Physiologically based extraction test (PBET)

Chemicals:

Gastric fluid:

- Pepsin
- Citric acid
- L(-)-Sodium malate
- Acetic acid
- Lactic acid
- Concentrated HCl

Small intestinal fluid:

- Pancreatin
- Bile salts
- Saturated solution of $NaHCO_3$

Equipment:

- Incubator (rotator in oven with temperature 37 °C)
- Water bath 37 °C
- Centrifuge
- Vials
- Filters

Procedure:

Day before test

- Weight reagents and samples, store in the fridge
- Turn on incubator

Procedure

- Preparation of gastric fluid solution (40 ml for sample, the amount is for 0.5 dm^3 of fluid)
- Mix in the beaker on magnetic stirrer these reagents:
    - 450 ml MQ water
    - 0.625 g pepsin
    - 0.25 g citric acid
    - 0.25 g L(-)-Sodium malate
    - 0.25 ml acetic acid
    - 0.21 ml lactic acid
- Add concentrated HCl for pH adjusting on 2.5 (add 0.6 ml and then slowly step by step add 0.05 ml).
- Transfer to volumetric flask and add water to volume 0.5 dm^3.
- Transfer gastric fluid to the vial and put to the water bath (37 °C) for 30 minutes.

Preparation of gastric phase extract

- 0.4 g dust + 40 ml gastric fluid
- Put into the incubator for 60 minutes
- Check and adjust pH in 15 and 45 minutes
- Take 8 ml of extract to conical vials, centrifugate for 10 min (3 000 rpm), filtrate to the vials and store in the fridge until solvent exchange

Preparation of small intestinal phase extract (the rest extract 32 ml)

- Adjust pH on 7 with saturated solution of $NaHCO_3$ (normally 1.2 ml, but in some cases of our dust samples it should be much more – 4 ml)
- Add reagents:
    - 20 mg pancreatin
    - 70 mg bile salts
- Put into the incubator for 4 hours (no pH adjusting is needed)
- Centrifugate for 10 min (3 000 rpm), filtrate to the vials and store in the fridge until solvent exchange

Solvent exchange

Chemicals:

- NaCl
- Acetic acid
- Dichloromethane
- Na_2SO_4

Equipment:

- Vials
- Pasteur pipettes

Procedure:

8 ml gastric phase extract

- Add:
    - 0.3 g NaCl
    - 0.03 ml acetic acid
    - 2 ml DCM
- Shake 3 min
- Collect DCM fraction
- Add 2 ml DCM and shake 3 min
- Collect DCM fraction
- Dehydration by Na_2SO_4, filtrate to the vials and set the volume to 10 ml and store in the fridge until clean-up

32 ml gastric and intestinal phase extract

- Add:
    - 1 g NaCl

    - 1 ml acetic acid
    - 4 ml DCM
- Shake 3 min
- Collect DCM fraction
- Add 4 ml DCM and shake 3 min
- Collect DCM fraction
- Dehydration by Na_2SO_4, filtrate to the vials and set the volume to 10 ml and store in the fridge until clean-up

Clean-up for flame retardants

Chemicals

- cleaned activated silica gel (activation for 12 hours at 150 ° C)
- cleaned non-activated silica gel
- sulfuric acid modified silica gel (22 ml concentrated H2SO4 + 50 g activated silica)
- hexane, DCM, nonane
- internal standards

Equipment

- glass column, internal diameter 1 cm
- 20 ml vial
- Pasteur pipette, cotton

Procedure

- prepare the separation column:
    - put a cotton on the bottom of the column
    - add about 1 cm high layer of cleaned activated silica gel, then 5 g of activated sulfuric acid-modified silica gel
    - gently tap the column with the stick
    - load 1-2 cm layer of non-activated silica gel at the top of the column, and then tap again
- transfer the sample to the column, wash the original vial at least 2x with 1 ml dichloromethane, add to the sample in the column
- elute with 30 ml of 50% dichloromethane in hexane into a 20 ml vial
- concentrate the sample under a gentle stream of nitrogen to 500 µl
- transfer the sample to a minivial, add 40 µl of nonane and evaporate to the final volume of 40 µl
- add internal standards
- carefully close the minivial and store it in the refrigerator

Determination of analytes by GC-MS

Analyte determination will be done by GC-MS.