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Adaptation of the quality by design concept in early pharmaceutical development of an intranasal nanosized formulation

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ABSTRACT

Regulatory science based pharmaceutical development and product manufacturing is highly recommended by the authorities nowadays. The aim of this study was to adapt regulatory science even in the nano-pharmaceutical early development. Authors applied the quality by design (QbD) concept in the early development phase of nano-systems, where the illustration material was meloxicam. The meloxicam nanoparticles produced by co-grinding method for nasal administration were studied according to the QbD policy and the QbD based risk assessment (RA) was performed. The steps were implemented according to the relevant regulatory guidelines (quality target product profile (QTPP) determination, selection of critical quality attributes (CQAs) and critical process parameters (CPPs)) and a special software (Lean QbD Software®) was used for the RA, which represents a novelty in this field. The RA was able to predict and identify theoretically the factors (e.g. sample composition, production method parameters, etc.) which have the highest impact on the desired meloxicam-product quality. The results of the practical research justified the theoretical prediction. This method can improve pharmaceutical nano-developments by achieving shorter development time, lower cost, saving human resource efforts and more effective target-orientation. It makes possible focusing the resources on the selected parameters and area during the practical product development.

1. Introduction

1.1. Pharmaceutical developments—new prospects

Research relating to nanodrugs is currently one of the most widely investigated areas of pharmaceutical technology. Until recently, the conventional procedure in such developments was the quality by testing (QbT) method, but this has become outdated today. In the QbT process, only the quality of the product is assured by the testing (raw material and drug substance testing, product manufacturing: in-process testing and end-product testing) and the quality is not guaranteed (Yu, 2008).

Rather than QbT, the new concept quality by design (QbD) is mostly preferred nowadays and is strongly recommended by regulatory agencies (the Food and Drug Administration, FDA, in USA, and the European Medicines Agency, EMA, in EU) (FDA, 2012; Chatterjee, 2011; EMA, 2014). According to the current regulatory science philosophy, QbD must be one of the key elements of the various pharmaceutical developments. Experiments in pharmaceutical technology should use the QbD concept in order to achieve a time- and cost-saving process ensuring a high-quality product.

The philosophy of the QbD concept can be summarized as a systematic, scientific, risk-based, holistic and proactive approach to pharmaceutical development so as to ensure the predefined quality (Nasr, 2006; Woodcock, 2004). The exact definition according to the International Conference on Harmonisation (ICH) Quality Guidelines is as follows: "QbD is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process

Abbreviation: API, active pharmaceutical ingredient; C_{max}, maximum plasma concentration; CQA, critical quality attribute; CPP, critical process parameter; EMA, European Medicines Agency; FDA, Food and Drug Administration; HA, sodium hyaluronate; ICH, International Conference on Harmonization; ISO, International Organization for Standardization; PEG, polyethyleneglycol; PVP, polyvinylpyrrolidone; QbD, quality by design; QbT, quality by testing; QTPP, quality target product profile; RA, risk assessment; REF, reference sample; T_{max}, the time until the maximum plasma concentration is reached after administration; TPP, target product profile.

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control, based on sound science and quality risk management" (EMEA/CHMP, 2009). The ICH guidelines for industry (Q8: Pharmaceutical Development, Q9: Quality Risk Management, and Q10: Pharmaceutical Quality System) reflect the current thinking and requirements of the national and international regulatory agencies (FDA and EMA) as well (EMEA/CHMP, 2009; EMA/CHMP, 2014a,b).

The steps in a QbD-guided pharmaceutical development are as follows: first, the researchers must accurately determine the target product profile (TPP) and its quality (quality target product profile, QTPP). The definition of the QTPP by the ICH Q8 is "a prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy, of the drug product" (EMEA/CHMP, 2009). In practice this means a preliminary estimation of various essential parameters, such as the mode of administration, strength, dosage form, therapeutic area and aim, release profile, etc.

Secondly, it is necessary to identify parameters which influence the QTPPs and to select those which do so critically. These are the critical quality attributes (CQAs), "physical, chemical, biological, or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality" (EMEA/CHMP, 2009). Besides the CQAs, the critical process parameters (CPPs) must be defined. A CPP is "a process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality" (EMEA/CHMP, 2009).

In practice, the identification of CQAs and CPPs means discussion and agreement among the professionals working on the project, and is based on their previous practical and literature knowledge and experience.

As a key parameter, the most important element of QbD-guided development is the risk assessment (RA) activity. RA may be initial and/or final. It may be repeated and refined, but in all cases it is essential. The RA procedure may also differ, but the achievement of good RA results can be priceless during the development process. RA can save considerable amounts of time and cost throught the whole research process. The official ICH definition is "A systematic process of organizing information to support a risk decision. It consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards" (EMA/CHMP, 2014a).

There are other elements of the QbD process, such as the design space and control strategy; ICH guidelines Q8 (R2) and Q10 (EMA/CHMP, 2014b) give more and detailed information. They are important elements of a complete medicine development, but are subsequent to the RA in the early development phase.

The ideology of the QbD approach can be summarized as quality cannot be tested into the product, it should be built in by design. In practice it means that the technical development (in scientific or industrial laboratory) should be started only after the theoretical development. This theoretical development is scientific knowledge (based on literary and practical experiences) and risk based which is resulted in prediction of the parameters that most strongly influence the final product and its quality. This prediction can help in design of experiments, in time management and in utilization of resources during the whole formulation process. This QbD approach can be used in every section of a pharmaceutical production process, from the early development (basic research) until the industrial manufacturing. The selected parameters (material attributes and process parameters) are in every case unique and different, and the results of the prediction depend on the target selected.

