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Original Article

A REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-PHOTO DIODE ARRAY ESTIMATION OF PROBENECID AND SULOPENEM ETZADROXIL IN BULK AND PHARMACEUTICAL DOSAGE FORM AND CHARACTERIZATION OF DEGRADANTS BY USING LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

M. DAVID RAJU[1*], PRAVEEN KUMAR PALIVELA[2], YESUPADAMU RAYINUTHALA[3]

[*1]Department of Chemistry, Parvathaneni Brahmayya Siddhartha College of Arts and Science, Vijayawada, AP, India. [2]Department of Chemistry, Sir C R Reddy College (Autonomous), Eluru, India. [3]Department of Chemistry, Government Degree College, Chebrole, Guntur District, AP-522212, India

[*]Corresponding author: M. David Raju; [*]Email: davidm201926@gmail.com

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ABSTRACT

Objective: This study presents a valid and dependable "Reverse Phase High-Performance Liquid Chromatography (RP-HPLC)" technique for the simultaneous quantification of sulopenem etzadroxil and probenecid in their pharmaceutical dose form, which indicates stability.

Methods: Probenecid and sulopenem etzadroxil were isolated by using an isocratic elution technique with a phenyl column (250 mm x 4.6 mm, 5µm) and a mobile phase consisting of acetonitrile and triethyl amine at a pH of 2.5, which was adjusted with a 0.1% formic acid buffer in a 60:40 ratio, and a flow rate of 1.0 ml/min, respectively. Sulopenem etzadroxil and probenecid were quantified using a 271 nm detection wavelength.

Results: At retention durations of 2.631 and 4.048 min, respectively, the peaks for probenecid and sulopenem etzadroxil were eluted with fine resolution. Both probenecid and sulopenem etzadroxil showed linear calibration curves with regression coefficients of 0.99998 and 0.99975 in the concentration range of 50-300 µg/ml respectively. Resolution of probenecid and sulopenem etzadroxil from its degradation-based chemicals demonstrated the sensitivity, precision, robustness, accuracy, and specificity of the proposed high-performance liquid chromatography method and also indicated stability. The degradation agents were identified by the use of LCMS in the research involving forced degradation.

Conclusion: The pharmaceutical dosage forms of probenecid and sulopenem etzadroxil were evaluated using the well-established high-performance liquid chromatography method, and the findings seemed adequate.

Keywords: Isocratic method, Development, Validation, RP-HPLC, Stability indicating

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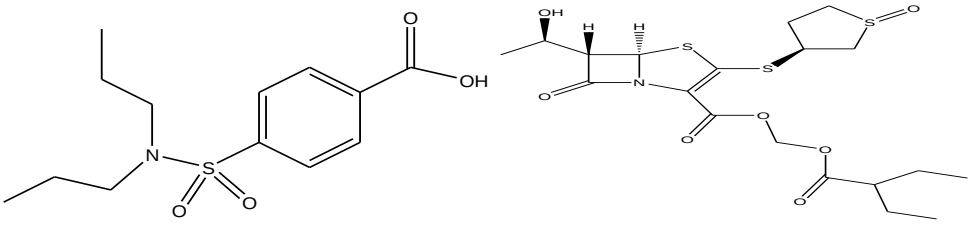
INTRODUCTION

Sulopenem, also known as CP-70,429, is an oral active thiopenem antibiotic derivative [1, 2] from the penem family. In conjunction with probenecid (Orlynvah brand name), it has been authorized for the treatment of simple UTIs [3, 4]. Research into its possible uses is continuous, and it has entered Phase III clinical trials on many times. It is being studied primarily for its ability to cure MDR UTIs. For the treatment of urinary tract infections in adult women caused by Escherichia coli [5, 6], klebsiella pneumoniae [7], or Proteus mirabilis [8] for whom there are few alternative oral antibiotic alternatives, the US Food and Drug Administration authorized a combination of sulopenem etzadroxil with probenecid. Iterum Therapeutics came up with the combo and sold it under the ORLYNVAH brand name.

An increase in the excretion of uric acid [9, 10] is the effect of the medicine probenecid, which is marketed under the trade name Probalan. Gout [11, 12] and hyperuricemia [13, 14] are the main indications of use. To increase the plasma concentration and duration of action of some medications, probenecid was created as a substitute for caronamide by competitively inhibiting renal excretion. When used with cidofovir, probenecid may protect the kidneys and raise the concentration of some antibiotics. Specifically, there is some data that suggests using intravenous cefazolin once day instead of three times with probenecid. Additionally, it has been used as a masking agent, which might aid athletes who use PEDs in evading drug testing. This medicine is often associated with mild side effects such as nausea, loss of appetite [15], dizziness [16], vomiting, headache, sore gums, or frequent urination. Thrombocytopenia [17], hemolytic anemia [18], leukemia and encephalopathy [19] are exceedingly uncommon but potentially fatal adverse effects. Uric acid kidney stones may be more likely to occur in patients using probenecid, at least in theory. A new sensitive stability-indicating RP-HPLC method for the evaluation of the combination of probenecid and sulopenem etzadroxil is suggested in this work. Estimating the pharmaceutical components probenecid, sulopenem, etzadroxil, and RP-HPLC is the goal of the investigation.



A B

Fig. 1: Structure of (A) Probenecid and (B) Sulopenem etzadroxil

Till date, only one HPLC method was available [20] in the literature. The aim of the study was to develop a new rapid and sensitive RP-HPLC method for the simultaneous estimation of probenecid and sulopenem etzadroxil and characterization of its degradants using LCMS/MS (Liquid Chromatographic Mass Spectrophotometer). We performed only assay and characterization of degradants only and we didn't go to further studies. Clinical relevance and regulatory data was available in USFDA.gov

MATERIALS AND METHODS

Chemicals

Merck India Ltd. of Mumbai, India, supplied the acetonitrile, HPLC-grade methanol, and water. We obtained the active pharmaceutical ingredients (APIs) of probenecid, sulopenem, and etzadroxil from Glenmark Mumbai. We used probenecid and sulopenem etzadroxil tablets, which are a formulation sample from Euphoria India Pharma, with a label claim of 500 mg each.

The instrumentation

We used an HPLC system from Waters Alliance, the e-2695, which has a quaternary pump, a PDA (Photo Diode Array) detector, and the Empower 2.0 software [20, 21].

Method optimization

The chromatographic conditions were optimized by experimenting with various ratios of phosphate buffer to acetonitrile in the mobile phase using isocratic mode. Nevertheless, in order to obtain satisfactory retention durations and improve resolution, the mobile phase composition was adjusted at each session. The active pharmaceutical components were shown to be more responsive after using an isocratic elution mobile phase consisting of acetonitrile and formic acid. The process was optimized by testing it with several stationary phases, including amino phenyl columns, C_8, and C_{18}. Based on these experiments, the peak morphologies using a 250 x 4.6 mm phenyl column with a 5 µ particle size, PDA detector were satisfactory. In order to achieve sufficient sensitivity, the mobile phase flow rate of 1 ml/min has been carried out at 271 nm. Probenecid and sulopenem etzadroxil had retention durations of about 2.631 and 4.048 min, respectively, with a tailing factor of 1.07 and 1.01, according to the circumstances mentioned above. Probenecid and sulopenem etzadroxil had 7253 and 8512 theoretical plates, respectively, indicating that the column produced the desired results. With a RSD (Relative Standard Deviation) of between 0.40 and 0.14 percent for six separate injections, the suggested method seems to be very accurate. The developed procedure was verified according to ICH (International Council for Harmonization) criteria.

