

Optimizing and Monitoring Solvent Quality for UV-Vis Absorption, Fluorescence and Charged Aerosol Detectors

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Key Words

Eluent Quality, Mobile Phase, UHPLC,
Liquid Chromatography

Goal

Provide guidance on how to find out if mobile phase quality is sufficient for application specific UV-Vis, fluorescence, and charged aerosol detection requirements. Give assistance in laboratory solvent quality monitoring and solvent cost control.

Introduction

Solvent quality has a large impact on laboratories using liquid chromatography (LC) methods. A minimum purity and filter grade is required to reliably operate an LC system. Low particle content is beneficial for the lifetime of LC pump check valves, pistons, and piston seals. The column depends even more on the absence of particles. Bottled high performance liquid chromatography (HPLC) solvents are typically filtered at least to the 0.22 μm level to remove bacterial contaminants in addition to any particulate matter. This is usually sufficient for conventional columns packed with 3 μm or 5 μm particles. When adding buffer salts to the mobile phase, further preparation may be required, e.g., centrifugation or filtering.

Ultra high performance liquid chromatography (UHPLC) often uses columns with sub-2 μm particles and 0.2 μm frits. UHPLC columns typically have narrow bores and smaller internal volumes that result in an increased risk of failure due to clogging with particles from HPLC grade solvents. In this case, every sample must be filtered through a frit with a porosity of 0.2 μm . In addition, a 0.2 μm porosity in-line filter is strongly recommended as a precautionary measure.¹

Beyond these precautions for the system mobile phase there are also detector- and application-related requirements. Optimizing the quality of mobile phase solvents can contribute to an improvement of the chromatographic or mass spectrometric properties of the analyte as well as the overall detection limits of the LC system.² To achieve lowest limits of detection (LOD) with optical detectors, the solvent should respond as little as possible to the selected wavelengths. Absorption or fluorescence of the mobile phase will result in a background signal that reduces the dynamic range of the detector, directly contributing to baseline noise, therefore adversely affecting the limit of detection.³ Charged aerosol detection uses an electrical analyzer together with HPLC.⁴ This detection method is based upon detecting charged particles rather than measuring individual ions in the gas phase that are differentiated upon mass-to-charge ratio (m/z).⁵ These charged particles can also contain residual mobile phase particulates after evaporation which may have an impact on the detector signal. In summary, the optical characteristics as well as the content and size of the particles are of highest importance to judge the suitability of solvents for HPLC or UHPLC. In contrast, LC/MS applications require solvents with low mass noise levels, minimal organic contamination, and minimal metal content.⁶

Solvent and instrument vendors may often recommend the best available solvent quality for HPLC or UHPLC analysis. Similarly, buffers, salts, and other mobile phase additives come in different reagent qualities. The use of high quality reagents in the mobile phase may result in quieter baselines.⁷ Laboratory managers however will question if it is really necessary to use the best quality. Is a medium solvent quality perhaps good enough for the needs of the application while sparing the laboratory budget? How can one make sure to identify lot-to-lot quality consistency?

The worldwide acetonitrile shortage in 2008–2009 pushed strategies to limit the consumption of solvents⁸ by reducing the particle size of the stationary phase carrier and shortening the column length, while adapting the linear velocity of the mobile phase to maintain separation efficiency.⁹ In addition to the limited availability of the solvent and the related increased purchase price, the acetonitrile shortage also compromised the solvent quality of some suppliers. How can a lab management make sure that these quality fluctuations are identified before valuable data is adversely affected?

In addition to the minimum LC requirements laid out earlier, this technical note answers the questions above by giving advice on how to optimize and monitor solvent quality. This may be used to identify specific application-related solvent requirements and to establish a laboratory-wide solvent monitoring system.

