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Determination of Cefuroxime Axetil in Tablet by HPLC, UV and First-Order Derivative Spectroscopy Methods & Plasma by UV Spectroscopy Method

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Abstract: It was aimed to develop and validate the UV spectroscopic, the first-order derivative spectroscopy, and the HPLC method for determination of cefuroxime axetil in bulk and tablets and also in spiked human plasma samples by UV spectroscopy method. In the spectroscopic analyses, the maximum absorbance of cefuroxime axetil in acetonitrile was obtained at 277 nm wavelength in the spectra. In first-order derivative spectroscopy method, two peaks were observed in spectra, a maximum at 258 nm and a minimum at 298 nm. 298 nm wavelength was used in the study. In HPLC-UV study, parameters were chosen as follows: C18 column, 0.1% acetic acid-acetonitrile (30:70; v/v) for mobile phase, 1.0 mL/min of flow rate, 280 nm of wavelength, 10 µL of injection volume and etodolac (2.5 µg/mL) as internal standard. Accuracy, precision, recovery, linearity and sensitivity parameters were determined for each of the three methods. Developed and validated methods were successfully applied on 4 tablets which are named as Cefaks, Cefurol, Aksef ve Enfexia. As a result, it is claimed that proposed method is sensitive, precise, accurate, and successfully used in quality control studies in the drug industry.

Keywords: Cefuroxime axetil, UV spectroscopy, first-order derivative spectroscopy, HPLC, tablet.

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1. INTRODUCTION

Cefuroxime axetil (1-acetoxyethyl ester of cefuroxime, CFA), commonly used in therapy, is a prodrug of cefuroxime, CFA), cefuroxime axetil whose chemical formula is (1RS)-1-[(acetyl)oxy] ethyl (6R,7R)-3-[(amino carbonyl oxy) methyl]-7-[[(Z)-2-(furan-2-yl)-2- (methoxy imino) acetyl]amino]-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-en-2-carboxylate (Figure 1), is a member of cephalosporins. Its closed formula is $C_{20}H_{22}N_4O_{10}S$ and its molecular weight is 510.48 g. It has white, a bitter taste, and a powdery appearance in an amorphous structure. Cefuroxime axetil is very soluble in methanol, ethyl acetate, and acetone but it is sparingly soluble in water, ethanol (96%), and ether (1). Cefuroxime axetil is an antibiotic. Natural or chemical substances formed by fungi or similar microorganisms, which have the power to stop the growth of microorganisms and other living things, and even kill them, are called "antibiotics"(2). Cefuroxime axetil is a second-generation cephalosporin that is active against a wide range of gram-positive and gam-negative organisms and resistant to most β-lactamases. Cefuroxime axetil has a wide usage area due to its strong antibacterial effect. Cefuroxime axetil is indicated for the treatment of infections caused by sensitive bacteria (3).

Cefuroxime shows its effect by inhibiting bacterial cell wall synthesis and stimulating autolytic enzymes. Cefuroxime axetil, like other cephalosporin's, is highly resistant to β-lactamases

and especially shows good activity on Escherichia coli, Klebsiella spp., Proteus mirabilis, Moraxella subgenus, Branhamellacatarrhalis, Neisseria gonorrhaeae, Streptococcus spp., Staphylococcus spp. And Haemophil. In addition, it shows good activity against all strains, including ampicillin-resistant strains in generally (4,5). Cefuroxime is bound to plasma proteins up to 50% and dose adjustment is required in case of renal failure (6). Studies on the direct quantification of cefuroxime axetil are very limited. In the literature reviews of cefuroxime axetil, which has a wide area of use as an antibiotic, scientific studies have been reached in the determination of plasma and pharmaceutical preparations (7,8). These methods in pharmaceutical preparations: spectrofluorometric method (9), HPLC (10-13), thin-layer chromatography and densitometry (14), capillary zone electrophoresis (15) and spectroscopy methods (16-19).

