ألخلاصة

أستخدمت في هذه الدراسة مشتقات طيف الأمتصاص لتقدير تركيز الأمبسلين بوجود عنصري النحاس الثنائي الشحنة والحديد الثلاثي الشحنة بصورة غير مباشرة بأستخدام التسخين.

ميل منحني المعايرة للأمبسلين المعاد عند الطول الموجي ٣٣٩ نانوميتر عند تسخينه لوحده لمدة ساعتان وبدرجة ١٠٠ مئوي عند المدى الخطي (٤-٢٠٠) جزء من المليون كان ٠,٠٠٨٢ ومعامل الأرتباط ٠,٩٩٩٩

وقد تم دراسة تأثير pH عند تعيين الامبسلين بالطول الموجي ٣٣٩ نانوميتر ولوحظ ان افضل pH هو ٨

إضافة النحاس الثنائي والحديد الثلاثي تأثير كبير في تعيين تركيز الامبسلين المعاد وذلك بسبب تداخل الاطوال الموجية لذلك فإن أستخدام طيف المشتقة الاولى عند الطول الموجي ٣٦٢ نانوميتر وطيف المشتقة الثانية عند الطول الموجي ٣٣٨ نانوميتر لتقدير كمية الامبسلين حيث إن ميل منحني المعايرة لطيف المشتقة الاولى ذات المدى الخطي (٤-٢٠٠) جزء من المليون كان ٠,٠٠٥٢ ومعامل التصحيح ٠,٩٩٩٩ وميل منحني المعايرة لطيف المشتقة الثانية ذات المدى الخطي (٤-٢٠٠) جزء من المليون كان ٠,٠٠٨٣ ومعامل الأرتباط ٠,٩٩٩٩ تأثير كبير في تلافي مشكلة تداخل الأطوال الموجية.

أما عند إضافة كل من أيونات الصوديوم ، البوتاسيوم ، الكالسيوم و الزنك الى الأمبسلين المعاد فلا يوجد أي تداخل عند أستخدام الطريقة الطيفية.

Summery

Refluxed ampicillin trihydrate was determined using conventional and derivative UV absorption spectrophotomerty.

The calibration curve for refluxed ampicillin trihydrate at 339 nm was found to have a linear range extended from 4 to 200 ppm with relative standard deviation for the slope of 0.0082 and correlation coefficient of 0.9999 with detection limit of 0.5 ppm.

The effect of pH on the determination of ampicillin trihydrate at this wavelength was investigated. The best pH was found to be 8.

Addition copper(II) and iron(III) have a large effect on the determination of ampicillin trihydrate due to their overlapped spectra. Therefore, first and second derivative spectrum at 362 and 338 nm, respectively, were used to overcome this problem.

The calibration curve for the first derivative spectra has a linear range of (4-200) ppm with relative standard deviation of the slope of 0.0052 and correlation coefficient of 0.9999 and the calibration curve for the second derivative spectra has a linear range of (4-200) ppm with a relative standard deviation of the slope of 0.0083 and correlation coefficient of 0.9999 in the presence of Cu(II) and Fe(III).

Na^{+}, k^{+}, Ca^{+2} and Zn^{+2} have shown no interaction when added to refluxed ampicillin trihydrate solution as shown by spectrophotometric method.

Drug is any substance used in a pharmaceutical product that is intended to modify or explore physiological systems or pathological states for the benefit of the recipient [(1)]. Pharmaceutical product is a dosage form containing one or more drugs along with other substances. Many drugs are either organic acids or bases. Various reasons determine whether drugs should be used in form of salts that may include [(1)]:

(a) modification of physicochemical properties, such as solubility, stability, photosensitivity, and organoleptic characteristics, (b) improvement of bioavailability through modification of the absorption, increase of potency, and extension of effect, and (c) reduction of toxicity.

Drugs are used for one or more of the following purposes: (a) provision of materials lacking in the organism: for example, vitamins, mineral salts, protein hydrolysates, and hormone, (b) prevention of disease or an infection: for example, sera and vaccines, (c) fight against an infection: for example, chemotherapeutics including antibiotics, (d) temporary blocking of normal function: for example, general and local anesthetics and oral contraceptives, (e) correction of a deranged function: 1) dysfunction: for example, cardiotonics for treatment of congestive heat failure, 2) hypofunction: for example, hydrocortisone for treatment of suprarenal insufficiency, 3) hyperfunction: for example, methyldopa in arterial hypertension. (f) detoxification of the body: for example, antidotes, and (g) diagnostic auxiliary agents: for example, rediopaque compounds.

The effects that drugs may cause result in a complex pattern of processes in which various factors intervene. Three phases may be observed in drug action: the pharmaceutical phase, a pharmacokinetic phase, and a pharmacodynamic phase as described in figure(1).

During the pharmaceutical phase, which is also called *the phase of exposition* where the disintegration of the form in which drug is administered. The fraction of the dose that is available for absorption constitutes a measure for the pharmaceutical availability [(2)].

During the pharmacokinetic phase, the absorption, distribution, metabolism and excretion of the drug occur. That fraction of the dose that reaches the general circulation is a measure of the biological availability.

The pharmacodynamic phase comprises the process of interaction of the drug with receptor. This interaction results in a stimulus which after a series of chemical and biochemical phenomena produces the expected biological effect.

Drugs and other strange chemical compounds that penetrate the living organism are either stored in the body or be eliminated after a period of time. While in the interior of the organism, they may continue intacting or undergoing chemical transformation, giving the following types of compounds: less active, more active and with similar or different activity. This process of chemical alteration of drugs inside a living organism is called *drug metabolism* or *drug biotransformation* [(3)].

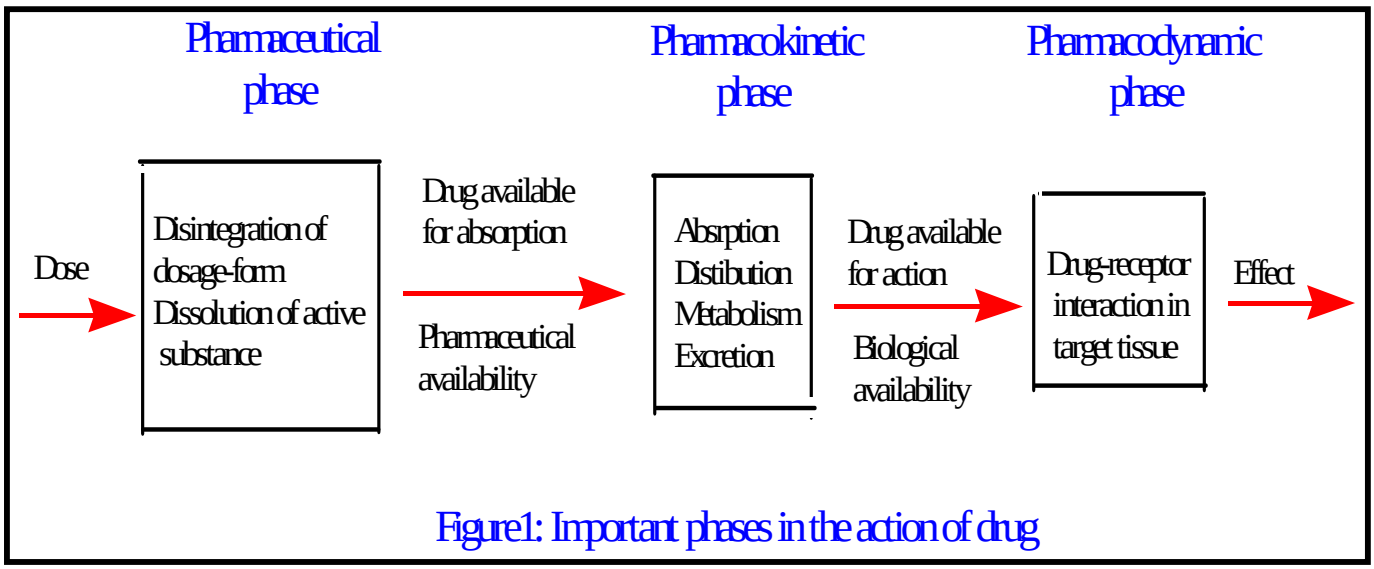

Figure1: Important phases in the action of drug

(1.1) Drug Interaction:

When taken concomitantly as drugs, chemical substances may interact with the following consequences: (a) an additive or synergic effect, when both present the same pharmacodynamic action, (b) loss of effect, when both exhibit opposite action, and (c) influence of one drug on the activity of the other one, changing its absorption, distribution, metabolism, or excretion [(4)].

The absorption of a drug may be diminished by the simultaneous administration of another drug which forms with the first a less soluble complex in the gastrointestinal tract the distribution of drugs is governed by their higher or lower binding to plasma proteins. The unbound protein is the biologically active one; the simultaneous administration of two drugs that have a high

affinity for protein may result, in that one displaces the other from its binding to protein, thereby potentiating the displaced drug [5].

For example, clofibrate is strongly bound to protein and therefore enhances the effects of oral anticoagulants by displacing them from protein binding sites.

(1.2) Classification of Drugs:

Drugs can be classified according to various criteria. They are classified according to: (a) chemical structure, (b) pharmacological action, (c) therapeutic use, (d) anatomic therapeutic chemical structure (ATC), and (e) mechanism of action at the molecular level [6].

Classification following the third criterion, therapeutic use, is very similar to the pharmacological classification and in many cases, identical with it. Attempts to classify drugs according to their mechanism of action at the molecular level cannot include all drugs, because many of them their mechanism is yet unknown. It is possible to divide a great number of drugs into the following classes: drugs acting on enzymes, as suppressors of gene function, by metabolic antagonism, on biological membranes, and drug acting by their physicochemical properties [7].

(1.3) Ampicillin Trihydrate:

Ampicillin Trihydrate (4-Thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid-6-(2-amino-2-phenylacetamido)-3,3-dimethyl-7-oxo), $C_{16}H_{19}N_3O_4S.3H_2O$, is a white crystalline powder with molecular weight of 403.5, melts in the range (198-200°C).

It is slightly soluble in water, and dissolves in dilute acids and alkaline solutions because of the presence of (NH_2) and (COOH) groups as shown in figure(2). However, it is insoluble in alcohol, ether and in fatty oils [8-10].

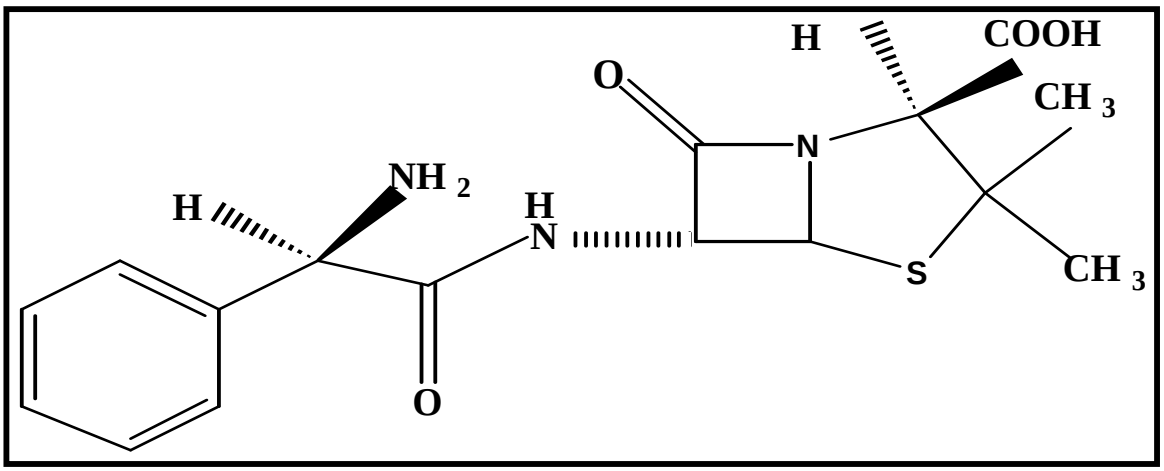

Figure2. **Ampicillin Trihydrate**

Ampicillin is an antibiotic in the class of drugs called Penicillin [8-10]. It is abroad-spectrum semi-synthetic penicillin that is effective in the treatment of gram-positive and gram-negative bacterial infections produced by *Streptococcus*, *Bacillus anthracis*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *Escherichia coli*. This antibiotic is used in the treatment of upper respiratory tract infections, genital and urinary tract infections, and otitis media in children [11].

(1.4) Dosage:

Ampicillin may be administered by oral or parenteral route. It is advisable to reserve the parenteral form of ampicillin for moderate to severe infection. Determine dosage is administrating according to sensitivity of causative micro-organism and severity of infection [12].

Symptoms of penicillin overdose include neuromuscular hypersensitivity (e.g., agitation, hallucinations, asterixis, encephalopathy, confusion, and seizures) [13].

Prolonged used of antibiotics may promote the overgrowth of non-susceptible organisms, including fungi when super-infection occur, appropriate increases should be taken. In prolonged therapy, and particularly with high dosage regiments, periodic evaluation of the renal, hepatic, and hematopoietic system is recommended [13].

(1.5) Pharmacokinetics:

Ampicillin is not degraded by gastric acid; oral absorption is incomplete but adequate. It is partly excreted in bile and reabsorbed-enterohepatic circulation occurs. However, primary channel of excretion is kidney [14].

(1.6) Drugs Affect Ampicillin Action:

Some drugs interfere with penicillin derivatives and may decrease the effects of ampicillin and prevent it from properly treating the infection; hydrocortisone inactivates ampicillin if mixed in the solution [10].

By inhibiting colonic flora, it may interfere with deconjugation and enterohepatic cycling of oral contraceptives (failure of oral contraception). Probenecid retards renal excretion of ampicillin. Some other drugs which may interfere are:

a) Cholestyramine (Questran) or Colestipol (Colestid);

b) Another antibiotic (for the same or different infection) such as erythromycin, tetracycline, minocycline, doxycycline.

Ampicillin may decrease the effectiveness of birth control pill. This will used to protect against pregnancy but it increases the effects of methotrexate and a dose adjustment during therapy with ampicillin will needed. It also increases

the side effects of allopurinol (zyloprim) and may cause a rash while Probenecid (Benemid) increases the effects of ampicillin [15].

(1.7) Determination Methods of Ampicillin:

A variety of techniques have been utilized for the determination of ampicillin concentration including liquid chromatography and ultraviolet-visible methods [16].

(1.7.1) Chromatographic methods:

Traditionally, microbiological methods have been used for analysis of antibiotics; these methods are time-consuming and normally non-specific [17].

At present, high performance liquid chromatography (HPLC) is the most important analytical separation method for the determination of antibiotics [18]. The determination of some antibiotics presents some difficulties because of their thermal instability. The HPLC method at room temperature is preferred [19].

Hartmann [20] described the analysis of some penicillins and cephalosporins by reversed phase liquid chromatographic method for closely related structure. The influence of eluent pH and NaCl concentration on the resolution of ampicillin and epicillin was discussed. The method can be applied

to the selective analysis of synthetically produced antibiotics and their pharmaceutical preparations. Because of the high sensitivity (10^{-7}g/cm^3) the method can also be used for the analysis of penicillin's in physiological fluids. The stationary phase was Nucleosil 100-5 CN, 250 x 3 mm id; eluent: 0.05 M phosphate buffer pH 7 in CH_3OH (7:3.6, v/v); at flow rate: 3.2 ml/min and detection: UV 220 nm.

