



Scholars Research Library

Der Pharmacia Lettre, 2015, 7 (12):114-121
(<http://scholarsresearchlibrary.com/archive.html>)



Stability indicating RP-HPLC method development and validation for the simultaneous estimation of candesartan cilexetil and hydrochlorothiazide in bulk and tablet dosage form

Kavitha Kotthireddy* and B. Rama Devi

Department of Chemistry, College of Engineering, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, Telangana State, India

ABSTRACT

The main aim of the current research work was to develop simple, rapid, robust, accurate and more precise stability indicating RP-HPLC method for simultaneous estimation of candesartan cilexetil and hydrochlorothiazide in active pharmaceutical ingredient and in tablet dosage form. The RP-HPLC analysis was carried out on an enable C18 (Kromasil, ODS, 5 μ , 250 X 4.5 mm) column with a mobile phase of methanol-acetonitrile-disodium hydrogen phosphate (0.01 M; pH adjusted to 2.5 with orthophosphoric acid) in the ratio of 20:30:50 (v/v/v). UV detection was performed at 240 nm. The method was found to be linear over a concentration range of 10-500 μ g/ml and 0.4-25 μ g/ml for candesartan cilexetil and hydrochlorothiazide respectively. The limit of detection was found to be as 2.5 μ g/ml and 100ng/ml for candesartan cilexetil and hydrochlorothiazide respectively. The limit of quantitation was found to be as 10 μ g/ml and 100ng/ml for candesartan cilexetil and hydrochlorothiazide. Recovery was found to be in the range 99-100% and precision less than 1%. Developed method was successfully applied for routine analysis of pure drug and pharmaceutical formulations. Forced degradation studies were performed for candesartan cilexetil and hydrochlorothiazide. The drugs were degraded in acidic, basic and oxidative conditions. The peaks of degraded products were well resolved from the actual drug. The developed method was simple, rapid, accurate, precise and stability indicating for the simultaneous estimation of candesartan cilexetil and hydrochlorothiazide in active pharmaceutical ingredient and in tablet dosage form.

Keywords: Candesartan cilexetil; Hydrochlorothiazide; RP-HPLC, Degradation, Validation

INTRODUCTION

Stability indicating methods have become an important aspect of analytical method validation and a part of US FDA requirements [1]. The stability of a drug product or a drug substance is a critical parameter which may affect purity, potency and safety. Changes in drug stability can risk patient safety by formation of a toxic degradation product(s) or deliver a low dose than expected. Hence it is essential to know the purity profile and behavior of a drug substance under various environmental conditions [2-3]. Candesartan cilexetil, 2-ethoxy-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl)methyl}-1H-1,3-benzodiazole-7-carboxylic acid, is an antihypertensive, used in the treatment of hypertension. The drug acts on the rennin-angiotensin system by two ways to decrease the total peripheral resistance. First it blocks the binding of angiotensin II to AT₁ receptor in vascular smoothmuscle causing vascular dialation. Second it inhibits sympsthetic norepinephrine production further reducing blood pressure [4-5]. Hydrochlorothiazide, 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide is a diuretic used in combination with candesartan cilexetil to treat hypertension [4-5]. New marketed formulation in combination of Candesartan cilexetil 32mg and hydrochlorthiazide 25 mg is commercially available as tablet (Atacand® HCT) for the treatment of edema and hypertension

Very few methods were published for the simultaneous estimation of candesartan cilexetil and hydrochlorothiazide involving HPLC techniques [6-11]. The references for the stability studies of candesartan cilexetil and hydrochlorothiazide in combination were not found. Hence we made an attempt to develop and validate a stability indicating method for the simultaneous estimation. The aim of the present study was to develop and validate a stability indicating method for the simultaneous estimation of candesartan cilexetil and hydrochlorothiazide. The degradation studies were performed and the degradant peaks were well resolved from the drug peak.

MATERIALS AND METHODS

1.1. Chemicals:

Candesartan cilexetil [Fig 1(a)] and hydrochlorothiazide [Fig 1(b)] were obtained as gift samples from Mylan laboratories (hydereabad). Methanol, acetonitrile of HPLC grade were obtained from Sigma Aldrich. Disodium hydrogen phosphate, ortho phosphoric acid were of analytical reagent grade procured from Finar chemicals limited. Atacand HCT tablets were purchased from local market.

