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Method Development and Validation of Hydrochlorothiazide and Quinapril in bulk and tablet dosage form by RP-HPLC

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Abstract: A RP–HPLC chromatographic method was developed and validated for the determination of Quinapril and Hydrochlorothiazide in bulk powder and in pharmaceutical formulations. Quinapril and Hydrochlorothiazide can be separated on Zorbax Eclipse XDB, C18 column (150 x 4.6 mm, 5 µm) at 30 °C using Acetonitrile: Phosphate buffer, pH 4.5 was adjusted with o-phosphoric acid in the ratio of 35:65 v/v as a mobile phase at flow rate of 0.9 mL min^{-1} and detected at 210 nm. The retention time of Quinapril and Hydrochlorthiazide was found to be 2.099 min and 5.537 min respectively. The validation of the proposed method was carried out for specificity, linearity, accuracy, precision, LOD, LOQ and robustness. Calibration was linear over a range of 50-300 µg mL^{-1} and 31.25–187.5 µg mL^{-1} with correlation coefficient of 0.999 for Quinapril and Hydrochlorthiazide, respectively. The robustness of the method was evaluated by deliberately altering the chromatographic conditions. The method developed can be applicable for quality control analysis.

Keywords: Quinapril; Hydrochlorothiazide; RP-HPLC; method development; 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. QUI is an angiotensin-converting enzyme inhibitor (ACE inhibitor) used in the treatment of hypertension and congestive heart failure. Hydrochlorothiazide (HCTZ) **(Figure 1)** is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7- sulphonamide. It 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. The review of literature revealed that various analytical methods involving Spectrophotometry UV-Visible,[1,2] Ion-pair HPLC,[3] UPLC-MS/MS,[4] HPLC,[5,6] HPTLC[7] have been developed for HCTZ in pharmaceutical dosage forms and biological fluids individually or in combination with other drugs.

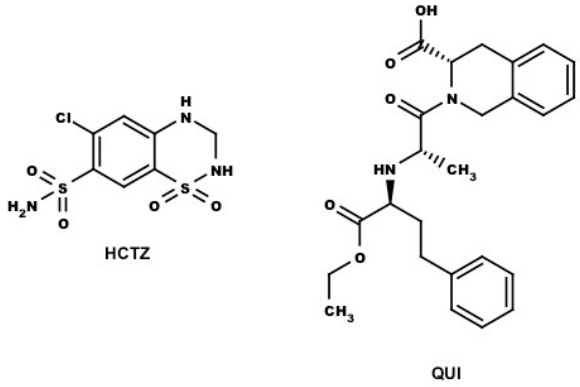

Figure 1. Structure of Hydrochlorthiazide (HCTZ) and Quinapril (QUI)

To the best of our knowledge, there are two methods reported by RP-HPLC for this combination.[8,9] So, the present paper describes a simple, accurate and precise method for simultaneous estimation QUI and HCTZ in combined pharmaceutical formulation by RP-HPLC method. The developed method was validated in accordance with ICH Guidelines.[10,11] The developed method has been successfully employed for the assay of QUI and HCTZ in their combined dosage form.

2. Result and Discussion

2.1. Method Development

2.1.1. Detection of wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. Drug solution containing 20 µg mL^{-1} of QUI and 12.5 µg mL^{-1} of HCTZ were prepared separately in 100 mL volumetric flask and made up the volume with methanol. The above solutions were scanned from 400 nm to 190 nm and their spectra were overlaid. The wavelength selected was 210 nm as both the drugs showed significant absorbance at this wavelength (**Figure 2**).

