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# QUANTIFICATION OF SPIRONOLACTONE AND ITS IMPURITIES PRESENT IN PHARMACEUTICAL DOSAGE FORMS BY STABILITY-INDICATING HPLC METHOD

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**ABSTRACT:** Spironolactone belongs to a group of medicine known as aldosterone antagonist used as sparing diuretic, Spironolactone (aldactone) is potassium -aspiring diuretic agent and has half-life of about 16 hours. Spironolactone one diuretics agents which increases renal excretion of water and solutes (mainly sodium salt). It is used mainly in the treatment of refractory edema in patients with congestive heart failure nephrotic syndrome, or hepatic cirrhosis. On its own, spironolactone is only a weak diuretic, but it can be combined with other diuretics. Spironolactone is 7α-acetyle thio-3-oxo-17α-pregn-4-ene-21,17β-carbolactone. Its molecular formula is $C_{24}$ $H_{32}O_4S$ having a molecular weight 416.58 gm/mole. The possible related compound (degradants and process-related impurities) as Spironolactone related compound –A of Spironolactone having chemical names are S-((2'R, 7R, 8R, 9S, 10R, 13S, 14S)-10, 13-Dimethyl-3, 5'-dioxo-1, 2, 3, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16-tetradecahydro-5'H-spiro[cyclopenta[a]phenanthrene-17,2'-furan]-7-yl) ethanethioate.

**INTRODUCTION:** Spironolactone is primarily used to treat fluid build-up due to heart failure, liver scarring, or kidney disease. It is also used in the treatment of high blood pressure, low blood potassium that does not improve with supplementation, early puberty in boys, acne and excessive hair growth in women and as a part of transgender hormone therapy in transfeminine people [1, 2]. Extended review revels that various analytical methods based on HPTLC, HPLC-APCI-MS [2, 3, 4] UPLC have been developed for determination of spironolactone pharmaceutical dosage form.

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According literature survey there is no validated stability indicating HPLC method for spironolactone solution [2, 5, 6]. This paper describes the Validating method for the related substances of Spironolactone solution for Known Impurities. For the Method Validation of Related substances of spironolactone to study validating parameter like specificity, Linearity, Precision [7, 8 9 10], Stability in analytical solution [11], also describes the parameter like Accuracy, Robustness [12, 13, 14]. The main advantage of this paperis to analyse spironolactone solution degradants

## Experimental:

**Chemical and Reagents:** Spironolactone Working Standards Were Obtained from Callidus Research Laboratories. Pvt. LTD, Pune. Water for HPLC, Ammonium acetate, Glacial Acetic Acid, Ammonia Solution, Acetonitrile HPLC grade, Methanol HPLC Grade, Hydrochloric acid Emparta

Grade, Sodium Hydroxide Emparta Grade, Hydrogen Peroxide, Spironolactone 25mg/5ml oral Solution was formulated and obtained from Callidus Research Laboratories Pvt. Ltd. Water used was obtained by using Millipore MilliQ Plus water purification system.

**Instrumentation:** The Thermo Scientific HPLC system with a photodiode array detector (PDA). The Output Signal was monitored and processed using the Chromo Leon Software. Hydrolysis studies were carried out in a water bath (Bio=Techniques India). Photostability studies were carried out in a photostability chamber (Mack Pharmatech). Thermal Stability Studies were performed in a dry-air oven (Bio-Techniques India).

**Chromatographic Condition:** Thermo scientific HPLC system Comprises with Online Degasser, auto injector, column compartment, photodiode array detector and the system was controlled through Chromo Leon Software.

The Chromatographic Separation was achieved on Waters X Bridge, C18,4.6 x 250mm, 5µ column, using mobile phase consisting Mobile Phase A: ammonium acetate (pH7.20) and Mobile Phase B: HPLC Grade Methanol, at a flow rate 0.9ml/min.

The Column Compartment temperature was set at 40°C and the sampler temperature was set at 25°C and the injection volume 10 µl. The UV spectrum spironolactone shows absorption maximum at 254nm. Typical HPLC chromatogram were extracted at this wavelength.

