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RESEARCH ARTICLE

Stability-Indicating RP-HPLC Method for the Simultaneous Estimation of Spironolactone and Furosemide in Bulk and Combined Pharmaceutical Formulations

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Abstract: Development and validation of a rapid, precise, and stability-indicating reversed-phase high-performance liquid chromatographic method enables the simultaneous estimation of spironolactone and furosemide in bulk forms and commercial solid oral dosage units. Chromatographic resolution is successfully achieved on a reversed-phase C18 column (250 mm×4.6 mm, 5 µm) utilizing an optimized isocratic mobile phase consisting of methanol and 0.1% aqueous acetic acid (60:40 v/v). The mobile phase is maintained at a standardized flow rate of 0.7 mL/min, with spectrophotometric detection at an isosbestic wavelength of 254 nm. Under these optimized parameters, furosemide and spironolactone resolve cleanly with baseline separation at retention windows of approximately 4.7 minutes and 6.3 minutes, respectively. Validation of the developed method performed in accordance with International Council for Harmonisation Q2(R1) regulatory guidelines confirm robust linearity across working concentration boundaries of 10- 50 µg/mL for furosemide and 25-125 µg/mL for spironolactone. Mean analytical recoveries range from 99.08%- 100.21% showing exceptional system accuracy devoid of chemical interference from common diagnostic tablet excipients. Forced degradation studies conducted under hydrolytic, oxidative, photolytic, and thermal stressors verify the stability-indicating capacity of the assay, as all generated degraded products resolve from the intact active therapeutic molecules. The validated method is highly reproducible and suitable for implementation within routine industrial quality control protocols and long-term shelf-life stability evaluations.

Keywords: Spironolactone; Furosemide; RP-HPLC; Stability-Indicating; Method Validation.

1. Introduction

The co-administration of loop diuretics and potassium-sparing aldosterone antagonists represents a well-established clinical paradigm in the therapeutic management of congestive heart failure, hepatic cirrhosis-induced ascites, and refractory essential hypertension [1]. Furosemide (4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid) acts fundamentally upon the thick ascending limb of the loop of Henle to inhibit the Na^+-K^+-$2Cl^-$ cotransporter, thereby inducing profound natriuresis and diuresis [2]. However, prolonged monotherapy frequently precipitates severe hypokalemia, driving cardiotoxic risks. To overcome this deleterious effect, spironolactone (7α-acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone) is given along with furosemide to competitively antagonize mineralocorticoid receptors within the distal convoluted tubule, preserving homeostatic serum potassium levels while potentiating overall diuretic efficacy [3].

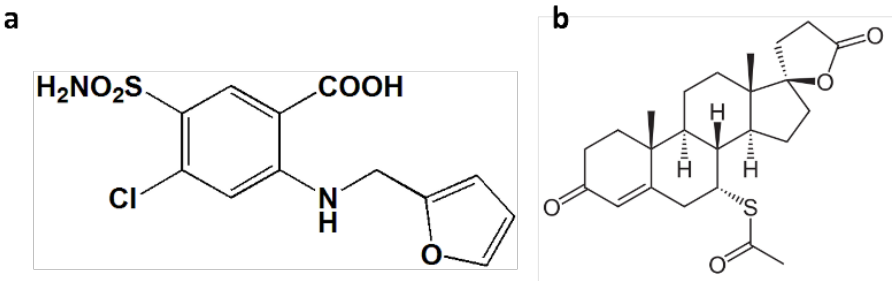

Figure 1. Structures of a. Furosemide and b. Spironolactone

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The therapeutic prevalence of combined formulations such as Furowise Plus tablets, the availability of highly selective, validated, and streamlined analytical tools is vital for pharmaceutical quality manufacturing and regulatory compliance [4]. Classical spectrophotometric methods often fall short when resolving multi-component matrices without intensive chemometric processing due to mutual spectral overlap [5]. Reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with ultraviolet detection serves as the gold standard for high-throughput operational assays, yet many conventional protocols suffer from protracted retention runtimes, hazardous mobile phase constituents, or a lack of proven stability-indicating capacity [6].

A stability-indicating method is explicitly defined by regulatory bodies as a validated quantitative analytical procedure that can accurately detect changes over time in the chemical properties of an active pharmaceutical ingredient, allowing distinct separation of the intact parent analytes from low-level degradation products, related impurities, and matrix excipients [1, 6]. This work presents the development, optimization, and validation of an isocratic RP-HPLC-UV assay meant for the efficient separation and quantification of spironolactone and furosemide. The inclusion of systematic forced degradation satisfies global current Good Manufacturing Practice mandates, ensuring the method's applicability for routine batch release testing and real-time stability monitoring [1, 4].

2. Materials and Methods

2.1. Materials and Reagents

Certified reference standards of furosemide and spironolactone active pharmaceutical ingredients were obtained as gift samples from Reliable's Shree Industrial Training Centre and Research Laboratory (RSITC), Jalgaon, India. Commercial multi-dose tablets, marketed under the brand name Furowise Plus, were purchased from a local medical retail outlet. High-performance liquid chromatography grade methanol and acetic acid, alongside analytical grade hydrochloric acid, sodium hydroxide, and hydrogen peroxide, were purchased from Merck India Ltd. High-purity pure water was filtered through a 0.45 μm membrane filter before laboratory use to eliminate microscopic particulate matter, ensuring suitability for high-sensitivity chromatographic analysis.

2.2. Instrumentation and Chromatographic Conditions

Chromatographic method development and system validation were performed using Agilent Technologies 1100 Series HPLC system equipped with a G1310A isocratic pump, an auto-injector, a thermostatted column compartment, and a variable wavelength ultraviolet detector, automated via ChemStation version 10.01 software [7, 8]. Isocratic analyte distribution was achieved using a reversed-phase C18 stationary column (250 mm×4.6 mm internal diameter, particle size 5 µm). The optimized mobile phase consisted of methanol and 0.1% aqueous acetic acid blended in a precise 60:40 (v/v) ratio. The aqueous mobile component was maintained at pH 2.8 via acetic acid concentration control to stabilize ionic equilibria. The mobile phase was filtered and degassed prior to system introduction [9]. Analytical flow was fixed at 0.7 mL/min under a column temperature of 33°C, with an injection volume of 20 μL and spectrophotometric monitoring maintained at 254 nm.

2.3. Preparation of Stock and Working Standard Solutions

Primary standard composite stock solutions were prepared by accurately weighing 50 mg of spironolactone reference standard and 20 mg of furosemide reference standard into a single 10 mL volumetric flask. The mixed analytes were dissolved in a localized volume of HPLC-grade methanol, subjected to a brief sonication window to ensure total sample dissolution, and diluted to the target mark with methanol. This initial dissolution step produced a stock solution containing fixed tracker levels of 5000 µg/mL spironolactone and 2000 µg/mL furosemide. Working standard solutions were sequentially generated by accurate volumetric pipetting of the primary stock solution, taking aliquots from 0.05 to 0.25 mL and diluting to 10 mL with the prepared mobile phase to establish exact linear ranges of 25- 125 µg/mL for spironolactone and 10- 50 µg/mL for furosemide [10].