Validation procedure

The following analytical characteristics were evaluated according to the ICH Q2 (R1) guidelines: system appropriateness, precision, specificity, accuracy, linearity, robustness, LOD (Limit of Detection), LOQ (Limit of Quantification), forced deterioration, and stability [22, 23]. (International Conference on Harmonization, or ICH)

Preparation of buffer

Use 0.1% formic acid to bring the pH of 1 liter of 0.1% triethylamine solution down to 2.5 and then filter the mixture using 0.22 micrometer filter paper.

Chromatographic conditions

Using a mobile phase of acetonitrile and triethylamine at pH-2.5 adjusted with 0.1% formic acid in a 60:40 ratio, and a phenyl (250x4.6 mm, 5 µ) column operating at a flow rate of 1.0 ml/min, the HPLC analysis was carried out using a reverse-phase HPLC system with isocratic elution mode.

Diluent: Ethanol.

Preparation of the standard solution

A 20 mg probenecid and 20 mg sulopenem etzadroxil standard solution was made by dissolving the two drugs in 10 ml of an ethanol solvent mix. The resulting solution had a concentration of 200 µg/ml. To further dilute, add diluents to 1 ml to 10 milliliters.

Preparation of the sample solution

The 45 mg sample, which is equal to 20 mg of probenecid and 20 mg of sulopenem etzadroxil (the label claim 500 mg of probenecid and 500 mg of sulopenem etzadroxil), was dissolved in 10 ml of an ethanol solvent mix to create a sample solution with a concentration of 200 µg/ml for each drug further dilute, add diluents to 1 ml to 10 ml.

RESULTS

The separation of active pharmaceutical components posed the greatest analytical problem during the process of developing a new technique. Optimizing the chromatographic conditions allowed for excellent results. The stability indicating technique for these medications utilizing HPLC has not been disclosed yet; nonetheless, sulopenem etzadroxil is an antibiotic prodrug and probenecid is a uricosuric drug. Therefore, in accordance with the ICH Guideline, a new approach has been created and verified.

The validity criteria were set up in accordance with the guidelines provided by the ICH [24-26].

System suitability

Table 1 displays the reported tailing and plate count values as well as the system appropriateness injecting standard solution and USP (United States of Pharmacopeia) values [27, 28].

Specificity

The interference was examined by separately analyzing the placebo, standard, and sample solutions in this test procedure [29]. The graphic below demonstrates that the active components were clearly separated from the blank and their excipients, and that the primary peak was unaffected by the placebo. Therefore, it is a targeted approach.

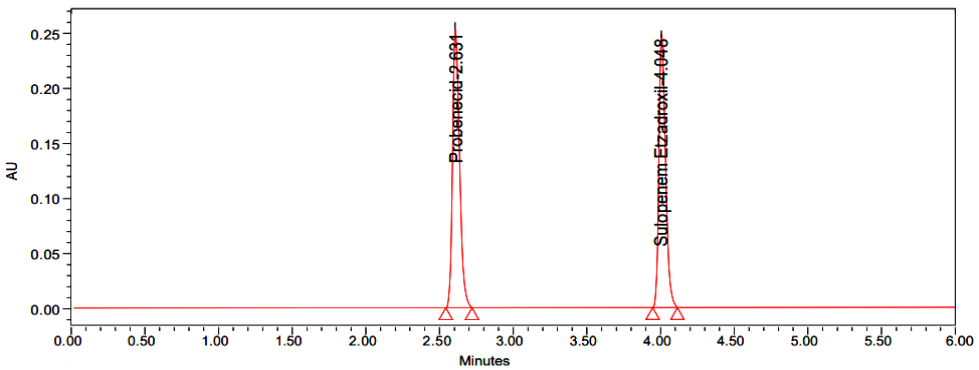


Fig. 2: Chromatogram of standard

Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Drug name	
		Probenecid	Sulopenem etzadroxil
USP Plate Count	Not Less Than 2000	7251	8536
USP Tailing	Not More Than 2.0	1.07	1.01
USP Resolution	Not Less Than 2.0	-	6.35
% RSD	Not More Than 2.0	0.40	0.14

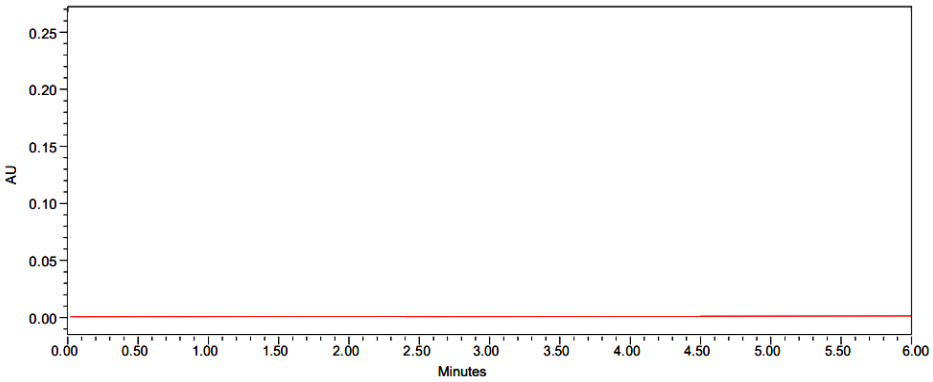


Fig. 3: Chromatogram of blank

Linearity

In this study, we tested probenecid and sulopenem etzadroxil for linearity of area response. The linear peak response areas were determined for probenecid and sulopenem etzadroxil by chromatography of solutions with concentrations ranging from 50 to 300 µg/ml. Fig. 4 shows the calibration curves for probenecid and sulopenem etzadroxil as well as the regression line equation and regression coefficient.

Accuracy

Probenecid and sulopenem etzadroxil assays in spiked samples were used to assess accuracy according to the suggested approach. The samples were supplemented with three different concentrations of probenecid and sulopenem etzadroxil standards: 50%, 100% and 150% [30, 31]. Table 3 displays the outcomes.

Precision

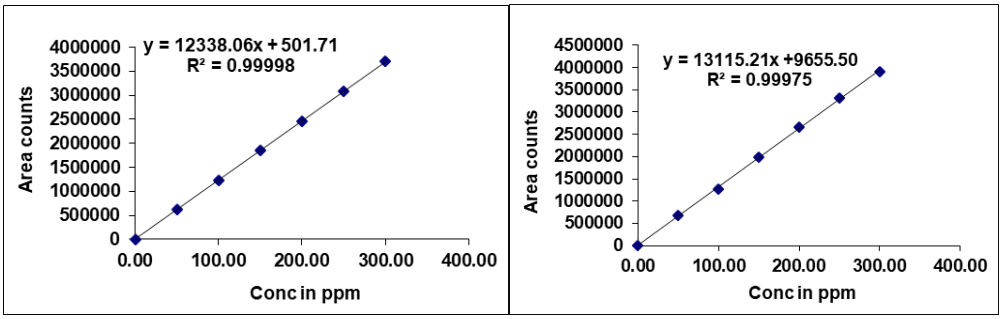
Precision was checked by measuring the same solution of probenecid and sulopenem etzadroxil six times throughout the day, with a concentration of 500 µg/ml each. Precision was confirmed by measuring the RSD of the peak regions of probenecid and sulopenem etzadroxil, while accuracy was confirmed by conducting % content tests of the two drugs. Table 4 displays these outcomes.

