International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Stability Indicating Rp-Hplc Method Development and Validation for Simultaneous Analysis of Quinapril and Hydrochlorthiazide in Bulk and Tablet Dosage Form

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Abstract: ***A simple, rapid, accurate, specific and sensitive RP-HPLC method has been developed and validated for the simultaneous estimation of Quinapril (QUI) and Hydrochlorthiazide (HCTZ) in bulk drug and pharmaceutical dosage form. The chromatographic separation was performed on thermoscientific Inertsil ODS C18 column (150mm×4.6mm, 5µm particle size) using a mobile phase of phosphate buffer: acetonitirle (26:74v/v), at a flow rate of 1.2ml/min at ambient temperature with the detection wave length at 210nm. The retention times of QUI and HCTZ were 3.76 min and 2.31 min respectively. The linearity was performed in the concentration range of 25-150 µg/ml (QUI) and 31.25-187.5 µg/ml (HCTZ) with a correlation coefficient of 0.999 respectively. The percentage purity of Quinapril and Hydrochlorthiazide was found to be 99.8 and 100.4% w/v respectively. The proposed method has been validated for specificity, linearity, precision, accuracy and robustness were within the acceptance limit according to ICH guidelines and the developed method was successfully employed for routine quality control analysis in the bulk and combined pharmaceutical dosage forms.***

Keywords: Quinapril, Hydrochlorthiazide, RP-HPLC, Validation

1. Introduction

Quinapril (Qui) (Figure 1) is chemically (3S)-2-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino}propanoyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. Quinapril is an angiotensin-converting enzyme inhibitor (ACE inhibitor) used in the treatment of hypertension and congestive heart failure. Quinapril inhibits angiotensin converting enzyme, an enzyme which catalyses the formation of angiotensin II from its precursor, angiotensin I. Angiotensin II is a powerful vasoconstrictor and increases blood pressure through a variety of mechanisms. Due to reduced angiotensin production, plasma concentrations of aldosterone are also reduced, resulting in increased excretion of sodium in the urine and increased concentrations of potassium in the blood [1] - [2].

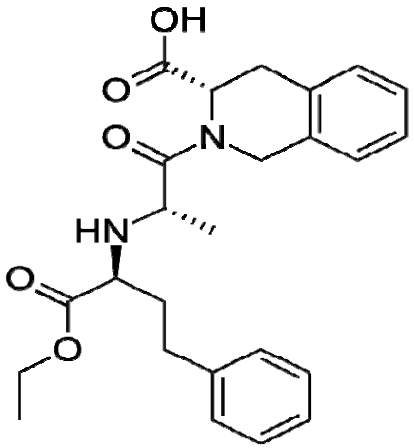

Figure 1: Chemical structure of Quinapril

Hydrochlorothiazide (HCTZ) (Figure 2) is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1{6},2,4-benzothiadiazine-7- Sulphonamide is a diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance. Hydrochlorothiazide is frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones [3]-[4].

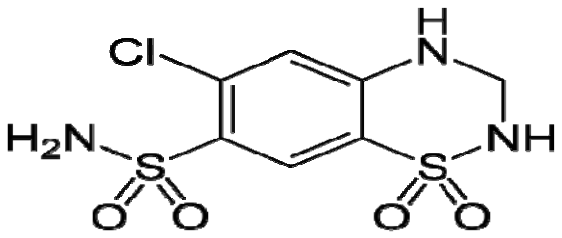

Figure 2: Chemical structure of Hydrochlorthiazide

Literature survey reveals[5]-[10] that few UV spectrophotometric methods, HPLC methods, Ion pair HPLC method, HPTLC method has been reported for the estimation of Quinapril and Hydrochlorthiazide. The aim of present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Quinapril and Hydrochlorthiazide in bulk and pharmaceutical dosage form as per ICH guidelines Q_2 (R1)[11]-[14].

2. Materials and Methods

2.1 Instrumentation

Different kinds of equipments viz Analytical weighing balance (shimadzu AUX 200), HPLC System (WATERS

2695– PDA Detector), Column C18 (150×4.6 mm, 5 µm), Injector (Rheodyne, 20µl), Sonicator (SONICA 2200MH), pH meter, Vacuum filter pump (model XI 5522050 of Millipore), Millipore filtration kit, mobile phase reservoir, Water bath, Sample Filtration assembly and glassware's were used throughout the experiment.

