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INSTITUTE OF SCIENCE AND TECHNOLOGY

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INTERNSHIP REPORT



FROM

(NOVEMBER 29, 2023 to DECEMBER 19, 2023)

SUBMITTED BY-

SHWETA.D

42910023

IInd - M.Sc. CHEMISTRY

December 19, 2023

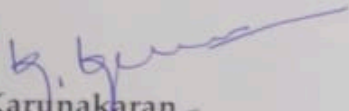


CERTIFICATE

This is to certify that **Ms. SHWETA D, (Reg.No.42910023) M.Sc, (Chemistry)** second year student from Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Rajiv Gandhi Salai, Chennai-600 119, Tamil Nadu, underwent **Internship** at our API Business Unit - Alathur during the period from **November 29, 2023** to **December 19, 2023**. During the above said tenure, we found her satisfactory to the job assigned to her by our Organization.

We wish her success in her career.

For Orchid Pharma Ltd.


K Karunakaran -
Asst.Manager-Human Resources

Preface

The requirement of practical knowledge by internship is met by professional training and ability of correlating theoretical knowledge to practical exercises under the proper guideline of the related professional staff. The training equips the students with skill, knowledge and professional values sufficient for building professionalism in them. This also guides the students to flourish in their career by imparting the ability of unending learning process. My university (Sathyabama Institute of Science and Technology, Chennai) has therefore been seized with the need of developing practical guideline which would help in providing experience to students to meet the future needs of the industry. In this regard I have been given an opportunity to experience 15 days internship at **ORCHID CHEMICALS & PHARMACEUTICALS LIMITED (ALATHUR)**. During this time, I have visited different sections of the company and I have been guided properly and I am very glad to compile what I have learnt in this report.

Acknowledgement

I would like to express my sincere gratitude to everyone who supported me throughout my internship and the creation of this report.

I am deeply thankful to **Mr. D. SARAVANAN (Manager)**, **Mr. K. SUNDRA KUMAR (Assistant Manager)**, **Mr. R. Prasanna (Human Resources)**, **Mr. S. Kathir (Executive of PD Lab)** for their invaluable guidance, mentorship, and unwavering support during my internship tenure. Their expertise and encouragement have been instrumental in shaping my learning experience.

I also extend my appreciation to the entire **ORCHID CHEMICALS & PHARMACEUTICALS LIMITED (ALATHUR)** team for providing me with a nurturing environment to learn and grow professionally. I am grateful for the opportunities I received to apply my skills and gain hands-on experience in full 15 days.

Furthermore, I am thankful to my university (**SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY**) for their continuous support and encouragement throughout this internship period.

Lastly, I am grateful to my family and friends for their unwavering support and understanding during this journey.

Thank you all for being part of this incredible learning experience.

Sincerely,

SHWETA.D

ABSTRACT

This report contains detailed information about each and every single process, equipment, plant investigated by me during the internship. In this report all the types of equipment are mentioned and the ones being used in the industry are especially distinguished. All the operating parameters of a specific plant are specified. A brief study on the flow path of Profile of the company, safety measures, Process of Batch distillation Process, preparing Cefazolin Sodium using latest instruments and reactors. Second phase of the internship contains High-Performance liquid chromatography .

TABLE OF CONTENTS

INTRODUCTION

COMPANY OVERVIEW

OBSERVATION AND EXPERIENCE

GAS CHROMATOGRAPHY

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

ION EXCHANGE CHROMATOGRAPHY

PREPARATION PROCEDURE

CONCLUSION



Established in 1992 as an export - oriented unit (EOU), Orchid Pharma Ltd. (Orchid) is a vertically integrated company spanning the entire pharmaceutical value chain from discovery to delivery with established credentials in research, manufacturing and marketing. They today rank among the top 50 pharmaceutical companies in India and enjoy a multi-therapeutic presence across segments like anti-infective, anti-inflammatory, central nervous system (CNS), cardio vascular segment (CVS), nutraceuticals and other oral and sterile products. Our pharmaceutical solutions include active pharmaceutical ingredients , finished dosage forms. They are globally present across 40+ countries through alliances and partnerships with globally reputed majors.

