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High-temperature open tubular liquid chromatography coupled to atmospheric pressure chemical ionisation mass spectrometry

Lena M. Nyholm, Per J.R. Sjöberg, Karin E. Markides*

Department of Analytical Chemistry, Uppsala University, P.O. Box 531, S-751 21 Uppsala, Sweden

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Abstract

A micro heated nebuliser interface was constructed and used for coupling of open tubular liquid chromatography at 150°C to atmospheric pressure chemical ionisation mass spectrometry. For the chromatography, 50 μm I.D. columns were utilised at a flow-rate of 0.1–1.6 μl/min. The efficiency loss due to the MS detector, including the interface, was found to correspond to only 5% for a 10-m column. The MS detector was shown to be mass flow sensitive at these low flow-rates, with a detection limit of 3 pg/s for 7,8-benzoquinoline. This corresponds to a concentration of 1 μ*M* using a flow-rate of 1 μl/min. In addition, the use of a temperature gradient in combination with MS detection is demonstrated. Important parameters for the performance of the interface such as temperature, orifice potential and organic modifier, as well as the choice of buffer salt in the mobile phase are also discussed. The MS detector was used to study the stability of the thermally labile compound bromocriptine in high-temperature chromatography. The experiments suggest that even thermally labile analytes can be separated with high-temperature open tubular liquid chromatography.

Keywords: Interfaces; Temperature gradients; Bromocriptine; Aromatic compounds

1. Introduction

Liquid chromatography is a very selective separation technique with possibilities to alter the selectivity through the use of a large number of mobile phases. When open tubular columns are used, the inherent selectivity of liquid chromatography can be combined with high efficiency, as described by Golay in his classic paper [1]. To realise these efficiencies the column, however, needs to be very narrow, only a few micrometers in diameter [2], due to the low diffusion coefficients of analytes in liquids. The value of the diffusion coefficients can be increased if the separation is performed at high temperature [3]. The theoretical advantages of high-temperature open tubular liquid chromatography (HT-OT–LC) have been discussed by Erni [4], and were verified experimentally by Liu et al. [5] and Takeuchi et. al. [6]. By increasing the temperature wider columns can be used while maintaining high efficiencies. Moreover, the analysis time is shortened while the sensitivity is improved. Separations showing one million plates, in less than one hour, have, for example, been demonstrated at 200°C for 50 μm I.D. columns [5].

The high-temperature conditions used in reversed-phase HT-OT–LC, i.e., water–methanol or water–acetonitrile mobile phases at temperatures up to 200°C, have unfortunately proved harmful to the stationary phase in the column [7]. While the stabili-

*Corresponding author.

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ty of open tubular columns for HT-OT–LC recently has been studied and also improved [8], the question regarding the stability of the solutes has not yet been fully addressed. The UV detector most often used does not give information about changes in the structure of the solute, unless dramatic degradation of the solutes occurs. Such information could, however, be obtained by coupling the HT-OT–LC system to an MS detector. The intensity of the quasi-molecular ion of the analyte in the mass spectra could then be monitored as a function of the temperature in the chromatographic system.

A more important reason for coupling HT-OT–LC to a mass spectrometer is to provide a tool for sample identification which is not only based on the comparisons of retention times with those of standard substances. Atmospheric pressure chemical ionisation (APCI), introduced by Horning et al. [9], is a suitable technique for coupling of HT-OT–LC to MS detection as it is a soft ionisation technique and is better suited for the hydrophobic compounds used in HT-OT–LC than, for example, electrospray (ES) [10]. While conventional LC has been interfaced to APCI [11,12] using flow-rates of 0.1 to 1 ml/min, we are, not aware of any coupling of open tubular LC to APCI–MS using flow-rates below 1 μl/min. Although APCI is considered a soft ionisation technique, heat is required for the vaporisation of the mobile phase. This heat could be harmful to thermally labile compounds which could make a study of the thermal degradation in the HT-OT–LC system more difficult. ES, on the other hand, does not require the application of heat and would probably cause less degradation for thermally labile compounds. ES is, unfortunately, not suitable for ionisation of the hydrophobic compounds best suited for HT-OT–LC, as it is best used with ions and polar compounds [10].

An additional advantage of coupling HT-OT–LC to an MS detector is that the use of temperature gradients is facilitated. Temperature gradients can easily be performed in the narrow open tubular columns used in HT-OT–LC and is an interesting alternative to solvent gradients [13]. Temperature gradients in LC coupled to MS detection were, for example, proposed by Yoo et al. [14] as a way to minimise problems associated with changes in the mobile phase viscosity and surface tension as a result of alterations in the composition of the mobile phase. This approach relies on the fact that the temperature in the transfer line between the column and the MS is kept constant. The mass-to-charge ratios (m/z) and the abundance of fragment ions can also be affected by changes in the mobile phase composition [14].

