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# Static and scanning array detection in capillary electrophoresis–mass spectrometry

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## ABSTRACT

An array detection system based on position- and time-resolved ion counting was evaluated for capillary electrophoresis–mass spectrometry using continuous-flow fast atom bombardment and a liquid-junction coupling. Peptides with molecular masses up to 3200 were measured. A 100–1000-fold improvement over conventional detection was demonstrated by applying the array detector in scanning and static modes. Absolute detection limits in the range 1–5 fmol are achievable.

## INTRODUCTION

The coupling of capillary electrophoresis (CE) with mass spectrometry (MS) yields an extremely powerful analytical tool, as it combines a very efficient separation method with a very selective detector. Interfacing CE with MS has been reported using existing high-performance liquid chromatographic (HPLC)–MS interfaces such as electrospray[1,2], ion spray[3] or continuous-flow fast atom bombardment (CF-FAB)[4–7]. These approaches are attractive because the "soft" ionization characteristics open up the possibility of analysing polar high-molecular-mass compounds. Further, the above interfaces have optimum flow-rates in the lower μl/min range and are more compatible with CE than with conventional HPLC. However, a make-up flow is essential to increase the flow-rate from the nl/min range to the *ca.* 5–20 μl/min needed for

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successful operation of these interfaces. The post-capillary addition of a make-up flow is achieved either by a coaxial solvent flow[2,7] or by means of a so-called liquid-junction coupling, which is in fact a low-dead-volume T-piece between the separation capillary and the transfer capillary[3–6]. The latter approach may result in a considerable loss of efficiency[5].

In this study, a CF-FAB interface was applied in the coupling of CE and MS. The make-up flow is achieved by the application of a liquid-junction coupling. The make-up flow is also used to add the necessary non-volatile FAB matrix, *e.g.*, glycerol[5].

Maintaining the efficiency during CE–MS coupling is an important goal which not only requires optimization of the liquid-junction coupling, but also demands extreme performance of the MS detection system with respect to sensitivity and scanning speeds. Moreover, the limited injection volume in CE, typically in the range 5–20 nl for a 1 m × 75 μm I.D. fused-silica capillary, requires detection at very low levels.

In principle, high efficiencies are beneficial for a mass-flow sensitive detector, although peak standard deviations of a few seconds require rapid scanning over a broad mass range. This makes the overall situation problematic. For sensitivity reasons array detection is an interesting approach in CE–MS. However, because the detection must be performed over a wide mass range, array detection in the scanning mode is of more general interest. Therefore, the application of a PATRIC (position- and time-resolved ion counting) detector[8] in CE–MS has been evaluated. The PATRIC detector operates in the ion counting mode. For each event the current central mass, the ion arrival position and the ion arrival time at the focal plane detector are registered, thus permitting scanning operation[8]. Scanning is not possible with array detectors based on photodiodes[8,9].

## EXPERIMENTAL

Capillary electrophoresis was performed with an 800 mm × 75 μm I.D. fused-silica capillary (SGE, Ringwood, Victoria, Australia) applying a potential of 20 kV, supplied by a Model RR100-1.5P power supply (Gamma High Voltage Research, Mt. Vernon, NY, U.S.A). A volume of typically 20 nl was injected hydrodynamically. A laboratory made liquid-junction coupling, described in detail elsewhere[5], was used for the addition of the make-up fluid (0.025% trifluoroacetic acid and 16.6% glycerol in water) and for the coupling of the CE capillary with the CF-FAB interface. The transfer capillary between the liquid-junction coupling and the CF-FAB probe tip was an 840 mm × 100 μm I.D. fused-silica capillary, resulting in a flow-rate of *ca.* 14 μl/min. A newly designed CF-FAB target was used with a gold-plated channel[10], yielding almost instant stability under the described conditions.

A Finnigan MAT (Bremen, F.R.G.) Model 900 double-focusing mass spectrometer equipped with both a normal secondary electron multiplier (SEM) and a PATRIC detector was used. The array detector was used in the static and scanning modes. For the experiments comparing the different detection modes in the analysis of three β-endorphin fragments, the resolution was set at 1000. Resolutions of 1000 and 2000 were used in the measurements of 15 pmol of galanin and 2 pmol of magainin, respectively.

