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Simultaneous determination of lisinopril and hydrochlorothiazide related impurities in lisinopril and hydrochlorothiazide combined tablet dosage forms using HPLC

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ABSTRACT

The present work describes a simple, sensitive and highly specific reverse phase high performance liquid chromatographic method for the simultaneous determination of impurities of lisinopril and hydrochlorothiazide from their combined dosage form. Symmetry C_{18} column and a mobile phase comprising of 1-hexane sulphonic acid sodium salt, triethylamine, orthophosphoric acid, acetonitrile and methanol has been used for this method. developed method is selective for the impurities of lisinopril and hydrochlorothiazide. The validation elements investigated showed that the method has acceptable specificity, recovery, linearity, solution stability and method precision. Acceptable robustness indicates that this method remains unaffected by small but deliberate variations, which are described in ICH guidelines.

KEYWORDS

Lisinopril;
Hydrochlorothiazide;
Impurities;
Method development;
Method validation.

INTRODUCTION

Lisinopril is chemically (S)-[N^2-[(*S*)-1-carboxy-3-phenylpropyl]-L-lysyl]-L-proline dihydrate. Molecular formula is $C_{21}H_{31}N_3O_5 \cdot 2H_2O$ and structural formula is given in figure 1(A). It is an angiotension converting enzyme (ACE) inhibitor used for the treatment of hypertension, heart failure and acute myocardial infarction.

Hydrochlorothiazide is chemically 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide1,1-dioxide. Molecular formula is $C_7H_8ClN_3O_4S_2$ and structural formula is given in figure 1(B). It is diuretic and used for the treatment of hypertension. The serious side effects of hypertension and renal failure associated with administration of higher doses of lisinopril alone can be eliminated by the use of diuretic like hydrochlorothiazide in combination with lisinopril[1].

Various analytical methods of HPLC[2,3-5] and UV [6,7] have been reported in the literature for the assay of lisinopril and hydrochlorothiazide individually and in combination. Lisinopril and hydrochlorothiazide drug substances are official in USP[8,9] and EP[10,11]. Lisinopril tablets and hydrochlorothiazide tablets are individually official in USP[12,13] and BP[14,15]. However they are not selective for determination of impurities in lisinopril and hydrochlorothiazide combined formulations. The literature survey reveals that there is no official method reported for the simultaneous determination of impurities of both these drugs from their combined tablet formulation. Hence a single method was developed for simultaneous determination of lisinopril impurities and hydrochlorothiazide impurities in lisinopril and hydrochlorothiazide tablets. The developed method was vali-

