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Summary of the Ph.D. thesis

Application of Analytical Quality by Design (AQbD) approach in *in vitro* release test (IVRT) method development in the case of semisolid topical formulations

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1. INTRODUCTION

To ensure the success of a semisolid drug delivery system, it is crucial to understand the mechanisms and target location of the action. The permeation process begins with the release of the Active Pharmaceutical Ingredient (API), known as liberation. The API then penetrates the stratum corneum or other skin layers, partitions within the epidermal environment, and finally exerts its effect at the target site. An *in vitro* release test (IVRT) is designed to characterize the steady-state drug release rate from a semisolid formulation. Changes in the parameters of the IVRT method can alter the measured release rate. IVRT is a well-established technique for analyzing semisolid dosage forms. The drug release rate and its retention from the topical product are critical factors in developing the desired effect. During the early development phase of a new or generic semisolid product, IVRT is particularly useful for detecting the effects of microstructural changes in the product that may affect the API's bioavailability.

International Council for Harmonization (ICH) guideline Q8 (R2) on pharmaceutical development defined the quality by design (QbD) approach as “a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management”. The goal of pharmaceutical drug development is to design and manufacture a quality product that consistently meets expected performance standards. Quality cannot be tested into the product; it must be built into the product during the development process. The concept of Analytical Quality by Design (AQbD) is an extension of QbD. Its main aim is to develop a robust, fit-for purpose and well-understood method that also delivers the intended performance during its lifecycle. Using AQbD principles while developing analytical methods can ensure the quality of both new and generic products from an analytical point of view. Furthermore, applying these principles also improves the reliability of the drug product, hence it plays an important role in ensuring patient safety.

2. EXPERIMENTAL AIMS

The aim of my thesis is the practical implementation of an AQbD based IVRT method development and using this concept, the comparison of the different apparatuses for IVRT. The

first AQbD guidelines were published in 2022; until then, only the QbD ICH Q8-Q11 guidelines were available for pharmaceutical development.

1. In the first part of my Ph.D. work, it was showed how the concept of QbD could be applied in the early stages of IVRT method development with United States Pharmacopeia (USP) apparatus IV (with semisolid adapter). At this time, the USP apparatus IV with semisolid adapter (SSA) was included in the USP <1724> general chapter, but since then, it has been removed from it. Thenabouts, an AQbD guideline has not been available yet. During the work we aimed to:
    - ✓ demonstrate the AQbD concept and its implementation in early stage of IVRT analytical method development;
    - ✓ define of Analytical Target Profile (ATP) and determination of Critical Method Attributes (CMAa);
    - ✓ identify and collect the (Critical) Method Parameters (MPs) with the help of the Ishikawa diagram;
    - ✓ establish initial risk assessment and prioritize a cause–effect relationship between CMAas and (C)MPs and reduce the number of possible critical parameters with the help of the Failure Mode Effects Analysis (FMEA) (determine the high-risk parameters of the *in vitro* drug release studies);
    - ✓ application of 2^3 full factorial design for mapping the possible impact of independent factors (CMPs) on each other;
    - ✓ update the FMEA table after factor screening study.
2. In 2022 USP 1220 general chapter was published and in 2023 ICH Q14 on analytical procedure development was made available and the ICH Q2(R2) on the validation of analytical procedures were adopted by Committee for Human Medicinal Product (CHMP). Together, the new ICH Q14 and the revised ICH Q2(R2) documents outline the proposed analytical development procedure and its validation for the analytical life cycle management approach. The second part of my Ph.D. work was to implement ICH Q14 guideline and USP <1220> general chapter. The aims were:
    - ✓ to establish targets in ATP with the help of international recommendations for IVRT studies;
    - ✓ to compare four IVRT apparatuses with the assistance of the ATP;

- ✓ to compare our own measurement results with the data found in the literature,
- ✓ to select a suitable IVRT apparatus for development of an analytical procedure.

