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Comparison of HPLC and UV Spectrophotometric Methods for the Determination of Cefaclor monohydrate in Pharmaceutical Dosages

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Abstract
This paper describes the development and evaluation of a HPLC and UV spectrophotometric methods to quantify Cefaclor Monohydrate in Oral suspensions and Capsules. HPLC analysis were carried out using a C_{18} Knauer column and a mobile phase composed of Triethylamine: methanol: Acetonitrile: water (2: 10: 20: 68)v\v%, with a flow rate of 1.0 mL/min and UV detection at 265 nm. For the spectrophotometric analysis, water was used as solvent and the wavelength of 264 nm was selected for the detection. Both methods were found to quantify Cefaclor monohydrate in Oral suspensions and Capsules accurately. Therefore HPLC and UV methods presented the most reliable results for the analyses of Oral suspension and Capsules.

Introduction

Cefaclor monhydrate (CAS 56238-63-2) (Figure 1) is a second generation cephalosporin with high antibacterial activity; it has enhanced in vitro activity against clinically important Gram- positive and Gram-negative microorganisms (1). The chemistry of cephalosporins has been widely explored because of their extensive medical applications (2). Several analytical procedures are available in literature for the analysis of antimicrobial. These methods are spectrophotometry (3–13), high performance liquid chromatography (14–19), capillary electrophoresis (20), fluorimetry (21–24), polarography (25–29),titrimetry (30), and bioassay (31–32). Spectrophotometric assay for determination of other cephalosporins as ceftazidime has been described (33) but no method for Cefaclor monohydrate had been previously described.

The purpose of this study was to develop and validate analytical methods to quantify Cefaclor monohydrate in Capsules & Oral suspensions, using HPLC and UV spectrometry. The results obtained by these methods were statistically compared, by using analysis of variance (ANOVA). In addition, the reliability and feasibility of them were evaluated focusing on routine quality control analysis.

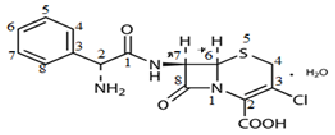

Figure 1. Structure of Cefaclor monohydrate

Experimental

Reagents and materials

Cefaclor monohydrate reference standard was kindly donated by Parabolic Indian Ltd. The Capsules and Oral Suspensions were purchased from Medico Labs-Homs-Syria and Oubari Company-Aleppo-Syria. Ultra Pure Water was purified by using a Millipore system (Bedford, MA). Methanol, Acetonitrile, and Triethylamine (HPLC grade) was obtained from Merck (Fairfield, OH).

Instruments and analytical conditions

All HPLC measurements were made on a Waters 1525 Binary HPLC Pump, consisting of a 7725i manual injector with a 20 µL loop (Rheodyne, Torrance, CA), integrated UV detector UV–vis (Milford, MA). The system employed a 250 mm × 4.6 mm C18 column Wat 054275 (Milford, MA) and particle size of 5 µm guard column. The detector was utilized at 265 nm and UV spectra from 200 to 400 nm were recorded on line for peak identification. The mobile phase consisted of Triethylamine: methanol: Acetonitrile: Ultra Pure water (2: 10: 20: 68)v\v%, at a flow rate of 1.0 mL/min. The injection volume was 20 µL. Ultraviolet spectrophotometric analyses were carried out on a UV-Vis Shimadzu UV mini 1240 (Shimadzu, Kyoto, Japan) spectrophotometer, in a 1 cm quartz cubette. The wavelength of 264 nm was selected for the quantitation of Cefaclor monohydrate and the measurements were obtained against water as a blank.

Preparation of standard and sample solutions

The standard stock solutions were prepared by dissolving 10 mg of Cefaclor monohydrate reference standard in 10 mL of water to get a concentration of 1 mg/mL. An aliquot of 100 µL of the obtained solution was transferred to a 10 mL volumetric flask. The volume was adjusted with Ultra Pure water for spectrophotometric and

chromatographic analysis, resulting in solutions of 10 µg/mL.

The sample solutions were prepared by dissolving 10 mg of Cefaclor monohydrate powder for Capsules or Oral suspensions in 10 mL of water to get a concentration of 1 mg/mL. An aliquot of 100 µL of this solution was transferred to a 10 mL volumetric flask. The volume was adjusted with water for spectrophotometric analysis or mobile phase for chromatographic analysis, to obtain a solution at 10µg/mL of Cefaclor.

