

# **HPLC solvents and mobile phase additives**

The following is intended to be a guide for the choice of solvent and mobile phase additives. Of course the choice of solvents for LC will be dictated primarily by the required separation but there are some guidelines that need to be followed. These are selected examples which have been divided up into three categories; commonly used, unsuitable and other less common ones. In all cases degassed solvents are necessary for LC-MS operation. This is achieved by sonication, helium sparging or vacuum membrane degassing.

## **Commonly used solvents**

### **Solvents**

- Water
- Acetonitrile
- Methanol

These common reverse phase LC solvents are ideal for LC-MS. When using high percentages of water, the source and probe temperature usually need to be raised to aid desolvation in the source.

# Commonly used solvents

## Additives

- **Acetic acid**
- **Formic acid**

If the pH of the mobile phase needs to be reduced to enhance LC separations then acetic and formic acid are the most suitable. Formic acid is stronger than acetic acid and therefore less needs to be added to reach a required pH. Addition of acids can suppress ionisation in negative mode ion analysis, weakly acidic compounds may not form  $[M-H]^-$  in acidic conditions.

- **Ammonium hydroxide (ammonia solution)**

If the pH of the mobile phase needs to be increased to enhance LC separations then ammonium hydroxide (ammonia solution) is suitable. If analyzing weakly acidic compounds in negative ion mode there will be no suppression of ionization. In fact ionization can be enhanced as the mobile phase is not acidic.

- **Ammonium acetate**

Ammonium acetate is a volatile salt which is often used to buffer mobile phases. It is better to use as little ammonium acetate as possible up to a maximum of 0.1 M. It is often used to replace phosphate buffers, which should be never used with LC-MS interfaces.

# Unsuitable solvents / additives

- **Non-volatile salts**

These must not be used as these can crystallise in the source and prevent the mass spectrometer from functioning (blocking skimmers, etc.). The most common non-volatile salts used are phosphates, these should be substituted with a volatile salt such as ammonium acetate/formate.

- **Surface active agents/detergents**

These can be suppress the ionisation of other compounds. Detergents, by their very nature are concentrated at the surface of a liquid. This causes problems with electrospray as the ionisation relies on the evaporation of ions from the surface of a droplets. The detergent therefore suppresses the evaporation of other ions. Surfactants are only used when they are being analysed themselves, not as additives to LC mobile phases.

- **Inorganic acids**

Inorganic acids (e.g. sulphuric acid or phosphoric acid) should be not used.

# Others

## Solvents

- **Normal phase solvent**

Normal phase solvents such as dichloromethane, hexane and toluene have been used but not regularly than reverse phase solvents. These can only be used with APCI and not electrospray which needs a polar mobile phase for ionisation.

- **Propan-2-ol (IPA), 2-methoxyethanol, ethanol etc.**

These have all been used with LC-MS but their use is limited to specific applications.

# Others

## Additives

- **Trifluoroacetic acid (TFA)**

This is frequently used for peptide and protein analysis. Levels below even 0.1% can cause suppression of sensitivity in positive ion mode. TFA may completely suppress ionisation in negative ion mode.

- **Triethylamine (TEA)**

This may suppress the ionisation of less basic compounds in positive ion mode (as TFA is readily ionised to give a  $[M+H]^+$  ion at  $m/z$  102). In negative mode analysis, TEA enhances ionisation of other compounds as TEA is basic. This is a particularly useful additives for the analyses of nucleic acids.

- **Tetrahydrofuran (THF)**

This can causes reduced performance which can be counteracted by post-column addition of ammonium acetate. It has no effect in APCI.

**Caution** – Do not use a concentration of THF greater than 5% with PEEK tubing. THF causes swelling in the PEEK tubing and consequently presents a risk.

# Summary of Additives

- **Do not use:** Non volatile salts (phosphahtes, etc.)  
Surfactants, inorganic acids (sulphuric, phosphoric etc.), ammonium acetate ( $>0.1$  M), normal phase solvents in electrospray.
- **Avoid (+ve ion):** trifluoroacetic acid (TFA) (0.1%).
- **Avoid (-ve ion):** organic acids, e.g. acetic acid, formic acid, trifluoroacetic acid (TFA).
- **Use (+ve ion):** acetic and formic acid, ammonium acetate ( $<0.1$  M).
- **Use (-ve ion):** trimethylamine (TEA), ammonium hydroxide (ammonia solution), ammonium acetate ( $<0.1$  M)