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Simultaneous RP-HPLC determination of hydrochlorthiazide and telmisartan in pharmaceutical preparations

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ABSTRACT

A simple, fast and precise reversed phase high performance liquid chromatographic method is developed for the simultaneous determination of Hydrochlorthiazide and Telmisartan using Ramipril as an internal standard. Chromatographic separation of the two drugs was performed on a Inertsil ODS-3V, C_{18} column (250mm × 4.6 mm, 5μm) as stationary phase with a mobile phase comprising of Buffer*: Acetonitrile (60:40) Buffer*: 0.05M KH_2PO_4+0.1% Triethylamine, pH to 4.3 with Orthophosphoric Acid, filtered and degassed, at a flow rate of 1.5mL/min,column temperature at 40^0C and UV detection at 210nm. The proposed method was validated for linearity, accuracy, precision, LOD, LOQ. Linearity, accuracy and precision were found to be acceptable over the ranges of 128-192μg/mL for Telmisartan, 40-60μg /mL for Hydrochlorthiazide. It can be conveniently adopted for routine quality control analysis.

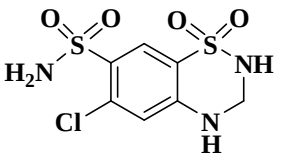
KEYWORDS

ICH guidelines;
Validation;
Column liquid chromatography;
Pharmaceutical preparations;
Hydrochlorthiazide;
Telmisartan;
Ramipril.

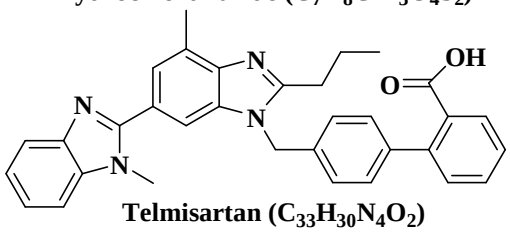
INTRODUCTION

Hydrochlorthiazide is 2H-1,2,4-Benzothiadiazine-7-sulfonamide,6-chloro-3,4-dihydro-,1,1-dioxide. 6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide, $C_7H_8ClN_3O_4S_2$, Hydrochloro thiazide is a thiazide diuretic. The thiazides are moderately potent diuretics which act at the proximal end of the distal tubule causing a decrease in reabsorption of electrolytes and an increase in excretion of sodium and chloride ions with accompanying water loss. The hypotensive effect is possibly due to a decrease in peripheral resistance. They are generally not effective in adults with a creatinine clearance of < 30ml/min. Telmisartan is 4'[(1,4'-Dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'-yl)methyl][1,1'-biphenyl]-2-carboxylic acid, $C_{33}H_{30}N_4O_2$. Telmisartan is an insurmountable angiotensin II subtype-1 (AT1) receptor antagonist.

The structures of these two drugs are shown in figure 1. One such combination contains 12.5mg of Hydrochlorthiazide and 40mg of Telmisartan. It is widely



Hydrochlorthiazide ($C_7H_8ClN_3O_4S_2$)



Telmisartan ($C_{33}H_{30}N_4O_2$)

Figure 1: Structures of hydrochlorthiazide and telmisartan

used as combination therapy against hypertension. The literature revealed no method was available for simultaneous determination of these two drugs in such pharmaceutical preparations by HPLC. Therefore an HPLC method was developed for determination of Hydrochlorthiazide and Telmisartan from their combined dosage form[5-12]. The method described is simple, fast, precise and accurate for simultaneous determination of Hydrochlorthiazide and Telmisartan from pharmaceutical preparation.

Chemicals and reagents

Standards were supplied from Hetero Labs Ltd., Mumbai, India. TELPRES H manufactured by Piramal Healthcare Limited, India was procured from the market. Acetonitrile, Triethylamine and orthophosphoric acid were from Qualigens. Double distilled water was employed throughout the work. All dilutions were performed in standard volumetric flasks.

