PORTON

Enabling Technology

Comprehensive Analytical R&D and QC Expertise in Pharmaceutical Development

Hui Chen, Ph.D.
CEO & GM of Porton J-STAR

Abstract

This white paper details our extensive capabilities in Analytical Research and Development (R&D) and Quality Control (QC) for pharmaceutical drug development. It highlights our expertise in method development, structure elucidation, and method troubling. Our goal is to ensure the highest standards of quality and efficiency in drug development and manufacturing.

Introduction

In drug development CMC (Chemistry, Manufacturing, and Controls), we must address several common challenges, including demanding timelines, tight budgets, and stringent regulatory requirements. This white paper outlines our approaches for analytical development and method validation, detailing how we effectively balance these challenges.

Structure Elucidation is a key component of our analytical team: Structure Elucidation is the process of determining the order or arrangement of atoms and the composition of a chemical compound. This process is critical in our services to help analyze the known and unknown components of a client's purchase order. We have also built a world-class team for structure elucidation, equipped with state-of-the-art instruments that can address the most challenging structure elucidation issues.

Analytical Method Development / Validation Approaches

Analytical method development / validation is a dynamic process that must be tailored to the specific properties of the compound and the stage of drug development. Our approach ensures that methods are fit for purpose and adaptable to the evolving requirements of drug development.

Fast Pace & Cost-efficient Method Development

1. Generic HPLC methods are employed for quick evaluation of reaction completion during the initial stage of process development, minimizing the cost of analytical development and sample testing.

2. Retrospective Approach to Method Development

- Objective: Enhance method development efficiency by applying a retrospective approach.
- Approach: Start with the development of the Drug Substance (DS) method, then incorporate starting materials and intermediates to evaluate overall method suitability. Optimize the method as needed.

3. Harmonize Testing of Regulatory Starting Materials (RSM), Intermediates, and DS.

- Objective: Simplify and streamline testing processes by harmonizing the analytical methods for RSM, intermediates, and DS.
- Approach: Develop a unified method that can effectively analyze all stages, reducing redundancy and chances of human error, and improving efficiency.

4. Method for In-Process Control Testing

- Objective: Maximize instrument efficiency by using a shorter version of DS method for in-process controls.
- Approach: Utilize a shorter mobile phase gradient with the same column and mobile phase combination/compositions to minimize error and maximize efficient use of each instrument.

5. Evaluate Method Specificity During Process Development

- Objective: Ensure adequate specificity of analytical methods throughout the process development.
- Approach: Continuously assess and adjust conditions to ensure they provide the necessary specificity for the current process.

6. Conduct Forced Degradation to Evaluate Method Specificity

- Objective: Confirm method specificity by evaluating its ability to detect degradants.
- Approach: Perform forced degradation studies under acidic hydrolysis, basic hydrolysis, oxidation, thermolysis, photolysis to generate degradants at the range of 5-20% at least under one of the conditions and ensure that adequate specificity is provided by the method. Identify potential degradation products and ensure the method can accurately quantify them.

7. Method development for compounds without UV chromophore

- Objective: Provide adequate sensitivity for compounds without UV chromophore
- Approach: Use HPLC with Charged Aerosol Detector (CAD) to enhance the sensitivity of compounds with weak or no UV chromophore. Expensive experiences have been accumulated from different projects to deliver the most robust and reliable HPLC-CAD method.

8. Method development for Drug Product (DP) IPC, Release Testing and Stability Study

- Objective: Simplify and streamline testing processes by harmonizing the analytical methods between DS, DP and equipment cleaning verification
- Approach: Analytical development for DP and DS is conducted in the same group to capture all of the history of the method development conducted for DS testing and remove any gaps that may occur during the method transfer between DS and DP.