2.4. Selection of Wavelength

To identify the optimal detection wavelength for simultaneous monitoring without sacrificing analyte sensitivity, the prepared working standard solutions were scanned individually over a wavelength spectrum of 200–400 nm against a clean methanol blank. Furosemide showed an individual ultraviolet absorbance maximum (λ_{max}) at 272 nm, while spironolactone exhibited a distinct maximum absorbance peak at 242 nm. Overlay processing of the spectra showed a well-defined isosbestic cross-over point at 254 nm, where both therapeutic compounds exhibit identical relative absorbance indices. Selecting 254 nm as the uniform analytical detection wavelength eliminated spectral interference, neutralized background noise from baseline shifts, and ensured sharp, well-resolved peak profiles suitable for simultaneous quantitative estimation.

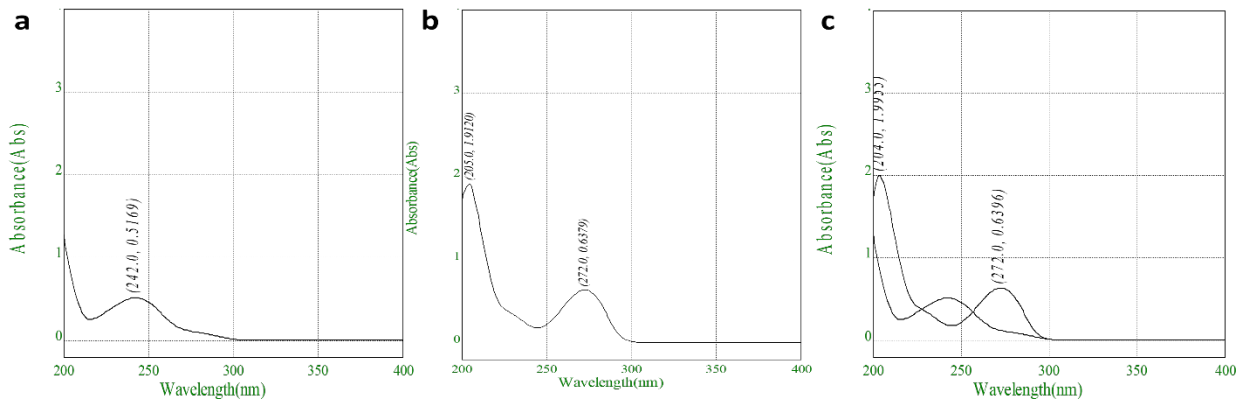


Figure 2. UV absorption spectrum of a. SPI (20 µg/mL) in methanol showing specific band maxima at 242 nm. b. FUR (20 µg/mL) in methanol outlining maximum spectral transmission at 272 nm. c. selected isosbestic wavelength at 254 nm.

2.5. Method Development and Mobile Phase Optimization Trials

Systematic trial-and-error optimization was carried out to establish robust method conditions that achieve baseline separation between the target loops and potassium-sparing agents within a concise elution window [11]. Mobile phase parameters were iteratively changed across five distinct trials:

- Trial 01: Utilizing a mobile phase composition of methanol and 0.1% aqueous acetic acid in an 80: 20(v/v) ratio caused rapid, co-eluting peaks with poor baseline separation and low symmetry.

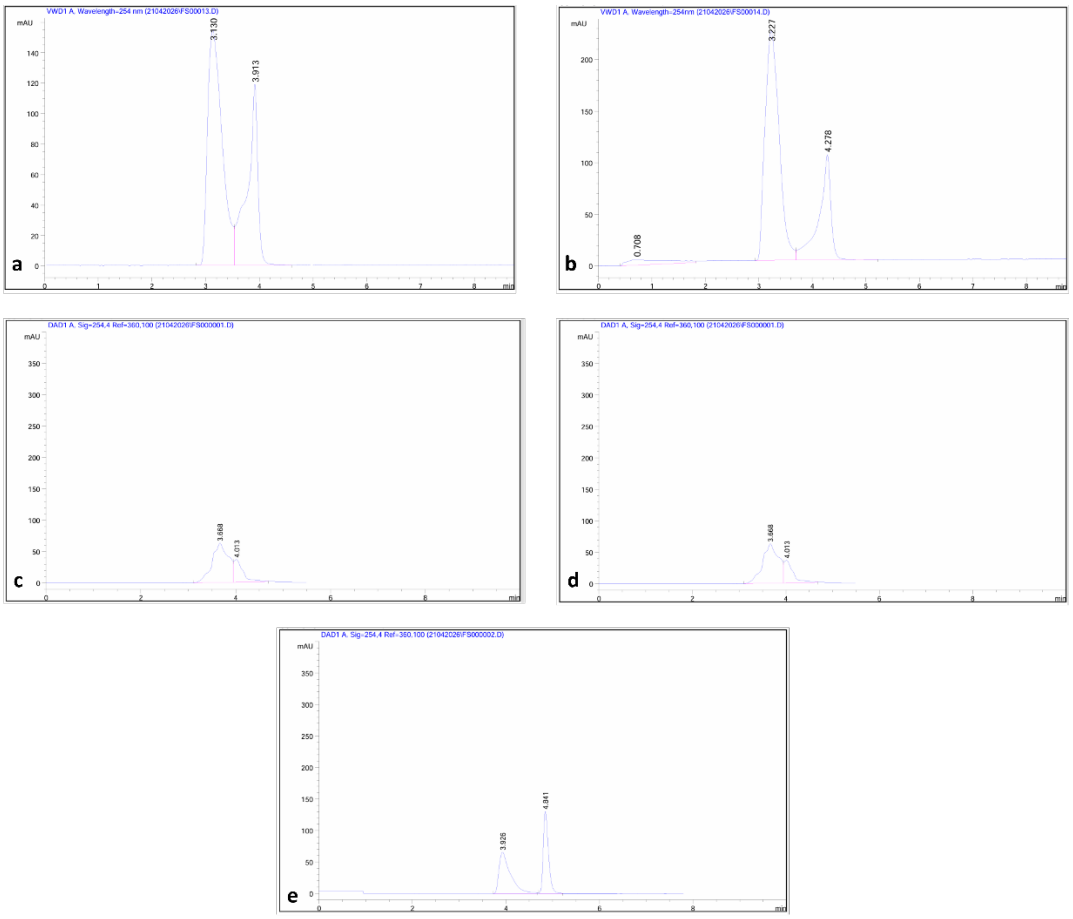


Figure 3. Chromatogram of a. Trial 1, b. Trial 2, c. Trail 3, d. Trial 4, and e. Trial 5

- Trial 02 & 03: Decreasing the organic phase fractions sequentially to 70:30 and 65:35 (v/v) enhanced peak retention parameters, but the column matrix yielded poor theoretical plate counts and unsatisfactory peak forms.
- Trial 04: Transitioning to a composition of 60:40 (v/v) improved structural resolution, but initial injection cycles suffered from poor system equilibration, causing suboptimal peak symmetry for the early-eluting analyte.
- Trial 05: Maintaining the mobile composition at 60:40 (v/v) with full column equilibration at 33°C delivered optimal chromatographic performance, generating symmetric peak profiles, stable retention markers at approximately 4.7 minutes for furosemide and 6.3 minutes for spironolactone, high theoretical plate counts, and baseline peak resolution.