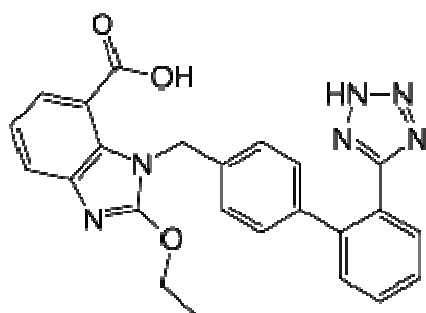


Fig 1(a)

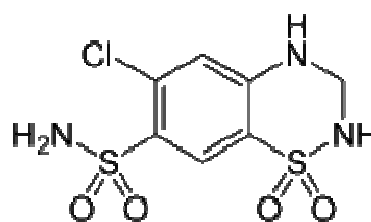


Fig 1(b)

1.2. HPLC system:

The chromatographic apparatus was of shimadzu model and software used was of LC-Solutions. The system was equipped with constant flow pump and UV detector. The chromatographic separation was carried out on enable C18 (Kromasil, ODS, 5 μ , 250 X 4.5 mm) column with a mobile phase of methanol-acetonitrile-disodium hydrogen phosphate (0.01 M; pH adjusted to 2.5 with orthophosphoric acid) in the ratio of 20:30:50 (v/v/v). UV detection was performed at 240 nm. Flow rate was maintained at a rate of 1 ml/min. The pH of the buffer was adjusted with orthophosphoric acid. The mobile phase was freshly prepared, filtered through 0.45 μ m filter and degassed prior to use.

1.3. Preparation of standard stock solutions:

Standard stock solutions were prepared by transferring 100 mg of candesartan cilexetil and 100 mg of hydrochlorothiazide separately into 100 ml volumetric flasks. To the flasks mobile phase was added and sonicated to dissolve the sample and the volume was made up to the mark using mobile phase. From these stock solutions working standards were prepared such that a single sample contains both the drugs over a concentration range of 10-500 μ g/ml of candesartan cilexetil and 0.2-25 μ g/ml of hydrochlorothiazide.

1.4. Preparation of sample solution:

Twenty tablets were weighed and powdered. Tablet powder equivalent to 32 mg of candesartan cilexetil and 25 mg of hydrochlorothiazide was accurately weighed and transferred into 100 ml volumetric flask and dissolved in 20 ml of mobile phase and sonicated for five minutes. The volume was made upto the mark using mobile phase. This is then filtered through 0.45 μ m membrane filter to obtain clear solution. This stock sample solution was diluted quantitatively with mobile phase to obtain the suitable working sample solutions for chromatographic measurements.

1.5. Method validation:

Specificity of the method was investigated by analyzing the blank and the sample solutions. Specificity was established by the lack of interfering peaks at the retention time for candesartan cilexetil and hydrochlorothiazide.

Linearity was tested at eight different concentrations over a range of 10-500 μ g/ml of candesartan cilexetil and 0.2-25 μ g/ml of hydrochlorothiazide. The calibration curves were established and the correlation coefficient values were calculated.

The limit of quantitation and limit of detection values for both the drugs were calculated.

The accuracy and precision of the method were determined by using analytical samples in three replicates over three concentrations of candesartan cilexetil and hydrochlorothiazide for five days.

Robustness of the method is investigated by making deliberate changes in parameters like flow rate (± 0.2 ml), composition of mobile phase (± 2 ml) and wavelength (± 2 nm).

Assay of the method was performed by analyzing the marketed formulation. Twenty tablets were weighed and powdered. Tablet powder equivalent to 500 μg of candesartan cilexetil and 25 μg of hydrochlorothiazide was dissolved in mobile phase and required concentration was prepared. These samples were analysed and percentage assay was calculated.

1.6. Stability studies:

Stress degradation studies are performed for both the drugs in acidic, basic, thermal and oxidative conditions.

