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Stability indicating RP-HPLC method development and validation for estimation of antihypertension class of drugs lisinopril and hydrochlorothiazide

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Abstract

The anticipated study defines the simultaneous assessment of lisinopril and hydrochlorothiazide in the drug substance and drug product by reverse-phase high-performance liquid chromatography (RP-HPLC). This method was developed by using inertsil ODS C18 (250 x 4.6 mm, 5 µm) column and a mobile phase consisting of 0.01M ammonium phosphate buffer and acetonitrile in the ratio of 70:30%v/v. 0.01M ammonium phosphate buffer pH was adjusted to 5.0 with the help of orthophosphoric acid. The UV detection wavelength was set at 211 nm and the retention time of lisinopril and hydrochlorothiazide were found to be 3.01 and 5.8 min, respectively. Linearity of lisinopril and hydrochlorothiazide were observed in the range of 2.5-15 µg/ml and 6.25-37.5 µg/ml, respectively. The reported R^2 values were 0.997 and 0.998 for lisinopril and hydrochlorothiazide, respectively. The mean % recovery was 100.56% and 99.61% for lisinopril and hydrochlorothiazide, respectively. As per ICH guidelines, Q1A (R2) stability analysis was performed by subjecting drugs to various stress conditions such as acidic, alkaline, oxidation, thermal and photolytic degradation and results were stating that degradation in oxidative conditions was more as compared with others.

1. Introduction

Lisinopril chemical name is known as N^2-[1-carboxy-3-phenylpropyl]-L-lysyl-L-proline (Figure 1 depicting the chemical structure of lisinopril). Lisinopril act as ACE inhibitor so incredibly helpful in the management of hypertension.

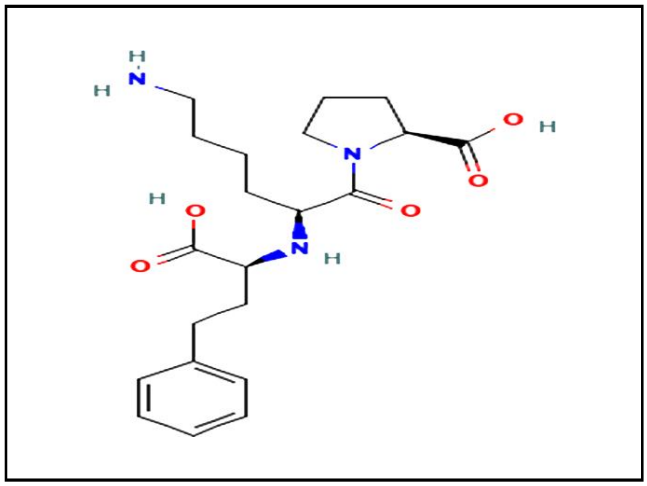

Figure 1: Lisinopril chemical structure.

Hydrochlorothiazide chemical name is 6-chloro-1, 1/dioxo-3,4/dihydro 2H-1,2,4/benzothiazidine 7-sulfonamide (Figure 2 depicting the structure of hydrochlorothiazide). As a thiazide diuretic, hydrochlorothiazide works to reduce the quantity of water in the body by decreasing urine flow, which lowers blood pressure.

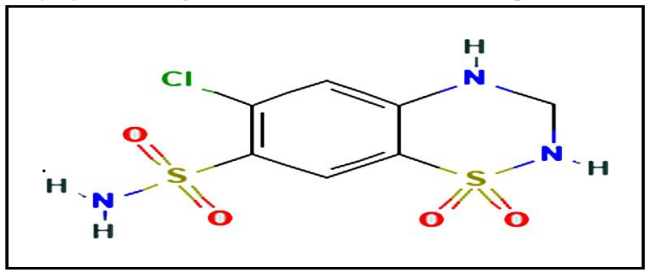

Figure 2: Chemical structure of hydrochlorothiazide.

Lisinopril and hydrochlorothiazide were announced on the market in a combined dosage form which is widely used in the treatment of hypertension (Aziz *et al*., 2016). The literature review reveals that five HPLC (Akiful Haque, 2018; Deepali *et al*., 2012; Girish *et al*., 2021; Mohan Sharma, 2012; Vania *et al*., 2013; Vikas Chander and Mohan Sharma, 2012) methods were reported on lisinopril and hydrochlorothiazide in the drug substance and drug product. The further literature survey is stating that one HPTLC method (Sagar *et al*., 2021) of lisinopril with another combination, one HPLC method (Kushal Ramdas *et al*., 2020) on hydrochlorothiazide with another combination, and two review articles (Khairi *et al*., 2020; Wajiha *et al*., 2016) on lisinopril alone and one RP-HPLC stability indicating method (Urupina and Bazi, 2016) on hydrochlorothiazide alone were found. The main benefits of RP-HPLC methods are cost-effective, flexibility and a small amount of the sample can also be analyzed. The main objective of the analytical method validation is to show that the suggested method, as determined by carefully thought-out experimental studies, is suitable for the intended usage.

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2. Materials and Methods

2.1 Materials

A pure standard sample of lisinopril and hydrochlorothiazide were procured from Richer Pharmaceuticals, Hyderabad. Formulation of listril plus (5 mg of lisinopril and 12.5 mg of hydrochlorothiazide) was procured from the local market. All the chemicals such as HPLC grade acetonitrile, hydrochloric acid, hydrogen peroxide, phosphoric acid procured from Merck and ammonium phosphate buffer, sodium hydroxide were procured from Ranchem.

