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'Quality by Design' approach for the analysis of impurities in pharmaceutical drug products and drug substances

Amandine Dispas [a, b], Hermane T. Avohou [a], Pierre Lebrun [c], Philippe Hubert [a], Cédric Hubert [a, *]

[a] University of Liège (ULiege), CIRM, Laboratory of Pharmaceutical Analytical Chemistry, Liège, Belgium
[b] University of Liège (ULiege), CIRM, Laboratory for the Analysis of Medicines, Liège, Belgium
[c] Arlenda S.A., Liège, Belgium

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ABSTRACT

The pharmaceutical industry is highly regulated by quality policies. The concept of risk management is strongly integrated into the quality assurance system to ensure pharmaceuticals' quality and patients' safety. In the context of quality control, the detection of impurities in raw materials and finished products is a major concern. It can be challenging for analytical scientists to meet specificity/selectivity and sensitivity requirements. Obviously, separation techniques are widely used for the detection of impurities but the method development required to achieve Analytical Target Profile (ATP) concerns is often challenging. Therefore, to ensure pragmatic and systematic methods development and simultaneously manage the risk associated with analytical methods, the principles of Quality by Design (QbD) should be applied. This paper provides an overview of QbD principles and statistical strategies (mainly DoE-DS approach) which can be applied to impurity detection methods, as well as a review of the literature where QbD has been applied to these types of analytical methods.

1. Introduction

Quality assurance is a major concern in the pharmaceutical industry, as described by Good Manufacturing Practices (GMP) requirements. This concept should be present through the whole pharmaceutical product lifecycle to ensure product quality and GMP compliance. This includes the management of (among others) environment, equipment, procedures and staff, as well as all kinds of materials/reagents/references or data and deliverables. Nonetheless, it is now largely recognized that one important component of quality assurance system is the management of risk associated with the pharmaceutical product. As stated in ICH Q9, "*An effective quality risk management approach can further ensure the high quality of the drug (medicinal) product to the patient by providing a proactive means to identify and control potential quality issues during development and manufacturing. Additionally, use of quality risk management can improve the decision making if a quality problem arises.*" [1]. This can be achieved by the introduction of control procedures in line with the risk incurred throughout the process.

Consequently, an in-depth scientific knowledge of the quality of the product is essential. Such a level of understanding is only acquired by the application of a systematic approach to pharmaceutical development, as facilitated by the Quality by Design (QbD) strategy [2].

The QbD concept, initially introduced for manufacturing processes, can be described in four steps [3]:

- determination of patient requirements, namely, the Quality Target Product Profile (QTPP);
- design and development of the manufacturing process;
- risk assessment and definition of the manufacturing Design Space (DS); and
- implementation of a control strategy.

An important step in the manufacturing process lifecycle consists of quality control activities either during or after production. Depending on the manufactured product, analysts may face challenges in developing analytical methods fit for their intended purposes, especially in the field of impurities control. Over the

* Corresponding author.
E-mail address: chubert@uliege.be (C. Hubert).

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course of many years, scientists have acquired detailed knowledge about analytes and their chromatographic/electrophoretic behaviour. Retention or migration mechanisms for liquid, gas, supercritical fluid chromatography or electrophoresis are deeply understood today, facilitating the management of complex samples. Two distinct approaches are observed in analytical development [4,5]:

- the use of empirical strategy to select a suitable experimental condition as quickly as possible; or
- management of the development through a risk-based strategy, facilitating an extensive knowledge of the analytical method.

Prior knowledge is the fundamental keystone of both analytical method development strategies. Development commonly relies on One-Factor-At-a-Time (OFAT) or the trial-and-error approach, expressed by Quality-by-Testing (QbT). This methodology is widespread because it is (wrongly) believed to yield a suitable and faster answer. Although such an approach could be applied to relatively simple problems, it can be inappropriate in cases of complex samples or difficult separations (i.e., impurities analysis). Indeed, it has been repeatedly demonstrated that a QbT approach does not provide any proper characterization of analytical methods. An accurate understanding of how factors such as mobile phase, background electrolyte composition, pH, gradient time (and so on) affect the peak retention/migration time, and how method uncertainties affect the peaks' separation are not managed [6]. Moreover, such a development does not allow systematic assessment of robustness throughout the development process or even to meet USP requirements [7,8] or pharmaceutical guidelines [1,2,9] regarding risk management.

The second approach is the one recommended by the authors. This approach can be defined as Analytical Quality by Design (AQbD) strategy, which is not explicitly discussed by ICH Q8. In this context, EMA and FDA introduced some guidelines to implement the concept defined in ICH Q8 in the field of analytical methods [10,11]. The Analytical Target Profile (ATP) and Method Operable Design Region (MODR) were introduced as parallel analytical concepts to QTPP and DS, defined specifically for manufacturing processes. In the present paper, MODR is described as the combination of Design of Experiments (DoE) and computation of a probabilistic DS (that has quantifiable risk). The DoE-DS strategy is systematically used in this paper to refer to MODR, in order to be coherent with referenced research papers. The DS represents a specific area of the experimental domain gathering a set of experimental conditions where the desired quality is achieved while heeding inevitable uncertainties, i.e., a robust experimental domain. From a quality assurance point of view, working within the DS does not represent change of method [2]. One of the key concepts of this strategy is the continuous improvement of the chosen method by means of experience acquired or new data collected throughout its lifecycle. Consequently, AQbD strategy is increasingly being adopted since it allows an earlier understanding of method and guarantees the determination of a wider set of experimental conditions [12–15].

