

Quantitative analysis of two penicillins in oral dosage form using modern high-performance liquid chromatography method

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Abstract

Context: An accurate and sensitive high-performance liquid chromatography-ultraviolet (HPLC-UV) assay method was developed for the determination of two penicillins in oral dosage form to compare the amount of active ingredients of cloxacillin (drug 1) and flucloxacillin (drug 2). **Objective:** The objective of the study was to develop a simple, sensitive, accurate, and kinetic spectroscopic method for the measurement of two penicillins in the form of oxacillin sodium monohydrate in oral dosage pharmaceutical product. **Materials and Methods:** Monohydrate capsules (500 mg) were purchased from two different commercial companies and oxacillin sodium monohydrate analar was used as a test formulations. Oxacillin sodium monohydrate concentrations were analyzed by HPLC-UV system at $\lambda = 230$ nm. The separation was achieved using the Ion Pac zorbax 300-SCX Agilent Column, 5 μ m, 4.6 \times 250 mm. The mobile phase consisted of water:acetonitrile:methanol (40:30:30), all with 10 mM formic acid at pH = 4.8. The comparison study for the two penicillin formulations was assessed by calculating the peak height. The standard oxacillin sodium monohydrate and other penicillins were eluted at a flow rate of 1.0 ml/min. **Results:** The recoveries were ranged within 91.0–100% and the linearity were ranged (0.3–1.5) μ g/ml, (n=5) with $R^2 \geq 0.9992$ while the relative standard deviation were (RSD) \pm 0.492–0.583 at room temperature 25°C. The detection lower limit of quantification was 4.44 μ g/ml and lower limit of detection was 1.46 μ g/ml. **Conclusion:** The proposed method was successfully applied to the determination of the drug in the pharmaceutical products and the validation of the statistical data. The results were compared to the reference method, and they showed good compatibility. There was no significant difference between the values detected by the new method and the classical method.

Key words: Penicillin, oxacillin, cloxacillin, flucloxacillin, quantitative analysis

INTRODUCTION

An isocratic liquid chromatographic method with ultraviolet (UV) detection at 230 nm is described for the determination of oxacillin sodium monohydrate injection. Chromatographic separation of two drugs was achieved on the reversed-phase column Agilent Zorbax-SCX-C18 (5 μ m, 250 mm \times 4.6 mm). The developed liquid chromatographic method offers a symmetric peak shape.^[1-4]

Oxacillin C₁₉H₁₈N₃NaO₅S [(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 chemical structure of oxacillin, which is an antibiotic widely used to treat diseases in humans. In this paper, a

solid-phase extraction method with a high-performance liquid chromatography (HPLC-UV) is shown for the determination of oxacillin. The reversed-phase column “Agilent Zorbax-SCX-C18 (5 μ m, 250 mm \times 4.6 mm)” was used in the method. The performance of the solid-phase extraction procedure on trace residues is quantitatively evaluated by HPLC-UV.^[5-7]

β -lactam antibiotics represent some of the most important antibacterial agents used in humans. However, serious

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reactions are known to occur in some individuals exposed to β -lactam antibiotics, and as a result, these compounds are carefully monitored in pharmacy practices. Maximum residue limits for oxacillin in a variety of drugs are established worldwide and are generally in the range of 0.3–1.5 $\mu\text{g}/\text{ml}$. These regulations require the detection limit and quantification limit by HPLC-UV.^[8-10] The detection limit and quantification limit show the clarity property of the present experimented new method. The structures and chemical constants for the compounds used in this study are shown in Table 1.

Oxacillin sodium is semisynthetic 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 HPLC.^[11-14]

The HPLC-UV spectrophotometry usually needs trace quantities of the analyses, so it is appropriate for the analysis of pharmaceuticals and biological fluids. This method is very expensive because it requires long and tedious pretreatment of the samples and laborious cleanup procedures before analysis. Therefore, it is necessary to develop a simple and suitable analytical method for the determination of oxacillin sodium. It is known that the UV-visible spectrophotometry is the technique of choice in research laboratories, hospitals, and pharmaceutical industries due to its low cost and inherent simplicity.^[15-19]

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 oxacillin sodium monohydrate as standard and two commercial penicillin antibiotic drugs.

MATERIALS AND METHODS

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.^[20]

Equipment

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

- LKB Pump 2150–HPLC, Bromma.
- IonPac column Zorbax 300-SCX Agilent Column, 5 μm , 4.6 \times 250 mm (P/N 880952-704) from the USA was chosen for separation of antibiotic drugs.
- Metrohm system with 100 μl electric injection valve as sample Volume.
- A PD 303 UV Detector single beam (Japan) equipped with an 18 μl flow cell (Helma UK)
- Data logger LabJack U12 acquisitions (Ocean Controls, Australia).
- Personal computer supplied with modifying 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 monohydrate capsules from two companies.

