Expert tip: Column regeneration

A common reason for reduced column performance is unwanted adsorption of contaminants on the stationary phase. This may result in:

- increased back pressure
- altered retention times
- peak shape deformation.

To prevent adsorption of contaminants it is recommended that guard columns should be used and suitable sample preparation, e.g. by filtration should be carried out.
In order to prevent or to improve already affected column performance by unwanted adsorption on the stationary phase, different cleaning steps can be applied to effectively regenerate the column. As contaminants usually accumulate at the column head, cleaning should always be performed in the reverse flow direction. A minimum of 20 column volumes of cleaning solvent per regeneration step is recommended. The cleaning solvent(s) should be chosen in accordance with the characteristics of the known impurities as well as the stability of the stationary phase. An overview of suitable solvents which are appropriate for different contaminants is given in Table 1. Further recommendations are given in the instructions of column producers, e.g. the “care and use instructions”.
The efficiency of regeneration can be increased at elevated temperatures. Considering the solvent(s) which are used as well as the temperature stability of the stationary phase, this can be in the range of 40 °C – 90 °C.

Table 1: Suitable solvents for column regeneration.

Contaminant			
salts	**non-polar substances**	**polar substances**	**proteins**
• water and aqueous / organic mixtures	• acetonitrile • isopropanol • tetrahydrofuran • dichloromethane • chloroform	• water and aqueous / organic mixtures • methanol • tetrahydrofuran	• injection of dimethyl sulfoxide • gradient from 10 % to 90 % B with A = 0.1 % TFA in water and B = 0.1 % TFA in acetonitrile

The following practical examples show the efficiency of column regeneration by cleaning.

A) Reduction of back pressure

In a quality control application for an active ingredient, an increase in back pressure of 180 bar was observed after several injections. For regeneration, column cleaning in the reverse flow direction was performed initially using an acetonitrile/water mixture and finally Isopropanol. This resulted in the back pressure returning to the expected value according to the column inspection report (CIR) and the column could be used again (see table 2).

Table 2: Reduction in back pressure.

Step	Pressure [bar]
column inspection report	55
before regeneration	227
after cleaning with ACN/H_2O (60/40)	85
after cleaning with IPA	58

B) Reduction of peak broadening and tailing

During analysis of a local anaesthetic, column performance deteriorated as a result of peak broadening and increased tailing after 75 injections.
As part of the further investigation, the column was retested using the conditions of the column inspection report. Afterwards, different cleaning steps were performed with 20 column volumes each in the reverse flow direction:

1) acetonitrile
2) isopropanol
3) isopropanol / THF (90/10)

After each step, the column was retested to determine the regeneration efficiency.
Peak width and tailing could be efficiently reduced by the cleaning steps (see table 3). Particularly effective was the third cleaning step which included THF (see figure 1). This indicated contamination of the stationary phase by hydrophobic substances.

Table 3: Reduction of tailing.

Step	Test compounds	Retention time	Tailing Faktor
column inspection report	uracil	1.44	
	methyl benzoate	4.12	1.08
	naphthalene	8.30	1.02
before cleaning steps	uracil	1.45	
	methyl benzoate	4.08	1.40
	naphthalene	8.08	1.40
after step 1	uracil	1.48	
	methyl benzoate	4.10	1.20
	naphthalene	8.14	1.20
after step 2	uracil	1.48	
	methyl benzoate	4.12	1.13
	naphthalene	8.16	1.13
after step 3	uracil	1.48	
	methyl benzoate	4.14	0.98
	naphthalene	8.13	1.05

