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Development and Validation of Analytical Method for Simultaneous Estimation of Cilnidipine and Chlorthalidone in their Combined Dosage Form

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Abstract

A simple, rapid and precise reverse phase high performance liquid chromatographic method was developed for the Simultaneous Estimation of Cilnidipine and Chlorthalidone in their combined dosage form. The chromatographic separation was achieved on Zorbax Bonus RP (250×4.6) mm, 5 µ column with an isocratic mixture of 0.05 M KH_2PO_4 Buffer (pH 6.5) : Methanol in the ratio of 50:50 v/v, respectively. The mobile phase was kept at a flow rate of 1.0 ml/min with injection volume of 20µl and wavelength of detection 225nm at room temperature. The retention times for Chlorthalidone and Cilnidipine was found to be 8.107±0.1min and 4.337±0.1min, respectively. The linearity was obtained in the range of 50-150µg/ml for both Chlorthalidone and Cilnidipine with correlation coefficient 0.9993 and 0.9996, respectively. The proposed method was found to be linear, accurate, precise, stable, robust and specific and was successfully applied for the determination of investigated drugs in combined dosage form.

Keywords

Cilnidipine, Chlorthalidone, RP-HPLC Method, Validation

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INTRODUCTION

Cilnidipine (INN) is a calcium channel blocker. Cilnidipine is the novel calcium antagonist accompanied with L-type and N-type calcium channel blocking function [1]. Chlortalidone (INN/BAN) or chlorthalidone (USAN) is a diuretic drug used to treat hypertension, originally marketed as Hygroton in the USA. It is described as a thiazide diuretic (or, rather, a thiazide-like diuretic because it acts similarly to the thiazides but does not contain the benzothiadiazine molecular structure)[2].

From the literature survey, it was found that there are methods available for combination of Cilnidipine (CIL) and Telmisartan, CIL and Olmesartan and CIL and Metoprolol.[3-5]

In addition to above, there are methods available for Chlortalidone (CHLOR) in compendia like IP, BP and USP by TLC and HPLC.[6-8]

It was found that there are methods available for combination of CHLOR and atenolol, CHLOR and Telmisartan, CHLOR and azilsartan and CHLOR and Metroprolol[9-12].

The aim of this project work is to development and validation of analytical method for estimation of Cilnidipine and Chlorthalidone in combined dosage form in Bulk drug and pharmaceutical formulation. To develop and validate stability indicating HPLC method for estimation of Cilnidipine and Chlorthalidone in combined dosage form according to ICH guideline and application of the method for estimation of drug in synthetic mixture/formulation[13].

MATERIALS AND METHODS

Equipment

Chromatographic separation was performed on HPLC System – Prominent Shimadzu, PDA detector equipped with a solvent delivery pump, sample injector and column thermostat. Empower system software was applied for data collecting and processing.

Chemicals and Reagent

1. Acetonitrile (AR Grade)
2. Water (HPLC Grade)
3. Acetic acid (AR Grade)
4. Methanol (HPLC Grade)

Nexovas CH Tablets (10 + 12.5) manufactured by Macleods Pharma were procured from local market. Reference standard of Cilnidipine and Chlorthalidone were obtained from Vaibhav Laboratory.

Preparation of Solutions

Preparation of buffer solution (pH 6.5)

Accurately weighed 1.84 grams of sodium di-hydrogen phosphate monohydrate were added in 1liter of beaker containing 1liter Milli-Q water. Solution was properly mixed. Add 1% of tri ethylamine and pH was adjusted to 6.5 with the help of 1% of ortho phosphoric acid. Finally solution was filtered through Whatmann no. 1 filter paper.

Mobile phase preparation

Selected solvents were mixed in required proportions either with water, other solvents or buffer and sonicated for specified time and then filtered through 0.45µ whatmann filter paper for further use.

Sample diluent: use mobile phase as diluent.

