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New Quantitative Visible (VIS) Spectrophotometric Analysis of pure Oxacillin in a Pharmaceutical: A Statistical Study of Linear Regression

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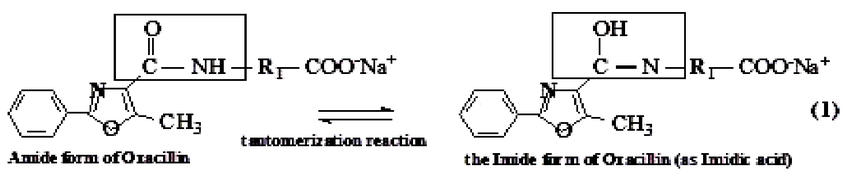
INTRODUCTION & AIM

Oxacillin is a penicillinase-resistant, narrow-spectrum beta-lactam crystalline antibiotic of the penicillin class, resistant to beta-lactamases . Oxacillin actually is a penicillin beta-lactam antibiotic from the second generation, frequently used in the treatment of bacterial infections caused by susceptible, usually gram-positive, organisms. Oxacillin has in vitro activity against gram-positive and gram-negative aerobic and anaerobic bacteria. The bactericidal activity of Oxacillin results from the inhibition of cell wall synthesis and is mediated through Oxacillin binding to penicillin binding proteins (PBPs). Oxacillin, through its β-lactam ring, covalently binds to penicillin-binding proteins, which are enzymes involved in the synthesis of the bacterial cell wall. This binding interaction interferes with the transpeptidation reaction and inhibits the synthesis of peptidoglycan, a prominent component of the cell wall. By decreasing the integrity of the bacterial cell wall, it is thought that oxacillin and other penicillin kill actively growing bacteria through cell lysis. Oxacillin is stable against hydrolysis by a variety of beta-lactamases, including penicillinases, and cephalosporinases and extended spectrum beta-lactamases. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, Oxacillin inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that Oxacillin interferes with an autolysin inhibitor. Oxacillin is stable against hydrolysis by a variety of beta-lactamases, including penicillinases, and cephalosporinases and extended spectrum beta-lactamases. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, Oxacillin inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that Oxacillin interferes with an autolysin inhibitor. **. The main purpose of this paper was to develop and apply a new spectrophotometric method in the visible (VIS) field for the analysis of pure Oxacillin from various pharmaceutical samples.**

METHOD

As with other members of the penicillin family, the chemical structure of oxacillin features a 6-aminopenicillanic acid nucleus with a substituent attached to the amino group. The 6-aminopenicillanic acid nucleus consists of a thiazolidine ring attached to a β-lactam ring, which is the active moiety responsible for the antibacterial activity of the penicillin family. The substituent present on oxacillin is thought to impart resistance to degradation via bacterial β-lactamases. **The objectives of the research** were represented by: the initiation and optimization of a new and accurate dosing method for Oxacillin evaluation from various differerent samples; comparison of the obtained results with the *Romanian Pharmacopoeia and the European Pharmacopoeia Xth Edition Rules* regarding the maximum percentage alloowed deviations compared to from the official stated amount of pure Oxacillin on solid film-coated capsule of a pharmaceutical product; and linearity analysis of the Oxacillin dosing method, according to the official protocols of *Good Pharmaceutical Practice.*

Method: Oxacillin reacted completely with 1.10 Phenanthroline 0.2% alcoholic solution in the presence of ferric **chloride $FeCl_3$, (6%), aqueous solution, which led to the quantitative synthesis of an intense bright yellow complex with a faint orange tint, after heating the solutions for 20 minutes at 70 ºC constant temperature. After gradually cooling and resting in the dark for 20 minutes, the intense bright yellow colored synthetized complex was then spectrophotometrically determined at λ = 420 nm corresponding to its absorption maximum wavelength, in relation to double-distilled water as a blank.**



