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Method Development and Validation of Hydrochlorothiazide, Amlodipine Besylate and Telmisartan in Tablet Dosage Form by RP-HPLC Method

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ABSTRACT

A simple, selective, rapid, precise and economical reverse phase high-pressure liquid chromatographic method has been developed as per ICH nomination for the simultaneous estimation of Hydrochlorothiazide, Amlodipine besylate and Telmisartan in tablet dosage form. The separation method was carried out by using a mobile phase consisting of 0.02M sodium dihydrogen phosphate, methanol and acetonitrile in the ratio 30:35:35.The detection was carried out by using UV – Visible SPD 20 A at 240nm.The column was phenominex Gemini C18 (250×4.6mm×5µ).The flow rate was selected as 1.5ml/min. The retention time of Hydrochlorothiazide, Amlodipine besylate and Telmisartan was found to be 2.2, 3.7 and 6.1 respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification and system suitability. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Keywords: RP-HPLC, Hydrochlorothiazide, Amlodipine besylate, Telmisartan, Validation.

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INTRODUCTION

Hydrochlorothiazide is chemically designated as 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide, 1-dioxide.It is a first-line diuretic drug. Amlodipine is chemically as (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate.It is used to treat pressure. Telmisartan is chemically 4'-[(1, 4'-dimethyl-2'-propyl [2, 6'-bi-1H-benzimidazol] - 1'-yl) methyl]-[1, 1'-biphenyl]-2-carboxylic acid. It is used to treat pressure [1, 3]. Fixed dose combination containing Hydrochlorothiazide 12.5 mg, Amlodipine besylate 5 mg, Telmisartan 40 mg in the tablet form is available in the market. Literature survey reveals that there was no method established for the RP-HPLC method development for this combination [5-8]. The present work describes the development of validated RP-HPLC method, which can quantify these components simultaneously from a combined dosage form. The present RP-HPLC method was validated following the ICH guidelines [2].

MATERIALS AND METHODS

Reagents and chemicals

Acetonitrile HPLC grade was procured from E.Merch Ltd, Mumbai. Methanol and orthophosphoric acid AR grade were procured from Qualigens Fine Chemical, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. Reference standard of Hydrochlorothiazide, Amlodipine besylate and Telmisartan were procured from Reltsen pharmaceutical Ltd Pondi.

Apparatus and Chromatographic conditions

Chromatographic separation was performed on HPLC-Shimadzu module prominence with SDP 20A detector. Column-Phenominex Gemini(250×4.6mm×5µ) was used for the separation. Mobile phase of buffer 0.02M sodium dihydrogen phosphate with pH 5.5, methanol and acetonitrile were mixed in the ratio 30:35:35, filtered through 0.45µ membrane filter, degassed and used for the separation. The flow rate was 1.5ml/min and inject volume was 20µL with detection at 240nm and analysis was performed at ambient temperature.

Preparation of standard solution

Weigh accurately about 199.8mg of Telmisartan, 32.5mg Amlodipine besylate and 62.7mg Hydrochlorothiazide working standard to a 100ml volumetric flask. Add about 30ml of methanol .Dissolve it completely and sonicate it. Make up to 100ml with methanol. Take 5ml from the above flask and make up to 100ml with mobile phase.

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Preparation of sample solutions

Weigh accurately 20 tablets **(**Telzen-AmH) equivalent to 843.6mg to a 100ml volumetric flask. Add about 30ml of methanol to dissolve it completely and sonicate for 10min with intermediate shaking Make up to 100ml with methanol and filter through 0.45µ GHP filter. Further dilute 5ml with 100ml mobile phase.

Assay method

With the optimized chromatographic condition, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of Hydrochlorothiazide, Amlodipine besylate and Telmisartan was found to be 2.2, 3.7 and 6.1 respectively. This procedure was repeated for the sample solution obtained from the formulation. The concentration of the drug was calculated using the following formulae.

$$\%\text{Assay} = \frac{\text{AT} \times \text{Std dilution} \times \text{Potency} \times \text{Average Wt of the tablet}}{\text{Std Area} \times \text{Sample Dilution} \times 100} \times 100$$

MEHOD VALIDATION

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in table 1.From data obtained, added recoveries of standard drugs were found to be accurate.

