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Commentary

Pharmaceutical evaluation of early development candidates "the 100 mg-approach"

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Abstract

Early development candidates are often selected for pre-clinical and clinical development based primarily on pharmacological and toxicological data. In order to choose the best compounds from a biopharmaceutical point of view, physicochemical parameters such as solubility, dissolution rate, hygroscopicity, lipophilicity, pK_a, stability, polymorphism and particle characteristics need to be evaluated as early as possible and above all with the highest accuracy. However, the low amounts of drug substance available in early development often compromise data quality, and therefore, hamper an early pharmaceutical assessment. This article summarises the Aventis approach on early pharmaceutical compound profiling with the aim of providing a high quality assessment requiring not more than 100 mg of drug substance. In particular, the evaluation criteria, process and miniaturised analytical technology that can be applied for this purpose are discussed.

Keywords: Physicochemical characterisation; Pharmaceutical evaluation; Salt selection; Miniaturisation; Pre-formulation

1. Introduction

Development cycle times are continuously challenged in order to remain competitive in an ever increasing competitive environment. Due to modern high-throughput technologies such as combinatorial chemistry and pharmacological screening the number of new chemical hits intended for pre-clinical and clinical development increased tremendously over the recent years (Gaviraghi et al., 2001). However, in addition to the increased speed of "pharmacological" compound selection, there is a strong need to optimise and choose the best compounds from a pharmaceutical "developability" point of view (Venkatesh and Lipper, 2000). In other words, to decide as early as possible on the most promising candidate and the right physical form for the intended route of administration.

Abbreviations: BA, bioavailability; BCS, biopharmaceutical classification system; CE, capillary electrophoresis; DMPK, drug metabolism and pharmacokinetics; DSC, differential scanning calorimetry; DVS, dynamic vapour sorption; eADMET, early absorption, distribution, metabolism, elimination and toxicology; FaSSIF, fasted state simulating intestinal fluid; FeSSIF, fed state simulating intestinal fluid; GIT, gastro-intestinal tract; HSM, hot-stage microscopy; HTS, high-throughput screening; (HP)LC, (high pressure) liquid chromatography; ICH, International Conference on Harmonisation; Mini-FTC, miniaturised-flow through cell; PK, pharmacokinetics; PSD, particle size distribution; RH, relative humidity; SGA, spectral gradient analysis; SSA, specific surface area; XRPD, X-ray powder diffraction

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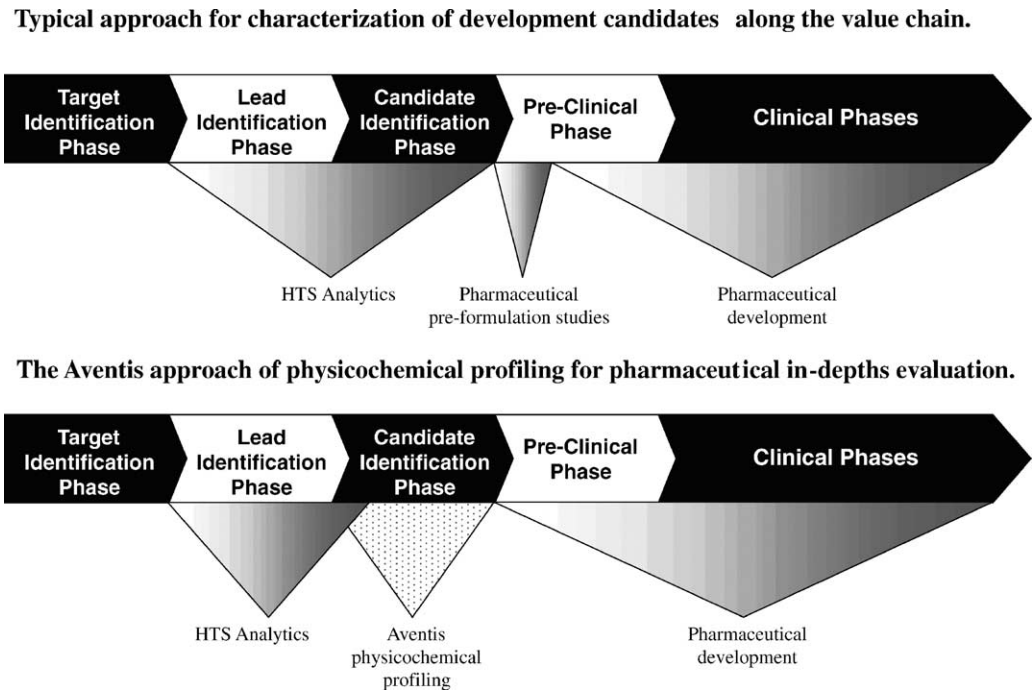

Fig. 1. Development value chain.