Intraday precision

On the same day, six separate samples containing 200 µg/ml of probenecid and 200 µg/ml of sulopenem etzadroxil were examined [32-34]. In order to determine the mean, standard deviation, and percentage of reliability, peak regions were computed.

Table 2: Linearity of probenecid and sulopenem etzadroxil

S. No.	Probenecid		Sulopenem etzadroxil	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
1	50.00	621753	50.00	685508
2	100.00	1221560	100.00	1278268
3	150.00	1862175	150.00	1989596
4	200.00	2466503	200.00	2662998
5	250.00	3087600	250.00	3318987
6	300.00	3698881	300.00	3903202
the correlation coefficient		0.99998		0.99975
Slope		12338.06		13115.21
intercept		501.71		9655.50



A **B**

Fig. 4: Calibration plots of (A) Probenecid (B) Sulopenem etzadroxil

Table 3: Results of accuracy of (A) Probenecid and (B) Sulopenem etzadroxil

A

Level (%)	Sample peak area	Amount of standard added (µg/ml)	Amount recovered	% Recovery	SD	% RSD
50	1221560	10	9.91	99.5	0.85	0.85
	1242558	10	10.08			
	1230567	10	9.98			
100	2469284	20	20.03	99.5	0.62	0.62
	2439286	20	19.79			
	2449381	20	19.87			
150	3695881	30	29.98	99.6	0.48	0.48
	3689792	30	29.93			
	3662896	30	29.71			

Data are given as mean+SD (n=3), SD – Standard Deviation

B

Level (%)	Sample peak area	Amount of standard added (µg/ml)	Amount recovered	% Recovery	SD	% RSD
50	1341854	10	10.02	100.1	0.14	0.14
	1338399	10	9.99			
	1341452	10	10.01			
100	2689215	20	20.07	100.0	0.34	0.34
	2674277	20	19.96			
	2673081	20	19.95			
150	3944586	30	29.44	98.6	0.60	0.61
	3956257	30	29.53			
	3990942	30	29.79			

Data are given as mean+SD (n=3)

Table 4: Intraday precision results of probenecid and sulopenem etzadroxil

S. No.	Probenecid			Sulopenem etzadroxil		
	Conc. (µg/ml)	Area	Percent recovery	Conc. (µg/ml)	Area	Percent recovery
1	200	2416891	98.1	200	2626795	98.1
2		2461789	99.9		2684321	100.3
3		2465810	100.1		2673873	99.9
4		2454608	99.6		2694855	100.7
5		2449396	99.4		2662664	99.5
6		2471312	100.3		2672798	99.8
Mean		2453301	99.6		2669218	99.7
SD		19470.81	0.789		23505.35	0.895

Data are given as mean+SD (n=6)

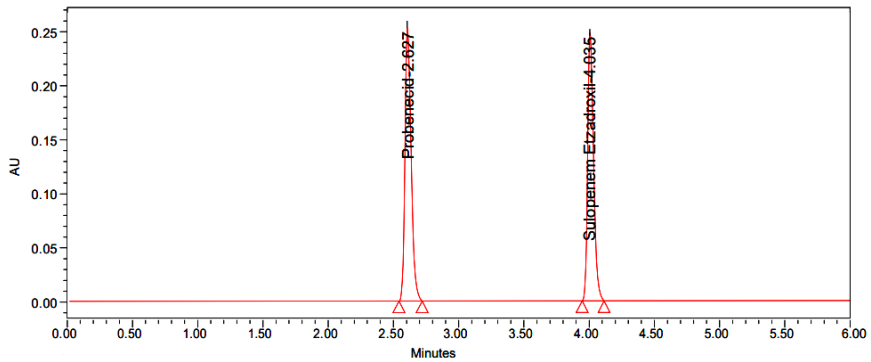


Fig. 5: Chromatogram of method precision

Intermediate precision

On different days, different equipment was tested, and six distinct versions of the sample solution were examined by the researchers. We have computed the peak areas that will be used to ascertain the mean percent RSD values. The outcomes are shown in the table that follows [35].

Inter-day precision

On separate days, six separate solutions containing probenecid and sulopenem etzadroxil were tested. Each solution had 200µg/ml of each compound. The mean, standard deviation, and percentage of reliability were determined by calculating the peak regions. Results showed that the current procedure was accurate, with RSD values below 2% and percentage assay values around 100%. Table 5 displays the outcomes.

LOD and LOQ

The limit of detection and limit of quantification were assessed using a signal-to-noise ratio. The LOQ for probenecid was 10 times the baseline noise, whereas the LOD for sulopenem etzadroxil was 3 times the baseline noise.

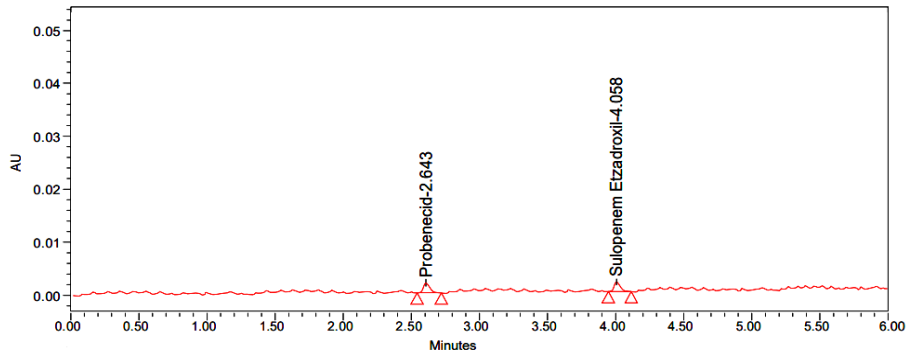
Table 5: Inter-day outcomes of accuracy of probenecid and sulopenem etzadroxil

S. No.	Probenecid				Sulopenem etzadroxil			
	Day-1 Area counts	Day-1 % recovery	Day-2 Area counts	Day-2 % recovery	Day-1 Area counts	Day-1 % recovery	Day-2 Area counts	Day-2 % recovery
1	2452889	99.6	2481362	100.8	2661868	99.4	2663021	99.5
2	2473794	100.5	2412754	98.0	2683677	100.2	2645817	98.8
3	2435815	98.9	2463958	100.1	2675424	99.9	2631025	98.3
4	2454611	99.7	2447845	99.4	2684435	100.3	2655248	99.2
5	2439397	99.1	2462305	100.0	2669579	99.7	2685479	100.3
6	2481324	100.8	2450187	99.5	2671873	99.8	2683054	100.2
Mean	2456305	99.8	2453069	99.6	2674476	99.9	2660607	99.4
SD	18177.32	0.753	23087.93	0.944	8653.98	0.331	21217.3	0.783

