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Scientific Journal
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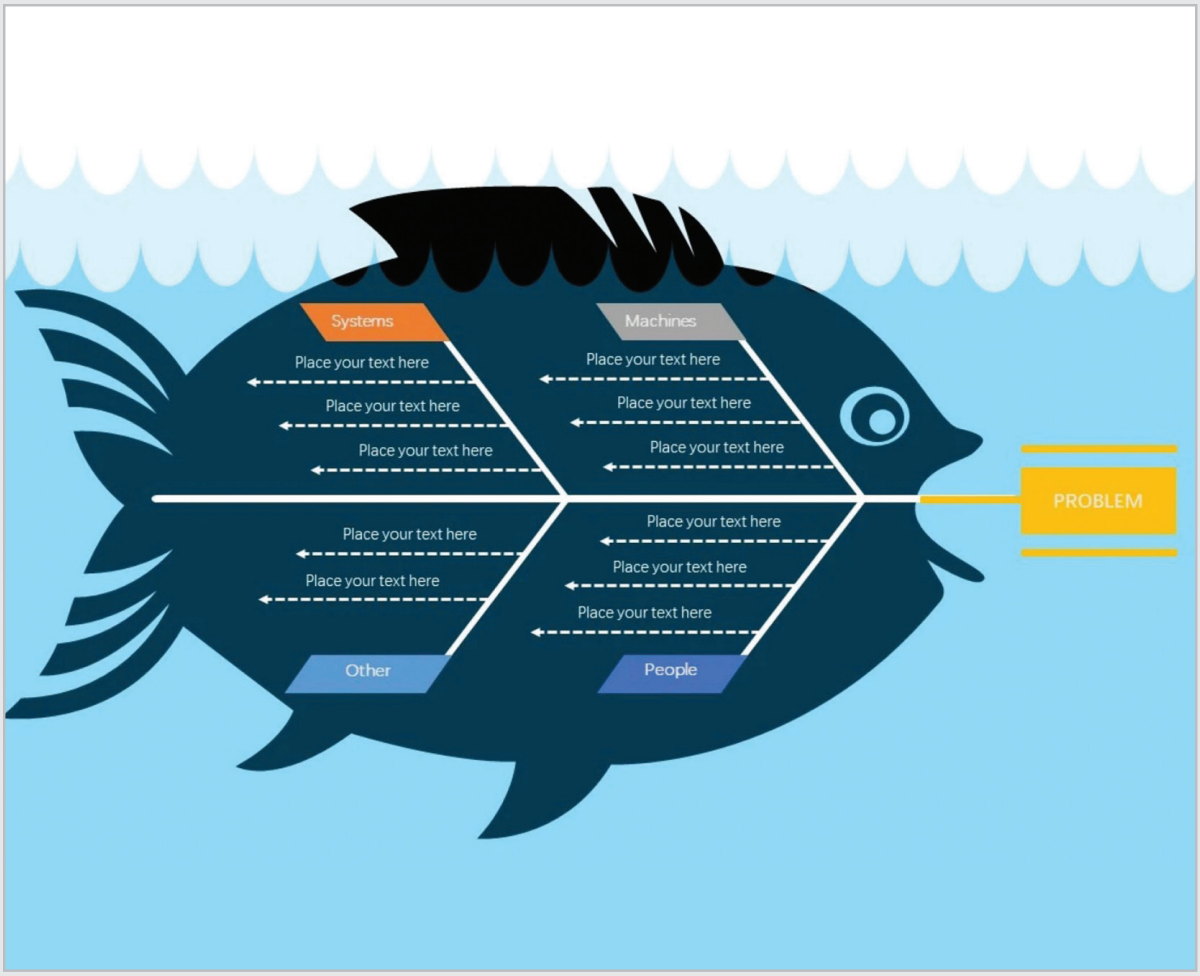
Acta Pharmaceutica Hungarica

Ishikawa-diagram



APH 2020;90:153–204. **ISSN 0001-6659**

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4
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Contents

Acta Pharmaceutica Hungarica: www.aph-hsps.hu

„Acta Pharmaceutica Hungarica" Scientific papers of the Hungarian Society for Pharmaceutical Sciences
Published by the Hungarian Society for Pharmaceutical Sciences, represented by *Prof. Éva Szökő*
Gyulai Pál u. 16., Budapest, 1085 Hungary, Phone: (+36-1) 235-09-99;
E-mail: office@aph-hsps.hu
Subscibtion: Hungarian Society for Pharmaceutical Sciences, Gyulai Pál u. 16., Budapest, 1085 Hungary by international banktransfer
(OTP bank account number: 11708001-20530530) – Mailing address: 1447 Budapest, Pf. 480
Informations for international banktransfer:
Account holder: OTP Bank Ltd. – Nádor utcai Kereskedelmi Banki Centrum
Account Number: 11708001-20530530
Bankadress: H-1051 Budapest, Nádor u. 6.
IBAN: HU20 1170 8001 2053 0530 0000 0000
Swift Code (BIC): OTPVHUHB
Subscibtion fee: HUF 6000 + HUF 300 VAT
Published quaterly
Typesetting: *Csaba Oláh*
Printing: ColorToys Bt.

Advances in drug release investigations: Trends and developments for dissolution test media

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Received: *Received: 2 July 2020 / Accepted: 17 July 2020*

Abstract: *Dissolution research started more than a century ago in the field of physical chemistry and went through several significant developments since. Yet, the explicit attention in drug-related dissolution has only started growing in the 1950s, after the researchers realized that drug release from orally administered solid pharmaceutical formulations significantly influences the bioavailability. Researches show that solubility and permeability of the drugs are key factors in the correlation between the in vitro examination and the in vivo determinations (IVIVC). This review aims to summarize the most relevant developments in chronological order, ranging from simple disintegration studies to biorelevant methods. Biorelevant methods can be used to recognize the effects of food on dissolution, as well as to identify solubility limitations and stability issues. The development of a biologically relevant dissolution method for oral dosage forms needs to take the physiological conditions of the gastrointestinal (GI) tract into account that may influence the drug dissolution. This review presents the simplest dissolution media, the composition of biorelevant media simulating gastrointestinal fluids, and the latest updates of the field.*

Keywords: drug dissolution, bioavailability, biorelevant methods, biorelevant media, gastrointestinal (GI) tract

Introduction

Dissolution tests in the pharmaceutical industry are not only used for the prediction of the expected bioavailability but also for quality control purposes. In product development, dissolution tests are used to select the labeled preparation, while in research to determine the influence of the critical manufacturing parameters (for example binder effect, mixing effect, granulation process, excipients) [1]. Predicting the in vivo bioavailability of the drug is extremely important to provide the best modeling of the digestive system. Dissolution methods should simulate the biological system in which the tablet passes through media of varying pH, ionic strength and bile acid content until it reaches the site of dissolution and absorption. Laboratory imaging of these conditions is particularly important in case of formulations where the dissolution of the active pharmaceutical ingredient (API) in the stomach is not advantageous and therefore provided with a gastroprotective coating. The digestive tract consists of three important parts: stomach, small intestine and large intestine [2]. Its diverse conditions have a major influence on the in vivo dissolution profile of the API [3]. The digestive system excretes various substances (e.g. water, enzymes, surfactants, hydrochloric acid) that amongst others determine the pH, buffer capacity and molarity [4]. All of these factors influence the dissolution and absorption of the API [5]. The stomach and the small intestine play vital role in the dissolution process, while primarily the latter is crucial for the absorption in most cases. Absorption can also happen in the large intestine, but its role therein isgenerally less important than the small intestines.

The Biopharmaceutical Classification System (BCS)

In terms of bioavailability, active pharmaceutical ingredients can be described with two main physicochemical properties: solubility and permeability. Experiments demonstrate that these are key factors in the correlation of in vitro drug dissolution and in vivo bioavailability [6] [7]. According to BCS, the drugs can be categorized into four basic groups based on their solubility and permeability (**Figure 1**) [6].

An active ingredient is considered to be highly soluble when its highest dose is soluble in max. 250 mL aqueous medium at pH 1.0-7.5, otherwise the active ingredient is poorly soluble [8]. The 250 mL volume estimate is derived from the typical bioequivalence testing protocols that require the patients to take the drug with a glass of water [9].

DOI: 10.33892/aph.2020.90.155-169

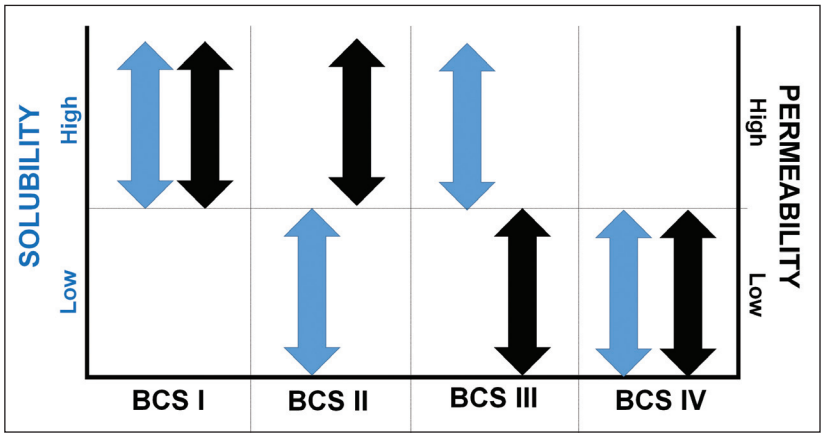

Figure 1 *The Biopharmaceutical Classification System.*

The European Medicines Agency has defined the pH to be in the range of 1.2 and for solubility testing. The pH of the medium must be measured before and after the addition of the active ingredient [10]. In the case of poorly water-soluble active ingredients, the solubility may be enhanced by the use of solubilizers. Furthermore, according to recent studies, the addition of formulation auxiliaries may also alter the permeability of the active ingredient [11].

During the early stages of developing drug candidates, it is possible to change the physicochemical properties of a molecule by modifying its chemical structure. The addition of polar substituents [12] or the formation of salts [13] can improve poor water solubility, while lipophilicity can be changed by e.g. the addition of fluoric substituents. In many cases, it is not possible to alter the chemical structure of a drug candidate because it may also alter its therapeutic effect, metabolism, toxicity or synthesis. Therefor specific formulation methods might provide a better solution to overcome poor water solubility.

The classification of permeability is based directly on the Human Intestinal Absorption of the API and is considered to be good above 85% [7]. A drug substance is considered highly permeable if the extent of intestinal absorption is 90% or higher. Otherwise the drug substance is considered poorly permeable [7] [14] [15]. Based on the BCS, a drug is classified as rapidly dissolving when at least 85% of the labeled amount of the active ingredient is dissolved in 30 min in USP (United States Pharmacopeia) Apparatus I at 100 rpm or in USP Apparatus II at 50 rpm in maximum 900 mL of each of the following media: a.) acidic media, such as 0.1 N HCl or USP simulated gastric fluid (SGF) without enzymes, b.) a pH 4.5 buffer, and c.) a pH 6.8 buffer or USP simulated intestinal fluid (SIF) without enzymes. Otherwise the drug product is considered to be a slowly dissolvable product [14] [16].

According to literature, an increasing proportion of the new drug molecules, estimated to be about 70%, are poorly water-soluble BCS Class II drugs, which sets a major challenge for both pharmaceutical researchers and developers. At least 30% of the drugs currently on the market also fall into this category [17].

The evolution of dissolution tests

Dissolution tests are one of the most essential techniques in the pharmaceutical industry. Not only used for development and quality control purposes, but the in vitro drug release measurements have always aimed to forecast the in vivo performance of pharmaceutical products [1]. From the simple disintegration assays the technique has greatly evolved, as its also enables the conduction of dissolution studies of immediate release (IR) products for quality control purposes [18].

The first dissolution studies were reported by Noyes and Whitney in 1897, who studied the dissolution of benzoic acid and lead chloride, two compounds well known for their poor solubility. The materials were placed inpl glass cylinders that were submerged into water tanks. The cylinders were rotated with constant speed at constant temperature. The authors noticed proportional correlation between the rate of dissolution and the difference between the instantaneous concentration C at time t. The saturation solubility C_s and k are constant (**Eq. 1**) [18] [19]:

(1) The Nernst- Brunner equation, published in 1904, is based on the diffusion layer concept and Fick's second law (**Eq. 2**).

(2) where , D is the diffusion coefficient, h is the thickness of the diffusion layer and V is the volume of the dissolution medium.

Later more alternative explanations were available. Danckwerts' classic surface renewal model was presented in 1951. It gives a quantitative description of gas absorption at the surface of a turbulent liquid. According to this model, the gas–liquid interface, where the absorption occurs, is continuously rejuvenated by fresh liquid elements

arriving from the liquid bulk [20]. In 1961 Higuchi reviewed the interfacial barrier model and due to the high activation energy level of the interfacial transport, he considered ite as the limiting step, rather than diffusion through the film [21].

In 1934 there was a disintegration test for tablets that used water as medium at 37 C° and periodical shaking. It was the first test that was published in Pharmacopeia Helvetica [22]. Several other disintegration methods were developed (using tubes [23], meshes [24], etc.) that tried to use rather biorelevant conditions, like simulated gastric fluid as disintegration media [25].. In 1948 Filleborn introduced an artificial stomach model that simulated the in vivo conditions, including the pH, peristalsis, fed state, volume of the gastric juice, and the hydrostatic pressure [26]. Notwithstanding the advances in in vitro dissolution, the tests were not used widely until the early 1950s.

Although a lot of experiments on physicochemical dissolution were carried out, the first official dissolution tests were accepted by the British Pharmacopoeia in 1945, and later, in 1950 by the USP [27]. It was only in the early 1950s that scientists began to recognize that the importance of dissolution – absorption rates for oral drugs [22]. Assuming that the drug is rapidly absorbed from the gastrointestinal tract, dissolution may be the rate-limiting step that controls its appearance in the bloodstream. This finding was first published by Edwards in 1951 [28]. He assumed that if aspirin tablets were dissolved in the stomach, the intestine would be the factor influencing the absorption into the bloodstream [22].

However, in 1957 Nelson was the first to explicitly link the in vitro dissolution rate with the blood levels of orally administered theophylline salts [29]. Only in the mid 1960s was the impact of dissolution on the therapeutic effect of orally administered drugs recognized. Two reports were published in 1963 [30] and 1964 [31] that drew attention to the lack of clinical efficacy of the two brands of tolbutamide marketed in Canada. The tablets showed much slower disintegration and release compared to other brands [32]. Such observations have also been confirmed with other tablets, like chloramphenicol and diphenylhydantoin [5]. In 1968 Martin et al. reported significant differences in the bioavailability of diphenylhydantoin, chloramphenicol, and sulfisoxazole-beading products of different brands [33].

The most serious bioavailability problems were reported in the United Kingdom and the United States (1971), and in Australia and New Zealand with phenytoin (1968). In the first case, a sevenfold difference was found in digoxin serum levels between the different formulations. This observation prompted the Food and Drug Administration (FDA or USFDA) to investigate the dissolution of 44 digoxin products of 32 manufacturers. Large differences were detected in the dissolution profiles in the in vitro assays [19] [34]. In the latter case, the phenytoin containing IR product, increased toxicity was observed when lactose was used as excipient instead of calcium sulfate [35]. The lower phenytoin absorption in the original case was attributed to precipitation of insoluble calcium phenytoin salt [36]. However, in 1979 Chapron et al. found no calcium-induced effect on the bioavailability of phenytoin when tested with calcium gluconate. These results indicate that the presence of lactose lead to the undesired increase in plasma concentrations that reached beyond the narrow therapeutic range (10-20 μg/mL). He considered that the highly hydrophile lactose increased the dissolution rate of phenytoin and thereby the bioavailability and consequently higher plasma concentrations [37].

This period pointed out the critical relationship between solubility and bioavailability. As a result, dissolution tests became one of the elemental tools for quality control purposes. As a consequence of these developments, the basket-stirred-flask test (USP Apparatus I, designed by M. Pernarowski) was accepted in 1970 as an official dissolution tester apparatus by the United States Pharmacopoeia (USP) and the National Formulary (NF) [1] [18] [27] [22] [38]. Since then the dissolution test was improved, standardized and calibration tests were also launched.

Pharmacopoeial Methods

Tablet disintegration devices have evolved into dissolution test devices, that usually contain 6-8 vessels. In addition to the samples blank or standard samples may also be used.

The rotating basket method first appeared in 1971. At the beginning of each test, the sample is placed in the basket made of stainless steel or equivalent. This method is generally used to measure capsules since this dosage form would float after being thrown into the dissolution medium. Nowadays, the official pharmacopoeial (Ph.Eur; USP; JP) baskets with standard size and design are made of stainless steel. Unfortunately, the long-

term use of these baskets in acidic or other corrosive media can damage them, thus causing the dissolution tests to fail. Pure gold coating up to 2.5 μm thickness is allowed by the USP for use in acid media or in case the active drug reacts with the plain steel basket. PTFE (polytetrafluorethylene), FEP (fluorinated ethylene propylene), PFA (perfluoralkoxy alkane) coatings are ideal for the protection of components when highly corrosive or solvent conditions exist.

The paddle method (USP Apparatus II) was introduced in 1978 [27] [22]. These devices are either made of stainless steel or another inert material, but inert surface coating is also approved. This test method is mainly applied on tablets that sink to the bottom of the vessel before mixing. When the Apparatus II is used for testing certain dosage forms, such as hard-gelatin capsules [39], sticky tablets or slowly disintegrating tablets, a sinker is needed to prevent the sample from floating [27]. Placing the samples in sinkers (for example 8 mesh basket sinker Japanese pharmacopeia size, spiral capsule sinker, O-ring style sinker, U-type sinker, etc.) may resolve these concerns allowing the use of the paddle apparatus [40]. In dissolution testing, according to the U.S. Pharmacopeia, typically a nonreactive stainless steel wire helix is used when normally floating dosage forms are tested. In 1987 Soltero et al. fabricated, tested and classified various new sinker designs. Four classes of sinkers were defined: (a) Longitudinal sinkers contact the dosage forms on the long axis; (b) Lateral sinkers wrap around the capsule or contact with it in the middle; (c) Screen enclosures are either cage-shaped and hold the entire capsule or circular and placed on top of the capsule; (d) Internal weights consist of two steel ball bearings [39].

While the use of sinkers may solve floating problems, their final position in the dissolution vessel may vary, thus contributing to deviance in test results. These can be eliminated by using the suspended stationary basket (for example felodipine stationary basket). Reproducibility and accuracy problems have been reported in connection with the USP Apparatus II. The primary source of variability is in the conventional cylindrical vessel, especially under the rotating paddle. The new peak vessels effectively displace the unstirred cone, forcing the tested API into the region of appropriate hydrodynamics, where the whole product is constantly and uniformly exposed to the medium [41].