In pharmaceutical industry, increased awareness of the relevance of physicochemical parameters for development (e.g. "Rule of 5", Lipinski et al., 1997) resulted in an extensive application of analytical characterisation tools in recent years. Most physicochemical characterisation approaches are more or less divided into two phases (Streng, 1997; Curatolo, 1998):

(a) early analytical high-throughput screening (HTS);
(b) complete pharmaceutical pre-formulation studies.

During lead and early candidate identification (Fig. 1), analytical HTS technologies (Kerns, 2001) and in-silico prediction tools (Blake, 2000; Clark and Pickett, 2000; Walters and Murcko, 2002) are used for selected parameters to support compound optimisation programs. Techniques such as nephelometry (Bevan and Lloyd, 2000) and computational approaches are excellent tools to provide approximate values for those programs, however, they do not provide an adequate accuracy for a conclusive pharmaceutical assessment.

Because the candidate molecules are often available in limited amounts and with a low degree of purity, accurate pharmaceutical pre-formulation studies usually take place after candidate selection, during pre-clinical or even early clinical development phases. The risk of this approach is that developability issues might be discovered after expensive and/or time consuming studies have been performed.

Therefore, it is desirable to start a pharmaceutical in-depth evaluation program soon after lead identification. However, for generating high quality data sets with drug substance quantities in the lower milligram-range, miniaturised or optimised analytical technologies, customised processes and appropriate evaluation criteria need to be established.

2. Evaluation process and assessment

At Aventis, a two-step approach in which the level of analytical accuracy and output is adapted to the intended use and compound availability has been established.

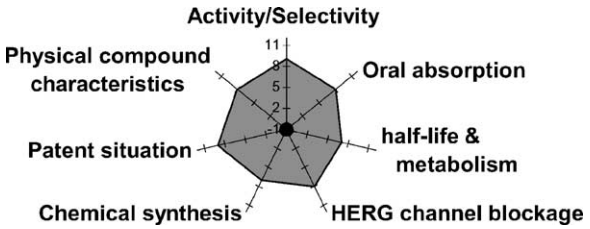

Fig. 2. Multi-parameter optimisation of a lead (Wess et al., 2001).

2.1. Analytical high-throughput screening (HTS)

During lead and early candidate identification a high-throughput approach is applied to provide preliminary physicochemical characterisation. The latter focuses in particular on the requirements of early absorption, distribution, metabolism, elimination and toxicology (eADMET) experiments (i.e. $CaCo_2$ permeability, metabolic stability) and pharmacological assays. Therefore, the physicochemical key parameters pK_a, log D, and turbidimetric solubility are measured. These data are part of the pre-selection process (Fig. 2, Wess et al., 2001) in which the compounds are assessed in comparison to an optimal lead profile.

Rank ordering of these data (e.g. less versus better solubility within a series of compounds) might be sufficient to guide the chemist during the chemical compound optimisation program. However, immediately after pre-selection of 2–10 promising candidates the pharmaceutical in-depth evaluation program needs to be started.

2.2. Pharmaceutical in-depth evaluation program ("the 100 mg-approach")

The physicochemical parameters are selected with regard to the most important aspects of pharmaceutical developability, in particular for oral administration: permeability, solubility, stability and formulation feasibility aspects (Fig. 3). Moreover, commonly used classification systems like Lipinski's Rule of 5 or the biopharmaceutical classification system (BCS) (Amidon et al., 1995) are taken into account.

2.2.1. Solid-state properties

The majority of candidates are solids at room temperature. Therefore, solid-state characterisation is the most prominent aspect of this approach, in which the following properties are investigated:

- specific surface area,
- particle size distribution,
- hygroscopicity,

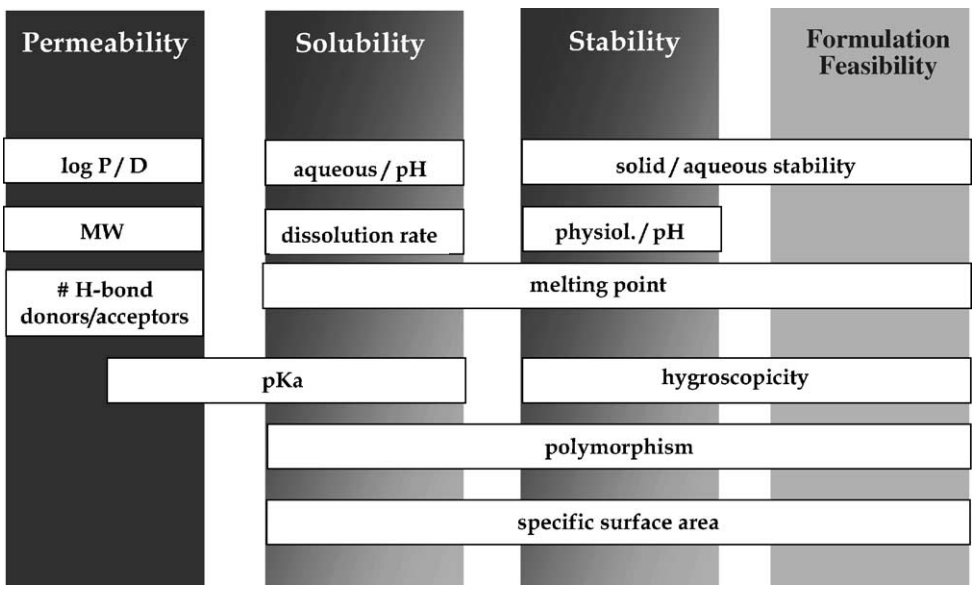

Fig. 3. Relevance of physicochemical parameters.

Table 1
Score card and pharmaceutical evaluation criteria

Test parameter	Technique	Evaluation[a]		
		+	0	−
Solubility in pH 1.2–8.0, FaSSIF, FeSSIF	Shake flask/LC	>100 μg/ml	>10 μg/ml	<10 μg/ml
Dissolution rate (for oral development only)	Mini-FTC	Only for comparison of different salts, modifications, batches with different specific surface areas, etc		
Lipinski's Rule of 5 (for oral development only)		Meets all	Does not meet 1	Does not meet two or more
BCS		Class I	Class II	Classes III, IV
Stability in solution	LC			
Simulated "in-vivo" stability for oral development: pH 1.2–8.0, FaSSIF, FeSSIF in darkness		<2% degradation	2–5% degradation	>5% degradation
Stress stability solid-state	LC			
60 °C/dry		<3% degradation	3–5% degradation	5% degradation, ≥ then storage at + 40 °C
Sensitivity to humidity (60 °C/100% RH)		<10% degradation	10–20% degradation	20% degradation, ≥ then storage at + 40 °C/75%RH
Sensitivity to light (ICH suntest)		<3% degradation	3–5% degradation	>5% degradation
Polymorphism tendency	XRPD, DSC	1 phase	2 or 3 phases	>3 phases or no crystalline
Melting point	DSC, HSM	>120 °C	80–120°C	<80 °C
Hygroscopicity (the whole range up to 95% RH is considered; the limits are given for 60% RH)	DVS	<2%	2–5% if no stable hydrate is formed	>5% if no stable hydrate is formed

[a] For rationale see Section 3.

- polymorphism tendency and melting point,
- stress stability at solid-state,
- dissolution rate.

2.2.2. Solution properties

The parameters listed below determine the characteristics of a compound in solution. They are useful for the development of liquid dosage forms and to understand the pharmacokinetic (and pharmacological) results of eADMET studies:

- dissociation constants,
- solubility as a function of pH,
- partition coefficients as a function of pH,
- stability in solution.

All compounds are characterised and assessed according to a pharmaceutical evaluation score card (Table 1). For the in-depth evaluation program approximately 100 mg of each pre-selected candidate are synthesised with strong focus on purity. Based on experience a purity of 95% should be available to ensure a high quality pharmaceutical data set.

2.2.3. Salt selection

All promising acids and/or bases undergo a salt screening in which a 96 well plate equipment is used for crystallisation experiments (Bastin et al., 2000) with pharmaceutically acceptable counterions (Bowker, 2002). Crystallisation is evaluated by birefringence microscopy and confirmed by X-ray powder diffraction (XRPD). About 50 mg of each confirmed crystalline salt are synthesised and an additional in-depth characterisation is performed for those parameters which might change with the salt form of a given acid or base (Table 2).

2.2.4. Assessment

The physicochemical profile together with the results of the eADMET testing finally lead to an integrated biopharmaceutical and technical developability assessment for candidate selection (Fig. 4).