2.2 Method

2.2.1 Instruments

The HPLC system used was waters with model (e2685) prominence equipped with a UV detector. The chromatogram was recorded and peaks were quantified through PC-based Empower-2 software.

2.2.2 Mobile phase preparation

In order to prepare the mobile phase, acetonitrile and 0.01M ammonium phosphate buffer were mixed in a 30:70% v/v ratio. Orthophosphoric acid was used to make a 0.01M ammonium phosphate buffer with a pH of 5.0.

2.2.3 Preparation of stock solution for lisinopril standard (500 µg/ml)

50 mg of lisinopril was weighed and placed into a 100 ml volumetric flask. Diluent was added and the mixture was sonicated for 30 min before being made up to the mark with mobile phase.

2.2.4 Preparation of stock solution for hydrochlorothiazide standard (1250 µg/ml)

Weighed out 125 mg of pure hydrochlorothiazide standard into a volumetric flask with a 100 ml capacity added 50 ml of diluent to dissolve them, and then diluted it with mobile phase to the appropriate concentration for analysis.

2.2.5 Mixed working standard solution preparation

10 ml of each standard stock solution were added to a 100 ml volumetric flask and the volume was then made up with mobile phase (50 µg/ml of lisinopril, 125 µg/ml of hydrochlorothiazide).

2.2.6 Sample stock preparation

Weighing the contents of 20 tablets, the average weight was calculated. They were finely ground into a powder using a glass mortar. Then transferred powder sample quantitatively equivalent to 5 mg of lisinopril and 12.5 mg of hydrochlorothiazide then put a powder sample into a 50 ml volumetric flask, equivalent to 5 mg of lisinopril and 12.5 mg of hydrochlorothiazide and then added 25 ml of diluent, sonicated and made up to the mark with the mobile phase. To get concentrations of approximately 10 µg/ml of lisinopril and 25 µg/ml of hydrochlorothiazide, the solution was further filtered through 0.45 µ membrane filter paper and with 10 ml of the filtrate put to a 100 ml volumetric flask and brought up to the required level with diluent.

3. Results

3.1 Method development and optimized chromatographic conditions

After a number of trials, which included altering the mobile phase ratios with various solvents and varying columns with variable mobile phase flow rates, the method was optimized. Table 1 displays the optimal chromatographic conditions and Figure 3 illustrates an optimized chromatogram of lisinopril and hydrochlorothiazide.

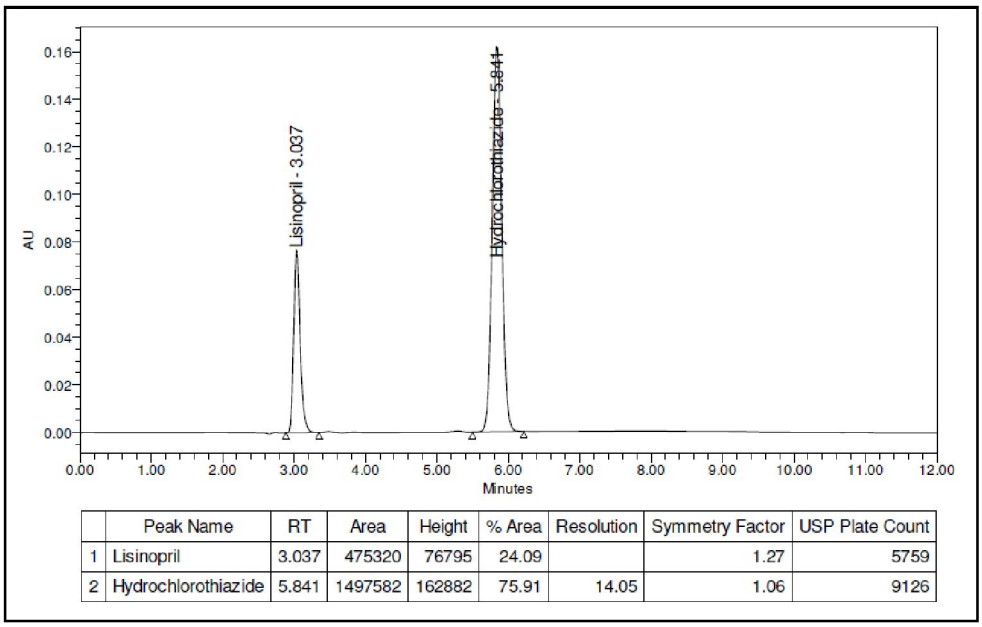
3.2. Method validation

3.2.1. Linearity

By plotting a graph between concentration and peak area, the linearity was determined. The test was carried out by using a working standard preparation, from which 0.5-3.0 ml were transferred into a series of 10 ml volumetric flasks and diluted up to the mark with mobile phase to achieve final concentrations of 2.5-15 µg/ml of lisinopril and 6.25-37.50 µg/ml of hydrochlorothiazide (Linearity results and linearity curves of lisinopril are shown in Table 2 and Figure 4 and for hydrochlorothiazide, they are shown in Table 3 and Figure 5, respectively).

Table 1: Optimized chromatographic conditions

Stationary phase	Inertsil ODS C_{18} (250 × 4.6 mm, 5 µm) column
Mobile phase	Ammonium phosphate buffer: Acetonitrile (70:30% v/v)
Buffer pH	Adjusted to 5.0 with diluted phosphoric acid
Flow rate/min	1ml
Column temperature	30^0C
Wavelength	211 nm
Diluent	Mobile phase
Elution	Isocratic
Injection volume	10 µl



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	Lisinopril	3.037	475320	76795	24.09		1.27	5759
2	Hydrochlorothiazide	5.841	1497582	162882	75.91	14.05	1.06	9126

Figure 3: Optimized chromatogram.

