

# Sample preparation & Solvent considerations for UHPLC-UV-MS

## 1. Sample purity

- A purified sample has the highest chance of a positive identification.
- All samples should be filtered through a 0.45 µm or finer filter just prior to analysis, especially if they are cloudy or display visible precipitates
- Label your sample vial with sample I.D.
- Samples should be prepared in the user's lab.
- Use only the standard 2mL autosampler sample vials with proper septa (Available from Chem Stores). Wrong sample vial could damage the autosampler management system.

## 2. Sample Preparation/Clean up \*

### Why is it important?

- Improves detection, method robustness and reliability
- Protects the instrument detection system from contamination
- Minimizes instrument downtime

### You must filter your sample if it is cloudy or displays visible particulates.

- Use a 0.2 µm syringe filter for sub 2.7 µm columns (UHPLC) and 0.45 µm filters for normal particle size columns (HPLC)
- Filter size of 13 mm for a 0-10 mL sample and 25 mm for a 10-100 mL sample volume.
- Choose the right filter for your sample:
  - Nylon: broad solvent compatibility, aqueous and organic materials
  - PTFE: ideal for HPLC, UHPLC sample preparation and excellent solvent resistance
  - PES (polyethersulfone): highest flow rates, ideal for ion chromatography, low protein binding for biological samples
  - PVDF (polyvinylidene): broad chemical compatibility, fast flow, low protein binding for biological samples; low UV absorbing extractables for HPLC and UHPLC.

\*resources outlining the various types of sample preparation are numerous:

<https://www.agilent.com/en-us/training-events/eseminars/sample-preparation>

## 3. Analytes Concentration

- Min concentration: 0.02 - 0.2 mg/mL
- Max concentration: 0.5 mg/mL for molecules below 1,000 Da, and 0.1 mg/mL for larger ones. Greater concentrations may cause system carryovers and source contamination, with loss of performance.
- Most suitable solvents are water, methanol, and acetonitrile. If DCM is required, try to use some (even up to 50%) of the recommended solvents in each sample vial.
- Total sample volume can vary from 0.3 - 1.5 mL. If you have less sample volume than 0.3 mL, ask for assistance.
- Do not overfill the vials, fill each vial up to 90 % only.

#### 4. Solvent, choice of solvents for mobile phase preparation

- Store solvents in borosilicate bottles; rinse bottle with desired solvent before refilling it; ensure bottle did not get contaminated with detergent from the dishwasher
- Do not use parafilm or other plastic films to cover solvent reservoirs
- Use only HPLC or higher-grade solvents, no need to filter HPLC grade solvents
- Avoid algae/microbial growth:
  - change water-based solvents daily and organic solvents every several days; do not top up solvents
  - add some organic to aqueous mobile phases to inhibit growth
  - avoid light exposure by storing water-based solvents in brown bottles
- ACN: filter with 0.45  $\mu\text{m}$  filters and store in brown bottles (ACN can polymerize under UV)
- If possible, samples should be submitted as solutions dissolved in methanol, water, acetonitrile or isopropanol since they will be injected into a stream of these liquids. Methanol would be the default solvent if no solvent information is specified.
- Any solvent used to dissolve the sample must be miscible to one of the recommended solvents
- Buffers and salt solutions need to be filtered (0.45 or 0.2  $\mu\text{m}$ )

#### 5. Additives

##### **Additives you should avoid!**

- Alkali salts and non-volatile buffers such as phosphate, borate, citrate buffers that cause signal suppression and ionization interference
- Polyethylene glycol (PEGs), plastics and plasticizers suppress the signal and clutter the spectrum
- Detergents suppress ionization and compete for ionization, in particular Triton and SDS
- Inorganic acids are corrosive
- Additives compete for ionization. Additives are carbohydrates, glycerol, EDTA, Urea,  $\text{GnHCl}$
- Non-volatile solvents such as DMSO and DMF
- Trifluoroacetic acid (TFA) to some extent suppresses (+) ion electrospray at levels exceeding 0.01% and greatly suppresses (-) ion spray
- Triethylamine (TEA) causes an intense  $[\text{M}+\text{H}]^+$  at  $m/z$  102 and suppresses (+) ion electrospray of less basic compounds
- Tetrahydrofuran (THF) is highly flammable and reacts with PEEK tubing.

##### **Additives you could use:**

- Dilute volatile buffers (1-20 mM): ammonium formate, acetate and carbonate
- Dilute volatile acids for positive ion mode (formic and acetic acid) (max 0.5-1% formic acid)
- Dilute volatile bases for negative ion mode (triethyl amine, ammonium hydroxide, ammonium formate at 10mM or less, ammonium acetate at 10 mM or less).