A State of the Art in Analytical Quality-by-Design and Perspectives in Characterization of Nano-enabled Medicinal Products

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Abstract

Quality-by-Design (QbD) guidance is a risk-based and proactive approach to drug development proposed in the mid 2000s and now widely used in the pharmaceutical field in compliance with the ICH Q8-Q11 guidelines. Analytical Quality by Design (AQbD), introduced in 2010, is the adaptation of the QbD paradigm for the development of analytical methods. AQbD aims at optimizing the accuracy and robustness of analysis results by identifying and controlling critical analytical variables and method parameters over the entire protocol, including biological sample preparation, measurement technology and statistical analysis. Nevertheless, much remains to be done for a clear understanding and an efficient implementation of this new paradigm in practice. The first objective of this review is to propose a global clarification of the Analytical Quality by Design approach by reviewing its terminology and steps and by clarifying its relationships with the well-established QbD paradigm and ICH guidelines. Two new templates of documents have been proposed: a form designed for the definition of the analytical target profile and a connection matrix between expected metrological properties and analytical attributes. Finally, the open challenges in the characterization of nano-enabled medicinal products are examined from the AQbD angle.

Keywords: Analytical Quality-by-Design, Physico-Chemical Characterization, Nanoparticle, Nanomedicine.

1. Introduction

Nanomaterials have become a considerable area of interest in the medical field for the development of new diagnostic and therapeutic tools as innovative solutions to unmet clinical needs [1]. Until now, nanomaterials in the pharmaceutical field have been mainly exploited as nano-carriers of poorly soluble drugs, to increase their bioavailability and delivery, increasing their therapeutic effects and reducing detrimental side-effects linked to systemic toxicity. During their marketing approval process, nano-enabled medicinal products are evaluated with the same process that applies to all products. Therefore, they must meet the same safety and quality criteria applied to all drug products that do not contain nanomaterials. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [1] has proposed different guidelines for the validation of analytical procedures (ICH Q2)[2], stability testing (ICH Q1A & Q1B)[3][4], chemical analysis and determination of impurities (ICH Q3A)[5]. In parallel, this

[1] https://www.ich.org/

committee has also proposed a set of guidelines (ICH Q8 to Q12) dedicated to the application of Quality-by-Design to drug development[6][7][8][9][10].

Due to the complex nature of these products, additional attributes must be taken into consideration for the evaluation of their quality, and safety, as well as for the determination of their pharmacokinetic profiles. For certain classes of nanomedicine, such as liposomes, polymeric micelles or iron-based colloidal products, the regulatory information requirements associated with the presence of nanomaterials in a drug product have been compiled in guidance documents released by the European Medicines Agency (EMA), Food and Drug Administration (FDA) and Ministry of Health Labour and Welfare (MHLW) [11] [12].

One early key step of the development of nano-enabled medicinal products is to characterize their physical and chemical properties, which generally include measurements of material attributes such as size and size distribution (polydispersity), morphology, surface charge, crystallinity, chemical composition, the analysis of surface properties, drug loading and importantly, the assessment of their chemical and physical stability in biological media. In this area, several European projects, such as EUNCL and REFINE , have been successfully contributing to define robust testing strategies for the physical-chemical (PCC) analysis and for the physico-chemical, morphological and topographical (PCMT) characterization of nano-enabled medicinal technologies. Starting with assisting the translational of nanomedicine products the European Nanomedicine characterization laboratory (EUNCL), jointly working with the NCI-Nanotechnology Characterization Lab, has developed robust characterization protocols integrated in a holistic and transdisciplinary testing infrastructure covering a comprehensive set of preclinical characterization assays (physical, chemical, and biological testing) enabling researchers to fully understand the administration, biodistribution, metabolism, pharmacokinetics, safety profiles and immunological effects of several typologies of nanomedicine products. Both characterization laboratories promoted a multistep approach tailored on a case-by-case basis to measure the physical and chemical properties of nanomedicine in single and complex relevant media, stressing the importance of implementing adapted tools such as orthogonal approaches of incremental complexity, robust standard operating procedures (SOPs) and quality controls. Similar initiatives have now been launched in the field of nano-enabled medical technologies, as part of the European Open Innovation Test Bed that offers services for every step of the value chain, including preclinical and clinical testing. This is the case of the European projects SAFE-N-MEDTECH[2] and TBMED[3].

Furthermore, the development of a regulatory science framework on nano-enabled medicinal products and medical devices from the REFINE project[4], assisted by the development of an informed and robust Regulatory Science Framework for the risk-benefit assessment of those nanotechnologies, has started by identifying the regulatory needs in material characterization [11]. Then, by mapping the technological solutions to address those needs, the consortium began performing interlaboratory comparisons to assess their suitability and robustness. Currently, a starter set of standard operating procedures covering the immunological assessment and genotoxicity of nano-enabled medici-

[2]https://safenmt.eu/

[3]https://www.tbmed.eu/

[4]http://refine-nanomed.eu/

nal products is under assessment under dedicated interlaboratory tests comparison studies. Other initiatives, driven by metrological institutes including ASTM E56 and ISO TC229, are developing standard methods for the physical and chemical characterization of nano-enabled medicinal products and medical devices [13], as well as performing interlaboratory exercises to test their robustness [12].

The intermediate precision and reproducibility of physicochemical and biological data has often proved very unsatisfactory. The factors affecting the quality and completeness of data on nanomaterials need to be considered with the objective of improving their reliability [14]. Among these factors, let us mention in particular: (i) the availability of standard reference materials, (ii) the deployment of reliable and robust SOPs suited to the preclinical characterization of nanoparticles (NP) used in medical applications, (iii) the selection of smart combination of methods and testing conditions to design a robust and informative testing cascade, that could be realistically transferred in an industrial setting during the R&D phase. The variety of methods and tools used for this purpose prevents any robust conclusion and sometimes introduces confusion and uncertainty.

While sample preparation and testing conditions have to be controlled and standardized for comparative and quality purposes, at the same time they need to be representative of the clinical application of choice (dose, administration route, monitoring and other), which is often not trivial and must be selected on a case-by-case basis [15]. Importantly, sample preparation should not induce artefacts, *e.g.* material aggregation, that could affect the analysis. Due to the incremental complexity of the systems analyzed *vs* standard pharmaceuticals and medical devices, the characterization of nano-enabled medicinal products often requires the use of complementary assays to support the results. Nonetheless, these have to be planned and not become a burden on the developer. Issues such as which methods to be used, and how many assays to perform to guarantee a robust characterization without rendering the experimental complexity too onerous for companies, are all to be considered and accounted for during early product assessment.

