipients and degradation products were observed. The proposed stability-indicating RP-HPLC method could be opt for routine quality control studies of quinapril and hydrochlorthiazide combined dosage forms.

Keywords: HPLC, Quinapril, Hydrochlorthiazide, Stability indicating, Forced degradation.

INTRODUCTION

Hydrochlorothiazide [1-4] is a diuretic belonging to the thiazide class of drugs. Hydrochlorothiazide is used in the treatment and management of congestive heart failure, hypertension, symptomatic edema, renal tubular acidosis, diabetes insipidus, osteoporosis, hypoparathyroidism and the avoidance of kidney stones. Hydrochlorothiazide prevents fluid retention in the body by reducing the electrolytes reabsorption from the renal tubules. Chemically, hydrochlorothiazide is designated as 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide (Fig. 1).

Quinapril [5-7] 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. 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. Chemically, quinapril is (3*S*)-2-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenyl-butan-2-yl]-amino]propanoyl]-3,4-dihydro-1*H*-isoquinoline-3-carboxylic acid (Fig. 1).

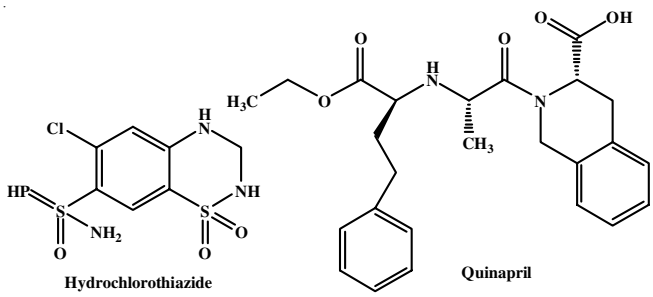

Fig. 1. Chemical structures of selected drugs

Hydrochlorothiazide (Fig. 1) is used in the treatment of hypertension either alone or in combination with other antihypertensives like angiotensin converting enzyme inhibitors and β-blockers. The combination of hydrochlorothiazide and quinapril is used to treat high blood pressure [8]. The lowering of high blood pressure helps in prevention of kidney problems, strokes and heart attacks.

Various analytical methods (Table-1) such as second-order-derivative spectrophotometry [9], ratio spectra derivative spectrophotometry [10], Continuous wavelet transform method [11], chemometric method [10,11], dual wavelength spectrophotometry [12], Fourier transform infrared spectroscopy [12], high performance thin layer chromatography [13], liquid chromatography-tandem mass spectrometry [14], high performance liquid chromatography [15-19], ion pair high performance liquid chromatography [20] and stability indicating high performance liquid chromatography [12,21,22] have been reported for quantitative determination of quinapril and hydrochlorothiazide simultaneously in pharmaceutical and biological samples. Most of the above reported methods [9,11,13-20] were not stability indicating. Disadvantages of the reported stability indicating methods [12,21,22] 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 this article, we report a rapid, reliable and fully validated RP-HPLC method with photodiode array detection for the precise and accurate determination of hydrochlorothiazide and quinapril concentrations in bulk and combined tablet dosage form.

EXPERIMENTAL

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.

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.

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 auto sampler. Data collection and processing was done using Waters Empower 2.0 software.

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 µ); 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.

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 upto mark using the same solvent. The stock standard solution was further diluted with mobile phase to obtain working standard solutions in a range from 10-30 µg/mL quinapril and 6.25-18.75 µg/mL hydrochlorothiazide.

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 in thrice. Calibration curves (peak area response of the analyte *versus* the nominal concentration of analyte) were fitted by least squares linear regression.