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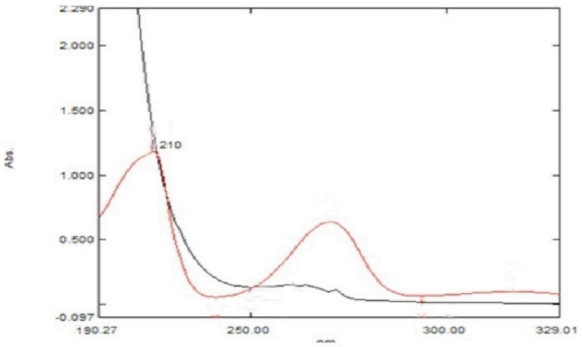

Figure 2. Overlaid spectra of HCTZ (—) and QUI (—)

2.1.2. Preparation of standard stock solution for QUI and HCTZ

Standard stock solution of pure drugs were prepared separately by dissolving 20 mg of QUI with 7 mL diluent into a 10 mL clean dry volumetric flask and make up to 10 mL with mobile phase [(ACN:PB$^{4.5}$)$_{35:65}$]. Pipette out 1 mL and make up to 10 mL to get a final concentration of 200 µg mL^{-1} (stock solution A). Dissolve 12.5 mg of HCTZ with 7 mL diluent into a 10 mL clean dry volumetric flask and made up to 10 mL (1250 µg mL^{-1}) with mobile phase (name). Pipette out 1 mL (1250 µg mL^{-1}) solution and dilute to 10 mL to get a final concentration of 125 µg mL^{-1} (stock solution B). A series of standard solution of HCTZ from stock solution A and QUI from stock solution B were prepared to obtain the concentration of 31.25 - 187.5 µg mL^{-1} and 50-300 µg mL^{-1}, respectively (**Figure 3**).

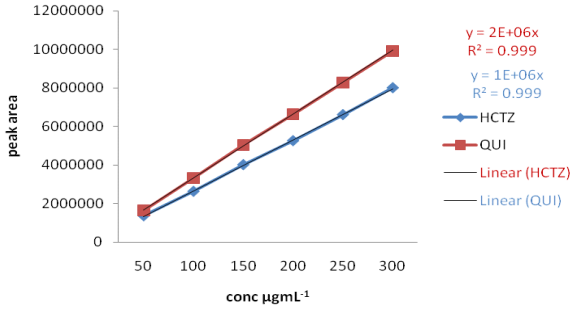

Figure 3. Calibration curve of HCTZ and QUI

2.1.3. Preparation of standard solution containing mixture of QUI and HCTZ

Pipette out 1 mL each from stock solution A of QUI and stock solution B of HCTZ in to a 10 mL volumetric flask and made up to volume with mobile phase [(ACN:PB$^{4.5}$)$_{35:65}$] to get a mixed standard solution (QH$_s$) containing 20 µg mL^{-1} of QUI and 12.5 µg mL^{-1} of HCTZ as final concentration.

2.1.4. Preparation of test solution containing mixture of QUI and HCTZ

Twenty tablets were weighed and crushed. An accurately weighed quantity of powder equivalent to 50 mg of QUI was transferred to 10 mL volumetric flask and dissolved in 7 mL of diluent. It was then sonicated for 25 min, further the volume was made up with mobile phase [(ACN:PB$^{4.5}$)$_{35:65}$] and filtered. From the filtered solution 2 mL was pipetted out into a 10 mL volumetric flask and was made up to 10 mL with mobile phase [(ACN:PB$^{4.5}$)$_{35:65}$] to get test solution (QH$_t$).

2.1.5. Chromatographic method for the determination of QUI and HCTZ

With optimized chromatographic condition a steady base line was recorded with mobile phase [(ACN:PB$^{4.5}$)$_{35:65}$] that was followed by the analysis of the sample solutions (QH$_s$ and QH$_t$). A 10 µL quantity of sample solution was injected and the chromatogram was recorded in triplicate. The retention times of HCTZ and QUI in QH$_s$ and QH$_t$ were found to be 2.097, 5.541 and 2.099, 5.537 min, respectively (**Figure 4**). Content of HCTZ and QUI in tablet was calculated by comparing mean peak area of sample with that of the standard. Concentrations of both drugs were calculated. Representative chromatogram of the test was shown in **Table 1**.

