(19) **DANMARK**

(10) **DK/EP 1050061 T4**

(12) Oversættelse af ændret europæisk patentskrift

Patent- og
Varemærkestyrelsen

(72) Opfinder: **KRUTCHINSKY, Andrew, N., 36B-99 Roslyn Road, Winnipeg, Manitoba R3L 0G4, Canada**
LOBODA, Alexandre, V., 230-140 Baylor Avenue, Winnipeg, Manitoba R3T 3P4, Canada
SPICER, Victor, L., 63 Point West Drive, Winnipeg, Manitoba R3T 5H1, Canada
ENS, Erich, W., 27 Warrendale, Winnipeg, Manitoba R2M 5B8, Canada
STANDING, Kenneth, G., 60 Kings Drive, Winnipeg, Manitoba R3T 3E5, Canada

(74) Fuldmægtig i Danmark: **NORDIC PATENT SERVICE A/S, Bredgade 30, 1260 København K, Danmark**

(54) Benævnelse: **Spektrometer forsynet med pulseret ionkilde og transmissionsanordning til dæmpning af ionbevægelse og fremgangsmåde til anvendelse deraf**

(56) Fremdragne publikationer:
WO-A-99/30350
DE-C- 19 511 333
US-A- 5 689 111
KRUTCHINSKY A N ET AL: "Collisional Damping Interface for an Electrospray Ionization Time -of-Flight Mass Spectrometer" JOURNAL OF THE AMERICAN SOCIETY FOR MASS SPECTROMETRY, vol. 9, no. 6, 1 June 1998 (1998-06-01), page 569-579 XP004122896 ISSN: 1044-0305 cited in the application
EP-A2- 0 409 362
DE-C1- 19 629 134
GB-A- 2 315 592
US-A- 4 535 235
US-A- 4 963 736
US-A- 5 117 107
US-A- 5 373 156
L. HE ET AL.: 'Detection of Oligonucleotides by External Injection into an Ion Trap Storage/Reflectron Time-of-

flight Device' RAPID COMMUNICATIONS IN MASS SPECTROMETRY vol. 11, 1997, pages 1440 - 1448

Finnigan MAT Product Leaflet "Triple Stage Quadrupole" (1981)

Finnigan MAT "Finnigan Topic 8161 (1981)

H. R. MORRIS ET AL.: 'High Sensitivity Collisionally-activated Decomposition Tandem Mass Spectrometry on a Novel Quadrupole/Orthogonal-acceleration Time-of-flight Mass Spectrometer' RAPID COMMUN. MASS SPECTROM vol. 10, 1996, pages 889 - 896

M. GUILHAUS ET AL.: 'Perfect Timing: Time-of-flight Mass Spectrometry' RAPID COMMUNICATIONS IN MASS SPECTROMETRY vol. 11, 1997, pages 951 - 962

V. MLYNSKI ET AL.: 'Matrix-assisted Laser/Desorption Ionization Time-of-flight Mass Spectrometer with Orthogonal Acceleration Geometry: Preliminary Results' RAPID COMMUNICATIONS IN MASS SPECTROMETRY vol. 10, 1996, pages 1524 - 1530

Baykut, 45th ASMS Conf. (1997)

S. GUAN ET AL.: 'Enhanced Mass Resolving Power, Sensitivity, and Selectivity in Laser Desorption Fourier Transform Ion Cyclotron Resonance Mass Spectrometry by Ion Axialization and Cooling' ANALYTICAL CHEMISTRY vol. 65, no. 13, 1993, pages 1753 - 1757

L. SCHWEIKHARD ET AL.: 'Quadrupolar excitation and collisional cooling for axialization and high pressure trapping of ions in Fourier transform ion cyclotron resonance mass spectrometry' INTERNATIONAL JOURNAL OF MASS SPECTROMETRY AND ION PROCESSES vol. 120, 1992, pages 71 - 83

Dworschak et. al., Abstract, 44th ASMS Conf. (1996), Paper WOA 10:30

DESCRIPTION

FIELD OF THE INVENTION

[0001] This invention relates to mass spectrometers and ion sources therefor. More particularly, this invention is concerned with pulsed ion sources and the provision of a transmission device which gives a pulse ion source many of the characteristics of a continuous source, such that it extends and improves the application of Time of Flight Mass Spectrometry (TOFMS) and that it additionally can be used with a wide variety of other spectrometers, in addition to an orthogonal injection time-of-flight mass spectrometer.

BACKGROUND OF THE INVENTION

[0002] Ion sources for mass spectrometry may be either continuous, such as ESI (electrospray ionization) sources or SIMS (secondary ion mass spectrometry) sources, or pulsed, such as MALDI (matrix-assisted laser desorption/ionization sources). Continuous sources have normally been used to inject ions into most types of mass spectrometer, such as sector instruments, quadrupoles, ion traps and ion cyclotron resonance spectrometers. Recently it has also become possible to inject ions from continuous sources into time-of-flight (TOF) mass spectrometers through the use of "orthogonal injection", whereby the continuous beam is injected orthogonally to the main TOF axis and is converted to the pulsed beam required in the TOF technique. This is most efficiently carried out with the addition of a collisional damping interface between the source and the spectrometer, and this is described in the following paper, having four authors in common with the present invention (Krutchinsky A.N., Chernushevich I.V., Spicer V.L., Ens W., Standing K.G., Journal of the American Society for Mass Spectrometry, 1998, 9, 569-579).

[0003] On the other hand, pulsed sources, MALDI sources for example, have usually been coupled directly to TOF mass spectrometers, to take advantage of the discrete or pulse nature of the source. TOF mass spectrometers have several advantages over conventional quadrupole or ion trap mass spectrometers. One advantage is that TOF mass spectrometers can analyze a wider mass-to-charge range than do quadrupole and ion trap mass spectrometers. Another advantage is that TOF mass spectrometers can record all ions simultaneously without scanning, with higher sensitivity than quadrupole and ion trap mass spectrometers. In a quadrupole or other scanning mass spectrometer, only one mass can be transmitted at a time, leading to a duty cycle which may typically be 0.1%, which is low (leading to low sensitivity). A TOF mass spectrometer therefore has a large inherent advantage in sensitivity.

[0004] However, TOF mass spectrometers encounter problems with many widely used sources which produce ions with a range of energies and directions. The problems are particularly acute when ions produced by the popular MALDI (matrix-assisted laser desorption/ionization) technique are used. In this method, photon pulses from a laser strike a target and desorb ions whose masses are measured in the mass spectrometer. The target material is composed of a low concentration of analyte molecules, which usually exhibit only moderate photon absorption per molecule, embedded in a solid or liquid matrix consisting of small, highly-absorbing species. The sudden influx of energy is absorbed by the matrix molecules, causing them to vaporize and to produce a small supersonic jet of matrix molecules and ions in which the analyte molecules are entrained. During this ejection process, some of the energy absorbed by the matrix is transferred to the analyte molecules. The analyte molecules are thereby ionized, but without excessive fragmentation, at least in the ideal case.

[0005] Because a pulsed laser is normally used, the ions also appear as pulses, facilitating their convenient measurement in a time-of-flight spectrometer. However, the ions acquire a considerable amount of energy in the supersonic jet, with velocities of the order of 700 m/s, and they also may lose energy through collisions with the matrix molecules during acceleration, particularly in high accelerating fields. These and similar effects lead to considerable peak broadening and consequent loss of resolution in a simple linear time-of-flight instrument, where the ions are extracted from the target nearly parallel to the spectrometer axis. A partial solution to the problem is provided by a reflecting spectrometer, which partially corrects for the velocity dispersion, but a more effective technique is the use of delayed extraction, either by itself or in combination with a reflector. In delayed extraction, the ions are allowed to drift for a short period before the accelerating voltage is applied. This technique partially decouples the ion production process from the measurement, making the measurement less sensitive to the detailed pattern of ion desorption and acceleration in any particular case. Even so, successful operation requires careful control of the laser fluence (i.e. the amount of power supplied per unit area) and usually some hunting on the target for a favorable spot. Moreover, the extraction conditions required for optimum performance have some mass dependence; this complicates the calibration procedure and means that the complete range of masses cannot be observed with optimum resolution at any given setting. Also, the technique

1

has had limited success in improving the resolution for ions of masses greater than about 20,000 Da. Moreover, it is difficult to obtain high performance MS-MS data in conventional MALDI instruments because ion selection and fragmentation tend to broaden the fragment peak width. The present inventors have realized that these problems can be overcome by abandoning the attempt to maintain the original pulse width, producing instead a quasi-continuous beam with superior characteristics, and then pulsing the injection voltage of the TOF device at an independent repetition rate.

