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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF HYDROCHLOROTHIAZIDE AND OLMESARTAN MEDOXOMIL BY RP-HPLC IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, accurate and precised method was developed for the simultaneous estimation of hydrochlorthiazide and olmesartan medoxomil in formulation by RP-HPLC method. Buffer in this method was 0.01N Na_2HPO_4 of pH 4 used with the combination of acetonitrile in the ratio of 45:55 as mobile phase. 10µl of sample was injected and the mobile phase was run for 7min with flow rate of 1ml/min through BDS 250mm column maintained at 30°C. Wavelength optimized was 257nm. Hydrochlorthiazide and olmesartan were eluted with good resolution of 7.11. Retention times of hydrochlorthiazide and olmesartan were 2.4min and 3.8min respectively. Other system suitability parameters like tailing factor and plate count were within the limits. According to the ICH guidelines this method was validated. There was no placebo interference observed resulting that this method was specific, %RSD obtained for hydrochlorthiazide and olmesartan were 0.6% and 0.7%. On plotting the linearity graph for hydrochlorthiazide and olmesartan linearity equations obtained were y = 12341x + 864.8 and y = 11251x + 1599 respectively. Correlation coefficient was 0.999. % Average recovery was calculate and fount to be 100.05% for hydrochlorthiazide and 100.02% for olmesartan. % Labled amount of both drugs were found to be 99.77% and 99.96% for hydrochlorthiazide and olmesartan. The developed method was validated as per ICH guidelines. As run time was decreased this is economical and simple method that can be used in the regular analysis.

Keywords: Hydrochlorthiazide, Olmesartan medoxomil and RP-HPLC.

INTRODUCTION

Hydrochlorothiazide is a thiazide diuretic used in the treatment of hypertension. Molecular formula of hydrochlorthiazide was $C_7H_8ClN_3O_4S_2$. Hydrochlorthiazide is soluble in methanol. IUPAC name was 6-chloro-1, 1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide. pKa was 7.9. Olmesartan is angiotensin II receptor antagonist used in the hypertension. Molecular formula of olmesartan was $C_{29}H_{30}N_6O_6$. IUPAC name was (5-methyl-2-oxo-2*H*-1, 3-dioxol-4-yl) methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl) phenyl] phenyl} methyl) -1 *H*-imidazole-5-carboxylate. Olmesartan is practically insoluble in water and sparingly soluble in methanol. pKa was 5.57. According to literature review there were some HPLC works includes, Ashok kumar et al., the retention times were 5.074 & 7.242 min for olmesartan medoxomil and hydrochlorothiazide, respectively. Kusum Lata et al., the retention times were 7.2 & 4.1min for olmesartan medoxomil and hydrochlorothiazide, respectively. Maitreyi Zaveri et al., the retention times were 4.0 & 7.2min for hydrochlorothiazide and olmesartan medoxomil, respectively. Raja et al., the retention times were 3.0 & 4.3min for hydrochlorothiazide and olmesartan medoxomil. Sivasakthi et al., the retention times were 3.1 & 4.3min for hydrochlorothiazide and olmesartan medoxomil. Suryadevara et al., the retention times were 3.6 & 4.3min for hydrochlorothiazide and olmesartan medoxomil.

EXPERIMENTAL WORK

Materials and reagents: Bulk Hydrochlorthiazide and olmesartan were gift samples by spectrum pharma research solutions, HPLC grade water and Acetonitrile were from Merk, and Olmezest H is a formulation of combined dosage form from sun pharmaceuticals.

Instruments: High performance liquid chromatography of waters 2695 with quaternary pumps, Auto sampler and PDA detector. Software integrated with the HPLC was Waters software Empower 2. Double beam Labindia UV spectrophotometer integrated with UV Win 5 software, Labman ultra sonic cleaner and Denver Digital balance.

Preparation of Buffer: (0.1%OPA) Accurately weighed 1.41gm of Di-sodium hyrogen Ortho phosphate in to a 1000ml of Volumetric flask add about 900ml of hplc grade water was added and sonicated to degas, finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 4 with dil. Ortho phosphoric acid.

Preparation of standard working solution: Weighed accurately 12.5mg and 20mg of hydrochlorthiazide and olmesartan in to two 10ml volumetric flasks separately. Small quantity of methanol was added firstly in to two volumetric flasks and sonicates to completely dissolve the drugs and made up with buffer and acetonitrile in the ratio of 40:60. From the both stock solutions 1ml was transferred to a single 10ml volumetric flask and made up with diluents.

