CHAPTER ONE
INTRODUCTION

1.1 Cephalosporins

Brotzu in 1948 isolated and identified the mould cephalosporin acremnium which he had found growing in the sea near sewer outlet of the Sadiman coast. It's association with sewage and Sardinia, where typhoid fever is common, suggested to him that it might elaborate an antibiotic effective against the typhoid bacillus. Accordingly, he sent a sample of the mould to Florey in Oxford, where Abraham and Neuton discovered that the culture medium, in which it was grown, contained three distinct antibiotics. One of these was pencillin N (or cephalosporin N); it is also produced by cephalosprin salosynnematum. Another was steroid compound, named cephalosprin P which is active only against gram positive organisms. The third was called cephalosporin C which had a similar antibacterial spectrum to that of pencillin. The cephalosporin currently in use includes cephalothin, cephaloridine, cephalozin, cephaloglcin, cephalexin, cephradine and cefuroxime. The cephalosporin are active against several gram-positive and some gram-negative bacteria, including penicillin resistance and penicillin-sensitive staphaureus, E.coli, mirablis and salmonella and shigella species. Cephradine has been shown to be active against actinomyces isratic.In general cephalosporins combine the specific anti bacterial active of the pencillinase-resistant pencillins with some of the broad spectrum active of ampicillin (Bowman and Rand, 1980).

The cephalosporins consist of fused β-lactam dihydrothiazine two ring –systen ,known as 7- amino cephalasporanic acid (ACA) , the quantitative analysis of these compounds give rise to many problems . These difficulties are due to the chemical instability of β-lactam nucleus. (Van krimpen.,*et al*. 1987). The β-lactam ring present in these drug molecules has

been shown to be enormously liable to neucleophylic attack in presence of acid and alkaline or even neutral molecules.

Several methods for quantitative estimation of β-lactam antibiotics have been based on measurement of colour reaction of their degradation products. (Nkeoma et al .,2007). There are several methods described for the determination of cephalosporins in aqueous solutions, e.g., GLC, formal titration, colorimetry, liquid chromatography, iodometry, reaction with hydroxylamine, and polarography. These methds are not generally used routinely for analysis because of limited sensitivity or if sensitive enough they are less practical,(Yu .,et al . 1977).

Most of cephalosprins determination methods depend upon either degradation products or formation of derivatives, e.g.,

1. **Fluorometric:** generally have no native fluorescent properties therefore a drivatzation procedure is necessary to optain such properties. (Jusko, 1971).
2. **Ultraviolet spectroscopy:** cephalosporins have a UV absorption maximum in the range of 250-270 nm due to dihydrothiazine ring, which disappears when β-lactam ring opened,(Holl, 1975).Also Application of degradation procedure in sulphate buffer is required.(Rogic , 1984).
3. **Colorimetry:** Cephalosporins do not react quantitatively with hydroxylamine which is described for this method. The reaction can be accelerated by alkaline (pH > 12), but side reactions occure at these pH values , modifications of assay were published ,(Mays,1975 and Kulo, 1976) . an automated method was adopted regarding the instability of the formed derivative . Other colorimetric assay has been developed using in general a degradation procedure and coloring compound, (Kirschaum, 1974).
4. **Gas chromatography:** Cephalosporins are not volatile and decompose on heating. Gas is not a method of choice. The degradation product can be

chromotographed by GC after derivatization, (Thorpe,1974 , Fujimoto, 1975 and Hughe, 1976).

5. Titrimetric analysis: iodometric assay has been applied to cephalosporins with less success due to instability of alkaline degradation products.(Alicino .,1946) .Using other nucleophilic reagents for the opening of β ring, e.g., hydroxylamine, the method was still subjected to more problem (Okada,1965 and Frantz ,1976) .The method is still used by BP 80.

Another well –known titration was introduced by (Korbl, 1973) the assay involves two potentiometric methods with a mecury (11) solution, (Korbl, 1973). The mercurimetric titration suffers from the same problem for cephalosporins as iodometric assay. The shape of the potentiometric curve is irregular .Korbol and Pospisilova described a mercumetric titration in pyridine using hydroxylamine as a nucleophile, (Korbl, 1983). The procedure is not applicable to all cephalosporins , errors varying from minus 25% to plus 60% were reported,(Heintz ,1985), other reported titration procedure included an oxidation with iodate , (Grime, 1979) ,and total degradation followed by titration of the librated sulfide ,(Fogg , 1982) .

Despite the fact that instrumental methods are usually much faster than purely chemical procedures and normaly much applicable at concentrations too small to be amenable to be determined by classical methods, yet many instruments are expensive and their use will only be justified if numerous samples have to be analyzed. With most instrumental methods it is necessary to carry out a calibration operation using a reference sample of known concentration. The exact analytical data for this alternative procedure calibration is normally carried out by the use of classical chemical methods. (Vogels, 1978).

Most of instrumental methods have low accuracy, e.g., spectrophotometric accuracy range from 0.5 to 5%,(James., *et al*.,1988).

Due to above mentioned reasons seeking for methods that give accurate results and require cheapest and readily available apparatus was one of the

objectives of this study, e.g., titrimetric, potentiometic and conductometric methods.

Several classes of organic compounds are sufficiently acidic or basic, under certain conditions, to be determined by direct titrations, a few of the compounds that can be determined directly in this manner are, amine, amides, ammonium hydroxide, acids, enols, and phenols.

Cephalosporins contain carboxylic groups in their structures they are considered as organic acids ,they are fairly strong acids due to the adjacent electronegative group, with pK_a approximately 1.7- 2.6 , (Yamana , 1976). Therefore may be determined quantitatively titimeterically, potentiometrically and conductometrically in the same way as organic acids.

Two of cephalosporins drugs were chosen for this study, because they are extensively widely used antibiotics in clinical practices

1- Cephalexin

2- Amoxicillin

1.2 Potentiometry

When a metal is immersed in a solution containing its own ion M^{n+}, then an electrode potential, E, is established, the value of which is given by Nernst equation.

$$E = E^{o} + (RT/nF) \ln a M^{n+}$$

E = electrode potential of metal M

E^{o}= electrode potential

R = the gas constant

F = the Faraday constant

T = the absolute temperature

n = the valency of the ion concerned

Where E^{o} is a constant, the standard electrode potential of the metal. E can be measured by combining the electrode with a reference electrode (commonly a standard Calomel electrode). Measuring the e.m.f. of the

resultant cell. It follows that knowing the potential E_r of the reference electrode , we can deduce the value of the electrode potential E, provided that the standard electrode potential E^o of the given metal is known, we can proceed to calculate the ion activity M^{n+} in the solution for a dilute solution as the measured ionic activity will be virtually the same as the ionic concentration, and for stronger solution, given the value of the ionic activity coefficient, we can convert the measured ionic activity to the corresponding concentration.

This procedure for using a single measurement of electrode potential to determine the concentration of an ionic species in solution is referred to as direct potentiometey. The electrode whose potential is dependant upon the concentration of the ion to be determined is termed the indicator electrode and when, as in the case above, the ion to be determined is directly involved in the electrode of the first kind. It is also possible, in appropriate case, the concentration of an ion, which is not directly concerned in the electrode reaction to be measured by direct potentiometry, This involves the use of an electrode of a second kind an example of which is the a silver chloride electrode which is formed by coating a silver wire with sliver chloride.

1.2.1 Potentiometric titration

It is a titration procedure in which potentiometric measurements are carried out in order to locate the end point,(Vogel,1978).

The procedure involves measurement of e.m.f. between two electrodes; an indicator electrode the potential of which is a function of the concentration of the ion to be determined, and reference electrode of constant potential.
Accurate determination of the e.m.f. is critical, in potentiometric titrations, absolute potentials or potentials with respect to standard half-cell are not usually required, and measurements are made whilst the titration is in process.

The equivalence point of the reaction will be revealed by a sudden change in potential in the plot of e.m.f. reading against the volume of titrating solution, Any method that detects this abrupt change of potential may be used

one electrode must maintain at constant, but not necessary known, potential; the other electrode must serve as an indicator of the changes in ion concentration, and must respond rapidly.

The solution must of course, be stirred during the titration. (Vogel, 1978).

1.3 Coductometry

1.3.1 General consideration

Ohm's law states that the current 1 (Amperes) flowing in a conductor is directly proportional to the applied electromotive force E (volts) and inversely proportional to the resistance R (ohms) of the conductor.

$$I = E/R$$

The reciprocal of the resistance is termed the conductance (G).This is measured in reciprocal Ohms (mho) for which the name siemens (S) has been proposed. The resistance of a homogenous sample of length L, and cross-section a, is given

$$R = \rho.1/a$$

Where ρ is a characteristic property of the material termed resistivity (formerly called specific assistance).

In SI unit(I)and(a)are measured respectively in meters and square meters, so that ρ refers to meter cube of material, and ρ:R.a/ℓ is measured in Ohm. Meters. Hitherto, resistivity measurement has been made in terms of centimeter cube of substance giving P the units Ohm cm.

The reciprocal of resistivity is the conductivity K (formerly specific conductivity), which in SI unit, is the conductivity of one meter cube of substance and has the units mho/*cm* (or S cm^{-1}) but P measured in Ohm cm, then K will be measured in mho cm^{-1} (or S cm^{-1}).

The conductivity of an electrolyte solution at any temperature depends only on the ion present, and their concentrations. When a solution of an electrolyte is diluted, the conductivity will decrease since fewer ions are present per cm of solution to carry the current. If all the solution is placed between two electrodes 1 cm apart and large enough to contain the whole of

the solution, conductance will increase as the solution is diluted, this is due to decrease in inter-ionic effect for strong electrolytes and to an increase in the degree of dissociation for weak electrolyte.

1.3.2 The basic of conductometric titration

The addition of a solution of an electrolyte to another, under condition that no change of volume occurs will affect the conductance of the solution according to whether or not an ionic reaction occurs.

If no ionic reaction occurs, such as in addition of one sample salt to another (eg potassium chloride to sodium nitrate), the conductance simply increases, if ionic reaction occurs, the conductance may either increases or decreases. In the addition of a base to a strong acid, the conductance decreases owing to the replacement of the hydrogen ion of high conductivity by alkali ions of low conductivity. This is the principle underlining conductometric titration, i.e, the substitution of ions of one conductivity by ions of another conductivity.

During the process of neutralization, precipitation, etc., changes in conductivity may generaly be employed in determining the end points as well as the progress of the reaction.

The conductivity is measured after each addition of small volume of reagent, and the volumes obtained are plotted to give a graph, which consists of two straight lines intersecting at the equivalent point.

The accuracy of the method is greater the more acute angle of intersection and the more nearly the points of the graph on the straight-line.

1.3.3 Apparatus and measurements

Conductivity cell for coductometric titration may be of any kind that lends itself to thorough stirring of the contents (preferably by mechanical means) and permits periodical addition of reagents, it may be necessary to place the cell in a large vessel of water in order to maintain consistancy of temperature but in most circumstances the cell may be used at ambient temperature of the laboratory, the condcutivity cell containing the solution

under test is made of insoluble glass or fused silica and must be thoroughly washed and steamed before use, the shape of the cell varies as shown in Fig 1.2.

The electrodes are generally of thick platinum foil, firmly fixed in position and coated with a layer of platinum black to decrease polarization effects, (Brown, 1972).

The conductivity of solution is obtained by measuring its resistance using a modified Wheatstone bridge circuit as shown Fig 1. 1.Because direct current cause back e.m.f. Due to polarization, a rapid x alternating current be used, and a telephone receiver or oscilloscope is used in the Wheatstone bridge circuit to detect the balance point.

The variable resistance and the position of x along a wire A B are changed until no current passes through the detector, at this balance point

$$\frac{\text{Resistance of conductivity cell}}{\text{Resistance of variable resistance}} = \frac{\mathbf{AX}}{\mathbf{XB}}$$

And the value of the resistance of conductivity cell is obtained.

From the measured resistance of solution in a given cell the resistivity and hence the conductivity could be obtained, if the cross sectional area of the electrode and the distance between them, L were known. Such measurements would, however be very difficult to make, and they can be avoided by making use of what is known as the cell constant. For a given cell both Land a are constants and the ratio of L/a, is called the cell constant

$$\text{Conductivity} = \frac{1}{\text{Measured resistance}} \times \text{cell constant}$$

The cell constant can be obtained from the cell dimension but it is more commonly measured by using a solution such as M/10 potassium chloride whose conductivity is known. Once measured, the cell constant for a cell is fixed so long as the physical dimensions of the cell are not altered in any way. To insure this, electrodes, in conductivity cell must be rigidly fixed in their relative positions,(Brown ,1972).

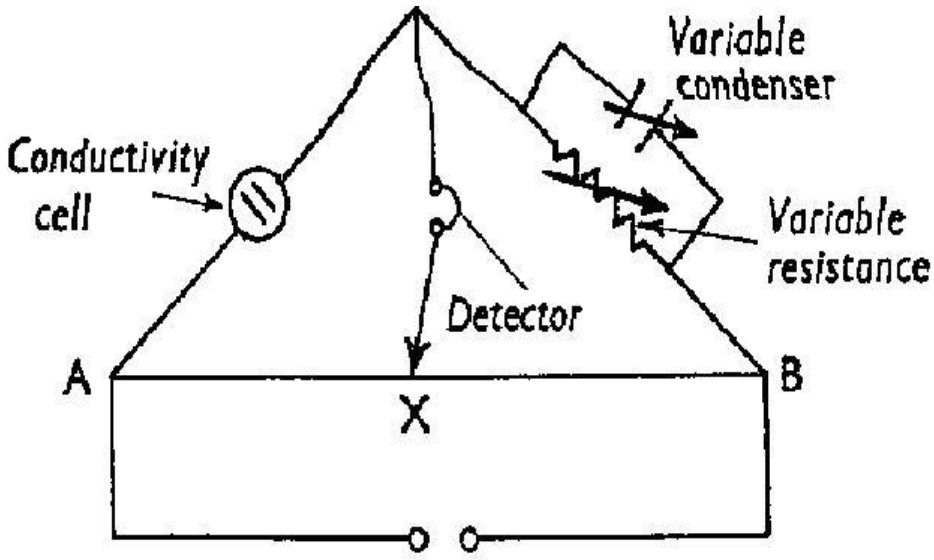


Fig 1.1 Wheatstone Bridge Circuit For Measuring Conductivity

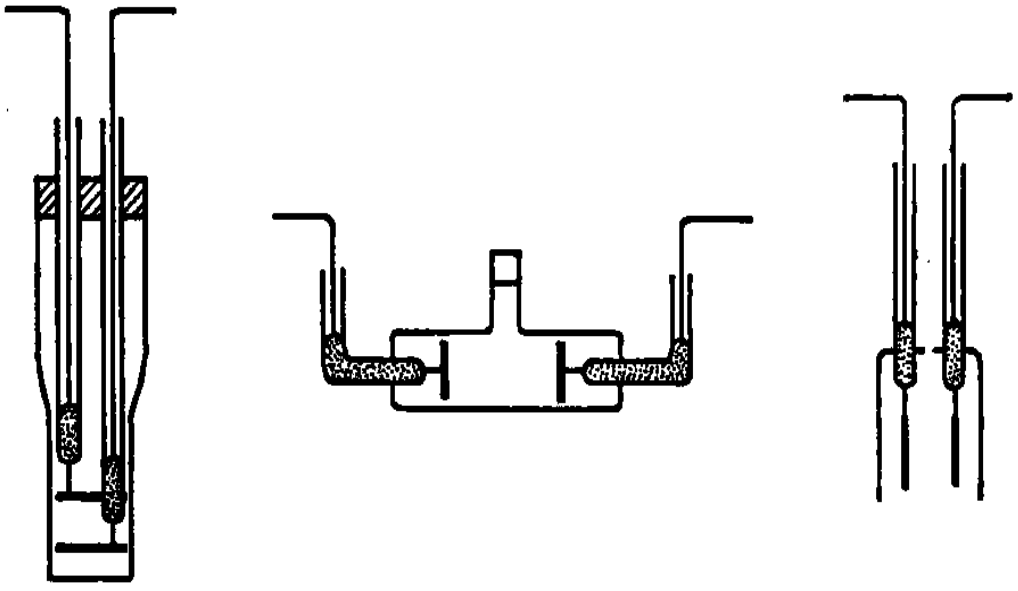


Diagram 1.2 Conductivity Cells

1.4 Chromatography

Chromatography is the general name given to the method by which two or more compounds in a mixture physically separate themselves by distributing themselves between two phases.

1- A stationary phase which can be solid or a liquid supported on a solid
2- A mobile phase either gas or a liquid which flows continuously rounding the stationary phase.

The separation of individual components results primary from difference of their affinity for the stationary phase, (Henry., *et al.* 1978).

The great importance of chromatography lies in its available advantage over other analytical methods, it can be used successfully to separate mixture of substances which are very similar physically and chemically, e.g., (amino acids, hydrocarbons, sugars, phenols, rare earth metals) and which necessitate lengthly and ledaus separation process when the usual physical and chemical methods are employed, it can be adopted to apply to very small quantities of materials making it possible to detect the presence of only micro grams of substances, (John Hicks ,1973).

1.4.1 Types of chromatography

1- Paper chromatography

Is special field of liquid chromatography in which the stationary liquid is a film of water adsorbed on a mat. Other stationary liquids can be used as well. It is practically used for separating and identifying traces of compounds.

2- Thin layer chromatography

Is similar to paper chromatography except that the paper is replaced by a glass or plastic plate coated with a thin layer of alumina, silica gel, or other powdered material,(Rorgert., *et al* .1976).

3-Column chromatography is also called elution chromatography since the separated compounds are eluted from the column.

Column chromatography and thin- layer chromatography are similar in principle, compounds in the mixture to be separated are partioned between a solid adsorbent (stationary phase) and a solvent (the mobile), that flows past the solid adsorbent into the stationary phase and the less the compound is dissolved in the moving liquid the slower the compound will migrate along the stationary phase in the direction of solvent flow.

In column chromatographic the adsorbent is packed into a glass column and the solvent flows through the column past the adsorbent particles, (Michae., *et al.* 1980).

1.4.2Quantitative analysis

Chromatography owes its enormous growth in part to its speed, simplicity and relativity low cost, its wide applicability as a separating tool, however, has become so wide spread because it can also provide quantitative information about the spreaded species.Quantitivate chromatography is based upon a comparison of either the hight or area of the analyte peak with that of one or more standards. If conditions are properly controlled both of these parameters vary linearly with concentration, Skoog and Holler (1992).

1.4.3 Methods for quantitative analysis

1- Internal standard

The technique is the addition of a known amount of standard that has a different retention time, but structurally related, to a known amount of sample, this mixture is then prepared, for subsequent injection into the chromatographic column. From the peak area of the standard and component of interest, the composition may be determined.

2- External standard

In this technique standard solutions covering the desired range are prepared, then equal amount are chromatographed and the peak area obtained. A calibration curve of composition verus area is prepared next.

The same volume of sample is chromatographed and the peak area calculated.The area of unknown should fall between two points on calibration curve so that the composition may determine by interpolation.

3-Standard addition

This technique is a combination of external and internal methods. In this approach best used for one or two components .First, chromatograph the sample under optimum conditions. Then a known amount of the desired components is added and the mixture chromatographed again. From these results the composition may be calculated.

1.4.4 Detectors

The detector is a device that supplies an output signal in response to the presence of samples. It is connected to the outlet of the column to monitor the column effluent in the real time. The detector can be the most sophisticated, and one of the most expensive components of chromatographic system.

There are many types of detectors.

1- Fixed wave length uv absorption detectors

2- Variable wave length uv detectors

3- Infrared detectors

4- Fluorescence detectors

5- Refractive index detectors

6- Flame ionization detecter

7- Thermal conductivity detecter

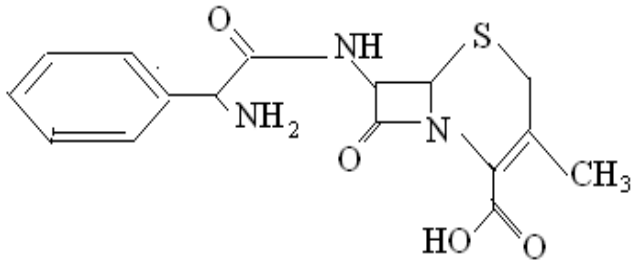
(Edward., *et al* . 1978)

CHAPTER TWO

LITERATURE REVIEW

2.1 Cephalexin

2.1.1structure



2.1.2Action and use : Antibiotic

2.1.3 Preparation

Cephalexin capsule

Cephalexin oral suspension

Cephalexin tablets

2.1.4 Defination

Cephalexin contains not less than 95 percent and not more than the equivalent 101 percent of (6R,7R)-7-[®-2-amino-2-phenyl acetamido]-3methyl-8-oxo-5-thio-1-azobicyclo [4.2.0] oct-2-ene-2-carbocilic acid, calculated with reference to the anhydrous substance, British Pharmacopoeia (1999).

2.1.5 Characters

White or almost white, crystalline powder, soluble in about 100 parts of water practically insoluble in alcohol and ether. British Pharmacopoeia (1999).

Also cephalexin is soluble, 1 in 30ml of 0.2M hydrochloric acid and also in a solution of dilute alkaline,(Jakson., *et al*. 1989).

2.1.6 Methods of determination of cephalexin

2.1.6.1 The chromatographic method

The chromatographic behavior of some cephalosporin has been studied on synthetic inorganic ion-exchanger(stannic oxide) layer using citrate and borate buffer as mobile phase ,several ternary and quaternary separation have been achieved for quantitative separation ,solution of cephalosporin were prepared in demineralized water.

Solutions of different cephalosporins were mixed, spotted by means of a micro syring and developed with selected mobile phase. A pilot plate was run simultaneously to facilitate exact poisoning on the spot on the working plate. The regions containg the cephalsporins were scraped from the plate, added to demineralized water,then filtered. The clear solution containing the cephalosporin content of each spot was then analysed by standard spectrophotometric method(Nabi., *et al.*2004).

A high performance liquid chromatographic (HPLC) procedure for measurement of five orally administered cephalosporins (cefiximme, cefactor, cefadroxil, cephalexin, and cephradine) in 0.1ml of human serum was develobed. Serum protein is precipitated with acetonitrile, the sample is centrifuged, and the supernatant is everporated under nitrogen, the residue is reconstituted in 0.1ml of mobile phase and 50 to 85mL of this is injected into a reversed phase Altex ultea sphere octyl(C_8) column. The five cephalosporins, are reversed by elution with a pH 2.6 mobile phase of methanol/mono basic phosphate buffer. (20/80 by vol), flow rate 2ml/min the column effluent is monitored at 240nm. Cefixime serve as the internal standard for the analysis of other compounds, cephalxin as the internal standard for cefixime.(Joy., *et al* .1987).

Asimple, precise and rapid reversed phase high performance liquid chromatographic method was developed by (Shindle., *et al.*1994). For the simultaneous determination of cephalexin and probene acid in tablets, on

Novapk C_{18}(4micro) column using water:methanol: actonitrile: glacial actic acid (50:20:30:1) as mobile phase. The flow rate was 1.5ml/min and effleuent was monitored at 254nm.

A rapid and accurate high speed liquid chromatographic method was developed for determination of cephalexin in human plasma and urine. The method involves filtration of urine specimens and methanol extraction of plasma samples followed by HPLC seperation on bonded reversed phase column utilizing mobile phase of methanol-water containing acetic acid. The quantitaty of uv response at 254nm covered a wide range of cephalexin concentrations down to 0.5µg/ml(Terumichi Nakagawa., *et al.* 1978).

A new high performance liquid chromatographic method was developed by Lee and Lee(1990), using a column –switching technique for simultaneous determination of cephalexin, cefuroxime, cefoxitin and cephaloridine in plasma. The plasma samples are injected onto a pre column packed with corasil Rpc18 (37-50µM) after simple dilution with internal standard solution in 0.01Macetate buffer (PH3.5).The method show excellent precison with good sensitivity with a detection limit of 0.5µg/L.

A thin layer chromatographic method using fluorescamine detection was described for quantitation of antibiotic, cephradine, cofactor, cephalexin and cefaroxil. The repeatability, sensitivity and detectability of the method is compared with those of o-phthalaldehyde fluorescence emission and uv absorption, (Huquette., *et al.*1985).

Rapid and simple method for the determination of cephalexin,cephalothin (Na salt) and cephradin without perior separation from their alkaline-induced degradation product is presented by measuring the concentration of the initial drug spectrophotometrically directly without interference of degradation product.(EL-Yazbi., *et al.* 1985).

2.1.6.2 Spectrophotometric methods

1- A new sensitive and simple spectrophotometric method for the determination of cephalexin (CEX) and ampcillin (AB-PC) was

established using O-hydroxyhydroquinone phathalin (Qn-Ph) and palladium(11) [Pd(11)] at low concentration of acetyl trimethyammonium chloride (CTAC) in weak acidic media. This method is based on the fact that the intensity of the absorption peak of [Qn.Ph-Pd(11)] complex at 630nm is deceased significantly by addition of CEX or AB-PC, and the decrease in the absorbance is proportional to the concentration of (EX or AB-PC).The method was applied to the determination of CEX or AB-PC in pharmaceutical preparation (Moriitsuo., *et al.* 1982).

2. A simple, accurate, sensitive and validated method was developed for spectrophotometric determination of cephalexin by (Dalia R. El-wasseif ,2007).

The method involves the reation of cephalexin with 2-cyanoacetamide in presence of 33%ammonia solution. The formed fluorescent product exhibited a maximum fluorescence intensity at 439nm after excitation at 339nm.

3-Vanadophosphoric acid in acidic medium was proposed as a modified reagent for the spectrophotometric determination of cephalexin sodium, cefazolin sodium and cefolaxime in a pure sample and in pharmaceutical preparation. The method is based on hydrolysis of cephalosporin and subsequent oxidation with vanadophosphoric acid the resulting solution exhibits maximum absorption at about 516nm. The method was applied to the determination of the drug In pharmaceutical preparations. The method proposed is accurate and precise(Alaa and Sayed ,2000).

4. A photometric extraction method was developed for determination of cephalexin. Cephalexin is hydrolysed for 5 minutes in sulphuric acid medium on a steam-bath, the hydrolytic product forming a coloured compound with ninhydrin in 25% H_2SO_4. This compound is extracted with $CHcl_3$ and its absorbance measured at 520nm. (Papazova., *et al.* (2005).

2.1.6.3 Other methods

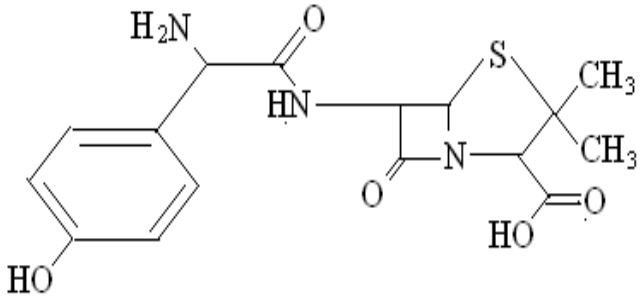
1- Electrolysis of glutathione and cephalexin was studied in 0.1M carbonate buffer pH 9.2 with boron dropped diamond thin-film electrode by cyclic voltammetry. It was found that a well resolved and irreversible oxidation voltamgram was obtained for glutathione cephalexin should a discernible. For cephalexin the detection limit was 5.0μM.(Orawon*., et al* .2001).

2- A flow injection (FI) method using the tris (2,2-bipyridyl) ruthenium(11) $[Ru(bpy)_3]^{2}$- potassium permanganate chemiluminescence (CL) was developed for the rapid and sensitive determination of cephalosporins such as cefoxitin, cefazolin, cephalexin, cofactor and cefoperzone. The method is based on the CL reation of cephalosporin, and$[Ru(bpy)_3]^{2}$ with potassium permanganate in the presence of perchloric acid, catalyzed by Mn(11) under the optimum condition, (Chalermporn., *et al* .2005).

3-An NMR method was developed to determine quantitatively the presence of cephalexin in cephradine. The method is applicable to the chemical itself as well as to capsules and oral suspension formulation. The determination is based on the NMR signal arising from the five aromatic protons of the cephalexin in molecule. Integration of this signal relative to a signal from cephradine provides the data necessary to determine the percentage of cephalexin present, (Walken., *et al* .2006).

2.2 Amoxcillin trihydrate

2.2.1 Structure



2..2.2 Action and use: Antibacterial

2.2.3 Preparations

1- Amoxcillin capsules
2- Amoxcilln oral suspension
3- Co-amoxcilav tablet

2.2.4 Defination

Amoxcillin trihydrate contains not less than 95.0% and not more than the equivalent of 100.5 percent of (2S,5R,6R)-6-[®-2-amino-2-(4-hydroxyphenyl)acetoamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxcylic acid, calculated with reference to the anhydrous substance.

2.2.5 Characters

White or almost white, crystalline powder, slightly soluble in water and in alcohol, practically insoluble in ether and in fatty oil, soluble in dilute acids and dilute solution of alkali hydroxide,(British pharmacopoeia,1999).

It's solubility 1 in 400ml of water, 1 in 100ml of ethanol, I in 200ml of methanol, practically insoluble in chloroform, (Jakson.,*et al.* 1989).

Amoxcillin is a ß-lactam antibiotic that belongs to the group of penicillin. It is extremely active against both gram-positive and gram-

negative micro organsims, including several pathogenic enteric, (Dousa., *et al.*2005).

2.2.6 Methods for determination of amoxicillin

2.2.6.1Chromatographic methods

A new HPlC-RP method was developed by (Pery-Lozano.,*et al.* 2006). For stability evaluation of amoxicillin in granular premixes. The method is based on monitoring of the degradation product formed during study, using a Nucleosil 120 C_{18} column and gradient elution, the mobile phase consists of a mixture of methanol and buffer solution pH 3±0.05. The flow rate was 1.750ml mn^{-1}. The detector was set at 230nm.

A liquid chromatographic Landem mass spectroscopic (LC-MS/MS) method for determination of amoxicillin (AMO) in animal feed was developed. The method was used to examine the quantity requirement for product intended in corporation into animal feeding stuffs. After the adition of the internal standard (Ampicillin), the medical feed samples were examined using a 0.01M potassium dihydrogen phosphate buffer solution (pH5) followed by a centrifugation and filtration step. An appropriately diluted aliquot of the extract was analysed on a PLRP-S polymeric column (150mm x2.1mm i.d., 100Å) using a mixture of 0.1% formic acid in water and acetonitile as the mobile phase. Gradient elution was performed at a flow rate of 0.2ml. The mass spectrometer was used in the positive electro spray ionization ms/ms mode,(De Baere Ptrick, 2007).

Gama and Dusi,(2003), used liquid chromatography (LC) with fluorescence detection, for determination of amoxicillin in animal feed. After pre-derivtization with a 7% formaldehyde solution, the method is very specific and sensitive, the derivtization step makes the sample preparation produces more complex but it is time consuming.

A liquid chromatographic (LC) with ultra violet detection method for determination of amoxicillin in feed stuff was developed by Dousa and

Hasmamova (2005). The method was simple and fast, but it is less sensitive for the determination of amoxicillin at subtherapeutic levels.

Arapid, simple, accurate, sensitive and reproducible high performance liquid chromatographic (HPLC) method for quantitation of amoxicillin in human plasma using cefadroxil as an internal standard (IS) has been developed and validated. The procedure involves an ultra filtration step prior to reversed –phase liquid chromatography. The drug and the IS were eluted from symmetery C_{18} stainless column (5µm 150x4.6mmI.D.) at room temperature with a mobile phase consisting of methanol 75 mm potassium dihydrogen phosphate buffer solution (10:90, v/v) (pH adjusted to 3.0 with phosphoric acid), at flow rate of 1.5mm^{-1}. The effeluent was monitor using a uv detector set at 228nm, (Malar, 2006).

Accurate, precise, sensitive HPLC assay was developed for the determination of amoxicillin in human plasma sample, to compare the bioavailability of two amoxicillin capsules. Amoxicillin conteration were analysed by combined reversed phase liquid chromatography and uv (λ=229nm). Amoxicillin and cefradoxil (internal standard) were extracted from the plasma by addition of cold methanol. The separation was achieved using the Lichrosorb 15µmC_{18} reversed phase column at room temperature. The mobile phase cosisted of 95% phosphate buffer 0.01mol/L, pH =4.8 and 5% acetonitrile mixture,(Luis Renato Pires Abteu., *et al.* 2003).

The chromatographic behavior of amoxicillin, ampcillin cephalexin, cloxacillin, doxycycline tetracycillin erythromycin, gentamycin, streptomycin and co-trimoxazile has been studied on thin layers of titanic silicate. Inorganic ion-exchange with organic, aqueous, and mixed aqueous organic phase-rapid separation of one antibiotic from numerous other antibiotics has been achieved. Antibiotics were detected with appropriate reagents (1%w/v) ninhydrin in ethanol was used to locate amoxicillin, ampcillin,cephalexin, cloxacillin, gentamycin and co-trimoxazole and

5%(w/v) potassium dichromate in concentrated H_2SO_4 was used to locate streptomycin, erythromycin, tetracycline and doxycycline,(Husain.,*et al.* 2004).

A reversed-phase column liquid chromatographic method was developed by Mei-Chic Hus and Pei-Wen Hus (1992). For the assay of amoxicillin and its preparation. The mobile phase, which was pumped through a reversed- phase column /µ Bondapak C_{18} with an isocratic folw rate of 1.5 ml/mim. The detector was set at 254nm. Chromatography was performed at room temperature and injection of 20µl of all solution, to be analysed, was made.The mobile phase, which was methanol- 1.25% acetic acid (20:80v/v) the mobile phase was filtered and degassed with ultrasonic bath perior to use.

A comparison was made between liquid chromatography (LC) methods and a microbial inhibition (MI) method for the determination of amoxicillin and ampicillin in milk cows dosed with the drugs. The LC methods using formaldehyde and salicyaldehyde were applied in the detection respectively.The LC salicylaldehyde was also applied to mixed samples and the results were in agreement with those determined separately. (Cathrina., *et al*.1997).

A rapid and specific high- pressure liquid chromatographic (HPLC) assay was developed for the simultaneous determination of amoxicillin and it's penicilloic acid metabolite in urine or after dilution with water-methanol (85:15).They were separated by several-phase chromatography and quantitated spectrofluormetrically following post column derivatzation with fluorescamine, (Lolee., *et al*. 1978).

A quick routine analytical procedure for the identification and quantification of premixture of amoxicillin was developed and tested by Dousa and Husmanova, (2005), using reversed phase high performance liquid (HPLC) this method proves to be selective and can be used in the

routine analysis, for the distinction between amoxicillin and other penicillin, which have similar structure.

(Hoizey., *et al*. 2002; and Yoon 2004) used reversd phase HPLC for the simultaneous determination of amoxicillin and clavulanic acid in human plasma, where Hoizey used HPLC with ultraviolet detection and Yoon improved the method by employing HPLC combined with mass spectroscopy, both methods were simple and accurate.

2.2.6.2 Spectrophotometric methods

A simple and sensitive spectrophotometric method was designed for determination of amoxicillin. The method is based on nucleophilic substitution reaction to measure the pink compound produce by the reaction of amoxicillin with sodium 1, 2 naphoquinone -4- sulfonate in pH 9.0 buffer solution. The stiochiometric ratio of the comound is 1:1 and it's maximum absorption wave length is at 468nm,(Li and Yang, 2006).

UV- spectrophotometric (UV) and high performance liquid chromatographic method (HPLC) were described for the determination of amoxicillin and clavulamic acid in pharmaceutical preparations. Spectrophotometrically, amoxicillin was determined by measuring the absorption values at 320nm in buffer $CuSO_4$ solution (pH 5.2) and at 313nm in imidazol solution (pH 6.8) for clavulanic acid. HPLC depend upon using a reversed phase RP_{18} column at ambient temperature with a mobile phase consisting of methanol-phosphate solution pH4.4 (4:96) at flow rate 1ml-min^{-1} (· Quantitation was achieved by UV detection at 220 nm(Ly Thuong, 2004).

A batch and flow injection analysis (FIA) spectrophotometric method have been developed by (Mouayed, *et al.*2005),for the determination of amoxicillin (AMX) in aqueous solution and pharmaceutical preparation. The methods are based on the reaction of AMX with N, N di methyl phenyl di amine in the presence of potassium hexacyano ferrte(111) in alkaline medium. The water soluble blue product measured at λ_{max} 660nm.

Spectrophotometric method for the determination of amoxicillin in pharmaceutical product was developed. The method is based on measurement of organic-red water soluble product formed by reaction between amoxicillin and 4-amino anti-pyrine in the presence of alkaline potassium ferric cyanide(111) at 507nm, (Chalermporn., *et al* .2005).

A simple, sensitive and accurate spectrophotometric method for the determination of ampicillin, amoxicillin trihydrate and cefazolin sodium.The procedure is based on the formation of Prussian blue (PB) complex. The reaction between acid hydrolysis product of antibiotics ($T=60^0$) with mixture of Fe^{+3} and hexacyano ferrate(111) ions was evaluated for spectrophotometric determination of the mentioned drugs. The maximum absorbance of coloured complex occurs at λ=700nm, (Khalil Farhadi., *et al*.2002).

Two simple, rapid and sensitive spectrophotometric procedures were developed for determination of amoxicillin and cefadroxil. The methods are based on the selective oxidation of the drug with N-bromosuccinimide or N-chlorosuccinimide in alkaline medium to give an intense yellow product (λmax=395nm) the methods were applied to the analysis of pharmaceutical formulations containing amoxicillin, (Gamal,1996).

2.2.6.3 Other methods

A sesitive flow injection method was applied successfully to determine amoxicillin in pharmaceutical preparations, human urine and serum without any pretreatment procedure, with recovery from (90%-110%), (Xiaofeng Xie and Zhenghua Song, 2006).

Hernandez, Borrull and Calull (1999) developed a method using capillary electrophoresis in order to determine amoxicillin content in animal plasma samples.

The amount of amoxicillin in pharmaceutical formulation was determined using spectra of diffuse reflectance infrared Fourier transform

spectroscopy (DRIFTS)., in association with partial least squares (PLS) regression(Graciele Parisotto., *et al*.2007).

CHAPTER THREE

MATERIALS , METHODS and RESULTS

3.1 Cephalexin

3.1.1 Direct titration of cephalexin

3.1.1.1Cephlexin monohydrate

3.1.1.1.1 Reagents

1- 0.1M Oxalic acid

2- 0.0094 M NaOH solution

3- Cephalexin solution (1.00g/250 ml distilled water)

3.1.1.2 Elie cephalexin capsule

3.1.1.2.1 Reagents

1-0.02814M NaOH solution

2- Elie cephalexin capsules solution

3- Phenelophthalin indicator

3.1.1.3 Changahi cephalexin capsule

3.1.1.3.1 Reagents

1- 0.02814M NaOH solution

2- Changahi Cephalexin capsules solution

3- Phenelophthalin indicator

3.1.1.4 Amepharma cephalexin capsule

3.1.1.4.1 Reagents

1- 0.02814M NaOH solution

2- Amipharma cephalexin capsules solution

3- Phenelophthalin indicator

3.1.1.5 Wafra cephalexin capsule

3.1.1.5.1 Reagents

1-0.01M NaOH solution

2-Wafra cephalexin capsules solution

3- Phenelophthalin indicator

3.1.1.6 General apparatus

1- 50ml burette

2- 20ml pipette

3- 100ml conical flasks

3.1.1.7General Procedure

Three aliqouts of 20 ml of cephalexin monohydrate were taken in three different conical flasks , two drops of ph.ph were added to each, then titrated with 0.0094 M NaOH solution.

weights of 1.0714 g, 1.0634 g, 1.0418 g and 1.077 g of Elie,Changahi, Amipharma and Wafra cephalexin capsules were taken respectively, which repectively contain 1.0026 g ,0.988 g ,0.989 g and 0.9952 g of pure cephalexin each was completely dissolved in 200 ml of distilled water with aid of magnetic stirer and magnetic rod, and transferred to 250 ml volumetric flask, completed up to the mark with distilled water and filtered.

Two portions of 10 ml aliquot were taken into two different conical flasks, two drops of ph.ph indicator were added , and titrated with NaOH solution related to each.

3.1.1.8 Results of direct titration methods with NaOH

3.1.1.8.1 Cephalexin monohydrate

The volume of 0.0094M NaOH required for nentralization is 22.2ml

$$Q{-}COOH + NaOH \rightarrow Q{-}\,C\,OONa + H_2O$$

1 mole 1 mole

mmoles of cephalexin monohydrate = mmoles of NaOH

= 22.2X0.0094 = 0.20868 mmoles

These mmoles presentin 20ml of cephalexin monohydrate solution

Number mmoles that present in 250 ml of cephalexin monohydrate solution

$$= \frac{0.20868X250}{20}$$

Therefore weight of cephalexin monohydrate = $\frac{2.6085X\ 365.4}{1000}$ = 0.953 g

% of cephalexin monohydrate = $\frac{0.953X100}{1.00}$ = 95.3%

3.1.1.8.2 Elie cephalexin capsule

The volume of 0.02814M NaOH required for nentralization is 3.65ml

−COOH + NaOH → Q −COONa + H_2O

1 mole 1 mole

mmoles of Elie cephalexin capsules = mmoles of 0.02814 M NaOH = $V_{NaOH}XM_{NaOH}$

= 3.65×0.02814 = 0.1027mmoles

These mmoles were contained in10 ml of Elie cephalexin capsules solution

mmoles that contained in 250 ml of Elie cephalexin capsules solution =

$$\frac{0.1027\times250}{10} = 2.5675\text{mmoles}$$

Weight of Elie cephalexin capsules = mmoles of it × M wt = 2.5675×365.4 = 938.165mg =0.938165g

% of Elie cephalexin capsules = $\frac{0.9382\times100}{1.0026}$ = 93.9%

3.1.1.8.3 Amipharma cephalexin capsules

The volume of 0.02814M NaOH required for nentralization is 3.5ml

Q −COOH + NaOH → Q −COONa + H_2O

1 mole 1 mole

mmoles of Amipharma cephalexin capsules = mmoles of 0.02814M NaOH

$=V_{NaOH}XM_{NaOH}$ $= 3.5\times0.02814$

= 0.09849 mmoles

These mmoles were contained in10 ml of Amipharma cephalexin capsules Solution

mmoles that contained in 250 ml of Amipharma cephalexin capsules solution = $\frac{0.09849 \times 250}{10}$ = 2.46225 mmoles

Weight of amipharma cephalexin capsules = mmoles of it × M wt =

2.46225 ×365.4 = 89907615 mg =0.8997615 g

% of amipharma cephalexin capsules = $\frac{0.89976 \times 100}{0.9892}$ = 90.9%

3.1.1.8.4 Changahi cephalexin capsules

The volume of 0.02814M NaOH required for nentralization is 3.55ml

Q −COOH + NaOH → Q −COONa + H_2O

1 mole 1 mole

mmoles of Amipharma cephalexin = mmoles of 0.02814m NaOH

$=V_{NaOH}XM_{NaOH}$

= 3.55×0.02814 = 0.0999 mmoles

These mmoles were contained in10 ml of Amipharma cephalexin capsules solution

mmoles that contained in 250 ml of Amipharma cephalexin capsules solution

= $\frac{0.0999\times250}{10}$ = 2.4975 mmoles

Weight of Amipharma capsule cephalexin capsules = mmoles of it × M wt =

2.4975×365.4 = 912.59 mg =0.91259 g

% of Amipharma cephalexin capsules = $\frac{0.91259\times100}{0.9883}$ = 92.34%

3.1.1.8.5 Wafra capsule cephalexin

The volume of 0.01M NaOH required for nentralization is 9.85ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of Amipharma cephalexin capsules= mmoles of 0.01M NaOH

$=V_{NaOH}XM_{NaOH}$ $= 9.85\times0.01$

= 0.0985mmoles

These mmoles were contained in10ml of Amipharma cephalexin capsules solution

mmoles that contained in 250 ml of Amipharma cephalexin capsules solution

$= \frac{0.0985\times250}{10}$ = 2.4625 mmoles

Weight of Amipharma cephalexin capsules = mmoles of it × M wt =

2.4625 ×365.4 = 899.7975mg =0.8997975 g

% of Amipharma cephalexin capsules $=0 \frac{.8998\times100}{0.9952} = 90.4$ %

3.1.2 Back titration methods with NaOH solution

3.1.2.1 Cephalexin monohydrate

3.1.2.1.1 Reagents

1- cepfalexin solution (0.5079g/250ml of distilled water)

2- 0.01944 M NaOH solution

3- 0.02198 M Hcl solution

4- Methyl red indicator

3.1.2.2 Elie Cephalexin capsule

3.1.2.2.1 Reagents

1- Elie capsules cephalexin soluation

2- 0.02814 M NaOH solution

3- 0.02364 M Hcl solution

4- Methyl red indicator

3.1.2.3 Changahi cephalexin capsules

3.1.2.3.1 Reagents

1- Changahi cephalexin capsulessolution

2- 0.02814 M NaOH solution

3- 0.02364 M Hcl solution

4- Methyl red indicator

3.1.2.4 Amipharma cephalexin capsule

3.1.2.4.1 Reagents

1- Amipharma capsules cephalexin solution

2- 0.02814 M NaOH solution

3- 0.0247 M Hcl solution

4- Methyl red indicator

3.1.2.5 Wafra capsules cephalexin

3.1.2.5.1 Reagents

1- Wafra capsules cephalexin soluation

2- 0.01M NaOH solution

3- 0.0135M Hcl solution

4- Methyl red indicator

3.1.2.5.2 General apparatus

1- 25 ml burette

2- 25 ml pippite

3- Conical flasks

4- Magnetic stirrer and magnetic rod

3.1.25.3 General procedure

Two aliquots of 25 ml of cephalexin monohydrate solution were taken into two different conical flasks; 25 ml of 0.01904 M Na OH solution were added to each, also 2 drops of methyl red indicator were added and then titrated with the 0.02198 M Hcl solution.

A weight of 1.0714 g, 1.0634 g, 1.0418 g and 1.0772 of Elie, Chinghai, Ampharma and wafra, cephalexin capsules respectively which respectively contain 1.0026 g, 0.9883 g0.9892 g and 0.9952 g of pure cephalexin were each completely dissolved in about 200 ml of distilled water with aid of magnetic stirrer and magnetic rod and transferred to 250 ml volumetric flask, completed up to the mark with water and filtered.

Two portions of 10 ml aliquot were taken into a 100 ml conical flask, 20 ml of related NaOH solution were added to each, two drops of methyl red indicator were added and titrated with related Hcl solution.

3.1.2.6 The blank titration for cephalexin monohaydreate

Two aliquots of 25 ml of 0.01944 M sodium hydroxide solution were taken into two different conical flasks; 2 drops of methyl red indicator were added to each then titrated with 0.02198 M Hcl solution.

3.1.2.7 Blank titration for others cephalexin

Two portions of 20 ml of related NaOH solution were taken into two different 100 ml conical flask, two drops of methyl red indicator were added to each then titrated with related Hcl solution.

3.1.2.8 Back titration methods results

3.1.2.8.1Cephalexin monohydrate

The volume of 0.02198 M Hcl required to neutralize 0.01944M NaOH after that consumed by the sample is 22.25ml.

The volume of 0.02198M Hcl required to neutralize the blank (0.01944M NaOH) was 23.55ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

$NaOH + Hcl \rightarrow Nacl + H_2O$

1 mole 1 mole

mmoles of cephalexin monohydrate = mmoles of the blank − mmoles of NaOH that remained after that consumed by the samples.

mmles of the blank (b) = mmoles of NaOH reacted with it

$= V_b X M_b$ $= 23.55 \times 0.02198 = 0.51729$m moles

mmoles of NaOH remained after that consumed by the sample = mmoles of Hcl used in the titration with it

Therefore mmoles of NaOH remained

$= V_{HcL} X M_{HcL} = 22.25 \times 0.02198 = 0.489055$ mmoles

mmloes of cephalexin monohydrate= m moles of blank − mmoles of the NaOH remained in from the sample

$= V_b X M_b - V_s X V_s = (23.55 \times 0.02198) - (22.25 \times 0.02198)$

$= 0.517629 - 0.489055 = 0.028574$

Weight of cephalexin monohydrate

$= 0.028574 \times 365.4 = 10.441$ mg $= 0.010441$g

These weight in 25 ml of cephalexin monohydrate solution

Therefore the weight containedin 250 ml of cephalexin monohydrate solution

$= 0.010441 \times 250/25 = 0.10441$

% of cephalexin monohydrate $= \frac{0.10441 \times 100}{0.5079} = \%\ 20.55$

3.1.2.8.2 Elie Cephalexin capsules

The volume of 0.02364 M Hcl required to neutralize 0.02814M NaOH after that consumed by the sample is 10.95ml.

The volume of 0.02364M Hcl required to neutralize the blank (0.02814M NaOH) was 11.05ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1mole 1mole

$NaOH + Hcl \rightarrow Nacl + H2O$

1 mole 1 mole

mmoles of Elie cephalexin capsules =mmoles of the blank − mmoles of 0.02814 M NaOH solution that remained after that consumed by the sample

mmoles of the blank = mmoles 0.02364 M Hcl that reacted with it

$= V_{Hcl} X M_{Hcl} = 11.05 \times 0.02364 =$ 0.2612 mmoles

mmoles of 0.02418m NaOH that remained after that consumed by the sample

=mmole of Hcl used in titration with it solution

$=V_{Hcl}$ X M_{Hcl}= 10.95×0.02364 =0.25886 mmoles

Since mmoles of Elie cephalexin capsules = mmoles of the blank − mmoles of 0.02364 M Hcl that reacted with it

Then mmoles of Elie cephalexin capsules

= 0.2612 − 0.25885 = 0.00235 mmoles

These mmoles were contained in10 ml of cephalexin solution

mmoles of Elie cephalexin capsules contained in 250 ml of solution =

$$\frac{0.00235\times250}{10} = 0.05875 \text{ mmoles}$$

Weight of Elie cephalexin cpsules = mmoles of it x M wt

= 0.05875x 365.4 = 21.5mg mg

% of Elie cephalexin capsules $=\frac{0.0215\text{x } 100}{1.0026} = 2.14\%$

3.1.2.8.3 Amipharma Cephalxin capsules

The volume of 0.02364 M Hcl required to neutralize 0.02814M NaOH after that consumed by the sample is 22.6ml

The volume of 0.02364M HcL required to neutralize the blank (0.02814M NaOH) was 22.75ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

$NaOH + Hcl \rightarrow Nacl + H_2O$

1 mole 1 mole

mmoles of Amipharma cephalexin capsules=mmoles of the blank − mmoles of 0.02814 M NaOH solution that remained after that consumed by the sample.

mmoles of the blank = mmoles 0.0247 M Hcl reacted with it

$V_{Hcl}XM_{Hcl}$= 22.75×0.0247 = 0.56193 mmoles

mmoles of 0.02418 M NaOH that remained from that consumed by the sample = $V_{Hcl}XM_{Hcl}$ = 22.6×0.0247 =0.55822 mmoles

Since m moles of Amipharma cephalexin capsules = mmoles of the blank − mmoles of 0.0247 M NaOH that remained after that consumed by the sample

Then mmoles of Amipharma cephalexin capsules

= 0.5619 − 0.55822 = 0.00371mmoles

These m moles were contained in10 ml of cephalexin solution

mmoles of Amipharma cephalexin capsules contained in 250 ml of solution =

$$\frac{0.00371\times250}{10} = 0.09275 \text{ mmoles}$$

Weight of Amipharma cephalexin capsules= mmoles of it x M wt

= 0.09275 x 365.4 = 33.89 mg = 0.03389 g

% of Amipharma cephalexin capsuls $= \frac{0.03389 \times 100}{0.9892} = 3.43\%$

3.1.2.8.4 Changahi Cephalexin capsules

The volume of 0.02364 M Hcl required to neutralize 0.02814M NaOH after that consumed by the sample is 10.95ml

The volume of 0.02364M Hcl required to neutralize the blank (0.02814M NaOH) was 11.15ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1 mole 1 mole

$$NaOH + Hcl \rightarrow Nacl + H_2O$$

1 mole 1 mole

mmoles of Changahi cephalexin capsules =mmoles of the blank − mmoles of 0.02814 M NaOH solution that remained after that cosumed by the sample

mmoles of the blank = mmoles 0.02364 M HCl reacted with it

$V_{Hcl}XM_{Hcl}$ = 11.15×0.02364 = 0.26359 mmoles

mmoles of 0.02841 M NaOH remained after that consumed by the sample = $V_{Hcl}XM_{Hcl}$= 10.95×0.0236 =0.25886 mmoles

Since mmoles of Changahi cephalexin capsules = mmoles of the blank − mmoles of 0.02814 M NaOH that remained after that cosumed by the sample the sample

Then mmoles o Changahi cephalexin capsules

= 0.26359 − 0.25886 = 0.0047 mmoles

These mmoles were contained in10 ml of cephalexin solution

mmoles of Changahi cephalexin capsules contained in 250 ml of the solution

$$= \frac{0.0047\times250}{10} = 0.11755 \text{ mmoles}$$

Weight of Changahi cephalexin capsules = mmoles of it x M wt

= 0.1175x 365.4 = 42.935 mg = 0.042935 g

% of Changahi cephalexin capsules $= \frac{0.042935\text{x } 100}{0.9883} = 4.34\%$

3.1.2.8.5 Wafra Cephalexin capsules

The volume of 0.0135 M Hcl required to neutralize 0.02814M NaOH after that consumed by the sample is 14.55ml

The volume of 0.0135M Hcl required to neutralize the blank (0.01M NaOH) was 14.85ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1 mole 1 mole

$$NaOH + Hcl \rightarrow Nacl + H_2O$$

1mole 1mole

mmoles of Wafra cephalexin capsules =mmoles of the blank − mmoles of 0.01 M NaOH solution that remained after that consumed by the sample

mmoles of the blank = mmoles 0.0135 M Hcl reacted with it

$= V_{Hcl}XM_{Hcl} = 14.85\times0.0135 =$ 0.2005 mmoles

mmoles of 0.01 M NaOH remained after that consumed by the sample = $V_{Hcl}XM_{Hcl} = 14.55\times0.0135 =$0.1964 mmoles

Since mmoles of Wafra cephalexin capsules = mmoles of the blank − mmoles of 0.01M NaOH that remained after that consumed by the sample

Then mmoles of Wafra cephalexin capsules = 0.2005 − 0.1964 = 0.0041 mmoles

These mmoles were contained in10 ml of cephalexin solution

mmoles of Wafra cephalexin capsules contained in 250 ml of solution =

$$\frac{0.0041\times250}{10} = 0.1025 \text{ mmoles}$$

Weight of Wafra cephalexin capsules = mmoles of it x M wt

= 0.1025x 365.4 = 37.45 mg = 0.03745g

$$\% \text{ of Wafra cephalexin capsules} = \frac{0.0375\text{x } 100}{0.9952} = \% 3.77$$

3.1.3 Conductometeric titration of cephalexin with NaOH solution

3.1.3.1 Cephalexin monohaydreate

3.1.3.1.1 Reagents

1- Cephalexin monohaydreate solution (0.25g/250 ml distilled water)

2- 0.025 M NaOH solution

3.1.3.2 Elie Cephalexin capsule

3.1.3.2.1 Reagents

1- Elie capsules cephalexin solution

2- 0.2814 M NaOH solution

3.1.3.1 Changahi Cephalexin capsule

3.1.3.1.1 Reagents

1- Changahi capsules cephalexin solution

2- 0.2814 M NaOH solution

3.1.3.4 Amipharma Cephalexin capsule

3.1.3.4.1 Reagents

1- Amipharma capsules cephalexin solution

2- 0.2814 M NaOH solution

3.1.3.5 Wafra Cephalexin capsule

3.1.3.5.1 Reagents

1- Wafra capsules cephalexin solution

2- 0.284 M NaOH solution

3.1.3.5.2 General apparatus

1) 50 ml pipitte
2) 50 ml measuring cylinder
3) Magnetic stirrer and magnetic rod
4) Conductometer
5) 100 ml beaker

3.1.3.5.3 General procedure

A volume of 50 ml of cephalexin solution was taken into 100 ml beaker then titrated coductometrically with 0.025 M NaOH solution wich was added in intervals of 1ml portion and stirred with the magnetic stirrer and the conductivity was recorded after each addition as shown in' Table (3.1). A graph of conductivity corrected via the volume of NaOH solution was plotted, and neutralization volume of NaOH solution was found from the graph, Fig No (3.1) then the amount of cephalexin was calculated according to that volume.