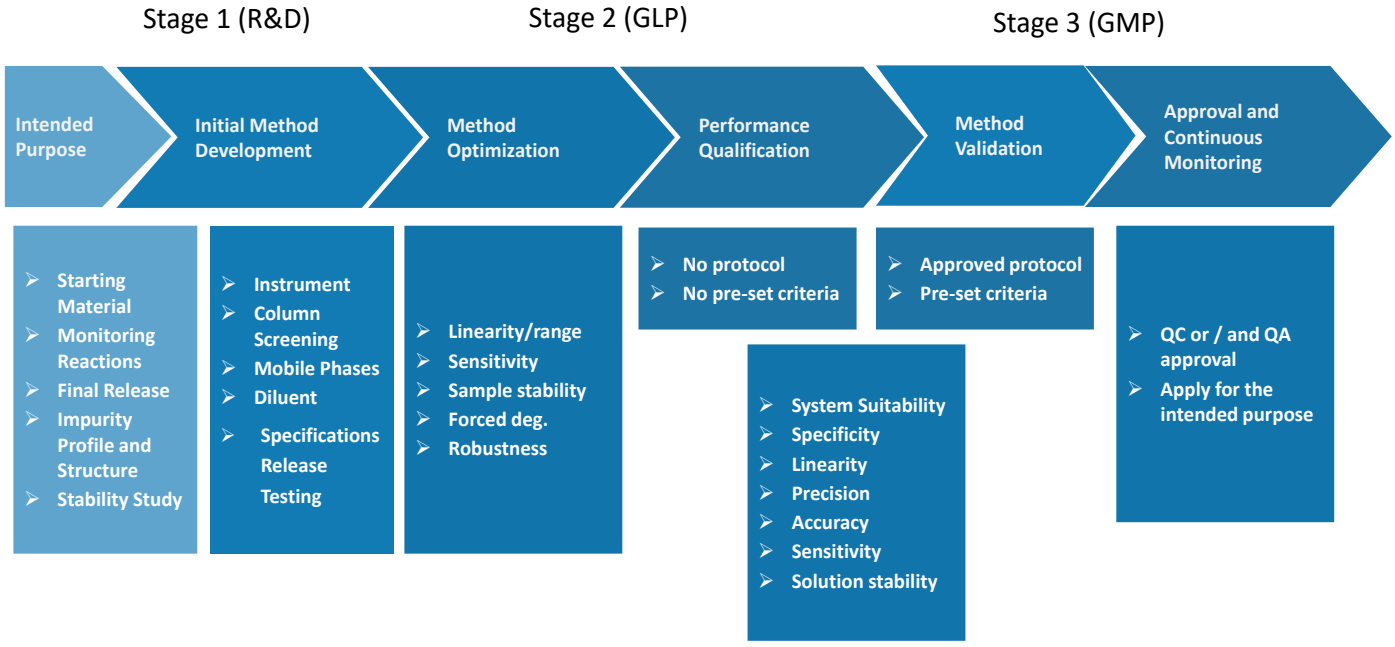
9. Application of quantitative NMR

- Objective: Provide a quick quantitative method for both main and minor components with minimum method development.
- Application 1: Internal calibration method by adding a known commercial RS (e. g. 1,3,5-trimethoxybenzene) to testing article for quantitation when reference standard of the compound under testing is not available.
- Application 2: External calibration method by using an RS of the known component that needs to be quantitated (e. g. a reference standard of TFA for quantitation of residual TFA in a testing article). This provides an alternative quick approach to quantitate compounds that are challenging by a typical chromatographic method. The compounds may include but are not limited to compounds with poor responses, poor retention, or with poor stability in solution.
- Application 3: Relative quantitation of two components by converting from molar% to weight% from proton NMR integration.

Phase Appropriate Approach

The comprehensiveness of analytical method development and validation highly depends on the phase of drug development. As an integral part of drug development, analytical development typically happens from scratch and simultaneously with process development. Below is an illustration of how the phase appropriate approach is applied at the different phase of drug development from the initial route scouting R&D stage to toxicological batch delivery to GMP production. The critical considerations have evolved from the initial reaction monitoring purpose to impurity profiling to impurity profiling, method robustness and regulatory satisfactory. At each stage, a few critical parameters should be evaluated to ensure scientific soundness of the method and regulatory satisfaction if required.

Phase Appropriate Approach:



Impurity Isolation and Structure Elucidation

Multidisciplinary team including both analytical scientists and organic scientists, and comprehensive instrumentation (cryoprobe NMR, HRMS, Flush chromatography, Prep-HPLC, SFC, etc.) and orthogonal analysis by UV, MS and NMR are the key for highly confident structure elucidation results. We routinely conduct structure elucidation for reference standards and related impurities in support of process development, GMP delivery and fate and purge experiments. We have also accomplished standalone challenging structure elucidation projects as the case studies presented below.

Case Study 1:
A thorough detective-like work on DP excipient impurity that was contaminated by another product line.

An impurity was found from an excipient of a drug product.

- The impurity is totally unrelated to the excipients
- The impurity was enriched and isolated by well-designed purification strategies.
- The obtained sub-milligram impurity was analyzed by HRMS and 500 MHz NMR with a cryoprobe.
- The structure of the impurity was proposed after extensive analyses and HRMS fragmentation and 2D NMR spectra.
- The proposed structure is that of a known drug.
- The identity of the impurity was confirmed by comparing it with the known drug.
- The source of the impurity was from contamination of another product line, which was verified by the production history of manufacturing.

Case Study 2:
Find a Needle in Haystack: A leachable impurity in DP Sample

- An impurity was found in an oral suspension drug product, which is not related to drug substance and all excipients.
- Separation of the impurity from DP sample is challenging and time-consuming.
- After thorough studies, the plastic bottle container for drug product was believed to be the culprit as the leachable source of the impurity.
- Sub-milligram of the impurity was obtained by purification, enrichment and extraction from the plastic bottle.
- Based on MS and NMR data, the structure of the impurity was proposed and further verified by comparing with reference. This is a new leachable impurity that has never been reported previously.