The β-endorphin fragments were a gift from Organon (Oss, The Netherlands). Galanin and maganin were purchased from Bachem Feinchemicalien (Bubendorf, Switzerland). Trifluoroacetic acid was purchased from Merck (Darmstadt, F.R.G.) and glycerol from Lamers and Pleuger ('s Hertogenbosch, The Netherlands). These solvents were of analytical-reagent grade.

## RESULTS AND DISCUSSION

### *Comparison of different detection modes*

The analysis of β-endorphin fragments by CE–MS using a liquid-junction coupling and a CF-FAB interface has been reported previously[5]. In this study, the three fragments 6–13, 8–15 and 10–17 with molecular masses of 857, 882 and 900, respectively, were selected for a comparative study of the different detection modes, *viz.*, conventional detection by means of an SEM detector, scanning array detection and static array detection by means of the PATRIC detector. In the last instance the ions in a fixed mass range of 8% around a central mass, in this case of *m/z* 875, are detected. The analysis was performed with 5 pmol of each β-endorphin fragment.

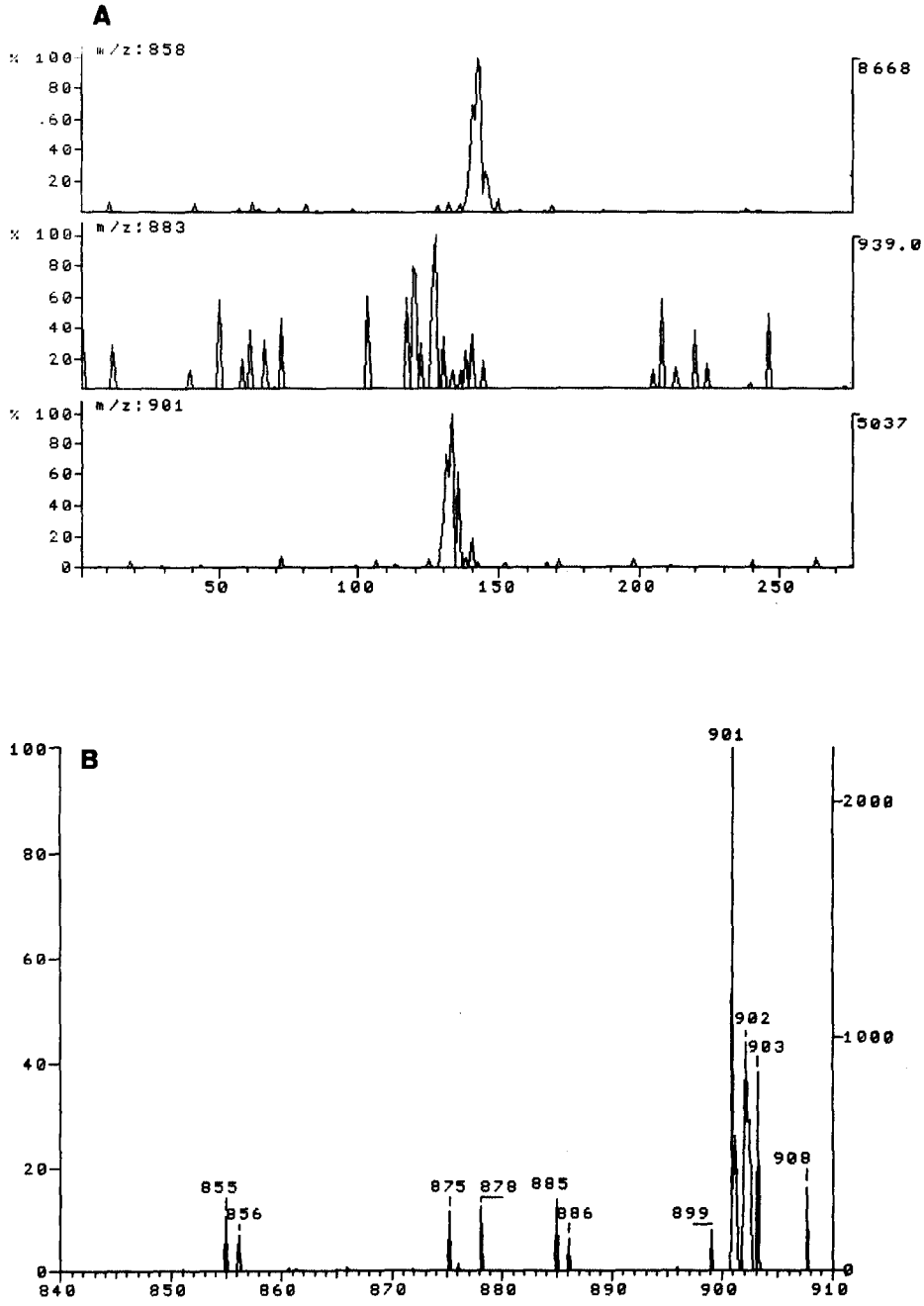
With the SEM detector, scanning over a broad mass range (*m/z* 300–1000) at the 5-pmol level was not successful. Therefore, the mass range in this mode was reduced to *m/z* 840–910 using a cycle time of 1 s. The reconstructed mass chromatograms of the *m/z* values of the protonated molecules are given in Fig. 1A together with a spectrum of the fragment 8–15 in Fig. 1B.