Candesartan cilexetil and hydrochlorothiazide standard solutions of 100 $\mu\text{g}/\text{ml}$ are prepared and subjected to acidic, basic and oxidative conditions. Thermal degradation was performed by direct exposure of the bulk drug at 60°C for about seven days.

RESULTS AND DISCUSSION

3.1. Method development:

Chromatographic method development began with the selection of wavelength and optimization of mobile phase composition.

3.1.1. Selection of wavelength:

The samples of candesartan cilexetil and hydrochlorothiazide were prepared in the concentration of 10 $\mu\text{g}/\text{ml}$. These samples were scanned in the range of 200-400nm using UV-visible spectrophotometer. From the overlain spectra [Fig 2] the detection wavelength was selected as 240 nm.

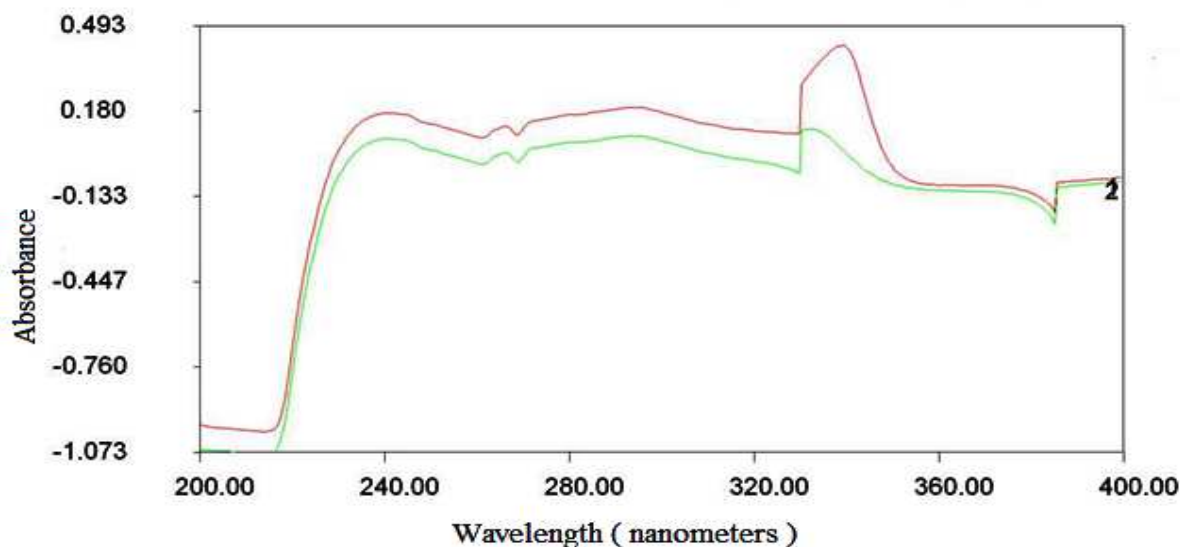


Fig 2: Overlain spectra of candesartan cilexetil and hydrochlorothiazide

3.1.2. Optimization of mobile phase:

The feasibility of several mixtures of solvent such as methanol and acetonitrile using different buffers such as formic acid, potassium dihydrogen ortho phosphate with variable pH range 3-5 was tested for complete resolution of candesartan cilexetil and hydrochlorothiazide. enable C18 (Kromasil, ODS, 5 μ , 250 X 4.5 mm) column with a mobile phase of methanol-acetonitrile-disodium hydrogen phosphate (0.01 M; pH adjusted to 2.5 with orthophosphoric acid) in the ratio of 20:30:50 (v/v/v) provided very good resolution and peak shape for candesartan cilexetil and hydrochlorothiazide (Fig. 3)