In this paper, an interface for coupling HT-OT–LC to APCI–MS at flow-rates between 0.1 and 1.6 μl/min is described. The interface is characterised with respect to extra column peak broadening, linearity, detection limit and flow dependence by considering the interface, ion source and mass detector as a detector unit. The coupling of HT-OT–LC to APCI–MS is used to study the possibilities of utilising temperature gradients in combination with MS detection without affecting the ionisation conditions. The stability of the thermally labile bromocriptine in the high-temperature chromatographic system is also studied.

2. Experimental

2.1. HT-OT–LC instrumentation

An LC pump, 2150, LKB (Pharmacia, Uppsala, Sweden), was used to deliver the flow, while injections were made with an injection valve, C6W Valco Instruments (Houston, TX, USA), equipped with a 10-μl sample loop and a 30 cm×0.25 cm I.D. stainless steel preheating tube. The preheating tube was heated separately in a laboratory-made oven. A 1/16-inch tee, 0.01-inch bore from Valco and an ss-4R3 A-EP valve (Nupro, Willoughby, OH, USA) were used to split the flow in a ratio of 1:1000. The columns were prepared from open tubular fused-silica capillaries, 50 μm I.D.×190 μm O.D. (Polymicro Technologies, Phoenix, USA). The column and the split tee were placed in a temperature programmable SFC oven, Lee Scientific Series 600 SFC/GC (Dionex, Sunnyvale, CA, USA). A 1.5 m×15 μm I.D. (145 μm O.D.) deactivated [15] fused-silica capillary (Polymicro Technologies) was coupled to the end of the column both as a restrictor, (i.e., to maintain the mobile phase as a liquid at high temperatures) and as a transfer line to the MS. The coupling was made in the chromatographic oven with a zero dead volume connection made in a

union, (SGE, Austin, TX, USA) using a single graphitised vespel ferrule, as illustrated in Fig. 1a. The transfer line was then inserted into the ion source probe of the MS. A UV, μPeak monitor, Pharmacia (Uppsala, Sweden) based on the use of fibre optics was used for on-column detection. The UV detector cell [16] was placed in the oven as depicted in Fig. 1a. Optical fibres with a diameter of 200 μm (Polymicro Technologies), were employed for the detection.

2.2. MS interface

The interface used for coupling of high-temperature open tubular LC to APCI–MS is depicted in Fig. 1b. The interface was based on a previously published supercritical fluid chromatography–MS interface [17] which was further modified to allow a more efficient vaporisation of the liquid mobile phase prior to the ionisation. The transfer line tip was positioned approximately 0.5 mm outside the 250 μm I.D. fused-silica nebulisation capillary, through which there was a flow of 0.3 l/min of nebulisation gas (synthetic air FID quality, AGA, Stockholm, Sweden, unless stated otherwise). Surrounding the nebulisation capillary there was a larger, 700 μm I.D.×850 μm O.D., fused-silica capillary, which was holding a heating wire coil to which a controlled voltage could be applied with a laboratory-made device. Between the nebulisation capillary and the wider capillary there was a flow of auxiliary gas, synthetic air FID quality, (AGA, Stockholm, Sweden) at a flow-rate of 0.5 l/min. The protecting polyimide layer was burned off the tips of

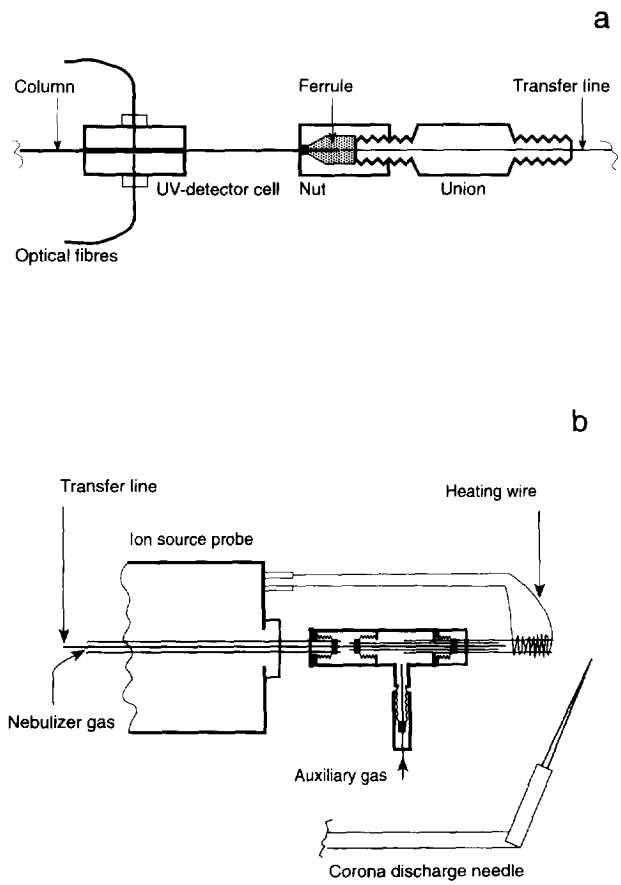

Fig. 1. (a) The on-column UV detector, positioned 6 cm from the column outlet, and the connection between the column and the transfer line. (b) The APCI-MS-interface.

the fused-silica capillaries entering the interface since disturbances from the polyimide otherwise can occur as described by Thomas et al. [18]. The ion source probe was directed 5 mm off axis relative to the orifice, to avoid injection of solvent vapour into the orifice. The ion source probe was either cooled to the ambient temperature, i.e. ca. 25°C, or heated to a pre-set constant temperature. This allowed temperature gradients to be run without affecting the conditions in the mass spectrometer.

