Journal of Carcinogenesis
Covering Genesis to Therapeutics
Journal of Carcinogenesis , ISSN: 1477-3163
2025; Vol 24: Issue 3, https://carcinogenesis.com/

Simultaneous Determination Of Probenecid And Sulopenem Etzadroxil Using RP-UPLC With Pda Detector

P. Srividya[1], P. Bharath[1], P. V Surendra Gupta[1], B. Sree Ramudu[2], D. Ramachandran[1,*]

[1] Department of chemistry, University College of Sciences, Acharya Nagarjuna University, Nagarjuna Nagar - 522 510, Guntur (Dt), A.P.
[2] Department of Chemistry, Adikavi Nannaya University Campus, Tadepalligudem -534101, Email: sreeram.elm.chem@gmail.com, Ph No: 9491126997
Corresponding author Email: ramchandran.anu22@gmail.com

ABSTRACT

Objective: The present study aimed to verify the cancer healing medications (Probenecid and Sulopenem Etzadroxil) by means of a UPLC (Waters Acquity) apparatus that had a PDA detector, and to separate them.
Methods: The isocratic RP-UPLC technique for the quantitative measurement of Probenecid and Sulopenem Etzadroxil is characterized by its simplicity, selectivity, validation, and well-defined stability. The Acquity UPLC BEH chemistry Shield RP-18 column, with dimensions 50x2.1 mm and 1.7 micron, was used in the chromatographic technique. The mobile phase consisted of a mixture of acetonitrile and 0.1% perchloric acid (30+70). The elution method was isocratic. Using the PDA detector, the instrumental parameters were adjusted at a 272 nm wavelength and a flow rate of 0.2 ml/min.
Results: Sulopenem Etzadroxil had a limit of detection (LOD) of 0.5 µg/ml and a limit of quantification (LOQ) of 2.0 µg/ml, whereas Probenecid had limits of 0.5 µg/ml and 2.0 µg/ml. An R2 value greater than 0.999 indicates that the calibration charts were linear. While validating the technique, we checked its recovery, specificity, linearity, accuracy, robustness, and ruggedness, and all were within acceptable ranges. We followed the rules set forth by the International Conference on Harmonization (ICH) when we validated the suggested technique.
Conclusion: In an experimental setting, the suggested approach should work quickly, easily, practically, and cheaply. It has several applications in stability testing, including regular examination of industrial samples and medication sample quality verification.

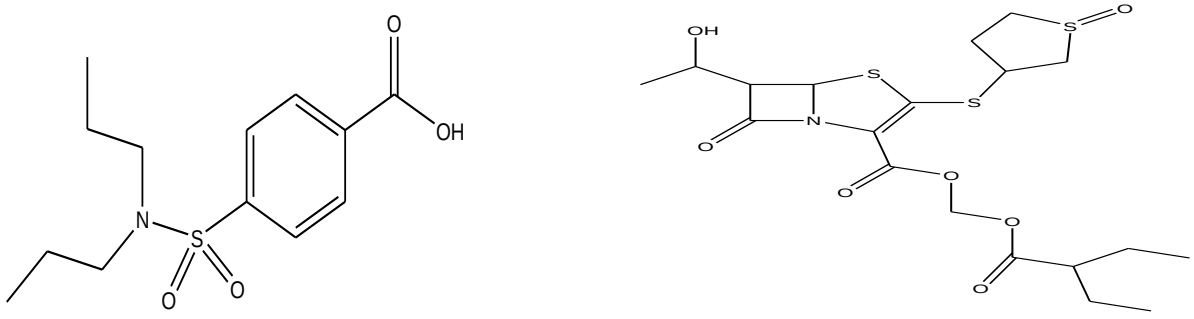
Key words: Probenecid, Sulopenem Etzadroxil, RP-UPLC, Development, Validation.

How to Cite: D. Ramachandran, et al (2025) Simultaneous Determination Of Probenecid And Sulopenem Etzadroxil Using RP-UPLC With Pda Detector., *Journal of Carcinogenesis*, *Vol.24, No.3*, 850-857.

INTRODUCTION

One of the antibacterial [1, 2] penem drugs, sulopenem etzadroxil, is a prodrug of sulopenem. The time-dependent inhibition of bacterial cell wall formation is a property shared by other beta-lactam antibacterials. Sulopenem, also known as CP-70,429, is an oral active thiopenem antibiotic that belongs to the penem family. There has been continuous investigation into its possible uses, particularly in the treatment of urinary tract infections [3, 4], and it has reached Phase III clinical trials on many times. To maximize antibiotic exposure, it is given with Probenecid. Vaginal yeast infections [5, 6], nausea, vomiting, diarrhea, gout [7, 8], and headaches were the most prevalent adverse effects. Caution should be used in the event of hypersensitivity responses, diarrhea caused by Clostridioides difficile [9, 10]. Patients who are using ketorolac tromethamine, have uric acid kidney stones [11, 12], or blood dyscrasias should not use SE/probenecid.
The medicine probenecid, which is marketed under the trade name Probalan, has the effect of increasing the excretion of uric acid in the urine. Hyperuricemia [13, 14] and gout are its main indications for 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 it comes to treating gout and hyperuricemia, probenecid is where it's at. 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. Nausea, vomiting, lack of appetite, lightheadedness, headache, painful gums, or increased urination frequency are among the mild side effects that may occur with this medicine. Very seldom do patients have potentially fatal adverse effects such as thrombocytopenia [15, 16], hemolytic anemia [17, 18], leukemia, and encephalopathy [19]. Uric acid kidney stones may be more likely to occur in patients using probenecid, at least in theory. You can see both drug structures in picture 1. Probenecid and Sulopenem Etzadroxil are pharmaceutical components that will be separated using RP-UPLC in this investigation.

