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Department of Pharmaceutical Technology

Ph.D. Thesis

Pharmaceutical development of co-crystals

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1 INTRODUCTION

Co-crystals are solids that are crystalline materials composed of two or more molecules in the same crystal lattice. Formation of co-crystals can solve several pharmaceutical issues raised during preformulation and formulation development e.g. by solubility, dissolution, bioavailability, chemical stability, decreasing hygroscopicity modulation. Formation of co-crystals could be a new path to improve physico-chemical and biopharmaceutical properties of medicines. One of the most difficult pharmaceutical formulation tasks is to improve the absorption of a weak base with poor and pH-dependent solubility properties; however, some combined chemical and formulation approaches give the possibility to reach this goal. Usually applied chemical tools are the salt and/or co-crystal formation, while the pharmaceutical approaches are micronization, nanonization, and elaboration of lipidic and amorphous formulations. However, to reach the targeted pharmacokinetic/pharmacodynamic (PK/PD) profiles, synergies of different chemical and pharmaceutical tools are needed.

2 AIMS

The aims of this thesis are

- to explore and apply the synergies among chemical and pharmaceutical tools in case of a development of pharmaceutical co-crystals on the example of SAR1 compound (origin molecule of Sanofi),

- to show the benefits of early cooperation among discovery and development scientists in the field of Early Drug Formulation (EDF),

- evaluate the results of preformulation from pharmaceutical processability point of view,

- access the usefulness of flow through dissolution technique in the area of Early Drug Formulation and co-crystal development,

- elaborate a practical guidance for scientists to formulate co-crystals as active pharmaceutical ingredients (API).

2.1 EARLY DRUG FORMULATION

The targets of EDF are

- supply discovery studies with classical or enabling formulations to ensure robust drug safety (and de-risk toxicological concerns), efficacy and pharmacokinetic measurements,

- early assessment of physico-chemical and biopharmaceutical properties of APIs that are amenable to downstream development,

- support early go/no go decision on discovery candidates.

Solutions are developed for early studies if it is possible. In that case absorption is not effected with particle size, polymorphic form of the API. Inadequate exposure may lead to poor efficacy and could lead to rejection of a potential blockbuster. Suspensions are supplied for late studies to mimic in vivo conditions after administration of a standard tablet or suspension formulation. Solubility properties of the possible new APIs determine the type of the elaborated formulations. If the solubility of the API is more than 100 µg/ml classical, aqueous solutions and suspensions are developed however if the solubility goes below 10 µg/ml only enabling formulations such as nanodispersions, lipic, cyclodextrin containing and amorphous formulations can support PK/PD and toxicological studies. If the solubility of the API is between 10 and 100 µg/ml a selection is needed between classical and enabling formulations based on the predicted doses.

2.2 FLOW THROUGH DISSOLUTION

Flow through dissolution technique is a well known approach from early 1970s elaborated for low solubility BCS II and BCS IV type active pharmaceutical ingredients and for their drug products. This is a suitable tool for evaluating and comparing active pharmaceutical ingredients and formulations but it is also used to explore special issues related to new chemical entities, salts and co-crystals. The main limitation of classical basket or paddle type

dissolution instruments is the sink condition requirement, because there is a high risk to reach quickly the super saturated concentration in a permanent one liter dissolution media, furthermore sometimes it is not suitable to reach the sink condition for active pharmaceutical ingredients, which are practically insoluble in aqueous solutions. In contrast to the past, when the majority of research compounds had a relatively small molecular weight and acceptable solubility, the number of larger and less soluble molecules showing permeability and/or solubility-limited absorption has increased during the past years. The opened type flow through dissolution technique (Figure 1), being a dynamic system, is closer to the in vivo status of the body, than the static-type classical paddle and basket apparatuses. The dissolved active pharmaceutical ingredient is removed and collected from the cells of the FTDE and this process provides the possibility for dissolution of a new portion of the solid material modeling absorption and elimination.

