Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 26s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Development And Validation of Stability Indicating Rp- Hplc Method for Simultaneuos Estimation of Cephalexin and Clavulanic Acid

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Cite this paper as: Khushbu Maru, Darshit Ram, Sheetal Buddhadev, (2025) Development And Validation of Stability Indicating Rp- Hplc Method for Simultaneuos Estimation of Cephalexin and Clavulanic Acid, *Journal of Neonatal Surgery*, 14 (26s), 673-681

ABSTRACT

The combined dosage form of cephalexin and clavulanic acid was tested using a stability- indicating RP-HPLC technique. The combined dosage form of cephalexin and clavulanic acid was tested using a stability-indicating rp-hplc technique.The measurement wavelength was found to be 228 nm. Linearity, accuracy, precision, robustness, LOD, and LOQ have all been verified for the approach. The concentration ranges of 62.5–187.5 µg/ml for clavulanic acid and 37.5–112.5 µg/ml for cephalexin were found to be linear. Using sodium dihydrogen phosphate monohydrate buffer (pH:5.0 with OPA): methanol in a 65:35% v/v ratio as the mobile phase, the rp-hplc approach showed better separation and asymmetry on an Intersil OCD C18 (250 x 4 mm, 4 µm) packed column at a flow rate of 1 ml/min. It was discovered that the retention times for cephalexin and clavulanic acid were 4.216 and 2.189 minutes, respectively. Drug products were subjected to oxidation, photolytic, thermal, acid, and base degradation during a stability study using forced degradation. Under the specified stress conditions, cephalexin and clavulanic acid were shown to degrade by 7–10%. The devised method, which can be employed for simultaneous estimation of cephalexin and clavulanic acid in their tablet dose form, was straightforward, precise, and specific

Keywords: *Cephalexin, clavulanic acid, RP-HPLC, method development, stability study, force degradation and validation.*

1. INTRODUCTION

C16H17N3O4S cephalexin (6R,7R) -7-[(2R)-2-amino-2-phenylacetamido] 3.-methyl -8-oxo- 5-thia-lazabicyclo [4.2.0]: Oct-2-ene-2-carboxylic acid is an antibiotic that is beta-lactam and a member of the first-generation cephalosporin class[1-6]. Its structure includes a beta-lactam ring (Fig 1). It treats bacterial infections in a variety of body parts. It is a member of the group of drugs called cephalosporin antibiotics. It's a solid with crystals[7-10]. It dissolves slowly in room temperature water, although it dissolves well in methanol, acetone, and ethyl acetate.[11-13]

Clavulanic acid C8H9NO5(2R,3Z,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxalazabicyclo[3.2.0]heptane-2-carboxylic acid is contain a beta-lactam ring in its structure(Fig 1) that binds in an irreversible fashion to beta-lactamases, preventing them from inactivating certain beta-lactam antibiotics, with efficacy in treating susceptible gram-positive and gram-negative infections.it is freely soluble slightly soluble in ethanol and acetone. A review of the literature also reveals that the spectrophotometric approach is used for combination dose forms. Nevertheless, no technique for the simultaneous RP-HPLC quantification of these two medications has been published [14-17].

Stress testing is necessary to clarify the inherent stability properties of the active ingredient, according to the International Conference on Harmonisation (ICH) guideline[18-19] titled "Stability testing of new drug substances and products." Effectively resolving the drug and its breakdown products is the optimal stability-indicating technique.

Therefore, it is relatively difficult for pharmaceutical analysts to apply an analytical methodology to evaluate cephalexin and clavulanic acid concurrently, in the presence of its degradation products. Thus, it was believed that research into the stability of cephalexin and clavulanic acid in acidic, basic, oxidative, UV, and photolytic environments was essential.

This study presents a validated stability-indicating HPLC method for estimating cephalexin and clavulanic acid simultaneously in the presence of their breakdown products. For regular measurement of cephalexin and clavulanic acid in combined dosage form, the suggested approach is straightforward, precise, repeatable, stability-indicating, and appropriate. The procedure was verified to be in accordance with ICH recommendations [20].