Equipment System

- Thermo Scientific™ Dionex™ UltiMate™ 3000 Quaternary Rapid Separation System including:
 - SR-3000 Solvent Rack
 - LPG-3400RS Quaternary Pump
 - DAD-3000RS Diode Array Detector
 - FLD-3400RS Fluorescence Detector with Dual-PMT
- Thermo Scientific™ Dionex™ Corona™ *ultra* RS Charged Aerosol Detector
- Thermo Scientific™ Dionex™ Viper™ Fingertight Fitting System
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System 7.1. software, SR2

Conditions

Column:	No column was used to make sure it did not influence the results. A Thermo Scientific™ Dionex™ nanoViper™ (75 µm i.d. × 350 mm) capillary is used to create a suitable backpressure (≥ 20 bar).
Eluents:	Water (H ₂ O), of different grades: <ul style="list-style-type: none"> – Purified water, de-ionized by an ion-exchange cartridge – Ultra-pure lab water, 18.2 MΩ·cm at 25 °C – LC/MS-grade water Methanol (MeOH), UHPLC- and LC/MS-grade, various vendors Acetonitrile (ACN), LC/MS-grade, various vendors
Flow Rate:	1 mL/min, isocratic flow
Run Time:	6.5 min
Diode Array Detector Settings	
UV Wavelength:	3D-field acquisition, 190 nm to 800 nm
Bunch Width:	1 nm
Band Width:	1 nm
Slit Width:	Narrow
Data Collection Rate:	25 Hz
Response Time:	0.2 s
Fluorescence Detector Settings	
Flow Cell Temp.:	45 °C
Lamp Mode:	Standard
Data Collection Rate:	20 Hz
Response Time:	0.4 s
Sensitivity:	5
PMT:	1 (Standard)

Table 1. Fluorescence detector settings.

Step	Time (min)	Command	Excitation (nm)	Emission (nm)	Variable Emission (nm)
	0	Auto Zero	219	330	280
1	0.5	Clear Auto Zero			
	1	Auto Zero	247	400	370
2	1.5	Clear Auto Zero			
	2	Auto Zero	232	443	435
3	2.5	Clear Auto Zero			
	3	Auto Zero	238	390	370
4	3.5	Clear Auto Zero			
	4	Auto Zero	294	396	370
5	4.5	Clear Auto Zero			
	5	Auto Zero	350	397	370
6*	5.5	Clear Auto Zero			

* For the evaluation of water, only steps 1–5 are used.

Charged Aerosol Detector Settings

Nebulizer Temp.:	30 °C
Data Collection Rate:	20 Hz
Filter Constant:	7 (0–3.5 min) 0 (3.5–6.5 min)

Experimental

Solvent Quality for Diode Array Detectors

The principle of UV-Vis spectroscopy is based upon the molecular absorption of light at a specific wavelength. In LC, both the active compound of interest and the mobile phase can be UV-Vis active. Organic solvents used in LC often contain organic contaminants absorbing in the range of 190–250 nm. This can cause fluctuating baselines and higher noise levels, influencing the baseline drift and LOD. Hence, the absorption behavior of the mobile phase is of general importance. A single blank run with 3D-field acquisition is typically enough to obtain all required information, UV-Vis spectra, and baselines.

Figure 1 shows the baselines of two methanol samples of nominal comparable qualities. The data was obtained with a diode array detector at 220 nm. The short-term noise is comparable for both samples, but the blue baseline shows high fluctuation caused by a high level of UV-active contaminants. In comparison, the black baseline shows almost no fluctuations. Please note that the short-term noise is pronounced as the detection conditions were selected to be sensitive to solvent quality differences.

This is also illustrated by the spectra of these methanol samples shown in Figure 2. The blue spectrum shows high absorption (up to 1 mAU) in the range of 200–250 nm, while the spectrum of the other sample (black) behaves as expected of a high grade eluent and shows no major absorption at any wavelength.

In conclusion, the low-quality eluent shown is not suitable for wavelengths between 200 nm and 250 nm but provides optical characteristics comparable to the high-quality solvent with wavelengths longer than 250 nm.

While this test can evaluate the presence of UV-active contaminants in the eluent, it does not provide information about residual particles that are invisible to UV detection. Lower quality solvents tend to have higher amounts of these particles that may cause higher baseline noise and may clog in-line filters and columns.

