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OPEN-TUBULAR LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY USING DIRECT LIQUID INTRODUCTION

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SUMMARY

The prospects for increasing the speed and resolving power of liquid chromatographic separations depend strongly on the feasibility of detecting solutes within effective volumes of the effluent of about 1 nl or less. Mass spectrometry, with a low detection limit, minimum sample size requirement and adjustable selectivity, is one of the first choices under consideration for dealing with this problem.

This paper describes some work in which direct liquid introduction (DLI) in combination with chemical ionization (CI) was applied. Diaphragms of about 4 μm I.D. were used, necessitating the application of a make-up flow in order to achieve jet formation. The nature of the make-up liquid in turn can be used to control the CI conditions. Peak broadening effects of such a set-up were studied by insertion of fused-silica capillaries as a dummy column. The results obtained with 5 and 10 μm I.D. columns show that standard deviations in terms of the volume of column effluent can be brought down to 0.1–1 nl or less. These low standard deviations obtained suggest the necessity for reconsideration of the calculations of Knox and Gilbert on the relative potential performance of open-tubular and packed column liquid chromatography. It is shown that with the present performance of the system, capillary columns of 5 μm I.D. and a few metres long would be applicable with no significant peak distortion, which indicates that open-tubular columns can be competitive under an even wider range of conditions than was assumed before.

The detection limits of the system look very promising. Without any optimization of the CI conditions, etc., 1–10 pg of solute could be detected. Further work is needed to extend the concentration range to the ppb values necessary for applications of open-tubular liquid chromatography.

INTRODUCTION

Open-tubular liquid chromatography (OT-LC) in capillaries smaller than 100 μm I.D. was introduced in 1978 by Tsuda and Novotny[1] after some pioneering research by Nota *et al.*[2]. Theoretical considerations by Knox and Gilbert[3] showed the necessity for drastically miniaturizing the detection cells in order to make OT-LC superior to packed columns: detection of the solutes in a volume of about 1 nl is

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required. Sensitive UV detection in such a small volume has been discussed[4,5] and it appears that the possibilities are very limited unless perhaps lasers are used as a radiation source. Therefore, research was directed towards other detection principles, such as (laser-induced) fluorescence[6,7] and electrochemical detection[8,9].

OT-LC would be of prime interest for separations in which many theoretical plates should be available. In these complex mixtures the constituents would probably differ widely in type and concentration. High sensitivity, low contribution to peak variance and as universal a response as possible are therefore prerequisites for OT-LC detection. Mass spectrometry is a highly sensitive technique, giving the possibility of both universal and selective detection. As also very small amounts of material are needed, mass spectrometry (MS) is one of the first choices for OT-LC detection. However, interfaces for OT-LC and MS described in the literature are either very difficult to construct[10] or not well documented[11]. Therefore, a further study of interfacing of OT-LC and MS appeared important.

In our laboratory, two types of interfaces are currently under study. The results obtained with a simple capillary inlet interfacing of OT-LC with a conventional electron-impact MS will be reported soon[12]. In this paper, interfacing of OT-LC by means of direct liquid introduction (DLI)[13–17] with chemical-ionization MS is discussed and attention is focused on extra-column peak variance contributions.

EXPERIMENTAL

Chromatography

The solvent delivery system for both the make-up liquid (see Results and discussion for an explanation) and the mobile phase was built using conventional LC