Preparation of standard stock solution of API

About 10 mg of Cilnidipine and of 12.5 mg Chlorthalidone were accurately weighed and transferred in to a 10 ml volumetric flask and diluted up to 10 ml with methanol. After that the solution was sonicated for 15 min to dissolve compounds.

Preparation of working standard solution

1 ml of standard stock solution was taken and transferred in to 10 ml volumetric flask and diluted up to 10 ml with methanol. From which, 0.1 ml of solution was taken into 10 ml volumetric flask and diluted up to 10 ml with methanol. This gave solution containing 12.5 µg/ml of Chlorthalidone and 10 µg/ml of Cilnidipine respectively.

Preparation of sample solution

20 tablets were weighed and triturated and equivalent weight of powder containing 12.5 mg of Chlorthalidone and 10 mg of Cilnidipine were weighed accurately and transferred in to a 20 ml volumetric flask and diluted up to 20 ml with methanol .After that the solution was sonicated for 15 min to dissolve compounds and further dilutions same as working standard solution were made to prepare solution containing 12.5 µg/ml of Chlorthalidone and 10 µg/ml of Cilnidipine respectively.

RESULTS AND DISCUSSION

Selection of wavelength

Detection wavelength was selected by taking an overlain spectrum of individual solutions of 12.5 µg/ml of Chlorthalidone and 10 µg/ml of Cilnidipine in Methanol by UV spectrophotometer. The wavelengths i.e. λ_{max} of Chlorthalidone (216.00 nm), λ_{max} of Cilnidipine (241.73 nm) and isoabsorptive wavelength (225 nm) were obtained from the spectra and isoabsorptive wavelength (225 nm) was selected for further method development.

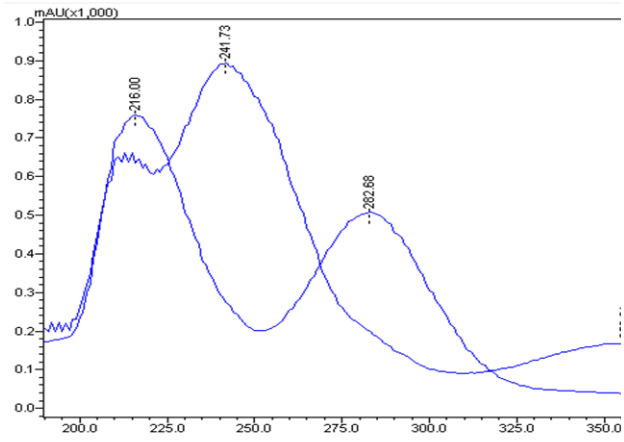

Figure 1 Selection of Wavelength

Linearity

Linear regression data for the calibration plot revealed good linear relationship between area and concentration over the range 50-150 µg/ml for both (Table 1).

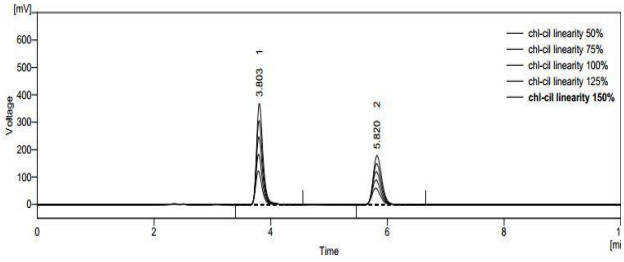

Figure 2 Chromatogram for calibration curve of CIL and CHLOR

Table 1 Linearity data for CIL & CHLOR for RP-HPLC

Sr no.	Concentration (µg/mL)		Area ±SD (n=3)	
	CIL	CHLOR	CIL	CHLOR
1.	10	12.5	930.276	607.125
2.	15	18.75	1394.179	909.35
3.	20	25	1882.144	1229.926
4.	25	31.25	2240.866	1529.931
5.	30	37.5	2886.651	1940.541

Table 2 Statistical data* for Cilnidipine and Chlorthalidone by HPLC method

PARAMETERS	RESULT	
	CIL	CHLOR
Linear Range(µg/ml)	50-150	50-150
Slope	95.18	52.59
Intercept	-36.952	-71.591
Standard deviation of intercept	71.83459408	36.57862837
Limit of Detection (µg/ml)	2.490587944	2.29529328
Limit of Quantitation (µg/ml)	7.547236193	6.955434183

Precision

The precision of this method is determined by Intraday and Interday precision. The %RSD was found less than 2, this indicate that the method is precise. The result of precision study are shown in Table 3.

