JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Chlorthalidone, Cilnidipine and Irbesartan in their Combined Marketed Dosage Form

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Article history:

Received 25 March 2017
Accepted 03 April 2017
Available online 10 Jan 2017

Citation:
Maurya N. D., Parajapati Y., Sakhareliya B., Development and Validation of RP-HPLC Method for Simultaneous Estimation of Chlorthalidone, Cilnidipine and Irbesartan in their Combined Marketed Dosage Form **J Pharm Sci Bioscientific Res**. 2017. 7(2):193-199

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(www.jpsbr.org)

ABSTRACT:

A Simple, rapid, economical, precise and accurate Reverse Phase Method High Performance Liquid Chromatography(RP – HPLC) Method For Simultaneous Estimation of Cilnidipine, Chlorthalidone, and Irbesartan in their combined dosage form has been developed. The RP- HPLC method was developed for the simultaneous estimation of Cilnidipine, Chlorthalidone, Irbesartan in their combined dosage form development method has been achieved. The separartion was attained by Column – C18 (25 cm 0.46 cm) Hypersil BDS and Ammonium Acetate buffer 0.05M (pH 3.0): Acetonitrile (60:40 v/v)as mobile phase at a Flow rate 1 ml per minute , Detection was carried out of wavelength of 226 nm retention time of Cilnidipine, Chlorthalidone, Irbesartan were found to be 6.923 min, 4.097 min, 2.967 min respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Cilnidipine 0.5-1.5 µg/ml , for Chlorthalidone 0.625-1.875 µg/ml, for Irbesartan 15-45 µg/ml. The percentage recovery obtained for Cilnidipine, Chlothalidone, Irbesartan were found to be in range 99.93 ± 0.70 , 99.41±0.82, 99.47 ±1.17 respectively. Developmet method was found to be accurate, precise and rapid for simultaneous estimation of Cilnidipine, Chlorthalidone and Irbesartan in their combined dosage form.

KEY WORDS: RP – HPLC, Hypersil BDS and Ammonium Acetate buffer, Acetonitrile, Cilnidipine, Chlorthalidone and Irbesartan, Simultaneous Estimation

INTRODUCTION [1-10]

1.1 INTRODUCTION TO DISEASES: [1]

Hypertension is the most common cardiovascular disease. As many as 50 million people in the United States have systolic and/or diastolic blood pressure above 140/90 mm Hg. Elevated arterial pressure causes pathological changes in the vasculature and hypertrophy of the left ventricle. As a consequence, hypertension is the principal cause of stroke, leads to disease of the coronary arteries with myocardial infarction and sudden cardiac death, and is a major contributor to cardiac failure, renal insufficiency, and dissecting aneurysm of the aorta.

Hypertension is defined conventionally as blood pressure 140/90; this serves to characterize a group of patients who carry a risk of hypertension-related cardiovascular disease that is high enough to merit medical attention. However, from the standpoint of health promotion, it should be noted that the risk of both fatal and nonfatal cardiovascular disease in adults is lowest with systolic blood pressures of less than 120 mm Hg and diastolic less than 80 mm Hg; these risks increase progressively with higher levels of both systolic and diastolic blood pressure.

CLASSIFICATION OF ANTIHYPERTENSIVE AGENTS

1) Diuretics:

a) Thiazides: e.g. 2338B, Chlorthiazide, Clopamide

b) Loop diuretics: e.g. Frusemide, Ethacrinic acid

c) Potassium sparing diuretics: e.g. Spironolactone, Amiloride

2) Drug acting on sympathetic system

a) Centrally acting: e.g. Clonidine, Guanabenz

b) Catecholamine deplaters: e.g. Reserpine

c) Adrenergic receptor blockers:

- Beta blockers: e.g. Atenolol, Metoprolol, Propranolol
- Alpha blockers: e.g. Tolazoline, Prazosin, Terazosin
- Alpha and beta blockers: e.g. Labetolol, Carvedilol

d) Adrenergic neurone blocker: e.g. Guanethidine, Bretylium

3) Ca++channel blockers: e.g. Verapamil, Nifedipine, Amlodipine.