The theoretical scoring and classifying of parameters according to the QbD approach as a modern quality managements technique can help in pharmaceutical technological development processes in focusing the efforts, therefore in saving time and money.

In the present paper, the authors study the support of regulatory science in contemporary pharmaceutical technological early developments. The aim was to demonstrate that the QbD concept and RA can reduce the research costs through the prediction of results, based on designation of the parameters that exert the greatest effect on the final and desired product quality.

To model the procedure of a QbD-based early development an analgesic immediate-release and quick-effect formula was selected as target product, and a nano-meloxicam-containing nasal formula (nasal gel) was chosen, as model active agent and model dosageform.

1.2. Nanosystems in pharmaceutical technology and nasal delivery

The International Organization for Standardization (ISO) has developed definitions of various terms relating to nanotechnology. In the ISO nomenclature there are two groups of "nanomaterials": "nanoobjects" and "nanostructured materials". On the basis of Scientific Committees and International Organizations, the European Commission defines a nanomaterial as "any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less" (Lövestam and Rauscher et al., 2010).

Nanotechnology, as a new technological process in pharmaceutical manufacturing, can offer solutions to certain disadvantages, such as solubility problems. The solubility enhancement of poorly-soluble drug substances is one of the most important tasks in pharmaceutical technology from the aspects of generic and prepharmacon formulation.

There are several techniques with which to produce nanosized drug materials that can be differentiated between bottom–up and top–down technologies (Raval and Patel, 2011; Ravi Kumar et al., 2004; Ambrus et al., 2009; Tobar-Grande et al., 2015; Kürti et al., 2011; Radacsi et al., 2014). The bottom–up technologies start from molecules which are dissolved and precipitated (crystallized) in a controlled fashion to yield the desired particle size. Top–down methods achieve nanodimensions with different size reduction procedures. Co-grinding is a top-down disintegration procedure of preparing nanoparticles by grinding the drug with one or more excipients. Co-grinding is organic solvent-free, and therefore is economically and environmentally desirable green technology. Control of the influencing factors (e.g. the duration of grinding, the rotation rate, the material and volume of the grinding pot, the material and number of the grinding balls, the excipients, and the drug/excipient ratio) during the co-grinding process is very important (Vogt et al., 2008; Tozuka et al., 2011).

Nanotechnology offers increased bioavailability, improved absorption and the potential of drug targeting, e.g. nasal administration of the drug.

Nasal delivery is a prominent route for drug administration whereby local, systemic and central nervous effects can be achieved, and nasal drug formulations are therefore very important the modern pharmaceutical technological developments (Zaman et al., 2013). As regards the frequently applied local formulations, nasal administration may be an alternative to the intravenous route. The advantages of nasal delivery include a large absorption surface, an epithelial barrier with many pores, the abundance of capillaries, avoidance of the liver first-pass metabolism and easy use for patients. The development of nasal gels containing a nanosized active pharmaceutical ingredient (API) can solve the bioavailability problems of poorly water-soluble drugs. Particle engineering, the choice of the type of excipients and the technological process are very important factors in the development of these products.

A nasal gel formulation and delivery can be made more effective through the use of excipients, which can promote mucosal absorption, e.g. sodium hyaluronate (HA) (Kürti et al., 2013; Wang et al., 2011).

1.3. QbD in pharmaceutical nano-development

Although nanomedicine development is highly focused (Etheridge et al., 2013; Ali et al., 2013; Gómez-Gaete et al., 2007), regulatory challenges and uncertainties remain (Gaspar, 2009). No exact regulatory criteria or QbD requirements have been found that are dedicated to nanosystems. The medicinal products containing a nanosized API that are on the market were developed and licensed by applying the classical authorization requirements and protocol (Bawa, 2013). As the use of QbD is strongly required now by the authorities for the licensing of new medicinal products, it should be applied even in the early drug development stage.

Not many papers are to be found in the current scientific literature as concerns the QbD concept in nano-research. Tőke demonstrated the importance of QbD in the development of an immunotherapeutic nanoproduct (Tőke et al., 2010). Bragagni made use of the elements of the concept the research on niosomes (Bragagni et al., 2014). The investigations by Raza et al. (2013) revealed the importance of systematic optimization of the development of solid-lipid nanoparticles, and Verma et al. (2009) utilized the QbD approach in the preparation of nanosuspensions.

2. Materials and methods

2.1. Materials

Meloxicam, a water-insoluble nonsteroidal anti-inflammatory and analgetic drug, was chosen as model active ingredient; it was purchased from EGIS Ltd. (Budapest, Hungary). The particle size was around 50 μm. Meloxicam has low solubility in water (4.4 μg/ml, i.e 1.2×10^{-8} mol/l) and a low dissolution rate, which are limiting factors for absorption. The average diameter of starting meloxicam particles was 2643.6 ± 2629.1 nm, which was measured by scanning electron microscopy (SEM), image analyzer technique.

The excipients polyvinylpyrrolidone (PVP) K25 and C30 were purchased from BASF (Ludwigshafen, Germany); polyethyleneglycol (PEG) 6000 and 20,000 were from Sigma–Aldrich Chemie GmbH, Germany. HA (Mw: 1400 kDa) was from Gedeon Richter. A physical mixture of the drug and the polymer in appropriate ratio was prepared as a reference sample (REF). PVP is an amorphous material in structure and has no melting properties in this case, but is able to help in particle size reduction. Types of PVP (K25 and C30) differ in their molecular weight and viscosity. The molecular weight of PVP–K25 is about 34,000 and PVP–C30 has a higher molecular weight, about 58,000. Their viscosity is relating to the molecular weight so the viscosity of PVP–C30 is higher, than for K25. PEG is a semicrystalline grinding excipient with low melting point which could melt due to friction work. PVP is a stabilizer agent, its present can help in prevention of agglomeration. The numbers of PEG (6000 and 20,000) refer to the molecular weight of the polymer.

