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Original Research Paper

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF CHLORTHALIDONE AND IRBESARTON IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A Simple precise and accurate method was developed and validated for the simultaneous analysis of chlorthalidone and irbesarton in tablet formulations. The method has been shown adequate separation of the two ingredients from each other. The chromatographic separation was achieved on a reverse phase column C_{18} (250 mm x 4.6 mm, 5µ), in a mobile phase consisting of 0.02 M ammonium phosphate buffer (adjusted to pH 5.5 with triethyl amine), acetonitrile and methanol in the ratio (40:40:20, v/v/v) at a flow rate of 1 ml/min with UV detection at 220 nm. This new method was validated, which include assay determination, accuracy, precision, selectivity, linearity and range, robustness and ruggedness. The current method demonstrates good linearity over range of 40-60% µg/ml of chlorthalidone with r^2 =0.9991 and in the range of 480 - 720 % µg/ml of irbesarton with r^2 = 0.9990. The average recovery of the method is 99.22 % µg/ml and 102.28 % µg/ml for chlorthalidone and irbesarton, respectively. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operator indicating that the method was found to be sufficiently robust and rugged. A simple, accurate, precise RP-HPLC method was developed and validated for the simultaneous determination of chlorthalidone and irbesarton in tablet formulation.

Keywords: Melatonin, Zolpidem, Simultaneous equation method, Method Validation, ICH guidelines.

INTRODUCTION

Chlorthalidone (CHL) is oral diuretic oral antihypertensive agent which is chemically described as (RS) 2-chloro-5-(1-hydroxy-3-oxo-2, 3-dihydro-1H-isoindol-1-yl) benzene-1-sulfonamide (figure 1a).[1] Irbesartan (IBS) is an Angiotensin II blocker is indicated for the treatment of hypertension, chemically 2- butyl-3-[[2-(1H-tetrazol-5-yl)[1, 1- biphenyl]-4-yl] methyl1, 3-diazaspiro [4, 4] non-1-en-4one (figure 1b).[7] Literature survey revealed that CHL can be estimated by spectrophotometry[2], RP-HPLC[3-5] and by HPTLC[6] individually and in combination with other drugs. Several methods have been described for the determination of IBS by UV Spectrophotometry[8,9], HPLC[10,11], UPLC[12] and HPTLC[13] individually and in combination with other drugs. However, there is no analytical method was reported for the simultaneous estimation of CHL and IBS in combined dosage form. Our study, attempts to develop an accurate, precise, specific, linear, simple, rapid, and cost effective analytical method for the simultaneous

estimation CHL and IBS in combined tablet dosage forms by RP- HPLC method and to validate the method as per International Conference on Harmonization (ICH) guidelines[14], and FDA guidelines.[15]

MATERIALS AND METHODS

Instrumentation and Chromatographic Conditions

The HPLC system, used for the method development (Shimadzu corporation Kyoto Japan) LC-2010AT HPLC pump, SPD-20A UV detector, Rheodyne injector and Spinchrome software. Chromatographic separation was performed isocratically at room temperature using C_{18} column (Enable C_{18} G) (250 mm x 4.6mm, 5µ) as a stationary phase with mobile phase composition of 0.02 M Ammonium phosphate buffer (pH 5.5 was adjusted with triethyl amine), acetonitrile and methanol (40:40:20,v/v/v) at a flow rate of 1 mL/ min. The injection volume was 20 µL. Eluent was monitored by a UV detection at 220 nm.

Chemical and Reagents

HPLC grade acetonitrile and methanol were purchased from Merck India Limited (Mumbai, India). Water HPLC grade was obtained from a Milli-Q water purification system was used throughout the analysis. Analytical regent grade triethylamine was purchased from fine chemicals (Mumbai, India), pure drug sample of CHL, % purity 99.36% and IBS, % purity 99.77% were kindly supplied as a gift sample by GKN New Pharma, Pondicherry. These samples were used without further purification. The tablet formulation (ESART/CT) was used for analysis (containing CHL 12.5 mg and IBS 150 mg).