Journal of Carcinogenesis , ISSN: 1477-3163
2025; Vol 24: Issue 3, https://carcinogenesis.com/



A **B**

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

No UPLC and HPLC procedures have been published in the literature as of yet. Therefore, we came up with a way to measure Probenecid and Sulopenem Etzadroxil at the same time. The in vitro approach was used to estimate the combined medicines using the developed UPLC method.

MATERIALS AND METHOD

Chemicals: In Mumbai, India, at Merck India Ltd., we bought acetonitrile, perchloric acid, and water. Cadila Healthcare Ltd. of Ahmedabad was contacted to acquire the active pharmaceutical ingredients (APIs) of Probenecid and Sulopenem Etzadroxil.

The Instrumentation: Using a photo diode array detector (model 2998) [22, 23] and an empower 2.0 data management system, this investigation used Waters Acquity liquid chromatography [20, 21].

Method optimization: In order to find the best chromatographic conditions, we tried using isocratic and gradient modes with mobile phases that varied the ratio of phosphate buffer to acetonitrile. Nevertheless, in order to obtain satisfactory retention durations and improve resolution, the mobile phase composition was adjusted at each session. Isocratic elution with acetonitrile and a 0.1% perchloric acid buffer was ultimately chosen since it increases the responsiveness of the active pharmaceutical components. Several stationary phases, including C18 and phenyl, RP-18 columns, were evaluated throughout the technique optimization process. Based on these tests, the Acquity UPLC BEH chemical Shield RP-18 column (50x2.1 mm, 1.7 micron) and PDA detector produced rather excellent peak shapes. In order to achieve sufficient sensitivity, the mobile phase flow rate was set at 272nm. Probenecid and Sulopenem Etzadroxil had retention durations of around 0.736 and 1.715 minutes, respectively, with a tailing factor of 1.02 and 1.18, as determined by the aforementioned parameters. Probenecid and Sulopenem Etzadroxil had 9456 and 8841 theoretical plates, respectively, indicating that the column was successful. It seems to be very exact according to the suggested method. The developed procedure was verified according to ICH criteria.

Validation procedure

Analytical metrics like system appropriateness, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation, and stability were verified in accordance with ICH Q2 (R1) criteria [24, 25].

Preparation of buffer: Perchloric acid, dissolved in 1 milliliter of HPLC water, was filtered via 0.22 micron filter paper.

Chromatographic conditions: Acquity UPLC BEH chemistry Shield RP-18 column, 50x2.1 mm, 1.7 micron, and a flow rate of 0.2 ml/min were used in the UPLC analysis, which was carried out using a reverse phase UPLC system with isocratic elution mode utilizing a mobile phase of acetonitrile and 0.1% perchloric acid.

Diluent: Acetonitrile.

Preparation of the standard stock solution: The following was made in a 10 ml volumetric flask: 50 mg of Probenecid and 50 mg of Sulopenem Etzadroxil as a standard stock solution. A 0.22-micron syringe filter was used for the filtration process. We got Probenecid and Sulopenem Etzadroxil at standard stock solution concentrations of 5000 µg/ml and 5000 µg/ml, respectively. To get a standard solution with concentrations of 500 µg/ml of Probenecid and 500 εg/ml of Sulopenem Etzadroxil, dilute 1 ml of the stock solution with 10 ml.

Preparation of the sample stock solution: A fine powder was obtained by triturating five tablets of Sulopenem Etzadroxil and Probenecid, which were precisely weighed. A 10 ml volumetric flask was used to dissolve 114.2 mg of sample (equivalent to 50 mg of Probenecid and 50 mg of Sulopenem Etzadroxil). After diluting the solution with diluent, it was ultrasonicated for 10 minutes. A syringe filter with a pore size of 0.22 micron was used to filter the tablet sample stock solution even further.

RESULTS AND DISCUSSION

Separating active pharmaceutical components was the primary analytical hurdle in developing a new technique. The chromatographic conditions were fine-tuned to ensure optimal performance.

System suitability: Table 1 displays the results of the USP tailing and plate count as well as the retention time of standard solution in System suitability [28-31].

Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Probenecid		Sulopenem Etzadroxil	
		Mean	Std dev	Mean	Std dev
USP Plate Count	NLT 2000	9483	0.05623	8869	0.00427
USP Tailing	NMT 2.0	1.05	0.00741	1.15	0.00659
USP Resolution	NLT 2.0			11.36	0.01253
Retention time	NLT 2.0	0.742	0.00534	1.721	0.04875

Mean±SD (n=6)

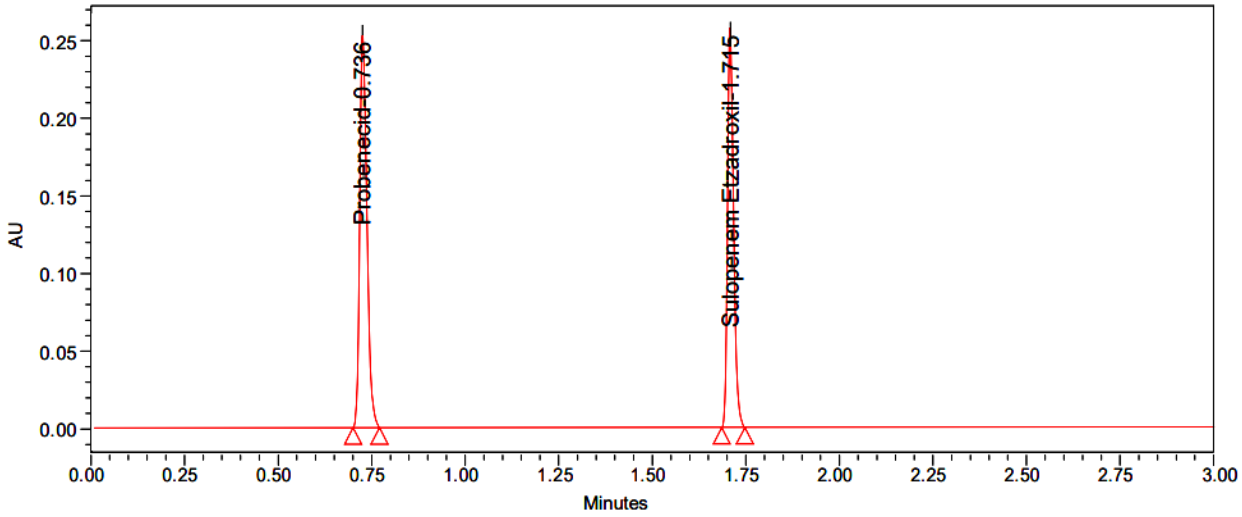


Fig. 2: Chromatogram of system suitability

Specificity: Separate analyses of the sample, standard, and placebo solutions were used to determine the level of interference in this test [32, 33]. 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.

