IJIRR

International Journal of Information Research and Review
Vol. 03, Issue, 04, pp. 2220-2228, April, 2016

Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR CILAZAPRIL AND HYDROCHLORTHIAZDIDE IN THE COMBINED PHARMACEUTICAL DOSAGE FORM

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ARTICLE INFO

Article History:

Received 17th, January 2016
Received in revised form
14th, February 2016
Accepted 29th, March 2016
Published online 27, April 2016

Keywords:

Cilazapril,
Hydrochlorthiazide,
RP-HPLC,
Validation.

ABSTRACT

The study describes the stability indicating RP-HPLC method for simultaneous estimation of cilazapril and hydrochlorthiazide in pharmaceutical dosage form has been developed. The proposed RP-HPLC method was performed with reversed phase C18 column (Luna C18 column) equilibrated with mobile phase comprising of Methanol: Buffer (60:40, v/v, pH 4.0 adjusted by using 5% solution of orthophosphoric acid) was used. The flow rate was maintained at 1 mL/min. as it gave a good peak shape and resolution. A linearity response was observed in the concentration range of 0.5 - 40 µg/mL for cilazapril and 5 - 60 µg/mL for hydrochlorthiazide respectively. Limit of detection and limit of quantification for cilazapril are 0.17µg/Ml and 0.5 µg/mL and for hydrochlorthiazide are 1.3µg/Ml and 4.1 µg/mL, respectively. The recoveries found to be 97.67 % - 98.90% and 97.60%-99.06% for cilazapril and hydrochlorthiazide respectively. The stability indicating method was developed by subjecting the drugs to stress conditions such as acid and base hydrolysis, oxidation, and photo- and thermal degradation and the degraded products formed were resolved successfully from the samples. The result shows that the proposed method is suitable for the routine quality control analysis of simultaneous determination of cilazapril and hydrochlorthiazide in bulk and pharmaceutical dosage form.

INTRODUCTION

Cilazapril is an angiotensin-converting enzyme inhibitor used in the treatment of hypertension and congestive heart failure. It competes with angiotensin I for binding at the angiotensin-converting enzyme, blocking the conversion of angiotensin I to angiotensin II. As angiotensin II is a vasoconstrictor and a negative feedback mediator for renin activity, lower angiotensin II levels results in a decrease in blood pressure, an increase in renin activity, and stimulation of baro-receptor reflex mechanisms. Chemically it is7-[[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]-6-oxo-1,2,3,4,7,8,9,10-octahydropyri dazino[1,2-a]diazepine-4-carboxylic acid (fig.1). Hydrochloro thiazide is a diuretic agent often used to treat high blood pressure and swelling due to fluid buildup Other uses include diabetes insipidus, renal tubular acidosis. For high blood pressure it is often recommended as a first line treatment. It reduces blood volume by acting on the kidneys to reduce sodium (Na^+) reabsorption in the distal convoluted tubule. Chemically, it is 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide (Fig. 2).

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Through literature survey reveals that analysis methods based on different techniques reported for the determination of cilazapril in pure and pharmaceutical formulations. Various methods for determining levels of cilazapril in biological samples have been developed including: enzyme immunoassay in serum or plasma, LC/positive ion tandem MS method in human plasma, HPLC with amperometric and photometric detection in urine, capillary zone electrophoresis in urine and gradient RP-high performance liquid chromatography in the presence of hydrochlorothiazide in combined dosage form. So an attempt was made to report a simple, rapid, sensitive, accurate and precise RP-HPLC method to estimate cilazapril and hydrochlorothiazide in pharmaceutical dosage form. HPLC methods are useful in the determination of drugs in pharmaceutical formulations especially those containing more than one active components. Therefore, the aim of this work was to develop a relatively simple HPLC method for simultaneous quantification of cilazapril and hydrochlorot hiazide without require to sample pre-treatment. This paper describes the development and validation of reliable, simple, stable and economic reverse phase HPLC assay, using UV detection for the simultaneous determination of cilazapril and hydrochlorothiazide. The method appears to be suitable for

quality control in pharmaceutical industry due to its sensitivity, simplicity, selectivity and lack of excipients interference.

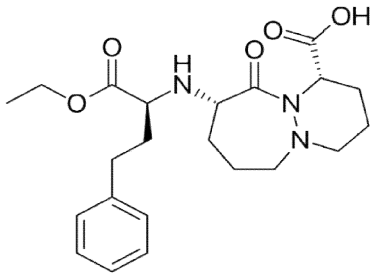

Fig. 1. chemical structure of Cilazapril

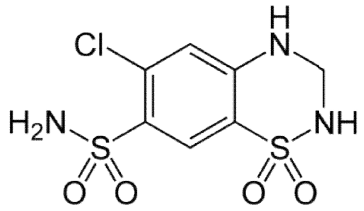

Fig. 2. chemical structure of Hydrochlorthiazide

MATERIALS AND METHOD

Chemicals and Reagents

Analytically pure Cilazapril (Pharmaceutical grade) was obtained as generous gift sample from the Hetero Drugs Limited, Hyderabad, India and Hydrochlorthiazide (Pharmaceutical grade) was obtained as generous gift sample from the Cadilla Healthcare Limited, Gujarat, India. Tablet formulation of combined dosage form was purchased from local market. Methanol, Acetonitrile, potassium dihydrogen phosphate, orthophosphoric acid, formic acid, and ammonia used for mobile phase preparation were of HPLC grade, Merck, Mumbai, India. Hydrochloric acid, Sodium hydroxide and hydrogen peroxide used for stress degradation studies were of analytical reagent grade, CDH Chemicals, Delhi, India. Calibrated micropitte were used for purpose for measurement and transfer. De-ionized water prepared using Milli-Q plus purification system, Millipore (Bradford, USA) was used throughout the study. All other chemical used were analytical grade.