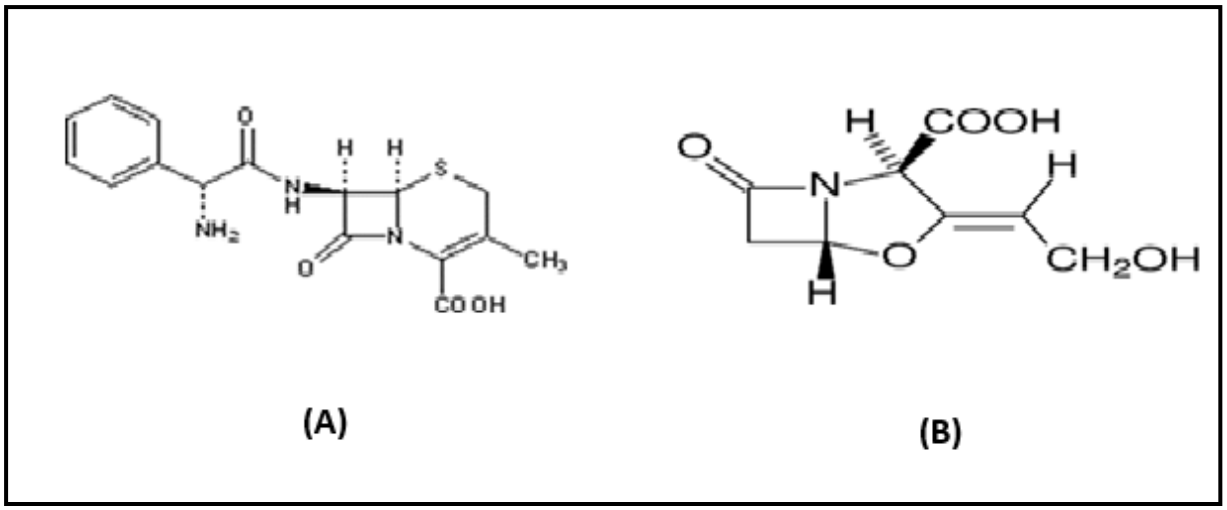


Fig 1. (A): Structure of Cephalexin and (B) Structure of Clavulanic acid

2. MATERIALS AND METHODS

SPIL Pvt. Ltd. provided pharmaceutical-grade CEPH and CLV as gift samples, respectively. The commercially available tablet formulation, Sporidex CV 750 ER Sun Pharmaceutical Industries Limited, India, had 750 mg of CEPH and 125 mg of CLV as indicated on the label. We bought HPLC-grade acetonitrile from Finar Chemicals Ltd. in Ahmedabad, India. Finar Chemicals Ltd. supplied the HPLC-grade methanol (Ahmedabad, India). Merck Ltd. (Mumbai, India) supplied the GR grade potassium dihydrogen orthophosphate. Merck Ltd. Supplied the sodium hydroxide (AR grade) (Mumbai, India). We bought GR-grade hydrogen peroxide from Merck Ltd. in Mumbai, India. Merck Ltd., Mumbai, India, supplied the AR grade hydrochloric acid that was utilized.

The Shimadzu LC-2010C HT HPLC system had an Inertsil OD C18 (250 x 4 mm, 4 µm) column. (CP 124S, Sartorius, Germany) Analytical Balance Toshniwal Process Instrument Pvt. Ltd., Ajmer, India, offers an ultrasonic bath (Fast clean). The digital pH meter manufactured by Electroequip Pvt. Ltd. in Delhi, India In this work, a hot air oven (Labline, Maharashtra, India) was utilized.

MOBILE PHASE PREPARATION:

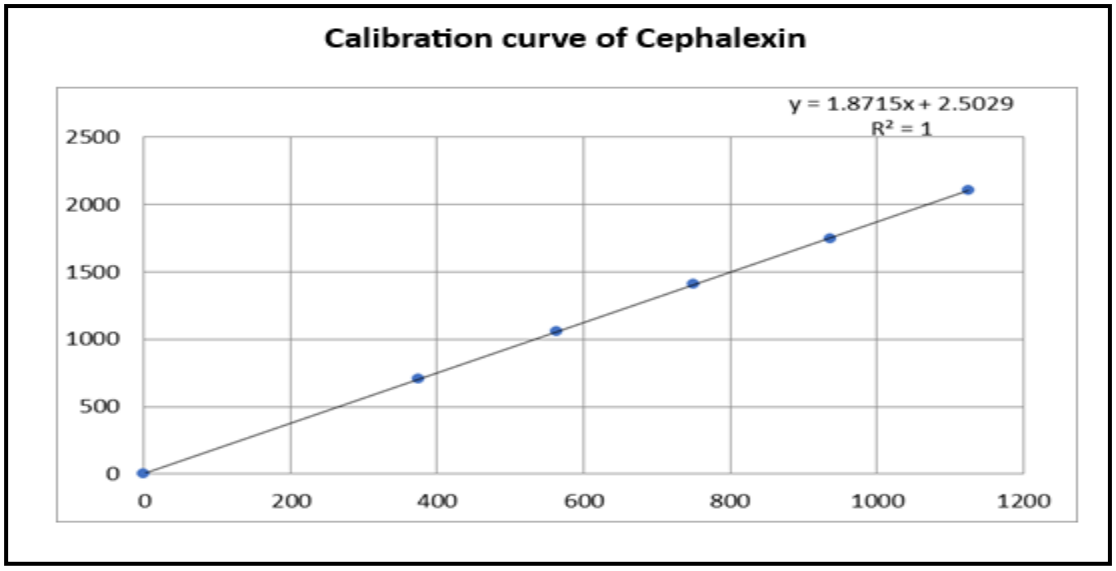
Using a degassed mixture of (65:35 v/v) pH 5.0 buffer and methanol as the mobile phase, the pH was adjusted to 5.0 ± 0.05 using a diluted ortho-phosphoric acid solution after 7.8 g of sodium dihydrogen phosphate monohydrate was dissolved and diluted to 1000 ml with water.