Data are given as mean+SD (n=6)

Table 6: LOD and LOQ for probenecid and sulopenem etzadroxil

Probenecid				Sulopenem etzadroxil			
LOD		LOQ		LOD		LOQ	
Concentration 0.06µg/ml	s/n 3	Concentration 0.2µg/ml	s/n 10	concentration 0.06µg/ml	s/n 3	Concentration 0.2µg/ml	s/n 10



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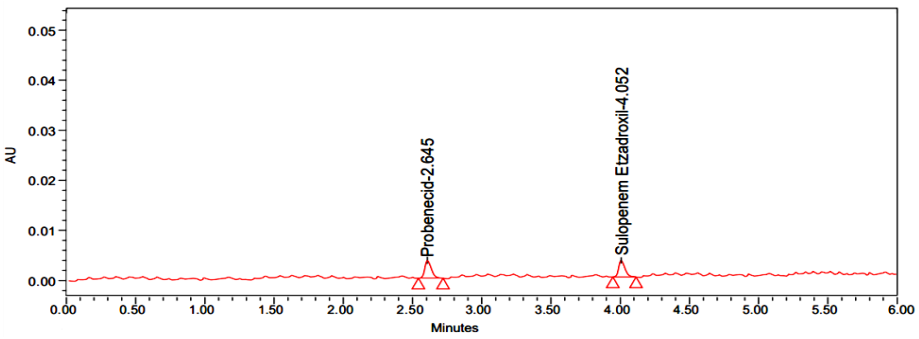


B

Fig. 6: Chromatogram of (A) LOD and (B) LOQ

Table 7: Robustness data of (A) Probenecid and (B) Sulopenem etzadroxil

A

Parameter	Condition	Peak area±SD, % RSD (n = 3)
Flow rate (±10%)	0.9	2295841± 5148.23, 0.78
	1	2475128± 5231.15, 0.32
	1.1	2751023± 3620.47, 0.57
Mobile Phase composition (±10%)	54:46	2104536± 6107.23, 0.61
	60:40	2451208± 5247.13, 0.26
	66:34	2985641± 4174.05, 0.95
pH Variation (±0.2)	2.3	2455879±5178.58, 1.12
	2.5	2415638±6325.21, 0.98
	2.7	2385641±4518.12, 1.47

Data are given as mean+SD (n=3)

B

Parameter	Condition	Peak area±SD, % RSD (n = 3)
Flow rate (±10%)	0.9	2561325± 1875.13, 0.63
	1	2685471± 2745.31, 0.84
	1.1	2884501± 1203.09, 1.24
Mobile phase composition (±10%)	54:46	2285715± 3621.42, 0.48
	60:40	2653204± 2736.36, 0.41
	66:34	3069340±1546.85, 1.64
pH Variation (±0.2)	2.3	2694857±1975.45, 0.63
	2.5	2634781±2659.31, 0.56
	2.7	2599472±3620.07, 1.01

Data are given as mean+SD (n=3), In the optimized method, we cannot mention the column temperature. So, we should not perform column temp variation in robustness.

Robustness

The strength was evaluated by measuring the peak area of a solution containing 200µg/ml of probenecid and sulopenem etzadroxil, with significantly altered parameters in the HPLC test. According to references [36, 37], table 7 displays the modified parameters and the subsequent peak regions.

Degradation studies

The medicine probenecid and sulopenem etzadroxil was partially degraded by subjecting the sample to different forced degradation settings. Studies of forced degradation have carried out to find out that the method is suitable for products of degradation [38]. Furthermore, the investigations describe the drug's instability under certain circumstances, which allows for the implementation of safeguards during formulation to prevent such instability [39].

Acid degradation

Using 1N HCl (Hydrochloric acid), the acid degraded 13.7% of probenecid and 12.1% of sulopenem etzadroxil. Two degradation products (DP) were formed, namely DP1 and DP 4.

Alkali degradation

Using 1N NaOH (Sodium Hydroxide), the base degraded 12.5% of probenecid and 11.8% of sulopenem etzadroxil. Two degradation products were formed, namely DP2 and DP 5.

Peroxide degradation

Using 10% H_2O_2 (Hydrogen Peroxide), degraded 15.9% of probenecid and 3.0% of sulopenem etzadroxil. Only one degradation product was formed, namely DP3.

Reduction degradation

Using 10% NaHSO3 (Sodium bisulphite), degraded 2.5% of probenecid and 10.2% of sulopenem etzadroxil. Only one degradation product was formed, namely DP6.

Thermal degradation

The sample was degraded to 2.1% of probenecid and 2.9% of sulopenem etzadroxil in thermal oven. No degradation products were formed.

Photolytic degradation

The sample was degraded to 2.4% of probenecid and 1.2% of sulopenem etzadroxil in photolytic chamber. No degradation products were formed.

Hydrolysis degradation

The sample was degraded to 1.7% of probenecid and 0.9% of sulopenem etzadroxil in hydrolysis condition (Addition of 1 ml of HPLC water only). No degradation products were formed.

All degradation results are tabulated in table 8. These degradants were characterized by using LCMS.

Table 8: Forced degradation results of probenecid and sulopenem etzadroxil

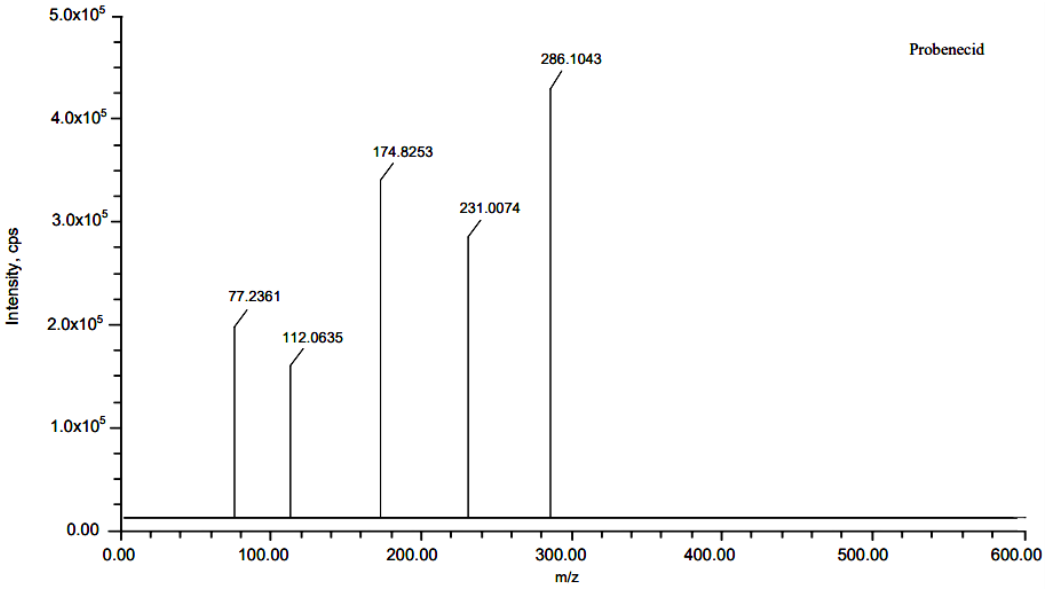
Degradation condition	Probenecid		Sulopenem etzadroxil		Number of DPs formed
	% Assay	% Deg	% Assay	% Deg	
Control degradation	100	0	100	0	-
Acid degradation	86.3	13.7	87.9	12.1	DP 1 and DP 4
Alkali degradation	87.5	12.5	89.2	11.8	DP 2 and DP 5
Oxidation degradation	84.1	15.9	87	3.0	DP 3
Reduction degradation	97.5	2.5	96.8	10.2	DP 6
Hydrolysis degradation	98.3	1.7	99.1	0.9	-
Thermal degradation	90.9	2.1	97.1	2.9	-
Photo degradation	97.6	2.4	98.8	1.2	-

