



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE

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PHARMACEUTICAL CHEMISTRY INTERNSHIP REPORT



FROM

(NOVEMBER 29, 2023 to DECEMBER 19, 2023)

SUBMITTED BY-

VASEEKARAN. A. S

42910025

IInd - M.Sc. CHEMISTRY



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DEPARTMENT OF CHEMISTRY

This is to certify that this Internship Report is the bonafide work of **VASEEKARAN. A. S,**
Reg.No. 42910025 who carried out the internship entitled "**PHARMACEUTICAL**
CHEMISTRY INTERN" under our supervision from **OCTOBER TO NOVEMBER.**

Head of the Department

Dr. J. KARTHIKEYAN MSc., PhD

Submitted for Viva-voce Examination held on **06/01/2024.**

Internal Examiner

Dr. T. ANAND
DEPARTMENT OF
CHEMISTRY

External Examiner

Dr. P. PRIYADHARSHINI
INTERNATIONAL RESEARCH

December 19, 2023



CERTIFICATE

This is to certify that **Mr. VASEEKARAN A S, (Reg.No.42910025) 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 him satisfactory to the job assigned to him by our Organization.

We wish him success in his 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,

VASEEKARAN. A. S

About Orchid pharma

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. A multi-therapeutic presence across segments like anti-infectives, anti-inflammatory, central nervous system (CNS), cardio vascular segment (CVS), nutraceuticals and other oral and sterile products. Our pharmaceutical solutions include active pharmaceutical ingredients (API), finished dosage forms. We are globally present across about 50 pharmaceutical companies in India through alliances and partnerships with globally reputed majors.

Vision

Enriching Lives through Innovation in Healthcare

Mission

"Discovery to Delivery"

Orchid is a vertically integrated pharmaceutical company with established research, manufacturing and marketing capabilities across multi-therapeutic domains. We successfully leverage our penchant for science and technology to create niche products and manufacturing platforms leading to attractive growth.



Our 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 we are present. In the years to come, driven by a highly competent and motivated team, we will move from strength to strength in the key domains of API, and global generics.

On March 31, 2020, the resolution plan under CIRP has been implemented and the Board has been re-constituted with the members nominated by Dhanuka Laboratories Limited, Haryana, India, the resolution applicant.

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CEFAZOLIN PREPARATION

INTRODUCTION:

Cefazolin is a widely used antibiotic belonging to the class of medications known as first-generation cephalosporins. It's primarily utilized for the treatment of bacterial infections caused by susceptible organisms.

Cefazolin works by inhibiting the synthesis of bacterial cell walls. It achieves this by binding to and disrupting the formation of peptidoglycan, a key component of the bacterial cell wall. This interference weakens the cell wall structure, ultimately leading to the death of the susceptible bacteria.

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 μ m 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 μ m 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 μ g/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.



HIGH-PRESSURE LIQUID CHROMATOGRAPHY:

High-Performance Liquid Chromatography (HPLC) is an analytical technique used to separate, identify, and quantify individual components within a mixture. It operates on the principle of chromatography, a method used to separate different components based on their interaction with the stationary phase and mobile phase within a column.

Here's an in-depth explanation of the key components and process involved in HPLC:

Components of HPLC:

Mobile Phase: This refers to the solvent or mixture of solvents used to carry the sample through the chromatographic system. It is usually a combination of water and organic solvents like methanol or acetonitrile.

Stationary Phase: It is a crucial part of the separation process and is contained within the column. The stationary phase can be made of various materials like silica, polymers, or other chemically modified substances. The choice of stationary phase depends on the specific compounds being analyzed.

Column: The column is packed with the stationary phase material. It provides the separation based on the interactions between the sample components and the stationary phase.

Injector: This component is responsible for introducing the sample into the mobile phase stream. It ensures precise and consistent injection of the sample onto the column.

Detector: The detector monitors the separated components as they elute from the column. Various detectors are used in HPLC, including UV-Vis detectors, fluorescence detectors, mass spectrometers, and more. The detector generates signals that are used to create chromatograms, displaying the separation of components.

Pump: The pump delivers the mobile phase through the system at a constant flow rate and pressure, which is essential for the efficiency and reproducibility of the separation.

Principle of Operation:

Sample Injection: A small amount of the sample is injected into the mobile phase stream. The sample travels through the column containing the stationary phase.

Separation: As the mobile phase carries the sample through the column, each component interacts differently with the stationary phase based on factors like polarity, size, and chemical properties. This results in differential retention times within the column.

