ORIGINAL ARTICLE

Quantitative Analysis of Cephradine using the Modern High-performance Liquid Chromatographic Method

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Abstract

Context: Cephradine is the most important type of antibiotics used vary widely. Analysis of these antibiotics is the challenge because of their sensitivity and instability to different conditions. **Objective:** The present article is extended to find out HPLC method. **Materials and Methods:** The Standard Solution 100 µg/ml Cephradine was prepared by dissolving accurately weight 100 mg of Cephradine in 1000 ml methanol and the range 2.5-12.5 µg/ml was prepared by serial dilution of the sample with methanol. HPLC-UV system with Ion Pac column Zorbax 300-SCX Agilent Column; 5µm, 4.6×250 mm used for analysis for Cephradine in formulations capsules. The chromatographic conditions used for the analysis as well as analytical parameters study carried out with experimental conditions. HPLC method is the most common method for the analysis of Cephradine in formulation and in biological fluids, the several analytical procedures have been described for analysis of Cephradine in different pharmaceutical the formulations. **Result and Discussion:** During the study analytical parameters studied such as range, linearity, precision, accuracy, *LLOD, LLOQ*. Cephradine as formulation capsules was almost stable at room temperature up to 2-3 days in the aqueous medium at pH between 4 and 5. The focus of the study of analysis of Cephradine in formulation capsules is used for the determination of various Cephradine for supported by budding researchers. **Conclusion:** This study suggested a simple, easy and highly sensitive method using standardized samples obtained from the best international companies. Comparisons were made with different commercial samples of cephradine antibiotics. The actual ratios of cephradine were determined in commercial pharmaceuticals.

Key words: Antibiotic drugs, cephradine capsules formulation, high-performance liquid chromatography-ultraviolet protocol

INTRODUCTION

Cephradine($C_{16}H_{19}N_3O_4S$)is(6*R*,7*R*)-7-[(*R*)-2-amino-2-(1,4-cyclohexadien-1-yl) acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, which is a first-generation antibiotic of the semisynthetic cephradine series. show in Figure 1: (a and b)

SYNTHESIS

1,4-cyclohexadiene rings are nearly as planar as benzene rings, but of greatly different reactivity, a cephalosporin was synthesized with such a moiety. The Birch reduction is an organic reaction which is particularly useful in synthetic organic chemistry. The reaction was reported in 1944 by the Australian chemist Arthur Birch (1915–1995) working in the Dyson Perrins Laboratory at the University of Oxford, building on earlier work by Wooster and Godfrey published in 1937. It converts aromatic compounds having a benzenoid ring into a product, 1,4-cyclohexadiene, in which two hydrogen atoms have been attached on opposite ends of the molecule. It is the organic reduction of aromatic rings in liquid ammonia with sodium, lithium, or potassium and an alcohol, such as ethanol and tetra-butanol. This reaction is quite unlike catalytic hydrogenation, which usually reduces the aromatic ring all

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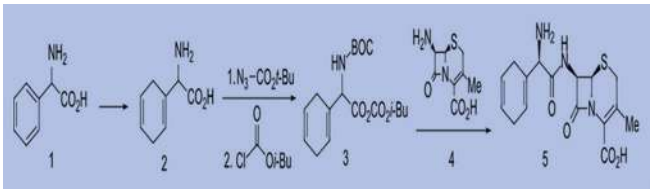
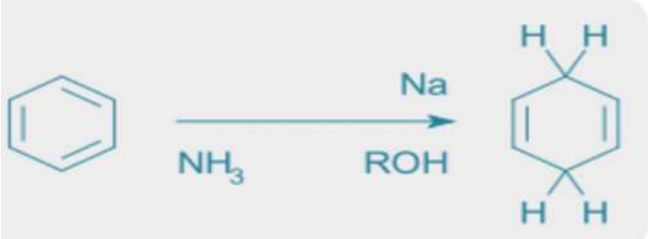
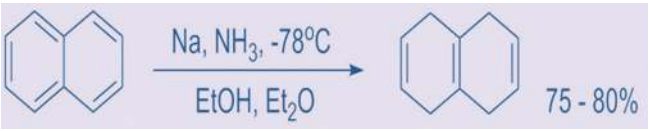
the way to a cyclohexane.[1-6]