DISCUSSION

According to a review of the relevant literature, just one HPLC [40] technique has been documented up to this point. To estimate probenecid and sulopenem etzadroxil, we have devised a rapid, accurate, and reliable HPLC method. Our HPLC approach is the first of its kind to simultaneously quantify probenecid and sulopenem etzadroxil in both bulk and tablet forms. The existing HPLC: probenecid and sulopenem etzadroxil analytical procedure was validated while considering the ICH requirements [41, 42]. The elution of probenecid and sulopenem etzadroxil was not affected by the tablet formulation excipients or the mobile phase components. The selectivity chromatograms (fig. 2, 3 and 5) corroborated the selectivity [43].

To separate probenecid and sulopenem etzadroxil, the chromatographic technique is mostly used. With the use of organic modifiers such as acetonitrile and methanol in the mobile phase and different stationary phases such as C_8, C_{18} and Phenyl as well as different mobile phases containing buffers such as formic acid, tri ethyl amine, and water with pH values ranging from 2 to 4, different trails were created using standard solution. The results showed that the phenyl column, mobile phase triethylamine buffer, and acetonitrile produced rather excellent peak shapes for probenecid and sulopenem etzadroxil. The flow rate was set at 1.0 ml/min. Probenecid and sulopenem etzadroxil were successfully separated using a phenyl column with a length of 250 mm, an inner diameter of 4.6 mm, a particle size of 5 µm, tri ethyl amine as

a buffer, and acetonitrile as the organic phase. The separation was carried out using an isocratic program of 40:60, with a post run time of 6 min and a detection wavelength of 271 nm. The outcomes were as follows given the aforementioned circumstances: With a tailing factor of 1.01 and a total of 7247 theoretical plates (N), the retention time of probenecid was 2.631 min, and the percentage RSD [44, 45] for six replicate injections was 0.40%. On the other hand, sulopenem etzadroxil had a retention time of 4.048 min, a total of 8587 theoretical plates (N), and a percentage RSD of 0.14%. Accelerated degradation experiments show that probenecid and sulopenem etzadroxil are easily broken down in environments with high temperatures, ultraviolet light, acidity, and oxidation. Using a photodiode array detector on Waters HPLC, the peak purity of stressed samples of probenecid and sulopenem etzadroxil was examined. It was found that all of the stress samples had a purity angle below the purity threshold, indicating that the analyte peaks were homogeneous. Our goal in doing this research was to find a novel characterisation HPLC technique that is sensitive, specific, and applicable to the analysis of probenecid, sulopenem etzadroxil.



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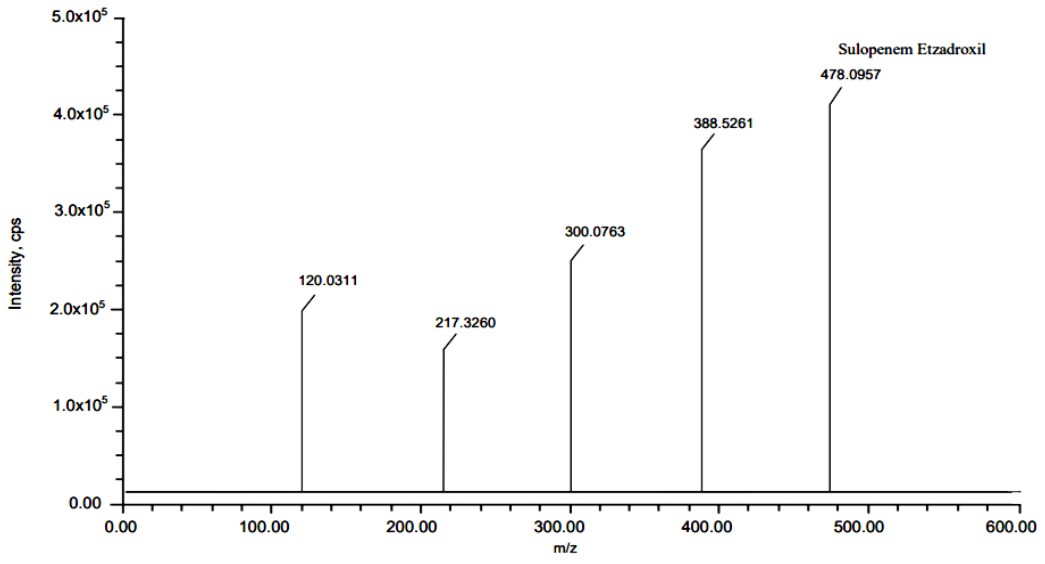

B

Fig. 7: MS Spectra of (A) Probenecid and (B) Sulopenem etzadroxil

Scheme 1

The process of fragmentation of probenecid degradation product 1 of m/z-303.0696 was observed under acidic degradation condition. The spectrum exhibited prominent fragment ions at m/z-203.9648 ($C_6H_{15}N$ loss), m/z-140.0029 (SO_2 loss). Mass spectrometry and precise mass measurements both lent credence to the suggested structures.

Scheme 2

The process of fragmentation of probenecid degradation product 2 of m/z-307.0854 was observed under alkaline degradation condition. The spectrum exhibited prominent fragment ions at m/z-207.9806 ($C_6H_{15}N$ loss), m/z-144.0187 (SO_2 loss). Mass spectrometry and precise mass measurements both lent credence to the suggested structures.

