Tips and tricks

Ion chromatography

Monitoring and maintaining column performance

One of the basic requirements for ensuring reliable chromatographic analyses is a high-performance separation column. Users of IC should regularly check the performance of their column. If a drop in performance becomes apparent, steps can be taken in good time to restore or maintain the proper functioning of the column. Find out here how you can assess column performance, which parameters you should monitor, and which measures you can take to ensure excellent column performance.

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First-time use of a new column

When you use a column for the very first time, we recommend that you check its initial performance. The *Certificate of Analysis* (CoA for short), which you receive with every purchase of a Metrohm column, is your source of reference here. Record a chromatogram and use the analysis conditions specified in the CoA, which include flow rate, temperature, eluent, and analyte concentration. The column's performance can be evaluated by comparing some of the result parameters with the values listed in the CoA, for example, retention time, theoretical plates, asymmetry, resolution, peak height, and peak area.

Regular monitoring of column performance

Columns that are already in use should be monitored regularly too. We recommend carrying out these tests with check standards under application conditions, as performance can vary depending on the type of analysis and associated analysis conditions as well as the instrumental setup. If a drop in performance is observed, the requirements of the application are crucial in deciding whether it can still be used. How to determine column performance based on five performance indicators is described in the following. You will also find out how you can prevent or rectify a decline in performance.

Counterpressure

Monitor the counterpressure: First, when you use your new column for the first time, save the counterpressure under the analysis conditions of your application as a reference value («common variable» in MagIC Net). Then use the user-defined results to monitor the difference between the initial counterpressure and the one for the current determination.

If an increase in the counterpressure compared against its initial value can be identified, this indicates that particles have been deposited in the guard column or separation column. If this increase is higher than 1 MPa, action must be taken. First, you should check which of the columns is affected. If the guard column is contaminated, it should be replaced. If the separation column is affected, first rinse it for several hours in the reverse flow direction. If this doesn't help, you should consider replacing the column. This will be essential if the maximum permitted counterpressure for the column is reached.

Retention time

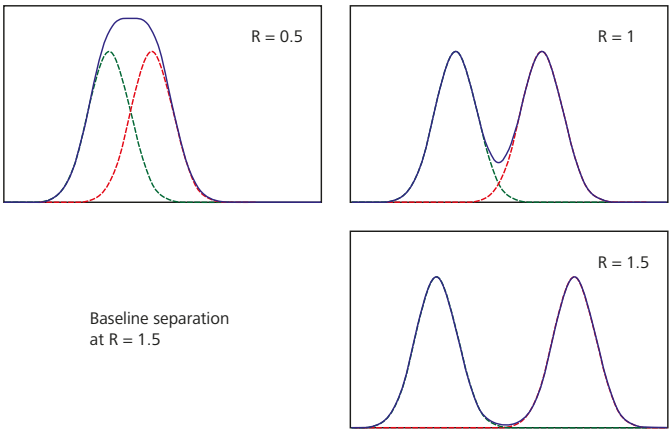
To track the change in the retention time, the retention time of the last peak is monitored in the chromatogram. Sulfate, for example, is suitable for this, as it usually elutes right at the end. Here too, work with a common variable to save the initial value.

Unstable retention times can be caused by carbon dioxide from the air or air bubbles in the eluent. These problems can easily be resolved (see Table 1, p. 31). The column may also have lost capacity. The column's capacity loss can be caused by high-valency ions. The column should then be regenerated in accordance with the column leaflet to remove any contamination. If this doesn't lead to any improvement, you should consider replacing the column depending on the requirements of the application, particularly in the event of progressive capacity loss.

Resolution

Monitor the chromatographic resolution by comparing measurements from a predefined check standard with an initial reference value. If the resolution is $R > 1.5$, the signal is baseline-separated (see figure below). However, in the case of high matrices and therefore peaks that are more widely spread, the resolution has to be higher to ensure baseline separation.

If a loss of resolution occurs, first make sure that it is not caused by the eluent or the IC system. Once this has been ruled out, the adsorptive effect of contaminations in the guard column or separation column may be responsible for the loss of resolution. A contaminated guard column should be replaced. If the cause of the problem is in the separation column, this should be regenerated in accordance with the column leaflet to free it from any organic or inorganic contamination. If the loss of resolution progresses, replacement is inevitable.