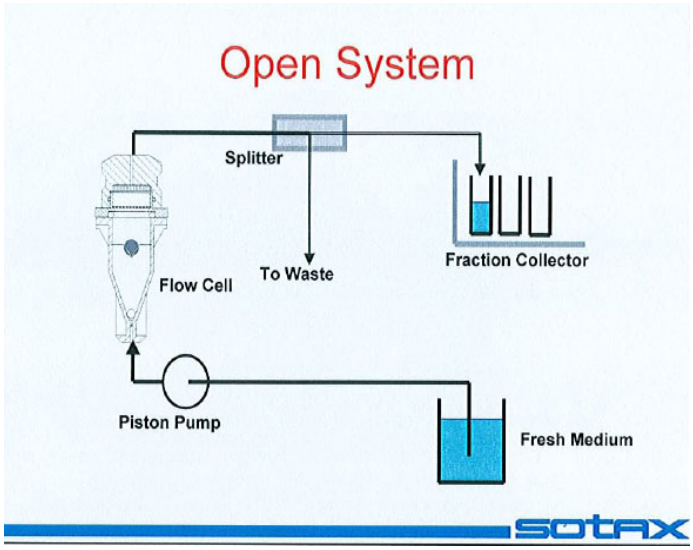

Figure 1 - Opened type flow through dissolution equipment

3 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Active pharmaceutical ingredient in the field of co-crystal development

Three active pharmaceutical ingredients were evaluated and compared. These are: SAR1 as a weak base, its di-HCl salt and its co-crystal with fumaric acid.

3.1.2 Pharmaceutical excipients

Cremophor ELP was ordered from BASF. Cremophor ELP, a purifed grade of Cremophor EL was specially developed for sensitive active ingredients, as the higher purity was found to improve their stability. Tween 80, lactic acid, citric acid, Span 85, PEG 200, sodium hydroxide were purchased from Merck while Eudragit L100-55 was ordered from Evonic. Some pharmaceutical excipients such as mannitol, sulfobuthyl β cyclodextrin, vitamin E TPGS, PVP K25, sodium docusate, Miglyol 812 N, sodium dodecyl sulfate, methyl cellulose, HPMC, crospovidone, microcrystalline cellulose, magnesium stearate and colloidal silica anhydrous were ordered from the internal warehouse of Sanofi.

3.2 METHODS

3.2.1 Pharmaceutical methods

3.2.1.1 Early drug formulation phase

Manufacturing of the exploratory formulations were performed on a laboratory scale using 20 to 100 g batch size. Eight formulations were prepared and tested in oral animal pharmacokinetic studies.

Formulation 1 was a suspension containing micronized SAR1 in methylcellulose and Tween 80 vehicle. Micronized material was manufactured on laboratory scale spiral jet mill and nanosuspension was prepared by Elan type nanotechnology (F2).

The API was fully dissolved in lactic acid before preparation of the Miglyol 812 N based w/o

emulsions (F3 and F4 formulations). The lactic acid solution was combined with 5 % sulfobutyl β cyclodextrin in case of F3 formulation while F4 formulation contained 5 % Span 85 to avoid free base precipitation at intestinal pH.

The weak base containing suspension formulation was prepared in a mortar with pestle (F5 formulation). SAR1 free base was suspended with 5 % Cremophor ELP first, followed by lactic acid, 20 % aqueous solution of vitamin E TPGS and PEG 200 were added to the suspension. pH adjustment to 4.0 was performed with NaOH solution.

The weak base and citric acid containing formulation was prepared with a classical wet granulation process. One portion of the elaborated stock granule was diluted with 2 portions of 0.6% methyl cellulose solution before administration to animals (F6 formulation).

A stabilized, amorphous solid solution preparation was initiated from the joint N-methyl-pyrrolidine solution of SAR1 weak base and Eudragit L100-55. The partially amorphous SAR1 was dosed in 2 % sodium dodecyl sulphate containing 0.5% methyl cellulose suspension.

The co-crystal of SAR1 with fumaric acid was suspended with Cremophor ELP firstly before dilution with 0.6% methyl cellulose solution to protect co-crystal from dissociation (F8 formulation).