TABLE No. 1 ACCURACY (Recovery Studies)

Drugs	Concentration	Avg area	Amt recovery	%Recovery	Mean	SD	%RSD
Hydrochlorothiazide	80	281.11	9.93	99.33	98.99	0.53	0.5
	100	348.74	12.47	98.38			
	120	421.49	14.89	99.28			
Amlodipine besylate	80	351.96	4.01	100.48	99.78	0.79	0.79
	100	433.08	4.94	98.91			
	120	525.15	5.99	99.95			
Telmisartan	80	4592.02	31.8	99.38	99.18	0.44	0.44
	100	5699.17	39.47	98.68			
	120	6896.15	47.76	99.50			

SYSTEM SUITABILITY STUDIES

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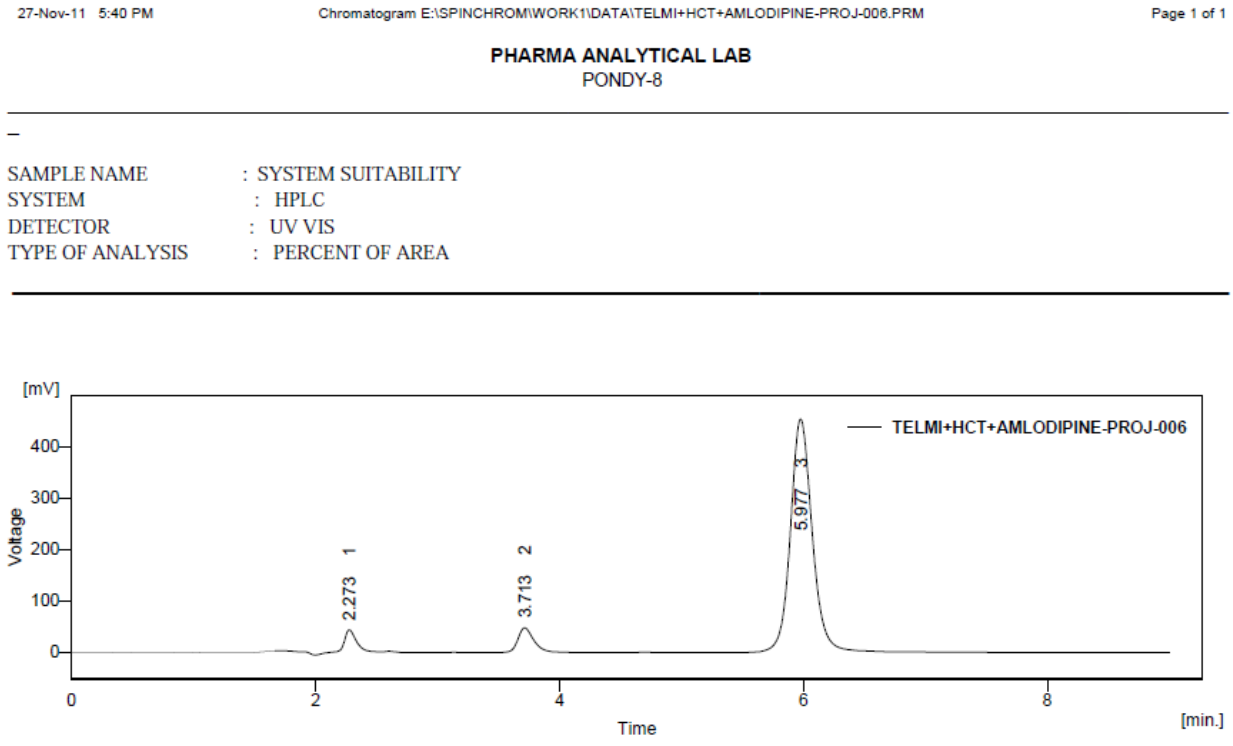
The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and presented in table 2 and fig1. The values obtained demonstrated the suitability of the system for the analysis of the drug combinations. System suitability parameter may fall within ±3% standard deviation range during routine performance of the method.