Table 2: Linearity results of lisinopril

S. No	Concentration (µg/ml)	Peak area
1	2.5	117712
2	5	237303
3	7.5	356218
4	10	474682
5	12.5	594692
6	15	712834

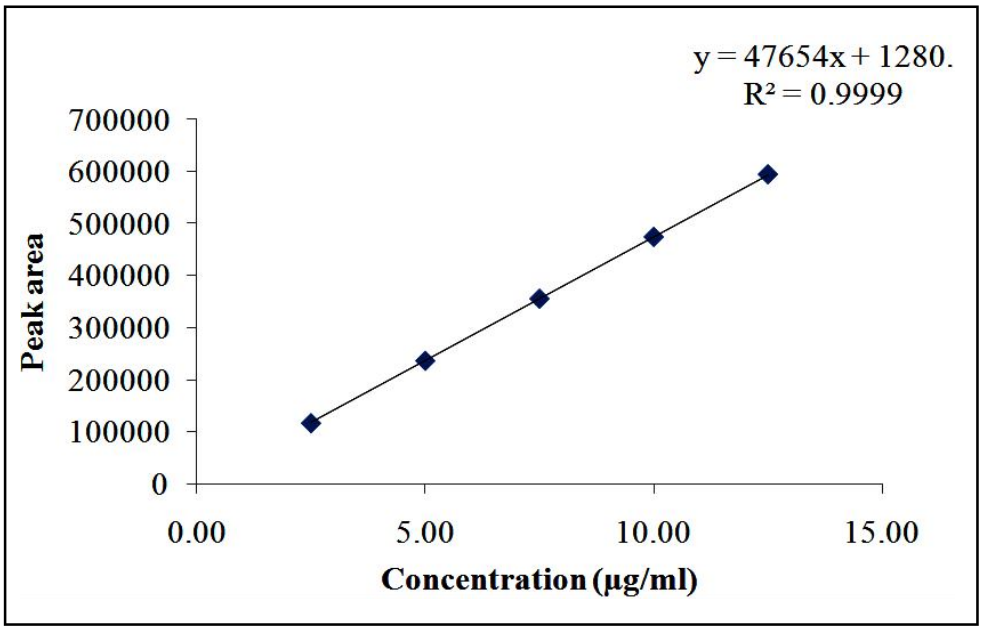


Figure 4: Linearity curve of lisinopril.

Table 3: Linearity results of hydrochlorothiazide

S. No.	Concentration (µg/ml)	Peak area
1	6.25	371814
2	12.50	749505
3	18..75	1125135
4	25.0	1501617
5	31.25	1877683
6	37.50	2252975

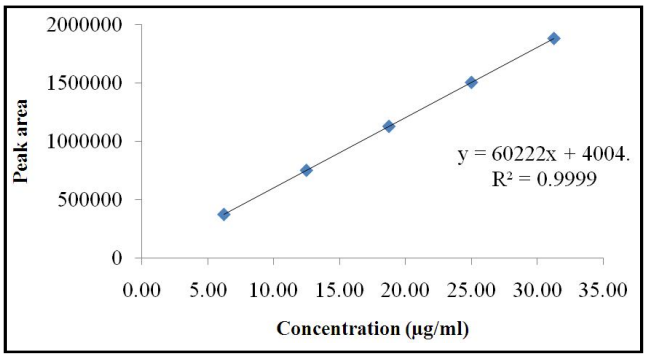


Figure 5: Linearity curve of hydrochlorothiazide.

3.2.2 Precision

It was evaluated at 2 levels such as system and method precision.

3.2.2.1 System precision

System precision is merely an assessment of the HPLC setup. The per cent RSD was calculated after injecting six duplicates of a mixed standard solution containing 10 µg/ml of lisinopril and 25 µg/ml of hydrochlorothiazide and the outcomes were determined to be acceptable. Results for system precision are displayed in Table 4.

Table 4: System precision results

S. No	Lisinopril		Hydrochlorothiazide	
	RT (min)	Peak area	RT (min)	Peak area
1	3.055	474324	5.836	1498460
2	3.044	468939	5.850	1479139
3	3.041	470051	5.847	1483822
4	3.037	475320	5.841	1497582
5	3.035	474396	5.837	1498803
6	3.035	475586	5.836	1502602
Average	3.041	473103	5.841	1493401
SD	0.008	2859.953	0.006	9508.692
% RSD	0.25	0.60	0.10	0.64

3.2.2.2 Method precision (Repeatability)

Lisinopril and hydrochlorothiazide sample preparation were injected in six replicates with known concentrations and the % RSD was determined and confirmed to be within limits. The results method precision is displayed in Table 5.

Table 5: Results of method precision

S. No	Lisinopril		Hydrochlorothiazide	
	RT (min)	Peak area	RT (min)	Peak area
1	3.025	474335	5.844	1478421
2	3.025	475945	5.845	1479442
3	3.028	472145	5.844	1463412
4	3.035	475115	5.865	1474541
5	3.022	474277	5.875	1475512
6	3.045	475145	5.865	1475615
Average	3.0300	474494	5.856	1474490.5
SD	0.0086	1304.225	0.014	5744.734
% RSD	0.283	0.275	0.233	0.390

3.2.3 Accuracy

Three levels, including 80%, 100% and 120%, of the known concentration of the sample solution were prepared and each of these was injected three times. The recovery results were computed using the formulas provided below in order to determine the accuracy test. The findings of recovery investigations are shown in Table 6.