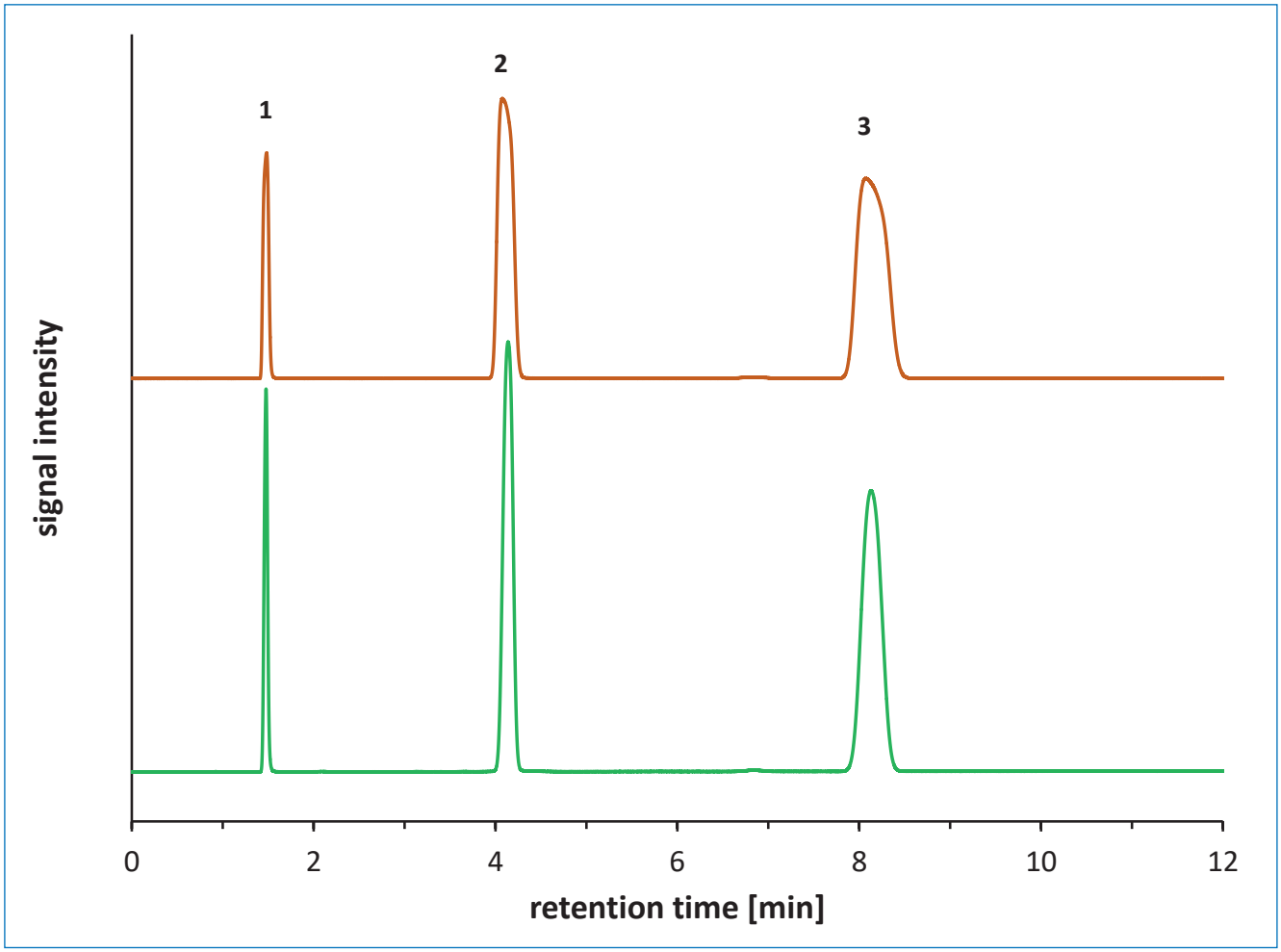

Figure 1: Chromatogram before cleaning steps (top) and after cleaning step 3 (bottom); 1 = uracil, 2 = methyl benzoate, 3 = naphthalene.

C) Elimination of peak splitting and retention time changes

During analysis of a non-steroidal anti-inflammatory drug, peak splitting and changes in retention times were observed after several weeks of use. As part of the further investigation, the column was retested according to the conditions of the column inspection report (before cleaning, see figure 2 top). Cleaning with 20 column volumes of acetonitrile in the reverse flow direction was performed which eliminated both peak splitting as well as the retention time changes (after cleaning, see figure 2 bottom).

Table 4: Elimination of peak splitting and retention time changes.

