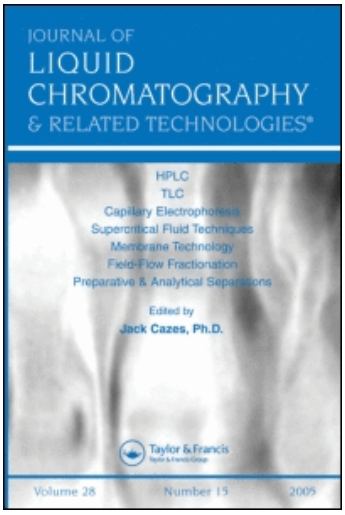
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Trace Analysis by Microbore HPLC

Sj. Van Der Wal[a]
[a] Varian Instrument Group, Walnut Creek, California

To cite this Article Van Der Wal, Sj.(1986) 'Trace Analysis by Microbore HPLC', Journal of Liquid Chromatography & Related Technologies, 9: 8, 1815 — 1828
To link to this Article: DOI: 10.1080/01483918608076720
URL: http://dx.doi.org/10.1080/01483918608076720

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(8), 1815-1828 (1986)

TRACE ANALYSIS BY MICROBORE HPLC

Sj. van der Wal
Varian Instrument Group
2700 Mitchell Drive
Walnut Creek, California 94598

Downloaded At: 15:55 24 January 2011

ABSTRACT

The potential and problems of trace analysis by microbore HPLC with on-column concentration and the benefits of additional column-switching in several modes are discussed.

An example of the performance of an on-column concentration column-switching microbore HPLC system is shown.

INTRODUCTION

For concentration sensitive detectors the minimum detectable quantity, MDQ_n, is proportional to the cross-sectional area (A) of the column unless the extra-column bandbroadening ($\sigma_{v,ex}$) is significant relative to the bandbroadening due to the column ($\sigma_{v,c,n}$):

$$MDQ_n = A \epsilon H_n (1+k'_n) \sqrt{\pi 2 N_c} C_{min,n} \left(1+\frac{\sigma^2_{v,ex}}{\sigma^2_{v,c,n}}\right) \quad (1)$$

Presented at the IXth I.S.C.L.C., Edinburgh (UK), July 1-5, 1985

Copyright © 1986 by Marcel Dekker, Inc. 0148-3919/86/0908-1815$3.50/0

Microbore chromatography is thus an attractive technique for trace analysis. Trace level analytes can be obscured, however, by sample compounds that overload the column.

Mass overloading by major components of the sample for trace analysis and volume overloading by the sample may be obviated by:

A on-column concentration (OCC)

B column switching

Both techniques accomplish a pre-separation of main compounds and the trace compounds of interest, converting the sample into a micro-column compatible fraction.

Downloaded At: 15:55 24 January 2011

EXPERIMENTAL

Injection valves were a 1 μl internal loop model 7410 (Rheodyne, Cotati, CA, USA) a 70 nl C-4W (Valco, Houston, TX, USA) and a CV-6-UHPa-N60 (Valco) with a 10 μl or a 1200 μl external loop. The switching valve in Figure 1 was a model CV-6-UHPa-N60 (Valco) and as a prime/purge valve model 02-0120 (SSI, State College, PA, USA) was used.

The HPLC systems were a model LC 5500 with a UV-200 absorbance detector, equipped with a microbore flowcell (Varian, Walnut Creek, CA, USA), operated in the split-flow mode[1] and a model LC 8500 (Varian) syringe type pump (Pump 1, Figure 1).

530 and 320 μm fused-silica columns of 70 to 400 mm length were packed at Varian with 3 μm reverse phase silica (Micropak SP C18-3, Varian). The concentration column in Figure 1 was a 20 x 2 mm stainless-steel column obtained from Upchurch (Oak Harbor, WA, USA), dry-packed with 5 μm polar bonded phase silica (Micropak SP CN-5, Varian). The Rheodyne 7302 in-line filter was equipped with a 0.2 μm pore size Fluoripore™ (Millipore, Bedford, MA, USA) insert[2] in addition to the standard stainless steel filter.

