Masters Thesis Information Showcase
Fall Semester-2026
Year 2026

Department: Chemistry
Programme: **Industrial Chemistry**
Specialization: **Industrial Chemistry**

1	**Title of the Thesis** Analytical Quality by Design (aQbD) Assisted Development and Validation of a Robust RP-HPLC Method for the Quantification of Resmetirom
2	**Abstract** (min 300 to 500 words) The global burden of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) and Metabolic Dysfunction-Associated Steatohepatitis (MASH) has historically grown without the support of targeted interventions. Resmetirom's recent approval as a novel selective thyroid hormone receptor-beta (TR-β) agonist marks a pivotal regulatory advancement. Consequently, this breakthrough has created an immediate industry-wide demand for the development of stringent, compliant, and dependable quality control protocols. Conventional method development in chromatography tends to rely extensively on empirical "One-Factor-at-a-Time" (OFAT) approaches, which frequently result in fragile operational setups that are highly sensitive to slight variations in environmental or instrumental conditions. This study addresses those specific gaps by developing a structured validation framework for a novel RP-HPLC technique. Aimed at the precise measurement of Resmetirom, the method was developed entirely within modern Analytical Quality by Design (aQbD) parameters to ensure long term reliability. A prospective Analytical Target Profile (ATP) was established based on the drug's lipophilic and electronic characteristics (log P $\approx$ 3.5; pKa = 3.82), identifying peak symmetry (tailing factor, Tf, column performance (theoretical plates, N), and elution dynamics (retention time, Rt as the critical quality attributes (CQAs). An initial qualitative hazard assessment was constructed using an Ishikawa diagram, which methodically categorized multi-dimensional operational risks into six primary areas. High-risk factors were then ranked through Failure Mode and Effects Analysis (FMEA) and Pareto analysis, pinpointing the critical method parameters (CMPs) needing multivariate optimization: mobile phase composition (ratio of organic modifier), pH of the aqueous phase, and volumetric flow rate. To model the multivariate design space, a 15-run Box-Behnken experimental design combined with Response Surface Methodology (RSM) is utilized. Second-order quadratic polynomial models were constructed for each monitored CQA and verified using Analysis of Variance (ANOVA), demonstrating excellent statistical fit ($R^2 > 0.99$) and negligible lack-of-fit values. Numerical optimization using desirability functions identified the ideal robust operational coordinates: an isocratic mobile phase consisting of Acetonitrile and an acidic aqueous buffer solution, maintained at a standardized flow rate through a stationary phase C18 column with UV spectrophotometric detection executed at λmax = 210. Triplicate experimental verification runs confirmed the predictive accuracy of the polynomial equations, with negligible deviation from the modeled response surface. To ensure compliance, the optimized method

	underwent formal validation according to ICH Q2(R2) standards. Over a concentration range spanning 10.00-30.00µg/mL}, the assay exhibited strong linearity; this is supported by the regression equation y = 38,791x + 125.4 and an exceptional R2 value of 0.9997. The method demonstrated high sensitivity, establishing highly restricted limits of detection (LOD) and quantification (LOQ) based on strict Signal-to-Noise (S/N) thresholds. System precision and accuracy criteria were easily satisfied, yielding intra- and inter-day %RSD consistently ≤ 1.0% and baseline recovery metrics spanning 98.0-102.0%. Deliberate, minor perturbations to critical variables during robustness testing produced no statistically significant shifts in system suitability parameters, confirming the extreme reliability of the defined design space. In conclusion, this research successfully bridges advanced statistical optimization with stringent regulatory compliance. By transitioning from historical empirical workflows to an asset-lifecycle aQbD strategy, this study provides a highly reproducible, robust, and validated analytical platform perfectly suited for routine pharmaceutical quality control, high-throughput manufacturing assays, and forward-looking ultra-high-performance liquid chromatography (UHPLC) transfer protocols for Resmetirom.
3	**Impact on SDG# 3, 9 & 12** (min 500 words) The implementation of an Analytical Quality by Design (aQbD) framework for the quantification of Resmetirom directly aligns with and drives several United Nations Sustainable Development Goals (SDGs), primarily SDG 3: Good Health and Well-being, SDG 9: Industry, Innovation, and Infrastructure, and SDG 12: Responsible Consumption and Production. By transitioning away from antiquated empirical methods toward a statistically driven quality lifecycle, this research generates significant impacts across clinical healthcare, high-tech pharmaceutical manufacturing, and ecological sustainability. **Direct Realization of SDG 3:** Good Health and Well-being Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) and its severe progression, Metabolic Dysfunction-Associated Steatohepatitis (MASH), present a massive global epidemiological challenge. Until the clinical approval of Resmetirom, therapeutic options were dangerously limited, leading many cases to advance toward irreversible cirrhosis and liver failure. By building a high-precision, hyper-robust Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method, this research establishes the core infrastructure needed for reliable drug monitoring. The extreme validation metrics achieved (recovery rates of 98.0%-102.0% and a linearity coefficient of R^2 value of 0.9997 guarantee that patients receive perfectly dosed, chemically pure pharmaceuticals. Eliminating batch-to-batch variability directly enhances patient safety, drastically minimizes adverse toxicological risks, and supports global health metrics by lowering mortality rates linked to chronic metabolic liver diseases. **Advancement of SDG 9:** Industry, Innovation, and Infrastructure Traditional pharmaceutical quality control relies on "One-Factor-at-a-Time" (OFAT) methodologies that generate unstable analytical states prone to frequent system failures.

