ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/25oct1325

Chemo-Metric Optimization for Robust and Green Chromatographic Methods

Faith O. Osabuohien[1]

[1]Chemistry, University of New Haven, West Haven, CT, United States of America.

Publication Date: 2025/11/05

Abstract: Developing chromatographic methods usually encounters challenges of achieving analytical robustness and adhering to the principles of greenness through Green Analytical Chemistry (GAC). Traditional One-Variable-at-a-Time (OVAT) optimization techniques often fail to capture complex factor interactions, resulting in inefficient methods and subsequent environmental impacts. This paper introduced chemometrics, which encompasses multivariate analysis (MVA), Design of Experiments (DoE), and Monte Carlo simulations as a data-driven and systematic solution. Chemometric optimization helps to identify Critical Method Parameters (CMPs) and facilitates the development of Method Operable Design Region (MODR), within which analytical performance is reliable and consistent. The integration of GAC metrics, such as the Analytical GREEnness (AGREE) calculator, would promote sustainability through chemometric approaches by reducing solvent consumption, lowering energy demands, minimizing waste generation, and shorter analysis times. Thus, the relationship between chemometrics and GAC provides a framework for developing efficient, robust, and environmentally responsible chromatographic methods that comply with regulatory expectations and Quality-by-Design (QbD) principles.

Keywords: *Chemometric Optimization, Robust Chromatographic, Green Chromatographic, Pharmaceuticals, One-Variable-at-a-Time, Green Analytical Chemistry.*

How to Cite: Faith O. Osabuohien (2025). Chemo-Metric Optimization for Robust and Green Chromatographic Methods. *International Journal of Innovative Science and Research Technology*,10(10), 2459-2465. https://doi.org/10.38124/ijisrt/25oct1325

I. INTRODUCTION

Chromatography, including HPLC and UHPLC, is central to pharmaceutical analysis, research, and quality control, due to its characteristic high resolution and efficiency (Khalid et al., 2024). These techniques are primary instruments, supporting the precise determination of drug quality and content through advanced detection systems such as UPLC-MS/MS and principles of Quality by Design (QbD) (Madhuri et al., 2024). They are also integral to the assessment of drug efficacy, safety, and detecting impurities (Gupta et al., 2022). UHPLC helps to improve resolution and analysis time, while trends such as automation and miniaturization are largely important for broadening future applications (D'Atrie et al., 2018; Khalid et al., 2024).

Meanwhile, traditional One-Variable-at-a-Time (OVAT) methods have proven inadequate in pharmaceutical analysis due to its failure to capture interactions, and often resulting in non-robust methods that are prone to routine and small variations (Venkatachalam et al., 2021). Trinanes et al. (2020) observed that this gap can be addressed using a novel approach that adopts desirability functions for assessing robustness or optimization in liquid chromatography by evaluating peak position variations because of operational variable changes. Moreover, the stochastic variability in gradient chromatography can be addressed with multi-objective process optimization algorithm, which consequently helps to improve robustness by taking care of environmental variables (Freier & von Lieres, 2018). This typically involves iterative optimizations and adopts Gaussian process regression models, although it fails to adequately address the limitations of the OVAT method.

Besides, traditional chromatographic methods consume large amounts of hazardous organic solvents, producing significant waste and raising safety and environmental concerns (Moussa Yabre, Ferey, & Gaudin, 2018). Green analytical chemistry is being adopted as a means to replace traditional methods with cleaner alternatives, and address these issues, with the aim of reducing environmental impact and enhancing safety. Nakov et al. (2020) wrote that the development of green chromatographic techniques using pure water or more eco-friendly solvents is crucial for pharmaceutical analysis. Thus, chemometrics aims at bridging the robustness and greenness gaps in chromatography, essentially fostering sustainable pharmaceuticals through techniques such as PCA, cluster analysis, and ANN, which help in predicting and optimizing processes while selecting green materials (Bystrzanowska & Tobiszweski, 2020).

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/25oct1325

➢ *Aim*

This paper aims to review and demonstrate the important role of chemometric optimization techniques in the development of robust and green techniques, which align with the principles of Green Analytical Chemistry (GAC).

