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DIRECT ELECTRICALLY HEATED SPRAY DEVICE FOR A MOVING BELT LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY INTERFACE

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SUMMARY

A direct electrically heated capillary spray device for the deposition of liquid chromatographic eluents onto a moving belt interface for liquid chromatography–mass spectrometry (LC–MS) has been constructed. This system shows significant advantages over hot propellant gas driven or cartridge heated sprayers, especially when eluents with high water content and high flow-rates are used. Various parameters have been examined to achieve optimum conditions with respect to capillary wall temperature, peak variance and analyte yield during deposition. Reversed-phase high-performance liquid chromatographic separations with standard bore columns (4.6 mm I.D.) demonstrate the enhanced possibilities of the moving belt interface for on-line high-performance LC–MS.

INTRODUCTION

The advantages of the moving belt interface for the combination of liquid chromatography and mass spectrometry (LC–MS) have been reported[1–3]. Attempts to use soft ionization techniques like secondary-ion mass spectrometry (SIMS) and fast atom bombardment (FAB) have been made[4,5]. Transfer of column eluent to the surface of the moving belt with a heated gas spray device or a cartridge system results in reasonable transfer efficiency. Extra column band broadening has been shown to be low for these devices[6–8]. However, when used with reversed-phase high-performance liquid chromatography (RP-HPLC) and standard columns (4 or 4.6 mm I.D.), either the propellant gas or the heating cartridge had to be heated to temperatures as high as 300°C. In spite of these harsh conditions the maximum throughput of aqueous eluents has been generally limited to about 0.5 ml/min with mobile phases containing more than 50% water. Thus, in order to maintain a flow of 1–1.5 ml/min, required for rapid separation conditions, it has often been necessary to split off at least part of the eluent. Alternatively, for eluents with high water content, microbore columns can be used. These are not, however, always convenient, especially with standard instrumentation.

In order to overcome these problems, we constructed, with inexpensive and

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readily available parts, a simple but efficient direct electrically heated spray device. In this context, it is interesting to point out that recently a thermospray device was modified from cartridge to direct electrical heating for similar reasons[9].

EXPERIMENTAL

Mass spectrometry

A Finnigan (San Jose, CA, U.S.A.) 4021B mass spectrometer equipped with a moving belt LC–MS interface with Kapton® belts was used in all experiments. The mass spectrometer was operated either in the electron impact (EI) or chemical ionization (CI) (0.25 Torr NH_3 or 0.2 Torr CH_4) mode with a source temperature of 200 or 250°C. The original fixed speed motor of the belt drive mechanism was substituted with a variable-speed motor (Bodine, Chicago, IL, U.S.A.).

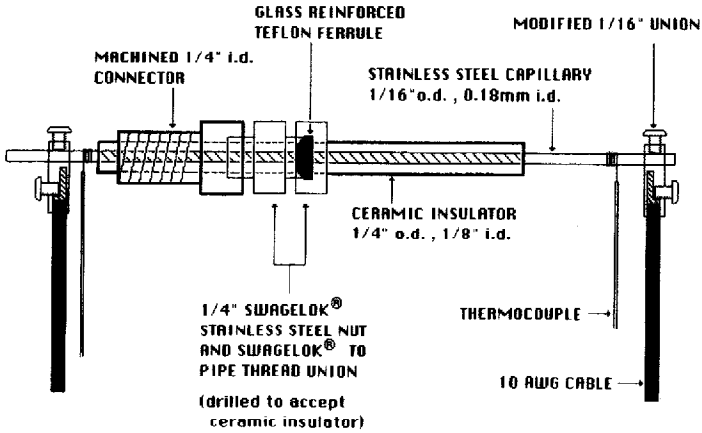


Fig. 1. Schematic diagram of the moving belt interface with direct electrically heated spray device. (Not drawn to scale).