HPLC Instrumentation

The analysis of drugs was carried out on a LC20-AD made by Shimadzu Corporation Kyoto Japan equipped with gradient HPLC pump with detector LC20-AD UV-Visible detector and Rheodyne universal injector 7725. Reverse phase HPLC column Luna C18 column 5µm particle size, length and internal diameter of 250X4.6 mm were used.

Chromatographic Conditions

A reversed phase C_{18} column (Luna C18 column) equilibrated with mobile phase comprising of Methanol: Buffer (60:40, v/v, pH 4.0 adjusted by using 5% solution of orthophosphoric acid) was used. The flow rate was maintained at 1 ml/ min. A 20µl of sample was injected using a fixed loop, and the total run time was 10 min.

Preparation of Mobile Phase

The mobile phase was comprising of Methanol: phosphate buffer (60:40 v/v, pH 4.0 adjusted by using 5% solution of orthophosphoric acid). The buffer solution was prepared by dissolving accurately weighing 27.12 g of potassium dihydrogen phosphate and dissolving water and dilute with water to 1000 ml. The 400 ml of buffer solution was mixed with 600 ml of methanol and the pH was adjusted to 4.0 ± 0.2 using Ortho phosphoric acid. The solution was filtered using Whatman filter paper (No. 1). The solution was sonnicated for 10 min for degassing prior to use in an ultrasonic bath.

Diluent Preparation

There was mobile phase used as a diluent.

Preparation of standard stock solution (*Standard stock solution (1000µg/ml)*)

The 25 mg of Cilazapril (CIL) were accurately weighed and transferred to 25 ml volumetric flask containing few ml (10 ml) of methanol. The flasks were sonicated for 2 minutes to dissolve the solids and volume was made up to the mark with diluent to obtain a standard solution containing 1000µg/ml Cilazapril (CIL). The 25 mg of Hydrochlorthiazide (HCT) were accurately weighed and transferred to separate 25 ml volumetric flask containing few ml (10 ml) of methanol. The flasks were sonicated for 2 minutes to dissolve the solids and volume was made up to the mark with diluent to obtain a standard solution containing 1000µg/ml Hydrochlorthiazide (HCT).

Preparation of sample solution

Twenty tablets weighed accurately and finely powdered. A powder quantity equivalent to10 mg CIL and 25 mg HCT was accurately weighed and transferred to 100 ml volumetric flask containing few ml (60 ml) of methanol. Flask was sonicated for 10 min and volume was made up to the mark with methanol. The above solution was filtered in another 50 ml volumetric flask through Whatman filter paper (No. 41) and volume was made up to the mark with the same solvent. From the above solution aliquot of 1 ml was transferred to a 10 ml volumetric flask and the volume was made up to the mark with the mobile phase to obtain a solution containing 10 µg/ml of CIL (and 20 µg/ml of HCT). The solution was sonicated for 10 min. This solution of CIL and HCT was injected as per the above chromatographic conditions and peak area was recorded. The amounts of both the drugs were calculated by keeping these values to the straight line equation of calibration curve.

RESULTS AND DISCUSSION

Method Validation

When a method has been developed it is important to validate it to confirm that it is suitable for its intended purpose. The validation tells how good the methods are, specifically whether it is good enough for the intended application.

Table 1. Optimization of mobile phase

Mobile phase	Observation
Methanol: Water (90:10)	Broad Peak of CIL and HCT
Acetonitrile:Water (40:60)	Both peak were co-eluted and resolution is poor
Acetonitrile:Water (10:90)	Both the peaks were well resolved but retention time was not satisfactory.
Acetonitrile:Methanol:Water	Separation is poor and more retention time
Methanol :buffer (80:20)	Both the peaks were well resolved but retention time was not satisfactory.
Methanol :buffer (70:30)	Both the peaks were well resolved but retention time was not satisfactory.
Methanol :buffer (50:30)	Both the drugs were well resolved with but poor asymmetric factor.
Methanol :buffer (50:30) and pH adjusted is 3.00	Both the drugs were separated with improve asymmetric factor but resolution is poor.
Methanol :buffer (50:30) and pH adjusted is 4.00	Both the drugs were separated with improve asymmetric factor and good resolution is observed.
Methanol :buffer (50:30) and pH adjusted is 5.00	Both the drugs were well resolved but asymmetric factor is poor.

