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How Could QbD Address the R&D Challenges of 'Nose-to-Brain' Liposomal Resveratrol Formulations?

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Abstract: Trans-resveratrol, due to its antioxidant property, has the potential to be successfully applied in the prevention and the treatment of neurological disorders (Parkinson's and Alzheimer's disease). Nevertheless, its traditional administration (intravenous, oral) and bioavailability are limited by its physical-chemical characteristics (solubility, chemical instability, sensitivity to heat, UV-light and pH). The 'nose-to-brain' application, as an alternate administration route, represents a way to reach the brain without the limitations of the blood-brain barrier, while the use of nano-sized drug delivery systems, like the liposomes, can overcome the developmental and therapeutic issues of the formulations. This research paper shows the application of Risk Assessment, the key element of the Quality by Design mindset, in the development of a liposomal resveratrol-containing formulation with brain target and nasal administration. The study intends to demonstrate the definition of the quality target product profile, the selection of the critical factors, and the application of the RA to get a detailed view on the critical parts of the development process. On these terms, the factors with the most significant impact on the product quality among the critical material attributes (phospholipids, active pharmaceutical ingredient (API) content, cholesterol ratio, surface modification), furthermore the production process (temperature, oxidation and light protection) were identified; subsequently, an RA-based liposome preparation process was described. The formulation procedures of 'nose-to-brain' liposomal systems loaded with drugs with many limiting factors meet several risks; however, the adaption of the QbD tools helps to focus on the aimed final product quality and achieve effective experimental designs.

Keywords: liposome; resveratrol; nose-to-brain; Quality by Design; Risk Assessment

1. Introduction

Trans-resveratrol (RSV) (3,4',5-trihydroxy stilbene) (Figure 1) is a polyphenolic phytoalexin compound that has been studied in a broader scale to prevent and treat diseases due to its several biological effects [1]. Many beneficial properties of the compound are known, such as antioxidant, antidiabetic, cardioprotective, immunomodulatory, antitumor, antihypertensive, anti-inflammatory, platelet aggregation inhibitor, anti-ageing and anti-obesity effects [1–3]. RSV effectively attacks the extracellular reactive oxygen species (ROS) and is, therefore, a potent antioxidant compound. The use of RSV is recommended to prevent and treat chronic diseases such as metabolic disorders, tumours and neurodegenerative disorders [1,4]. It has been demonstrated in a rat model that RSV-containing lipid-based nanocarrier systems ameliorate β-amyloid plaques mediated memory impairments in Alzheimer's disease [5]. The outcome of the RSV therapy is the result of different processes (reduction of NF-κB (nuclear factor-kappa B)), inhibition of apoptosis, lipid peroxidation, aggregation and hyperphosphorylation of τ-proteins, reduction of ROS-induced toxicity and

number of β-amyloid plaques) [4]. The beneficial effects of RSV in Parkinson's disease also relate to its antioxidant effect, as the molecule reduces the number of ROS and decrease the apoptotic cell damages caused by oxidative stress [4]. It has also been shown that liposomal RSV has a more substantial protective effect on the dopaminergic neurons of *Striatum nigra* in rats with Parkinson's disease than the free drug [6]. Different conventional oral formulations have been developed in the pharmaceutical industry to exploit the therapeutic properties of RSV [1]; however, the application of free RSV in treatments is limited by its low bioavailability, metabolism, and rapid elimination from the systemic circulation. The biological half-life of the compound is also short [3]. Two geometric isomers of RSV are known, trans- and cis-RSV, which two have different biological activities. The isomerisation process occurs due to exposure of UV or visible light [7]. The trans-RSV is the more biologically active [7] and more stable isomer [8], however, its practical use is significantly limited by its low water solubility, UV-light, and heat sensitivity [1].

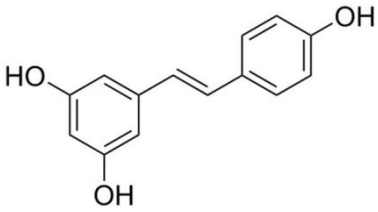

Figure 1. Structural formula of trans-resveratrol.

