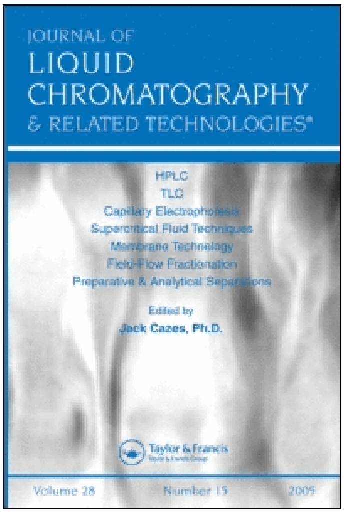

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EVALUATION OF COLUMN EFFICIENCY BY SEVERAL METHODS

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ABSTRACT

Four ways of calculating column efficiency were studied. A moments and a half-height based method appear most sensitive to monitor column 'lifetime' and column damage, respectively.

INTRODUCTION

Several methods for estimating the efficiency of chromatographic columns have recently been reviewed (1). Methods using statistical

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moments are the fundamentally correct way of measuring efficiency since they make no assumptions on the peak shape.

Chesler and Cram determined the error in moment calculations as a function of baseline noise and number of datapoints per peak (2) and found that an exponentially modified gaussian function (EMG) is a reasonably good approximation of an experimental chromatographic band (3). Andersen and Walters studied the effect of baseline drift on moments calculations of the EMG and proposed an empirical non-summation method that is more precise and almost as accurate as the common summation methods over a limited range of assymmetries (4).

The characterization of EMG's by half-height, peak area/height ratio and two to five sigma methods was investigated by Bidlingmeyer and Warren (1). These methods are simpler but less accurate than the method of Andersen and Walters. Although a 'good chromatographic peak should resemble an EMG it is not clear that the most accurate method to describe the EMG is also the most sensitive and reliable method to monitor a change in column or system performance.

We calculated the efficiency of columns and extra-column bandbroadening of the chromatographic system by plotting the variance of the chromatographic bands versus the corrected square of the reciprocal elution volume (Huber plot (6)), analogous to the method of Kok et al. (7), but based on moments measurements and on the half-height, four sigma and 95 % area, respectively. This method has the potential of not only indicating the column performance but also the bandbroadening outside the retentive part of the system (the column proper) although for extra-column bandbroadening (the hardware column) replacing the column by a short capillary (9) may be more convenient and precise (10, 11). Using the Huber plots in evaluating column 'lifetime', column damage and column end fittings is the subject of this paper.

EXPERIMENTAL

The chromatographic system was the same as used before (11).
A model LC 5500 with UV-200 absorbance detector (Varian) was used as a liquid chromatograph. The columns were a 40 x 4.6 mm MicroPak SP Ultra Fast C18-3 (column A) and a 33 x 4.6 mm 3 µm reverse phase High Speed column (column B).
The mobile phase was ca. 45 % acetonitrile (Burdick + Jackson, Muskegon, MI, USA), 55 % water.

Four Column endfittings were used:

1. AT, the top fitting of column A consisted of a flat-bottomed 1/4 to 1/16" union with a two-layer wire screen. The wide-mesh layer serves as a flow distributor while the fine mesh part retains the packing.

2. AE, the exit fitting of column A, a 1/4 to 1/16" union with a pressed-in 1/8" diameter 0.5 µm pore size frit.

3,4. BT,BE top and exit fittings of column B, flat-bottomed 1/4 to 1/16" unions with a ca. 1 µl cone containing a six-spoke flow distributor and a 4.6 mm frit to retain the packing.

NOTES ON ALGORITHMS

The chromatograms have been acquired on VISTA 402 data systems and transferred to an HP1000 minicomputer where they were processed using experimental programs developped in-house.

The first part of the processing is an automated high performance peak sensing, which determines the quality of the peaks and their baseline. Baseline determination is critical for any moment evaluation. Curve fitting methods have been used in the past (12, 13), but they require more extensive computations.

The width of the valid peaks was evaluated after baseline correction by four means:

1 - Second moment: true statistical moment. The 'correct' way to do it, but very sensitive to noise.

2 - Width at half-height: 2.355 sigma for a gaussian peak (Interpolated).