Table 6: Recovery studies

Analyte	% concentration level	% recovery
Lisinopril	80	99.00
	100	100.56
	120	100.78
Hydrochlorothiazide	80	100.19
	100	99.61
	120	99.75

3.2.4 Robustness

Robustness is the capacity of an analytical procedure to remain unaffected by little changes to its parameters. The test procedure's robustness results demonstrated that it was unaffected by minor, deliberate modifications to the procedure's parameters, such as flow rate (0.2 ± ml/min) and column oven temperature (50ºC).

3.2.5 Limit of detection and Limit of quantification (LOD and LOQ)

According to reports, lisinopril's LOD and LOQ values are 0.11µg/ml and 0.33 µg/ml, respectively. According to reports, the LOD and

LOQ for hydrochlorothiazide are 0.21 µg/ml and 0.66 µg/ml, respectively. It was completed utilising the following formulas.

LOD = 3.3 × standard deviation/slope and LOQ = 10 × standard deviation/slope

3.2.6 Assay

Lisinopril and hydrochlorothiazide assay results were compared to the corresponding labeled quantities and provided in a Table 7. The lack of extra peaks in the chromatogram obtained for the analysis of the marketed formulation showed that the tablet's excipients did not interfere with the analysis in any way. Lisinopril and hydrochlorothiazide both tested positive with percentages of 100.57% and 100.12%, respectively.

$$\text{Assay (\%)} = \frac{\text{mg / tablet}}{\text{label claim}} \times 100$$

Table 7: Assay of lisinopril and hydrochlorothiazide

S.No.	Component	Label claim (mg/tab)	Amount found (mg/tab)	% purity
1	Lisinopril	5 mg	5.03	100.57
2	Hydrochlorothiazide	12.5 mg	12.52	100.12

3.2.7 Specificity

It was done by injecting blank, lisinopril and hydrochlorothiazide standard solution, lisinopril and hydrochlorothiazide mixed sample. The findings showed that this method was unique because no peaks were seen at the retention times of lisinopril and hydrochloro thiazide. Figures 6, 7, 8, 9 and 10 are blank chromatogram, specificity chromatogram for standard lisinopril, standard hydrochlorothiazide, chromatogram for mixed standards and mixed sample, respectively, Table 8 is results of specificity.

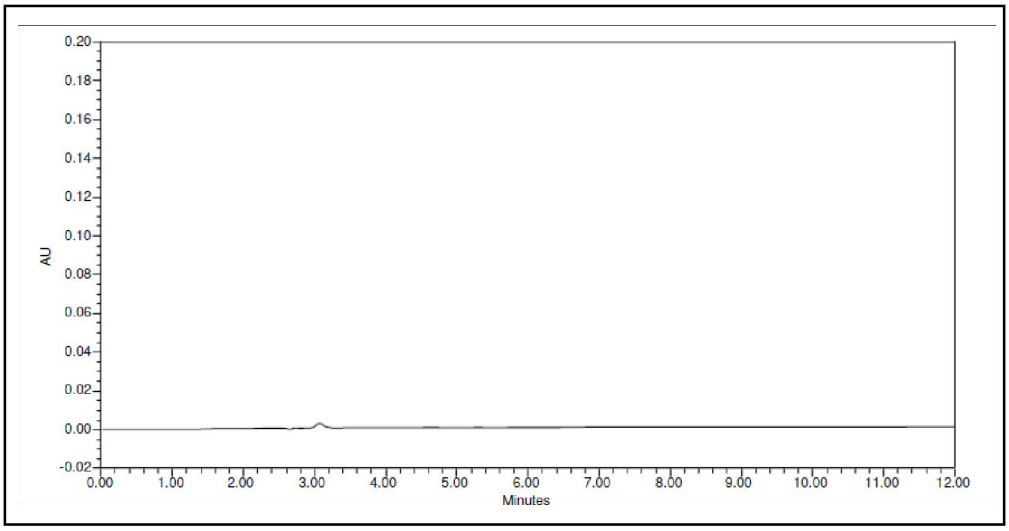
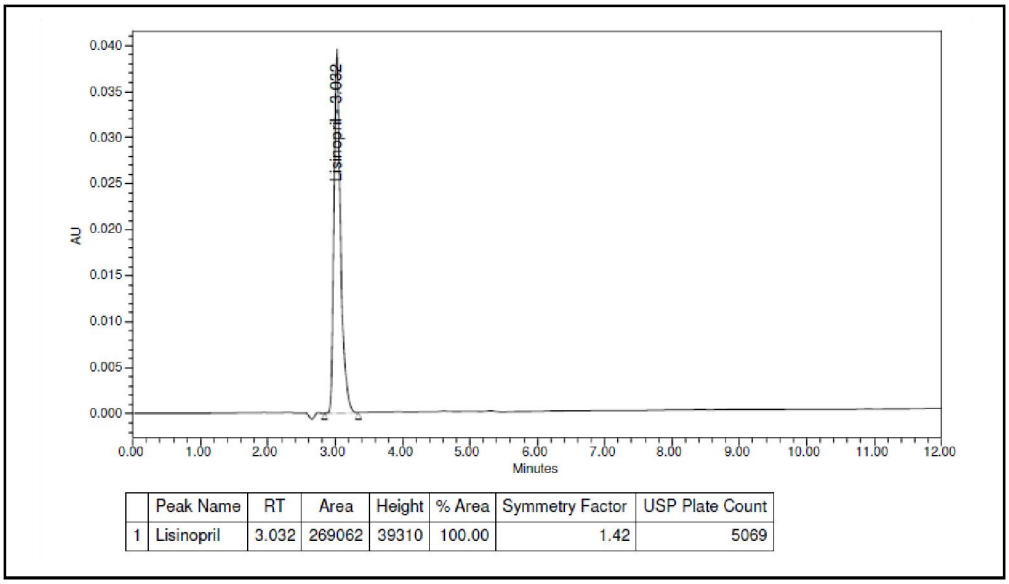
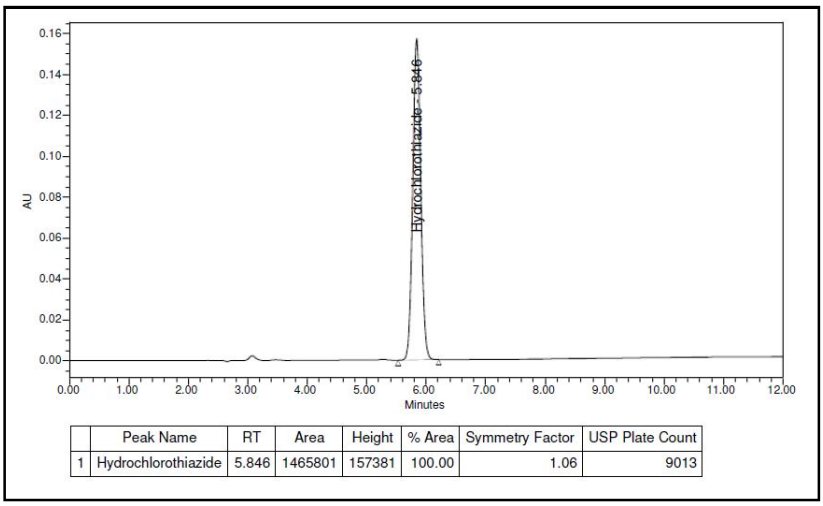


