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METHOD DEVELOPMENT, VALIDATION AND SIMULTANEOUS ESTIMATION OF QUINAPRIL AND HYDROCHLORTHAIZIDE IN TABLET DOSAGE FORM BY USING RP-HPLC

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ABSTRACT
A simple, accurate, precise method was developed for the simultaneous estimation of the Quinapril and HCTZ in Tablet dosage form. Chromatogram was run through Inertsil ODS column (250mm: 4.6mm, 5µ). Mobile phase containing Buffer & Acetonitrie in the ratio of (45:55) was pumped through column at a flow rate of 0.8ml/min. Buffer used in this method was ortho phosphoric acid Temperature was maintained at 30°C. Optimized wavelength for Quinapril and HCTZ was 210nm. Retention times of Quinapril and HCTZ were found to be 2.17min and 3.4min. % RSD of the Quinapril and HCTZ were and found to be 0.65 and 0.42 respectively. % Recover was Obtained as 99.91 and 100.02 for HCTZ and Quinapril respectively. LOD, LOQ values are obtained from regression equations of Quinapril and HCTZ were 0.67, 2.03 and 0.5, 1.52 respectively. Regression equation of HCTZ is y = 20044x + 4078, and of Quinapril is y = 317877x + 2711 Regression co-efficient was 0.999.

KEYWORDS: HCTZ, Quinapril, RP-HPLC.

INTRODUCTION

Quinapril is the ethyl ester prodrug of the non-sulfhydryl angiotensin converting enzyme inhibitor quinaprilat. It is used to treat hypertension and heart failure. ACE inhibitors are commonly used as a first line therapy in the treatment of hypertension, along with thiazide diuretics or beta blockers. Angiotensin II constricts coronary blood vessels and is positively inotropic, which under normal circumstances, would increase vascular resistance and oxygen consumption. This action can eventually lead to myocyte hypertrophy and vascular smooth muscle cell proliferation. Angiotensin II also stimulates production of plasminogen activator inhibitor-1 (PAI-1), increasing the risk of thrombosis.[1-4] IUPAC name (3*S*)-2-[(2*S*)-2-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino] propanoyl]-3,4-dihydro-1*H*-isoquinoline-3-carboxylic acid. Quinapril is soluble in water (95 mg/ml at 25° C), ethanol (95 mg/ml at 25° C), and DMSO (18 mg/ml at 25° C). Melting Point: 120-130° C.

Hydrochlorothiazide is the most commonly prescribed thiazide diuretic. It is indicated to treat edema and hypertension. Hydrochlorothiazide use is common but declining in favour of angiotensin converting enzyme inhibitors. Many combination products are available containing hydrochlorothiazide and angiotensin converting enzyme inhibitors or angiotensin II receptor blockers. IUPAC name 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide.

Hydrochlorothiazide, USP is a white, or practically white, crystalline powder which is slightly soluble in water, freely soluble in sodium hydroxide solution, in n-butylamine, and in dimethylformamide; sparingly soluble in methanol; insoluble in ether, in chloroform, and in dilute mineral acids.

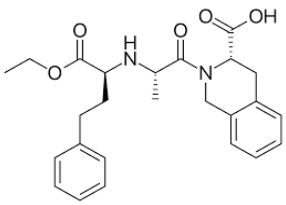

Figure 1: Structure of Quinapril.

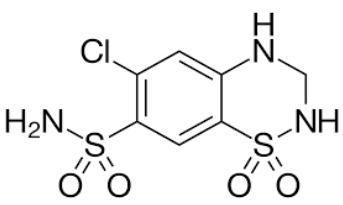

Figure 2: Structure of Hydrochlorothiazide.

The literature survey revealed that There are Various analytical methods were carried out for the estimation of Quinapril and Hydrochlorothiazide as a single or combined with other drugs in pharmaceutical dosages Literature survey reveals that the retention time for the simultaneous estimation of Quinapril and Hydrochlorothiazide is more. Hence the present study, we had made an attempt to develop simple, accurate, precise, less time consuming and with less retention time using RP-HPLC for the simultaneous estimation of Quinapril and Hydrochlorothiazide in bulk and pharmaceutical dosage form by RP-HPLC.[3-15] To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS

Chemicals and Reagents: HCTZ and Quinapril were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 210 nm with Inertsil ODS column (250mm: 4.6mm, 5µ) dimensions at 30 ^{0}C temperature. The optimized mobile phase consists of Buffer & Acetonitrie in the ratio of (45:55). Flow rate was maintained at 0.8 ml/min.

PREPARATION OF SOLUTIONS

Preparation of phosphate buffer solution

1ml of Ortho Phosphoric acid was diluted to 1000ml with HPLC grade water.

Preparation of mobile phase

Methanol, Buffer and Acetonitrile were mixed in the ratio of 65:30:5 and sonicated for 20minutes, Filtered with 0.45 µ membrane filter.

Preparations of working standard solution

Accurately Weighed and transferred 10mg of Quinapril and 12.5mg of Hydrochlorothiazide 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.

