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Journal of Chromatography A, 854 (1999) 119–130

JOURNAL OF CHROMATOGRAPHY A

Preliminary comparison of precursor scans and liquid chromatography–tandem mass spectrometry on a hybrid quadrupole time-of-flight mass spectrometer

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Abstract

Recent mass spectrometry instrumentation developments include the appearance of novel hybrid tandem instrumentation, Q-TOF, consisting of a quadrupole mass analyzer (MS1) and a time-of-flight (TOF) analyzer. The TOF analyzer is not scanned, but collects all fragment ions entering the analyzer at a given time. Thus, the typical precursor scan experiment cannot be performed. Instead, a full MS–MS spectrum can be acquired for each mass passed by MS1. Appropriate data manipulation, i.e. extracted ion current chromatograms, can correlate specific fragment ion formation to the parent ion. Precursor scanning and LC–MS–MS are compared on a Q-TOF instrument for the determination of protein modifications, including acetylation and phosphorylation. Model peptides used for phosphopeptide detection were generated from a mixture of β-casein. Model acetylated peptides were generated from a mixture of acetylated substance P1–9 and substance P1–11. The results were then applied to a more complex mixture, a digest of HIV-p24. Results indicate that precursor scanning is useful for screening, but that LC–MS–MS has a sensitivity advantage and is less susceptible to suppression effects. LC–MS–MS, therefore, appears to be better for the detection of trace components in complex mixtures.

Keywords: Mass spectrometry; Peptides; Substance P; HIV-p24

1. Introduction

Mass spectrometry has become an increasingly important technique in the determination of post-translational and/or chemical modifications of proteins through a combination of proteolysis and tandem mass spectrometry (MS–MS) [1]. Many of these modifications, e.g. phosphorylation and glycosylation, yield MS–MS spectra that contain ions characteristic of the modifying group. Carr and co-workers [2–4] and Ding et al. [5] have reported experiments in which the orifice potential is stepped during a scan so that fragment ions are observed at lower mass (high orifice potential) while molecule ions can be observed at higher mass (low orifice potential). This approach was applied to the identification of glycosylated, phosphorylated, sulfated and acylated peptides. Precursor scanning, also known as parent ion scanning, has long been a standard approach in tandem mass spectrometry and takes the stepped orifice potential approach one step further in that the specific precursor molecule ion can be determined when more than one component at a time is passed into the ion source [2,6]. Precursor scan-

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00479-3

ning has also been used in conjunction with nanospray [7] to distinguish ions due to peptides from background ions based on the appearance of specific immonium ions characteristic of peptides [8]. In these experiments, typically performed on a tandem quadrupole mass, the second mass spectrometer is set to transmit only the characteristic ion of interest, while the first mass spectrometer is scanned. Any ion that decomposes in the collision cell to yield the ion of interest will give rise to a signal. In addition to specificity, precursor scans are also characterized by increased sensitivity because the instrument is set to monitor only the ions of interest.

Recent mass spectrometry instrumentation developments include the appearance of novel hybrid tandem instrumentation, Q-TOF, consisting of a quadrupole mass analyzer as MS1 and a time-of-flight (TOF) analyzer as MS2 with orthogonal ion injection into MS2 [9]. MS2, a TOF analyzer, is not scanned, but collects all fragment ions entering the analyzer at a given time with the mass-to-charge ratio of the ion being determined by the time it takes the ion to reach the detector. Thus, the typical precursor scan experiment as carried out on a tandem quadrupole or magnetic sector instrument, in which MS2 is set to pass only selected ions, cannot be performed. An alternative experimental protocol does exist, however, in which MS1 is stepped to sequentially pass ions of increasing mass through the quadrupole mass filter into the collision cell with a full MS–MS spectrum being acquired at each step of MS1 (Fig. 1). Depending on the quadrupole mass resolution settings, the mass range of ions passed through the quadrupole can be varied. Appropriate data manipulation, i.e. extracted ion current chromatograms, can correlate specific fragment ion formation to the parent ion.

There are several potential advantages and disadvantages of this approach. Bateman et al. [10] have calculated that the sensitivity of precursor scans in this mode is approximately 2% of that obtained with a triple quadrupole instrument, when a single precursor ion is selected. The time to successfully complete the experiment for a single selected ion can also be significantly longer for a Q-TOF of than for a triple quadrupole instrument. A major potential advantage, however, is that full scan MS–MS spectra are acquired of all ions covered by the mass range scanned by MS1, including immonium ions and ions resulting from sequence-specific fragmentations. Thus, the second experiment of obtaining the full scan MS–MS spectrum of ions of interest may not be necessitated, and the data set can be probed at a later date, even after several months or years, for a different set of specific fragment ions.

Our laboratory has several projects that involve identification of sites of post-translational modification or chemical modification on proteins using proteolysis combined with mass spectrometric detection. With the recent availability of a hybrid Q-TOF mass spectrometer and of software capable of carrying out automated precursor scans, we have begun to investigate precursor scans on this instrument and to compare them to data-dependent LC–MS–MS on the same instrument.

2. Experimental

2.1. Acetylation of substance P1–9, substance P1–11, and HIV-p24

Acetylation of the lysine-ε-amino and N-terminal amino groups of substance P1–9, P1–11 (Sigma, St. Louis, MO, USA) and HIV-p24 (Austral Biologicals, San Ramon, CA, USA) were performed as previous-

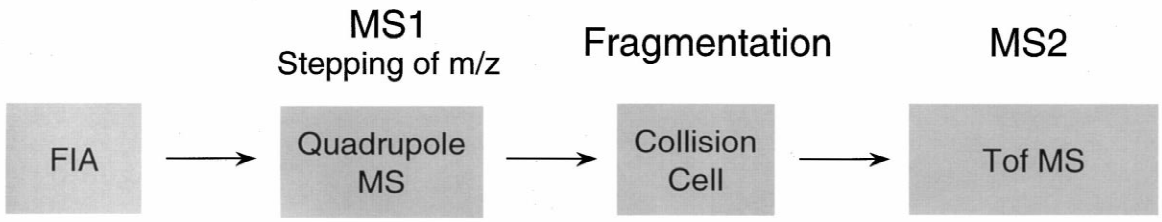

Fig. 1. Precursor scanning using Q-TOF.

ly described [11] with acetic anhydride in 200 m*M* NH_4HCO_3. Reactions were carried out for 30 min at 22°C and the pH due to the hydrolysis of the anhydride was maintained at 6.5 by addition of 25% NH_3. An approximately equimolar mixture of substance P1–9 and P1–11 was acetylated by addition of a 500-fold molar excess of acetic anhydride/amino group at a peptide concentration of 1 μg/μl. The acetylated peptides were desalted and the buffer was exchanged for a methanol–water mixture (50:50, v/v) by solid-phase extraction using a C_{18} Sep-Pak cartridge (Waters, Milford, MA, USA) following the manufacturer's instructions.

