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Solubility Check in FaSSIF or FeSSIF by HPLC

Equilibrium solubility is the amount of drug that is naturally dissolved (without influence of solvent or Supersaturation). The results from this experiment enable you to see if there are any major solubility limitations for your compound. You can also compare and benchmark the solubility between different compounds or batches of compound without the interference of time or solvent. FaSSIF solubility is generally more important than FeSSIF because it is preferred if drugs can be taken without food. FeSSIF is important for drugs with a narrow therapeutic window or poorly soluble lipophilic drugs if you want to know if a fatty meal could change their solubility and bioavailability.

Stage: Pre-clinical compounds where you want data to identify if there are likely to be solubility limitations (i.e. solubility less than 0.1 mg/ml).

Tried and tested

As every compound behaves slightly differently, we cannot guarantee this exact methodology will work in all cases. However, this method has been tested rigorously in our own labs and should work for the majority of compounds. Specifically the HPLC is a general method that may have to be modified for some compounds.

For successful results: use proper, calibrated equipment.

Equipment needed

- 5-10 mL glass vials, sealable (e.g. W225293 Wheaton, US)
- 0.20/0.22 µm, Ø 13 mm, PVDF syringe filter
- Balance for accurately weighing about 10 mg
- Small magnetic rod that fits inside vial (e.g. 3x9 mm)
- SIF Powder 2.5 L bottle
- Buffer (see SIF Powder Preparation Protocol)
- HPLC method suitable for your compound

Time needed: about 1.5 days

Actual labor effort: about 3 hours

Estimated drug requirements: about 30 mg

Recommended number of replicates: N = 3 (i.e. do the experiments on 3 independent samples)

How to measure the equilibrium solubility of compounds in FaSSIF or FeSSIF

1. Prepare FaSSIF or FeSSIF (15 minutes)

See SIF Powder Preparation Protocol on www.biorelevant.com

2. Set up solubility experiment (about 10 minutes and 24 hours waiting time)

- Accurately weigh 10 mg of your compound into the glass vial
- Add 5 ml of either FaSSIF or FeSSIF at 25 °C*
- A magnetic rod should be used to stir the suspension continuously
- Close or seal the glass vial to prevent evaporation
- Stir for 24 hours

3. Filtration (15 minutes)

- Filter the suspension of drug in FaSSIF or FeSSIF through a 0.22 µm pore size PVDF filter using a syringe (Inject-F, Braun Germany). If a 0.22 µm filter is not working (blocking of the filter), try a 0.45 µm filter but make sure your filtrate is clear

  *If there is no undissolved API and it is chemically stable, your solubility in the investigated medium is higher than 2 mg/ml and you probably won't have any solubility issues *in vivo**

4. Analysis

If you don't have an HPLC method, the following method might be helpful:

a. HPLC (about 1 hour)

The HPLC system might consist of an Agilent Series 1200 equipped with a Photodiode Array Detector (Palo Alto, CA, USA).

Conditions:

- A versatile column that can be used is a SunFire™ C18 (75 × 4.6 mm, 2.5 µm) from Waters (Milford, MA, USA)

- A gradient HPLC method using Water+0.1 % Formic Acid (Mobile Phase A) and Acetonitrile+0.1 % Formic Acid (Mobile Phase B) according to the following table can be recommended:

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	85	15
8.0	5	95

13.0	5	95
13.1	85	15
15.0	85	15

- A flow rate of 1.2 mL/min and a column oven temperature of 40°C could be used
- Check Linearity of your compound over the investigated concentration range

b. Sample analysis

- Analyze your sample by HPLC. If the concentration is too high, dilute your filtrate with a suitable organic solvent in which your drug dissolves, preferably Methanol

Other tips….

Want to find out more about solubility?

Particularly for pH sensitive compounds (e.g. acids), it can be really helpful to also measure solubility in the blank buffer alone. This control measurement gives an insight into the role of Mixed Micelles on the solubility or whether the solubility is mainly pH dependent.

Temperature

*25°C is easier if routinely used and analysis is moved from lab to lab. 37°C is less practical but can be highly informative because it is closer to *in vivo* conditions. Whichever condition you choose, for consistency and comparison across a set of compounds or batches it is recommended to stick to the same temperature.