Fig. 1 contrasts QbT and QbD approaches. In this representation, “Method understanding?” is presented as the link between QbT and QbD methodology. Indeed, having an understanding of the method is the key concept that differentiates both approaches. In the latter, the knowledge space resulting from a first optimization phase (called “DS 1”) could be further explored. Indeed, despite the fact that working within “DS 1” already ensures that all separations obtained are acceptable, a subsequent optimization phase could be performed using a second DoE (called “DoE 2”) resulting in a second DS (called “DS 2”). In this refined DS, new constraints arising during the method lifecycle are managed. This refined DS is also a robust area where the desired quality is achieved. Consequently, QbD strategy may be considered as a learning process [5].

The Analytical Quality by Design (AQbD) concept is defined similarly to the QbD approach for manufacturing processes. Therefore, AQbD also includes four main steps:

- determination of the required analytical method performances (the ATP);
- determination of relevant method parameters and quality attributes (the screening phase);
- optimization of the method and assessment of risk by defining the analytical DS (the robust optimization); and
- implementation of a control strategy for the continuous improvement of the method.

2. Discussion

2.1. Overcoming barriers to implement Quality by Design (QbD) in analytical method development

The starting point for the development of a separative method is the selection of method parameters. For readability purposes the discussion below will focus on the liquid chromatography technique, but the same could also apply to other separation techniques. Specific problems exist for the screening of chromatographic factors, leading to some reluctance to use DoE in the industry. One of these issues is that some early-studied principal factors, such as stationary phase and solvent, are qualitative. Consequently, the number of parameters to be considered in statistical modelling explodes, preventing the elaboration of sparse and efficient experimental designs. Another issue is that, for different stationary phases the chromatographic interactions of continuous factors (pH, etc.) might be so different that simple models cannot cope with all column-to-factor interactions. However, in the case of models with higher order interactions (complex models) it is not possible to keep the number of experiments low.

Therefore, during screening, the authors advise the fixing of qualitative factors such as the type of stationary and mobile phases based on scientific knowledge of the molecules and any relevant impurities. Indeed, it is very unlikely that these parameters will change during routine use. Then, during method optimization for robustness, quantitative factors such as pH, mobile phase composition or gradient time can be studied.

If necessary, pre-tests on qualitative factors can be conducted. Usually, the chosen column(s) will be those optimizing peak shapes, time of analysis and selectivity. Knowledge of the interactions between the stationary phase, the mobile phase and the molecule of interest should of course be included in such studies.

2.2. Difficulties related to impurities analysis

In the analytical method development, impurities have always been a matter of particular importance because of several difficulties discussed below.

2.2.1. Availability of material

Some impurities (i.e., degradation and manufacturing impurities) might be difficult to obtain in sufficient quantities to run a DoE. Forced degradation of raw materials or finished products is an option, but it should be noted that this does not provide proof that all possible impurities observed in the future will be present in the sample.