In the studies based on spectroscopic analyses, cefuroxime axetil was derivatized by reacting with different reagents (1-nitroso-2 naphthol and sodium hydroxide; bromothymol blue) and analyzed by spectrophotometric method (16,17). Pritam et al. analyzed cefuroxime axetil in eye drops in HCl medium with spectrophotometer (18). Game et al., (19) on the other hand, developed a UV spectrophotometric method in 0.1 M HCl medium and a derivative spectrophotometric method in 0.1 M NaOH medium for the determination of cefuroxime axetil in tablets.

The aim of this study is to develop and validate UV spectroscopic, first-order derivative spectroscopy and HPLC methods for the determination of the active ingredient cefuroxime axetil in bulk and tablets, as well as UV spectroscopy methods for the determination of cefuroxime axetil in plasma. Quantitative determination of cefuroxime axetil in spectroscopy methods was performed without derivatization. This provides convenience in the determination method.

2. MATERIALS AND METHODS

2.1. Reagents and Chemicals

Cefuroxime axetil and etodolac was obtained from Nobel Ilac A.Ş. (Turkiye). Acetonitrile (HPLC grade), phosphoric acid (Merck), diethyl ether (Merck) and acetic acid (Fluka) were purchased. The deionized water was made fresh every day and filtered (0.45 m). 4 commercial tablets (Cefaks, Cefurol, Aksef and Enfexia) were obtained in the community pharmacy (Erzurum, Turkiye). Human plasma was obtained from Erzurum Kızılay Blood Bank.

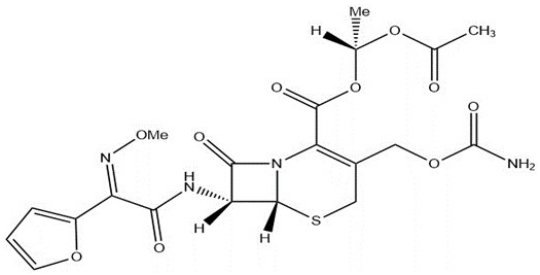

Figure 1: Chemical structure of cefuroxime axetil.

2.2. Apparatus and Method Conditions

A Thermospectronic double-beam UV-Visible spectrophotometer (HEλIOSβ) with a fixed slit width 2 nm and a data processing system was used. UV and first-order derivative spectra (N=6. Δλ=4.0 nm) of sample solutions were recorded in 1 cm quartz cells between wavelength ranges of 200-300 nm at a scan speed of 280 nm/min and derivation interval (Δλ) 21.0 nm. Maximum absorbance was observed at 277 nm and measurements were taken under these conditions. In first-order derivative spectroscopy methods, two peaks were observed in the spectrum, a maximum at 258 nm and a minimum at 298 nm.

HPLC System (Agilent Technologies 1200 Series), Degasser (Agilent Technologies), Pump (Agilent Technologies), Column (C18, 5µm, 250 x 4.6mm) (Phenomenex Bondolone USA), Auto Simpler (Agilent Technologies) and Computer (HP). HPLC conditions of work: UV detector (280 nm), deionized water (0.1% acetic acid): acetonitrile (30:70, v/v) of mobile phase, 25 °C of column temperature, 1.0 mL/min of flow rate and 10 µL of injection volume.

2.3. Preparation of Stock Solution, Standard Working Solutions, and Quality Control Solutions

The stock solution of cefuroxime axetil (100 µg/mL) was prepared by dissolving 10 mg of cefuroxime axetil in 100 mL of acetonitrile. The standard working solutions were prepared in 0.3, 0.5, 1.0, 3.0, 6.0, and 12. µg/mL concentrations for HPLC analysis and 1.0, 2.0, 4.0, 6.0, 8.0,10.0, 15.0, 20.0, 25.0, 30.0 and 35.0 µg/mL concentrations for spectroscopy methods from stock solution and also the quality control (QC) solutions were prepared at 0.4, 4.0, and 8.0 µg/mL concentrations for HPLC method and at 1.5, 4.5, 9.5, and 30.0 µg/mL concentrations for spectroscopic methods. The QC samples were analyzed for validation assessment.