A two-step acetylation/trideuteroacetylation was employed to modify HIV-p24 which was diluted with 200 m*M* NH_4HCO_3 to a final concentration of 33 ng/μl. First, a 10 000-fold molar excess of acetic anhydride was used for partial modification using the reaction conditions described above. Subsequently, a 100 000-fold molar excess of hexadeuteroacetic anhydride (Aldrich, Milwaukee, WI, USA) was added to obtain complete modification. Acetylated HIV-p24 was purified by HPLC using a binary pumping system (model 1100) and a model 1100 UV detector (both from Hewlett-Packard, Waldbronn, Germany). Purification was carried out on a Protein C_4 (250× 4.6 mm, 10 μm particle size) reversed-phase column (Vydac, Hesperia, CA, USA) using as solvents 90% acetonitrile and water, both containing 0.1% TFA (trifluoroacetic acid), at a flow-rate of 1 ml/min.

2.2. Enzymatic digestion of HIV-p24

For the enzymatic digestion of acetylated HIV-p24, the HPLC-purified protein (approximately 100 μg) was redissolved in 20 μl acetonitrile-deionized water (50:50, v/v). The acetonitrile concentration was reduced to 10% by the addition of 80 μl of 50 m*M* NH_4HCO_3, giving a final volume of 100 μl. Proteolytic digestion was performed by adding 15 μl immobilized trypsin bead slurry (Porozyme, PerSeptive Biosystems, Framingham, MA, USA) and incubating for 4 h at 37°C. The end plug was then removed from the compact reaction column (USB Specialty Biochemicals, Cleveland, OH, USA), and the sample digest was pushed through the column into an Eppendorf tube.

2.3. Enzymatic digestion of β-casein

A tryptic digestion of β-casein (Sigma) was performed by adding 10 μl of immobilized trypsin bead slurry (PerSeptive Biosystems) to 800 μg of the protein dissolved in 800 μl of 25 m*M* ammonium hydrogencarbonate buffer, pH 8.3, in a compact reaction column (USB Specialty Biochemicals). Digestions were allowed to proceed for 2 h under the conditions described above.

2.4. Mass spectrometer

All experiments were performed on a Micromass Q-TOF mass spectrometer (Micromass, Altrincham, UK), equipped with both a nebulized nanospray electrospray source (Fig. 2), a nanospray electrospray source for use with nanovials, and MassLynx software. For nanovial sample introduction, approximately 2 μl of the sample was placed into a Micromass Type A nanospray needle. The needle voltage used was 1500 V; the cone voltage was 30 V.

In the nebulized nanospray source, nitrogen gas is introduced coaxially as the nebulizing gas. Sample introduction can be either by flow injection analysis (FIA) or by packed capillary HPLC. In the FIA experiments, injections were performed by placing the end of a 50 μm I.D. capillary column into the sample vial which is inside a stainless steel pressure vessel. Samples were forced onto the column by pressurizing with helium [12]. The helium pressure was adjusted to give a flow-rate of ~200 nl/min. The end of the capillary tubing was connected to the entrance tubing of the nebulized nanospray interface with a PTFE sleeve. Samples were diluted to 0.01 μg/μl with acetonirile–water (50:50), both containing 0.1% formic acid, before FIA analysis.

Mobile phase flow through the packed capillary column was generated using a Gilson Gradient HPLC system and controller (Gilson, Middleton, WI, USA), operating at 0.5 ml/min. The HPLC system consisted of Gilson model 305 and 306 pumps, a model 811C manometric module, and a model 805 mixing chamber. Flow through the column was reduced to approximately 200–300 nl/min using an LC Packings Acurate Splitter (LC Packings, Amsterdam, The Netherlands). The flow from the splitter was connected to an LC Packings FAMOS automatic

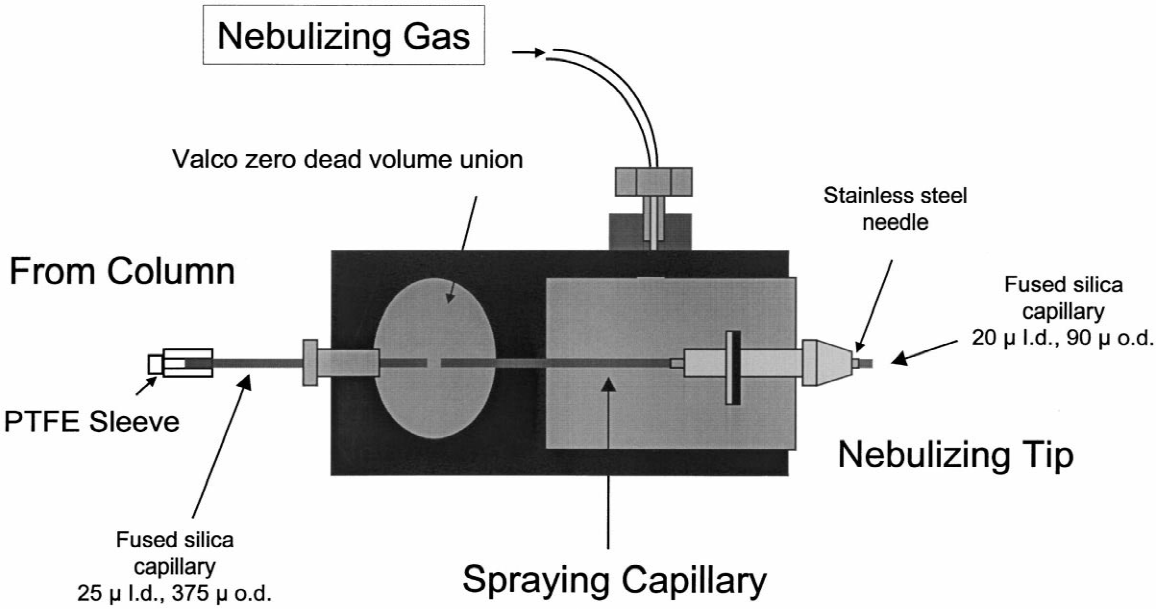

Fig. 2. Nebulized nanospray electrospray source diagram.

injector. A 1-m length of 50 μm I.D. capillary was used between the injector to the front end of the capillary column. The extra length of fused-silica before the column packing allowed easy manipulation of the column and nanospray stage. The column used for these separations was a 15-cm Hypersil C_{18} ('Pepmap') column, packed with 5 μm particles, from LC Packings. The tail of the column was connected to the entrance tubing of the nebulized nanospray interface with a PTFE sleeve (Fig. 2). A water–acetonitrile gradient was employed for the separations, with both mobile phases containing 0.1% formic acid. Gradients used were from 5 to 95% acetonitrile, with a 5-min hold at the initial conditions.