Their integrated business model enables us to cater to business opportunities throughout the value chain, from research to delivery of drugs across therapeutic segments. Our niche product basket helps us maintain an edge over our peers in the markets where they are present. In the years to come , driven by a highly competent and motivated team, they will move from strength to strength in the key domains of API, and global generics

WHAT WE DID:

On first we go to know about the safety class and then we learned about instrumentation of HPLC, GC, polarimeter and then how they make and preparation of cefazolin sodium in the Pharmaceutical limited.

PURPOSE OF THE INTERNSHIP:

Various reasons for selecting ORCHID Pharma company for the internship are as follows:

- To gain detailed knowledge about chemical applications and analysis, lab equipments making bulk products of tablet product and also to get the experience of field work.
- To get the technical ideas by trouble shooting the case studies done by organisation. This helps to become a part of project and helps to apply the knowledge in future profession.

PRODUCTION REVIEW

- They were using Methyl di chloride, Tetra hydro fluorine, Toluene, Dimethyl acetylene, ethyl acetylene as more than 50 solvents.
- Cephalexin, Cephaloridine., Cefixime etc. as like they are creating 150 types of tablet
- They were using two type of reactors called gas line reactor and SSR reactor
- Gas line reactor for acid solution and SSR reactor for base solution.
- They also used +7", -10", -25', -40", -70", in three utilities. =7" is used for only watering other " for cooling methanol.

PROCESS DEVELOPMENT LAB

POLARIMETER

- A Polarimeter is a scientific instrument used to measure the angle of rotation caused passing polarised light through an optically active substance
- The machine used in industries is JASCOP 1020
- Polarimetry is a type of qualitative and quantitative technique, used for optically active compounds.
- The tendency of molecules to rotate the plane of polarised light (clockwise or anticlockwise) and the extent of rotation is measured.
- Thus polarimeter can be used to identify and estimate the compounds.



PHARMACEUTICAL APPLICATIONS

- To determine the product purity by measuring specific rotation and optical rotation of :Amino acids, Amino sugar , Analgesics , Antibiotics, Cocacine, Dextrose, Diuretics , Steroids, Serums Transquilizers, Vitamins etc .
- For raw material inspection of : Camphors, Citric acid, Glyceric acid gums , Lavender oil, Lemon oil, Spearmint oil.

GAS CHROMATOGRAPHY

Gas Chromatography is a widely used analytical technique used to separate & analyze the gaseous & volatile compounds. In 1952, Modern Gas Chromatography was invented by James & Martin. Since early 1950's this technique was first used for the separation of amino acids now GC has large number of applications as this technique is rapid & has a great sensitivity. Both qualitative & quantitative analysis can be done through GC. Even minute quantity sample can be analyzed through GC. In gas chromatography, the sample is dissolved in a solvent and vaporized in order to separate the analytes. The sample is distributed between two phases: a stationary phase and a mobile phase. The mobile phase is a chemically inert gas such as helium, nitrogen etc. Gas chromatography is one of the unique forms of chromatography that does not need the mobile phase for interacting with the analyte. The stationary phase is either a solid adsorbent, termed gas-solid chromatography (GSC), or a liquid on an inert support, termed gas-liquid chromatography (GLC). The criteria for the compounds to be analyzed in GC is volatility & thermostability.