Weights of 1.0775 g, 1.0634 g, 1.0418 g and 1.038 g of Elie, Changahi, Amipharma, and Wafra cephalexin capsules respcetively, which respectively contains 1.008g,0.9883g,0.9892g and 0.9952g of pure cephalexin each were dissolved in about 200 ml of distilled water with aid of magnetic stirrer and magnetic rod, transferred to 250 ml volumetric flask. Completed up to the mark with distilled water and filtered.

50 ml of aliquot were taken into 100 ml beaker, it's conductivity was measured, then the related NaOH solution was added in portions of 0.2 ml and the conductivity was measured after each addition and stirring

and recorded as shown in' tables (3.2,3.3,3.4,3.5) , a graph of corrected conductivities against volume of NaOH solution was plotted as shown in' Figs (3.2, 3.3, 3.4, 3.5,). The amount of cephalexin capsules was calculated for each.

3.1.3.6 Results of conductometeric titration with NaOH

3.1.3.6.1cephalexin monohydrate

The volume of 0.025 M NaOH from the graph is 5.7 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of cephalexin monohydrate = mmoles of 0.025m NaOH

$= V_{NaOH} X M_{NaOH} = 5.7 \times 0.025 = 0.1425$ mmoles

These m moles are contained in 50ml of cephalexin monohydrate solution

mmoles of cephalexin monohydrate that contained in 250 ml cephalexin monohydrate solution

$$= \frac{5.7 \times 0.025 \times 250}{50} = 0.7125 \text{ mmoles}$$

Weight of cephalexin monohydrate

= mmoles ×M wt = 0.7125× 365.4 =260mg =.260g

$$\% \text{ of cephalex monohydrate} = \frac{0.260 \times 100}{0.25} = 104.0\%$$

Table 3.1 Conductometreic titration of 50ml cephalexin monohydrate with 0.025M NaOH

Vol. of NaOH/ml	Ω / ms	Ω $(V_o+V)/V_o$ ms
0.00	0.01345	0.01345
1	0.0409	0.0417
2	0.0749	0.0778
3	0.1037	0.1099
4	0.1335	0.144
5	0.1611	0.1772
6	0.2040	0.228
7	0.2810	0.320
8	0.3670	0.4257
9	0.4460	0.526
10	0.5170	0.620
11	0.6000	0.732
12	0.6600	0.818
13	0.7320	0.922
14	0.8010	1.025
15	0.8690	1.1297
16	0.9300	1.2276
17	0.9880	1.323
18	1.0450	1.421
19	1.0950	1.511
20	1.1470	1.6058

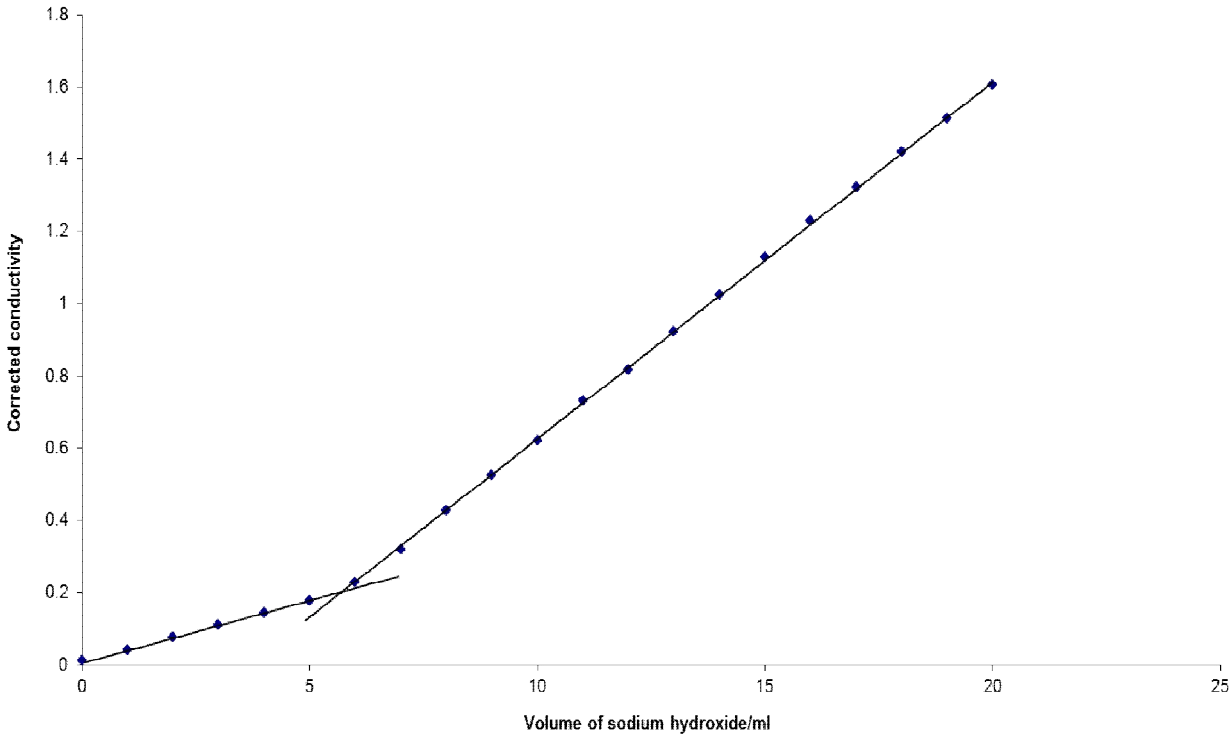


Fig 3.1 Conductmetric titration of cephalexin monohydrate with 0.025M NaOH

3.1.3.6.2 Elie cephalexin capsules

Volume of 0.2814 M NaOH solution from the graph is 2.08 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of Elie cephalexin capsules = m moles 0.2814 M NaOH

$= V_{NaOH} \times M_{NaOH}$ $= 2.08 \times 0.2814$ = 0.5853 mmoles

These mmoles were contained in 50 ml of Elie cephalexin capsules solution

mmoles that contained in 250 ml of the solution $= \frac{0.5853 \times 250}{50}$

= 2.927 mmoles

Weight of Elie cephalexin capsules = mmoles×M wt

= 2.927×365.4 =1069.53 mmoles

% of Alie cephalexin capsules $= \frac{1069.53 \times 100}{1000 \times 1.008} = 106.1\%$

Table 3.2 Conductometeric titration of 50ml Elie Cephalexin capsule with 0.2814M NaOH

Vol.of(NaOH/ml)	Ω /ms	$\Omega(V_o+V)/V_o$ ms
0.0	0.0545	0.0545
0.2	0.136	0.1365
0.4	0.213	0.2147
0.6	0.283	0.2860
0.8	0.358	0.3637
1.0	0.432	0.4406
1.2	0.512	0.5240
1.4	0.574	0.5900
1.6	0.637	0.6573
1.8	0.719	0.7448
2.0	0.789	0.8205
2.2	0.979	1.0220
2.4	1.197	1.2450
2.6	1.433	1.5075
2.8	1.665	1.7580
3.0	1.873	1.9850
3.2	2.090	2.2230

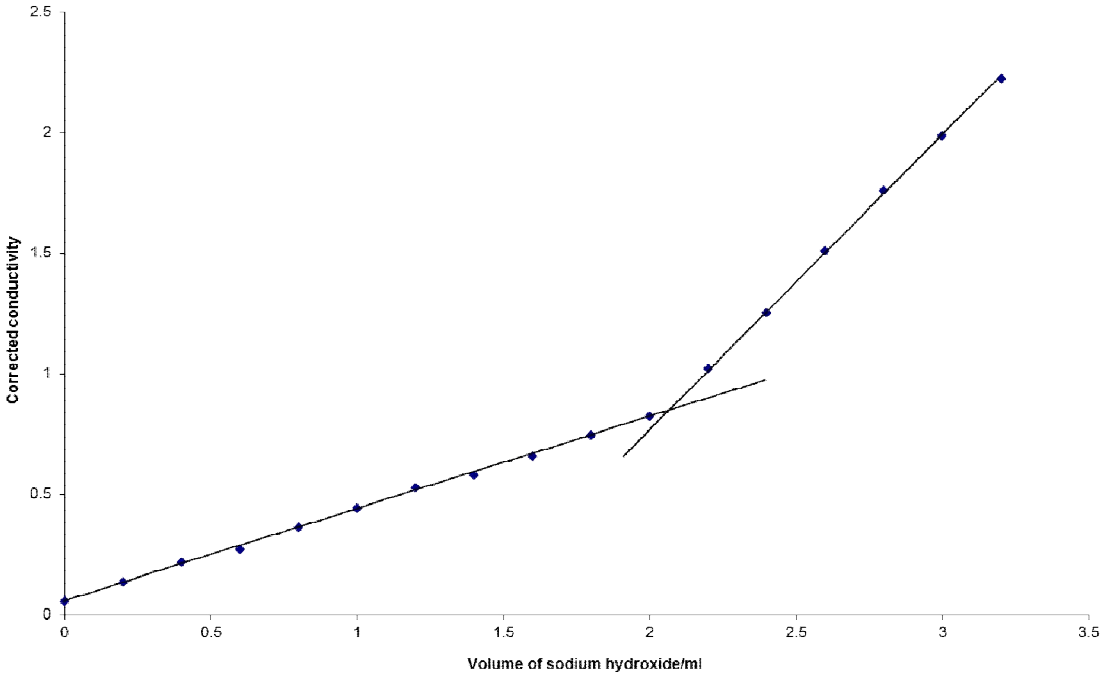


Fig 3.2 Conductmetric titration of Elie cephalexin capsules with 0.2814M NaOH

3.1.3.6.3 Amipharma cephalexin capsules

Volume of 0.2814 M NaOH solution from the graph is2.1 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of Amipharma cephalexin capsules = mmoles 0.2814 M NaOH

$=V_{NaOH}XM_{NaOH}$ $=2.1\times0.2814$ $= 0.59094$ mmoles

These mmoles were contained in 50 ml of Amipharma cephalexin capsules solution

mmoles that contained in 250 ml of the solution of Amipharma cephalexin capsules $= \frac{0.59094\times250}{50}$ $= 2.9547$ mmoles

Weight of Amiphama cephalexin capsules

= mmoles×m Mt = 2.9547×365.4 =1079.65 moles

% of Amipharma cephalexin capsules $= \frac{1079.65\times100}{1000\times0.9892} = 109.1\%$

Table 3.3 Conductometeric titration of 50ml Amipharma cephalexin capsules with 0.2814M NaOH

Vol.of(NaOH/ml)	Ω /ms	$\Omega(V_o + V) / V_o$ ms
0.0	0.0615	0.0615
0.2	0.1338	0.1343
0.4	0.213	0.2147
0.6	0.286	0.2890
0.8	0.351	0.3560
1.0	0.432	0.4406
1.2	0.504	0.5160
1.4	0.582	0.5980
1.6	0.658	0.6790
1.8	0.727	0.7530
2.0	0.792	0.8240
2.2	1.023	1.0680
2.4	1.281	1.3420
2.6	1.511	1.5890
2.8	1.744	1.8410
3.0	1.976	2.0940

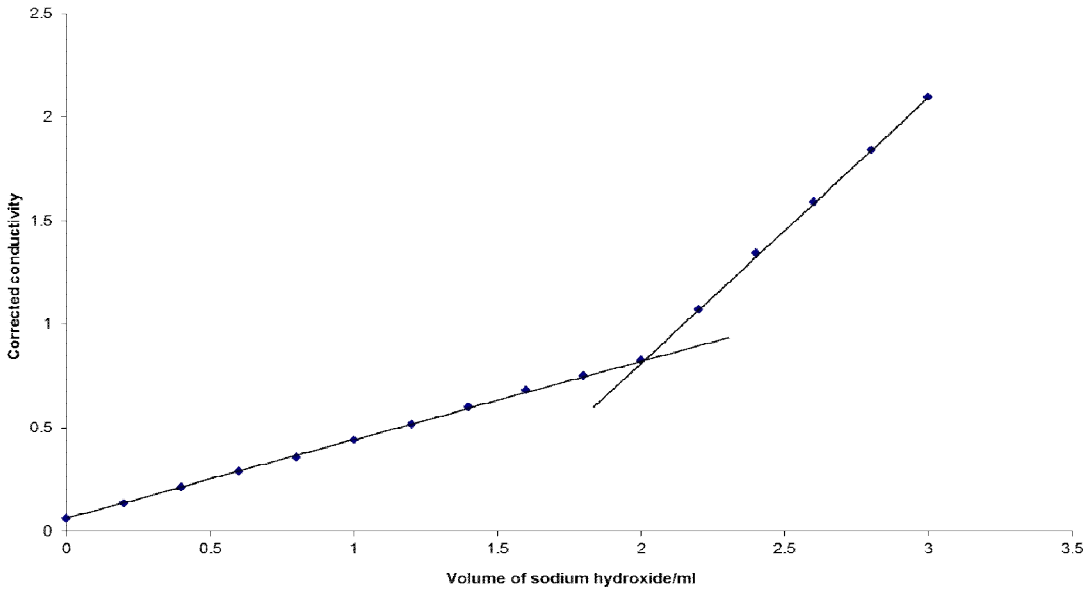


Fig 3.3 Conductometric titration of Amipharma cephalexin capsules with 0.2814M NaOH

3.1.3.6.4 Changahi cephalexin capsules

Volume of 0.2814 M NaOH solution from the graph is 1.96 ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1 mole 1 mole

mmoles of Changahi cephalexin capsules = mmoles 0.2814 M NaOH

$=V_{NaOH}XM_{NaOH}$ $=1.96\times0.2814$ = 0.5515 mmoles

These mmoles were contained in 50 ml of Changahi cephalexin capsules solution

mmoles that contained in 250 ml of the solution of Changahi cephalexin capsules $= \frac{0.5515\times250}{50}$ = 2.7575 mmoles

Weight of Changahi cephalexin capsules

= mmoles×M wt = 2.7575×365.4 =1007.59 moles

% of Changahi cephalexin capsules $= \frac{1007.59\times100}{1000\times0.9882} = 101.96\%$

Table 3.4 Conductometeric titration of 50ml Changahi cephalexin capsules with 0.2814m NaOH

Vol.of(NaOH/ml)	Ω /ms	$\Omega(V_o + V) / V_o$ ms
0.0	0.0736	0.0736
0.2	0.153	0.1536
0.4	0.240	0.2419
0.6	0.311	0.3147
0.8	0.371	0.3769
1.0	0.471	0.4800
1.2	0.531	0.5437
1.4	0.605	0.6219
1.6	0.675	0.6966
1.8	0.763	0.7900
2.0	0.838	0.8715
2.2	1.023	1.1080
2.4	1.062	1.3810
2.6	1.318	1.6210
2.8	1.792	1.8920
3.0	2.010	2.1306

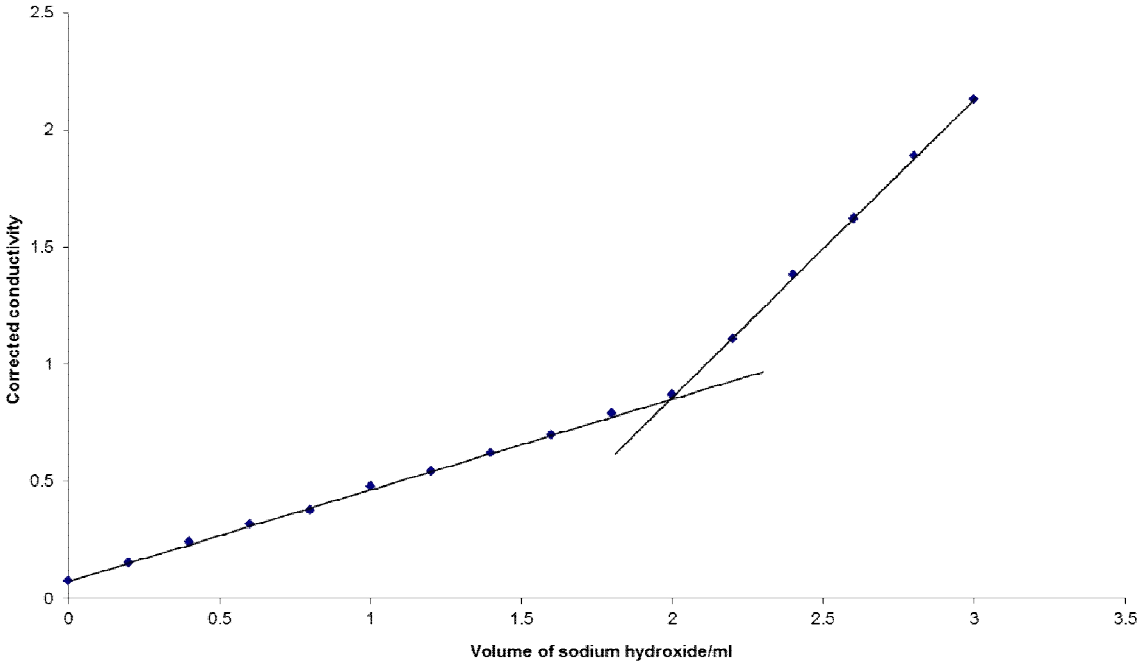


Fig 3.4 Conductmetric titration of Shangahi cephalexin capsules with 0.2814M NaOH

3.1.3.6.5 Wafra cephalexin capsules

Volume of 0.284m NaOH solution from the graph is2.1 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of Wafra cephalexin capsules = mmoles 0.284 M NaOH

$=V_{NaOH} X M_{NaOH}$ $=2.1\times0.284$ = 0.5964mmoles

These mmoles were contained in 50 ml of Wafra cephalexin capsules solution

mmoles that contained in 250 ml of the solution of Wafra cephalexin capsules $= \frac{0.5964\times250}{50} = 2.982$mmoles

Weight of Wafra cephalexin capsules

= mmoles×M wt = 2.982×365.4 =1089.6. mmoles

% of Wafra cephalexin capsules $= \frac{1089.6\times100}{1000\times0.959}$ = % 113.6

Table 3.5 Conductometeric titration of 50ml Wafra cephalexin capsules

0.284M NaOH

Vol.of (NaOH/ml)	Ω /ms	$\Omega(V_o + V) / V_o$ ms
0.0	0.043	0.0483
0.3	0. 156	0.1565
0.6	0. 283	0.2863
0.9	0.392	0.3990
1.2	0.503	0.5150
1.5	0.606	0.6240
1.8	0.710	0.7355
2.1	0. 874	0.9110
2.4	1.138	1.1930
2.7	1.443	1.5200
3.0	1.744	1.8490
3.3	2.040	2.1750
3.6	2.310	2.4760

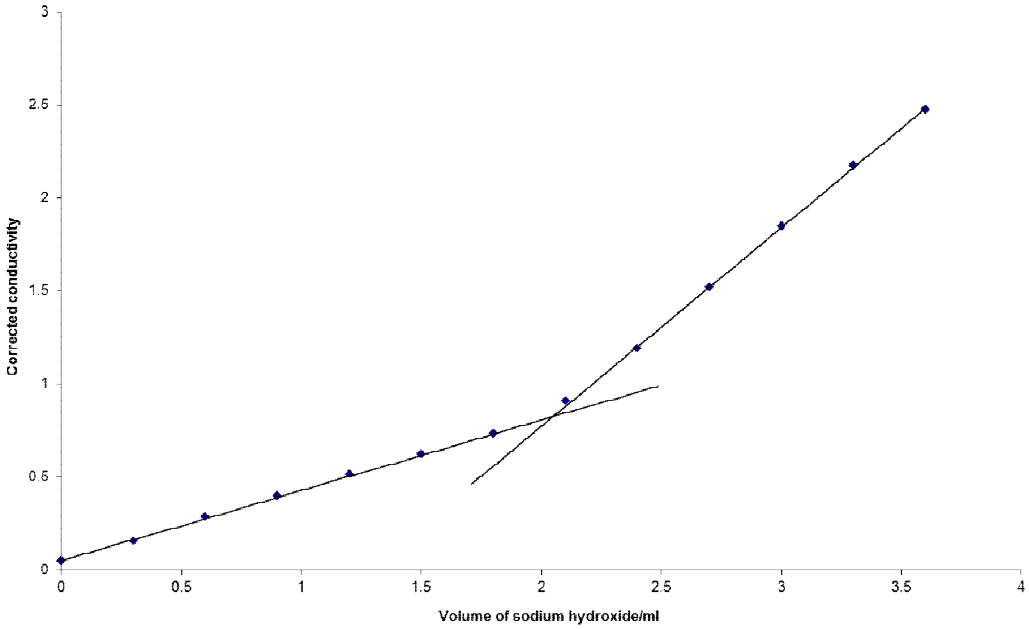


Fig 3.5 Conductmetric titration of Wafra cephalexin capsules with 0.284M NaOH

3.1.4 Conductometerictitration of cephalexin with NH4OH solution

3.1.4.1Cephalexin monohydrate

3.1.4 .1.1 Reagents

1- Cephalexin monohydrate solution (0.5 g/250 ml distilled water)

2- 0.0935 M NH_4OH solution

3.1.4.2 Elie cephalexin capsules

3.1.4.2.1 Reagents

1-Elie cephalexin capsules solution

2- 0.220 M NH_4OH solution

3.1.4.3 Changahi cephalexin capsules

3.1.4.3.1 Reagents

1-Changahi cephalexin capsules solution

2- 0.220 M NH_4OH solution

3.1.4.4 Amipharma cephalexin capsules

3.1.4.4.1 Reagents

1-Amipharma cephalexin capsules solution

2- 0.220 M NH_4OH solution

3.1.4.5 Wafra cephalexin capsules

3.1.4.5.1 Reagents

1-Wafra cephalexin capsules solution

2-0.250 M NH_4OH solution

3.1.4.5.2 General apparatus

1- 50 ml pipette

2- 50 ml measuring

3- Magnetic stirrer and magnetic rod

4- conductometer

5- 100 ml beaker

3.1.4.5.3 General procedure

A volume of 50 ml of aliquot was taken into 100 ml beaker, then titrated with 0.0935 M NH_4OH solution. The conductivity was measured first, then NH4OH was added in a portion of 0.2 ml, the conductivity was measured after each addition and stirring and recorded as shown' in table (3.6). A graph of corrected conductivities virus volume of NH_4OH solution was plotted graph the end point was detected from the same graph as shown' in Fig. (3.6), and the amount of cephalexin was calculated.

Weights of 1.0714 g, 1.0634 g, 1.0418 g and 1.038 g of Elie, Changahi, Amipharma and Wafra cephalexin capsules respectively, which respectively contains 1.0026g , 0.9883g , 0.9892g and 0.959g of pure cephalexin , each were dissolved in about 200 ml of distilled water with aid of magnetic stirrer and magnetic rod, transferred to 250 ml volumetric flask. Completed up to the mark with distilled water and filtered.

50 ml of aliquot were taken into 100 ml beaker, it is conductivity was measured, then the related NH_4OH solution was added in portions of 0.2 ml and the conductivity was measured after each addition and stirring and recorded as shown' in tables (3.7,3.8,3.9,3.10), a graph of corrected conductivities against volume of NH_4OH solution was plotted as shown' in Figs (3.7,3.8,3.9,3.10). The amount of cephalexin capsules was calculated for each.

3.1.4.6 Results of conductometeric titration of cephalexin with NH_4OH

3.1.4.6.1Cephalexin monohydrate

The volume of 0.0935 M NH_4OH from the graph is 2.925 ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1mole

mmoles of cephalexin monohydrate =m moles of 0.0935m NH_4OH

$= V_{NH4OH} X M_{NH4OH} = 2.925 \times 0.0935 = 0.272$mmoles

These mmoles were contained in 50 ml of cephalexin monohydrate solution

mmoles of cephalexin monohydrate that contained in 250 ml of the solution cephalexin monohdrate

$$= \frac{0.272 \times 250}{50} = 1.360 \text{ mmoles}$$

Weight of cephalexin monohydrate = mmols of it x it is M wt

$$= 1.360 \times 36504 = 497.9 \text{ mg}$$

$$\% \text{ of cephalexin monohydrate} = \frac{497.9 \times 100}{1000 \times 0.5} = 99.6\%$$

Table 3.6 Coductometeric titration of 50ml cephalexin monohydrate with 0.0935M NH_4OH

Vol .of NH_4OH/ml	Ω /ms	$\Omega(V_o+V)/V_o$ ms
0.0	0.0229	0.0229
0.4	0.0698	0.0703
0.8	0.0131	0.1332
1.2	0.1892	0.1937
1.6	0.245	0.2520
2.0	0.299	0.3109
2.4	0.351	0.3678
2.8	0.391	0.4128
3.2	0.415	0.4416
3.6	0.428	0.4588
4.0	0.431	0.4655
4.4	0.435	0.4783
4.8	0.437	0.4789
5.2	0.439	0.4847
5.6	0.440	0.4893
6.0	0.441	0.4939

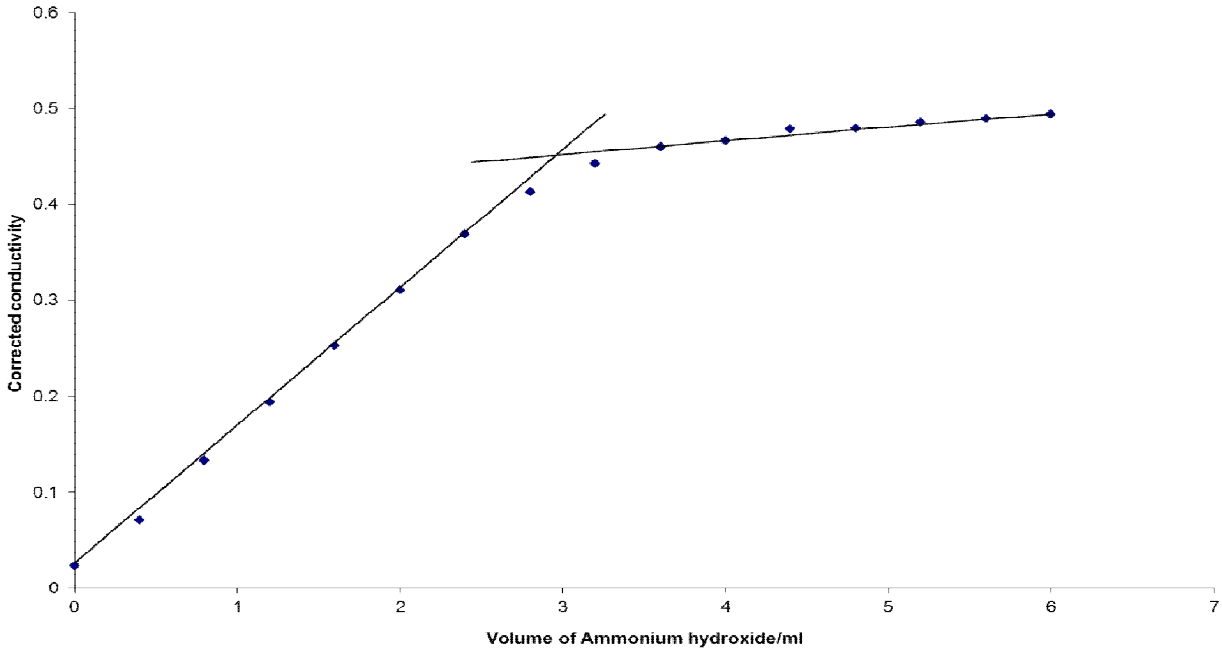


Fig 3.6 Conductmetric titration of cephalexin monohydrate with 0.0935M NH_4OH

3.1.4.6.2 Elie cephalexin capsules

The volume of 0.220 M NH_4OH from the graph is 2.52 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of Elie cephalexin capsules = mmoles oh 0.220 M NH_4OH = $V_{NH4OH} \times M_{NH4OH}$ $=2.52 \times 0.220$ = 0.5544 mmoles

These mmoles were contained in 50 ml of Elie cephalexin capsules solution

mmoles of Elie cephalexin capsules that contained in 250 ml of the solution of Elie cephalexin capsules

$$= \frac{0.5544 \times 250}{50} = 2.772 \text{ mmoles}$$

Weight of Elie cephalexin capsules = mmoles×M wt

= 2.772×36504 = 1012.89 mmoles

% of Elie cephalexin capsules $= \frac{1012.89 \times 100}{1000 \times 1.0026} = 101.02\%$

Table 3.7 Conductometeric titration of 50ml Elie cephalexin capsule with 0. 220M NH_4OH

Vol of (NH_4OH/ml)	Ω / ms	$\Omega(V_o + V) / V_o$ ms
0.0	0.0609	0.0609
0.2	0.1394	0.1399
0.4	0.2150	0.2167
0.6	0.288	0.2914
0.8	0.359	0.3647
1.0	0.440	0.4488
1.2	0.506	0.5181
1.4	0.583	0.5993
1.6	0.655	0.6759
1.8	0.715	0.7410
2.0	0.775	0.8060
2.2	0.833	0.8696
2.4	0.890	0.9327
2.6	0.936	0.9846
2.8	0.962	1.0158
3.0	0.982	1.0490
3.2	0.996	1.0597
3.4	1.004	1.0770
3.6	1.006	1.0784
3.8	1.010	1.0825
4.0	1.011	1.0918

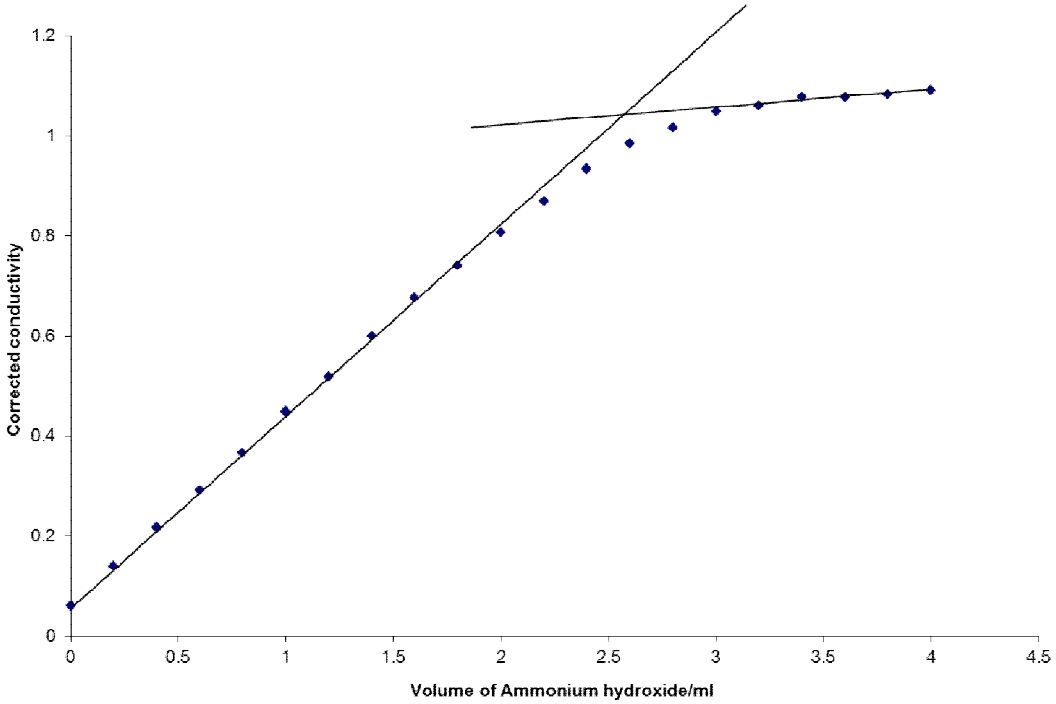


Fig 3.7 Conductometeric titration of Elie cephalexin capsule with 0.220M NH_4OH

3.1.4.6.3 Amipharma cephalexin capsules

The volume of 0.220m NH_4OH from the graph is 2.56 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of Amipharma cephalexin capsules

= mmoles of 0.220 M NH_4OH =

$V_{NH4OH} \times M_{NH4OH}$ $=2.56 \times 0.22$ = 0.5632 mmoles

These mmoles were contained in 50 ml of Amipharma cephalexin capsules solution

mmoles of Amipharma cephalexin that contained in 250 ml of the solution of Amipharma cephalexin capsules

$$\frac{0.5632 \times 250}{50} = 2.816 \text{ mmoles}$$

Weight of Amiphama cephalexin capsules = mmoles×M wt

= 2.816 ×36504 = 1028.97 mmoles

$$\% \text{ of Amipharma cephalexin capsules} = \frac{1028.97 \times 100}{1000 \times 0.9892} = 104.02\%$$

Table 3.8 Conductometeric titration of 50ml Amipharma cephalexin capsule with 0.220M NH_4OH

Vol of (NH_4OH/ml)	Ω / ms	$\Omega(V_o+V)/V_o$ ms
0.0	0.064	0.0647
0.2	0.135	0.1360
0.4	0.214	0.2160
0.6	0.288	0.2910
0.8	0.361	0.3667
1.0	0.427	0.4355
1.2	0.493	0.5048
1.4	0.562	0.5777
1.6	0.625	0.6450
1.8	0.696	0.7210
2.0	0.747	0.7760
2.2	0.808	0.8430
2.4	0.852	0.8928
2.6	0.884	0.9290
2.8	0.908	0.9588
3.0	0.927	0.9826
3.2	0.935	0.9948
3.4	0.940	1.0090
3.6	0.946	1.0130

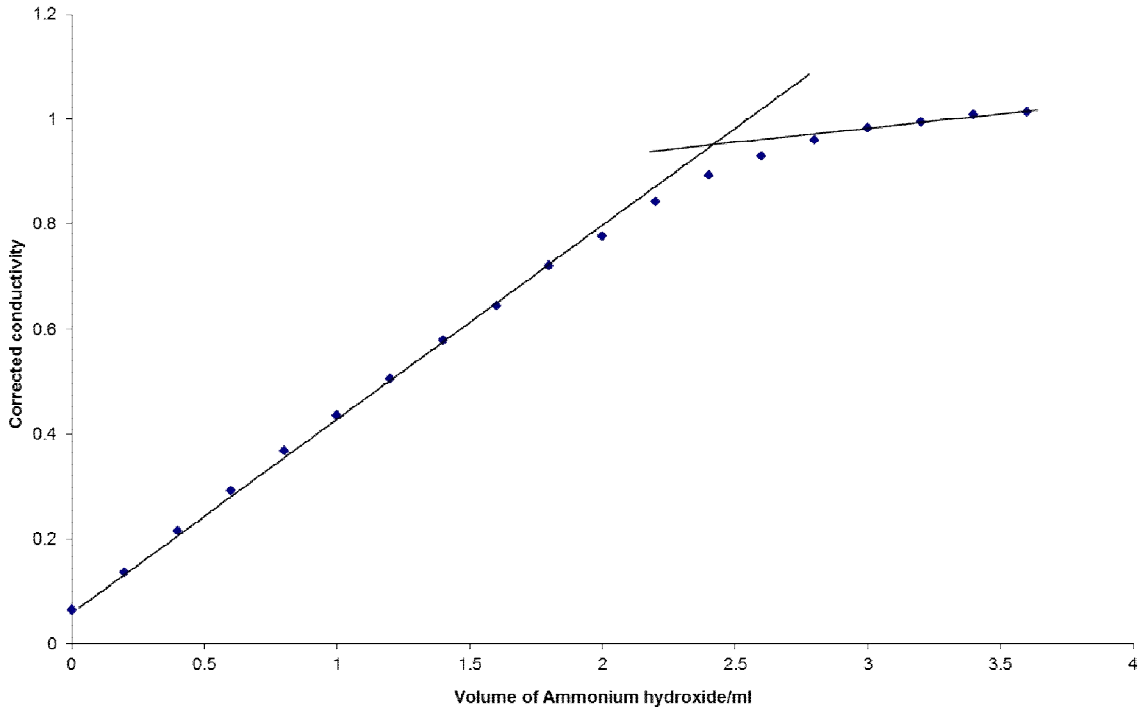


Fig 3.8 Conductometeric titration of Amipharma cephalexin capsule with 0.220 M NH_4OH

3.1.4.6.4 Changahi cephalexin capsules

The volume of 0.220 M NH_4OH from the graph is 2.52 ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1 mole 1 mole

mmoles of Changahi cephalexin capsules =mmoles of 0.220 M NH_4OH

$= V_{NH4OH} \times M_{NH4OH}$ $=2.52 \times 0.220$ $= 0.5544$ mmoles

These mmoles were contained in 50 ml of Changahi cephalexin capsules solution

mmoles of Changahi cephalexin capsules that contained in 250 ml of the solution Changahi cephalexin capsules

$$\frac{0.5544 \times 250}{50} = 2.772 \text{ mmoles}$$

Weight Changahi cephalexin capsules = mmoles×M wt

$= 2.772 \times 365.4 = 1012.89$ mmoles

$$\% \text{ of Changahi cephalexin capsules} = \frac{1012.89 \times 100}{1000 \times 0.9883} = 102.5\%$$

Table 3.9 Conductometeric titration of 50ml Changahi cephalexin capsules with 0.220M NH_4OH

Vol of (NH_4OH/ml)	Ω / ms	$\Omega(V_o + V) / V_o$ ms
0.0	0.072	0.0721
0.2	0.156	0.1560
0.4	0.223	0.2247
0.6	0.301	0.3046
0.8	0.376	0.3820
1.0	0.451	0.4600
1.2	0.520	0.5350
1.4	0.576	0.5920
1.6	0.649	0.6697
1.8	0.712	0.7376
2.0	0.777	0.8080
2.2	0.737	0.8738
2.4	0.893	0.9358
2.6	0.927	0.9750
2.8	0.949	1.0020
3.0	0.967	1.0250
3.2	0.988	1.0290
3.4	0.975	1.0400
3.6	0.982	1.0520
3.8	0.988	1.0630

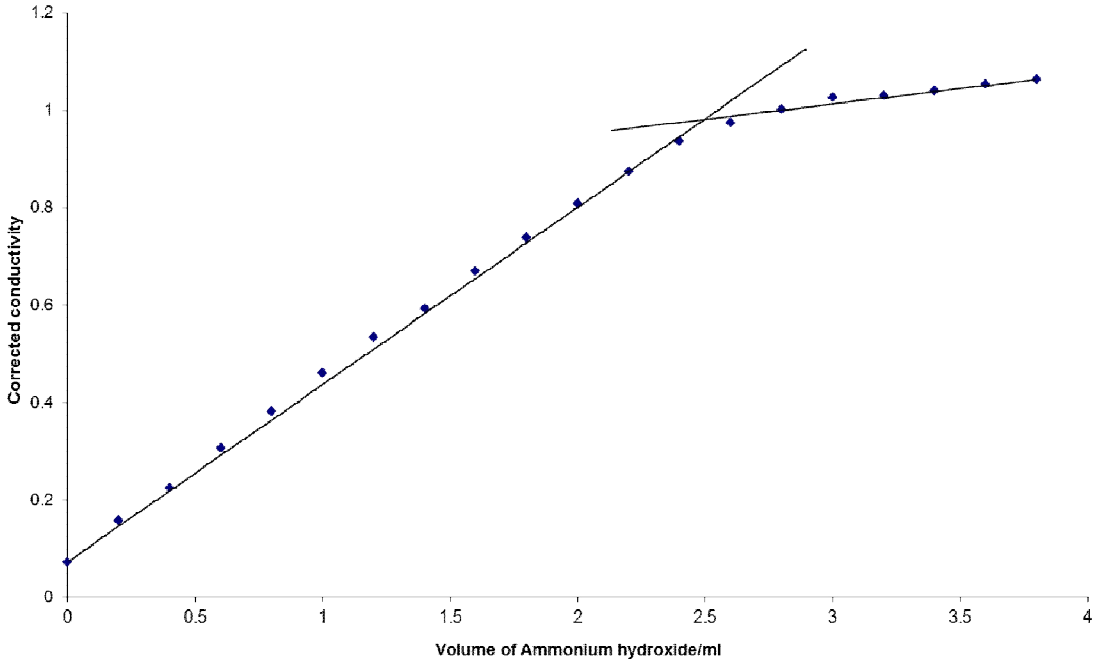


Fig 3.9 Conductometeric titration of Changahi cephalexin capsules with 0.220M NH_4OH

3.1.4.6.5 Wafra cephalexin capsules

The volume of 0.25M NH_4OH from the graph is 2.12ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1mole 1mole

mmoles of Wafra cephalexin capsules = mmoles NH_4OH = $V_{NH4OH} \times M_{NH4OH}$ $=2.12\times0.25$ = 0.53 mmoles

These mmoles were contained in 50ml of Wafra cephalexin capsules solution

mmoles of Wafra cephalexin capsules that contained in 250ml of the solution of Wafra cephalexin capsules

$$\frac{0.53\times250}{50} = 2.65 \text{ mmoles}$$

Weight of Wafra cephalexin capsules = 2.65x365.4 = 968.3 mg

% of Wafra cephalexin capsules cephalexin capsules

$$= \frac{968.3\times100}{1000\times0.959} = 100.97\ \%$$

Table 3.10 Conductometeric titration of 50ml Wafra cephalexin capsules with 0.25M NH_4OH

Vol of (NH_4OH/ml)	Ω / ms	$\Omega(V_o + V) / V_o$ ms
0.0	0.047	0.0471
0.3	0.164	0.1650
0.6	0.286	0.2890
0.9	0.404	0.4100
1.2	0.521	0.5330
1.5	0.641	0.6600
1.8	0.743	0.7700
2.1	0.818	0.8520
2.4	0.866	0.9076
2.7	0.885	0.9330
3.0	0.895	0.9490
3.3	0.903	0.9630
3.6	0.906	0.9710
3.9	0.910	0.9810

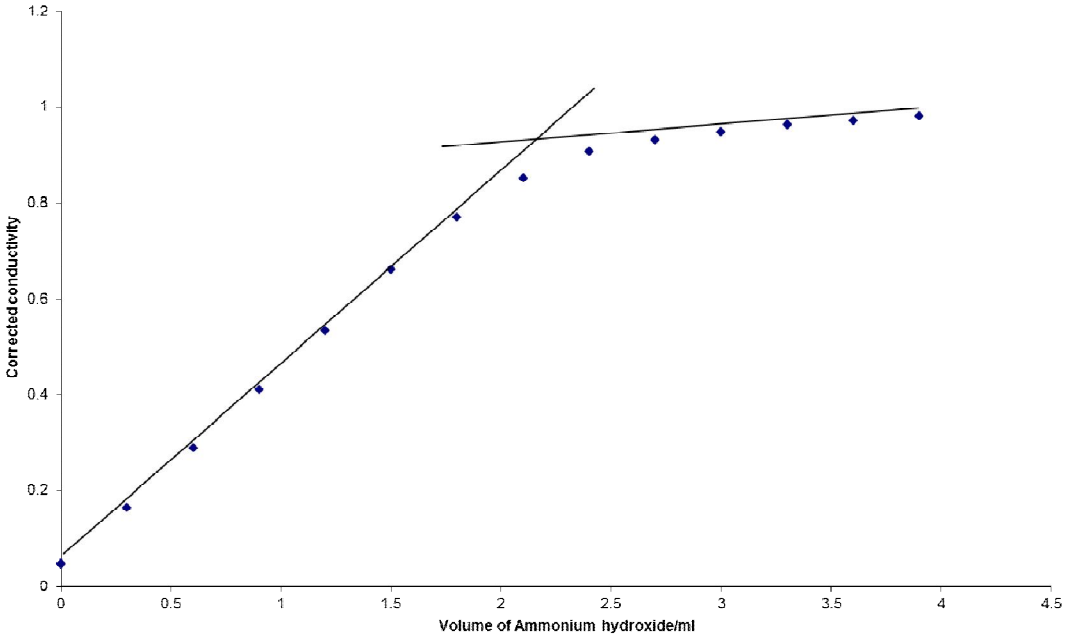


Fig 3.10 Conductometeric titration of Wafra cephalexin capsules with 0.25M NH_4OH

3.1.5 Potentionmetric titration of cephalexin with NaOH solution

3.1.5.1 Cephalexin monohydrate

3.1.5.1.1 Reagents

1- Cephalexin solution (0.25 g/250 ml of distilled water)

2- 0.025 M NaOH solution

3.1.5.2 Elie cephalexin capsules

3.1.5.2.1 Reagents

1-Elie cephalexin capsules solution

2- 0.2814 M NaOH solution

3.1.5.3 Changahi cephalexin capsules

3.1.5.3.1 Reagents

1- Changahi cephalexin capsules solution

2- 0.2814 M NaOH solution

3.1.5.4 Amipharma cephalexin capsules

3.1. 5.4.1 Reagents

1-Amipharma cephalexin capsules solution

2- 0.2814 M NaOH solution

3.1.5.5 Wafra cephalexin capsules

3.1.5.5.1Reagents

1-Wafra cephalexin capsules solution

2- 0.284 M NaOH solution

3.1.5.5.2 General apparatus

1- 50 ml measuring cylinder

2- 50 ml pipette

3- pH meter

4- Magnetic sirrer and magnetic rod

5- 100 ml beaker

3.1.5.3 General procedure

An aliquod of 50 ml of cephalexin solution was taken into 100 ml beaker then titrared potentiometerically with 0.025 M NaOH solution. NaOH solution was added in 1 ml portions and stirred after each addition of NaOH solution and the pH value was recorded after each addition and also the calculated ($\Delta pH/\Delta V$) ,table (3.11). Graphs of pH values virus volumes of NaOH and ($\Delta pH/\Delta V$) virus volume were plotted as shown' in Fig (3.11,3.12) and the amount of cephalexin was calculated.

Weights of 1.0714 g. 1.0634 g, 1.0418 g and 1.0380 g of Elie, Changahi, Amipharma and Wafra cephalexin capsules respectively which respectively contains 1.0026g ,0.9883g , 0.9892g and 0.959g each were dissolved in about 200 ml of distilled water with aid of magnetic stirrer and magnetic rod, transferred to 250 ml volumetric flask. Completed up to the mark with distilled water and filtered.

50 ml of aliquot from each were taken into 100 ml beaker, it's pH was measured, then the related NaOH solution, the pH of the solution was measured after each addition and stirring and also the calculated ($\Delta pH/\Delta V$) as shown' in Tables(3.12,3.13,3.14,3.15) Graphs of pH values against the volume of NaOH added and ($\Delta pH/\Delta V$) against the volume of NaOH were plotted.