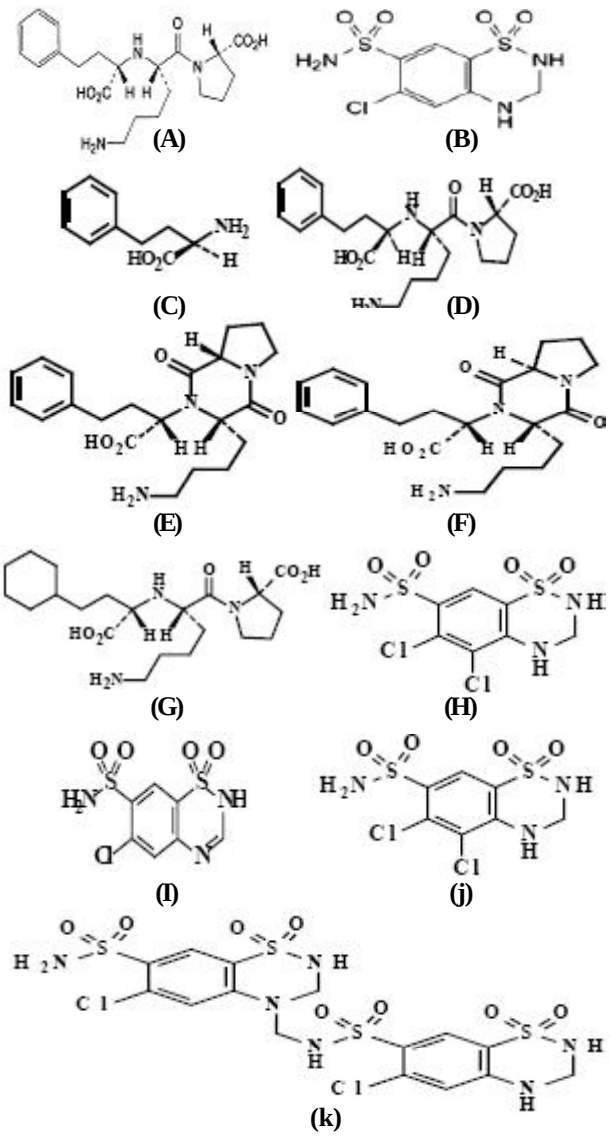

Figure 1: Lisinopril, hydr ochlorothiazide and lisinopril and hydrochlorothiazide related impurities

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The impurities of lisinopril are (2RS)-2-amino-4-phenylbutanoic acid; (2S)-1-[(2S)-6-amino-2-[[(1R)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrole-2-carboxylic acid [lisinopril R,S,S-isomer];2S)-2-[(3S,8aS)-3-(4-aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-4-phenylbutanoic acid[S,S,S-diketopiperazine]; (2S)-2-[(3S,8aR)-3-(4-aminobutyl)-1,4-dioxohexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-4-phenylbutanoic acid[R,S,S-diketopiperazine]; (2S)-1-[(2S)-6-amino-2-[[(1S)-1-carboxy-3-cyclohexylpropyl] amino] hexanoyl]pyrrole-2-carboxylic acid[cyclohexyl analogue].

The impurities of hydrochlorothiazide are 4-amino-6-chlorobenzene-1,3-disulphonamide[salamide]; 6-chloro-2H-1,2,4-benzothiadiazine-7-sulfonamide -1,1-dioxide[Chlorothiazide]; 5-chlorohydrochlorothiazide; 6-chloro-N-[(6-chloro-7-sulphamoyl-2,3-dihydro-4H-1,2,4-benzothiadiazin-4-yl1,1-dioxide)methyl]-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide[Hydrochlorothiazide dimer]. The structures of the above mentioned impurities are serially given in figure 1(C-K).

EXPERIMENTAL

Materials and equipment

Lisinopril and hydrochlorothiazide tablets were manufactured by Aurobindo Pharma Ltd. Working standards and impurities were prepared by Chemical Research Department of Aurobindo Pharma Research Center. Acetonitrile, methanol, orthophosphoric acid, triethylamine, 1-hexane sulphonic acid sodium salt were procured from Merck (India) Limited and water was obtained from an in-house USP quality water purification system. Reagents and solvents used were analytical or HPLC grade and were used without further purification.

The chromatographic system consisted of an HPLC (Waters 2695 separation module equipped with 2996 photo diode array detector) system, with symmetry C_{18} (4.6×150 mm) 5µm particle size column.

Chromatographic conditions

Mobile phase flow rate was 1.5 ml/min. UV Detection was at 215 nm and column oven temperature was 50°C. Gradient pump mode was used. The time program was as follows, time (min) A(v/v):B(v/v). $T_{0.01}$/92:8, $T_{10.0}$/92:8, T_{40}/80:20, T_{45}/92:8,T_{55}/92:8.

Preparation of analytical solutions

1. Mobile phase

Hexane sulphonic acid buffer (pH 2.4)[1.0g of 1-Hexane sulphonic acid sodium salt was dissolved in 1 litre water, 5 ml of Triethylamine was added and adjusted to pH 2.4±0.1 with Orthophosphoric acid and filtered through 0.45µ membrane filter] has been used as mobile phase A. Acetonitrile and methanol in the ratio of 90:10 v/v used as Mobile phase B.

2. Preparation of diluent

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1.0 g of 1-hexane sulphonic acid sodium salt was dissolved in 1 litre water, 5 ml of triethylamine was added and adjusted to pH 3.0 ± 0.05 with orthophosphoric acid. Filtered through 0.45µ membrane filter.

