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BELT-SPEED PROGRAMMING, A NEW TECHNIQUE FOR PEAK COMPRESSION IN LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY AND SUPERCRITICAL-FLUID CHROMATOGRAPHY–MASS SPECTROMETRY WITH MOVING-BELT INTERFACES

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SUMMARY

The mass spectrometer is a mass-flow-sensitive detector. Improvement of the detection limit in target compound analysis can be obtained by increasing the analyte mass-flow into the spectrometer. This can be achieved by applying peak compression methods. Belt-speed programming is a peak compression method that can be applied with the moving-belt interface for liquid chromatography–mass spectrometry. Peak compression is obtained by increasing the belt speed after the deposition of a chromatographic peak on to the belt. This paper describes preliminary results of belt-speed programming in both liquid chromatography–mass spectrometry and supercritical-fluid chromatography–mass spectrometry, with the latter case being the most effective. At present, the gain in mass flow is limited by the belt speed range available with the interface and by the inefficiency of the thermal desorption process.

INTRODUCTION

Although combined liquid chromatography–mass spectrometry (LC–MS) is nowadays used on a routine basis, it still lacks the sensitivity normally achived in combined gas chromatography–mass spectrometry (GC–MS). In general, the detection limits in LC–MS are in the picogram to nanogram range, depending on the type of analyte, the instrumentation and the operating conditions[1]. Optimization of experimental parameters does not often result in sufficiently low detection limits. Therefore,

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other ways of improving the detection limits in target compound analysis are investigated.

Because the mass spectrometer is a mass-flow-sensitive detector, an improvement in detection limits can be obtained by means of an increase in the mass flow. One of the methods applicable in this respect is the phase-system switching (PSS) approach, which was introduced for the post-chromatographic elimination of non-volatile additives in mobile phases[2,3]. In applying the PSS method it is possible to obtain an increased mass-flow as a result of peak compression effects[4].

In this paper another method for increasing the mass-flow to the mass spectrometer is presented, the so-called belt-speed programming (BSP). In contrast to PSS this method can only be used with a moving-belt interface. In BSP the chromatographic peak of interest is deposited or collected on the belt at a very low belt speed, while the actual mass analysis is performed at high belt speed. After collection of the peak on the belt the belt speed is increased, resulting in an increased mass-flow. The BSP method can be applied in LC–MS, and also in combined supercritical-fluid chromatography–mass spectrometry (SFC–MS). Results from both LC–MS and packed-column SFC–MS are reported here.

THEORETICAL

In the discussion on the theoretical aspects of BSP a Gaussian peak shape has been assumed, despite the fact that the usual peak asymmetry of real LC peaks will also influence the peak heights and thereby the detection limits. A chromatographic peak can be characterized by the peak S.D. σ_v, the concentration of the analyte at the peak maximum C_{max}, and the retention volume V_r. Furthermore, it has been assumed that the moving-belt interface does not cause any additional band-broadening[5,6].

In a mass-flow-sensitive detector the signal is directly proportional to the mass-flow, which can be expressed as:

$$dm/dt = C(t)F$$

in which $C(t)$ is the concentration at the end of the column and F is the flow-rate. At the peak maximum, $C(t)$ is equal to the maximum concentration C_{max}. The moving-belt interface is based on solvent removal. Therefore, C_{max} is ill-defined and difficult to use in this case. The use of the peak standard deviation instead of C_{max} is very attractive and valid because of the inverse relation with C_{max}.

The initial σ_v (in units of volume) is transformed by the flow-rate F to σ_t (in units of time) at the outlet of the chromatographic system:

$$\sigma_t = \frac{\sigma_v}{F}$$

Deposition of the peak on the belt moving at a certain speed v_1 and the subsequent evaporation of the solvent gives a σ_z (in units of length):

$$\sigma_z = \sigma_t v_1 = \sigma_v \frac{v_1}{F}$$

If the analyte on the belt is introduced into the spectrometer without changing the belt speed v_1, the σ_t is:

$$\sigma_{t,MS} = \frac{\sigma_z}{v_1} = \sigma_t = \frac{\sigma_v}{F}$$

If, however, the belt speed is increased to v_2 prior to introduction of the analyte into the MS, the σ_t becomes:

$$\sigma_{t,MS} = \frac{\sigma_z}{v_2} = \sigma_t = \frac{v_1}{v_2} = \sigma_v \frac{v_1}{v_2 F}$$

It can be seen that the effect of the belt-speed programming is a reduction of the peak standard deviation. As a result of the inverse relationship between C_{max} and σ this leads to an increase of the mass flow.