3. MATERIALS AND METHODS

3.1 Diclofenac sodium cream and hydrogel

For IVRT studies Diclofenac sodium cream and hydrogel were used as model product, both magistral semisolid products were prepared in our laboratory. The matrix of the topical hydrogel containing diclofenac sodium included purified water, HPMC (hydroxypropyl methylcellulose), and propylene glycol. The excipients of the cream that contained diclofenac were purified water, cetostearyl alcohol, castor oil, polysorbate 60, methylparaben, and white petrolatum. Diclofenac sodium salt was obtained from Molar Chemicals Ltd. (Halásztelek, Hungary). Hypromellose, polysorbate 60, castor oil, white petrolatum, cetostearyl alcohol, methylparaben, and propylene glycol were provided by Hungaropharma Ltd., (Budapest, Hungary).

3.2 For IVRT and Ultra-High Performance Liquid Chromatography (UHPLC) measurements

Sodium chloride, di-sodium hydrogen phosphate dihydrate, and sodium hydroxide were obtained from Molar Chemicals Ltd. (Halásztelek, Hungary). Potassium dihydrogen phosphate was purchased from Thomasker (Budapest, Hungary). The water used was purified and deionized with ELGA PURELAB Chorus 1 (ELGA LabWater Headquarters, Lane End, UK). Orthophosphoric acid was acquired from Merck (Darmstadt, Germany). Methanol was purchased from Honeywell International Inc. (Charlotte, NC, United States of America (USA)). The receptor medium for the IVRT test were pH 7.4 Phosphate-buffered saline (PBS), pH 6.9 PBS, pH 7.9 PBS, pH 7.4 PBS + NaCl, and pH 7.4 PBS–NaCl. For the IVRT measurement, artificial ME (a mixture of nitrocellulose and cellulose acetate) membrane filters with a diameter of 25 mm (pore size of 0.22 μm), with a diameter of 47 mm (pore size of 0.22 μm), and 47 mm ME membrane (pore size of 0.45 μm), 25 mm Polyethersulfone (PES) membrane (pore size of 0.45 μm) were employed. ME membrane were provided by Labex Ltd. (Budapest, Hungary), Millipore PES membrane was supplied by Merck (Darmstadt, Germany). Before

each IVRT measurement, the ME membrane filters were soaked in the receptor medium for 30 min.

3.3 Static Vertical Diffusion Cell (Franz Cell)

The vertical diffusion cell system (Teledyne Hanson Co., Chatsworth, CA, USA), containing 6 cells (diffusional surface area: 1.767 cm^2) and equipped with an autosampler (Hanson Microette Autosampler System), was used to model the *in vitro* drug release from diclofenac sodium topical hydrogel and cream. Approximately 320 mg of the drug product was placed onto the 25 mm diameter cellulose membrane with a pore size of 0.22 µm (Labex Ltd., Budapest, Hungary). A receptor medium of 7 mL, with a pH of 7.4 ± 0.05 or pH 7.9 ± 0.05 PBS, was chosen and maintained at 32 ± 0.5 ◦C during the measurements. The stirring rate was set to 400 rpm. Samples of the acceptor medium (800 µL) were collected at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min and then analyzed using UHPLC. The volume of the replacement medium was 1.1 mL of pH 7.4 PBS or pH 7.9 PBS.

3.4 USP Apparatus II with Immersion Cell (USP II)

The USP Apparatus II dissolution test system (Vision® G2 Elite 8, Teledyne Hanson Co., Chatsworth, CA, USA) was used with a 0.53 mL immersion cell (Teledyne Hanson Co., Chatsworth, CA, USA). The use of this cell (Model B) is described in USP general chapter <1724>. The immersion cell, with a membrane surface of 1.77 cm^2, containing diclofenac cream or hydrogel (size of sample: 600–700 mg), was placed in a 150 mL flat-bottom vessel. The 150 mL receptor medium at pH 7.4 PBS or pH 7.9 PBS was applied at 32.0 ± 0.5 ◦C. The mini spin-paddle stirrers were set to stir at 250 rpm. Samples of 1.0 mL were taken from the acceptor medium at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min time points. No medium replacement occurred, as this had been accounted for during the calculation of the IVRT results.

