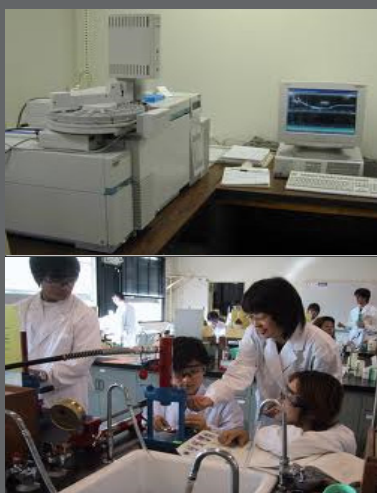


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# Development and Validation of a Liquid Chromatographic Method for the Determination of Cefdinir Residues on Manufacturing Equipment Surfaces

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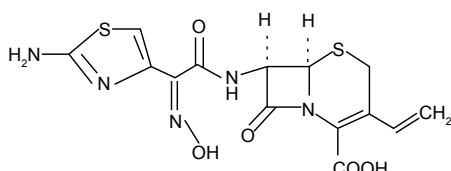
## Abstract

The cleaning validation procedure for the manufacturing equipment surfaces of cefdinir was done using cotton swabs moistened with the extraction solution (900 ml of water and 3.0 ml phosphoric acid and then adjusting the pH to  $7.0 \pm 0.05$ ). The HPLC method was validated on a LC system using Waters (USA) Symmetry - C<sub>18</sub> (250 mm×4.6 mm×5 μm) at 25°C in the presence of a mobile phase composed of acetonitrile: pH 7 buffer (85-15) as at flow rate of 1.0 ml/min and an injection volume of 20 μl over the concentration range 14.5-74.5 μg mL<sup>-1</sup>. UV detection was made at 254 nm. The detection limit (DL) and quantification limit (QL) were 0.7 and 2.2 μg mL<sup>-1</sup>, respectively. The intra-day and inter-day precisions, expressed as relative standard deviation (R.S.D.), were below 2.00%. The recoveries were 98.68, 101 and 102.28% for three concentration levels with an average recovery of 100.65%.

**Keywords:** Cefdinir; HPLC-UV; Cleaning validation; Residues; Swab analysis

## Introduction

Good manufacturing practice dictates that the equipment should be to manufacture pharmaceuticals must be in a clean and orderly manner [1]. The same equipment may be used for processing in different products, the cleaning procedure validation describe responsibilities, facilities, cleaning strategies. It is of great importance to evaluate carefully the material to be used. Chemically, Cefdinir is [6R-[6α, 7β (Z)]]-7-[[[(2-amino-4-thiazolyl) (hydroxyimino)acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (Scheme 1).



Non-extractive visible spectrophotometric method was proposed for the determination of cefdinir based on the formation of donor-acceptor complex between Cefdinir and Fe in a buffered medium [2-8].

The goal of this study was to develop and validate a simple analytical method for the determination of trace levels of Cefdinir residues in production area equipment. The method validated considering accuracy, selectivity, precision, linearity, and limits of detection (LOD) and quantification (LOQ).

## Experimental

### Reagent and chemicals

Cefdinir reference standard of United States Pharmacopoeia (USP) was bought from Sigma, United States. A fixed dose combination (FDC) was obtained from manufacturer, Cefdin Capsules 300 mg (Novartis) produced by SB-Egypt. Disodium EDTA (EDTA), 85% phosphoric acid, potassium hydroxide, Tetrabutyl ammonium hydroxide (25% aqueous) (TBAH) and acetonitrile were of chromatographic grade and were purchased from Merck company, Germany. All chemicals and water used were of HPLV analytical reagent grade.

A buffer of pH 7.0 was prepared by dissolving 16 mg of (EDTA) with 8.3 ml of (TBAH) in 800 ml of water then adjusting to pH 7.0 ±

0.05 with phosphoric acid and mix.

Alpha Swab polyester on a propylene handle-TX714A (ITW Texwipe, Mahwah, USA) have been used during samples analysis.