Preparation of sample working solution: 10 tablets were weighed and average weight was calculated. 10 tablets were powdered and weight equivalent to 62.5mg hydrochlorthiazide and 100mg olmesartan was transferred to 25ml volumetric flask, small quantity of methanol was added and sonicates to dissolve the drug content and made up with diluents. Filtered 0.5ml from the sample stock solution was transferred to 10ml volumetric flask and made up with diluents.

Chromatographic conditions: 10µl of working solution was injected in to the channel through which mobile phase composition of sodium phosphate buffer and Acetonitrile (45:55), pumped with a flow rate of 1ml/min. Column used in this method was BDS 250mm column maintained at 30°C temperature. Wavelength optimized was 257nm and run time was 7min.

Method Validation: The method passed all the system suitability parameters so the method was set for validation according to the ICH guidelines including the parameters below.

Specificity: Specificity indicates whether the method estimates the drug without the interference of placebo contents in the formulation. Blank, Placebo, and Samples were prepared, injected and retention times in these three chromatograms were compared for interference.

Linearity: Six linearity concentrations 31.25ppm, 62.5ppm, 93.75ppm, 125ppm, 156.25ppm, 187.5ppm of hydrochlorthiazide and 50ppm, 100ppm, 150ppm, 200ppm, 250ppm, 300ppm of olmesartan were prepared from the standard stock solutions by taking 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml form both stack solution in to a 10ml volumetric flask and made up with diluents.

Precision:

Repeatability: Precision was to measure the closeness of the agreement in the multiple sampling of working solution form a stock solution and was reported in %Relative standard deviation.

Intermediate Precision: It was also known as, day-day precison, system-system precsion or analyst-analyst precision. six sample working solutions were prepared form the sample stock solution and were injected on the next day and %RSD was calculated between intraday and interday agreements.

Accuracy: Sample solutions of three levels were prepared. Accuracy 50% (62.5ppm of hydrochlorthiazide and 100ppm of olmesartan), accuracy 100% (125ppm of hydrochlorthiazide and 200ppm of olmesartan) and accuracy 150% (187.5ppm of hydrochlorthiazide and 300ppm of olmesartan) and were injected. The results were compared to 100% Standard working solution and %Recovery was calculated.

LOD: Limit of detection is the lowest concentration of the drug that can be detected at the detector level without necessary quantification. S/n ratio is 3:1.

LOQ: Limit of quantification is the lowest concentration of the drug that can be quantified with an accuracy and precision. S/n ratio is 10:1.

Robustness: Small changes in the optimized method like 10% flow plus and minus, 10% of acetonitrile plus and minus, 5°C temperature plus and minus. Six changes were made and reported whether the results found were precised.

Assay: Assay of the formulation was performed using the developed method. Direct comparison method was used, results obtained from the sample working solution were compared to the standard working solution results.

RESULTS AND DISCUSSIONS

In the method development process, many trials were done by changing columns, mobile phase combination, buffers, pH, flow rate, but all the system suitability parameters were satisfactory with the optimized method in which column used was BDS (250mm:4.6mm, 5µ), through which mobile phase composition of di-sodium hydrogen phosphate and acetonitrile(45:55) with flow rate 1ml/min. The column was maintained at 30°C and the optimized wavelength was 257nm. Hydrochlorthiazide and olmesartan were eluted with good resolution of 7.11. Retention times of hydrochlorthiazide and olmesartan were 2.4min and 3.8min respectively. Other system suitability parameters like tailing factor and plate count were within the limits. According to the ICH guidelines this method was validated. There was no placebo interference observed resulting that this method was specific, %RSD obtained for hydrochlorthiazide and olmesartan were 0.6% and 0.7%. On plotting the linearity graph for hydrochlorthiazide and olmesartan linearity equations obtained were y = 12341x + 864.8 and y = 11251x + 1599 respectively. Correlation coefficient was 0.999. % Average recovery was calculate and fount to be 100.05% for hydrochlorthiazide and 100.02% for olmesartan. % Labled amount of both drugs were found to be 99.77% and 99.96% for hydrochlorthiazide and olmesartan.