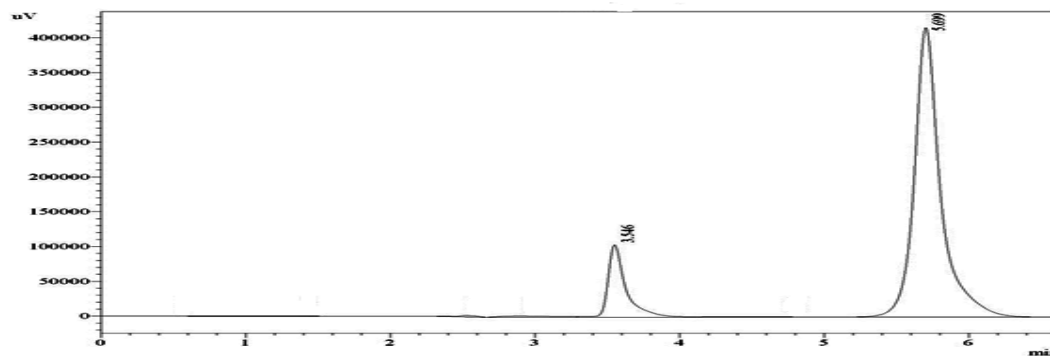


Fig 3: Chromatogram showing simultaneous estimation of candesartan cilexetil (Rt 5.89) and hydrochlorothiazide (Rt 3.54)

3.2. Method validation:

3.2.1. Specificity and Sensitivity:

Chromatograms of sample solution (tablet sample) and blank were analysed and no interferences were found at the retention time of candesartan cilexetil and hydrochlorothiazide. Representative chromatograms of blank and sample are shown in Fig 4. LOD and LOQ values are determined and reported in Table-1.

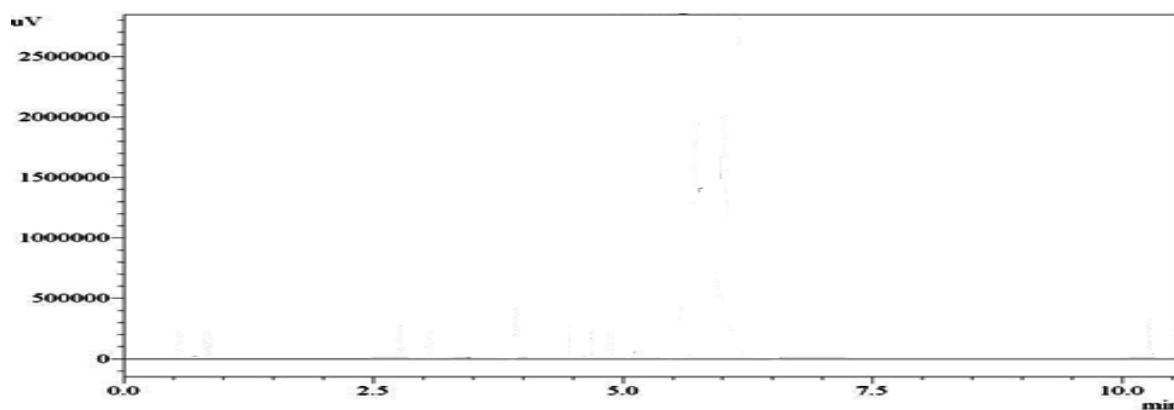


Fig 4(a): Chromatogram of blank sample

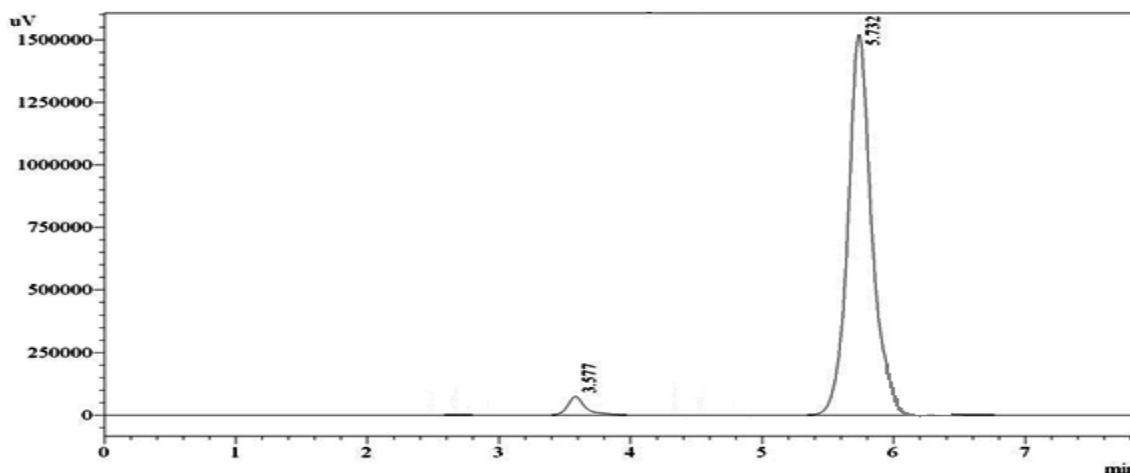


Fig 4(b): Chromatogram of sample solution (marketed formulation)