The reciprocating cylinder method (USP Apparatus III), based on the disintegration of tablets and capsules, appeared in 1991 [42] [43]. This method is used for modified (sustained or delayed) release formulations. However, it simulates the vital physicochemical parameters and is undoubtedly more effective than USP Apparatus I-II for making in vivo predictions [44] [45], the method was still extremely labor-intensive and there were limitations with regards to automatization [18] [42].

Later, in 1995, the USP Apparatus IV was introduced as a flow-through cell method designed to mimic the bowel movement by pulsating flow. The design allows the medium to vary continuously thereby inducing the pH gradient, thusthe effect of this gradient and the continuous removal of the medium can be investigated [46]. This device also enables the dissolution testing of sustained-release formulations as the product always contacts with the fresh medium as if absorbed from the intestinal tract. The in vitro-in vivo correlations (IVIVC) of the poorly soluble compounds have been made easy with this flow-through apparatus [47].

Sampling is also an important issue for dissolution studies. Today's modern equipments are capable of sampling, which means it is removed, filtered, and collected from a specific location at a given time. It is possible to measure the dissolved drug in the sample on-line with a spectrophotometer connected to the system. To ensure the accuracy of the measurements rapid testing is inevitable along with the integrity of the equipment. Since the insoluble or yet undissolved particles (API or excipient) in the medium mustn't leave the vessels, the applied filters are of great importance in dissolution testing. In general, the filters are made of UMWH PE (ultra-high-molecular-weight polyethylene), but on occasions when chemical compatibility is an issue, PVDF (polyvinylidene fluoride or polyvinylidene difluoride) filters can also be used. PVDF exhibits increased chemical resistance and compatibility among thermoplastic materials and known for its inert resistance (**Figure 2**).

Pharmacopoeias also regulate the procedure of the dissolution tests. For example, two pharmacopoeial approaches are listed in the European Pharmacopoeia for the examination of delayed-release solid dosage forms. The tablets are stirred for two hours in (a) 750 – 750 mL or (b) 1000 – 1000 mL 0.1M degassed hydrochloric acid (HCl) at

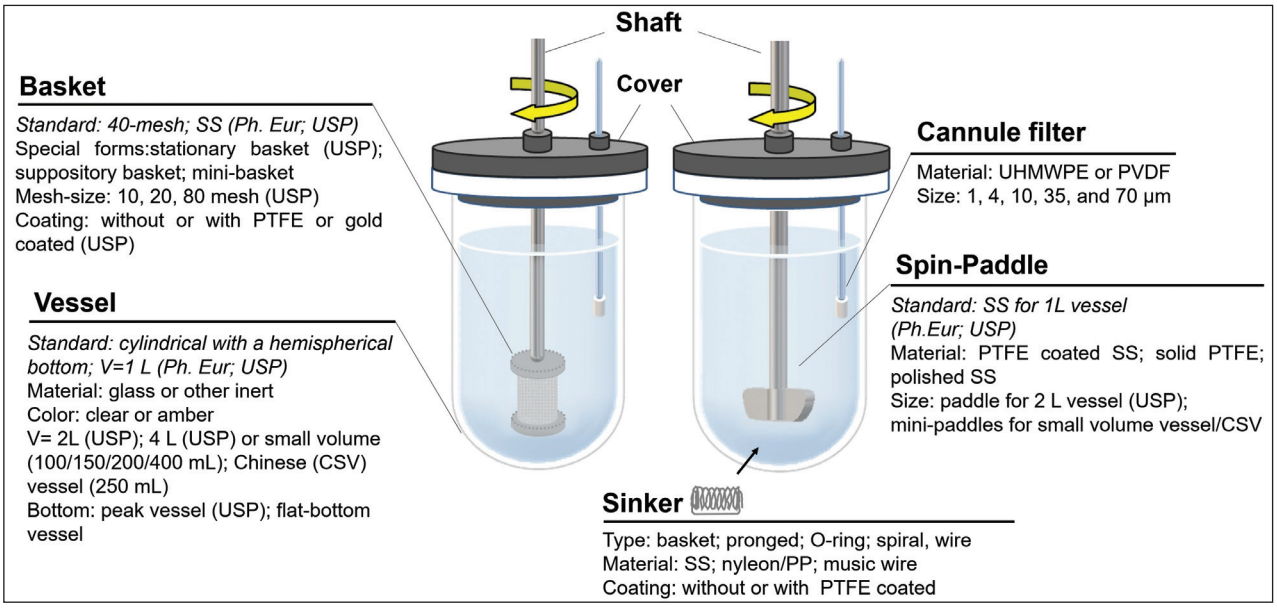

Figure 2 *USP Apparatus I-II and various accessories. (SS: stainless steel; PTFE: polytetrafluorethylene; UHMWPE: ultra-high-molecular-weight polyethylene; PVDF: polyvinylidene fluoride or polyvinylidene difluoride)*

37±0.5 C°. Afterward, the pH is raised by adding buffer solutions: (a) 250 mL 0.02M trisodium phosphate dodecahydrate solution is added to the dissolution vessel. If necessary, the pH is adjusted to 6.8±0.05 with 2 M hydrochloric acid or 2 M sodium hydroxide solution. (b) After two hours of dissolution in 0.1 M HCl, the hydrochloric media is drained from the vessels and replaced with 1000 mL pH 6.8 phosphate buffer. The buffer solution is prepared by mixing 0.1 M HCl solution (3 parts) and 0.20 M aqueous solution of trisodium phosphate dodecahydrate (1 part). If necessary, the pH is adjusted to 6.8±0.05 with 2 M hydrochloric acid or 2 M sodium hydroxide solution. The solution exchange can also be accomplished by replacing the acidic vessel with new vessels previously filled with the 1000 mL of degassed pH 6.8 buffer solution and thermostated to 37±0.5 C° and transferring the baskets. In both cases, the devices are used for additional 45 minutes or a specified period. Then a sample of the solution is removed from the bulk and analyzed by suitable qualitative and quantitative tests [48].

In vitro forecasting methods

Bicarbonate buffers are often used for in vitro predictions as they simulate the physiological pH ranges (between pH 5.0 and 8.4) of luminal fluids present in the digestive tract well. The pH of the bicarbonate buffer is the result of a complex and dynamic interaction among the bicarbonate ions, the carbonic acid and the dissolved and solvated carbon dioxide, as well as the partial pressure above the solution [49]. Liu et al. developed the bicarbonate system by modifying Hanks' buffered saline (pH 7.4) [50], that has too high pH but has too low buffer capacity compared to human intestinal fluids [51]. Therefore, this buffer was modified to create a pH 6.8 bicarbonate buffer with higher buffer capacity. The Hanks' solution is primarily a bicarbonate buffer in which hydrogen carbonate (HCO_3^-) and carbonic acid (H_2CO_3) are present simultaneously and carbon dioxide ($CO_{2(aq)}$) is formed by the dissociation of the latter [52] [53] (**Eq. 3**).

(3) The buffer pH can be adjusted by altering the acid (H_2CO_3) and conjugate base (HCO_3^-) concentration corresponding to the Henderson-Hasselbalch equation (**Eq. 4**):

(4) Bicarbonate buffers are less commonly used in the solubility studies of solid formulations. The reason lies in the thermodynamic instability of hydrogen carbonate solutions due to the complex equilibrium between the various ions [49]. Spontaneous CO_2 loss from the solution results in irregularly increases in pH. To keep the pH at the desired level either the CO_2 loss must be completely avoided, or the leaked CO_2 must be replaced by quantitative substitution, so an appropriate amount of gas has to be introduced to obtain the required pH again [54].

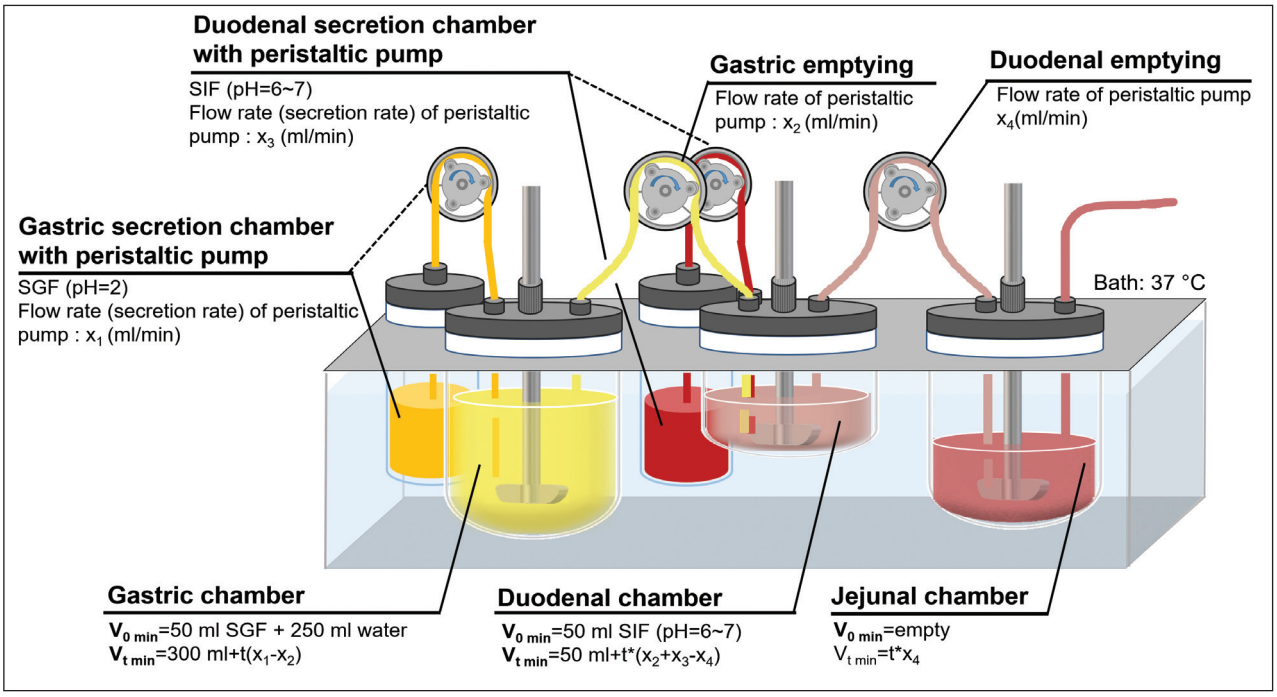

Figure 3 *The GIS dissolution model.*

To avoid difficulties, Al-Gousous et al. developed a phosphate-based dissolution method (pH 6.5 phosphate buffer) that resulted in similar dissolution profile compared to the carbonate system [55]. This method provides a better biopredictability in the fed state. The dissolution behavior of enteric polymers is more complex than the small molecules, therefore such approaches have been used where the molarity of phosphate gave similar disintegration times with the physiological bicarbonate buffer [55].

Biorelevant forecasting methods

Although the parameters and the biorelevant media can simulate the gastrointestinal tract better, the biorelevant methods provide a more complex but less feasible way of studying the dissolution behavior of drugs. [56]. In these cases, smaller volumes (250mL) of biorelevant fluids (e.g. FaSSIF-Fasted State Simulated Intestinal Fluid, FeSSIF-Fed State Simulated Intestinal Fluid, FaSSGF- Fasted State Simulated Gastric Fluid, FeSSGF- Fed State Simulated Gastric Fluid) are used to simulate the composition of fluids occurring at the different parts of the digestive tract. This way the experiments can provide a more accurate picture of in vivo dissolution [57] [58] [59] [60]. These media also model buffer capacity and surfactants (bile acids, lecithin) at exact pH levels. However, the quantitative prediction of in vivo performance directly from the dissolution test results can be challenging since various physiological parameters (e.g. gastric emptying, permeability through the intestinal membrane, transit time, pH, etc.) can also influence the bioavailability [61]. In general, the three most important absorption sites are modeled: stomach, duodenum and jejunum. The pH values of the media representing these sites are 2.0, 6.5 and 6.8. The stirring of the medium is ensured by mixing elements and the test is kept at constant temperature (37±0.5 C°).

Some methods that can be used to predict the behavior of the human body, such as the ASD (Artificial Stomach-Duodenum), a digestive system simulator that models three parts of the gastrointestinal tract: the stomach, the duodenum and the jejunum [62] [63] (**Figure 3**).The precision pumps provide the fluid supply for gastric and intestinal secretion, as well as the transfer of chamber content between the gastric-duodenum and duodenum-jejunum. The speed of the pumps and the liquid temperature are adjustable. Furthermore, the pH can be controlled while the rotation speed of paddles is kept constant [64].

Gut-on-a chip

The small intestine serves as a barrier against orally administered drugs, so it is highly important to predict its function at drug development.

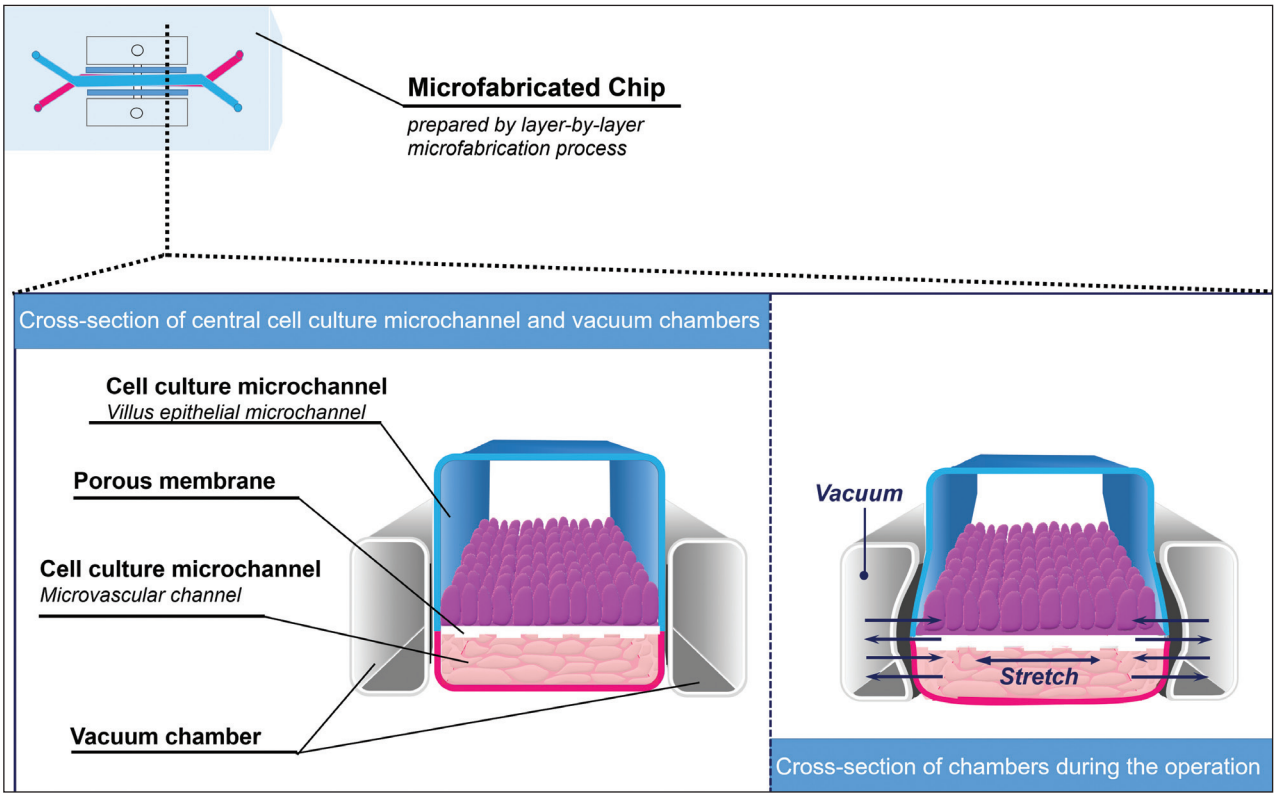

Figure 4 *Gut-on-a chip device.*

The biggest problem of animal models is that they can not always predict the processes that take place in the human body [65] [66]. This poses particular problems with the management of nutrient metabolism, transport, and oral absorption [67]. For these reasons, there has been a growing interest in developing an in vitro model of human intestinal function, including cell culture systems using transwell filter inserts that allow transepithelial inhibition. None of the existing in vitro intestinal models summarize the mechanically active intestinal microenvironment (e.g., peristaltic movements and intraluminal fluid flow) [68]. To evaluate this feature, Kim et al. developed a gut-on-a chip device (**Figure 4**) with an optical sensor system that allows continuous stimulation of Caco-2 cells with physiological stimuli, such as shear stress and cyclic mechanical stress, that stimulate peristaltic movements in vivo. The gut-on-a chip device was made by chip microfabrication using a flexible material, polydimethylsiloxan (PDMS) polymer with an adaptation of a soft lithographic technique. Therefore, the device has two independent compartments separated by a microporous membrane on which small intestinal model (Caco-2) cells were cultured.

Cyclic aspiration caused by peristaltic movements of the human gut was performed with a computer-controlled vacuum tube. The authors demonstrated that the in vitro model reproduced the results of the previously described animals and humans, thus concluding that the gut-on-a chip device could be used to analyze intestinal pathophysiology and dissect disease in vitro [69].

Components of the dissolution test media

The FDA Dissolution Database describes a large number of the different media from the simplest medium (water) or buffer solutions to solutions with added surfactants, organic solvents and enzymes [70]. The pH values of the most common dissolution media according to the database are in the range of 1.0- 7.5 (ionic strength similar to USP). Their pH values align with the corresponding sections of the GI tract, thus they are considered physiologically relevant [70]. In fasted state the gastric emptying time is approximately 30 minutes [71], in fed state it takes longer, about 2 hours [72]. This period varies greatly in healthy adults (20-53 years) and healthy children aged 0-5 years [73].

Conforming to the database, various acidic aqueous solutions can be used as solvents. The

most commonly used sour media is hydrochloric acid (0.1M, 0.01M, 0.001M), but phosphoric acid (0.01M) is also widely used [70]. To simulate the intestinal fluid (SIF) a medium at pH 6.8 should be used. As the source of water highly influences basic test conditions, like the pH or surface tension, water is not recommended choice for dissolution medium.Likewise, owing to both the active and inactive ingredients, the circumstances may also change during the dissolution test itself [74]. Besides, its low buffering capacity is also disadvantageous. For water-insoluble or sparingly water-soluble drugs the use of surfactants, such as sodium lauryl sulfate (SLS) [4], Tweens (polysorbate 20 and 80), lauryl dimethylamine oxide (LDAO), Triton X [75], Brij 35 (polyoxyethylene lauryl ether) or cetyl trimethyl ammonium bromide (CTAB) is common [70]. Artificial surfactants can interfere with salt formation of weak bases, thus dissolution can be affected in an artefactual way [76]. The incidental use of higher pH should be justified and should not exceed pH 8.0 in principle [74]. Small intestine transit time is nearly similar in children [77] and healthy adults in fed state, it is about 7.5 hours [78], in fasted state it is much shorter, approximately 2.5 hours [79].