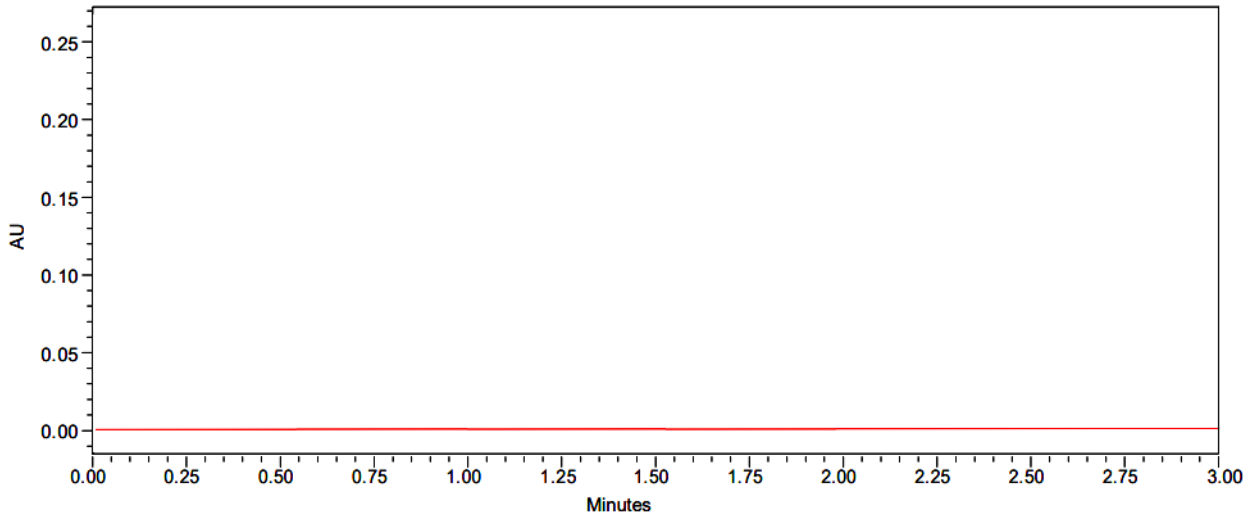


Fig. 3: Chromatogram of blank

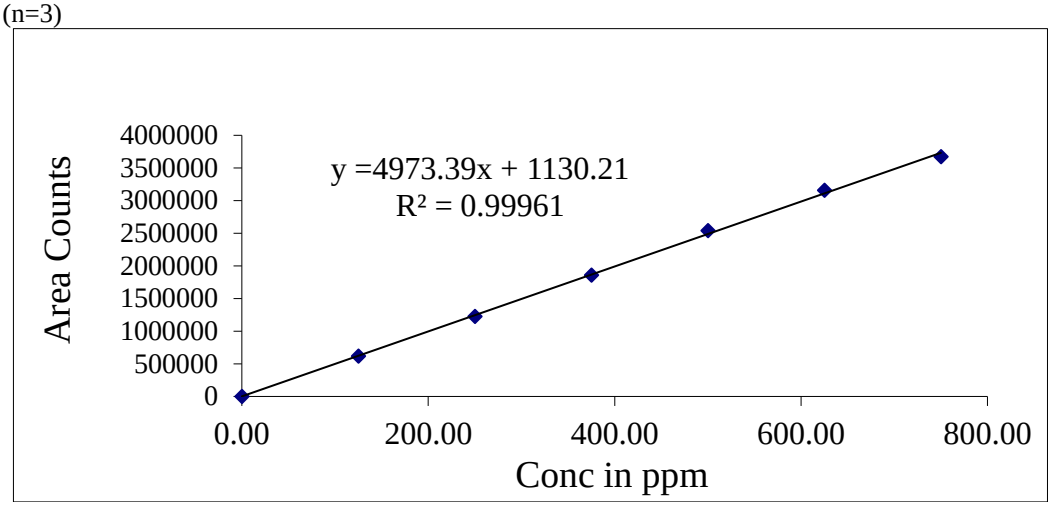
Linearity: Probenecid and Sulopenem Etzadroxil have each had their linearity peak areas assessed at 25, 50, 75, 100, 125, and 150 percent, respectively, throughout a range of concentrations [34–36]. The linearity test was carried out for Probenecid and Sulopenem Etzadroxil within the concentration range of 125-750µg/ml. The correlation values were above 0.999.

Table 2: Linearity of Probenecid and Sulopenem Etzadroxil

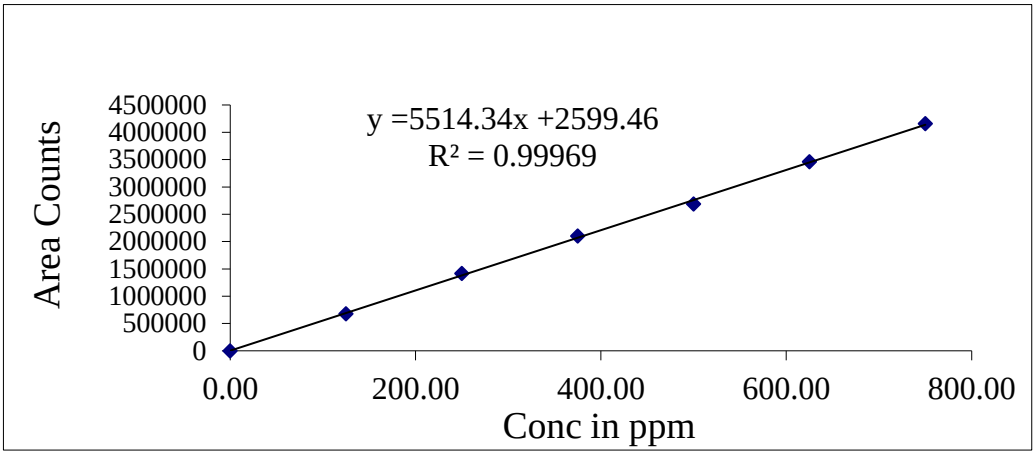
S. No	Conc µg/ml	Probenecid area count	Conc.µg/ml	Sulopenem Etzadroxil area count
1	125	615496	125	676240

2	250	1226387	250	1415769
3	375	1856524	375	2101547
4	500	2537458	500	2686341
5	625	3154816	625	3458246
6	750	3672389	750	4155207
Correl coef		0.99961		0.99969
Slope		4973.39		5514.34
intercept		1130.21		2599.46

Mean±SD (n=3)



A



B

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

Accuracy: The method's accuracy was tested three times by evaluating sample solutions of active pharmaceutical ingredients at 50%, 100%, and 150% of each concentration within the allowed range. The measured percentage recoveries were determined to be within the specified limit. The devised approach was shown to be accurate and reliable. Table 3 displays the outcomes.

Table 3: Results of accuracy

S. No	% Level	Probenecid		Sulopenem Etzadroxil	
		Mean % Recovery	Std dev	Mean % Recovery	Std dev
1	50	100.2	0.7010	99.9	0.7900
2	100	99.7	0.8970	100.2	0.6010
3	150	100.5	0.2480	100.3	0.3920

Mean±SD (n=3)

Precision: For the technique precision research, six separate samples were prepared and injected into the UPLC system with concentrations of 500 ppm of Probenecid and 500 ppm of Sulopenem Etzadroxil.

