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# HPLC METHOD DEVELOPMENT AND VALIDATION OF SPIRONOLACTONE IN TABLET DOSAGE FORMS IN PRESENCE OF IMPURITIES AND DEGRADANTS

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**Keywords:**

Isocratic HPLC, Canrenone, β-isomer, limit of detection, Forced degradation

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**ABSTRACT:** An isocratic reversed-phase HPLC method was developed and validated to estimate and separate spironolactone and its related substances in its tablet dosage forms. Estimation and separation were achieved on Symmetry C-8 analytical column (150X3.9) mm, 5µm, using Water, Tetrahydrofuran (THF), and Acetonitrile (ACN) in the ratio of 77:21:2 v/v as mobile phase at the flow rate of 20 µl. UV photodiode array (UV PDA) detector detects the peaks at the wavelength of 254 and 283 nm. Validation studies were carried out for specificity, accuracy, precision, limit of detection (LOD), and quantitation (LOQ). Validation studies revealed that the method is simple, reliable and reproducible. The limit of detection and limit of quantitation for spironolactone, canrenone, and β-isomer was achieved 0.018% and 0.053%, 0.022% and 0.66% and 0.028%, and 0.084%, respectively. Forced degradation studies were carried out for stability testing, and system suit parameters were also performed to test the method.

**INTRODUCTION:** Spironolactone **Fig. 1** is a synthetic γ- lactone steroid structure similar to aldosterone, a natural adrenocortical hormone [1]. It is a competitive antagonist of mineralocorticoid receptor, thus inhibiting mineralocorticoids' functions. Due to the inhibition of mineralocortecoid activity it increases the excretion of sodium and water and retains potassium. As it improves potassium retention, it is used as potassium-sparing diuretic [2]. Anti mineralocorticoid activity of spironolactone makes it suitable for use in the treatment of hypertension, heart failure [3], hypokalemia, conn's syndrome [4], primary aldosteronism [5], hirsutism [6] and complications of liver cirrhosis [7].

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Spironolactone is a prodrug rapidly metabolized in the liver. The active metabolites of spironolactone are Canrenone and 7-α- methylthiospironolactone [8].

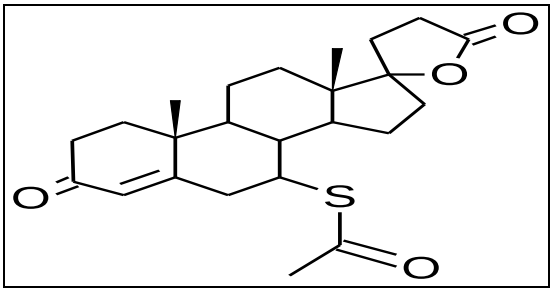

**FIG. 1: STRUCTURE OF SPIRONOLACTONE**

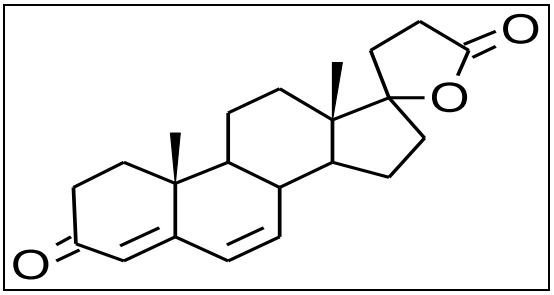

**FIG. 2: STRUCTURE OF CANRENONE**

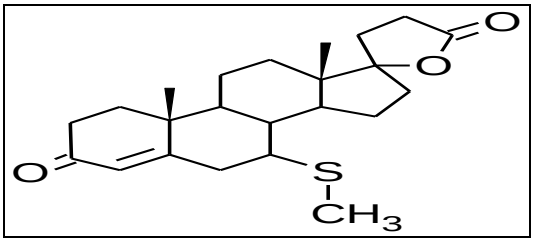

**FIG. 3: 7-A METHYLTHIOSPIRONOLACTONE**

An isocratic reversed-phase HPLC method was developed and validated to determine Spironolactone and its related substances in the tablet dosage form.

## MATERIALS AND METHODS:

### Chemical and Reagents:

Dipharma provided spironolactone, canrenone, and β-isomer standards. Canrenone and β-isomer were also obtained from Dipharma. Tablets containing 25mg, 50mg, and 100mg of spironolactone were purchased from the market.

HPLC grade acetonitrile and tetrahydrofuran were obtained from Merck Ltd (Mumbai) and water (HPLC grade) was obtained from Milli-Q RO water purification system.

All other reagents used in the study were of analytical grade.

**Instrument and Chromatographic Condition:** The chromatographic system that was employed for the development and validation of the method for related substances was the waters Acquity model with UV-PDA detector and Empowered software.

### Selection of Chromatographic Conditions:

**Wavelength Selection:** Various wavelengths were tested, and best response was reflected at 283 nm for spironolactone and canrenone and 254 nm for β-isomer.