➢ *Objectives:*

- To compare the traditional One-Variable-at-a-Time (OVAT) approach with the Design of Experiments (DoE) and their efficiencies
- To demonstrate the application of chemometrics tools, including Monte Carlo simulation and DoE, to define a robust Method Operable Design region (MODR)
- To integrate Green Analytical Chemistry principles with modern metrics, such as the AGREE calculator, into the workflow
- To describe the application of these different strategies using a conceptual case study for a chromatographic technique.

II. ONE-VARIABLE-AT-A-TIME (OVAT) AND CHEMOMETRICS

➢ *One-Variable-at-a-Time (OVAT) Optimization*

One-Variable-at-a-Time (OVAT) refers to the process of varying an independent factor or variable at a time while keeping others constant in order to observe its effect on the response while repeating for other variables in the right sequence (Farinini, 2024; Fernandes, 2024). The approach is highly intuitive and simple, but has many drawbacks. First, inter-relations of factors are often ignored, such that OVAT fails to capture the joint impact of two factors on a response in a non-additive way.

Second, OVAT is ineffective for exploring *k* factors at m levels each, where more experiments than multifactorial designs are required (Williamson et al., 2022) since each factor must be varied per level separately, and all combinations are not captured. Also, there is a high risk of arriving at a false optimum or sub-optimal as the best OVAT settings may be local per other factors being at fixed levels with little to no robust perturbations (Caroco, 2019). For instance, small temperature or flow changes that interact with pH may impact the retention or resolution while OVAT may not be able to disclose such sensitivities (Farinini, 2024). Again, OVAT is inadequate for supporting nuanced mapping of the behavior of the method over a continued space, implying that robust operating spaces are unknown (Fernandes, 2024).

➢ *Fundamentals of Design of Experiments (DoE)*

Design of Experiments (DoE) refers to a simultaneous and structured experimentation approach, where multiple factors are varied per a pre-defined experimental design. The purpose is to quantify their main and interactive effects on at least one response (Kariminejad et al., 2024). The core concepts of DoE include models, interactions, responses, and factors. Models are polynomial or statistical models relating the factors to their responses, which are fitted to the data from the experiments (Jankovic, Chaudhary, & Goia, 2021). Interactions refer to the combined effect of at least two factors acting together, which may be distinct from the totality of their individual effects.

According to Antony (2023), DoE helps to identify and quantify interactions. Factors include organic modifier proportion, flow rate, and temperature, while responses are critical quality or analytical attributes including retention time, resolution between specified peaks, tailing, and peak shape. Taylor et al. (2023) explained that the key function of DoE is its potential to build a comprehensive mathematical model describing responses' behavior across the experiment rather than at isolated points. This aligns with the response prediction under untested combinations of factors, identification of robust operating conditions that are less sensitive to small factor variations, and efficient optimization. DoE is the foundation of Quality by Design (QbD) in ICH and other regulatory guidance for developing analytical methods (Beg et al., 2019).

III. A CHEMO-METRICS FOR ROBUSTNESS: DEFINING THE METHOD OPERABLE DESIGN REGION (MODR)

One way to utilize a chemometrics technique is in enhancing robustness by defining the Method Operable Design Region (MODR). This can be carried out in the following steps.

➢ *Screening: Identifying Critical Method Parameters (CMPs)*

Screening is the first phase in a chemometric approach, aimed at distinguishing the parameters with the most significant impacts on the critical analytical attributes (CQAs) while excluding or focusing less on non-significant ones. Screening reduces burdens and concentrates optimization where it matters. Research shows that common designs used in screening are Placket-Burman designs and Fractional Factorial designs (Beg, 2021). Placket-Burman designs are economical, enabling the evaluation of a large number of factors in a few runs – and identifying main effects, but not substantially reliable for higher-order interactions (McGrath, Xu, & Taylor, 2024), while Fractional Factorial designs are believed to be more robust when certain interaction effects are suspected. The designs examine a part of the possible combinations, getting rid of a few information on interactions, but adding efficiency (Haviari & Mentre, 2024).

