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RP-HPLC METHOD FOR SIMULTATANEOUS DETERMINATION OF IRBESARTAN, LOSARTAN, HYDRO-CHLOROTHIAZIDE AND CHLORTHALIDONE–APPLICATION TO COMMERCIALLY AVAILABLE DRUG PRODUCTS

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ABSTRACT

A simple, precise and stability-indicating HPLC method was developed and validated for the simultaneous determination of anti-hypertensive drugs Irbesartan, Losartan, diuretics Hydrochlorothiazide and Chlorthalidone. The separation was achieved on Hypersil BDS (Length 250 mm × Diameter 4.6 mm Particle size 5 µm) column with gradient flow. The mobile phase at a flow rate of 1.0 mL min^{-1} consisted of 0.05 M sodium dihydrogen phosphate buffer and acetonitrile (Gradient ratio). The UV detection was carried out at 220 nm. The method was successfully validated in accordance to ICH guidelines. Further, the validated method was applied for commercially available pharmaceutical dosage form.

INTRODUCTION: The parent guideline on drug stability testing Q1A (R2) issued by International Conference on Harmonization (ICH) stipulates stress studies to be carried out on a drug in order to establish the drug's inherent stability characteristics [1-2]. Literature studies show various analytical methods reported for the estimation of individual, binary or tertiary combination of anti-hypertensive drugs or in combination with diuretics [3-12]. Recently, HPLC method with fluorescence detection for simultaneous determination of chlorthalidone, valsartan and fluvastatin from human plasma has been reported [13].

The HPLC-MS/MS method for simultaneous estimation of atenolol, bisoprolol, hydrochlorothiazide, chlorthalidone, salicylic acid, enalapril and its active metabolite enalaprilat, valsartan and fluvastatin is also reported [14]. However, so far, no method was reported for the simultaneous determination in combination for Irbesartan, Losartan, Hydrochlorothiazide, Chlor-thalidone and its application to pharmaceutical samples. An attempt was made in this study to develop a rapid, economical, precise and accurate stability-indicating assay method for simultaneous estimation of Irbesartan, Losartan, Hydrochlorothiazide and Chlorthalidone in tablet formulation.

The proposed method is rapid, simple, accurate, and reproducible, and can be successfully employed in the routine analysis of both these drugs simultaneously, in tablet dosage form.

MATERIAL AND METHODS:

Chemicals and Reagents: Drug substances were provided by Sharon Biomedicine, India. All the chemicals and reagents sodium hydroxide, hydrochloric acid, monobasic sodium dihydrogen phosphate, hydrogen peroxide (30 %) were used of Analytical grade. While acetonitrile was procured from Merck (Germany). A Millipore Milli Q plus water purification system (Milford, USA), was used to prepare distilled water (>18 µΩ). The commercially available drug products were used as LOSAR 50 Tablets (Losartan - 50 mg, Unichem Laboratories, India), LOSAR H Tablets (Losartan - 50 mg and Hydrochlorothiazide - 12.5 mg; Unichem Laboratories, India).

Instruments: Integrated HPLC system, Ultimate 3000 manufactured by Dionex (Germany) was used for method development and method validation. This system comprised of a quaternary gradient pump, auto sampler, column oven and a photodiode array detector. PC installed Chromeleon software was used to record and integrates the chromatograms. The analysis was carried out at ambient temperature. Photostability studies were performed in a photostability chamber, from Thermolab (India).

Chromatographic Conditions: Hypersil BDS (Length 250 mm × Diameter 4.6 mm Particle size 5 µm) analytical column from Thermo Fischer Scientific Inc was used as a stationary phase. The flow rate was 1.0 mL min^{-1} and the detector was set at 220 nm. The volume of the sample solution injected was 10 µL. The gradient mobile phase consisted of 0.05 M sodium dihydrogen phosphate buffer and acetonitrile (Mobile Phase-B) with the gradient as mentioned in (**Table 1)**. A membrane filter of 0.45 µm porosity was used to filter and degas the mobile phase.

