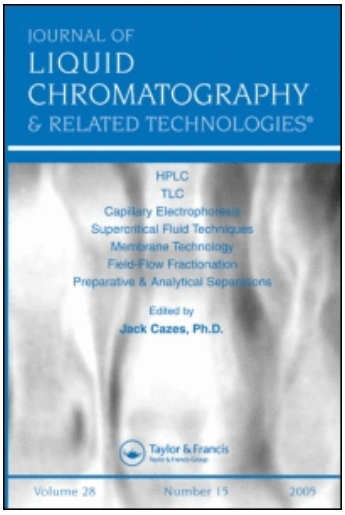


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A VALIDATED STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFPODOXIME PROXETIL AND POTASSIUM CLAVULANATE IN BULK AND TABLET DOSAGE FORM

A. B. Thomas[a]; S. B. Dighe[a]; R. K. Nanda[a]; L. P. Kothapalli[a]; S. N. Jagdale[a]; A. D. Deshpande[a]
[a] Department of Pharmaceutical Chemistry, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, India

Online publication date: 19 November 2010

To cite this Article Thomas, A. B. , Dighe, S. B. , Nanda, R. K. , Kothapalli, L. P. , Jagdale, S. N. and Deshpande, A. D.(2010) 'A VALIDATED STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFPODOXIME PROXETIL AND POTASSIUM CLAVULANATE IN BULK AND TABLET DOSAGE FORM', Journal of Liquid Chromatography & Related Technologies, 33: 18, 1689 — 1703
To link to this Article: DOI: 10.1080/10826076.2010.519255
URL: http://dx.doi.org/10.1080/10826076.2010.519255

Journal of Liquid Chromatography & Related Technologies, 33:1689–1703, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826076.2010.519255

A VALIDATED STABILITY INDICATING HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFPODOXIME PROXETIL AND POTASSIUM CLAVULANATE IN BULK AND TABLET DOSAGE FORM

A. B. Thomas, S. B. Dighe, R. K. Nanda, L. P. Kothapalli, S. N. Jagdale, and A. D. Deshpande

Department of Pharmaceutical Chemistry, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, India

□ *Cefpodoxime proxetil (Cef) and Potassium clavulanate (Pot.clav.) are used in the treatment of acute otitis media, typhoid fever, pharyngitis and tonsillitis. A simple, selective and stability indicating HPTLC method has been established for the simultaneous analysis of Cef and Pot.clav. in pharmaceutical formulations. The method uses aluminum-backed silica gel 60F_{254} HPTLC plates as stationary phase with toluene:methanol:chloroform:acetonitrile [4:3:2:1.5 (v/v/v/v)] as mobile phase with densitometric analysis at 278.0 nm. This system was found to give compact bands for Cef and Pot.clav. with* Rf *values 0.72 ± 0.02 and 0.33 ± 0.02 respectively. Linear relationships were obtained between response and amount of drug with high correlation coefficients (r^2) in the range 250–3500 ng band^{-1} for Cef ($r^2 = 0.9951$) and 100–4500 ng band^{-1} for Pot.clav. ($r^2 = 0.9930$). Inter and intraday RSD values for Cef were 0.523% and 0.530% respectively and 0.401% and 0.355% for Pot.clav. respectively. Total recoveries of Cef and Pot.clav. from the tablet formulations were 99.78% and 99.96% respectively. The LOD and LOQ were 24.29 and 73.60 ng band^{-1} for Cef, 16.7 and 50.61 ng band^{-1} for Pot.clav. respectively. Cef and Pot.clav. were subjected to forced degradation studies. The degradation products obtained were well resolved from the pure drugs with significantly different* Rf *values. As the method could effectively separate the drugs from its degradation products, it can be used for stability-indicating analysis.*

Keywords cefpodoxime proxetil, forced degradation, high-performance thin-layer chromatography, potassium clavulanate, stability-indicating method, validation

Address correspondence to Prof. A. B. Thomas, Assistant Professor, Department of Pharmaceutical Chemistry, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune 411 018, India. E-mail: dypharmachem@yahoo.co.in

INTRODUCTION

Cefpodoxime proxetil (Cef) is chemically (RS)-1(isopropoxycarbonyloxy) ethyl (+)-(6R, 7R)-7-[2-(2-amino-4-thiazolyl)-2-{(Z)methoxyimino}-acetamido]-3-methoxy methyl-8-oxo-5-thia-1-azabicyclo oct-2-ene-2-carboxylate and is official in United State Pharmacopoeia and British Pharmacopoeia.[1,2] Potassium clavulanate (Pot.clav.) is monopotassium (Z)-(2R, 5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo heptane-2-carboxylate and is official in British Pharmacopoeia.[2] Cef is active against a wide-spectrum of Gram-positive and Gram-negative bacteria. The bactericidal activity of Cef results from its inhibition of cell wall synthesis. It is used in the treatment of acute otitis media, typhoid fever, pharyngitis, and tonsillitis. The similarity in chemical structures allows Pot.clav. to act as a competitive inhibitor of beta-lactamases secreted by certain bacteria that help to restore the antimicrobial activity of Cef.[3–5]

