OPEN ACCESS
Eurasian Journal of Analytical Chemistry
ISSN: 1306-3057
2017 12(2):127-149
DOI 10.12973/ejac.2017.00159a

New Stability Indicating Method for the Simultaneous Determination of Impurities Present in Candesartan Cilexetil and Hydrochlorothiazide Tablets by Ultra Performance Liquid Chromatography with Photo Diode Array Detector

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Received 2 July 2016 ▪ Revised 9 August 2016 ▪ Accepted 9 August 2016

ABSTRACT
A simple, rapid Reverse phase – Ultra Performance liquid chromatography (RP-UPLC) method was developed and validated for the simultaneous determination of thirteen known potential impurities present in Candesartan cilexetil and Hydrochlorothiazide fixed dose combination drug product. Chromatographic separation attained using 0.1% Perchloric acid in water and acetonitrile as Mobile phase-A and B respectively. The components were efficiently separated in Acquity UPLC HSS T3, 100 mm x 2.1 mm with 1.8 µm particle size column. Flow gradient elution mode with initial flow rate of 0.5 $mL.min^{-1}$ followed by 0.6 $mL.min^{-1}$ was used. The impurities were quantified at a working wavelength of 220 nm. The developed method was validated as per International Conference on Harmonization (ICH) recommendations for specificity, linearity, precision, ruggedness, accuracy, sensitivity (Limit of Detection & Limit of Quantitation) and robustness. The present stability indicating method is having shorter run time which is helpful for fast analysis of samples during quality control testing with reduced solvent consumption in a cost and time effective approach.
Keywords: RP-UPLC, candesartan cilexetil, hydrochlorothiazide, acquity UPLC HSS T3, ICH

INTRODUCTION

Candesartan cilexetil (CAN) is a drug used for treating high blood pressure (hypertension) [1]. It belongs to the class of drugs called angiotensin receptor blockers (ARBs). Angiotensin, formed in the blood by the action of angiotensin converting enzyme (ACE), is a powerful

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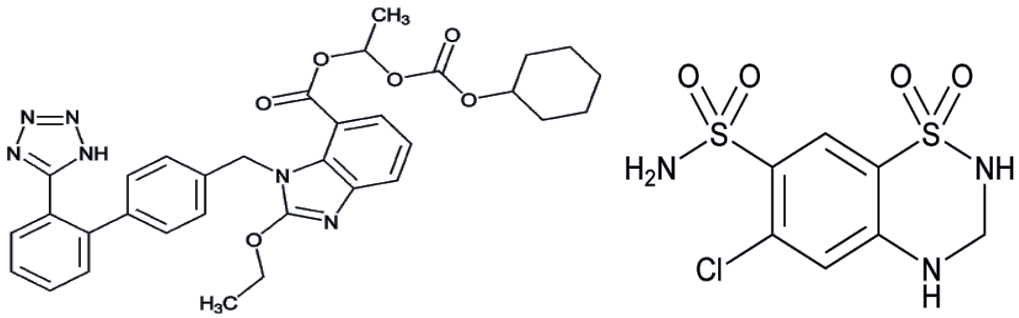


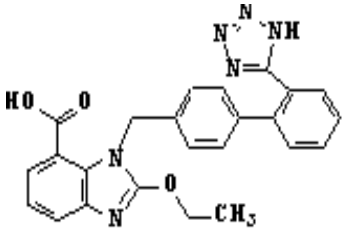
CAN **HCT**

Figure 1. Structures of CAN and HCT

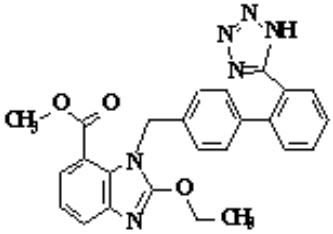
chemical that attaches to angiotensin receptors found in many tissues but primarily on smooth muscle cells surrounding blood vessels. Angiotensin's attachment to the receptors causes the muscle cells to contract and the blood vessels to narrow (vasoconstrict) which leads to an increase in blood pressure [2]. CAN is classified as an angiotensin II receptor type 1 antagonist which is widely used in treatment of diseases like hypertension, heart failure, myocardial infarction and diabetic nephropathy. CAN causes reduction in blood pressure and is used in treatment of hypertension. It is also used in the treatment of congestive heart failure and given as prophylaxis to reduce the severity and duration of migraine. CAN is also available in a combination formulation with a low dose thiazide diuretic, invariably hydrochlorothiazide, to achieve an additive antihypertensive effect [3].

Hydrochlorothiazide (HCT) is a thiazide diuretic that helps prevent the body from absorbing too much salt, which can cause fluid retention. HCT treats fluid retention (edema) in people with congestive heart failure, cirrhosis of the liver, or kidney disorders, or edema caused by taking steroids or estrogen. This medication is also used to treat high blood pressure (hypertension) [4].

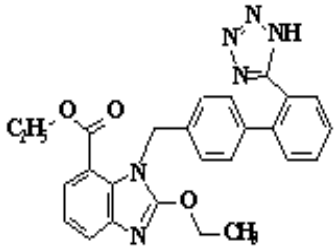
CAN and HCT tablets are a fixed dose combination (FDC) product with the brand name of Atacand HCT tablets in US market. The available three strengths are 16 mg/12.5 mg, 32 mg/12.5 mg and 32 mg/25 mg. The combination of CAN and HCT is an effective treatment for patients with hypertension. The combination of these two agents showed an excellent adverse event profile. This combination, usually at doses of 16 and 12.5 mg respectively, which has been shown to be more effective in lowering blood pressure than either agent alone and to be capable of reducing blood pressure to a similar or greater extent than the combination of HCT with an angiotensin-converting enzyme (ACE) inhibitor or another angiotensin II antagonist, without loss of the placebo-like tolerability that angiotensin II antagonist have when used alone [5-8].



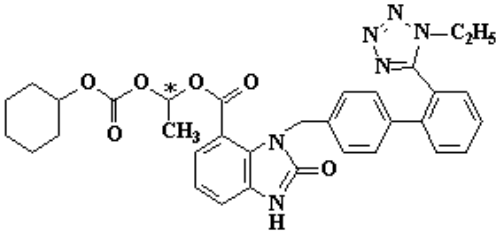
CAN-Imp-1
(Candesartan Acid)



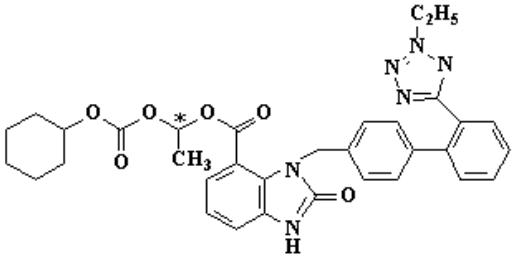
CAN-Imp-2
(Candesartan Methyl ester)



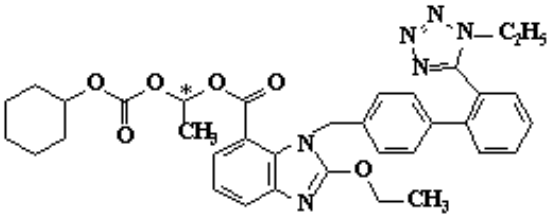
CAN-Imp-3
(Candesartan Ethyl ester)



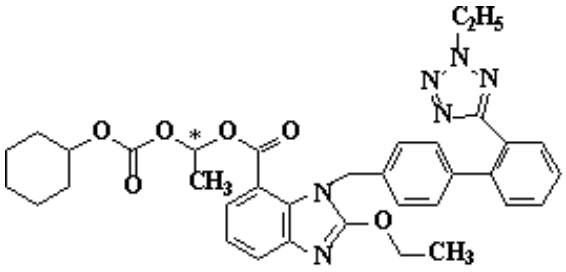
CAN-Imp-5*
(Desethyl N1-Ethyl Candesartan Cilexetil)



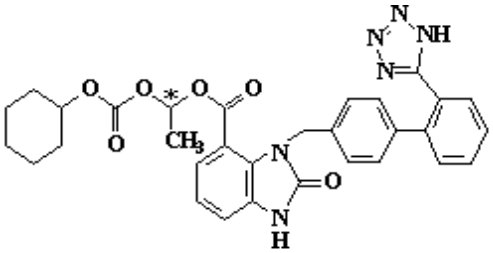
CAN-Imp-6*
(Desethyl N2-Ethyl Candesartan Cilexetil)



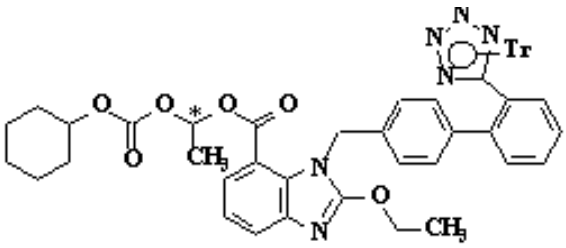
CAN-Imp-7*
(N1-Ethyl Candesartan Cilexetil)



CAN-Imp-8*
(N2-Ethyl Candesartan Cilexetil)



CAN-Imp-4
(Desethyl Candesartan Cilexetil)



CAN-Imp-9
(Trityl Candesartan Cilexetil)

*****Pair of isomers*

Figure 2. Structures for impurities of HCT

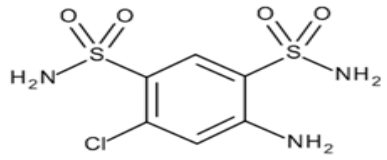
CAN is chemically described as (±)-1-Hydroxyethyl 2-ethoxy-1-[p-(o-1H-tetrazol-5ylphenyl) benzyl]- 7-benzimidazole carboxylate, cyclohexyl carbonate (ester). It is a white to off-white powder. It is practically insoluble in water and sparingly soluble in methanol. Candesartan cilexetil is a racemic mixture containing one chiral center at the cyclohexyloxycarbonyloxy ethyl ester group. The empirical formula is $C_{33}H_{34}N_6O_6$ and Molecular weight is 610.67. The chemical name of HCT is 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4- benzothiadiazine-7-sulfonamide.

