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## RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF AMLODIPINE BESYLATE, VALSARTAN, TELMISARTAN, HYDROCHLOROTHIAZIDE AND CHLORTHALIDONE: APPLICATION TO COMMERCIALY 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 Amlodipine Besylate, Valsartan, Telmisartan and diuretics Hydrochlorothiazide and Chlorthalidone. The separation was achieved on Cosmosil PAQ (150 mm × 4.6 mm) 5 μm column with gradient flow. The mobile phase at a flow rate of 1.0 mL min<sup>-1</sup> 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<sup>1</sup>.

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<sup>3-11</sup>.

Recently, HPLC method with fluorescence detection for simultaneous determination of chlorthalidone, valsartan and fluvastatin from human plasma has been reported<sup>12</sup>. While, the HPLC method has been developed and validated for quantification of losartan, telmisartan, and valsartan in human urine sample<sup>13</sup>.

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<sup>14</sup>.

However, so far, no method was reported for the simultaneous determination in combination for Hydrochlorothiazide, Chlorthalidone, Amlodipine besylate, Valsartan, Telmisartan, 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 Hydrochlorothiazide, Chlorthalidone, Amlodipine besylate, Valsartan, Telmisartan 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 ( $>18 \mu\Omega$ ). The commercially available drug products were used as VALZAAR 40 (Valsartan - 40 mg, Torrent Pharma, India), TELMA H (Telmisartan - 40 mg and Hydrochlorothiazide - 12.5 mg; Glenmark, India), TAZIOC (Telmisartan - 40 mg and Amlodipine- 5 mg, USV, 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:** Cosmosil PAQ (150 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ) analytical column from Nacalai Tesque INC, Japan was used as a stationary phase. The flow rate was 1.0 mL  $\text{min}^{-1}$  and the detector was set at 220 nm. The volume of the sample solution injected was 10  $\mu\text{L}$ . 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  $\mu\text{m}$  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
8	40	60
11	20	80
15	20	80
18	70	30

**Standard and Test solutions:** Weighed accurately about 50 mg of each Hydrochlorothiazide, Chlorthalidone, Amlodipine besylate, Valsartan, Telmisartan reference standard into 50 ml volumetric flask. Added to it 12.5 ml methanol and sonicated to dissolve. Diluted this solution up to volume with diluent (Buffer: ACN, 70:30 v/v).

Pipette out 5.0 ml of this solution into 50 ml volumetric flask and diluted to volume with diluent. (100  $\mu\text{g mL}^{-1}$  each of Hydrochlorothiazide, Chlorthalidone, Amlodipine besylate, Valsartan, Telmisartan). Similarly, the test solutions were prepared at same concentration using same diluents. (100  $\mu\text{g mL}^{-1}$  of each).

**Method Development:** A variety of mobile phases were investigated in the development of a stability-indicating LC method for the analysis of Hydrochlorothiazide, Chlorthalidone, Amlodipine besylate, Valsartan, and Telmisartan 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 100  $\mu\text{g mL}^{-1}$  in diluent was scanned on PDA from 190 nm to 400 nm. The maximum wavelength were observed Hydrochlorothiazide (224 nm and 271 nm), Chlorthalidone (197 nm), Amlodipine besylate (194 nm and 238 nm), Valsartan (202 nm), Telmisartan (200 nm, 226 nm and 296 nm). However detection was carried out at 220 nm on basis of higher response (Table 2 and Fig. 1).

**TABLE 2: AREA RESPONSE OF PEAKS AT DIFFERENT WAVELENGTHS**

Wavelength (nm)	HTZ	CL	AB	VS	TS
220	7007699	4889135	1519197	3659099	6826700
230	4460150	3605191	1740202	3082518	6792775
240	485333	1906409	1963812	2174204	4314868
250	737294	803379	1280204	1894671	2995497
260	2215073	468808	590182	1611626	2748410
280	2563998	321762	108100	593051	2496815
300	538401	0	67946	93976	3253137