3. Preparation of system suitability stock solution

Accurately weighed quantities of about 5 mg of chlorothiazide and about 3 mg of lisinopril R-S-S-isomer were transferred into a 50 ml clean, dry volumetric flask, dissolved in 5 ml of methanol and made up to volume with methanol.

4. Preparation of system suitability solution

Accurately weighed quantities of about 45 mg of lisinopril and 50 mg of hydrochlorothiazide working standards were transferred into a 50 ml clean and dry volumetric flask. 5 ml of methanol was added and sonicated for 5 minutes to dissolve the drug. To this was added 2.5 ml of system suitability stock solution, mixed, made up to volume with diluent and then filtered through 0.45µ membrane filter paper. The above solution yielded a concentration of 5µg/ml of chlorothiazide, 3µg/ml of R.S.S isomer,900µg/ml of lisinopril and 1000µg/ml of hydrochlorothiazide.

5. Preparation of standard solution

Accurately weighed quantities of about 45 mg of lisinopril and 50 mg of hydrochlorothiazide working standards were transferred into a 100 mL clean, dry volumetric flask. 5 mL of methanol was added, sonicated for 5 minutes to dissolve the drug and made upto volume with diluent. This solution was further serially diluted with diluent to obtain concentration of 4.5µg/ml of lisinopril and 5µg/ml of hydrochlorothiazide.

6. Preparation of sample solution

Twenty tablets were crushed to a fine powder. A quantity of powder equivalent to about 50 mg of hydrochlorothiazide was transferred to a 50 ml volumetric flask. 5 ml of methanol was added, the solution was sonicated for 15 minutes and made up to volume with diluent. The solution was centrifuged at 5000 rpm for 10 minutes. The supernatant solution filtered through 0.45µ membrane filter. This solution yielded the concentration of 1000µg/ml of hydrochlorothiazide and 800µg/ml of Lisinopril.

7. Preparation of all impurity mix solution

The impurities namely salamide, chlorothiazide, 2-amino-4-phenyl butanoic acid, 5 chlorohydrochloro thiazide, lisinopril R,S,S isomer, hydrochlorothiazide dimer, S,S,S diketopiparazine, RSS diketopiperazine, cyclohexyl analogue were prepared in diluent to obtain a concentration of 5µg/ml of each impurity.

8. Solutions for testing degradation and specificity

5 M Sodium hydroxide, 5 M hydrochloric acid and 30% hydrogen peroxide solutions were used to induce degradation of lisinopril and hydrochlorothiazide tablets. Separately weighed and transferred tablets powder equivalent to about 50 mg of hydrochlorothiazide into three different 50 ml volumetric flasks. To each flask 5ml of methanol was added and sonicated for 5minutes, 20 ml of diluent was added and sonicated for 15 minutes. For the first flask 5 ml of 5 M hydrochloric acid was added and heated on water bath at 85°C for 15 minutes. For the second flask 5 ml of 5 M sodium hydroxide was added and heated on water bath at 85°C for 30 minutes. For the third flask 5 ml of 30% hydrogen peroxide was added and heated on water bath at 85°C for 30 minutes. All three flasks were cooled to room temperature, neutralized and made up to the mark with diluent.

The effect of light on the stability of lisinopril and hydrochlorothiazide was studied for tablets powder by exposing to low intensity UV lamp for 12,000 Lux/288 hours.

9. Solutions for testing linearity and range

Standard solutions used for testing the linearity of calibration plots for lisinopril, hydrochlorothiazide and their related substances were at concentration levels of about 0.2 to 15µg/ml.

10. Solutions for testing accuracy

Sample solutions of lisinopril and hydrochlorothiazide and their impurities were prepared by transferring sample equivalent to about 50 mg of hydrochlorothiazide and three quantities of impurities, respectively, in the range of 0.06 to 0.9 mg. Diluent was then added to make the volume up to the mark into three 50 ml volumetric flasks.

