Ph.D. Thesis

Pharmaceutical development of co-crystals

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Publications

I. **Venczel, M**.; Szvoboda, I; Podányi, B.; Valente, D.; Menegotto, J.; Pintye-Hódi, K.; Ujhelyi G., Formulation possibilities of a weak base with a narrow solubility range, Cryst. Growth Des., 2012, 12 (3) pp 1101-1110, DOI: 10.1021/cg200462g.

II. Ujhelyi, G.; **Venczel, M**.; Bajdik, J.; Kónya, M.; Vajdai, A., Classical and innovative technologies in pharmaceutical development. Journal of Hungarian Chemists 2010. LXV. 9.

III. Tóth, A.; Csernák, L., Koritsánszky, K.; Salamon, E.; **Venczel, M**.; Végeli, E., Stabilized pharmaceutical compositions and process for the preparation thereof. 1998. WO1998026765.

IV. Jakab, B.; Kálmánné, M.I.; Tóth, A.; Ujhelyi, G.; Vas, J.; **Venczel, M**., Stable pharmaceutical composition comprising pravastatin sodium. 2003. WO/2003/063836.

V. **Venczel, M**.; Budavári, Z; Szabó, A.; Pintye-Hódi, K.; Ujhelyi G., Co-crystal integrity and pharmaceutical role of Cremophor ELP, accepted for publication by International Journal of Research in Pharmaceutical Sciences in 2012.

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Abstract

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Presentations

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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 [1]. Formation of co-crystals can solve several pharmaceutical issues raised during preformulation [2] and formulation development e.g. by solubility, dissolution, bioavailability, chemical stability, decreasing hygroscopicity modulation [3, 84]. Formation of co-crystals could be a new path to improve physico-chemical and biopharmaceutical properties of medicines [5, 6, 7]. One of the most difficult pharmaceutical formulation tasks is to improve the absorption of a weak base with poor and pH-dependent solubility properties [8]; however, some combined chemical and formulation approaches give the possibility to reach this goal [10]. 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) [10, 11],

- to show the benefits of early cooperation among discovery and development scientists in the field of Early Drug Formulation (EDF) [12],

- evaluate the results of preformulation from pharmaceutical processability point of view [13],

- access the usefulness of flow through dissolution technique in the area of Early Drug Formulation and co-crystal development [14],

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

3 LITERATURE SURVAY

3.1 CO-CRYSTALS

Pharmaceutical co-crystals should be attractive to the pharmaceutical industry because they offer multiple opportunities to modify the chemical and/or physical properties of an API without making or breaking covalent bonds [15, 16, 17].

3.1.1 Historical survey

Co-crystals are a long known but little explored alternative to the traditionally known forms of APIs. Higuchi et al. described the formation of molecular complexes of methylated xantines with p-aminobenzoic acid, salicylic acid, acetylsalicylic acid and p-hydroxybenzoic acid [18], [19]. Solubility properties of the new co-crystal forms depends on the solubility of theirs components. The solubility ratio of the co-crystal / drug is approximately 1 if the co-crystal former has ten times higher solubility than the drug itself. However high aqueous solubility of co-crystal forms can lead to rapid convervation and hinter performance [20] that is why one of the main role of formulation experts is to work in a strong collaboration with chemical and analytical experts to protect physical integrity of co-crystals during the formulation work.

3.1.2 Designing of co-crystals

Co-crystals are supramolecular homo- (I and III) or heterosynthons (II and IV) presented on Figure 1. Carboxylic acid moieties represent one of the most commonly studied functional groups in crystal engineering and they exist in 30 of the 100 top-selling prescription drugs in the USA. Carboxylic acids therefore represent an excellent starting point for crystal engineering of pharmaceutical co-crystals. Moreover the alcohol-amine and alcohol-pyridine supramolecular heterosynthons are also well established in crystal engineering [15, 21, 22].

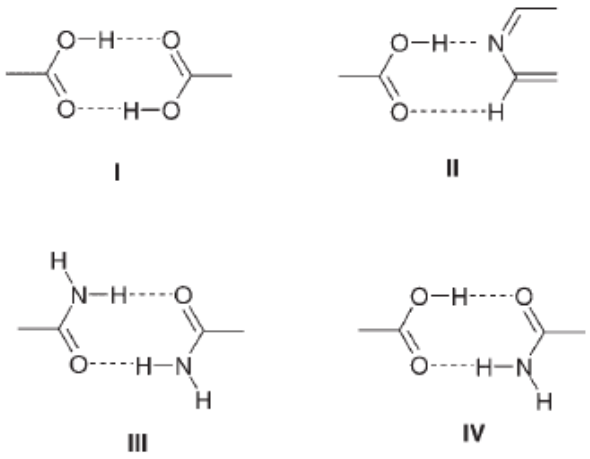


Figure 1: Supramolecular homo- and heterosynthons of co-crystals

3.2 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 [23, 24],
- early assessment of physical and biopharmaceutical properties of APIs that are amenable to downstream development [25, 26],
- support early go/no go decision on discovery candidates [27].

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 [28, 29]. Solubility properties of the possible new APIs [30] determine the type of the elaborated formulations showed on Figure 2. 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 [31, 32]. Since the available API quantities are limited in early discovery phase from a few mgs to grams formulation experts have to explore innovative solutions to supply animal studies with robust and stable formulations. Early classification of new candidates according to the Biopharmaceutics Classification System (BCS) [9] is a useful tool for decision making in early development [33].

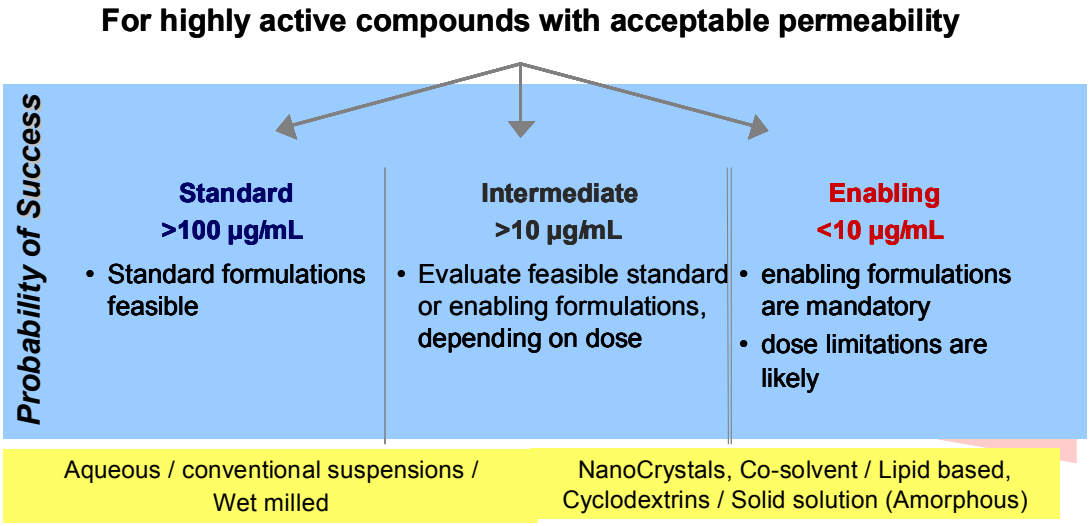


Figure 2: Translation of BCS (Biopharmaceutical Classification System) into internal Formulation strategy

3.3 FLOW THROUGH DISSOLUTION

Flow through dissolution technique is a well known approach from early 1970s elaborated for low solubility BCS II and BCS IV [34, 35] 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 Flow Through Dissolution Equipment (FTDE) is used for research and development studies mainly [36] but pharmacopoeias also make it possible to elaborate a method on FTDE for routine analysis. Preparing FTDE for initiating a study is slightly a longer process than in the case of classical dissolution equipment, but

researchers can reach significant results even if only a few mgs of the new chemical entities are available. 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 [37]. The opened type flow through dissolution technique (Figure 3), 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. It is possible to combine the spectroscopic imaging and flow through dissolution technique to improve the possibilities for investigating the release of poorly soluble APIs from pharmaceutical tablets [38].

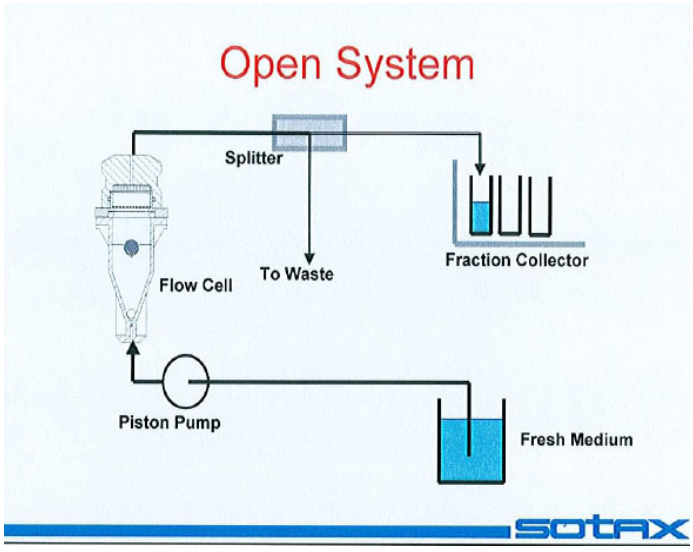


Figure 3 - Opened type flow through dissolution equipment

4 MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 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. All API study batches were manufactured in laboratory scale from 10 g to 30 g. Resynthesis batch of the fumaric acid co-crystal was manufactured in 0.7 kg scale.

4.1.2 Active pharmaceutical ingredient in the area of flow-through dissolution

An origin molecule of Sanofi coded as a „C" model material was used for FaSSIF (fasted state simulated intestinal fluid) and FeSSIF (fed state simulated intestinal fluid) dissolution study. The same „C" model API and its sevaral salt forms were applied to show the benefits of flow through dissolution during salt selection studies.

4.1.3 Buffers

Buffer solutions were prepared according to the USP and Ph. Eur. recommendations [39, 40].

4.1.4 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 [41]. 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.

4.2 METHODS

4.2.1 Chemical manufacturing

4.2.1.1 SAR1 as a fumaric acid co-crystal

The reactor was charged with acetone (12 L), SAR1 base Form III (592 g, 1.29 mol) and fumaric acid (600 g, 5.16 mol). The slurry was stirred at room temperature for 24 hours, the crystals were filtered off, washed with water (1 L) and ethanol (1 L), and dried in a vacuum at 80°C for five hours. Yield: 723 g (94.0%) pale yellow powder [42]. The purity of the product was: 98.9% (HPLC).

4.2.1.2 SAR1 as a dihydrochloride salt

SAR1 (29 g) was added to methanol (1370 ml) under nitrogen. The suspension of API was stirred in Ultra-Turrax system for 30 minutes at room temperature. The concentrated hydrochloric acid (8.03 ml, 2.3 eq) diluted in 25 ml of methanol was added to the mixture in 30 minutes. The slurry was obtained in yellow color. The stirring was maintained overnight at room temperature. The cake was rinsed with methanol after filtration and dried under vacuum at 30°C. Measured molar ratio was 1.95.

4.2.2 Analytical methods

The analysis of samples was performed on Agilent 1100 type HPLC equipment with gradient method to evaluate solubility and chemical stability of APIs and formulations as well. HPLC parameters were: Purospher STAR 5 µm C18, 125 mm x 4.0 mm column. The HPLC analysis was performed at room temperature, with 10 to 50 µl injection volume and with 1.0 ml/min flow rate. The A eluent composition was: Acetonitrile : pH=2.5 buffer solution (100:900). Preparation of the buffer solution: 10 mM NaH_2PO_4 x $2H_2O$, its pH was set to pH=2.5 with H_3PO_4. The B eluent was acetonitrile. The ratio of the A eluent was: 100:100:35:35:100 at 0, 2, 17, 24 and 25 minutes. The samples were analyzed at 225 nm with UV detector. The concentrations of the standard calibration curve were: 6, 10, and 14 µg/ml. Bioassay was performed with a bioanalytical method: exploratory LC-MS/MS method form plasma as a matrix. LOQ was : 1 ng/mL. The stoichiometry of the prepared co-crystal was checked by 1H

NMR spectroscopy. The sample was dissolved in DMSO-D6. The 1H NMR spectrum was recorded at 400.13 MHz on a Bruker DRX-400 spectrometer using 30° pulse length and 10 s relaxation delay. Solid state characterization of the drug substance forms were performed by solid state 15N NMR spectroscopy and XRPD. During the pharmaceutical processability phase the analysis of dissolution samples was performed by an Agilent 8453 type spectrophotometer. Samples were measured at 342 ± 2 nm undiluted (90 and 120 minutes dissolution) or after 200-fold (until 20 minutes dissolution), 40-fold (30 minutes dissolution) or 60-fold (until 60 minutes dissolution) dilution with dissolution medium as acetate buffer.

4.2.3 Pharmaceutical methods

4.2.3.1 Early drug formulation phase

Manufacturing of the exploratory formulations were performed on a laboratory scale using 20 to 100 g batch size. Qualitative and quantitative compositions of the formulations are summarized in Table 1. Eight formulations were prepared and tested in oral animal pharmacokinetic studies [43].

Formulation 1 was a suspension containing micronized SAR1 in methylcellulose and Tween 80 vehicle. Micronized material was manufactured on laboratory scale spiral jet mill [44] and nanosuspension was prepared by Elan type nanotechnology [45, 46, 47, 85] (F1 and F2 formulations).

The API was fully dissolved in lactic acid (0.5 g and 1.5 g SAR1 base was solved in 28 g lactic acid) before preparation of the Miglyol 812 N based w/o emulsions (F3 and F4 formulations) [48]. The lactic acid solution was combined with 5 % sulfobutyl β cyclodextrin [49] 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.

Table 1: Formulation approaches

Name of the formulation	Type of the formulation	Composition of the formulations	Concentration of SAR1	Administration volumes by oral route
WEAK BASE				
F1: Micronized API containing formulation	Microsuspension	**Micr. API : 0.6% MC sol. : Tween 80** 0.5 : 99 : 0.5 % 1.5 : 98 : 0.5 %	5.0 and 15.0 mg/ml	20 ml/kg
F2: Nanomilled API containing formulation	Nanosuspension	**Nan. API : PVP K25 : DOSS : Tween 80 : Water** 0.5 : 3 : 0.15 : 0.4 : 95.95 % 1.5 : 3 : 0.15 : 0.4 : 94.95 %	5.0 and 15.0 mg/ml	20 ml/kg
F3: Lactic acid + Cyclodextrin (CD) containing formulation	w/o emulsion	**API : Lactic acid : CD : Miglyol** 0.5 : 24 : 5 : 70.5 % 1.5 : 28 : 5 : 65.5 %	5.0 and 15.0 mg/ml	20 ml/kg
F4: Lactic acid + Span 85 containing formulation	w/o emulsion	**API : Lactic acid : Span85 : Miglyol** 0.5 : 24 : 5 : 70.5 % 1.5 : 28 : 5 : 65.5 %	5.0 and 15.0 mg/ml	20 ml/kg
F5: Lactic acid + permeability enhancers + solubilisation	suspension	**API : Crem.ELP : Lactic a.: Vitamin E TPGS : NaOH sol. 3M : PEG 200** 1 : 5 : 8.86 : 30 : 6 : 49.14 % 3 : 5 : 8.86 : 30 : 6 : 47.14 %	10.0 and 30.0 mg/ml	10 ml/kg
F6: Citric acid containing stock granule	suspension	**API + Citric acid containing granule: 0.6% MC sol.** 3 : 30.3 : 66.7 % 9 : 30.3 : 60.7 %	10.0 and 30.0 mg/ml	10 ml/kg
F7: Partially amorphous API containing formulation	suspension	**API : Eudragit L100-55 : 0.6% MC sol. : SDS** 1: 1.38 : 95.62 : 2 % 3: 4.14 : 90.86 : 2 %	10.0 and 30.0 mg/ml	10 ml/kg

Table 1 (cont.): Formulation approaches

CO-CRYSTAL WITH FUMARIC ACID				
F8: Permeability enhancer, solubiliser and co-crystal protector containing formulation	suspension	**API : Crem.ELP : 0.6% MC sol.** 1 : 5 : 94 % 3 : 5 : 92 %	10.0 and 30.0 mg/ml	10 ml/kg

The weak base and citric acid containing formulation was prepared with a classical wet granulation process. The excipients of the internal phase were: citric acid, mannitol, microcrystalline cellulose, HPMC and crospovidone. Water was used as a granulation liquid. The components of the external phase were: colloidal anhydrous silica and magnesium stearate. 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 [50]. A drop dispersion was performed with water followed by the centrifugation of the suspension and washing with water. Filtration and drying was done at 100 °C for 4 hours (F7 formulation). 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) [51].

4.2.3.2 Pharmaceutical processability phase

Manufacturing of the different formulations were performed in Mi-pro miniaturized high shear granulator (Pro-C-ept) [52]. The speed of the impeller was 500 rpm while the chopper rpm was 3000. Four experimental compositions were manufactured in 30g miniaturized scale with 10% API load (Table 2). The integrity of the co-crystal was studied from granules. Loss on drying values were measured at 105°C until 20 minutes three times during the manufacturing process: after mixing of the internal phase without Cremophor ELP, after the

wet granulation process and after drying. Comparable loss on drying results were reached for the internal phase and after the drying process.

Tabletting was performed on Korsch excentrical tabletting machine with 3-15 kN pressure force [53, 54]. Flat, rimmed tablets were pressed with 30-35 N hardness. The diameter of the tablets were 6 mm. The temperature of the plant was 21°C and the relative humidity was 23 %.

4.2.4 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.

4.3 TEST METODS

4.3.1 Dissolution study

Experimental dissolution work was carried out in opened, Sotax type flow through dissolution and Hanson type paddle dissolution equipment. The temperature of the media was 37.0 ± 0.5 °C. Dissolution samples were collected by a fraction collector for both dissolution techniques followed by HPLC and spectrophotometric analysis. Samples were collected for up to 60 and 120 minutes.

4.4 STATISTICAL EVALUATION

Statistical evaluation was performed on FaSSIF/FeSSIF dissolution results of “C” model material. Dissolution curves were compared at P=0.95 confidence level.

Table 2: Formulation compositions and function of ingredients

Formulations	Function of ingredients	P1	P2	P3	P4
Internal phase					
SAR1 *fumaric acid co-crystal*	active pharmaceutical ingredient	10 %*	10 %*	10 %*	10 %*
mannitol	diluent	49 %	49 %	49 %	49 %
microcrystalline cellulose	diluent	25 %	25 %	25 %	25 %
Hypromellose	binder	5 %	5 %	5 %	5 %
croscarmellose sodium	disintegrant	4 %	4 %	4 %	4 %
Cremophor ELP	surfactant solubiliser	5 %	5 %	5 %	5 %
granulation liquid	-	water	water + Cremophor ELP	water	water
position of water	-	Added to the internal phase	added to the internal phase	added to the active directly	added to the internal phase
position of Cremophor ELP	-	Last excipient of the internal phase	part of the granulation liquid	last excipient of the internal phase	added to the active directly
External phase					
stearyl fumarate sodium	glidant	2 %	2 %	2 %	2 %
Total	-	100 %	100 %	100 %	100 %
Mass of tablets	-	100 mg	100 mg	100 mg	100 mg

* expressed as free base, fumaric acid parts are corrected from quantity of the diluents

5 RESULTS AND DISCUSSION

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

5.1.1 Physico-chemical and biopharmaceutical properties of the candidates

SAR1 was evaluated as a model compound (Fig 4) planned for use in the oncology area. The measured Caco-2 permeability [55] value of SAR1 was 32 x 10^{-7} cm/s, which indicates a potentially good *in vivo* permeability [56].

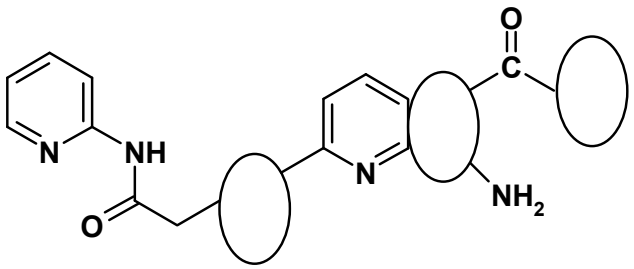


Figure 4: SAR1 as a model active pharmaceutical ingredient (API)

The evaluation of the key and critical physico-chemical and biopharmaceutical properties of this API are summarized in Table 3 [57], together with these data of its di-HCl salt [58, 59] 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 (Fig 5). 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 6. 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 7 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.

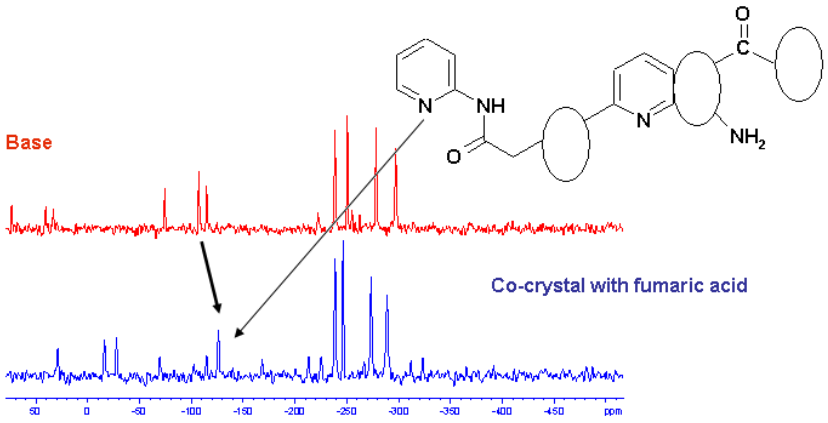


Figure 5: Solid phase NMR spectum of SAR1 base and its co-crystal with fumaric acid

Table 3: Critical physico-chemical and biopharmaceutical properties of SAR1 as a function of crystal form

Parameters	**Issues**	**Proposed solutions**
	Weak Base	
pKa_1=2.9 pKa_2=3.5	Salt formation with strong acids Chemical stability of the formulation cannot be ensured	Focus on co-crystal formation
Bioavailability	Poor, below 2 %	Decrease particle size and evaluate permeability enhancers and amorpization
Equilibrium solubility	Strongly pH dependent	Solubilisation
	„di-HCl" salt of the weak base	
Stoichiometry	Not stoichiometric	Stop the development
Physico-chemical stability	unstable	Stop the development
Bioavailability	Moderate bioavailability 28 % 100 mg/kg 5.9 % 300 mg/kg	Focus on salt like candidates such as co-crystals
	Co-crystal of the weak base	
The character of the drug substance in the drug product	Integrity of the co-crystal during drug product manufacturing	Follow the co-crystal integrity during the manufacturing process Avoid pharmaceutical excipients harmful for co-crystal integrity
Equilibrium solubility	Dependent strongly on pH	Solubilisation

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 [60]. 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 [61]. 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.

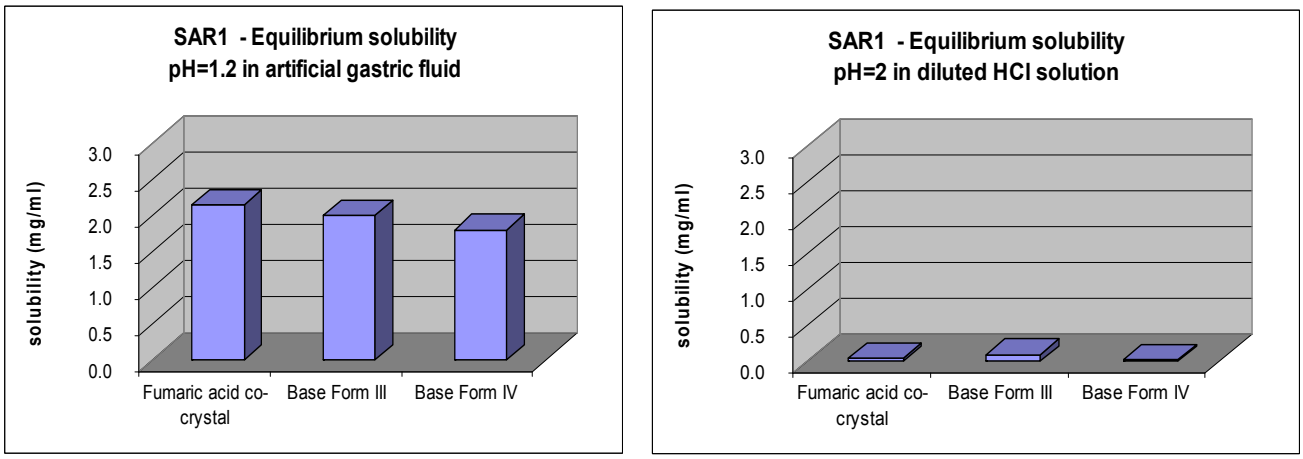


Figure 6: Equilibrium solubility results of SAR1 free base and that of the fumaric acid co-crystal form

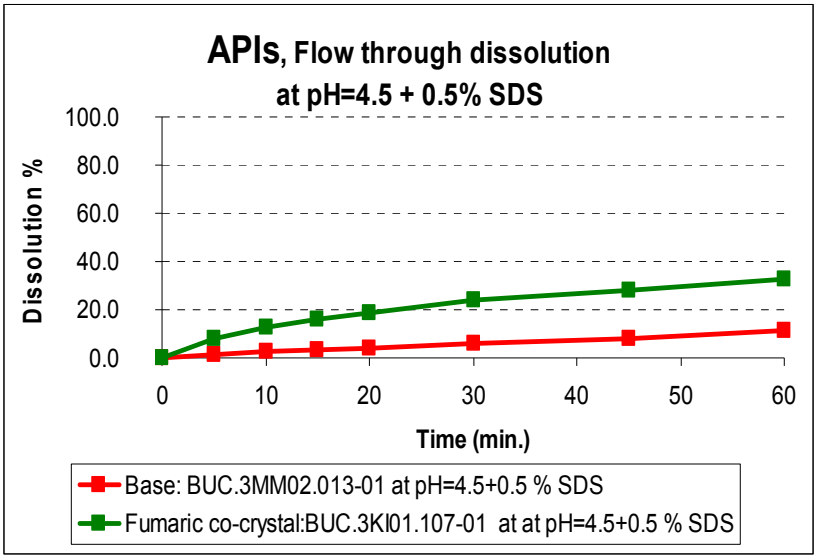


Figure 7: Comparative flow through dissolution results of SAR1 free base and that of the fumaric acid co-crystal form

5.1.2 Selected formulations for pharmacokinetic evaluation on rat animal model

Based on the physico-chemical and biopharmaceutical evaluation of the candidates (the weak base and the co-crystal) (Table 3) eight formulations (Table 1) were prepared for a PK evaluation. The role of each excipients are summarized in Table 4. The administration volumes were decreased from 20 mL to 10 mL in rat animal model to increase the tolerability of the formulations. To compensate for the lower dose volume, the concentrations of SAR1 in formulations F5, F6, F7 and F8 were doubled from 5 and 15 mg/ml to 10 and 30 mg/ml. Chemical stability of the formulations were monitored by HPLC. Formulations were stored at 5°C during the course of the study to ensure chemical stability [62, 63]. The total degradation observed at 5 °C after 2 weeks was below 2%, which is acceptable for a Discovery animal study.