**Analytical Procedure:**

**Preparation of Diluted Standard Solution:** Weigh and transfer accurately about 20 mg of Spironolactone working/reference standard in to a 50 mL volumetric flask. Add 35 mL of diluent and sonicate to dissolve. Dilute to volume with diluent and mix well. Pipette 5 mL of this solution in to 50 mL volumetric flask and make up volume to 50 mL with diluent and mix well. Further dilute 5 mL of this solution to 100 mL with diluent and mix well.

**Preparation of Placebo Solution:** Pipette out 5 mL of Placebo for Spironolactone 25 mg/5ml Oral Solution corresponds to 25 mg of Spironolactone into 25 mL volumetric flask. Add 15 mL diluent and shake well. Dilute to volume with diluent and mix well. Fill in to HPLC vial for further analysis.

**Preparation of Sample Solution:** Pipette out 5 mL sample of Spironolactone 25 mg/5ml Oral Solution equivalent to 25 mg of Spironolactone into 25 mL volumetric flask. Add 15 mL diluent and shake well. Dilute to volume with diluent and mix well. Fill in to HPLC vial for further analysis.

**Procedure:** Inject the Blank (diluent), Blank in single, Diluted standard solution in six replicates, Placebo solution and Sample solution in single in the sequence. Record the chromatograms. Find out the system suitability [15, 16] parameters as given below, from the System suitability solution and diluted standard solution chromatogram. Measure the area counts of impurity peaks. Examine the blank and placebo chromatogram for any extraneous peaks and disregard any corresponding peaks observed in the chromatogram of sample solution.

**Gradient Programme Elution:** (Blank, Placebo and Sample solution).

| Time (min) | % Mobile Phase A | % Mobile Phase B |
|---|---|---|
| 00.00 | 50 | 50 |
| 50.00 | 50 | 50 |
| 55.00 | 20 | 80 |
| 62.00 | 20 | 80 |
| 65.00 | 50 | 50 |
| 70.00 | 50 | 50 |

## RESULT AND DISCUSSIONS:

**Method Validation:** The validation of the developed method was done according to ICH guidelines with respect to system suitability, specificity, linearity, accuracy, precision, solution stability and robustness [17, 18, 19].

**TABLE 1: FORCE DEGRADATION STUDY**

| Sr. no. | Degradation | Degradation Condition | Total Imp % (A) | % Assay by assay method (B) | Total mass (C) = A+B | Mass Balance |
|---|---|---|---|---|---|---|
| 1 | Control | As such | 0.26 | 100.9 | 101.2 | NA |
| 2 | Acid | 0.02 N HCl / 0.5 mL / RT/ 30 minutes | 0.27 | 100.2 | 100.5 | 99.3 |
| 3 | Base | 0.02 N NaOH /0.5mL / RT / 5 | 15.11 | 87.9 | 103.0 | 101.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | minutes | | | | |
| 4 | Peroxide | 1% $H_2O_2$ / 2 mL / RT / 28 hours | 0.30 | 100.3 | 100.6 | 99.4 |
| 5 | Control | As such | 0.30 | 100.5 | 100.8 | NA |
| 6 | Thermal | 105°C – 48 hours | 0.22 | 100.4 | 100.6 | 99.8 |
| 7 | Humidity | 40°C/75% RH – 72 hours | 0.25 | 98.9 | 99.2 | 98.4 |
| 8 | Photolytic Control | Exposed with Wrapping Aluminum foil at 1.2 million lux hours and UV energy 200-watt hours/square meter | 0.27 | 101.6 | 101.9 | 101.1 |
| 9 | Photolytic Primary pack | 1.2 million lux hours and UV energy 200-watt hours/square meter. | 0.28 | 100.9 | 101.2 | 100.4 |
| 10 | Photolytic Clear Glass | 1.2 million lux hours and UV energy 200-watt hours/square meter | 1.76 | 98.2 | 100.0 | 99.2 |

**System Suitability:** System suitability tests were used to verify that the proposed method produced acceptable results with high reproducibility. System suitability is analyzed in terms of tailing factor (must be <2.0), theoretical plate counts (should be >2000), retention time, *etc*. According to the results presented, theoretical plate, tailing factor, %RSD. He proposed HPLC method fulfils these requirements within the accepted limits.