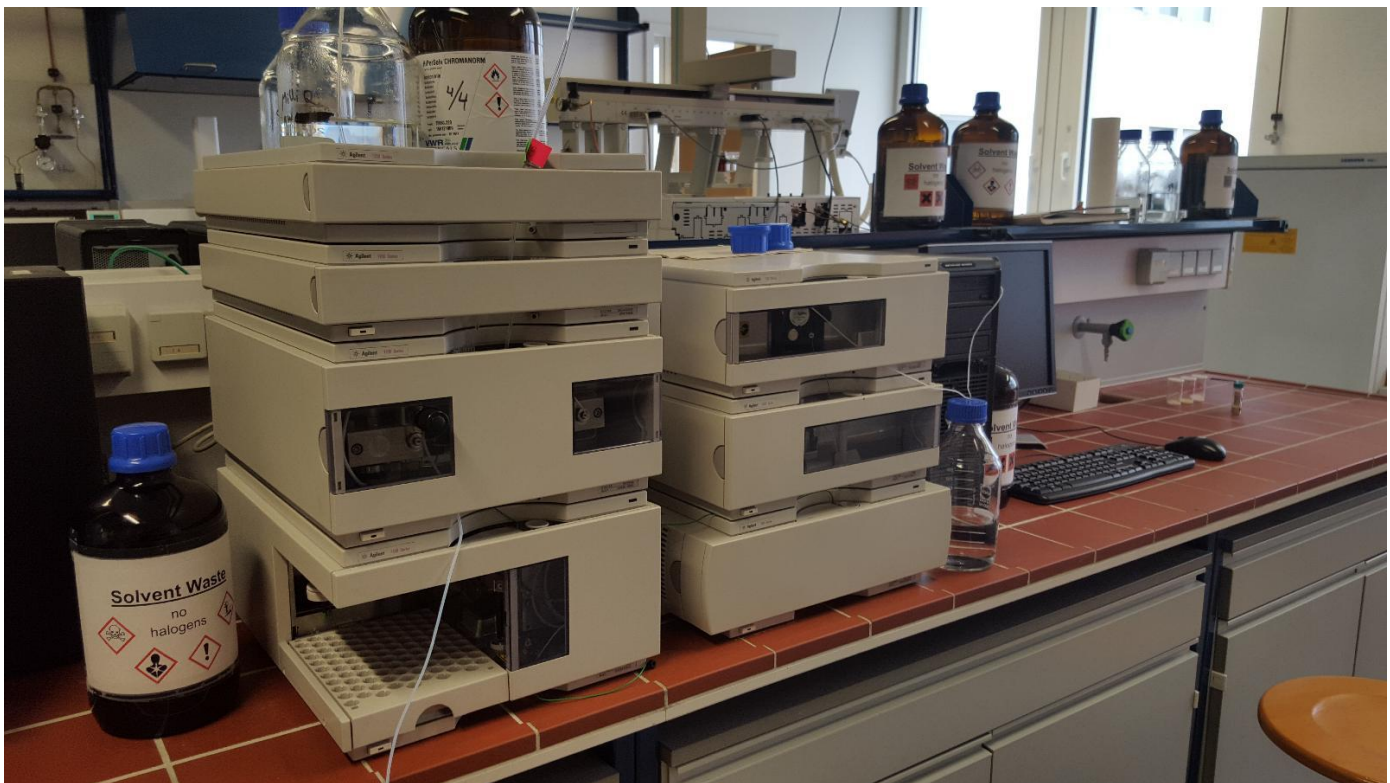
Detection: The separated components exit the column and pass through the detector. The detector records the signal generated as each component elutes from the column, producing peaks on a chromatogram.

Data Analysis: The chromatogram displays peaks corresponding to individual components. The area and height of the peaks are used to quantify the amount of each compound present in the sample.

Applications of HPLC:

- Pharmaceutical analysis (drug development, purity testing)
- Environmental analysis (pollutant detection)
- Food and beverage analysis (nutrient analysis, contaminants)
- Clinical diagnostics (detection of biomarkers)
- Chemical research and analysis

HPLC is a versatile and widely used analytical technique due to its high sensitivity, selectivity, and ability to separate a wide range of compounds in various industries and research fields.



GAS CHROMATOGRAPHY

Gas Chromatography (GC) is another powerful analytical technique used for separating, identifying, and quantifying volatile and semi-volatile compounds within a sample mixture. It operates on the principle of chromatography, similar to HPLC, but with a different mobile phase (gas instead of liquid) and specific components tailored for gas-based analysis.

Components of Gas Chromatography:

Injector: The injector introduces the sample into the GC system. The sample is vaporized and injected into the carrier gas stream.

Carrier Gas: Unlike HPLC's liquid mobile phase, GC uses an inert gas such as helium, nitrogen, or hydrogen as the carrier gas. This gas moves the sample through the column.

Column: The column is a long, coiled tube usually coated with a stationary phase, such as a liquid or solid material. The interaction between the sample components and the stationary phase in the column causes separation based on their different affinities.

Oven: The oven maintains a controlled temperature, crucial for the separation process. Different temperatures can impact the separation efficiency and selectivity.