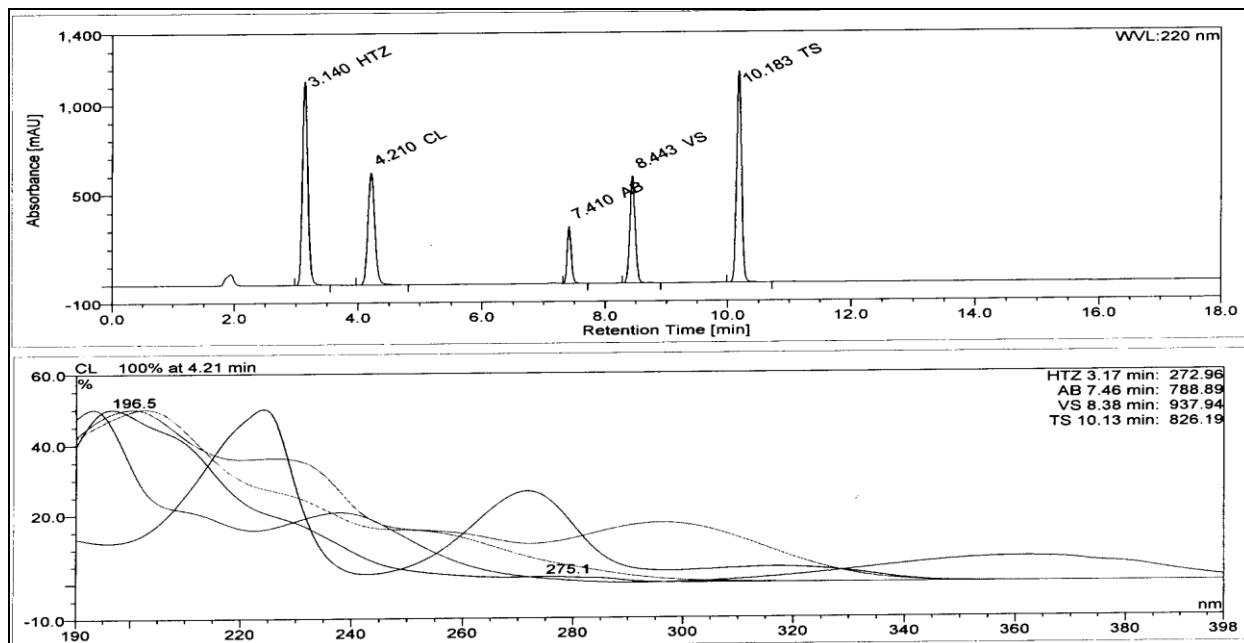


FIG. 1: UV SPECTRUM FOR DRUG SUBSTANCES IN DILUENTS

**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].

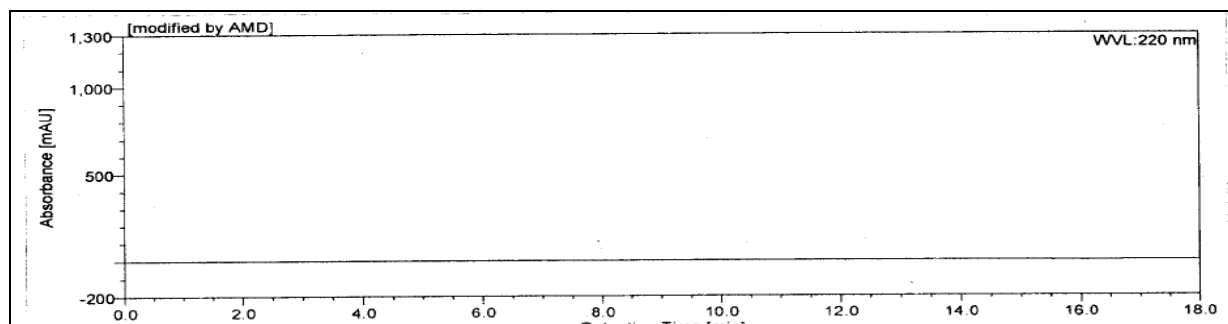
#### Specificity- Forced Degradation Study:

- **Acid and Base Hydrolysis:** Forced degradation study was conducted on 10 mg drug powder of each drug substances by exposing with 4ml of 1N hydrochloric acid/sodium hydroxide for 5 days at room temperature. Then neutralized with acid or base (when necessary) and dilute up to 20 ml with diluent. Pipette out 5 ml of this solution in to 50 ml volumetric flask and diluted up to 50 with diluent.
- **Oxidation:** Forced degradation study was conducted on drug substances by exposing with 1% H<sub>2</sub>O<sub>2</sub> and dilute up to 20 ml with diluent. Pipette out 5 ml of this solution in to 50 ml