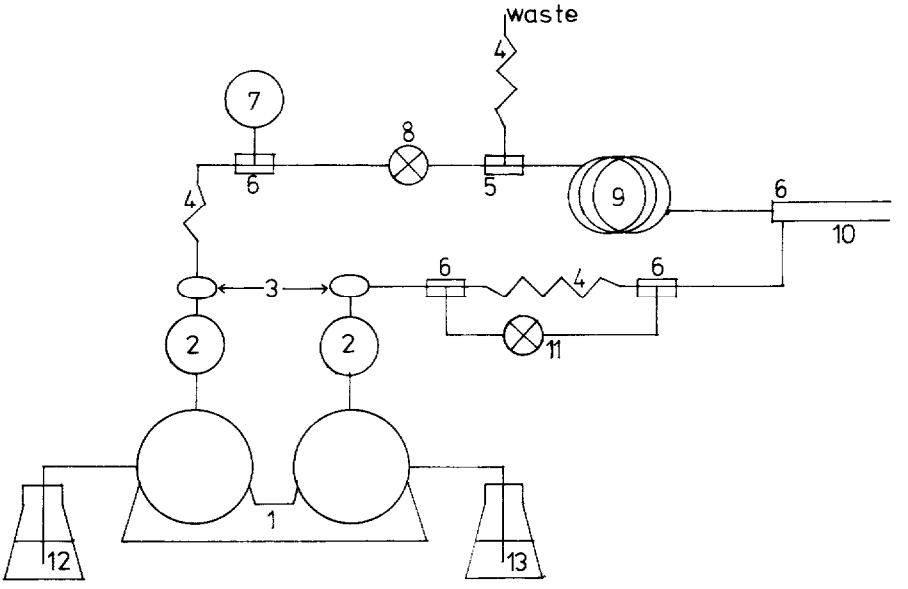

Fig. 1. Schematic diagram of the solvent-delivery system using in OT-LC and DLI. 1, Dual head pump; 2, pressure gauge; 3, 0.5-μm line filter; 4, fused-silica capillary restrictions; 5, split injection T-piece; 6, T-piece; 7, column pressure gauge; 8, injection valve with 10-μl loop; 9, open-tubular column; 10, DLI probe; 11, fast pumping line with valve; 12, mobile phase; 13, make-up liquid.

pumps. It consisted of a dual-head pump (Type DMP 1515, Orlita, Giessen, F.R.G.), which was actually used as two separate constant-pressure pumps using appropiate fused-silica (SGE, Ringwood, Australia) capillary restrictions (Fig. 1). One of the pump heads delivered the make-up liquid (0.2–0.6 μl/sec) and the other the mobile phase for the open-tubular column. The system is capable of delivering stable flow-rates. Variation of the peak residence time within a series was less than 0.5% (R.S.D.). For sample injection an injection valve (Type 7020, Rheodyne, Berkeley, CA, U.S.A.) with a 10-μl loop was used. A splitting device, similar to those described by other workers (*e.g.*, ref. 18) was used. Line filters (0.5 μm) (Nupro, Willoughby, OH, U.S.A.) were included in both pumping lines.

As open-tubular columns, various lengths of unmodified fused-silica capillaries (25, 10 and 5 μm I.D.) were used.

Mass spectrometry

The mass spectrometer was a Finnigan 3100 GC–MS system (Finnigan, San Jose, CA, U.S.A.), the vacuum system of which was modified to allow differential pumping between the analyser and the ion source housing[19]. The source housing was evacuated using a 0.3 m^3/sec oil diffusion pump (Edwards, Crawley, U.K.) and a 0.035-m^2 active surface area cryogenic pump. The installation of butterfly valves made possible easy cleaning of the cryopump without turning off the vacuum system. The pressure in the source housing was monitored with a Edwards Type 8 Penning ionization tube.

The laboratory-built DLI probe is shown schematically in Fig. 2. The open-tubular column fits inside the probe and ends in a small cylindrical chamber (0.3 mm I.D.) about 1 mm from the diaphragm. The diaphragm was a small nickel plate with a 4 ± 2 μm hole (custom-made by Veco, Eerbeek, The Netherlands).

The DLI probe fits into a laboratory-build direct insertion inlet, evacuated by a rotary forepump (Alcatel, Paris, France) equipped with an Edwards Type FL20K foreline trap.

For mass spectrometric detection, one of the channels of the four-channel programmable multiple ion monitor (PROMIM) of the mass spectrometer was used.

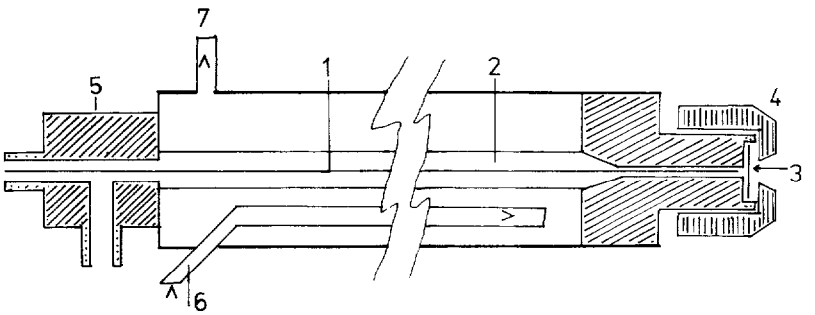

Fig. 2. Schematic diagram of the laboratory-built DLI probe (10 mm O.D.). 1, Fused-silica open-tubular column; 2, make-up liquid; 3, 4-μm nickel diaphragm enclosed between two PFTE spacers; 4, stainless-steel nut; 5, modified Swagelock 1/16 in. T-piece; 6, cooling water in; 7, cooling water out.