**TABLE 2: EVALUATION OF ACCURACY:**

| Amount spiked | % Recovery | |
|---|---|---|
| | Spironolactone | Spironolactone Related compound A |
| LOQ | 87.4 | 95.0 |
| 50% | 94.9 | 96.8 |
| 100% | 97.6 | 92.2 |
| 150% | 106.9 | 97.2 |
| % RSD | 7.41 | 2.94 |

**Specificity:** The specificity of the method was checked by injecting solution containing excipient without drug substances, and no chromatographic interference was observed from the excipient at the retention time of the analyte peaks. The specificity of the method was also checked by performing the stressed study and no interference from degradation products at the retention time of Spironolactone was found. Stress studies were performed for Spironolactone 25mg/5ml oral Solution to provide an indication of the stability-indicating property and specificity of the proposed method summarised in **Table 3**. Intentional degradation was attempted to determine the stress conditions of heat (50°C), photolytic sunlight for ~1.2 million lux h and UV light, both at shorter and longer wavelengths for ~200 Wh/$m^3$, acid (0.02 N HCl refluxed at RT° for 30min), base (0.02 N NaOH refluxed at RT for 5MIN) and oxidation (1% $H_2O_2$ refluxed at room temperature for 28h) and for humidity, the study was for 72 h at 40°C and 75% relative humidity (RH).

**Linearity:** Linearity of the assay method was tested from LOQ% to 200% (Spironolactone 4.0 µg $ml^{-1}$, 10µg $ml^{-1}$) and of analyte. The calibration graphs were obtained by plotting peak area ratios against the concentration of the drugs.

**Precision:** The repeatability of the analytical method was evaluated by analyzing six test samples solutions of Spironolactone oral solution during the same day, under the same experimental conditions. Intermediate precision was evaluated by analyzing six test solutions on different days. Precision was expressed as a relative standard deviation of percentage found to be well within the limit. Hence, the method was found to be precise **Table 3A** and **B** [20, 21, 22].

**TABLE 3A: PRECISION AND RUGEDNESS: UNSPIKED SAMPLE**

| Sample | % Impurity at RRT | | | | | | % Total Impurity |
|---|---|---|---|---|---|---|---|
| | Unk. Imp. at RRT 0.51 | Unk. Imp. at RRT 0.69 | Unk. Imp. at RRT 1.50 | Unk. Imp. at RRT 1.65 | Unk. Imp. at RRT 1.94 | Unk. Imp. at RRT 1.98 | |
| Precision-1 | 0.020 | 0.029 | 0.040 | 0.064 | 0.030 | 0.043 | 0.23 |
| Precision-2 | 0.029 | 0.029 | 0.035 | 0.069 | 0.030 | 0.038 | 0.23 |
| Precision-3 | 0.027 | 0.028 | 0.038 | 0.060 | 0.026 | 0.041 | 0.22 |
| Precision-4 | 0.026 | 0.027 | 0.037 | 0.078 | 0.025 | 0.044 | 0.24 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Precision-5 | 0.028 | 0.028 | 0.028 | 0.083 | 0.025 | 0.039 | 0.23 |
| Precision-6 | 0.027 | 0.025 | 0.040 | 0.071 | 0.024 | 0.046 | 0.23 |
| Ruggedness-1 | 0.022 | 0.021 | 0.029 | 0.052 | 0.039 | 0.041 | 0.20 |
| Ruggedness-2 | 0.020 | 0.021 | 0.034 | 0.065 | 0.027 | 0.042 | 0.21 |
| Ruggedness-3 | 0.018 | 0.022 | 0.031 | 0.053 | 0.036 | 0.044 | 0.20 |
| Ruggedness-4 | 0.016 | 0.023 | 0.032 | 0.063 | 0.030 | 0.049 | 0.21 |
| Ruggedness-5 | 0.021 | 0.022 | 0.032 | 0.059 | 0.027 | 0.050 | 0.21 |
| Ruggedness-6 | 0.020 | 0.023 | 0.035 | 0.058 | 0.033 | 0.042 | 0.21 |
| Cumulative % Mean | 0.02 | 0.02 | 0.03 | 0.06 | 0.03 | 0.04 | 0.22 |
| Cumulative % SD | 0.004 | 1.254 | 3.135 | 2.983 | 2.373 | 1.738 | 0.562 |
| Cumulative %RSD | 19.18 | 12.53 | 11.73 | 14.45 | 15.74 | 8.14 | 5.32 |

