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Some aspects of peak broadening in particle-beam liquid chromatography–mass spectrometry

A. P. TINKE, R. A. M. VAN DER HOEVEN, W. M. A. NIESSEN*, U. R. TJADEN and J. VAN DER GREEF

Division of Analytical Chemistry, Center for Bio-Pharmaceutical Sciences, P.O. Box 9502, 2300 RA Leiden (Netherlands)

ABSTRACT

The particle-beam interface has recently been introduced for coupling liquid chromatography and mass spectrometry. The coupling of a particle-beam interface to a Finnigan MAT TSQ-70 triple quadrupole instrument is described. A compound-dependent peak broadening in the interface has been observed. In this paper various experiments are described to investigate some of the sources of peak broadening. For this purpose, the transfer efficiency of the particle-beam interface has been measured, and the potential of volatility-enhancing derivatization procedures has been explored. The detection of 40 pg of the pentafluorobenzyl derivative of palmitic acid is demonstrated.

INTRODUCTION

The particle-beam (PB) interface is a relatively new interface for coupling liquid chromatography and mass spectrometry (LC–MS), which has been commercially available since 1988 [1–3]. In a PB interface the column effluent is pneumatically nebulized in a heated desolvation chamber at nearly atmospheric pressure. The analyte molecules in the solvent stream nucleate to form submicrometre particles, which are selectively separated from the solvent vapour molecules in a two-stage momentum separator and subsequently transported to the mass spectrometry ion source. The particles evaporate on collision at the heated ion source wall and the released molecules are ionized and mass analysed. The PB interface enables the on-line LC–MS acquisition of electron-impact (EI) and solvent-independent chemical ionization (CI) spectra as well as fast atom bombardment (FAB) [1–5] spectra, although the latter is not well documented. Compared to other successful LC–MS interfaces applicable to conventional LC splitless columns, the PB interface is easier to operate than the moving-belt interface [6], which can also yield EI and CI spectra, but its applicability range is limited compared to the thermospray interface [7], which cannot generate EI spectra.

During the evaluation of a PB interface in this laboratory it was found that significant peak broadening in the PB interface can be observed. The extent of peak

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broadening appeared to be compound-dependent. Various experiments were performed to determine the sources of peak broadening. The results of these experiments are reported here.

EXPERIMENTAL

Equipment

A Hewlett-Packard (Palo Alto, CA, USA) 59980A PB interface with a pneumatic nebulizer was coupled to a Finnigan MAT (San José, CA, USA) TSQ-70 mass spectrometer with a standard EI–CI source using a laboratory-made stainless-steel transfer tube, introduced into the vacuum through the gas chromatography (GC)–MS inlet flange. A schematic diagram of the interface is given in Fig. 1. A Busch (Virginia Beach, VA, USA) Model RA 0025 and an Edwards (Crawley, Sussex, UK) E2M18 mechanical pump were used at the first and second stage of the momentum separator, respectively.

An LKB (Bromma, Sweden) Model 2150 LC pump was used for the delivery of a mobile phase of 80% methanol in water at a flow-rate of 0.5 ml/min. The sample solutions were injected with a Rheodyne (Cotati, CA, USA) Model 7125 injection valve equipped with a 20-μl sample loop.

Chemicals

Methanol (p.a.) and acetonitrile (p.a.) were obtained from Baker (Deventer, Netherlands). Clobazam [7-chloro-1-methyl-5-phenyl-1H-1,5-benzodiazepine-2,4-(3H,5H)-dione] (Fig. 2) (Hoechst, Frankfurt, Germany), pentafluorobenzyl bromide (PFBBr) (Sigma, St. Louis, MO, USA) and 0.2 *M* of trimethylanilinium hydroxide in methanol (Pierce, Rockford, IL, USA) were stored at −20°C. All other solutes were purchased from various commercial sources and were used without further purification.

