Determination of amount of cloxacillin and fluxacillin using new HPLC method and using oxacillin as standard

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Abstract:

An accurate, precise and sensitive HPLC assay method was developed for the determination of 2 penicillin in Oral dosage form, to compare the amount of active ingredients of cloxacillin (Drugs 1) and flucloxacillin (Drugs 2) monohydrate capsules (500mg) from two different commercial companies formulations and Oxacillin sodium monohydrate analar as a test formulation. Oxacillin sodium monohydrate concentrations were analyzed by HPLC-UV System at (λ=230 nm). The separation was achieved using the Ion Pac zorbax 300-SCX Agilent Column; 5µm, 4.6×250 mm. The mobile phase consisted of a Water/Acetonitrile/Methanol (40:30:30), all with 10 mM Formic acid at pH=4.8. The comparison study of two pencillins formulations was assessed by calculating the peaks height. The standard Oxacillin sodium monohydrate and other pencillins eluted at a flow rate of 1.0 ml/min. The recoveries were rang within 91.0-100% Linearity rang (0.3 -1.5) µg/ml, (n=5) with $R^2 \geq 0.9992$ and RSD ±0.492-0.583at room temperature 25°C. The detection limit of quantification (LLOQ) was 4.44µg/ml and Lower limit of detection (LLOD) 1.46µg/ml . There was no significant difference between the values detected by the new method and the classical method.

Introduction:

An isocratic liquid chromatographic method with UV detection at 230 nm is described for determination of Oxacillin sodium monohydrate injection. Chromatographic separations of two drugs was achieved on the reversed phase column Agilent Zorbax- SCX- C18 (5 µm, 250 mm × 4.6 mm). The developed Liquid Chromatographic method offers symmetric peak shape[1].

Oxacillin $C_{19}H_{18}N_3NaO_5S$ [(sodium;(2S,5R,6R)-3,3-dimethyl-6-[(5-methyl-3-phenyl-1,2-oxazole-4-carbonyl)amino]-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate] (Figure. 1) is the resistant Oxacillin is antibiotics widely used to treat diseases in humans. In this paper, a solid phase extraction method with a high performance liquid chromatograph (HPLC-UV) is shown for the determination of Oxacillin. In the method, the reversed phase column Agilent Zorbax- SCX- C18 (5 µm, 250 mm × 4.6 mm) . The performance of the solid phase extraction procedure on trace residues is quantitatively evaluated by HPLC-UV.

ß-lactam antibiotics Oxacillin represent some of the most important antibacterial agents used in humans. However, serious reactions are known to occur in some individuals exposed to Oxacillin as a result, these compounds are carefully monitored in pharmaceutical drugs. Maximum residue limits (MRLs) for Oxacillin in a variety of drugs are established worldwide and are generally in the range of 0.3-1.5 µg/ml. These regulations require detection and quantification by HPLC-UV. This application will show the development of a sample extraction and cleanup method and the quantification by the reversed phase IC The structures and chemical constants for the compounds used in this study are shown in Table 1.

Oxacillin sodium is semi synthetic penicillin. It is available commercially as the monohydrate sodium salt which occurs as a fine, white, crystalline powder that is odorless or has a slight odor. It is freely soluble in water and has a pKa of about 2.8.

Numerous analytical methods have been reported in the scientific literature for the determination of Oxacillin sodium monohydrate. These methods are based on Spectrophotometric high performance liquid chromatography.