Downloaded At: 15:55 24 January 2011

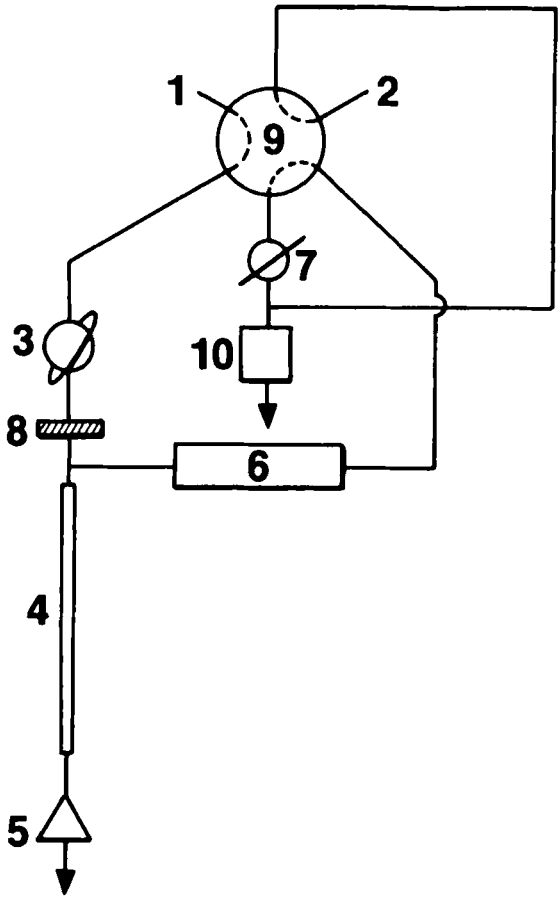


Figure 1 Microbore column-switching system for trace analysis. 1 pump 1; 2 pump 2; 3 injection valve; 4 analytical column; 5 detector; 6 concentration column; 7 prime/purge valve; 8 in-line filter; 9 switching valve; 10 constant pressure device.

As a constant pressure device a Tescom 26-1721-44-043 pressure regulator (Tescom, Minneapolis, MN, USA) was employed. Extra-column variance due to injection was determined by linear regression of peak variance vs the square of the reciprocal elution volume for anthracene with 215-400 mm long 0.32 mm diameter columns and 60 - 90% acetonitrile/40 - 10% water as mobile phases. The validity of this method is indicated by the split-injection results and was verified by a method[3] analogous to that of Kok et al.[4]

The fluorescence detector was a Fluorichrom (Varian) with 220I and 7- 60 filters and modified as described elsewhere[3]. Test compounds were obtained from Aldrich (Milwaukee, WI, USA) and Chem Service (West Chester, PA, USA) and solvents from Burdick and Jackson (Muskegon, MI, USA).

Downloaded At: 15:55 24 January 2011

RESULTS AND DISCUSSION

A On-column concentration

The use of a sample solvent in which the compounds of interest have a very high capacity factor is described extensively in the literature[5,6] and is a standard technique on commercial clinical HPLC analyzers for concentration of the sample after on-line extraction.[7]

OCC reduces the contribution of the injection to the total variance of the chromatographic bands. This contribution can be measured by eliminating the detector contribution and the hydraulic connection lines. Fused-silica columns can be connected directly into injection valves as shown in Figure 2.