[0006] Although coupling to a TOF instrument is used as an example above, problems also arise in coupling MALDI and other pulsed sources to other types of mass spectrometer, such as quadrupole (or other multipole), ion trap, magnetic sector and FTICRMS (Fourier Transform Ion Cyclotron Resonance Mass Spectrometer). Further, it is also desirable to be able to couple MALDI or other pulsed sources to tandem mass spectrometers, e.g. a triple quadrupole or a quadrupole TOF hybrid instrument, which allows MS/MS of MALDI ions to be obtained. Standard MALDI instruments cannot be configured to carry out high performance MS/MS. The dispersion in energy and angle of ions produced by a MALDI source, or similar source, accentuates the difficulty of ion injection. Also, because the residence times of ions in most other types of mass spectrometer are considerably longer than in TOF instruments, the large space charge in the pulse can introduce additional problems. These instruments are all designed to operate with continuous sources, so conversion of the pulsed source to a quasi-continuous one solves most of the problems.

[0007] German Patent DE 19511333 (corresponding to published British application GB 2,299,446A) discloses a mass spectrometer including a continuous, electrospray ion source that injects ions into a capillary. The ions pass through chambers at progressively decreasing pressures, and pass through a hexapole ion guide to an ion trap 12. The ion trap 12 serves to confine the ions in a configuration suitable for outpulsing into the flight tube of a time-of-flight section. There is no teaching or suggestion that a pulse source could be used.

[0008] US 5,373,156 discloses a method and device for the mass-spectrometric examination of organic ions. A neodymium YAG laser 1 produces a light pulse lasting about 10 microseconds. A focal point is produced on one side of a foil 4, which is covered on the opposite side with a thin application of the substance under examination. The ions shaken off the foil are decelerated in a friction chamber 23 due to collisions with hydrogen atoms acting as a friction gas and admitted into the friction chamber 23 via inlets 5 and 7. A skimmer 10 feeds the slowed ions to the skimmer opening, the ions then being carried along into the next chamber 24. The ions are then directed into the chamber of the mass spectrometer by the potential of a skimmer 15. An ion-optical lens 17 delays the ions and focuses them on the inlet opening of an RF quadrupole ion trap 18, where the ions are slowed by a damping gas and caught. For examination of the ions, the ion trap is operated with a scanning method in which the ions are ejected mass-sequentially through holes in an end cap. The ejected ions are measured with an ion detector 19.

[0009] US 5,689,111 discloses an ion storage time-of-flight mass spectrometer comprising a continuously operating ion source 10. The ions are introduced into a first stage pumping region 20, formed into a beam by a multipole ion guide and collimated and transferred into the pulsing region 26 of the time-of-flight mass analyzer. The mass analyzer is operating in an orthogonal injection mode and employs a pulsed electric field between a repeller lens 23 and a draw-out lens 24. The pulsed electric field establishes the start time for the measurement of the flight time distribution of the ions arriving at the detector 36, the flight time being related to the mass to charge ratios of the ions. In the continuous mode embodiment the ions emitted from the ion source 10 are directly fed through the ion guide into the time-of-flight mass analyzer. In the storage mode embodiment, on the other hand, the ions are first stored in the ion guide by means of a potential well in the longitudinal direction of the ion guide and then emitted from the ion guide into the time-of-flight mass analyzer by switching for a short duration the voltage on the exit electrode 15 thereby creating a leak of the potential well. After a variable delay t2 the electric field in region 26 of the mass analyzer is pulsed for a short period of time by the repeller plate 23 to accelerate the ions perpendicular to their original direction towards the flight tube 35 to be detected for mass analysis.

BRIEF SUMMARY OF THE PRESENT INVENTION

[0010] Accordingly, it is desirable to provide an apparatus and method enabling a pulse source, such as a MALDI source, to be coupled to a variety of spectrometer instruments, in a manner which more completely decouples the spectrometer from the source and provides a more continuous ion beam with smaller angular and velocity spreads.

[0011] More particularly, it is desirable to provide an improved TOF mass spectrometer with a pulsed ion source, in which the energy spread in the ion beam is reduced, in which the source is more completely decoupled from the spectrometer than in existing instruments, in which problems resulting from ion fragmentation are reduced, enabling new types of measurement, and in which the results obtained from the mass spectrometer and its ease of operation are consequently improved.

[0012] It is also desirable to provide a TOF mass spectrometer with both continuous and pulsed sources, for example both ESI and MALDI sources, so either source can be selected.

[0013] In accordance with the present invention, there is provided a mass spectrometer system according to claim 1.

[0014] As time of flight mass spectrometers require a pulsed beam, conventional teaching is that a pulsed source should be coupled maintaining the pulsed characteristics. However, the present inventors have now realised that there are advantages to, in effect converting a pulsed beam into a continuous, or at least quasi-continuous, beam, and than back into a pulsed beam. The advantages are: improvement in beam quality through collisional damping; decoupling of the ion production from the mass measurement; ability to measure the beam current by single-ion counting because it is converted from a few large pulses to many small pulses, for example from about 1 Hz. to about 4 kHz, or a factor of 4,000; compatibility with a continuous source, such as ESI, offering the possibility of running both sources on one instrument.

[0015] Preferably, the ion source provides the analyte for ionization by radiation, and there is provided a source of electromagnetic radiation, more preferably a pulsed laser, directed at the ion source, for generating radiation pulses to cause desorption and ionization of analyte molecules.

[0016] Advantageously, the ion source comprises a target material composed of a matrix and analyte molecules in the matrix, the matrix comprising a species adapted to absorb radiation from the radiation source, to promote desorption and ionization of the analyte molecules.

[0017] Preferably, the transmission device comprises a multipole rod set. There can be two or more multipole rod sets and means for supplying different RF and DC voltages to the rod sets.

[0018] Collisional damping can also be accomplished in a chamber where no RF field is present providing there is enough buffer gas pressure. In this case ions with reduced velocities can be moved to the exit of the chamber by gas flow drag or a DC electrostatic field. Combinations of electrostatic fields, RF fields and gas flow can also be implemented in a collisional damping chamber.

[0019] Another advantage of the invention is that the collisional cooling of the ions helps to reduce the amount of fragmentation of the molecular ions. It is usually desirable to produce a simple mass spectrum containing only ions representative of molecular species. In typical MALDI ion sources, therefore, the laser power must be carefully optimized so that it is close to the threshold of ionization in order to reduce fragmentation. The inventors have observed, however, that the presence of a gas around the sample surface greatly assists in reducing fragmentation, even at relatively high laser power. Presumably this is due to the effect of collisions with gas molecules which remove internal energy from the desorbed species before they can fragment. This means that the laser power can be increased in order to improve the ion signal strength, without causing excessive decomposition.

[0020] The inventors have observed that the amount of fragmentation is decreased as the pressure is increased up to at least approximately 1 torr (approx. 130 N/m^2). Higher pressures may be even more advantageous, but electric fields may be required to avoid clustering reactions at higher pressure.

[0021] The mass spectrometer system can include a continuous ion source, and means for selecting one of the pulsed ion source and the continuous ion source, and this then provides the characteristics of two separate instruments in one instrument. The two ion sources can comprise a MALDI source and an ESI source.

[0022] Another aspect of the present invention provides a method according to claim 23.

[0023] The gas pressure of the damping gas can be in the range from about 10^{-4} Torr (approx. 0.013 N/m^2) up to at least 760 Torr (approx. 1.013×10^5 N/m^2). Preferably, step (3) comprises providing an RF rod set within the transmission device. Further, a DC field can be provided between the ion source and the spectrometer to promote movement of ions towards the spectrometer.

[0024] The method can include providing two or more rod sets in the ion transmission device, and operating at least one rod set with a DC offset to enable selection of ions with a desired mass-to-charge ratio. A potential difference can be provided between two adjacent rod sets sufficient to accelerate ions into the downstream rod set, to cause collisionally induced dissociation in the downstream rod set.

[0025] When a pulsed laser is used, for each laser pulse, a plurality of pulses of ions are delivered into the time-of-flight mass spectrometer.

[0026] The ions can first pass through one or more differentially pumped regions that provide a transition from the pressure at the ion source to pressure in the spectrometer. The ion source may be at atmospheric pressure or at least at a pressure substantially higher than that in downstream quadrupole stages and in the mass spectrometer. At least one of these regions can be without any rod set and ion motion towards the mass spectrometer is then driven by gas flow and/or an electrostatic potential.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

[0027] For a better understanding of the present invention and to show more clearly how it may be carried into effect, reference will now be made, by way of example, to the accompanying drawings, which show preferred embodiments of the present invention and in which:

Figure 1 shows a block diagram of a mass spectrometer system;

Figure 2 is a schematic diagram showing a MALDI-TOF mass spectrometer with orthogonal injection of the MALDI ions into the spectrometer through a collisional damping interface (quadrupole ion guide) according to the present invention;

Figure 3 shows a mass spectrum of a mixture of several peptides and proteins leucine-enkephalin-Arg (Le-R), substance P (Sub P), melittin (ME), CD4 fragment 25-58 (CD4), and insulin (INS)) produced in the spectrometer of Figure 2;

Figure 4 shows plots of transit times through the interface for different ions;

Figure 5 shows a mass spectrum of substance P;

Figure 6 shows a mass spectrum of a tryptic digestion of citrate synthase;

Figure 7A shows a schematic of part of spectrometer of Figure 2, showing the collisional interface and indicating applied voltages;

Figures 7B, 7C and 7D show different operating regimes of the mass spectrometer of Figure 2;

Figures 8A, 8B, 8C, and 8D are mass spectra obtained from substance P recorded in the different operation regimes, according to Figures 7B, 7C, and 7D;

Figure 9 shows the behaviour of the ion current from a single target spot as a function of time; and

Figure 10 shows schematically combined ESI and MALDI sources for a mass spectrometer.

