

Solvent quality- a guarantee to avoid ghost peaks in gradient HPLC

In many industries, 'control' is essential in production and analysis. One of the most common methods used is reversed-phase HPLC or UHPLC. However, there are several factors that can harm your analysis such as contamination from the HPLC system or using low quality consumables and reagents. These factors can cause ghost peaks, introduce impurities and ruin your HPLC or UHPLC analyses. Quality and batch-to-batch solvent reproducibility are one of the key parameters to avoid poor quality results.



The presence of ghost peaks is an issue because these non identified peaks are close to peaks of interest, and can deliver incorrect results in quantitative analyses when they overlap peaks of interest. Uncertainty in data quality and reliability is of course the result.

With the increase in UV detector sensitivity in recent years, detection has become possible at very low concentrations, bringing the problem of ghost peaks into the spotlight.

It's clear that when ghost peaks suddenly appear, scientists are not so comfortable, and in many cases, they return to less efficient isocratic methods that are ultimately more time-consuming and costly.

THERE ARE MANY POSSIBLE CAUSES WHY GHOST PEAKS MAY BE OBSERVED IN GRADIENT HPLC, YOU WILL FIND SOME OF THEM WITH OUR RECOMMENDATIONS

1. Ghost peaks from instruments

One reason for the creation of ghost peaks is the transition of the sample from the autosampler to the instrument, especially when impurities are adsorbed to the inside and outside surfaces of the needle dipped into the vial. Those substances, which are not eliminated even after needle rinsing, are carried over to the next analysis and appear as ghost peaks.

2. Ghost peaks from samples

If an impurity in the sample has the same absorption wavelength as the compound of interest, it results in a ghost peak.

Our recommendations are:

- Make a test using a blank injection
- Change the sample preparation process (extraction, filtration or reagent quality during sample preparation)
- When the appearance of a ghost peak is due to degradation of the sample, use an autosampler with a vial cooling feature
- When using a UV detector, if the source of the ghost peak is from dissolved oxygen in the sample solvent, you need to reduce the amount of dissolved oxygen in the mobile phase

3. Ghost peaks from mobile phases

In HPLC gradient elution, some substances can be retained in the column at the start of analysis, and then when they are eluted with the organic solvent, they can create some ghost peaks making separation difficult.

Ghost peaks can also be generated from the mobile phase in several ways.

- If the mobile phase is not in a closed container, you can observe a dissolution of organic substances from the air
- Mobile phase vial contamination, due to topping-off existing mobile phase with new mobile phase over a long period of time, instead of preparing a fresh bottle each day or for each set of samples
- Use of contaminated organic solvent and/or water to prepare a mobile phase
- 'Old' contaminated mobile phase used before being replaced



3.1. Water

Water used for gradient HPLC must be as pure and fresh as possible. It should ideally be free from trace organics, inorganics and particulates.

- Fortunately, different purification systems are available to provide such quality. So, the water should not be an issue

3.2. Inorganic impurities in water

Modern reversed-phase HPLC columns use ultra-pure silica that is very low in trace metal ions. The performance of the column relies on preserving this low metal ion content.

- Water used for gradient HPLC must be fully deionised and purified, not only for the sake of the column, but also because of the effect that the ionic content can have on the gradient baseline
- You can use a high purity HPLC gradient water controlled in different specifications linked to the HPLC gradient elution

3.3. Organic solvents

Sometimes, some impurities in an organic solvent can be concentrated on the reversed-phase HPLC column and then eluted by a trace enrichment and focusing mechanism. These impurities usually come from the organic solvent.

In fact, the range of quality between different suppliers, and even between product batches can be very significant.

To illustrate this problem, here are some examples.

Huge volumes of acetonitrile for HPLC are produced every year from the acrylonitrile industry. The number of impurities in acetonitrile can be important, despite many purification processes before becoming a 'finished' product filled in bottle. The range of impurities includes a lot of molecules such as acetamide, ethyleneimine, methylamine, ... acrolein, oxazole, methacrylonitrile, butanedinitrile, pentanedinitrile, di-*t*-butylmethylphenol, butylated hydroxytoluene, glutaronitrile, succinonitrile and ammonia. Depending of the purification process, it is not surprising that the quality of acetonitrile is not sufficient to be suitable for gradient HPLC.

Same story with HPLC grade THF, (unstabilised) still not pure enough for gradient HPLC. If BHT (2,6-di-*t*-butyl-4-methylphenol) stabilised THF is used, BHT is easily observed as a well-formed ghost peak typically midway through the gradient.

IN SUMMARY, SOLVENTS OF SUITABLE QUALITY FOR GRADIENT HPLC ARE AVAILABLE, BUT IT IS IMPORTANT TO CARRY OUT SOME TESTS TO BE SURE OF THE RIGHT QUALITY FOR YOUR APPLICATION

3.4. Reagents and additives for mobile phases

Ghost peaks can also be generated by buffer reagents.

If an additive is suspected, eluents should be made up without additives to test and solve the problem. Phosphates and acetates are available in a wide range of purities, and many are not suitable for gradient HPLC.

- Filter the aqueous buffer component solution with an appropriate filter
- The quality of some additives, like trifluoroacetic acid, is also variable as the reagent oxidises with age. Old TFA, often exhibiting a pale brown colour, shows a considerable ghost peak

TEST YOUR HPLC SOLVENTS TO BE SURE OF THE QUALITY BATCH-TO-BATCH

3.5. Phthalates, plastic additives and airborne organics as ghost peaks

Eluents for gradient HPLC should never be kept in plastic containers. The plastic may contain softeners, antioxidants, stabilisers or colours that may leach out of the material and cause ghost peak problems. Ghost peak impurities can be generated from polyethylene and polypropylene containers into pure water after only 1 hour. For gradient HPLC, it's mandatory to avoid the use of cylinders, pipettes and wash bottles in plastic material. Phthalate esters are semi-volatile liquids used as plasticisers in resins and polymers.

Other organic compounds known to be present in indoor air, include surfactants, fire retardants, antioxidant polishes, volatile cleaning products and odorants. Some ghost peaks can also be produced directly from these sources.

3.6. Contamination by mobile phase glassware

Glassware can represent another potential risk with an active surface as it can be difficult to remove adsorbed contaminants, particularly detergent residues.

- 10 rinses of the glassware with tap water and 10 rinses with deionised water will obtain satisfactory results

Key points

- Try to use pre-mixed solvents
- Use fresh eluents with salts
- Filter your HPLC solvents
- Use high quality water (18,2 MOhm, <5 ppb TOC or water gradient grade for HPLC)
- Use good quality buffers and solvents
- Control the pH of mobile phases
- Control glassware cleaning

Description	1 l glass bottle	2,5 l glass bottle	4 l glass bottle	5 l aluminium bottle
Acetonitrile Reag. Ph. Eur., USP, ACS gradient grade suitable for UPLC and UHPLC	20060.290	20060.320	-	20060.360
Acetonitrile Reag. Ph. Eur., USP, ACS super gradient grade suitable for UPLC and UHPLC	83639.290	83639.320	83639.400	83639.360
Methanol Reag. Ph. Eur. gradient grade for HPLC	20864.290	20864.320	20864.400	20864.360
Methanol ACS, Reag. Ph. Eur., super gradient grade for HPLC, UPLC and UHPLC	85681.290	85681.320	85681.400	85681.360
Water super gradient grade for HPLC for UPLC and UHPLC	83650.290	83650.320	-	-