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Method Development and validation of Losartan Potassium and Hydrochlorothiazide by RP-HPLC

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ABSTRACT- A rapid, sensitive, and accurate RP-HPLC method was developed for the identification and quantification of Hydrochlorothiazide and Losartan Potassium using a Waters HPLC system equipped with a PDA detector. Chromatographic separation was achieved on an Inertsil ODS-C18 column (250 × 4.6 mm, 5 µm particle size) with a flow rate of 1.0 mL/min. The mobile phase consisted of degassed and filtered Acetonitrile–Water (70:30, v/v). Detection was performed at 254 nm, providing reliable resolution and measurement of both analytes.

INDEX TERMS: Losartan Potassium, Hydrochlorothiazide, RP-HPLC

I. INTRODUCTION

Hyzaar® is a fixed-dose combination of losartan potassium and hydrochlorothiazide. This medication is used to treat high blood pressure (hypertension). The specific components work together to lower blood pressure and reduce the risk of associated health problems like strokes and heart attacks.

Losartan potassium (6-7) is an angiotensin II receptor blocker (ARB). It works by blocking a substance in the body (angiotensin II) that causes blood vessels to tighten. This action relaxes and widens blood vessels, which lowers blood pressure and increase the supply of blood and oxygen to the heart.

Hydrochlorothiazide (8) is a thiazide diuretic, or "water pill". It helps the kidneys remove extra water and salt from the body through urine. This decreases the overall fluid volume in the body, which helps to lower blood pressure.

II. MATERIALS AND METHODS

Preparation of Stock solution: 100 mg of Losartan Potassium and 100 mg of Hydrochlorothiazide API standards were accurately weighed and are transferred into a two separate neat and dry 100 ml volumetric flasks and dissolved in 100ml of mobile phase. The mixture was then sonicated for 20 minutes to obtain 1000PPM.

Preparation of working standard solution: From the stock solutions of both standards, each 4 ml was pipetted out and transferred in to 100ml volumetric flasks, made up to 100 ml with mobile phase and sonicated for10 minutes, to get 40PPM of Losartan Potassium and Hydrochlorothiazide.

III. RESULTS AND DISCUSSION

Method validation: Validation parameters include specificity, linearity, range, accuracy, precision, limit of detection, limit of quantification, robustness and assay (1-5).

Specificity: Specificity is the ability to assessing equivocally the analyte in the presence of components which may be expected to be present. Typically, these components include impurities, degradants, matrix etc. Blank solution and standard solutions of Losartan Potassium (40µg/ml) and Hydrochlorothiazide (40µg/ml) were injected into the HPLC system. The peak purity data of Losartan Potassium and Hydrochlorothiazide were compared. There should not be any interference at the retention time of the main peaks.

Linearity: Linearity for the drugs Losartan Potassium and Hydrochlorothiazide (9-12) was determined by preparing the standard solutions at six concentrations levels in the range of 20-70µg/ml of Losartan Potassium and 20-70µg/ml of Hydrochlorothiazide from stock solution. The linearity charts of Losartan Potassium and Hydrochlorothiazide was shown in the figure no 2&3. The correlation coefficient was found to be 0.9999 and 0.9992 for Losartan Potassium and Hydrochlorothiazide respectively. Linearity results were tabulated in table 2.

Accuracy: Accuracy was performed by spiking known amounts of standard solution to sample solution at three different concentrations levels (50%, 100%, 150%) and there by analyzed for %RSD which should not be more than 2.0.The % recovery was calculated and the results was reported in table no. 3 & 4.

Precision: The precision (13-16) of the analytical method was studied by injecting six replicates of standard containing 40µg/ml of Losartan Potassium

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and 40µg/ml of Hydrochlorothiazide which were injected into HPLC system. The % RSD was calculated and the results were reported in the table no.5 & 6.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection was defined as the concentration which yields a signal - to – noise ratio 3:1 where as the limit of quantification was calculated to be the lowest concentration that could be measured with signal - to – noise ratio10:1. LOD and LOQ were calculated from slope and standard deviation. The results were tabulated in table no. 7.

Robustness: The smallest deliberate changes in method like change in flow rate are made but there were no predictable changes in the results and are in the range as per ICH guidelines. Conditions like decrease in flow rate (0.8 ml/min), increase in flow rate (1.2 ml/min) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. % RSD was found to be within the limits and results were tabulated in table no. 8.

