

## Application of Analytical Quality by Design (AQbD) in Developing Robust Methods for Herbicide Analysis-A Case Study on Metribuzin

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### ABSTRACT

Analytical Quality by Design (AQbD) applies Quality by Design principles to analytical method development, aiming to design methods that are robust, well-understood, and life cycle managed. Herbicide residue analysis (monitoring active compounds and metabolites in formulations, food, soil, and water) presents challenges of trace-level detection, matrix complexity, and regulatory limits. Metribuzin — a widely used triazinone herbicide — has multiple analytical procedures reported (UV/vis spectrophotometry, HPLC-UV, LC-MS/MS, GC-MS/MS, electrochemical and sensor-based approaches). This review synthesizes AQbD theory and tools, surveys analytical methods for metribuzin, and proposes an AQbD-driven workflow tailored to herbicide analysis with practical examples from the literature. By combining Analytical Target Profile (ATP) definition, risk assessment, systematic Design of Experiments (DoE), method operable design region (MODR) determination, and control strategy, AQbD offers a structured path to methods that meet regulatory and environmental surveillance needs while improving robustness and transferability. The review highlights existing applications of DoE/optimization in metribuzin analysis, identifies gaps where AQbD could add value (e.g., multi-residue LC-MS workflows and metabolite quantification), and outlines a recommended AQbD case study plan for future thesis work on metribuzin analysis.

**Keywords:** Analytical Quality by Design (AQbD), metribuzin, herbicide analysis, Design of Experiments (DoE), method operable design region (MODR), LC-MS/MS, HPLC, spectrophotometry.

### I. INTRODUCTION

Quality by Design (QbD) is a systematic, science-based approach originally developed for pharmaceutical product and process design.

Analytical Quality by Design (AQbD) extends QbD principles to analytical procedures: define what the method must achieve (Analytical Target Profile, ATP), identify critical method attributes (CMAs) and critical method parameters (CMPs), use risk assessment and Design of Experiments (DoE) to build understanding, and define an operable region in which the method reliably meets ATP across its lifecycle. AQbD moves analytical development from trial-and-error to knowledge- and risk-based design, enabling robust methods that are easier to transfer between laboratories and better suited to regulatory expectations for method control and lifecycle management<sup>[1,3]</sup>

Herbicide analysis, including monitoring residues in formulations, foodstuffs, soil, and water, requires sensitive, selective, and rugged methods capable of quantifying parent compounds and transformation products at low levels in complex matrices. Metribuzin (4-amino-6-tert-butyl-3-(methylthio)-s-triazin-5(4H)-one) is a pre- and post-emergence herbicide used widely in many crops; it also produces polar metabolites (e.g., DA, DK, DADK) that require monitoring because of mobility and environmental relevance. Analytical tasks for metribuzin typically include formulation content analysis, residue monitoring in crops/soil/water, and degradation/metabolite profiling — each with different ATPs (e.g., LOQ requirements, specificity, throughput). Representative methods reported in the literature span UV/vis spectrophotometry and derivative/spectrophotometric techniques, RP-HPLC with UV detection, GC-MS/MS and LC-MS/MS for trace residue and metabolite analysis, electroanalytical methods, and novel sensor approaches<sup>[7]</sup>.

Given the varying needs (from routine QC of formulations to ultra-trace environmental monitoring), AQbD offers a powerful framework for developing metribuzin analytical methods that

are traceable, robust, and validated against a clear ATP.

## II. ANALYTICAL QUALITY BY DESIGN — CORE COMPONENTS AND TOOLS

### 2.1 Analytical Target Profile (ATP)

ATP is a concise statement of the method's purpose and performance requirements (e.g., analytes, matrices, required LOQ/LOD, accuracy, precision, specificity, throughput). ATP drives the selection of technique and defines the acceptance criteria used across DoE and validation.

