RESEARCH ARTICLE

Comprehensive RP-HPLC Analysis of Sulopenem Etzadroxil and Probenecid: Development, Validation and Stability Studies

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ABSTRACT
A highly precise and validated RP-HPLC method was established for the simultaneous estimation of Sulopenem Etzadroxil and Probenecid in both pure form and pharmaceutical formulations, including stability studies.The RP-HPLC analysis was performed using a Hyperclone 5µ BDS C18 column with an isocratic mobile phase of Acetonitrile and Ammonium Formate Buffer (40:60, pH 3.0). Detection was carried out at 272 nm with a 1.0 mL/min flow rate, 10 µL injection volume, and a 5-minute runtime. The method, validated as per ICH guidelines, exhibited excellent linearity (25–150 µg/mL) with high correlation coefficients. Accuracy studies demonstrated recoveries of 100.7% for Sulopenem Etzadroxil and 99.9% for Probenecid. The robustness evaluation indicated minimal variation in retention time and peak area under slight changes in chromatographic conditions. Sulopenem Etzadroxil exhibited maximum degradation under thermal, hydrolytic, and peroxide conditions, while Probenecid showed notable degradation under peroxide, acid, and photolytic stress. Minimal degradation was observed under alkali and reduction conditions for both drugs.
Keywords: RP-HPLC, Sulopenem Etzadroxil, Probenecid, Method Validation, Stability Studies, ICH Guidelines.
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INTRODUCTION

Sulopenem Etzadroxil (Fig.1) is an orally bioavailable prodrug of sulopenem, a broad-spectrum β-lactam antibiotic effective against multidrug-resistant bacteria, including ESBL-producing strains. It hydrolyzes to sulopenem, inhibiting PBPs and bacterial cell wall synthesis, leading to bacterial lysis.[1,2]. Probenecid, a uricosuric agent, manages hyperuricemia by inhibiting renal uric acid reabsorption and enhances β-lactam antibiotics by inhibiting OAT1 and OAT3, prolonging their half-life. Though not antibacterial, probenecid improves antibiotic efficacy in severe infections and plays a key role in antimicrobial therapy [3-5]. Several analytical methods have been reported for Probenecid estimation like LC-MS/MS [6,7], and UV-Visible spectroscopic method [8,9] RP-HPLC [10].

MATERIALS AND METHODS

Equipments

The instruments used in this study included a Waters Alliance HPLC system (e2695) with Empower 2.0 software, an Eutech pH meter, a Sartorius weighing balance, and a UV-1700 UV/VIS spectrophotometer. Additionally, Borosil pipettes, beakers, and burettes were utilized, along with a Unichrome UCA 701 ultra sonicator and an isocratic model pump to support sample preparation and analysis.

Chemicals

The chemicals and reagents used in the study included Acetonitrile (HPLC grade, Rankem), Formic Acid (AR grade, Analytical Reagents), HPLC grade Water (Milli-Q or equivalent), and Ortho Phosphoric Acid (AR grade, Analytical Reagent). These high-purity reagents ensured the reliability and accuracy of the analytical method

Standard and sample Preparation

For standard solution preparation, weigh 10 mg each of Sulopenem Etzadroxil and Probenecid, dissolve in a diluent, and dilute to 10 mL (stock solution). Pipette 1 mL of this stock into a 10 mL flask and dilute to obtain 100 ppm of each drug. For the sample solution, weigh 24.24 mg of the sample, dissolve in a diluent, sonicate for 30 min, and dilute to 10 mL. Pipette 1 mL of this stock into a 10 mL flask, dilute, and filter through a 0.45-micron filter to obtain 100 ppm of each drug.

