CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.7533311

Available online at: http://www.iajps.com *Research Article*

DEVELOPMENT AND VALIDATION OF A RP - HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF SPIRONOLACTONE AND HYDROCHLOROTHIAZIDE IN PURE AND PHARMACEUTICAL DOSAGE FORM

H. Padmalatha
Gyana Jyothi College of Pharmacy

Abstract:
New method was established for simultaneous estimation of Spironolactone and Hydrochlorothiazide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Spironolactone and Hydrochlorothiazide by using Inertsil C18 (4.6mm ×250mm, 5µm particle size), flow rate was 1.0 ml/min, mobile phase ratio was (55:45% v/v) Methanol: Phosphate buffer pH 4.8 (pH was adjusted with ortho phosphoric acid), detection wavelength was 282nm. The instrument used was WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector. The retention times were found to be 1.688mins and 3.282mins. The % purity of Spironolactone and Hydrochlorothiazide was found to be 99.86%. The system suitability parameters for Spironolactone and Hydrochlorothiazide such as theoretical plates and tailing factor were found to be 7586, 1.69 and 6235 and 1.58, the resolution was found to be 10.85. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Spironolactone and Hydrochlorothiazide was found in concentration range of 100µg-500µg and 30µg-70µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.112% and 100.16%, %RSD for repeatability was 0.1702 and 0.043 respectively. The precision study was precise, robust, and repeatable. The LOD value was found to be 2.1µg/ml and 1.28µg/ml, and LOQ value was 6.3µg/ml and 3.84µg/ml for Spironolactone and Hydrochlorothiazide respectively. The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Spironolactone and Hydrochlorothiazide in pharmaceutical dosage form.

Keywords: *Spironolactone, Hydrochlorothiazide, RP-HPLC, Simultaneous estimation.*

Corresponding author:
H. Padmalatha,
Principal,
Gyana Jyothi College of Pharmacy
Gyana Jyothi Nagar, Uppal, Hyderabad
Telangana, India.
E-mail: Padmalatha.malthar@gmail.com

QR code

Please cite this article in press ***H. Padmalatha , Development And Validation Of A RP - HPLC Method For The Simultaneous Determination Of Spironolactone And Hydrochlorothiazide In Pure And Pharmaceutical Dosage Form.,*** *Indo Am. J. P. Sci, 2022; 09(12).*

INTRODUCTION:

Spironolactone is indicated for the treatment of New York Heart Association Class III-IV heart failure, management of edema in cirrhotic adults not responsive to fluid and sodium restrictions, primary hyperaldosteronism short-term preoperatively, primary hyperaldosteronism long-term in patients with aldosterone producing adrenal adenomas that are not candidates for surgery or patients with bilarteral micro/macronodular adrenal hyperplasia, as an add-on therapy in hypertension, and in nephrotic syndrome when treatment of the disease as well as fluid and sodium restriction with other diuretics is inadequate. Spironolactone has antiandrogenic activity which leads to many of its off label uses. Spironolactone is used off label in the treatment of hirsutism, female pattern hair loss, and adult acne vulgaris.[1] Spironolactone is also frequently used for its antiandrogenic effects in transgender female patients due to its low cost and reducing male-pattern hair growth.[2] IUPAC name 4-(acetylsulfanyl)-9a,11a-dimethyl-2,3,3a,3b,4,5,7,8,9,9a,9b,10,11,11a-tetradecahydrospiro[cyclopenta[a]phenanthrene-1,2'-oxolane]-5',7-dione. Molecular weight is 416.5 g/mole. Molecular formula is $C_{24}H_{32}O_4S$.