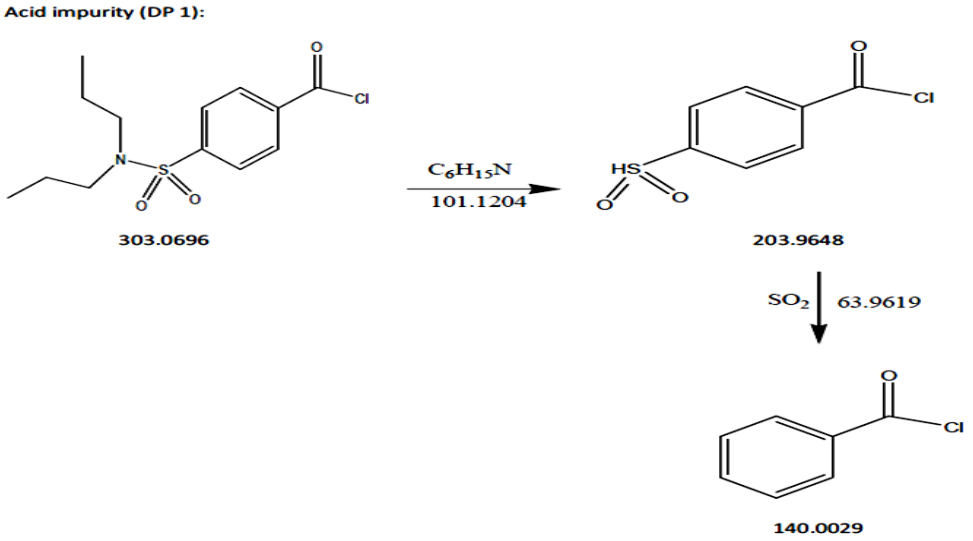

Fig. 8: Fragmentation mechanism of DP 1

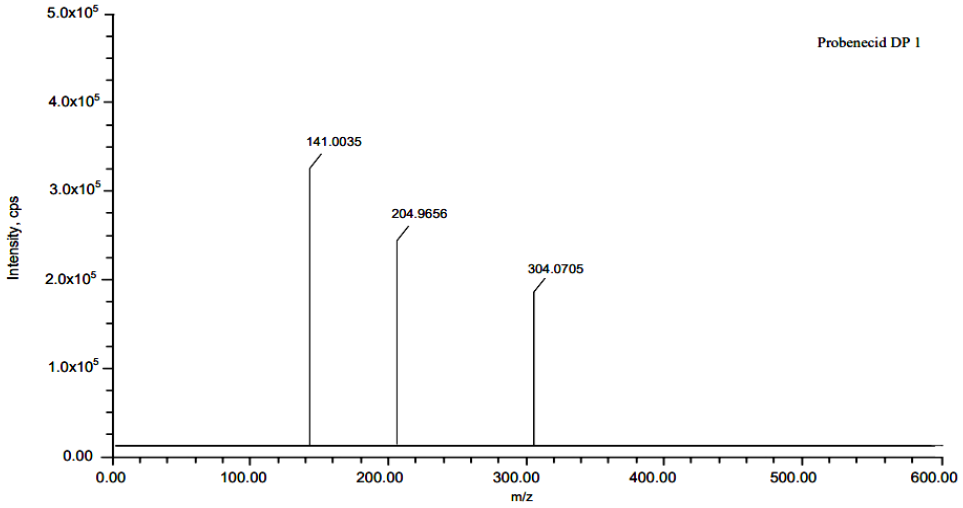

Fig. 9: Mass spectra of DP 1

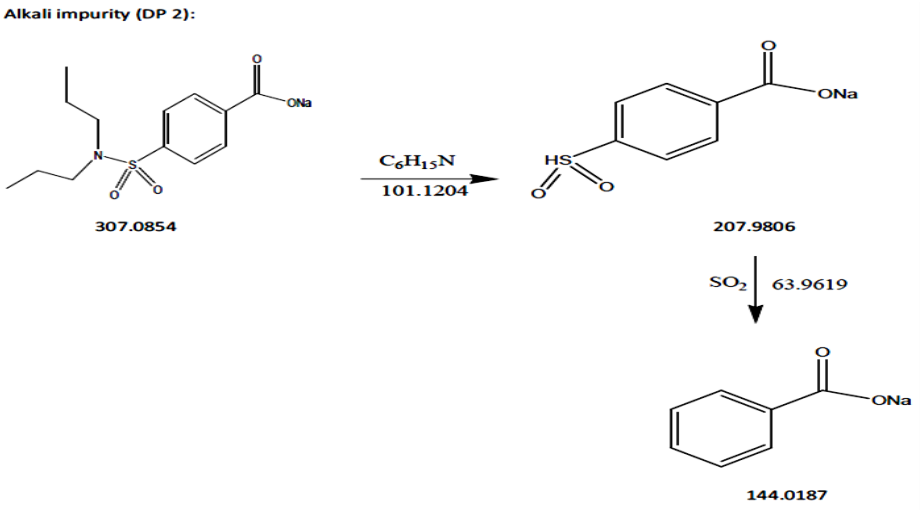

Fig. 10: Fragmentation mechanism of DP 2

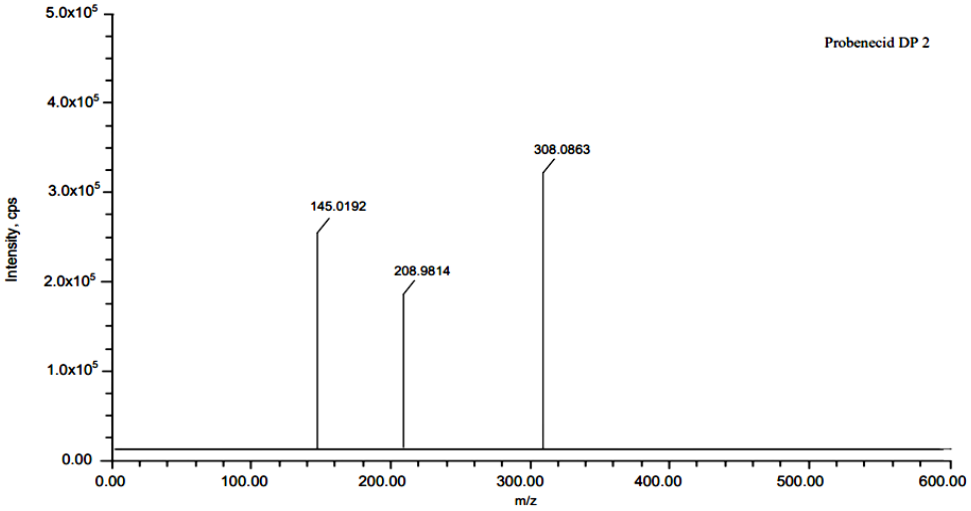

Fig. 11: Mass spectra of DP 2

Scheme 3

The process of fragmentation of probenecid degradation product 3 of m/z-301.0984 was observed under peroxide degradation condition. The spectrum exhibited prominent fragment ions at m/z-185.9987 ($C_6H_{15}NO$ loss), m/z-122.0368 (SO_2 loss). Mass spectrometry and precise mass measurements both lent credence to the suggested structures.