Solvent Quality for Fluorescence Detectors

Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation. LC fluorescence detectors typically use UV-Vis light of a selected wavelength to excite the analyte. Fluorescence-active substances do not convert the absorbed energy into heat, but re-emit some of it as red-shifted light. This light is detected by a fluorescence detector. In contrast to absorption spectroscopy where small differences in light

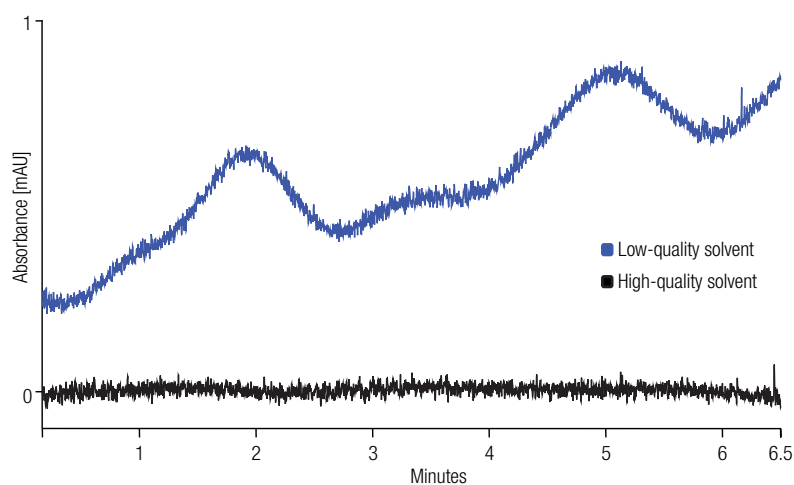


Figure 1. Comparison of the baseline behavior of two methanol samples at 220 nm (nominal LC/MS grade). Blue: low-quality solvent, black: high-purity solvent.

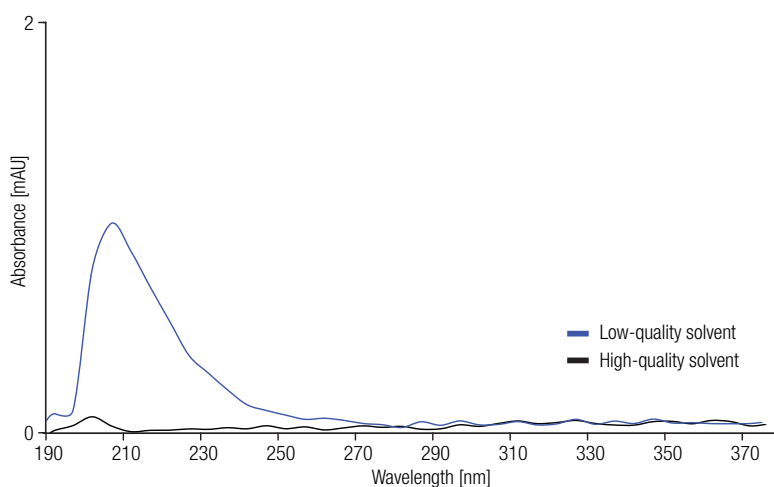


Figure 2. Comparison of the UV-spectra of two methanol samples (nominal LC/MS grade). Blue: low-quality solvent, black: high-quality solvent. The low-quality solvent shows strong absorption at 200–250 nm.

intensity have to be detected, a fluorescence detector measures low levels of light at a wavelength longer than the excitation. In general, fluorescence detection has different requirements for solvent quality compared to the almost ubiquitous UV detection.⁶ Solvent manufacturers typically offer specific solvents for fluorescence applications. These are supposed to provide a low content of fluorescing contaminants or particles that may cause background fluorescence and stray light (autofluorescence), and thus increase noise and reduce the dynamic range of the detector.

In many cases, selecting fluorescence- or LC/MS-grade solvents should serve the need for low background fluorescence, but it does not automatically mean that an eluent is qualified for any fluorescence measurement. It also does not indicate that a solvent is not suitable because it is not fluorescence (or LC/MS) grade. Even more important than the grade of the solvent is the compatibility with the specific application. As fluorescence is highly specific, the wavelength settings applied during the analysis should be considered for evaluation of the mobile phase.

A good example of how the analysis can be influenced by contaminants is shown in Figure 3. The autofluorescence of water with different grades is evaluated at five different excitation/emission settings. These were selected, as they mimic the detection conditions of a real application to detect five polycyclic aromatic hydrocarbons (PAHs). It is quite common to set both excitation and emission wavelengths individually for each analyte to increase the specificity and the sensitivity of the measurement. The steps in Figure 3 are generated by testing the wavelength pairs: first, after an Autozero and second, after a ClearAutoZero command. The latter is used to release the baseline signal which will display the background fluorescence caused by the solvent. This autofluorescence would otherwise be subtracted by the Autozero in standard detector operation. For general applications, this subtraction is reasonable, but in the case of autofluorescence monitoring it may conceal the source of increased baseline noise. The higher this response, the higher the fluorescing of the solvent (and possibly included contaminations), reducing the suitability of the solvent. Purified water that was de-ionized by an ion-exchange cartridge is used as the eluent in the following experiments in order to showcase differences in the autofluorescence examples.