**TABLE 3B: PRECISION AND RUGEDNESS: SPIKED SAMPLE**

| **Sample** | **% Impurity at RRT** | | | | | | **% Total Impurity** |
|---|---|---|---|---|---|---|---|
| | **Impurity-A at RRT 1.24** | **Unk. Imp. at RRT 0.69** | **Unk. Imp. at RRT 1.49** | **Unk. Imp. at RRT 1.66** | **Unk. Imp. at RRT 1.94** | **Unk. Imp. at RRT 1.98** | |
| Precision-1 | 0.496 | 0.025 | 0.035 | 0.063 | 0.025 | 0.043 | 0.69 |
| Precision-2 | 0.507 | 0.025 | 0.029 | 0.081 | 0.026 | 0.036 | 0.70 |
| Precision-3 | 0.501 | 0.028 | 0.036 | 0.083 | 0.029 | 0.044 | 0.72 |
| Precision-4 | 0.512 | 0.025 | 0.029 | 0.072 | 0.025 | 0.038 | 0.70 |
| Precision-5 | 0.545 | 0.028 | 0.030 | 0.071 | 0.028 | 0.040 | 0.74 |
| Precision-6 | 0.558 | 0.026 | 0.027 | 0.075 | 0.029 | 0.037 | 0.75 |
| Ruggedness-1 | 0.563 | 0.024 | 0.037 | 0.061 | 0.031 | 0.037 | 0.75 |
| Ruggedness-2 | 0.559 | 0.023 | 0.040 | 0.057 | 0.026 | 0.044 | 0.75 |
| Ruggedness-3 | 0.556 | 0.022 | 0.036 | 0.064 | 0.025 | 0.040 | 0.74 |
| Ruggedness-4 | 0.559 | /’0.023 | 0.038 | 0.065 | 0.023 | 0.032 | 0.74 |
| Ruggedness-5 | 0.546 | 0.023 | 0.036 | 0.059 | 0.022 | 0.033 | 0.72 |
| Ruggedness-6 | 0.563 | 0.023 | 0.037 | 0.070 | 0.020 | 0.037 | 0.75 |
| Cumulative % Mean | 0.54 | 0.02 | 0.03 | 0.07 | 0.03 | 0.04 | 0.73 |
| Cumulative % SD | 0.026 | 1.481 | 3.006 | 2.357 | 1.733 | 2.221 | 0.739 |
| Cumulative %RSD | 4.89 | 8.16 | 12.51 | 12.07 | 11.81 | 10.40 | 3.11 |

*Impurities Level Observed LOQ to 0.1 % NMT 20.0, *Impurities Level Observed More than 0.1 % NMT 15.0, *Total Impurities Observed in more than 0.1% NMT 15.0.

**Accuracy:** Accuracy was evaluated for determining analytes in a solution prepared by the standard addition method. The experiment was carried out by adding a known amount of component corresponding to three concentrations levels 50%, 100% and 150% of the target analyte concentration in placebo solution. The samples were prepared in triplicate at each level.

The solutions were then analyzed as per the proposed method. The quantification of added analyte (% weight/weight) was carried out by using an external standard of the corresponding main drug prepared at the analytical concentration. The recovery values for Spironolactone are as shown in **Table 2**. The established quantification range is from 50 % to 150 % for Spironolactone target analytic concentration.