The amount of cephalexin of each was calculated from end points obtained from the graphs as shown' in Figs [(3.13,3.14) . (3.15,3.16) , (3.17,3.18) , (3.19,3.20)],

3.1.5.6 Results of potentionmetric titration of cephalexin with NaOH

3.1.5.6.1 Cephalexin monohydrate

1- From the graph of pH/V the neutralization volume of 0.02 M NaOH is 5.8 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1mole 1 mole

mmoles of cephalexin monohydrate = mmoles oh 0.025 M NaOH

$= V_{NaOH} X M_{NaOH} = 5.8 \times 0.025 = 0.145$

These mmoles were contained in 50 ml of cephalexin monohydrate solution

mmoles that contained in 250 ml of the solution

$= 0.145 \times 250/50 = 0.725$ mmoles

Weight of cephalexin monohydrate = mmoles of it × M wt

$= 0.725 \times 365.4 = 264.915mg = 0.265g$

$$\% \text{ of cephalexin monohydrate} = \frac{0.265 \times 100}{0.25} = 106.00\%$$

2- From ΔpH/ ΔV the neutralization volume of 0.025 M NaOH is5.755ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of cephalexin monohydrate = mmoles of 0.025 M NaOH

$= V_{NaOH} X M_{NaOH} = 5.755 \times 0.025 = 0.144$ mmoles

These mmoles were contain in 50 ml of cephalexin monohydrate solution

mmoles that contain In 250 ml of cephalexin monohydrate solution

$= 0.0.144 \times 250)/50 = 0.720$ mmoles

Weight of cephalexin monohydrate = mmoles of it × M wt

$= 0.720 \times 365.4 = 263.1mg$

$$\% \text{ of cephalexin monohydrate} = \frac{263.1 \times 100}{1000 \times 0.25} = 105.2\%$$

Table 3.11 Potentiometeric titration of 50ml cephalexin monohydrate with 0.025M NaOH

Vol. of NaOH/ml	pH	δ pH/δ V	Vol.of NaOH/ml	pH	δ pH/δ V
0.00	4.021		7.00	10.148	
		1.961			0.969
1.00	5.982		8.00	10.483	
		0.762			0.335
2.00	6.744		9.00	10.574	
		0.416			0.136
3.00	7.160		10.00	10.672	
		0.454			0.098
4.00	7.614		11.00	10.768	
		0.380			0.096
5.00	7.994		12.00	10.855	
		0.1185			0.087
6.00	9.179				

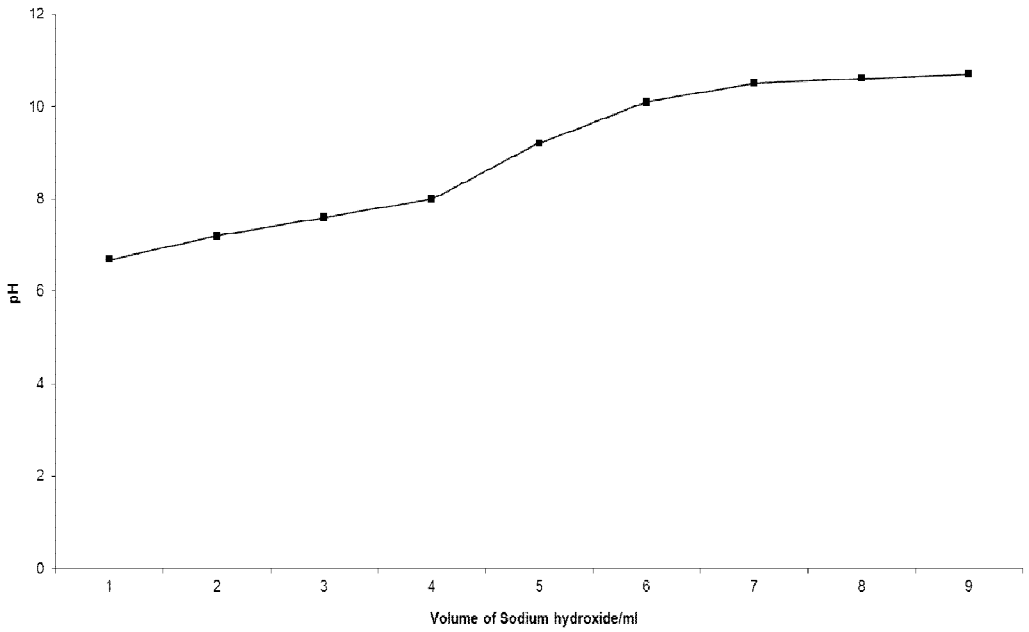


Fig 3.11 Potentiometeric titrationof 50ml cephalexin monohydrate with 0.025MNaOH-1

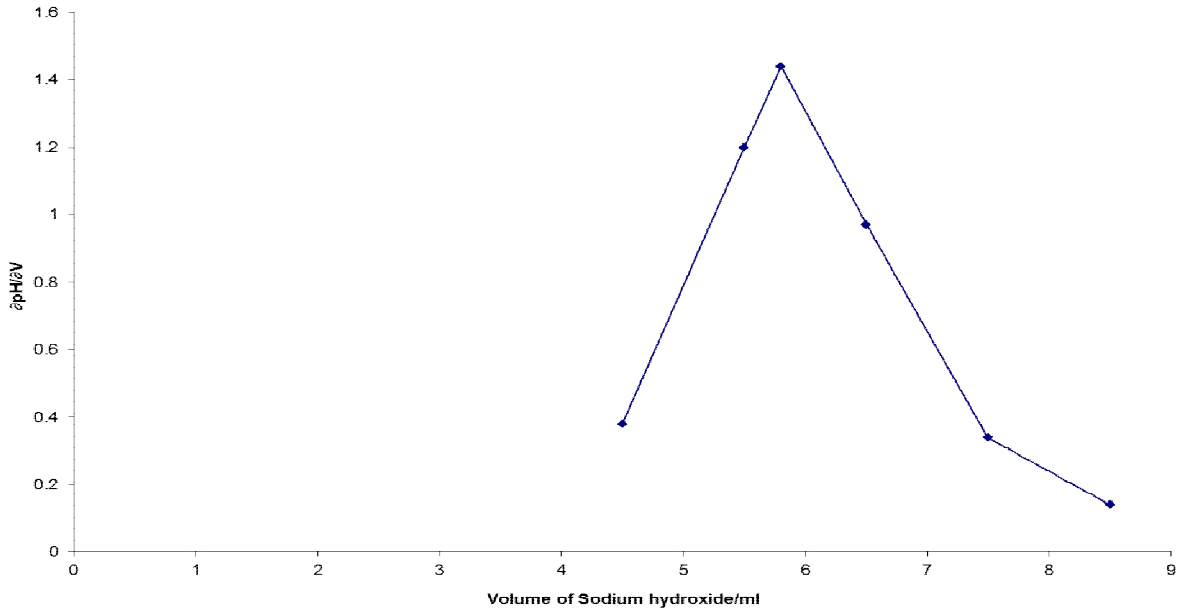


Fig 3.12 Potentiometeric titration of 50ml cephalexin monohydrate with 0.025M NaOH-2

3.1.5.6.2 Elie cephalexin capsules

1- From of pH/V the volume of 0.2814 M NaOH is2.00ml

Q −COOH + NaOH → Q −COONa + H_2O

1 mole 1 mole

mmoles of Elie cephalexin cpsules = mmoles of 0.2814 M NaOH

= $V_{NaOH} \times M_{NaOH}$ = 2×0.2814 = 0.5628 mmoles

These mmoles were contained in 50 ml of Elie cephalexin capsules solution mmoles of Elie cephalexin capsules that contained in 250 ml of the solution of Elie cephalexin capsules

= 0.5628×250/50 = 2.8140

Weight of Elie cephalexin capsules = mmoles×M wt

= 2.814×365.4 = 1028.265 mg = 1.0283g

% of Elie cephalexin capsules = 1.0283×100/ 1.0026 = 102.56%

2- from the graph of δ pH/ δ V the volume of 0.2814 M NaOH is 1.95 ml

mmoles of Elie cephalexin capsules = mmoles of 0.2814 M NaOH = $V_{NaOH} \times M_{NaOH}$ = 1.95×0.2814 = 0.54873 mmoles

These mmoles were contained in 50 ml of Elie cephalexin capsules solution mmoles of Elie cephalexix capsules that contained in 250 ml of the solution of Elie cephalexin capsules

$$= \frac{0.54873 \times 250}{50} = 2.74365 \text{ mmoles}$$

Weight of Elie cephalexin capsules = mmoles×M wt

= 2.74365×365.4 = 1002.53 mg = 1.00253g

$$\% \text{ of Elie cephalexin capsules} = \frac{1.00253 \times 100}{1.0026} = 99.99\%$$

Table 3.12 Potentiometeric titration of 50ml Elie cephalexin capsule with 0.2814M NaOH

Vol. of NaOH/ml	pH	δ pH/δ V	Vol.of NaOH/ml	pH	δ pH/δ V
0.0	4.656		1.8	8.164	
		4.03			1.34
0.4	6.268		1.9	8.298	
		1.2075			10.4
0.8	6.751		2.0	9.338	
		1.0172			9.28
1.2	7.158		2.1	10.266	
		1.21			3.95
1.5	7.521		2.3	11.056	
		1.55			2.216
1.6	7.676		2.5	11.499	
		1.78			0.5525
1.7	7.854		2.9	11.72	
		3.1			

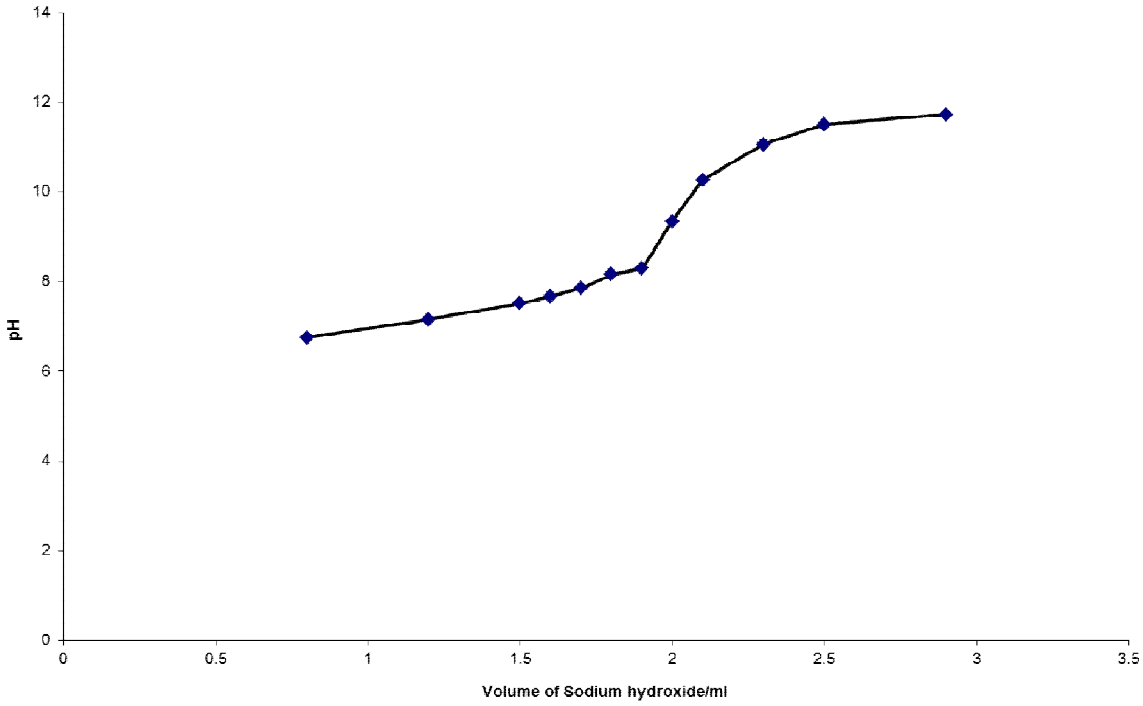


Fig 3.13 Potentiometeric titration of 50ml Elie cephalexin capsule With 0.2814M NaOH-1

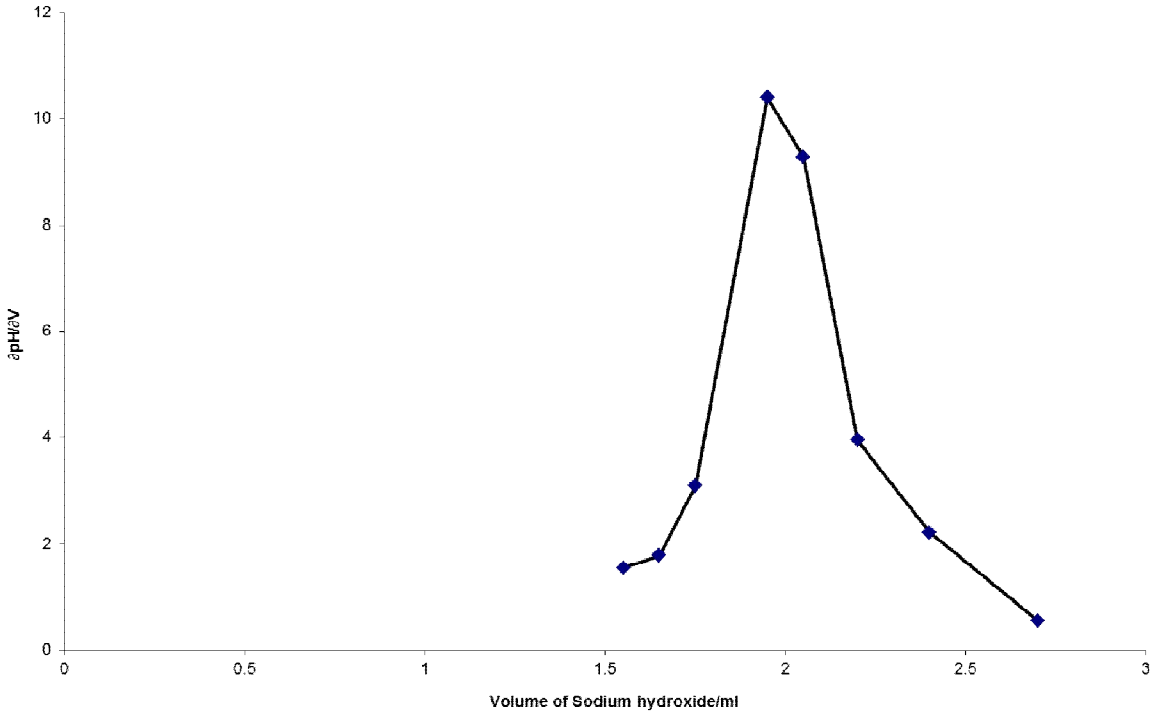


Fig 3.14 Potentiometeric titration of 50ml Elie cephalexin capsule with 0.2814M NaOH-2

3.1.5.6.3 Amipharma cephalexin capsules

1- From of pH/V the volume of 0.2814 M NaOH is1.9 ml

Q −COOH + NaOH → Q −COONa + H_2O

1 mole 1 mole

mmoles of Amipharma cephalexin capsules = mmoles of 0.2814 M NaOH

= $V_{NaOH} \times M_{NaOH}$ = 1.9 ×0.2814 = 0.53466 mmoles

These mmoles were contained in 50 ml of Amipharma cephalexin capsules solution

mmoles of cephalexin that contained in 250 ml of the solution of Amipharma cephalexin capsules

= 0.53466×250/50 = 2.67 mmoles

Weight of Amipharma cephalexin capsules = mmoles×M wt

= 2.67 ×365.4 = 974.6mg = 0.9746g

% of Amipharma cephalexin capsules = 0.9746×100/ 0.9892= 98.52%

2- From the graph of δ pH/ δ V the volume of 0.2814 M NaOH is 1.95ml

mmoles of cephalexin = mmoles of 0.2814 M NaOH

= $V_{NaOH} \times M_{NaOH}$ = 1.95×0.2814 = 0.54873 mmoles

These mmoles were contained in 50 ml of Amipharma cephalexin capsules solution

mmoles of Amipharma cephalexin capsules that contained in 250 ml of the solution $= \frac{0.54873 \times 250}{50}$ = 2.744 mmoles

Weight of Amipharma cephalexin capsules = mmoles×M wt

= 2.744×365.4 =1002.658mg =1.00268 g

% of Amipharma cephalexin capsules $= \frac{1.002658 \times 100}{0.9892}$ = 101.36%

Table 3.13 Potentiometeric titration of 50ml Amipharma cephalexin capsule with 0.2814MNaOH

Vol. of NaOH/ml	pH	δ pH/δ V	Vol.of NaOH/ml	pH	δ pH/δ V
0.0	5.829				3.39
		1.4775	1.8	8.420	
0.4	6.420				7.67
		1.30	1.9	9.187	
0.8	6.940				10.2
		1.0375	2.0	10.207	
1.2	7. 355				2.523
		1.055	2.3	10.964	
1.4	7.566				0.9975
		1.33	2.7	11.363	
1.6	7.832				0.435
		2.49	3.1	11.537	
1.7	8.081				

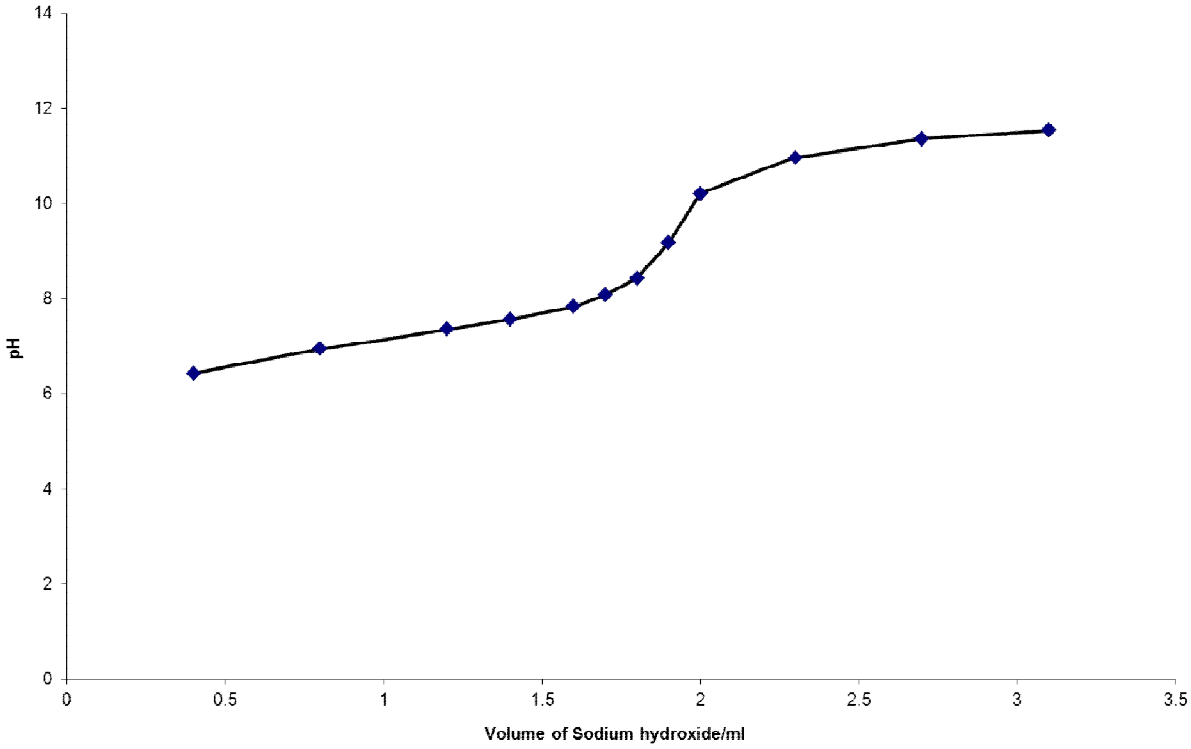


Fig 3.15 Potentiometeric titration of 50 ml Amipharma cephalexin capsule with 0.2814MNaOH -1

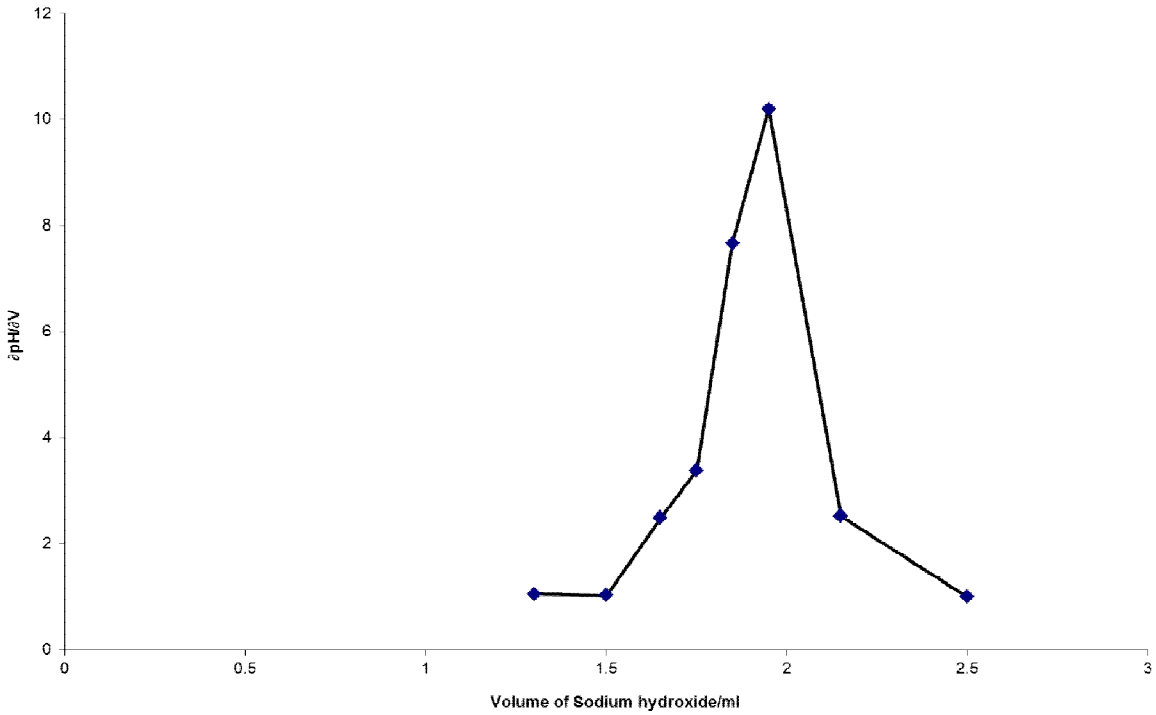


Fig 3.16 Potentiometeric titration of 50 ml Amipharma cephalexin capsule with 0.2814MNaOH -2

3.1.5.6.4 Changahi cephalexin capsules

1- From of pH/V the volume of 0.2814 M NaOH is1.9 ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1 mole 1 mole

mmoles of Changahi cephalexin capsules = mmoles of 0.2814 M NaOH = $V_{NaOH} \times M_{NaOH}$ = 1.96 ×0.2814 = 0.5515 mmoles

These mmoles were contained in 50 ml of Changahi cephalexin capsules solution

mmoles of Changahi cephalexin capsules that contained in 250 ml of the solution of Changahi cephalexin capsules

$$= \frac{0.5515 \times 250}{50} = 2.76 \text{ mmoles}$$

Weight of Changahi cephalexin capsules = mmoles×M wt

= 2.76 ×365.4 = 1007.671 mg = 1.007671g

% of Changahi cephalexin capsules= 1.007671×100/ 0.9883 = 101.96%

2- from the graph of δ pH/ δ V the volume of 0.2814 M NaOH is 1.95 ml

mmoles of Changahi cephalexin capsules = mmoles of 0.2814 M NaOH

= $V_{NaOH} \times M_{NaOH}$ = 1.95×0.2814 = 0.54873 m moles

These mmoles were contained in 50 ml of Changahi cephalexin capsules solution

mmoles of Changahi cephalexin capsules that contained in 250 ml of the solution of Changahi cephalexin capsules

$$= \frac{0.54873 \times 250}{50} = 2.744 \text{ mmoles}$$

Weight of Changahi cephalexin capsules= mmoles×M wt

=2.744×365.4 = 1002.53mg = 1.00253 g

% of Changahi cephalexin capsules = $\frac{1.002553 \times 100}{0.9883}$ = 101.42%

Table 3.14 Potentiometeric titration of 50ml Changahi cephalexin capsules with 0.2814M NaOH

Vol. of NaOH/ml	pH	δ pH/δ V	Vol.of NaOH/ml	pH	δ pH/δ V
0.0	4.948				3.81
		3.06	1.8	8.397	
0.4	6.172				6.24
		1.4775	1.9	9.022	
0.8	6.763				11.58
		1.1575	2.0	10.18	
1.2	7. 226				6.82
		0.64	2.1	10.862	
1.4	7.482				1.842
		0.71	2.3	11.231	
1.6	7.766				0.72
		2.5	2.7	11.519	
1.7	8.016				0.535
			3.1	11.733	

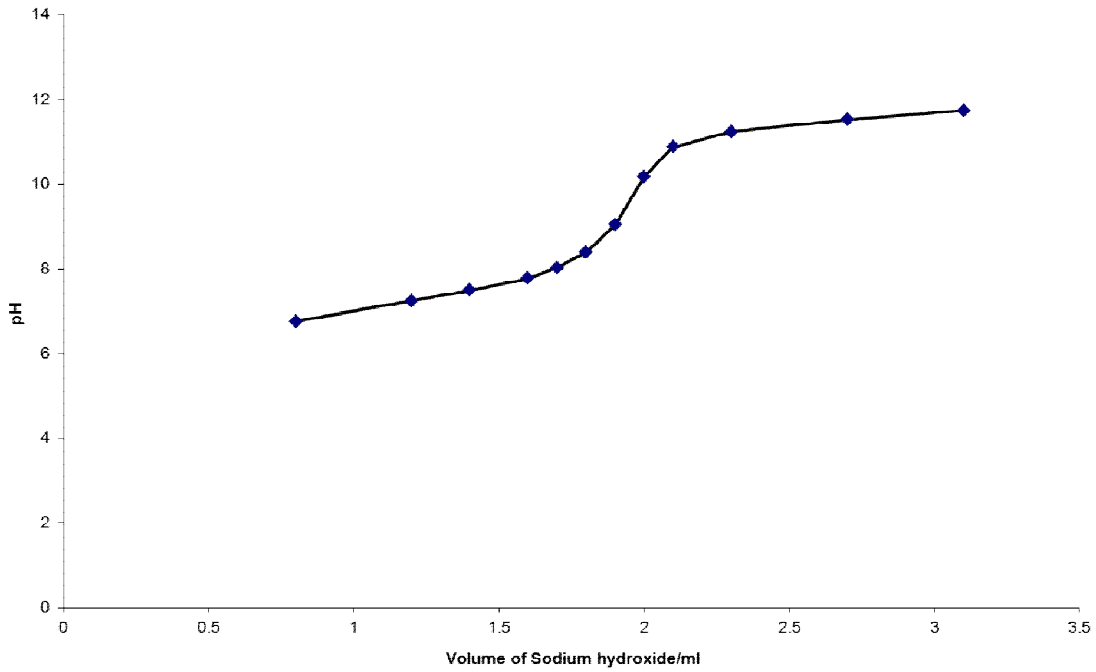


Fig 3.17 Potentiometeric titration of 50 ml Changahi cephalexin capsules with 0.2814M NaOH -1

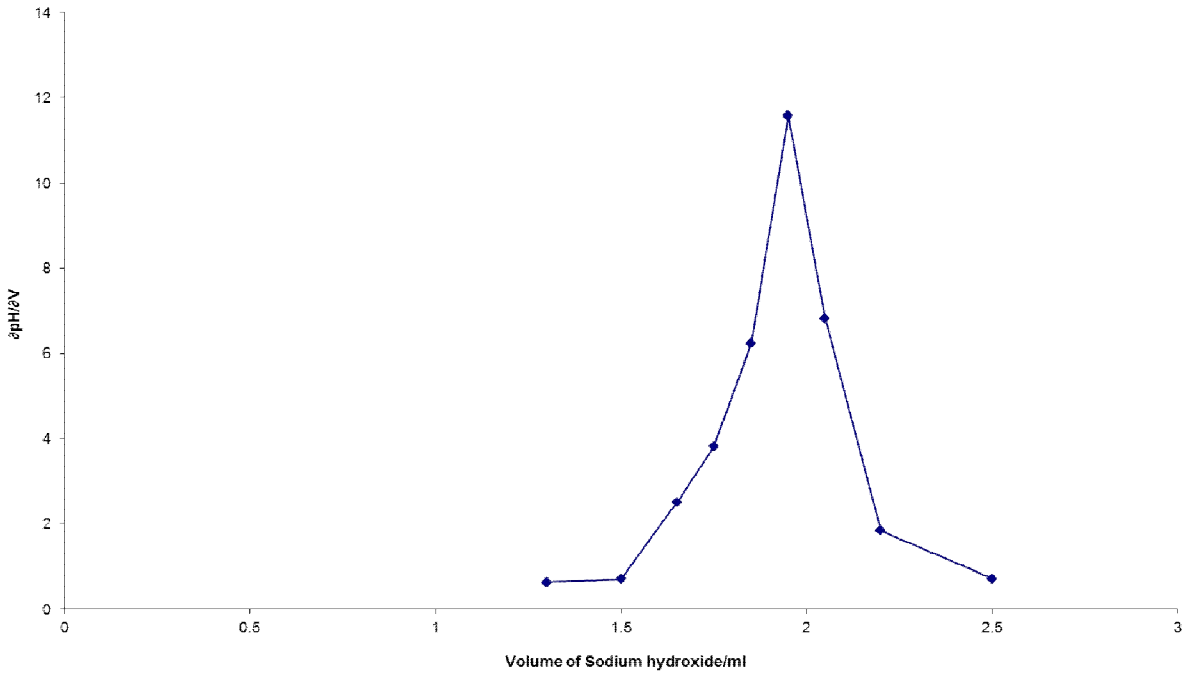


Fig 3.18 Potentiometeric titration of 50 ml Changahi cephalexin capsules with 0.2814M NaOH -2

3.1.5.6.5 Wafra cephalexin capsules

1- From of pH/V the volume of NaOH is 1.95 ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1 mole 1 mole

mmoles of Wafra cephalexin capsules = mmoles of NaOH

$= V_{NaOH} \times M_{NaOH}$ $= 1.95 \times 0.284$ $= 0.5538$ mmoles

These mmoles were contained in 50 ml of Wafra cephalexin capsule solution

mmoles of Wafra cephalexin capsules that contained in 250 ml of the solutionof Wafra cephalexin capsules

$$\frac{= 0.5538 \times 250}{50} = 2.769 \text{ mmoles}$$

Weight of Wafra cephalexin capsules = mmoles×M wt

$= 2.769 \times 365.4$ = 1011.79 mg = 1.01179 g

% of Wafra cephalexin capsules = 1.0118×100/0.959 = 105.5 %

2- from the graph of δ pH/ δ V the volume of NaOH is1.96 ml

mmoles of Wafra cephalexin capsules = mmoles of

$= V_{NaOH} \times M_{NaOH}$ $= 1.96 \times 0.284$ $= 0.5566$ mmoles

These mmoles were contained in 50 ml of Wafra cephalexin capsules solution

mmoles of Wafra cephalexin capsules that contained in 250 ml of the solution of Wafra cephalexin capsules

$$\frac{= 0.5566 \times 250}{50} = 2.783 \text{ mmoles}$$

Weight Wafra of cephalexin capsules = mmoles×M wt = 2.7834×365.4

= 1016.91mg = 1.01691g

% of Wafra cephalexin capsules $\frac{= 1.0169 \times 100}{0.959} = 106.03$ %

Table 3.15 potentiometeric titration of 50ml wafra cephalexcin capsules with 0.284M NaOH

vol. of NaOH/ml	pH	δ pH/δV/V	Vol.of NaOH/ml	pH	δpH/δv /V
0.00	5.219				6.06
		2.11	2.0	9.151	
0.5	6.274				5.26
		1.093	2.1	9.677	
0.8	6.252				3.43
		1.06	203	10.363	
1.1	7.070				1.48
		0.94	2.6	10.806	
1.4	7.354				0.743
		1.4	2.9	11.020	
1.6	7.648				0.39
		2.15	3.4	11.224	
1.7	7.863				
		2.99			
1.8	8.082				
		4.63			
1.9	8.545				

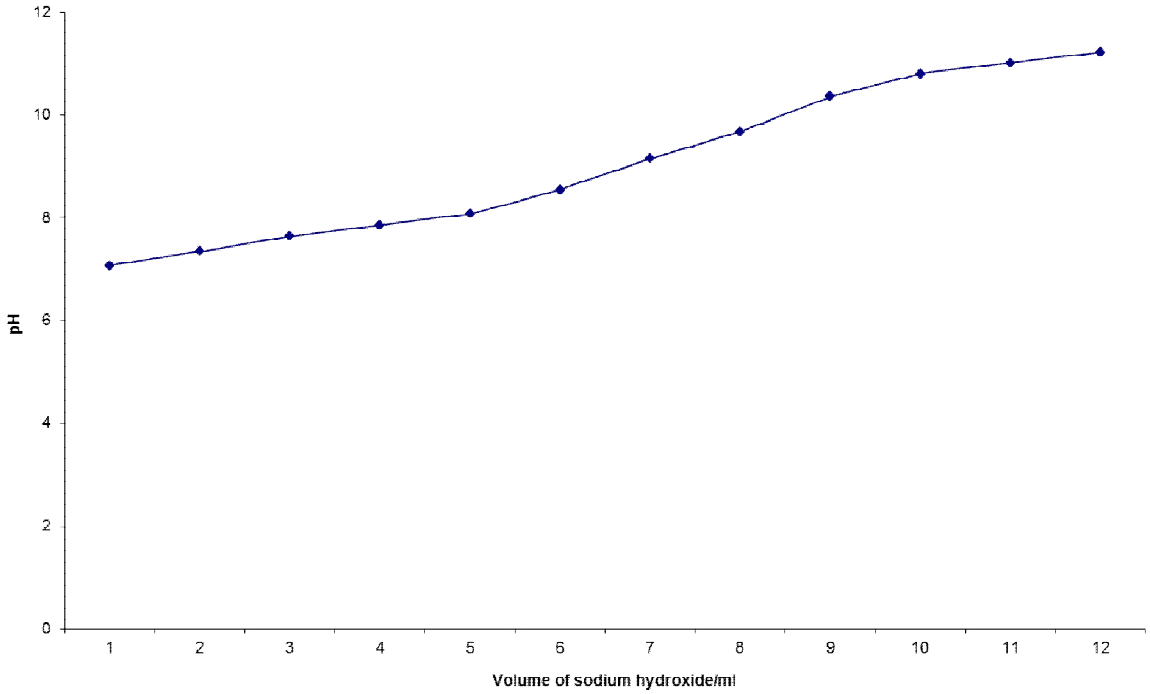


Fig 3.19 potentiometeric titration of 50 ml wafra cephalexcin capsules with 0.284M NaOH -1

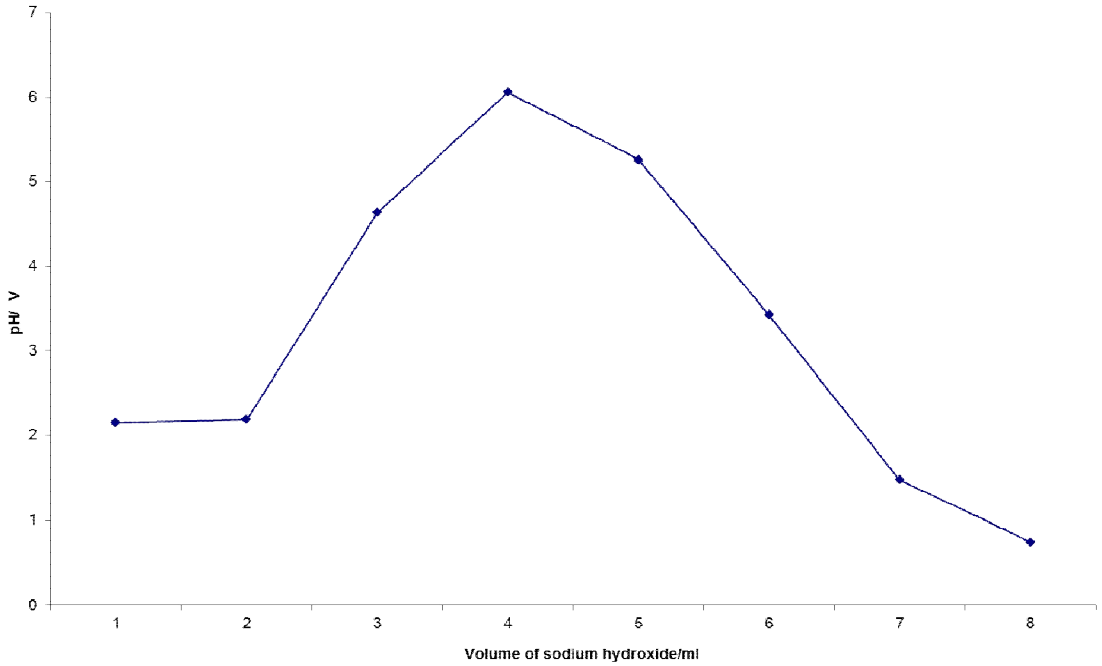


Fig 3.20 potentiometeric titration of 50 ml wafra cephalexcin capsules with 0.284M NaOH -2

3.1.6 Spectrophotometeric determination of Cephalexin

3.1.6 .1 Reagents

1-100µg/Ml solution ofcephalexin

2- 0.007M 4-aminoantipyrine (AP) solution

3- 0.008m NaOH solution

4- 0.016m K_3FeCN_6 solution

3.1.6.2Apparatus

Spectrophotometer (Jenway-6505Uv/Vis)

3.1.6.3 Procedure

2.0ml of 100µg/ml standard cephalexin solution,4.0ml of 0.007M aminoantipyrine, 4.0ml of 0.008M NaOH were mixed with 2.0ml of 0.016M potassium ferri cyanide in 25mlvolumeteric flask and diluted to the mark with distilled water, The maximum absorption wavelength of the cephalexin 4-APcomplex was determined.

2.0ml of cephalexin4-AP complex was prepared by taking 2.0ml of 100µg/ml standard amoxicillin solution into 25ml volumetric flask 4.0mLof 0.007M 4AP, 4.0mlof 0.008M NaOH solution,and 2.0ml of K3FeCN6 solution were added and the volume was completed to the mark with distilled water . Serial dilutions of that complex was done to give different concentrations cephalxin solutions, and the absorbance of each was recorded, and the calibration curve was plotted as shown in Fig (3.21).

Weights of 0.027g, 0.0273g, 0.02684g and 0.030g were taken respetively from (Amipharma, Wafra, Elie and Changahi cephalexin capsule each of which was dissolved with aid of magnetic stirrer in distilled water, transfared into 250ml volumetric flask, completed to the mark with distilled water to give a solution of 100µg/mlof amoxicillin and filtered.

1.0 ml from each were taken into 25ml volumetric flask ,2.0ml of 0.007M 4-AP solution , 2.0ml of 0.008M NaOH solution and 1.0ml of 0.016M K_3FeCN_6 solution were added ,the volume was completed up to

the mark with distilled water,the solutions were successively diluted twice to(50:50) percent with distilled water to give a solution of (2.14, 2.2, 2.15 and 2.4)µg/ml of each respectively, and the absorbance of each solution was measured then the amount and the percentage of cephalexin sample were found from the calibration curve.

3.1.6.4 Results of spectrophotometric method

The standard curve data

Concentrationof Cephalexin (µg/ml)	0.25	0.5	1.0	2.0	4.0
Absorbance	0.046	0.079	0.144	0.276	0.520

The maximum absorption wave length (λ) was 419nm

From the equation

$$Y = a + bc$$

Y = Absorbance

a = Intercept

b =Slope

c = Concentration

Results of cephalexin samples

Cephalexin samples	Absorbance	Weight/µg	%
Amipharma	0.246	1.864	81.7
Elie	0.244	1.85	85.97
Wafra	0.233	1.765	84
Changahi	0.239	1.811	75.4

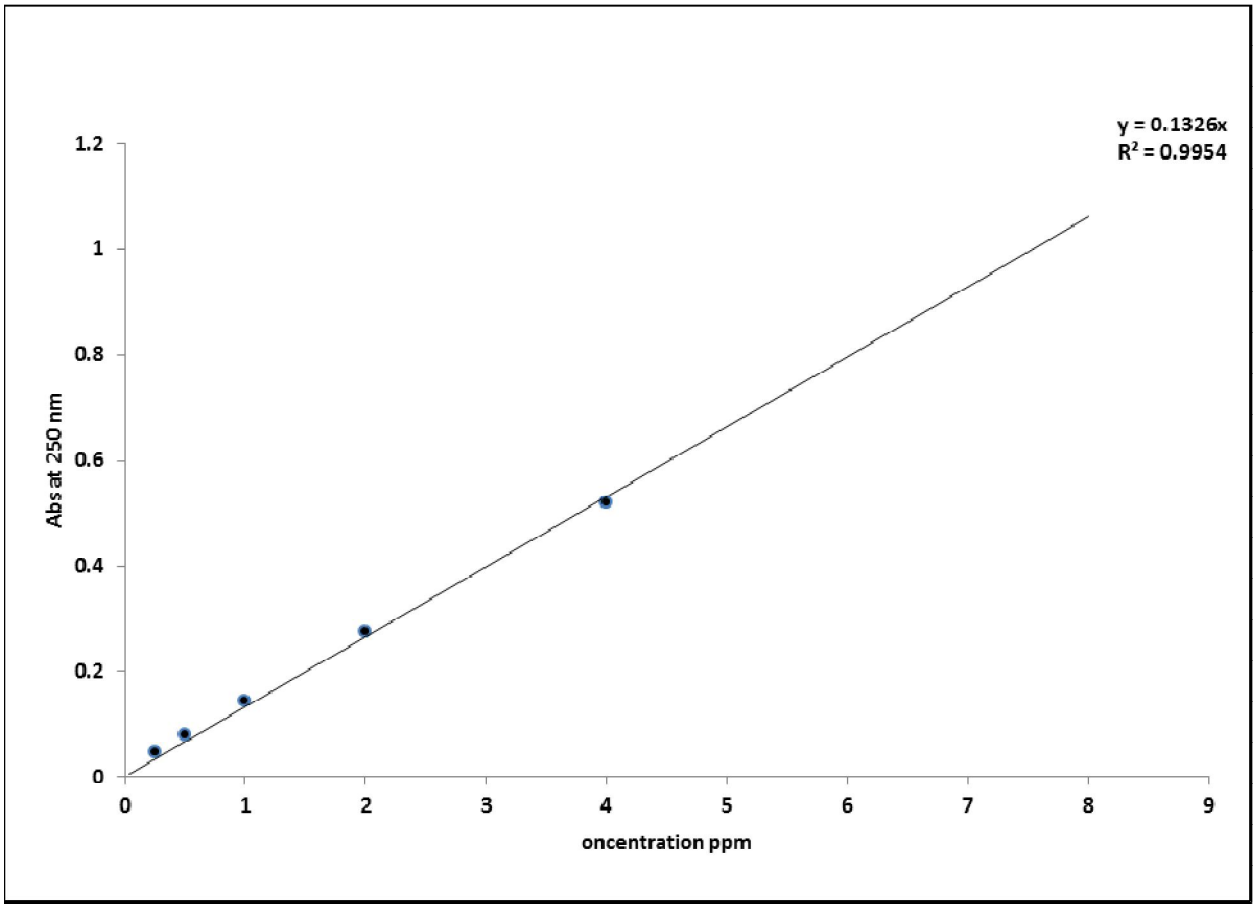


Fig 3.21 Standard Cephalexin monohydrate calibration curve of Spectrophotometric method

3.1.7 Detemination of cephalexin using high performance liquid chromatography (HPLC)

3.1.7.1 Reagents

1- Cephalexin solution.

2- Mobile phase (2 volume of methanol, 5 volume of acetonityle, 10 volume of 13.6 g /ℓ solution of potassium dihydrogen phosphate and 83 volume of distilled water.

3.1.7.2 Apparatus

1- HPLC apparatus. Shimadzu Quto Japan, with with two LC-10 ADVP liquid chromotograph and DGU 14A degaser pump. SIL 10 ADVP auto injector. SPD- M10A VP diode array detecter and C TO 10 ASV column oven.

2-Separation column (Shimpack-ODS), 15 cm length ,4.6mm internal diameter and 5µm (particale size). Flow rate 1ml /minute. Oven temperature 30^{o}C

3- Spectrophotometer detector.

3.1.7.3 Procedure

A weight of 0.05 g of cephalexin(standard) was dissolved in a little amount of distilled water, transferred to 50 ml volumetric flask and completed up to the mark with water, others solutions of 0.4mg/ml ,0.24ml/ml and 0.10mg/ml concentrations were made . Spectrophotometer detector was set at 254nm, cephalexin (standard) solutions were injected , chromotograph was recorded, and calibration curve was plotted as shown in Fig (3.22).

Amount of 0.0118 g, 0.010 g , and 0.0101 g of cephalexin capsules from ,Amipharma , Wafra and Elie , respectively , which respectively contains 0.0108 gm, 0.0079g and 0.0091g were each dissolved in a little amount of distilled water, transferred to 50 ml volumetric flask and completed to the mark with distilled water , and chromotographed.

3.1.7.4 Result of HPLC determination of Cephalexin

3.1.7.4.1 Amipharma capsule cephalexin

Weight of the sample in50 ml of solution =0.0108 g

Replicates	1st r	2nd r	3rd r	Average
Concentrations Obtained	0.228 mg/ml	0.225 mg/ml	0.225 mg/ml	0.226 mg/ml

Therefore the weight of Amipfarma capsule cephalexin obtained

= 0.226 X 50/1000 =0.0113g

The percentage of Amipharma capsule cephalexin

=0.0113x100/0.0108 = 104.63%

3.1.7.4.2 Elie capsule cephalexin

Weight of the sample in50 ml of solution =0.0079 g

Replicates	1st r	2nd r	3rd r	Average
Concentrations Obtained	0. 158m g/ml	0.158 mg/ml	0.158 mg/ml	0.158 mg/ml

Therefore the weight of Elie capsule cephalexin obtained

= 0.158 X 50/1000 =0.0079 g

The percentage of Elie capsule cephalexin

= 0.0079x100/0.00788 = 100.25 %

3.1.7.4.3 Wafra capsule cephalexin

Weigh of the sample in50 ml of solution = 0.0091g

Replicates	1st r	2nd r	3rd r	Average
Concentrations Obtained	0. 179 mg/ml	0.179 mg/ml	0.179 mg/ml	0.179 mg/ml

Therefore the weight of Wara capsule cephalexin obtained

= 0.179 X 50/1000 = 0.00895 g

The percentage of Wafra capsule cephalexin

= 0.00895 x 100/0.0091 = 98.35 %

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User: System
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{Sample Description} : Cefalexin- std1-Rep1(0.1mg/ml)

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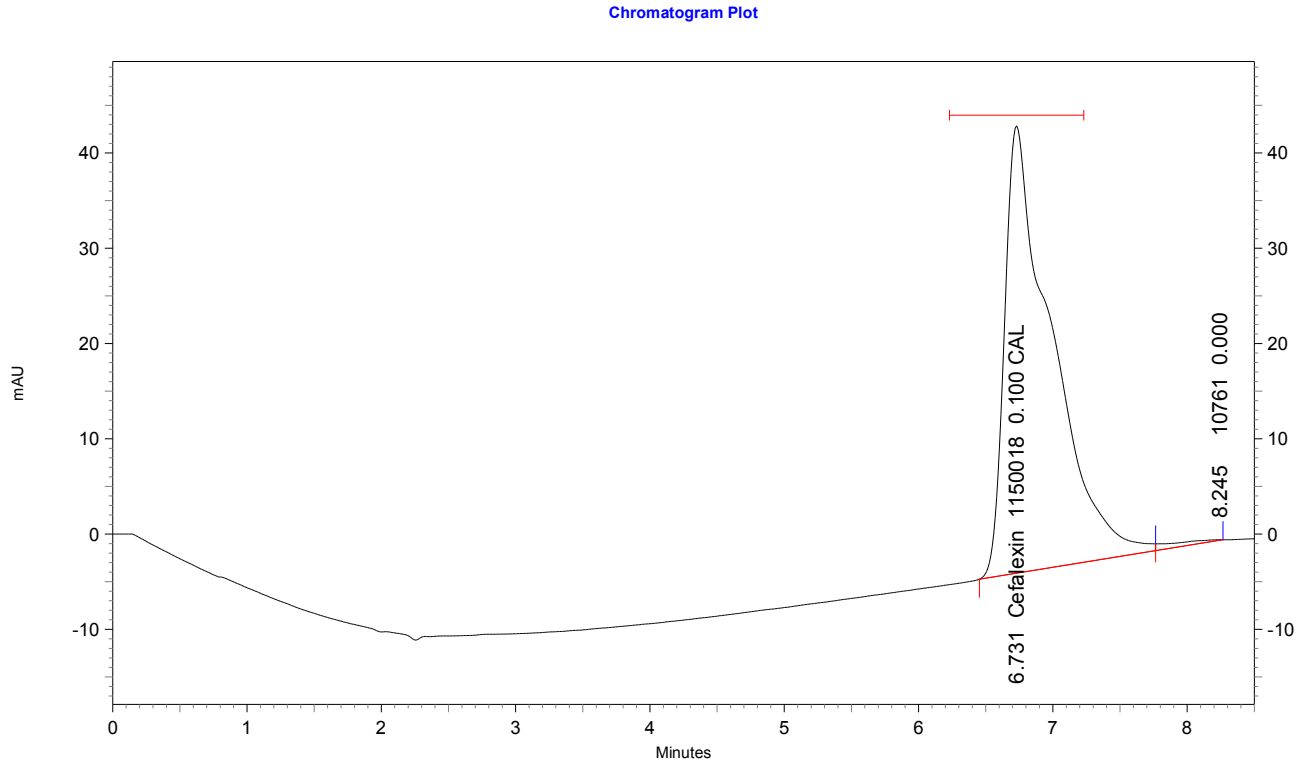

Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	6.731	1150018	0.100 CAL	mg/ml

Chromotogram plot 3.1 (R1.1) standard cephalexin monohydrate

Data Name: C:\CLASS-VP\Cefalexin1- std1-Rep2.1

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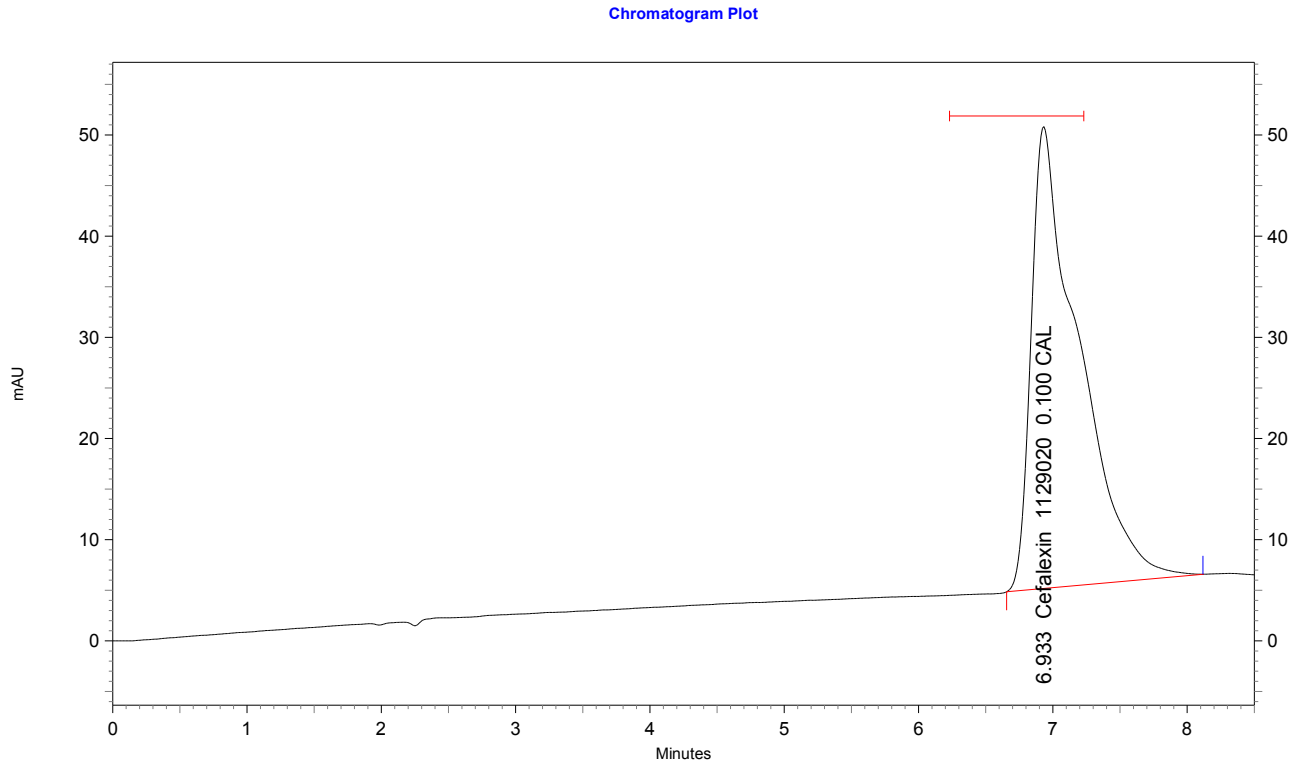
{Sample Description} : Cefalexin- std1-Rep2(0.1mg)



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	6.933	1129020	0.100 CAL	mg/ml

Chromotogram plo 3.2 (R1.2) standard cephalexin monohydrate

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Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

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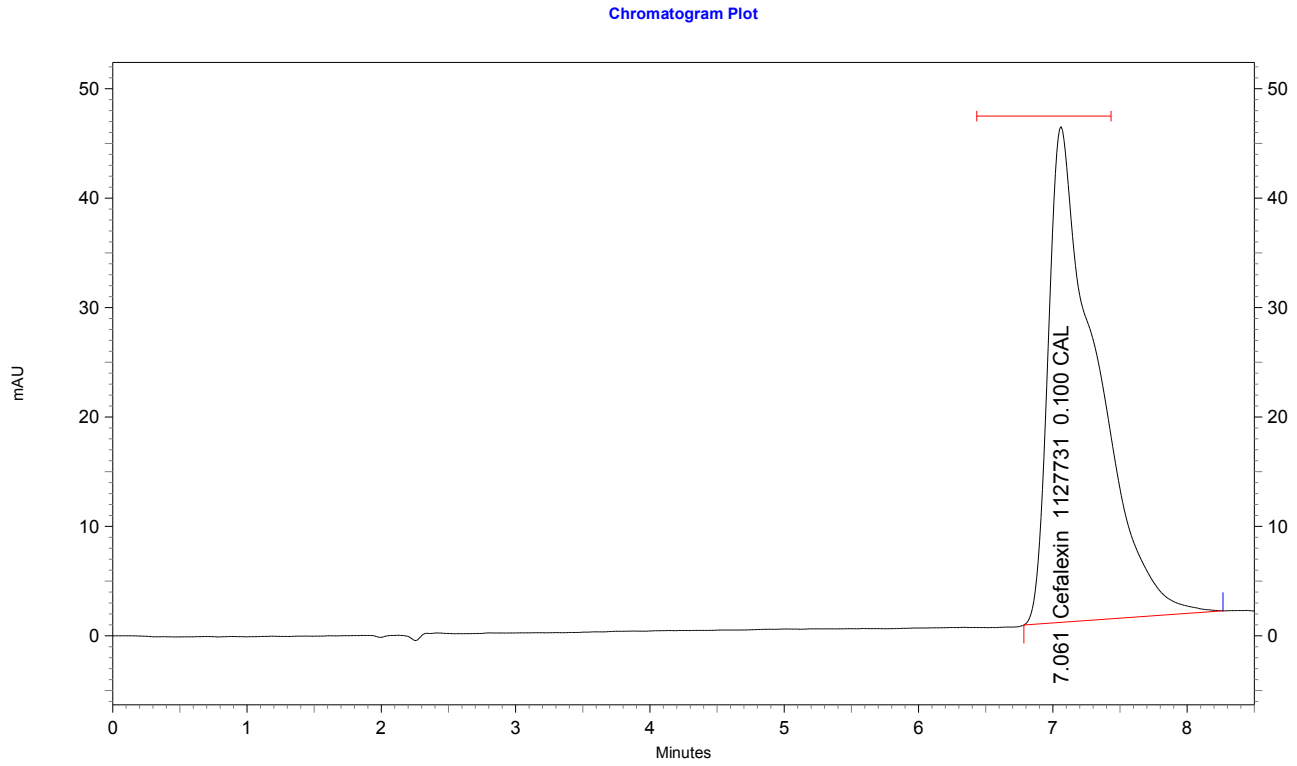
{Sample Description} : Cefalexin- std1-Rep3(0.1mg)



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	7.061	1127731	0.100 CAL	mg/ml

Chromotogram plot 3.3 (R1.3) standard cephalexin monohydrate

Data Name: C:\CLASS-VP\Cefalexin1- std2-Rep1.2

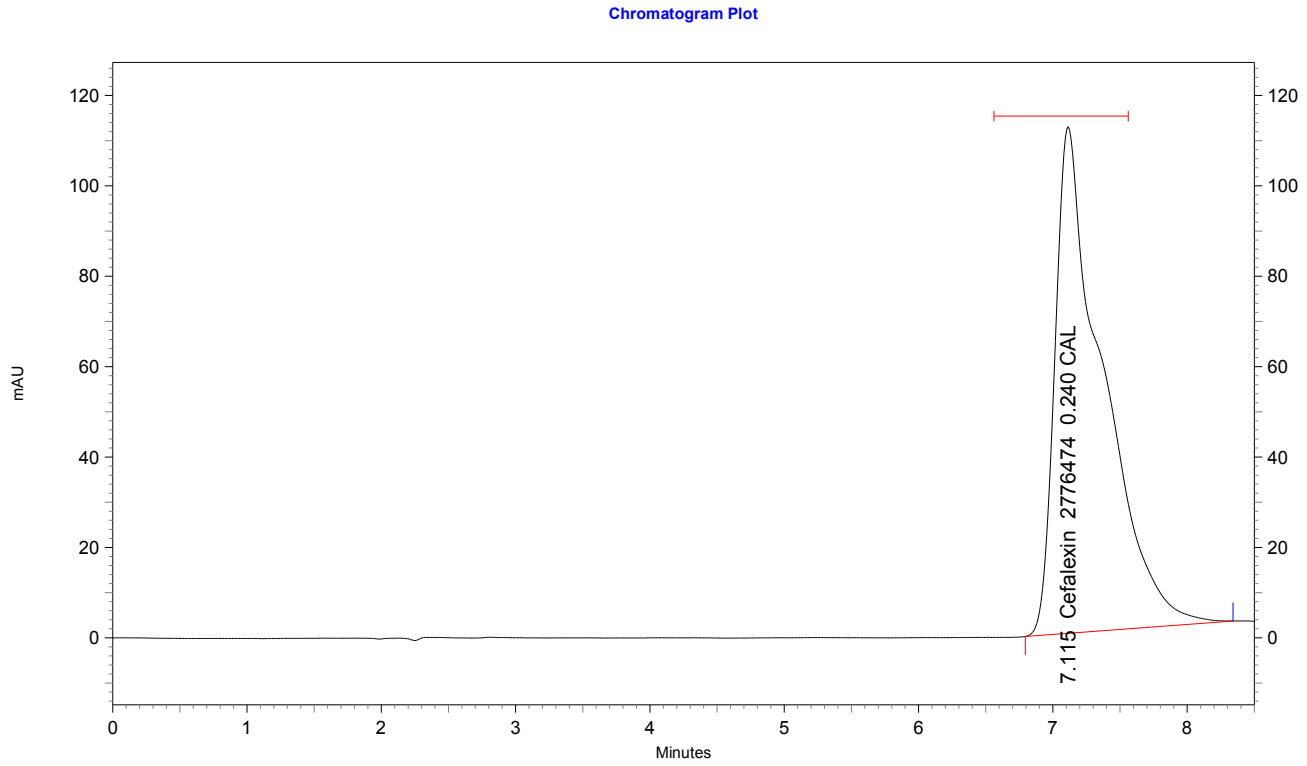
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 7:03:27 PM

