

## Assay of Ampicillin in capsule Dosage Form Manufactured by Different Pharmaceutical Manufacturing Factories available in Iraq.

\*Rehab Alrubaiy

\* NCDRC (National Center for Drugs control & drug Researches) Ministry of health, Baghdad, Iraq

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Corresponding Author email:

rehabalrubaiy@yahoo.com

### Abstract:

The assay of Ampicillin is carried out by HPLC (High performance liquid chromatography) system to calculate the weight of the active constituent of the drug in this dosage form. In this work we study six samples of Ampicillin 250 mg capsule manufactured by different pharm-

aceutical companies available in Iraq to evaluate the content of drug in this dosage form. This has been achieved by making calibration curve of different concentrations of stock solution, using standard ampicillin tri hydrate (Reference Standard Ampicillin) USP, we obtained different reading of the area under the peak (AUP) which followed straight – line equation.

**Key words:** Assay, (HPLC) High performance liquid chromatography, (AUP) Area under the peak, Ampicillin Trihydrate (AMP. Tri. H).

تقييم عقار الامبسيلين بالشكل الصيدلاني الكبسول مصنع من عدة مصانع دوائية مختلفة متوفرة في العراق.

رحاب حسين الربيعي

المركز الوطني للرقابة والبحوث الدوائية/وزارة الصحة / بغداد. العراق

### الخلاصة:

تقييم عقار الامبسيلين بالشكل الصيدلاني الكبسول يتم بواسطة نظام (التحليل الكروماتوغرافي العالي الكفاءة للسوائل) والذي بواسطته يمكن تحديد وزن المادة الفعالة للدواء في أي شكل دوائي معتمد. في هذه الدراسة تم اخذ ست عينات على شكل كبسول من عقار الامبسيلين 250 ملغم من شركات صيدلانية مختلفة متوفرة في العراق لمقارنة محتوى الدواء في هذا الشكل الصيدلاني. اجريت الدراسة بتشكيل منحنى التحديد الناتج من استخدام محاليل قياسية بتراكيز مختلفة باستعمال المعيار المرجعي للامبسيلين ثلاثي جزيئات الهيدروجين حسب دستور الادوية الامريكي لتعطي قراءات مختلفة لمساحة ما تحت القمة كل واحد منها تتبع معادلة الخط المستقيم .

**الكلمات المفتاحية:** تقييم، التحليل الكروماتوغرافي عالي الكفاءة للسوائل، مساحة ما تحت القمة، امبسيلين ثلاثي جزيئات الماء.

### Introduction:

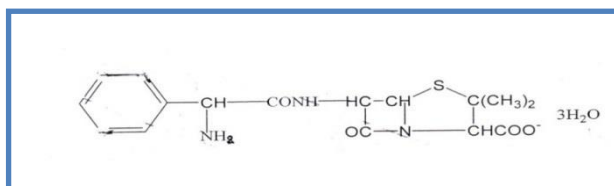
Ampicillin is abroad–spectrum antibiotic derived from penicillin nucleus 6-aminopenicilanic acid (6APA) chemically it is 6- (D (-) a-amino phenyl acetamido pencillanic acid or D (-) a- amino benzyl

pencillin (penbritin polycillin, omnipen, Amcil and principen <sup>(1)</sup> Its formulated as

capsules or vial (IV, IM) of 250, 500mg ,1000mg and suspension of 125, 250 mg/5ml <sup>[2]</sup>

Metabolism: Ampicillin is metabolized to penicilloic acid which is excreted in urine and some excreted in feces Elimination: about 20-40% of orally administered dose may be excreted unchanged within 6hr, by IV administration about 60-80 is excreted in urine within 6 hr. [3]

Ampicillin name is 6-(2-amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo (3.2.0) heptane-2-carboxylic acid [4]



**Figure (1): Chemical structure of ampicillin tri hydrate [4]**

$\lambda$  (max) UV spectrum in aqueous acid :257 nm, 262nm, 268nm, 300nm.[5]

Ampicillin white crystalline powder, practically odorless, stable at room temperature, occurs as the trihydrate and Ampicillin sodium. [14]

Decomposition point: (199-202C°) [5]

Solubility: 0.25 % solution in water has a pH3.5-5.5 its water solubility 1:150 & 1:250 in absolute ethanol. It dissolves in diluted solutions of acids and of alkali hydroxides. [6]