In the assessment possible obstacles for development are disclosed and the most suitable candidate for development is recommended according to the

Table 2
Pharmaceutical in-depth evaluation of early candidates and their salts: physicochemical parameters tested and amount of substance required

Test parameters	Pharmaceutical evaluation program	Salt screening[a]
Particle size/specific surface area	☒	☒[b]
Hygroscopicity	☒	☒
Polymorphism tendency	☒	☒
Solid-state stress stability	☒	☒
Dissolution rate	☐	☒
Dissociation constant	☒	☐
Solubility	☒	☒
Partition/distribution coefficient	☒	☐
Stability in solution	☒	☐
Amount of substance	100 mg	50 mg[b]

[a] In case that the complete data set is available for the free acid/base.
[b] SSA (BET) is usually determined for the selected salt if a suspension is used in PK studies. This measurement, which requires approximately 100 mg, is performed with the batch used in the respective PK study.

identified pharmaceutical evaluation criteria (Table 1). The assessment is divided into three parts:

- expert statement and recommendation for development,
- one page parameter scorecard for all candidates (+/0/−),
- list of all experimental data for each candidate.

2.2.5. Formulations

In order to link the in-depth evaluation data of a candidate with the results of the pharmacokinetics (PK) experiments, eADMET formulations—in particular the suspensions for bioavailability (BA) studies in animals—are investigated regarding physicochemical aspects such as particle size distribution (PSD), polymorphism, stability and percentage dissolved. These data are not part of the score card ranking but are considered in the expert's final assessment.

3. Pharmaceutical in-depth evaluation program ("the 100 mg-approach"): analytical technologies and evaluation criteria

Since drug substance quantities of 50–100 mg are not sufficient to run a full pharmaceutical characterisation program in the traditional way, miniaturised or specialised analytical technologies are applied to provide high quality data sets (Table 3). It is important to get as much information as possible from a

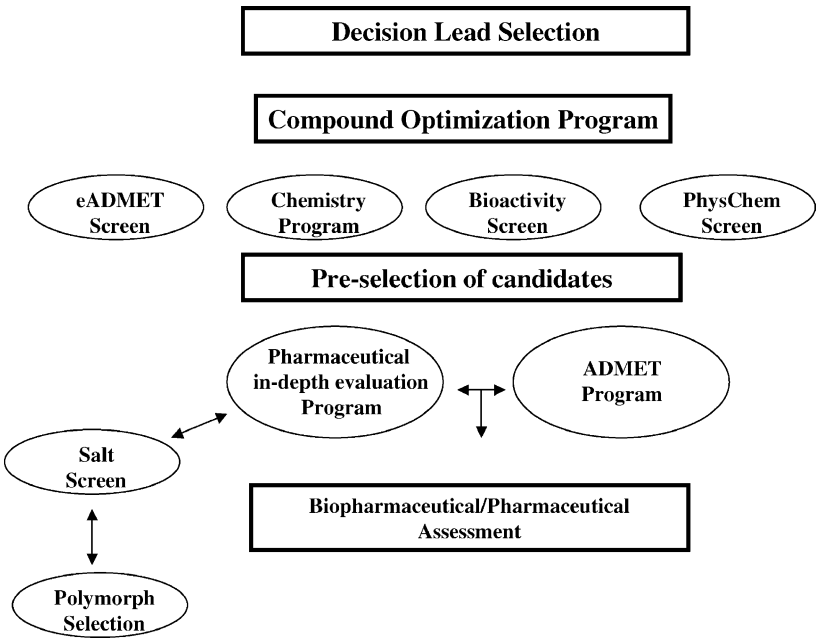

Fig. 4. Evaluation process.

Table 3
Amount of compound required for customised/miniaturised technologies

Parameter	Technology	Quantities (mg)
Dissociation constant	CE	1
Dissolution rate	Mini-FTC	10
Hygroscopicity	DVS	3
Particle size distribution	Microscope	1
Partition/distribution coefficient	LC	1
Polymorphism tendency	XRPD	10
Stress stability at solid-state	HPLC	5
Solubility	HPLC	6
Stability in solution	HPLC	6
Specific surface area	Monosorb	100

testing system, but throughput needs to be considered as well. Therefore, a workflow has been developed to ensure the most effective sample use for evaluation of various physicochemical parameters in a period of approximately 21 days (Fig. 5).