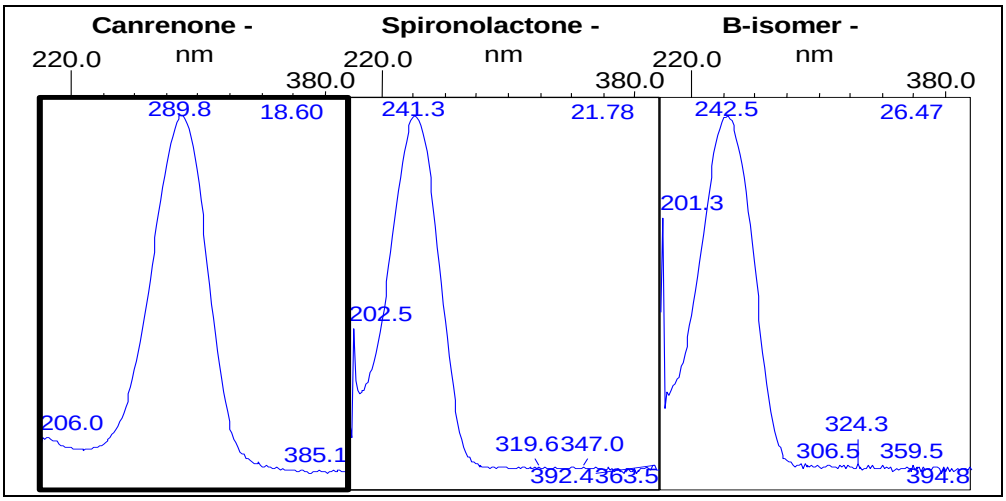


**FIG. 4: CHROMATOGRAM FOR WAVELENGTH SELECTION FOR CANRENONE, SPIRONOLACTONE AND B-ISOMER**

**Buffer and pH Selection:** Water, THF, and ACN were used in various proportions during trials, and finally, Water: THF: ACN in the ratio of 77:21:2 was selected.

**Column Selection:** Initially, trials were carried out on various columns like kromasil C-8(150*4.6)5 µm, Symmetry C-8 (150*4.6), 5µm etcetera but finally, good resolution between Canrenone and main peak (spironolactone) was achieved on symmetry C-8(150*3.9), 5µm column.

**Diluent Selection:** Diluent was kept the same as mobile phase, *i.e* Water: THF: ACN in the ratio of 74:18:8.

**Test concentration & injection volume:** Concentration of the test solution and the standard solution were kept at 2500 ppm and 7.5 ppm, respectively, while injection volume was kept 20µl.

**TABLE 1: CONDITIONS FOR HPLC**

| HPLC Column | Symmetry C-8 (150X3.9) mm, 5µm |
|---|---|
| Injection Volume | 20µl |
| Flow Rate | 1 ml/min |
| Column Temperature | 40ºC |
| Sample Temperature | 20ºC |
| Run Type | Isocratic |
| Mobile Phase | Water: THF: ACN :: 77:21:2 |
| Wavelength | 254nm, 283nm |

Chromatographic analysis was performed on Symmetry C-8 (150 mm X 3.9 mm), 5 µm column, which was maintained at a temperature 40°C. The mobile phase consisting of water: tetrahydrofuran: acetonitrile in the ratio of 770:210:20 was used at a flow rate of 1ml/min, and the injection volume was kept 20 µl for the same. For the standard solution, the run time was kept 40 min, while for sample run time was kept 75 min. Detection was performed at 254 nm and 283 nm wavelengths.

**Preparation of Solutions:**

**Mobile Phase Preparation:** For the preparation of the mobile phase, Milli-Q water was filtered through a 0.2 µm membrane filter and then mixed well with water, tetrahydrofuran, and acetonitrile in a ratio of 770:210:20. The mobile phase thus prepared was then sonicated for 5 minutes to degas the mixture and then used as mobile phase.

**Diluent Preparation:** Diluent was prepared by filtering Milli-Q water through a 0.2 µm membrane filter. Water was then mixed well in composition water: Tetrahydrofuran: Acetonitrile in the ratio of 740:180:80 and finally degassed in ultrasonic bath.

**Standard Preparation:** Spironolactone, canrenone, and β-isomer standard stock solutions were prepared in 10 ml volumetric flask by dissolving 2.5 gm of each standard in 1ml THF and then making up the volume up to 10 ml with the diluent. Following this, 3 ml of each stock solution was transferred to a 100 ml volumetric flask, and their volume was made upto100 ml with the diluents.

**Preparation of System Suitability Solution:** Standard solution was used to evaluate system suitability parameters.

**Preparation of test Solution:** Average weight of ten tablets was determined and was crushed to a fine powder. The sample powder equivalent to about 62.5 mg of spironolactone was accurately weighed and transferred to 25 ml volumetric flask, and 2.5 ml tetrahydrofuran were added to it. Then the solution was degassed for 5 minutes on an ultrasonicator. 15 ml diluent was added to this reaction mixture and again sonicated for 30 minutes with intermittent shaking in cool water. The volume of the reaction mixture was made up of the diluents, and finally, the prepared test solution was filtered through 0.2 µm nylon membrane filter.