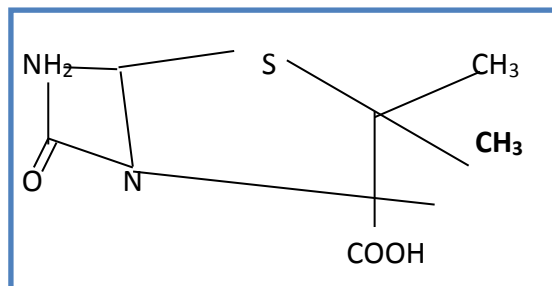
mode of action:  $\beta$  – Lactam antibiotic (such as penicillins, cephalosporins and penam) These are validated inhibitors for eight of fourteen enzymatic steps in bacterial cell wall. [7,8]

Stability: It is hydrolyzed rapidly by ring opening of  $\beta$  lactam ring, because four membered rings is joined to five or six membered ring a weaker bond exist between carbon and nitrogen of  $\beta$  lactam [9]

It is used to treat common urinary tract infection, some respiratory infection, and bacterial meningitis in children, uncomplicated gonorrhea and endocarditis as prophylaxis for dental procedures. [10] Ampicillin agent of choice against

Haemophilus influenza [10] but in combination with sulbactam.

Incomplete absorption together with excretion of effective concentration in bile may contribute to the effectiveness of ampicillin in treatment of salmonellosis and shigellosis [11,12]



**Figure (2) 6APA 6-aminopenicillanic acid**

### Chromatography:

Chromatography is the components mixture separation depending on selective distribution of these components between mobile and stationary phases. The first step in all types of chromatography involves adsorbing the sample on to some material called stationary phase, second phase (the mobile) moved across the stationary phase according to the properties of the two phases and the mixture components. This mixture will be separated according to the rate of components removing from stationary phase by mobile phase. [13] The use of HPLC leads to decline the use of gas chromatography (GC) as a quantitative technique in the analysis of drug. [14]

In HPLC technique the liquid mobile phase moves under pressure through a stainless-steel column containing 3- 10um in diameter particles of stationary phase. Analyte is loaded onto the head of the column via loop separation in the stationary phase. All components of mixture need different amount of the mobile phase in order to go out the column. [15] Analysis of formulation are not simple but when compared to analysis of drug in biological system fluids or elucidation of complex drug degradation, the presence of some difficulties the main

potential interference in analysis of a formulation are preservatives, colorants and degradation products of the formula. [16,17]

**Materials and Instruments:**

HPLC apparatus (advanced scientific instrument) consist of manager type 5000 PUMP type 1000 (KNAUERL/ Germany). (KNAUERL/ Germany). Detector type 2800 Auto sampler/chrom Gate 3900 (KNAUERL/Germany). L1 column (25 cmx4.6cm) stainless steel column Solvent Chamber PH meter: Metrohm, 644 Switzerland Water bath vibrator: KARL KOLB NO. D-6072 TYPE 254 West Germany METTLER TOLEDO204 Switzerland Balance:Melting point apparatus: Thomas Hoover England UV-Visible Spectrophotometer:CARY 100 CONC VARIAN VARLA AUSTRALIA IR Spectrophotometer back:Back USA

**Conditions of Assay using HPLC:**

1. Stationary phase: (Knauer, Germany) consist of 10 ml of 1M monobasic potassium phosphate and 1ml of 1N acetic acid, dilute to 1000ml with water, mix to adjust the pH to 5.

2. Stainless steel column C18 (ODS) Octadecyl silane with a dimension of (4.6mm x23cm) that containing 5 to 10 um packing (L1)
3. Flow rate 1.5 ml/mint
4. Column temperature: room temperature.
5. Sample conc.: 0.1 mg/UL
6. Injected volume: 20uL
- 7.The mobile phase: Prepared by a suitable distilled water, acetonitrile 1M mono basic potassium phosphate, and 1N acetic acid (909:8:10:1): [18]

-Preparation of Standard solution and calibration curve:

Dissolve an accurate amount of USP Ampicillin RS in mobile phase to obtain a solution with conc. of (1mg/ml). This stock solution shacked and sonicated to achieve complete dissolution, use this stock solution promptly after preparation (18). Then we transferred different volumes from the stock solution into 5ml volumetric flask, then diluted to 5 ml with mobile phase to prepare solutions of different concentrations we prepare another standard of (1.2 mg/ml) concentration Then each one of dilution injected into HPLC apparatus. The result was obtained using the area under the peak (AUP) method as shown in table (2).