Method Development

Initial trials with Luna Phenyl Hexyl (250x4.6mm, 5µ) using ACN and 0.1% HCOOH as the mobile phase at different ratios (80:20 and 70:30) resulted in unacceptable retention times and broad peaks. Further modifications, including the use of ammonium formate buffer (pH 3.0) with orthophosphoric acid (OPA), improved peak resolution; however, tailing and unknown peaks were observed in trials conducted with different mobile phase ratios (50:50 and 45:55). For precise separation and

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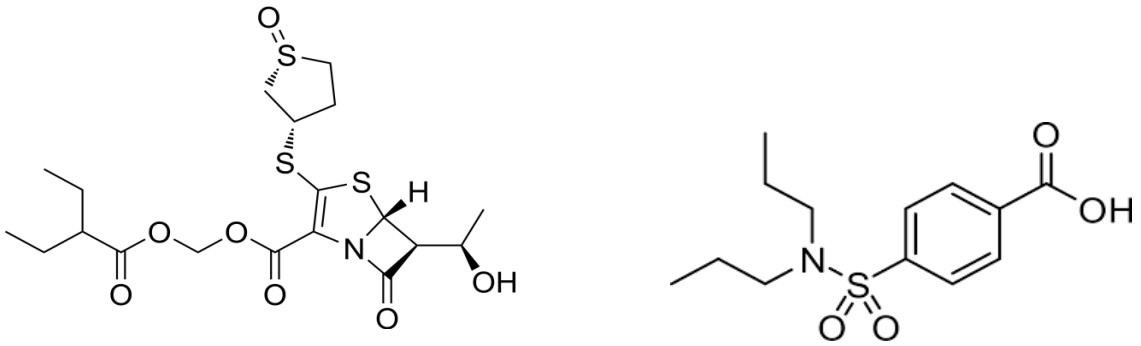

Figure1: Chemical structure of Sulopenem Etzadroxil and Probenecid

enhanced system suitability, the method employed a Hyperclone 5µ BDS C18 130°A (250 × 4.6 mm, 5 µm) column, utilizing an ACN and AFB (40:60, pH 3.0) mobile phase. The optimized parameters included detection at 272 nm, a 1.0 mL/min flow rate, a 10 µL injection volume, and a streamlined 5-minute runtime. The system suitability results showed well-defined peaks with retention times of 2.403 min for Sulopenem Etzadroxil and 3.336 min for Probenecid, with acceptable plate counts, tailing factors, and resolution (6.21). The %RSD values (0.22% and 0.62%) confirmed the method's precision and reliability.

Method validation

The method validation tests such as selectivity, sensitivity, range, specificity, linearity, accurateness were studied for the evaluation of the method validity. Additionally, the approach was tested for Sulopenem Etzadroxil and Probenecid estimation in bulk and pharmaceutical formulations.[11-14].

Specificity

To demonstrate specificity, representative chromatograms need to be utilized, and extracted components must be labeled consistently. An appropriate level of investigation must be applied to critical separations in chromatography. The components' resolution that elute nearest to each other could be employed to assess the specificity of a significant separation. Analytical procedures with higher levels of specificity may compensate for less specificity of individual procedures

Linearity, Precision

Linearity is recommended to evaluate the method such that the obtained results produce a proportional response

