Quantitative analysis of Cephardine using the modern HPLC method

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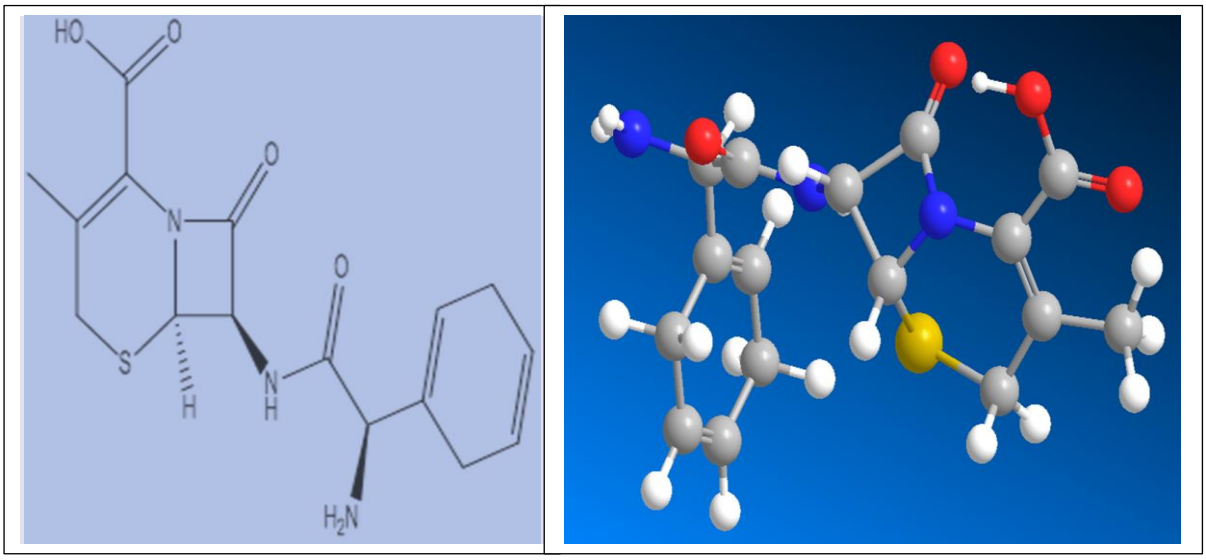
ABSTRACT:

Cephradine are the most important type of antibiotics used vary widely. Analysis of these antibiotics is challenge because of their sensitivity and instability to different conditions. The present article is extended to find out HPLC method 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. 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 aqueous medium at pH between 4 and 5. The focus of study of analysis of Cephradine in formulation capsules are useful for the determination of various Cephradine for supported by budding researchers.

Keywords: *Cephradine capsules formulation, HPLC-UV protocol, antibiotic drugs.*

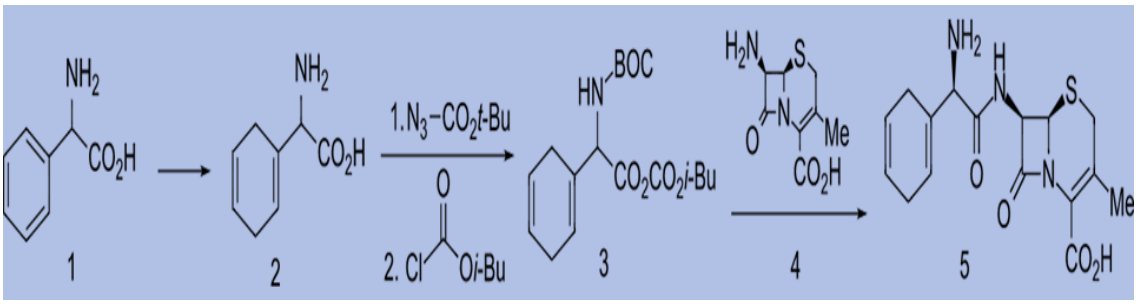
Introduction

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.