Intraday precision: On the same day, six separate solutions containing Probenecid and Sulopenem Etzadroxil were tested. Each solution had a concentration of 500µg/ml. In order to determine the mean, standard deviation, and percentage of reliability, peak regions were computed. Listed in table 4 below are these findings [37, 38].

Inter-day precision: Alternately referred to as Intermediate clarity. On separate days, six separate sample solutions containing Probenecid (500µg/ml) and Sulopenem Etzadroxil (500µg/ml) were examined. The mean, standard deviation, and percentage relative standard error were determined by calculating the peak regions [39]. Results showed that the current procedure was accurate, with RSD values below 2% and percentage assay values around 100%.

Table 4: Precision results of Probenecid and Sulopenem Etzadroxil

S No	% Assay			
	Probenecid		Sulopenem Etzadroxil	
	MP	IP	MP	IP
1	99.8	99.9	99.3	99.4
2	99.4	100.1	99.2	100.5
3	99.9	99.0	100.6	98.6
4	99	99.4	99.8	99.7
5	100.6	100.4	100.5	100.5
6	100.5	99.4	99.9	99.7
Mean (n=6) ± SD	99.9± 0.619	99.7± 0.522	99.9± 0.585	99.7± 0.917
%RSD (n=6)	0.62	0.52	0.59	0.72

Mean±SD (n=6)

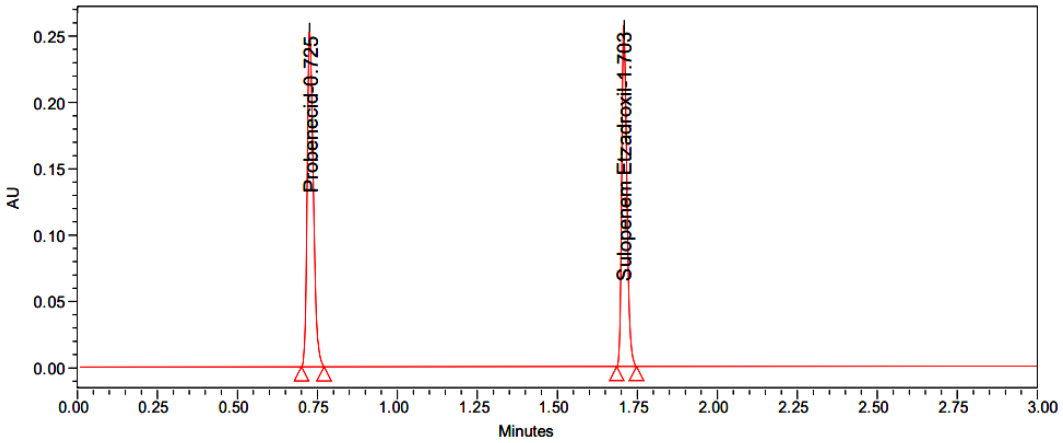


Fig. 5: Chromatogram of method precision

LOD and LOQ: The limits of detection (LOD) for Probenecid and Sulopenem Etzadroxil are 0.5 µg/ml and 3 respectively, with respect to the signal-to-noise ratio (s/n). With a s/n value of 10, the LOQ concentration for Probenecid and Sulopenem Etzadroxil are 2.0 µg/ml. Following the ICH criteria, the approach has been verified [40, 41].

Table 5: LOD and LOQ for Probenecid and Sulopenem Etzadroxil

Probenecid				Sulopenem Etzadroxil			
LOD		LOQ		LOD		LOQ	
Concentration	s/n	Concentration	s/n	concentration	s/n	Concentration	s/n
0.5 µg/ml	3	2.0 µg/ml	10	0.5 µg/ml	3	2.0 µg/ml	10

Robustness: Experimental parameters were chosen to assess the stability of a previously formed system under deliberately changed circumstances, including changes to the organic percentage of the mobile phase and the flow rate [42–44]. Results were shown in Table 7, which show that the robustness of Probenecid and Sulopenem Etzadroxil was determined to be within the limit.

Table 6: Robustness data of Probenecid and Sulopenem Etzadroxil

Parameter name	Probenecid % Assay		Sulopenem Etzadroxil % Assay	
	Mean	Std dev	Mean	Std dev
Flow minus (0.18 ml/min	99.9	0.702	99.3	0.513
Flow plus (0.22 ml/min)	99.5	0.85	99.5	0.404
Organic minus (27:73)	99.8	0.656	99.6	0.426

Organic plus (33:67)	99.4	0.351	99.7	0.547

RSD- Relative standard deviation; All the values are presented as Mean±SD (n=3)

Degradation studies: The medicine Sulopenem Etzadroxil and Probenecid was partially degraded by subjecting the sample to different forced degradation settings. Research on forced deterioration [45, 46] has shown that it works well for degradation products [47, 48]. In addition, the investigations outline the drug's instability under certain circumstances, which allows for the implementation of safeguards during formulation to prevent such instability.