Case Study 3:
Structure Elucidation of a Complex ADC Linker-Payload

- The molecular weight of the linker-payload is close to 2k Dalton.
- Three pairs of restricted rotation of C-N bonds produce 8 potential rotamers, resulting in broad features and significant overlap in the spectra.
- 500 MHz NMR with a Cryoprobe was used to obtain NMR data.
- Extensive 2D NMR experiments including COSY, TOCSY, ROESY, HSQC, and HMBC were used for the successful structure elucidation of the complex ADC linker-Payload structure.

Analytical Method Trouble Shooting

Analytical method trouble shooting constantly happens as part of in-house method development to meet different stages of method requirements. In addition, we also take standalone analytical method trouble shooting projects for methods developed by a third party. Below are the case studies we have conducted recently for this type of work.

Case Study 1:
Resolve the discrepancy of HPLC testing results from two HPLC methods

- Two methods were developed independently by two CROs at different stages of the drug development.
- A new impurity at 0.91% was observed under the new conditions but not under the initial conditions.
- The two methods were set up at different pH mobile phases
- The structure of the new impurity was proposed by HRMS and it's the dimer of DS.
- pKa evaluations show that the dimer impurity exists in different protonation states under the "old method" and "new method" conditions. A new protonated form of the dimer was predicted under the "new method" condition. The results explained the shift of RRT for this "new" impurity from 1.25 to 0.93 when the "new method" was applied.

Case Study 2:
HPLC Method optimization for specificity and sensitivity

- Method was provided to understand one of the degradants by forced degradation.
- Coelution was observed by LC-MS when following provided conditions
- Severe peak tailing and broadening were observed for the main peak, and it appeared the column was overloaded in the original method order to achieve the required sensitivity.
- Method robustness issue was observed due to weak ionic strength of the acidic additive.
- Optimization included adjusting UV wavelength, reducing sample size, increasing mobile phase ionic strength, and alternative column screening.
- The impurity profiles were compared between the provided conditions and the optimized conditions for a few representative samples.
- A strong justification was provided for the method change followed by a fit-for-purpose method validation.
- A few more new impurities were observed under the optimized conditions with the structure elucidation from HRMS for the new observed impurities.

Case Study 3:
USP Method optimization for testing Polyethylene Glycol (PEG)

- An apparent Out-of-speciation was observed when establishing the in-house capability to test residual ethylene oxide in PEG by the current USP/NF and Ph.Eur methods.
- Further investigation showed that this OOS was caused by coelution of ethylene oxide with methyl formate.
- GC-MS was used for impurity identification for this OOS investigation.
- The current USP/NF and Ph.Eur methods were optimized to separate the two coeluting peak, ethylene oxide and methyl formate.
- The work was published on “Analytical Method Development, Validation, and Out-Of-Specification (OOS) Investigations for Polyethylene Glycol (PEG), Journal of Pharmaceutical and Biomedical Analysis (2023), 235, 115613”.

Reference Standard Certification

The desirable quality, adequate quantity and associated cost are the typical considerations when preparing a reference standard for DS testing. A few approaches are taken to ensure the achievement of the above goals.

- In-house SOP to ensure consistent practice for RS certification, usage and distribution.
- Calculate the amount of RS and associated cost based on the intended application and the current process to make RS, and plan accordingly.
- Conduct in-process-control testing to ensure the desired quality of RS.
- Conduct comprehensive structure elucidation by HRMS, NMR and FTIR.
- Conduct the solid-state characterization by XRPD, DSC, single crystal X-Ray.
- Test related impurities by HPLC, enantiomeric impurity / diastereomeric impurities by chiral HPLC.
- Test volatile components and water content by GC and KF.
- Test inorganic impurities by Residue on ignition (ROI).
- Conduct Q-NMR as an orthogonal way to evaluate purity calculated from mass balance.
- Other tests as deemed necessary based on the structure of RS.

Stability Study

A stability indicating method is developed and ready in use prior to stability study.

- In-house SOPs for stability chambers controlling and monitoring, and stability studies.
- Stability chambers at all five ICH conditions available.
- Photo stability chamber set at ICH light limit exposure for forced degradation study.
- Specific protocol for each stability study and approved by client.
- Flexible testing plan with spare samples to provide extension or termination of a study as needed.
- World-class team for impurity identification and degradation pathway understanding as needed.

Conclusions

This article outlines the general practices and workflows that demonstrate our competence and cost-efficient approach in every aspect of analytical method development. Additionally, we present several case studies that showcase our expertise in structure elucidation and troubleshooting of analytical methods at all stages.

As an essential component of CMC, we are the ideal partner for your analytical and QC needs. Our confidence in our analytical team and the service we can provide will help to streamline our clients' needs in an efficient and effective process.

Hui Chen, Ph.D.

CEO & GM of PORTON J-STAR

Over 20+ years of experience in Analytical development in pharmaceuticals and cosmetics, analytical strategies and solutions from the early development to regulatory submission.

hui.chen@jstar-research.com

Click here to follow the author on LinkedIn.