LC Troubleshooting

Solvent Selection, Part III — Solvent Life and Degradation

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In this third installment of a series about solvents, the authors discuss potential problems related to the deterioration of solvents and the resulting chromatographic difficulties.

Testing Ether Solvents for Peroxides

Many procedures are available to test for peroxides in ether solvents. A simple test is to shake a 1:1 (v/v) mixture of the ether sample and 10% aqueous potassium iodide and look for color. No color or a faint yellow tint indicates little peroxide contamination, and a dark yellow or brown color indicates significant levels of peroxides. See reference 3 for a detailed discussion of peroxides in solvents.

In the two previous installments of this series (1,2), we discussed important decisions to make regarding the choice of solvents for particular applications. This month we discuss problems that arise when solvents degrade. Unlike wines that improve with age, solvents degrade over time. Manufacturers of solvents for liquid chromatography (LC) have had to contend with solvent degradation issues for years, but many users have been unaware of these problems. Now, however, new analytical techniques and lower detection wavelengths and limits have all served to highlight the issues associated with solvent stability. Three areas are of particular importance. First, degradation products in unstabilized solvents can rapidly build up and interfere with an analysis. Second, stabilizers that are added to control solvent degradation also can interfere with the analysis. Finally, safety can become a concern if solvent degradation products are strongly reactive or toxic.

Manufacturers add stabilizers to many solvents to prevent or slow degradation. Users frequently can choose between stabilized or nonstabilized solvent, and, in some cases, they can choose between different stabilizers for a particular solvent. Some solvents do not require stabilizers; others may need stabilization but are not available with a suitable stabilizer for a particular application. This article will discuss specific solvents that are known to degrade and which stabilizers are available to minimize degradation. The effect of stabilizers on practical LC will be highlighted.

John Dolan
LC Troubleshooting Editor

CHLORINATED SOLVENTS

Chlorinated solvents such as chloroform and methylene chloride are widely used for gas chromatography and normal-phase LC. The susceptibility to, and rate of, degradation depend on both the solvent type and storage conditions. Solvent manufacturers add stabilizers to chlorinated solvents to improve solvent stability and ease of use for routine applications.

Chlorinated solvents are susceptible to free-radical formation:

$$\mathrm{RCl} + h\nu \rightarrow \mathrm{R}\cdot + \mathrm{Cl}\cdot$$
$$\mathrm{Cl}\cdot + \mathrm{HR}\cdot \rightarrow \mathrm{HCl} + \mathrm{R}:$$
$$\mathrm{R}\cdot + \mathrm{R}: \rightarrow \mathrm{RR}\cdot$$

Light, heat, and oxygen can initiate formation of free radicals that generate decomposition products such as phosgene, hydrogen chloride, and oligomers.

Many chromatographers use chloroform without considering the potential problems that can result from decomposition. Chloroform can generate free-radical decomposition products in a process that begins as soon as purification is complete. However, many additives can stabilize this decomposition process. For example, alcohols and olefins are the most successful and widely used stabilizers today. Olefins act as free-radical scavengers and remove the decomposition products (for example, hydrogen chloride). Alcohols inhibit free-radical formation in chloroform, but they must be present at high levels (1%) to be effective. Although amylene is the most common chloroform additive, chloroform is also available with ethanol — a combination originally developed and primarily used for normal-phase methods. Ethanol will change the polarity of the mobile phase and thus may affect the analysis. For this reason, methods that use chloroform in a mobile phase should clearly state whether ethanol is included as a preservative.

ETHERS

The two most widely used solvents that contain ethers are tetrahydrofuran and ethyl ether (diethyl ether). Light, heat, and oxygen cause all ether-containing solvents to form free radicals that lead to peroxide formation. Tetrahydrofuran and ethyl ether are of particular interest because of their widespread use and rapid peroxide formation. Peroxide formation in the mobile phase raises several concerns. The explosive nature of peroxides makes safety the most serious issue when handling any peroxide-containing solvent. Another concern is the interaction of peroxide with the analytes of interest or the column packing. The simplest way to avoid these problems is to purchase solvents in unit quantities small enough to ensure their use (or disposal) within approximately 30 days after opening. Alternatively, and as a safety measure, the peroxide level of the solvent can be checked before use (see "Testing Ether Solvents for Peroxides").