RESULTS AND DISCUSSION

Interface design

Liquid jet formation has been extensively studied for both direct liquid introduction[10,13–15] and other purposes[20,21]. In the kinetically near to optimal[3] operation of OT-LC, flow-rates between 0.1 and 10 nl/sec are expected. Forming a liquid jet with such a small flow-rate would require diaphragms considerably smaller than 1 μm I.D. (*i.e.*, 0.04–0.8 μm)[14]. Such diaphragms are difficult to fabricate and will become blocked very easily. Therefore, we decided to add a larger auxiliary liquid stream, the make-up liquid, to the column effluent in order to form the liquid jet. We can then use larger diaphragms. One of the disadvantages of using a make-up liquid is that we cannot avoid a small dead volume at the outlet side of the column (see Fig. 2). The contribution to peak variance of this volume, which actually acts as a mixing chamber, is governed by three design parameters: length and inner diameter of the chamber and the ratio of make-up liquid and column flow-rates. In practice, the length of the chamber cannot be made smaller than about 1 mm. The inner diameter of the mixing chamber has a large influence on the contribution to peak variance but cannot be smaller than the outer diameter of the fused-silica column (0.25 mm). Therefore, in our design the mixing chamber has a volume of about 70 nl. In order to keep the contribution to peak variance due to this volume sufficiently small, the total flow-rate through the mixing chamber should be higher than 0.2 μl/sec. Therefore, diaphragms of about 4 μm I.D. were selected, requiring make-up liquid flow-rates greater than 0.1 μl/sec. In practice, we used 0.2–0.5 μl/sec, giving a pressure of 0.01–0.03 Pa in the source envelope. As the LC flow-rate is negligibly small compared with the make-up liquid flow-rate, it is expected that the contribution to peak variance (σ_t) of the mixing chamber will depend on the make-up liquid flow-rate but not on the column flow-rate. In this design we can also select the CI reagent gas composition independent of the LC mobile phase by choosing an appropriate make-up liquid composition, provided that the two liquids are readily miscible.

Liquid jet formation

Before actually starting the experiments on interfacing OT-LC and MS with DLI, the liquid jet formation was studied both in still air and in a laboratory-built vacuum testing chamber. In these experiments special attention was paid to the pressures necessary to form a liquid jet (the "jet pressure"). Variation of the jet pressure in our system would inevitably result in variations in the linear velocity of the mobile phase through the column. As the jet pressure is proportional to the square of the flow-rate[14] and the linear velocity in the column is directly proportional to the pressure drop over the column, a linear relationship between linear velocity in the column and the square of the make-up liquid flow-rate is to be expected. This was confirmed experimentally.

Stable liquid jets were obtained with various solvents using the inexpensive diaphragms described. As has been noted by other workers (*e.g.*, ref. 14), sudden changes in the direction of the liquid jet can occur. In the literature there has been some dispute about the causes of these disturbances[14,22,23]. As our column did not contain any packing material, blocking of the diaphragm or directional changes of the jet are not necessarily caused by loss of column material[22], although dissolution

of column material has recently been confirmed in high-performance liquid chromatography–inductively coupled argon plasma atomic emission spectroscopy studies[24]. Directional changes mostly had no detectable effect on the jet pressure. The jet pressure can be constant over a long period of time even with frequent injections of concentrated solutions of low-volatility solutes such as sucrose.

Plate height versus linear velocity curves

Extra-column peak variance contributions have the greatest impact on unretained peaks. Moreover, measuring the peak variance of the unretained peaks facilitates a comparison of experimental values with theory (Golay equation for unretained peaks). In most experiments methanol was used as the mobile phase and toluene as the solute. The diffusion coefficient of the latter in the mobile phase was calculated using the Wilke–Chang equation[25].