Table 1. Method Development Trials for Furosemide and Spironolactone

Trial No.	Mobile Phase Composition (v/v)	Flow Rate (mL/min)	Injection Volume
Trial 01	Methanol : 0.1% Acetic Acid (80:20)	0.7	20 µL
Trial 02	Methanol : 0.1% Acetic Acid (70:30)	0.7	20 µL
Trial 03	Methanol : 0.1% Acetic Acid (65:35)	0.7	20 µL
Trial 04	Methanol : 0.1% Acetic Acid (60:40)	0.7	20 µL
Trial 05	Methanol : 0.1% Acetic Acid (60:40)	0.7	20 µL

3. Results

3.1. Method Validation

The optimized chromatographic methodology was systematically validated according to the unified specifications set forth by the International Council for Harmonisation (ICH Q2(R1)) regulatory guidelines [1, 11]. The parameters evaluated to prove method validity include linearity, range, sensitivity (Limit of Detection and Limit of Quantification), accuracy (percent recovery), precision (repeatability, intraday, and interday variation), specificity, ruggedness, and robustness [1, 4].

3.1.1. Linearity

The system response was tested dynamically over five discrete concentrations to show a mathematical relationship between analyte concentration and the integrated spectrophotometric detector peak area [2, 6]. Linear regression processing confirmed an excellent fit over the targeted working boundaries of 10-50 μg for furosemide and 25-125 μg/mL for spironolactone [1, 2].

Table 2. Linear Regression for Furosemide

Concentration (µg/mL)	Absolute Injection Area-01 (mAU·s)	Absolute Injection Area-02 (mAU·s)	Calculated Mean Peak Area (mAU·s)	Standard Deviation (σ)	Relative Standard Deviation (%RSD)
10	655.95	654.94	655.45	0.71	0.11
20	1087.09	1088.34	1087.72	0.88	0.08
30	1494.80	1497.57	1496.19	1.96	0.13
40	1936.40	1936.96	1936.68	0.40	0.02
50	2302.94	2300.81	2301.88	1.51	0.07

Table 3. Linear Regression for Spironolactone

Concentration (µg/mL)	Absolute Injection Area-01 (mAU·s)	Absolute Injection Area-02 (mAU·s)	Calculated Mean Peak Area (mAU·s)	Standard Deviation (σ)	Relative Standard Deviation (%RSD)
25	1854.89	1855.58	1855.24	0.49	0.03
50	2977.54	2978.78	2978.16	0.88	0.03
75	4153.68	4163.13	4158.41	6.68	0.16
100	5323.13	5321.82	5322.48	0.93	0.02
125	6565.55	6556.07	6560.81	6.70	0.10

For furosemide, the mean chromatographic peak area advanced predictably from 655.45 mAU·s at the lower boundary to 2301.88 mAU·s at the upper limits [2]. Spironolactone showed a proportional increase from 1855.24 mAU·s up to 6560.81 mAU·s [2]. Regression lines calculated using least-squares modeling yielded correlation coefficients (r^2) meeting strict acceptance boundaries ($r^2 > 0.999$), indicating precise detector sensitivity across the analytical range [2]. The calculated relative standard deviations (%RSD) across all concentration points remained low, ensuring reproducibility [2].

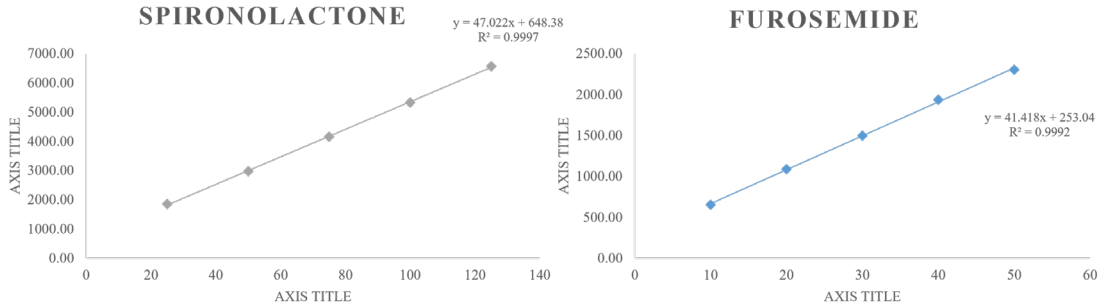


Figure 4. Standard calibration curves for spironolactone and furosemide.

3.1.2. Sensitivity (LOD and LOQ)

Method sensitivity limits were determined mathematically using the standard deviation of the response (σ) and the calculated calibration slope (S) obtained from the linear regression equations, satisfying international ICH validation expectations [2]. The average standard deviation across baseline injections was fixed at 1.09 [2]. Using the regulatory formulas:

$$\text{LOD} = \frac{3.3 \times \sigma}{S}$$

$$\text{LOQ} = \frac{10 \times \sigma}{S}$$

The calculated Limit of Detection (LOD) was found to be 0.0870 μg/mL, and the calculated Limit of Quantification (LOQ) was found to be 0.2636 μg/mL [2]. These low values confirm that the method is highly sensitive and capable of detecting and quantifying minor baseline changes in trace analyte concentrations during sample processing [2].

3.1.3. Recovery

Method accuracy was evaluated through recovery studies utilizing the standard addition technique across three concentration points: 80%, 100%, and 120% of the target formulation levels [2, 6]. Pre-analyzed tablet matrices were fortified with known quantities of pure active reference standards to measure percentage recovery and assess potential excipient interference [2, 6]. The average recovery values obtained spanned 99.63%-100.10% for furosemide and 99.08%-100.21% for spironolactone [2]. The standard deviations and tight relative standard deviations (%RSD < 0.6%) achieved across both analytes confirm excellent precision and accuracy, proving that the formulation excipients do not interfere with peak recovery [2].