11. Solutions for testing precision and stability

Sample solutions of lisinopril and hydrochlorothiazide and their impurities were prepared by transfer-

ring sample equivalent to about 50 mg of hydrochlorothiazide and impurities (0.20mg of each lisinopril related impurities and 0.25 mg of each hydrochlorothiazide impurities) into 50 ml volumetric flask. Diluent was then added to make the volume up to the mark. The above solutions were also used to study the effect of various method parameters and for solution stability.

12. Placebo solution

Placebo solution was prepared by an accurately weighed portion of the Placebo powder equivalent to about 50 mg of hydrochlorothiazide into a 50 mL clean, dry volumetric flask. Added 5 mL of methanol and sonicated for 5 minutes. To this 30 mL of diluent was added and again sonicated for 15 minutes. Diluted up to the mark with diluent. Centrifuged this solution at 5000 rpm for 10 minutes. Supernatant solution was filtered through 0.45µ membrane filter.

13. Chromatographic procedure and calculation

20µL of the system suitability solution, Standard solution, dilutent, placebo solution and sample solution and were injected into the chromatographic system. Recorded the chromatograms at the wavelength of 215 nm. The resolution between lisinopril R,S,S isomer & lisinopril and also between chlorothiazide and hydrochlorothiazide was above 1.5. The RSD (Relative Standard Deviation) of the peak areas of lisinopril and hydrochlorothiazide for six replicate injections of standard solution was not more than 5.0%.

RESULTS AND DISCUSSIONS

Method development and optimization

The method evaluation for determination of impurities was initiated using the monograph methods for individual drugs. The USP monograph method for lisinopril tablets could not detect the hydrochlorothiazide dimer impurity. The EP monograph method for hydrochlorothiazide drug substance could not detect the lisinopril impurities. The monograph methods were also modified for its capability of simultaneous determination. Lisinopril and hydrochlorothiazide had different PKa values (Lisinopril 3.3 and hydrochlorothiazide 8.7).

During the method development, different parameters were attuned to obtain an acceptable resolution between the impurities with acceptable recoveries and to satisfy the HPLC system suitability and to use it as a stability indicating method. These parametres include : flow rate(0.5-1.5ml/min^{-1}), column temparature (30-50°), different types of columns like C_{18} and C_8 ,different types of buffers pH ranging from 2.0 to 5.0 and various organic modifiers and mixtures of methanol, acetonitrile with different ratios.Based upon the results obtained by our experimental trials we optimized the method. Some of critical parameters of optimisation are as below.

Column: Optimized to symmetry C_{18}, 5µ (150×4.6 mm) where in satisfactory baseline separation was achieved.

Mobile phase: The peak due to hydrochlorothiazide was not retained irrespective of the change in the buffer and gradient programme. Hence introduction of ion pair reagent was required. The pH of mobile phase was optimised to 2.4 ± 0.1 for seperation of all the impurities due to lisinopril and hydrochlorothiazide.

Maximum absorbance for lisinopril was at 215 nm and for hydrochlorothiazide was at 270 nm. At 215 nm, it was found that response of lisinopril and hydrochlorothiazide impurities was found to be satisfactory. So we have optimized nm to 215.

Method validation

1. Specificity

All impurity solutions of lisinopril and hydro chlorotiazide were prepared and injected to conform the retention times. Sample solutions were prepared with and without spiking of impurities and injected into the HPLC.

The peak purity data of lisinopril and hydrochlorothiazide peaks in control sample and spiked sample (known impurities spiked sample) indicated that the peaks are homogeneous and have no co-eluting peaks indicating speficity of the method. The data is presented in TABLE 1. Further these samples were subjected to LC-MS (PE SCIEX-API 2000) analysis. Based on the observed m/z values 285.5 (Salamide), 295.5 (Chloro thiazide), 297.5 (Hydrochlorothiazide), 179.0 (2-Amino-4-phenyl butanoic acid), 332.0 (5-Chloro hydrochlorothiazide), 405.0 (Lisinopril), 405.0 (Lisinopril R,S,S-isomer), 607.0 (Hydrochlorothiazide dimer), 387.0(S,S,S-Dikdetopiperazine), 387.0 (R,S,S-Dikdetopiperazine) and 411.0 (Cyclohexyl analogue) it was confirmed that the method is specific.