3.1. Specific surface area

During development the specific surface area (SSA) is often used as a quality check for batch release. In the candidate identification phase when only few batches are available, this aspect is not relevant. However, the SSA might have a direct impact on the dissolution rate, and therefore, on the oral BA of a compound. To be in a position to compare the results of oral animal PK studies with future human PK trials, the SSA is measured during pharmaceutical in-depth evaluation. Analytical principle is the quantification of the N_2 volume that can be adsorbed on the surface of a compound (BET). A miniaturised flow method is applied for which an absolute surface area of not more than 0.1 m^2 is necessary in contrast to the volumetric method that needs an absolute surface area of 1 m^2. Since both methods provide equivalent results (Table 4), it was possible to

Table 4
Comparison flow vs. volumetric BET method

Aventis research compound	Flow method (Monosorb) (m^2/g)	Volumetric method (Nova 2000) (m^2/g)
A	2.5	2.8
B	0.7	0.6
C	4.6	5.1
D	3.3	3.3
E	1.0	1.2

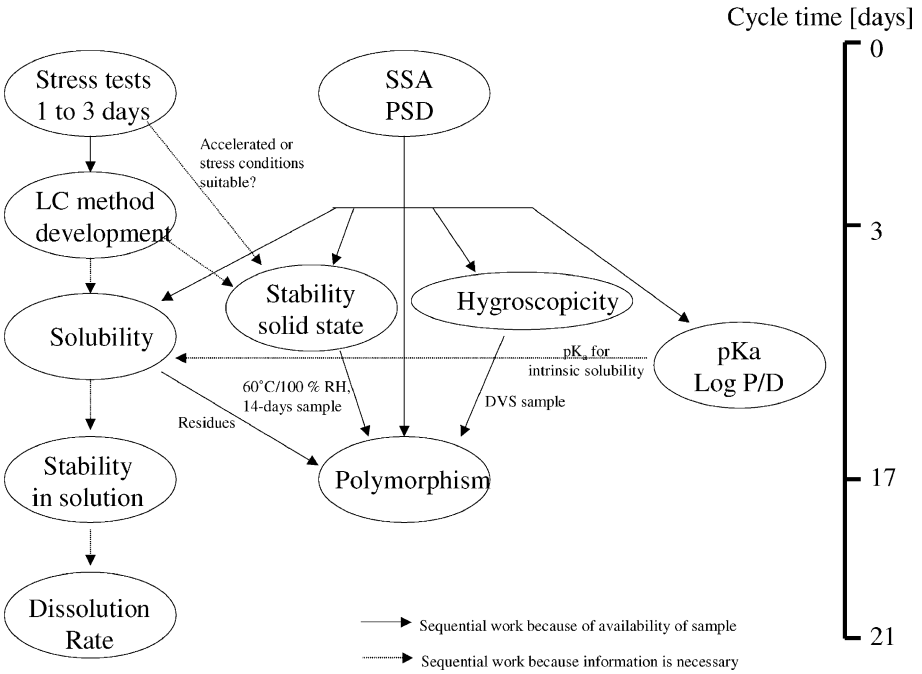

Fig. 5. Workflow for sample analysis.

reduce the required amounts from 1 g to about 100 mg. The major advantage in conducting SSA analysis is that the test is non-destructive and the material can be used for other investigations.

3.2. Particle size distribution

The particle size distribution of a batch is determined at least twice during pharmaceutical in-depth evaluation, in solid-state and in suspension. The latter is necessary, because many compounds for oral animal PK studies are dosed as suspensions, and information is required whether the PSD has been changed during processing. Since SSA cannot be measured in suspension, PSD is the only parameter that enables a correlation between particle size, dissolution and bioavailability in PK studies.

There are many analytical methods available (Streng, 1997). Optical microscopy is applied, because it is quick, simple, independent of the matrix, requires only 1 mg of material, and delivers results of sufficient accuracy for the intended purpose.

Both, SSA and PSD, are not assessed with the score card system, but the values obtained are taken into account to discuss PK and dissolution rate data.

3.3. Hygroscopicity

The amount of water ad-/desorbed can have a significant impact on solid-state stability as well as on formulation aspects. Though hygroscopicity is a function of relative humidity (RH) and temperature, both variables are not varied during pharmaceutical in-depth evaluation. Dynamic vapour sorption (DVS), which requires very little amount of compound (about 3 mg), is used to determine the hygroscopicity at +25 °C, a temperature which is most important for compound handling. After DVS testing, the samples are investigated by X-ray powder diffraction in order to get information on whether humidity triggered modification change has taken place.

Issues are not to be expected with a water uptake of not more than 2% at 25 °C/60% RH. Without formation of a stable hydrate water uptake of more than 5% at this condition will probably require major efforts in formulation development and compound handling (Callahan et al., 1982).

3.4. Polymorphism tendency and melting point

The physical and chemical properties of a compound are strongly dependent on its form in solid-state. It is generally recognised that most organic compounds exist in several solid-state forms (Henck et al., 1997; Grunenberg, 1997). Therefore, the aim of the first investigations is to assess potential for polymorphism tendency.