TABLE 1: MOBILE PHASE GRADIENT FOR CHROMATOGRAPHIC METHOD

Time (min)	% 0.05 M sodium dihydrogen phosphate buffer	% Acetonitrile
0	70	30
3	70	30
5	60	40
15	20	80
18	70	30
20	70	30

Standard and Test solutions: Weighed accurately about 50 mg of each Irbesartan, Losartan, Hydrochlorothiazide and Chlorthalidone reference standard into 50 ml volumetric flask. Added to it 20.0 ml methanol and sonicated to dissolve. Diluted this solution up to volume with diluent (Buffer: Methanol 30:70 v/v). Pipette out 5.0 ml of this solution into 50 ml volumetric flask and diluted to volume with diluent. (100 µg mL^{-1} each of Irbesartan, Losartan, Hydrochlorothiazide and Chlorthalidone).Similarly, the test solutions were prepared at same concentration using same diluents. (100 µg mL^{-1} of each).

Method Development: A variety of mobile phases were investigated in the development of a stability-indicating LC method for the analysis of Irbesartan, Losartan, Hydrochlorothiazide and Chlorthalidone drug substances. The suitability of mobile phase was decided on the basis of selectivity and sensitivity of the assay, stability studies and separation among impurities formed during forced degradation studies.

- **Wavelength Selection:** The individual drug substance solution at concentration of 100µg mL^{-1} in diluent was scanned on PDA from 190 nm to 400 nm. The maximum wavelength were observed Hydrochlorothiazide (224 nm and 271 nm), Chlorthalidone (196 nm), Irbesartan (202 nm) and Losartan (197 nm and 251 nm). However detection was carried out at 220 nm on basis of higher response **(Fig. 1 and Table 2).**