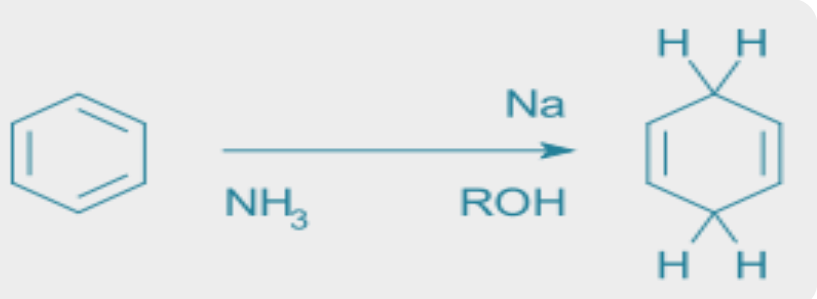


1.Synthesis:

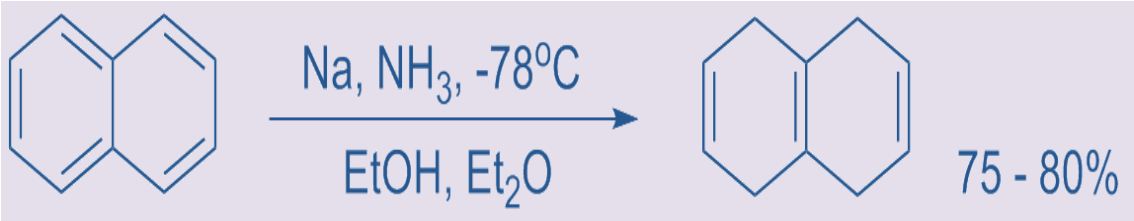
1,4-cyclohexadiene rings are nearly as planar as benzene rings but of greatly different reactivity, a cephalosporin was synthesized with such a moiety. The Birch reduction is an organic reaction which is particularly useful in synthetic organic chemistry. The reaction was reported in 1944 by the Australian 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-cyclohexadienes, 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 tert-butanol. This reaction is quite unlike catalytic hydrogenation, which usually reduces the aromatic ring all the way to a cyclohexane,[1][2][3][4][5][6] .



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



An example is the reduction of naphthalene [9].



2. Evolution 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 formulation which may impart 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 quantitative assay of cephardine along with assessment of potency of a Cephradine capsules formulated.

There are various methods used for the analysis of Cephradine in the various forms like chromatographic, UV and electrophoresis; but the most important in all methods is HPLC-UV method [10][11][12].

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

The HPLC chromatographic method having high limit of detection value so it's very preferred. HPLC technique can provide valuable tool which generating high pure compound and HPLC has ability to analyze both volatile and nonvolatile compounds with ultra-trace level may be employed in pharmaceutical research. Many antibiotics contain ionizable group can be analyzed by ion exchange chromatographic methods. High resolving power of HPLC serves as a particularly important method for isolation and purification of antibiotics [13][14].

High-Performance Liquid Chromatographic Methods (HPLC) is most common method for the analysis of cephardine in formulation and in biological fluids, the several analytical procedures have been described for analysis of cephardine in different pharmaceutical formulations.

There are various HPLC methods are reported for the analysis of a single cephardine in pharmaceutical drugs. All these methods present a unique preparatory and chromatographic protocol. Several methods have been used for the analysis of cephardine which analysis in High Performance Liquid Chromatography method that is very accurate and sensitive [15][16][17] .

Very sensitive HPLC methods have been developed for the detection and quantitation of cephardine in a variety of biological matrices. The determination of cephardine 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 method used largely for the purpose of cephardine analysis. yet, the reversed phase chromatography is fairly recommended for the analysis of cephardine 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; 5µm, 4.6×250 mm). Prior to final selection of the column, column length, diameter, particle size, pore size and carbon load should be taken in 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 ultraviolet (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 prior to routine application [18][19][20][21].

Aim of Study: The main aim of this study was to develop an efficient anew method for HPLC-UV system for determination of Cephardineas 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; 5µm, 4.6×250 mm (P/N 880952-704) from USA was chosen for separation anti-biotic drugs.
- Metrohme 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 accquisions (Ocean control/Australia).
- Personal Computer Supplied with modify software programs / cvi programs UV.
- Printer (EPSON/ Japan).

pH meter (Hana-Italy).