It is a white, or practically white, crystalline powder which is slightly soluble in water, but freely soluble in sodium hydroxide solution. The empirical formula of HCT is $C_7H_8ClN_3O_4S_2$ and its molecular weight is 297.74 Chemical structures were illustrated in **Figure 1**.

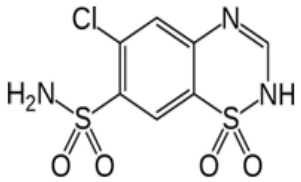
From the study of literature, it was found that, various analytical methods like UV, HPLC, and UPLC methods are available for determination of CAN and its impurities in its individual dosage forms [9-14]. HPLC method for determining HCT in pharmaceutical dosage forms was reported [15]. Different analytical methods using HPLC, HPTLC and UPLC are mentioned for simultaneous determination of CAN and HCT in its combined pharmaceutical dosage forms and in biological samples [16-23] and also HPLC method reported for determination of impurities in CAN and HCT in combined dosage form [24]. However, there are no UPLC methods available for the simultaneous determination of CAN, HCT impurities in their fixed dosage forms. Also, drug product of this FDC is official in United States Pharmacopeia. However, the related substances method given by HPLC with a long run time of 70 minutes for monitoring seven impurities. However, the developed method is by UPLC technique for monitoring 13 impurities with a short run time of 26 minutes. Since UPLC offers better selectivity, sensitivity, fast analysis, eco-friendly due to less solvent consumption, RP-UPLC equipment was selected for the simultaneous determination of CAN and HCT impurities in the combination drug product.

Total thirteen specified impurities are present for both CAN and HCT out of which, nine impurities (including two pairs of isomers) related to CAN and four impurities are due to HCT. Structures were given in **Figure 2** and **Figure 3**.

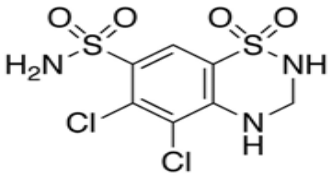
Present scenario in pharmaceutical industry requires fast analytical methods to deliver the products within stringent timelines to meet customer requirements. This can be accomplished by the development of shorter runtime chromatographic methods especially for the estimation of related substances which require longer runtimes to achieve desired separation. Also, these methods should be sensitive enough to detect low level impurities and sufficiently stability indicating for the assessment of shelf life stability for pharmaceutical dosage forms. Hence, it was proposed to develop the related substances method by UPLC to meet the specific targets. Forced degradation studies were conducted to check the stability indicating power of the developed method by producing a degradation profile similar to that what would be observed in a formal stability study under ICH conditions. Forced degradation



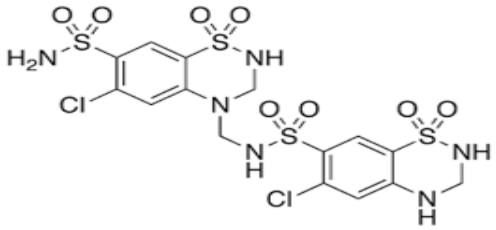
HCT-Imp-1 (Salamide)



HCT-Imp-2 (Chlorothiazide)



HCT-Imp-3 (5-Chloro Hydrochlorothiazide)



HCT-Imp-4 (Hydrochlorothiazide Dimer)

Figure 3. Structures for impurities of HCT

studies are able to determine the intrinsic stability of drug substance in formulation and useful to establish degradation pathways of drug substances and drug products. These studies can solve stability related problems by differentiating degradation products that are related to drug product from those which are generated from placebo matrix present in the formulation. The present method was validated as per the requirements of United States Pharmacopeia (USP) and ICH [25-31].

EXPERIMENTAL

Chemicals and reagents

CAN and HCT drug substances, impurities of CAN and HCT, Atacand HCT (Candesartan cilexetil /Hydrochlorothiazide) tablets were provided by Aurobindo Pharma limited. Acetonitrile of UPLC grade, Perchloric acid and Ortho phosphoric acid of AR grade were purchased from Merck chemicals. Ultrapure water is prepared by using Millipore Milli-Q plus water purification system.

Instrumentation

The experimental work was carried out on Waters-Acquity UPLC system with high pressure binary gradient pump, column oven, Photo diode array detector, Auto injector, Computer with Empower-2 software for data acquisition. The main drug components along with their impurities were separated using Acquity UPLC HSS T3, (100 mm x 2.1 mm id) with 1.8 µm particle size column.

Summary of UPLC method optimization

The method is intended for the simultaneous determination of impurities present in both CAN and HCT in their fixed dose combination product. The critical step in the development

Table 1. Gradient program

Time (min)	Flow rate (mL.min^{-1})	% Acetonitrile	% Buffer
0.01	0.5	7	93
5	0.5	25	75
10	0.5	40	60
15	0.6	55	45
18	0.6	70	30
20	0.6	80	20
23	0.6	90	10
23.5	0.5	7	93
26	0.5	7	93

is to separate both active ingredients along with their respective impurities with satisfactory resolution and at reduced runtime. The developed method should be sensitive to determine the impurities even at lower levels to ensure safety and efficacy of drug product.

From the structures, it was found that, both the drugs are containing functional groups of basic nature. Due to this fact, there will be interaction of these groups with acidic residual silanol groups left in the column. This effect is more prominent while using mobile phase at alkaline pH and leads to peak tailing. To avoid this, it is necessary to acidify the mobile phase to protonate the residual silanols and helps to improve peak symmetry. Also, HCT and its impurities HCT Imp-1 and HCT Imp-2 are highly polar in nature and do not have sufficient retention in the column with buffers such as phosphate and acetate. In order to retain these impurities, mobile phase should contain ion pair reagents such as sodium octane sulfonate. However, there are disadvantages with these long chain ion pair reagents under UPLC system. Also, they require longer equilibration times and not suitable for gradient elution. Considering these facts, trials were taken with 0.1% perchloric acid in water for mobile phase. Perchloric acid is a strong acid and acts as smaller ion pair reagent. It generates perchlorate ion in the mobile phase which will neutralize the positively charged basic polar analytes and helps to retain them. Hence the same was preferred for mobile phase buffer preparation. Acetonitrile was used as organic solvent which is a strong eluent and elutes non polar impurities of CAN.

HCT and Impurities are polar in nature. Impurities of CAN are non-polar in nature and some of the impurities are mid polar in nature. Hence, gradient elution mode was adopted with higher buffer composition for initial gradient steps followed by high percentage of acetonitrile for eluting highly non polar impurity namely CAN Imp-9. Initial flow rate of 0.5 mL.min^{-1} used to retain the polar impurities of HCT which gradually increased to 0.6 mL.min^{-1} to elute late eluting non polar peaks of CAN. After taking different trials, the gradient program (**Table 1**) which can separate all the impurities with satisfactory resolution was finalized. To have symmetric peak shapes and to reduce the column back pressure, column oven temperature is fixed at 45°C. With these chromatographic parameters, resolution between critical pair of impurities and main drug components is found to be at satisfactory level of more than 1.5. Hence the same were adopted in the finalized methodology.

Table 2. Elution order

Name of the component	Retention time	Relative Retention time	USP Resolution
HCT- Imp-1	1.77	0.10	--
HCT- Imp-2	2.03	0.12	1.56
HCT	2.32	0.13	1.79
HCT- Imp-3	3.89	0.22	10.46
HCT- Imp-4	5.60	0.32	11.83
CAN- Imp-1	9.11	0.52	23.94
CAN- Imp-2	11.90	0.69	18.36
CAN- Imp-3	12.92	0.74	6.23
CAN- Imp-4	15.16	0.87	13.30
CAN- Imp-5	16.69	0.96	9.67
CAN	17.37	1.00	4.55
CAN- Imp-6	17.94	1.03	4.13
CAN- Imp-7	19.00	1.09	7.77
CAN- Imp-8	20.17	1.16	8.33
CAN- Imp-9	23.28	1.34	21.59

Column selection also plays very important role in the separation of required components ranges from polar, mid polar to nonpolar. Among different columns tried, Acquity HSS T3 (100 mm x 2.1 mm), 1.8 µm particle size column shows optimum separation between all the desired peaks. HSS T3 column contains tri functional C18 alkyl phase bonded which provides more retention of polar compounds and compatible to higher compositions of aqueous mobile phase. This T3 column is more effective than conventional end capped columns, and has shown better retention of polar impurities. Hence the same column was finalized for the entire study.

The placebo matrix in the drug product may interact with the impurities which lead to poor recovery of impurities. Considering this issue, diluent pH was kept at acidic side. Since drug components are soluble in organic solvents, a degassed mixture 0.1% ortho phosphoric acid in water and acetonitrile in the ratio of 50:50 v/v is fixed as diluent and found satisfactory solubility for impurities of CAN and HCT.

For better responses of impurities, concentrations of HCT and CAN were fixed at 250 µg/mL and 320 µg/mL respectively. Based on ICH limit for drug products, impurity solutions were prepared at a level of 0.4% for and 0.5% for HCT and CAN impurities respectively and injected along with drug components to check the retention times and relative retention times. (**Table 2**).

Spectral data obtained from Photo Diode Array detector shows that, majority of impurities of HCT and CAN exhibit wavelength maxima at about 220 nm (**Figure 4** and **Figure 5**). Hence, the same wavelength of 220 nm has been chosen for quantification of impurities. For sufficient area counts, 4 µL injection volume has been chosen and found precise area counts for impurities as well as main drugs.