	This thesis inherently advances scientific innovation by embedding multi-variable Response Surface Methodology (RSM) via a 15-run Box-Behnken experimental design directly into the method development lifecycle. This represents a structural upgrade from reactive testing to prospective, built-in quality engineering. The defining of a predictable mathematical "Design Space" builds a highly resilient infrastructure within the analytical laboratory. Because the method map is mathematically verified via Analysis of Variance (ANOVA), it allows for seamless adjustments to environmental fluctuations without requiring time-consuming re-validation. This future-proof architecture actively facilitates effortless technology transfers to advanced, high-throughput Ultra-High-Performance Liquid Chromatography (UHPLC) platforms, advancing modern technological capabilities in pharmaceutical manufacturing. **Contribution to SDG 12:** Responsible Consumption and Production. Conventional chromatographic development relies heavily on trial-and-error, a process that consumes massive volumes of hazardous organic chemical solvents and produces substantial laboratory waste. By using a disciplined, multivariate aQbD approach, the number of exploratory experimental iterations was strictly minimized to just 15 optimized runs. This drastically reduced the consumption of toxic mobile phase reagents like Acetonitrile during the method optimization phase. Furthermore, because the validated design space prevents unexpected chromatographic system failures (such as peak tailing or un-reproducible retention shifts), the rate of aborted quality control runs during commercial manufacturing is dropped to near zero. Minimizing analytical re-runs directly decreases chemical waste streams, reduces the overall carbon footprint of pharmaceutical quality operations, and promotes sustainable resource usage. This establishes a highly reproducible blueprint for green chemistry in routine pharmaceutical testing.
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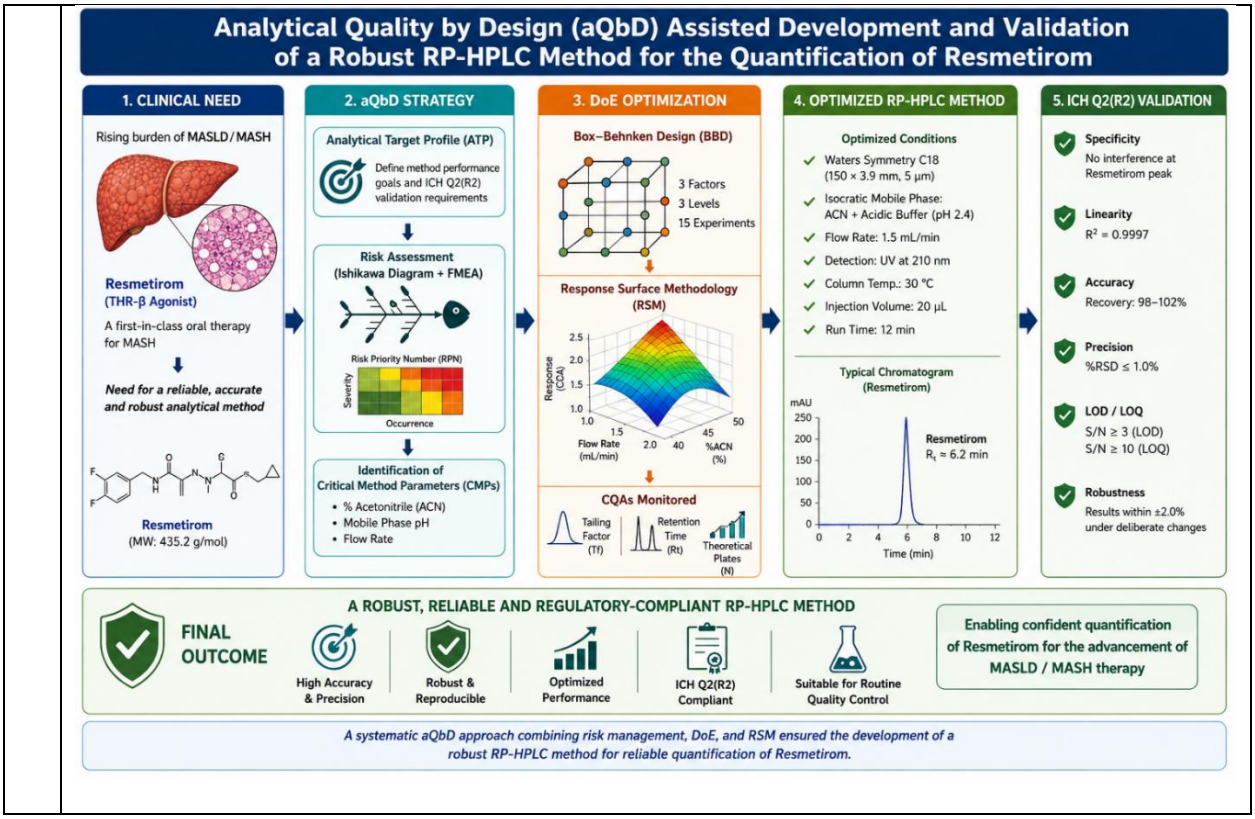
6	**Graphical Abstract**