2.2. Methods

2.2.1. Definition of the TPP and QTPP

The QTPP forms the basis of product development design. It should include patient-relevant product performance and professional characteristics. Considerations for QTPP selection can be found in ICH guideline Q8, e.g. the route of administration, dosage form, delivery system, pharmacokinetic and product quality criteria (e.g. sterility, stability and drug release), etc. (EMEA/CHMP, 2009). The selection of TPP and QTPP demands careful planning and considerations and the agreement of the participants. QTPP is unique in every case, depending on the targeted aims.

2.2.2. Determination of CQAs

The identification of potential CQAs means the selection of those characteristics which influence the product quality and can be studied and controlled. They are generally associated with the drug substance, excipients, intermediates (in-process materials) and drug product. Potential drug product CQAs should be derived from the QTPP and/or prior knowledge. The determination and selection of CQAs in this case was also based on prior knowledge and data from the relevant literature.

2.2.3. Determination of CPPs

Those process parameters whose variability affects the CQAs and the desired quality must be selected. These are the CPPs, selection of which requires a knowledge of the different

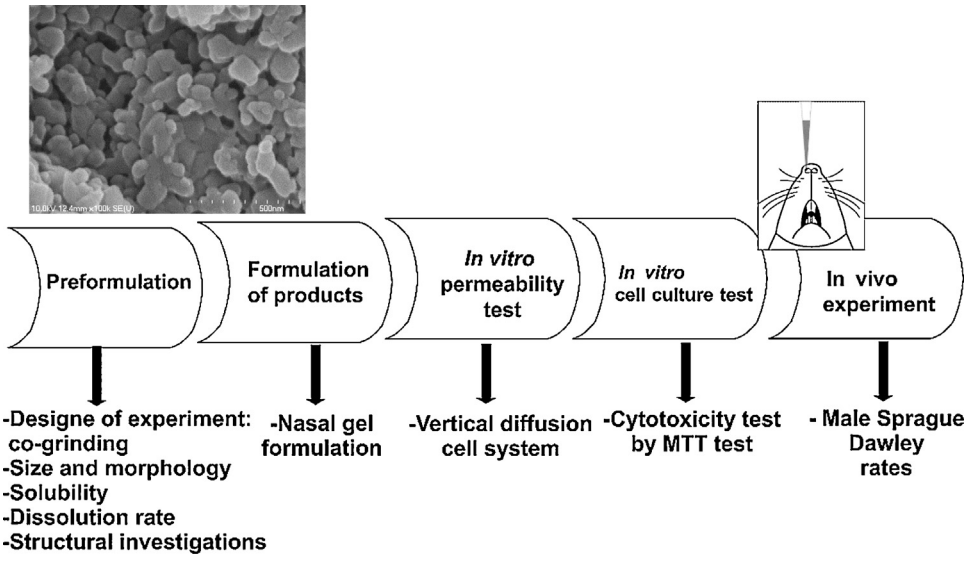

Fig. 1. Steps of a nasal investigational protocol for screening innovative formulations.

production methods and their applicability. The CPP selection is based on prior knowledge, previous practical investigations and data from the relevant literature. In this study, previous research findings led to the selection of co-grinding as an appropriate way to produce nanosized meloxicam.

The process variables of co-grinding were the following: the material of the grinding pot and the grinding balls was silicon nitride (Si_3N_4), the volume of the grinding container was 80 ml, the number of the grinding balls was 25 and their diameter was uniformly 10 mm. The time of grinding was in each case 2 h. The composition of samples was varied during the study, different drug-carrier ratios (1:0.5, 1:1 and 1:2) was investigated. The amount of meloxicam was 1.0 g in each experiment, which was ground together with the calculated amount of polymer additive.

2.2.4. RA

As a valuable science-based process (EMA/CHMP, 2014a), RA can aid in identifying which material attributes and process parameters potentially influence the product CQAs. On the basis of prior knowledge and initial experimental data, RA tools can be used to identify and rank parameters (e.g. process, equipment and input materials) with the potential of impacting on product quality.

The initial RA was performed with Lean QbD Software® from the qbdworks.com (2014 QbDWorks LLC., Fremont, USA) (Anon,

Table 1
The selected QTPPs, CQAs and CPPs, their targets, justifications and explanations.

