



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

Internship Report

Submitted in partial fulfillment of the requirements for the award of
Master of Science in Chemistry.

Submitter By:

VIGNESH A,
MSc., Chemistry,
Reg# 42910027.

Department of Chemistry
Sathyabama Institute of Science and Technology,
Chennai – 119

Done at,
Kamgo Technologies Private Limited
Kottivakkam, Chennai.





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DEPARTMENT OF CHEMISTRY

This is to certify that this Internship Report is the bonafide work of **VIGNESH. A,**
Reg.No 42910027 who carried out the internship entitled "**Technical Aspects of Column Chromatography**" under our supervision from **05th OCTOBER TO 20th OCTOBER 2024.**

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 CENTRE



KAMGO TOWERS
KAMGO TECHNOLOGIES INDIA PVT. LTD.,
NO.141, NEHRU NAGAR, 9TH LINK STREET
KOTTIVAKKAM, CHENNAI - 600041
MOB: 9176625411
Kamgotech@gmail.com / Info.kamgotech@gmail.com
GSTIN: 33AAHCK5507E1ZU

Date: 02-05-2023

TO WHOMSOEVER IT MAY CONCERN

This is to certify that Vignesh. A has successfully completed Technical Aspects of Column Chromatography project in our organization from 5th Oct 2023 to 20th Oct 2023. He was highly motivated and hardworking. He worked sincerely at his tasks and did a very good job.

We wish him great success in his future endeavours.

For KAMGO TECHNOLOGIES INDIA PVT LTD

V. Gopinath
Authorised Signatory

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 **KAMGO TECHNOLOGIES INDIA PRIVATE LIMITED**. 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 heartfelt gratitude to all those who have contributed to the successful completion of my internship.

I am highly indebted to **Mr. GOPINATH. V (Managing Director)** for his guidance, constant supervision and kind concern, providing necessary information regarding the internship.

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I would like to express my deepest gratitude towards **Mr. RATHINAGIRI. A (DGM-QC, Apotex Pharmachem India Pvt Limited, Bangalore)** and my parents for providing me with moral support and concerned authorities at **Kamgo Technologies** and all the staffs for their kind co-operation, which helped me in the completion of internship.

VIGNESH A

16/10/2023

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Chapter 1

Company Profile

1.1 Introduction

Established in the year 2018 in Chennai, India.

Kamgo Technologies India Private Limited is a recognized market leader in providing comprehensive laboratory solutions to the analytical, life sciences, food, CRO industries in South India. They have operations in Chennai, Bangalore and Hyderabad. We represent world-leading analytical column brands, Consumables, instruments. They also partner with them to provide value added and cost-effective services to their esteemed clients.

They have a dedicated and knowledgeable sales team which understands the customers' needs and their pain points and supports them with their optimum and economical solutions. They have well equipped and adequately staffed offices to handle end-to-end business functions such as sales, services, logistics, distribution, warehousing, accounts, etc. With the help of their application lab in Chennai, they can immediately handle any kind of analytical column troubleshooting for their clients.

They work closely with laboratory testing team and clients to understand specific needs which enables us to design appropriate water treatment systems and distribution. High-purity solvents will give you the performance you need with minimal risk of contaminants, while maximizing the sensitivity and detecting power of instrumentation.

1.2 Company's Mission Statement

To become one of the solutions for all analytical needs of the science community, build long relationships with their clients with the help of quality service. To provide solutions to the new challenges in separation and purification faced in the industry.

1.3 Company's Vision

Kamgo Technologies India Private Limited has received sustainable growth and good recognition from customers in the recent years. We believe that in the forthcoming years KTPL becomes one of the leading techno commercial organizations with a strong emphasis on science for setting quality standards through analytical and separation science and providing best value products, services and solutions to their customers.

1.4 Safety and Environment Management

Kamgo Technologies is a very responsible company. It cares greatly about the environment as well as the satisfaction of its customers. For this reason, they have adopted almost all national and international quality and environmental safety policies.

1.5 Manufacturing Products

- Filtration Cups: Pre-sterilized, single-use devices for efficient microbial analysis in pharmaceutical API and formulation industries.
- Specimen Container: Container made up of polystyrene, cap crafted from high-density polyethylene, available in both sterile and non-sterile options, suitable for collection.
- Dispobags: Autoclave bags designed for waste disposal, ideal for disposing of pathological, clinical or contaminated materials.
- Spoon/Spatula: Available in both sterile and non-sterile options, featuring a flat surface and small scoop. Ideal for weighing dry powders.
- Type 1/DM Water

Ultra-Pure Water: High purity and UV transmission for column and detector protection, ensuring reproducibility.