### Equipment

Chromatographic separation was performed on Agilent 1100 series liquid chromatographic system consisted of a quaternary pump, an automatic injector, a column oven and multiwavelength detector G1315A, all 1100 Series from Agilent Technologies, with HP Chemstation software. Symmetry-C<sub>18</sub> analytical column (250 mm×4.6 mm, 5 μm, Waters USA) have been used in HPLC separation, in the sample preparation procedure, ultrasonic instrument (China) and Orion Ross combination pH electrode (Model 81-02) was used for all pH measurements.

### Chromatographic conditions

The mobile phase consisted of 850 ml of buffer of pH 7.0 mixed with 150 ml Acetonitrile. The mobile phase solution was filtered through 0.45 μm membrane filter (Millipore) and degassed prior to use. Extraction solution consisted of 900 ml of water and 3.0 ml phosphoric acid and then adjusts to pH  $7.0 \pm 0.05$  with Potassium Hydroxide and mix.

All chromatographic experiments were performed in isocratic mode. The mobile phase was pumped at flow rate of 1.0 ml min<sup>-1</sup> with 20 μl injection volume. The column temperature was at 25°C. UV detection was performed at λ 254 nm.

### Standard solution preparation

Standard stock solution was prepared by weighing 10 mg of

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Cefdinir standard and transferring into a 200 ml volumetric flask, 100 ml of diluting solvent was added and the content of flask was sonified for 15 min. the solution in the flask was diluted to volume with diluting solvent. An aliquot of 10 ml was diluted to 100 ml and the final concentration being 0.005 mg/ml.

### Sample solution preparation

The selected surfaces (10 cm×10 cm) of stainless steel, previously cleaned and dried, were sprayed with 350 µL of stock standard solution (the stock solution of standard was prepared by accurately weighing 100 mg of Cefdinir reference standard and transferring into a 200 ml volumetric flask, Approximately 100 ml of diluting solvent was added and content of flask was sonified for 15 min. the solution in the flask was diluted to volume with diluting solvent) the final concentration being 0.018 mg/ml.

The background control sample was prepared from the extraction solvent.

## Results and Discussion

### Acceptance limit calculation

The calculated limit per surface area (LSA) in the case of Cefdinir was 1.75 µg /swab pro 100 cm<sup>2</sup>. A stainless steel surface area of 10 cm×10 cm was chosen for practical reasons.

### Optimization of the chromatographic conditions

For analysis the combination of water, tetraheptylammonium hydroxide, (EDTA), buffer 7, Phosphoric Acid and Acetonitrile is frequently used as the mobile phase. The amount of Acetonitrile was varied 12.0% to 20.0%, wavelength detector (λ) was varied 250 nm to 258 nm and flow rate varied 0.8 ml/min to 1.1 ml/min. The sufficient tailing factor and plate number were achieved with the proposed mobile phase (16 mg of (EDTA) with 8.3 ml of (TBAH) in 800 ml of water then adjust to pH 7.0 ± 0.05 with phosphoric acid and mix) at flow rate 1.0 ml/min. Wavelength 254 nm was selected for detection. The calibration curve obtained at 254 nm showed good linearity. Regarding the chromatographic procedure, different C18 columns were evaluated but the Symmetry C18 5 µm (250×4.6 mm) was preferred to improve

the plate number and tailing factor. The analysis was performed at 25°C to improve the tailing factor and plate number.

### Optimization of the sample treatment

Different quantities of Cefdinir have been spiked and placed into tubes. 10 ml of pH 7.0 as extraction solvent and sonification time of 5 min were the optimum conditions. This technique was applied in the subsequent work. The samples were calculated by the following equation:

$$\frac{\text{Area of sample} \times \text{Standard wt}_{(\text{mg})} \times \text{Potency of st } (\%) \times 10 \times 10}{\text{Area of Standard} \times 200 \times 100 \times 100}$$

Then the equation can be simplified to:

$$\frac{\text{Area of sample} \times \text{Standard wt}_{(\text{mg})} \times \text{Potency of st } (\%)}{\text{Area of Standard} \times 20000}$$