Scanning array detection was performed over a much wider mass range, *viz. m/z* 300–1000, with a cycle time of 1 s. The reconstructed mass chromatograms and the spectrum of the 8–15 fragments from this analysis are given in Fig. 1C and D.

Fig. 1E and F give the results obtained with static array detection using the full 8% window, *i.e.*, detecting over the mass range *m/z* 840–910, with an accumulation time of 1 s.

When the reconstructed mass chromatograms in Fig. 1A, C and E are compared, a considerable improvement in the signal-to-noise ratio is observed on going from scanning with SEM detection via scanning with array detection to the static array detection mode. In the last instance the three peaks are clearly observed in the trace of *m/z* 883. The last peak can be attributed to the 8–15 fragment; the protonated molecule of this peptide loses a molecule of water on FAB. One of the other peaks is the β-endorphin fragment 6–13, and the third peak at this *m/z* ratio is probably due to an impurity in the sample.

The improvement in the quality of the spectrum in the series Fig. 1B, D and F, which represent single spectra without averaging, is also evident from the signal-to-noise ratio and the isotope cluster. The number of ions detected for the peak at *m/z* 901 in a single spectrum of fragment 8–15 in the different modes is 16, 480 and 16 000, respectively. In evaluating these figures it must be kept in mind that in the scanning array detection mode a broader mass range was scanned (*m/z* 300–1000) than with the conventional detector (*m/z* 840–910). Taking this into account, a 100-fold improvement for the scanning array mode and a 1000-fold improvement for the static array mode are observed. The data compared are all based on one spectrum and a cycle time of 1 s. This means for the static array mode that the absolute detection limit for the peptides investigated is in the 1–5-fmol range, and even lower, if all spectra during a CE peak are averaged.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939.0
m/z:901
5037
B
901
2000
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1000
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908
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880
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Fig. 1.

