

## Stability of mobile phase in oligonucleotide analysis with LC-MS

Ion-pair reversed-phase liquid chromatography (IP-RP) combined with MS is a common method for the analysis of oligonucleotides. Alkylamines in combination with a fluoroalcohol are ideal as eluents to ensure MS sensitivity in addition to LC separation.

The most commonly used pair is triethylamine (TEA) with 1,1,1,3,3,3-hexafluoroisopropanol (HFIP). However, ageing of the mobile phase is a common problem with these ion pairing agents. Loss of MS sensitivity can occur within a short period of time.



However, especially with large sample sequences, it is not always practical to prepare fresh mobile phase every day. Instead, it is worth taking a closer look at the reasons for mobile phase ageing and taking steps to prevent it:

- Alkylamine oxidation
- Evaporation of HFIP
- Formation of alkylamine micellar aggregates

While alkylamine oxidation has the greatest influence, the other factors are negligible.

## Amine oxidation

### The influence of pH

The oxidation of alkylamines is directly related to the pH of the mobile phase. A high pH value of 10 favours oxidation, whereas at a neutral pH value no oxidation occurs. Therefore, the ageing of the mobile phase caused by alkylamine oxidation can be influenced by its pH.

#### **Solution:**

The mobile phase should preferably be kept at neutral pH. If this is not possible, the pH value should be kept as low as possible.

### The influence of solvents

The solvent in which the alkylamine is dissolved also plays an important role in amine oxidation. In particular, protic solvents promote the oxidation process.

#### **Solution:**

Use the ion pairing agent only in a polar aprotic solvent such as acetonitrile. This can be implemented with a quaternary system as follows: The ion pairing agent is removed from lines A and B, while an additional line C contains ion pairing agent in acetonitrile, which is intended for the ion pair supply.

### The influence of oxygen exposure

Amines such as tributylamine oxidise when exposed to air, for example through open bottles or simply through cap openings.

#### **Solution:**

To control air exposure, the use of bottle caps with reduced airflow is recommended. This reduces amine oxidation and additionally the evaporation of volatile ion pairing agents such as HFIP.

#### **Reference**

Guimaraes G. J. et al. (2023) Mobile Phase Aging and Its Impact on Electrospray Ionization of Oligonucleotides, *J. Am. Soc. Mass. Spectrom.*, 34, 2691-2699