MATERIALS AND METHOD:

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

Reagents and standards:

- Acetonitrile; HPLC grade, BDH Chem. LTD 7526- 13
- Methanol; HPLC grade, BDH M/ 405/9 LTD 610098 Cas 58-33-1.
- Formic acid; BDH M/ 231/202LTD 12526 Cas 98-142-2
- Commercial Cephardine Capsules from two companies.
- Analar Cephardine powder as standard Sigma-Aldrach Germa.
- The Stock Standard Solution 100 µg/ml Cephardine was prepared by dissolving accurately weight 100mg of Cephardine in 1000 ml methanol which was purchased from Aldrach 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.
- Cephardine 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 ^{o}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 elunt at 1.2ml/min. Cephardine capsules or standard were

manually injected with Metrohm electronic injection valve fitted with 100µl loop in 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, 5µm ,4.6×250 mm (p/N880952-704) was used as a separation column. APD 303UVdetector 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. A data logger lab jack-Ocean control/ Australia. Personal computer and printer were handling the data of the home made system. A symmetrical Peaks height is corresponding to the Cephardine concentration of standards and sample concentrations[23][24][25].

RESULTS:

All the experiment results for the determination of Cephardin were included in table 1., which show the instrument specification, the method used in the estimation and the optimum conditions of the separation process.

Table.1. Method Parameters

Parameters	***Conditions***
Description Column	Ion Pac zorbax 300- SCX Agilent Column; 5µm, 4.6×250 mm (P/N 880952-704)
System Suitability Requirement	USP Tailing Factor @ 5 %Peak Height 1.12 Plates 10270
Isocratic Mobil phase	Water / Methanol / 0.5 M Sodium acetate / 0.7 N Acetic acid (782 : 15 : 3) pH=4.8.
Test sample	Cephardine capsules were diluted in the mobile phase
Detection System	UV detection
Maximum Wavelength	254 nm
Flow Rate	1.2 mL / min
Temperature	25 °C
Pressure Background	150 Bar
Run Time	14 min
Injection Volume	100 µL

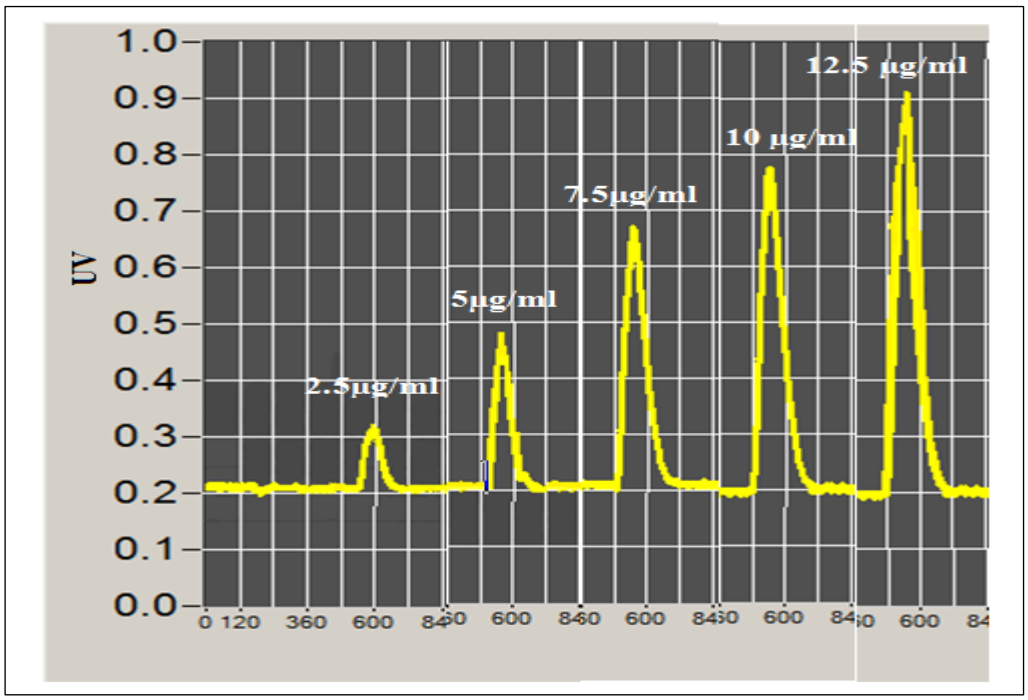


Figure 2: Chromatogram Calibration curve of Cephardine in concentrations[(2.5 5.0, 7.5, 10.0 and 12.5)µg/ml] and Peaks height [(13, 28, 45, 58 and 68)mm] Respectively.