Preparation of CEPH stock solutions:

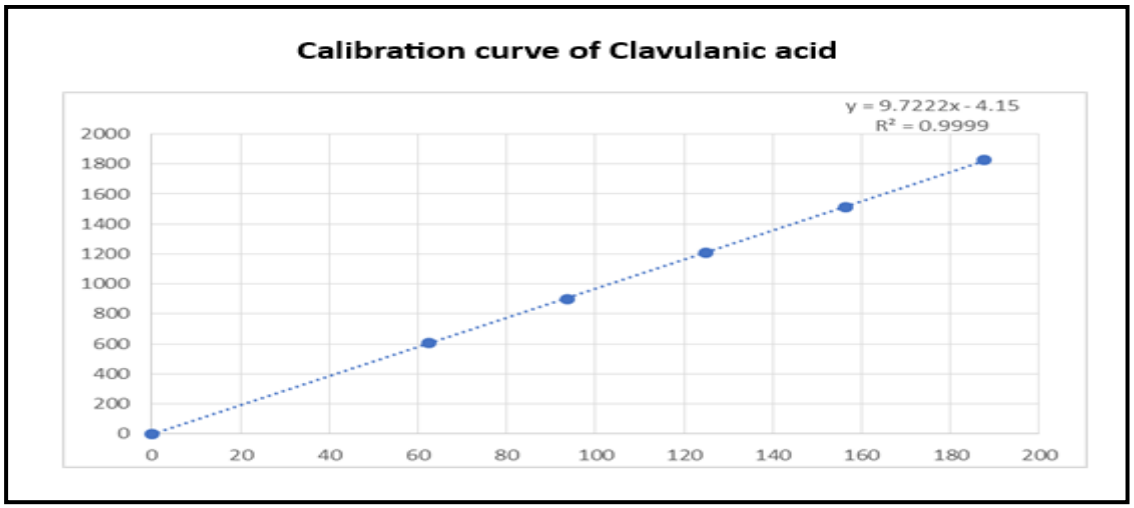
After bringing the solution down to room temperature, methanol was added to bring it up to volume.

Getting the clavulanic acid stock Solution:

Approximately 1.25 mg of precisely weighed CLV pure powder was added to a 5 mL volumetric flask. To dissolve it, 4 mL of methanol was added and sonicated. After the solution had cooled to room temperature, methanol was added to bring it up to volume.



(A)



(B)

Fig 2. (A): Calibration curve for Cephalexin and (B): Calibration curve for Clavulanic acid

Analysis of marketed formulation:

Twenty tablets were finely ground after being precisely weighed. A 50 mL volumetric flask was filled with a weighed amount of powder equal to 750 mg CEPH and 125 mg CLV, which was then dissolved in 40 mL of methanol. After 30 minutes of sonication, methanol was added to bring the final volume up to par. Whatman filter paper number 41 was used to filter the mixture. The final solution with CEPH (750 µg/mL) and CLV (125 µg/mL) was then obtained by diluting 0.5 mL of the solution with 10 mL of mobile phase.

Table 1: Linear data for calibration curve

Parameters (units) (µg/ml)	**Cephalexin**	**Clavulanic acid**
Linearity range	37.5 – 112.5	62.5 – 187.5
R2	1	0.9999
Slope	1.8715	9.7222

Validation of the Method:

The analysis's accuracy, linearity, LOD, LOQ, precision, and robustness were all checked against the ICH guidelines.

Method precision:

The research of CEPH and CLV's intraday and interday precision involved estimating the corresponding responses three times on the same day and three separate days for concentrations of 750 µg/ml and 125 µg/ml, respectively.

Limits of Quantitation and Detection:

The limits of quantitation (LOQ) and detection (LOD) were calculated using the following formulas: LOQ is equal to 10(SD)/S, and LOD is equal to 3.3(SD)/S, where SD is the response standard deviation and s is the average slope of the calibration curve. CEPH and CLV were found to have LODs of 0.118 µg/ml and 0.616 µg/ml, respectively. The LOQs for CEPH and CLV were found to be 2.752 µg/ml and 0.525 µg/ml, respectively.

Tests for system suitability:

A system suitability calculation was completed before each validation parameter was examined. Throughout the entire inquiry, the values of the system appropriateness findings were documented; the outcomes are displayed in table 2.

Table 2: Validation and SST parameters results

Parameters (units)	Cephalexin	Clavulanic acid
Linearity range µg/ml	37.5-112.5	62.5– 187.5
Correlation coefficient	1	0.9999
LOD µg/ml	0.616	0.118
LOQ µg/ml	2.752	0.525
Interday Precision (%RSD)	0.39%	0.52%
Intraday Precision (%RSD)	0.45%	0.20%
Robustness	Robust	Robust
Theoretical plates	7894	8934
Retention time	4.216	2.189
Tailing factor	1.85	1.45

Robustness:

A change in each of the following parameters was made, and the impact on the system's appropriateness was noted. The mobile phase flow rate changed to 0.9 and 1.1 millilitres per minute (± 10%). Mobile phase ratio change (about 2% absolute) as buffer: methanol (67:33), buffer: methanol (53:47), Temperature changes in column ovens from ± 5°C absolute to 20°C and 30°C.