**RESULT AND DISCUSSION:**

**Method Development and Optimization:** Many trials were performed to achieve better resolution and separation. Various mobile phases were tried in different compositions in isocratic mode to separate the peaks. The trials were started with water and methanol as a mobile phase, but peak separation was not achieved between β- isomer, and canrenon, and some unknown peaks also appeared. So, the mobile phase was changed to Water: THF: ACN in the ratio 74:18:8, which leads to better separation but still, the main peak was not base to base separated. Then, mobile phase composition was optimized to achieve the resolution, and the optimized method was validated, but during base degradation studies, an unknown impurity peak interferes with the spironolactone peak. Many trials were performed to separate the main peak from the unknown peak. Finally, a well-separated chromatogram was attained with the mobile phase composition Water: THF: CAN in the ratio of 77:21:2.

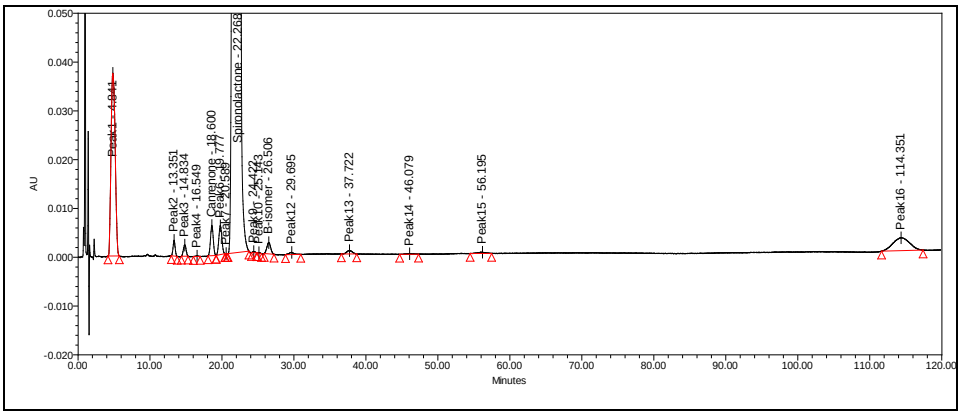


**FIG. 5: CHROMATOGRAM OF SPIRONOLACTONE**

**TABLE 2: CHROMATOGRAPHIC PARAMETERS FOR ABOVE CHROMATOGRAM**

| S. no. | Name | Retention Time | Area | % Area | USP Resolution | USP Tailing | USP Plate Count | Purity1 Flag |
|---|---|---|---|---|---|---|---|---|
| 1 | Peak1 | 4.841 | 1503256 | 1.58 | na | 1.16 | 343 | Yes |
| 2 | Peak2 | 13.351 | 64164 | 0.07 | 10.70 | 1.03 | 9815 | No |
| 3 | Peak3 | 14.834 | 61252 | 0.06 | 2.30 | 0.97 | 6564 | Yes |
| 4 | Peak4 | 16.549 | 2915 | 0.00 | 2.33 | 0.85 | 3755 | No |
| 5 | Canrenone | 18.600 | 165063 | 0.17 | 2.84 | 1.01 | 10567 | No |
| 6 | Peak6 | 19.777 | 174831 | 0.18 | 1.53 | 1.02 | 9779 | Yes |
| 7 | Peak7 | 20.589 | 491 | 0.00 | 1.56 | 1.01 | 123353 | No |
| 8 | Spironolactone | 22.268 | 92858136 | 97.30 | 2.60 | 0.70 | 7127 | No |
| 9 | Peak9 | 24.422 | 5468 | 0.01 | 2.62 | 1.13 | 21682 | No |
| 10 | Peak10 | 25.143 | 2499 | 0.00 | 1.23 | 1.20 | 44061 | No |
| 11 | B-isomer | 26.506 | 85749 | 0.09 | 1.71 | 1.02 | 10791 | No |
| 12 | Peak12 | 29.695 | 21190 | 0.02 | 2.89 | 0.99 | 8675 | No |
| 13 | Peak13 | 37.722 | 37192 | 0.04 | 6.02 | 1.02 | 10076 | No |
| 14 | Peak14 | 46.079 | 8051 | 0.01 | 6.10 | 0.96 | 11456 | No |
| 15 | Peak15 | 56.195 | 18804 | 0.02 | 6.04 | 0.91 | 6005 | No |
| 16 | Peak16 | 114.351 | 427205 | 0.45 | 17.40 | 1.03 | 10392 | No |

## Method Validation:

**Specificity:** For performing specificity studies, the chromatogram of diluent was used as blank and compared with the chromatogram of spironolactone sample spiked with all the known impurities. It is depicted in the chromatogram that all the peaks are well separated with good resolution.

