RESEARCH ARTICLE
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Simultaneous Determination of Cefdinir and Clavulanic Acid in Tablets by Using High-Performance Liquid Chromatographic Methods

ABSTRACT

Background: A novel, rapid, and easy liquid chromatographic method was developed and validated for the simultaneous measurement of clavulanic acid and cefdinir in tablets.

Methods: Reverse phase C18 column was used for the chromatographic separation, isocratic application of methanol-20.0 mM phosphate buffer (pH 3) (35:65, v/v) was used as the mobile phase system.

Results: For clavulanic acid and cefdinir, the linearity ranges were determined to be 7.5-35.0 µg/mL and 18.0-84.0 µg/mL, respectively. It was calculated that the limits of quantification and detection for cefdinir and clavulanic acid, respectively, were 1.91 and 0.63 µg/mL and 1.33 and 0.44 µg/mL. Clavulanic acid and cefdinir were found to have mean recoveries of 96.645-104.26% and 96.60-98.52%, respectively. The suggested techniques were effectively used to identify commercially available tablets, and the outcomes were statistically compared using the Student's *t* and Fischer's *F* tests pharmacopeia methods. It was discovered that, at 95% CIs, there is no discernible difference between the approaches' mean values and SDs.

Conclusion: The method that has been devised is easy to use, rapid, accurate, repeatable, and safe for routinely determining the simultaneous presence of clavulanic acid and cefdinir in tablets.

Keywords: Cefdinir, clavulanic acid, HPLC, simultaneous determination, validation

INTRODUCTION

Cefdinir, whose chemical name is (-)-(6R,7R)-7-[2-(2-Amino-4-thiazolyl) glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 7^2-(Z)-oxime (Figure 1a), is a broad-spectrum, semi-synthetic, third-generation cephalosporin used orally. It has been found to be effective against oxacillin-sensitive *Staphylococcus aureus* and coagulase-negative staphylococci, *Streptococcus pneumoniae*, *S. pyogenes*, *Escherichia coli*, and *Moraxella catarrhalis*. It is also highly effective against *Haemophilus influenzae*, *Klebsiella pneumoniae*, *K. oxytoca*, *Proteus mirabilis*, and *P. vulgaris*. It is used for the treatment of various upper respiratory tract infections, including skin and soft tissue infections, chronic bronchitis, otitis media, pharyngitis, pneumonia, sinusitis, and tonsillitis in adults and children.[1-3]

Clavulanic acid, whose chemical name is (2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid; (2R,3Z,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo [3.2.0] heptane-2-carboxylic acid (Figure 1b), is a clavam derivative beta-lactamase inhibitor antibiotic obtained from Streptomyces clavuligaris. Clavulanic acid usually does not have a pronounced antibacterial effect alone. Since the production of beta-lactamase enzymes in bacteria is the most important and general factor in resisting penicillins, clavulanic acid has been used to increase the potency of penicillins and to expand their spectrum of action. However, with a new combination, it has been started to be used with cefdinir for a similar effect. The presence of clavulanic acid in the combination of cefdinir-clavulanic acid protects cefdinir from being broken down by

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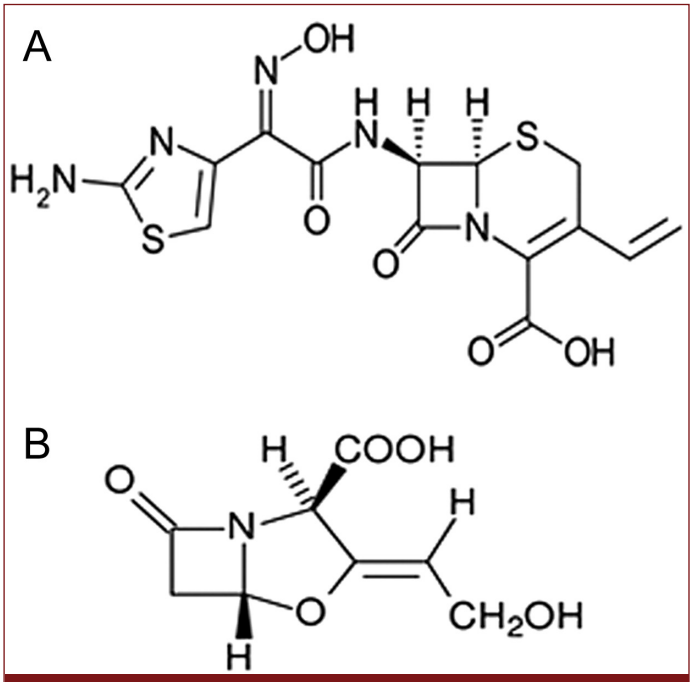