Standard Solutions and HPLC Conditions

Standard stock solution was prepared by dissolving 25.6 mg of CHL and 300.4 mg of IBR dissolved in 100 ml of mobile phase. Working standard solution was prepared by diluting 5 ml of standard solution to 25 ml with mobile phase to obtain a known concentration of 50 µg /ml of CHL and 600 µg/ml of IBR. Sample stock solutions of the formulation (Brand Name- ESART/CT) was prepared by dissolving a quantity of the powdered tablet equivalent to 300 mg of IBR in 100 ml of mobile phase and further diluted to get the same concentration as in the standard solution.

RESULTS AND DISCUSSION

Method Development

Preliminary studies involved trying C_{18} and C_8 columns and testing several mobile phase containing buffers like phosphate and acetate with different pH (4 to 8) and using organic modifiers like acetonitrile, methanol. For the effective separation CHL and IBS C_{18} (250x4.6mm, 5µ) column was selected with mobile phase composition by ammonium phosphate buffer (adjusted pH 5.5 with triethylamine) acetonitrile and methanol (40:40:20, v/v/v) at) at a flow rate of 1 ml/min and a detection wavelength of 220 nm afforded the best separation of these analytes, (figure 2 and table 2). In this developed method for assay of CHL and IBS no internal standard because no extraction or separation step was involved. Peak shape of the CHL and IBS were found to be symmetrical in this optimized chromatographic conditions. Good separation is seen as retention times of CHL and IBS were 6.183 and 9.98 min, respectively, with resolution factor greater than 5. The results of the analysis of the tablet formulations are reported in (table 1)

Method Validation

After method development, the validation of the developed method has been performed in accordance with ICH guidelines which include accuracy, precision, selectivity, linearity and range, limit of detection and limit of quantization, robustness and ruggedness.

Linearity and range

To evaluate linearity of the method, different concentrations of the two analytes in the range of 40-60 µg/ml for CHL and 480-720 µg/ml for IBS were analyzed and the linearity between the peak area and the concentration was examined for each analyte. The results obtained show that the current method is linear for the ranges specified above with a correlation coefficient were found to be above 0.9990, (figure 3a &3b), (table 2).

Accuracy (Recovery)

The accuracy of the method was determined by recovery study, which was determined by spiking the sample with 80%, 100% and 120% concentration raw material. The mixture was analysed by the proposed method the experiment was performed in triplicate and recovery (%) RSD and standard error of mean (SEM) were calculated. Results have shown that the RSD was found to be less than 2 %, (table 3).

Precision

System precision

The system precision of this method was evaluated by the calculating % RSD of the peak areas of six replicate injections of the standard solution, which was found to be 1.09 % and 0.320% for CHL and IBS respectively, (table 4).

Method precision

The method precision of this method was evaluated by the calculating % RSD of the peak areas of six replicate injections of the sample solution, which was found to be 1.10 % and 0.23 % for CHL and IBS (table 4). These results showed that the current method is repeatable.

Selectivity

Selectivity of the current method was demonstrated by good separation of the two analytes from each other. Furthermore, excipient of the tablet formulation did not interfere with the active ingredients of the drug product.

Limit of detection and Limit of quantization

Limit of detection (LOD) and limit of quantization (LOQ) were estimated from the signal-to-noise ratio. The detection limit was determined as the lowest concentration level resulting in a peak area. With signal-to-noise ratio of 3. The quantization limit was determined as the lowest concentration level that provided a peak area with signal-to-noise ratio of 10, (table 2).

Robustness and ruggedness

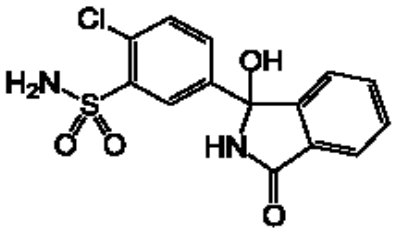
Robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. Robustness of the current method was investigated by analysing samples of the drug product using the same chromatographic conditions, method development but with a small change in the following chromatographic parameters (a) flow rate: 0.8 and 1.2 ml/min instead of 1.0 ml/min, (b) detection wavelength 218 and 222 nm instead of 220 nm (c) pH 5.3 and pH 5.7 instead of pH 5.5 %. The RSD of CHL and IBS were calculated under these conditions and found to be less than 2 %, (table 5). The ruggedness of the current method was estimated by carrying out the experiment as per proposed method in duplicates using different column and analyst on different days. The method was carried out on INERTSIL-ODS C_{18} and phenomenex-Gemini C_{18} column. It was observed that were no marked changes in the chromatograms and the RSD was found to be less than 2 % CHL and IBS, respectively which demonstrated that the RP-HPLC method developed was sufficiently robust for normal expected variations in chromatographic conditions (table 6).

