*Available online at* www.pelagiaresearchlibrary.com

Pelagia Research Library

**Der Pharmacia Sinica, 2012, 3 (3):327-331**

Der Pharmacia Sinica
**ISSN: 0976-8688**
**CODEN (USA): PSHIBD**

# Mercurimetric Titration of Some Beta Lactam Antibiotics in Bulk and Pharmaceutical Products

**Hamad M. I. Hasan[1], Ibrahim H. Habib[1,2*] and Suad K. Omar[3]**

*[1]Faculty of Science, Omar Al- Mukhtar University, Al- Beida, Libya*
*[2]National Research Centre, Applied Organic Chem. Dept., Dokki, Cairo, Egypt*
*[3]Faculty of Science, Omar Al- Mukhtar University, Al- Beida, Libya*

---

**ABSTRACT**

*A simple, sensitive and rapid method is described for the determination of some derivatives of beta lactam antibitics in bulk drug and in tablet dosage forms. In this method, ampicillin and amoxicillin are first degraded in 1 M NaOH medium for 20 min, acidified with nitric acid then titrated volumetrically with mercuric ions in the presence of few drops of diphenlycarbazone. The method is applicable over 0.3-2 µg/ml range of antibiotics and the stoichiometric reaction between antibiotic and mercury is found to be 2:3, respectively. Intra-day and inter-day precision and accuracy for the developed methods were evaluated. The method is successfully applied to the assay of antibiotics in capsule and injection formulations and the results were statistically compared with those of a reference method. No interference was observed from common capsule excipients.*

---

## INTRODUCTION

Ampicillin and amoxicillin are broad spectrum penicillin antibiotics [1] and their basic nucleus is 6-aminopenicillanic acid, which consists of a highly strained and reactive thiazolidine ring linked to β-lactam ring. Because of various electron donor groups ($CO_2^-$, R-S-R, $NH_2$, etc.) available in this side chain, they exhibited acid-base and complexing properties. In neutral aqueous solutions, the penicillin derivatives have ability to form complexes as monodentate either with monovalent cations, e.g. Ag(I) [1], divalent cations, e.g. Cd(II) [2], Cu(II) [3], Zn(II) [2], or trivalent cations, e.g. Al(III) [4]. On the other hand, copper(II) ions have also ability to form 1:2 and 1:3 complexes but only with acidic hydrolytic products of penicillin [3,5]. The same results are obtained when Hg(II) cations form 1:1 and 1:2 complexes with alkaline degraded products of penicillins [6,7]. All these stochiometric calculations were detected either by spectrophotometric or potentiometric techniques. In the present work, Hg(II) form 3:2 complex by simple and rapid volumetric titration as elucidated below.

## MATERIALS AND METHODS

### *Materials*

All chemicals used were of analytical grade. Standard solutions of ampicillin and amoxicillin (Aldrich, with purity 99% and 98%, respectively) were prepared by dissolving 50 mg in 10 ml methanol and completed to 100-ml measuring flask with double distilled water. Standard stock solution of mercuric nitrate was prepared by placing appropriate amount of ultra pure mercury (Merck) in least volume of diluted nitric acid, waiting for the complete

reaction and then completing with double distilled water to the mark obtaining finally 0.1 M solution. Solutions of sodium hydroxide 1 M; nitric acid 1 *M;* ethanolic solution of 2,4 diphenylcarbazone 0.2% were also prepared.

***General Procedure***

Place a portion of 0.3-2.1 mg/ml antibiotic solution in a 100-ml conical flask containing 1 ml of 1 M sodium hydroxide solution and leave aside for 10 min at room temperature. Add 2 ml of 1 M nitric acid and 2-3 drops of 0.2% diphenylcarbazone. Titrate against 0.001 M mercuric nitrate solution till the end point from yellow to deep violet color. Repeat the experiment three times and make blank under the same condition but without the active ingredient. Calculate the amount of the ingredient per sample expressing in milligram from the following general equation:

$$w = M_{Hg}(V_{Hg} - V_{blank})MW_{drug} = mg$$

where $MW_{drug}$ is the molecular weight of ampicillin and amoxicillin (394.41 and 238, respectively).
$M_{Hg}$ and $V_{Hg}$ are the molarity and volume of mercuric nitrate in milliliters required to complex with degraded product of amoxicillin or ampicillin.

