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FOURIER TRANSFORM- ION CYCLOTRON MASS RESONANCE SPECTROSCOPY

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Abstract

Mass spectrometry is essentially a technique for "weighing" molecules. Mass spectrometry is based upon the motion of a charged particle, called an ion, in an electric or magnetic field. Mass spectrometry relies on the formation of gas-phase ions (positively or negatively charged) that can be isolated electrically (or magnetically) based on their mass-to-charge ratio (*m/z*). Where as in Fourier transform ion cyclotron mass resonance spectroscopy (FTICR-MS) the m/z ratio measurement of an ion is based upon the ion's motion or cyclotron frequency in a magnetic field. Ions are detected by passing near detection plates and thus differently from other mass detectors/analysers in which ions are hitting a detector (at different times or places),The ions are trapped in a magnetic field combined with electric field perpendicular to each other (Penning trap). They are excited to perform a cyclotron motion. The cyclotron frequency depends on the ratio of electric charge to mass (m/z) and strength of the magnetic field.This spectrometric analysis can provide important information about the analytes, including their structure, purity, and composition.

***Keywords*:** weighing molecules, ion, mass-to-charge ratio, cyclotron frequency.

1. Introduction [1-3]

Mass spectrometry relies on the formation of gas-phase ions (positively or negatively charged) that can be isolated electrically (or magnetically) based on their mass-to-charge ratio (*m/z*).For FTICR-MS the m/z ratio measurement of an ion is based upon the ion's motion or cyclotron frequency in a magnetic field. Ions are detected by passing near detection plates and thus differently from other mass detectors/analysers in which ions are hitting a detector (at different times or places),The ions are trapped in a magnetic field combined with electric field perpendicular to each other (Penning trap) . They are excited to perform a cyclotron motion. The cyclotron frequency depends on the ratio of electric charge to mass (m/z) and strength of the magnetic field. Applications of FTICR-MS include identifying and quantitating pesticides in water samples, it identifying steroids in athletes, determining metals at ppq (Parts Per Quadrillion) levels in water samples, looking for life on Mars, determining the mass of an 28Si atom with an accuracy of 70 ppt, and studying the effect of molecular collision angle on reaction mechanisms. This is an essential technique for "weighing" molecules. FTICR-MS differs significantly from other mass spectrometry techniques in that the ions are not detected by hitting a detector such as an electron multiplier but only by passing near detection plates. Additionally the masses are not resolved in space or time as with other techniques but only by the cyclotron (rotational) frequency that each ion produces as it rotates in a magnetic field. Thus, the different ions are not detected in different places as with sector instruments or at different times as with time-of-flight instruments but all ions are detected simultaneously over some given period of time. In FT-ICR MS, resolution can be improved by increasing the strength of the magnet (in teslas) or by increasing the detection duration.

2. Principle

In the simplest mass spectrometer, organic molecules(gas) are bombarded with electrons (high energy electron beam 70ev) by using tungsten or rhenium filament and converted to high energy positive charged ions (molecular ions), which can break up into smaller ions (fragment ions) the loss of an electron from a molecule leads to radical cation. This process can be represented as $M - M^+$ (molecular ion) (loss of electron) the molecular ion M^+ decomposes to pair of fragments, which may be either a radical cation. Where as in FTICR-MS, Ions can be generated in an external ion source outside of the magnet and transferred into the cell, or volatile substances can be ionized within the cell. The ions are confined in the cell by the strong magnetic field and by an electric field created by the

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cell's two end-cap electrodes. The trapped ions describe a circular orbit with a characteristic orbital frequency, also known as the ion cyclotron frequency ω_c where,

$$\omega_c = \frac{qB}{m}$$

B_0 is the magnetic flux density and m/q is the mass-to-charge ratio of the ion. On applying an excitation signal with a frequency ω_c, the ions will absorb energy and the ion ensemble will describe a coherent cyclotron motion with a larger orbital radius. The ion packet induces an image current in a pair of detector electrodes. The Fourier transform is a mathematical tool to convert the induced transient signal from the time domain to the frequency domain, which directly gives the mass-to-charge ratio of the ion.