3.2.1.2 Pharmaceutical processability phase

Manufacturing of the different formulations were performed in Mi-pro miniaturized high shear granulator (Pro-C-ept). Four experimental compositions were manufactured in 30g miniaturized scale with 10% API load. The integrity of the co-crystal was studied from granules. Tabletting was performed on Korsch excentrical tabletting machine with 3-15 kN pressure force. Flat, rimmed tablets were pressed with 30-35 N hardness.

3.2.2 Animal studies

Species are male rats. Approximate weight at initiation of dosing was between 210-270 gs. The age of rats at initiation of dosing was 7 weeks.

4 RESULTS AND DISCUSSION

4.1 EARLY DRUG FORMULATION - FORMULATION POSSIBILITIES OF A WEAK BASE WITH A NARROW SOLUBILITY RANGE

4.1.1 Physico-chemical and biopharmaceutical properties of the candidates

SAR1 was evaluated as a model compound (Fig 2) planned for use in the oncology area. The measured Caco-2 permeability value of SAR1 was 32 x 10^{-7} cm/s, which indicates a potentially good *in vivo* permeability.

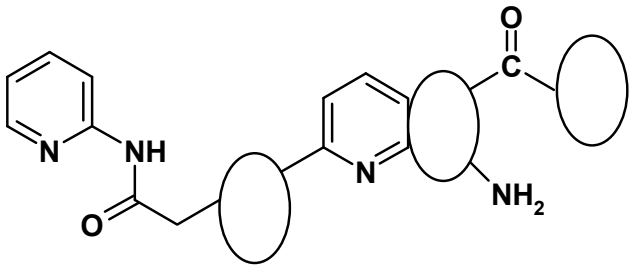

Figure 2: SAR1 as a model active pharmaceutical ingredient (API)

The evaluation of the key and critical physico-chemical and biopharmaceutical properties of this API were done together with these data of its di-HCl salt and the SAR1 fumaric acid (1:1) co-crystal. The co-crystal formation was done between the pyridine nitrogen and carboxylate group of the fumaric acid verified by ss NMR data. The API, as a weak base, shows salt formation with strong acids such as hydrochloride acid only, the presence of which causes the hydrolysis of the amide bond and the formation a 2-amino-pyridine and the corresponding carboxylic acid. Based on the above mentioned acidic hydrolysis of the API, the chemical stability of SAR1 and potential formulations in the presence of HCl is not suitable. Another issue of the dihydrochloride salt was that stoichiometric salt formation was not feasible. However, the manufactured HCl salt showed promising oral absorption and bioavailability. In a rat model, over 100 mg/kg oral dose, the exposure did not increase proportionally with the dose and biovailabilty ranged between 28 % after 100 mg/kg dose and 5.9 % after 300 mg/kg dose. Based on the chemical instability and stoichiometry issue, the di-HCl salt was not suitable for development but showed the potential of focusing on co-crystals as a way to

improve oral bioavailability. The weak base itself showed excellent physico-chemical stability, but very poor oral bioavailability in rat animal model. Based on the very low (below 2%) bioavailability of the base, particle size reduction using micronization and nanomilling was explored as formulation options. Futhermore the use of permeability enhancers as pharmaceutical excipients, in-situ salt formation with wet granulation, and amorphization were explored. One factor to consider in the observed low oral bioavailability is the strong pH dependence of SAR1 aqueous solubility. The equilibrium solubility is 2 mg/ml at pH=1.2 in artificial gastric fluid which decreases to below 0.05 mg/ml at pH=2.0 at 37°C presented on Figure 3. However significant difference was measured at pH=4.5 in 0.5 % SDS containing acetate buffer between the SAR1 co-crystal and base forms. Figure 4 shows the better dissolution kinetic of the co-crystal form. This better dissolution kinetic of the SAR1 co-crystal form as an API was correlated during the development with faster dissolution results on prototype formulation and with the best PK results from the evaluted eight formulatins.