$V_{blank}$ is the volume of mercuric nitrate due to the blank

***Procedure for the analysis of pharmaceutical products***

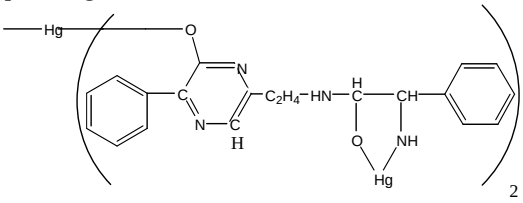
The following formulations containing ampicillin I as sodium salt in vials or as trihydrate II in capsules or suspension and amoxicillin II are purchased from local pharmaceutical stores and used in the present investigation: PAN Ampicillin vials (Panpharma S.A., France), Epicocillin vials (EIPICO, Egypt), Penodil capsules (Remedica, Cyprus), Maymox capsule (GMPMSCO, Great Jamahiriya), Amoxil vials (UNIMED, Tunisia) and Amoxicillin capsules (Hemofarm, Serbia).

An amount of vial/capsule/suspension powder was accurately weighed and transferred into a 100-ml calibrated flask, 10-ml of methanol was added and shake for moments. Then, the volume was diluted to the mark with double distilled water, the content was mixed well and filtered using a Whatman No. 42 filter paper. A convenient aliquot (*e.g.*, 3 ml) of the filtrate was analyzed by the titrimetry as described earlier.

## RESULTS AND DISCUSSION

It is well known that β lactam antibiotics having α-amino group on the side chain (aminopenicillins, such as ampicillin and amoxicillin) are degradable in alkaline media into pyrazine derivatives [10]. Toyozo et al [10] have proved by HPLC technique that 2-hydroxy-3-phenyl-6-ethylpyrazine is ready to complex with divalent mercuric ions. In the present work, and as shown in figure 1, the relationships between taken and found concentrations of I and II are linear over the concentration range of 0.3-2 µg/ml with correlation coefficients of 0.999, confirming the reaction proceeds stoichiometrically in molar ratio 2:3.

The structural formula of the complex might be as shown below:




The degradation reaction was found to be completed and quantitative in 20 min and continuous degradation beyond this time up to 60 min had no effect on the stoichiometry in condition to restrict to the proposed concentration range of 0.3-2 µg/ml.

