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ANALYTICAL QUALITY BY DESIGN IN CHROMATOGRAPHIC METHOD DEVELOPMENT

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Abstract

Analytical quality by design (AQbD) applies the principles of ICH Q8, Q9 and Q10 to the development of chromatographic procedures, replacing trial-and-error optimisation with defined goals, risk assessment, designed experiments and a documented operating region. This narrative review summarises published work on the analytical target profile, critical method attributes, screening and response-surface designs, design space construction, robustness testing and lifecycle management. Reported applications show clearer understanding of method behaviour and fewer failures on transfer, although most are single-laboratory demonstrations. Regulatory expectations are still evolving, and shared standards for design space verification are lacking. This is a review of published literature and reports no new experimental data.

1. INTRODUCTION

Liquid chromatography is the workhorse of pharmaceutical quality control. Assay, related substances, dissolution and stability testing all lean on it, and a method that behaves badly costs time in every laboratory that inherits it. For many years development followed a familiar pattern: pick a column and mobile phase from experience, change one factor at a time until the peaks look acceptable, then validate against the usual performance characteristics [1]. The approach works, in the sense that most methods reach validation. What it hides is how the method responds to small changes, and that hidden behaviour tends to surface later as failed system suitability, awkward transfers or out-of-specification investigations [2, 3].

Analytical quality by design (AQbD) borrows the reasoning of pharmaceutical development set out in the ICH Q8(R2), Q9 and Q10

guidelines and in the quality-by-design literature [4, 5, 6] and turns it on the analytical procedure instead of the product. The method is treated as something with a purpose, a set of inputs that can be varied and a set of outputs that must stay inside limits. Early proposals appeared in the pharmaceutical literature around 2007 and 2010 [2, 3], and several reviews have since mapped the field [7, 8, 9]. Regulators have also moved: a draft ICH guideline on analytical procedure development was released for consultation in 2022, alongside a revision of the validation guideline [10, 11]. This review follows the sequence of an AQbD study, from goals to lifecycle, and notes where the published evidence is strong and where it is thin.

The wider case for this shift was made in the general literature on pharmaceutical quality by design. Yu argued that quality should be built into a product through understanding of the process rather than confirmed by end-product testing alone [12], and the later overview by Yu and colleagues described how regulators came to expect knowledge of that kind in submissions [13]. Analytical procedures were slower to receive the same treatment, partly because pharmacopoeial methods were treated as fixed and partly because the consequences of a poor method seemed less visible than those of a poor process. Yet the data on which every batch is released come from the method, so a weak method undermines the whole system of control, whichever way the manufacturing process is described.

A short word on terminology helps, since the field uses several overlapping phrases. Quality by design, enhanced approach and lifecycle approach are not identical, though they are often used as if they were. In this review, AQbD means a development process that starts from stated performance requirements, uses risk assessment to choose what to study, relies on multivariate experiments rather than single-factor changes, and ends in a defined region of operation with a control strategy [14, 15]. A method developed with a design of experiments but without any stated target would not count. That distinction matters because several published papers describe only the statistical step and still claim the whole label.

2. TARGET PROFILE AND CRITICAL ATTRIBUTES

Every AQbD study begins with the analytical target profile (ATP), a statement of what the procedure must achieve. A good ATP is written in terms of the decision the result supports, for example that an impurity at a stated level must be quantified with a stated maximum uncertainty, rather than in terms of a technique. This keeps the choice of column, detector or gradient open and puts the weight on the performance needed [14, 16]. The concept is borrowed from the quality target product profile of quality by design for products [4], and the analogy is useful: the ATP is to the method what the product profile is to the formulation.

From the ATP follow the critical method attributes (CMAs), the measurable properties of the separation that decide whether the target is met. For a stability-indicating assay these are usually resolution between the active ingredient and its closest degradant, peak tailing, retention time window and, for quantitation, accuracy and precision at the working level [9, 14]. Choosing too many attributes buries the study in responses; choosing too few can miss a failure mode. Published examples tend to settle on two to four chromatographic responses, with resolution of the critical pair nearly always among them [17, 18].

Method scouting comes next, and it is where analyst experience still carries the most weight. Column chemistry, organic modifier, pH range and detection wavelength are narrowed using the properties of the analytes and prior knowledge of similar separations [9, 19]. AQbD does not remove this judgement. What it asks is that the reasoning be written down, so that a reviewer can see why a C18 phase with a phosphate buffer was carried forward and a phenyl phase was not. In the better-documented studies this record is a short table of options considered, the reason each was kept or dropped, and the evidence behind the decision [8, 20].