System Suitability

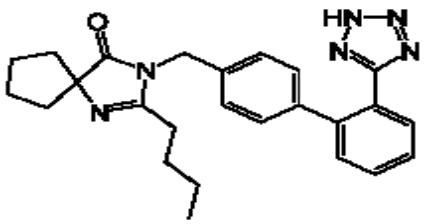
To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factors, theoretical plates, repeatability and resolution.

CONCLUSION

A simple, accurate, precise RP-HPLC method was developed and validated for the simultaneous determination of CHL and IBS in combines tablet form.



(1a) Chlorthalidone



(1b) Irbesartan

Figure 1: Structure of Chlorthalidone and Irbesartan

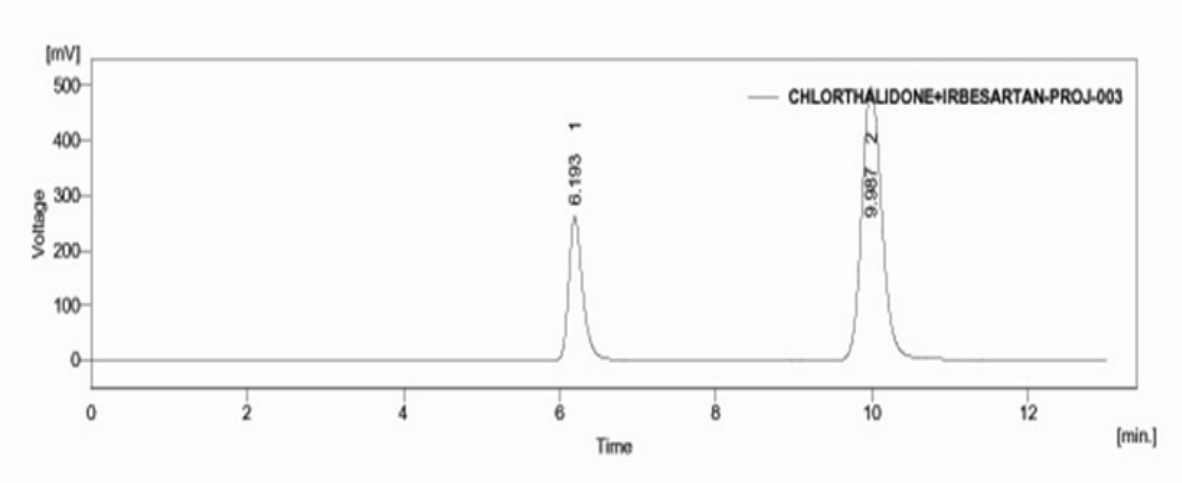


Figure 2: The typical chromatogram of Chlorthalidone and Irbesartan in tablet dosage form