Figure 6: Blank chromatogram.



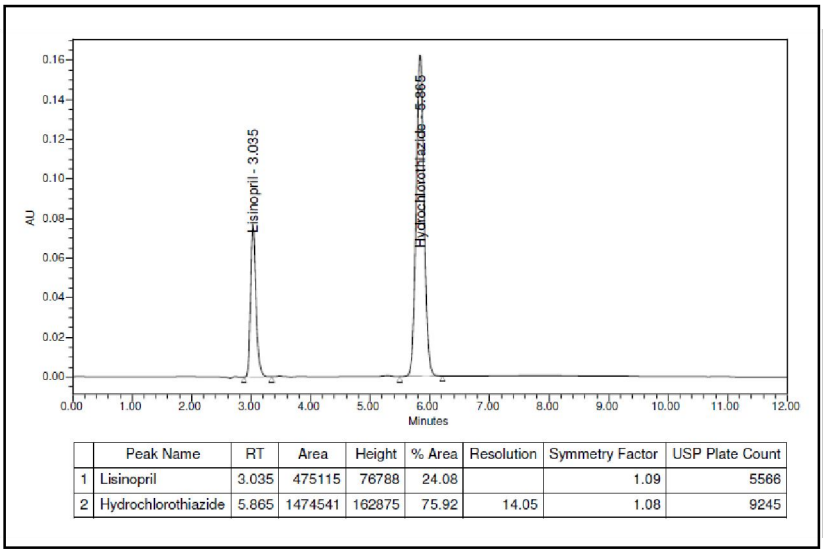
	Peak Name	RT	Area	Height	% Area	Symmetry Factor	USP Plate Count
1	Lisinopril	3.032	269062	39310	100.00	1.42	5069

Figure 7: Specificity chromatogram for lisinopril standard.



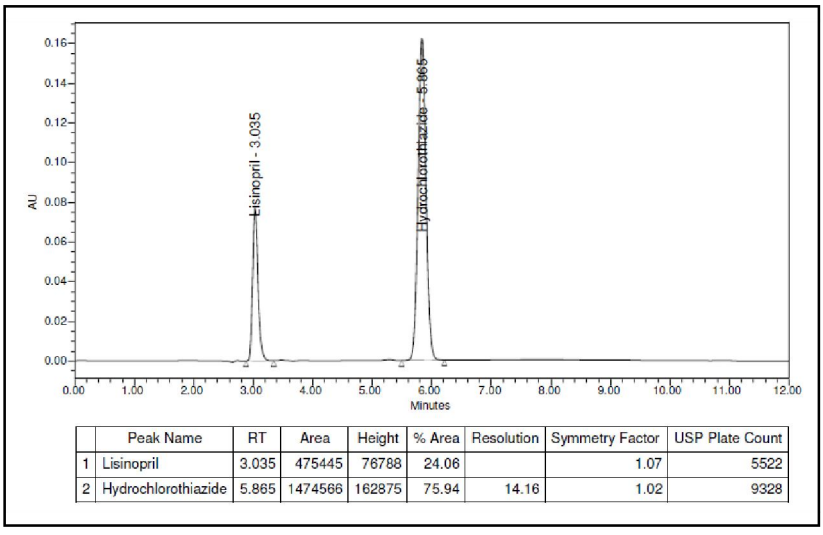
	Peak Name	RT	Area	Height	% Area	Symmetry Factor	USP Plate Count
1	Hydrochlorothiazide	5.846	1465801	157381	100.00	1.06	9013

Figure 8: Specificity chromatogram of hydrochlorothiazide standard.