Preparation of Sample solution

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred 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 pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Procedure

20µL of the standard, sample are injected into the chromatographic system and the areas for HCTZ and Quinapril peaks are measured and the %Assay are calculated by using the formulae.

METHOD

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 0.8 ml/min to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Inertsil ODS column (250mm: 4.6mm, 5µ), the mobile phase of composition Buffer: ACN (45:55), pH- 3.5 was allowed to flow through the column at a flow rate of 1 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine HCTZ and Quinapril in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method

Linearity: Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the Stock solutions of HCTZ and Quinapril are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 31.25ppm, 62.5ppm, 93.75ppm, 125ppm, 156.25ppm, 187.5ppm of HCTZ and 25ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm of Quinapril. The results are shown in figure 6 and 7.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150% and 50%, 100%, 150% Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for HCTZ and Quinapril and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day,

different analyst, different instrument. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.3 ml/min to 0.4 ml/min. The results are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 8.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION

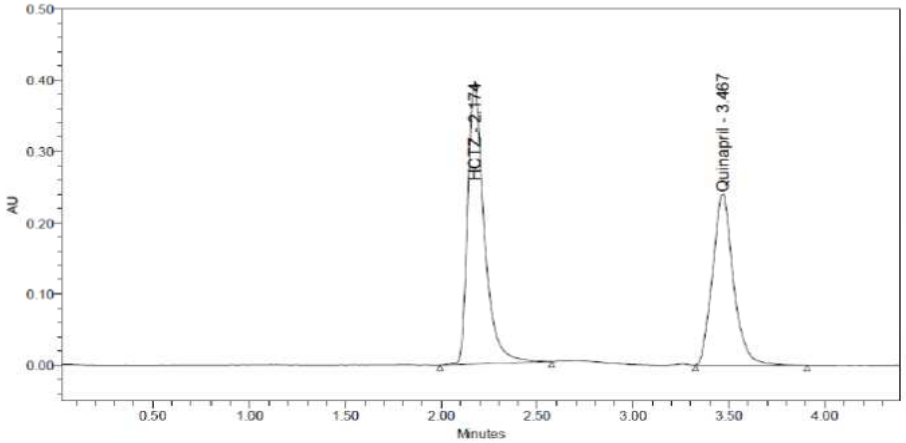

Figure 3: Standard chromatogram.

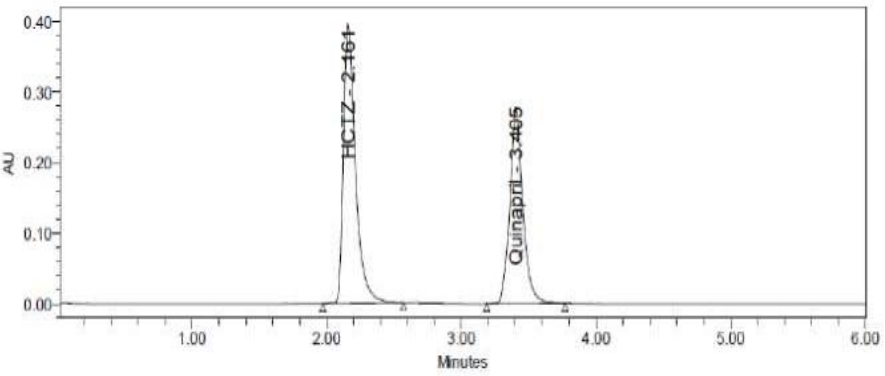

Figure 4: Sample chromatogram.

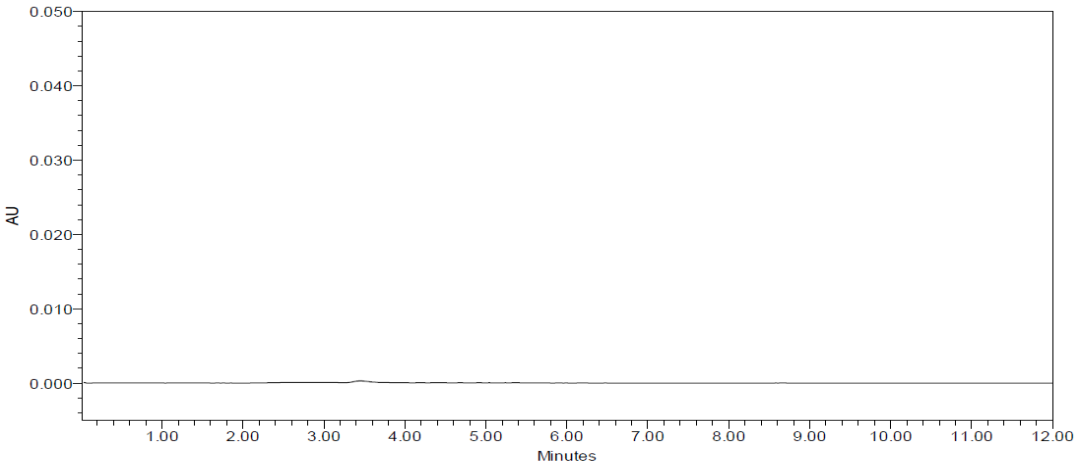

Figure 5: Blank chromatogram.

