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Study of the Analytical Lifecycle Phases within Supercritical Fluid Chromatography Methodologies

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Abstract: *Recently, there has been a significant increase in the overall interest surrounding Supercritical Fluid Chromatography (SFC) across multiple fields, with pharmaceutical analysis being particularly noteworthy. To the best of our knowledge, contemporary SFC has not yet been implemented as a routine procedure for drug quality control. None of the SFC methods that have been reported were found to completely fulfill all stages of the analytical method lifecycle. In light of this, the purpose of the current contribution is to present a comprehensive summary of the existing and previous accomplishments pertaining to SFC techniques, focusing specifically on this lifecycle and its subsequent stages. As a result, the discussions that were incorporated were appropriately organized, with a particular focus on the lifecycle of the analytical method as defined by the International Conference on Harmonisation (ICH). A review of recent and significant scientific publications in the domain of analytical SFC is presented, along with discussions on method validation, instrumental evolution, qualification strategies, and method development methodologies*

Keywords: Analytical lifecycle, Method development, Optimization

I. INTRODUCTION

To extend gas chromatography to solid and ionic compounds, Lovelock introduced supercritical fluid as the mobile phase in 1958 [1]. While studying porphyrin separation, Klesper, Corwin, and Turner invented Supercritical Fluid Chromatography (SFC), formerly Dense Gas Chromatography, in 1962 [2] [3]. After HPLC superseded it, this method had a turbulent existence [4]. Before a few years, SFC equipment had poor dynamic range, UV sensitivity, and robustness [5]. SFC was unsuitable for pharmaceutical quality monitoring due to these drawbacks. In order to improve SFC devices, makers sought sensitivity and reproducibility. The 2010s saw new SFC instruments developed and marketed [6]. These instruments allow measurement of sub-2 µm particles in the Ultra High Performance SFC (UHPSFC) range [7, 8]. Modern technologies also benefit SFC: The method has advantages over liquid chromatography: 1. orthogonal selectivity, 2. high chromatographic efficiency, 3. high throughput, 4. compatible with various detectors, 5. quick equilibration, 6. inexpensive analysis per sample, 7. low solvent consumption, and 8. little waste [9]. This method can replace LC [6] [10]-[14], making it beneficial in green analytical chemistry [4]. Environmental friendliness may not be the major reason SFC is adopted [15]. The major economic motive is to decrease garbage, which is costly to manage and treat [16]. Cost-effectiveness and greenness contribute to environmental safety in sustainability, which assesses economic implications of processes or technology [17].

SFC has grown in popularity in the recent decade because of these benefits. Many areas, notably the pharmaceutical business, are using it more, especially research. Drug screening, impurity profiling, drug analysis, quantitative applications, bioanalysis, metabolic/metabolomics research, chiral separations, and drug development benefit from SFC [26]. [27]. Pharmaceuticals, petroleum, food, polymer, and industrial processes utilize SFC. SFC methods have several applications, but their lifespan descriptions have not been published. Analytical methods must go through the lifespan from inspiration to regular usage. Strangely, most SFC research only complete a few steps of this cycle. SFC may be seldom used. Modern SFC tools and industrial secrecy constraints are unique.

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This page discusses pharmaceutical analytical technology lifecycles. Since frequent usage is advised but not documented, method development and validation will be prioritized. This study examines SFCs in pharmaceutical analysis to answer the question, "Can contemporary SFCs withstand the entire lifecycle of an analytical procedure?" The method lifecycle stages will influence disputes, particularly those related to regular SFC method application, despite the absence of definition.

Analytical Instrument Qualification in SFC

Scientific and Regulatory Context

Operational integrity must be assessed to qualify the analytical equipment. Typically, it has three levels: 1) Performance, 2) Installation, and 3) Operational Qualification assess, record, and report on the instrument's appropriateness. Public health concerns make the pharmaceutical business one of the most strictly regulated worldwide. Several regulatory agencies provide rules for product quality maintenance and improvement to assure pharmaceutical quality control (QC). Instrument certification before any analytical test is required by these recommendations to ensure data dependability. The USP emphasizes that a certified instrument is the foundation of data quality. To get reliable, reproducible, and meaningful data, one must use the right instrument and check its capabilities via frequent System Suitability Tests (SSTs) and certification. Such criteria are necessary in science to ensure instrument data repeatability and technique transfer.