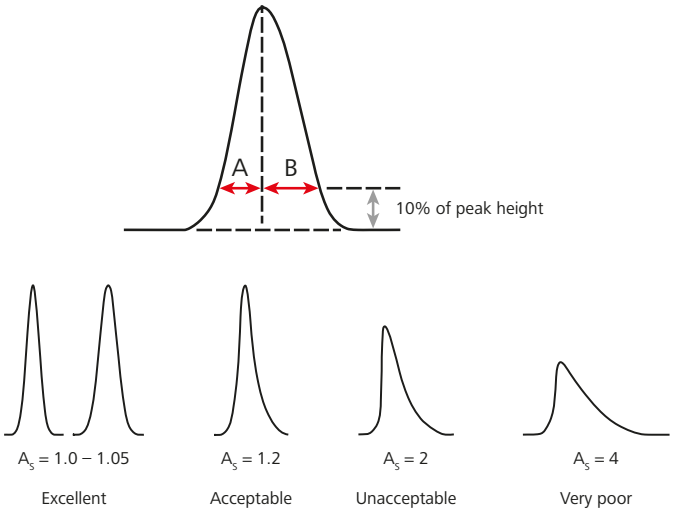
Baseline separation at R = 1.5

Theoretical plates
Save the initial number of theoretical plates in MagIC Net as a common variable. Usually, the last eluting peak is used – in anion chromatograms, sulfate would be a suitable candidate, for example. Use the user-defined results to track the development of the theoretical plates.

A decrease in the theoretical plates can suggest dead volume in the IC system (see Table 1). A low number of theoretical plates may also be observed if the column has been overloaded by a high salt concentration in the sample matrix, for instance. If the theoretical plates decrease by more than 20%, this indicates that column performance is declining. Depending on the requirements of the application, action may need to be taken. If the guard column is the reason for the drop in performance, it should be replaced. If the problem is with the separation column, we recommend regenerating the column in accordance with the column leaflet to eliminate any organic or inorganic contamination. If this doesn't help, you should consider replacing the column, particularly if a trend toward lower theoretical plates is observed.

Asymmetry
Determine the initial asymmetry of the analytes by measuring a predefined check standard under the analysis conditions of your application. Save it as a common variable. Then use the user-defined results to observe the development of asymmetry over time. The maximum acceptable values for the asymmetry vary depending on the analyte. For example, calcium and magnesium peaks initially present relatively high asymmetry values.

Asymmetry is defined as the distance from the centerline of the peak to the descending side of the peak (B in the figure) divided by the distance from the centerline of the peak to the ascending side of the peak (A in the figure), where both distances are measured at 10% of the peak height.



$A_S > 1$ means tailing and $A_S < 1$ means fronting. Optimum chromatography is achieved with peak asymmetries as close as possible to 1. As a general rule, column performance is declining when asymmetry is $A_S > 2$ or $A_S < 0.5$. Depending on the requirements of the application, measures have to be taken in this case in order to improve symmetry and to enable better integration. The reason for high asymmetry values may be down to the ion chromatograph – due to dead volume, for example. If this is not the case, it is important to find out whether the asymmetry is caused by problems with the guard column or with the separation column. If the guard column causes the asymmetry, it should be replaced. If it is the separation column, it should first be regenerated in accordance with the column leaflet to remove any organic or inorganic contamination. If this doesn't help, you should consider replacing the column. If a trend toward higher asymmetry values can be observed, replacement is unavoidable.

Table 1. Preventing and correcting performance loss in IC columns

Indicator	Cause	Preventive and corrective measures
Increasing counterpressure	Particles on the guard column	Replace the guard column.
	Particles on the separation column	Rinse out the separation column in the reverse flow direction: • Place the column outlet in a beaker. • Rinse out the separation column for approx. one hour. • Reinstall the separation column in the flow direction.
	Particles in the sample	Sample preparation, e.g., remove particles through Inline Ultrafiltration
Shortened retention time	Carbonate in the eluent	Carbon dioxide from the air affects the carbonate/hydrogen carbonate balance in the eluent. A carbonate/hydrogen carbonate eluent weakens over time; a hydroxide eluent strengthens. • Always tightly seal eluent bottles and bottles containing eluent concentrate. • Always use a CO_2 adsorber.
	Air bubbles in the eluent	Air bubbles make the eluent flow unstable. The counterpressure is an indicator of unstable flow. It should remain stable within a range of ± 0.1 MPa. • Deaerate the high-pressure pump. • Use an eluent degasser.
	Capacity loss in the column due to high-valency ions	Regenerate the column as per the column leaflet to remove any inorganic deposits.
Resolution loss	Eluent too old or produced incorrectly	Eluents should be freshly prepared. Make sure that they are produced correctly and particularly that carbonate and hydrogen carbonate are not confused.
	Adsorptive effect of the contamination deposited in the guard column	Replace the guard column.
	Adsorptive effect of the contamination deposited in the separation column	Regenerate the column as per the column leaflet to remove any organic or inorganic deposits.
Loss of theoretical plates	Guard column contaminated	Replace the guard column.
	Separation column contaminated	Regenerate the column as per the column leaflet to remove any organic or inorganic deposits.
	Separation column overloaded	The separation column can be overloaded by factors such as a high salt content in the sample matrix. • Dilute the sample. • Inject less sample.
	Dead volume in the IC system	• Check that all capillaries have a diameter ≤ 0.25 mm; if they don't, replace the capillaries. • Check that all of the capillaries have been installed correctly. The installation process is described step by step in the «IC Maintenance» multimedia guide.
Asymmetry	Dead volume or contamination on the guard column	Replace the guard column.
	Separation column contaminated	Regenerate the column as per the column leaflet to remove any organic or inorganic deposits.

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