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/25oct1325

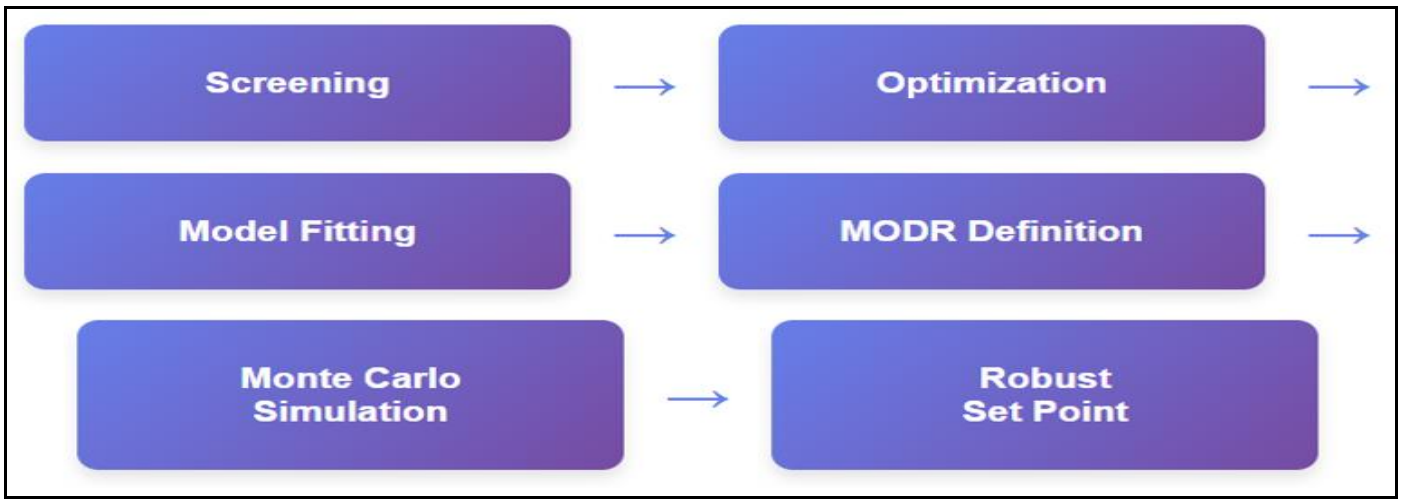

Fig 1 Pictorial Representation of the Chemometric Workflow for Defining a Method Operable Design Region (MODR)

From Figure 1, the outcome of this process is a reduced set of critical method parameters. For instance, it is possible to start with flow, temperature, pH, gradient slope, etc. while screening show that only gradient slope and pH have large effect size or are significant for retention time, resolution, etc.

- *Optimization: Modeling and Mapping the Response Surface*

After identifying the significant parameters, the optimization stage proceeds to using designs that enable exploration of non-linear or quadratic interactions and effects. The two most common designs are Box-Behnken Design (BBD) and Central Composite Design (CCD). CCD involves center points, axial points, and factorial points for estimating curvature (Tamandani & Hashemi, 2022), which tends to require advanced experimental runs while giving more information about the response surfaces' nonlinear shape in the extremes of factor ranges. On the other hand, BBD is more efficient per number of runs for the same number of factors since it avoids extreme corner points, which may be riskier or harder to run, although it may also have less information about very low or high factor levels and their behavior (Szpisjak-Gulyas et al., 2023).

A predictive mathematical model, which is usually a quadratic polynomial, is built from the data of these optimization designs, relating critical method parameters (CMPs) such as temperature, pH, and gradient time to responses. The response surface fosters visualization of the change in response over the experiment, which highlights optima, trade-offs, and ridges among responses (Yabre et al., 2020).