3.2.2. Linearity:

Calibration standards were prepared in the concentration of 10-500 $\mu\text{g/ml}$ of candesartan cilexetil and 0.2-25 $\mu\text{g/ml}$ of hydrochlorothiazide. These samples were injected into the chromatographic system and the peak areas were recorded. The calibration curves for candesartan cilexetil and hydrochlorothiazide were plotted. The calibration curve for candesartan cilexetil [Fig 5(a)] was linear over the concentration range of 10-500 $\mu\text{g/ml}$ and that for hydrochlorothiazide [Fig 5(b)] was linear over the range of 0.4-25 $\mu\text{g/ml}$. The correlation coefficients for both the drugs were found to be 0.998 and 0.999. The regression equation and linearity details were reported in **Table 1**.

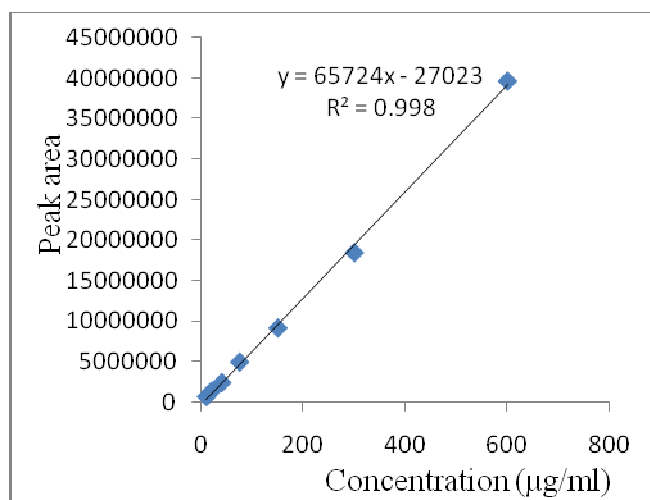


Fig 5(a): Calibration curve of candesartan cilexetil mesylate

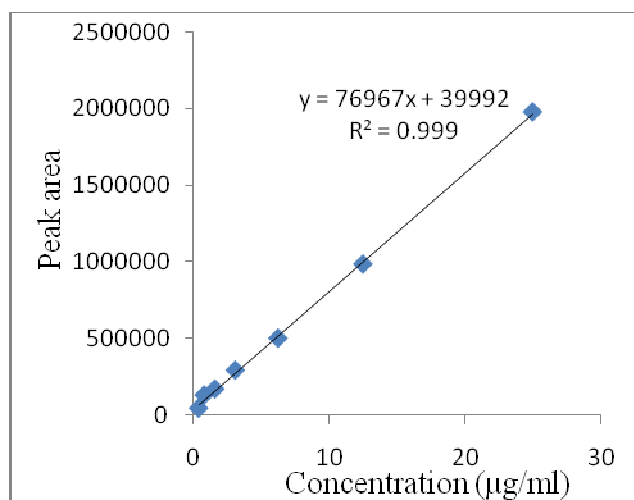


Fig 5(b): Calibration curve of hydrochlorothiazide

Table 1: Analytical performance parameters for linearity

Parameter	Candesartan cilexetil mesylate	Hydrochlorothiazide
Linear range	10 – 500 µg/ml	0.4 – 25 µg/ml
Slope	65724	76702
R ² value	0.998	0.999
LOQ	10 µg/ml	0.4 µg/ml
LOD	3.1 µg/ml	0.1 µg/ml

3.2.3.Precision:

Precision of the method was determined by performing intraday and interday precision. Intraday precision was determined by three replicate analyses of three concentrations (10, 75 and 500 µg/ml of candesartan cilexetil mesylate, 0.4, 3.1, 25 µg/ml of hydrochlorothiazide) within a day. Interday precision was performed for three days by three replicate injections of three samples in a day. Intraday and interday precision values are presented in **Table 2**.