Fukuda and colleagues reviewed the use of experimental design across pharmaceutical

and analytical quality by design and made a point that deserves repeating: statistical tools are the means, not the end [21]. A study can run a perfectly good central composite design and still fail the spirit of AQbD if the responses were chosen casually. The sensible order is therefore to settle the ATP and the attributes first and only then to ask which design suits the number of factors involved. Where the order is reversed, the design often drives the choice of responses, and the resulting model describes whatever was easy to measure rather than what the user of the result needs to know.

Sample and system characteristics need to be part of this early picture too. Degradation pathways, forced-degradation profiles, analyte pKa values and likely interfering excipients all shape which attributes are critical [18, 20]. Studies that skip forced degradation and rely on a clean standard mixture frequently find, once real samples arrive, that the critical pair is different from the one they optimised. The same is true of impurity methods, where the peak that limits the separation may be a minor component that only appears in aged material. Collecting this information before experiments are designed is cheap compared with repeating them [20, 22].

Choice of detection deserves a mention, since it is easily taken for granted. Diode-array detection with peak purity assessment gives some assurance that the critical pair is truly resolved, and mass spectrometric detection can confirm identity in forced-degradation samples [18, 20]. Neither is a substitute for chromatographic selectivity, but both change what counts as an acceptable resolution. A method with orthogonal confirmation of peak purity can tolerate a slightly smaller resolution value than one relying on a single wavelength. This trade is rarely stated in the literature, yet it affects the acceptance limits set on the attributes and so the size of the eventual design space [9, 18].

The ATP also gives a rational basis for acceptance limits on the CMAs. Rather than adopting a resolution of 2.0 because it is customary, the analyst can ask what resolution is needed for the integration error on the minor peak to stay within the uncertainty allowed by the target [16, 23]. This sounds obvious, yet in practice limits are often inherited from pharmacopoeial monographs and applied without examination. Where the ATP is quantitative, the limits can be justified from first principles, and, importantly, they can be revisited if the intended use of the method changes [10, 16].

3. RISK ASSESSMENT AND EXPERIMENTAL DESIGN

Risk assessment turns the long list of possible method variables into a short list worth studying. Tools from ICH Q9 [5], mainly Ishikawa diagrams and failure mode and effects analysis, are applied to instrument settings, mobile phase preparation, column, sample handling and data processing. Each factor is ranked by its likely influence on the CMAs and by how well it can be controlled in routine use. The output is not a result but a prioritisation: factors judged high-risk go into experiments, while the rest are fixed or monitored [3, 5, 15]. Critics point out that these rankings are subjective and vary with the team, and that is a fair complaint, but the exercise at least exposes assumptions that would otherwise stay private [8].

Screening designs come first. Fractional factorial and Plackett-Burman designs allow many factors to be tested in a small number of runs, which is valuable when each gradient run takes half an hour [24, 25]. The aim is only to separate the few factors that matter from the many that do not. A typical liquid chromatography screen might vary column temperature, buffer pH, gradient time, flow rate, organic modifier proportion and wavelength, and conclude that pH, gradient slope and temperature drive resolution while the others are minor [17, 26]. The price of economy is aliasing, so screening results are best treated as a guide to the next round instead of a final answer.

Factor ranges are the quiet determinant of whether a design succeeds. Too narrow and the model describes a flat region with no information on failure; too wide and it covers combinations that nobody would run, or conditions at which the column degrades. Good practice sets ranges from prior

knowledge and a few scouting experiments, so that the lower end approaches failure of the critical attribute and the upper end remains physically sensible [9, 17]. For pH, the working range of the stationary phase and the pKa of the analytes set the limits. For temperature, the stability of the analytes in the autosampler and on the column matters. Writing the reasons for each range into the study record is a small step that pays off at review.

Once the vital few are known, optimisation designs describe how responses change across the factor range. Central composite and Box-Behnken designs are the usual choices, fitting second-order polynomial models that capture curvature and interactions [27, 28, 29]. Box-Behnken designs need fewer runs for three or four factors and avoid extreme corner combinations, which can be an advantage when a high pH and high temperature together would damage a silica column [28]. Central composite designs extend beyond the studied range and give better estimates of curvature. Both are widely used in published HPLC work, and the choice often reflects convenience and software defaults as much as principle [26, 29].