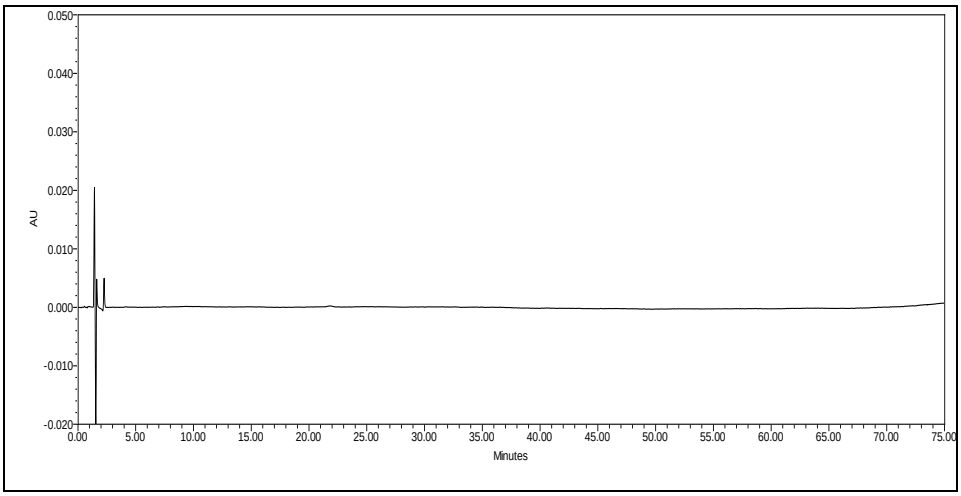


**FIG. 6: CHROMATOGRAM FOR BLANK SAMPLE**

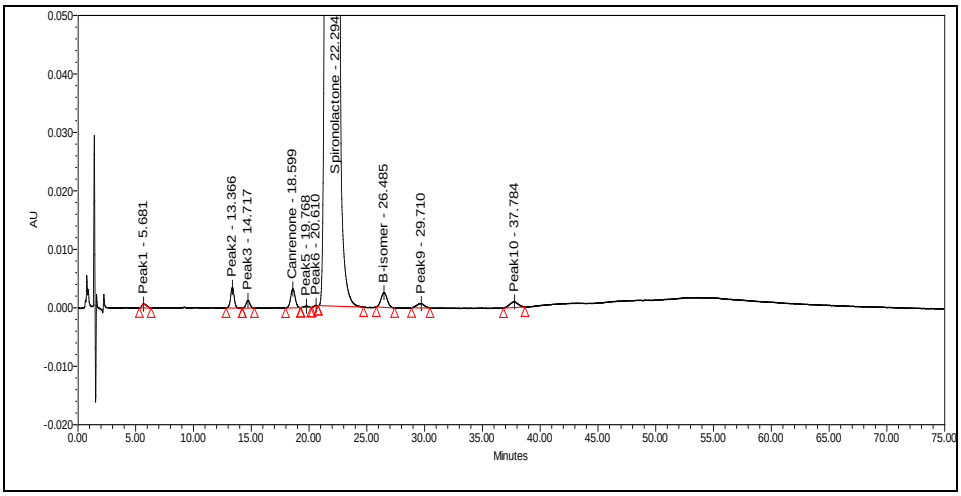


**FIG. 7: CHROMATOGRAM FOR SPIKED SAMPLE**

**TABLE 3: COMPONENTS OF SPECIFICITY**

| S. no. | Name | Retention Time | Area | % Area | USP Resolution | USP Tailing | USP Plate Count | Purity 1 Flag |
|---|---|---|---|---|---|---|---|---|
| 1 | Peak1 | 4.682 | 25770 | 0.03 | na | 1.50 | 690 | No |
| 2 | Peak2 | 13.400 | 74759 | 0.08 | 14.18 | 1.03 | 9434 | No |
| 3 | Peak3 | 14.773 | 31028 | 0.03 | 2.31 | 1.06 | 9916 | No |
| 4 | Canrenone | 18.699 | 87440 | 0.09 | 5.73 | 1.02 | 10555 | No |
| 5 | Peak5 | 19.892 | 7578 | 0.01 | 1.70 | 0.90 | 15472 | No |
| 6 | Peak6 | 20.667 | 1420 | 0.00 | 1.12 | 0.87 | 11606 | No |
| 7 | Spironolactone | 22.417 | 97956323 | 99.56 | 1.92 | 0.69 | 6769 | No |
| 8 | B-isomer | 26.690 | 97850 | 0.10 | 4.01 | 1.03 | 10633 | No |
| 9 | Peak9 | 29.917 | 39560 | 0.04 | 2.63 | 0.89 | 7089 | No |
| 10 | Peak10 | 38.037 | 64219 | 0.07 | 5.26 | 1.04 | 11151 | No |

## Linearity:

### Injection Linearity:

For Injection linearity studies, a test solution of 31.9 ppm was prepared and injected with different injection volumes (10, 20, 30, 40, and 50 µl). A linear graph was obtained between peak area and injection volume using regression analysis. It indicates that the developed method is linear.