Simone et al. [21] described the development and validation of a reversed-phase liquid chromatographic method for the determination of cetirizine dihydrochloride in oral formulations. LC analysis was performed on a reversed-phase C18 column (250 × 4.6 mm id, 5 µm particle size). The mobile phase was 1% orthophosphoric acid solution, pH 3.0–acetonitrile (60 / 40, v/v), pumped at a constant flow rate of 1.0 ml/min. Measurements were made at a wavelength of 232 nm. The calibration curves were linear over the range of 10–30 µg/ml (r^2 = 0.9999). The relative standard deviation (RSD) values for intraday precision were 0.94 and 1.43% for tablets and compounded capsules, respectively. Recoveries ranged from 97.7 to 101.8% for tablets and from 98.4 to 102% for compounded capsules. No interferences from the excipients were observed. Because of its simplicity and accuracy, the method was suitable for routine quality-control analysis for cetirizine in tablets and compounded capsules.

Martin et al. [22] used liquid chromatographic method for quantitative determination of rabeprazole in coated tablets. The system consisted of a

Hypersil Keystone Betabasic C8 column (250 × 4.6 mm, 5 µm particle size), an isocratic acetonitrile–water (35 / 65) mobile phase at a flow rate of 1.0 ml/min, and a diode array detector set at 282 nm. The method showed a good linearity in the concentration range of 10–70 µg/ml. The detection limit was 0.80 µg/ml. The method has a good relative standard deviation 1.03. Accuracy was also evaluated, and the results were satisfactory. The mean recovery was 101.61%. The analysis of a placebo mixture demonstrated the method was also specific.

Ana et al. [23] used liquid chromatographic method for the determination of the antihistamine fexofenadine. Although widely used in the treatment of allergic diseases, fexofenadine is not listed in any pharmacopeia, and there are few methods in the literature for its quantitation in pharmaceutical dosage forms. In this work, a LiChrospher 100 RP-18 (250 × 4.0 mm, 5 µm) column was used as the stationary phase and acetonitrile–5 mM ammonium acetate buffer (50 / 50, v/v) at pH 3.2 was the mobile phase. Through the evaluation of the analytical parameters, it was shown that the method was linear (r = 0.9999) at concentrations ranging from 20.0 to 80.0 µg/ml, relative standard deviation [RSD] value = 0.77%), accurate (mean recovery = 99.05%). The detection and quantitation limits are 0.3409 and 1.033 µg/ml, respectively. These low values show the good sensitivity of the proposed method.

Tushar et al. [24] used a simple, fast, specific, and precise reversed-phase liquid chromatographic method for the determination of Cefdinir in its different

dosage forms, i.e., capsules and suspensions. The method was developed and optimized by analyzing the placebo preparation, formulations, and degraded samples of the drug substance according to the International Conference on Harmonization. The proposed method can successfully separate the drug from degradation products formed under stress conditions along with pharmaceutical ingredients such as preservatives. The developed method was used to determine cefdinir in capsules and suspensions. The developed method was found to be linear for a concentration range of 6–14 µg/ml. Average recoveries obtained with the method were 99.3 ± 0.4 and 99.6 ± 0.4% for suspensions and capsules, respectively.

Thorburn et al. [25] used reversed phase HPLC with UV-Visible detection for the simultaneous identification and determination of ampicillin and some of it degradation products following their extraction from an intramammary separation used in veterinary medicine. Extraction of the intramammary into petroleum ether and partition of the penicillin into the mobile phase gave a mean recovery of 100.8% for ampicillin with coefficient of variation of 1.0. Using optimized HPLC conditions ampicillin eluted in approximately 2 minutes independently of their degradation products.

White et al. [26] used (HPLC) for the detection of linezolid in human serum, the mobile phase was 1% *ortho*-phosphoric acid, 30% methanol, 2 g/L heptane sulphonic acid, pH 5. UV detection was used (λmax 254 nm). Samples were prepared by mixing with acetonitrile and an injection volume of 20 µl was used.

Linearity and accuracy were investigated, the detection limit and recovery of linezolid from serum were determined. In addition, the stability of linezolid, stored under a variety of conditions, was assessed and the retention time of linezolid was 6.5 min. The intra- and inter-day reproducibility was good and the assay was linear across the therapeutic range. Serum recovery was 100% at all concentrations tested. There was no significant interference with the linezolid peak. Linezolid was demonstrated to be stable. This rapid assay was ideal for clinical laboratories with basic HPLC equipment.

Shawky [27] used HPLC method for the determination of nanogram quantities of 5 broad-spectrum structurally related –lactam antibiotics (cefazolin, cefadroxil, cephalexin, cephradine and ampicillin) in solution using C18 reversed-phase column and an aqueous mobile phase containing isopropyl alcohol and acetic acid. Relative resolution between the antibiotic peaks ranged from 1.7 to 5.9 for all peaks. Chromatographic retention times are 2.97, 3.92, 4.57, 5.37 and 6.56 min for cefazolin, cefadroxil, cephalexin, ampicillin and cephradine, respectively.

Accuracy, precision, linearity, and long term analytical reproducibility were determined by statistical analysis [28].

Anna et al. [29] used HPLC method for the quantitative determination of gliclazide and repaglinide in pharmaceutical formulations. Determination was performed using a mobile phase containing acetonitrile phosphate buffer (pH 2.1; 60/40, v/v), and UV detection at 225 nm. Repaglinide was used as an

internal standard for gliclazide determination and for repaglinide assay. The method was validated with respect to linearity, precision, accuracy, and specificity. The calibration graphs ranged from 0.015 to 0.09 mg/ml for gliclazide and 0.06 to 0.36 mg/ml for repaglinide. Relative standard deviation values for the standard solutions were 0.7 and 1.01% for gliclazide and 0.78 and 0.93% for repaglinide, respectively. Total recoveries of gliclazide and repaglinide from the laboratory-prepared mixtures were 99.82±0.58 and 101.5±0.46% for gliclazide and repaglinide, respectively. Finally, the method was applied for the quality control of commercial gliclazide and repaglinide tablets. Total recovery was 100.40±0.35 and 104.46±0.23% for gliclazide and repaglinide, respectively.

Hérida et al. [30] used a sensitive, precise, and specific HPLC method for the determination of gatifloxacin in tablets. The method was validation parameters yielded good results. The HPLC separation was carried out by reversed-phase chromatography on a C18 absorbosphere column (250 × 4.6 mm id, 5 µm particle size) with a mobile phase composed of acetic acid 5%-acetonitrile-methanol (70 / 15 / 15, v/v/v) pumped isocratically at a flow rate of 1.0 ml/min. The calibration graph for gatifloxacin was linear from 4.0 to 14.0 µg/ml; the relative standard deviation was less than 1.05%

(1.7.2) Spectrophotometric methods:

One of the most common methods of analytical measurement of ampicillin was by spectrophotometric means. Spectrophotometric techniques utilize the property of selective absorption of radiant energy by chemical substances.

Photometric methods whether UV, visible, or IR, are characterized by their sensitivity and selectivity. The visible and UV regions are usually of greater practical application of drugs because the molar absorptivity exhibited are usually of high order of magnitude than those in the IR. Thus greater sensitivity can be obtained at these spectra region [(31)].

In the medical fields, the visible and ultraviolet region can be used for the analysis of enzymes, hormones and steroids [(11)]. These measurements can be used in the diagnosis of many symptoms of diseases. In pharmacy, it can be used to measure the purity of drugs during manufacturing and of the final products [(32)].

(1.8) Derivative Method:

The development of a satisfactory analytical method to measure any drug selectively in pharmaceutical preparations is an important task. Often many measurement techniques that is sensitive but lack selectivity toward complex samples. There has always been an interest in sensitive techniques to improve the measurement selectivity. Among the most conceptually simplicity of these methods is the derivative spectrophotometry.

Derivative spectrophotometric method is an analytical technique of a great utility for extracting both qualitative and quantitative information from spectra composed of unresolved bands. It was introduced more than forty years ago, and has demonstrable advantages for the solution of specific analytical problems [33]. In recent years, the introduction of electronic differentiation by a micro-computer interfaced with spectrophotometer has made possible the plotting of the first, second or higher order derivatives of a spectrum with respect to wavelength.

This technique has improved the resolution of overlapping absorption bands against broader band. Therefore, the use of derivative spectrophotometry is not restricted only to special cases, but can be applied whenever quantitative study of normal spectra is difficult [34]. Derivative spectrophotometry offers a convenient solution to a number of well defined analytical problems, such as

resolution of multi-component system; minimize the effect of sample turbidity, matrix background and enhancement of spectral details [35].

A derivative spectrogram shows a peak or valley corresponding to every inflection point in the normal spectrum, giving greatly enhanced resolution [36]. The principle of operation is based on measurement of the changes in intensity or absorbance, manually or automatically by certain instrument. The approach is based on the idea that the wavelength scan rate, (dλ/dt) is constant, then the derivative of the intensity with respect wavelength, dI/dλ proportional to the derivative of intensity with respect to time, dI/dt, which is measured by means of its electronic differentiation [37]:

$$\mathbf{(dI/d\lambda) = (dI/dt) / (d\lambda/dt)}$$

For a single-peak spectrum, the first derivative is plot of the gradient dA/dλ of the absorption envelope vs. wavelength and features a maximum and a minimum. The vertical distance between these is amplitude, which is proportional to the analyte concentration.

Theoretically, dA/dλ is zero at λ max for the band in the normal spectrum. The second derivative spectrum $d^2A/d\lambda^2$ vs. λ has two maximum with a minimum between them, at λ max of the normal absorption.
In principle, both peak-heights (measured from $d^2A/d\lambda^2 = 0$) are proportional to the analyte concentration [38].

The amplitude D_n of the nth derivative is related to the nth power of the inverse of the band-width w, of the normal spectrum [39].

$$D_n \alpha (1/W)^n$$

Thus, for two bands A and B of equal absorbance but different width, the derivative amplitude of the sharper bands (A, for example) is greater than that of the broader (B) by factor that increase with increasing derivative order:

$$(D_n, A)/ (D_n, B) \alpha (WB/WA)^n$$

For this reason, the use of derivative spectra can increase the sensitivity of minor spectral feature. For quantitative analysis, if beer's law is obeyed for the normal spectrum, the following equation can be obtained [40].

$$(d^nA/d\lambda^n) = (d^n\varepsilon/d\lambda^n) \times LC$$

Where A = absorbance

ε = molar absorptivity

L = cell path-length

C = molar concentration of the analyte

This forms the basis for analytical determinations.

An inconvenience of the derivative technique is that the signal-to noise ratio (S/N) becomes worse for progressively higher orders [41]. Practical derivative technique includes some degree of smoothing to control the increase in noise. The effect of smoothing depends on two variables:

a- The smoothing ratio which is the ratio of the width of the smoothing peak to the number of data points corresponding to the peak full width at half-maximum, and

b- The number of times that the smoothing is done.

In general, the selection of the optimum smoothing ratio depends on the purpose for which the derivative technique is used [42].

Derivative spectrophotometry has several applications in different fields. It has proved particularly useful in eliminating matrix interferences.

In the medical field, it can be used for the analysis of enzymes, vitamins, hormones, and steroids [32]. These measurements are used in the diagnosis of diabetes, kidney damage, and myocardial infection.

Tariq et al. [43] used derivative spectrometry technique to measure carbamazepine (anticonvulsant drug) in human serum for patients receiving this drug. Second derivative spectrum at 258nm was chosen for this purpose. The presences of other drugs, that are given concurrently, were not observed at the selected wavelength. The calibration curve used for this method has a wide linear range of 2-30 µg/ml.

Riyadh et al. [44], measured atenolol, a medical drug administrated commonly for the treatment of arterial hypertension, angina pectoris and cardiac arrhythmias, in the presence of other drugs. Frusemide and amiloride are antihypertensive drugs that are usually given concurrently with atenolol. They have a large effect on the determination of atenolol due to overlapped

spectra. Therefore derivative absorption spectrometry technique to overcome this problem, first, second, and fourth derivative spectra at 233nm, 229nm, and 279nm, respectively, were used for this purpose.

The calibration curves for the first, second, and fourth derivative spectra have a linear range of 0.1-100 ppm, 0.5-200 ppm, and 2-500 ppm, respectively.

In pharmacy, derivative technique has been used to measure the purity of drugs during manufacture and of final product. In agriculture, it has been applied for the determination of pesticides on plants, in polluted rivers, and in fish and animals that eat or drink polluted food [(32)].

Martin et al. [(45)] used derivative absorption spectrometry technique to determine chlorpyrifos and diazinon in the presence of fenvalerate and triflumuron. Second derivative at 308 nm and third derivative at 304 nm was chosen for this purpose. The calibration curve used for this method has a linear range of 1-150 ppm.

Galera et al. [(46)] used absorption and first derivative absorption pectra to the simultaneous determination of three insecticides atrazine, diuron, and chlorpyrifos in mixtures. The proposed method was applied satisfactorily to the determination of these insecticides in groundwater and soils.

Diaz et al. [(47)], applied derivative absorption spectrophotometry technique to measure N-(1-naphthyl)phthalamic acid (naptalam) and its metabolites, N-(1-naphthyl)phthalamide and 1-naphthylamine in mixtures. The normal and first derivative absorption spectra of mixtures were used to perform the optimization

of the calibration curve. The proposed method was applied to the determination of analytes in river water at the ppb level.

First derivative spectrophotometry was also used for the simultaneous determination of o-nitrophenol and p-nitrophenol by Toral et al. [48], The analytes were separated from samples by liquid-liquid extraction into the 1,2-dichloroethane organic phase and subsequently evaluated directly by derivative spectrophotometry.

The determination ranges were found to be between 0.115 and 3 ppm and 0.130 and 3 ppm for o-nitrophenol and p-nitrophenol, respectively. The proposed method was applied successfully to the determination of these compounds in fruit juices.

Baranowska et al. [49] used first order derivative spectra for the determination of bromacil and the second order ones for the determination of hexazinone in their mixtures. Metoxuron was determined in the presence hexazinone by normal or derivative spectrophotometry.

First order derivative spectrophotometry was also applied for the determination of promazine and simazine in the presence of bromacil, hexazinone and metoxuron. In the three component mixture it was possible to analyze metoxuron in the presence of bromacil and hexazinone using first order derivative and bromacil in the presence of metoxuron and hexazinone applying the second derivative spectra.

Nevin et al. [50] studies the derivative spectrophotometry for the simultaneous determination of phenazopyridine hydrochloride with ampicillin trihydrate in binary mixtures. The analytical signals were 324.9 nm and 283.6 nm in the phenazopyridine hydrochloride with ampicillin trihydrate in mixture. The first derivative of the mixture solution in methanol was used. Calibration graphs were established for 2.0-18.0 $\mu g.mL^{-1}$ phenazopyridine hydrochloride and 4.0-32.0 $\mu g.mL^{-1}$ ampicillin trihydrate in binary mixture.

Khadiga et al. [51] used spectrophtometry for determination of cefalozidine (I), cefotaxime (II), and cefotaxime sodium (III) in the presence of their degradation products, first derivative (D1) was used.

The D1 absorbance was measured at 268.6, 306, and 228.6 nm for I, II, and III, respectively. The method determine I, II, and III in concentration ranges of 5-150, 5-135, and 5-140 g/ml, respectively with corresponding mean accuracies of 99.7±0.8, 100±0.7, and 99.8±0.8%. The method determines the drug in the presence of up to 90% degradation products for I and II and up to 80% for III.

Liang [52] studied the direct UV detection to determine the association constants between lectins and saccharides but the interaction was always between the labeled carbohydrates rather than the truly underivatized carbohydrates and lectins. In order to directly detect saccharides during the study on the interaction of glucose and its derivatives with lectins the detection at wavelength of 195 nm has been employed. The UV allowed the detection of these compounds at less than 4 mmol/ml level, and quantification by the peak

area method allowed reproducible determination of them at least at their respective concentration range. The method is characterized by its simplicity, rapidity and reproducibility and should be useful for the analysis of the interaction of glucose and its derivatives with lectins.