**Stability in Analytical Solution:** Prepared diluted standard solution, system suitability solution, placebo solution, controlled sample solution of Spironolactone and Spironolactone Solution sample spiked with Spironolactone Impurity D at stability specification level *i.e.* at 0.20 % level (Prepared spiked sample as mentioned in 100 % accuracy level sample). Stored these samples at 25°C and 2-8°C and established the solution stability. Injected and analyzed these solutions continuously on hourly basis at a regular interval till the solution complies with acceptance criteria at least 48 Hrs. **Table 4A and 4B.**

**TABLE 4: MOBILE PHASE STABILITY: RETENTION TIME FOR DILUTED STANDARD**

| Sr. no. | Time in (hours) about | Spironolactone | |
|---|---|---|---|
| | | Retention Time (min) | % Difference with Initial |
| 1 | 0.0 | 22.537 | NA |
| 2 | 6.0 | 22.108 | -1.90 |
| 3 | 11.0 | 21.282 | -5.57 |
| 4 | 15.0 | 21.028 | -6.70 |
| 5 | 19.0 | 22.577 | 0.18 |
| 6 | 22.0 | 22.215 | -1.43 |
| 7 | 26.0 | 22.522 | -0.07 |
| 8 | 29.0 | 22.160 | -1.67 |
| 9 | 33.0 | 22.537 | 0.00 |
| 10 | 36.0 | 22.205 | -1.47 |
| 11 | 40.0 | 22.650 | 0.50 |
| 12 | 43.0 | 22.248 | -1.28 |
| 13 | 47.0 | 22.573 | 0.16 |
| 14 | 50.0 | 22.217 | -1.42 |
| 15 | 54.0 | 22.583 | 0.20 |
| 16 | 57.0 | 22.208 | -1.46 |
| 17 | 60.0 | 22.595 | 0.26 |
| 18 | 63.0 | 22.240 | -1.32 |

**TABLE 4A: SOLUTION STABILITY AT 25°C: SPIRONOLACTONE DILUTED STANDARD**

| Sr. no. | Time in (hours) about | Spironolactone | |
|---|---|---|---|
| | | Response (Area) | % Difference with Initial |
| 1 | 0.0 | 41010 | NA |
| 2 | 6.0 | 41141 | 0.32 |
| 3 | 11.0 | 41492 | 1.18 |
| 4 | 15.0 | 40670 | -0.83 |
| 5 | 19.0 | 41481 | 1.15 |
| 6 | 22.0 | 41426 | 1.01 |
| 7 | 26.0 | 40495 | -1.26 |
| 8 | 29.0 | 41667 | 1.60 |
| 9 | 33.0 | 42316 | 3.18 |
| 10 | 36.0 | 42735 | 4.21 |
| 11 | 40.0 | 43156 | 5.23 |
| 12 | 43.0 | 41140 | 0.32 |
| 13 | 47.0 | 41643 | 1.54 |
| 14 | 50.0 | 42197 | 2.89 |
| 15 | 54.0 | 41650 | 1.56 |
| 16 | 57.0 | 44572 | 8.69 |
| 17 | 60.0 | 43074 | 5.03 |
| 18 | 63.0 | 43344 | 5.69 |

**TABLE 4B: SOLUTION STABILITY AT 25°C: SPIRONOLACTONE: SPIKE SAMPLE**

| Sr. no. | Time in (hrs.) about | Impurity at RRT 1.96 | | Impurity at RRT 1.98 | | Spironolactone | |
|---|---|---|---|---|---|---|---|
| | | Area | % Diff. with Initial | Area | % Diff. with Initial | Area | % Diff. with Initial |
| 1 | Initial | 6101 | NA | 7155 | NA | 20273912 | NA |
| 2 | 18.0 | 5605 | -8.13 | 7747 | 8.27 | 20299848 | 0.13 |
| 3 | 24.0 | 6128 | 0.44 | 7834 | 9.49 | 20568538 | 1.45 |
| 4 | 31.0 | 5966 | -2.21 | 7647 | 6.88 | 20552136 | 1.37 |
| 5 | 38.0 | 5560 | -8.87 | 7484 | 4.60 | 20593778 | 1.58 |
| 6 | 45.0 | 5875 | -3.70 | 6946 | -2.92 | 20684076 | 2.02 |
| 7 | 51.0 | 5787 | -5.15 | 6715 | -6.15 | 20799150 | 2.59 |
| 8 | 57.0 | 5983 | -1.93 | 7588 | 6.05 | 20754430 | 2.37 |

**Robustness:** To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method (e.g., flow rate, column temperature, pH of the buffer solution, organic composition, wavelength).