Table 1: Method parameters

Parameters	Conditions
Description column	IonPac Zorbax 300-SCX Agilent Column; 5 μm , 4.6 \times 250 mm (P/N 880952-704)
System suitability requirement	USP Tailing Factor at 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 monohydrate 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

Table 2: The reproducibility of peak height and t_R of oxacillin sodium monohydrate

Representative samples and drugs ($\mu\text{g/mL}$)	Peak height (mm)	$\pm\text{RSD}\%$	t_R (min)	$\pm\text{RSD}\%$
0.3	7	0.492	11	0.324
0.6	13	0.522	11	0.355
0.9	20	0.583	11	0.310
5 $\mu\text{g/mL}$ for Drugs (1)	83	0.517	11	0.307
5 $\mu\text{g/mL}$ for Drugs (2)	84	0.510	11	0.351

RSD: Relative standard deviation, t_R : Retention time

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

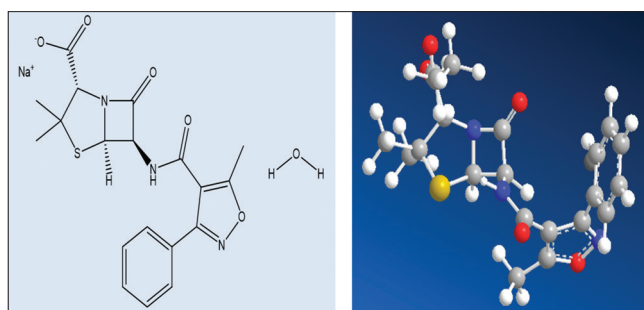
R^2	0.9992
Std Er ^a	0.638
Std Er Est ^b	0.586
Intercept	0.7
Slope	21
LLOD $\mu\text{g/mL}$	1.46
LLOQ $\mu\text{g/mL}$	4.44
MDL (standard) $\mu\text{g/mL}$ ($\text{SD} \times t_{95\%}$) at ($n=5-1$)	0.02037
MDL (sample) $\mu\text{g/mL}$ ($\text{SD} \times t_{95\%}$) at ($n=5-1$)	0.02133

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

- Analar oxacillin powder as standard, Sigma-Aldrich, Germany.
- The stock standard solution 100 $\mu\text{g/ml}$ oxacillin sodium monohydrate was prepared by dissolving accurately weight 100 mg of oxacillin sodium monohydrate in 1000 ml methanol which was purchased from Aldrich 33/8467-LTD.
- A working solution in the range 0.3–1.5 $\mu\text{g/ml}$ 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, and 100 mg of this powder accurately weighs and dissolved in 1000 ml of methanol.

Procedure

Under a temperature of 25°C and pressure of 120 bar, all chromatography experiments were carried out by HPLC-UV chromatography system, which consisted of LKB-pump model; 2150-HPLC that pumping the eluent at 1 ml/min. Cloxacillin and flucloxacillin monohydrate capsules or standard was manually injected with Metrohm electronic injection valve fitted with 100 μl loop in the eluent of water:acetonitrile:methanol (40:30:30), all with 10 mM formic acid at pH=4.8. IonPac column Zorbax 300-SCX Agilent, 5 μm , 4.6 \times 250 mm (p/N880952-704), was used as a separation column. APD 303UV detector single beam spectrophotometer (Japan), equipped with 18 μl flow cell

**Figure 1:** Structure of oxacillin sodium monohydrate

(Helma UK), was used to measure the UV signal at 230 nm of the separated species. A data logger LabJack, Ocean Controls, Australia. Personal computer and printer were handling the data of the homemade system. A symmetrical peak height is corresponding to the oxacillin concentration of standards and sample concentrations.^[21-24]

RESULTS AND DISCUSSION

In the present study, a new HPLC method for quantitative analysis of two penicillins in oral dosage form was developed and validated. The result of two penicillins under the established conditions is listed in Table 1, and a method of the standard calibration was used to obtain the calibration curve for oxacillin sodium monohydrate, by plotting the concentration versus the peak height of asymmetrical peaks. It is penicillin in ear over the range of 0.3–1.5 $\mu\text{g/ml}$ oxacillin sodium monohydrate. The IC system supplies with column temperature evaluating in the range of 25–45°C in five-degree steps. As expected, increased column temperature reduced retention time (t_R) and led to a good baseline for standard solutions and sample separation in chromatographic diagrams. However, maintaining the constant temperature in the IC system is difficult. Hence, 25°C was selected to be used in future work.^[25] Table 2 lists the R^2 and slope of the curve, which are 0.9992 and 21.0, respectively.^[26] The reproducibility of the method was estimated by injection of 0.3, 0.6, and 0.9 $\mu\text{g/ml}$ represented standard and two commercial drugs into eluent. Excellent relative standard deviation (RSD) % for t_R and peak height was obtained as shown in Tables 2 and 3. The lower limit of detection (LLOD) and lower limit of quantitation (LLOQ), LLOD = 3.3 SD/S and LLOQ = 10 SD/S, are the concentrations that give the