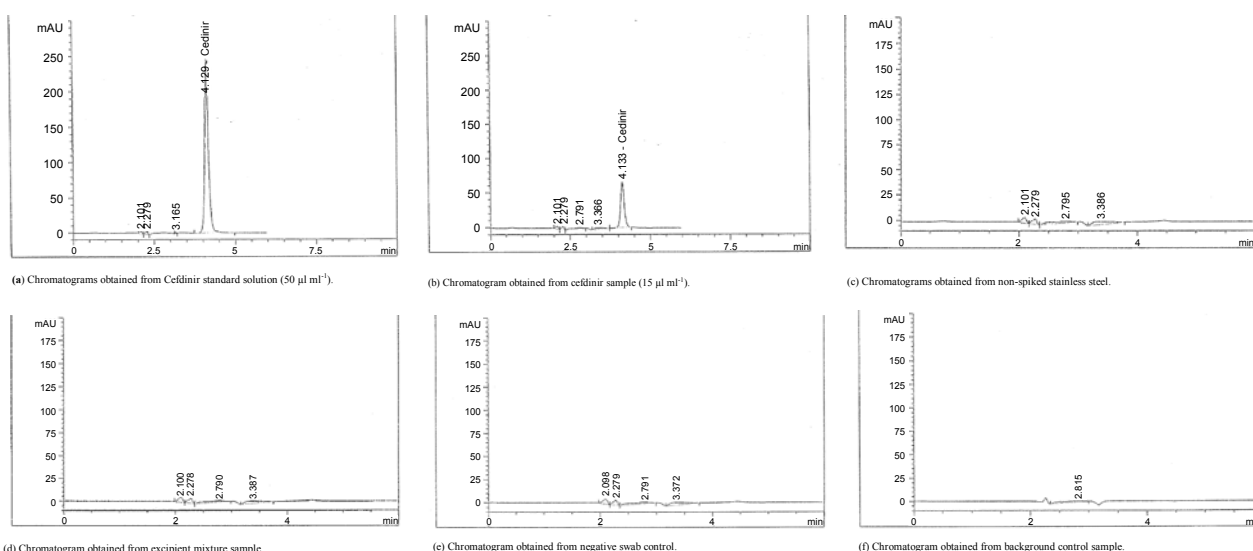
### Validation of the method

**System suitability:** In all cases relative standard deviation (RSD) of the peak areas was <2.0%, the average number of theoretical plates per column was >5800 and the USP tailing Factor ≤ 1.5.

**Specificity:** The specificity of the method was checked by injection the Cefdinir standard, Cefdinir sample, the background control sample, the negative swab control, un-spiked stainless steel 10 cm×10 cm plate swabbed as described, four standard solutions after storage under destructive condition (80°C for 24 hrs), (in 0.05 M Hydrochloric Acid for 24 hrs), (in 0.05 M Sodium Hydroxide for 24 hrs) and (in 3% H<sub>2</sub>O<sub>2</sub> for 24 hrs). Cefdinir has chromatographic resolution more than 1.5 from other peaks. The results are shown in figures 1a-1f.

**Linearity:** Linearity of the method has been studied by analyzing standard solutions at seven different concentration levels in the range from 15-74.5 µg mL<sup>-1</sup> with triplicate determination at each level. The calibration curve values of intercept, slope and correlation coefficient for Cefdinir are presented in table 1.

**Limit of detection (LOD) and Limit of quantification (LOQ):**



**Figure 1:** Chromatograms obtained from (a) Cefdinir standard solution, 50 µl ml<sup>-1</sup>, (b) Cefdinir sample 15 µl ml<sup>-1</sup>, (c) non-spiked stainless steel, (d) excipient mixture, (e) negative swab control and (f) background control sample.



Statistical Parameters	Values
Concentration Range $\mu\text{g mL}^{-1}$	15.0 - 74.0
Regression equation	$Y=38229X-7.3419$
Coefficient of Determination	0.9999
S(a)-error in intercept	0.39

**Table 1:** Linear regression data in the analysis of Cefdinir.

Amount added $\mu\text{g mL}^{-1}$	Amount found $\mu\text{g mL}^{-1}$	95% confidence interval %	Recovery %	RSD % n=6
14.89	14.70	97.1-100.3	98.68	1.88
49.65	50.06	101.2-102.4	101	0.69
74.47	76.17	102.26-102.30	102.28	0.02