Literature survey reveals that several methods such as U.V. spectroscopy,[6–9] HPLC,[10–18] and HPTLC[19,20] have been reported for the estimation of the individual drugs as well as in combination with other drugs. There are, however, no reports of analytical methods for determination of the degradation profiles of these drugs when used in combination. The objective of the present work was to develop a stability indicating HPTLC method for analysis of Cef and Pot.clav. in bulk and marketed formulation.

EXPERIMENTAL

Materials and Reagents

Analytical pure samples of Cef (% purity −99.98) and Pot.clav. (% purity −98.70) (Emcure Pharmaceuticals, Pune, India) were used in the study. The pharmaceutical dosage form used in this study was a Cepodem XP 325 tablet (Ranbaxy Pharmaceuticals, India) procured from a local market and labeled to contain 200 mg of Cef and 125 mg of Pot.clav. per tablet. The solvents and chemicals used in the study were of analytical grade (Qualigens Fine Chemicals, Mumbai).

METHOD

Preparation of Stock Solutions

Standard stock solutions (1000 μg mL^{-1}) of Cef and Pot.clav. were prepared by dissolving 100 mg of each drug in 100 mL of methanol:water mixture (60:40 %v/v) separately.

Selection of Mobile Phase

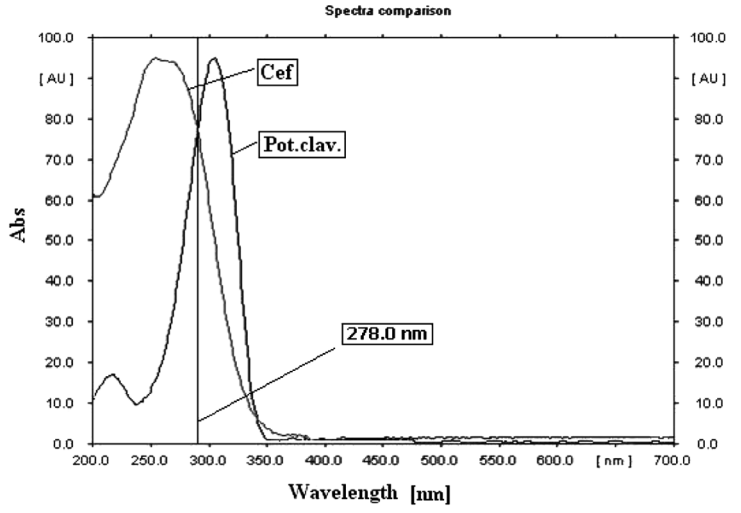
Pure drug of Cef and Pot.clav. were applied on aluminum-backed HPTLC plates coated with silica gel $60F_{254}$ and run in different solvent systems. Different mobile phase systems like toluene:methanol, chloroform: methanol, and toluene:acetonitrile were tried in order to determine the best conditions for the effective separation of Cef and Pot.clav. The mobile phase consisting of toluene:methanol:chloroform:acetonitrile [4:3:2:1.5 (v/v/v/v)] was selected as it gave compact spots with high resolution of Cef and Pot.clav. Observed peaks were well defined and symmetrical with minimal tailing.

Selection of Analytical Wavelengths

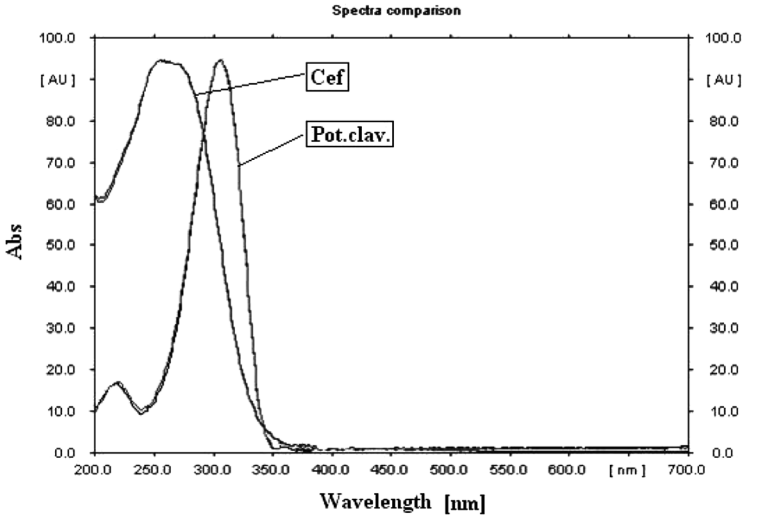
By appropriate dilutions of the standard stock solutions with methanol: water mixture (60:40 %v/v), various concentrations of Cef and Pot.clav. were prepared and applied on silica gel plates, separately. The plates were scanned in the densitometer in the wavelength range of 200–700 nm and their overlain spectra were obtained. From the overlain spectra (Figure 1A), it was observed that both Cef and Pot.clav. exhibited strong absorbance at about 278.0 nm, which was selected as the analytical wavelength for further analysis.