4) Drugs acting on rennin angiotensin system:

a) Drugs block rennin release: e.g. Clonidine, Ramikiren

b) Drugs inhibit angiotensin converting enzyme: e.g. Captopril, Ramipril, Lisinopril.

c) Drugs inhibit angiotensin- II: e.g. Saralasin

d) Drugs inhibit angiotensin- I receptors: e.g. Losartan

5) Vasodilators: e.g. Hydralazine, Minoxidil, Diazoxide

1.2. INTRODUCTION TO ANALYTICAL METHOD [2-6]

Pharmaceutical products formulated with more than one drug, typically referred to as combination products. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. The development and validation of analytical methods [Spectrophotometric, High performance liquid chromatography (HPLC) & High performance thin layer chromatography (HPTLC)] for drug products containing more than one active ingredient. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products.

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing ones. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

1.2.1. INTRODUCTION TO HPLC METHOD

Liquid chromatography (LC) is a physical separation technique conducted in the liquid phase. A sample is separated into its constituent components (or analytes) by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). For example, the flowing liquid can be an organic solvent forced through the column at high speed and the stationary phase can be porous silica particles packed in a column. The modern form of column chromatography has been called high performance, high Pressure, high-resolution and high-speed liquid chromatography. HPLC is a modern form of LC that uses small-particle columns through which the mobile phase is pumped at high pressure.

High-performance liquid chromatography (HPLC), sometimes called high-pressure liquid chromatography, is a separation technique based on a solid stationary phase and a liquid mobile phase.

There are different modes of separation in HPLC:

1) Normal phase mode.
2) Reversed phase mode.
3) Ion exchange chromatography.
4) Reverse phase ion pair chromatography.
5) Affinity chromatography and
6) Size exclusion chromatography.

Parameters that are affected by the changes in chromatographic conditions:

1. Resolution (Rs).
2. Capacity factor (k').
3. Selectivity (α).
4. Column efficiency (N).
5. Peak asymmetry factor (As).

1.3. DRUG PROFILE [8-10].

1.3.1CHLORTHALIDONE [8]

Chlorthalidone official in USP30–NF25, IP 2010. Description of Chlorthalidone is a diuretic drug used to treat hypertension. Chemical Formula of chlorthalidone C14H11ClN2O4S.its mol.weight 333.766 gm/mol and IUPAC Name is (RS)-2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzene-1-sulfonamide in Antihypertensive agent and Diuretic Categories. soluble in methanol ,slightly soluble ethanol (95 per cent) , alkali hydroxides,Practically insoluble in water, in ether and in chloroform. Melting point 238-240° C, Log P-1.27, Water solubility 5.28e-02 mg/L (at 25 °C) . Mechanism action of Chlorthalidone inhibits sodium ion transport across the renal tubular epithelium in the cortical diluting segment of the ascending limb of the loop of Henle. By increasing the delivery of sodium to the distal renal tubule, Chlorthalidone indirectly increases potassium excretion via the sodium-potassium exchange mechanism.

1.3.2 CILNIDIPINE [9]

Cilnidipin not official in any pharmacopoeia. Description of Cilnidipine is a dual L-/N-type calcium channel protein inhibitor and blocker. Chemical Formula -C27H28N2O7, Mol. Weight -492.52 g/mol. its IUPAC Name -O3-(2-methoxyethyl)O5-[(E)-3-phenylprop-2-enyl]2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine- 3,5-dicarboxylate. Categories Anti hypertensive, Soluble in ethanol (20 mg/ml), water (≤ 2 mg/ml), and methanol, Melting point 110 °C, CAS NO - 132203-70-4, Log P - 5.42. Mechanism action of Cilnidipine Blocks the calcium channel and due to it, it dilates both arteriole & venules as a result the pressure in the capillary bed is reduces.