To obtain true through-column detection, two millimeter of the fused- silica column packed with 3 μm reverse phase silica was used as a detector cell.[1] Peak-broadening due to dispersion in the detector cell becomes negligible and "optical" peakbroadening proportional to $(1+k'_a)$ so that total variance, σ_t^2, is:

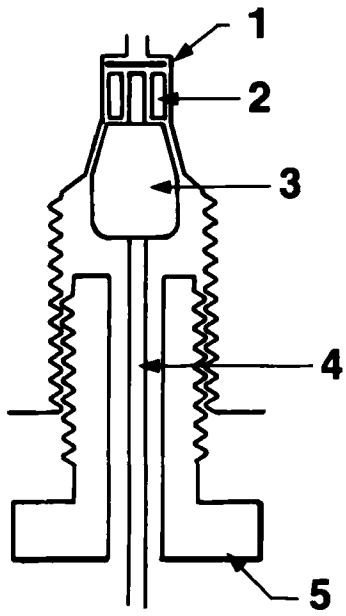


Figure 2 Connection of the injection valve to the fused-silica column. 1 0.5 μm pore size screen frit; 2 teflon washer; 3 1/16" to 0.5 mm vespel ferrule; 4 fused-silica column; 5 1/16" nut

Downloaded At: 15:55 24 January 2011

$$\sigma_{t,n}^2 = \sigma_{t,c,n}^2 + \sigma_{t,od,n}^2 + \sigma_{t,i}^2$$
$$= \frac{(1+k_n)^2}{F^2}\left(\frac{V_c^2}{N_c} + \frac{V_d^2}{\Psi_1^2}\right) + \sigma_{t,i}^2 \qquad (2)$$

The variance due to injection, $\sigma_{t,i}^2$, can be expressed in volume units as:

$$\sigma_{v,i}^2 = \frac{V_i^2}{\Psi_2^2} + \sigma_{v,occ}^2 \qquad (3)$$

Figure 3 indicates an experimental value for Ψ_2 of two for the indicated valve and flowrate range. The different injection volumes were calculated from the time interval of injection. The residual injection contribution, $\sigma_{v,occ}$, is mainly due to mixing in the ports of the valve (e.g. see split-flow injection, Figure 4).

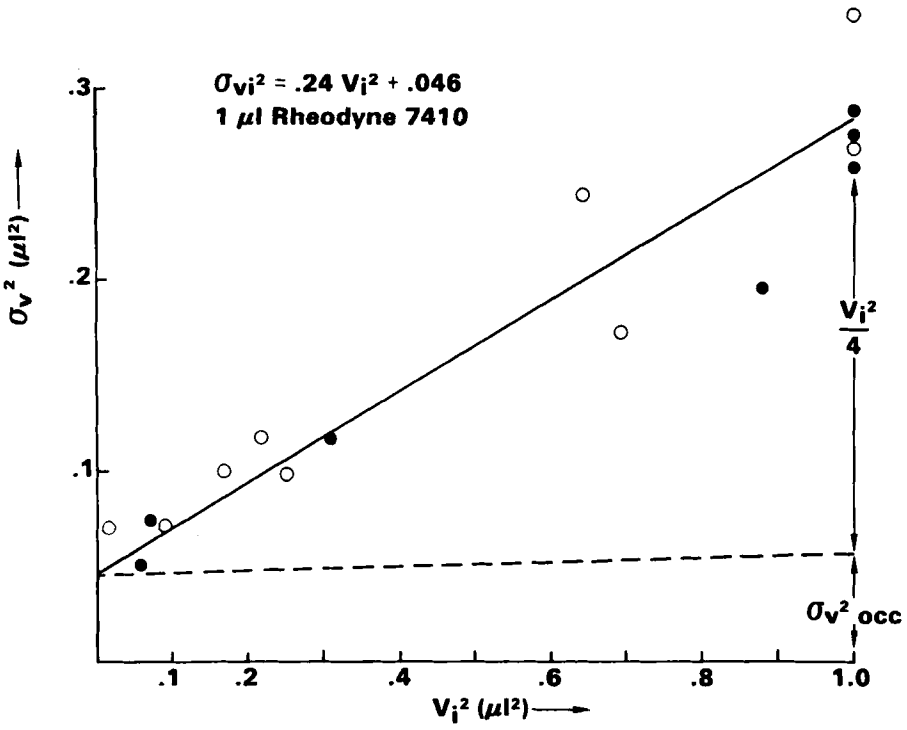


Figure 3 Variance due to injection. See experimental; flowrate: ○ 5 μl/min; ● 2.5 μl/min.

