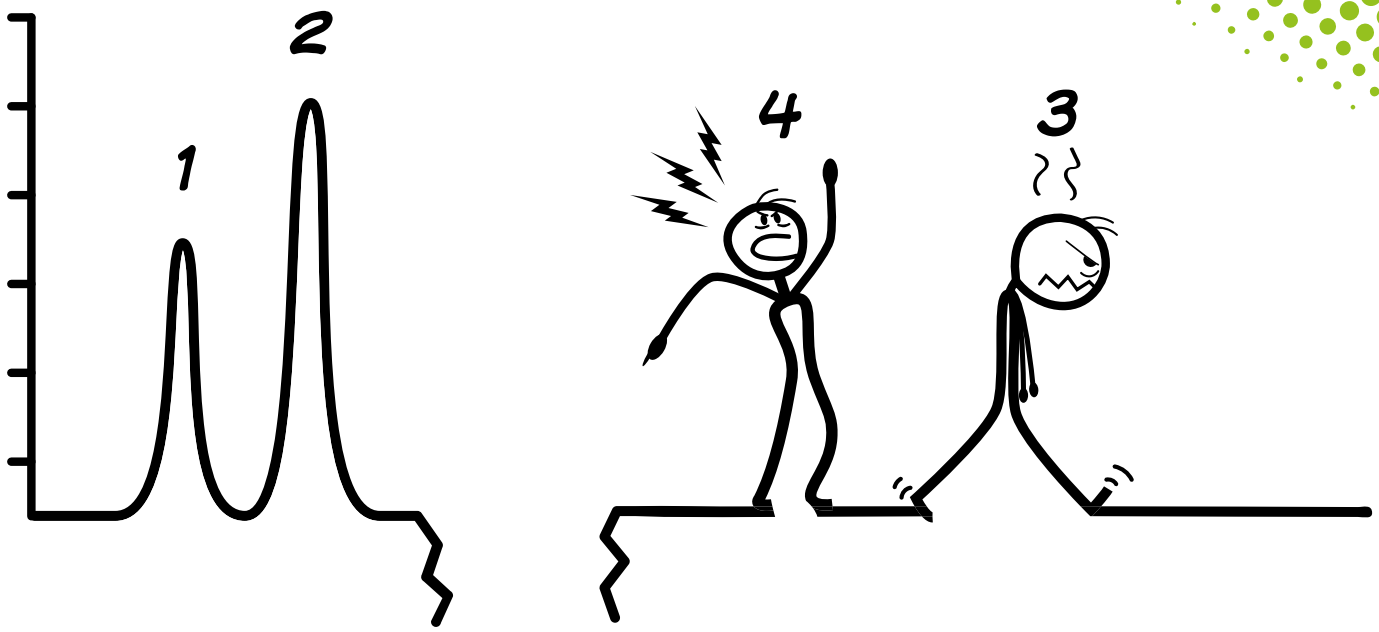
When Retention Times take a Walk

Stable mobile Phase without Gradient Control?
Simple Solutions for a complex Problem.



It's all about the Mixture!

Anyone who runs their HPLC or UHPLC with **solvent mixtures** will sooner or later face the problem of **evaporation**. The mixing ratio influences the resolution of the analyte, separation performance and selectivity. Evaporation changes the mixture, and your retention times take a little "walk" through the chromatogram. Sometimes a big one, depending on the resolution of your timeline.

Gradient systems were invented so that your peaks are where they are expected to be. Gradient control continuously adjusts the mixing ratio of the solvent mixture during the run to optimize separation and resolution.

Challenges of the gradient method

However, this achievement also entails some risks, and the decision as to whether a gradient system can or must be used has to be carefully considered.

High effort: Gradient systems require extensive **calibration** and **optimization** to find the best mixing curve - and consistent monitoring once you've found it. This increases the technical and time effort.

Contamination and cleaning: Gradient changes can lead to layer formation in the system. The consequences are contamination and time-consuming cleaning.

Costs: Gradient systems are significantly more expensive to purchase and operate because they require special pumps, mixers, detectors and complex control software.

Retention time variability: Adjusting the mixing ratio offers a lot of flexibility to optimize the resolution - but this flexibility also means variances in the retention times themselves. This makes it particularly difficult to compare analytical results.