1.3.3 IRBESARTAN[10]

Irbesartan Official in USP30-NF25. Description of Irbesartan is an angiotensin receptor blocker (ARB) used mainly for the treatment of hypertension. It competes with angiotensin II for binding at the AT1 receptor subtype. Chemical Formula -C25H28N6O, Mol. Weight -428.53 g/mol. its IUPAC Name- 2-butyl-3-({4-[2-(2-H-1,2,3,4-tetrazol-5-yl) phenyl]phenyl}methyl)-1,3-diazaspiro[4.4]non-1-en-4-one, Categories Angiotensin Receptor Blocker. Slightly Soluble inn Alcohol and methylene Chloride and Practically in soluble in Water. Melting point - 180-181 °C, CAS NO- 138402-11-6 , Log P - 5.5. Mechanism action of Irbesartan is a non peptide tetrazole derivative and an angiotensin II antagonist that selectively blocks the binding of angiotensin II to the AT1 receptor.

Table .1 Introduction to Marketed Formulation

Brand Name	Dosage form	Contents with strength	Manufacturer
Clindasartan-CH	**Tablet**	Chlorthalidone(6.25mg) Cilnidiline(5 mg) Irbesartan (150 mg)	Globus Labs

2. EXPERIMENTAL WORK

2.1 Analytical Method Development And Validation For Simultaneous Estimation of Chlorthalidone ,Irbesartan and Cilnidipine By RP-HPLC.

2.1.1 Apparatus and Instruments:

- ✓ Model: LC- 20AT
- ✓ Column: C_{18} (25 cm × 0.46 cm) Hypersil BDS
- ✓ Injector: 20µL fixed loop.
- ✓ Detector: SPD 20 A UV Detector
- ✓ Software: Spinchrome
- ✓ Analytical balance: Electronic analytical balance (shimadzu)
- ✓ Corning volumetric flasks and pipettes

2.1.2 Reagents and Materials:

- ✓ Chlorthalidone was procured from Oasis laboratory .
- ✓ Irbesartan was procured from Oasis laboratory
- ✓ Cilnidipine was procured from Oasis laboratory
- ✓ Water
- ✓ Methanol
- ✓ Acetonitrile
- ✓ Hydrogen phosphate

2.1.3 Chromatographic conditions :

- ✓ **Column**: C_{18} (25 cm × 0.46 cm) Hypersil BDS
- ✓ **Mobile Phase**: Acetate Buffer (pH 3.0); Acetonitrile (60:40)
- ✓
- ✓ **Table .3 Intraday and Interday studies of Chlorthalidone, Irbesartan and Cilnidipine**
- ✓ **Detection Wavelength**: 226 nm
- ✓ **Run time**: 9 min
- ✓ **Injection volume** : 20.0 µl

3.Method validation

3.1 Linearity and Range

The linearity for Chlorthalidone, Cilnidipine and Irbesartan were assessed by analysis of combined standard solution in range of 0.625-1.875 µg/ml,0.5-1.5 µg/ml and 30-45 µg/ml respectively. Correlation co-efficient for calibration curve Chlorthalidone, Cilnidipine and Irbesartan was found to be 0.993, 0.991, 0.990 respectively.

The regression line equation for Chlorthalidone, Cilnidipine and Irbesartan are as following:

For Chlorthalidone: **y = 631.5x - 7.333** and For Irbesartan: **y = 32.26 - 21.12** and for Cilnidipine **y = 1159x - 40.51**

3.2 Precision

I. Repeatability

The data for repeatability of peak area measurement for Chlorthalidone(1.25 µg/ml), Cilnidipine (1 µg/ml) and Irbesartan (30 µg/ml), based on six measurements of same solution of Chlorthalidone(1.25 µg/ml), Cilnidipine (1 µg/ml) and Irbesartan (30 µg/ml). The % RSD for Chlorthalidone, Irbesartan and Cilnidipine were found to be 0.716, 0.383 and 1.189 respectively.