Where several responses compete, a single compromise has to be found. Desirability functions convert each response into a scale from zero to one and combine them into an overall score [30], which is simple, transparent and available in most design software. Its weakness is that it gives a single optimum with no sense of uncertainty. Multiple-response approaches based on posterior predictive probability address this by calculating the chance that all responses meet their limits at once [31, 32], and they feed directly into design space construction. Another family of methods models retention directly from a few scouting gradients, then uses simulation software to explore the separation without further runs [17, 33, 34].

Run order is a small detail with large consequences. Randomising the sequence of experiments protects against drift in column condition, mobile phase ageing and detector response, which would otherwise be mistaken for factor effects. Where randomisation is impractical, for instance because a column oven needs a long time to equilibrate between temperature settings, runs can be grouped in blocks and the block treated as a factor in the analysis. Replicated centre points spread through the sequence act as a check on drift, since a steady change in their responses shows that the system is moving. Tutorials on experimental design in chromatography treat these points as routine, although many application papers do not say how the runs were ordered [24, 26].

Reviews of response surface methodology in analytical chemistry stress a few habits that carry over directly to chromatography [35]. Coded factor levels make coefficients comparable across factors measured in different units. Residual plots show whether variance is constant across the range, and transformation of a response, for example taking the logarithm of a retention factor, can rescue a model that otherwise fits poorly. Interaction terms are frequently the interesting part, since the effect of pH on resolution often depends on the organic modifier fraction when ionisable analytes are involved. Authors who report only the final equation and the optimum deprive readers of these diagnostics, and the omission is one reason why published designs are hard to reproduce or to judge [29, 35]. A short supplementary table of coefficients, error estimates and lack-of-fit results would cost little.

The published evidence on designs and models is organised in table 1, which lists representative approaches with their reported role and the chromatographic problem they were used to address. The pattern is consistent: screening designs reduce the factor list, response-surface designs describe it, and multiple-response methods or retention modelling convert the model into an operating region. The distribution of the table entries across categories is shown in fig. 1. The balance towards experimental design reflects what has been published, not necessarily what is best practice, since design-space-oriented and lifecycle work is newer and still less frequently reported [8, 9, 20].

Qualitative factors such as column chemistry and organic modifier type need separate

handling, because they cannot be placed on a continuous scale. Many published studies simply fix the column after scouting, and a few include it as a categorical factor in a screening design, which is more informative but multiplies the number of runs [15, 26]. Column-to-column and lot-to-lot variability is a particular worry, since it is a source of variation that analysts cannot choose to remove. Including at least two lots of the chosen column in confirmation experiments, as some groups do, gives an early signal of sensitivity that single-lot studies miss completely [22, 25].

4. DESIGN SPACE AND CONTROL STRATEGY

The design space is the combination of method parameters within which the CMAs have been shown to meet their limits. The term was first used for manufacturing processes [4]; its analytical counterpart is sometimes called the method operable design region (MODR). The practical meaning is straightforward: if the method is run anywhere inside the region, it should meet the ATP, and moving within the region is not a change that needs fresh regulatory notice [4, 23]. Reaching that claim requires more than overlaying contour plots. It requires a probability statement about meeting the limits, because a point on the edge of a contour has about a fifty per cent chance of failing once ordinary variation is added [23, 31].

Table 1: Representative tools used in analytical quality by design for chromatographic methods

Tool or approach	Reported role in chromatographic method development	Ref.
Ishikawa diagram with FMEA ranking	Lists candidate method variables and ranks them by likely effect on critical attributes before any experiment.	[3, 5]
Plackett-Burman design	Screens many factors in few runs to identify the pH, temperature and gradient settings that matter most.	[24, 25]
Fractional factorial design	Screens factors with some aliasing; results guide the later optimisation stage.	[17, 26]
Box-Behnken design	Fits second-order models with fewer runs than central composite designs and avoids extreme corners.	[27, 28]
Central composite design	Describes curvature and interactions across the factor range for resolution and retention responses.	[26, 29]
Desirability function	Combines several responses into a single score to pick a compromise optimum.	[29, 30]
Posterior predictive probability	Estimates the chance that all responses meet limits together, supporting a probabilistic design space.	[31, 32]
Retention modelling simulation software	Predicts separation from a few scouting runs and maps resolution of the critical pair.	[17, 33, 34]
Simulated robustness testing	Replaces many laboratory runs with model-based variation around the working point.	[25, 34]
Method operable design region	Defines the parameter zone in which the target profile is expected to be met.	[4, 23]