When a positive potential is applied, as the molecule are positively charged, they get, repelled and travel with a great speed in straight path.

i,e potential energy = kinetic energy of molecule

$eV = ½ mv^2$

e - Charged ion
V- Accelerating velocity
m – mass
v – Velocity after acceleration

When a magnet or electric field is applied, the positive charged fragments travelling in a straight path, now travels in a carved path, when they travel in carved path under the influence of magnetic field, the fragments are separated into different masses because of radius of

$m/e \alpha r^2$

r – radius of path

Therefore mass α (radius of path ion)

Since e = 1 (unit of positive charge)

In recent years, FT-ICR has proven itself to be an excellent mass analysing technique, providing ultra-high resolving power, high mass accuracy and tandem and higher- order tandem mass spectrometry [$(MS/MS)^n$] capability through non-destructive detection. Advances in electronics and computers together with the development of electro spray ionisation (ESI) have further improved the performance of FTICR mass spectrometry systems and have made it possible to analyse biological macromolecules.

3. Instrumentation [4-10]

A basic mass spectrometer contains following parts

1.) Vacuum pump
2.) Sample introduction device
3.) Ionization source
4.) Mass analyzer or ion separator
5.) Ion detector

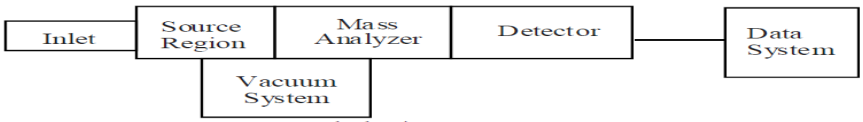

Figure 1: Block diagram of a mass spectrometer

1.) Vacuum pump

✓ To ensure the filament does not burn out.
✓ Helps to vaporize the sample to be vaporized
✓ Prevents ions, once formed being lost by collision with atmospheric gas
✓ Removes the sample from the instrument after analysis

2.) Sample introduction: (inlet system): The inlet system is used to introduce sample (milligram or nanogram) into the spectrometer. With the help of inlet system sample is converted to gaseous ions (system contains means for volatilizing solid or liquid samples). A sample studied by mass spectroscopy may be gas, liquid or solid. The sample should be finally converted to vapour state to obtain stream of molecules that must flow into the ionization chamber.

3.) Ionization source: From the inlet system sample is introduced into ionization chamber, where a beam of electrons is bombarded with the molecules of samples, to convert the molecules into the ionized form. The ion sources used in mass spectroscopy is classified into two methods, 1.) Gas phase source 2.)Desorption source These source impart high or sufficient energy to analyte molecule, so that they are left in a highly excited energy state. Relaxation then involves rupture of bonds, producing fragment ions that have M/e ratio less than molecular ion.

4.) Mass analyzers: This is the part where separation of ions according to their mass is observed. An ideal mass analyzer should be capable of distinguishing between minute mass differences and should allow passage of sufficient number of ions to yield readily measurable ion currents. Also should have high resolution (R) and high transmission of ions (I).In order to undergo FT-ICR, a powerful mass analyzer where a sample must first be ionized. That means turning the liquid sample into a gas while applying a charge to it, making each of the atoms or molecules under study a positively or

negatively charged particle (ion). The charge is critical to allowing the cyclotron's magnet to determine its mass.

5.) Detectors: Detection of ions is based upon their charge or momentum. For large signals a faraday cup is used to collect ions and measure the current. Older instruments used photographic plates to measure the ion abundance at each mass to charge ratio. Most detectors currently used amplify the ion signal using a collector similar to a photomultiplier tube. These amplifying detectors include: electron multipliers, channeltrons and multichannel plates. The gain is controlled by changing the high voltage applied to the detector. A detector is selected for its speed, dynamic range, gain, and geometry. Some detectors are sensitive enough to detect single ions.

Along with these above mentioned there are also a horizontal bore magnet is aligned with a metal frame which supports a vacuum system, ion sources and an inlet system for the introduction of volatile samples. From the main vacuum chamber a titanium tube leads into the centre of the magnet and ends with a cell where the ions are stored. The metal frame runs on tracks and can easily be moved away from the magnet to provide access to the cell. The instrument is controlled by and data is retrieved and analysed on a single Silicon Graphics O2 or Indy (Warwick) workstation. A number of remote workstations are used for subsequent data analysis.