{Sample Description} : Cefalexin- std2-Rep1(0.24mg)



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	7.115	2776474	0.240 CAL	mg/ml

Chromotogram plot 3.4 (R2.1) standard cephalexin monohydrate

Data Name: C:\CLASS-VP\Cefalexin1- std2-Rep2.2

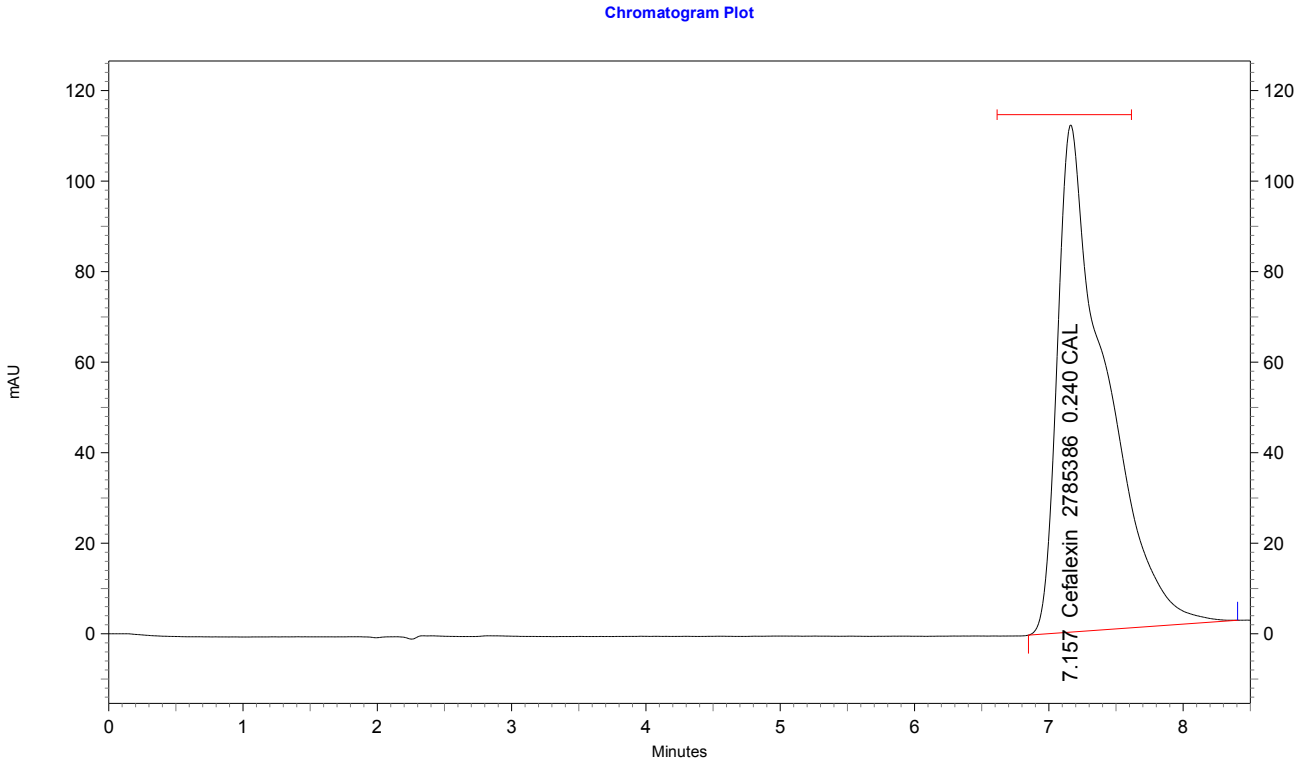
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 7:14:09 PM

{Sample Description} : Cefalexin- std2-Rep2(0.24mg)



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	7.157	2785386	0.240 CAL	mg/ml

Chromotogram plot 3.5 (R2.2) standard cephalexin monohydrate

Data Name: C:\CLASS-VP\Cefalexin1- std2-Rep3.2

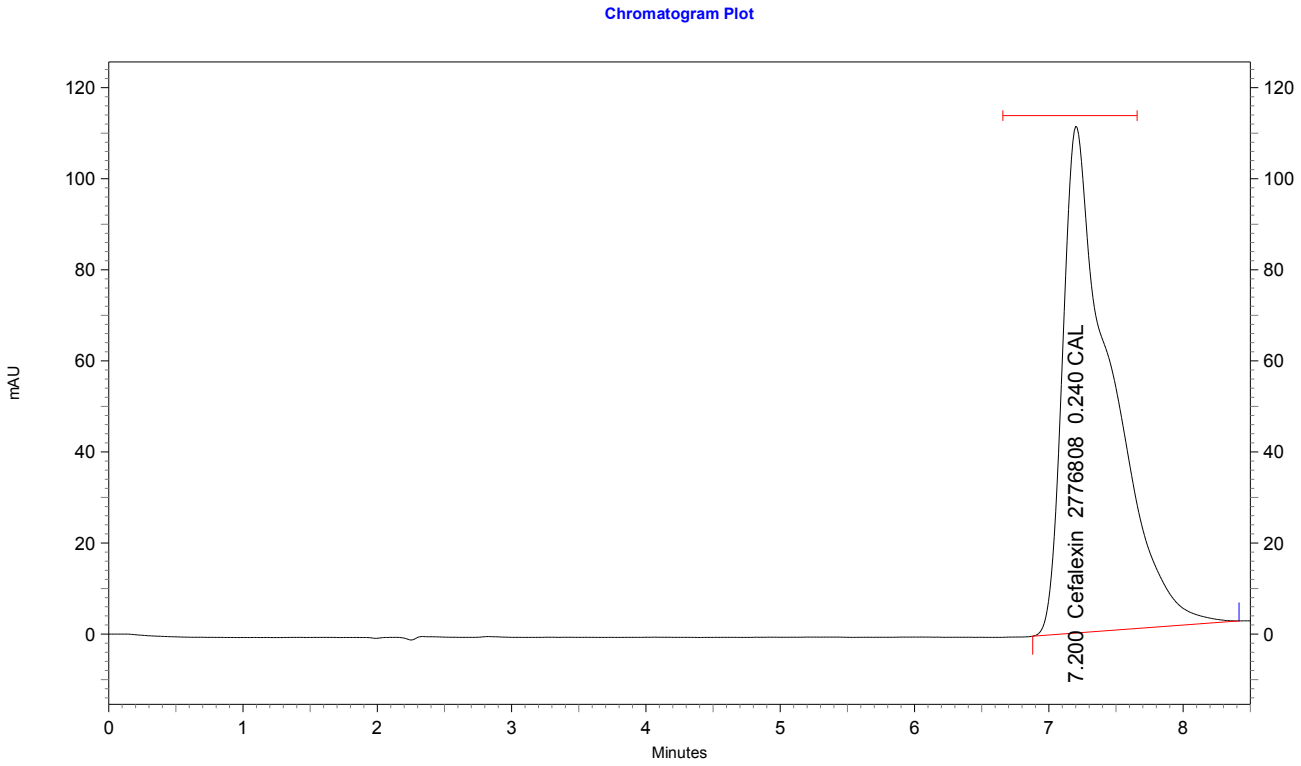
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 7:24:53 PM

{Sample Description} : Cefalexin- std2-Rep3(0.24mg)



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	7.200	2776808	0.240 CAL	mg/ml

Chromotogram plot 3.6 (R2.3) standard cephalexin monohydrate

Data Name: C:\CLASS-VP\Cefalexin1- std3-Rep1.1

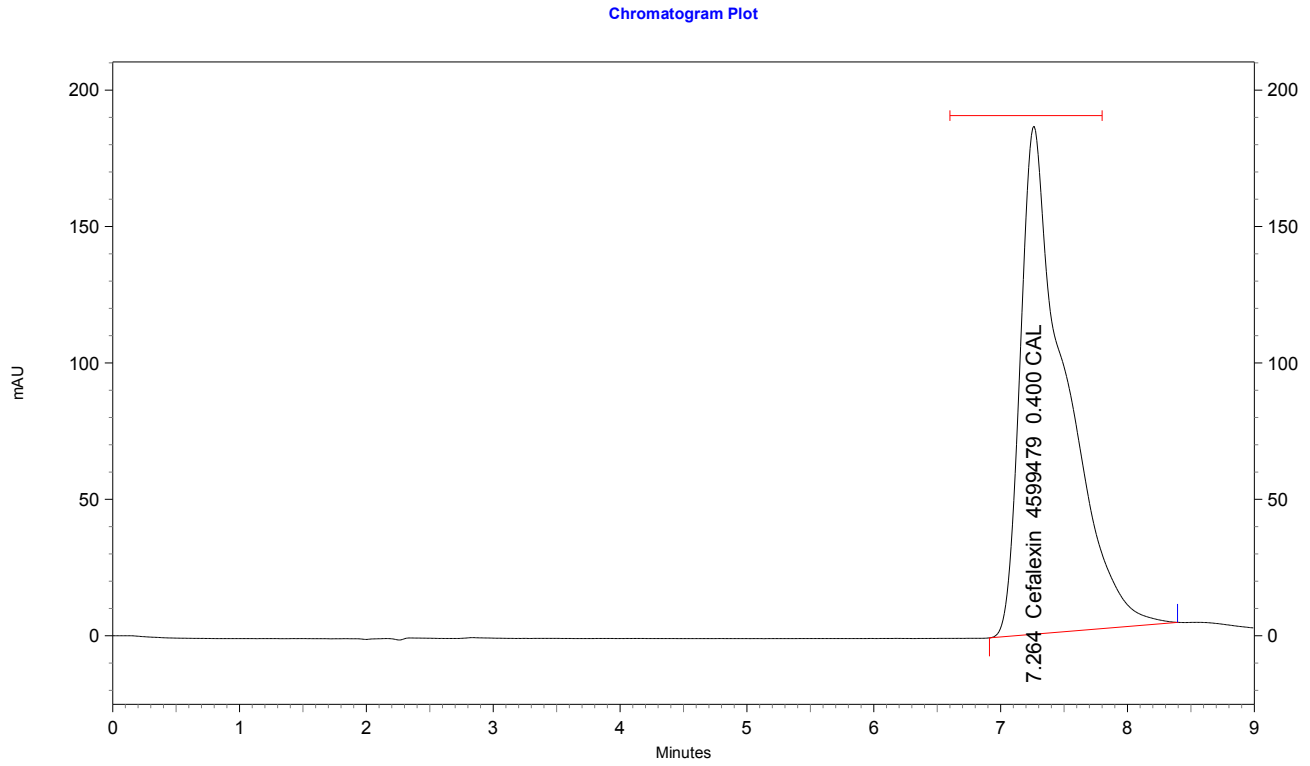
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 7:35:37 PM

{Sample Description} : Cefalexin- std3-Rep1(0.4mg)



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	7.264	4599479	0.400 CAL	mg/ml

Chromotogram plot 3.7 (R3.1) standard cephalexin monohydrate

Data Name: C:\CLASS-VP\Cefalexin1- std3-Rep2.1

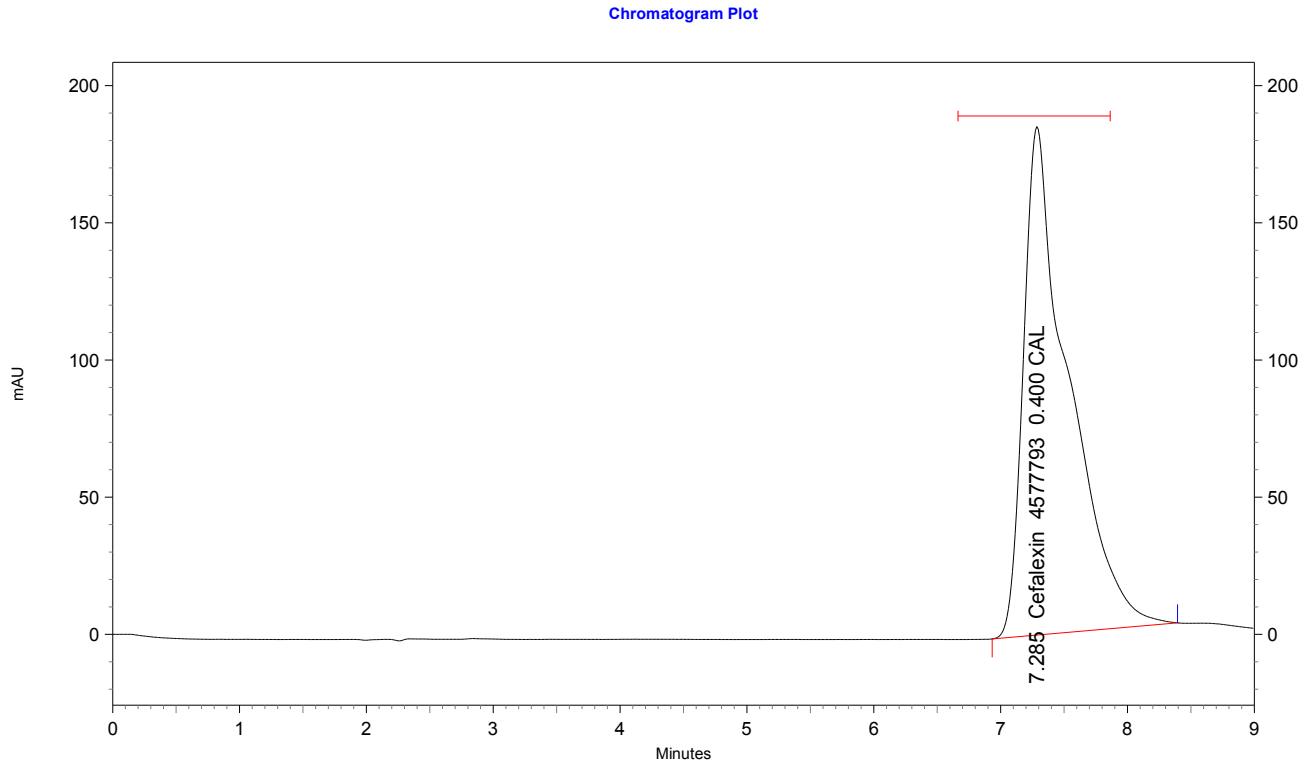
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 7:46:20 PM

{Sample Description} : Cefalexin- std3-Rep2(0.4mg)



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	7.285	4577793	0.400 CAL	mg/ml

Chromotogram plot 3.8 (R3.2) standard cephalexin monohydrate

Data Name: C:\CLASS-VP\Cefalexin1- std3-Rep3.1

Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

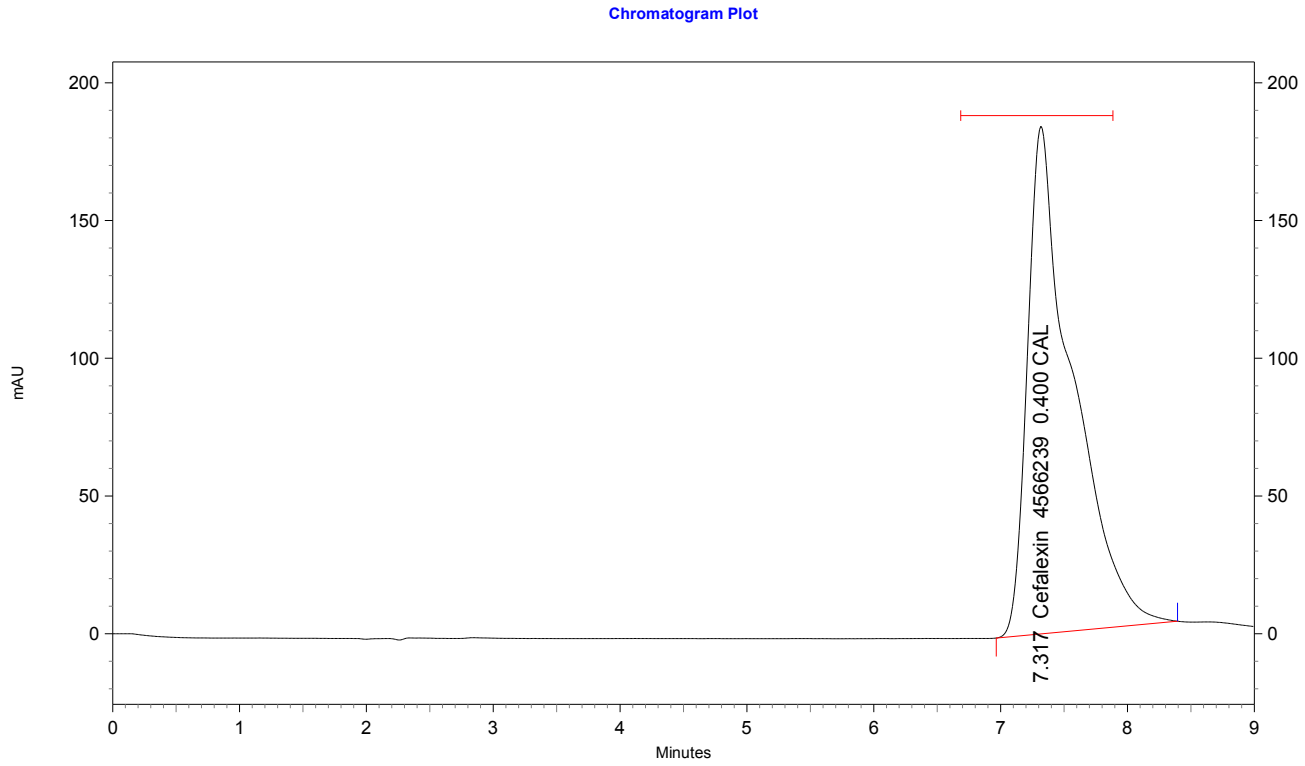
Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 7:57:08 PM

{Sample Description} : Cefalexin- std3-Rep٣(0.4mg)

.



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	7.317	4566239	0.400 CAL	mg/ml

Chromotogram plot 3.9 (R3.3) standard cephalexin monohydrate

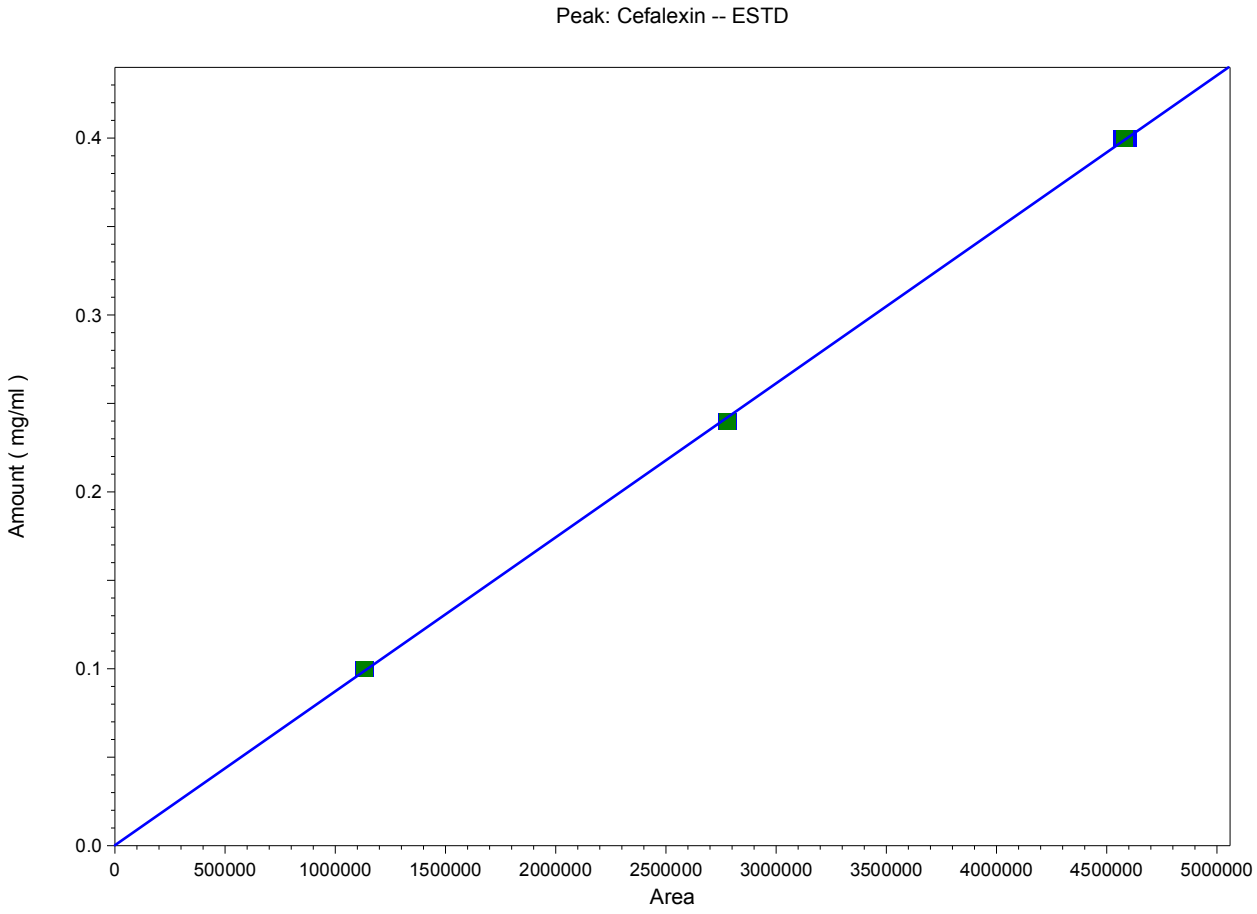


Fig 3.22 standard cephalexin monohydrate calibration curve of chromatographic method

Data Name: C:\CLASS-VP\Cefalexin1- ami-Rep1

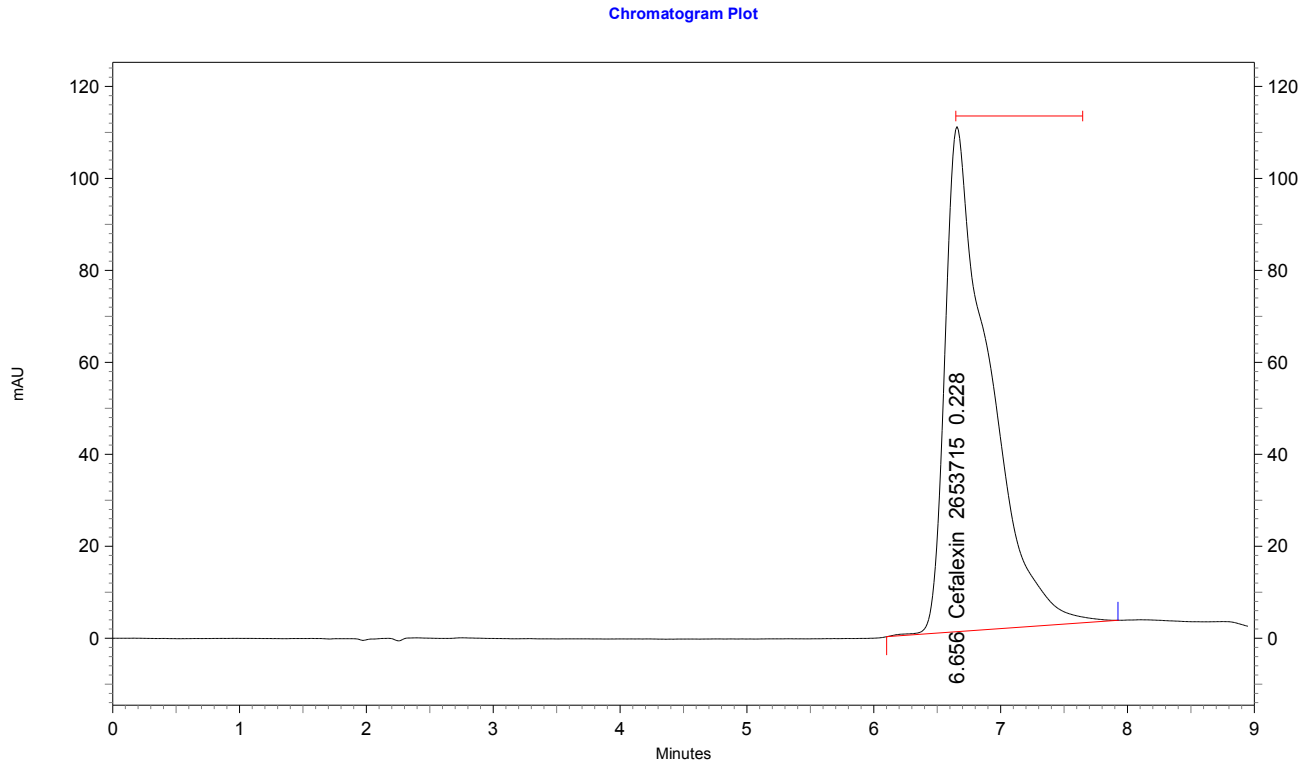
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 10:31:38 AM

{Sample Description} : Amipharma Cefalexin Rep1



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	6.656	2653715	0.228	mg/ml

Chromotogram plot 3.10 (R1) Amipharma cephalexin capsules

Data Name: C:\CLASS-VP\Cefalexin1- ami-Rep2

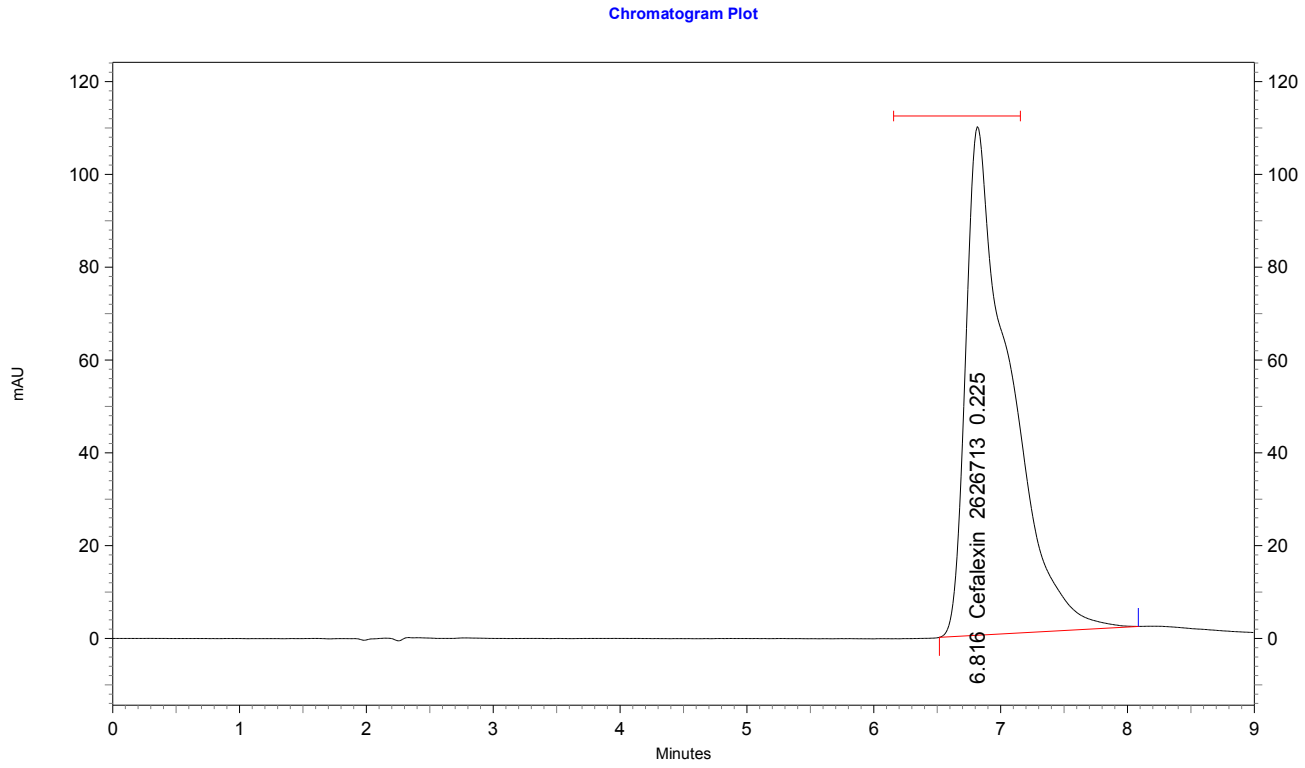
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 10:43:09 AM

{Sample Description} : Amipharma Cefalexin Rep2



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	6.816	2626713	0.225	mg/ml

Chromotogram plot 3.11 (R2) Amipharma cephalexin capsules

Data Name: C:\CLASS-VP\Cefalexin1-ami-Rep3

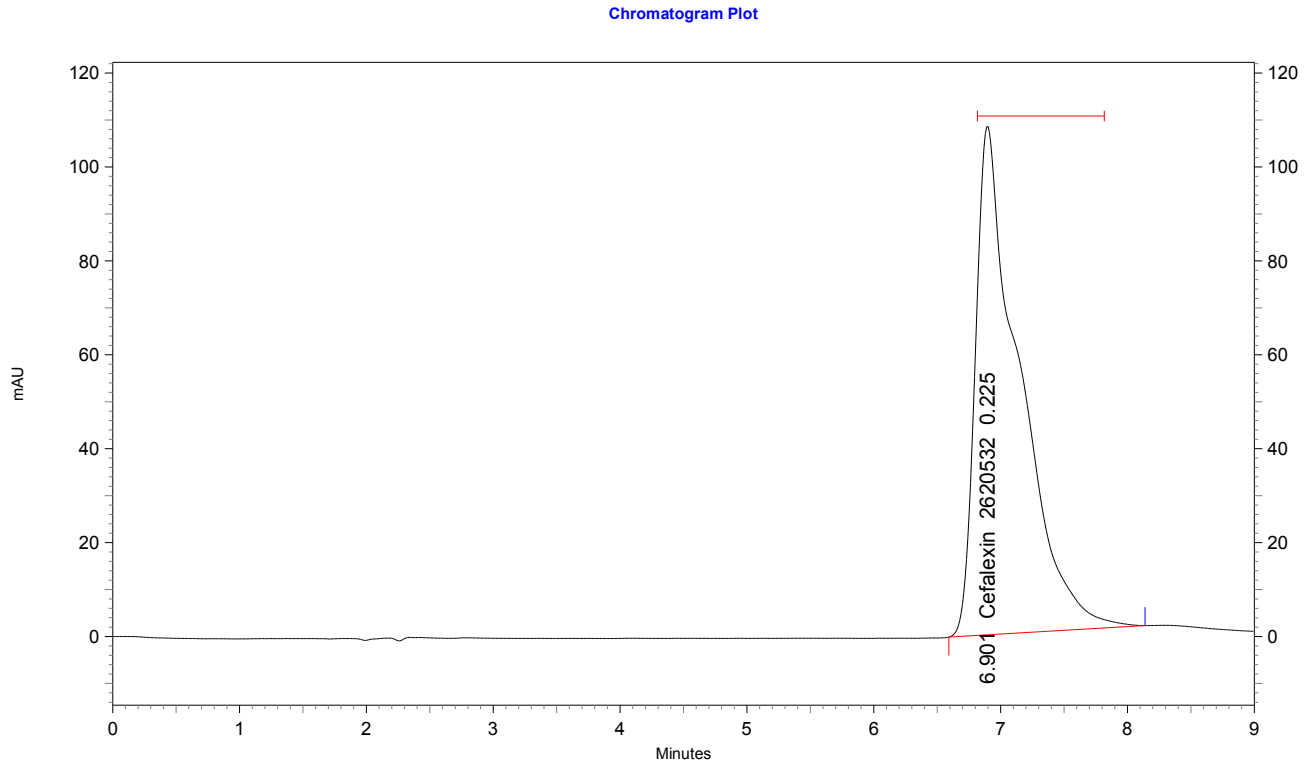
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 10:53:52 AM

{Sample Description} : Amipharma Cefalexin Rep3



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	6.901	2620532	0.225	mg/ml

Chromotogram plot 3.12 (R3) Amipharma cephalexin capsules

Data Name: C:\CLASS-VP\Cefalexin1- eli-Rep1

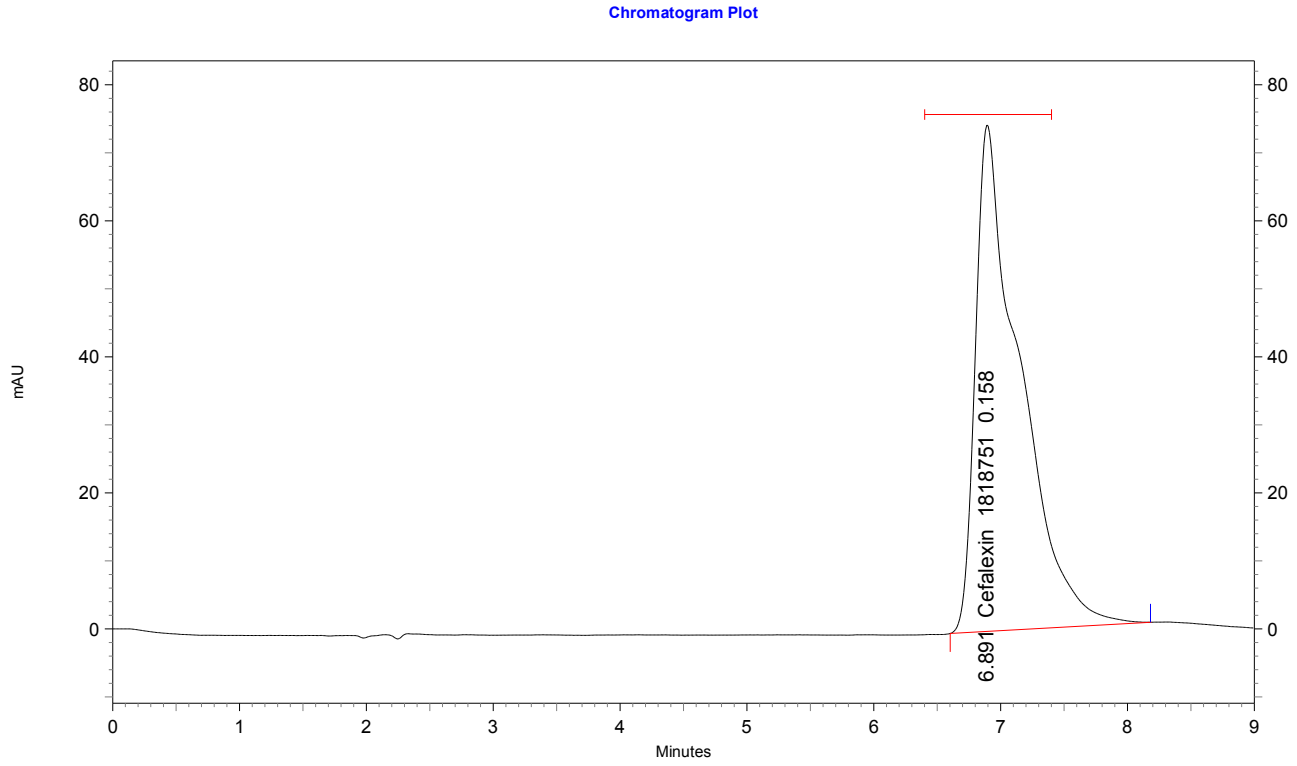
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 11:04:36 AM

{Sample Description} : Eli Cefalexin Rep1



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	6.891	1818751	0.158	mg/ml

Chromotogram plot 3.13 (R1) Elie cephalexin capsules

Data Name: C:\CLASS-VP\Cefalexin1- eli-Rep2

Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

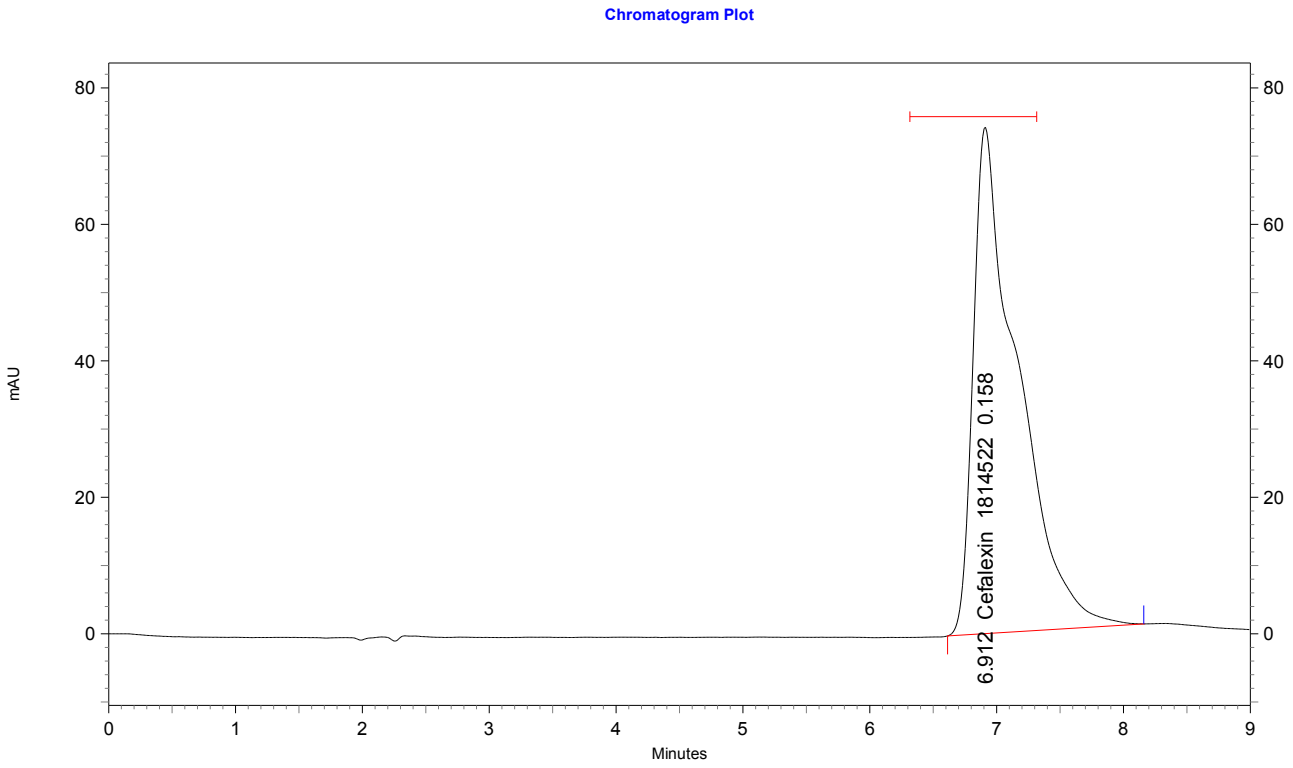
Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 11:15:13 AM

{Sample Description} : Eli Cefalexin Rep2

.



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	6.912	1814522	0.158	mg/ml

Chromotogram plot 3.14 (R2) Elie cephalexin capsules

Data Name: C:\CLASS-VP\Cefalexin1 -eli-Rep3

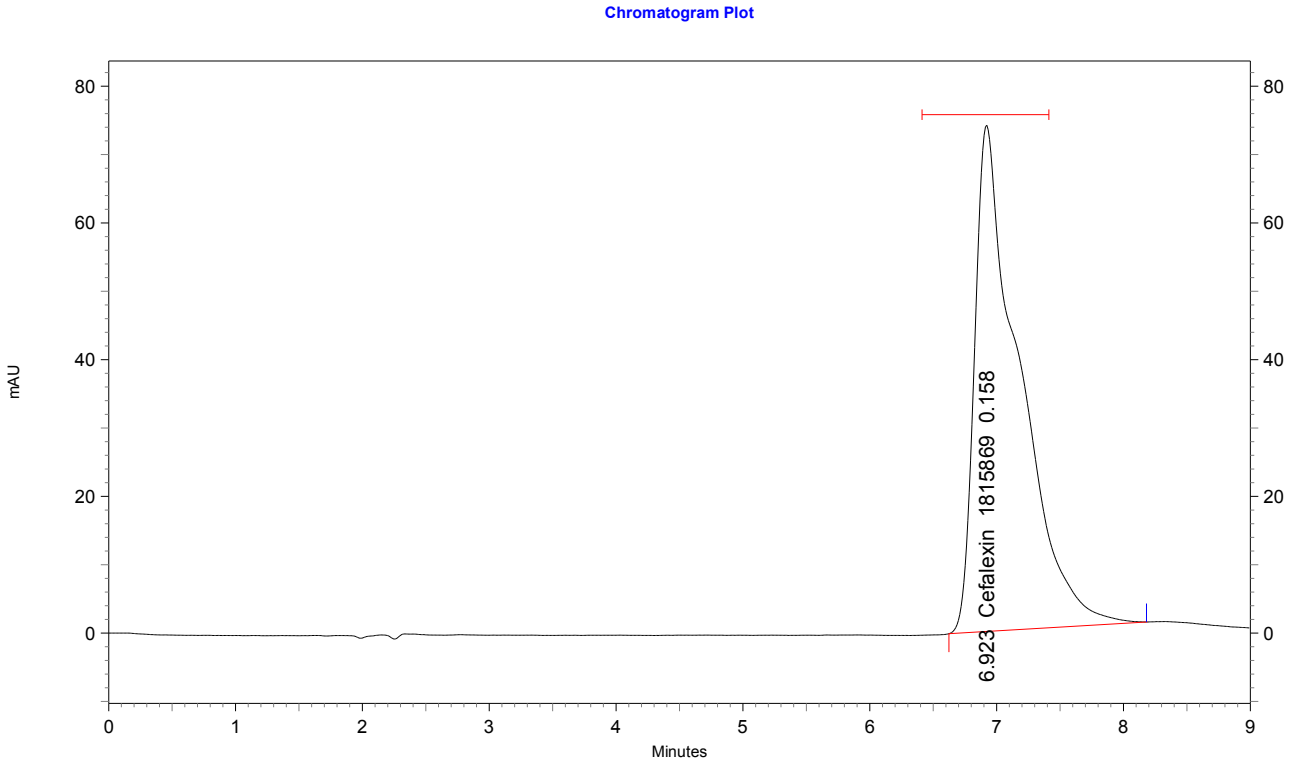
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 11:25:59 AM

{Sample Description} : Eli Cefalexin Rep3



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	6.923	1815869	0.158	mg/ml

Chromotogram plot 3.15 (R3) Elie cephalexin capsules

Data Name: C:\CLASS-VP\Cefalexin1- wafra-Rep1

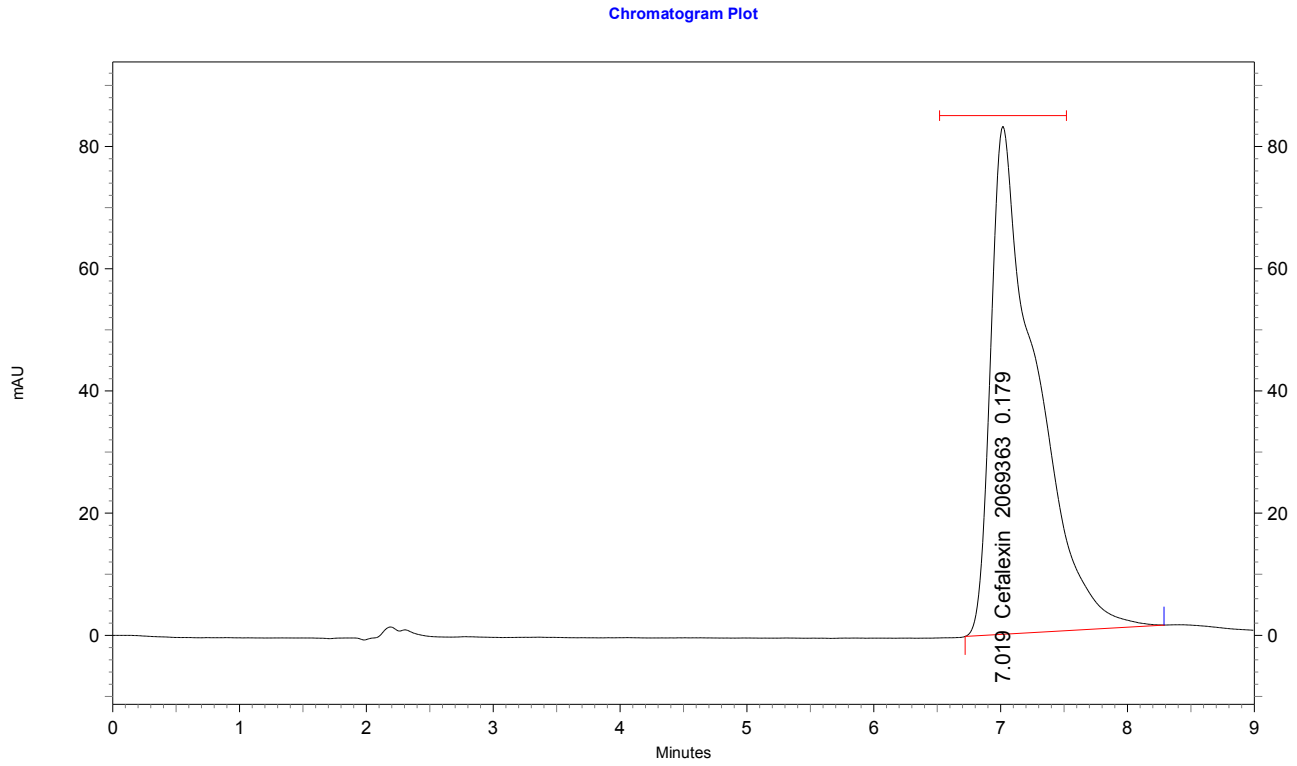
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 11:36:42 AM

{Sample Description} : Wafra Cefalexin1 -Rep1



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	7.019	2069363	0.179	mg/ml

Chromotogram plot 3.16 (R1) Wafra cephalexin capsules

Data Name: C:\CLASS-VP\Cefalexin1- wafra-Rep2

Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

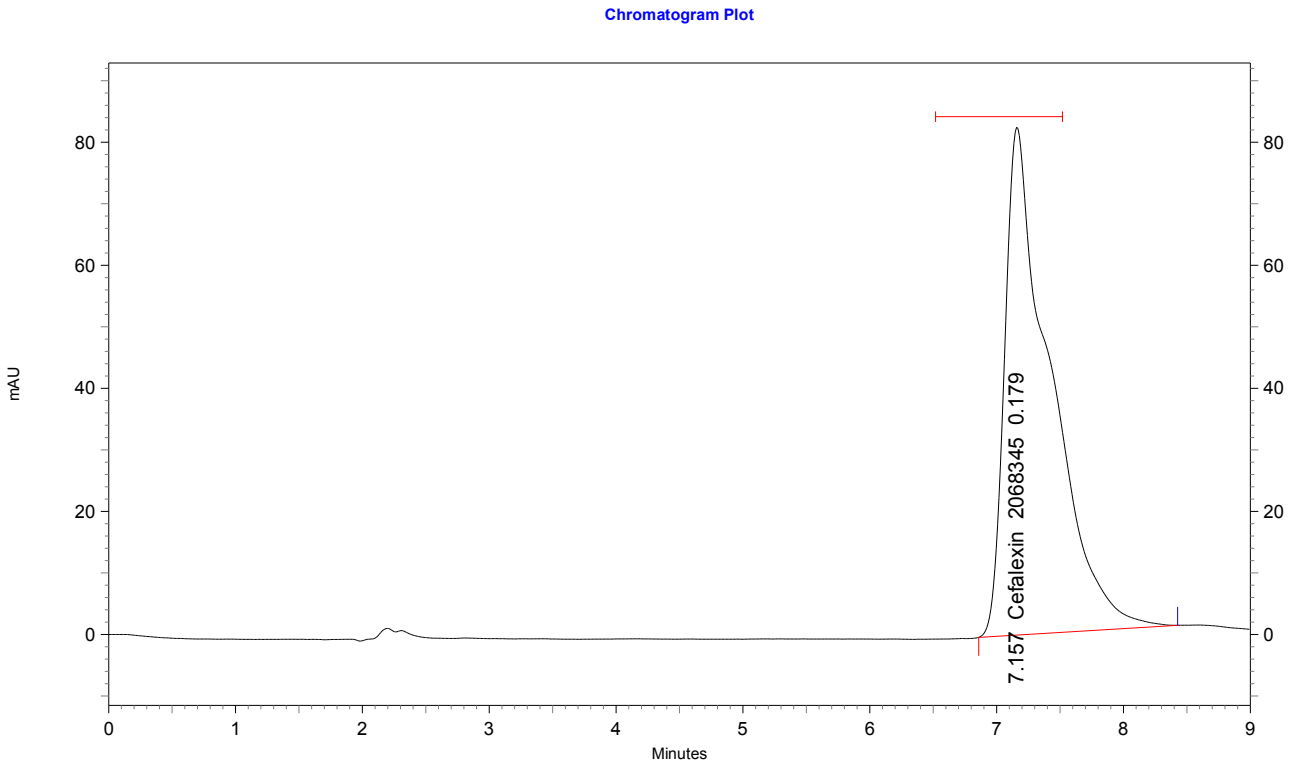
Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 11:47:30 AM

{Sample Description} : Wafra Cefalexin1 -Rep2

.



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	7.157	2068345	0.179	mg/ml

Chromotogram plot 3.17 (R2) Wafra cephalexin capsules

Data Name: C:\CLASS-VP\Cefalexin1- wafra-Rep3

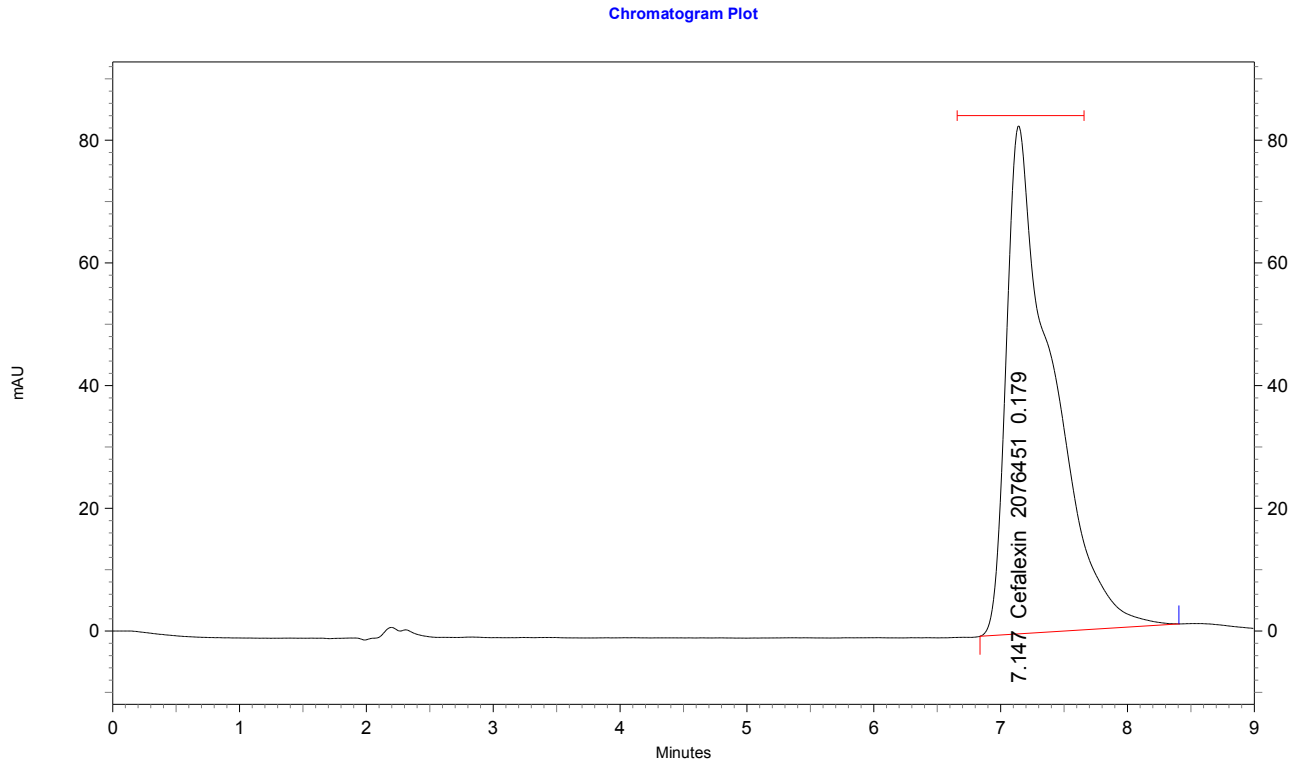
Method Name: C:\CLASS-VP\Methods\cefalexin cups.met

Sample ID: Cefalexin

User: System

Acquired: 11/1/2010 11:58:13 AM

{Sample Description} : Wafra Cefalexin1 -Rep1Rep3



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Cefalexin	7.147	2076451	0.179	mg/ml

Chromotogram plot 3.18 (R3) Wafra cephalexin capsules

3.2 Amoxicillin

3.2.1 Amoxicillin trihydrate

3.2.1.1 Direct titration of Amoxicillin trihydrate

2.2.1.1.1Reagents

1- 0.00924 M NaOH solution

2- Amoxicillin trihydrate

3- Phenelophthilin indictor

3.2.1.2 Amipharma amoxicillin capsules

3.2.1.2.1 Reagents

1- 0.00921M NaOH solution

2- Amipharma amoxicillin capsules solution

3- Phenelophthalin indicator

3.2.1.3 Changahi amoxicillin capsules

3.2.1.3.1 Reagents

1- 0.00917 M NaOH solution

2- Changahi amoxicillin capsules solution

3- Phenelophthalin indicator

3.2.1.4 Wafra amoxicillin capsules

3.2.1.4.1Reagents

1-0.02814 M NaOH solution

2-Wafra amoxicillin capsules solution

3- Phenelophthalin indicator

3.2.1.5 G.M. amoxicillin capsules

3.2.1.5.1 Reagents

1-0.00917M NaOH solution

2- G.M. amoxicillin capsules solution

3- Phenelophthalin indicator

3.2.1.6 General apparatus

1- 50ml burette

2- 20ml pipette

3- 100ml conical flasks

3.2.1.7 General procedure

A weight of 0.4998g of amoxicillin tri hydrate was completely dissolved in 200 ml of distilled water with aid of magnetic stirrer and magnetic rod, transferred to 250 ml volumetric flask, completed up to the mark with distilled water and filtered.