DISCUSSION:

1. Effect of Column type, elunt Concentration and Retention Time:

Ion Pac Zorbax 300-scx, 5 µm 4.6×250mm column was recommended as a suitable and efficient separation column for Cephardine and samples. It can be detect by using UV detector at λ_{max} 254 nm with mixture of elunt consist 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 Cephardine Sodium monohydrate, the linear gradient ranged between 10-11 minutes for each injection and one peak appearance in Chromatogram.

The distinct peak cause of good method sensitivity to determination Cephardine Sodium monohydrate; But some ringing peaks refer to very small concentration of CO_2 dissolve in eluent [27][28].

2. Effected 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 retention time and led to good baseline for the separation Chromatogram of the standards and samples. But due to difficulties of maintaining temperature stability in the constructed home- made IC system. So 25 °C was selected to be used in future work.

3. Method performance (linearity, Reproducibility and Detection Limits): Under the established conditions listed in Table 1, a method of the standard calibration was used to obtain the calibration curve for Cephardine, by plotting the concentration versus the peak height of asymmetrical peaks. It is linear over the range (2.5-12.5) µg/ml Cephardine. 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 RSD% for retention time (t_R) and peak height were obtained as shown in Table 2 and 3.3 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 [29]. This can be detected and verified by the divided of standard deviation of response (SD) by the slope of calibration curves (S) By using the single-sided student's test method (at the 95% confidence limit) for five consecutive injections of 7.5 µg/ml of Cephardine sample and standard, the values of LLOD and LLOQ were 1.32 µg mL^{-1} and 4.30 µg mL^{-1} respectively.

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

Representative samples and drugs (µg mL^{-1})	Peaks height (mm)	±RSD%	Retention Time (t_R) minutes	±RSD%
٢,٥	١٣	±0.354	١٠	±٠,٢٠١
٥,٠	٢٨	±0.310	١٠	±٠,٢٢٠
٧,٥	٤٥	±0.377	١٠	±٠,٢٠٠
5 µg mL^{-1} for Drugs (1)	٢٦	±0.383	١٠	±٠,٢٩٨
5 µg mL^{-1} for Drugs (2)	٢٤	±0.401	10	±٠,٢٩٨

Table 3: Regression statistics of the proposed method with LLOD, LLOQ, Intercept and Slope	
R^2	٠,٩٩٥٠
Std Er[a]	٠,٥٢٧
Std Er Est[b]	٠,٤٧٥
Intercept	٠,١٩٠
Slope	٥,٦٢٢
LLOD µg mL^{-1}	1.32
LLOQ µg mL^{-1}	٤,٣٠
MDL(standard) µg mL^{-1} (SD×t 95%) at n= (5-1)	٠,٠١٠٢٦
MDL(sample) µg mL^{-1} (SD×t 95%) at (n= 5-1)	٠,٠١٠٢٢