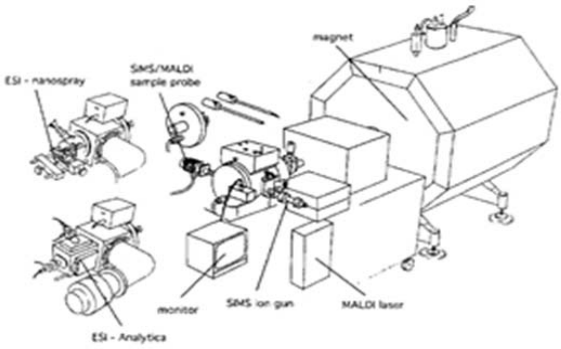

Figure 2: FTICR- Mass Spectrometer

4. Working [11-16]

4.1 Pumps and vacuum: The vacuum system is a differentially pumped, oil-free, ultra-high vacuum system capable of sustaining a base pressure of below 5×10^{-9} mbar using either turbomolecular pumps backed by mechanical rough pumps or cryogenic pumps (Warwick). The ESI source is equipped with a fore pumping system which includes one mechanical pump and a 250 Ls turbomolecular pump. The system is compatible with a wide range of reagent and buffer gases. The part of the system within the magnet bore can be cooled and heated from ambient temperature up to 150°C for baking or for blackbody infrared radiative dissociation (BIRD) experiments. A gate valve separates the cell side of the vacuum system from the source side allowing ion source changes or maintenance without disturbing the ultra-high vacuum.

4.2 Magnet: The magnet is a 9.4T central-field, superconducting (Nb_3Sn) magnet (Magnex Ltd, UK) with passive shielding. The magnet system includes a He and N_2 monitor, an emergency quench unit, transfer lines for liquid He and liquid N_2 and a line for recycling He. The cryogen consumption is <15 L N_2 (liquid) / 24 h and <1.5 L He (liquid) / 24 h. About 90% of the He is recycled in-house for the magnet. The room temperature bore is 160 mm. The field homogeneity at the centre of the magnet within a cylindrical volume of 30 mm diameter and 60 mm length has been measured to be <8.2 ppm peak to-peak and <3.4 ppm within a 15 mm diameter cylindrical volume of the same length. The passive shielding surrounding the magnet consists of an iron cage with a weight of 11 metric tonnes. The shield reduces the outside fringing fields to <5 gauss radially at the outer surface of the magnet shield and <10 gauss axially at 2 m from the centre of the magnet.

4.3 The cell: The INFINITY cell is of cylindrical geometry and consists of one pair of plates used for detection and, offset by 90°, a second pair of plates that is used to introduce radio frequency excitation pulses. Two plates with annular entrance holes cap the cell. The cell is equipped with the SIDEKICK ion accumulation system and a quadrupolar excitation axialisation (QEA) system. The cell electronics include a pulse shaping system for generation of frequency shots, correlated frequency sweeps, on-resonance and sustained off-resonance irradiation (SORI) for collision induced dissociation (CID) experiments, electronics for dynamical trapping of ions and a preamplifier and detection electronics for direct and heterodyne-mode detection. Outside the cell is an electron gun for internal electron impact (EI) ionization and chemical ionisation (CI) of volatile substances. This electron gun can also be used for electron capture dissociation (ECD). The electron gun can be removed and replaced with a laser for infrared multiphoton dissociation (IRMPD) experiments.

4.4 Data acquisition and control: Data are acquired through a 12-bit fast (10 MHz, broad-band mode) and a 14/16-bit slow (400 kHz, heterodyne mode) digitizer, with an acquisition memory of 1 Mbytes. Data is subsequently transferred to either a Silicon Graphics workstation or an Indy workstation (Warwick) via a dedicated Ethernet connection between the acquisition computer and the workstation. The workstation is equipped with a second Ethernet card for external communication. The system is controlled by and data is retrieved and analysed with the Bruker XMASS™ software package, presently at version 5.0.6.

4.5 Ion sources and sample introduction [20]: The mass spectrometer is equipped with four external ion sources and an electron gun for internal (in cell) electron impact (EI) ionisation and low-pressure chemical ionisation (CI). The four external sources are an electrospray ionisation (ESI) source, matrix-assisted lasers desorption/ionisation (MALDI) source, a secondary ion mass spectrometry (SIMS) ion source and a switchable EI/CI source.

The ESI source (Analytica, Branford, CT, USA) is equipped with an inlet glass capillary (15 cm length and 0.5 mm i.d. with platinum capping at both ends). The spraying needle is a stainless steel capillary with 0.1 mm i.d. N_2 nebulising gas is used to assist the spray, and a counter-flow of heated drying gas is used to promote solvent evaporation from the electrosprayed droplets. The electrospray source is fitted with a holder for nanospray needles and a small aperture stainless steel cap for the glass capillary. Ions are accumulated in a computer-controlled hexapole ion trap behind a skimmer, and are pulsed into the spectrometer. The sample is infused using a syringe pump (ATI Orion, model Sage™ 361 or Cole Parmer 74900 series).

