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(54) Title: METHOD OF AND APPARATUS FOR SELECTIVE COLLISION–INDUCED DISSOCIATION OF IONS IN A QUADRUPOLE ION GUIDE

(57) Abstract

A method and apparatus are provided for selective collision–induced dissociation of a substance, by resonance excitation of ions. An ion stream is supplied into a quadrupole ion guide operated at elevated pressure with a buffer gas. In addition to a radio frequency field for guiding ions through the ion guide, an extra field or other excitation is provided. This field is selected to cause resonance excitation of parent ions of interest. These ions would then gain kinetic energy and undergo enhanced collision–induced dissociation with a buffer gas. This generates fragment ions, so that the resultant ion stream, containing remaining parent ions and fragment ions can be analysed in a suitable analyzer. The method essentially enables the two steps of selection of a particular parent ion and generation of fragment ions by collision–induced dissociation to be carried out in a single step, giving a simpler apparatus and enhanced efficiency.

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Title: METHOD OF AND APPARATUS FOR SELECTIVE COLLISION-INDUCED DISSOCIATION OF IONS IN A QUADRUPOLE ION GUIDE

FIELD OF THE INVENTION

This invention relates to a mass spectrometer, and more particularly relates to collision-induced dissociation (CID) in a tandem mass spectrometer or in an ion guide.

BACKGROUND OF THE INVENTION

Radio frequency (RF) only multipole spectrometers, more particularly quadrupole spectrometers, are widely applied in mass spectrometry and nuclear physics, due to their ability to transport ions with minimal losses. During such transportation of the ions, the initial ion positions and velocities change, but the total phase space volume occupied by the ion beam remains constant (see Dawson, Quadrupole mass spectrometry and its applications). However, if a buffer gas is introduced into the ion guide, a dissipative process occurs, due to ion molecule collisions, and this enables an ion beam to be focused onto the quadrupole axis after the initial velocities have been damped.

Collisional quadrupole or other multipole devices have been used as an ion guide providing an interface between an ion source and a mass spectrometer, or alternatively as a collision cell for collision-induced dissociation (CID) experiments. As a straightforward interface, collisional damping reduces the space and velocity distributions of the ions leaving the ion source, thus improving the beam quality. For CID experiments, primary ions having relatively large velocities enter the multipole and collide with buffer gas molecules, so collision-induced dissociation takes place. The multipole helps to keep both primary ions and fragment ions, resulting from the collision-induced dissociation, close to the axis and to deliver them to the exit for further analysis. Collisions inside the multipole spectrometer again act to reduce the space and velocity distribution of the ion beam.

Ion motion in a perfect quadrupole field is governed by Mathieu's equation (See Dawson as cited above); ions oscillate around the quadrupole axis at an appropriate fundamental frequency which is determined by their m/z and quadrupole parameters, and is independent of ion position and velocity. If the frequency of any periodic forces acting on ions coincides with the ion fundamental frequency, then resonance excitation takes place. Similar resonance excitation is widely applied in quadrupole ion trap or in ion cyclotron resonance mass spectrometers (R.E. March, R.J. Hughes, Quadrupole storage mass spectrometry, 1989, John Wiley&Sons).

These properties of spectrometers have been employed in many ways. Thus, in U.S. provisional patent application 60/046,926 filed May 16, 1997, there is disclosed a high pressure MS-MS system. This was intended to provide improvements to a conventional triple quadrupole mass spectrometer arrangement, employing two precision quadrupole mass spectrometers separated by an RF-only quadrupole which is operated as a gas collision cell. The first mass spectrometer is used to select a specific ion mass-to-charge ratio (m/z), and to transmit the selected ions into the RF-only quadrupole or collision cell. In the RF-only quadrupole collision cell, some or all of the parent ions are fragmented by collisions with the background gas, commonly argon or nitrogen, at a pressure of up to several millitorr. The fragment ions, along with any unfragmented parent ions are then transmitted into the second precision-quadrupole which is operated in a mass resolving mode. Usually, the mass resolving mode of this second spectrometer is set to scan over a specified mass range, or else to transmit selected ion fragments by peak hopping, i.e. by being rapidly adjusted to select specific ion m/z ratios in sequence. The ions transmitted through this spectrometer are detected by an ion detector. A problem with this conventional arrangement is that the two mass resolving quadrupoles are required to operate in the high vacuum region (less than 10^{-5} torr), while the intermediate collision cell operates at a pressure up to several millitorr. That earlier invention was intended to simplify the apparatus and eliminate the necessity for separate RF-only and resolving spectrometers at the input to the apparatus. Instead, a single quadrupole is provided, operating in the RF-mode to act as a high pass filter. Additionally, this quadrupole is provided with an AC field, which can be identified as a "filtered noise field", which contains a notch in the frequency range corresponding to the mass of an ion of interest. This notch can be moved, to select and separate desired ions.

