Summary

Cephalexin is a medical drug administrated commonly for the treatment of infections.

Two methods were examined for determination of cephalexin based on conventional and derivative UV absorption spectrophotometry.

The calibration curve at 262 nm has linear range extended from (1.5-100) ppm with relative standard deviations for the slope of 0.91 and correlation coefficient of 0.9993. The effect of pH on the determination of cephalexin at this wavelength was also investigated. The best pH for its determination was found to be 6.7.

The effect of the presence of some metal ions that found in human body on the cephalexin determination was studied. We noticed that there are interference between metal ions and cephalexin measurements. The metal ions Na^{+}, K^{+}, Mg^{2+}, Ca^{2+} were found that have no interference but Cu^{2+}, Fe^{3+} can interfere with cephalexin.

Derivative absorption spectrophotometry was developed for determination of cephalexin in the presence of some metal ions that interfere with cephalexin.

First, second, and third derivative spectra at 317 nm, 337 nm and 291nm, respectively for Fe^{3+}, were used for this purpose.

First, second, and third derivative spectra at 285 nm, 238 nm and ٢٢٢nm, respectively for Cu^{2+}, were used for this purpose.

The calibration curves for the first, second and third derivative spectra have a linear rang (2-50) ppm, (2-50) ppm and (2-40) ppm with relative standard deviation for the slope of 1.38, 0.32 and 3.47 with correlation coefficient of 0.9993, 0.9996, 0.9990, respectively, for cephalexin determination in the presence of Fe^{3+}.

The calibration curves for the first, second and third derivative spectra have a linear rang (2-50) ppm, (2-50) ppm and (2-40) ppm with relative standard deviation for the slope of 3.22, 0.57 and 4.47 with correlation coefficient of 0.9992, 0.9998, 0.9994, respectively, for cephalexin determination in the presence of Cu^{2+}.

No effect of pH on the determination of cephalexin using derivative methods was found.

الخلاصة

يستخدم العقار الطبي السيفالكسين عادة في علاج الالتهابات لانه يستخدم كمضاد حيوي للبكتريا.

تم استخدام طريقتين لتقدير تركيز السيفالكسين اعتمادا على الطريقة التقليدية وطريقة المشتقة الطيفية وكانت منحني المعايرة في الطول الموجي ٢٦٢ نانوميتر ذات مدى خطي يمتد من (١,٥-١٠٠) جزء من المليون وكان الانحراف المعياري النسبي لميل الخط المستقيم ٠,١٩ ومعامل التصحيح ٠,٩٩٩٣ .

كما تم دراسة تأثير الاس الهيدروجيني pH على تقدير السيفالكسين عند الطول الموجي المذكور. وجد ان افضل رقم هيدروجيني لتقدير السيفالكسين هو ٦,٧.

وقد استخدم تأثير بعض العناصر الموجودة في جسم الانسان على العقار الطبي السيفالكسين وملاحظة هل هناك تداخل بينها وبين السيفالكسين. وقد وجد ان عناصر ال Na^{+}، K^{+} Mg^{2+}، Ca^{2+} لاتتداخل بينما Cu^{2+}، Fe^{3+} تتداخل مع السيفالكسين عند اطيافها الجزيئية. لذلك تم أستخدام مشتقة طيف الامتصاص لتقدير السيفالكسين في حالة تداخل اي من العناصر المذكورة مع السيفالكسين لغرض التغلب على هذه المشكلة.

وقد أستخدمت أطياف المشتقة الاولى والثانية والثالثة عند الاطوال الموجية ٣١٧و ٣٣٧ و٢٩١ نانوميتر على التوالي لهذا الغرض بالنسبةFe^{3+} .

وكانت منحنيات المعايرة لطيف المشتقة الاولى والثانية والثالثة ذات مدى خطي من (٢-٥٠) و(٢-٥٠) و(٢-٤٠) جزء من المليون، وكان الانحراف المعياري النسبي لميل الخط المستقيم

١,٣٨ و٠,٣٢ و ٣,٤٧ ومعامل التصحيح ٠,٩٩٩٣ و٠,٩٩٩٦ و٠,٩٩٩٠ على التوالي بالنسبة لعنصر الحديد Fe^{3+}.

وقد أستخدمت أطياف المشتقة الاولى والثانية والثالثة عند الاطوال الموجية ٢٨٥و ٢٣٨ و٢٢٢ نانوميتر على التوالي لهذا الغرض بالنسبةCu^{2+} .

وكانت منحنيات المعايرة لطيف المشتقة الاولى والثانية والثالثة ذات مدى خطي من (٢-٥٠) و(٢-٥٠) و(٢-٤٠) جزء من المليون، وكان الانحراف المعياري النسبي لميل الخط المستقيم ٣,٢٢ و 0.57 و٤,٤٧ ومعامل التصحيح ٠,٩٩٩٢ و٠,٩٩٩٨ و٠,٩٩٩٤ على التوالي بالنسبة لعنصر النحاسCu^{2+} .

وقد لوحظ عدم تأثير pH في تعين السيفالكسين بواسطة طريقة المشتقة الطيفية بسبب ان pH للسيفالكسين هي نفس pH الوسط الذي اعمل به.

Abbreviations

D1	First Derivative
D2	Second Derivative
D3	Third Derivative
DS	Derivative Spectrophotometric
FID	Flow Injection Detector
FIIA	Flow Injection Immunoanalysis
HPLC	High Performance Liquid Chromatography
LC	Liquid Chromatography
M	Mass
O-Phen	O-Phenanthroline
RSD	Relative Standard Deviation

S/N	Signal to Noise ratio
TLC	Thin Layer Chromatography
UV	Ultraviolet

Acknowledgments

I wish to express my deepest gratitude and appreciation to my supervisor **Dr. Shahbaz A. Maki** for his patient, supervision and encouragement during the course of my study.

I am sincerely thankful to **Assist. Prof. Dr. Afaf AL-Derzi, Dr. Salman A., and Dr. Nabil S. Nassori** and to my college for the financial support and to the department of chemistry at AL-Nahrain University for all the facilities that they offered to me during my research.

Finally, I would like to thank my parents, brothers, husband Ammar, Mariam and Bashaier as well as my friends, for the support and encouragement.

Republic of Iraq
Ministry of Higher Education
and Scientific Research
AL-Nahrain University
College of Science
Department of chemistry

Quantitative Analysis of Cephalexin Antibiotic by using Normal and Derivative spectrophotometric Methods

A thesis
Submitted to the Collage of Science at AL-Nahrain University as a Partial Fulfillment of Requirement of the Degree of Master of Science in Chemistry

By:
Dalia Mahmoud Jamil
(B.Sc ٢٠٠٣)

(July) 2006 **١٤٢٧ هـ**

CHAPTER ONE

Introduction

An antibiotic is a drug that kills or slows down the growth of bacteria. Antibiotics are one class of antimicrobials, a larger group that also includes anti-viral, anti-fungal, anti-parasitic drugs. They are relatively harmless to the host, and therefore can be used to treat infections.

The term originally describes only those formulations derived from living organism, but is now applied also to synthetic antimicrobial, such as the sulfonamide. Unlike previous treatments for infections, which included poisons such as strychnine, antibiotics were labeled “magic bullets” drugs, which targeted disease without harming the host.

Antibiotics are not effective in viral, fungal and other non-bacterial infections. Individual antibiotics vary widely in their effectiveness on various types of bacteria. Some specific antibiotic target either *gram-negative* or *gram-positive bacteria*, and others are wide-spectrum antibiotics. The effectiveness of individual antibiotics varies with the location of the infection and the ability of the antibiotic to reach that site. Oral antibiotics are the simplest approach when effective, with intravenous antibiotics reserved for more serious cases.

Antibiotic may sometimes be administered topically, as with eye-drop or ointment (1). β-lactam antibiotic are very widely used in veterinary medicine for the treatment and prevention of disease. The development of antibiotic resistant bacteria cannot be underestimated (2). Although the use of antibiotics as chemotherapeutic gent is relatively

recent when compared with some other areas of medicine, the existence of such substances has been recognized for many decades (3).

Two major antibiotic families, penicillin and cephalosporin, share the same structural component, the β-lactam ring. The fact that penicillins and cephalosporin exhibit some cross-sensitivity (i.e. persons allergic to penicillin are likely to exhibit allergic sensitivity to some cephalosporin) indicates that this shared component is clinically important. Drugs from both families are inactivated by the enzyme β-lactamase, which is produced by some resistant pathogens (٤).

Most antibiotics were discovered in the United States, but from 1960 on, Japan has been the major contributor. Only 300 to 400 antibiotics, however, are used in medicine, because, in addition to other reasons, most antibiotics are devoid of selective toxicity, being equally toxic to both parasite and host (5).

1.1 Classification of Antibiotics

Antibiotics are classified depending not only on chemical structure but also in their mechanisms of action, antibacterial spectra origin, and other points. In spite of the variety of chemical structure involved, most antibiotics appear to arise from a limited number of biogenetic themes, and may be divided into three major groups according to their potential derivation from amino acids, sugars, and acetate or propionate units(6).

Antibiotics can also be classified by the organisms against which they are effective, and by the type of infection in which they are useful.

Although there are many antibiotics, the majority come from only a few types of drugs (7).

1.2 Cephalexin

Cephalexin, -5-thia-1-aza-bicyclo[4.2.0] octa-2-ene-2- carboxylic acid, 7-[2-mino-2-phenyl acetamido] -3-methyle-8-oxo, (Fig.1) is a white or almost white, crystalline powder, molecular weight 365.4 g/mol. It is soluble in water, particularly insoluble in alcohol and ether (8, ١2).

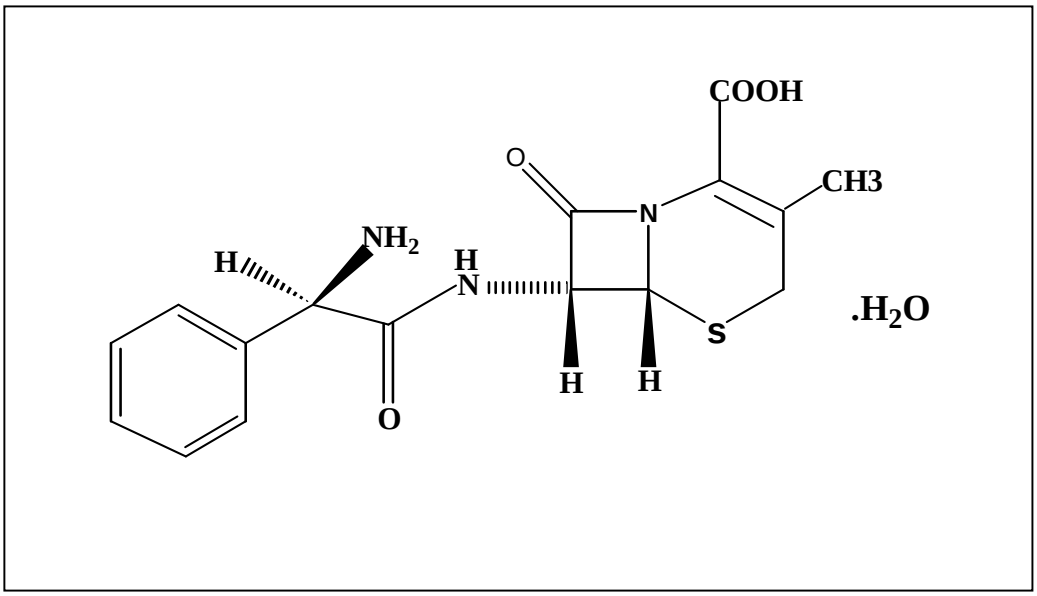

Fig.1. Structure of Cephalexin monohydrate

Cephalexin belongs to the class of antibiotic cephaloporins. They are similar to penicillin in action and side effects. Bacteria susceptible to cephalexin include *staphylococcus aureus, streptococcus penicillin, haemophilus influenza, E. coli* and several other (13).