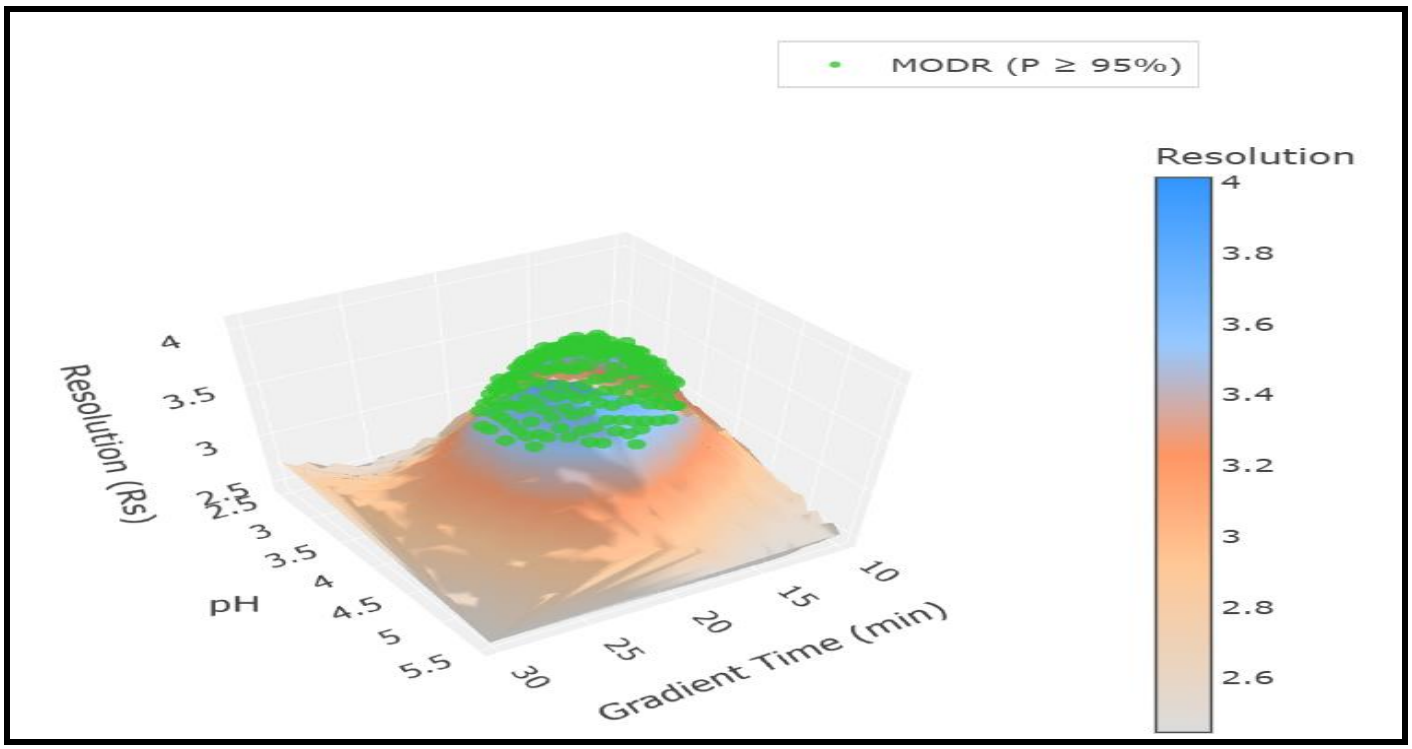

Fig 2 A 3D Illustration of Response Surface Modelling

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/25oct1325

➢ *Establishing the Method Operable Design Region (MODR)*

The Method Operable Design Region (MODR) is the multi-dimensional region in the critical method parameters (CMP) landscape within which the CQAs are acceptable and meet specifications, even while slightly varying the parameters. This involves the use of fitted models to describe regions of factor combinations to ensure that the CQAs meet the criteria (Jagan et al., 2021). However, such criteria are fixed, for instance, retention time </= limit, resolution >/= 2.0. Failure is possible at boundary regions when parameter drift occurs.

Uncertainty in model coefficients, variation in CMPs, and experimental error are accounted for using Monte Carlo simulations (Heibelmann et al., 2019). When thousands of random CMP combinations are simulated within pre-defined ranges, with the assumption that distributions for uncertainty and factor variation, the probability that CQAs are within specification and define the boundaries of probabilistic failure, such as a factor combination is acceptable with a >/= 95% chance of meeting the criteria. This, in turn, generates a robust MODR with safe operating conditions under variation.

