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Simultaneous Determination of Candesartan and Hydrochlorothiazide in combined Pharmaceutical Dosage form by New RP-HPLC Method

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ABSTRACT

A simple, accurate reverse phase high-performance liquid chromatographic method was developed and validated for the simultaneous estimation of Candesartan and Hydrochlorothiazide in bulk and pharmaceutical dosage forms. Chromatography was carried out by using Chromosil C-18,column having 250 x 4.6mm internal diameter with a mixture of MeOH : THF:0.1 % O.P.A 85:05:10 (V/V/V) as mobile phase. Determination of the different analytical parameters such as linearity, precision, accuracy, and specificity, limit of detection (LOD) and limit of quantification (LOQ) was done. The calibration curve was found to be linear for each analyte in the desired concentration range. The percentage of recovery was found to be 99.95 and 99.46 for Candesartan and Hydrochlorothiazide respectively. The proposed method is highly sensitive, precise and accurate, which was evident from the LOD value of 0.06 and 0.02 9ppm for Candesartan and Hydrochlorothiazide respectively and hence the present method can be applied successfully for the quantification of active pharmaceutical ingredient (API) content in the combined formulations of Candesartan and Hydrochlorothiazide.

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INTRODUCTION

Candesartan is an angiotensin II receptor antagonist used mainly for the treatment of hypertension. The prodrug candesartan cilexetil is marketed by AstraZeneca and Takeda Pharmaceuticals, commonly under the trade names Blopress, Atacand, Amias, and Ratacand. As all angiotensin II receptor antagonists, candesartan is indicated for the treatment of hypertension. Results from the CHARM study in the early 2000s demonstrated the morbidity and mortality reduction benefits of candesartan therapy in congestive heart failure.[1] Thus, while ACE inhibitors are still considered first-line therapy in heart failure, candesartan can be used in combination with an ACE to achieve improved mortality and morbidity vs. an ACE alone and additionally is an alternative in patients intolerant of ACE inhibitor therapy.

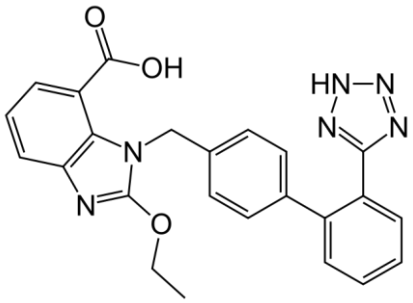


Figure1: Structure of Candesartan

Hydrochlorothiazide is frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones. [6] It is also sometimes used for hypercalciuria, Dent's disease and Ménière's disease. For diabetes insipidus, the effect of thiazide diuretics is presumably mediated by a hypovolemia-induced increase in proximal sodium and water reabsorption, thereby diminishing water delivery to the ADH-sensitive sites in the collecting tubules and reducing the urine output. Thiazides are also used in the treatment of osteoporosis. Thiazides decrease mineral bone loss by promoting calcium retention in the kidney, and by directly stimulating osteoblast differentiation and bone mineral formation [7] is a first-line diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance. [5] Hydrochlorothiazide is a calcium-sparing diuretic, meaning it can help the body get rid of excess water while still keeping calcium.

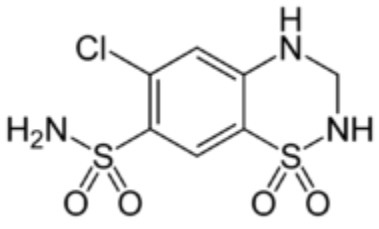


Figure 2: Structure of Hydrochlorothiazide

ISSN: 0975-8585

MATERIALS AND METHODS

Chemicals and Reagents

Candesartan and Hydrochlorothiazide as pure standard reference drugs were purchased from ZEN Pharma, Ahmadabad and pharmaceutical formulation from local market were used for this present study. Water, Acetonitrile, methanol and Orthophosphoric acid, Tri Ethyl Amine (all HPLC grade) were purchased from Merck Specialties Private Limited, Mumbai, India.

Instrumentation

To develop a HPLC method for quantitative estimation of Hydrochlorothiazide and Candesartan, an isocratic PEAK HPLC instrument with Hypersil C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software. UV-2306 Spectrophotometer was used for wavelength checking. Denver analytical Balance was used to weigh the drug.