In the HPLC study, etodolac (2.5 µg/mL) was used as the internal standard (IS).

2.4. Preparation of Plasma Samples

The plasma removed from -20 °C was allowed to dissolve at room temperature. An appropriate amount of cefuroxime axetil solutions at final concentrations of 1-30 µg/mL were spiked in 0.5 mL

of blank plasma and mixed and then extracted. After extraction, the plasma working solutions at 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 30 µg/mL concentrations and the plasma quality control solutions at 1.5, 4.5, and 9.5 µg/mL concentrations were obtained.

2.5. Extraction Process of Cefuroxime Axetil from Plasma

0.5 mL of plasma was placed in centrifuge tubes and 0.5 mL of 1 M phosphoric acid was added to each of these tubes. Plasma was vortexed for one minute. 1 mL diethyl ether was added. Plasma was vortexed for three minutes and then centrifuged. The organic phase was taken. Evaporated under nitrogen gas at room conditions. Finally, it was dissolved in 4 mL of acetonitrile.

2.6. Preparation of Tablet Solutions

Application of the method to real samples was carried out by using 4 commercial tablets (Cefaks, Cefurol, Aksef and Enfexia) that contains cefuroxime axetil. In this study, 10 tablets from each formulation were weighed, mean values of them were recorded. Then, these tablets were grinded and blended until they became powder. A certain amount of this mixture was taken to be cefuroxime axetil weighing 1 tablet and put into a 100 mL volumetric flask. It was solved in acetonitrile and then its volume was completed to 100 mL. Final solutions were filtered, the new solutions at the concentrations of tablet samples to be used in the study were prepared and their concentrations were determined.

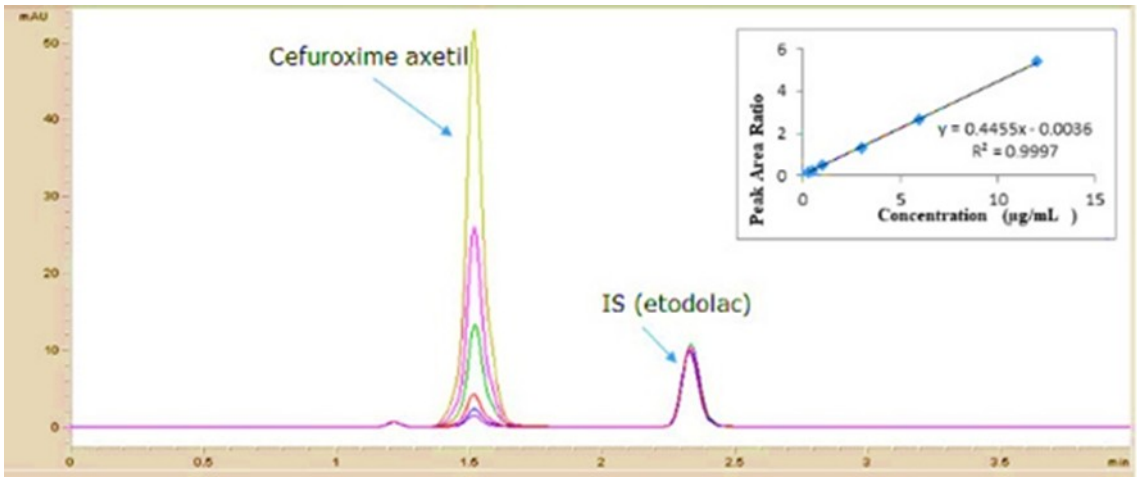

Figure 2: HPLC chromatogram of standard solutions (0.3, 0.5, 1.0, 3.0, 6.0, and 12 µg/mL) of cefuroxime axetil containing IS (etodolac:2.5 µg/mL).