Determination of transfer efficiency

The skimmer set, transfer tube and a CI ion volume were cleaned thoroughly and washed with 5 ml of methanol (later used as blanks) before installation. The source pressure was adjusted to *ca.* 700 mPa of air and the temperature to 45°C (or to

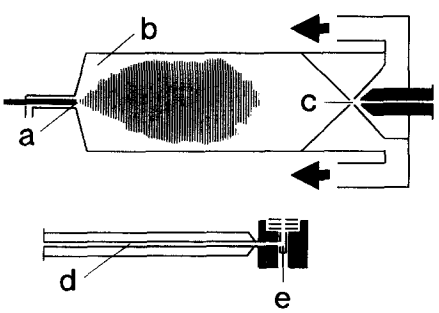

Fig. 1. Schematic diagram of the PB interface. a = Nebulizer; b = desolvation chamber; c = momentum separator; d = transfer tube; e = ion source with ion volume.

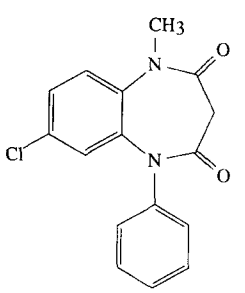

Fig. 2. Structure of clobazam.

250°C in some other experiments). Clobazam (10 μg) was injected in the column bypass mode. After 2 min, the transfer tube and the ion volume were washed separately with 5 ml of methanol. The amounts of clobazam collected in the two fractions were determined by high-performance liquid chromatography with ultraviolet (UV) detection against a series of standard solutions. The possibility of evaporative losses in the high vacuum during the procedure was excluded; a *ca.* 90% recovery was found for clobazam after storage of the ion volumes, at which clobazam was deposited, in the ion source for a similar time periode and under similar conditions.

Derivatization of fatty acids

Fatty acids were converted into the corresponding pentafluorobenzyl derivatives [8] by adding 2 ml of an aqueous solution of 0.1 mol/l tetrabutylammonium hydrogensulphate in 0.2 mol/l sodium hydroxide solution and 40 μl PFBBr to 0.5 mg of fatty acid in 2 ml of dichloromethane. After shaking for 20 min the water layer was discarded, the dichlotomethane layer was diluted with methanol and an aliquot was injected onto a 100 × 3 mm I.D. C_8 column for LC–MS detection in the negative-ion chemical ionization (NCI) mode.

RESULTS AND DISCUSSION

The PB interface was evaluated in this laboratory for various reasons. Previously, considerably effort has been put into the application of various LC–MS interfaces in both qualitative and quantitative bioanalysis. Other interfaces applied so far, *i.e.* thermospray, moving-belt and continuous-flow FAB, were successful in many applications, but also showed limitations in either the information content of the spectra for qualitative analysis or the determination limits for quantitative analysis. The PB interface is especially attractive with respect to qualitative analysis because of its ability to acquire on-line EI spectra, whereas its potential in quantitative analysis is at first sight less promising, but on the other hand has hardly been evaluated systematically. In addition, the PB interface would be most helpful for the automated acquisition of series of EI spectra from a variety of samples. In comparison with the moving-belt interface, which has similar potential, the PB interface is more robust.

From the first experiments, it appeared that with some analytes significant peak broadening is observed. As an example, the UV peak and the EI-MS peak after a column bypass injection of 2 μg of clobazam are given in Fig. 3. The peak width at the base is 4.8 s in the UV trace (asymmetry factor at 10% of the height of 1.3) and 23.2 s in the MS trace (asymmetry factor of 2.8). In principle, possible sources of peak broadening are the nebulizer, the desolvation chamber, the momentum separator, the transfer tube and the ion source. It was decided to study the effects of the transfer tube and the TSQ-70 ion source first, *i.e.* the parts differing from the Hewlett-Packard system. In this way, it would also be possible to evaluate the transfer efficiency of the PB interface. The transfer efficiency is defined here as the ratio of the amount of analyte collected in the ion source to the amount of analyse injected in the mobile phase. Transfer efficiencies between 0.05 and 0.70 have been claimed [9,10] for the various commercially available PB interfaces.