2.3. MS

The interface was coupled to a triple quadrupole Sciex API III$^+$ mass spectrometer equipped with a point to plane corona discharge ion source. The discharge current was 1–2 μA and the potential was set to 650 V at the interface plate, 35 V at the orifice (unless stated otherwise) and 30 V at the entrance quadrupole. The curtain gas, dry nitrogen of 99.9999% purity (6.0 AGA), was heated to 50°C. The collision gas was Argon 99.9999% pure (6.0 AGA).

2.4. Chemicals

Methanol of HPLC gradient grade was supplied by Merck (Darmstadt, Germany) while acetophenone was obtained from Fluka (Buchs, Switzerland). The polycyclic aromatic compounds were kindly donated by Dr. Milton Lee, Brigham Young University, UT, USA. Bromocriptine was a kind gift from Sandoz, Basle, Switzerland.

2.5. Chromatographic conditions

The 1.5 m×50 μm I.D. open tubular fused-silica column used was deactivated with cyanopropylhydrosiloxane according to the procedure described by Markides et al. [15]. The column was then coated with 49% *n*-octyl-, 1% *n*-octenylpolymethylsiloxane stationary phase of a film thickness of 0.4 μm and dynamically crosslinked with 5.0% dicumylperoxide and azo-*tert*.-butane [8]. The mobile phase used was a mixture of methanol–water (3:7), unless stated otherwise, and the temperatures used for the separations ranged from 25°C to 150°C. Linear temperature gradients were performed in two steps, 50°C to 120°C at a rate of 10°C/min, followed by 120°C to 150°C at a rate or 30°C/min. The temperature was, finally, held at 150°C for 10 min before cooling the oven to 50°C prior to the next run.

2.6. Linearity studies

To study the linearity of the MS detector, injections of a large sample volume, 50 μl, were performed and the signal was measured and corrected for the background signal. In these experiments, an untreated fused-silica capillary, 65 cm×50 μm I.D., was employed as the column. No splitting of the flow was used. To minimise variations in the signal due to flow-rate variations, the flow-rate was kept constant at 1.0 μl/min using an HPLC pump PU 980 (Jasco, Tokyo, Japan) operated at constant pressure. The sample employed, 7,8-benzoquinoline, was dissolved in the mobile phase, i.e., methanol–water (8:2). Selected ion monitoring (SIM) was employed to measure the intensities of *m/z* 180 corresponding to the $[M+H]^+$ ion, and the isotopic ions, *m/z* 181 and 182, respectively. Multiple reaction monitoring (MRM) was performed by selecting *m/z* 180 for fragmentation followed by monitoring of the two most abundant ions at *m/z* 152 and 127, respectively. The collision energy was 35 eV and the collision gas thickness was $220 \cdot 10^{13}$ molecules/cm^2.

3. Results and discussion

3.1. Peak broadening

To study the peak broadening due to the interface, the UV detector was coupled on-column and positioned 6 cm from the end of the column, prior to the LC–MS interface. The peak broadening due to the transfer line and MS detector could then be evaluated by comparing the peaks obtained with the UV and MS detectors. The UV and MS detector responses for 2 ng 7,8-benzoquinoline using an untreated fused-silica capillary as the column are compared in Fig. 2. The difference between the retention times in the two detectors corresponds to the delay time in the transfer line.

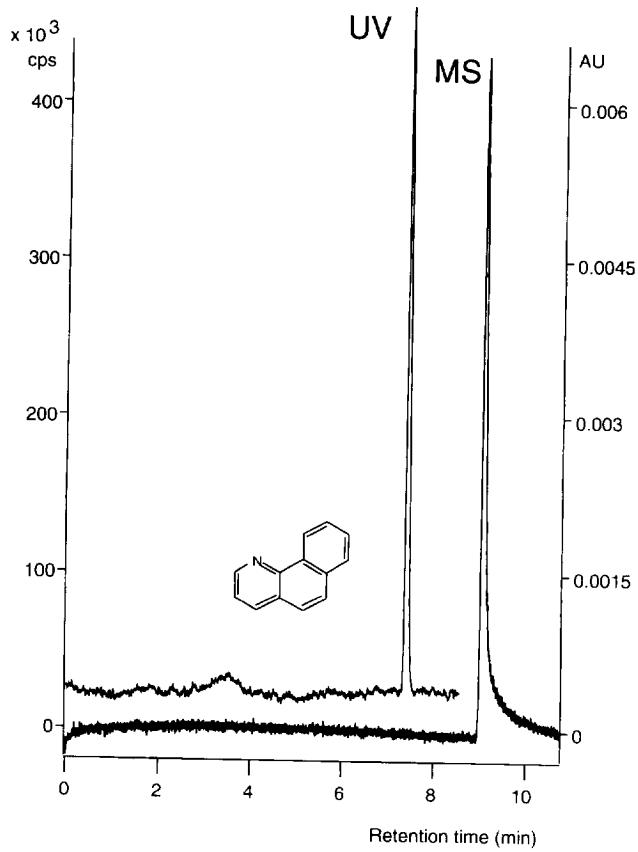

Fig. 2. Comparison of UV and MS detector responses for an injection of 2 ng 7,8-benzoquinoline. Conditions: oven temperature, 150°C (isothermal); methanol–water (3:7) mobile phase; flow-rate, 0.45 μl/min; UV detection wavelength, 254 nm; MS–SIM, m/z 180.