The extra-column contribution, $\sigma_{v,ec}$, using OCC with different valves and injection volumes is shown in Figure 4. A 150 x 0.32 mm fused-silica column of 10,000 theoretical plates will give a 0.1 μl^2 peak variance, $\sigma_{v,c,n}^2$, of a compound with a capacity factor of three. If the demand is made that $\sigma_{v,ec}$ is smaller than $\sigma_{v,c,n}$ then the injected volume should be less than 3 μl, or half the column volume.

No OCC is necessary with this valve and column combination when injecting less than 100 nl.

A useful diagnostic tool in evaluating column performance is split- flow injection, in which the split is made between the injector and the column[8]. Negligible

Downloaded At: 15:55 24 January 2011

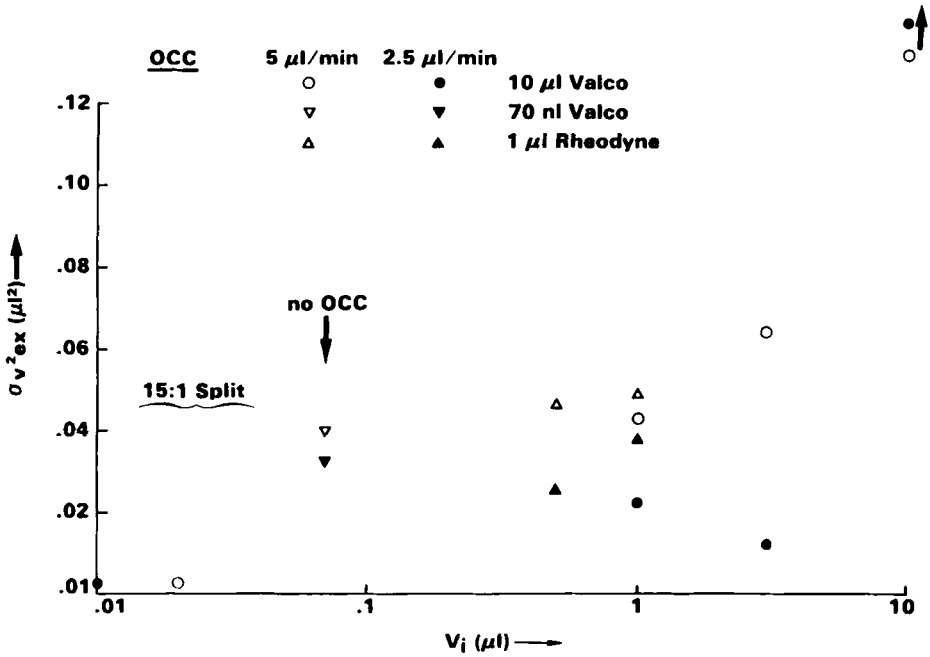


Figure 4 Extra-column variance in a microbore system with and without on- column concentration.

Downloaded At: 15:55 24 January 2011

extra-column contribution to the overall peakbroadening is observed (see Figure 4) when combining split-flow injection, elimination of transfer lines and through-column detection.

Problems encountered with single microbore column OCC operation:

1. Instability of the column bed. Injection valve material deposits on the top of the column, which decreases local permeability and depresses the top of the column bed.
2. With larger samples and smaller diameter columns injection time is non-negligible:

$u = 1\ mm/s \quad V_i = 40\ \mu l$

Column I.D. (mm):	1.0	0.3	0.1
Δt_i (min):	.5	5	50

B Column Switching

In addition to OCC column switching can extend the sample volume and mass range that can be analyzed by microbore column chromatography[4].