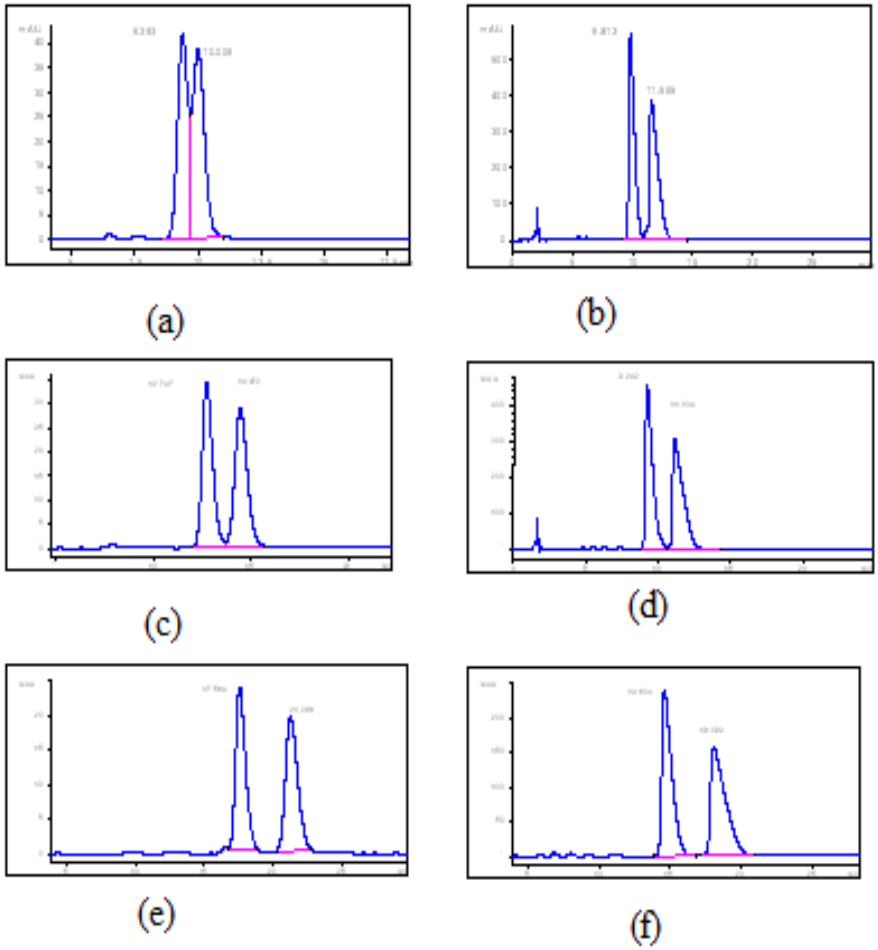

Fig. 3. Optimization of Mobile phase

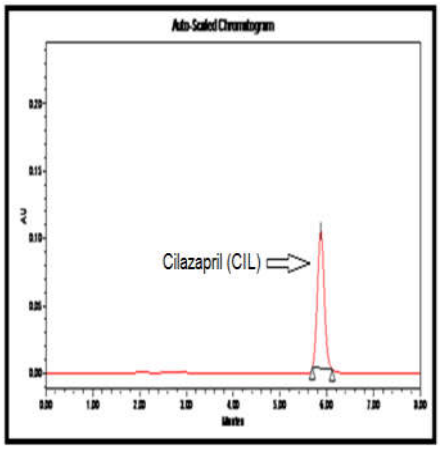

Fig. 4. Chromatogram of standard solution of Cilazapril (CIL) (20µg/ml)

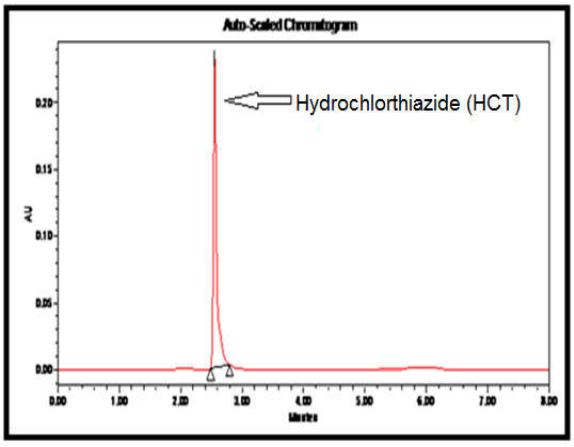

Fig. 5. Chromatogram of standard solution of and Hydrochlorthiazide (HCT) (20µg/ml)

The main criterion is to develop successful stability indicating HPLC method for determination of Cilazapril (CIL) and Hydrochlorthiazide (HCT) in bulk and pharmaceutical dosage form. The method should be able to determine drug and degradation products in single run and should be accurate, reproducible, robust, stability indicating, free of interference from degradation products/impurities and straightforward enough for routine use in quality control laboratory. Optimization of mobile phase was performed based on resolution of the drugs and degradation products, asymmetric factor and number of theoretical plates obtained for Cilazapril (CIL) and Hydrochlorthiazide (HCT).

Regulatory agencies recommend the use of stability-indicating methods (USFDA, 2000) for the analysis of stability samples (Bakshi, M and Singh, S., 2002). Stress studies are required in order to generate the stressed samples, method development, and method validation (Swartz, M., Krull, I., 2005). In order to separate Cilazapril (CIL) and Hydrochlorthiazide (HCT) and degradation products produced under stress conditions, different mobile phases were tried and adjusted to obtain a rapid and simple method with a reasonable run time, suitable retention time, and a sharp peak. Several mobile phases were tried to accomplish good separation of Cilazapril (CIL) and Hydrochlorthiazide (HCT) (Table 1) in bulk and pharmaceutical dosage form.

Several mobile phases of methanol, acetonitrile, water and buffer was tried in different composition shown in fig 3. Good separation was achieved by using the mobile phase containing Methanol: 0.025M Potassium Di-hydrogen Phosphate Buffer pH 4 (60: 40) at 286nm was found to be satisfactory and gave two well resolved peaks for Cilazapril (CIL) and Hydrochlorthiazide (HCT). The retention time for Cilazapril (CIL) and Hydrochlorthiazide (HCT) was found to be 5.85 minute and 2.56 minute, respectively. The retention time of Cilazapril (CIL) and Hydrochlorthiazide (HCT) was confirmed separately by injecting the same concentration and same chromatographic condition shown in fig. 4 and fig. 5 the overlain chromatogram has been shown in fig.12.

The 3D chromatogram of Cilazapril (CIL) and Hydrochlorthiazide (HCT) is shown in fig. 7. The blank chromatogram is shown in fig. 8. Satisfactory separation was obtained with the mobile phase prepared by mixing Methanol: 0.025M Potassium Di-hydrogen Phosphate Buffer pH 4 (60: 40) gave good result with adequate resolution. The retention time of was found to be Cilazapril (CIL) and Hydrochlorthiazide (HCT) was found to be 5.85 ± 0.05 minute and 2.56 ± 0.05 minute. The method was validated for linearity, precision, accuracy, limit of detection, limit of quantification, ruggedness.

Selection of the analytical wavelength

The UV spectra of Cilazapril (CIL) and Hydrochlorthiazide (HCT) standard solution was scanned between 200-400nm which showed that the drug Cilazapril (CIL) and Hydrochlorthiazide (HCT) absorbed appreciably at 256 nm, so the same was selected as the detection wavelength for the study (Fig. 9).