Table 1. Analysis of marketed formulations

Commercial formulation	Ingredients	Label amount (mg)	Amount present (mg)	% purity
Accupril -H	QUI	20	19.8	98%
	HCTZ	12.5	12.25	97%
D 19	QUI	20	19.5	97.5%
	HCTZ	12.5	12.05	96.4%

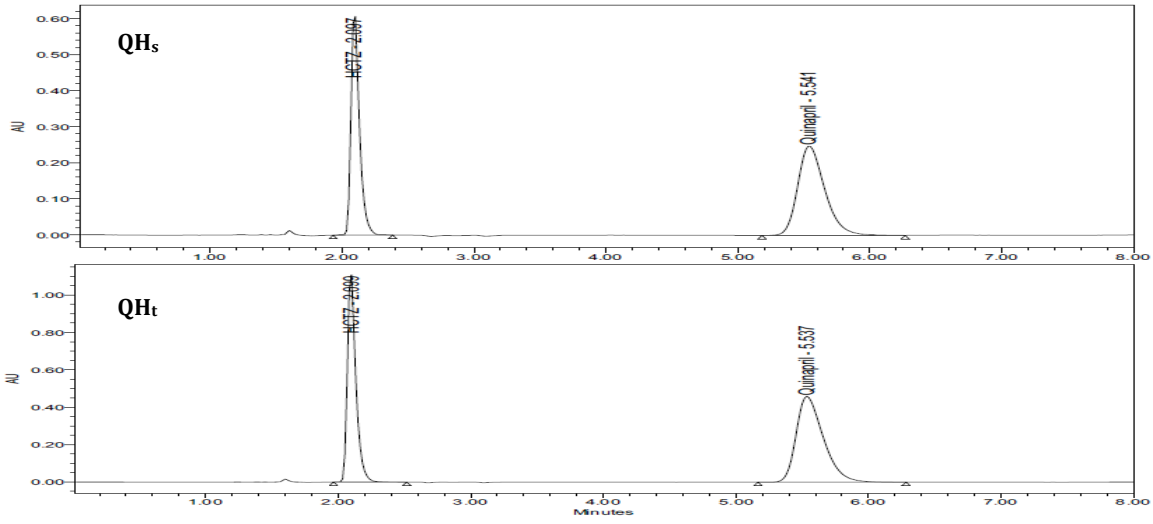

Figure 4. Chromatogram of samples containing mixture of HCTZ and QUI in QH$_s$ and QH$_t$

2.2. Method Validation

2.2.1. Linearity and Range

The linearity of the method was determined at five concentration levels ranging from 31.25-187.50 µg mL^{-1} for HCTZ and 50-300 µg mL^{-1} for QUI. The calibration curve was constructed by plotting response factor against concentration of drugs.

2.2.2. Accuracy

To ascertain the accuracy of the proposed method recovery studies were carried out by standard addition method, adding known amount of each drug to the pre analyzed tablet at three levels 50%, 100% and 150% of the label claim. Recovery studies were carried out in triplicate and the percentage recovery and standard deviations, which are within acceptance limits as shown in **Table 2**.

Table 2. Accuracy studies

Amount Spiked	Amount added[a] (Amount recovered)[a]		% Estimated[b] (%RSD)	
	HCTZ	QUI	HCTZ	QUI
50%	6.25 (6.22)	10 (10.35)	99.72±0.73 (0.73)	100.92±0.6 (0.65)
100%	12.5 (12.6)	20 (20.17)	100.82±0.42 (0.42)	100.82±0.47 (0.47)
150%	18.75 (18.89)	30 (30.33)	100.81±0.61 (0.61)	101.11±0.12 (0.12)

[a]Values are in mg; [b]Mean±SD of triplicate

2.2.3. Precision

Precision was the measure of the degree of repeatability of an analytical method under normal operation and it was normally expressed as the relative standard deviation for a statistically number of samples. Precision should be performed at three different levels: repeatability, intermediate precision and reproducibility, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and %RSD. This shows that the percentage RSD is not more than 2% the chromatogram as shown in **Table 3 & 4**.