Several drug delivery systems have already been developed in nanotechnology to apply RSV effectively; however, the traditional administration routes were targeted in those cases. The intranasal route of administration, one of the alternative drug delivery methods, allows treating the brain areas by bypassing the blood-brain barrier (BBB), thus providing an opportunity for targeted therapy. In addition to the local and systemic drug delivery routes, the nasal cavity and the intranasal administration route provide the opportunity to deliver drugs to the brain areas as well. The intranasally administered drugs could absorb into the systemic circulation, reach the CNS through the BBB, accumulate in organs and tissues, or can be eliminated from the body. The other way is when the drug enters the brain tissue directly from the nasal cavity by bypassing the BBB directly along the olfactory nerve [9]. The BBB and the cerebrospinal fluid barrier make it significantly more difficult to deliver drugs to the CNS effectively; therefore, this is the most significant limitation of the medical treatment of the brain. APIs used to treat neurodegenerative disorders and administered into the blood circulation must cross the barrier of BBB to give their effect, but that prevents hydrophilic substances above 600 Da from entering the systemic circulation [10]. This limiting factor is one of the reasons why many diseases affecting CNS do not have an appropriate treatment yet. One of the research topics of today is this so-called 'nose-to-brain' drug delivery pathway that, bypassing the BBB. Higher brain concentrations of small lipophilic drugs were observed after intranasal administration than with intravenous application. This limited but direct transport to the CNS through the nasal mucosa happens by bypassing the blood-brain barrier due to the unique anatomical and physiological properties of the olfactory epithelium [11]. The nasal mucosa allows faster and higher level absorption than the gastrointestinal (GI) administration due to its higher permeability and neutral pH. Drugs that can be degraded by GI enzymes can also be used in this way. The 'nose-to-brain' pathway provides a useful route of administration for drugs that can provide adequate effects even at low doses and for those only minimal bioavailabilities can be achieved at oral administration [9], even if several factors influence the absorption of intranasally administered drugs and the development of the concentration required to achieve their therapeutic effect.

Over the years, various micelle-, liposome-, and other nanoparticle-based carrier systems [9] have been developed to transport polyphenols [1,2] in order to increase their solubility,

bioavailability, and absorption [4]. Among them, liposomes are one of the most successful delivery systems for drug delivery [3]. These nano-sized, spherical vesicles are bounded by one or more bilayers composed of phospholipids and cholesterol [12]. The lipid film has a similar structure to the natural cell membrane, with beneficial properties such as biocompatibility, delayed drug release, biodegradability, safety, and potential for industrial scale-up [3]. Different compositions, sizes, charges, and preparation methods are known for liposomes; however, their specific, both hydrophilic and hydrophobic segments containing structure makes these vesicles promising candidates for drug delivery [12].

An organised design is needed to unite the knowledge and the requirements when the goal of the research is to formulate a nano-sized, liposome-based drug delivery system when not just the compound itself but the administration route and the carrier have their limitations as well. The Quality by Design (QbD) concept is a knowledge and risk assessment-based quality management approach, used mainly in the pharmaceutical industry [13,14], but also can be extended and applied in the pharmaceutical early research and development (R&D) phase [15]. The QbD is a holistic and systemic way of improvements, where the primary focus came on the profound preliminary target product design; therefore the theoretical design phase is extended, and it is based on prior knowledge (from literature and previous research) and risk estimation. This accurate design and especially the implementation of the Risk Assessment (RA) help the proper set up of the practical experiments. A QbD method guided development has several steps. The first step is the definition of the Quality Target Product Profile (QTPP), which is a prospective summary of the quality characteristics of the product that ideally will be achieved. The next step is the selection of the parameters that have a critical influence on the QTPP. These are the Critical Quality Attributes (CQAs) maintaining the safety and efficacy of the product. Other critical factors are classified as the Critical Material Attributes (CMAs) related to the substances and the Critical Process Parameters (CPPs) related to the selected production method. The key element of a QbD-guided development is the RA as it ranks the CQAs and the CPPs by their critical effect on the targeted product quality. The Design of Experiments (DoE) [16] can be set up based on the results of the RA, which means that the practical experiments are planned and executed according to the most relevant influencing factors (CQAs, CPPs). The determination of the product's Design Space (DS) [17] can be performed in the next step. The DS has remarkable benefits as modifications within the DS do not mean changes in the product quality. The following steps of the QbD method are the improvement of the Control Strategy and the planning of the Continuous Improvement, which have relevance from the perspective of the pharmaceutical industry. The QbD and RA-based development and screening can be especially useful in early pharmaceutical developments of complex and sensitive drugs or systems with special considerations, furthermore ensure more effective and targeted experiments [18–23].