Proper choice of stationary and mobile phases in two-dimensional chromatography allows for alleviation of mass overloading by exploiting the differential characteristics of trace and main sample constituents.

Figure 5 shows four microbore systems of increasing complexity and versatility.

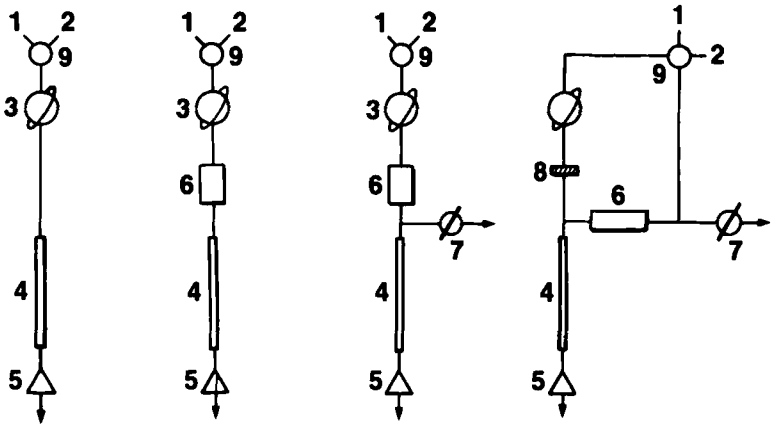
In system B debris of the injection valve is filtered on a wide-bore concentration column resulting in less incidence of clogging than system A. For large samples this system is still slow and all components of the sample eventually reach the detector.

System C has the possibility of quickly eluting undesired compounds to waste[4]. This scheme with a wide-bore concentration column has not been applied here because peakbroadening and fractionation on the concentration column can be reduced by backflushing this column (System D).

In system D the filter between the injection valve and the columns does not contribute appreciably to the peakbroadening since on both columns OCC is applied and the filter is not in the flowpath when backflushing the concentration column.

A modification of system D was built (system E) and its hydraulic schematic is depicted in Figure 1. Incorporating a constant pressure device allowed more gentle and precise operation of this switching system.

Downloaded At: 15:55 24 January 2011



System	A	B	C	D
Advantage	Simplest	Simple		
Disadvantage	Debris Slow	Slow Gradient	Gradient	Complex

Figure 5 Schematics of microbore HPLC trace analysis systems. Numbers as in Figure 1.

Downloaded At: 15:55 24 January 2011

The variance of chromatographic bands can be expressed as:

$$\sigma^2_{v,c,n} = L\ H\ \epsilon^2\ A^2\ (1+k_n)^2 \tag{4}$$

The capacity factor in the injection solvent is very large so only the top of the concentration column is used and its effective length, L_1^{eff}, is:

$$L_1^{eff} = \frac{\Delta t_i\ F_o}{\epsilon_1\ A_1\ (1+k'_{0,n})} \tag{5}$$

The total peak variance in system E becomes then:

$$\sigma^2_{v,n} = \left\{ \sigma^2_{v,i} \left[\frac{1+k'_{1,n}}{1+k'_{0,n}} \right] + (H_{1,0}+H_{1,1})\ \epsilon_1\ A_1\ \Delta t_i\ F_o\ \frac{(1+k'_{1,n})^2}{(1+k'_{0,n})} + \sigma^2_{bf} \right\}$$