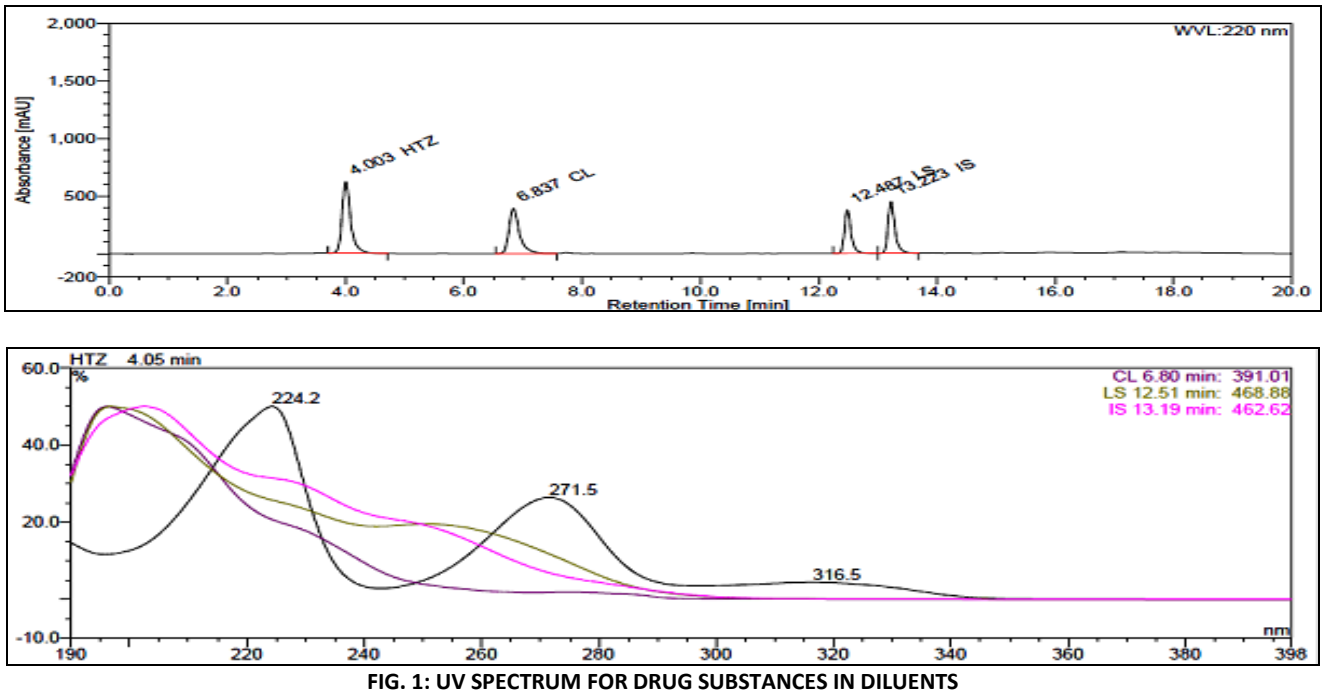


FIG. 1: UV SPECTRUM FOR DRUG SUBSTANCES IN DILUENTS

TABLE 2: AREA RESPONSE OF PEAKS AT DIFFERENT WAVELENGTHS

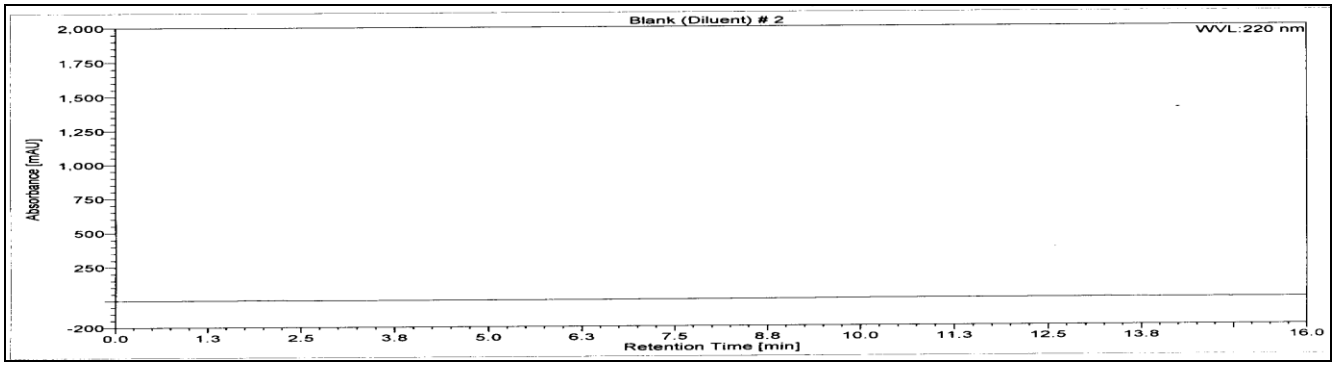
Wavelength (nm)	HTZ	CL	LS	IS
220	106.077	79.797	49.645	61.321
230	66.306	58.102	41.734	55.227
240	8.362	31.149	34.415	42.270
250	11.057	12.610	34.975	36.237
260	33.829	7.420	31.756	25.729
280	37.587	5.323	11.189	8.309
300	8.007	0.161	1.056	1.269

Method Validation: The optimized chromatographic conditions were validated by evaluating specificity-Forced degradation, linearity, precision, accuracy, robustness and system suitability parameters in accordance with the ICH guideline Q2 (R1) [2].

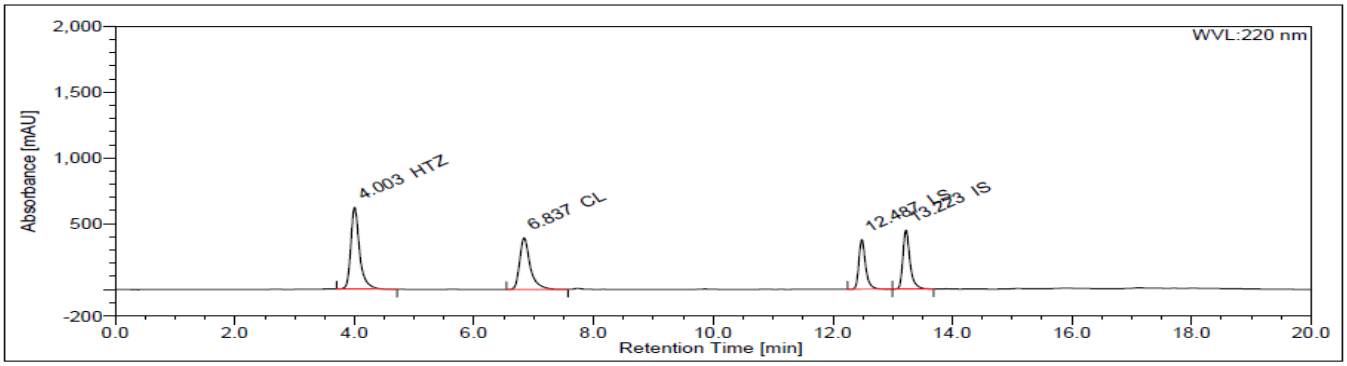
Specificity- Forced Degradation Study:

- **Acid Hydrolysis:** Forced degradation study was conducted on 5 ml stock solution of standard solution containing 1000 µg mL^{-1} of each drug substances by exposing with 5ml of 1N hydrochloric acid for 1 hour at room temperature. Then neutralized with base and dilute up to 50 ml with diluent.
- **Base Hydrolysis:** Forced degradation study was conducted on 5 ml stock solution of standard solution containing 1000 µg mL^{-1} of each drug substances by exposing with 5ml of 1N sodium hydroxide for 30 minutes at room temperature. Then neutralized with acid and dilute up to 50 ml with diluent.
- **Oxidation:** Forced degradation study was conducted on 5 ml stock solution of standard solution containing 1000 µg mL^{-1} of each drug substances by exposing with 5ml of 5% H_2O_2 for 3 hours at room temperature. Then dilute up to 50 ml with diluent.
- **Thermal Degradation:** Solid drugs powder was kept in dry oven at 105°C for 24 hours.
- **Photolysis:** Photolysis studies were carried out on solid drugs, their combination and their dosage form. The sample in a petri plate was spread as a thin layer (1 mm) and exposed to light in a photostability chamber. The method's analytical data were collected at a single wavelength of 220 nm. Additional PDA detector data were collected for the peak purity evaluation.

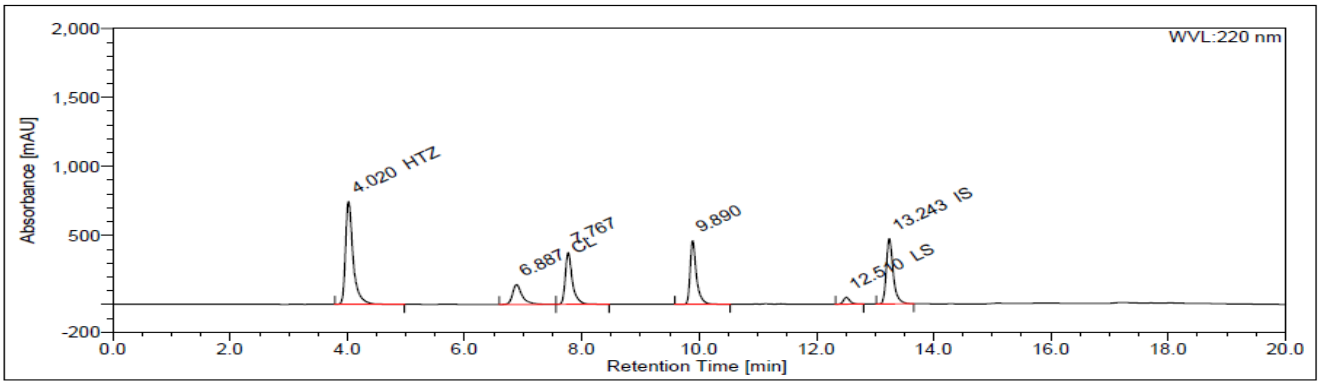
The chromatograms were extracted for Peak purity and demonstrated as in **(Fig. 2a, b, c, d, e, f, g and Table 3).**