HPLC Water: Ideal for reproducible separations due to high UV transmittance, low particle count, acidity, alkalinity, and evaporation residue.

DM Water: Suitable for various lab applications, commonly used in regulated and demanding settings.

1.6 Marketed Products

- Zodiac Life Sciences
- Ametek BrookField Viscometers
- Sartorius
- Shinwa Chemical Industries
- Cyber Lab Vials
- Sterinox System
- Kamgo-Lab Water

Chapter 2

Water Treatment Plant

2.1 Introduction

Every laboratory has need for Purified Water. Purified water has very range of uses in chemical, microbial and biochemical laboratories. From glassware washing to autoclave filling, it is essential to have good quality purified water.

2.1.1 Sterile Environment Requirements for Pharmaceutical Water

The pharmaceutical industry adheres to stringent standards for the purity and sterility of water used in the manufacturing and testing of pharmaceuticals products. Sterile water is essential for preventing microbial contamination, ensuring the safety and efficacy of drugs.

High purity solvents and reagents give us the performance we need minimizing the risk of contaminants that can limit accuracy while maximizing instrument sensitivity and detection power in key applications: UHPLC and LC/MS. It is ideal for cutting edge applications, such as proteomics, pharmacokinetics, clinical research and drug discovery.

2.1.2 Types of Pharmaceutical-Grade water

Pharmaceutical-grade water is classified into various categories based on its intended use and purity requirements.

- **LC-MS Grade water:** Water with exceptionally low levels of impurities, suitable for high-performance liquid chromatography (HPLC and mass spectrometry (MS) applications.
- **HPLC-Grade water:** Water with minimal impurities that can interfere with HPLC analysis.
- **ICP-MS Grade water:** Water with extremely low levels of trace metals, essential for inductively coupled plasma mass spectrometry (ICP-MS) applications.
- **IC-Grade water:** Water with minimal ionic contaminants, suitable for ion chromatography (IC) analysis.
- **Pyrogen-free water:** Water free of pyrogens, endotoxins derived from gram-negative bacteria, which can cause adverse immune reactions.
- **Reagent water:** Water with low levels of impurities suitable for general laboratory use.

2.1.3 Components of an SS316L Water Treatment Plant

- Water inlet: The entry point for raw water from the source.
- Softener: Removes hardness-causing ions (calcium and magnesium) from the water.
- MGF (Multi-Gradient Filter): Removes suspended solids, particulate matter, and organic contaminants.
- Ultrafiltration skid: Employs a semipermeable membrane to remove dissolved impurities, including bacteria and viruses.
- UF permeates storage tank: Holds the purified water from the ultrafiltration process.
- PW generation system: Generates pure water for pharmaceutical applications.
- Control panel: Monitors and controls the operation of the water treatment system.
- Electric power supply: Provides electricity to operate the various components of the system.
- Multipurpose tank: Stores water for various purposes, such as system cleaning and maintenance.
- UV and mixed bed skid: Combines UV disinfection with mixed bed resin polishing to remove residual impurities and ensure sterility.
- Post ultrafiltration tank: Stores the final purified water before distribution to various points of use.
- UV feed tank: Supplies the UV disinfection system with purified water.

Principle for UV sterilization

All forms of life known on the earth exist based on DNA and RNA for reproduction and existence. DNA and RNA are both composed of 4 kinds of chemical substance units.

A--Adenine

T--Thymine

C--Cytosine

G--Guanine (the basic component for the nucleic acid)

In time of cell reproduction, the long chains inside.

DNA are opened when Unit A in each chain will look for and connect with Unit T. Each long chain can reproduce an identical chain to the chain freshly split, restore the complete DNA before splitting and serve as the foundation for the new-borne cells. However, ultraviolet at a wavelength of 240-270nm can break the capacity of DNA to produced protein and reproduction. Once the DNA and RNA of bacteria and viruses are damaged, their capacity to produce protein and reproduction are both lost. As the bacteria and viruses usually have a very short life cycle.



The effective of UV sterilization is often expressed in killing ratio, e.g., 99.9%. Namely, 99.9% of the original bacteria have been killed and only 0.1% exists, or, 1/1 000 has survived. As 1000 is the 3 in 103 or in log, it is habitually to express the killing ratio with 3log. The killing ratio of ultraviolet depends on two factors: 1, ultraviolet intensity (I); and 2, irradiation time (T).

2.2 Experimental Methodology

For sophisticated laboratory applications, a combination of methods is usually utilized, beginning with some pre-treatment such as water softening followed by reverse osmosis filtration, UV treatment and deionization for higher grades.