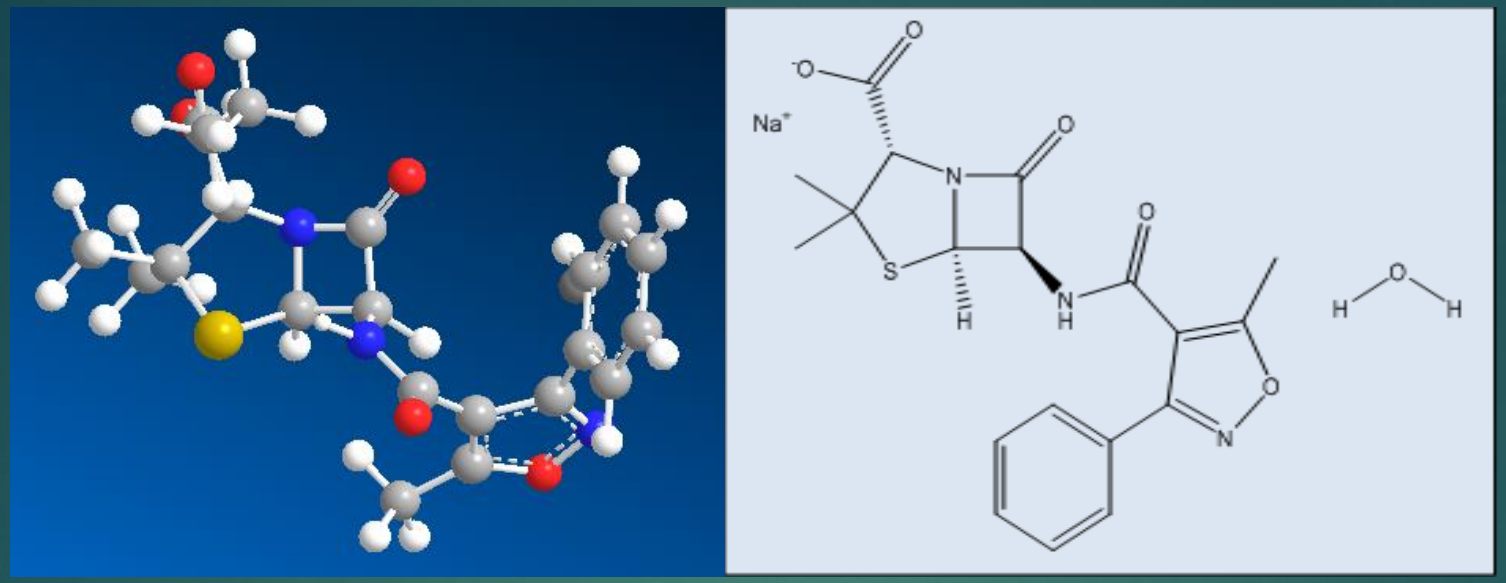

Figure 1. Structure of Oxacillin Sodium monohydrate

The determination as in biological fluids normally requires the use of trace analysis techniques such as High Performance Liquid Chromatography (HPLC-UV) Spectrophotometry, these method is very expensive because it require long and tedious pretreatment of the samples and laborious clean up procedures prior to analysis. Therefore, it is necessary to develop a simple and suitable analytical method for the determination of Oxacillin sodium. UV-Visible spectrophotometry is the technique of choice in research laboratories, hospitals and pharmaceutical industries due to its low cost and inherent simplicity.

Goal:

The main aim of this study w as to develop an efficient anew method for HPLC-UV system for determination of Oxacillin Sodium mono hydrate as standard and two commercial penicillin in antibiotic drugs.

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

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).

Reagents and standards:

- Acetonitrile; HPLC grade, BDH Chem. LTD 7177- 48
- Methanol; HPLC grade, BDH M/ 405/17 LTD 116967 Cas 67-56-1.
- Formic acid; BDH M/ 231/202LTD 12526 Cas 34-44-2
- Commercial Oxacillin Sodium mono hydrate Capsules from two companies.
- Analar Oxacillin powder as standard Sigma-Aldrach Germa.
- The Stock Standard Solution 100 µg/ml Oxacillin Sodium mono hydrate was prepared by dissolving accurately weight 100mg of Oxacillin Sodium mono hydrate in 1000 ml methanol which was purchased from Aldrach 33/8467-LTD.
- A working solution in the range 0.3-1.5 µg/ml was prepared by serial dilution of this stock solution with methanol.
- cloxacillin and flucloxacillin monohydrate 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 120 bar all chromatography experiments were carried out by HPLC-UV chromatography system, which consisting LKB pump 2150-HPLC pumping the elunt at 1ml/min. cloxacillin and flucloxacillin monohydrate capsules or standard were manually injected with Metrohm electronic injection valve fitted with 100µl loop in eluent of Water/Acetonitrile/Methanol (40:30:30), all with 10 mM Formic acid at pH=4.8. 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 230 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 Oxacillin concentration of standards and sample concentrations.