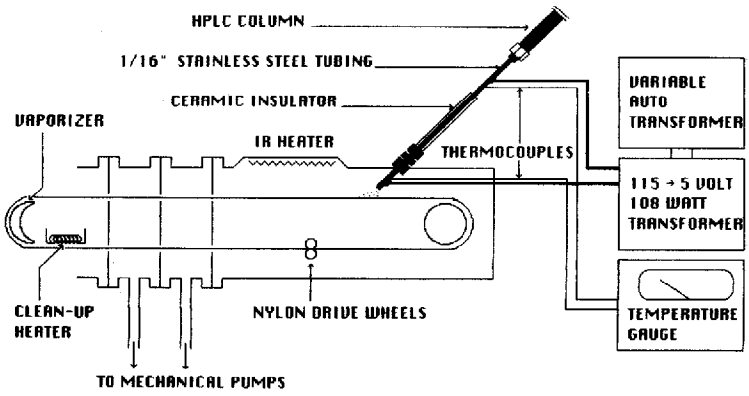


Fig. 2. Schematic diagram of the spray device.

Spray device

Schematic diagrams of the direct electrically heated spray device are shown in Figs. 1 and 2. A variable autotransformer, adjustable from 0 to 130 V a.c., is connected to the line voltage side of a high-power step down transformer (input 115 V, output 5 V, max. 100 W). The secondary side is connected via two 2.5 mm diameter insulated copper wires (*ca.* 30 cm long) to homemade clamps. The clamps are mounted to the 1/16 in. stainless-steel HPLC capillary (0.18 mm I.D.). In order to reduce the influence of ambient temperature fluctuations, the capillary, when connected to the belt housing via modified Swagelok® fittings, was shielded with a 15-cm ceramic insulator (1/4 in. O.D., 1/8 in. I.D.; Omega, Stamford, CT, U.S.A.).

A distance of 15–20 cm between the two clamps proved to cover all the necessary temperature ranges; for practical reasons a 0.5-cm portion at the tip of the capillary was not subjected to electrical heating. Thermocouples (iron/constantan) were attached at the two cable connectors, and the temperature was monitored by means of a thermocouple direct reading meter.

The temperature of the capillary was adjusted to produce a constant mist or a slightly wet spray by variation of the output voltage of the autotransformer.

It is important to emphasize that, in order to maintain chromatographic performance, it was critical to have a slightly wet spray. A dry spray invariably resulted in poor peak shape and sample loss. Wetness was checked off-line by means of a mirror placed at a distance of about 1 cm from the tip of the capillary and on-line by visual inspection of the produced spot on the belt. An optimum spray shape was achieved by flat-cut capillaries, whereas pointed tips tended to produce droplets. The optimum distance between sprayer tip and belt was 3–5 mm. Similar to previous investigations[8], an angle of 30–45° between capillary and belt resulted in optimum peak shape and amount of deposition. The solute transfer efficiency was determined by first spraying a peak from the chromatographic system onto the belt and then comparing this peak area with that obtained by direct deposition of the same amount of sample on the belt with a syringe. The syringe was assumed to provide a 100% transfer of solute to the belt.

HPLC

Liquid chromatography was performed with a Waters (Milford, MA, U.S.A.) M 6000A solvent delivery system, a Model 660 solvent programmer and a Rheodyne (Cotati, CA, U.S.A.) 7125 injection valve (20 μl loop). A Kratos (Ramsey, NJ, U.S.A.) Spectroflow 773 variable-wavelength UV detector was used at 210 and 254 nm for LC–UV studies. The columns used were Partisil ODS-3 (250 × 4.6 mm, I.D., particle size 5 μm) from Whatman (Clifton, NJ, U.S.A.) and Altex Ultrasphere Octyl (250 × 4.6 mm, I.D., particle size 5 μm) from Beckman (Berkeley, CA, U.S.A.). Xanthines and trichothecene-mycotoxins were purchased from Sigma (St. Louis, MO, U.S.A.), the pregnene-steroids from Steraloids (Wilton, NH, U.S.A.). Solvents were HPLC grade, purchased from Baker (Phillipsburg, NJ, U.S.A.).

RESULTS AND DISCUSSION

The goal of this work was to study the applicability of the spray device, especially for "critical" eluents containing salt and high amounts (> 50%) of water up

to flow-rates corresponding to normal-bore HPLC. For the purpose of this work, the following issues were considered of primary importance and were evaluated: (a) the contribution of the sprayer and the sprayer–belt combination to the chromatographic peak variance, (b) the transfer efficiency from the HPLC to the mass spectrometer, (c) the reproducibility of the deposition over long periods of time and (d) the stability of the MS high vacuum when polar solvents were sprayed on the belt.

