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Binding of one lipophilic compound to the Precellys tubes with ceramic beads during tissue homogenization

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CONTEXT

To study lipophilic compounds glass vials are widely used in labs. We were aiming to quantify the loss of one **lipophilic compound** in the tissue homogenization process using Precellys tubes with ceramic beads. For that, we selected one compound that shown to bind plastic extensively. The compound info is: small molecule; neural compound; MW>500; experimental / theoretical LogP >3; low water solubility.

MATERIAL

- Precellys 24 homogenizer coupled with Cryolys.
- Precellys lysing kit: 03961-1-003 (CK14 - 2mL tubes).
- 34 mg of blank human kidney tissue.
- 200µL of buffer (same buffer used in compound extraction, non-organic and contains no protein homogenization reagent) .
- Stock concentration of the lipophilic compound: 0.125µg/mL, 2µg/mL, 20µg/mL and 100µg/mL.

PROTOCOL

- Preparation of a kidney homogenate at 34 mg/2000µL.
- Take 200µL of kidney homogenate into 4 Precellys lysing tubes CK14 and in 4 glass vials as a control group.
- Add 10µL of each stock concentration of the compound to 200µL of kidney homogenate in the Precellys lysing tubes and glass vials.
- Samples were vortexed and let equilibrated at room temperature for 15 min.
- Homogenization using Precellys tubes with 15 seconds of vortexing twice at 6000 rpm with 10 seconds break using Cryolys with dry ice and acetone to cool the unit.
- Take 150µL out from the Precellys tubes or the glass tubes for compound extraction and LC-MS/MS analysis.

RESULTS

Both compound extraction and LC-MS/MS analytical methods were validated and published previously. We assume that the binding of the compound to glass tubes is minimal and thus use glass tubes as controls.

The percentage loss is 19.3 ± 10.9 % (mean ± SD), ranging from 9.3% to 34.5%. The data from the 2µg/mL group may be an outlier due to the big deviation from the other concentration groups. The percentage loss from the other three concentration groups is 14.2 ± 4.8 % (mean ± SD), ranging from 9.3% to 18.9%.

The percentage lost due to Precellys tubes binding does not seem to depend on compound concentrations which is good for calculation that may account for the loss of the drug at an unknown concentration.

The standard curves for the 3 conditions are shown in the figure 1.

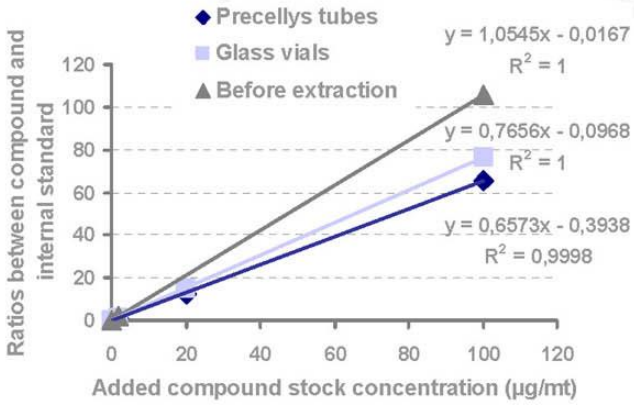

Figure 1: Standards curves

CONCLUSION

We concluded that the combo **Precellys 24 homogenizer / Cryolys** and the **Precellys lysing kits CK14_2mL** are adequate in the application of tissue homogenization of one **lipophilic compound** in our research given its great consistency in homogenizing different tissue samples and acceptable loss of lipophilic compound (~15% or less) to Precellys tubes with ceramic beads.

03712-810-DU067

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