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 1.11 Plates 2590-2975**
Isocratic Mobil phase	**Water/Acetonitrile/Methanol (40:30:30), all with 10 mM Formic acid at pH=4.8.**
Test sample	**Oxacillin Sodium mono hydrate cloxacillin and flucloxacillin monohydrate capsules were diluted in the mobile phase**
Detection System	**UV detection**
Maximum Wavelength	**230 nm**
Flow Rate	**1.0 mL / min**
Temperature	**25 °C**
Pressure Background	**120 Bar**
Run Time	**17 min**
Injection Volume	**100 µL**

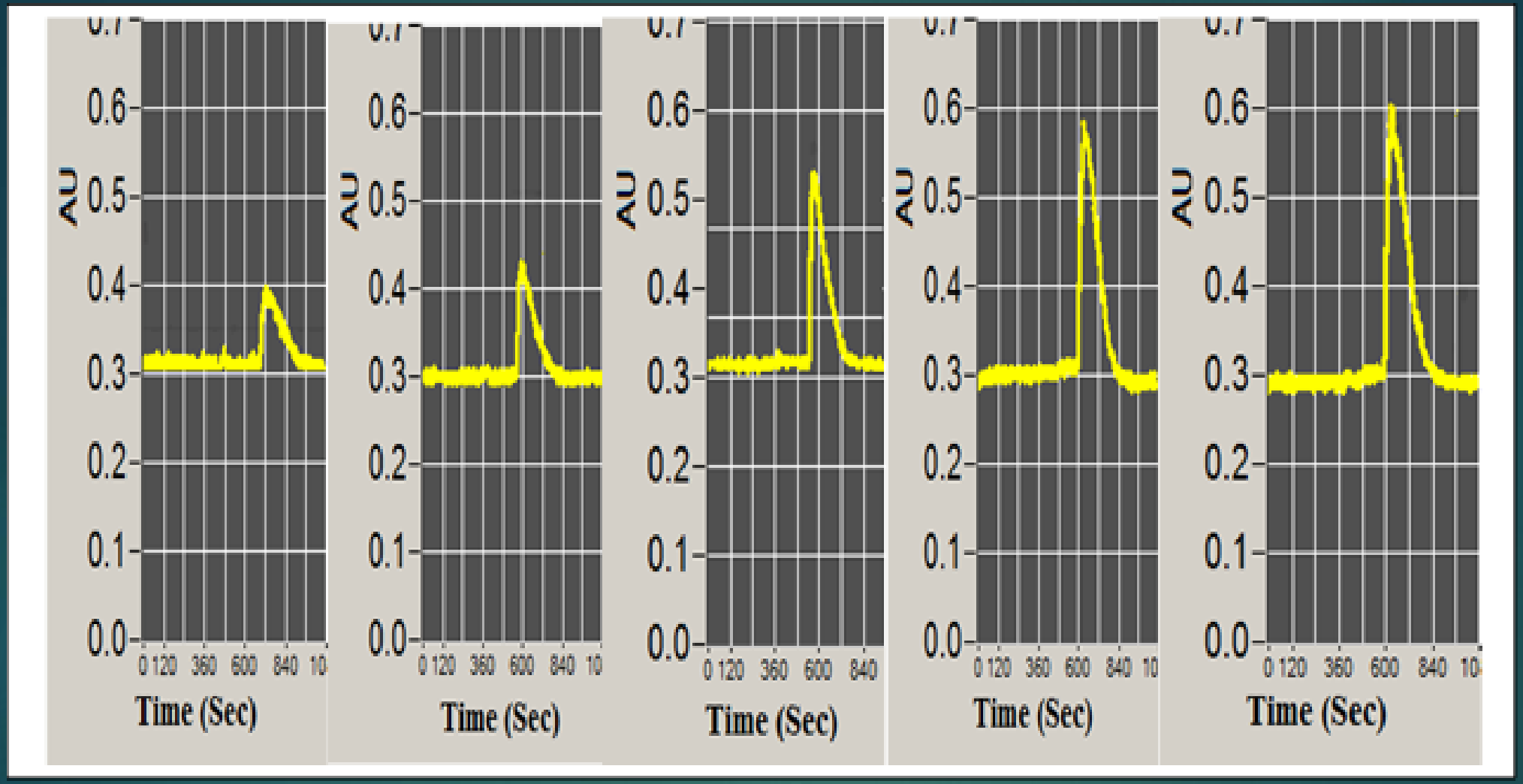

Figure 2: Chromatogram Calibration curve of Oxacillin Sodium monohydrate in concentrations[(0.3, 0.6, 0.9, 1.2and 1.5)µg/ml] and Peaks height [(7, 13, 20, 27and 30)mm] Respectively.

RESULT AND 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 Oxacillin and samples. It can be detect by using UV detector at λ_{max} 230 nm with mixture of elunt consist Water/Acetonitrile/Methanol (40:30:30), all with 10 mM Formic acid at pH=4.8, which can be freshly prepared.

Figure 2 shows that the column has high efficiency to separate Oxacillin Sodium monohydrate, the linear gradient ranged between 11 minutes for each injection and one peak appearance in Chromatogram .The distinct peak cause of good method sensitivity to determination Oxacillin Sodium monohydrate; But some ringing peaks refer to very small concentration of CO_2 dissolve in eluent.