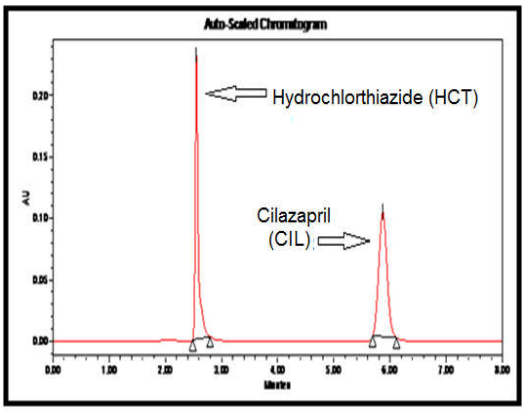

Fig. 6. Chromatogram of standard solution of Cilazapril (CIL) and Hydrochlorthiazide (HCT) in mix standard

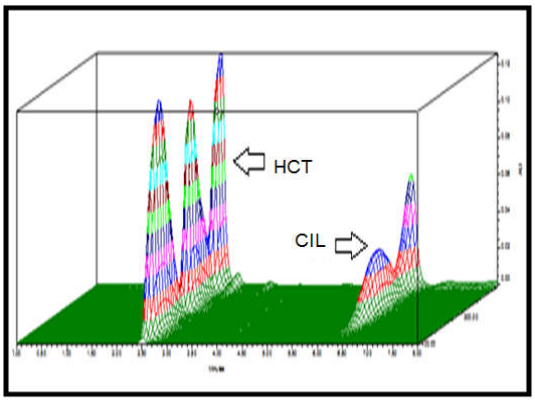

Fig. 7. 3D Chromatogram of Cilazapril (CIL) and Hydrochlorthiazide (HCT) in mix standard

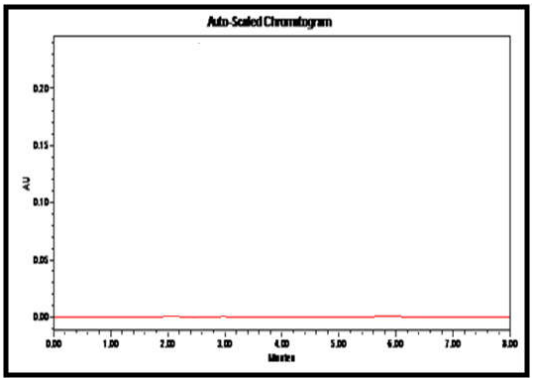

Fig 8. Chromatogram of blank

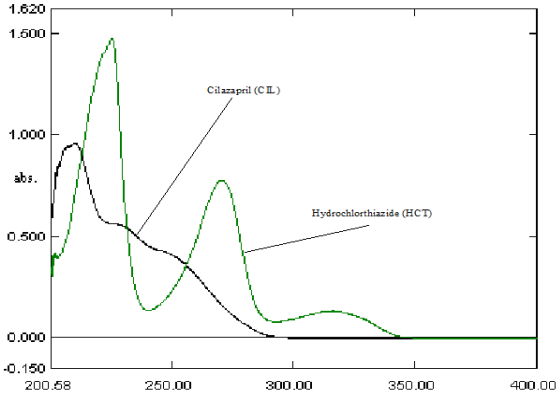

Fig. 9. UV spectrum of Cilazapril (CIL) and Hydrochlorthiazide (HCT)

Validation of the Proposed Method

The optimized method was validated with respect to the following parameters. The validation was performed as per the ICH guidelines. The most common validation parameters will be briefly described below. The method was validated for linearity, precision, accuracy, limit of detection, limit of quantification, ruggedness.

Linearity and Range

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range. The linearity and range of the method was determined by plotting a calibration curve over the concentration range of 0.5-40 µg/ml for Cilazapril (CIL) and 5-60 µg/ml for Hydrochlorthiazide (HCT), respectively. The calibration curve was constructed by plotting peak areas versus concentrations of 0.5 - 40 µg/ml for Cilazapril (CIL) and 5 - 60 µg/ml for Hydrochlorthiazide (HCT), respectively shown in fig.10 and fig.11. The data of regression analysis of the calibration curves are shown in table 2 and table 3. The regression equation was found to be y = 43554x + 58900 and correlation coefficient was found to be 0.996 for Cilazapril (CIL). The regression equation was found to be y= 1865x - 1970 and correlation coefficient was found to be 0.998 for Hydrochlorthiazide (HCT). Each response was the average of three determinations. The overlain chromatogram of Cilazapril (CIL) in the concentration range of 0.5-20µg/ml and Hydrochlorthiazide (HCT) in the concentration range of 5 - 60 µg / ml in mix standard is shown in fig. 13.

Table 2. Result of calibration curve for CIL

Concentration (µg/ml)	Area Mean^n ± S.D.	CV
0.5	54418.50 ± 0931.50	1.59
1	71123.00 ± 0717.83	1.00
5	273246.0 ± 1407.68	0.51
10	531448.0 ± 0957.25	0.18
20	993421.0 ± 1682.76	0.16
40	1761636 ± 0874.53	0.10

Table 3. Result of calibration curve for HCT

Concentration (µg/ml)	Area Mean^n ± S.D.	CV
5	16894.50 ± 0241.41	1.42
10	35540.70 ± 0440.08	1.23
20	138660.0 ± 0825.50	0.59
40	458601.0 ± 4153.54	0.90
50	847557.0 ± 1298.05	0.15
60	1727219 ± 5812.23	0.33