For all these reasons, the need to establish well-defined and safe methods, has been a growing aspect of research. Analytical Quality by Design (AQbD), introduced in 2010, is the application of the QbD concept, strongly recommended by FDA and EMA, to analytical method development [16]. AQbD aims at optimizing accuracy and robustness of analysis results by identifying and controlling critical quality variables and risk factors over the complete protocol, including biological sample preparation, measurement technology and statistical analysis. Over the last decade, a few studies have tried to provide a framework of this new concept, such as Peraman *et al.* [17], Jayagopal *et al.* [18] and G.Vogt *et al.* [19] through article reviews. However, as this paradigm is quite new in the field, it still suffers from a lack of clarity regarding the implementation workflow as well as consistent definitions of its compounds. Our review work, presented here, focuses on three objectives: (i) providing a clear workflow of AQbD while clarifying its position in relation to the QbD parent process; (ii) proposing more precise and generic content concerning two key documents produced at the beginning of the AQbD process; (iii) identifying new questions and challenges concerning the characterization of nanomedicine products.

With these aims in mind, the review is organized as follows. Section 2 introduces the concepts of Quality by Testing, Quality by Design and Safety by Design to assess risks during the development of new pharmaceutical products. Section 3 describes in detail the QbD and AQbD workflows by explaining their constitutive steps and key milestones.

Section 4 focuses on the lacks and weaknesses of the current AQbD approach and proposes two document templates associated with the first two stages of AQbD. Section 5 examines the AQbD needs to develop robust standards of protocols for the off-line and on-line characterization of nanomaterials.

2. New bricks in Quality Control

Historical background. The concept of "quality control" was introduced in 1924 by W. A. Shewhart [20], in which he notably introduced tools for statistical process control like control charts. He is also recognized as the author of the quality cycle: Plan-Do-Check-Act, which would be popularized in the 1950s by W. E. Deming. The latter collaborated a lot in post-war Japan with J. J. Duran, inventor of the Pareto Diagram, also a specialist in quality control and at the origin of the name *Quality by Design* [21]. Both have made it possible to create in Japan a dynamic centered on quality with, on the one hand academics such as K. Ishikawa, inventor of the 5M diagram, and G. Taguchi who democratized the methodology of experimental design, and on the other hand manufacturers such as T. Ohno and E. Toyoda within the Toyota automobile company which became one of the pioneering companies in terms of quality by design. A few years later, this proactive approach of quality gave rise to two paradigms: (1) Lean Manufacturing promoted by J. Krafcik [22] by restructuring the concept of *muda* invented by the Japanese a few years earlier and (2) the Six-Sigma approach based on the "Define, Measure, Analyze, Improve, Control" cycle, initiated by the Motorola company. These two trends ultimately gave rise to a synthesis entitled Lean Six Sigma in the early 2000s. All these methods only address quality of the manufacturing process, without considering the development of innovative products. The Design for Six Sigma paradigm was proposed in the early 2000s to address this absence.

At that time, FDA officials drew on all this work to reform the way drugs were developed. A founding paper was published in 2004 [23], which would lead to the international directive ICH Q8[5] and the official birth of the pharmaceutical Quality by Design, revised in 2009 [6]. It includes a circular methodological structure and statistical risk assessment tools to ultimately reduce development delays and costs while optimizing the quality of pharmaceuticals from earlier steps of development.

Before QbD, quality assessments were based on the Quality-by-Testing (QbT) approach, where product compliance with ISO standards was monitored only after the process design and drug manufacturing. Unfortunately, when problems are detected then, it is usually too late to fix either the produced batch or the process which can take multiple cycles before getting it right as illustrated in Figure 1. In [24], Dispas *et. al.* proposed another diagram comparing the content of the QbT and QbD paradigms. Conversely to QbT, QbD handles risks from the early development steps, before process design and drug manufacturing. The overarching goal of Pharmaceutical QbD is to minimize development delays and maximize the added value of the product, while extra benefits include not only increased efficiency and quality of the manufacturing process, but also the reduction of production costs and facilitated regulatory approval.

QbD & SbD. The Safe-by-Design (SbD) paradigm addresses the identification and reduction of environmental and health risks in the early steps of product development. Already applied in many different industrial areas, it was

[5] https://www.ich.org/page/quality-guidelines

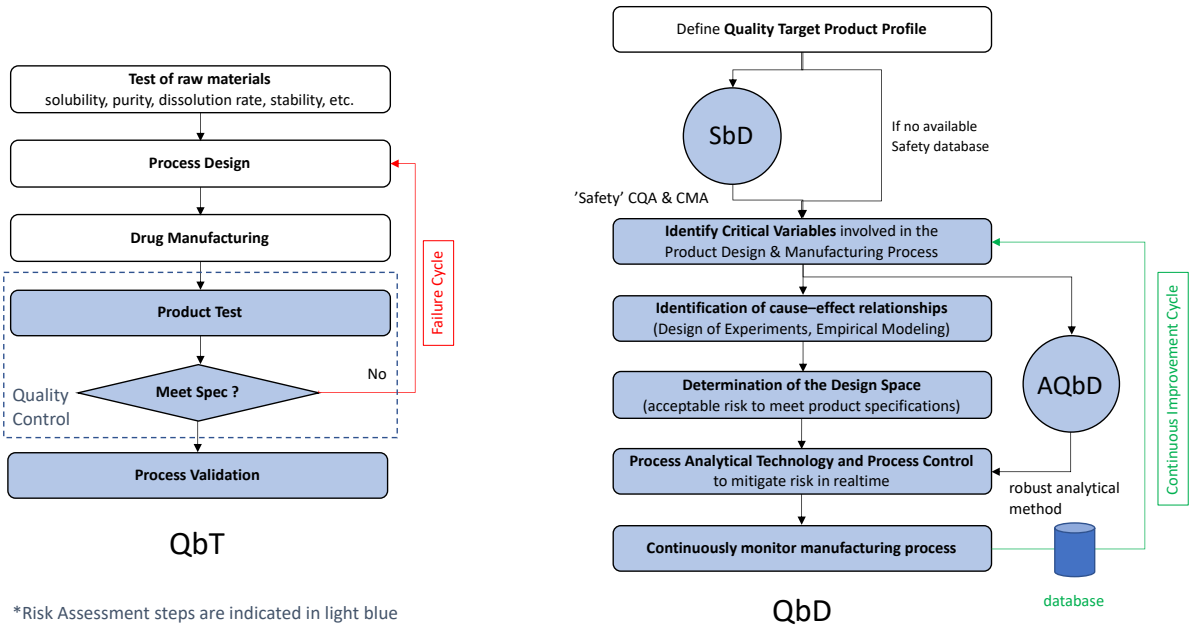

Figure 1: Quality by Testing vs Quality by Design. Steps involving risk assessment are indicated in light blue. CQA: Critical Quality Attributes, CMA: Critical Material Attributes