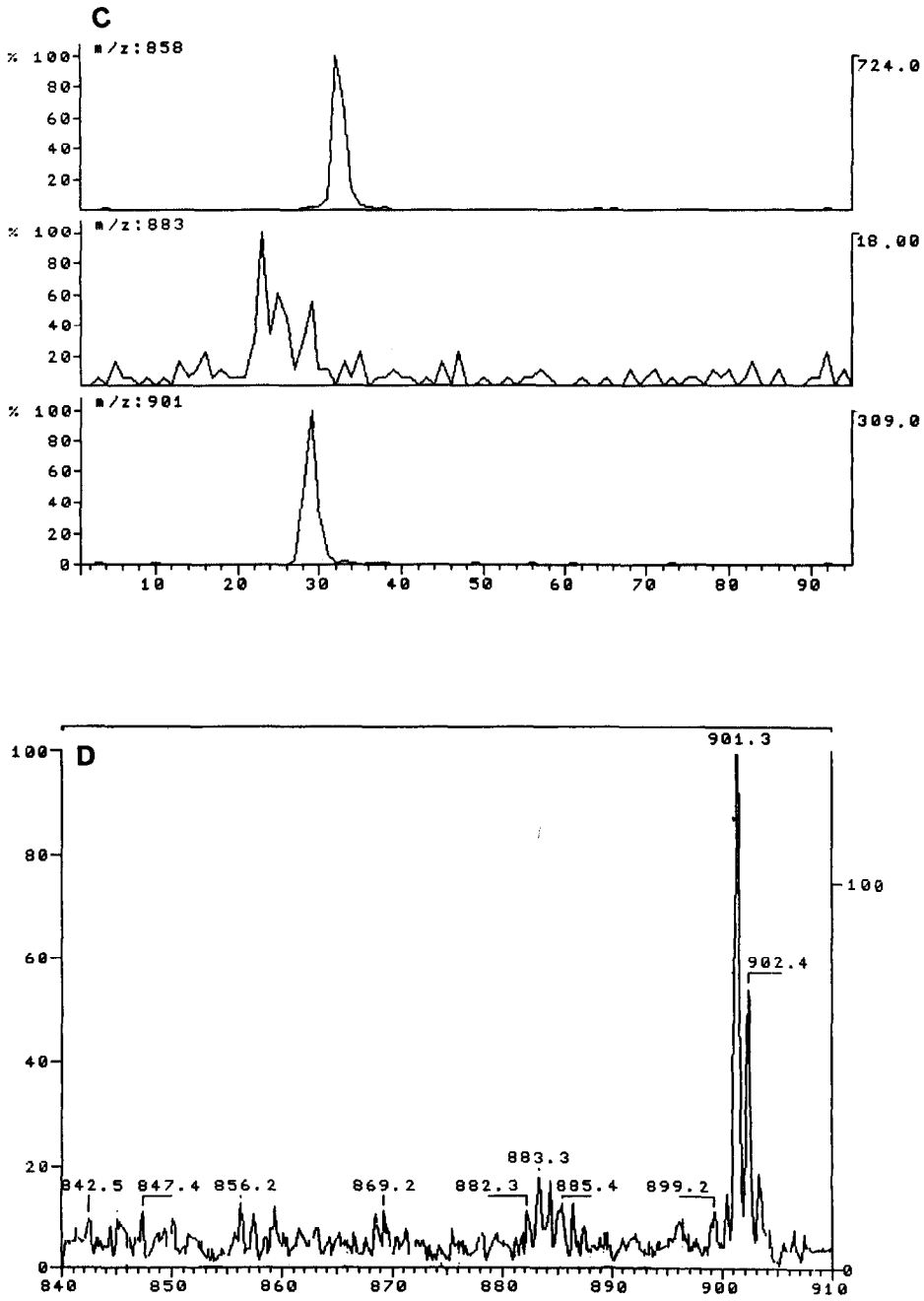


Fig. 1.