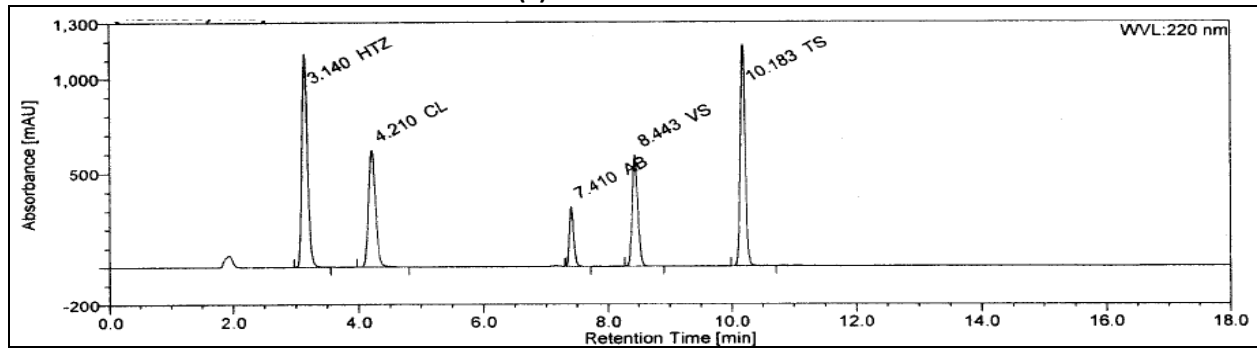
volumetric flask and diluted up to 50 with diluent.

- **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.

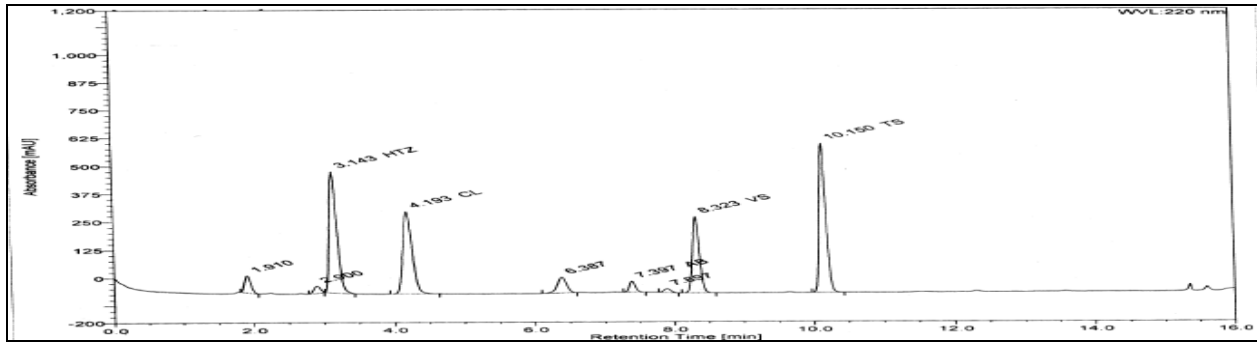
The chromatograms were extracted for Peak purity and demonstrated as in (Fig. 2a, b, c, d, e, f, g and Table 3).