The „luck“ factor

Let's assume we had all of these factors under control and cost wasn't an issue. All of this assumes that absolutely identical conditions constantly exist around our solvent. Anyone who has ever relied 100% on the weather forecast knows: it doesn't always work out well.

When it comes to the weather, we can occasionally hope for luck. If we get wet once, it's not the end of the world. However, anyone who has responsibility in analytics should never rely on the luck factor.

A test: stable Mixtures without Gradient Control

Since HPLC often involves organic compounds, we took a closer look at the behavior of some **PAHs (polycyclic aromatic hydrocarbons)** for our test. The aim was to find out whether and how a methanol-water mixture as a solvent affects the analysis if it is left "to its own devices" for some time.

Framework Conditions

For our test we chose the following HPLC configuration:

HPLC system: VWR HITACHI LaChrom Elite® with diode array detector. Isocratic pump conditions with premixed mobile phase. Control software: EZChrom Elite™.

HPLC column: Purospher® RP-18e (5 µ), 125 x 4 mm.

Procedure

1. Four bottles (A to D) were filled with the **identical** methanol-water mixture (80/20) as solvent.

2. At the beginning, a reference chromatogram with samples of the three PAHs **chrysene, naphthalene and pyrene** was recorded using the mixture from **bottle A**.

3. Immediately afterwards, bottle A was **completely closed** as a **reference mixture**., using the included GL45 screw cap with PTFE seal.

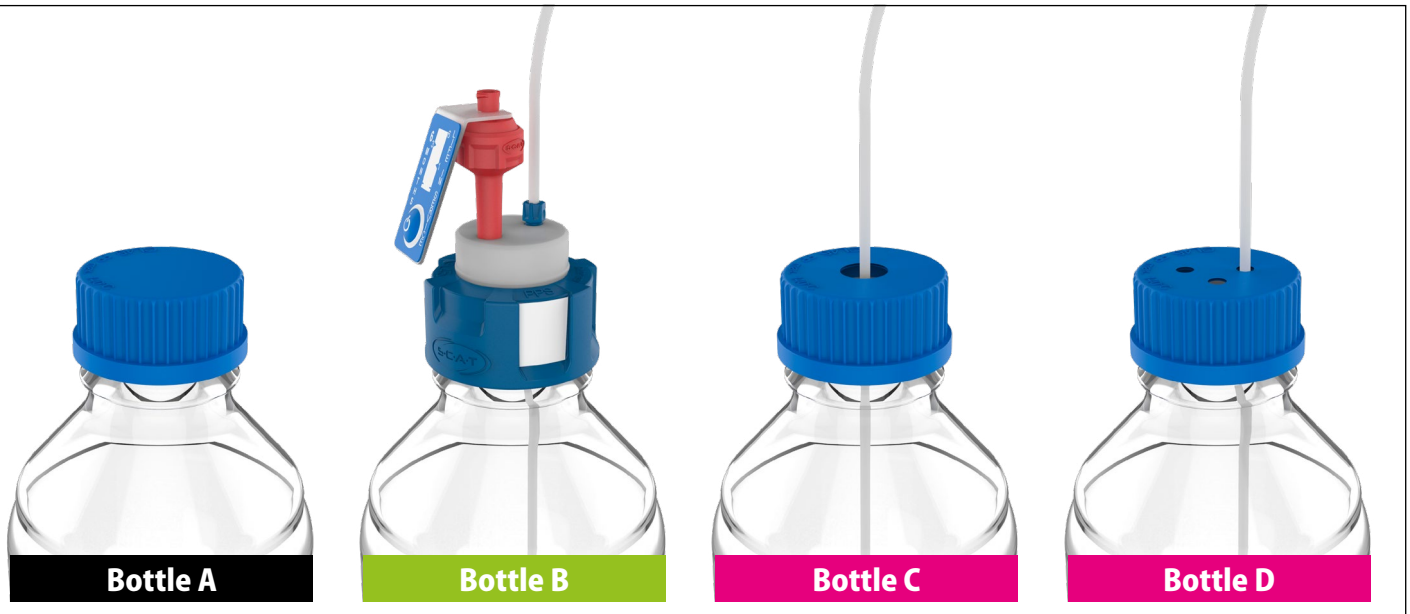
4. All bottles were weighed to track changes in mass.