The contribution to peak broadening from the interface was found to be 12 nl ($n=14$, R.S.D.= 17%) at flow-rates between 0.15 to 1.6 μl/min. This corresponds to an efficiency loss of less than 5% for an unretained compound using a 10-m column, at a flow-rate of 1 μl/min and a temperature of 150°C. The corresponding loss for a retained compound will be even lower. The contribution from the interface was calculated from Eq. (1) [19], where σ^2_{MS} and σ^2_{UV} denote the peak variances in the MS and UV detector, respectively.

$$\sigma_{interface} = \sqrt{\sigma^2_{MS} - \sigma^2_{UV}} \quad (1)$$

The on-column UV detector was considered not to contribute to the extra column peak broadening as the detector cell volume was less than 0.4 nl. The theoretical peak broadening was calculated from the Golay equation [1] using a diffusion coefficient of $4 \cdot 10^{-9}$ m^2/s [13].

The efficiency losses for a slightly retained solute utilising a significantly shorter column, 1.5 m, at different flow-rates are summarised in Table 1. The smallest loss, about 10%, was seen for a flow-rate of about 1.3 ml/min. The loss of efficiency for this column is larger than the 5% calculated for an

Table 1
Efficiency loss as a function of flow-rate

F (μl/min)	Efficiency loss (%)	n	R.S.D. (%)
0.2	26	3	10
0.6	20	2	20
1.1	17	3	1
1.3	10	2	1
1.6	17	3	30

Conditions: 1.5-m column; 150°C.

unretained compound using a 10 m long column, as any dead volume, associated with the interface, will have a larger influence on the overall peak broadening for a shorter column.

The reason for the loss of efficiency could be dispersion in the transfer line or disturbances in the flow pattern as a result of the different dimensions of the column and the transfer line. Other factors which could contribute are the acquisition rate of the MS [20], the nebulisation, ionisation and the transport of ions in the mass spectrometer. Our results suggest that the change in dimensions could be a likely explanation, since the contribution to the peak broadening from the 1.5 m×15 mm I.D. transfer line was calculated to be only 1–4 nl (the highest value was obtained at the highest flow-rate of 1.6 μl/min) while a value of 12 nl was obtained from the experimental data. The extra column peak broadening caused by the transfer line was calculated, in volume units, from Eq. (2), derived from the Golay equation [1] assuming $k'=0$ and neglecting the first term.

$$\sigma^2_{\text{tube}} = \frac{LF^2 d_c^2}{96 D_m u} \qquad (2)$$

where L denote the length of the capillary, F the flow-rate, d_c the capillary internal diameter, D_m the diffusion coefficient in the mobile phase and u the linear velocity.

It can thus be concluded that the volume of the transfer line is of minor importance in this set-up. A further confirmation that the peak broadening is not due to diffusion in the transfer line is the fact that the efficiency loss remained unchanged after raising the temperature in the transfer line to 70°C. In Eq. (2), it is seen that small diffusion coefficients should lead to more peak broadening, implying that the transfer line should be kept at a high-temperature.

The cause for the peak broadening can hence be either processes in the MS or the difference in dimension between the column and the transfer line. Since the ratio between the cross section areas for the column and the transfer line was as large as 11, a significant disturbance of the flow profile due to this effect seems very probable. This makes peak broadening in the MS less likely as the primary cause for the loss of efficiency.

3.2. Linearity and detection limit

The detection limit and linear range of a detector are of significant importance in quantitative analyses based on MS detection. The detection limit for the present MS detector, determined as the amount of sample generating a signal level three times that of the standard deviation of the noise, was found to be 1 μ*M* for 7,8-benzoquinoline using *m/z* 180 in SIM mode. This corresponds to a mass flow of 3 pg/s. The signal at *m/z* 180 originated from the quasi-molecular ion, $[M+H]^+$, which was the only abundant ion under the experimental conditions employed in this study. As is seen in Fig. 3, the upper limit of the linear range was approximately 30 μ*M* for evaluations based on *m/z* 180. Larger concentrations than 100 μ*M* were not considered relevant for analysis. A larger linear range, of about two orders