The compound is zwitterions. The isoelectric point of cephalexin is approximately 4.5 to 5 (14).The pKa's of this drug are (5.2 and 7.3) (15). Discovered in 1948 from a fungus found in Sardinia (16).

Cephalexin has a D-phenylglycyl group as substituent at the 7-amino position and unsubsituted methyl group at the 3- position (17).

1.3 Pharmacokinetics

Cephalexin is rapidly absorbed with a mean peak plasma level about 18 mcg/ml at one hour; elimination half- life of cephalexin is 0.7 hour (18).

Cephalexin in plasma showed sharper and higher peaks than those for cephadroxil. Half-life and renal clearance remained independent of dose (19).

Cephalexin is exerted in the urine by glomerular filtration and tubular secretion. In vitrotests demonstrate that the cephalosporins are bactericidal because of their inhibition of cell-wall synthesis. Cephalexin has been shown to be active against most strains of microorganisms both in vitro and in clinical infection as described in the indications and usage of the drug (20).

1.4 Methods of Cephalexin determination:-

Methods developed for the determination of four alpha-amino cephalosporin, namely cefaclor, cefadroxil, cephalexin and cephradin. The reaction of the target compounds with fluorescamine at a specific at pH 7.8 to 8.4 (21).

Flurescence spectroscopy is an especially useful technique for the study of classes of compounds with relatively high flurescence yields. As is well documented in the flurescence literature for organic molecules in solution or on solid surfaces, one measure transitions from the lowest vibrational level, υ=0, of the first electronic excited singlet state to various vibrational levels of the electronic ground state (22)

An electrochemical method for quantifying beta-lactam antibiotics (cephalexin and ampicillin) and their hydrolysis products is described using cyclic voltammetry at the water/ nitrobenzene interface in a four-electrode system. The various hydrolysis products as well as the ionized antibiotics were studied in voltammetric transfer from water to nitrobenzene using the method of the interface between two immiscible electrolyte solutions (ITIES). It was concluded that this electrochemical method is suitable for the quantification of beta-lactam antibiotics and their hydrolysis products (23).

A sensitive, accurate and rapid flow injection analysis (FID) method for the determination of cephlexin, cefotaxime, cefaclor, cefixime, ceftriaxone, cefuroxime, and ceftioxime, has been reported. Cephalexin was digested for 15 min with 0.1 M NaOH at 80 ^{O}C and then oxidize with Fe^{3+} in sulfuric acid medium**.** The produced Fe^{2+} is then complexed with *O*-phenanthroline (*O*-phen) in citrate buffer at pH 4.2 to form the red complex, Fe (*O*-phen) 3(2+), which exhibits an absorption maximum at 510 nm. Variables such as acidity, reagent concentrations, flow rate of regents and other flow injection parameters were optimized to produce the most sensitive and reproducible

results. The method was successfully applied to the analysis of pharmaceutical preparations. Excellent agreement between the results of the proposed and the official methods was obtained (24).

A fully automated stand-alone flow injection immunoanalysis (FIIA) device for the determination of cephalexin in milk has been developed. The system is based on principles of flow-through immunoassays and on sequential addition of the assay components to an immunoreactor. Protein G is immoblised on the surface of the immunoreactor serving as affinity matrix for the polyclonal anti-cephalexin antibodies (25). A cephalexin-alkaline phosphatase conjugate is mixed with the analyte-containing sample and binds in a competitive manner to the corresponding antibodies in the immunoreactor. After substrate addition enzymatically, the generated *p*-aminophenol is detected at a carbon electrode at +150 mV vs.Ag/AgCl. One assay cycle takes 16 min including regenerations without significant loss of signal height.

Due to the high specificity of the anti-cephalexin antibodies, other β-lactam antibiotics like penicillin, amoxicillin and cloxacillin do not interfere in the measurements, even when added at 10 mg/40mg. To deactivate alkaline phosphatase present in milk, samples are heat-treated for 3 min prior to measurements. Cephalexin recoveries from two milk samples were 90 and 110%. The detection limit in milk is 1 microgram (mean relative standard deviation of 3%), less than the maximum residue level of 4 micrograms per Kg.

The device is suitable for fast quantitative data generation from consecutively measured samples and thus adds to analytical screening methods (26).

The analysis of cephlexin in pharmaceutical samples show the precision of UV absorbance of intact and acid degraded cephaloporins. High performance liquid chromatography and lodometric methods have used for analysis of cefoxitin, cefotaxime, cephazolin and cephalexin. To obtain the calibration graphs, the analytical signal used

were: absorbance, first derivative absorbance, second derivative absorbance and pH-point standard additions method by using absorbance values at two selected wavelengths as analytical signal. These methods and calibration graphs were also used for the determination of cephalexin in pharmaceutical samples (27).

1.4.1 Chromatographic methods

The chromatographic technique has been widely used for the separation and identification of many sample components. Thin Layer chromatography (TLC) and high performance liquid chromatography (HPLC) techniques have already made a significant contribution to pharmaceutical, biochemical, clinical and environmental analysis (28).

Cephalexin was analyzed by LC as reported by Tamai et al. (29), with slight modification. The analytical column was a spherisorb C_{18} (250 by 4.6mm) column. The mobile phase was methanol-0.1M ammonium acetate (25/75; vol/vol) at the flow rate 1ml.min^{-1}, and the absorbance was monitored at 262nm.

Thomas et al. (30) used HPLC quantification of the serum extract by using three different chromatographic systems. The first elution pattern developed from a serum extract of didnosine, lamivudine, and stavudine added into serum at concentrations near C_{max}. The second elution pattern developed from a serum extract to which nevirapine and zidovudine has been added to concentrations near C_{max}. The third elution pattern developed from a serum extract to which delavirdine, indinavir, nelfinavir, ritonavir, and saquinvir has been added to concentrations near C_{max}.

Prasad et al. (31), used components in aliquots (50µL) of the solutions of colistin methanesulfonate (CMS) prepared in water were separated on a steal strong-anion-

exchange (SAX) column (250 by 4.6 mm inside diameter) packed with 5- μm-diameter spherisorb, through which a mobile phase consisting of acetonitrile: 1.2mM sodium sulfate (70:30 vol/vol) was pumped at 1ml/min. The eluent was monitored for its UV absorbance at 210 nm.

1.4.2 Spectrophotometric methods

A spectrophotometric methods have been described for the quantification of individual compounds in capsules containing either probenecid and ampicillin or probenecid and cephalexin (32).

Nagaraja et al. (33), determine the imipramine hydrochloride, desipramin hydrochloride, clomipramin hydrochloride, and trimipramin meleate belonging to dibenzazepin class of the drugs. This method depends on the interaction of diazotized p-phenylene diamin dihydrochloride with the drug in sulfuric acid medium. The resulting chromphore was measured at 565 nm and was stable for about 2.5 hr.

1.4.2.1 Ultraviolet Spectrophotometric Method

This technique is used to distinguish between chemical forms, such as substituted aromatics, conjugated olefins, vitamin and aldehydes.

Ultraviolet analysis has been used to studies chemical components, such as vitamins and hormones. These measurements are used in the diagnosis of diabetes, kidney

damage, and myocardial interaction, among other ailments. In the medical field, it can be used to measure the purity of drugs during manufacture and of final product (34).

Bebawy et al. (35), used quantitative determination of cephalexin by conversion of this drug into piperazine-2, 5 dion derivatives by heating in alkaline sorbitol zinc ion solution for (10-25) min. at 90 ^{o}C and subsequent treatment of these derivatives with 0.1N sodium hydroxide to obtain highly absorbing product with λ_{max} at 345 nm at zero order absorption curve.

1.4.2.2 Derivative Method

The development of a satisfactory analytical method to measure any compound selectively in sample mixture is an important task. Often many measurement techniques are sensitive but lack selectivity toward complex samples. There has always been an interest in sensitive technique to improve the measurement selectivity. Among the most conceptually simple of these methods is the derivative spectrophotometry (36).

Derivative spectrophotometry is an analytical technique of a great utility for extracting both qualitative and quantitative information from spectra of unresolved bands.

It was introduced more than forty years ago (37), and has demonstrable advantages for the solution of specific analytical problems. 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 wave length (38). This technique improved the resolution of overlapping absorption bands against broader bands.

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 (39).

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 back ground and enhancement of spectral details (40). It can be used in quantitative analysis when it is desired to measure the concentration of obscured peak buried by large overlapping or interfering peaks.

A derivative spectrogram shows peak or valley corresponding to every infection point in the normal spectrum giving greatly enhanced resolution (41).

The principle of operation of this technique 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, ($d\lambda/dt$) is constant, then the derivative of the intensity with respect to time, dI/dt, which is measured by means of its electronic differentiation (42):

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

For a single-peak spectrum, the first derivative is a plot of the gradient $dA/d\lambda$ of the absorption envelope vs. wavelength and features a maximum and a minimum the vertical distance between these is the amplitude, which is proportional to the analyte concentration. Theoretically, $dA/d\lambda$ 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 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 (43).

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 (44):

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

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

$$D_n, A/D_n, B\ \alpha\ (W_B/W_A)^n$$

For this reason, the use of derivative spectra can increase the detection sensitivity of minor spectral feature. For quantitative analysis, if Beer's law is obeyed for the normal spectrum, the following equation can be obtained (45).

$$d^nA/d\lambda^n = d^n\varepsilon/ d\lambda^n\ Lc$$

Where A= absorbance

ε= molar absorptivity

L= cell path-length

C= molar concentration of the analyte

This forms the basis for analytical determination.