Although, the conduction of in vivo assays may often be opposed for ethical reasons, technical limitations or monetary consideartions, a better understanding of the infantile digestive process would be greatly informative and helpful. Learning the process of infant formula disintegration in the infantile digestive system is a key in the development of new neonatal formulas with additional health benefits [80]. In recent years there was a little progress in the development of biorelevant media for suitable pediatric dissolution tests [81]. Predictive biopharmaceutical methods representing the in vivo drug dissolution in children would be of huge benefit for early formulation screening and influence assessment.

Simulated gastric fluids

In the fasted state the simplest dissolution medium is Simulated Gastric Fluid (SGF). This pepsin-containing hydrochloric acid has a pH value of 1.2, and its surface tension approximately equals to water [82]. To reduce its surface tension the use of surfactants, like SLS have been proposed [4]. Human stomach pH varies considerably (1.2-6.4) among individuals due to the prandial state, the quality and quantity of the consumed food. Typically the pH for fasted fluid ranges between 1.2 and 2.7 [83] [84] [85] [86] [87] [88], for fed gastric fluid it can range from 3.0 to 6.4, depending on the stage of digestion [85] [86].

In 2005, Vertzoni et al. developed a medium to simulate the fasted state of gastric fluid , which reflects the actual gastric composition according to published physiological data [89]. FaSSGF (pH = 1.6) contains physiologically relevant amounts of pepsin, bile salts and lecithin to obtain in vivo-like surface tension.

The content of the fed stomach is complex and its physicochemical properties change constantly over time as secretion, digestion and gastric emptying proceeds, therefor milk and/or nutritional liquid products can only be used to simulate initial gastric conditions in the fed state. Homogenized long-life milk (3.5% fat) is recommended as a solvent for simulating nutritional conditions [58] [90] [91] [92] [93]. The FeSSGF contains equal amounts of UHT-milk and acetate buffer. The mixture is stirred with a magnetic stirrer while the pH is adjusted to 5.0 with 0.1 N HCl. This medium can be used to predict food effects [94]. The pH in the stomach usually rises due to the buffering ability of the meal and initially may be up to pH 7.0, depending on the composition of the meal. After several hours of continued selection of gastric acid, the pH is reduced to the initial value [95] [85] [96].

In 2017 Passannanti et al., developed a dynamic in vitro digestive system based on infant physiology, including the oral, gastric and small intestinal phases of digestion to estimate the digestibility of rice starch- and rice cream-based baby foods [97]. In another study, cow- and soya milk formulas were used to simulate the gastric fluid in neonates [98] (**Table 1**).

Simulated small intestinal fluids

The most straightforward way to represent the small intestinal conditions is by using Simulated Intestinal Fluid (SIF), a pancreatin-bearing medium with pH = 6.8. Generally it is used for quality control puropses and its suitability for IVIVC is restrained. For example, the effect of food on the absorption of a drug cannot be predicted [99]. In the small intestine, the pH gradually increases between the duodenum and the ileum [2] [100] [101]. At the beginning of the transit, the pH changes rather slowly [102]. In the fasting state, the small intestine has a pH between 6.0-7.2, that decreases

Table 1 *Adult and paediatric biorelevant media (V=1L)* [58] [70] [71] [72] [76] [77] [78] [88] [93] [97]

	Fasted- state Simulated Gastric Fluid				Fed-state Simulated Gastric Fluid		
	Neonate	Infant	Adult	Adult in-vivo data [95] [105] [106]	Neonate-cow formula	Neonate-soy formula	Adult
Pepsin (mg/mL)	0.015	0.025	0.1	~0.8	-	-	-
Sodium taurocholate (μM)	20	60	80	~0.8	-	-	-
Lecithin (μM)	5	15	20	-	-	-	-
NaCl (mM)	34.2	34.2	34.2	68±29	100.35	94.79	237.02
Acetic acid (mM)	-	-	-	-	7.25	7.25	17.12
Sodium acetate (mM)	-	-	-	-	64.65	64.65	29.75
Milk:buffer ratio	-	-	-	-	1:1	1:1	1:1
pH	1.6	1.6	1.6	1.4-2.1	5.7	5.7	5.0
Buffer capacity (mmol/l/ΔpH)	-	-	-	41.0±6.0	15	15	25
Osmolality (mOsmol/kg)	120.7±2.5	120.7±2.5	120.7±2.5	191±36	340	240	400±10
Gastric emptying time (h)	~0.5				~1.0		~2.0

	Fasted-state simulated intestinal fluid		**Fed- state simulated intestinal fluid**						
	FaSSIF	FaSSIF-V2	Neonate-breastfed	Neonate-cow formula	Infant-cow formula	Adult	"Early"	"Middle"	"Late"
Sodium taurocholate (mM)	3	3	2.5	2.5	7.5	15	10	7.5	4.5
Lecitin (mM)	0.75	0.2	0.5	0.5	1.5	3.75	3	2	0.5
Dibasic sodium phosphate (mM)	28.65	-	-	-	-	-	-	-	-
Maleic acid (mM)	-	19.12	55.02	55.02	55.02	-	28.6	44	58.09
Sodium hydroxide (mM)	8.7	34.8	81.65	81.65	81.65	101	52.5	65.3	72
Sodium chloride (mM)	105.85	68.62	95	111.73	107.35	173	145.2	122.8	51
Glyceryl monooleate (mM)	-	-	5	6.65	5	-	6.5	5	1
Sodium oleate (mM)	-	-	0.8	1.06	0.8	-	40	30	-
Acetic acid (mM)	-	-	-	-	-	144	-	-	0.8
pH	6.5	6.8	5.8	5.8	5.8	5.0	6.5	5.8	5.4
Buffer capacity (mmol/l/ΔpH)	12	10	25	25	25	76	25	25	15
Osmolality (mOsmol/kg)	270±10	180±10	330±10	330±10	390±10	635±10	400±10	390±10	240±10
Small intestinal transit time (h)	~2.5		~8.0			~7.5			

after a meal to a pH of about 5.0- 6.0, since the food is mixed with the gastric juices that reduces the initial alkaline pH of the small intestine [103] [55]. After reaching the colon, the pH decreases to approximately 5.0 [100] [102].

In 1998 Dressman et al. introduced a medium to simulate the fasting state in the small intestine based on physiological data [4] [58]. In addition to pH, osmolality and buffer capacity, FaSSIF takes the solubility of intestinal fluids into account. FaSSIF (pH = 6.5) contains sodium taurocholate and phospholipids (4:1). In 2008 Jantratid et al. came

up with the updated composition of FaSSIF. FaSSIF-V2 contains reduced amount of lecithin (0.75 mM in FaSSIF to 0.2 mM), it has lower osmolality and the phosphate buffer is substituted to maleate buffer (Table 1) [94].

The use of bicarbonic media in the small intestine requires attention as carbon dioxide introduction is necessary to maintain the pH level, buffer capacity, ionic strength and osmolality [104]. Phosphate buffer is used instead to avoid the incidental pH instability.

The fed state simulating fluid (FeSSIF) was introduced for better simulation of the small intestinal surroundings [4] [58]. FeSSIF has a pH of 5.0, the osmolality and buffer capacity were adjusted to approach the in vivo data like FaSSIF. In fed state (FeSSIF) the sodium taurocholate and phospholipids concentrations are higher than in fasted state (FaSSIF) [105].

Based on novel studies, Jantratid et al. have revised the composition of FeSSIF as data indicated the rather slow pH decrease in the jejunum (upper part of the small intestine) after food intake [94]. The updated media (FeSSIF-V2) also contains various lipolysis products (glyceryl monooleate and sodium oleate).

So called snapshot media were developed to mirror the influence of digestive processes on the composition of fed state gastric media. "Early", "middle" and "late" phases represent the different time-frames of the composition of the small intestine after the ingestion. FeSSIF-V2 is suggested as a representative medium for the conditions in the small intestine [94]. Table 1 also includes the media used to simulate the intestinal fluids of neonates and infants [98] as well as the transit time of the small intestine [77] [78] [79].

Simulated colon fluids

The colon can also serve as an absorption site, an alternative drug delivery route to the systemic circulation. Although the absorption from the colon is less significant than from the small intestine, the systemic drug delivery via the colon has its advantages: namely the prolonged retention time, the relatively low enzyme secretion and the direct delivery to the systemic circulation avoiding the hepatic first-pass effect. Previous studies have shown that the pH in the initial part of the large intestine ranges with time and the composition of food intake from 4.8 to 7 [106]. In 2005 Fotaki et al. [107] developed a Simulated Colonic Fluid (SCoF) based on the pH values and short-chain fatty acid concentration according to available physiological data. SCoF has slightly acidic pH (pH 5.8). In 2010 Vertzoni et al. developed media that simulates the physicochemical characteristics of the ascending colon in the fed and fasted states [108]. Fasted state simulated colonic fluid (FaSSCoF) contains tris/maleate buffer solution and the pH is adjusted to 7.8. The same components are present in the fed state simulated colonic fluid (FeSSCoF) but in different concentrations and the pH is adjusted to 6.0 [106]. Significant differences can be observed in the colon transit times of different age groups. While in children this period is aabout 17.5 hours long [77], in adults it takes remarkably more, around 39 hours [109] whilein the elderly (75-80 years) this can extend even up to 66 hours [110] (Table 2).

Comparison of in vitro dissolution profiles

Among the numerous model-dependent and model-independent methods investigated for dissolution profile comparison, the use of fit factors are the simplest. The method was introduced by Moore and Flanner and nowadays it is recommended by several global regulatory authorities. It is a model-independent mathematical approach to analyze the dissolution curves applying the similarity factor f_2 [111] [112]. The method compares the dissolution values (in percentage) of the reference and sample preparation at time and can be described by the following equation (**Eq. 5**).

(5) where n is the number of sampling points, R_t is the cumulative percentage of the drug dissolved in the reference product at, T_t is the cumulative percentage dissolved at the selected times of the test product [113].

Profile tests and f_2 calculations are not necessary if more than 85% of the drug is dissolved in 15 minutes. If the similarity factor, f_2 is higher than 50, the dissolution profiles can be considered similar.

Table 2 Components of SCoF (V=1 L) [76] [108] [110] [111]

	SCoF
Acetic acid (mM)	170
NaOH (mM)	157
pH	5.8
Osmolality (mOsmol/kg)	295
Buffer capacity (mmol/l/pH)	29.1
Ionic strength	0.16
Transit time (h)	Children: ≈17.5Adult: ≈39

Conclusions

The dissolution methods used in quality control tests are simple, standardized tests that are well regulated by the pharmaceutical authorities. However, the conditions of these studies are often far from biologically relevant conditions, their in vivo predictability is limited. Biorelevant dissolution methods are used as in vitro representatives of in vivo performance. The updated biorelevant dissolution media simulate the physiological in vivo and in vitro hydrodynamic conditions accurately and also attempt to mimic the luminal hydrodynamics. These media can be used to predict the performance of different formulations and the effect of food in vivo. The proper selection of in vitro circumstances that closely simulate the in vivo conditions can lead to successful predictions of in vitro- in vivo correlation (IVIVC) for oral formulations.

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Overview of the causes and management of drug shortages in the United States and in Hungary

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Received: *22 October 2020 / Revised: 17 November 2020 / Accepted: 1 December 2020*

Abstract: *Drug shortages are a multifactorial international concern that are increasingly reported all over the world. A continuously rising number of cases could be observed since 2001 in the United States, but the issue is particularly significant since 2009. In Hungary, the same increasing tendency was observed; while in 2012 464 shortages occurred, in 2020 1466 cases were reported. According to the US Food and Drug Administration, there are three root causes behind the shortages. This paper aims at conducting a comprehensive overview of literature, exploring these principal causes and additional factors deriving from them in detail and thereby explaining how each of these contributes to shortages. Furthermore, it will illustrate how the lack of sufficient information due to an imperfect warning system also exacerbates the issue. As the problem affects every stakeholder in the supply chain from patients to Marketing Authorisation Holders, each party should be involved in the development and implementation of mitigating strategies that can provide the basis of policy measures. The article highlights how international trends both in terms of affected therapeutic areas and causes of shortages are reflected in the Hungarian markets; therefore, similar approaches could be adapted to tackle the issue domestically and deliver enduring solutions.*

Keywords: *drug shortages, literature overview, causes and impacts of drug shortages, global tendencies, prevalence of shortages in Hungary*

1. Introduction

During the second decade of the twenty-first-century people's mindsets have become increasingly global, with the need for immediate access to a variety of goods. However, the frequency and severity of medicine shortages in almost all member states of the European Economic Area (EEA) and in the United States (US) have substantially intensified only in the last couple of years. Some medicinal products are simply not available on a certain market even though there is sufficient economic power to pay them [1]. Drug shortages are a state when the available or calculated claim for medicines does not meet adequately at the end-user level. Causes are multiple and diverse among countries [2]. There are a complex set of factors underlying such shortages but various stakeholders of the supply chain of human medicine all agree that they mainly derive from the following root causes, according to Figure 1:

- *Logistical [procurement of excipients] and regulatory challenges*
- *Maintaining mature Quality Management Systems*
- *Business and Economic Issues [3,4].*

These factors can negatively affect patient recovery as delayed medicines and the use of replacement treatments are more likely to cause medication errors. Drug shortages also have a detrimental impact on the finances and personnel management of health care sites [5]. As a result, physicians cannot manage their patient's therapy adequately or clinical pharmacists must provide an alternative treatment, which requires their substantial extra time and effort [6]. The issue of medicine shortages must be resolved as soon as possible, especially when "critical medicines" are affected [7]. The aim of this review is to develop a comprehensive overview of the problem with an emphasis on global occurrence [8]. Figure 2 shows the annual cases of new shortages identified through the last 2 decades in the US. [9].

2. Literature Review

To gain a thorough understanding of the topic a search through Google Scholar and Pub Med was conducted between 19th September 2020 and 12th October 2020. The most frequent keywords and terms used included the followings: *drug shortage as a global challenge*, *shortage impacts on stakeholders*, *causing factors of shortages*, *generics market*, *commonly affected products by shortages*, *handling of shortages*, *advanced protocols for shortages*. Additional informa-

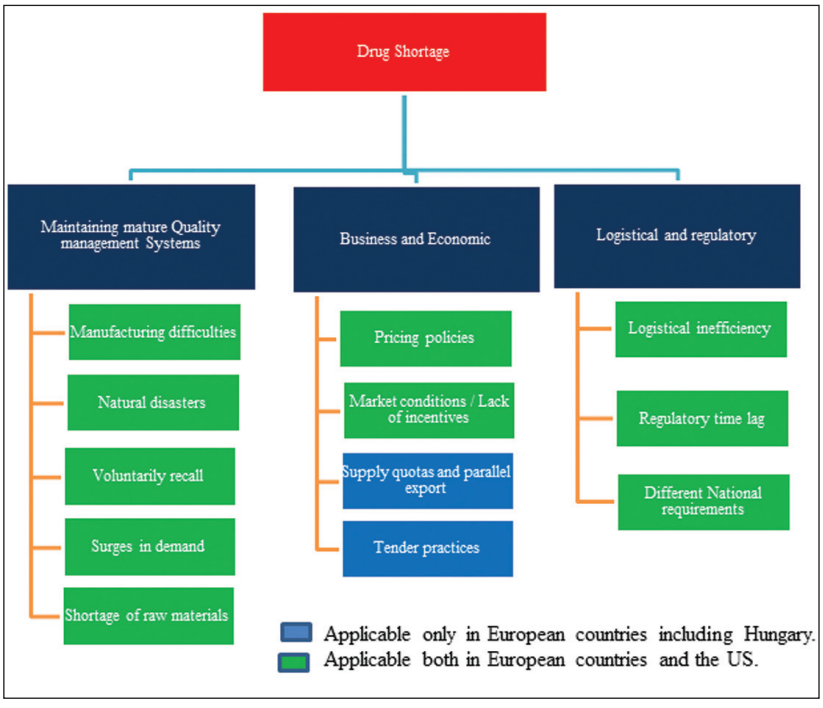

Figure 1 *Potential root causes of shortages and derived factors [3,4].*

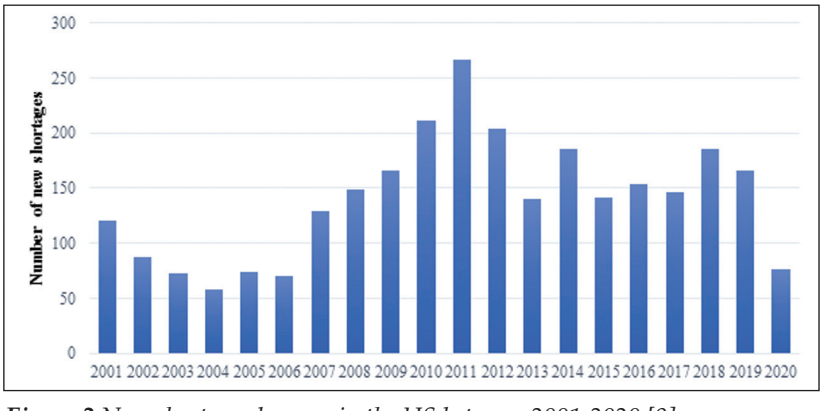

Figure 2 *New shortages by year in the US between 2001-2020 [9].*

tion has been collected from the American Society of Health-System Pharmacists (ASHP) website, the European Association of Hospital Pharmacist (EAHP) report and the National Institute of Pharmacy and Nutrition (OGYEI), and Food and Drug Administration(FDA) database. The following professional journals have also served as a source: The New England Journal of Medicine, The Lancet Oncology, The Oncologist, Mayo Clinic Proceedings, Journal of Parenteral and Enteral Nutrition, Journal of Oncology Practice, Journal of the American Medical Association, American Journal of Health-System Pharmacy: AJHP: official journal of the American Society of Health-System Pharmacists, American Journal of Pharmaceutical Education. To obtain relevant statistical data regarding annual new shortages in the United States between 2001 and 2020 and the therapeutic percentage of shortages between 2013 and 2019, the University of Utah information system and database was used.

Drug shortage is a state when the available or calculated claim for medicines does not meet demand adequately at the end-user level despite sufficient financial resources being available [1]. The problem has continuously been growing in the recent decades and the World Health Organization (WHO) reported more than 20 countries to be affected worldwide in 2011[10]. Despite this being a global challenge, there is no single common definition to exactly determine the state of "drug shortage"[11]. Based on an Institute for Healthcare Informatics survey conducted in November 2011 in the US, there are five disease areas highly exposed to this problem, these being oncology, anti-infectives, cardiovascular, central nervous system, and pain management medications [7] [12]. Unfortunately, drug shortages show an increasing trend based on the data of the ASHP reports from 2013 [13] and 2020 [9]. The same tendency can be observed in Hungary. In 2012, 464 reported shortages were reported [14], while the „List of Product Shortages" published by the National Institute of Pharmacy and Nutrition in 2020 contains 1466 products being short in supply [15]. This Hungarian data is based on MAHs legally obliged reports towards the Authority [16]. Lack of sufficient information due to an imperfect warning system contributes to the issue to a great extent [17]. According to US law, MAHs should notify the FDA in advance if they intend to discontinue supply [18], however nor administrative neither financial burdens are in prospect if they do so [17]. In case the notification does not happen, there is no chance to prepare healthcare systems for all direct and indirect consequences of drug shortages [19]. Manufacturers suggest that if they would be notified about the expected discontinua-

tion of production of other MAHs, they would be able to increase production accordingly. However, this would require a much better flow of information among supply chain members [20].