Figure 11 shows a MALDI-QqTOF mass spectrometer utilizing a collisional damping interface including extra ion manipulation stages which are added between the interface and the time-of-flight mass spectrometer;

Figure 12 shows mass spectra obtained on a MALDI-QqTOF of Figure 11 in a single MS and MS-MS modes;

Figure 13 shows an alternative collisional damping setup for the MALDI-QqTOF mass spectrometer of Figure 11, where ion velocities are partially damped in a region without RF fields;

Figure 14 shows an experimental apparatus which was used to investigate the effect of pressure and electric field strength on the MALDI ion current; and

Figure 15 is a graph showing the total ion current produced by MALDI source shown in Figure 14 as a function of voltage difference applied at different pressures in the chamber.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0028] The first embodiment shown in Figure 1 is a block diagram of a general mass spectrometer system. Here 1 represents any sort of pulsed ion source (for instance MALDI), 2 is a collisional focusing chamber or region filled with a buffer gas and with a multipole 3 driven at some RF voltage. This is followed by an optional manipulation stage 4 and then a mass analyzer 5. The collisional ion guide 3, in accordance with the present invention, spreads the pulsed ion beam in time, and improves its beam

quality (i.e. space and velocity distributions) by damping the initial velocity and focusing the ions toward the central axis. The beam is then quasi-continuous and may enter an optional manipulation stage 4, where ions can be subjected to any sort of further manipulation. Finally the resultant ions are analyzed in the mass analyzer 5.

[0029] A simple example of further manipulation in stage 4 is dissociation of the ions by collisions in a gas cell, so that the resulting daughter ions can be examined in the mass analyzer. This may be adequate to determine the molecular structure of a pure analyte. If the analyte is a complex mixture, stage 4 needs to be more complicated. In a triple quadrupole or a QqTOF instrument (as disclosed in A. Shevchenko et al, Rapid Commun. Mass Spectrom. 11, 1015, (1997)), stage 4 would include a quadrupole mass filter for selection of a parent ion of interest and a quadrupole collision cell for decomposition of that ion by collision-induced dissociation (CID). Both parent and daughter ions are then analyzed in section 5, which is a quadrupole mass filter in the triple quadrupole, or a TOF spectrometer with orthogonal injection in the QqTOF instrument. In both cases stages 1 and 2 would consist of a pulsed source and a collisional damping ion guide.

[0030] It will be appreciated that the collisional focusing chamber 2 is shown with a multipole rod set 3, which could be any suitable rod set, e.g. a quadrupole, hexapole or octopole. The particular rod set selected will depend upon the function to be provided.

[0031] Alternatively, a radio frequency ring guide could be used for the collisional focusing device, and ion creation could be performed within the volume defined by the radio frequency field in order to contain the ions.

[0032] Figure 2 shows a preferred embodiment of a MALDI-TOF mass spectrometer 10 according to the present invention. The spectrometer 10 includes a conventional MALDI target probe 11, a shaft seal chamber 12, pumped in known manner, and a target installed in the target-holing electrode 13. A mixture of the sample to be investigated and a suitable matrix is applied to the sample probe following the usual procedure for preparing MALDI targets. A pulsed laser 14 is focused on the target surface 15 by lens 16, and passes through a window 17. The laser beam is indicated at 20, and the laser is run at a repetition rate of anywhere from below a few Hz to tens of kHz, more specifically in this embodiment tested at a rate of 13 Hz. An inlet 18 is provided for nitrogen or other neutral gas. Each laser shot produces a plume or neutral and charged molecules. Ions of the sample analyte are produced and entrained in the plume which expands into vacuum chamber 30, which contains two quadrupole rod sets 31 and 32. Chamber 30 is pumped by a pump (not shown) connected to port 34 to about 70 mTorr (approx. 9.33 N/m^2) but the pressure can be varied over a substantial range by adjusting the flow of gas through a controlled leak valve 18. Pressures of up to 1 atmosphere (1.013 x 10^5 N/m^2) could also be used in the ion generation region, by putting the ionization region in a chamber which is upstream of chamber 30, and providing a small aperture through which ions are pulled into chamber 30. Lower pressures could be used, and an important characteristic is the product of pressure and rod length. Thus, a total length × pressure value of at least 10.0 mTorr-cm (approx. 1.33 N/m^2-cm) could be used, although a value of 22.5 mTorr-cm (approx. 3.0 N/m^2-cm), as in U.S. patent 4,963,736, is preferred. The gas in chamber 30 (typically nitrogen or argon or other suitable gas, preferably an inert gas) will be referred to as a damping gas or cooling gas or buffer gas.

[0033] In the embodiment tested, the quadrupole rod sets 31 and 32 were made of rods 4.45 cm in length and 11 mm in diameter, and were separated by 3 mm, i.e. the spacing between rods on adjacent corners of the rod set. The quadrupoles 31 and 32 are driven by a power supply which provides operating sine wave frequencies from 50 Hz to 2 MHz, and output voltages from 0 to 1000 volts peak-to-peak. Typical frequencies are 200 kHz to 1 MHz, and typical voltage amplitudes are 100 to 1000 V peak-to-peak. Both quadrupoles are driven by the same power supply through a transformer with two secondary coils. Different amplitudes may be applied to the quadrupoles by using a different number of turns in the two secondary coils. D.C. Bias or offset potentials are applied to the rods of quadrupoles 31 and 32 and to the various other components by a multiple-output power supply. The RF quadrupoles 31 and 32, with the damping gas between their rods can be run in an RF-only mode, in which case they serve to reduce the axial energy, the radial energy, and the energy spreads, of the ions which pass through it, as will be described. This process substantially spreads the plume of ions out along the ion path, changing the initial beam, pulsed at about 13 Hz, into a quasi-continuous beam as described in more detail below. The first quadrupole 31, can also be run in a mass-filtering mode by the application of a suitable DC voltage. The second quadruple 32 can then be used as a collision cell (and an RF-guide) in collision-induced dissociation experiments (see below).

[0034] From chamber 30, the ions pass along an ion path 27 and through a focusing electrode 19 and then pass through orifice 38, into a vacuum chamber 40 pumped by a pump (not shown) connected to a port 42. There, the ions are focused by grids 44 through a slot 46 into an ion storage region 48 of a TOF spectrometer generally indicated at 50.

[0035] In known manner, ions are extracted from the storage region 48 and are accelerated through a conventional accelerating column 51 which accelerates the ions to an energy of approximately 4000 electron volts per charge (4 keV). The ions travel in a

direction generally orthogonal to the ion path 27 between the ion storage region, through a pair of deflection plates 52. The deflection plates 52 can serve to adjust the ion trajectories, so that the ions are then directed toward a conventional electrostatic ion mirror 54, which reflects the ions to a detector 56 at which the ions are detected. The ions are detected using single-ion counting and recorded with a time-to-digital converter (TDC). The accelerating column 51, plates 52, mirror 54 and detector 56 are contained in a main TOF chamber 58 pumped to about 2×10^{-7} Torr) $(2.67 \times 10^{-3}$ N/m^2) by a pump (not shown) connected to a port 60.

