SOLUBILITY TOOLBOX FOR SUCCESSFUL DESIGN OF DRUG CANDIDATES

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Introduction

INVENTIVA is a fully integrated Drug Discovery company based in Dijon (France) dedicated to the finding of novel drug development candidates. The chemistry team (>30 Scientists) provides an extensive panel of services encompassing medicinal chemistry, synthetic & parallel synthesis, Computer-Assisted-Drug-Design, and analytical & purification.

Physical properties play a crucial role in the success of a drug candidate [1]. Compounds with suboptimal physical properties like low solubility not only hamper the reliability of *in vitro* and *in vivo* assays, but also add significant burden to drug development. Even though thermodynamic solubility of lead compounds has always been measured by the Medicinal Chemists, the arrival of combinatorial chemistry leading to large libraries of screening compounds and small amount of the available samples has generated the need for development of rapid, high throughput, accurate, low consumption, automated solubility measurement techniques [2]. Typically the concept of kinetic solubility has been introduced [3] to answer this demand, however some limitations of this concept have been exemplified [4,5] such as the influence of the residual DMSO used for the initial solubilization or the fact that the starting point is not the solid state but the DMSO solution. To overcome these issues new developments have focused on thermodynamic-like approaches, also called “pseudo-thermodynamic” [6]. Based upon all these techniques, INVENTIVA has set up a full Solubility Toolbox containing three generic protocols.

Other important parameters in Medicinal Chemistry are partition coefficients (logP/logD). Even if they are systematically predicted using diverse in-silico tools, it is always valuable to benchmark these predictions against experimental measurements. We therefore added logD measurement option to our toolbox. It is measured via direct or indirect techniques.

As of today, our Physchem team provides on a daily basis the support to our Medicinal Chemistry team.

Kinetic

Protocol :

Sample / compound consumption	From stock DMSO solution/1.2 mg
Solubility range	5µM to 100µM
Principle	Dilution to PBS pH 7.4, allow for precipitation, filtering out and quantitation
Incubation time	30 min
Readout Reference solution	Ultra Violet(UV) PBS/ACN (50/50)
Residual solvent	1.3% DMSO
Throughput	20 compounds / 3h
Replicates & quantification	4 replicates 1 calibration point (50µm)
Requires	Good UV chromophores DMSO soluble compounds (10, 5 or 2.5 mM)
Acceptance criteria Caffeine is our positive control	UV spectrum quality (fig1) Caffeine : absorbance for reference>0,7AU Solubility for test solution >110µM No more than 1 outlier out of mean +/- 15% Test solutions :min absorbance 0,2AU No more than 1 outlier out of mean +/- 15%

Strengths :

- High throughput
- Easy to run
- Automation
- Visual check of ref solutions
- Ability to detect diffusion due to nanoparticles

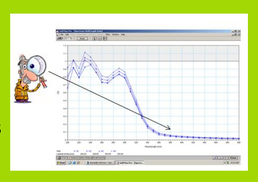


Fig 1 : UV spectrum is inspected for absence of diffusion

Weaknesses :

- Sensitive to compound purity. Integrity is checked by LCMS
- Sample consumption (~ 1.2mg)
- Limited solubility range
- Kinetic data, presence of residual DMSO
- Restricted to short incubation time
- Typical outliers, pitfalls : compound can precipitate in reference solution; low UV absorbance

Abbreviations

ACN : acetonitrile, DMSO : dimethylsulfoxyde, PBS : Phosphate Buffer Solution, DPI : 4,5-diphenylimidazole

High Throughput

Thermodynamic

Protocol :

Sample / compound consumption	From stock/ 2mg
Solubility range	5µM to 1mM
Principle	Addition of PBS pH7.4 into sample tube, incubation, filtering out and quantitation
Incubation time	24h
Readout Reference solution	HPLC-UV or HPLC-MS 50/25/25 (ACN/DMSO/PBS)
Residual solvent	none
Throughput	5 compounds / 4h over 2 days
Replicates & quantification	3 injections of test solution 4 calibration points
Requires	/
Acceptance criteria	Calibration curve : not less than 3 calibration points r^2 >0.975 Test solutions no more than 1 outlier out of mean +/- 15%