EXPERIMENTAL

Liquid chromatography

The HPLC system consisted of a Model 2150 HPLC pump (LKB, Bromma, Sweden) and a Model 7125 injector (Rheodyne, Berkeley, CA, U.S.A.), and was used in flow injection mode, *i.e.* direct injection in a liquid stream without a chromatographic column. The mobile phase was analytical grade methanol (Merck, Darmstadt, F.R.G.) with a flow-rate of 0.2 ml/min unless stated otherwise. Cholesterol (30 ng) was used as a test compound by injecting 20 μl of a solution containing 1.5 ng/μl of cholesterol. The solvent was deposited on the belt with the standard deposition needle.

Two series of experiments were performed, *i.e.* reference measurements at a constant belt speed for depositing and mass analysis, and the actual BSP measurements.

Supercritical-fluid chromatography

The laboratory-built SFC system consisted of slightly modified commercially available modules. A detailed description of the SFC instrument is given elsewhere[7]. SFC was performed with a Rosil C_{18} packed column (150 mm × 4.6 mm I.D., 8-μm packing) and a mobile phase consisting of carbon dioxide modified with 2% analytical grade methanol (Merck) at a flow-rate of 2 ml/min unless stated otherwise. Diuron (62.5 ng) was used as a test compound by injecting 5 μl of a solution containing 12.5 ng/μl of diuron. A crimped stainless-steel capillary was used as a restrictor for the SFC system and as a spray device for mobile phase deposition. Freezing of the restrictor owing to expansion of the supercritical fluid is prevented by installing a small heating element at the tip[8].

Two series of experiments were performed, *i.e.* reference measurements at a constant belt speed for depositing and mass analysis, and the actual BSP measurements.

Mass spectrometry

The system used was a H-SQ 30 Hybrid (BEQQ) mass spectrometer (Finnigan MAT, Bremen, F.R.G.) linked to a SS-300 data system and equipped with a moving-belt interface (Finnigan MAT). The source temperature was 200°C.

The control electronics of the moving-belt interface used in this study only supports belt speeds between 2.0 and 4.5 cm/s. Theoretically this will give a maximal gain in mass flow of a factor of 2.2, which for validation of the BSP method is rather low. Therefore, it was desirable to increase the belt-speed range available on the moving-belt interface. By installing a variable resistor in the belt-speed control electronics, the belt speed could be regulated continuously between 1.0 and 4.5 cm/s.

For the HPLC experiments with cholesterol the spectrometer was operated in electron impact mode (EI, 70 eV). The operating conditions of the moving-belt interface were: solvent evaporator temperature, 150°C; sample evaporator setting, 5.

For the SFC experiments with diuron the spectrometer was operated in EI mode 70 eV). The operating conditions of the moving belt interface were: solvent evaporator temperature, 60°C; sample evaporator setting, 5.

Detection was performed in both cases in MID mode (resolution 1000), on masses m/z 368 and m/z 386 for cholesterol or m/z 232 for diuron.

Data evaluation

For the interpretation of the results, $\sigma_{t,MS}$ values are approximated by means of the area method:

$$\sigma_{t,MS} = \frac{A}{h\sqrt{2\pi}}$$

where A *and* h are the peak area and the peak height, respectively[9]. The accuracy of this approximation depends on peak asymmetry[9], but is nevertheless satisfactory for this study.

For the evaluation of the BSP results, the ratios of the peak areas, the peak heights and the peak S.D. as obtained with and without BSP are compared with the ratio of the belt speeds used in BSP. Theoretically a ratio of peak areas of 1.0 is expected, while the ratio of peak heights with and without BSP is equal to the ratio of the belt speeds v_2 and v_1 in BSP, and the ratio of peak S.D. with and without BSP is equal to the ratio of the belt speeds v_1 and v_2.

RESULTS AND DISCUSSION

Moving-belt interface characteristics

By calibration of the belt speed it was found that the actual belt speed can be regulated between 0.6 to 3.9 cm/s instead of the indicated 1.0–4.5 cm/s. Since belt speeds in the range 0.6–1 cm/s frequently lead to irregular belt speeds and even stopping of the belt, because the drive mechanism was not designed for such low belt speeds, those belt speeds cannot be used reliably. Actual belt speeds will be indicated in the text from now on.