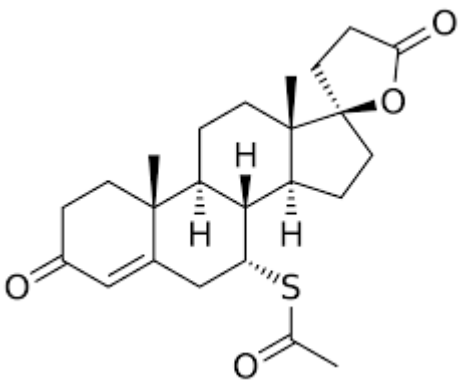

Figure 1: Structure of Spironolactone

Hydrochlorothiazide is indicated alone or in combination for the management of edema associated with congestive heart failure, hepatic cirrhosis, nephrotic syndrome, acute glomerulonephritis, chronic renal failure, and corticosteroid and estrogen therapy. Hydrochlorothiazide is also indicated alone or in combination for the management of hypertension.[3] Hydrochlorothiazide is transported from the circulation into epithelial cells of the distal convoluted tubule by the organic anion transporters OAT1, OAT3, and OAT4.[4] From these cells, hydrochlorothiazide is transported to the lumen of the tubule by multidrug resistance associated protein (MRP4).[4] Normally, sodium is reabsorbed into epithelial cells of the distal convoluted tubule and pumped into the basolateral interstitial by a sodium-potassium ATPase, creating a concentration gradient between the epithelial cell and the distal convoluted tubule that promotes the reabsorption of water.[5] IUPAC name 6-chloro-1,1-dioxo-3,4-dihydro-2H-1lambda6,2,4-benzothiadiazine-7-sulfonamide.

Molecular weight is 297.7. Molecular formula is $C_7H_8ClN_3O_4S_2$.

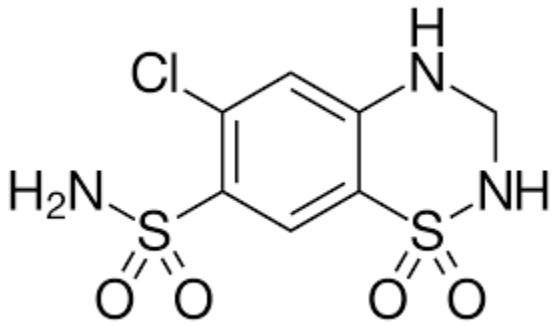

Figure 2: Structure of Hydrochlorothiazide

The literature survey revealed that There are very few methods reported in the literature for analysis of Spironolactone and Hydrochlorothiazide. Which are UV-Spectroscopic method[6,7] , RP-HPLC[8,9] method and LC-MS[10] method Only one RP-HPLC methods has been reported so far for simultaneous estimation of both the drugs[11] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Simultaneous estimation of Spironolactone and Hydrochlorothiazide in API and Pharmaceutical dosage form, attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Tamsulosin and Dutasteride. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Spironolactone and Hydrochlorothiazide in API and Pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form. To apply the developed method for the simultaneous estimation of Spironolactone and Hydrochlorothiazide in API and Pharmaceutical dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Spironolactone and Hydrochlorothiazide were obtained as a gift sample from sura training lab, Hyderabad. NaH_2PO_4 was

analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 282 nm with column Phosphate Buffer (pH-4.8): Methanol (55:45% v/v), dimensions at 35^0C temperature. The optimized mobile phase consists of. Flow rate was maintained at 1 ml/min and run time for 6 min.

Preparation of solutions:
Preparation of mobile phase: Accurately measured 500 ml (50%) of HPLC Methanol and 350 ml of Acetonitrile (35%) and 150 ml of Water (15%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Diluent Preparation:
Accurately measured 450 ml (45%) of HPLC Methanol and 550 ml of Phosphate Buffer (55%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.

Preparation of the Spironolactone and Hydrochlorothiazide standard solution:

Preparation of standard solution: (Spironolactone)
Accurately weigh and transfer 10 mg of Spironolactone, working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.

Preparation of standard solution: (Hydrochlorothiazide)
Accurately weigh and transfer 10 mg of Hydrochlorothiazide working standard into a 10ml of clean dry volumetric flasks add about 7ml of diluent and sonicate to dissolve and removal of air completely and make volume up to the mark with the diluent.
Further pipette 3ml of Spironolactone, 0.5ml of Hydrochlorothiazide from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of Sample Solution:
Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Spironolactone, Hydrochlorothiazide sample into a 10ml clean dry volumetric flask and add about 7ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
Procedure:
Further pipette 1.2ml of Spironolactone, Hydrochlorothiazide from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 6 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Inertsil ODS C 18 column (4.6 x 250mm, 5µm), the mobile phase of composition Phosphate Buffer (pH-4.8): Methanol (55:45% v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Spironolactone and Hydrochlorothiazide in their pharmaceutical dosage form. The result obtained for Spironolactone and Hydrochlorothiazide was comparable with the corresponding labeled amounts and they were shown in Table-2.