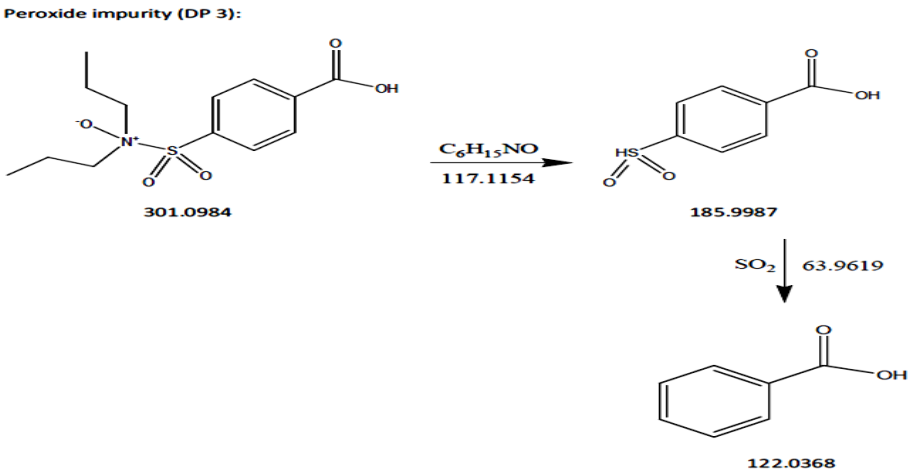

Fig. 12: Fragmentation mechanism of DP 3

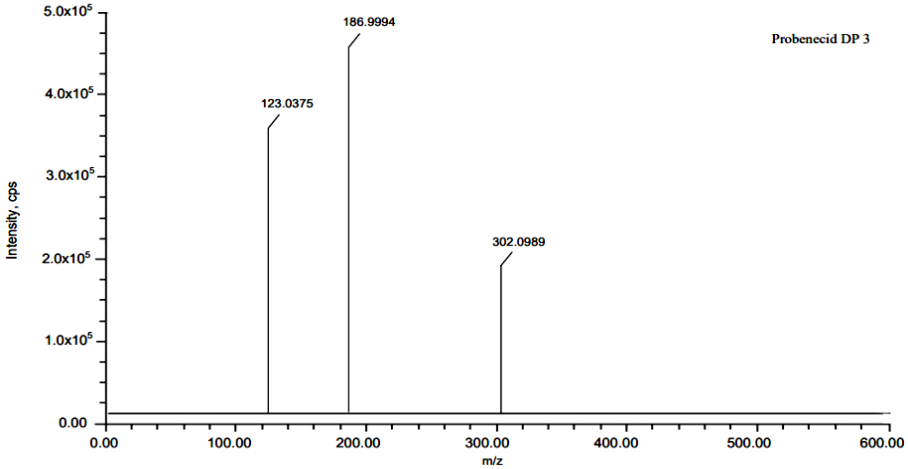

Fig. 13: Mass spectra of DP 3

Scheme 4

The process of fragmentation of sulopenem etzadroxil degradation product 4 of m/z-382.9723 was observed under acid degradation condition. The spectrum exhibited prominent fragment ions at m/z-280.9583 (C_4H_8OS loss), m/z-196.9372 ($C_4H_8O_2$ loss), m/z-63.0143 ($C_3H_3ClO_2S$ loss). Mass spectrometry and precise mass measurements both lent credence to the suggested structures.

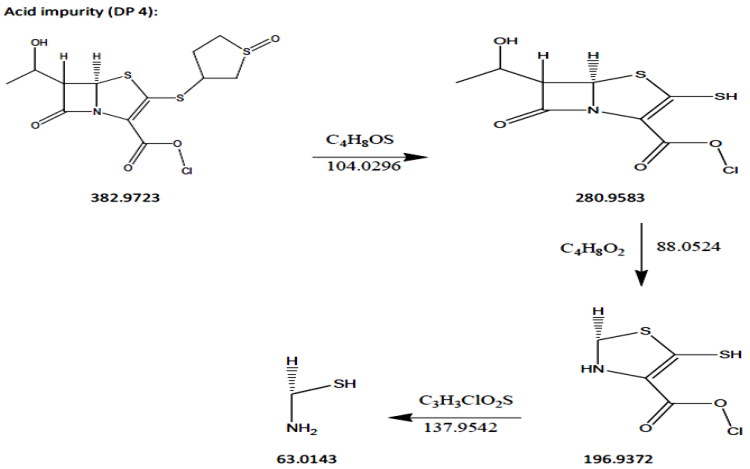

Fig. 14: Fragmentation mechanism of DP 4

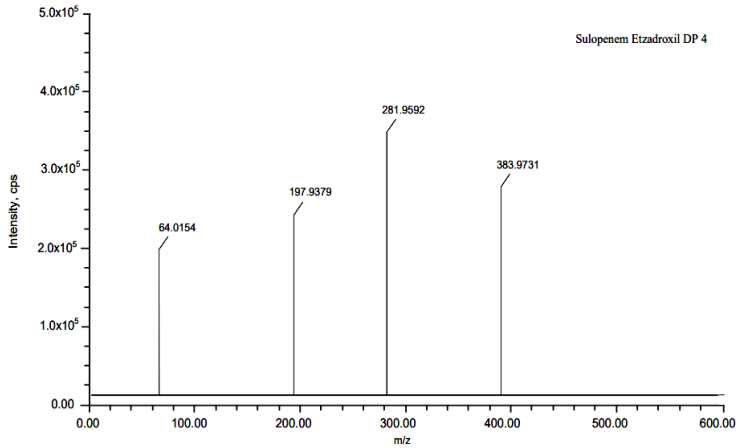

Fig. 15: Mass spectra of DP 4

Scheme 5

The process of fragmentation of sulopenem etzadroxil degradation product 5 of m/z-370.9932 was observed under alkali degradation condition. The spectrum exhibited prominent fragment ions at m/z-268.9792 (C_4H_8OS loss), m/z-184.9581 ($C_4H_8O_2$ loss), m/z-63.0143 ($C_3H_3NaO_2S$ loss). Mass spectrometry and precise mass measurements both lent credence to the suggested structures.