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	Lisinopril	3.035	475115	76788	24.08		1.09	5568
2	Hydrochlorothiazide	5.865	1474541	162875	75.92	14.05	1.08	9245

Figure 9: Specificity chromatogram of mixed standard.

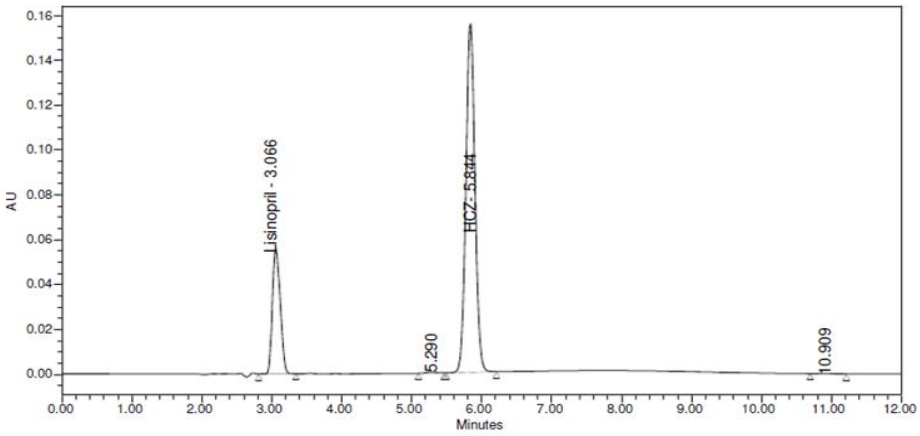


	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	Lisinopril	3.035	475445	76788	24.06		1.07	5522
2	Hydrochlorothiazide	5.865	1474566	162875	75.94	14.16	1.02	9328

Figure 10: Specificity chromatogram of mixed sample.

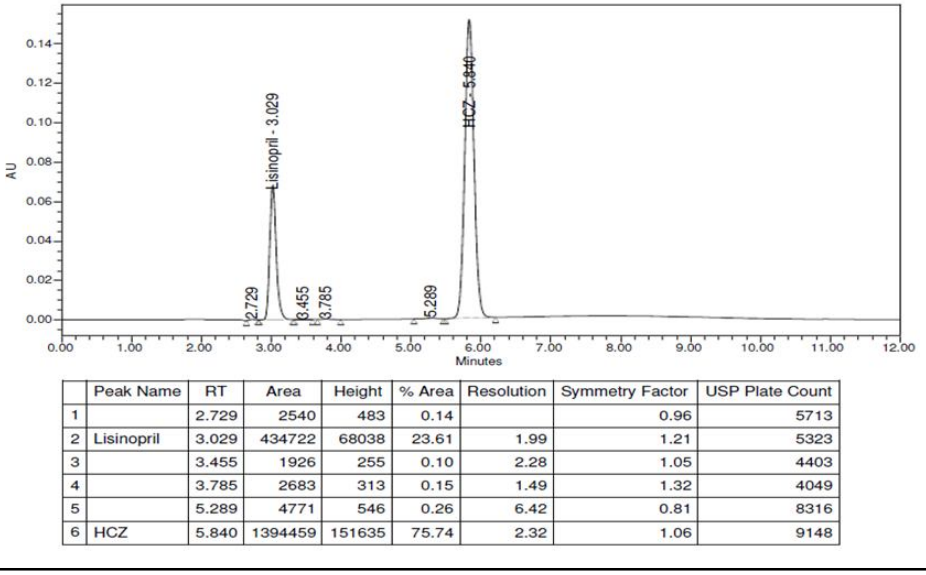
Table 8: Results of specificity

Sample name	Lisinopril		Hydrochlorothiazide	
	RT (min)	Peak area	RT (min)	Peak area
Blank	Not detected	Not detected	Not detected	Not detected
Lisinopril standard	3.032	269062	Not applicable	Not applicable
Hydrochlorothiazide standard	Not applicable	Not applicable	5.846	1465801
Mixed standard	3.035	475115	5.865	474544
Mixed sample	3.035	475445	5.865	9328



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1	Lisinopril	3.066	435279	55683	23.63		1.17	3183
2		5.290	4770	557	0.26	10.12	0.88	8562
3	HCZ	5.844	1400334	155913	76.03	2.40	1.04	9643
4		10.909	1387	102	0.08	17.18	1.70	15498

Figure 11: Chromatogram of acidic degradation.