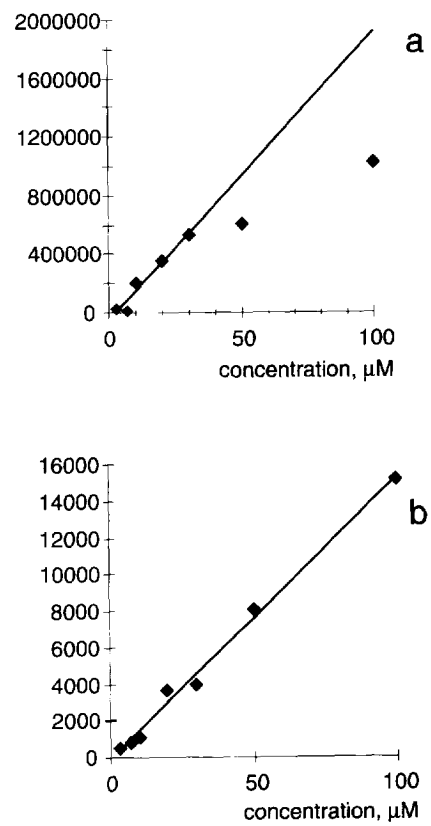

Fig. 3. Linear ranges for 7,8-benzoquinoline based on (a) *m/z* 180 and (b) the isotopic ion *m/z* 182. Conditions: methanol–water (8:2) mobile phase, flow-rate 1 μl/min.

of magnitude, was, on the other hand, found for the less intense isotopic ion corresponding to m/z 182. As is also seen in Fig. 3, the advantage of the larger linear range for the isotopic ion at m/z 182 is, however, offset by a lower sensitivity. The reasons for the limited linear range and the differences between the different m/z ratios are not fully understood. Narrow linear ranges in mass spectrometric experiments are, however, not uncommon and can be caused by, e.g., limitations in the ion counting [21], insufficient background correction [22], depletion of reagent ions in the ion source [21,23] and space charge effects [24].

An indication that the narrow linear range was not coupled to the ion counting detector is that the linear range was found to be small also in MRM mode experiments, although the signal levels in these experiments were much lower compared to in the SIM experiments, discussed above. There was also no improvement in the detection limit. In these multiple reaction monitoring mode experiments, in which the disturbance from other species than the analyte can be minimised, the first quadrupole was adjusted to allow the passage of ions with m/z 180. These ions then underwent collision induced fragmentation in a high pressure collision cell with subsequent analysis of the two most abundant fragments, 152 and 127, in the third quadrupole. These results thus imply that the narrow linear range is most likely caused by imperfections in the ionisation step, i.e., the formation of $[M+H]^+$ or transmission of ions through the first quadrupole. Further studies, which are beyond the scope of this investigation, are however, required to reach a full understanding of this complex phenomenon.

3.3. Dependence on flow-rate

The dependence of the signals of MS detectors on the flow-rate of a liquid carrying the analyte has been studied frequently [25,26] and it has been found that the detector can be either concentration or mass flow-rate sensitive depending on the conditions employed. Andrien et al. [25] thus reported an APCI–MS to be a concentration sensitive detector under high flow-rate conditions (i.e., 0.1–2 ml/min), while Hopfgartner et al. [26] found both concentration and mass flow sensitive behaviour in ion spray depending on the flow-rate. In this study, the MS detector unit could be characterised as mass flow sensitive at flow-rates between 0.1 and 1.6 μl/min by considering the interface, ion source and the mass detector as a detector unit for the chromatographic system. In Fig. 4, the flow-rate dependence of the UV and MS peak area is compared for injections of solutes of the same concentration but for different flow-rates. It is clearly seen that the MS (volume based) peak area increases almost linearly with the flow-rate while the dependence of the UV peak area, on the other hand, is characteristic of a concentration sensitive detector. This implies that to obtain reproducible MS results, care must be taken to mini-

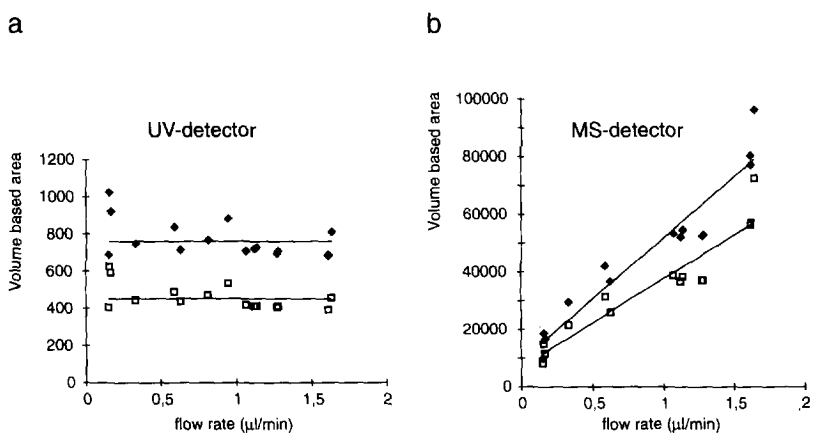

Fig. 4. Volume-based peak area as a function of flow-rate in (a) the UV and (b) the MS detector. Conditions: mobile phase, methanol–water (3:7); (□) 7 ng 5,6-benzoquinoline and (◇) 5 ng 7,8-benzoquinoline.

mise variations in the flow-rate. The mass flow dependence also means that the sensitivity of the MS detector may be improved by the use of higher flow-rates, or by adding a make up flow of additives that enhance the ionisation, providing, of course, that the detector unit still remains mass flow sensitive.