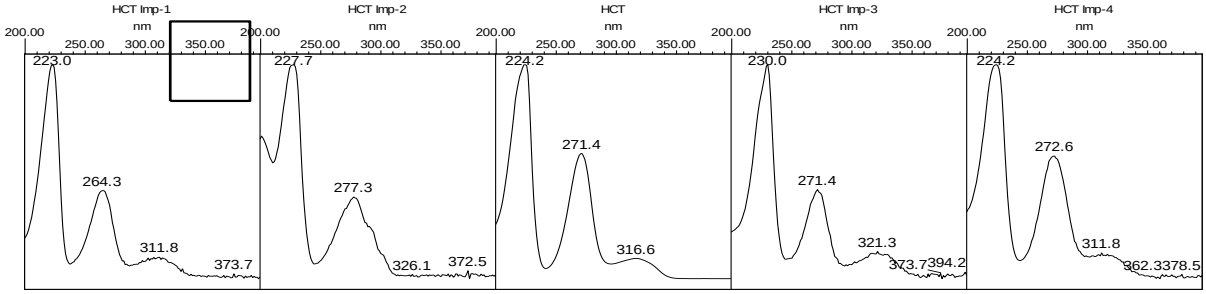


Figure 4. Spectra for HCT impurities

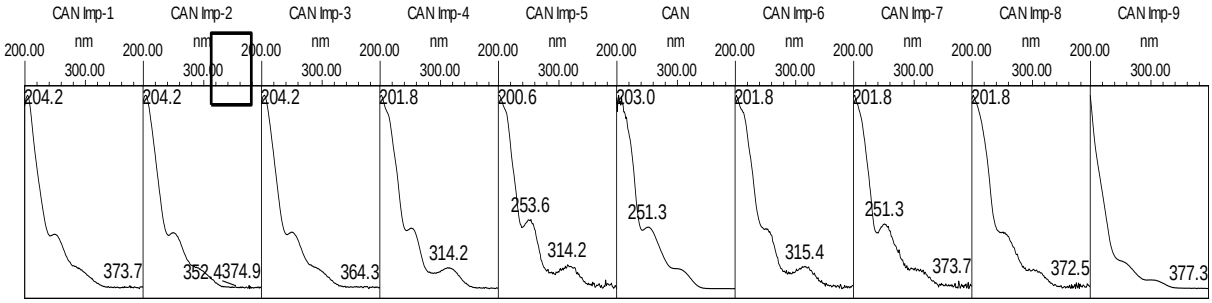


Figure 5. Spectra for CAN impurities

Preparation of standard solution

Initial Standard stock solution of HCT and CAN (0.4 mg/mL of HCT and 0.5 mg/mL of CAN) was prepared by dissolving in diluent. This stock solution was further diluted to obtain a concentration of 1.28 µg/mL for HCT and 1.6 µg/mL for CAN. All impurities were prepared by initially dissolving in an appropriate amount of acetonitrile, followed by using diluent at desired concentration levels for validation purpose.

Preparation of sample solution

Weighed and crushed not less than 10 tablets. Transferred an accurately weighed portion of sample powder, equivalent to about 32 mg of Candesartan cilexetil into a 100 mL clean, dry volumetric flask, added about 70 mL of diluent and sonicated for about 20 minutes, at room temperature (25°C), with intermediate shaking. Diluted to volume with diluent and mixed. Filtered the sample solution through a 0.22 µ filter (Millipore PVDF or Nylon mdi).

System suitability criteria

The below system suitability criteria was adopted from standard solution.

1. The column efficiency as determined from CAN peak is not less than 25000 plate count
2. The Tailing factor for CAN and HCT peaks is not more than 2.0.
3. RSD for peak areas of six injections of the standard solution is not more than 5.0%.

Table 3. Chromatographic system suitability data

Name of the component	Theoretical plates	Tailing factor	% RSD for replicate injections
HCT	--	1.09	0.8
CAN	150000	1.08	0.6

System suitability results obtained in the finalized method are tabulated in (**Table 3**).

Reporting of unknown impurities for quantification purpose

a) Specified impurities of CAN and HCT will be reported using respective diluted standard solutions.
b) Unspecified impurities of CAN and HCT will be identified from the degradation data of individual drug components of CAN and HCT with placebo reported accordingly.
c) Other unspecified impurity for which is not matching with the above data shall be identified by spectra using Photo diode array detector and reported accordingly.
d) Any other unspecified impurities which are unaccountable shall be reported using diluted standard of lower label claim component (i.e., HCT).

Analytical method validation

Atacand HCT (Candesartan cilexetil /Hydrochlorothiazide) tablets 32 mg/25 mg has been considered for the method validation of developed method. The validation parameters such as Specificity, Forced degradation, Precision, Ruggedness, Sensitivity (Limit of detection and Limit of Quantification), Linearity, Range, Accuracy, solution stability and Robustness were performed as per ICH general recommendation.

Specificity and Stress studies

The specificity of the related substances method was evaluated by injecting diluent, placebo used in sample matrix, standard and sample with thirteen potential impurities in presence of Atacand HCT tablets at a test concentration of 320 µg/mL for CAN and 250 µg/mL for HCT and its corresponding degradation products (**Figure 6a** to **6f**). The stress conditions used for degradation study are Acid hydrolysis (1 N HCl / 5 mL/ 25°C / 30 minutes), Base hydrolysis (1 N NaOH / 5 mL / 25°C / Immediately), Oxidation (30% H_2O_2 /5 mL/ 85°C / 60 minutes), Thermal (105°C /48 hours), Humidity (95%RH / 48 Hours) and Photolytic (white fluorescent 1.2 million lux hours UV 200 watt hr/m^2 for 7 days).

Precision and Intermediate precision

The precision of the method was checked by injecting six individual preparations of CAN/HCT tablets spiked with 0.4% level for HCT- Imp-1,2,3 and 4; 0.5% for CAN-Imp-1, 2, 3, 4, 5&6 (two isomers), 7&8 (two isomers) and 9 against sample test concentration of 250 µg/mL and 320 µg/mL for HCT and CAN respectively. The percentage RSD for % w/w of each impurity is calculated. The intermediate precision (Ruggedness) of the method was

evaluated by different analyst using different column and different UPLC instrument on different day.

Sensitivity

For the establishment of Limit of detection (LOD) and Limit of Quantitation (LOQ) levels, a series of test solutions were prepared from 1 to 150% with respective impurity specification level by diluting the impurity stock solution to the required concentration. Linearity curves were drawn by plotting concentration versus area of the individual impurity. From these plots, LOD and LOQ were predicted from the formulae $3.3\sigma/S$ and $10\sigma/S$ respectively where σ is the standard deviation of the response and S is the slope of the linearity curve. Precision was performed at predicted LOD and LOQ values and finalized the levels.

Linearity and Range

Linearity curves were plotted from the finalized LOQ level to 150% of the impurity specification level. The correlation coefficient, slope and Y-intercept of the Linearity curve are calculated for each impurity.

Accuracy

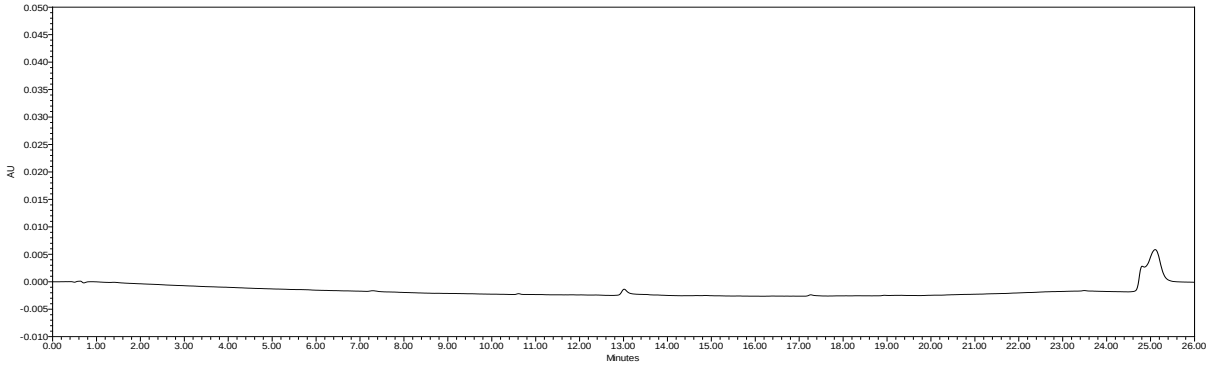
A known amount of the impurity stock solutions were spiked to the samples at LOQ concentration, 50%, 100% and 150% of the analyte concentration. The % w/w of recoveries for all the impurities was calculated. Each concentration level is prepared for triplicate preparation.

Solution Stability

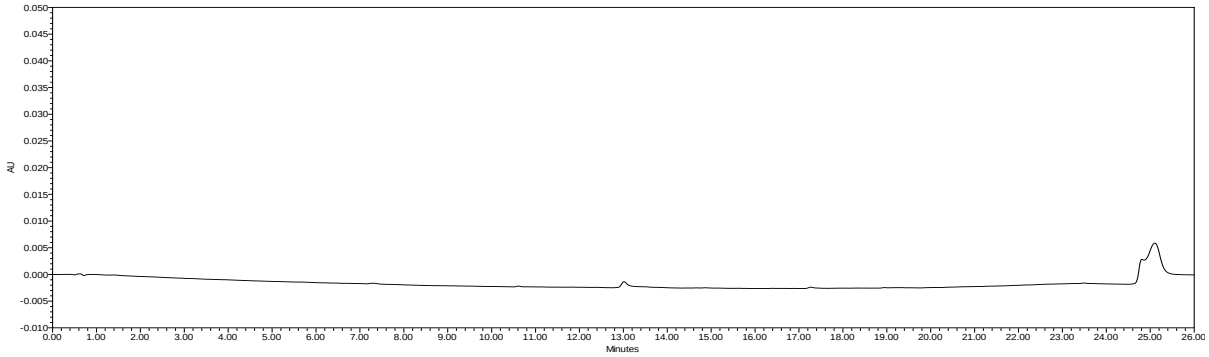
In order to demonstrate the stability of both reference and sample solutions, these solutions were injected immediately after preparation and at periodical intervals by maintaining at room temperature or cooler temperature.