Plate height *versus* linear velocity curves for 25, 10 and 5 μm I.D. fused-silica capillaries were obtained. Various methods exist for the evaluation of peak shapes and the determination of peak standard deviations. We calculated peak standard deviations from the second central moment of the peaks or from the peak width at 0.6 of the height. Fig. 3 shows the measured plate height *versus* linear velocity curve for a 6.82 m × 25 μm I.D. capillary, together with the theoretical line calculated according to the Golay equation. Measured values for 25 μm I.D. capillaries agree well with the theoretical values.

For flow-rates between 1.5 and 13 nl/sec, the volume standard deviation of the extra-column region is 2–5 nl. Its contribution to the total standard deviation is only 5% of the column peak standard deviation (see Table I).

From these results for the 6.82 m × 25 μm I.D. column it was expected that the extra-column region would make significant contributions to the total peak variance when short 10 or 5 μm I.D. columns are used. A linear velocity (u) *versus* plate height (H) curve for a 2.03 m × 10 μm I.D. column is shown in Fig. 4, and that for a 1.81 m × 5 μm I.D. column in Fig. 5. Measured extra-column contributions to the total peak variance for these columns are summarized in Table I.

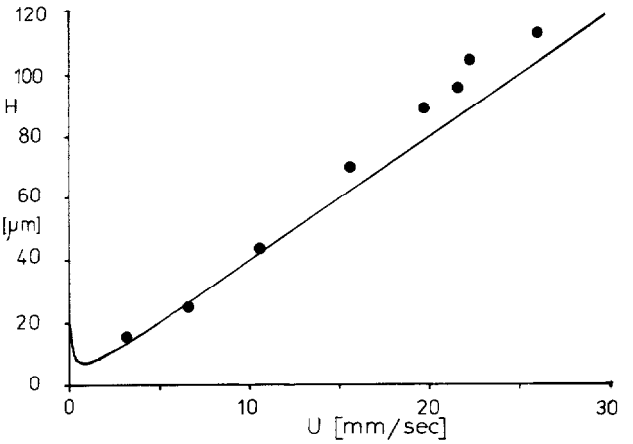

Fig. 3. Plate height *versus* linear velocity curve for a 25 μm I.D. open-tubular column. Column length 6.82 m; $k' = 0$; mobile phase, methanol; sample, toluene. ●, Experimental; line, theoretical.

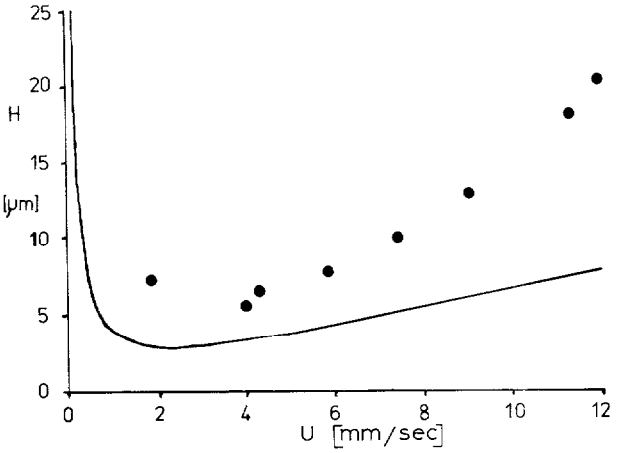

Fig. 4. Plate height *versus* linear velocity curve for a 10 μm I.D. open-tubular column. Column length, 2.03 m; $k' = 0$; mobile phase, methanol; sample, toluene. ●, Experimental; line, theoretical.

It is important to discuss the consequences of these figures. First, these results show that extra-column peak variance contributions below 1 nl can easily be achieved with the described DLI interface for OT-LC–MS. For the experiment with the 5 μm I.D. column the measured peak variance was even smaller than 70 pl. Knox and Gilbert[3] showed that for separations for which more than 30,000 plates are required, OT-LC would be superior to packed columns when the detector peak variance contributions could be reduced to below 1 nl. It appears, therefore, that values below the lower limit of 1 nl which they assumed in their calculations can easily be reached in practice.