Table 4: Role of the chemical and pharmaceutical excipients

Name of the excipients	**Role of excipients within the formulations**
Chemical excipients	
Fumaric acid [64]	Co-crystal former
Pharmaceutical excipients	
Cremophor ELP [41]	Protect the dissolved API from precipitation, improve permeability and protect the integrity of the co-crystal
Docusate Sodium (DOSS) [64]	Secondary stabilizer of the nanosuspension
Eudragit L100-55 [50]	Support amorphization of the API
Citric acid [64]	Provide acidic microenvironmental pH of the granule
Lactic acid [64]	Solvent of the API
Methyl cellulose (400 mPa.s) [64]	Diluent/suspending agent

Table 4 (cont.): Role of the chemical and pharmaceutical excipients

Name of the excipients	**Role of excipients within the formulations**
Pharmaceutical excipients	
Miglyol [66]	Diluent for emulsion and permeability enhancer
NaOH, 3M solution	pH adjustment to 4.0
PEG 200 [66]	Diluent and permeability enhancer
PVP K25 [65]	Primary stabilizer of the nanosuspension
Span 85 [67]	Surfactant, protect the dissolved API from precipitation, permeability enhancer
Sodium dodecyl sulphate (SDS) [64]	Surfactant, protect the dissolved API from precipitation and improve permeability
Sulfobutyl β cyclodextrin [68, 69]	Improve permeability of the API / solubiliser
Tween 80 [70, 71]	Surfactant, protect the dissolved API from precipitation and improve permeability
Vitamin E TPGS [72]	Protect the solved API from precipitation and improve permeability

5.1.3 Pharmacokinetic results

The measured exposure (AUC) results were summarized in Table 5 and its graphic representation can be seen in Fig. 8.

Table 5: 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

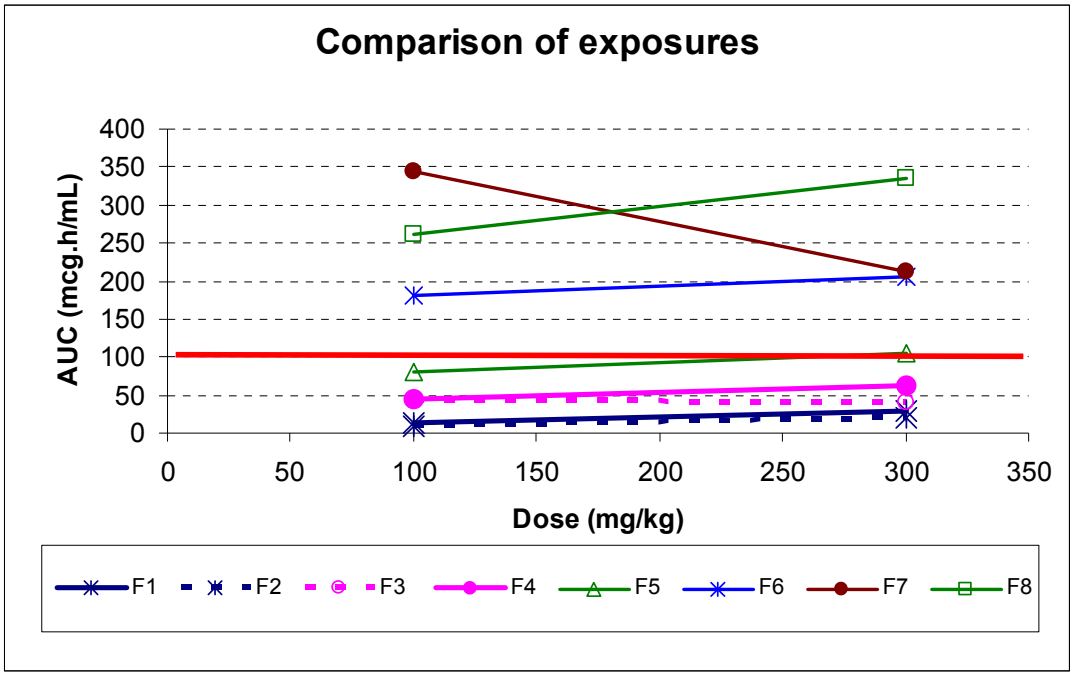


Figure 8: AUC function of dose.

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

5.1.4 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) [3, 73, 74]. The measured AUC results were promising with the partially amorphous base containing formulation (F7) [75] 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. The targeted exposure was reached with the citric acid containing formulation as well (F6) however it was kept as a back-up option based on the complexity of the formulation for toxicological studies. Encouraging absorption was reached with the base containing suspension formulation (F5). A very slight increase in terms of exposure was observed using the emulsion formulation containing sulfobutyl β cyclodextrin complex (F3). Adding a surfactant to the emulsion formulation (F4) rather than cyclodextrin lead in a slight increased in exposure (F3). The decrease of the particle size of the base form to micrometer or a nanometer ranges did not increase the oral exposure (F1 and F2 formulations). The results suggest that rapid precipitation of the free base at intestinal pH negated any improvement in rate of dissolution afforded by the reduction in particle size.

5.1.5 Evaluation of the formulation approaches based on the PK results

5.1.5.1 Micronized and nanomilled API containing formulations:F1 and F2

Decreased particle sizes of the API (weak, crystalline base) below 20 μm (d90) for micronized and 400 nm (d90) for nanoformulations did not reach the targeted oral exposure of 100 mcg.h/mL. Fig. 9 compares the dissolution curves for the micronized weak base and for the nanoformulation at two pH values. According to the dissolution curves, the nanoformulation showed 100 % dissolution at pH=1.2 in artificial gastric fluid within 10 minutes. Contrary to

the nanoformulation, the free base dissolved below 40 % within the same time period. The dissolution was significantly decreased at pH=6.8 in both formulations based on pH dependent solubility of SAR1. The equilibrium solubility of the free base at pH=1.2 in artificial gastric fluid at 37°C is 2.0 mg/ml, which is decreased to 0.05 mg/ml at pH=2 buffer solution. Based on the observed low exposure results in Fig. 8 independently on doses the particle size reduction was not effective. It is expected that irrepective of particle size, such a weak base would precipitate with a change in pH leading to poor exposure that is why differences were not measured for micronized and nanonised formulations.

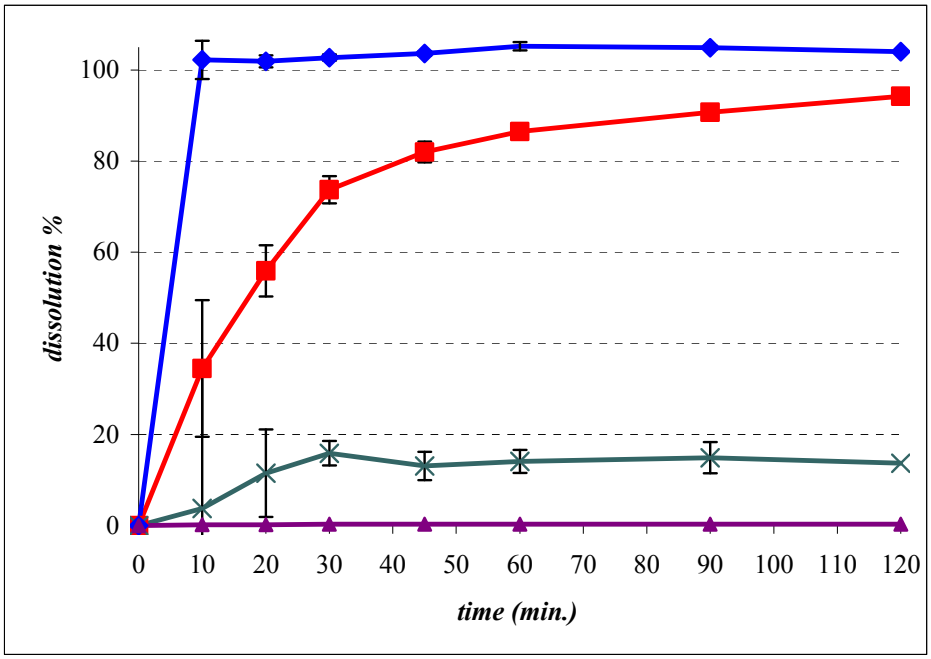


Figure 9: Comparative dissolution data of base and nanoformulation.

Micronized base: pH 1.2, Micronized base: pH 6.8, Nanoformulation: pH 1.2
Nanoformulation: pH 6.8

5.1.5.2 Evaluation of the w/o emulsion formulations:F3 and F4

F3 and F4 formulations were prepared as oil based emulsions because of the high hydrophobicity of SAR1 and preparation of an aqueous solution was not feasible even with surfactant. Dilution of the dissolved SAR1 in lactic acid was feasible with an excipient with high apolarity such as Miglyol [76]. An increase in exposure was measured in contrary to the

micronized and nanonized formulations but the exposure results did not fulfill the targeted AUC value (100 mcg.h/ml). Increased exposure with F3 and F4 formulations compared to F1 and F2 formulations is purely a solubility enhancement effect due to addition of cyclodextrine as well alteration of microenvironmental pH due to lactic acid. Better exposure of F4 formulation is related to Span 85, which is prevented the dissolved API from precipitating. The observed very slow absorption is related to the oily character of the formulation, the presence of SAR1 in the samples of plasma was analysed at 48 hours also after the oral administration.

5.1.5.3 Comparison of the emulsion and suspension formulations: F4 and F5

Interestingly, around a 2-fold higher exposure was reached with the suspension formulation containing permeability enhancers and solubiliser (F5) over the emulsion formulation where the base was dissolved totally in lactic acid (F4). The explanation could be the very narrow good solubility range (2.0 mg/ml) of the API. If the API arrives as an emulsion formulation into stomach there is a high risk for quick precipitation. Solubilisation with Span 85 in an oil based emulsion probably was not enough to prevent the base from precipitating at higher pHs than 1.2 under *in vivo* conditions. The expectation was confirmed by *in vitro* precipitation study. F4 formulation was diluted at 37°C with pH=4.5 acetate and pH=6.8 phosphate buffers in 1:2 (formulation : buffer) ratio. A milky type precipitation was observed within 2 minutes [77]. F5 formulation is complex and number of effects might be occuring such as solubility enhancement with vitamin E TPGS and PEG 200 and microenvironmental pH changes with lactic acid.

5.1.5.4 Evaluation of the citric acid containing formulation: F6

The main objective of preparing the base and citric acid containing formulation was to prepare in-situ salt or co-crystal during the wet granulation process. Based on the PK curves of Fig. 8 to reach the targeted exposure was feasible however XRPD shows no evidence of salt or co-crystal formation. Based on the complexity of the citric acid containing formulation a separate study was decided to explore the scientific background of the good *in vivo* performance.

5.1.5.5 Partially amorphous API containing formulation: F7

The exposure with F7 formulation presented in Fig. 8 is definitely due to an increase in apparent solubility due to amorphous nature of the formulation [78, 79]. Manufacturing of the amorphous SAR1 formulation was designed according to the physico-chemical properties of the API. SAR1 showed good solubility in N-methylpyrrolidone, which was used to dissolve SAR1 during the preparation of this formulation. The free base has a very high melting temperature (ca. 300°C), which means a high cohesive self-assembly, and it requires an excipient for the physical stabilization of the amorphous phase. As the free base is able to form co-crystals with acidic co-formers, an acidic polymer (Eudragit) was selected as a stabilizer. To avoid the precipatation of the API upon delivery, a gastro resistant system was selected. Based on the characterization of the partially amorphous formulation, some traces of API crystals were measured by X-ray powder diffraction [80] (Fig. 10). Further crystallization was not detected upon storage. The increase in exposure using this formulation is likely due to an increase in the kinetic solubility of the API an amorphous material.

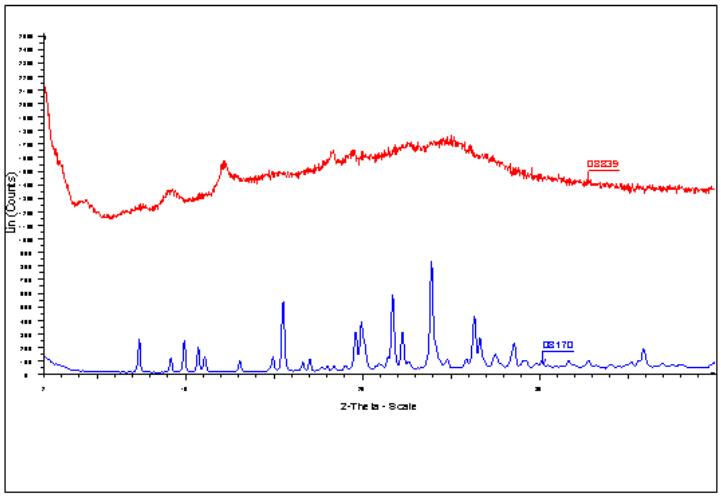


Figure 10: XRPD patterns of the crystalline free base (sample number: 08170) and its amorphous formulation with Eudragit L100 55 (sample number: 08839)

5.1.5.6 Comparison of the base and co-crystal containing formulations:F8

The XRD powder diffraction study of the solid obtained in the reaction of SAR1 and fumaric acid indicated that this is a crystalline compound. The ^{1}H NMR spectrum of the solid dissolved in DMSO-D_6 proved that it contains SAR1 and fumaric acid in 1:1 molar ratio. An extended part of the spectrum can be seen in Fig. 11 (signal of 1 hydrogen atom of SAR1 can be seen on the left while signal of 2 hydrogen atoms of fumaric acid can be seen on the right in the spectrum). The comparison of its solid state ^{15}N NMR spectrum with those of SAR1 base proved that this is a co-crystal of SAR1 and fumaric acid (Fig. 5) [81, 82].

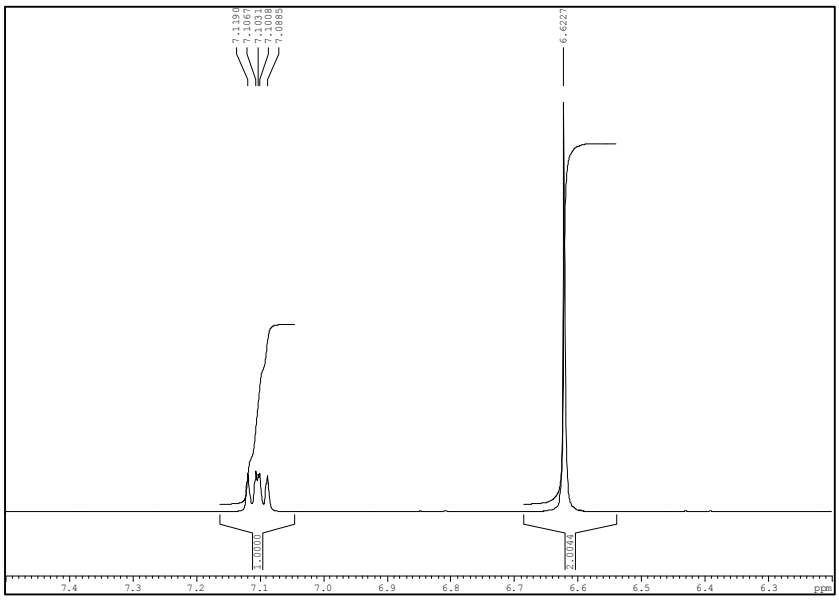


Figure 11: 1H NMR spectrum of fumaric acid co-crystal of SAR1 base

The particle size distribution of the API in a co-crystal form was 0.96 µm at d(90), 2.66 at d(50) and 10.90 at d(90) measured by lased diffraction [83].

For better understanding of why the co-crystal containing formulation achieved the best oral exposure results among all of the formulations, a complementary *in vitro* kinetic solubility study was initiated at 37 °C in artificial gastric fluid without pepsin (pH=1.2) and in USP buffer (pH=6.8) solution. The study design is summarized in Table 6. The kinetic solubility study was initiated with the free base, the fumaric acid co-crystal and with formulations

containing both. The same pharmaceutical composition was applied as in formulation (F8) which provided the best PK results. The candidates were mixed together with Cremophor ELP, included as a surfactant, permeability enhancer and co-crystal protector. The mixture was then diluted with the 0.6% methyl cellulose solution. There is no significant difference between the kinetic solubility results at pH=1.2 (Fig. 12) of the free base and formulated base. On the contrary, differences in dissolution behavior were identified for the co-crystal. The co-crystal itself and the co-crystal containing formulation reached higher, 2.5 mg/mL concentration compared to the free base containing suspensions (1.3 – 1.5 mg/mL). This higher concentration decreased significantly for the co-crystal formulated as a simple suspension within 1.5 units. However, the high concentration of the co-crystal was maintained for the co-crystal formulated with Cremophor ELP. Cremophor ELP is a polyoxyethylene castor oil from chemical point of view, which has hydrophil and lipophil parts as well. The integrity of the co-crystal was protected against aqueous microenvironment and dissociation with the lipophil, castor oil part of Cremophor ELP. These results, the sustained higher concentration of the co-crystal, plus the pharmaceutical composition which stabilizes the co-crystal from precipitation, are responsible together for the better *in vivo* performance of the co-crystal containing formulation. As it is shown in Fig. 13 no significant differences were observed between the solubility curves of the base and co-crystal containing suspensions and pharmaceutical compositions at pH=6.8. According to the good exposure results with a co-crystal containing formulation the extended good solubility at pH=1.2 is enough to provide the targeted exposure (Fig. 8).

Table 6: In vitro solubility studies at 37°C in artificial gastric fluid (pH=1.2) and at pH=6.8 in phosphate buffer solution

APIs	**APIs Alone**	**5% Cremophor ELP containing classical suspension formulation with 0.6% Methyl cellulose**
SAR1 (base)	X	X
SAR1B (fumaric acid co-crystal)	X	X

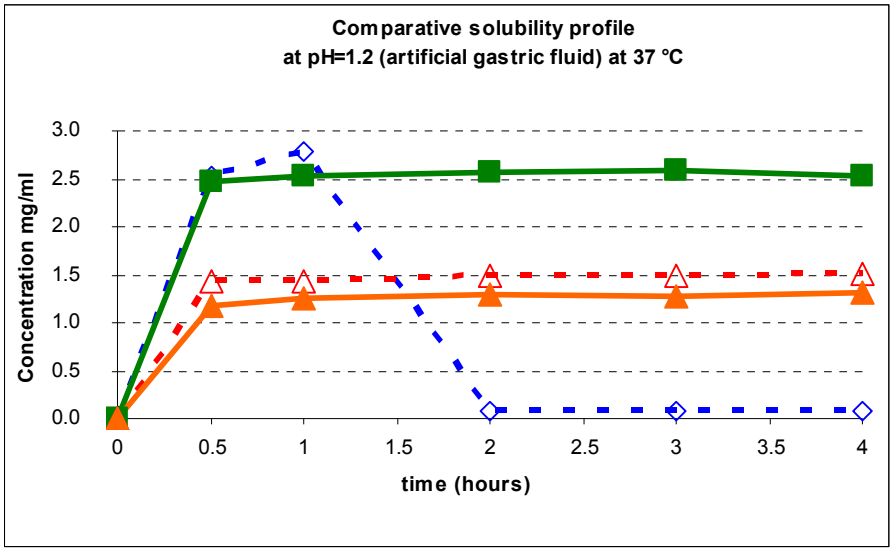


Figure 12 - Concentration time profile at pH=1.2 for the SAR1 base and co-crystal as an APIs and in formulations at 37 °C.

SAR109511B: co-crystal as an API, SAR109511B: co-crystal in methyl cellulose + Cremophor ELP suspension formulation, SAR109511: base as an API, SAR109511: base in a formulationin methyl cellulose + Cremophor ELP suspension formulation

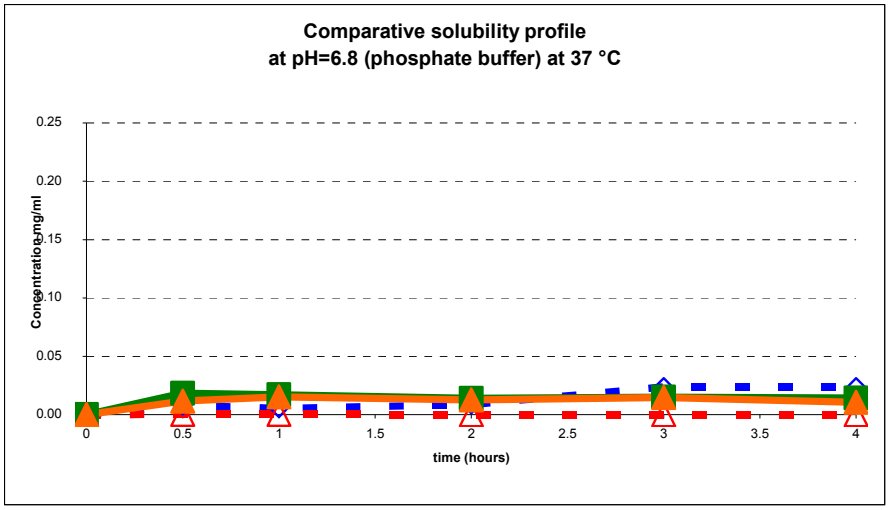


Figure 13 - Concentration time profile at pH=6.8 for the base and co-crystal as an APIs and in formulations at 37 °C.

SAR109511B: co-crystal as an API, SAR109511B: co-crystal in methyl cellulose + Cremophor ELP suspension formulation, SAR109511: base as an API, SAR109511: base in a formulationin methyl cellulose + Cremophor ELP suspension formulation

5.1.6 Conclusions

Based on the physico-chemical and biopharmaceutical evaluation of the API candidates formulation possibilities for toxicology and for first in man were determined. An increase of the very low bioavailability of the weak base was feasible with permeability enhancers, surfactants, acidic excipients, amorphization, and with fumaric acid co-crystal formation. The fumaric acid co-crystal was selected for development [84] 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. Cremophor EL is a well known pharmaceutical excipient for oral and intraveneous formulations. Serious side effects reported with Cremophor EL intravenous formulations [86,

87] were not observed when Cremophor ELP was administered by the oral route in the rat models at 5% concentration. Similar good toxicological results were presented by BASF, the manufacturer of Cremophor EL in 2008 [41]. The integrity of the co-crystal within the formulation is essential to reach better bioavailability via faster dissolution kinetics. Bioavailability increase of a poorly soluble weak base was feasible based on the collaborative work among chemists, analysts and formulation experts.

5.2 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. Cremophor ELP is commonly used as solubiliser and is known to ensure the integrity of the co-crystals [10]. Cremophor ELP has been demonstrated to be a well tolerated pharmaceutical excipient via oral route [86, 87]. SAR1 fumaric acid co-crystal was used as model active pharmaceutical ingredient in the present study. Increased bioavailability of fumaric acid co-crystal versus the free base was confirmed in a pharmacokinetic study [10]. The integrity of the fumaric acid co-crystal of SAR1 active pharmaceutical ingredient was studied after a wet granulation process [88, 90] 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. The composition and function of each formulation are summarized in Table 2.

5.2.1 P1 formulation

API and the excipients of the internal phase were sieved through 0.63 mm sieve size. Cremophor ELP was added to the internal phase as last excipient and granulation was performed with water. Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sive size and finally stearyl fumarate sodium of the external phase was added to the granules.

5.2.2 P2 formulation

The active and the excipients of the internal phase were sieved on 0.63 mm sieve size. Cremophor ELP was added to the granulation liquid. Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sieve size and finally the excipient of the external phase was added to the granules.

5.2.3 P3 formulation

Water was added directly to the active followed by the excipients of the internal phase. Cremophor ELP was added to the internal phase as the last excipient. Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sieve size and finally the excipient of the external phase was added to the granules.

5.2.4 P4 formulation

Cremophor ELP was added directly to the active followed by the excipients of the internal phase. Granulation was performed with water. Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sive size and finally the excipient of the external phase was added to the granules.

5.2.5 Reference formulation

A suspension formulation was prepared as reference to the solid experiments. For the 10 mg/ml concentrated suspension formulation API was manually suspended in a mortar in methyl cellulose water solution.

5.2.6 Evaluation of XRPD and dissolution results

In a previous work, it was shown that Cremophor ELP can have protective effects against rapid dissociation of fumaric acid co-crystal of SAR1 as active pharmaceutical ingredient [10]. Cremophor ELP was included in the formulations at three different positions. It was the last excipient of the internal phase in two cases (P1 and P3), one time it was added directly to the API (P4) and one time it was the part of the granulation liquid (P2). Different addition orders of water within the formulations were investigated as well. In order to evaluate the effect on the integrity of the co-crystals, water, as standard granulation liquid, was added to the internal phase in three cases (P1, P2, P4) and in one case it was added directly to the active (P3). The crystallinity of API in the granules was examined by XRPD (Figure 14). The appearance of a peak at ~12.0° 2Θ not related to any starting phase was observed with different intensities in the granules. This new peak corresponds to a disproportionated free base observed in the reference formulation (10 mg/ml SAR1 suspension). Based on the results of our studies the fumaric acid to API ratio was shown to decrease in parallel with the intensity increase of peak 12.0° 2Θ in the XRPD pattern of centrifuged suspension samples. It suggests that in the granulated samples a minor part of the API disproportionates to base and fumaric acid. The appearance of the disproportionated phase in the granules are represented by the intensity % of peak 12.0° 2Θ compared to peak 11.6° 2Θ (Table 7). The most intense change was observed in the P3 sample, where the API was mixed with water in a mortar before granulation, which is similar to the preparation of the suspension. The highest level of co-crystal integrity was measured for P2 and P4 formulations where the SAR1 was granulated with the mixture of water and Cremophor ELP (P2) and when Cremophor ELP was added directly to SAR1 (P4).

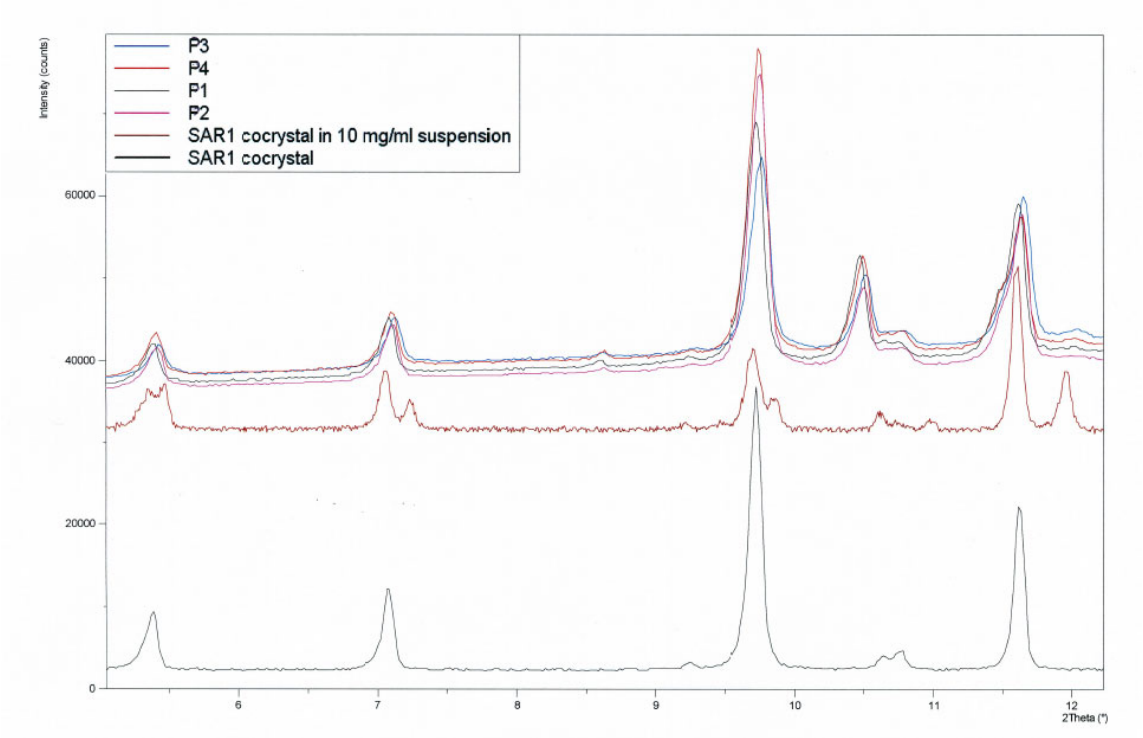


Figure 14: XRPD patterns of the test formulations P1 – P4 compared to SAR1 co-crystal and SAR1 co-crystal reference suspension formulation

Table 7: Intensity % of peak 12.0° 2Θ compared to peak 11.6° 2 Θ (100%)

Samples	Intensity %
P1	4.0
P2	2.9
P3	6.7
P4	4.3
SAR1 co-crystal	0.0

When the dissolution kinetics were measured, about 10% dissolution decrease were observed with P2 compared to the P1 formulation (Table 8 and Fig. 15). The difference in dissolution among the P2, P4 and P1, P3 formulations is significant at P=0.95 confidence level. A slight

decrease in dissolution could have a negative impact on bioavailability that is why it is proposed to increase the content of the disintegrant within the formulation when Cremophor ELP is used.