II. Intraday precision

The % R.S.D. for Intraday precision was found to be 0.468-0.831. for Chlorthalidone and 0.153-0.377 for Irbesartan and 0.693-1.419 for Cilnidipine

III. Inter day precision

The % R.S.D. for inter day precision was found to be 0.236-0.978. for Chlorthalidone and 0.198-0.805 for Irbesartan and 0.384-1.029 for Cilnidipine

3.3 Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. Percentage recovery for Chlorthalidone was 99.41-99.93 %, and Irbesartan, it was found to be in range of 99.47-99.83%. and for Cilnidipine 99.77-99.93%

Table .2 Repeatability data for Chlorthalidone, Irbesartan and Cilnidipine

DRUG	CONCENTRATION(µg/ml)	%RSD
Chlorthalidone	1.25	0.716
Irbesartan	30	0.3863
Cilnidipine	1	1.189

Table .3 Intraday and Interday studies of Chlorthalidone, Irbesartan and Cilnidipine

DRUG	CONCENTRATION (µg/ml)	%RSD	
		INTRADAY	INTERDAY
Chlorthalidone	0.625	0.831	0.175
	1.25	0.817	0.878
	1.875	0.468	1.625
Irbesartan	15	0.153	0.403
	30	0.377	0.206
	45	0.199	0.463
Cilnidipine	0.5	1.419	0.817
	1	1.032	1.044
	1.5	0.693	1.065

Table. 4 System suitability for Chlorthalidone ,Cilnidipine and Irbesartan

Parameters	Data observed		
	Irbesartan	Chlorthalidone	Cilnidipine
Theoretical plates per	6020	7239	6021
Symmetry factor	1.450	1.370	1.574
Resolution	6.540	10.288	

Table. 5 LOD and LOQ for Chlorthalidone, Cilnidipine and Irbesartan

Parameters	Chlorthalidone	Cilnidipine	Irbesartan
LOD	0.156 µg/ml	0.142 µg/ml	4.402 µg/ml
LOQ	0.473 µg/ml	0.432 µg/ml	13.340 µg/ml

Table. 6 Analysis of marketed formulation

Tablet	Clindasartan-CH		
Label claim	Chlorthalidone (6.25mg)	Irbesartan (150mg)	Cilnidipine (5mg)
Assay	98.11±0.194	99.08±0.900	100.99±0.600

(% of label claim*) Mean ± S. D.

Table .7 Recovery data for Chlorthalidone

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	0.625	0.5	0.493	98.694	99.93 ± 1.13
2		0.625	0.5	0.505	100.924	
3		0.625	0.5	0.501	100.160	
4	100 %	0.625	0.625	0.617	98.688	99.41 ± 0.82
5		0.625	0.625	0.627	100.302	
6		0.625	0.625	0.620	99.239	
7	120 %	0.625	0.75	0.749	99.892	99.42 ± 0.49
8		0.625	0.75	0.742	98.922	
9		0.625	0.75	0.746	99.446	

Table .8 Recovery data for Irbesartan

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	15	12	11.896	99.132	99.78 ± 0.74
2		15	12	11.956	99.634	
3		15	12	12.070	100.585	
4	100 %	15	15	14.897	99.312	99.47 ± 1.17
5		15	15	15.106	100.709	
6		15	15	14.757	98.383	
7	120 %	15	18	18.056	100.312	99.83 ± 0.49
8		15	18	17.879	99.329	
9		15	18	17.972	99.845	

Table .9 Recovery data for Cilnidipine

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	10	8	7.922	99.025	99.77 ± 1.28
2		10	8	8.100	101.249	
3		10	8	7.923	99.033	
4	100 %	10	10	9.923	99.232	99.93 ± 0.70
5		10	10	10.063	100.634	
6		10	10	9.993	99.933	
7	120 %	10	12	12.031	100.262	99.77 ± 50
8		10	12	11.913	99.272	
9		10	12	11.974	99.784	

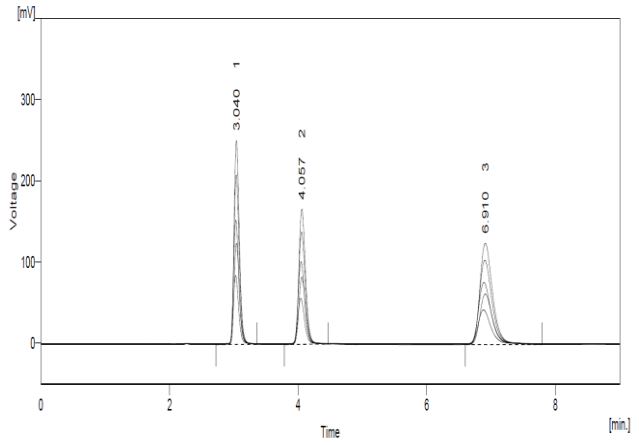

Figure 1 Overlay chromatogram of different concentrations of mixtures of Chlorthalidone ,Cilnidipine and Irbesartan