Figure 1. Chemical structures: (a) cefdinir and (b) clavulanic acid.

beta-lactamase enzymes and extends the effect spectrum of cefdinir to include many normally resistant bacteria.[1]

When previously developed methods for the determination of these 2 drug substances were examined, it was seen that there were many methods for their determination individually.

These methods, which allow the determination of both cefdinir[4-17] and clavulanic acid[18-30] alone, are generally based on high-performance liquid chromatography (HPLC) or spectrophotometric methods. However, as a result of our research, only 1 study was found for the simultaneous analysis of clavulanic acid and cefdinir in tablets, which also includes another antibiotic substance, cefixime.[31]

Since simultaneous active ingredient analysis has advantages in terms of time and cost, simultaneous analyses are always needed in routine analyses of combined drug forms, instead of analyzing each active ingredient individually. Since effervescent tablets of cefdinir and clavulanic acid are currently used in treatment, this study intended to develop a new, faster, and simpler HPLC method for the simultaneous analysis of clavulanic acid and cefdinir in effervescent tablets. The recommended method has been validated, and its applicability to the analysis of drug substances in tablets has been tested.

MATERIAL AND METHODS

Chemicals

Cefdinir, clavulanic acid, and their pharmaceutical preparation (Elucef Plus ®) (containing 300 mg of cefdinir and 125 mg of clavulanic acid per tablet) were provided by Deva (İstanbul, Türkiye). Liquid chromatography grade methanol, analytical purity sodium hydroxide, ortho-phosphoric acid (85%), and potassium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany). Ultrapure water from the Elga brand water system was used in the research laboratory.

Solutions

Cefdinir and clavulanic acid were dissolved in phosphate buffer (pH 7) and methanol, respectively, to prepare 1 mg/mL stock solutions. In order to prepare calibration samples, the stock solutions of clavulanic acid and cefdinir were diluted to final concentrations of 7.5-35.0 µg/mL and 18-84 µg/mL, respectively.

To prepare a 20 mM (pH 3) phosphate buffer, a 0.2 M KH_2PO_4 solution was first prepared. This solution was adjusted to pH 3 with orthophosphoric acid and then diluted 10-fold with water to obtain a 20 mM buffer.

High-Performance Liquid Chromatography System

The liquid chromatographic system equipped with an autosampler, a column oven compartment, and a DAD was used in the study. The device used in the analysis was the LC 20A system of the Shimadzu brand (Kyoto, Japan). Chromatographic conditions were used for the separation, with a Teknokroma C18 (250 × 4.6 mm, 5 µm i.d.) column and a flow rate of 1.0 mL/min. Cefdinir and clavulanic acid were detected at 210 nm. Isocratic application of methanol:20.0 mM phosphate buffer (pH 3) (35:65, v/v) was used as the mobile phase system.

Procedure for Tablets

Effervescent tablet (Elucef Plus®) containing 300 mg of cefdinir and 125 mg of clavulanic acid was dissolved with distilled water in a 100 mL volumetric flask and filled to its volume. It was kept in an ultrasonic bath for 5 minutes. The mixture was then filtered with blue-banded filter papers. Thus, a tablet stock solution containing 3000 µg/mL cefdinir and 1250 µg/mL clavulanic acid was prepared. It was diluted with mobile phase from the tablet stock solution to obtain a final concentration of 30 µg/mL cefdinir and 12.5 µg/mL clavulanic acid before the injection.

