CHAPTER 1 INTRODUCTION

1.1 Research Background

Fat soluble vitamins, including vitamins A, D, and E, are essential micronutrients that play critical roles in maintaining human health. Vitamin A supports vision, immune function, and cellular growth; Vitamin D regulates calcium metabolism and bone health; while Vitamin E acts as a potent antioxidant, protecting cells from oxidative damage. Unlike water soluble vitamins, these compounds are lipophilic, stored in adipose tissue and the liver, and require specialized analytical methods due to their low solubility in aqueous solutions and susceptibility to degradation. Accurate quantification of fat-soluble vitamins is therefore vital for ensuring nutritional adequacy, pharmaceutical quality, and food safety (Zhang et al., 2022).

In Indonesia, the demand for fat soluble vitamins has grown significantly in recent years, driven by the expansion of fortified foods, dietary supplements, and pharmaceutical products. The fortified food and beverage industry including dairy products, cereals, and nutritional drinks has increasingly incorporated vitamins A, D, and E to meet consumer expectations for functional nutrition. Rising awareness of health and wellness, combined with regulatory encouragement from the Indonesian National Agency of Drug and Food Control (BPOM), has created a dynamic market where laboratories are under pressure to deliver rapid, reliable, and cost-efficient testing of vitamin content. This trend highlights the need for analytical methods that not only meet scientific standards but also align with business efficiency and regulatory compliance (Supriyono et al., 2020).

Figure 1.1 vitamins A, D, and E are obtained from diverse food sources and contribute to vision, bone health, and antioxidant protection (Infographic Website, 2024).

High Performance Liquid Chromatography (HPLC) remains the gold standard for vitamin analysis because of its sensitivity, reproducibility, and ability to separate complex mixtures. In

HPLC, column characteristics such as length, internal diameter, and particle size directly influence analytical performance. Among these, particle size is a critical determinant of resolution, speed, and solvent consumption. Traditionally, laboratories have relied on 5 µm columns, which provide reliable separation but often require longer run times and higher solvent volumes. Advances in column technology have introduced 3.5 µm alternatives, which promise improved resolution, faster analysis, and reduced solvent usage. These improvements have direct implications for laboratory operations: higher throughput, lower chemical costs, and enhanced competitiveness in a growing market (Kumar et al., 2023).

Figure 1.2 Waters ACQUITY Arc UHPLC system and XBridge column used in this study (adapted from Waters Corporation, 2024).

In laboratory operations, cost efficiency has become increasingly critical due to rising expenses in solvents, consumables, instrument maintenance, and regulatory compliance. One effective strategy to reduce operational costs without compromising analytical quality is through column transfer specifically, upgrading from standard 5 µm HPLC columns to more efficient 3.5 µm columns. The smaller particle size in 3.5 µm columns enhances chromatographic resolution, allowing for better separation of closely eluting peaks and reducing the need for repeat injections or method adjustments (Kumar et al., 2023; Agilent Technologies, 2023). These columns also deliver faster run times, enabling laboratories to process more samples per day, thereby increasing throughput. In addition, 3.5 µm columns typically consume less mobile phase per run, which lowers monthly chemical costs and reduces waste disposal fees (Ispiryan et al., 2024).

These technical improvements translate directly into business benefits. Reduced solvent usage and fewer repeat analyses contribute to significant cost savings. Faster analysis cycles improve

time efficiency, allowing analysts to dedicate more resources to other tasks or clients. Shorter runs also minimize instrument wear, extending column life and reducing downtime and maintenance expenses. Importantly, if the method transfer is validated under USP <621> Chromatography (USP, 2023), ICH Q2(R2) Validation of Analytical Procedures (ICH, 2022), and BPOM's 2021 guideline on analytical method validation, laboratories can adopt 3.5 µm columns without triggering costly revalidation or compliance delays.

1.2 Problem Statement

Although High Performance Liquid Chromatography (HPLC) is widely recognized as the gold standard for fat soluble vitamin analysis, most laboratories in Indonesia continue to rely on conventional 5 µm columns. These columns provide reliable separation but are associated with longer run times, higher solvent consumption, and increased operational costs. Advances in column technology have introduced 3.5 µm alternatives that promise improved resolution, faster analysis, and reduced solvent usage. However, despite these advantages, the adoption of 3.5 µm columns remains limited, largely due to concerns about regulatory compliance, investment costs, and the lack of evidence based studies linking technical improvements to business outcomes (Kumar et al., 2023).

The Indonesian pharmaceutical and food testing industries face additional challenges that exacerbate cost pressures. High dependency on imported raw materials around 90% of active pharmaceutical ingredients (APIs) are sourced from abroad, mainly China and India raises production costs and exposes laboratories to currency fluctuations (Jakarta Globe, 2024; Healthcare Asia Daily, 2025). Regulatory requirements from BPOM demand strict validation and compliance, which can slow down the adoption of new analytical technologies. Infrastructure limitations and unequal distribution of advanced equipment across regions create inconsistency in testing capacity, while competitive market pressures require laboratories to balance scientific accuracy with cost efficiency (Supriyono et al., 2020). With evidence indicating that operational costs must be reduced by at least 20–30**%** to remain sustainable (Statista, 2025). As a result, laboratories are caught between the need to maintain analytical precision and the necessity to reduce costs in order to remain sustainable.

This will highlight the gap in this situation while the technical benefits of smaller particle size columns are well documented, there is insufficient evidence connecting with these improvements to measurable business advantages such as cost savings, throughput, and return on investment in the Indonesian context. Without such evidence, laboratories may hesitate to

adopt 3.5 µm columns, missing opportunities to enhance efficiency and competitiveness. Therefore, this study seeks to bridge the gap by evaluating both the analytical and economic impacts of column transfer, providing laboratories with evidence-based recommendations that align with regulatory requirements and industry needs.