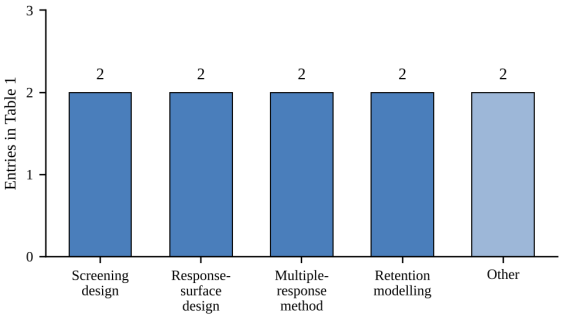


Fig. 1: Number of entries in table 1 by type of tool

Two broad ways of building a design space appear in the literature. The first overlays contour plots of each response and shades the zone where all pass. It is easy to explain and quick to produce, and it was the approach in many early papers [17, 32]. The second uses Monte Carlo simulation on the fitted models, with realistic variability in factors and residual error, to estimate the probability of success across the factor space; the design space is then the region where that probability

exceeds a chosen threshold [23, 31]. Hubert and colleagues compared a conventional stability-indicating method with one redeveloped through AQbD and found the latter easier to defend and to transfer [22]. The probability approach asks more of the analyst but returns something that matches the meaning of the term.

How the success threshold is chosen deserves comment. Some authors require a probability of 95 per cent or more that all attributes meet their limits, others use 90 per cent, and a few give no threshold at all [23, 31]. The choice is a business and risk decision as much as a statistical one: a release assay that guards patient safety justifies a stricter threshold than a method used for in-process information. Whatever the number, it should be justified from the ATP and the consequence of failure, in line with the principles of ICH Q9 [5]. Without this link, the probability becomes another number copied from a previous study, and the reasoning that AQbD is meant to bring is lost.

Retention modelling offers a shortcut for chromatographic problems. If retention times are measured at two or three gradient times, temperatures and pH values, software can predict the whole separation and map resolution of the critical pair across the factor space. Molnár and co-workers described this idea for design space exploration in HPLC [33], and the later work of Monks and colleagues applied it to explore robustness before validation [17]. The advantage is a large reduction in laboratory runs and a map that covers the entire space instead of a fitted polynomial. The disadvantage is that models are only as good as the retention mechanism they assume, so ionisable compounds near their pKa and mixed-mode retention can be predicted poorly [17, 34].

Within the design space the analyst chooses a working point, often called the method operable point, and its location matters. Placing it at the centre of the largest robust region gives the most protection against drift in mobile phase pH, temperature or composition [17, 23]. Placing it near an edge to save run time may satisfy the target on paper but leaves little margin. Some groups define the point by maximising the distance from the nearest failing region or by maximising the success probability [31, 32]. Whichever rule is used, it should be written down, since the point can then be defended after a column lot change or an instrument replacement.

The regulatory standing of an analytical design space is less settled than that of its manufacturing equivalent. The FDA guidance on analytical procedures and methods validation describes expectations for validation and for method changes, but does not set out a separate pathway for movements within a design space [29]. Companies that have used the concept generally describe the region in the application and commit to the control strategy, relying on a documented quality system and a lifecycle control strategy to manage changes inside it [6, 29]. Until the final development guideline is issued, practice will vary and sponsors may reasonably wish to discuss their proposals with regulators at an early stage [10].

The control strategy completes the picture. It lists the controls that keep the method inside its design space during routine use: system suitability criteria tied to the CMAs, specifications for columns and reagents, defined preparation steps and, where useful, in-use monitoring of critical pair resolution [5, 6, 16]. In a well-built strategy, system suitability is not a generic list copied from a pharmacopoeia but a short set of tests chosen because failure in one of them signals movement towards the edge of the design space. This is a quiet but real improvement on typical practice. Test limits that were derived from data are easier to explain to inspectors than limits that were adopted by habit [14, 16, 29].