To determine the second moment (variance) M_2 of the peak, we assumed an exponentially modified Gaussian shape and used the approximation formula (eqn. 1) of Foley and Dorsey[10]

$$M_2 = \frac{W_{0.1}^2}{1.764\,(B/A)^2 - 11.15\,(B/A) + 28} \quad (1)$$

where $W_{0.1}$ = peak width at 10% height, B/A = asymmetry factor at 10% height.

Off-line studies

Fig. 3 shows the relationship between eluent flow through the capillary and the wall temperature of the non-shielded capillary (15 cm between electrical connections) needed to obtain a slightly wet spray. For flow-rates between 0.2 and 2.0 ml/min the temperature near the outlet tip had to be maintained at or slightly above the actual boiling point of the mobile phase. This was accomplished by adjustment of the transformer voltage. In correct operation the maximum temperature at the tip never exceeded this value by more than 30°C. A typical sign for clogging of the capillary was, besides the increased back-pressure of the system, a much higher temperature necessary to produce an optimum spray (often 100°C above boiling point).

The selected examples of eluents and flow-rates cover the whole range of normal and reversed-phase separations with narrow bore (2 mm I.D.) and standard

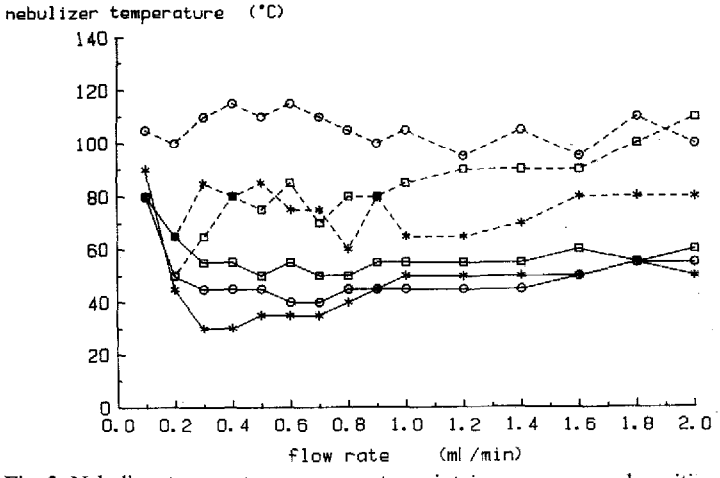


Fig. 3. Nebulizer temperature necessary to maintain proper spray deposition. (Measured 5 mm from the tip of a non-shielded 1/16 in. O.D., 0.18 mm I.D. stainless-steel capillary). (*—*) Dichloromethane, (○—○) hexane, (□—□) methanol, (*---*) methanol–water (1:1), (○---○) water, (□---□) 20% methanol containing 50 m*M* Na_2HPO_4.

columns (4–4.6 mm I.D.). Even the vaporization of a common buffer containing eluent (20% methanol, 50 m*M* disodium hydrogen phosphate) was readily accomplished. When the desired wet spray was produced, nearly no deposition of salt at the tip was observed. The spray at 10 mm distance from the tip had a diameter of about 3 mm. These were ideal dimensions for the deposition on the belt.

On-line measurements

Vacuum stability. Fig. 4 shows the result of a study where the capillary wall temperature of the nebulizer was varied to accommodate changes in the water content of the mobile phase. The high vacuum of the mass spectrometer was kept constant at $1 \cdot 10^{-6}$–$2 \cdot 10^{-6}$ Torr (a typical MS vacuum with the interface connected, but without LC eluent deposition is about $8 \cdot 10^{-7}$ Torr, EI mode). The result was a more or less linear relationship of heater temperature (measured at the tip of the spray) *versus* water content. The data again show that, with this approach, a fast and efficient vaporization can be performed at rather low temperatures. In addition, there was no formation of droplets on the belt and hence no pressure surges in the mass spectrometer due to incomplete evaporation of the solvent.