Detector: GC employs various types of detectors, including Flame Ionization Detectors (FID), Thermal Conductivity Detectors (TCD), Electron Capture Detectors (ECD), and Mass Spectrometers (MS). These detectors measure the concentration of compounds eluting from the column and generate signals used to create chromatograms.

Data System: A data system processes the detector signals and generates chromatograms, which represent the separation of compounds over time.

Principle of Operation:

Sample Introduction: The sample is vaporized and introduced into the carrier gas stream using the injector.

Separation: The carrier gas carries the sample through the column. As the sample travels through the column, different compounds interact with the stationary phase, causing separation based on their affinities.

Detection: As separated compounds exit the column, they pass through the detector. The detector generates signals proportional to the concentration of each compound, producing peaks on the chromatogram.

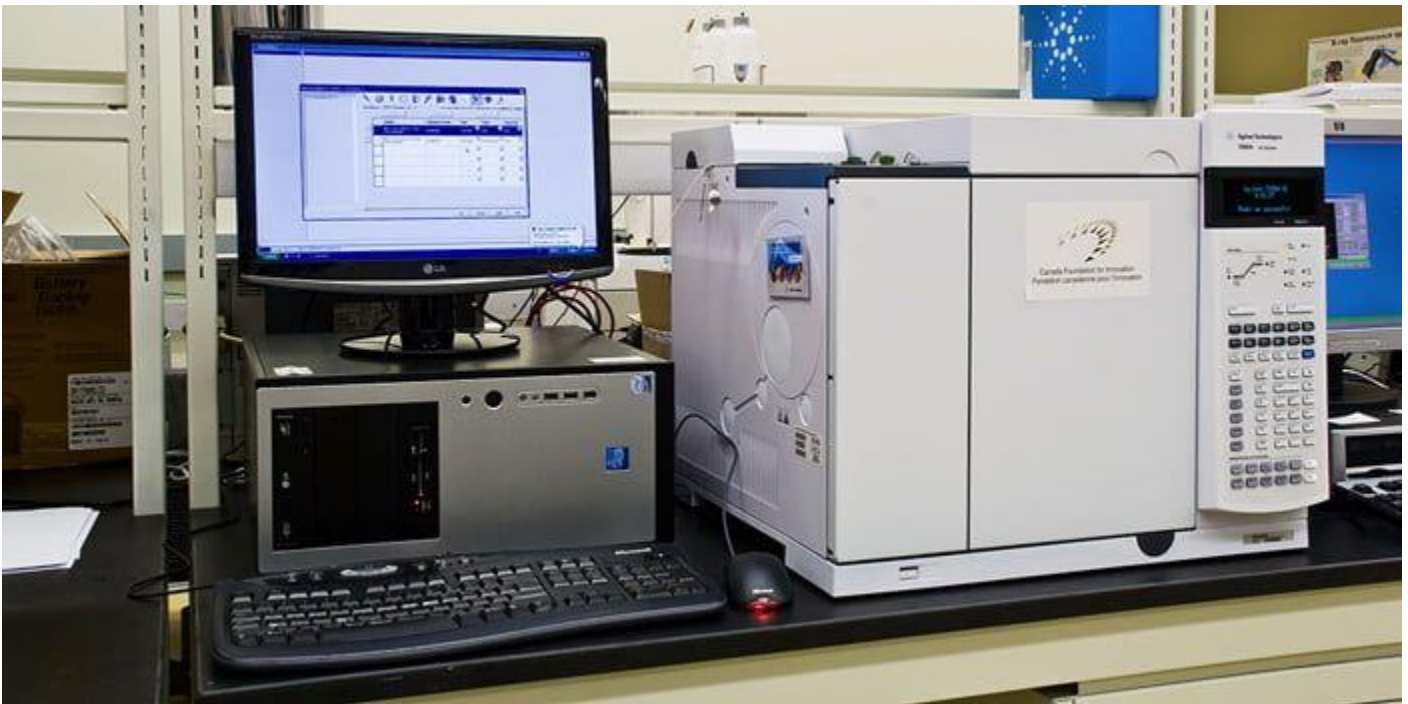
Data Analysis: The peaks on the chromatogram represent individual components. The area and height of these peaks are analysed to determine the identity and quantity of each compound in the sample.

Applications of Gas Chromatography:

- Environmental analysis (air, water, soil contaminants)
- Pharmaceutical analysis (drug purity, composition)
- Petrochemical industry (analysis of hydrocarbons)
- Forensic science (analysis of trace evidence)

- Food and beverage analysis (flavor and fragrance analysis)

Gas Chromatography is highly versatile, offering excellent separation efficiency and sensitivity, making it a valuable tool in various industries for analysing complex mixtures of volatile compounds. The choice between HPLC and GC depends on the nature of the sample and the specific compounds being analysed.



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.