Strengths :

- Golden reference technique for thermodynamic solubility
- Non sensitive to compound purity
- Able to check compound integrity over 24 hours
- Visual check of reference solutions
- Adsorption upon material is circumvented by filtration of large volumes

Weaknesses :

- Low automation, limited throughput

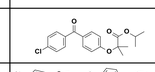
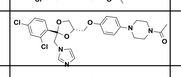
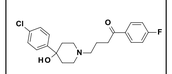
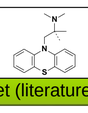
Compound	Structure	Thermodynamic solubility	
		Literature	Experimental (pH 7.4)
Fenofibrate		<1µM	<2µM
Ketoconazole		3µM	3µM
DPI		25µM	14µM
Haloperidol		37µM	36µM
Promethazine		55µM	56µM

Table 1: validation set (literature is retained if conditions are detailed)

Limited amounts in DMSO solution

Pseudo-thermodynamic

Protocol :

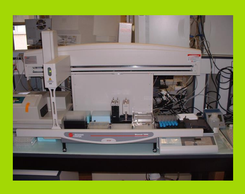
Sample / compound consumption	From stock DMSO solution/0,2mg
Solubility range	5µM to 200µM
Principle	Evaporation of DMSO in 96W-plate, addition of PBS pH7.4, incubation, centrifugation and quantitation
Incubation time	24h
Readout Reference solution	Ultra Violet(UV) or HPLC-UV PBS/DMSO (50/50)
Residual solvent	none
Throughput	20 compounds / 4h over 2 days
Replicates & quantification	3 replicates for test solutions , 6 calibration points
Requires	Very good UV chromophores beyond 265nm (DMSO) DMSO soluble compounds 10mM
Acceptance criteria caffeine is our the positive control DPI is used for control chart survey	Calibration curve : Not less than 4 calibration points r^2> 0.975 test solutions no more than 1 outlier out of mean+/- 15% Caffeine solubility should be >=180µM DPI from 10µM to 30 µM

Strengths

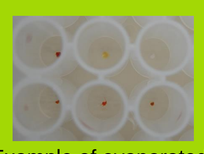
- Compound consumption: neglectable
- Thermodynamic-like data (no cosolvent)
- Range of interest in Drug Discovery
- High throughput in UV readout
- Automation

Weaknesses :

- Sensitive to compound purity
- Unable to detect degradation over 24 h
- HPLC-UV quantitation can be used to check the purity of compounds
- Typical outliers, pitfalls : compound remaining stuck to the well sublimation or degradation of compound during drying (40°C)



Biomek 3000 liquid handler



Example of evaporated plate

Results / Discussion

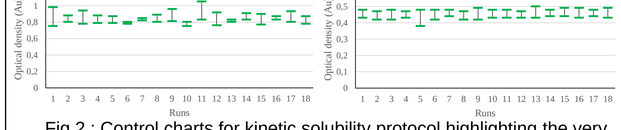


Fig 2 : Control charts for kinetic solubility protocol highlighting the very good reproducibility of the technique (caffeine)

The kinetic protocol is basically a high-throughput protocol which is very stable (fig2) and robust. Data quality remains excellent as illustrated in fig3. This example illustrates that tiny structural differences can be easily discriminated.

