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MECHANIZED OFF-LINE COMBINATION OF MICROBORE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND LASER MASS SPECTROMETRY

JOSEF F. K HUBER*, TADEUSZ DZIDO* and FRANZ HERESCH
Institute of Analytical Chemistry, University of Vienna, Waehringerstrasse 38, A-1090 Vienna (Austria)

SUMMARY

Laser desorption mass spectrometry is shown to be very suitable for a mechanized off-line combination with liquid chromatography. In the procedure described, a simple mechanical device provides fractionwise collection of the chromatographic effluent at flow-rates up to 0 5 ml/min. The peak broadening effect of this module is very low because of the particular design of the sample holder used. Up to 30 chromatographic fractions have been monitored by mass spectrometry, the fraction sizes being in the order of one half of the peak standard deviation

INTRODUCTION

The combination of different analytical unit operations requires, in general, a mutual adjustment of their individual operating conditions and this compromise can lead to a loss in performance For optimum results the integrity of the unit operations involved should be maintained as far as possible The question of whether or not to couple different unit operations in real-time (provided modes are technically feasible) is dependent on their time compatibility which should be evaluated before any experimental approach to coupling

As to the combination of chromatography and mass spectrometry (MS), it is necessary not only to minimize extra-column sample dispersion effects, restrictions in the choice of phase systems and ionization modes, etc , but also to maintain the full range of tractable compounds With liquid chromatography (LC)–MS, in contrast to gas chromatography (GC)–MS, the limiting factor is the mass spectrometry because of its low (or even zero) sensitivity for many substances that can be handled by LC, especially macromolecules In order to cope with non-volatile and thermolabile compounds and, in consequence, with low ion currents (signal/noise ratios), large mass ranges, etc., the MS measurement should not be limited in time, particularly when not only structural confirmation is required and more sophisticated

* Permanent address Department of Inorganic and Analytical Chemistry, Medical Academy, Lublin, Poland

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and time-consuming techniques such as high-resolution scanning and MS–MS have to be used. In this analytical situation, which may be classified as "non-routine", the MS measurement should be independent of the chromatographic speed

Based on such considerations, an off-line LC–MS system is proposed in which the linking steps, namely (i) collection of the LC effluent and (ii) MS measurement, are mechanized. A multi-sample holder specially designed for quantitative laser desorption mass spectrometry[1] is used for localized storage of the LC fractions In this paper the performance of the set-up is reported with emphasis on its extra-column peak broadening effect using a microbore column. Microbore packed columns are increasingly applied for on-line LC–MS coupling[2], especially with direct liquid introduction[3–7], and offer several advantages such as low flow-rate, low sample dilution, etc.

As a model problem for the evaluation of the proposed off-line LC–MS coupling, the separation and detection of L-3,4-dihydroxyphenylalanine (L-DOPA) and dopamine (DA) was investigated mainly because of the possibility of using electrochemical detection as a reference[8,9] which is known to give negligible peak broadening[10]

EXPERIMENTAL

Microbore high-performance liquid chromatography (HPLC)

The chromatographic conditions for the liquid chromatographic separation of L-DOPA and DA were as follows: high-performance liquid chromatograph (Model S-101, Siemens, Karlsruhe, G.F R.), stainless-steel column (200 × 1 mm), packed with 10-μm octyl silica (LiChrosorb RP-8; E Merck, Darmstadt, G.F R.), mobile phase, 0.1 *N* aqueous HNO_3 + NaOH to pH 2 8, flow-rate 5–30 μl/min, room temperature, injection device, pneumatic sampling valve (Model 60 AH, Valco Instruments, Houston, U.S.A) with 0.5-μl loop, injection solvent 0 1 *N* aqueous trichloroacetic acid, reference detector, modified electrochemical detector with glassy carbon electrode (Model LC-2A, Bioanalytical Systems, West Lafayette, U.S.A), as described[11].

Laser MS

Mass spectrometric detection was carried out on a magnetic sector instrument (MS 902, AEI-Kratos, Manchester, Great Britain) adapted for pulsed laser desorption[12,13]. The measurement conditions and procedures for quantitative work were as described earlier[1] The indented stainless-steel rod used as sample holder is shown in Fig. 1. Each indentation is loaded with a fraction of the chromatographic effluent. The indentations were designed for two particular tasks; (i) to allow laser desorption or related surface ionization techniques, (ii) to enable localized deposition of liquids. Depending on the surface tension of the liquid it is possible, for instance, to overload an indentation having a volume of 1 mm^3 as listed in Table I.