3.5 USP Apparatus IV: Flow-Through Cell with Semisolid Adapter

A semisolid adapter or insertion cell (diffusional surface area: 1.54 cm^2) was used with USP apparatus IV (Sotax CE7 smart with CY 7 piston pump, Sotax Corporation, USA) to model the *in vitro* drug release from diclofenac sodium topical hydrogel. The donor compartments of the

semi-solid adapters (available in different sizes: 400 µL, 800 µL and 1200 µL) were filled with topical product, afterwards the ME membranes (pore size 0.45 or 0.22 µm) were fitted into the screw constraint and were placed over the surface of the sample compartments. The adapters with the membrane facing down were loaded into the 22.6 mm tablet cells prefilled with glass beads (1 mm glass beads). The USP IV apparatus (from 30-Nov-2023 this USP IV apparatus is no longer found in the USP chapter <1724>) was used in an "open loop" configuration. The receptor mediums were deaerated, the flow rate was 2 mL/min, 4 mL/min or 8 mL/min. The test temperature was 32 ± 0.5 °C and samples were collected (Sotax C 615 fraction collector, Sotax Corporation, USA) at 30, 60, 120, 180, 240, 300 and 360 min or 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min. We used 400 µL and 1200 µL semi-solid adapters for the IVRT development.

3.6 Flow-Through Diffusion Cell (FTDC)

Our flow-through diffusion cell, uniquely designed in our laboratory and equipped with a syringe pump, is an open-system diffusion cell suitable for measuring *in vitro* diffusion and skin penetration. This apparatus features only one measuring block, a spiral diffusion cell with a volume of 875 µL, allowing for one parallel measurement at a time. To ensure the leak-free assembly of the device, a 0.22 µm ME membrane with a 15.5 mm diameter and a 1.76 cm^2 diffusional surface area, (Labex Ltd., Budapest, Hungary) was applied for the measurements. The IVRT time spanned 6 h, with eleven fractions of the pH 7.4 PBS receptor fluid being manually collected at 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min, respectively, and then measured by UHPLC. The flow rate under the membrane was adjusted to approximately 2 mL/min and 4 mL/min, with the system temperature set to 32°C.

3.7 Description of the Ultra-High-Performance Liquid Chromatography Analysis method

The concentration of diclofenac sodium was determined using a Waters Acquity I-Class UHPLC system with a photo diode array (PDA) detector set to the wavelength of 240 nm. Chromatographic separation was performed using an Acquity UPLC BEH (ethylene bridged hybrid) UHPLC column (2.1 mm X 50 mm, 1.7 µm, 130Å, Waters Corporation, Milford, MA, USA), the temperature was maintained at 40°C and the mobile phase was a mixture of methanol

and potassium dihydrogen phosphate buffer (pH 2.5; 20 mM) (36 / 64 v/ v). The potassium dihydrogen phosphate buffer was filtered through a 0.22 µm filter. Degassing of the mobile phase was achieved by the ultrasonication of the eluent up to 5 min. The run time was set to 3 min. The flow rate was 0.45 mL/min. For calibration, the injection volume was 2 µL, but during the measurement of the IVRT samples, different volumes were used depending on the diclofenac sodium concentration in the samples. For each in vitro release study, calibration was established in the concentration range of 4 to 100 µg/mL ($R^2 \geq 0.995$).