Analytical Quality by Design (aQbD) Assisted Development and Validation of a Robust RP-HPLC Method for the Quantification of Resmetirom
1. CLINICAL NEED
Rising burden of MASLD / MASH
Resmetirom
(THR-β Agonist)
A first-in-class oral therapy for MASH
Need for a reliable, accurate and robust analytical method
Resmetirom
(MW: 435.2 g/mol)
2. aQbD STRATEGY
Analytical Target Profile (ATP)
Define method performance goals and ICH Q2(R2) validation requirements
Risk Assessment (Ishikawa Diagram + FMEA)
Risk Priority Number (RPN)
Severity
Occurrence
Identification of Critical Method Parameters (CMPs)
% Acetonitrile (ACN)
Mobile Phase pH
Flow Rate
3. DoE OPTIMIZATION
Box–Behnken Design (BBD)
3 Factors
3 Levels
15 Experiments
Response Surface Methodology (RSM)
Response (CQA)
Flow Rate (mL/min)
%ACN (%)
CQAs Monitored
Tailing Factor (Tf)
Retention Time (Rt)
Theoretical Plates (N)
4. OPTIMIZED RP-HPLC METHOD
Optimized Conditions
Waters Symmetry C18 (150 × 3.9 mm, 5 µm)
Isocratic Mobile Phase: ACN + Acidic Buffer (pH 2.4)
Flow Rate: 1.5 mL/min
Detection: UV at 210 nm
Column Temp.: 30 °C
Injection Volume: 20 µL
Run Time: 12 min
Typical Chromatogram (Resmetirom)
mAU
Resmetirom
Rt ≈ 6.2 min
Time (min)
5. ICH Q2(R2) VALIDATION
Specificity
No interference at Resmetirom peak
Linearity
R² = 0.9997
Accuracy
Recovery: 98–102%
Precision
%RSD ≤ 1.0%
LOD / LOQ
S/N ≥ 3 (LOD)
S/N ≥ 10 (LOQ)
Robustness
Results within ±2.0% under deliberate changes
FINAL OUTCOME
A ROBUST, RELIABLE AND REGULATORY-COMPLIANT RP-HPLC METHOD
High Accuracy & Precision
Robust & Reproducible
Optimized Performance
ICH Q2(R2) Compliant
Suitable for Routine Quality Control
Enabling confident quantification of Resmetirom for the advancement of MASLD / MASH therapy
A systematic aQbD approach combining risk management, DoE, and RSM ensured the development of a robust RP-HPLC method for reliable quantification of Resmetirom.