2.2. Chemicals and solvents

The working standard of Quinapril and Hyrochlorthiazide were provided as gift samples from Cipla Pvt. Ltd. Mumbai (Maharashtra, India), Cadila Pharmaceuticals, Ahmadabad (Gujarat, India) respectively. The market formulation ACUPIL-H tablets 22.5 mg of (10 mg Quinapril and 12.5mg Hydrochlorothiazide) were procured from local market. HPLC grade water, methanol [HPLC Grade] and Acetonitrile [HPLC Grade] were purchased from E.Merck (India) Ltd, Mumbai, India. Potassium dihydrogen phosphate, orthophosphoric acid of AR grade was obtained from S.D. Fine Chemicals Ltd, Mumbai, India.

3. Chromatographic Conditions

The column used was thermoscientific Inertsil ODS C18 column (150mm×4.6mm, 5µm particle size) for analytical separation. The mobile phase consisted of an aqueous solution of Potassium dihydrogen phosphate buffer (pH 4) and Acetonitrile in the ratio of (26:74%v/v). The flow rate was adjusted to 1.2ml/min. The instrument was operated at an ambient temperature. The detection was achieved at 210nm using photo diode array. The injection volume was 20µl capacity.

3.1. Preparation of analytical solutions:

3.2. Preparation of 0.01 M phosphate buffer (pH 4.0):

Accurately weighed 1.36gm of potassium dihydrogen phosphate was dissolved in 1000ml of HPLC grade water and mixed using ultrasonicator and filter through 0.45µ membrane filter and the resulting solution pH was adjusted to 4.0 with the help of dilute orthophosphoric acid.

3.4. Preparation of mobile phase:

Mix a mixture of above buffer 260 ml (26%), 740ml of acetonitrile (HPLC grade-74%) and degas in ultrasonic for 5 min and filter through 0.45µ filter under vaccum filteration.

3.5. Preparation of diluent:

Acetonitirle and Water (50:50) was used as diluent.

3.6. Preparation of standard stock solution:

Accurately weighed and transferred 10mg of Quinapril and 12.5mg of Hydrochlorthiazide working standards into a 10 ml clean dry volumetric flask, add 7ml of diluent , sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

3.7. Preparation of sample solution (marketed formulation):

5 tablets were weighed and the average weight (54.8mg was calculated and the sample weight observed is 53.8mg which is having an equivalent to 10mg of Quinapril and 12.5mg of Hydrochlorthiazide, hence 54.8mg of powder (sample) is taken into a 25 ml volumetric flask, 20ml of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.5ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluent.

4. Method development and validation by RP-HPLC:

The suggested analytical method was validated according to ICH guidelines Q2 (R1) with respect to certain parameters such as specificity, linearity, precision, accuracy, robustness and system suitability.

4.1. Linearity

Express ability to obtain test results where directly proportional to the concentration of analyte in the sample. The linearity of the method was established by a spiking a series of sample mixtures of Quinapril and Hydrochlorthiazide of six different concentration levels 20-150µg/ml (Quinapril) and 31.25-187.5µg/ml (Hydrochlorthiazide) are injected in to the HPLC system. Construct the calibration curves for the standard solutions by plotting their response ratios (ratios of the peak of the analytes) against their respective concentrations linear regression was applied and slope-a, intercept-b, correlation coefficient-R2 and standard error (SE) were determined.

4.2. Accuracy

Accuracy was determined in terms of percentage recovery the accuracy study was performed for 50%, 100% and 150% for Quinapril and Hydrochlorthiazide. Standard and sample solutions are injected in to HPLC system in triplicate and percentage recoveries of Quinapril and Hydrochlorthiazide are calculated. The area of each level was used for calculation of % recovery.

4.3. Precision

Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions.

Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision/ruggedness (it show the degree of reproducibility of test results obtained by analyzing the sample under variety of normal test conditions such as analyst, instruments).

In order to determine precision, six independent sample solution preparations from a single lot of formulation 10µg/ml for Quinapril and 12.5µg/ml for Hydrochlorthiazide was injected into HPLC system, the

retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained which are found to be within the specified limits.

4.4. Intermediate precision

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It is checked that the results are reproducible under differences in conditions, analysts and instruments and hence the proposed method was found to be rugged.

4.5. Limit of detection (LOD):

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD is calculated from the formula

$$LOD = 3.3\sigma / S$$

Where, σ = standard deviation of the response, S = slope of calibration curve.

4.6. Limit of quantitation (LOQ):

The limit of quantitation is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ is calculated from formula;

$$LOQ = 10\sigma / S$$

Where, σ = standard deviation of the response, S = slope of calibration curve

4.7. Robustness:

Robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like flow rate (±5%) and change in wave length (±5nm). The robustness was performed for the flow rate variations from 0.8ml/min to 1.2ml/min and the method is robust only in less flow condition and even by change in the mobile phase ±5%.