[0036] The use of orthogonal-injection of MALDI ions from source 13 into the TOF spectrometer 50 has some potential advantages over the usual axial injection-geometry. It serves to decouple the ion production process from the mass measurement to a greater extent than is possible in the usual delayed-extraction MALDI. This means that there is greater freedom to vary the target conditions without affecting the mass spectrum, and the plume of ions has more time to expand and cool before the electric field is applied to inject them into the spectrometer. Some improvement in performance might also be expected because the largest spread in velocities is along the ejection axis, i.e. the ion path 27, normal to the target, which in this case is orthogonal to the TOF axis. However, orthogonal injection of MALDI ions into the TOF 50 without collisional cooling has several problems which appear to make the geometry impractical, namely:

1. (1) The radial energy distribution, while much smaller than the axial energy is still sufficient to cause substantial spreading and expansion of the beam as it leaves the quadrupole rod set 32 and travels toward the TOF axis. The spatial spread of the beam along the TOF axis limits the resolution. The effect can be reduced with collimation but only at a significant sacrifice in sensitivity; a collimating slit must be placed sufficiently far from the TOF axis to avoid distorting the extraction field, and so the target must be placed far enough from the collimation slit to produce a reasonably parallel beam;

2. (2) The axial velocity of the ions, i.e. velocity along the ion path 27, in the plume is largely independent of mass which means the energy is mass dependent. Since the axial energy determines the direction of the trajectory after acceleration into the TOF spectrometer, instrumental acceptance (or acceptance by the TOF spectrometer) is mass dependent; i.e. there is mass discrimination. The same effect is observed when ESI ions are injected without collisional cooling as explained in detail in the prior publication mentioned above; and

3. (3) The width of the axial energy distribution is comparable in magnitude with the axial energy itself, so the beam spreads out along its axis by an amount comparable to the separation between the target and the TOF axis. The size of the aperture which admits ions from the storage region into the spectrometer must clearly be much smaller than this to maintain a uniform extraction field, particularly if a slit is placed between the target and the TOF axis. This further reduces the sensitivity.

[0037] In delayed extraction MALDI in the usual axial geometry, i.e. not the orthogonal configuration shown, acceptance is nearly complete, and while the largest velocity spread is along the TOF axis, the well-defined target-plane perpendicular to the TOF axis allows a combination of time-lag focusing (delayed extraction with optimized values of delay and applied voltage) and electrostatic focusing (optimized value of the reflector voltage) in an ion mirror to produce resolution well above 10,000 in some cases.

[0038] Experiments carried out by the present inventors suggest that competitive resolution could not be obtained with an acceptable signal using orthogonal injection, unless collisional cooling according to the present invention is employed. Moreover, some disadvantages of delayed-extraction MALDI -- the dependence of optimum extraction conditions on mass, and the more complex calibration required -- are still present in orthogonal injection MALDI without cooling although to a lesser extent than with axial injection.

[0039] The introduction of an RF quadrupole or other multipole with collisional cooling of the ions between the MALDI target and the orthogonal injection geometry avoids the problems described above while offering additional advantages. These are detailed below with reference to the remaining figures.

[0040] By reducing the radial energies of the ions, an approximately parallel beam can be produced, greatly reducing the losses that result from collimation before the ions enter the storage region. This allows the use of a larger entrance aperture to the TOF spectrometer 50, further reducing losses. By reducing the axial energies of the ions, and then reaccelerating them to a uniform energy, the mass discrimination mentioned above is not present.

[0041] The uniform energy distributions of the ions after cooling remove any mass dependence on the optimum extraction conditions and allow the simple quadratic relation between TOF and mass to be used for calibration with two calibrant peaks. Figure 3 shows a spectrum of an equimolar mixture of several peptides and proteins from mass 726 to 5734 Da in an α-cyano-4-hydroxy cinnamic acid matrix. The spectrum was acquired in a single run and shows uniform mass resolution (M/ΔM$_{FWHM}$) of

about 5000 throughout the mass range. Using a simple external calibration with substance P and melittin, the mass determination for each of the molecular ions is accurate within about 30 ppm. Here, the peaks for the various substances are identified as: peak 60 for Leucine-enkephalin; peak 61 for substance P; peak 62 for Melittin; peak 63 for CD4 fragment 25-28; and peak 64 for insulin. All peaks are identified both on the overall spectrum and as an enlarged partial spectrum. The resolution demonstrated in Figure 3 is rather close to the resolution obtainable with the same instrument using an ESI source. In the present embodiment, the entrance orifice was made slightly larger than normally used in ESI, approximately 1 mm diameter as compared to a normal diameter of around 1/3 mm, to make adjustments easier in the preliminary experiments. This does not appear to have been necessary so it is reasonable to expect improved resolution if a smaller orifice is used. Resolution up to 10,000 has been obtained with ESI ions in the same instrument and in the MALDI-QqTOF instrument described below.

[0042] The decreasing relative intensity of the molecular ions with mass is to some extent a reflection of the decreasing detection efficiency with increasing mass. Detection efficiency depends strongly on velocity, which decreases with mass for singly-charged ions at a given energy. In this embodiment the energy of singly-charged ions is only about 5 keV (compared to 30 keV in typical MALDI experiments), so the detection efficiency limits the practical range of application to less than about 6000 Da. The relative intensities of the molecular ion peaks in Fig. 3 is consistent with that observed from the same sample when analyzed in a conventional MALDI experiment using 5 kV acceleration. The detection efficiency in the present embodiment can be increased by increasing the voltage which accelerates the ions into the spectrometer, or by increasing the voltage on the detector.

[0043] As mentioned above, the collisional cooling spreads the ions out along the ion beam axis changing the initial beam pulsed at 13 Hz into a quasi-continuous beam. This is illustrated in Figure 4 which shows the count rate as a function of time after the laser pulse; i.e. the distribution of transit times through the ion guide. The width of the time distribution is on the order of 20 ms which represents an increase in the time spread by a factor of at least 10^7 as each laser pulse is about 2 ns long. It will be appreciated that it is not necessary to produce a time distribution of the order of 20 ms; for example the quasi-continuous pulse could be as short as 0.1 ms. Dispersion along the axis is a disadvantage in orthogonal-injection MALDI without cooling, but with the present invention, since optimum extraction conditions do not depend on the time delay after the laser shot, multiple injection pulses into the TOF storage region 48 can be used for each laser shot. In the present embodiment, 256 injection pulses into the TOF storage region 48 were used for every laser shot. The losses are then determined by the duty cycle of the instrument which in this case is about 20%. The duty cycle is the percentage of the time that ions can be injected from the storage region into the TOF spectrometer; here, it effectively means the fraction of the time, the TOF storage region 48 is available to accept ions. A quasi-continuous beam is in fact an advantage in this mode of operation. Approximately 10^4 to 10^6 ions are ejected from the target probe with every laser shot at a repetition rate of 13 Hz, but as a result of spreading along the beam axis or ion path 27 (and some losses) approximately 2 to 5 ions are injected into the instrument with every injection pulse less than one ion on average of a particular species. This allows single-ion counting to be used with a TDC (Time to Digital Converter), which makes the combination of high timing resolution (0.5 ns) and high repetition rate (essential for maximum duty cycle) technically much simpler than using a transient recorder which is necessary in conventional MALDI experiments. In addition, the use of single-ion counting eliminates problems with detector shadowing from intense matrix peaks, and problems with peak saturation which require attention in conventional MALDI because of the strong dependence of the signal on laser fluence and the shot-to-shot variation. Finally, single-ion counting places much more modest demands on the detector and amplifier time resolution because the electronic reduction and digitization of the pulse is quite insensitive to the detector pulse shape.

[0044] In Figure 4, four graphs are shown of the count rate against time, for leucine-enkephalin shown at 70, substance P shown at 72, Melittin shown at 74 and insulin shown at 76. Additionally, for each of these substances, graphs or spectra 71, 73, 75 and 77, are inserted showing normal TOF spectra, similar to Figure 3.

[0045] Assuming 10^4 ions of a single molecular ion species are produced with each laser shot, the transmission efficiency of the RF-quadrupole is in the range of 10 %. Taking account of the duty cycle, about 2 % of the ions produced at the target are detected in the mass spectrometer. This represents significant losses compared to the conventional axial MALDI experiment in which transmission is probably 50% or more. However, from the point of view of data rate, the losses can be compensated to a large extent by the higher repetition rate and higher fluence of the laser. In these experiments, the repetition rate was 13 Hz, but can easily be increased to 20 Hz with the current laser, or in principle up to at least 100 Hz before the counting system becomes saturated. In contrast, the usual MALDI experiment is run at about 1 or 2 Hz. The laser fluence in a conventional MALDI experiment must be kept close to threshold to achieve the best performance, the threshold being the minimum energy necessary to cause vaporization of the sample to produce a useful signal using a conventional transient recording with analog to digital conversion. In the present invention, the laser fluence can be increased to the fluence at which the ion production process saturates. As the quadrupole serves to smooth out the ion burst produced by the laser, a short intense burst of ions can be accepted. From the point of view of absolute sensitivity, it seems that the independence of the spectrum on laser conditions (see below) allows more efficient usage of the sample deposited on the target. Using fluence several times higher than threshold

produces ions until the matrix is completely removed from the target probe. Figure 5 shows that the practical sensitivity achieved with substance P is in the same range as that obtainable with conventional MALDI. Five femtomoles of substance P were applied to the target using 4HCCA as the matrix. The left hand side of the spectrum is indicated at 80, and the right hand side is shown enlarged by a factor of 44 as indicated at 81. A portion of this spectrum is shown enlarged at 82 showing the molecular ion (MH^+).