5. ROBUSTNESS, VALIDATION AND LIFECYCLE

Robustness has traditionally been tested at the end of development, with a small set of deliberate variations run on the finished method. Experience shows how late this is. If the method turns out to be fragile, much of the work is wasted. AQbD moves robustness assessment forward so that the method is built to be robust rather than found to be so [25, 36]. Dejaegher and Vander Heyden discussed

how the factors, ranges and responses for a robustness study should be chosen, and how the same screening designs used in development can serve for testing [25, 36]. Simulated robustness studies based on retention models have been compared with experimental ones and found to agree well for the cases studied [34].

Conventional validation remains necessary, and AQbD does not replace it [1]. What changes is how the results are interpreted. Specificity, linearity, accuracy and precision are shown across the design space instead of at one set of conditions, and the earlier development data can be cited as evidence of robustness [1, 16]. Some authors describe this as a link between development and validation which was missing before. The revised validation guideline and the draft development guideline released in 2022 both point in this direction, although the drafts were still under consultation at the time of writing and details may change [10, 11].

The lifecycle view extends the story past validation. A method has a development stage, a qualification stage in which it is shown to perform in its working laboratory, and a continued verification stage during routine use. The ATP is the thread running through the stages, because results from routine use can be judged against it [14, 16]. Trending of system suitability data, control charts for critical pair resolution and a formal process for deciding when a change needs revalidation all fall within this stage. Published examples of continued verification are scarce compared with development studies, and that is an honest sign of how recently the lifecycle concept has been taken up [16, 20].

Trending turns the lifecycle idea into a daily habit. A control chart of critical pair resolution, plotted from system suitability injections, shows slow movement long before a result fails, and an alert limit set inside the limit derived from the ATP gives the laboratory time to act. The pattern of the drift is informative. A gradual fall across a column's life suggests ageing of the stationary phase, whereas a sudden step usually points to a change in mobile phase preparation or in the column lot. Distinguishing the two keeps investigations short and avoids needless revalidation. None of this needs special software, only a record that someone reads on a regular schedule and a written rule for what follows an alert [14, 16].

Training and organisational habits matter for the lifecycle view as much as the technical content. Laboratories that keep method development in one team and routine testing in another often lose the knowledge that development generates, because the documentation passed on is a procedure and not an understanding. The ATP, the risk assessment and the design space report are the documents that carry that knowledge across the boundary [14, 16]. Where they are kept and read, the quality unit can use them to judge deviations; where they are not, a surprising peak shape becomes an investigation from scratch. This is an organisational benefit that does not appear in method papers but probably accounts for much of the practical value [15, 20].

Method transfer is where the benefits are most easily seen, at least in principle. A receiving laboratory with a different instrument, a different dwell volume or a different column lot is a source of exactly the variation that a design space is meant to absorb. Case studies report smoother transfers when the sending laboratory supplies the design space and the reasoning behind it rather than only a standard operating procedure [15, 20, 22]. Regulatory guidance on validation expects transfer documentation, but does not yet describe how a design space should be communicated [29]. Until it does, each company is developing its own convention, which weakens the benefit of the idea.

Impurity methods have received particular attention, since they combine tight resolution requirements with low-level quantitation. Dispas and colleagues reviewed AQbD for impurity analysis and noted that the critical pair is often two structurally close compounds whose separation is sensitive to pH and organic modifier [20]. In such cases a design space may be narrow, and the useful contribution of AQbD is to show clearly how narrow it is. A method that operates safely in a small region and is controlled accordingly

can be better than a nominally wider method that no one has examined. Karmarkar and co-workers described a stability-indicating method developed this way, with the design space and the control of the critical pair explicitly documented [18].

6. LIMITATIONS AND FUTURE DIRECTIONS

A fair reading of the literature must acknowledge its weaknesses. Most AQbD papers are demonstrations by one group on one analyte, and few offer a direct comparison against a conventionally developed method on the same problem. Where such comparisons exist, they favour AQbD, but publication bias is likely [8, 22]. The effort needed is also real. Screening, optimisation and confirmation add experimental work upfront, and the saving arrives later in avoided failures, which are hard to count. For a method that will be used once, the investment is difficult to justify; for a release assay run for a decade, it clearly is [9, 15].

Questions of scale and cost deserve honest treatment. Smaller laboratories, especially in developing regions, may not have access to the software, the instrumentation for automated column and solvent switching, or the statistical support that many published AQbD studies assume [9, 15]. A pragmatic version of the approach, with a written target, a short risk assessment, a screening design and a modest response-surface design on the few critical factors, is within reach of most laboratories, and still produces a better-understood method than one-factor-at-a-time work [3, 21]. It would help the field if more papers described such low-cost versions and reported what they cost in time and materials.