EXPERIMENTAL

Method development and optimization of chromatographic conditions

To develop a suitable LC method for the analysis of Hydrochlorthiazide and Telmisartan in their combined dosage form, different mobile phases were tried. The criteria employed for selecting the mobile phase for the analyses of the drugs were cost involved, time required for the analysis, better separation of drugs. Chromatographic separation was preformed with Waters Alliance system performance liquid chromatography having HPLC quarternary pump, equipped with auto sampler and a photo-diode array detector. The uv spectrum of all the three drugs were scanned on photo diode array detector for selecting the working wavelength. Peak purity of all the three drugs were checked using photo diode array detector. Chromatograms and data were recorded by means of Empower software. An Inertsil ODS3, C18 column (250mm × 4.6 mm, 5 µm particle) was used for the analysis.

The mobile phase comprising of Buffer* : Acetonitrile (60:40) Buffer* : 0.05M KH_2PO_4+0.1% Triethylamine, pH to 4.3 with Orthophosphoric Acid, filtered and degassed., at a flow rate of 1.5mL/min,column temperature at 40^{0}C and UV detection at 210nm, 20 µL

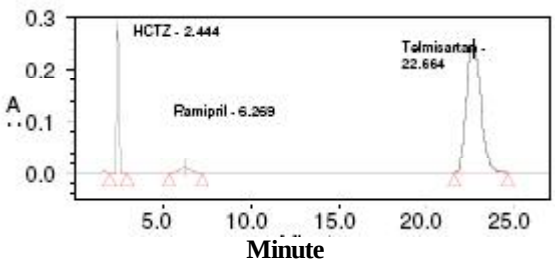


Figure 2 : Chromatogram of telmisartan and hydrochlor thiazide with Ramipril(internal standard) in standard preparation

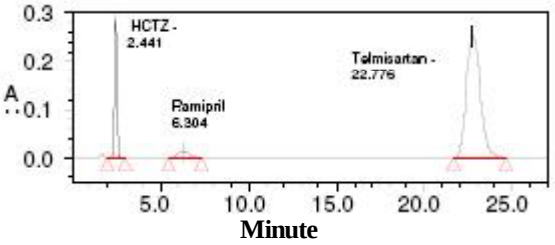


Figure 3 : Chromatogram of Telmisartan and hydrochlor thiazide with Ramipril(internal standard) in sample preparation

of sample was injected in the chromatographic system and for simultaneous determination of the two drugs.

A typical HPLC chromatogram for simultaneous determination of Hydrochlorthiazide and Telmisartan from pharmaceutical formulation is shown in figures 2 and 3.

A typical HPTLC chromatogram for simultaneous determination of Hydrochlorthiazide and Telmisartan from pharmaceutical formulation is shown in figures 1 and 2.

Preparation of standard stock solutions

The stock solution of Hydrochlorthiazide (500 µg/mL) was prepared by dissolving 50.0 mg of Hydrochlorthiazide (99.7 %) in mobile phase in a standard 100mL volumetric flask (solution A). Internal standard (Ramipril) stock solution (1000µg/mL) was prepared by dissolving 100.0 mg of Ramipril in mobile phase in a 100mL standard volumetric flask(stock B).

Working standard solution

Telmisartan (160µg/mL) was prepared by adding 40.0 mg of Telmisartan (99.5 %) into a 250ml volumetric flask, into this flask transfer 25.0 mL of stock solutions A and 25ml of stock solution B and add mobile phase to dissolve Telmisartan and dilute up to the

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mark with mobile phase.

Sample preparation

Transfer about 1 Tablet weight equivalent powder, accurately weighed to a 250 ml volumetric flask; add 200 ml of diluent sonicate for 15 minutes and dilute to volume with mobile phase.

RESULTS AND DISCUSSION

System suitability

System suitability tests are used to verify that the reproducibility of the equipment is adequate for the analysis to be carried out. System suitability tests were performed as per the USP 31 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 20-µL standard solutions of Hydrochlorthiazide and Telmisartan of strengths 50µg/mL and 160µg/mL respectively using Ramipril as an internal standard in five replicates. The RSD values of Hydrochlorthiazide and Telmisartan were 0.5 and 0.7 respectively. The RSD values were found to be satisfactory and meeting the requirements of USP 31 (RSD less than 2.0 %). Theoretical plates, resolution, tailing factor were determined and are presented in TABLE 1.