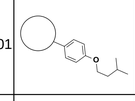
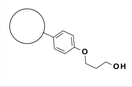
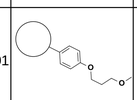
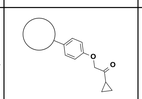
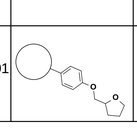
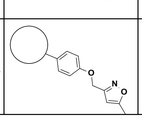
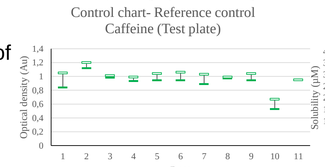
Compound ID	Chemical Structure	MW	Formula	Kinetic Solubility	Compound ID	Chemical Structure	MW	Formula	Kinetic Solubility
IV1[redacted]5-01		338	C22 H30 N2 O	55µM	IV[redacted]4-01		326	C20 H26 N2 O2	>100µM
IV1[redacted]0-01		340	C21 H28 N2 O2	74µM	IV[redacted]3-01		350	C22 H26 N2 O2	73µM
IV[redacted]1-01		352	C22 H28 N2 O2	90µM	IV1[redacted]5-01		363	C22 H25 N3 O2	97µM

Fig 3 : Structure-Property-Relationship established over 6 closely related compounds by Kinetic protocol

Whereas determination of aqueous solubility by thermodynamic approach is not suitable for high throughput, particularly when the amount of compounds is limited or when the compounds are prepared by parallel synthesis, Kinetic protocol offers a first option easy to set up and to automate. As an alternative, the Pseudo-thermodynamic protocol, as introduced by Y.W.Alelyunas et al, is able to deliver a very good compromise between compound consumption, throughput and data quality (thermodynamic-like data).

However this protocol requires a high level of expertise as several steps are critical and must be carefully developed :

- DMSO evaporation,
- stirring of plates,
- withdrawing of centrifugated solutions



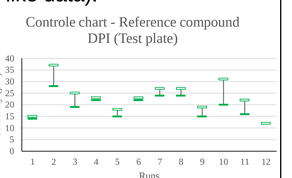


Fig 4 : control charts for Pseudo-thermodynamic protocol exhibiting the good reproducibility of the technique.

All the protocols can be performed at different pH and in various media.

The required equipment is limited to a liquid handling platform equipped with a UV-plate reader and completed by one HPLC-UV-MS instrument.

Log D

Log D is of particular interest when measured at physiological pH of 7.4 . Measurement at low pH for acids or high pH for basic compounds is also worth considering as it corresponds to the LogP value of the neutral chemical species. To this aim we have developed two HPLC-based indirect techniques, whereas LogD pH of 7.4 is measured by conventional shake-flask technique.

Conclusion

These diverse tools are all combined within a unique platform manned by two technical experts. Depending on the stage of the research program, the optimal combination of data is defined. For early stage programs Kinetic solubility is preferred with indirect measurement of logP whereas for more advanced compounds, Pseudo-thermodynamic is used as generic. The features of the compounds also influence the selection of the technique (UV-absorbing, expected solubility, sensitivity to drying…). Finally, for the most advanced compounds, thermodynamic solubility and logD pH of 2.5 and 7.4 remain the gold standard.

[1] Lipophilicity and related molecular properties as determinants of pharmacokinetic behavior, B.Testa, P.A.Carrupt, University of Lausanne, CHIMIA, **2000**, 54, N°11, 672-677.
[2] A high-throughput screening method for the determination of aqueous drug solubility using Laser nephelometry in microtiter plates, C.D.Bevan, GlaxoWellcome, Analytical Chemistry, Vol 72, N°8, April 15, **2000**.
[3] Development of new experimental tools for fast determination of solubility and lipophilicity , Thesis B.Bard, University of Geneva, **2008**
[4] Optimizing solubility : kinetic versus thermodynamic solubility temptations and risks, C. Saal, Merck KGaA, European Journal of Pharmaceutical Sciences, 47, **2012**, 589-595
[5] A highly automated assay for determining the aqueous equilibrium solubility of Drug Discovery compounds, M.C.Wenlock, AstraZeneca R&D Charnwood, Journal of Laboratory Automation, **2011**, 16, 276-284.
[6] Application of dried-DMSO rapid throughput 24h equilibrium solubility in advancing discovery candidates”, Y.W.Alelyunas, AstraZeneca pharmaceuticals LP, European Journal of Pharmaceutical Sciences 37, **2009**, 172-182.

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