Table 8: XRPD and dissolution results of the four test formulations

Formulations	P1	P2	P3	P4
Integrity of SAR1 *fumaric acid co-crystal* by XRPD method	P1 and P4 same level of integrity	highest level of integrity	lowest level of integrity	P1 and P4 same level of integrity
Dissociation of SAR1 *fumaric acid co-crystal* by XRPD method	signs of the dissociated co-crystal	signs of the dissociated co-crystal	highest level of dissociation	signs of the dissociated co-crystal
Dissolution profiles	reference profile	≈ 10 % decrease	comparable with F1	≈ 10 % decrease

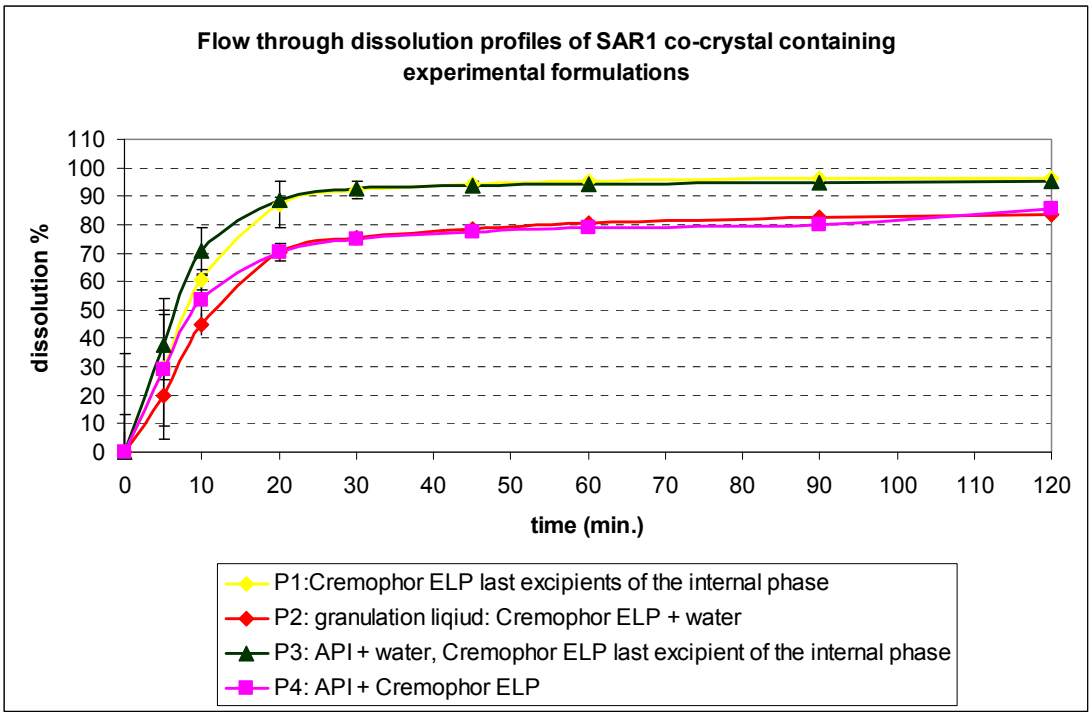


Figure 15: Flow through dissolution profiles of SAR1 co-crystal formulations P1 – P4

5.2.7 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 [89]. 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. Four experimental formulations were manufactured to study the influence of water and Cremophor ELP order of addition in the formulation process. 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. Dissolution profiles of Cremophor ELP containing formulations needs to be monitored regularly as Cremophor ELP has an effect both on co-crystal integrity and on dissolution kinetics. However from biological effect point of view, ensuring co-crystal integrity is more important than a slightly lower dissolution profile [90].

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

5.3.1 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 [91, 92]. Physical integrity of SAR1 co-crystal after the micronization study was confirmed by XRPD analysis [93]. The classical paddle type and flow through dissolution techniques were compared on exploratory formulations. The compositions are summarized in Table 9.

Table 9 - Compositions of the co-crystal containing formulations

Batch numbers		P-0230209	P-0250509
API and excipients	Function of the excipients	Wet granulation process / mini formulation	
		%	
SAR1 fumaric acid co-crystal, not micronized	API	6.3 d(90)=15.7 µm	-
SAR1 fumaric acid co-crystal, micronized		-	6.3 d(90)=3.9 µm
Mannitol	Diluent	53.3	53.3
Microcrystalline cellulose PH 101		22.9	22.9
HPMC	Binder	5.0	5.0
Crospovidone Type A	Disintegrant	5.0	5.0
Cremophor ELP	Surfactant and permeability enhancer	5.0	5.0
Mg stearate	Lubricant	2.0	2.0
Colloidal silica anhydrous	Glidant	0.5	0.5
200 mg granule contains 10 mg API (expressed in base)		100 %	100 %

5.3.1.1 Discriminative dissolution method development

To establish a discriminative dissolution method, the formulations outlined in Table 9 were tested firstly in the flow through dissolution equipment [14] to find the best method, and then in the classical paddle type equipment using the chosen medium [94]. 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.

Flow through dissolution was conducted with a 4.0 mL/min flow rate in the powder cell, which has five mL volume. The cumulative flow through dissolution curves are summarized in the Fig. 16. Significant differences between micronized and not micronized SAR1 fumaric acid co-crystal containing formulations were not measured at pH=1.2 and 6.8 with 0.5 % sodium dodecyl sulfate however at pH=4.5 with 0.5 % sodium dodecyl sulfate the difference was significant for the formulations. Based on the flow through dissolution results, acetate buffer at pH=4.5 with 0.5% sodium dodecyl sulfate was selected as a potential discriminative

dissolution method to evaluate the prototype formulations in the paddle USP2 dissolution equipment. The USP2 dissolution measurement was performed with 250 and 500 ml volumes at 50 rpm. These volumes were selected to avoid the co-crystal from dissociation. Since the fumaric acid part of the co-crystal has a high solubility in water there is a potential risk for the co-crystal to be physically unstable and precipitate as the free base before complete dissolution. This is a potential risk under in vivo conditions as well that is why protection of the co-crystal form is important within the formulations. Dissolution experiments validated the concept and classical dissolution curves did not show any differences at pH=4.5 between micronized and unmicronized containing formulations. The classical dissolution curves are shown in Fig. 17. No significant differences between dissolution curves were observed in 250 and 500 mL. Disintegration of the co-crystal to base + fumaric acid is quick in large aqueous volumes. Significance of the protection of co-crystals from dissociation were published by other scientists also based on in vitro dissolution results [89]. Flow through dissolution technique is mandatory to support formulation development based on the in vitro results.

5.3.1.2 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. Interesting similar results were published by other authors for nanoparticles containing formulations [95]. The data showed the flow-through cell to be unequivocally the most robust dissolution method for the nanoparticulate system. Furthermore, the dissolution profiles conform closely to the classic Noyes–Whitney model, indicating that the increase in dissolution rate as particles become smaller results from the increase in surface area and solubility of the nanoparticles.

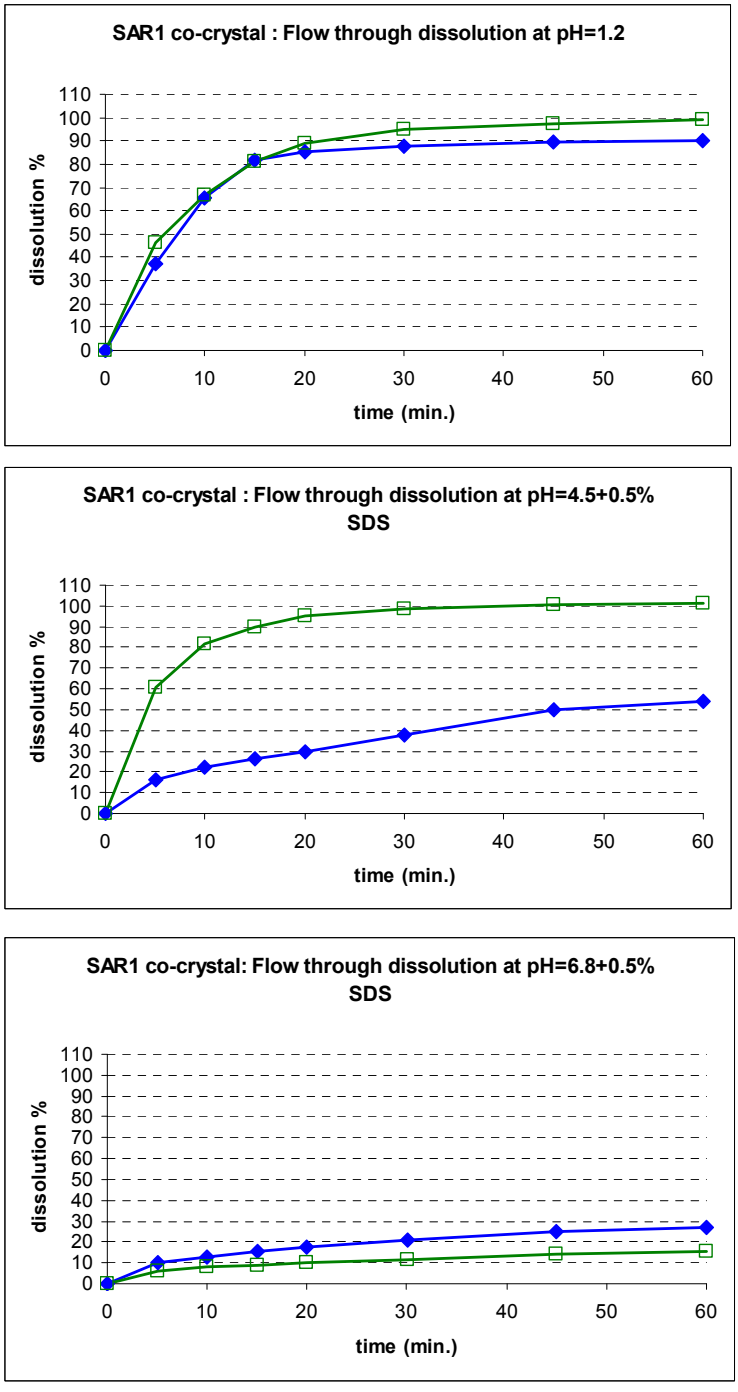
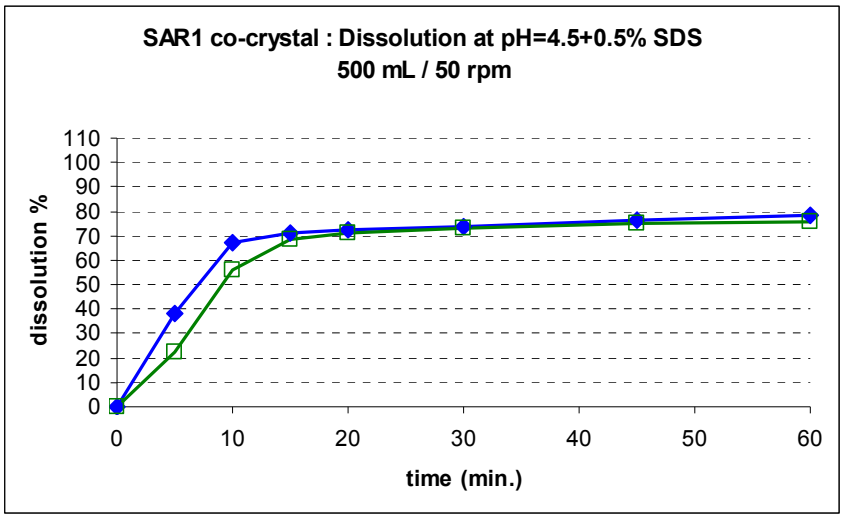


Figure 16: Flow through dissolution curves of co-crystal containing prototype formulations



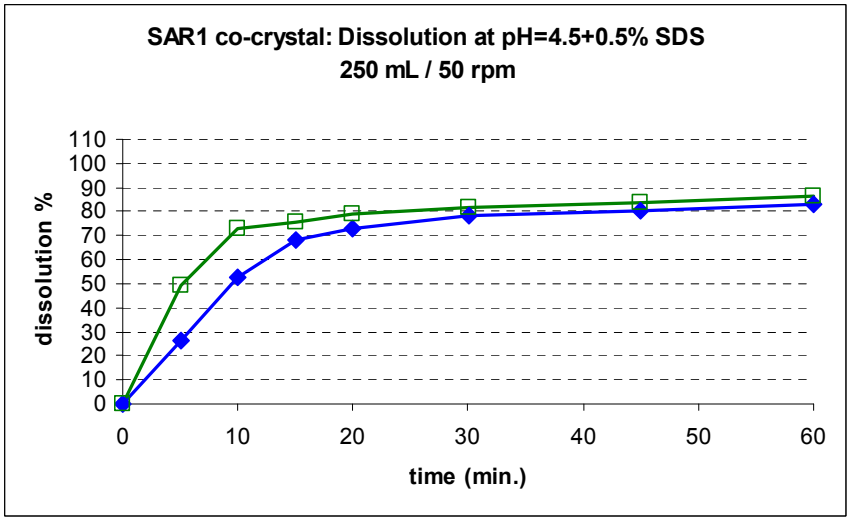


Figure 17: Classical dissolution curves of prototype formulations

5.3.2 Flow through dissolution on the area of FaSSIF/FeSSIF dissolution

The food effect prediction for „C" model as a fumarate salt was performed on the opened type flow through dissolution equipment, which most closely models in vivo conditions. The most frequently used media are the fasted and fed simulated small intestinal fluids (FaSSIF and FeSSIF) developed by Galia et al [96], [97, 98]. Since the studied salt met fresh dissolution media during the dissolution study the results are closer to the dynamic system of the in vivo

conditions, than to the static-type classical dissolution techniques. The dissolution results, the calculated reaction speed constants and the evaluation of the kinetic of the dissolution process are summarized in Table 10 while the dissolution curves are presented in Figures 18.

5.3.2.1 Conclusions

1.7 times higher absorption (average of FeSSIF/FaSSIF ratio) is expected based on the in vitro flow through dissolution results after high fat containing breakfast, which means a slight risk for food effect. FeSSIF:FaSSIF ratio measurement is a standard measurement during preformulation studies, but if the ratio is based on equilibrium solubility or on classical dissolution measurements there is high risk for much higher differences during clinical studies, because classical approaches do not calculate with the dynamic circumstances of the human body. Based on the above mentioned facts FTDE is proposed to measure the FeSSIF:FaSSIF ratio.

Table 10: FaSSIF, FeSSIF Flow through dissolution results of „C" model material

Dissolution medium FaSSIF, C_0=1 mg						
Time (min.)	Dissolved %	Dissolved mg	Remaining mg (C)	Reaction speed constants		Evaluation of the kinetic
				Zero order $k=\frac{C_0 - C}{t}$	First order $k=\frac{2.303}{t}\log\frac{Co}{C}$	
5	0.02	0.0002	0.9998	0.0040	0.0000	Lag Time
10	3.64	0.0364	0.9636	0.3640	0.0037	
30	35.32	0.35323	0.6468	1.1774	0.0145	First order
45	48.48	0.48477	0.5152	1.0773	0.0147	
60	57.74	0.57743	0.4226	0.9624	0.0144	
90	65.40	0.65397	0.3460	0.7266	0.0118	
120	72.98	0.72977	0.2702	0.6081	0.0109	

Table 10 (cont.): FaSSIF, FeSSIF Flow through dissolution results of „C" model material

Dissolution medium FeSSIF, C_0=1 mg						
5	0.04	0.0004	0.9996	0.0080	0.0001	Lag Time
10	9.07	0.0907	0.9093	0.9073	0.0095	
30	63.04	0.6304	0.3696	2.1012	0.0332	First Order
45	76.54	0.7654	0.2346	1.7010	0.0322	
60	87.33	0.8733	0.1267	1.4555	0.0344	
90	93.95	0.9395	0.0605	1.0439	0.0312	
120	100.51	1.0051	-0.0051	0.8376	-	

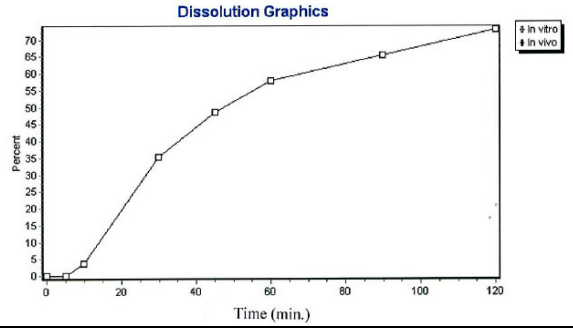
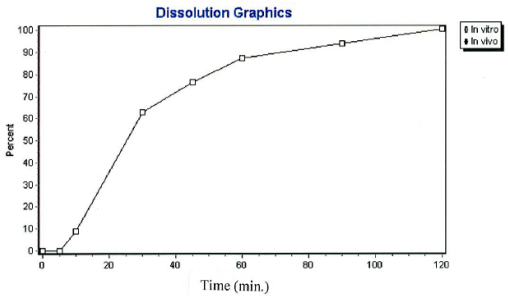
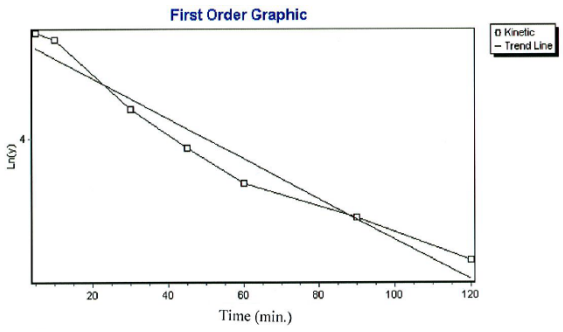
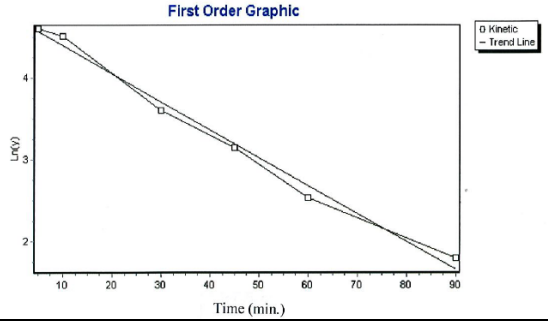
FaSSIF Flow through dissolution curves of „C" model material	FeSSIF Flow through dissolution curves of „C" model material
	
	

Figure 18: FaSSIF, FeSSIF dissolution curves and first order graphic of "C" model material

5.3.3 Flow through dissolution on the field of salt selection studies

The flow through dissolution study was performed on two salts (fumarate and di-sulfate) and on the base form of the „C" model. The results are presented in Figure 19. The dissolution study was prepared at pH=1.2, 3.0, and 7.4 furthermore at pH=7.4 with 0.5 % Tween 80. Based on the flow through dissolution curves it can be seen that the dissolution behavior of the tested two salt forms and the base form is similar (they have decreasing solubility from pH=1.2 to 7.2), however fumarate salt has the best dissolution rate at pH=7.4 when Tween 80 was measured into the dissolution medium. This fact was used during the formulation development of the fumarate salt of the „C" model.

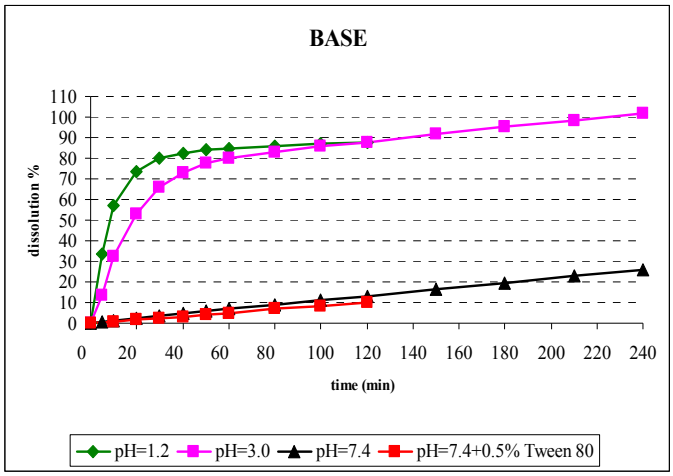
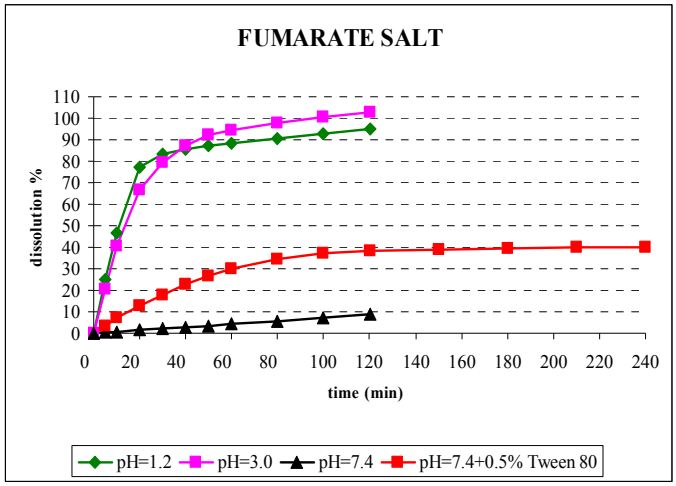


Figure 19: Comparative flow through dissolution curves of „C" model material



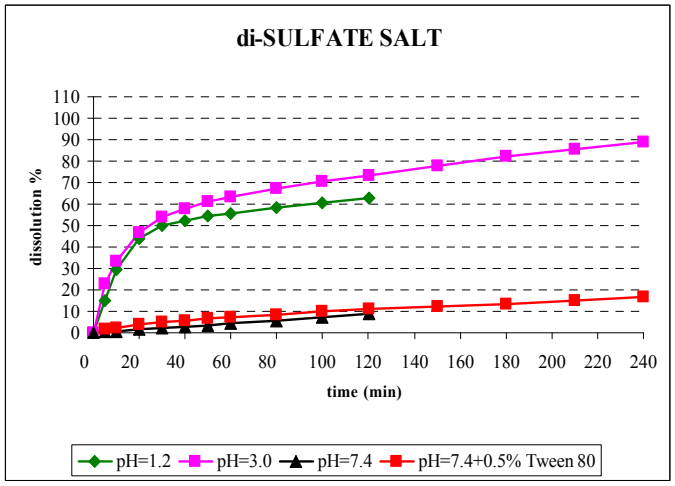


Figure 19 (cont.): Comparative flow through dissolution curves of „C" model material

5.4 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.

6 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) [99] 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. Small decrease in dissolution results is expected coming from the effect of the oily part of Cremophor ELP [49]. Double effect of Cremophor ELP on co-crystal integrity and small decrease in dissolution is not separatable.

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 [100] 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. The opened-type FTDE represents the dynamic system of the human body in a better way than the classical paddle or basket methods that is why FTDE has a definitely higher role during the Discovery and Preclinical studies, in particular for BCS II and IV type candidates. Based on the results it can be stated that flow through dissolution techniques has mandatory to study the dissolution properties of co-crystal API containing formulations.

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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ANNEX

Related articles

I.

CRYSTAL GROWTH & DESIGN

Article

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Formulation Possibilities of a Weak Base with a Narrow Solubility Range

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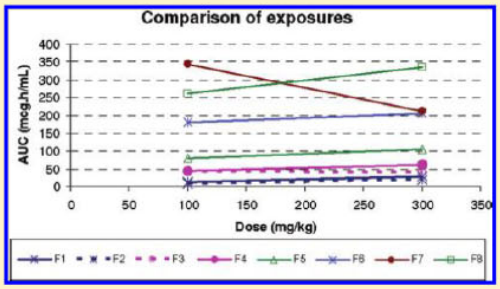
ABSTRACT: The target of this article is to summarize the chemical and pharmaceutical (formulation) approaches that were found to be suitable for increasing the bioavailability of a model weak base (SAR1) with a very narrow good solubility range at physiologically relevant pH.[1] As part of the preformulation and formulation development to support toxicological and first in man studies of a free base (SAR1), several formulation approaches, including particle size reduction, emulsions, permeability enhancers, amorphous dispersions, salt formation, and co-crystal formation, were screened by in vitro dissolution methods and in vivo pharmacokinetic (PK) studies to evaluate and rank formulation performance. From the PK studies, it was observed that a suspension formulation containing a SAR1 fumaric acid (1:1) co-crystal provided the best oral exposure. Sensitivity of the co-crystal to physical disintegration into base and fumaric acid was solved by including Cremophor ELP as a solubility enhancer, surfactant, and co-crystal protector. This formulation was well-tolerated in rat. A flow-through dissolution method was more discriminating than the paddle type dissolution equipment for evaluation of co-crystal containing solid formulations.



1. INTRODUCTION

1.1. General Introduction. 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.[2] 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 profiles, synergies of different chemical and pharmaceutical tools are needed. The aim of this article is to explore and apply the synergies among chemical and pharmaceutical tools.

1.2. Physicochemical and Biopharmaceutical Properties of the Candidates. SAR1 was evaluated as a model compound (Figure 1) planned for use in the oncology area. The measured Caco-2 permeability value of SAR1 was 32×10^{-7} cm/s, which indicates a potentially good in vivo permeability.

The evaluation of the key and critical physicochemical and biopharmaceutical properties of this API is summarized in Table 1, together with the 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 ssNMR data (Figure 6). 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-aminopyridine and the corresponding carboxylic acid. On the basis of the above-mentioned acidic hydrolysis of the API, the chemical stability of SAR1 and potential formulations in the presence of HCl is not suitable.

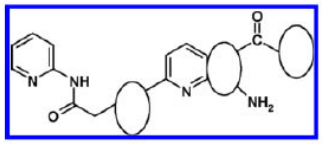


Figure 1. SAR1 as a model active pharmaceutical ingredient (API).