The MALDI source consists of an UV (337 nm) N_2 laser with optics, filters, attenuators, a manually-controlled sample probe and a CCD camera with a monitor for visual monitoring of the sample. The SIMS ion source has a pulsed 25 keV Cs^+ ion gun and uses a similar sample probe to the MALDI source.

The external EI/CI source uses a direct inlet probe (DIP) that can be heated up to 400°C. Alternatively, a dedicated port for introduction of volatile liquids and gases can be used for EI/CI. On the Uppsala instrument, the SIMS source is always mounted. Switching between the external ion sources (MALDI, EI/CI or ESI) is relatively easy, as only the front flange of the source chamber has to be changed.

4.6 Computer-controlled fast valves for gas introduction: Experiments that require introduction of gas, two computer-controlled fast gas-inlet valves are provided. By opening an inlet valve connecting the cell and a volume of collision gas (e.g. Ar or CO_2 at a pressure of 5 mbar) for ~10 ms, the pressure in the cell is raised to a level (~10^{-7} mbar) suitable for those experiments.

5. Modifications [18-19]

A new electrospray has been assembled to allow lower flow rates to be used. This source has a stainless steel needle (0.1 mm i.d.) etched to a conical shape. This needle is connected to a syringe through a short piece of Teflon tubing. The sample is loaded to the spraying end of the needle. No nebulising or drying gas is used. Flow rates are between 0.2 and 0.4 µL min^{-1}. A further improvement to the electrospray source is the fitting of a computer controlled shutter. This A new electrospray source has been assembled in shutter essentially closes the spectrometer so that no more ions are accumulated in the hexapole ion trap, allowing investigation of multipole storage-assisted dissociation (MSAD). A further advantage is that contamination of the source is reduced. The flexibility of the XMASS program has permitted the compilation of experimental pulse programs controlling not only this shutter, but also the internal EI gun used to perform ECD experiments.

Negative-ion detection mode [21-24]: Switching from positive-ion detection to negative-ion detection mode can be easily achieved by changing the polarity of the ion source and transfer optics. Routine or advanced experiments can be performed either in the positive or in the negative mode, e.g. broadband and heterodyne detection, resonant and sustained off resonance irradiation CID.

5.1 Performance characteristics [25-30]

Mass-to-charge range: Since the cyclotron frequency is inversely proportional to mass, the sampling rate and the bandwidth of the electronics set a lower mass limit. With a 9.4 T magnetic field and a sampling frequency of 10 MHz, the lower mass limit is m/z ~ 29 according to the Nyquist criterion. C2H5+ ions (m/z = 29) formed using methane regent gas in the external chemical ionisation source have been detected. The highest detected m/z value to date is singly-charged ubiquitin (m/z = 8565) formed by MALDI.

Resolving power: Resolving power, here defined as $m/\Delta m_{FWHM}$, in excess of 4,000,000 (magnitude mode) has been achieved on a fragment ion at

m/z=130.9916 (C_3F_{5+}) from perfluorotributyl-amine (PFTBA) using external EI ionisation and heterodyne detection (Figure 2), although it must be born in mind that, in this context, measurement of a single peak does not provide a very useful figure of merit because of peak coalescence.

High mass performance: Electrosprayed protein A (45 kDa) and bovine serum albumin (BSA, 66 kDa) with charge states ranging from 30+ to 55+, have been fully isotopically resolved with a resolving power of over 150,000 for the 50+ charge state in broad-band mode (600 summed spectra). No ions were ejected prior to detection. Apotransferrin (77 kDa) has been successfully detected in broadband mode using ESI Electrosprayed streptokinase,[24] a 47.3 kDa protein, has been completely isotopically resolved with a resolving power over 150,000 in broadband mode for the 36+ charge state.

Mass accuracy: Mass accuracy in the ESI mode has been shown to be of the order of a few ppm over the m/z range 200-2000. The m/z scale is externally calibrated using a Hewlett Packard pre made calibrant for ESI (G2421A) which generates seven peaks, viz. m/z = 118.0868, 322.0487, 622.0295, 922.0103, 1521.9719, 2121.9335 and 2721.8951. The software allows two- or three-point calibration formulae with linear or quadratic fitting to the calibration peaks. Typically, a calibration formula $m/z = a / f + b / f^2 + c$ is used, where f is the cyclotron frequency and a, b and c are calibration constants. In the analysis of tryptic digests, more than 100peptides have been identified in one spectrum where the distribution of mass measurement error was approximately normal with a standard deviation of 1.7 ppm (external calibration).