According to the FDA, there are three root causes of shortages [3]. The first is business and economic issues. There are no incentives to market barely profitable drugs [21], resulting from strong price competition, the so-called "race to the bottom pricing" [22]. This can be traced back to basic economic principles, as companies rather invest their profit into drugs which promise a higher return [21] instead of old generics [23]. Another aspect of the first root cause is the highly concentrated generic market, [24] caused by the numerous mergers and acquisitions of companies since 1980 in the US [24]. As an example, the sterile injectable drugs for oncology are marketed only by three Marketing Authorization Holders (MAHs) [7]. Moreover, parallel distribution, a very controversial field which refers to business transactions between wholesalers registered in different markets, discussed in detail in section 2.1, can also potentially explain the first root cause. Tendering practice as "single winner approach" introduces the risk that the chosen company may not be able to supply the right quantity in the right time [4].

The second root cause according to FDA reports is the obligation to maintain the quality management as laid down in the Good Manufacturing Practice (GMP) [25]. To meet every standard, MAHs tend to remove batch for every suspected deviation from the GMP, which practice is referred to as voluntary recall [26]. From this root cause also derives to a more severe issue, manufacturing and quality problems, which were attributed to around 64% of shortages in 2017 [27]. This may primarily be due to subcontractors located on another continent, mostly in Asia [28]. To mitigate this issue, the FDA issued guidance on how to select proper subcontractors [29]. Another reason behind manufacturing bottlenecks can be that MAHs often use the same equipment to produce different drugs [20]. Furthermore, the shortage of raw materials can also often lead to inability to maintain the quality management, thereby resulting in shortages of medicine [3]. This is due to the reason that MAHs do not only depend on manufacturers, but also on suppliers located in great geographical distances, [30] mostly in China and India [31].

The third root cause [3] is logistical and regulatory challenges which are also in connection with outsourced activities [32]. What regards logistics, pharmacies and hospitals obtain medicines from wholesalers, and the highly restricted conditions of storing, selling and delivering drugs often cause delivery delays [33] "Just-in-time " stock management system also contributes short supply [34]. Despite occurring rarely, natural disasters or pandemics can cause large shortages if they destroy manufacturing capacity [3] or spike up demand [4]. If the level of demand raised over regular expectations, [37] MAH may not be able to keep up production capacity [38]. On the regulatory side, the decision to contract out any task results in high bureaucratic and administrative burdens towards different authorities which are becoming increasingly strict [39]. MAHs also report that the increasing regulatory control is generally detrimental to their ability to meet demand as planned [17].

Regardless of their cause, shortages have a grave impact on every stakeholder [5]. Often they can be managed using alternative medicinal products, [40] [18], however, this may reduce patient safety due to various reasons, [41] [42] cause reputation damage for MAHs, hinder healthcare professionals from carrying out their tasks, [43] and increase labor cost [44] [45] because additional effort is needed to find the alternative therapy [46]. Besides, alternative medication often has a higher cost [45]. A prospective risk assessment plan is necessary to minimize all the above-mentioned impacts [8].

Healthcare institutions should be ready to mitigate the impact of shortages even before they occur. They should implement a shortage management strategy in advance, particularly to avoid the risk of making unethical triage decisions when already facing the shortage [18]. Healthcare institutions have to create and maintain an interdisciplinary expert committee who can make quick decisions, handle potential shortages, and minimize their impact [18]. A resource allocation committee should be delegated to monitor resources in the facility, [47] while if a specific therapeutic area is affected, an expert from that particular field should be involved [48]. The triage procedure must be adapted to shortages, and it should be based on patient characteristics after any conflict of interest has been excluded [49]. This also helps to avoid the necessity for professionals to make triage calls on their own when the amount of available medications is limited. Healthcare institutions should create formal guidelines in advance on how to identify and approve alternatives [50].

There are some complex cases when it is not possible to change the original agent [51]. It is in any case important to ensure through predefined procedures that priority is given to those who are younger or sicker [48] [49]. The main goal is to not distinguish particular patients and always follow the principles of justice, beneficence, and nonmaleficence [52]. Drug shortage management starts when the first signal arrives from the market, which launches a chain reaction [17]. Operational assessment should be carried out, clarifying the expected duration of shortage and the available inventory, converting inventory count into common measurement units if necessary [18]. Furthermore, therapeutic assessment is needed to identify available alternative therapies and identify affected patient groups [49]. In Hungary, the most critical aspect is the communication between supply chain players [53]. Often pharmacists behind the counter need to improvise which may cause mistrust between them and the patients, resulting in professional reputation loss [18]. If a certain drug cannot be provided, the first step is checking replacement therapies on the Hungarian Competent Authority's website. If replacements are not available, the pharmacist notifies the prescriber to claim alternative therapy. The second step should be taken by wholesale distributors, who play an intermediary role between pharmacies and MAHs, and have the capability to identify and collect signals from the market which predict an approaching shortage [54]. They should apply for "contingent approval" and/ or "individual approval of OGYEI" that both grant an authorization to import from abroad [55]. Additionally, the physician has an alternative option to prescribe medicine non-registered in Hungary to avoid possible life-threatening consequences [56]. However, it is the MAHs' responsibility by law [57] to not only market medicinal products on a particular market but also prepare a shortage strategy in advance to mitigate potential impacts of a drug shortage. MAHs should also be responsible for accommodating unforeseen circumstances [17].

2.1. Causing factors behind drug shortages

Despite not being an explicit root cause, the lack of sufficient information due to an imperfect warning system contributes to the issue of drug shortages to a great extent [17]. In the US drug manufacturers must inform the Competent Authority (FDA) 6 months in advance in case they intend to stop the production of a certain medicinal product [18]. However, there is nor financial neither administrative fines for missing the report towards the FDA [58] .The competent authority cannot mandate a MAH to produce a particular product. Without sufficient warning of upcoming discontinuation, there is no chance to prepare healthcare systems for all direct and indirect consequences of drug shortages [19]. Some MAHs do voluntarily alert the FDA about upcoming shortages. If all other MAHs would be notified in advance that a drug shortage is anticipated, they probably would be able to increase production to obviate it [17]. Wholesalers and other actors entitled to administer medicines for patients have also been inconsistent in providing information to health systems in relation to approaching drug shortages [2]. Drugs being short in supply are often only noted when patients walk into the pharmacy and try to purchase medicine, but it is no longer available. The pharmacist behind the counter should have reliable and timely information to manage patients therapy [59]. In case the particular medicinal product being in shortage is produced only by one MAH who would be the only source for wholesalers or hospitals, the situation is more severe [1].

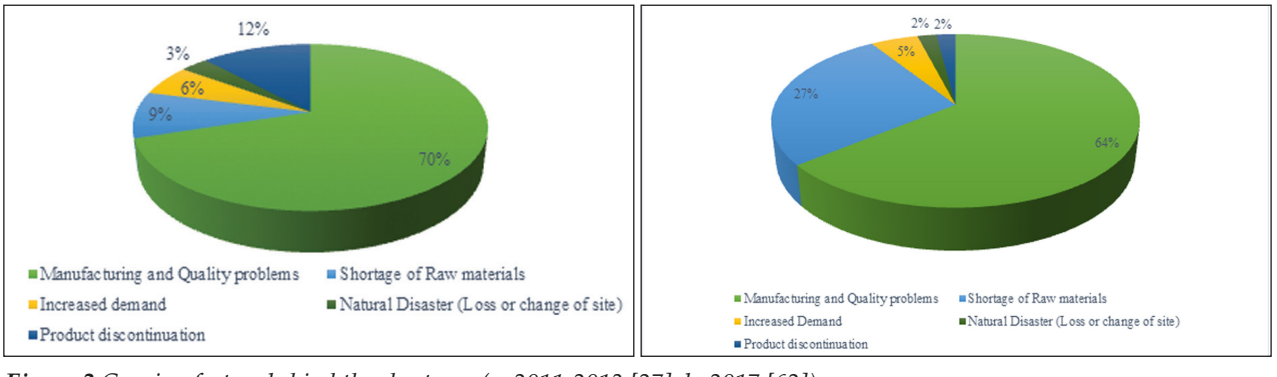

Figure 3 *Causing factors behind the shortages (a: 2011-2013 [27], b: 2017 [62])*

The statistics in Figure 3/a are based on the information of the MAHs reported towards the authority regarding the cause of occurred drug shortages. Table 1 reflects what is the proportion of different causes among all reported cases. Increased demand refers to a growth in the request for a particular product compared to its traditional amount of usage e.g.: COVID-19 [60]. The manufacturing and quality problems involve both delays due to capacity issues and voluntary recall due to bacterial contamination or any other foreign matter found in the current batch [61]. The analysis of the FDA data indicates that 6% of the shortages reported between January 1, 2011, and June 30, 2013, were due to increased demand and 3% because of natural disasters [27]. According to recent data (current as of 22-Oct-2019) by FDA (see Figure 3/b) reports increased demand to account for 5% and natural disasters for 2%, indicating little change in the proportion of these causes over the years [62]. On the other hand, the proportion of "manufacturing and quality problems" which decreased significantly (from 70% to 64%), while product discontinuation causes were 12% of all shortages in 2017, compared to 2% in 2011-2013. Furthermore, the shortage of raw materials also shows a significant increase (from 9% to 27%) [27] [62].

2.1.1 Root Cause 1 – Business and economic issues

Lack of incentives to market barely profitable medicines

MAHs frequently remove less profitable products from their portfolio potentially causing short supply [21]. Causes of these decisions could be the poor profit, a new generic product arrives on the market, anticipated clinical demand, patent expiration, drug-approval status, increased regulatory requirements, the expense to correct manufacturing problems, or mergers [17]. If market conditions limit the profitability of MAHs they are undermotivated to continue the production of the given product and invest more money in improving its quality [4]. There is strong price competition in the pharmaceutical market, also referred to as " race to the bottom in pricing" [22]. This practice does not motivate MAHs to invest in the production of older generic drugs which have little potential returns [21]. The essential medicines are frequently generics, therefore relatively cheap to purchase, however, not easy to produce [23]. There is an open competition on the pharmaceutical market for generic medicines, which results in lower prices and reduces the return on investment of producers and marketers. As another economic factor, mergers and acquisitions of pharmaceutical companies since the 1980s often lead to decreased product quantity and lines or due to shifting production to another facility, further contributing to shortages [24]. Cost-containment actions such as reference pricing, payback mechanism or discounts, introduced by some countries to control public expenditures on pharmaceuticals also limit profitability and thereby the incentives of MAHs to produce certain medications. Such economic pressure on MAHs also motivates them to store lower quantities or extract from the market [4].

Parallel distribution

Occasionally, parallel distribution is also mentioned as a potential cause for medicine shortages, however, it is a highly controversial aspect. Parallel distribution refers to business transactions between wholesalers registered in different markets, which leverage the difference in prices among their countries [63]. This is made possible by the concept of a common EU market without domestic borders is embedded in articles 34–36 of the Treaty on the Functioning of the EU. If manufacturers of a country produce the quantity necessary to meet the needs of their market, but a wholesaler exports a proportion of the products to a foreign market where they can gain a higher profit, a shortage will occur in the domestic market [64]. In response to this ethically questionable practice of wholesalers, MAHs introduced quotas to reduce the possible amount of exported products. Quotas control the amount of certain medicinal products available to countries to limit parallel trade. However, if quotas are not set at right levels to cover domestic demand, they can also contribute to drug shortages [38].

Tendering Practices

Public tendering is used to increase price competition and thereby reduce purchase prices. In 2009, 18 EU member states used tendering practices for procuring medicinal products mostly for hospitals. In Hungary "Act CXLIII of 2015 on public procurement" regulates tender practices [65]. The single winner approach introduces the risk that the chosen company may not be able to supply the

right quantity in the right time of the product, which is then sanctioned by fines. To mitigate this issue, when selecting the winner of the tender, besides the most important criteria, the price, the second alternative criteria should be the availability of the medicine. The single winner approach leaves hospitals highly dependent on one firm, which could be resolved by including second or third winners based on such multifactorial criteria, who could automatically supply if the first winner is unable to do so [4].

2.1.2 Root Cause 2 - Maintaining mature quality management systems.

The pharmaceutical market does not acknowledge nor reward MAHs' effort of investing in quality management systems [3]. All MAHs have to meet regulatory requirements like the GMP which is set forth as a prerequisite of being permitted to operate on the pharmaceutical market. Mature quality systems conform to GMP principles and is based on performance and patient-focused approach, incorporating technology, statistical process control, and planning activities to make sure medicines are produced and supplied in a transparent manner [25].

Voluntarily recall

Recall refers to not marketing a specific batch of products because of a lack of confidence in safety or any other defect [26]. These recalls may have a fast and severe effect on the accessibility especially if certain drugs are marketed only by a few MAHs. A dilemma may arise if it is predicted that a voluntary recall will cause a drug shortage [66]. To resolve this dilemma, OGYEI may authorize the MAH to place particular batch onto the market to assure continuous supply even if it differs only formally from the marketing authorization guidelines but is otherwise safe to market. The procedure and the relating issues are described in 35§ of Decree No. 52 of 18 November, 2005 of the Minister of Health [67].

Manufacturing difficulties

According to FDA investigation, around 64% and 70% of shortages were attributed to manufacturing and quality problems. Pharmaceutical manufacturers often subcontract production to third parties in geographically distant locations to take advantage of significantly production costs of developing countries. Consequently, the physical manufacturing facility and company headquarters do not coincide [28]. Every party involved in the production process is responsible to work according to GMP, creating an additional responsibility for MAHs, who have to ensure that subcontractors also comply [29]. To ensure that these global guidelines are met, the new API regulation was introduced in 2013 in the EU. As a result, MAHs and regulatory authorities need to perform more personal audits over their contractors [68].

Bottlenecks can arise at various stages of the production process, for example, due to aged and inefficient equipment, the lack of competent workforce or resources shifted towards business areas with higher profit potential such as research and development. MAHs often use the same equipment to produce various products, therefore it is complicated to raise the level of production of one product without causing supply issues and delays for another [20].

Shortage of raw materials

On several occasions, disruptions in the supply chain of the raw materials bring forth shortages. The difficulties arising in the procurement of excipients and active substances has been highlighted as one of the principal reasons behind medicine shortages [17]. Shortages of raw materials have a critical impact on the supply chain because even if multiple MAHs are producing the same medicinal product, they may have only one source of raw materials. Consequently, any interruption in the phase of procurement of raw materials can greatly affect the availability of the finished product [17]. This issue can also be tied back to the geographical concentration of production. Historically, the manufacturing of drugs for US citizens has been inland based [31], however, the import of raw materials particularly for active pharmaceutical ingredients (APIs) is continuously on the rise, making the stability of these channels essential for the whole supply chain [31] (see Figure 4). As of August 2019, only 28% of manufacturers procured Active Pharmaceutical Ingredients (APIs) as raw materials from domestic market, while the remaining 72% has been imported from overseas [31].

As materials are largely procured from non-European countries such as China or India, the stable

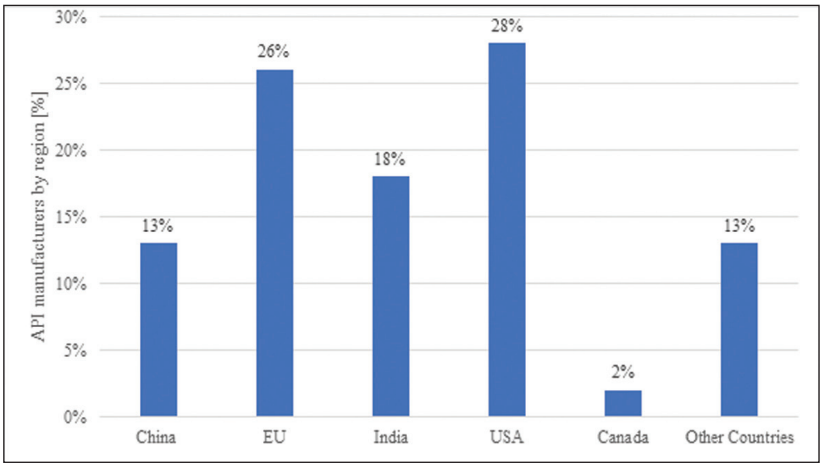

Figure 4 *Global percentage of API manufacturers by region, Aug 2019 [31].*

drug supply of developed countries is highly dependent on these dominant producers [17].

In addition, there is a low number of suppliers capable of meeting quality standards required by EU or USA legislation, and many of them are affected by operative risks of countries with high occurrence of natural disasters, armed conflicts, or political instability. Problems also may arise due to, trade arguments, damage during transport, changing climate conditions, or a decreased yield of plants that are a source for materials [17].

2.1.3 Root Cause 3 - Logistical and regulatory challenges

Through the past decades, the pharmaceutical supply chain became higly complex and disintegrated as numerous industries have transmitted a higher portion of their production overseas [31]. Normally markets would respond to an upcoming shortage by raising production level, however logistical and regulatory burdens resulting from the overly complex supply chain often limits the ability and speed of MAHs in raising production. If MAHs want to modify production capacity they may have to obtain approvals from many different competent authorities and/or may have to find new API suppliers [4].

Supply chain difficulties

Most hospitals and pharmacies who are entitled to provide medicines for patients procure through wholesale distributors. Every procedure along the supply chain is highly regulated, therefore any small deviation from normal procedure may result in shortages. Post marketing surveillance and market approval requirements induce MAHs to only provide to those wholesalers who ensure strict compliance with their contract terms and procedures. Most MAHs and distributors use "just-in-time" stock management, which allows a lower amount of goods "on-hand". Benefits include optimized cashflow and storage capacity [34]. This inventory strategy is a widely-used practice among pharmaceutical companies, however, it results in higher exposure to unforeseen shortages. Delivery issues can also contribute to short supply, especially when too many pharmacies or hospitals in a certain region are using the same source as distributor [4].

Natural disasters

Natural disasters may reduce product availability drastically. Production or shipment capacity can be significantly cut back by fires, hurricanes, tornadoes, and floods. If a certain manufacturer is a sole-source of a product, a long-term shortage might occur because of the damage to manufacturing facilities. Sometimes disasters generate shortages as the treatment of victims creates an unexpected spike in the demand for certain medicines [5].

Supply and demand issues

Level of demand for a certain drug rises over general expectations due to a various reasons , such as a new indication being approved regarding the product, a disease being in high spread, or growing media attention around a product [5] MAHs are often simply not able to keep up with drastically increased demand and they are not able to exceed their capacity e.g.: COVID-19 [60].