The original reaction reported by Arthur Birch in 1944 used sodium and ethanol. Alfred L. Wilds later discovered that lithium gives better yields. Furthermore, the use of tetra-butyl alcohol has become common. The reaction is widely used in synthetic organic chemistry.[7,8]



An example is the reduction of naphthalene.[9]



EVOLUTION OF METHODOLOGY

Cephradine is available in different dosage forms such as capsule, dry suspension, and IV injection. According to the previous reports, cephradine itself tends to be quite stable at pH 4-5, but it is extremely important to know the compatibility of the drug and its excipients in the formulation which may import the stability and effectiveness of the drugs. It is also noted that the excipients may be different from different manufacturers which may affect the stability. This paper describes a quantitative assay of cephradine along with the assessment of the potency of a cephradine capsules formulated.

There are various methods used for the analysis of cephradine in the various forms such as chromatographic, ultraviolet (UV), and electrophoresis, but the most important in all methods is the high-performance liquid chromatography (HPLC)-UV method.[10-12]

HPLC-UV methods are fast, but it requires elevated temperature, it may cause thermal degradation of drugs, and to avoid that, it requires derivatization to improve volatility and to improve chromatographic behavior. Hence, these methods are not applicable for antibiotics.

The method HPLC-UV has very low detection limits so this technique has the ability to estimate the archeological samples from pharmaceutical drugs. HPLC can be a valuable tool in the analysis and evaluation of pharmaceutical, organic, and high-volatility samples and for this reason can be used in many pharmaceutical research. Many antibiotics contain ionizable group which can be analyzed by ion exchange chromatographic methods. The high-resolving power of HPLC serves as a particularly important method for the isolation and purification of antibiotics.[13,14]

HPLC methods are the most common method for the analysis of cephradine in formulation, and in biological fluids, the several analytical procedures have been described for the analysis of cephradine in different pharmaceutical formulations.

There are various HPLC methods reported for the analysis of a single cephradine in pharmaceutical drugs. All these methods present a unique preparatory and chromatographic protocol. Several methods have been used for the analysis of cephradine which analyzes in HPLC method that is very accurate and sensitive.[15-17]

Very sensitive HPLC methods have been developed for the detection and quantitation of cephradine in a variety of biological matrices. The determination of cephradine is an issue that has significant importance from the healthy, social, and economic point of view. Despite the presence of many separation techniques, HPLC occupies a major rank in this regard that it is deemed as one of the methods used largely for the purpose of cephradine analysis. Yet, the reversed-phase chromatography is fairly recommended for the analysis of cephradine due to the ease of sample preparation, best reproducibility and detectability, lower cost, and less sample preparation. The most universal and versatile column is a bounded octadecyl silica column (Ion Pac column Zorbax 300-SCX Agilent Column; 5 µm, 4.6 mm × 250 mm). Before final selection of the column, column length, diameter, particle size, pore size, and carbon load should be taken into account. The use of HPLC is turned to be more familiar as the advent of diode array, and multi-wavelength detectors have enhanced the selectivity of the method by giving UV absorption profiles and derivative spectral data for each peak in the chromatogram. The analyst may apply the quantitative HPLC-described below-method despite the large variety of stationary and mobile phases. This method is applied for its own best performance. All methods should be properly validated and/or verified before the routine application.[18-21]

The aim of the study

The main aim of this study was to develop an efficient new method for HPLC-UV system for the determination of cephradine as standard and two commercial penicillin in antibiotic drugs.