5. Bottles B, C and D were connected to the HPLC in **different ways** using capillaries (outside diameter: 3.2mm, inside: 1.6mm). A **hermetically sealed** system (SCAT Safety Caps) was only used for **bottle B**. The remaining bottles were sealed using **conventional** methods (see Figure 1).

6. The bottles remained connected to the HPLC for **31 days** under laboratory conditions at room temperature (22°C). Except bottle A, which remained permanently closed as a reference mixture.

7. After 31 days, the bottles were weighed again and the separation of the three PAHs was repeated under **identical HPLC conditions** using the mobile phases from bottles B, C and D.

Fig. 1: *Setup of the solvent bottles in the test*

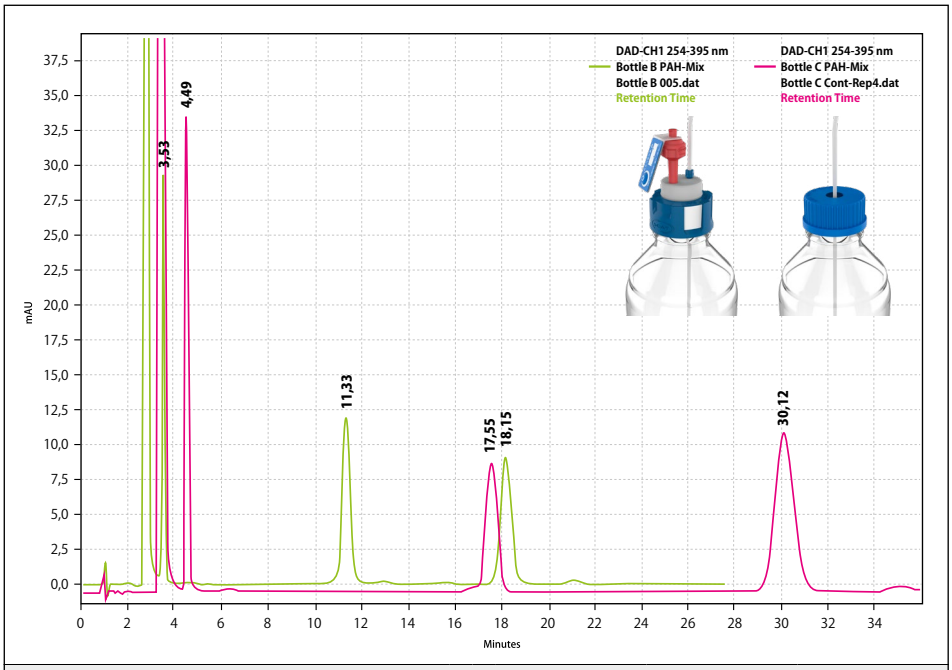


Bottle A was completely closed as a **reference mixture** after recording the reference chromatogram at the beginning of the experiment.

Bottle B was **hermetically sealed** with a SCAT Safety Cap. Airtight capillary connection and ventilation valve for pressure equalization.

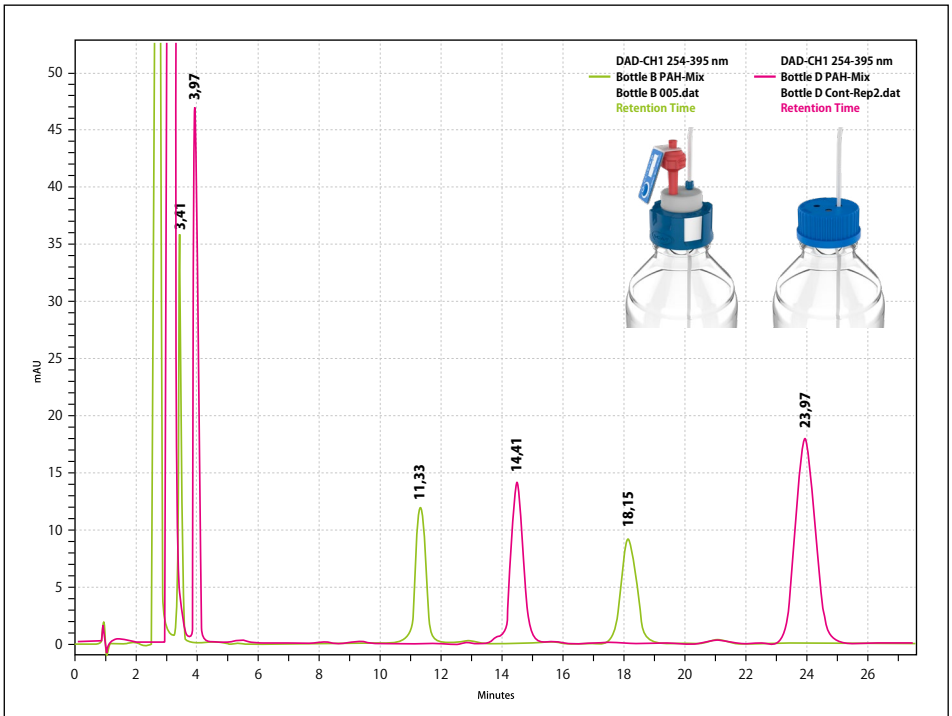
For bottle C, the capillary was passed through a **normal hole** (10 mm) in a standard cap. An open area of approximately 0.785 cm^2 was created.