VALIDATION

Proposed method was validated to ICH Q2 (R1) Guideline.[32] Selectivity of the methods was investigated with a mixture of commonly used effervescent tablet excipients.

The linearity range of the proposed method was determined to be 7.5-35.0 µg/mL and 18.0-84.0 µg/mL for clavulanic acid and cefdinir, respectively (n = 6).

The limits of quantification (LOQ) and limits of detection (LOD) were calculated using the following formulas: LOQ = 10SDa / b; LOD = 3SDa / b (SDa is the SD of the intercept and *b* is the slope).

The standard addition method was used to determine the accuracy of the developed method. It was applied by adding cefdinir and clavulanic acid standard solutions in 4 different concentrations to a tablet solution at concentrations of 18.0 µg/mL cefdinir and 7.5 µg/mL clavulanic acid.

In precision studies, standard solution samples were prepared at concentrations of 18, 48, and 84 µg/mL for cefdinir and concentrations of 7.5, 20, and 35 µg/mL for clavulanic acid. In intra-day analyses, 6 replicates of samples at all 3 concentrations were analyzed during the day, and in inter-day analyses, 3 replicates of samples at all 3 concentrations were analyzed on different days.

For the applicability of the method, the validated method was used to determine Elucef Plus® (300 mg of cefdinir and 125 mg of clavulanic acid) effervescent tablets. Using the Student's *t*-test and the variance ratio *F*-test, the method's computed values for cefdinir[33] and clavulanic acid[34] were statistically compared with those obtained using USP pharmacopeia methods.

RESULTS

Specificity and Separation

At the beginning of the method development studies, different stationary and mobile phases were tested by examining the solubility and polarity properties of the substances. It was observed that the most efficient separation was achieved on a Teknokroma C18 column (250 × 4.6 mm I.D., 5 µm particles) with a methanol:20.0 mM phosphate buffer (pH 3) (35:65, v/v) mobile phase system. Under these chromatographic conditions, cefdinir and clavulanic acid were determined to be 5.6 and 3.8 minutes, respectively. Representative chromatograms are shown in Figure 2. It was demonstrated that there is no interference from a mixture of tablet excipients and the mobile phase in the chromatograms.

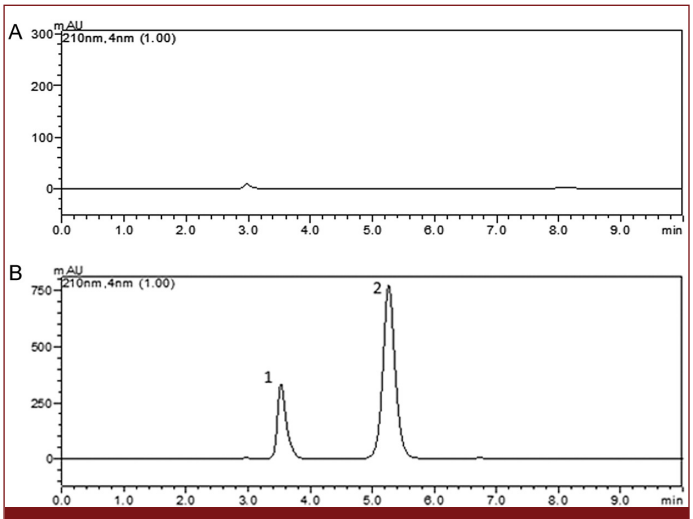

Figure 2. HPLC chromatograms obtained with the developed method: (a) tablet excipients mixture sample, (b) standard solution of clavulanic acid (35 µg/mL) (1) and cefdinir (84 µg/mL) (2).