Acid degradation: Degradation of Probenecid was 11.1% and that of Sulopenem Etzadroxil was 12.5% when acidic conditions were used at 1N HCl.

Alkali degradation: At 1N NaOH, alkali degradation was performed on Probenecid (9.3% degradation) and Sulopenem Etzadroxil (10.9% degradation).

Peroxide degradation: Degradation of 13.3% for Probenecid and 16.5% for Sulopenem Etzadroxil was seen when peroxide degradation was performed at a concentration of 10% hydrogen peroxide.

Reduction degradation: Degradation of Probenecid and Sulopenem Etzadroxil was 3.9% and 3.5%, respectively, in the reduction degradation process.

Thermal degradation: In thermal degradation the standard was degraded to 3.4% of Probenecid and 0.2% of Sulopenem Etzadroxil.

Photolytic degradation: In photo degradation the standard was degraded to 1.6% of Probenecid and 1.5% of Sulopenem Etzadroxil.

Hydrolysis degradation: In hydrolysis degradation the standard was degraded to 2.8% of Probenecid and 1.8% of Sulopenem Etzadroxil.

All degradation results are tabulated in table 9.

Table 9: Forced degradation results of Probenecid and Sulopenem Etzadroxil

Degradation condition	Probenecid % Deg		Sulopenem Etzadroxil % deg	
	Mean	Std dev	Mean	Std dev
Control degradation	0	0	0	0
Acid degradation	11.1	0.00634	12.5	0.04395
Alkali degradation	9.3	0.00527	10.9	0.00317
Peroxide degradation	13.3	0.06329	16.5	0.00263
Reduction degradation	3.9	0.04186	3.5	0.00854
Hydrolysis degradation	2.8	0.00594	1.8	0.00457
Thermal degradation	3.4	0.00307	0.2	0.02985
Photolytic degradation	1.6	0.02845	1.5	0.07849

Data expressed as Mean±SD (n=3)

CONCLUSION

In this paper, probenecid and sulopenem etzadroxil are quantitatively determined using isocratic RP-UPLC technology, which is simple, selective, verified, and well-defined. The proposed method was found to be fast, simple, feasible, and affordable in the assay condition, as all the degradation products formed during the stress conditions and active pharma ingredients were well separated. The peaks were also resolved from each other and had an appropriate retention time. Consequently, the technology that was developed for use in stability testing may be used to everyday manufacturing sample analysis and to ensure that medication samples are of high quality during stability studies.

ACKNOWLEDGEMENT

The authors are grateful to the Acharya Nagarjuna University college for providing them with the resources they needed to perform this study.

CONFLICTS OF INTEREST

None

AUTHORS CONTRIBUTION

Sugandha Kumar has collected the literature, information about the drug and carried out the research samples and prepared the manuscript. Rambabu checked the data and reviewed the article.

REFERENCES

1. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 2022; 399: 629–55. doi:10.1016/S0140-6736(21)02724-0.