3.8 Establishing the Analytical Target Profile (ATP), the Critical Method Attributes (CMAas) and Critical Method Parameters (CMPs)

The initial phase of the analytical method development involved the establishment of the ATP for the IVRT measurement. As defined, "an ATP consists of a description of the intended purpose, appropriate details on the product attributes to be measured and relevant performance characteristics with associated performance criteria". The ATP should encompass the definition of the analyte and the product, including details such as dosage form, strength, matrix components, and route of administration. It should also cover aspects like range, acceptable bias, and precision (maximum allowable combined bias or target measurement uncertainty). "Target measurement uncertainty (TMU) is the maximum acceptable uncertainty in the reportable result that must be achieved by the analytical procedure". "Once the acceptable probability of making an incorrect decision of compliance has been established and a decision rule has been defined, the target measurement uncertainty is decided". On the basis of our prior method development knowledge and data, CMAas are derived from the ATP. CMAas are the elements of method performance which need to be measured and/or evaluated to ensure that the desired data will be provided. CMAas are analogous to Critical Quality Attributes (CQAs) in drug development. After the definition of CMAas, all the MPs can be summarized systematically with the help of an Ishikawa diagram. The aim of this method is to summarize all influencing factors during a brainstorming session, and then to categorize and to visually represent MPs.

3.9 Establishing Failure Mode Effects Analysis (FMEA)

In the risk matrix, we can estimate the effect and the risk of the MPs with regard to the method performance. The outcomes of an FMEA are the risk priority numbers (RPNs). RPNs are calculated by multiplying occurrence (O), severity (S), and detection (D) indexes. O is the occurrence of failure or the likelihood of an event occurring. S is the severity scale that could be based on the impact that the sources of variability have on the analytical procedure measurement (ability to meet the ATP criteria). D is detectability or the ease with which a failure mode can be detected. On the basis of RPNs, low risk is from 1 to 29, medium risk is from 30 to 59 and the high risk is from 60 to 125. The MPs which were classified as medium or high risk in the FMEA should be considered as critical method parameters (CMPs).

3.10 Design of Experiments (DoE) for IVRT Method

The DoE method is a modelling tool for assessing possible interactions between the factors influencing the drug development process and, thus, the quality of the final product. CMPs must be chosen as independent variables and CMAas as dependent variables in the factorial design process. After the FMEA and the preliminary experiments, a 2^3 full factorial design was performed for the optimization of an IVRT method for a diclofenac sodium hydrogel formulation.

4. RESULTS

4.1. Experiment part I

4.1.1 Definition of ATP and Determination of CMAas

In order to support the development of the formulation on the analytical side, we need adequate analytical methods that should guarantee the quality of the product. Accordingly, the IVRT should be sensitive to the changes and alterations in the formulations, and the analytical measurements must be able to accurately and precisely quantify the API in IVRT samples. Therefore, we defined these targets in the ATP (Table 1) and then CMAas (Table 2) were derived from ATPs. The ATP should lead the selection of analytical technology: UHPLC is a more reliable and widely used technique and it is capable of satisfying the ATP requirements.

Table 1 Analytical target profile of diclofenac sodium topical gel

ATP element	Target
Target sample (Product name)	Diclofenac sodium 1% topical gel
API Name	Diclofenac sodium
Dosage strength	1 % (10 mg/g)
Dosage forms	Hydrogel
Route of administration	Topical
Matrix	Propylene glycol (50 %), HPMC (1.5%), purified water (47.5%)
Packaging	Plastic tube
Regulatory specification	ICH, EMA (European Medicines Agency), FDA (Food and Drug Administration)
Release / *In vitro* release test	The release tests should be sensitive to relevant changes in the ingredients and process parameters. Adequate release efficiency, release profile and reproducibility. Meets regulatory requirements. Precision RSD ≤ 10 % (6 parallel).
Analytical measurements	Analytical measurements: The procedure must be able to accurately quantify diclofenac sodium in IVRT samples over the range of 25 – 200 % of the nominal concentration with an accuracy of 2.0 %