Short columns, especially with the 10 and 5 μm I.D. capillaries, were used in these experiments. These figures allow the calculation of the column length (L^*) at which the extra-column peak variance contribution for an unretained peak would be

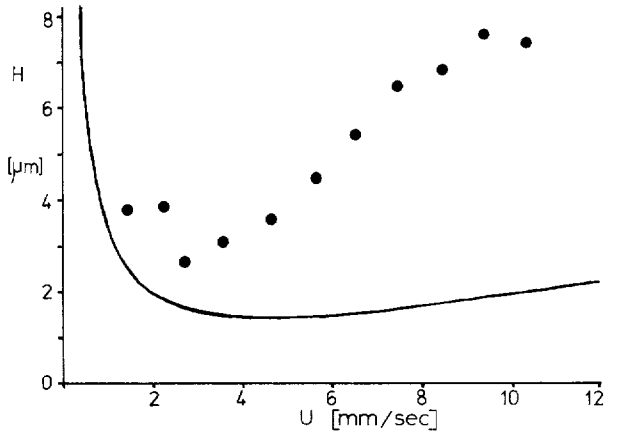

Fig. 5. Plate height *versus* linear velocity curve for a 5 μm I.D. open-tubular column. Column length, 1.81 m; $k' = 0$; mobile phase, methanol; sample, toluene. ●, Experimental; line, theoretical.

TABLE I

EXPERIMENTAL EXTRA-COLUMN PEAK VARIANCE CONTRIBUTIONS IN OT-LC–DLI-MS

d_c = Column diameter; L = column length; F_c = column flow-rate; $\sigma_{t,ex}$ = time standard deviation of the extra column region; $\sigma_{v,ex}$ = volume standard deviation of the extra column region; σ_{tot} = measured total standard deviation; σ_{col} = calculated column standard deviation; % contr. = % contribution.

d_c (μm)	L (m)	F_c (nl/sec)	$\sigma_{t,ex}$ (sec)	$\sigma_{v,ex}$ (nl)	% contr.*
25	6.82	12.9	0.30	3.9	5
		1.7	0.91	1.5	5
10	2.03	0.94	0.42	0.39	63
		0.32	0.53	0.17	33
5	1.81	0.203	0.29	0.059	95
		0.054	0.49	0.027	27

* % contr. is calculated with $\sigma_{tot} = [1 + (\%\ \text{contr.})]\sigma_{col}$.

reduced to the generally accepted 5% of the column peak variance[26]. These column lengths are summarized in Table II. As we worked at pressures smaller than 5 MPa, the column can indeed be made longer.

Retention will lead to larger peak variances and reduce the influence of extra-column peak variance contributions. The capacity factors ($k'_{5\%}$) at which the extra-column contribution has been reduced to below 5% were also calculated and are shown in Table III. We assumed diffusion coefficients in the mobile and stationary phases of $1 \cdot 10^{-9}$ and $1 \cdot 10^{-11}$ m²/sec, respectively, and calculated the film thickness from a phase ratio of 0.05 (as in reversed-phase LC).

The plate numbers expected with these columns for both unretained peaks (N^0) and retained peaks ($N^{k'}$) (with the calculated capacity factor) are also given in Table III. Although retention gives a dramatic decrease in the plate number compared with the plate number with unretained peaks, we are still able to achieve high plate numbers in a short time. For example, the 1.81 × 5 μm I.D. column would give 162,000 plates in only 19 min [144 plates per sec ($k' = 0.7$), which is fairly high].

TABLE II

CONSEQUENCES OF MEASURED EXTRA-COLUMN PEAK VARIANCE CONTRIBUTIONS: COLUMN LENGTH

The calculated column length, L^*, applies for $k' = 0$ with methanol as mobile phase and toluene as solute. For further explanation, see text.

d_c (μm)	L (m)	u (mm/sec)	$\sigma_{t,ex}$ (sec)	L^* (m)
25	6.82	22.7	0.40	7.1
10	2.03	11.9	0.42	4.9
		4.1	0.53	3.1
5	1.81	10.4	0.29	7.7
		2.7	0.49	2.7

TABLE III

CONSEQUENCES OF MEASURED EXTRA-COLUMN PEAK VARIANCE CONTRIBUTIONS: CAPACITY FACTOR

Calculated using the Golay equation with $D_m = 1 \cdot 10^{-9}$ and $D_s = 1 \cdot 10^{-11}$ m²/sec; d_f based on 0.05 phase ratio. d_f = Film thickness; D_m = diffusion coefficient in the mobile phase; D_s = diffusion coefficient in the stationary phase. For further explanations, see text.

d_c (μm)	L (m)	u (mm/sec)	$\sigma_{t,ex}$ (sec)	N^0	$k'_{5\%}$	$N^{k'}$
25	6.82	22.7	0.40	41,500	0	41,500
10	2.03	11.9	0.42	80,500	0.7	13,000
		4.1	0.53	289,000	0.4	75,500
5	1.81	10.4	0.29	233,500	1.0	31,500
		2.7	0.49	734,500	0.7	162,000

Sources of extra-column peak variance contributions

Selective ion monitoring on the m/z 93 ion of toluene was used for the plate height *versus* linear velocity curves. The low-pass filter frequency of the PROMIM must be selected with care, otherwise very tailing peaks will result (asymmetry factors of about 3 or even more). For the peaks measured in the H *vs.* u curves, asymmetry factors between 1.09 and 1.25 were found.