Two portions of 10 ml of aliquot were taken into two different 100 ml conical flask, two drops of ph.ph indicator were added, and then titrated with 0.009244 M NaOH solution.

Weights of 0.472 g, 0.4977 g, 0.4999 g and 0.5031 g of Amipharma, Changahi, Wafra and G.M. amoxicillin capsules were taken respectively, which repectively contained 0.4189 g ,0.424 ,0.4576 g and 0.43378 g of pure amoxicillin; each amount was completely dissolved in 200 ml of distilled water with aid of magnetic stirrer and magnetic rod, transferred to 250 ml volumetric flask, completed up to the mark with distilled water and filtered.

Two portions of 10 ml aliquot were taken into two different conical flasks; two drops of ph.ph indicator were added, and titrated with NaOH solutions, that had specified molarity.

3.2.1.8 Results of direct titration methods

3.2.1.8.1 Amoxcillin trihydrate

The volume of 0.009244M that required to neuttalize the Amoxicillin trihydrate sampte is 5.15ml

$Q{-}COOH + NaOH \rightarrow Q{-}C\,OONa + H_2O$

1 mole 1 mole

mmoles of amoxicillin trihydrate = m moles of 0.009244M NaOH

= 5.15X0.009244 = 0.0476 mmoles

These mmoles contain in 10ml of amoxicillin trihydrate

Therefore mmoles that contained in 250 ml of amoxicillin trihydrate

$$= \frac{0.0476X250}{10} = 1.19 \text{ mmoles}$$

Therefore weight of amoxicillin tri hydrate $= \frac{1.19 \text{ X } 419.4}{1000} = 0.4991g$

% of amoxicillin trihydrate $= \frac{0.4991X100}{0.4998} = 99.81\%$

3.2.1.8.2 Amipharma Amoxicillin capsules

The volume of 0.009211M that required to neuttalize the Amifarma Amoxicillin capsule solution sample was 3.65ml

Q −COOH + NaOH → Q −COONa + H_2O

1mole 1mole

mmoles of Amipharma amoxicillin= mmoles of 0.009211M NaOH

$=V_{NaOH}XM_{NaOH} = 3.65\times0.009211 = 0.04007$mmoles

These mmoles were contained in10ml of Amipharma amoxicillin capsule solution

mmoles that contained in 250ml of Amipharma amoxicillin capsule solution

$$= \frac{0.04007 \times 250}{10} = 1.0017\text{mmoles}$$

Weight of Amipharma amoxicillin capsule = mmoles of it × M wt

$= 1.0017\times419.4 = 420.0$ mg $=0.420$ g

% of amipharma amoxicillin capsule $= \frac{0.420\times100}{0.4189} = 100.3\ \%$

3.2.1.8.3 Changahi Amoxicillin capsules

The volume of 0.00917M that required to neuttalize Changahi Amoxicillin capsule solution sample was 4.75ml

Q −COOH + NaOH → Q −COONa + H_2O

1mole 1mole

mmoles of Changahi amoxicillin capsules = mmoles of 0.009211M NaOH

$=V_{NaOH}XM_{NaO} = 4.75\times0.00917 = 0.0435575$mmoles

These m moles were contained in10 ml of Changahi amoxicillin capsule solution

mmoles that contained in 250ml of Changahi amoxicillin capsule solution =

$$\frac{0.043557\times250}{10} = 1.08894\text{mmoles}$$

Weight of Changahi amoxicillin capsule = mmoles of it × M wt

$$= 1.08897\times419.4 = 456.7\text{ mg} = 0.4567\text{ g}$$

% of Changahi amoxicillin capsules $\frac{0.4567\times100}{0.424} = 107.7\%$

3.2.1.8.4 G .M Amoxicillin capsules

The volume of 0.00917M that required to neuttalize G.M Amoxicillin capsule sample is 4.35ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1mole

mmoles of G.M amoxicillin capsule= mmoles of 0.00917MNaOH

$=V_{NaOH}XM_{NaOH}$ $= 4.35\times0.00917$ $= 0.0398895$mmoles

These mmoles were contained in10ml of G.M amoxicillin capsule solution

mmoles that contained in 250ml of G.M amoxicillin capsulesolution =

$$\frac{0.0398895\times250}{10} = 0.99724\text{ mmoles}$$

Weight of G M capsule amoxicillin = mmoles of it × M wt =

$0.99724\times419.4 = 418.2424$mg $= 0.4182424$ g

% of G.Mamoxicillin capsules = $\frac{0.4182424\times100}{0.43378} = 96.42\%$

3.2.1.8.5 Wafra amoxicillin capsules

The volume of 0.02814M that required to neuttalize Wafra Amoxicillin capsule sampleis 3.55ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1mole

mmoles of Wafra amoxicillin capsules = mmoles of 0.02814M NaOH

$=V_{NaOH}XM_{NaOH}$ = 3.55×0.02814 = 0.099897mmoles

These mmoles were contained in10 ml of Wafra amoxicillin capsule solution

mmoles that contained in 250 ml of Wafra amoxicillin capsule solution =

$$\frac{0.099897\times250}{25} = 0.99897\text{mmoles}$$

Weight of Wafra amoxicillin capsule= mmoles of it×M wt

0.99897×419.4 = 418.96mg = 0.41896 g

% of Wafra amoxicillin capsules = $\frac{0.41896\times100}{0.4576}$ = 91.6%

3.2.2 Back titration methods with sodium hydroxide solution

3.2.2.1 Amoxicillin trihydrate

3.2.2.1.1 Reagents

1- Amoxicillin trihydratesolution.

2-0.009211 M NaOH solution

3- 0.009336 M Hcl solution

4- Methyl red indicator

3.2.2.2 Amipharma amoxicillin capsules

3.2.2.2.1 Reagents

1- Amipharma amoxicillin capsule soluation

2- 0.009244 M NaOH solution

3- 0.009336 M Hcl solution

4- Methyl red indicator

3.2.2.3 Changahi amoxicillin capsules

3.2.2.3.1 Reagents

1- Changahi amoxicillin capsule solution

2- 0.00921 M NaOH solution

3- 0.009336 M Hcl solution

4- Methyl red indicator

3.2.2.4 Wafra amoxicillin capsules

3.2.2.4.1 Reagents

1-Wafra amoxicillin capsule solution

2- 0.02814 M NaOH solution

3- 0.01967 M Hcl solution

4- Methyl red indicator

3.2.2.5 G.M amoxicillin capsules

3.2.2.5.1Reagents

1- G.M amoxicillin capsule solution

2- 0.00917 M NaOH solution

3- 0.009336 M Hcl solution

4- Methyl red indicator

3.2.2.6 General apparatus

1- 25 ml burette

2- 25 ml pipette

3- Conical flasks

4- Magnetic stirrer and magnetic rod

3.2.2.7 General procedure

A weight of 0.4998g of amoxicillin trihydrate was dissolved in a little amount of distilled water and transferred to 250 ml volumetric flask and completed to the mark with water .Two portions of 20 ml of aliquot were taken into two different conical flasks, 20 ml of 0.009244 M NaOH solution were added to each, 2 drops of methyl red indicator were added to each, then titrated with 0.009336 M Hcl.

3.2.2.7.1 Blank titration procedure for amoxicillin trihydrate

Two portion of 20 ml of 0.009244 M NaOH solution were taken into different conical flasks, to witch 2 drops of methyl red indicator were added to, then titrated with 0.009336 M Hcl.

A weight of 0.472 g, 0.4977 g, 0.4999 g and 0.5031 of Amipharma, Changahic, Wafra and G.M. amoxicillin capsules respectively which respectively contain 0.4189 g, 0.424 g0.4576 g and 0.43378 g of pure amoxicillin were each completely dissolved in about 200 ml of distilled water

with aid of magnetic stirrer and magnetic rod and transferred to 250 ml volumetric flask, completed up to the mark with water and filtered.

Two portions of 10 ml aliquot were taken into a 100 ml conical flask, 20 ml of related NaOH solution were added to each, two drops of methyl red indicator were added and titrated with related Hcl solution.

3.2.2.7.2 Blank titration procedure for other amoxicillins

Two portions of 10 ml of aliquot were taken into two different 100 ml conical flask, two drops of methyl red indicator were added to each then titrated with related Hcl solution.

3.2.2.8 Results of Back titration methods

3.2.2.8.1 Amoxicillin trihydrate

The volume of 0.009336M that required to neutralize 0.009211M NaOH that remained after that consumed by the the sample is 19.65ml.

The volume of 0.009336M Hcl that required to neutralize the 0.009211NaOH M

blank is 19.75ml.

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1mole 1mole

$NaOH + Hcl \rightarrow Nacl + H_2O$

1mole 1mole

mmoles of amoxicillin trihydrate = mmoles of the blank − mmoles of NaOH that remained after that consumed by the sample

mmoles of NaOH neutralized the blank = mmoles of the blank (b)

$= V_b X M_b = 19.75 \times 0.009336 = 0.84386$ mmoles

mmoles of NaOH that remained after that consumed by the sample

$= V_{Hcl} X M_{Hcl} = 19.65 \times 0.009336 = 0.1834524$mmoles.

Since mmoles of amoxicillin trihdrate = mmoles of blank − mmoles of the sample

$= V_b X M_b - V_s X V_s$

$= (0.009336 \times 19.75) - (0.009336 \times 19.65)$

= 0.184386− 0.1834524=0.0009336 mmoles

These mmoles were contained in 20ml of amoxicillin trihddrate solution

mmoles that contained in 250ml of the solution

$$= \frac{0.0009336 \times 250}{20} = 0.01167 \text{ mmole}$$

Weight of amoxicillin trihydrate = 0.01167× 419.4= 4.894mg = 0.004894g

$$\% \text{ of amoxicillin trihydrate} = \frac{0.004894 \times 100}{0.4998} = \% 0.98$$

3.2.2.8.2 Amipharma Amoxicillin capsules

The volume of 0.009336M that required to neutralize 0.009244M NaOH that remained after that consumed by the sample is 19.35ml

The volume of 0.009336M Hcl that required to neutralize the 0.009244M NaOH blank is19.75ml.

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1mole

$$NaOH + Hcl \rightarrow Nacl + H_2O$$

1mole 1mole

mmoles of Amipharma amoxicillin capsule = mmoles of the blank − mmoles of 0.009244M NaOH solution that remained after that consumed by the sample

mmoles of the blank = mmoles 0.009336 m Hcl reacted with it

$= V_{Hcl}XM_{Hcl} = 19.75 \times 0.009336 =$ 0.18438 mmoles

mmoles of 0.009244M NaOH that remained after that consumed by the sample that reacted with 0.009336 M Hcl solution = $V_{Hcl}XM_{Hcl}$ = 19.35×0.009336 =0.1806516 mmoles

Since mmoles of Amipharma amoxicillin capsule = mmoles of the blank − mmoles of 0.009244M NaOH that remained after that consumed by the sample.

Therefore mmoles of Amipharma amoxicillin capsule

= 0.184386- − 0.1806516 = 0.0037344 mmoles

These mmoles were contained in10ml of Amipharma amoxicillin capsule solution

mmoles of Amipharma amoxicillin capsules contained in 250ml of solution =

$$\frac{0.00235\times250}{10} = 0.009336 \text{ mmoles}$$

Weight of Amipharma amoxicillin capsule = mmoles of it xM wt

= 0.009336x 419.4 = 39.155184mg = 0.039155184g = 0.0392g

% of Amipharma amoxicillin capsules $= \frac{0.0392\text{x } 100}{0.4189} = 9.35\%$

3.2.2.8.3Changahi Amoxicillin capsules

The volume of 0.009336M that required to neutralize 0.00921M NaOH that remained after that consumed by the the sam,ple is19.35ml

The volume of 0.009336M Hcl that required to neutralize the 0.00921NaOHM blank is 19.65ml.

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1mole

$$NaOH + Hcl \rightarrow Nacl + H_2O$$

1mole 1mole

mmoles of Changhi amoxicillin capsules=mmoles of the blank − mmoles of 0.00921M NaOH solution that remained after that consumed by the sample sample

mmoles of the blank = mmoles 0.00933mHcl reacted with it

$= V_{Hcl}XM_{Hcl} = 19.65\times0.00933 =$ 0.1833 mmole

mmoles of 0.00921m NaOH that remained after that consumed by the sampl and reacted with 0.00933m Hcl solution =

$V_{Hcl}XM_{Hcl} = 19.35\times0.009336 = 0.18053$ m moles.

Since mmoles of Changahi amoxicillin capsule = mmoles of the blank − mmoles of 0.00921 NaOHm that remained after that consumed by the sample.

Therefore mmoles o Changshi amoxicillin capsule = 0.18433 − 0.18053 = 0.00277mmoles.

These mmoles were contained in10ml of Changahi amoxicillin capsule solution

mmoles o Changahi amoxicillin capsule contained in 250ml of solution =

$$\frac{0.00277\times250}{10} = 1.162\text{mmoles}$$

Weight of Changahi amoxicillin capsules = mmoles of it xM wt

=1.161 x 419.4= 29.43mg = 0.02943g

$$\%\text{ of Changahi amoxi cillin capsules} = \frac{0.02943\text{x }100}{0.423} = 6.96\%$$

3.2.2.8.4 G.M Amoxicillin capsule

The volume of 0.009336M that required to neutralize 0.009171M NaOH that remained after that consumed by the sample is 19.55ml

The volume of 0.009336M Hcl that required to neutralize the 0.00917 NaOH M blank is 19.65ml.

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1mole

$$NaOH + Hcl \rightarrow Nacl + H_2O$$

1mole 1mole

mmoles of G.M amoxicillin capsules = mmoles of the blank − mmoles of 0.00917M NaOH solution that remained after that consumed by the sample and reacted with 0.009336m Hcl

mmoles of the blank = mmoles 0.009336mHCLreacted with it

$= V_{Hcl}XM_{Hcl} = 19.65\times0.009336 =$ 0.1835 mmoles

mmoles of 0.009171M NaOH that remained after that consumed by the sample and reacted with 0.009336m Hcl solution =

$V_{Hcl}XM_{Hcl} = 19.55\times0.009336 = 0.1825$ mmoles

Since mmoles of G.m amoxicillin capsule = mmoles of the blank − mmoles of 0.009171M NaOH that remained after that consumed by the sample and reacted with 0.009336M Hcl solution =

Therefore mmoles of G.M amoxicillin capsule= 0.1835− 0.1825 = 0.001mmoles.

These m moles were contained in10ml of amoxicillin solution

mmoles of G.M amoxicillin capsule contained in 250ml of solution =

$$\frac{0.001\times250}{20} = 0.0125 \text{ mmoles}$$

Weight of G.M amoxicillin capsule = mmoles of it xm wt

=0.0125x 419.4= 5.24mg = 0.00524g

% o G.M amoxicillin sapsule $= \frac{0.02943 \text{x } 100}{0.43378} = 5.88\%$

3.2.2.8.5 Wafra Amoxicillin capsules

The volume of 0.01967M that required to neutralize 0.0281M NaOH that remained after that consumed by the sample is 35.45ml

The volume of 0.01967M Hcl that required to neutralize the 0.00281NaOHM blank is 35.75ml.

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1mole 1mole

$NaOH + Hcl \rightarrow Nacl + H_2O$

1mole 1mole

mmoles of Wafra amoxicillin capsules = mmoles of the blank − mmoles of 0.02814NaOH solution that remained after that cosumed by the sample and reacted with 0.01967m Hcl

mmoles of the blank = mmoles 0.01967M Hcl reacted with it

$= V_{Hcl}XM_{Hcl} = 35.75\times0.01967 = 0.7032$ mmoles

mmoles of 0.02814M NaOH that remained from the sample and reacted with 0.01967M Hcl solution = $V_{Hcl}XM_{Hcl} = 35.45\times0.01967= 0.6976$moles

Since mmoles of Wafra amoxicillin capsule = mmoles of the blank − mmoles of 0.02814M NaOH that remained from the sample and reacted with0.01967M Hcl solution

Therefore mmoles of Wafra amoxicillin capsule = 0.7032−0.6976 = 0.0059 mmoles

These mmoles were contained in10 ml of Wafra amoxicillin capsule solution

mmoles of Wafra amoxicillin capsule contained in 250ml of solution =

$$\frac{0.0059\times 250}{10} = 0.1475\text{mmoles}$$

Weight of Wafra amoxicillin capsule = mmoles of it xM wt

$$=0.1475\times 419.4 = 61.862\text{mg} = 0.061862\text{g}$$

$$\%\text{ of Wafra amoxicillin capsule} = \frac{0.061862\times 100}{0.4576} = 13.52\%$$

3.2.3 Conductometeric titration of amoxicillin with NaOH solution

3.2.3.1 Amoxicillin trihydrate

3.2.3.1.1 Reagents

1- amoxicillin tri hydrate solution (0.4889 g/250 ml distilled water)

2- 0.09244 M NaOH solution

3.2.3.2 Amipharma amoxicillin capsule

3.2.3.2.1 Reagents

1- Amipharma amoxicillin capsule solution

2- 0.09211 M NaOH solution

3.2.3.3 Changahi amoxicillin capsule

3.2.3.3.1 Reagents

1- Changahi amoxicillin capsule solution

2- 0.0917 M NaOH solution

3.2.3.4 Wafra amoxicillin capsule

3.2.3.4.1 Reagents

1- Wafra amoxicillin capsule solution

2- 0.0745 M NaOH solution

3.2.3.5 G.M amoxicillin capsule

3.2.3.5.1Reagents

1- G.M amoxicillin capsule solution

2- 0.0917 M NaOH solution

3.2.3.6 General apparatus

1) 50 ml pipette
2) 50 ml measuring cylinder
3) Magnetic stirrer and magnetic rod
4) conductometer
5) 100 ml beaker

3.2.3.7 General procedure

A volume of 50 ml of aliquot was taken into 100 ml beaker the conductivity of was measured, then 0.09244 M NaOH solution was added in a portion of 0.2 ml and the conductivity of the mixture was measured after each addition and stirring and recorded as shown' in Table (3.16) Then the concentration and the amount of the amoxicillin were calculated from the graph as shown' in Fig (3.23).

A weight of 0.4270 g, 0.4977 g, 0.4999 g and 0.5031 g of Amipharma, Changahi, Wafra and G.M amoxicillin capsules which contain 0.4189 g, 0.424 g, 0.4276 g and 0.43378 g of pure cephalexin was dissolved in about 200ml of distilled water with aid of magnetic stirrer and magnetic rod, transferred to 250 ml volumetric flask. Completed up to the mark with distilled water and filtered.

A volume of 50 ml of aliquot were taken into 100 ml beaker, it is conductivity was measured, then the related NaOH solution was added in portions of 0.2 ml and the conductivity was measured after each addition and stirring as shown' in tables (3.17,3.18,3.19,3.20). A graph of corrected conductivities against volume of NaOH solution werw plotted sa shown' in Figs (3.24,3.25,3.26,3.27). The amount of amoxicillin capsules were calculated for each.

3.2.3.8 Results of conductometeric titration methods with NaOH

3.2.3.8.1 Amoxicillin trihdrate

The volume of 0.09244M NaOHm from the graph is 2.6ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1mole

mmoles of amoxcillin = mmoles of 0.09244M NaOH

$= V_{NaOH} X M_{NaOH}$ $= 0.09244 \times 2.6 = 0.240344$ m moles

These m moles are contained in 50ml of amoxicillin trihydrate solution

mmoles of amoxicillin trihdrate that contained in 250ml of the solution

$$= \frac{0.09244 \times 2.6 \times 250}{50} = 1.20172 \text{mmoles}$$

Weight of amoxicillin trihydrate = mmoles ×M wt = 1.20174× 419.4=504mg =0.504g

$$\% \text{ of amoxicillin trihydrate} = \frac{0.504 \times 100}{0.4994} = 100.9\ 0\%$$

Table 3.16 Conductometreic titration of 50ml amoxcillin trihydrate with.0.09224M NaOH

Vol. of NaOH/ml	Ω / ms	Ω $(V_o + V_o)/V$ ms
0.00	0.01536	0.01536
0.20	0.0291	0.0292
0.40	0.0482	0.0486
0.60	0.0742	0.0751
0.80	0.0932	0.0947
1.00	0.1140	0.1162
1.20	0.1361	0.1394
1.40	0.1535	0.1573
1.60	0.1728	0.1783
1.80	0.1953	0.2023
2.00	0.215	0.2236
2.20	0.235	0.2453
2.40	0.254	0.2662
2.60	0.271	0.285
2.80	0.296	0.3125
3.00	0.321	0.3572
3.20	0.337	0.3586
3.40	0.369	0.399
3.60	0.396	0.4245
3.80	0.424	0.4562
4.00	0.451	0.487

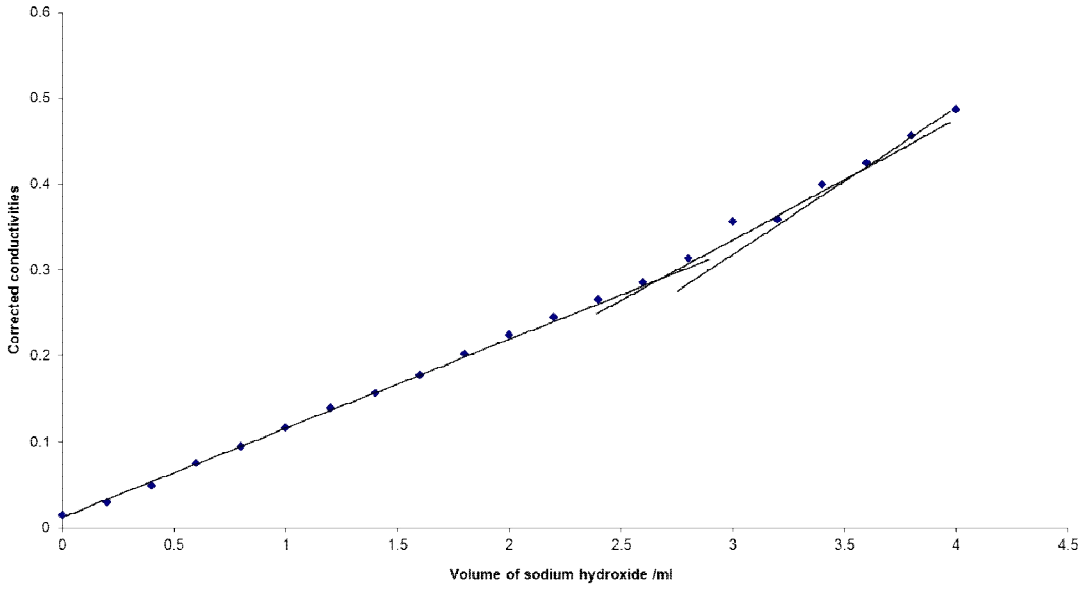


Fig 3.23 Conductometreic titration of amoxcillin tri hydrate with 0.09224M NaOH

3.2.3.8.2 Amipharma Amoxicillin capsules

Volume of 0.09211 M NaOH solution from the graph is 2.34ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1mole 1mole capsules = mmoles 0.09211 M NaOH

$=V_{NaOH}XM_{NaOH}$ $=2.34 \times 0.09211$ = 0.21554 mmoles

These mmoles were contained in 50ml of Amipharma amoxicillin capsules solution

mmoles that contained in 250ml of the solution

$$= \frac{0.21554 \times 250}{50} = 1.07769 \text{ mmoles}$$

Weight of Amipharma capsules = 1.07769×419.4 = 451.982m g

$$\% \text{ of Amipharma moxicillin capsules} = \frac{451.982 \times 100}{1000 \times 0.4189} = 107.9\ \%$$

Table 3.17 Conductometeric titration of 50ml Amipharma amoxicillin capsule with 0.09211M NaOH

Vol.of(NaOH/ml)	Ω /ms	Ω $(V_o + V_o)/V$ ms
0.00	0.033	0.033
0.20	0.052	0.0534
0.40	0.0736	0.074
0.60	0.1077	0.1089
0.80	0.1316	0.1337
1.00	0.1575	0.1606
1.20	0.1825	0.1867
1.40	0.209	0.2148
1.60	0.233	0.2404
1.80	0.254	0.263
2.00	0.282	0.2932
2.20	0.303	0.3163
2.40	0.328	0.3437
2.60	0.365	0.3839
2.80	0.386	0.4076
3.00	0.420	0.4452
3.20	0.454	0.4830
3.40	0.485	0.5179
3.60	0.529	0.5670
3.80	0.546	0.5874
4.00	0.578	0.6242

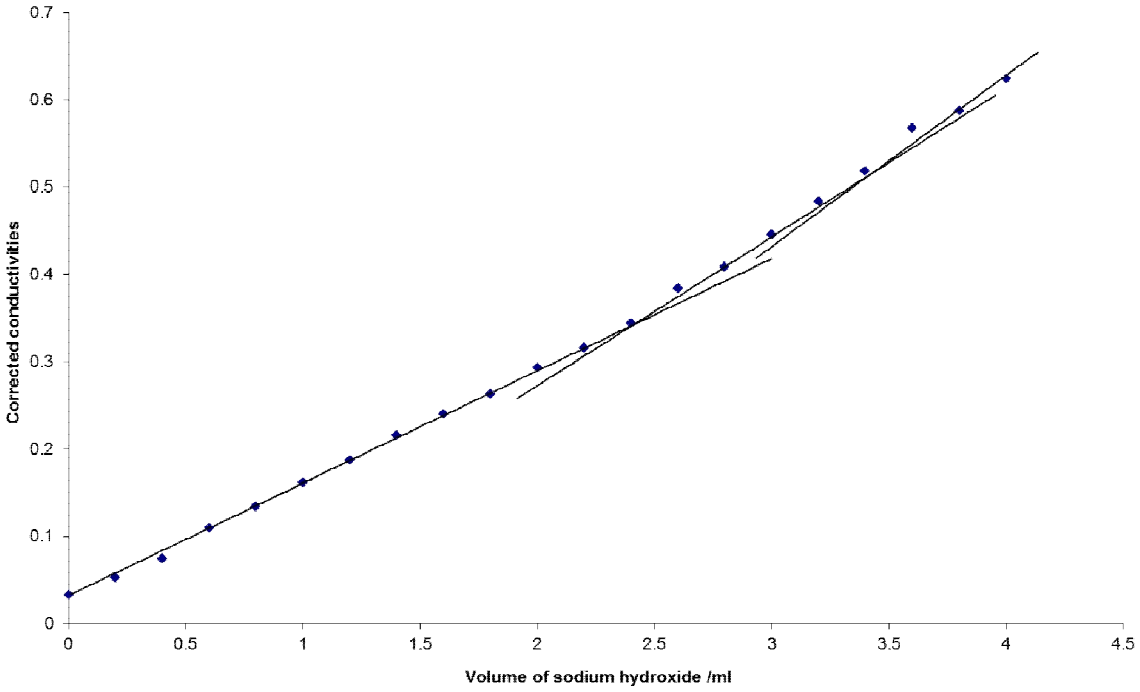


Fig 3.24 Conductometeric titration of Amipharma amoxicillin capsule with 0.09211M NaOH

3.2.3.8.3 Changahi Amoxicillin capsules

Volume of 0.0917 M NaOH solution from the graph is 2.4ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1mole 1mole

mmoles of amoxicillin = mmoles 0.0917M NaOH

$=V_{NaOH}XM_{NaOH}$ $=2.4 \times 0.0917$ = 0.22 mmoles

These mmoles were contained in 50 ml Changahi moxicillin capsules solution

mmoles that contained in 250ml of the solution

$$= \frac{0.22 \times 250}{50} = 1.1 \text{ mmoles}$$

Weight of Changahi amoxicillin capsules Wt= $1.1 \times 419.4 = 461.34$mg

$$\% \text{ of Changahi moxicillin capsules} = \frac{461.34 \times 100}{1000 \times 0.424} = 108.81\%$$

Table 3.18 Conductometeric titration of 50ml amoxicillin Changahi capsule with 0.0917 NaOH

Vol.of(NaOH/ml)	Ω /ms	Ω $(V_o + V_o)/V$ ms
0.00	0.0379	0.0379
0.30	0.0663	0.06669
0.60	0.1113	0.1126
0.90	0.1458	0.1484
1.20	0.1801	0.1844
1.50	0.216	0.2224
1.80	0.250	0.259
2.10	0.287	0.2990
2.40	0.318	0.3332
2.70	0367	0.3868
3.00	0.406	0.430
3.30	0.450	0.4797
3.60	0.497	0.5327
3.90	0.541	0.5831
4.20	0.592	0.6417
4.50	0.640	0.6976
4.80	0.693	0.7595
5.10	0.741	0.8165

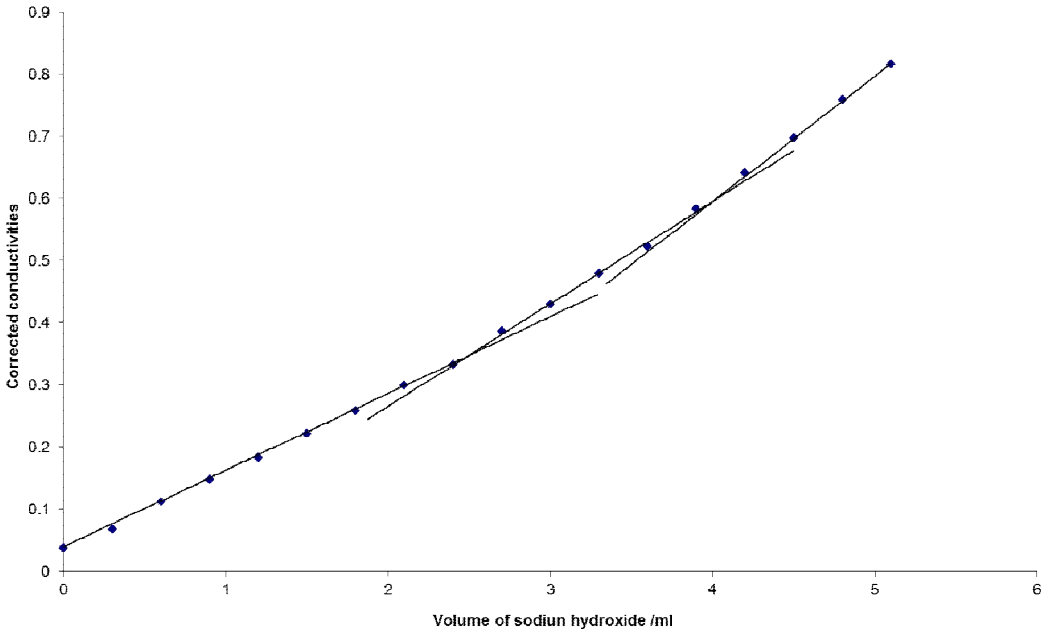


Fig 3.25 Conductometeric titration of amoxicillin Changahi capsule with0.0917M NaOH

3.2.3.8..4 G.M amoxicillin capsules

Volume of 0.0917 M NaOH solution from the graph is 2.5ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1mole

mmoles of amoxicillin = mmoles 0.0917M NaOH

$=V_{NaOH}XM_{NaOH}$ $=2.5 \times 0.0917$ $= 0.22925$ mmoles

These mmoles were contained in 50ml of G.M amoxicillin capsule solution

mmoles that contained in 250ml of the solution

$$= \frac{0.22925 \times 250}{50} = 1.14625 \text{ mmoles}$$

Weight of G.M amoxicillin capsles = $1.14625 \times 419.4 = 480.74$

$$\% \text{ of G.M moxicillin capsules} = \frac{480.74 \times 100}{1000 \times 0.43378} = 110.8\%$$

Table 3.19 Conductometerictitration of 50ml G M amoxicillin capsules with 0.0917MNaOH

Vol.of(NaOH/ml)	Ω /ms	Ω $(V_o + V_o)/V$ ms
0.00	0.0378	0.0378
0.30	0.0723	0.0727
0.60	0.1075	0.10889
0.90	0.1436	0.14618
1.20	0.1794	0.1837
1.50	0.220	0.2266
1.80	0.256	0.2652
2.10	0.291	0.3032
2.40	0.322	0.3344
2.70	0.367	0.3868
3.00	0.413	0.43778
3.30	0.462	0.4925
3.60	0.5090	0.5456
3.90	0.554	0.5972
4.20	0.598	0.6482
4.50	0.651	0.709
4.80	0.698	0.765

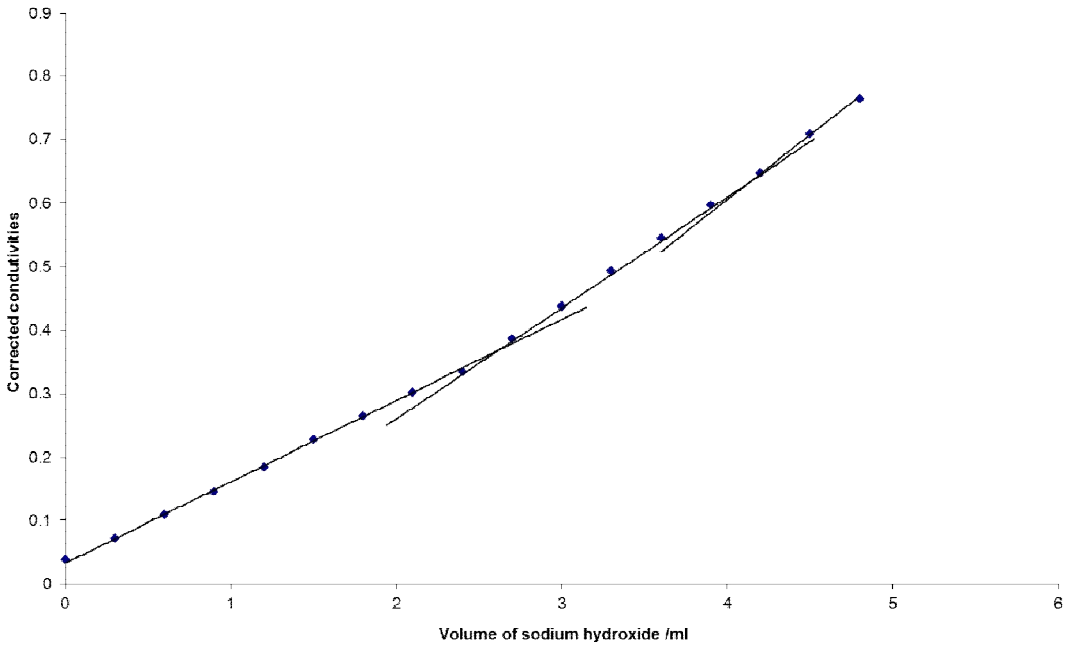


Fig 3.26 Conductometeric titration of G M amoxicillin capsules with 0.0917MNaOH

3.2.3.8. 5 Wafra Amoxicillin capsules

Volume of 0.0645M NaOH solution from the graph is 2.76ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1mole

mmoles of Wafra amoxicillin capsules = mmoles 0.0745 NaOH

$=V_{NaOH}XM_{NaOH}$ $=2.76 \times 0.7645$ $= 0.20562$mmoles

These mmoles were contained in 50 ml of Wafra amoxicillin capsules solution

mmoles that contained in 250ml of the solution

$$= \frac{0.20562 \times 250}{50} = 1.03\text{mmole}$$

Weight of Wafra amoxicillin capsules = $1.0281 \times 419.4 = 431.$ mg

$$\% \text{ of Wafra moxicillin capsules} = \frac{431.85 \times 100}{1000 \times 0.4276} = 94.4\%$$

Table 3.20 Conductometric titration of 50ml Wafra amoxicillin capsules with 0.0745M NaOH

Vol.of(NaOH/ml)	Ω /ms	Ω $(V_o + V_o)/V$ ms
0.00	0.0395	0.0395
0.30	0.0617	0.062
0.60	0.0882	0.0892
0.90	0.1134	0.1154
1.20	0.1376	0.1409
1.50	0.1625	0.1673
1.80	0.1885	0.1953
2.10	0.208	0.2167
2.40	0.231	0.242
2.70	0.253	0.2666
3.00	0.277	0.2936
3.30	0.308	0.328
3.60	0.324	0.3473
3.90	0.361	0.3891
4.20	0.389	0.4216
4.50	0.415	0.4523

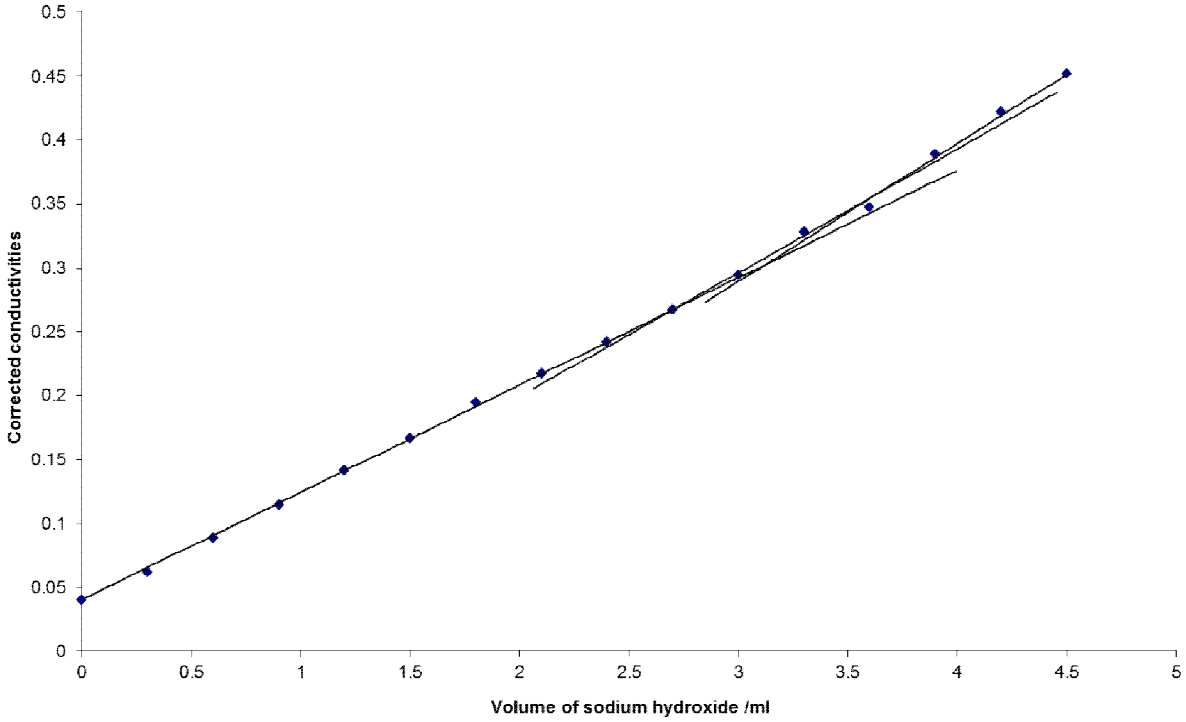


Fig 3.27 Conductometric titration of Wafra amoxicillin capsules with 0.0745M NaOH

3.2.4 Conductometeric titration of amoxicillin with NH_4OH solution

3.2.4.1 Amoxicillin trihydrate

3.2.4.1.1 Reagents

1- Amoxicillin trihydrate solution (0.4994 g/250 ml distilled water)

4- 0.0794 M NH_4OH solution

3.2.4.2 Amipharma amoxicillin capsules

3.2.4.2.1 Reagents

1- Amipharma amoxicillin capsules solution

2- 0.07883 M NH_4OH solution

3.2.4.3 Changahi amoxicillin capsules

3.2.4.3.1 Reagents

1-Changahi amoxicillin capsules solution

2- 0.0725 M NH_4OH solution

3.2.4.4 Wafra amoxicillin capsules

3.2.4.4.1 Reagents

1- Wafra amoxicillin capsules solution

2- 0.1202 M NH_4OH solution

3.2.4.5 G.M amoxicillin capsules

3.2.4.5.1 Reagents

1- G.M amoxicillin capsules solution

2- 0.07886 M NH_4OH solution

3.2.4.6 General apparatus

1- 50 ml pipette

2- 50 ml measuring

3- Magnetic stirrer and magnetic rod

4- conductometer

5- 100 ml beaker

3.2.4.7 General procedure

A volume of 50 ml of amoxicillin tri hydrate aliquot was taken into 100 ml beaker and the conductivity of the aliquot was measured.

Then 0.0794 M NH_4OH solution was added in a portion of 0.2ml and the conductivity of the mixture was measured after each addition and stirring and recordered as shown' in table(3.21) then the concentration and the amount of amoxicillin was calculated from the end point obtained from the graph as shown' in Fig (3.28).

A weight of 0.5613 g, 0.4977 g, 0.4999 g and 0.5031 g of Amipharma, Changahi, Wafra and G.M. amoxicillin capsules which contain 0.4981 g, 0.4240 g, 0.4276 g and 0.43378 g of pure amoxicillin each was dissolved in about 200 ml of distilled water with aid of magnetic stirrer and magnetic rod, transferred to 250 ml volumetric flask. Completed up to the mark with distilled water and filtered.

A volume of 50 ml of aliquot were taken into 100 ml beaker, it is conductivity was measured, then the related NH_4OH solution was added in portions of 0.2 ml and the conductivity was measured after each addition and stirring as shown' in tables(3.22,3.23,3.24,3.25). Graphs of corrected conductivities against volume of NH4OH solution were plotted as shown' in Figs (3.29,3.30,3.31,3.32). The amount of cephalexin capsules was calculated for each.

3.2.4.8 Results of conductometric titration method with NH_4OH

3.2.4.8.1 Amoxicillin trihydrate

The volume of 0.0794m NH_4OH from the graph is 2.88 ml

$$Q-C\,OOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1mole

mmoles of amoxicillin =mmoles of 0.0794M NH_4OH

$= V_{NH4OH} \times M_{NH4OH}$ $= 0.0794 \times 2.88 = 0.228672$mmoles

These mmoles were contained in 50 ml of amoxicillin trihydrate solution

mmoles of amoxicillin trihydrate that contained in 250 mlof the solution

$$= \frac{0.228672 \times 250}{50} = 1.14336 \text{ mmoles}$$

Weight of amoxicillin trihydrate = mmols of it x it is Mwt

=1.14336 ×419.4 =479.525mg

% of amoxcillin trihydrate $= \frac{479.525 \times 100}{1000 \times 0.4994} = 96.02\%$

Table 3.21 Coductometeric titration of 50ml amoxicillin trihydrate with0.0794MNH_4OH

ol .of NH_4OH/ml	Ω /ms	Ω $(V_o + V_o)/V$ ms
0.00	0.114	0.1148
0.20	0.1752	0.1759
0.40	0.208	0.20966
0.60	0.229	0.2317
0.80	0.252	0.2560
1.00	0.273	0.2785
1.20	0.304	0.31129
1.40	0.330	0.3392
1.60	0.361	0.37255
1.80	0.386	0.39989
2.00	0.409	0.4254
2.20	0.431	0.44996
2.40	0.452	0.47337
2.60	0.475	0.4997
2.80	0.491	0.51849
3.00	0.505	0.5353
3.20	0.515	0.54796
3.40	0.524	0.55963
3.60	0.535	0.5735
3.80	0.545	0.5864
4.00	0.554	0.5983

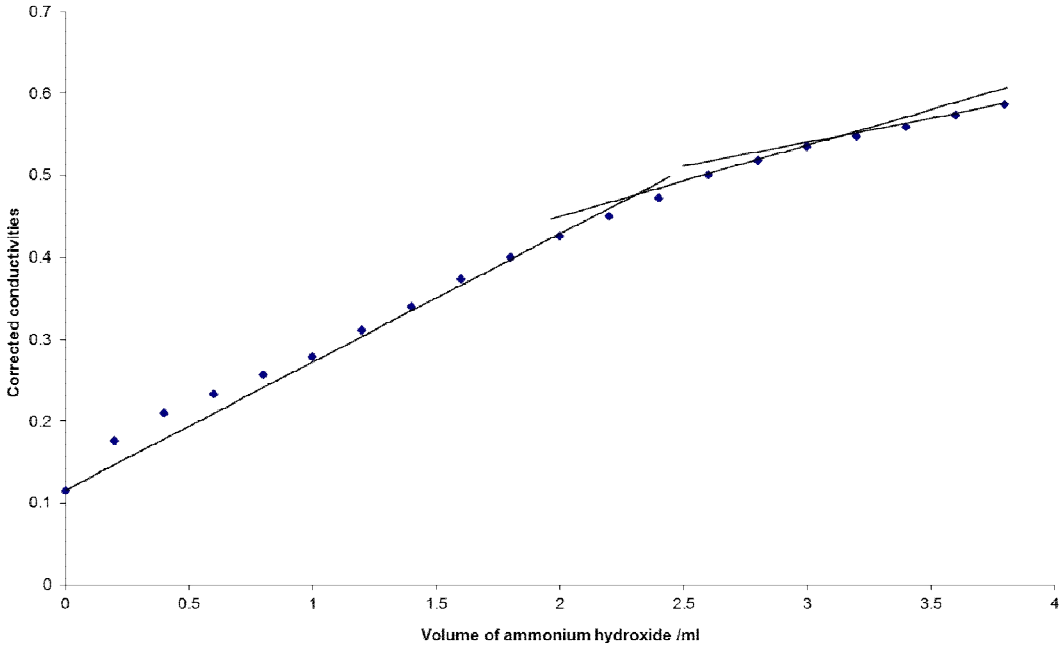


Fig 3.28 Coductometeric titration of amxcillin tri hydrate with 0.0794M NH_4OH

3.2.4.8.2 Amipharma Amoxicillin capsules

The volume of 0.07883 MNH_4OH from the graph is 2.98 ml

Q -COOH + NaOH → Q −COONa + H_2O

1mole 1mole

mmoles of Amipharma amoxicillin capsules =mmoles of 0.07883 M NH_4OH

= $V_{NH4OH} \times M_{NH4OH}$ =2.98 ×0.07883 = 0.23491 mmoles

These mmoles were contained in 50ml of Amipharma amoxicillin capsules solution

mmoles of Amipharma amoxicillin capsules that contained in 250ml of the solution

$$\frac{0.23491 \times 250}{50} = 1.17455 \text{mmoles}$$

Weight of Amipharma amoxicillin capsules = mmoles×M wt

= 1.17455×419.4 =492.63mmoles

% of Amipharma amoxicillin capsules $= \frac{492.63 \times 100}{0.4981} = 98.90\%$

Table 3.22 Conductometeric titration of 50ml Amipharma amoxicillin capsules With0.07883M NH_4OH

Vol of (NH_4OH/ml)	Ω / ml	Ω (V_o +V_o)/V ms
0.00	0.0392	0.0392
0.30	0.0802	0.08078
0.60	0.1251	0.1266
0.90	0.1882	0.1915
1.20	0.218	0.223
1.50	0.257	0.2647
1.80	0.302	0.3128
2.10	0.343	0.3475
2.40	0.377	0.395
2.70	0.419	0.4416
3.00	0.444	0.4706
3.30	0.461	0.491
3.60	0.481	0.5156
3.90	0.492	0.5303
4.20	0.505	0.545
4.50	0.509	0.5548
4.80	0.516	0.5655

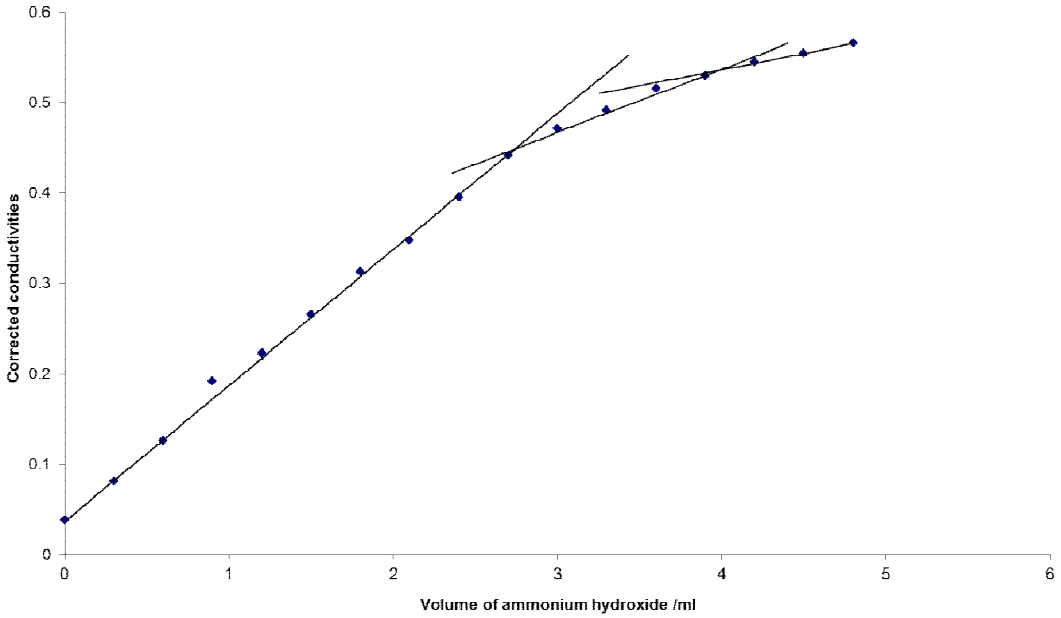


Fig 3.29 Conductometeric titration of Amipharma amoxicillin capsules amoxicillin with 0.07886M NH_4OH

3.2.5.8.3 Changahi Amoxicillin capsules

The volume of 0.07883 M NH_4OH from the graph is3.02 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1mole 1mole

mmoles of amoxicillin=mmoles of 0.07883 M NH_4OH

$= V_{NH4OH} \times M_{NH4OH}$ $=3.0\times0.0725$ $= 0.2175$mmoles

These mmoles were contained in 50ml of Changahi amoxicillin capsules solution

mmoles of Changahi amoxicillin capsules that contained in 250ml of the solution

$$\frac{0.2175 \times 250}{50} = 1.0875 \text{mmoles}$$

Weight of Changahi amoxicillin capsules = mmoles×M wt

$= 1.0875 \times 419.4$ $=456.0975$

% of Changahi amoxicillin capsules $= \frac{456.0975\times100}{1000\times0.4276} =$ % 106.66

Table 3.23 Conductometeric titration of 50ml Changahi amoxicillin capsule with 0.0723M NH_4OH

Vol of (NH_4OH/ml)	Ω / ml	Ω (V_o +V_o)/V ms
0.00	0.0399	0.0399
0.30	0.0702	0.0706
0.60	0.0926	0.0937
0.90	0.1342	0.1366
1.20	0.1754	0.1796
1.50	0.212	0.218
1.80	0.247	0.25589
2.10	0.281	0.2928
2.40	0.317	0.3322
2.70	0.362	0.3815
3.00	0.393	0.41658
3.30	0.417	0.4445
3.60	0.436	0.48776
3.90	0.455	0.4904
4.20	0.468	0.5073
4.50	0.481	0.52429
4.80	0.489	0.5359

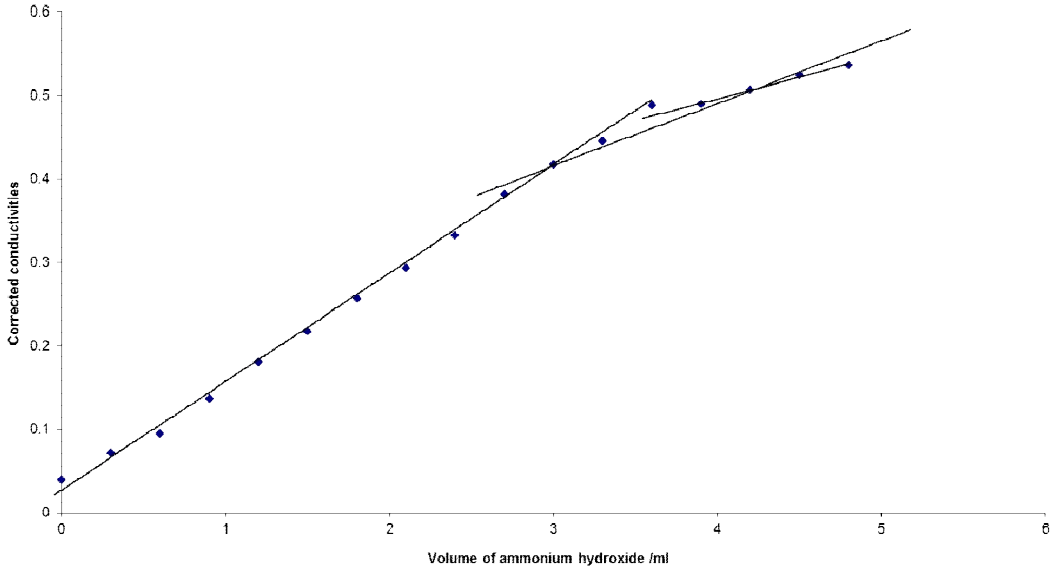


Fig 3.30 Conductometeric titration of Changahi amoxicillin capsule with 0.0723M NH_4OH