Another issue of the dihydrochloride salt was that stoichiometric salt formation was not feasible. However, the manufactured HCl salt showed promising oral absorption and

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Table 1. Critical Physicochemical and Biopharmaceutical Properties of SAR1 as a Function of Crystal Form

parameters	issues	proposed solutions
	Weak Base	
pK_{a1} = 2.9	Salt formation with strong acids	focus on cocrystal formation
pK_{a2} = 3.5	Chemical stability of the formulation cannot be ensured	
bioavailability	poor, below 2%	decrease particle size and evaluate permeability enhancers and amorpization
equilibrium solubility	strongly pH dependent	solubilization
	"di-HCl" Salt of the Weak Base	
stoichiometry	not stoichiometric	stop the development
physicochemical stability	unstable	stop the development
bioavailability	moderate bioavailability	focus on salt-like candidates such as co-crystals
	28% 100 mg/kg	
	5.9% 300 mg/kg	
	Co-Crystal of the Weak Base	
the character of the drug substance in the drug product	integrity of the cocrystal during drug product manufacturing	follow the cocrystal integrity during the manufacturing process avoid pharmaceutical excipients harmful for co-crystal integrity
equilibrium solubility	dependent strongly on pH	solubilization

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 a 100 mg/kg dose and 5.9% after a 300 mg/kg dose. On the basis of 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 physicochemical stability, but very poor oral bioavailability in rat animal model. On the basis of 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 was 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.

Measured pK_a values of SAR1 are pK_{a1} = 2.9, pK_{a2} = 3.5 as a divalent base. If the pK_a values are close to one another, the below mentioned equation describes the solubilization process:

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

where C is the concentration, and S is the solubility.

A logarithmic solubilization slope of 2.0 corresponds to a dramatic 100-fold change in solubility with each one unit change in a pH.[3]

Because of basic compounds with sharp pH-dependent solubilities, such compounds solubilized in the gastric fluid are very likely to precipitate after the solution empties from the stomach into the small intestine.[4]

On the basis of 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 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.

2. MATERIALS AND METHODS

2.1. Materials. *2.1.1. Active Pharmaceutical Ingredients.* 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. All API study batches were manufactured in laboratory scale from 10 to 30 g. Resynthesis batch of the fumaric acid co-crystal was manufactured in 0.7 kg scale.

2.1.2. Buffers. Buffer solutions were prepared according to the USP and Ph Eur recommendations.

2.1.3. 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.[6]

Tween 80, lactic acid, citric acid, Span 85, PEG 200, and 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.

2.2. Methods. *2.2.1. Chemical Manufacturing. 2.2.2.1. SAR1 as a Fumaric Acid Co-Crystal.* The reactor was charged with acetone (12 L), SAR1 base Form III (592 g, 1.29 mol), and fumaric acid (600 g, 5.16 mol). The slurry was stirred at room temperature for 24 h, and the crystals were filtered off, washed with water (1 L) and ethanol (1 L), and dried in a vacuum at 80 °C for 5 h.

Yield: 723 g (94.0%) of pale yellow powder. The purity of the product was 98.9% (HPLC).

2.2.1.2. SAR1 as a Dihydrochloride Salt. SAR1 (29 g) was added to methanol (1370 mL) under nitrogen. The suspension of API was stirred in Ultra-Turrax system for 30 min at room temperature.

The concentrated hydrochloric acid (8.03 mL, 2.3 equiv) diluted in 25 mL of methanol was added to the mixture in 30 min. The slurry was obtained in yellow color. The stirring was maintained overnight at room temperature. The cake was rinsed with methanol after filtration and dried under vacuum at 30 °C. Measured molar ratio was 1.95.

2.2.2. Analytical Methods. The analysis of samples was performed on Agilent 1100 type HPLC equipment with gradient method to

Table 2. Formulation Approaches

name of the formulation	type of the formulation	composition of the formulations	concentration of SAR1	administration volumes by oral route
		Weak Base		
F1: micronized API containing formulation	microsuspension	**micr. API: 0.6% MC sol.: Tween 80**	5.0 and 15.0 mg/mL	20 mL/kg
		0.5: 99: 0.5%		
		1.5: 98: 0.5%		
F2: nanomilled API containing formulation	nanosuspension	**nan. API: PVP K25: DOSS: Tween 80: water**	5.0 and 15.0 mg/mL	20 mL/kg
		0.5: 3: 0.15: 0.4: 95.95%		
		1.5: 3: 0.15: 0.4: 94.95%		
F3: lactic acid + cyclodextrin (CD) containing formulation	w/o emulsion	**API: lactic acid: CD: miglyol**	5.0 and 15.0 mg/mL	20 mL/kg
		0.5: 24: 5: 70.5%		
		1.5: 28: 5: 65.5%		
F4: lactic acid + Span 85 containing formulation	w/o emulsion	**API: lactic acid: Span85: Miglyol**	5.0 and 15.0 mg/mL	20 mL/kg
		0.5: 24: 5: 70.5%		
		1.5: 28: 5: 65.5%		
F5: lactic acid + permeability enhancers + solubilization	suspension	**API: Crem. ELP: lactic a.: vitamin E TPGS: NaOH sol. 3 M: PEG 200**	10.0 and 30.0 mg/mL	10 mL/kg
		1: 5: 8.86: 30: 6: 49.14%		
		3: 5: 8.86: 30: 6: 47.14%		
F6: citric acid containing stock granule	suspension	**API + citric acid containing granule: 0.6% MC sol.**	10.0 and 30.0 mg/mL	10 mL/kg
		3: 30.3: 66.7%		
		9: 30.3: 60.7%		
F7: partially amorphous API containing formulation	suspension	**API: Eudragit L100−55: 0.6% MC sol.: SDS**	10.0 and 30.0 mg/mL	10 mL/kg
		1: 1.38: 95.62: 2%		
		3: 4.14: 90.86: 2%		
		Co-Crystal with Fumaric Acid		
F8: permeability enhancer, solubilizer and cocrystal protector containing formulation	suspension	**API: Crem. ELP: 0.6% MC sol.**	10.0 and 30.0 mg/mL	10 mL/kg
		1: 5: 94%		
		3: 5: 92%		

evaluate solubility and chemical stability of APIs and formulations as well.

HPLC parameters were Purospher STAR 5 μm C18, 125 mm × 4.0 mm column. The HPLC analysis was performed at room temperature, with 10−50 μL injection volume and 1.0 mL/min flow rate. The A eluent composition was acetonitrile/pH = 2.5 buffer solution (100:900). Preparation of the buffer solution: 10 mM $NaH_2PO_4 \cdot 2H_2O$, its pH was set to pH = 2.5 with H_3PO_4.

The B eluent was acetonitrile. The ratio of the A eluent was 100:100:35:35:100 at 0, 2, 17, 24, and 25 min.

The samples were analyzed at 225 nm with UV detector. The concentrations of the standard calibration curve were 6, 10, and 14 μg/mL.

Bioassay was performed with a bioanalytical method: exploratory LC-MS/MS method form plasma as a matrix. LOQ was 1 ng/mL.

The stoichiometry of the prepared co-crystal was checked by ^{1}H NMR spectroscopy. The sample was dissolved in DMSO-d_6. The ^{1}H NMR spectrum was recorded at 400.13 MHz on a Bruker DRX-400 spectrometer using 30° pulse length and 10 s relaxation delay. Solid state characterization of the drug substance forms was performed by solid state ^{15}N NMR spectroscopy and x-ray powder diffraction (XRPD).

Experimental dissolution work was carried out in opened, Sotax type flow-through dissolution and Hanson-type paddle dissolution equipment. The temperature of the media was 37.0 ± 0.5 °C. Dissolution samples were collected by a fraction collector for both dissolution techniques followed by HPLC analysis. Samples were collected for up to 60 and 120 min.

2.2.3. Pharmaceutical Methods. Manufacturing of the exploratory formulations were performed on a laboratory scale using 20−100 g batch size. Qualitative and quantitative compositions of the formulations are summarized in Table 2.

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 a laboratory-scale spiral jet mill, and nanosuspension was prepared by Elan type nanotechnology[5] (F1 and F2 formulations).

The API was fully dissolved in lactic acid (0.5 and 1.5 g of SAR1 base was dissolved in 28 g of 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 the case of F3 formulation while the 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 was 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. The excipients of the internal phase were citric acid, mannitol, microcrystalline cellulose, HPMC, and crospovidone. Water was used as a granulation liquid. The components of the external phase were colloidal anhydrous silica and magnesium stearate. One portion of the elaborated stock granule was diluted with two 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. A drop dispersion was performed with water

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followed by the centrifugation of the suspension and washing with water. Filtration and drying were done at 100 °C for 4 h (F7 formulation). The partially amorphous SAR1 was dosed in 2% sodium dodecyl sulfate containing 0.5% methyl cellulose suspension.

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

2.2.4. Animal Studies. Species are male rats. Approximate weight at initiation of dosing was between 210 and 270 g. The age of rats at initiation of dosing was 7 weeks.

3. RESULTS AND DISCUSSION

3.1. Selected Formulations for Pharmacokinetic Evaluation (PK) on Rat Animal Model. On the basis of the physicochemical and biopharmaceutical evaluation of the candidates (the weak base and the co-crystal) (Table 1), the below mentioned formulations (Table 2) were prepared for a PK evaluation. The role of each excipient is summarized in Table 3.

Table 3. Role of the Chemical and Pharmaceutical Excipients

name of the excipient	role of excipient within the formulations
	Chemical Excipients
fumaric acid	co-crystal former
	Pharmaceutical Excipients
Cremophor ELP[6]	protect the dissolved API from precipitation, improve permeability and protect the integrity of the co-crystal
docusate sodium (DOSS)	secondary stabilizer of the nanosuspension
Eudragit L100-55	support amorphization of the API
citric acid	provide acidic microenvironmental pH of the granule
lactic acid	solvent of the API
methyl cellulose (400 mPa·s)	diluent/suspending agent
Miglyol[7]	diluent for emulsion and permeability enhancer
NaOH, 3 M solution	pH adjustment to 4.0
PEG 200[8]	diluent and permeability enhancer
PVP K25	primary stabilizer of the nanosuspension
Span 85[9]	surfactant, protect the dissolved API from precipitation, permeability enhancer
sodium dodecyl sulfate (SDS)	surfactant, protect the dissolved API from precipitation and improve permeability
sulfobutyl β cyclodextrin[10]	improve permeability of the API/solubilizer
Tween 80[11]	surfactant, protect the dissolved API from precipitation and improve permeability
vitamin E TPGS[12]	protect the solved API from precipitation and improve permeability

The administration volumes were decreased from 20 to 10 mL in the rat animal model to increase the tolerability of the formulations. To compensate for the lower dose volume, the concentrations of SAR1 in formulations F5, F6, F7, and F8 were doubled from 5 and 15 mg/mL to 10 and 30 mg/mL.

Chemical stability of the formulations were monitored by HPLC. Formulations were stored at 5 °C during the course of the study to ensure chemical stability. The total degradation observed at 5 °C after 2 weeks was below 2%, which is acceptable for a Discovery animal study.

3.2. Pharmacokinetic Results. The measured exposure (AUC) results were summarized in Table 4 and its graphic representation can be seen in Figure 2.

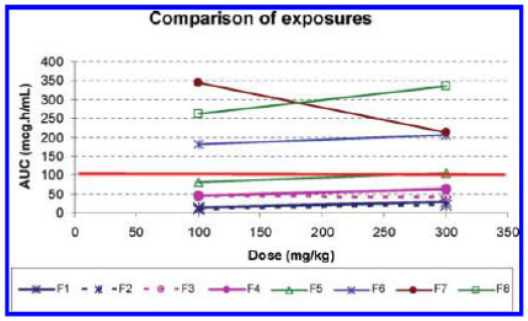

Figure 2. AUC function of dose. F1: base micronized, F2: base nanonized, F3: base + lactic acid + CD, F4: base + lactic acid + Span 85, F5: base + lactic acid + permeability enhancer + solubilization, F6: citric acid containing stock granule, F7: partially amorphous API, F8: cocrystal + permeability enhancer + solubilizer + co-crystal protector.

3.3. Evaluation of PK Results. The targeted exposure (100 μg·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).[13−15] The measured AUC results were promising with the partially amorphous base containing formulation (F7)[16] 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.

The targeted exposure was reached with the citric acid containing formulation as well (F6); however, it was kept as a back-up option based on the complexity of the formulation for toxicological studies.

Table 4. Pharmacokinetic Parameters

	AUC (μg·h/mL)							
dose (mg/kg)	base micronized	base nanomilled	base + lactic acid + CD	base + lactic acid + Span 85	base + lactic acid + permeability enhancers + solubilization	citric acid containing stock granule	partially amorphous API	cocrystal + permeability enhanser + solubilizer
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

Encouraging absorption was reached with the base containing suspension formulation (F5).

A very slight increase in terms of exposure was observed using the emulsion formulation containing sulfobutyl β cyclodextrin complex (F3). Adding a surfactant to the emulsion formulation (F4) rather than cyclodextrin led to a slight increase in exposure (F3).

The decrease of the particle size of the base form to micrometer or nanometer ranges did not increase the oral exposure (F1 and F2 formulations). The results suggest that rapid precipitation of the free base at intestinal pH negated any improvement in the rate of dissolution afforded by the reduction in particle size.

3.4. Evaluation of the Formulation Approaches Based on the PK Results. *3.4.1. Micronized and Nanomilled API Containing Formulations:F1 and F2.* Decreased particle sizes of the API (weak, crystalline base) below 20 μm (d90) for micronized and 400 nm (d90) for nanoformulations did not reach the targeted oral exposure of 100 μg·h/mL. Figure 3

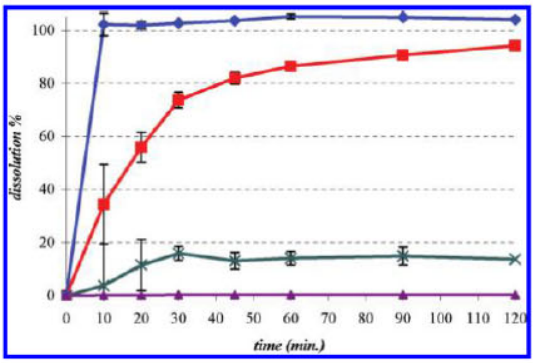

Figure 3. Comparative dissolution data of base and nanoformulation. Red solid squares: micronized base: pH 1.2. Green X's: micronized base: pH 6.8. Blue solid diamonds: Nanoformulation: pH 1.2. Purple solid triangle:nanoformulation: pH 6.8.

compares the dissolution curves for the micronized weak base and for the nanoformulation at two pH values. According to the dissolution curves, the nanoformulation showed 100% dissolution at pH = 1.2 in artificial gastric fluid within 10 min. Contrary to the nanoformulation, the free base dissolved below 40% within the same time period. The dissolution was significantly decreased at pH = 6.8 in both formulations based on pH-dependent solubility of SAR1.

The equilibrium solubility of the free base at pH = 1.2 in artificial gastric fluid at 37 °C is 2.0 mg/mL, which is decreased to 0.05 mg/mL at pH = 2 buffer solution. On the basis of the observed low exposure results in Figure 2 independently of doses the particle size reduction was not effective. It is expected that irrepective of particle size, such a weak base would precipitate with a change in pH leading to poor exposure; that is why differences were not measured for micronized and nanonized formulations.

3.4.2. Evaluation of the w/o Emulsion Formulations: F3 and F4. F3 and F4 formulations were prepared as oil based emulsions because of the high hydrophobicity of SAR1, and preparation of an aqueous solution was not feasible even with surfactant. Dilution of the dissolved SAR1 in lactic acid was feasible with an excipient with high apolarity such as Miglyol. An increase in exposure was measured in contrast to the micronized and nanonized formulations, but the exposure results did not fulfill the targeted AUC value (100 μg·h/mL).

Increased exposure with F3 and F4 formulations compared to F1 and F2 formulations is purely a solubility enhancement effect due to addition of cyclodextrine as well as alteration of microenvironmental pH due to lactic acid. Better exposure of F4 formulation is related to Span 85, which is preventing the dissolved API from precipitating. The observed very slow absorption is related to the oily character of the formulation; the presence of SAR1 in the samples of plasma was analyzed at 48 h also after oral administration.

3.4.3. Comparison of the Emulsion and Suspension Formulations: F4 and F5. Interestingly, around a 2-fold higher exposure was reached with the suspension formulation containing permeability enhancers and solubilizer (F5) over the emulsion formulation where the base was dissolved totally in lactic acid (F4). The explanation could be the very narrow good solubility range (2.0 mg/mL) of the API. If the API arrives as an emulsion formulation into the stomach, there is a high risk for quick precipitation. Solubilization with Span 85 in an oil based emulsion probably was not enough to prevent the base from precipitating at higher pHs than 1.2 under in vivo conditions. The expectation was confirmed by an in vitro precipitation study. F4 formulation was diluted at 37 °C with pH = 4.5 acetate and pH = 6.8 phosphate buffers in a 1:2 (formulation: buffer) ratio. A milky type precipitation was observed within 2 min.

F5 formulation is complex and a number of effects might be occurring such as solubility enhancement with vitamin E TPGS and PEG 200 and microenvironmental pH changes with lactic acid.

3.4.4. Evaluation of the Citric Acid Containing Formulation: F6. The main objective of preparing the base and citric acid containing formulation was to prepare in situ the salt or co-crystal during the wet granulation process. On the basis of the PK curves of Figure 2 to reach the targeted exposure was feasible; however, XRPD shows no evidence of salt or co-crystal formation. On the basis of the complexity of the citric acid containing formulation, a separate study was conducted to explore the scientific background of the good in vivo performance.

3.4.5. Partially Amorphous API Containing Formulation: F7. The exposure with F7 formulation presented in Figure 2 is definitely due to an increase in apparent solubility due to the amorphous nature of the formulation.

Manufacturing of the amorphous SAR1 formulation was designed according to the physicochemical properties of the API. SAR1 showed good solubility in N-methylpyrrolidone, which was used to dissolve SAR1 during the preparation of this formulation.

The free base has a very high melting temperature (ca. 300 °C), which means a high cohesive self-assembly, and it requires an excipient for the physical stabilization of the amorphous phase. As the free base is able to form co-crystals with acidic coformers, an acidic polymer (Eudragit) was selected as a stabilizer. To avoid the precipatation of the API upon delivery, a gastro resistant system was selected. On the basis of the characterization of the partially amorphous formulation, some traces of API crystals were measured by XRPD (Figure 4). Further crystallization was not detected upon storage. The increase in exposure using this formulation is likely due to an

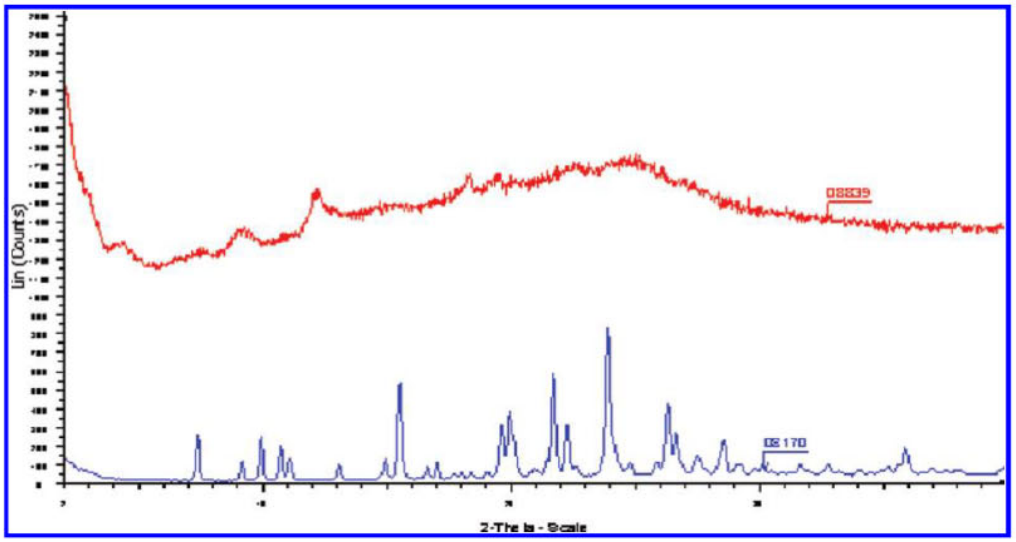

Figure 4. XRPD patterns of the crystalline free base (sample number: 08170) and its amorphous formulation with Eudragit L100 55 (sample number: 08839).

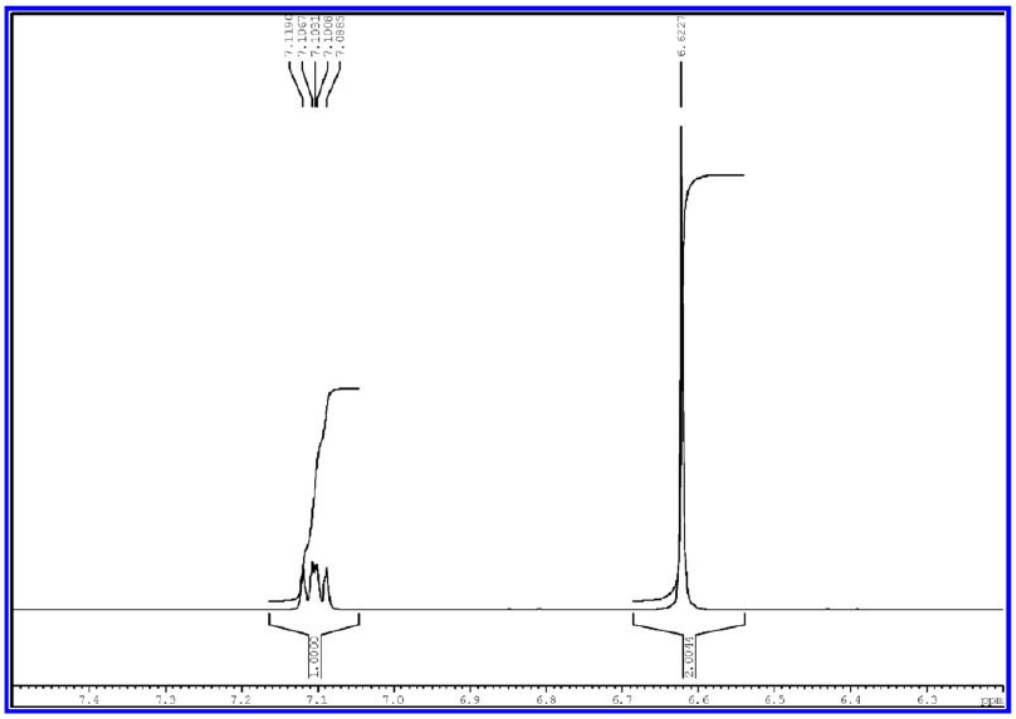

Figure 5. ^{1}H NMR spectrum of fumaric acid co-crystal of SAR1 base.

increase in the kinetic solubility of the API, an amorphous material.

3.4.6. Comparison of the Base and Co-Crystal Containing Formulations: F8. The XRPD study of the solid obtained in the reaction of SAR1 and fumaric acid indicated that this is a crystalline compound. The ^{1}H NMR spectrum of the solid dissolved in DMSO-d_6 proved that it contains SAR1 and fumaric acid in a 1:1 molar ratio. An extended part of the

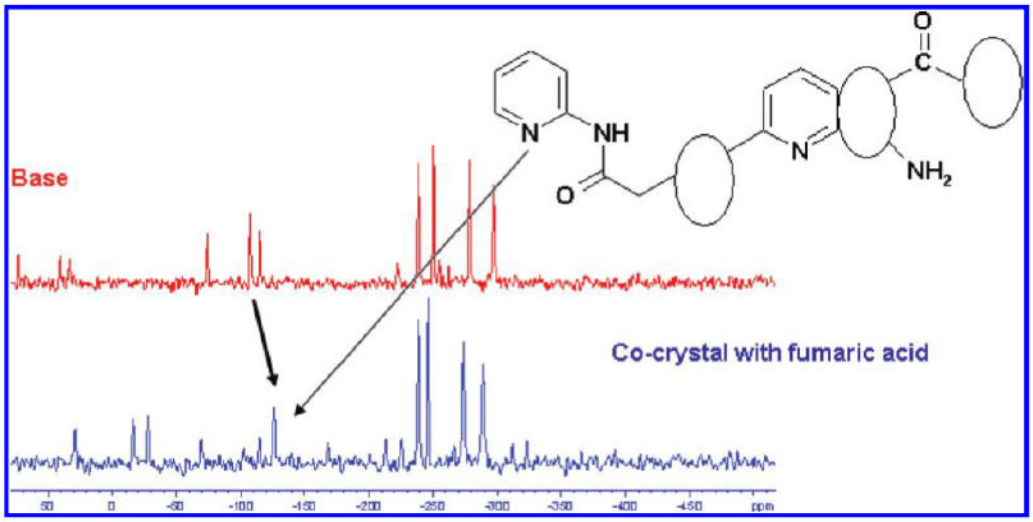

Figure 6. Solid phase NMR spectrum of SAR1 base and its co-crystal with fumaric acid.

spectrum can be seen in Figure 5 (signal of one hydrogen atom of SAR1 can be seen on the left, while signal of two hydrogen atoms of fumaric acid can be seen on the right in the spectrum). The comparison of its solid state ^{15}N NMR spectrum with those of SAR1 base proved that this is a co-crystal of SAR1 and fumaric acid (Figure 6).[17]

The particle size distribution of the API in a co-crystal form was 0.96 μm at d(90), 2.66 at d(50), and 10.90 at d(90) measured by laser diffraction.

For better understanding of why the co-crystal containing formulation achieved the best oral exposure results among all of the formulations, a complementary in vitro kinetic solubility study was initiated at 37 °C in artificial gastric fluid without pepsin (pH = 1.2) and in USP buffer (pH = 6.8) solution. The study design is summarized in Table 5. The kinetic solubility

Table 5. In Vitro Solubility Studies at 37°C in Artificial Gastric Fluid (pH = 1.2) and at pH = 6.8 in Phosphate Buffer Solution

APIs	APIs alone	5% Cremophor ELP containing classical suspension formulation with 0.6% methyl cellulose
SAR1 (base)	X	X
SAR1B (fumaric acid cocrystal)	X	X

study was initiated with the free base, the fumaric acid co-crystal, and with formulations containing both. The same pharmaceutical composition was applied as in formulation (F8) which provided the best PK results. The candidates were mixed together with Cremophor ELP, included as a surfactant, permeability enhancer, and co-crystal protector. The mixture was then diluted with the 0.6% methyl cellulose solution.

There is no significant difference between the kinetic solubility results at pH = 1.2 (Figure 7) of the free base and formulated base. On the contrary, differences in dissolution behavior were identified for the co-crystal. The co-crystal itself and the co-crystal containing formulation reached a higher, 2.5 mg/mL concentration compared to the free base containing suspensions (1.3–1.5 mg/mL). This higher concentration decreased significantly for the co-crystal formulated as a simple suspension within 1.5 units. However, the high concentration of the co-crystal was maintained for the co-crystal formulated with Cremophor ELP. Cremophor ELP is a polyoxyethylene castor oil from the chemical point of view, which has hydrophil and lipophil parts as well. The integrity of the co-crystal was protected against the aqueous microenvironment and dissociation with the lipophil, castor oil part of Cremophor ELP.

These results, the sustained higher concentration of the co-crystal, plus the pharmaceutical composition which stabilizes the co-crystal from precipitation, are together responsible for the better in vivo performance of the co-crystal containing formulation.

As it is shown in Figure 8 no significant differences were observed between the solubility curves of the base and co-crystal containing suspensions and pharmaceutical compositions at pH = 6.8. According to the good exposure results with a co-crystal containing formulation, the extended good solubility at pH = 1.2 is enough to provide the targeted exposure (Figure 2).