3. Results and discussion

3.1. Precursor scanning parameter determination

In the precursor scan mode, MS1 can be stepped in varying mass windows. Thus, MS1 could pass ions every u, every 5 u, etc. The mass resolution of MS1 is also variable over the range from less than 1 u passed through the quadrupole to approximately a 10–15-u mass range being passed into the collision hexapoles. Thus, if an appropriate resolution setting is used, all ions can be probed using mass steps of 5 u or greater. Because the TOF analyzer has a high duty cycle, short MS2 data acquisition times, i.e. 1–2 s, are typical. The primary parameters of the precursor scan mode, including MS1 mass steps, MS1 resolution, and MS2 data acquisition time, were investigated using an acetylated peptide (acetylated substance P), a digest of an acetylated protein (HIV-p24), and a digest of a phosphorylated protein (β-casein).

3.2. Acetylated substance P

Initial experiments were performed on an acetylated mixture of substance P1–9 and substance P1–11 using nanospray sample introduction. A series of parent ion scans were acquired with the quadrupole set to 1-, 5- and 10-u steps (Fig. 3). The initial resolution was set to pass a 3.5-u window. Similar total ion current (TIC) chromatograms (Fig. 3) and extracted ion current (EIC) chromatograms of the acetylated lysine immonium ion (Fig. 4) were observed for 1- and 5-u steps. In the case of a 5-u step at this resolution, even though there is a 1.5-u

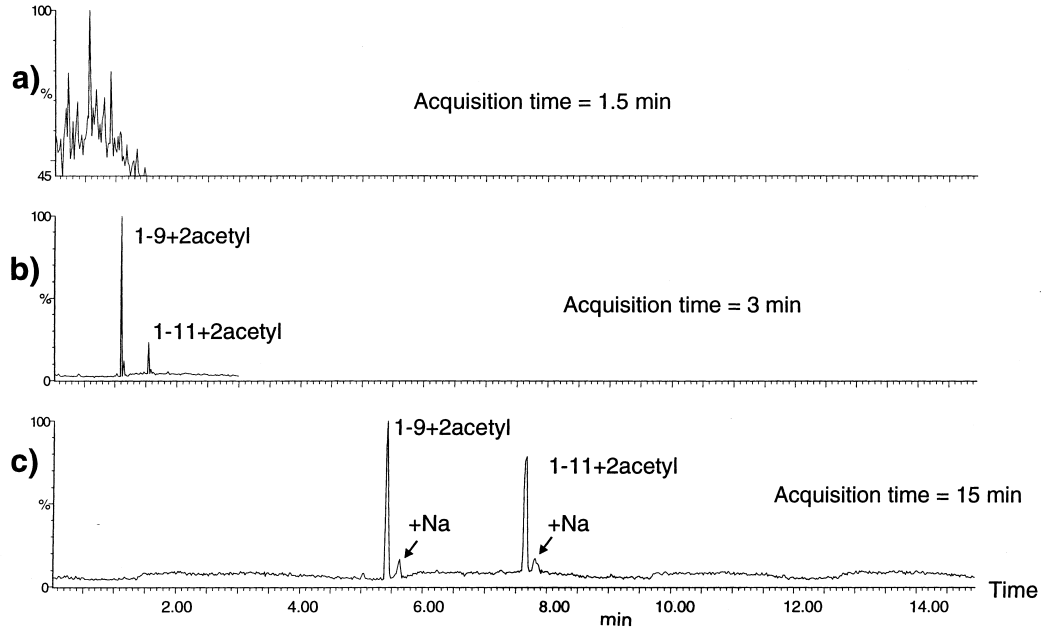

Fig. 3. Precursor scanning of a mixture of acetylated substance P1–9 and acetylated substance P1–11. Total ion current chromatograms, acquired with MS1 mass windows (steps) of (a) 10 u, (b) 5 u, and (c) 1 u, over the mass range 300–1100 u. Solution concentration, 0.01 μg/μl. Resolution, 3.5 u.

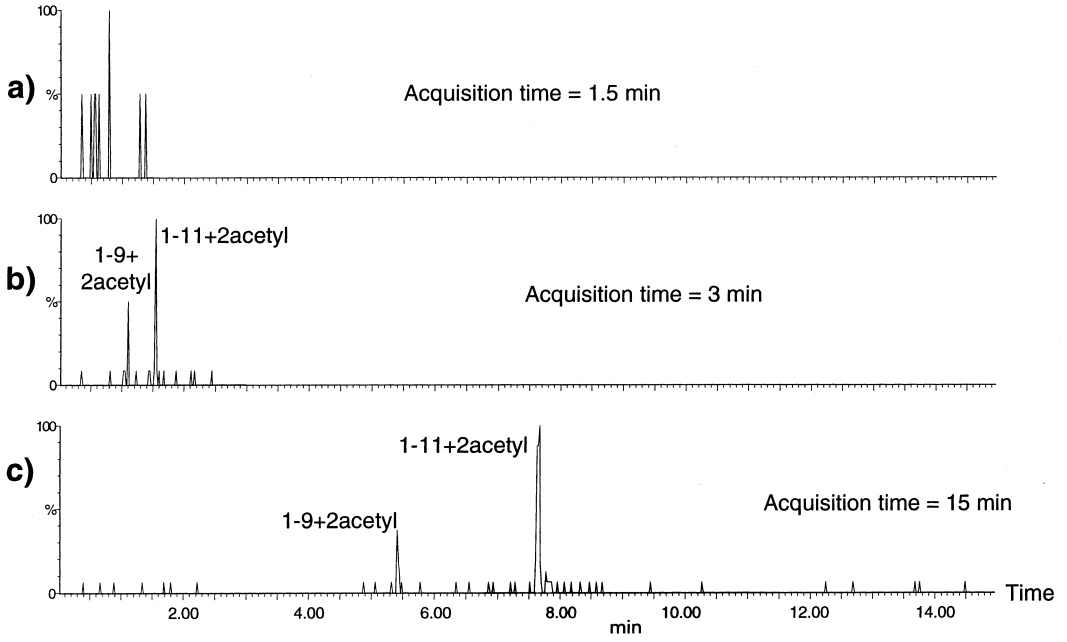

Fig. 4. Precursor scanning of a mixture of acetylated substance P1–9 and acetylated substance P1–11. Extracted ion current chromatograms of m/z 143, the immonium ion of acetyllysine, acquired with MS1 mass steps of (a) 10 u; (b) 5 u; and (c) 1 u, over the mass range 300–1100 u. Solution concentration, 0.01 μg/μl. Resolution, 3.5 u.