3.4. Temperature gradients

Temperature gradients are interesting in HT-OT–LC as they can be used as an alternative to solvent gradients to shorten the analysis times for late eluting compounds. One problem is, however, that the UV detector cell, commonly employed in HT-OT–LC, has to be placed in the oven to avoid losses of efficiency [27,28]. During a temperature gradient, the refractive index of the mobile phase will then change and consequently cause a shift in the baseline [29]. This is illustrated in Fig. 5 which compares the response from the UV and MS detector during a temperature gradient run designed for a separation of some polycyclic aromatic hydrocarbons. As is seen in the figure, background in the MS detector remains practically unchanged during the temperature gradient indicating that the temperature of the mobile phase reaching the interface to the mass spectrometer do not change during the experiment. With the MS detector, temperature gradients can thus be employed without effecting the baseline level. Although UV detection "on-column" on the narrow transfer line would eliminate the drift in the background, it is not recommended due to the reduced sensitivity. As is also seen in Fig. 5, the MS detector provided the highest sensitivity for the first four compounds while the UV detector provided the highest sensitivity for the very non-polar and highly UV absorbing chlorobenzenes and phenanthrene, (i.e., peaks 5–7). The chlorobenzenes and phenanthrene could not be detected in the MS most likely as a result of poor ionisation.

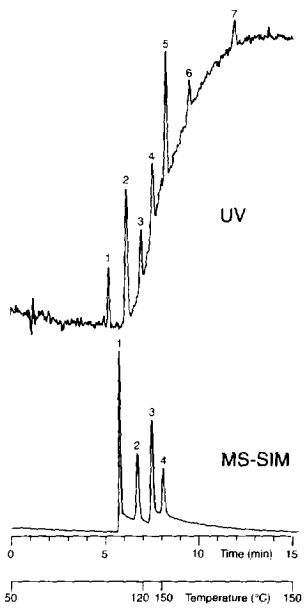

Fig. 5. Temperature programmed separation in OT–LC with UV and MS–SIM detection. Conditions: mobile phase, methanol–water (3:7); flow-rate, 0.42 μl/min; UV detection wavelength, 210 nm; MS–SIM, *m/z* 121, 122, 153 and 180. Peak identity: (1) 2 ng acetophenone, (2) 3 ng 5,6-benzoquinoline, (3) 2 ng N,N-dimethylaniline, (4) 2 ng 7,8-benzoquinoline, (5) 10 ng chlorobenzene, (6) 3 ng 1,2-dichlorobenzene and (7) 1 ng phenanthrene.

Peak tailing was observed in the MS detector for polar compounds such as acetophenone and N,N-dimethylaniline, see Fig. 5. At present we can offer no full explanation for the peak tailing but adsorption on surfaces in the ion source or a re-circulation of sample vapour into the ionisation region are considered possible candidates. Adsorption in the LC system and in the transfer line can, most likely, be excluded as additions of 1 m*M* triethylamine (TEA) to the mobile phase, in an attempt to minimise sample interactions with surface silanol groups, proved ineffective. N,N-Dimethylaniline is known to interact with surface silanol groups in LC columns. Higher concentrations of TEA were not considered as problems with plugging of the transfer line occurred already at the 1 m*M* level. Deactivation of the transfer line did not improve the peak tailing for the polar compounds showing that the interactions with silanol groups were indeed negligible. Less tailing was, however, seen when an ion source exhaust pump was used to remove gaseous species from the ion source. This seems to support the hypothesis of a re-circulation of sample vapour into the ionisation region. The influence of the pump could, however, also be interpreted on the basis of an adsorption equilibrium involving, for instance, metal surfaces in the ion source. In this case, the peak tailing would also be improved with the pump since the influence of the adsorption would be minimised

by a lowering of the concentration of the adsorbing species in the gaseous phase. At present, we have no means of differentiating between the two possible phenomena, adsorption or re-circulation. A thorough investigation of this problem was also considered beyond the scope of the present work. It is, however, clear that the ion source exhaust pump should always be employed when peak tailing is considered to be a significant problem. In the present study, the advantage of reduced peak tailing must, unfortunately, be weighted against the fact that the use of the pump resulted in a significantly increased noise level at the low flow-rates used (i.e., ca. 1 μl/min). In the present work, the latter disadvantage was considered more serious and the exhaust pump was therefore not routinely employed.