The transfer efficiency can be measured in the TSQ-70 system relatively easily, because the EI–CI ion source is equipped with replaceable ion volumes. By decreasing

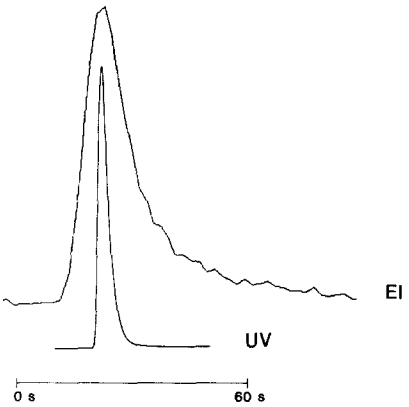

Fig. 3. Illustration of the peak broadening in the PB interface [2 μg column bypass injection of clobazam (mol. wt. = 300)].

the ion source temperature to avoid losses due to analyte evaporation, the amount of sample transferred to the ion source can be measured. For each experiment a thoroughly cleaned skimmer set, transfer and CI ion volume were installed. The sample, 0.5 mg/ml clobazam in methanol, was injected and after a pre-set period of time the ion volume was removed and the amount of clobazam collected in the ion volume was measured. Typical transfer efficiencies measured under these conditions were 2%. As this figure was unexpectedly low, it was decided to measure the amount of clobazam collected in the transfer tube as well, which was 19% of the injected amount. Apparently, the particle beam from the momentum separator does not behave as a narrow parallel beam of particles, as was stated by Winkler *et al.* [2], but significantly diverges, allowing the particles to collide with the transfer tube surface. It can also be concluded that for clobazam only 21% of the injected amount passes the momentum separator, *i.e.* significant sample losses take place in the PB interface.

Under normal operating conditions, the temperature of the transfer tube is higher due to thermal conductance from the ion source, which is typically at 250°C. Therefore, a more efficient evaporation of collided particles from the tube wall is anticipated at a higher temperature. It was decided to measure the amount of clobazam collected in the transfer tube while the ion source was kept at 250°C. This amount was 5% of the injected sample, which means that the effective transfer efficiency for clobazam to the ion source is *ca.* 16% under normal operating conditions.

From these data two conclusions were drawn. Firstly, the transfer tube must be as short as possible. Independent heating of the transfer tube might be helpful as well. The implementation of a short heated transfer tube in this experimental set-up is currently under investigation. Therefore, further studies on the transfer efficiency were postponed. Secondly, the divergence of the particle beam and the resulting collisions and subsequent sample evaporation at the transfer tube wall will lead to peak broadening, the extent of which depends on the sample volatility and the wall temperature. Similarly, the evaporation step in the ion source on particle collision prior to the ionization will lead to peak broadening, the extent to which also depends on the sample volatility and the wall temperature. At this stage, it was decided to