Peak variance. In order to determine the contribution of the different parts of the on-line chromatographic system to peak broadening, we measured the second moments (variances) of the weakly polar solute fluorene and the more polar caffeine. This measurement was made by injecting 2 μl of solutions of caffeine (380 ng/μl, belt vaporizer 225°C) and fluorene (100 ng/μl, belt vaporizer 155°C) via the chromatographic system with and without a column and comparing these results with those of the LC–UV combination (Table I). As we were particularly interested in the extra-column effects, the capacity factors (k') of both compounds were artificially low to emphasize the external peak broadening.

Table I shows that extra-column effects for fluorene, measured by flow injection, are in the order of 250 μl^2. This variance of the belt-MS detected peak is in conformity with earlier studies[1]. For fluorene the contribution of the LC–MS interface to peak broadening is, within the limits of experimental error, the same as that

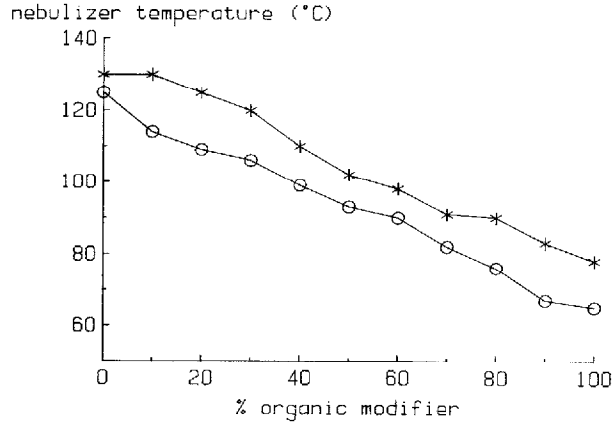


Fig. 4. Nebulizer temperature necessary to maintain MS-vacuum. Technique: flow injection; flow-rate: 1 ml/min; belt speed: 3 cm/min; high vacuum MS-source: $1 \cdot 10^{-6}$–$2 \cdot 10^{-6}$ Torr. (*) Acetonitrile–water, (○) methanol–water.

TABLE I

COMPARISON OF SECOND MOMENTS (VARIANCES) OF LC–MS AND LC–UV PEAKS

	Caffeine (μl^2)	*Fluorene* (μl^2)
Injection without column MS detection	920 ± 140*	250 ± 30**
Injection with column MS detection	3190 ± 470*,***	1890 ± 170**,§
Injection with column UV detection	2330 ± 80*,***	2220 ± 170**,§

* Eluent: 50% acetonitrile, 1 ml/min.
** Eluent: 100% acetonitrile, 1 ml/min.
*** k' value = 0.42.
§ k' value = 0.54.

of the UV detector. The caffeine peaks, however, appear broadened by LC–MS.

In order to localize the source of the increased variances for caffeine, we spotted a solution containing equal amounts of fluorene and caffeine on the belt via a hypodermic syringe. When the variance was measured in units of time, effectively the same values were observed for the two substances. Moreever, since the LC–UV values are identical, the experimental data in Table I suggest some discrimination in the spray deposition process as a function of analyte polarity, resulting in broader peaks for the more polar caffeine in LC–MS. This is supported in part by the experiment described below, where both compounds were analyzed using the same mobile phase, thus eliminating individual solvent polarity effects.

As the relative contribution of extracolumn effects to the total bandbroadening decreases with increasing k' values, the larger LC–MS peak width of the more polar compound, determined at the very low k' value of 0.4, can be tolerated in systems designed for real separations.

Belt speed. The influence of the belt speed on the variance was studied in a flow injection experiment. Fluorene and caffeine were coinjected and spray deposited at varying belt speeds (vaporizer temperature: 225°C); the results are shown in Fig.

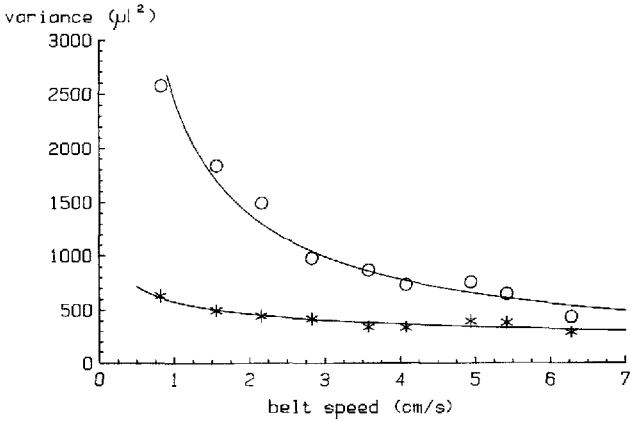


Fig. 5. Variance as a function of the belt speed. Technique: flow injection; eluent: 100% acetonitrile; flow-rate: 1 ml/min; detection via MS in EI mode. (*) Fluorene, (O) caffeine.