Instrumentation and Chromatographic Conditions

The chromatographic analysis was performed on 10 cm × 10 cm aluminum-backed HPTLC plates coated with 250 μm layers of silica gel $60F_{254}$ (E. Merck, Darmstadt, Germany, supplied by Merck India, Mumbai, India). The plates were prewashed with methanol and activated at 110°C for 15 min prior to chromatography. The samples were applied as 6 mm wide bands, under a continuous flow of nitrogen, using Camag Linomat V (Muttenz, Switzerland). Sample was applied with a 100 microlitre syringe (Hamilton, Bonaduz, Switzerland) at a constant application rate of 150 nL s^{-1} and the distance between adjacent bands was 10 mm. The mobile phase consisting of toluene:methanol:chloroform: acetonitrile [4:3:2:1.5 (v/v/v/v)] was used for the development of the chromatograms. For linear ascending development, a twin-trough glass chamber 20 cm × 10 cm (Camag, Muttenz, Switzerland) previously saturated with mobile phase for 30 min at room temperature (25 ± 2°C) was employed. The length of each chromatogram run was 80 mm. After chromatographic development, plates were air dried and densitometric scanning was performed at 278.0 nm (Figure 1A) with a Camag HPTLC scanner III operated



(A)



(B)

FIGURE 1 (A) Standard overlain spectra of Cef and Pot.clav. measured from 200 to 700 nm. (B) Spectra comparison of standard and tablet sample of Cef and Pot.clav. mearsured from 200 to 700 nm.

in reflectance–absorbance mode and controlled by WinCATS software (Version 1.4.3.6336). The slit dimensions were 5×0.45 mm and the scanning speed was 20 mm s^{-1}. The source of radiation used was a deuterium lamp emitting continuous UV spectra between 190–400 nm. Concentrations of the compounds chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression.

TABLE 1 Assay of Cef and Pot.clav. in Cepodem XP 325 Tablet (Ranbaxy Pharmaceutical, India)

Drug	Label claim (mg/tablet)*	Amount found (mg)	Drug content (%)*	RSD (%)
Cef	200	196.56	99.28	0.503
Pot.clav.	125	124.21	99.37	0.552

*Denotes average of six estimations.

Development of Calibration Curves

Amount of standard solutions equivalent to 250–3500 ng band^{-1} of Cef and 100–4500 ng band^{-1} of Pot.clav. were applied to the prewashed HPTLC plates and the plates were developed and scanned under the aforementioned chromatographic conditions. Calibration plots were constructed by plotting peak areas against the corresponding amount of the drugs.

Estimation of Marketed Formulation

Twenty tablets (Cepodem XP 325, containing 200 mg Cef and 125 mg Pot.clav. per tablet, manufactured by Ranbaxy Pharmaceuticals, New Delhi, India) were weighed and crushed to fine powder. An accurately weighed

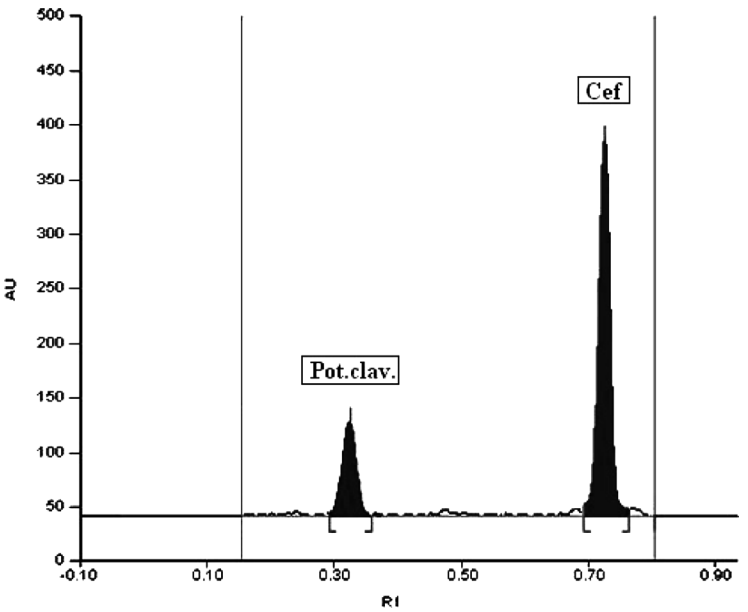


FIGURE 2 Densitogram of Cef and Pot.clav. in tablet formulation. Pot.clav. *Rf* value – 0.33. Cef *Rf* Value – 0.72.