brought into the field of nanotechnology to deal with the uncertainty of the potential harmful effects on humans and the environment. SbD was extensively tested and applied within the Flagship European project NANoREG[6] and then complemented by the European ProSafe project with the preparation of industrial cases for regulation. This concept is new and not yet included in ICH, EMA, or FDA guidelines. Nevertheless, SbD could be used in QbD in the same way as the Failure Mode, Effects and Criticality Analysis method to assess the criticality of some attributes related to safety outcomes. Used in this way, as illustrated in Figure 1, SbD can be integrated as a key component of QbD to pre-identify critical safety attributes.

QbD & AQbD. The successful implementation of QbD relies on efficient process analytical technology and process control strategy. The latter is used to continuously mitigate risks and maintain the operating point in the normal operating region, yet this goal cannot be reached without a reliable measurement of critical process parameters. In practice, the latter are often estimated on-line by means of spectroscopic technologies, such as Raman or Near Infrared probes. A biased estimation of those parameters could lead to inaccurate tracking of the real operating point and therefore the risk of being out of specification without knowing it. To avoid this situation, we need to develop robust analytical methods, which is the subject of a dedicated QbD paradigm entitled 'Analytical Quality by Design'. More details on this subject can be found in ICH Q2 and in the draft version of ICH Q14 [25].

QbD with regard to Assurance/Control Quality. If quality assurance relates to how a process is performed or how a product is made, and if quality control deals with the inspection aspect of quality management, then QbD can be

[6]http://www.nanoreg.eu/

described as an integrated vision of those two quality aspects. On the one hand, through the concept of "design space", QbD aims to identify regions of critical material attributes and process parameters meeting the quality requirement with an acceptable level of risk. On the other hand, QbD involves process analytical technology (PAT) and testing procedures to monitor critical quality attributes and check their compliance with the initial quality requirements.

3. QbD and AQbD

In this section, after recalling the main concepts of QbD, we will present those of AQbD and how they are linked. Figure 2 shows two quality circles, in green for the QbD and in red for AQbD. The AQbD cycle is inspired by that proposed by Deidda *et al.* in [26] with an additional step dedicated to the continuous improvement of the method. Those two circles are nested at two levels. The metrological requirements specified at the beginning of the AQbD cycle are directly related to the choice of critical quality attributes in the QbD process. Secondly, the AQbD results are directly used in the implementation of the process analytical technologies in QbD [27]. It should be noted that several studies have proposed other constitutive diagrams of the AQbD process. This is notably the case in Parr and Schmidt's review published in 2018 [28].

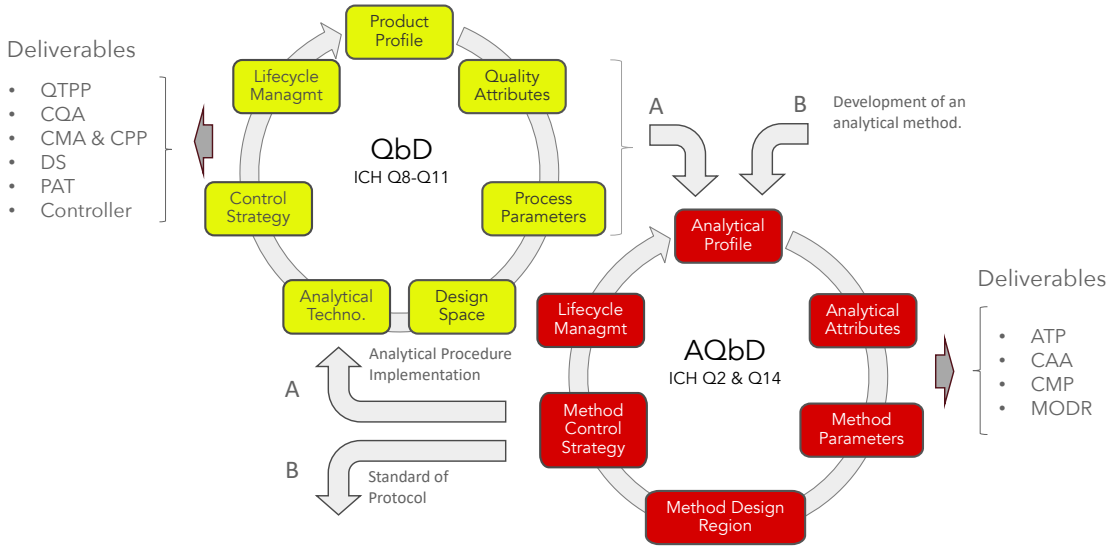

Figure 2: A: AQbD cycle (red) is used as a complement of the QbD cycle (green) to develop a robust and reproducible analytical procedure associated with the process analytical technology. B: AQbD is used independently to develop a standard of protocol for a given characterization/detection technique. QbD deliverables: QTPP (Quality Target Product Profile), CQA (Critical Quality Attributes), CMA/CPP (Critical Material Attributes/Critical Process parameters), DS (Design Space). AQbD deliverables: ATP (Analytical Target Profile), CAA (Critical Analytical Attributes), CMP (Critical Method Parameters), MODR (Method Operable Design Region)

3.1. Basic reminders on QbD

The first step of QbD is to define the Quality Target Product Profile (QTPP) of the ideal drug product we wish to develop. Herein, quality encompasses efficacy as well as safety aspects. This includes information like the intended use, dosage form & appearance, route of administration, stability, physical attributes, purity, sterility and water content, but

it can also include business information about the targeted market, as emphasized in [29]. Its content must be regularly updated throughout the development process.

The second step aims at defining the Critical Quality Attributes (CQA) of the product. A Quality Attribute is a physical, chemical, biological or microbiological property or characteristic that must be kept within an appropriate range to ensure the desired product quality defined in the QTTP. Criticality of quality attributes is defined with respect to several areas of interest:

- product efficacy: is the quality attribute a relevant metric of therapeutic benefits?
- product safety: is the quality attribute a relevant metric of adverse effects?
- product quality: is the quality attribute a relevant metric of reliability, stability or any other perceived quality of the product?
- regulatory compliance: is the quality attribute a relevant metric of compliance with respect to laws, standards or regulatory specifications?