'gap' between steps which is not sampled, the major components were detected. This is probably because a 3.5-u window will pass at least some of the isotope peaks into the collision cell. The TIC (Fig. 3a) and the EIC (Fig. 4a) for the 10-u steps did not show any signal at an $S/N>2$. Reducing the resolution from 3.5 to 7 u did not improve the EIC. When the wider mass window, e.g. 7 u, is passed into the collision cell, increased background signals are passed into the cell, leading to poor S/N. From this set of experiments, it can be concluded that incrementing the quadrupole (MS1) window in 5-u steps with moderate mass resolution provides suitable data. The advantage of 5-u step windows over 1-u step windows is a decrease in the total analysis time, and ultimately the amount of sample consumed.

In these experiments, a 0.01-μg/μl solution was sprayed through a nanospray vial at a flow-rate of approximately 1 μl/h. For a 3-min experiment, this translates to approximately 500 fmol consumed, and, for a 15-min experiment, to 2.5 pmol. Thus, sample consumption is low compared to standard flow injection analysis at 3–5 μl/min. As discussed above, full MS–MS spectra were obtained in these experiments. The quality of the data can be seen from Fig. 5, where the MS–MS spectra of the higher mass component, doubly acetylated substance P1–11 (m/z 716), obtained at 1-u steps (Fig. 5a) and 5-u steps (Fig. 5b) are shown, respectively. Roepstorff–Fohlman nomenclature is used for the fragment ions [13].

3.3. Acetylated HIV-p24

A more complex example is that of the tryptic digest of acetylated and trideuteroacetylated HIV-p24. The protein HIV-p24 is a *gag* protein from the core of the human immunodeficiency virus (HIV) and has a molecular mass of 25 565 u. In these experiments, surface accessible lysine residues were probed by acetylating the protein under non-denaturing conditions. Initially, the protein is partially

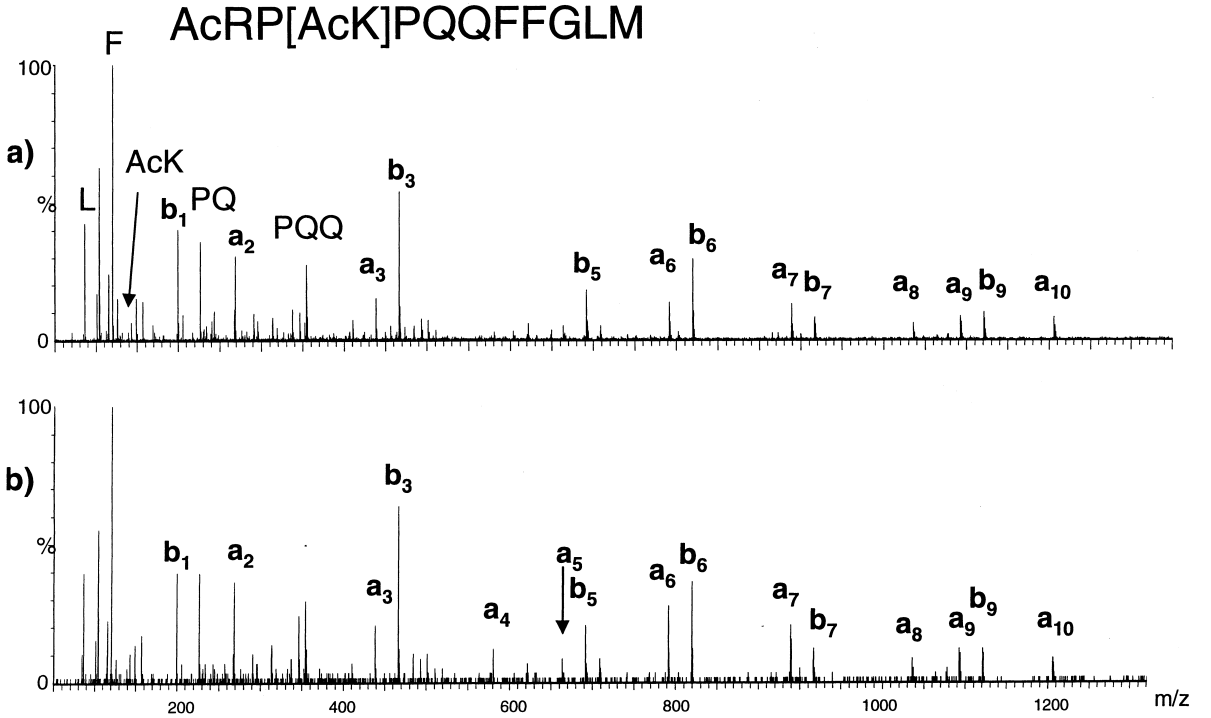

Fig. 5. Comparison of MS–MS spectra of doubly acetylated substance P1–11 from precursor scans, acquired with MS1 mass steps of (a) 1 u and (b) 5 u. Resolution, 3.5. u.

acetylated with acetic anhydride and is subsequently subjected to complete acetylation with hexadeuteroacetic anhydride. This procedure, after tryptic digestion, leads to a mixture of acetylated and trideuteroacetylated derivatives of the same peptides. The ratios of the relative ion abundances of the acetylated and trideuteroacetylated lysines in the peptide can be correlated with surface accessibilities of these lysine residues in the protein [7].

Using the nebulized nanospray source with FIA, MS1 steps of 1 u, and a mass resolution of 4 u, precursor scanning of the digestion mixture resulted in the TIC and EICs for the immonium ions of acetyllysine (m/z 143) and trideuteroacetyllysine (m/z 146) shown in Fig. 6. Due to the complexity of this mixture, MS1 steps of 1 u were used to provide more accurate determination of the mass of the parent ion. Although a number of acetyllysine-containing components could be identified from the EIC traces, the overall S/N is relatively low ($<$5:1). Due to the low noise level associated with this instrument, ions with an $S/N>2$ are significant. Spectra obtained from this experiment will be discussed in more detail in comparison with LC–MS–MS results below.