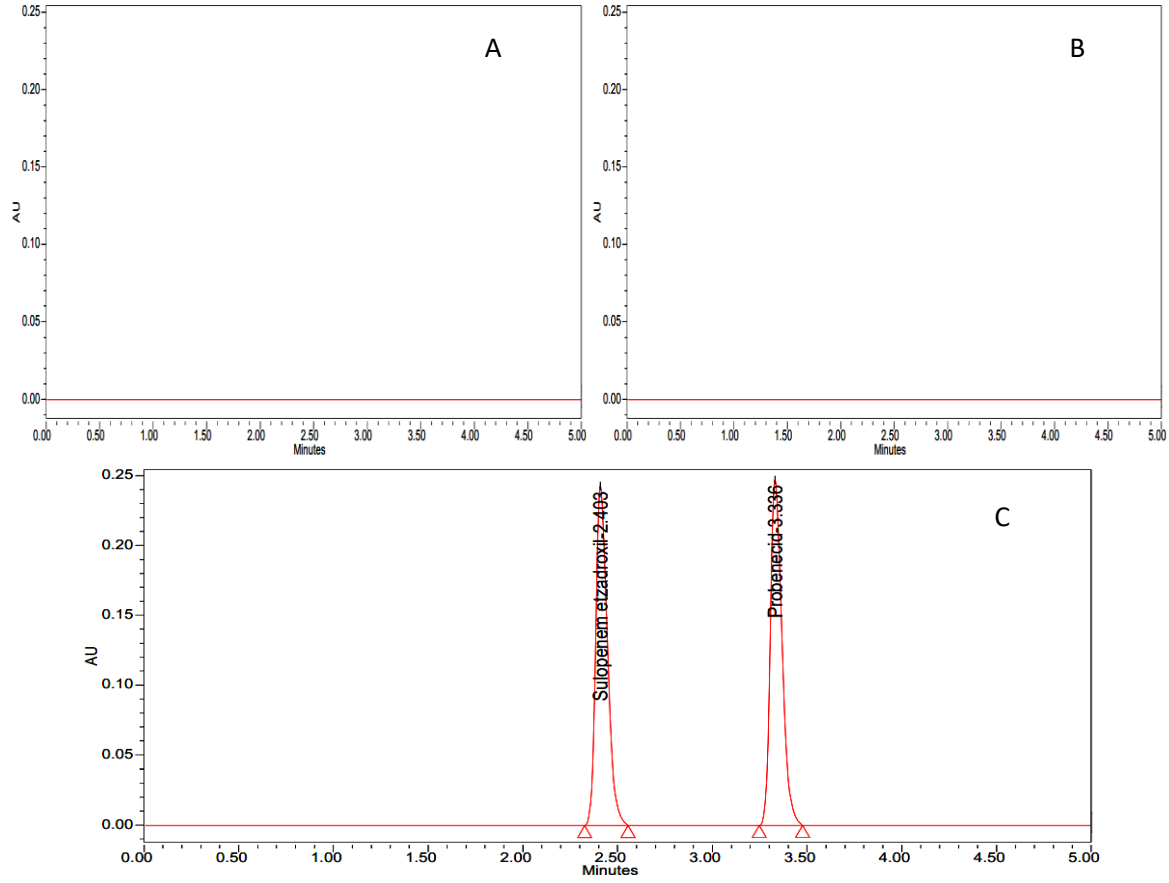

Figure 2: Representative chromatogram of A: Blank B: Placebo C: Standard Chromatogram

Table 1: Results of linearity for Sulopenem Etzadroxil & Probenecid

S.No	Sulopenem Etzadroxil		Probenecid	
	Conc.(µg/ml)	Peak area	Conc.(µg/ml)	Peak area
1	25	746411	25	756537
2	50	1492822	50	1513074
3	75	2239232	75	2269611
4	100	2985643	100	3026148
5	125	3732054	125	3782685
6	150	4378465	150	4439222
Regression equation	y = 29427.86x +17857.21		y =29832.9x + 17857.14	
Slope	29427.86		29832.9	
Intercept	17857.21		17857.14	
R^2	0.99982		0.99983	

Table 2: Method Precision for Sulopenem Etzadroxil & Probenecid

S. No.	Area for Sulopenem Etzadroxil	Area for Probenecid
1	2965841	3026148
2	2934150	3058942
3	2957484	3014846
4	2956312	3024671
5	2975417	3042146
6	2944310	3052148
Average	2955586	3036484
Standard Deviation	14759.494	17299.502
%RSD	0.50	0.57

over the desired range of analyte concentrations. Linearity was determined by regression analysis, which involves recording average areas of triplicate injections of 25-150 µg/mL for Sulopenem Etzadroxil and 25-150. µg/mL for Probenecid. Plot a linearity graph with concentration against peak area and for Sulopenem Etzadroxil and Probenecid correlation coefficient values were acceptable fits for the data of regression line. All the drugs exhibit proportional response with a good coefficient of correlation of 0.999.

Repeatability is the ability of the procedure to generate the results over a short time interval under identical conditions. Intermediate precision is the precision attained over an extended time in a single laboratory but with different analysts, different columns, different reagents etc.