An inconvenience of the derivative techniques is that the signal-to-noise ratio (SNR) becomes worse for progressively high orders (46). Practical derivative techniques

include some degree of smoothing to control the increase in noise. The ratio of the (SNR) of the unsmoothed nth derivative $(SNR)_n$ to that of the unsmoothed normal SNR$(SNR)_0$ is given by (47):

$$(SNR)_n/(SNR)_0 = \alpha_n C_n r^{(n+0.5)}\sqrt{M}$$

Where C_n is a constant which increases with derivative order n, and α_n is the attenuation factor of the nth derivative for a smoothing ratio r. Values of $(SNR)_n/(SNR)_0$ have been calculated as a function of r. The SNR ratio of all derivatives, including the zeroth, increase with r, but tend to converge at high r values.

Therefore the effect of smoothing depends on two variables:

(a) The smoothing ratio which is the ratio of the width of the smoothed peak to the number of mass (M) of data point corresponding to the peak full width at half-maximum, and

(b) The number of times that the smoothing is done (38).

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

This technique has the following advantages; it eliminates fluctuations of the light source and results in a better S/N (48).

Derivative spectrophotometry has several applications in different fields. It has proved particularly useful in eliminating matrix interference (38).

In the medical field, it can be used for the analysis of enzyme, vitamins, hormones, and steroids (34).

Derivative spectrophotometry has been applied in the analysis of many pharmaceutical formulations (49-56).

Derivative spectroscopy extracts more information contained within a radiation intensity spectral distribution than is accessible through direct spectroscopy techniques because of the specific measurement of the derivatives of that distribution (57).

The first derivative spectrophotometric method has been established for the determination of either cephalexin or cephradine in human urine (34).

Because of their importance in therapy, the determination of some drugs in analytical problem in the quality control of the pharmaceutical industry. DS is an analytical technique of great utility for resolving drug mixtures with overlapping spectra.

Moreover, DS has been applied successfully to the determination of the drugs in the presence of their degradation products.

In determination of an individual drug, drug additives and drug decomposition both interfered. Therefore, utilizing DS for assay of individual drug allowed elimination of undesirable interferences as compared with normal spectrophotometry (58).

Riyadh (59), used first derivative absorption spectra to the determination of atenolol in two pharmaceutical drugs. Nevado et al. (60), used the determination of furaltadone and chloramphenicol in two pharmaceutical products were possible using first derivative spectrophotometry. Bedair et al. (60) have been proposed first and second derivative pectrophotometric methods for the determination of glibenclamid, mebeverine hydrochloride and clopamide.

1.5 Aim of the work:

The aim of this work was to use the derivative spectrophotometric method for the determination of cephalexin in pharmaceutical formulation.

The cephalexin absorption spectrum overlapped with the spectra of some metals, which made it difficult to measure without physical or chemical separation. The use of derivative spectra may solve this problem.

The method was involved determination of the drug in the presence of some metals.

Chapter One

Introduction

Chapter Three

Results & Discussion

Chapter Two

Experimental Part

The determination of cephalexin using UV absorption spectrophotometry was studied in this work. The UV spectrum of cephalexin has shown an absorption maximum at 262nm with molar absorptivity of 7880.6 $L.mol^{-1}. cm^{-1}$. The calibration curves was obtained using range of concentration from (1.5-100) ppm at wavelength 262nm Fig. 10 shows the linear calibration curve for cephalexin at this wavelength.

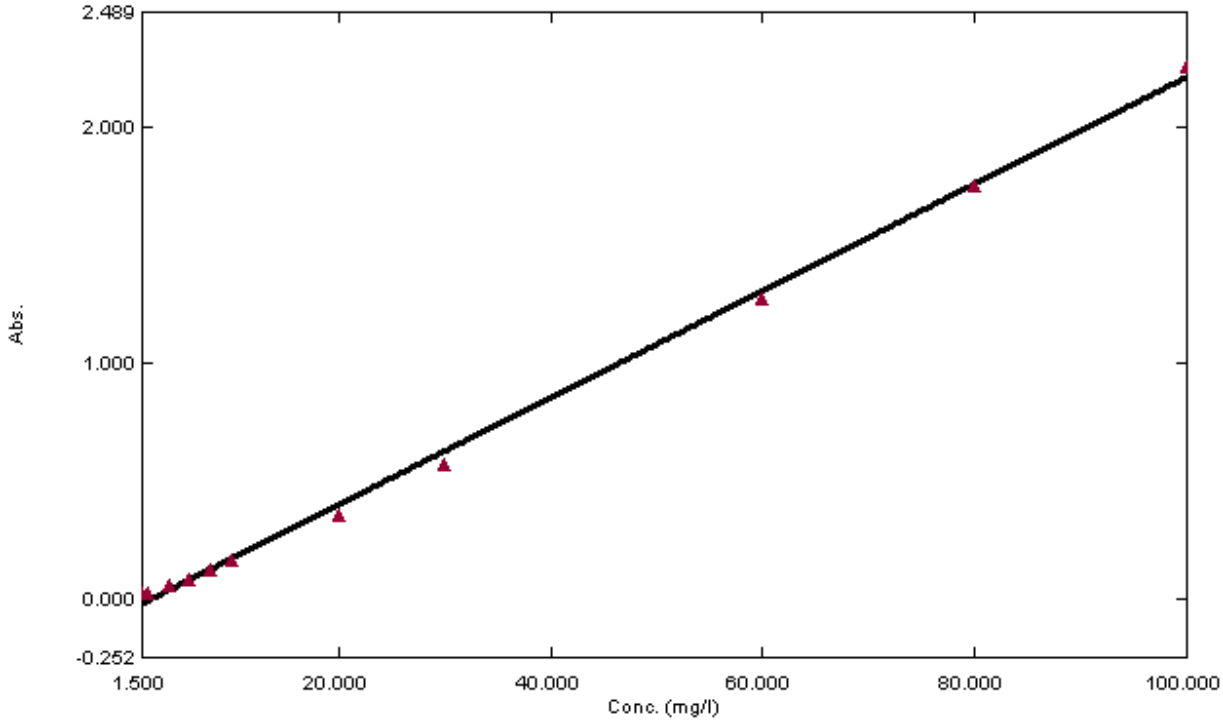

Fig. 11. Calibration curve of normal spectrum of cephalexin at 262nm.

A linear equation has been obtained in the range from (1.5-100) ppm, with correlation coefficient (R) of 0.9993. The slope of the linear calibration curve was 0.02154.

The absorbance of these solutions were measured. The concentrations were calculated using the linear equation. It was found that the relative error was averaged -0.61% and the recovery was averaged of 98.74% as listed in Table 1.

Table 1. Calibration curve parameters

λ_{max}, nm	**262**
Linearity range (ppm)	**1.5-100**
Reg. Eq. Y= | ax+b |	**Y=0.02155X-0.03398**
Corr. Coef. (r)	**0.9993**
RSD%	**±0.91**
Error%	**-0.61**
Recovery%	**98.74**

The utility of the method was tested on three different manufactures for cephalexin capsules; the result found in cephalexin capsules is shown in Table (2).

Table 2. Determination of cephalxin in capsules using normal spectrophotometer.

Pharmaceutical	Samara	Aki	India
Listed (g)	**0.5**	**0.25**	**0.5**
measured (g)	**0.5265**	**0.273**	**0.5438**
Error%	**2.65**	**2.3**	**4.38**

The effect of pH on the determination of cephalexin by normal spectrophotometer was investigated. The absorbance at 262 nm as a function of pH for solutions containing 40ppm cephalexin is shown in Fig.11. The Fig. shows no major influence of pH in the determination of cephalexin especially at pH above 4.5 which is the natural value of pH of cephalexin solution.

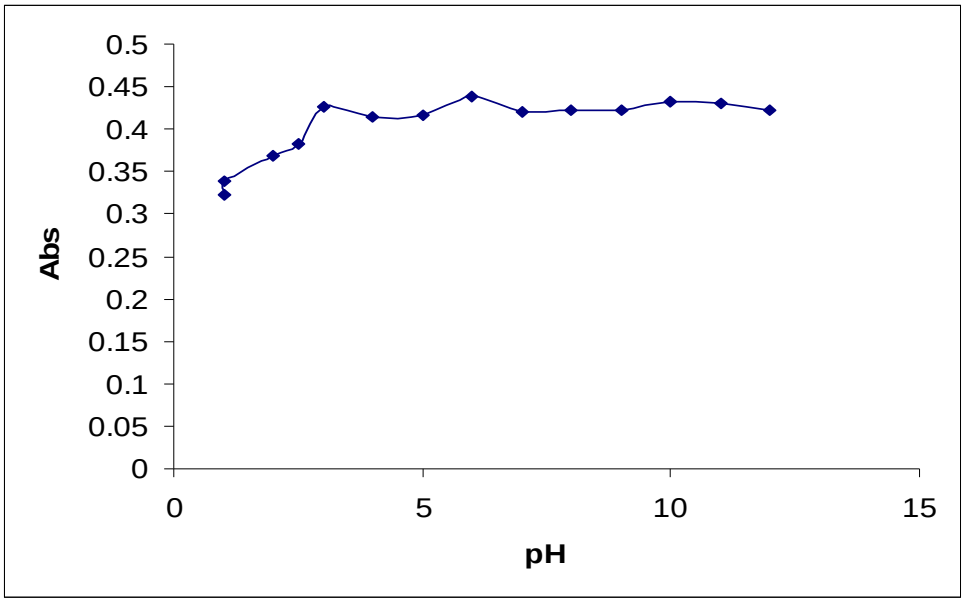

Fig. 12. Effect of pH at 262nm.

The effect of interferences of some metal ions such as sodium (I), potassium (I), cupper (II), magnesium (II), calcium (II) and iron (III), which may be present in human blood, on the determination of cephalexin was also studied. The absorbance of series of standard solutions containing different amounts of interfering metals ion with fixed concentration of cephalexin were measured. The results are listed in Table (3).

These results indicated that sodium (I), potassium (I) , calcium (II), magnesium (II) ions have no apparent effect on the determination of cephalexin at 262 nm wavelength.

Ferric (III) and cupper (II) ions have shown a high interference on determination of cephalexin at this wavelength as illustrated from overlapped spectra shown in Fig. 12.