Sensitivity: Angiotensin concentrations down to 1 nM (200 attomole consumed during the data acquisition period of 4 s) have been detected using the home-built ESI source and a spraying solvent comprising $CH_3OH + H_2O + HOAc$ (199 : 99 : 2 v/v). Low (20-40 attomole) amounts consumed during data acquisition of bradykinin, bradykinin fragment 1-5, leucine enkephalin and [Arg^8]-vasopressin have been detected simultaneously using "fairy dust" nanospray and a spraying solvent comprising $CH_3CN + H_2O + HOAc$ (99:99:2v/v).

6. Applications [25-35]

The work and research conducted on the FT-ICR mass spectrometer are organised by a steering committee, which is open for co-operation and joint projects together with other groups. A few of the current research works are described below. The FT-ICR facility at Warwick is a national service facility for collaborative projects with other groups, as selected by a Management Advisory Panel.

Peptides and proteins: ESI FT-ICR tandem mass spectrometry (MS/MS) provides both a sensitive and selective method for detection of peptides. In co-operation with other groups, neuropeptides present in small amounts are analysed. Biological samples have been analysed using liquid separation methods (capillary electrophoresis and micro-scale high performance liquid chromatography) coupled with FT-ICR mass spectrometry. Biological applications, particularly the study of non-covalent interactions of biological molecules and protein conformation, are priority for the FT-ICR. Evidence of non-covalent dimerisation of calmodulin has been reported.

Peptide fragmentation: In order to investigate novel approaches to peptide fragmentation, the internal electron gun was reprogrammed and used for electron capture dissociation. Another more recent approach to peptide dissociation is multipole-storage assisted dissociation (MSAD), where ions are accumulated and fragmented in the hexapole ion trap in the ESI source.

Oligonucleotides

Negative-ion detection has been applied for the study and characterisation of oligonucleotide complexes on the instrument. A mass spectrum of the Dickerson Drew dodecamer with a nominal mass of 7267 Da is shown in Figure 5, where both single- and double-stranded DNA are observed. It has been found that the nanospray ion source (Analytica, Bradford) can be effective for negative-ion detection. This is partially due to the smaller number of vari-ables associated with nanospray compared with the number associated with standard ESI. Extra parameters to be adjusted in standard ESI, compared with nanospray, are the drying and needle gases, the flow rate and the extra voltages associated with the spraying process.

Synthetic polymers: A wide range of polymers has been studied using ESI FT-ICR

Eg: **PEG:** Poly-ethylene glycol 3500 mass spectrum obtained with nano-electrospray ionisation using a 20 µM solution prepared using 50 : 50 water + methanol with the addition of 1% 1 mM NaOH**.**

Fullernes: ESI FT-ICR has been successfully used for the first time to characterise fullerenes and fullerene derivatives with the combined benefits of the gentle ionisation process, high resolution and high mass-accuracy. Using the tandem

capability of laser desorption/ionisation with FT-ICR, complexes of fullerenes with helium have been produced by resonant excitation of the fullerene ions in helium gas. The laboratory-frame collision energy was not determined accurately, but would have been approximately 3 keV.

6.1 Recent advanced applications:

- For complex mixtures or unknown analytes, ultra high mass resolution is a necessary prerequisite for ultra high mass accuracy (sub ppm) because each peak must be fully resolved before mass can be assigned uniquely.
- Assignment of element composition (metabolomics, fossil fuels, environmental mixtures) and amino acid composition is determined.
- -post translational modification of peptides and proteins and mapping of protein binding sites by Helium/Deuterium exchange followed by enzyme cleavage.
- - Another place that FTICR-MS is useful is in dealing with complex mixtures since the resolution (narrow peak width) allows the signals of two ions of similar mass to charge (*m/z*) to be detected as distinct ions.