Multiple tools can be used to assess criticality of quality attributes, most of which are exposed in the ICH-Q9 guidelines. It can be just a summary Risk Assessment Chart with a simple traffic light color to indicate the rank of the criticality (red for critical, amber for potentially critical and green for non-critical). In this case, the criticality level of each CQA is assessed by expert staff. A more sophisticated tool is the Failure Mode & Effect Analysis (FMEA, see IEC 60812) technique. FMEA has been around since the 1940s with the objective to identify potential failures occurring during the manufacturing, assembly and design processes. During this analysis, a team of experts has to sort the risks from highest to lowest according to an RPN scale. The RPN (risk priority number) accounts for the likelihood of failure, its severity and its detectability. It is calculated by multiplying these three attributes and allows for rank failures. However, its applicability to assess criticality of Quality Attributes is not entirely proved since a physical/chemical/biological property is not exactly like a failure. For instance, the notions of occurence and detectability for a QA are often unclear and difficult to objectively estimate. All these risk assessment tools rely on declarative information, which can be drawn from different sources: reports on previous studies and investigations, experience, product history, regulatory feedback, quality reviews, adverse effect reports, recalls, or databases, such as the OECD database on the safety of manufactured nanomaterials [7].

In the third step, two categories of risk factors (Material Attributes and Process Parameters) are firstly enumerated and secondly ranked according to a criticality scale of impact on the critical quality attributes. Unlike the latter, these input variables can be manipulated and used as control levers to finally ensure that CQA specifications are met. Their criticality level is assessed either through an FMEA approach or after the application of specific experimental designs [30] able to screen and analyze critical effects with a limited number of assays.

The mathematical relationship between quality outcomes (CQA) and input factors (CMA and CPP) is experimentally determined in a fourth step using specific statistical methods for the design of experiments and empirical modeling.

[7] http://www.oecd.org/env/ehs/nanosafety/publications-series-safety-manufactured-nanomaterials.htm

In practice, response surface equations, a class of polynomial models, are often used to describe the links between input and output variables. This type of model enables us to compute a Design Space: a risk mapping tool used to identify the operating region of quality. The Design Space can be divided into three regions of probability:

- the Out Of Specification (OOS) region, in which the probabilities of meeting the technical requirements on CQA are too small. In such a situation, deeper investigations are required to understand the reasons for unacceptance;
- the Proven Acceptance Region (PAR) , in which the probabilities of meeting the technical requirements are acceptable but some adjustments should be made to access the NOR;
- the Normal Operating Region (NOR) corresponds to the desired region where CQA have a high degree of probability of complying with their quality specifications;

The fifth item introduces the technological chapter of QbD, beginning with the Process Analytical Technology (PAT), *i.e.* the equipment and software able to measure off- or on-line critical attributes and process parameters [31]. This data provides real-time information about the location of the operating point within the Design Space. Near-infrared spectroscopy-in-line, Raman in-line, chemical imaging, particle imaging and measurement, solid state nuclear magnetic resonance, powder X-ray diffraction or tetrahertz pulsed imaging are all examples of PAT implemented in the pharmaceutical industry. Some emerging PAT addressing nanomaterial specific properties are technologies for online sizing, such as spatially resolved dynamic light scattering (SR-DLS) provided by the NanoFlowSizer [32] (for more details see Section 5).

The sixth QbD component addresses the question of the real-time adaptation of some process parameters so as to maintain critical quality attributes within regions of quality despite variations of background variables. Some solutions implemented to solve that control issue are state feedback control, statistical or Bayesian process control [33, 34].

The last component of the QbD paradigm deals with the management and storage of production data in secured databases. This data can be used in a second round to refine the product knowledge and so to continuously improve its quality.

3.2. Main steps of AQbD

Introduced in 2010 in a position paper [16], AQbD extends the QbD concept to the development of robust and cost-effective analytical methods. In a sense, AQbD introduces risk management in the ICH Q2 validation guidelines of analytical procedures [2]. AQbD can be regarded as a subset of QbD but can also be used independently. For the latter, AQbD can be useful to support the development and interlaboratory testing of methods for the characterization of the drug substances and impurities. The development of the AQbD approach [35] was motivated by several goals: to harmonize vision and terminology between different quality management areas, and more particularly those concerned by the development of innovative analytical methods; to facilitate lab-to-lab transfers; to understand how variation in some protocol parameters can affect analytical results; to incorporate techniques and methods of risk analysis and to meet and maintain method performances within desired metrological specifications.

3.2.1. Analytical Method Target Profile (ATP)

Analytical Quality-by-Design starts with the definition of an analytical method target profile (ATP). The proper use of this ATP is to ensure that the method under development fits with the targeted purpose [27]. Attention should be paid to the fact that ATP is initially associated with some CQA, CPP or CMA variables that must be measured in a QbD cycle for the implementation of a process analytical technology, as emphasized in Figure 2. Nevertheless, it could also be used to optimize and validate the standards of protocol for some specific analytical methods. Whatever the targeted application, the operational goal of the method and end-user requirements should be clearly expressed [19]. Moreover, since several competing methods may be candidates for measuring quality attributes, Borman *et al.* have recently proposed a structured approach to select the most appropriate analytical technology.

As stated earlier, so far there has been no guidelines to writing the ATP form. Its content depends on the authors' knowledge and expertise [27]. Nevertheless, at least six constitutive elements are essential:

- Definition of the intended application or AQbD scopes: either for the PAT implementation in a QbD framework or for the optimization of standards of protocol (SOP) independently of any QbD implementation. This could be the CQA, CMA or CPP of a QbD study, in which a Process Analytical Technology has to be implemented, or biological, chemical or physical properties concerned by a SoP has to be developed and validated. Table 1 presents an example of ATP template applied to Basso et al. case study. The objective is to develop an analytical procedure to measure two CQA: drug loading and encapsulation efficiency.

- Which variables are to be measured by the analytical method? In Table 1, the determination of drug loading and encapsulation efficiency requires the measurement of three concentrations: Atorvastatin calcium, curcumin and celecoxib. These three quantities are called measurands[8] thereafter. It can happen that the CQAs and the measurands are the same, especially when the CQAs are directly measurable.

- Which method(s) is/are able to measure the selected critical variables? A reversed-phase high performance liquid chromatography is used in [36] to measure concentrations of curcumin, atorvastatin calcium and celecoxib.