**TABLE 4: COMPONENTS FOR LINEARITY**

| Injection | RT (min) | Inj. Vol. (µL) | Area | $R^2$ |
|---|---|---|---|---|
| Inj-1 | 22.268 | 10 | 65787438 | 0.999 |
| Inj-2 | 22.268 | 20 | 165717521 | |
| Inj-3 | 22.268 | 30 | 267474670 | |
| Inj-4 | 22.268 | 40 | 371221678 | |
| Inj-5 | 22.268 | 50 | 464389120 | |

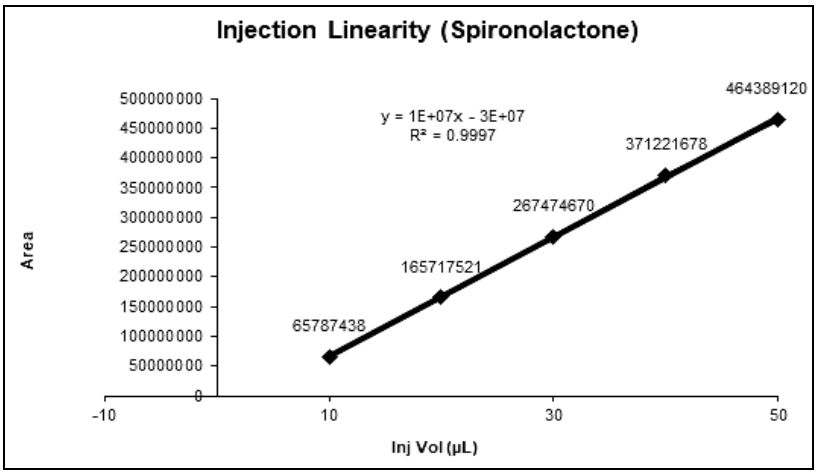


**FIG. 8: GRAPH DEPICTING INJECTION LINEARITY**

**Detector Linearity:** Test solutions were prepared in eight concentrations and injected into the HPLC column. The result obtained depicts that the test solutions are linear between 40- 160% of analyte concentration.

**TABLE 5: DETECTOR LINEARITY**

| Conc. Inj. (ppm) | Vol Injected (µL) | Area | Average Area |
|---|---|---|---|
| 15.9 | 20 | 28949889 | 28904466 |
| | 20 | 28749689 | |
| | 20 | 29013819 | |
| 25.5 | 20 | 46329068 | 46029068 |
| | 20 | 45429068 | |
| | 20 | 46329068 | |
| 31.9 | 20 | 58081853 | 57788809 |
| | 20 | 57121824 | |
| | 20 | 58162749 | |

| | | | |
|---|---|---|---|
| 51.0 | 20 | 91632831 | 92390721 |
| | 20 | 92798212 | |
| | 20 | 92741121 | |
| 63.8 | 20 | 116163707 | 114393707 |
| | 20 | 117153707 | |
| | 20 | 109863707 | |
| 102.0 | 20 | 185716272 | 185885335 |
| | 20 | 186016321 | |
| | 20 | 185923412 | |
| 127.5 | 20 | 232145340 | 232673904 |
| | 20 | 233255129 | |
| | 20 | 232621243 | |
| 255.0 | 20 | 463990997 | 463003586 |
| | 20 | 459189881 | |
| | 20 | 465829881 | |

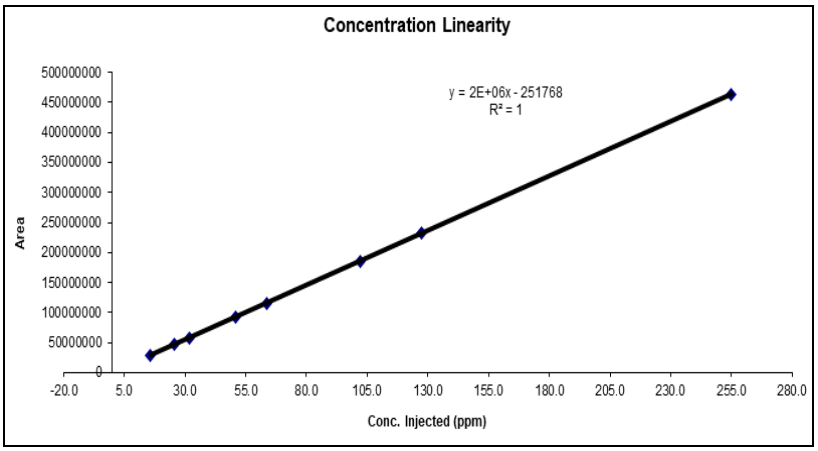


**FIG. 9: GRAPH DEPICTING DETECTOR LINEARITY**

**Accuracy:** The accuracy of the developed method was determined by injecting the test solution sample in triplicates in three different levels of 80%, 100%, and 120% of the assay concentration, *i.e.*, a total of nine determinations of each level. Percentage recovery for spironolactone was obtained as 100.18, 100.39, and 100.20, respectively.