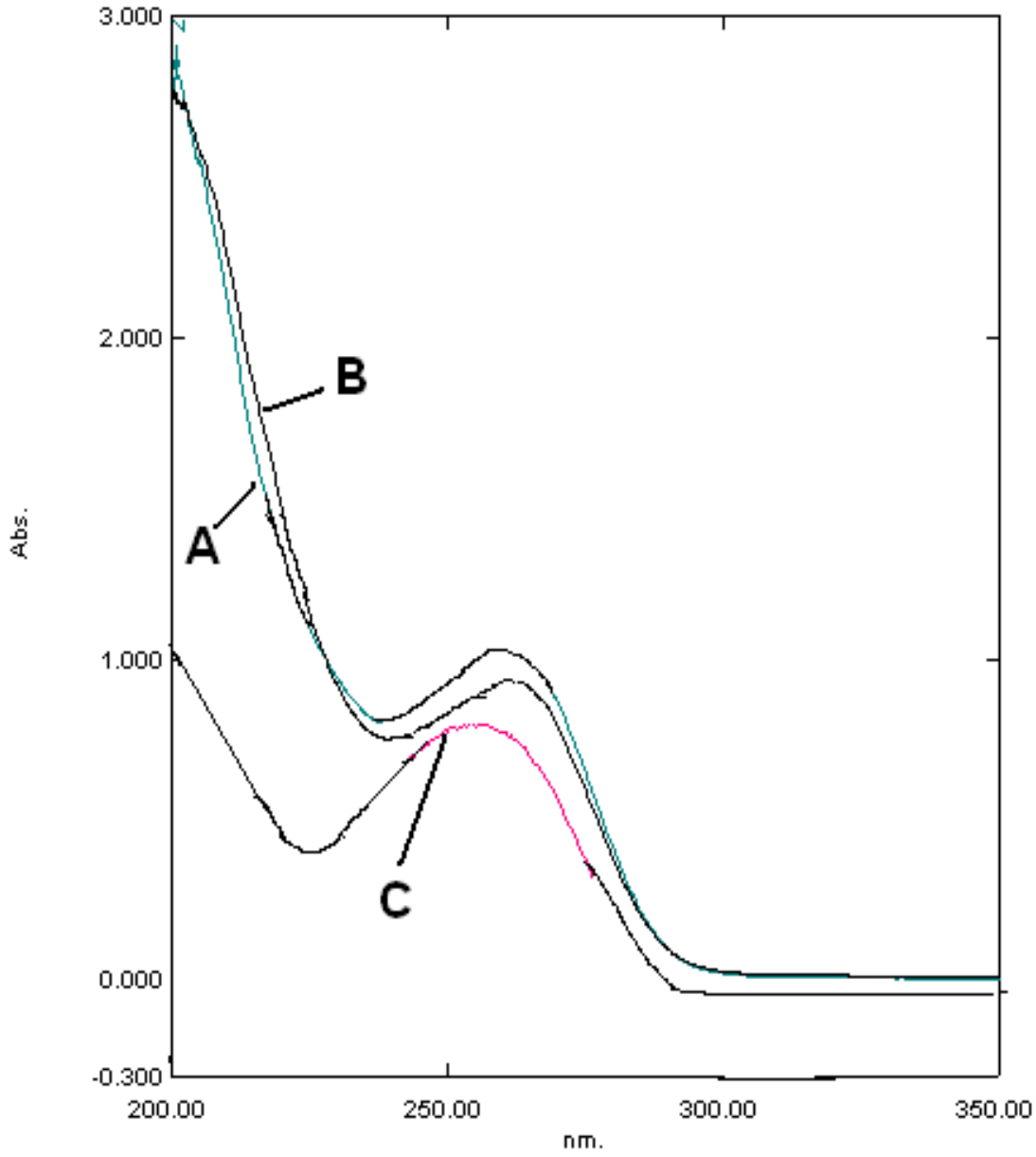

Fig. 13. Spectrum of A) Cephalexin, B) Cephalexin + Cu^{2+} , C) Cephalexin+ Fe^{3+}

Table 3. Effect of interfering material on the determination of 100 ppm cephalexin.

Interfering ions	Conc. of interfering ions (ppm)	Error%
Na^+	2-80	-٠,٦٣
K^+	2-10	-١,٤٣
Mg^{2+}	2-10	-٢,١٣
Ca^{2+}	2-10	-١,٠٥
Fe^{3+}	2-40	+٩,٧٩
Cu^{2+}	2-50	+٦,٤

It is found wide range of concentrations of interfering ions (Na^+, K^+, Mg^{2+}, Ca^{2+}) do not effect on the determination of cephalexin (at 100ppm).

These results indicated that Cu^{2+}, Fe^{3+} have a large influence on the determination of cephalexin at 262nm, with relative error greater than 5%.

Therefore, the concentration of cephalexin can not be determined by direct absorbance measurement in the presence of Cu^{2+}, Fe^{3+}. The derivative spectrophotometric method was studied to assist in solving this problem.

Second order for cephalexin to be determined in the presence of Cu^{2+}, Fe^{3+}, was found suitable.

Changing the number of derivatized points of the first (D1), second (D2), and third (D3) absorption spectra has shown a large effect on the shape of the spectra. At low delta value a noisy generated spectra was obtained. However at high delta value degradation of spectra points was the result. The best result was obtained at delta 4.

The best wavelength chosen for the complex cephalexin with Fe^{3+} for the first, second, and third derivative spectra were at 317nm, 337nm, 291nm, respectively, because they did not measure during the derivatizing Fe^{3+} spectrum**.**

The best wavelength chosen for the complex cephalexin with Cu^{2+}, for the first, second, and third derivative spectra were at 285nm, 238nm, 222nm, respectively, because they did not measure during the derivatizing Cu^{2+}spectrum**.**

.

3.1. Determination of Cephalexin in presence Fe^{3+}

The determination of cephalexin in presence of Fe^{3+} can be measured by derivative spectrophotometry in the linear range 2-50 ppm for 317 nm, 337 nm and 291 nm, as shown in Fig. 14, 15, 16, respectively.

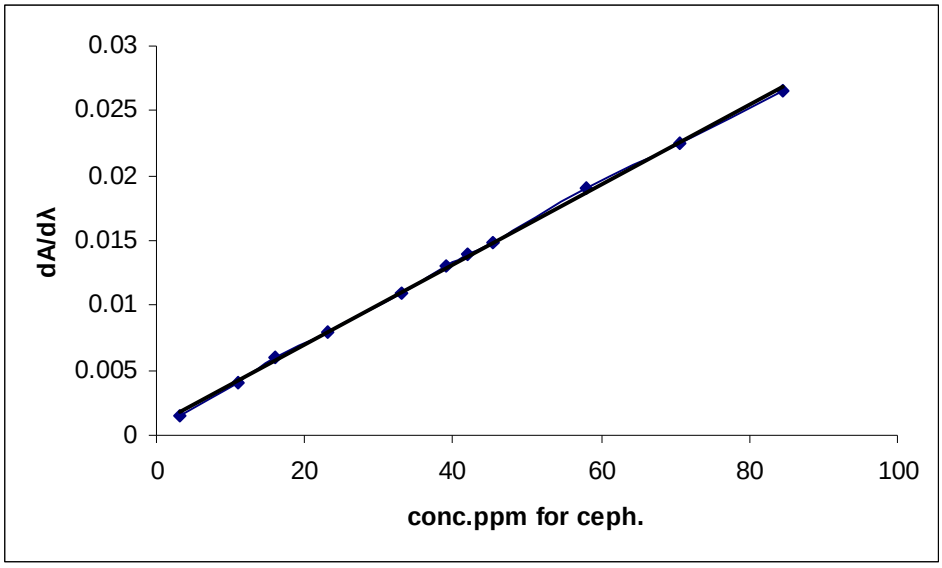

Fig.14. Calibration curve of cephalexin using first derivative spectrophotometry in presence of Fe^{3+} at 317 nm

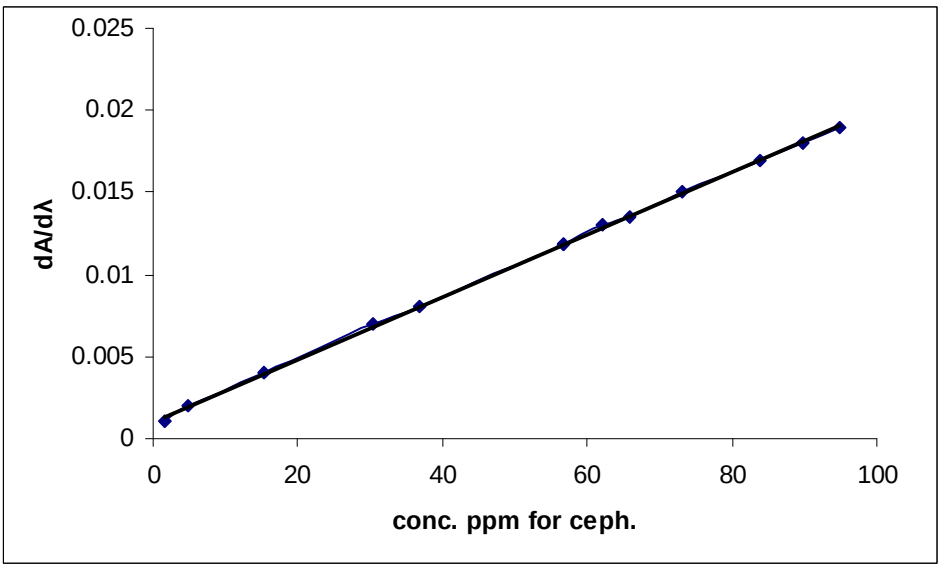

Fig.15. Calibration curve of cephalexin using second derivative spectrophotometry in presence of Fe^{3+} at 237 nm

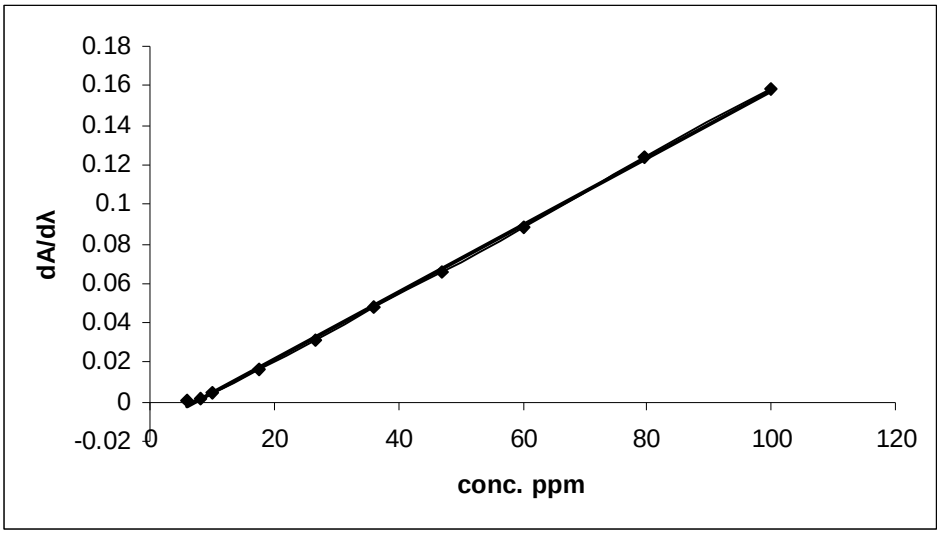

Fig.16. Calibration curve of cephalexin using third derivative spectrophotometry in presence of Fe^{3+} at 291 nm

The linear relationship between amplitude of the D1, D2, D3 spectrum and concentration of cephalexin at 317, 337 and 291 nm respectively.