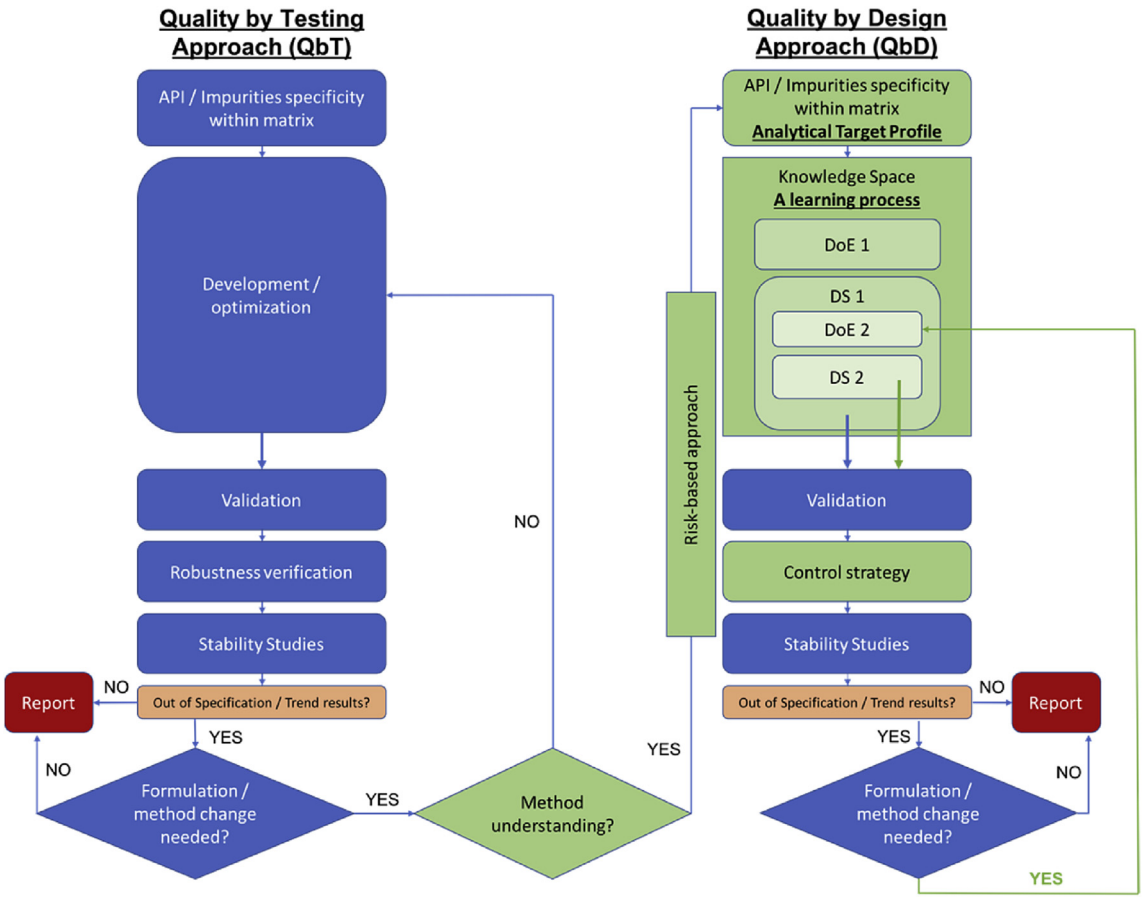

Fig. 1. Comparison of Quality by Testing (QbT) approach (Left) and Quality by Design (QbD) approach (Right).

2.2.2. Degradation of the material during experiments

Degradation of the material during the several runs that comprise the DoE can occur. In this case, peak width and height could evolve during the experiments, adding difficulties in compound identification, as well as noise in the tracking of the retention times.

2.2.3. Change of formulation

During the lifecycle of a drug, it is often necessary to adapt its formulation. This is typically done to improve long-term stability, hence potentially reducing the number of impurities. This can also lead to new compounds being present in the chromatogram, increasing the risk of co-elution at a fixed chromatographic setting.

2.2.4. Active Principle Ingredient (API) and impurities behaviour

Because impurities generally have molecular structures that are close, or related to, the API, they also tend to show similar chromatographic behaviour. It is of paramount importance in the choice of the stationary phase to use all possible knowledge to choose a column that will eventually allow a full separation. However, these separations often remain limited, i.e., they do not appear as robustly as needed. Fortunately, as their behaviour remains correlated, they tend to stay separated even when undergoing separative process variability, as illustrated in Fig. 2. On the left, the two compounds are fairly different, thus inducing independence in their chromatographic behaviour. On the right, the API and impurity dependencies allow the separation to remain under analytical process variability. This shows how critical it is to account for these correlations between peaks when attempting to model the chromatographic behaviour, and to meet the ICH Q8 definition of DS [2].

2.2.5. Concentration levels

One of the main issues is the concentration levels and especially the difference between API and impurities concentration (i.e., factor 100–1000) is usual). Furthermore, the impurity level might be very low. It means that a stability indicating method must be able to track them. Nevertheless, it is the very purpose of the DoE to develop such a method. Spiking the sample to increase impurities concentration can be an option, when possible (see “availability of material”).

2.3. Analytical Quality by Design (AQbD) and risk management

In order to obtain a flexible method that can overcome the difficulties described above, it is necessary to develop DS that express a high probability of meeting specification(s) with a high robustness. This means that the range of chromatographic settings defining the DS must be large enough to allow the method to be updated easily when needed. Though a large DS might seem costly to develop, the cumulative cost of OFAT development (and with it, completely redeveloping an assay due to a new impurity or a change of formulation) is tremendously higher when all multivariate combinations and interactions of input variables are not known precisely.

2.3.1. Analytical Target Profile

As described above, AQbD starts with the definition of the ATP, which is analogous to the QTPP in a product's QbD approach [16]. Furthermore, Critical Methods Attributes (CMAs) could be introduced as an analytical equivalent to the Critical Quality Attributes (CQAs) defined in ICH Q8. The ATP describes the purpose and scope