Regulatory Issues

Another reason behind drug shortages can be the increasing control by the regulatory authorities. However, FDA regulatory bodies do not agree with this view as they identify manufacturing difficulties are the main causing factor [17]. Regulatory-related shortages are reported when FDA officers find non-compliance with GMP in the MAHs'

Table 1 *Comparing Root causes of shortages in EU/Hungary and the US [3] [4].*

Root causes	Derived shortage factor	USA	EU including Hungary
Economic related	Market conditions / Lack of incentives	Economic incentives favour highly profitable drugs, therefore MAHs might discontinue the production of those with low profit margin.	
	Tender practices	Not applicable.	Single winner structure leaves hospitals highly dependent on one firm, who might not be able to meet the whole demand.
	Supply quotas and parallel export	Not applicable.	Due to exporting to more profitable markets, discrepancy arises between the volume manufacturers release on a given market and the ability of the wholesalers to satisfy patients' needs from the said market.
Manufacturing and quality	Manufacturing difficulties	Manufacturer cannot provide sufficient quantity or quality.	
	Natural disasters	Production facilities have to discontinue production and/or have to meet increased the demand for particular medicinal products which would be essential to treat disaster victims.	
	Voluntarily recall	The supply of medicines could be significantly reduced because of GMP issues.	
	Surges in demand	Due to unpredicted increases in the use of a particular product, supply cannot meet the market needs e.g.: COVID-19.	
	Shortage of raw materials	Due to limited availability of ingredients production is forced to be lower than normal manufacturing capacity e.g.: Valsartan case.	
Logistical and regulatory	Logistical inefficiency	Medicine would be available however patients are unable to acquire due to various reasons.	
	Regulatory time lag	Medicine wait for MA renewal.	
	Different National requirements	Specific requirements by authorities e.g.: specific label requirements, pharmacovigilance system, and FMD regulation.	

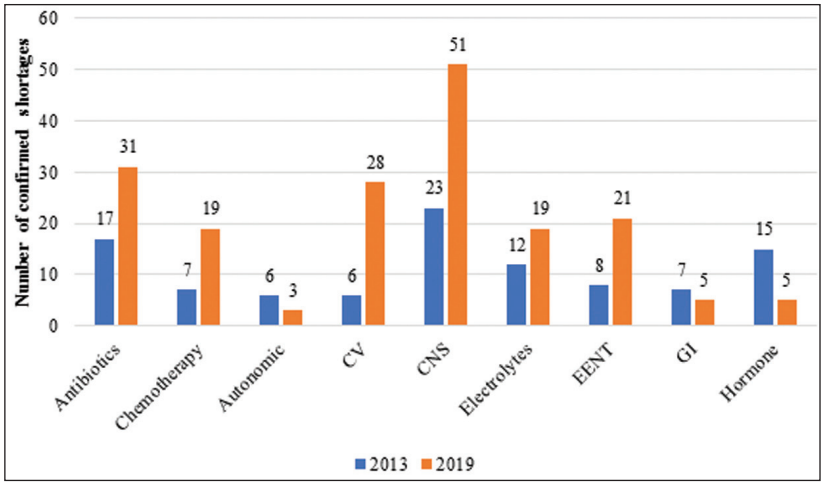

Figure 5 *Affected drug classes with a shortage between 2013-2019 [9] [13]. CV: Cardiovascular, CNS: Central Nervous System, EENT: Eye, Ear, Nose and Throat, GI: Gastrointestinal*

facility or on their subcontractors' site [5]. Depending on the outcome of the inspection, the authority may seize involved products and schedule another inspection of the facility until the violation has been corrected. Further regulatory related issue if medicine that was previously approved on a certain market and its Marketing Authorization was invalidated, pending for MA approval or a medicine previously approved must wait for MA renewal so called "regulatory time lag"[4].

2.2 Shortages as a global challenge

Drug shortages became a global concern in the past decades both in Europe, and overseas (the USA and Canada). The World Health Organization confirmed that shortages occurred in more than 20 countries worldwide in 2011 [10]. It is complicated to classify and assess the issue because of the scarcity of publicly available data and different definitions between countries for drug shortage [11]. The Institute for Healthcare Informatics (IMS) in November 2011 identified five disease ar-

Table 2 *Reported causes of shortages by MAHs in Hungary from 13th January 2020 till 20th January 2020 [15]*

Commercial considerations	922	63%
Manufacturing problems	395	27%
Logistics and warehousing problems	47	3%
Problems with the availability of active substances	43	3%
Administrative problems	34	2%
Unexpected increase in demand	21	1%
Other reasons	4	0%
	1466	100%

eas which are extremely exposed to drug shortages, meaning that 63% of all cases are reported in these categories. These areas were oncology, anti-infectives, cardiovascular, central nervous system and pain management, and generic injectables. The same five disease areas which are most often exposed to shortages have also been confirmed in 2013 [13] and in 2019 [9].

However, Figure 5 illustrates that some disease areas became more commonly affected between 2013-2019 with shortage for example chemotherapy products (from 7 to 19), cardiovascular products (from 6 to 28), central nervous system products (from 23 to 51) EENT products (from 8 to 21), electrolytes products (from 12 to 19). On the other hand, there are some disease areas with a lower occurrence of shortage in 2019, for example, autonomic products (from 6 to 3), gastrointestinal products (from 7 to 5), and hormones (from 15 to 5).

The same trend regarding the affected therapeutic classes was also observed in Hungary based on a research project conducted between 15th of May and 30th of June 2019 [69]. The survey was completed by 42 clinical pharmacists and highlighted the following groups: Blood and Blood-Forming Organs (52.4%), Cardiovascular system (50%), Antineoplastic agents (47.6%), Anti-infectives for systematic use (38.1%), and Nervous system (38.1%) [69].

2.2.1 Prevalence of shortages in Hungary

The „List of Product Shortages" published by the National Institute of Pharmacy and Nutrition shows an increasing trend of shortages. In 2020, 1466 products were reported by the OGYEI [15], while in 2012 "only" 464 cases were listed [14]. The Hungarian pharmaceutical market usually reflects European trends, and this aspect is no exception. In Hungary, only those products make it to this list that have been reported by MAHs themselves as per their legal obligation: *„In the event where a marketing authorization holder intends to temporarily suspend the distribution of a specific medicinal product in the territory of Hungary or wishes to discontinue the distribution of such medicinal product, the wholesalers of medicinal products engaged under contract and the government body for pharmaceuticals must be notified accordingly at the time of delivery of the last production batch to the medicinal product wholesaler, in any case at least two months before the scheduled suspension or termination of distribution, and shall be liable to provide a supply of the medicinal products in question until the date estimated for the suspension or termination of distribution in the quantity required to cover demand as estimated from previous turnover data. The government body for pharmaceuticals shall verify the availability of supplies notified by the marketing authorization holder in the quantity estimated to satisfy demand"* [16].The reporting process includes the filing of a specific Shortage Report Form on which the marketing MAHs are obliged to indicate not only the specific causes of the shortage but also the estimated period it will last for, and the methods by which the product could be substituted. Table 2 summarizes the causes of shortages as reported by the marketing authorization holders for the 1466 products on the list of January 19th, 2020:

The section of the Shortage Report Form on the specific causes of shortages can be individually edited on a case-by-case basis by MAHs. Based on this reporting system, it is possible to conclude that shortages in Hungary occur due to the above listed 6 reasons among which commercial considerations and manufacturing problems account for 90% of all the shortages.

2.3. Impact of drug shortages and strategies to mitigate them

Regardless of the original cause of a drug shortage, it has a grave impact on every stakeholder in the supply chain [17]. Shortages would severely affect the effectiveness of patient therapy and could simoultaneosly hinder healthcare professionals from doing their jobs effectively. It would force them to take on additional work, as the working hours spent mitigating the effect of shortages has already tripled between 2004 and 2010. A shortage is always associated with a higher level of risk due to the discontinuation of regular care and using alternative substitution that are less safe and cause a higher occurrence of medication

errors [5]. This is because physicians can be forced to prescribe an alternative drug they may not be familiar with [43]. When working with unfamiliar substances health care professionals may miscalculate the dosage, thereby increasing the occurrence of overdosing or underdosing. Moreover, when patients are "triaged", the medicines for which there is no known alternative substitute are preserved for those who are suggested to have the best medical prognosis, thereby reducig the quality of care some patients receive [17] .

To mitigate such severe impacts, drug shortage management has to start when the first piece of information arrives pertains to approaching interruption. The appearance of a potential drug shortage launches a "chain reaction" from procurement until final therapeutic decision making. The drug shortage team has to measure the expected operational and therapeutic impacts on patient care to develop a final action plan for its approval and implementation [5]. A designated person should be responsible to perform the operational assessment, which should cover all details of approaching shortage like estimate available stock on hand, and the availability of an alternative supplier or alternative therapy [70]. In practice, this is usually the responsibility of the chief hospital pharmacist.

The designated person in charge of drug shortages management can contact the MAHs responsible for marketing affected products, distributors, FDA, the Centers for Disease Control and Prevention (CDC), and other sources to identify the cause of the interruption and its expected duration. In case the shortage has not already been reported by MAHs and made available on the FDA and ASHP shortage websites as well, the appointed person ahould report the supply interruption. These reports for example when the product will be available again meaning great help to hospitals and persons how entitled to provide medicines for patients to develop strategies [18].

After the fact of shortage has been confirmed, the clinical pharmacist officer has to assess "on-hand stock" and estimate the period it can last for. Pharmacy Department has to assess available stock and analyze based on past usage proportions whether the inventory stock can last to cover demand during the expected short supply period.

The Therapeutic Assessment can be adjusted by the same appointed person who conducted the Operational Assessment. The main target to identify alternative therapies and to assess the affected patient groups [18].

In case a drug is critically low in stock and existing alternatives for certain patient groups are highly undesirable, it may be required to prioritize the dispense of the drug for these patient groups. Prioritization criteria can be based on the medication-use evaluation data upon prescribing and administration trends. Further criteria can be the characteristic of therapeutic application (curative vs. palliative). Triage practice for such scenarios should be developed in advance by an interdisciplinary team and follow strict ethical considerations [49].

Available therapeutic alternatives should be procured and stockpiled in advance under the management of the Pharmacy Department, who has to ensure sufficient supplies to meet all patient needs.

3. Conclusions

The most crucial point of maintaining a strictly regulated pharmaceutical supply chain is protecting patient safety by legally assuring that both the highest quality and sufficient quantity of medicines will be available to satisfy patients' needs. Drug shortages may compromise this ambition, while they can also reduce the effectiveness of the healthcare system in general, thereby causing further risks for patient safety from multiple aspects. Even though drug shortages may arise due to different causes, the consequences in the supply chain are almost identical despite the initial trigger. As root causes and associated factors underlying the shortages are well-described in literature, this should be an easily avertable issue. Nevertheless, shortages remain a global problem, as a continuously rising number of cases were observed over the last decades both internationally and in Hungary. The most affected therapeutic areas being critical medications, drug shortages should be considered a current health crisis, and therefore a comprehensive solution must be adopted in the coming years to tackle this phenomenon. Firstly, a comprehensive and effective reporting system should be adopted to ensure every stakeholder is notified about the shortage in time. Almost all European member states obligate stakeholders to notify a specific institution according to Directive 2001/83/EC Article 81 and 23a of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use [57]. Table 3 demonstrates that how many kind of reporting systems exist at the same time in European territory. Even though they have the common aim of obtaining the same

Table 3 *Comparing reporting systems in Europe [1] [71] [72]*

Country	Reportable products	Frequency of database updating	Short Description
Austria	Unlimited	Weekly	The voluntary list provided by MAHs regarding their products.
Belgium	If unavailability poses a risk to public health/ no therapeutic alternative available	Daily	MAHs have to report by law any shortage that will last for 2 weeks.
Croatia	Only reimbursed products	Monthly	A list is available for download at website.
England	Products for community pharmacy only	Monthly	Pharmacists via direct email.
Estonia	Unlimited	On-call basis	It changes with new information every day.
France	If unavailability poses a risk to public health/ no therapeutic alternative available	Daily	Pharmacists and hospitals
Germany	If unavailability poses a risk to public health/ no therapeutic alternative available	In cases assumed to be of special interest to healthcare professionals	MAHs should report to different institutions. List is often not up to date, and not all shortages are listed here because it is not mandatory.
Hungary	Unlimited	Weekly	Based on MAHs information also have a national website where they propose a solution for substitutions.
Italy	Unlimited	Weekly	MAHs, health care providers, health departments, patients, or associations.
Latvia	Unlimited	Daily	Anyone (hospital, pharmacy, patient) could use this website to report.
Norway	Unlimited	Weekly	Both Norwegian Authority and the national centre of shortage of drugs in hospital has webpages open to everyone.
Poland	Unlimited	At least bimonthly	Chef Pharmaceutical Inspectorate collects data from the pharmacist who are obliged to report online by giving details of nature of shortage.
Spain	Unlimited	On-call basis	MAHs or health authorities of autonomous communities.
Netherlands	Unlimited	Daily	MAHs, wholesalers, pharmacists.

information, they have been created based on different logics and they operate in different ways, therefore they are not comparable to each other.

Secondly, transparent and effective communication would be favourable not only from MAHs towards the Authority but also among other stakeholders in the supply chain. New communication platforms should be created, which can provide an appropriate information gathering system while also protecting business interests and the integrity of the supply chain. Moreover, healthcare institutions should designate a person or team responsible for developing and implementing an agile strategy to prevent and mitigate the effect of drug shortages. Finally, if communication issues have been resolved among stakeholders, special inventory measures can be taken to protect scarce resources and ensure the disposal of medicines in the most reasonable manner. In conclusion, the drug shortage problem demands a quick and enduring solution to avoid unnecessary burden on patients, extra workload of health care professionals, and to reduce to use of expensive alternatives.

Future perspectives and possible solutions

The impact of drug shortages is still not fully identified. Future studies should move from char-

acterizing the problem towards developing a solution to reduce or even eliminate the occurrence and so impacts of drug shortages. It would be a great step ahead if we could assess shortages based on the severity of their impact on patients. It would be cost-effective to measure such impact severity using the same method in all member states at the same time [73].

On the regulatory side, a *„Supportive Attitude Practice"* from the competent authority would be a great step to avoid post-approval changes hindering the availability of a product. For example, different pack sizes at the national level based on Marketing Authorization and multi-country packages should be accepted in case of a confirmed shortage of a medicine.

From an economic perspective, incentives would be necessary to ensure that life-saving drugs remain on the market even when sufficient profits are no longer achievable. Reasonable market conditions including foreseeable pricing, payback and reimbursement mechanism would provide help to keep MAHs on a particular market or willing to enter the market. Concentration of markets should also be avoided, as in case more than one MAHs supply a product there is lower chance for a shortage to occur.

To mitigate manufacturing and quality-related issues, policymakers should reward MAHs who are investing in manufacturing reliability and quality, as this ensures the supply medicines continuously for patients.

Finally, to improve communication channels, MAHs have to communicate with competent authorities when anticipating or experiencing a shortage to speed up the transmission of relevant information to all stakeholders, and thereby allow for the preparation of impact mitigation strategies as soon as possible.

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Non-antibiotic compounds affecting the growth of urinary pathogens during urine culture: a preliminary *in vitro* study

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Received: *14 July 2020 / Revised: 10 August 2020 / Accepted: 11 August 2020*

Introduction: *Urine samples are one of the most frequently submitted samples for culture to clinical microbiology laboratories, exceeding the number of most of the other clinical sample types. Various non-antibiotic pharmaceutical compounds may have inhibitory properties on bacteria, as many of these agents accumlate in/eliminated through urine.*
Aims: *The aim of our present study is to screen various non-antibiotic group pharmacological agents in vitro for their potential to augment the viability of pathogenic bacteria in urine samples.*
Methods: *Sixty (n=60) pharmacological agents were tested during our experiments. Bacillus subtilis ATCC 6633, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603 (ESBL-producing) and Staphylococcus aureus ATCC 29213 were the bacterial strains utilized in this study. Detection of inhibitory activity among the tested compounds was performed on Mueller-Hinton plates, using disk diffusion method.*
Results: *Nineteen (n=19) compounds presented with various levels of inhibitory activity on the tested bacterial strains (four compounds for K. pneumoniae, seven compounds on E. coli and sixteen compounds on S. aureus). The compounds showed the highest levels of inhibitory activity on B. subtilis ATCC 6633, which is one of the main bacterial strains used for the screening of the 'intrinsic' antibacterial activity of urine.*
Conclusion: *During urinalysis, all possible confounding variables must be taken into consideration, which may distort the culture results of routine laboratories. Our results suggest that further experiments, involving additional pharmacological agents is warranted, to establish the full extent of their influence on the appropriate culture of urine samples.*

Keywords: *urinary tract infections; urinalysis; intrinsic antibacterial activity; non-antibiotics; antimicrobials; drug repurosing; disk diffusion; Bacillus subtilis*

1. Introduction

Urinary tract infections (UTIs) are one of the most common infectious pathologies worldwide (following lower respiratory tract infections and gastrointestinal infections) [1,2]. From the standpoint of public health, UTIs represent an important factor or morbidity and mortality, affecting both patients in primary care and tertiary care settings [3]. In fact, according to some estimates, around 50-60% of women in the age range of 20—40 years experience a UTI at least once during their lifetime, while nosocomial UTIs may represent 25–50% of hospital-acquired infections overall [4]. The diagnosis and management of UTIs, and the corresponding lost working days associated with these infections also have a significant economic consequence, estimated to be around 3-5 billion US dollars annually [5,6]. Uncomplicated UTIs are principally associated with members of the intestinal flora, with *Escherichia coli* representing 50-90% of these etiologies [7,8]; the spectrum of pathogens assicoated with nosocomial infections is more diverse, including non-fermenting Gram-negative bacteria, Gram-positive cocci (*Staphylococcus aureus*, *S. saphrophyticus*, *Enterococcus* spp.) and *Candida* spp [9-11]. UTIs are associated with a variety of clinical signs and symptoms, including the burning sensation in the genitourinary region, strong and persistent urge to urinate, small volume of voided urine, urinary incontinence, pelvic pain, fever and nausea/vomiting [12]. Additionally, the color and consistency of the voided urine may be also subject to changes (cludy, red, bright pink, bloody, and foul-smelling urine) [12,13].