3.2.4.8.4 G.M Amoxicillin capsules

The volume of 0.07883 M NH_4OH from the graph is 2.79 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1mole 1mole

mmoles of G.M amoxicillin capsules=mmoles of 0.07883 M NH_4OH

$= V_{NH4OH} \times M_{NH4O}$ $=2.79 \times 0.07886$ $= 0.22$ mmoles

These mmoles were contained in 50 ml G.M moxicillin capsules solution

mmoles of G.M amoxicillin capsules that contained in 250ml of the solution

$$\frac{0.22 \times 250}{50} = 1.1 \text{ mmoles}$$

Weight of G.M amoxicillin capsules = mmoles×M wt $1.1 \times 419.4 = 461.3$ mg

% of G.M amoxicillin capsules $= \frac{461.3 \times 100}{1000 \times 0.43378} = 106.3\%$

Table 3.24 Conductometeric titration of 50ml G.M amoxicillin capsule with 0.07886M NH_4OH

Vol of (NH_4OH/ml)	Ω / ml	Ω (V_o +V_o)/V ms
0.00	0.0366	0.0366
0.30	0.0715	0.0719
0.60	0.1184	0.1198
0.90	0.1568	0.1596
1.20	0.1991	0.2038
1.50	0.236	0.243
1.80	0.271	0.2807
2.10	0.307	0.3199
2.40	0.333	0.3489
2.70	0.378	0.3984
3.00	0.399	0.4229
3.30	0.417	0.4445
3.60	0.433	0.4642
3.90	0.445	0.4797
4.20	0.457	0.495
4.50	0.465	0.5068
4.80	0.474	0.5195

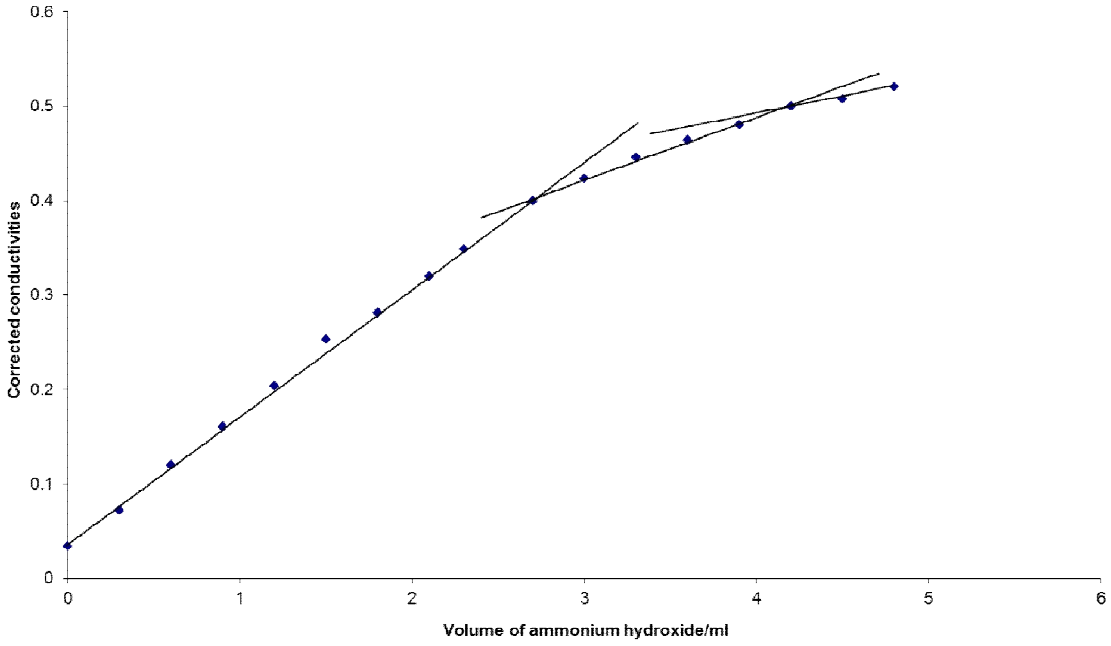


Fig 3.31 Conductometeric titration of G.M amoxicillin capsule with 0.07886M NH_4OH

3.2.4.8.5 Wafra Amoxicillin capsules

The volume of 0.1202M NH_4OH from the graph is1.68ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1mole

mmoles of Wafra amoxicillin capsules =mmoles of 0.1202 m NH_4OH

$= V_{NH4OH} \times M_{NH4OH}$ $=1.68\times0.1202$ $= 0.202$ mmoles

These mmoles were contained in 50ml of Wafrra amoxicillin capsules solution

mmoles of Wafra amoxicillin capsules that contained in 250ml of the solution

$$\frac{0.202 \times 250}{50} = 1.101 \text{mmoles}$$

Weight of Wafra amoxicillin capsules = mmoles×M wt 1.101×419.4 =423.6mg

$$\% \text{ of Wafra amoxicillin capsules} = \frac{423.6\times100}{1000\times0.4276} = 99.7\%$$

Table 4.25 Conductometeric titration of 50 Wafra amoxicillin capsules with 0.1202M NH_4OH

Vol of (NH_4OH/ml)	Ω / ml	Ω (V_o +V_o)/V ms
0.00	0.0389	0.0389
0.20	0.0879	0.08825
0.40	0.1300	0.1310
0.60	0.1632	0.1651
0.80	0.1998	0.2029
1.00	0.236	0.2407
1.20	0.275	0.2816
1.40	0.313	0.3217
1.60	0.343	0.3539
1.80	0.368	0.3812
2.00	0.393	0.4087
2.20	0.411	0.429
2.40	0.428	0.4485
2.60	0.440	0.4628
2.80	0.451	0.4762
3.00	0.460	0.4876
3.20	0.468	0.4979
3.40	0.475	0.5073

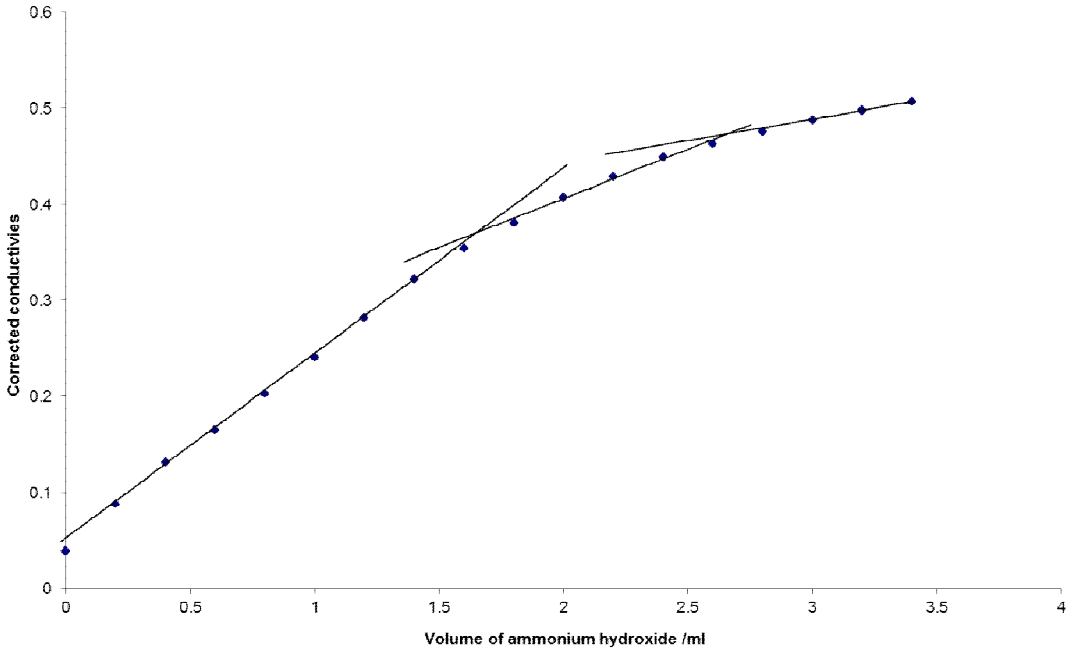


Fig 3.32 Conductometeric titration of Wafra amoxicillin capsules with 0.1202M NH_4OH

3.2.5 Potentionmetric titration of amoxicillin with NaOH

3.2.5.1 Amoxicillin trihydrate

3.2.5.1.1 Reagents

1- Amoxicillin trihydrate solution (0.4994 g/250 ml of distilled water)

2- 0.09244 M NaOH solution

3.2.5.2 Amipharma amoxicillin capsules

3.2.5.2.1 Reagents

1- Amipharma amoxicillin capsules solution

2- 0.09211 M NaOH solution

3.2.5.3 Changahi amoxicillin capsules

3.2.5.3.1 Reagents

1- Changahi amoxicillin capsules solution

2- 0.09211 M NaOH solution

3.2.5.4 Wafra amoxicillin capsules

3.2.5.4.1 Reagents

1- Wafra amoxicillin capsules solution

2- 0.0745 M NaOH solution

3.2.5.5 G.M. amoxicillin capsules

3.2.6.5.1 Reagents

1- G.M. amoxicillin capsules solution

2- 0.0917 M NaOH solution

3.2.5.6 General apparatus

1- 50 ml measuring cylinder

2- 50 ml pipette

3- pH meter

4- Magnetic sirrer and magnetic rod

5- 100 ml beaker

3.2.5.7 General procedure

An aliquod of 50 ml of amoxicillin solution was taken into 100 ml beaker then titrared potentiometerically with 0.09244 M NaOH solution. NaOH

solution was added in portions and stirred after each addition of NaOH solution, the pH value was recorded after each addition and (ΔpH/ΔV) valueswere calculated as shown, in Table (3.26) values were calculated. Graphs of pH values virus volumes of NaOH and (ΔpH/ΔV) virus volume were plotted as shown, in Figs (3.33,3.34) and the amount of cephalexin were calculated.

A weight of 0.4720 g. 0.4977 g, 0.4999 g and 0.5031 g of Amipharma, Changahi, Wafra and G.M. amoxicillin capsules respectively which contain respectively 0.4189 g, 0.424 g, 0.4576 g and 0.4337g of pure amoxicillin were dissolved in about 200 ml of distilled water with aid of magnetic stirrer and magnetic rod, transferred to 250 ml volumetric flask. Completed up to the mark with distilled water and filtered.

A volume of 50 ml of aliquot from each were taken into 100 ml beaker, it is pH was measured, then the related NaOH solution was added and the pH of the solution was measured after each addition and stirring and (ΔpH/ΔV) values were calculated as shown, in Tables (3.27,3.28,3.29,3.30) Graphs of pH values against the volume of NaOH added, and (ΔpH/ΔV) against the volume of NaOH.

The amount of amoxicillin of each was calculated from end points Obtained fom the graphs as shown, in Figs [(3.35,3.36) (3.37,3.38) (3.39,3.40) (3.41,3.42)].

3.2.5.8 Results of potentiometeric titration method with NaOH

3.2.5.8.1 Amoxicillin trihdrate

1- From of pH/V the volume of 0.09244 M NaOH is 2.35ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1 mole 1 mole

mmoles of amoxicillin trihydrate = mmoles of 0.09244 M NaOH

$= V_{NaOH} \times M_{NaOH} = 2.35 \times 0.09244 = 0.217$ mmoles

These m moles were contained in 50 ml of amoxicillin trihydrate solution

mmoles of amoxicillin trihydrate that contained in 250 ml of the solution of amoxicillin trihdrate

$$= \frac{0.24 \times 250}{50} = 1.085 \text{mmole}$$

Weight of amoxicillin trihydrate = mmoles×M wt

= 1.085×419.4 = 455.049 mg = 0.455049g

% of amoxicillin tri hydrate = 0.455049×100/ 0.4994 = % 91.12

2- from the graph of ΔpH/ ΔV the neutralization volume of 0.09244 M NaOH is 2.35 ml

$$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$$

1mole 1 mole

mmoles of amoxicillin tri hydrate = mmoles of 0.09244 M NaOH

$= V_{NaOH} X M_{NaOH}$ = 2.35×0.09244 =0.217mmoles

These mmoles were contained in 50 ml of amoxicillin trihydrate solution

mmoles that contained in 250 ml of amoxicillin trihydrate solution

$$= \frac{0.0.217 \times 50}{2\ 50} = 1.085 \text{ mmoles}$$

Weight of amoxicillin trihydrate = mmoles of it × M wt

= 1.085×419.4 = 455.049mg

$$\% \text{ of amoxicillin trihydrate} = \frac{455.049 \times 100}{1000 \text{ x } 0.4994} = \% 91.12$$

Table 3.26 Potentiometeric titration of 50ml amoxicillin trihydrate with 0.09244M NaOH

Vol. of NaOH/ml	PH	δpH/δv/ v	Vol.of NaOH/ml	pH	δ pH/δV /V
0.00	5.151		2.5	8.351	
		2.364			1.45
0.50	6.333		2.6	8.496	
		1.248			1.15
1.00	6.957		2.7	8.11	
		0.788			1.09
1.50	7.351		2.8	8.720	
		0.797			1.23
1.80	7.590		3.0	8.965	
		0.925			1.646
2.00	7.775		3.5	9.363	
		1.42			0.85
2.10	7.917		4.0	9.788	
		1.16			0.58
2.20	8.033		5.0	10.368	
		0.73			0.637
2.30	8.106		6.0	11.005	
		1.54			
2.40	8.260				
		0.91			

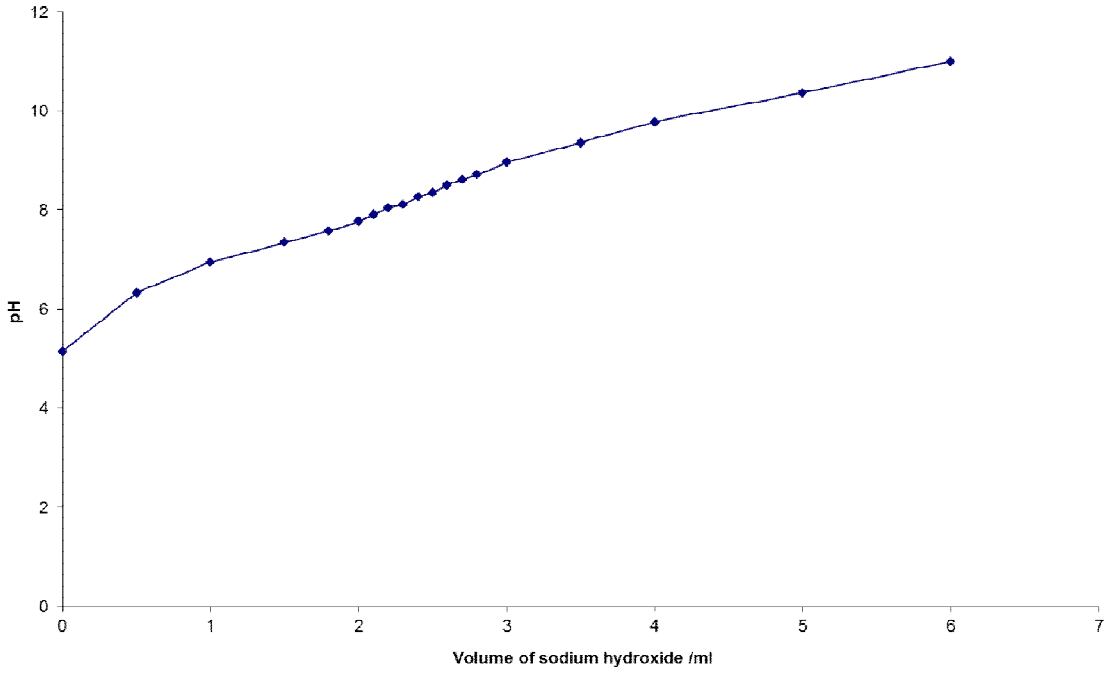


Fig 3.33 potentiometeric titration of 50ml amoxicillin trihydratewith 0.09244MNaOH-1

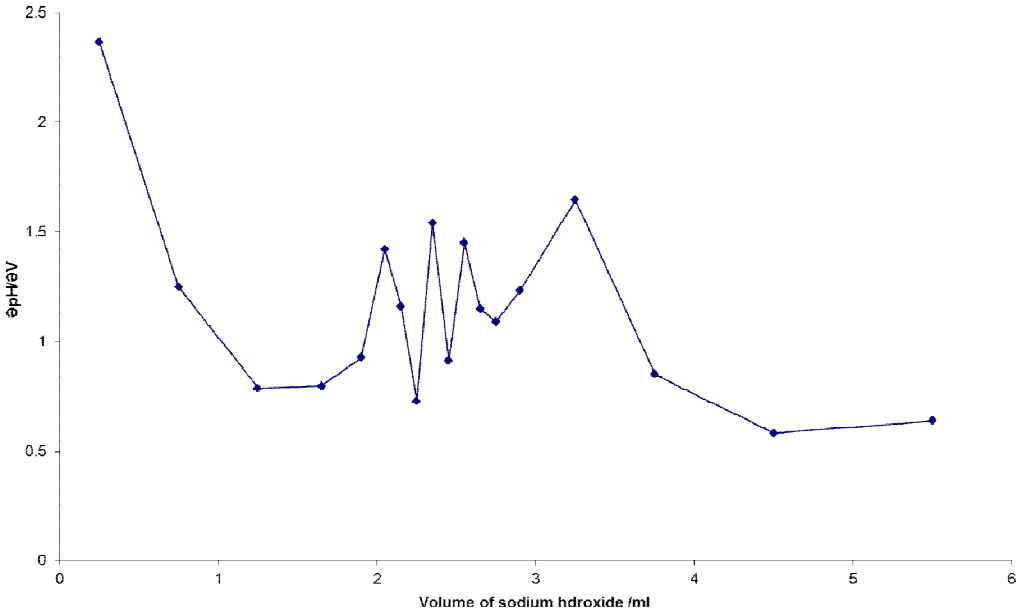


Fig 3.34 potentiometeric titration of 50ml amoxicillin trihydrate with 0.09244M NaOH-2

3.2.5.8.2 Amipharma amoxicillin capsules

1- From of pH/V the volume of 0.09211 M NaOH is2.45ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of Amipharma amoxicillin capsules = mmoles of 0.092 M NaOH

$= V_{NaOH} X M_{NaOH}$ $= 2.45 \times 0.09211 = 0.226$ mmoles

These m moles were contained in 50 ml of Amipharma amoxicillin capsules solution

mmoles that contained in 250 ml of Amipharma amoxicillin capsules solution

$$= \frac{0.226 \times 50}{2\ 50} = 1.115 \text{mmoles}$$

Weight of Amipharma amoxicillin capsules = mmoles of it × M wt

$= 1.115 \times 419.4 = 467.63$mg

% of Amipharma amoxicillin capsules $= \frac{467.63 \times 100}{1000 \times 0.4189}$ % 111.6

2- from the graph of ΔpH/ ΔV he neutralization volume of 0.09211M NaOH is 2.4 ml

mmoles of Amipharma amoxicillin capsules = mmoles of 0.09211 M NaOH

$= V_{NaOH} \times M_{NaOH}$ $= 2.4 \times 0.09211 = 0.2211$ mmoles

These mmoles were contained in 50 ml of Amipharma amoxicillin capsules solution

mmoles of Amipharma amoxicillin capsules that contained in 250 ml of the solution

$$= \frac{0.2211 \times 250}{50} = 1.1055 \text{ mmole}$$

Weight of Amipharma amoxicillin capsules = mmoles×M wt

$= 1.1055 \times 419.4 = 463.65$ mg $= 0.0.46365$g

% of amipharma amoxicillin capsules = 0.46365×100/ 0.4189 = %110.7

Table 3.27 Potentiometeric titration of 50ml Amipharma amoxicillin capsule with 0.09211M NaOH

Vol. of NaOH/ml	pH	δ pH/δv /v	Vol.of NaOH/ml	pH	δ pH/δV /V
0.00	4.92				0.826
		2.356	3.5	9.648	
0.50	6.098				0.764
		1.78	4.0	10.030	
1.00	6.988				0.512
		0.986	4.5	10.286	
1.50	7.481				0.705
		0.98	5.5	10.991	
1.70	7.677				0.413
		1.02	6.5	11.404	
1.90	7.881				0.333
		1.215	7.5	11.737	
2.10	8.124				0.166
		0.855	8.5	11.903	
2.30	8.295				0.093
		1.405	9.5	11.996	
2.50	8.576				0.102
		1.003	10.5	12.098	
2.70	8.877				0.06
		0.716	11.5	12.158	
3.00	9.235				

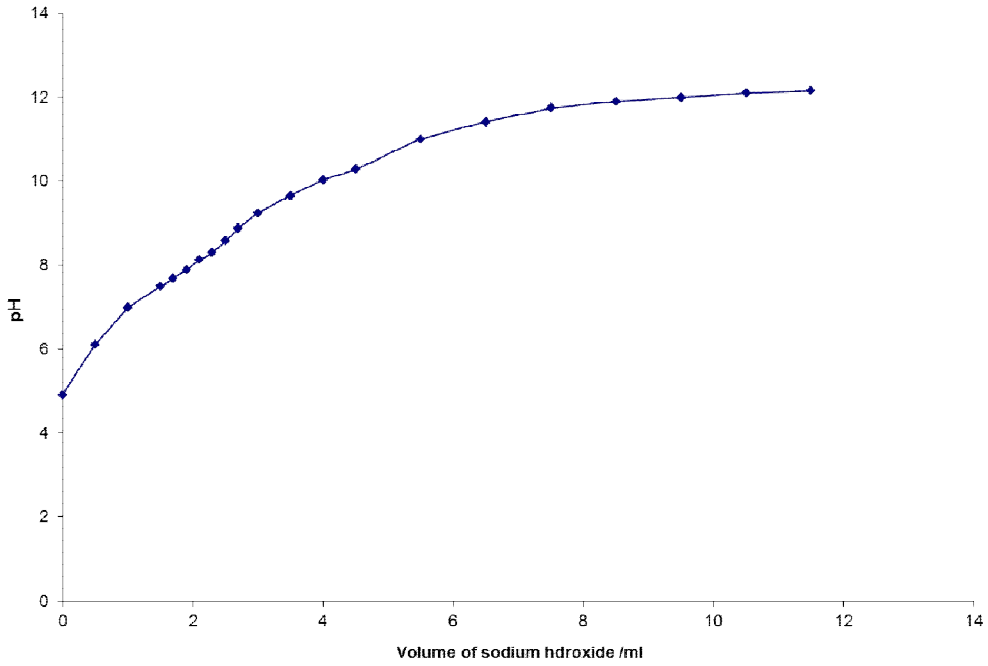


Fig 3.35 Potentiometeric titration of 50ml Amipharma amoxicillin capsule with 0.09211M NaOH -1

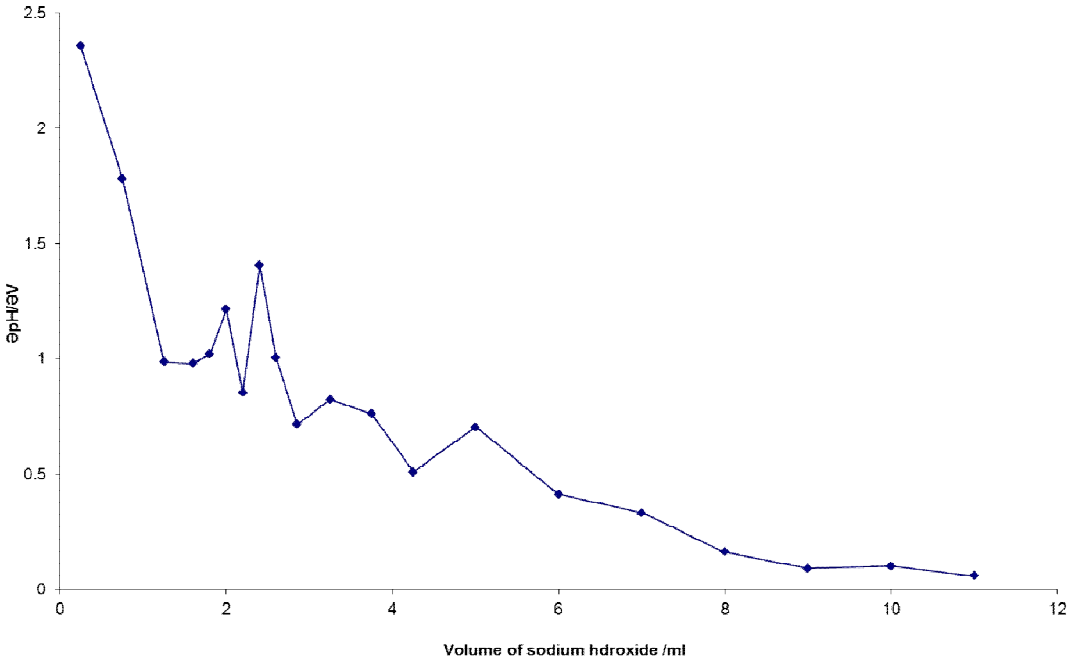


Fig 3.36 Potentiometeric titration of 50 ml Amipharma amoxicillin capsule with 0.09211M NaOH -2

3.2.5.8.3 Changahi amoxicillin capsules

1- From of pH/V the volume of 0.09211 M NaOH is 2.02 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of Changahi amoxicillin capsules = mmoles of 0.09211 M NaOH = $V_{NaOH} \times M_{NaOH}$ $= 2.02 \times 0.09211$ $= 0.1861$ mmoles

These m moles were contained in 50 ml of Changahi amoxicillin capsules solution

Therefore mmoles that contained in 250 ml of Changahi amoxicillin capsules solution

$$= \frac{0.1861 \times 250}{50} = 0.9305 \text{mmole}$$

Weight of Changahi amoxicillin capsules = mmoles×M wt

$= 0.9305 \times 419.4 = 390.252$mg $= 0.390252$g

%of changahi amoxicillin capsules $=0.390252 \times 100/0.424$ = % 92.04

2- From the graph of $\Delta pH/\Delta V$ the neutralization volume of 0.09211M NaOH is 2.0 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1mole 1 mole

mmoles of Changahi amoxicillin capsules = mmoles of 0.092 M NaOH= $V_{NaOH} X M_{NaOH}$ $= 2.0 \times 0.09211$ $=0.18422$mmoles

These mmoles were contained in 50 ml of Changahi amoxicillin capsules solution

Therefore mmoles that contained in 250 ml of Changahi amoxicillin solution

$$= \frac{0.18422 \times 50}{2\ 50} = 0.9211 \text{ mmoles}$$

Weight of Changahi amoxicillin capsules = mmoles of it × M wt

$= 0.9211 \times 419.4 = 386.31$ mg

% of Changahi amoxicillin capsules $= \frac{386.31 \times 100}{1000 \text{ x } 0.424}$ % 91.1

Table 3.28 Potentiometeric titration of 50ml Changahi amoxicillin capsule with 0.09211M NaOH

Vol. of NaOH/ml	pH	δ pH/δv /v	Vol.of NaOH/ml	pH	δ pH/δV /V
0.00	4.001				1.04
		2.644	3.0	8.735	
0.50	5.323				0.966
		2.576	3.5	9.218	
1.00	6.611				0.512
		1.422	4.0	9.474	
1.50	7.322				0.97
		1.025	4.5	9.959	
1.70	7.527				0.492
		0.48	5.0	10.105	
1.90	7.623				0.444
		1.47	5.5	10.427	
2.10	7.917				0.52
		0.605	6.0	10.687	
2.30	8.038				
		0.885			
2.50	8.215				

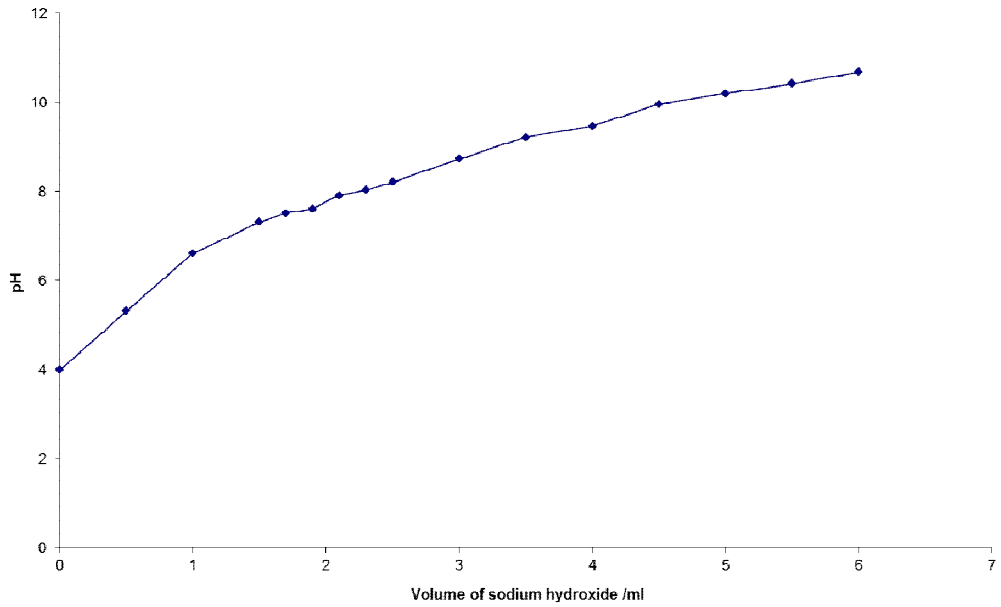


Fig 3.37 Potentiometeric titration of 50ml Changahi amoxicillin capsule with 0.09211M NaOH -1

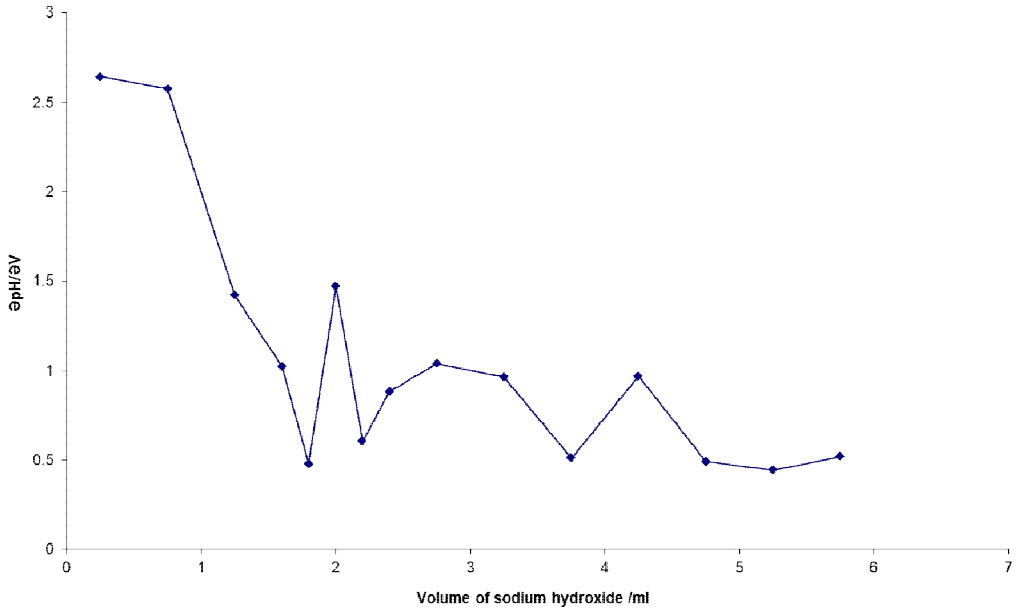


Fig 3.38 Potentiometeric titration of 50ml Changahi amoxicillin capsule with 0.09211M NaOH -2

3.2.5.8.4 G.M amoxicillin capsules

1- From of pH/V the volume of 0.0917 M NaOH is 2.25ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of G.M amoxicillin capsules = mmoles of 0.0917 M NaOH

$= V_{NaOH} \times M_{NaOH} = 2.25 \times 0.0917 = 0.2063$mmoles

These mmoles were contained in 50 ml of G.M amoxicillin capsules solution

Therefore mmoles that contained in 250 ml of G.M amoxicillin capsules solution

$= 0.2063 \times 250/50 = 1.0315$mmole

Weight G.M of amoxicillin capsules = m moles×M wt

$= 1.0315 \times 419.4 = 432.6$ mg $= 0.4326$g

% of G.M amoxicillin capsules $= 0.4326 \times 100 / 0.4337 = \%\ 99.75$

2- from the graph of $\Delta pH/\Delta V$ the neutralization volume of 0.0917M NaOH is 2.1 ml

$Q-COOH + NaOH \rightarrow Q-COONa + H2O$

1mole 1 mole

mmoles of G.M amoxicillin capsules = mmoles of 0.0917 M NaOH= $V_{NaOH} X M_{NaOH} = 2.1 \times 0.0917 = 0.19257$mmoles

These mmoles were contained in 50 ml of G.M amoxicillin capsules solution

Therefore mmoles that contained in 250 ml of G.M amoxicillin capsules solution

$$= \frac{0.19257 \times 50}{2\ 50} = 0.96285 \text{ mmoles}$$

Weight of G.M amoxicillin capsules = mmoles of it × m wt

$= 0.96285 \times 419.4 = 403.82$ mg

$$\%\text{ of G.M amoxicillin capsules} = \frac{403.82 \times 100}{1000 \text{ x } 0.4337} = \%\ 93.11$$

Table 3.29 Potentiometeric titration of 50ml GM amoxicillin capsules with 0.0917M NH_4OH

Vol. of NaOH/ml	pH	δpH/δv / v	Vol.of NaOH/ml	pH	δ pH/δV / V
0.00	4.738				1.17
		2.88	2.8	8.690	
0.50	6.178				1.77
		1.58	3.0	8.964	
1.00	6.968				0.79
		0.958	3.2	9.122	
1.50	7.447				1.086
		0.9066	3.5	9.448	
1.80	7.719				0.378
		0.5	4.0	9.637	
2.00	7.819				0.746
		0.975	4.5	10.010	
2.20	8.014				0.508
		1.35	5.0	10.264	
2.40	8.284				0.55
		0.86	5.5	10.539	
2.60	8.456				

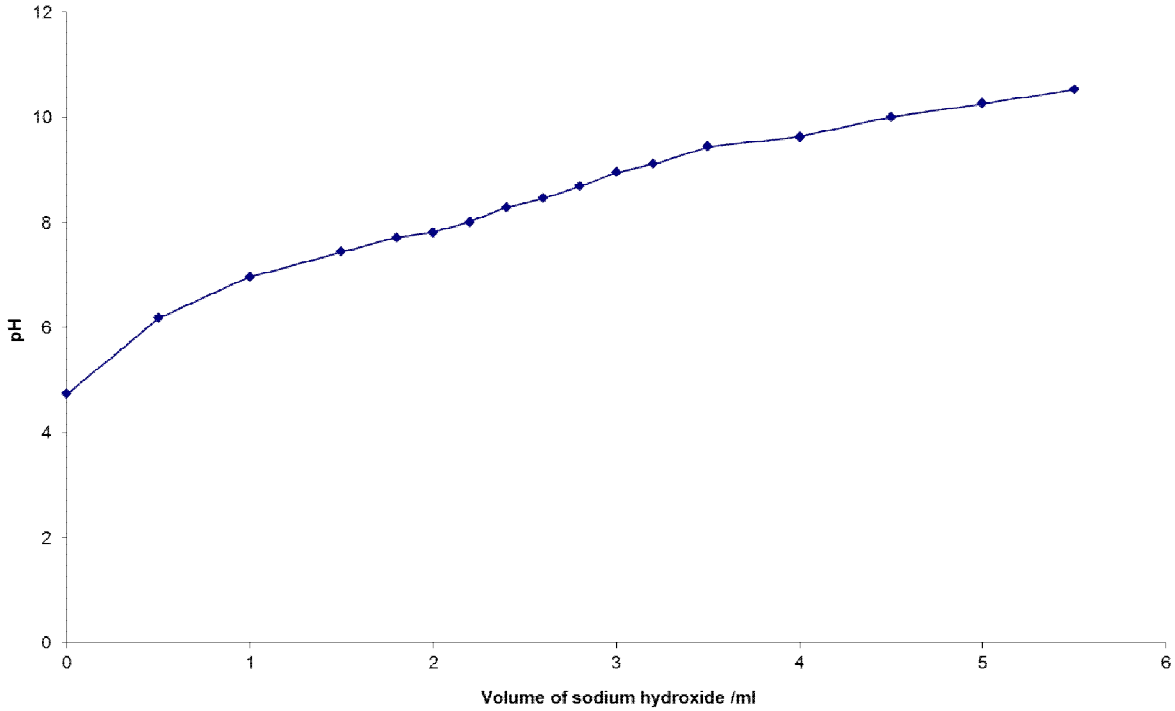


Fig 3.39 Potentiometeric titration of 50ml G.M amoxicillin capsules with $0.0917MNH_4OH$ -1

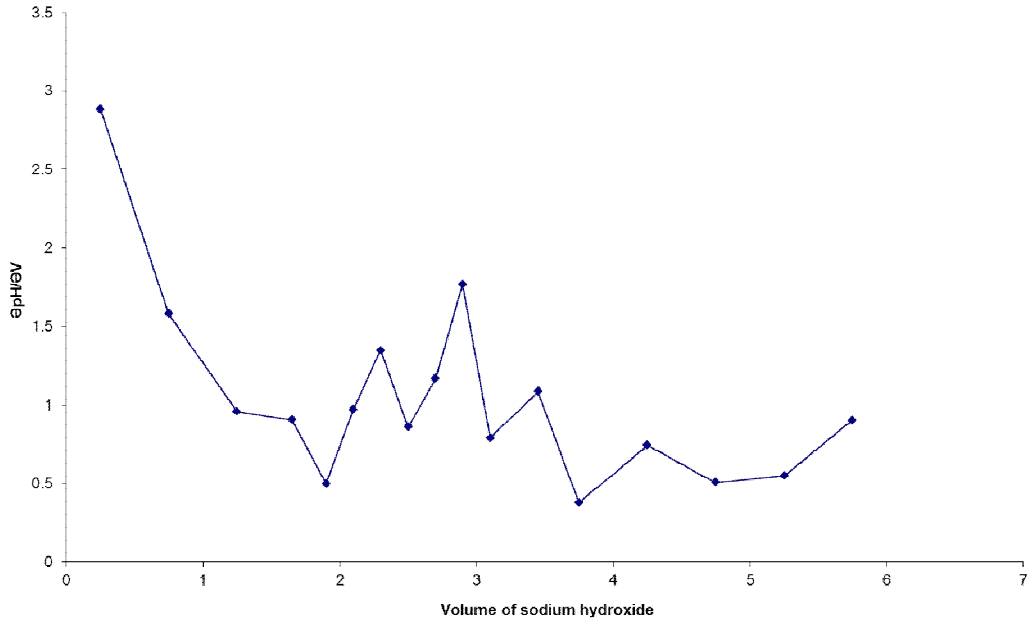


Fig 3.40 Potentiometeric titration of 50ml GM amoxicillin capsules with 0.0917MNH_4OH -2

3.2.5.8.5 Wafra amoxicillin capsules

1- From of pH/V the volume of 0.0745 M NaOH is 2. 45ml

$Q-COOH + NaOH \rightarrow Q-COONa + H_2O$

1 mole 1 mole

mmoles of Wafra amoxicillin capsules = mmoles of 0.0745 M NaOH= $V_{NaOH} X M_{NaOH}$ $= 2.45 \times 0.0745$ $= 0.1825$ mmoles

These mmoles were contained in 50 ml of Wafra amoxicillin capsules solution

Therefore mmoles that contained in 250 ml of Wafra amoxicillin capsules solution

$$= \frac{0.1825 \times 250}{50} = 0.9125 \text{ mmoles}$$

Weight of Wafra amoxicillin capsules = mmoles of it × M wt

$= 0.9125 \times 419.4 = 382.7$ mg

% of Wafra amoxicillin capsules $= \frac{382.7 \times 100}{1000 \times 0.4576} = \%\ 83.62$

From the graph of ΔpH/ ΔV the neutralization volume of 0.0745M NaOH is 2.5 ml

mmoles of Wafra amoxicillin capsules = mmoles of 0.0917 M NaOH

$= V_{NaOH} \times M_{NaOH} = 2.5 \times 0.0745 = 0.1863$ mmoles

These mmoles were contained in 50 ml of Wafra amoxicillin capsules solution

Therefore mmoles that contained in 250 ml of Wafra amoxicillin capsules solution

$$= \frac{0.1863 \times 250}{50} = 0.9315 \text{mmole}$$

Weight of Wafra amoxicillin capsules = mmoles×M wt

$= 0.9315 \times 419.4 = 390.67$ mg $= 0.39067$g

% of Wafra amoxicillin capsules $= 0.39067 \times 100 / 0.4576 = \%\ 85.4$

Table 3.30 Potentiometeric titration of 50ml wafra amoxicillin capsules with 0.0745M NaOH

Vol. of NaOH/ml	pH	δ pH/δV/V	Vol.of NaOH/ml	pH	δpH/δv /V
0.00	5.2				0.61
		1.856	2.8	8.219	
0.50	6.128				0.60
		1.722	3.1	8.399	
1.00	6.986				0.68
		0.678	3.6	8.739	
1.50	7.328				0.954
		0.5166	4.1	9.216	
1.80	7.483				0.564
		675	4.6	9.498	
2.00	7.618				0.622
		0.590	5.1	9.809	
2.20	8.738				0.36
		0.69	6.1	10.171	
2.40	8.847				0.47
		1.25	7.1	10.641	
2.60	8.097				

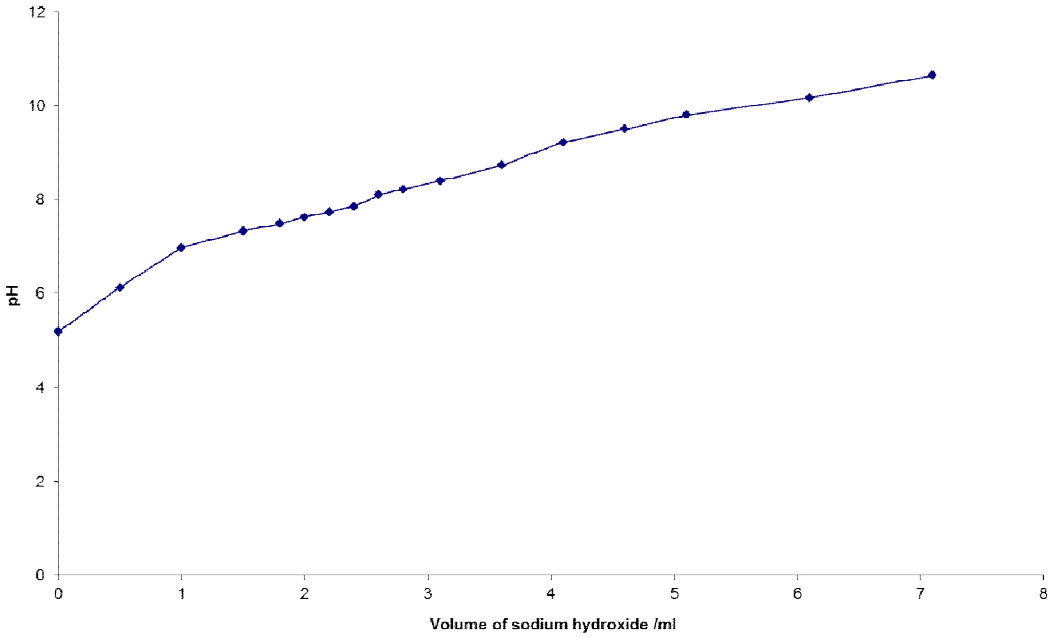


Fig 3.41 Potentiometeric titration of 50ml wafra amoxicillin capsules with 0.0745M NaOH -1

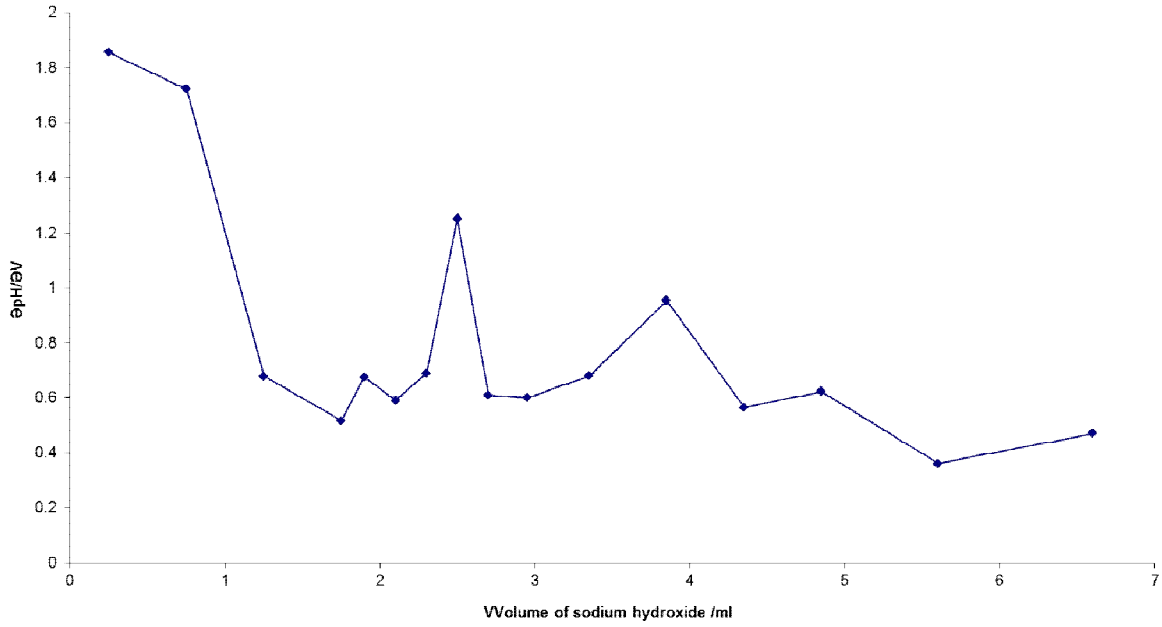


Fig 3.42 Potentiometeric titration of 50ml wafra amoxicillin capsules with 0.0745M NaOH -2

3.2.6 Spectrophotometeric determination of amoxicillin

3.2.6.1 Reagents

1- 100µg/Ml solution of amoxicillin
2- 0.1M potassium ferric cyanide (111) $K_3Fe(CN)_6$ solution in 0.01MNaOH solution
3- 0.1Mm 4-aminopyrine (4-AP) slolution
4- 0.007M 4-AP solution
5- 0.008M NaOH solution
6. 0.016M $K_3Fe(CN)_6$ solution

3.2.6.2 Apparatus

Spectrophotometer (Jenway – 6505 UV/ Vis)

3.2.6.3 Procedure

2.0 ml of 100µg/mLstandard amoxicillin solution and 4.0mL of 0.1M potssium ferric cyanide (111) in 0.01M NaOH were mixed with 4.0ml of 0.1M aminoantipyrine (4-AP) solution in 25ml volumeteric flask and diluted to the mark with distilled water. The maximum absorption wavelength of the amoxicillin 4-APcomplex was determined after sucessive dilution, twise times of the complex solution to give 50:50 percent dilution.

2.0ml of amoxicillin 4-AP complex was prepared by taking 2.0ml of 100µg/mL standard amoxicillin solution into 25ml volumetric flask 4.0ml of 0.007M 4AP, 4.0 ml of 0.008 MNaOH solution,and 2.0mlof $K_3Fe(CN)_6$ solution were added and the volume was completed to the mark with distilled water . Serial dilutions of that complex was done to give different amoxicillin concentrations solutions, and the absorbance of them were recorded , and calibration curve was plotted as shown in Fig(3.43)

Weights of 0.0284g, 0.0296g, 0.0291g and 0.0293g were taken, respectively, from Amipharma, G.M, Wafra and Changahi amoxicillin capsule .each of which was dissolved separately with the aid of amagnetic stirrer in distilled water, transfared into 250ml volumetric flask and completed to the mark with distilled water and filtered.

2.0 ml from each, were taken into 25ml volumetric flask ,4.0ml of 0.007M(4-AP) solution ,4.0ml of 0.008MNaOH solution and 2.0ml of 0.016M $K_3Fe(CN)_6$ solution were added and the volume was completed up to the mark with distilled water ,the solution was successively diluted triple times to (50:50) percent to give a solution of 1.14 ,1.2 ,1.16 and 1.17µg/ml for each resectively and the absorbance of these solutions were measured; Then, the amount and the percentage of the samples were calculated.

3.2.6.4 Results of Spectrophotometric determination of amoxicillin

The standard curve data

Concentrationof amoxicillin(µg/ml)	0.25	0.5	1.0	2.0	4.0
Absobance	0.02	0.034	0.067	0.143	0.265

The maximum absoption wave length (λ) was 520nm

From the equation

$$Y = a + bc$$

Y = Absorbance

a = Intercept

b = Regression

c = Concentrion

Samples results

Amoxicillin samples	Absorbance	Weight/µg	%
Amipharma	0.058	0.87	75.9
G.M	0.062	0.93	77.1
Wafra	0.061	0.91	78.5
Changahi	0.059	0.88	75.3

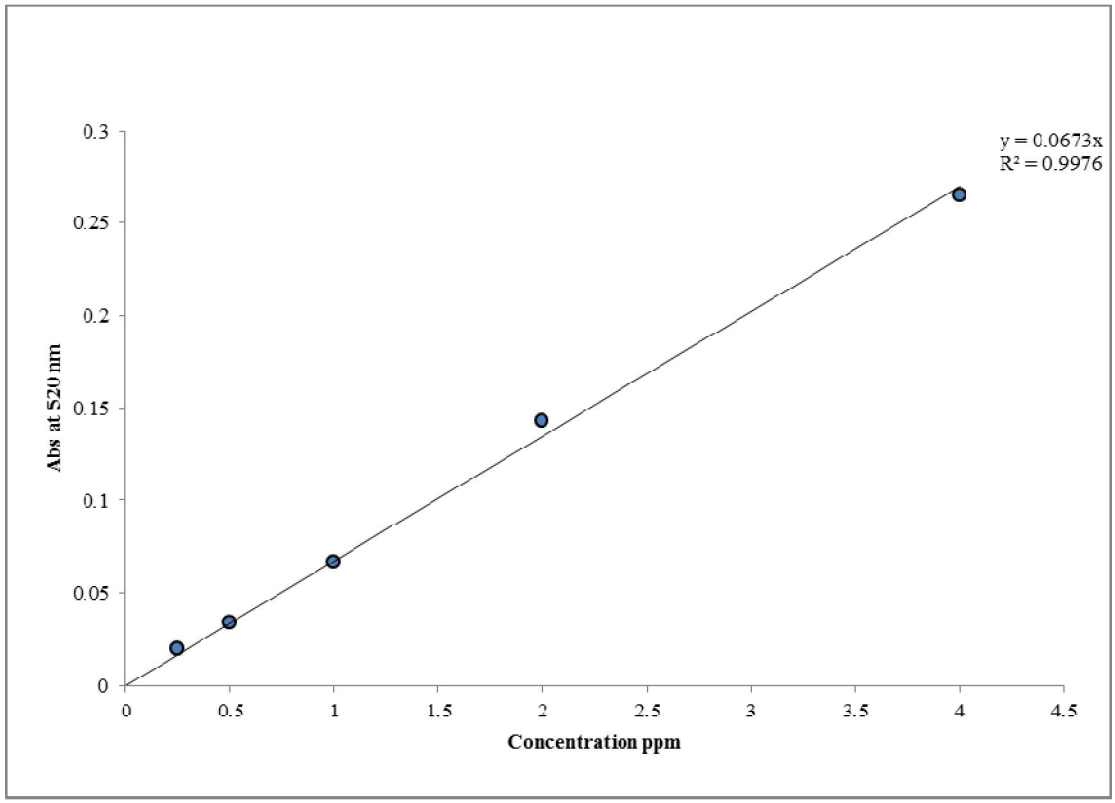


Fig 3.43 Standard Amoxicillin trihydrate calibration curve of spectrophotpmetric method

3.2.7 Determination of amoxicillin using HPLC

3.2.7.1 Reagent

1-Amoxcillin trihydrate solutions

2- Mobile phase (A):

A mixture of (1 volumes of acetonitrile and 99 volume of buffer solution pH 5)

3- Mobile phase (B):

A mixture of 20 volumes of acetonitrile and 80 volumes buffer solution pH 5.

4- Buffer 5 solution (prepared as follows, to 250ml of 0.2 molar potassium dehydrate phosphate, dilute sodium hydroxide solution was added until pH was obtained, Then diluted to 1000 ml with distilled water).

3.2.7.2 Apparatus

1- HPLC apparatus. Shimadzu Quto Jpan ,with two LC-10 ADVP liquid chromotograph and DGU 14A degasser pump . SIL 10 ADVP auto injector. CTO 10 ASV column oven.

2- Separation column (Shimpack-ODS), 15 cm length, 4.6mm internal diameter and 5μm (particale size). Flow rate 1ml /minute. Oven temperature $30C^0$

3- Spectrophotometeric (Ultraviolt) detector.

3.2.7.3 Procedure

A weight of 0.05 g of amoxcillin trihydrate (standard) was dissolved in a small amount of a mixture solution of mobile phase A and mobile phase B (ratio A: B of 92:8) in 50ml volumetric flask and completed to the mark with the same mobile phases mixture, other solutions of concentrations 0.4mg/ml, 0.24 mg/ml and 0.1 mg/ml were prepared from that solution. Spectrophotometric detector was set at 254 nm,the amoxicillin trihydrate (standard) solutions were injected and chromotographed,a curve of peak area against concentration was plottedas shown in Fig (3.44).