In Figure 3, the following behavior is observed:

- For the first wavelength pair, the purified water creates a signal of ~400,000 counts. This signal is about four times the response of the ultra-pure lab water and the LC/MS grade water.
- At the third wavelength pair, the signal level caused by the purified water is comparable to its response in the first setting, whereas the fluorescence of the other water samples is even three times higher. The purified water causes a level of background fluorescence that is only 1.2 times the signal of the LC/MS and the ultra-pure lab water. This means that the relative deviation of the different water qualities is smaller compared to the first setting.
- Examining the fifth wavelength pair, the samples response level is far lower than that of the first wavelength setting (~85% lower). However, the relative difference between the samples is more significant than with wavelength setting one. The fluorescence of the purified water is five times higher than the response level of the other water samples. This again demonstrates the strong dependency of the background fluorescence on the wavelength settings. The highest relative background fluorescence difference is present under lowest overall response conditions.

A general conclusion of this test is that the purified water shows the highest background fluorescence throughout all wavelength pairs. Ultra-pure lab water and LC/MS-grade water provide comparable results with typically much less autofluorescence than the purified water. This demonstrates that the use of LC/MS-grade water and ultra-pure lab water is clearly better for the applied conditions. The relative difference of the response however varies and depends on the wavelength pair selection.

The spike at 2 minutes is a result from the switch of the wavelength settings. This is caused by the rise of the signal to the response level of the next wavelength setting before the AutoZero command is executed.

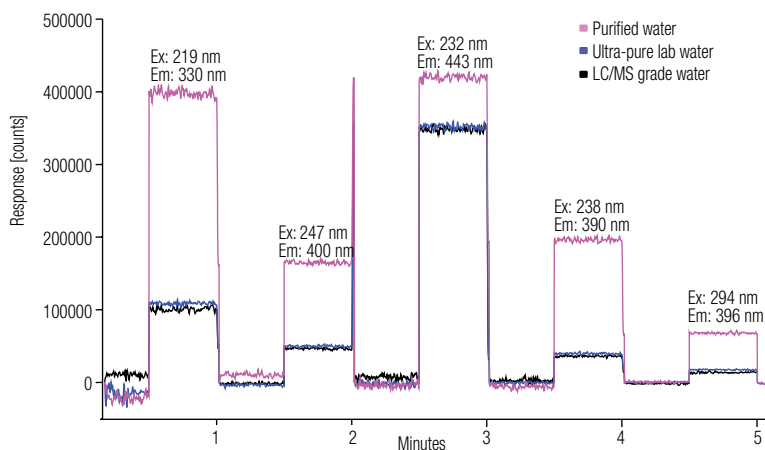


Figure 3. Comparison of the fluorescence baseline behavior of different grade water samples (Pink: purified water, blue: ultra-pure lab water, black: LC/MS grade water). Five wavelength pairs are evaluated using the "ClearAutoZero" command.

Figure 4 demonstrates that differences can also be found in more realistic scenarios. It shows the fluorescence behavior of four methanol solvent samples of different grades and suppliers at six wavelength settings.

- For the first wavelength pair, from 0.5 min to 1 min, all solvent qualities show a high response. The highest response, and thus the highest contamination, is caused by the UHPLC-grade sample with a fluorescence of $\sim 8.4 \times 10^6$ counts. In comparison to the solvent that performed best at these settings, this is equivalent to a 60% higher signal level. The LC/MS grade solvents of different suppliers show generally less response, but their compatibility with this wavelength setting also differs by up to 14% compared to the sample with the lowest fluorescence level, the LC/MS grade eluent from supplier B. Since the baseline noise is proportional to the autofluorescence of the sample, it has a direct impact on the LOD. An increase of the background fluorescence by a factor of two impairs the LOD by a factor of two as well.
- At an excitation wavelength of 247 nm and a corresponding emission wavelength of 400 nm (second wavelength pair), the overall response level is more than 95% lower. The ranking of the eluent samples differs from the first wavelength pair setting. In this case, the LC/MS-grade solvent from supplier C shows the highest background fluorescence (3.3×10^5 counts). The LC/MS-grade solvents from supplier A and B provide similar response and perform slightly better than supplier C. Although all samples show a low response, the relative response differences are as for the first wavelength pair. The autofluorescence of the sample from supplier C is approximately 50% higher than the response of the MeOH sample from supplier A (best performance). This means that the signal is even higher than the response of the UHPLC-grade solvent, which is $\sim 40\%$ higher than the sample with the lowest fluorescence.
- With the third wavelength pair, all samples show almost the same low level of background fluorescence and are therefore similarly suitable. When only interested in this wavelength pair, even the lowest solvent quality would be as good as the higher purity samples.