CONCLUSION

A simple, accurate and precise rp-hplc method was developed for the simultaneous estimation of hydrochlorthiazide and olmesartan medoxomil in formulation. This method can be applied for routine quality control of hydrochlorthiazide and olmesartan medoxomil in pharmaceutical industries.

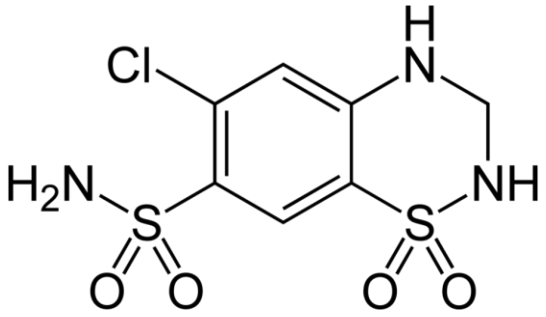

Fig.1: Hydrochlorthiazide

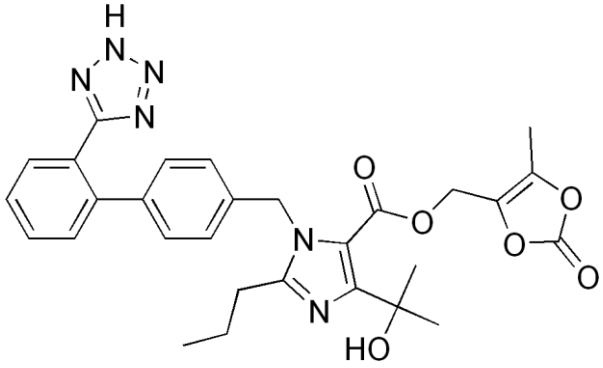

Fig.2: Olmesartan medoxomil

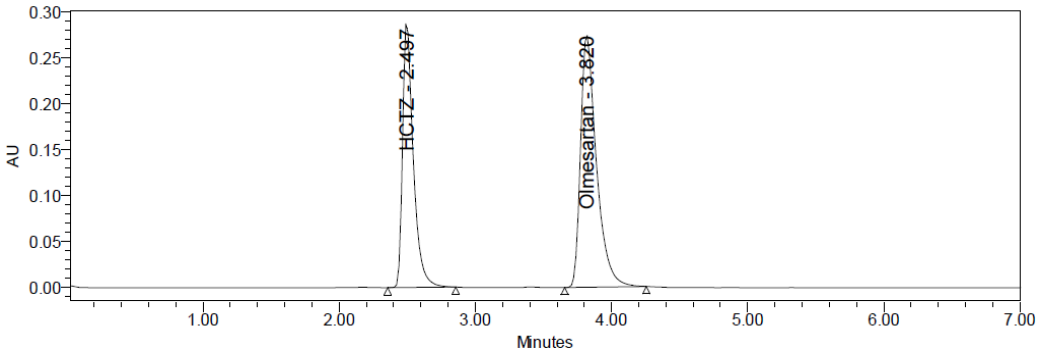

Fig.3: Chromatogram of sample solution

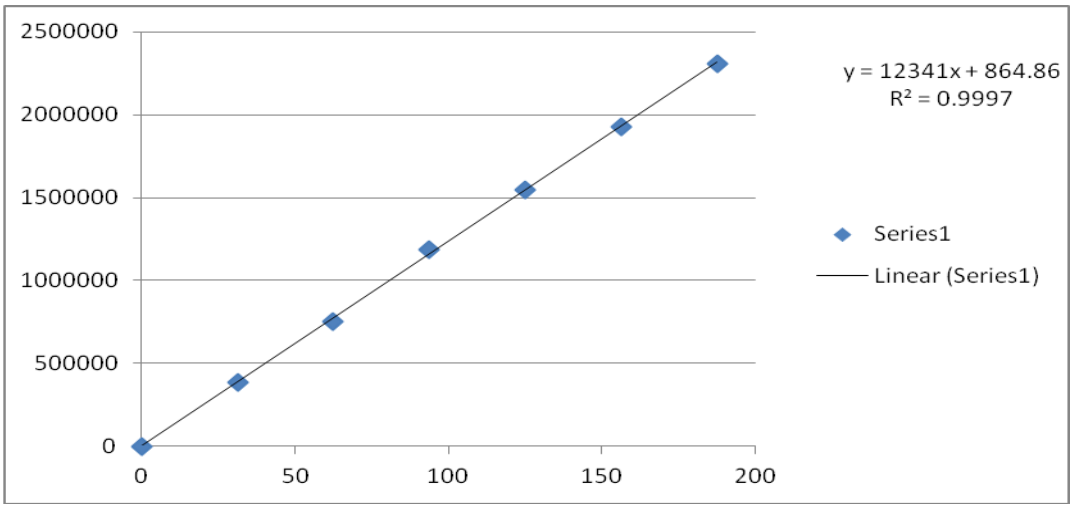

Fig.4: Calibration curve of hydrochlorthiazide

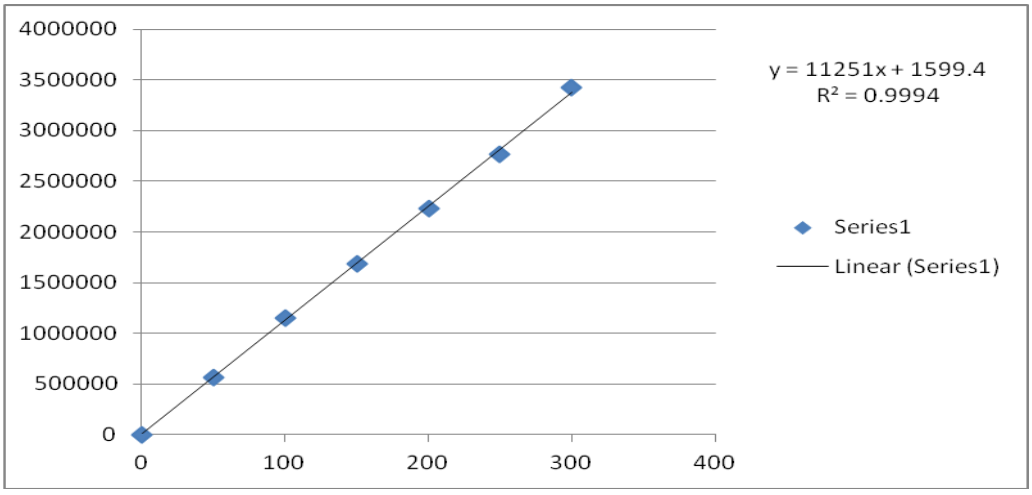

Fig.5: Calibration curve of olmesartan medoxomil

Table.1: Validation parameters

Parameters	Hydrochlorthiazide	Olmesartan
Recovery	100.05%	100.02%
Repeatability	0.72	0.64
Inter day precision	0.6	0.7
LOD	0.23ppm	0.47ppm
LOQ	0.70ppm	1.42ppm
Specificity	Specific	Specific
Robustness	1.7	0.9
Solvent stability	Stable for 24 hrs	Stable for 24 hrs

Table.2: Calibration Data

Parameters	Hydrochlorthiazide	Olmesartan