When evaluating contributions from the injector and detector system, we also evaluated peak shapes using peak deconvolution methods based on the exponential modified gauss (EMG) model of a chromatographic peak[27,28]. It is well known that many extra-column peak variance contributions can be characterized by an exponential time constant[29].

It can be shown that with the 25 μm I.D. capillary the small deviations from theory are due to some exponential peak broadening effect, which is flow dependent.

With the 10 and 5 μm I.D. capillaries, peak deconvolution methods also show exponential peak broadening, but these effects cannot completely explain the deviations from theory found. Only about 40–70%, depending on the flow-rate, of the extra-column peak variance contribution can be attributed to an exponential broadening effect. Note that volume peak broadening contributions of about 80 pl for the 10 μm I.D. and about 20 pl for the 5 μm I.D. are the subject of this discussion.

The deconvolution methods that we used are not expected to give completely correct results when there is more than one significant exponential peak broadening in the system. Nevertheless, the use of these methods broadened our insight into the nature of the peak broadening processes in our system.

Other explanations for the deviations from theory are possible. The deviations can be explained in terms of retention or in terms of deviations from the stated inner diameters. However, retention of toluene in a phase system consisting of pure silica as the stationary phase and methanol as the mobile phase does not seem likely. Curve fitting (with $H = a + b/u + c^*u$) or linear regression ($H = a + c^*u$) was used to estimate the extent of deviation in the diameter necessary to explain the described effects. The deviations thus found, *viz.*, around 40% for the 10 μm I.D. and around 80% for the 5 μm I.D. capillaries, are not in agreement with other experiments with the same type of capillaries but using other detection principles[7,12].

Evaluation of the extra-column peak variance contributions showed an important feature, namely that the contribution appears to be flow dependent. As the peak variance contribution of the mixing chamber at the top of the DLI probe is expected to be independent of the column flow-rate, it must be the injection volume that makes the most significant contribution to peak variance. High splitting ratios (1:3000 with the 25 μm I.D. capillary and even 1:280,000 with the 5 μm I.D. capillaries) were used, but still the contribution to peak variance was too great. Therefore, it is expected that a reduction in the injection volume will give even better prospects for the interfacing of OT-LC and MS described here.

Further work on the estimation and calculation of various peak variance contributions in the extra-column region is still in progress.

Detection limits

In developing a detector system for OT-LC, two problems must be solved. First, we must meet the requirement of small extra-column peak variance, and second, the sensitivity must be high. So far we have focused our attention on the peak variance contributions. Sensitivity in DLI-MS is known to be greatly influenced by the composition of the CI reagent gas and by the distance of the probe from the ion source. Optimization of these parameters by adjusting the source pressure and temperature, the geometry of the desolvation chamber and the distance between the diaphragm and the ion source is currently in progress. For volatile solutes, such as toluene and aniline, we measured detection limits (based on a signal-to-noise ratio of three) of 1–10 pg, which seems low, but one must realize that concentration detection limits are of the order of 1–10 μg/ml. At least 1000-fold lower detection limits must be reached in order to make the coupling of OT-LC and MS by the proposed DLI interface really interesting for the complete analysis of complex mixtures.

CONCLUSION

The coupling of open-tubular liquid chromatography and mass spectrometry has been studied. To make OT-LC superior to packed column LC extra-column peak variance contributions must be reduced to below 1 nl. The results presented here show that this is possible with the proposed DLI interface. The next part of our work will be concerned with the detection limits. So far we have found absolute detection limits of about 10 pg (concentration detection limits of 10 μg/ml), with almost no system optimization in terms of ion-source pressure or temperature or distance of DLI probe and ion source. We expect to gain at least two orders of magnitude in detection limits. Further work is in progress.

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