[0046] Figure 6 shows the spectrum 85 obtained from a tryptic digest of citrate synthase again showing the uniform mass resolution over the mass range; the inset 86 shows the spectrum obtained from 20 fmoles applied to the target.

[0047] These results indicate that the performance of the invention for peptides is comparable to conventional MALDI experiments but with the advantage of a mass-independent calibration, and a simple calibration procedure. However, the most important advantages result from the nearly complete decoupling of the ion production from the mass measurement. In a conventional MALDI experiment, the location of the laser spot on the target and the laser fluence and location must be carefully selected for optimum performance, and these conditions are typically different for different matrices and even for different target preparation methods. The situation was improved with the introduction of delayed extraction but even so, many commercial instruments have implemented software to adjust laser fluence, detector gain, and laser position, and to reject shots in which saturation occurs. None of these techniques are necessary with the present invention. The performance obtained shows no dependence on target or laser conditions. The laser is simply set to maximum fluence (several times the usual threshold) and left while the target is moved to a fresh position occasionally. This means that alternative targets can easily be tried (including insulating targets), and alternative lasers with different wavelengths or pulse widths can be used.

[0048] The decoupling of the ion production from the mass measurement also provides an opportunity to perform various manipulations of the ions after ejection but before mass measurement. One example is parent ion selection and subsequent fragmentation (MS/MS). This is most suitably done with an additional quadrupole mass filter as described below, but even in the present embodiment of Figure 2, some selectivity and fragmentation is possible.

[0049] Figures 7A, 7B and 7C show three different modes of operation of the instrument shown in Figure 2. The reference numerals of Figure 2 are provided along the z axis to indicate correspondence between potential level and the different elements of the apparatus. Voltages for the quadrupole sections 31, 32 are indicated respectively at U_1 (t) and U_2 (t).

[0050] Figure 7A shows the simple collisional ion guide mode that was used in obtaining the results shown in Figures 4-6. Here the same amplitudes of RF voltage and no DC offset voltages are applied to different sections of the quadrupole. Potential differences in the longitudinal direction are kept small to minimize fragmentation due to CID.

[0051] Figure 7B shows a mass filtering mode, which is analogous to the same filtering mode implemented in conventional quadrupole mass filters. Here a DC offset voltage V is added to the first section of the quadrupole to select an ion of interest, while the second section again acts as an ordinary ion guide since there is no CID because of the small potential difference between the sections. The amplitude of the voltage applied in the second quadrupole section 32 is only one third of the voltage applied in the first section 31.

[0052] Figure 7C is an MS-MS mode which differs from the mode of Figure 7B by a higher potential difference between the quadrupole sections 31,32, so ions are accelerated in that region and enter the second section with high kinetic energy, the additional energy being indicated as Δ collision energy. In that case the second section acts as an collision cell and parent ions are decomposed there by collisions with the buffer gas (CID). Again, the amplitude of the RF voltage in the second section is only one third of the amplitude of the RF voltage in the first section, which allows daughter ions much lighter than the parent ions to have stable trajectories and to be transmitted through the second quadrupole.

[0053] Figure 8 shows examples of the spectra obtained in the different modes illustrated in Figure 7, and in particular gives an example of possible beam manipulation. All the spectra were acquired using the same initial sample.

[0054] Figure 8A is a mass spectrum where ions were cooled in a collisional focusing ion guide (the mode of Figure 7A).

[0055] Figure 8B is an example where ions of interest were selected in the first quadrupole 31 and cooled in the second quadrupole 32 section (the mode of Figure 7B). Once ions of interest have been selected, they can be used for fragmentation in CID to obtain detailed information on composition and structure.

[0056] Figure 8C presents an MS/MS spectrum of substance P obtained in this way. Molecular ions of substance P are selected in the first quadrupole section and fragmented by collisions in the second quadrupole section (according to the mode of Figure

7C). The potential difference, Δ collision energy, between the first and second quadrupoles was 100V. The intensities of the fragment ions were small in comparison with intensity of the primary ion so the region inside dotted lines is expanded by a factor of 56. Figure 8D shows the spectrum obtained in the same mode but where the potential difference between the quadrupoles 31, 32 was 150 V. In this case, more fragment ions are observed and the parent ion peak is substantially reduced.

[0057] Figure 9 shows how long a signal from the same spot on a MALDI target can last. In this experiment, a given spot was irradiated by a series of shots from the laser, running at 13 Hz. The laser intensity was two or three times the "threshold" intensity. On average the sample lasted for about one minute. The shape of the curve suggests that the laser shots dig deeper and deeper into the sample until it is exhausted. At that point the laser irradiates the metallic substate, so no signal is observed.

[0058] In the past it has not been possible to use both continuous sources, such as electrospray ionization (ESI), and pulsed sources, such as MALDI, in the same instrument, which would have significant advantages. To the inventors' knowledge, the only successful ESI-TOF instruments to date have been the orthogonal injection spectrometers (by the present inventors, Dodonov, and now the commercial machines by PerSeptive and others), so it appears that orthogonal injection is necessary for ESI-TOF, with or without collisional damping, although the former improves the situation, as detailed in Krutchinsky A.N., Chernushevich I.V., Spicer V.L., Ens W., Standing K.G., Journal of the American Society for Mass Spectrometry, 1998, 9, 569-579 . Up to now, attempts to put MALDI on an orthogonal injection instrument have been without collisional damping (for example by the present inventors and by Guilhaus' and both gave unpromising results). The present invention enables two such sources to be available in one instrument. Here, the MALDI probe 11 in Figure 2 can be replaced by an ESI source to enable measurement of ESI spectra in the instrument. The instrument would then be essentially the same as the one illustrated in the paper Krutchinsky A.N., Loboda A.V., Spicer V.L., Dworschak R., Ens W., Standing K.G., Rapid Commun. Mass Spectrom. 1998,12, 508-518 . This change could of course be carried out by actually taking off one source and replacing it by the other, but a number of more convenient arrangements can be provided.

[0059] For instance Figure 10 shows a further embodiment where the electrospray ion source 94a is attached to the input of a collisional damping interface 92, including a quadrupole, or other multipole, rod set 93. A MALDI ion source 94b is introduced on a probe 95 that enters from the side, and can be displaced in and out; for this purpose, a shaft end 96 is slidingly and sealingly fitted into the housing of the collisional interface 92. The MALDI ion source 94b is similar to the one shown in Figure 2 except in this case the sample is deposited onto a flat surface machined on the side of the probe shaft 95, instead of onto the end of a cylindrical probe. The sample is irradiated by a laser with corresponding optics, generally indicated at 97, and ions are transmitted to a spectrometer indicated at 98. When the ESI source (940) is operating, shaft 96 is pulled out far enough to clear the path of the ESI ions. When the MALDI ion source 94b is operating the shaft 96 is inserted back so the MALDI target 94b is in the central position.

[0060] Presently, MALDI and ESI techniques are often considered to be complementary methods for biochemical analysis, so many biochemical or pharmaceutical laboratories have two instruments in use. Obviously there are significant benefits of combining both ion sources in one instrument, as in the embodiments above. In particular, the cost of a combined instrument is expected to be little more than half the cost of two separate instruments. In addition, similar procedures for ion manipulation, detection and mass calibration could be used, since the ion production is largely decoupled from the ion measurement. This would simplify the analysis and processing of the separate spectra and their comparison.

[0061] The ability the use both MALDI and ESI sources on a single instrument is not restricted to the spectrometer shown in Figure 1, but is applicable to any mass spectrometer with a collisional damping interface. In particular it is applicable to the QqTOF instrument discussed above and described in more detail below.

[0062] While specific embodiments of the invention have been described, it will be appreciated that a number of variations are possible within the scope of the present invention. Thus, the apparatus could include a single multipole rod set as shown in Figure 1, or two rod sets as shown in Figure 2. While quadrupole rod sets are preferred, other rod sets, such as hexapole and octopole are possible, and the rod set can be selected based on the known characteristics of the different rod sets. Additionally, it is possible that three or more rod sets could be provided. Further, while Figure 2 shows the two rod sets, 31 and 32 provided in a common chamber, the rod sets could, in known manner, be provided in separate chambers operating at different pressures, to enable different operations to be preformed. Thus, to perform conventional mass selection, there could be one chamber operating at a very low pressure so that there is little or no collisional activity between the ions and the damping gas. Further, the pressure of the gas could be varied, between different chambers, to meet the requirements for collisional damping, where a relatively large number of collisions are desired as opposed to collision induced fragmentation, where excessive collisions are not desirable.

[0063] Reference will now be made to Figure 11. For simplicity and brevity, components common with the apparatus or

spectrometer of Figure 2 are given the same reference numeral, and a description of these components is not repeated.