Mahgoub et al. [53] used a normal spectrophotometric method for the resolution of binary mixtures of ampicillin sodium and sulbactam sodium. In aqueous solution, zero-order spectra were subjected to interferences, so first derivative spectro photometry was used to enhance the spectral details allowing the determination of ampicillin from the signal at the zero-crossing point for sulbactam sodium at 268 nm. In 0.1N sodium hydroxide, sulbactam sodium was determined from the absorbance at 260 nm with negligible contribution from ampicillin. Also, sulbactam sodium was determined without interference using first- and second-derivative spectra in a 0.1N sodium hydroxide at 276 nm and 284 nm, respectively. The method is rapid, simple, did not require a separation step and allows the determination of each drug without interference from the other.

Mostafa et al. [54] used first-derivative spectrophotometric (D1) method to determine pyritinol dihydrochloride (I) in the presence of its precursor (II) and its degradation product (III) with 0.1N hydrochloric acid as a solvent. Linear relationships were obtained in the ranges of 6-22 µg/ml. It was possible to determine pyritinol dihydrochloride in its pure powdered form with an accuracy of 100.36±1.497% for the (D1) method.

Khadiga [55] used first-derivative spectrophotometric method for the simultaneous determination of ternary mixtures of caffeine (A), 8-chlorotheophylline (B), and chlorphenoxamine hydrochloride (C) in bulk powder and dosage forms. In the first-derivative spectrophotometric technique (D1), calibration curve was linear in the range of 4–20 µg/ml for A, B, and C (r = 0.9992, 0.9994, and 0.9976, respectively). The measurements were carried out at 212, 209.2, and 231.4 nm for A, B, and C, respectively. The percentage recoveries were 99.1 ± 0.89, 100.1 ± 0.95, and 100.1 ± 1.0, respectively.

Maria et al. [56] developed a method for the simultaneous determination of *N*-butylscopolamine bromide and oxazepam in pharmaceutical formulations using first-order derivative spectrophotometry. Acetonitrile was selected as the solvent in which both compounds showed well-defined bands. Both analytes showed good stability in this solvent when solutions of the analytes were exposed to light and temperatures between 20° and 80°C. The linear range of determination was found to be 2.5×10^{-7} to 8.0×10^{-5} mol/L for *N*-butylscopolamine and 7.1×10^{-8} to 8.0×10^{-5} mol/L for oxazepam. A very good relative standard deviation of 0.2% was observed for *N*-butylscopolamine and oxazepam. The proposed method was applied to the determination of these drugs in pharmaceutical formulations (capsules).

Sawsan et al. [57] used a rapid, simple, and selective method for the determination of etodolac. The method depends on complexation of etodolac with

copper (II) acetate and iron (III) chloride followed by extraction of complexes with dichloromethane and then measuring the extracted complexes spectrophotometrically at 684 and 385 nm of Cu(II) and Fe(III), respectively. Different factors affecting the reaction, such as pH, concentrations, and time, were studied. By using Job's method of continuous variation, the mole ratio method, and elemental analysis, the stoichiometry of the reaction was found to be in the ratio of 1:2 and 1:3, metal:drug in the case of Cu(II) and Fe(III), respectively. The method obeys Beer's law in a concentration range of 2.00 to 9.00 and 0.50 to 2.00 mg/ml of Cu(II) and Fe(III), respectively. The stability of the complexes formed was also studied, and the reaction products were isolated for further investigation. The complexes have apparent molar absorptivities of about 32.14 ± 0.97 and 168.32 ± 1.12 L. mol^{-1}. cm^{-1}. for Cu(II) and Fe(III), respectively. The suggested procedures were successfully applied to the analysis of pure etodolac and its pharmaceutical formulations. The validity of the procedures was further ascertained by the method of standard additions, and the results were compared with other reported spectrophotometric methods and showed no significant difference in accuracy and precision.

Ali et al. [58] used a rapid, simple, and sensitive differential kinetic method for the determinations of acetaminophen (also known as paracetamol) and salicylamide. The method was based on their oxidation reaction by Fe^{3+} ion in the presence of 1,10-phenanthroline as indicator. The reactions can be monitored spectrophotometrically by measuring the increase in the absorbance

of the solution at 510 nm. Two steps were selected one in which only paracetamol was oxidized by Fe^{3+} ion and the other in which both drugs were oxidized by Fe^{3+} ion. The method allowed the simultaneous determination of paracetamol and salicylamide at concentrations between 0.5–20 and 1–40 µg/ml with relative standard deviations of ±3.47 and ±2.58%, respectively. The method was applied to the simultaneous determination of paracetamol and salicylamide in human serum and pharmaceutical formulations.

Jan et al. [(59)] used derivative spectrophotometry for the determination of indomethacin and 5-methoxy-2-methyl-3-indoleacetic acid as its possible impurity in Metindol injections. At the selected wavelengths, 233.04 and 284.65 nm, no interference between the components determined was observed. Under the established experimental conditions, recoveries of the particular components were from 96.14 to 98.17%. Linearity was maintained over a broad range of concentrations, from 11.88×10^{-3} to 35.64×10^{-3} mg/ml for indomethacin and 0.4 to 1.2 mg/ml for 5-methoxy-2-methyl-3-indoleacetic acid. The limit of detection was found to be 6.0×10^{-3} mg/ml for indomethacin and 0.04×10^{-3} mg/ml for 5-methoxy-2-methyl-3-indoleacetic acid. The limits of quantitation were found to be 10.0×10^{-3} mg/ml and 0.20×10^{-3} mg/ml respectively.

Patricio et al. [(60)] determines attapulgite and nifuroxazide in pharmaceutical formulations by first-and second-derivative spectrophotometry, respectively. In order to obtain the optimal conditions for nifuroxazide stability, studies of

solvent, light, and temperature effects were performed. The results showed that a previous hydrolysis of 2h in 1.0×10^{-1} M NaOH solution is necessary in order to obtain stable compounds for analytical purposes. Subsequently, the first-and second-derivative spectra were evaluated directly in the same samples. The attapulgite determination was carried out using the first derivative at 278.0 nm and the nifuroxazide determination, using the second derivative at 282.0 nm. The determination ranges were 5.7×10^{-6}–1.0×10^{-4} and 3.7×10^{-8} –1.2×10^{-4} M for attapulgite and nifuroxazide, respectively. Relative standard deviation values of 1.2 and 3.0% were observed for attapulgite and nifuroxazide, respectively. The ingredients commonly found in commercial pharmaceutical formulations did not interfere. The proposed method was applied to the determination of these drugs in tablets.

Andrés et al. [(61)] determined dapsone and pyrimethamine by first-order derivative spectrophotometry. Acetonitrile was used as a solvent to extract the drugs from the pharmaceutical formulations, and the samples were subsequently evaluated directly by derivative spectrophotometry. The simultaneous determination of both drugs was performed by the zero-crossing method at 249.4 and 231.4 nm for dapsone and pyrimethamine, respectively. The linear range of determination for the drugs was from 6.6×10^{-7} to 2.0×10^{-4} and from 2.5×10^{-6} to 2.0×10^{-4} mol/L for dapsone and pyrimethamine, respectively. The excipients of commercial pharmaceutical formulations did not interfere in the analysis.

Nasr et al. [62] used spectrophotometric method for the determination of josamycin in its dosage forms. The method was based on oxidation of the drug with alkaline potassium permanganate at room temperature for a fixed time of 20 min and measuring the produced green color at 611 nm. The absorbance–concentration plot was rectilinear over the range of 2–10 µg/ml with minimum detectability of 1.0 µg/ml. The determination of josamycin by fixed concentration and the rate-constant methods were also feasible with the calibration equations obtained, but the fixed-time method proved to be more applicable. The procedure was applied successfully to commercial tablets, and statistical analysis showed that the results compared favorably with those obtained by reference methods.

Amr et al. [63] used spectrophotometric method for analysis of 3 antihistaminic drugs, acrivastine (I), mequitazine (II), and dimethindene maleate (III). The method was based on reaction of the drugs with 7,7,8,8-tetracyanoquinodimethane (TCNQ) in acetonitrile to form highly stable colored products that were measured at 750, 766, and 844 nm for I and II, and 480 and 618 nm for III. Beer's law was obeyed in the ranges of 5–60 µg/ml for I, 5–50 µg/ml for II, and 10–70 µg/ml for III. The optimum assay conditions and their applicability to the determination of the cited drugs in pharmaceutical formulations were described.

Aim of The Work :

The aim of this work was to use spectrophotometric method based on derivative spectra to determine ampicillin trihydrate.

The normal absorption spectrophotometry was found not applicable in the determination of ampicillin directly; therefore, refluxed ampicillin trihydrate gave better results.

The method was also involved the study of interference that might occur in the determination of ampicillin trihydrate in the presence of metals such as Na^{+}, K^{+}, Zn^{+2}, Ca^{+2}, Cu^{+2} & Fe^{+3}.

Addition copper(II) and iron(III) have a large effect on the determination of ampicillin due to their overlapped spectra. Therefore, first and second derivative spectrum at 362 and 338 nm, respectively, were found to overcome this problem.

The ampicillin trihydrate absorption spectra make it difficult to measure without any physical or chemical separation. The use of derivative spectra may solve this problem.

(3.1) Determination of the Wavelength of Ampicillin Alone:

(3.1.1) Before Using Reflux:

The determination of ampicillin trihydrate using UV absorption spectroophotometry was studied in this work. The UV spectrum of ampicillin trihydrate has shown no observed maximum wavelength in the wavelength range (200-400) nm, as shown in figure(3):

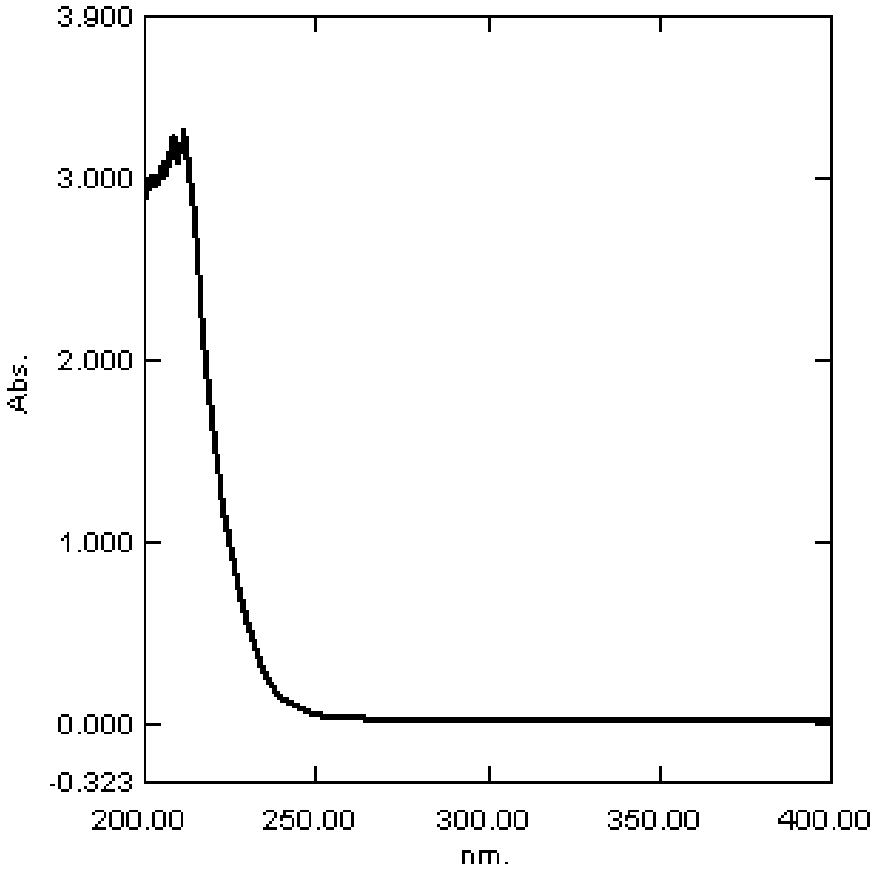

Figure3: Spectrum of 100 ppm ampicillin solution

(3.1.2) After Using Reflux:

Upon refluxing [(65)] under 100°C the ampicillin solution color changed from colorless to pale yellow. The UV spectrum has shown an absorption maximum at 339 nm with concentration range (4-200) ppm and the molar absorptivity of ampicillin at this wavelength was 3173.4 L. mol^{-1}. cm^{-1}, as shown in Figure(4).

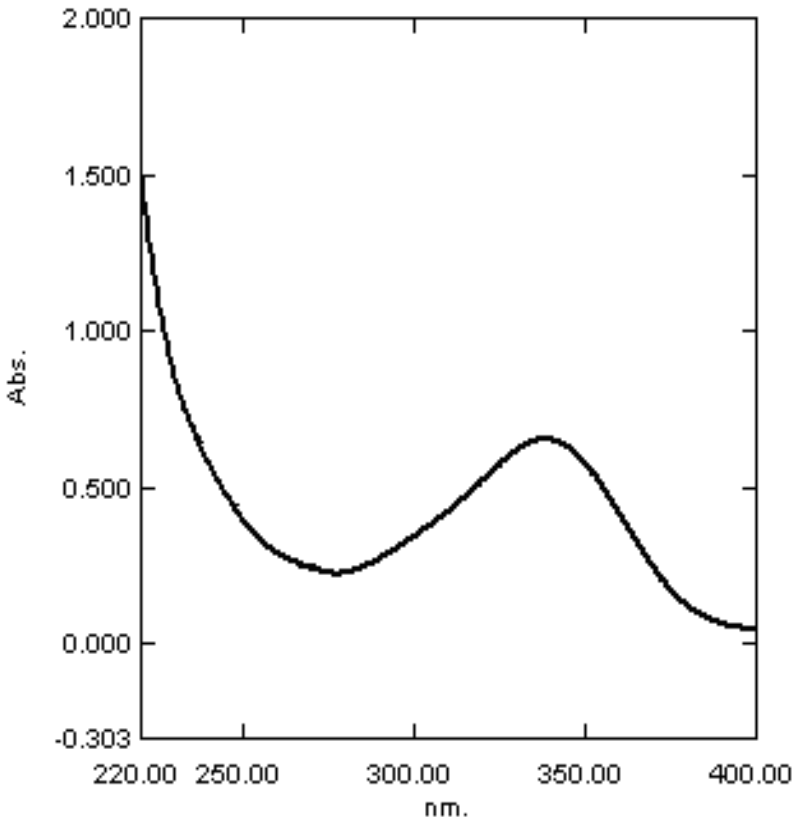

Figure4: Spectrum of 100 ppm ampicillin after refluxing

(3.2) Determination of the Effect of Refluxed Time:

The refluxing of the drug solution performed at different period of time until reaching maximum absorbance in two hours. The absorbance remained constant after complete conversion as indicated from reaching two hours, as shown in figure(5):

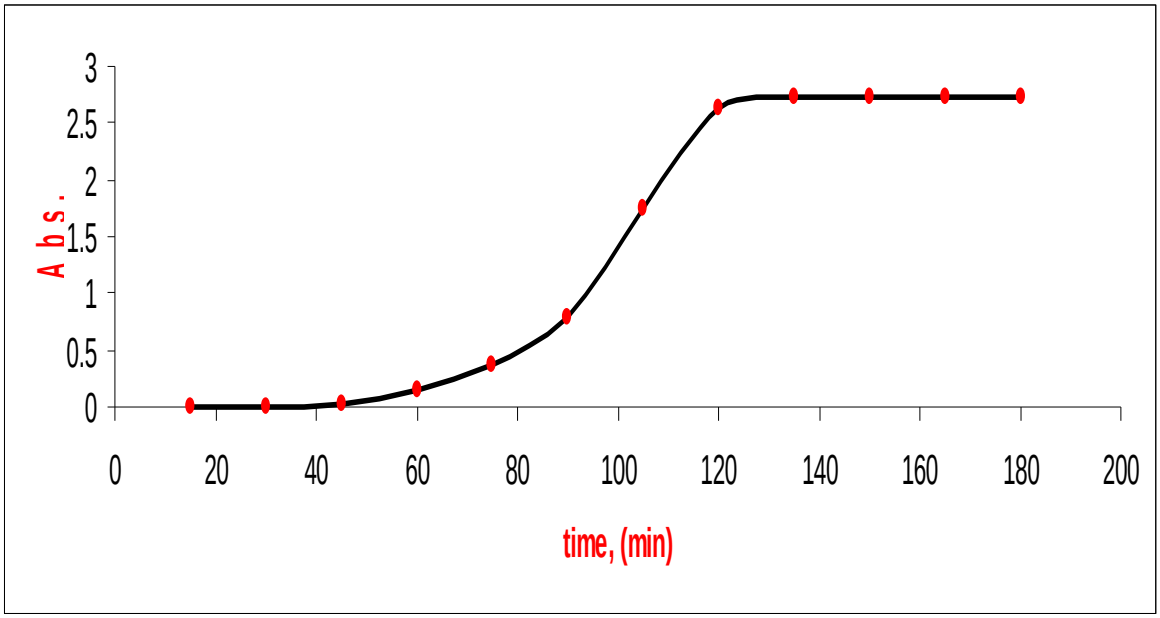

Figure5: Variation of time with absorbance during refluxing ampicillin solution

(3.3) Study the Effect of pH on Ampicillin by Normal Spectroscopic Method:

The effect of pH on the determination of heated ampicillin by this method was investigated. The absorbance at 339 nm as a function of pH of solutions containing 100 ppm of ampicillin is shown in Figure(6). The effect of pH on the absorbance of ampicillin is pronounced and the optimum value was found at pH 8.