In bottle D, **3 holes** (3.2 mm each) were drilled. The connecting capillary was passed through hole 1. Bores 2 and 3 remained open. An open area of approximately 0.212 cm2 was created.



Day 31	Napthalene	Pyrene	Chrysene
Reference / Bottle B	3,530	11,330	18,150
Bottle C	4,490	17,550	30,120
Delta (min.)	**+ 0,960**	**+ 6,220**	**+ 11,970**
Interpolated: Change per day (minutes)	**+ 0,031**	**+ 0,201**	**+ 0,386**

Fig. 2: *Retention time shift with eluent from bottle C after 31 days.*



Day 31	Napthalene	Pyrene	Chrysene
Reference / Bottle B	3,410	11,330	18,150
Bottle D	3,970	14,410	23,970
Delta (min.)	**+ 0,560**	**+ 3,080**	**+ 5,820**
Interpolated: Change per day (minutes)	**+ 0,018**	**+ 0,099**	**+ 0,188**

Fig. 3: *Retention time shift with eluent from bottle D after 31 days.*

Test Results

Mass changes (Table 1)

1. Bottles A (reference eluent) and B (with SCAT Safety Cap) showed **no significant mass changes**.

2. Bottles C and D experienced **significant mass/liquid loss**. Since liquid was neither removed nor added, this loss is due to evaporation, with experience showing that the solvent portion of the mixture evaporates more quickly than the aqueous portion. The ratio of water and methanol in the vapor may have varied over the test period because mixtures of water and methanol form azeotropes.

	Mass Change (g)			
	A	**B**	**C**	**D**
Day 1	457,45	539,26	724,14	715,08
Day 31	457,43	539,26	672,45	687,36
+/- (g)	**- 0,02**	**0,00**	**- 51,69**	**- 27,72**
+/- (%)	**- 0,004**	**0,000**	**- 7,138**	**- 3,876**

Table 1: *Mass changes*

Change in retention times (Fig. 2 and 3)

1. The eluent from bottle B (with SCAT Safety Cap) leads to almost **identical** retention times of the PAH test compounds without significant shifts compared to the reference chromatogram.

2. The eluent from bottles C and D provides a **significant shift** or increase in retention times compared to the reference chromatogram. In this case, a purely retention time-based identification of the compounds would be **impossible**.

Assuming a **linear** progression of the evaporation of the mobile phase, it becomes clear that **changes in the retention time** can be expected even after using partially open bottles for **one day**.