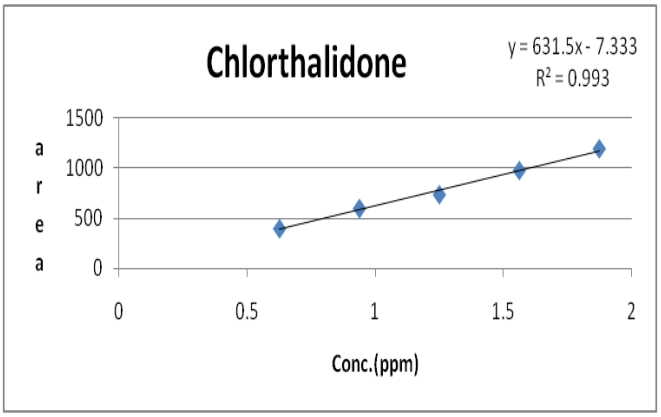

Figure 2 Calibration Curve of Chlorthalidone (0.625-1.875 µg/ml).

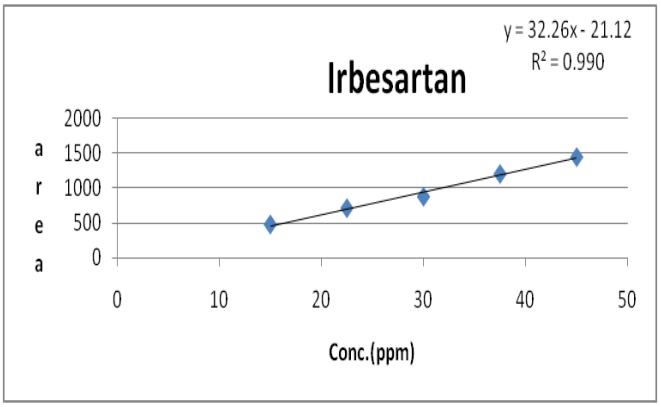

Figure 3 Calibration Curve of Irbesartan (15-45 µg/ml).

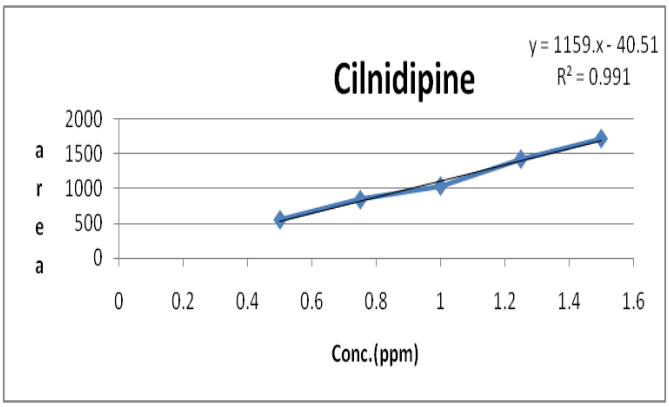

Figure 4 Calibration Curve of Cilnidipine (0.5-1.5 µg/ml).

4. CONCLUSION

- ✓ A simple, specific, accurate and precise RP-HPLC method has been developed and validated as per ICH guideline for Simultaneous Estimation of Irbesartan, Chlorthalidone and Cilnidipine In Their Combined Dosage Form.
- ✓ Validation parameters like Linearity, Accuracy, Precision, Robustness, System suitability, Specificity were tested.
- ✓ Observation of all these parameters leads to the point that developed RP-HPLC method is linear, accurate, precise, specific and robust
- ✓ .It can be successfully adopted for routine quality control analysis of Irbesartan, Chlorthalidone and Cilnidipine in Combined dosage form without any interference from common excipients and impurity.
- ✓ This method can now transfer to utilize for routine laboratory analysis and assay of Irbesartan, Chlorthalidone and Cilnidipine In Their Combined Dosage Form.

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