Stress study by Forced degradation:

The CEPH and CLV underwent forced degradation experiments in the following conditions: acidic, basic, oxidative, photolytic, and thermal.

Acid decomposition studies were performed by transferring required ml of stock solution (1.5 mg/mL & 250 µg/mL of CEPH and CLV respectively) to 5 ml of volumetric flask. Two ml of

0.1 N HCl solutions was added and mixed well and kept for 5 hrs at Room temperature. After time period 2 ml of 0.1 N NaOH was added to neutralize the solution and volume was fill up with M.P to get 750 µg/mL & 125 µg/mL respectively for CEPH and CLV. The final solution along with acid degradation blank solution were analyzed under the proposed chromatographic conditions and chromatograms were recorded.

Base decomposition studies were performed by transferring required ml of stock solution (1.5 mg/mL & 250 µg/mL of CEPH and CLV respectively) to 5 ml of vol. flask. 2 ml of 0.1 N NaOH solutions was add and mixed well and kept it for 5 hrs at Room temperature. After time period two ml of 0.1 N HCl was added to neutralize the solution and volume was made up

with the mobile phase to get 750 µg/mL & 125 µg/mL respectively for CEPH and CLV.

To conduct oxidative decomposition, 5 ml of volumetric flask was filled with the necessary amount of stock solution (1.5 mg/mL and 250 µg/mL of CEPH and CLV, respectively). After thoroughly mixing in two millilitres of 3% H2O2 solutions, the mixture was allowed to sit at room temperature for five hours. Following the time interval, the volume was adjusted for the mobile phase to obtain 750 µg/mL for CEPH and 125 µg/mL for CLV.

Photo decomposition studies were performed by transferring required ml of stock solution (1.5 mg/mL & 250 µg/mL of CEPH and CLV respectively) to 5 ml of volumetric flask (having transparent glass). Transparent glass Volumetric flask was kept in UV Chamber for 24 hrs. After time period make up the volume with mobile phase to get 750µg/mL & 125µg/mL respectively for CEPH and CLV.

A 5 mL volumetric flask containing 7.5 mg and 1.25 mg of the Thermal Degradation Standard (CEPH and CLV, respectively) was placed in an oven set to 80°C for 24 hours. Once the flask was taken out and allowed to cool to room temperature, 5 mL of methanol was added, and 1 mL of this solution was made up to 2 mL with mobile phase to obtain 750 µg/mL & 125 µg/mL for CEPH and CLV, respectively.

3. RESULT AND DISCUSSION

The sodium dihydrogen phosphate monohydrate buffer (pH 5.0 with OPA) that makes up the mobile phase: Methanol was optimised at a ratio of 65:35 (%v/v) at a flow rate of 1 ml/min, which produced Response and RT optimized with better separation and asymmetry (Fig 3[A & B]). and wavelength obtained at 228 nm (CEPH and CLV). The peak of CEPH and CLV was found well separated at 4.216 min, 2.189 min. It was discovered that the calibration curves(Fig 2) for CEPH and CLV were linear over the ranges of 37.5–112.5 µg/ml and 62.5–187.5 µg/ml, respectively. In Table 1, the calibration curves are displayed. The suggested approach was successfully used to calculate the dosage forms of CEPH and CLV in tablets. The combination produced the following outcomes shown in (Fig 3 A & B).