A comparation between some parameters using these three derivative orders is shown in table 4.

Table 4. Calibration curves of cephalexin in presence Fe^{3+} as interference using derivative methods

Derivative order **λmax, nm**	**D1** **317**	**D2** **337**	**D3** **291**
Linearity range (ppm)	1.5-100	2-100	4-100
Reg. Eq. **Y= | aX+b |**	Y= 0.0017x - 0.013	Y= 0.0003x + 0.0007	Y=0.0002x + 0.001
Corr. Coef. (r)	0.9993	0.9996	0.9990
RSD%	±1.38	±0.32	±3.47
Error%	+2.2	-1.78	-4.26
Recovery%	103.36	98.45	96.67

A linear equation was obtained from (1.5-100), (2-100) and (4-100) ppm, relative standard deviation (RSD) and recovery of the linear calibration curve using first, second

and third derivative absorption spectra are Y= 0.0017x - 0.013, Y= 0.0003x + 0.0007, Y=0.0002x + 0.001; 1.38, 0.32, 3.47; and 103.36, 98.45, 96.67, respectively. From the above parameters it seems that the signal measurement using second derivative spectrum at 337 nm would give better results than the measurement using first and third derivative spectra at 317 nm and 291 nm, respectively.

The applicability of the derivative method has been appraised through the assay of cephalexin in three different manufacturers of capsules, and results are listed in Table (5).

The commercial available of cephalexin (Samara, Aki, India), that used locally to control wide range, were tested using UV spectrophotometric method, with derivative techniques.

Table 5. Calibration curves of cephalexin in capsules with Fe^{3+} using derivative methods

Pharmaceutical	Samara			Aki			India		
D, order	D1	D2	D3	D1	D2	D3	D1	D2	D3
Listed (ppm)	100	100	100	100	100	100	100	100	100
Measured(ppm)	98.2	95.5	102.9	117.7	101.8	101.2	111.8	100.1	102.2
Error%	+1.8	+4.5	-2.9	-17.7	-1.8	-1.2	-11.8	-0.1	-2.2

3.2. Determination of Cephalexin in presence Cu^{2+}

The determination of cephalexin in presence of Cu^{2+} can be measured by derivative spectrophotometry in the linear range 2-50 ppm at 285 nm, 238 nm and 222 nm, for first, second and third respectively as shown in Fig. 17, 18, 19, respectively**.**

The linear relationship between amplitude of the D1, D2, D3 spectrum and concentration of cephalexin at 285, 238, 222 nm respectively.

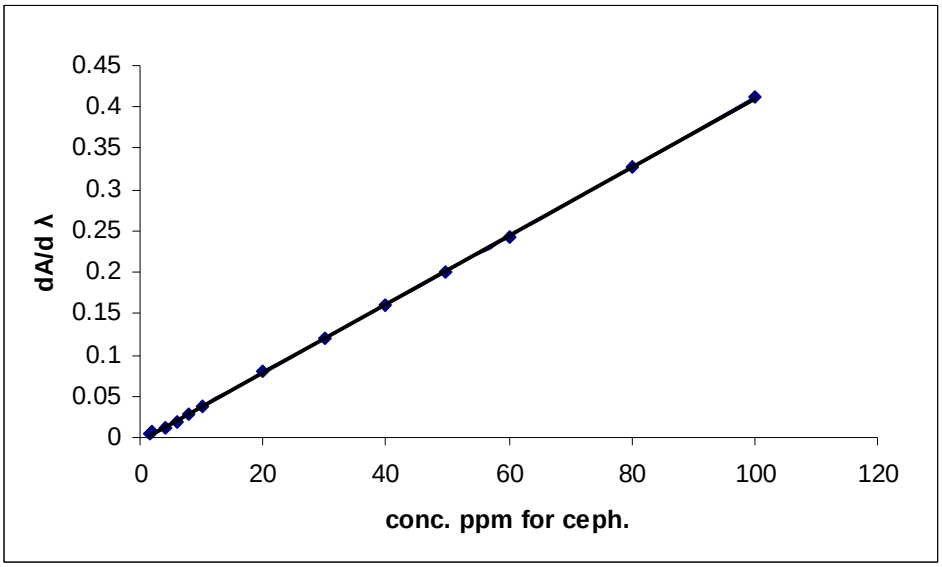

Fig.17. Calibration curve of cephalexin using first derivative spectrophotometry in presence of Cu^{2+} at 285 nm

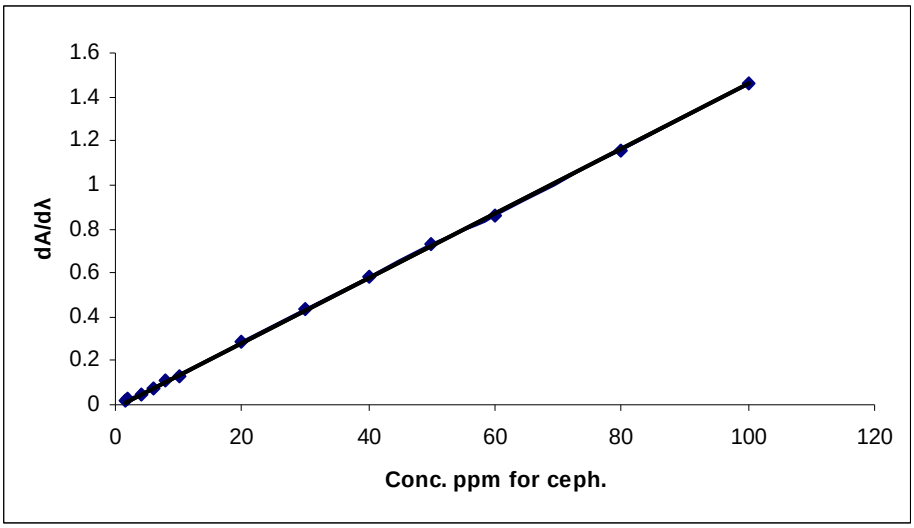

Fig.18. Calibration curve of cephalexin using second derivative spectrophotometry in presence of Cu^{2+} at 238 nm

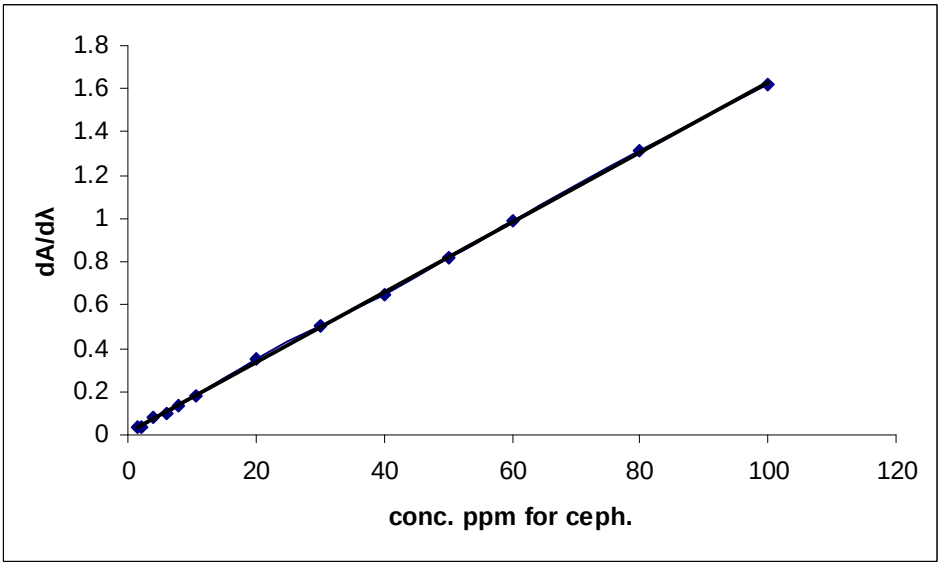

Fig.19. Calibration curve of cephalexin using third derivative spectrophotometry in presence of Cu^{2+} at 222 nm

The linear relationship between amplitude of the D1, D2, D3 spectrum and concentration of cephalexin at 285, 238 and 222 nm respectively.

A comparation between some parameters using these three derivative orders is shown in Table 6.

Table 6. Calibration curves of cephalexin in presence Cu^{2+} as interference using derivative methods

Derivative order **λmax, nm**	**D1** **285**	**D2** **238**	**D3** **222**
Linearity range (ppm)	1.5-100	2-100	4-100
Reg. Eq. **Y=|aX+b|**	Y=0.0151X-0.0226	Y=0.0145X-0.0347	Y=0.0042X-0.012
Corr. Coef. (r)	0.9992	0.9998	0.9994
RSD%	±3.22	±0.57	±4.47
Error%	+2.3	-1.46	-2.38
Recovery%	102.03	97.33	94.70

A liner equation was obtained from (1.5-100), (2-100) and (4-100) ppm, the slope , relative standard deviation (RSD) and recovery of the linear calibration curve using first, second and third derivative absorption spectra are Y=0.0151X-0.0226, Y=0.0145X-0.0347, Y=0.0042X-0.012; 0.0151, 0.0145, 0.0042; 3.22, 0.57, 4.47; and 102.03, 97.33, 94.70,

respectively. From the above parameters it seems that the signal measurement using second derivative spectrum at 238 nm would give better results than the measurement using first and third derivative spectra at 285 nm and 222 nm, respectively.

The applicability of the derivative method has been appraised through the assay of cephalexin in three different manufacturers of capsules, and results are listed in Table (7).

Table 7. Calibration curves of cephalexin in capsules with Cu^{2+} using derivative methods

Pharmaceutical	Samara			Aki			India		
D, order	D1	D2	D3	D1	D2	D3	D1	D2	D3
Listed (ppm)	100	100	100	100	100	100	100	100	100
Measured(ppm)	97	94.4	104.1	109.3	99.8	101.3	105.2	101.4	101.6
Error%	+3	+5.6	-4.1	-9.3	+0.2	-1.3	-5.2	-1.4	-1.6

No effect of pH on the determination of cephalexin in presence of the Fe^{3+} and Cu^{2+} ions using the derivative method because the pH of cephalexin is the same pH of this solution.

The effect of Fe^{3+} on the determination of cephalexin by these derivative methods was investigated, and the results are shown in Table (8).

Table 8. Effect of interfering Fe^{3+} on the determination of 100 ppm cephalexin using derivative methods.

Interfering Metal	Conc. Of Interfering Metal, ppm	Error%		
		Derivative order, λmax nm		
		D1,317	D2,337	D3,291
Fe^{3+}	2	+2.3	+4.39	-3.61
	4	-0.21	+1.69	-8.61
	6	-1.31	+0.49	+2.29
	8	-3.21	-0.41	+2.19
	10	-4.01	-1.91	+1.79
	20	-6.11	-7.11	+5.69
	30	-11.81	-10.51	+6.3
	40	-12.41	-11.31	+7.19
	50	-17.11	-17.11	+7.78

Table (8) shows that there is no effect of Fe^{3+} on cephalexin determination using second derivative at 337 nm except above of 20 ppm Fe^{3+}.