Table 4. Recovery Study Results for Furosemide

Quality Tier Level (%)	Formulation Baseline Conc. (µg/mL)	Pure Active Standard Added (µg/mL)	Absolute Measured Response Area	Measured Analyte Level Found (µg/mL)	Individual Recovery Performance (%)	Mean Calculated Recovery (%)	Standard Deviation (σ)	Relative Standard Deviation (%RSD)
80%	10	8	999.70 997.72	8.03 7.98	100.40 99.80	100.10	0.42	0.42
100%	10	10	1080.33 1078.97	9.98 9.95	99.79 99.46	99.63	0.23	0.23
120%	10	12	1164.27 1163.12	12.01 11.98	100.05 99.82	99.93	0.16	0.16

Table 5. Recovery Study Results for Spironolactone

Quality Tier Level (%)	Formulation Baseline Conc. (µg/mL)	Pure Active Standard Added (µg/mL)	Absolute Measured Response Area	Measured Analyte Level Found (µg/mL)	Individual Recovery Performance (%)	Mean Calculated Recovery (%)	Standard Deviation (σ)	Relative Standard Deviation (%RSD)
80%	25	20	2765.25 2767.12	20.02 20.06	100.11 100.31	100.21	0.14	0.14
100%	25	25	2989.54 2987.36	24.79 24.75	99.17 98.98	99.08	0.13	0.13
120%	25	30	3222.38 3233.60	29.74 29.98	99.15 99.94	99.55	0.56	0.57

3.1.4. System Precision

Method precision was evaluated across three distinct categories: system repeatability, same-day intraday variation, and day-to-day interday variation [2, 6].

Intraday Precision: Intraday precision was assessed by performing duplicate injections of three separate working standard mixtures (20,30, and 40 µg/mL for furosemide; 50, 75 and 100 µg/mL for spironolactone) at scheduled intervals within a single operational day [2, 6]. The observed retention times remained highly stable under the optimized conditions [2]. The calculated relative standard deviations (%RSD) for furosemide were 0.45%, 0.41%, and 0.05% across its respective concentration tiers [2]. For spironolactone, the observed intraday precision values were 0.06%, 0.02%, and 0.16% [2]. The individual quantified recovery indices remained near 100%, indicating excellent instrument repeatability [2]. The tables below present the summarized intraday precision.

Table 6. Intraday Precision Results for Furosemide

Concentration Tier (µg/mL)	Absolute Injection Area Metrics	Calculated Mean Response Area	Measured Component Found (µg/mL)	Measured Formulation Found (%)	Standard Deviation (σ)	Relative Standard Deviation (%RSD)
20	1087.28 1080.40	1083.84	20.06	100.32	4.86	0.45
30	1498.29 1489.68	1493.99	29.97	99.89	6.09	0.41
40	1936.90 1938.39	1937.65	40.68	101.71	1.05	0.05

Table 7. Intraday Precision Results for Spironolactone

Concentration Tier (µg/mL)	Absolute Injection Area Metrics	Calculated Mean Response Area	Measured Component Found (µg/mL)	Measured Formulation Found (%)	Standard Deviation (σ)	Relative Standard Deviation (%RSD)
50	2986.77 2989.22	2988.00	49.76	99.52	1.73	0.06
75	4167.90 4168.97	4168.44	74.86	99.82	0.76	0.02
100	5319.55 5331.46	5325.51	99.47	99.47	8.42	0.16

Interday Reproducibility: Interday reproducibility was evaluated by performing replicate injections of the same concentration series across separate operational days [2, 6]. The observed compound retention windows remained highly constant, confirming the long-term reliability of the chromatographic method [2]. The calculated day-to-day relative standard deviations (%RSD) for furosemide were 0.12%, 0.22%, and 0.15% [2]. For spironolactone, the interday %RSD indicators were 0.23%, 0.08%, and 0.11% [2]. These low variance parameters indicate that the system is stable and robust for routine daily batch analysis [2]. The tables below show the results for interday reproducibility across the period.

Table 8. Results of Interday Precision for Furosemide

Concentration (µg/mL)	Absolute Injection Area	Calculated Mean Response Area	Amount Found (µg/mL)	Measured Formulation Found (%)	Standard Deviation (σ)	Relative Standard Deviation(%RSD)
20	1084.12 1085.95	1085.04	20.09	100.46	1.29	0.12
30	1495.21 1490.56	1492.88	29.94	99.81	3.28	0.22
40	1926.42 1930.46	1928.44	40.46	101.15	2.86	0.15

Table 9. Results of Interday Precision for Spironolactone

Concentration (µg/mL)	Absolute Injection Area	Mean Response Area	Amount Found(µg/mL)	Measured Formulation Found (%)	Standard Deviation (σ)	Relative Standard Deviation(%RSD)
50	2981.12 2990.90	2986.01	49.72	99.43	6.92	0.23
75	4165.46 4170.45	4167.96	74.85	99.81	3.53	0.08
100	5317.56 5325.56	5321.56	99.39	99.39	5.65	0.11

3.1.5 Specificity and Selectivity

Method specificity was evaluated by chromatographing standard active components in the presence of common tablet formulation excipients [2, 6]. Examination of the resulting profiles showed clean, symmetrical baseline separation for both compounds, with no interfering peaks detected within the designated analyte retention windows [2]. The calculated active label claims fell well within target regulatory parameters, confirming that common industrial tablet filler matrices do not interfere with quantitative analysis [2].

Table 10. Specificity and Formulation Excipient Selectivity

Compound	Nominal Target Conc. (µg/mL)	Individual Measured Absorbance Area	Calculated Mean Response Area	Measured Level Recovered (µg/mL)	Nominal Claim (%)	Standard Deviation (σ)	Relative Standard Deviation (%RSD)
Furosemide (FUR)	20	1090.09 1087.34	1088.72	20.21 20.15	101.07 100.74	1.945	0.179
Spironolactone (SPI)	50	2979.54 2980.18	2979.86	49.58 49.59	99.16 99.19	0.453	0.015

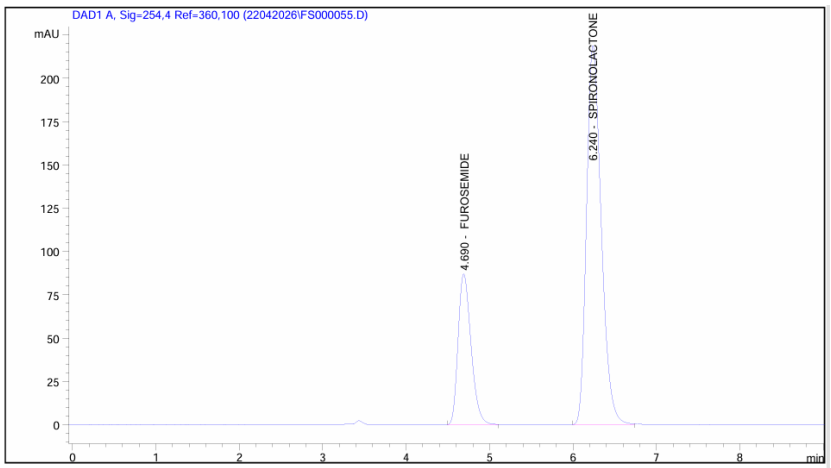


Figure 5. Chromatogram of specificity at 20+ 50 µg/mL

3.1.6 System Repeatability

System repeatability was verified through consecutive injections of a standard reference mixture (20 µg/mL furosemide and 50 µg/mL spironolactone) under identical chromatographic configurations [2, 6]. The recorded peak properties, symmetry indices, and theoretical plate counts showed exceptional system precision, with low variance indicating suitable equipment performance for routine quality testing [2].