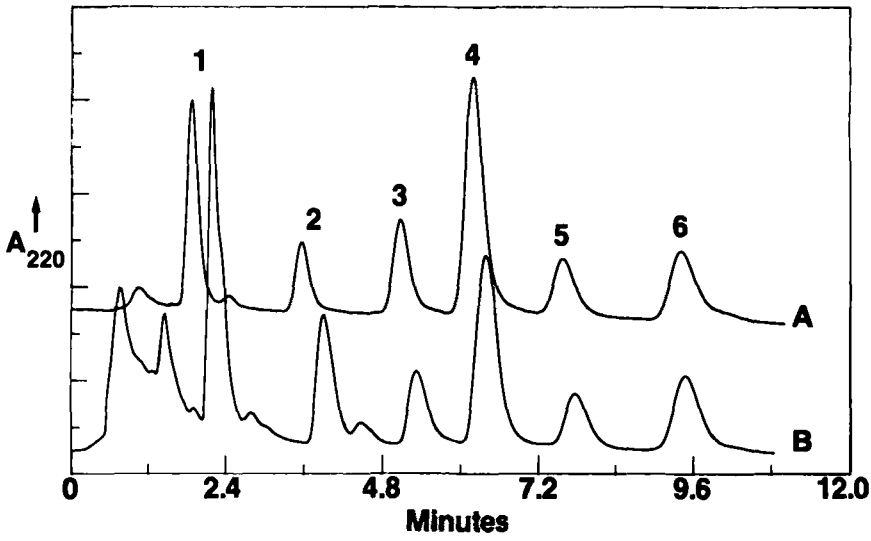


Figure 6 Trace analysis of a PNA sample. A: one microliter injected with OCC; B: one milliliter injected with the valve and eluent program of Table 2. For peak identity see Table 1.

$$\times \frac{(1+k'_{3,n})^2}{(1+k'_{2,n})^2} + H_2\ L_2\ \epsilon_2^2\ A_2^2\ (1+k_{3,n})^2 \qquad (6)$$

The contributions of the three parts of the first right hand side term of equation 6 relative to the second term are of interest and can be evaluated by differential measurement.

An example of the potential of system E is given in Figure 6 and Table 1.

A mixture of polynuclear aromatics was prepared and one microliter was injected using OCC, and eluted from the 100 x .53 mm fused-silica column with 75% acetonitrile, 25% water at 40 μl /min flowrate (curve A, Figure 6).

The same sample was diluted thousandfold with water and introduced from a 1200 μl loop in system E with the valve and solvent sequence of Table 2. Note that

Downloaded At: 15:55 24 January 2011

TABLE 1

Loss of Resolution in System E

Compound	Annotation (Fig. 6)	$k'_{2,n}$	R_A (σ)	R_B (σ)	R_B/R_A (%)
fluorene	1	.8			
benz (a) anthracene	2	2.4	4.67	2.55	55
benzo (b) fluoranthene	3	3.9	2.95	1.92	65
benzo (a) pyrene	4	5.0	1.86	1.30	70
dibenzo (a,h) anthracene	5	6.3	2.07	1.60	77
indeno (1,2,3,c,d) pyrene	6	8.1	2.31	1.79	77

Downloaded At: 15:55 24 January 2011

TABLE 2

Valve and Solvent Program for PNA Analysis

Time (min)	Mode	Solvent	Flowrate (ml/min)	Valve 7	Valve 9
0	equilibration	1	1	open	-
5	injection	1	1	open	-
6	backflush	2	1	open	+
6.1	elution	2	.04	closed	+

solvent 1 : water

solvent 2 : 75% acetonitrile, 25% water

before the injection the system was equilibrated for five minutes with weak eluent (water).

The resulting chromatogram is curve B in Figure 6. Chromatogram B was started 3.5 minutes after elution to demonstrate more clearly that even when using the backflush mode a slight gradient is created. A 20 - 40% loss in resolution over the useful capacity factor range is indicated in Table 1. Sample loading time, however, is reduced relative to one column OCC from 25 to one minute, and total cycle time is cut in half.

Addition of weak mobile phase between the concentration column and the analytical column[9] is attractive since the increase in variance due to dilution is more than offset by concentration during OCC - especially when pH changes are employed. In system E this could be accomplished via the large loop of the injection valve and an extra pressure setting on device #10 (Figure 1), while leaving valve #7 in the open position during OCC on the analytical column.