Measured pKa values of SAR1 are: pKa_1=2.9, pKa_2=3.5 as a divalent base. If the pKa values are close to one another the below mentioned equation describes the solubilisation process:

$$\text{Log } C_B^{-2} = \log S_{HBH} + 2 \text{ (pH - pKa)}$$

where:

C is the concentration.

S is the solubility.

A logarithmic solubilisation slope of 2.0 corresponds to a dramatic one-hundred-fold change in solubility with each one unit change in a pH. Due to basic compounds with sharp pH-dependent solubilities, such compounds solubilised in the gastric fluid are very likely to precipitate after the solution empties from the stomach into the small intestine. Based on the very narrow good solubility range of the candidates, the use of surfactants in the formulations was investigated to enhance in vivo dissolution and create the possibility of a relevant in vivo exposure. A further complication in developing suitable formulations for SAR1 was the high aqueous solubility of the fumaric acid used as the co-crystal former. In the case of co-crystals there are only hydrogen bonds between the parent compound and the co-crystal former. If the co-crystal was instilled into a highly aqueous environment during the manufacturing of the

formulation, without any protecting effect, it would likely cause the loss of the integrity (dissociation) between the parent and the co-crystal former. This dissociation could have an impact on the biological advantage of administering a co-crystal.

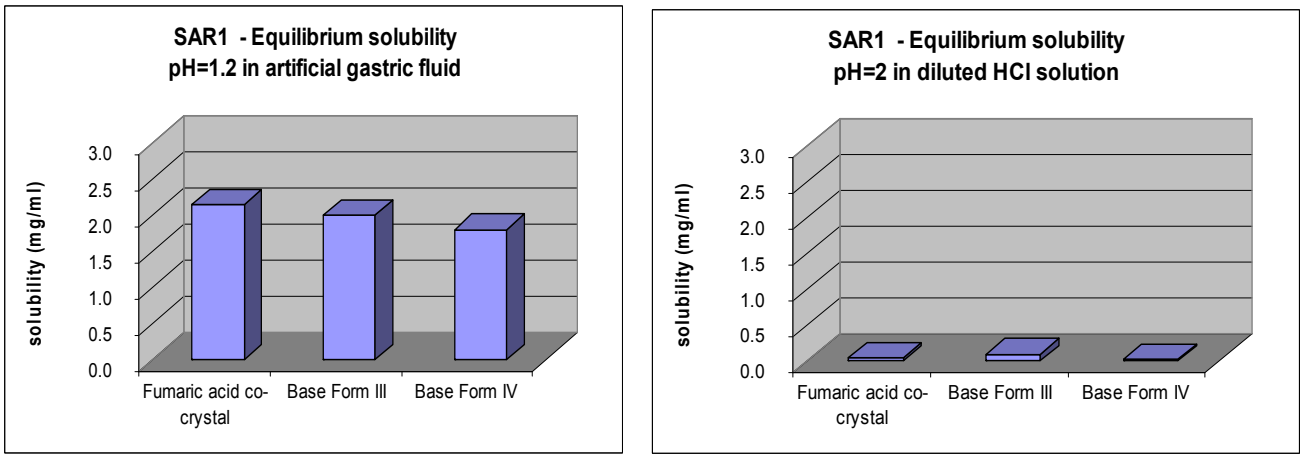

Figure 3: Equilibrium solubility results of SAR1 free base and that of the fumaric acid co-crystal form