Experimental Condition

Flow rate of the mobile phase was changed from 0.5 – 1.5 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0 ml/min flow rate was ideal for the successful elution of the analyte. The HPLC system was hence operated using an isocratic mode at a flow rate of 1.0 ml/min at 25 ± 2ºᶜ. For analysis the most suitable mobile phase was found to be MeOH: THF: 0.1 % O.P.A 85:05:10 (V/V/V) Methanol. Detection was carried out at wavelength of 272 nm.

Preparation of Mobile Phase

For the preparation of mobile phase suitable for the present determination methanol, THF and O.P.A of HPLC grade were mixed, filtered and degassed in such a way that the final volume consisted of these in the ratio 85:05:10 respectively, whose pH was adjusted to 4.8.

Preparation of mixed standard solution

Candesartan and Hydrochlorothiazide (1mg/ml) standard stock solutions were prepared using methanol as a solvent. Aliquots of mixed standard solutions of Candesartan and Hydrochlorothiazide were diluted in mobile phase to get a final concentration of 5-35ppm.

Preparation of sample solution of pharmaceutical formulation

ISSN: 0975-8585

Pharmaceutical form containing 16 mg of Candesartan and 12.5 mg of Hydrochlorothiazide was weighed and dissolved in 25 ml of methanol and sonicate for 15 min. Using methanol the volume was made up to 50 ml and filtered through 0.45µ membrane filter. The final mixed sample solution corresponding to 40 ppm of Candesartan and 31.25 ppm of Hydrochlorothiazide was prepared.

Recording of chromatograms

After stabilization of the base line with the optimized chromatographic conditions standard solutions containing 5-35 ppm of Candesartan and Hydrochlorothiazide were injected and the corresponding chromatograms were recorded. Retention time of Candesartan and Hydrochlorothiazide were found to be 1.6 and 2.6 mins respectively. Likewise for sample solution chromatograms were recorded. Calibration curves were plotted using peak area retentions of standard drug peaks against concentration of corresponding standard solutions.

RESULTS AND DISCUSSION

Method validation

The method was validated by determining linearity, precision, accuracy, specificity, ruggedness and robustness by analyzing 5-35 ppm of both Candesartan and Hydrochlorothiazide.

Mobile phase	MeOH : THF:0.1 % O.P.A 85:05:10 (V/V/V)
Pump mode	Isocratic
A.P.I Conc.	Candesartan – 20 PPM Hydrochlorothiazide - 20 PPM
pH	4.8
Diluents	Mobile phase
Column	C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	272 nm
Injection Volume	20 Ml
Flow rate	1.0 mL/min
Run time	10 minutes
Retention Time	Candesartan – 1.6 Hydrochlorothiazide – 2.6

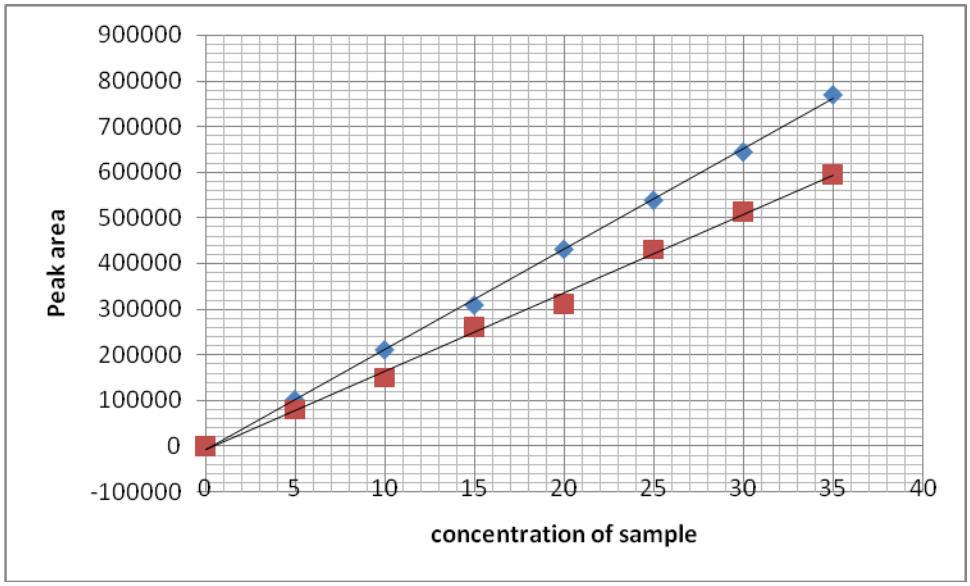
Table 1: Optimized chromatographic conditions for estimation of Candesartan and Hydrochlorothiazide

Linearity

The linearity of the response for Candesartan and Hydrochlorothiazide assay method was determined by preparing and injecting standard solutions of Candesartan and Hydrochlorothiazide. The linear regression data for the calibration curves indicate that the

ISSN: 0975-8585

response is linear over the concentration range studied with correlation coefficient (r^2) value, slope and intercept as shown in table 3.