- Which equipment/technology could be implemented? Basso *et al.* used a LC-2010C HT liquid chromotograph (Shimadzu Co., Kyoto, Japan) equipped with a quaternary pump, a CTO-10AS oven and a SPD-M2OA detector.

- What are the metrological properties sought? The ICH Q2(R1) guideline defines several criteria to validate analytical procedures: accuracy, repeatability, intermediate precision, specificity, detection limit, quantitation limit, linearity, range, robustness and associated quantitative specifications. This same directive lists four types of application for which the validation criteria change. In our example, the measurement of concentrations corresponds to a quantitative test of analytes in samples of drug substance (fourth column, in gray, in Table 1). In this case, ICH Q2(R1) recommends using nine specific criteria to validate the method.

[8] ISO 10241:1992 - International terminology standards: particular quantity subject to measurement

- What are the references of publications or guidelines explaining the material and method used to measure the same critical variables?

We propose gathering all these characteristics in a new ATP template presented in Table 1. Its content relies on information provided by Basso *et al.* [36] for the selection and optimization of a reversed-phase high performance liquid chromatography (RP-HPLC) method to simultaneously quantify curcumin, atorvastatin calcium and celecoxib.

Table 1: An example of ATP template applied to Basso *et al.* case study. The specifications shown in this table have been arbitrarily added as illustrative examples.

	Target	Information					
Intended Application	Measurement of two Critical Quality Attributes in the QbD development of lipid nanoparticles : drug loading and encapsulation efficiency						
Measured Variables	Concentration of Atorvastatin calcium (ATO), curcumin (CUR) and celecoxib (CXB)	The method shall quantify ATO, CUR and CXB co-encapsulated in bulk and entrapped forms of lipid nanopaticles both for routine and stability analysis					
Metrological Properties	**Property**	**Application**				**Selection**	**Specification ?**
		Identification	**Impurities Limit**	**Impurities Quantitative**	**Assay Potency**		
	Accuracy	-	-	+	+		RRMSE<0.1
	Repeatability	-	-	+	+		relative standard deviation < 2%
	Intermediate Precision	-	-	+	+		relative standard deviation < 2%
	Reproducibility	-	-	+	+		relative standard deviation < 2%
	Specificity	+	+	+	+		
	Detection Limit	-	+	-	-		
	Quantitation Limit	-	-	+	-		
	Linearity	-	-	+	+		RRMSE<0.1
	Range	-	-	+	+		0.5–100 mg mL-1
	Robustness	-	-	+	+		
	(-) means that this characteristic is not mandatory to be assessed in this application and (+) means that this property shall be evaluated						
Method	Reversed-phase high performance liquid chromatography	RP-HPLC is an analytical technique able to separate highly lipophilic substances with a fast analysis, small sample volume, and low organic solvent consumption.					
Equipment	Liquid chromatograph	A quaternary pump is used to get a precise mixing of mobile phase solvents, and a photodiode array is used to detect more than one substance over a wide range of wavelengths.					
Ref.	Basso *et al.* [36]						

3.2.2. Critical Analytical Attributes

The second main step of the AQbD approach deals with the identification of Critical Analytical Attributes (CAA) of the analytical procedure, *i.e.* the metrics or response variables, which characterize the metrological performances of the analytical method [37]. In our study case[36], the retention time, peak area, peak resolution and tailing factor have been considered as analytical attributes. The first two are used to identify the type of analyte and to estimate its concentration respectively. In chromatographic method development, other examples of CAA are the critical resolution, run time,

efficiency, method selectivity, time of analysis, precision and robustness [38]. The identification of critical analytical attributes is similar to that of critical quality attributes (CQAs) in QbD [39]. These attributes are all the more critical as they are potentially directly or indirectly linked to the metrological validation criteria listed in Table 1. In other words, their values can invalidate the method of analysis. In Table 2, we have proposed a matrix template to more clearly identify the connections between the metrological validation criteria used in the ATP and the measurable characteristics corresponding to analytical attributes. If one of these attributes plays an active role in the evaluation of one of the metrological criteria then it becomes critical (red) for this criterion and non-critical (green) otherwise. As soon as an attribute has at least one red box in Table 2 it is considered critical and it is therefore advisable to specify the associated validation threshold.

To illustrate its content, we have here also borrowed the example of Basso's study. This table presents the expected metrological characteristics defined in the ATP form in rows and four Analytical Attributes in columns. For each of the four attributes, we applied the criticality rule stated above. Consider the example of the Peak area attribute and its potential impact on the linearity criterion. Let us note Y the true and unknown concentration (measurand) and X the peak area. From an experimental dataset, a nonlinear relationship between them was identified:

$$Y = f(X) + E, \tag{1}$$

where $E \sim \mathcal{N}(0, s^2)$ is a random variable describing effects of the experimental errors and measurement noise. A predicted value $\hat{Y}$ of the analyte concentration is:

$$\hat{Y} = f(X) \tag{2}$$

and the linearity criterion is here described by the relative root mean squared error:

$$RRMSE = \frac{\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_i - \hat{Y}_i(X)\right)^2}}{\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_i - \bar{Y}\right)^2}}, \tag{3}$$

where i is the index of collected data and $\bar{Y}$ is the mean of the observed data. The peak area X is explicitly involved in the linearity criterion and becomes thus a critical analytical attribute. A threshold of 0.1 was arbitrarily chosen for the RRMSE as an example in Table 1.

3.2.3. Critical Method Parameters

Critical Method Parameters (CMP) are high-risk instrumental parameters that contribute to each method performances and thus could have a critical impact on the variability of the selected CAA [40]. They concern factors associated with the sample preparation, reference standard, reagent preparations, equipment operating conditions and generation of the calibration curve, as well as formulae for the calculation, *etc.* They can be divided into two groups: (i) controllable factors - concentration, amount, potency range over to quantify the analyte – defined as fixed factors that can be monitored in a quality control sample and (ii) random factors, which represent uncontrolled sources of variability, *e.g.* operator, equipment, reagent batch [41]. Supplementary information may need to be considered (prior experience with similar techniques, regional or geographic limitations, availability of experimenters, supplies, specific

Table 2: Qualitative correlation matrix used for the identification of critical analytical attributes. Criticality of analytical attributes is evaluated with respect to the metrological properties previously selected in the analytical target profile. The content of the boxes was arbitrarily chosen based on the Basso *et al.* case study