This study aimed to apply the risk-focused QbD approach in the development phase of the research project and to establish a development process for a resveratrol-containing liposomal formulation with brain target and nasal administration.

2. Experiments

As the first step, the screening of the literature, the collection of the information and the previous experiences were made as a knowledge space development. The steps of the QbD-, and RA-based development process were done in the followings. The research group defined the QTPP of the product, collected the critical parameters related to the quality of the targeted product (CQAs), material characteristics (CMAs), and production (CPPs), rated their independence from each other in a 3-grade (low, medium or high level) scale. The initial RA was performed with the QbD LEAN Software® (QbDWorks LLC, USA) and the results of the calculations were interpreted in the point of the experiments view. The thin-film hydration liposome preparation method [24] was chosen and investigated as a preparation process. The main points of this method are the formation of the lipid

film from phospholipids and cholesterol under low pressure, the hydration of the thin film, and the modification of the layer number and vesicle size [25].

3. Results

3.1. Ishikawa Diagram

The information collected from literature screening and experimental knowledge were combined, investigated and systematised in an Ishikawa diagram [26] (Figure 2) to illustrate the science and requirements of the development process together.

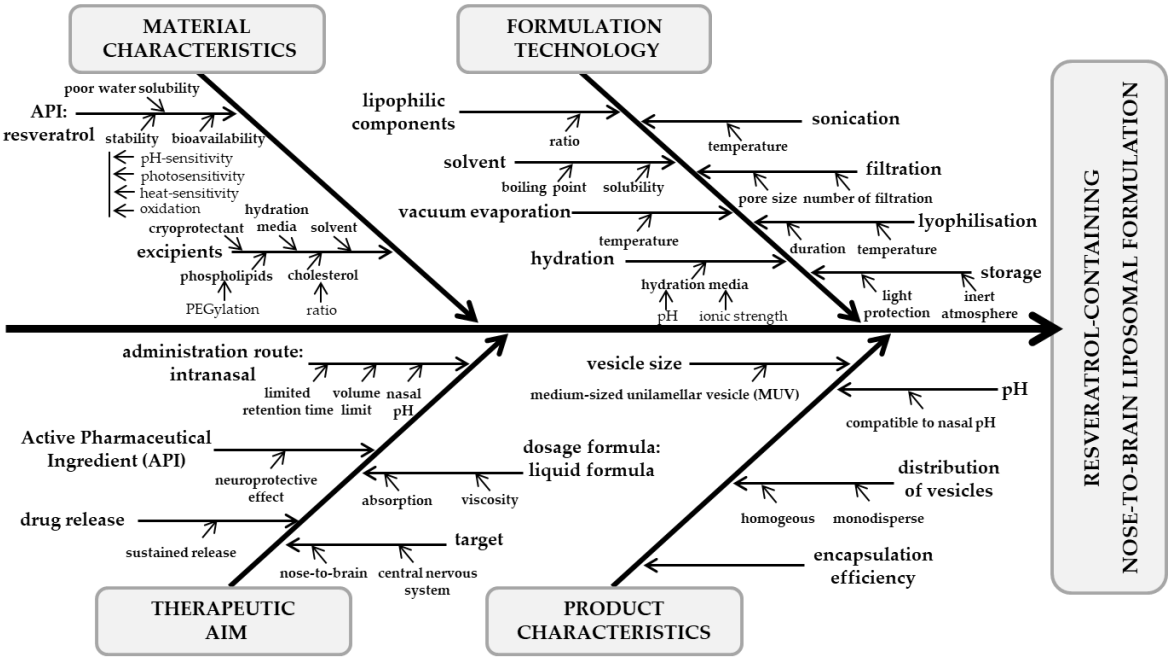

Figure 2. Ishikawa diagram for the evaluation of the cause-and-effect relationships in the trans-resveratrol containing 'nose-to-brain' liposomal formulation.