3.2.4.Accuracy:

Accuracy of the method was determined by recovery studies (standard addition method). Recovery was performed at three levels i.e. 50%, 100%, 150% of those expected by spiking a previously analysed sample solution with standard drug solution. The samples were analysed for three repeated times and the peak areas were recorded. Percentage recovery values were presented in **Table 2**.

Table 2: Intraday, inter day precision and accuracy for candesartan cilexetil and hydrochlorothiazide

Sample	Concentration (µg/ml)	Precision (%RSD)		Accuracy (%recovery)
		Intraday	Interday	
Candesartan cilexetil mesylate	10	0.3	0.7	99.7
	75	1.0	0.3	99.7
	500	0.5	0.4	100.05
Hydrochlorothiazide	0.4	0.3	0.6	98.5
	3.1	0.2	0.5	98.6
	25	0.4	0.8	99.8

3.2.5.Robustness:

Robustness of the method is determined by making deliberate changes in the parameters like flow rate, wavelength, mobile phase ratio. The samples are analysed by changing the parameters and the peak areas are recorded. The values of robustness are presented in **Table 3**.

Table 3: Robustness values of candesartan cilexetil and hydrochlorothiazide

Parameter		Candesartan cilexetil mesylate	hydrochlorothiazide
Flow rate (ml/min)	0.9	0.49%	0.7%
	1.1	0.61%	0.8%
Mobile phase (MET:ACN:BUFFER)	20:20:60	1.2%	1.41%
	25:25:50	1.26%	1.46%
Wavelength (nm)	238	0.25%	0.82%
	242	0.3%	0.84%

3.3. Assay:

The developed method was successfully applied for the marketed formulation. The assay was performed and found to be 99.55% for candesartan cilexetil and 99.68% for hydrochlorothiazide.

3.4. Stability studies:

Stability studies of both the drugs were carried out in acidic, basic, oxidative and thermal conditions.

3.4.1. Acid degradation studies:

To the standard solutions 5 ml of 1N HCl is added. These samples are kept at room temperature for seven days and analysed at every 24h. The degradation details are presented in **Table 4**. The degradation peak is well resolved from the drug peaks [Fig 6 (a)].

3.4.2. Base degradation studies:

To the standard solutions 5 ml of 1NaOH is added. These samples are kept at room temperature for seven days and analysed at every 24h. The degradation details are presented in **Table 4**. The degradation peak is well resolved from the drug peaks [Fig 6 (b)].

3.4.3. Oxidative degradation:

To the standard solutions 5 ml of 3% H₂O₂ is added. These samples are kept at room temperature for seven days and analysed at every 24h. The degradation details are presented in **Table 4**. The degradation peaks are well resolved from the drug peaks [Fig 6 (c)].

3.4.4. Thermal degradation:

Accurately weighed sample of about 100 mg of candesartan cilexetil and hydrochlorothiazide were transferred into separate vials. These vials are kept in hot air oven at 60⁰c for about seven days. These samples are analysed for every 24h for seven days. Both the drugs remained stable [Fig 6(d)] in the above conditions for seven days.