Table 4. Statistical analysis data of calibration curve

Parameters	CIL	HCT
Linear Range (µg/ml)	0.5 – 40	5 – 60
Slope	43554	1865
Intercept	58900	1970
Regression Coefficient (r^2)	0.9960	0.9980
Standard deviation of slope	49.88	273.05
Standard deviation of intercept	497.24	1065.76
Limit of Detection (µg/ml)	0.17	1.3
Limit of Quantitation (µg/ml)	0.5	4.1

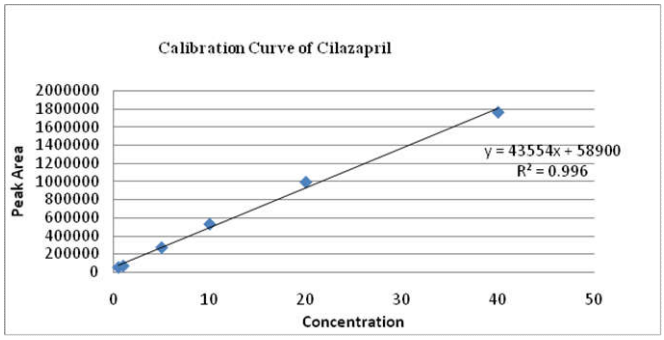

Fig. 10. Calibration curve of CIL in mix standard

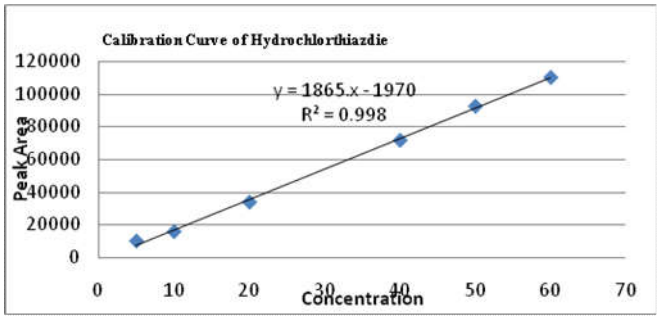

Fig. 11. Calibration curve of HCT in mix standard

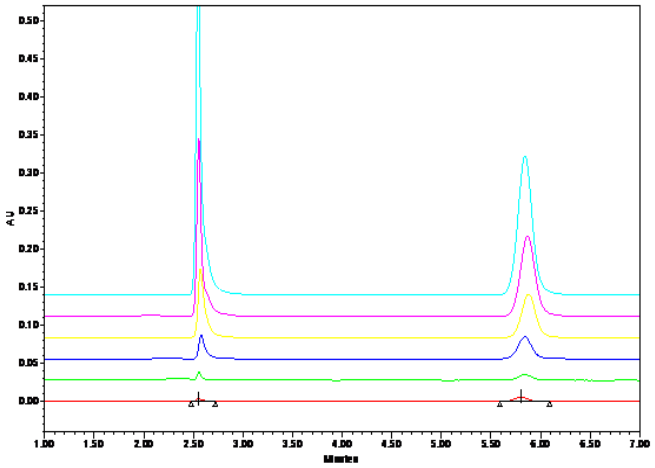

Fig.12. Overlain chromatogram of CIL (0.5-20µg/ml) and HCT (5-60 µg/ml) in mix standard

Precision

The repeatability of developed method was determined by analyzing 10µg/ml CIL solution six times on the same day. The percentage CV was found to be 0.18. The repeatability of developed method was determined by analyzing 20µg/ml HCT solution six times on the same day. The percentage CV was found to be 0.90. The results of repeatability data are shown in Table 5. The results of the intermediate precision (Intraday precision and Interday precision) experiments are shown in Table 6 for CIL. Replicate analyses of three different concentrations 0.5, 5 and 40µg/ml of CIL solutions showed good reproducibility. The CV of intraday and interday studies was found to be 0.12–1.5% and 0.13–1.63% respectively for CIL. The results of the intermediate precision (Intraday precision and Interday precision) experiments are shown in Table 7 for HCT. Replicate analyses of three different concentrations 0.02, 1 and 20µg/ml of HCT solutions showed

good reproducibility. The CV of intraday and interday studies was found to be 0.07–1.18% and 0.45–1.94% respectively for HCT. The developed method was found to be precise and repeatable on the basis of the mean CV values for the repeatability and intermediate precision studies which were < 1.7 for CIL and < 2 % for HCT respectively. Reproducibility of the developed method was determined by two different analysts under the same chromatographic condition and on same liquid chromatography instrument for the CIL and HCT at 10 µg/ml and 20µg/ml concentration level respectively. The effect on the peak was evaluated by applying the F-test. There was no significant difference was found indicating that the developed method was reproducible. The reproducible results are shown in table 8 and table 9 for CIL and HCT respectively.

Table 5. Repeatability study

Concentration	CIL 10 (µg/ml)	HCT 20 (µg/ml)
	531329	46015
	532994	46096
Peak	531929	46025
Area	530142	46002
	531319	45015
	530975	46006
Mean	531448	45860
SD	957.251	415.54
CV	0.18	0.90

CV: Coefficient of variation

Table 6. Intraday and Interday Precision study for CIL

Intraday Precision		
Conc. (µg/ml)	(Area ± S.D) (n=3)	CV
0.5	58582,00 ± 0884.55	1.50
5	273618.7 ± 1524.04	0.55
40	1761701 ± 2083.15	0.12
Interday Precision		
0.5	58681.33 ± 0957.25	1.63
5	273446.0 ± 1959.66	0.71
40	1762966 ± 2391.61	0.13

n=Three determination

Table 7. Intraday and Interday Precision study for HCT

Intraday Precision		
Conc. (µg/ml)	(Area ± S.D) (n=3)	CV
5	9794.5 ± 198.64	1.18
20	33660 ± 745.81	0.53
60	110219 ± 267.64	0.07
Interday Precision		
5	9729.67 ± 332.51	1.94
20	33574.3 ± 1060.96	0.76
60	118018 ± 917.78	0.45

n=Three determination

Table 8. Reproducibility data for CIL (10 ppm)