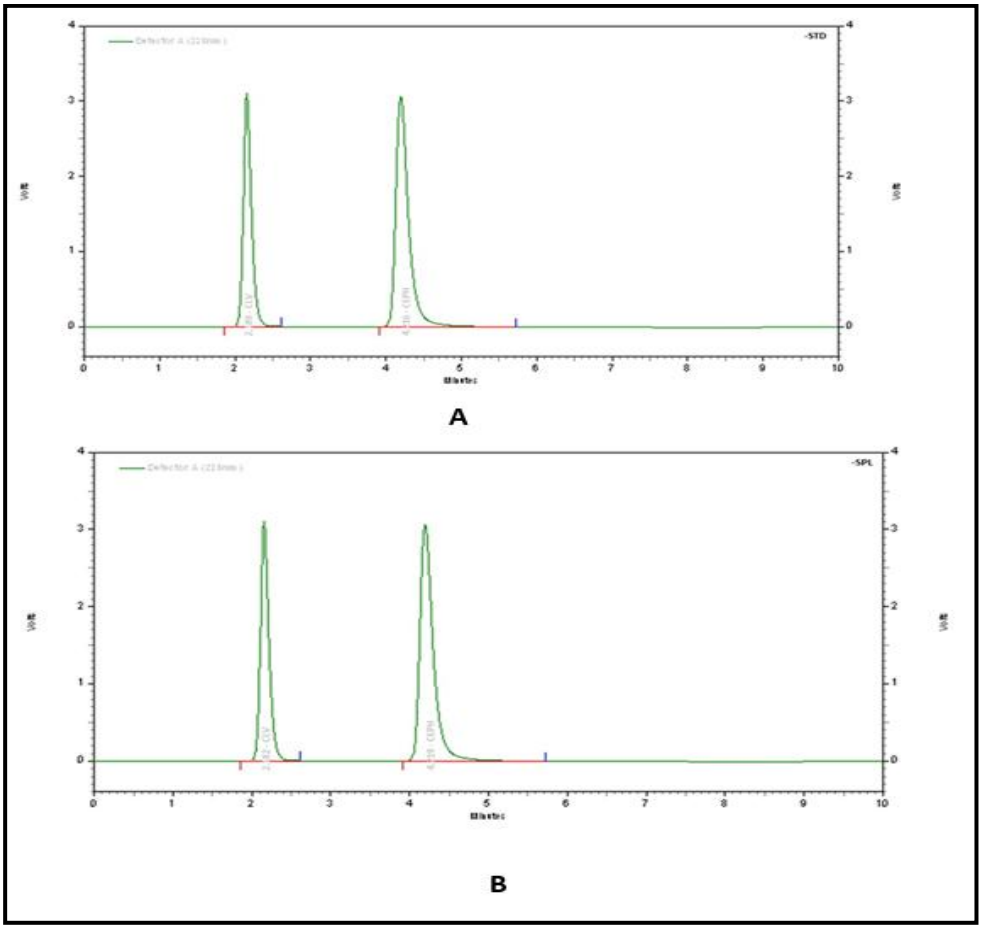


Fig 3. (A) : Chromatograms of CEPH and CLV (B): Chromatogram of mixture cephalexin and clavulanic acid (B) and chromatogram of marketed formulation of CEPH and CLV(B).

The results showed that the LOD for CEPH and CLV was 0.118 µg/ml and 0.616 µg/ml, respectively. whereas CEPH and CLV have LOQs of 2.752 µg/ml and 0.525 µg/ml, respectively. (Table 2) provides a summary of the validation and system suitability test parameter results. (Table 2) [Fig 9] displays the results of the medications' robustness evaluation.

According to the degradation study, the medication breaks down, as seen by the peak's diminished regions. By comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of both medications under non-degradation conditions, the percentage of degradation was determined.

For force deterioration, use 3% v/v H_2O_2, 0.1 N HCl, and 0.1 N NaOH for five hours. 24 hrs in uv chamber, and 24 hrs in oven at 80°C for thermal degradation were completed. Under the specified conditions, the percentage of degradation for CEPH and CLV in their tablet dosage form was determined to be between 10% and 20% utilizing the improved HPLC technique (Fig 4-8). Additionally, (Table 3) contains CEPH and CLV deterioration statistics.

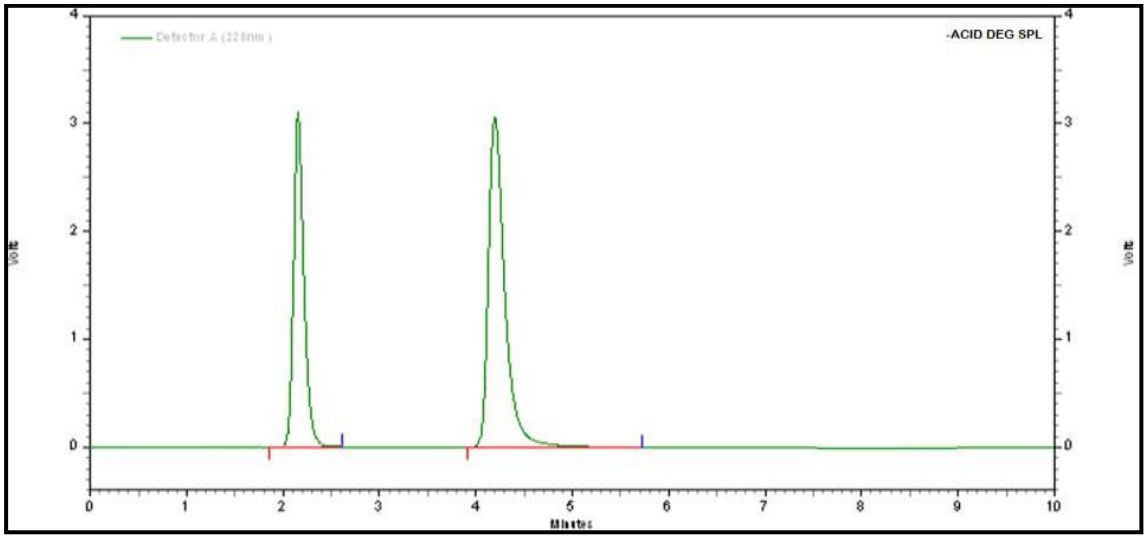


Fig 4. Chromatogram of 0.1 N HCl for 5 hr for acid degradation of sample CEPH and CLV