Optimized Wavelength	257nm	257nm
Linearity range	31.25ppm-187.5ppm	50ppm-300ppm
Intercept	864.8	1599
Slope	12341	11251
Correlation Coefficient	0.999	0.999
Linearity Equation	y = 12341x + 864.8	y = 11251x + 1599

Table.3: Robustness Data

Parameters	Hydrochlorthiazide	Olmesartan
Flow minus	0.14	0.10
Flow Plus	0.16	0.20
Mobile phase minus	0.52	0.30
Mobile phase plus	0.06	0.08
Temperature minus	0.10	0.09
Temperature Plus	0.04	0.18

Table.4: Recovery Data

Parameters	Hydrochlorthiazide			Olmesartan			
Level of Recovery	50%	100%	150%	50%	100%	150%	
%Recovery	100.21	99.92	100.03	99.95		100.15	99.98
STDEV	0.41	0.90	0.15	0.76	0.54	0.59	
%RSD	0.41	0.90	0.15	0.76	0.53	0.59	

Table.5: Assay table

	Lable claim		Amount recovered		% Assay	
Formulation	HCTZ	OLME	HCTZ	OLME	HCTZ	OLME
Olmezest H	12.5	20	12.47	19.99	99.77%	99.96%

Table.6: System suitability table

Parameters	Hydrochlorthiazide	Olmesartan
Retention time	2.4±0.3min	3.8±0.3min
Plate count	4953	5021
Tailing Factor	1.51	1.55
Resolution		7.11
%RSD	0.6	0.7

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