Urine samples (more commonly clean-catch/midstream and catheter-specimen urine) are one of the most frequently submitted samples for culture to the clinical microbiology laboratories, exceeding the number of most of the other clinical

DOI: 10.33892/aph.2020.90.185-191

sample types [14]. Clean-catch urine samples are an inexpensive and non-invasive without the risk of complications; although contamination of the sample with the normal flora or the distal urethrea is a risk, the appropriate instruction of patients regarding hygienic considerations and sample collection is usually adequate for appropriate samples to be attained [15]. Nevertheless, collection of urine by using a single catherer is a more appropriate method to use to avoid contamination in hospitalized patients [1,2,15]. Bacteriological culture of urine samples on non-selective or chromogenic media (frequently coupled with the use of nitrite and leukocyte-esterase tests or a hemocytometer) is the gold standard method in the etiological diagnosis of UTIs. The interpretation of culture results (usually $\geq 10^5$ colony forming units/mL corresponding to singificant bacteriuria) from urine samples provide little or no challenge to clinical microbiologists [16]. Based on data from the literature, 50-70% of urine cultues are culture-negative, while out of the positive urine samples, 40-50% of isolated bacteria are relevant urinary pathogens [17]. Sample procurement, time elapsed before sample processing and expertise of the staff are all relevant factors in establishing the etiology of UTIs. However, some additional factors may influcence the results of succesful interpretation of urine cultues. It it well-known that microbiological sampling should preferably be carried out before the administration of antibiotics, as these drugs may lead to false negative results (inhibiting or significantly reducing bacterial growth), misleading clinicians and microbiologists [18]. To screen for this, routine microbiology laboratories often perform ancillary tests with pan-susceptible bacterial strains (e.g., *Bacillus* spp., *E. coli*) to assess the intrinsic antibacterial activity of the urine samples [19]. If these tests prove to be positive, clinical microbiologists may observe different rules during interpretation of culture results.

Nevertheless, there is increasing evidence that various non-antibiotic pharmaceutical compounds may also have inhibitory properties on bacteria [20]; as a part of drug repurposing advances, several drugs have also been screened for their antimicrobial properties [21]. In addition, the pharmacokinetic properties of these drugs should also be taken into consideration, as many of these agents accumlate in/eliminated through urine, thus, they may possess the potency to adversely affect the growth of uropathogenic bacteria [22]. Therefore, the aim of our present study is to screen various non-antibiotic group pharmacological agents *in vitro* for their potential to augment the viability of pathogenic bacteria in urine samples or their growth on culture media during urinalysis.

2. Materials and Methods

2.1. Chemicals

Sixty (n=60) pharmacological agents, encompassing a wide variety of different chemical structures and mechanisms of action were tested during our experiments: acetylsalicylic acid (Sigma-Aldrich; Budapest, Hungary; will be listed as SA in the subsequent text), acetaminophen (SA), acetyl-cysteine (Teva Pharmaceuticals; Petah Tikva, Israel; will be listed as TPh in the subsequent text), acyclovir (TPh), allopurinole (SA), amantadine (SA), ambroxol (TPh), atorvastatin (SA), atracurium (SA), azelastine (SA), bleomycin (TPh), cisplatin (TPh), celecoxib (Pfizer Hungary Ltd.; Budapest, Hungary), cetirizine (SA), chlorpromazine (SA), chloroxazone (SA), cidofovir (SA), clotrimazole (TPh), cyclophosphamide (Baxter; Deerfield, IL, United States), diclofenac (SA), doxorubicin (TPh), enalapril maleate (SA), etodolac (SA), famotidine (SA), fluconazole (SA), fluoxetine (SA), gemcitabine (TPh), guaifenesin (SA), indomethacin (Sanofi; Paris, France; will be listed as SP in the subsequent text), imipramine (SA), ivermectin (SA), metamizole-sodium (SF), mebendazole (Richter Pharmaceuticals; Budapest, Hungary; will be listed as RPh in the subsequent text), lidocaine (SA), metoprolol succinate (SA), paclitaxel (TPh), prazozin (SA), metformin (SA), methotrexate (Ebewe Pharma, Unterach am Attersee, Austria), prilocaine (SA), promethazine (SA), risperidone (SA), simvastatin (SA), sitagliptine (SA), suxamethonium (SA), terbinafine (GlaxoSmith-Kline Hungary Ltd., Budapest, Hungary), thioridazine (SA), topotecan (SA), valsartan (SA), verapamil (TPh), vincristine (TPh), xylomethazoline (SA), Vitamin B_1 (EGIS Pharmaceuticals; Budapest, Hungary; will be listed as EGIS in the subsequent text), Vitamin B_6 (EGIS), Vitamin B_{12} (RPh), Vitamin C (SA), Vitamin D (EGIS), Vitamin E (SA), Vitamin K (SA) and 5-fluorouracil (TPh). The compounds were chosen on a basis of being substrates of the organic cation transporter-2 (OCT2/SLC22A2), organic anion transporters 1 and/or 3 (OAT1/SCL22A6 and OAT3/SCL22A8) and multi-antimicrobial extrusion protein (MATE), which are all relevant transporters in

the renal elimination of various pharmacological agents [23]. The list of relevant substrates was acquired from the DrugBank database (https://www.drugbank.ca/).

Pharmaceutical compounds were dissolved in phosphate-buffered saline, with the exception of simvastatin and atorvastatin, which were dissolved in dimethyl sulfoxide (DMSO), in addition to Vitamin D and Vitamin K, which were dissolved in acetone and 70% ethanol, respectively. The final concentration of the tested compounds was set at 100 μg/mL in the experiments.

2.2. Bacterial strains

The following bacterial strains were used during our growth inhibition experiments: *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 (ESBL-producing) and *Staphylococcus aureus* ATCC 29213.

2.3. Culture media, paper disks

Bacterial strains were maintained on blood agar and eosine methylene blue plates (bioMérieux, Marcy-l'Étoile, France). Inhibitory activity of the tested compounds was investigated on Mueller-Hinton agar plates (bioMérieux, Marcy-l'Étoile, France).

Filter paper disks (7.0 mm in diameter, Whatman 3MM) were impregnated with the solutions of the tested compounds. Ciprofloxacin (5 μg), meropenem (10 μg) and trimethoprim/sulfamethoxazole (1.25/23.75 μg) disks (Liofilchem, Abruzzo, Italy) were used in the control experiments.

2.4. Inhibitory activity of non-antibiotic drugs

Detection of inhibitory activity among the tested compounds was performed on MHA plates, containing *B. subtilis* ATCC 6633 spores [22,24,25]. A maximum of 6 sterile filter paper discs (impregnated with 10 μL of the solutions of the solutions of different the tested compounds) were placed on MHA, containing a *B. subtilis* spore suspension (250 μl per 1 liters). Control strains (*S. aureus*, *E. coli* and *K. pneumoniae*) were plated on MHA agar conventionally, and the sterile filter paper discs were placed on the inoculated plates. The plates were incubated at 37 °C in an air thermostat. The inhibitory activity of the tested compounds was assessed semi-quantitatively; the zone of inhibition around the disks impregnated with the solutions of the tested compounds were recorded after 16–18 h of incubation, using a caliper (expressed as milimeters ± standard deviation [SD]). Any measureable zone of inhibition was considered as positive [22,24,25]. DMSO (at 2 V/V% concentration) was used as a negative control for the tested compounds, while ciprofloxacin, meropenem and trimethoprim/sulfamethoxazole disks were used as positive controls. All experiments were performed in triplicate.

3. Results

Out of the 60 tested pharmacological agents, nineteen (n=19) compounds presented with various levels of inhibitory activity on the tested bacterial strains. The results of our disk diffusion inhibitory experiments are presented in *Table 1.* Out of the nineteen compounds, four compounds (atracurium, doxorubicin, lidocaine, thioridazine) showed measurable inhibition zones on *K. pneumoniae* ATCC 700603 (ranging between 2-6 mm), while seven compounds (atracurium, celecoxib, chlorpromazine, doxorubicin, imipramine, lidocaine, thioridazine) showed inhibitory activity on *E. coli* ATCC 25922 (with zone diameters ranging between 1-7 mm*). S. aureus* ATCC 29213 was more susceptible to the inhibitory activity of the tested drugs (zone diameters ranging between 4-14 mm; for 16 out of the 19 compounds), with the exception of allopurinole, methotrexate and verapamil). The compounds showed the highest levels of inhibitory activity on *B. subtilis* ATCC 6633, which is one of the main bacterial strains used for the screening of the 'intrinsic' antibacterial activity of urine; with zone diameters ranging between 4 mm (allopurinole) and 22 mm (thioridazine). All tested reference antibiotics showed zone diameters for the respective bacterial strains, which corresponded to the 'susceptible' therapeutic category (based on EUCAST v. 9.0 breakpoints). 2 V/V% DMSO did not show any inhibitory activity during the experiments.

4. Discussion

UTIs are a major publich health and economic burden to healthcare infrastructres worldwide, therefore the correct determination of the etiological agents in these infections in of utmost importance [1-3, 5, 11, 25, 26]. During urinalysis, all possible confounding variables must be taken into consideration, which may distort the culture re-

Table 1 *Inhibitory activity of tested pharmaceutical compounds (results expressed as mm ± SD)*

	Bacillus subtilis **ATCC 6633**	***Escherichia coli*** **ATCC 25922**	***Klebsiella pneumoniae*** **ATCC 700603**	***Staphylococcus aureus*** **ATCC 29213**
Allopurinole	4 ± 1	Ø	Ø	Ø
Atorvastatin	11± 2	Ø	Ø	8 ± 2
Atracurium	14 ± 2	5 ± 1	3 ± 1	6 ± 1
Bleomycin	16 ± 2	Ø	Ø	8 ± 3
Celecoxib	20 ± 3	1 ± 1	Ø	14 ± 2
Chlorpromazine	17 ± 3	3 ± 1	Ø	10 ± 2
Clotrimazole	15 ± 2	Ø	Ø	5 ± 2
Doxorubicin	18 ± 3	5 ± 2	5 ± 1	8 ± 2
Etodolac	15 ± 3	Ø	Ø	7 ± 1
Fluconazole	17 ± 1	Ø	Ø	7 ± 2
Imipramine	9 ± 2	3 ± 3	Ø	4 ± 2
Ivermectin	14 ± 3	Ø	Ø	8 ± 3
Lidocaine	17 ± 4	7 ± 2	6 ± 1	10 ± 3
Mebendazole	16 ± 1	Ø	Ø	12 ± 2
Methotrexate	10 ± 2	Ø	Ø	Ø
Promethazine	7 ± 2	Ø	Ø	6 ± 3
Simvastatin	13 ± 2	Ø	Ø	10 ± 2
Thioridazine	22 ± 4	5 ± 1	2 ± 1	9 ± 3
Verapamil	6 ± 3	Ø	Ø	Ø
Ciprofloxacin (5 μg)	24 ± 3	27 ± 3	26 ± 2	26 ± 2
Meropenem (10 μg)	29 ± 2	24 ± 1	23 ± 1	24 ± 1
Trimethoprim/sulfamethoxazole (1.25/23.75 μg)	16 ± 3	19 ± 1	18 ± 2	16 ± 2

Ø: no inhibition zones were observed

sults of routine laboratories. These may include issues during sample procurement and time elapsed before sample has been processed (i.e. the pre-analytical phase), however, troubleshooting must also encompass steps in the analytical phase [27]. The chemical composition of urine clearly affects the viability and species-composition of bacteria, for example, if the pH of the urine shifts in either directions, it may inhibit or potentiate the replication of several microorganisms [26,27]. Many natural compounds and constituents of our diet have well-known antibacterial properies (e.g., ajoene [28], betulinic acid [29], cranberry juice [30], curcumin [31], essential oils [32], horse raddish [33], pepper [34], resveratrol [35] and zeaxantin [36]), which may influence bacterial viability in urine. Nevertheless, the relevance of non-antibiotic compounds in this regard must not be underestimated [20,21,37]; this is especially true in case of older patients, whom many drugs are simulatenously prescribed [38]. In our study, nineteen out of the sixty tested pharmacological agents presented with growth inhibitory properties on the tested bacterial strains. With the inclusion of *S. aureus*, *E. coli* and *K. pneumoniae* in the study, we aimed to assess the relevance of these drugs in decreasing the viability of pathogenic bacteria in urine; in contrast, the *B. subtilis* strain is predominantly used to provide information on the antibacterial activity of the urine sample itself. While 4-16 compounds (depending on the bacterial strain) showed growth inhibitory activity on the reference strain, n=19 drugs inhibited the growth of *B. subtilis* in the disk diffusion tests to various extents. This experiental result may point out that in addition to antibiotics, non-pharmacological agents may also be responsible to „positive" tests, when assessing the antibacterial activity of the urine samples received, depending on the concentration, in which they are available in the urine

[22]. Similarly to our results, the potential antibacterial activity of azole antifungal agents [39], antracyclines [40], phenothiazines [41], local and general anesthetics [42], peripherially acting muscle relaxants [43], non-steroidal anti-inflammatory drugs [44] and statins [45] were already demonstrated by studies in different settings. However, other studies also highlighted the antibacterial properties of acetyl-salicylic acid [46], allopurinole [47], various cardio-vascular medications [48], and several vitamins (A, C, D and K) [49-52]; this was not demonstrated in our *in vitro* settings.

5. Conclusions

In conclusion, the aim of our present study was produce *in vitro* data on the possible role of non-antibiotic pharmacological agents, as inhibitors of growth during urinalysis, i.e. the culture of urine samples on bacteriological media, if a UTI is suspected. Our results show that a wide variety of structurally unrelated drugs may have the potential to inhibit the growth of urinary pathogens, or *B. subtilis*, a commonly used microorganism in ancillary tests. Although the methodology used during our experients (disk diffusion) offers only preliminary, semi-quantitative results and the experiments were carried out in a select group of bacteria, our results suggest that further experiments, involving additional pharmacological agents is warranted, to establish the full extent of their influence on the appropriate culture of urine samples.

Acknowledgements

None.

Funding

M.G. was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. M.G. would also like to acknowledge the support of the ESCMID's "30 under 30" Award.

Conflicts of interest

The author declares no conflict of interest, monetary or otherwise.

Ethical considerations

None.

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Implementation of Patient Reported Outcome Measures (PROMs) in QbD based formulation development in ophthalmology

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Received: *2 October 2020 / Revised: 4 November 2020 / Accepted: 4 November 2020*

Abstract: *Development of drug delivery systems for chronic disorders needs a complex thinking in order to ensure the quality of the product. A multidisciplinary approach of pharmaceutical technology, regulatory and behavioral sciences on the basis of the Quality by Design methodology can be a proper tool for this to handle formulators', patients', and also doctors' needs in therapy planning in case of chronic ophthalmologic disorders. According to the present state-of-the-art", patient perceptions are collected in the form of the "Patient Reported Outcome Measurements" during the clinical trials, but no feedback is given to the formulation development in order to take these aspects into consideration when designing a new product. This work aims to link the key performance indicators from patients' point of view to the pharmaceutical development and show a new approach to product development by evaluating the patient and formulator aspect as critical quality attributes within the classical Quality by Design workflow. This study can be the basis of the formulation design and development of a new ophthalmic formulation as it revealed the patient critical needs and requirement.*

Keywords: Patient Reported Outcome, Ophthalmology Disorder, Early development and formulation, QbD

Introduction

Patient Reported Outcome Measurements

According to competent authorities (European Medicines Agency (EMA) and Food and Drug Administration (FDA) the term Patient Reported Outcome (PRO) is an umbrella nomenclature, which covers single and multi - dimension measures as well in connection with the general health status of the patients, satisfaction with the treatment, adherence to the treatment, symptoms and Health Related Quality of Life (HRQoL) [1,2]. In addition, PROs evaluate all the subjective perceptions of the patients, obtained directly from them [3]. These feedbacks offer information to the health care team to find the possible intervention for health status improvement, to develop the individualized therapy and also could be useful for the researchers or academics during the early development process [4,5]. Patient Reported Outcome Measurements (PROMs) are performed mostly via self-reported questionnaires. Generic and disease specific questionnaires are used for detecting PROs [6]. Importance of PROMs are documented, in the field of clinical trials are used in several years [7]. The competent authorities require to use PROs for the authorization of a new pharmaceutical drug or a new indication. HRQoL of life presents a specific subset of PROs. The definition of HRQoL based on the World Health Organization's (WHO) health definiton is: *„a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity"* [8].

Almost all chronic disorders mean life long treatment for the affected patients. To adapt for a long term therapy and the life style changes is quite a big challenge, and patient adherence to treatment and the persistence in long term, are essential for a successful therapy. Based on this fact, it is important to take into account the patients perceptions from the very beginning at the early development phase, to fulfill the Patient Centered Care and the ensure the HRQoL. According to the World Health Organization's (WHO) Quality of life Questionnaire (WHOQoL) the influencing factors are divided into 4 domains: (1) Physical health (e.g.: mobility, pain and discomfort, work capacity), (2) Psychological (e.g.: negative, positive feelings, religion, personal beliefs), (3) Social relationships (e.g.: social support, sexual activity), (4) Environment (e.g.: financial resources, transport, freedom) [8]. These dimensions are covering all relevant factors of HRQoL and could be useful to separate the influencing factors from the patients' point of view.

Formulation aspects of ocular drug delivery systems

Development of an ocular drug delivery system is a great challenge in the field of pharmaceutical research and development, as human eyes are indispensable for normal daily activities. Therapy of ocular diseases is a complex task due to the complex anatomical and physiological barriers, patient-compliance and the unique physicochemical attributes of several active ingredients (API) applied in ophthalmology. Eyes are made up of two anatomical segments, the anterior segment is from the cornea to the lens, while the posterior segment contains the lens, vitreous humour, retina, sclera, and the optic nerve. The human cornea consists of five layers: the lipophilic epithelium, Descemet's membrane, the hydrophilic stroma, Bowman's layer and the lipophilic endothelium. Lipophilic agents can permeate through the epithelium by passive diffusion, while the diffusion of hydrophilic drugs is restricted because of the tight junctions of the epithelium layer. Meanwhile the thickest layer of cornea, the stroma is hydrophilic, therefore the diffusion rate of lipophilic API is slower there, while hydrophilic compounds can pass it freely. For optimal transcorneal permeation, a balance is needed in the lipophilic-hydrophilic characteristics in case of the given drug delivery system. Physiological barriers, which are formed by the complex anatomical structure, defend the eye from external agents. Tear film includes a lipid layer, water and mucin, which protects conjunctiva and cornea. Cornea and conjunctiva also act as a barrier, which mainly restrict the penetration of API to the anterior tissues. Blood Aqueous Barrier is partly permeable for compounds with low molecular weight. Because of the tight junctions of retinal blood vessels and retinal pigment epithelium, the Blood Retinal Barrier blocks the drug penetration from systemic circulation, application of oral and intravenous dosage forms are limited, because large doses are needed for proper healing, which results not targeted presence of API and increase the possibility of unwanted side-effects. Moreover, the anatomical blockade, reflex mechanisms (blinking, increased lachrymal secretion) are induced after any external stimulus, therefore the precorneal elimination accelerates the drainage of applied formulation from the ocular surface [9,10].

Considering the mentioned restrictions in ocular drug delivery, ensuring optimal and successful therapy is an exceptionally hard challenge. In the case of chronic diseases of eye, the target of therapy is mainly the posterior segment. Nowadays, invasive routes like, intravitreal and subconjunctival injections are the most conventional methods, although non-invasive innovations are published to reach the posterior segment, besides the therapy of ailments at the anterior segment of eye. Topical administration is the most favourable self-applicable method, which does not need expert assistance. Mainly eye-drops, inserts semisolid formulations and contact lens are used As dosage forms [11].