Table 9. Effect of interfering Cu^{2+} on the determination of 100 ppm cephalexin using derivative methods.

Interfering Metal	Conc. Of Interfering Metal, ppm	Error%		
		Derivative order, λmax nm		
		D1,285	D2,238	D3,222
Cu^{2+}	2	+0.8	-0.5	+1.8
	4	+0.9	-1.7	-0.9
	6	+0.2	+4.0	+2.7
	8	-3.1	+9.5	+5.4
	10	-4.3	+10.2	+9.5
	20	-4.9	+11.4	+13.4
	30	-5.1	+13.2	+15.1
	40	-6.7	+16.1	+17.0
	50	-8.4	+19.8	+25.2

Table (9) shows that there is no effect of Cu^{2+} on cephalexin determination using second derivative at 238 nm except above of 8 ppm Cu^{2+}.

Conclusion

The normal absorption spectrophotometry was found not applicable in the determination of cephalexin in the presence of some metals such as Cu^{2+}, Fe^{3+} because of the overlapped spectra of these metals with cephalexin.

Derivative absorption spectrophotometry was found to be a convenient technique for determing the cephalexin in the presence of these metals over awide range of their concentration at pH 6.7.

Second derivative spectrum at 337, 238 nm for Fe^{3+} and Cu^{2+}, respectively, was chosen for the determination of cephalexin in the presence of some metal ions and in the commercial drugs.

Derivative methods give good results in the determination of cephalexin.

Suggested for Future Work

1- Study the effect of other drugs such as cloxacillin and amoxicillin on cephalexin determination using derivative absorption spectrophotometry.

2- Determination the degradiation of cephalexin in human serum using derivative and other analytical separation techniques such as HPLC**.**

3- Utilization of derivative absorption spectrophotometry for the determination of the other drugs.

4- Study of the interaction of cephalosporin and penicillin with separated by derivative spectrophotometry.

Examining Committee's Certification

We, the Examining Committee, certify that we read this thesis and have examined the student (**Dalia Mahmoud Jamil)**, in its contents and that, in our opinion; it is adequate as a thesis for the Degree of Master of Science, in Chemistry.

Chairman

Signature:

Name: **Dr. M.I. Al-Joboury**

Date:

Member

Signature:

Name: **Dr. Nabil S. Nassori**

Date:

Member

Signature:

Name: **Dr. Emad A. Yousif**

Date:

Member& Supervisor

Signature:

Name: **Dr. Shahbaz A.Maki**

Date:

Approved for the College Committee of Graduate Studies

Signature:
Name: **Assist. Prof. Dr. LAITHABDUL AZIZ AL-ANI**
Address: Dean of the College of Science AL-Nahrin University
Date:

CHAPTER TWO

EXPERIMENTAL PART

2-1 Instruments and Equipments

The instruments used in this work are:

1- Double-beam UV –Visible spectrophotometer model (UV-1650 PC) Shimadzu/ (Japan), interfaced with computer via a shimadzu UV-probe data system program.

2- pH meter Orion expandable ion analyzer model (EA 940) equipped with a glass combination electrode.

2-2 Chemicals

- **Cephalexin monohydrate standard was a gift from the State**

Company for Drug Industries and Medical Appliances (Samara-IRAQ-SDI).

2-3 Preparation of standard solutions

1- Standard solution of (100 ppm) cephalexin was prepared by dissolving 0.01 g in a small amount of deionized water and then diluted to 100 ml with deionized water.

More diluted solution was prepared by subsequent dilution of the stock solutions to 80, 60, 50, 40, 30, 20, 10, 8, 6, 4, 2, 1.5 ppm cephalexin solution.

These solutions were kept in cold and dark place until use; they were stable under these conditions.

2- Solutions of 40 ppm were prepared at different pH solution ranging from (1 to 12). The spectra of these solutions were scanned from 200-400 nm.

3- Standard solutions of 1.5 to 100 ppm cephalexin monohydrate were prepared at pH= 6.7 using phosphate buffer, the absorbance of these solution were measured at 262nm.

4- Stock solutions of 100 ppm sodium(I), potassium(I), ferric(III), magnesium(II), calcium(II) and cupper(II) ions were prepared by dissolving 0.025 g sodium chloride, 0.019 g potassium chloride, 0.03 g ferric chloride, 0.084 g magnesium chloride and 0.024 g calcium chloride, 0.025 g cupper sulfate, respectively, and diluted to 100 ml deionized water. Other standard solutions for the above ions were prepared from the stock solutions.

2-4 Preparation of pharmaceutical samples

2-4-1 CAPSULES SAMPLES:-

Two types of capsules were used to determine the concentration of cephalexin:-

1- Samara: - (cephalexin capsules BP 250mg), one pill of this capsule was grinded and dissolved in deionized water and completed in volumetric flask to (100ml) and the absorbance was measured for this solution.

2- India: - APKEF-250 (cephalexin capsules BP 250mg), ajanta pharma limited; factory: 31-0,MIDC area, Chikalthana,Aurangbad 431210, Charkhop, Kandivli(W), Mumbai 400067: one pill of this capsule was grinded and dissolved in deionized water and complete in volumetric flask to (100ml) and the absorbance was measured for this solution.

٣- Aki:- (cephalexin capsules BP 100mg), Ajanta pharma limited; one pills of this capsule was grinded and dissolved in deionized water and complete in volumetric flask (100ml) and the absorbance was measured for this solution .

The cephalexin spectrum was then derivatized by the instrument microprocessor to the first, second and third orders. The derivatized points (delta) were then changed from (1-8) for each order.

Delta 1 represents derivatizing all successive points, while delta 8 take the different between 9^{th} point and 1^{st} point and so on.

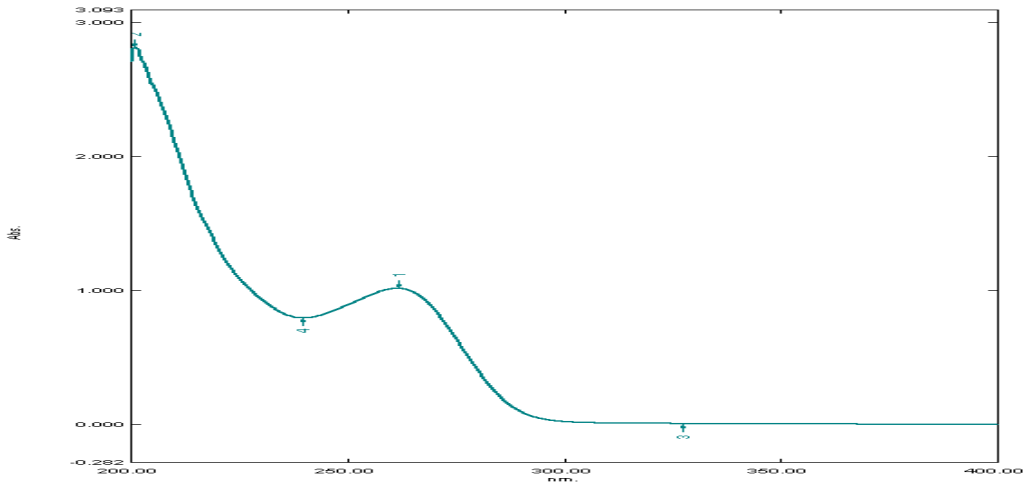

Fig.2. spectrum of 50 ppm Cephalexin using Normal Spectrophotometer

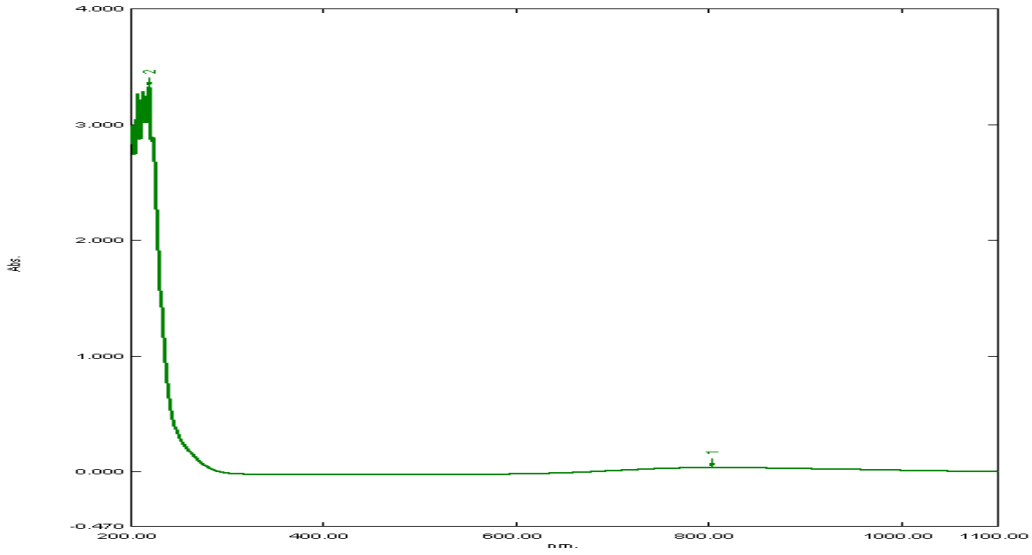

Fig.3. spectrum of 100 ppm Cu^{2+}

The wavelength of Cu^{2+} only is present in 816nm, while its complex with cephalexin, the wavelength is shifted to the lower wavelength.

Figures 4, 5 and 6 shows the first, second and third derivative for Cu^{2+} ion and cephalexin (curve A for cephalexin and curve B for Cu^{2+} ion).