Table 1: System suitability parameters.

Property	HCTZ	Qnlnnpi-U
Retention time (tR)	2.174 = 03 min	3.4(57±0.3min
Theoretical plates (N)	3162 = 163.48	5671± 1(53.48
Tailine factor (T)	1.58 = 0.117	1.34=0.117

Table 2: Assay results for HCTZ and Quinapril.

S. No.	HCTZ %Assay	Quinapril %Assay
1	100.38	99.36
2	99.13	99.98
3	100.72	100.17
4	99.44	100.09
5	99.88	100.50
6	99.91	100.02
AVG	99.91	100.02
STDEV	0.65	0.42
%RSD	0.65	0.42

Table 3: Calibration data of HCTZ and Quinapril.

S. No.	Concentration	Response	Concentration	Response
1	0	0	0	0
2	31.25	652346	25	461223
3	62.5	1266327	50	908455
4	93.75	1870418	75	1331810
5	125	2477581	100	1783305
6	156.25	3115270	125	2197232
7	187.5	3800454	150	2722315

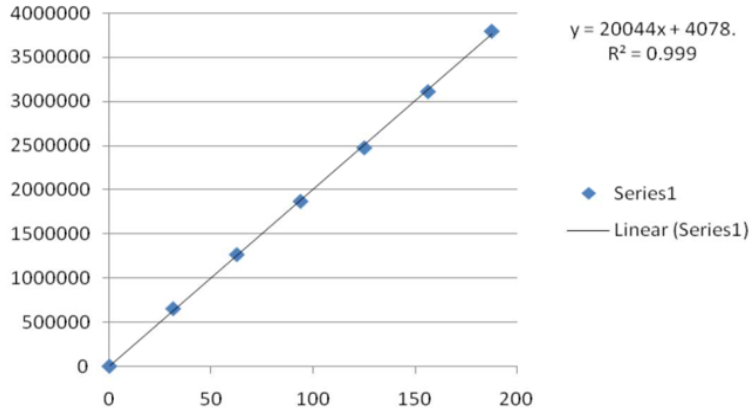

Figure 6: Calibration curve of HCTZ.

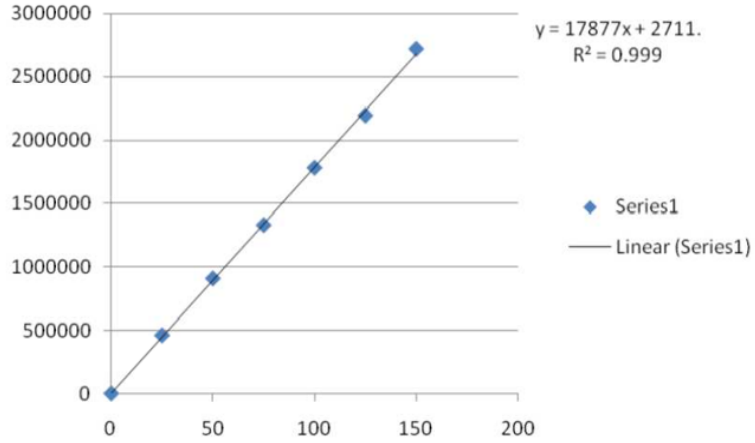

Figure 7: Linearity graph for Quinapril.

Table 4: Showing accuracy results for HCTZ and Quinapril.

Sample	Amount Taken	Amount Recovered	Recovery (%)	% RSD
HCTZ	62.5	62.41	99.87	0.81
	125	124.68	99.75	0.41
	1 87.5	187.81	100.17	065
Quinapril	50	50.13	100.27	082
	100	99 68	99.68	0 75
	150	150.31	100.21	065

Table 5: Precision results for HCTZ and Quinapril.

Sr. No.	HCTZ	Quiuapril
1	2427807	1826545
2	2397565	1837939
3	2436009	1841406
4	2405206	1839909
5	2415832	1847465
Mean	2416484	1838653
SKI Dev.	15772	7647
%RSD	0.65	0.42

Table 6: Intermediate precision results for HCTZ and Quinapril.

Sr. No.	HCTZ	Quinapril
1	2409895	1845685
2	2437895	1833515
3	2409312	1838087
4	2396554	1847364
5	2419122	1852085
Mean	2414556	1843347
Std. Dev.	15320	7454.71
%RSD	0.63	0.40

Table 7: Robustness results of HCTZ and Quinapril.

S.NO	Robustness condition	HCTZ %RSD	Quinapril %RSD
1	Flow minus	0.3	0.3
2	Flow Plus	0.4	0.4
3	Mobile phase minus	0.8	0.9
4	Mobile phase Plus	0.7	0.9
5	Temperature minus	0.8	0.9
6	Temperature Plus	0.4	0.3

Table 8: LOD, LOQ of HCTZ and Quinapril.

	HCTZ	Quinapril
LOD (µg/mL)	0.35956	0.084037
LOQ (µg/mL)	1.08958	0.254658

CONCLUSION

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of HCTZ and Quinapril in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of HCTZ and Quinapril in pure and its pharmaceutical dosage forms.

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