TABLE 1: Result of system suitability

Parameters	Ramipril (IS)	Hydrochlorthiazide	Telmisartan
Resolution	5.20	-	12.0
Tailing factor	1.16	1.06	1.21

TABLE 2: Results of linearity

Analyte	Slope (mean)	Intercept (mean)	Correlation coefficient (r^2) (n=7)
Hydrochlorthiazide	0.0087	0.157	0.9996
Telmisartan	0.0035	0.125	0.9997

TABLE 3: Results of assay experiment

	Hydrochlorthiazide	Telmisartan
Drug found in mg/mL (mean)	12.46	40.21
Mean %	99.68	100.53
RSD	0.55	0.46

TABLE 4: Accuracy of the method

Analyte	Initial conc. (mg)	Conc. added (mg)	Total conc. (mg)	Conc. found (mg)	RSD (%) n= 3	Recovery (%)	% Bias
Hydrochlorthiazide	12.5	0	12.5	12.3	0.22	98.4	+0.78
	12.5	1.25	13.75	13.78	0.29	100.21	+0.22
	12.5	2.50	15.00	14.95	0.35	99.67	+0.45
	12.5	3.75	16.25	16.20	0.42	99.69	+0.42
Telmisartan	40	0	40.0	39.8	0.24	99.50	+0.44
	40	4.0	44.0	43.8	0.28	99.55	+0.38
	40	8.0	48.0	47.6	0.42	99.17	+0.28
	40	12.0	52.0	51.5	0.37	99.04	+0.68

Linearity

Linearity was evaluated by analysis of working standard solutions of Hydrochlorthiazide and Telmisartan of seven different concentrations[2,3]. The range of linearity was from 40 - 60 µg/mL for Hydrochlorthiazide and 1280-192 µg/mL for Telmisartan. The peak area ratio and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained for the two pharmaceuticals are represented in TABLE 2. The result shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area ratio and concentration of each drug.

Limit of detection and limits of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were established at signal-to-noise ratio of 3:1 and 10:1 respectively[2,3]. The LOD and LOQ of Hydrochlorthiazide and Telmisartan were experimentally determined by six injections of each drug. The LOD of Hydrochlorthiazide and Telmisartan were found to be 0.5µg/mL *and* 0.1µg/mL respectively. The LOQ of Hydrochlorthiazide and Telmisartan were found to be 5.0µg/mL and 1.0µg/mL respectively.

Precision

Repeatability was studied by carrying out system precision. System precision was determined from results for six replicate injections of the mixed standard solutions[3]. The relative standard deviations were less than 2% for the two drugs. Method precision was determined from results from ten independent determinations at 100% of the test concentrations of Hydro chlorthiazide and Telmisartan in the product. The RSD were 0.62 and 0.38 respectively. Refer TABLE 3.

Accuracy

To study accuracy of the method, recovery experiment was carried out by applying the standard addition

method. A known quantity of each drug substance corresponding to 100%, 110%, 120% and 130% of the label claim of each drug was added, to determine if there are positive or negative interferences from excipients present in the formulation[4]. Each set of addition was repeated three times .The accuracy was expressed as the percentage of analytes recovered by the assay. TABLE 4 lists the recoveries of the drugs from a series of spiked concentrations. The results indicate the method is highly accurate for simultaneous determination of the three drugs.

DISCUSSION AND CONCLUSION

Several mobile phases such as water-methanol, water-acetonitrile in different ratios were tried but good peak shape and good resolution between Hydrochlorthiazide, Ramipril and Telmisartan was observed using the mobile phase mentioned in chromatographic conditions. The method after being completely validated showed satisfactory data for all the method validation parameters. The method was found to be specific. The low values of %RSD for Method precision suggested that the method is precise. Linearity evaluated for the analyte peak showed a good linear response over a wide range of concentration. The linearity, precision, accuracy of the method proves that the method is specific, accurate,easily reproducible and can be used for simultaneous determination of Telmisartan and Hydrochlorthiazide from pharmaceutical preparations.

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