TABLE No. 2: Analytical Parameters

System suitability parameters	Hydrochlorothiazide	Amlodipine besylate	Telmisartan
RSD	0.714	0.78	0.4771
Tailing factor	1.323	1.515	1.200
No. of theoretical plates	2516	4090	5679
Resolution	-	6.964	8.238
LOD(mcg/mL)	54.13	117.37	90.69
LOQ(mcg/mL)	164.03	355.68	274.84
Retention Time	2.2	3.7	6.1

Figure no 1: Chromatograms



Column Performance Table (From 50% - TELMI+HCT+AMLODIPINE-PROJ-006)

	Reten. Time	Asymmetry [-]	Efficiency [th.pl]	Resolution [-]
1	2.273	1.323	2516	-
2	3.713	1.515	4090	6.964
3	5.977	1.200	5679	8.238

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SPECIFICITY

There is no interference in the standard peak of Hydrochlorothiazide, Amlodipine besylate and Telmisartan. It shows that developed analytical methods was specific in the tablet dosage form of the drug and were presented in table 3.

TABLE No.3 Specificity studies of Hydrochlorothiazide, Amlodipine besylate and Telmisartan in dosage form

Sample	Hydrochlorothiazide		Amlodipine besylate		Telmisartan	
	Avg area*	%Drug Content	Avg area*	%Drug Content	Avg area*	%Drug Content
Standard	352.914	100.18	440.46	99.67	5745.002	99.48

N* =6 Determination of average area

PRECISION

a) Method Precision

The method precision of the method was established by carrying out the analysis of analyte (n=6) using the proposed method. The low value of relative standard deviation showed that the method was precise. The results obtained were presented in table 4.

TABLE No.4: Method precision of Hydrochlorothiazide, Amlodipine besylate and Telmisartan in dosage form

Drugs	%Assay	%RSD of Assay(n=6)
Method precision of Hydrochlorothiazide, Amlodipine besylate and Telmisartan in dosage forms		
Hydrochlorothiazide	**100.18**	**1.003**
Amlodipine besylate	**99.67**	**0.752**
Telmisartan	**99.48**	**0.472**

b) System precision

The system precision of the method was established by six replicate injections of the standard solution containing both the analytes of interest. The percentage RSD was calculated and presented in table 5.

LINEARITY

Linearity of the method was determined at five concentration levels ranging from 20 to 30µg/ml for Hydrochlorothiazide, 10 to 15 µg/ml for Amlodipine besylate and 80 to 120 µg/ml for Telmisartan using for pure drug and the result were tabulated in table 6.The slope and intercept value for calibration curve was Y=3.540,S=3.121(R^2=0.9980) for Hydrochlorothiazide, Y=4.438,S=1.889(R^2=0.9980) for Amlodipine besylate and Y=58.68,S=31.93(R^2=0.9990) for Telmisartan.Fig 2,3and4.

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TABLE No.5: System precision of Hydrochlorothiazide, Amlodipine besylate and Telmisartan in dosage form

S.No	Area peak of Hydrochlorothiazide	Area peak of Amlodipine	Area peak of Telmisartan
1.	351.28	436.242	5782.986
2.	350.350	437.912	5711.245
3.	355.758	441.782	5759.766
4.	354.557	439.163	5737.489
5.	355.189	441.728	5759.686
6.	350.350	445.989	5718.843
Mean	352.914	440.46	5745.002
SD	2.52	3.46	27.41
%RSD	0.714	0.78	0.4771

TABLE No.6: Linearity of Hydrochlorothiazide, Amlodipine besylate and Telmisartan in dosage form

Hydrochlorothiazide		Amlodipine besylate		Telmisartan	
Conc (mcg/mL)	Peak area	Conc (mcg/mL)	Peak area	Conc (mcg/mL)	Peak area
20	298.163	10	389.904	80	4812.217
22.5	337.369	11.25	404.205	90	5347.697
25	352.422	12.5	435.665	100	5691.882
27.5	381.944	13.75	478.482	110	6343.275
30	408.070	15	531.231	120	7038.987