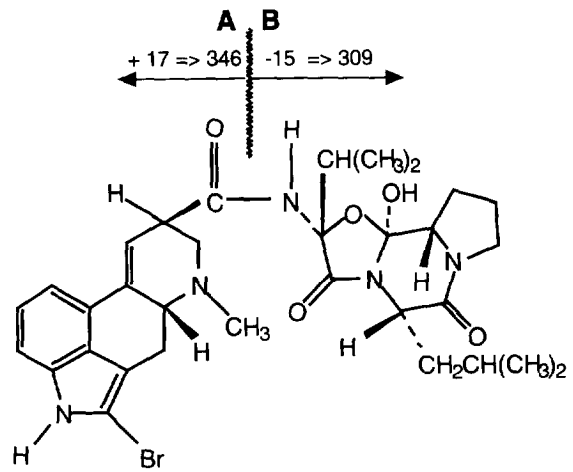

Fig. 7. Structure of bromocriptine indicating the two fragments **A** and **B**.

3.5. Other aspects

When using a MS detector coupled to LC, attention should be paid to the influence of the chromatographic conditions on the performance of the MS detector. The composition of mobile phase can, for instance, influence the ionisation conditions and may thus affect cluster formation and fragmentation. This will thus be of importance for both the sensitivity and the structural information that can be obtained in an MS experiment. An example of the dependence of the nature of the MS spectra on the composition of the mobile phase is given in Fig. 6. In this figure, spectra for bromocriptine are compared for methanol or acetonitrile as the organic mobile phase modifier. As depicted in Fig. 7, bromocriptine easily disso-

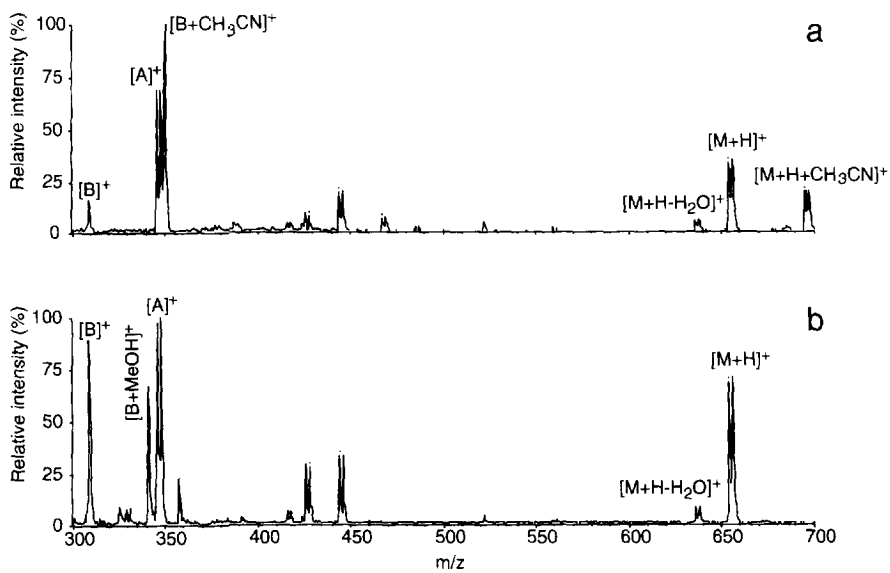

Fig. 6. Mass spectra obtained after an injection of 200 ng bromocriptine using (a) acetonitrile–water (4:6) or (b) methanol–water (3:7) as the mobile phase at a flow-rate of 0.4 μl/min.

ciates in two major fragments referred to as **A** and **B** below. As seen in Fig. 6, the intensity of the $[M+H]^+$ ion is smaller when acetonitrile is used instead of methanol. This can be explained by the fact that the $[M+H]^+$ ion forms a cluster with acetonitrile but not with methanol (see Fig. 6). The fragment **A** forms clusters with acetonitrile and to a lower extent also with methanol.

The difference in potential between the orifice and the entrance quadrupole, the drift potential, can be tuned to change the fragmentation and background level. At higher drift potentials, the background is decreased due to dissociation of water clusters [30] but fragmentation of the analyte ions also occur. This is demonstrated for bromocriptine in Fig. 8 where the quasi-molecular ion $[M+H]^+$ at *m/z* 654 is shown to exhibit its maximum signal at a drift potential of 10 V. When the signal corresponding to the $[M+H]^+$ ion starts to decline at higher voltages, the signal corresponding to the ion having lost water, at *m/z* 636, increases. The fragment ion **A**, at *m/z* 309 forms a weak cluster with methanol, seen at *m/z* 341, which can be broken up at relatively low drift potentials.

Increased relative intensity of base line noise due to the addition of a buffer salt in the mobile phase can be a problem when coupling LC to MS. Fouda et al. [22], for example, observed problems already at as low concentrations as 5 m*M* of ammonium acetate. Another potential problem is plugging of the transfer line capillaries. We have, nevertheless, carried out experiments with 10 m*M* ammonium acetate/ammonia solutions of pH 10 for several hours without any such problems. When the aqueous component of the mobile phase included a 50 m*M* acetic buffer of pH 5.0 and the heating wire was positioned directly around the nebulising capillary, plugging of the transfer line tip was, however, a frequent problem. It was found that the position of the transfer line tip relative to the nebulisation capillary and heating wire was crucial. The heat evolved has to be enough to vaporise both the mobile phase and the sample, thus creating a uniform spray, while too much heat will cause sample degradation and eventually plugging of the transfer line tip. Adsorption of the sample was observed when too little heat was used. The adsorbed material could be rapidly desorbed by increasing the temperature.