Assay: Assay was conducted on marketed formulation and mean % assay was found. The results were tabulated in table no. 9.

Table1: Optimised Chromatographic Conditions

Parameter	**Method**
Stationary Phase (column)	Inertsil -ODS C18 (250 x 4.6 mm, 5 µ)
Mobile Phase	Acetonitrile and water (70:30)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	8 min
Temperature in the column (°C)	Ambient
Volume of injection loop (µl)	20
Wavelength of detection (nm)	254nm
Drug RT (min)	3.244min for Losartan Potassium and 4.679 for Hydrochlorothiazide

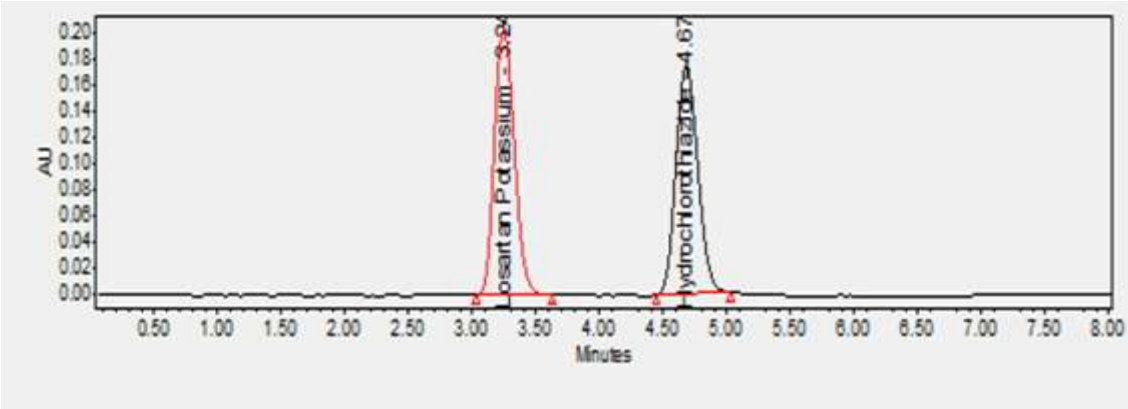

Figure 1: Optimized Chromatogram

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Table 2: Linearity data of Losartan Potassium and Hydrochlorothiazide

Losartan Potassium		Hydrochlorothiazide	
Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
20	1311086	20	1686945
30	1933713	30	2531191
40	2555753	40	3332140
50	3173113	50	4215742
60	3811126	60	4986245
70	4441218	70	5387642