Equipment

Chromatography experiments were carried out by HPLC-UV Chromatography consisting of:

- LKB Bump 2150–HPLC, Bromma.
- Ion Pac column Zorbax 300-SCX Agilent column; 5 µm, 4.6 mm × 250 mm (P/N 880952-704) from the USA was chosen for separation antibiotic drugs.
- Metrohm electric injection valve with 100 µL loop fitted in.
- A PD 303 UV Detector single beam (Japan) equipped with an 18 µl flow cell (Helma. UK.).
- Data logger LabJackU12 acquisions (Ocean control/ Australia).
- Personal Computer Supplied with modifying software programs/CVI programs UV.
- Printer (EPSON/Japan).

pH meter (Hana-Italy).

MATERIALS

All solvents and reagents were of analytical grade unless indicated otherwise, and all experiments were performed with deionized water (18.2 Ω-cm) resistivity at 25°C.[22]

Reagents and standards

- Acetonitrile; HPLC grade, BDH Chem. LTD 7526-13.
- Methanol; HPLC grade, BDH M/405/9 LTD 610098Cas58-33-1.
- Formic acid; BDH M/231/202LTD 12526 Cas98-142-2
- Commercial cephradine capsules from two companies.
- Analar Cephradine powder as standard Sigma-Aldrich, Germany.
- The Stock Standard Solution 100 µg/ml cephradine was prepared by dissolving accurately weight 100 mg of cephradine in 1000 ml methanol which was purchased from Aldrich 49/1586-LTD.
- A working solution in the range 2.5–12.5 µg/ml was prepared by serial dilution of this stock solution with methanol.
- Cephradine capsules as samples were prepared by powdering 10 capsules (500 mg) for each one, 100 mg of this powder accurately weights and dissolved in 1000 ml of methanol.

Procedure

Under a temperature of 25°C and pressure of 150 bar, all chromatography experiments were carried out by HPLC-UV chromatography system, which consisting LKB pump 2150-HPLC pumping the eluent at 1.2 ml/min. Cephradine capsules or standard was manually injected with Metrohm electronic injection valve fitted with the 100 µl loop in the eluent of water/methanol/0.5 M sodium acetate/0.7 N acetic acid (782:15:3) pH = 4.8, all with 10 mM formic acid at pH = 4–5. Ion Pac column Zorbax 300-SCX Agilent, 5 µm, 4.6 mm × 250 mm (p/N880952-704), was used as a separation column. APD 303UV detector single-beam spectrophotometer (Japan), equipped with 18 µl flow cell (Helma UK), was used to measure the UV signal at 254 nm of the separated species. Personal computer and printer were handling the data of the homemade system. Asymmetrical peak height is corresponding to the cephradine concentration of standards and sample concentrations.[23-24]

RESULTS

Under the established conditions listed in Table 1, a method of the standard calibration was used to obtain the calibration curve for cephradine, by plotting the concentration versus the peak height of asymmetrical peaks. It is linear over the range (2.5–12.5) µg/ml cephradine. Table 2 lists the R^2 and slope of the curve, which are 0.9950 and 5.622, respectively.

The reproducibility of the method was estimated by injection of a 2.5, 5.0, and 7.5 µg/ml represented standard and two commercial drugs into eluent. Excellent relative standard deviation (RSD) % for retention time (t_R) and peak height were obtained as shown in Tables 2 and 3. Lower limit of detection (LLOD) and quantitation (LLOQ), LLOD = 3.3 SD/S and LLOQ = 10 SD/S, are the concentrations that give the signal-to-noise ratio of 3:1 or 10:1, respectively.[25] This can be detected and verified by the divided of SD of response by the slope of calibration curves (S). Using the single-sided student's test method (at the 95% confidence limit) for five consecutive injections of 7.5 µg/ml of cephradine sample and standard, the values

Table 1: Method parameters

Conditions	Parameters
Ion pac Zorbax 300-SCX agilent column; 5 µm, 4.6 mm×250 mm (P/N 880952-704)	Description column
USP tailing factor @ 5% peak height 1.12	System suitability requirement
Plates 10270	
Water/methanol/0.5 M sodium acetate/0.7 N acetic acid (782:15:3) pH=4.8.	Isocratic mobil phase
Cephardinecapsules were diluted in the mobile phase	Test sample
UV detection	Detection system
254 nm	Maximum wavelength
1.2 mL/min	Flow rate
25°C	Temperature
150 Bar	Pressure background
14 min	Run time
100 µL	Injection volume