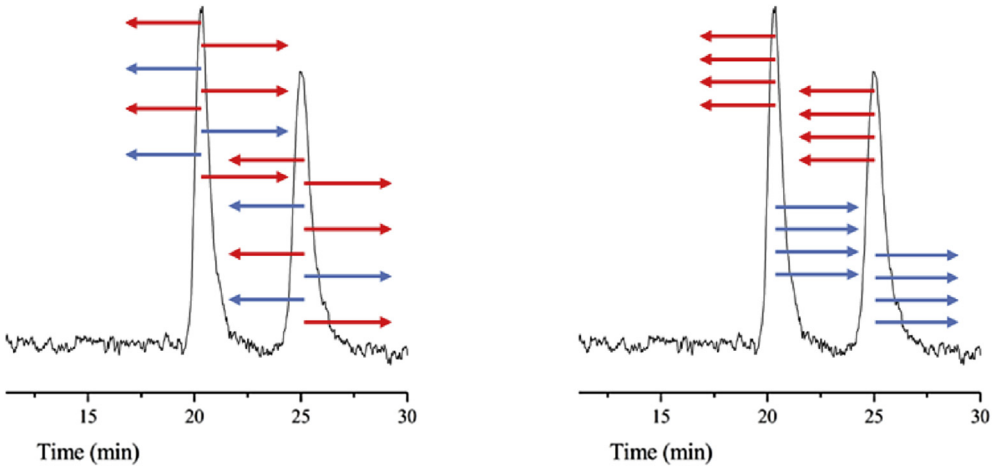

Fig. 2. Impact of peak retention correlation when two peaks behave independently (Left) or not, as in the case of API and impurity (Right).

of the method along with a set of performance criteria including: the parameters to be measured; the CMAs of the reportable results; their specifications and quality levels [3,15]. For example, in chromatography, CMAs could be the separation criterion (defined as the difference between the end of a first peak and the beginning of the second peak of the critical pair) [14].

2.3.2. Risk assessment

Based on the ATP, the most appropriate analytical techniques are selected and then risk-assessed to identify and prioritize potential factors that can affect their performances. To do so, devoted tools such as flow-charts and Ishikawa diagrams may be used to split the method into key sequences, and to identify and characterize potential risks associated with them. These risks can be prioritized with appropriate tools such as Failure Mode and Effects Analysis (FMEA), and then grouped into controlled factors, potential noise factors and CMPs [3,14,15]. Controlled and uncontrolled noise factors should be identified in order to perform a method ruggedness study, focused on uncontrolled factors [17].

2.3.3. Design of Experiments (DoE) and Analytical Design Space (DS)

Potential CMPs such as method and instrumental factors are further investigated for robustness using the statistical DoE and multivariate analysis. DoE is a critical step in understanding the method's operation and defining control strategies. Contrary to the OFAT approach, DoE is a structured, cost-effective and cost-efficient method to organize experiments and to determine the simultaneous effects and interactions of multiple CMPs on the CMAs. The ultimate objective of DoE is the definition of the analytical DS, which is the operating ranges of CMPs that guarantee quality results. This can ideally be derived from the multivariate statistical analysis of the DoE outputs.

2.3.3.1. Design of Experiments (DoE). DoE involves first the choice of appropriate experimental design(s). Generally, two types of designs might be used, depending on the number of CMPs to be tested and the complexity of the mathematical relationships between the CMAs and the CMPs. Although prior scientific knowledge should allow selection of the riskiest factors, if a large set of CMPs are identified, screening designs might first be needed to screen out those having negligible effects on the CMAs. The most commonly used screening designs are the popular Plackett–Burman or fractional factorial designs. Another common class of designs are the orthogonal designs or D-optimal designs and the more recent definitive screening designs [18]. Given their very low cost in term of experiments, such designs generally enable only limited understanding of interactions among CMPs, and thus provide insufficient method optimization. Consequently, optimization designs such as the central composite, the Box–Behnken and I-optimal designs are then used to find the combination of relevant CMPs that predicts the optimal CMAs with good precision [14,19]. The interested reader is invited to read specialized textbooks [20–22] for details on adequate DoE for DS.

2.3.3.2. Analytical Design Space (DS). The DS concept is intimately linked with the QbD approach. ICH Q8 defines the DS for pharmaceutical processes as the "*multidimensional combination and interaction of input variables that have been demonstrated to provide assurance of quality*". Applied to analytical methods, the DS may be defined as the set of all combinations of a method's input variables that have been proven to provide assurance of the quality of the data produced by the method [3,14,15]. In practice, the analytical DS corresponds to the range of operating conditions where future CMAs of the analytical method are within acceptance limits, with a high level of assurance. If the DS is large, the method can be considered robust; this is because changes of operating conditions within the DS will not degrade the quality of the results. It must be stressed that the concept of quality assurance underscores the need to indicate the likelihood of the method producing acceptable results [6,23,24], for instance expressed as a joint probability of meeting specifications for all CMAs.