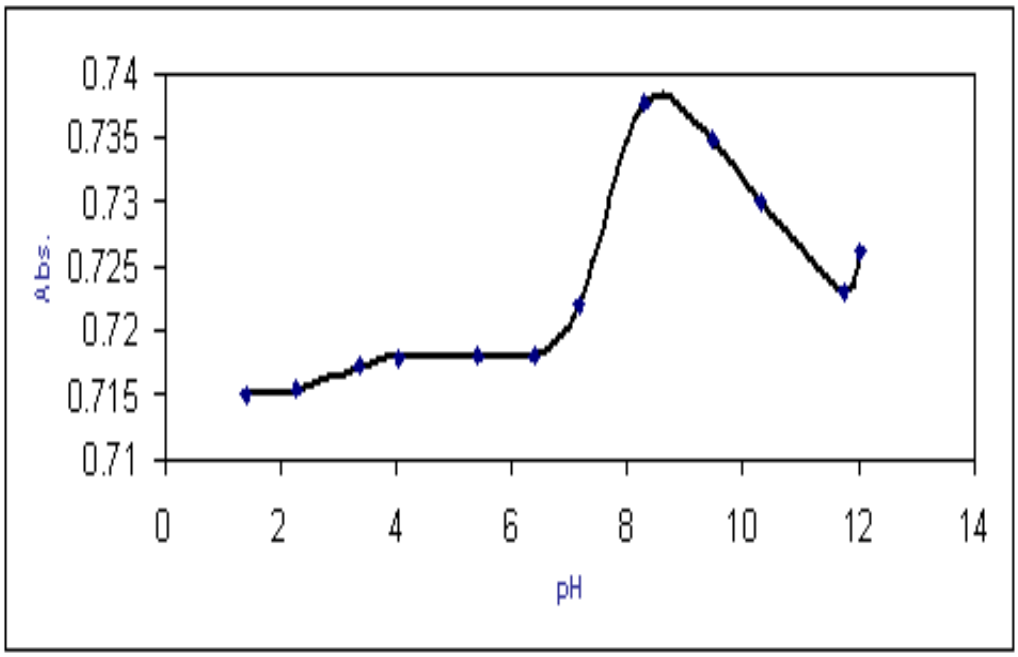

Figure6: Effect of pH at 339 nm

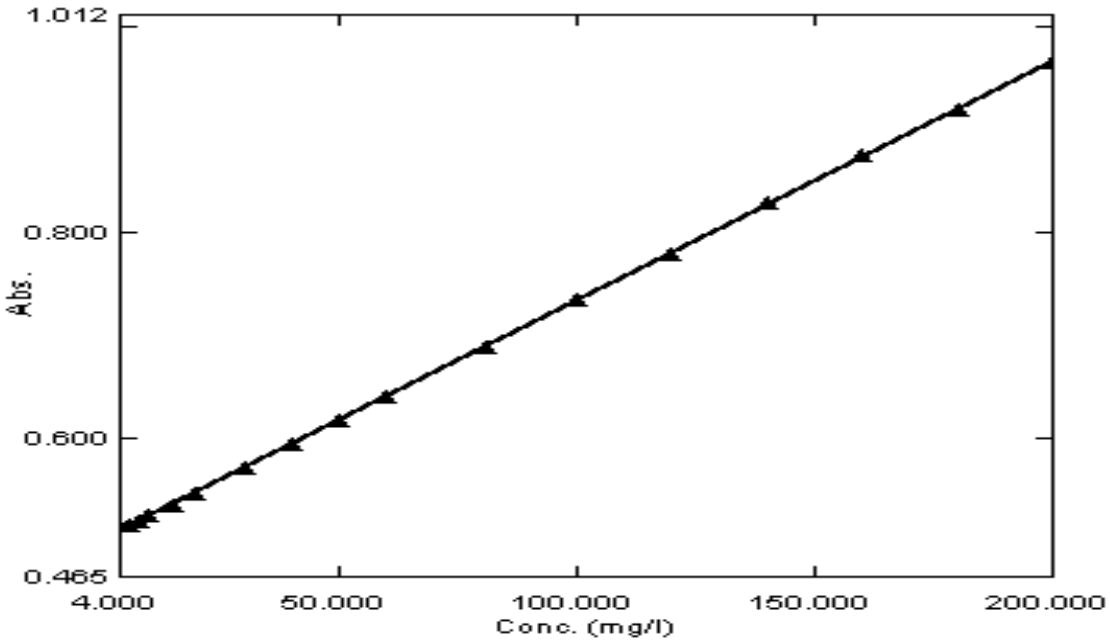

Figure7: Calibration curve of ampicillin at pH 8

Figure(7) shows the calibration curve of ampicillin at 339 nm at pH 8. The linear equation was: Y=0.00233X + 0.50085 and the resulted correlation coefficient was around one.

(3.3.1) Effect of pH on Ampicillin by Derivative Spectroscopic Method:

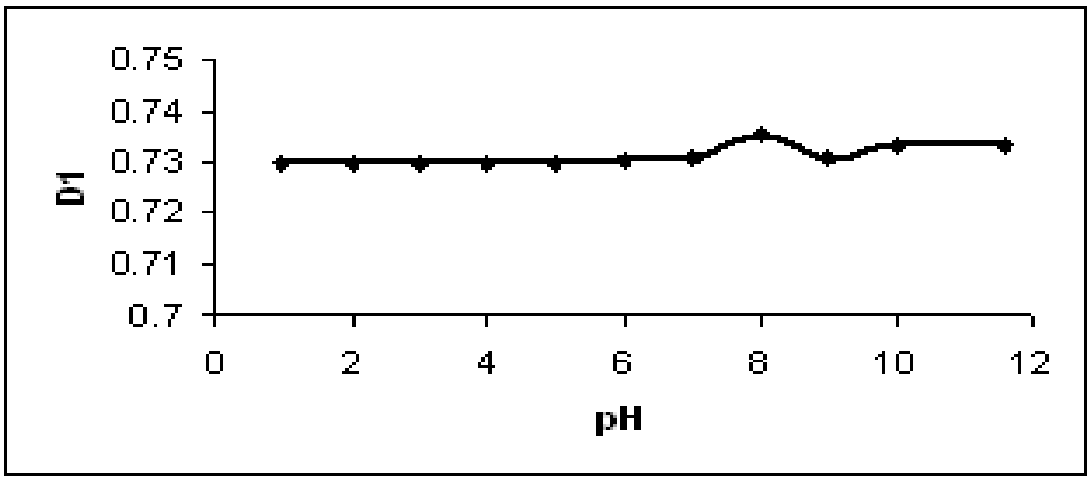

Figure8: Effect of pH using first derivative method

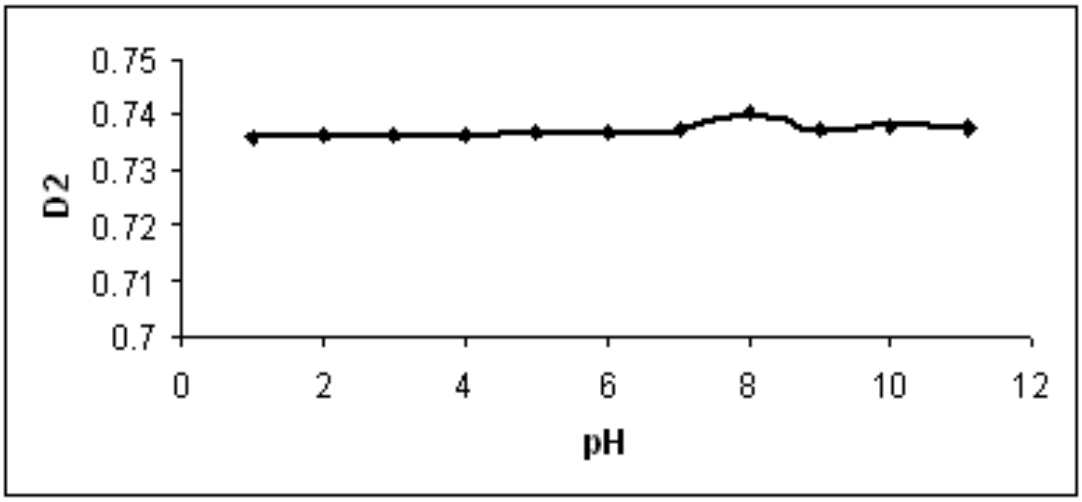

Figure9: Effect of pH using second derivative method

Figure(8) and (9) show the magnitudes of first and second derivative maxima at 362 and 338 nm, respectively, as a function of the pH of solution containing 100 ppm of ampicillin. These figures show that the first and second derivative amplitude remain constant over the pH range of 1 to 7 but littile higher at pH 8 therefore, pH 8 was chosen in this study.

(3.4) Study The Proposed Refluxed Ampicillin Structure:

The reflux of ampicillin solution changed ampicillin (figure1) to another form as shown in the proposed reaction in figure(10) [(64)]. This may be described by FTIR spectrum which explains the disappearance of cyclic carbonyl group at 1772 cm^{-1} as shown in figure(11) and changed to carboxyl group at 1658 cm^{-1} as shown in figure12.