2.2.1 Description of the SS316L Water Treatment Plant

Stainless Steel UV Lamp Sterilizer Water Treatment System

UV sterilizers are used both inside and outside the main stainless steel, polished body to strengthen internal and external UV illumination of high purity quartz assembled, with special ultraviolet lamp treatment, with a strong bactericidal, long life, stable and reliable, the bactericidal effect Rate $\geq 99\%$, life 8000 hours, has been widely used in medicine, food, beverage, life, electronics and other fields.



The SS316L water treatment plant utilized in this study consisted of the components mentioned above. The plant was designed and operated in accordance with Good Manufacturing Practices (GMP) to ensure the production of high-quality pharmaceutical-grade water.

2.2.2 Water Quality Monitoring Procedures

Regular monitoring of various water quality parameters was conducted to ensure compliance with pharmaceutical standards. Parameters such as conductivity, total organic carbon (TOC), endotoxin levels, and microbial contamination were monitored using appropriate analytical methods.

2.2.3 Analytical Methods for Water Quality Assessment

Water samples were collected at various stages of the treatment process and analyzed using standard analytical techniques. Conductivity was measured using a conductivity meter, TOC was determined using a TOC analyzer, endotoxin levels were assessed using the Limulus Amebocyte Lysate (LAL) test, and microbial contamination was evaluated using plate counting methods.

2.3 Results

2.3.1 Evaluation of Water Quality Parameters

The monitoring results consistently demonstrated that the water produced by the SS316L water treatment plant met all pharmaceutical-grade water specifications. Conductivity, TOC, and endotoxin levels were within acceptable limits, and microbial contamination was undetectable.

2.3.2 Assessment of Microbial Control

The UV disinfection system effectively eliminated microorganisms, ensuring sterile water production. The absence of detectable microbial contamination in the final product confirmed the efficacy of the treatment process.

2.3.3 Monitoring of Pyrogen Levels

The SS316L water treatment plant effectively removed endotoxins, ensuring pyrogen-free water production. The LAL test results consistently showed no detectable endotoxin levels in the final product.

2.4. Conclusion

The SS316L water treatment plant effectively produced high-quality pharmaceutical-grade water, meeting all relevant standards for LC-MS grade water, HPLC-grade water, ICP-MS grade water, IC-grade water, pyrogen-free water, and reagent water. The plant's consistent performance in maintaining water quality parameters and eliminating microbial contamination demonstrates its suitability for pharmaceutical applications.

Chapter 3

HPLC Columns

3.1 Introduction

3.1.1 Internship Objectives

- My internship aims to:
- Gain a comprehensive understanding of HPLC columns and phase interactions: This includes learning about different types of HPLC columns, their functionalities, and how they interact with various compounds.
- Develop expertise in protocols and managing solvent waste systems: This involves understanding the components of a solvent waste system, implementing safety protocols, and adhering to environmental regulations for solvent waste disposal.
- Refine customer interaction and sales techniques: This focuses on building rapport with clients, effectively presenting products and services, addressing objections, and closing deals.
- Gain practical experience in sales and customer service: This includes applying theoretical knowledge to real-world scenarios by visiting pharmaceutical companies and pitching them HPLC columns, water purification systems, and related services.
- Develop professional communication and interpersonal skills: This involves interacting effectively with colleagues, clients, and other stakeholders in a professional setting.

3.1.2 Internship Overview

This internship provided me a valuable opportunity to learn from industry professionals, gain hands-on experience with real-world applications of HPLC technology, and develop essential skills in sales, customer service, and professional communication. The internship involved a combination of theoretical learning, practical training, and real-world exposure through client visits and project assignments.

3.2 HPLC Columns and Phase Interactions

3.2.1 Types of HPLC Columns

HPLC stands for High-Performance Liquid Chromatography, a versatile and powerful technique used to separate and identify compounds in a mixture. Imagine it as a microscopic maze where different molecules travel at different speeds based on their size and interactions with the maze walls.

1. **Sample Injection:** A small sample of the mixture is injected into the HPLC system.
2. **Mobile Phase:** A liquid solvent, called the mobile phase, flows through a narrow column packed with tiny, porous beads called the stationary phase.
3. **Separation:** Different molecules in the sample interact with the stationary phase at different strengths. Some molecules stick more tightly to the stationary phase, slowing them down, while others pass through more quickly.
4. **Detection:** As the molecules emerge from the column, they pass through a detector that measures their presence.
5. **Signal Generation:** The detector generates a signal, typically a peak on a chart, corresponding to each molecule as it emerges from the column. The height and position of each peak provide information about the identity and concentration of the molecule.
6. **Data Analysis:** The peaks are analyzed to identify the compounds present in the sample and their relative amounts. This information can be used to understand the composition of the sample, diagnose medical conditions, or monitor environmental pollutants.