The deposition of a typical reversed-phase mobile phase, *e.g.* 50% methanol in water, with a flow-rate of 0.6-1.0 ml/min on a moving-belt running at a low belt speed,

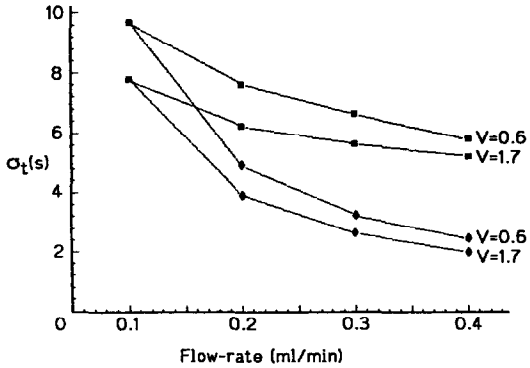

Fig. 1. Influence of the flow-rate on the peak S.D. (σ_t) at belt speeds of 0.6 and 1.7 cm/s, with (■) the experimental and (◆) the theoretical values, as calculated from the peak S.D. at a flow-rate of 0.1 ml/min.

will give problems with respect to the evaporation of the mobile phase and will often lead to a deteriorated peak shape. For BSP the belt speed for deposition will preferably be in the range 0.6–1.5 cm/s. In order to study the influence of the flow-rate on the peak S.D. at low belt speeds the flow-rate was varied in the range 0.1–0.4 ml/min at belt speeds of 0.6 and 1.7 cm/s, with a solvent evaporator temperature of 100°C. Fig. 1. shows the experimental and theoretical curves for these experiments. The results indicate that a high flow-rate, relative to the belt speed, causes significant additional band-broadening, which is probably due to a reduced storage capacity of the belt, resulting in severe back-mixing at the deposition needle. It has been observed that this back-mixing effect is negligible when a spray deposition device is used instead of the needle deposition device[10].

Another important factor determining the interface performance is the desorption of the analyte as a function of the belt speed at a given flow-rate and sample evaporator setting. As a measure of the desorption efficiency, the peak heights and areas as calculated by the data system were used. The result of these experiments, shown in Fig. 2, agrees with theory. There is an almost linear relationship, with a negative slope, between belt speed and the mass flow entering the ion source. These results seem to be unfavourable for a successful application of the BSP method.

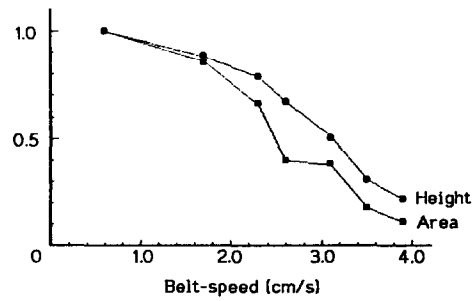

Fig. 2. Normalized (■) peak area and (●) peak height for cholesterol (*m/z* 386) as a function of the belt speed (methanol, flow-rate 0.6 ml/min, sample evaporator setting 5, and solvent evaporator temperature 100°C).

Belt-speed programming

In order to accomplish a successful BSP experiment three important parameters have to be known: (1) the retention time of the compound; (2) the width (in units of time) of the chromatographic peak; (3) the transport time of the chromatographic peak to the mass spectrometer at the deposition belt speed. Proper collection of peaks is possible only when the total peak width is smaller then the tranport time of the chromatographic peak. Otherwise, the front of the peak will enter the spectrometer before belt-speed programming is applied.

Demonstrating BSP in combination with HPLC and SFC, Figs. 3 and 4 show the mass chromatograms that have been obtained with either a constant belt speed or BSP for HPLC and SFC, respectively. The improvements in peak heights and peak S.D. obtained with BSP in LC and in SFC are summarized in Table I and II.

By comparing the ratio of belt speeds (v_2/v_1) with that of peak S.D. (σ_1/σ_2), the conclusion can be drawn that, with respect to peak S.D., experimental values in BSP agree with the theory described above. With respect to peak heights, the agreement between theoretical and experimental values is less accurate. The deviations observed can be attributed to a decrease of the desorption efficiency at higher belt speeds (see below). This can be concluded from the fact that the observed ratios of peak areas (A_2/A_1) lie between 0.6 and 0.7, whereas theoretically, a value of 1.0 is predicted: a signal loss of *ca.* 30% is occurring. Correcting the observed peak heights for this loss [see column $(h_2/h_1)_{corr}$ in Tables I and, II], assuming a linear relation between peak height and area, yields ratios of peak heights that are almost identical with the ratios of peak S.D.