The flow rate for mobile phase was 1ml/min; to study the effect of flow rate on resolution it was changed to 0.8ml/min and 1.0ml/min. to study effect of pH it was changed to 7.0 and 7.4 instead 7.2. The effect of column temperature was studied at 50°C and 40°C.

The effect of organic concentration was studied by changing the concentration to ±10%. The effect of wavelength was studied at 249 nm and 259 nm. The Changes in chromatographic parameters (i.e., theoretical plates and tailing factor) were evaluated for the studies. In all the deliberately varied chromatographic conditions, the chromatogram for system suitability [15, 16] solution showed satisfactory results with no significant changes in chromatographic parameters for assay and dissolution are shown in **Table 6A** and **6B.**

**Filter Equivalency:** Prepared sample solution of Spironolactone 25mg/5ml Oralsolution by spiked with spironolactone Related Compound A at stability level i.e.50%level. Filtered it in triplicate and filter in triplicate through one or more, different types of filters, such as Nylon 0.45 µ, PVDF 0.45µ filters by discarding first 2-3 ml of filtrate and then further analyzed into the HPLC **Table 5A** and **5B** [23, 24, 25].

**TABLE 5A: FILTER EQUIVALENCY: FOR SPIRONOLACTONE 25 MG/5ML ORAL SOLUTION SAMPLE**

| Sample | Impurity A (RRT 1.24) | % Unknown Impurities at RRT | | | | | % Total Impurities |
|---|---|---|---|---|---|---|---|
| | | 0.68 | 1.51 | 1.67 | 1.97 | 2.00 | |
| Unfiltered Sample-1 | 0.532 | 0.027 | 0.048 | 0.070 | 0.044 | 0.035 | 0.756 |
| Unfiltered Sample-2 | 0.521 | 0.028 | 0.037 | 0.065 | 0.041 | 0.039 | 0.731 |
| Unfiltered Sample-3 | 0.534 | 0.028 | 0.038 | 0.062 | 0.033 | 0.038 | 0.734 |
| 0.45µ Nylon-1 | 0.532 | 0.023 | 0.043 | 0.059 | 0.035 | 0.037 | 0.730 |
| 0.45µ Nylon-2 | 0.537 | 0.027 | 0.043 | 0.058 | 0.038 | 0.037 | 0.741 |
| 0.45µ Nylon-3 | 0.543 | 0.030 | 0.043 | 0.061 | 0.027 | 0.043 | 0.748 |
| % RSD | 1.39 | 8.55 | 9.17 | 6.90 | 16.60 | 7.55 | 1.37 |
| 0.45µ PVDF-1 | 0.53 | 0.03 | 0.03 | 0.07 | 0.03 | 0.04 | 0.74 |
| 0.45µ PVDF-2 | 0.52 | 0.03 | 0.04 | 0.06 | 0.03 | 0.04 | 0.72 |
| 0.45µ PVDF-3 | 0.52 | 0.03 | 0.04 | 0.08 | 0.03 | 0.05 | 0.76 |
| % RSD | 1.40 | 4.64 | 14.08 | 12.24 | 18.92 | 13.79 | 2.02 |

**TABLE 5B: FILTER EQUIVALENCY FOR SPIRONOLACTONE STANDARD SOLUTION**

| Sample No. | Area of Spironolactone | | |
|---|---|---|---|
| | Unfiltered | 0.45µ Nylon | 0.45µ PVDF |
| 1 | 40381 | 40813 | 41163 |
| 2 | 40921 | 39433 | 40079 |
| 3 | 41112 | 40447 | 40397 |
| Mean | 40805 | 40231 | 40546 |
| % Correlation with Unfiltered | NA | 98.6 | 99.4 |