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TABLE 1: Specificity-peak purity results

Name of the compound	RT (min)	RRT	Peak Purity	
			Purity angle	Purity threshold
Salamide	2.923	0.13	0.143	0.373
Chlorothiazide	3.870	0.17	0.125	0.331
Hydrochlorothiazide	4.420	0.20	0.088*	0.286*
2-Amino-4-phenyl butanoic acid	9.789	0.44	5.228	6.325
5-Chloro hydrochlorothiazide	11.132	0.50	0.496	0.818
Lisinopril	22.276	1.00	0.192	0.336
Lisinopril R,S,S-isomer	24.028	1.08	6.414	7.452
Hydrochlorothiazide dimmer	25.075	1.13	1.295	1.555
S,S,S-Diketopiperazine	34.454	1.55	4.923	6.777
R,S,S-Diketopiperazine	35.211	1.58	5.430	7.400
Cyclohexyl analogue	36.912	1.66	15.993	21.093

RT-Retention Time, RRT-Relative retention time, *Obtained from Spiked sample (20+25mg / Tablet)-diluted

TABLE 2: Forced degradation

Degradation mechanism	Degradation Condition	% Area*	% Degradation	Lisinopril Peak Purity		Hydrochlorothiazide Peak Purity	
				Purity angle	Purity threshold	Purity angle	Purity threshold
-	Un degraded	99.44	-	0.170	0.396	0.037	0.277
Acid degradation	5M HCl /85°C 15 min	83.06	16.5	0.439	1.163	0.025	0.264
Base degradation	5M NaOH/ 85°C 30 min	74.32	25.3	0.108	0.499	0.096	0.299
Peroxide degradation	30% H_2O_2/ 85°C/30 min	88.45	11.1	0.220	0.561	0.198	0.565
Thermal degradation	105°C/288 Hours	97.45	2.0	0.119	0.358	0.029	0.263
Photolytic degradation	12,000 Lux/ 288 Hours	99.64	Nil	0.083	0.347	0.026	0.273
Humidity degradation	92% RH/ 25°C/288 Hours	99.51	Nil	0.084	0.320	0.035	0.287

***Sum of % area of hydrochlorothiazide and % area of lisinopril**

TABEL 3: Linearity and range

Name of the compound	Spec. Levels(%)	Range µg/mL	R^2	Slope	Intercept	RSQ	RF	LOD (%w/w)	LOQ (%w/w)
Salamide	1.0	0.213-15.350	0.9999	72191	-1297	3193	0.81	0.008	0.015
Chlorothiazide	1.0	0.224-15.389	0.9998	48592	263	4523	1.20	0.011	0.022
Hydrochlorothiazide	-	0.326-10.534	0.9994	58363	14762	7675	-	-	-
2-Amino-4-phenyl butanoic acid	0.3	0.283-3.599	0.9983	20228	1356	1578	1.04	0.036	0.057
5-Chloro hydrochlorothiazide	0.5	0.373-8.189	0.9998	34868	1530	1882	1.67	0.017	0.036
Lisinopril	-	1.314-5.520	0.9991	21060	-3696	-	-	-	-
Lisinopril R,S,S-isomer	0.3	0.946-3.626	0.9989	16321	-2352	874	1.29	0.059	0.117
Hydrochlorothiade dimer	1.0	0.774-15.656	0.9993	40743	11674	9052	1.43	0.021	0.043
S,S,S-Diketopiperazine	1.5	1.268-18.282	0.9956	15504	2440	10051	1.36	0.081	0.173
R,S,S-Diketopiperazine	0.3	1.201-3.640	0.9994	15767	-1765	596	1.34	0.051	0.118
Cyclohexyl analogue	0.3	1.065-3.879	0.9957	9734	-1789	1109	2.16	0.067	0.111

Spec. : Specification, RSQ: Residual Sum of Squares, RF : Response Factor, LOD: Limit of Detection, LOQ: Limit of Quantification