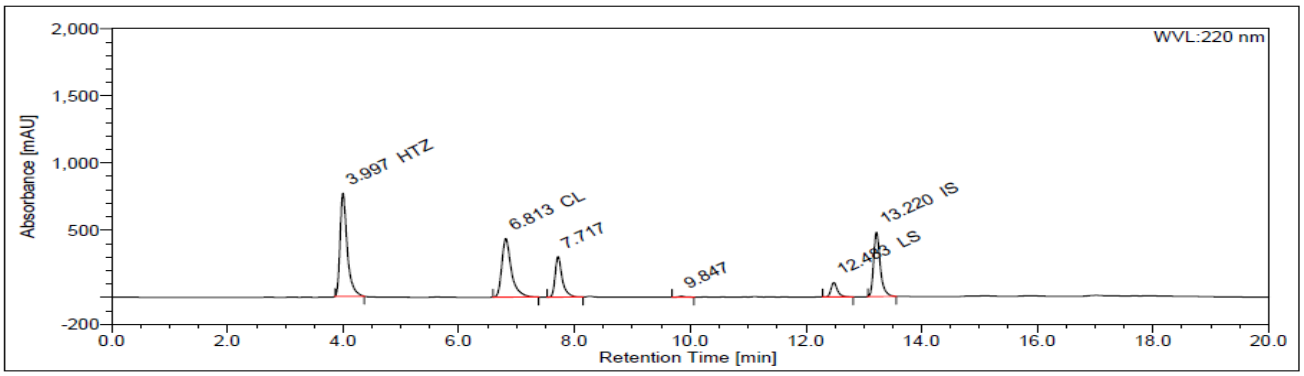
(a) BLANK SOLUTION



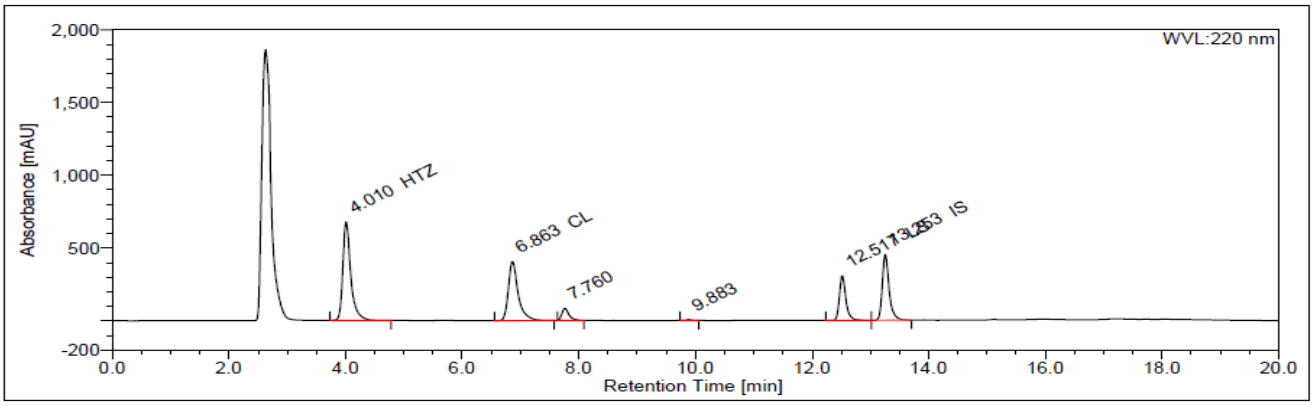
(b) STANDARDS SOLUTION



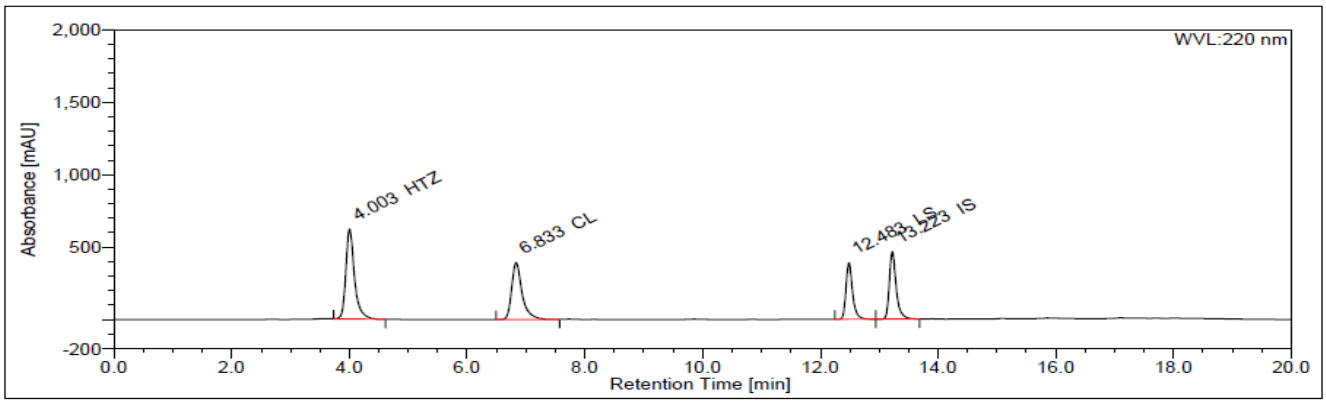
c) SPECIFICITY-ACID HYDROLYSIS



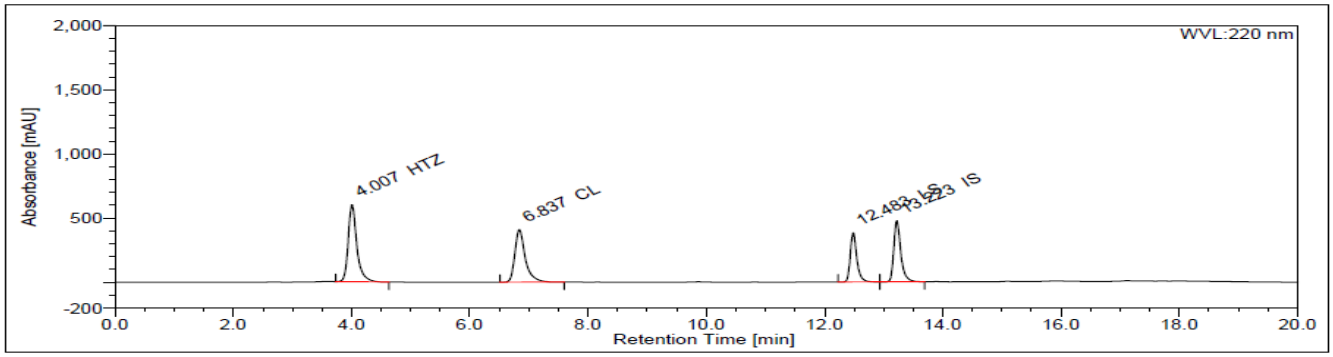
d) SPECIFICITY-ALKALINE HYDROLYSIS



e) SPECIFICITY-PEROXIDE OXIDATION



f) SPECIFICITY PHOTOLYTIC DEGRADATION



g) SPECIFICITY-THERMAL DEGRADATION

FIG. 2: CHROMATOGRAMS FOR STRESSED CONDITIONS

TABLE 3: RESULTS FOR FORCED DEGRADATION STUDY

Degradation Condition		HTZ	CL	IS	LS
Acid hydrolysis	% Degradation	0.00	66.11	0.00	86.99
	Peak purity	1000	1000	1000	1000
Alkaline hydrolysis	% Degradation	0.00	0.00	2.87	72.28
	Peak purity	1000	1000	1000	1000
Peroxide Oxidation	% Degradation	0.00	1.26	0.82	19.85
	Peak purity	1000	1000	1000	1000
Photolytic degradation	% Degradation	0.00	0.00	0.00	0.00
	Peak purity	1000	1000	1000	1000
Thermal degradation	% Degradation	2.72	0.00	0.00	0.15
	Peak purity	1000	1000	1000	1000

Linearity: Standard stock solution of the drug was diluted to prepare linearity standard solutions in the concentration range of 10 –150 µg mL^{-1} for all Hydrochlorothiazide, Chlorthalidone, Irbesartan and Losartan. Three sets of such solutions were prepared. Each set was analyzed to plot a calibration curve. Slope, intercept and coefficient of determination (r^2) of the calibration curves were calculated to ascertain linearity of the method **(Fig. 3 and Table 4).**