After administration, the drug has to pass the hydrophilic tear film barrier. From the precorneal area the elimination of eye drop is through the nasolacrimal drainage to the systemic circulation. The possibilities for permeation pathway from the tear film are the corneal and non-corneal routes. In the case of corneal pathway, the drug meets the layer formed by lipophilic corneal epithelial cells. Penetration of hydrophilic molecules are limited there, meanwhile lipophilic active ingredients permeate easily by transcellular passive diffusion. Under the epithelial multilayer, the hydrophilic stroma restricts the permeation of lipophilic drugs. The lipophilic endothelial monolayer is permeable for macromolecules, compared with the epithelium. The conjunctival scleral (non-corneal) route is the other possible permeation pathway after passing the tear film barrier, where the permeation of active ingredient mostly depends on the molecular weight. To reach the posterior segment, the formulation needs to pass the complex anterior segment. The opposite directional secretion of aqueous humour also limits the permeation. Using novelties like cyclodextrins, liposomes, nanoparticles, nano lipid carrier systems, polymer micelles and mucoadhesive polymers can overcome these difficulties [12,13,14,15,16].

Considering the fact, that eyes are one of the most sensitive organs in human body, the applied formulation must meet the physiological requirements. Preparation must be done in aseptic environment, sterility of dosage form must be ensured during the therapy and parameters like pH, osmolality, surface tension and viscosity must be optimized to avoid side-effects [12,17,18].

Application of the Quality by Design methodology in product development

The Quality by Design (QbD) approach of the developments is generally used in the pharmaceuti-

cal industry and its application was forced by the regulatory authorities. The QbD method realizes a modern quality management thinking, as it is a risk and knowledge based systemic and holistic development model, described in ICH Q8 (R2), Q9 and Q10 documents [19,20,21]. It focuses on profound preliminary design, taking into consideration of all stakeholder's needs and requirements from the initial step. The stakeholders are: the patient, the pharma industry and the regulatory authority and they have different requirements for having finally a product with proper quality, safety and efficacy profile [22,23,24,25]. The steps of a QbD based product development include the following:

1. Definition of Target Product Profile (TPP) and its quality indicators (Quality Target Product Profile, QTPP). This usually comprises therapeutic requirements and other quality demands (e.g. dissolution profile, stability aspects, etc.).
2. Identification of Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs) which have critical influence on the desired final product. The selection of the CQAs and the CPPs should be based on previous scientific experience and knowledge from relevant literature sources.
3. Risk Assessment (RA) is a systematic process of organizing information to support a risk decision and is the key activity of the QbD based methodology. RA can be initial, repeated and final and RA results help to aim attention on the most critical influencing factors and avoid profitless efforts in later phases of the development process.
4. The next steps of the QbD approach are: the Design of the Experiments (DoE) based on the RA results, performing of the experiment and establishment of the Design Space (DS), the control strategy, and finally considering the possibility of the continuous improvements from the whole process point of view.

The challenges in case of the pharmaceutical formulation of an ophthalmic product, associated with special characteristics of the eye, and the crucial effects on the patients, suffering from chronic eye disorders gave the basic to determine these two different stakeholders' expectations and needs from their own point of view. There are a lot of standardized technological parameters which cannot be altered just because the patients are unsatisfied with the product or with the therapy but also couple of these parameters could be changed according to patients' perceptions and expectations. The research team hypothesized that determination of these factors could provide feedback to researchers for improving the formulation procedure. Implementing patients' aspects to the early development process granted the think of Patient Centered Care from the beginning and ensure the way to improve HRQoL and Patient adherence to treatment.

Based on these facts, the main aim of the research work was to improve the development by means of the QbD based methodology. This tool compares the patients' aspects and expectation to researchers' aspects, and also handles the pharmaceutical technology parameters in case of an ophthalmic product on a risk-based approach.

Materials and Methods

Evaluation of Patient Reported Outcomes

The PROMs were selected according to those chronic ophthalmic disorders, which can be treated by means of eye drops (glaucoma, chronic dry eye syndrome). Based on the evaluation these measures were selected based on the influencing factors which are crucial for the improvement of HRQoL in case of patients affected by chronic ophthalmic disorders, mentioned abo ve[26,27,28,29,30,31,32,33,34,35]. These factors were classified according to HRQoL's dimension of WHO.

Definition of the QTPP and the Knowledge Space Development

QTPP forms the basis of the product development design. It is a prospective summary of the quality characteristics of the product that ideally will be achieved which include patient-relevant product performance and regulatory based professional requirements. The QTPP selection was based with careful planning and consideration the relevant needs and special requirements in chronic ophthalmic disorders. This collection and systemic evaluation of the influencing factors is called as "Knowledge Space Development" [21]. The defined QTPP contains the following elements: 1. eye discomfort (itching, redness, smarting, tearing, dryness, irritation, swelling) 2. anxiety 3. daily routine 4. health literacy 5. social support 6. work capacity.

Determination of CQAs

The identification of potential CQAs means the selection of those characteristics which influence the final product's performance and quality. These critical quality parameters were defined from patient outcomes point of view.

The following CQAs were selected: 1. Life-long therapy 2. Topical administration route 3. Dosage form (eye drop) 4. Local effect 5. Dissolution profile (residence time) 6. Device to the administration 7. Microbiological stability 8. Physicochemical stability.

Determination of CPPs

CPPs come generally from the production method. In this special case the targeted observation process aimed the Medical Product Application.

In this patient focused theoretical research the selected CPPs are: 1. Storage (temperature), 2. Regimen (frequency of the administration), 3. Device applicability 4. Long-term stability, 5. Long-term sterility, 6. Application without decreased vision 7. Hygienic circumstances, 8. Mobile application (alarm system).

Risk Assessment

The RA was performed using Lean QbD Software (QbD Works LLC., Fremont. CA, USA, qbdworks.com). According to the design of the software, the connections between QTPP elements, the CQAs and CPPs were thoroughly evaluated. The interdependence between QTPPs and CQAs, as well as between CQAs and CPPs was structured and evaluated one by one, then rated on a three-level scale. This scale reflects the impact of the parameters' interaction on the product as high (H), medium (M) or low (L). The probability of the occurrence of the critical factors was also estimated using the same three-grade scale. As the output of the RA evaluation, Pareto diagrams were generated showing the ranked parameters according to their critical effect on the aimed product.

Ishikawa diagram

The Ishikawa, cause – effect, or fishbone diagram is a widely used quality improvement method. Ishikawa diagram illustrates possible causes of a problem and in sorts ideas into categories. According to the expected effect, all the factors can be summarized and grouped as inputs or causes. It is advised to form 4-6 major cause categories and based on these, the minor causes are classified [36].

For the visualization of the selected influencing factors in case of CQAs and CPPs, Ishikawa diagrams were set up as well.

For determining the influencing factors as CQAs in case of a chronic ophthalmic disorder (effect), four major causes were selected according to WHO's HRQoL classification: (1) Physical Health (2) Psychological (3) Environment (4) Sociological Relationship.

To achieve the optimal ophthalmic formulation (the effect for selecting CPPs), the next dimensions, causes were determined: (1) Stability (2) Formulation (3) Efficiency (4) Active Ingredient (5) Preparation (6) Patient Adherence.

Results and Discussion

This research work evaluated the key intervention possibilities in chronic ophthalmic disorders from the patients' point of view for finding the increasing point of the adherence in this life - long treatment needing situation.

First, the QTPPs were identified, as follow: patients who suffer in chronic eye disorder and need life-long therapy, the aimed administration route was topical, and the selected dosage form was the solution (eye drop). The expected effect was a local effect and an intermediate dissolution of the active ingredient is needed, as the residence time on the eye is limited to the physiological environment and state. The device was also the element of the QTPP, as it should protect the formula and helps in preservation of the microbial and physicochemical stability. The long-term protection of the microbial and physicochemical stability has financial advantages and helps in the every-day life of the patient if the medicinal product has no special requirement for storage, handling etc. So, the QTPP elements were: (1) Life-long therapy (2) Topical administration route (3) Dosage form (eye drop) (4) Local effect (5) Dissolution profile (6) Device to the administration (7) Microbiological stability and (8) Physicochemical stability (Table 1).

It should be note that there are some essential requirements of the QTPP if the target dosage form is a solution, namely an eye drop. These essential quality requirements are strictly regulated by the physiological needs of the topical application and the researchers have to meet the pharmaceutical stan-

Table 1 *The selected QTPP elements, their target, justification and explanation*

QTPP element	Target	Justification	Explanation	
Therapeutic indication	Chronic eye disorder	Globally more than 253 million people suffers vision impairment	Therapeutic indication is a suggested QTPP by the ICH Q8	**Investigated in the RA of this study**
Target population	Patients, who need life-long therapy	Life-long therapy determined the patents' everyday life, decrease the HRQoL and leads to non-adherence patients' behavior	Target patient group is a suggested QTPP by the ICH Q8 in the clinical settings	
Administration route	Topical (eye)	The topical use avoids systematic effects and drug-drug interactions. Administration of drug by avoiding first-pass-metabolism, Blood Retinal Barrier and Blood- Aqueous Barrier. Expert competence is not needed for application	The route of administration has to be evaluated as a QTPP according to the ICH Q8 guideline	
Dosage form	Solution (eye drop)	Local irritation is decreased permeability of drug is increased, compared with suspension formulations	Dosage form is an essential QTPP element by the ICH Q8	
Site of activity	Local	Local effect is usually a general requirement of products for eye treatment. It is influenced by the solubility properties of the active pharmaceutical ingredient (API), the mucosal adsorption and wettability.	It is critically related to the quality, safety and efficacy of the medicinal product. Being a QTPP is a therapeutic requirement	
Dissolution profile	Immediate release	Immediate effect is usually a critical expectation for locally administered products. The residence time of the formula is limited on the surface.	It is critical from the patients' point of view	
Device	Proper to eye administration	Easy application, dose reproducibility are the main requirements. It is also linked to the microbial stability of the product.	It is critically related to the application safety and product quality	
Microbial stability	Long term microbial stability	Antimicrobial stability is essential in ocular drug delivery, considering of sensitivity of human eyes	It is critically related to the application safety and product quality	
Physicochemical stability	Long term physicochemical stability	It is critically related to the efficient and safe application of medicinal product	Default quality requirement	
pH	pH=7-9 pH=5-9	pH= 7-9 (optimal) pH= 5-9 (acceptable, not painful)	Default quality requirement	**Not investigated in the RA (strict regulated factors)**
Viscosity	30mPa*s	Should be under 30 mPa*s	Default quality requirement	
Osmolality	300mosm/kg	Should be close to isotonic level	Default quality requirement	
Surface tension	43mN/m	Surface tension of tear is about 43 mN/m. It should be similar in the product because of optimal spreadability and therapeutic effect	Quality requirement	

Table 1: The selected QTPP elements, their target, justification and explanation

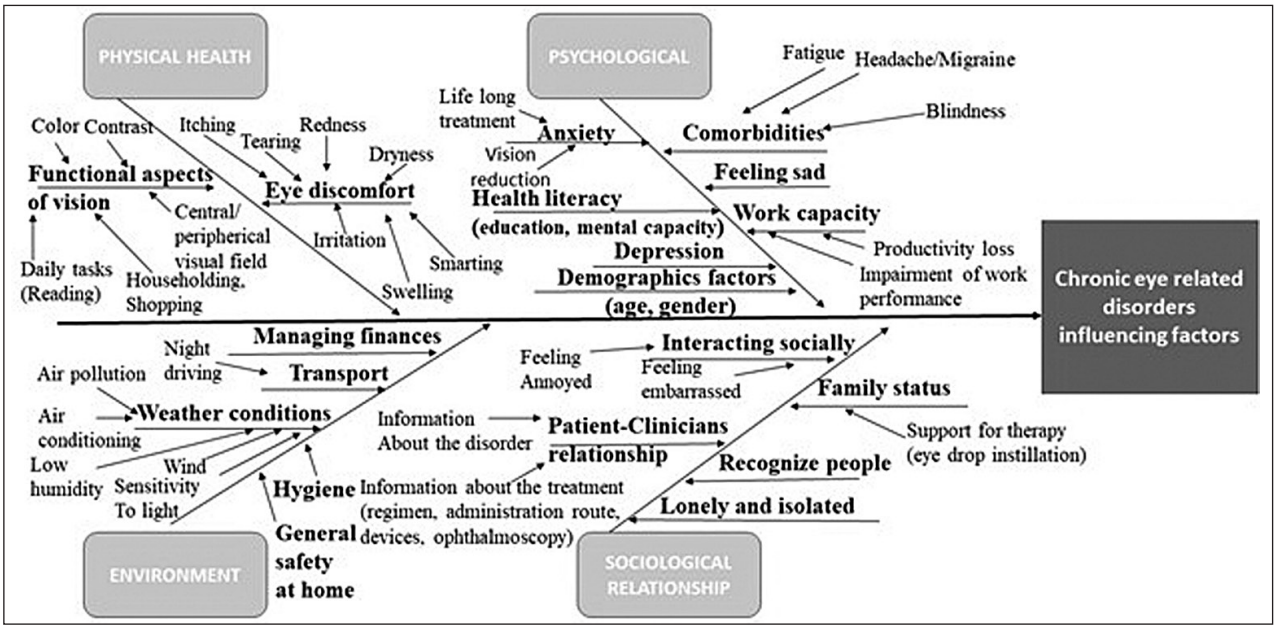

Figure 1 *Ishikawa diagram of influencing factors related to the chronic eye disorders*

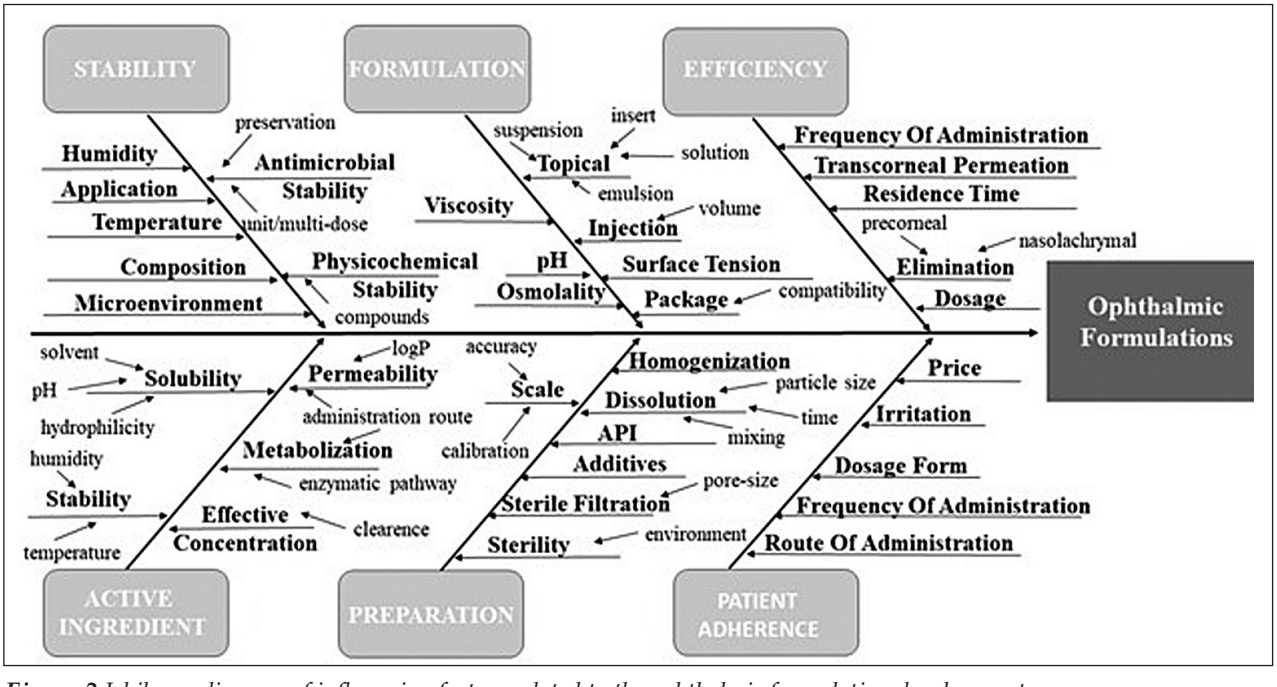

Figure 2 *Ishikawa diagram of influencing factors related to the ophthalmic formulation development*

dards. These are: (1) pH (2) Viscosity (3) Osmolality (4) Surface tension. However, these factors were not part of the RA because one and all would mark with "high" impact without reference to have or not have connection to selected CQAs and/or CPPs. If these parameters would be part of the assessment mentioned bias in the results. All relevant QTPPs are visualized in the following table (Table 1).

After the previous and profound QTPP determination cause-effects diagrams (Figure 1, Figure 2) were set up for the visualization of the most relevant influencing factors.

As it can be seen in Figure 1, a chronic eye disorder affects every aspects of patients' everyday life. Hard to compare the single effects according to their severity on patients' life. According to this fact was summarizes all parameters which could cover every part of the affected patients' life. If some of these factors damaged, supposed to lead ineffectiveness in the ophthalmic therapy.

QTPP – CQA

CQA	QTPP	(H) Life-long therapy	(H) Topical administration route	(M) Dosage form (eye drop)	(H)Local effect	(M) Dissolution profile (residence time)	(H)Device to the administration	(H) Microbiological stability	(H)Physicochemical stability
Eye discomfort	28%	High	High	Low	Low	Low	Low	High	High
Anxiety	14%	High	Medium	Medium	Low	Low	Low	Low	Low
Daily routine	12%	High	Low	Low	Low	Low	Low	Low	Low
Health literacy	13%	Medium	Medium	Medium	Low	Low	Medium	Medium	Medium
Social support	16%	High	Medium	Medium	Low	Low	Medium	Low	Low
Work capacity	18%	Medium	Medium	Low	Low	Low	Low	High	High

CQA – CPP

Process		Drug Product Application Process							
CQA	CPP	Storage (temperature)	Regimen (frequency of the administration)	Device applicability	Long-term stability	Long-term sterility	Application without decreased vision	Hygienic circumstances	Mobile application (alarm system)
		17%	30%	9%	6%	6%	21%	21%	12%
Eye discomfort	28%	Low	High	Low	Low	Low	High	High	Low
Anxiety	14%	Low	High	Medium	Low	Low	Medium	Low	Low
Daily routine	12%	High	Medium	Low	Low	Low	High	Low	Medium
Health literacy	13%	High	Medium	Medium	Medium	Medium	Low	High	High
Social support	16%	Medium	Medium	Low	Low	Low	Low	Medium	Low
Work capacity	18%	Medium	High	Medium	Low	Low	High	Low	Medium

OCCURANCE

CPPs	CPP Occurence	CPP Severity	CPP relative occurence	Occurence/ Severity
1. Storage (temperature)	High	17%	20%	1.22
2. Regimen (frequency of the administration)	High	30%	20%	0.68
3. Device applicability	Medium	9%	7%	0.79
4. Long-term stability	Medium	6%	7%	1.18
5. Long-term sterility	High	6%	20%	3.54
6. Application without decreased vision	Low	0%	2%	-
7. Hygienic circumstances	High	21%	20%	0.98
8. Mobile application (alarm system)	Low	12%	2%	0.19

Figure 3 *Results of the interdependence rating between CQAs and QTTPs as well as between CQAs and CPPs together with the occurrence of the CPPs*

The formulation aspects can be seen in the form of an Ishikawa diagram in Figure 2. The diagram shows the influencing factors related to development of ocular drug delivery systems, although all of the interactions are difficult to represent with this method. Stability, efficiency, patient adherence, composition and preparation are considered as the main groups of the diagram.