Validation

The optimized spectrophotometric and chromatographic methods were completely validated according to the procedures described in ICH guidelines Q2(R1) for the validation of analytical methods (34).

Linearity

Standard solutions containing 1000 µg/mL of Cefaclor monohydrate in water were prepared, in triplicate. Aliquots of these solutions were diluted in water. Eight different concentrations, corresponding to 1.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0 and 60µg/mL of Cefaclor (for UV analysis) and Twelve different concentrations, corresponding to 0.1, 0.5, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0 and 80.0µg/mL of Cefaclor (for HPLC analysis). Calibration curves with concentration versus peak area or absorbance were plotted for each method and the obtained data were subjected to regression analysis using the least squares method.

Precision

The intra-day precision was evaluated by analyzing six samples (n = 6), at the test concentration of 10 µg/mL, using the UV and the HPLC methods. Cefaclor monohydrate contents and the relative standard deviations (RSD) were calculated.

Accuracy

Cefaclor monohydrate reference standard was accurately weighed and added, at three different concentrations. At each concentration, sample were prepared in triplicate and the recovery percentage was determined by UV and HPLC methods.

Robustness

The robustness of the method was determined by the variation of the analyst and mobile phase flow rate.
The flow rate was checked in 0.8 mL to 1.0 mL.

Analysis of Cefaclor monohydrate powder for Capsules& Oral Suspension

Samples of Medaclor, Oraclor were analyzed by the validated HPLC and UV methods. The sample solutions for the HPLC and UV analyses were prepared as described previously. The Cefaclor monohydrate contents were determined by using the two methods and the obtained results were statistically compared by using ANOVA test and Tukey's multiple comparison test, applied at 0.05 significance level.

Results and Discussion

During the chromatographic method development, Ultra Pure Water showed to be a more adequate organic solvent than Methanol, regarding the Cefaclor monohydrate retention. A typical chromatogram obtained is as shown by Figure 2.

After the evaluation of the Cefaclor monohydrate UV spectrum in various solvents (Ultra Pure water, methanol, (Ultra Pure Water: Methanol) (50:50)v\v%, hydrochloric acid 0.1M, and sodium hydroxide 0.1 M) In the range of 200–400 nm (Figure 3), the wavelength of 264 nm was chosen due to the adequate molar absorptivity of Cefaclor monohydrate in this region and to minimize possible interference from other compounds and solvents in the samples.

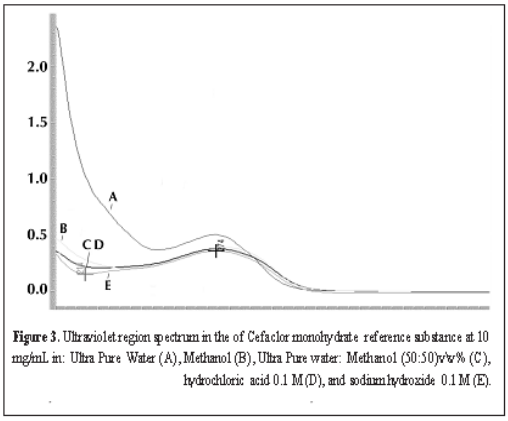

Figure 3. Ultraviolet-region spectrum in the of Cefaclor monohydrate reference substance at 10 mg/mL in: Ultra Pure Water (A), Methanol (B), Ultra Pure water: Methanol (50:50)v/v% (C), hydrochloric acid 0.1 M (D), and sodium hydroxide 0.1 M (E).

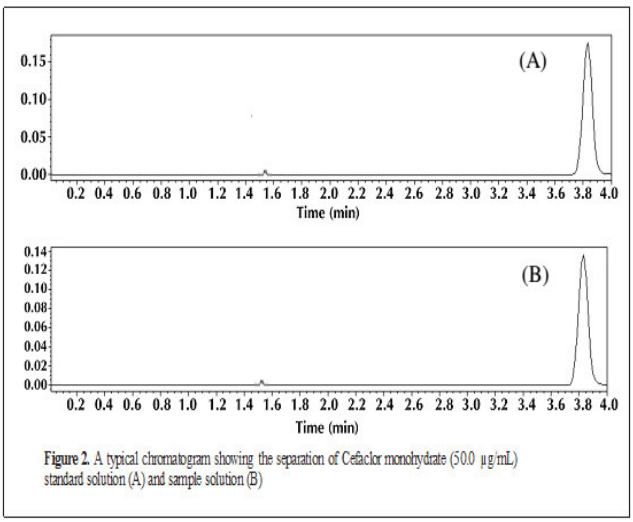

Figure 2. A typical chromatogram showing the separation of Cefaclor monohydrate (50.0 µg/mL) standard solution (A) and sample solution (B)

A linear relationship was found between the Cefaclor monohydrate concentrations and the response of both HPLC and UV methods. The regression analysis data are presented in Table I. High regression coefficient (r2) values were obtained (0.9995 and 0.9996, respectively). A random pattern of the regression residues was found and no significant deviation of linearity was detected in the assayed range.