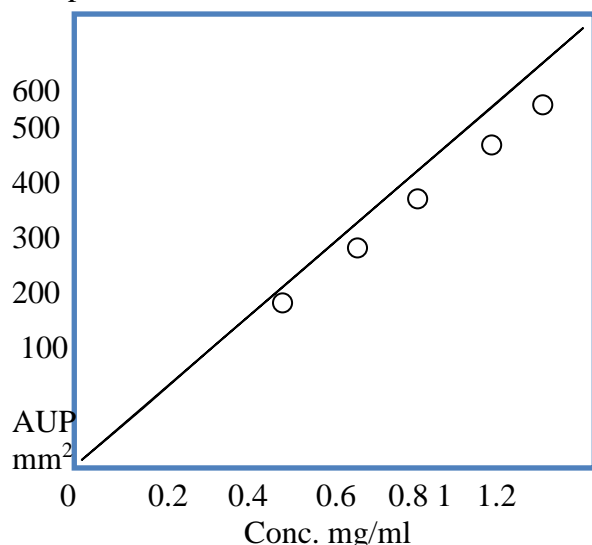
**Table (2): Dilutions of Ampicillin and their peak area.**

volume of stock solution	concentration (X- axis)	AUP mm2 (y-axis)
0ml	0 mg/ml	0.00
2ml	0.4mg/ml	185
3ml	0.6mg/ml	280
4ml	0.8mg/ml	372
5ml	1mg/ml	464
6ml	1.2mg/ml	555

We draw the calibration curve by plotting the concentration of ampicillin versus the peak area in figure (3) The calibration curve follows the equation of straight-line (y=a + bx) This equation that was used in the calculation is re arranged to:

Y=1.6+462 R=0,999969 The highly significant linear correlation of the area under the peak and the conc.is indicated by the high value of (r&r<sup>2</sup>) which are close to the highest value of perfect correlation, this ensures the accuracy of the work.

: amp-ESTD-UV detectors 2500 Peak



**Figure (3): calibration curve of**

**Ampicillin.**

The chromatogram of (0.1mg/ml) concentration of the solution of Ampicillin trihydrate RS and Ampicillin of other six pharmaceutical companies appears on figures and all are identical.

**Assay preparation:**

The content of 20 capsules was removed and weighed to determine the average weight per capsule.

Transfer amount of Ampicillin, equivalent to 100 mg of anhydrous ampicillin to a 100ml volumetric flask add about 75ml of diluent, shake and sonicate if necessary, to

achieve complete dissolution then complete the volume to 100 ml by diluent, use this solution promptly after preparation. Each one of the six samples were tested by using the same condition that was used in external standard in HPLC system to get AUP. The equation of straight line used to calculate Ampicillin concentration and the weight of (AMP.Tri. H) of each sample. <sup>[19,20]</sup>

**Results:**

From the data obtained each AUP was obtained, we can estimate the weight, percentage of error and the recovery percent compared to the standard which is 250 mg also we can calculate the relative standard deviation percent (RSD% or CV) as shown in table (4).

The concentration in (mg / ml) of Ampicillin in solution of each sample can be obtained from the straight – line equation  $Y = 1.6 + 462x$

$$X \text{ (mg/ ml)} = \frac{y - 1.6}{462}$$

Calculated weight of Ampicillin T.H. of each sample = concentration Of Amp.Tri H.X100x2.5

$$\text{Error \%} = \frac{\text{Calc. wt.}}{\text{std. wt.}} \times 100$$

$$\text{Recovery \%} = \frac{\text{Calc. wt.}}{\text{std. wt.}} \times 100$$

$$\text{RSD\%} = \frac{\text{Standard Deviation}}{\text{mean of AUP reading}} \times 100$$

**Table (4): The weight of Amp. T.H., errors %recovery % and RSD%**

No	Drug source	weight of Amp. T.H. of each sample	Errors%	Recovery %	Standard deviation	RSD% or CV.
1	AMPICILIN	270.75	8.3 %	108%	10.04	1.997%
2	JEDACILLIN	249.125	- 0.4 %	99.6%	9.245	1.995%
3	AMPICINAL	252.75	1%	101%	9.950	2.010%
4	ACAMPIN	235.5	0.8 %	99.2%	8.74	1.999%
5	JULPHAPEN	250.5	0.09 2%	100.09%	9.28	1.998%
6	APCILLIN	267	6.1 %	106.132 %	9.390	2.002%

## Discussion

Depending on above information, we can conclude the following:

1. The difference allowed for all capsules tested are within the range of maximum percentage.
2. Ampicillin capsule have quantitative percentage of 90-120, depending on USP 2009, and these results are accepted.
3. HPLC is multipurpose technique which includes separation, drug's analysis and identification.
4. Depending on results gained, Julphapen (Julphar) has nearly one hundred percent of recovery, expressed in table no. (5)
5. Also, from the result obtained it was found that Ampicillin (SDI) is the one of highest weight\*.
6. HPLC technique can be applied to the drug assay in all dosage forms the HPLC technique.
7. HPLC can be utilized for quantities and qualities estimation of drug in vivo and in vitro as a facility in biochemical and pharmaceutical approach.
8. The HPLC technique can be used for separation of enantiomers and diastereoisomers drug molecules.

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