[0064] Here, the MALDI target is provided at 100 and generates an ion beam indicated at 102. The MALDI target 100 is located in a differentially pumped chamber 104 connected to a pump as indicated at 106 in known manner. A first rod set Q0 is located in the chamber 104. An aperture and an interquad aperture plate 108 provides communication through to a main chamber 110. Again, in known manner a pump connection is provided at 112.

[0065] Within the main chamber 110, there is a short rod set 111, sometimes referred to as "stubbies", provided for the purpose collimating the beam. A first quadrupole rod set in the chamber 110 is indicated at Q1 and a second rod set at Q2.

[0066] The rod set Q2 is located in a collision cell 114 provided with a connection, indicated at 116, for a collision gas.

[0067] On leaving the collision cell 114, ions pass through a grid and then an aperture into the storage region 48 of the TOF instrument, again indicated at 50. Here, a TOF instrument 50 is provided with a liner 118 around the flight region.

[0068] Here, the differentially pumped chamber 104 is maintained at pressure of around 10^{-2} torr. The main chamber 110 is maintained at a pressure of around 10^{-5} torr, while the collision cell 114 is maintained at a pressure of around 10^{-2} torr. In known manner, the pressure in the collision cell 114 can be controlled by controlling the supply of nitrogen to it through connection 116.

[0069] Here, collisional damping of ions generating from the MALDI target 100 is accomplished by the relatively high pressure in the differentially pumped chamber 104. Ions then pass through into the quadrupole rod set Q1, which can be operated to mass select a desired ion.

[0070] The mass selected ion is then passed to the collision cell 114, and the rod set Q2; potentials are such that ions enter the rod set Q2 with sufficient energy to effect collision induced dissociation. The fragment ions generated by this CID are then passed into the TOF instrument 50 for analysis.

[0071] Typical spectra obtained in a MALDI-QqTOF instrument are presented in Figure 12. The spectrum shown in Fig. 12a was obtained when Q1 was operated in a wide band mode, so all ions produced in the MALDI ion source were delivered to the TOF mass analyzer. Three peaks (121, 122, 123) in Fig 12a correspond to ions of leucine-enkephalin, substance P and mellitin respectively. When Q1 is operated in selection mode, the spectrum shown in Fig 12b is observed. Here Q1 was set to select only ions of substance P (peak 122) located at m/z around 1347.7. Note that no other peaks or background were observed in the mass spectrum, as conditions in Q1 prevented transmission of other ions. Figure 12c shows the result of selection at substance P (peak 122) and collisional induced dissociation of the substance P ions. In this case Q1 was set to selection mode as in Fig 12b but the potential difference between Q0 and Q2 was increased to promote CID. The peaks observed in the lower mass region are fragments of the substance P ions.

[0072] Referring now to Figure 13, again, like components are given the same reference numerals as in Figures 11 and 2.

[0073] In Figure 13, the MALDI source is indicated at 130 and the ion beam at 132. Here, a sampling cone 134 was placed between the MALDI source 130 and the rod set Q0. This effectively separates the differentially pumped region into a first differentially pumped region 136 and a second differentially pumped region 138. These differentially pumped regions 136, 138 are provided with respective connections 137 and 139 to pumps.

[0074] As before, a short set of rods or stubbies 140 together with a rod set Q1 are provided in a chamber here indicated at 142.

[0075] The alternative collisional damping setup of Figure 13 has been implemented in MALDI-QqTOF instrument but can be used with any configuration of collisional RF ion guides such as the simpler geometry described earlier and shown in Figure 2. In the Figure 13 configuration, some collisional damping is accomplished in the first region or chamber 136 where almost no RF field is present. Nitrogen is supplied to this chamber 136, as in Figure 2, and is also supplied to the chamber 104 in Figure 11; this is comparable to Figure 2, although the nitrogen connection is not shown in these later figures. The pressure in this first differentially pumped region or chamber 136 is typically higher than in the second differentially pump chamber 138 and ions are dragged towards the entrance of Q0 by the combination of a DC field and the gas flow. In spite of the higher pressure, no significant change in the spectrum was observed. The signal Intensity in this configuration was essentially the same as in the configuration shown in Fig 11, provided the diameter of the cone opening was larger than 1mm. With a smaller diameter opening, the signal intensity drops down, presumably because the size of the opening becomes smaller than the diameter of the ion beam.

[0076] Figure 14 shows an apparatus used to study the effect of pressure and electric field on the intensity of the signal produced by MALDI. MALDI ions are generated at a target 150 by a pulsed UV-laser beam 152. The laser beam 152 passed through a lens 154 and a window 156, as in the spectrometer configurations described above. The window 156 is provided i n a chamber 158, whose internal pressure can be varied in known manner (connections for pumps, etc. are not shown). A potential difference U between the target 150 and a collector electrode 162 is provided by a power supply 160.

[0077] Thus, ions generated at the target 150 travel, as indicated at 164, to the collector electrode 162. An approximately homogeneous electric field is established in the region between the target 150 and the collector electrode 162. The field strength is proportional to the applied potential difference U. The distance between the target and collector was about 3 mm. The laser was operated at 20 Hz and the total ion current was measured using an amplifier 166.

[0078] Figure 15 shows the dependence of the total ion current produced by MALDI at different pressures inside the chamber 158 as a function of the voltage applied between the target 150 and the collector electrode 162 shown in Fig. 14. It is apparent that ion yield decreases with increasing pressure, and there is a significant drop in yield between 14 and 47 Torr. However, the drop in yield can be recovered by raising the electric field strength.

[0079] These results indicate that the MALDI technique can be used at any desirable pressure, even out of the range in which RF collisional multipoles can be implemented. Collisional damping of the ions can be accomplished at least partially in the region with no RF field adjacent to the sample target. The inventors believe that similar dependence of pressure and electric field can be observed in some other pulsed ion sources and these ionization techniques can be also used with collisional damping at higher pressures.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- DE19511333 [0007]
- GB2299446A [0007]
- US5373156A [0008]
- US5689111A [0009]
- US4963736A [0032]

Non-patent literature cited in the description

- **KRUTCHINSKY A.N.CHERNUSHEVICH I.V.SPICER V.L.ENS W.STANDING K.G.**Journal of the American Society for Mass Spectrometry, 1998, vol. 9, 569-579 [0002] [0058]
- **A. SHEVCHENKO et al.**Rapid Commun. Mass Spectrom., 1997, vol. 11, 1015- [0029]
- **KRUTCHINSKY A.N.LOBODA A.V.SPICER V.L.DWORSCHAK R.ENS W.STANDING K.G.**Rapid Commun. Mass Spectrom, 1998, vol. 12, 508-518 [0058]

PATENTKRAV

1.   Massespektrometersystem (10) omfattende:

en ionkilde (1; 11, 14, 15) til tilvejebringelse af
analytioner;

et massespektrometer (50);

en ionbane (27), der strækker sig mellem ionkilden (1;
11, 14, 15) og massespektrometret (50); og

en iontransmissionsanordning, der er anbragt i
ionbanen (27) og har en dæmpningsgas i det mindste i
en del af ionbanen (27), kendetegnet ved, at ionkilden
omfatter en pulseret ionkilde (1; 11, 14, 15), hvorved
ioner udsættes for kollisionsdæmpning, og at der
bevirkes konvertering af ionpulser fra ionkilden til
en kvasikontinuerlig ionstråle (102; 132),

hvor massespektrometret omfatter et ortogonalt
flyvetids-massespektrometer, hvorved den
kvasikontinuerlige ionstråle (102; 132) træder ind i
det ortogonale flyvetids-massespektrometer og pulseres
for at konvertere den kvasikontinuerlige ionstråle
(102; 132) tilbage til ionpulser.

2.   Massespektrometersystem (10) ifølge krav 1, hvor
dæmpningsgassen har et tryk i området fra ca. 0,013
N/m^2 (10^{-4} Torr) op til ca. 1,013 x 10^5 N/m^2 (760
torr).

3.   Massespektrometersystem (10) ifølge krav 1 eller 2,
hvor dæmpningsgassen er tilvejebragt i nærheden af den
pulserede ionkilde (1; 11, 14, 15) og har et
tilstrækkeligt tryk til at bevirke reduktion af

energispredningen af ioner, som emitteres fra ionkilden.

4. Massespektrometersystem (10) ifølge krav 1, 2 eller 3, hvor iontransmissionsanordningen indbefatter en RF ionleder (3; 31, 32; 93), og dæmpningsgassen er tilvejebragt i RF ionlederen (3; 31, 32; 93).

5. Massespektrometersystem (10) ifølge krav 4, hvor RF ionlederen er et RF multipol-stangsæt (3; 31, 32; 93), og hvor produktet af trykket af dæmpningsgassen inden i RF multipol-stangsættet (3; 31, 32; 93) gange længden af stængerne i RF multipol-stangsættet (3; 31, 32; 93) er mindst 1,33 N/m^2 – cm (10,0 mTorr-cm).