Lisinopril and hydrochlorothiazide tablets and its placebo were subjected to stress conditions (Acid, base, peroxide, thermal, photolytic, humidity degradations) and solutions were prepared with respective

TABLE 4 : Precision results

Lisinopril (20mg) + Hydrochlorothiazide (25mg) Strength										
Name of the compound	S1	S2	S3	S4	S5	S6	Mean	SD	% RSD	95% Confidence interval
Salamide	0.550	0.539	0.537	0.554	0.549	0.547	0.546	0.007	1.3	± 0.007
Chlorothiazide	0.672	0.664	0.671	0.661	0.684	0.672	0.671	0.008	1.2	± 0.008
2-Amino-4-phenyl butanoic acid	0.821	0.819	0.819	0.826	0.818	0.939	0.840	0.048	5.7	± 0.050
5-Chloro hydrochlorothiazide	0.526	0.506	0.520	0.528	0.511	0.494	0.514	0.013	2.5	± 0.014
Lisinopril R,S,S-isomer	0.668	0.674	0.666	0.730	0.681	0.674	0.682	0.024	3.5	± 0.025
Hydrochlorothiade dimer	0.496	0.503	0.496.	0.492	0.490	0.535	0.502	0.017	3.4	± 0.018
S,S,S-Diketopiperazine	0.760	0.757	0.753	0.764	0.751	0.754	0.757	0.005	0.7	± 0.005
R,S,S-Diketopiperazine	0.603	0.610	0.606	0.602	0.608	0.603	0.605	0.003	0.5	± 0.003
Cyclohexyl analogue	0.555	0.521	0.559	0.552	0.514	0.523	0.537	0.020	3.7	± 0.021

stressed samples. Each stressed sample was injected into the HPLC.

From the placebo chromatogram, it was concluded that no peak was observed at the retention times of known impurities, lisinopril and hydrochlorothiazide peaks. Hence it can be concluded that no interference was found due to placebo for the determination of impurities of lisinopril and hydrochlorothiazide in lisinopril and hydrochlorothiazide tablets. Also in the forced degraded samples it was observed that the purity sample angle is less than purity threshold. The data is presented in TABLE 2.

2. Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ values of each related substance were predicted from a separate linearity data performed at lower concentration. Each predicted concentration was verified by preparaing the solutions at about predicted concentration and injected each solution six times into the HPLC.

The LOQ value for each impurity is well below the 50% of specification level, indicating that the method is sufficiently precise for the quantification of the lisinopril and hydrochlorothiazide impurities in lisinopril and hydrochlorotihiazide tablets.

3. Linearity

A series of solutions were prepared using lisinopril and hydrochlorothiazide working standards and their related substances at concentration levels from limit of quantification (LOQ) to 150% of specification level and each solution was injected to the HPLC.

The correlation coefficient is more than 0.990 for lisinopril, hydrochlorothiazide and impurities of lisinopril and hydrochlorothiazide. Hence, the response of lisinopril, hydrochlorothiazide and each impurity are linear from LOQ to 150% of specification level. The LOD, LOQ and linearity data are presented in TABLE 3.

Method precision and ruggedness

Six sample solutions were prepared individually using same batch of lisinopril and hydrochlorothiazide tablets powder spiked with impurities at 0.5% level and each solution was injected to the HPLC. To check the ruggedness, separate set of six sample solutions with impurities spiked at 0.5% level was prepared by different analyst, on a different day, using different column and HPLC system. The data of precision and ruggedness is presented in TABLES 4 and 5. The % RSD of all the impurities were well within 10%. Hence the method is precise and rugged.

6. Accuracy

Method accuracy was determined by injecting solutions in triplicate using lisinopril and hydrochlorothiazide tablets powder spiked with impurities at levels 50%, 100% and 150% of specification level. Data is presented in the TABLE 5.