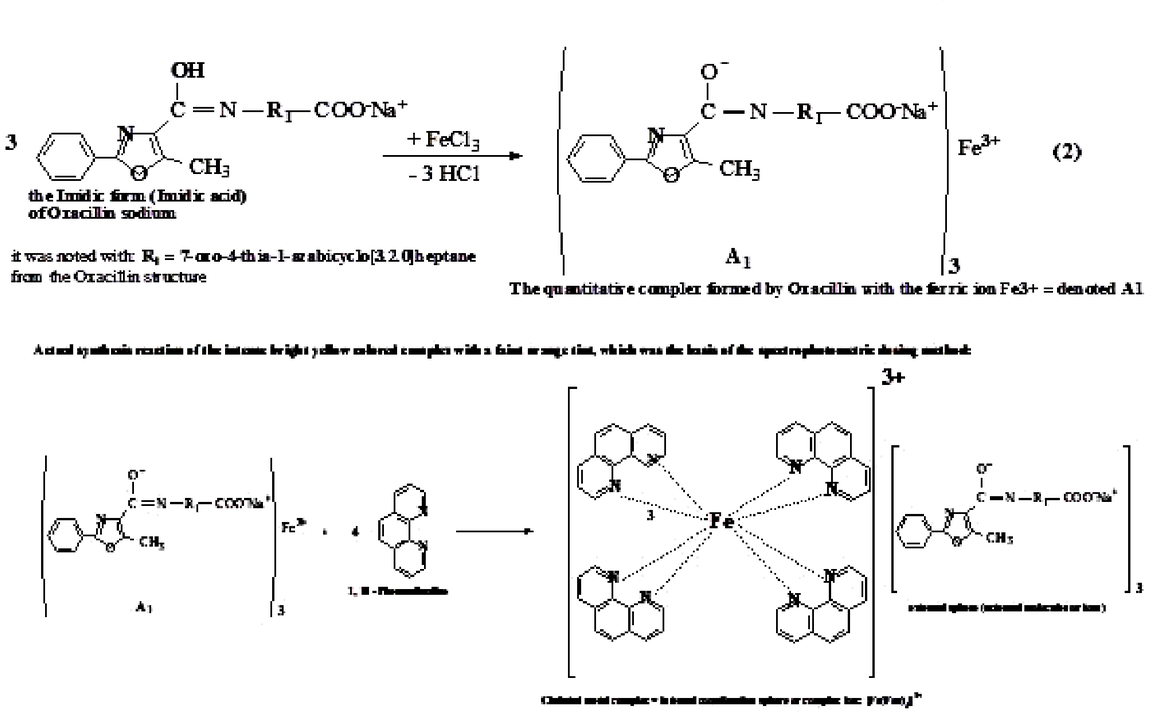
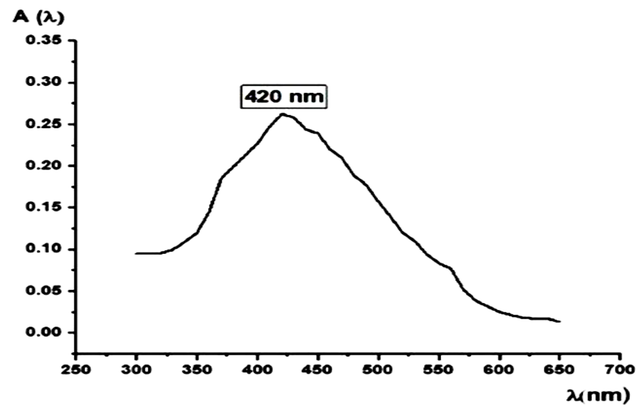


Fig. 1 Spectrophotometric dosing method of Oxacillin with 1-10 Phenanthroline in the Visible (VIS) field