The Current Case of SFC

Many SFC framework papers concern instrument certification for highly regulated businesses. Even though most of these publications are old, they may be relevant for contemporary SFC systems. Anton & Siffrin showed that three packed column SFC systems fulfill 15-year-old ICH and "cGMP" drug product and substance analysis criteria. The authors supplied generic qualification methods and tables with ICH-compliant technique validation criteria (LOD, LOQ, Precision, Linearity, etc.). The temperature sensor, pressure gauge, flow meter, and other instruments required to verify such qualities are assessed. The equipment's high repeatability and flow rate variations may impair transferring technique performance, according to the observation report.

Sun said in 2011 that SFC instruments are approved for pharmaceutical product development, proving regulatory conformity. HPLC certification is used for UV detector calibration, injector qualification, temperature monitoring, and gradient composition verification since current SFC is influenced by LC. Huber also wrote a primer on SFC equipment certification to current standards (GxP, ISO, FDA, USP). In carefully controlled conditions, any current packed SFC instrument may succeed using these articles' requirements. This study was updated by Hicks et al. to evaluate the Agilent 1260-Aurora A5 and Waters UPC2 fusion systems' chiral analytical capabilities in GMP industrial No protocols for analyzing or monitoring existing (UHP) SFC systems were provided, unlike previous studies.

Flow Rate Measurement in SFC

Sun presented a novel SFC flow rate measurement method after studying GMP SFC instrument certification. With its compressible, thick, non-viscous nature, SFC mobile phase flow rate may be tougher to detect and validate than LC. The scientists used acetone, UV detection, and a calibrated sample loop (1 or 5 ml, depending on flow rate) to replace the column. After data gathering, flow rate is computed. Since it requires no instrumental changes and is simple, this technique is interesting. The drawback is that flow rate measurement is required for analysis. The Tarafder & Guiochon workgroup recommended modifying mobile phase flow rate measurement and expression. They advised mass flow rate instead of volumetric flow rate since the former fluctuates throughout the apparatus's channels while the latter is almost constant throughout the SFC system. High fluid compressibility, room temperature, and instrument design impact volumetric flow rate, causing fluctuations. This workgroup published a second study on adapting a Coriolis Flow Meter (CFM) to their SFC devices (Waters UPC2, TharSFC, and Jasco). This SFC equipment modification accurately measured mass flow rate live. A flow meter to measure CO2 consumption was needed initially due to the pump's 150-bar maximum working pressure. Pump piston reciprocation produced a noisy signal. Averaging the data indicated a lower flow rate than the instrument's software.

Enmark et al. monitored molecular phase flow to confirm Tarafder's findings using the same CFM. The findings are consistent, however a pulsation damper between the flow meter and pump may reduce signal and data processing noise.

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This flow meter must be approved since pump pulsations and connection leaks may bias it. GMP regimes need instrument requalification (IQ, OQ, and PQ) after change.

II. METHOD DEVELOPMENT

Quality by Testing and Quality by Design

The lifetime of analytical techniques begins with method development. In this stage, analysts address analytical problems (e.g., chemical separation, identification) while addressing restrictions and needs (e.g., matrix effect, composition, runtime reduction, sample degradation, detection limit). Quality by Testing (QbT) and the new Quality by Design (QbD) methodologies are two important developments in analytical science technique development. Univariate and multivariate techniques approach the analytical issue differently. Numerous documented SFC techniques confirm that QbT was the usual "trial and errors" approach used by most analysts for years. This nomenclature was used to refer to what was not QbD, which employs chemometric tools and risk management to improve product quality from the start.