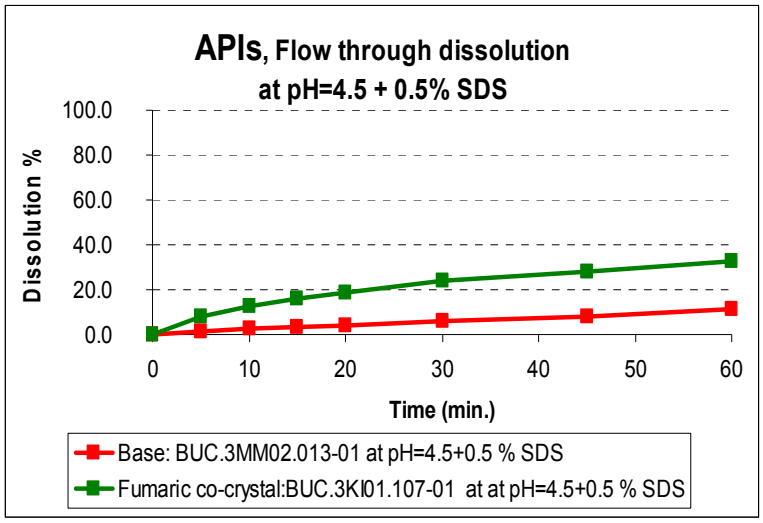

Figure 4: Comparative flow through dissolution results of SAR1 free base and that of the fumaric acid co-crystal form

4.1.2 Pharmacokinetic results

The measured exposure (AUC) results were summarized in Table 1 and its graphic representation can be seen in Fig. 5.

Table 1: Pharmacokinetic parameters

Dose (mg/kg)	base micronised	base nanomilled	base + lactic acid + CD	base + lactic acid + Span 85	base + lactic acid + permeability enhancers + solubilisation	Citric acid containing stock granule	partially amorphous API	co-crystal + permeability enhanser + solubiliser
	AUC (µg.h/ml)							
Code of the formulation	F1	F2	F3	F4	F5	F6	F7	F8
100	14	10	41.6	44.0	81.5	180	344	262
300	30	19	40.2	63.1	104.0	205	212	335

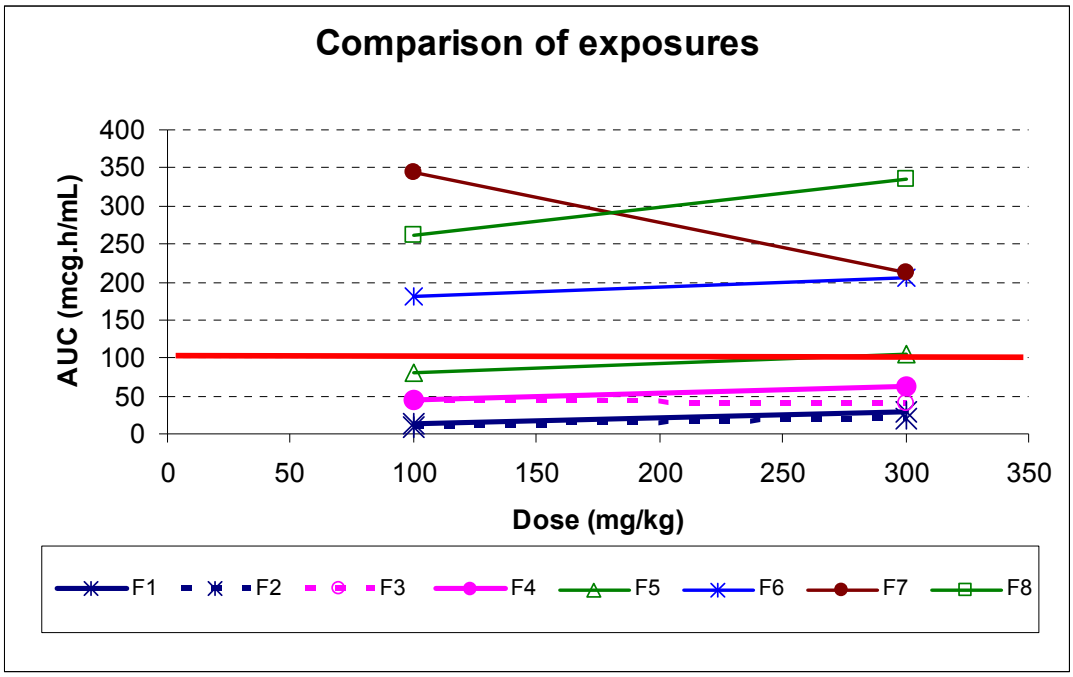

Figure 5: AUC function of dose.