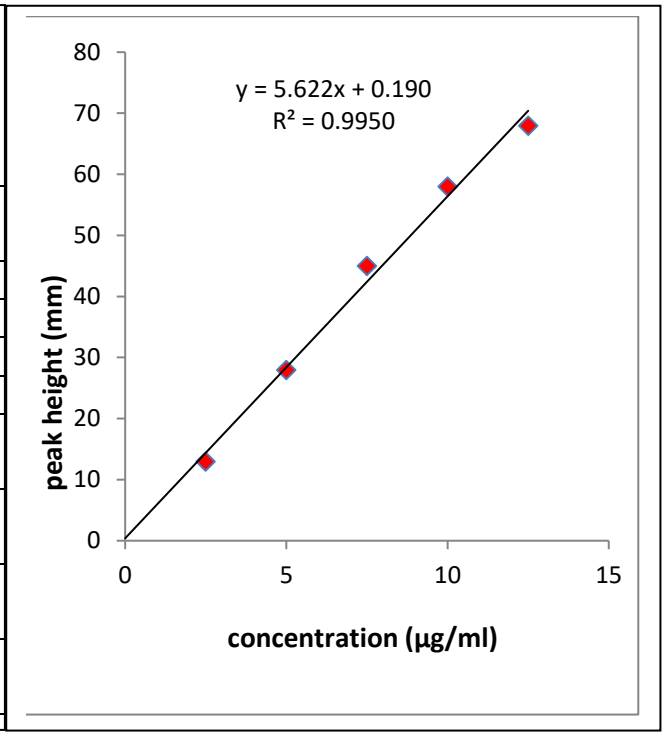
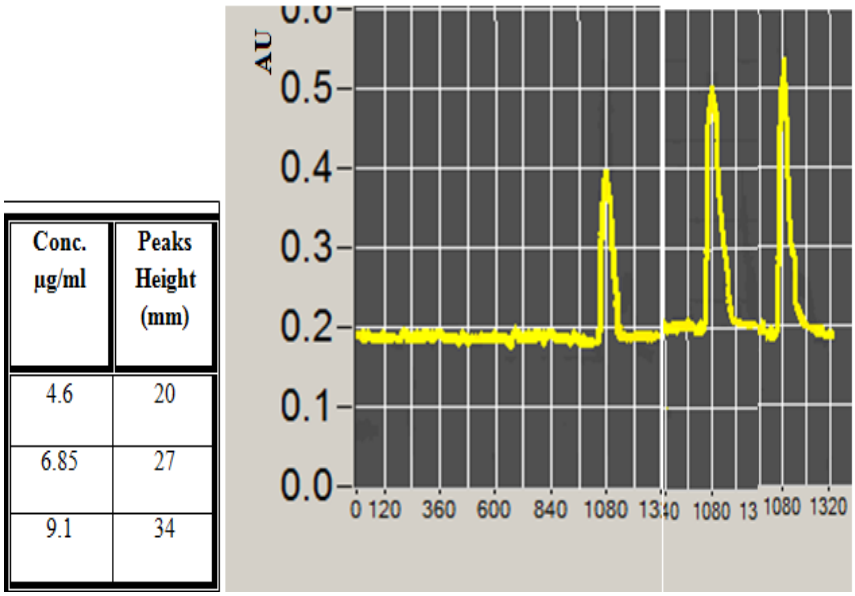