Assay of quinapril and hydrochlorothiazide in tablets: Twenty tablets were weighed accurately. The average weight was calculated. Tablets were crushed into 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

TABLE-1
PERFORMANCE OF REPORTED AND PROPOSED HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS

Method	Detection wavelength (nm)	Run time (min)	Drug	Linearity (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	RSD (%)	Recovery (%)	Ref.
RP-HPLC	210	10	Qui	50-300	3.1	9.1	0.12-0.21	100.82-101.11	[15]
			Hyd	31.25-187.5	3	9.8	0.05-0.26	99.72-100.82	
RP-HPLC	220	7	Qui	50-150	0.0592	0.1793	1.284	99.70-101.50	[16]
			Hyd	50-150	0.0509	0.1543	0.067	98.80-100.40	
RP-HPLC	210	10	Qui	25-150	0.44	2.3	0.24-0.46	99.91-100.09	[17]
			Hyd	31.25-187.5	0.15	0.76	0.23-0.56	99.72-100.11	
RP-HPLC	211	8	Qui	2-30	0.0195	0.0639	0.506-0.652	98.40-101.00	[18]
			Hyd	1.25-18.75	0.0030	0.0098	1.304-1.611	100.23-102.50	
RP-HPLC	225	15	Qui	5-30	0.547	1.659	0.5	99.98	[19]
			Hyd	6-37	0.578	1.751	0.4	99.24	
Ion-pair HPLC	220	6	Qui	30-150	0.05	0.4	1.373	102.20-102.50	[20]
			Hyd	30-150	0.02	0.1	0.776	99.66-99.67	
Stability indicating RP-HPLC	210	8	Qui	25-150	0.3978	1.2055	0.4	99.66-100.42	[21]
			Hyd	31.25-187.5	0.9245	3.552	0.6	99.78-100.20	
Stability indicating RP-HPLC	216	12	Qui	40-200	0.35	1.06	1.03-3.14	99.52-100.14	[22]
			Hyd	25-125	0.61	1.85	0.75-1.77	100.37-102.78	
Stability indicating RP-HPLC	215	8	Qui	2-10	0.60	1.83	0.91-1.99	100.71	[12]
			Hyd	2.5-12.5	0.54	1.65	0.80-1.89	99.96	
Stability indicating RP-HPLC	210	6	Qui	10-30	0.045	0.149	0.120	99.70-100.21	Present study
			Hyd	6.25-18.75	0.0214	0.0715	0.080	100.51-100.52	

Qui = Quinapril; Hyd = Hydrochlorothiazide

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. 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 upto 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.

Stability of 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 conditions using 0.1 N HCl (sonication for 30 min at room temperature), basic conditions using 0.1 N NaOH (sonication for 30 min at room temperature), oxidative conditions using 30 % H_2O_2 (sonication for 30 min at room temperature), photolytic (direct exposure of tablet powder to sun light for upto 24 h) and thermal (exposure of tablet powder to 105 °C for 30 min in oven) [23]. 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.

RESULTS AND DISCUSSION

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 it was used in the entire analysis. 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 (Fig. 2).

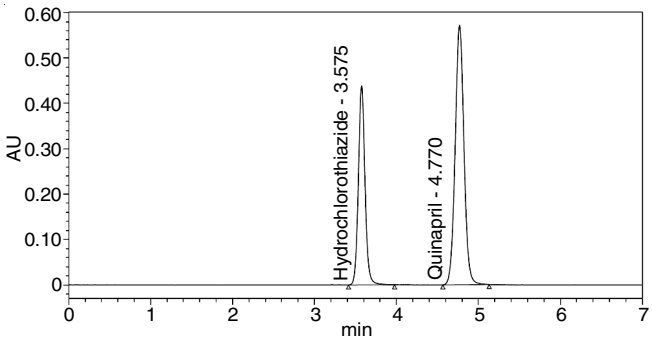

Fig. 2. Chromatogram of well separated peaks of quinapril and hydrochlorothiazide with optimized chromatographic conditions

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 [24].

To evaluate the system suitability of the method, the peak area, plate count, tailing factor resolution and retention time of five replicate injections of 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-2. The values indicating the good performance of the system.

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 while 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 ($R^2 > 0.999$).

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 [24]. The detection limit and quantitation limit for quinapril are 0.045 and 0.149 µg/mL and for hydrochlorthiazide 0.021 and 0.071 µg/mL, respectively.