		Analytical Attributes			
	does this attribute play a critical role in the evaluation of this validation criterion ? (NA/not critical/critical)	Retention time	Peak area	Peak resolution	Tailing factor
Analytical Target Profile	**Metrological Criterion**				
	Accuracy	critical	critical	not critical	not critical
	Repeatability	critical	critical	not critical	not critical
	Intermediate Precision	critical	critical	not critical	not critical
	Reproducibility	critical	critical	not critical	not critical
	Specificity/Selectivity	not critical	not critical	critical	not critical
	Sensitivity	not critical	critical	not critical	not critical
	Detection Limit	NA	NA	NA	NA
	Quantitation Limit	NA	NA	NA	NA
	Linearity	not critical	critical	not critical	not critical
	Range	not critical	critical	not critical	not critical
	Robustness	critical	critical	not critical	not critical

technologies needed in the laboratories), to support the studies and cycle-time requirements regarding process operations [16]. A quality control strategy generally begins with a quasi-exhaustive enumeration of method parameters that could potentially affect each analytical attribute. The initial list of method parameters can be reduced and prioritized through a risk assessment process, *e.g.* FMEA or statistical design of experiments, which specifies their potential criticality [39] and so highlight those requiring special attention [42].

3.2.4. Identification of Method Operable Design Region (MODR)

The Method Operable Design Region (MODR) is the analytical design space, *i.e.* a diagram describing the regions of CMP values for which there is an acceptable probability of complying with CAA specifications. This diagram is built from a mathematical model, itself obtained from experimental data. The MODR can be regarded as equivalent to the design space in QbD. Its determination often requires the implementation of statistical methods and tools for the design of experiments and the multivariate analysis of datasets [30]. As for the concept of design space, MODR can be broken down into several distinct regions, as previously described in Section 3.1.

3.2.5. Control Strategy

The control strategy consists in defining the action plans to be applied to the CMPs to guarantee that the analytical method meets the performance requirements and that it complies with the pre-established specifications [19, 43, 44, 18, 35]. To achieve this, it takes into consideration multiple sources of data, collected during the method development phase and provided by DoE, robustness studies, stability assays, compatibility tests and a method verification process [17] with the aim of predicting the capability of the procedure to meet the CAA specifications [18]. Moreover, a

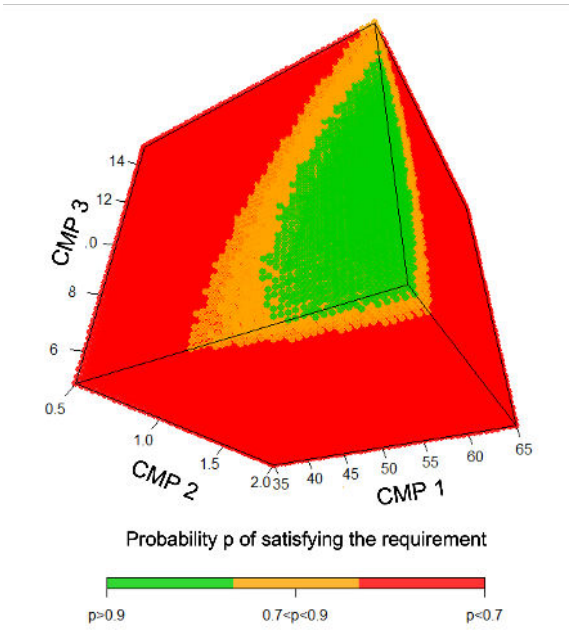

Figure 3: Example of Method Operating Design Region for three method parameters (CMP1 to CMP3). OOS (red); PAR (orange); NOR (green)) are the three constitutive regions defined in Section 3.1. To compensate random variations and drifts of the analytical method, a correction action shall be performed on Critical Method Parameters to keep the operating point in the NOR region

control strategy can, if designed properly, help identify failure modes as well as ensure the prevention of erroneous results [43]. It should also include environmental and instrument control systems. For instance, laboratory controls can be critical when handling thermal, humidity or photo-sensitive samples[44]. Consequently, an efficient control strategy enables the enhancement of knowledge sharing and the development of accurate and robust methods, which leads to greater operative flexibility taking into account all regulatory oversight, as well as saving significant resources due to the real-time testing [18]. However, analysis bias can arise and grow due to the effects of uncontrolled background factors. Subsequently, the control strategy must be updated throughout the lifecycle of the analytical method [44]. For instance, Panda *et al.*[45] and Deidda *et al.* [46] employed control charts with acceptance limits applied to Critical Method Variables involved in liquid chromatography methods.

3.2.6. Lifecycle Management and Continuous Improvement

During the development and use of an analytical method, a lot of data is generated. This last step addresses the issues of identification, acquisition, synchronization, organization, storage and access to this data. The challenge is to use it to refine our knowledge and improve both the accuracy and robustness of the method.

4. Lacks and Confusion

This section addresses current general areas of weakness hindering an efficient application of AQbD. Indeed, as with QbD, AQbD is neither a magic formula nor a plug-and-play technology. If any step of this approach is not properly applied, the method development can diverge from the pre-set objectives and therefore lead to a non-optimized method

[39]. In other terms, this risk-based development approach can itself generate risks in cases of wrong application. This was another factor motivating for the current development of the ICH Q14 guidelines.

4.1. Incompleteness of ICH-Q2(R1)

Up to now, the ICH Q2(R1) guideline has been the only harmonization document devoted to the validation of analytical procedures. It aims to demonstrate that analytical procedures meet their intended purpose and considers four common types of analytical methods applied for the development of classical drugs: impurity identification, impurity content, control of impurities and tests of the active moiety in drug substance samples. This same document stresses that the listed tests do not cover the whole range of properties to be considered. For instance, robustness is not listed in the table yet should be considered at an appropriate stage in the development of the analytical procedure.

Moreover, with the introduction of Process Analytical Technologies in QbD, new needs have emerged. The analysis of the chemical and physical properties of biopharmaceuticals, including nano-enabled medicinal products, involves the online processing of spectral data or of complex particle distribution information, and requires new multivariate computational methods, a category of data analysis for which no ICH validation guideline yet exists. That was one of the reasons a group of experts were motivated to revise ICH Q2(R1) in 2018 to work on a new project of ICH Q14 guidelines devoted to Analytical Procedure Development [25].

Furthermore, as its name indicates, this directive was initially designed to validate analytical methods, not develop them. It is therefore not surprising to see the same disadvantages as QbT. More precisely, no risk assessment procedure is included in ICH Q2.