Table 3. Method of precision

S.NO	HCTZ		QUI	
	Rt (min)	Peak area (n=3)	Rt (min)	Peak area (n=3)
1	2.099	4939962	5.463	6461393
2	2.101	4939495	5.487	6565316
3	2.105	4979099	5.490	6442481
Mean		4952852		6489730
SD		22731.77		66138.86
%RSD		0.05		0.21

Table 4. Intermediate precision

Parameter	Inj	HCTZ		QUI	
		Rt (min)	AUC (n=3)	Rt (min)	AUC (n=3)
Intraday Precision Analyst-1	1	2.079	4947356	5.481	6349954
	2	2.083	4936963	5.484	6364801
	3	2.084	4962116	5.501	6353063
	Mean		4948812		6355939
	%R.S.D		0.26		0.12
Interday Precision Analyst-2	1	2.084	4942883	5.504	6363923
	2	2.090	4933657	5.521	6344797
	3	2.093	4953878	5.577	6356320
	Mean		4943473		6355013
	%R.S.D		0.20		0.15

2.2.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. LOD and LOQ for both the drugs were calculated by using the values of slopes and intercepts of the calibration curves as shown in **Table 5**.

Table 5. Limit of detection and quantification

Contents	HCTZ	QUI
LOD (S/N)	3	3.1
LOQ (S/N)	9.8	9.1

2.2.5. Robustness

Robustness of the proposed method was ascertained by deliberately changing the chromatographic conditions such as change in flow rate of the mobile phase (±0.1 mL min^{-1}), change in composition of the mobile phase. Effect of change in chromatographic parameters on resolution and tailing factor of peak was studied. The condition with variation and their result were shown in **Table 6**.

Table 6. Robustness studies

	Flow rate (mL^{-1}) Peak Area			
	HCTZ		QUI	
	1.2 (mL)	1.4 (mL)	1.2 (mL)	1.4 (mL)
	4951359	5004839	5681577	6457829
	4985132	4995256	5736681	6451940
	4968245	5000048	5709129	6454885
Mean	4968245	5000048	5709129	6454885
SD	16886.5	4791.5	27552	2944.5
%RSD	0.34	0.10	0.48	0.05
	Mobile phase Peak Area			
	HCTZ		QUI	
	30 : 70	30 : 70	35 : 65	35 : 65
	5004839	4997275	6457829	6480049
	4995256	4998272	6451940	6480896
	5000048	4997773	6454885	6480472
Mean	5000048	4997773	6454885	6480472
SD	4791.5	498.5	2944.5	423.5
%RSD	0.10	0.01	0.05	0.01

2.2.6. System suitability studies

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ±2% standard deviation range during routine performance of the method the chromatogram as shown in **Table 7** & **Figure 5**.

Table 7. System suitability parameters

S. No.	Parameters	HCTZ	QUI
1	Theoretical plates	5136	3516
2	Symmetric factor	1.5	1.7
3	Resolution	6.9	6.3
4	Tailing factor	1.02	1.29

3. Experimental

Materials and methods: Standard samples of QUI and HCTZ were provided as a gift samples from Pfizer Limited, Mumbai. The marketed formulation ACCUPRIL-H & D-19 tablets containing 20 mg and 12.5 mg of QUI and HCTZ, respectively were procured from local market. HPLC grade water and acetonitrile were