The selectivity of the method was confirmed by comparison of the chromatograms of placebo (Fig. 3a), mobile phase blank (Fig. 3b), tablet sample (Fig. 3c) and working standard (Fig. 3d) 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 hydrochlorthiazide.

TABLE-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

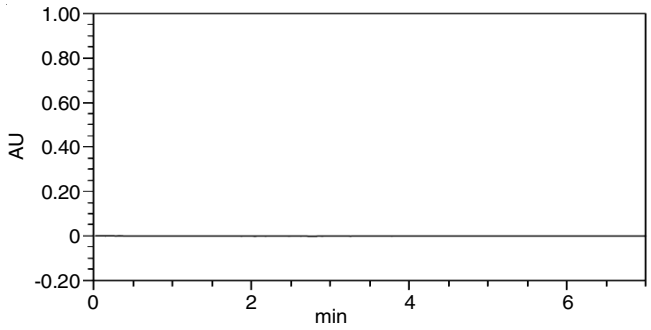

Fig. 3a. Chromatogram of placebo

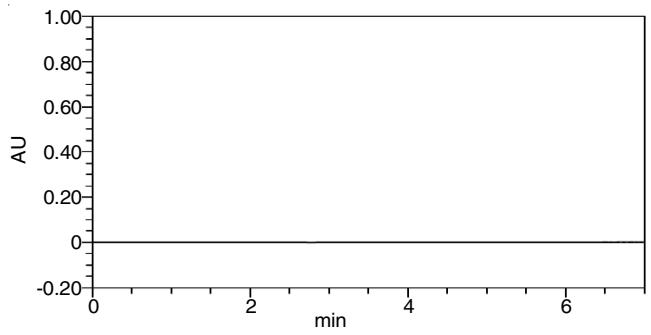

Fig. 3b. Chromatogram of mobile phase blank

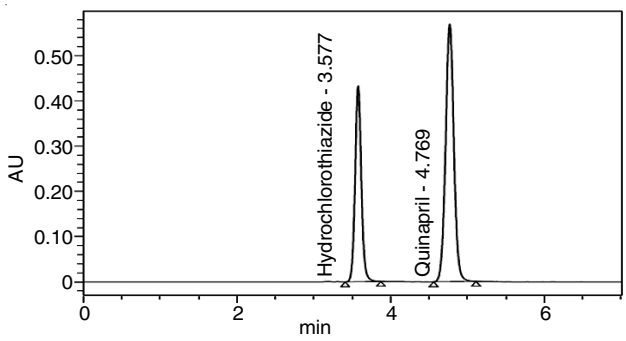

Fig. 3c. Chromatogram of tablet sample (20 µg/mL quinapril and 12.5 µg/mL hydrochlorothiazide)

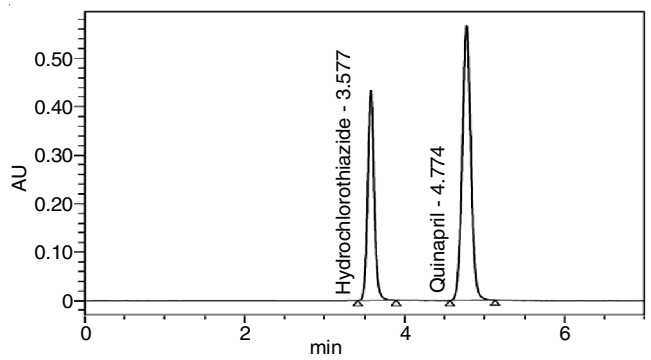

Fig. 3d. Chromatogram of working standard (20 µg/mL quinapril and 12.5 µg/mL hydrochlorothiazide)

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. The chromatograms under various degradation conditions are shown in Fig. 4a-4e. The results of degradation studies are given in Table-3.