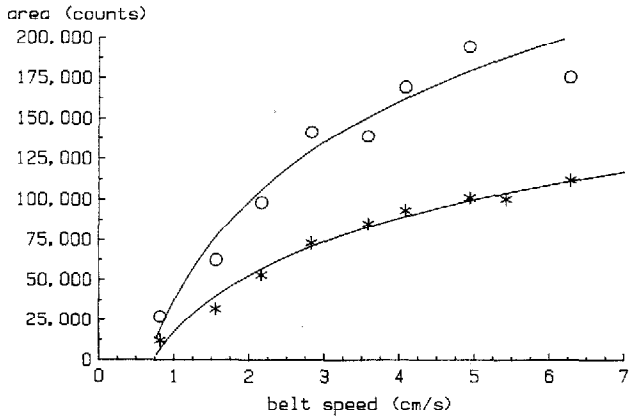


Fig. 6. Peak area as a function of the belt speed. Technique: flow injection; eluent: 100% acetonitrile; flow-rate: 1 ml/min; detection via MS in EI mode. (*) Fluorene, (O) caffeine.

5. It is apparent in this experiment that there is nearly no influence of belt speed on the performance of fluorene. On the other hand, for caffeine the peak width decreases dramatically as a function of belt velocity. When the belt speed operated was faster than 4 cm/s, the peaks were generally subjected to an increased asymmetry. For the typical operational belt speed of about 3 cm/s used in this work, the peak variances for these two compounds of widely varying polarity are still different. However, this was found to be generally acceptable, particularly when considering the high selectivity of the MS detector.

Transfer efficiency. The amount of substance detected increases with increasing belt speed in an apparently parabolic function (Fig. 6). The weaker signals seen at slower belt speeds are believed to be a result of the increase in time available for evaporation and thus loss of solvent and solute in the vacuum locks. Similar tendencies were reported previously with the gas driven nebulizer[8].

Critical to the transport process is the wetness of the eluent sprayed onto the belt. High water-containing solvents required a more careful adjustment of the spray

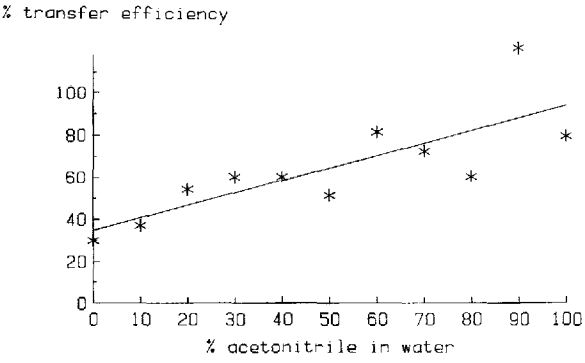


Fig. 7. Transfer efficiency of caffeine as a function of water content. Technique: flow injection; flow-rate: 1 ml/min; belt speed: 3 cm/min; amount: 500 ng each.

temperature. A very wet surface produced a maximum amount of deposition, but added additional tailing and peak shape problems.

Fig. 7 shows the result of an experiment, in which the transfer efficiency of caffeine as a function of water content was determined. With acetonitrile as eluent, nearly 100% of the injected compound could be transferred from the eluent stream