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Validation

To illustrate the applicability of the proposed analytical method, all three methods were validated according to the International Conference on Harmonization Conference (ICH) for approval of analytical procedures using several parameters (20). All statistical calculations were performed with excel functions and formulas.

3.1.2. Selectivity

When the chromatograms of standard solutions were examined, it was observed that the retention times of cefuroxime axetil and etodolac (IS) were 1.5 and 2.3 minutes, respectively. It was determined that the standard working solutions of cefuroxime axetil gave maximum absorbance at 280 nm for HPLC method (Figure 2) and at 277 nm for UV spectroscopic method (Figure 3). In first-order derivative spectroscopy method, two peaks were observed in the derivative spectrum: a maximum at 258 nm and minimum at 298 nm. In the study, the measurements were carried out at a wavelength of 298 nm (Figure 4). To obtain the calibration curve values for plasma study, the appropriate amounts of the previously prepared standard stock solution of cefuroxime axetil were spiked in 0.5 mL of human plasma and they were mixed and extracted. The spectra of the extracted solutions by UV spectroscopy method were taken and their absorbance value was read (Figure 5). When the spectrum and chromatogram of all three methods were examined, it was observed that there was no interfering substance.

3.1.3. Linearity and working range

Linearity was determined with 11 different standard solutions in the concentration range of 1.0-35.0 µg/mL for UV spectroscopy and first order derivative spectroscopy methods and with 6 different standard solutions in the concentration range of 0.3-12.0 µg/mL for HPLC method and also the linearity was determined 7 different extracted plasma solutions in the concentration range of 1.0 – 30.0 µg/mL for the UV spectroscopic method. The calibration curve was derived by plotting the absorbance values against

the solution concentrations in the spectroscopy methods. In the HPLC method, the calibration curve was derived by plotting the peak area ratios against the solution concentrations. Correlation coefficients and equations of the line were obtained by regression analysis of the calibration curves (Table 1).

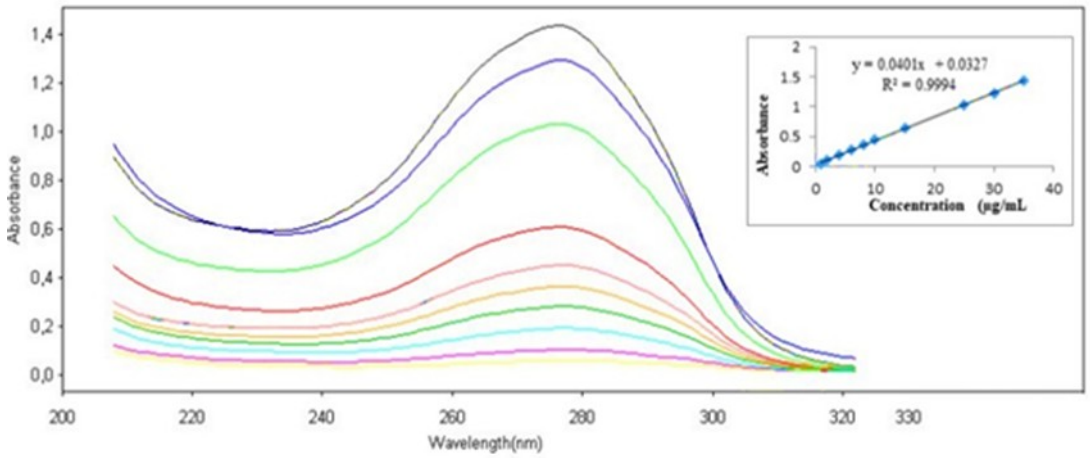

Figure 3: UV spectrum of standard solutions (1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 20.0, 25.0, 30.0, and 35.0 µg/mL) of cefuroxime axetil.