Statistical skill is another constraint. Software has made designs and desirability plots easy to produce, and with it the risk of misuse has grown: inappropriate factor ranges, missing replicates, extrapolation outside the studied space and models accepted on fit statistics alone [26, 29]. Training analysts in the basics of experimental design is arguably a prerequisite for useful AQbD, and the shortage of such training may be a bigger barrier than any regulatory one. Hibbert's tutorial review of experimental design in chromatography remains a good starting point for this purpose [26].

Future work should address the gaps already noted. Agreed approaches for verifying that a design space holds in a second laboratory would help, as would shared reporting standards so that studies can be compared. Links with greener method assessment, with biologics and with multi-attribute methods are developing but are still thinly documented. Regulatory clarity should improve once the 2022 draft guidelines are finalised [10, 11], though the final wording may differ from the drafts. Finally, it would be useful to see long-term follow-up of methods developed under AQbD, reporting how often they needed change and how that compares with older methods. Without such data, the claim of fewer failures remains plausible rather than proven [16, 20].

7. CONCLUSION

Analytical quality by design offers a sensible way to make chromatographic methods less fragile. Its core ideas are simple: state what the method must do, find out by designed experiments what influences it, and describe the region in which it is safe to operate. The published literature supports the benefits in terms of understanding, flexibility inside a defined region and easier transfer [9, 15, 22]. Screening and response-surface designs are well established, and probabilistic design spaces are increasingly seen [23, 31].

The evidence has limits. Most reports are single-laboratory demonstrations, comparisons with conventional development are few, and lifecycle follow-up is rare [16, 20]. Regulatory guidance is still being finalised [10, 11]. For practitioners, the sensible course is to adopt the parts that clearly help, especially a written target profile, risk-based factor selection and probability-based design spaces, and to keep records that allow the next analyst to see why the method looks the way it does [14, 20].

8. ABBREVIATIONS

AQbD-Analytical quality by design, ATP-Analytical target profile, CMA-Critical method attribute, FMEA-Failure mode and effects

analysis, HPLC-High-performance liquid chromatography, ICH-International Council for Harmonisation, MODR-Method operable design region, pKa-Acid dissociation constant (negative logarithm)

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10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

12. REFERENCES

1. Rozet E, Ziemons E, Marini RD, Boulanger B, Hubert P. Quality by design compliant analytical method validation. Anal Chem 2012;84:106–12.
2. Schweitzer M, Pohl M, Hanna-Brown M, Nethercote P, Borman P, Hansen G, *et al.* Implications and opportunities of applying QbD principles to analytical measurements. Pharm Technol 2010;34:52–59.
3. Borman P, Nethercote P, Chatfield M, Thompson D, Truman K. The application of quality by design to analytical methods. Pharm Technol 2007;31:142–152.
4. Pramod K, Tahir MA, Charoo NA, Ansari SH, Ali J. Pharmaceutical product development: a quality by design approach. Int J Pharm Investig 2016;6:129–38.
5. ICH Harmonised Tripartite Guideline. Quality risk management Q9. Geneva: ICH; 2005.
6. Hubert P, Nguyen-Huu JJ, Boulanger B, Chapuzet E, Chiap P, Cohen N, *et al.* Harmonization of strategies for the validation of quantitative analytical procedures: a SFSTP proposal, part I. J Pharm Biomed Anal 2004;36:579–86.
7. Vogt FG, Kord AS. Development of quality-by-design analytical methods. J Pharm Sci 2011;100:797–812.
8. Orlandini S, Pinzauti S, Furlanetto S. Application of quality by design to the development of analytical separation methods. Anal Bioanal Chem 2013;405:443–450.
9. Tome T, Zigart N, Casar Z, Obreza A. Development and optimization of liquid chromatography analytical methods by using AQbD principles: overview and recent advances. Org Process Res Dev 2019;23:1784–1802.
10. ICH Harmonised Guideline. Analytical procedure development Q14, draft version for public consultation. Geneva: ICH; 2022.
11. ICH Harmonised Guideline. Validation of analytical procedures Q2(R2), draft version for public consultation. Geneva: ICH; 2022.
12. Yu LX. Pharmaceutical quality by design: product and process development, understanding, and control. Pharm Res 2008;25:781–791.
13. Yu LX, Amidon G, Khan MA, Hoag SW, Polli J, Raju GK, *et al.* Understanding pharmaceutical quality by design. AAPS J 2014;16:771–783.
14. Peraman R, Bhadraya K, Padmanabha Reddy Y. Analytical quality by design: a tool for regulatory flexibility and robust analytics. Int J Anal Chem 2015;2015:868727.
15. Sangshetti JN, Deshpande M, Zaheer Z, Shinde DB, Arote R. Quality by design approach: regulatory need. Arab J Chem 2017;10:S3412–S3425.
16. Jackson P, Borman P, Campa C, Chatfield M, Godfrey M, Hamilton P, *et al.* Using the analytical target profile to drive the analytical method life cycle. Anal Chem 2019;91:2577–2585.
17. Monks K, Molnár I, Rieger HJ, Bogáti B, Szabó E. Quality by design: multidimensional exploration of the design space in high performance liquid chromatography method development for better robustness before validation. J Chromatogr A 2012;1232:218–230.
18. Karmarkar S, Garber R, Genchanok Y, George S, Yang X, Hammond R. Quality by design (QbD) based development of a stability indicating HPLC method for drug and impurities. J Chromatogr Sci 2011;49:439–446.
19. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. 3rd ed. Hoboken: Wiley; 2010.
20. Dispas A, Avohou HT, Lebrun P, Hubert P, Hubert C. Quality by design approach for the analysis of impurities in pharmaceutical drug products and drug substances. Trends Anal Chem 2018;101:24–33.
21. Fukuda IM, Pinto CFF, Moreira CS, Saviano AM, Lourenço FR. Design of experiments (DoE) applied to pharmaceutical and analytical quality by design (QbD). Braz J Pharm Sci 2018;54:e01006.