4.8. System Suitability

System suitability tests were carried out on freshly prepared standard stock solutions of Quinapril and Hydrochlorthiazide and it was calculated by injecting standards six replicates at 6 min interval and the values were recorded.

4.9. Specificity and forced degradation studies

The specificity and forced degradation studies was carried out to determine whether there are any interference of any impurities (presence of components may be unexpected to present) in retention time of analytical peak, forced degradation studies are carried out by using 0.1M HCl, 0.1M NaOH, heat and UV light.

4.9.1. Acid degradation

To 1ml of stock solution Quinapril and HCTZ, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain 100µg/ml&125µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

4.9.2. Alkali degradation

To 1 ml of stock solution Quinapril and HCTZ, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60°C. The resultant solution was diluted to obtain 100µg/ml&125µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

4.9.3. Oxidative degradation

To 1 ml of stock solution of Quinapril and HCTZ, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 100µg/ml&125µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

4.9.4. Thermal induced degradation

The standard drug solution was placed in oven at 105°C for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml&125µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

4.9.5. Photolytic degradation

The photochemical stability of the drug was also studied by exposing the solution to UV Light by keeping the beaker in UV Chamber for 7hr in photo stability chamber. for HPLC study, the resultant solution was diluted to obtain 100µg/ml&125µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

5. Results and Discussions

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of Quinapril and Hydrochlorthiazide. In order to get the optimized RP-HPLC method various mobile phases and columns were used. From several trials final method is optimized with the following conditions

The mobile phase consisted of phosphate buffer (pH-4) and acetonitrile in the ratio of (26:74v/v) and the column used was Inertsil ODS C18 (150mm×4.6mm, 5µm particle size). The flow rate was adjusted to 1.2ml/min. the instrument was operated at an ambient temperature with the detection wave length 210nm. The retention times of QUI and HCTZ were 3.76 min and 2.31 min respectively. All the chromatograms are shown in **Fig. 3 to 6.**