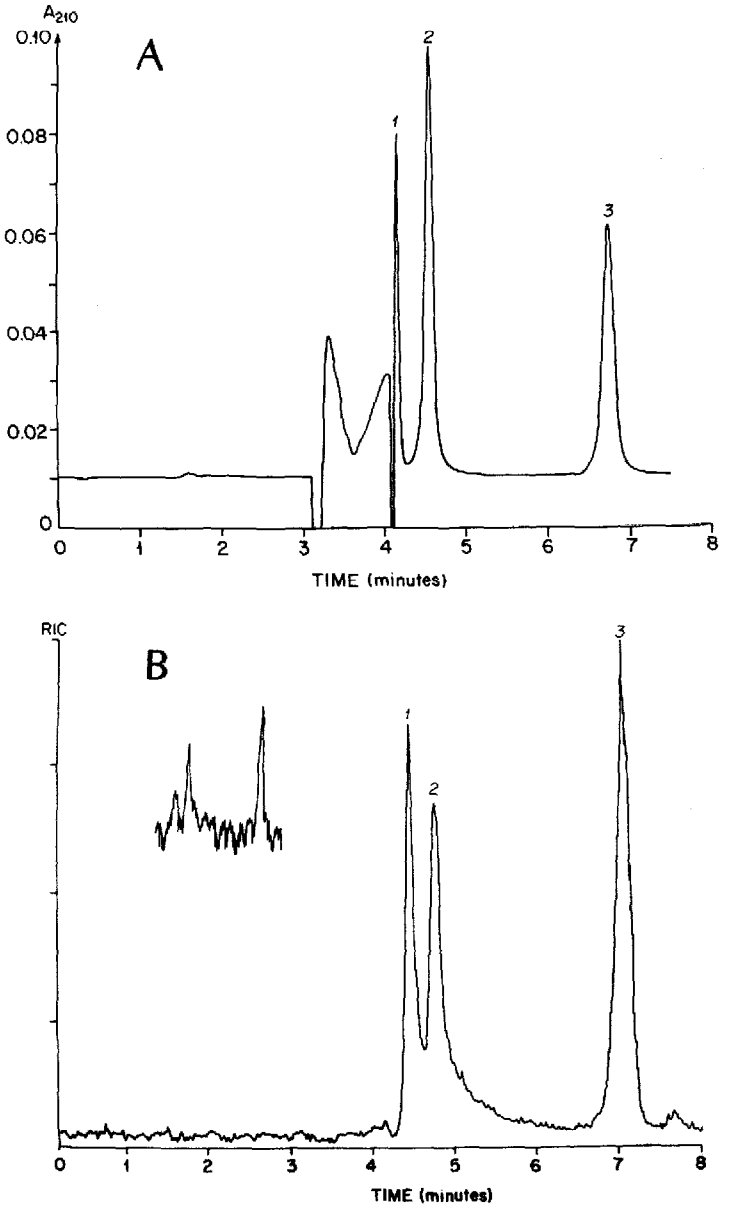


Fig. 8. HPLC separation of (3) caffeine, (2) theophylline and (1) theobromine. (A) LC–UV (210 nm); (B) LC–MS (EI–MID at *m/z* 181 and 194. Column: Partisil ODS3 5 μm; eluent: acetonitrile–water (20:80, v/v); flow-rate: 1 ml/min; amount: 100 ng each. Inset in Fig. 8B: 10 ng each.

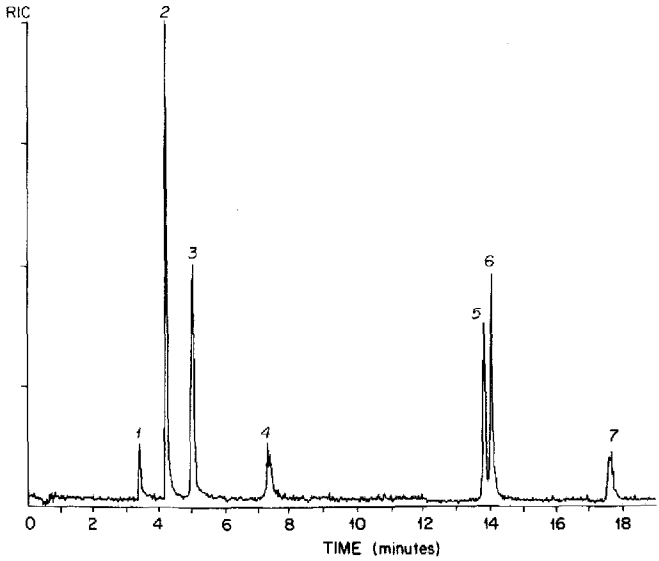


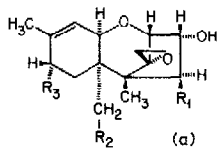
Fig. 9. HPLC-separation of trichothecene–mycotoxins. Column: Partisil ODS3 5 μm; solvent program: 6 min isocratic acetonitrile–water (20:80, v/v), 1 min gradient to acetonitrile–water (50:50, v/v), isocratic acetonitrile–water (50:50, v/v); flow-rate: 1 ml/min; scan range: 265–335 and 419–491 a.m.u.; NH_3-pos. CI; amount: 2 μg each. See Table II for compound listing.