Amounts of 0.0115 g ,0.0114 g , 0.0116 g and 0.0102 g of amoxicillin capsules of Amipharma , G.M. Changahi and Wafra, containing 0.0099 g ,0.0095 g , 0.0099 g and .0086 g of pure amoxicillin respectively, each was dissolved in 50 ml of a solution mixture of mobilephase (A) and mobile phase(B) in the ratio of 92:8, and chromotographed

3.2.7.4 Results of HPLC determination of amoxicillin

3.2.7.4.1 Amipharma capsule amoxicillin

Weight the sample in50 ml of solution = 0.0099 g

Replicates	1st r	2nd r	3rd r	Average
Concentrations Obtained	0.215 mg/ml	0.214 mg/ml	0.215 mg/ml	.2147mg/ml

Therefore the weight of Amipfarma capsule amoxicilln obtained

= 0.2167 X 50/1000 =0.0107g

The percentage of Amipharma capsule amoxicillin

=0.0107x100/0.0099 = % 108.48

3.2.7.4.2 G.M capsule amoxicillin

Weight the sample in50 ml of solution = 0.0095g

Replicates	1st r	2nd r	3rd r	Average
Concentrations Obtained	0. 198m g/ml	0.199 mg/ml	0.199 mg/ml	0.158 mg/ml

Therefore the weight of G.M capsule amoxicilln obtained

= 0.1987 X 50/1000 =0.00935 g

The percentage of G.M capsule amoxicillin

=0.009935x100/0.0095 = % 104.58

3.2.7.4.3 Changahi capsule amoxicillin

Weight the sample in50 ml of solution =0.0099 g

Replicates	1st r	2nd r	3rd r	Average
Concentrations Obtained	0.217 mg/ml	0.214mg/ml	0.215 mg/ml	0.2153 g/ml

Therefore the weight of Changahi capsule camoxicillin obtained

= 0.2153X 50/1000 =0.010765 g

The percentage of Changahi capsule amoxicillin

=0.010765x 100/0.0099 = % 108.74

3.2.7.4.4 Wafra capsule amoxicillin

Weight the sample in50 ml of solution =0.0086 g

Replicates	1st r	2nd r	3rd r	Average
Concentrations Obtained	0.184 mg/ml	0.185mg/ml	0.185 mg/ml	0.1847 g/ml

Therefore the weight of Wafra capsule amoxicillin obtained

= 0.1847X 50/1000 =0.009235 g

The percentage of Wafra capsule amoxicillin

=0.009235x 100/0.0086 = % 107.4

Data Name: C:\CLASS-VP\Amoxicillin -std1-Rep1

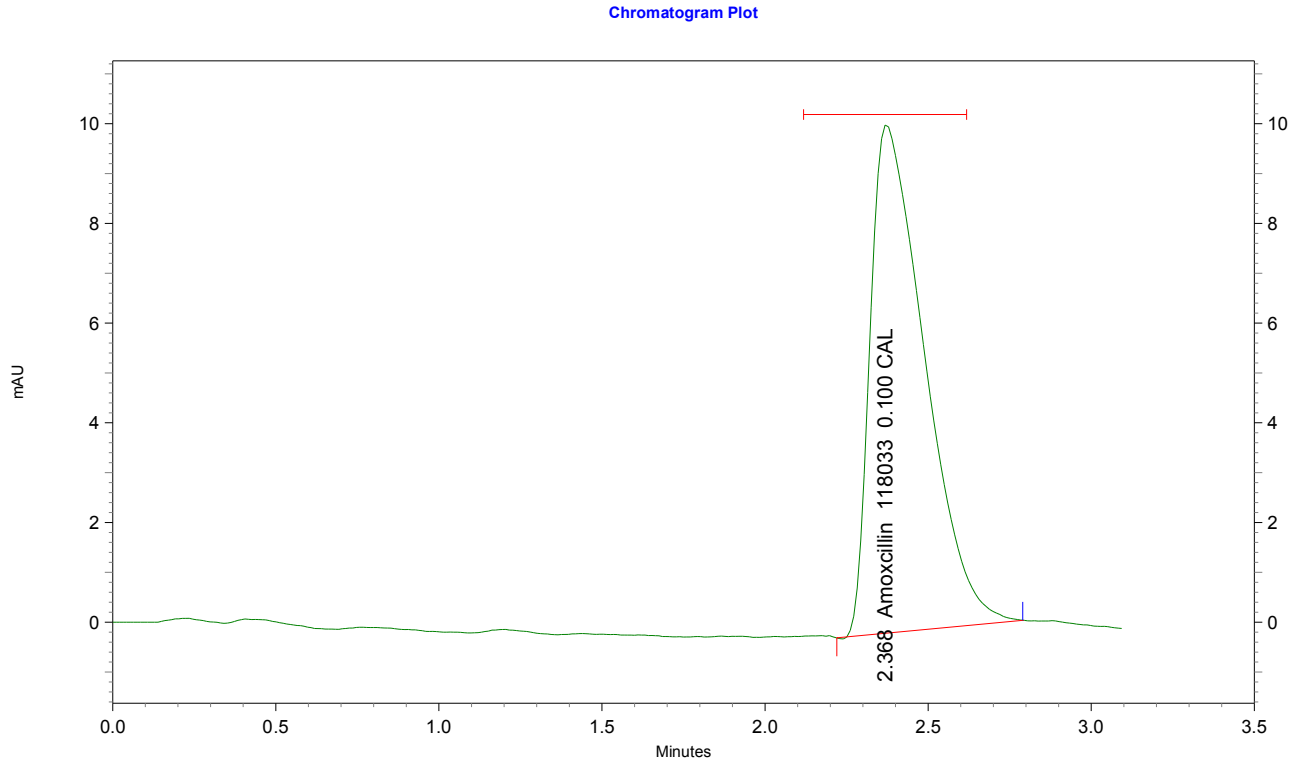
Method Name: C:\CLASS-VP\Methods\amoxellin.met

Sample ID: Amoxicillin

User: System

Acquired: 10/31/2010 5:27:54 PM

Sample Description : std1-Rep1(0.1mg/ml)



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxcillin	2.368	118033	0.100 CAL	mg/ml

Chrotogram plot 3.19 (R1.1) Standard Amoxicillin Tri hydrate

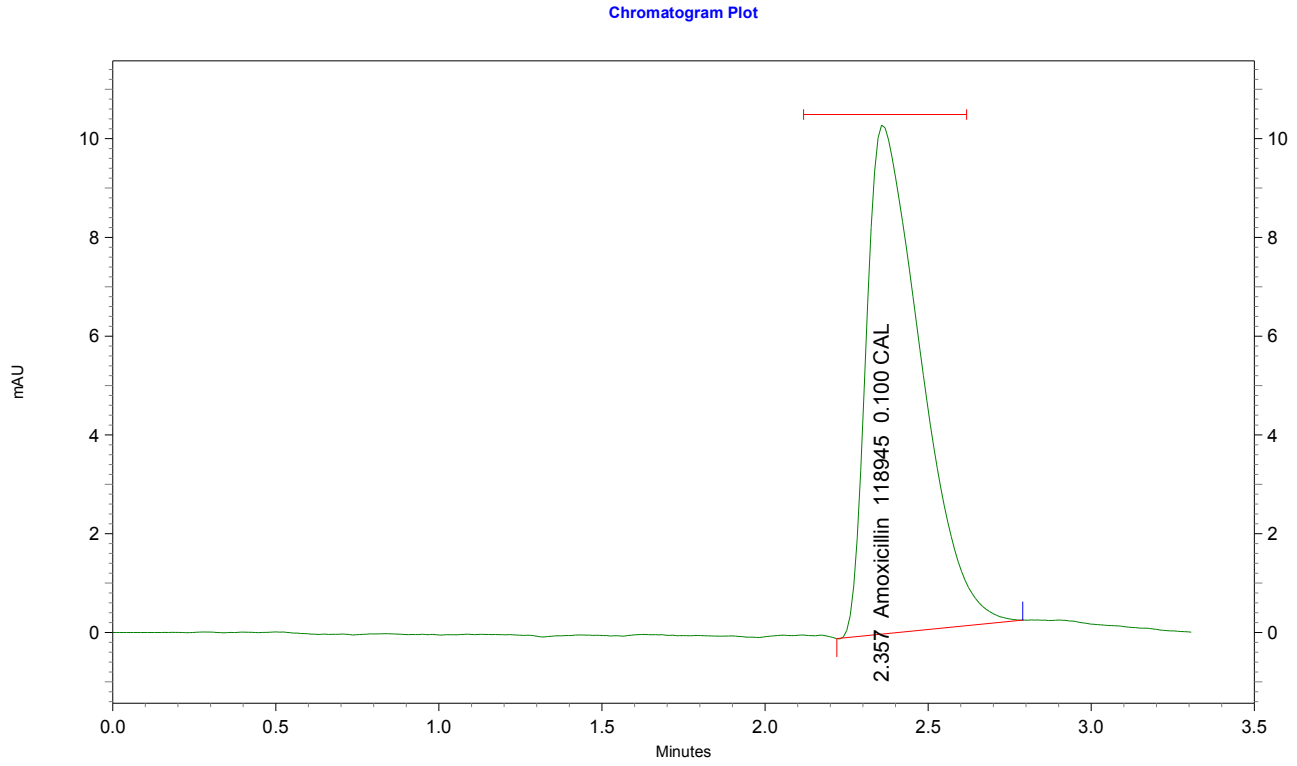
Method Name: C:\CLASS-VP\Methods\amoxellin.met

Sample ID: Amoxicillin

User: System

Acquired: 10/31/2010 5:32:39 PM

{Sample Description} : **std1-Rep2**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.357	118945	0.100 CAL	mg/ml

Chrotogram plot 3.20 (R1.2) Standard Amoxicillin trihydrate

Data Name: C:\CLASS-VP\Amoxicillin -std1-Rep3

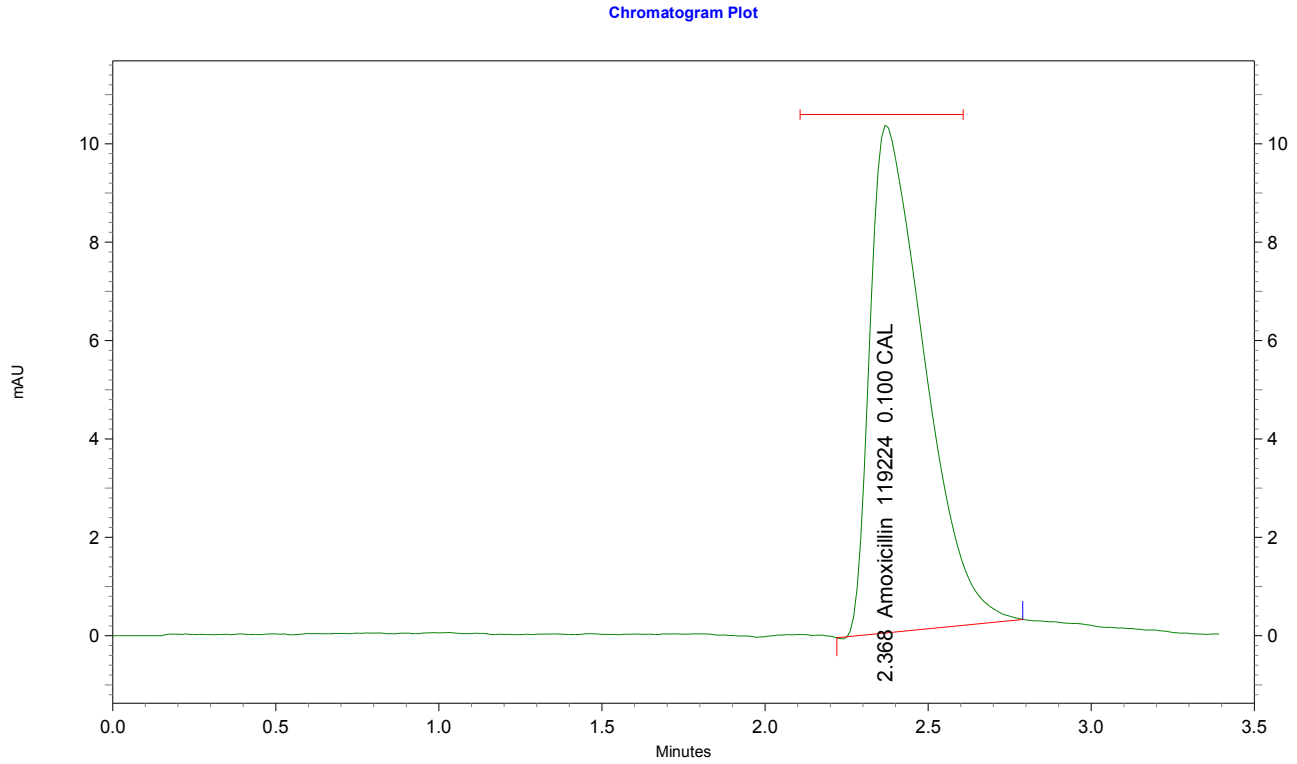
Method Name: C:\CLASS-VP\Methods\amoxellin.met

Sample ID: Amoxicillin

User: System

Acquired: 10/31/2010 5:37:42 PM

{Sample Description} : **std1-Rep3(0.1mg/ml)**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.368	119224	0.100 CAL	mg/ml

Chrotogram plot 3.21 (R1.3) Standard Amoxicillin Tri hydrate

Data Name: C:\CLASS-VP\Amoxicillin -std2-Rep1

Method Name: C:\CLASS-VP\Methods\amoxellin.met

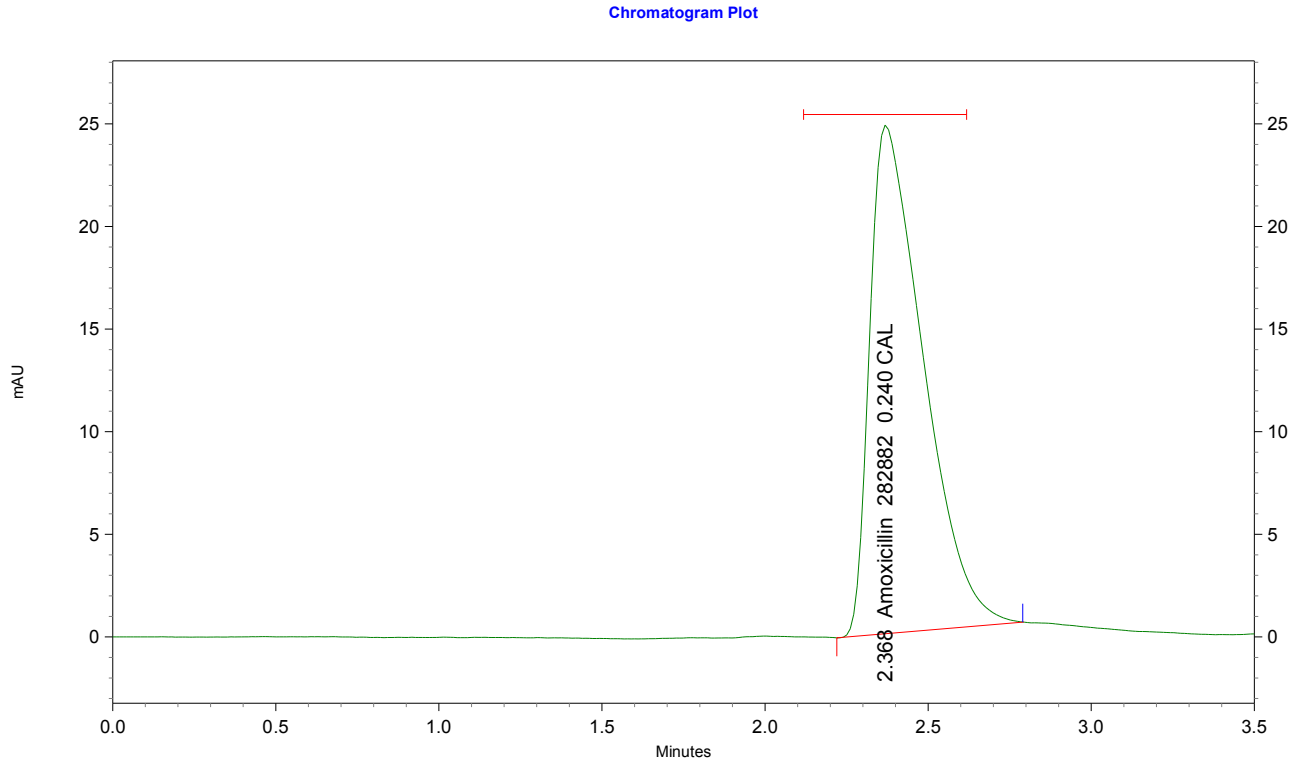
Sample ID: Amoxicillin

User: System

Acquired: 10/31/2010 5:42:51 PM

{Sample Description} **: std2-Rep1 (0.24mg/l)**

.



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.368	282882	0.240 CAL	mg/ml

Chrotogram plot 3. 22 (R2.1) Standard Amoxicillin Tri hydrate

Data Name: C:\CLASS-VP\Amoxicillin -std2Rep2

Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

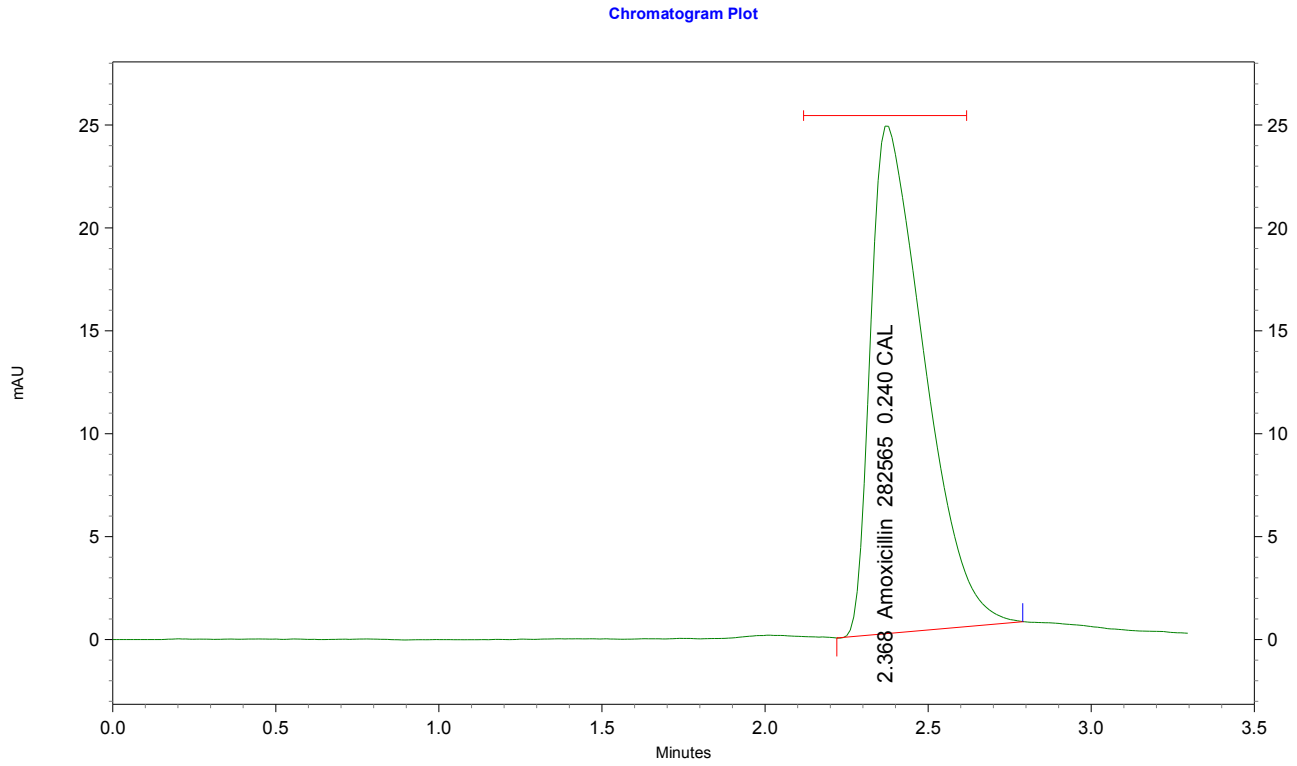
Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 5:48:10 PM**

{Sample Description} **: std2Rep2**

.



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.368	282565	0.240 CAL	mg/ml

Chrotogram plot 3.23 (R2.2) Standard Amoxicillin Tri hydrate

Data Name: **C:\CLASS-VP\Amoxicillin -std2-Rep3**

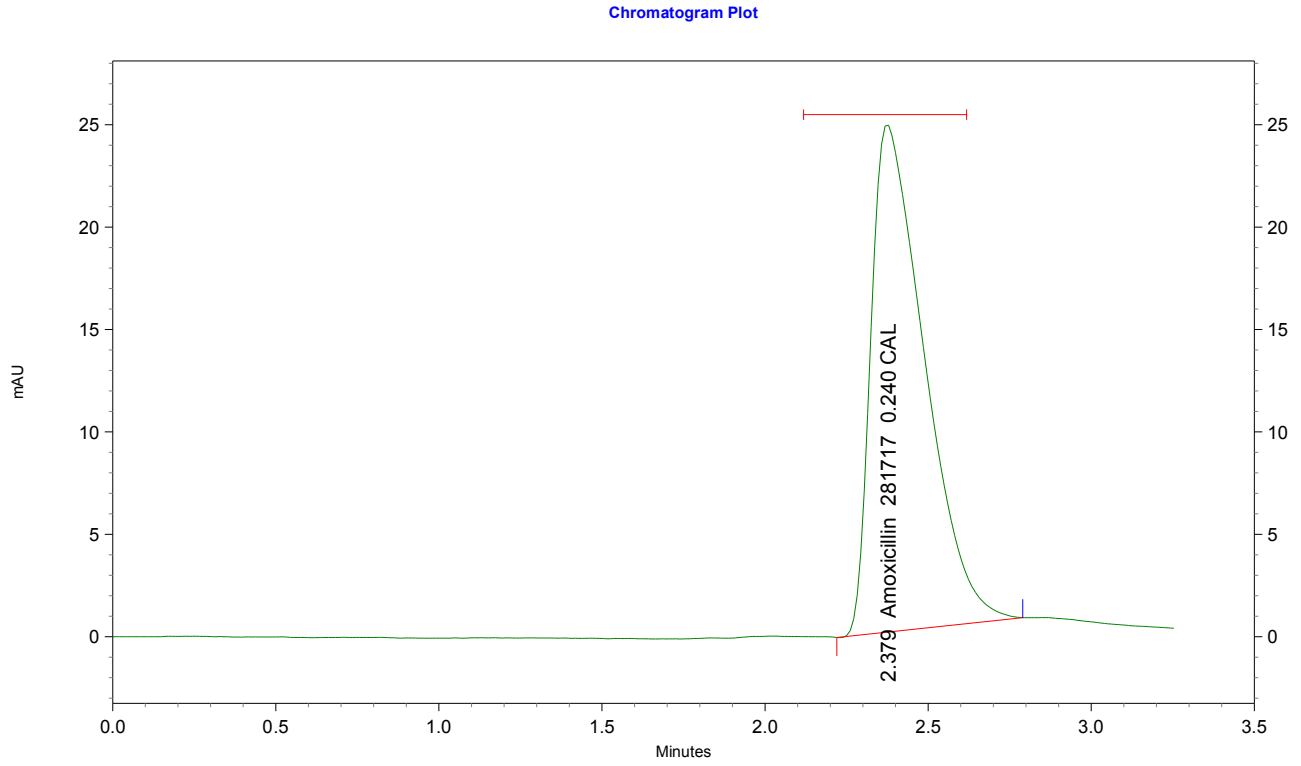
Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 5:53:13 PM**

{Sample Description} **: std2-Rep3**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.379	281717	0.240 CAL	mg/ml

Chrotogram plot 3.24 (R2.3) Standard Amoxicillin Tri hydrate

Data Name: **C:\CLASS-VP\Amoxicillin -std3-Rep1**

Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

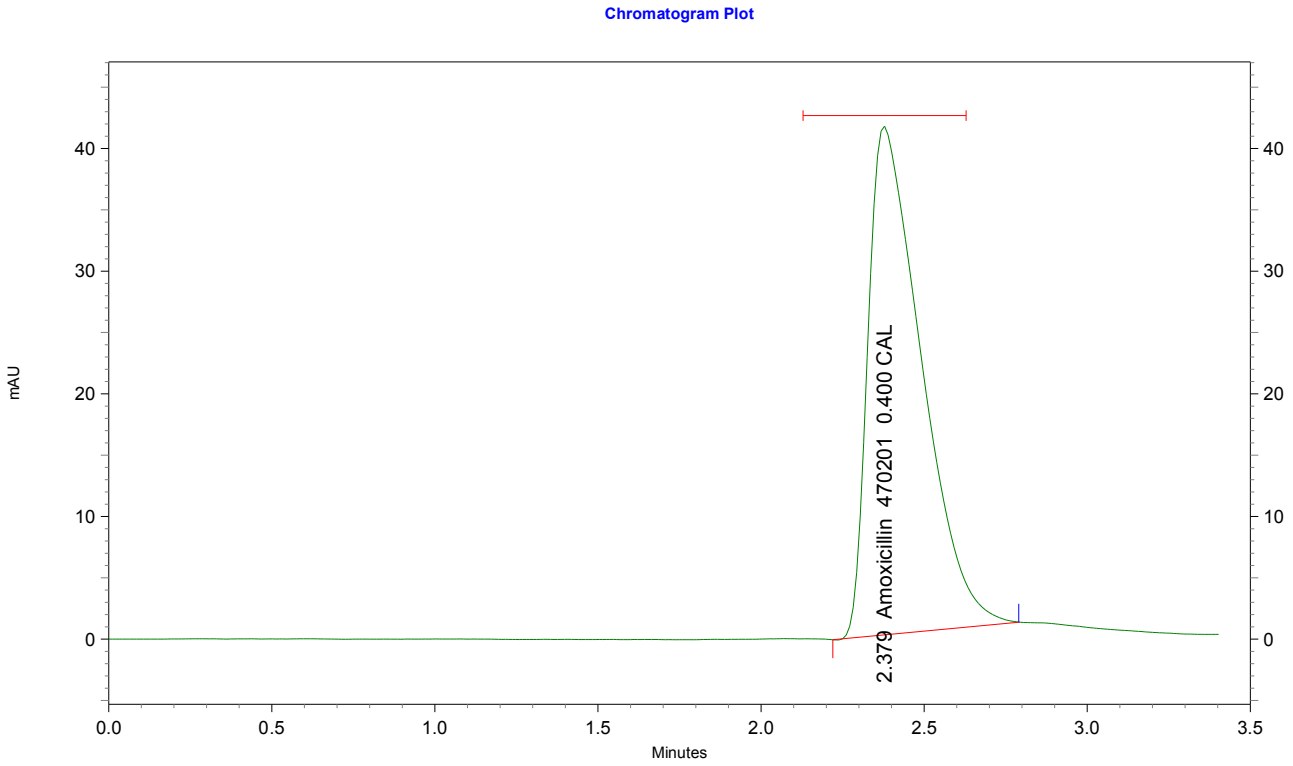
Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 5:58:13 PM**

{Sample Description} **: std3-Rep1(0.4mg/ml)**

.



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.379	470201	0.400 CAL	mg/ml

Chrotogram plot 3.25 (R3.1) Standard Amoxicillin Tri hydrate

Data Name: **C:\CLASS-VP\Amoxicillin -std3-Rep2**

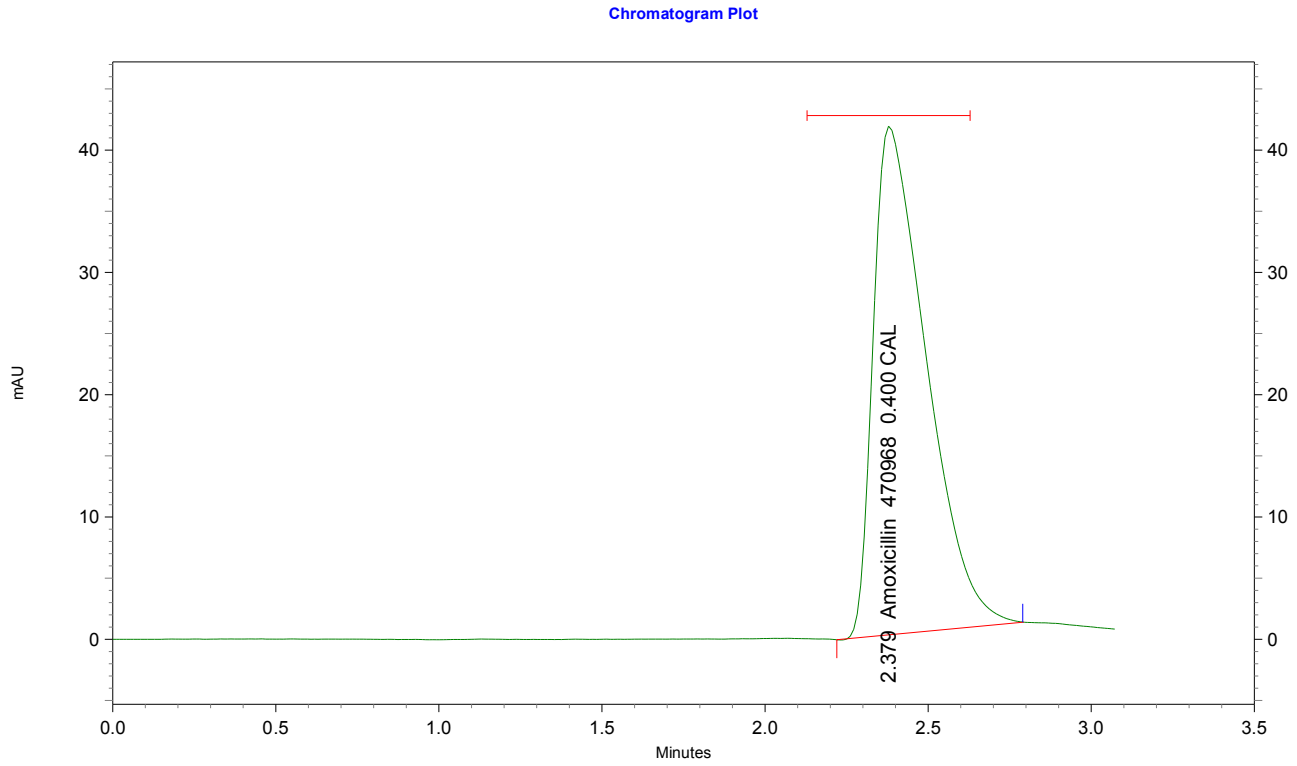
Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 6:03:21 PM**

{Sample Description} **: std3-Rep2**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.379	470968	0.400 CAL	mg/ml

Chrotogram plot 3.26 (R3.2) Standard Amoxicillin Tri hydrate

Data Name: **C:\CLASS-VP\Amoxicillin -std3-Rep3**

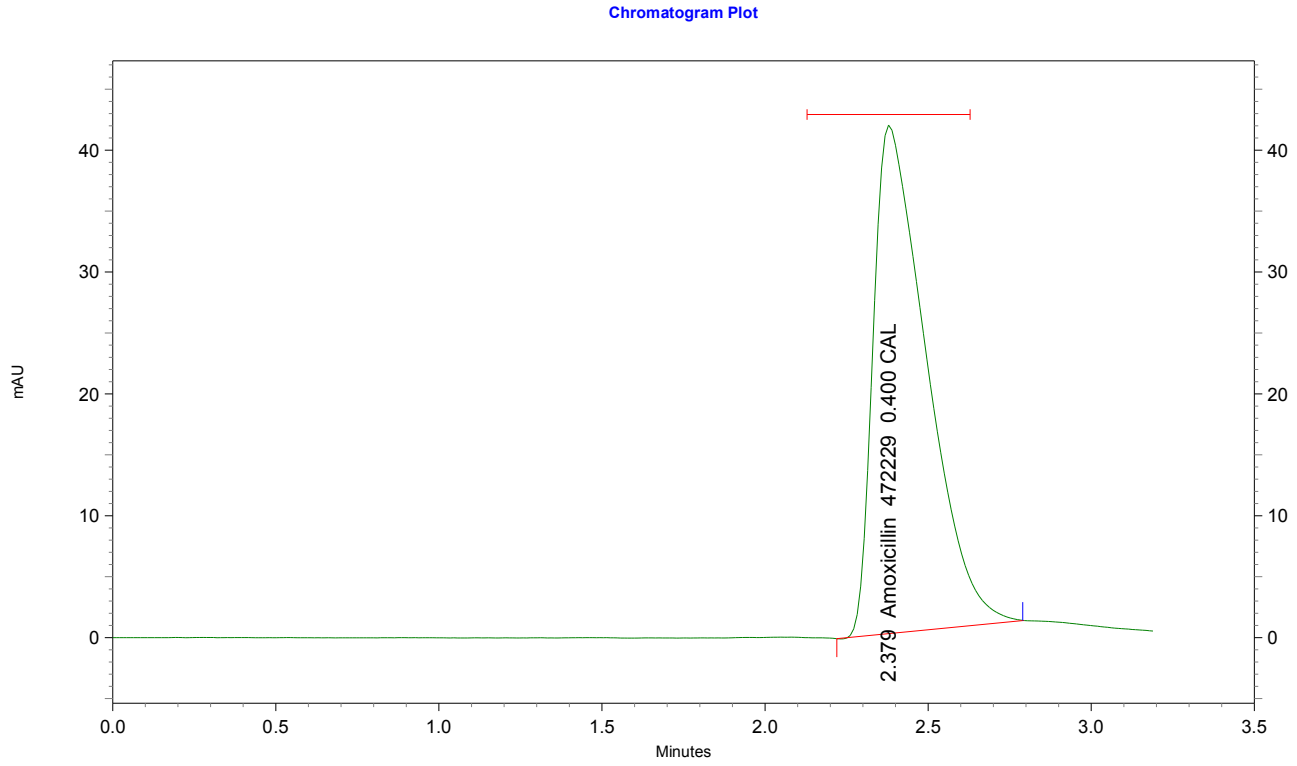
Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 6:08:10 PM**

{Sample Description} **: std3-Rep3**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.379	472229	0.400 CAL	mg/ml

Chrotogram plot 3.27 (R3.3) Standard Amoxicillin Tri hydrate

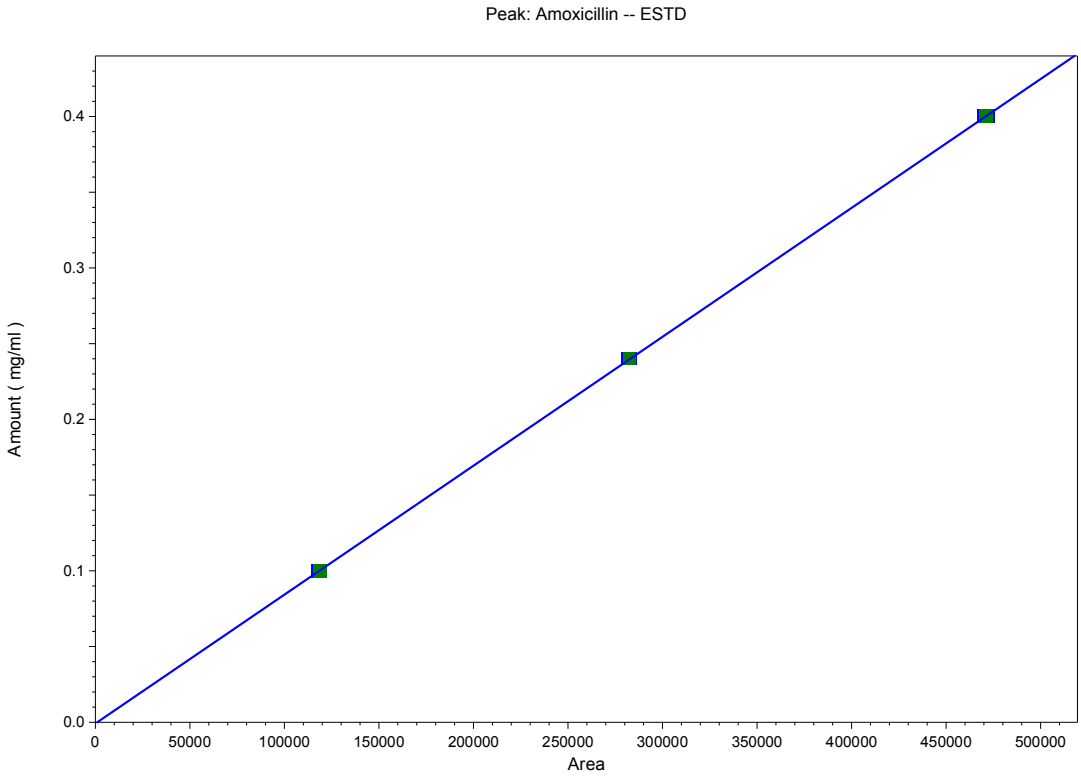


Fig 3.44 Chromatographic standard amoxicillin tri hydrate curve of chromatographic method

Data Name: **C:\CLASS-VP\Amoxicillin- ami2-Rep1**

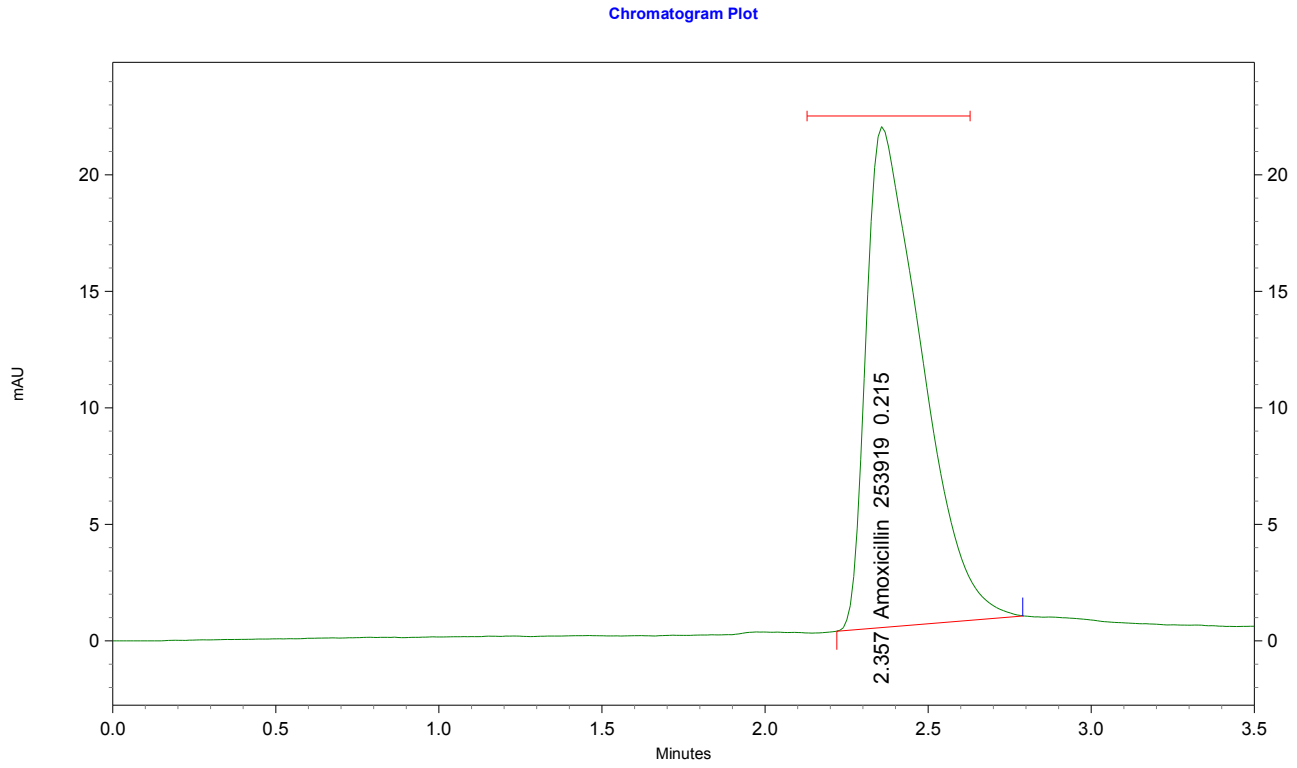
Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 3:45:24 PM**

{Sample Description} **: Amipharma Amoxicillin rept1**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.357	253919	0.215	mg/ml

Chromatogram plot 3.28 (R.1) Amipharma amoxicillin capsules

Data Name: **C:\CLASS-VP\Amoxicillin ami2-Rep2**

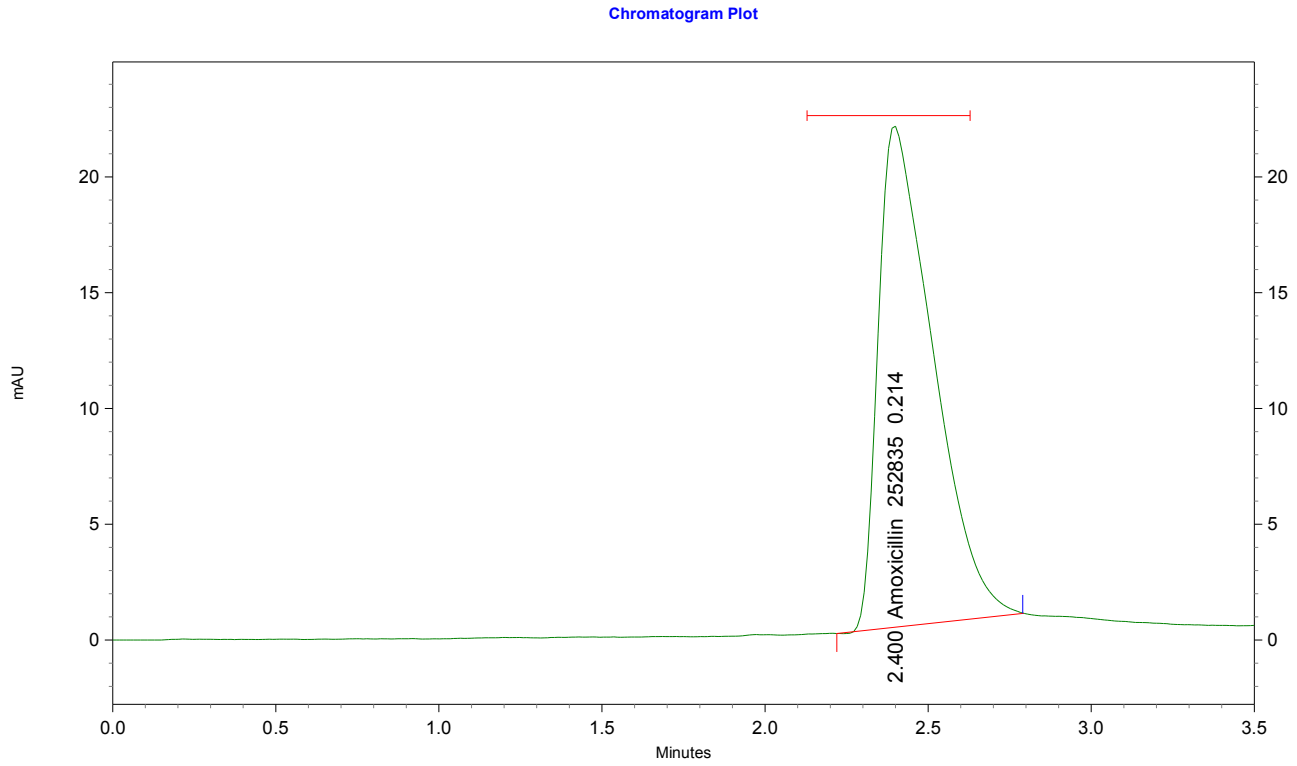
Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 3:51:03 PM**

{Sample Description}**:Amipharma Amoxicillin rept2**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.400	252835	0.214	mg/ml

Chromatogram plot 3.29 (R.2) Amipharma amoxicillin capsules

Data Name: **C:\CLASS-VP\Amoxicillin ami2-Rep3**

Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

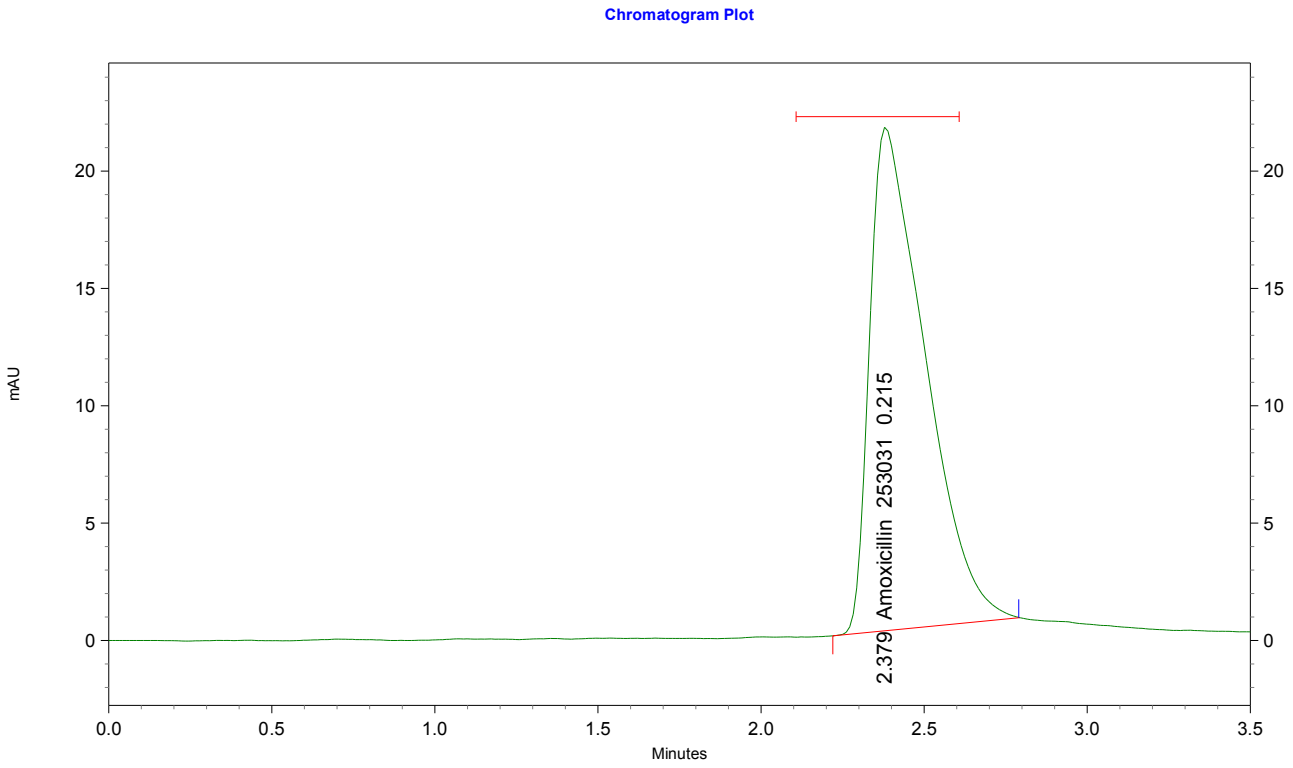
Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 3:56:50 PM**

{Sample Description} **: Amipharma Amoxicillin rept3**

.



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.379	253031	0.215	mg/ml

Chromatogram plot 3.30 (R.3) Amipharma amoxicillin capsules

Data Name: **C:\CLASS-VP\Amoxicillin -G.M-Rep1**

Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

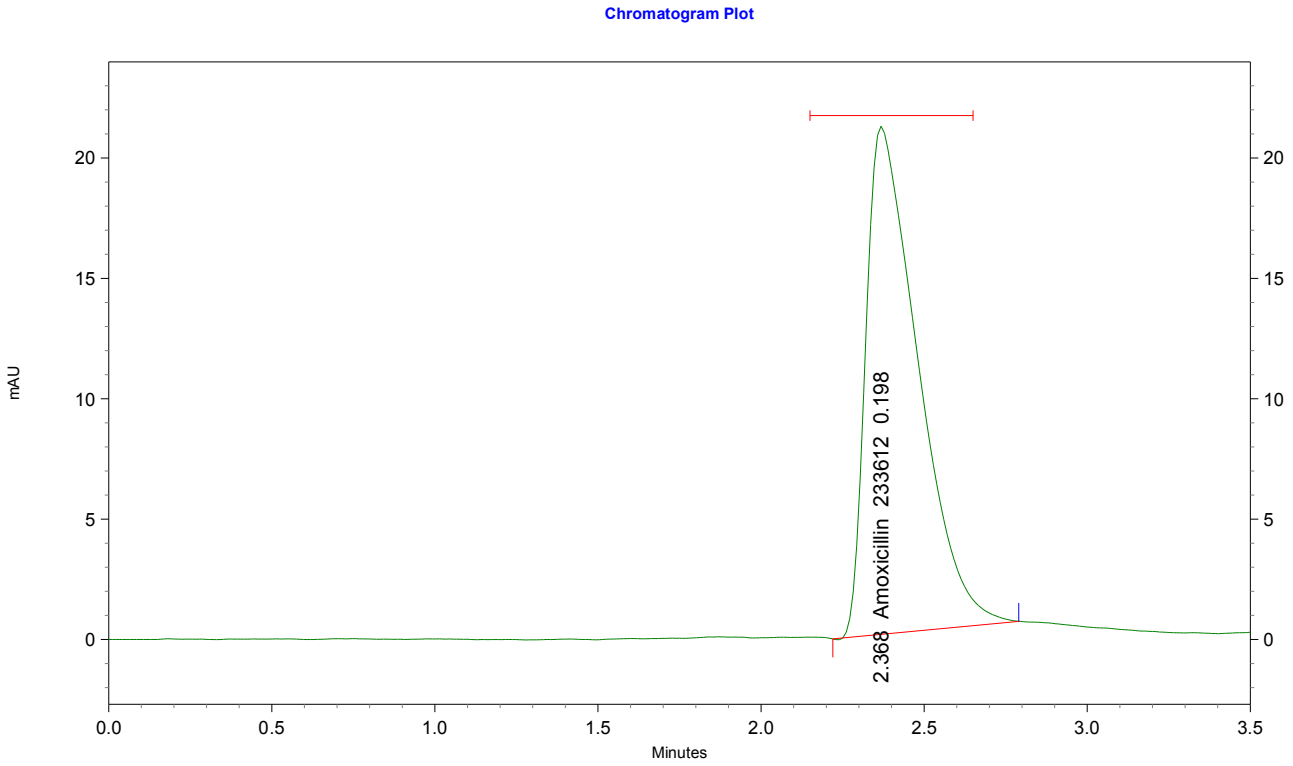
Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 4:47:47 PM**

{Sample Description} **:G.M Amoxicillin rept1**

.