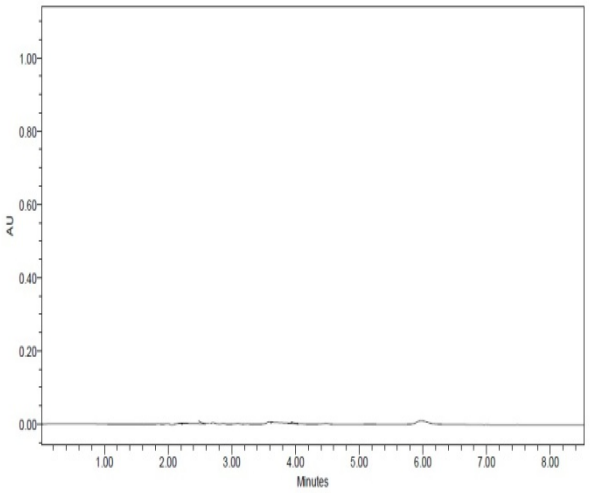

Figure 3: Blank Chromatogram

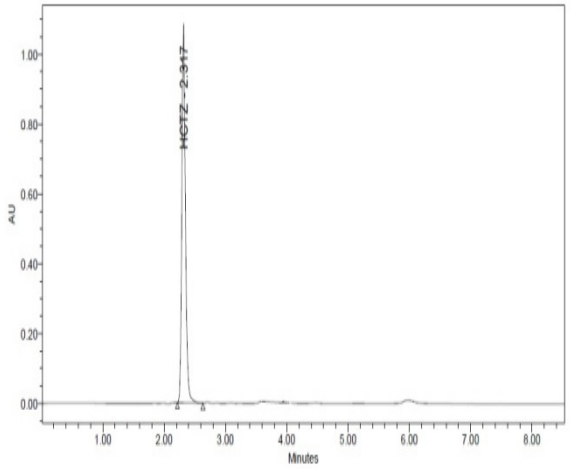

Figure 4: Chromatogram of standard Hydrochlorthiazide solution

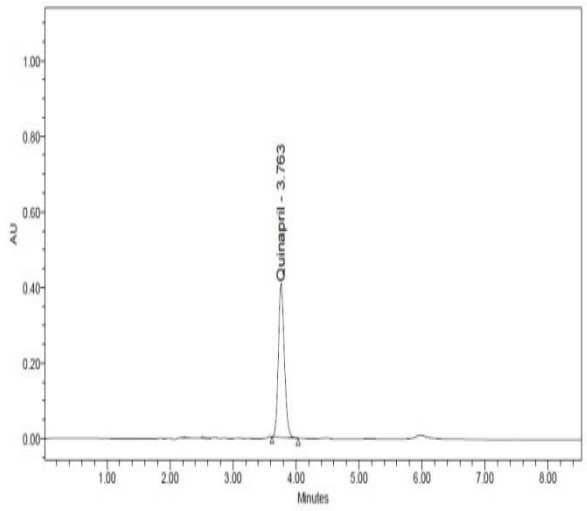

Figure 5: Chromatogram of Standard Quinapril solution

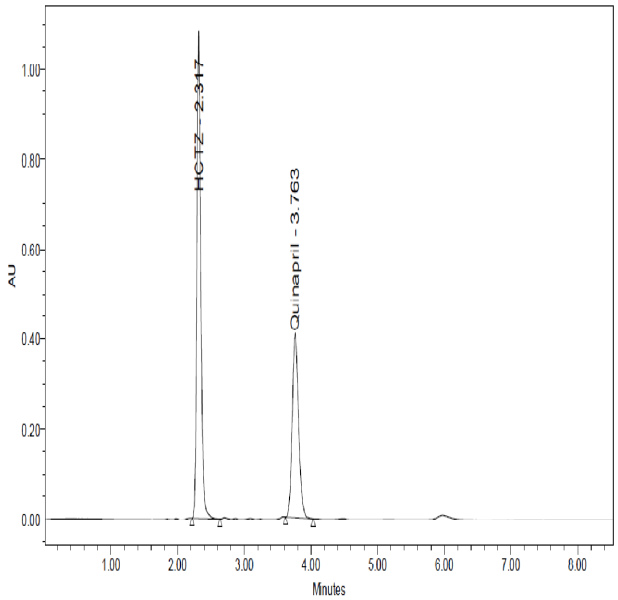

Figure 6: Chromatogram of sample solution of marketed formulation

The linearity was determined as linearity regression of clamied analyte concentration of the range 25-150 µg/ml (Quinapril) and 31.25-187.5 µg/ml (Hydrochlorthiazide). The calibration curve obtained was linear as shown in **Fig .7** and the correlation coefficient was found to be 0.999 for both the compounds.

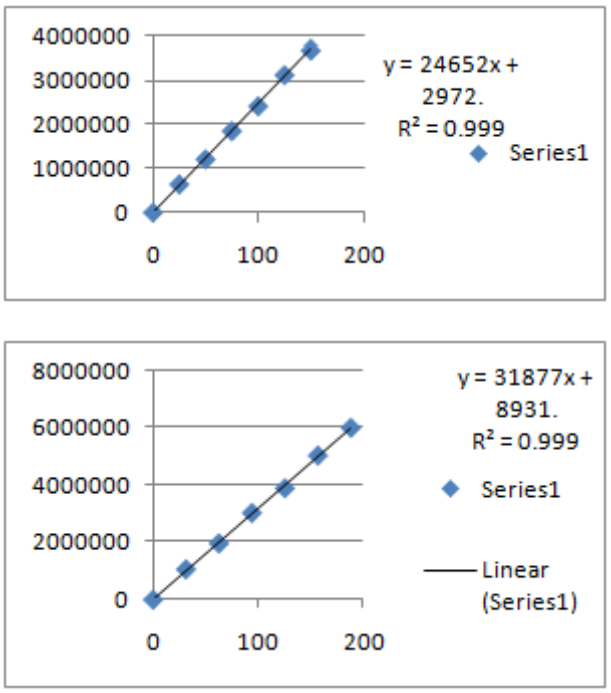

Figure 7: Linearity plot of Quinapril and Hydrochlorthiazide

The precision of the method was ascertained from determination of peak areas of six replicates of sample solution. The % Relative Standard Deviation for method precision presented in **Table No. 1** was found to be 0.4 and 0.6 for Quinapril and Hydrochlorthiazide respectively.

Table 1: Method precision values for Quinapril and Hydrochlorthiazide

Injections	Quinapril		Hydrochlorthiazide	
	Rt	Area	Rt	Area
1.	3.701	2329811	2.312	3858045
2.	3.755	2310078	2.316	3861869
3.	3.758	2327970	2.316	3822300
4.	3.765	2310931	2.322	3861043
5.	3.772	2325524	2.323	3805808
6.	3.775	2307049	2.323	3850295
Avg		2318561		3843227
Std. Dev.		10259		23584.2
%RSD		0.4		0.6

The accuracy study was performed in 50%, 100%, 150%. The percentage recovery was determined for Quinapril and Hydrochlorthiazide and was found to be 100.10 and 100.03% presented in **Table No. 2 & 3.**