RESULTS AND DISCUSSION:

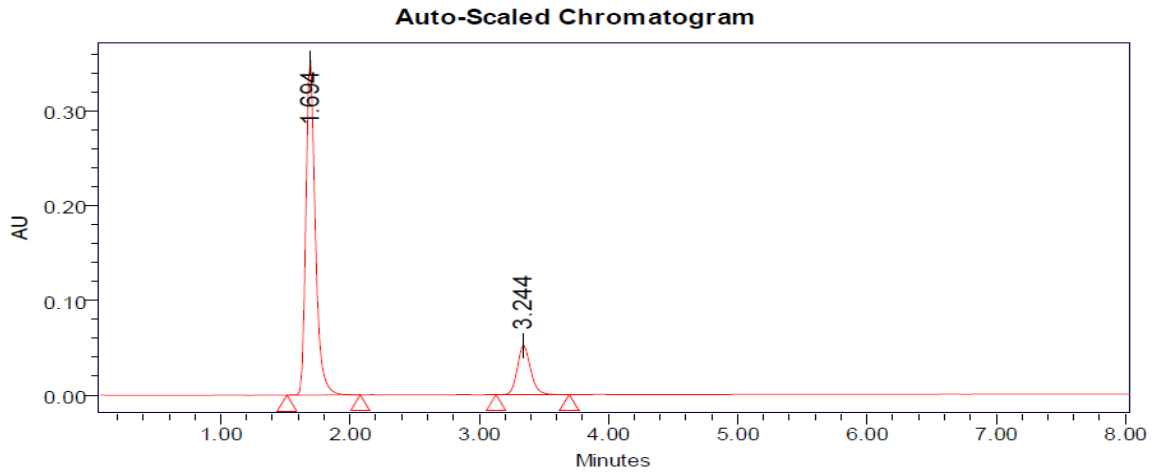

Figure 3: Standard chromatogram

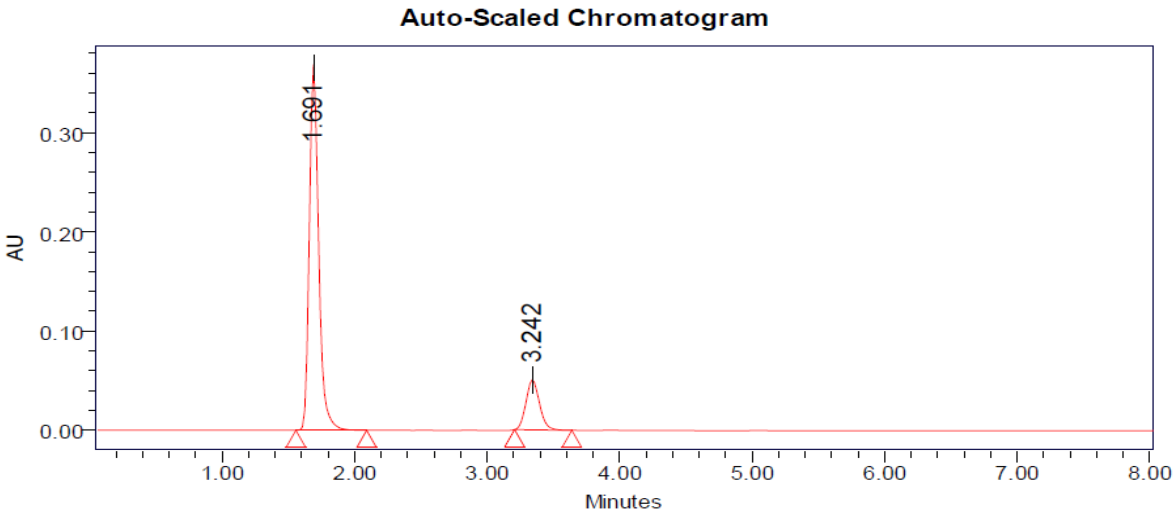

Figure 4: Sample chromatogram

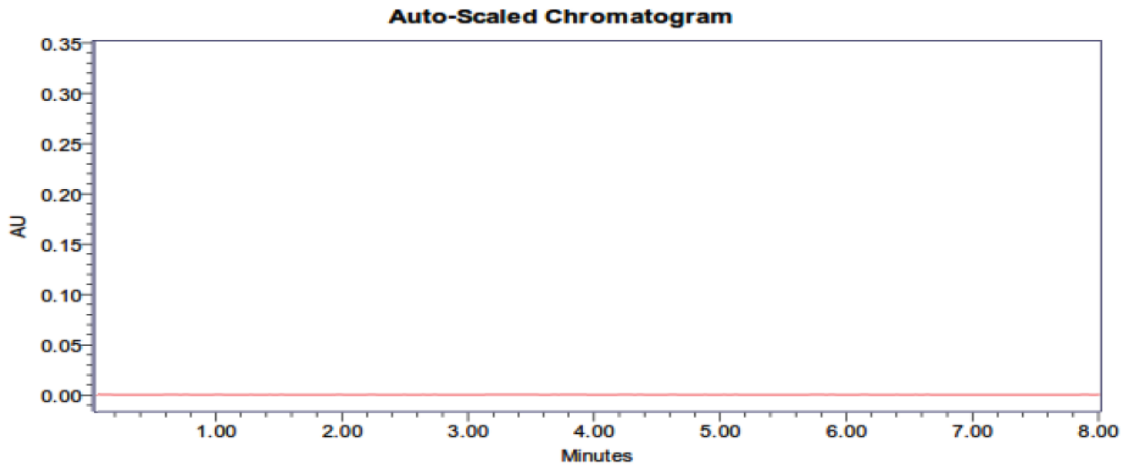

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	Spironolactone	Hydrochlorothiazide
Retention time	1.688	3.282
USP Plate count	7586	6235
USP Tailing	1.69	1.58

Table 2: Assay results for Spironolactone and Hydrochlorothiazide

	Label Claim (mg)	% Assay
Spironolactone	80	99.86
Hydrochlorothiazide	20	99.86

Linearity: The linearity study was performed for the concentration of 100ppm to 500ppm and30 ppm to 70 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The resulte are shown in table 3.

Table 3: Linearity results for Spironolactone and Hydrochlorothiazide

Spironolactone		Hydrochlorothiazide	
Concentration(µg/ml)	**Area**	**Concentration(µg/ml)**	**Area**
100	585985	30	268764
200	1182468	40	356958
300	1768785	50	445631
400	2326852	60	535186
500	2856874	70	624698
Correlation coefficient	0.999	Correlation coefficient	0.999