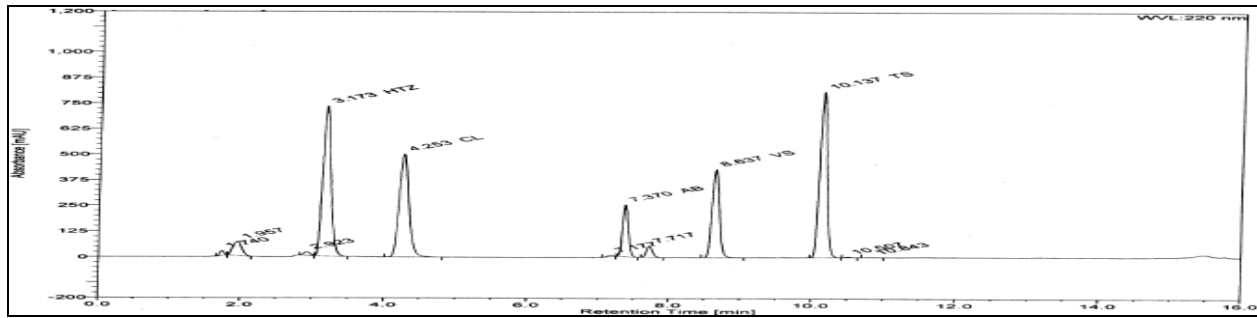
(a) BLANK SOLUTION



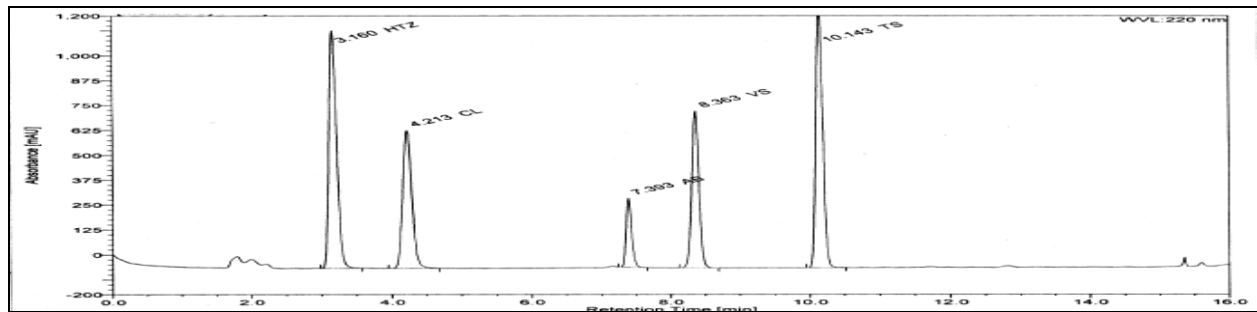
(b) STANDARD SOLUTIONS



c) SPECIFICITY-ACID HYDROLYSIS



d) SPECIFICITY-ALKALINE HYDROLYSIS



e) SPECIFICITY-PEROXIDE OXIDATION

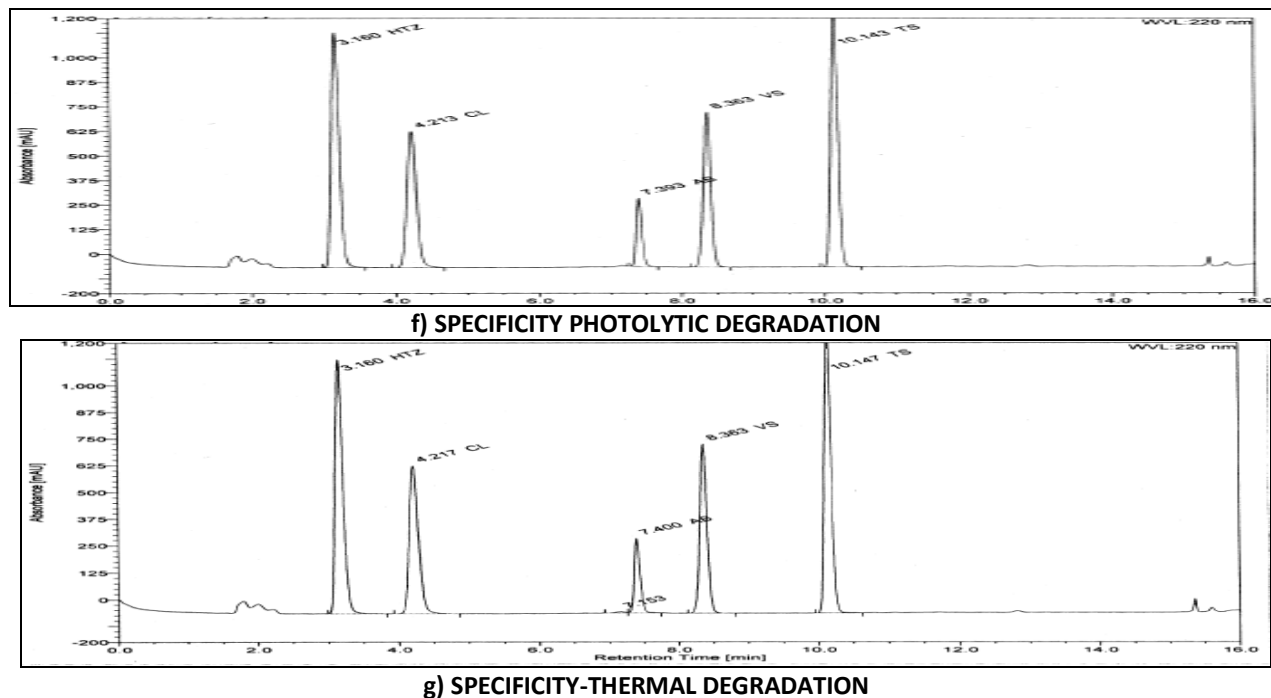


FIG. 2 - CHROMATOGRAMS FOR STRESSED CONDITIONS

TABLE 3: RESULTS FOR FORCED DEGRADATION STUDY

Degradation Condition		HTZ	CL	AB	VS	TS
Acid hydrolysis	% Degradation	13.0	12.5	77.6	4.0	11.4
	Peak purity					
Alkaline hydrolysis	% Degradation	9.1	11.2	17.7	10.2	10.7
	Peak purity					
Peroxide Oxidation	% Degradation	23.2	22.8	25.0	19.1	19.3
	Peak purity					
Photolytic degradation	% Degradation	0.8	2.6	8.6	0	1.6
	Peak purity					
Thermal degradation	% Degradation	0.0	2.2	11.6	0	3.0
	Peak purity					

**Linearity:** Standard stock solution of the drug was diluted to prepare linearity standard solutions in the concentration range of 2 –150 µg mL<sup>-1</sup> for all Hydrochlorothiazide, Chlorthalidone, Amlodipine besylate, Valsartan, and Telmisartan. 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).

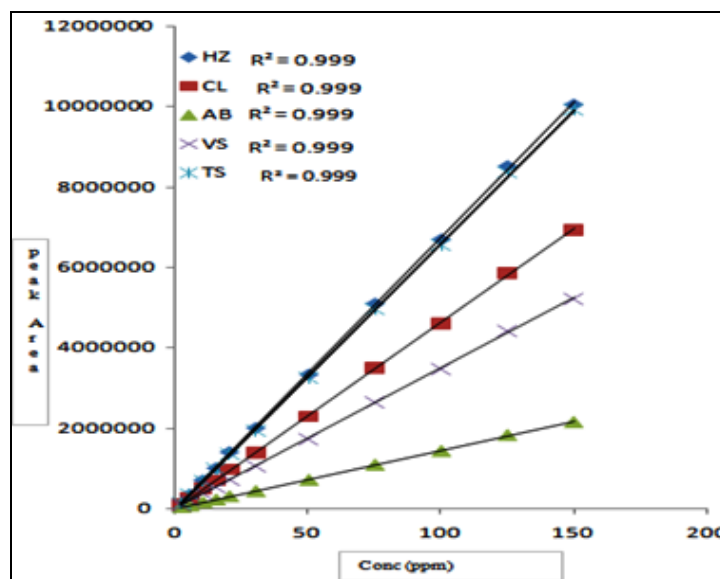


FIG. 3: LINEARITY CURVE WITH CORRELATION CO-EFFICIENT

**TABLE 4: SLOPE, INTERCEPT & REGRESSION COEFFICIENT**

Drug substance	Slope	Intercept	Regression( $r^2$ )
Hydrochlorothiazide	67343	22504	0.9998
Chlorthalidone	46363	11002	0.9998
Amlodipine Besylate	14528X	1280	0.9998
Valsartan	34968X	6424	0.9998
Telmisartan	66206X	3469.4	0.9998

**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 flow rate (altered by  $\pm 0.2$  mL min<sup>-1</sup>), wavelength (altered by  $\pm 0.3$  nm), and column oven temperature (altered by  $\pm 5^\circ\text{C}$ ) and pH of buffer in mobile phase (altered by  $\pm 0.2$ ). These chromatographic variations were evaluated for resolution between all drug substances (**Table 8 to 11**).

**Solution Stability:** To assess the solution stability, standard and test solutions were kept at 25 °C (laboratory temperature) for 24 hrs (**Table 12**).