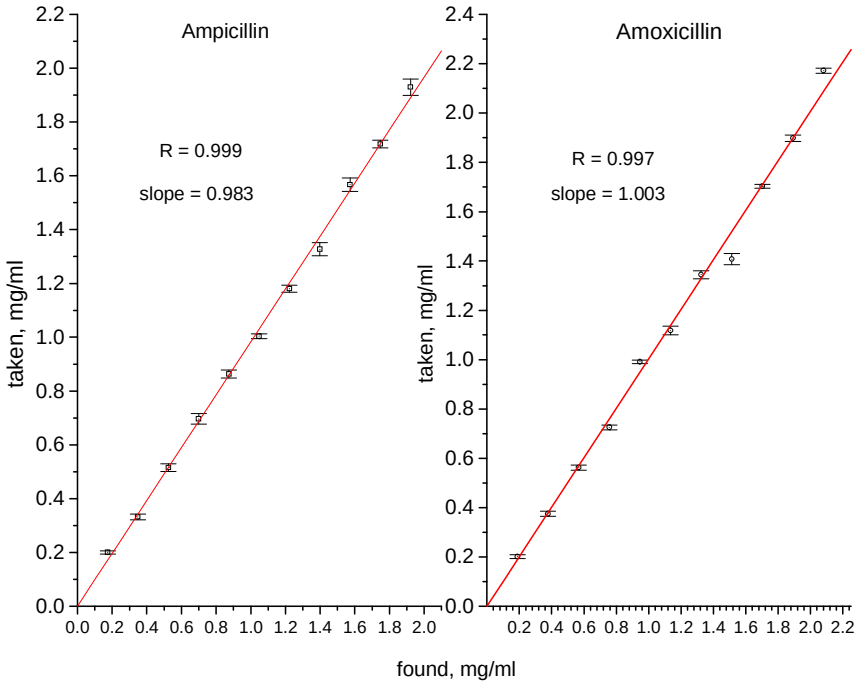



**Figure 1: Correlation coefficient of taken versus found amounts of ampicillin and amoxicillin**

*Precision and accuracy*

Analysis of variance ANOVA, two-way analysis without replication, enables the separation of two different sources of variation: the intra- and inter-day variations [11]. The repeatability (intra-day) and reproducibility (inter-day) of the results obtained by means of the proposed titremetric procedure are examined for different concentrations of ampicillin and amoxicillin on the same day and on two successive days, respectively. Table 1 and 2 show no significant difference between two sources of content variation (intra- and inter-day precision represented by rows and columns, respectively) at the 95% confidence level. Mean recoveries of 100.45±2.62% (n = 12) on the same day and on two success days were achieved, that indicating high accuracy and precision of the proposed procedure and is suitable for quality control of penicillins.

Intra-day precision was assessed from the results of six replicate analyses on pure drug solution. The mean values and relative standard deviation (RSD) values for replicate analyses at eleven different concentration levels were calculated. To evaluate the inter-day precision, analysis was performed over a period of two days, using the same stock solutions kept in refrigerator.

The accuracy of the methods was determined by calculating the percentage deviation observed in the analysis of pure drug solution and expressed as the relative error. Tables 1 and 2 summarize the intra-day precision and accuracy data for the assay of I and II in pure drug solution by the proposed method and they were within 2.0%. The inter-day RSD was <2.4%.

**Table 1: Analysis of variance due to intra- and inter-days variations for the titremetric determination of ampicillin**

| mg/ml taken | 1st day | 2nd day | Mean |
|---|---|---|---|
| 0.175 | 105.33 | 103.85 | 104.59 |
| 0.349 | 97.11 | 98.05 | 97.58 |
| 0.524 | 99.93 | 97.39 | 98.66 |
| 0.699 | 98.17 | 102.81 | 100.49 |
| 0.874 | 99.98 | 97.79 | 98.885 |
| 1.048 | 99.56 | 97.27 | 98.415 |
| 1.223 | 98.78 | 100.63 | 99.705 |
| 1.398 | 98.95 | 97.36 | 98.155 |
| 1.572 | 97.89 | 99.55 | 98.72 |
| 1.747 | 98.8 | 97.7 | 98.25 |
| 1.922 | 102.18 | 99.18 | 100.68 |
| Average | 99.70 | 99.23 | 99.47 |
| SD | 2.29 | 2.30 | 1.96 |
| ANOVA | | | |
| *Source Variation* | $F_{ca}$ | | $F_{th}$ |
| Rows (df=10) | 2.653 | | 2.978 |
| Columns (df=1) | 0.410 | | 4.965 |

**Table 2: Analysis of variance due to intra- and inter-days variations for the titremetric determination of amoxicillin**

| mg/ml taken | 1st day | 2nd day | Mean |
|---|---|---|---|
| 0.189 | 106.35 | 104.88 | 105.615 |
| 0.378 | 97.35 | 102.23 | 99.79 |
| 0.568 | 102.47 | 98.35 | 100.41 |
| 0.757 | 97.51 | 95.82 | 96.665 |
| 0.946 | 102.4 | 100.39 | 101.395 |
| 1.135 | 101.88 | 98.94 | 100.41 |
| 1.324 | 99.81 | 102.37 | 101.09 |
| 1.514 | 99.8 | 96.19 | 97.995 |
| 1.703 | 100.71 | 97.43 | 99.07 |
| 1.892 | 97.28 | 100.21 | 98.745 |
| 2.081 | 104.07 | 103.49 | 103.78 |
| Average | 100.88 | 100.03 | 100.45 |
| SD | 2.92 | 2.99 | 2.54 |
| ANOVA | | | |
| *Source Variation* | $F_{ca}$ | | $F_{th}$ |
| Rows (df=10) | 2.879 | | 2.978 |
| Columns (df=1) | 0.881 | | 4.965 |