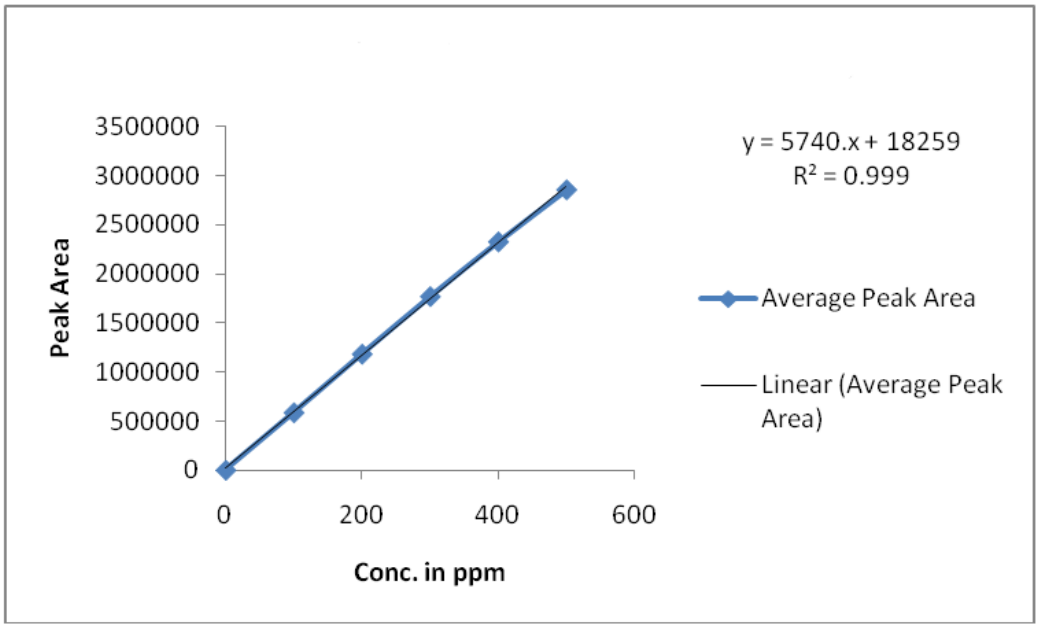

Figure 4: Linearity graph for Spironolactone

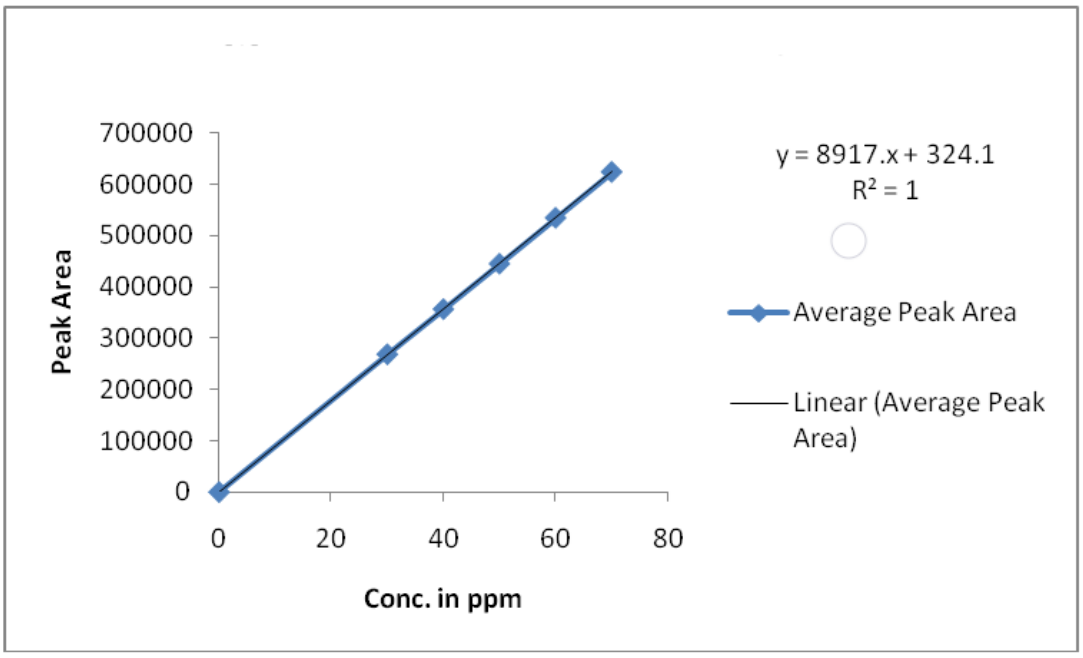

Figure 5: Linearity graph for Hydrochlorothiazide

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added and calculate the individual recovery and mean recovery values. The results are shown in table 4,5.

Table 4: Showing accuracy results for Spironolactone

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	879537	150	150.048	100.032	100.112%
100%	1743252	300	300.521	100.172	
150%	2609693	450	450.598	100.132	

Table 5: Showing accuracy results for Hydrochlorothiazide

%Concentration (at specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	224271	25	25.114	100.456%	100.16%
100%	445748.3	50	49.952	99.904%	
150%	670006.3	75	75.101	100.134%	

Precision Studies: precision was caliculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The resulte are shown in table 6.

Table 6: Precision results for Spironolactone and Hydrochlorothiazide

S. No	Spironolactone	Hydrochlorothiazide
1	1658254	426598
2	1658952	426589
3	1654857	426985
4	1659854	426587
5	1653298	426515
Mean	**1657043**	**426654.8**
Std.dev	**2820.29**	**187.5692**
%RSD	**0.1702**	**0.043963**

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The resulte are shown in table 7 and 8.

Table 7: Intermediate precision resultes for Spironolactone and Hydrochlorothiazide on day 1:

S. No	Sample Area 1	Sample Area 2
1	1665985	436598
2	1662598	436855
3	1668484	436598
4	1664598	436587
5	1663579	436741
6	1664587	432659
Mean	**1664972**	**436006.3**
Std. Dev.	**2060.327**	**1643.285**
% RSD	**0.123745**	**0.376895**

Table 8: Intermediate precision resultes for Spironolactone and Hydrochlorothiazide on day 2:

Injection	Area for Spironolactone	Area for Hydrochlorothiazide
Injection-1	1648598	415985
Injection-2	1642587	415267
Injection-3	1649852	415986
Injection-4	1648754	415265
Injection-5	1645289	415874
Injection-6	1647581	415632
Average	**1647110**	**415668.2**
STD Deviation	**2699.291**	**337.2106**
%RSD	**0.16388**	**0.081125**