3.5. Exploratory Dissolution Experiments. *3.5.1. Dissolution Study of the co-crystal Containing Formulations.* 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. The classical paddle type and flow-through dissolution techniques were compared on exploratory formulations. The compositions are summarized in Table 6.

3.5.2. Discriminative Dissolution Method. To establish a discriminative dissolution method, the formulations outlined in Table 6 were tested first in the flow-through dissolution equipment[18] 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.

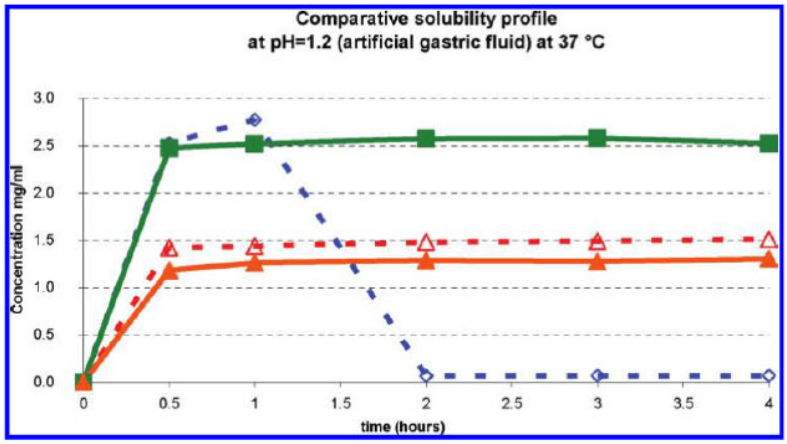

Figure 7. Concentration time profile at pH = 1.2 for the SAR1 base and co-crystal as an API and in formulations at 37 °C. Blue open diamond: SAR109511B: co-crystal as an API. Green solid square: SAR109511B: cocrystal in methyl cellulose + Cremophor ELP suspension formulation. Red open triangle: SAR109511: base as an API. Orange solid triangle: SAR109511: base in a formulationin methyl cellulose + Cremophor ELP suspension formulation.

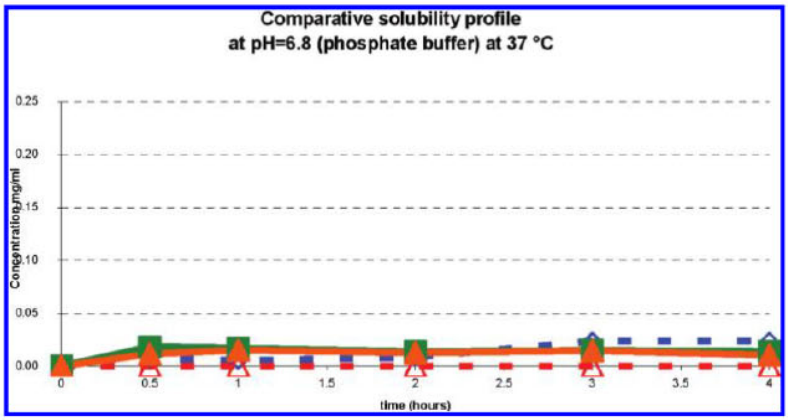

Figure 8. Concentration time profile at pH = 6.8 for the base and co-crystal as an API and in formulations at 37 °C. Blue solid squares: SAR109511B: co-crystal as an API. Green solid squares: SAR109511B: co-crystal in methyl cellulose + Cremophor ELP suspension formulation. Red open triangles: SAR109511: base as an API. Orange solid triangles: SAR109511: base in a formulationin methyl cellulose + Cremophor ELP suspension formulation.

Flow-through dissolution was conducted with a 4.0 mL/min flow rate in the powder cell, which has five milliliter volume. The cumulative flow-through dissolution curves are summarized in the Figure 9. Significant differences between micronized and not micronized SAR1 fumaric acid co-crystal containing formulations were not measured at pH = 1.2 and 6.8 with 0.5% sodium dodecyl sulfate; however at pH = 4.5 with 0.5% sodium dodecyl sulfate the difference was significant for the formulations.

On the basis of the flow through dissolution results, acetate buffer at pH = 4.5 with 0.5% sodium dodecyl sulfate was selected as a potential discriminative dissolution method to evaluate the prototype formulations in the paddle USP2 dissolution equipment.

The USP2 dissolution measurement was performed with 250 and 500 mL volumes at 50 rpm. These volumes were selected to prevent the co-crystal from dissociation. Since the fumaric acid part of the co-crystal has a high solubility in water, there is a potential risk for the co-crystal to be physically unstable and precipitate as the free base before complete dissolution. This is a potential risk under in vivo conditions as well; that is why protection of the co-crystal form is important within the formulations. Dissolution experiments validated the concept and classical dissolution curves did not show any differences at pH = 4.5 between micronized and unmicronized containing formulations. The classical dissolution curves are shown in Figure 10.

No significant differences between dissolution curves were observed in 250 and 500 mL.

Disintegration of the co-crystal to base + fumaric acid is quick in large aqueous volumes.

Table 6. Compositions of the Co-Crystal Containing Formulations

Batch numbers		P-0230209	P-0250509
API and excipients	Function of the excipients	Wet granulation process / mini formulation	
		%	
SAR1 fumaric acid co-crystal, not micronized	API	6.3 d(90)=15.7 μm	-
SAR1 fumaric acid co-crystal, micronized		-	6.3 d(90)=3.9 μm
Mannitol	Diluent	53.3	53.3
Microcrystalline cellulose PH 101		22.9	22.9
HPMC	Binder	5.0	5.0
Crospovidone Type A	Disintegrant	5.0	5.0
Cremophor ELP	Surfactant and permeability enhancer	5.0	5.0
Mg stearate	Lubricant	2.0	2.0
Colloidal silica anhydrous	Glidant	0.5	0.5
200 mg granule contains 10 mg API (expressed in base)		100 %	100 %

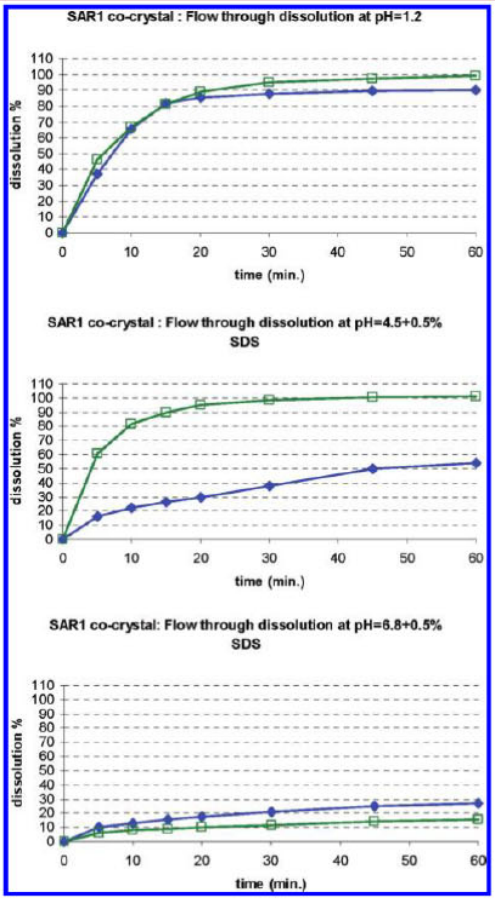

Figure 9. Flow-through dissolution curves of prototype formulations. Blue solid diamond: SAR1 co-crystal: P-0230209: not micronized API. Green open squares: SAR1 co-crystal: P-0250509: micronized API.

Significance of the protection of co-crystals from dissociation were published by other scientists also based on in vitro dissolution results.[19]

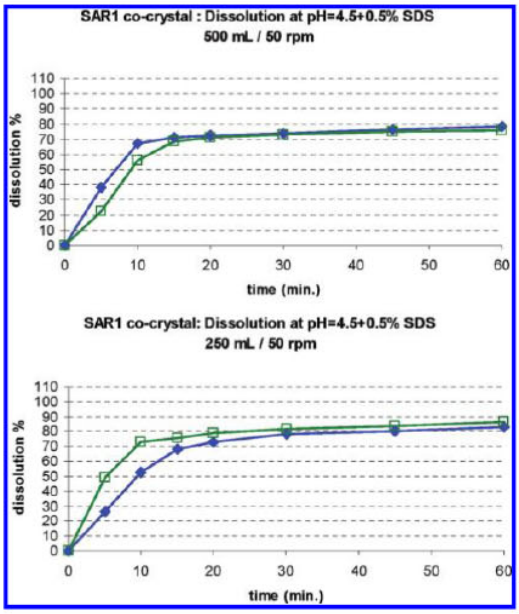

Figure 10. Classical dissolution curves of prototype formulations. Blue solid diamonds: SAR1 co-crystal: P-0230209: not micronized API. Green open squares: SAR1 co-crystal: P-0250509: micronized API.

The flow-through dissolution technique is mandatory to support formulation development based on the in vitro results.

4. CONCLUSION

On the basis of the physicochemical and biopharmaceutical evaluation of the API candidates formulation possibilities for toxicology and for first in man were determined. An increase of the very low bioavailability of the weak base was feasible with permeability enhancers, surfactants, acidic excipients, amorphization, and fumaric acid co-crystal formation. 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, solubilizer, and co-crystal protector with its castor oil part provided the best oral exposure in a rat model. Cremophor EL is a well-known pharmaceutical excipient for oral and intraveneous formulations. Serious side effects reported with Cremophor EL intravenous formulations[20−22] were not observed when Cremophor ELP was administered by the oral route in the rat models at 5% concentration. Similar good toxicological results were presented by BASF, the manufacturer of Cremophor EL in 2008.[6]

The integrity of the co-crystal within the formulation is essential to reach better bioavailability via faster dissolution kinetics.

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 coformer.

Bioavailability increase of a poorly soluble weak base was feasible based on the collaborative work among chemists, analysts, and formulation experts.

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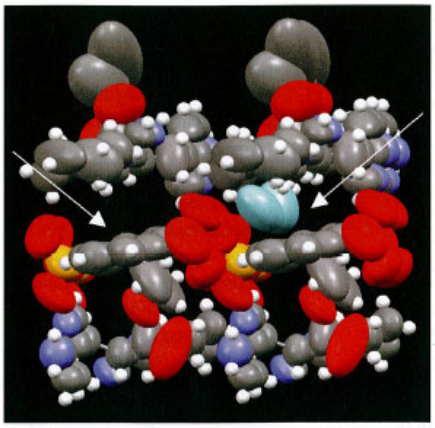
II.



3. ábra. Az SA molekula egykristály-szerkezetének csatornákra merőleges vetülete, amely egy szomszédos üres és egy vízmolekulákat (világoskék színnel jelölve) tartalmazó csatornát mutat

anyag egykristályszerkezetének megjelenítése látható a csatornákra merőleges vetületből.

A vízmolekula kémia környezetéről SCXRD hiányában szilárd fázisú NMR-méréssel kaphatunk információt. A topotechan-HCl 3 és 5 mól vízzel is kristályosodhat [2], az első három mól rácshidrátként stabilan kötődik a szerkezetbe, a további vizet viszont már csatornahidrátként veszi fel (a csatornahidrát-viselkedést a DVS- és a termoanalitikai vizsgálatokkal igazolták). A trihidrát és pentahidrát fázisokról készült ^{13}C és ^{15}N szilárd fázisú NMR-vizsgálatok eredményének összehasonlításából megállapították, hogy a gyengén kötött csatornavíz a laktongyűrű és gyűrűs amid funkciós csoportok környezetét befolyásolják leginkább.

A csatornahidrátok jellemzésének fontossága abban rejlik, hogy jósolható legyen a hatóanyag viselkedése a gyógyszer-előállítás különböző fázisaiban, a kristályosítástól a tablettázásig. Ugyanis a kristályvíz elvesztése fizikai stabilitási problémákhoz vezethet [2]; amorfizálódás léphet fel, ami befolyásolhatja a kémiai stabilitást is, továbbá a könnyen változó víztartalom dozírozási nehézségeket is okozhat.

KÖSZÖNETNYILVÁNÍTÁS

A szerzők köszönetet mondanak Winfried Heyse, Harald Schweitzer, Bruno Baumbgartner és Norbert Nägel kollékáknak (sanofi-aventis, Frankfurt) az egykristály-röntgenfelvételért.

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ÖSSZEFOGLALÁS

Szabó András–Molnár-Gábor Dóra–Bokotey Sándor: **Csatornahidrát típusú gyógyszerhatóanyagok jellemzése**

A csatornahidrát típusú vegyületek változó víztartalmuk miatt körültekintő szilárd fázisú jellemzést igényelnek, hogy a gyógyszer-technológiai folyamatok során jósolható legyen a viselkedésük. A cikk célja, hogy rövid áttekintést adjon az alkalmazható analitikai technikákról és az általuk megszerezhető szerkezeti információkról, néhány irodalmi és saját vegyület eredményei alapján.

Ujhelyi Gabriella–Venczel Márta–Bajdik János–Kónya Magdolna–Vajdai Attila

■ sanofi-aventis/Chinoin, K+F Gyógyszerfejlesztés

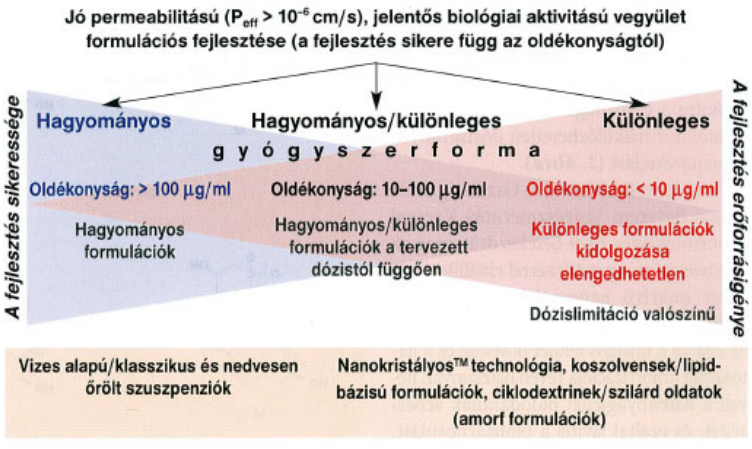
Klasszikus és innovatív technológiák a Gyógyszerfejlesztésben

A Chinoin Gyógyszerfejlesztésének tevékenysége, amely átível az originális molekulákkal történő kutatási tevékenységtől a generikus fejlesztésig, az 1920-as évekig nyúlik vissza.

Jelen formulációs fejlesztőmunkánk egyaránt magában foglalja a felfedező kutatás gyógyszerjelölt vegyületeinek kiválasztási támogatását, az eredeti molekulák klinikai vizsgálataihoz kifejlesztett formulációkat, a korábbi Chinoin-termékek formulációs megújítását és generikus gyógyszerek formulációinak kidolgozását.

Korai formulációs kísérletek. Fontos, hogy a kiválasztásra kerülő gyógyszerjelölt vegyületek olyan formulációban kerüljenek vizsgálatra, amely biztosítja a hatóanyag optimális felszívódását és toxikológiai szempontból jól tolerálható. Mivel a kutatás e korai szakaszában csak néhány milligramm hatóanyag áll rendelkezésre formulációs célokra, nincs lehetőség egy gyógyszerforma fizikai paramétereinek – pl. a hatóanyag szemcseméretének és polimorf formájának – meghatározására. Az *in vivo* farmakoló-

1.ábra. A formulációs stratégia kiválasztása a hatóanyag oldékonysága alapján [1]

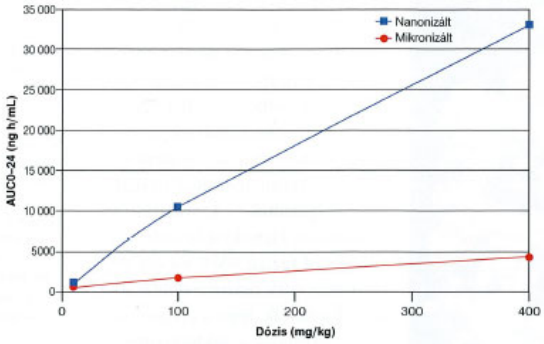


giai és ártalmassági vizsgálatokra, együttműködve a felfedező kutatás kutatóival, a gyógyszerészeti segédanyagokra vonatkozó és a hatóanyagról már rendelkezésre álló tudás birtokában oldatos formulációkat dolgozunk ki.

Formulációs stratégia. A formulációfejlesztés irányvonalát a várható klinikai dózis mellett alapvetően a hatóanyag fizikokémiai és biofarmáciai tulajdonságai határozzák meg. Amennyiben a hatóanyagjelölt vízoldékonysága nagyobb, mint 100 µg/ml, klasszikus gyógyszerformák, azaz orális adagolás esetében tabletták és kapszulák kidolgozása az elsődleges cél **(1. ábra)**. Minél kisebb egy hatóanyagjelölt vízoldékonysága, annál nagyobb a valószínűsége annak, hogy a terápiás hatás biztosításához különleges, innovatív gyógyszerforma kidolgozására van szükség [1]. Utóbbi esetekben a megfelelő biológiai hozzáférhetőség biztosítására megoldás például a hatóanyagot nanoméretű részecskék formájában tartalmazó formulációk alkalmazása. Ilyen irányú fejlesztéseink során őrlési és elektroszpinning [2] technológiákkal állítjuk elő a nanoméretű szemcséket tartalmazó hatóanyagot, amelynek stabilitását speciális hordozórendszerekkel biztosítjuk.

Az elmúlt években sikeresen alkalmaztuk a Nanokristályos™ technológiával készült nanokristályos diszperziót a biohasznosulás optimálására preklinikai toxikológiai állatkísérletekben, valamint később a diszperzióból készült tablettát a humán klinikai vizsgálatokban. Az állatkísérletes *in vivo* farmakológiai eredmények azt mutatták, hogy egy adott hatóanyagjelölt szemcseméretének csökkentése jelentősen növelte a biohasznosulást: a kiindulási 7%-ról mikronizált hatóanyag esetében 24%-ra, nanokristályos hatóanyagnál pedig 100%-ra növelve azt. A nanokristályos hatóanyag biztosította a toxikológiai és biztonsági vizsgálatokhoz nélkülözhetetlen dózisarányos hatásnövekedést **(2. ábra)**.

A Budapesti Műszaki és Gazdaságtudományi Egyetem Vegyészmérnöki Karával együttműködve 2009-ben kezdtük meg az elektroszpinning módszerrel előállított polimer mátrixú nanoszálak alkalmazását rosszul oldódó hatóanyag fejlesztésére. Ez az eljárás a fajlagos felület növelése és a hatóanyag amorfizációja révén hatékonyan növeli a hatóanyagjelölt oldódásának sebességét, és ezáltal javítja a biohasznosulást.



2. ábra. Egy hatóanyag mikronizált és nanonizált változatát tartalmazó készítményeinek AUC_{0-24} vs. dózis görbéi

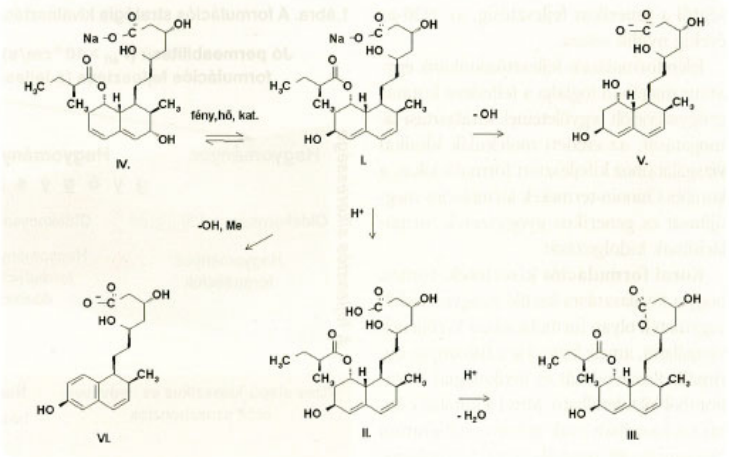
További előnye az elektroszpinning eljárásnak, hogy akár nagy molekulák, fehérjék formulálására is alkalmas, mivel az eljárás során magát a hatóanyagot nem éri extrém behatás, például magas hőmérséklet, nyomás vagy nyíróerő.

Az innovatív lehetőségek alkalmazása mellett különös figyelmet fordítunk a hatóanyag felszívódás szempontjából legmegfelelőbb sójának vagy kokristályos formájának kiválasztására is [3]. A különböző só és/vagy kokristály formák összehasonlítását átfolyó cellás kioldókészüléken [4] végezzük, amelynek a fejlesztésben betöltött fontos szerepét az eddigi *in vivo* eredmények is megerősítették.

Nemcsak a különleges gyógyszerformák kidolgozása igényel innovatív megoldásokat, hanem a klasszikus formák fejlesztési és előállítási lehetőségei is jelentős fejlődést mutatnak. A fejlesztés kezdeti lépéseiben a korlátozott mennyiségű hatóanyag miatt a technológiák miniatürizálását alkalmazzuk. A modern eszközök alkalmazásával lehetőség nyílik már kis mennyiségű hatóanyagból megkapnunk azokat az információkat, amelyek az egyre kiterjedtebb preformulációs vizsgálatok eredményeivel együtt megalapozzák a tudományos alapokon nyugvó és gyorsan kivitelezhető fejlesztést. Munkánk során alkalmazzuk a matematikai elveken nyugvó tervezési módszereket is, amelyek segítségével a gyártási folyamat jobban megérthetővé és gyorsabban optimálhatóvá válik, és lehetőséget biztosíthatunk ún. „Quality by design" megközelítés alkalmazására.

Életciklus-kiterjesztés. Gazdasági szempontból kiemelt gyári érdek, hogy a gyógyszerpiacon elismert és jól bevált készítményeink gyógyszerformái mindenkor megfeleljenek a kor követelményeinek, továbbá korszerűbb kiszerelési formákkal a betegek elvárásait is kielégítsük. Az évek során megújult többek között a No-spa tabletta, a Polyvitaplex és a Neo-Bilagit filmtabletta. Utóbbit 1925-ben vezették be a terápiába. A legutóbbi, 2001-es felújítása során a fejlesztés egyik szempontja a korszerűtlen és energiaigényes cukordrazsírozás filmbevonásra való felváltása volt. Ugyanez a váltás a kor követelményeinek megfelelően felújított összetett vitaminkészítményünk, a Polyvitaplex filmtabletta esetében is megtörtént. Mindkét említett készítmény törzskönyvi megújításához értelemszerűen a minősítéshez és stabilitásvizsgálathoz használandó teljes analitikai arzenált is korszerűsí-

3. ábra. Pravasztatin-Na bomlási útvonalai



tenünk kellett, ami – többkomponensű készítményekről lévén szó – összetett analitikai fejlesztést igényelt.

Egyre gyakrabban merül fel igény kombinációs készítmények kifejlesztésére. Ezen a területen az utóbbi évtizedekben egyik legjelentősebb fejlesztésünk a drotaverin-, paracetamol- [5] és a drotaverin-, paracetamol-, kodeintartalmú tabletták, valamint a drotaverin- és ibuprofentartalmú combokészítmény, amelyeket No-Spalgin és Algoflex M néven törzskönyveztek. A szelegilinhidroklorid hatóanyag esetében transzdermális formulációt dolgoztunk ki [6].

Generikus fejlesztés. Vállalatcsoportunkon belül a Chinoin Gyógyszerfejlesztése foglalkozott generikus gyógyszerek szilárd gyógyszerfomáinak (tabletta, kapszula) fejlesztésével is, amely összetett feladat. Analitikus, technológus, farmakokinetikai, szabadalmi és törzskönyvezési szakemberek összehangolt munkájára van szükség egy stabil, bioegyenértékű készítmény fejlesztéséhez. Az analitikai és bioekvivalencia-vizsgálatok elengedhetetlenek ahhoz, hogy hitelesen igazoljuk a generikum egyenértékűségét az originális készítménnyel.

A Gyógyszerfejlesztés életében az 1999 és 2004 közötti időszak rendkívül sikeres volt. A fejlesztőmunka eredményeként 6 év alatt számos terméket fejlesztettünk és 33 terméket üzemesítettünk. Vállalatcsoportunk az általunk fejlesztett generikus készítményekkel jelenleg is képviseli magát a gyógyszerpiacon, például enalapril [7] és lisinopril hatóanyag-tartalmú tablettákkal [8], fluoxetintartalmú kapszulával és az egyébként bomlásra rendkívül hajlamos pravasztatintablettával [9], amelynek stabilizált formáját dolgoztuk ki.

A pravasztatin tartalmú tabletta két hatáserősségének kifejlesztésére 21 hónap alatt került sor oly módon, hogy az általunk kidolgozott készítmény komoly feltalálói sikereket hozott.

Találmányunk alapja az a felismerés, hogy a pravasztatin karboxil- és hidroxilcsoportjával kelátkomplexet képző fémion, például a kalciumion megakadályozza a molekula szigmatrop átrendeződését, ezáltal a vegyület stabilizálható **(3. ábra).** Ezt a felismerést kihasználva kalcium-laktát alkalmazásával sikerült stabil készítményt kidolgoznunk.

Összegzés. A vállalatcsoporton belül a Chinoin gyógyszerfejlesztési egysége a klasszikus és innovatív technológiák célirányos alkalmazásával, a hatóanyagok fizikokémiai tulajdonságainak felderítésével a hazai egyetemekkel és nemzetközi társegységekkel szoros együttműködésben biztosítja a megfelelő hatékony gyógyszerformákat a terápiás célok megvalósításához.

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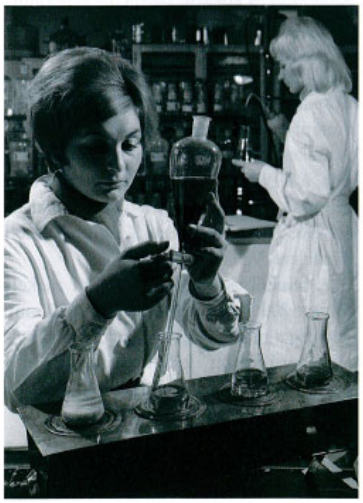
ÖSSZEFOGLALÁS

Ujhelyi Gabriella–Venczel Márta–Bajdik János–Kónya Magdolna–Vajdai Attila:
Klasszikus és innovatív technológiák a Gyógyszerfejlesztésben

Az összeállításban röviden áttekintjük a Gyógyszerfejlesztés originális és generikus fejlesztésben, valamint az életciklus-kiterjesztésben betöltött szerepét. Az originális fejlesztés tekintetében az utóbbi években nyújtott tevékenységek közül a preformulációs aktivitásokat, a korai formulációs kísérleteket, az innovatív technológiákat és az újszerű szemléleteket magában foglaló speciális formulációs stratégiák néhány jellemző példáját mutatjuk be. A Gyógyszerfejlesztés több évtizeden keresztül igen sikeres nemzetközi szintű generikus fejlesztési tevékenységet is folytatott, amelynek néhány kiemelkedő eredménye szintén a kézirat részét képezi.

Földi Zoltán, az első olyan vegyészmérnök, aki kiemelkedő munkássága elismeréseként akadémiai tagságban részesült

Laborbelső az 1970-es években



Tablettázó a 60-as, 70-es években

III.

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(54) Title: STABILIZED PHARMACEUTICAL COMPOSITIONS AND PROCESS FOR THE PREPARATION THEREOF

(57) Abstract

The invention relates to pharmaceutical compositions of enalapril maleate stabilized by maleic acid.