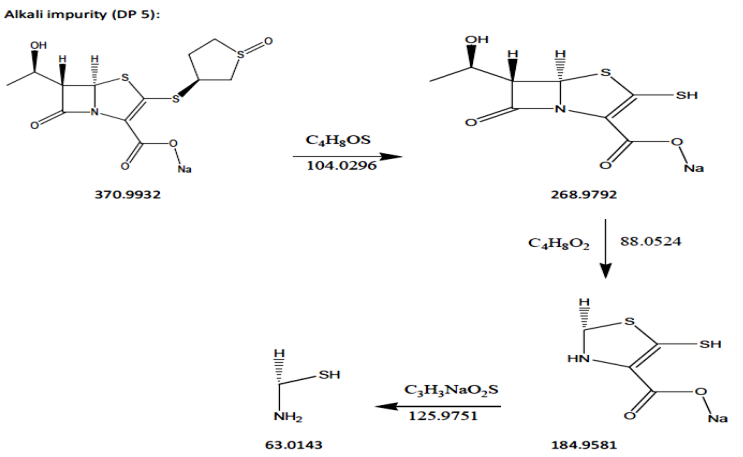

Fig. 16: Fragmentation mechanism of DP 5

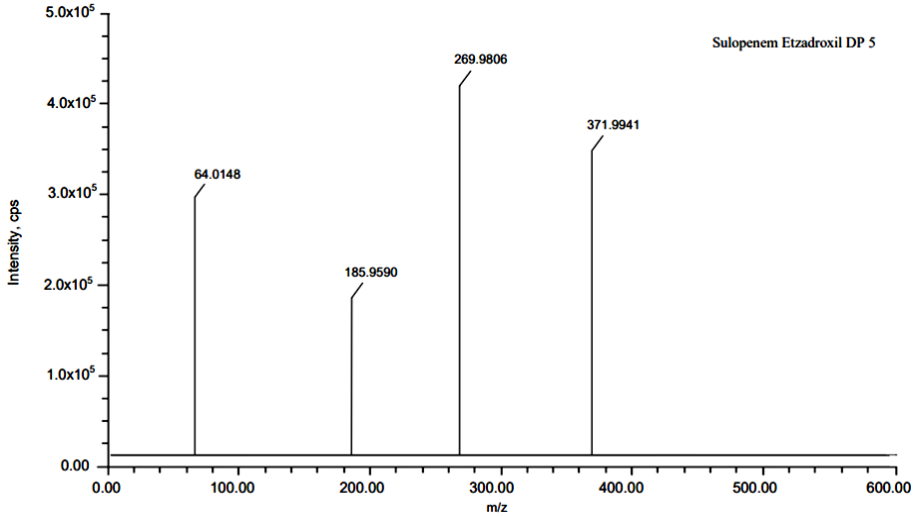

Fig. 17: Mass spectra of DP 5

Scheme 6

The process of fragmentation of sulopenem etzadroxil degradation product 5 of m/z-450.95 was observed under reduction degradation condition. The spectrum exhibited prominent fragment ions at m/z-348.9361 (C_4H_8OS loss), m/z-264.9149 ($C_4H_8O_2$ loss), m/z-118.9863 ($CHNaO_5S$ loss). Mass spectrometry and precise mass measurements both lent credence to the suggested structures.

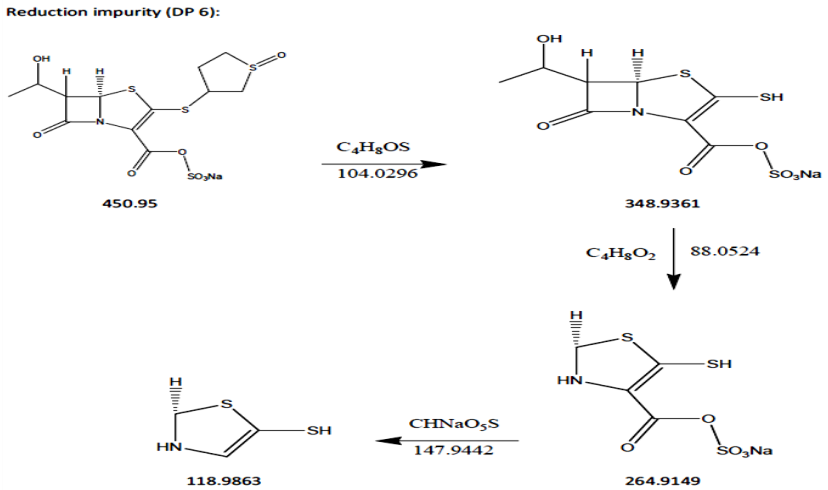

Fig. 18: Fragmentation mechanism of DP6

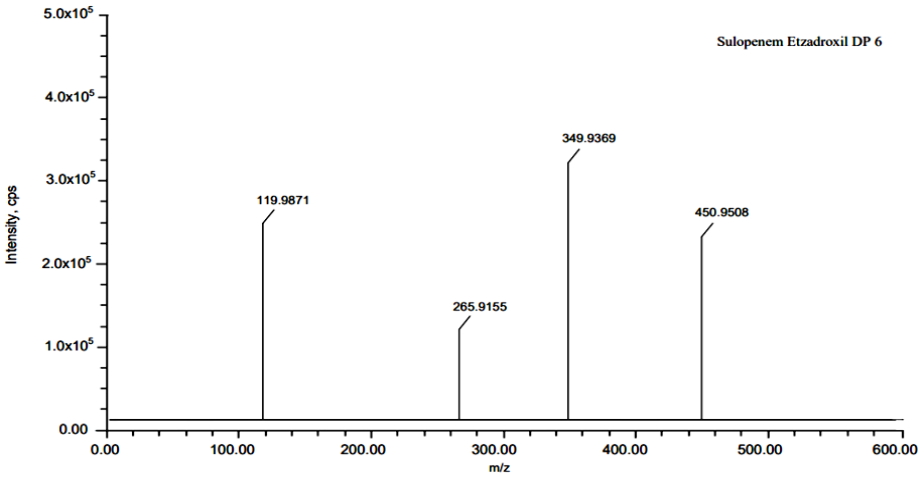

Fig. 19: Mass spectra of DP 6

Table 9: LC-MS/MS data of probenecid and it's degradation products and some major fragments

	Molecular formula	Calculated mass	Observed mass	Mass error (ppm)	Major fragment ions
Probenecid	$C_{13}H_{19}NO_4S$	285.1035	285.1043	2.805999	77.2361, 112.0635, 174.8253, 231.0074
DP 1	$C_{13}H_{18}ClNO_3S$	303.0696	303.0705	2.969615	141.0035, 204.9656
DP 2	$C_{13}H_{18}NNaO_4S$	307.0854	307.0863	2.930781	145.0192, 208.9814
DP 3	$C_{13}H_{19}NO_5S$	301.0984	301.0989	1.660587	123.0375, 186.9994

Table 10: LC-MS/MS data of sulopenem etzadroxil and it's degradation products and some major fragments

	Molecular formula	Calculated mass	Observed mass	Mass error (ppm)	Major fragment ions
Sulopenem Etzadroxil	$C_{19}H_{27}NO_7S_3$	477.095	477.0957	1.467213	120.0311, 217.3260, 300.0763, 388.5261
DP 4	$C_{12}H_{14}ClNO_5S_3$	382.9723	382.9731	2.088924	64.0154, 197.9379, 281.9592
DP 5	$C_{12}H_{14}NNaO_5S_3$	370.9932	370.9941	2.425920	64.0148, 185.9590, 269.9806
DP 6	$C_{12}H_{14}NNaO_8S_4$	449.95	449.9508	1.777975	119.9871, 265.9155, 349.9369

We got ppm error values like the above only. We calculated these errors using https://warwick.ac.uk/fac/sci/chemistry/research/barrow/barrowgroup/calculators/mass_errors/ sheet. Not all LCMS fig. (e. g., fig. 12–14) contain peak structure annotations within the graphic itself—some rely solely on captions – We can't understand this sir

CONCLUSION

The proposed research work is found to be promising and less time-consuming, with minimum amount of solvent utilization for method development. The developed method proved that the method is specific, accurate, precise, and robust for probenecid and sulopenem etzadroxil. Stress degradation studies revealed that probenecid and sulopenem etzadroxil withstand thermal, photo and hydrolysis conditions. At the same time, acidic, alkali, oxidative degradation occurred for probenecid and acidic, alkali, reduction degradation occurred for sulopenem etzadroxil. The developed method and obtained statistical data manifested that designed protocol is simple, rapid and economical for the estimation of probenecid and sulopenem etzadroxil APIs (Active Pharmaceutical Ingredient) and pharmaceutical formulation. Forced degradation products were characterized by using LCMS. Both drugs were not toxic, already stated that *in vitro* data is available in USFDA. gov. This method uses very less run time and organic phase composition was 60% very less cost for analysis and eco eco-friendly nature.

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AUTHORS CONTRIBUTIONS

Praveen Kumar has collected the literature and information about the drugs, David Raju carried out the research samples and prepared the manuscript.

CONFLICTS OF INTEREST

Declared none

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