4.2. Confusions in terminology

It is critical to examine the terms and definitions of its components to facilitate the AQbD implementation. Indeed, a lot of different acronyms have been employed by authors in their AQbD applications, and any confusion systematically leads to application errors and, eventually, the impossibility of reaching the initial requirements. We have analyzed the terminology used in 37 reviewed articles. With the objective of clearly exposing the various terms used by the authors, a confusion matrix was elaborated, and presented in supplementary material. Each cell of this table contains the number of occurrences in which row and column attributes are used simultaneously. If QbD and AQbD terms were used without any confusion between each other, this matrix would be bloc-diagonal, but the presence of significant non-diagonal terms in each AQbD and QbD blocks emphasizes numerous confusions among the 37 articles.

Firstly, it can be observed that the term “AQbD” is not systematically used to describe the development of analytical procedures. In contrast, the most employed acronym is “ATP” to designate the specification term of the analytical method to be developed. This is not so surprising, since most of the published articles were focused on the elaboration of the Analytical Target Profile. Nevertheless, ATP is also often associated with QbD and CQA, while CQA is more used within AQbD applications than CAA, which fosters mix-ups between the two terms. The interconnections between QbD and AQbD may partly explain this result. The same observation applies to CPP and CMA used instead of CMP. Finally, the concept of MODR is relatively well incorporated in the AQbD implementation, even if it is also often used in conjunction with the term QbD. This contingency analysis emphasizes the need to better clarify and

define AQbD notation in order to obtain homogenization in the scientific community. This could be achieved through the elaboration of new regulatory agencies' documents, as suggested by Jackson *et al* [27]. Additional inconsistencies in the terminology are reported in supplementary material[47].

4.3. Differences in content

So far, there has been no general methodology for applying AQbD. All authors have proposed their own application modalities according to their knowledge and interpretation of the AQbD paradigm [48][46]. In addition, it has been emphasized that AQbD implementation has not always been carried out in the same manner in the past. In particular, various authors did not use the typical ICH-Q2 validation characteristics in the same stage of AQbD. Most of them incorporated these metrological requirements in the terminal validation step of AQbD. In [39, 40] however, the ICH-Q2 requirements, *e.g.* the precision, lower limit of quantification and dosing range of the analytical method are introduced at the beginning in the elaboration of the ATP document to specify the expected measurement performances. Furthermore, depending on to the authors, the ATP document was exposed in multiple formats: with tables in [36, 45, 49, 39] [50] or in simple texts in [44, 43, 48, 37, 51, 46]. Very recently in [27], Jackson *et al.* has detailed through three examples how to precisely fill in the ATP application form.

4.4. Differences in implementation

In practice, the number of steps involved in the different AQbD implementations varies substantially. The simplest mode of applying AQbD was composed of solely three steps in [52], in which Awotwe-Otoo *et al.* applied it to develop a robust reverse phase-HPLC method. In [44, 37], the authors implemented a four-step AQbD procedure for sample preparation and the improvement of a Liquid Chromatography coupled to Mass Spectrometry stability-indicating method, respectively. One additional stage is considered in [51, 45, 46] for the optimization and validation of high-performance liquid chromatography techniques. The majority of AQbD schemes rely on a six-step process as in [43, 39, 49, 53]. Similarly, E. Rozet *et al.* have proposed in [40] a generic six-step AQbD approach, but they also introduced a probabilistic interpretation and a Bayesian estimation of the Method Operable Design Region. In [48] a seven-step QbD approach was carried out for the development of analytical separation methods by including in particular a method scouting in the AQbD workflow. These differences in the number and content of steps illustrate variabilities in implementation. However, most of them share the same key phases, including: ATP elaboration, CAA and CMP identification but also MODR determination associated with a control strategy implementation. The observed differences are essentially due to steps that have been more or less detailed and split up in some applications. Nevertheless, we also noted the scattered introduction of specific tasks, such as method scouting, MODR robustness analysis, method validation and data monitoring. As a consequence, the elaboration of a golden standard path will be welcomed by the scientific community to harmonize its implementation in practice.

5. Perspectives in nanopharmaceutical characterization

5.1. Applications to off-line characterization analysis

In the field of nanopharmaceuticals, due to the presence of nanocarriers and their specific properties, additional critical quality attributes *vs* classical pharmaceutical products must be monitored. This applies to (at least) three nano-specific aspects: (i) the physical properties of the particles, including their size/size distribution, surface charge, morphology and particle concentration, (ii) the spatially resolved chemical structure inside the core of the particles, where the structure and crystallinity play a key role in synergy with the whole chemical composition and presence of impurities and (iii) the particle surface and its interactions with the biological environment surrounding the particles, *e.g.* particle-proteins interactions. Moreover, when the nanoparticles are loaded with an active pharmaceutical ingredient (API), be it a small molecule or large biological entity such as nucleic acids, the determination of the loading, stability and release of the API from the nanocarrier is of utmost importance. As an additional layer of complexity, there is a huge variety of nanoparticle classes in the field of nano-pharmaceuticals, *e.g.* polymeric particles, metallic particles, metal oxide and lipid-based nanoparticles. Each specific class may require adaptation of the characterization methods.

Even within a certain particle class, depending on the properties to be characterized such as size, shape, aggregation, the structure of the core and/or the surface properties, significant variations may be observed. Given this constitutive heterogeneity, a systemic analytical approach is required able to develop accurate and robust characterization methods to address the numerous technical, methodological and regulatory gaps in the field of nanopharmaceuticals[54, 55]. These gaps greatly vary depending on the type of quality attributes to be measured. In some cases, there is simply no methodological solution available, *e.g.* for the determination of particle surface properties. In other cases, methods are available only for certain nanomaterial classes and there is a need to expand their applicability to additional nanotechnological platforms [12]. Such adaptation is not only required for a physical-chemical analysis of the nanopharmaceuticals but also in case of *in vitro* methods for the toxicological and/or biocompatibility assessments that have been developed for small-molecule drugs, and this might require special considerations and additional controls when used with nanomaterials. A similar situation applies for the determination of the formulation sterility and endotoxin content, where, for example, standardized LAL assays suffer from interference from the nanomaterials and may generate invalid results. In the field of medical devices containing nanomaterials, a specific standard: ISO/TR 10993-22:2017 Biological evaluation of medical devices — Part22: Guidance on nanomaterials [56] was proposed to provide a general guidance on the additional parameters to take into account while testing nanomaterials, although, in practice, the analytical solutions to do so are insufficient, nonexistent or not yet validated.