RESULTS & DISCUSSION



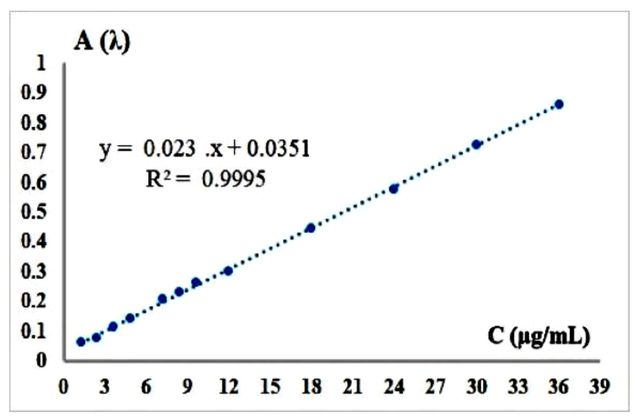


Fig. 2 Absorption Spectrum of Bright yellow intense dye quantitatively formed by Oxacillin, following the reaction with 1,10 – Phenantrolin and Fig. 2 Calibration Graph obtained for standard solutions of Oxacillin (1.20 µg/mL - 36.00 µg/mL)

Table 1. Measured mean absorbances values depending on the standard solutions concentrations of Oxacillin sodium (1.20 µg/mL - 36.00 µg/mL)

Nr. det.	mL working solution Oxacillin sodium,. 300 µg/mL	mL $FeCl_3$, 6 % solution	mL alcoholic solution 1,10-Fenantrolină, 0,2 %	C (µg/mL)	A (λ)
1.	0,1	0,1	0,1	1.20	0,064
2.	0,2	0,2	0,2	2,40	0,081
3.	0,3	0,3	0,3	3,60	0,117
4.	0,4	0,4	0,4	4,80	0,145
5.	0,6	0,6	0,6	7,20	0,208
6.	0,7	0,7	0,7	8,40	0,233
7.	0.8	0.8	0.8	9,60	0,264
8.	1,0	1,0	1,0	12,00	0,302
9.	1,5	1,5	1,5	18,00	0,447
10.	2,0	2,0	2,0	24,00	0,579
11.	2,5	2,5	2,5	30,00	0,725
12.	3,0	3,0	3,0	36,00	0,864

Table 2 Oxacillin Sample Solution Preparation and the Dosing Values

unknown Sample Solution of Oxacillin®	Ap'	Cp' (µg/mL)	µg Oxacillin sodium / solid capsule	mg Oxacillin sodium / solid capsule
	0,140	4,56087 µg/mL	491308,004 µg	491,308 mg
	$FeCl_3$ 6%	1,10 - Phenanthroline, 0,2%		
0,3 mL sample	0,3 mL	0,3 mL		

Table 3. Linear Regression Analysis Parameters - Oxacillin Dosing Method

Linear Regression Statistics parameters (Regression Statistics)	
Multiple R (Correlation Coefficient)	0.999752
R Square (R^2 = Linear Regression coefficient)	0.999504
Adjusted R Square (R^2)	0.999455
Standard Error of the regression line (SE)	0.006130
Observations	12
Detection Limit (LOD) = (3. SE) / Slope = (3 . 0,006130) / 0.023 = 0,7996 µg/ mL.	LOD = 0,7996 µg/ mL
Quantitation Limit (LOQ) = (10. SE) / Slope = (10 . 0,006130) / 0.023 = 2,6652 µg/ mL	LOQ = 2,6652 µg/ mL

Slope was 0,023 deduced from Fig 2. (y = 0,023.x + 0,0351 or A(λ) = 0,023 Cp.(µg/mL) + 0,0351). Standard Error of the regression line (SE) = 0.006130 (Table 3)

CONCLUSION

As a result of the experiment, **491.308 mg** of pure Oxacillin/solid capsule of pharmaceutical product were obtained, which corresponded to a percentage content of **98.262 %** determined by pure sodium Oxacillin. This amount was very close to the reference value, officially indicated by the manufacturer, which was **500 mg pure Oxacillin on capsule**. The relative percentage deviation (relative procedural error) compared to the official allowed value (500 mg)was **only 1.738 %** and it fell perfectly within the official normal and admitted percentage error values indicated by the Romanian and European Pharmacopoeias X Th Edition **(± 5 %).**

FUTURE WORK / REFERENCES

1. https://go.drugbank.com/drugs/DB00713, accessed Thursday 12.09.2024, at 18.00 hour .
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