HPLC columns are the heart of HPLC analysis, separating compounds based on their interactions with the stationary phase. Common types of HPLC columns include:

- **Reverse-phase (RP) columns:** The most widely used type, employing a hydrophobic stationary phase and a hydrophilic mobile phase.
- **Normal-phase (NP) columns:** Utilize a hydrophilic stationary phase and a hydrophobic mobile phase, suitable for separating polar compounds.
- **Ion-exchange (IEX) columns:** Separate compounds based on their ionic charge, employing charged stationary phases.
- **Size-exclusion (SEC) columns:** Separate macromolecules based on their size, using a stationary phase with pores of varying sizes.

Also, there are different types of columns used by pharma and industries which has their own selectivity, some of them are,

Perp Columns: Preparative Chromatography is an important stage in liquid chromatography employed for scale ups, isolation of Proteins, Peptides or Impurities and Purification of samples in pharma and life science industries.

Carb Columns: Analysis of Carbohydrates, Sugar and Sugar alcohols in different formulations.

Ghost Buster Columns: Contaminants from a variety of sources such as mobile phase solvents, mobile phase containers, flow liners, pumps, filters, gradients mixers etc. cause unknown peaks called Ghost Peaks that appear in chromatogram interfering with the target peaks making the gradient analysis unreliable.

This causes resolution of ghost peaks difficult.

Selectivity of the columns can be understandable by USP Classification given below.

[Source: Zodiaclifesciences]

Series	USP Classification	Particle size	Pore Size (Å)	Carbon Loading %	End - Cap	pH Range
ZODIAC C18	L1	3,5,10	100	24	Yes	1.5-10
ZODIAC C18 (1)	L1	3,5,10	190	12.9	Yes	1.5-10
ZODIAC C18(AQ)	L1	3,5,10	120	17	Yes	1.5-10
ZODIAC C8	L7	3,5,10	100	15	Yes	1.5-10
ZODIAC C8(1)	L7	3,5,10	190	7.4	Yes	1.5-10
ZODIAC C8(AQ)	L7	3,5,10	120	10	Yes	1.5-10
ZODIAC Phenyl	L11	3,5,10	100	14	Yes	1.5-8
ZODIAC Phenyl (1)	L11	3,5,10	190	7.4	Yes	1.5-8
ZODIAC PFP	L43	3,5,10	190	15	Yes	2-8
ZODIAC Amino	L8	3,5,10	100	10	Yes	2-7
ZODIAC Amino (1)	L8	3,5,10	190	8	Yes	2-7
ZODIAC RP Amide	L60	3,5,10	120	15	Yes	2-8
ZODIAC Cyano	L10	3,5,10	100	15	Yes	2-7.5
ZODIAC (1) Cyano	L10	3,5,10	190	12	Yes	2-7.5
ZODIAC Silica	L3	3,5,10	100	0	No	2-6
ZODIAC Silica (1)	L3	3,5,10	190	0	No	2-6

3.2.2 Understanding Phase Interactions in HPLC

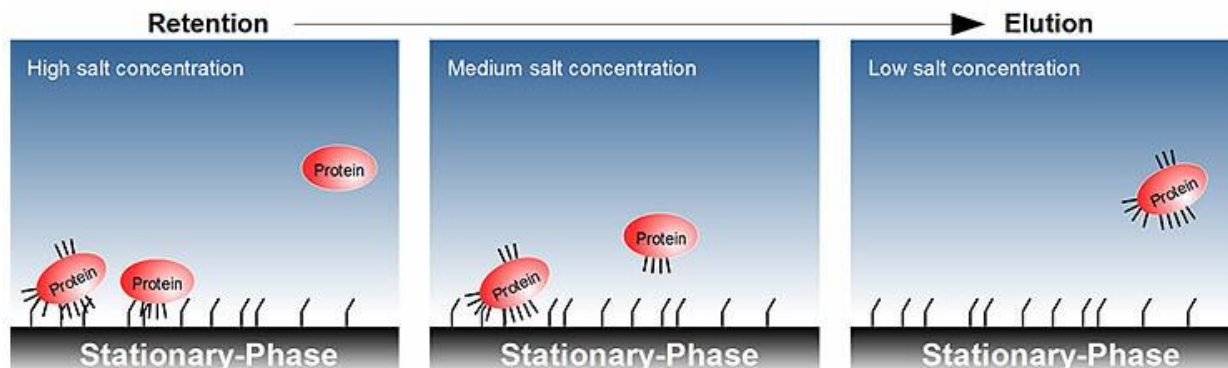
Phase interactions are the driving force behind separation in HPLC, influencing how long a compound stays in the column (retention) and when it elutes. Understanding these interactions is essential for optimizing separation conditions and achieving accurate results.