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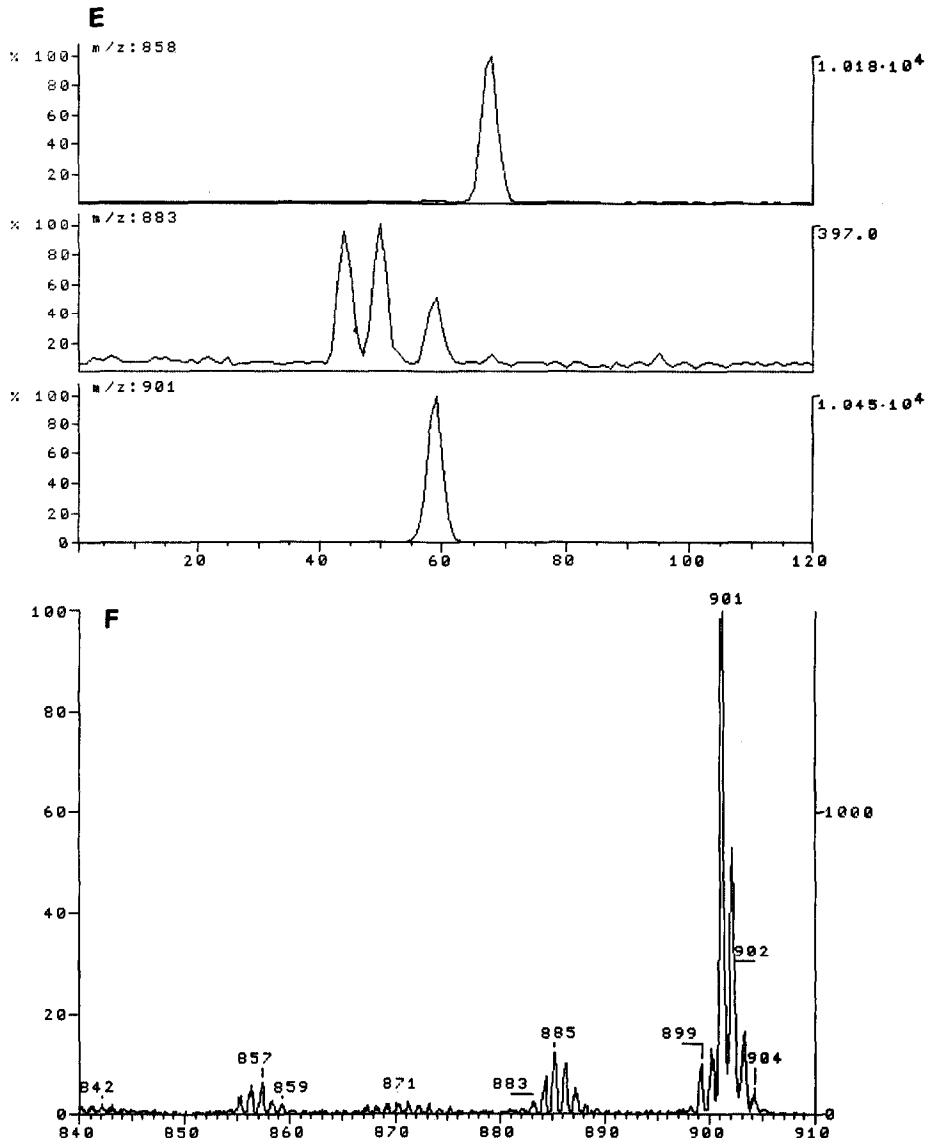


Fig. 1. (A, C and E) Reconstructed mass chromatograms (time in s) from the CE–MS analysis of the β-endorphin fragments 6–13, 8–15 and 10–17 and (B, D and F) CF-FAB mass spectra of the β-endorphin fragment 8–15. Data acquired with 1 s per scan. A and B, electron multiplier in scanning mode (*m/z* 840–910); C and D, scanning array detection (*m/z* 300–1000); E and F, static array detection with 8% window (*m/z* 840–910).

*Static array detection of peptides with molecular mass 2000–3000*

Continuous-flow FAB has been used very successfully in the detection of peptides in the mass range below 2000. Above this range hardly any data have been reported. From our own experiences the sensitivity at higher mass is lower than expected on the basis of comparing conventional FAB with CF-FAB at low mass. For