The outcome of this is a statistical basis for setting control limits for the method, selecting working points, and regulatory documentation. For instance, ICH Q8/Q9 requires that method robustness be proved while justifying the operable region, and thus ensures that routine deviations do not lead to method failure. In context, Abud et al. (2022) carried out a study on analytical quality by design using Montel Carlo simulation over grid combinations of flow rate, gradient time, and temperature to compute a 90% probability of MODR for meeting criteria.

IV. THE CONCEPT OF GREENNESS: INTEGRATING SUSTAINABILITY INTO METHOD DEVELOPMENT

➢ *Principles of Green Analytical Chemistry (GAC)*

Green Analytical Chemistry (GAC) is guided by some principles designed to minimize the health and environmental impact of analytical approaches (Sajid & Plotka-Wasylka, 2022). Those relevant to chromatographic method development are the use of safer, less hazardous, or benign reagents and solvents, prevention of waste by reducing the number of steps and minimizing reagent and solvent use. Consequently, this minimizes sample size while avoiding or reducing derivatization, energy efficiency, in situ or real time analysis when possible, and safe handling & reduction of exposure to harmful chemicals (Zou, Tang, & Li, 2024). Through these principles, developers are forced to think beyond the mere goal of reaching performance targets, but also toward minimizing operator risk and environmental footprint.

➢ *Assessing Greenness*

Several tools have been developed to assess the greenness of an analytical method. The analytical eco-scale is a quantitative tool that assigns a penalty for parts of the method that reduce greenness, including solvent volume, solvent toxicity, energy usage, and subtracts from a base to produce a score (Hammed V., 2023). This supports comparison, but lacks comprehensiveness (Imam & Abdelrahman, 2023). Also, the National Environmental Methods Index (NEMI) involves the use of a simple pictogram which is divided into parts and reflects toxicity, corrosiveness, and the generation of hazardous waste. However, the coarse or binary grading limits discrimination, while it also fails to consider the extent of impact. According to Pena-Pereira, Wojnowski, & Tobiszewski (2020), the AGREE Calculator (Analytical GREEnness Metric Approach) is the most current technique, which operationalizes the twelve (12) principles of GAC, mapping each principle to a score of 0-1, facilitating weighting, and consequently producing a pictogram demonstrating method performance on each principle and an overall score. This is often used for comparison, holistic assessment, and for making specific and objective trade-offs.

➢ *Green Chromatography Strategies*

Greenness can be practicalized and applied to real life situations by using strategies such as the use of elevated temperatures to reduce phase viscosity (Knoop, J.E. et al, 2023), which also allows for faster flow rates or lower back-pressure while reducing run time and preserving separation (Aly & Gorecki, 2019). In addition, solvent replacement has proven effective, involving the use of more renewable or less toxic solvents such as isopropanol or ethanol in the place of methanol or acetonitrile, or at least reducing the portion of organic modifier (Joshi & Adhikari, 2019). Another common way to ensure green chromatography is method transfer to UHPLC or shorter columns, which uses smaller particle sizes, resulting in shorter analysis times and sharper peaks, implying less energy and solvent use per analysis (Aly & Gorecki, 2019).

V. CASE STUDY APPLICATIONS

Consider a scenario where an analytical laboratory seeks to develop a reverse phase UHPLC (RP-UHPLC) method to assess a drug substance and its impurities in a pharmaceutical formulation. The following steps will be required:

➢ *Step #1: What is the Goal and Risk Assessment?*

CQAs are identified, where resolution between the drug peak and each impurity must be >/= 2.0, run time is as short as </= 10 minutes for throughput, and acceptable peak asymmetry with tailing factor </= 1.5.

CMPs are selected per prior knowledge, including gradient slope or time, mobile phase pH, flow rate, column temperature, and organic modifier composition.

➢ *Step #2: Screening Design of Experiments (DoE)*

A Fractional Factorial design is used to vary the four CMPs at multiple levels to determine the main effects and some interactions.

Results indicate that pH and gradient time have the largest impact on drug resolution, while flow and temperature

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/25oct1325

have smaller influences, although they are still observed for robustness.