The laser desorption spectra of L-DOPA and DA exhibit mainly pseudomolecular signals such as $[M + alkali]^+$, $[M - H + alkali_2]^+$ and $[M + H]^+$ The intensity ratio of protonated *vs.* cationized species is highly dependent on the chemical environment and pretreatment of the sample Calibration experiments were made with compounds dissolved in the LC eluent Due to the acidic pH of 2.8, a $[M + H]^+/[M + Na]^+$ ratio of about 10 was observed in this case.

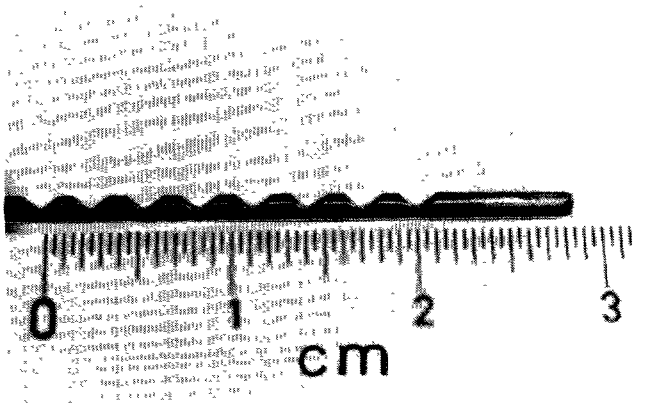

Fig 1 Indented sample holder

For detection, measurements were performed in scanning and in selected ion monitoring modes (using accelerating voltage alternation). Since a pulsed ion source is involved, both types of operation are equivalent and have to be pulse-synchronized. For details see ref. 13.

Mechanized fractionation

The mechanized discontinuous (fractionwise) collection of LC effluent requires two simultaneous, but independent, operations; (i) shift of the storage device; (ii) actual sampling. In the mechanized apparatus shown in Fig. 2 these operations are both made by means of a screw rod (R1)driven by a motor (M). The periodic deposition of effluent is effected by the helical motion of rods R1 and R2 (onto which the sample holder S is mounted excentrically). Each turn thus brings a new indentation into the chromatographic outlet where the liquid (droplet accumulated since the previous indentation) is collected. The appropriate shift along direction Z is determined by the indentation of the sample holder (see Fig. 1) to which thread T corresponds. The time interval, Δt_f, between collections is independently regulated only via the speed of the motor.

TABLE I

CORRELATION BETWEEN SURFACE TENSION, γMM), AND MAXIMUM VOLUME, ΔV_{max}, ACCOMMODATED PER INDENTATION FOR DIFFERENT SOLVENTS

(a) Manual loading (from syringe), (b) mechanized loading (from chromatographic outlet) at sampling rate of 20 min^{-1}

Solvent	$\gamma_{20°C}$ (dyn cm) (ref. 14)	$\Delta V_{max}(\mu l)$	
		(a)	(b)
Water	73	13	5.8
Water–methanol (1:1, v/v)	35	8	4.5
Water–acetonitrile (1:1, v/v)	31	7	3.8
Acetonitrile	29	5	2.9
Methanol	23	1.5	1.4

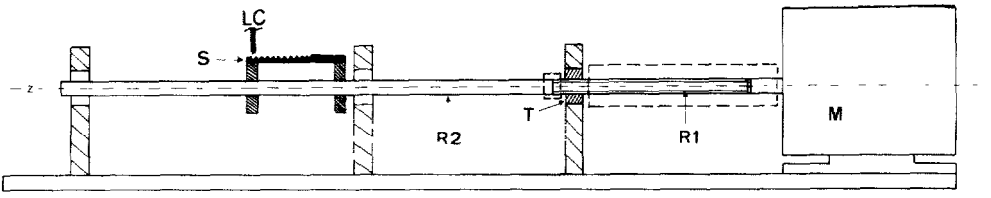

Fig 2 Apparatus for automated storage of LC fractions M = motor, R1 = screw rod; R2 = rod connected to R1, T = thread, S = sample holder, LC = chromatographic outlet, Z = rotation axis

In this way the chromatographic effluent is gradually stored in the indentations, *i.e.*, in one sample holder or in a number of them placed in series (on R2), which can then be transferred to the mass spectrometer. The solvent is usually removed just after deposition. If necessary, *e.g.*, in case of labile compounds, the fractions may be kept in solution until insertion into the spectrometer

For measurement, the sample holder(s) were attached to the direct probe and manually inserted into the mass spectrometer as described[1].