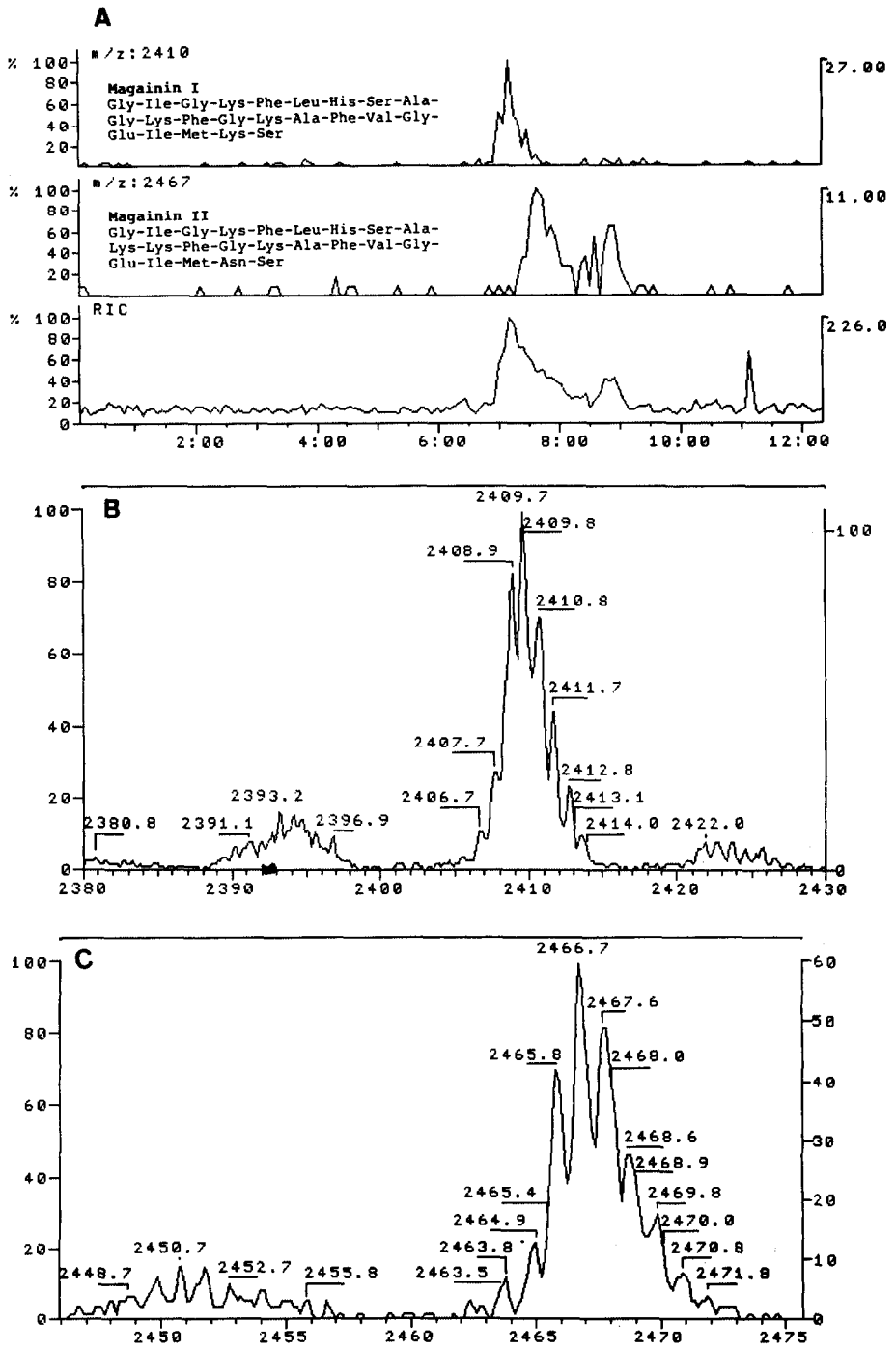


Fig. 2. (A) Reconstructed total ion and mass chromatograms (time in min) from CE–MS analysis of magainin I and II (static array detection). Data acquired with 1 s per scan. (B) CF-FAB mass spectrum of magainin I (average molecular mass of the protonated molecule = 2410.9). (C) CF-FAB mass spectrum of magainin II (average molecular mass of the protonated molecule = 2467.9).

this reason, static array detection was used to measure the performance of CF-FAB in combination with CE in the mass range 2000–3300.