Instrumentation

Generally, all the chromatographs (GSC or GLC) consists of six basic components:

Sample injection system:

A sample port is necessary for introducing the sample at the head of the column. A calibrated microsyringe is used to transfer a volume of sample through a rubber septum and thus into the vaporization chamber. Most of the separations require only a small fraction of the initial sample volume and a sample splitter is used to direct excess sample to waste. Commercial gas chromatographs involves the use of both split and splitless injections when alternating between packed columns and capillary columns. The vaporization chamber is typically heated 50 °C above the lowest boiling point of the sample and subsequently mixed with the carrier gas to transport the sample into the column

2. CARRIER GAS

A carrier gas plays a vital role in GC. It should be inert, dry & free of oxygen. Helium, Nitrogen, argon & hydrogen gases are used as carrier gas depending upon the desired performance & detector being used. Carrier gas is supplied at high pressure & is passed to instrument at a rapid & reproducible rate.

3. Separation column

Open tubular columns or capillary columns & packed columns are used in GC. The first type of capillary column is a wall-coated open tubular (WCOT) column and the second type is a support-coated open tubular (SCOT) column. WCOT columns have a thin layer of the stationary phase coated along the column walls. In SCOT columns, the column walls are first coated with a thin layer of adsorbant solid, such as diatomaceous earth, a material which consists of single-celled, sea-plant skeletons. The adsorbant solid is then treated with the liquid stationary phase. While SCOT columns are capable of holding a greater volume of stationary phase than a WCOT column due to its greater sample capacity, WCOT columns still have greater column efficiencies. One of the most popular types of capillary columns is called the coated Fused Silica open tubular column.

4. Column Oven or Thermostat chambers:

The thermostat oven are there to control the temperature of the column to conduct precise work. The oven can be operated in two manners: isothermal programming or temperature programming. In isothermal programming, the temperature of the column is held constant throughout the whole separation. In the temperature programming method, the column temperature is either increased continuously or in steps as the separation progresses.

5. Detectors:

Most common types of detectors used in GC are: Mass Spectrometer, Flame ionization detector (FID), Electron capture detector (ECD), Thermal conductivity detector (TCD), Atomic emission detector (AED), Photoionization detector (PID), Chemiluminescence detector. Detector is present at the end of the column & gives the quantitative measurement of the components of the mixture as they elute in combination with the carrier gas.

6. Amplification & Recorder system:

These are the last & final components of GC instrumentation. These are meant to record the signals that come from the detector. These use special electronic circuits the process & amplify the signals so as to display in an understandable graphical format that represents several peaks of the constituents of the sample under analysis.

APPLICATIONS

Identification of hazardous compounds in waste dumps.

1. Quantification of drugs & their metabolites in blood & urine for both pharmacological & forensic applications.
2. Identification of reaction products.
3. Quantification of pollutants in drinking & waste water.
4. Analysis of industrial products for quality control.
5. Skin sample analysis.
6. RNA isolation.
7. Astro chemistry & geochemical search



ION EXCHANGE CHROMATOGRAPHY



Ion exchange chromatography can be defined as a reversible process in which ions of same sign are exchanged between solid and liquid, a highly insoluble body in contact with it. The solid is known as an ion exchanger and no substantial organic polymer has been used as ion exchanger [1, 2]. It is also known as cation-anion exchange chromatography. Ion exchange chromatography, which is also known as adsorption chromatography, is a useful and popular method due to its

- High capacity,
- High resolving power,
- Mild separation conditions,
- Versatility and wide spread applicability,

Tendency to concentrate the sample relatively low

PROPERTIES OF ION EXCHANGE CHROMATOGRAPHY:

The most common properties of all ion exchangers which have been used in analysis are:

- It should be almost insoluble in water and organic solvents, like benzene, carbon tetrachloride, ether etc.
- It should be complex in nature and in fact they are polymeric

It should contain active or counter ions that will leads to exchange reversibility with other ions in a surrounding solution without any substantial change in the material

ADVANTAGES:

- * **Detectability:** Useful for the detection of many inorganic salts and also for the detection of organic ions with poor UV absorptivity likes alkyl amines or sulfonates.
- * **Preparative separations:** Usually preferred because of the availability of volatile buffers. Volatile buffer makes the removal of mobile phase easier.
- * Useful to resolve very complex samples i.e., in the case of multi-step separation.
- * Useful for separation of mixture of biological origin, in organic salts and some organometallics.
- * Provides long life to resin
- * Cheap maintenance
- * Environmental friendly because it deals only with substance occurring in water.