**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 13**).

**TABLE 5: RECOVERY FROM COMMERCIALY AVAILABLE SAMPLES**

Valzaar 40 Tablets VS 40 mg Torrent Pharma		Telma H Tablets HTZ 12.5mg & TS 40mg Glenmark Pharma Ltd.		Tazioc Tablets TS 40mg & AB 12.5mg USV Ltd	
Level	Recovery (%) For VS	Recovery (%) For HTZ	Recovery (%) For TS	Recovery (%) For TS	Recovery (%) For AB
50 %	99.8	102.0	100.5	99.2	103.0
	101.0	100.9	99.4	98.7	102.3
	99.0	101.2	99.8	99.7	102.5
100 %	100.3	102.7	101.2	99.7	103.4
	99.5	100.7	98.6	99.6	103.1
	100.3	102.6	100.8	99.0	102.6
150 %	100.3	101.2	99.4	99.9	103.4
	99.0	100.8	99.2	99.2	102.8
	98.9	101.3	99.7	99.4	102.5
<b>Avg.</b>	<b>99.8 %</b>	<b>101.4</b>	<b>99.8</b>	<b>99.4</b>	<b>102.8</b>
<b>RSD</b>	<b>0.75</b>	<b>0.82</b>	<b>0.83</b>	<b>0.38</b>	<b>0.38</b>

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

% Recovery	% Assay				
	HTZ	CL	AB	VS	TS
Level – 50%	102.2	101.7	100.1	101.2	100.3
	100.1	99.6	99.1	100.7	98.2
	102.7	99.8	100.3	100.1	101.0
Level – 100%	102.5	100.5	98.6	101.5	101.0
	102.8	101.5	98.8	99.6	101.4
	102.5	101.9	99.5	100.4	101.2
Level – 150%	102.7	100.8	99.6	101.2	100.9
	101.9	99.9	101.3	99.3	100.4
	100.9	100.5	99.0	99.7	99.1
<b>Average</b>	<b>102.0</b>	<b>100.7</b>	<b>99.6</b>	<b>100.4</b>	<b>100.4</b>
<b>RSD</b>	<b>0.94</b>	<b>0.84</b>	<b>0.85</b>	<b>0.79</b>	<b>1.09</b>

\*Chlorthalidone and Amlodipine Besylate drug substances spiked with Valzaar and Telma H drug products.

**TABLE 7: PRECISION**

Sr. No.	Repeatability					Intermediate Precision				
	HTZ	CL	AB	VS	TS	HTZ	CL	AB	VS	TS
1	101.0	100.7	99.5	99.6	99.8	101.8	99.6	99.6	97.7	100.0
2	100.8	101.6	100.9	99.2	101.8	101.3	98.8	100.2	98.9	101.0
6	101.3	99.1	98.7	99.1	98.5	100.9	100.5	101.8	101.1	99.5
4	100.3	99.7	99.7	99.8	100.3	99.1	99.7	99.8	100.0	98.2
5	100.1	99.8	100.6	99.6	100.8	98.1	98.7	98.1	101.3	101.8
6	101.2	99.5	100.6	101.7	100.2	101.2	100.2	100.5	100.3	101.4
<b>Avg.</b>	<b>100.8</b>	<b>100.1</b>	<b>100.0</b>	<b>99.9</b>	<b>100.2</b>	<b>100.4</b>	<b>99.6</b>	<b>100.0</b>	<b>99.9</b>	<b>100.3</b>
<b>RSD</b>	<b>0.48</b>	<b>0.92</b>	<b>0.85</b>	<b>0.96</b>	<b>1.07</b>	<b>1.49</b>	<b>0.77</b>	<b>1.19</b>	<b>1.37</b>	<b>1.35</b>
<b>Average for Precision and Intermediate precision</b>						<b>100.6</b>	<b>99.8</b>	<b>100.0</b>	<b>99.9</b>	<b>100.3</b>
<b>RSD for Precision and Intermediate precision</b>						<b>1.04</b>	<b>0.83</b>	<b>1.00</b>	<b>1.13</b>	<b>1.17</b>

TABLE 8: CHANGE IN FLOW RATE ( $\pm 0.2$  ML MIN<sup>-1</sup>)

