Where did my analyte go?

Coping with poor solubility and non-specific binding

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Poor solubility and non-specific binding

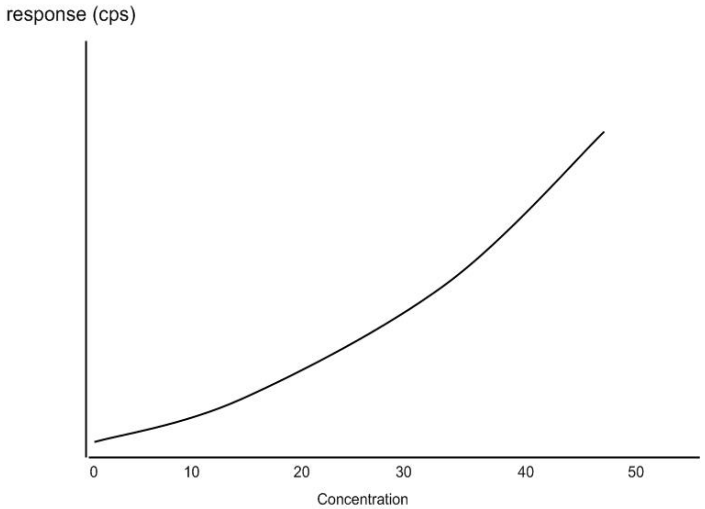
- **Often overlooked issues**
- **Prominent in low-protein matrices, *i.e.* urine, CSF**
- **Can cause:**
  > Non-linear standard lines
  > Inaccuracy of QC's
  > Assay variability
  > Loss of sensitivity

- **Difficult to identify**
- **Difficult to resolve**

Non-specific binding

- **Adsorption of analyte onto the surface area of the container**
- **Higher impact at lower analyte concentrations**
- **Non-linear calibration line**
- **Adsorption depends on:**

  > Analyte properties
  > Temperature
  > Matrix / solvent
  > Container



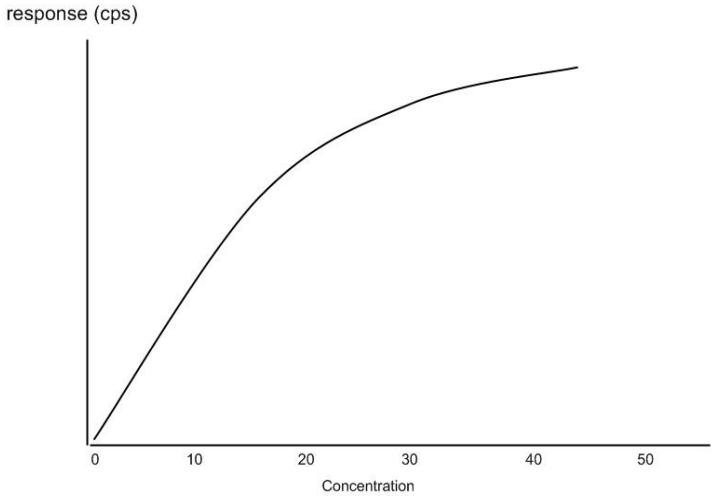
Poor solubility

- **Poor dissolution of analyte in solvent**
- **Higher impact at higher analyte concentrations**
- **Non-linear calibration line**
- **Analyte variability from a single well**
- **Solubility depends on:**