	Target	Justification	Explanation
QTPP elements			
Therapeutic indication	Analgesia	Meloxicam, a well-known NSAID, with analgesic and anti-inflammatory effect. The size of the starting meloxicam particles was 2643.6 ± 2629.1 nm	Clinical setting is a QTPP by the ICH Q8, where the indication is essential
Target patient population	Adults	NSAIDs main target group, several states needed effective and quick pain relief (e.g. migraine)	Clinical setting is a QTPP by the ICH Q8, which includes the patent population
Route of administration	Nasal	No first pass effect, relatively high absorption surface area, highly vascularized, easy to administrate	It is a suggested QTPP by the ICH Q8
Site of activity	Systemic effect	Nasal mucosa absorption of the active agent and systemic analgesic effect by blood circulation	It is critically related to the quality, safety and efficacy. Being QTPP is a therapeutic requirement.
Nasal gel with nanosized API	Around 100 nm particle size	Needs small particles for effective absorption on nasal mucosa	The dosage form is a suggested QTPP by the ICH Q8 and an administrational requirement
Dissolution profile	Immediate release	Dissolution have to be <15 min. Nasal mucosa juice is replaced in every 10–15 min.	It is critically related to quality, safety and efficacy, affecting the bioavailability and pharmacokinetics it is suggested as being QTPP by tha ICH Q8
Production method	Co-grinding	According to preformulation studies and literature simple and organic solvent free method for production nanosized systems.	The efficient process with consistent yield can select as QTPP (Awotwe-Otoo et al., 2012). It meets the environmental requirements
CQAs			
Excipients	PVP, PEG, HA	They can help in nanosize-stabilization, aggregation prevention, etc. PVP is amorphous with no melting properties. PVP K25 (higher viscosity, the molecular weight is about 34,000) and PVP–C30 (about 58,000 molecular weight, lower viscosity) was used. PEG has a semicrystalline structure, with low melting point. The molecular weight of the PEGs are different (6000–20,000).	ICH Q8 suggests them as being CQAs. Excipient are critically related to the dissolution profile of the final product.
Size/surface area (SA) (nano)	Nanosize (around 100 nm)	Large specific surface area, increased dissolution rate, solubility and permeability, passive diffusion	Critically related to the administration route (membrane diffusion etc.), to the local or systemic therapeutic effect and influences the drug release
Appearance	Dry powder in gel	Better stability, cost effective and simple application	Influences the patient compliance
Dissolution	<15 min	Effective nasal mucosa absorption	Critically related to efficacy
Toxicity/irritation	Non-toxic, and non-irritative	No negative and side effects on nasal mucosa and nasal cavity.	Critically related to safety
Structure (Cryst./Amorph.)	Amorphous	Miss of crystal lattice, increased absorption, lower dissolution time	Critically related to efficacy
Stability	No visible sign of instability, aggregation or precipitation in 15 s	dry powder is stable, there is only few seconds between nasal gel formulation (from powder) and nasal administration	Critically related to safety, efficacy and quality
Permeability	Effective absorption	High maximum plasma concentration (C_{max}) in short time correlated with intravenous application	Critically related to efficacy, influences the administration route, the site of activity, the bioavailability, etc.
Solubility	Well soluble on nasal pH	Nasal liquid pH 4.5–6.5	Critically related to efficacy and safety
CPPs[a]			
Composition (sample compounds, additives, ratios)	Decreased and stabilized size, increased dissolution rate	Amount and type of co-grinding additives (it influences the water solubility, amorphous and semi- crystalline structure etc. Different drug-carrier ratios (1:0.5, 1:1 and 1:2) were tested, where the amount of drug was constantly 1 g in each cases).	Critically related to efficacy and quality
Rotation time	Nanosized particles	Sufficient time for reaching the nanosize, but no negative effects. For this purposes the grinding time was equally 2 h.	Critically related to quality
Rotation speed	Nanosized particles	Reaching the desired nanosized product.	Critically related to quality
Grinding parameters	Standard	Grinding parameters means the grinding conditions, including the pot shape/size, etc. There were no changes in grinding parameters settled in previous production processes: silicon nitride (Si_3N_4) grinding pot (80 ml) and grinding balls (no. 25, diameter: 10 mm).	Related to quality

[a] Co-grinding for production of nano-scale drug.

2014). According to the structure of the software, the connections between the QTPPs, CQAs and CPPs were evaluated.

The interdependence between the QTPPs and CQAs, just as between the CQAs and CPPs, was structured, evaluated one by one and rated on a three-level scale. This scale reflects the impact of their interaction on the product as high (H), medium (M) or low (L).

The probability of occurrence is necessary for the analysis and was performed with the Acquisition Risk Management protocol by a manual mapping approach (Engert and Lansdowne, 1999). For the probability rating, a 0–10 scale was used, and the values were estimated in accordance with the agreement of each participant.

2.2.5. Design and preparation of a product for nasal use containing nanosized meloxicam

A nasal delivery investigation protocol was applied for the development from the preformulation up to the in vivo experiments (Fig. 1). During the sample preparation procedures, pharmaceutical excipients (PEG 6000, or 20,000 or PVP K-25 or C-30) in different ratios were preliminarily tested and a three-level full factorial design was applied to find the optimal process parameters of the co-grinding procedures for the production of nanosized meloxicam particles in uniform size distribution. The powder samples were analyzed to control the micrometric properties, the structure, the solubility and the in vitro dissolution rate. HA was applied to form a nasal gel sample for permeability, cytotoxicity and in vivo experiments. Fig. 1 lists those methods which formed part of the development protocol (Kürti et al., 2011; Zaman et al., 2013).

2.2.6. Testing methods of the developed nanosized product

2.2.6.1. Particle size and surface morphology. The particle size and the surface morphology of the MEL particles were visualized by scanning electron microscopy (SEM). Samples were fixed onto a metallic stub with double-sided conductive tape (diameter 12 mm, Oxford Instruments, UK). Images were taken in secondary electron image mode on a Hitachi S-4700 Type II instrument (Japan) at an acceleration voltage of 10 kV. MEL particle diameter distributions were obtained by analyzing SEM images with the ImageJ software environment.

2.2.6.2. Evaluation of physical state. The physical state of MEL in the different samples was evaluated by X-ray powder diffraction (XRPD) analysis. Diffraction patterns were analyzed with a Miniflex II X-ray diffractometer (Rigaku Co., Japan), where the tube anode was Cu with K α = 1.5405 Å. The pattern was collected with a tube voltage of 30 kV and a tube current of 15 mA in in-step scan mode (4°/min).

2.2.6.3. Solubility testing. The solubility of MEL was determined at physiological conditions (pH 7.4, 37 °C) by addition of an excess of the drug to the solvent, after which the mixture was stirred on a magnetic stirrer for 8 h, then filtered (Minisart SRP 25, Sartorius, Germany), and the content of dissolved drug was analyzed spectrophotometrically at 362 nm (Unicam UV/vis spectrophotometer, Germany).