Figure 4 illustrates that the level of background fluorescence and stray light of the different samples is highly dependent on the wavelength setting. This directly influences the signal-to-noise ratio as presented in Figure 5. A high level of autofluorescence (blue, above) results in a higher noise compared to the black baseline at the bottom of the scheme. The difference in the background fluorescence levels is proportional to the change of the baseline noise. Hence, a solvent with a five times higher autofluorescence typically results in a fivefold decrease of the signal-to-noise ratio (S/N).

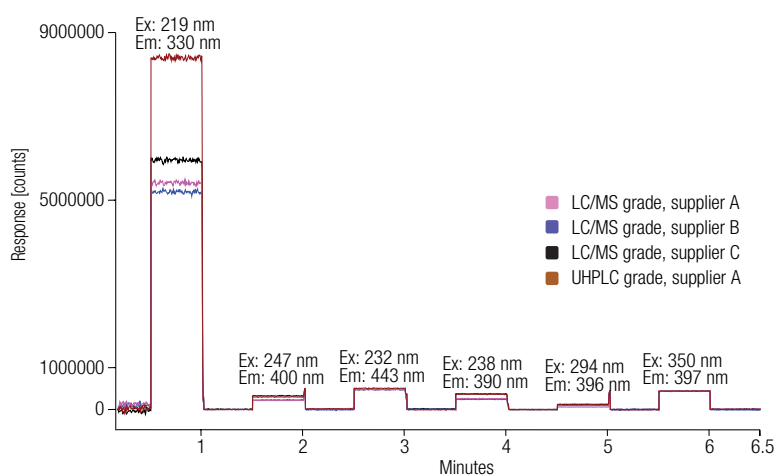


Figure 4. Comparison of the fluorescence baseline behavior of four methanol samples with pink/blue/black: LC/MS grade of different suppliers and brown: UHPLC grade. Six wavelength pairs are evaluated using the "ClearAutoZero" command.

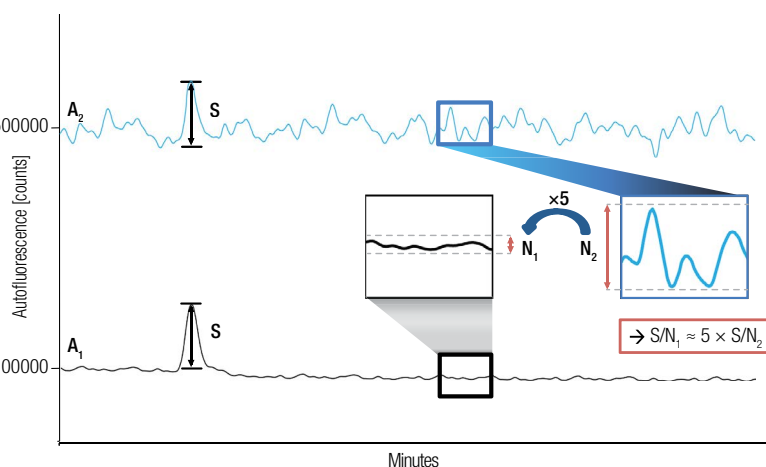


Figure 5. Scheme of the influence of different autofluorescence on the S/N.

Consequently, choosing the solvent with the lowest background fluorescence is essential for achieving the best S/N and LOD. Nevertheless, it may be an option to use the lower-grade solvent if the influence on the measurement is negligible for the application, e.g., when an ultra-low LOD is not essential. Trading nominal solvent quality for an acceptable baseline noise increase has the potential for substantially reducing solvent costs. A good practice would be to check the compatibility of the solvent prior to running the application in the lab with the described procedure. If the lot of the eluent has changed, it is recommended to check the suitability of the solvent with this quick test.