2. Theuretzbacher U, Piddock LJ. Non-traditional Antibacterial Therapeutic Options and Challenges. Cell Host & Microbe 2019; 26: 61–72. doi:10.1016/j.chom.2019.06.004
3. Harding C, Mossop H, Homer T, Chadwick T, King W, Carnell S, et al. Alternative to prophylactic antibiotics for the treatment of recurrent urinary tract infections in women: multicentre, open label, randomised, non-inferiority trial. BMJ 2022; 376: e068229. doi:10.1136/bmj-2021-0068229
4. Kwok M, McGeorge S, Mayer Coverdale J, Graves B, Paterson DL, et al. Guideline of guidelines: management of recurrent urinary tract infections in women. BJU Int 2022; 130: 11–22. doi:10.1111/bju.15756
5. Phillips NA, Bachmann G, Haefner H, Martens M, Stockdale C. Topical Treatment of Recurrent Vulvovaginal Candidiasis: An Expert Consensus. Women's Health Rep 2022; 3: 38–42. doi:10.1089/whr.2021.0065
6. Nyirjesy P, Brookhart C, Lazenby G, Schwebke J, Sobel JD. Vulvovaginal Candidiasis: A Review of the Evidence for the 2021 Centers for Disease Control and Prevention of Sexually Transmitted Infections Treatment Guidelines. Clin Infect Dis 2022; 74: 2–8. doi:10.1093/cid/ciab1057
7. Zhang Y, Chen S, Yuan M, et al. Gout and Diet: A Comprehensive Review of Mechanisms and Management. Nutrients 2022; 14: 3525. doi:10.3390/nu14173525
8. McKenzie BJ, Wechalekar MD, Johnston RV, et al. Colchicine for acute gout. Cochrane Database Syst Rev 2021; 2021: CD006190. doi:10.1002/14651858.CD006190.pub3
9. Drewes JL, Chen J, Markham NO, Knippel RJ, Domingue JC, Tam AJ, et al. Human Colon Cancer-Derived Clostridioides difficile Strains Drive Colonic Tumorigenesis in Mice. Cancer Discov 2022; 12: 1873–85. doi:10.1158/2159-8290.CD-21-1273
10. Cymbal M, Chatterjee A, Baggott B, Auron M. Management of Clostridioides difficile infection: Diagnosis, Treatment, and Future Perspectives. Am J Med 2024; 137: 571–6. doi:10.1016/j.amjmed.2024.03.024
11. Abufaraj M, Xu T, Cao C, et al. Prevalence and Trends in Kidney Stone Among Adults in the USA: Analyses of National Health and Nutrition Examination Survey 2007-2018 Data. Eur Urol Focus 2021; 7: 1468–75. doi:10.1016/j.euf.2020.08.011
12. Stamatelou K, Goldfarb DS. Epidemiology of Kidney Stones. Healthcare 2023; 11: 424. doi:10.3390/healthcare11030424
13. Yanai H, Adachi H, Hakoshima M, Katsuyama H. Molecular Biological and Clinical Understanding of the Pathophysiology and Treatments of Hyperuricemia and Its Association with Metabolic Syndrome, Cardiovascular Diseases and Chronic Kidney Disease. Int J Mol Sci 2021; 22 (17): 9221. doi:10.3390/ijms22179221
14. Si K, Wei C, Xu L, Zhou Y, Lv W, Dong B, et al. Hyperuricemia and the Risk of Heart Failure: Pathophysiology and Therapeutic Implications. Front Endocrinol (Lausanne) 2021; 12: 770815. doi:10.3389/fendo.2021.770815
15. Ahmed I, Majeed A, Powell R. Heparin induced thrombocytopenia: diagnosis and management update. Postgrad Med J 2007; 83: 575–82. doi:10.1136/pgmj.2007.059188
16. Estcourt LJ, Malouf R, Hopewell S, Doree C, Van Veen J, et al. (Cochrane Haematological Malignancies Group). Use of platelet transfusions prior to lumbar punctures or epidural anaesthesia for the prevention of complications in people with thrombocytopenia. Cochrane Database Syst Rev 2018; 2018: CD011980. doi:10.1002/14651858.CD011980.pub3
17. Eisa Mahmoud S, Mohamed Shehab F, Ibrahim Firyal, Shariff Khalid, Sadik Nagham, Nashwan Abdulqadir, Yassin Mohamed A. Paroxysmal Nocturnal Hemoglobinuria with Glucose-6-Phosphate Dehydrogenase Deficiency: A Case Report and Review of the Literature. Case Rep Oncol 2019; 12: 838–44. doi:10.1159/000503817
18. Hill Anita, Hill Quentin A. Autoimmune hemolytic anemia. Hematology 2018; 2018: 382–9. doi:10.1182/asheducation-2018.1.382
19. Carapancea Evelina, Cornet Marie-Coralie, Milh Mathieu, De Cosmo Lucrezia, Huang Eric J, et al. Clinical and Neurophysiologic Phenotypes in Neonates With BRAT1 Encephalopathy. Neurology 2023; 100: e1234 – e1247. doi:10.1212/WNL.0000000000206755
20. Subrahmanyam Talari, Anuradha V, Komala sai prathyusha A. New Validated RP-HPLC Method for Cisplatin and Topotecan in API and Vaccine Form and its Stress Studies. Int J Res Pharm Sci 2021; 12: 808-14.
21. Syed Rafi, Kantipudi Rambabu. Stability indicating validated HPLC method for the determination of Aceclofenac and Misoprostol in bulk and Pharmaceutical formulation. Int J Res Pharm Sci 2020; 11: 7848-53.
22. Bhavani P, Prasada Rao K, Mohan S. Novel validated reversed phase high performance liquid chromatography method for determination of glucosamine, diacerein, and methyl sulfonyl methane in micro sample rat plasma and its application to pharmacokinetic and dissolution studies. Asian J Pharm Clin Res 2020; 13: 50-63.
23. Shivani C P, Maheshwari D G. Development and validation of UV spectrometric and HPLC method for estimation of escitalopram oxalate and Sulopenem Etzadroxil dihydrochloride in combined dosage form. AJPTI 2016; 4: 59-70.
24. Naresh Kumar D. S, Patel D. Stability indicating chromatographic method development and validation for the simultaneous estimation of escitalopram oxalate and Sulopenem Etzadroxil in its pharmaceutical dosage form by HPLC. WJPR 2017; 6: 549-66.
25. Supriya T, Naresh D, Vijaya Kumar G, Haneer MA. Stability indicating RP-HPLC method development and validation for simultaneous estimation of escitalopram and Sulopenem Etzadroxil pure and marketed formulation. Asian J Pharm Res 2018; 8: 4-10.