During salt screening, birefringence microscopy is used as a quick and simple method to estimate whether a compound is crystalline or not. Nevertheless, this technique is not suitable for an in-depth evaluation, because change in morphology or crystal habit as seen under microscope does not necessarily mean that there is a change in the crystal packing or arrangement of the molecules in the unit cell. On the contrary, changes in crystallinity do not necessarily lead to visible changes in morphology. Therefore, miniaturised capillary X-ray powder diffraction is applied, supported by data from differential scanning calorimetry (DSC) and hot-stage microscopy (HSM). XRPD as the first choice does not require much material and is an excellent method for polymorph detection. A three step XRPD approach is performed:

1. initial measurement,
2. temperature-dependent measurement (T-XRPD) showing potential polymorph changes during heating, e.g. manufacturing conditions,
3. miniaturised solvent-screen using solvents with different polarities, i.e. methanol, acetic acid ethylester and acetone.

For the whole investigation about 10 mg of a compound is needed.

If this miniaturised XRPD approach with three solvents and one temperature cycle leads to more than three phases or if all attempts to obtain crystalline material fail, issues during development are to be expected.

DSC and HSM tend to overestimate the number of polymorphs. However, they are fast, provide the melting point, require only small quantities of material (3 and 1 mg, respectively), and are suitable to confirm the XRPD results (i.e. T-XRPD). With regard to solid formulation development the melting point of a synthetic substance should not be below +80 °C. For candidates

with melting points below + 80 °C salt formation is recommended.

At this stage, not all polymorphs will be found nor a final statement on the thermodynamically most stable modification at room temperature can be given. However, during the pre-clinical phase, when more compound is available, the appropriate crystalline form will be determined or confirmed.

3.5. Stress stability in solid-state

Preliminary information on the stability is needed to understand if a compound can be developed and how it must be handled and stored. Stress stability studies are performed to get a timely impression of the influence of temperature, humidity, and light. In addition, these tests are used for LC method development (Fig. 5) and to investigate the degradation pathway with LC/MS. For chiral compounds all investigations are done with regard to chemical and chiral stability.

The following stress conditions are applied using a miniaturised approach:

1. +60 °C for 3, 7 and 14 days,
2. +60 °C/100% RH for 3, 7 and 14 days,
3. artificial sunlight according to International Conference on Harmonisation (ICH) for 1 day.

For each condition approximately 0.2–0.3 mg of compound are required.

If under the above mentioned conditions strong degradation occurs and/or if the melting point is close to the stress temperatures, accelerated conditions (+40 °C, +40 °C/75% RH for 7, 14, 28 days) are considered. Candidates with more than 5% degradation at these accelerated conditions are expected to raise issues.

Because heat and humidity can change the modification, a sample stored at +60 °C/100% RH is also investigated by XRPD after 2 weeks.

Based on the stability results obtained, preliminary storage directions like “store protected from light” or “store protected from humidity” are set.

Important information for development is also derived from the degradation pathway. For example, is the degradation related to high temperature only or is it likely to occur already at long-term storage conditions? Does hydrolysis observed at +60 °C/100% RH correspond to the degradation pathway determined in the experiments described in Section 3.10? Hence, sensitive structural elements are identified very early which can be fed back to the chemical compound optimisation program.

3.6. Dissolution rate

The dissolution rate in physiological media can be a critical parameter for the bioavailability of a compound. In a particular solvent, the dissolution rate is influenced by a compound’s modification, purity, PSD, and specific surface area. Typically, it is not possible to standardise these parameters for different development candidates. Therefore, the dissolution rate is primarily evaluated for salt and polymorph selection of a given NCE. Based on solubility data, the most critical, and therefore, discriminating medium is selected. A miniaturised-flow through cell (mini-FTC) system (apparatus four according to USP XXIV equipped with six microparticle/implant cells; HPLC analysis) is used to determine the dissolution rates of powders with very small amounts of drug substance (approximately 1.5 mg/cell). It has been shown that the method can give important information to predict the in-vivo behaviour of salts in comparison to their mother compound. An example where a sodium salt has been compared with the free acid is given in Fig. 6. Both compounds were granulated and tested in-vivo and in-vitro (fasted state simulating intestinal fluid, FaSSIF medium). The in-vivo results indicated a superiority of the sodium salt over the free acid

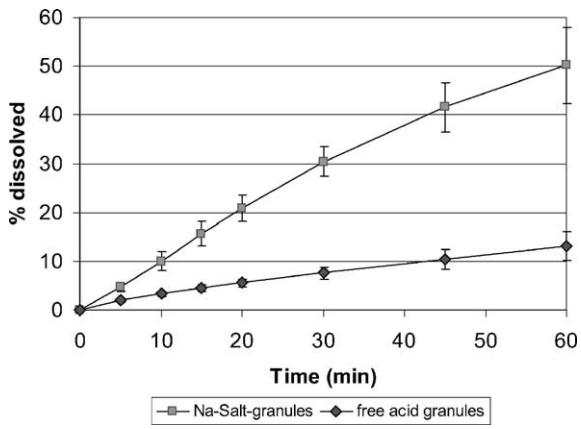

Fig. 6. Dissolution profiles of a sodium salt vs. free acid granules of an Aventis development compound ($n = 6$).