Signal losses of *ca.* 80% are observed at high belt speeds (*i.e.* 4.0 cm/s), when the influence of the belt speed on the signal at a constant flow-rate and a constant sample

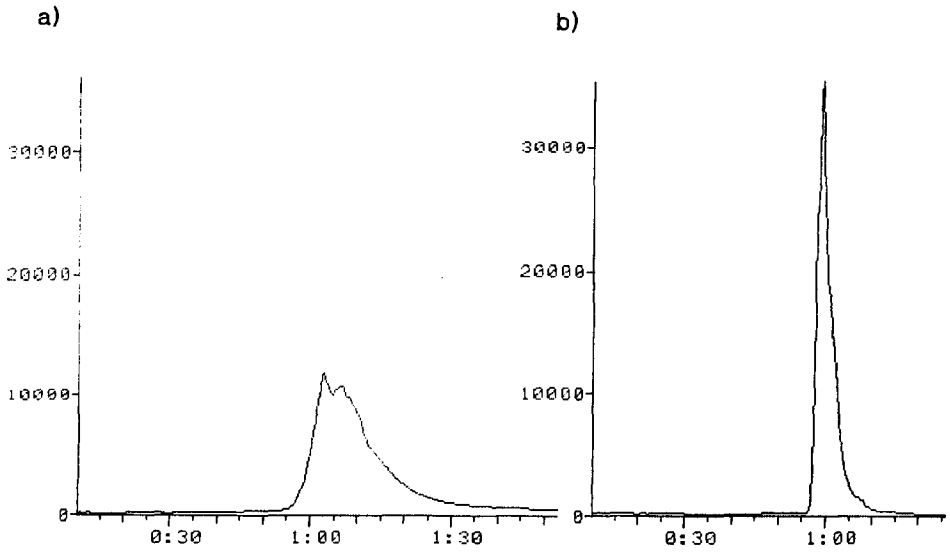

Fig. 3. Mass chromatograms from LC/MS of cholesterol (*m/z* 386). (a) Normal operation with belt speed 1.2 cm/s; (b) belt-speed programming with deposition rate 1.2 cm/s and desorption rate 4.0 cm/s.

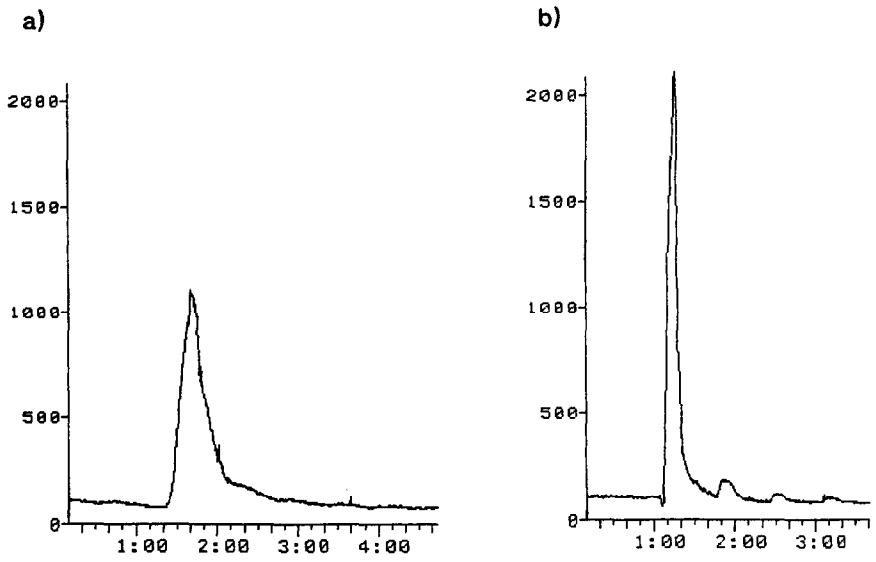

Fig. 4. Mass chromatograms from SFC–MS of diuron (*m/z* 232). (a) Normal operation with belt speed 3.6 cm/s; (b) belt-speed programming with deposition rate 1.2 cm/s and desorption rate 3.6 cm/s.