UV: Ultraviolet

Table 2: The reproducibility of peak height and t_R of cephardine

±RSD%	tR min	±RSD%	Peaks height (mm)	Representative samples and drugs (µg/mL)
0.201±	10	±0.354	13	2.5
0.220±	10	±0.310	28	5
±0.200	10	±0.377	45	7.5
0.298±	10	±0.383	26	5 µg/mL for drugs (1)
0.298±	10	±0.401	24	5 µg/mL for drugs (2)

tR: Retention time, RSD: Relative standard deviation

Table 3: Regression statistics of the proposed method with LLOD, LLOQ, intercept, and slope

0.995	R^2
0.527	Standard error
0.475	Standard error estimate
0.19	Intercept
5.622	Slope
1.32	LLOD
	µg/mL^{-1}
4.3	LLOQ
	µg mL^{-1}
0.01026	MDL (standard) µg mL (SD×t_{95}%) at *n*= (5-1)
0.01022	MDL (sample) µg/mL (SD×t_{95}%) at (*n*=5-1)

LLOD: Lower limit of detection, LLOQ: Lower limit of quantitation

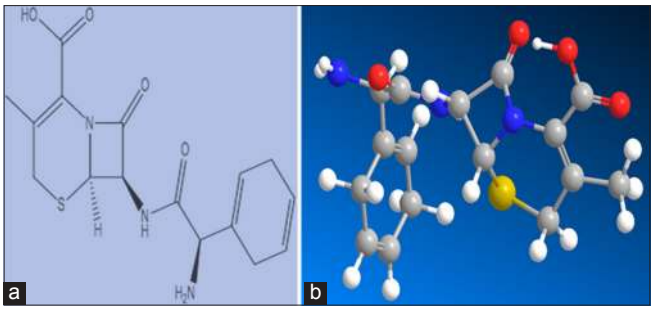


Figure 1: (a and b) Structure of cephardine

of LLOD and LLOQ were 1.32 µg/mL and 4.30 µg/mL, respectively.

DISCUSSION

The analytical performance that studied done by HPLC-UV method for separation and estimation of the cephradine sample, performed through some factors such as the column type, eluent, concentration, tR, and temperature effect, these factors directly affect the accuracy and precision of the results that obtained from the HPLC measurements for different concentrations of the cephradine sample.

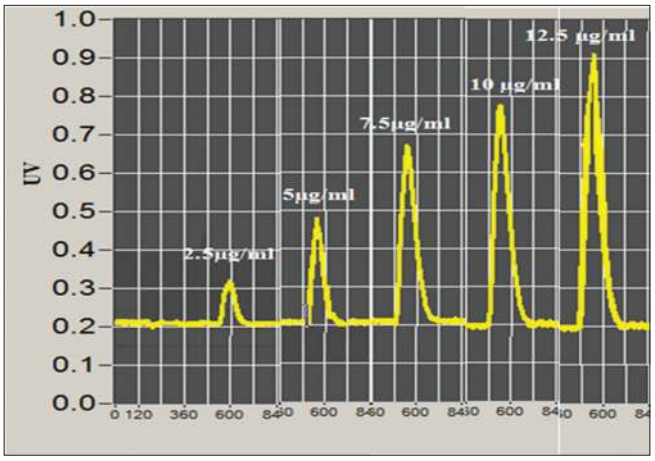


Figure 2: Chromatogram calibration curve of cephardine in concentrations (2.5, 5.0, 7.5, 10.0, and 12.5 µg/ml) and peak height (13, 28, 45, 58, and 68 mm), respectively