2.2.6.4. Dissolution study. The extent of dissolution of MEL was studied in 50 ml phosphate buffer (PBS) at physiological conditions

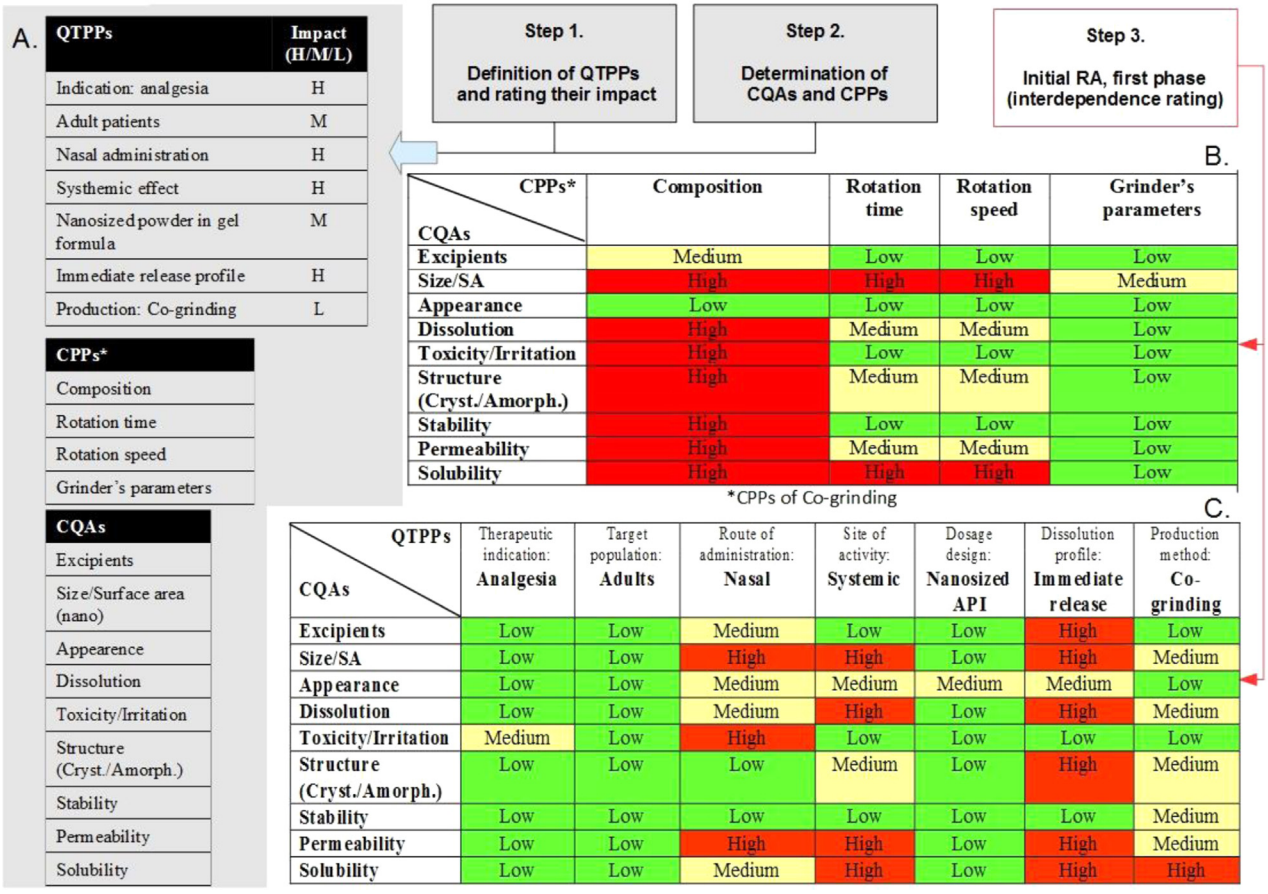

Fig. 2. Selected QTPPs, CQAs and CPPs and their interdependence rating in the initial RA process.

with Pharmatest equipment (Germany). In vitro permeability studies were performed with a vertical Franz—diffusion cell system (Microette Topical and Transdermal Diffusion Cell System, Hanson Research, USA) containing six cells at physiological conditions.

2.2.6.5. Cell culture model. MEL nanoparticles were tested on a cell culture model of the nasal epithelium (Kürti et al., 2013), RPMI 2650 human nasal epithelial cell line (ATCC, USA) was applied for toxicity and permeability assays. Cells were grown in Eagle's minimal essential medium (Invitrogen, USA) supplemented with 10% foetal bovine serum (Lonza, Switzerland).

2.2.6.6. In vivo tests. During the in vivo measurements a dose of 60 μg per animal was administered to the right nostril of male Sprague–Dawley rats ($n = 5$) via a polyethylene tube by using a syringe. During drug administration the animals were narcotized by isoflurane. Blood samples were withdrawn from the tail vein before and at 5, 15, 30, 60, 120, 360 and 1440 min post-dosing, respectively. To a 50 μl of plasma sample, 100 μl acetonitrile containing piroxicam (internal standard at 30 ng/ml concentration) was added and the mixture was vortex-mixed for 30 s. Supernatant was obtained by centrifugation of the mixture for 25 min at 16100 × *g* at 4 °C. The resulted supernatant was injected into the LC–MS/MS system for analysis.