TABLE 1
Accuracy of the different methods applied to EMG's
(Tau = expon. decay, Sigma = standard dev. of Gaussian)

Tau/Sigma	0	.5	.75	1.	1.5
1 True sigma	1.	1.12	1.25	1.41	1.80
2 From W 1/2	1.	1.09	1.16	1.23	1.37
3 From W 13,5 %	1.	1.10	1.19	1.30	1.54
4 From W 95 %	1.	1.12	1.25	1.41	1.76

3 - Width at 13.53 % of height: 4 sigma for a gaussian peak (Interpolated)

4 - Minimal width at 95.46 % of area: 4 sigma for a gaussian peak (Interpolated). If the width is minimal, is means dA/dt is the same in both sides, i.e. the height is the same (↔ 13.53 % of height for a gaussian).

RESULTS AND DISCUSSION

A summary of the experiments is given in table 2.

Each number is the average of three determinations. The CV's of 21 or 15 series of 3 experiments are given in table 2. The result values and the CV's indicate that the 95 % area method resembles more closely the moment method and the 4 sigma method more the half-height method in accuracy and precision.
We will therefore focus on the two extremes, the moment method and the half-height method to determine their usefulness.

Column lifetime

Examples of two different columns are given in figure 1.
Column A is stable during the 11000 no-sample injections run (11); column B seems stable based on half-height calculation but loses more than half its performance according to moments-based efficiency.
It is clear that the moments method is in this case more discriminating than the half-height method.

TABLE 2
Summary of the Experimental Data

Experiment	Column	Methods Moments		95 % area		4 sigma		half height	
		σ_{ex}^2 $(\mu l)^2$	N_∞	σ_{ex}^2 (μl^2)	N_∞	σ_{ex}^2 (μl^2)	N_∞	σ_{ex}^2 (μl^2)	N_∞
n = 500	A	380	1800	310	1860			350	3960
n = 10900	A	500	2470	380	2410			220	4230
n = 500	B	420	4190					240	5010
n = 11000	B	240	1750	180	1750	160	3810	140	4920
before damage type 1	B	260	1940	190	1910	140	3830	110	4860
after damage	B	250	1980	190	1810	130	2320	110	2680
after 5' rest*	B	240	1750	180	1750	160	3810	140	4920
before damage type 2	B	220	2110	180	2010	140	3460	120	4570
after damage	B	270	1750	230	1680	110	2020	30	2100
after 5' rest*	B	330	1990	260	1970	150	3600	120	4640
Fitting AT AE	B	700	1110	540	1070	50	2140	40	3130
BT BE	B	730	1970	600	1740	180	3280	100	3980
AT BE	B	500	1970	380	1870	130	3260	80	4320
AT AE	B	560	1040	480	1080	90	1760	80	3010
CV (%)	n'=21	21	9	21	8			12	3
CV (%)	n'=15					16	4		

* performance when leaving the column for five minutes without flow after damage

n is number of no-sample injections
n' is number of triplicate experiments

Column damage

Two cases of double peaking due to high flowrate and pressure pulsations were studied. The first case is barely visible and is not detected by the moments method but leads to a significant decrease in half-height-based efficiency. Even in a more severe case the double peaking is not indicated by the moments method (figure 2).

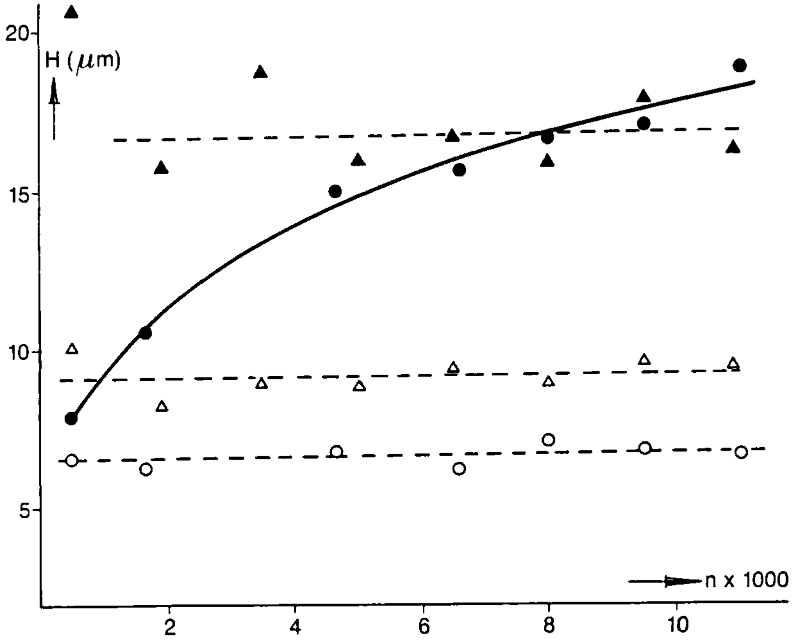

FIGURE 1: Theoretical plateheight as a function of number of no-sample injections (n).
Open symbols: half-height method ; closed symbols: moments method. Circles: column B , triangles: column A. For details see experimental section.