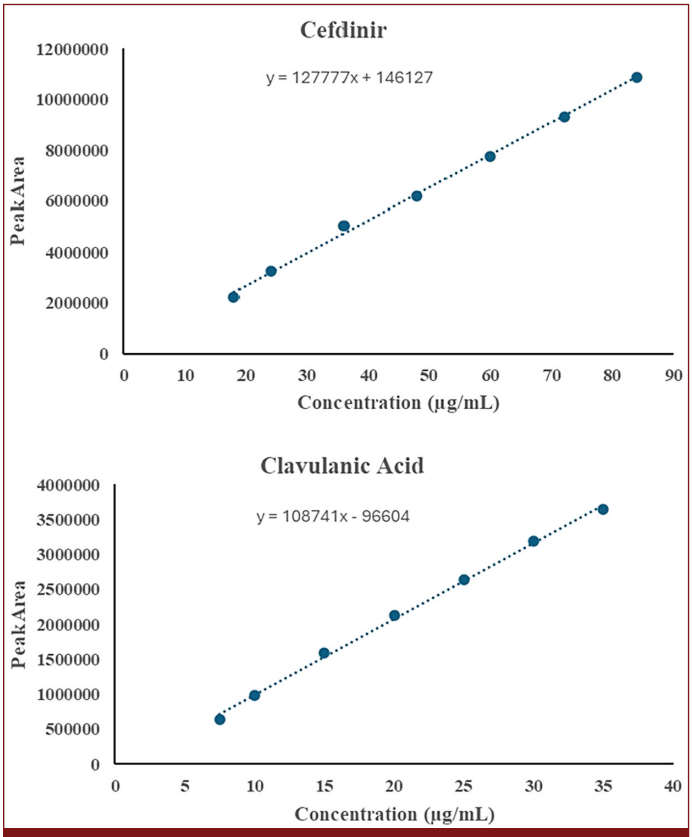

Figure 3. Calibration curves of clavulanic acid (7.5-35.0 µg/mL) and cefdinir (18.0-84.0 µg/mL).

Linearity Range and Sensitivity

Linearity ranges were studied between the range of 7.5 and 35.0 µg/mL and 18.0-84.0 µg/mL for clavulanic acid and cefdinir, respectively. The mean regression equations were as follows: A = 127777 C + 146127.2 (r = 0.9987) for cefdinir and A = 108741 C – 96604.2 (r = 0.9988) for clavulanic acid, with C representing the concentration of the compounds (µg/mL) and A representing the peak area. The results for linearity from the proposed method are presented in Figure 3 and Table 1. The LOQ and LOD values were calculated as 1.91 µg/mL and 0.63 µg/mL for cefdinir and 1.33 µg/mL and 0.44 µg/mL for clavulanic acid, too.

Table 1. Some Validation Parameters of Developed Method

	Cefdinir	Clavulanic Acid
Linearity (µg/mL)	18.0-84.0	7.5-35.0
Regression equation	*A* = 127 777 *C* + 146 127.2	*A* = 108 741 *C* – 96 604.2
Slope ± SD	127 777 ± 425	108 741 ± 1252
Intercept ± SD	146 127.2 ± 28 002	96 604.2 ± 12 815
Correlation coefficient (r) (mean)	0.9987	0.9988
LOD (µg/mL)	0.63	0.44
LOQ (µg/mL)	1.91	1.33

Table 2. Statistical Evaluation of Analysis Results with Comparison Methods

	Cefdinir		Clavulanic Acid	
Statistical Value	HPLC Method[a]	USP Pharmacopeia Method	HPLC Method[a]	USP Pharmacopeia Method
Mean ± SD	300.08 ± 0.438	299.28 ± 2.49	125.086 ± 0.047	125.074 ± 0.056
Recovery (%)	100.02	99.81	100.43	100.37
RSD (%)	0.12	0.67	0.23	0.28
Confidence limits	299.64-300.52	296.8-301.76	125.04-125.13	125.02-125.13
Student's *t*-test **	0.334		0.087	
F-test**	0.031		0.06	

**$P = .05$, $t = 2.23$, $F = 5.05$.
[a]Elucef Plus tablet® (300 mg cefdinir and 125 mg clavulanic acid).

Precision and Recovery

Percentage RSD values, which are the expression of method precision, were calculated to be between 0.1 and 1.55% for cefdinir and between 0.06 and 1.98% for clavulanic acid.