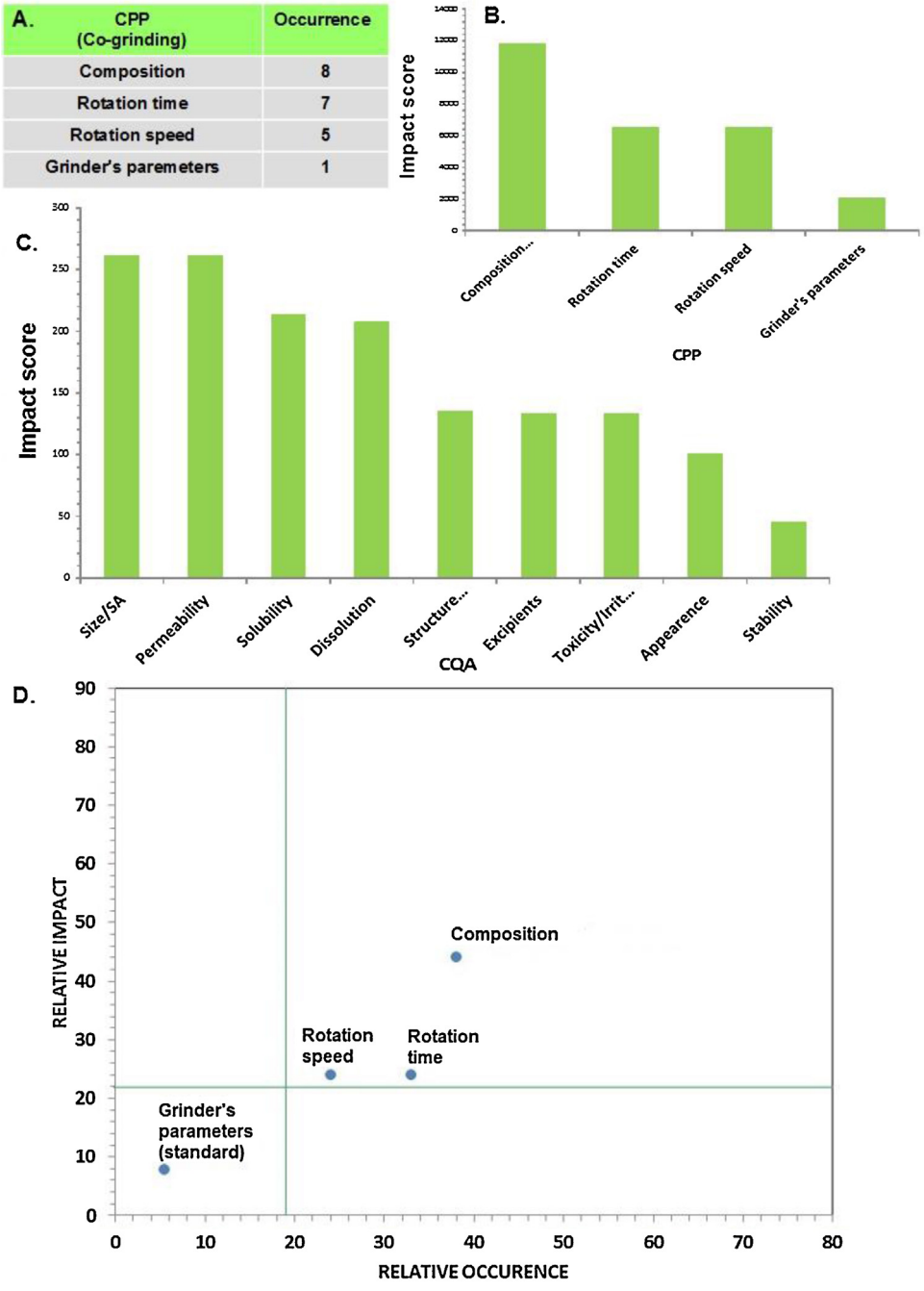

Fig. 3. Estimated occurrence of CPPs and the graphical results of the RA.

3. Results

3.1. RA and priority sorting

As described above, the targeted profile was an immediate-release and rapid-effect analgesic formula containing meloxicam. According to these product quality expectations, scientific knowledge and previous research findings, the QTPPs were determined as the initial step of the QbD process. The CQAs and CPPs were then defined. Table 1 lists the QTPPs, CQAs and CPPs with selected targets and their justification. The table also presents short explanations about the classification of elements selected in the different groups.

The selection of QTPPs was in accordance with the relevant guideline and literature. The ICH Q8 guideline for industry recommends the defining of the QTPPs as it relates to quality, safety and efficacy. On the other hand, the selection of the CQAs is based on the drug substance, excipients, intermediates and drug product characteristics. CQAs are typically those aspects which affecting critically the product quality, drug release and stability etc. but they are in each case derived from the QTPPs. The relevant literature is not completely unified with selection criteria of CQAs and there could be find discrepancies. For example the dissolution profile is suggested as being QTPP by the ICH Q8 and it is a therapeutic requirement of a dosage form. Actually, the dissolution rate of the API, or its toxicity which can be an outcome of excipient selection and production process also can be selected as CQA (Zidan et al., 2014). The CQA selection in this study was derived from the QTPPs, based on the ICH Q8 guideline and the literature.

After the QTPP definition (Fig. 2, Step 1) and selection of CQAs and CPPs (Fig. 2, Step 2), the RA was the following element of this QbD-based product design and development. RA is a key step which needs an estimation of the impact of QTTPs on the desired product quality. This impact estimation, based on the prior agreement of researchers, is indicated in Fig. 2A. The initial RA was performed with Lean QbD Software® (Anon, 2014). Use of the application is clear; its first phase is the interdependence rating (Fig. 2, Step 3) between the CQAs and CPPs (Fig. 2B) and between the CQAs and QTPPs (Fig. 2C) on the three-level scale. These interactions and their estimated impact on the desired product quality are shown in Fig. 2.