evaporator temperature is studied. This signal loss at higher belt speed can be attributed to a decreased desorption efficiency. The chromatographic peak is spread over a rather large belt area containing, relatively, more active sites on the belt material. In order to minimize these losses it is necessary to apply higher sample evaporator temperatures. However, higher sample evaporator temperatures cannot be used at low belt speeds, because they lead to destruction of the belt. As a result the BSP experiments are performed at values of the sample evaporator temperature that are lower than the optimum values at the belt speed of desorption. Owing to a long response time of the sample evaporation heater, there was no improvement when the

TABLE I
SUMMARY OF THE RESULTS OF BSP IN COMBINATION WITH HPLC

v (cm/s)	Area ($/10^5$)	Height ($/10^4$)	σ(s)
1.2	1.6	1.2	5.6
1.2–>4.0	1.3	3.2	1.4
1.7	2.1	1.7	4.9
1.7–>4.0	1.3	3.0	1.7

v_1–>v_2 (cm/s)	v_2/v_1	A_2/A_1	h_2/h_1	σ_1/σ_2	$(h_2/h_1)_{corr}$
1.2–>4.0	3.3	0.7	2.8	4.0	4.0
1.7–>4.0	2.4	0.6	1.8	2.9	3.0

TABLE II
SUMMARY OF THE RESULTS OF BSP IN COMBINATION WITH SFC

v (cm/s)	*Area (/10⁴)*	*Height (/10³)*	*σ(s)*
3.6	2.1	1.1	8.1
1.2–>3.6	1.5	2.0	2.9

v_1–>v_2 *(cm/s)*	v_2/v_1	A_2/A_1	h_2/h_1	σ_1/σ_2	$(h_2/h_1)_{corr}$
1.2–>3.6	3.0	0.7	1.9	2.8	2.7

sample evaporator setting was changed to higher values at the same time as the change in belt speed. The losses due to insufficient desorption are less in BSP than in an experiment with constant belt speed. The main difference between BSP and the constant speed experiment (as described above) is that in the latter the chromatographic peak is spread over a rather large belt area, while in BSP it is deposited on only a small area of the belt, containing fewer active sites.

Another unsolved limitation is the belt-speed range supported by the moving-belt interface. The maximum gain in mass flow that can be obtained is about a factor of 4 when the desorption is complete. Increasing this gain requires a wider range of belt speed, *i.e.* 0.5–10.0 cm/s. A belt speed of 10.0 cm/s can be used successfully only when the desorption process is significantly improved. Also the belt speed at the lower end is of great importance because it not only effects the gain in mass-flow but also the maximum time for peak collection, which at present is *ca.* 60 s. At this point it is interesting to compare results from HPLC and SFC. As a result of the differences in the volatilities of the mobile phases, the flow-rate and the peak deposition belt speed are strongly related in HPLC, whereas this is not the case in SFC. Very low deposition belt speeds are only interesting in the case of SFC. It should even be possible, at low modifier contents of the supercritical mobile phase, to collect the chromatographic peak in SFC with zero belt speed. Assuming that it is possible to collect a chromatographic peak with a total width of 20 s on 1 cm of the belt, desorption and introduction into the spectrometer at a belt speed of 5.0 cm/s will result in a peak width of 20 ms in the spectrometer. In terms of mass flow this example represents a gain of 100. As indicated above, other means of desorption than presently available on the interface are necessary to perform an experiment with zero belt speed.

A factor of great importance for quantitative analysis is the reproducibility and linearity of the BSP method. The variance in peak height and area between repeated experiments are comparable with those obtained under normal operation of the moving-belt interface. The linearity of the method has not yet been investigated, but it is expected to be similar to that of normal operation of the moving-belt interface[12].

CONCLUSION

It is demonstrated that belt-speed programming is capable of improving the sensitivity in LC–MS and SFC–MS, although the gain is not as great as expected

theoretically. The major reasons are the incomplete desorption of the compound at low sample evaporator temperatures and the slow response of the sample evaporator to a change in the settings. For useful application of BSP, a desorption method with an instantaneous response to a change in the desired desorption power is required. Laser desorption and fast atom bombardment are two techniques that meet this requirement.

Another conclusion is that with the available moving-belt interface the gain in mass flow is limited because of the limited belt-speed range. This is true even if the moving-belt interface is adapted to support very low (*ca.* 0.5 cm/s or less) and high (greater than 5.0 cm–s) belt speeds. This is especially the case for BSP in combination with HPLC because the flow-rates in conventional HPLC are incompatible with very low belt speeds. For SFC, such a limitation is not present and consequently a large gain in mass flow can be obtained with BSP.

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