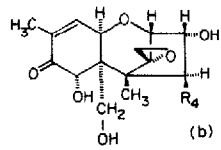
to the belt (compared to direct spotting on the belt). Even with pure water, 35% of the injected substance could be deposited with acceptable peak shape.

Reproducibility. Injections of single components to determine the reproducibility of the peaks were usually performed ten times. Reproducibilities of about 5–10% over long periods of time were achieved with any given solvent system following optimization of the spray conditions. However, changes in solvent composi-

TABLE II

STRUCTURES OF THE SEPARATED TRICHOTHECENES





Peak	*Core structure*	R_1	R_2	R_3	R_4
1 T-2 tetraol	a	OH	OH	OH	
2 Nivalenol	b				OH
3 Deoxynivalenol (DON)	b				H
4 Verrucarol	a	OH	OH	H	
5 4,15-Diacetoxyscirpenol (DAS)	a	$OCOCH_3$	$OCOCH_3$	H	
6 HT-2 toxin	a	OH	$OCOCH_3$	$OCOCH_2CH(CH_3)_2$	
7 T-2 toxin	a	$OCOCH_3$	$OCOCH_3$	$OCOCH_2CH(CH_3)_2$	

tion usually necessitated a reoptimization which affected the analyte recovery. These effects were particularly pronounced at high organic modifier content, where the acceptable range between a too dry and a too wet belt surface was broader, and are reflected in the poor correlation coefficient in the plot of Fig. 7. Thus, while gradients conducted at high organic modifier content may suffer from poorer reproducibility in sample recovery, this effect works to an advantage when operating at the normally more critical high water region of gradient LC.