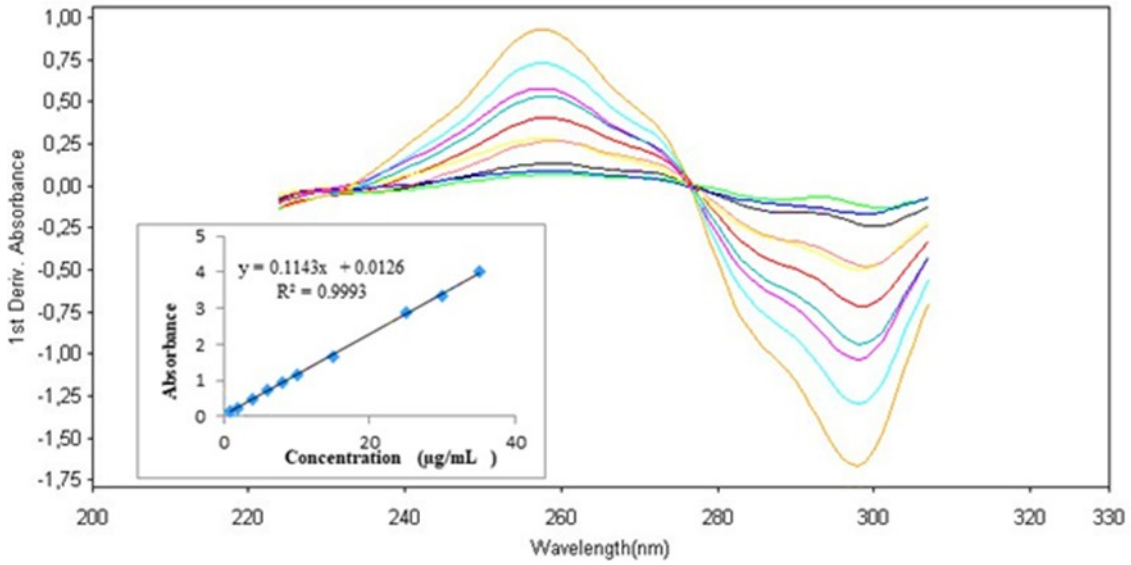

Figure 4: First-order derivative spectrum of standard solutions (1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 20.0, 25.0, 30.0, and 35.0 µg/mL) of cefuroxime axetil.

3.1.4. Precision and accuracy

Precision and accuracy were determined by intra-day and inter-day experimental. Intra-day precision and accuracy; QC solutions at 4 different concentrations (1.5, 4.5, 9.5, and 30.0 µg/mL) for UV and first-order derivative spectroscopy methods and 3 different concentrations (0.4, 4.0, and 8.0 µg/mL) for HPLC method were determined by measuring six replicates on the same day. Inter-day precision and accuracy were determined by analyzing the same samples over the 6 following days.

Intra-day and inter-day precision and accuracy were given with the relative standard deviation (RSD%) and the relative error (RE%), respectively. Obtained results for precision and accuracy were found to be acceptable (Table 2).