Robustness

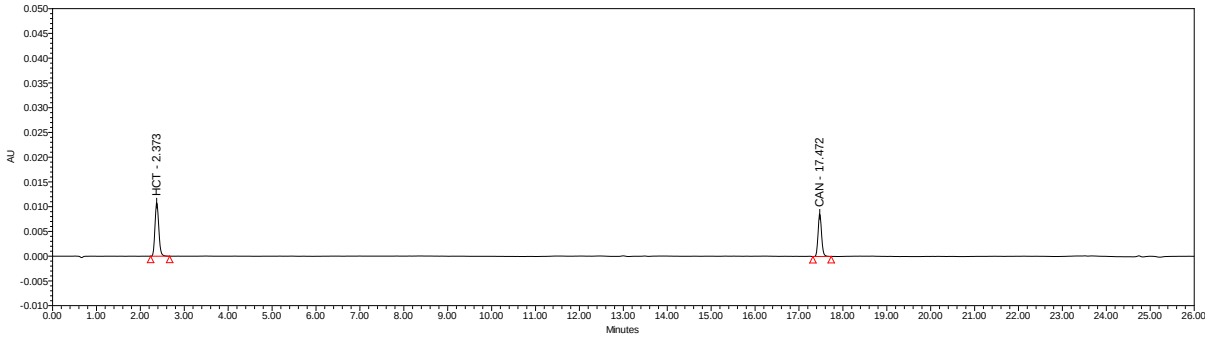
To determine the robustness of the developed method, experimental conditions are deliberately changed and the impact of the variation on each impurity was evaluated. The flow rate of the mobile phase is 0.50 and 0.60 mL/min. To study the effect of flow rate ± 0.05 mL/min unit was changed i.e., 0.45 & 0.55 mL/min and 0.55 & 0.65 mL/min. The effect of column temperature (actual 45°C) is studied at 40°C and 50°C. For gradient programme variation, the composition of mobile phase-B was changed by ± 2% absolute. For wavelength variation, ± 5 nm was changed from the working wavelength i.e., 220 nm.



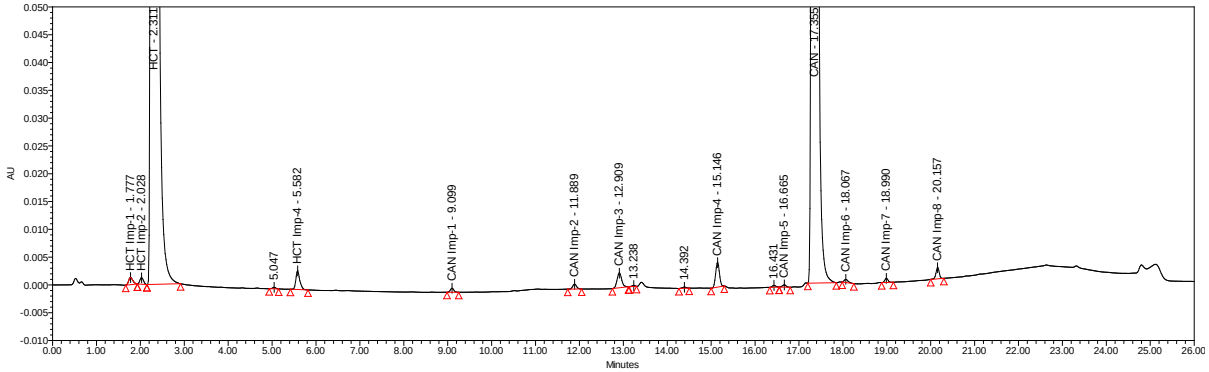
(a) Diluent



(b) Placebo

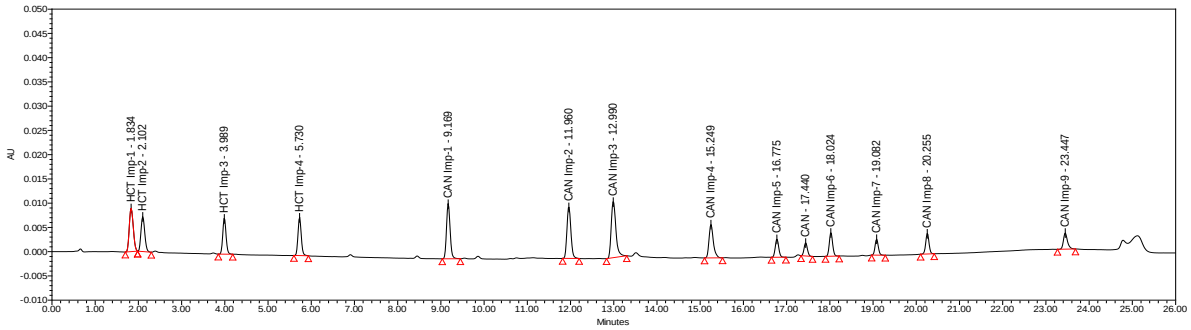


(c) Standard

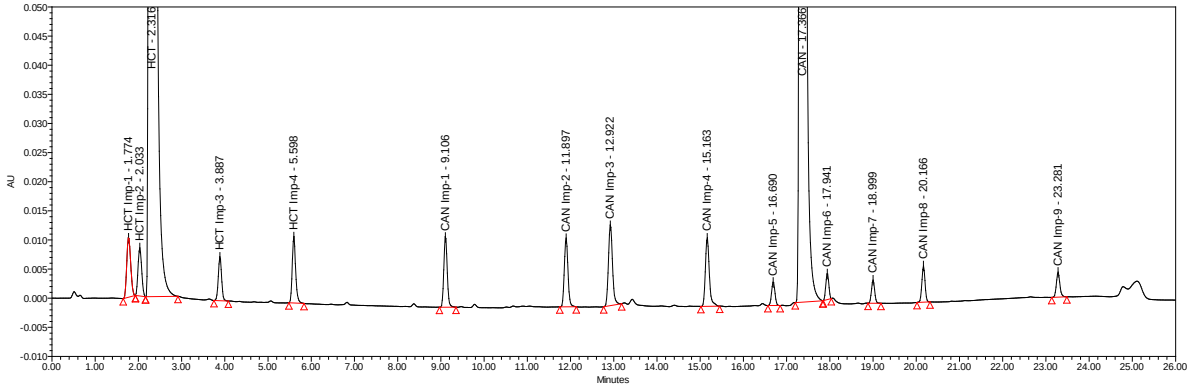


(d) Control sample

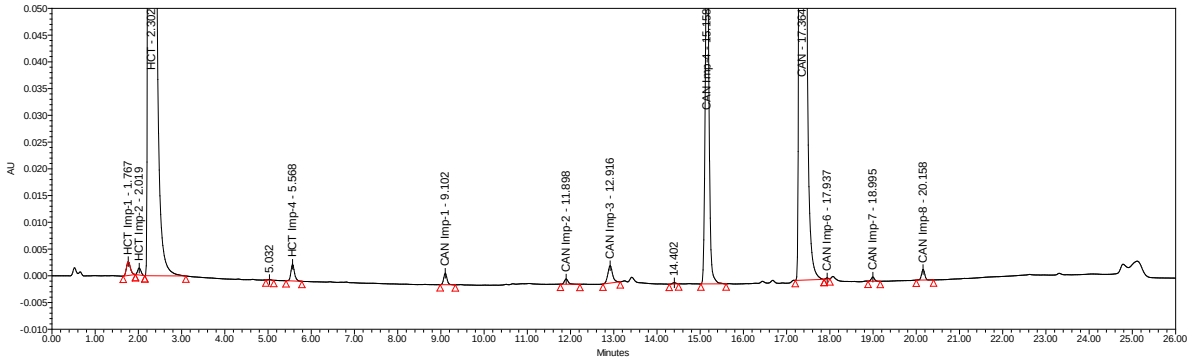
Figure 6. Typical chromatograms of diluent, placebo, standard solution, control sample