F1: base micronised, F2: base nanonised, F3: base + lactic acid + CD, F4: base + lactic acid + Span 85, F5: base + lactic acid + permeability enhancer + solubilisation, F6: citric acid containing stock granule, F7: partially amorphous API, F8: co-crystal + permeability enhancer + solubiliser + co-crystal protector

4.1.3 Evaluation of PK results

The targeted exposure (100 mcg.h/mL) was reached with three formulations (F6, F7 and F8) after 100 mg/kg and 300 mg/kg single oral dose and also with formulation F5 after 300 mg/kg dose only. With formulation F7 a decrease in exposure was observed between 100 and 300 mg/kg. For the other formulations a lack of dose proportionality was also observed but not a decrease. On average, the best exposure, at both doses, was reached with the fumaric acid co-crystal containing formulation (F8). The measured AUC results were promising with the partially amorphous base containing formulation (F7) as well but because of a scalability issue and the decrease in terms of exposure at higher dosage, the co-crystal containing formulation got the first priority.

4.1.4 Conclusions

Based on the physico-chemical and biopharmaceutical evaluation of the API candidates formulation possibilities for toxicology and for first in man were determined. The fumaric acid co-crystal was selected for development however the strongly pH dependent solubility profile and high water solubility of the co-crystal former caused further issues. Sensitivity of the co-crystal to physical disintegration such as dissociation in solution into base and fumaric acid was solved by the addition of Cremophor ELP to the formulation. The use of 5% Cremophor ELP, included in the formulation as a permeability enhancer, solubiliser and co-crystal protector with its castor oil part provided the best oral exposure in a rat model.

4.2 PHARMACEUTICAL PROCESSABILITY - CO-CRYSTAL INTEGRITY AND PHARMACEUTICAL ROLE OF CREMOPHOR ELP

The target of pharmaceutical development is to administer pharmaceutical co-crystals in formulations, in which the integrity of the co-crystal is ensured as much as possible. Most preferred granulation process from industrial manufacturing point of view is the wet granulation. The aim of this study was to evaluate how the physical integrity of the co-crystal during a high shear wet granulation process is affected. In addition, the influence of Cremophor ELP on physical stability and dissolution was studied. The integrity of the fumaric acid co-crystal of SAR1 active pharmaceutical ingredient was studied after a wet granulation process with four formulations containing the same qualitative and quantitative composition.

Standard pharmaceutical excipients, particularly water and Cremophor ELP were used in different addition order to evaluate the robustness of the manufacturing process.

4.2.1 Conclusions

Keeping the integrity of co-crystals as pharmaceutical ingredients after the manufacturing process is essential to ensure advantages like faster dissolution kinetic and higher bioavailability. As the physical interaction between the active and its co-crystal former, these pharmaceutical co-crystals are sensitive to rapid or slow dissociation in aqueous microenvironment. Based on XRPD results higher integrity of the active as co-crystal was measured when granulation process was performed with the mixture of Cremophor ELP and water. Fast dissolution kinetic were obtained with all formulations containing the co-cystal form. This suggests that Cremophor ELP is a suitable pharmaceutical excipient to increase the physical stability of co-crystals and to ensure a positive effect on bioavailability.

4.3 FLOW THROUGH DISSOLUTION - A USEFUL TOOL FROM DISCOVERY PHASE TO PRECLINICAL DEVELOPMENT

4.3.1 Flow through dissolution on the field of co-crystal development

Target of the study was to elaborate a dissolution method that is able to distinguish between formulations prepared with different particle size distributions of SAR1 fumaric acid (1:1) co-crystal. Physical integrity of SAR1 co-crystal after the micronization study was confirmed by XRPD analysis. The classical paddle type and flow through dissolution techniques were compared on exploratory formulations.

4.3.1.1 Discriminative dissolution method development

To establish a discriminative dissolution method, the formulations were tested firstly in the flow through dissolution equipment to find the best method, and then in the classical paddle type equipment using the chosen medium. Flow through dissolution was performed at three different pHs:

- pH=1.2 artificial gastric fluid without pepsin,
- pH=4.5 acetate buffer plus 0.5 % sodium dodecyl sulfate,

- pH=6.8 phosphate buffer plus 0.5 % sodium dodecyl sulfate to ensure the requirement of the sink conditions.