Analyst 1 Area ± S.D (n = 3)	Analyst 2 Area ± S.D (n = 3)	Result of F-test	Inference
532084 ± 843.253	530812 ± 605.193	0.51	No Significant Difference

* At 95% confidence interval, (F-Tabulated = 9.28)

Table 9. Reproducibility data for HCT (20 ppm)

Analyst 1 Area ± S.D (n = 3)	Analyst 2 Area ± S.D (n = 3)	Result of F-test	Inference
33457 ± 441.396	33745 ± 710.03	2.58	No Significant Difference

* At 95% confidence interval, (F-Tabulated = 9.28)

Accuracy

The recovery of the method was carried out by the standard addition to the preanalysed test sample at three different concentration levels 50%, 100% and 150%. Triplicate determinations were made at each concentration level. The accuracy of the method was determined by calculating recoveries of 5, 10, 15 µg/ml CIL and 2.5, 5, 7.5 µg/ml HCT in the preanalysed concentration of 10 µg/ml CIL and 5 µg/ml HCT by method of standard addition. The recoveries found to be 97.67 % - 98.90% and 97.60%-99.06% for CIL and HCT, respectively. The result of the method is indicating good accuracy for chromatographic method. The accuracy result shown in Table 10.

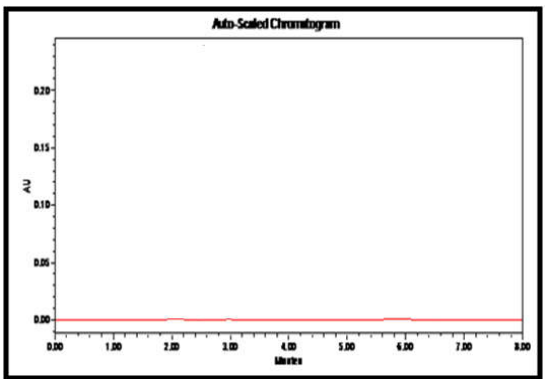

Fig.13. Chromatogram of diluents

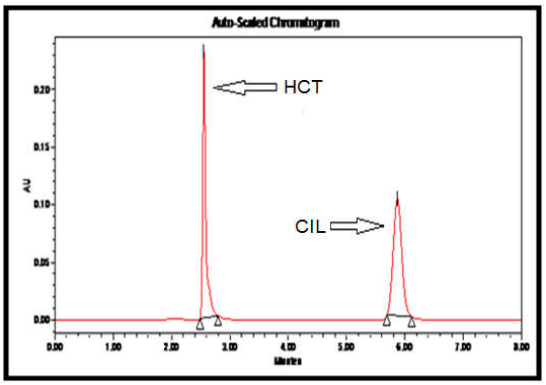

Fig 14. Chromatogram of standard solution of Cilazapril (CIL) and Hydrochlorthiazide (HCT)

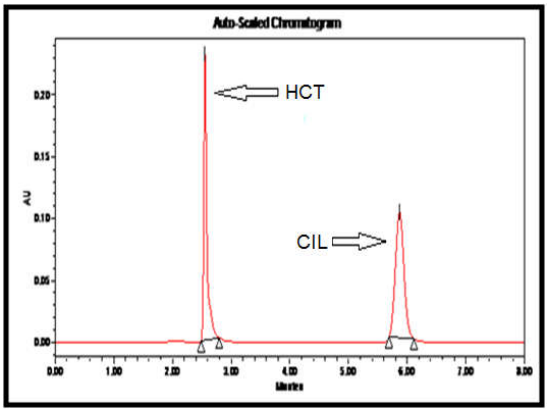

Fig. 15. Chromatogram of Test solution of Cilazapril (CIL) and Hydrochlorthiazide (HCT)

Table 10. Accuracy study

% Level	Amount Added (µg/ml)		Amount Recovered (µg/ml)[a]		% Recovered ± S.D.	
	CIL	HCT	CIL	HCT	% CIL	%HCT
0	10+0	5+0	4.88	9.76	97.60 ± 0.52	97.67 ± 0.78
50	10+5	5+2.5	7.44	14.81	98.16 ± 1.04	98.90 ± 0.36
100	10+10	5+5	9.90	19.83	98.33 ± 0.57	98.16 ± 1.04
150	10+15	5+7.5	12.41	24.90	99.06 ± 0.60	98.20 ± 0.72

a=Average of Three determination

Table 11. System suitability test parameters

PARAMETER	CIL	HCT
Retention time (min)	2.56	5.85
Resolution	6.58	
Asymmetric factor	1.10	1.11
Theoretical Plate count	3630.69	4739.82

Table 12: Robustness study for Cilazapril (CIL) and Hydrochlorthiazide (HCT)

Parameters	Change in condition	Cilazapril (CIL) (10 µg/ml)		Hydrochlorthiazide (HCT) (20 µg/ml)	
		Area [n]± SD	CV	Area [n] ± SD	CV
Flow rate (ml/min)	0.9	485714.4±2654.71	0.55	441936.2±3626.96	0.82
Changed	1.1	485830.4±2750.91	0.57	442484.8±2553.22	0.58
Flow rate (ml/min) Used	1	487150.3±1387.16	0.28	443638.3±2260.44	0.51
pH of mobile phase changed	4.5	485482.3±2784.77	0.57	441050±3110.72	0.71
	3.5	488281.6±3755.75	0.57	442738±2745.80	0.62
pH of mobile phase used	4	485061.60±2416.51	0.49	439502.6±0730.95	0.16
Mobile phase proportion	Methanol:buffer (65:35)	485699.9±3192.43	0.66	442358.2±3240.55	0.73
Changed	Methanol:buffer (55:45)	486526.6±3916.08	0.80	442738.0±4010.99	0.91
	Methanol:buffer (60:40)	484307.5±2788.50	0.57	444749.6±1899.69	0.42