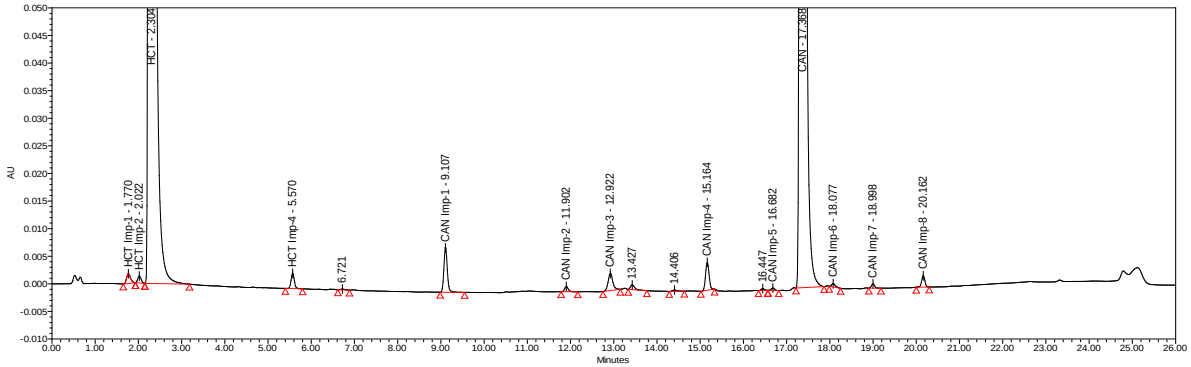
(e) Impurity mixture



(f) Sample spiked with impurities

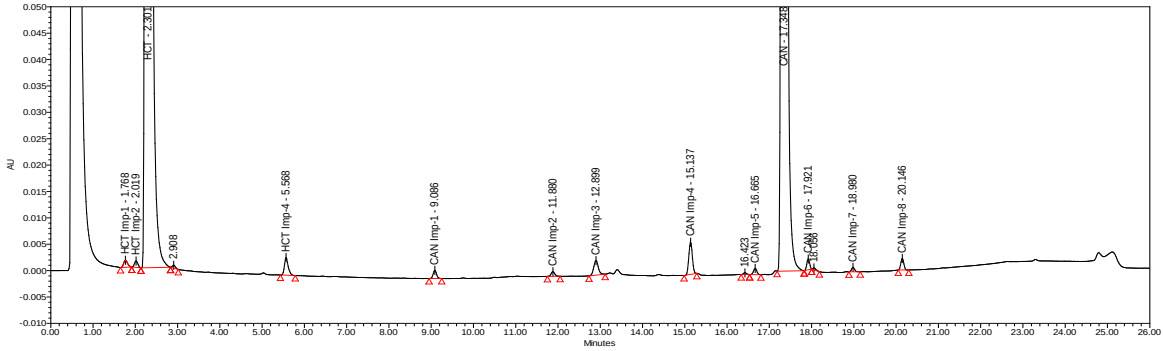


(g) Acid stress sample

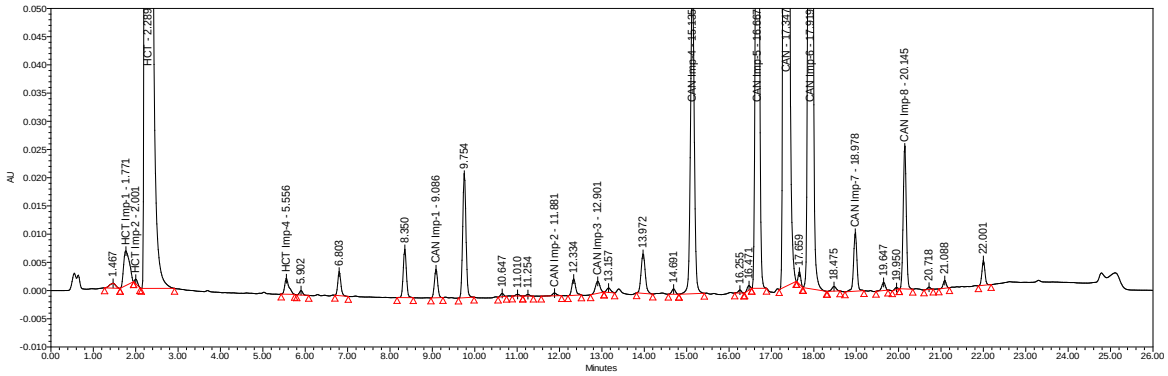


(h) Alkaline stress sample

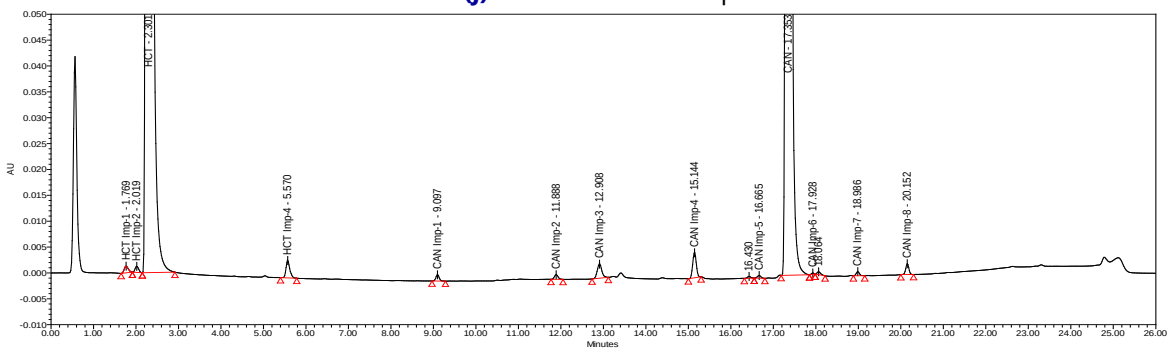
Figure 6. (continued) Typical chromatograms of impurity mixture, spiked, stress sample



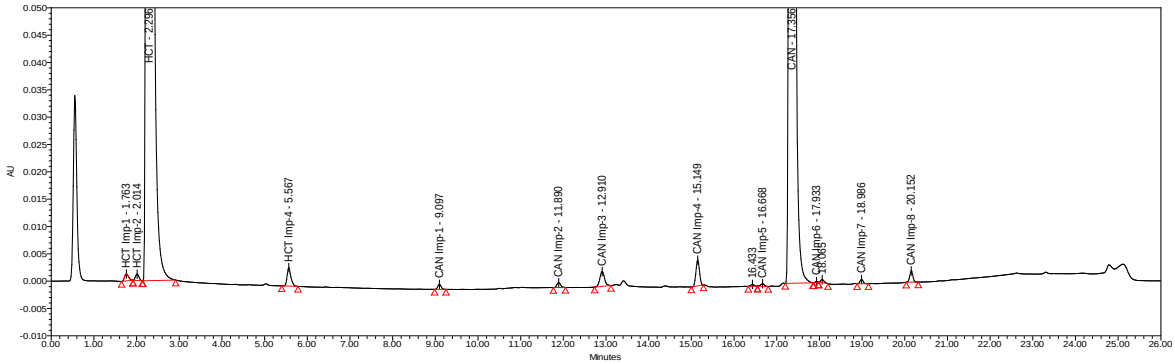
(i) Oxidation stress sample



(j) Thermal stress sample

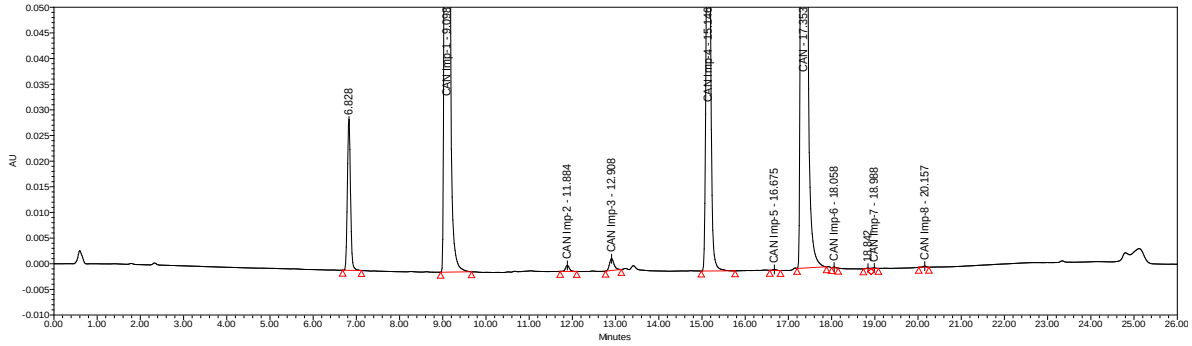


(k) Humidity stress sample

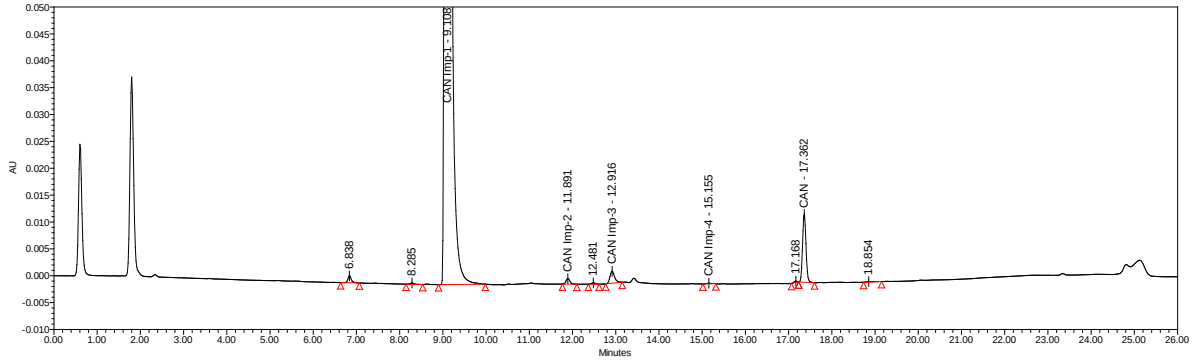


(l) Photolytic stress sample

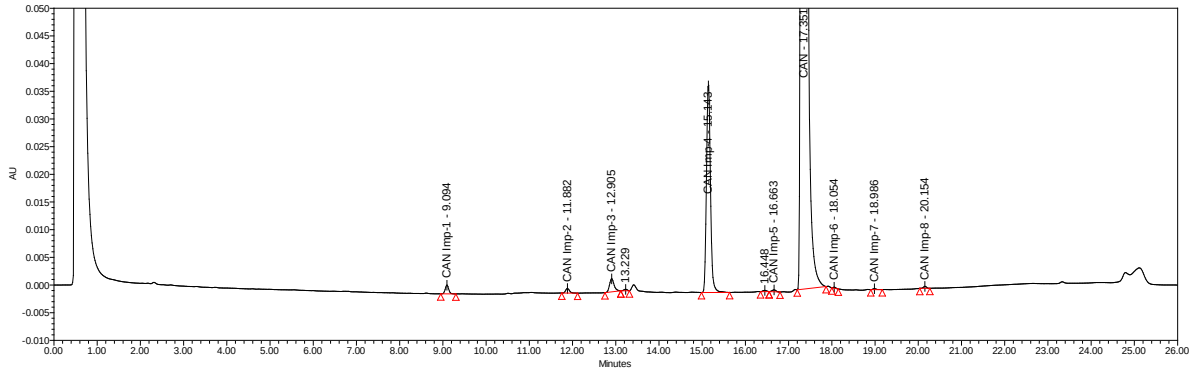
Figure 6. (continued)