A mathematical formalism to compute a DS that is fully compliant with this requirement of quality assurance, as stated in ICH Q8, has been proposed by Peterson [6] as:

$$\mathrm{DS} = \left\{ \tilde{\boldsymbol{x}} \in \chi : \Pr\left(\tilde{\boldsymbol{Y}} \in \mathbf{A} \middle| \tilde{\boldsymbol{x}}, \mathrm{data} \right) \geq \pi_0 \right\}$$

where $\tilde{\boldsymbol{x}}$ is a vector of CMPs, χ is the experimental domain, DS is the design space, $\Pr(\cdot)$ stands for the posterior probability of an event, $\tilde{\boldsymbol{Y}}$ is a vector of predicted CMAs, $\mathbf{A}$ is the subspace defined by the acceptance limits for the CMAs, π_0 is the minimum quality level, and data denotes the data used to build the model. In other words, the DS includes any point of the experimental domain whose predicted CMAs meet the specifications with at least a pre-specified probability π_0, given the data.

An effective approach to obtain predictions $\tilde{\boldsymbol{Y}}$ of the CMAs is to generate Monte Carlo samples from the joint posterior predictive distribution of the CMAs [23,24]. This predictive distribution is

derived from a Bayesian (multi-response) multiple regression of the CMAs as functions of the CMPs as proposed by Peterson [6] and Lebrun et al. [24]. Thus, this distribution accounts for correlations among CMAs and uncertainties related to both model parameters and common cause variation. In cases where the modelled responses differ from the CMAs, the predictive distribution of the CMAs can subsequently be computed as function of the multivariate predictive distribution of the modelled responses based on the Monte Carlo samples in a Monte-Carlo error propagation scheme. The Bayesian approach for multi-response optimization of methods is strongly recommended, due to is recognized high performance. The reader is referred to the textbook of del Castillo [21] for details on its rationale and technical implementation.

A widespread alternative method to compute the DS is the overlapping mean responses, which determines the DS as the subspace of CMPs' domain where the estimated mean responses of individual CMAs are all within specifications. However, this approach has several flaws that have been extensively demonstrated by many studies [6,14,19,23,24]. In brief, firstly, the models used do not account for correlations among multiple CMAs and uncertainties about unknown model parameters. Secondly, the predicted CMAs are mean values. Consequently, even if mean responses meet specifications, it is well-established that individual future runs of the method will not necessarily be within acceptance limits. Therefore, the DS-based mean responses may include operating conditions with low assurance of quality results, and therefore this approach does not produce a DS compliant with QbD expectations. However, such approaches are widely used by the scientific community.

2.3.4. Control strategy (CS)

Following the DS computation, it is recommended to define a control strategy (CS). The goal of the CS is to ensure that the chosen method performs as intended in routine use. Performance parameters for routine monitoring can be derived from the outcomes of the DS analysis. These parameters are known as validity tests or system suitability tests (SST).

2.4. Quality by Design (QbD) compliant methods for the determination of drug impurities: a critical review

The control of impurities during the manufacture of drugs involves two main steps: the control of raw materials before drug manufacturing; and the control of finished products before batch release. This two-stage control process requires selective, specific and sensitive analytical techniques. In this context, separation techniques are generally used (chromatography and electrophoresis) with UV or mass spectrometer detectors. Liquid chromatography (LC) is generally considered the gold-standard analytical technique involved in the raw material control, as described in the Pharmacopeias (EP, USP, BP). However, some of the standardized methods coming from the Pharmacopeia could be considered obsolete and several alternatives are now proposed in the literature. Among those techniques, optimized LC, capillary electrophoresis (CE) and its related techniques and, more recently, supercritical fluid chromatography (SFC) present several advantages such as efficiency, speed, sensitivity, etc.

Separation of API and its related impurities is highly challenging because of the similarity between the chemical structures of the targeted analytes. Moreover, the concentration level of API in comparison with impurities could lead to a really wide peak. In this context, method development using DoE is now generally used. Furthermore, as described above, the pharmaceutical regulatory requirements and advantages of QbD methodology have led to an increase in use of the QbD compliant analytical method for the determination of impurities. As explained previously, it is important to note that method development that does not take prediction error and its propagation into account does not allow the performance of QbD compliant optimization. Indeed, this approach does not manage the risk. Consequently, methodology involving mean response surfaces obtained using DoE to define optimal condition could not be considered as a QbD compliant approach. Consequently, some methods described in the literature as 'QbD approach' are not truly QbD methodology and are not discussed in the present paper. Several research groups focus their work on QbD implementation; their main papers related to detection of impurities are summarized in Table 1.

An illustrated flowchart of QbD compliant methods for detection of impurities is presented in Fig. 3. The first step of a QbD compliant method is the definition of method objectives by means of ATP. When dealing with detection of impurities, the ATP is mainly focused on method selectivity to ensure a complete separation between API, related and unknown impurities, and eventually excipients. A second objective is also to reach the required method sensitivity regarding the tolerance (e.g. 0.1% of API). Regarding published methods [5,25–42], method selectivity is the main target extensively studied during method development.