n= Average of Three determination

Table 13: Solution stability study

Time	Area of CIL (10 µg/ml)	Area of HCT (5 µg/ml)	% Amount Drug Found [n]	
			CIL (10 µg/ml)	HCT (20 µg/ml)
0 hr.	490442.68	449630.94	98.97	99.67
4.0 hrs.	489572.44	447942.88	98.76	99.34
8.0 hrs.	486526.60	444566.76	98.00	98.45
24.0 hrs.	484786.12	442878.70	97.65	98.00
48.0 hrs.	483480.76	441190.64	97.34	97.67

n= Average of Three determination

Table 14: Assay results of marketed formulation

Formulation	Drug	Amount Taken(µg/ml)	Amount Found[n] (µg/ml)	%CIL ±SD	%HCT ±SD
Inhibace Plus	CIL	10	9.85	98.46 ± 0.47	98.38 ± 0.68
(Tablet)	HCT	25	24.92		

n= Average of Three determination

Table 15: Force Degradation Study by Proposed Method

Condition	Time	% Recovery of cil	% Recovery of hct	Retention time of degradants
Alkaline hydrolysis (0.1N NaOH)	1 hr	99.38	38.8	3.33 min
Acidic hydrolysis (0.1N HCl)	2 hr	99.76	99.88	--------
Oxidation (3% H_2O_2)	2 hr	99.77	99.72	--------
Dry heat (80 °C)	4 hr	99.75	99.82	--------
Wet Heat (Boiling Water bath)	2 hr	99.35	99.76	-------
Sun light	72 hr	99.66	99.66	--------
UV radiation (254nm)	2hr	99.34	99.29	----
UV radiation (365nm)	2hr	99.25	99.87	----

Limit of detection and limit of quantitation

The detection limits for CIL and HCT were found to be 0.17µg/ml and 1.3µg/ml, respectively, while quantitation limits were found to be 0.5µg/ml and 4.1 µg/ml, respectively. The values of LOD and LOQ of CIL and HCT respectively indicate the sensitivity of proposed method.

System Suitability Tests

The system-suitability tests are integral part of gas and liquid chromatography. They are used to verify that the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. System suitability tests were carried out on freshly prepared standard stock solution of CIL and HCT and parameters obtained are summarized in table 11.

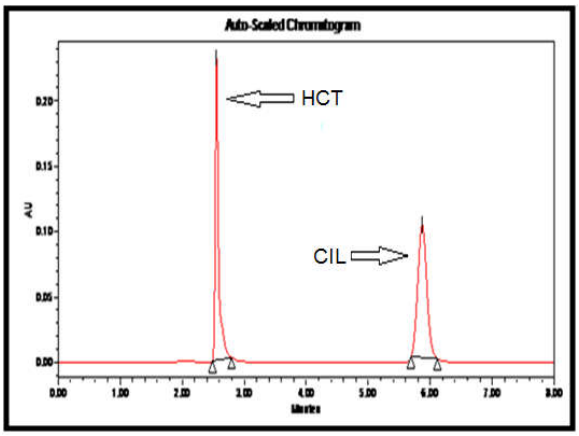

Fig. 16. Chromatogram of marketed formulation of Cilazapril (CIL) and Hydrochlorthiazide (HCT)

Specificity and Selectivity

The specificity study was carried out to check the interference from the excipients used in the formulations by preparing synthetic mixture containing both the drugs and excipients. The chromatogram showed peaks for both the drugs (CIL and HCT) without any interfering peak and the estimation of both the drugs were found to be satisfactory. Test solution is prepared by mixing of CIL and HCT with the tablet powder excipients. Specificity is proven by comparing the chromatogram of diluent, standard solution and test preparation solution to show that there was no any interference of excipients with the peak of Cilazapril (CIL) and Hydrochlorthiazide (HCT), as shown in fig.13, 14 and 15.

Robustness

Robustness of the proposed method was evaluated after deliberate changes in the analytical parameters, such as changing the composition of the mobile phase, flow rate, and pH of the mobile phase over small ranges. The optimize condition of mobile phase is Methanol: 0.025M Potassium Di-hydrogen Phosphate Buffer pH 4 (60: 40) and flow rate is 1ml/min. The small changes in pH are at 3.5 and 4.5, small changes in flow rate is 0.9 and 1.1 ml/min and mobile phase composition is methanol: buffer (65:35), and (55:45) is evaluated. After small changes in this parameter effect on the peak area of Cilazapril (CIL) and Hydrochlorthiazide (HCT) was determined. The method was found to be robust, as small but deliberate changes in method parameters have no detrimental effect on the method performance as shown in table 12.

Solution Stability

The solution stability study revealed that Cilazapril (CIL) and Hydrochlorthiazide (HCT) solutions were stable for 48 h without detectable degradation. The percentage amount of both the drugs was found to be satisfactory (table 13).

Analysis of marketed formulation

The developed RP-HPLC method was successfully applied for the estimation of Cilazapril (CIL) and Hydrochlorthiazide (HCT) in marketed dosage form. Marketed formulations were analyzed using proposed method which gave percentage recovery of more than 97.0 for Cilazapril (CIL) and Hydrochlorthiazide (HCT) (Table 14). No interference from the excipients present in the marketed tablet formulation was observed which is shown in fig.16

Forced degradation study

Forced degradation or stress testing was undertaken to demonstrate specificity when developing stability-indicating methods. The ICH guideline entitled "Stability Testing of New Drug Substances and Products" requires stress testing to be carried out to elucidate the inherent stability characteristics of the active substances (ICH-Q1A (R2), 2003). The stability assay methods are gaining importance for the evaluation of active pharmaceutical substance.The forced degradation studies reveal that both drugs are stable in normal condition. Summary of degradation study of Cilazapril (CIL) and Hydrochlorthiazide (HCT) in mix standard solution has been sown in table 15. For the developed RP-HPLC methods, no interferences from the products of stress-testing studies, diluents, impurities, and excipients were observed, indicating a high degree of specificity of this method for the determination of Cilazapril (CIL) and Hydrochlorthiazide (HCT) in bulk and in pharmaceutical formulations.