Table 2: Recovery studies for Quinapril

Concentration (at Specification level)	Area	Amount added (mg)	% Recovery	Mean Recovery
50%	1157023	5	99.66%	100.10%
100%	2331568.3	10	100.42%	
150%	3157464.3	15	100.23%	

Table 3: Recovery studies for Hydrochlorthiazide

%Concentration (at Specification level)	Area	Amount added (mg)	% Recovery	Mean Recovery
50%	1915385	6.25	100.12%	100.03%
100%	3833762	12.5	100.20%	
150%	5727339.3	18.75	99.78%	

The results of LOD and LOQ are shown in table No. 4

Table 4: LOD & LOQ of Quinapril and Hydrochlorthiazide

Drug	LOD	LOQ
Quinapril	0.3978	1.2055
Hydrochlorthiazide	0.9245	3.552

The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength, column, temperature and flow rate as presented in **Table No. 5**. Theoretical plates and tailing factor were observed and found to be 7765 and 7586 (theoretical plates) and 1.17 (plate count) for both compounds.

Table 5: List of Robustness values for Quinapril and Hydrochlorthiazide

Parameters	Adjusted to	Average area		Rt	
		QUI	HCTZ	QUI	HCTZ
Flow rate	0.8ml/min	3367497	5896322	2.594	3.307
	1ml/min	2654104	4764616	2.594	2.131
	1.2ml/min	2798561	4348961	4.041	2.352
Mobile phase composition	ACN: Buffer(55:45)	2392847	3719853	5.062	2.661
	ACN: Buffer(65:35)	2782206	4317940	4.490	2.409
	ACN: Buffer(70:30)	2798561	4348961	4.041	2.353

The system suitability parameters like theoretical plates (N), tailing factor (T) were calculated and were found to be more than 2000 and not more than 2 and ascertained that proposed RP-HPLC method was accurate and precise as presented in **Table No. 6**.

Table 6: *system suitability parameters for Quinapril and Hydrochlorthiazide*

S. No.	Parameters	Quinapril	Hydrochlorthiazide
1.	Average area	2679176	4365322
2.	Retention time(min)	3.763	2.317
3.	Tailing factor	1.17	1.17
4.	USP Plate count	7586	7765

Forced degradation studies were performed to evaluate the stability indicating properties of the method. Intentional degradation was carried out by exposing of samples to stability conditions such as Hydrolytic degradation under acidic condition (using 2N HCl at 60°C), Hydrolytic degradation under alkaline condition (using 2N NaOH, at 60°C), Oxidative degradation (by using 20% w/v of H2O2) Thermal induced degradation (by placing in oven at 105°C for 6hrs), Photolytic degradation (exposed to UV lamp in photostability chamber providing illumination for 7hr). The results were shown in **Table No. 7&8.**

Table 7: Forced degradation study of Quinapril

S. No	*Degradation Studies*	*Retention Time*	*Area*	*USP Plate Count*	*USP Tailing Factor*	*Purity Angle*	*Purity Threshold*
1.	Hydrolytic degradation under acidic condition	3.492	32790	11399	0.8	1.472	3.472
2.	Hydrolytic degradation under basic condition	3.883	15028	6542	1.4	1.472	3.472
3.	Thermal induced degradation	3.480	281291	9530	0.8	1.215	1.354
4.	Oxidative degradation	3.48	35246	11691	0.8	1.54	4.105
5.	Photolytic degradation	3.714	3758127	7663	1.2	0.243	0.417

Table 8: Forced degradation study of Hydrochlorthiazide

S. No	*Degradation Studies*	*Retention Time*	*Area*	*USP Plate Count*	*USP Tailing Factor*	*Purity Angle*	*Purity Threshold*
1.	Hydrolytic degradation under acidic condition	2.847	5601	9466	1.2	7.472	8.823
2.	Hydrolytic degradation under basic condition	3.480	281291	9530	0.8	1.215	1.354
3.	Thermal induced degradation	2.839	60591	9864	1.1	2.207	2.710
4.	Oxidative degradation	2.838	6013	11088	1.0	1.36	3.105
5.	Photolytic degradation	2.322	2288266	7663	1.2	0.398	0.398

6. Summary and Conclusion

The proposed method was found to be simple, precise, accurate and rapid for determination of Quinapril and Hydrochlorthiazide from API and pharmaceutical dosage form. The method was validated for parameters like specificity, linearity, accuracy, precision, robustness and system suitability values were found to be within limits. The method has significant advantages, in terms of shorter analysis time, selectivity and accuracy than previously reported. The validation study indicates that method can be considered suitable for carrying out quality control and routine determination of Quinapril and Hydrochlorthiazide in bulk and pharmaceutical dosage form.

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