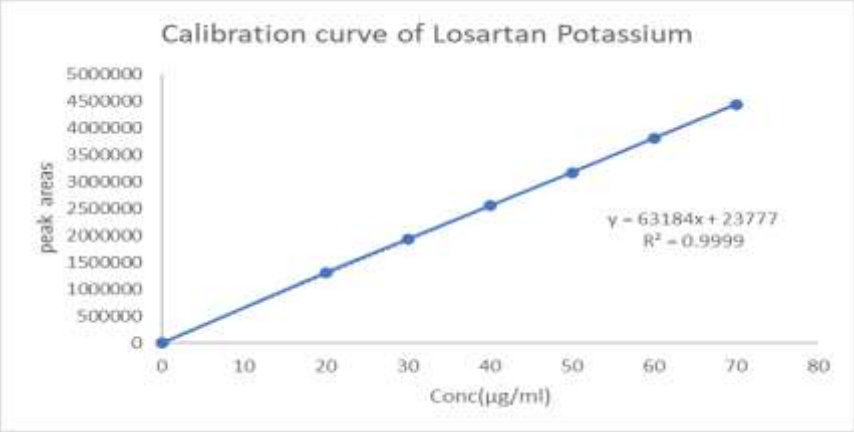

Figure 2: Calibration curve of Losartan Potassium

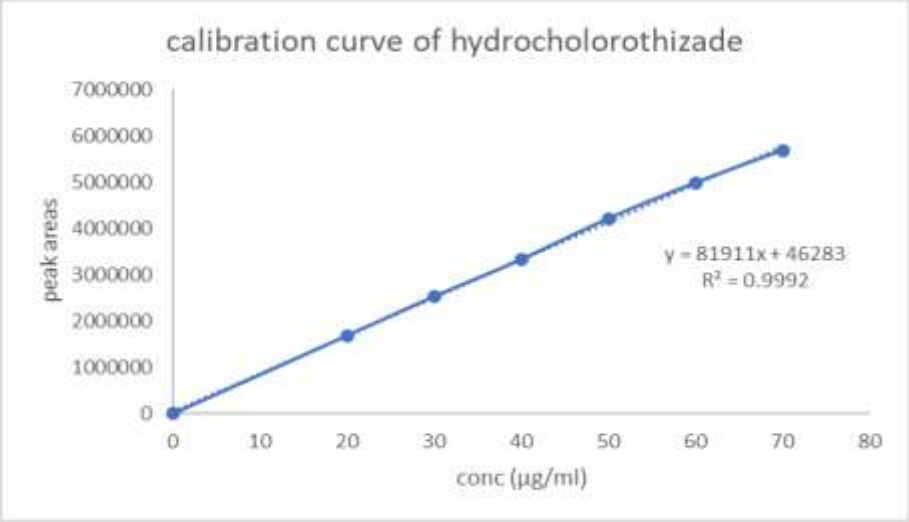

Figure 3: Calibration curve of Hydrochlorothiazide

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Table 3: Accuracy Data of Losartan Potassium

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% - 1	20	20.01	100.01	**MEAN**	99.92
50% - 2	20	19.98	99.99		
50% - 3	20	19.92	99.90	**%RSD**	0.543
100 % - 1	40	39.98	99.95	**MEAN**	99.94
100 % - 2	40	39.87	99.88		
100% - 3	40	39.85	99.12	**%RSD**	0.878
150% - 1	60	59.92	99.90	**MEAN**	100.07
150% - 2	60	60.02	100.04		
150% - 3	60	60.02	100.04	**%RSD**	0.896

Table 4: Accuracy Data for Hydrochlorothiazide

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% - 1	20	19.98	99.97	**MEAN**	99.97
50% - 2	20	19.90	99.92		
50% - 3	20	20.03	100.01	**%RSD**	0.818
100 % - 1	40	39.98	99.92	**MEAN**	99.96
100 % - 2	40	40.05	100.02		
100% - 3	40	40.10	100.09	**%RSD**	0.687
150% - 1	60	59.98	99.89	**MEAN**	99.96
150% - 2	60	59.99	99.99		
150% - 3	60	59.98	99.98	**%RSD**	0.897

Table 5: System Precision data of Losartan Potassium and Hydrochlorothiazide

S. No	Peak areas of Losartan Potassium	Peak areas of Hydrochlorothiazide
1	2556345	3324328
2	2555348	3330564
3	2555472	3336780
4	2553487	3316879
5	2552785	3334217
Mean	2554687	3328554
SD	1488.264	8030.278
% RSD	0.058256	0.241254

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Table 6: Method Precision data of Losartan Potassium and Hydrochlorothiazide

S. No	Peak areas of Losartan Potassium	Peak areas of Hydrochlorothiazide
1	2555431	3334540
2	2556384	3365742
3	2554663	3358701
4	2552786	3348970
5	2556428	3338756
6	2555786	3338950
Mean	2555246	3347610
SD	1371.822	12472.72
% RSD	0.053686	0.372586

Table 7: LOD and LOQ data of Losartan Potassium and Hydrochlorothiazide

Drug Name	LOD (µg/ml)	LOQ (µg/ml)
Losartan Potassium	0.04	0.13
Hydrochlorothiazide	0.07	0.197

Table 8: Robustness data of Losartan Potassium and Hydrochlorothiazide

S No	Drug Name	Condition	Peak area	% RSD
1	Losartan Potassium	Decreased Flow rate of 0.8 ml/min	2461396	1.177
2		Increased Flow rate of 1.2 ml/min	2668021	0.868
3	Hydrochlorothiazide	Decreased Flow rate of 0.8 ml/min	3235555	0.715
4		Increased Flow rate of 1.2 ml/min	3423008	0.031

Table 9: Assay data Losartan Potassium and Hydrochlorothiazide

S. No	Peak area of Losartan Potassium	% Assay	Peak area of Hydrochlorothiazide	% Assay
1	2555934	100.23	3338541	99.67
2	2559846		3330681	
3	2553886		3334697	
4	2556364		3338751	
5	2557450		3339674	
6	2558478		3338738	

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IV. CONCLUSION

The developed RP-HPLC method was validated as per ICH guidelines. All the system suitability parameters were within the range as stated by ICH guidelines. Interference peaks were not observed in blank, standard and sample chromatogram. Hence simple, precise and accurate, sensitive, specific and robust method was developed and validated. This can be used in quality control department with respect to routine analysis.