3.4. β-Casein

Phosphorylation of serine, threonine, and tyrosine residues is involved in cellular signal transduction, and identification of phosphorylated residues is an important application of mass spectrometry in biology. The most common method of detecting the presence of phosphorylated peptides has been to perform precursor scanning of m/z 79/97 (PO_3^- and $H_2PO_4^-$) under negative ion conditions [1]. To test the applicability of the corresponding precursor scans using the Q-TOF, a tryptic digest of phosphorylated β-casein was studied. The TIC and EICs for m/z 79 and 97 are shown in Fig. 7a–c. Precursors of m/z 79 and 97 are observed at masses corresponding to multiply-charged ions of tetraphosphorylated tryptic peptide T1–2. The full scan mass spectrum of this digest is shown in Fig. 7d. This spectrum also is dominated by ions due to tetraphosphorylated T1–2. A major difference between the precursor scan TIC

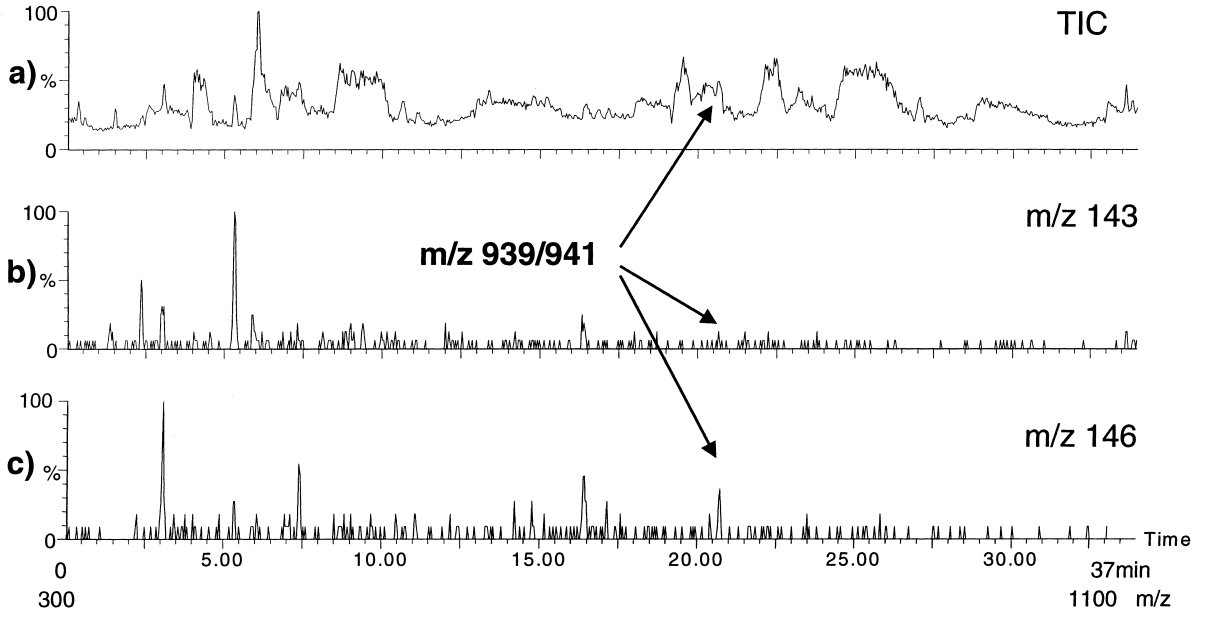

Fig. 6. Precursor scanning of a tryptic digest of acetylated HIV-p24, showing (a) the total ion current chromatogram, (b) the EIC chromatogram for the immonium ions of acetyllysine (m/z 143), and (c) the EIC chromatogram for the immonium ions of trideuteroacetyllysine (m/z 146). Positive ion nebulized nanospray; 1-u steps; collision energy, 30 eV; mass range, 350–1300 u.

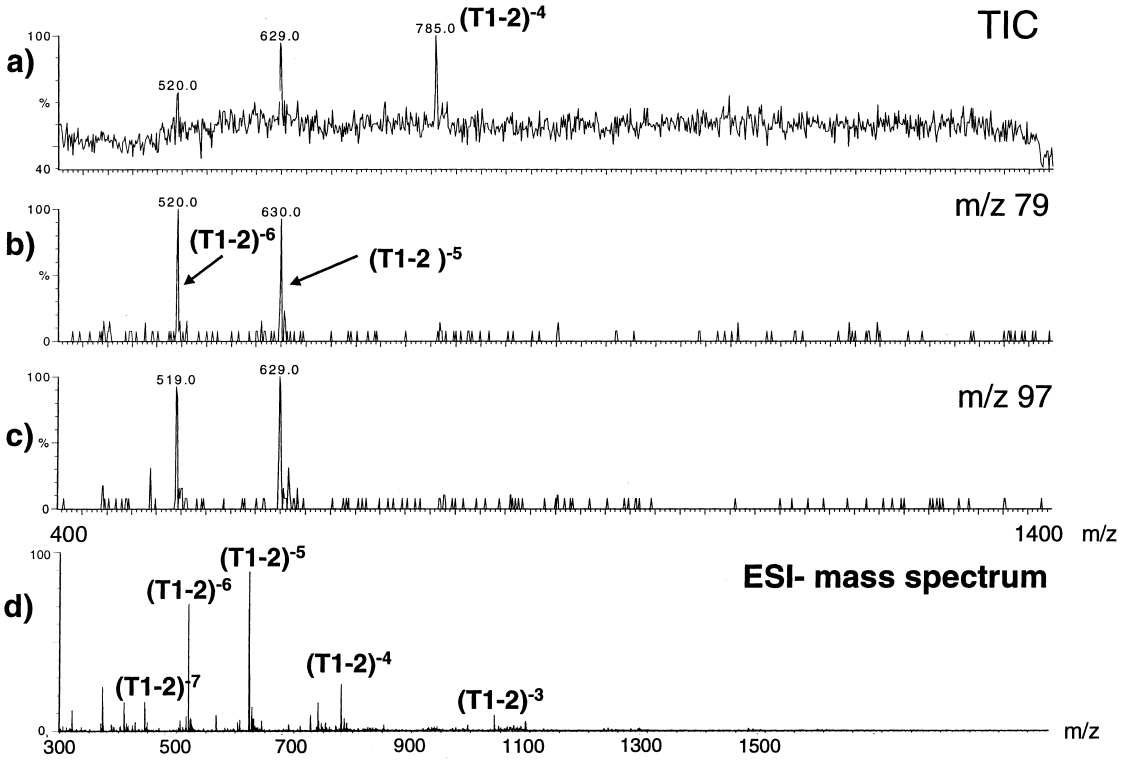

Fig. 7. Precursor ion scanning of a tryptic digest of β-casein, showing (a) the total ion current and the extracted ion chromatograms for (b) m/z 79 and (c) m/z 97, acquired in the negative ion mode. The full scan negative ion mass spectrum of the digest is shown in (d). The mass range scanned was from 400 to 1400 u; collision energy, 40 eV.