In case of a modified backflush eluent $(1+k'_{2,m})$ in equation 6 is replaced by:

$$\frac{F_4+F_2}{F_2} \cdot \frac{(1+k'_{2,m})^2}{(1+k'_{4,m})}$$

CONCLUSIONS

Sample volumes up to half the column volume can be analyzed by microbore chromatography by means of on-column concentration.

For samples of at least hundred times the column volume an on-column concentration column switching system can be used with a minor loss of resolution but a substantial decrease in analysis time relative to single column microbore chromatography with on-column concentration.

Downloaded At: 15:55 24 January 2011

SYMBOLS

A	cross-sectional area of the column
A_1	cross-sectional area of the concentration column
A_2	cross-sectional area of the analytical column
$C_{min,n}$	minimum detectable concentration of n by the detector
F	flowrate on the column during elution
F_o	flowrate on the column during loading of the sample
F_4	flowrate of the backflush additive
F_2	flowrate of the backflush eluent
H_n	theoretical plateheight of the column for compound n
$H_{1,0}$	theoretical plateheight of the concentration column in the loading eluent
$H_{1,1}$	theoretical plateheight of the concentration column in the backflush eluent
H_2	theoretical plateheight of the analytical column
k'_n	capacity factor of compound n
$k'_{0,n}$	capacity factor of compound n on the concentration column in the loading eluent
$k'_{1,n}$	capacity factor of compound n on the concentration column in the backflush eluent
$k'_{2,n}$	capacity factor of compound n on the analytical column in the backflush eluent
$k'_{3,n}$	capacity factor of compound n on the analytical column in the final eluent
$k'_{4,n}$	capacity factor of compound n on the analytical column in the modified backflush eluent
L_2	length of the analytical column
L_1^{eff}	effective length of the concentration column
MDQ_n	minimum detectable quantity for compound n

Downloaded At: 15:55 24 January 2011

N_c	number of theoretical plates of the column for compound n
Δt_i	duration of injection of the sample
u	linear velocity of the eluent on the column
V_i	injected sample volume
ϵ	porosity of the column
Ψ_1, Ψ_2	geometrical constants
σ_{bf}	variance of the extra-column bandbroadening occurring during backflushing in system E
$\sigma_{t,n}, \sigma_{v,n}$	variance of the total bandbroadening
$\sigma_{t,od,n}$	bandbroadening due to non-ideal optics
$\sigma_{t,i}, \sigma_{v,i}$	variance of the bandbroadening due to sample injection
$\sigma_{t,c,n}, \sigma_{v,c,n}$	variance of the bandbroadening on the column for compound n
$\sigma_{v,ec}$	variance of the extra-column bandbroadening

Downloaded At: 15:55 24 January 2011

ACKNOWLEDGEMENTS

The author would like to thank R. E. Majors and R. A. Simpson for their editorial advice, and Mrs. M. Bobinecz and Mr. D. Brown for their help in preparation of the manuscript.

REFERENCES

1. Sj. van der Wal and F. J. Yang, J.H.R.C + C.C. 6 (1983) 216
2. Sj. van der Wal, L.C. Mag., 3 (1985) 488
3. Sj. van der Wal, submitted for publication to J. Chromatogr.
4. W. Th. Kok, U.A. Th. Brinkman, R. W. Frei, H. B. Hanekamp, F. Nooitgedacht and H. Poppe, J. Chromatogr. 237 (1982) 357
5. J. N. Little and G. J. Fallick, J. Chromatogr. 112 (1975) 389
6. J. Lankelma and H. Poppe, J. Chromatogr. 149 (1978) 587
7. S. J. Bannister, Sj. van der Wal, J. W. Dolan and L. R. Snyder, Clin. Chem., 27 (1981) 849
8. F. J. Yang, J. Chromatogr. 236 (1982) 265
9. W. Lindner, presentation, VIIth Int. Symp. on Column Liquid Chromatography, Baden-Baden, GFR, 1983.