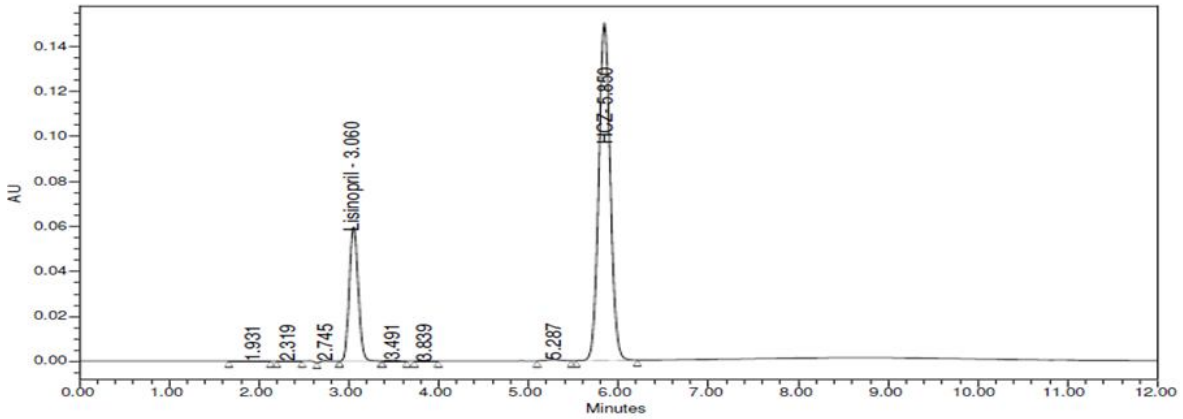


	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1		2.729	2540	483	0.14		0.96	5713
2	Lisinopril	3.029	434722	68038	23.61	1.99	1.21	5323
3		3.455	1926	255	0.10	2.28	1.05	4403
4		3.785	2683	313	0.15	1.49	1.32	4049
5		5.289	4771	546	0.26	6.42	0.81	8316
6	HCZ	5.840	1394459	151635	75.74	2.32	1.06	9148

Figure12: Chromatogram of alkali degradation.

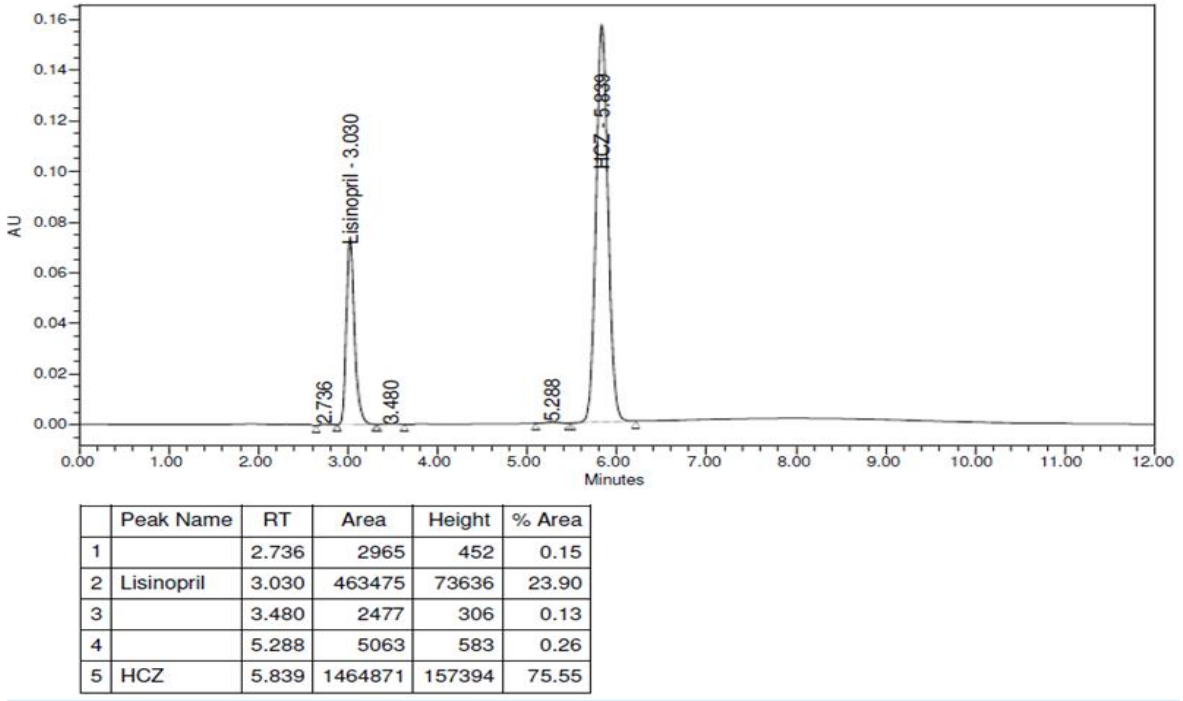
3.3 Stability studies

Degradation investigations were carried out in a various stress conditions including thermal, oxidative, photolytic, acidic and alkali. The presence of oxidative conditions was found to cause the sample to degrade more, while the presence of thermal conditions caused the sample to degrade less. Figures 11, 12, 13, 14, and 15 shows chromatograms of acidic, alkaline, oxidative, thermal, and photolytic degradation respectively and degradation studies summarized in Table 9.