**TABLE 6A: ROBUSTNESS EVALUATION OF THE DEVELOPED HPLC METHOD**

| Robustness Criteria | Condition | RRT Related Compound | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Unknown Impurities at RRT | | | | | | |
| | | 0.51 | 0.69 | 1.50 | 1.65 | 1.94 | 1.98 | % Total Imp |
| Control (Ruggedness) | - | 12.24 | 3.41 | 6.70 | 8.59 | 14.65 | 8.41 | 1.95 |
| Flow Rate (0.90 mL/min ± | 0.80 mL/min | BLQ | 6.37 | 10.34 | 12.46 | 14.18 | 8.68 | 2.23 |
| 0.1 ml/min | 1.00 mL/min | BLQ | BLQ | 10.38 | 9.12 | 13.64 | 15.70 | 8.26 |
| Column Temperature (45°C | 40°C | BLQ | 3.11 | 8.90 | 7.84 | 13.37 | 8.41 | 10.96 |
| ±5°C) | 50°C | BLQ | 7.58 | 10.50 | 8.72 | 13.60 | 9.48 | 1.98 |
| Control (Method Precision) | - | 11.63 | 5.04 | 12.59 | 11.99 | 9.45 | 6.85 | 2.37 |
| Change in Wavelength (254 | 249 nm | 16.45 | 12.68 | 14.66 | 14.85 | 9.52 | 11.13 | 9.46 |
| nm ±5 nm) | 259 nm | BLQ | BLQ | 14.75 | 15.91 | 12.49 | 6.32 | 12.97 |
| Change in pH of buffer | pH 7.00 | BLQ | 4.90 | 11.48 | 11.75 | 9.48 | 8.65 | 6.66 |
| solution (7.20 ± 0.2 units) | pH 7.40 | 11.63 | 7.39 | 12.02 | 11.92 | 11.08 | 7.27 | 7.08 |

**TABLE 6B: ROBUSTNESS OF HPLC METHOD PARAMETERS-SYSTEM SUITABILITY**

| Robustness Criteria | Condition | System Suitability Criteria | | | | |
|---|---|---|---|---|---|---|
| | | Retention Time (min) | Theoretical Plates | Tailing Factor | % RSD STD | % RSD BKTS STD |
| Control (Ruggedness) | - | 22.917 | 12175 | 1.00 | 1.23 | 2.70 |
| Flow Rate (0.90 mL/min ± 0.1 ml/min | 0.80 mL/min | 26.195 | 12498 | 0.99 | 2.65 | 3.23 |
| | 1.00 mL/min | 20.823 | 12300 | 0.99 | 1.04 | 3.60 |
| Column Temperature | 40°C | 26.685 | 7933 | 0.99 | 2.09 | 2.00 |
| (45°C ±5°C) | 50°C | 20.520 | 11647 | 1.02 | 1.23 | 1.60 |
| Control (Method Precision) | - | 22.773 | 7838 | 1.10 | 2.03 | 2.90 |
| Change in Wavelength | 249 nm | 22.403 | 6661 | 1.14 | 2.06 | 2.09 |
| (254 nm ±5 nm) | 259 nm | 22.407 | 5988 | 1.22 | 1.93 | 3.03 |
| Change in pH of buffer | pH 7.00 | 22.772 | 8102 | 0.99 | 1.84 | 1.69 |
| solution (7.20 ± 0.2 units) | pH 7.40 | 23.138 | 8988 | 0.98 | 0.88 | 1.00 |

STD- Standard; BKTS-Bracketing Standard

**DISCUSSION:** The results from the method development indicate that the planned method conditions are optimum and best fit for the analysis of the spironolactone and its impurities. As all the peaks of the interest were eluting at <15 min. The prolonged runtime was given to wash out the inactive ingredients if any were retained on the column. Based on the nature of the sample, one can reduce the runtime accordingly.

The forced degradation study results prove the method's stability-indicating capability. The lower LOD and LOQ values show the higher sensitivity of the method. The results from the method validation study prove that the proposed analytical method is precise, accurate, linear and specific.

The precision, linearity and accuracy results prove the range of analytical method from LOQ to 150% of the target specified concentration for Spironolactone and its four impurities.

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**CONFLICTS OF INTEREST:** Nil

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