It is now well known that QbD encompasses DoE strategy; the definition of DoE is therefore also a crucial step in reaching the method objective. To determine DoE factors (CMP), a screening step using adequate screening design is generally used and recommended in the field of drug manufacturing (e.g. process optimization, etc.). Regarding analytical method development, the use of screening design could be considered a marginal practice. Indeed, empirical experiments are generally mandatory to provide to the analyst some preliminary results/information. In the specific case of chromatography, stationary phases (column) selection is performed before the definition of the DoE for two main reasons [34,42]. Firstly, stationary phase is a qualitative factor requiring the DoE to be performed integrally on each column and leading quickly to a huge number of experiments. Secondly, performing exactly the same DoE on different columns is not always relevant. It is also important to notice that some software [37] extrapolates data from one stationary phase to another, which is not chromatographically relevant. In the field of electrophoretic techniques, the selection of separation mode (e.g., CZE, MEKC, MEEKC, etc.) should also be determined before establishing method optimization design [25–32]. Afterwards, continuous factors are investigated, such as pH, organic content, gradient time, temperature, voltage, etc. When the analyst selects too many factors (CMP), a screening design is really interesting to help opt for the most relevant/influential ones before starting method optimization with maximum 3–4 factors [25–32]. In the literature, some applications dealing with Fusion software proposed a screening step with totally different CMP than those selected for the optimization step. A two-steps optimization denomination should be more adequate but shows also that this approach is not fully QbD compliant [37].

The response(s) to be studied should also be selected before starting the DoE. This corresponds to the CMAs, making it possible to fulfil the method objective. Method selectivity is the core business for the determination of impurity, and various CMAs could be selected: resolution Rs [25–28,30–32,37–40], selectivity α [33,35] or separation S [5,29,34,36,41,42]. A discussion about the advantages and drawbacks of these responses (Rs, S) regarding mathematical modelling is beyond the scope of this review and can be found in more specific papers [43]. Nevertheless, as mentioned in Table 1, analysts use several strategies: the CMA is applied to one of several critical pair(s) or to the whole panel of analytes. Moreover,

Table 1
An overview of QbD compliant method developments for detection of impurities.

Ref.	Analytes	Technique	Methodology to select CMP and CQA	Method optimization DoE	CMP	CQA	Statistical approach to define DS	Software
[25]	Amitriptyline and 4 impurities	Solvent-modified MEKC	Ishikawa diagram Screening design	Doehlert design 23	Voltage *n*-butanol concentration ACN concentration Urea concentration	Rs of two critical pairs Analysis time	Sweet spot plots to fix one factor (V) and compute DS on the three others Monte Carlo simulations	Modde
[26]	Drugs formulation: Captopril and 1 impurity – Hydrochlorothiazide and 3 impurities	Cyclodextrin- and solvent-modified MEKC	Preliminary empirical experiments Screening symmetric matrix	Central composite design 29	BGE composition: - pH - sodium cholate concentration - *n*-butanol concentration Voltage	Rs of two critical pairs Analysis time	Sweet spot plots and Monte-Carlo simulations	
[27]	Metformin and 5 impurities	Cyclodextrin-modified MEKC	Preliminary empirical experiments Screening asymmetric matrix	Doehlert design 23	Injection time CD concentration Buffer concentration pH	Rs of two critical pairs Analysis time		
[28]	Glibenclamide and 2 impurities	CE	Preliminary empirical experiments Screening symmetric matrix	Box–Behnken design 27	Voltage Injection time Buffer concentration pH	Rs of one critical pair Efficiency Analysis time		
[29]	Almotriptan and 3 impurities	MEEKC	Preliminary empirical experiments Screening asymmetric matrix	D-optimal 62	Temperature Voltage Buffer concentration Buffer pH Microemulsion composition: % aqueous phase, % oil phase, % surfactant	S of two critical pairs Analysis time		
[30]	Zolmitriptan and 5 impurities	CZE	Preliminary empirical experiments Screening symmetric matrix	Box–Behnken design 15	Buffer concentration pH Temperature	Rs of two critical pairs Analysis time Efficiency		
[31]	Diclofenac and 5 impurities	Cyclodextrin-modified MEEKC	Preliminary empirical experiments Screening design (D-optimal 16 exp.)	D-optimal 36	Voltage Buffer pH CD concentration Microemulsion composition: % aqueous phase, % oil phase, % surfactant	Rs of one critical pair Analysis time		
[32]	Levosulpiride and enantiomeric impurity	Dual-cyclodextrin chiral CE	Preliminary empirical experiments Screening asymmetric matrix	Doehlert design 23	pH sCD concentration nCD concentration Voltage	Rs Analysis time		
[33]	Bilastine and 2 degradation impurities	HILIC	Preliminary empirical study HILIC theoretical knowledge	Box–Behnken design 15	ACN content (%) Ammonium acetate concentration pH	Selectivity of critical pair Analysis time		
[34]	Olanzapine and 7 impurities	HILIC	Chromatographic screening Preliminary empirical study	Rechtschaffen design 10	Temperature Initial aqueous phase content (%) Gradient time	S between two critical pairs		

(*continued on next page*)

Table 1 (*continued*)