(Fig. 7a) and the full scan mass spectrum (Fig. 7d), compared with the EIC traces (Fig. 7band c), is the relative abundance of the $(M\text{-}4H)^{4-}$ ion. The higher abundance of this ion in the TIC trace than in the EIC traces was probably due to insufficient collision energy to decompose the 4− ion. Future versions of the software are scheduled to have the capability of doing multiple collision energies which will eliminate this problem. These results, however, demonstrate the applicability of precursor ion scanning in the negative ion mode to the identification of phosphorylated peptides.

4. LC–MS–MS

LC–MS–MS experiments using data-dependent switching were carried out on the same compounds for comparison with the precursor scanning data. In the data-dependent switching mode, the instrument is triggered to perform MS–MS on a series of ions in the mass spectrum when these ions have reached a certain preselected abundance. A family of MS–MS 'chromatograms' (corresponding to the most abundant ion in the preceding MS, the second most abundant ion, etc., respectively) is generated which can then be searched for a particular characteristic mass. The data system may be programed to save only those scans where the abundance conditions are met, which gives a somewhat artificial appearance to the chromatogram, but saves considerably on disk space. For comparison with precursor ion scanning, data were obtained for the same three samples used above.

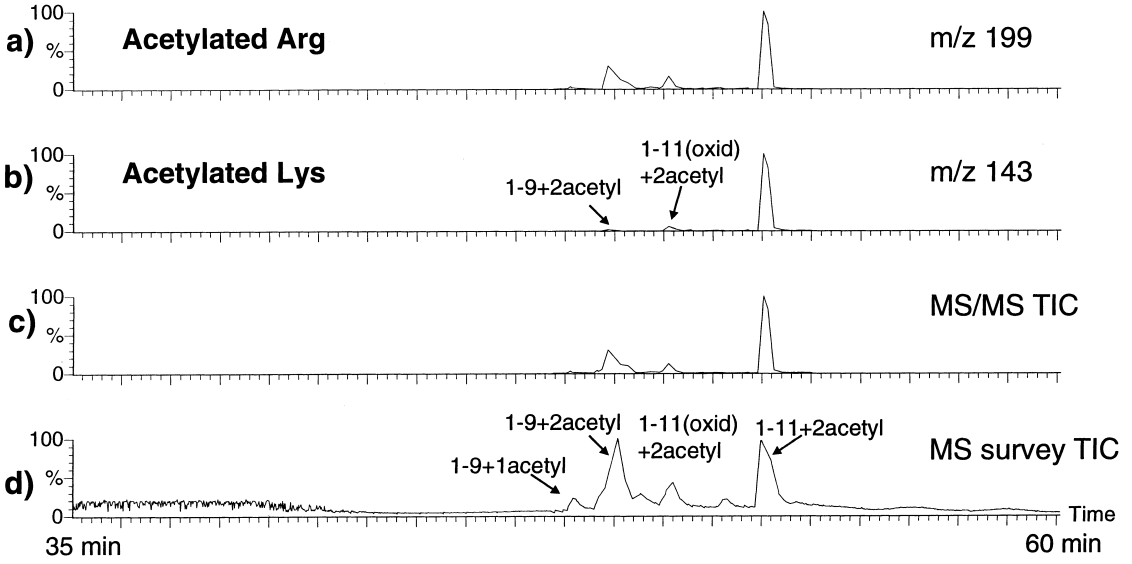

Fig. 8. LC–MS–MS of acetylated substance P1–9 and 1–11, showing (a) the EIC chromatogram for the b_1 ion of acetylarginine (m/z 199); (b) the EIC chromatogram for the immonium ion of acetyllysine (m/z 143); (c) the total ion current MS–MS chromatogram; and (d) the total ion current chromatogram. Solution concentration, 0.01 μg/μl; 0.2 μl injected.

4.1. Acetylated substance P

Fig. 8a–c show a set of MS–MS chromatograms which were generated by performing data-dependent switching during an LC–MS analysis (Fig. 8d) of the mixture of acetylated substances P. In Fig. 8a,b, the MS–MS data from this mixture has been searched for the mass of the b_1 ion from acetylated arginine (the N-terminal amino acid of both substances P) m/z 199, and the immonium ion of acetylated lysine m/z 143. The HPLC peaks of components containing these immonium ions can be identified, and the MS–MS spectra of these components can be displayed. The component eluting at 52.5 min, seen to contain both acetylated residues, was identified as substance P1–11+2 acetyl groups based on its MS–MS spectrum. Comparing the EIC of m/z 143 obtained by precursor scanning (Fig. 3c) with that obtained from the LC–MS–MS analysis (Fig. 8b), one can see that the two most abundant components can be observed by both techniques, but that the lower abundance components are observed only in the LC–MS–MS run. This is consistent with the higher instantaneous concentration of analyte delivered to the source using LC due to the concentrating effect of the LC relative to FIA.