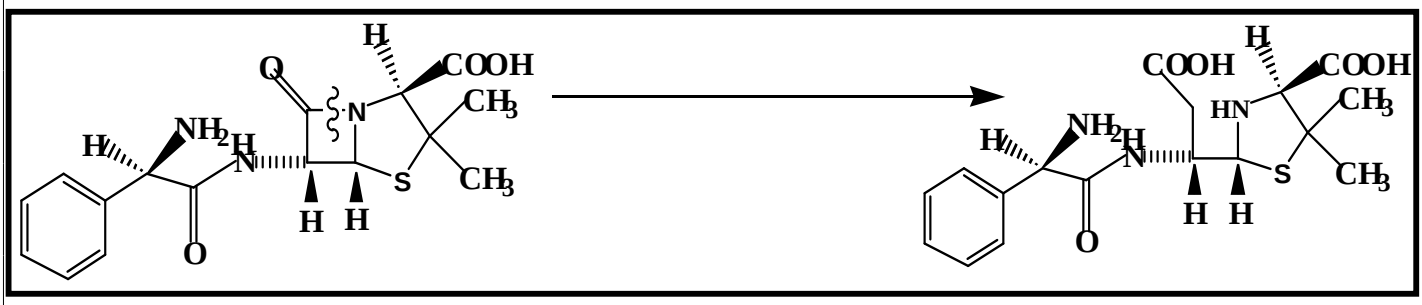

Figure10: Proposed ampicillin conversion upon refluxing

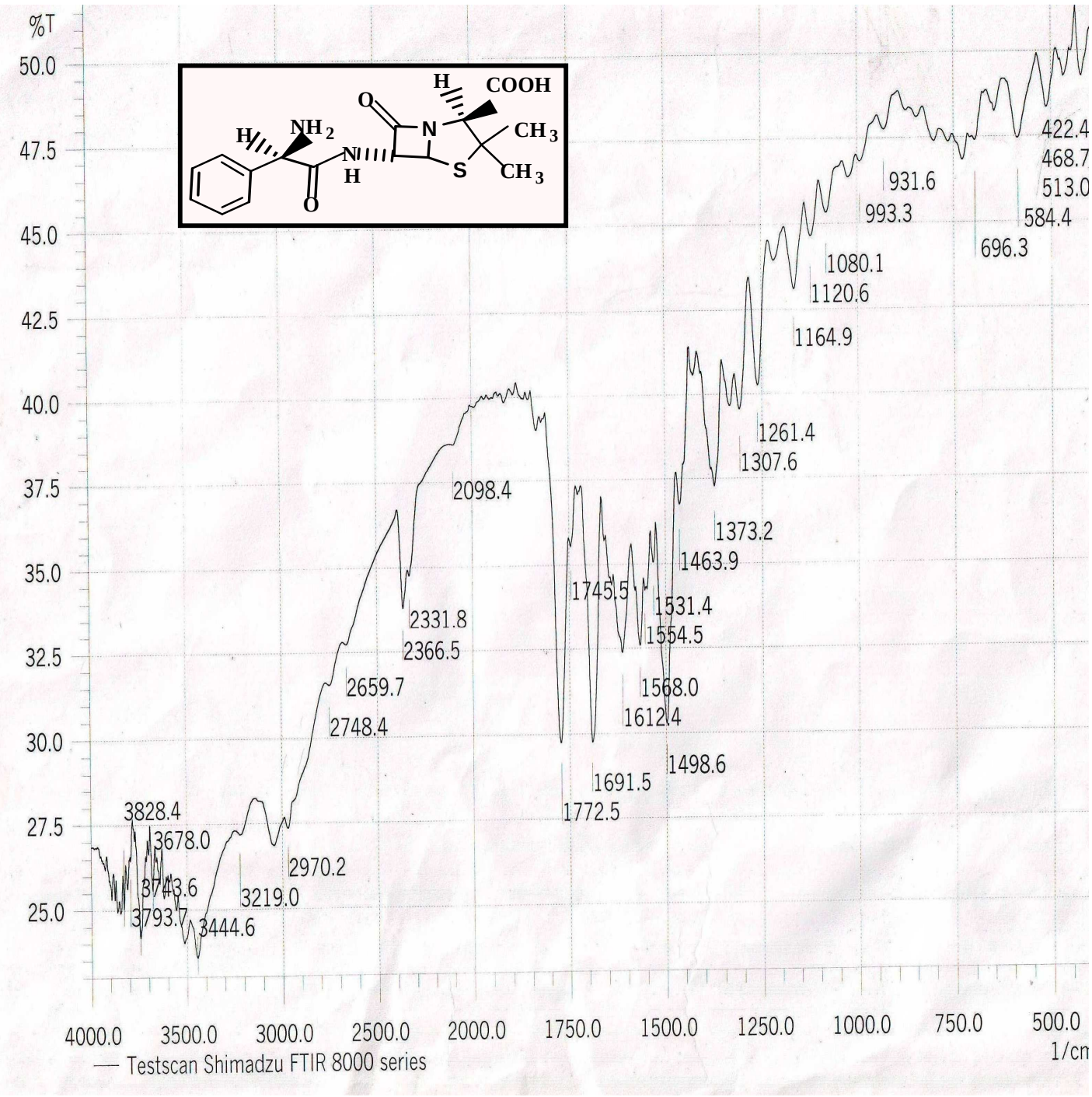

Figure11: FTIR spectrum of standard ampicillin

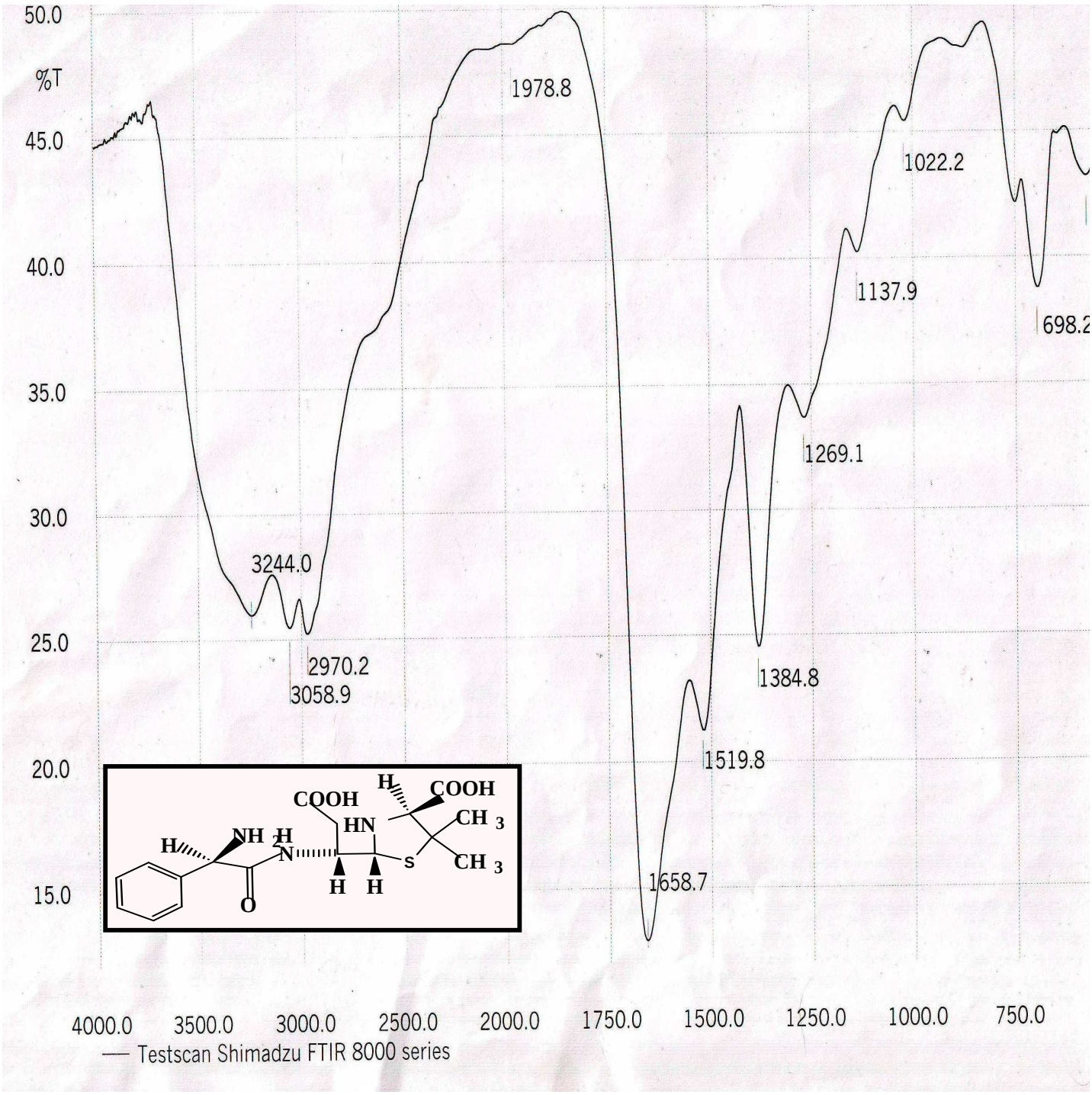

Figure12: FTIR spectrum of ampicillin after refluxing

TLC-technique was also used to identify the conversion product. Water was used as an eluent with iodine vapor which showed one spot as shown in figure(13).

Figure13: TLC- analysis of product(s) after refluxing ampicillin.

(3.5) Effect of Variation of Ampicillin Concentration on Absorption:

Calibration curve was constructed using range of concentration of 4 to 200 ppm of heated ampicillin at 339 nm as shown in figure(14):

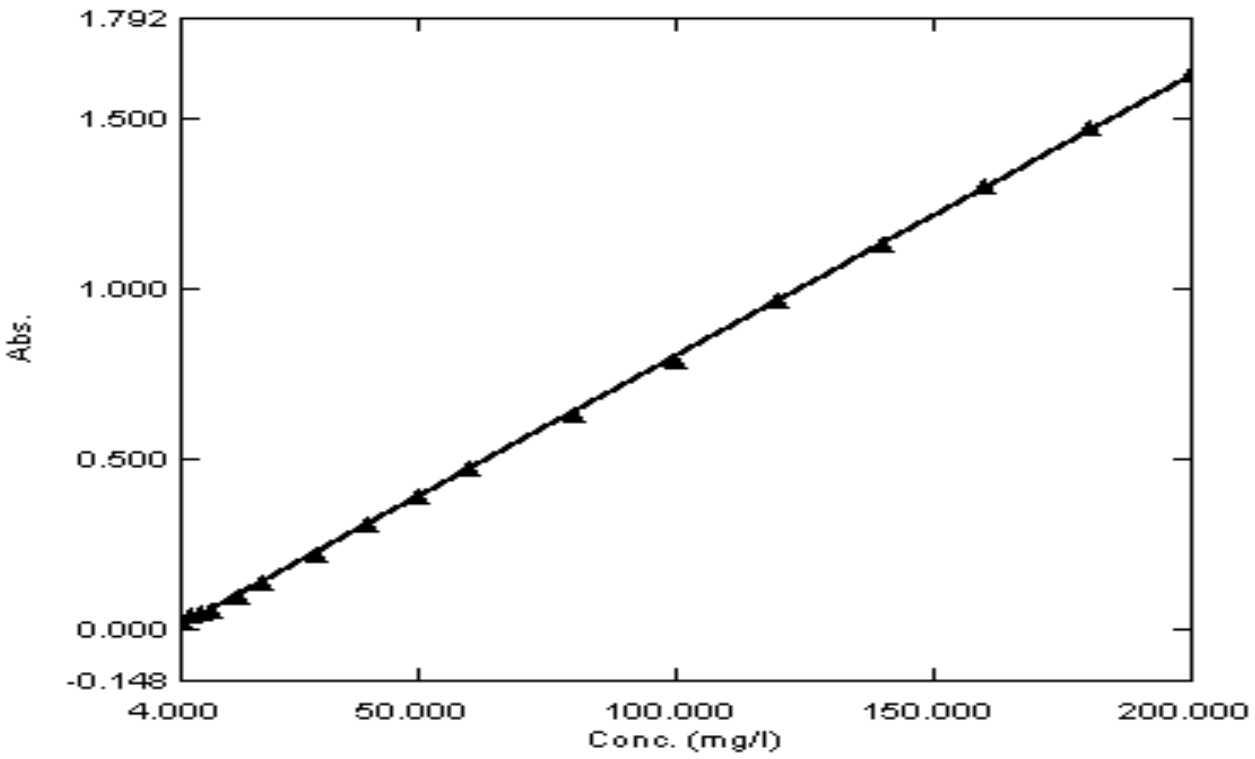

Figure14: Calibration Curve of ampicillin Trihydrate at 339nm

The calibration curve parameters for reluxed ampicillin is listed in table(1).

Table 1. Calibration curve parameters:

λmax, nm	**339**
Linear range (ppm)	**4-200**
Reg. Eq. Y=|aX + b|	**Y=0.00822X - 0.01945**
Corr. Coef. (r)	**0.9999**
RSD%	**±1.268**
Relative Error%	**-2.56**
Recovery%	**97.44**

The linear equation, relative standard deviation (RSD) and recovery of the linear calibration curve using 339nm were: Y=0.00822X - 0.01945, ±1.268 and 97.44, respectively and the detection limit was 0.5 ppm with correlation coefficient of 0.9999.

(3.5.1) Determination of Ampicillin in Capsule by Normal Spectroscopic Method:

The utility of the method was tested on two different manufactures for ampicillin capsules; the recovery amount of ampicillin capsules is shown in table (2):

Table 2. Determination of ampicillin in capsules:

Pharmaceutical	Ampicillin capsule (Sammara)	Ampicillin capsule (India)
λmax, nm	339	339
listed (g)	0.250	0.500
Found (g)	0.248	0.524
Recovery%	99.04	104.74
Relative Error%	-0.96	-4.74

(3.6) Study the Coordination of Ampicillin with Cations:

(3.6.1) Using Continuous Variation Method:

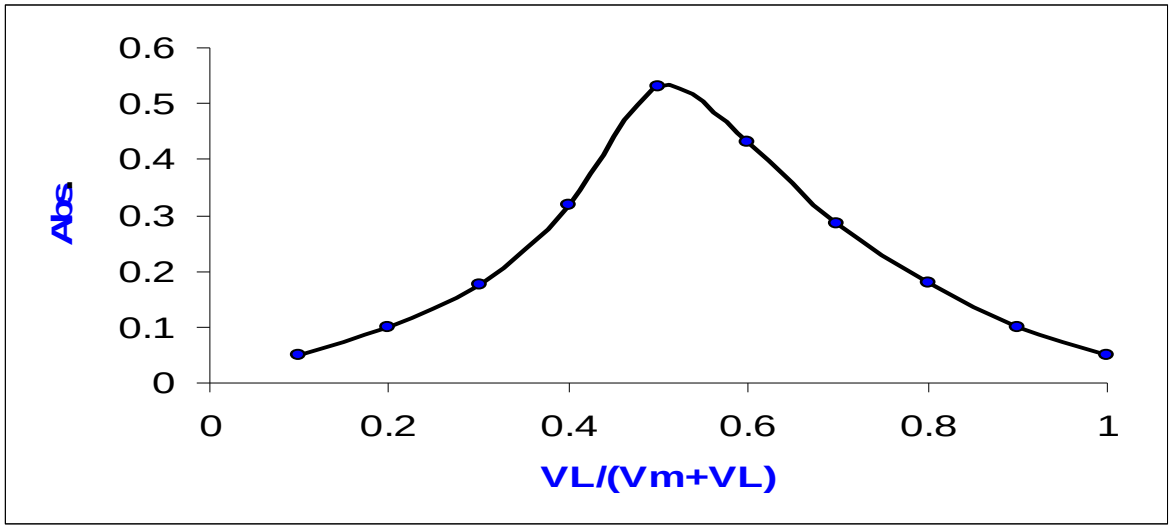

Figure15: Continuous variation method using Cu^{2+}.

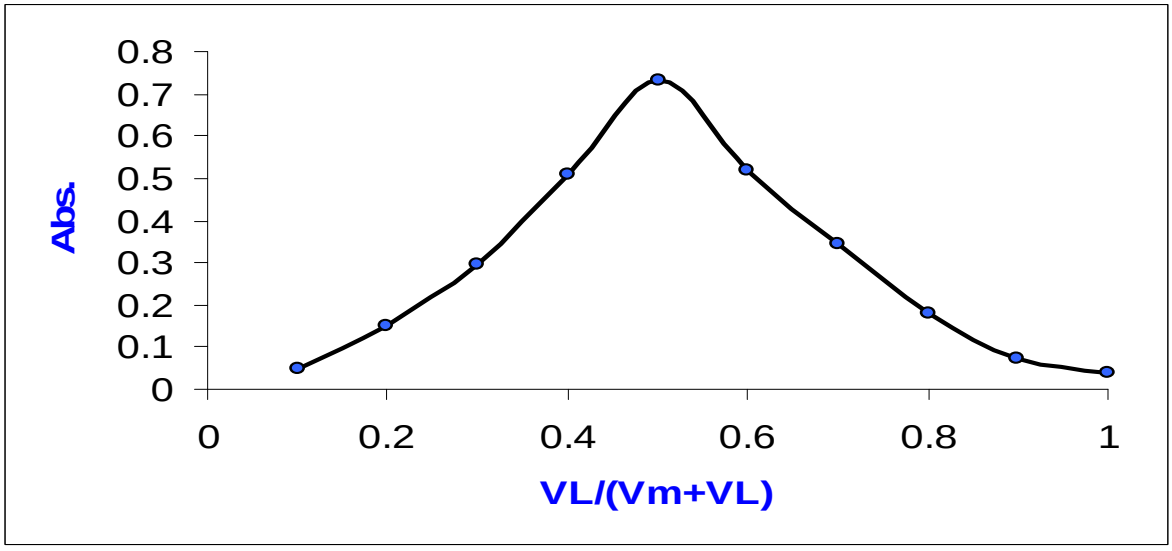

Figure16: Continuous variation using Fe^{3+}.

(3.6.2) Using Mole Ratio Method:

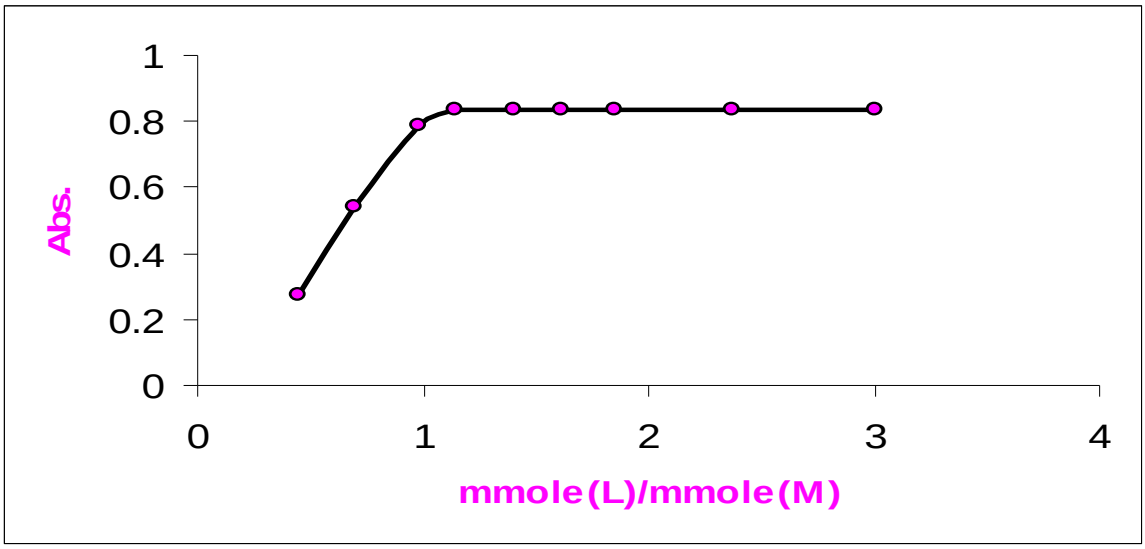

Figure17: Mole ratio method using Cu^{2+}.