  > Analyte properties

  > Temperature

  > Matrix / solvent



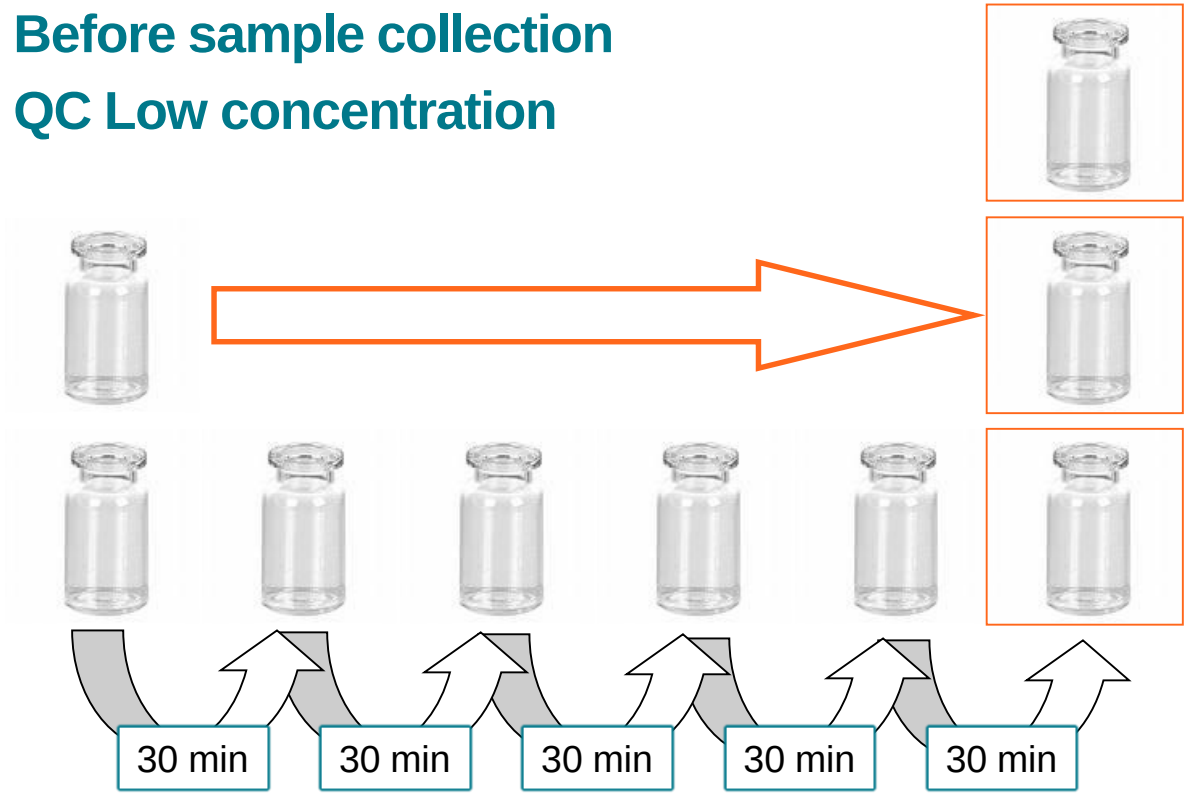
Non-specific binding

Non-specific binding

- **Analyte adsorbs onto container surface**
  > Electrostatic interactions
  > Non-specific interactions
- **Binding may be affected by:**
  > Matrix (ionic strength, pH, lipids and protein content)
  > Container material
  > Container brand or quality
  > Freeze/thaw or long term storage

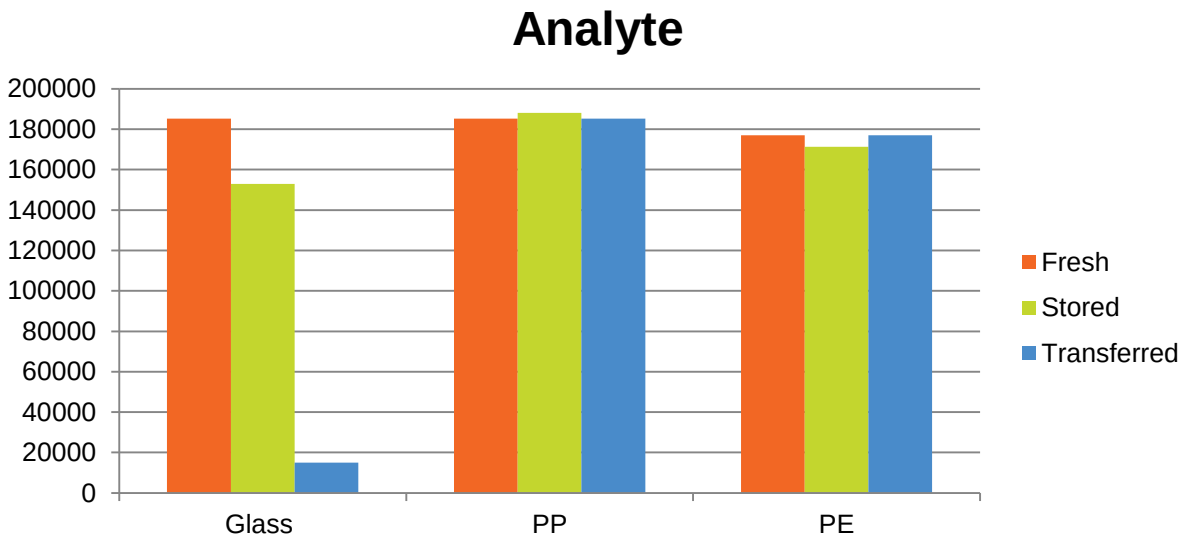
Non-specific binding - assessment

- During method development
- Before sample collection
- QC Low concentration



Non-specific binding - assessment

- Glass, polypropylene, polyethylene
- Water, dark urine, light urine



Non-specific binding - solutions

- **Change of container**
  - > Simplest solution
  - > Same material different container
  - > Eppendorf LoBind® - modified PP
  - > Wheaton® AntiBIND™ - modified PP surface

- **Addition of organic**
  - > MeOH, MeCN, DMSO, DMF or IPA
  - > Decrease urine polarity and therefore increase analyte solubility
  - > Solvent may attach to binding sites?
  - > % solvent needs to be optimised

Non-specific binding - solutions

- **Addition of acid / buffers**

  > Decreases electrostatic charge from surface?

