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Compound selection for development – Is salt formation the ultimate answer? Experiences with an extended concept of the "100 mg approach"

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ABSTRACT

In order to select the best candidates for development, physicochemical criteria such as solubility, chemical and physical stability, hygroscopicity, and thermal characteristics need to be evaluated as early as possible and balanced against other important criteria such as pharmacology or pharmacokinetics. It could be shown, that our miniaturized pharmaceutical profiling concept ("100 mg approach"), is capable to reliably identify potential development issues of drug candidates, which, therefore, can be approached early on. Salt formation is a well established strategy to improve unfavorable properties, in particular poor solubility. This article describes our stepwise approach on salt screening, including selection criteria, and summarizes the observations we had during compound investigation. Considering a data base of 337 compounds (salts and uncharged substances), experiences with various counterions evaluated over the last 10 years are discussed. We realized that salt formation usually improves poor solubility of a given candidate, but this is often at the cost of other attributes being relevant for pharmaceutical development. Surprisingly, in more than 50% of all cases the "free form" was finally selected after carefully weighing all compound characteristics.

Therefore, we conclude that an early salt selection strategy is of utmost importance to predict potential development issues and to enable the provision of alternative physical forms. However, salt formation itself is not necessarily the best solution to meet all development requirements. The selection of a free form (acid or base) in combination with advanced formulation strategies should always be considered, sometimes as best compromise.

1. Introduction

High attrition rates are commonly observed during the development of new molecular entities (NME). Besides unacceptable toxicity and poor efficacy in preclinical and clinical studies, this is mainly due to the lack of desirable physicochemical and biopharmaceutical attributes, all of which contribute to increasing drug development costs (Wilding, 2002).

Since high-throughput screening with DMSO stock solutions have been introduced in research, there is a trend to pre-select large, lipophilic and poorly water-soluble lead structures (Curatolo, 1998; Lipinski et al., 1997) which also tend to be amorphous. Consequently, most prominent physicochemical issues of the final development candidates selected are poor solubility and dissolution rate which frequently result in insufficient bioavailability (Ku and Dulin, 2010; Lipinski, 2000).

While various pharmaceutical companies are dealing differently with these challenges, it is generally acknowledged to evaluate development related aspects already during the research phase (e.g. Chen et al., 2006; Curatolo, 1998; Gardner et al., 2004; Huang and Tong, 2004; Saxena et al., 2009; Venkatesh and Lipper, 2000). In 2004, the "100 mg approach" was published introducing a miniaturized analytical in-depth characterization concept as an additional element of the overall candidate selection process (Balbach and Korn, 2004). Potential development issues of candidates are detected and measures, such as salt formation, can be initiated early.

This article focuses now on the subsequent steps of the "100 mg approach" which requires not more than milligram amounts of a given candidate and is divided into three parts:

Abbreviations: DSC, Differential Scanning Calorimetry; DVS, Dynamic Vapor Sorption; FaSSIF, Fasted State Simulating Intestinal Fluid; FeSSIF, Fed State Simulating Intestinal Fluid; (HP)LC, (High Pressure) Liquid Chromatography; ICH, International Conference on Harmonization; NMR, Nuclear Magnetic Resonance; RH, Relative Humidity; SGFSP, Simulated Gastric Fluid sine Pepsin; TGA, Thermogravimetric Analysis; (T-)XRPD, (Temperature-resolved) X-ray Powder Diffraction.

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1. Choice of salt formers
2. Salt screen and confirmation of salt formation
3. In-depth characterization

Pre-defined selection criteria, which are of utmost importance to reliably anticipate potential development issues, have been systematically improved. In addition, considering a data base of 337 compounds with various counter ions and physical forms, our experience gained over the last 10 years is discussed.

2. Materials and methods

2.1. Choice of salt formers

For the formation of a stable salt, it is widely accepted that there should be a minimum difference of about 2 pK_a units between the candidate and its counter ion (Semin et al., 2010; Stahl, 2003; Tong and Whitesell, 1998), in particular when the drug substance is a very weak acid or base. As pK_a values can be determined with only minimum amounts of drug substance, potential salt forming agents can be easily predicted (Brittain, 2007). However, although pK_a values might differ sufficiently, it is often observed that crystalline and/or stable salts cannot be obtained (Stephenson et al., 2011). On the opposite, exceptions may be found where complexes between the drug substance and a counter ion are formed, despite small differences in the pK_a values (Peresypkin et al., 2008). Consequently, the formation of a crystalline form needs to be confirmed in an experimental setting. Besides acidity or basicity of the ionisable group, other aspects, such as general safety of the counter ion, have to be ensured.

