Development of a Partially Automated Solubility Screening (PASS) Assay for Early Drug Development

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ABSTRACT: A medium-throughput, compound-saving, thermodynamic solubility assay for early drug development was developed. Solid compound suspended in heptane was used for simple, time-saving, and flexible compound distribution into 96-well plates, with minor risk to generate new physical forms during dispensing. Low volume, well-stirred incubation vessels were generated by using a combination of V-shaped wells, well caps, and vertically inserted stir bars. This allowed solubility determination up to 100 mg/mL in 40–80 μL volumes in aqueous and nonaqueous, low- and high-viscosity solvents. After removal of residual solid through syringe filters mounted on microtiter plates, the filtrate was quantified by ultra performance liquid chromatography (UPLC) using a 1.2 min gradient. Combined with a robotic liquid handling system, throughput was 45 samples per hour and >600 solubility measurements per week. Results from the partially automated solubility screening (PASS) assay correlated well with reported solubility values ($r^2 = 0.882$). The PASS assay is useful for compound-saving, thermodynamic solubility measurement at the discovery–development interface where maximal solubility in many commonly used solvents needs to be determined. PASS results provide a basis for the identification of formulation strategies, the selection of appropriate excipients, and for the prediction of the potential *in vivo* behavior of compounds.
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Keywords: preformulation; excipients; solubility; formulation; formulation vehicle; automation; high-throughput technologies; HPLC (high-performance/pressure liquid chromatography)

INTRODUCTION

Drug solubility is a crucial factor for absorption in the gastrointestinal tract, and compounds with too low aqueous solubility often have inappropriate pharmacokinetic properties and carry a higher risk to fail during early or late development. Therefore, phase appropriate solubility measurements are performed along the drug discovery and development process, the assays and their focus varying with the phase.

In drug discovery, *in silico* prediction and high-throughput screening (HTS) of aqueous solubility of compounds have become a fully integrated part of the screening cascade. Assays must be high-throughput, rapid, inexpensive, and should work with small quantities.[1,2] Therefore, "kinetic" solubility methods such as turbidimetric,[1] nephelometric,[3,4] and direct UV[5] are preferred that work with DMSO stock solutions of compounds used in many discovery set-ups (Tab. 1). Stocks are added to aqueous buffers and precipitated or dissolved

Abbreviations: PASS, partially automated solubility screening; HTS, high-throughput screening; NMP, *N*-methyl-2-pyrrolidone; UPLC, ultra performance liquid chromatography.

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Table 1. Differences in the Requirements for Solubility Measurement in Various Phases

	Drug Discovery	Clinical Candidate Selection	Drug Development
Compound			
Number	>100	1–10	1
Amount	<0.2 g	0.2–1 g	>1000 g
Solid	Amorphous/crystalline (not characterized)	Amorphous/crystalline (characterized)	Crystalline (polymorphs characterized)
Assay			
Compound	In solution (DMSO)	Solid	Solid
Solvents	1–5 (aqueous only)	20–100	10–50
Range	0.001–1 mg/mL	0.001–100 mg/mL	0.001–300 mg/mL
Time scale	Minutes	24–48 h	24–48 h
Pellet	Not analyzed	Analyzed (not always feasible)	Analyzed
Type	Kinetic	Thermodynamic	Thermodynamic
Detection	UV, Turbidity	HPLC	HPLC
Focus	Early *in vivo* structure-activity relationship, lead optimization	First screening and development of formulations for PK-, PD-, and toxicity-studies, compound selection for development	Formulation development, dissolution, regulatory affairs

drug is determined. Known and accepted limitations of this set-up are that (a) the solid state properties (purity, amorphous vs. crystalline, particle size, polymorphism) of starting materials and/or precipitates are not characterized, (b) apparent and not true solubility is determined since precipitation is kinetically driven, and (c) the measurable solubility range is limited to less than 1 mg/mL since residual DMSO may affect drug solubility.[1,2]

In contrast, highly validated thermodynamic (equilibrium) solubility assays are used in pharmaceutical development. Assays are slow, have low throughput, and are used for in-depth solubility studies in a few selected solvents up to several hundreds of milligrams (Tab. 1). Compounds are available in large quantities, have well-defined solid state properties, and are added as solid in excess to the solvent. After agitation for 24–48 h to reach equilibrium, solubility is determined after removal of residual compound. Examples are the saturation shake-flask,[6] pHStat,[7] potentiometric[8] and calorimetric methods.[9] For high-quality experimental data, changes in pH, particle size, polymorphism, stability, and excess of solids need to be considered.[10,11]

At the interface between discovery and development, the clinical candidate selection phase, solubility assays have to meet the needs of both drug discovery and development. In particular for poor solubility compounds, extended solubility studies in both aqueous and nonaqueous pharmaceutically relevant solvents are required to identify promising preformulation approaches for the subsequent PK, PD, and toxicological studies. Ideally, thermodynamic assays covering the range up to 50–100 mg/mL would be ideal to identify those excipients and formulation approaches early on that could also meet the solubility requirements for the typically more demanding toxicity studies performed later on (Tab. 1). In reality, this is often not possible. Compound availability is still limited and either many solvents may be tested at low concentrations or a few selected ones at high drug load in standard solubility assays. Since the first approach may not allow discrimination between solvents and the second one may overlook potential formulation opportunities, formulators often use combined, sequential approaches, their outcome and duration a lot depending on the formulator's experience. Therefore, we developed a fast, reliable, miniaturized, medium-throughput, semi-automated assay for the selection of vehicles for preclinical studies that allows parallel, thermodynamic solubility measurements in aqueous and nonaqueous solvents up to 100 mg/mL with minimal compound requirements. The development, applications, and limitations of the assay are presented.