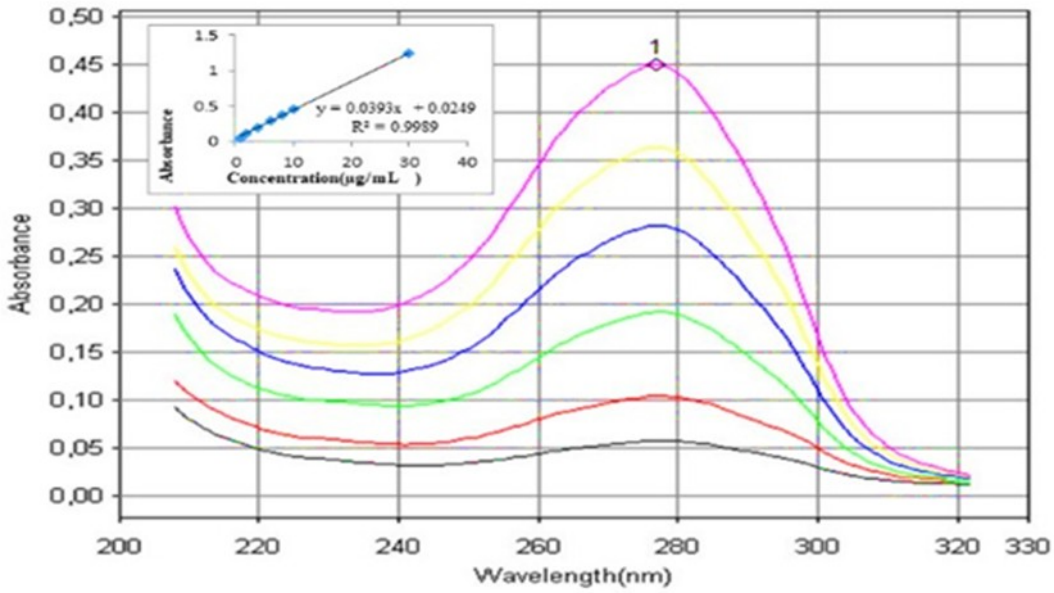

Figure 5: UV spectrum of extracted solutions (1.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 30.0 µg/mL) from plasma of cefuroxime axetil.

Table 1: Statistical analysis values of calibration curves derived from cefuroxime axetil standard working solutions and plasma samples.

	HPLC method	**UV spectroscopic method**	**First-order derivative spectroscopy method**	**UV spectroscopic method for plasma**
Concentration (µg/mL)	0.3-12.0	1.0-35.0	1.0-35.0	1.0-30.0
λ (nm)	280	277	298	277
LRA[a]	0.4455x+0.0036	0.0401x + 0.0327	0.1143x + 0.0126	0.0393x + 0.0249
Sa	2.0×10^{-2}	2.4×10^{-3}	3.9×10^{-4}	8.3×10^{-4}
Sb	3.8×10^{-2}	8.3×10^{-2}	3.42×10^{-3}	7.3×10^{-3}
R^2	0.9998	0.9997	0.9996	0.9994

aBased on six calibration curves; λ: Wavelength, LRA: Regression Equation, Sa: Standard Error of Slope, Sb: Standard Error of Intercept, R2: Regression Coefficient.

Table 2: Precision and accuracy of the proposed methods (n=6).

Method	**λ (nm)**	**Added (µg/mL)**	**Intra-day**			**Inter-day**		
			Average± SD (µg/mL)	**Precision RSD%**	**Accuracy RE%**	**Average± SD (µg/mL)**	**Precision RSD%**	**Accuracy RE%**
UV spectroscopy	277	1.5	1.40±0.02	1.42	-6.66	1.41±0.02	1.42	-6.00
		4.5	4.37±0.02	0.46	-2.89	4.42±0.02	0.45	-1.78
		9.5	9.22±0.01	0.23	-2.95	9.43±0.02	0.21	-0.74
		30.0	31.02±0.09	0.30	3.40	30.10±0.09	0.30	0.33

First-order derivative spectroscopy	298	1.5	1.43±0.01	0.69	-4.67	1.45±0.01	0.41	-3.33
		4.5	4.37±0.01	0.23	-2.89	4.40±0.01	0.23	-2.22
		9.5	9.33±0.01	0.11	-1.79	9.43±0.01	0.11	-0.74
		30.0	29.38±0.02	0.07	-2.07	29.31±0.02	0.07	-2.30
UV spectroscopy in plasma	277	1.5	1.46±0.03	2.05	-2.67	1.51±0.05	3.31	0.67
		4.5	4.35±0.03	0.69	-0.03	4.36±0.03	0.69	-3.11
		9.5	9.48±0.03	0.32	-0.21	9.58±0.03	0.31	0.84
HPLC	280	0.4	0.41±0.01	2.43	2.50	0.42±0.01	2.38	5.00
		4.0	4.07±0.04	0.98	1.75	4.05±0.03	0.74	1.25
		8.0	8.26±0.01	0.12	3.25	8.28±0.06	0.72	3.50

λ: wavelength; RSD: Relative Standard Deviation; RE: Relative Error.