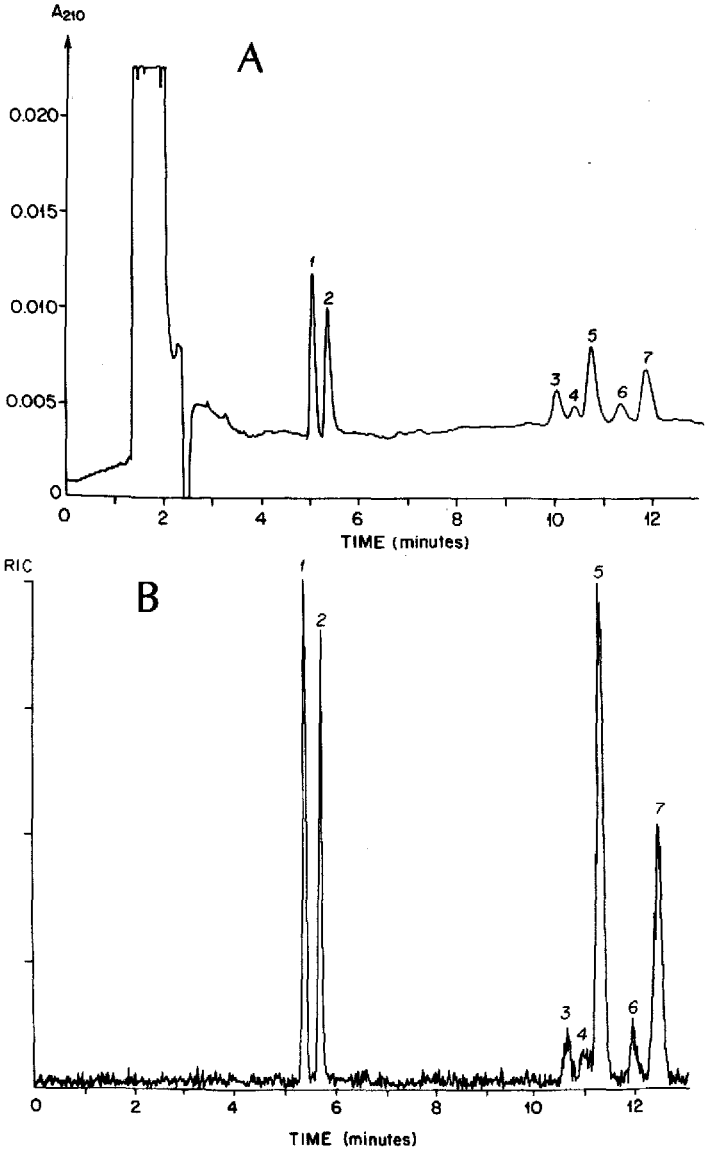


Fig. 10. HPLC-separation of pregnene steroids. (A) LC–UV (210 nm), (B) LC–MS (scan range: 310–337 a.m.u.); CH_4-pos. CI. Column: Ultrasphere octyl 5 μm; eluent: acetonitrile–water (62:38, v/v); flow-rate: 1 ml/min; amount: 40 ng each. See Table III for compound listing.

LC–MS applications

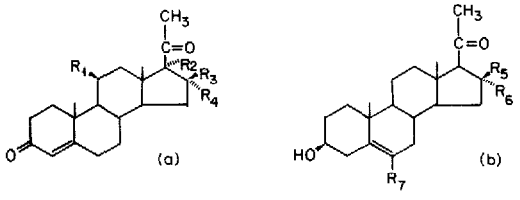
In order to demonstrate the applicability of the deposition system, we examined several chromatographic separations in the reversed-phase mode with different amounts of water in the eluents. Fig. 8 compares LC–MS with LC–UV analysis of theobromine, theophylline and caffeine, 100 ng each, at a flow-rate of 1 ml/min on a C_{18} column with acetonitrile–water (20:80, v/v). Whereas the UV diagram showed severe interference with system peaks, the MS system was free of any additional signals. Detection limits in the electron ionization multiple ion detection mode (EI–MID) were in the low nanogram range (inset in Fig. 8, 10 ng each).

A more difficult separation is shown in Fig. 9: trichothecene–mycotoxins of different polarity (Table II) were separated by means of a step gradient ranging from 20 to 50% acetonitrile. Detection was performed in the positive CI mode (0.25 Torr NH_3) with scanning in the mass ranges 265–335 and 419–491 a.m.u. The change in sprayer temperature in order to maintain optimum deposition and peak shape, regardless of the significant change in solvent composition could easily be performed manually. Compared to the UV-detected system, where a gradient in the low UV-range could not be performed, neither a drift in baseline nor a change in the noise level could be observed during the LC–MS run.

Finally, Fig. 10 demonstrates an application for steroids of the pregnene type (Table III). MS and UV chromatograms are again compared. Detection limits for the two detector systems are comparable in this case. However, the distinct difference in the ratios of the principal ion peaks of the LC–MS mode permit differentiation of these structurally similar compounds.

TABLE III

STRUCTURES OF THE SEPARATED PREGNENES



Peak	*Core structure*	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1 4-Pregnen-11β-ol-3,20-dione	a	OH	H	H	H			
2 4-Pregnen-17α-ol-3,20-dione	a	H	OH	H	H			
3 4-Pregnen-16α-methyl-3,20-dione	a	H	H	H	CH_3			
4 5-Pregnen-6-methyl-3β-ol-20-one	b					H	H	CH_3
5 5-Pregnen-16α-methyl-3β-ol-20-one	b					H	CH_3	H
6 4-Pregnen-16β-methyl-3,20-dione	a	H	H	CH_3	H			
7 5-Pregnen-16β-methyl-3β-ol-20-one	b					CH_3	H	H

CONCLUSIONS

This work has demonstrated that LC–MS via the moving belt interface can be easily performed at normal HPLC flow-rates even with eluents containing high amounts of water. It is demonstrated that the eluent of standard bore columns can be totally deposited on the belt, regardless the composition of the liquid. The capillary temperatures necessary to nebulize the eluent can be maintained slightly above the actual boiling point, thus minimizing thermal degradation of material. Due to the small mass being heated, the system reacts very fast to external adjustments in temperature and allows rapid optimization of the spray and even manual adjustment when solvent composition is changing. As the only "critical" component of the spray device, the capillary, is easily exchangeable, even clogging due to particles from the chromatographic packing and overheating has a minor influence on the efficiency of the system.

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