Recoveries, which are one of the expressions of method accuracy, were found in the range of 96.60%-98.52% and 96.045%-104.26% for cefdinir and clavulanic acid, respectively.

Determination of Clavulanic Acid and Cefdinir in Tablets

The developed method was applied to tablet analysis as described above (Figure 4) The comparison of the results obtained with the developed method in terms of means *t*-test (Student *t*-test) and the comparison in terms of SDs were performed by applying the *F* test (Fisher's test). As seen in Table 2, the calculated *t*- and *F*-values were found to be lower than the values reported in the relevant tables. It was found that there was no significant difference between the developed HPLC method and comparison methods at the 95% probability level.

DISCUSSION

The developed and validated method is faster, and the sample preparation phase is much easier compared to the previous method[31] that included simultaneous analysis of these substances.

The linearity ranges of the method were chosen to be suitable for analyzing lower amounts of clavulanic acid and higher amounts of cefdinir, in accordance with the amounts in the effervescent tablets. The total analysis time of the method, which analyzes both substances with high accuracy and precision, has been completed in 6 minutes, and the sample preparation has taken only 5 minutes.

In addition, the results obtained with the developed method have been compared with those obtained from the pharmacopoeia methods of both substances using *t* and *F* tests, and thus the accuracy and precision of the developed method have been once again confirmed.

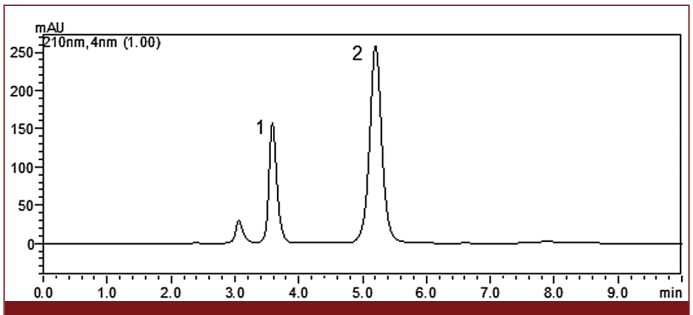

Figure 4. HPLC chromatograms obtained with tablet solution of clavulanic acid (12.5 mg) (1) and cefdinir (30 mg) (2).

CONCLUSION

In this study, an easy-to-use, fast, reproducible, and highly reliable method for the simultaneous determination of clavulanic acid and cefdinir in tablets was developed and validated. Considering the contribution of simultaneous analyses to the pharmaceutical industry in terms of cost, time, and labor force, it is apparent that the developed method is quite advantageous. The validated method can be used in routine pharmaceutical analysis in a reliable, fast, and practical way for the simultaneous determination of cefdinir and clavulanic acid in tablets.

Ethics Committee Approval: No ethics committee approval is required as the study used tablet analysis method.

Informed Consent: No informed consent is required as the study used tablet analysis method.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.E.T.; Design – S.E.T.; Supervision – S.E.T.; Resources – A.R.A., S.E.T., G.E.K.; Materials – S.E.T., A.R.A.; Data Collection and/or Processing – S.E.T., A.R.A., G.E.K.; Analysis and/or Interpretation – S.E.T., A.R.A.; Literature Search – A.R.A., S.E.T., G.E.K.; Writing – S.E.T., G.E.K.; Critical Review – S.E.T.

Declaration of Interests: The authors have no conflict of interest to declare.

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