It deserves mention that when double peaking, due to alternative flow paths, is severe, the extra-column bandbroadening becomes meaningless since the effective column length, plateheight and the relative contribution of the pathways is not known.

Column endfittings

The four available types of fittings were exchanged using the same column. The BT fitting left a < .1 mm thin gap, which explains the slightly higher extra-column variance in table 2 using this fitting.

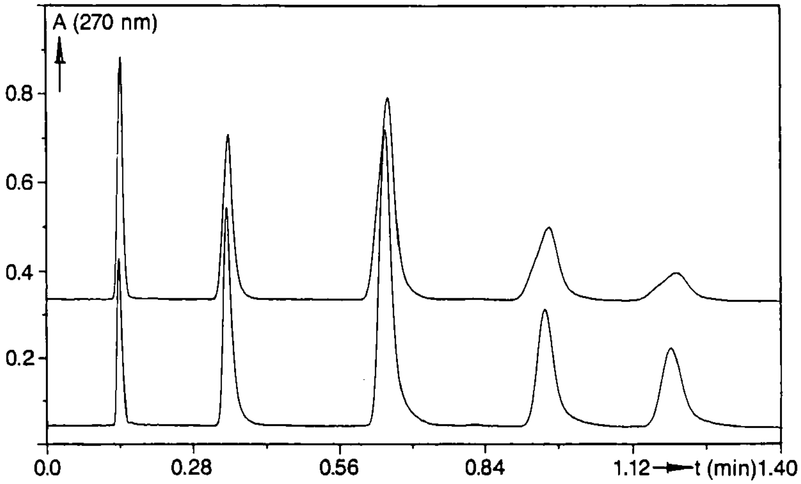

FIGURE 2: Example of column damage and restoration. This is case #2 of table 2. A is the double peaking chromatogram, B the chromatogram after five minutes with no backpressure or flow through the column.

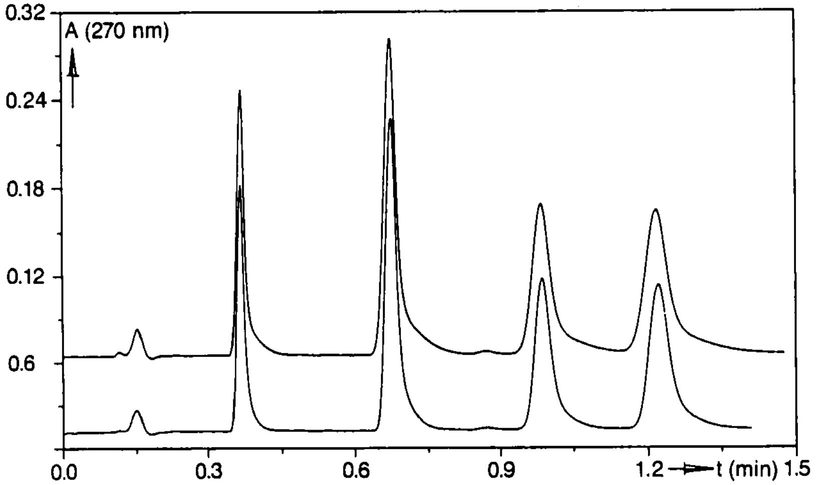

FIGURE 3: Illustration of the dependence of column efficiency on endfitting design.
A. Column B with AT and AE fittings.
B. Column B with AT and BE fittings.

A more than 50 % increase in efficiency is obtained by replacing AE by BE. The extra column variance shows that no extra dead volume was present in fitting AE. It can be assumed that the distributor in BE eliminates stagnant corners of the column that may be present using the AE fitting with its 1/8" frit.

By replacing the original fittings at the end of the endfitting experiments, equivalent performance with the starting experiment was obtained.

CONCLUSION

Although moment methods are the fundamentally correct way of evaluating column efficiency, The application of a moment method to a split peak theoretically does nog produce meaningful results. The experiments reported here show that it is prudent to use half-height data in addition to moments for monitoring column efficiency in practice because in the case of column damage investigated here, the half height method is clearly more discriminating.

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