Table 11. Results of Method Repeatability

Component	Nominal Concentration (µg/mL)	Measured Injection Responses	Calculated Mean Peak Area	Calculated Level Found (µg/mL)	Calculated Recovery Yield (%)	Standard Deviation (σ)	Relative Standard Deviation (%RSD)
Furosemide (FUR)	20	940.44 939.86	940.15	16.59	82.97	0.41	0.04
Spironolactone (SPI)	50	2697.36 2696.01	2696.69	43.56	87.13	0.95	0.04

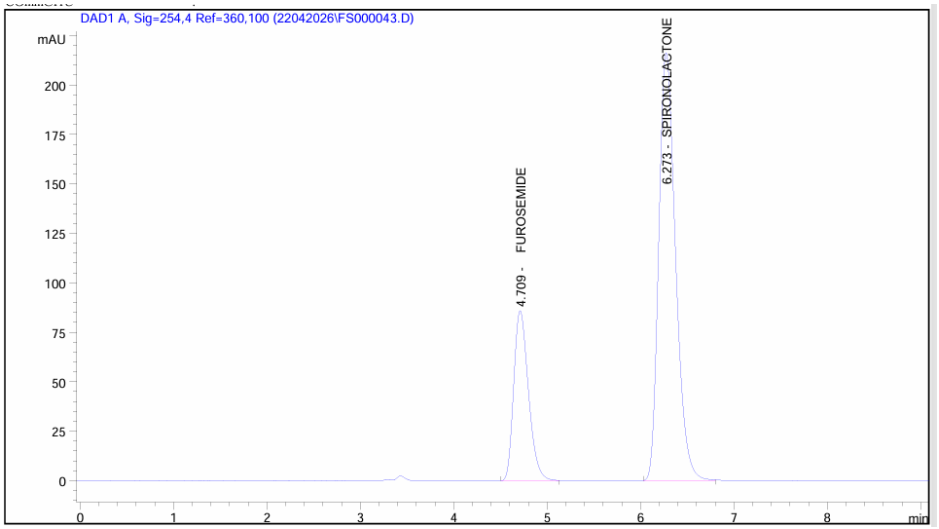


Figure 6. Chromatogram of Repeatability (Injection 01)

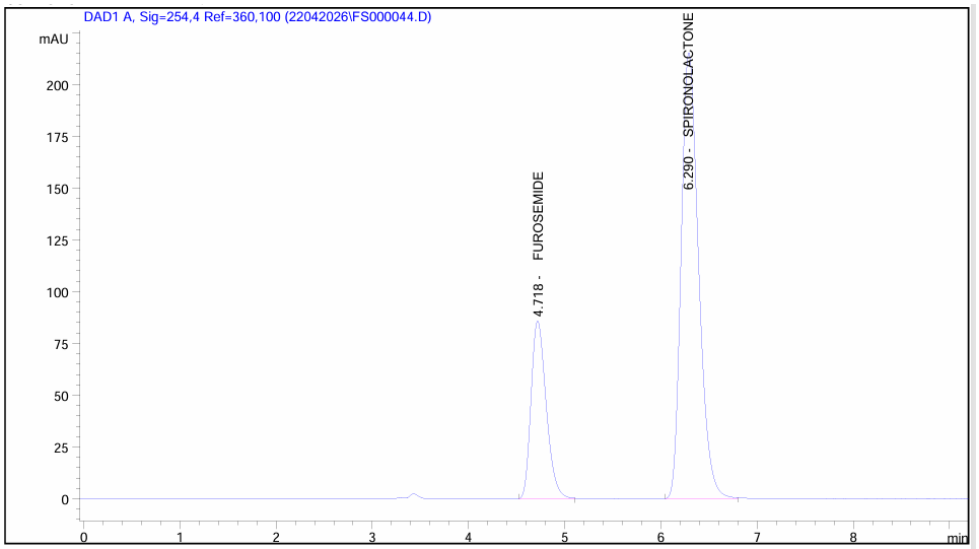


Figure 7. Chromatogram of Repeatability (Injection 02)

3.1.7 Analytical Ruggedness

Method ruggedness was evaluated by performing identical analyses under varying experimental conditions, including multiple analysts working with different reagent batches [2, 6]. The results indicated that minor variations in operator handling did not significantly affect the integrated peak areas or retention windows [2]. The mean recovery performance met standard industrial acceptance guidelines, and low %RSD metrics verified that the methodology is rugged and reliable for routine deployment across different quality control laboratories [2].

Table 12. Inter-Analyst Ruggedness

Target Analyte Active	Apparent Matrix Conc. (µg/mL)	Absolute Area Responses Recorded	Calculated Mean Response Area	Measured Amount Found (µg/mL)	Quantified Label Recovery (%)	Standard Deviation (σ)	Relative Standard Deviation (%RSD)
Furosemide (FUR)	30	1493.97 1491.98	1492.98	29.97 29.92	99.89 99.73	1.407	0.094
Spironolactone (SPI)	75	4187.46 4142.80	4165.13	75.27 74.32	100.36 99.09	31.579	0.758

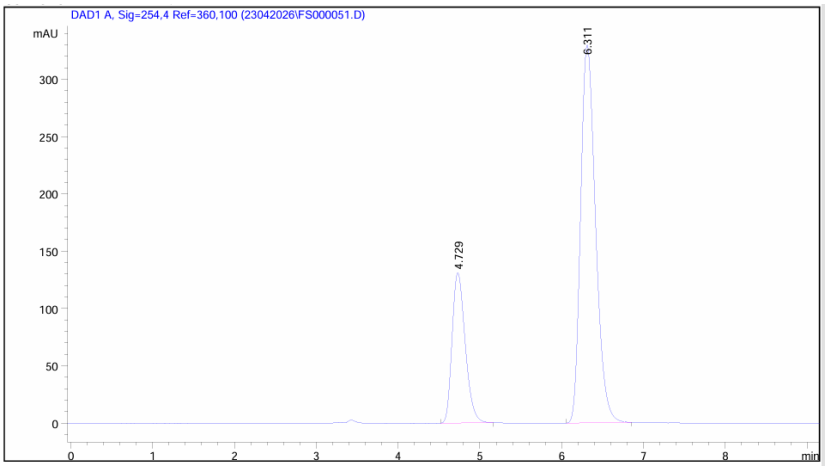


Figure 8. Chromatogram of Ruggedness

3.1.5. Analytical Robustness

Method robustness was evaluated by introducing small, deliberate changes to key chromatographic parameters, specifically the mobile phase organic ratio (±1%) and the spectrophotometric optical wavelength (±1 nm) [2, 6]. Under all modified conditions, system performance remained stable, with minimal variations observed in peak resolution, symmetry, or theoretical plate count [2]. The low calculated %RSD values show that the method can accommodate minor operational fluctuations without compromising quantification accuracy [2].