THEORY:

The principle of separation is by reversible exchange of ions between the ions present in the solution and those present in the ion exchange resin. Ion exchange separations are mainly carried out in columns packed with an ion exchanger. There are two types of ion exchanger, namely

1. Cationic exchangers
2. Anionic exchanger

Cationic exchangers:

It possesses negatively charged groups and these will attract positively charged groups. These exchangers are also called acidic ion exchange materials since their negative charges result from the proteolysis of acidic groups.

Anionic exchangers:

It has positively charged groups, which will attract negatively charged molecules. This exchanger is termed as basic ion exchange materials since their positive charges generally result from the association of protons with basic groups. Based upon the affinity of ions towards the matrix the ions like cation and anion are separated. The ions that have less

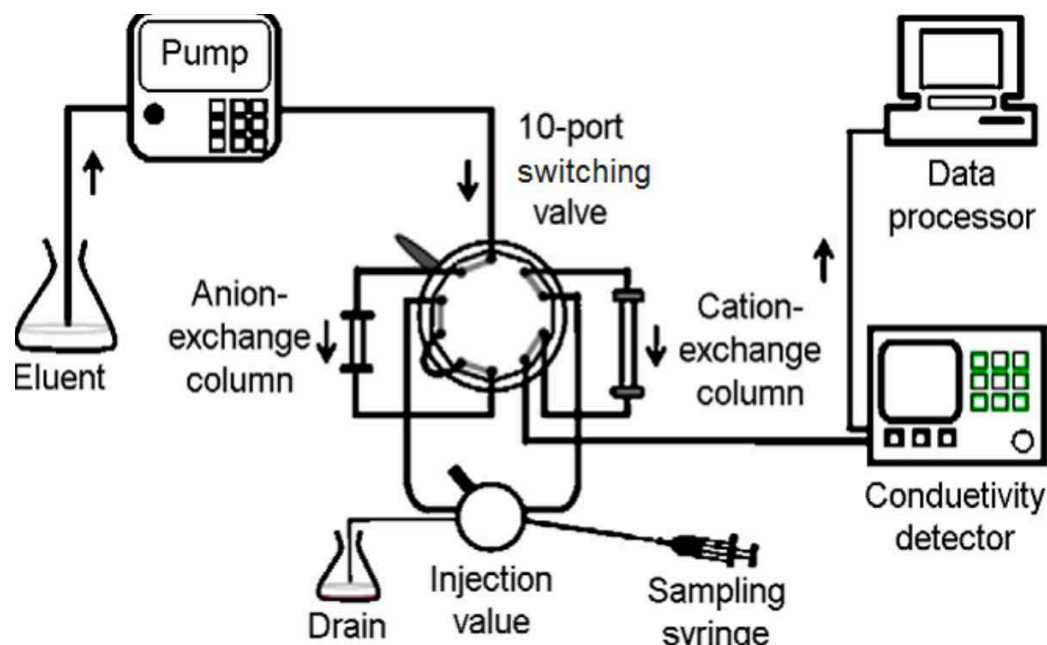
affinity towards matrices will elute first and the ions that have more affinity towards matrices it will elute later.

The actual ion exchange mechanism is thought to be composed of five distinct steps:

1. Diffusion of the ion to the exchanger surface. This occurs very quickly in homogeneous solutions.
2. Diffusion of the ion through the matrix to the exchanger site. This is dependent upon the degree of cross linkage of the exchanger and the concentration of the solution.
3. Exchange of ions at the exchange site occurs.