Stabilized pharmaceutical compositions and process for the preparation thereof

The present invention relates to novel stabilized pharmaceutical compositions, process for their preparation and the use of maleic acid as a stabilizer. The active ingredient of the above compositions is enalapril maleate which is a potent angiotensin-converting enzyme inhibitor, and it is useful in the treatment of hypertension.

Enalapril, its salts and the process for their preparation are described in the European Patent Application publ. No. EP-012401 A1.

As it is known, many compounds that inhibit ACE (Angiotensin-Converting Enzyme) have poor stability either in form of free acids or salts if they are in a pharmaceutical dosage form. These compounds easily decompose first of all by hydrolysis and intramolecular cyclization, but the amount of other decomposition products not identified in many cases may be also significant. This is particularly true in case of enalapril and its maleate salt.

Main decomposition products of enalapril are shown in Fig. demonstrating that the decomposition is due to hydrolytic and cyclization processes.

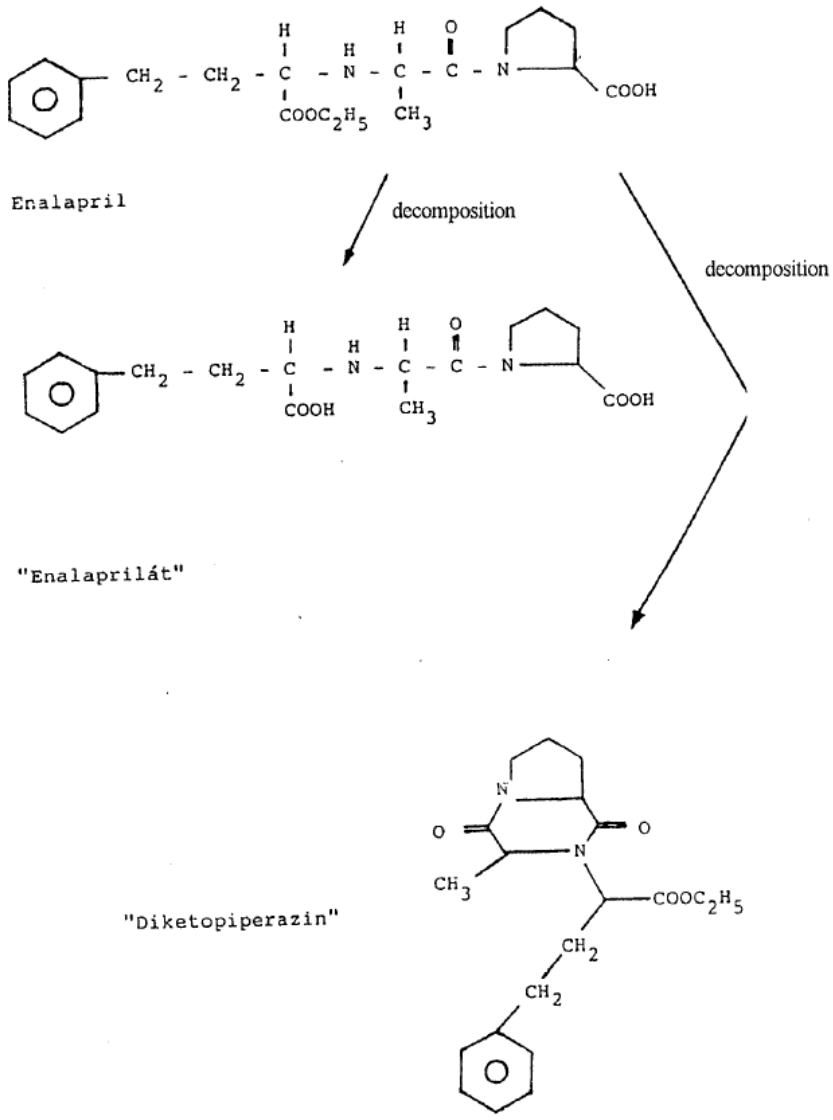
The diketopiperazine (DPK) is the internal cyclization product and the diacid (enalaprilat ET) is the product of ester hydrolysis.

A lot of solutions have been elaborated to stabilize angiotensin-converting enzyme inhibitors, among them enalapril salts in pharmaceutical compositions.

According to the European Patent Application publ. no. EP-0 280 999 A2, magnesium carbonate shows a stabilizing effect in pharmaceutical products containing saccharides, e.g. lactose and quinapril.

According to the European Patent Application publ. no. EP-0 545 194 A1, enalapril is transformed into its sodium salt. The enalapril sodium salt in pharmaceutical preparations is said to be more stable than the enalapril maleate salt.

Fig.


Enalapril
decomposition
decomposition
"Enalapriláť"
"Diketopiperazin"

United States Patent no. 5.562.921 describes that the enalapril maleate salt extensively decomposes in the presence of commonly used vehicles, filling substances, lubricants or disintegrating agents in many pharmaceutical products for example in the pharmaceutical dosage forms containing microcrystalline cellulose, calcium phosphate or magnesium stearate.

It is described in EP-A-099239 and EP-B-0264887 that ascorbic acid may be used as an antioxidant or colour stabilizing agent in case of ACE-inhibitors.

The aim of our invention is to prepare pharmaceutical formulations of high stability which contain enalapril maleate with commonly used filling substances (e.g. lactose, mannitol, sorbitol) lubricant (e.g. magnesium stearate) and disintegrating agents (e.g. starch) and in which the amount of decomposition products is low even in case of long-term storage, thus ensuring a longer expiration time and in the same time a high quality.

It has been found that if enalapril maleate is transformed into pharmaceutical formulations by applying commonly used filling substance (e.g. filling substance of saccharide type) and maleic acid stabilizer, an extremely stable enalapril formulation is obtained. This is true even if magnesium stearate or other compounds are used as lubricants, affecting the stability of enalapril maleate .

At realizing our invention, we have successfully applied for example mono- or disaccharides, water-free lactose, lactose monohydrate or DC (direct compression) lactose as filling substances, starches or partly hydrolysed starches, or crospovidone (polyvinylpolypyrrolidone) as disintegrating agents, magnesium stearate, hydrogenated vegetable oil or talc as lubricants, and maleic acid as stabilizer, in addition to the currently used colouring and binding agents, e.g. ferric oxide and povidone (polyvinylpyrrolidone). Further auxiliary substances applicable for these purposes are enumerated in the Hungarian Pharmacopoeia or in the European Pharmacopoeia.

One of the preferred variant forms of our invention is the tablet or the granules for filling capsule consisting of enalapril maleate, maleic acid, lactose, starch, partly hydrolysed starch and magnesium stearate, and optionally colouring and binding agents.

Our invention also relates to the process for the preparation of the above pharmaceutical formulations. During this process, granules to be compressed in tablets or to be filled into capsules are prepared by wet granulation using aqueous solution of maleic acid.

During one of the favourable implementations of the above process, dry enalapril maleate, lactose, starch and partly hydrolysed starch are mixed, and then their mixture is granulated using aqueous solution of maleic acid used as granulation liquid. Of course, ingredients may be mixed in other sequences. The granules obtained are dried and classified, and then compressed into tablets with magnesium stearate added.

Tablets according to our invention can be prepared by direct tabletting, i.e. mixing enalapril maleate with all other auxiliary substances and with maleic acid used as stabilizing agent, and by compressing the mixture in tablets.

Pharmaceutical products (dosage forms) prepared according to our invention may preferably contain enalapril maleate in 0.1-25 weight %, lactose in 30-95 weight %, starch and partly hydrolysed starch in 6-80 weight %, maleic acid in 0.1-10 weight %, lubricant in 0.1-5 weight % and colouring and binding agents in 0.01-5 weight %.

Preferred dosage forms are tablets and granules which contain enalapril maleate in 1.5-15 weight %, lactose in 65-90 weight %, starch and/or other disintegrating agents in 5-15 weight %, binding and colouring agents in 3-7 weight %, pregelatinized starch in 1-4 weight %, maleic acid in 1-5 weight % and lubricants in 0.1-1.5 weight %. Most preferred unit dosage forms are tablets with 75-300 mg tablet mass having above preferred compositions.

Further details of our invention are shown by the examples below, without limiting our claims to the examples.

Examples

Example 1

100 g of enalapril maleate, 3930 g of lactose monohydrate, 380 g of corn starch, 120 g of pregelatinized starch were homogenized. 24 g of maleic acid was dissolved in 1000 ml of purified water. The homogenized powder mixture was granulated with slowly (10-15 min) added aqueous solution of maleic acid. The wet granules were dried (at 40-50°). The dried granules were homogenized with 20 g of magnesium stearate (for 10-20 min).

The homogenized granules were tabletted and tablets having 115 mg total mass and containing 2.5 mg of enalapril maleate were obtained.

Example 2

150 g of enalapril maleate, 5934 g of lactose monohydrate, 570 g of corn starch, 180 g of pregelatinized starch were homogenized. 36 g of maleic acid was dissolved in 1500 ml of purified water. The homogenized powder mixture was granulated with slowly (10-15 min) added aqueous solution of maleic acid. The wet granules were dried (at 40-50°). The dried granules were homogenized with 30 g of magnesium stearate (for 10-20 min).

The homogenized granules were tabletted.

Example 3

200 g of enalapril maleate, 3300 g of lactose monohydrate, 300 g of corn starch, 100 g of pregelatinized starch were homogenized. 48 g of maleic acid was dissolved in 950 ml purified water. The homogenized powder mixture was granulated by slowly (10-15 min) added aqueous solution of maleic acid. The wet granules were dried (at 40-50°). The dried granules were homogenized with 36 g of magnesium stearate (for 10-20 min).

The homogenized granules were tabletted.

Example 4

250 g of enalapril maleate, 1890 g of lactose monohydrate, 188 g of corn starch, 68 g of pregelatinized starch were homogenized. 60 g of maleic acid was dissolved in 600 ml of purified water. The homogenized powder mixture was granulated with slowly (10-15 min) added aqueous solution of maleic acid. The wet granules were dried (at 40-50°). The dried granules were homogenized with 45 g of magnesium stearate (for 10-20 min).

The homogenized granules were tabletted and tablets having 200 mg total mass and containing 20 mg of enalapril maleate were obtained.

Example 5

200 g of enalapril maleate, 3300 g of lactose monohydrate, 300 g of corn starch, 100 g of pregelatinized starch, 48 g of maleic acid were homogenized. The homogeneous powder mixture was granulated with slowly (10-15 min) added purified water (950 ml). The wet granules were dried (at 40-50°). The dried granules were homogenized with 36 g of magnesium stearate (for 10-20 min).

The homogenized mixture was tabletted.

Example 6

3300 g of lactose monohydrate, 300 g of corn starch, 100 g of pregelatinized starch were homogenized. 48 g of maleic acid was dissolved in 1100 ml of purified water. While stirring, 200 g of enalapril maleate was added. The homogeneous powder mixture was added to the suspension. The wet granules were dried (at 40-50°). The dried granules were homogenized with 36 g of magnesium stearate.

The homogenized granules were tabletted.

Example 7

250 g of enalapril maleate, 4200 g of lactose monohydrate, 370 g of corn starch, 120 g of pregelatinized starch were homogenized. 45 g of maleic acid was dissolved in 1300 ml of purified water. While stirring, 120 g of polyvinylpyrrolidone was added to the pure solution. The

homogenized powder mixture was granulated with slowly (10-15 min) added aqueous solution of maleic acid and polyvinylpyrrolidone. The wet granules were dried (at 40-50°). The dried granules were homogenized with 36 g of magnesium stearate (for 10-20 min).
The homogenized granules were tabletted.

Example 8

250 g of enalapril maleate, 60 g of maleic acid, 45 g of magnesium stearate, 120 g of polyvinylpyrrolidone, 120 g of pregelatinized starch were homogenized. 370 g of corn starch, 4200 g of lactose monohydrate were added to the homogeneous mixture and the mixture was homogenized again (for 15-20 min).
The homogeneous mixture was tabletted.

Example 9

200 g of enalapril maleate, 1600 g of lactose monohydrate, 1600 g of corn starch, 250 g of pregelatinized starch, 100 g of polyvinylpyrrolidone, 150 g of talc were homogenized. 50 g of maleic acid was dissolved in 1000 ml of purified water. The homogenized powder mixture was granulated with slowly (10-15 min) added aqueous solution of maleic acid. The wet granules were dried (at 40-50°). The dried granules were homogenized with 36 g of magnesium stearate.
The homogenized granules were tabletted.

Example 10

200 g of enalapril maleate, 250 g of pregelatinized starch were homogenized, 100 g of polyvinylpyrrolidone, 150 g of talc, 50 g of maleic acid and 40 g of magnesium stearate were homogenized. To the homogeneous mixture 1600 g of lactose and 1600 g of corn starch were added. The mixture was homogenized (for 15-20 min).
The homogenized mixture was tabletted.

Example 11

100 g of enalapril maleate, 1700 g of lactose monohydrate, 40 g of crospovidone, 110 g of maize starch and 2 g of ferrous oxide red were homogenized. 48 g of maleic acid was dissolved in 1200 ml of purified water. The homogenized powder mixture was granulated with slowly (10-15 min) added aqueous solution of maleic acid. The wet granules were dried at 40-50°C. The dried granules were homogenized with 10 g of magnesium stearate for 20 min.. The homogenized granules were tabletted and tablets having 200 mg total mass and containing 10 mg of enalapril maleate were obtained.

CLAIMS

1. Stable pharmaceutical composition, characterized in that, it contains enalapril maleate as active substance, maleic acid as stabilizing agent and one or more auxiliary substances.

2. Composition according to claim 1, characterized in that, it contains enalapril maleate in 1.5-15 weight %, filling substances in 65-90 weight %, disintegrating agents in 6-20 weight %, maleic acid in 1-5 weight %, binding and colouring agents in 3-7 weight % and lubricants in 0.1-1.5 weight %.

3. Composition according to claim 1, characterized in that, it contains mono- or disaccharides as filling substance, starches and/or crospovidone as disintegrating agent, stearate salts or esters, or hydrogenated vegetable oils as lubricants.

4. Composition according to claim 1, characterized in that, it contains enalapril maleate as active substance, maleic acid as stabilizing agent, lactose as filling substance, starch and crospovidone as disintegrating agent, magnesium stearate, hydrogenated vegetable oil or talc as lubricant, povidone as binding agent and optionally ferric oxide as colouring agent.

5. Composition according to claim 1, characterized in that, it contains enalapril maleate as active substance, maleic acid as stabilizing agent, lactose monohydrate as filling substance, starch as disintegrating agent, magnesium stearate as lubricant and optionally ferric oxide as colouring agent.

6. Composition according to claim 1, characterized in that, the dosage form is a tablet.

7. Composition according to claim 6, characterized in that, the dosage from is a tablet having 75-300 mg of tablet mass.

8. Composition according to claim 1, characterized in that, the dosage form is a capsule filled with granules.

9. Process for the preparation of composition according to claim 1, characterized in that, wet granulation is used.

10. Process according to claim 9, characterized in that, wet granulation is carried out with aqueous solution of maleic acid used as stabilizing agent.

11. Process according to claim 9, characterized in that, the enalapril maleate, lactose, disintegrating and colouring agents are mixed, dried, aqueous solution of stabilizing maleic acid is added, the mixture is wet granulated, the obtained granules are dried, classified, mixed with lubricant and compressed into tablets.

12. Use of maleic acid to stabilize enalapril maleate in a pharmaceutical composition as defined in anyone of claims 1 to 8.

13. Use of maleic acid as stabilizing agent in the manufacture of a pharmaceutical composition containing enalapril maleate as defined in anyone of claims 1 to 8.

14. Composition according to claim 1, characterized in that, it is in a commercial package in the form of orally applicable dosage form together with instructions for its administering.

IV.

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(54) Title: STABLE PHARMACEUTICAL COMPOSITION COMPRISING PRAVASTATIN SODIUM

(57) Abstract: The invention relates to a stable pharmaceutical composition containing pravastatin sodium salt and a salt of lactic acid.

STABLE PHARMACEUTICAL COMPOSITION COMPRISING PRAVASTATIN SODIUM

The invention relates to stable pharmaceutical compositions. The compositions according to the invention contain as active ingredient pravastatin-sodium, which is a HMG CoA reductase inhibitor, and its structure and manufacturing process is described among others in U.S. Patent No. 4 346 227.

Pravastatin-sodium according to formula (I) contains a β,δ-dihydroxy-valerianic-acid group. It is known, that δ-hydroxy-carboxylic acids form a six-membered stable lactone ring. At lower pH regime (pH= 6-7) one of the possible degradation products of pravastatin is the lactone derivative of the formula (III), formed by condensation.
Pravastatin as an acid is nearly as strong as acetic acid (pK= 4,9), so it can go through hydrolysis.
The product of the hydrolysis might be the non-dissociated form of formula (II), which can also be favorable for the formation of the lactone ring. An other possible degradation product is the 3-hydroxy isomer of the formula (IV), formed from a 6-hydroxy-compound of the formula (I) through sigmatrop rearrangement affected by heat and light.

For the above reasons to ensure the stability of the pravastatin containing solid pharmaceutical compositions pravastatin is used in the form of its stable salts, preferably in the form of its sodium salt, and/or a basifying agent is used to impart a desired pH of at least 9, preferably 9,5 to an aqueous dispersion of the composition (EP 0 336 298 B1). As basifying agent magnesium oxide, aluminium oxide, an alkali metal hydroxide such as sodium hydroxide, potassium hydroxide or lithium hydroxide, or alkaline earth metal hydroxide or oxide such as calcium hydroxide or magnesium hydroxide or magnesium oxide are mentioned.
According to the solution described in WO 00/35425 to ensure the basic environment the pravastatin sodium contains a small amount of a buffering agent, preferably less than 1%, most preferably 0,3%, based on the weight of the active substance. To neutralise the acidifying effect of carbon dioxide the final composition may contain an additional amount (preferably 10-20 %, based on the weight of the composition) of buffering agent. As buffering agents sodium or potassium citrate, sodium phosphate, dibasic sodium phosphate, calcium carbonate, hydrogen phosphate, phosphate, sulphate, sodium or magnesium carbonate, sodium ascorbinate, benzoate, sodium or potassium carbonate,

lauryl sulphate are mentioned and the use of sodium citrate and dibasic sodium phosphate are exemplified.

Disadvantage of the above solutions is, that at pH=9-10 additional decomposition products may be formed. So through hydrolysis of the iso-butyric acid, compounds of the formula (V), and - as a result of a Cannizzaro like process induced by hydroxyl ions being present in a higher concentration because of the higher pH value - the decomposition product of the formula (VI).

The aforesaid decomposition is presented in Scheme 1, which contains Figures 1 to 6. Figure 1 shows Formula (I), Figure 2 shows Formula (II), figure 3 shows Formula (IV), figure 4 shows Formula (IV), figure 5 shows Formula (V), and figure 6 shows Formula (VI).

The aim of the present invention is to provide an oral pharmaceutical composition containing as active ingredient pravastatin sodium with high stability, in which the amount of decomposition products is low even after a long storage.

It has been found that if in an oral pharmaceutical composition containing pravastatin sodium as active ingredient a lactate salt of a metal capable for chelate-complex formation is used in an appropriate amount, a composition with high stability can be obtained even for longer storage.

The invention is based on the recognition, that the carboxyl and hydroxyl groups of pravastatin can be stabilised with the lactate salt of a metal capable for chelate-complex formation, preferably with calcium-lactate. This phenomenon might be explained by the fact, that the carboxylate anion formed by the hydrolysis initiated by the humidity content of the air and the other excipients of the composition forms a week chelate-complex with the metal ion capable for chelate-complex formation, e.g. with the calcium ion of calcium lactate. Thus the negativity of the carboxylate anion is decreased; the anion is stabilised through declining the proton admission ability. The calcium-lactate hinders the sigmatrop rearrangement because of its chelate-complex forming capability. Due to the lower pH-value the hydroxyl ion concentration and so also the chance of taking place of a Cannizzaro process is decreasing. It can be assumed further, that the carboxylate ion shaded by the calcium lactate and the proton localised by the monosaccharides, with proton

acceptor property such as mannitol, lactose etc. used as fillers can accomplish just one hydrogen bridged binding, in which the spheric hindrance can also play a role.

The present invention relates to a stable pharmaceutical composition containing pravastatin sodium as active substance, lactate salt of a metal capable for chelate-complex formation as stabilising agent and one or more auxiliary substances.

The composition according to the invention preferably contains the active substance in an amount of 1.0-20 weight %, the stabilising agent in an amount of 1,0-25 weight %, filling and/or carrying substances in an amount of 0-15 weight %, surface active agent in an amount of 0,1-12 weight %, disintegrating agent in an amount of 0,1-12 weight % and lubricants in an amount of 0-5 weight %.

The pH value of the composition according to the invention is 6,5-8,5, preferably 7,0-8,5.

As stabilising agent calcium lactate or magnesium lactate, preferably calcium lactate may be used.

In the stable pharmaceutical compositions of the inventions ordinary used fillers such as monosaccharides, such as mannitol, lactose, fructose, pre-treated starch, microkristalline cellulose, calcium carbonate, disintegrants such as crospovidone, croscaramellose sodium, lubricants such as magnesium stearate, aluminium stearate, zinc stearate, surface active agents such as hydrogenated vegetable oils can be used.

The stable pharmaceutical compositions of the inventions can be prepared by known methods in that pravastatin sodium is mixed with the stabilising agent and the auxiliary substances and the mixture thus obtained is granulated and/or pressed to tablets.

Further details of the invention are shown in the examples without restricting the scope claimed to the examples.

Example 1

Composition:	(mg)
Pravastatin sodium	20,0
Mannitol	70,0
Pre-treated starch	20,0
Microcristalline cellulose	31,0
Crospovidone	3,0
Ca lactate	20,0
Ca carbonate	5,0
Mg stearate	1,0
Hydrogenated ricinus oil	30,0
Total:	200,0

Table 1

	Starting value	1 month 50 °C	1 month 50 °C/76 % RH
PH	8,3	8,3	8,2
Active material	20,1 (100 %)	19,8 (98,5 %)	19,7 (98,0 %)

Heat treatment in the case of all of the samples:

1 month at 50 °C in a closed glass vessel with cap,

1 month at 76% RH on 50 °C in an open glass vessel.

Technology used in the case of all of the samples:

The substances are previously passed through a sieve and after homogenisation tablets are formed from the mixture by direct tablet formation.

Example 2

Composition:	(mg)
Pravastatin sodium	20,0
Mannitol	90,0
Microcristalline cellulose	35,0
Crospovidone	8,0
Ca lactate	20,0
Ca carbonate	5,0
Mg stearate	2,0
Hydrogenated ricinus oil	20,0
Iron oxide	0,2
Total:	200,20

Table 2

	Starting value	1 month 50 °C	1 month 50 °C/76 % RH
PH	8,5	8,5	8,3
Active material	20,7 (100 %)	20,0 (96,6 %)	19,7 (95,2 %)

Example 3

Composition:	(mg)
Pravastatin sodium	40,0
Mannitol	140,0
Microcristalline cellulose	62,0
Pre-treated starch	40,0
Crospovidone	6,0
Ca lactate	40,0
Ca carbonate	10,0
Mg stearate	2,0

Hydrogenated ricinus oil	60,0
Total:	400,0

Table 3

	Starting value	1 month 50 °C	1 month 50 °C/76 % RH
PH	8,1	8,4	8,5
Active material	38,7 (100 %)	39,5 (102 %)	39,4 (101,8 %)

Example 4

Composition:	(mg)
Pravastatin sodium	40,0
Mannitol	190,0
Microcristalline cellulose	50,0
Crospovidone	16,0
Ca lactate	40,0
Ca carbonate	10,0
Mg stearate	8,0
Hydrogenated ricinus oil	50,0
Iron oxide	0,4
Total:	404,4

Table 4

	Starting value	1 month 50 °C	1 month 50 °C/76 % RH
PH	8,4	8,4	8,5
Active material	38,8 (100 %)	39,2(101,0%)	38,6(99,5%)

Example 5

Composition:	(mg)
Pravastatin sodium	10,0
Mannitol	35,0
Pre-treated starch	13,5
Microcristalline cellulose	30,0
Crospovidone	1,5
Ca lactate	7,5
Ca carbonate	2,0
Mg stearate	0,5
Total:	100,0

Table 5

	Starting value	1 month 50 °C	1 month 50 °C/76 % RH
PH	7,9	7,8	7,6
Active material	9,8 (100 %)	9,7 (98,9 %)	9,6 (98,0 %)

Example 6

Composition:	(mg)
Pravastatin sodium	10,0
Mannitol	45,0
Microcristalline cellulose	17,5
Crospovidone	4,0
Ca lactate	10,0
Ca carbonate	2,5
Mg stearate	1,0
Hydrogenated ricinus oil	10,0
Total:	100,0

Table 6

	Starting value	1 month 50 °C	1 month 50 °C/76 % RH
PH	7,8	7,9	7,5
Active material	10,2 (100 %)	10,1 (99,0 %)	10,0 (98,0 %)

Example 7

Composition:	(mg)
Pravastatin sodium	10,0
Mannitol	47,5
Microcristalline cellulose	12,5
Crospovidone	4,0
Ca lactate	10,0
Ca carbonate	2,5
Mg stearate	2,0
Hydrogenated ricinus oil	12,5
Total:	101,0

Table 7

	Starting value	1 month 50 °C	1 month 50 °C/76 % RH
PH	7,6	7,7	7,5
Active material	9,9 (100 %)	9,7 (98,0 %)	9,6 (97,0 %)

Claims

1) A stable pharmaceutical composition, characterised in that, it contains pravastatin sodium as active substance, lactate salt of a metal capable for chelate-complex formation as stabilising agent and one or more auxiliary substances.

2) The composition according to claim 1, characterised in that, it contains the active substance in an amount of 1.0-20 weight %, the stabilising agent in an amount of 1,0-25 weight %, filling and/or carrying substances in an amount of 0-15 weight %, surface active agent in an amount of 0,1-12 weight %, disintegrating agent in an amount of 0,1-12 weight % and lubricants in an amount of 0-5 weight %.

3) The composition according to claim 1 having a pH value of 6,5-8,5, preferably 7,0-8,5.

4) The composition according to claim 1, characterised in that, it contains as stabilising agent calcium lactate or magnesium lactate, preferably calcium lactate.

5) Use of calcium lactate for stabilising a pharmaceutical composition containing as active ingredient pravastatin sodium.

6) Process for the preparation of a pharmaceutical composition according to claim 1, characterised in that pravastatin sodium is mixed with the stabilising agent and the auxiliary substances and the mixture thus obtained is granulated and/or pressed to tablets in a manner known per se.

V.

Co-crystal integrity and pharmaceutical role of Cremophor ELP

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Abstract

Co-crystals are sensitive to dissociation in aqueous microenvironment losing their effects on bioavailability increase before oral administration. The integrity of the fumaric acid co-crystal of SAR1 active pharmaceutical ingredient (API) 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. Slight dissociation of fumaric acid co-crystal was measured by XRPD in all cases, lowest dissociation was observed when Cremophor ELP was added to the granulation liquid. Dissolution profiles of the formulations were analysed by flow through dissolution method. The in vitro dissolution profile of the experimental formulation showing the best co-crystal integrity was approximately 10% lower compared to the formulation with the highest integrity.