The influence of sample concentration on fragmentation, cluster formation and abundance of ions also has to be taken into account if quantitative analyses are to be performed. This is particularly true when extensive fragmentation and cluster formation are present. The intensity of the $[M+H]^+$ ion of bromocriptine, relative to the fragment $[\mathbf{B}+CH_3CN]^+$ was, for example, decreased from 35% to 6% upon a tenfold reduction of the bromocriptine concentration.

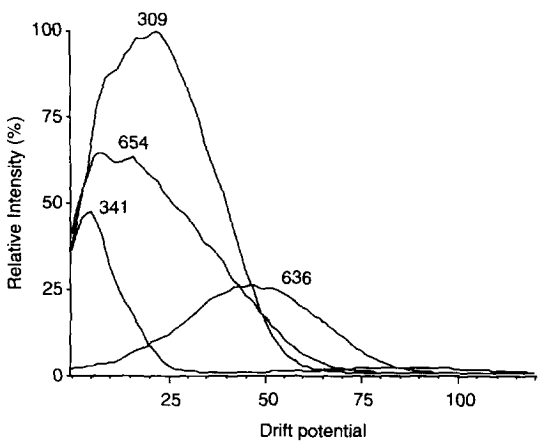

Fig. 8. Influence of drift potential on clustering and fragmentation of bromocriptine. Conditions: mobile phase, methanol–water (3:7); flow-rate, 0.4 μl/min.

3.6. Solute stability

To study the thermal degradation in the present chromatographic system, experiments were performed with the thermally labile compound bromocriptine [19]. In these experiments, the MS detector was thus used to monitor the intensity of the quasi-molecular ion signal as a function of the temperature in the chromatographic system. The mass spectra were obtained from the one and only peak present in the total ion chromatogram. Care was taken to remove oxygen from the mobile phase by continuous degassing with helium purging. As is seen in Fig. 9, the quasi-molecular ion was present at the same relative intensity at an oven temperature of 150°C as for room temperature separations. This indicates that the oven temperature does not contribute significantly to the thermal decomposition of bromocriptine, despite the appearance of a new peak at *m/z*

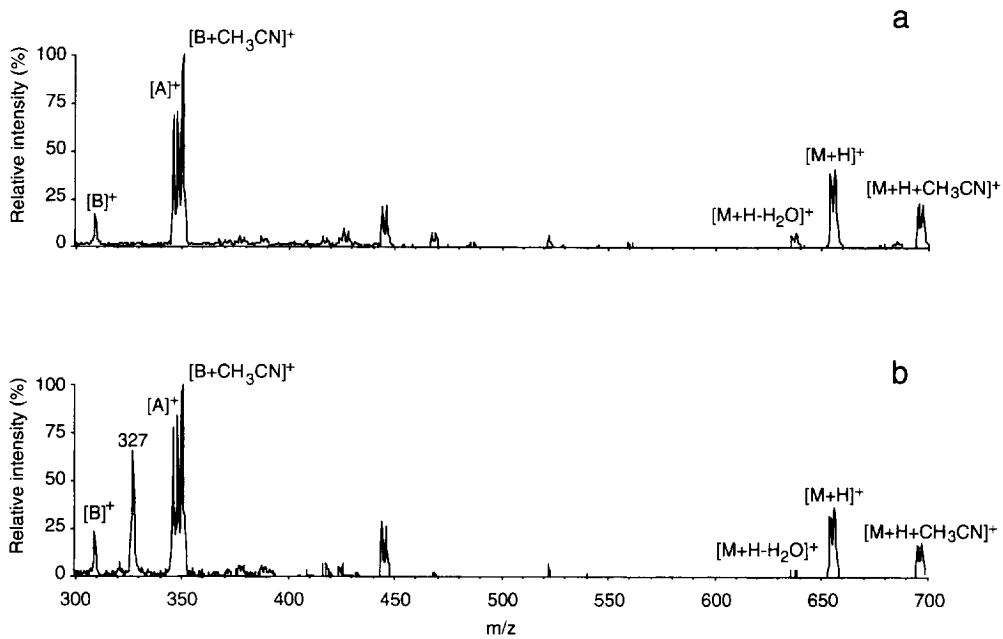

Fig. 9. Mass spectra obtained after injections of 200 ng bromocriptine at an oven temperature of (a) 25°C and (b) 150°C, respectively. Conditions: mobile phase, acetonitrile–water (4:6); flow-rate, 0.4 μl/min.

327. A difference in the degradation of bromocriptine was neither seen when nitrogen, rather than synthetic air, was used as the nebulisation gas, indicating that the extent of oxidative degradation of bromocriptine by oxygen was small. The limited thermal degradation for bromocriptine in HT-OT–LC hence suggests that this technique may also be used for thermally sensitive compounds. The reason for this is most likely that the analyte is exposed to the high temperatures for a relatively short period of time [3].

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