Table 2. Critical method attributes of diclofenac sodium topical gel

CMAa parameters	Target	Justification
Release efficiency in 6 hours	Q (6 h) ≥ 70 %	IVRT is a fundamental tool to identify formulation factors that influence the release of the API, an effective method to monitor lot-to-lot changes and stability studies during development. A draft guideline on the quality and equivalence of topical products described this criterion.
Characterize the release profile	6 time points should be obtained in the linear portion of the drug release profile	
RSD % of the released API amount of the 6 parallel samples at given sampling points	RSD ≤ 10 % (6 parallel)	RSD values below 10 % are considered as an indication of the good reproducibility of the IVRT method.
Accuracy	Between 98-102 %	In the case of UHPLC measurements, the weak point of the true value determination is accuracy.
System suitability test of the chromatography system	USP Plate Count: N ≥ 3000	There is a need for a chromatography system in which the API can properly separate from the matrix components. The plate count has a fundamental impact on the extent of measurement error through the peak's capability of being integrated. Therefore, the chromatography method should be suitable within the purpose to detect the API in IVRT samples at 25 % of the nominal concentration.

4.1.2. Identification of the Method Parameters using the Ishikawa Diagram

According to prior knowledge, our next step was to systematically collect all the MPs that could influence a failure concerning the IVRT method. For this, we used the Ishikawa diagram as a risk assessment tool to identify potential variables that could have an impact on CMAas. With

the help of the Ishikawa diagram, more than 100 method parameters were identified that can influence the method performance and the quality of the method's results.

4.1.3 Initial Risk Assessment using FMEA (Effects of MPs on CMAas)

During the FMEA analysis, the possible effects of MPs on CMAas were investigated. The analysis was carried out in the case of all the MPs one by one. The initial risk assessment aims to identify the potential CMPs (that were assigned the highest RPNs), which will be investigated during the preliminary experiments. Based on the literature data and our prior method development knowledge, the highest risks (RPN $\geq$ 60) were identified using FMEA, including osmolality, the pH of the media, membrane type, rate of flow, sample weight (volume of the SSA), individual flow rate of cells, API%, and the composition of the product. During the screening process, we examined the impact of only the highest scoring parameters on the CMAs independently from each other as a preliminary study.

4.1.4 2^3 Full Factorial Design for the IVRT Method

The high-risk CMPs must be chosen as independent variables and CMAas as dependent variables in the factorial design process. In our case the preliminary risk assessment, the flow rate was not a critical parameter, but considering our previous experiences, the sample volume and the flow rate may have a combined effect; therefore, we examined the flow rate as an independent variable in our factorial design. The flow rate (X1), volume of SSA (X2), and the pH of the medium (X3) were chosen as independent factors and the *in vitro* release rate (IVRR) (Y1) and release efficiency in 6 h (Y2) were dependent factors. With the preliminary experiments, the flow rate (mL/min) was not found to be a CMP, although, apart from the main effects, two-way or/and three-way interactions can be significant. From the results of 2^3 full factorial statistical analysis (n = 5 per analysis) the main factors X2 (the volume of the SSA) and X3 (pH) exert a significant effect ($p < 0.05$) on Y1 (IVRR). In other respects, the statistical analysis shows that only one main factor X2 (the volume of the SSA) has a significant effect ($p < 0.05$) on release efficiency in 6 h (Y2). The other factors did not have a significant effect on Y2.

4.1.5 Summary of experimental part 1

In the first part of my Ph.D. study, it was showed how the concept of AQbD can be applied in the early stages of IVRT method development in the case of USP apparatus IV. After defining the ATP and selecting CMAs (at least 70% of the active substance applied is released after 6 h, six time points should be obtained in the linear portion of the drug release profile, and the relative standard deviation in the computed released amount of the six vessels was less than or equal to 10%), an initial risk assessment was carried out: with the help of the Ishikawa diagram, more than 100 method parameters were identified that can influence method performance and the quality of the results. FMEA was used to reduce the number of possible parameters down to eight factors: osmolality, the pH of the medium, membrane type, the rate of flow, sample weight (volume of the SSA), the individual flow rate of cells, API%, and the composition of the product. During the screening process, we examined the impact of these parameters on CMAs independently of each other. These CMPs (the pH of the medium and the sample weight of the product) were given as independent variables in the factorial design.