Solvent manufacturers use several procedures to minimize the degradation of ether solvents. One method is to remove one or more of the three sources of free-radical formation. Manufacturers minimize the exposure to light and oxygen during bottling by blanketing the solvent with nitrogen to remove oxygen, and by using amber bottles to shield the solvent from UV light. This packaging technique protects the solvent until the bottle is opened, at which time the solvent is exposed to oxygen. Manufacturers add stabilizers to further extend solvent life. Because tetrahydrofuran begins to form peroxides as soon as the solvent is exposed to oxygen, manufacturers have added the antioxidant butylated hydroxytoluene (BHT) to scavenge the oxygen and prevent the peroxides from forming. This stabilizer has been very effective, but BHT has an aromatic functional group that is a strong UV absorber at 280 nm. Consequently, BHT-preserved tetrahydrofuran is difficult to use in LC methods that call for UV detection, so most users buy tetrahydrofuran without stabilizer. As mentioned above, buying small unit volumes (for example, 1-L bottles) will help ensure that the solvent is used before peroxides become a problem. For best results, dispose of any unused solvent 30 days after the bottle is opened, and use or dispose of any unopened bottles within a year of purchase. Another option is to reblanket the solvent with nitrogen, a procedure that can extend solvent life for as long as six months.

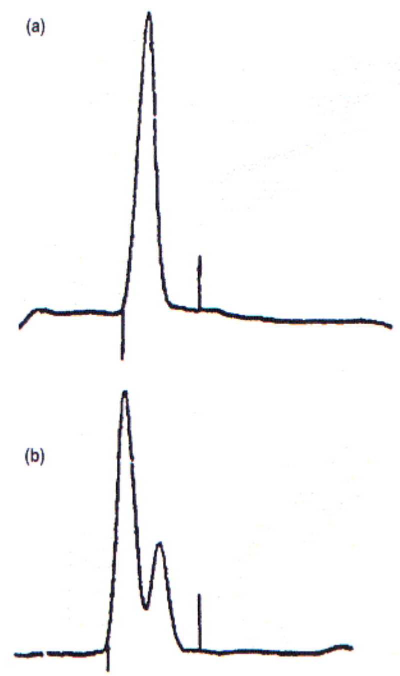

FIGURE 1: Chromatograms of analyte using (a) fresh and (b) aged tetrahydrofuran in the mobile phase. See text for details.

Increased UV absorption is a common chromatographic symptom of peroxide formation in tetrahydrofuran. Less common is a chemical reaction between the peroxides and analytes (Figure 1). As shown in Figure 1a, an analysis had been performed successfully, then was set aside for several weeks. The method was restarted using the tetrahydrofuran that had been left on the instrument. The chromatogram showed a second peak as depicted in Figure 1b. Troubleshooting revealed that the peroxide level in the tetrahydrofuran was greater than 80 ppm. Replacing the degraded solvent with a fresh bottle of tetrahydrofuran solved the problem.

Ethyl ether is stabilized with ethanol, and, as is the case with ethanol-preserved chloroform, the polarity of the solvent is changed by the additive. Consider the implications of the added ethanol when selecting ethyl ether for a chromatographic application.

KETONES

Most chromatographers find ketones such as acetone and methyl ethyl ketone to be stable and easy to use. New applications and lower detection limits have created conditions in which the effects of solvent degradation may be important. Ketones degrade via the aldol condensation. For acetone, this involves the condensation of the ketone with the enol tautomer, which is always present at trace levels:

$$\mathrm{CH_3{-}COH{=}CH_2 + CH_3COCH_3 \rightarrow (CH_3)_2COH{-}CH_2{-}CH_2COCH_3}$$

When ketones are exposed to heat, the aldol condensation reaction occurs, but the byproducts (dimers that may further react to form larger polymers) seldom are noticed in LC analyses. Another potential interaction can occur between ketone degradation products and amine-containing analytes or stationary phases (for example, aminopropyl bonded phases). These effects should be considered when selecting carbonyl solvents as mobile-phase components.

HYDROCARBON SOLVENTS

Hexane and other hydrocarbon solvents are common mobile-phase components for normal-phase LC applications. Hydrocarbon solvents normally are perceived to be stable, so their degradation seldom is considered a potential source of chromatographic artifacts. In rare instances, the degradation of the solvent can cause artifactual peaks or increased baseline noise.