Accuracy

Samples were arranged in triplicates by sample solution at known concentrations of Sulopenem Etzadroxil and Probenecid at 50%, 100%, and 150% spike levels. The peak area values observed in each analysis were compared with the corresponding standard and % recovery of Sulopenem Etzadroxil and Probenecid was calculated. The % recovery in the limit of 98-102 % was considered as acceptable as per the guidelines.

Robustness

It is evaluated to know the change in system suitability parameters in response to variations in flow rate, mobile phase, and temperature. % RSD was calculated for evaluation of the parameters and found to be less than 2% which satisfies the acceptance criteria.

Assay

To determine the concentration of the drug, inject 10 µL of both the standard and sample solutions into the RP-HPLC system and measure the peak areas for Probenecid and Sulopenem Etzadroxil. The sample concentration is determined by comparing it to the standard peak area and employing an appropriate calculation formula.

The formula for Assay

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Degradation Studies

Degradation studies were conducted under various stress conditions for Sulopenem Etzadroxil and Probenecid. Acid and alkali degradation involved treatment with 1N HCl and 1N NaOH, respectively, followed by neutralization and dilution. Oxidative and reductive degradation were performed using 10% H_2O_2 and 10% sodium bisulfite, while thermal and photolytic degradation involved exposure to 105°C for 6 hours and photo-stability conditions for 6 hours, respectively. Hydrolysis degradation was carried out using HPLC-grade water, and all degraded samples were diluted, filtered, and analyzed using the optimized HPLC method.

RESULTS AND DISCUSSION

Optimization of Method

A validated RP-HPLC approach was designed for the concurrent estimation of Sulopenem Etzadroxil and Probenecid, employing a Waters HPLC system with an autosampler and PDA detector. The method employed a Hyperclone 5µ BDS C18 130°A (250x4.6mm, 5µm) column, ensuring effective separation of both analytes. The mobile phase, consisting of ACN and AFB (pH 3.0) in a 40:60 ratio, was delivered at a flow rate of 1 mL/min, providing stable peak resolution. A 10 µL injection volume was used for analysis, with detection carried out at 272 nm for optimal sensitivity. The run time was set at 5 minutes, allowing for rapid and efficient analysis. The method was performed under ambient temperature conditions (25°C), ensuring practicality for routine laboratory use. The developed isocratic mode of

Table 3: Accuracy results of Sulopenem Etzadroxil and Probenecid

%Concentration(at specification Level)	Sulopenem Etzadroxil		Probenecid	
	% Recovery	Mean Recovery	% Recovery	Mean Recovery
50%	100.8	100.7	99.6	99.9
	99.8		99.7	
	101.4		100.5	
100%	100.8	100.9	99.6	100.2
	101.7		100.7	
	100.1		100.1	
150%	100.9	100.9	99.6	99.8
	100.2		100.3	
	101.6		99.5	