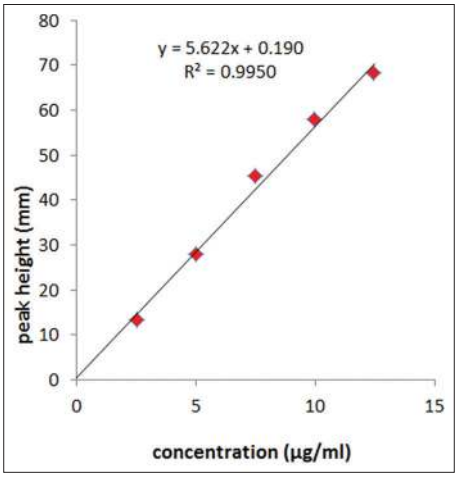


Figure 3: Calibration curve for cephradine

Parameters of the obtained results such as accuracy and precision indicate the success of the chromatographic method that used in the separation and estimation of cephradine. Factors affecting analytical performance can be discussed in detail as follows:

Effect of column type, eluent concentration, and t_R

Ion Pac Zorbax 300-scx, 5 µm 4.6 × 250 mm column was recommended as a suitable and efficient separation column for cephradine and samples. It can be detected using UV detector at λ_{max} 254 nm with the mixture of eluent consisting water/methanol/0.5 M sodium acetate/0.7 N acetic acid (782:15:3) pH = 4.8, all with 10 mM Formic acid at pH = 4.8, which can be freshly prepared.[26]

Figure 2 shows that the column has high efficiency to separate cephradine sodium monohydrate, the linear gradient ranged between 10 and 11 min for each injection and one peak appearance in chromatogram.

Table 4: Method accuracy for cephardine recoveries obtained by HPLC-UV system

Recovery% ±RSD%	Found by classical method (µg/ml)	Recovery% ±RSD%	Found concentration (µg/mL)	Taken concentration (µg/mL)
100±0.281	2.5	0.354±96.0	2.4	2.5
102±0.310	5.1	0.310±100	5	5
99.6±0.465	7.47	0.377±98.0	7.35	7.5
99.5±0.317	9.95	0.317±101.0	10.1	10
97.6±0.342	12.2	0.387±100	12.5	12.5
102±0.341	5.1	0.383±100	5	5 µg/ml drug (1)
102±0.371	5.1	0.401±97.6	4.88	5 µg/ml drug (2)

HPLC-UV: High-performance liquid chromatography-ultraviolet, RSD: Relative standard deviation

Table 5: Intra- and interday precision and accuracy of standard analysts (*n*=5)

Interday		Intraday		
Recovery%±RSD%	Found (µg/mL)	Recovery%±RSD%	Found (µg/mL)	Taken concertation (µg/mL)
88.8±0.400	2.22	0.354±96.0	2.4	2.5
89.6±0.401	4.48	0.310±100	5	5
101.0±0.372	7.6	0.377±98.0	7.35	7.5
100.0±0.490	10	0.317±101.0	10.1	10
98.4±0.340	12.3	0.387±100	12.5	12.5