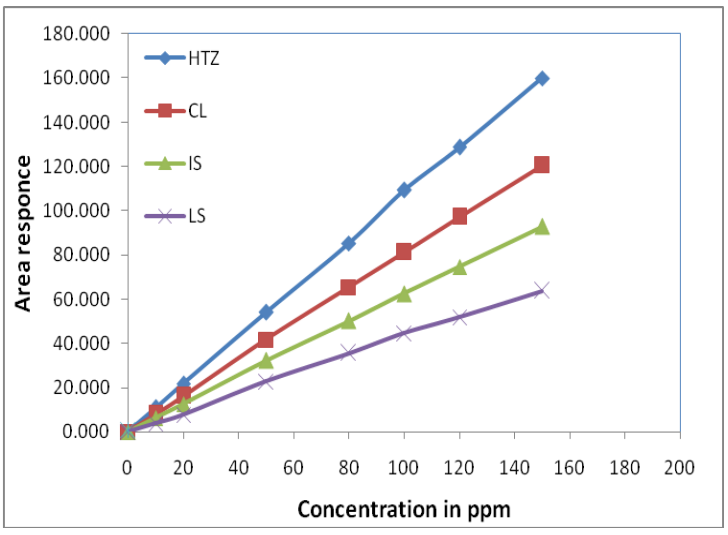


FIG. 3: LINEARITY CURVE WITH CORRELATION CO-EFFICIENT

TABLE 4: SLOPE, INTERCEPT AND REGRESSION COEFFICIENT

Drug substance	Slope	Intercept	Regression (r^2)
Hydrochlorothiazide (HTZ)	1.069	0.473	1.000
Chlorthalidone (CL)	0.752	0.228	1.000
Irbesartan (IS)	0.769	0.043	1.000
Losartan (LS)	0.701	-0.226	0.999

Recovery: Recovery of the method was determined by analyzed the drug products and synthetic mixture of drug products with 50%, 100% and 150% levels. These mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery (%); RSD (%) were calculated **(Table 5 and 6).**

Precision: The precision of the proposed method was evaluated by carrying out six independent assays of test samples. RSD (%) of six assay values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different analyst on another instrument **(Table 7).**

Robustness: The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were wavelength (altered by ±0.3 nm), column oven temperature (altered by ±5°C) and pH of buffer in mobile phase (altered by ±0.2). These chromatographic variations were evaluated for resolution between all drug substances **(Table 8 to 10).**

TABLE 5: RECOVERY FROM COMMERCIALLY AVAILABLE SAMPLES

% Recovery	LOSAR Tablets LS 50mg Unichem Lab Ltd	LOSAR H Tablets LS 50mg and HTZ 12.5mg Unichem Lab Ltd	
	Recovery (%) For LS	Recovery (%) For HTZ	Recovery (%) For LS
50 %	101.41	98.52	98.71
	99.02	99.61	99.93
	98.08	101.51	101.82
100 %	100.20	101.15	101.12
	98.79	99.08	99.16
	98.84	98.45	98.49
150 %	99.97	98.46	98.37
	99.06	98.15	98.12
	100.94	100.51	100.44
Avg	**99.59**	**99.49**	**99.57**
RSD	**1.11**	**1.28**	**1.33**

TABLE 6: RECOVERY ON SYNTHETIC MIXTURE OF ALL FIVE DRUG SUBSTANCE

% Recovery	% Assay			
	HTZ	CL	IS	LS
Level – 50%	99.61	98.49	99.84	98.25
	101.45	98.37	99.45	100.12
	100.36	100.85	101.58	99.21
Level – 100%	100.56	101.87	101.61	99.22
	99.09	101.69	101.79	97.65
	98.32	99.85	101.13	97.11
Level – 150%	98.91	101.11	100.58	97.50
	99.48	100.56	98.78	98.03
	99.79	98.20	99.10	98.84
Average	**99.79**	**100.11**	**100.42**	**98.41**
RSD	**0.97**	**1.47**	**1.18**	**0.99**

***** Chlorthalidone and Irbesartan drug substances spiked with Losar H drug products.

TABLE 7: PRECESSION

Sr. Nos.	% Assay - Repeatability				% Assay- Intermediate Precision			
	HTZ	CL	IS	LS	HTZ	CL	IS	LS
1	99.74	99.49	99.66	99.31	99.17	99.67	99.74	99.77
2	99.42	99.18	100.15	99.61	99.81	99.20	100.19	99.89
6	99.60	99.26	99.85	99.71	99.65	99.18	99.91	99.81
4	99.24	99.37	99.97	99.02	99.80	99.84	99.54	99.59
5	99.81	99.57	99.90	98.94	99.23	99.67	100.01	99.57
6	99.49	99.59	99.88	99.61	99.18	99.67	99.69	99.42
Avg	**99.55**	**99.41**	**99.91**	**99.37**	**99.47**	**99.54**	**99.85**	**99.68**
RSD	**0.21**	**0.17**	**0.16**	**0.33**	**0.31**	**0.28**	**0.24**	**0.18**
Average for Precision and Intermediate precision					**99.51**	**99.47**	**99.87**	**99.52**
RSD for Precision and Intermediate precision					**0.26**	**0.23**	**0.19**	**0.30**