2. Effected Column Temperature on the separation: The IC system supply with Column temperature evaluating in the range 25-45^{o}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 ^{o}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 Oxacillin Sodium mono hydrate, by plotting the concentration versus the peak height of asymmetrical peaks. It is linear over the range (0.3-1.5) µg/ml Oxacillin Sodium mono hydrate. Table 2 lists the R^2 and slope of the curve, which are 0.9992 and 21.0 respectively.

The reproducibility of the method was estimated by injection of a 0.3, 0.6 and 0.9 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**.** 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. 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 0.9 µg/ml of Oxacillin Sodium mono hydrate sample and standard, the values of LLOD and LLOQ were 1.46 µg mL^{-1} and 4.44 µg mL^{-1} respectively.

Table 2: The reproducibility of peak height and t_R of Oxacillin Sodium mono hydrate

Representative samples and drugs (µg mL^{-1})	Peaks height (mm)	RSD% ±	Retention Time (t_R) minutes	RSD%±
٠,٣	٧	±0.492	١١	±٠,٣٢٤
٠,٦	١٣	±0.522	١١	±٠,٣٥٥
٠,٩	٢٠	±0.583	١١	±٠,٣١٠
5 µg mL^{-1} for Drugs (1)	٨٣	±0.517	١١	±٠,٣٠٧
5 µg mL^{-1} for Drugs (2)	٨٤	±0.510	11	±٠,٣٥١

Table 3: Regression statistics of the proposed method with LLOD, LLOQ, Intercept and Slope

R^2	0.9992
Std Er.	0.638
Std Er Est.	0.586
Intercept	0.7
Slope	21.0
LLOD µg mL^{-1}	1. 46
LLOQ µg mL-	4.44
MDL(standard) µg mL^{-1} (SD×$t_{95\%}$) at n= (5-1)	0.02037
MDL(sample) µg mL- (SD×$t_{95\%}$) at (n= 5-1)	0.02133