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1ml/min. The Wavelength varied from 243nm to 247nm. The resulte are shown in table 9,10,11,12

Robustness results for Spironolactone

Table 9: Organic Composition results for Spironolactone:

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	7365	1.62	1.868
Actual Flow rate	1	7586	1.69	1.688
More Flow rate	1.2	7254	1.61	1.544

Table 10: Wavelength variation results for Spironolactone:

		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6284	1.51	3.621
Actual Flow rate	1	6235	1.58	3.282
More Flow rate	1.2	6168	1.56	2.998

Robustness results for Hydrochlorothiazide

Table 11: Flow variation results for Hydrochlorothiazide:

Flow Rate (ml/min)		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6284	1.51	3.621
Actual Flow rate	1	6235	1.58	3.282
More Flow rate	1.2	6168	1.56	2.998

Table 12: Organic Composition results for Hydrochlorothiazide:

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	50:50	6182	1.54	3.621
Actual organic phase	55:45	6235	1.58	3.282
More organic phase	60:40	6322	1.56	2.302

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 13.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

Table 13: LOD, LOQ of Spironolactone and Hydrochlorothiazide

Drug	LOD	LOQ
Spironolactone	2.10	6.30
Hydrochlorothiazide	1.28	3.84

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Spironolactone and Hydrochlorothiazide in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Spironolactone and Hydrochlorothiazide in pure and its pharmaceutical dosage forms.

REFERENCES:

1. Kim GK, Del Rosso JQ: Oral Spironolactone in Post-teenage Female Patients with Acne Vulgaris: Practical Considerations for the Clinician Based on Current Data and Clinical Experience. J Clin Aesthet Dermatol. 2012 Mar;5(3):37-50. (PubMed ID 22468178)
2. Millington K, Liu E, Chan YM: The Utility of Potassium Monitoring in Gender-Diverse Adolescents Taking Spironolactone. J Endocr Soc. 2019 Apr 4;3(5):1031-1038. doi: 10.1210/js.2019-00030. eCollection 2019 May 1. (PubMed ID 31065620)
3. FDA Approved Drug Products: Hydrochlorothiazide Oral Tablets
4. Hasegawa M, Kusuhara H, Adachi M, Schuetz JD, Takeuchi K, Sugiyama Y: Multidrug resistance-associated protein 4 is involved in the urinary excretion of hydrochlorothiazide and furosemide. J Am Soc Nephrol. 2007 Jan;18(1):37-45. doi: 10.1681/ASN.2005090966. Epub 2006 Nov 29. (PubMed ID 17135398)
5. Gamba G: The thiazide-sensitive Na+-Cl- cotransporter: molecular biology, functional properties, and regulation by WNKs. Am J Physiol Renal Physiol. 2009 Oct;297(4):F838-48. doi: 10.1152/ajprenal.00159.2009. Epub 2009 May 27. (PubMed ID 19474192)
6. Dinc E, Balenu D. Continuous wavelet transform and chemometric methods for quantitative resolution of a binary mixture of quinapril and hydrochlorothiazide in tablets. Jou.braz.chem.soc2007;18:962-8.
7. Kowalczuk D, Hopkala H. Application of derivative spectrophotometry for simultaneous determination of quinapril and hydrochlorothiazide in the combination tablets. Journal of AOAC Int. 2004;87:847–51.
8. Valizadeh H, Zakeri-Milani P, Islambulchilar Z, Tajerzadeh H. A simple and rapid high-performance liquid chromatography method for determining furosemide, hydrochlorothizde and phenol Red: Applicability to intestinal permeability studies. Journal of AOAC Int 2006;89:88.
9. Huang T, He Z, Yang B, Shao L, Zheng X, Duan, Simultaneous determination of captopril and hydrochlorothiazide in human plasma by reverse-phase HPLC from linear gradient elution. J Pharm Biomed Anal 2006;41:48-55
10. Vonaparti A, Kazanis M, Panderi I. Development and validation of liquid chromatographic electro spray ionization mass spectrometric method for the determination of benazepril, benzeprilat and hydrochlorothiazide in human plasma. Journal of Mass Spectrum 2006;41:593-605
11. Maha A. Hegazy, Fadia H. Metwaly, M. Abdelkawy, and Nada S. Validated chromatographic methods for determination of Hydrochlorthiazide and spironolactone in pharmaceutical formulation in presence of impurities and degradants. Journal of Chromatographic Science,49;2011;129-135.