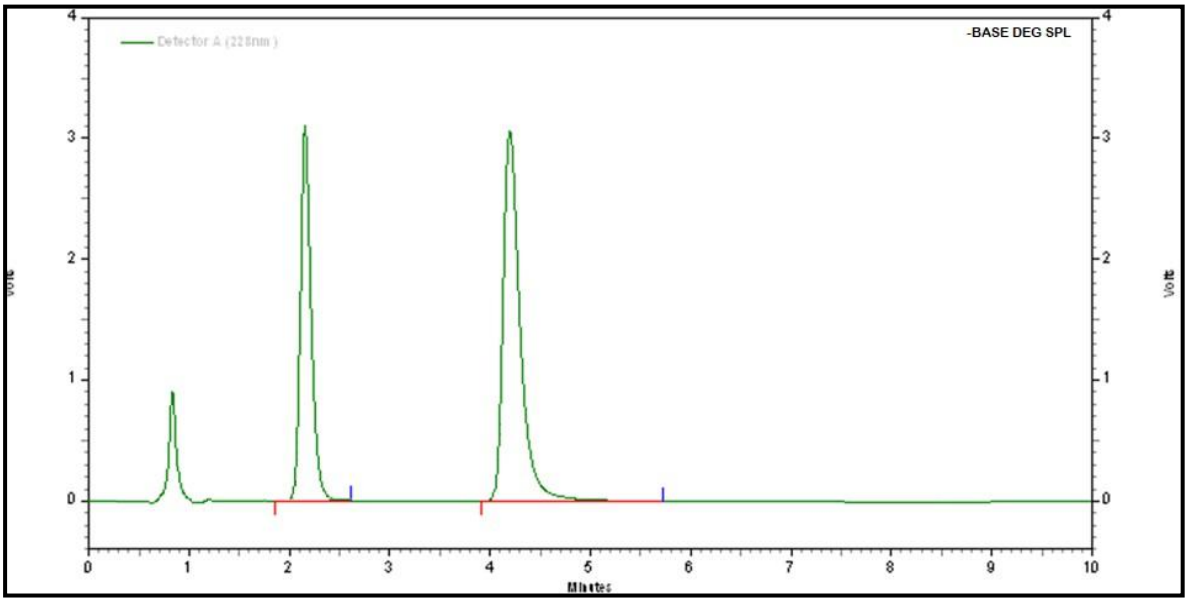


Fig 5. Chromatogram of 0.1 N NaOH for 5 hr for base degradation of sample CEPH and CLV

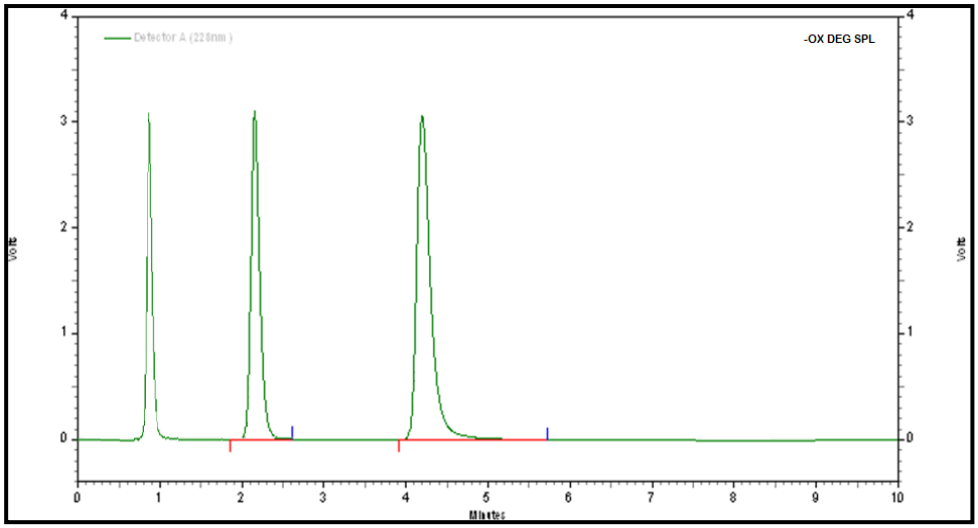


Fig 6. Chromatogram of 3% H2O2 for 5 hr for oxidation degradation of sample CEPH and CLV

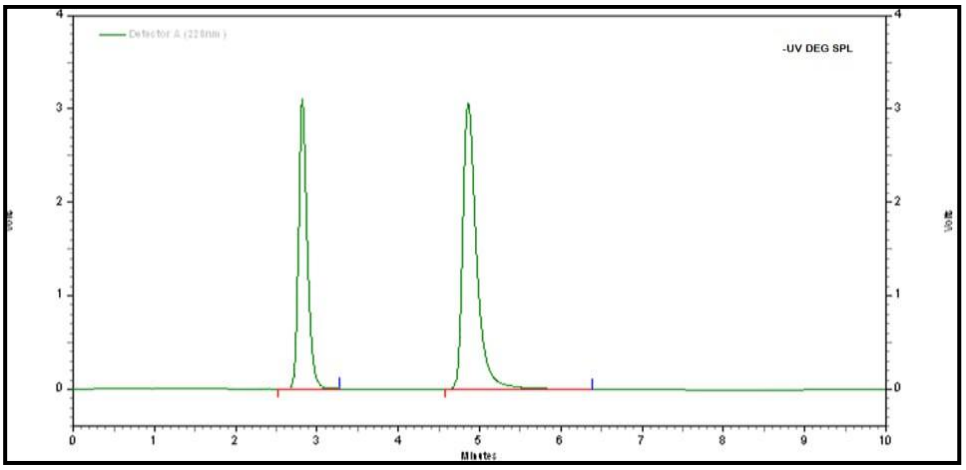


Fig 7. Chromatogram of UV Chamber for 24 hr for photo degradation of sample CEPH and CLV.