Figure no 2: Calibration curve of Hydrochlorothiazide

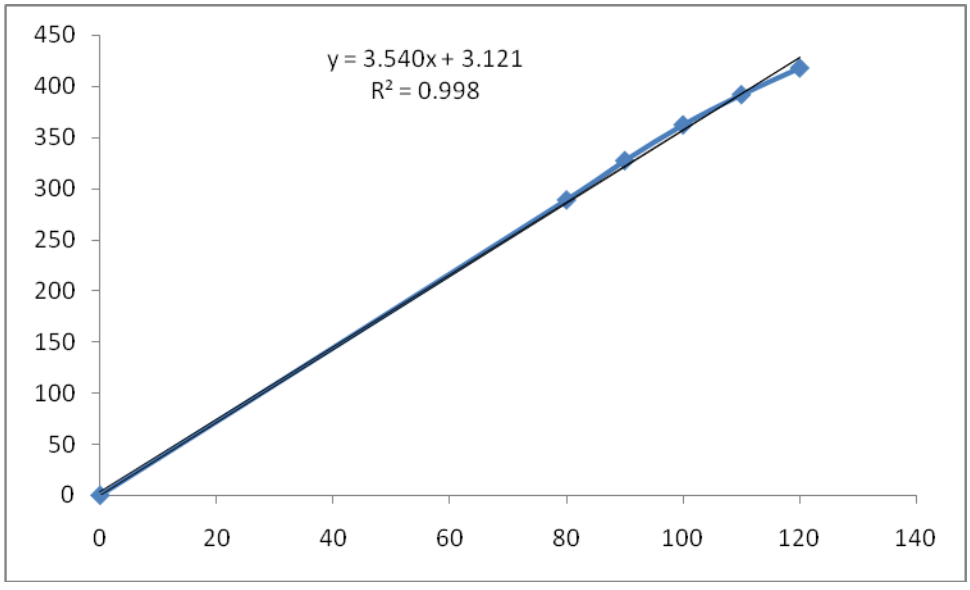

Figure no 3: Calibration curve of Amlodipine besylate

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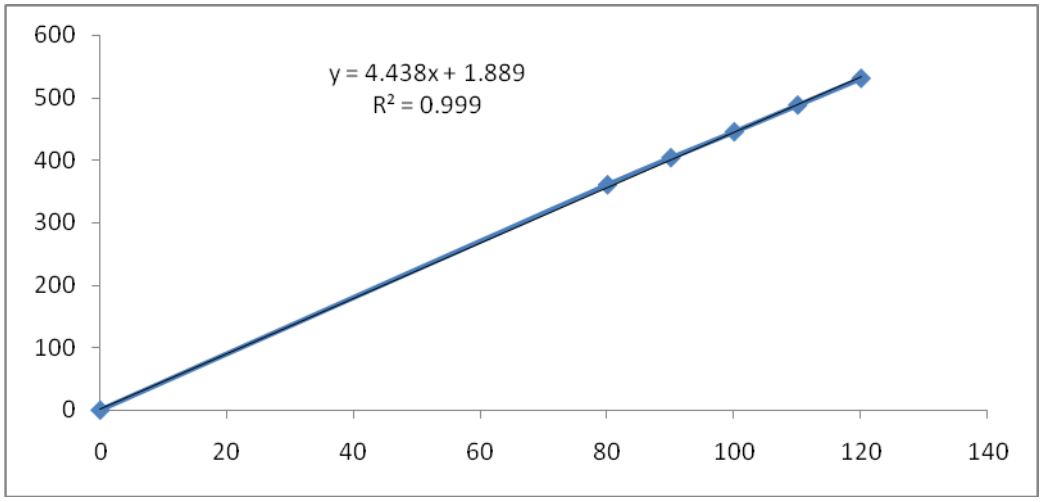
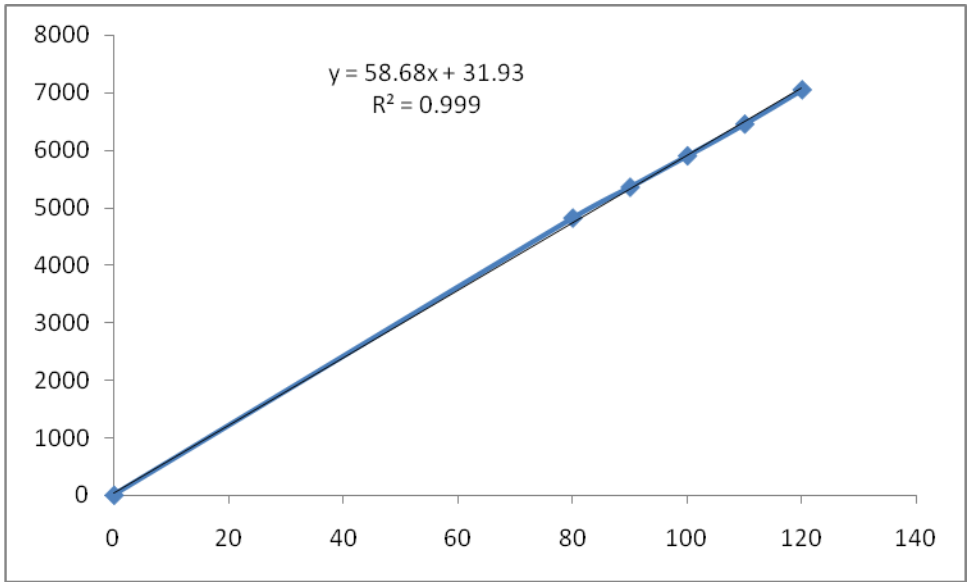