Alkali hydrolysis

Forced degradation of Cilazapril (CIL) and Hydrochlorthiazide (HCT) in basic medium was performed in the solution of 0.1 N NaOH heated at 80ºC for 1 hr.

Acid hydrolysis

Forced degradation of Cilazapril (CIL) and Hydrochlorthiazide (HCT) in acid medium was performed in the solution of 0.1 N HCl heated at 80ºC for 2 hr. Acid hydrolysis study showed that CIL and HCT were stable in acidic condition.

Neutral hydrolysis

Forced degradation of Cilazapril (CIL) and Hydrochlorthiazide (HCT) in neutral medium was performed in the solution of water heated at 80ºC for 3 hr. Neutral hydrolysis study showed that Cilazapril (CIL) and Hydrochlorthiazide (HCT) were stable in neutral condition.

Oxidative stress degradation

Oxidative stress degradation of Cilazapril (CIL) and Hydrochlorthiazide (HCT) was performed in the solution of 3% H_2O_2 heated at 80ºC for 2 hr. Oxidative stress degradation study showed that Cilazapril (CIL) and Hydrochlorthiazide (HCT) were stable in the solution of 3% H_2O_2.

Thermal degradation

Dry heat degradation of Cilazapril (CIL) and Hydrochlorthiazide (HCT) was studies in oven at 80^{o}C for 4 hrs. Dry heat degradation study showed that Cilazapril (CIL) and Hydrochlorthiazide (HCT) were stable at 80^{o}C and no additional peak in HPLC chromatogram. Wet heat degradation of Cilazapril (CIL) and Hydrochlorthiazide (HCT) was studies

in boiling water bath for 2 hrs. Wet heat degradation study showed that Cilazapril (CIL) and Hydrochlorthiazide (HCT) were stable at 80^{o}C and no additional peak in HPLC chromatogram.

Photo degradation study

Photo degradation of CIL and HCT was studies in sunlight for 72 hrs. Photo degradation study showed that CIL and HCT were stable in sunlight.

Furthermore, a stress degradation study in direct UV radiation was performed by exposing the solid drugs of CIL and HCT and their mixture to UV radiation at 254 and 365 nm for 2 h at room temperature.

Conclusion

Thus the proposed stability indicating RP-HPLC method for the simultaneous determination of Cilazapril (CIL) and Hydrochlorthiazide (HCT) in tablet dosage form was accurate, precise, linear, reliable, simple, economic and robust. The method has several advantages, including simple mobile phase, low solvent consumption, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The method can be used for routine analysis of marketed products of Cilazapril (CIL) and Hydrochlorthiazide (HCT) in combined tablet formulation.

REFERENCES

Anthony, D. Woolf and Bruce Pfleger, 2003. Burden of major musculoskeletal conditions, Theme Papers, Bulletin of World Health Organization, 81(9), 646-656.

Beckett A. H. 1997. In Practical Pharmaceutical Chemistry Part II; CBS Pubmethers and Distributors, 4th Edn; 275-337.

Bhatt, P. A, Hamrapurkar, P. D and Shigwan, H. S. 2013. Development and Validation of Stability Indicating RP-HPLC method for LNC in Bulk Drug, *Int. J. Res. Pharm. & Biomed Sci,* 4(3), 959-966.

Bhavsar, S. M., Patel, C. N. 2010. Validated RP-HPLC method for simultaneous estimation of LNC and THC in solid dosage form, *J. Chem. Pharm. Res*.., 2(2), 563-572.

Bhusari, V. K and Dhaneshwar, S. R, 2012. Development of validated Stability-indicating HPLC assay method for DKP, *Int. J. Pharm. Pharm Sci*, 4(1), 321-326.

Cowart, J.B., Taylor A.A. 2012. *Curr. Hypertens. Rep*. 14, 324.

Dinc, E., Baleanu, D. 2002. *J. Pharm. Biomed. Anal*. 30, 715.

Gupta, A.K., Arshad, S., Poulter, N. R. 2010. Hypertension 55, 399.

Hillaert, S., De Grauwe, K., Van den Bossche W. 2001. *J. Chromatogr*., A 924, 439.

ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology, Q2 (R1), 1(2005).

Kowalczuk, D., Pietraú, R., Hopka≥a, H. 2004. Chromatographia 60, 245.

Martin, P.D., M.J. Warwick, A.L. Dane, S.J. Hill, P.B. Giles, P.J. Phillips and E. Lenz, 2003. *Clin. Ther*., 25, 2822.

Prejbisz, A., Januszewicz, A. 2012. Fixed dose combination drugs (Polish). Medical Education, Warszawa.

Sylwia, K. Paszun, Beata, J. Stanisz and Agnieszka Gradowska, 2013. *Acta Poloniae Pharmaceutica ñ Drug Research*, Vol. 70 No. 6 pp. 1079ñ1085, ISSN 0001-6837.

Sylwia, K. Paszun, Beata. Stanisz and Wojciech Pawlowski, 2012. *Acta Poloniae pharmaceutica Drug Research*, Vol. 69 No. 2 pp. 193ñ201, ISSN 0001-6837.

The Merck Index, 13th Edn., Merck Research Laboratories, Merck & Co., White House Station, NJ, USA. 3949 (2004).

Welling, S.G. 1986. Biopharm. *Drug Dipos*., 7, 501.

Yoshioka, S., Stella, V.J. 2002. Stability of drugs and dosage forms. *Kluwer Academic Publishers*, New York.

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