The recovery results are within 90-110%. Hence the developed method has an acceptable level of accuracy for the determination of lisinopril and hydrochlo-

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TABLE 5: Ruggedness results

Lisinopril (20mg) + Hydrochlorothiazide (25mg) Strength										
Name of the compound	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**Mean**	**SD**	**% RSD**	**95% Confidence interval**
Salamide	0.497	0.495	0.496	0.490	0.499	0.501	0.496	0.004	0.8	± 0.004
Chlorothiazide	0.808	0.801	0.789	0.794	0.800	0.794	0.798	0.007	0.9	± 0.007
2-Amino-4-phenyl butanoic acid	0.514	0.522	0.519	0.527	0.540	0.514	0.523	0.010	1.9	± 0.010
5-Chloro hydrochlorothiazide	0.526	0.506	0.512	0.518	0.513	0.525	0.517	0.008	1.5	± 0.008
Lisinopril R,S,S-isomer	0.740	0.711	0.738	0.716	0.717	0.730	0.725	0.012	1.7	± 0.013
Hydrochlorothiade dimer	0.629	0.632	0.640	0.630	0.633	0.621	0.631	0.006	1.0	± 0.006
S,S,S-Diketopiperazine	0.460	0.446	0.417	0.438	0.484	0.455	0.450	0.022	4.9	± 0.023
R,S,S-Diketopiperazine	0.454	0.401	0.426	0.425	0.441	0.431	0.430	0.018	4.2	± 0.019
Cyclohexyl analogue	0.544	0.542	0.465	0.521	0.558	0.500	0.522	0.034	6.5	± 0.036