3.2. Quality Target Product Profile and Critical Quality Attributes

The defined profile (QTPP) and quality requirements (CQAs) of the targeted product are summarised in Table 1.

Table 1. QTPP and CQAs of the trans-resveratrol-containing 'nose-to-brain' liposomal formulation.

QTPP	Details	CQAs	Details
indication	neuroprotection	morphology of liposomes	spherical conventional vesicles
target patient population	ageing population	size of vesicles	medium-sized unilamellar vesicles (MUV)
route of administration	‚nose-to-brain'	surface modification	polyethylene glycol (PEG) chains
dosage form	aqueous solution; lyophilised plaques	polydispersity	monodisperse formulation
drug target	dopaminergic neurons; β-amyloid plaques	zeta potential	highly charged
drug release	sustained release	phase transition	formulation-suitable,

		temperature	trans-resveratrol decreases the value
viscosity	enough but not too viscous	API content	trans-resveratrol -containing vesicles
osmolarity	tolerable	position of the API	in the lipophilic double membrane
pH	suitable for intranasal administration and trans-resveratrol stability	encapsulation efficiency	high value
stability	stable formulation		
homogeneity	homogenous formulation		

3.3. Critical Material Attributes and Process Parameters

The influence of the collected material characteristics (CMAs) and settings of the manufacturing process (CPPs) on the QTPP and CQAs were compared and rated on a 3-grade scale. After mathematical calculations on the provided data, the software generated a relative severity-relative uncertainty diagram (Figure 3).

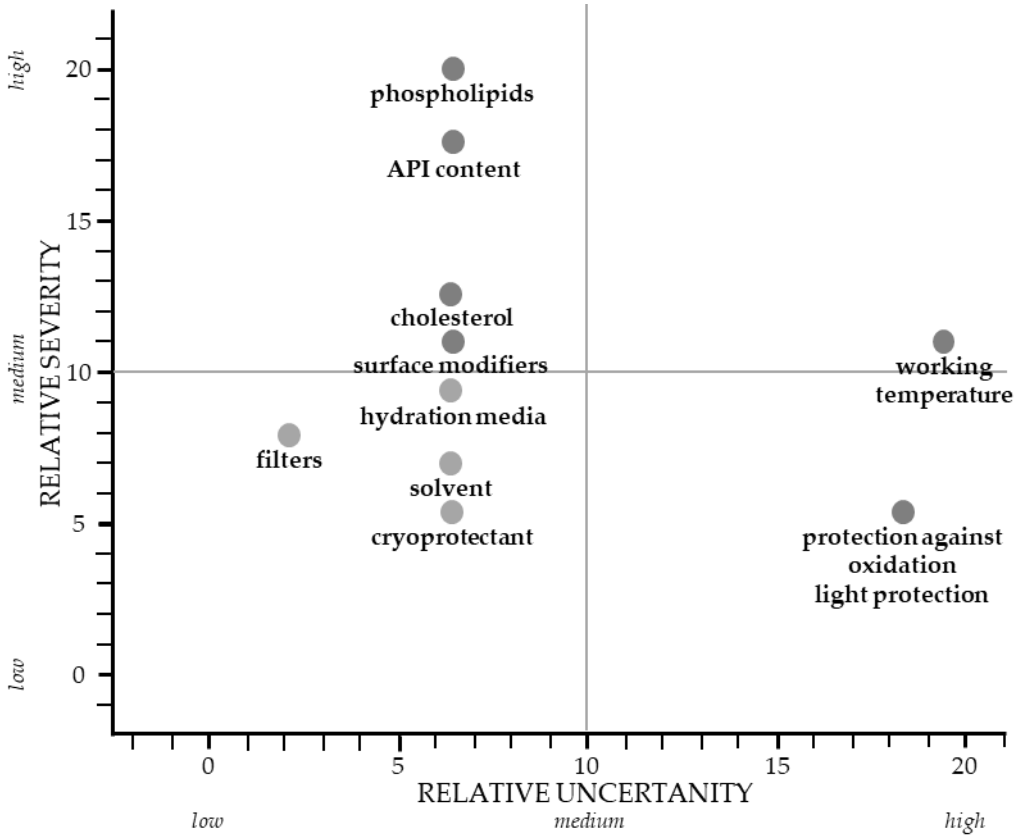

Figure 3. Relative severity-relative uncertainty diagram presenting the CMAs and CPPs of the formulation as a result of the RA.