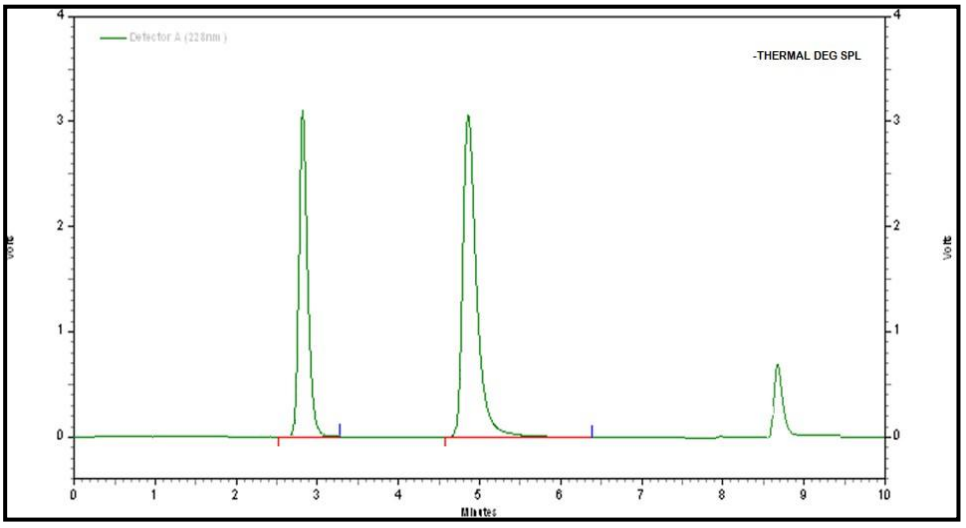


Fig 8. Chromatogram of 80°C for 24 hr in oven for thermal degradation of sample CEPH and CLV

TABLE 3: DEGRADATION STUDIES FOR CEPH AND CLV

Degradation condition	Time(hr)	% Degradation	
		CEPH	CLV
Acid (0.1 N HCl)	5 hr	7.26	6.28
Base (0.1 N NaOH)	5 hr	4.04	5.70
Oxidation (3% H2O2)	5 hr	4.89	5.48
Photolytic (Uv chamber)	24 hr	3.96	1.74
Thermal (80°C in oven)	24 hr	7.88	5.69

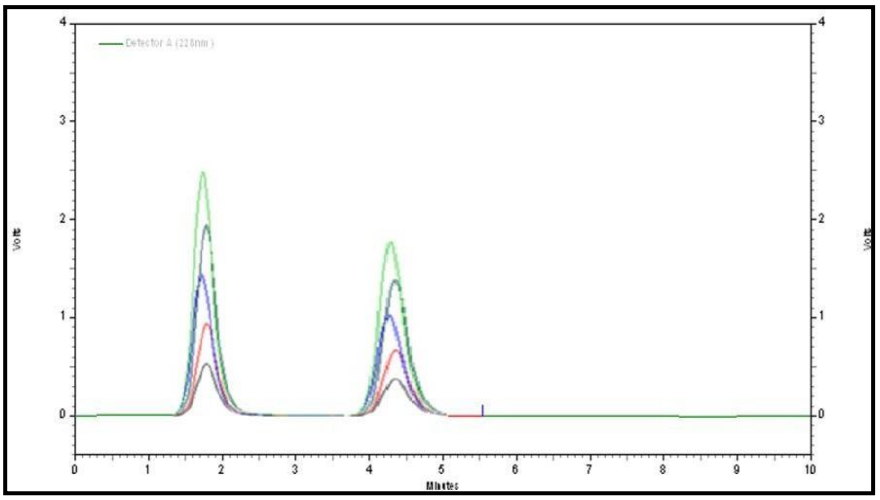


Fig 9. Overlay Uv spectra of Cephalexin and Clavulanic acid

4. CONCLUSION

Using ICH guidelines, a stability-indicating RP-HPLC technique was created and validated for the simultaneous quantification of CEPH and CLV medication. The accuracy, precision, robustness, linearity, and repeatability of the procedure have been demonstrated. It was discovered that the devised method was easy to use, sensitive, and selective for analysing CEPH and CLV together without any excipient interference. In particular, the approach uses all of the degradants produced throughout the forced degradation study to assess both medicines. CEPH and CLV assay results for the combined dose form utilising the suggested approach were 99.72 ± 0.12% and 99.73 ± 0.35%, respectively. The result shows that the method is appropriate for studying the stability of CEPH and CLV under a variety of forced degradation circumstances, including thermal, oxidative, photolytic, base, and acid degradation.