Table 5
Comparison of the pK_a results obtained with CE during in-depth evaluation and SGA during HTS analytics with those of a reference (values that were not measurable with the given method are marked with "–")

Compound	CE	SGA	Reference (Dinnendahl and Fricke, 2003)
Chinidine	4.0	4.0	4.0
	8.2	–	8.6
Furosemide	3.5	3.5	3.9
	10.9	10.2	7.5
Ketoconazole	3.0	3.2	2.9
	6.1	–	6.5
Metoclopramide	9.2	9.3	9.4
Phenytoin	8.1	–	8.3
Warfarin	5.0	4.8	5.0

by a factor of 3.8–5.1 (absolute BA, in dogs). From the in-vitro dissolution results a factor of 3.8 (Fig. 6, values after 60 min) was predicted.

3.7. Dissociation constant

The degree of ionisation is a critical parameter from a physiological point of view and for formulation development. The spectral gradient analysis (SGA) method used during HTS is an excellent tool for fast track analysis, however, the method has some limitations (Kerns, 2001) for compounds with:

- poor solubility in the low μg/ml range,
- a UV chromophore more than four bonds distant from the ionisable moiety,
- more than one, however, close pK_a values.

Therefore, during in-depth evaluation the dissociation constants are re-determined using capillary electrophoresis (CE). This technique requires very little amount of compound (about 1 mg), is suitable for substances with limited solubility, does not require co-solvents, is characterised by high accuracy (Table 5), and facilitates the assignment of pK_a values to basic and acidic centres. Moreover, the method indicates the pH range in which a compound is either not charged or in zwitterionic form. Possible absorption issues in the gastro-intestinal tract (GIT) might, therefore, be disclosed.

3.8. Solubility as a function of pH

Solubility as a function of pH is one of the most important physicochemical parameters. Dissolution rate, and therefore, absorption rate usually correlate with the solubility of a compound and a pH-solubility profile is also required to develop an optimum liquid dosage form. In the candidate identification phase the exact dosing range in man is usually not known. The solubility related evaluation criteria (Table 1) for compounds intended for oral administration, are therefore, based on a presumed dose of 25 mg and BCS criteria. As soon as more information is available the limits are adjusted accordingly. Moreover, in the assessment the amount of impurities and the crystallinity of the compound is considered.

During HTS, solubility is determined with the turbidimetric approach using DMSO stock solutions. Unfortunately, the values obtained have a limited accuracy due to possible over-saturation triggered by DMSO (Kerns, 2001). As a consequence, additional tests are carried out in aqueous media with the shake flask method at +25 °C and +37 °C, in which the medium is added to an excess of compound. The pH is checked and the solubility is measured with the stability indicating LC method developed with stressed samples (see sample flow Fig. 5). For this approach, several authors (Agharkar et al., 1976; Higuchi et al., 1979; Streng et al., 1984; Hintz and Johnson, 1989) describe equilibration periods of some days. Taking into account kinetic aspects these periods might be reasonable. However, considering the mean transit time of a compound in the upper GIT (Theodorakis et al., 1980; Malagelada et al., 1984; Davis et al., 1986; Meyer et al., 1988; Dressman et al., 1998) as well as across the proximal and transversal colon (Dressman et al., 1998) the values are determined in USP buffers pH 1.2, 4.5, 6.8 and 8.0 after 4 and 24 h.

To guide i.v. formulation development, the solubility in water is measured as well and the resulting pH value is documented. In order to get information on the extent of transformation to a salt or another polymorph, XRPD studies are carried out with the precipitates.

Because many lipophilic compounds show food-effects, additional studies in FaSSIF and fed state simulating intestinal fluid (FeSSIF) media (Galia et al., 1998) are executed. A solubility ratio of 5:1

(FaSSIF:FeSSIF) or more indicates a negative food effect, a ratio of 1:5 a positive food effect. Moreover, an increased solubility in FeSSIF (pH 5.0) and FaSSIF (pH 6.5) compared to USP buffer pH 4.5 and 6.8, respectively, reveal a tendency to dissolve in lipophilic media which is important information for formulation development.