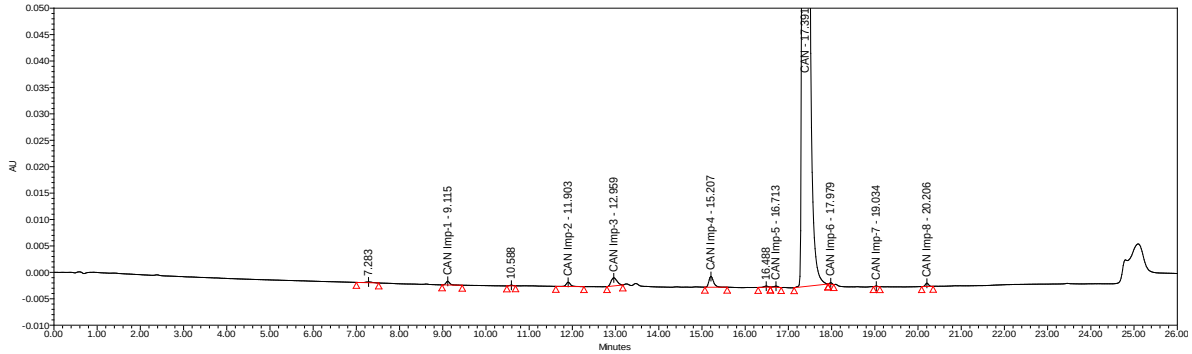
(m) Placebo with CAN acid stress



(n) Placebo with CAN alkaline stress

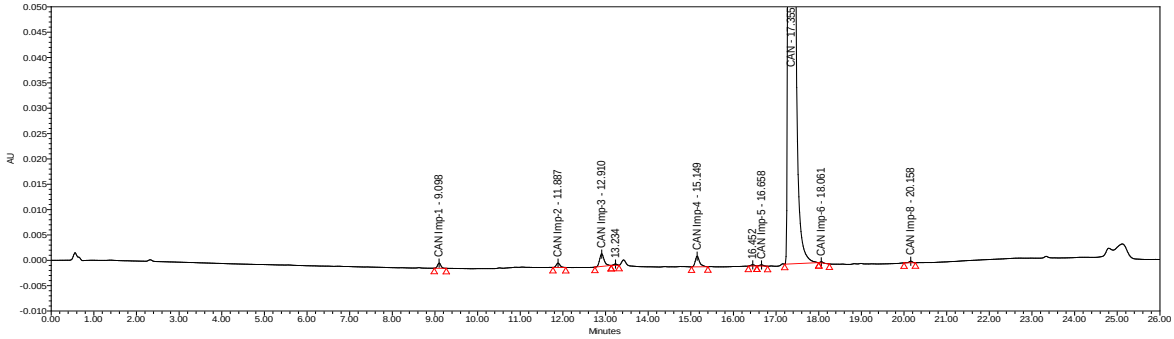


(o) Placebo with CAN oxidation stress

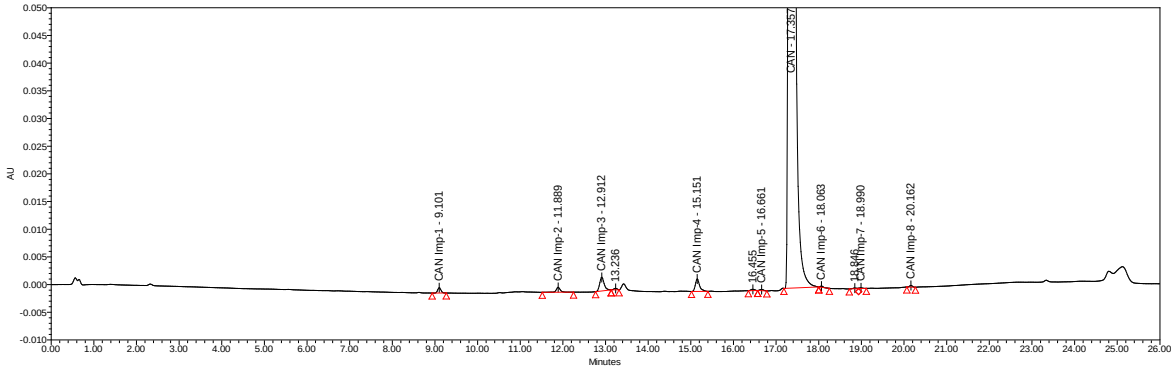


(p) Placebo with CAN thermal stress

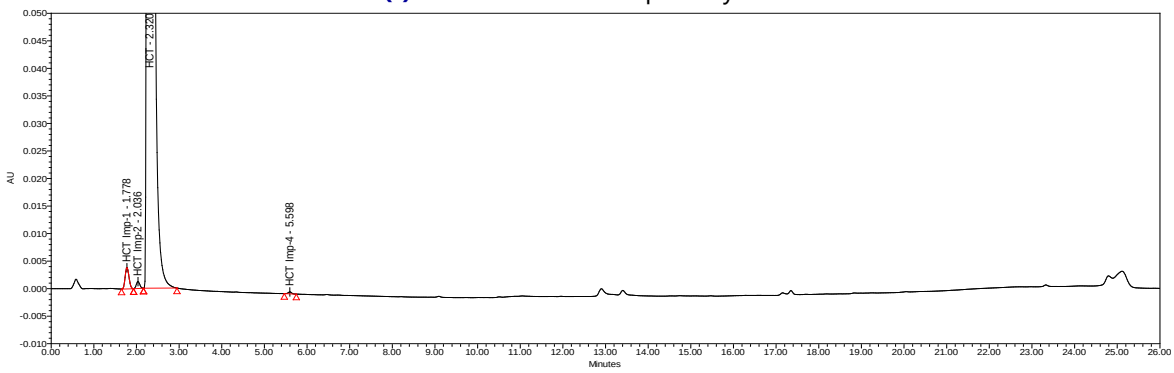
Figure 6. (continued)



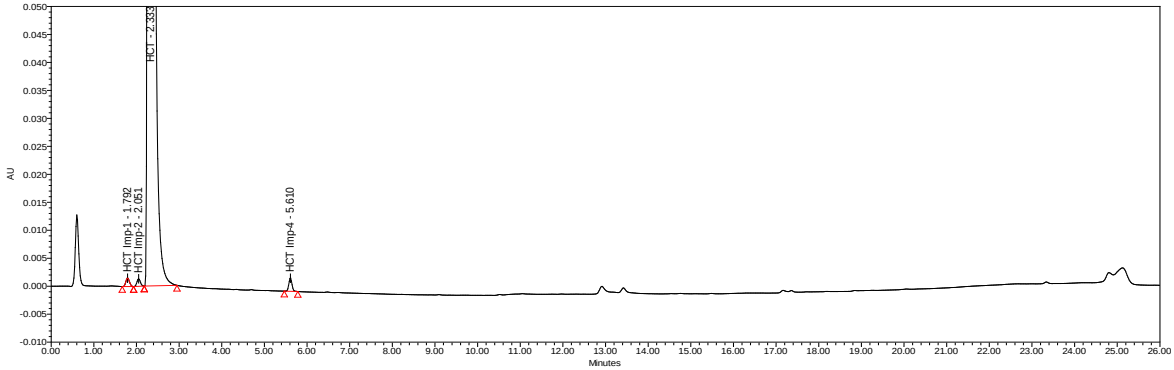
(q) Placebo with CAN humidity stress



(r) Placebo with CAN photolytic stress

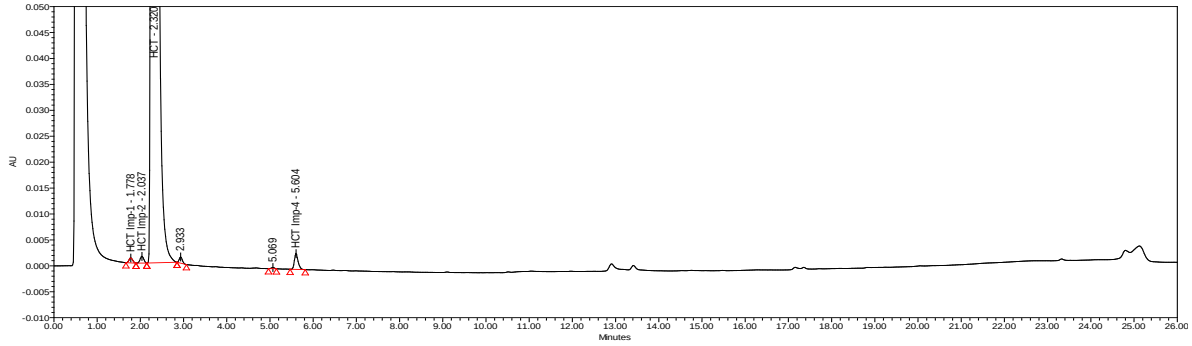


(s) Placebo with HCT acid stress

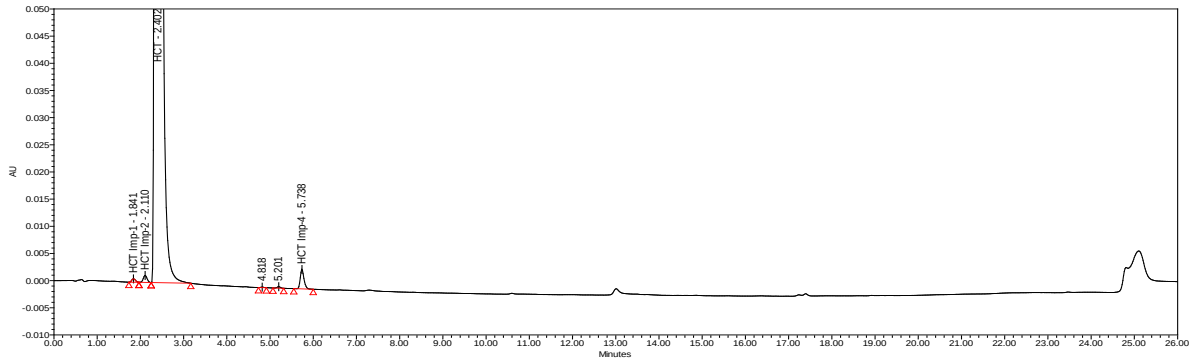


(t) Placebo with HCT alkaline stress

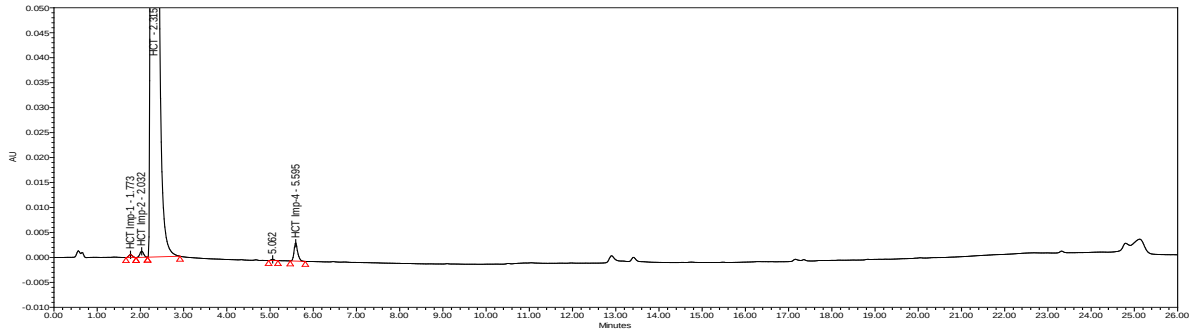
Figure 6. (continued)



