

Best Practices for Addressing Problems Associated With Unstable Solvents in an (U)HPLC Environment

Introduction

Introduction

This technote provides an overview on how to operate safely with unstable solvents in an (U)HPLC environment and how to avoid damage to the instrument or separation column.

Corrosion can occur in stainless steel systems if free halides, hydroperoxides, free radicals or strong, oxidizing acids are present. There are several solvents where, under non-ideal conditions, reactions can occur and these harmful compounds are generated. Prevent formation of reactive substances when using the following solvents:

- Solutions of alkali halides and their respective acids (for example lithium iodide, potassium chloride, and so on)
- High concentrations of inorganic acids like sulfuric acid and nitric acid, especially at higher temperatures
- Halogenated, organic solvents or mixtures which form radicals and/or acids (for example chloroform, methylene chloride)
- Chromatographic grade ethers, which can contain peroxides (for example THF, dioxane, diisopropyl ether)
- Solvents containing strong complexing agents (for example EDTA)

In addition, ethers and cyclic ethers are readily degraded in the presence of oxygen. The hydroperoxides that are formed are hazardous to some components within the instrument and may cause malfunctions.

The stability of the solvents can be maximized by control of the environmental conditions in the solvent reservoir. The exclusion of moisture, light and air is the most effective measure. If there is a ready source of inert gas such as nitrogen or argon, connect an additional line to the reservoir to maintain a blanket of dry inert gas in the headspace of the bottle. The flow rate of this gas is not substantial, as only a low flow rate of the liquid is being removed. In general, a gas flow of only a few cm³ per minute is more than adequate.

Some reactions are catalyzed by heat or light. Prevent exposure to direct sunlight or the proximity of heat sources. It is recommended to use dark solvent bottles only.

A detailed technical note about stabilizers can be found at the Sigma Aldrich web site:

<https://www.sigmaaldrich.com/DE/de/technical-documents/technical-article/chemistry-and-synthesis/reaction-design-and-optimization/stabilizer-systems>

Typically, it is highly recommended to filter all solvents prior to use. We do not recommend this for reactive solvents (except for buffers or modified solvents), because pouring in the open atmosphere may cause degradation during initial handling before use within the chromatographic system.

Changing Solvents

NOTE

When using a stabilized solvent, such as THF with BHT or ethanol, filtering does not pose the risk of unwanted reactions. See [Solvent Recommendations](#) on page 6.

Ideally, use the solvent in the original bottle in which it was delivered. If necessary, keep it closed with a matching lid with two holes that can accommodate the solvent line and the inert gas line to prevent free air circulation within the container. Agilent solvent lines are approximately 1.6 mm in outside diameter.

Using small solvent bottles (≤ 1 L) will allow consumption of the solvent in reasonable time, preventing the solvent from aging and thus unwanted reactions. Use a reduced flow (~ 0.1 mL/min) through the channels, when the system is not in use. Do not recirculate or recycle solvent during idle instrument times.

NOTE

Stabilizers are depleted over time and it is very difficult to monitor their concentration. Dispose of stabilized solvents after recycling maximum three times.

Some reaction products can pose a significant risk with increased concentrations that can occur during open storage, for example when fractions are collected and evaporate. Hydroperoxides can act as contact explosives and self-detonation of residual solvent material has been reported.

Changing Solvents

Even if the environmental conditions for the HPLC/GPC instruments stay unchanged, the evolution of compounds and polymers can require a change in operating parameters. Before changing from water to organic solvents, buffers must be completely washed out of the column, capillaries, and detectors (including the reference cell of the RI Detector). Verify that the new solvents/mobile phases are miscible with the previously used solvents/mobile phases. If these solvents are not miscible, flush the entire system with an intermediate set of miscible solvents. This measure prevents buffers from salting out and/or miscibility issues.

Impact on Hardware

When potentially harmful compounds come in contact with the hardware, corrosion can occur. Not only can stainless steel be attacked but also other parts of the systems are affected. Even if only high-quality, inert material is used in the flow path, several system components show signs of wear ahead of time when exposed to corrosive contaminants regularly.

Pump

Solvent Lines

Standard solvent lines can get cloudy and brittle after a long exposure to reactive solvents. To prevent this, it is recommended that solvent lines with PN G7120-68070 be used.

Solvent Selection Valve

The material in the solvent selection valve is resistant to a large variety of different solvents but degradation can lead to problems with sealing, whereby a crossflow between port one and two of the same channel can be observed. If the solvent selection valve is not needed for the application, bypassing the valve should be considered.

When using solvents with a low boiling point, such as DCM, MeOH, Hexane or THF, sometimes small gas bubbles are generated inside the solvent selection valve. These gas bubbles will be removed by the degasser.