Ref.	Analytes	Technique	Methodology to select CMP and CQA	Method optimization DoE	CMP	CQA	Statistical approach to define DS	Software
[35]	Pramipexole and 5 impurities	RP-HPLC	Preliminary empirical study	D-optimal 29	Stationary phase Salt type Salt concentration % ACN Temperature	Retention of first and last eluting peaks Selectivity	Retention factor modelling to predict the CQAs and Monte-Carlo simulations	Design-Expert and Matlab
[36]	Dabigatran etexilate mesilate and 10 impurities	RP-HPLC	Preliminary empirical study	Box–Behnken design 16	Gradient time % ACN initial % ACN final	S	Retention times modelling to predict the CQAs and Monte-Carlo simulations	
[37]	Drugs formulation: Naproxen and 6 impurities – Sumatriptan and 5 impurities	RP-UHPLC	Fractional factorial design (pH – stationary phase – organic modifier) (44 experiments)	Fractional factorial design 20	Flow rate Temperature Gradient time	Rs Number of peaks	Process capability index using Monte-Carlo simulations	Fusion
[38]	HIV-tritherapy: lamivudine, abacavir, dolutegravir and 26 impurities	RPLC	Previous methods used in the lab	Full factorial design + complementary experiments to reach central composite design 19	pH Temperature Mobile phase part B %	Rs of 6 critical pairs RT of 2 compounds S of 2 critical pairs	Overlay of response surface and Monte-Carlo simulations	Devize
[39]	Pramipexole and 4 impurities	RPLC	Based on compendial method (European Pharmacopeia)	Full factorial design 21	Gradient time pH Temperature Independent eluent study: ionic strength and ion pairing concentrations	Rs	Retention times modelling Rs prediction	DryLab
[40]	Terazosin and 10 impurities	RP-UHPLC-MS	Preliminary empirical study	Full factorial design 12	Gradient time Temperature Ternary eluent composition			
[5]	Confidential API and 4 impurities	RPLC-MS	Preliminary empirical study	D-optimal mixture design 33	Mobile phase composition: MeOH/ACN/buffer Temperature	S	Retention times modelling Bayesian model and Monte-Carlo simulations to obtain the distribution of S	JMP and R
[41]	Cholecalciferol (vitamin D3) and 3 impurities	SFC	Preliminary empirical study	Central composite design 35	Temperature % EtOH final Gradient time			
[42]	Salbutamol sulphate and 5 impurities	SFC	Preliminary empirical study Chromatographic screening	Central composite design 27	% MeOH initial Gradient time Pressure Temperature			