The precision data obtained for the evaluated methods are demonstrated in Table II. Both methods presented RSD values lower than 2.0%, assuring a good precision.

Accuracy (Table II) was investigated by means of a standard addition experiment. Both chromatographic and spectrophotometric methods exhibited mean recoveries (n = 9) close to 100% demonstrating an adequate accuracy.

The difference in the retention time, the peak area and the analyst (for a given Cefaclor monohydrate concentration) caused by the aforementioned minor alterations were insignificant (Table II).

Table I. Overview of the Linearity Data Obtained for Cefaclor monohydrate by the Chromatographic and Spectrophotometric Methods

Regression parameters	HPLC	UV
Regression coefficient (r^2)	0.9995	0.9996
Slope ± standard error	0.199 ± 0.20	0.025 ± 0.0017
Intercept ± standard error	0.205 ± 0.11	0.006 ± 0.010
Relative standard error (%)	1.13	1.78
Concentration range (µg/mL)	0.1-80.0	1.0–60.0
Number of points	12	8

Table II. Validation Paramaters of the Evaluated Methods for Cefaclor monohydrate Determination

Validation parameters	HPLC	UV
Intra-day precision, n = 6 (RSD%)	1.13	1.78
Accuracy, n = 9 (mean recovery, %) (10 µg/mL)	100.10	100.82

Table III. Robustness of the HPLC Method for Cefaclor monohydrate by Varying the Analyst

Analyst	Area	Mean ± SEM	RSD(%)
1	691545 682258 685912 691089 699896 680178 691563	688479 ± 0.27	0.72
2	613157 599899 651955 630085 618799	634243 ± 1.25	3.31

RSD = relative standard deviation
SEM = standard error mean

Table IV. Robustness of the HPLC Method for Cefaclor monohydrate by Varying the Mobile Phase Flow Rate

Flow (mL/min)	Area	Mean ± SEM	RSD (%)
0.8	691545 682258 685912 691089 699896 680178 699595	688479 ± 0.27	0.72
1.0	692278 695982 698189 699396 690578	696003 ± 0.14	0.38

RSD = relative standard deviation.
SEM = standard error mean.

Table V. Cefaclor monohydrate Contents in Injectable Samples Obtained by HPLC and UV (n = 6)

Cefaclor monohydrate content (%) ± S.D.		
Sample	HPLC	UV
Mediaclor 500mg\capsules	99.84 ± 0.24	99.49 ± 0.62
Oraclor 250mg\5ml	101.74 ± 0.36	100.99 ± 0.92

S.D.: standard deviation.

Analysis of Capsules & Oral suspensions Cefaclor monohydrate

The validated chromatographic and spectrophotometric methods were applied to the analysis of Cefaclor

monohydrate in Medaclor, Oraclor (Table III). ANOVA test revealed a statistically significant difference between the results obtained for injectable samples, from the distinct methods, at a confidence level of 0.05. Chromatographic analysis showed to be the most sensitive and selective method, and might be applied successfully for Cefaclor monohydrate trace analysis and quantitation in biological matrices. We cannot discharge, however, the analyses time and cost. The spectrophotometric method is clearly less expensive and requires shorter analysis time, besides the ease of handling and lower residues generation.
Since the use of Cefaclor monohydrate as a potent antimicrobial drug is widespread, the development and validation of simple and reliable methods are essential to assure the quality of the raw materials and pharmaceutical formulations marketed nowadays. A simple method to identify and precisely quantify these drugs may be an important tool to avoid treatment inefficacy and development of resistance due to the exposition to sub therapeutic doses (35).

Conclusion
HPLC and UV spectrophotometry were found to be adequate methods to quantify Cefaclor monohydrate in Capsules & Oral suspensions solutions; the chromatographic and spectrophotometric methods presented the most reliable results. Since these methods are fast and simple, they may be successfully applied to quality control analyses, with the aim of quantifying and identifying Cefaclor monohydrate in pharmaceutical products.

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