**Table 2:** Precision and accuracy of the results obtained from swabbed plates spiked with Cefdinir.

Chromatographic parameter	RSD %	Tailing factor	Plate count	Sample Result (ppm)
1) Wavelength (nm)				
250	0.27	1.2	5869	1.70
252	0.28	1.2	5874	1.30
254	0.42	1.2	5866	1.30
256	0.45	1.2	5870	1.30
258	0.42	1.2	5873	1.30
2) Flow rate ( $\text{ml min}^{-1}$ )				
0.8	0.09	1.2	28089	1.45
0.9	0.05	1.2	6695	1.50
1	0.42	1.2	5866	1.30
1.1	0.07	1.1	6016	1.40
3) Column temperature ( $^{\circ}\text{C}$ )				
20	0.27	1.1	6254	2
23	0.18	1.1	6435	1.4
25	0.42	1.2	5866	1.32
27	1.98	1.1	6632	1.62
4) % of Acetonitrile content in the mobile phase				
12.0%	0.69	1.0	6092	2
15.0%	0.42	1.2	5866	1.3
18.0%	1.16	1.4	10988	2.3
20.0%	1.75	1.5	14231	1.5

**Table 3:** Effect of different chromatographic parameters.

The LOD and LOQ for Cefdinir were found to be 0.7 and  $2.2 \mu\text{g mL}^{-1}$ , respectively.

**Precision and accuracy:** Precision and accuracy were also inspected after storage for 24 hours at room temperature  $25^{\circ}\text{C}$  with 1.6% and 0.30% difference in results for standard & samples respectively.

**Filter evaluation:** Samples and standard solutions of Cefdinir prepared as per analysis method, filtered with Millipore millex-HV-PVDF  $0.45 \mu\text{m}$  and millex-PTFE-  $0.45 \mu\text{m}$ , and then compared to the unfiltered samples. The Millipore millex- HV-PVDF  $0.45 \mu\text{m}$  and millex-PTFE- $0.45 \mu\text{m}$  pore size syringe filter were qualified for use with filter evaluation ratio 100.1% and 100.30 for Cefdinir standard with PVDF and PTFE filter respectively. For samples the filter evaluation ratio 100.8% and 100.3% for PVDF and PTFE filter respectively (Table 2).

## Robustness

In order to test the robustness of the HPLC-UV method, the effect of different chromatographic parameters on the resolution and the concentration of cefdinir from cleaning samples, was estimated. The

Serial no.	Location description	Results (ppm)
1	Inner surface of V- end point	13.0
2	Inner surface of the cover	13.0
3	Inner surface of the right side	1.01
4	inner surface of the left side	1.01
5	inner surface of the discharging opening	1.44

**Table 4:** Determination of Cefdinir in actual swab samples collected from  $100 \text{ cm}^2$  swabbed areas from different locations of the equipment train (V- Blender).

amount of acetonitrile in the mobile phase was varied from 12% to 20%, the flow rate was varied from  $0.8 \text{ ml min}^{-1}$  to  $1.1 \text{ ml min}^{-1}$ , column temperature was varied from  $20^{\circ}\text{C}$  to  $27^{\circ}\text{C}$  and the wavelength detector ( $\lambda_{\text{max}}$ ) was varied from 250 nm to 258 nm. The results obtained have been showed in table 3.

## Assay of swab samples collected from different locations within the equipment train

The residual of cefdinir have been analyzed by the proposed method, results obtained are presented in table 4.

## Conclusion

In the present study an HPLC-UV method is proposed for the determination of cefdinir residues on manufacturing equipment surfaces applying a wipe test procedure using a cotton swab. The LOD and LOQ for cefdinir were found to be 0.7 and  $2.2 \mu\text{g mL}^{-1}$ , respectively. Linearity of the method was studied by analyzing standard solutions at seven different concentration levels range from  $15\text{-}74.5 \mu\text{g mL}^{-1}$  with triplicate determination at each level. The Coefficient of determination is 0.9999. The method is selective, linear, precise and accurate with a RSD less than 2.0 and the recoveries obtained from the stainless steel surfaces were 99.0-102.0% without interferences from the cotton swab. Stability studies show that the Cefdinir samples are at least, stable over the investigated 24 hours.

## Acknowledgment

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