Table 4: Method accuracy for oxacillin sodium monohydrate recoveries obtained by HPLC-UV system

Taken concentration ($\mu\text{g/mL}$)	Found concentration ($\mu\text{g/mL}$)	Recovery \pm RSD%	Found by classical method ($\mu\text{g/ml}$)	Recovery \pm RSD %
0.3	0.29	96.6 \pm 0.492	0.3	100 \pm 0.390
0.6	0.6	100 \pm 0.522	0.6	100 \pm 0.421
0.9	0.85	94.4 \pm 0.583	0.87	96.6 \pm 0.576
1.2	1.1	91.6 \pm 0.517	1.1	91.6 \pm 0.428
1.5	1.5	100 \pm 0.510	1.5	100 \pm 0.455
5 $\mu\text{g/ml}$ drug (1)	4.8	96 \pm 0.581	4.9	98 \pm 0.455
5 $\mu\text{g/ml}$ drug (2)	4.9	98 \pm 0.499	4.9	98 \pm 0.482

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

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

Taken concentration ($\mu\text{g/mL}$)	Intraday		Interday	
	Found ($\mu\text{g/mL}$)	Recovery \pm RSD%	Found ($\mu\text{g/mL}$)	Recovery \pm RSD%
0.3	0.29	96.6 \pm 0.492	0.28	93.3 \pm 0.501
0.6	0.6	100 \pm 0.522	0.58	96.6 \pm 0.512
0.9	0.85	94.4 \pm 0.583	0.84	93.3 \pm 0.483
1.2	1.1	91.6 \pm 0.517	1	83.3 \pm 0.502
1.5	1.5	100 \pm 0.511	1.3	92.8 \pm 0.451

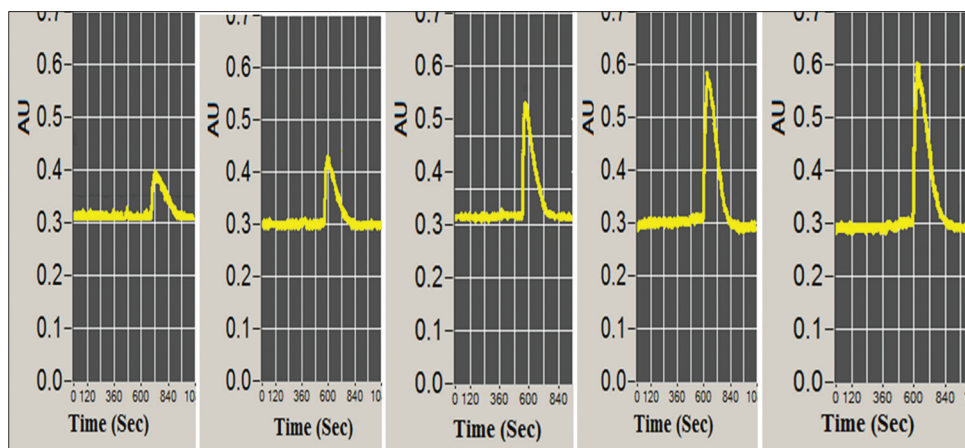


Figure 2: Chromatogram calibration curve of oxacillin sodium monohydrate in concentrations (0.3, 0.6, 0.9, 1.2, and 1.5 $\mu\text{g/ml}$) and peak height (7, 13, 20, 27, and 30 mm), respectively

signal-to-noise ratio of 3:1 or 10:1, respectively. It can detect and verify the validity of the results through the (SD) values and the response of the slope (S) of the calibration curves. Using the single-sided Student's t-test method (at the 95% confidence limit) for five consecutive injections of 0.9 $\mu\text{g/ml}$ of oxacillin sodium monohydrate sample and standard, the values of LLOD and LLOQ were 1.46 $\mu\text{g/mL}$ and 4.44 $\mu\text{g/mL}$, respectively.^[27] IonPac Zorbax 300-SCX, 5 μm 4.6 \times 250 mm column, was recommended as a suitable and efficient separation column for oxacillin and samples. It can be detected using UV detector at λ_{max} 230 nm with a mixture of eluent that consisting of water:acetonitrile:methanol (40:30:30), with 10 mM of formic acid at pH = 4.8, which can be freshly prepared.^[28]