3.1.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

In UV and first-order derivative spectroscopy methods, a series of standard solutions were prepared at smaller concentrations than the smallest concentration on the calibration curve and the absorbance values of those solutions were monitored by six times. The RSD% values of these samples were determined. The concentration with a RSD% value less than 20% was defined as the LOD value, and the concentration value less than 10% was defined as the LOQ value. LOQ and LOD values of the proposed methods were 1.00 µg/mL and 0.40 µg/mL, respectively. For the plasma study, LOQ and LOD values of UV spectroscopy method were 1.01 µg/mL and 0.45 µg/mL, respectively.

In HPLC method, the lowest concentrations were assayed where the signal /noise ratio was at least 3, this concentration was described as LOD. The LOQ was defined as a signal/noise ratio of 10. Under these chromatographic conditions, LOQ and LOD values for proposed method were calculated as 0.10 and 0.03 µg/mL, respectively.

3.1.6. Recovery

Analytic recovery from the tablet formulations was carried out the standard addition method. For this purpose, the tablet solutions of Cefaks, Cefurol, Aksef ve Enfexia tablets as described in the Section 2.5 were prepared at a concentration of 10 µg/mL, and the absorbance of these tablet solutions was measured by the available spectroscopy methods. Then, the QC solutions in three different concentrations (1.5, 4.5 and 9.5 µg/mL) were added separately to the tablet solutions and the absorbance of these total solutions obtained by the present methods were measured again.

For HPLC method, the solutions of Cefaks, Cefurol, Aksef ve Enfexia tablets at a concentration of 2.5 µg/mL was prepared. The chromatogram was taken and the peak area was determined. Then, the QC solutions in 3 different concentrations (0.4, 4.0, and 8.0 µg/mL) were added separately in tablet solutions and the chromatograms of these solutions were taken by HPLC.

The analytical recovery values of proposed methods were calculated with the formula:

$$\frac{the\ value^{*}\ of\ total\ solutions - the\ value^{*}\ of\ tablet\ solution}{the\ value^{*}\ of\ added\ QC\ standard\ solutions}\ x\,100$$

value* = absorbance or peak area of our measurement

The average analytical recovery value of tablets (four different tablets) of UV spectroscopy, first-order derivative spectroscopy and HPLC methods was determined as 100.1, 100.3, and 99.9%, respectively.

In the recovery study in plasma samples, the certain concentrations of standard working solution of cefuroxime axetil were spiked to the

blank human plasma, they were mixed and extracted (according to Section 2.3). The absorbance of the obtained extracts was read at 277 nm. The percent recovery values were calculated by proportioning the absorbance value of the standard solution at the same concentration with the absorbance values of the extracted solution. The mean recovery value from plasma was determined as 99.8%.

3.2. Discussion

For an analytical method to be of value, it must be developed and validated by studying parameters such as accuracy, precision, reproducibility, selectivity, specificity, sensitivity and short analysis time. Before starting on work in an analytical study, it is necessary to conduct a good literature review about the study. Therefore, we conducted a good

literature review in our study. In the literature review, many studies were found about the cefuroxime axetil. In the current study, UV spectroscopy, first-order derivative spectroscopy and HPLC methods for determined of cefuroxime axetil in tablet formulations and plasma were developed and validated.