Table 13. Analytical Robustness Results

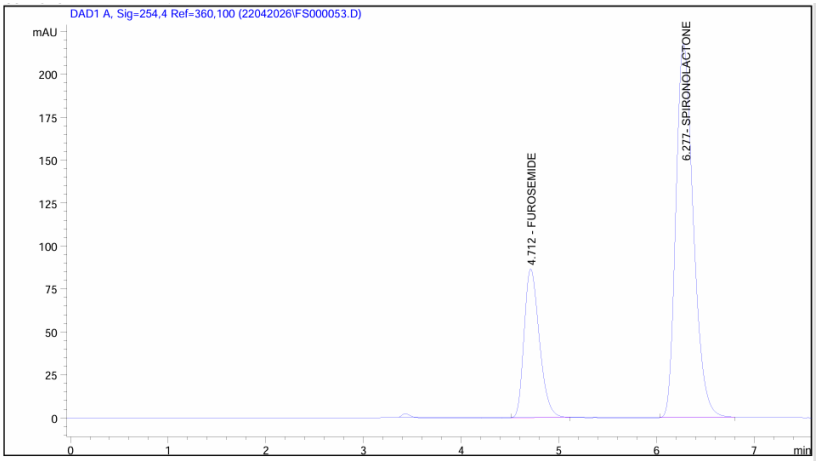
Experimental Robustness Variable	Compound Evaluated	Altered Parameter	Concentration Level (µg/mL)	Calculated Mean Area	Target Standard Deviation (σ)	Calculated Relative Variance (%RSD)
Mobile Phase Composition Ratio Modification	Furosemide (FUR)	Methanol : Buffer (61:39 v/v)	30	1459.40 1465.16	0.16 0.11	0.01 0.01
	Spironolactone (SPI)	Methanol : Buffer (59:41 v/v)	75	4184.50 4179.44	1.40 1.58	0.03 0.04
Detection Optical Wavelength Adjustment	Furosemide (FUR)	Methanol : Buffer (61:39 v/v)	30	1510.70 1424.46	1.65 2.03	0.11 0.14
	Spironolactone (SPI)	Methanol : Buffer (59:41 v/v)	75	4348.40 4007.71	2.44 3.56	0.06 0.09

3.2 Quantitative Batch Assay of Commercial Formulations

The validated isocratic method was applied to quantify spironolactone and furosemide in commercial multi-dose solid oral tablets (Furowise Plus) [2, 6]. Sample processing was completed according to the established sample extraction protocol [6]. The resulting chromatograms exhibited sharp, symmetrical parent peaks with reproducible retention times [2]. Quantitative evaluation yielded mean active formulation levels of 100.71% for furosemide and 99.21% for spironolactone relative to their label claims [2]. The low relative standard deviations and clean peak separation show that the assay is highly accurate and suitable for routine manufacturing quality control and batch release testing [2].

Table 14. Assay of Commercial Dosage Formulations

Targeted Component Profile	Labelled Conc. (µg/mL)	Replicate Injection Responses	Calculated Mean Peak Area	Measured Concentration (µg/mL)	Final Calculated Label Assay (%)	Standard Deviation (σ)	Relative Standard Deviation (%RSD)
Furosemide (FUR)	20	1086.01 1088.05	1087.03	20.12 20.17	100.58 100.83	1.442	0.133
Spironolactone (SPI)	50	2980.48 2981.08	2980.78	49.60 49.61	99.20 99.23	0.424	0.014



Figures 9. Assay of Commercial Batch Formulation

3.2. Forced Degradation and Stability-Indicating Evaluations

To confirm the stability-indicating capability of the developed method, mixed standard solutions were subjected to forced degradation studies under various stress conditions, including acidic, alkaline, neutral hydrolytic, oxidative, photolytic, and thermal environments [12, 13]. Chemical baseline checks were completed to evaluate individual compound stability and verify the baseline resolution of stress-induced degradants from the parent drug peaks [14].

3.2.1. Acid-Induced Hydrolytic Stress (0.1 N HCl, 2 Hours)

Subjecting the sample solution to 0.1 N hydrochloric acid for 2 hours at room temperature resulted in significant degradation of both analytes [2, 6]. Furosemide showed higher susceptibility to acid-catalyzed hydrolysis, with 15.23% degradation observed, leaving 84.77% of the active compound intact [2]. Spironolactone exhibited similar reactivity under acidic conditions, undergoing 13.00% degradation, with 87.00% of the parent peak area remaining [15]. The chromatograms displayed well-resolved, distinct peaks for both surviving drug molecules alongside minor, low-level hydrolytic degradation products, with no peak co-elution observed [16].

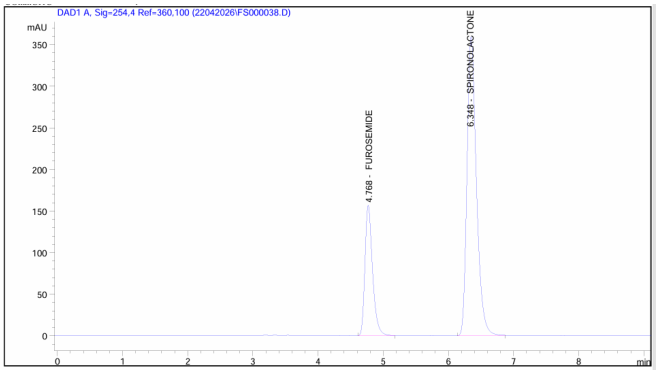


Figure 10. Chromatogram of forced degradation after 2 hour in 0.1 N HCl (Acidic condition)

3.2.2. Base-Induced Hydrolytic Stress (0.1 N NaOH, 2 Hours)

Under basic hydrolytic conditions (0.1 N NaOH exposure for 2 hours at room temperature), the two analytes displayed highly distinct stability profiles [2, 6]. Furosemide was highly susceptible to base-catalyzed cleavage, exhibiting 11.44% degradation, with 88.56% of the parent compound remaining [2]. In contrast, spironolactone proved highly stable in basic media, undergoing only 3.09% structural breakdown, with 96.91% of its initial peak area preserved [2]. The appearance of secondary degradation bands in the baseline area confirmed base-induced breakdown, particularly for furosemide, while demonstrating the method's capacity to completely resolve the surviving parent molecules from adjacent degradant peaks [2].