Effects of Phase Interactions:

- **Selectivity:** Different compounds interact with the stationary phase and mobile phase to varying degrees. This leads to the separation of different compounds based on their specific interactions, allowing for the identification and quantification of individual components in a mixture.
- **Resolution:** The ability to distinguish between closely related compounds depends on the difference in their retention times. Optimizing phase interactions ensures sufficient resolution for accurate analysis.

- **Peak shape:** The shape of a peak in the chromatogram can provide information about the purity of the compound and the efficiency of the separation. Tailing peaks indicate poor resolution or excessive interaction with the stationary phase, requiring adjustments to phase interactions.

Hydrophobic Phase Interaction:



Optimizing phase interactions is crucial for:

- Developing efficient and reproducible HPLC methods.
- Achieving accurate separation and quantification of target compounds.
- Troubleshooting and resolving issues related to separation performance.

3.2.3 Applications of Different HPLC Columns

By understanding the properties of our target compounds and the desired separation, we can choose the right HPLC column for the job. The right choice ensures efficient and accurate results.

- **RP (Reverse Phase) Columns:** These are like the Swiss Army knife of HPLC columns. They're versatile and work well for a wide variety of compounds, making them a good starting point.
- **NP (Normal Phase) Columns:** Think of these as the specialists for polar compounds. They excel at separating compounds that are attracted to water, like sugars and alcohols.
- **IEX (Ion Exchange) Columns:** Imagine magnets attracting charged particles. These columns use charged surfaces to separate compounds based on their electrical charge, perfect for analyzing proteins and other biological molecules.

- SEC (Size Exclusion) Columns: Think of these as sieves with different hole sizes. They separate macromolecules like proteins and polymers based on their size, allowing you to analyze their molecular weight distribution.

3.3 Customer Interactions and Sales Techniques

3.3.1 Building Rapport and Understanding Customer Needs

Effective customer interactions involve:

- Active listening: Understand customer needs and challenges.
- Empathy: Demonstrate understanding and willingness to help.

3.3.2 Effective Presentation of Products and Services

Tailor product presentations to customer needs:

- Highlight relevant features and benefits.
- Provide clear and concise information.

3.3.3 Handling Objections and Closing the Sale

Address customer objections professionally:

- Listen attentively and identify the root cause of the objection.
- Provide clear and persuasive responses to address the objection.
- Propose solutions that align with customer needs.

3.4 Sales Visits to Pharmaceutical Companies

3.4.1 M&M Pharma: Sales of Water and Columns

- Highlight product quality and compliance with pharmaceutical standards.
- Emphasize expertise in HPLC columns and water purification systems.
- Demonstrate understanding of M&M Pharma's specific needs and requirements.

3.4.2 Axxelent Pharma: Sales of Water

- Focus on the purity and consistency of the water purification system.
- Emphasize the ability to meet Axxelent Pharma's specific water quality requirements.
- Highlight cost-effectiveness and long-term value.

3.4.3 Madras Pharma: Sales of Columns

- Demonstrate expertise in selecting the right HPLC columns for Madras Pharma's applications.
- Provide technical support and guidance in column selection and usage.
- Offer competitive pricing and attractive sales terms.

3.5 Conclusion

3.5.1 Key Learning from the Internship

This internship provided a valuable opportunity to learn and gain practical experience in various aspects of HPLC technology, sales, and customer service.

My internship at Kamgo Technologies equipped me with a solid foundation in the field. I gained valuable knowledge of HPLC columns, phase interactions, and solvent waste management practices. This theoretical understanding was complemented by practical skills in HPLC analysis and data interpretation, allowing me to confidently navigate the basic principles of this technology.

Beyond technical knowledge, the internship significantly enriched my professional development. I honed my customer interaction and sales techniques, enabling me to effectively engage with potential clients and achieve success through real-world sales presentations. These experiences fostered strong communication and interpersonal skills, essential for navigating professional relationships.

On a personal level, the internship nurtured my confidence and independence, empowering me to tackle challenges with initiative and autonomy. Collaborative efforts with colleagues significantly improved my teamwork, critical thinking, and problem-solving abilities. Additionally, I developed effective time management and organizational skills, allowing me to prioritize tasks and optimize my workflow.

Thank you.