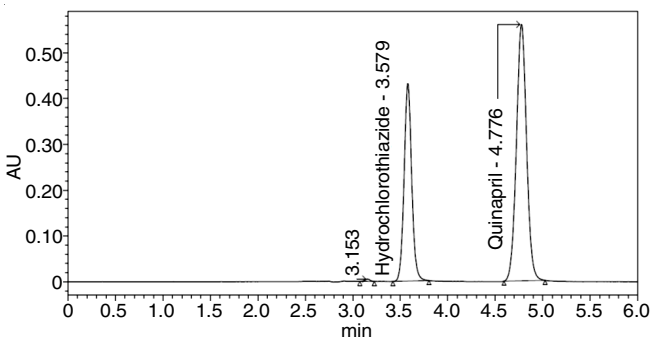

Fig. 4a. Chromatogram of hydrochlorothiazide and quinapril under acidic degradation

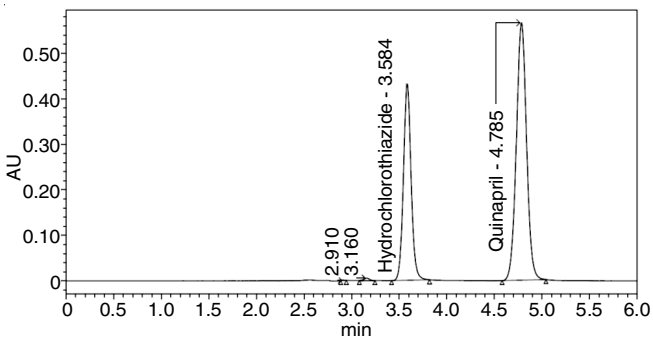

Fig. 4b. Chromatogram of hydrochlorothiazide and quinapril under basic degradation

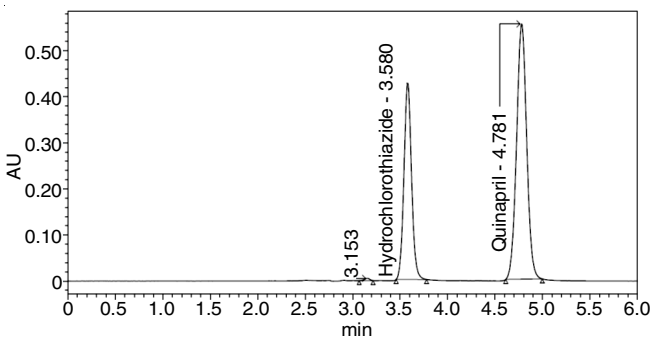

Fig. 4c. Chromatogram of hydrochlorothiazide and quinapril under oxidative degradation

TABLE-3
STRESS TESTING RESULTS OF HYDROCHLOROTHIAZIDE AND QUINAPRIL

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

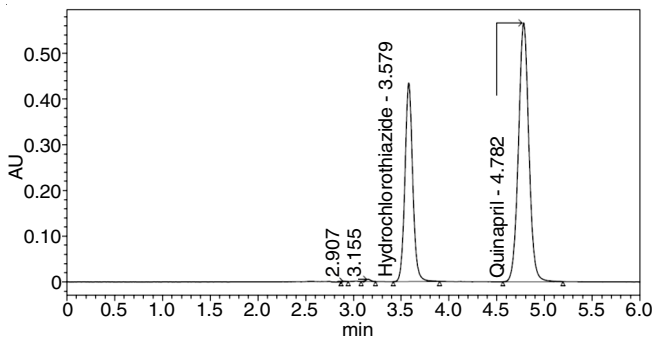

Fig. 4d. Chromatogram of hydrochlorothiazide and quinapril under thermal degradation