6. Massespektrometersystem ifølge krav 4 eller 5, hvor RF ionlederen og dæmpningsgassen er tilvejebragt i nærheden af den pulserede ionkilde.

7. Massespektrometersystem (10) ifølge krav 1, 2, 3, 4 eller 6, som indbefatter i det mindste et af et RF multipol-stangsæt (3; 31, 32; 93) og et ringsæt, hvor ionbanen (27) strækker sig derigennem, og hvor dæmpningsgassen er tilvejebragt i mindst et af RF multipol-stangsættet (3; 31, 32; 93) og RF ringsættet, når det er til stede.

8. Massespektrometersystem ifølge krav 1, hvor længden af ionbanen (27) med dæmpningsgassen og dæmpningsgassens tryk er tilstrækkeligt til at konvertere ionpulser til kvasikontinuerlig ionstråle (102; 132).

9. Massespektrometersystem (10) ifølge et hvilket som helst af de foregående krav, som indbefatter et første differenstrykkammer (136), hvor den pulserede ionkilde (1; 11, 14, 15) er tilvejebragt i det første differenstrykkammer (136).

10. Massespektrometersystem (10) ifølge et hvilket som
helst af kravene 1 til 8, som indbefatter et første
differenstrykkammer (136), hvor den pulserede ionkilde
(1; 11, 14, 15) er tilvejebragt i det første
differenstrykkammer (136), et andet
differenstrykkammer (138), der er anbragt mellem det
første differenstrykkammer (136) og massespektrometret
(50), og en skimmer (134) mellem det første (136) og
det andet (138) differenstrykkammer til opretholdelse
af en trykdifferens mellem det første og andet
differenstrykkammer (136; 138).

11. Massespektrometersystem (10) ifølge krav 9, hvor det
første differenstrykkammer (30, 104, 136) indbefatter
et RF multipol-stangsæt (3; 31, 32; 93), der er
konfigureret til at virke som en ionleder.

12. Massespektrometersystem (10) ifølge krav 10, hvor det
andet differenstrykkammer (138) indbefatter et RF
multipol-stangsæt (Q0), der er konfigureret til at
virke som en ionleder.

13. Massespektrometersystem (10) ifølge et hvilket som
helst af kravene 1 til 7, som indbefatter en
masseanalysator (31, Q1) og en kollisionscelle (32;
114), der er tilvejebragt før massespektrometret (50)
og i ionbanen (27), hvor masseanalysatoren (31, Q1)
indbefatter et multipol-stangsæt (31, Q1), der er
konfigureret til at udvælge en præcursorion, og
kollisionscellen (32; 114) er forsynet med en
dæmpningsgas under anvendelsen til at bevirke
fragmentering af udvalgte præcursorioner til dannelse
af fragmentioner til analyse i massespektrometret
(50).

14. Massespektrometersystem (10) ifølge krav 13, hvor kollisionscellen (114) er tilvejebragt som et fra masseanalysatoren (Q1) adskilt kammer.

15. Massespektrometersystem (10) ifølge et hvilket som helst af de foregående krav, hvor den pulserede ionkilde (1; 11, 14, 15) omfatter en overflade (15), der indeholder analytmolekyler, og en pulseret laser (14), der er rettet mod overfladen (15) til tilvejebringelse af laserpulser for at bevirke ionisering af analytmolekylerne.

16. Massespektrometersystem (10) ifølge krav 15, hvor overfladen (15) indeholder et targetmateriale, som er sammensat af en matrix og analytmolekyler i matricen, hvor matricen omfatter en species, der absorberer stråling fra den pulserede laser (14) for at fremme desorption og ionisering af analytmolekylerne.

17. Massespektrometersystem (10) ifølge et hvilket som helst af de foregående krav, som yderligere indbefatter en kontinuerlig ionkilde (94a) og et organ til udvælgelse af en af den kontinuerlige ionkilde (94a) og den pulserede ionkilde (94b).

18. Massespektrometersystem (10) ifølge krav 17, som indbefatter i det mindste en af: en flerhed af pulserede ionkilder (94b) og en flerhed af kontinuerlige ionkilder (94a), hvor organet til udvælgelse muliggør udvælgelse af en hvilken som helst af de kontinuerlige og pulserede ionkilder.

19. Massespektrometersystem (10) ifølge krav 1, som indbefatter et udvælgelsesorgan til at bevirke masseudvælgelse af en præcursorion og en kollisionscelle for at bevirke kollisionsfremkaldt dissociation af præcursorioner til dannelse af

fragmentioner, hvor udvælgelsesorganet og kollisionscellen er anbragt i ionbanen (27) før massespektrometret (50).

20. Massespektrometersystem (10) ifølge krav 19, hvor udvælgelsesorganet omfatter en ionfælde til at bevirke både masseudvælgelse af en præcursorion og kollisionsfremkaldt dissociation.

21. Massespektrometersystem (10) ifølge krav 19, hvor kollisionsfremkaldt dissociation bevirkes af en af ultraviolet eller infrarød stråling eller af overfladefremkaldt dissociation.

22. Massespektrometersystem (10) ifølge et hvilket som helst af de foregående krav, hvor der også bevirkes i det mindste en af;

    en reduktion i energispredningen af ioner, der emitteres fra ionkilden,

    i det mindste delvis undertrykkelse af uønsket fragmentering af analytioner; og

    spredning af ioner rumligt og tidsmæssigt langs ionbanen.

23. Fremgangsmåde til frembringelse af ioner langs en ionbane (27), der strækker sig gennem en iontransmissionsanordning til et massespektrometer (50), til analyse, kendetegnet ved, at fremgangsmåden omfatter trinene til:

    (1) tilvejebringelse af en pulseret ionkilde (1; 11, 14, 15) som ionkilden,

(2)   frembringelse af ionpulser fra den pulserede
      ionkilde (1; 11, 14, 15),

(3)   forsyning af iontransmissionsanordningen med en
      dæmpningsgas i det mindste i en del af ionbanen
      (27) for at bevirke kollisionsdæmpning af
      ionbevægelse og for at bevirke konvertering af
      ionpulser fra ionkilden til en kvasikontinuerlig
      ionstråle (102; 132;

(4)   placering af ionbanen (27) vinkelret i forhold
      til flyvetids-massespektrometrets (50) akse;

(5)   passage af den kvasikontinuerlige ionstråle (102;
      132) i alt væsentligt kontinuerligt ind i
      flyvetids-massespektrometret (50); og pulsering
      af ionerne i flyvetids-massespektrometret (50)
      for at bevirke masseanalyse.

24. Fremgangsmåde ifølge krav 23, hvor gassen
     tilvejebringes ved et tryk i et område fra ca. 0,013
     N/m^2 (10^{-4} Torr) op til ca. 1,013 x 10^5 N/m^2 (760
     Torr).

25. Fremgangsmåde ifølge krav 23 eller 24, som omfatter
     tilvejebringelse af dæmpningsgas i nærheden af
     ionkilden (1; 11, 14, 15) og med et tryk, der er
     tilstrækkeligt til at bevirke en reduktion i
     energispredningen af ioner, som emitteres fra
     ionkilden (1; 11, 14, 15).

26. Fremgangsmåde ifølge krav 23, 24 eller 25, hvor
     gastrykket er et sådant, at ioner, der emitteres fra
     ionkilden (1; 11, 14, 15) dæmpes tilstrækkeligt til
     betydeligt at reducere uønsket fragmentering af ioner.

27. Fremgangsmåde ifølge et hvilket som helst af kravene 23 til 26, som indbefatter tilvejebringelse af en RF ionleder (3; 31, 32; 93) langs ionbanen (27) og tilvejebringelse af et gastryk i RF ionlederen.

28. Fremgangsmåde ifølge krav 27, hvor RF ionlederen er et RF multipol-stangsæt, og gastrykket er et sådant, at et multiplum af længden af RF ionlederen (3; 31, 32; 93) gange gastrykket er mindst 1,33 N/m^2 - cm (10,0 mTorr-cm).

29. Fremgangsmåde ifølge et hvilket som helst af kravene 23 til 28, som indbefatter gennemføring af ionerne langs ionbanen (27) gennem i det mindste et af et RF multipol-stangsæt (3; 31, 32; 93) og et RF ringsæt.

30. Fremgangsmåde ifølge krav 23, som yderligere omfatter: tilvejebringelse af længden af den nævnte del af ionbanen (27), der er forsynet med dæmpningsgassen, og et tryk af dæmpningsgassen på en sådan måde, at ionpulserne konverteres til en kvasikontinuerlig stråle.

31. Fremgangsmåde ifølge et hvilket som helst af kravene 23 til 30, som indbefatter tilvejebringelse af et differenstrykområde (104), der strækker sig fra ionkilden (1; 11, 14, 15) og indbefatter en RF ionleder, som omfatter et multipol-stangsæt (3; 31, 32; 93), hvor fremgangsmåden indbefatter opretholdelse af et ønsket tryk i differenstrykområdet (104) og drift af ionlederen for at samle og lede ioner langs ionbanen(27).