Graph 1: Calibration Plot for Candesartan and Hydrochlorothiazide

S.NO	CONCENTRATIONS	Candesartan peak area	Hydrochlorothiazide peak area
1	5	102456	80459
2	10	211731	150498
3	15	309854	260351
4	20	432056	311927
5	25	539487	430589
6	30	645208	512498
7	35	770332	594829

Table 2.

Parameters	Candesartan	Hydrochlorothiazide
Calibration range (ppm)	5-35	5-35
Slope	21932	17180
Intercept	-7424	-8016
Correlation coefficient (r^2)	0.9996	0.9993

Table 3: Regression analysis of the calibration curve

Precision

The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Candesartan and Hydrochlorothiazide combined test solution in the same equipment at a concentration value of 20 ppm on the same day. The experiment was repeated by assaying

freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak areas of the drugs were determined and precision as % RSD was reported.

S.NO	CONCENTRATION	**Candesartan peak area**	**Hydrochlorothiazide peak area**
1	20 ppm	432056	311927
2	20 ppm	433564	315896
3	20 ppm	435209	314087
4	20 ppm	434089	319064
5	20 ppm	431568	316689
6	20 ppm	430857	317453
		%R.S.D = 0.38	%R.S.D = 0.79

Table 4: Intraday precision

S.NO	CONCENTRATION	**Candesartan peak area**	**Hydrochlorothiazide peak area**
1	20 PPM	431089	316657
2	20 PPM	433964	325894
3	20 PPM	436871	316940
4	20 PPM	439658	316874
5	20 PPM	432596	310548
6	20 PPM	430578	309663
		%RSD = 0.806	%RSD = 1.81

Table 5: Interday precision

Parameters	**Candesartan**	**Hydrochlorothiazide**
Theoretical plates (N)	5923	20272
Retention time (min)	1.6	2.8
Tailing factor	1.53	1.47
LOD (ppm)	0.06	0.09
LOQ (ppm)	0.12	0.2
R.S.D. (%)	0.152	0.14