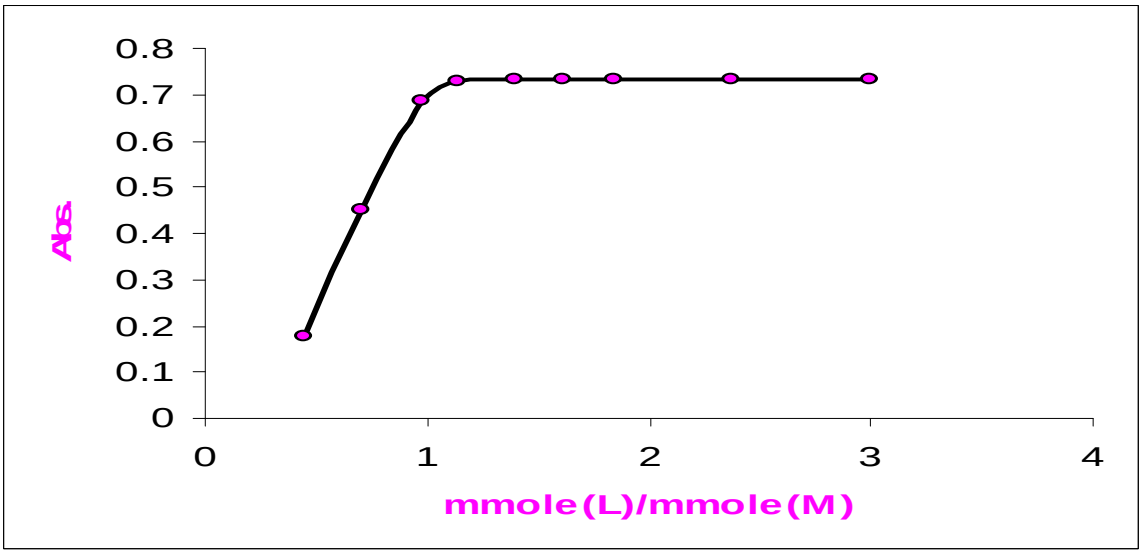

Figure18: Mole ratio method using Fe^{3+}.

Both methods show stochiometric composition of 1:1 Cu^{2+} or Fe^{3+}:ampicillin as shown in figures(15), (16), (17), and (18).

(3.6.3) The Effect of Some Metal Ions on the Determination of Spectrum Ampicillin:

The interference effect of some metal ions such as: sodium(I), potassium(I), calcium(II), zinc(II), copper(II) and iron(III), on the determination of ampicillin were studied.

The absorbances of series of standard solutions containing different amount of interfering materials with a fixed concentration of ampicillin were measured. The results of this study are listed in table(3).

Table(3): Effect of some cations on the determination of 80 ppm ampicillin

Cations	Conc. of cations (ppm)	Relative error% of the absorbance at λmax, 339 nm
Na^+	2	+3.47
	4	+3.41
	6	+3.36

	8	+3.33
	10	+3.25
	20	+2.87
	40	+2.05
	60	+1.28
	80	+0.46
K^+	2	+0.78
	4	+0.73
	6	+0.65
	8	+0.59
	10	+0.53
	20	+0.22
	40	-0.27
	60	-0.72
	80	-1.07
Ca^{2+}	2	+4.03
	4	+3.63
	6	+3.13
	8	+2.73
	10	+2.23

	20	+0.63
	40	-2.37
	60	-4.67
	80	-5.27
Zn^{2+}	2	+0.40
	4	+0.39
	6	-o.31
	8	-0.67
	10	-0.99
	20	-2.42
	40	-4.75
	60	-4.77
	80	-4.82
Cu^{2+}	2	+2.30
	4	+3.30
	6	-2.70
	8	-4.70
	10	-6.70
	20	-16.70
	40	-35.70

	60	**-51.70**
	80	**-69.70**
Fe^{3+}	**2**	**+31.00**
	4	**+30.50**
	6	**+29.60**
	8	**+27.80**
	10	**+25.90**
	20	**+24.10**
	40	**+13.50**
	60	**+9.30**
	80	**-28.30**

***Each result represents an average of at least four measurements.

These results indicated that four metal ions (Na^+, K^+, Ca^{2+} and Zn^{2+}) have no apparent effect on the determination of ampicillin at 339nm.
Copper(II) and iron(III) have shown a high interferences on determination of ampicillin at the studied wavelength as illustrated from overlapped spectra as shown in Figure(19).

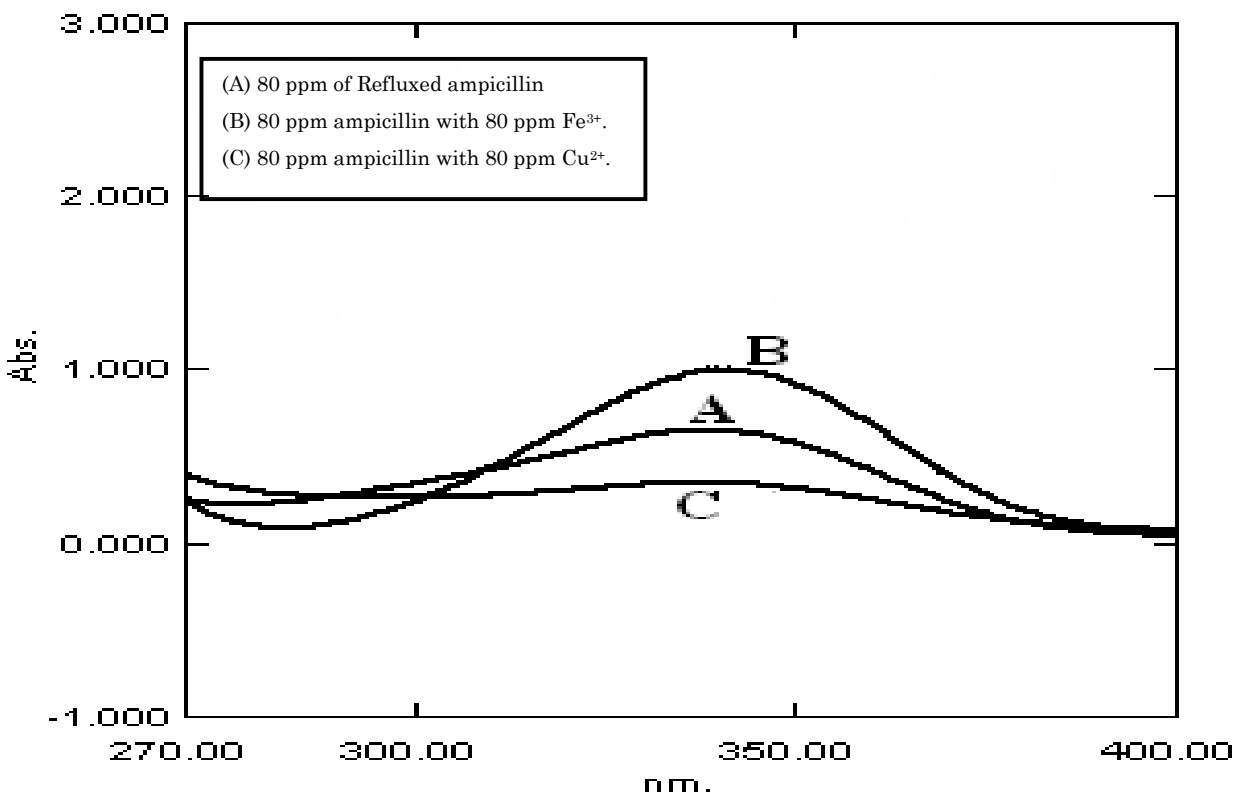

Figure19: Normal spectra of (A)80 ppm of Refluxed ampicillin ,(B)80 ppm ampicillin with 80 ppm Fe^{3+}, (C) 80 ppm ampicillin with 80 ppm Cu^{2+}

Figure(20) showed normal spectrum of Fe^{3+}, in which the maximum absorbance (613 nm) overlaps with that of ampicillin peak. When the Fe^{3+} mixed with ampicillin this wavelength is shifted to 345.5 nm as shown in figure19(curve B).

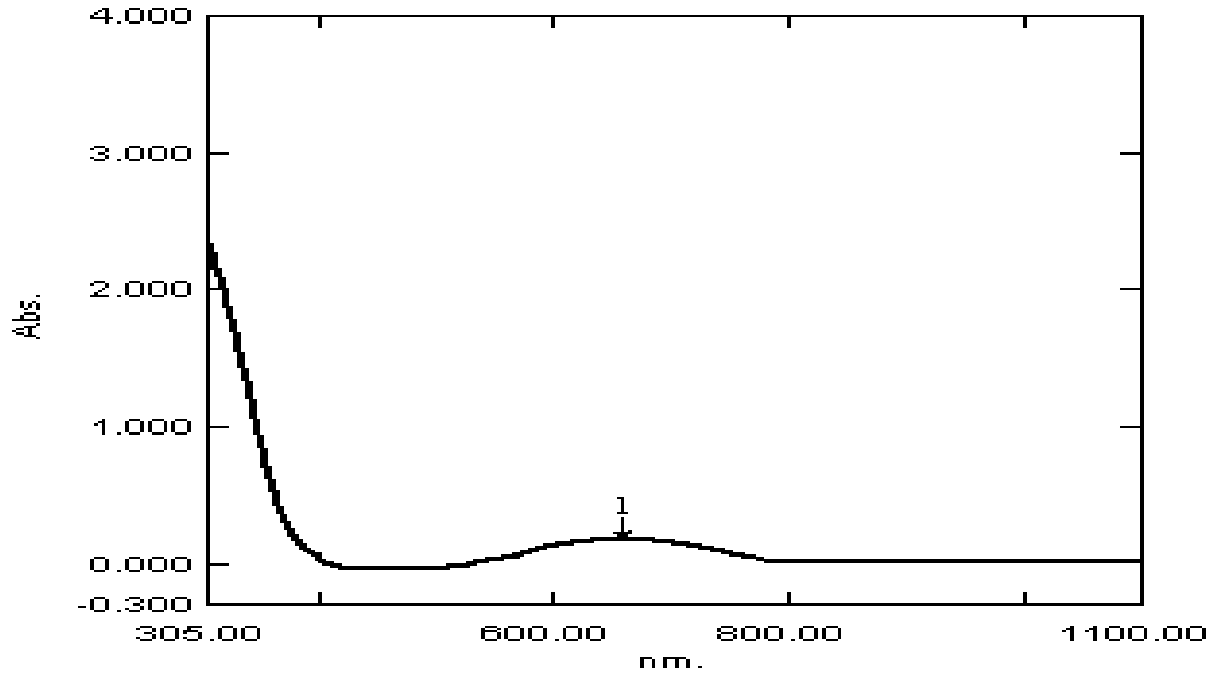

Figure20: Normal spectrum of 500 ppm Fe^{3+}.

In figure(21) the normal spectrum of Cu^{2+} shows a maximum absorbance at 803 nm when Cu^{2+} mixed with ampicillin the absorbance at 339.5 nm is also affected as shown in figure19(curve C).

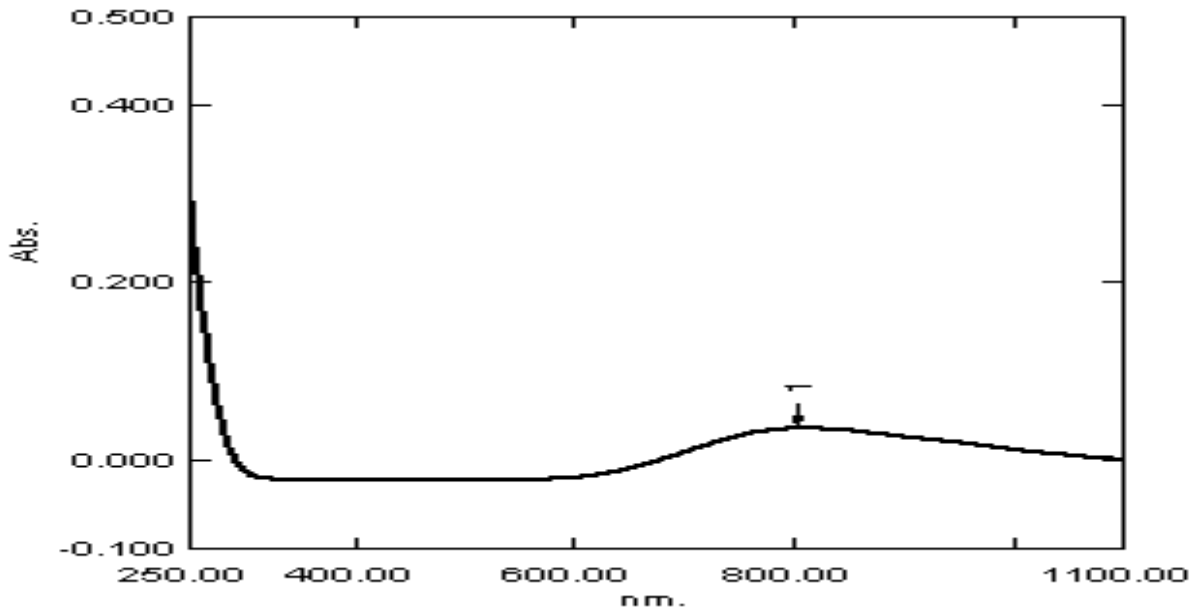

Figure21: Normal spectrum of 500 ppm Cu^{2+}.

From figure(19) a clear interferences observed when these two metal ions mixed with ampicillin

(3.7) Using the Derivative Method to Determine the Spectra of Ampicillin:

The concentration of ampicillin can not determined spectrophotometrically in the presence of copper(II) and iron(III). The derivative method was studied to assist in solving this problem. The absorption spectra for the above metal ions were derivatized to D1 and D2 order using delta 5, as shown in figures(22), (23), (24) and (25), respectively.

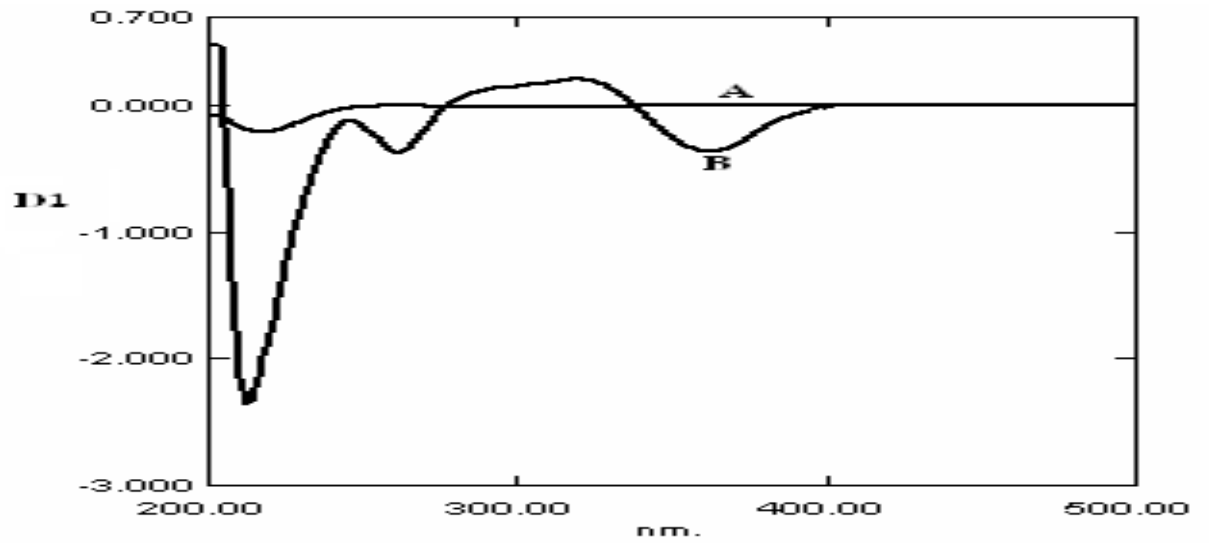

Figure22: First derivative spectra of (A) 80 ppm Fe^{3+} with (B) 80 ppm ampicillin