Waters UHPLC system equipped with a UV detector as the analytical platform were employed in this research. Although UHPLC is typically associated with sub-2 µm particles, in this research it is used to evaluate conventional 5 µm and newer 3.5 µm columns for fat soluble vitamin analysis.

In Indonesia, where many laboratories operate under tight budgets and face increasing sample volumes, adopting 3.5 µm columns offers a strategic advantage. It enables laboratories to meet growing market demands for vitamin testing while maintaining profitability and regulatory integrity. However, the Indonesian pharmaceutical industry also faces several challenges. High dependency on imported raw materials increases production costs and exposes laboratories to currency fluctuations. Regulatory requirements from BPOM demand strict compliance, which can slow down the adoption of new technologies if not properly validated. Limited infrastructure and uneven distribution of advanced analytical equipment across regions create disparities in testing capacity. Furthermore, competitive market pressures require laboratories to balance scientific accuracy with cost efficiency to remain sustainable(Universitas Airlangga, 2022).

With addressing these challenges, the transfer from 5 µm to 3.5 µm HPLC columns not only improves analytical performance but also strengthens the financial toughness of laboratories in Indonesia's pharmaceutical and food testing sectors.

1.3 Research Questions

1. As the result from the analytical performance, how do 3.5 µm HPLC columns compare with 5 µm columns in separating vitamins A, D, and E in terms of resolution, retention time, and solvent use?
2. From a business efficiency perspective, to what extent does switching to 3.5 µm columns lower operational costs and increase sample throughput? What is the expected return on investment (ROI), payback period, and from a financial management perspective, what is the Net Present Value (NPV) for laboratories adopting 3.5 µm columns?

3. Based on the regulatory compliance is it validated methods can be transferred from 5 µm to 3.5 µm columns without full revalidation under USP <621>, ICH Q2(R1), and BPOM? What regulatory steps must Indonesian laboratories take to ensure compliance during column transfer?
4. For industry challenges and adoption, What are the main barriers (cost, infrastructure, regulation) to adopting 3.5 µm columns in Indonesia? How do laboratory stakeholders (analysts, supervisors, managers) perceive the risks and benefits of adopting 3.5 µm columns?

1.4 Research Objectives

To compare the analytical and economic performance of 3.5 µm HPLC columns against conventional 5 µm columns in the analysis of fat-soluble vitamins (A, D, E), and to evaluate the regulatory feasibility of adopting newer column technologies in Indonesian laboratories.

Specific Objectives:

1. Analytical Dimension
   - Assess improvements in resolution, retention time, reproducibility, and solvent consumption when using 3.5 µm columns.
   - Evaluate the suitability of 3.5 µm columns for routine vitamin quantification in food and pharmaceutical samples.
2. Business Dimension
   - Measure the impact of column choice on throughput, solvent usage, and cost per analysis.
   - Calculate ROI and payback period for laboratories adopting 3.5 µm columns.
   - Evaluate the Net Present Value (NPV) of adopting 3.5 µm columns compared to 5 µm columns, ensuring that financial feasibility is assessed.
3. Regulatory Dimension
   - Determine whether method transfer can be achieved without full revalidation under USP <621>, ICH Q2(R1), and BPOM.
   - Ensure compliance with L/dp limitations to safeguard method validity.
4. Strategic Dimension
   - Provide evidence based recommendations for laboratories and distributors on column technology selection.

- Support sustainable laboratory management strategies in Indonesia's pharmaceutical and food testing sectors.

1.5 Scope and Limitation

1.5.1 Scope of the Study

This research evaluates the comparative performance of 3.5 µm and 5 µm HPLC columns in analysing fat-soluble vitamins A, D, and E. The study is situated within Indonesian pharmaceutical and food testing laboratories, emphasizing cost efficiency, regulatory compliance, and analytical accuracy. The scope includes:

1. Analytical evaluation leads to Resolution, retention time, reproducibility, and solvent consumption.
2. Economic assessment factor that will have Throughput, cost per analysis, solvent usage, ROI, payback period and Net Present Value (NPV).
3. From the Regulatory Review that make method transfer feasibility under USP <621>, ICH Q2(R1), and BPOM, including L/dp limitations.
4. For Strategic Insight in the upcoming time proving practical implications for laboratory stakeholders in balancing performance and cost.

1.5.2 Limitations of the Study

Despite its comprehensive scope, several limitations must be acknowledged

1. Technology scope that restricted to use HPLC method and the use of UHPLC as HPLC system ; excludes UPLC and other advanced techniques.
2. Analyte focus that limited only to fat-soluble vitamins A, D, and E; excludes water soluble vitamins and other compounds.
3. Laboratory conditions using UHPLC Instrument both in I3L Lab and ITB lab as a research area for doing the analysis and conducted under controlled settings, may not fully reflect variability across institutions or regions.
4. Financial estimation that deliver ROI and payback based on standard cost models; actual figures may vary with procurement and market conditions.
5. Regulatory interpretation that based on literature and expert review; does not involve direct regulatory submission.
6. Stakeholder input was obtained through unstructured observation in workshops; no full scale survey or qualitative interview study is conducted.

7. Baseline comparison uses prior 5 µm columns and existing HPLC/UHPLC instruments, giving a realistic foundation but limiting generalization to labs without such systems.
8. The study does not include market research or industry wide adoption analysis; results are confined to laboratory experiments and modeled financial outcomes..