Since the emergence of the AQbD approach, its applications in the characterization of nanomaterials remain very limited. However, two studies that have used as examples in the previous sections are noteworthy: Basso *et al.* [36] and Sylvester *et al.* [51]. Basso *et al.* used the AQbD approach to optimize a reversed-phase high performance liquid chromatography method to quantify curcumin, atorvastatin calcium and celecoxib in lipid-based nanoparticles, as described in Table 1, while Sylvester *et al.* implemented it to simultaneously detect curcuminoids and doxorubicin encapsulated in a liposomal formulation. In both cases, method operable design regions (MODR) are identified to

determine operating conditions in compliance with the expected specifications on the separation and quantification of multiple components (both excipients and the API) present in the nanoformulation. To our knowledge, no use of the AQbD approach can be found beyond assessing the nanoparticle composition *via* liquid chromatography. The lack of implementation of the AQbD in the field of nanopharmaceuiticals, despite all the identified methodological challenges in the field motivated the authors to write this review to better explain to the community the concept of the AQbD.

As final perspective, we would like to discuss the potential benefits of the AQbD for the future developments of analytical methods and standards of protocol suited to the characterization of nanopharmaceuticals [57]. Is beyond the scope of this review article to go further than providing a perspective on the application of the AQbD in the field of nanopharmaceuticals, that is in fact still in its infancy.

The development and harmonisation of asymmetric flow-field flow fractionation coupled with light scattering detection approaches (AF4-MALS-DLS) to measure particle size and physical stability of nanopharmaceuticals could be considered as an example to illustrate the potential role of the AQbD to support the development, validation and harmonisation steps [58]. AF4-MALS-DLS is aiming at measuring the physical properties and stability of nanopharmaceuticals, e.g. to determine batch-to-batch variability, stability *vs* storage and to isolate and characterize the particles when in complex biological media. It combines a fractionation step (AF4) and the online characterization of the size of the analyte with different online light scattering detectors (DLS and MALS). The NCI-NCL and EU-NCL identified this as a very versatile approach to answer multiple regulatory needs related to the preclinical development of nanopharmaceutical products, including liposomes, lipid based nanoparticles, metal oxide nanoparticles and extracellular vesicles. Therefore they jointly developed a standard operating procedure with the aim to guide the user to adopt best measurement practices while performing in laboratory method development, optimisation and adaptation of the measurement protocol to different nanomaterial classes[58]. At the same time, an overarching ISO technical specification[9] was drafted by the nanotechnology committee of the ISO229 to define the general specifications to evaluate the method applicability for the analysis of all kind of nanomaterials. The latter is a generic document that contains indication of the critical metrological properties to be considered during method validation, including the acceptable amount of mass recovery after the AF4 fractionation process, an acceptable threshold for repeatability and reproducibility of the main analytical attributes and the criteria for the data analysis associated to different potential instrumental configurations. Based on those general guidance documents, metrological institutes, regulatory agencies and academic experts involved in the characterization of nanopharmaceuticals felt the need to develop standards test methods based on AF4-MALS-DLS that are formulation specific. As a consequence, the ASTM E56 standardisation committee started to develop specific standard test methods associated to different kind of nanomaterial classes, including liposomal products[59] and lipid based nanoparticles for RNA delivery. In this context, AQbD was not considered as a supporting tool, but according to the authors it could have played a role at multiple levels. First, AQbD can support the formulation specific method optimisation by identifying the material attributes and method parameters that are specific to different material classes (liposomes vs LNPs) and by applying the MODR concept to the existing general

[9] https://www.iso.org/obp/ui/#iso:std:iso:ts:21362:ed-1:v1:en

analytical procedures and guidances. Second, during the in-lab validation or the larger interlaboratory comparison supporting the standard development, AQbD may help to minimize inter-laboratory deviations. Finally, the adoption of a planned set of control(s) for all possible variation(s) would assure that the ATP requirement is met during the analytical method transfer between labs as well as in routine use, guaranteeing a better reproducibility in interlaboratory comparison exercises and during method transfer between manufacturing sites [60].

5.2. Emerging nano-specific PAT technologies for online control purposes

AQbD could also be implemented both for optimizing online characterization and for the new emerging PAT technologies addressing nanomaterial specific properties. Online PAT technologies are traditionally associated with Raman and other spectroscopy techniques that mostly monitor the chemical composition and crystallinity of the proposed drug product. However, specific PAT technologies have also been developed very recently for online control of nanospecific properties, and could be hopefully considered in the future in the QbD of nanoformulation, through the application of AQbD. One example is emerging technologies for the online monitoring of particle size. The conventional way to monitor particle size (or other particle attributes) relies on an off-line mode in which a sample is firstly manually extracted then measured in a laboratory to estimate the targeted attribute, *e.g.* particle size. However, this approach introduces a significant delay between the measurement, impact assessment and any potential correction. Moreover, it can be performed only once the batch is complete. Consequently, it is impossible to fine-tune the processing parameters in real time during the manufacturing phase. Some emerging technologies for online sizing are currently being tested, such as spatially resolved dynamic light scattering (SR-DLS) provided by the NanoFlowSizer [32], or real time multiangle light scattering (RT-MALS) [61], which are able to characterize the size and molecular weight of turbid nano dispersions, enabling PAT analysis of the physical properties of nanomaterials. Both technologies have been tested on lipid based nanoparticles, polymeric particles and liposomes with very promising results, ensuring batch consistency by online monitoring for size of the entire batch or immediate discharge of the product if the particle size no longer meets specification. Nevertheless, the analysis of multidimensional data provided by those technologies requires the development of new chemometric methods.

6. Conclusion

In the ten years since its emergence, the Analytical Quality by Design method has been used in many applications devoted to the optimization of analytical methods. However, as exposed in this review, confusion still exists and can be problematic in the end for an approach designed to reduce risks of producing interlaboratory heterogeneity. Consequently, new guidelines, such as the current work on the ICH-Q14, could be a real asset for an optimal implementation of AQbD. In this spirit, this review has proposed some clarifications and homogenizations of AQbD steps and documents. More precisely, we propose a new template for the definition of the Analytical Target Profile and a qualitative correlation matrix to more rationally select the critical analytical attributes. The review has also investigated how the scope of AQbD scope could be expanded to nanomaterial characterization. Indeed, since its advent, AQbD methods have been mainly applied to classical drugs and to liquid chromatography techniques, but rarely to methods used to

measure nano-specific properties. Measurement technologies used in nanotechnology characterization such as AF4 separation-MALS-DLS, spatially resolved dynamic light scattering or real time multiangle light scattering have been identified as new application targets of AQbD. Is now the aim of the authors to disseminate and demonstrate the broader applicability of the AQbD for future pre-normalization and standardisation activities in the field of nanopharmaceuticals.

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