  > pH control

  - Urine pH varies between 4.5 and 8.
  - Change of pH can affect **ionisation state** of analyte and therefore adsorption/solubility

Non-specific binding - protein

- **Addition of protein**
  - **Bovine Serum Albumin (BSA)**

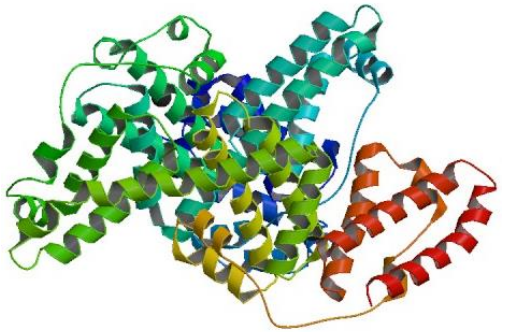
  > Serum albumin protein derived from cows

  > Mainly used with large molecules

  > Expensive

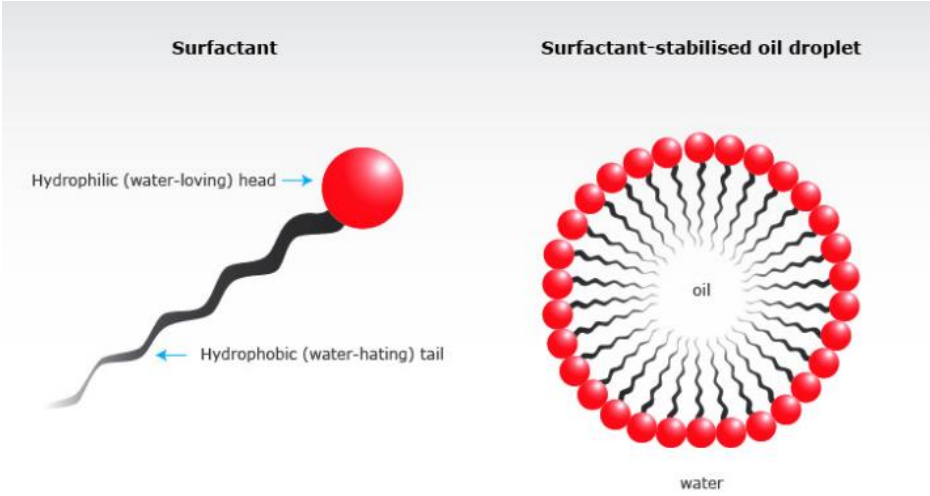
  > Can interfere with the extraction

  > Can contain biomarkers!!



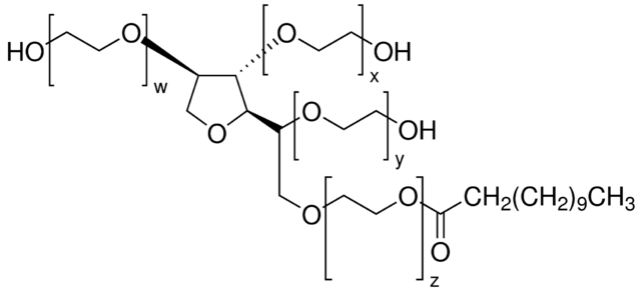
Non-specific binding - solutions

- **Addition of surfactants**
  - > Detergents
  - > Organic compounds that contain hydrophobic (*tails*) and hydrophilic groups (*heads*)
  - > Coat surface?
  - > Increase analyte solubility
  - > May reduce analyte sensitivity
  - > May be difficult to clean from MS