Then, with the Lean QbD Software®, the precise impact scores for each of the influencing parameters were calculated. These impact scores of the CQAs and CPPs are presented in Fig. 3. The results of the occurrence rating of the CPPs can also be seen (Fig. 3A).

Fig. 3B reveals that the sample composition has the highest relative impact score among the CPPs on the final product. In this case, the relative impact was about 12,000-scores high. This was classified as a first-line priority parameter. The parameters with the next highest influence among the CPPs were the rotation speed and the grinding time. These have a similar impact on the product quality, about half that of the composition (Fig. 3B). These parameters were graded as second-line priority ones. The parameters of the grinder, such as its volume, shape, material, etc., had no major effect, and were therefore included in the third-line priority group.

The impact scores of the CQAs (Fig. 3C) led to the following findings:

"Size" and "permeability" were the CQAs with the highest impact on the final product quality, and were classified as first-line priority CQAs. These properties demand the greatest attention during the design of the composition, selecting materials, excipients, etc., in the practical and theoretical development.

"Solubility" and "dissolution" were second-line priority CQAs. They also need relatively great attention during the design. Other examined CQAs (structure, toxicity, irritation, appearance and stability) proved to be third-line priority CQAs in this investigation.

The software-based graphical presentation of the relative impact and relative occurrence of the CPPs yielded the same results (Fig. 3D). This diagram has four parts. The lower quarter shows the elements with only low impact on the quality and with low occurrence. These include the grinder parameters, e.g. the pot parameters, the type of grinder, etc. Two other quarters relate to

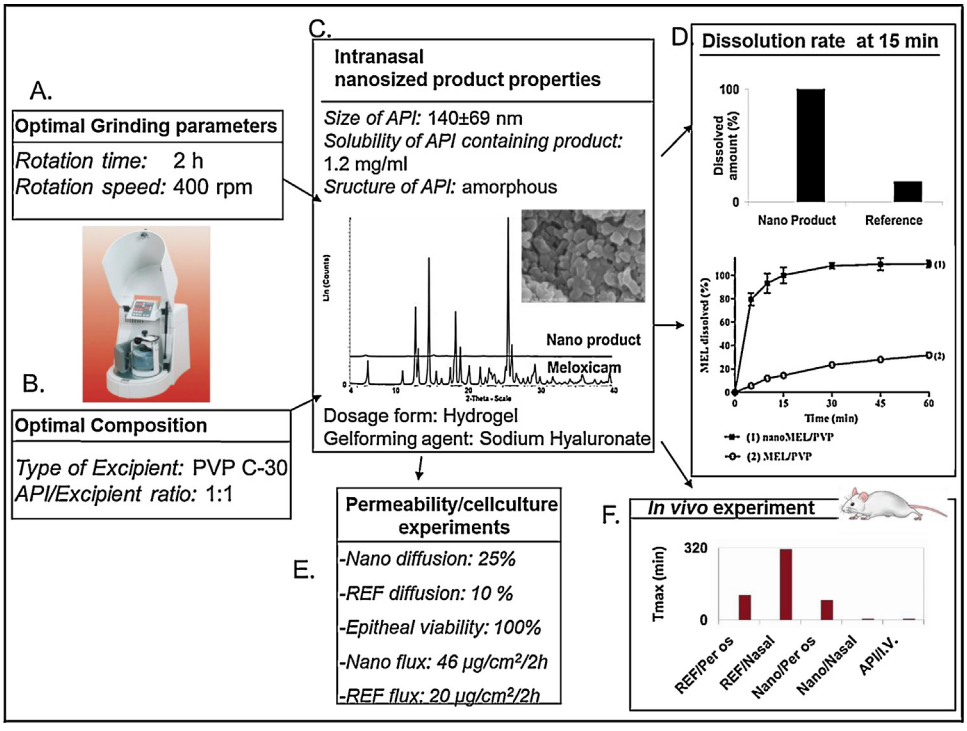

Fig. 4. Experimental production and characterization of intranasal nanosized product.

features with high relative impact, but low occurrence, or to low relative impact and high occurrence. There are no parameters in these quarters in the present case. The quarter listing features with high impact and high occurrence contains three parameters: the composition properties (with the highest impact and occurrence), and the grinder rotation speed and the grinding time. These are therefore the areas that require the greatest focus as concerns the experimental design and practice, with especial focus on the selection of the additives.

3.2. Comparison of the theoretical results and practical measurements

After the acquisition of the QbD-based theoretical RA and software prediction results, the experimental evaluation came into focus (Kürti et al., 2011; Zaman et al., 2013). The experimental results are presented in Fig. 4.

The optimal technological parameters required to achieve the nanosized drug are a grinding time of 2 h and a rotation speed of 400 rpm (Fig. 4A), with PVP C-30 in 1:1 mass ratio (Fig. 4B). Several factors were found to lead to the optimal dissolution properties of meloxicam nanoparticles: a particle size reduction (from 50 μm to 140 nm), an increased specific surface area, the presence of a hydrophilic polymer and the amorphous structure (Fig. 4C). The solubility and dissolution rate under nasal conditions (pH 7.4; 30 °C) differed markedly from those of the starting drug and physical mixtures (the API with PVP C-30) as REF (Fig. 4C,D). HA was applied as gel-forming material to formulate a nasal gel containing nanosized meloxicam (Fig. 4C). Diffusion was quicker from the formulation containing meloxicam nanoparticles and HA: 25% of the meloxicam diffused from the formulation containing nanosized meloxicam vs. 10% from the physical mixture during the first 2 h. The in vitro permeability results on a synthetic membrane and the cell-based assays suggest the potential usefulness of the pharmaceutical formulation containing nanosized meloxicam and HA for nasal delivery. MTT testing and a real-time assay demonstrated that the formulation containing meloxicam nanoparticles and HA is not toxic for human nasal epithelial cells. The flux of meloxicam through human RPMI 2650 cell layers was significantly higher in the case of the nasal formulation containing nanoparticles and HA (Fig. 4E).