A 2^3 full factorial design experiment was employed to assess the IVRR and the release efficiency in 6 h. After the examination, we re-evaluated the risks according to the results and recorded them in the updated FMEA table, thus narrowing the method parameters to CMPs. On the basis of our results, the amount of the product and the pH were clearly defined as critical parameters during the application of the AQbD approach. At least 70% diclofenac sodium release from the hydrogel (all parallel samples) was achieved within 6 h under all testing conditions; therefore, it meets the ATP requirements. The ATP is capable of satisfying the EMA guideline (draft guideline on quality and equivalence of topical products) criteria. On the other hand, the means of operation of USP apparatus IV allows more time points to be applied in order to meet the criterion “6 time points should be obtained in the linear portion of the drug release profile”. Summarizing the results of the first part of our experiments, a robust IVRT test can be developed using the USP apparatus IV, which complies with the international guidelines, but the effect of the pH of the medium and the sample weight on the IVRT results must be analyzed in each case.

4.2.Experiment part II

4.2.1. Definition of ATP for the IVRT

The ATP was defined (Table 3) for the IVRT method for the development of the topically used diclofenac sodium hydrogel and cream, as suggested by the ICH Q14 guideline and USP general chapter <1220>. The intended purpose of the development of the analytical method was the quantification of the diclofenac sodium API for an IVRT quality control (QC) test. The dosage strengths were 1% and 2%. Assuming a normal distribution, the calculation of target measurement uncertainty (TMU) (σ) can be described using the following formula:

$$\sigma = \frac{x - \mu}{z} = \frac{77\% - 70\%}{1.65} = 4.24\%, \qquad \text{if } Q_{final} = 70\% \quad (1)$$

where x is the upper specification limit of accuracy (in our case, it was 110%, so with 70% of Q_{final}, x value is 77%), μ is the true value (100%), and z is the coverage factor (two-tailed, 90% confidence level 1.65). After the IVRT apparatus was chosen and the initial Analytical Control Strategy (ACS) was established at the end of stage 1, it should be confirmed that the reportable values of the developed analytical procedure meet the ATP criteria.

Table 3. ATP of IVRT of cream and hydrogel that contain diclofenac sodium.

Attributes	Target	Justification
Accuracy	90–110%	The procedure must be able to accurately quantify diclofenac sodium in IVRT samples over the range of 50% to120% of the nominal concentration with accuracy and precision, ensuring that measurements fall within ± 4.24 of the true value with 90% probability.
Method Precision (measured at the last sampling point of the IVRT)	RSD (%) at the last time points ≤ 10%	
Linearity	$R^2 \geq 0.97$	
Range	± 20 % over the specified range	
Cumulative amount released at the end of the IVRT experiment	$Q_{final} \geq 70\%$	The duration of the IVRT should be sufficient to characterize the release profile, with data collected at six time points within the linear portion of the drug release profile. Ideally, at least 70% of the applied diclofenac sodium should be released.
Robustness	Mean slope ($\mu g*cm^{-2}*min^{-0.5}$) of an IVRT run with pH 7.4 and pH 7.9 medium, or 2 mL/min and 4 mL/min, should fall within ± 15%	The average slope of the IVRR of diclofenac sodium, measured with pH 7.4 and pH 7.9 medium and/or 2 mL/min and 4 mL/min (2*6 measurements), should not be deviated by more than 15% from the nominal method parameter settings.