32. Fremgangsmåde ifølge krav 31, som indbefatter: tilvejebringelse af et første differenstrykområde (136) i umiddelbar nærhed af ionkilden (130), tilvejebringelse af ionlederen (Q0) i et andet

differenstrykområde(138) i nærheden af det første
differenstrykområde (136) og tilvejebringelse af en
skimmer (134), der adskiller det første og andet
differenstrykområde (136; 138); og bevirkelse af at
ioner, som frembringes af en ionkilde (130), vandrer
langs ionbanen (132) fra det første
differenstrykområde (136) til det andet
differenstrykområde (138) ved hjælp af i det mindste
et af en gasstrøm og et elektrostatisk potential.

33. Fremgangsmåde ifølge et hvilket som helst af kravene
    23 til 29, som indbefatter tilvejebringelse af en
    masseanalysator, der indbefatter et multipol-
    stangsæt(31, Q1), og en kollisionscelle (32, 114), der
    indbefatter et multipol-stangsæt (32, Q2), hvor
    fremgangsmåden indbefatter overføring af ioner gennem
    masseanalysatoren for at udvælge præcursorioner,
    overføring af præcursorionerne ind i kollisionscellen
    (32, 114) for at bevirke kollisionsfremkaldt
    dissociation og derved danne fragmentioner og
    efterfølgende gennemføring af fragmentionerne ind i
    massespektrometret (50) til masseanalyse.

34. Fremgangsmåde ifølge krav 33, som indbefatter
    overføring af ionerne fra kollisionscellen (114)
    ortogonalt ind i en flyvetidsanordning (50) og
    pulsering af ioner i flyvetidsanordningen (50) for at
    bevirke masseanalyse.

35. Fremgangsmåde ifølge krav 33, som indbefatter
    overføring af ionerne gennem et yderligere
    massefilter, der indbefatter et firepolet stangsæt til
    udvælgelse af et masse-til-ladningforhold til
    detektering, og detektering af de udvalgte ioner.

36. Fremgangsmåde ifølge et hvilket som helst af kravene
    23 til 35, som indbefatter frembringelse af ioner ved

tilvejebringelse af en kilde for analytmolekyler og bestråling af kilden for analytmolekyler med en pulseret laserstråle (20) for derved at frembringe ionpulser.

37. Fremgangsmåde ifølge krav 23, 29 eller 35, som indbefatter frembringelse af ioner ved den pulserede ionkilde ved tilvejebringelse af en kilde for analytmolekyler og bestråling af kilden for analytmolekyler med en pulseret laserstråle for derved at frembringe ionpulser, og injicering af ionpulser i flyvetids-massespektrometret ved en hastighed, der er væsentligt større end frembringelseshastigheden for laserpulserne, hvorved hver ionpuls fra den pulserede ionkilde tilvejebringer en flerhed af injektionspulser for flyvetids-massespektrometret.

38. Fremgangsmåde ifølge krav 36 eller 37, som indbefatter tilvejebringelse af analytmolekylerne i et targetmateriale (15; 50) omfattende en matrix af en species, der er tilpasset til at absorbere stråling fra laseren (14) og analytmolekylerne, hvor fremgangsmåden omfatter bestråling af matricen med den pulserede laser (14), hvorved species absorberer laserstråling for at bevirke desorption og ionisering af analytmolekylerne.

39. Fremgangsmåde ifølge et hvilket som helst af kravene 23 til 38, som yderligere indbefatter tilvejebringelse af en kontinuerlig ionkilde (94a) og udvælgelse af en af den førstnævnte ionkilde (94b) for pulserede ioner og den kontinuerlige ionkilde (94a) for at frembringe ioner.

40. Fremgangsmåde ifølge krav 39, som yderligere indbefatter tilvejebringelse af i det mindste en af: en flerhed af pulserede ionkilder (94b) og en flerhed

- 10 -

af kontinuerlige ionkilder (94a), hvor fremgangsmåden yderligere omfatter udvælgelse af en hvilken som helst af de kontinuerlige og pulserede ionkilder til tilvejebringelse af ioner.

41. Fremgangsmåde ifølge krav 23, som indbefatter forud for gennemføring af ionerne ind i massespektrometret (50) udvælgelse af en præcursorion og bevirkelse af kollisionsfremkaldt dissociation af præcursorionerne til dannelse af fragmentioner og efterfølgende gennemføring af fragmentionerne ind i massespektrometret (50) til masseanalyse.

42. Fremgangsmåde ifølge krav 41, som indbefatter at bevirke masseudvælgelse af en præcursorion og kollisionsfremkaldt dissociation i en enkelt anordning.

43. Fremgangsmåde ifølge krav 41, som indbefatter at bevirke kollisionsfremkaldt dissociation ved hjælp af en af ultraviolet eller infrarød stråling eller af overfladefremkaldt dissociation.

44. Fremgangsmåde ifølge et hvilket som helst af kravene 23 til 43, hvor der bevirkes i det mindste en af:

en reduktion i energispredningen af ioner, der emitteres fra ionkilden;

i det mindste delvis undertrykkelse af uønsket fragmentering af analysandioner; og
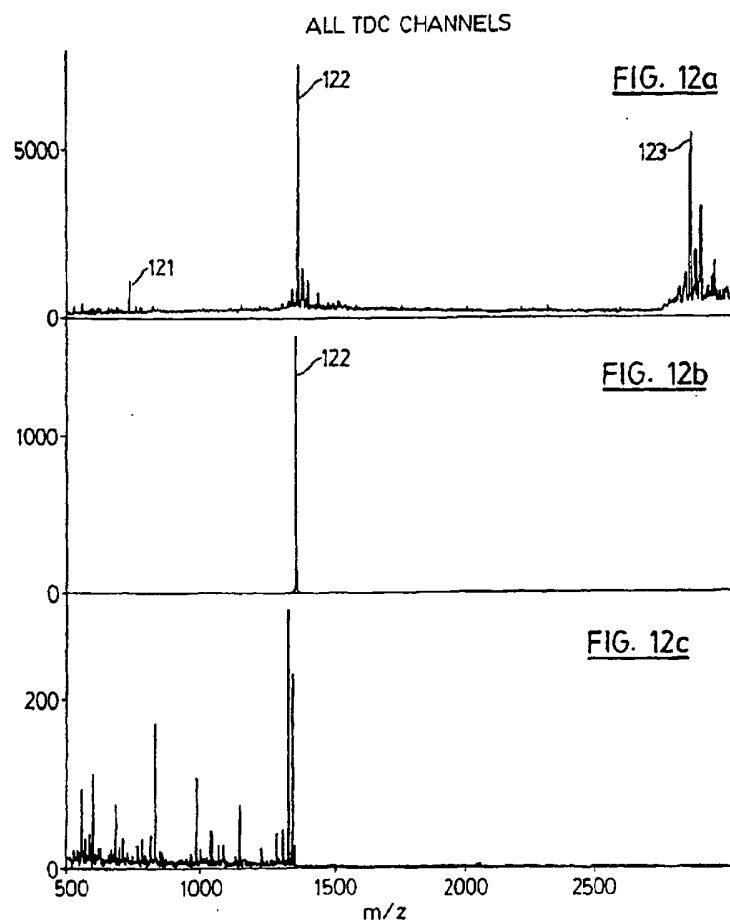
spredning af ioner rumligt og tidsmæssigt langs ionbanen.

DRAWINGS

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

SENSITIVITY FOR SUBSTANCE P. ACQUISITION TIME 1 MIN
5fM ON THE PROBE (1 µL OF 5nM SOLUTION)

FIG. 6

FIG. 7

$U_1(t) = A_1 SIN (\omega t)$
$U_2(t) = A_2 SIN \omega t$
$A_2 = A_1$

FIG. 7A

$U_1(t) = A_1 SIN (\omega t)+V$
$U_2(t) = A_2 SIN (\omega t)$
$A_2 = 1/3A_1$

FIG. 7B

$U_1(t) = A_1 SIN (\omega t)+V$
$U_2(t) = A_2 SIN (\omega t)$
$A_2 = 1/3A_2$

FIG. 7C

FIG. 8A

FIG. 8B

FIG. 8C

FIG. 8D

SUBSTANCE P IONS DESORBED FROM ONE SPOT OF THE
α-CIANO-HYDROXY-CINNAMIC ACID MATRIX

NUMBER OF IONS / 13 LASER SHOTS
OR NUMBER OF IONS / SEC

NUMBER OF LASER SHOTS ON ONE SPOT

FIG. 9

9

FIG. 10

FIG. 11

11

FIG. 12a

FIG. 12b

FIG. 12c

FIG. 13

FIG. 14

14

FIG. 15