26. Malathi S, Devakumar D. Development and validation of rp-hplc method for the estimation of escitalopram oxalate and Sulopenem Etzadroxil dihydrochloride in combined dosage form and plasma. Int J Pharm Pharm Sci 2021; 13: 61-6.
27. Mukta D. Naykode, Durgacharan A. Bhagwat, Swapnil D. Jadhav, Harinath N, et al. Analytical and bio analytical method for quantification of pure azilsartan, not its salt by RP-HPLC. Research J. Pharm. and Tech 2017; 10:708-14.
28. Mayanka Singh, Manoj Charde, Rajesh Shukla, Rita M.C. Determination of calcipotriene in calcipotriene cream 0.05% w/w by RP-HPLC method development and validation. Research J Pharm and Tech 2011; 4: 1219-23.
29. Asha Eluru, Surendra Babu K. A Study of Method Development, Validation and Forced Degradation for Simultaneous Quantification of Povidone Iodine and Ornidazole in Bulk and Pharmaceutical Dosage Form by Using RP-HPLC. IJPSR 2021; 12: 1217-22.
30. Senthil Rajan D, Muruganathan G, Shivkumar K, Ganesh T. Development and validation of hplc method for simultaneous quantification of vasicine, glycyrrhizin and piperine in poly herbal cough syrup. Int J Curr Pharm Res 2020; 12: 15-9.
31. Malak Y, Al-Bathish A. A, Gazy M. K. El-Jamal. Rp-hplc and chemometric methods for the determination of two anti-diabetic mixtures;metformin hydrochloride-canagliflozin and metformin hydrochloride-gliclazide in their pharmaceutical formulation. Int J Pharm Pharm Sci 2020; 12: 83-94.
32. Gadhvi M.P, Bhandari A, Suhagia B.N, Desai U.H. Development and validation of RP-HPLC method for simultaneous estimation of atazanavir and ritonavir in their combined tablet dosage form. Research J. Pharm. and Tech 2013; 6: 200-3.
33. Swati K, Abhishek P, Sushank S, Bothiraja C, Atmaram P. High-performance liquid chromatography for the simultaneous estimation of cefoperazone and sulbactam in rat plasma and its importance in therapeutic drug monitoring. Int J Pharm Pharm Sci 2020; 12: 92-7.
34. Gomathy S, Narenderan S.T, Meyyanathan S.N, Gowramma B. Development and validation of hplc method for the simultaneous estimation of apigenin and luteolin in commercial formulation. J Crit Rev 2020; 7: 4785-90.
35. Vijayakumari M, Balasekhar reddy Ch. Stability indicating validated hplc method for the determination of zanubrutinib in bulk and pharmaceutical dosage form. Asian J Pharm Clin Res 13: 2020; 159-62.
36. Abdul Raziq, Syed Umer Jan et al., Relative comparison of stability and degradation of methylcobalamine tablets of different brands at different storage settings. Int J App Pharm 2021; 13:171-5.
37. Rajakumari R, Sreenivasa Rao S et.al., Stress degradation studies and development of a validated RP-HPLC method for determination of Tiagabine in presence of its degradation products. Int J Pharm Pharm Sci 2016; 8: 230-6.
38. Charu Pandya P, Sadhana Rajput J. Development and validation of stability indicating method RP-HPLC method of Acotiamide. Int J Pharm Pharm Sci 2018; 10: 1-8.
39. Birva Athavia A, Zarna Dedania R et.al., Stability indicating HPLC method for determination of Vilazodone hydrochloride. Int J Curr Pharm Res 2017; 9: 123-9.
40. Tarekegn Tadesse Unade, Krishnamanjari Pawar A. A new stability indicating UPLC method for the determination of two anti diabetic drugs in combination: Applications to bulk and tablet formulation. Int J App Pharm 2022; 14: 192-9.
41. Manoranjani M. A study of method development, validation and forced degradation for simultaneous quantification of Cisplatin and Fluorouracil in bulk and pharmaceutical dosage form by RP-HPLC. J Pharm Sci & Res. 2021; 13 : 155-61.
42. Prasada Rao P T S R K. A study of method development and validation of Pemetrexed and Cisplatin using RP-HPLC. J Pharm Sci & Res. 2021; 13: 143-8.
43. Satya Dev T N V S S. A new selective method development and validation of Cabozantinib and Nivolumab using HPLC. J Pharm Sci & Res. 2021; 13: 188-92.
44. Manoranjani M. Assay method development and validation of Cilnidipine and Ramipril, Characterization of its degradants by using LC-MS/MS. Int J App Pharm. 2022; 14 : 276-85.
45. Prasada Rao P T S R K. HPLC method development and validation of Lercanidipine HCl and Atenolol, Characterization of its degradants by using LC-MS/MS. Int J App Pharm. 2022; 14: 125-34.
46. Satya Dev T N V S S, Chintalapudi Ramakrishna. A new related substances method development and validation of two anti-cancer drugs by using effective liquid chromatographic method. Int J App Pharm. 2022; 14: 116-24.
47. David Raju M. Simultaneous method development and validation of Choline salicylate and tannic acid using RP-HPLC in bulk and pharmaceutical dosage form. Int J App Pharm. 2022; 14 : 227-35.
48. David Raju M. Development and validation of HPLC method for the determination of Lasmiditan drug in bulk and tablet dosage form. J Pharm Sci & Res. 2021; 13: 170-3.