Solvent Quality for Charged Aerosol Detectors

The principle of charged aerosol detection is different from the two optical detection methods already mentioned. First, the column effluent is nebulized to form small droplets. These droplets are dried by evaporating the eluent. The evaporation residue then forms uncharged particles with sizes related to the concentration of the analyte. Charge is applied to these particles by the collision of positively charged nitrogen gas. The measured total charge of the particle is finally related to its size and therefore to the mass of the analyte. Charged aerosol technology is optimized for the detection of non- or semi-volatile compounds dissolved in the mobile phase and is considered to be a mass-sensitive form of detection. Therefore, the influence of liquid impurities (e.g., other solvents) is almost negligible for charged aerosol applications. One of the major solvent quality factors for charged aerosol detectors is the presence of non-volatile dissolved contaminants or particles. In addition, unstable solvents such as acetonitrile or tetrahydrofuran often include stabilizing agents that may also inversely affect baseline stability. By measuring the baseline noise of a blank chromatographic run it is possible to obtain qualitative data on the presence of interfering particulates after evaporation. Evaluate the baseline by using two

different filter constants: 7 (highest filter applied) and 0 (no filter applied). The two different filter constants are used to better visualize the differences between the tested solvents. The extent of the baseline noise allows a prediction about the level of non-volatile contaminants in the solvent.

In the following experiment, four methanol samples of different grade and vendors (one UHPLC-grade sample, three LC/MS-grade samples) are evaluated for their suitability for charged aerosol applications. UHPLC-grade solvents are typically filtered through a 0.1 μm filter, whereas LC/MS grade solvents are filtered to 0.2 μm level. Therefore, one would expect a lower content of particles remaining in solvents specialized for UHPLC and hence, lower noise levels. Figure 6 shows an overlay of the tested samples. The brown baseline was obtained with a UHPLC-grade eluent which shows pronounced disturbance and a high noise level due to a high level of contamination. The solvent's noise levels are factor 5–18 higher than for the tested LC/MS-grade solvents and are therefore clearly not suitable for an analysis requiring best S/N and low LOD, respectively. The pink, blue, and black baselines represent LC/MS-grade solvents with comparable quality. The noise created by these solvents differs by a factor of 2–4 compared to the best eluent.

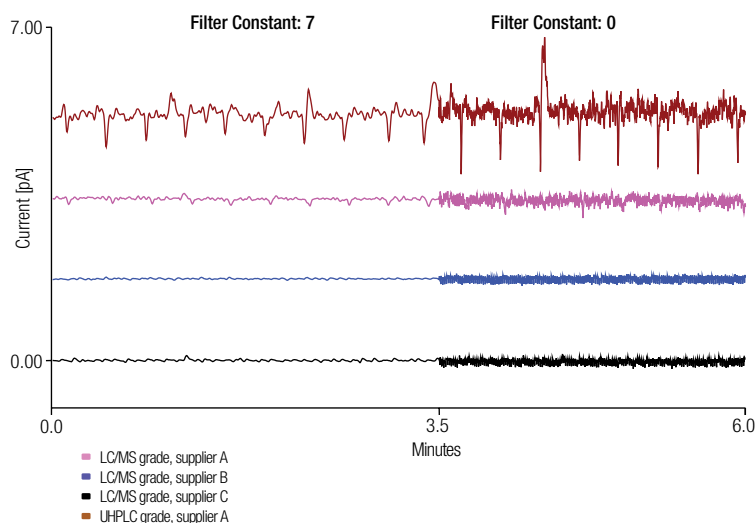


Figure 6. Comparison of the charged aerosol baseline behavior of four methanol samples of different vendors displayed in a stacked chromatogram; with pink/blue/black: LC/MS grade of different suppliers and brown: UHPLC grade. Two filter constants were used: 7 (0–3.5 min) and 0 (3.5–6.0 min).

It is also possible to visualize the influence of storage on the eluent quality and the lot-to-lot consistency. A new lot can quickly be tested whether its quality still meets the requirements. It is good laboratory practice to verify the quality of the eluents from time to time as it is likely that solvents, once opened, are contaminated by compounds and particles from the environment. Another factor is the growth of algae or bacteria possibly causing rapid degradation of water or buffers. Long-time exposure can lead to severe contamination of the system membranes (e.g., in inline degassers), which may amplify the effects on baseline current and background noise. Effects of storage and the deviation of the solvent quality between different lots are displayed in Figure 7. The pink and black baselines result from two acetonitrile samples of the same lot. The pink data represent acetonitrile that has been reopened and stored multiple times. By repetitive opening of the eluent bottles, the risk increases that contaminants are introduced and enriched. The black data shows acetonitrile of the same lot, opened directly before analysis. It is clearly displayed that the fresh acetonitrile shows 30–50% lower noise levels with both filter settings. The baseline noise has a direct impact on the signal-to-noise ratio. If best S/N is required, the solvent might no longer be suitable for analysis.