QbT Methodology and SFC

QbT uses "standard" starting circumstances to construct SFC. Column temperature is usually 40˚C, with back-pressure regulators set at 130-150 bars. [6] [9]. These figures attempt to reduce fluid compressibility and match pure carbon dioxide supercritical characteristics (73.8 bar, 31.1˚C). Column chemistry, flow rate, density (pressure and temperature), mobile phase composition (organic modifier and/or additives), and elution technique (isocratic or gradient) are also investigated. After that, each variable is investigated independently to reach a technique development aim, which may be challenging due to their reliance (pressure, temperature, mobile phase composition, etc.). Testing ended when chromatograms were excellent (peak separation, run length, peak shape). Unfortunately, the analytical method's success or failure is unclear, making flaws difficult to spot. Minor modifications may affect output quality. Univariate approach may delay method development by making agreement harder. After development and before method validation, further trials may be needed to verify approach resilience.

Xia et al. created UHPSFC autoblender. Multi-position proportioning valve of modifier pump was computer-modified. This device made modifiers and additives easy to get, addressing SFC pumps' binary design's time and solvent usage issues.

Method Development and SFC

Analytical SFC technology development is ongoing. Indeed, building the analytical approach is vital and may take time and effect its longevity. Many DoE and response surface creation and optimization examples without QbT and QbD are worth noting [12]. These papers describe full- or fractional SFC factorial and Box-Behnken experiments. These designs allowed analytical methods, SFC-MS detector hyphenation optimization, and experimental parameter evaluations. Several authors have presented alternate SFC technique development techniques. De Klerck et al. suggested general technique development to speed up (UHP) SFC chiral analysis method development, while Delahaye et al. demonstrated how stationary phase optimized selectivity predictions may estimate solute retention on various columns under isopycnic circumstances

Constant pressure vs. constant flow rate gradient elution was also conceptually addressed by De Pauw et al They modeled gradient elution fluid density. Simulations of constant pressure analysis showed a 40% time reduction over constant flow gradient elution. Due to lesser modifier concentrations' higher gradient start mobile phase velocity. To maintain system pressure, the fluid's decreased viscosity demands higher flow rates. Based on HPLC improvements, this approach offers faster SFC analysis, however the fluid's greater constant density may affect selectivity. Continuous pressure gradient elution must be difficult for compounds/separations impacted by mobile phase density, such as apolar molecules on silica-based stationary phases.

III. METHOD VALIDATION

Method Validation in the Field of SFC

A literature review found few method validation studies due to SFC's technical issues. Additionally, most SFC applications seem to be qualitative or preparatory, with just a tiny number quantitative. SFC techniques seem to be scaled up from analytical to preparative. Consider that a validation step is not needed for preparation in this case.

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Increasing SFC devices' sensitivity and robustness was difficult [4] [27]. Thus, ICH organic impurity traces analytical requirements were met at extremely low quantification levels of 0.05% of the active component. Considering SFC-UV's lower sensitivity than HPLC/UHPLC, several publications recommend using highly concentrated samples. This may not work if the materials are poorly soluble in injectable solvents or are available in small concentrations (like impurities). SFC has few authorized procedures, which is expected. Changes are occurring in SFC's numerous applications, testing its quantitative capabilities [10] [13] [23].

Validated SFC Methods

Several SFC and analytical testing showed no method validity. Few discussions indicated the acceptance criterion consensus gap. SFC pharmaceutical tablets were assessed. Concerns exist because intra-series RSD values are larger than inter-series ones at certain concentrations. One Student t-test indicated equal SFC and HPLC values. Drug quality control was adequate with SFC. Méjean et al. measure retinoids. RSD was high but repeatability was decent. This is even more worrisome as RSD is usually 5% and the final medical product standard is 95%–105% API. Thus, this method should make pharmaceutical product quality evaluation harder. Unlike SFC-UV, researchers discovered no retinoid sensitivity. Not surprising given chemicals' strong UV absorption.

Drug impurity profiles by Alexander et al. Though validation wasn't the goal, the method's impurity quantification was confirmed using many criteria. ICH Q2 (R1) states that technique validation certifies an analytical method for its intended usage. For drug substance identification, several studies allowed impurity RSD >10%. Possible causes: low impurity levels (<0.1% active) or difficulties separating pollutants. The 2001 "SFC-Validation paper" emphasizes robustness despite paragraph 5.1's early requirement. Multiple injections of the same solution generated peak area variability in two series. Second series RSD is lowest at 0.3% to 6.67%. Daily medication analysis was wrong; injection sequence mattered [5]. Injection error should minimize with internal standards like GC.