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.368	233612	0.198	mg/ml

Chromatogram plot 3.31 (R1) G.M amoxicillin capsules

Data Name: **C:\CLASS-VP\Amoxicillin- G.M-Rep2**

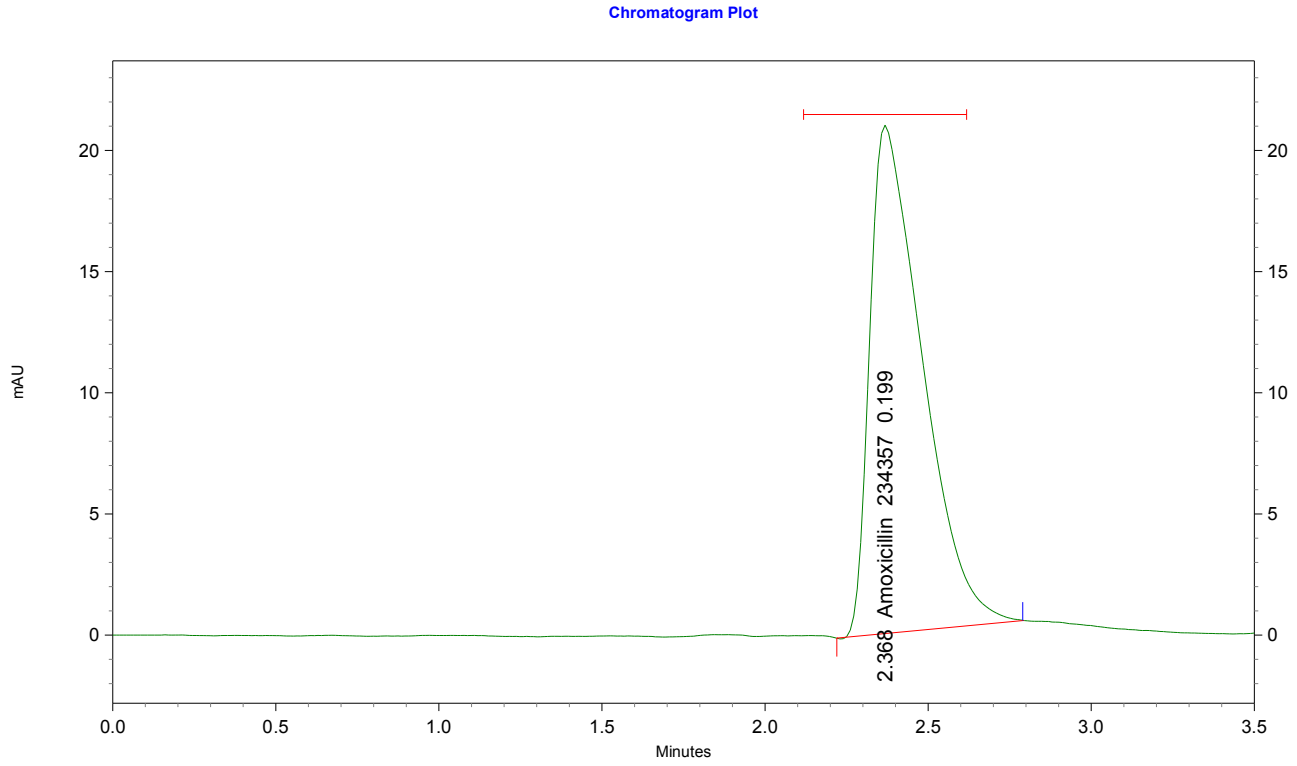
Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 4:53:34 PM**

{Sample Description} **: G.M Amoxicillin rept2**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.368	234357	0.199	mg/ml

Chromatogram plot 3.32 (R2) G.M amoxicillin capsules

Data Name: **C:\CLASS-VP\Amoxicillin -G.M-Rep3**

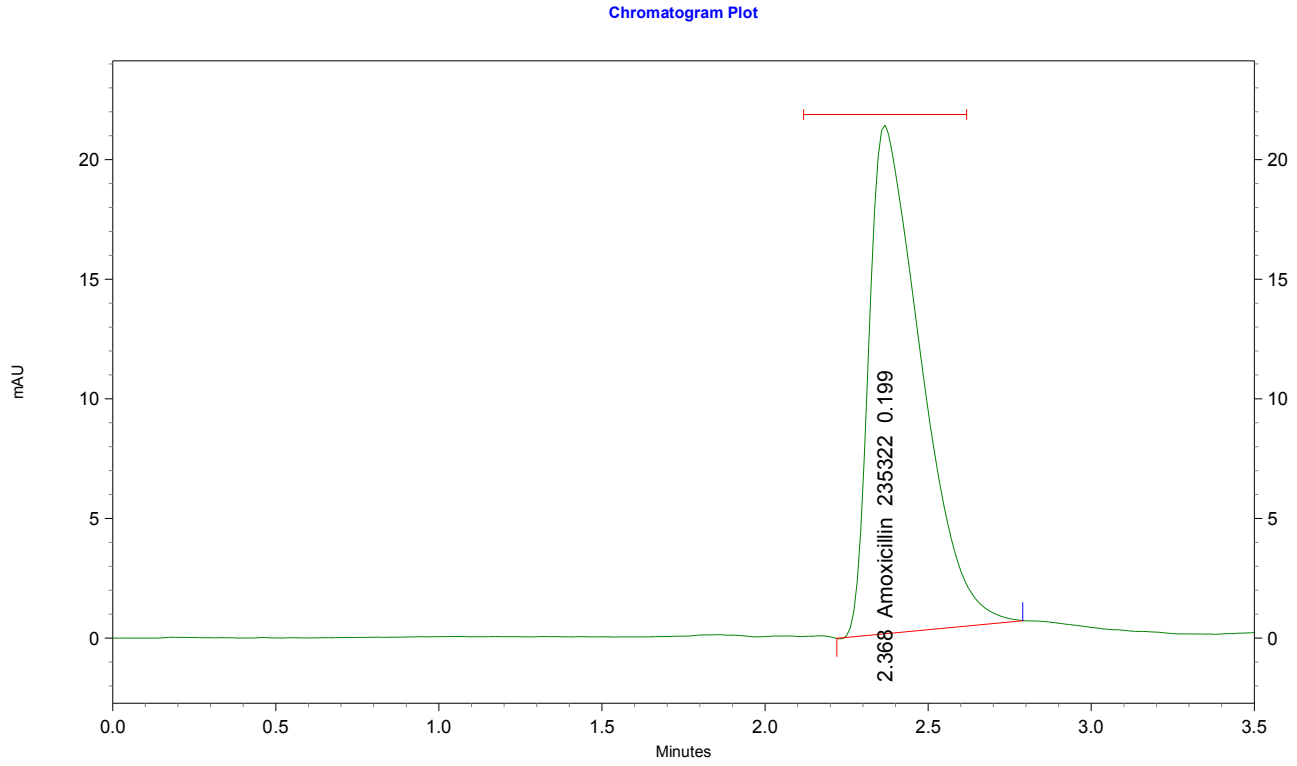
Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 4:59:16 PM**

{Sample Description} **:G.M Amoxicillin rept3**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.368	235322	0.199	mg/ml

Chromatogram plot 3.33 (R3) G.M amoxicillin capsules

Data Name: **C:\CLASS-VP\Amoxicillin- shang-Rep.1.1**

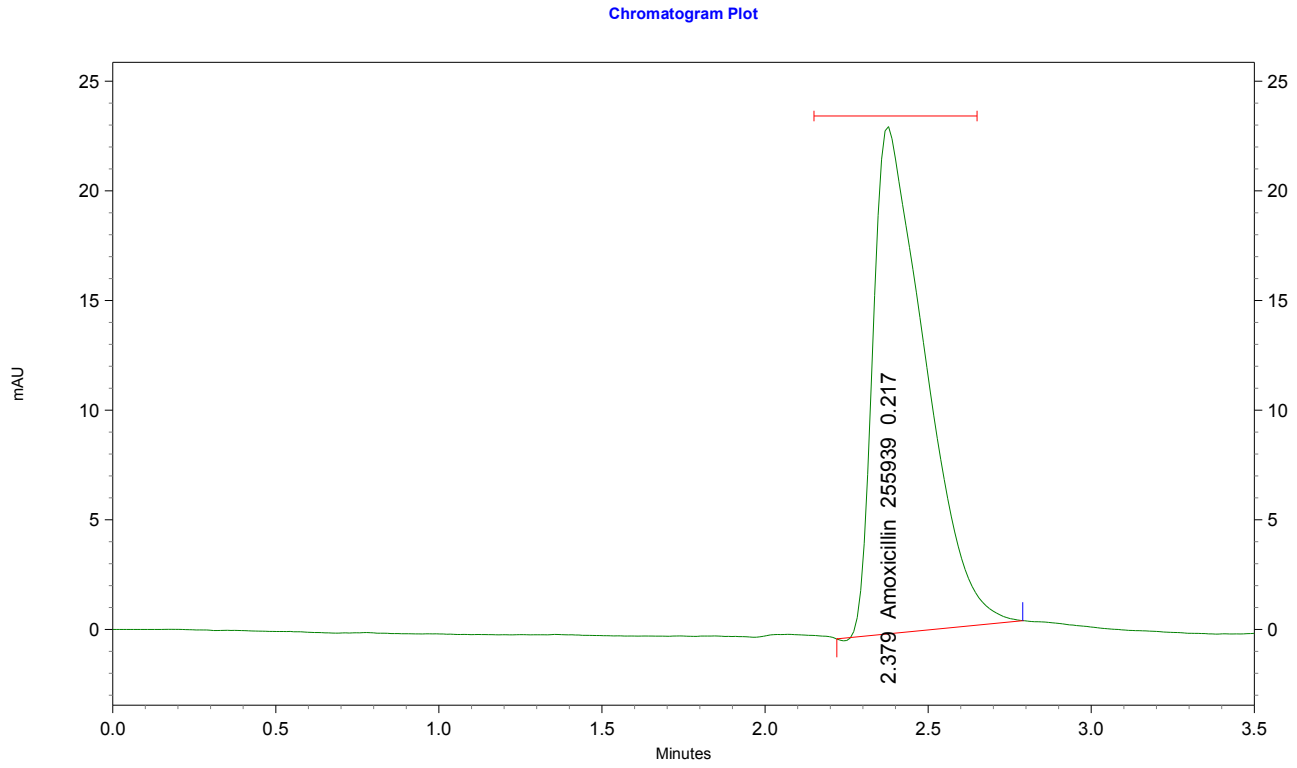
Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 4:42:08 PM**

{Sample Description}**:Shanghi Amoxicillin rept3**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.379	255939	0.217	mg/ml

Chromatogram plot 3.34 (R1) Changahi amoxicillin capsules

C:\CLASS-VP\Amoxicillin- shang-Rep2 Data Nam

Method Name: C:\CLASS-VP\Methods\amoxellin.met

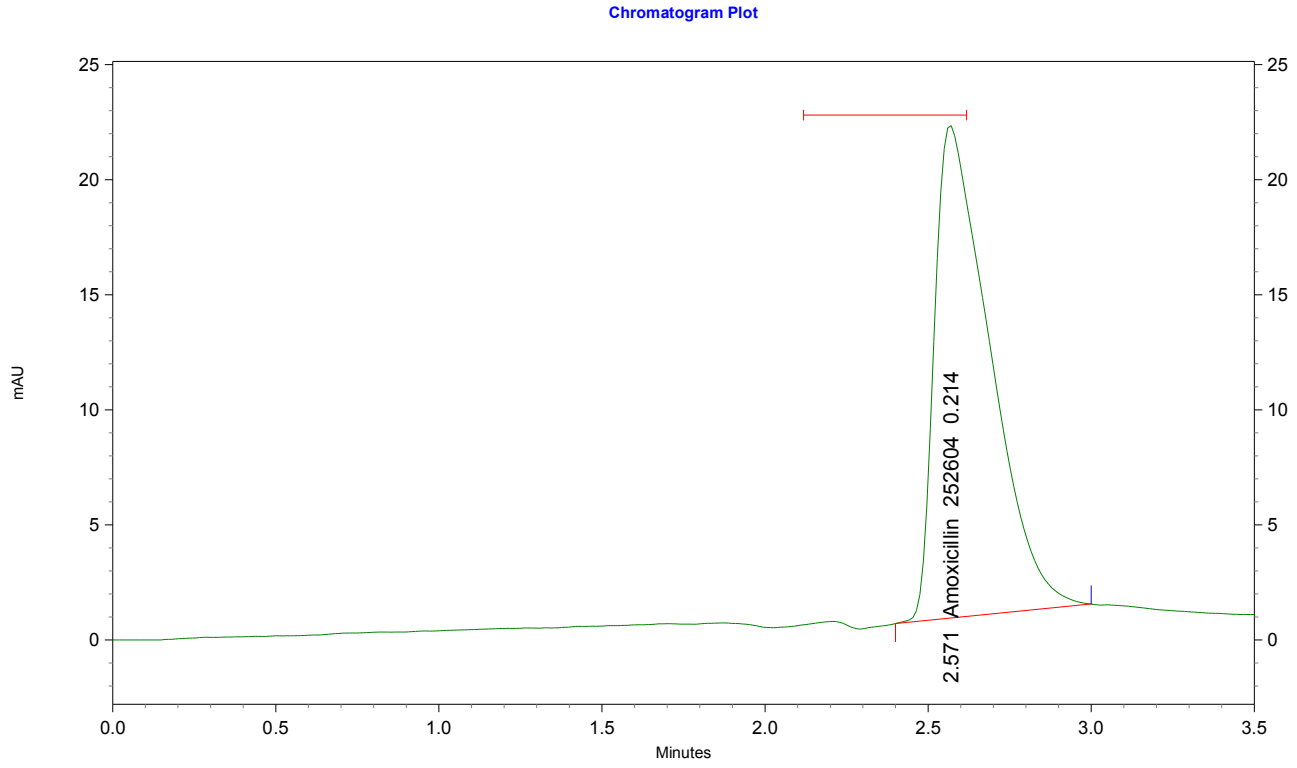
Sample ID: Amoxicillin

User: System

Acquired: 10/31/2010 4:29:43 PM

{Sample Description} **:** Shanghi Amoxicillin rept2

.



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.571	252604	0.214	mg/ml

Chromatogram plot 3.35 (R2) Changahi amoxicillin capsules

Data Name: C:\CLASS-VP\Amoxicillin -shang-Rep3

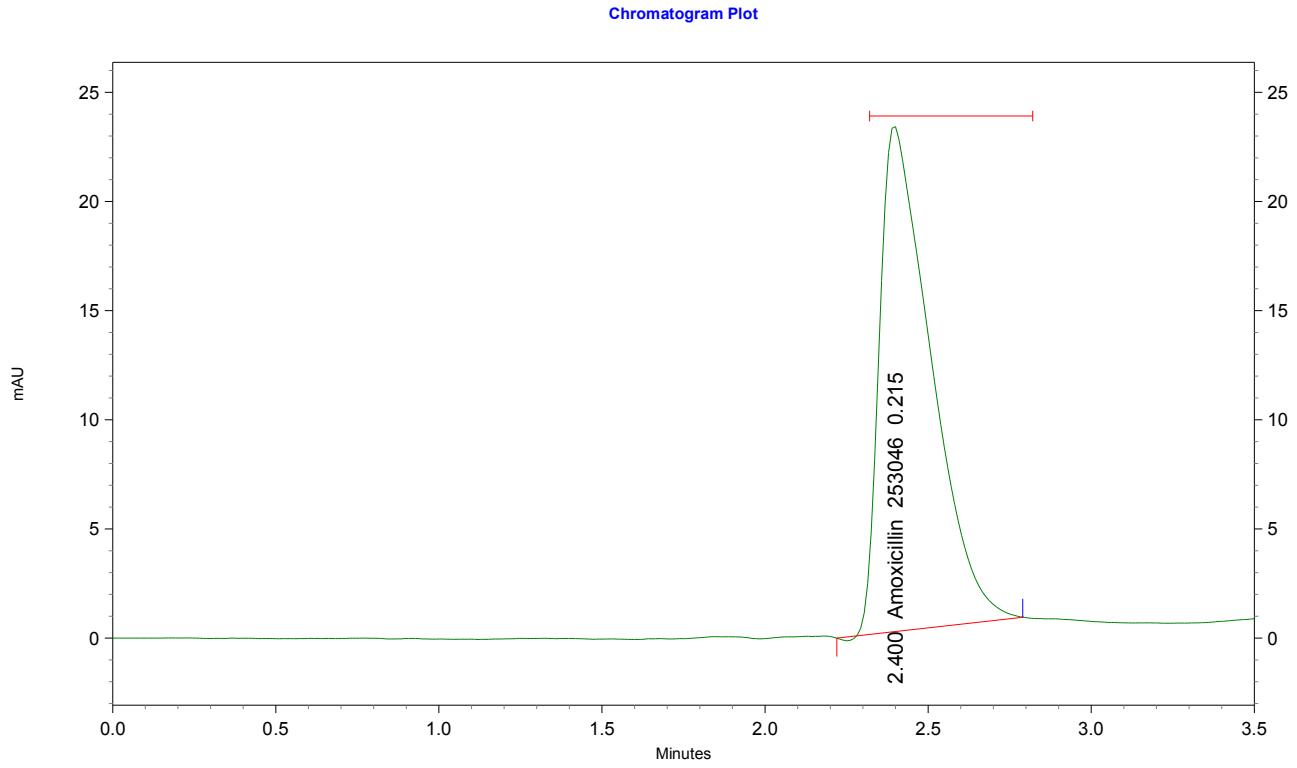
Method Name: C:\CLASS-VP\Methods\amoxellin.met

Sample ID: Amoxicillin

User: System

Acquired: 10/31/2010 4:36:21 PM

{Sample Description} **: Shanghi Amoxicillin rept3**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.400	253046	0.215	mg/ml

Chromatogram plot 3.36 (R3) Changahi amoxicillin capsules

Data Name: C:\CLASS-VP\Amoxicillin- wafra-Rep1

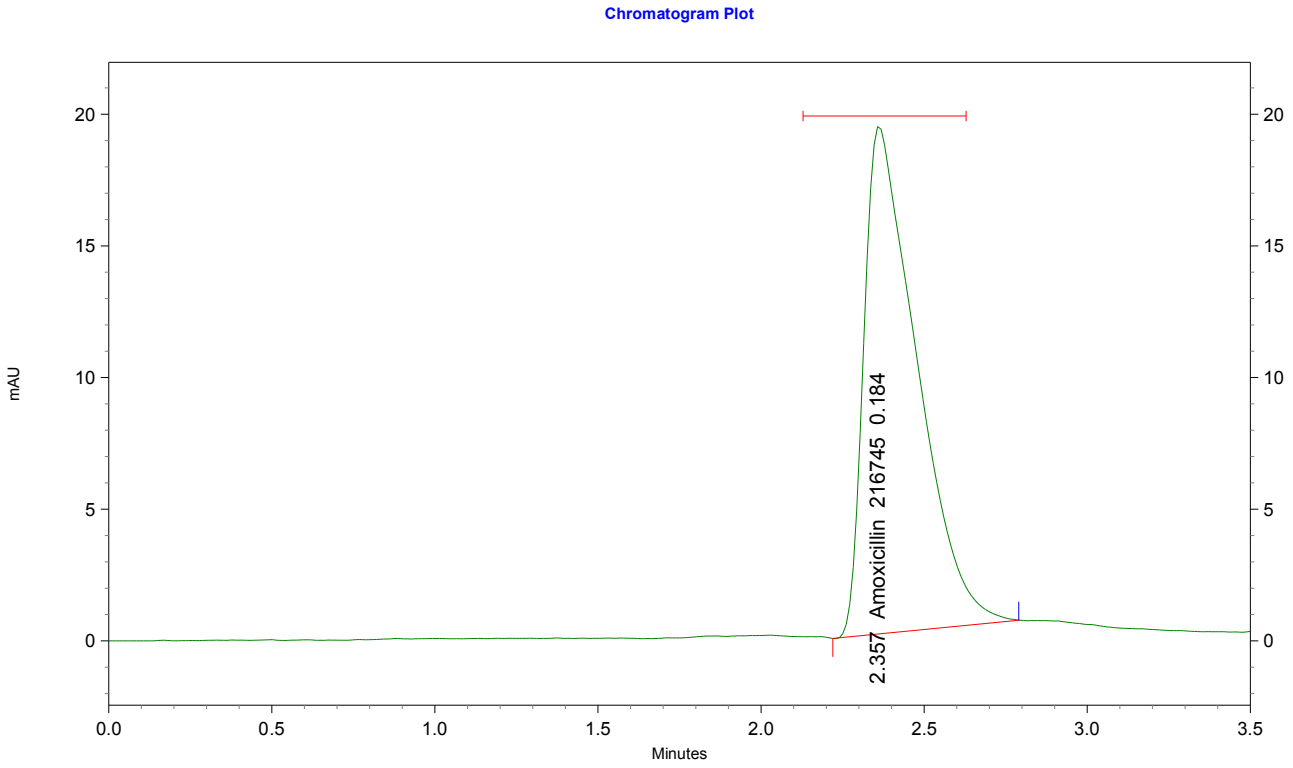
Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 4:02:32 PM**

{Sample Description}**:Wafra Amoxicillin rept1**



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.357	216745	0.184	mg/ml

Chromatogram plot 3.37 (R1) Wafra amoxicillin capsules

Data Name:C:\CLASS-VP\Amoxicillin- wafra-Rep2

Method Name:C:\CLASS-VP\Methods\amoxellin.met

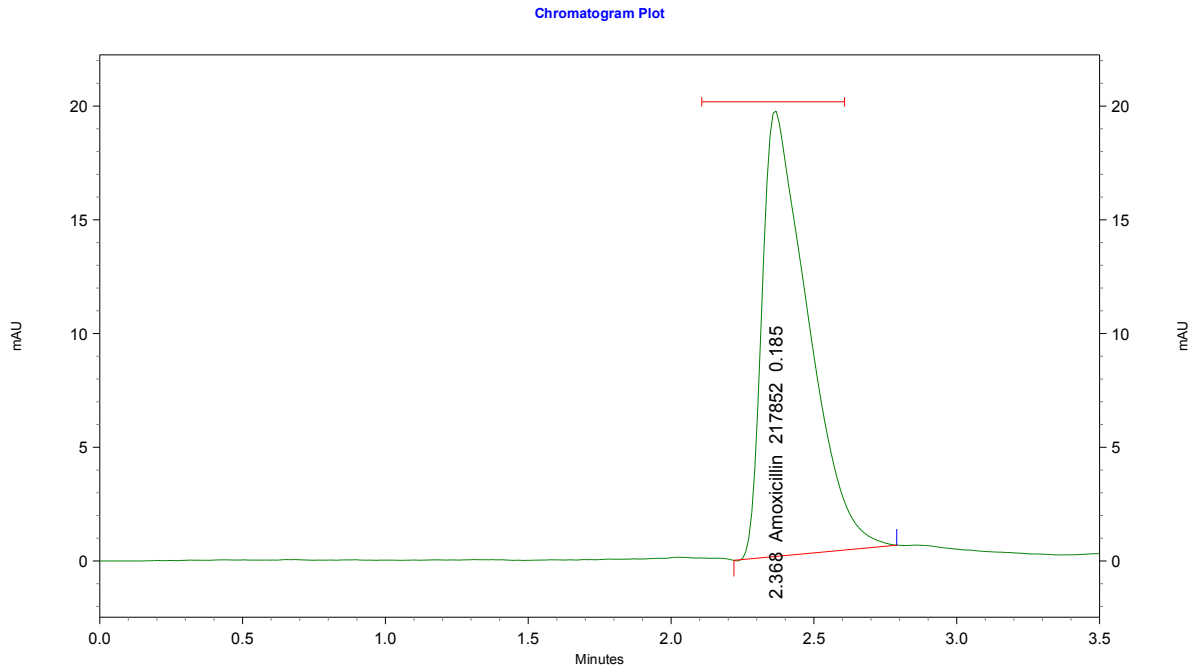
Sample ID: Amoxicillin

User: System

Acquired: 10/31/2010 4:08:10 PM

{Sample Description}**:Wafra Amoxicillin rept2**

.



Channel A

3: 254 nm, 8 nm

Name	Retention Time	Area	ESTD concentration	Units
Amoxicillin	2.368	217852	0.185	mg/ml

Chromatogram plot 3.38 (R2) Wafra amoxicillin capsules

Data Name: **C:\CLASS-VP\Amoxicillin -wafra-Rep3**

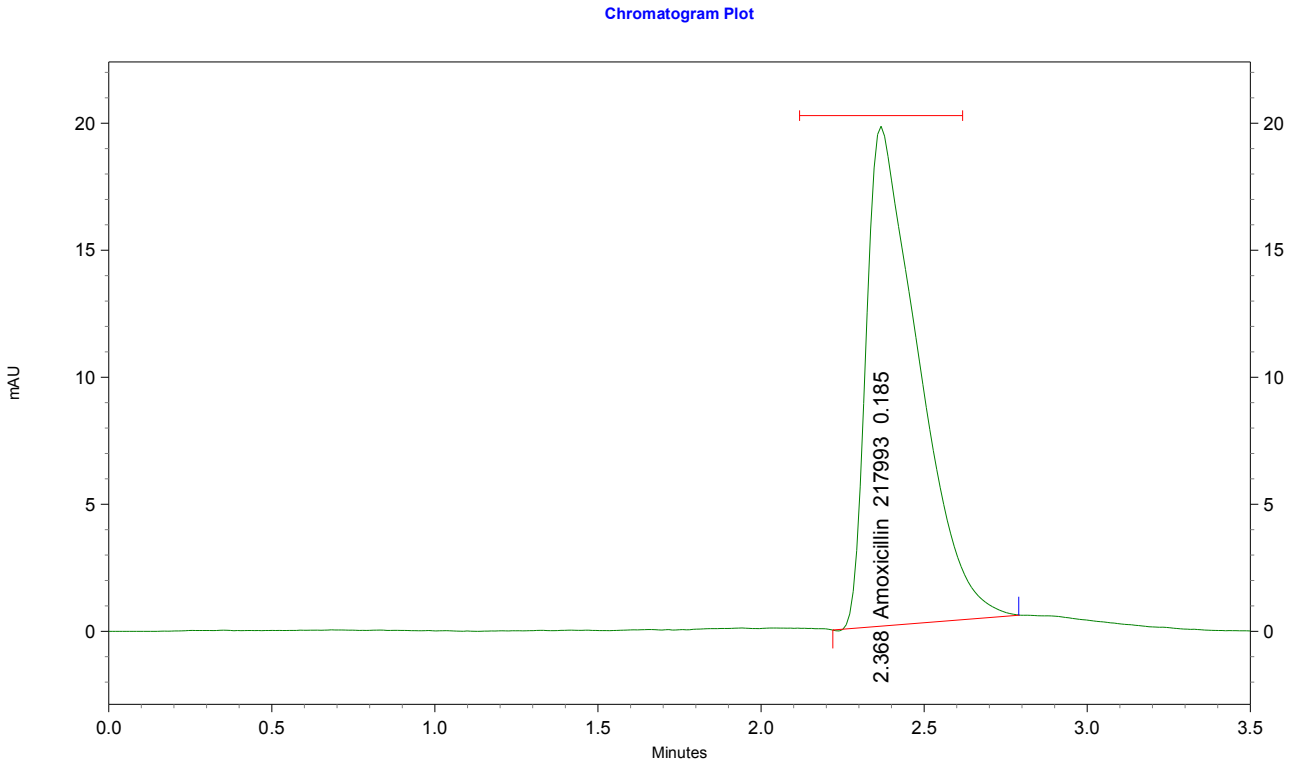
Method Name: **C:\CLASS-VP\Methods\amoxellin.met**

Sample ID: **Amoxicillin**

User: **System**

Acquired: **10/31/2010 4:13:52 PM**

{Sample Description}**:Wafra Amoxicillin rept3**



Chromatogram plot 3.38 (R3) Wafra amoxicillin capsules

CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

4.1 DISCUSSION

Data of cephalexin and amoxicillin , except back titration method data which shows very poor results were subjected twise to analysis of variance one for direct titration , conductometic, potentiometric, spectrophotometric and HPLC methods and the second for direct titration , conductometic, potentiometric using , computer program (SPSS). The analysis of variance was done according to the following statistical model.

$Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij} = observation

μ = overall mean

T_i = fixed effect of determination methods

e_{ij} = random error term

The statistical analysis results are as follows

4.1.1 Cephalexin

Table 4.1Analysis of variance for effect of different methods of determination cephalexin contents.

Sourceof variation	DF	Sum square	Meansum square	F	Significant level
Treatment	6	764.11	127.35	14.72	*
Error	14	121.10	8.65		
Total	20	1402.49			

* Significant at P < 0.001

Table (4. 1) reveales that there is significant differance ($p<0.001$) among methods in cephalexin amount.

Table 4.2 Means sepration of Cephalexin determination methods

Methods	Means	Standard deviation
Spectrophotometric	91.17^{a}	2.14
Direct titration	91.73^{a}	1.89
HPLC	101.15^{b}	3.12
Condutometric with NH_4OH	102.00^{b}	1.75
Potentiometric (pH/V)	102.17^{b}	3.5
Potentiometric (δpH/δV)	102.46^{b}	3.17
Conductometric with NaOH	109.6^{c}	3.77

Table(4.2) shows that conductometric titration with NaOH gave the highest value followed by the second derivative curve potentiometric method

(δpH/δV), the first derivative curve potentiometric method(pH/V), conductometric method with NH_4OH, direct titration method and HPLC method, in this order; however, while spectrophotometric method gave the lowest value.

Table 4.3 Analysis of variance for effect of different determination methods of cephalexin contents.

Source of variation	DF	Sum square	Mean sum square	F	Significant level
Treatments	4	565.35	141.34	6.06	*
Error	20	176.02	8.88.22		
Total	24	741.37			

* Significant at $P < 0.001$

Table (4.3). reveales that there is significant ($p<0.001$) among methods incephalexin amount.

Table 4.4 mean separation of Cephalexin determination methods

Methods	Means	Standard deviation
Direct titration	92.57^a	2.05
Condutometric with NH_4OH	101.62^b	10.69
Potentiometric (pH/V)	102.9^b	3.02
Potentiometric (δpH/δV)	102.8^b	2.65
Conductometric with NaOH	106.95^c	4.56

Table(4.4) shows that conductometric method with NaOH gavethe highest value

followed by potentiometric method (pH/V) ,potentiometric method(δpH/ δV), conductometric method with NH_4OH, in this order; the direct titration method gave the lowest value.

4.1.2 Amoxicillin

Table 4.5. Analysis of variance for effect of different determination methods of amoxicillin contents

Source of variation	DF	Sum square	Mean square	F	Significant level
Treatments	6	836.22	139.37	2.37	*
Error	21	1232.80	58.71		
Total	27	2069.02			

* Significant at $P < 0.05$

Table (4.5) reveales that there is significant ($p<0.05$) among methods in amoxicillin amount.

Table 4.6 means separation among Amoxicillin determination methods

Methods	Means	Standard deviation
Spectrophotometric	89.75^{a}	1.41
Potetiometric (δpH/δV)	95.08^{ab}	10.91
Potentiometric (pH/V)	96.75^{ab}	11.89
Direct titration	99.01^{ab}	6.80
Conductometric with NH_4OH	102.89^{b}	4.16
Conductometric with NaOH	105.48^{b}	7.48
HPLC	105.82^{b}	4.83

Table (4.6) shows that HPLC gave the highest value followed by conductometric method with NaOH, conductometric method with NH_4OH potentiometric method (pH/V), potentiometric method (δpH/δV), then direct titration method, in this order; however, the spectrophotometric method gave the lowest value.

Table (4.7). Analysis of variance for effect of different methods of determination amoxicillin contents with HPLC method-2

Source of variation	DF	Sum square	Mean square	F	Significant level
Treatments	4	354.47	88.62	1.44	NS
Error	20	1233.21	61.66		
Total	24	1587.68			

NS notsignificant at P >0.05

Th results revealed that there is significant ($p<0.001$) among methods inamoxicillin amount (Table 4.7).

Table (4.8) means separation among Amoxicillin determination methods

Methods	Means	Standard deviation
Potetiometric (δpH/δV)	94.29[a]	9.62
Potentiometric (pH/V)	95.63[a]	10.60
Direct titration	99.17[a]	5.90
Conductometric with NH_4OH	101.52[a]	4.74
Conductometric with NaOH	104.56[a]	6.80

Table (4.8) shows that conductometric method with NaOH gave the highest value followed by conductometric method with NH_4OH, direct titration method, potentiometric method (pH/V), in this order; however; the potentiometric method (δpH/δV) gave the lowest value

A significant difference was calculated for both cephalexin and amoxicillin results by direct titration, conductometric, potentiometric, spectrophotometric and HPLC methods.

A significant difference at level ($P < 0.001$) was calculatd for cephalexin results by direct titration, conductometrc and potentiometric methods .

No significant difference at level (P > 0.05) was calculated for results by direct titration, conductometric and potentiometric methods .

Statistically direct titration, coductometric, potentiometrc, spectrophotometric and HPLC methods, show symmetrical mean results.

The means results of direct titration, conductometric and potentiometric methds, show acceptable results than that of HPLC method.

The very poor results of back titration method of cephalexin and amoxicillin may be due to the degradation of these cephlosporins antibiotics, cephalexin and amoxicillin, with sodium hydroxide ion as shown, in Fig(4.1). The reactions of cephalexin with nucleophiles (Nu and OH) are similar to those penicillin (reactions 1 and 3). Page (1984) ,Boyed (1984), Bundgaard (1975), Boyed (1985) and Page and Protor(1984). which involve the opening of β-Lactam ring by hydroxyl ion (reaction 1) , Page (1984) , Hou and Poole (1971) , Levine (1960), proceeds via tetrahedral intermediate and result in the formation of 5Rbenzylpenicilloic acid in contrast to penicilloics acid the cephalosporoic acids. In cephalosporins with C_3 methylene-X substituents , where X has a leaving group ability ,a slightly modified ring opening is observed : reaction 4. The nucleophilic attack at the β-Lactam has been attended with expulsion of X and migration of of the double bond. Some authors claim that the expulsion was concerted with the nucleophilic attack. Boyed (1984), Coene et al (1984) ,Boyed (1985), others claim a stepwise process.Page(1984),PageandProctor(1984).At acertain reaction conditions,the group X is substitute by nucleophile. Indelicato *et al*.(1874),Bradshaw et al.(1968).

The presence of carboxyl, amide and amino groups these resemble dipeptide in acid base ; in agueous solutions, depending on pH, they can exist as a cation (H_2+) Zwitterion (HL+-) or anion (L-), Lapshin,(2009).

As the same manner as amino acids in solution at neutral pH, they pre-dominantly dipolar ions (or Zwitterions) rather than uniionized molecules. In

the dipolar form of an amino acid, the amino group is protonated (NH_3+) , and carboxyl group is dissociated (- COO^-) ; the ionization state of amino acid varies with pH .In acid solution the carboxyl group is unionized (-COOH) and the amino group is ionized (NH_3+). In alkaline solution, e.g., (pH 11), the carboxyl group is ionized (-COO-) and the amino group unionized (-NH_2),i.e., at some intermediate point , the dipolar ion shown is formed , here the hydrogen ion from the carboxyl group is not transferred to the solvent ,but is transferred internally to the –NH_2 group . This intermediate form is also called an inner salt or (zwitterions), the value of pH at this intermediate is called iso electric point, because at it the dipolar ion has no net charge, Wood et al. (1968) as shown in Fig (2) and Fig (3). The conductimetric titration method curves show one neutralization point for cephalexin and two, for amoxicillin; these two points indicate diprotic behavior of amoxicillin ,in agreement with Adel,(2005).These two points showed by amoxicillin are due to the fact amoxicillin contains a phenolic ring in its structure; Phenols not only react with sodium hydroxide, but also form intermolecular hydrogen bonding, Morison and Boyed (1973). This intermolecular hydrogen bonding facilitates the deprotonation of OH group of phenolic ring to react with sodium hydroxide solution causing the second point. For the same reason the potentiometric titration curves show two separate buffer regions for amoxicillin, and one buffer region for cephalexin.

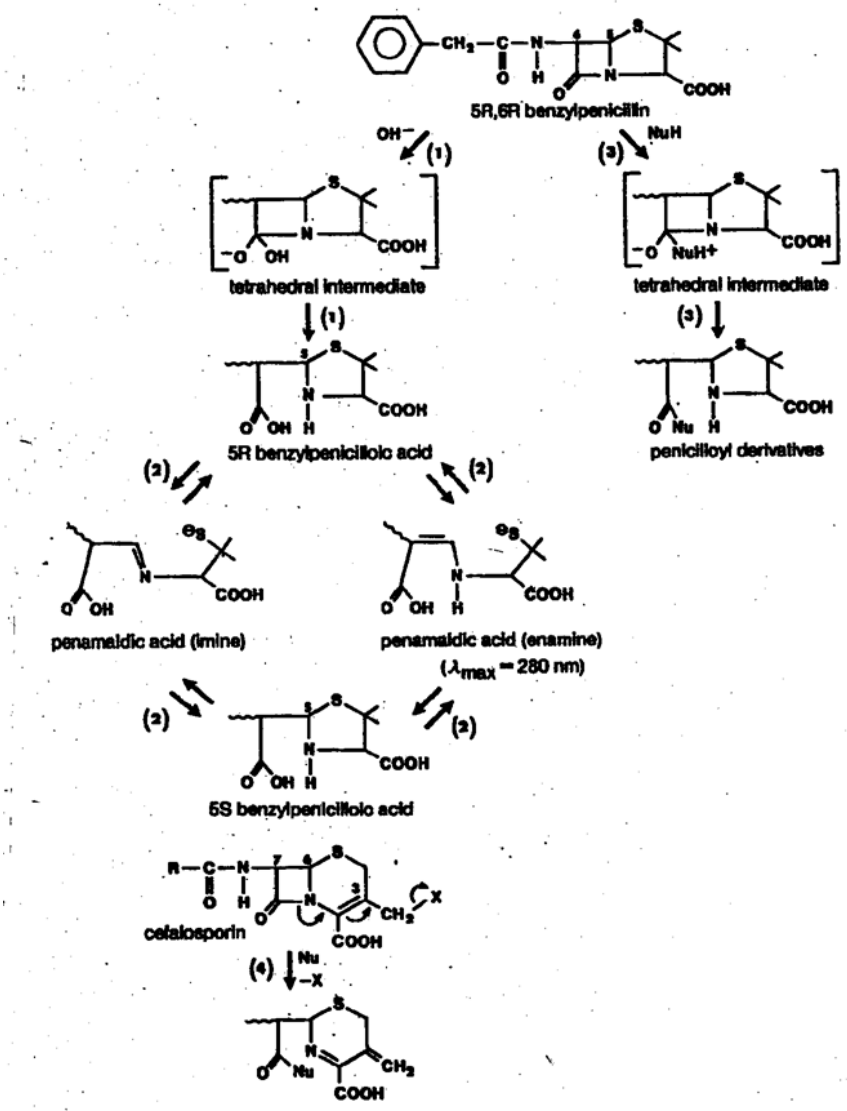


Fig (4.1): Reactivity and degradation of penicillin and cephalosporins in neutral and alkaline medium.

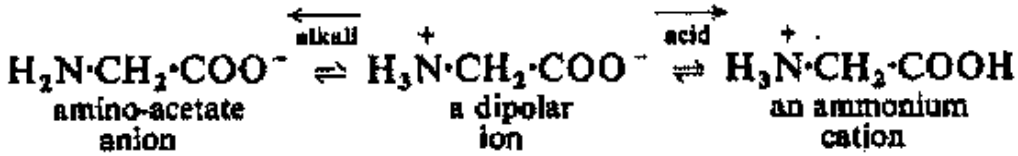


Fig (4.2) Reaction of Amino Acid with Acids and Bases

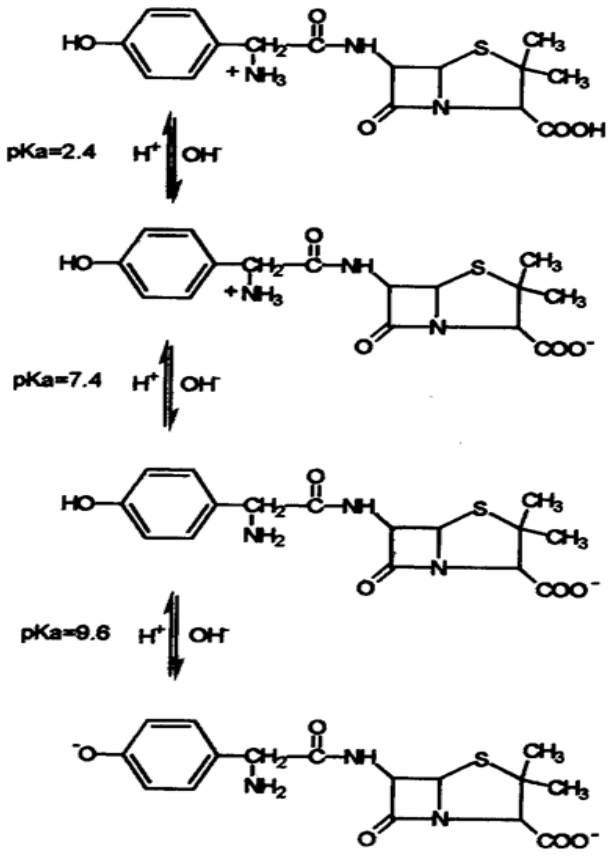


Fig (4.3) Ionic Species of Amoxicillin

4.2 Conclusion

Statistical analysis of this work shows that the results obtained by conductometric and potentiometric methods give comparable results to those obtained by the high performance liquid chromatography. They could be used as simple and cheap alternative quantitative methods for the determination of cephalexin and amoxicillin.

4.3 Suggestions for futher studies

Following the success of the analysis carried out on cephalexin and amoxicillin, using conductometric and potentiometric methods, the research work could be extended for the application of the developed technique for a range of pharmaceutical products such as penicillin and ampicillin.

REFERENCES

Alaa, S. Amin and Sayed, A. Shama. (2000). Vanado phosphoric acid as a modified reagent for the spectrophotometeric determination of certain cephalosporins and their dosage form. Monatshefte fur cheme,Vol.31,No.4,page313−319.

Bowman, W.c and Rand, M.j. (1980). Text book of pharmacolog 2nd edition. Black well scientific publication .Cabridge University. U.K.

Boyed DB. (1984) . Electronic structure of cephalosporins ad penicillin . 15 . inductive effect of the 3-position side chain in cephalosporins . j Med chem. 27: 63-3.

Boyed DB. (1985) . Elucidating the leaving group effect in the beta-lactam ring opening mechanism of cephalosporins . j Org chim 50:886-8 .

Bradshaw. Eard, S and Long AG. (1968). Cephalosporanic acids . Part v1. Action on primary and secondary aromatic amine on cephalosporanic acids. J Chem Soc [C] . 801-6.

British pharmacoepia. (1999).

Brown, G.I. (1972). Introduction to physical chemistry.

Bundgaard, H. (1975) .Chemical studies related to cephalosporins allergy.1. Kinetics of aminolysisof cephalosporons and effect of C_{-3} substituents on beta –lactam reactivety . Arch pharm chemi (soci) 94-123 .

Catharina,Y.W. Wenhonglluo, Veronical, C. Call and Herbert, F. Righter. (1997). Comparison of liquid chromatography with microbial inhibition Ass incurred amoxicillin and ampicillin residues in milk. J. Agric. Food, chem. 45(11) 4351-4356.

Chaleermporn Thongpoon.Boonsom Liawruangrath. Saisunee. Liawrungrath. Alan, R.Wheatly and Alan Townshend. (2005). Flow injection chemiluminescence determination of

cephalosporins using pharmaceutical preparation using tris(2,2−bipyridyl)ruthenium (11) - potassium pemangnate. Analytica Chemica Acta, Vol.553,issue 1-2,pages 123-133.

Chalrmporn Thongpoon, Kungwan Saekue, Wisanu Thngchai and Theeraphan Machan. (2007) 33rd Cogress on science and Technology of Thailand.

Coene B, Schanck A, Dereppe J-M, and Van Meerssche M. (1984). Substituent effects on reactivity and spectral parameters of cephalosporins. J M Chem :27;694-700.

Dalia.R.and El−wasseef.(2007). Spectroflurometyric determination of cephalexin in pharmaceutical preparations and spiked human urine using 2-cyanoacetamide.Spectroscopy letter, Vol.40,issue6,page 797−809.

De Baere,S and Patrick Backer. (2007). Quantitative determination of amoxicillin in animal feed using liquid chromatography with Tandem mass spectrometric.Anaytica chimica acta.

Dousa.M, Hosmanova.R. (2005). Pharm. Biomed.Anai. 37, 373- 377.

Dusa,M., Hosmanova, R.(2005). Rapid determination of amoxicillin in premixes by HPLC. J. Pharm,.Biomed. Anal. Amesterdam Vol.37, no .2, p 373-377.

Dusa,M.Hosmanova,R. (2005).J. Pharm.Biomed.Anal. 37,373.

Edward, L .Jonson and Robert Stevenson.(1978). Basic liquid chromatography. Varina association.

EL−Yazbi Fawzy and Barary Magda, H. (1985).Determination of some cphalosporins using derivatives pectrophotometery.Analytical lett, 18(B5), 629 40.

Fogg, AG. Abdalla, MA Henriques, HP.(1982). Titrimeteric determination of the yield of sulphide formed by alkaline degradation of cephalosporins . Analy. 107:449-52.

Frantz, BM .(1976). Iodometic and spectrophotometeric assays for cephradine after its hydrolsis with a beta-lactamase. J pharm sci .65:887-91.

Gamal,A.Saleh.(1996). Two selective spectrophotometeric method for determination of amoxicillin and cefadroxil. Analyst, 121,641-645,Do:10.

Gamba,V. and Dusi,G. (2006). Anal.Chem. A Cta, 493, 69.

Graciele Parisotto, Marco Flore Ferrao, Joao Carlos Furtado, Rolf Fredi Molz.(2007). Determination of amoxicillin content in powdered pharmaceutical formulations using Drifts and PLS. Brazilin journal of pharmaceutical sciences,Vol.43,No.1.

Grime, JK Tan, B.(1979). Direct titration oh antibiotics with iodate solution .Part 11:Some selected cephalosporins.Anal Chem Acta .105:36.

Heintz, B. Kruger, m.(1985). β-lactam antibaiotika: Queck-selbertitration oder hydroxatmethode? Dtsh Apoth Ztg . 125: 2594-602.

Heinz-Martin Kuss and Evelin Denkhaus. (2005). New method for determination of β-lactam anti biotics by means of diffuse reflectance soectoscopy using polyurethane foam as a sorbent.

Henry,H. Baver.,Gary,D.Chritain and James,E. Oreilly.(1978). Instrumental analysis. Allen and Bacon, inc, U.S.A.

Hernandez,M. Brrul,F. Calull,M.(1999). Determination of amoxicillin in plasma samples by capillary electrophoresis. J.Chromatogr.,B,Biomed. Sci.App., Amisterdam , Vol.731, no.2, p.309-315.

Hoizey,G.Lamiable,D.Frances,C.Trenque,T.Kaltenbach,M.and Millart,H.(2002). Simutaneous determination of amoxicillin and calvulanic acid in human plasma by Holc with UV detection. J.pharm biomed anal, Amesterdam,Vol.30, no.3, p.661-666.

Holl, W. O. Brien, M. Filan ,J. et al; (1975). Automated spectrrophotometeric assay of cephazolin .J pharm sci ; 64 : 1232-4.

Hou , JP. Poole ,JW. (1971) .Beta-lactam antibiotics: their phsiochemical properties and biological activities in relation to structure . J pharm Sci 60:503-32.

Hughe, DW.Vilim, A.Wilson,W .(1976). chemical and physical analysis of antibiotics. Part 11. Can J pharm sci . 11: 97-108.

Huquette, Fabe. Blanchin,Marie. Dominique, Lerner. Danand, Manddrous, Bernaditte. (1985). Determination of cephalexin utilizing thin layer chromatography with fluroescamine detection. Analyst (London),110(7) −775−8.

Husain,S.W, Ghoulipour,V. and Sepahrian,H. (2004). Chromtographic behaviour of anti biotics on thin layers of an inorganic ion-exchanger. Acta Chromatographia, No.14.

Indelicato ,JM.Norvisa, TT. Pfeffer, RR .Wheeler, WJ. and Wilham ,WL. (1974). Substiution effects upon the base hydrolysis of pencillins and cephalosporins. Competitve inter molecular nucleophilic amino attack in cephalosporins . J Med Chem :523-7.

Jakson, J.V., Mass, M.S. and Widdop, B.(1989). Clare isolation and identification of drugs. 2 nd edition, the pharmaceutical press, London.

James, D. Maglc, J.R. Stanly, R and Crouch. (1988). Spectrochemical analysis. Prentice Hall, Englewood cliff, New Jeresy 07632, United State of America.

John Hicks, M.A. (1973). Comperhensive chemistry .2nd edition. Great Britain, by Anchor press.

Joy, A. Mc Ateer, F. Hiltke, B Michael Sller, and Robert,F. Faulkner. (1987). Liquid chromatographic determination of five orally active cephalosporins, cefixime, cofactor, cefadroxil, cephalixin

and cephradine in humanserum. Clinical chemistry,Vol.33, No.10.

Jusko, WJ.(1971). Fluorimetric anaylsis of ampicillin in biological fluids .J pharm sci; 60: 728-32.

Khalil Farhadi. Sedigheh Ghalangahi. Ramin and Feridoum Salek Asgan. (2002). Spectrophotometeric determination of selected anti biotic using Prussian blue reagent. J.of Chinese chemical society ,49.6.(2002/12),993-997.

Kirschbaum, j.(1974). colorimetric determination of cephradine ,a cephalosporins antibiotic .J phram sci.63: 923-5.

Korb, J..Pospisilova, B. (1983). Mercumrimetric determination of cephalosporins . Cesk farm 1.32:6-11.

Kulo, AE. (1976). Automated system foraetermination ph cephalosporins and penicillin using hydroxylamine reagent. Farm Notisbl; 1: 1-7.

Lapshin, S. V . and Alekseev, V. G. (2009). Copper(11) Comlexation with Ampicillin . Amoicillin and Cephalexin, Russian journal of inorganic chemistry ,vol. 54 No.7, pp. 1066-1069.

Lapshin, S. V . and Alekseev V. G. (2009). Copper(11) Comlexation with Ampicillin , Amoicillin and Cephalexin, Russian journal of inorganic chemistry ,vol.54 No.7, pp. 1066-1069.

Lec,Y. and Lec, H.C. (1990). Anew high performance liquid chromatographic determinatrion of cephalexin ,cefuroxime ,cefoxitin and cephaloridine in plasma uing a colum- switching technique. Chromtographia,vol.30 ,No.1-2 ,80-84. Springer.

Levine, BB.(1960). Degradationof benzylpenicillin at pH 705 to d-benzylpenicilloic acid .Nature 186: 939-40 .

Li,Quanmin and Yang, Zhanjun.(2006). Study of spectrophometeric determinationof amoxicillin using sodium 1,2Naphthoquinone-4—sulfonate as the chemical deriviative chromatographic reagent. Analytical litters, Vol.39,No.4, pp.763-775(13).

Lo Lee. Lucius. D Arecont. Marvin, A. Brooks.(1978). High pressure liquid chromatographic determination of amoxicillin.

Lubert Styer (1988). Biochemistry, 3rd addition, United State.

Luis RenetoPires de Abreu. and Rodrigo Mas Ortiz. (2003).HPLC determination of amoxicillin comperative bio availability in healthy volunteers after a single dose a dminstratio.J.Phamaceut sci (www.utaberto.ca/~csps) 6(2):223-230.

Ly Thuong Kiet,(2004). Comparsion between UV and HPLC methods in the dosage o phaceutical products containing a mixture of 2 7components amoxicillin and clavulanic acid.Vietnam information for science and technology (VISTA).

Matar,K.M. (2006). Simple and rapid LC method for the determination of amoxicillin in plasma. J. chromatographia,Vol.64,No5-6, page 255-260.

Mays, DL. Bangert, FK. Cantrell ,WC. Evans ,WG. (1975). Hydroxylamine determination of cephalosporins . Anal chem. . 47: 2229-34.

Mei-Chich Hsu and Pei-Wen Hsu (1992). High-performance liquid chromatographic method for potency determination oh amoxicillin in commercial preparation and for stability studies. Anti microbial, Agents and chromatography, Vol.36, No.6, pages 1276-1279.

Michael, p. Doyl. Williams Mungall (1980). Expermintal organic chemistry.

Moriilsuo,FujitaYoshirazu and Sakaguchi Kimika. (1982). Spectrophotometeric determination of cephalexin and ampicillin using o-hydroxy hydroquinone phthalein and palladinm (11). Chemical and pharmaceutical bulletin,Vol.30,No.71,PP2599–2603.

Morison and Boyed (1973), organic chemistry, 3rd edition, Ally and Bacon.

Mouayed,Q. Al-Abachi, Hind Haddi and Anas,M. Ai-Abchi. (2005). Spectrophotometeric determination of amoxicillin by reaction with N,N-dimethyl-P-phenylene diamine and potassium hexacyanoferrate(111). Analytical chimica acta, v.554, issues.1-2, pages 184-189.

Nabi,S.A Lagi,E. and Ilam, A., (2004). Selective separation and determination of cephalosporins by LTC on stanic oxide layer. Acta chromatographia, No.14,

Nkeoma, N. Okoye Godwin I. C. Nwoked Nkechinyere, N. Ukwueze and Festu, B .C. Okoye.(2007). Spectrophotometric determination of some cephalosporin antibiotics using Prussian blue reaction ,Vol.2(8) pp 342-347,

Nkeoma, N. Okoye Godwin, I. C. Nwokedi Nkechinyere, N. Ukwueze and Festus, B .C. Okoye.(2007).Spectrophotometric determination of some cephalosporin antibiotics using Prussian blue reaction ,Vol.2(8) pp 342-347,

Okada, S. Hattori, K., Takono, T.(1965). An iodometric assay of some derivatives of aminocephalosporanic acid. Bull Chem soc Jpn . 38: 2186-7.

Orawon ChailApakui. Akira Fujishima. Pornpimol Tipthara. and Hatai Siriwongchai (2001). Electro analysis of Glutathione and cephalexin using the boro −doped diamond thin −film electrode applied to flow ijection analysis. Analytical science,Vol.17, supplement (2001), bJapan society for analytical chemistry.

Page, MI . Proctor Ph (1984) . Mechanism of beta-lactam ring opening in cephalosporins . J Am chem. Soc 106:3820-5 .

Page, MI.(1984) .The mechanisms of reactions of beta- lactam antibiotics . Acc Chem. Res ,17: 144-51 .

Papazova.P, Bontchev,P.R, and Kacarova,K. (2005). Aphotometric extraction method for determination of cephalexin. Micro chemical Acta,Vol.66,No.1−2.

Perez- Lozano, P. Garcia-Monlga,E.Orriols,A. Minarro.M.Tico,J.R and Sune-Nere,J.M. (2006). Stability evaluation of amoxicillin in solid premix veterinary formulation by monitoring the degradation products through anew HPLC analytical method. J. pharm.Biomed, analysis,42. 192-199.

Rogic, D . Mandic, Z.(1984). Spectrophotometric determination of cephalexin .Acta pharm Jugosi : 34; 51-7.

Rorbert, L. Pecsor,L. Donald Shields,Thomas Cagns.Ian,G. Mc Willian. (1976). Modern motheds of chemical analysis. 2nd edition. John Wely and son, printed U.S.A.

Shindle ,VM. Desia,BS. and Tendolkar NM. (1994). Simultaneous determination of cephlexin and probenacid from tablets by RP−HPLC. Inian journal of pharmaceutical science, Vol.56, issue.2, page 58−60.

Skoog, West Holler. (1992). Fundamental of analytical chemistry .5th edition.Saunder golden, sun burstseries, U.K.

Terumichi Nakagaw. Junhaginaka.Kiyoshi Yamaoka and Toyozounon (1978). High speed liquid chromatographic determination of cephalexin in humn plasma and urine. The journalof anti biotic , Vol.31,No.8.pp.769−775.

Van Krimpen ,P.C. Van Bennekom, W.P and Bult A. (1987). Physiochemical properties and analysis in pharmaceutical and biological matrices ,Vol. 9. Pharm Weekblad Scientific Edition

Vogel. (1978). Text book of quantitative analysis in organic chemistry. 4th addition, Longman group limited, London.

Walken,K.J. Zarembo,J.E. Staiger, D. B. and Postsmith,A. (2006). Nmr spectroscopic analysis of cephalexin in cephradine bulk drug and

dosage form. Journal of pharmaceutical science,Vol.67, issue.10,page1481-148

Wood ,C .W. Holliday, A .K . and Beer R .J . S (1968).organic chemistry, Butterworth and Co ,UK.

Xiaofengxie and Zhenghua Song.(2006). Ultra sensitive determinastion of amoxicillin using chemiluminescence with flow ijection analysis. Spectroscopy,Vol.20,No.1,Pages 37-43.

Yamana ,T. and Tsuji A.(1976). Comparative stability of cephalosporins in aqueous solutions : Kinetics and mechanisum of degradation .J pharm sci ,65: 1563-74 .

Yoon,K.H,Kim,H.J, Lee,S.Y, Kim,W. and Park,J.S. (2004). Simutaneous determination of amoxicillin and calavulanic acid in human plasma by HPLC-ESI Mass spectrometery. J.Chromatogr.,B,Biomed sci. Appl., Amesterdam, v.813,n.1-2,p.121-127.

Yu, A .B .C. Nightingale C.H. and Flanagan D. R. (1977) .Rapid sensitive Fluorometric of cephalosporin Antibiotics ,Vol.66 , No 2.