22. Hubert C, Lebrun P, Houari S, Ziemons E, Rozet E, Hubert P. Improvement of a stability-indicating method by quality-by-design versus quality-by-testing: a case of a learning process. J Pharm Biomed Anal 2014;88:401–409.
23. Rozet E, Lebrun P, Debrus B, Boulanger B, Hubert P. Design spaces for analytical methods. Trends Anal Chem 2013;42:157–167.
24. Lundstedt T, Seifert E, Abramo L, Thelin B, Nyström Å, Pettersen J, *et al.* Experimental design and optimization. Chemom Intell Lab Syst 1998;42:3–40.
25. Dejaegher B, Vander Heyden Y. Ruggedness and robustness testing. J Chromatogr A 2007;1158:138–157.
26. Hibbert DB. Experimental design in chromatography: a tutorial review. J Chromatogr B 2012;910:2–13.
27. Box GEP, Behnken DW. Some new three level designs for the study of quantitative variables. Technometrics 1960;2:455–475.
28. Ferreira SLC, Bruns RE, Ferreira HS, Matos GD, David JM, Brandão GC, *et al.* Box-Behnken design: an alternative for the optimization of analytical methods. Anal Chim Acta 2007;597:179–186.
29. US Food and Drug Administration. Guidance for industry: analytical procedures and methods validation for drugs and biologics. Silver Spring: FDA; 2015.
30. Derringer G, Suich R. Simultaneous optimization of several response variables. J Qual Technol 1980;12:214–219.
31. Peterson JJ. A posterior predictive approach to multiple response surface optimization. J Qual Technol 2004;36:139–153.
32. Debrus B, Lebrun P, Ceccato A, Caliaro G, Rozet E, Nistor I, *et al.* Application of new methodologies based on design of experiments, independent component analysis and design space for robust optimization in liquid chromatography. Anal Chim Acta 2011;691:33–42.
33. Molnár I, Rieger HJ, Monks KE. Aspects of the design space in high pressure liquid chromatography method development. J Chromatogr A 2010;1217:3193–3200.
34. Kormány R, Fekete J, Guillarme D, Fekete S. Reliability of simulated robustness testing in fast liquid chromatography, using state-of-the-art column technology, instrumentation and modelling software. J Pharm Biomed Anal 2014;88:329–337.
35. Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 2008;76:965–977.
36. Vander Heyden Y, Questier F, Massart DL. Ruggedness testing of chromatographic methods: selection of factors and levels. J Pharm Biomed Anal 1998;18:43–56.