The reconstructed total ion and the mass chromatograms for a CE–MS analysis of the two peptides magainin I and II are shown in Fig. 2A. Magainin I and II peptides, which originally were isolated from frog skin, have molecular masses of 2408.3 and 2465.3, respectively. Although the peptides differ in only 2 of the 23 amino acids, a separation is achieved in CE without any pretreatment of the separation capillary, and an impurity is also detected in the mass chromatogram of *m/z* 2467. The spectra obtained at the 2-pmol level with a resolution of 2000 are shown in Fig. 2B and C and are of good quality; a well defined isotope envelope is obtained.

In the analysis of the peptide galanin (average molecular mass 3211.6), the sensitivity is considerably lower. With a resolution of 1000 and injection of 15 pmol of the peptide, a good signal-to-noise ratio in the spectrum is observed, as shown in Fig. 3.

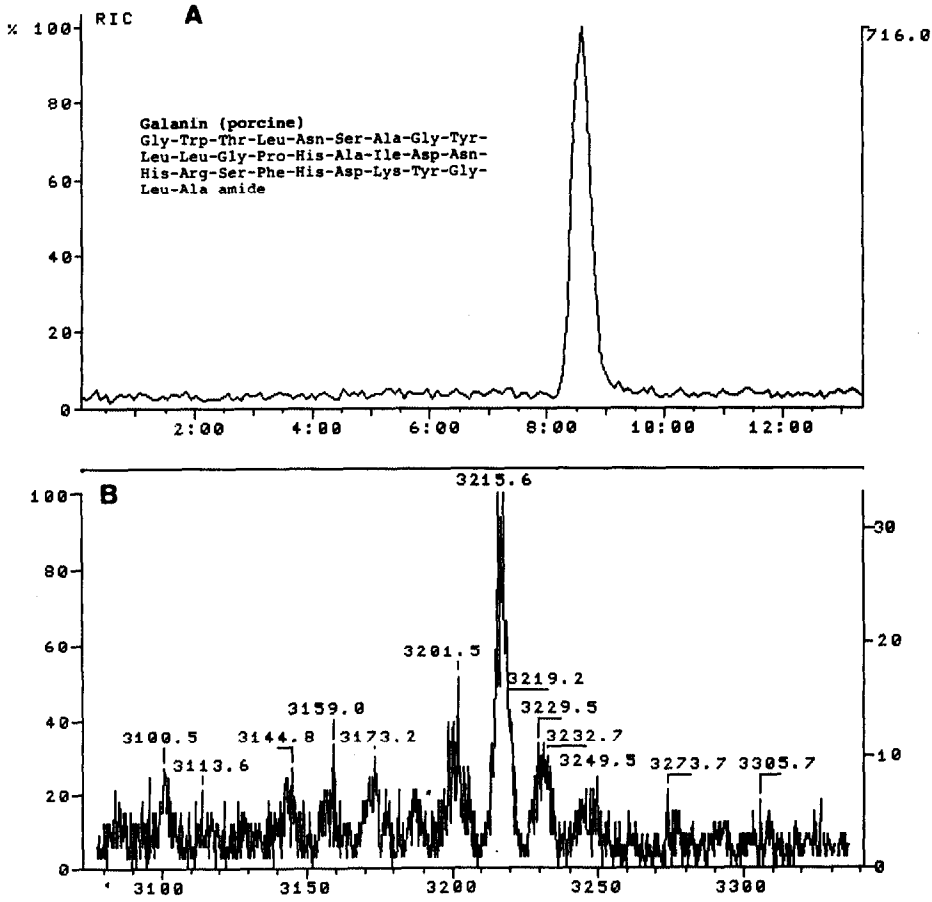


Fig. 3. (A) Reconstructed total ion chromatogram (time in min) and (B) CF-FAB mass spectrum of galanin (average molecular mass of the protonated molecule = 3211.6). Data acquired with 1 s per scan.

## CONCLUSION

The PATRIC array detection method has been found to be an important tool for the improvement of the sensitivity in mass spectrometric detection in CE–MS. The static detection mode is useful in target compound analysis and results in sensitivities comparable to those obtained by single ion monitoring, but information over a mass range of 8% around a central mass is obtained. Scanning array detection is a universal detection approach of major importance, as the gain in sensitivity can be of the order of 10–100-fold over a conventional SEM detector in combination with scanning.

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