Degasser

The materials used inside the degasser are highly versatile and can resist a variety of different solvents. The degassing efficiency will only suffer when the unit is exposed to reactive solvents over a long period of time. Since the tubing inside the degasser is highly permeable to air, a hazardous reaction can occur directly in the degasser chamber when no vacuum is applied and no fresh solvent is moved through the chamber. Volatile solvents permeating through the chamber tubing can condense down in the degasser pump and damage it.

It is advised to either flush out reactive solvents before turning off the pump or keep a small flow (~0.1 mL/min) through the degasser channels when the system is not in use.

Inlet Valves

Generally, the standard inlet and outlet valves should be used when running solvents with higher reactivity, but use an Active Inlet Valve when available. Using the N-Type inlet valves is only recommended when unmodified non-polar, aprotic solvents such as hydrocarbons (hexane, heptane, octane, benzene, toluene), ethylacetate, or alike, are used. These valves will corrode when used with protic, polar or reactive solvents.

Impact on Hardware

Pistons

The ceramic pistons used in 1290 Infinity and 1290 Infinity II pumps as well as in the 1260 Infinity II Flexible Pump can get damaged by acidified organic solvents, especially when phosphoric acid is used. In cases where these solvent combinations are used or where repeated fails of ceramic pistons are found, alternative sapphire pistons can be used.

Plunger Assembly Sapphire Supported (for classical, 5067-4695 and EM pump heads, 5067-6174).

Piston Seals

The standard pump seals for the respective pump type can be used as delivered usually. However, we recommend replacement of the PTFE-based (black) seals in G131x and G711x pumps with PE seals (pack of 2) (0905-1420), when running solvents with extremely low conductivity, such as hydrocarbons without additives.

Rotor Seals

The rotor seals in automatic purge valves can be comprised of different materials, either polyimide or PEEK blends. Depending on the reactive solvent, the rotor seal might be damaged after long exposure. This can cause leakages and pressure fluctuations. It is advised to either flush out reactive solvents or to keep a small flow through the pump when the system is not in use.

Sampler

Needle Seat

During the injection cycle the flow path is opened at the needle seat, thus exposure to air cannot be prevented at this point. The material in the needle seat can be affected by reactions of unstable solvents and get brittle after a long exposure. Signs of wear might be extended, carry over or leakages at the needle and an exchange of needle and needle seat would be required in that case.

Rotor Seals

The rotor seals in injection valves can have different materials, either polyimide or PEEK blends. Depending on the reactive compound, the rotor seal might be damaged after long exposure. This can cause leakages and variations in peak height. It is advised to either flush out reactive solvent or keep a small flow through the sampler, when the system is not in use.

Leak Sensors

The leak sensors are covered with a polymer. This polymer usually resists organic solvents - except at least DMSO, that tends to swell the polymer. The leak sensor therefore needs to be changed after getting in contact with DMSO. Generally, the leak sensors will activate a complete stop of the flow when a leak is detected in different parts of the HPLC/GPC system. The polymer covering the leak sensor usually is not damaged by the organic solvent, apart from DMSO which tends to swell the material. In the case of a DMSO leak, the sensor will activate the stop flow function, but it will be necessary to be replaced.

Solvent Recommendations

Halogenated Organic Solvents

Halogenated, mostly chlorinated, solvents are routinely sold with stabilizers to minimize degradation and side reactions and are frequently used for chromatographic and other applications. We highly recommend that only stabilized solvents be used.

The common stabilizers used for chlorinated solvents include some hydrocarbons, amylene and specifically for chloroform, ethanol may also be used. When considering an appropriate stabilizer, it is important to think about whether that stabilizer will have an impact on any of the chromatographic properties of the solvent. Hydrocarbon and amylene stabilizers do not significantly alter the polarity or selectivity of chlorinated solvents; however, ethanol, which is often used to stabilize chloroform, will increase the polarity of chloroform and may affect separations done on normal phase columns.

Usually chloroform is considered to be less toxic than DCM or HFIP. Unfortunately, polyamides and other difficult to dissolve polymers like PET are soluble at room temperature only in HFIP, so the use of this solvent cannot be avoided. Moreover, the vapors of these solvents tend to accumulate in the lab, so the efficacy of ventilation must be verified regularly. For HFIP, the sodium salt of TFA must be added (0.2 M) in order to obtain correct chromatography and avoid the adsorption of the polymer in the column.