Flow rate	% Assay				
	HTZ	CL	AB	VS	TS
Flow rate 0.8 mL min <sup>-1</sup>	101.7	99.2	99.4	97.6	99.8
	101.8	99.1	100.6	99.1	101.3
Flow rate 1.0 mL min <sup>-1</sup>	101.8	99.6	99.6	97.7	100.0
	101.3	98.8	100.2	98.9	101.0
Flow rate 1.2 mL min <sup>-1</sup>	101.4	99.0	99.1	97.2	99.5
	101.3	98.5	100.3	99.0	101.1
<b>Average</b>	<b>101.6</b>	<b>99.0</b>	<b>99.9</b>	<b>98.3</b>	<b>100.5</b>
<b>RSD</b>	<b>0.24</b>	<b>0.38</b>	<b>0.59</b>	<b>0.86</b>	<b>0.77</b>

TABLE 9: CHANGE IN WAVELENGTH

Wavelength	% Assay				
	HTZ	CL	AB	VS	TS
217 nm	101.8	99.4	99.6	97.9	100.3
	101.2	98.4	100.2	98.7	101.0
220 nm	101.8	99.6	99.6	97.7	100.0
	101.3	98.8	100.2	98.9	101.0
223 nm	101.8	99.4	99.6	97.5	100.0
	101.3	98.7	100.2	98.7	100.9
<b>Average</b>	<b>101.5</b>	<b>99.1</b>	<b>99.9</b>	<b>98.2</b>	<b>100.5</b>
<b>RSD</b>	<b>0.29</b>	<b>0.49</b>	<b>0.33</b>	<b>0.61</b>	<b>0.49</b>

TABLE 10: CHANGE IN COLUMN OVEN TEMPERATURE

Column oven temperature	% Assay				
	HTZ	CL	AB	VS	TS
20°C	101.5	98.9	99.3	97.4	99.6
	101.5	98.7	100.6	99.1	101.1
25°C	101.8	99.6	99.6	97.7	100.0
	101.3	98.8	100.2	98.9	101.0
30°C	101.4	99.0	98.9	96.6	99.5
	101.4	97.9	98.4	96.5	100.9
<b>Average</b>	<b>101.5</b>	<b>98.8</b>	<b>99.5</b>	<b>97.7</b>	<b>100.4</b>
<b>RSD</b>	<b>0.09</b>	<b>0.56</b>	<b>0.82</b>	<b>1.13</b>	<b>0.73</b>

TABLE 11: CHANGE IN PH OF BUFFER SOLUTION IN MOBILE PHASE

pH of buffer	% Assay				
	HTZ	CL	AB	VS	TS
pH 4.0	101.9	99.6	98.3	95.7	100.5
	101.5	98.9	101.1	98.5	101.3
pH 4.2	101.8	99.6	99.6	97.7	100.0
	101.3	98.8	100.2	98.9	101.0
pH 4.4	101.2	98.9	100.6	98.6	99.9
	101.2	98.6	102.5	99.3	100.9
<b>Average</b>	<b>101.5</b>	<b>99.1</b>	<b>100.4</b>	<b>98.1</b>	<b>100.6</b>
<b>RSD</b>	<b>0.30</b>	<b>0.43</b>	<b>1.41</b>	<b>1.32</b>	<b>0.56</b>

TABLE 12: RESULTS FOR SOLUTION STABILITY

Time (Hours)	% Assay				
	HTZ	CL	AB	VS	TS
Initial	101.6	99.2	99.9	98.3	100.5
3	102.0	99.4	100.1	98.5	100.7
6	102.1	99.5	100.1	98.3	100.7
9	102.3	99.8	100.9	98.6	100.9
12	101.6	99.2	100.2	98.2	100.7
18	101.6	99.2	100.9	98.7	101.0
24	102.3	99.8	100.9	98.6	100.9
<b>Average</b>	<b>101.9</b>	<b>99.4</b>	<b>100.4</b>	<b>98.4</b>	<b>100.8</b>
<b>RSD</b>	<b>0.33</b>	<b>0.25</b>	<b>0.43</b>	<b>0.17</b>	<b>0.17</b>

TABLE 13: CHROMATOGRAPHIC PARAMETERS OF SYSTEM SUITABILITY

Drug substances	RT (min)	Theoretical plates	Symmetry	Resolution	Peak purity
HTZ	3.14	6253	1.19	-	Passed
CL	4.21	6950	1.14	5.93	Passed
AB	7.41	56678	1.24	19.60	Passed
VS	8.44	44004	1.09	7.24	Passed
TS	10.18	72930	1.06	11.16	Passed

## 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, Amlodipine besylate, Valsartan, and Telmisartan peaks. PAQ (150 mm × 4.6 mm) 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 phosphate (pH 4.1) 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 Hydrochlorothiazide, Chlorthalidone, Amlodipine besylate, Valsartan, Telmisartan drug substances and drug products.