4. Discussion

4.1. Ishikawa Diagram

The Ishikawa diagram (Figure 2) was designed to organise the knowledge and the requirements what are needed to execute a successful experimental design. The fishbone diagram collects the most important material quality and formulation requirements, furthermore the therapeutic and target product-oriented aspects.

4.2. Quality Target Product Profile and Critical Quality Attributes

The profile of the target product (QTPP) and the quality requirements of the formulation (CQAs) are summarised in Table 1. The QTPP depends mainly on the therapeutic or clinical aims and requirements, the characteristics of the drug substance, the administration route and the dose of the drug. This collection of the properties is always unique for a formulation. The CQAs of the product are the physical, chemical, biological and microbiological properties that should reach the indicated quality, range or limit to ensure the consistent end-product quality. For example, the features of liposomes, the size, the size distribution and the surface charge of the vesicles, furthermore the API-related properties of the formulation can be a part of the CQAs. These factors are also always unique for the preparation and depend on the QTPP.

4.3. Critical Material Attributes and Process Parameters

The relative severity-relative uncertainty diagram (Figure 3), as a result of the RA, has shown that the liposome wall-forming agents, the quality of the hydration media and the solvent used in the formulation, the applied temperature, the oxidation and the protection against light are the riskiest steps and factors during the manufacturing process and should get the most attention. The results also mean that the quality of the product can be influenced the easiest by changing the parameters related to the wall-forming agents (ratio, quality) and the trans-RSV-content. At the same time, the most critical point of the manufacturing process is the working temperature, because its change has a great impact on the outcome. The effect of the CMAs and CPPs can be accurately investigated if some of the values are set on the same level, while the ones under the scope of the study are being changed according to the Design of the Experiment.

5. Conclusions

Even though trans-RSV displays several health-beneficial properties and possesses remarkably strong antioxidant activity, its positive effects are restricted by the characteristics of the compound itself. The paper showed the opportunities of a liposomal formulation containing trans-resveratrol as an active ingredient, designed for the ‘nose-to-brain’ administration route, the technological challenges of development and their possible solutions by applying the QbD approach during the design phase of the research. As a conclusion, we can say that the RA highlights the critical points of the formulation process, and in this way helps to achieve the targeted quality for the product. Applying the QbD approach to design pharmaceutical formulations is a functioning way to improve the development process of the pharmaceutical formulations by collecting the proper information, optimising and rationalising the required experiments and measurements. The determination of the critical features and parameters (QTPPs, CQAs, and CPPs) provides a holistic network of information that can be useful to achieve a more effective experimental design.

Abbreviations

The following abbreviations are used in this manuscript:

API	active pharmaceutical ingredient
BBB	blood-brain barrier
CMAs	Critical Material Attributes
CPPs	Critical Process Parameters
CQAs	Critical Quality Attributes
DOE	Design of Experiments
DS	Design Space
GI	gastrointestinal
MUV	medium-sized unilamellar vesicle
NF-κB	nuclear factor-kappa B
PEG	polyethilene glycol
QbD	Quality by Design
QTPP	Quality Target Product Profile
R&D	research and development
RA	Risk Assessment
ROS	reactive oxygen species
RSV	resveratrol

Author Contributions: Z.N., E.P., D.G.D. and I.C. took part in conceptualisation; Z.N., E.P. and I.C. contributed in methodology; Z.N. and E.P. worked with software; N.Z. and D.G.D. performed investigation; N.Z. did data curation; N.Z. wrote the paper; P.E., D.G.D. and I.C. supervised the work. All authors have read and agreed to the published version of the manuscript.

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