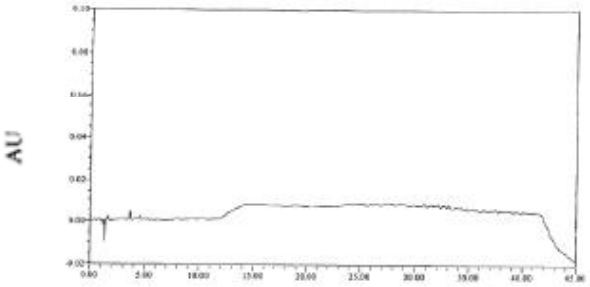

Figure 2 : Diluent chromatogram

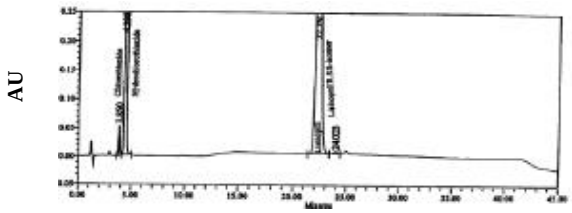

Figure 3: System suitability chromatogram

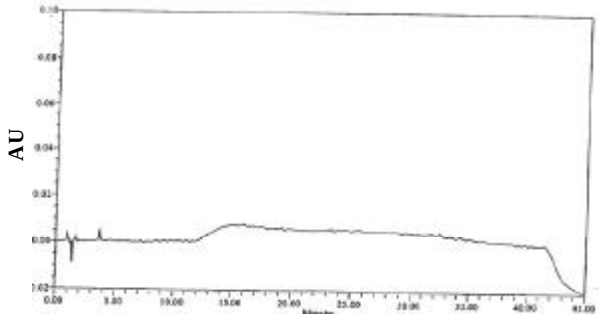

Figure 4 : Placebo chromatogram

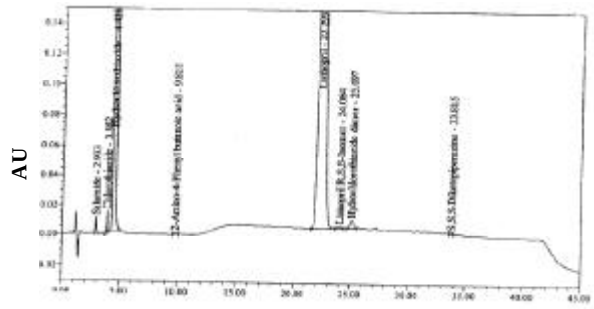

Figure 5 : Control sample chromatogram

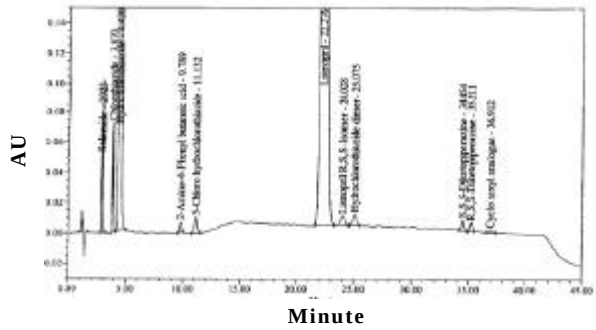

Figure 5 : Control sample chromatogram

rothiazide impurities in lisinopril and hhydrochloro thiazide tablets.

7. Robustness

System suitability solution and sample solution spiked with impurities at 0.5% level was prepared and injected to the HPLC at different variable conditions (Change of flow ± 10%, wavelength±5nm, organic in mobile phase ± 2%, column oven temperature ± 5°C and buffer pH ± 0.2 and 0.1). From the data of analysis of system suitability solution and sample solution spiked with all the impurities at above mentioned conditions, it was found that there is no effect on the relative retention times of impurities in the system suitability solution. The resolution of all the impurities at the deliberately varied conditions was also not altered. However it was observed that the impurities, cyclohexyl analogue and R,S, S-diketopiparazine coeluted at the pH variation. To further verify the pH effect, the buffer pH

TABLE 6: Accuracy results

Name of the compound	Quantity (% w/w) Added	Quantity found[a]	Recovery (%)	Bias[b] (%)
	0.501	0.493	98.4	-1.6
Salamide	1.001	1.034	103.3	3.3
	1.501	1.463	97.5	-2.5
Average±RSD			99.7±3.1	
	0.500	0.504	100.8	0.8
Chlorothiazide	0.999	1.003	100.4	0.4
	1.498	1.513	101.0	1.0
Average±RSD			100.7±0.3	
	0.152	0.156	102.6	2.6
2-Amino-4-phenyl butanoic acid	0.304	0.311	102.3	2.3
	0.456	0.473	103.7	3.7
Average±RSD			102.9±0.7	
	0.249	0.250	100.4	0.4
5-Chloro hydrochlorothiazide	0.498	0.493	99.0	-1.0
	0.747	0.737	98.7	-1.3
Average±RSD			99.4±0.9	
	0.149	0.150	100.7	0.7
Lisinopril R,S,S-isomer	0.299	0.307	102.7	2.7
	0.448	0.457	102.0	2.0
Average±RSD			101.8±1.0	
	0.491	0.505	102.9	2.9
Hydrochlorothiade dimer	0.981	1.014	103.4	3.4
	1.471	1.446	98.3	-1.7
Average±RSD			101.5±2.8	
	0.748	0.689	92.1	-7.9
S,S,S-Diketopiperazine	1.495	1.467	98.1	-1.9
	2.243	2.228	99.3	-0.7
Average±RSD			96.5±4.0	
	0.152	0.160	105.3	5.3
R,S,S-Diketopiperazine	0.304	0.311	102.3	2.3
	0.456	0.464	101.8	1.8
Average±RSD			103.1±1.8	
	0.149	0.147	98.7	-1.3
Cyclohexyl analogue	0.298	0.300	100.7	0.7
	0.447	0.443	99.1	-0.9
Average±RSD			99.5±1.1	

variation was reduced by ± 0.1 units and analysis was repeated. At this pH variation there was good separation of all the impurities, indicating the pH variation of mobile phase 2.4 ± 0.1 is suitable for analysis.

CONCLUSION

The simultaneous method developed can be used for determination of impurities of lisinopril and hydrochlorothiazide in combined formulation. It could be precisely used to estimate the impurities in stability sample solutions as per ICH conditions. Based on validation parameters performed, the method was found to be specific, linear, selective, precise and robust. All statistical values (Percentage recoveries, R.S.D., slope and intercept, LOD and LOQ) were within the acceptable limits.

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