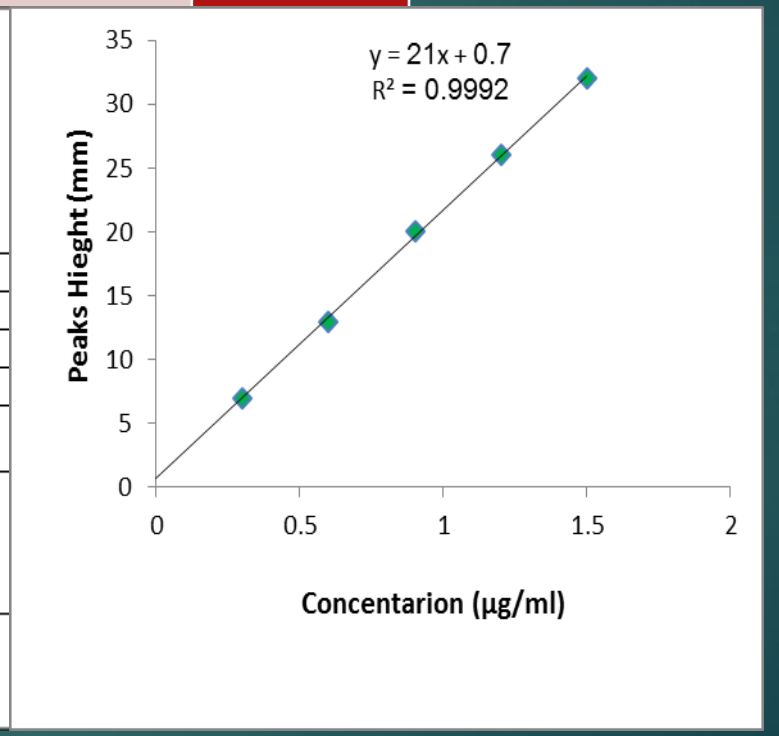

Figure 3: calibration curve for Oxacillin

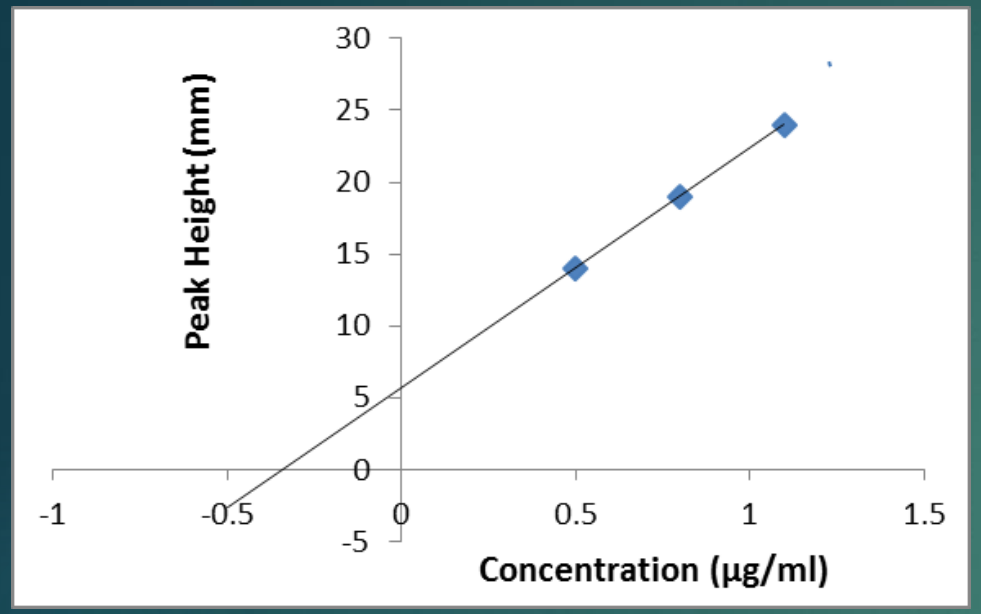

Figure 4: Standard additions for Oxacillin Sodium mono hydrate determination

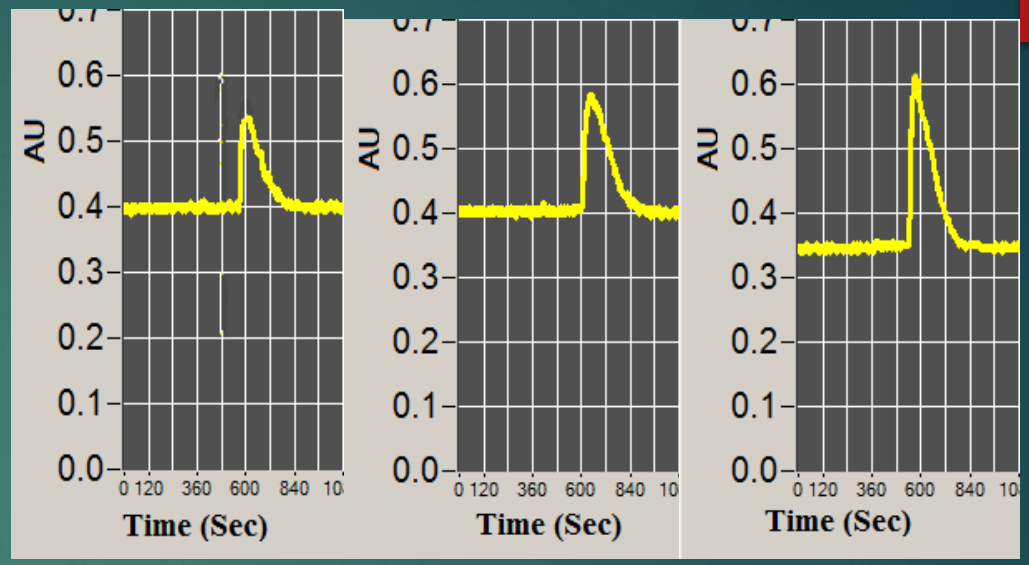

Figure 5: peaks of Standard additions Method in concentrations[(0.5, 0.8 and 1.1)] and Peaks height [(13, 19 and 25)mm] Respectively

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 Oxacillin Sodium monohydrate 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 %
٠,٣	٠,٢٩	±0.492٩٦,٦	0.3	100 ± 0.390
٠,٦٠	٠,٦٠	١٠٠±0.522	٠,٦٠	± 0.421 ١٠٠
٠,٩٠	٠,٨٥	٩٤,٤±0.583	٠,٨٧	± 0.57٩٦,٦
١,٢٠	١,١٠	٩١,٦±0.517	١,١٠	91.6 ± 0.428
١,٥٠	١,٥٠	١٠٠±0.510	١,٥٠	± 0.455١٠٠
5µg/ml Drug (1)	٤,٨٠	96 ± 0.581	٤,٩٠	98 ± 0.455
5µg/ml Drug (2)	٤,٩٠	98 ± 0.499	٤,٩٠	98 ± 0.482

Precision:

Precision of method, reported as % RSD, was estimated by measuring repeatability (intra-day assay) for five replicate injections for all concentrations of Oxacillin Sodium mono hydrate and two