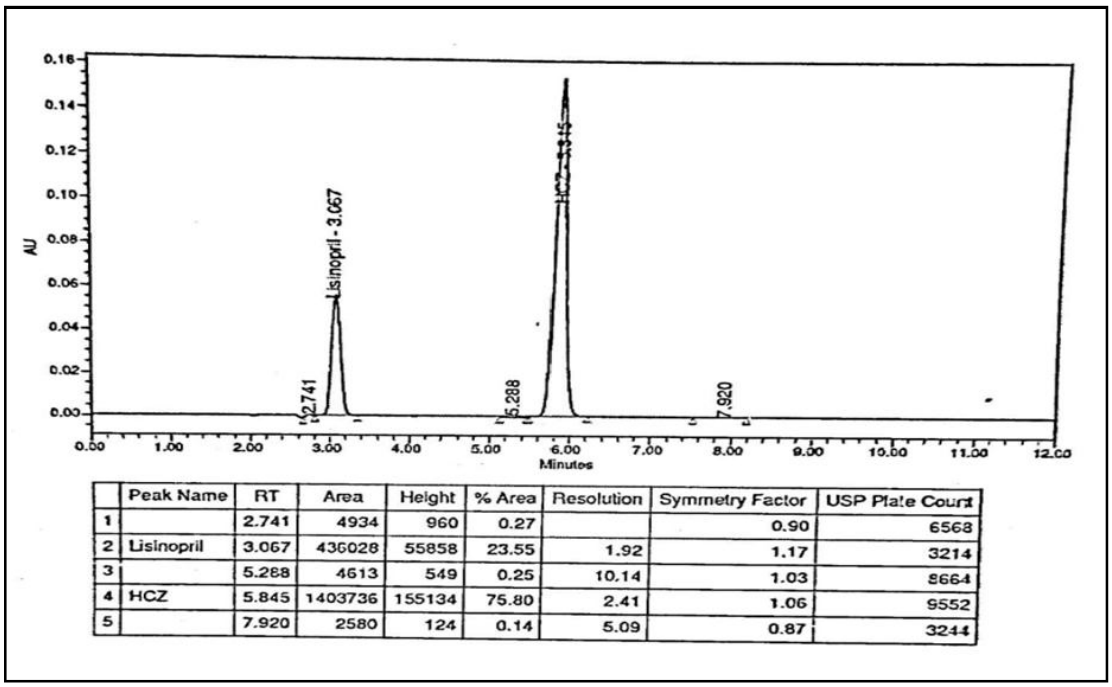
	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1		1.931	1363	85	0.08		0.85	401
2		2.319	310	34	0.02	1.05	1.24	218
3		2.745	4390	619	0.25	1.97	0.97	3498
4	Lisinopril	3.060	408067	59824	23.21	1.89	1.08	4524
5		3.491	2094	302	0.12	2.31	1.14	5520
6		3.839	1584	212	0.09	1.74	1.19	5655
7		5.287	5672	664	0.32	6.70	0.95	8603
8	HCZ	5.850	1334580	149742	75.91	2.45	1.05	9940

Figure13: Chromatogram of oxidative degradation.



	Peak Name	RT	Area	Height	% Area
1		2.736	2965	452	0.15
2	Lisinopril	3.030	463475	73636	23.90
3		3.480	2477	306	0.13
4		5.288	5063	583	0.26
5	HCZ	5.839	1464871	157394	75.55

Figure14: Chromatogram of thermal degradation.



	Peak Name	RT	Area	Height	% Area	Resolution	Symmetry Factor	USP Plate Count
1		2.741	4934	960	0.27		0.90	6568
2	Lisinopril	3.067	436028	55858	23.55	1.92	1.17	3214
3		5.288	4613	549	0.25	10.14	1.03	8664
4	HCZ	5.845	1403736	155134	75.80	2.41	1.06	9552
5		7.920	2580	124	0.14	5.09	0.87	3244

Figure 15: Chromatogram of UV degradation.

Table 9: Degradation studies at various conditions

Condition	Duration of stressed conditions (hours)	% of degradation	
		Lisinopril	Hydrochlorothiazide
Acidic (sample solution + 0.1N HCl)	24	7.6	5.17
Alkaline (sample solution + 0.1N NaOH)	24	7.71	6.19
Oxidative (sample solution + 3% H_2O_2)	2	13.38	10.20
Photolytic (at 254 nm)	5	7.44	5.57
Thermal (300^{0}C)	3	1.61	1.45

4. Discussion

To achieve quick and effective separation with appropriate system suitability parameters, various mobile phase compositions with acidic and alkaline buffers were tested. Finally, lisinopril, hydrochlorothiazide were successfully separated using a mobile phase ratio of 70:30% v/v of ammonium phosphate buffer and acetonitrile. The proposed approach underwent validation in accordance with ICH recommendations. Lisinopril and hydrochlorothiazide were discovered to have a linear relationship with concentration ranges of 2.5 to 15 µg/ml and 6.25 to 37.50 µg/ml, respectively with an R^2 value of 0.999 for both drugs. The % RSD values for both drugs were found to be <2 and results for the per cent recoveries were closer to acceptability requirements in the range of 98-102%, indicating that the suggested approach is more precise and accurate. The limit of detection and limit of quantification values were low, hence the developed method was sensitive. The approached method revealed that formulation was found to very pure which consist 100.57% of lisinopril and 100.12% of hydrochlorothiazide. The drugs present in sample were discovered to be significantly deteriorated under stress conditions such as acidic, alkali, thermal, photolytic (UV) and thermal, but more degraded under oxidative (hydrogen peroxide) and less degraded under thermal.

5. Conclusion

A successful stability indicating RP-HPLC method for the simultaneous determination of lisinopril and hydrochlorothiazide in the bulk and pharmaceutical dosage forms was established. The proposed method was found to be simple, specific, sensitive, accurate and precise. This proposed method offers less use of acetonitrile (30%), hence method was found to be cost-effective. This approach has a number of benefits, including affordability, sensitivity and dependability. This newly discovered method can be used in both industrial and quality control labs for routine analysis for the estimation of lisinopril and hydrochlorothiazide in the bulk and pharmaceutical dosage form. ICH guidelines Q1A (R2), Q2 (R2) and Q14 were referred for stability testing, validation, and analytical procedure development, respectively for estimation of lisinopril and hydrochlorothiazide.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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