The following example, as shown in Figure 7, deals with the question of solvent lot-to-lot consistency. The blue and black baselines represent the response of two acetonitrile samples of about the same grade and only differ by their lot number. At first glance, both samples seem to provide similar results in the range of 0.02–0.03 pA. It should be

no problem to use either of these if best S/N is not the main objective. If best S/N is most important, it makes sense to take a closer look at the relative difference of the baseline noise, as in this example.

For best LOD and highest reproducibility always choose the solvent with the lowest noise value. An eluent that might cause spiking due to its high content of contaminants or particulates after evaporation should not be used with a charged aerosol detector. Document the vendor, lot number, and particulate after evaporation of the solvent on a regular basis to ensure best charged aerosol detector conditions.

Conclusion

The purity of the mobile phase is essential for the quality of an HPLC analysis and even more for UHPLC analysis. The market offers a wide range of solvents optimized for UV-Vis and fluorescence, UHPLC or mass spectrometry applications. These mobile phases vary in their grade, suitability for specific applications, and in price. Although using the highest solvent quality is desirable, it may not always result in a measurable performance benefit. This work provided guidance whether the mobile phase quality was sufficient for the analysis.

With the examples shown it is possible to compare different suppliers and lot-to-lot consistency, to prove the solvent's suitability for UV-Vis absorbance, fluorescence, and charged aerosol applications and to monitor the quality of the solvent on a regular basis. The test procedures for optical detectors are application tailored and easily adaptable.

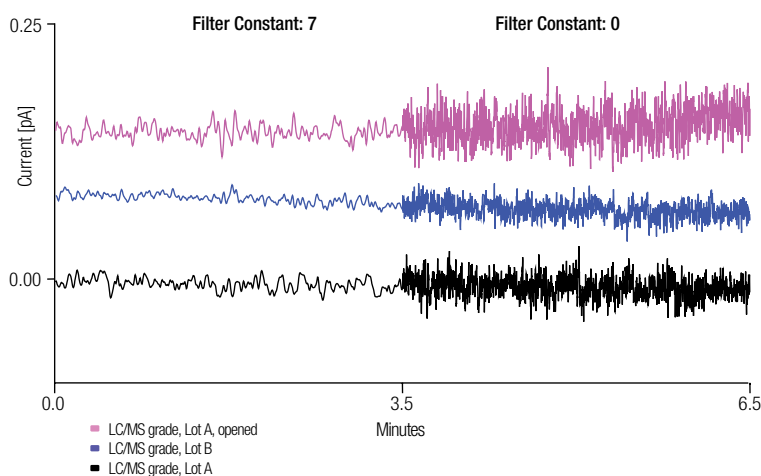


Figure 7. Comparison of the charged aerosol baseline behavior of acetonitrile samples of the same grade in a stacked way (pink: lot A, opened repeatedly; black: lot A, opened directly before analysis; blue: lot B). The lot-to-lot consistency and the contamination during storage are evaluated. Two filter constants are used: 7 (0–3.5 min) and 0 (3.5–6.5 min).

These experiments showed that for fluorescence and UV-Vis absorption detectors, the solvent suitability for an application strongly depends on the applied detection wavelength(s) and the required detection performance limits. A lower-eluent quality often causes a higher baseline noise and therefore adversely affects the S/N. However, it may be possible to measure without any disadvantages at the detection wavelength(s) that you are interested in. In a different scenario, the respective noise or signal-to-noise ratio may be good enough if trace level detection is not required. In both cases, the use of the highest-purity grade might not be necessary. Trading nominal solvent quality for an acceptable baseline noise therefore has a huge potential for saving solvent costs, particularly when a larger number of instruments are involved. The procedures in this work can also be used to quickly and easily check the solvent quality on a regular basis to ensure consistent performance, especially if the supplier or the lot has changed, or the solvent was potentially contaminated.

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