powder sample equivalent to 100 mg of Cef was weighed, transferred to a 100 mL volumetric flask, and dissolved in 70 mL methanol:water mixture (60:40 %v/v). The solution was sonicated for 30 min to allow for dissolution of the active components and the volume was made up to the mark with the same solvent. It was then filtered through Whatman filter paper No. 42. An aliquot of the sample solution (1 μL) containing 1000 ng of Cef and 625 ng of Pot.clav. was applied on HPTLC plates followed by chromatographic development with selected mobile phase under the optimized chromatographic conditions. Content of Cef and Pot.clav. were calculated by comparing peak areas of sample with that of the standards (Table 1). The densitogram of the tablet formulation is shown in Figure 2.

METHOD VALIDATION

The method was validated in accordance with ICH guidelines.[21]

Precision

Precision of the method was determined with the tablet samples. The precision was done to asses the repeatability of sample application and measurement of sample concentrations. The intra-day and inter-day precision was determined by repeating the assay six times in the same day for intra-day precision and on different days for inter-day precision studies.

Recovery Studies (Accuracy)

To check the accuracy of the proposed methods, recovery studies were carried out at 80, 100, and 120% of the test concentration as per ICH guidelines.

To perform recovery studies at 80% of the test concentration, a preanalyzed tablet sample containing 10 mg of Cef and 6.25 mg of Pot.clav. was weighed. To it, 8.0 mg of standard Cef and 5.0 mg of standard Pot.clav. each were added and mixed thoroughly. From it, sample powder containing the equivalent to 10 mg of Cef and 6.25 mg of Pot.clav. was weighed, transferred to a 100 mL volumetric flask, and dissolved in methanol:water mixture (60:40 %v/v). The contents were shaken in a sonicator for 30 min and the volume was made up to the mark with the same solvent. The solution was filtered through Whatmann filter paper No. 42 and then analyzed as per the procedure given for tablets. Similarly, to perform recovery studies at 100% and 120% of the test concentration, a preanalyzed tablet sample containing 10 mg of Cef and 6.25 mg of Pot.clav. each was weighed. To it, 10 mg of Cef and 6.25 mg of Pot.clav. (for 100%), and 12 mg of Cef and 7.50 mg of Pot.clav. (for 120%) were added separately and the contents

were mixed thoroughly. From each, sample powders containing equivalent to 10 mg of Cef and 6.25 mg of Pot.clav. were weighed and transferred to separate 100 ml volumetric flasks. The sample dilutions and analysis were performed as per the procedure given for tablets. The recovery studies were performed three times at each level of recovery.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were separately determined based on the standard deviation of the y-intercept and slope of the calibration curves. Also, the repeatability of measurements was validated at the LOD and LOQ levels. The LOD and LOQ for the proposed method were determined using Eqs. (1) and (2), respectively.

$$\mathrm{LOD} = \frac{3.3\sigma}{\mathrm{S}} \tag{1}$$

$$\mathrm{LOQ} = \frac{10\sigma}{\mathrm{S}} \tag{2}$$

where σ = Standard deviation of the response and S = Slope of calibration curve of analyte.

Robustness of the Method

To evaluate the robustness of the developed HPTLC method, deliberate variations were made in the mobile phase composition. Chromatograms were run with mobile phases of composition toluene:methanol:chloroform: acetonitrile (4.1:3:1.9:1.5 v/v), toluene:methanol:chloroform:acetonitrile (3.9:3:2.1:1.5 v/v), toluene:methanol:chloroform:acetonitrile (4:3.1:2: 1.4 v/v), and toluene:methanol:chloroform:acetonitrile (4:2.9:2:1.6 v/v). Chamber saturation period and development distance were varied in the range ±10%. Times from sample application to development and from development to scanning were also varied (0, 10, 20, and 30 min). The tablet sample solution containing 1 μg mL^{-1} of Cef and 0.625 μg mL^{-1} of Pot.clav. was employed to check the robustness of the method under different chromatographic conditions.

Specificity

The specificity of the method was ascertained by analyzing standard drug (1 μg per band of Cef and 0.625 μg per band of Pot.clav.) and tablet samples (1:0.625 μg per band of Cef and Pot.clav., respectively). The bands

for Cef and Pot.clav. in the sample were confirmed by comparing the *Rf* values and spectra of the sample band with that of standard. The peak purity of Cef and Pot.clav. was assessed by comparing the spectra at three different levels, i.e., peak start(S), peak apex(M), and peak end (E) positions of the band (Figure 1B). The specificity of the method was also evaluated by performing forced degradation studies.