**TABLE 6: RESULTS OF PERCENTAGE RECOVERY**

| S. no. | Percentage of spironolactone | % Recovery | Average |
|---|---|---|---|
| 1 | 80%/1 | 100.20 | |
| 2 | 80%/1 | 100.29 | 100.18 |
| 3 | 80%/1 | 100.06 | |
| 4 | 100%/1 | 100.64 | |
| 5 | 100%/2 | 100.51 | 100.39 |
| 6 | 100%/3 | 100.04 | |
| 7 | 120%/1 | 100.23 | |
| 8 | 120%/2 | 100.07 | 100.20 |
| 9 | 120%/3 | 100.31 | |

**Precision:** Readings for each injected sample are presented in the table and these findings are précised and % RSD was found to be 0.134%.

**TABLE 7: RESULTS FOR PRECISION**

| Injection No. | Inj. Vol. (µL) | RT (min) | Area | Average Area | Standard deviation | % RSD |
|---|---|---|---|---|---|---|
| Inj-1 | 20 | 22.268 | 92858136 | | | |
| Inj-2 | 20 | 22.268 | 92858223 | | | |
| Inj-3 | 20 | 22.268 | 92552831 | 92807461 | 124742.9589 | 0.134 |
| Inj-4 | 20 | 22.268 | 92858204 | | | |
| Inj-5 | 20 | 22.268 | 92858721 | | | |
| Inj-6 | 20 | 22.268 | 92858651 | | | |

**Limit of Detection (LOD) and Limit of Quantitation (LOQ):** The retention time for Spironolactone was 22 minutes; relative retention time, LOD, and LOQ for spironolactone, Canrenone, and β-isomer are mentioned in **Table 8.**

**TABLE 8: RESULTS FOR LOD AND LOQ**

| Name | Relative retention time (RRT) | Tentative LOD% | Tentative LOQ% |
|---|---|---|---|
| Spironolactone | 1.00 | 0.018% | 0.053% |
| Canrenone | 0.84 | 0.022% | 0.066% |
| β- Isomer | 1.19 | 0.028% | 0.084% |

**Robustness:** Robustness studies were performed to investigate the effect of small changes in temperature, flow rate, and pH of the mobile phase on the developed method. The data obtained from robustness studies are summarized in the table below, which shows that the method is robust.

**TABLE 9: ROBUSTNESS PARAMETERS**

| S. no. | Name | Retention Time | Area | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|
| **Control condition** | | | | | |
| 1 | Spironolactone | 22.268 | 92858136 | 0.70 | 7127 |
| 2 | β- Canrenone | 18.600 | 165063 | 1.01 | 10567 |
| 3 | β-isomer | 26.506 | 85749 | 1.02 | 10791 |
| **Flow minus** | | | | | |
| 1 | Spironolactone | 22.421 | 93018112 | 0.79 | 8325 |
| 2 | β- Canrenone | 18.701 | 183043 | 1.18 | 11467 |
| | β-isomer | 26.635 | 87271 | 1.23 | 9891 |
| **Flow plus** | | | | | |
| 1 | Spironolactone | 22.211 | 91758121 | 0.72 | 7998 |
| 2 | β- Canrenone | 18.217 | 154183 | 1.21 | 11152 |
| 3 | β-isomer | 26.124 | 83536 | 1.13 | 10985 |
| **pH minus** | | | | | |
| 1 | Spironolactone | 22.400 | 92997225 | 0.77 | 7343 |
| 2 | β- Canrenone | 18.642 | 176068 | 1.17 | 10929 |
| 3 | β-isomer | 26.689 | 86532 | 1.60 | 11232 |
| **pH plus** | | | | | |
| 1 | Spironolactone | 22.121 | 92947267 | 0.73 | 7235 |
| 2 | β- Canrenone | 18.465 | 174171 | 1.15 | 11371 |
| 3 | β-isomer | 26.322 | 83976 | 1.19 | 10826 |
| **Temperature minus** | | | | | |
| 1 | Spironolactone | 22.127 | 92725612 | 0.80 | 7245 |
| 2 | β- Canrenone | 18.434 | 164061 | 1.23 | 10534 |
| 3 | β-isomer | 26.356 | 85890 | 1.37 | 10667 |
| **Temperature plus** | | | | | |
| 1 | Spironolactone | 21.998 | 92846739 | 0.78 | 7234 |
| 2 | β- Canrenone | 18.113 | 16986 | 1.10 | 10673 |
| 3 | β-isomer | 26.204 | 85458 | 1.13 | 10538 |

**Forced Degradation Studies:** Forced degradation studies were performed as per ICH guideline ICHQ1A.

Forced degradation studies were performed to establish stability, indicating assay method under stressed conditions for spironolactone.