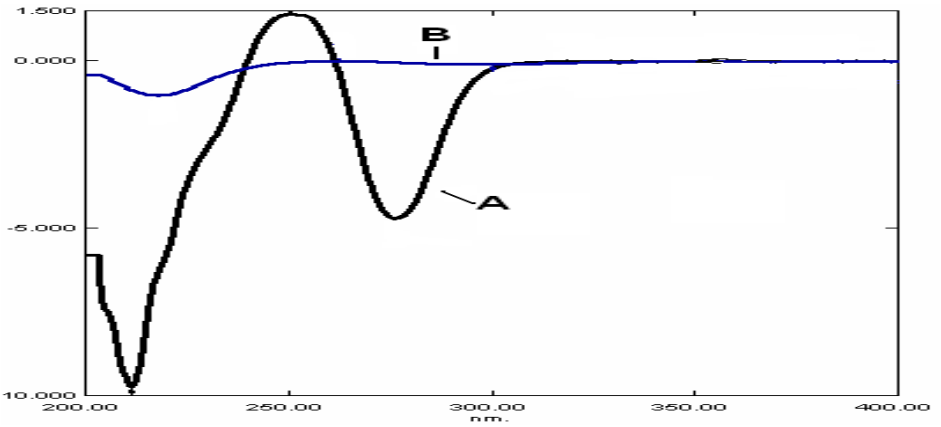

Fig.4. First derivative spectra for (A) 50 ppm Cephalexin (B) 50 ppm Cu^{2+}

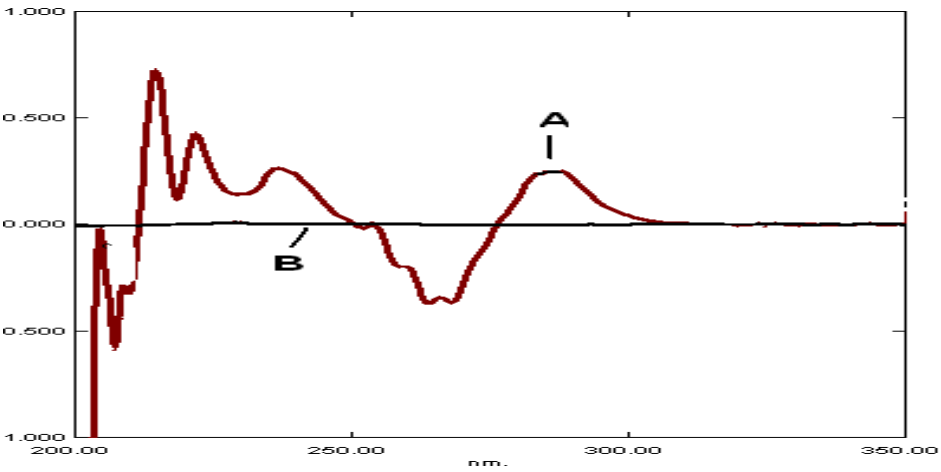

Fig.5. Second derivative spectra for (A) 50 ppm Cephlexin (B) 50 ppm Cu^{2+}

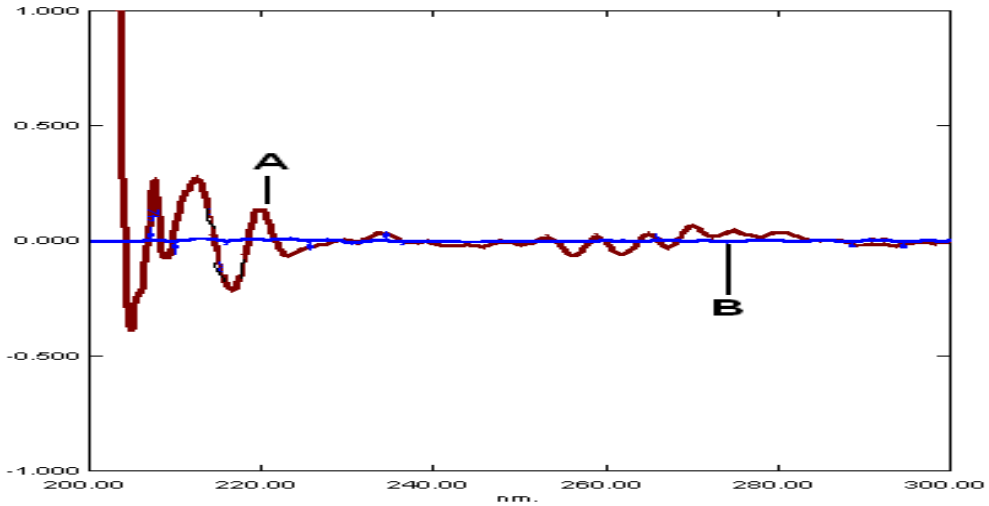

Fig.6. Third derivative spectra for (A) 50 ppm Cephlexin (B) 50 ppm Cu^{2+}

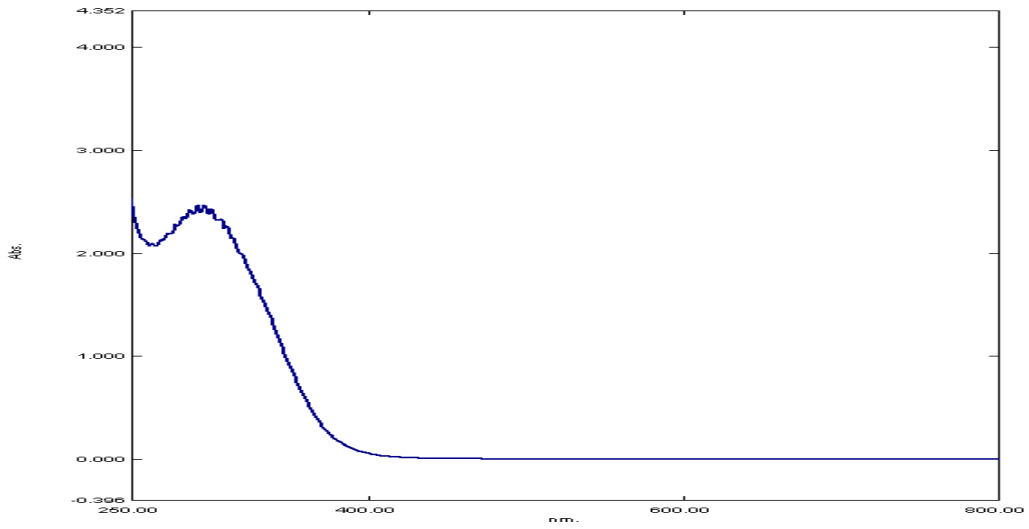

Fig.7. spectrum of 100 ppm Fe^{3+}

The wavelength of Fe^{3+} only is present in 292 nm; its complex with cephalexin, the wavelength is shifted to the lower wavelength.

Figures 7, 8 and 9 shows the first, second and third derivative for Fe^{3+} ion and cephalexin (curve A for cephalexin and curve B for Fe^{3+}ion).

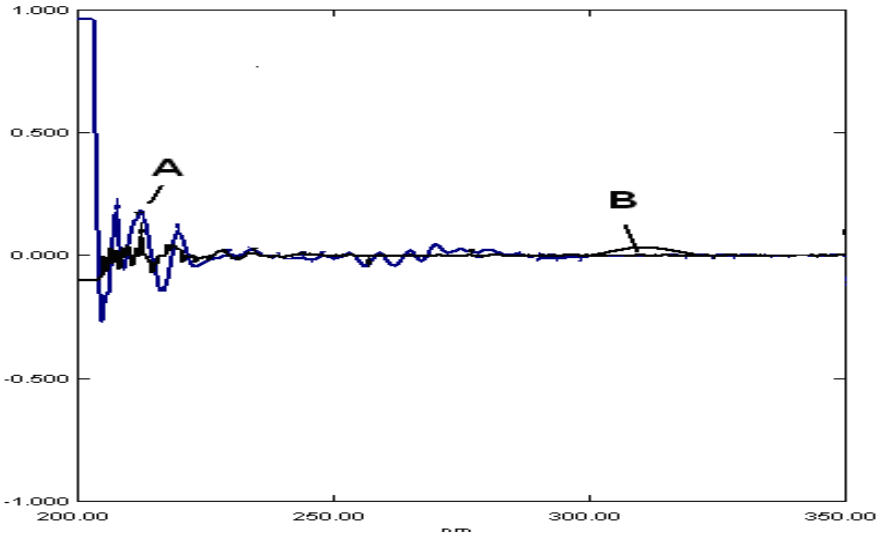

Fig.8. First derivative spectra for (A) 50 ppm Cephalexin (B) 50 ppm Fe^{3+}

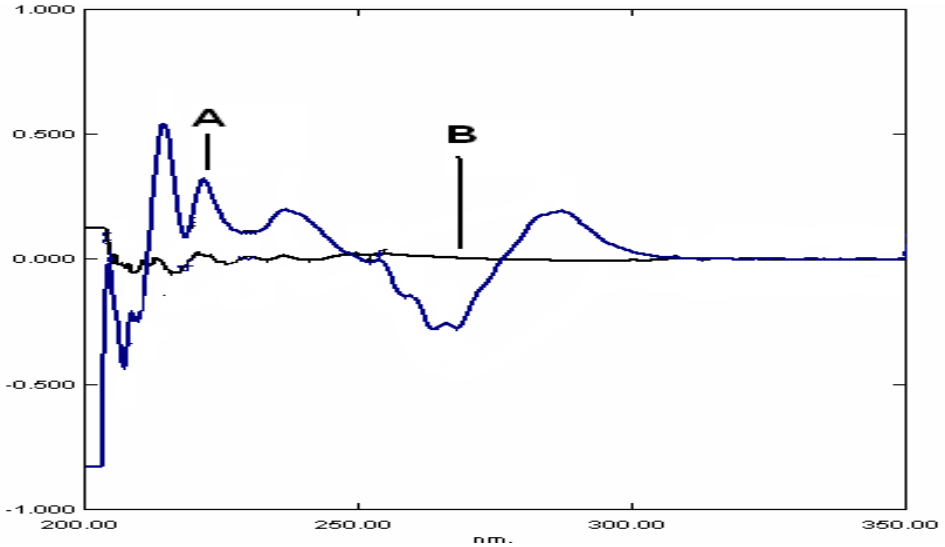

Fig.9. Second derivative spectra for (A) 50 ppm Cephlexin (B) 50 ppm Fe^{3+}

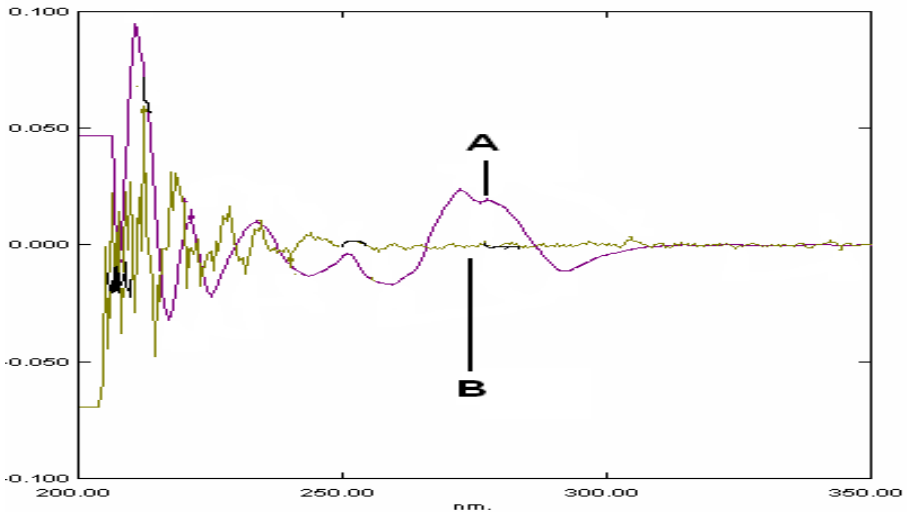

Fig.10. Third derivative spectra for (A) 50 ppm Cephlexin (B) 50 ppm Fe^{3+}

List of Figures

List of Figures

List of Tables

References

[1] Henry S., Peter M., and Stephen H. Buhner; Antibiotic resistance, June **Vol**. 68, No. 9, 2005.