This occur instantaneously in an equilibrium process: $\text{Resin} - \text{SO}_3\text{H} + \text{Na}^+ \text{Resins} - \text{SO}_3\text{Na} + \text{H}^+$ $\text{Resin-N}(\text{CH}_3)_3\text{OH} + \text{Cl}^- \text{Resin} - \text{N}(\text{CH}_3)_3\text{Cl} + \text{OH}^-$. Diffusion Of the exchanged ion through the exchanger to the surface 5. Selective desorption by the eluent and diffusion of the molecule into the external solution takes places.

INSTRUMENTATION



ION EXCHANGE TECHNIQUES

Two techniques are generally used to bring the solution in contact with ion exchange resins. They are:

1. Batch method
2. Column method

1. Batch method: This method involves single step equilibrium. The resin and the solution are mixed in a vessel until equilibrium is attained. The solution which is obtained filtered. In single step equilibrium of this type, only a single portion of the exchange capacity of the resin is utilized. This method is used for softening of water and the production of deionised or demineralised water

2. Column method:

Ion exchange chromatography is technique in which separation of the components of a mixture is based upon the different selective coefficients for the resin which leads to

different migration rates on an ion exchange column. Chromatography process is classified according to physical state of mobile and stationary phases, whether they are gases, liquid and gases. These are

- 1) Frontal analysis
- 2) Elution analysis and
- 3) Displacement development

APPLICATIONS

Ion exchange chromatography can be applied for the separation and purification of many charged or ionisable molecules such as proteins, peptides, enzymes, nucleotides, DNA, antibiotics, vitamins and etc. from natural sources or synthetic origin. Some of its applications are as follows

1. Separation of similar ions: The ion exchange chromatography is used for separation of similar ions as different ions undergo exchange reactions to different extent. E.g.: A mixture of H^+ , Na^+ , and K^+ can be separated by using cation exchange resin. Similarly Cl^- , Br^- , I^- can be separated by passing through basic anion exchanger. The apparatus used in the column method consists of a glass column fitted with a glass wool plug or a sintered glass disc at the lower end. An ordinary burette can also be used. The resin used should have small particle size and the diameter of the resin should be less than one tenth of the column. The slurry is slowly poured into the column containing some water. The resin is allowed to settle and then excess water is drained off.

2. Softening of hard water:

Hardness of water is due to the presence of Ca^{2+} , Mg^{2+} and other divalent ions may be removed by passing the hard water through the cation exchanger charged with Na^+ ions. Then the following exchange reaction takes place:

The Ca^{2+} , Mg^{2+} ions from water are retained on the column while Na^+ ions pass into the solution. These Na^+ ions are harmless for washing purpose. After using the ion exchange for a time, it becomes inactive. Percolating through it a concentrated solution of $NaCl$ when the following reverse reaction takes place can revive its activity

3. Complete demineralization of water:

This requires complete removal of ions i.e., both cations and anions. For this, water is passed through an acidic cation exchanger then metallic cations are exchanged with H^+ ions. The water obtained is then passed through a basic anion exchanger then the anions present in the water are exchanged by OH^- of the exchanger. The H^+ and OH^- ions which pass into solution combine to form unionized water [5, 12, and 14]. E.g.: Cation exchanger – Sulphonic acid resin is commonly used Anion exchanger – Strong basic resin is used

4. Purification of organic compounds:

Many natural products extracted in water have been found to contain ions originally present in water. Those ions can be removed by using ion exchange process

5. Separation of sugars:

This method is developed by Khym and Zill in 1951. Sugars are first converted into borate complex and the separation of borate complexes have been achieved quantitatively on columns by using ion exchange chromatography. In this, disaccharide can be separated from monosaccharide's and the individual compounds of hexose, pentose from the mixture can be resolved

6. Separation of amino acids:

Ion exchange methods can be used to separate the complex mixture of 18 amino acids obtained by the acid hydrolysis of proteins. The mixture of amino acids is first introduced on a very short column at pH 2 and eluted with 0.35 N sodium citrate buffers at pH 5.25. Acidic and neutral amino acids at first leave the column unseparated and after that other amino acids are separated. Similarly, a mixture of vitamins like vitamin B1, B2, B6, Niacin, Folic acid, B12 etc. can be separated using ion exchange technique.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