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REFERENCES

[1]. Majors RE. Recent advances in HPLC packings and columns. Journal of Chromatographic Science. 1980, 18(10), 488-511.

[2]. Swartz M. HPLC Detectors: A Brief Review. Journal of Liquid Chromatography & Related Technologies. 2010, 33(9-12), 1130-1150.

[3]. Sethi PD. High Performance Liquid Chromatography Quantitative Analysis of Pharmaceutical Formulations. New Delhi: CBS Publishers. 2001, 116-120.

[4]. Singh R. HPLC method development and validation-An Overview. Journal of Pharmaceutical Education & Research. 2013, 4(1).

[5]. T. Bhagyasree, N. Injeti, A. Azhakesan, U.M.V. Rao. A review on analytical method development and validation. International Journal of Pharmaceutical Research and Analysis. 2014, 4(8), 444-448.

[6]. https://pubchem.ncbi.nlm.nih.gov/compound/Losartan

[7]. K L Goa, A J Wagstaff. Losartan potassium: a review of its pharmacology, clinical efficacy and tolerability in the management of hypertension. Drugs. 1996, 51(5), 820-45.

[8]. https://pubchem.ncbi.nlm.nih.gov/compound/Hydrochlorothiazide

[9]. Sailaja and K. Veditha. Simultaneous estimation of losartan and hydrochlorothiazide in tablet dosage form by RP-HPLC. International Journal of Research in Pharmacy and Chemistry. 2022, 12(1), 47-54.

[10]. N.Santhi, S.S.Rajendran, S.Suresh Kumar, N.Vivekanandan. Development and Validation of RP-HPLC method for the Simultaneous Estimation of Losartan Potassium and Hydrochlorthiazide in bulk and tablet dosage form. International Journal of Pharmaceutical Research and Applications. 2022, 7(2), 254-261.

[11]. Jagadeeswararao V, Vasanth PM, Ramesh T, Ramesh M. Analytical method development and validation of losartan potassium and hydrochlorothiazide in combined dosage form by RP-HPLC. International Journal of Chem Tech Research. 2013, 5(6), 3007-3014.

[12]. Chaitali Thube, Jyoti Dhagude and P.Y.Pawar. Simultaneous estimation and validation of losartan potassium and hydrochlorothiazide in bulk and tablet dosage form by using different spectrophotometric method. Der Pharma Chemica. 2014, 6(2), 24-30.

[13]. Khan M. Rizwan, Shaikh Anis, Thaker A.K. Simultaneous determination and method development for assay of Losartan potassium and hydrochlorothiazide drugs in solid dosage form by RP-HPLC. Indian Journal of Pharmaceutical Science & Research. 2012, 2(1), 42-45

[14]. Sawaikar L, Usgaonkar K, Keny SM and Savaikar A. Simultaneous estimation of losartan potassium and hydrochlorothiazide in combined dosage form by UV Spectrophotometry. International Journal of Biology, Pharmacy and Allied Sciences. 2023, 12(6), 217-226.

[15]. Prasenjit Mondal, Nanappu Sravanthi, Adi Pooja, Peruka Harish, Nardela Swapna and Potu Appa Rao. A novel quantification method for the simultaneous estimation of Losartan Potassium and Hydrochlorothiazide in bulk and tablet dosage form using simultaneous equation method. Der Pharmacia Lettre, 2016, 8 (4), 91-95.

[16]. Md. Arif Hossen, Md. Ahsanul Haque, Irin Dewan, A. N. M. Hamidul Kabir, Md. Khalid Hossain and S. M. Ashraful Islam. Development and validation of RP-HPLC method for the simultaneous estimation of hydrochlorothiazide and losartan potassium in tablet dosage form. Dhaka Univ. J. Pharm. Sci. 2011, 10(1), 35-42.