Forced Degradation Studies

Standard stock solutions (1000 μg mL^{-1}) were prepared by dissolving 100 mg each of Cef and Pot.clav. in 100 mL of methanol:water mixture (60:40 %v/v) separately.

Acid-Induced Degradation

HCl (0.001 M, 5 mL) was added separately to 5 mL methanolic stock solutions of Cef and Pot.clav. in 25 mL volumetric flasks. The mixtures were refluxed at 40°C for 30 min and the volume was made up with methanol:-water mixture (60:40 %v/v) (200 μg mL^{-1}). The forced degradation was performed in the dark to exclude the possible photolytic effect of light. The resulting solutions (5 μL, 1,000 ng band^{-1} for Cef and 3.13 μL, 625 ng band^{-1} for Pot.clav.) were applied to HPTLC plates and the chromatograms were run uder optimized chromatographic conditions.

Base-Induced Degradation

NaOH (0.001 M, 5 mL) was added separately to 5 mL methanolic stock solutions of Cef and Pot.clav. in 25 mL volumetric flask. The mixtures were refluxed at 40°C for 30 min and the volume was made up with methanol: water mixture (60:40 %v/v) (200 μg mL^{-1}). The samples were then applied and analyzed as described in the acid induced degradation study.

Hydrogen Peroxide-Induced Degradation

H_2O_2(3%, 5 mL) was added separately to 5 mL methanolic stock solutions of Cef and Pot.clav. in 25 mL volumetric flasks. The mixtures were refluxed at 40°C for 30 min and the volume was made up with methanol: water mixture (60:40 %v/v) (200 μg mL^{-1}). The samples were then applied and analyzed as described in acid induced degradation study.

Wet Heat Degradation

For wet heat degradation study, 5 mL stock solution of each drug were transferred to 25 mL volumetric flasks separately. To each, 15 mL methanol:water mixture (60:40 %v/v) was added. The samples were

refluxed at 40°C for 30 min and the volume was made up with same solvent (200 $\mu g\,mL^{-1}$). The samples were then applied and analyzed under the optimized chromatographic conditions.

Dry Heat and Photo-Stability Studies

For dry heat and photo-stability studies, the standard powder drugs were placed in an oven at 40°C and photo-stability chamber (UV-light 2000 lux h) for 24 hr. Appropriate dilutions of stock solutions were prepared in methanol:water mixture (60:40 %v/v) and then analyzed under the optimized chromatographic conditions.

RESULTS AND DISCUSSION

A HPTLC method employing toluene:methanol:chloroform:acetonitrile (4:3:2:1.5 v/v/v/v) as the mobile phase with densitometric scanning at 278 nm was developed for Cef and Pot.clav. The *Rf* values for Cef and Pot.clav. were 0.72 and 0.33, respectively. The mean % content of Cef and Pot.clav. in tablet formulation were 99.28% and 99.37%, respectively, with low % RSD (less than 2%) (Table 1). The optical characteristics and validation data for the developed method are presented in Table 2. Also, the mean % recoveries of Cef and Pot.clav. were found to be between 98–102% and are presented in Table 3. The LOD were found to be 24.29 ng per band for Cef and 16.70 ng per band for Pot.clav. Similarly, the LOQ were 73.60 ng per band and 50.61 ng per band for Cef and Pot.-clav., respectively (Table 2). The results of the robustness study as shown in Table 2 indicated that the developed method is unaffected by small changes in method parameters and is robust.

Stability Indicating Study

Acid Induced Degradation Study

Pot.clav. was found to undergo acid degradation very rapidly. Degradation studies were performed under milder conditions by using 0.001 M HCl, refluxed at 40°C for 30 min. Pot.clav. showed degradation peaks at *Rf* values 0.22 and 0.41 (about 28% degradation). However, Cef was stable to acid degradation conditions (Figure 3).

Base Induced Degradation Study

In the base induced degradation study, Cef showed additional peaks at *Rf* values 0.48, 0.60 (about 31% degradation) while Pot.clav. exhibited an additional peak at *Rf* value 0.22 (about 14% degradation) (Figure 4).

TABLE 2 Optical Characteristics and Validation Data of Cef and Pot.clav

Parameters	Cef	Pot.clav.
Linearity (ng band^{-1}) Precision*	250–3500	100–4500
Interday (% RSD)	0.523	0.401
Intraday (% RSD)	0.530	0.355
LOD (ng band^{-1})*	24.29	16.70
LOQ (ng band^{-1})*	73.60	50.61
Regression Values*		
i. Slope	3483.5	2152.3
ii. Intercept	25.639	10.894
iii. Regression coefficient (r^2)	0.9942	0.9926
Robustness studies (% R.S.D.)		
i. Mobile phase composition (±0.1 mL)	0.305	1.131
ii. Chamber saturation period (±5 min)	0.269	1.515
iii. Development distance (±5 mm)	0.460	1.229
iv. Time from spotting to chromatography (0, 10, 20, 30 min)	0.323	1.122
v. Time from chromatography to scanning (0, 10, 20, 30 min)	0.248	0.602

*Denotes average of six estimations.