Key words: wet granulation; co-crystal integrity; flow through dissolution

1. Introduction

1.1.General introduction

The advantages of pharmaceutical co-crystals are better solubility and dissolution kinetic profiles than that of free base or acid forms [1, 2, 3, 4]. Using co-crystals within formulations gives the opportunity to increase oral bioavailability of APIs, especially when free acid or base forms show very low aqueous solubility such as BCS Class II and IV actives [5, 6]. 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 target 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. Cremophor ELP is commonly used as solubiliser and is known to ensure the integrity of the co-crystals [7, 8, 9, 11]. Cremophor ELP has been demonstrated to be a well tolerated pharmaceutical excipient via oral route [13, 14].

SAR1 fumaric acid co-crystal was used as model active pharmaceutical ingredient in the present study. Increased bioavailability of fumaric acid co-crystal versus the free base could be confirmed in a pharmacokinetic study [11].

2. Materials and Methods

2.1. Materials

2.1.1. Active pharmaceutical ingredient

SAR1, a co-crystal with fumaric acid, was used in the study as a model material (Fig.1).

As a weak base with pKa_1 of 2.9 and pKa_2 of 3.5 salt formation is only feasible with strong acids however, under strong acidic conditions, hydrolysis occurs. Salt formation process has not further been taken into consideration to avoid chemical degradation of the parent compound.

The batch of the fumaric acid co-crystal was manufactured in 0.7 kg scale. Particle size distribution of SAR1 showed 15.7 μm at D(90) measured by laser diffraction.

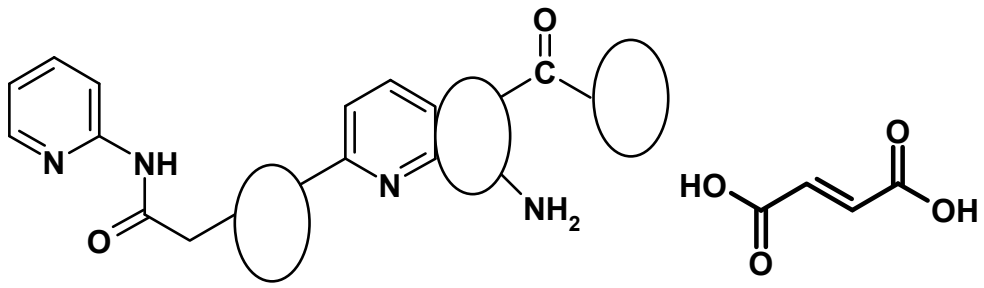

Fig 1: SAR1 fumaric acid co-crystal as a model active pharmaceutical ingredient (API)

2.1.2. Dissolution buffer

Acetate buffer solution, pH=4.5 with 0.5 % sodium dodecyl sulphate was prepared according to USP recommendations. Sodium dodecyl sulphate was ordered from Fluka. Discriminative properties of the dissolution method were evaluated in a separate study [11].

2.1.3. 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 [6].

Pharmaceutical excipients such as mannitol, microcrystalline cellulose, HPMC, croscarmellose sodium and stearyl fumarate sodium, all compliant to pharmacopeial requirements, were ordered from the internal warehouse of Sanofi. Excipients were selected based on results of chemical and physical compatibility pre-tests containing 1 % of active as the most sensitive concentration after 30 days storage at 50°C and 50°C, 75% R.H.

2.2. Methods

2.2.1. Chemical manufacturing of the co-crystal

The reactor was charged with acetone (12 L), SAR1 base Form III (592 g) and fumaric acid (600 g). The slurry was stirred at room temperature for 24 hours, the crystals were filtered off, washed with water (1 L) and ethanol (1 L), and dried in a vacuum at 80°C for five hours.

The obtained yield was 723 g (94.0%) as pale yellow powder. The purity of the product was 98.9% determined by HPLC.

2.2.2. Analytical methods

2.2.2.1. Dissolution method

Experimental dissolution work was carried out in an opened, Sotax type flow through dissolution equipment [10]. The temperature of the media was 37.0 ± 0.5 °C. Dissolution samples were collected by a fraction collector followed by a spectrophotometric analysis. Samples were collected up to 120 minutes. Flow through dissolution was performed on 100 mg mass tablets with 10% API load.

2.2.2.2. Spectrophotometric method

The analysis of dissolution samples was performed by an Agilent 8453 type spectrophotometer. Samples were measured at 342 ± 2 nm undiluted (90 and 120 minutes dissolution) or after 200-fold (until 20 minutes dissolution), 40-fold (30 minutes dissolution) or 60-fold (until 60 minutes dissolution) dilution with dissolution medium acetate buffer.

2.2.2.3. XRPD method

The X-ray diffractograms were recorded on a PANalytical X'PertPro diffractometer, using Cu-K_α (without monochromator) radiation. The granulated samples were loaded to a 25mm standard holder and measured in the range of 3- 35 ° 2Θ with 0.007°/min scan speed. Starting materials and the centrifuged suspension samples were measured on silicon zero background holder with 0.05°/min scan speed.

2.2.3. Pharmaceutical formulations

The composition and function of each formulation are summarized in Table 1.

2.2.3.1. High shear granulation

Manufacturing of the different formulations were performed in Mi-pro miniaturized high shear granulator (Pro-C-ept). The speed of the impeller was 500 rpm while the chopper rpm was 3000.

Four experimental compositions were manufactured in 30g miniaturized scale with 10% API load. The integrity of the co-crystal was studied from granules.

Table 1: Formulation compositions and function of ingredients

Formulations	Function of ingredients	F1	F2	F3	F4
Internal phase					
SAR1 *fumaric acid co-crystal*	active pharmaceutical ingredient	10 %*	10 %*	10 %*	10 %*
mannitol	diluent	49 %	49 %	49 %	49 %
microcrystalline cellulose	diluent	25 %	25 %	25 %	25 %
Hypromellose	binder	5 %	5 %	5 %	5 %
croscarmellose sodium	disintegrant	4 %	4 %	4 %	4 %
Cremophor ELP	surfactant solubiliser	5 %	5 %	5 %	5 %
granulation liquid	-	water	water + Cremophor ELP	water	water
position of water	-	Added to the internal phase	added to the internal phase	added to the active directly	added to the internal phase
position of Cremophor ELP	-	Last excipient of the internal phase	part of the granulation liquid	last excipient of the internal phase	added to the active directly
External phase					
stearyl fumarate sodium	glidant	2 %	2 %	2 %	2 %
Total	-	100 %	100 %	100 %	100 %
Mass of tablets	-	100 mg	100 mg	100 mg	100 mg

* expressed as free base, fumaric acid parts are corrected from quantity of the diluents

Loss on drying values were measured at 105°C until 20 minutes three times during the manufacturing process: after mixing of the internal phase without Cremophor ELP, after the wet granulation process and after drying. Comparable loss on drying results were reached for the internal phase and after the drying process.

2.2.3.1.1. F1 formulation

API and the excipients of the internal phase were sieved through 0.63 mm sieve size. Cremophor ELP was added to the internal phase as last excipient and granulation was performed with water. Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sive size and finally stearyl fumarate sodium of the external phase was added to the granules.

2.2.3.1.2. F2 formulation

The active and the excipients of the internal phase were sieved on 0.63 mm sieve size. Cremophor ELP was added to the granulation liquid. Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sieve size and finally the excipient of the external phase was added to the granules.

2.2.3.1.3. F3 formulation

Water was added directly to the active followed by the excipients of the internal phase. Cremophor ELP was added to the internal phase as the last excipient.

Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sieve size and finally the excipient of the external phase was added to the granules.

2.2.3.1.4. F4 formulation

Cremophor ELP was added directly to the active followed by the excipients of the internal phase. Granulation was performed with water. Drying of the wet internal phase was performed at 50°C until 45 minutes. Calibration of the granules was made on 1 mm sive size and finally the excipient of the external phase was added to the granules.

2.2.3.1.5. Reference

A suspension formulation was prepared as reference to the solid experiments.

For the 10 mg/ml concentrated suspension formulation API was manually suspended in a mortar in methyl cellulose water solution.

2.2.3.2. Tabletting process

Tabletting was performed on Korsch excentrical tabletting machine with 3-15 kN pressure force. Flat, rimmed tablets were pressed with 30-35 N hardness. The diameter of the tablets were 6 mm. The temperature of the plant was 21°C and the relative humidity was 23 %.

3. Results and Discussion

In a previous work, it was shown that Cremophor ELP can have protective effects against rapid dissociation of fumaric acid co-crystal of SAR1 as active pharmaceutical ingredient [11].

Cremophor ELP was included in the formulations at three different positions. It was the last excipient of the internal phase in two cases (F1 and F3), one time it was added directly to the API (F4) and one time it was the part of the granulation liquid (F2). Different addition orders of water within the formulations were investigated as well. In order to evaluate the effect on the integrity of the co-crystals, water, as standard granulation liquid, was added to the internal phase in three cases (F1, F2, F4) and in one case it was added directly to the active (F3).

The crystallinity of API in the granules was examined by XRPD (Figure 2).

The appearance of a peak at ~12.0° 2Θ not related to any starting phase was observed with different intensities in the granules. This new peak corresponds to a disproportionated free base observed in the reference formulation (10 mg/ml SAR1 suspension). Based on the results of our studies the fumaric acid to API ratio was shown to decrease in parallel with the intensity increase of peak 12.0° 2Θ in the XRPD pattern of centrifuged suspension samples. It suggests that in the granulated samples a minor part of the API disproportionates to base and fumaric acid.

The appearance of the disproportionated phase in the granules are represented by the intensity % of peak 12.0° 2Θ compared to peak 11.6° 2Θ (Table 2). The most intense change was observed in the F3 sample, where the API was mixed with water in a mortar before granulation, which is similar to the preparation of the suspension. The highest level of co-crystal integrity was measured for F2 and F4 formulations where the SAR1 was granulated with the mixture of water and Cremophor ELP (F2) and when Cremophor ELP was added directly to SAR1 (F4).

Table 2: Intensity % of peak 12.0° 2Θ compared to peak 11.6° 2 Θ (100%)

Samples	Intensity %
F1	4.0
F2	2.9
F3	6.7
F4	4.3
SAR1 co-crystal	0.0

When the dissolution kinetics were measured, about 10% dissolution decrease were observed with F2compared to the F1 formulation (Table 3 and Fig. 3). The difference in dissolution among the F2, F4 and F1, F3 formulations is significant at P=0.95 confidence level. A slight decrease in dissolution could have a negative impact on bioavailability that is why it is proposed to increase the content of the disintegrant within the formulation when Cremophor ELP is used.

Table 3: XRPD and dissolution results of the four test formulations

Formulations	F1	F2	F3	F4
Integrity of SAR1 *fumaric acid co-crystal* by XRPD method	F1 and F4 same level of integrity	highest level of integrity	lowest level of integrity	F1 and F4 same level of integrity
Dissociation of SAR1 *fumaric acid co-crystal* by XRPD method	signs of the dissociated co-crystal	signs of the dissociated co-crystal	highest level of dissociation	signs of the dissociated co-crystal
Dissolution profiles	reference profile	≈ 10 % decrease	comparable with F1	≈ 10 % decrease

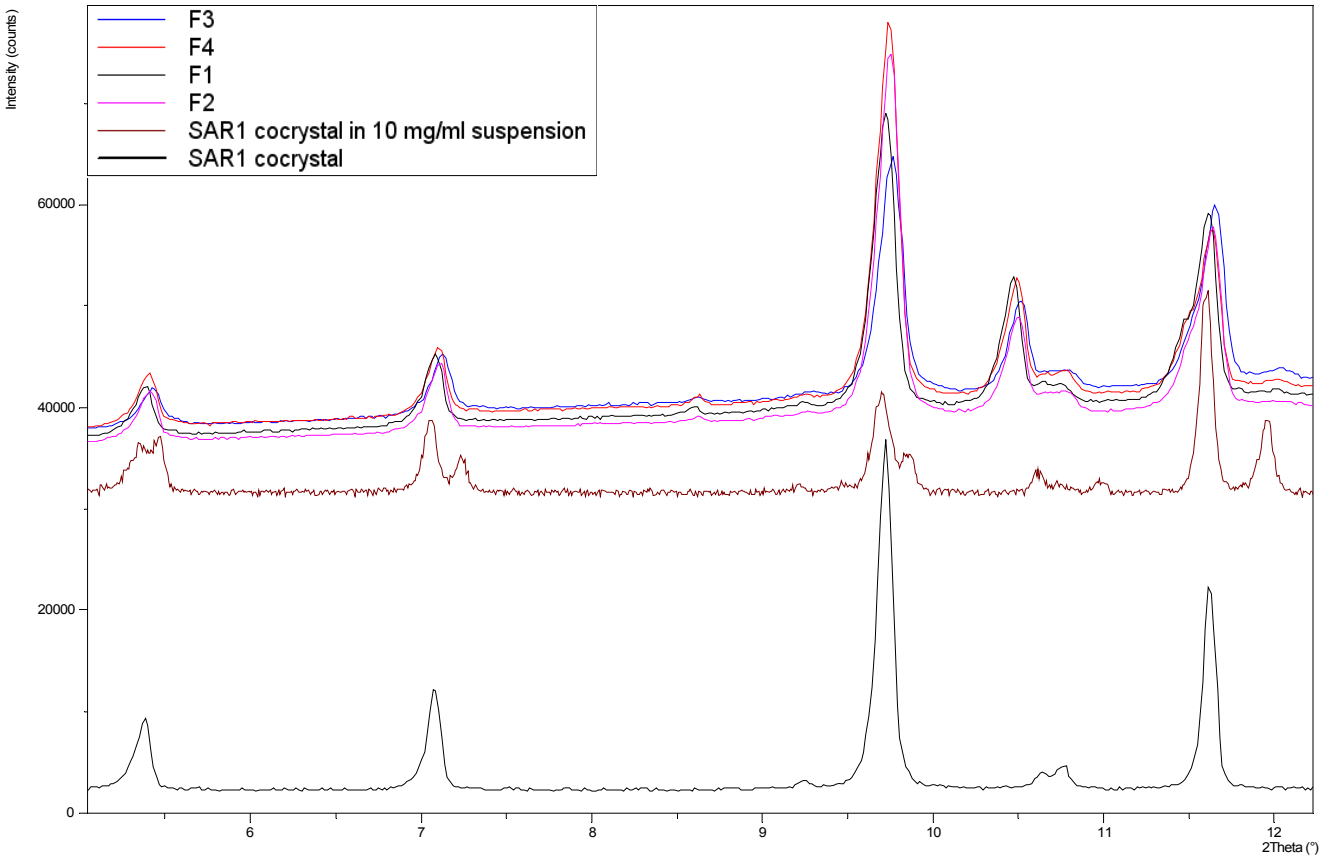

Fig 2: XRPD patterns of the test formulations F1 – F4 compared to SAR1 co-crystal and SAR1 co-crystal reference suspension formulation

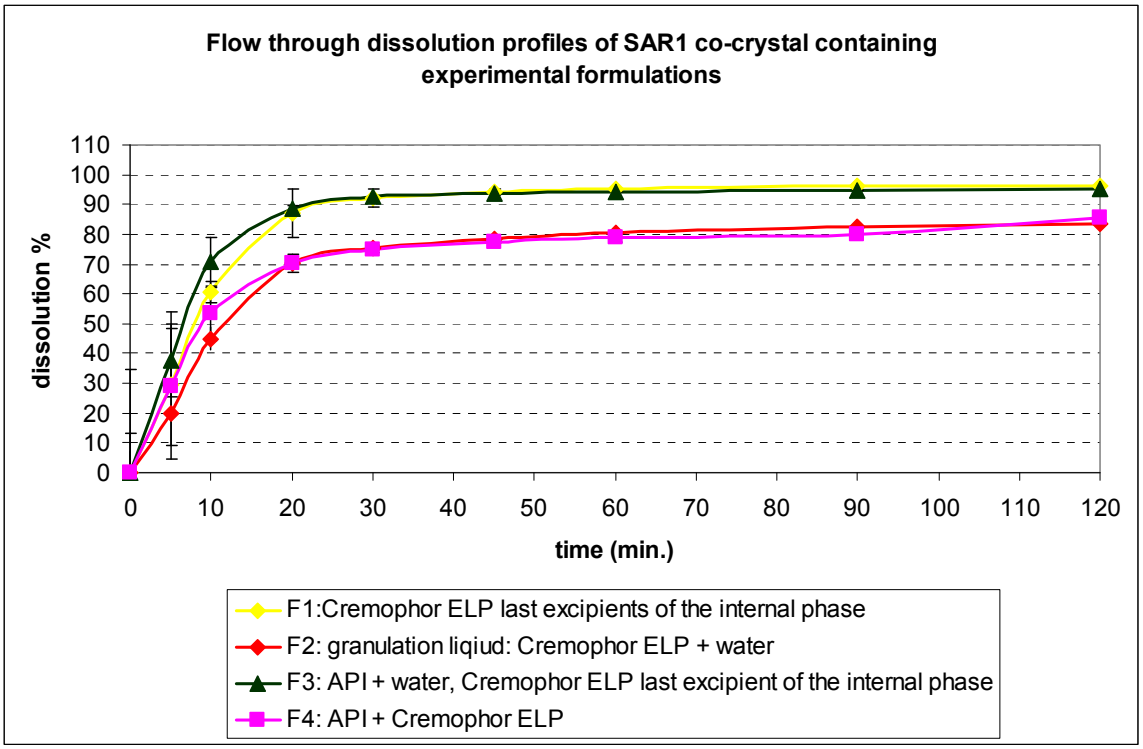

Fig 3: Flow through dissolution profiles of SAR1 co-crystal formulations F1 – F4

4. Conclusion

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 [12].

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. Four experimental formulations were manufactured to study the influence of water and Cremophor ELP order of addition in the formulation process.

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. Dissolution profiles of Cremophor ELP containing formulations needs to be monitored regularly as Cremophor ELP has both an effect on co-crystal integrity and on dissolution kinetics. However from biological effect point of view, ensuring co-crystal integrity is more important than a slightly lower dissolution profile.

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VI.

Flow through Dissolution - a Useful Tool from Discovery Phase to Preclinical Development

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Abstract

Flow through Dissolution technique is a well known approach from early 1970s elaborated for low solubility BCS II and BCS IV [1] 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 Flow through Dissolution Equipment (FTDE) is used for research and development studies mainly, but pharmacopoeias also make it possible to elaborate a method on FTDE for routine analysis. Preparing FTDE for initiating a study is slightly a longer process than in the case of classical dissolution equipment, but researchers can reach significant results even if only a few mgs of the new chemical entities are available. Furthermore the volume of the used dissolution medium is four times lower at least, which is an economic advantage, if the price of the dissolution media is rather high.

The aim of the article is to emphasize the potentials of the equipment during Discovery and Preclinical / Preformulation phase.

Key words: Flow through Dissolution, active pharmaceutical ingredients, solubility, solubility kinetic, solubility in FaSSIF and FeSSIF solutions

1. Introduction

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 [2].

The opened type Flow through Dissolution technique, 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.

It is possible to combine the spectroscopic imaging and Flow through Dissolution technique to improve the possibilities for investigating the release of poorly soluble APIs from pharmaceutical tablets [3].

2. Methods and Materials

2.1. Flow through Dissolution Technique

The experimental work was carried out on opened, Sotax type Flow Through Dissolution equipment. This type of equipment is designed both for on-line spectrophotometric and off-line HPLC analysis. On line spectrophotometric measurement is a suitable tool for routine analysis, when the dissolution kinetic profile is known from previous measurements. The off-line configuration is preferred for Discovery and Preclinical / Preformulation phase, when practically the evaluated candidates or salts have different dissolution kinetic profiles. The set flow rate was 4.0 ml/minutes in all cases to ensure suitable discriminative effect between the candidates; this is the lowest flow rate recommended by the European Pharmacopoeia and USP. The temperature of the media was 37.0 ± 0.5 °C. The scheme of the equipment is presented in Figure 1.

The fraction collector is designed with 60 ml tubes. Based on this the collection of all the fractions is possible with 15-minute sampling intervals. If the sampling interval is increased to 30 minutes, 50% of the fractions are collected automatically, which means that a representative sample is collected.

The end point of the performed studies was between 60 and 120 minutes. According to this the maximum necessary dissolution medium is between 240 and 480 mL / cells. This low quantity dissolution media is preferred when the price of the special media e.g. Fasted state simulating intestinal fluid (FaSSIF) and Fed state simulating intestinal fluid (FeSSIF) is high because good quality of lecithin and Na-taurocholate are proposed for the solution preparation. It is feasible to mimic the in vivo conditions with the media selector tool. In that case several solutions – from acidic to neutral – flow through the same cells. Some researchers put together the Flow through Dissolution Equipment with a Caco-2 cell to obtain some data on the absorption behavior of the active pharmaceutical ingredients, too [4].

As opened-type equipment was used for the trials the candidates met fresh dissolution media during the study providing a tool for studying new molecules with low aqueous solubility.

Different types of cells are available for testing powders, granules, solid dosage formulations and patches. The most appropriate cell for Discovery and Preclinical / Preformulation activities is the powder cell.

Since the particle size distribution of candidates has an impact on the solubility kinetic, it was measured by laser diffraction or by microscopic methods. The differences between particle size distributions are considered for the evaluation.

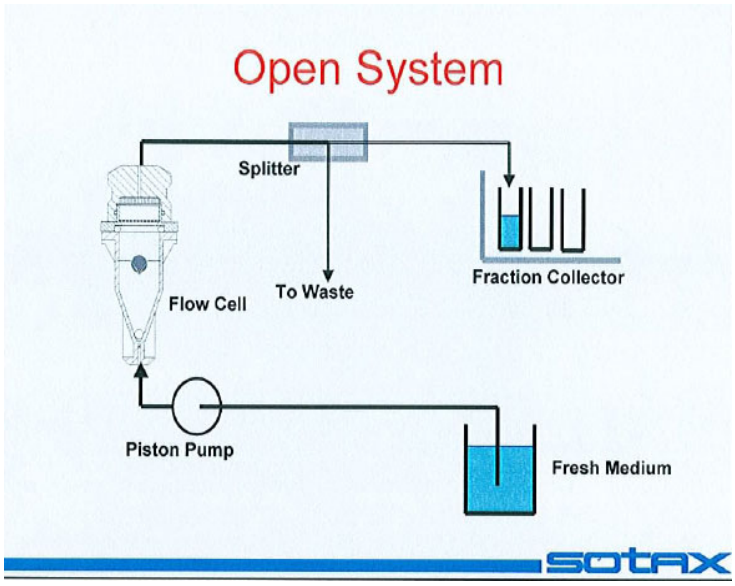


Figure 1

Opened type Flow through Dissolution Equipment

2.2. Comparison of the Classical Dissolution Technique with Flow through Method

During classical dissolution experiments the dissolution medium is practically permanent (except when a media replacement is performed during the sampling period, but this replacement is not comparable to the conditions of a flow through dissolution technique); that is why it is challenging to ensure the sink condition requirement in case of BCS Class II type active pharmaceutical ingredients; it is very often feasible with surfactants, only. Dissolution experiment performed with high concentration of surfactants decreases the correlation possibilities with in vivo conditions.

2.3. Materials

Some model active pharmaceutical ingredients with anti-inflammatory therapeutic effect were selected. These are:

- „A" model: one chemical structure was evaluated,
- „B" model: four different chemical structures were measured,
- „C" model:

  → two different salts and the base form of the active pharmaceutical ingredient were compared

  → FaSSIF and FeSSIF solubility was evaluated

Buffer solutions were prepared according to the USP and Ph Eur recommendations.

The FaSSIF and FeSSIF solutions were prepared based on the description of the USP working group [5] with high quality Lecithin and Na-taurocholate. Lecithin: the manufacturer is Lipoid, product name is Lipoid E PC S, the purchase order number is 108036-1/104.

Sodium taurocholate: the manufacturer is Prodotti Chimici e Alimentari, the purchase order number is 39778608. The analysis of samples was performed on Agilent 1200 type HPLC equipment with gradient method. HPLC parameters in case of the „A" and „B" model materials were: C_{18} XTerra column, 5µm, with 150 mm length and 4.6 mm diameter. The HPLC analysis was performed at 37 °C, with 20 µl injection volume and with 0.8 ml/min flow rate. The A eluent composition was: Water:Acetonitrile:Methane sulphonic acid (1000:25:1) while the B eluent composition was Water:Acetonitrile:Methane sulphonic acid (25:1000:1). The samples were analyzed at 220 nm with UV detector. The concentrations of the standard calibration curve were: 5, 10, and 25 µg/ml. HPLC parameters in case of the „C" model materials were: C_{18} XTerra column, 5µm, with 150 mm length and 4.6 mm diameter. The HPLC analysis was performed at 37 °C, with 10 µl injection volume and with 0.8 ml/min flow rate. The A eluent composition was: 5mM KH_2PO_4, 5mM K_2HPO_4 containing Water:Acetonitrile (950:50) while the B eluent was Acetonitrile. The samples were analyzed at 250 nm with UV detector. The concentrations of the standard calibration curve were: 20, 40, and 85 µg/ml.

3. Results and Discussion

3.1. Discovery Phase

Predicting before clinical testing how a drug will behave in humans requires a battery of sophisticated in vitro tests that complement traditional in vivo animal safety assessments [6]. The quantities of promising new candidates available for early pharmaceutical evaluation are usually limited between 10 to 20 mg during the lead optimization process. In order to choose the best compounds from biopharmaceutical point of view, physicochemical parameters such as solubility, dissolution rate, hygroscopicity, lipophilicity, pKa, stability, polymorphism and particle characteristics need to be evaluated as early as possible and, above all, with the highest accuracy [7]. From the point of view of the success of the research it has a high importance to initiate solubility kinetics studies in buffered and in biorelevant solutions (e.g. in FaSSIF, FeSSIF) to estimate the in vivo behavior of the compounds as early as possible. Two main types of evaluation exist during the early and late Discovery phases. One of them is the formulation support while the other is the early biopharmaceutical evaluation of new candidates. The particle size of the Discovery candidates was fine, below 20μm measured by optical microscope. During the comparison of several dissolution curves of different candidates the particle size was also measured and evaluated. This is essential based on the Noyes-Whitney equation as shown below, since dissolution rate depends both on the specific surface area and the particle size distribution.

$$\frac{dW}{dt} = \frac{DA(Cs - C)}{L} \qquad (1)$$

where:

$\frac{dW}{dt} =$ is the rate of dissolution.

A is the surface area of the solid.

C is the concentration of the solid in the bulk dissolution medium.

Cs is the concentration of the solid in the diffusion layer surrounding the solid.

D is the diffusion coefficient.

L is the diffusion layer thickness.

The greatest effect of particle size on absorption was simulated for low dose - low solubility drugs. In general, the sensitivity of absorption to particle size decreased with increasing dose or solubility. At a solubility of 1 mg/mL, particle size had practically no effect on the percentage of dose absorbed over the range of simulated doses (1–250 mg) [8].

3.1.1. Formulation Support with Flow through Dissolution Technique: testing of "A" model material

Formulation development during early drug discovery and lead optimization involves several challenges including limited drug supply, the need for rapid turnaround, and limited development time. It is also desirable to develop initial formulations that will be representative of final commercial formulations [9]. The target of Discovery is to screen many molecules as fast as possible. This activity can be helped, if the new candidates can be found in a solution formulation because in that case the physical characterization such as determining the polymorphic forms and measuring the particle size distribution within the suspension formulation is not necessary [10]. The basic knowledge required to elaborate a solution formulation is the pH dependent solubility profile of new candidates. The measurement of the pH dependent solubility properties of the new candidates is rather difficult, if the available quantity of the candidate is 10 mg only. Flow through Dissolution Equipment is a very good tool in that case, since there is a possibility to initiate the solubility kinetics study from 1 mg active pharmaceutical ingredient (API) per powder cells. The pH dependent solubility profile and pH range requirement of the administration route determine the pharmaceutical possibilities of the formulators. In the case of „A" model material the pH dependent solubility profiles were measured at three different pHs such as pH=1.2, 4.5 and 7.2. The cumulative flow through dissolution curves are presented in Figure 2. According to the curves it can be established that the dissolution profile of „A" model material is faster and better at pH=1.2 and 4.5 and it has a very low solubility at pH=7.2. This low solubility at pH=7.2 is really a pharmaceutical challenge since nasal administration route is planned and neutral pH was requested by pharmacologists. The formulation issue was solved with a low quantity of surfactant at pH=7.2. The tolerability of the formulation was tested on animal model with good results.