After establishing the crucial ATP, which includes relevant performance characteristics with the associated performance criteria, the second step involved the initial technology selection for IVRT method development.

4.2.2. The Selection of the IVRT Technology

In our case, multiple analytical technologies for IVRT were available in our laboratory, offering a choice from four IVRT technologies (static vertical diffusion cell, USP Apparatus II with immersion cell, USP Apparatus IV with semisolid adapter, and a flow-through diffusion cell).

The UHPLC measurement technique and method were chosen; however, the UV spectrophotometric analytical method could not be used due to the UV active-matrix component. If the development of the product and the analytical method development proceed simultaneously, a wealth of measurement data becomes available for selecting the analytical technology. In our case, there was minimal prior knowledge of the IVRR of diclofenac sodium from the hydrogel and cream matrix. In our situation, the IVRT method parameters of the USP II with immersion cell, USP IV with SSA, and the static vertical diffusion cell (Franz cell) meet the sink condition criterion, but the FTDC does not. In the case of the sink condition, although it should be noted that the volume of the receptor phase is, in most cases, a basic apparatus property, in our case only the FTDC could not fulfill this condition at the starting point of the IVRT. Nevertheless, in the case of FTDC (and USP IV with SSA), sustaining the continuous replacement of the fresh receptor medium ensures the correct sink conditions more easily throughout the experiment. Both flow-through cell apparatuses were used in an open-loop configuration.

4.2.3. In Vitro Test Results for the Cream and Hydrogel Containing Diclofenac Sodium

Creams and hydrogel also containing 1% and 2% of diclofenac sodium were measured by the "usual" IVRT parameters employed for the initial measurements involved using pH 7.4 PBS (without cosolvent or surfactant) for all four apparatuses. The results can be seen in the Table 4 and Table 5.

Table 4. Predefined acceptance criteria and results for 1% and 2% diclofenac cream in the selection of the IVRT method.

Apparatus	Attributes	Target	Results of 1% diclofenac cream		Results of 2% diclofenac cream	
Franz cell USP II, immersion cell USP IV, SSA	Method precision	RSD (%) at the last timepoint ≤ 10%	2.83	passed	4.06	passed
			4.50	passed	5.36	passed
			2.44	passed	1.91	passed
FTDC			16.10	failed	13.94	failed
Franz cell USP II, immersion cell USP IV, SSA	The cumulative amount released at the end of the IVRT experiment	$Q_{final} \geq 70\%$	49.29	failed	61.30	failed
			26.10	failed	38.00	failed
			30.02	failed	25.62	failed
FTDC			56.64	failed	42.96	failed
Franz cell USP II, immersion cell USP IV, SSA	Accuracy	90–110%	96.58	passed	-	-
			100.94	passed	-	-
			99.04	passed	-	-
Franz cell USP II, immersion cell USP IV, SSA	Robustness (pH)	Mean slope of an IVRT run with pH 7.4 and pH 7.9 medium should be within ± 15%	-13.85	passed	-0.68	passed
			-4.26	passed	-2.94	passed
			-15.93	failed	9.81	passed
USP IV, SSA	Robustness (flow rate)	Mean slope of an IVRT run with 2 mL/min and 4 mL/min flow rate should be within ± 15%	-6.55	passed	-0.15	passed
FTDC			-49.67	failed	-20.58	failed

Table 5. Predefined acceptance criteria and results for 1% and 2% diclofenac hydrogels in the selection of the IVRT method.