4.3.1.2 Conclusions

Flow through dissolution was found to be a good tool for screening co-crystal formulations, as the smaller volume of this technique eliminated the potential for dissociation between the API and co-former.

4.4 ELABORATE A PRACTICAL GUIDANCE FOR SCIENTISTS TO FORMULATE CO-CRYSTALS AS ACTIVE PHARMACEUTICAL INGREDIENTS

In presence study Cremophor ELP was ensured the physical integrity of SAR1 co-crystal as an API. Cremophor ELP could be a possible co-crystal protector for other co-crystals too however its effect on co-crystal integrity has to be analysed carefully in collaboration with chemical and analytical experts. Early drug formulation approach will be implemented into the research process to gain robust results during PK/PD investigations. Flow through dissolution equipment has mandatory to evaluate co-crystal containing formulations.

5 SUMMARY

Designing of pharmaceutical co-crystals is feasible among e.g. carboxylic acid, alcohol-amine and alcohol-pyridine moieties of the parent API and co-crystal formers. Co-crystals are sensitive to dissociation in aqueous microenvironment that is why a cooperation is needed among chemists, analysts and formulations experts to protect and monitor the physical integrity of these special APIs. Critical physico-chemical and pharmaceutical parameters of a co-crystal containing formulation development were explored. **Strong collaboration of CMC** (Chemical, Manufacturing and Control) experts ensured for the success of the study. Pharmaceutical co-crystals can provide a solution in case of bioavalailability issue justified on SAR1 co-crystal. The faster solubility and dissolution kinetic of co-crystals is responsible for higher absorption however keeping the integrity of the co-crystal as a pharmaceutical active ingredient is essential to reach the targeted effect and ensure the robustness of the formulation. The physical integrity of SAR1 co-crystal was protected with Cremophor ELP.

Early drug formulation has mandatory from lead selection phase to ensure PK/PD studies with robust formulations. **Pharmaceutical processability** of clinical formulations needs to be evaluated during formulation development. The results of the Early Drug formulation was evaluated from pharmaceutical processability point view on SAR1 co-crystal. The best sequence of Cremophor ELP and the granulation liquid as water was determined from the co-crystal integrity point of view. The best co-crystal integrity was reached, when the granulation process was performed with the mixture of Cremophor ELP and water.

Viewpoints of **Early Drug Formulation** were followed during the preparation of prototype formulations administered to animals. Solutions, suspensions and enabling formulations were manufactured during the Discovery phase.

Flow through dissolution technique is an excellent tool for evaluating several candidates of both Discovery and Preclinical phase in particular when low quantities are available from candidates for pharmaceutical evaluation. This technique is able to support the development of a discriminative dissolution method, even if it is unfeasible with a classical dissolution approach in 500 ml or 1000 ml dissolution medium.

As a future **guidance for scientists** we have to emphasise that pharmaceutical development of co-crystals is feasible with protective excipients such as Cremophor ELP to ensure the physical integrity and the therapeutic effect of these special active pharmaceutical ingredients.

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VI. **Venczel, M**.; Ujhelyi, G.; Sovány, T.; Pintye-Hodi, K. Flow through dissolution - a useful tool from discovery phase to preclinical development, submitted.

Abstract

I. **Venczel, M**.; Ujhelyi, G.; Sovány, T.; Pintye-Hódi, K., Flow through dissolution - a useful tool from discovery phase to preclinical development, Gyógyszerészet LIII:(2009/11 Suppl.I.) p. S49 Paper E-29.

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I. **Venczel, M**.; Ujhelyi, G.; Sovány, T.; Pintye-Hódi, K., Flow through dissolution - a useful tool from discovery phase to preclinical development, Pharmaceutical chemical and technological conference, September 2009

II. **Venczel, M**.; Ujhelyi, G.; Sovány, T.; Pintye-Hódi, K., Flow through dissolution - a useful tool from discovery phase to preclinical development, Congressus Pharmaceuticus Hungaricus XIV. November 2009