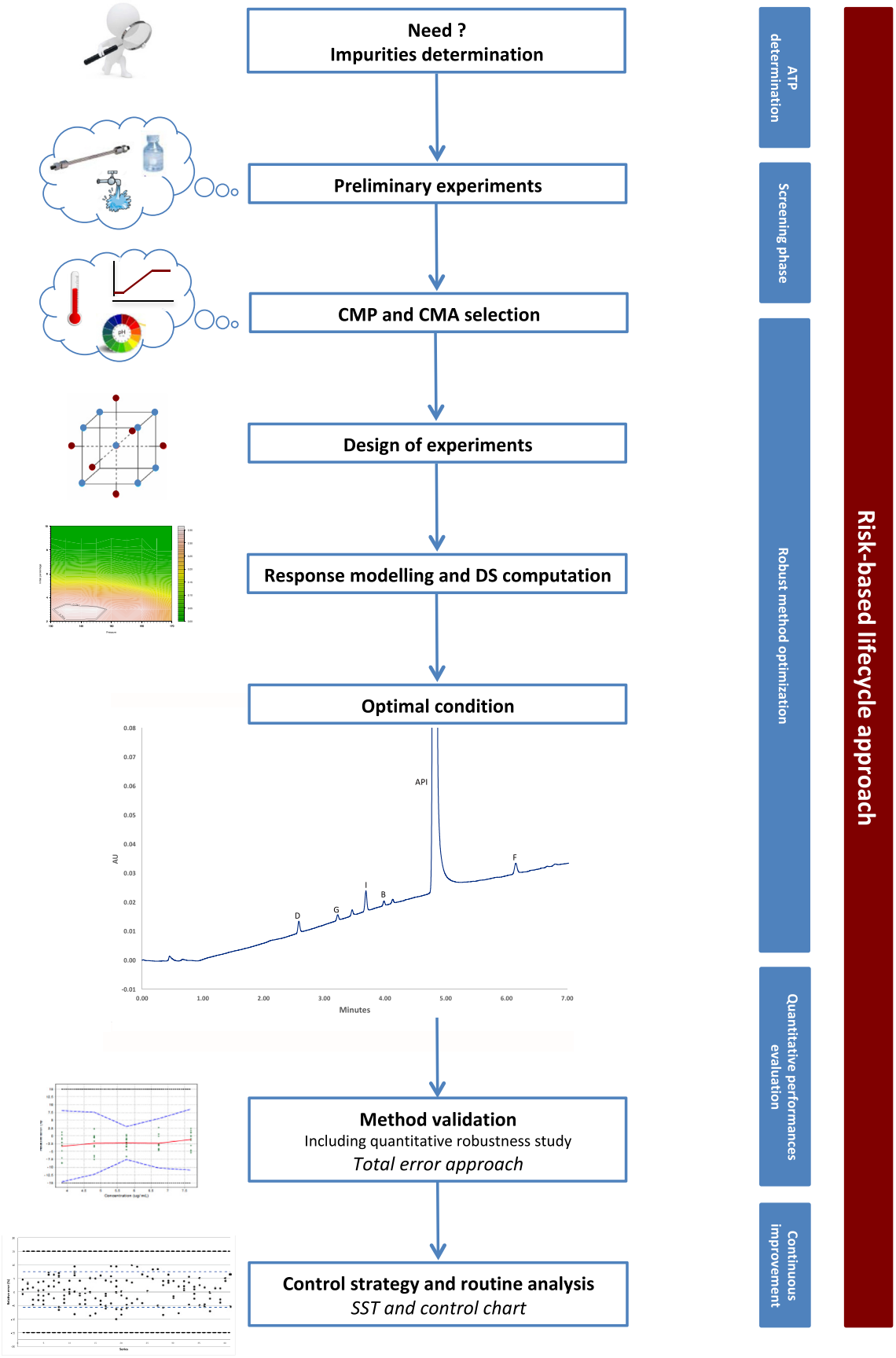

Fig. 3. Flowchart: QbD compliant methodology for the determination of impurities.

one or more CMA could be added, such as the analysis time, the peak efficiency, etc.

Following the definition of CMP and CMA, the DoE should be selected according to the method objective. As expected, the referenced methods [5,25–42] are most often used in design optimization. However, data treatment to model and compute the DS is one of the most interesting topics to discuss. Different strategies are published: the most popular one is to overlay contour plot (or sweet spot plot) for each CMA and then perform Monte-Carlo simulations to define the DS [25–34,38]. Using this strategy, the CMA modelling is performed independently by neglecting the interactions and could lead to method misunderstanding. Furthermore, the independent study of different CMPs is not relevant in reaching method knowledge/understanding and adequate DS computation [20]. Joint prediction is a really useful tool to simultaneously model all required CMAs, as described in several papers [5,41–45]. This statistical strategy is mandatory to reach an in-depth method understanding compliant with ICH Q8 requirements.

Regarding the definition of DS, the method robustness is directly assessed during method optimization. In this context, robustness testing is no longer required. Some papers propose robustness study not as a mandatory analytical method lifecycle step but as a demonstration of the reliability of the DS methodology. Even if an extensive robustness study is not required, it is a good practice to verify several DS conditions to ensure that the CMAs are met. It also important to note that the majority of published QbD compliant methods focus on qualitative CMAs (i.e., separation, resolution, etc.), so the method should be robust regarding qualitative criteria. Consequently, robustness of quantitative performances should be studied before or during method validation. More extensive discussion about method robustness study in the context of a risk-based approach can be found in a specific reference dealing with the determination of impurities [46].

Following the optimization step using AQbD strategy, the quantitative performance of the method should be evaluated by means of method validation. In this context, the SFSTP commission proposed a validation strategy focused on risk management and using a clear decision tool as recommended by ICH Q9 [1,19,20,47–50]. Using accuracy profile as a powerful decision tool has already been demonstrated for detecting impurities with LC or SFC methods [5,41,42]. The full integration of optimization and validation phases was also proposed in order to perform an evaluation of the quantitative performances of the whole robustness area [44]. This first demonstration is a really interesting perspective regarding pharmaceutical impurities applications. Considering this approach, the CMA could also be the quantitative performances expressed as trueness, precision or accuracy, with the ATP describing the minimal requirements for these criteria to achieve a method usable for product characterization and release.

3. Conclusion

The detection of impurities is a major concern for quality control of raw materials and finished products. Separation techniques are widely used for detection of impurities regarding selectivity and sensitivity requirements. To deal with close chemical structures and different concentration levels between API and impurities, a systematic approach to method development is advised. In this context, the authors proposed the AQbD strategy, which encompasses the use of DoE and the computation of a probabilistic DS. AQbD strategy facilitates in-depth understanding of method and risk management.

Different AQbD approaches are available in the literature and discussed above. As expected, electrophoresis and chromatography are reference techniques for detection of impurities. The method optimization is most often focused on separation of peaks. The research literature presents independent studies of different CMP. This methodology is not totally relevant to developing method knowledge and appropriate DS computation. Joint prediction is a really useful tool to simultaneously model all required CMAs, as described in several papers. Furthermore, AQbD compliant method optimization has to take into account the prediction error, and its propagation, in order to manage the risk. In this context, both DoE and the statistical approach used to compute the DS should be selected carefully.

Finally, API and impurities separation is not enough to properly fulfil the ATP. The method's quantitative performance should be evaluated by means of analytical method validation. The total error approach must be promoted with reference to pharmaceutical risk management requirements.

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