RESULTS AND DISCUSSION

Fraction size

The size of the effluent fractions collected, *i.e.*, their volume, ΔV_f, or duration, Δt_f, has to be optimized with respect to the chromatographic and MS integrity as mentioned earlier. In this context it is useful to relate these parameters to the chromatographic scale, namely to the volume or time standard deviations, σ_V or σ_t, of a peak, and to employ a relative fraction size, $\alpha = \Delta V_f/\sigma_V = \Delta t_f/\sigma_t$. It was shown that α has to be in the order of 0 5 if no information is to be lost[15], which will happen if α, is larger, whereas in the case of smaller values only redundant information may be gained. The MS information content, on the other hand, is mainly affected via the signal/noise ratio which depends on the sample level and therefore increases with relative fraction size, α. Due to the nature of the chromatographic process, σ_V and σ_t are functions of retention volume and retention time, respectively. In order to keep α constant, the fraction size (ΔV_f or Δt_f) has to be increased in the course of a chromatogram. This can be done by programming the motor of the mechanized fraction collector shown in Fig. 2. If only a limited number of chromatographic fractions are to be investigated (see Fig. 3), the size of the fractions may remain unchanged.

The working conditions for mechanized fractionation have to be adjusted to the chromatographic flow-rate, w, in such a way that the sampling rate, $1/\Delta t_f$ = motor revolutions per minute, is greater or equal to $w/\Delta V_{max}$. The values of ΔV_{max} were determined for various liquids and are given in Table I. So far, sampling rates up to 60 min^{-1} have been used which corresponds to a maximum flow-rate of almost 0.5 ml/min for aqueous effluent (see Table I).

In Fig 3 the results of electrochemical and MS detection are contrasted. The selected mass profiles (histograms) refer to the $[M + H]^+$ ions of L-DOPA and DA (m/z 198 and 154, respectively). As can be seen from the mass profile, L-DOPA also shows a weak signal at m/z 154 resulting from the loss of CO_2.

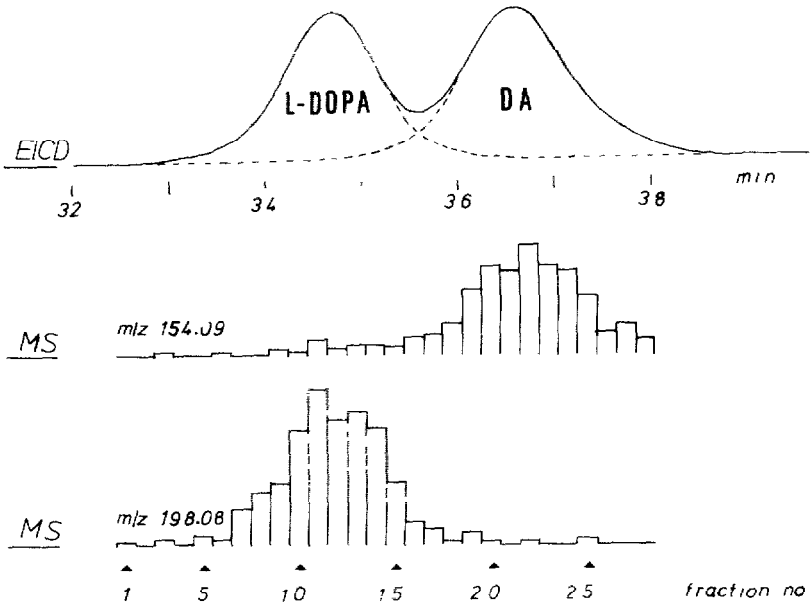

Fig 3 Electrochemical (EICD) *versus* MS peak profiles for L-DOPA and DA (100 ng each).