➢ *Step #3: Optimization Design of Experiments (DoE)*

A Central Composite Design (CCD) is constructed with pH and gradient time as factors, including the addition of axial points and center points to enable estimation of curvature. The response observed is evidenced by run time, tailing/asymmetry, and resolution between nearest peaks.

- *Next is to Fit a Quadratic Model:*

Resolution = *f (pH,* gradient time) = a + b1pH + b2(grad time) + b12(pH X grad time) + b11pH2 + b22(grad time)2.

The trade-off can be visualized with response surface plots, where an increase in gradient time will improve resolution, but increase run time, while pH balances resolution and may impact peak shape.

➢ *Step #4: MODR Definition and Greenness Evaluation*

Using a Monte Carlo simulation, the pH and gradient time distributions can be defined with pre-defined settings, such as expecting some variation in routine. The next step is to sample multiple combinations such as >/= 5000, propagate the uncertainty of the model from standard errors of coefficients and residual error, and calculate the probability that resolution >/=2.0 asymmetry </= target, and run time </= target. The region in factor space will be identified, especially where the success probability is >/=95%.

The two set points will be compared- that is, the Set Point A (traditional) versus Set Point B (Optimized using Chemometrics). For the traditional set point, higher proportion of organic modifier is used with longer gradient time to ensure resolution while run time is limited to approximately 12 minutes, and solvent consumption is high. In Set Point B, a point within the MODR center balances run time and resolution, for instance, slightly adjusted pH, shorter gradient time, lower proportion of organic modifier, slightly adjusted pH, lower solvent waste, and run time of approximately 8-9 minutes.

Both methods will be assessed with the AGREE calculator, where all relevant parameters, including volumes, solvent types, energy consumption, and number of steps, will be inputted, and Set Point B is expectedly to show a higher AGREE score, better overall score (closer to 1), greener pictogram, and meeting all CQAs to preserve robustness.

This scenario demonstrates that developers can achieve a robust and green method through DoE + MODR + greenness metrics instead of trading one off for another.

VI. DISCUSSION

From the case study, the use of DoE, MODR via Monte Carlo simulation, and screening provides a strong robustness and greenness synergy. In terms of greenness, the strategies help to reduce solvent waste, ensure safer solvent choices, lower run times, and reduce environmental & cost burden, while waste disposal, labor & energy savings are other advantages with the use of reagents (Hessel et al., 2022). However, major barriers include the need for adequate statistical training, especially as many analysts are more conversant with OVAT and casual assessment. Also, the time implication is significant as screening, optimization, and simulation stages consume more effort at the initial stage. Besides, resource limitations or instrument constraints may limit possible optimizations. Nonetheless, the long-term payoff in terms of method robustness, regulatory compliance, cost & environmental impact, and reproducibility is substantial.

VII. CONCLUSION AND FUTURE IMPLICATIONS

Chemometric optimization offers a scientifically robust framework for developing chromatographic techniques that are economically efficient and environmentally sustainable. In the transition from OVAT to structured Design of Experiments (DoE), defining MODR, and modelling response surfaces, method developers can make sure to see that methods maintain performance in routine variation. The integration of greenness metrics, including AGREE, allows for the making of safety, energy, and environmental concerns quantifiable design criteria.

In the future, integrating greenness metrics such as Eco-Scale and AGREE into chemometric software landscapes will streamline workflows. The use of machine learning (ML) and artificial intelligence (AI) may enhance predictive model accuracy, automate the selection of green, optimized, robust set points, and reduce the number of experiments. Regulatory expectations are transitioning toward demanding Quality-by-Design and sustainability in analytical methods, so chemometrics + GAC is critical for the future and essential for developers in environmental and food industries and pharmaceuticals.

REFERENCES

[1]. Abud, T. P., Augusto, A. A., Fortes, M. Z., Maciel, R. S., & Borba, B. S. (2022). State of the art Monte Carlo method applied to power system analysis with distributed generation. Energies, 16(1), 394.
[2]. Aly, A. A., & Górecki, T. (2019). Green chromatography and related techniques. In Green analytical chemistry: past, present and perspectives (pp. 241-298). Singapore: Springer Singapore.
[3]. Antony, J. (2023). Design of experiments for engineers and scientists. Elsevier.
[4]. Beg, S., Hasnain, M. S., Rahman, M., & Swain, S. (2019). Introduction to quality by design (QbD): fundamentals, principles, and applications. In Pharmaceutical quality by design (pp. 1-17). Academic Press.
[5]. Bystrzanowska, M., & Tobiszewski, M. (2020). Chemometrics for Selection, Prediction, and Classification of Sustainable Solutions for Green Chemistry—A Review, Symmetry, 12, 2055.