Keeping these prerequisites in mind, one set of pharmaceutically acceptable acids and bases frequently used in pharmaceutical industry is proposed (Table 1, cf. also e.g. Stahl and Wermuth, 2002). A backup set of acids is available in case no crystalline salt is formed with the first set. However, due to limited number of preferred bases no back-up set is in place for this class. Finally for special delivery approaches, additional salt formers (e.g. pamoic acid, hippuric acid) may be considered as long as they are pharmaceutically acceptable.

2.2. Salt screening and confirmation of salt formation

During discovery, the amount of drug substance available rarely exceeds the milligram range. Therefore, a microplate technique for preliminary salt screening (Bastin et al., 2000) was adapted and further miniaturized. Typically, 50–100 mg free base or acid are used for this screening step.

Table 1
Anion and cation set for salt screen.

Acids, 1st set	Acids, backup set	Bases
Acetic acid	D,L-Aspartic acid	NaOH
Adipic acid	Capric acid	KOH
Benzoic acid	Galactaric acid	Mg-acetate
Citric acid	Gluconic acid	Ca-acetate
Fumaric acid	Glucuronic acid	N-Methyl-glucamine
Hydrochloric acid	Glutamic acid	L-Lysine
Lactic acid	Glutaric acid	L-Arginine
L-Malic acid	2-oxo-Glutaric acid	Trometamol (Tris)
Phosphoric acid	Glycolic acid	Cholinhydroxide
Succinic acid	Lactic acid	
Sulfuric acid	Malonic acid	
L-Tartaric acid	Naphthoic acid	
	Palmitic acid	
	Propionic acid	
	Sebacic acid	
	Stearic acid	
	Trimellitic acid	
	Trimesic acid	

5 mM solutions of the free compound in methanol are placed into each well. Concentrated solutions of acids and bases are prepared and a few microliters of each are added sequentially to the wells leading to a final volume of 50 μl with about 0.15 mg of sample per well. By increasing the amount of acid/base corresponding to 1, 2 or 3 equivalents, the ratio sample/counter ion can be changed. A typical scheme is given in Fig. 1. The wells are inspected for the appearance of crystals using X-ray powder diffraction (XRPD). Crystallization can be promoted by evaporation of any excess solvent using a slow stream of dry nitrogen gas.

Once the counter ions which might lead to crystalline salts have been identified, about 10 mg per salt is provided to confirm viability and stoichiometry of the salts by means of Raman spectroscopy, XRPD and NMR.

2.3. In-depth characterization

For hits which have been confirmed as salts by XRPD as well as for the free form, scale-up experiments up to amounts of about 50–100 mg will be initiated and solid state characterization, stability studies and investigations on solubility as part of the in-depth characterization program are performed in a staged approach (Fig. 2).

2.3.1. In-depth characterization – stage 1

Successful up-scaling to crystalline material with a purity of at least 95% is key for the following physicochemical characterization. Investigations are performed to confirm crystallinity as well as to identify thermal properties, hygroscopicity and potential solvate formation.

2.3.1.1. Crystallinity. Miniaturized transmission XRPD is applied. Samples of 3–5 mg are subjected to the X-ray diffractometer.

In addition, Thermogravimetric Analysis (TGA) measurements to evaluate potential hydrate and solvate formation are performed by placing samples (about 5 mg) in open Al_2O_3 crucibles and heating at a rate of 10 °C/min up to 300 °C.

2.3.1.2. Melting point. The melting point is determined by means of differential scanning calorimetry. DSC samples in 40 μl aluminum crucibles with sealed lid and pinhole are heated in a nitrogen gas flow at a rate of 10 °C per minute from 25 °C to 350 °C.

Temperature-dependent XRPD (T-XRPD) is performed by measurements on a Stoe Stadi-P powder diffraction system equipped with a capillary oven and an image plate position sensitive detector (IP-PSD). The temperature is increased in steps of 5 °C from 30 to 300 °C.

2.3.1.3. Hygroscopicity. The rate and extent of moisture sorption under different humidity conditions are determined by means of Dynamic Vapour Sorption (DVS) which requires very little amount of compound (about 3 mg). Although hygroscopicity is a function of relative humidity and temperature, the latter variable is not varied during salt selection. Two cycles are run in which the relative humidity is stepped from 0% to 95% and back to 0%. After DVS testing, the samples are investigated by XRPD in order to get information on whether a humidity-triggered modification change has taken place.

2.3.2. In-depth characterization – stage 2

2.3.2.1. Chemical and physical stability. Data for pH-dependent stability, especially in the physiological relevant range, are typically available from pre-candidate profiling. During salt selection, solid