Hydrogen Peroxide Induced Degradation Study

In the oxidative degradation study, it was found that both Cef and Pot.clav. were labile to oxidative degradation exhibiting additional degradation peaks at *Rf* values of 0.74 (about 8% degradation) and 0.18 (about 22% degradation), respectively, as shown in Figure 5.

Wet Degradation Study

The wet degradation studies suggested that Pot.clav. was stable showing no degradation. However, Cef showed an additional peak at *Rf* value 0.67 (about 9% degradation) (Figure 6).

TABLE 3 Statistical Validation Data of Tablet Formulation and Recovery Studies

Component	Amount present (mg per tablet)	Amount Found* (%)	RSD* (%)	Recovery Level (%)	Initial Amount (ng band^{-1})	Amount Added (ng band^{-1})	Recovery** (%)	RSD** (%)
Cef	200	99.78	0.421	80	1000	800	99.94	0.144
				100	1000	1000	99.96	0.148
				120	1000	1200	99.45	0.970
Pot.clav.	125	99.96	0.398	80	625	500	100.03	0.374
				100	625	625	100.09	0.522
				120	625	750	99.75	0.297

*Denotes average of six estimations.

**Denotes average of three estimations at each level of recovery.

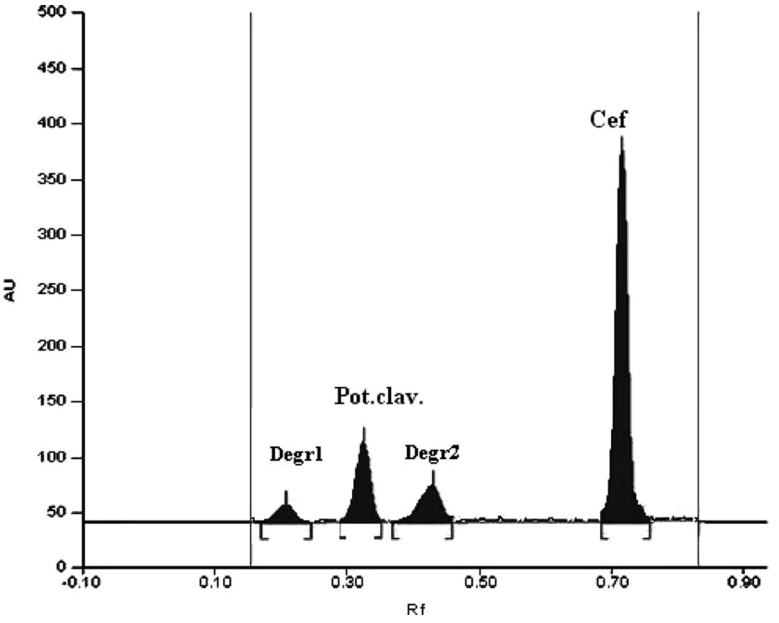


FIGURE 3 Acid degraded Densitogram of Cef and Pot.clav. Degr1 and Degr2 – Acid degradation peaks of Pot.clav. (*Rf* 0.22 and 0.417).

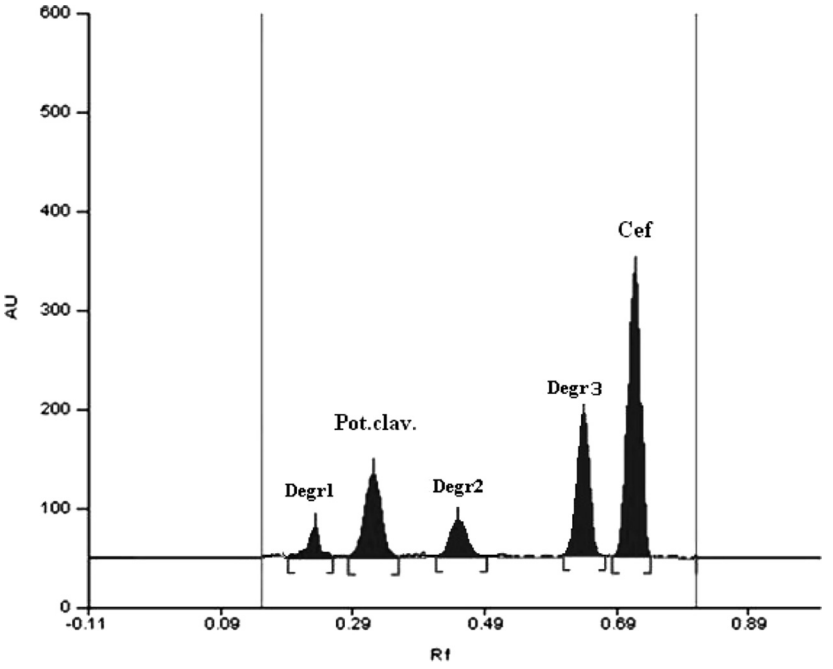


FIGURE 4 Base degraded Densitogram of Cef and Pot.clav. Degr1 – Base degradation peak of Pot.clav. (*Rf* 0.22). Degr2 and Degr3 – Base degradation peaks of Cef (*Rf* 0.48, 0.60).