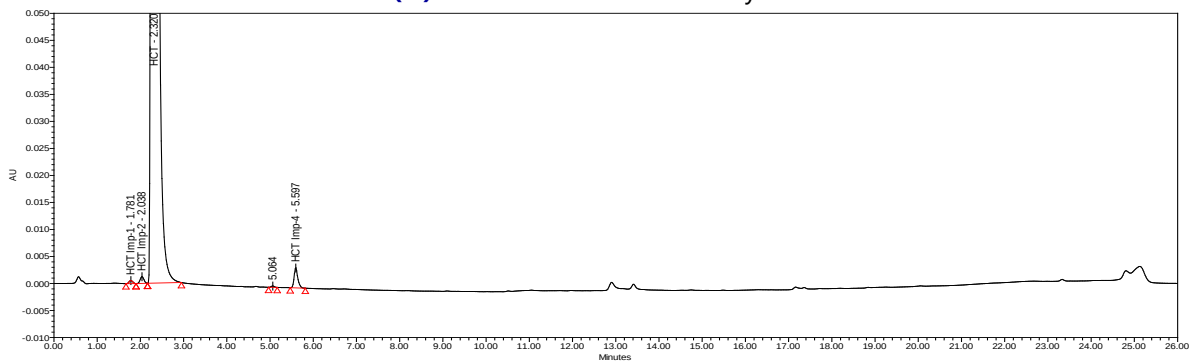
(u) Placebo with HCT oxidation stress



(v) Placebo with HCT thermal stress



(w) Placebo with HCT humidity stress



(x) Placebo with HCT photolytic stress

Figure 6. (continued)

Table 4a. Summary of forced degradation study for CAN

Stress condition	Time	% Assay of CAN	% imps+ %Deg. products CAN	mass balance (%Assay+ Imps+% Deg products of CAN	Major Appeared impurities
Acid hydrolysis (1N HCl / 5mL/ 25°C)	30 minutes	96.5	4.23	100.73	CAN-Imp-4
Base hydrolysis (1N NaOH / 5mL / 25°C)	Immediately	97.2	3.26	100.46	CAN-Imp-1 and Unspecified impurity at RRT 0.74.
Oxidation (30% H_2O_2 / 5mL/ 85°C)	60 minutes	98.5	0.97	99.47	CAN-Imp-4
Thermal (105°C)	48 hours	90.6	9.1	99.7	CAN-Imp-1, 4, 5&6 and 7&8 and Unspecified impurities at RRTs 0.39, 0.48, 0.56, 0.71 and 0.80
Humidity (95%RH)	48 hours	98.2	0.78	98.98	No major degradation
Photolytic (white fluorescent 1.2 million lux hours UV 200 watt hr/m^2)	7 days	98.7	0.76	99.46	No major degradation

RESULTS AND DISCUSSION

Specificity and Stress studies

Stress studies on Candesartan cilexetil and Hydrochlorothiazide tablets under different stress conditions suggested the following degradation behavior. (**Figure 6g** to **6l** and **Table 4a** & **Table 4b**).

Acid stress

In Acid hydrolysis stress condition, CAN Imp-4 and HCT imp-1 were formed in significant levels.

Alkaline stress

In base hydrolysis stress condition, CAN Imp-1 and HCT imp-1 were formed in significant levels.

Oxidative stress

In Peroxide stress condition, CAN Imp-4 formed in low level. HCT not degraded in this condition.

Table 4b. Summary of forced degradation study for HCT

Stress condition	Time	% Assay of HCT	% imps+ %Deg. products HCT	mass balance (%Assay+ Imps+% Deg products of HCT	Major Appeared impurities
Acid hydrolysis (1N HCl / 5mL/ 25°C)	30 minutes	95.5	3.57	99.07	HCT-Imp-1
Base hydrolysis (1N NaOH / 5mL / 25°C)	Immediately	95.8	3.07	98.87	HCT-Imp -1
Oxidation (30% H_2O_2 / 5mL/ 85°C)	60 minutes	97.8	1.52	99.32	No major degradation
Thermal (105°C)	48 hours	96.2	3.16	99.36	HCT-Imp -1
Humidity (95%RH)	48 hours	97.8	1.47	99.27	No major degradation
Photolytic (white fluorescent 1.2 million lux hours UV 200 watt hr/m^2)	7 days	97.2	1.49	98.70	No major degradation

Thermal stress

In thermal degradation, CAN Imp-5&6 impurity formed at higher levels. Significant levels of CAN Imp-7&8, CAN Imp-4 and CAN Imp-1were also generated. HCT significantly degraded to HCT Imp-1.

Humidity and Photolytic stress

There is no significant degradation observed for CAN and HCT.

Precision and Intermediate precision

The percentage RSD of %w/w for HCT-Imp-1, 2, 3, 4, CAN-Imp-1, 2, 3, 4, 5&6 (sum of two isomers), 7&8 (sum of two isomers) and 9 is 0.9, 0.8, 0.8, 0.7, 0.9, 0.6, 0.8, 1.0, 1.4, 0.9 and 2.4 respectively were obtained confirming that the test method is precise. The % RSD from the intermediate precision experiment for the same impurities is 0.6, 0.8, 0.8, 0.1, 0.2, 1.3, 0.7, 0.6, 1.2, 1.5 and 3.9 respectively which indicates that, the method is rugged. % RSD for the overall results of %w/w is found to be less than 15.0% between method precision and intermediate precision data.

Limit of detection and Limit of Quantitation

The levels of Limit of Quantification (%w/w) for HCT-Imp-1, 2, 3, 4 are 0.022, 0.024, 0.019, 0.021. Limit of Detection values (%w/w) for HCT-Imp-1, 2, 3, 4 are 0.011, 0.012, 0.010, 0.011. Levels of Limit of Quantification (%w/w) for CAN-Imp-1, 2, 3, 4, 5&6, 7&8 and 9 are

Table 5. Linearity table

Name of the component	Trend line equation	Range	Correlation coefficient	Intercept	Residual sum of squares
CAN	y = 28121x - 320	0.016-2.423	0.99998	-320	189
CAN-Imp-1	y = 45107x + 264	0.014-2.199	0.99997	264	316
CAN-Imp-2	y = 43141x + 380	0.016-2.380	0.99997	380	310
CAN-Imp-3	y = 40870x + 807	0.017-2.588	0.99995	807	395
CAN-Imp-4	y = 33336x + 180	0.014-2.119	0.99983	180	510
CAN-Imp-5&6	y = 30124x + 87	0.016-2.365	0.99993	87	327
CAN-Imp-7&8	y = 27535x – 61	0.015-2.212	0.99994	-61	251
CAN-Imp-9	y = 9939x + 619	0.016-2.418	0.99466	619	979
HCT	y = 48857x - 27	0.013-1.947	0.99996	-27	344
HCT-Imp-1	y = 51262x - 89	0.011-1.692	0.99997	-89	258
HCT-Imp-2	y = 35837x - 100	0.012-1.847	0.99998	-100	169
HCT-Imp-3	y = 40969x - 41	0.010-1.449	0.99997	-41	190
HCT-Imp-4	y = 40172x + 268	0.011-1.585	0.99992	268	312

0.023, 0.025, 0.027, 0.022, 0.024, 0.023 and 0.025. Limit of Detection values (%w/w) for CAN-Imp-1, 2, 3, 4, 5&6, 7&8 and 9 are 0.012, 0.013, 0.014, 0.011, 0.012, 0.012 and 0.013.

Linearity and Range

Calibration curves obtained by the least square regression analysis between peak area and concentration showed linear relationship with a correlation coefficient of greater than 0.995 over the calibration ranges tested. Linear calibration plot for the related substances method is obtained over the calibration range LOQ to 150%. The results show an excellent correlation obtained between peak area and concentration of Candesartan cilexetil, Hydrochlorothiazide and all the impurities. (**Table 5**).

Accuracy

Accuracy was assessed from three replicate determinations of four different levels including LOQ, 50%, 100% and 150% of the specification level of the impurities. The observed recovery results were found in the range between 90 to 110% with the RSDs lower than 5.0% demonstrating that the method is accurate within the desired range (**Table 6a**, **Table 6b**, **Table 6c** and **Table 6d**).

Solution Stability

The solution stability experiment data confirms that solutions of standard and sample are stable up to 24 hours at room temperature (25°C) and cooler temperature (6°C).