UV spectroscopy method is accepted as one of the preferred techniques for the quantification of pharmaceuticals in terms of high sensitivity, medium and high selectivity, high accuracy, certainty, facility, and comfortability (21). In spectroscopy methods, derivatization agents used in some other methods were not needed. A calibration curve with very good linearity was obtained without the need for any derivatization attempt, and a satisfactory study was observed based on the recovery values. In the UV spectroscopy study, cefuroxime axetil gave maximum absorbance at a wavelength of 277 nm. In first order derivative spectroscopic study; two peaks, a maximum at 258 nm and a minimum at 298 nm, were observed. Our study was carried out at a wavelength of 298 nm. It was observed that the proposed spectroscopic method was linear in the concentration range of 1.0-35.0 µg/mL. LOD and LOQ values proposed methods was found as 0.4 µg/mL, and 1.0 µg/mL. The intra-day and inter-day precision (RSD%) and accuracy (RE%) are lower than 1.5% and 7.0%, respectively. UV spectroscopy and first-order derivative methods was successfully applied to 4 commercial tablets for determination of cefuroxime axetil and the average analytical recovery values of UV spectroscopy and first-order derivative spectroscopy method were determined as 100.1% and 100.3%, respectively.

The amount of cefuroxime axetil in plasma was tried to be determined by UV spectroscopy method. It was observed that the method was linear in the concentration range of 1.0-35.0 µg/mL. LOD and LOQ values proposed method was determined as 0.45 µg/mL, and 1.0 µg/mL, respectively. The intra-day and inter-day precision and accuracy values were showed that lower than 3.5%. Recovery value from plasma was found as 99.8%.

Chromatography is widely used in the separation, identification and quantification of substances in a mixture (22). Between these methods; HPLC has more advantages such as accuracy, precision, repeatability, selectivity, sensitivity, recovery, having the opportunity for analysis with low-volume samples and getting results rapidly. For these reasons, HPLC has been used commonly in the drug industry for the quantification of tablets or the analysis of active substances in biological fluids. In this study, a new HPLC method was developed for determination of cefuroxime axetil in tablets. In the HPLC study, some parameters like temperature, column, mobile phase components and percentages may affect the run time. So, optimization of chromatographic conditions was needed to improve the distinction and acceptable results. In the study, the working parameters were optimized by trial-and-error method based on the literature. The reverse phase C18 column (5 µm, 250 x 4.6 mm) was used and different mobile phase mixtures (0.1% acetic acid-acetonitrile rate: 30:70, 20:80 and 10:90 v/v) tested. According to the results obtained, the value in which 0.1% acetic acid-acetonitrile rate (30:70, v/v) was determined as optimum. The column temperature, mobile phase flow rate, wavelength and injection volume were used as variable, 1.0 mL/ min, 280 nm and 10 µL, respectively, and also etodolac was used as IS (2.5 µg/mL). The concentration range of 0.3-12 µg/mL of HPLC method was determined to be linear. A calibration curve was obtained by plotting the ratio of the peak area of cefuroxime axetil to the peak area of IS (etadolac) against the concentration of each solution. The LOQ and LOD values of proposed method was found as 1.0 µg/mL and 0.3 µg/mL, respectively. The intra-day and inter-day precision (RSD%) and accuracy (RE%) were showed that lower than 2.5% and 5.0%, respectively. Average analytical recovery value from tablets was 99.9%.

The developed and validated methods were applied to determine the amount of active substance (cefuroxime axetil) in four different tablets (Cefaks, Cefurol, Aksef ve Enfexia tablets). The analysis time of our HPLC method is shorter than the others in the literature and it is a big superiority. Our method differs from the methods reported in the literature considering the chromatographic parameters used such as UV wavelength, column, temperature, detector, flow rate, preparation procedures of bulk and plasma samples.

4. CONCLUSION

In summary, UV spectroscopy, first-order derivative spectroscopy and HPLC methods were developed for the determination of the active substance of cefuroxime axetil in bulk and tablets. UV spectroscopy method was also developed and validated for the determination of cefuroxime axetil in plasma. Results showed that the methods are suitable for accuracy, precision, sensitivity, recovery, linearity, specificity, and selectivity parameters for determination of cefuroxime axetil. The methods can be applied for routine analysis of cefuroxime axetil in quality control assays and pharmaceutical preparations.

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