It is called high-performance liquid chromatography or high-pressure liquid chromatography, HPLC is primarily based on the use of a column that contains packing material. (Stationary phase), a pump that drives the mobile phase(s) across the column, and a detector that displays the molecule retention durations. The retention time is governed by the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) utilized [21]. The sample is usually added to the mobile phase stream and is slowed by chemical or physical interactions with the stationary phase. Gradient elution changes the mobile phase composition during the analysis. The gradient separates analyte mixtures based on the analyte's affinity for the mobile phase. The nature of the stationary phase and the sample influence the choice of mobile phase, additives, and gradient.

There are many types of HPLC, we will focus on only Normal phase-HPLC, Reversed HPLC, size exclusion-HPLC. In normal phase-HPLC, A polar stationary phase and a non-polar mobile phase are used. The polar stationary phase interacted with and held the polar analyte.

Adsorption strengths raise as analyte polarity increases, and the interaction between the polar analyte and the polar stationary phase prolongs elution time or retention factor. On the other hand, Reversed-HPLC has a non-polar stationary phase and a moderately polar aqueous mobile phase. it works on the principle of hydrophobic interactions, which are caused by repulsive forces between a polar solvent, a comparatively non-polar analyte, and a non-polar stationary phase. besides, size exclusion-HPLC or gel permeation

chromatography can separate based on particle size, it determines the tertiary and quaternary structures of proteins and amino acids. Also, the molecular weight of polysaccharides.

Applications of High performance liquid chromatography

In Pharmaceutical applications

- 1-evaluate of pharmaceutical product shelflife.
- 2-Identify active constituents in dosage forms.
- 3- develop pharmaceutical quality control Environmental.

In Environmental applications

- 1- Identify diphenhydramine in deposited samples.
- 2- Pollutant biomonitoring.

In Clinical

- 1- Estimation of bilirubin and bilivirdin levels in blood plasma in the presence of hepatic diseases.
- 2- Detection of endogenous neuropeptides in brain extracellular fluids.

Analytical procedure

Standard preparation

Approximately 50 mg of cefazolin sodium was weighed as the reference standard in a 100 mL volumetric flask, dissolved by sonication, and made up to the required volume with mobile phase, giving 500 µg/mL of cefazolin sodium as the stock standard solution used in further processing. For a working standard solution, 10 mL of stock standard solution was diluted in a 100 mL flask with the same diluent. Further dilutions were made to obtain the concentrations required.

Sample preparation

To make a stock sample of cefazolin sodium 500 µg/mL, 10 vials were emptied and the contents were mixed to obtain an evenly homogenized 50 mg sample, which was added to a 100 mL volumetric flask along with 50 mL of mobile phase. The sample was sonicated for 10 minutes, placed on a stirrer for 10 minutes, and the same diluent was added up to the mark level of the flask. This was used as the stock sample solution, 10 mL of which was diluted further in 100 mL of diluent to prepare a working sample solution. The sample was then filtered through 0.45 µm filter paper and injected into the HPLC system.

Preparation of serum sample for in vitro study

Blood samples were taken from healthy volunteers who were aged 20–30 years, not taking any medications, and not participating in any strenuous activity. All volunteers were informed of the aims and risks of the study, and written informed consent was obtained. Our ethics committee approved the study protocol. Blood was collected into an evacuated glass tube through an indwelling cannula in a forearm vein by a clinical laboratory technician. The blood was shaken and centrifuged at 10,000 rpm for 10 minutes to separate out the plasma. Next, 90 mL acetonitrile was added to 10 mL of plasma, and the mixture was centrifuged at 10,000 rpm for 10 minutes to remove the protein. The supernatant serum obtained was filtered through Whatman 42 filter paper and stored at -20°C. To make a working sample, 5 mL of the stock sample was put into a 50 mL flask and diluted with 10 mL of mobile phase, followed by addition of 25 mL of serum. The sample obtained was stirred for 10 minutes and the same diluent was then added up to the mark level of the flask. All samples prepared were filtered through 0.45 µm filter paper and injected in triplicate into the HPLC system. A blank serum sample was made and run to establish the specificity of the method in biological fluid.