Table 6: System suitability and validation parameters

ISSN: 0975-8585

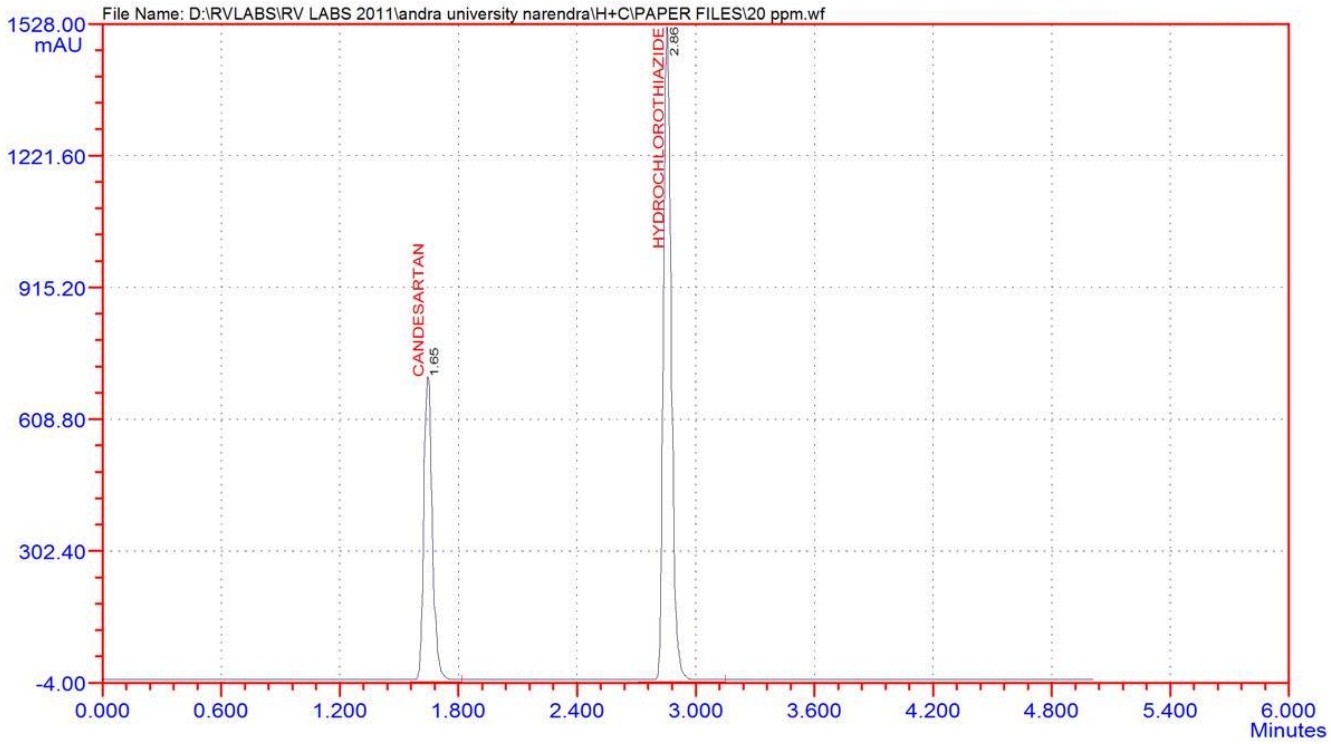


Figure 2: Typical chromatogram of standard Candesartan and Hydrochlorothiazide.

Recovery

The recovery of the standard solutions was done by adding them to pre analyzed sample solution at different levels i.e. 50%, 100%, and 150% separately to study the accuracy of the above method. The corresponding results were recorded.

S.NO	CONCENTRATION In ppm	Candesartan AMOUNT RECOVERED	Hydrochlorothiazide AMOUNT RECOVERED	% Candesartan RECOVERED	% Hydrochlorothiazide RECOVERED
1	10	9.96	9.91	99.6	99.1
2	20	19.89	19.96	99.45	99.8
3	30	30.24	29.85	100.8	99.5
				Avg Recovery =99.95	Avg Recovery =99.46

Table 7: Recovery of Candesartan, Hydrochlorothiazide

Specificity

Specificity was performed to exclude the possibility of interference with excipients in the region of elution of Candesartan and Hydrochlorothiazide. The specificity and selectivity of the method was tested under normal conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of Candesartan and Hydrochlorothiazide.

ISSN: 0975-8585

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

LOD and LOQ were determined from standard deviation of y-intercept of regression line and slope method as per ICH guidelines.

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

S.NO	PARAMETER	CONDITION	Candesartan peak area	% of change	Hydrochlorothiazide peak area	% of change
1	Standard	Standard conditions	432056	0.0	311927	0.0
2	Mobile phase	MeOH :THF:0.1 %O.P.A 75:05:20	432954	0.2	311482	0.14
3	Mobile phase pH	4.6	432628	0.13	311557	0.11
4	Wavelength	274 nm	432447	0.09	311691	0.07

Table 8: Robustness study

Analysis of marketed formulations

The validated HPLC method was adopted for the quantification of Candesartan and Hydrochlorothiazide in their combined pharmaceutical dosage form and the typical chromatograms of the formulation are shown in fig. The results of analysis are given in Table 9. The contents of the pharmaceutical dosage form were found to be in the range of 100±2% with RSD less than 2% which indicate suitability for routine analysis of Candesartan and Hydrochlorothiazide in pharmaceutical dosage form.

S.NO	Drug	Dosage	Sample Conc.	Amount of drug estimated	% of drug estimated
1	Candesartan	16 mg	40ppm	39.94ppm	99.85
2	Hydrochlorothiazide	12.5mg	31.25ppm	31.18 ppm	99.77

Table 9: Formulation

CONCLUSION

The developed method was validated and found to be simple, sensitive, accurate and precise. It was also proved to be convenient and effective for the determination of Candesartan and Hydrochlorothiazide in the pharmaceutical dosage form. The percentage of recovery shows

that the method is free from interference of the excipients used in formulation. Moreover, the lower solvent consumption along with the short analytical run time leads to cost effective chromatographic method. The mobile phase has no any type of salt buffers, then column works efficiently long time

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