Step	Test compounds	Retention time	Tailing factor
column inspection report	uracil	1.27	
	methyl benzoate	4.38	1.10
	naphthalene	9.06	1.05
before cleaning	uracil	1.33	
	methyl benzoate	8.38	1.49
	naphthalene	11.34	1.60
after cleaning	uracil	1.28	
	methyl benzoate	4.41	0.97
	naphthalene	9.11	0.97

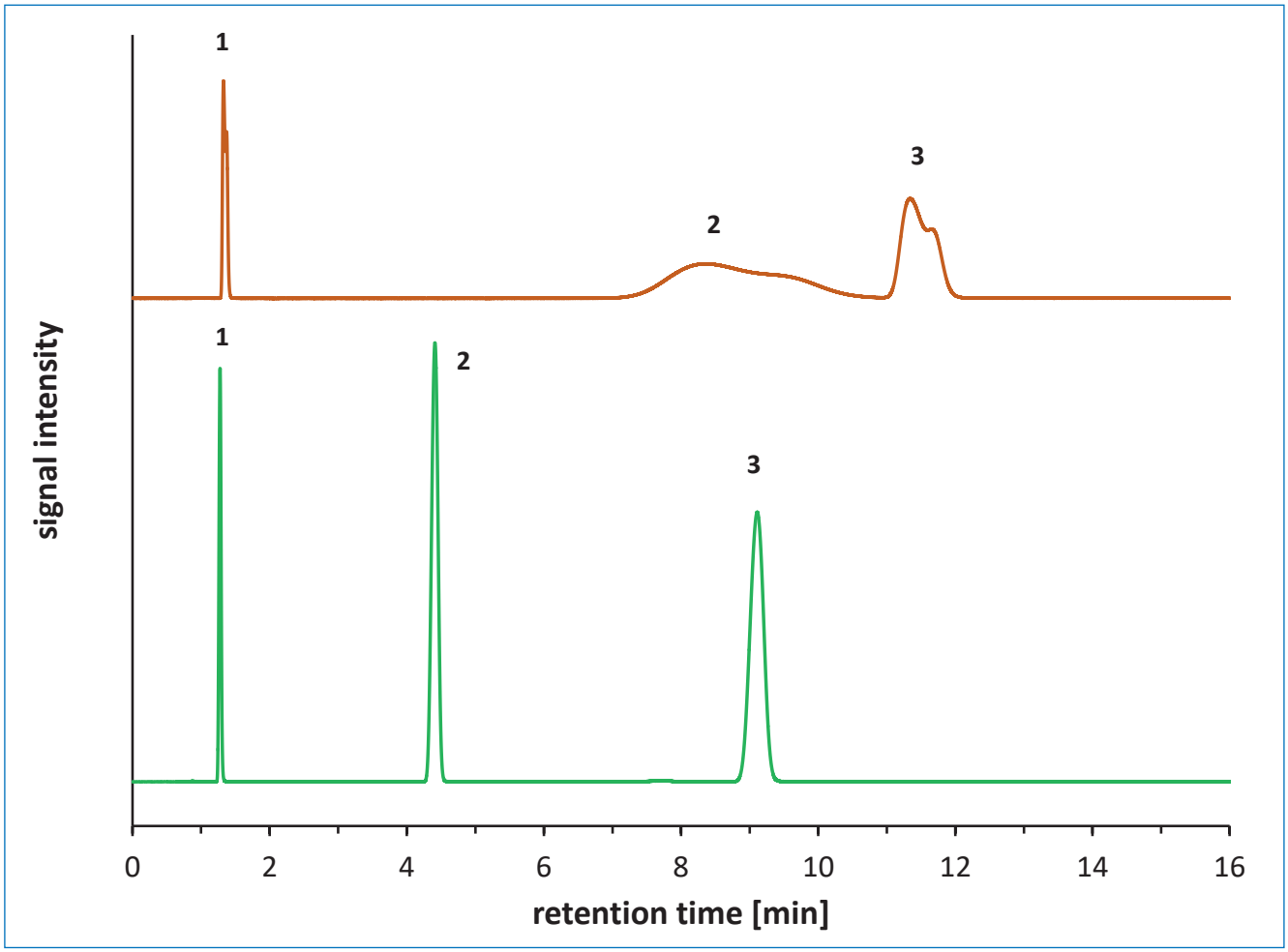

Figure 2: Chromatogram before cleaning (top) and after cleaning (bottom); 1 = uracil, 2 = methyl benzoate, 3 = naphthalene.

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