### 2.2 Risk assessment

Risk tools (e.g., Ishikawa/fishbone diagrams, FMEA) are used early to prioritize CMPs (column chemistry, mobile phase composition, pH, gradient, temperature, sample preparation variables) that potentially affect CMAs (retention, resolution, signal-to-noise, matrix effects).

### 2.3 DoE and experimental modelling

Screening designs (fractional factorial, Plackett–Burman) identify high-impact factors; optimization designs (central composite, Box–Behnken, D-optimal) model factor–response relationships and interactions. Response surface models enable prediction and visualization of robust regions.

### 2.4 Method Operable Design Region (MODR)

Based on DoE models and acceptance criteria, MODR is the multidimensional region of CMPs where the method meets ATP with acceptable robustness (probabilistic definitions can be used).

### 2.5 Control strategy and lifecycle management

After method characterization, a control strategy (setpoints, system suitability tests, monitoring plans) is implemented. AQbD also promotes continuous method performance monitoring and re-evaluation if changes occur.

AQbD benefits include fewer out-of-specification events, greater robustness during method transfer, more efficient validation, and better regulatory defensibility. Multiple contemporary reviews and consensus papers describe AQbD steps and success stories in chromatographic method development<sup>[11,14]</sup>.

## III. WHY HERBICIDE (AND METRIBUZIN) ANALYSIS BENEFITS FROM AQBD

Herbicide analysis faces several challenges that make AQbD especially useful:

- **Matrix complexity & low levels:** Food, soil, and water matrices contain components that interfere with analysis; methods must achieve low LOQs with acceptable robustness. LC–MS/MS methods are widely used for trace quantification; however, matrix effects and sample-prep variability complicate routine transfer<sup>[13]</sup>.
- **Multiple analytes/metabolites:** Metribuzin and its polar degradants need simultaneous detection and quantification across differing polarities and stabilities — requiring careful chromatographic and sample-preparation design<sup>[7]</sup>.
- **Regulatory scrutiny & well-defined limits:** Environmental and food safety agencies set strict residue limits and require validated methods; AQbD helps ensure that validated performance is maintained across labs/lifecycles<sup>[21]</sup>.

AQbD helps by formalizing ATPs (e.g., LOQ for parent and metabolites), systematically identifying CMPs that influence matrix effects, and by defining a MODR that ensures acceptable performance across expected variations (e.g., mobile phase grade, column batch variability, minor pH shifts).

## IV. REVIEW OF ANALYTICAL METHODS FOR METRIBUZIN AND EXAMPLES OF DOE/AQBD

This section summarizes representative metribuzin analytical methods and notes where DoE/AQbD approaches have been reported or could be applied.

### 4.1 UV/Vis and spectrophotometric methods

Several spectrophotometric procedures have been reported for metribuzin assay in formulations and simple matrices. Notably, methods using complexation (e.g., Cu(II) complex), derivatization with p-dimethylaminobenzaldehyde (DMAB), or extractive spectrophotometry have used factorial designs to optimize reaction conditions and sample-preparation variables (pH, reagent volumes, reaction time). These works demonstrate how even

simple techniques benefit from DoE for robust optimization<sup>[17,4]</sup>.

#### 4.2 HPLC-UV / RP-HPLC methods

RP-HPLC-UV methods have historically been used for formulation assay and residue work at moderate LOQs. Several validated HPLC-UV procedures for formulation QC (e.g., methods for “Tata Metri” formulations) are available; these generally use phosphate buffers and acetonitrile or methanol mobile phases. Such methods are amenable to AQBd: the chromatographic CMPs (organic content, pH, column type, temperature, flow) can be screened and optimized with DoE to maximize resolution, sensitivity, and run time efficiency<sup>[5]</sup>.