**Base Degradation Studies:** For base degradation studies 1 ml stock solution of spironolactone standard drug and sample drug were taken, and 0.2ml 0.1N NaOH was added and refluxed for half an hour at 60ºC temperature. 20µl of each sample was injected into HPLC column, and stability studies were performed.

From the chromatogram below, it is established that all the base degradation peaks of β- canrenone, β- isomer and spironolactone are well separated, and the purity of each peak is up to par.

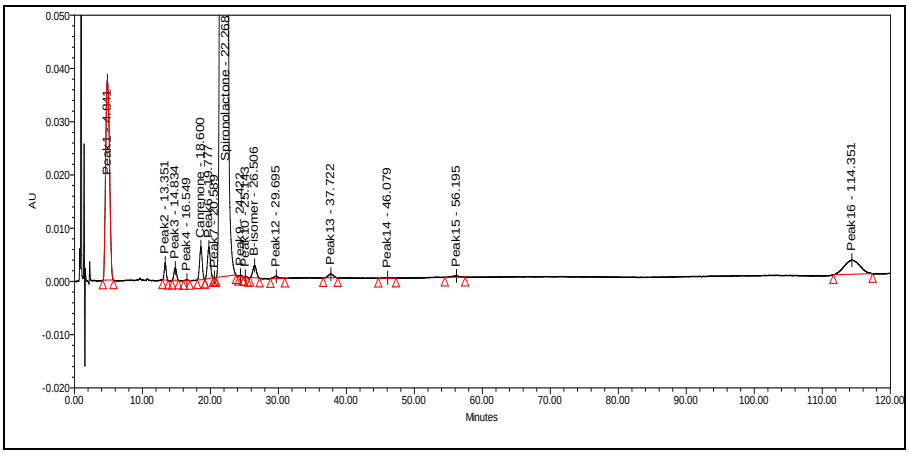


**FIG. 10: CHROMATOGRAM SHOWING BASE DEGRADATION STUDIES**

**TABLE 10: RESULTS FOR BASE DEGRADATION STUDIES**

| S. no. | Name | Retention Time | Area | % Area | USP Resolution | USP Tailing | USP Plate Count | Purity1 Flag |
|---|---|---|---|---|---|---|---|---|
| 1 | Peak1 | 4.841 | 1503256 | 1.58 | NA | 1.16 | 343 | Yes |
| 2 | Peak2 | 13.351 | 64164 | 0.07 | 10.70 | 1.03 | 9815 | No |
| 3 | Peak3 | 14.834 | 61252 | 0.06 | 2.30 | 0.97 | 6564 | Yes |
| 4 | Peak4 | 16.549 | 2915 | 0.00 | 2.33 | 0.85 | 3755 | No |
| 5 | Canrenone | 18.600 | 165063 | 0.17 | 2.84 | 1.01 | 10567 | **No** |
| 6 | Peak6 | 19.777 | 174831 | 0.18 | 1.53 | 1.02 | 9779 | Yes |
| 7 | Peak7 | 20.589 | 491 | 0.00 | 1.56 | 1.01 | 123353 | No |
| 8 | Spironolactone | 22.268 | 92858136 | 97.30 | 2.60 | 0.70 | 7127 | **No** |
| 9 | Peak9 | 24.422 | 5468 | 0.01 | 2.62 | 1.13 | 21682 | No |
| 10 | Peak10 | 25.143 | 2499 | 0.00 | 1.23 | 1.20 | 44061 | No |
| 11 | B-isomer | 26.506 | 85749 | 0.09 | 1.71 | 1.02 | 10791 | **No** |
| 12 | Peak12 | 29.695 | 21190 | 0.02 | 2.89 | 0.99 | 8675 | No |
| 13 | Peak13 | 37.722 | 37192 | 0.04 | 6.02 | 1.02 | 10076 | No |
| 14 | Peak14 | 46.079 | 8051 | 0.01 | 6.10 | 0.96 | 11456 | No |
| 15 | Peak15 | 56.195 | 18804 | 0.02 | 6.04 | 0.91 | 6005 | No |
| 16 | Peak16 | 114.351 | 427205 | 0.45 | 17.40 | 1.03 | 10392 | No |

**Thermal Degradation:** To establish thermal studies, 1ml of stock solution of standard drug and samples were exposed to a temperature of 105ºC for 72 h. 20µl of each solution was injected into the HPLC column for thermal studies. From the chromatogram, it has been concluded that all the chromatographic parameters obtained were within range.