Table 6a. Table for Accuracy study for HCT- Imp-1, HCT- Imp-2 and HCT- Imp-3

Sample spiked at level	HCT- Imp-1		% Acc	HCT - Imp-2		% Acc	HCT - Imp-3		% Acc
	Amount added (%w/w)	Amount recovered (%w/w)		Amount added (% w/w)	Amount recovered (%w/w)		Amount added (% w/w)	Amount recovered (%w/w)	
LOQ	0.0221	0.0223	100.9	0.0276	0.0281	101.8	0.0216	0.0226	104.6
50%	0.225	0.206	91.6	0.246	0.249	101.2	0.193	0.206	106.7
100%	0.451	0.454	100.7	0.492	0.489	99.4	0.386	0.418	108.3
150%	0.676	0.669	99.0	0.738	0.724	98.1	0.579	0.608	105.0

Table 6b. Table for Accuracy study for HCT- Imp-4, CAN- Imp-1 and CAN- Imp-2

Sample spiked at level	HCT- Imp-4		% Acc	CAN - Imp-1		% Acc	CAN - Imp-2		% Acc
	Amount added (%w/w)	Amount recovered (%w/w)		Amount added (% w/w)	Amount recovered (%w/w)		Amount added (% w/w)	Amount recovered (%w/w)	
LOQ	0.0192	0.0179	93.2	0.0226	0.0246	108.8	0.0245	0.0259	105.7
50%	0.211	0.218	103.3	0.226	0.244	108.0	0.245	0.262	106.9
100%	0.422	0.430	101.9	0.452	0.485	107.3	0.489	0.522	106.7
150%	0.633	0.639	100.9	0.678	0.715	105.5	0.734	0.774	105.4

Table 6c. Table for Accuracy study for CAN- Imp-3, CAN- Imp-4 and CAN- Imp-5&6

Sample spiked at level	CAN - Imp-3		% Acc	CAN - Imp-4		% Acc	CAN - Imp-5&6		% Acc
	Amount added (%w/w)	Amount recovered (%w/w)		Amount added (% w/w)	Amount recovered (%w/w)		Amount added (% w/w)	Amount recovered (%w/w)	
LOQ	0.0270	0.0289	106.9	0.0228	0.0216	94.7	0.0295	0.0311	105.4
50%	0.266	0.286	107.5	0.218	0.208	95.4	0.243	0.238	97.9
100%	0.532	0.568	106.8	0.436	0.422	96.8	0.486	0.494	101.6
150%	0.799	0.869	108.7	0.653	0.626	95.9	0.729	0.734	100.7

Table 6d. Table for Accuracy study for CAN- Imp-7 & 8 and CAN- Imp-9

Sample spiked at level	CAN - Imp-7&8		% Acc	CAN - Imp-9		% Acc
	Amount added (%w/w)	Amount recovered (%w/w)		Amount added (% w/w)	Amount recovered (%w/w)	
LOQ	0.0195	0.0183	93.8	0.0252	0.0270	93.3
50%	0.227	0.221	97.4	0.254	0.266	95.4
100%	0.455	0.457	100.4	0.508	0.537	94.6
150%	0.682	0.686	100.6	0.762	0.811	94.0

%Acc: %Accuracy

Robustness

Close observation of analysis results for deliberately changed chromatographic conditions such as Flow rate, column temperature, wave length and change of organic component in gradient programme revealed that there is no significant change observed in the relative retention times of the main analyte and their corresponding impurities illustrating the robustness of the developed method.

CONCLUSION

The proposed RP-UPLC method enables the separation and simultaneous quantitative determination of specified and unspecified impurities of CAN and HCT in CAN/HCT tablets. The developed method is validated as per ICH requirements. The stress studies indicated that method is selective and stability indicating. UV detection at 220 nm was found to be suitable without any interference from excipients. All the calibration curves obtained were found to linear with values of correlation coefficients greater than 0.995. LOD and LOQ values are well below the specification limits. Recovery tests confirmed the accuracy of the method. The proposed RP-UPLC method is fast, precise, accurate, sensitive and efficient.

ACKNOWLEDGEMENTS

The author wishes to thank the management of Aurobindo Research centre (A Division of Aurobindo Pharma Limited, Bachupally, Hyderabad, India) for supporting this work.

REFERENCES

1. Fogari, R., Mugellini, A., Derosa, G., & CANDIA (candesartan and diuretic vs. amlodipine in hypertensive patients) study group. (2011). Efficacy and Tolerability of Candesartan cilexetil/hydrochlorothiazide and Amlodipine in patients with poorly controlled mild-to-moderate essential Hypertension. *International Journal of Clinical Medicine, 2*, 64-68.
2. Heran, B. S., Wong, M. M. Y., Heran, I. K., & Wright, J. M. (2008). Blood pressure lowering efficacy of angiotensin receptor blockers for primary hypertension. *Cochrane Database of Systematic Reviews, 4*, Art. No. CD003822.
3. Asif, H., Sabir A., Moloy M., & Parminder S. B. (2011). A Review on Candesartan, Pharmacological and Pharmaceutical Profile. *Journal of Applied Pharmaceutical Science, 1*(10), 12-17.
4. Antonio, S., & Lorenzo, G. (2006). Thiazide Diuretics in the Treatment of Hypertension, An Update. *Journal of American Society of Nephrology, 17*(4), supplement 2, S25-S29.
5. Melian, E. B., & Jarvis, B. (2002). Candesartan cilexetil plus hydrochlorothiazide combination, a review of its use in hypertension. *Drugs, 62*(5), 787-816.
6. Mancia, G., Omboni, S., & CARDIO. (Candesartan combined with diuretic in hypertension) study group. (2004). Candesartan plus hydrochlorothiazide fixed combination vs previous monotherapy plus diuretic in poorly controlled essential hypertensive patients. *Blood Pressure supplement, 2*, 11-17.
7. Spratt, J, C. S., Webb, D. J., Shiels, A., & Williams, B. (2001). Effects of candesartan on cardiac and arterial structure and function in hypertensive subjects. *Journal of the Renin Angiotensin Aldosterone System, 2*, 227–32.

8. Campbell, M., Sonkodi, S., Soucek, M., & Wiecek, A. (2001). A candesartan cilexetil/hydrochlorothiazide combination tablet provides effective blood pressure control in hypertensive patients inadequately controlled on monotherapy. *Clinical and Experimental Hypertension, 23*(4), 345-355.
9. Basawaraj, P., Raghavedra, R. N. G, Suvarna, J., Upendra, K., & Mahesh, G. M. (2011). Estimation of Candesartan cilextil in bulk and tablet dosage forms by U.V Spectrophotometric method. *International Journal of Research in Ayurveda & Pharmacy, 2*(1), 204-206.
10. Srinivas, G., Kumar, K. K., Kanumula, G. V., Priya V. M., & Mukkanti, K. (2012). A Stability Indicating UPLC Method for Candesartan in Bulk Drug Samples. *American Journal of Analytical Chemistry, 3*, 704-709.
11. Peepliwal, A. K., Bonde, C. G., & Mohanraj, K. (2010). Bioanalytical method development and its validation for determination of candesartan cilexetil by high performance liquid chromatography with UV Detection. *Acta Pharm Sciencia, 52*, 247–53.
12. Mohan, A., Shanmugavel, S., Goyal, A., Venkataraman, B. R., & Saravanan, D. (2009). Identification, isolation and characterization of five potential degradation impurities in candesartan cilexetil tablets. *Chromatographia, 69*, 403–603.
13. Zhang, Y. D., Wei, X. H., Wang, A. F., Xu, Y. J., Zhu, X. H., & Luo, J. M. (2004). HPLC Determination of Candesartan Cilexetil and its Related Substances. *Central South Pharmacy, 2*(2), 80-83.
14. Kumar, N. D. A., Sudhakar, B. K., Gosada, U., & Sharma, N. (2012). A validated ultra high-pressure liquid chromatography method for separation of candesartan cilexetil impurities and its degradents in drug product. *Pharmaceutical Methods, 3*(1), 31–39.
15. Bhagwate, S., & Gaikwad, N. J. (2013). Stability Indicating HPLC Method for the Determination of Hydrochlorothiazide in Pharmaceutical Dosage form. *Journal of Applied Pharmaceutical Science, 3*(2), 88-92.
16. Veeranjaneyulu, D., Aneesha, A., & Nandakishore, A. (2013). Stability indicating RP-HPLC method for the simultaneous determination of candesartan cilexetil and hydrochlorothiazide in bulk and dosage forms. *Indian journal of research in pharmacy and biotechnology* issn, 2321-5674(print) issn, 2320 – 3471(online).
17. Singh, B., Lokhandae, R. S., Dwivedi, A., Sharma, S., & Dubey, N. (2014). Improved simultaneous quantitation of candesartan and hydrochlorthiazide in human plasma by UPLC–MS/MS and its application in bioequivalence studies. *Journal of Pharmaceutical Analysis, 4*(2), 144–152.
18. Erk, N. (2003). Simultaneous Analysis of Candesartan Cilexetil and Hydrochlorothiazide in Human Plasma and Dosage Forms Using HPLC with a Photodiode Array Detector. *Journal of Liquid Chromatography and Related Technologies, 26*, 2581–91.
19. Balamuralikrishna, K., & Syamasundar, B. (2010). Development and validation of high performance liquid chromatographic method for the simultaneous estimation of candesartan cilexetil and hydrochlorothiazide in combined tablet dosage form. *Der Pharma Chemica, 2*, 231–7.
20. Annapurnaa, M. M., Narendra, A., & Kumar, K. R. (2012). Liquid Chromatographic Method for Determination of Candesartan Cilexetil and Hydrochlorothiazide in Pharmaceutical Dosage Forms. *Journal of Drug Delivery & Therapeutics, 2*(2), 48-54.
21. Mehta, B. H., & Morge, S. B. (2008). HPTLC-Densitometric Analysis of Candesartan Cilexetil and Hydrochlorothiazide in Tablets. *Journal of Planar Chromatography, Modern TLC, 21*(2), 173-176.
22. Devanaboyina, N., & Satyanarayana, T. (2012). Simultaneous Determination of Candesartan and Hydrochlorothiazide in Combined Pharmaceutical Dosage Form by New RP- HPLC Method. *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 3*(1), 270-278.

23. Lakshmi Narasimham, Y. S., & Barhate Vasant, D. (2010). Development and validation of stability indicating UPLC method for the simultaneous determination of beta-blockers and diuretic drugs in pharmaceutical dosage forms. *Journal of Chemical Metrology, 4*(1), 1-20.
24. Eranki, R. J. V., Inti, G., Jayaraman, V., Vidiyala, S. R., & Sreeramulu, J. (2014). New stability indicating method for quantification of impurities in candesartan cilexetil and hydrochlorothiazide tablets by validated HPLC. International *Journal for Pharmaceutical Research Scholars, 3*(2), 100-112.
25. ICH. (2005). Validation of Analytical Procedures, Text and Methodology, Q2(R1).
26. ICH. (2005). Stability Testing of New Drug Substances and Products, Q1A(R2).
27. US FDA Guidance. (2000). Analytical Procedures and Methods Validation.
28. Official Methods of Analysis, 15th Ed. (1990). Association of Official Analytical Chemists International, Arlington, VA, XVII.
29. Validation of Compendial Methods <1225> (2012). The United States Pharmacopeia.
30. United States pharmacopeial Convention, 36th ed. (2012). The United States Pharmacopoeia, Rockville, MD.
31. Guideline for submitting samples and Analytical Data for Methods Validation, US Food and Drug Administration. (1987).