

Column Testing in Gas Chromatography. The Grob Test.

A Synopsis By Prof. Wolfgang Bertsch

The comprehensive column test procedure devised by Grob et al.[1,2] is now universally used by both column manufacturers and column users. The principal advantage of the Grob test is that it provides quantitative information about four important aspects of column quality: separation efficiency, adsorptive activity, acidity/basicity and the stationary phase film thickness. The experimental conditions are optimized for columns with a medium range of film thicknesses (0.08-0.4 micrometers) and column internal diameters of 0.25-0.35 mm. Although the separation number values obtained for wide bore or thick film columns may be slightly below their maximum values, for convenience the same experimental conditions are generally used for all column types. The Grob test mixture contains 12 compounds which encompass acidic/basic and polar/apolar structures. The test is performed under standardized conditions.

STANDARD EXPERIMENTAL CONDITIONS FOR PERFORMING THE GROB TEST

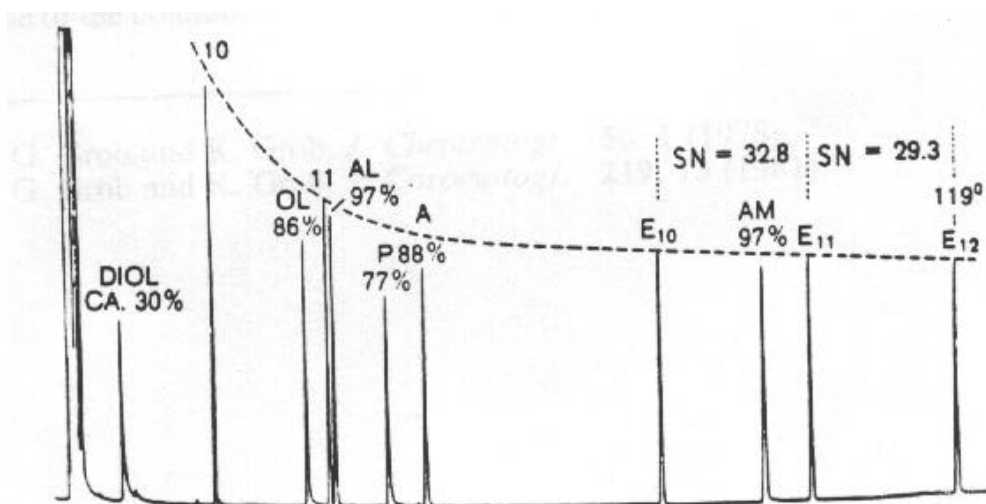
Column Length (m)	Hydrogen		Helium	
	CH4 elution (s)	Temperature Program C°/min)	CH4 elution (s)	Temperature Program C°/min)
10	20	5.0	35	2.5
15	30	3.3	53	1.65
20	40	2.5	70	1.25
30	60	1.67	105	0.84
40	80	1.25	140	0.63
50	100	1.0	175	0.5

STEPWISE PROCEDURE FOR PERFORMING THE GROB TEST

- Cool the column oven to below 40°C.
- Adjust the flow rate by measuring the gas holdup time for methane. Adjust the time to the standard time ($\pm 5\%$).
- Adjust the temperature program rate to the appropriate value given in the Table above.
- Inject the test mixture under conditions that allow ca. 2 ng of a single test substance to enter the column (e.g., 1 microliter with a split ratio of 1:20 to 1:50, depending on injector design).
- Immediately after injection, heat the oven to 40°C (for very thin films to 30°C) and start the temperature program.
- Within the temperature range in which the third ester is eluted (on most columns, 110-140°C), make two marks on the recorder chart noting the actual oven temperature.
- At the end of the run, inter- or extrapolate the elution temperature of the third ester.
- Draw the "100 % line" over the two alkanes and the three esters.
- Express the height of the remaining peaks as a percentage of the distance between the baseline and the 100% line.
- Determine S/N as an average of S/N E10/E11 and S/N E10/E11?

Please note that your (Fluka) standard does not contain either II (undecane) nor AL (nanonal). There are also slight differences in the elution pattern because your stationary phase is not exactly the same as shown in the figure.

The column efficiency is determined by the separation number obtained for the methyl esters of decanoic, undecanoic, and dodecanoic acids (E10, E11, E12). As the relative difference in the molecular sizes of the homologous pairs decreases with increasing molecular size, the first pair of methyl esters (E10/E11) provides a separation number value that is about 8% higher than that of the second pair (E11/E12). The average of the two values is normally used as a measure of the column separation efficiency.



An easy way to quantify the adsorptive and acid/base characteristics of a column is

Test chromatogram of an open tubular column according to the method of Grob. A line is drawn over the peaks of the nonadsorbed solutes. The peak height of the remaining peaks is determined as a percentage of the ideal peak height. In the absence of adsorption all peaks should reach the dotted line.

to measure the peak height as a percentage of that expected for complete and undisturbed elution. The two alkanes and three fatty acid methyl esters (non-adsorbing peaks) are connected at their apexes to provide the 100% line as shown in the Figure. The column activity is then quantified by expressing the height of the remaining peaks as a percentage of the distance between the baseline and the 100% line. It accounts for all types of peak distortion that occur in practice: peak broadening, peak tailing, irreversible adsorption and degradation. The alcohols, octanol and 2,3-butanediol, are used to measure adsorption by a hydrogen bonding mechanism. Acid/base inter-actions are assessed from the adsorptive behavior of 2,6-dimethylaniline, 2,6-dimethylphenol, dicyclohexylamine, and 2-ethylhexanoic acid. Probes with sterically hindered functional groups are used for this purpose in order to avoid adsorption by hydrogen bonding, which can complicate the interpretation of the interaction. 2-Ethylhexanoic acid and dicyclohexylamine provide much more stringent tests of acid/base behavior than the phenol and aniline. On nonpolar (or apolar) phases, even less than 1.0 ng of 2-ethylhexanoic acid may cause column overloading, resulting in a distorted (leading) peak. In this case, peak area as opposed to peak height must be used to quantify column interactions.

The film thickness is calculated from the elution temperature of methyl dodecanoate (E12). However, quantitation of film thickness requires a calibration of elution temperature against stationary phase thickness for all stationary phases of interest. A nonogram (not shown here) can then be constructed and the stationary phase film thickness can be obtained from the elution temperature of E12. The elution temperature of E12 is reproducible to within ± 1 °C if the standard conditions are kept within reasonable limits; in terms of film thickness this corresponds to a variation of 5 % or less.

The limitations of the Grob test are worthy of mention. It cannot be used to test columns coated with liquid phases of high melting point. The elution order of the test mixture is not the same on all stationary phases and the occurrence of peak co-elution cannot be eliminated entirely. The elution order of the test mixture on my common phases has been given by Grob. For phases not previously tested, or in cases where the elution order is in doubt, individual standards must be injected for peak identification. In the case of peak co-elution, the test mixture should be divided into groups of separated components and each group injected independently. Finally, the test is biased towards the measurement of adsorptive as opposed to catalytic activity. Catalytic activity causes time-dependent, concentration-independent losses of sample components. With increasing column temperature, adsorption decreases whilst catalytic/thermal decomposition increases. Although at high column temperatures adsorption may be of little importance compared to catalytic activity, the latter may not be observed in the Grob test. As catalytic activity is a fairly specific influence on particular solutes it may be necessary to customize an activity test for it based on the intended use of the column.

[1] K. Grob, G. Grob and K. Grob, *J. Chromatogr.* 156: 1 (1978).

[21] K. Grob, G. Grob and K. Grob, *J. Chromatogr.* 219: 13 (1981).