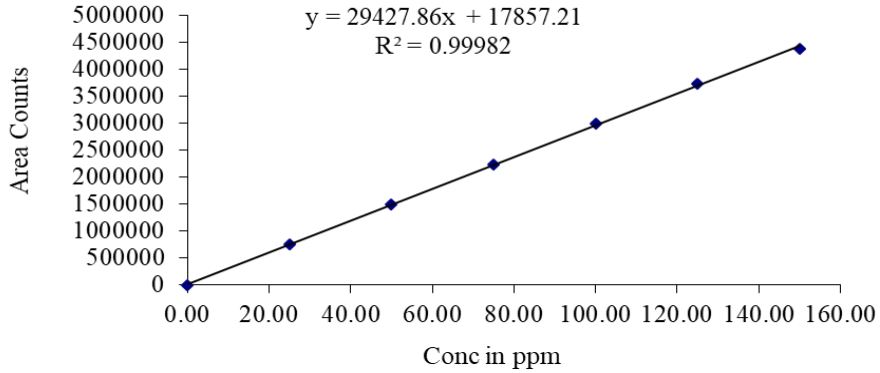

Figure 5: Regression curve for Sulopenem Etzadroxil

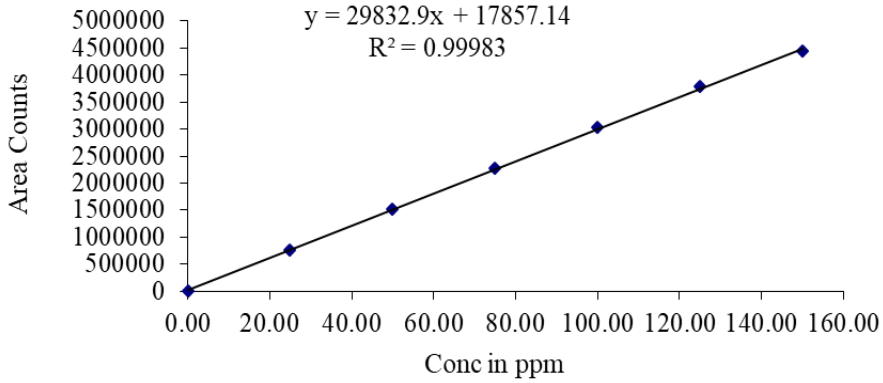

Figure 6: Regression curve for Probenecid

separation provided well-defined peaks with acceptable resolution, tailing factor, and theoretical plate count.

Specificity

In the presence of possible interfering compounds in the sample, specificity is the capacity to precisely identify and quantify the target components. It can be evaluated by analyzing chromatograms of the blank, standard, and sample to ensure no additional peaks appear, confirming that the method selectively detects the intended analytes. Specificity is depicted through the chromatograms presented in Figure 2.

Linearity

The linearity study showed a strong correlation between concentration and peak area for Sulopenem Etzadroxil (R^2 = 0.99982) and Probenecid (R^2 = 0.99983) over 25–150 µg/mL (Table 1). The regression equations confirm the method's linearity, as illustrated in Figures 5 and 6.

Method Precision

The method precision results showed %RSD values of 0.50% for Sulopenem Etzadroxil and 0.57% for Probenecid, indicating excellent repeatability and consistency of the RP-HPLC method as per ICH guidelines (Table 2).

Accuracy

Table 4: Forced Degradation results for Sulopenem Etzadroxil and Probenecid

Degradation studies	Sulopenem Etzadroxil		Probenecid	
	% Assay	% Deg	% Assay	% Deg
Control	100	0	100	0
Acid	96.7	3.3	86.3	13.7
Alkali	97.6	2.4	98.6	1.4
Peroxide	88.1	11.9	84.2	15.8
Reduction	95.9	4.1	96.3	3.7
Photolytic	96.3	3.7	88.7	11.3
Hydrolysis	87.1	12.9	97.0	3.0
Thermal	86.0	14.0	96.6	3.4