Ethers

Ethers are routinely sold with stabilizers to minimize degradation and side reactions and are frequently used for GPC applications. To avoid the buildup of peroxides, 200 – 500 ppm of BHT are added to the solvent. For RI detectors, this will have a minimum impact (a larger solvent peak being recorded).

When using ethers in conventional HPLC or other applications, BHT as additive might cause an increased baseline noise or absorbance due to its aromatic nature. In this case ≥ 1 % ethanol can be used as a stabilizer, too, but ethanol will increase the polarity of the solvent and alter the chromatographic results.

We highly recommend that only stabilized ethers should be used, but in some cases this is not possible. Consider the above recommend actions and ensure that no hazardous reaction products are generated. Limiting the solvent container size to a minimum and exchanging the solvent with fresh solvent daily is required.

Previously-opened bottles of solvent may not be of suitable quality for HPLC/GPC. The hydroperoxides contaminants that are formed when the solvent is in contact with oxygen are considerable UV absorbers and render the solvent useless after enough exposure time.

Examine the suitability of the solvent by measuring the UV background when the ether is introduced into the HPLC. Most common, with columns removed from the flow path, flush the instrument with methanol and perform an auto zero on the UV detector at 230 nm. Having done that, introduce the candidate bottle of ether and allow it to thoroughly flush through the system at a routine flow rate. Without auto zeroing the UV detector, observe the offset in UV that occurs as the ether fills and and equilibrates within the system. If the UV elevation of the baseline exceeds 0.3 absorbance units, the solvent may be questionable. If it exceeds one absorbance unit or higher, discard the solvent and open a fresh container to continue your work. It is not unusual for an aged or poorly-stored bottle of ether to exceed two absorbance units offset during this test.

Buffers and Modified Solvents

Although water does not attack HPLC instruments, it is rarely used without additives in HPLC or GPC. Both buffers (salt) or added acids can damage the pump and tubing, and usually it is recommended to avoid pHs <2.5 unless running Agilent's InfinityLab Bio-inert Solution. For pHs >10, the corrosive nature of the hydroxy ions for silica glass and some polymers should be considered. When working at these conditions, an exchange of the standard solvent inlet filters with PEEK or SST should be considered. When available, the rotor seals in the valves throughout the system should be replaced with PEEK seals to avoid leaks and carryover. For water-soluble polymers adding NaNO₃ (0.2 M) is necessary since most of them are not soluble in 100 % water at room temperature. Adding the salt modifies the temperature at which these polymers become soluble in water, thus making possible their dissolution at room temperature.

When using buffers or added acids, care should be taken to avoid the buildup of the crystallized salt. If precipitation is found in the solvent bottle, a fresh solution should be prepared and used. To prevent crystallization around the pump pistons, which can lead to malfunction of the pump piston/seals, the active seal wash option should be used.

Solvent Alternatives

Whenever possible, it should be considered to replace the most harmful solvents with a less dangerous alternative. Some recommendations can be found here:

| Undesirable solvents | Alternative |
|--|---|
| Pentane or hexane(s) | Heptane |
| Di-isopropyl ether, diethyl ether or THF | 2-MeTHF or MTBE or CPME |
| Dichloroethane or carbon tetrachloride | DCM, Chloroform |
| Dimethyl formamide, dimethyl acetamide | Acetonitrile |
| Dichloromethane | Ethyl acetate or heptane or Ethyl lactate |
| Benzene | Toluene |

We are currently reviewing and reworking some of our Application Notes to propose the use of a less harmful solvent alternative for these applications.

Also, solvent vendors are improving their portfolio with more sustainable and environmentally friendly solvent alternatives. Please see following information:

<https://b2b.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/234/922/sustainable-solvent-br5046en-mk.pdf>

Solvent Abbreviations

Acids

| | |
|------|---------------------------------|
| TFA | Trifluoroacetic acid |
| NaAc | Sodium acetate |
| EDTA | Ethylenediaminetetraacetic acid |

Ethers

| | |
|---------|--------------------------|
| THF | Tetrahydrofuran |
| MTBE | Methyl tert-butyl ether |
| 2-MeTHF | 2-Methyltetrahydrofuran |
| CPME | Cyclopentyl methyl ether |

Halogenated, organic solvents

| | |
|------|--------------------------------|
| HFIP | Hexafluoroisopropanol |
| DCM | Dichloromethane |
| TCM | Trichloromethane or chloroform |

Organic solvents

| | |
|------|-----------------------|
| DMF | N,N-dimethylformamide |
| DMSO | Dimethyl sulfoxide |
| MeOH | Methanol |

Stabilizer

| | |
|-----|--------------------------|
| BHT | Butylated hydroxytoluene |
|-----|--------------------------|

The information in this document also applies to Infinity II modules.

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