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/25oct1325

[6]. Caroço, R. F. (2019). Model-based Monitoring and Optimization of a Bio-based Process.
[7]. Degerman, M., Westerberg, K., & Nilsson, B. (2009). A Model-Based Approach to Determine the Design Space of Preparative Chromatography. Chemical Engineering & Technology, 32, 1195-1202.
[8]. Farinini, E. (2024). Use of Experimental Design and Multivariate Analysis for solving industrial problems.
[9]. Fernandes, F. A. N. (2024). Experimental design for chemometrics: best practices. In Chemometrics (pp. 39-59). Elsevier.
[10]. Freier, L., & von Lieres, E. (2018). Robust Multi-Objective Global Optimization of Stochastic Processes with a Case Study in Gradient Elution Chromatography. Biotechnology Journal, 13.
[11]. Gupta, M.K., Ghuge, A., Parab, M., Al-Refaei, Y., Khandare, A., Dand, N., & Waghmare, N. (2022). A comparative review on High-Performance Liquid Chromatography (HPLC), Ultra Performance Liquid Chromatography (UPLC) & High-Performance Thin Layer Chromatography (HPTLC) with current updates, Current Issues in Pharmacy and Medical Sciences, 35(4):224-228.
[12]. Hammed, V.: Solubility of CO2 in Paramagnetic Ionic Liquids. ProQuest Dissertations Publishing, North Carolina Agricultural and Technical State University (2023)
[13]. Haviari, S., & Mentré, F. (2024). Distributive randomization: a pragmatic fractional factorial design to screen or evaluate multiple simultaneous interventions in a clinical trial. BMC Medical Research Methodology, 24(1), 64.
[14]. Heiβelmann, D., Franke, M., Rost, K., Wendt, K., Kistner, T., & Schwehn, C. (2019). Determination of measurement uncertainty by Monte Carlo simulation. In Advanced Mathematical and Computational Tools in Metrology and Testing XI (pp. 192-202).
[15]. Hessel, V., Tran, N. N., Asrami, M. R., Tran, Q. D., Long, N. V. D., Escribà-Gelonch, M., ... & Sundmacher, K. (2022). Sustainability of green solvents–review and perspective. Green Chemistry, 24(2), 410-437.
[16]. Imam, M. S., & Abdelrahman, M. M. (2023). How environmentally friendly is the analytical process? A paradigm overview of ten greenness assessment metric approaches for analytical methods. Trends in Environmental Analytical Chemistry, 38, e00202.
[17]. Jagan, B. G. V. S., Murthy, P. N., Mahapatra, A. K., & Patra, R. K. (2021). Quality by Design (QbD): Principles, underlying concepts, and regulatory prospects. The Thai Journal of Pharmaceutical Sciences, 45(1), 54-69.
[18]. Jankovic, A., Chaudhary, G., & Goia, F. (2021). Designing the design of experiments (DOE)–An investigation on the influence of different factorial designs on the characterization of complex systems. Energy and Buildings, 250, 111298.
[19]. Joshi, D. R., & Adhikari, N. (2019). An overview on common organic solvents and their toxicity. J. Pharm. Res. Int, 28(3), 1-18.
[20]. Kariminejad, M., Tormey, D., Ryan, C., O'Hara, C., Weinert, A., & McAfee, M. (2024). Single and multi-objective real-time optimisation of an industrial injection moulding process via a Bayesian adaptive design of experiment approach. Scientific Reports, 14(1), 29799.
[21]. Khalid, M., Abubakar, Faizan, M., & Kumar, L. (2024). Role of Chromatography in Pharmaceutical Analysis: Trends and Future Perspectives. International Journal for Research in Applied Science and Engineering Technology.