4.2. β-Casein

Fig. 9b shows the reconstructed TIC for the LC–MS–MS of the tryptic digest of β-casein. These data were acquired in a data-dependent switching analysis where the data system has generated MS–MS spectra when specific abundance criteria have been met in the MS survey scan. Components whose MS–MS spectra include either m/z 79 or 97, ions characteristic of the phosphate group in the negative ion mode, elute at a retention time of 31–32 min (Fig. 9a). Other LC peaks, giving negative ion spectra, elute later in the chromatogram, and MS–MS data have been recorded for these components as well. An expanded view of the 31–32 min region is shown in the inset. At the collision energies used to produce abundant m/z 79/97 ions, the full-scan negative-ion MS–MS spectrum gives molecular ion information

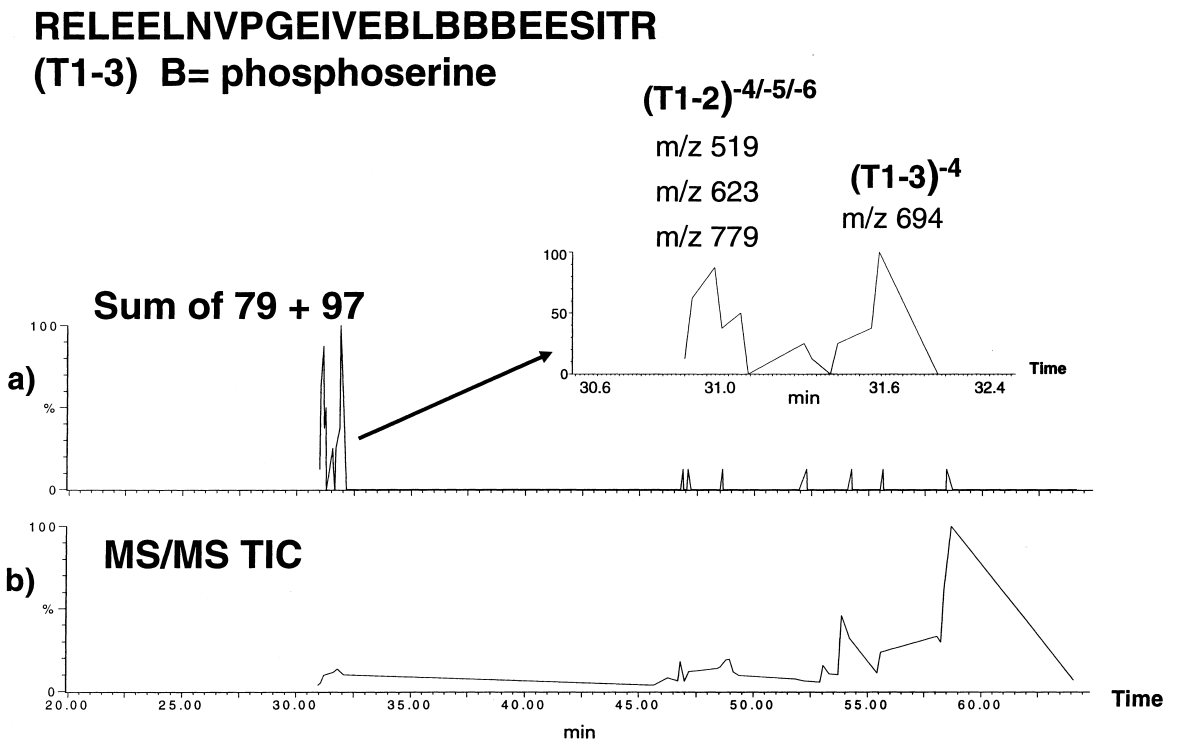

Fig. 9. LC–MS–MS of a tryptic digest of β-casein. (a) The extracted ion current chromatograms for the sum of m/z 79 and m/z 97 and (b) the MS–MS total ion current chromatogram, acquired in the negative ion mode. Solution concentration, 1 μg/μl; injection volume, 5 μl; collision energy, 40 V.

which can be used to identify the tryptic fragment, but is uninformative as to the amino acid sequence.

4.3. Acetylated HIV-p24

The results for the LC–MS–MS analysis of a more complicated mixture, the products of a tryptic digestion of acetylated and deuteroacetylated HIV-p24, are shown in Fig. 10. EIC chromatograms for the immonium ions of trideuteroacetylated lysine and acetylated lysine, obtained by searching the MS–MS TIC (Fig. 10c), are shown in Fig. 10a,b. The component which elutes at 19 min can be seen to contain both acetylated and trideuteroacetylated residues. Based on its MS–MS spectrum (Fig. 11a), this compound can be identified as amino acid residues 68–82. This is the same component which was pointed out in Fig. 6 (m/z 939/941). The S/N ratios of the peaks in the m/z 143 and 146 EIC traces for this component obtained from the LC–MS–MS experiment (Fig. 10a,b) are at least 20 times higher than that observed for the corresponding EICs from the precursor scan experiments (Fig. 6b,c). A comparison of the LC–MS–MS spectrum with the precursor ion MS–MS spectrum of this component is shown in Fig. 11. Even with the relatively poorer S/N observed for the acetyllysine immonium ion in the precursor scan mode, the MS–MS spectrum obtained in the precursor scan experiments compares well with that obtained in the LC–MS–MS experiments.

5. Conclusions

Precursor scanning using a Q-TOF mass spectrometer is relatively fast and useful data can be acquired at low resolution (a 3.5-u window). This is

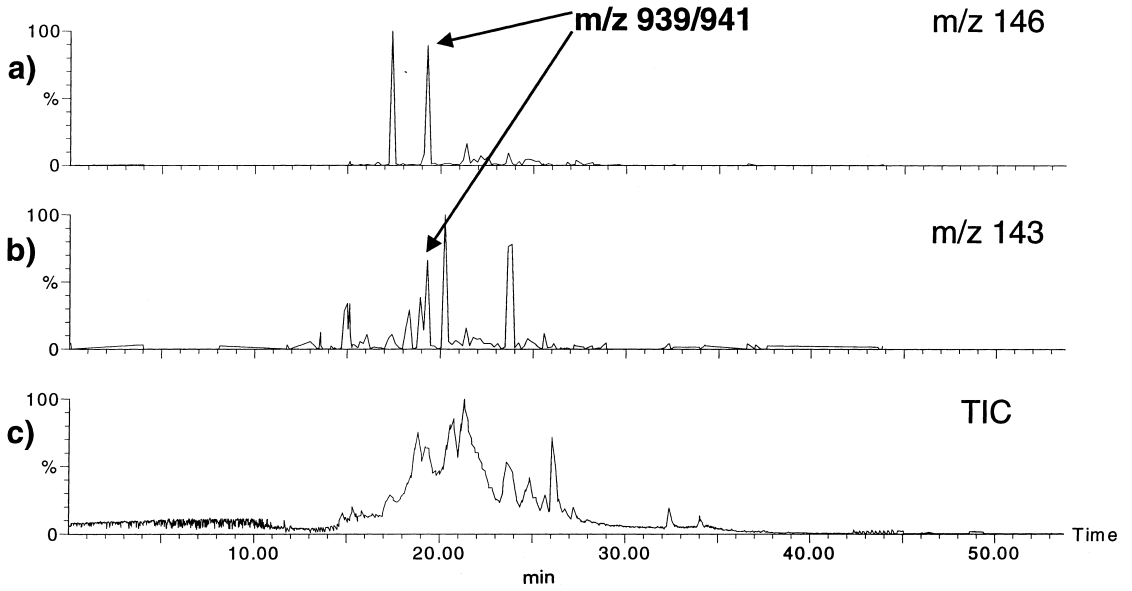

Fig. 10. LC–MS–MS of a tryptic digest of acetylated HIV-p24, showing (a) the reconstructed ion chromatgram for the immonium ion of trideuteroacetyllysine (m/z 146); (b) the reconstructed ion chromatogram for immonium ion of acetyllysine (m/z 143); and the (c) the total ion chromatogram. The injection volume was 1 μl. The data were acquired at a collision energy of 30 V.