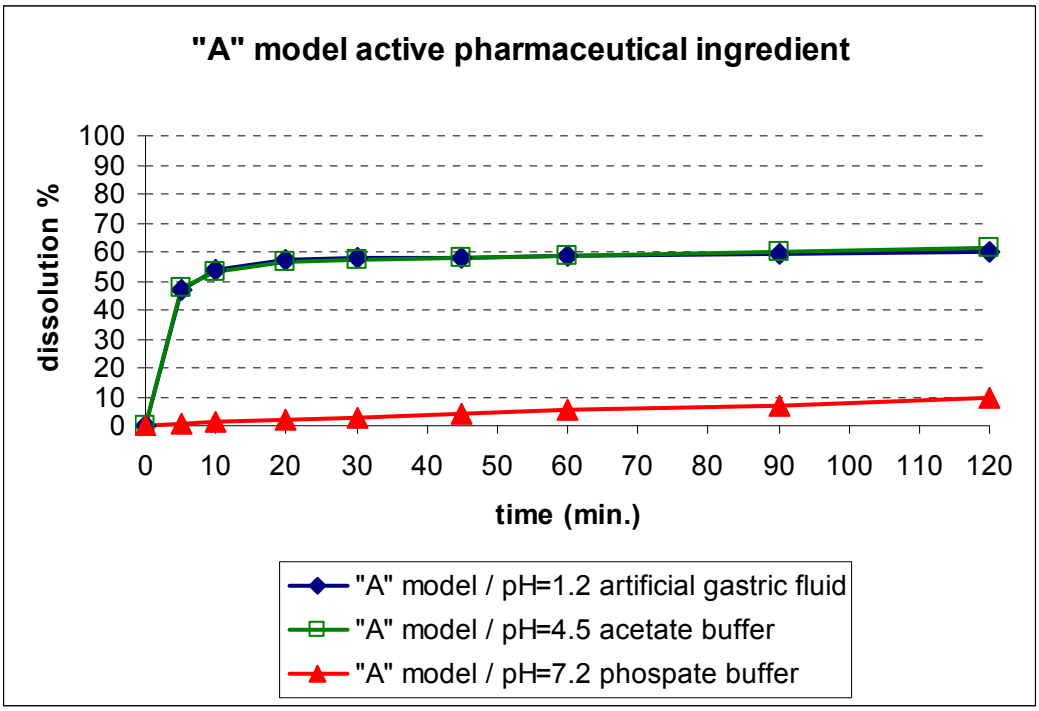


Figure 2
Cumulative dissolution curves of „A" model material

3.1.2. Biopharmaceutical Evaluation of Discovery Model Material with Flow through Dissolution Technique: testing of "B" model material

The objective was to select the lead candidate based on chemical and pharmaceutical assessment. This example illustrates that collaboration between chemists and pharmacists as early as possible is important to identify insoluble chemical scaffolds.

Even though the solubility can be estimated from computation of the effect on solubility of each functional group individually, the exact analysis of the solubility of the complete chemical structure is essential that is why an *in vitro* kinetics test at 37°C has been performed. Based on chemical stuctures of Figure 3 the best solubility kinetic profile was expected from the IVth chemical structure at pH 1.2 in artificial gastric fluid however the fastest dissolution kinetic was measured for the Ist chemical structure. Interesting results were shown by the IIIrd and the IVth structures with similar dissolution kinetics under acidic and almost neutral conditions as well.

The calculated or measured pKa values are available for candidates, if they can be ionized. Based on those values the possible absorption site of the non-ionized forms within the gastro-intestinal tract can be evaluated. The main targets of the early biopharmaceutical evaluation are to measure the pH dependent solubility profiles of the leads or scaffold structures and to support the candidates that have better solubility properties on the estimated absorption site. Different chemical structures of „B" model were evaluated based on the above mentioned considerations. The cumulative solubility

curves are presented in Figure 3. Cumulative curves of Figure 3 show that „B" model / IIIrd structure (has appropriate solubility both at pH=1.2 and 7.2, which is promising because the candidate can be found in a non-ionized form (suitable for absorption) at pH=7.2 under Fasted conditions. „B" model / Ist structure has an excellent solubility at pH=1.2 but we can not use this from pharmaceutical point of view because the candidate can be found 99.9% in ionized form.

Structure II has a pH independent absorption but its solubility is rather low at both pH=1.2 and 7.2, that is why this structure is not proposed for further development.

Structure IV has moderate and practically similar solubility properties at pH=1.2 and 7.2, however 36.5% of the candidate can be found in a non ionized form at pH=7.2 according to the calculated pKa value. Based on the above mentioned facts Structure IV has not been proposed for further development.

The Flow through Dissolution technique was an excellent tool, because the evaluation was available within a short time and it gave a good feedback to chemists and pharmacist as well.

According to the above mentioned approach the new, supported candidates were the ones that have acceptable aqueous solubility on the estimated place of the absorption. This is also very important from Preclinical development point of view, since the costs can be reduced if e.g. particle size decrease can be omitted based on the acceptable aqueous solubility property of the candidate. Naturally not only solubility but the absorption properties of the candidates are so important for the pharmacological effect.

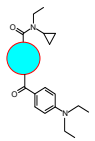
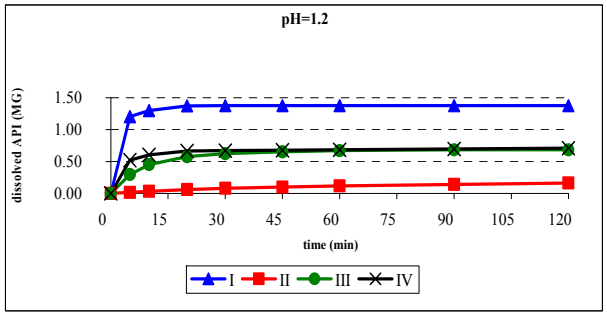
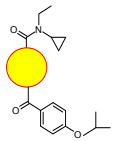
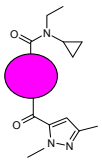
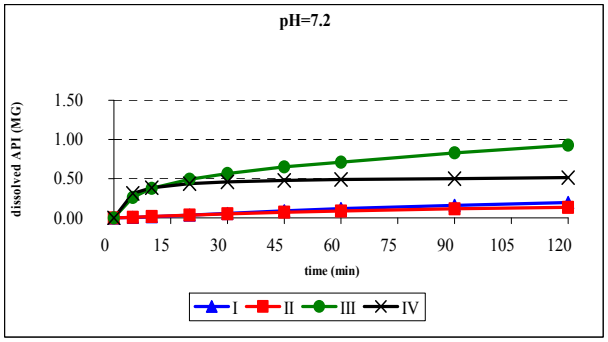
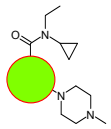
"B" project scaffold structures	pKa	Ratio of the non-ionized form		Flow through dissolution curves
		at pH=1.2	at pH=7.2	
		Fasted conditions		
I 	4.17±0.32 (calculated)	0.1 %	99.9%	
II 	non ionizable	pH independent absorption		
III 	0.69±0.33! (calculated)	76.4 %	100.0%	
IV 	7.44±0.42 (calculated)	0%	36.5%	

Figure 3

Selection of the best scaffold structure from biopharmaceutical point of view

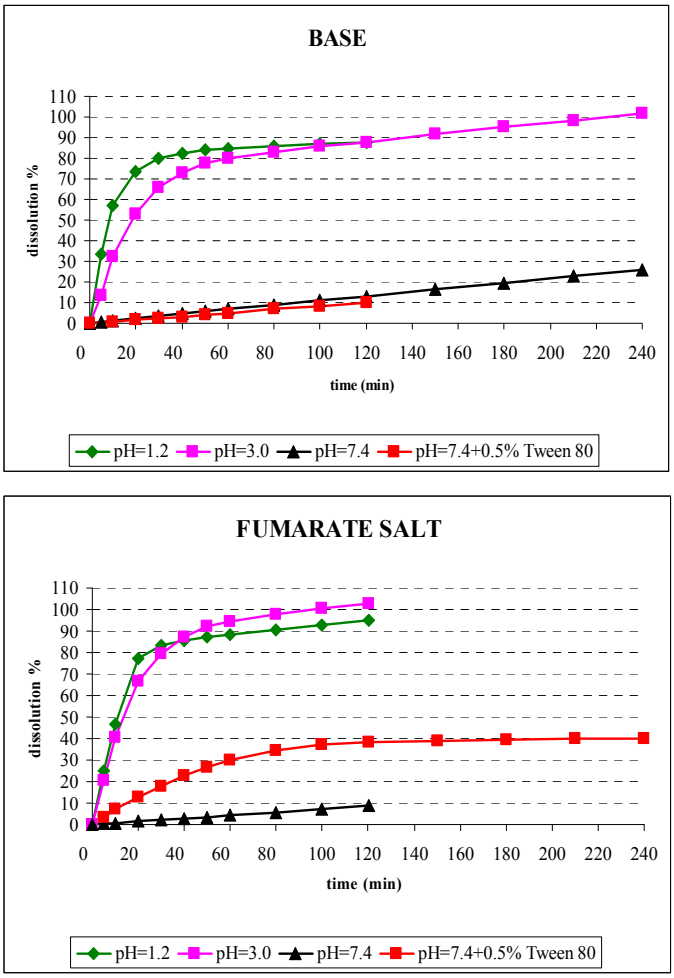
4. Preclinical Phase: testing of "C" model material

Higher quantities of the selected APIs are available for several Preclinical activities such as pharmaceutical evaluation of several salts, co-crystal [11] versus base and formulations.

4.1. Comparison of Several Salts or Co-crystals

The Flow through Dissolution study was performed on two salts (fumarate and di-sulfate) and on the base form of the „C" model. The results are presented in Figure 4.

The dissolution study was prepared at pH=1.2, 3.0, and 7.4 furthermore at pH=7.4 with 0.5 % Tween 80. Based on the flow through dissolution curves it can be seen that the dissolution behavior of the tested two salt forms and the base form is similar (they have decreasing solubility from pH=1.2 to 7.2), however fumarate salt has the best dissolution rate at pH=7.4 when Tween 80 was measured into the dissolution medium. This fact was used during the formulation development of the fumarate salt of the „C" model.



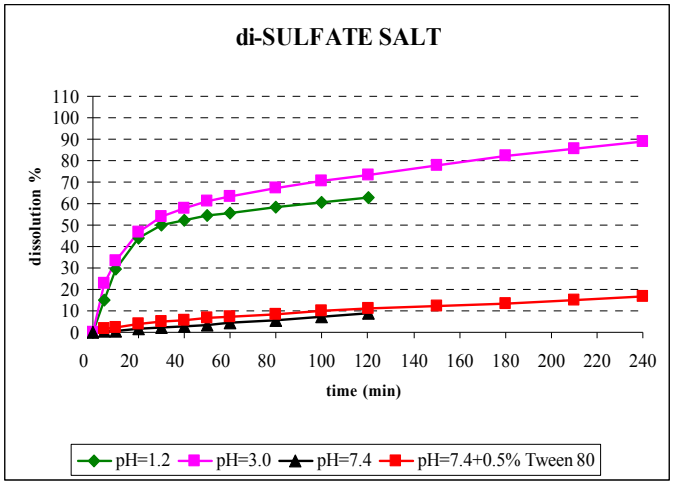


Figure 4

Comparative Flow Through Dissolution curves of „C" model material

4.2. Flow through Dissolution Study in FaSSIF and FeSSIF Solutions

The food effect prediction for „C" model as a fumarate salt was performed on the opened type Flow through Dissolution equipment, which most closely models in vivo conditions. The most frequently used media are the fasted and fed simulated small intestinal fluids (FaSSIF and FeSSIF) developed by Galia et al [12]. Since the studied salt met fresh dissolution media during the dissolution study the results are closer to the dynamic system of the in vivo conditions, than to the static-type classical dissolution techniques. The dissolution results, the calculated reaction speed constants and the evaluation of the kinetic of the dissolution process are summarized in Table 1 while the dissolution curves are presented in Figures 5 and 6.

4.2.1. Evaluation of the kinetic order of „C" model material

After some minutes of lag time a clear first kinetic order was confirmed for the Flow through dissolution in the FeSSIF medium (the correlation coefficient is 0.9889) graphically and with calculation. But the kinetic order in FaSSIF medium correlates with the first order between 30 and 60 minutes dissolution time and it has a pseudo-first order from 90 minutes dissolution time (the correlation coefficient is 0.9551). According to the results of Table 1 higher lecithin and Na-taurocholate content of FeSSIF medium ensures a first kinetic order of "C" model material but in case of the FaSSIF medium the dissolution of the API is limited in FaSSIF medium from 90 minutes dissolution time.

4.2.2. Evaluation of the food effect of „C" model material

1.7 times higher absorption (average of FeSSIF/FaSSIF ratio) is expected based on the in vitro Flow through Dissolution results after high fat containing breakfast, which means a slight risk for food effect.

FeSSIF:FaSSIF ratio measurement is a standard measurement during Preformulation studies, but if the ratio is based on equilibrium solubility or on classical dissolution measurements there is high risk for much higher differences during clinical studies, because classical approaches do not calculate with the dynamic circumstances of the human body. Based on the above mentioned facts FTDE is proposed to measure the FeSSIF:FaSSIF ratio.

Table 1

FaSSIF, FeSSIF Flow through Dissolution results of „C" model material

Dissolution medium FaSSIF, C_0=1 mg						
Time (min.)	Dissolved %	Dissolved mg	Remaining mg (C)	Reaction speed constants		Evaluation of the kinetic
				Zero order $k=\frac{C_0 - C}{t}$	First order $k=\frac{2.303}{t}\log\frac{Co}{C}$	
5	0.02	0.0002	0.9998	0.0040	0.0000	Lag Time
10	3.64	0.0364	0.9636	0.3640	0.0037	
30	35.32	0.35323	0.6468	1.1774	0.0145	First order
45	48.48	0.48477	0.5152	1.0773	0.0147	
60	57.74	0.57743	0.4226	0.9624	0.0144	
90	65.40	0.65397	0.3460	0.7266	0.0118	Pseudo-first order
120	72.98	0.72977	0.2702	0.6081	0.0109	
Dissolution medium FeSSIF, C_0=1 mg						
5	0.04	0.0004	0.9996	0.0080	0.0001	Lag Time
10	9.07	0.0907	0.9093	0.9073	0.0095	
30	63.04	0.6304	0.3696	2.1012	0.0332	First Order
45	76.54	0.7654	0.2346	1.7010	0.0322	
60	87.33	0.8733	0.1267	1.4555	0.0344	
90	93.95	0.9395	0.0605	1.0439	0.0312	
120	100.51	1.0051	-0.0051	0.8376	-	

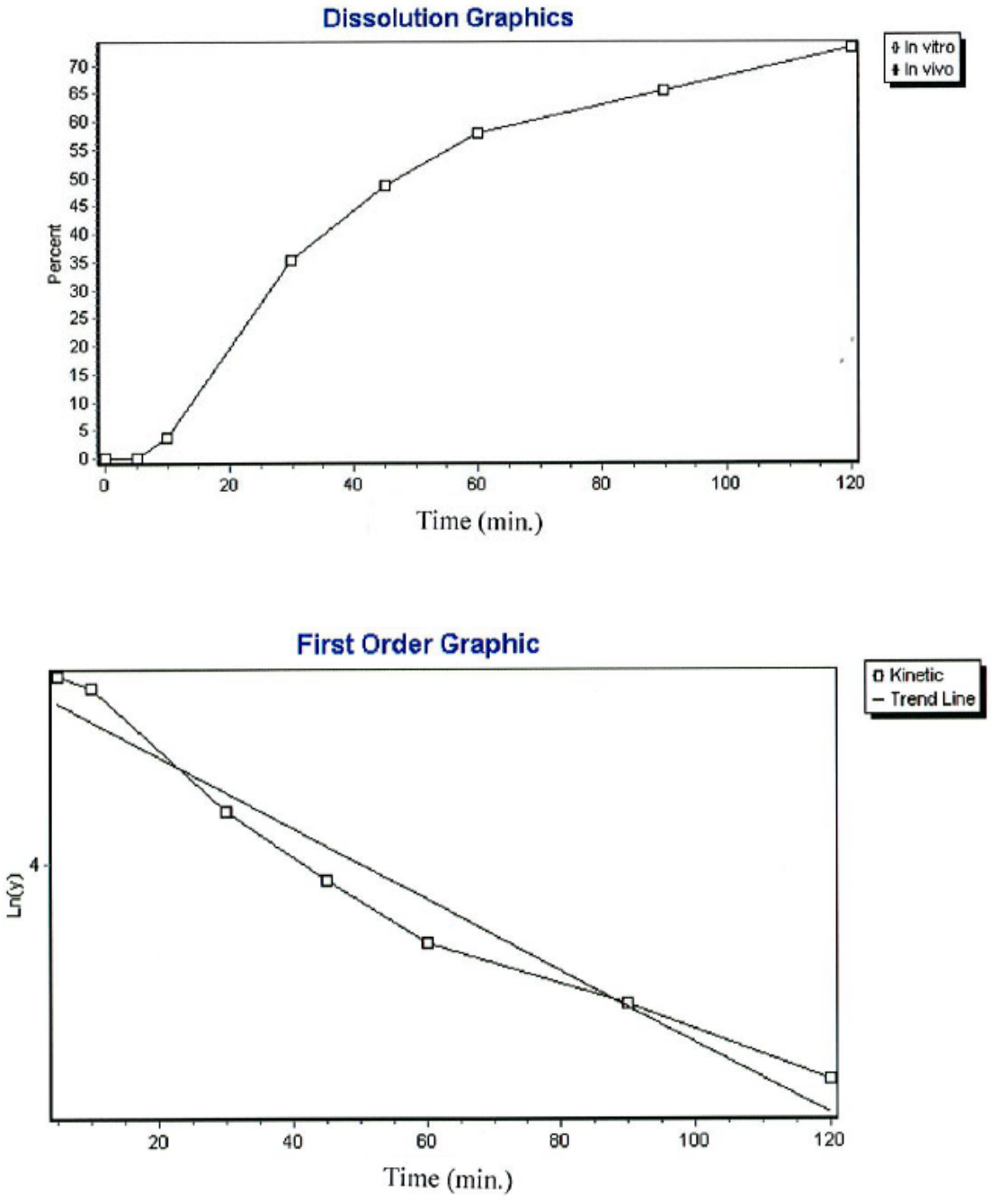


Figure 5

FaSSIF Flow through Dissolution curves of „C" model material

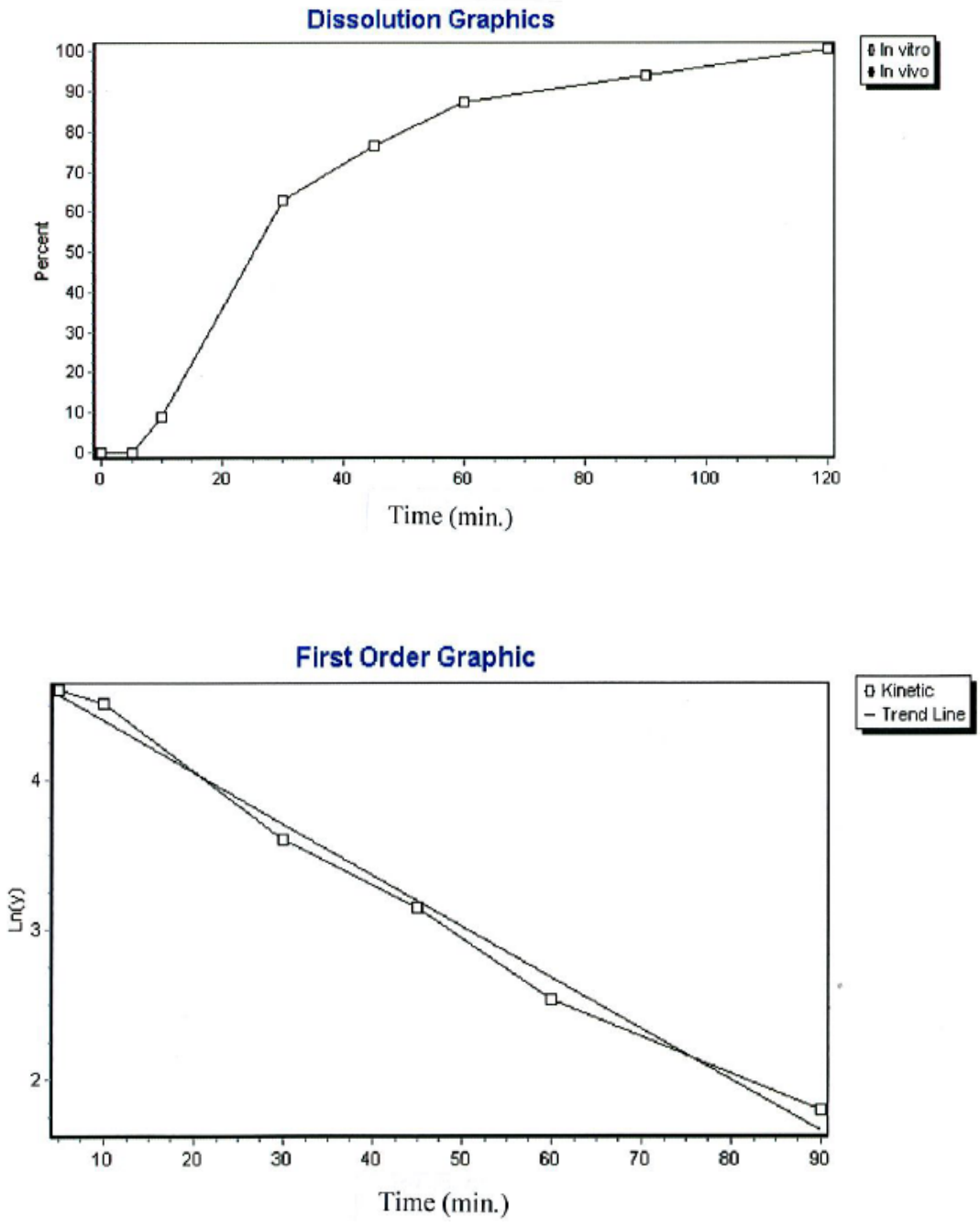


Figure 6

FeSSIF Flow through Dissolution curves of „C" model material

5. Conclusion

Based on the results it can be stated that the 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.

This approach provides a possibility for Preformulation experts to build the pharmaceutical knowledge into the molecules as early as possible. The basic pharmaceutical knowledge regarding the pH dependent solubility of the API was available within a short time with an HPLC analysis and the results gave the possibility to start the formulation approach.

The opened-type FTDE represents the dynamic system of the human body in a better way than the classical paddle or basket methods that is why FTDE has a definitely higher role during the Discovery and Preclinical studies, in particular for BCS II and IV type candidates.

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Related abstract

I.

E-29

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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 Flow through Dissolution Equipment (FTDE) is used for research and development studies mainly, but pharmacopoeias also make it possible to elaborate a method on FTDE for routine analysis. Preparing FTDE for initiating a study is slightly a longer process than in the case of classical dissolution equipment, but researchers can reach significant results even if only a few mgs of the new chemical entities are available. Furthermore the volume of the used dissolution medium is four times lower at least, which is an economic advantage, if the price of the dissolution media is rather high.

The aim of the article is to emphasize the potentials of the equipment during Discovery and Preclinical / Preformulation phase.

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PAT (Process Analytical Technology) – eredmények és lehetőségek

Fekete Pál

A PAT (Folyamat Elemzési Technológia) irányelvet végső formájában az FDA 2004 szeptemberében hozta nyilvánosságra. Annak ellenére, hogy a PAT módszerek alkalmazása más iparágakban már bizonyította a termékminőség javítására, a termelékenység fokozására és a költségek csökkentésére gyakorolt kedvező hatását, a gyógyszeriparban való széleskörű elterjedése mindmáig várat magára.

Az elmúlt időszakban pedig számos olyan mérési módszert dolgoztak ki, illetve újítottak meg, amelyek alkalmasak a gyógyszergyártás során a kiindulási anyagok, a közbenső termékek és a végtermékek kritikus minőségi paramétereinek mérésére, az adatok sokoldalú feldolgozására és a hagyományos minőségi követelményekkel való harmonizációjára. A PAT bevezetése ugyanakkor új ismereteket, új szervezeti formákat és szisztematikus kísérleti munkát igénylő feladat, ezért a kezdeti lelkesedést a kockázatok felsorolása váltotta fel. Mivel ezek jó része jogos volt, ez új lendületet adott a szabványosítható, robosztusabb, egyszerűbben kezelhető, olcsóbb mérő- és adatfeldolgozó rendszerek kifejlesztésére. A PAT módszerek bevezetésének bonyolultsága miatt azonban a legtöbb szakértő a kis lépésekben való haladás taktikáját javasolja.

Bár a PAT alkalmazása önkéntes, a bevezetésével késlekedő vállalatok könnyen versenyhátrányba kerülnek a minőség, a hatékonyság, a gyártási költségek csökkentése, az új termékek kifejlesztési időigénye, valamint a változtatások engedélyeztetése területén.

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PAT helyzet a parametrikus felszabadításban

Monori Csilla

A parametrikus felszabadítás alkalmával a késztermék felszabadítása speciális paraméterek esetében a késztermék rutinszerű vizsgálatának elhagyásával, a gyártásközi vizsgálatok eredményei és gyártási paraméterek ellenőrzése alapján történik. Jelenleg a parametrikus felszabadítás csak végső tartályukban sterilezett termékekre engedélyezett. Ezeknél a termékeknél a sterilitási vizsgálatnak mint leghosszabb ideig tartó vizsgálatnak elhagyása jelentősen lerövidíti a felszabadítási folyamat időtartamát, ami jelentős költségcsökkentő tényező. Ez azonban nem történhet a termékbiztonság rovására. Egy jól kidolgozott PAT rendszer a biztosítéka annak, hogy a felszabadító a döntéshozatalkor ne kerülhessen patthelyzetbe, és mindig az érvényben lévő forgalombahozatali engedélyben rögzítetteknek, valamint az aktuális GMP követelményeinek megfelelő terméket engedjen forgalomba.

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Kockázatelemzés, mint a Minőségügyi Rendszer része

Morvai Magdolna

A XXI. század minőségbiztosítási rendszerének alapja, a termék minőségét befolyásoló hatások elemzése, értékelése, azok kritikusságának besorolása, abból a célból, hogy a kockázatok csökkentésére, megelőzésére, s ezáltal a termék minőségének fenntartására megfelelő intézkedéseket lehessen meghozni.

Általánosan ismert, hogy a kockázatot az ártalom előfordulási és súlyossági valószínűségének kombinációjaként határozzák meg. Egy gyógyszertermék gyártása és felhasználása szükségszerűen több kockázati fokozatot foglal magában.

A hatékony kockázatirányítás biztosítja a gyógyszerter-