Peak broadening

As with any other part of a chromatograph, the evaluation of the mixing characteristics of the MS detector has to be based on an analysis of the peak variance. The variance, σ^2, of a recorded peak is the sum of different contributions

$$\sigma^2 = \sigma_S^2 + \sigma_C^2 + \sigma_D^2 \tag{1}$$

where σ_S^2 = variance of the sample volume and sampling device, σ_C^2 = variance of the chromatographic column and σ_{VC}^2 = variance of the detection system

The peak variance, σ_{VC}^2 in volume units, caused by the chromatographic column is given by[16]

$$\sigma_{VC}^2 = \varepsilon_m AHL(1 + \kappa)^2 \tag{2}$$

in which ε_m = fraction of the column volume filled by the mobile phase, m, A = cross-sectional area of the column, H = theoretical plate height of the column describing its mixing characteristics, L = length of the column and κ = capacity factor describing the retardation of the analyte. It can be seen from this equation that the contribution of the chromatographic column to the peak variance decreases proportionally with the cross-sectional area of the column. Therefore the extra-column contributions, σ_{VS}^2 and σ_{VD}^2, to the peak variance are crucial for the use of microbore columns.

On the other hand, microbore columns exhibit a high mass sensitivity since the peak height is given by[17]

$$c_{i\,max} = Q/(2\pi)^{\frac{1}{2}}\sigma_V \tag{3}$$

where $c_{i,\,max}$ = concentration maximum of the peak in the detector, Q = amount of analyte and σ_V is described by eqns. 1 and 2. In addition, microbore columns have a low flow-rate which is favourable for MS.

In LC–MS the detection system consists of the interface and the spectrometer. The contribution of the described off-line interface plus the mass spectrometer to the peak variance can be calculated by means of eqn 1.

Due to the extremely low dead-volume of the electrochemical detector and its low time constant it may be assumed that its contribution, σ_D^2, to the total peak variance is negligible. Assuming that the contribution of sampling, σ_S^2, is constant, it follows that the volume standard deviation for MS detection, $(\sigma_{VD})_{MS}$, is given by

$$(\sigma_{VD})_{MS} \approx \sqrt{(\sigma_V^2)_{MS} - (\sigma_V^2)_{ElCD}} \tag{4}$$

where $(\sigma_V^2)_{MS}$ and $(\sigma_V^2)_{ElCD}$ are the total volume variances of the peaks for MS and electrochemical detection, respectively.

It is evident from eqn 1 that σ_{VC}^2 and σ_{VS}^2 should be kept as small as possible when determining $(\sigma_{VD})_{MS}$. This implies high chromatographic performance (low theoretical plate height, low sample volume, low dead-volumes of injector and connecting lines) and low κ values of the investigated peaks. The σ_V (μl) data given in Table II were obtained for the L-DOPA peak separated at two different flow-rates with κ = 2.15. For MS detection the $[M + H]^+$ ion (m/z 198) was monitored.

The results listed in Table II indicate negligible peak broadening for the off-line MS detection. Independently of the experimental conditions (flow-rate, fraction size), the observed peak standard deviation only increases by 0.1–0.2 μl when applying off-line MS instead of electrochemical detection. The greater extra-column effect at low sample level (200 ng) clearly results from the reduced precision of quantitation (standard deviation *ca.* 25%). The actual detector contribution, $(\sigma_{VD})_{MS}$ calculated according to eqn 1, is given in column 6 of Table II and amounts to about 1.5 μl

TABLE II

PEAK WIDTH OBTAINED WITH ELECTROCHEMICAL (ElCD) OR OFF-LINE MASS SPECTROMETRIC (MS) DETECTION UNDER DIFFERENT CONDITIONS

Theoretical plate height, 34 and 62 μm at flow velocities of 0.27 and 0.83 mm/sec. Precision of the mean value of the peak standard deviation by off-line MS calculated from five measurements 0.2 μl for 1 μg, 0.5 μl for 0.2 μg

Flow-rate (μl/min)	*Flow velocity (mm/sec)*	*Amount injected (μg)*	κ	*Mean value of peak standard deviation (μl)*		*Contribution of MS detection $(\sigma_{VD})_{MS}$ (μl)*
				$(\bar{\sigma}_V)_{ElCD}$	$(\bar{\sigma}_V)_{MS}$	
8.7	0.27	1.0	0.24	4.6	4.7	0.9
			0.48	4.6	4.8	1.4
		0.2	0.48	4.6	5.7	3.3
28.0	0.83	1.0	0.31	6.1	6.3	1.7

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