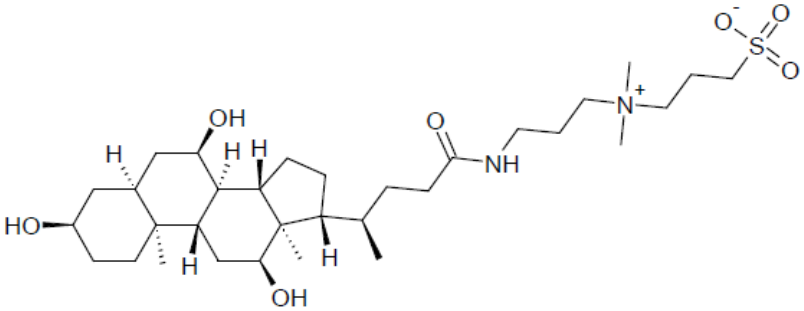


Non-specific binding - surfactants

- **Tween 20 & Tween 80**
  - > Most commonly used in our labs
  - > Small concentration required
  - > Cheap and non-toxic



- **CHAPS**
  - > Zwitterionic surfactant
  - > Used to solubilise biological macromolecules
  - > Expensive



Non-specific binding – solutions

- **Anti-adsorbing agents (AAA) - pre or post sample collection?**
- **Post-collection**

  > Ideal scenario

  > Reversible adsorption

Urine volume calculated from weight

Non-specific binding - post-collection

- **Advantages**
  > Same % of AAA added to each sample
  > Standards and QC's treated the same way
  > No need to correct sample concentrations
  > Urine collection bottles don't need to be pre-filled with chemicals

- **Disadvantages**
  > Primary containers may need to be shipped to the laboratories
  > Additional sample analysis time with sample pre-treatment

Non-specific binding – pre-collection

- **Pre-collection**

  > When non-specific binding is irreversible

Urine volume calculated from density

Non-specific binding – pre-collection

- **Advantages**

  > Easier sample pre-treatment

- **Disadvantages**

  > Not all AAA suitable (H_3PO_4, FA, etc)

  > Individual sample concentrations need to be corrected

  > A range of % AAA needs to be validated

Solubility

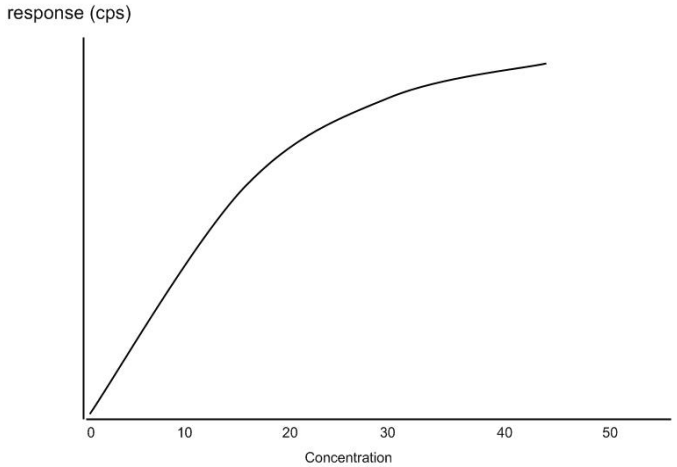
Solubility

- **A compound is soluble when it forms a homogeneous solution with a solvent**

- **Solubility depends on:**
  > Analyte properties
    - Molecules with a similar polarity to water will dissolve well in water
  > Temperature
    - Solids become more soluble as temperature increases
  > Matrix
    - pH and protein content

Poor solubility - assessment

- **Poor solubility of an analyte results in a solution that is not homogeneous**
- **Solution may look homogeneous**
- **More difficult assessment than non-specific binding**
  > Non-linear calibration lines
  > Poor CV's from repeat injections
- **Poor solubility may appear after storage**
  > Lower temperatures upon storage
  > Slow analyte precipitation



Poor solubility - solutions

- **Similar to non-specific binding**

- **Addition of organic**
  > MeOH, MeCN, DMSO, DMF or IPA
  > Decrease urine polarity and therefore increase analyte solubility
  > % solvent needs to be optimised

- **Addition of acid / buffers**
  > pH control
    - Urine pH varies between 4.5 and 8.
    - pH of urine can affect **ionisation state** of analyte and therefore adsorption/solubility

- **Addition of surfactants**

Poor solubility - solutions

- **Sonication**

  > Sound energy agitates particles using ultrasonic frequencies

  > Can be used to speed dissolution, by breaking intermolecular interactions **if the solute is soluble**

  > However:

  - **Supersaturated** solutions may be formed – precipitation/ crystallisation may occur upon storage
  - Prolonged sonication increases sample temperature – analyte stability?
  - Inconsistent results

  > SONICATION SHOULD BE AVOIDED

Conclusions

- Solubility and non-specific binding are difficult to identify
- Prominent in low protein matrices
- Always investigated during method development and prior to sample collection
- Interconnected issues – similar solutions