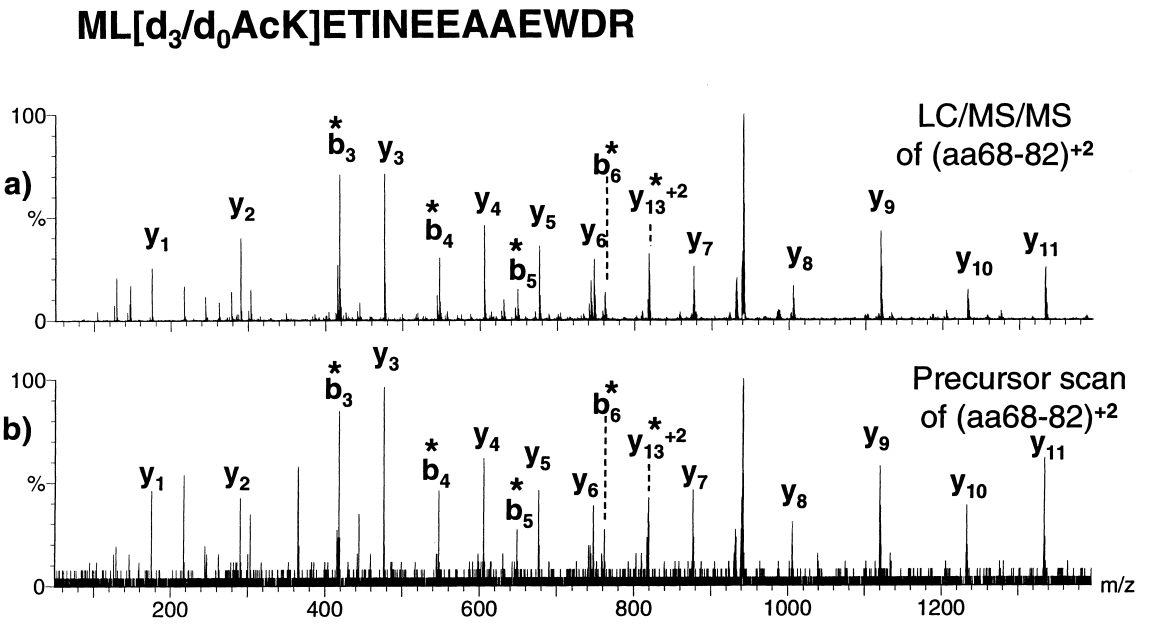

Fig. 11. MS–MS mass spectrum of the doubly-charged ions of m/z 939/941, a doublet due to the presence of [$^1H_3$]acetyllysine and [$^2H_3$]acetyllysine (acetyllysine-d_3) from a tryptic digest of acetylated HIV-p24 obtained from (a) an LC–MS–MS analysis, and (b) a precursor scanning experiment. Fragment ions denoted by an asterisk contain the acetyllysine-d_0/d_3 residue.

a good technique for screening for specific post-translational modifications, and yields good MS–MS spectra. The disadvantages associated with this technique are suppression effects and dynamic range effects, which may lead to missing the trace components of a mixture.

LC–MS–MS has the LC advantage of concentrating the components, so that they elute at a higher effective concentration than in the original sample. LC–MS–MS is also less subject to suppression and/or dynamic range effects, since fewer components coelute. A disadvantage of this technique is the need for more equipment (i.e. an HPLC system). Also, like other techniques involving liquid chromatography, such as conventional LC–MS, there is a longer analysis time, due to both the actual run time and the column re-equilibration time. LC–MS–MS also uses significant amounts of disk space, due to the large amount of data acquired (both MS and MS–MS scans).

Acknowledgements

This work was supported by a research stipend for C.B. from the Deutsche Forschungsgmeinschaft, Bonn, Germany.

References

[1] K.L. Busch, G.L. Glish, S.A. McLuckey, in: Mass Spectrometry/Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry, VCH, Weinheim, 1988.
[2] M.J. Huddleston, M.F. Bean, S.A. Carr, Anal. Chem. 65 (1993) 877.
[3] S.A. Carr, M.J. Huddleston, M.F. Bean, Protein Sci. 2 (1993) 183.
[4] M.J. Huddleston, R.S. Annan, M.F. Bean, S.A. Carr, J. Am. Soc. Mass Spectrom. 4 (1993) 710.
[5] J. Ding, W. Burkhart, D.B. Kassel, Rapid Commun. Mass Spectrom. 8 (1994) 94.
[6] S.A. Carr, M.J. Huddleston, R.S. Annan, Anal. Biochem. 239 (1993) 183.
[7] M. Wilm, M. Mann, Anal. Chem. 68 (1996) 1.
[8] M. Wilm, G. Neubauer, M. Mann, Anal. Chem. 68 (1996) 527.
[9] H.R. Morris, T. Paxton, A. Dell, J. Langhorne, M. Berg, R.S. Bordoli, J. Hoyes, R.H. Bateman, Rapid Commun. Mass Spectrom. 10 (1996) 889.
[10] R.H. Bateman, R. Carruthers, J.B. Hoyes, A.J. Giles, J.I. Langridge, K. Malone, R. Bordoli, in: Proceedings of the 46th Annual Conference on Mass Spectrometry and Allied Topics, Orlando, FL, 1 June 1998, p. 42.
[11] M.O. Glocker, C. Borchers, W. Fiedler, D. Suckau, M. Przybylski, Bioconj. Chem. 5 (1994) 583.
[12] L.J. Deterding, M.A. Moseley, K.B. Tomer, J.W. Jorgenson, Anal. Chem. 61 (1989) 2504.
[13] P. Roepstorff, J. Fohlman, Biomed. Mass Spectrom. 11 (1984) 601.