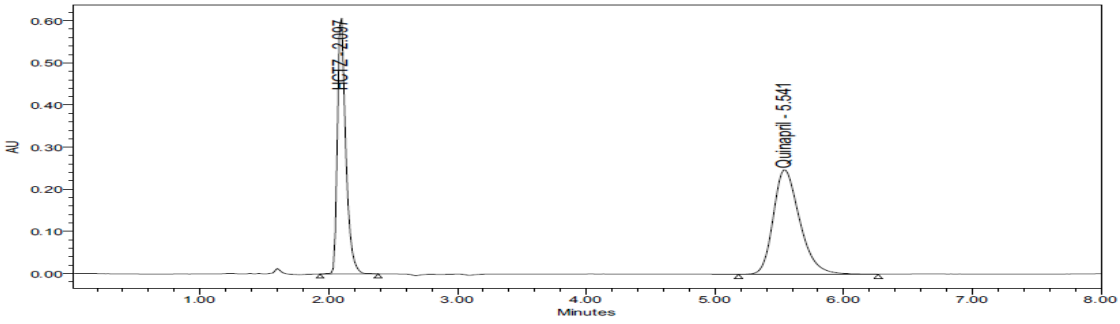

Figure 5. System suitability study for HCTZ and QUI

purchased from Merck, India. Weighing of samples was performed on Secura 224-1S Analytical balance (Sartorius, USA). Degassing of mobile phase using Branson 1510MTH Ultrasonic Cleaner (Balkowitsch Enterprises Inc., USA). Mobile phase pH was checked and adjusted using pH-meter. All chromatographic analyses were performed on HP Agilent 1100 (Agilent Technologies, USA) machine with Zorbax Eclipse XBD column (Agilent Tehnologies, USA). Empower 3 CDS (Waters, USA) has been used to plot chromatographic data. Excel 2007 (Mirosoft Office 2007, Microsoft) was used for plotting calibration curves.

3.1. Method development

3.1.1. Chromatographic conditions

Flow rate: 0.9 mL min^{-1}
Column : Zorbax Eclipse XDB, C18, 150 x 4.6 mm, 5µm.
Detector wave length: 210 nm
Column temperature: 30 °C
Injection volume: 10 µL
Run time: 10 min
Mobile phase (diluent): $[(ACN:PB^{4.5})_{35:65}]$

3.1.2. Preparation of buffer ($PB^{4.5}$)

Weigh accurately about 2.72 gm of Potassium dihydrogen orthophosphate (KH_2PO_4) and dissolved in 900 mL of milli-Q water in a 1000 mL beaker, sonicated and pH 4.5 was adjusted with orthophosporic acid and finally make up to the volume.

3.1.3. Preparation of mobile phase [$(ACN:PB^{4.5})_{35:65}$]

A 65 mL of potassium dihydrogen orthophosphate buffer of pH 4.5 and 35 mL of acetonitrile was taken into 100 mL volumetric flask, sonicated for 25 min and filtered.

3.2. Method validation

3.2.1. Specificity

It is evaluated by injecting the blank, placebo and the control sample solution prepared as per the proposed method to check for the interference if any peak at the retention time of QUI and HCTZ.

3.2.2. Linearity and Range

QUI and HCTZ standard stock solution was transferred to volumetric flask of 10 mL capacity. The volume was adjusted to the mark with methanol to give solutions containing 31.25 to 187.5 µg mL^{-1} HCTZ and 50 to 300 µg mL^{-1} QUI. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

3.2.3. Method precision

The repeatability was evaluated by assaying the sample solution for 6 times. Different concentration of QUI (31.25, 62.5, 93.75 µg mL^{-1}) and HCTZ (50, 100, 150 µg mL^{-1}) were used for the estimation of intraday and interday precision.

3.2.4. Accuracy

Accuracy was assessed by determination of the recovery of the method by addition of standard drug to the pre-quantified placebo preparation at 3 different concentration levels 50%, 100% and 150%, taking into consideration percentage purity of added bulk drug samples. Each concentration was analyzed 3 times and average recoveries were measured.

3.2.5. Intermediate precision (Ruggedness)

Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e, different analysts, laboratories, instruments, reagents, assay temperatures, small variations in mobile phase, different days etc. (*i.e.* from laboratory to laboratory, from analyst to analyst). Acceptance criteria for ruggedness, the %RSD for the area of five standard injections should not be more than 2%.

3.2.6. Robustness

As part of robustness, deliberate change in the flow rate and mobile phase composition was made to evaluate the impact on the method. The mixed standard solution is injected in two replicates and %RSD was calculated.

3.2.7. LOD and LOQ

LOD is the smallest concentration of the analyte that gives measurable response (signal to noise ratio of 3). The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10).

Abbreviations

ACN-Acetonitrile; **HCTZ**-Hydrochlorthiazide; **PB**-Phosphate buffer; **QUI**-Quinapril

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