#### 4.3 GC-MS/MS and LC-MS/MS for residues and metabolites

For trace residue and metabolite analysis in complex matrices, GC-MS/MS (with derivatization where needed) and LC-MS/MS are the methods of choice. Recent multi-residue LC-MS/MS methods include metribuzin among panels of hundreds of pesticides, often achieving ng/L or ng/g LOQs for water and food matrices. Method development here must address extraction efficiency (e.g., QuEChERS variants, ultrasonic or microwave-assisted extraction), matrix-matched calibration or isotopic internal standards, and chromatographic separation to reduce ion suppression. AQBd strategies — particularly DoE for extraction and chromatographic parameter optimization and MODR definition — can substantially reduce matrix effects and improve method ruggedness<sup>[6,13]</sup>.

#### 4.4 Electrochemical & sensor methods

Electroanalytical approaches (e.g., chronopotentiometry) and emerging sensor technologies (SPR, plasmonic sensors, nanoparticle-enhanced sensors) have been reported for metribuzin determination. These techniques have potential for field-deployable screening, and DoE can be applied to optimize sensor surface chemistry, pH, and other conditions to optimize limit of detection and selectivity<sup>[18]</sup>.

#### 4.5 Examples where DoE was used

The spectrophotometric metribuzin method that used  $2^3$  and  $2^2$  factorial designs to optimize reagent volumes, pH, and reaction time is a direct example of DoE application in herbicide analysis. More generally, literature reviews demonstrate the growing adoption of AQBd and

DoE in chromatographic method development, including pesticide analyses<sup>[11,17]</sup>.

### V. PRACTICAL EXAMPLES & NOTES FOR METHODS

- **Sample preparation:** For residues, QuEChERS-like acetonitrile extraction with dispersive SPE or SPE clean-up is common; optimization of extraction solvent ratio, salt composition, and dSPE sorbents via DoE can improve recovery and minimize matrix effects. EPA and Bayer analytical method documents for metribuzin provide validated extraction approaches that can be starting points for DoE optimization<sup>[22]</sup>.
- **Chromatography (LC):** For LC-MS/MS, consider using a reversed-phase column designed for polar analytes or mixed-mode columns when metabolites are particularly polar. Model retention as a function of organic content, pH, and temperature in DoE to identify robust separation conditions<sup>[13]</sup>.
- **Matrix effects:** Quantify matrix effects during DoE (e.g., matrix factor or post-column infusion experiments) and include matrix-matched calibration or isotopically labelled internal standards where feasible. Factor these strategies into ATP and MODR definitions.
- **Spectrophotometric methods:** For formulation QC or rapid screens, methods using complexation or derivatization have used factorial designs to optimize reagent volumes and pH; these are quick wins for demonstrating DoE competence in a thesis<sup>[17]</sup>.
- **Sensor & electrochemical methods:** These offer rapid screening; however, their ATPs differ (semi-quantitative vs quantitative). DoE can optimize sensor functionalization and measurement conditions, and AQBd principles still apply.

### VI. DISCUSSION

While DoE has been used in some metribuzin spectrophotometric and extraction optimization studies, comprehensive AQBd-driven LC-MS/MS methods specifically documented for metribuzin and its metabolites — with full MODR and lifecycle control strategy — are less common in the public literature. There is an opportunity to produce a thesis that not only validates a sensitive LC-MS/MS method for metribuzin/metabolites, but also documents an AQBd approach end-to-end: ATP, systematic risk assessment, multistage DoE,

MODR definition, validation, and transfer/robustness studies.

## VII. CONCLUSIONS

AQbD offers a structured, scientifically defensible approach to analytical method development that is particularly valuable for herbicide analysis where matrix complexity, regulatory requirements, and the need to quantify multiple analytes at trace levels create risks for inadequate performance. Metribuzin has an extensive analytical literature — ranging from spectrophotometry and HPLC to LC–MS/MS and GC–MS/MS — and examples exist where DoE has already improved method robustness. A dedicated AQbD-driven LC–MS/MS (or combined LC–MS/MS + HPLC–UV for different ATPs) approach for metribuzin and its metabolites would yield a method that is robust, transferable, and lifecycle-managed, satisfying both research and regulatory needs.

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