Also, chiral compound determination techniques were partly evaluated. Chiral compounds are tested by molecules or criteria. Chiral separation of 9 amide medicines by Xiang et al. A few compounds were authorized. Also, only linearity, repeatability, and LOQ were evaluated. Wang and colleagues assessed SFC orthogonality solely to RP-LC. This study explored technique selectivity. Data was lacking for further validation. A pharmaceutical R-timolol analytical technique was reported by Marley et al. [10]. Per ICH Q2 (R1), three standards solutions were injected and evaluated six times daily for two trial days to establish repeatability and intermediate accuracy. Six determinations at 100% of the test concentration (1.0% R-timolol for S-timolol) with the bracketing standard (1.0%) assessed accuracy. The quantitative impurity test met European Pharmacopeia (max 1.0% R-timolol in S-timolol). Earlier, Mukherjee reported chiral chemical separation. Assessing validation criteria utilizing ICH and FDA recommendations. But intra-day repeatability testing showed 9% RSD at numerous doses. Inter-day accuracy is determined incorrectly using intra-day mean.

Nováková et al. studied UHPSFC-MS/MS doping agents' LOD, LOQ, and linearity. A quantitative study and technique validation of oestrogen-containing topicals. They disclosed ICH Q2 (R1) validation criteria and frequent usage of their technique to quantify oestrogen steroids in samples. Ganzera tested UHPSFC isoflavone. Method performance was assessed using ICH criteria. Despite low development output, the approach accurately recovers isoflavones from diverse vegetal matrixes, including herbs, intra- and inter-day.

Application of the Total Error Approach to SFC Methods

A publication detailing completely verified UHPSFC and UHPLC methodologies for the analysis of amoxicillin powder in capsule form was recently published by Dispas et al. Unlike the results in other papers, they also showed the promise of UHPSFC as a quantitative and repeatable approach. Additionally, Dispas et al. demonstrated an interest in using a total error approach and robust optimization technique for method validation, which subsequently enabled them to create the UHPLC and UHSFC methods quickly and securely. Clearly, this example is very beneficial from an industrial standpoint (where fast throughput is vital).

Outcomes and Perspectives

Media highlight SFC's revival. The literature may include fresh SFC basic research and applications. By reviewing SFC literature, analytical method lifespan was analyzed. Many examples show how the SFC supports life cycle stages. SFC

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passed EMA, GxP, ICH, and other pharmaceutical requirements. Published SFC instrument certification criteria and methods were few. Empirical QbT seems to specify analytical technique development criteria. Recent research has shown that QbD can generate analytical methods like the SFC method that outperform empirical alternatives. Recently, several DoE-based method development examples were provided. Despite its unpopularity, this discovery reveals chromatographic method optimization.

SFC chemical quantification and instrument advances attract analysts. Modern SFCs are quantitative instruments for the highly regulated pharmaceutical business. Thus, its quantitative capability reports should boost. Incorrect adjustments (such as applying non-independent validation standards), inconsistent findings (such as high RSD values), and restricted method validation (assessing only a few criteria) make the validated method less trustworthy. Making findings more dependable and consistent requires work. Thus, regulatory authorities must establish validation approval standards. Undefined references allow everyone use validation results. There is no framework for discussing the approach's outcomes and performance. Decision-making utilizing the error and accuracy profile may improve method validation. It tests the analytical process and raises questions.

There are few robustness tests in literature. The technique's global weakness may explain this. More updated technology makes updating these scientific studies appealing. SFC articles did not cover transferring and using analytical SFC. This value should alter owing to instrument improvements and the agreement that SFC is better than NP-HPLC [10] [27]. SFC may replace NP-HPLC in several analytical applications owing to its advantages. The SFC was compared to pharmaceutical industry standards like RP-HPLC and UHPLC [27] [29]. These comparisons include the SFC, which is less responsive and adaptive [8] [18] [29].

Alternatively, green analytical chemistry employs SFC [4]. Consider SFC a “green analytical tool” for its extensive literature. SFC is mature and complements liquid and gas chromatography [19]. Reviewing scientific literature determined SFC. According to [9], important tasks remain. This review showed some targets achieved and trends continuing. More than ever, analytical SFC approaches are dying.

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