**Table 3. Assay of formulations by the proposed method**

| | Taken | Found*, % | | Reference | |
|---|---|---|---|---|---|
| | mg/ml | Mean | SD | Mean | SD |
| PAN AMPICILLIN | 0.86 | 98.37 | 3.05 | 99.54 | 2.72 |
| 500 mg/vial | 1.72 | 98.75 | 2.57 | 100.21 | 2.05 |
| EIPICOCILLIN | 0.86 | 100.42 | 1.85 | 99.67 | 2.31 |
| 1000 mg/vial | 1.72 | 100.76 | 3.35 | 99.01 | 3.22 |
| PENODIL | 0.48 | 101.17 | 4.16 | 100.21 | 3.27 |
| 500 mg/capsule | 0.96 | 99.95 | 3.13 | 100.35 | 2.86 |
| MAYMOX | 1.2 | 101.75 | 1.63 | 99.46 | 2.98 |
| 250 mg capsule | 2.4 | 100.06 | 0.91 | 100.68 | 2.46 |
| AMOXIL | 0.86 | 99.42 | 2.36 | 98.79 | 1.97 |
| 1 g/vial | 1.72 | 98.95 | 3.05 | 99.11 | 2.34 |
| AMOXICILLIN | 0.475 | 98.87 | 2.47 | 98.45 | 3.06 |
| 500 mg/capsule | 0.95 | 100.71 | 0.94 | 99.33 | 2.87 |
| *average of n=12 | | 99.93 | | 99.57 | |
| SD =standard deviation | | 1.069 | | 0.683 | |
| **$t_{theo}$=2.20 at df=11** | | 0.3327 | | | |
| **$F_{theo}$=2.82 at df=11,11** | | 2.45 | | | |

*Application to dosage forms*

The method has been applied to determine the cited ingredients in capsules and injection vials and the results were statistically compared with those obtained by HPLC method (12). Satisfactory results are obtained as depicted in table 3 with mean recovery and its standard deviation 99.93 and 1.1% respectively for some pharmaceutical products used. The calculated *t* and *F*-values were lower than the tabulated values at 95% confidence level, revealing that the proposed methods and the reference method have similar accuracy and precision.

## CONCLUSION

The proposed method is accurate to –1.6 to +1.3% and precise from 0.9 to 3.5% with a determinable range of 0.1–2.4 mg/ml. The results of the assay demonstrate that it can be used to determine the content uniformity of capsules, capsule to capsule variation, as well as the purity of antibiotic raw material. Besides the simplicity of the procedure, the titremetric method has the clear advantages of sensitivity comparable to that achieved by an expensive technique like HPLC.

## REFERENCES

[1] Rang HP, Dale MN and Ritter JM, *Pharmacology*, 3rd Ed., Chuchill Livingstone Publications, **1996**, pp 725.
[2] Alekseev V, Demskaya L, *Russian J. Coordination Chem.* **2007**, 33(3), 203
[3] Alekseev V, Milashs E, Larin S, Shigina O., *Russian J. Coordination Chem.* **2006**, 32(7), 469.
[4] Pilar Gutiérrez Navarro, Ana Márquez Garcia, Pedro Martinez de las Parras, *Polyhedron*, **1992**, 11(9), 1049.
[5] Alekseev V and Zamyslov V, *Russian J. Coordination Chem.* **2007**, 33(4), 254.
[6] Sher A, Veber M, Marolt-Gomišček M, Gomišček S, *International J. Pharmaceutics*, **1993**, 90(3), 181.
[7] Pospisilov B, Kubes J, *Pharmazie* , **1986**, 41(10), 705.
[8] Bo Karlberg and Ulf Forsman, Analytica Chimica Acta **1976**, 83, 309.
[9] *The British Pharmacopoea*, London **1998**, pp 1151.
[10] Toyozo U, Masada M, Yoshihiro K, Terumichi N, *Chem. Pharm. Bull*. **1981**, 29(5) 1344.
[11] Miller JC and Miller JN, *Statistics for Analytical Chemistry* , 3rd Ed , Ellis Horwood , Chichester. **1993**, pp 53.
[12] Ashnagar A and Gharib Naseri N, *E-Journal of Chemistry*, **2007**, 4(4), 536.