[22]. Knoop, J.E.; Hammed, V.; Yoder, L.D.; Maselugbo, A.O.; Sadiku, B.L.; Alston, J.R. Synthesis, characterization, and magnetic properties of lanthanide-containing paramagnetic ionic liquids: an Evan's NMR study. ACS Appl. Eng. Mater. 2023, 1, 2831-2846. DOI: 10.1021/acsaenm.3c00240
[23]. Madhuri, V., Pandreka, M.K., Gayatri, G., Yamini, M., Abhishek, G., Gope, E.R., Raghava, D., & Nageswara, R.K. (2024). Advances in High-Performance Liquid Chromatography (HPLC) and Ultra-Performance Liquid Chromatography (UPLC), Journal of Pharma Insights and Research.
[24]. McGrath, R. N., Xu, Y., & Taylor, A. (2024). Screening main and interaction effects in a Plackett-Burman design. Communications in Statistics-Simulation and Computation, 53(11), 5180-5200.
[25]. Nakov, N., Acevska, J., Brezovska, K., Petkovska, R., Kavrakovski, Z., & Dimitrovska, A. (2022). Possibilities and challenges of "green" chromatographic solutions, Macedonian Pharmaceutical Bulletin, 68(1):37-38.
[26]. Pena-Pereira, F., Wojnowski, W., & Tobiszewski, M. (2020). AGREE—Analytical GREEnness metric approach and software. Analytical chemistry, 92(14), 10076-10082.
[27]. rayudu, s. (2023). an exploration of high-performance liquid chromatography. international journal of scientific research.
[28]. Sajid, M., & Płotka-Wasylka, J. (2022). Green analytical chemistry metrics: A review. Talanta, 238, 123046.
[29]. Szpisják-Gulyás, N., Al-Tayawi, A. N., Horváth, Z. H., László, Z., Kertész, S., & Hodúr, C. (2023). Methods for experimental design, central composite design and the Box–Behnken design, to optimise operational parameters: A review. Acta Alimentaria, 52(4), 521-537.
[30]. Tamandani, M., & Hashemi, S. H. (2022). Central composite design (CCD) and Box-Behnken design (BBD) for the optimization of a molecularly imprinted polymer (MIP) based pipette tip micro-solid phase extraction (SPE) for the spectrophotometric determination of chlorpyrifos in food and juice. Analytical Letters, 55(15), 2394-2408.
[31]. Taylor, C. J., Pomberger, A., Felton, K. C., Grainger, R., Barecka, M., Chamberlain, T. W., ... & Lapkin, A. A. (2023). A brief introduction to chemical reaction optimization. Chemical Reviews, 123(6), 3089-3126.
[32]. Triñanes, S., Rodríguez-Mier, P., Cobas, C., Sanchez, E.F., Phan-tan-luu, R., & Cela, R. (2020). Robustness

ISSN No: -2456-2165 https://doi.org/10.38124/ijisrt/25oct1325

assessment in computer-assisted liquid chromatography procedures based on desirability functions. Journal of chromatography. A, 460439.

[33]. Venkatachalam, M., Shum-Chéong-Sing, A., Caro, Y., Dufossé, L., & Fouillaud, M. (2021). OVAT analysis and response surface methodology based on nutrient sources for optimization of pigment production in the marine-derived fungus Talaromyces albobiverticlius 30548 submerged fermentation. Marine drugs, 19(5), 248.

[34]. Williamson, E. M., Sun, Z., Mora-Tamez, L., & Brutchey, R. L. (2022). Design of experiments for nanocrystal syntheses: a how-to guide for proper implementation. Chemistry of Materials, 34(22), 9823-9835.

[35]. Yabré, M., Ferey, L., Somé, T. I., Sivadier, G., & Gaudin, K. (2020). Development of a green HPLC method for the analysis of artesunate and amodiaquine impurities using Quality by Design. Journal of Pharmaceutical and Biomedical Analysis, 190, 113507.

[36]. Zou, Y., Tang, W., & Li, B. (2024). Mass spectrometry in the age of green analytical chemistry. Green Chemistry, 26(9), 4975-4986.