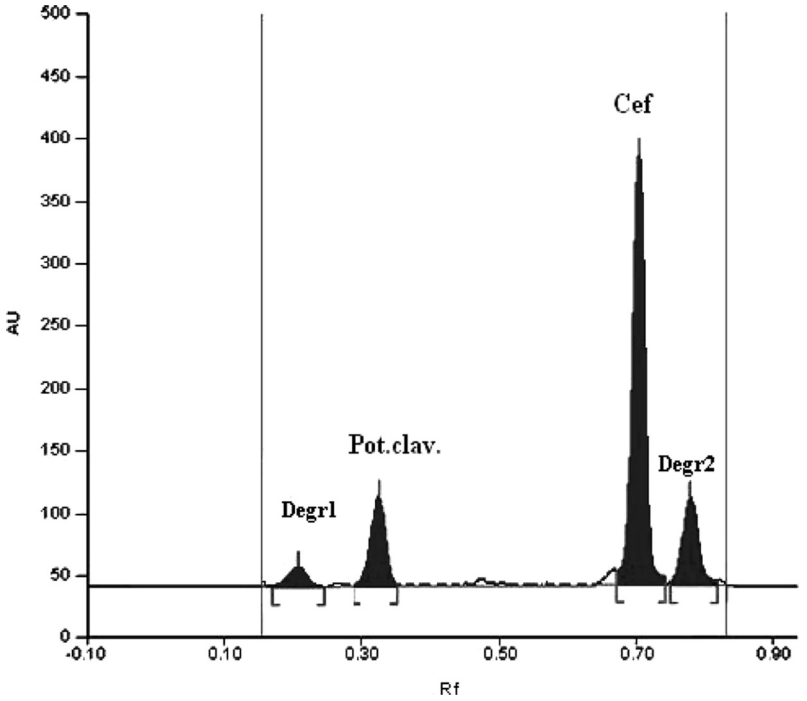


FIGURE 5 Oxidative degraded Densitogram of Cef and Pot.clav. Degr1 – Oxidative degradation peak of Pot.clav. (*Rf* 0.18). Degr2 – Oxidative degradation peak of Cef (*Rf* 0.74).

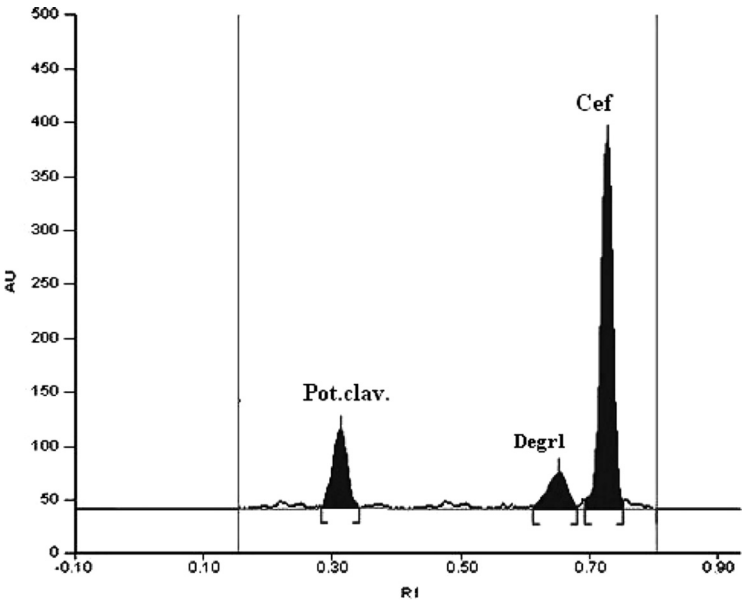


FIGURE 6 Wet degraded Densitogram of Cef and Pot.clav. Degr1 – Wet degradation peak of Cef (*Rf* 0.67).