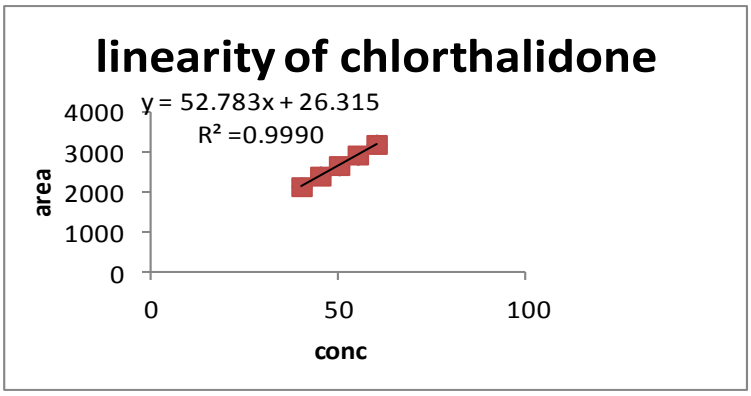


Figure (3a): Linearity of Chlorthalidone

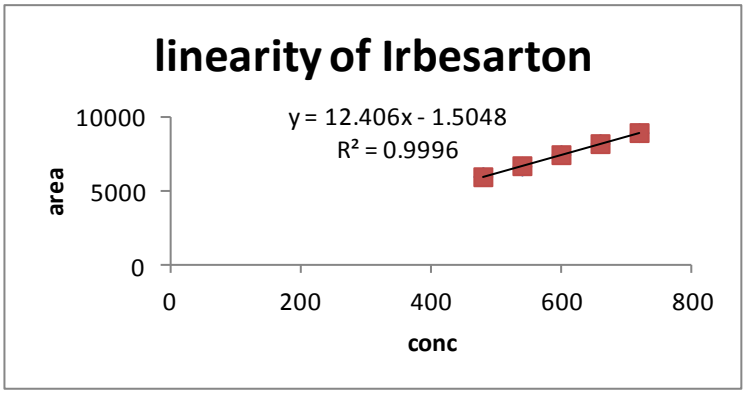


Figure (3b): Linearity of irbesarton

Figure 3 (a&b): Linearity curve of chlorthalidone and irbesarton

Table 1: The estimation of chlorthalidone and irbesarton in tablet dosage form

Drug	Label claim	Amount present	%Assay
Chlorthalidone	12.5mg	12.81±0.25	102.57±0.85
Irbesarton	150mg	149.34±0.25	99.15±0.97

Table 2: Chromatographic parameters of separated analysts

Content	Chlorthalidone	Irbesarton
Linearity range (mg/ml)	40-60	480-560
Slope	52.783	12.406
Intercept	26.315	1.5048
Regression co efficient	0.9990	0.9996
Limit of detection(µg/ml)	0.319	3.11
Limit of quantification(µg/ml)	0.96	9.44
Retention time (min)	6.193	9.087
Tailing factor	1.238	1.077
Resolution factor	-	10.070
Theoretical plates	6808	7770

Table 3: Accuracy (%recovery) of Chlorthalidone and Irbesarton in tablet formulation

Sr. No	Chlorthalidone				Irbesarton			
	Recovery	Avg area	Amount recovery	% Recovery	Recovery	Avg area	Amount recovery	% Recovery
1	80%	2135.814	10.20	102.00	80%	5972.268	119.82	99.86
2	100%	2656.831	12.70	101.50	100%	7417.469	148.83	99.22
3	120	3174.550	15.04	102.28	120%	8924.842	179.08	99.48