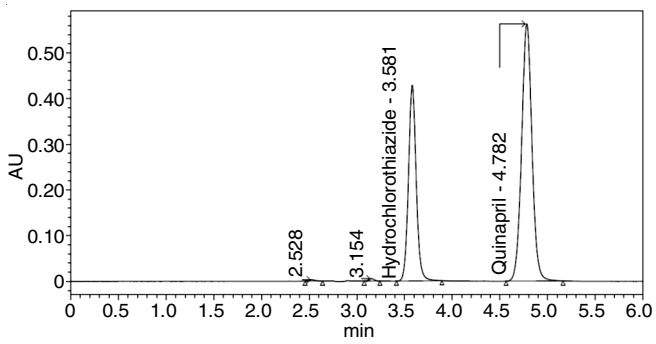

Fig. 4e. Chromatogram of hydrochlorothiazide and quinapril under photo degradation

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 upto 4.62 % and quinapril was degraded upto 6.82 %. Under basic stress hydrochlorothiazide was degraded upto 5.74 % and quinapril was degraded upto 5.94 %. Under oxidative stress hydrochlorothiazide was degraded upto 4.55 % and quinapril upto 5.24 %. Under thermal stress quinapril was degraded upto 7.38 % and hydrochlorothiazide was degraded upto 5.13 %. Under photolytic stress hydrochlorothiazide and quinapril were degraded upto 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 (Fig. 4a) and oxidative (Fig. 4c) degradation conditions. Two degradation products were produced in basic (Fig. 4b), photolytic (Fig. 4e) and thermal (Fig. 4d) degradation conditions. The developed RP-HPLC method well separated the degradation products from hydrochlorothiazide and quinapril peaks (Fig. 4a-4e). 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-3) 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.

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 quinarpril (0.120 %) and hydrochlorthiazide (0.080 %) lower than 1 %. The results are shown in Table-4.

TABLE-4
VALUES DETERMINED FOR THE PARAMETER PRECISION AND ACCURACY OF QUINARPRIL AND HYDROCHLORTHIAZIDE WORKING STANDARD

Sample No.	Quinarpril		Hydrochlorthiazide	
	Peak area	Recovery (%)	Peak area	Recovery (%)
1	2449497.0	99.990	4251921.0	99.410
2	2447469.0	99.900	4258602.0	99.560
3	2447132.0	99.890	4258583.0	99.560
4	2440264.0	99.610	4257847.0	99.540
5	2445867.0	99.840	4252767.0	99.430
6	2444370.0	99.780	4251679.0	99.400
Mean*	2445020.4	99.804	4255895.6	99.498
RSD	0.120	0.119	0.080	0.077

*Average of six values

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 quinarpril (99.804 %) and hydrochlorthiazide (99.498 %). The results are shown in Table-4.

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 hydrochlorthiazide in assay of accuracy was between 99.70-100.21 % and 100.51-100.52 % and 58.3 % (Table-5). Thus, the method can be considered accurate. The tablet excipients did not interfere with the assay of quinapril and hydrochlorthiazide.

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 °C), the effects on the system suitability parameters were examined. The system suitability parameters are within the recommended limits indicated robustness of the method (Table-6).

Conclusion

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 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

TABLE-5
VALUES OF RECOVERY TEST

Spiked level (%)	Quinarpril				Hydrochlorothiazide			
	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Mean* (%)	Added (µg/mL)	Found (µg/mL)	Recovery (%)	Mean* (%)
50	10.00	10.02	100.20	100.21	6.19	6.21	100.41	100.51
	10.00	10.03	100.26		6.19	6.22	100.54	
	10.00	10.02	100.17		6.19	6.22	100.57	
100	20.00	19.93	99.63	99.80	12.38	12.45	100.58	100.52
	20.00	19.97	99.85		12.38	12.43	100.47	
	20.00	19.98	99.92		12.38	12.44	100.52	
150	30.00	29.92	99.73	99.70	18.56	18.66	100.51	100.52
	30.00	29.89	99.62		18.56	18.67	100.58	
	30.00	29.93	99.76		18.56	18.65	100.46	

*Average of three values

TABLE-6
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 °C)	1.01	10495	7.14	1.05	10805	–
Temperature (25 – 5 °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	–

tablets or degradation products produced during degradation conditions. The proposed RP-HPLC method could be useful for quality control laboratories.

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