Table 3 intraday precision of Cilnidipine for RP-HPLC

Precision	CIL	CHLOR
Intraday	1.0846722	0.883836
Interday	0.970499	1.096422

Limit of Detection and Limit of Quantification (LOD and LOQ)

The sensitivity of method is described in terms of LOD and LOQ. LOD and LOQ values for CIL were found to be 2.490587944 µg/ml and 7.547236193 µg/ml and that for CHLOR were found to

be2.29529328 µg/ml and 6.955434183 µg/ml respectively.

Accuracy

The accuracy was evaluated by recovery or CIL and CHLOR at three different level (80, 100 and 120). The %Recovery found to be for CIL and CHLOR respectively, %RSD was found to be less than 2, ensuring that the method is accurate. The result of accuracy are shown in table 4.

Table 4 Accuracy of CIL and CHLOR

Formulation	%Recovery	Average	SD	%RSD
CIL	80%	99.82408397	1.326424845	1.328762351
	100%	99.7377608	0.825045513	0.827214794
	120%	99.72887845	0.648154161	0.649916224
CHLOR	80%	100.5955204	1.128288002	1.121608594
	100%	100.2416379	0.651974925	0.650403304
	120%	100.1968991	0.492200638	0.491233404

Repeatability

The experimental value obtained for the repeatability of CIL and CHOR in sample is present in Table 5. The result obtained shows %RSD less than 2, indicating good repeatability of method.

Table 5 Repeatability of Cilnidipine and Chlorthalidone

Sets	Area	
	CIL	CHLOR
AVERAGE	1873.890667	1225.42
SD	14.04676597	9.53925
% RSD	0.749604351	0.778447

Robustness

Robustness of the method was carried out by deliberately made small change in flow rate, pH of Mobile Phase and Mobile phase composition. The results are shown in table 6.

Table 6 Result of Robustness Study

Sr no.	Formulation	Parameter	Change	Average Area	SD	RSD
1.	CIL	Flow rate	+0.2	1829.193	21.08138	1.152496
			-0.2	1942.409	23.28028	1.198527
2		Mobile Phase	+2	1829.025	19.22056	1.050864
			-2	1918.851	29.12617	1.517897
3.		pH	+0.2	1781.874	29.163	1.636648
			-0.2	1918.219	33.0160	1.72118
1.	CHLOR	Flow rate	+0.2	1192.679	14.65904	1.229085
			-0.2	1272.617	10.51243	0.826048
2.		Mobile Phase	+2	1194.902	13.16788	1.102005
			-2	1257.079	14.97667	1.191387
3.		pH	+0.2	1167.354	14.06241	1.20464
			-0.2	1258.251	13.25166	1.053181

Specificity

Specificity was observed that diluents did not interfere with detection of CIL and CHLOR.

Label claim recoveries from tablets

The proposed method was evaluated in the assay of commercially available tablet containing CIL (10 mg) and CHLOR (12.5 mg). Three replicated determination were carried out on and accurately weighted

amount of tablet equivalent to 10 mg of CIL and 12.5 mg of CHLOR. The result of label claim studies are shown in table 7.

Table 7 Result of assay of Tablet formulation

	Label Claim	Avg %Assay	SD	%RSD
CIL	10 mg	99.97311571	0.12604	0.126074
CHLOR	12.5 mg	100.1124158	0.199865	0.199641

CONCLUSION

The developed RP-HPLC method was accurate, precise, reproducible and robust. It can be used as simultaneous determination of CIL and CHLOR in pharmaceutical dosage form. The method was validated as per ICH Guideline.

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