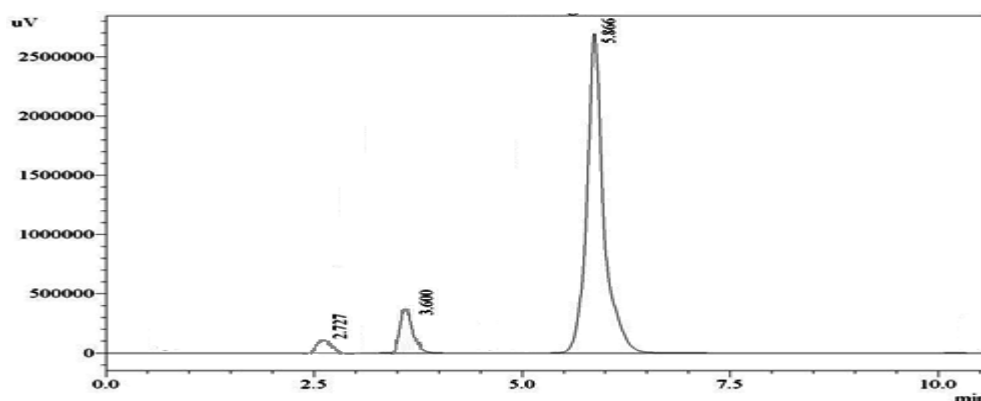


Fig 6(a): Chromatogram showing acid degradation of candesartan cilexetil and hydrochlorothiazide. (an additional peak is shown at Rt 2.7min which is the degradation peak of hydrochlorothiazide)

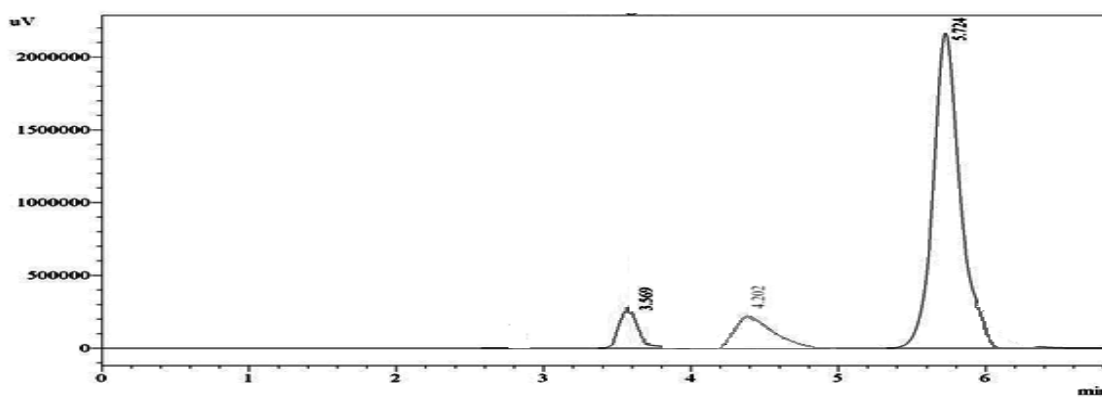


Fig 6(b): Chromatogram showing base degradation of candesartan cilexetil and hydrochlorothiazide (an additional peak is shown at Rt 4.3min which is the degradation peak of candesartan cilexetil mesylate)

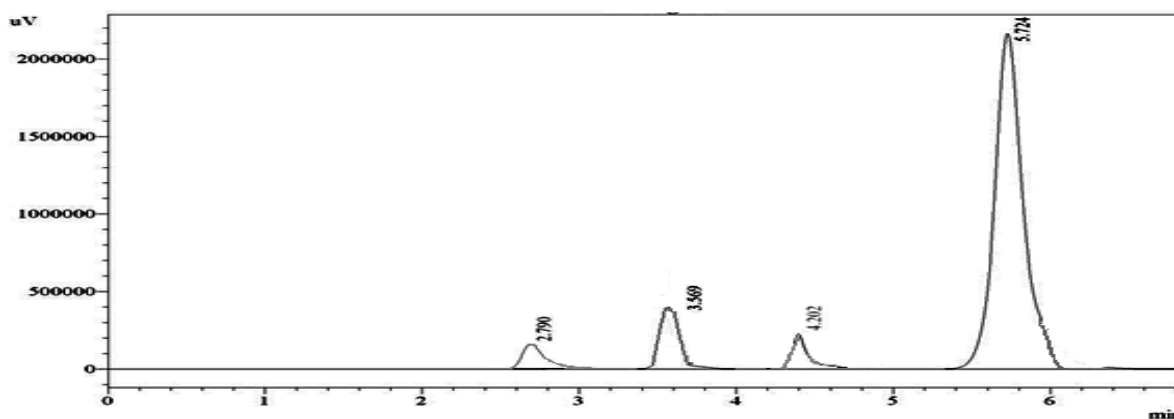


Fig 6(c): Chromatogram showing oxidative degradation of candesartan cilexetil and hydrochlorothiazide (two additional peaks are shown at Rt 4.2min which is the degradation peak of candesartan cilexetil and at Rt 2.7min which is the degradation peak of hydrochlorothiazide)