The previous visualization of the cause and effects relationships presented in the Figure 1 and Figure 2 helped in identification of the potential critical factors. So, the next step was the selection of the CQAs, based on the Figure 1. As there are originally determined and regulated critical factors (pH, viscosity, osmolality, surface tension), those critical quality factors were determined in this study as CQAs which could be modified according to patients' expectations and perceptions. The selected CQAs are "*patient focused*" quality attributes in our present case.

The identified CQAs were the following: (1) Eye discomfort (Itching, Tearing, Redness, Dryness, Irritation, Smarting, Swelling) (2) Anxiety (causing by life-long treatment and the vision reduction) (3) Daily routine, like householding, reading, shopping (4) Health Literacy, which is determined by education level and current mental capacity or status (5) Social support, first of all family members and friends (6) Work capacity, which could resulted as productivity loss or impairment of work performance.

The physical aspects like eye discomfort essentially influence the whole life, causes pain and overall physical disharmonies, which leads to negative attitude in some cases and could expand in psychological problem like, anxiety or depression. For people whose do not have vision problem, really hard to imagine that even performing the daily routine tasks have lot of difficulties, takes more time and could leads to misunderstanding, like patients do not recognize a familiar person or a family member, pay in the shop with wrong bank note or cannot find what they wanted to buy. These causes humiliation and presume that these patients will not leave their home after a while, especially if this come in younger ages. Besides, probable the problem will state at home as well. To perform the household or cooking will be more difficult. If the hygiene of the house is not enough sufficient, patients do not want to invite friends or family members, which reduced their social life. The situation is a little bit easier, if there are some family member or friends who can support the life of the affected, but unfortunately many patients are alone and do not have any support. The vision impairment affects not just private life but labour life as well. There is no work which can perform correctly without good vision. The loss of productivity and the reduction of the work performance from one side improved in negative feelings and from the other side in the long run could causes the loss of the work, which means lower monthly income and life quality reduction. For managing every kind of treatment crucial the patient's personal equation. The usual heath literacy belongs to a successful therapy output. If patients are not in adequately educated and also do not want to understand due to lack of interest in their own therapy, will not keep the defined treatment, loss some dose or overdose themselves, or do not use the device adequately, like the eye drop bottle, which is the determined device in our case.

All these factors escalate the problem and destroy the affected patients' entire life.

From the researchers' point of view, first of all the technological parameters determined the production of a drug, which was mentioned above as pharmaceutical standards In this case the production steps of an eye-drop formulation are fixed, the composition and preparation depend on the physicochemical attributes of active ingredients and additives. The final formulation need to meet the strict physiological requirements, such as pH, osmolality, viscosity and surface tension. The preparation must be done under aseptic environment to ensure a sterile product and proper microbiological stability during the storage and the application of the eye drop.

As the product production has severe defined elements in our present study "*the application of the medicinal product by the patient*" was identified as the process, and its critical attributes were identified as CPPs. The enumeration of the selected CPPs are: (1) Storage conditions, e.g. temperature, (2) Regimen, which is characterized by the frequency of the drug application (3) Device applicability, which is determined by the complexity of the drug application (4) Long-term stability (5) Long- term sterility (6) Application without decreased vision – this means the shortest time between the application and the perfect vision capacity to continue the daily routine, (7) Hygienic circumstances, e.g. clear hands, (8) Mobile application, which functions as an alarm system to pay attention to the application of the next dose.

The selected QTPPs, CQAs and CPPs were applied in the initial RA process. In the initial item of the RA the interdependence ratings were performed. The interdependence was evaluated step by step by each pair of the CQA and QTPP element, then by each pair of the CQA and CPP items. The effects of pairs by each other were estimated using the three-grade scale, as the potential effect can be rated as high, medium or low. Figure 3. presents graphically the results of the interdependence rating as part of the RA between QTPP elements and the CQAs as well as the CQAs and CPPs as. The CQAs and CPPs are also presented in Pareto charts (Figure 4), generated by the software, which shows also the numeric data of the selected critical factors and their ranking.

Figure 5 shows the relative severity –relative occurrence diagram. It has four quarters, which

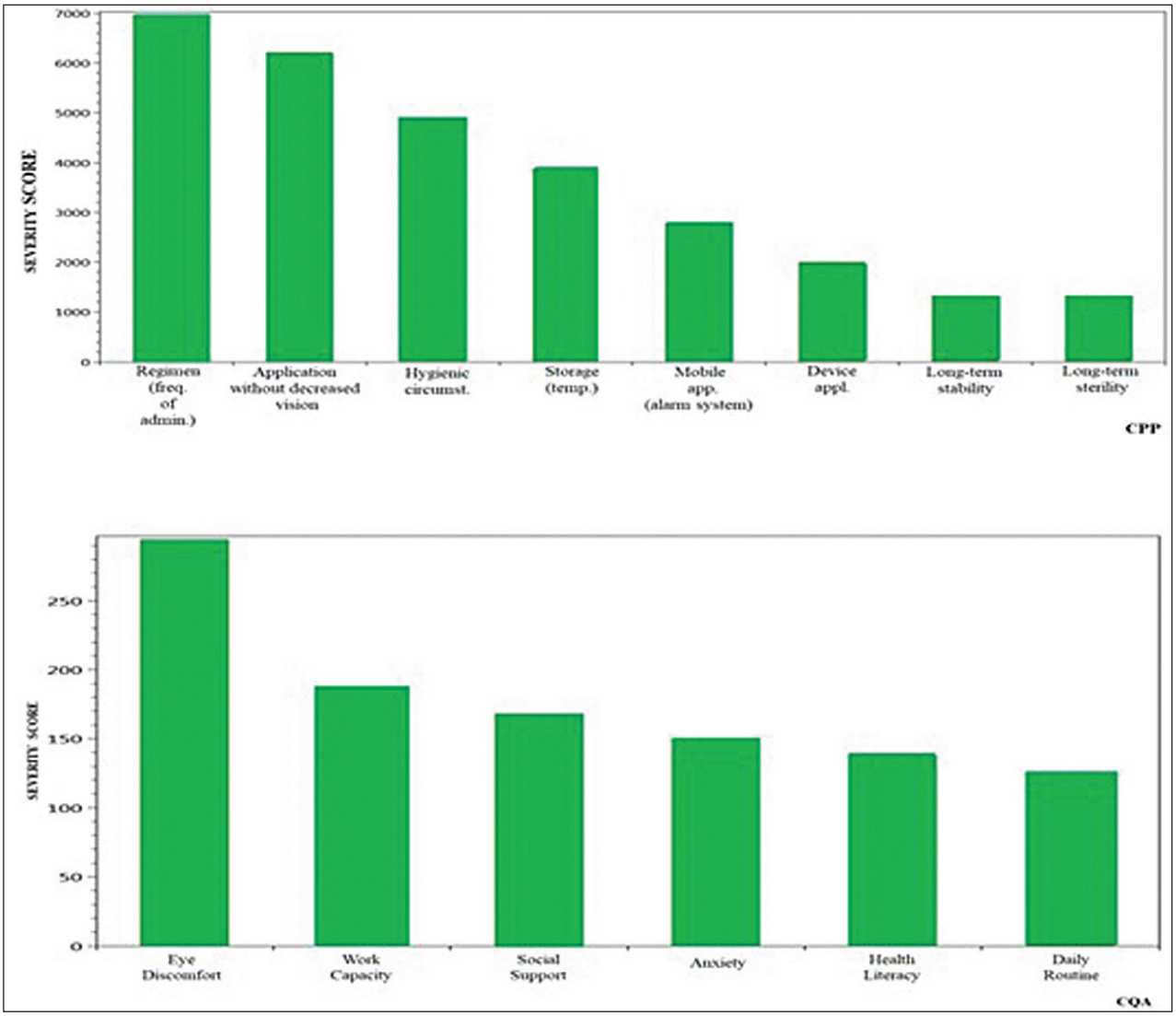

Figure 4 *Pareto charts according to numeric data of CQAs and CPPs*

present the estimated occurrence and the estimated severity of critical factors related to the application process from the patients' aspects point of view. The most important is the "relative high occurrence – relative high severity" quarter. In this study this quarter contains the factors like the regimen (the frequency of a product use), the hygienic circumstances (e.g. purity of hands and environment), and the storage conditions (temperature).

Conclusion

Our study delivered up those factors which are crucial from ophthalmic patients' point of view – based on commonly used disease specific questionnaires' items - and are worthy to take into consideration at the early development phase of formulation work. These are the essential elements which influence the pharmaceutical treatment in ophthalmology and are capable to improve the long-term patient adherence to treatment, resulting in an increased HRQoL. Besides, apart from the patients and the researchers, the health care providers, like ophthalmologist, also play crucial role in the treatment selection and optimization. Figure 6 summarizes the partners involved in the ophthalmology treatment. This figure also presages the completion of a further study, because the researcher and the patient aspects were evaluated in this present work.

If the storage condition, the frequency of the drug application and the comprehensive hygienic circumstances are highlighted during the formulation process, for example ensuring with primary wrapper change, or ensuring reduced application

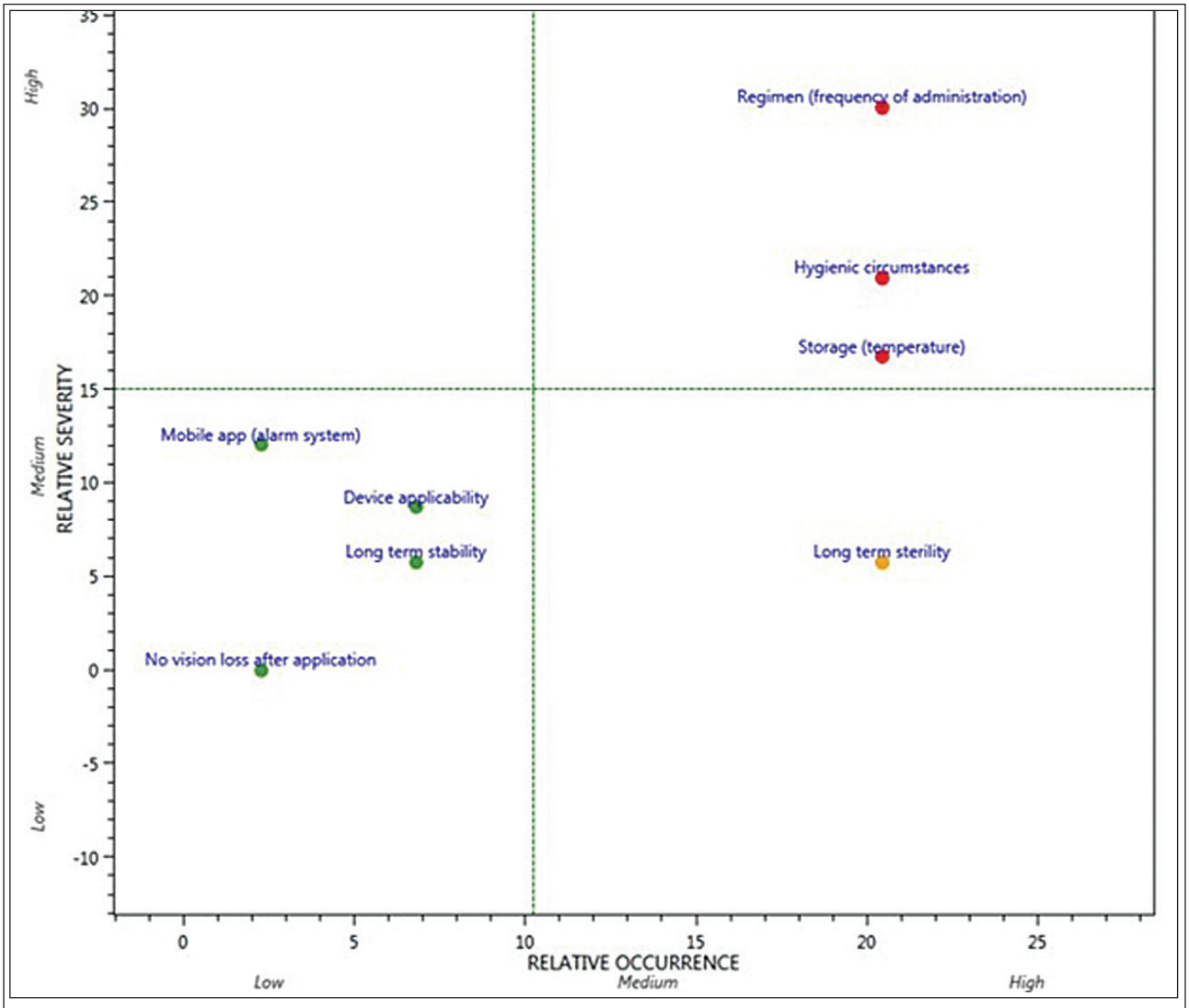

Figure 5 *The application process dependent relative-severity – relative occurrence, based on selected CQAs*

frequency, possible to help for patients during with drug application process, which was determined as the critical process in our study. However, our study has some limitations as well, which needed further observation and examination. First of all, the collected results need to apply during formulation process in practice and also important to ask the affected patients directly about their opinion to justify our results via self-reported questionnaires. The main aim of this study was to compare the patients' and the researchers' expectations and perceptions to give feedback for early development process via QbD based manner. This QbD based manner was achieved and RA was performed by using all affected parties' opinion. The research work is a method development which needs to be proved by further real-life experiments. However, the work aimed to develop a method which could be used as a basic for practical application. The most important outcome of the research is that if there is a concrete specific chronic eye disorder as a target, by using the QbD approach, the determination of the target product profile and its desired quality is possible in the first step. Then, based on the QTPP and related knowledge from the literature and practice the CQAs and CPPs can be identified. After performing the risk assessment, the design of experiments can be made and later the DoE-based experimental work the will be resulted in the determination of the design space. The information needed to the QbD based formulation-design can originate from the scientific literature and directly form patients via PROMs. In our specific case those questionnaires' items (more specifically the issues which were covered by the items) were used which are the most common regarding to chronic ophthalmic disorders. The presented

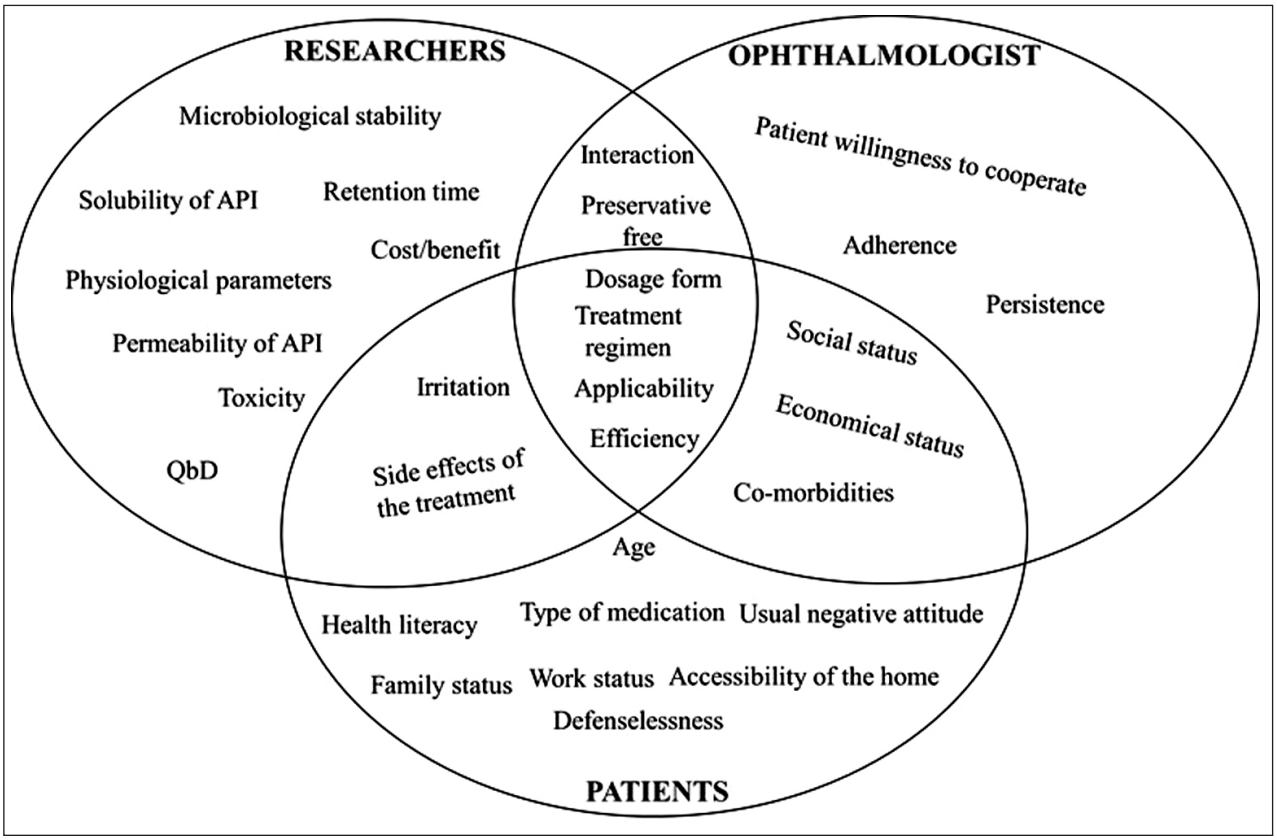

Figure 6 *Presage of all of the concerned parties' expectations*

method helps in systemization of the available information on a risk-based manner. By a further research, patients should be interviewed directly via questionnaires and the responses could be compared to technologists' and health care providers' point of view as well. The "Patient Centered Care"-approach can be provided from the very beginning, if the patients' needs and requirements are taken into consideration from the design phase of the pharmaceutical formulation and it is built in during the whole development process. In addition, by the presented risk-based method several useful results can be predicted to both of the parties, as this model can improve the satisfaction of the patients and can improve the success of the drug development.

Declaration

Competing Interest: No competing interest to declare.

Author Declaration: All authors have seen and approved the final version of the manuscript being submitted. This article is an original work, has not received prior publication and is not under consideration for publication elsewhere.

Funding Source: EFOP-3.6.1-16-2016-00008

Data availability: The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions: The University of Szeged, Faculty of Pharmacy, Doctoral School of Pharmaceutical sciences and the Institute of Pharmaceutical Technology and Regulatory Affairs made possible the scientific work.

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