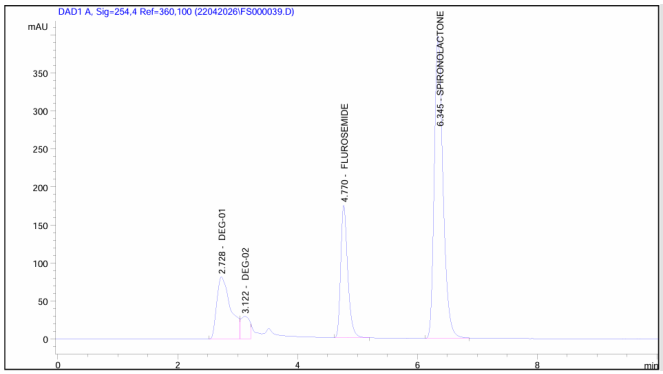


Figure 11. Chromatogram of forced degradation after 2 hour in 0.1 N NaOH (Alkaline condition)

3.2.3. Neutral Aqueous Hydrolytic Stress (Purified Water, 2 Hours)

Under neutral aqueous conditions, both compounds exhibited excellent chemical stability with minimal degradation observed over the 2-hour monitoring window [2, 6]. Furosemide maintained 97.72% of its initial peak area, representing a minor hydrolytic degradation level of 2.28% [2]. Spironolactone proved almost completely stable in neutral aqueous media, retaining 99.98% of its parent peak response with no measurable degradation [2]. These findings confirm that both drugs are chemically stable in neutral aqueous environments [2].

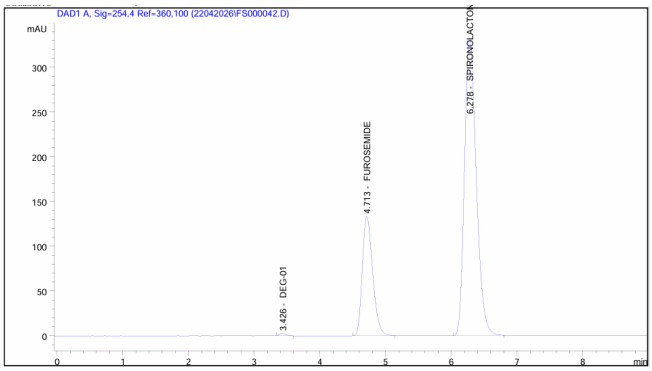


Figure 12. Chromatogram of forced degradation after 2 hours under neutral condition

3.2.4. Oxidative Stress (3% H_2O_2, 2 Hours)

Subjecting the sample to oxidative stress via exposure to 3% hydrogen peroxide for 2 hours at room temperature induced measurable breakdown in both therapeutic molecules [2, 6]. Furosemide showed 8.11% oxidative breakdown, leaving 91.89% of the active compound intact [2]. Spironolactone underwent 5.36% degradation, with 94.64% of its initial parent peak area preserved [2]. The resulting chromatograms displayed multiple small degradation product peaks cleanly resolved from the parent drug bands, confirming that the method can effectively separate oxidative degradation products [2].

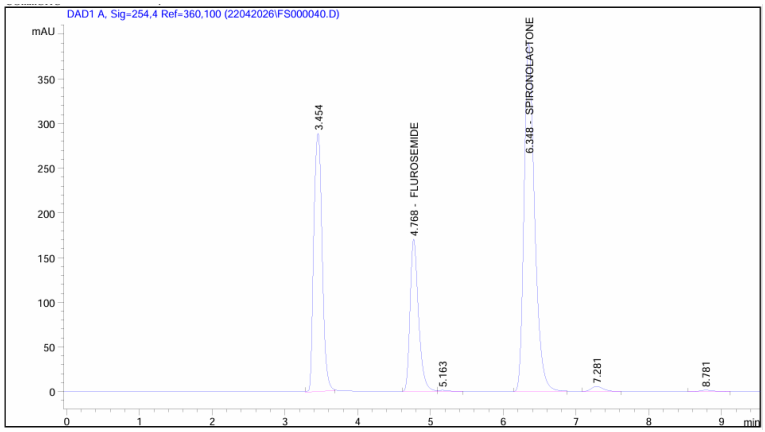


Figure 13. Chromatogram of forced degradation after 2 hour in 3% H_2O_2 (Oxidative condition)

3.2.5. Stress Degradation Kinetics

The tables below present the compiled remaining percentage values and actual degradation kinetics observed across the different stress environments.

Table 15. Forced Degradation Studies for Furosemide

Targeted Stress Condition Variable	Baseline Standard Peak Area	Residual Peak Area after Stress	Residual Parent Active Drug (%)	Quantified Degradation Performance (%)
Acid Hydrolytic Exposure (0.1 N HCl)	1494.80	1267.09	84.77	15.23
Alkaline Hydrolytic Exposure (0.1 N NaOH)	1494.80	1323.85	88.56	11.44
Oxidative Environmental Stress (3% H_2O_2)	1494.80	1373.64	91.89	8.11
Neutral Aqueous Media Dissolution	1494.80	1460.74	97.72	2.28

Table 16. Forced Degradation Studies for Spironolactone

Targeted Stress Condition Variable	Baseline Standard Peak Area	Residual Peak Area after Stress	Residual Parent Active Drug (%)	Quantified Degradation Performance (%)
Acid Hydrolytic Exposure (0.1 N HCl)	4153.68	3613.50	87.00	13.00
Alkaline Hydrolytic Exposure (0.1 N NaOH)	4153.68	4025.19	96.91	3.09
Oxidative Environmental Stress (3% H_2O_2)	4153.68	3931.24	94.64	5.36
Neutral Aqueous Media Dissolution	4153.68	4152.73	99.98	0.02

3.2.6. Photolytic Light Exposure (UV Radiation Stress)

Photolytic stability was evaluated by exposing standard mixtures to high-intensity ultraviolet radiation under controlled laboratory conditions [2, 6]. Both analytes showed strong resistance to photolytic degradation [2]. Furosemide maintained 99.33% of its original parent peak area after a 34-hour exposure window, corresponding to a minor degradation level of 0.67% [2]. Spironolactone underwent 1.49% degradation over a 24-hour exposure period, with 98.51% of its initial active peak response preserved [2]. The absence of significant degradation product peaks confirms that both compounds are highly stable under photolytic stress [2].