RSD: Relative standard deviation

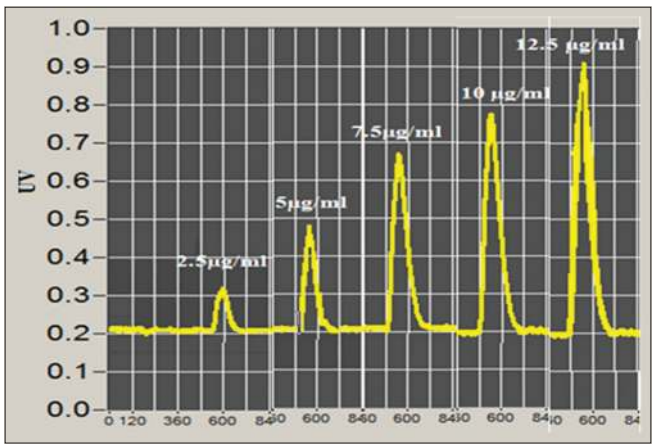


Figure 4: Standard addition method for cephardine determination

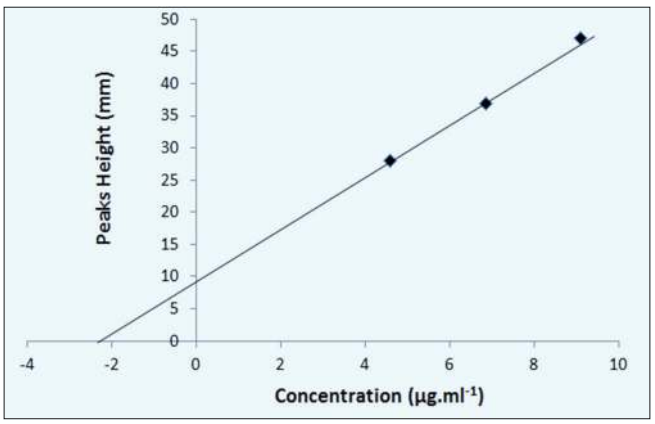


Figure 5: Peaks of standard additions method in concentrations (4.6, 6.85, and 9.1) and peaks height (20, 27, and 34 mm), respectively

Clear peaks indicate the accuracy and sensitivity of the method used in the determination of cephradine Sodium monohydrate. However, some ringing peaks refer to the very small concentration of CO_2 dissolve in eluent.[27,28]

Affected column temperature on the separation

The IC system supply with Column temperature evaluating in the 25°C in five-degree steps. As expected, increasing the column temperature decreased t_R and led to a good baseline for the separation chromatogram of the standards and samples. Due to difficulties in maintaining the temperature stability in the IC-UV system when changing the other parameters used in the measurement. Therefore 25 ° C was selected for use in future work

Accuracy

To evaluate the accuracy of the HPLC-UV System, recovery experiments were performed on three representative standards and two commercial drug samples. Standard additions method [Figure 5] was used for all of these determinations to avoid all the possible interferences. Table 4 summarizes all of these studies. A good agreement between the results was

obtained which clearly indicated that IC-UV System can be used for several applications.

Precision

The precision of the method, reported as % RSD, was estimated by measuring repeatability (intraday assay) for five replicate injections for all concentrations of cephradine and two samples The intermediate precision (interday variation) was also studied for 2 days using an intermediate concentration solution of cephradine and samples. The average recoveries were in the range (96.0–101) which thought to be an acceptable result. Table 5 summarizes all of these studies.

To evaluate the accuracy of the locally produced HPLC system, the recovery experiments on cephradine and representative samples were performed using a standard additive method for all these decisions to avoid the effect of overlaps. Figures 2-5 and Tables 2-5 show the data obtained and calculated using SPSS software programs (CVI). The average recoveries were in an acceptable range (96.0–101%) which clearly indicated that the method could be successfully used to determine cephradine samples. To determine the validity of the HPLC method, the same group of representative samples was analyzed by HPLC-UV and conventional methods. Good agreement between all the results obtained. These results indicate that the HPLC method can be used for several applications. Furthermore, the results of accuracy and accuracy were illustrated by intra-day and inter-day analysis values, which are considered as basic parameters in the analysis and estimation of cephradine as shown in Table 5, which included these results. These two factors have clearly demonstrated that the HPLC-UV method can be used to determine cephradine ratios in pharmaceuticals with precision and sensitivity. Cephradine mixing with other pharmaceuticals does not affect separation and estimation.

CONCLUSION

This work described HPLC System equipped with a UV detector for cephradine determination in two commercial pharmaceutical drugs. This developed method considered as simple, inexpensive and needs only a very small volume of the sample as well as used it's an ultraviolet detector makes this system very specific because of one peak in the chromatogram. In this application, there is no need for high sensitivity since the pharmaceutical drugs have a very low concentration. The method was validated as per the IC-UV guidelines and the developed method obeys Beer's law over the concentration range of 2.5–12.5 µg/mL for drugs.

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AUTHOR'S CONTRIBUTIONS

This research was done individually in the laboratories of the Faculty of Pharmacy/University of Basra and the laboratories of the College of Education for Pure Sciences.

This research was completed over 3 months with serious and continuous work, and therefore, excellent results were obtained in finding an easy and sensitive way to estimate the cephradine in some pharmaceuticals.

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