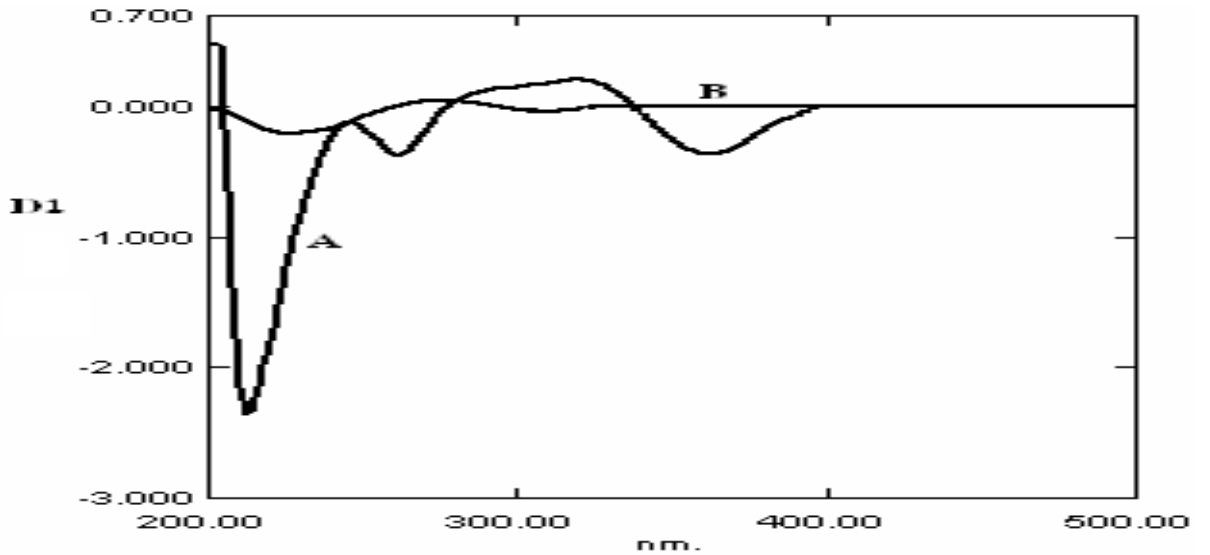

Figure23: First derivative spectra of (A) 80 ppm ampicillin with (B) 80 ppm Cu^{2+}

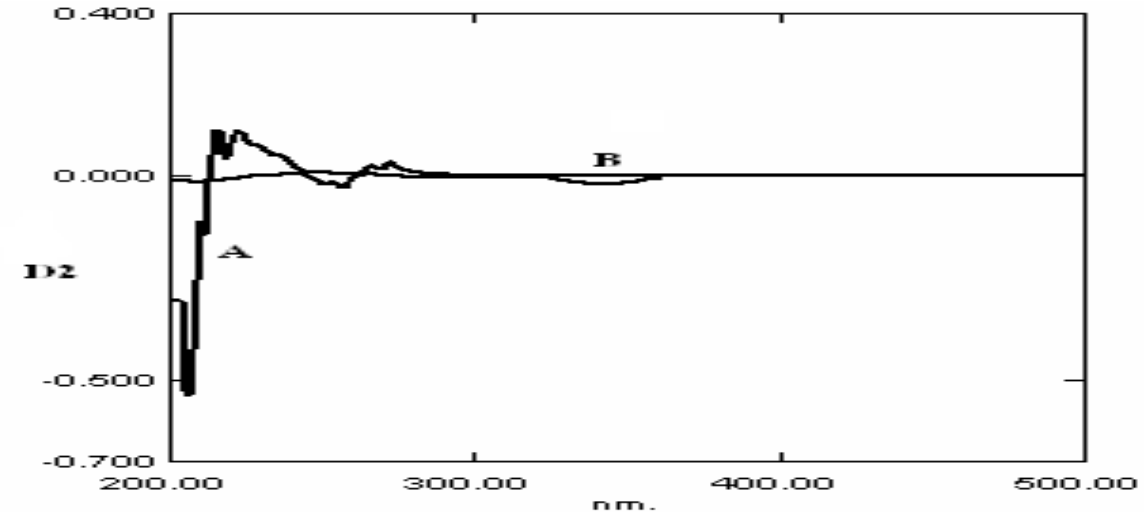

Figure24: Second derivative spectra of (A) 80 ppm ampicillin with (B) 80 ppm Fe^{3+}

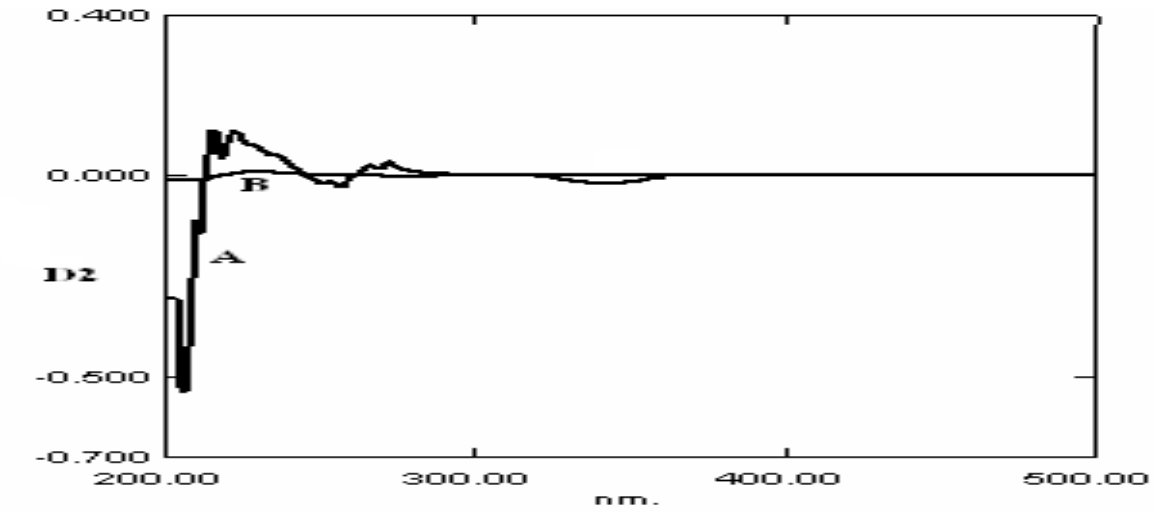

Figure25: Second derivative spectra of (A) 80 ppm ampicillin with (B) 80 ppm Cu^{2+}

The best wavelength obtained for the first derivative for the copper(II) and iron(III) was 362 nm while the second derivative was 338 nm.

Linear relationship between amplitude in the first and second derivative absorption spectra and the concentration of ampicillin was obtained are shown in figure(26) and (27), respectively. Dynamic linear equation has been found in the range (4-200) ppm

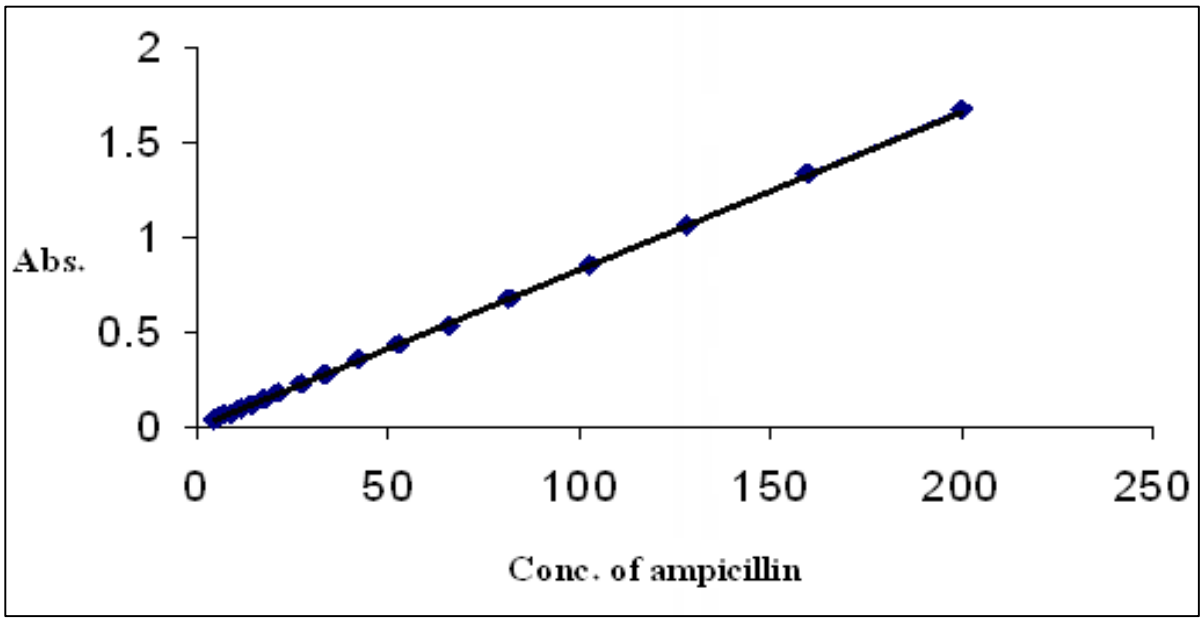

Figure26: Calibration curve of ampicillin using first derivative method in the presence of Fe^{3+} and Cu^{2+}.

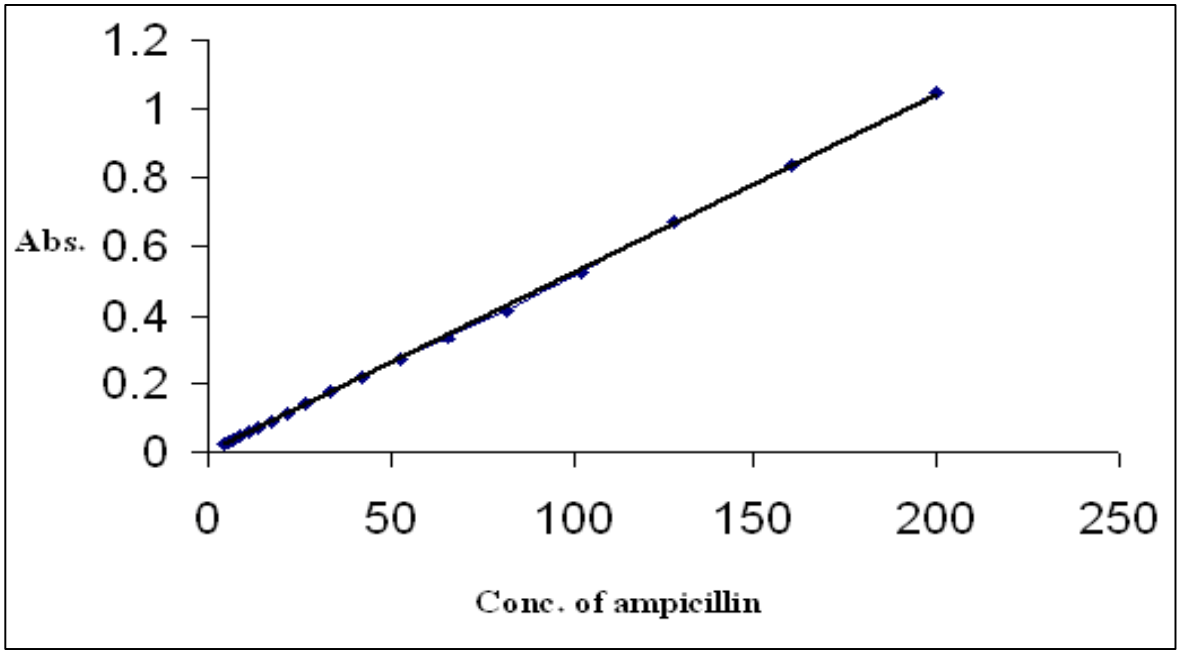

Figure27: Calibration curve of ampicillin using second derivative method in the presence of Fe^{3+} and Cu^{2+}.

A comparison between some parameters using these two derivative orders is shown in table(4):

Table(4): Calibration curves parameters of ampicillin in presence of Fe^{3+} and Cu^{2+} as interference using derivative methods.

Derivative oeder	D1	D2
λ, nm	362	338
Req. Eq. Y=|aX+b|	Y =| 0.0052X - 0.0026|	Y = |0.0083X - 0.0021|
Corr. Coeff. (r)	0.9999	0.9999
RSD%	±1.790	±1.589
Relative Error%	-1.72	-0.70
Recovery%	99.28	99.30

The linear equation, relative standard deviation (RSD), and recovery of the linear calibration curve using first and second derivative absorption spectra were Y =| 0.0052X - 0.0026|, Y = |0.0083X - 0.0021|, ±1.79, ±1.589; and 98.28, 99.3, respectively. From the above parameters it seems that the signal measurement using second derivative spectrum at 338 nm would give better results than the measurement using first derivative spectra at 362 nm in the presence of Cu^{2+} and Fe^{3+}.

(3.7.1) Determination of Ampicillin in Capsules:

The applicability of the derivative methods has been appraised through the assay of ampicillin in two different manufacturers of capsules, and results are listed in table (5):

Table(5): Determination of ampicillin in capsules as interference with Fe^{3+} and Cu^{2+} using derivative methods.

Pharmaceutical	Sammera		India	
D, order	D1	D2	D1	D2
listed (ppm)	200	200	500	500
Found (ppm)	196.70	199.20	488.46	498.68
Relative Error%	-1.65	-0.40	-2.31	-0.26

(3.7.2) The Effect of Cu^{2+} and Fe^{3+} on Ampicillin:

The effect of copper(II) and iron(III) on the determination of ampicillin concentration by these methods was investigated, as shown in table(6):

Table(6): Effect of interfering metals on the determination of 80 ppm ampicillin using derivative methods

Interfering Materials	Concentration of Interfering Materials	Relative Error%	
		Derivative order, λmax nm	
		D1,362	D2,338
Cu^{2+}	2	+0.187	+0.270
	4	+0.185	+0.240
	6	+0.184	+0.200
	8	+0.183	+0.160
	10	+0.180	+0.120
	20	+0.177	+0.010

	40	**+0.173**	**-0.14**
	60	**+0.171**	**-0.250**
	80	**+0.166**	**-0.340**
Fe^{3+}	**2**	**+0.326**	**+0.269**
	4	**+0.310**	**+0.249**
	6	**+0.284**	**+0.213**
	8	**+0.267**	**+0.189**
	10	**+0.242**	**+0.160**
	20	**+0.173**	**+0.064**
	40	**-0.160**	**-0.134**
	60	**-0.146**	**-0.326**
	80	**-0.319**	**-0.523**

***Each result represents an average of at least four measurements.

Conclusion :

The normal absorption spectrophotometry was found not applicable in the determination of ampicillin in the presence of some metals such as copper(II) and iron(III) because of the overlapped spectra of these compounds with ampicillin.

Derivative absorption spectrophotometry was found to be a convenient technique for the determination of ampicillin in the presence of those two metals over wide concentration range.

Second derivative spectrum at 338 nm was chosen for this purpose, it gave better results than first derivative spectra at 362 nm.

Other metals i.e. K^{+}, Na^{+}, Zn^{+2} and Ca^{+2} showed no detectable interferences with ampicillin spectra.

Future Work :

1. Study the effect of other penicillin and cephalosporin drugs such as cloxacillin and cephalixen on ampicillin trihydrate using derivative absorption spectrophotometry.

2. Study the effect of interferences drugs using other analytical techniques such as HPLC.

3. Determination of ampicillin trihydrate in human serum using derivative and other analytical techniques such as HPLC.

(2.1) Instruments and Equipment:

- Double-beam UV-Visible spectrophotometer model (UV-1650CP) Shemadzu\ (Japan) interfaced with computer via Shemadzu UV-probe data system program.

- Infrared spectrophotometer Shemadzu, FTIR-8000 _Japan).

- pH meter Orion expandable ion analyzer model (EA940) equipped with a glass combined electrode.

- Ultra Sonic device (ultrasonicator) for dissolving samples, (SONOREX), (W. Germany).

(2.2) Chemicals:

Ampicillin Trihydrate standard, ampicillin capsule 250mg were a gift from the State Company for Drug Industries and Medical Appliances (Samara-IRAQ-SDI).
Ampicillin capsule 500mg (Ajanta Pharmaceutical Limitted Company, India).
All other chemicals and reagents of analytical grade were obtained from Fisher, Fluka and BDH Companies.