Figure no 4: Calibration curve of Telmisartan



RUGGUDNESS AND ROBUSTNESS

The ruggedness of the method was determined by carrying out the experiment in different laboratories with different instruments like HPLC-Shimadzo module prominence. UV Visible SDP 20A detector. Column-Gemini (250×4.6mm×5µ) by different operators using different column of similar type. Robustness of the method was determined by slight change in the chromatographic condition. It is observed that there were no observed changes in the chromatograms, which demonstrated that RP-HPLC method is rugged and robust. The results were in the tables 7,8,9 and10.

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TABLE No.7: Method Ruggedness of Hydrochlorothiazide, Amlodipine besylate and Telmisartan in dosage form

Sample. No	% Assay of Hydrochlorothiazide	% Assay of Amlodipine besylate	% Assay of Telmisartan
Analyst – 1	98.9	102.0	99.76
Analyst – 2	99.2	100.1	98.99
Analyst – 3	99.7	101.4	99.7
Analyst – 4	99.6	101.3	99.5

TABLE No.8: Method Robustness of Hydrochlorothiazide in dosage form

Robustness Criteria	Avg peak area	SD	%RSD for Area
Change in flow +0.2	311.998	0.63	0.2
Change in flow -0.2	401.328	0.266	0.06
Change in wavelength by -2nm	303.177	0.196	0.064
Change in wavelength by +2nm	456.32	1.62	0.35

TABLE No.9: Method Robustness of Amlodipine besylate in dosage form

Robustness Criteria	Avg peak area	SD	%RSD for Area
Change in flow by +0.2	388.363	2.73	0.7
Change in flow by -0.2	499.26	8.93	1.78
Change in wavelength by -2nm	426.85	4.61	1.08
Change in wavelength by +2nm	449.45	7.56	1.68

TABLE No.10: Method Robustness of Telmisartan in dosage form

Robustness Criteria	Avg peak area	SD	%RSD
Change in flow by +0.2	5086.71	45.99	0.904
Change in flow by -0.2	6562.5	0.49	0.007
Change in wavelength by -2nm	5224.29	24.57	0.47
Change in wavelength by +2nm	6443.28	166.86	2.58

RESULTS AND DISCUSSION

HPLC method was developed. It was validated for the estimation of Hydrochlorothiazide, Amlodipine besylate and Telmisartan in tablet dosage form using HPLC Shimadzu Prominence with UV-Visible SDP 20A Detector and Phenominex Gemini C18 (250x4.6mm, 5µ) column, injection of 20 µl is injected and eluted with the mobile phase of sodium dihydrogen phosphate buffer, methanol and acetonitrile in the ratio 30:35:35, which was pumped at a flow rate of 1.5 ml at 240 nm. The peak of Hydrochlorothiazide, Amlodipine besylate and Telmisartan are found well separated at 2.2, 3.7 and 6.1 respectively. The developed method was validated for various parameters as per ICH guidelines like Accuracy, Precision, Linearity, Specificity, Ruggedness, Robustness, LOQ and LOD.

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CONCLUSION

The analytical method validation of Hydrochlorothiazide, Amlodipine besylate and Telmisartan by RP HPLC method was found to be satisfactory and could be used for the routine pharmaceutical analysis of Hydrochlorothiazide, Amlodipine besylate and Telmisartan.

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