samples The intermediate precision (inter-day variation) were also studied for two days using an intermediate concentration solution of Oxacillin Sodium monohydrate and samples. The average recoveries were in the range (91-100) which thought to be an acceptable result Table 5 Summarizes all of these studies.

	Intra-day		Inter-day	
Taken conc. ($\mu g\ mL^{-1}$)	Found ($\mu g\ mL^{-1}$)	Recovery% ±RSD%	Found ($\mu g\ mL^{-1}$)	**Recovery% ±RSD%**
٠,٣	٠,٢٩	٩٦,٦ ± 0.492	٠,٢٨	**± 0.501٩٣,٣**
٠,٦	٠,٦	١٠٠ ± 0.522	٠,٥٨	**± 0.512٩٦,٦**
٠,٩	٠,٨٥	٩٤,٤ ± 0.583	٠,٨٤	**± 0.483٩٣,٣**
١,٢	١,١	٩١,٦ ± 0.517	١,٠	**± 0.502٨٣,٣**
١,٥	١,٥	١٠٠ ± 0.511	١,٣	**± 0.451٩٢,٨**

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

CONCLUSION :

This work described HPLC System equipped with UV detector for Oxacillin Sodium mono hydrate 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 0.3-1.5 µg/mL for drugs.

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Conflict of interest:

there no conflict of interest.