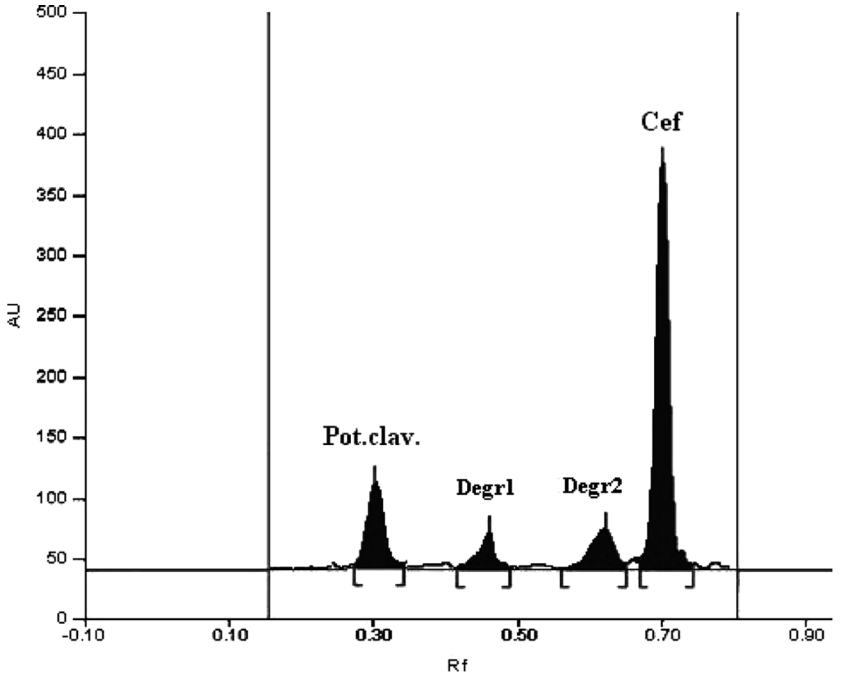


FIGURE 7 Dry degraded Densitogram of Cef and Pot.clav. Degr1 – Dry degradation peak of Pot.clav. (*Rf* 0.45). Degr2 – Dry degradation peak of Cef (*Rf* 0.60).

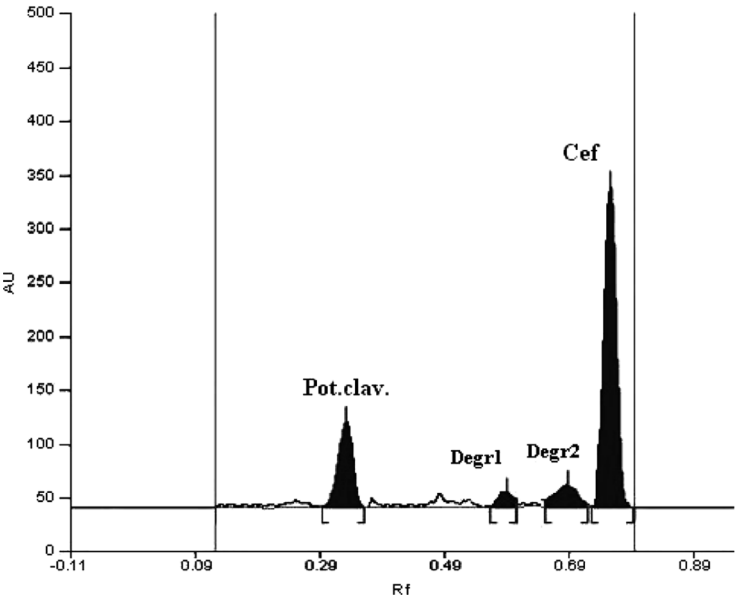


FIGURE 8 Photo degraded Densitogram of Cef and Pot.clav. Degr1 and Degr2 – Photo degradation peaks of Pot.clav. (*Rf* 0.54 and 0.65).

Dry Degradation Study

In the dry degradation study, it was found that both Cef and Pot.clav. were labile to dry degradation exhibiting additional degradation peaks at *Rf* values 0.60 (about 9% degradation) and 0.45 (about 18% degradation), respectively, as shown in Figure 7.

Photo Degradation Study

Pot.clav. was found to undergo photo degradation very rapidly. Pot.clav. showed degradation peaks at *Rf* values 0.54 and 0.65 (about 37% degradation). However, Cef was stable to photo degradation conditions (Figure 8).

CONCLUSION

The developed HPTLC technique is precise, specific, accurate, robust, and stability indicating. Validation studies indicated that the proposed method is suitable for the simultaneous estimation of Cef and Pot.clav. in bulk and in pharmaceutical formulation without any interference from the excipients. A stability indicating assay has been established following the recommendations of ICH guidelines. It can be employed for the simultaneous estimation of Cef and Pot.clav. and their degradation products in stability samples in the industry.

ACKNOWLEDGEMENTS

The authors express their gratitude to Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, MH, India, for providing necessary infrastructural facilities and to Emcure Pharmaceutical, India, for the generous gift samples of pure Cef and Pot.clav.

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