3.9. Partition/distribution coefficients as a function of pH

The ability of a compound to be absorbed is related to its partition/distribution coefficient (log P/D). After oral administration a drug will encounter pH values from about 1 (Dressman et al., 1990; Russell et al., 1993) up to 8. Over this wide range, the predominant species in solution, and therefore, the rate of absorption might change significantly. An important reason to measure the partition coefficient is also to aid formulation development of liquid dosage forms (i.e. emulsions or other multiple liquid-phase systems). The distribution of the compound in the multi-phase system will be directly related to its partitioning behaviour. In order to cover the entire range and to also calculate log P, the partition/distribution coefficient is determined by RP-HPLC at pH values which refer to the compounds pK_a. Log P/D is considered in the score card via Lipinski's Rule of 5 (see Table 1).

3.10. Stability in solution

Information on the stability of a compound in solution is needed to understand its characteristics under physiological conditions and to develop liquid dosage forms. The pH-dependent stability testing at +37 °C covers the pH range in the GIT using USP buffers pH 1.2, 4.5, 6.8 and 8.0. For compounds with low solubility (<5 μg/ml) acetonitrile up to 25% (v/v) is added. The incubation times are 2 (pH 1.2 only), 4 and 24 h.

In accordance with the stability testing at solid-state, all tests consider chiral stability and they are also used to investigate the degradation pathway with LC/MS in order to identify sensitive structural elements.

It is obvious that a drug should not degrade significantly in the GIT before being absorbed. Compounds with more than 5% degradation at a physiological pH within the investigated period are expected to raise issues in this respect.

In particular, strong light sensitivity can be a major challenge for development. Therefore, for i.v. formulation development the buffers pH 4.5, 6.8 and 8.0 are additionally incubated under the influence of light.

For the development of liquid formulations (e.g. suspensions, emulsions, solutions), in-use stability studies are performed. The studies orientate on the intended use, packaging, and application way.

4. Discussion and conclusion

In order to facilitate clinical development and to reduce attrition rate, a stronger and earlier focus on pharmaceutical developability issues during candidate selection is desired. Therefore, a pharmaceutical in-depth evaluation and salt screening program has been established to generate high quality data sets using miniaturised technology. In contrast to the early HTS approach, the main focus is on analytical accuracy, however, an adequate throughput has been achieved as well.

Miniaturisation has led to a reduction of drug substance needs from the gram to the lower milligram range. The success of this approach is not based on major technological, and therefore, financial investment. It is the sum total of many stepwise improvements regarding miniaturised or optimised technologies, appropriate evaluation criteria and customised processes.

Those physicochemical parameters known to give critical information on the pharmaceutical developability of a compound have been selected and evaluation criteria have been defined. The latter were continuously improved based on the gained development experience over the last few years. Nevertheless, it is important to stress that the evaluation criteria can only be considered as orientation and that the data for each project need to be discussed on case by case among the experts.

The described approach provides a high quality pharmaceutical evaluation package which on average requires not more than 50–100 mg of a compound. However, the primary pre-requisite for a successful miniaturised analytical evaluation is compound purity (target >95%). It could be shown that if "pure" compounds are evaluated, data quality is comparable to

data obtained with commonly used non-miniaturised equipment. Consequently, attempts are made to purify and crystallise all candidates to achieve the target purity. Possible issues can be detected or predicted before selecting a candidate, which is confirmed by the fact that since this approach has been introduced, no compound has failed due to unexpected physicochemical issues so far.

With the implementation of this approach, a high quality physicochemical data base has been established which will be continuously enlarged and scrutinised to possibly derive new developability rules, focusing on bioavailability and formulation aspects. The gained experience will also enable to further fine tune current evaluation criteria and will be used to develop and optimise in-silico tools, for which high quality data sets are mandatory.

Further improvement is required in the early selection of the thermodynamically most stable polymorph at room temperature, and in the crystallisation process for salt selection. New promising miniaturised automated systems focusing on crystallisation conditions are currently under evaluation and they might help to overcome this bottleneck in the near future.

New analytical approaches such as chip technology might be the next breakthrough to lower the drug substance needs. However, further miniaturisation of the available analytical technologies is currently not expected to keep the achieved level of data quality. Thus, our main focus will be on an increased throughput while keeping analytical accuracy.

Taking all together, it can be shown that with minimum amounts of pure drug substance high quality physicochemical data sets can be provided, which give important information on the pharmaceutical developability of a potential candidate molecule.

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