Degradation occurs when hydroperoxides are formed by the autooxidation of hydrocarbons in the presence of dissolved oxygen. Branched or cyclic hydrocarbon isomers will degrade faster because of the formation of a more stable hydroperoxide. Because the hydrocarbon is attacked by oxygen in a free-radical chain reaction, little oxygen is required. This process is similar to the free-radical reactions other solvents undergo in the presence of light, heat, oxygen, metal ions, or highly unstable sample molecules.

WATER

Pure water is a stable solvent, but it can become contaminated by its container during storage or by bacterial growth when exposed to air. Water stored in laboratory glassware or plasticware can leech contaminants from the containers. Figure 2 shows examples of the problems that can be encountered. Water used with LC methods should be purchased as HPLC grade or generated in the laboratory using a commercial water-purification apparatus designed to produce HPLC-grade water. The simplest way to avoid water problems is to use freshly purified water from a well-maintained water system and to make sure the reservoirs are sufficiently clean.

HANDLING LESS-STABLE SOLVENTS

Although it is best to avoid less-stable solvents when developing an LC method, some new and existing methods call for their use. When less-stable solvents such as ethers, ketones, and hydrocarbons are used, analysts should minimize potential problems. For example, when solvent manufacturers recommend using the product within six months of manufacture, remember that this timetable begins as soon as manufacturers ship the solvent and not when consumers purchase it. Container size is also important. For example, if tetrahydrofuran is

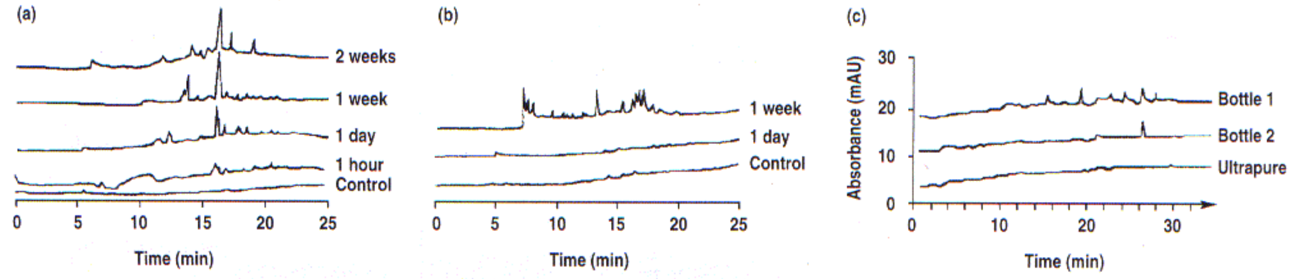

FIGURE 2: Gradient-elution chromatograms generated using water stored in (a) a polyethylene container, (b) laboratory glassware, and (c) open containers. Column: C18; gradient: 100% water to 100% acetonitrile in 20 min; flow rate: 1 mL/min. Reprinted from reference 4 with permission.

used at a rate of approximately 1 L per week, a case of 12 1-L bottles may be a wiser purchase than a case of four 4-L bottles. Each bottle will be open for a short period of time, thereby minimizing the potential for peroxide problems. The added convenience in solvent handling should offset the increased cost of smaller containers. Be sure to dispose of unused mobile phase regularly and avoid topping off a reservoir of old mobile phase with fresh solvent.

Further, consider the recommended expiration date that manufacturers list on each solvent bottle's label; the solvent may not be usable after that date has passed. Testing for peroxides and blanketing the solvent with an inert gas after removing solvent may be necessary to help extend the useful life of unstable solvents.

Also, solvents should be stored in a controlled environment. Store unopened bottles in a vented, fireproof storage cabinet at room temperature to minimize exposure to light, heat, and oxygen. Improper storage conditions such as extremely warm storage sheds can shorten solvent life.

Finally, worker safety always is a concern when using LC solvents. Be sure to be familiar with the information contained in the material safety data sheet (MSDS) for each solvent you use. If you have misplaced the MSDS or did not receive one, solvent suppliers will be happy to provide copies.

Unlike wines that improve with age, solvents degrade over time.

CONCLUSIONS

Most solvents will degrade over time, depending on their individual properties, the presence or absence of stabilizers, and storage conditions. When using less-stable solvents, pay careful attention to *all* the variables that affect solvent life. Thoughtful storage and careful handling can go a long way toward preventing practical problems with potentially unstable solvents. For details about specific solvents, consult solvent manufacturers or their literature such as that listed in reference 5.

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