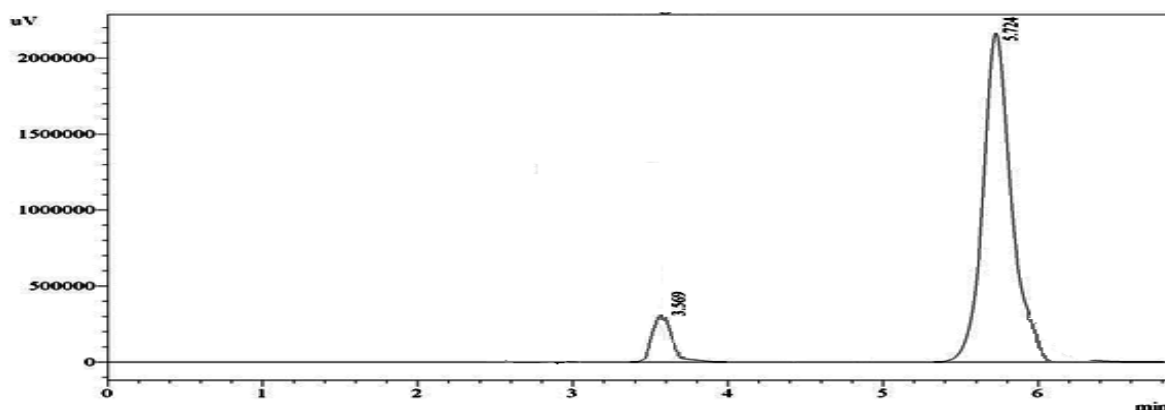


Fig 6(d): Chromatogram showing thermal degradation of candesartan cilexetil and hydrochlorothiazide (both the drugs are stable in thermal conditions)

Table 4: Degradation details of candesartan cilexetil and hydrochlorothiazide

S.No	Degradation Studies	Percentage Degradation	
		Candesartan cilexetil mesylate	Hydrochlorothiazide
1	Acid	Stable	11%
2	Base	13%	Stable
3	Oxidation	11.5%	12%
4	Thermal	Stable	Stable

CONCLUSION

The developed HPLC technique is specific, precise, accurate and stability indicating. The validation results are within acceptable limits. The developed method is successfully applied for the marketed formulation (tablet). Stability studies are performed and the degradation peaks are well resolved from the drug peaks with good peak shape. Hence the developed method can be employed as stability indicating method.

REFERENCES

- [1] MD Rockville, *United States Pharmacopoeia*, 25th ed., United States Pharmacopoeial Convention Inc, **2002**.
- [2] DR Hildegard. *Life science Technical bulletin*, **2011**, 1-4.
- [3] NGWA George. *Drug Delivery Technology*, **2010**, 1-4.
- [4] PJ Blankestijn, H Rupp. *Cardiovascular & Hematological Agents in Medicinal Chemistry*, sixth ed., **2008**.
- [5] WH Frishman, CL Angela, J Nawarskas. *The Current Cardiovascular Drugs*, second ed., **2007**.
- [6] PP Manisha, JW Sailesh, LK Ashish, GY Pramod. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **2012**, 3, 1228-1231
- [7] ABN Nageswara Rao, M Ojeyemi, J Jayathirtha Rao, JVLN Seshagiri Rao. *Der Pharmacia Lettre*, **2011**, 3, 318-325.
- [8] U Harsha, N Bhanubhai, N Chhaganbhai. *Pharm. Methods*, **2011**, 2, 143-147.
- [9] M Kamila, N Mondal, LK Ghosh. *Indian Journal of Chemical Technology* **2008**, 15, 194-196.
- [10] A Fawzy, El Yazbi, HH Hammud, GM Sonji. *International Journal of Applied Chemistry*, **2007**, 1, 1-12
- [11] XN Lia, HR Xua, WL Chena, GY Liub, NN Chua, CYub. *Journal of Chromatography B*, **2007**, 853, 47-53.