Table 17. Photolytic Degradation Studies

Drug Active Profile	Targeted Stress Degradation Environment	Initial Standard Peak Area	Post-Stress Measured Area	Residual Parent Active (%)	Actual Quantified Degradation (%)
Furosemide (FUR)	UV Radiation Stress Exposure (34 Hours)	1494.80	1484.80	99.33	0.67
Spironolactone (SPI)	UV Radiation Stress Exposure (24 Hours)	4153.68	4091.68	98.51	1.49

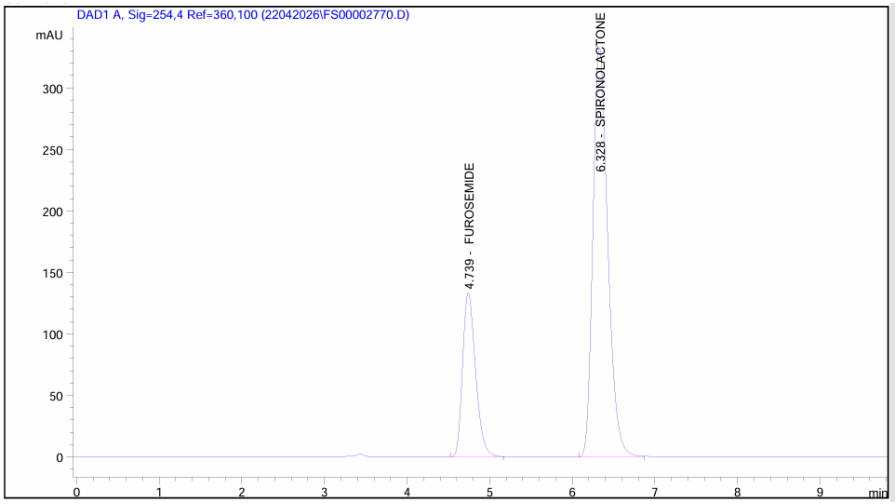


Figure 14. Chromatogram of photolytic degradation

3.2.7. High-Temperature Thermal Stress (60°C Dry Oven)

Thermal stability profiling was completed by exposing dry drug matrices to a constant temperature of 60∘C within a hot-air oven [2, 6]. Both diuretics showed strong thermal stability under these conditions [2]. Furosemide maintained 99.44% of its initial baseline response, undergoing a minor thermal breakdown of 0.56% [2]. Spironolactone exhibited similar stability with 99.69% of its initial active area preserved, representing a negligible degradation level of 0.31% [2]. The chromatographic parameters, including symmetry factors and theoretical plate counts, remained stable, confirming that both active compounds are highly resistant to dry thermal stress [17].

Table 18. Dry Heat Thermal Degradation

Drug	Targeted Stress Degradation Environment	Initial Standard Peak Area	Post-Stress Measured Area	Residual Parent Active (%)	Actual Quantified Degradation (%)
Furosemide (FUR)	High-Temperature Oven Stress (60°C)	1494.80	1486.45	99.44	0.56
Spironolactone (SPI)	High-Temperature Oven Stress (60°C)	4153.68	4140.78	99.69	0.31

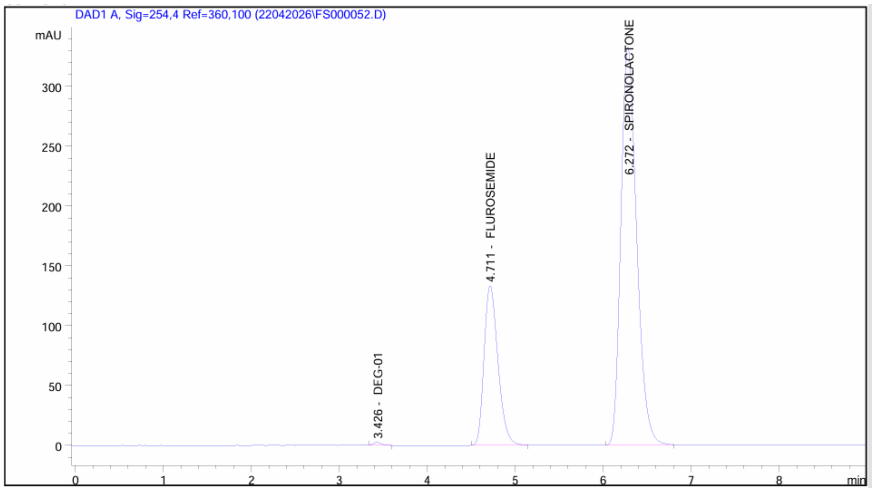


Figure 15. Chromatogram of Thermal degradation

The forced degradation studies show that furosemide is more susceptible to degradation under acidic and basic hydrolytic conditions, whereas spironolactone exhibits significantly greater chemical stability, particularly in basic and neutral media [2]. The overall degradation for this combined formulation can be summarized as:

Acidic Hydrolysis > Alkaline Hydrolysis > Oxidative Breakdown > Neutral Solvolysis > Photolysis ≈ Thermal Stress

The optimized chromatographic system successfully resolved all stress-induced degradation products from the parent drug peaks, confirming the stability-indicating capability of the developed method [2].

4. Conclusion

The developed isocratic reversed-phase high-performance liquid chromatographic method provides an efficient, dependable, and highly sensitive platform for the concurrent quantification and stability tracking of spironolactone and furosemide. By utilizing an optimized mobile phase of methanol and 0.1% aqueous acetic acid (60:40 v/v) over a premium C18 stationary column, baseline resolution (R_s = 6.60) is systematically maintained with short runtimes. Furosemide consistently elutes at approximately 4.7 minutes, followed closely by spironolactone at 6.3 minutes, enabling high-throughput sample batches without sacrificing thermodynamic resolution. Validation assays executed in strict accordance with the International Council for Harmonisation (ICH Q2(R1)) regulatory directives confirm that the framework is linear ($r^2 > 0.999$), precise (%RSD < 2.0% across repeatability, intraday, and inter-day cycles), and highly accurate, yielding mean standard addition recovery values between 99.08%-100.21%. The low analytical detection limits (LOD=0.0870 µg/mL) allow for the reliable determination of minor, trace-level impurities or process degradants. This research provides a validated, robust, and stability-indicating RP-HPLC-UV assay tailored for the concurrent quantitative analysis of spironolactone and furosemide in bulk matrices and multi-dose commercial pharmaceutical formulations. The experimental forced degradation confirm that the method is stability-indicating, demonstrating absolute baseline separation of all stress-induced degradation products derived from aggressive acid, base, peroxide, light, and dry-heat exposures away from the main therapeutic drug peaks.

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