(2.3) Preparation of Standard Solutions:

Stock solution of 200 ppm ampicillin was prepared by dissolving 0.02 g in 100ml deionized water. Other standard solutions were prepared by subsequent dilution of stock solution.

The ampicillin trihydrate before heating has shown no observed maximum wavelength, as shown in figure3, and the FTIR spectrum of standard ampicillin was obtained and shown in figure8.

Stock solution of 200 ppm potassium(I), sodium(I), calcium(II), zinc(II), copper(II), and ferric(III) ions were prepared by dissolving 0.0418g potassium chloride, 0.0508 g sodium chloride, 0.055 g calcium chloride, 0.0418 g zinc chloride, 0.05 g copper sulphate and 0.058 g ferric chloride, respectively, and diluted to 100ml deionized water. Other standard solutions for the above ions were prepared from the stock solution.

According to Graham L. study [(64)] ampicillin converted to a pale yellow upon heating due to rearrangement as shown in figure6.

Consequently, the ampicillin solution was refluxed for different period of time with ½ hr intervals and the absorbances of the resulting solutions were measured. Figure5 shows the absorbance of the resulted product during heating with time.

The spectrum of convertad ampicillin was measured at 339 nm as shown in figure4 and the FTIR spectrum of the proposed conversion structure was obtained and shown in figure8.

Continuous variation method has been used to study the stochiometric composition of converted ampicillin that coordinates with metal ions. Various volumes of 200 ppm ampicillin has been taken and they were: 25, 22.5, 20, 15, 12.5, 10, 7.5, 5, 2.5 and 0 ml and various volumes of 200 ppm Zn ion has been also taken; they were:0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5 and 25 ml. Each volume of the drug was mixed with the Zn ion solutions and then refluxed for about 2 hr. The resultant measurement is shown in figure15. The absorbances were measured at 339 nm.

The same procedure was repeated using Fe^{3+} as shown in figure17.

Mole ratio method was also used to verify the results of continuous variation method. A series of solutions of 4.96×10^{-4} M ampicillin were prepared by taking: 1, 1.3, 1.6, 1.9, 2.2, 2.5, 2.8, 3.1, 3.4 and 3.7 ml.

Each volume of the drug was mixed with 3 ml of 4.96×10^{-4} M Zn ion solution and the mixture was refluxed for about 2 hr. The absorbances then measured at 339 nm as shown in figure16.

The same procedure was repeated using Fe^{3+} as shown in figure18.

Standard solution of 100ppm of ampicillin at different pH value ranging from 1 to 12 were prepared using buffer solutions.

Standard solutions of 80ppm of ampicillin with different concentrations of sodium(I), potassium(I), calcium(II), zinc(II), copper(II) and ferric(III) ion solutions were prepared and refluxed for about two hours.

Stock solutions of 250 ppm and 500 ppm ampicillin trihydrate capsule obtained from two different manufacturers “Samara and India” were prepared by dissolving 0.0152g and 0.0293g respectively in 100ml deionized water.

The ampicillin trihydrate spectrum then derivatized by the instrument microprocessor to the first, second, third and fourth orders.

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ألأهداء

إلى التي زرعتني في الحياة بذرة .....

إلى من سهرت وأفنت سنين عمرها .....

إلى منبع التضحية .. بحر الحنان .. حضن الأمان .....

إلى رمز المحبه والعطاء .....

أمي الغالية

إلى من عانى لكي أصبح .....

إلى الشمعه التي أذابها التعب .....

أبي العزيز

إلى البسمة التي تملئ حياتي ونجماً يتلألأ في سمائي .....

إلى القلب الحنون والمعطاء .....

أخي الحبيب

إلى الذين شاطروني أفراحي وأحزاني وكانوا موضع ثقتي .....

إلى الذين جمعتني معهم لحظات جميلة .. إلى من وجدت فيهم التضحيه والوفاء...

صديقتي بشائر.

مريم

Abbreviations

D1	First derivative
D2	Second derivative
D3	Third derivative
D4	Forth derivative
HPLC	High Performance Liquid Chromatography
hr	hour
IR	Infrared Spectroscopy
LC	Liquid Chromatography
L	Litter
M	Molarity
mg	milligram
ml	millilitter
nm	nanometer
min	minute
No.	number
ppm	Part per million
RSD	Relative Standard Deviation
S/N	Signal to Noise Ratio
TLC	Thin layer chromatography
UV	Ultraviolet
Vis	Visible
Vm	Volume of Metal ion
VL	Volume of Legand
λ	Wavelength

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References

(1) Andrejus K.; "Essentials of Medicinal Chemistry", 2nd ed., **9**, 7 (1988).

(2) White A., Handler A., and Smith p.; "Principles of Biochemistry". New York, McGraw-Hill Company, **18**, 13 (1984).

(3) Norbert W.; "Fundamental of Clinical Chemistry", 20th ed., W.B. Standard Company, New York, **34**, 645 (1999).

(4) Hustad S.; "Clinical Chem.", **45**, 862 (1999).

(5) Codoner A., and Medina P.; "Spectrochemica Acta Part", **49**, 32 (1993).

(6) Wright A.,"Ampicillin", Mayo Clin Proc, **74**(3), 290 (1999).

(7) Eric R.; "Blood Drugs and Other Analytical Challenges", **7**, 645 (1987).

(8) "British Pharmacopoeia on CD-ROM", Version 2, Copyright by Crown, Ltd., Norwich, 64 (1998).

(9) "The Merck Index on CD-ROM", Copyright by Merck Co., Inc., White ho., USA, 1999.

(10) "British Pharmacopoeia on CD-ROM", Version 4, Copyright by Crown, Ltd., London, 84 (2000).

(11) Hamid S.and Beg A., "Ampicillin Bioavailability and Pharmacokinetics" *Pol J.* Pharmacol Pharm **39**, 337 (1987).

(12) Attel O., Ali A. and Ali H.," Effect of Ampicillin Trihydrate ". *J.* Antimicrob Chemother **39**, 523 (1997).

(13) Blaser J and Zinner S. "The Study of Antibiotic Activities". *J.* of Prog Drug Res **31**, 349 (1987).

(14) Donowitz G.; "Beta-Lactum Antibiotics", N Engl. *J.* Med*,* **318**, 419 (1988).

(15) Zinner S., Blaser J., Stone B. and Groner M., "Use of Kinetic Model to Study Antibiotic Combinations" *J.* Antimicrob Chemother **15**, 221 (1990).

(16) Analytical Methods Committee, Analyst **25**, 353 (2000).

(17) Vega M., Garcia G., Saelzer R., and Villegas R.; "HPTLC Analysis of Antibiotics"*, J.* of Chromatogr, **7**, 159 (1994).

(18) Meyer V.; "Practical High-Performance Liquid Chromatography"*,* 3rd ed., 309 (1998).

(19) Veronica A., Loredana S., Rodica G., and Hajnalka F.; "HPLC Determination of Some Antibiotics", **43**, 29 (2003).

(20) Hartmann V.; "Application of HPLC to the Analysis of Penicillins and Cephalosporins, Chromatographia", **9**, 66 (1995).

(21) Simone G., Lisiane B., Isabel F., Marcelo D., and Silvia H.; "Liquid Chromatographic Determination of Cetirizne in Oral Formulations", *J.* of AOAC International, **88**(2), 424 (2005).

(22) Martin S., Cássia V., and Clésio S.; "New Liquid Chromatographic Method for Determination of Rabeparazole Sodium in Coated Tablets", *J.* of AOAC International, **87**(4), 842 (2004).

(23) Ana R., Clésio S., Júulia M., and Elfrides E.; "Development and Validation of Liquid Chromatographic Method for Fexofenadine Hydrochloride in Capsules", *J.* of AOAC International, **87**(5), 1093 (2004).

(24) Tushar N., Gunta S., and Kilambi P.; "Determination of Cefdinir by Stability-Indicating Liquid Chromatographic Method", *J.* AOAC International, **88**(6), 1661 (2005).

(25) Thorburn B., O'Callaghan M., Franklin S., and Ayilng J.; "High-Performance Liquid Chromatographic Analysis of Ampicillin and Cloxacillin", 1st ed., **340**, 53 (1991).

(26) White L., Tobin C., and Sunderland J.; "High-Performance Liquid Chromatography Assay for Linezolid in Human Serum, Journal of Antimicrobial Chemotherapy", **48**, 605 (2001).

(27) Shawky A.; "Simultaneous Liquid Chromatographic Analysis of the ß–Lactam Antibiotics Cefazolin, Cefadroxil, Cephalexin, Cephradine and Ampicillin in Solution", *J.* of AOAC international, **83**(3), 436 (2000).

(28) Ptyde, A., and Gibert; "Application of HPLC", Chapman and Hall, London, **68**, 77 (1979).

(29) Anna B., Anna G., and Hanna H.; "Development and Validation of New HPLC Method for the Determination of Glicazide and Repaglinide in Pharmaceutical Formulations", *J.* of AOAC International, **89**(2), 319 (2006).

(30) Hérida R., and Cristiani C.; "Determination of Gatifloxacin in Tablet Preparations by HPLC", *J.* of AOAC International, **89**(3), 642 (2006)

(31) Van M.; "Analytical Methods for Residue Analysis", Zweig, G., e.d, Academic Press, New York, **44**, 60 (1981).

(32) James V., W.R.; "Undergraduate Instrument Analysis", Macel Dekker, Inc., pp. 215, (1987).

(33) Morrison, J.D.; *J.* Chem. Phys., **21**, 1767 (1953).

(34) O'Haver, T.C.; Clin. Chem., **25**, 1548 (1979).

(35) Cottrel, C.T.; Anal. Proc., **19**, 43 (1982).

(36) Fix, G.L., and Pollak, D.J.; Anal. Chem., **52**, 1589 (1980).

(37) O'Haver, T.C.; Anal. Chem., **51**, 91A (1979).

(38) Hager, R.N.; Anal. Chem., **45**, 1131 (1973).

(39) Fell, A.F., and Smith G., Anal. Proc., **19**, 28 (1982).

(40) Fell, A.F., Jarvie, D.R., and Stewart, M.J., Clin. Chem., **27**, 286 (1981)

(41) O'Haver, T.C., Anal. Chem., **19**, 22 (1982).

(42) Rojas, F.S., Ojeda, C.B., and Pavon, J.M., Talanta, **35**, 753 (1988).

(43) Tariq M., Shahbaz A., and Yahya Y.; "Iraqi *J.* of Chemistry" **26**, 471 (2000).

(44) Ryadh M., Shahbaz A., and Nabil S.;" Derivative Absorption Spectrophotometry for the Determination of Atenolol in Pharmaceutical Drugs", M.Sc. thesis, 7, 2001, Department of Chem., Al-Nahrain Univ.

(45) Martin G., Shahbaz A. and Nabil S.;" Determination of Chlorpyrifos and Diazinon Insecticides Using Derivatives Absorption Spectrophotometric Method", M.Sc. thesis 14, 2002, Department of Chem., Al-Nahrain Univ.

(46) Galera, M.M., Vidal, J.L., Frenich, A.G., and Parrilla, P., Analyst, **199**, 1189 (1994).

(47) Diaz, T., Valenzuela, M., and Salinas, F.; "Fresenius Journal of Analytical Chemistry", **350**, 692 (1994).

(48) Toral, M., Richter, P., Cavieres, M., and Gonzalez, W.; Environ. Monit. Asses. **54**, 191 (1999).

(49) Branowska, I., and Pieszko, C.; Chemia Analyticzna, **45**, 583 (2000).

(50) Nevin E., and Gülay G.; "Resolution of Two Component Mixtures in Pharmaceutical Formulations Containing Phenazopyridine Hydrochloride with Ampicillin Trihydrate by Spectra Derivative", **35**, 83 (2002).

(51) Khadiga K., Lories I., and Laila A.; "Stability Indicating Spectrophotometric Method for Determination of Some Cephalosporines", *J.* of AOAC International, **83**(4), 601 (2000).

(52) Liang Y.; "Direct UV Detection of Glucose and Its Derivatives", *J.* of Pharm. Biomed. Anal., **18**, 905 (2003).

(53) Mahgoub H., and Aly F.; "UV Spectrometric Determination of Ampicillin Sodium and Sulbactam in Two Component Mixtures", *J.* of Pharm. Biomed. Anal., **17**, 1273 (1998).

(54) Mostafa A., Mohammed A., Mohammed G., and Mohammed F.; "Stability-Indicating Methods for Determination of Pyritinol Dihydrochloride in the Presence of Its Precursor and Degradation Product by Derivative Spectrophotometry", *J.* of AOAC International, **88**(1), 80 (2005).

(55) Khadiga M., "Simultaneous Determination of Caffine, 8-Chlorotheophylline and Chlorphenoxamine Hydrochloride in Ternary Mixtures by First Derivative Spectrophotometric", *J.* of AOAC International, **88**(4), 1126 (2005).

(56) Maria I., Marcelo A., and Sandra L.; "Simultaneous Determination of N-Butylscopolamine and Oxazepam in Pharmaceutical Formulations by First-order Derivative Spectrophotometry", *J.* of AOAC International, **88**(4), 1173 (2005).

(57) Sawsan M., Yasser S., Fadia H., and Kareem M.; "Spectrophotometric Study of Etodolac Complexes with Copper(II) and Iron(III)", *J.* of AOAC International, **88**(6), 1637 (2005).

(58) Ali R., Abbas A., and Nahid S.; "Simultaneous Spectrophotometric Determination of Paracetamol and Salicylamide in Human Serum and Pharmaceutical Formulations", *J.* of AOAC International, **88**(6), 1695 (2005).

(59) Jan K., Mariusz S., and Wlodzimierz R.; "Application of Derivative Spectrophotometry to Simultenous Determination of Indomethacin and 5-Methoxy-2-methyl-3-indoleacetic Acid in Metindol Injections", *J.* of AOAC International, **87**(3), 592 (2004).

(60) Patricio L., Maximilliano P., and Pablo R.; "Determination of Attapulgite Nifuroxazide in Pharmaceutical Formulations by Derivative Spectrophotometry", *J.* of AOAC International, **87**(6), 1323 (2004).

(61) Andrés T., César S., and Pablo R.; "Simultaneous Determination of Dapsone and Pyrimethamine by Derivative Spectrophotometry in Pharmacetical Formulations", *J.* of AOAC International, **86**(2), 241 (2003).

(62) Nasr Y., Abdulrahman A., Fathalla B., and Kamal E.; "Spectrophotometric Determination of MAcrolide Antibiotic Josamycin in Formulations", *J.* of AOAC International, **86**(3), 484 (2003).

(63) Amr. M., Samah S., and Hyam M.; "Spectrophotometric Determination of Some Antihistaminic Drugs using 7,7,8,8-Tetracyanoquinodimethane (TCNQ)", *J.* of AOAC International, **89**(1), 46 (2006).

(64) Graham L., "Medical Chemistry", 2nd ed., **8**, 491, 2004.

(65) Khallow K., "Peparation and Characterization of Complexes of Ampicillin, Iraqi *J.* of Chemistry, **27**, 925 (2001).

Chapter One

Introduction

Chapter Two

Experimental Part

Chapter Three

Results & Discussion

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جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم الكيمياء

إستعمال المشتقات الطيفية لتحليل المضاد الحيوي أمبسلين لوحده وبوجود بعض العناصر

رسالة مقدمة
الى كلية العلوم في جامعة النهرين كإستكمال جزئي لمتطلبات نيل درجة الماجستير في علوم الكيمياء

من قبل:

مريم يعقوب يوسف
بكلوريوس كيمياء(٢٠٠٣)

رمضان (١٤٢٧) أيلول (٢٠٠٦)