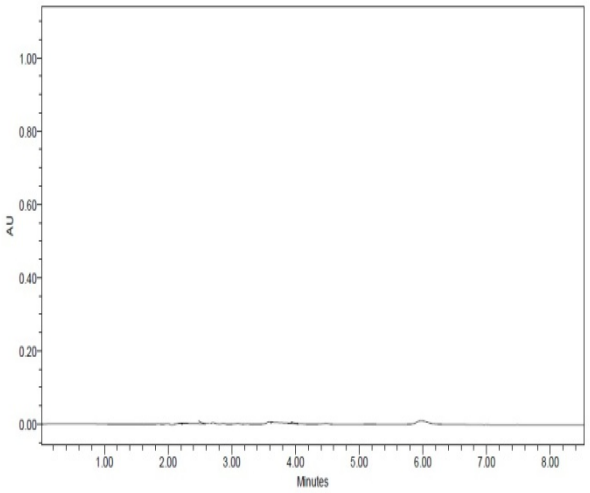

Figure 3: Blank Chromatogram

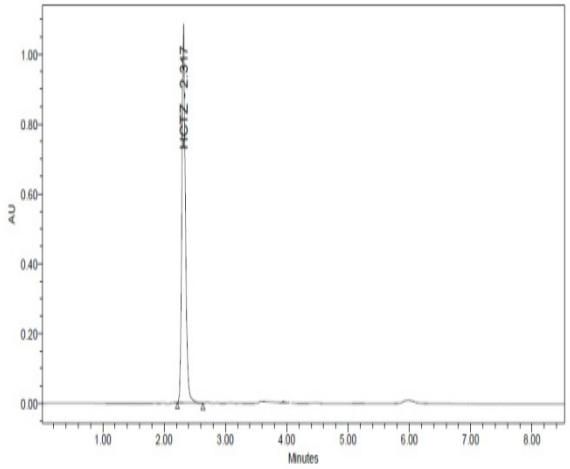

Figure 4: Chromatogram of standard Hydrochlorthiazide solution

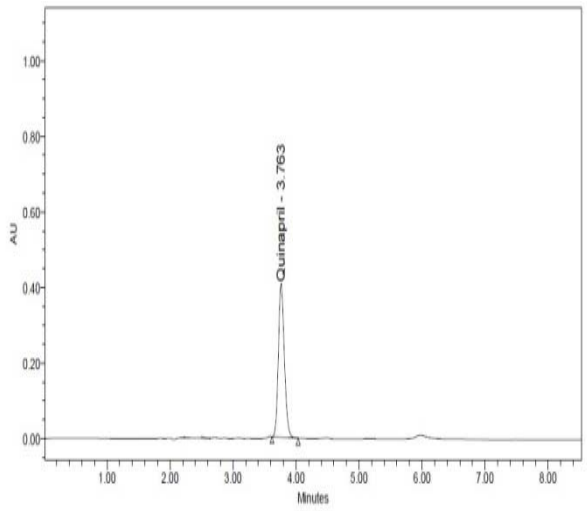

Figure 5: Chromatogram of Standard Quinapril solution

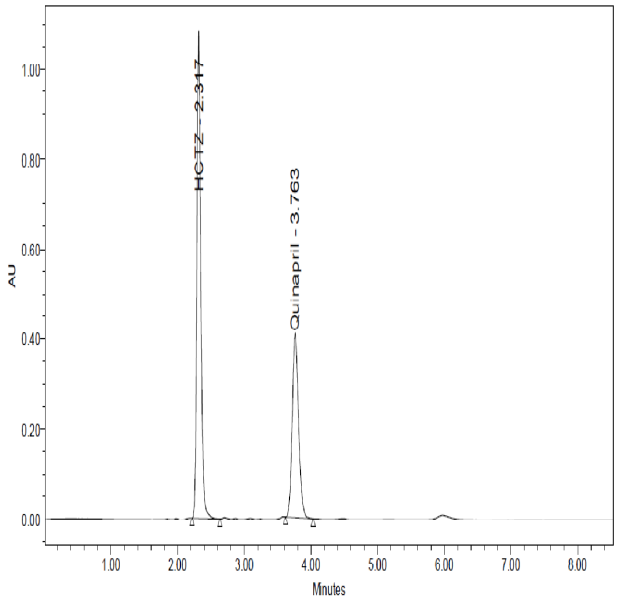

Figure 6: Chromatogram of sample solution of marketed formulation

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.

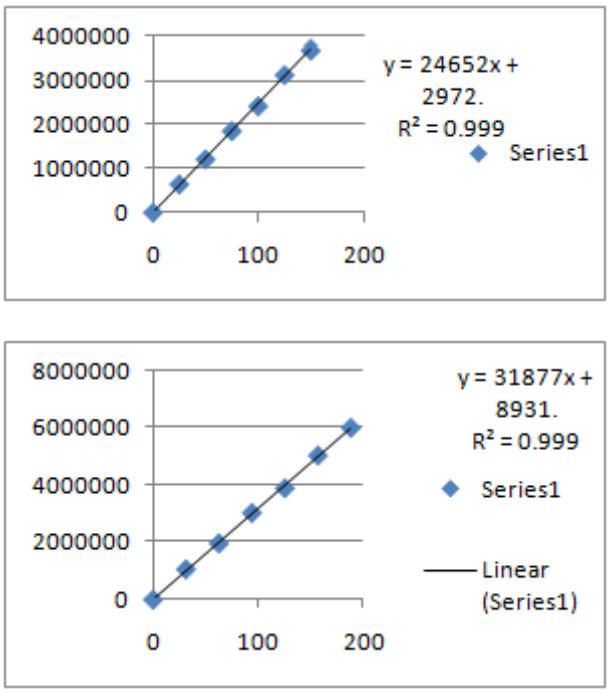

Figure 7: Linearity plot of Quinapril and Hydrochlorthiazide

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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