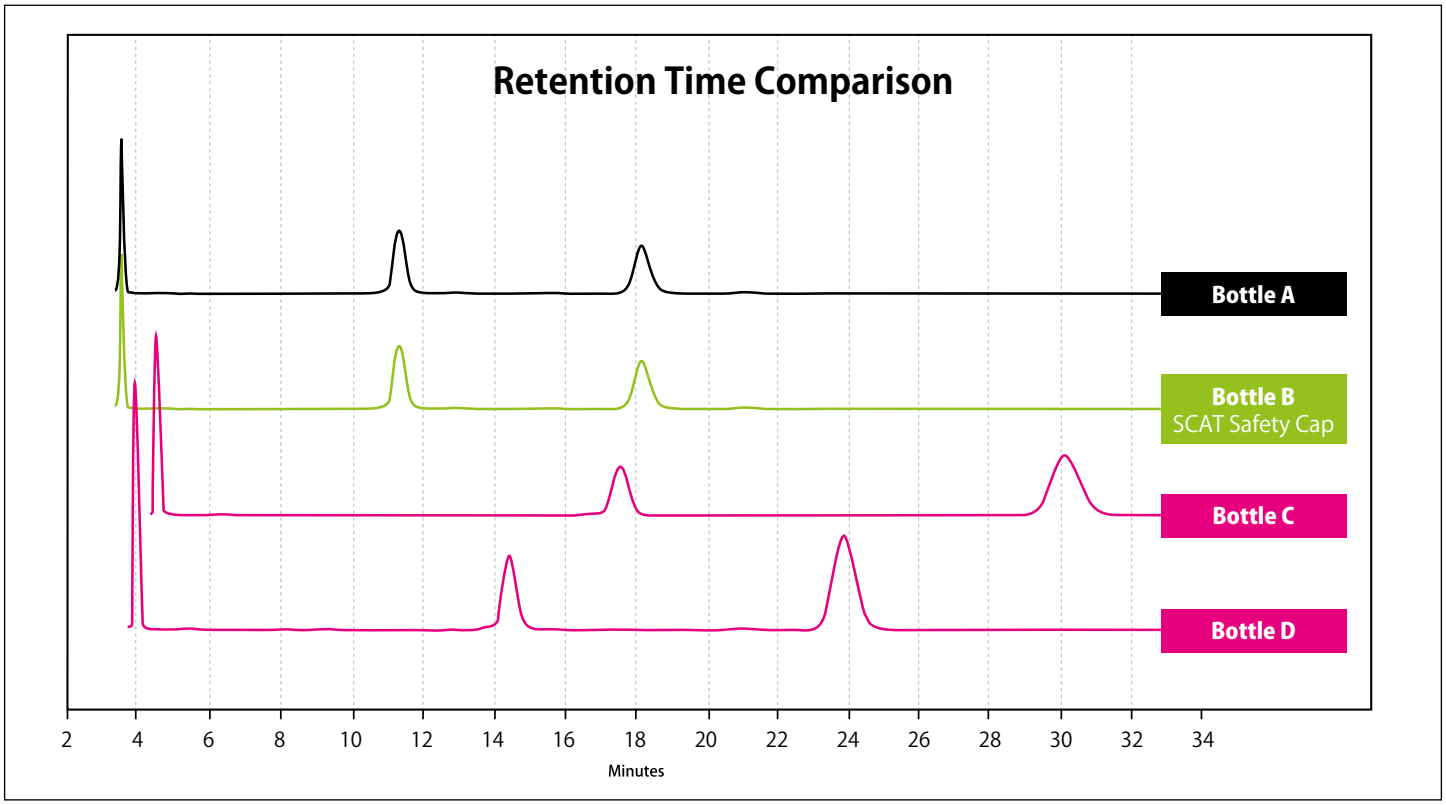


Fig. 4: *Comparison of all retention times after 31 days.*

Advantages of hermetically sealed eluents

Reproducibility and consistency: A hermetically sealed eluent prevents evaporation and thus ensures consistent mixing ratios. This means that the retention times remain constant across different analyzes and the results are more reproducible.

Stability: Changes in environmental conditions, such as temperature and pressure, have no effect on the mixing ratio, resulting in more stable analyzes overall.

Lower method complexity: A solid eluent requires less technical setup, calibration and maintenance compared to gradient systems.

Reduced sample preparation: The constant retention times eliminate the need to re-determine them for each analysis, saving time and effort.

Better comparability: The consistent retention times make it easier to compare results across different time points, which is particularly important in long-term studies or quality controls.

Exceptions

Of course, it can happen that you need gradient control for your analysis in **any case**. For example, if factors **other than the eluent** influence the behavior of your sample during the analysis. The interaction between the analyte and the column ultimately depends on many conditions.

However, a hermetically sealed eluent saves you a lot of considerations and **relieves** your gradient control of a **critical factor.** This leaves more time for perfect adjustment of the other parameters of your method.

Conclusion

To put it briefly: yes, a stable mobile phase can be achieved **without** gradient control, with significantly **less effort** and **lower costs.**

While gradient systems offer the flexibility to adapt the mixing ratio to the sample, **hermetically sealed eluents** have clear advantages in terms of reproducibility, stability and lower effort. Especially in laboratories that rely on high **precision** and **reproducibility**, the use of a solid eluent leads to more accurate and reliable results.

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