[2] Els D., Hendrik D. Ruyck, and Roland V. Renterghem; Confirmatory Assay for the Simultaneous Detection of Penicillin and Cephalosporin in Milk using LC/MS-Ms {e.daeseleire@clo.fgov.be}

[3] Douglas M. Considine; Chemical and Process Technology Encyclopedia, **Vol.** 55, No. 125, 1974.

[4] Center for Disease Control and prevention {WWWonder.ccd.gov}.

[5] European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infections Diseases (ESCMID); **Vol**. 4, May 2003.

[6] Motta R.N., Oliveira M.M., Maglhaes P.S., Dias A.M., Aragao L.p.; Brazilian Journal of Infections Diseases, **Vol**. 7, No. 2, 2003.

[7**]** Antibiotics **{**Medicine Consumer Health}, 2006.

[٨] Andrejus Korolkovas; Essentials of Medicinal Chemistry, 2**nd** edition, **Vol**.778, 1988.

[٩] "European Pharmacopoeia on CD-ROM", 3rd ed., 1998.

[١٠] British Pharmacopoeia, **V**ol. 1, 1993.

[1١] "Martindale the extra-pharmacopoeia on CD-ROM", Royal Pharmaceutical Society, 1996.

REFERENCES

[١٢] The Pharmaceutical Codex: Principles and Practice of Pharmaceutics, 12th ed., the Pharmaceutical Press, London, 1994.

[١٣] Jay Marks., M.D., and Omudhome Ogbru; Medicine Net.com, **Vol.**30, 2004.

[١٤] National Committee for Clinical Laboratory Standards: Methods for Dilution antimicrobial susceptibility tests for bacteria that grow Aerobically; 3rd edition, **Vol**. 13, No. 25, 1993.

[١٥] "The Merk Index on CD-ROM", Copyright by Merk Co., Inc., White ho., USA, 1999.

[١٦] Van't Riet K, Tramper J.; and basic bioreactor design, New York: Marcel Dekker, **Vol**. 15, 1991.

[١٧] Simerville, M.D., William C. Maxted, M.D., and John J. Pahira, M.D.; American Family Physician, **V**ol. 71, No. 6, 2005.

[18] Welling PG, Selen JG, Kwok-F, Johnson CA; Pharmacokinetic Comparison of Cephalexin and Cephadroxil using HPLC assay Procedures; **Vol.** 6, No.147-157, 1996.

[19] Barbhaiya RH; Department of Metabolism and Pharmacokinetics, 1996.

[20] Laurel A. Elisenhauer, Lynn W. Nichols, Roberta T. Spencer, and Frances W. Bergan; Clinical Pharmacology and Nursing management, 5th edition, No. 87, 1998.

[21] Hefnawy M., EL- Shabrawy Y, and Belal F; Journal of Pharmacology Biomedicine Analysis, **V**ol. 21, No. 4, 1999.

REFERENCES

[22] Delyle East wood and Russell L. Lockheed; "Environmental Systems And Technologies", **V**ol. 6, No. 211- 215, 1994.

[23] Basae L. and Vanysek P.; J. of Biomedicine Analysis, **V**ol. 19, No. (1-2), 1999.

[24] AL-Momani I.; J. of Pharmacology Biomedicine Analysis, **V**ol. 25, No. (5-6), 2001.

[25] Meyer U., Zhi Z., Loomans E. and Spener F; Analyst, **V**ol. 124, No. 11, 1999.

[26] Gallo M., Campins F., Sevillano C.; J. of Pharmacology Biomedicine Analysis, **V**ol. 29, No. 3, 2002.

[27] Ptyde, A., and Gibert,"Application of HPLC", Chapman and Hall, London, 1979.

[28] Tamai, I., H.Y.Ling, S.M. Timbul, J. Nishikido, and A. Tsuji; stereospecific absorption and degradiation of cephalexin, J.Pharm. Pharmacol; **V**ol. 40, No. 320-324, 1988.

[29] Jian Li., Robert W. and Roger L., "Antimicrobial Agents and Chemotherapy" **Vol**. 47, No. 4, 2003.

[30] Thomas P., Zelalem T. and Robert E., "Clinical Chemistry" Vol. 45, No. (1465-1476), 1999.

[31] Prasad-BP; Rao-A, Kumar-Y, Mathur-SC and Rathore-YK, spectraophotometric estimation of Probencid- ampicillin and Probenecid- cephalexin in combined dosage form; Central Indian Pharmacopoeia; **V**ol. 32, No. 451-453, 1995.

REFERENCES

[32] Nagaraja P., Silwadi MF. and Syed AA.; Selective Spectrophotometric of some dibenzazepine drugs, Vol. 33, No. 2913-2926, 2000.

[33] James W. Robinson; Undergraduate Instrumental Analysis, 4th edition 1987.

[34] Bebawy Li., Kelani K. and Fttah LA., "Spectrocopy"; **Vol**. 30, No. 331-343, 1997.

[35] Chadburn B.P., "Anal. Proc., **V**ol. 19, No. 42, 1982.

[36] Morrison, J. D., J. Chem. Phys., **V**ol. 21, No. 1767, 1953.

[37] Rojas, F.S., Ojeda, C.B. and Pavon and J.M.; Talanta, **V**ol. 35, No. 753, 1988.

[38] O'Haver, T.C., Clin. Chem., **V**ol. 25, No. 1548, 1979.

[39] Cottrel, C.T., Anal. Proc., **V**ol. 19, No. 43, 1982.

[40] Fix, G.L., and Pollak, D.J., Anal. Chem., **V**ol. 52, No. 1589, 1980.

[41] O'Haver, T.C., Anal. Chem., **V**ol. 51, No. 91A, 1979.

[42] Hager, R.N., Anal. Chem., **V**ol. 45, No. 1131, 1973.

[43] Fell, A.F. and Smith, G., Anal. Proc., **V**ol. 19, No. 28, 1982.

[44] Fell, A.F., Jarvie, D.R., and Stewart, M.J., Clin. Chem., **V**ol. 27, No. 286, 1981.

[45] O'Haver, T.C., Anal. Proc., **V**ol. 19, No. 22, 1982.

[46] O'Haver, T.C. and Begley, T.; Anal. Chem., **V**ol. 53, No. 1876, 1981.

[47] Hager, R. N., Anal. Chem., **V**ol. 45 No. 1131A, 1975.

[48] O'Haver, T.C., Clin. Chem., **V**ol. 25(9), No. 1548-53, 1979.

[49] Gursoy, A. and Senyucel, B.; J. Microencapsulation, **V**ol; 14, No. 769, 1997.

REFERENCES

[50] Nuran, O. and Aysegul, K.; J. Pharm. Biomed. Anal., **V**ol. 16, No. 337, 1997.

[51] Hassan, S. M. and Davidson A. G.; J. Pharm. Phrmacol., **V**ol. 36, No. 7, 1984.

[52] Kitamura, K. and Majima R.; Anal. Chem., **V**ol. 55, No. 54, 1988.

[53] Such, V., Traveset, J. and Gonzalo, R.; Anal. Chem., **V**ol. 52, No. 412, 1980.

[54] Davidson, A.G. and Elsheik, H.; Analyst, **V**ol. 107, No. 879, 1982.

[55] Lawrence, A.H. and MacNeil, J.D.; anal. Chem., **V**ol. 54, No. 2385, 1982.

[56] Abdel-Hamid M., Mahrous Ms., Daabees HG. Beltagy YA.; Journal Of Clin. Pharm. Ther., **V**ol. 17, No. 2, 1992.

[57] Christenson, R.H. and McGlothin C.D.; Anal. Chem., **V**ol. 54, No. 2015, 1982.

[58] Riyadh M.; "Derivative Absorption Spectrophotometery for determination of Atenolol in Pharmaceutical Drugs"; 2001.

[59] Nevado, J.J., Flores, J.R. and Pardo, M.L.; Fres. J. Anal. Chem., **Vol.** 349, No. 756, (1994).

[60] Bedair, M.M., Korany, M.A., Ebdel-Hay, M.A. and Gazy, A.A.; Analyst, **Vol**. 115, No. 449, (1990).

References

Contents

Contents

Chapter Two: Experimental Part

Chapter Three: Results and Discussion

Supervisior Certification

I certify that this thesis was prepared under my supervision at the Department of Chemistry, Collage of Science, and AL- Nahrain University as partial requirements for the **Degree of Master of Science in Chemistry.**

Signature:
Supervisor: Dr. Shahbaz A. Maki
Date:

In view of the available recommendations, I forward this thesis for debate by the Examining Committee.

Signature:
Name: Assist. Prof. Dr. Afaf Al-Derzi
Head of Chemistry Department
College of Science
AL-Nahrain University

بسم الله الرحمن الرحيم (١)

الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ(٢)الرَّحْمَنِ الرَّحِيمِ(٣)مَالِكِ يَوْمِ الدِّينِ(٤)إِيَّاكَ نَعْبُدُ وَإِيَّاكَ نَسْتَعِينُ(٥)اهْدِنَا الصِّرَاطَ الْمُسْتَقِيمَ(٦)صِرَاطَ الَّذِينَ أَنْعَمْتَ عَلَيْهِمْ غَيْرِ الْمَغْضُوبِ عَلَيْهِمْ وَلا الضَّالِّينَ(٧)

(صدق الله العظيم)

الاهداء

الى الشمعة التي أحترقت لتنير دربي.....
الى من غرس في نفسي الامل.....
الى القلب الكبير....

والدي العزيز

الى من بنت فاعلى الله مقامها....
الى من زرعت فحصدت طيب ثمارها....
الى من سهرت وافنت سني عمرها....

أمي الغالية

الى من تقر بهم عيني....
الى من برؤيتهم يزول همي....
الى من من حبهم أستمد عزمي....

أخوتي الاحباء

الى القلب الذي يخفق حبا ووفاء....
الى نصفي الاخر.....

عمار

داليا

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

التحليل الكمي للمضاد الحيوي سيفالكسين باستخدام الطرق الطيفية الاعتيادية ومشتقاتها

رسالة مقدمة
الى كلية العلوم في جامعة النهرين كجزء من متطلبات نيل درجة الماجستير في علوم الكيمياء

من قبل:
داليا محمود جميل
بكلوريوس كيمياء(٢٠٠٣)

(تموز)٢٠٠٦
١٤٢٧هـ