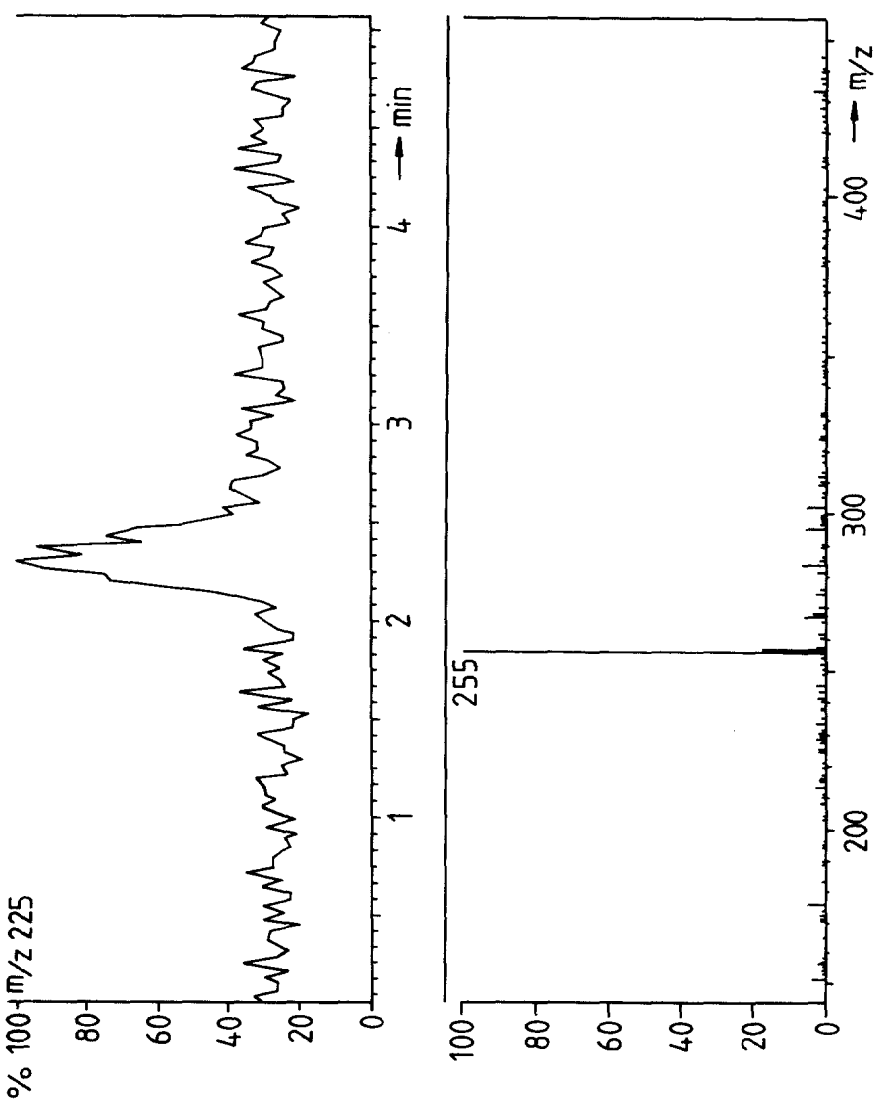

Fig. 4. (upper) Selected ion chromatogram of 40 pg of the pentafluorobenzyl derivative of palmitic acid (mol. wt. = 256) after injection of the reaction mixture on a 100 × 3 mm I.D. C_8 column and monitored under ammonia–NCI conditions. (lower) NCI spectrum of 400 pg of the pentafluorobenzyl derivative of palmitic acid.

evaluate the potential of volatility-enhancing derivatization reactions. Reactions for a wide variety of compounds are available from GC–MS practice [11].

To test the hypothesis of the influence of sample volatility in a more systematic way, the signals obtained from column bypass injections of a series of fatty acids and their methyl esters were compared. In general, the sensitivity for the fatty acids was not very good. Injections of *ca.* 100 μg of palmitic or stearic acid were necessary to achieve signals in scanning mode with a reasonable signal-to-noise ratio. The peak width at the base was 27.3 s (asymmetry factor at 10% of the height of 4.2). In contrast, for methyl palmitate and methyl stearate, 100 times higher signal-to-noise ratios were achieved on the injection of *ca.* 1 μg. Significantly less peak broadening and more symmetric peaks are observed; the peak width at the base is 12.0 s (asymmetry factor of 2.3) The methylation of carboxylic acids to enhance their volatility can also be readily performed on-line, as demonstrated by Vouros and co-workers [12,13] in a post-column ion-pair liquid–liquid extraction system, where the carboxylic acids are extracted as ion pairs with the trimethylanilinium (TMA) counter ion to the organic phase. The fatty acid–TMA ion pair decomposes on heating to form the methyl esters [12,13]. Preliminary experiments with this approach indicate significant improvements in signal-to-noise ratios and peak shape for some fatty acids.

The demonstrated potential of volatility enhancing derivatization procedures to improve the analyte detection in PB LC–MS also opens the possibility of a broad application of other potentially more sensitive ionization methods, such as electron-capture NCI. In Fig. 4, the NCI spectrum of 400 pg of the pentafluorobenzyl derivative of palmitic acid and the on-column chromatogram of 40 pg of the derivative are shown. The peak at m/z 255 is formed in a dissociative electron capture.

In conclusion, it can be stated that a more profound knowledge of the processes determining the operation of the PB interface will enable improvements of the interface performance. Further studies along this line are presently being performed in this laboratory to enhance the applicability of the PB interface.

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