TABLE 8: CHANGE IN WAVELENGTH

Wavelength	% Assay			
	HTZ	CL	IS	LS
217 nm	99.18	99.76	99.57	99.47
220 nm	99.65	99.17	99.19	99.39
223 nm	99.85	99.47	9956	99.48
Average	**99.58**	**99.47**	**99.44**	**99.45**
RSD	**0.34**	**0.30**	**0.22**	**0.05**

TABLE 9: CHANGE IN COLUMN OVEN TEMPERATURE

Column oven temperature	% Assay			
	HTZ	CL	IS	LS
20°C	99.11	99.33	99.56	99.36
25°C	99.65	99.17	99.19	99.39
30°C	99.14	99.50	99.61	99.77
Average	**99.30**	**99.33**	**99.45**	**99.51**
RSD	**0.31**	**0.17**	**0.23**	**0.23**

TABLE 10 - CHANGE IN PH OF BUFFER SOLUTION IN MOBILE PHASE

pH of buffer	% Assay			
	HTZ	CL	IS	LS
pH 2.3	99.37	99.68	99.20	99.50
pH 2.5	99.65	99.17	99.19	99.39
pH 2.7	99.22	99.45	99.30	99.64
Average	**99.41**	**99.43**	**99.23**	**99.51**
RSD	**0.22**	**0.26**	**0.06**	**0.13**

Solution stability: To assess the solution stability, standard and test solutions were kept at 25°C (laboratory temperature) for 24 hrs **(Table No. 11).**

TABLE 11: RESULTS FOR SOLUTION STABILITY

Time (Hours)	% Assay			
	HTZ	CL	IS	LS
Initial	99.85	100.09	99.82	98.40
5	98.80	99.24	99.37	98.24
8	99.96	100.09	100.30	98.58
12	99.57	99.72	99.62	98.69
18	99.40	99.46	100.19	88.93
24	96.57	97.75	99.34	80.59

System Suitability: The system suitability parameters with respect to theoretical plates, tailing factor, repeatability and resolution between peaks of all drug substances were defined **(Table No.12).**

TABLE 12: CHROMATOGRAPHIC PARAMETERS OF SYSTEM SUITABILITY

Drug substances	RT (min)	Theoretical plates	Symmetry	Resolution	Peak purity
HTZ	4.03	5288	1.66	11.36	1000
CL	6.92	9259	1.60	21.13	1000
LS	12.60	40643	1.33	2.93	1000
IS	13.34	42797	1.35	-	1000

RESULTS AND DISCUSSION:

HPLC method development: The maximum absorption wavelength of the reference drug solution and of the forcefully degraded drug solution was found to be 220 nm. This was observed from the UV absorption spectra (Fig. 1) and was selected as detection wavelength for LC analysis. The main objective of this chromatographic method was separation of degraded impurities from all drugs. Forced degradation study revealed a critical separation of closely eluting impurity formed from the Hydrochlorothiazide, Chlorthalidone, Irbesartan and Losartan peaks. Hypersil BDS (Length 250 mm × Diameter 4.6 mm, Particle size 5 µm) helped in resolving all peaks as the column had carbon loading approx 11% against conventional ODS. This effect was observed by using the mobile phase 0.05 sodium hydrogen phosphates (pH 2.5) and acetonitrile in the gradient ratio.

Summary of Validation Parameters: The assay test method is validated for Specificity, Linearity, Precision, Accuracy (Recovery), Stability of Analytical Solution and Robustness and was found to be meeting the predetermined acceptance criteria.

The validated method is Specific, Linear, Precise, Accurate and Robust for determination of assay of Irbesartan, Losartan, diuretics Hydrochlorothiazide and Chlorthalidone drug substances and drug products. Hence this method can be introduced into routine and stability analysis for the assay of Irbesartan, Losartan, diuretics Hydrochlorothiazide and Chlorthalidone drug substances.

CONCLUSION: The stability indicating RP-HPLC assay method was developed and validated for simultaneous determination of Irbesartan, Losartan, diuretics Hydrochlorothiazide and Chlorthalidone drug

substances and drug products. The method was found to be simple, specific, Precise and Robust and can be applied for the routine and stability analysis for commercially available formulation.

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