Our in vivo results with meloxicam nanoparticles indicated that the maximum plasma concentration ($C_{max)}$ was reached within 90 min in the case of an oral liquid formulation and 5 min in the case of the nasal formulation. The time at which the maximum plasma concentration was reached (T_{max}) after oral or nasal application was nearly 2 h, in comparison with more than 5 h for the REF samples. The plasma kinetics of nasally applied meloxicam nanoparticles and intravenously injected material were similar: C_{max} was reached by 5 min (Fig. 4F).

The results of these experiments mirrored and correlated with the RA results, e.g. the type of grinding additive had a significant effect.

4. Discussion

The design and development of a new medicinal product involve three requirements. The desired product quality must satisfy the industrial, the patient (or customer) and the regulatory demands (Fig. 5A). The industrial and patient expectations are as important as the regulatory ones, but this article relates to the adoption of regulatory science in the early pharmaceutical technological development and deals with this stage of the requirements. The regulatory-based QbD is built up from ICH quality guidelines as shown in the scheme (Fig. 5B). In accordance with the regulatory expectations and rules, the elements and

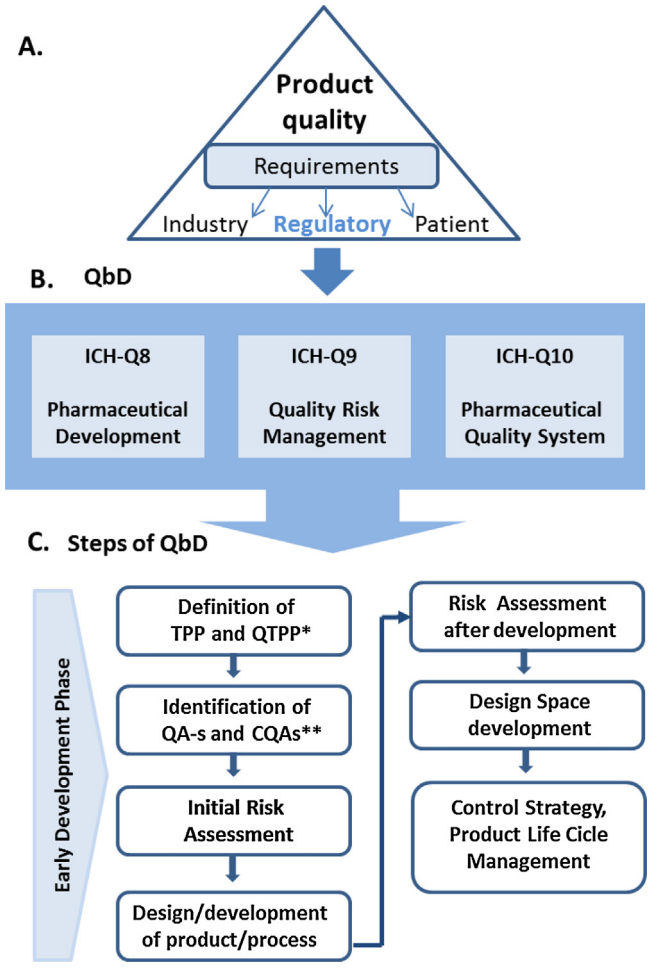

Fig. 5. Quality requirements and elements with the steps and roadmap of the regulatory based QbD (Charoo et al., 2012).

practical steps of a QbD-guided pharmaceutical technological development are presented in Fig. 5C.

The present investigation related to the support of regulatory science in the contemporary pharmaceutical technological early development and preformulation stage. A QbD concept-based RA was performed. The model target product was an analgesic formula containing meloxicam for adults with immediate release and a rapid systemic effect. Nasal administration and a nanosized active agent were selected for these purposes. The results of the RA with novel software (Lean QbD Software®) provided a theoretical indication of the parameters (CPPs and CQAs) with the greatest influence and effects on the desired product quality.

The sample composition was found to be the parameter with the greatest effect among the CPPs, and was classified as a first-line priority CPP. The grinding duration and rotation speed were ranked as second-line priority CPPs. In the further evaluation, size and permeability were classified as first-line priority CQAs. Solubility and dissolution were listed as second-line priority CQAs. These factors have the highest impact, and must be focused on in the design of the practical research in order to attain the desired product, i.e. a nanosized product with appropriate dissolution, solubility and permeability properties.

The practical research justified the theoretical prediction. Our experimental results reflected and correlated with the RA results. The optimum technological parameters for the attainment of a nanosized drug are a grinding time of 2 h and a grinding rotation speed of 400 rpm, with PVP C-30 in 1:1 mass ratio. The plasma

kinetics of the meloxicam in the prepared formulation containing a gel-forming agent (HA) were similar to those of an intravenous injection, and the maximum plasma concentration was reached in 5 min.

5. Conclusions

This study has confirmed that a QbD-based experimental design and RA can help to reduce the practical aspects of the early development research in pharmaceutical technology by predicting the parameters that most strongly influence the final quality. This QbD-based prediction can result in a shorter development time, lower costs, fewer needs for human resources and more effective target orientation. These can be of considerable importance in developments which are expensive, time-consuming and complex, e.g. nano-technological experiments. Our model example demonstrates the applicability and relevance of QbD in the early stages of pharmaceutical development. We plan investigations with the aim of improvement of the integration and extension of the QbD in such developments.

Conflict of interests

The authors declare that they have no competing interests.

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