
KALADRUG-R: Laboratory SOP#17

Preparation of stock solutions of reference drugs for *in vitro* application



Date of preparation: October 25th 2010

Author: University of Antwerp

Standard Method

The standard solvent for the preparation of stock solutions is dimethylsulfoxide (DMSO) as it dissolves both polar and non-polar compounds and is miscible with a wide range of organic solvents and water. In particular cases, for example for compound stability reasons or if good water-solubility is known, other solvents such as milliQ water or phosphate buffered saline (PBS) can be used. Stock solutions should be prepared in millimolar (mM) concentrations, except if the molecular weight is not known, for example, for natural extracts or in case of drug mixtures (in mg/ml).

Method: Preparation of 20 mM stock solution

- Weigh exact amount of the drug (about 4 – 5 mg, one decimal place)
 - o Use an analytical scale (range 0.1 mg)
 - o Use hermetically closed vials (i.g. Eppendorf tubes, glass tubes with screw cap).
 - o Use clear labels (preferably printed on adhesive tape, covered with plastic).
 - Drug identification, concentration, date, person identification
 - o Do not fix on a predefined mass (remove/add).
- Calculate the volume of DMSO to obtain a concentration of 20 mM (Formula)

$$\left(\frac{\text{weight (mg)} \times 1000}{\text{Molecular weight } \left(\frac{\text{g}}{\text{mol}}\right) \times \text{stock concentration (20 mM)}} \right) \times 1000 = \text{Volume } (\mu\text{L})$$

- Add the volume of DMSO
 - o Do not pipette up and down (residues on pipette tip).
 - o Mix by vortexing for 1 minute.
 - o If not dissolved: use a shaking/rotating instrument for 1 hour
 - o If not dissolved: use a sonicating water bath for 5 minutes (take possible heating into consideration).
 - A fine suspension can be achieved if no complete dissolution. Take care to vortex each time the stock solution is used.
 - o If not dissolved: use an ultrasonic rod (place on ice to prevent heating).
- Keep stock solutions in DMSO at room temperature and avoid contact with water since DMSO is highly hygroscopic. It is preferable to divide large volumes of stock solution over small-volume aliquots.

Remarks

- To ensure optimal solubility, fresh DMSO should be used. DMSO is hygroscopic and repeated opening and closing of the stock bottle will enhance this. Divide the stock bottle over different smaller containers (max 50 ml tubes) and discard them after use.
- DMSO should be kept at room temperature, but closed from air.
- Solutions in water and PBS should be kept at -20°C.
- Stock solutions are preferably kept in the dark (aluminum foil, dark vials).
- Storage period: depending upon stability of the compound – usually not known.
- Final in-test concentrations of *in vitro* experiments should not exceed 0.5% to 1% DMSO because of toxicity (dependent on the test model).

Practical examples for current anti-leishmania reference drugs:

Antimony:

- **Sb^V**: Keep dry stock in the dark at 4°C.
Make a stock solution of 5.12 mg/ml in PBS.
Weigh about 51.2 mg – add PBS (about 10ml)
Put at 37°C for 1 hour until complete dissolution: clear liquid.
Keep at -20°C in small aliquots (1 ml) in the dark.
Frozen aliquots may be kept for max 3 months
 $C_{12}H_{17}O_{17}Sb_2.3Na$ total molecular weight: 745.725 g/mol including 243.5g Sb^V
1 g SSG contains $243.5/745.725 = 0.3265g$ Sb^V equivalents
In-test concentration (10 µl in 210 µl): 242.9 µg/ml → 77 µg/ml eq.
- **Sb^{III}**: Keep dry stock in the dark at 4°C / Make a stock solution of 5.12 mg/ml in PBS.
Weigh about 51.2 mg – add PBS (about 10ml)
Sb^{III} is completely soluble in PBS, no further dissolving steps are needed.
Keep at -20°C in small aliquots (1 ml) in the dark.
Frozen aliquots may be kept for max 3 months
In-test concentration (10 µl in 210 µl): 242.9 µg/ml → 88 µg/ml eq.

Miltefosine (MIL)

Keep dry stock at room temperature / make a stock solution of 20mM in water.
Complete dissolution after vortexing.
Keep stock solution at 4°C

Paramomycin sulphate (PMM)

Keep dry stock at room temperature / make a stock solution of 20mM in water.
Keep stock solution at 4°C
Paramomycin sulphate: $C_{23}H_{47}N_5O_{18}S = 713.707$ g/mol
Paramomycin: $C_{23}H_{45}N_5O_{14} = 615.634$ g/mol
Stock solution 20 mM → 25 mg + 2030 µl