Fig. 2. Calibration curve of cephardine



Conc. µg/ml	Peaks Height (mm)
4.6	20
6.85	27
9.1	34

Figure 4: Standard addition method for Cephardine determination

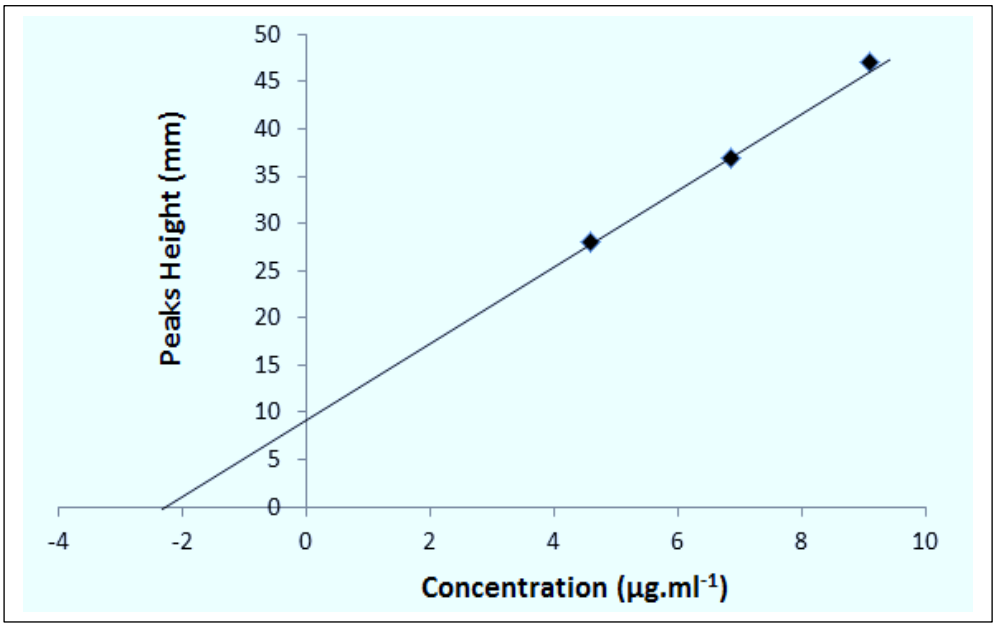


Figure 5: peaks of Standard additions Method in concentrations[(4.6, 6.85 and 9.1)] and Peaks height [(20, 27 and 34)mm] Respectively

Accuracy: To evaluate the accuracy of the HPLC-UV System. A recovery experiments were performed on three representative standards and two commercial drug samples. Standard additions method (Fig 5) was used for all of these determinations in order to avoid all the possible interferences. Table 4 summarized 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.

Table 4: method accuracy for Cephardine recoveries obtained by HPLC-UV system

Taken Conc. (µg mL^{-1})	Found conc. (µg mL^{-1})	Recovery% ± RSD %	Found by classical Method (µg/ml)	Recovery% ± RSD %
٢,٥	٢,٤٠	٩٦,٠±0.354	2.50	100 ± 0.281
٥,٠	٥,٠٠	١٠٠±0.310	٥,١٠	١٠٢ ± 0.310
٧,٥	٧,٣٥	٩٨,٠±0.377	٧,٤٧	٩٩,٦ ± 0.465
١٠,٠	١٠,١٠	١٠١,٠±0.317	٩,٩٥	99.5 ± 0.317
١٢,٥	١٢,٥٠	١٠٠±0.387	١٢,٢	٩٧,٦ ± 0.342
5µg/ml Drug (1)	٥,٠٠	100 ± 0.383	٥,١٠	102 ± 0.341
5µg/ml Drug (2)	٤,٨٨	97.6 ± 0.401	٥,١٠	102 ± 0.371

Precision: Precision of method, reported as % RSD, was estimated by measuring repeatability (intra-day assay) for five replicate injections for all concentrations of Cephardine and two samples The intermediate precision (inter-day variation) were also studied for two days using an intermediate concentration solution of Cephardine 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.

Table5: Intra and inter-day precision and accuracy of standard analysts (n=5).

Taken conc. (µg mL^{-1})	Intra-day		Inter-day	
	Found (µg mL^{-1})	Recovery% ±RSD%	Found (µg mL^{-1})	Recovery% ±RSD%
٢,٥	٢,٤٠	٩٦,٠±0.354	٢,٢٢	٨٨,٨ ± 0.400
٥,٠	٥,٠٠	١٠٠±0.310	٤,٤٨	٨٩,٦ ± 0.401
٧,٥	٧,٣٥	٩٨,٠±0.377	٧,٦٠	١٠١,٠ ± 0.372
١٠,٠	١٠,١٠	١٠١,٠±0.317	١٠,٠	١٠٠,٠ ± 0.490
١٢,٥	١٢,٥٠	١٠٠±0.387	١٢,٣	٩٨,٤ ± 0.340

CONCLUSION

This work described HPLC System equipped with UV detector for Cephardine determination in two commercial pharmaceutical drugs. This developed method offer simple, inexpensive and needs only a very small volume of the sample and using a UV detector makes this system very specific due to 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 IC-UV guidelines and the developed method obeys beer's law over the concentration range of 2.5-12.5 µg/mL for drugs.

Acknowledgements

The student thank for all Professors in Discussion Committee and thank the staff of the pharmaceutical Chemistry laboratories in the College of pharmacy, University of Basrah. The student also give thanks

and appreciation to the supervisor of working and writing this humble Research.

Conflict of interest: there no conflict of interest for any one.

Funding of research: the research was funded by the researcher himself.

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