	Attributes	Target	1% hydrogel results		2% hydrogel results	
Franz cell	Method Precision	RSD (%) at the last timepoint ≤ 10%	3.74	passed	3.00	passed
USP II, immersion cell			4.43	passed	7.78	passed
USP IV, SSA			1.40	passed	0.88	passed
FTDC			6.32	passed	2.11	passed
Franz cell	The cumulative amount released at the end of the IVRT experiment	$Q_{final} \geq 70\%$	92.40	passed	91.74	passed
USP II, immersion cell			86.47	passed	86.86	passed
USP IV, SSA			94.23	passed	100.57	passed
FTDC			104.61	passed	98.74	passed
Franz cell	Accuracy	90–110%	94.64	passed	-	-
USP II, immersion cell			102.00	passed	-	-
USP IV, SSA			97.08	passed	-	-
Franz cell	Robustness (pH)	Mean slope of an IVRT run with pH 7.4 and pH 7.9 medium should be within ± 15%	-4.31	passed	-3.57	passed
USP II, immersion cell			4.62	passed	8.85	passed
USP IV, SSA			6.24	passed	-9.49	passed
USP IV, SSA	Robustness (flow rate)	Mean slope of an IVRT run with 2 mL/min and 4 mL/min flow rate should be within ± 15%	-0.48	passed	-8.90	passed
FTDC			-6.31	passed	-23.93	failed

4.2.4. Summary of experimental part 2

In the second part of Ph.D. study four IVRT apparatuses were compared (USP Apparatus II with immersion cell, USP Apparatus IV with semisolid adapter, static vertical diffusion cell, and a new, in-house-developed flow-through diffusion cell) with the help of the ATP. The new ICH Q14 guideline and the USP general chapter <1220> were implemented to define an ATP for IVRT. The results of the preliminary IVRT experiments of the cream which contains diclofenac sodium showed that the release of the diclofenac sodium from the cream matrix was slow, as measured by all four IVRT apparatuses, and the $Q_{final} \geq 70\%$ criterion described in the ATP was not met. Therefore, the IVRT measurement should be at least 12 h long. Based on our results, both of the USP II with immersion cell and the USP IV with SSA were appropriate to measure diclofenac sodium hydrogel and cream. The USP II with immersion cell apparatus will

be the best choice to develop an analytical IVRT method for diclofenac sodium cream, and the USP II with immersion cell apparatus and/or USP IV with SSA is best for diclofenac sodium hydrogel.

5. SUMMARY

The aim of my Ph.D. work was to implement AQbD concept in practice in the early stages of IVRT development and apparatus selection. During my Ph.D. work, the following findings were established:

- ✓ We have implemented the AQbD concept in early stage of IVRT analytical method development.
- ✓ We have defined Analytical Target Profile (ATP) and determination of Critical Method Attributes (CMAas) for IVRT.
- ✓ We have identified and collected the (Critical) Method Parameters (MPs) for IVRT with the help of the Ishikawa diagram.
- ✓ We have established initial risk assessment and prioritize a cause–effect relationship between CMAas and (C)MPs and reduced the number of possible critical parameters for IVRT.
- ✓ We have established targets in ATP with the help of international recommendations for IVRT studies in order to compare four IVRT apparatuses.
- ✓ On the basis of our experiments, we have selected a suitable IVRT apparatus for development of an analytical procedure.

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

ARTICLES

I. **Réka Szoleczky**, Mária Budai-Szűcs, Erzsébet Csányi, Szilvia Berkó, Péter Tonka-Nagy, Ildikó Csóka, Anita Kovács
Analytical Quality by Design (AQbD) Approach to the Development of In Vitro Release Test for Topical Hydrogel
Pharmaceutics, 14(4), 707 (2022), Q1, IF: 5.40

II. **Réka Szoleczky**, Anita Kovács, Szilvia Berkó and Mária Budai-Szűcs
An Analytical Target Profile for the Development of an In Vitro Release Test Method and Apparatus Selection in the Case of Semisolid Topical Formulations
*Pharmaceutics, 16(3), 313 (2024), Q1, 4.90**

PRESENTATIONS

I. **Szoleczky Réka**; Budai-Szűcs Mária; Kovács Anita: Analytical Quality by Design (AQbD) approach to the development of in vitro release test for topical hydrogel. IV. *Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science; Szeged, Hungary, January 19-21, 2022.*