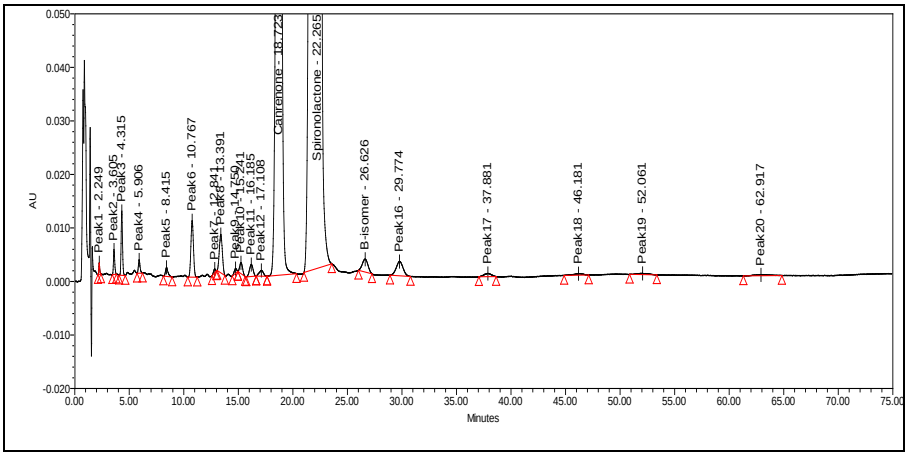


**FIG. 11: CHROMATOGRAM FOR THERMAL DEGRADATION**

**TABLE 11: PARAMETERS OF THERMAL DEGRADATION**

| S. no. | Name | Retention Time | Area | % Area | USP Resolution | USP Tailing | USP Plate Count | Purity1 Flag |
|---|---|---|---|---|---|---|---|---|
| 1 | Peak1 | 2.249 | 12429 | 0.02 | NA | 1.23 | 3482 | No |
| 2 | Peak2 | 3.605 | 38479 | 0.05 | 7.19 | 1.18 | 4499 | Yes |
| 3 | Peak3 | 4.315 | 104892 | 0.13 | 3.08 | 1.11 | 5649 | Yes |
| 4 | Peak4 | 5.906 | 27153 | 0.03 | 6.08 | 1.28 | 7133 | Yes |
| 5 | Peak5 | 8.415 | 27101 | 0.03 | 6.70 | 1.46 | 6630 | No |
| 6 | Peak6 | 10.767 | 170748 | 0.21 | 5.24 | 1.04 | 10038 | No |
| 7 | Peak7 | 12.841 | 8916 | 0.01 | 5.21 | 0.79 | 20457 | No |
| 8 | Peak8 | 13.391 | 138244 | 0.17 | 1.23 | 1.06 | 10341 | Yes |
| 9 | Peak9 | 14.750 | 14335 | 0.02 | 2.82 | 0.83 | 18492 | Yes |
| 10 | Peak10 | 15.241 | 38124 | 0.05 | 1.04 | 1.19 | 14005 | No |
| 11 | Peak11 | 16.185 | 51358 | 0.06 | 1.66 | 0.95 | 10297 | Yes |
| 12 | Peak12 | 17.108 | 28893 | 0.04 | 1.31 | 0.95 | 7231 | Yes |
| 13 | Canrenone | 18.723 | 6851222 | 8.34 | 2.10 | 0.96 | 10401 | No |
| 14 | Spironolactone | 22.265 | 74347235 | 90.51 | 4.04 | 0.73 | 8032 | No |
| 15 | B-isomer | 26.626 | 86860 | 0.11 | 4.39 | 1.01 | 11975 | No |
| 16 | Peak16 | 29.774 | 117675 | 0.14 | 2.86 | 1.06 | 10599 | No |
| 17 | Peak17 | 37.881 | 28126 | 0.03 | 6.28 | 0.99 | 15241 | No |
| 18 | Peak18 | 46.181 | 16897 | 0.02 | 4.45 | 0.83 | 10013 | No |
| 19 | Peak19 | 52.061 | 15857 | 0.02 | 2.62 | 1.07 | 7126 | No |
| 20 | Peak20 | 62.917 | 16850 | 0.02 | 4.51 | 1.04 | 8280 | No |

**CONCLUSION:** A simple, linear, accurate, precise, and robust HPLC method for the estimation and separation of Spironolactone and degradants (Canrenone and β-isomer) in tablet dosage forms was developed and validated as per ICH guidelines. Various analytical parameters like mobile phase, mobile phase composition, diluents, column temperature, type of column, and flow rate were tested. Best peak separation between Spironolactone, Canrenone, and β-isomer and peak purity of all the peaks were achieved with mobile phase Water: Tetrahydrfuran: Acetonitrile: 77:21:2, symmetry column C-8 (150mmX3.9mm) 5µm, column temperature 40ºC, flow rate 1.0ml/min, wavelength 254 nm and 283 nm. The parameters like linearity, accuracy, precision, and robustness were evaluated. Forced degradation studies were also performed to separate the peaks of spironolactone and beta canrenone. The developed method is specific and can quantify spironolactone in presence of the degradent products. Therefore, the method can be successfully used in routine work for the quantification of spironolactone in tablet dosage form available on the market.

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