Preparation of sample for study of drug degradation

First, 5 mL samples of stock solution were added to four separate 50 mL volumetric flasks. Next, 15 mL of degrading agent was added to each flask individually, with the exception of one flask, to which diluent (mobile phase) only was added. The degrading agents included 0.1 N HCl, 0.1 N NaOH, and 10% H₂O₂, and the same diluent was added to each flask up to the mark level. In one experiment, all four samples were placed in a water bath at 60°C for one hour, and in another experiment, the samples were analyzed without heat. The samples were then filtered through 0.45 µm filter paper and injected into the HPLC system. Force degradation data are

Stability studies

For the stability studies, commercially available parenteral samples were stored in accelerated conditions of temperature, ie, 40°C with 75% relative humidity, and in ambient conditions of 30°C temperature with 65% relative humidity, in an environmental chamber for 6 months. The stability protocol

Validation of methods

International Conference on Harmonization guidelines were used for the method validation studies. Various parameters, including specificity, linearity, range, accuracy, robustness, and intraday and interday precision were evaluated. To study linearity, 20 dilutions were prepared from a stock standard solution with a drug content range of 10%–200%. A standard calibration curve was generated using regression analysis.

Precision was investigated by identifying any intraday and interday variations in the results of the testing method used for cefazolin sodium in the concentration range of 40–60 µg/mL. Precision was determined by analyzing a homogeneous bulk sample against a freshly prepared standard daily three times daily at 8-hour intervals for 3 consecutive days. To determine accuracy, the reference standard for cefazolin sodium was spiked at three different concentration levels into samples for injection and into serum. Samples were prepared in triplicate for each concentration and percent recovery was determined. The limits of detection and quantification for the method were established from a linearity curve with formula (limit of detection, $3 \sigma/S$, and limit of quantification, $10 \sigma/S$) where σ is the standard deviation and S is the slope. This empirical formula was used to obtain the limit of quantification as shown in the chromatogram.

To study the robustness of the method, injectable cefazolin sodium formulation samples were assayed with intentional variation in the method parameters, including chromatographic conditions, mobile phase, flow rate, and temperature. Suitability of the system was established by calculating the percent relative standard deviation of repeat injections and analyzing the symmetry of the peak and theoretical plates of the column.

Results and discussion

Development of an HPLC method and its validation are important issues for any drug entering the marketplace, and is a regulatory requirement for quality assurance. Cefazolin sodium is very vulnerable to hydrolysis, so stability studies have an important role in this context. However, very few methods are available for effective assessment of the stability of cefazolin sodium. Calorimetry gamma irradiation, and electrophoresis can be used to indicate stability, but are not widely used, especially at an industrial level. Chromatographic methods developed by Mayer and cannot assess stability. Lalitha et al developed an HPLC method for assessment of stability, but this cannot be used to quantify cefazolin sodium in physiological fluid. However, good results for recovery in studies involving human serum and a low limit of quantification make this method useful for pharmacokinetic studies and routine quality control purposes because it is simple to process, has a short run time, does not require complicated instrumentation, and uses inexpensive reagents.



CONCLUSION

To conclude, this internship experience with the ORCHID Pharmaceutical company has been an illuminating journey, offering first hand insights into the intricate workings of the industry. Navigating the intersection of theory and practice has not only enhanced my understanding of Pharmaceutical operation but has also cultivated a profound appreciation for the collaborative efforts driving innovations. As I transition from this internship, I carry forward a wealth of knowledge, practical skills and a new found passion for contributing to the dynamic landscape of Pharmaceutical advancement.