Table 4: Precision studies

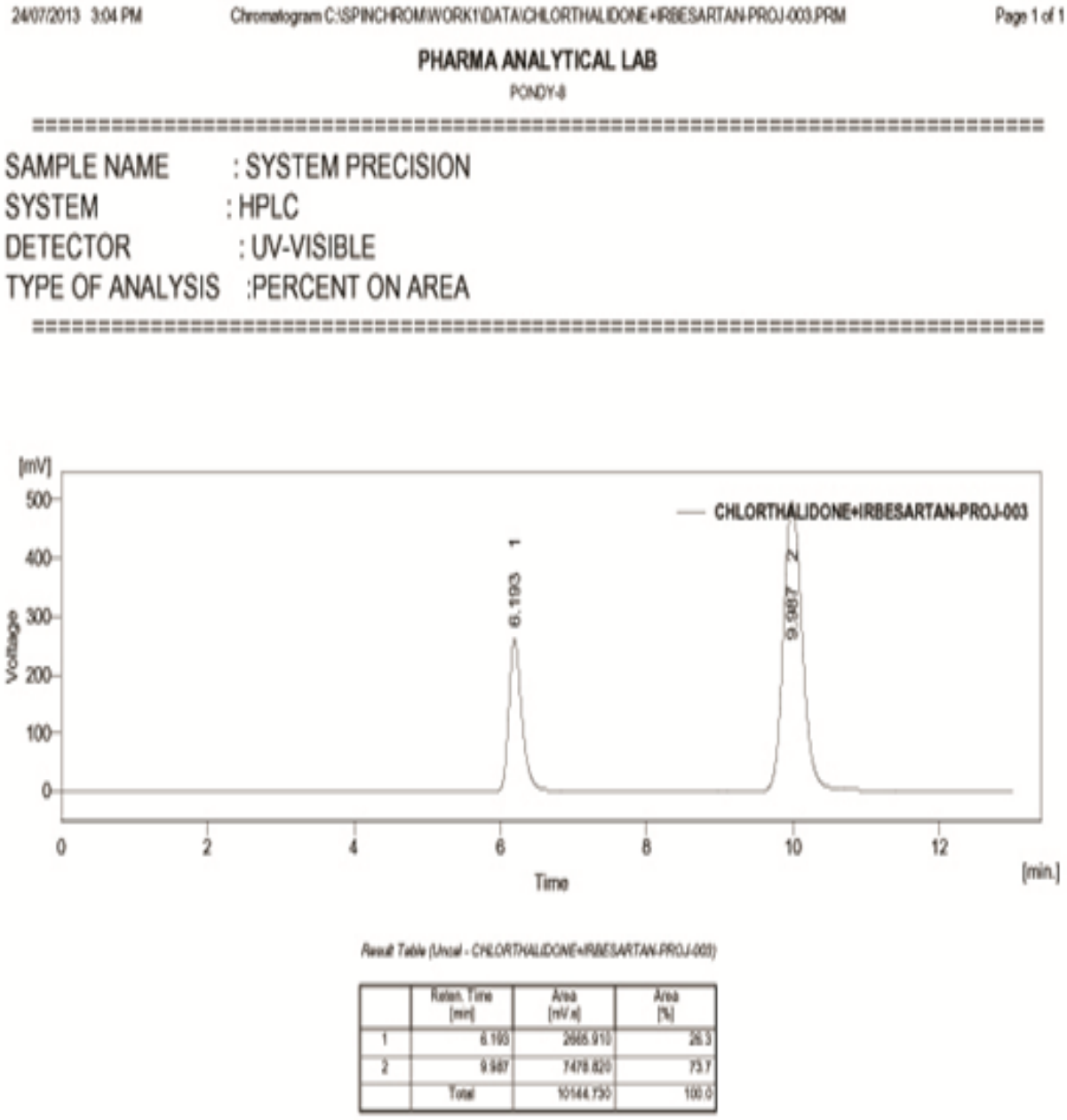
Method precision								
Drug	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Assay 6	Average	%RSD
CHR	101.28	101.62	99.28	101.62	102.63	101.74	101.42	1.10
IBS	99.52	99.49	99.34	99.86	99.46	99.35	99.55	0.23
System precision	Area 1	Area 2	Area 3	Area 4	Area 5	Area 6	Average	% RSD
CHR	2613.570	2686.169	2668.785	2613.573	2678.472	2648.888	2659.171	1.09
IBS	7465.607	7431.874	7437.245	7465.606	7443.832	7489.610	7453.633	0.32

Table 5: Robustness of Chlorthalidone and Irbesarton %RSD at different flow rates, different nm and different pH

Chlorthalidone			Irbesarton	
Parameter	Average area	%RSD	Average area	%RSD
Flow rate(0.8)	3415.66	0.867	8957.37	0.207
Flow rate(1.2)	2477.52	0.605	7097.57	0.491
Wavelength(218)	2221.69	0.180	7274.63	0.089
Wavelength(222)	3634.78	0.144	8629.77	0.080
pH (5.3)	3320.80	0.662	8855.41	0.122
pH (5.7)	2676.18	0.740	6855.13	0.164

Table 6: Ruggedness of the drug on different days and different analyst

Day 1	Chlorthalidone (%)	Irbesarton (%)
Analyst 1, Inst 1	99.61	99.07
Analyst 2, Inst 1	99.90	99.00
Analyst 1, Inst 2	100.32	99.25
Analyst 2, Inst 2	99.20	99.59
Day 2 Analyst 1, Inst 1	101.68	99.52
Analyst 2, Inst 1	99.28	99.46
Analyst 1, Inst 2	101.62	99.49
Analyst 2, Inst 2	101.74	99.82



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