ACKNOWLEDGMENTS

The authors thankful to Faculty of Pharmacy, Noble University, Junagadh for providing the facilities to carry out the research work.

CONFLICTS OF INTEREST: No conflict of interest was declared by the authors

REFERENCES

[1] Mohan H., Textbook of Pathology; 6th Edn; Jaypee Brothers Medical Publisher(P) LTD, New Delhi, 2010, pp 646.

[2] Tripathi K.D., Essential of medical pharmacology; 7th Edn; Jaypee Brothers medical publisher(P) LTD, New Delhi, 2013,pp 579.

[3] Narsu kumari k., rajiya sd, bhulakshmi a. (2019). Analytical method development and validation of cephalexin by rp-hplc method. Journal of emerging technologies and innovative research, 6(12), 696–699.

[4] Vyas a, shukla s. S, patel r, pandey r, jain v, singh d, nagori b. P. Development and validation of spectrophotometric method for estimation of cephalexin in bulk and tablet dosage forms. Orient j chem 2011;27(1)

[5] Jeswani, r. M., sinha, p. K., topagi, k. S., & damle, m. C. (2009). A validated stability indicating hptlc method for determination of cephalexin in bulk and pharmaceutical formulation. International journal of pharmtech research, 1(3), 527-536.

[6] Sanjeevarani, a., rajesh, t., kumar, g. V., & haneef, m. A. Analytical method development and validation for simultaneous estimation of cephalexin and bromhexine hcl in pure and pharmaceutical dosage form by rp-hplc.

[7] Rao, a. L., prasanthi, t., & spandana, u. S. (2017). Stability indicating rp-hplc method development and validation for the analysis of cephalexin and bromhexine in pharmaceutical dosage form. International journal of research in ayush and pharmaceutical sciences, 137-147.

[8] Prasad, g. V., sravani, s., ishaq, b. M., madhu, m., munna, s., & gopinath, c. (2013). Development and validation of uv-spectrophotometric method for determination of cephalexin. Asian journal of research in chemistry, 6(5), 490-494.

[9] Hussein, R. F., & Hammami, M. M. (2014). Determination of cephalexin level and stability in human plasma by fully validated rapid HPLC analysis. WJPPS, 3(12), 20- 31.

[10] Gawande, V. T., Bothara, K. G., & Marathe, A. M. (2017). Stress studies and identification of degradation products of cephalexin using LC–PDA and LC– MS/MS. Chromatographia, 80(10), 1545-1552.

[11] Eldin, A. B., Ismaiel, O. A., Hassan, W. A. F. F. A., & Shalaby, A. B. D. A. L. L. A. (2015). Development and validation of stability indicating green HPLC-UV method for determination of cephalexin in pharmaceutical dosage forms and human urine using micellar mobile phase. International Journal of Pharmacy and Pharmaceutical Sciences, 7(9), 122-127.

[12] Khan, M. N., Ahmad, J., Jan, M. N., Gulab, H., & Idrees, M. (2016). Development and validation of a new spectrophotometric method for the determination of cephalexin monohydrate in pure form and pharmaceutical formulations. Journal of the Brazilian Chemical Society, 27(5), 912-918.

[13] von Ahn, A., Dallegrave, A., & dos Santos, J. H. Z. (2022). Evaluation of the Cefalexin Drug Degradation Profile in Pharmaceutical Capsule Forms Based on Forced Degradation Studies. Chromatographia, 85(3), 263-279.

[14] Fawaz, S., Merzouk, M., Barton, S., & Nabhani-Gebara, S. (2021). Stability of amoxicillin and clavulanic acid in separate containers for administration via a y- site. Drug design, development and therapy, 3979-3984.

[15] Addotey, J. N., Awudzi, L., & Adosraku, R. K. (2014). Stability studies on reconstituted amoxicillin-clavulanic acid oral powder by HPLC method development and quantification. International Journal of Pharmaceutical Science and Practice, 3(1), 1- 12.

[16] Atici, E. B., Yazar, Y., Ağtaş, Ç., Ridvanoğlu, N., & Karlığa, B. (2017). Development and validation of stability indicating HPLC methods for related substances and assay analyses of amoxicillin and potassium clavulanate mixtures. Journal of Pharmaceutical and Biomedical Analysis, 136, 1-9.

[17] Zalewski, P., Cielecka-Piontek, J., & Paczkowska, M. (2014). Development and validation of stability-indicating HPLC method for simultaneous determination of meropenem and potassium clavulanate. Acta Pol Pharm, 71, 255-60.

[18] International conference on Harmonization, Harmonized Tripartite Guideline, Stability Testing of New Drug Substances and Products(Revision 2), CPMP/ICH Q1A(R2), 2003.

[19] ICH, Q2B, Harmonized Tripartite Guideline, Validation, In: Proceedings of The International Convention On Quality For The Pharmaceutical Industry, Toronto, Canada, And September, 2002.

[20] ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva 1996.

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