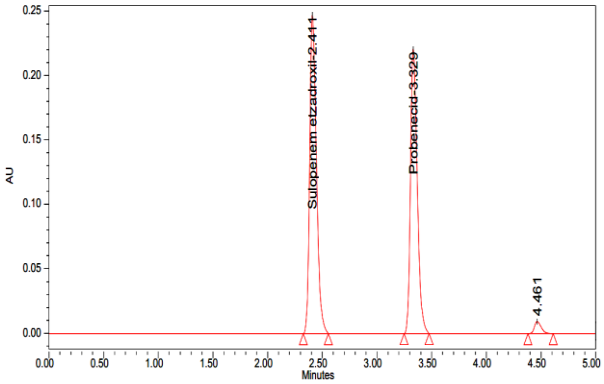

Figure 6: Acidic degradation

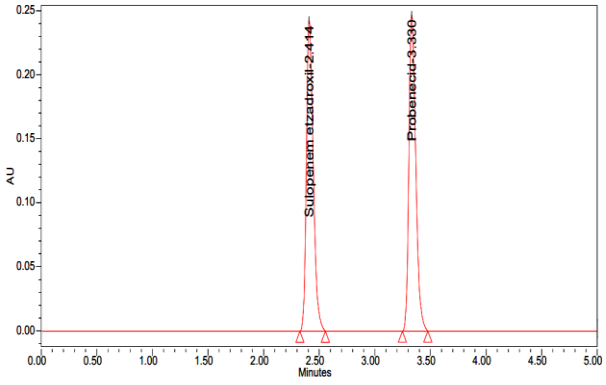

Figure 7: Alkali degradation

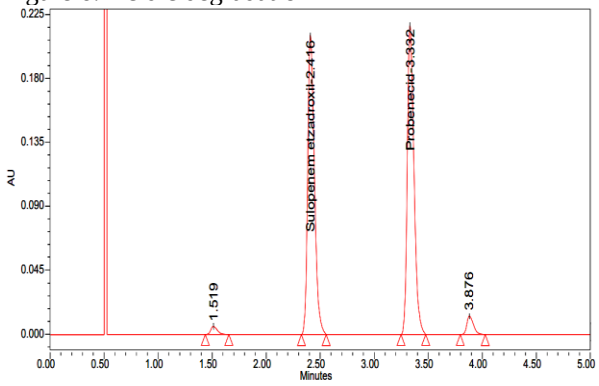

Figure 8: Peroxide degradation

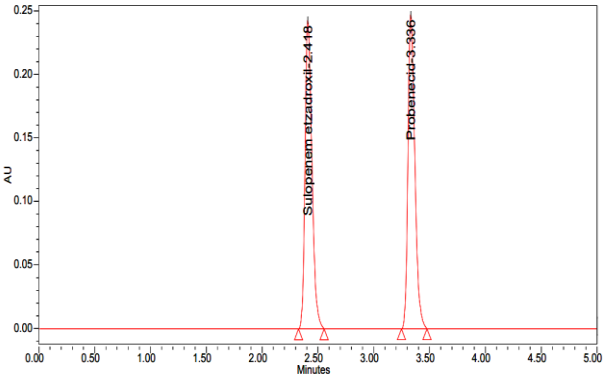

Figure 9: Reduction Degradation

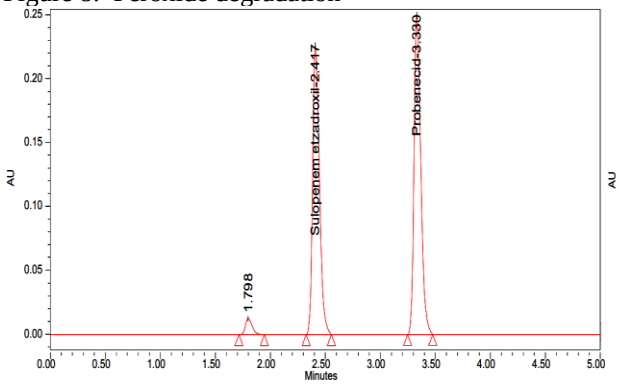

Figure 10: Thermal degradation

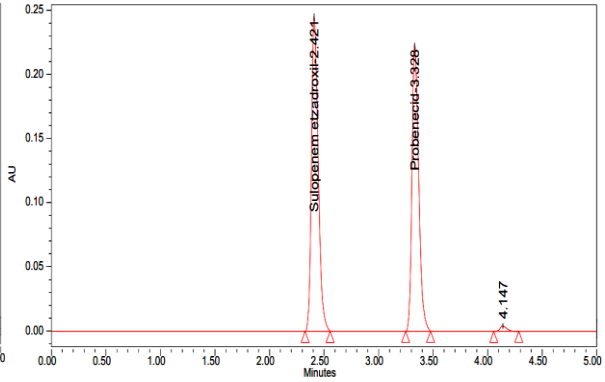

Figure 11: Photolytic degradation

The accuracy study of Sulopenem Etzadroxil and Probenecid at 50%, 100%, and 150% concentration levels demonstrated mean recovery values of 100.7% and 99.9%, respectively, indicating the method's accuracy (Table 3).