Figure 2 shows that the column has high efficiency to separate oxacillin sodium monohydrate, and the linear gradient ranged between 11 min for each injection and one peak appearance in chromatogram. The reason for obtaining characteristic peaks is the use of HPLC-UV method, which is an easy and highly sensitive method in the estimation of various pharmacological Samples, especially penicillin derivatives; however, some ringing peaks refer to very small concentration of CO_2 dissolved in eluent.^[29] To evaluate the accuracy of the HPLC-UV system, the recovery was studied through the application of the standard additive method. Therefore, recovery trials were performed on three representative criteria and two commercial drug samples [Figures 4 and 5]. The standard additive method was used

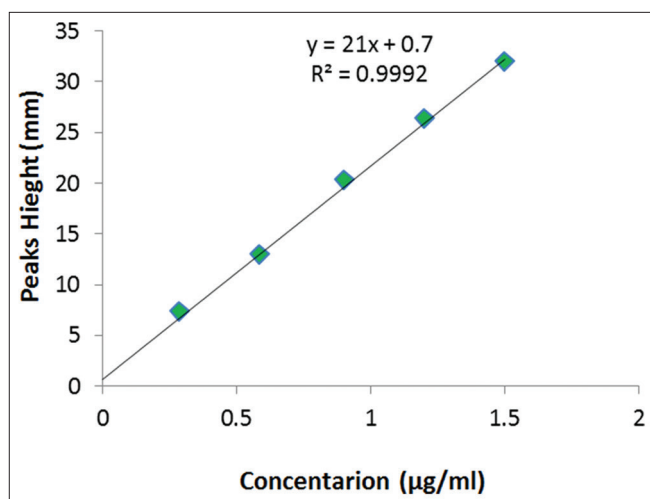


Figure 3: Calibration curve for oxacillin

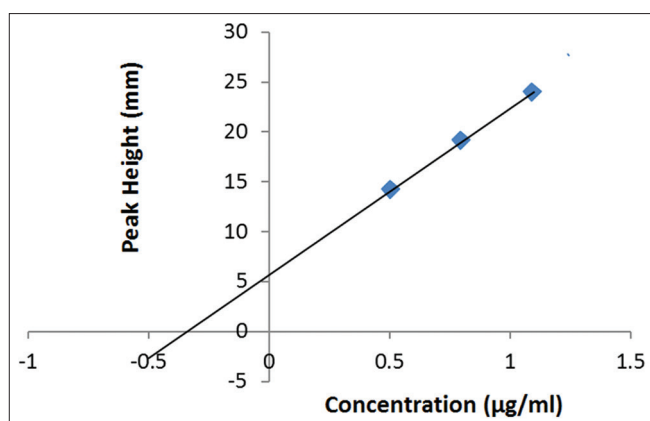


Figure 4: Standard additions for oxacillin sodium monohydrate determination

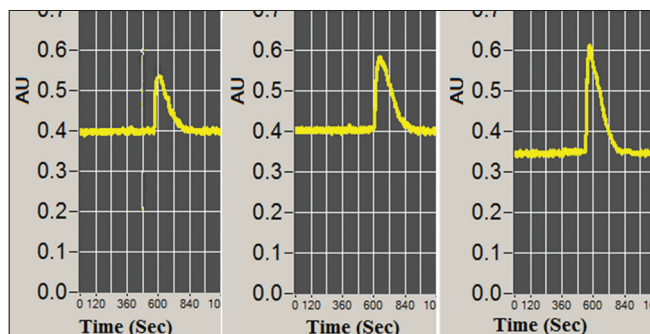


Figure 5: Peaks of standard addition method in concentrations (0.5, 0.8, and 1.1) and peak height (13, 19, and 25 mm), respectively

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.^[30,31] Precision of method, reported as % RSD, was estimated by measuring repeatability (intraday assay) for five replicate injections for all concentrations of oxacillin sodium monohydrate and two samples. The intermediate

precision (interday variation) was also studied for 2 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.^[2,32]

CONCLUSION

This work described HPLC system equipped with UV detector for oxacillin sodium monohydrate determination in two commercial pharmaceutical drugs. This developed method consider 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 scheme of chromatogram, shown in [Figure 3]. 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 0.3–1.5µg/mL for drugs.

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AUTHOR'S CONTRIBUTIONS

This research was done individually in the laboratories of the College of Pharmacy, University of Basrah. This research was completed over a period of 2 months with serious and continuous work, and therefore, excellent results were obtained in finding an easy and sensitive method to the analysis of two penicillin drugs in oral dosage form.

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