References

1. M.B. Comisarow and A.G. Marshall, Chem. Phys. Lett. 25, 282 (1974).
2. M.B. Comisarow and A.G. Marshall, Chem. Phys. Lett 26, 489 (1974).
3. A.G. Marshall and L. Schweikhard, Int. J. Mass Spectrom. Ion Processes 118/119, 37 (1992).
4. N.M.M. Nibbering, Analyst 117, 289 (1992).
5. C.L. Holliman, D.L. Rempel and M.L. Gross, Mass Spectrom. Rev. 13, 105 (1994).
6. A.G. Marshall, C.L. Hendrickson and G.S. Jackson, Mass Spectrom. Rev. 17, 1(1998).
7. R.B. Cody, R.C. Burnier, B.S. Freiser, Anal. Chem.54, 96 (1982).
8. J.W. Gauthier, T.R. Trautman and D.B. Jacobson, Anal. Chim. Acta 246, 21 (1991).
9. J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong and C.M.Whitehouse, Mass Spectrom. Rev. 9, 37 (1990).
10. K.D. Henry, E.R. Williams, B.H. Wang, F.W. McLafferty, J. Shabanowitz and D.F. Hunt, Proc. Natl. Acad. Sci. USA 86, 9075 (1989).
11. K.D. Henry and F.W. McLafferty, Org. Mass Spectrom. 25, 490 (1990).
12. P. Caravati and M. Allemann, Org. Mass Spectrom. 26, 514 (1991)
13. V.C. Dale, J.P. Speir, G.H. Kruppa, C.C. Stacey, M. Mann and M. Wilm, Biochem. Soc. Trans. 24, 943 (1996).
14. G. Savard, S. Becker,G. Bollen, H.-J. Kluge, R.B.Moore, T. Otto, L. Schweikhard, H. Stolzenberg and U. Wiess, Phys. Lett A 158, 247 (1991).
15. S. Guan, H.S. Kim, A.G. Marshall, M.C. Wahl, T.D. Wood and X. Xiang, Chem. Rev. 94, 2161 (1994).
16. R.A. Zubarev, N.L. Kelleher and F.W. McLafferty, J.Am. Chem. Soc. 120, 3265 (1998).
17. J. Axelsson, M. Palmblad, K. Håkansson and P.Håkansson, Rapid. Commun Mass Spectrom. 13, 474(1999).
18. R.L. Woodlin, D.S. Bomse and J.L. Beauchamp, J. Am. Chem. Soc. 100, 3248 (1978).
19. W.D. Price, P.D. Schnier and E.R. Willliams, Anal.Chem. 68, 859 (1996).
20. J. Axelsson, K. Håkansson, M. Palmblad and P.Håkansson, Rapid. Commun Mass Spectrom. 13, 1550 (1999).
21. K. Sannes-Lowery, R.H. Griffey, G.H. Kruppa, J.P. Speir and S. Hofstadler, Rapid. Commun MassSpectrom. 12, 1957 (1998).
22. K. Håkansson, J. Axelsson, M. Palmblad and P.Håkansson, J. Am. Soc. Mass Spectrom. 11, 210 (2000).
23. A.G. Marshall and S. Guan, Rapid. Commun MassSpectrom. 10, 1819 (1996).
24. H.J. Cooper, A.J.R. Heck, M. von Raumer. H. Dalton and P.J. Derrick, unpublished results.
25. A.J.R. Heck and P.J. Derrick, Eur. Mass Spectrom. 4, 181 (1998).
26. X. Feng, A.E. Giannakopulos, J. Wallace and P.J. Der- rick, Rapid Commun Mass Spectrom. 14, 719 (2000).
27. D. Barnidge, S. Nilsson and K.E. Markides, Anal. Chem.71, 4115 (1999).
28. D.L. Smith and Z.Q. Zhang, Mass Spectrom. Rev. 13,411 (1994).
29. J.A. Loo, Mass Spectrom. Rev. 16, 1 (1997).
30. T.D. Veenstra, Biophys. Chem. 79, 63 (1999).
31. B.N. Pramanik, P.L. Bartner, U.A. Mirza, Y.H. Liu and A.K. Ganguly, J. Mass. Spectrom. 33, 911 (1998).
32. A.J.R. Heck and P.J. Derrick, Anal. Chem. 69, 3603 (1997).
33. M. Blomqvist, J. Bergquist, A. Westman, K. Håkansson, P. Håkansson, P. Fredman and R. Ekman, Eur. J. Biochem. 263, 312 (1999).
34. D. Lafitte, A.J. Heck, T.J. Hill, K. Jumel, S.E. Harding and P.J. Derrick, Eur. J. Biochem. 261, 337 (1999).
35. M.P. Barrow, X. Feng, J.I. Wallace, O.V. Boltalina, R. Taylor, P.J. Derrick and T. Drewello, in preparation.