Robustness

At a lower flow rate (0.9 mL/min), Sulopenem Etzadroxil and Probenecid showed retention times of 2.569 and 3.516 min with peak areas of 2,741,583 and 2,935,148, respectively, while at a higher flow rate (1.1 mL/min), the retention times decreased to 2.277 and 3.201 min with peak areas increasing to 3,041,278 and 3,258,410. Similarly, with a lower organic phase ratio (18:82), retention times increased to 2.738 and 3.686 min with

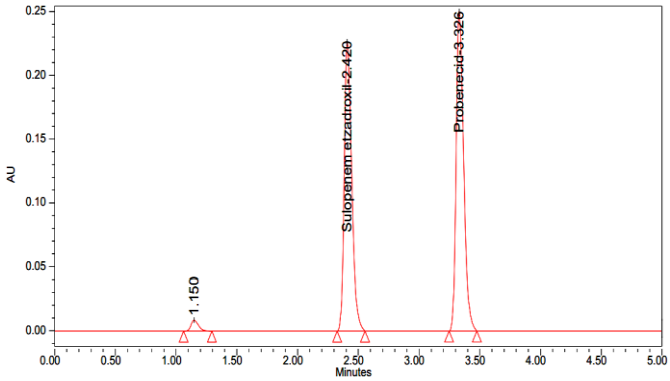

Figure 12: Hydrolysis degradation

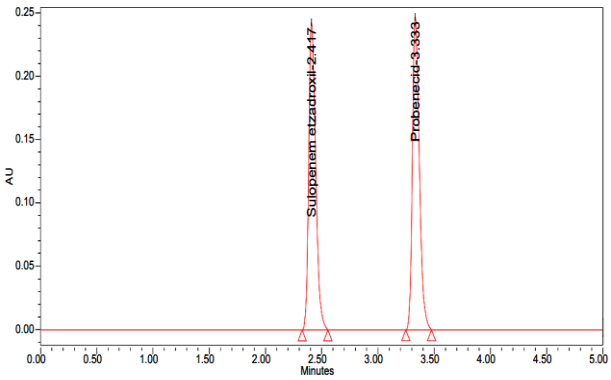

Figure 13: Control degradation

reduced peak areas, whereas increasing the organic phase (22:78) resulted in decreased retention times of 2.058 and 3.117 min with higher peak areas. The method remained robust, as minor variations did not significantly impact peak integrity.

Assay

The assay of 100.9% forSulopenem Etzadroxil and 100.0% for Probenecid, confirms the accuracy and reliability of the method.

Stress studies

The forced degradation study demonstrated the stability of Sulopenem Etzadroxil and Probenecid under various stress conditions. Sulopenem Etzadroxil showed the highest degradation under thermal (14.0%) and hydrolytic (12.9%) conditions, while Probenecid exhibited significant degradation under peroxide (15.8%) and acid (13.7%) stress. Minimal degradation was observed under alkali and reduction conditions for both drugs. Photolytic degradation was notable for Probenecid (11.3%) but lower for Sulopenem Etzadroxil (3.7%). The results confirm the method's stability-indicating capability. Degradation chromatograms are presented in Figures 7 to 13.

CONCLUSION

A validated RP-HPLC method was successfully developed for the simultaneous estimation of Sulopenem Etzadroxil and Probenecid in pure and pharmaceutical dosage forms, ensuring high precision, accuracy, and robustness. The method employed a Hyperclone 5µ BDS C18 column with an optimized mobile phase composition, delivering well-resolved peaks within a short retention time. Validation parameters demonstrated excellent linearity, precision, and accuracy, confirming the method's reliability. Robustness studies indicated minimal impact of minor variations in chromatographic conditions, ensuring method consistency. Forced degradation studies established the method's stability-indicating capability, confirming its suitability for detecting degradation products under stress conditions. This RP-HPLC method provides an efficient and reliable analytical tool for routine quality control and stability assessment of Sulopenem Etzadroxil and Probenecid formulations.

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