Funding of research:

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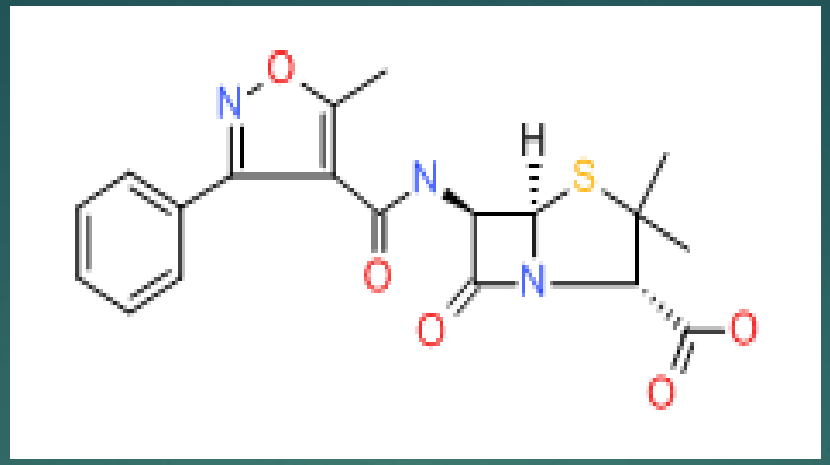
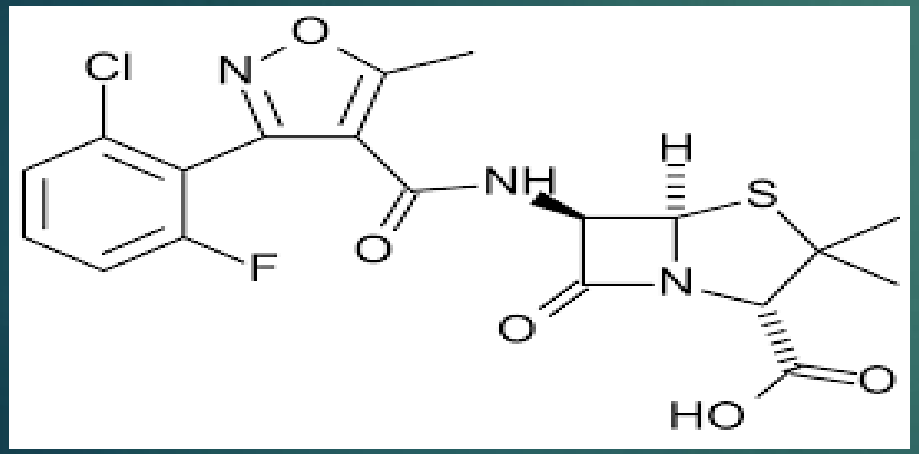
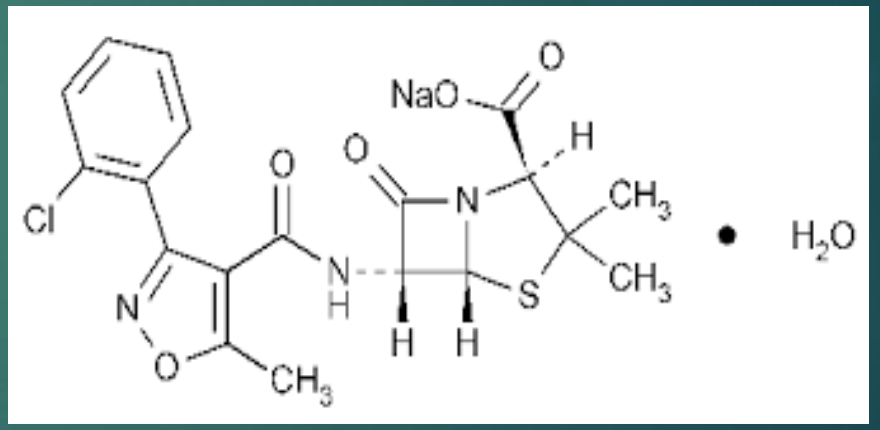


Oxacillin



Flucloxacillin



Cloxacillin

Thanck you for attention