Hence, this method can be introduced into routine and stability analysis for the assay of Hydrochlorothiazide,



Chlorthalidone, Amlodipine besylate, Valsartan, and Telmisartan drug substances.

**CONCLUSION:** The stability indicating RP-HPLC assay method was developed and validated for simultaneous determination of Hydrochlorothiazide, Chlorthalidone, Amlodipine besylate, Valsartan, Telmisartan 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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#### REFERENCES:

1. ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Stability Testing of New Drug Substances and Products Q1A (R2), 2003.
2. ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of analytical procedure: Text and Methodology Q2 (R1), 2005.
3. Ashok K, Sathya A, Senthil K, Patil S, Prathap N B, Lokesh SB, Gopa V: Simultaneous Estimation of Olmesartan medoxomil and Hydrochlorothiazide by RP-HPLC Method from Combined Dosage Form. *Int J Res Pharm Sci* 2010; 1(1): 24-27.
4. Joshi SJ, Pradnya A, Karbhari S I, Bhoir KS, Bindu D Chhanda D: RP-HPLC method for simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet formulation. *Journal of Pharmaceutical and Biomedical Analysis* 2010; 52(3): 362-371
5. Krishnaiah CH, Reddy AR, Ramesh K, Mukkanti: Stability-indicating UPLC method for determination of Valsartan and their degradation products in active pharmaceutical ingredient and pharmaceutical dosage form. *Journal of Pharmaceutical and Biomedical Analysis*: 2010; 53 (3): 483-489.
6. Ashok K, Sathya A, kumar KS, Patil S and Chhanda D: RP-HPLC method for simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet formulation. *Journal of Pharmaceutical and Biomedical Analysis*, 2010; 52 (3): 362-371.
7. Feng G, Mengliang Z, Xiangyong C, Zhonghua W, Yantong S and Jingkai G: Simultaneous quantitation of hydrochlorothiazide and metoprolol in human plasma by liquid chromatography–tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* 2010; 52(1): 149-154.
8. Shah R P and Saranjit S: Identification and characterization of a photolytic degradation product of telmisartan using LC–MS/TOF, LC–MS, LC–NMR and on-line H/D exchange mass studies. *Journal of Pharmaceutical and Biomedical Analysis* 2010; 53(3):755 761.
9. Chi-YL and Chia-HF: Quantization of Irbesartan and major proteins in human plasma by mass spectrometry with time-of-flight analyzer. *Journal of Pharmaceutical and Biomedical* 2011; 54(1):100-105.
10. [10] Chitlange SS, Kiran B and Sakarkar DM: Stability Indicating RP- HPLC Method for Simultaneous Estimation of Valsartan and Amlodipine in Capsule Formulation. *Asian J Research Chem* 2008;1(1): 32-35
11. Wankhede SB, Tajne MR, Gupta KR and Wadodka SGRP-HPLC method for simultaneous estimation of telmisartan and hydrochlorothiazide in tablet dosage form. *Indian Journal of Pharmaceutical Science Research* 2007; 69(2):298-300.
12. Oskar Gonzalez *et al*: Optimization and validation of a SPE-HPLC-PDA-fluorescence method for the simultaneous determination of drugs used in combined cardiovascular therapy in human plasma. *Journal of Pharmaceutical and Biomedical Analysis* 2009; 50(4): 630-639.
13. María DR, Brunetto, Yaritza C, Sabrina C, Dina T, Yelitza D, Fernando O, Carlos A, Máximo G, José ME and Víctor CM: Determination of losartan, telmisartan, and valsartan by direct injection of human urine into a column-switching liquid chromatographic system with fluorescence detection. *Journal of Pharmaceutical and Biomedical Analysis* 2009;50(2):194-199.
14. Gonzalez O, Iriarte G, Rico E, Ferreirós N, Maguregui MI, Alonso RM and Jiménez: RM.LC-MS/MS method for the determination of several drugs used in combined cardiovascular therapy in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010; 15(28): 2685-92.

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