Other older proposals can be found, for example, in U.S. Patent 5,420,425 (Bier et al. and assigned to Finnigan Corporation). This relates to an ion trap mass spectrometer, for analyzing ions. It has electrodes shaped to promote an enlarged ion occupied volume. A quadrupole field is provided to trap ions within a predetermined range of mass to charge ratios. Then, the quadrupole field is changed so that trapped ions with specific masses become unstable and leave the trapping chamber in a direction orthogonal to the central axis of the chamber. The ions leaving the spectrometer are detected, to provide a signal indicative of their mass-to-charge ratios. One method that is taught in this patent is to first introduce ions within a predetermined range of mass-to-charge ratios into the chamber and subsequently change the field to select just some ions for further manipulation. The quadrupole field is then adjusted so as to be capable of trapping product ions of the remaining ions, and the remaining ions are then dissociated or reacted with a neutral gas to form those product ions. Subsequently, the quadrupole field is changed again, to remove, for detection, ions whose mass-to-charge ratios lie within the desired range, which ions are then detected.

The first technique taught above is complex, and requires a number of separate quadrupoles or the like, and the ability to move the ions sequentially through the different quadrupole sections. The technique taught in the Finnigan patent is complex and requires a number of steps. Also, it is concerned with ion traps and not a flow quadrupole. Accordingly, it is desirable to provide one technique which, in one device, readily enables ions of a selected mass-to-charge ratio to be subject to collision-induced-dissociation (CID) or fragmentation, so that the fragments can be transported further for subsequent analysis. It is desirable to provide this in a single device, since movement of ions from one device to another inevitably leads to some losses. Similarly, the techniques of the Finnigan patent work with pulse ion sources, but attempts to use them for continuous ion flow, for instance from an electrospray ion source, will lead to inefficiencies. In this field, spectrometers are frequently used to analyze small samples, and often, high efficiency is required, if any reliable reading or measurement is to be obtained.

SUMMARY OF THE PRESENT INVENTION

In accordance with a first aspect of the present invention, there is provided a method of analyzing a substance, the method comprising the steps of:

(1) ionizing the substance to generate a stream of ions;

(2) supplying the stream of ions to a quadrupole ion guide;

(3) providing a buffer gas in the ion guide;

(4) applying a radio frequency field by a quadrupole ion guide to maintain desired ions in a stable trajectory through the ion guide;

(5) in addition to the radio frequency field applied in step (4), applying a periodic change to the ion guide to cause resonance excitation of ions having a selected m/z ratio whereby the selected ions acquire increased kinetic energies resulting in enhanced collision-induced dissociation with the buffer gas; and

(6) analyzing the ion spectrum after fragmentation.

The selected ions preferably are subject to resonance excitation by one of: application of an additional field in the quadrupole, either by being applied to the existing rod set or by application to extra electrodes or rods provided for that purpose; amplitude modulation of the radio frequency field applied by the quadrupole; frequency modulation of the radio frequency field applied by the quadrupole; and periodic variation in the quadrupole radius, the resonance excitation being at a frequency different from the frequency of the radio frequency field.

Further, it is possible for the method to include two or more excitations applied by the quadrupoles, with the second and other excitations chosen to cause resonance excitation of one of an additionally selected parent ion and fragment ions.

With a buffer gas in a quadrupole there is an excitation threshold below which all energy acquired over one excitation period dissipates in collisions. So, the value of the threshold reflects the collision properties of the excited ions, and thus the ion cross-section and mobility could be measured.

In accordance with another aspect of the present invention, there is provided an apparatus, for analyzing a substance by resonance excitation of selected ions and selective collision-induced dissociation, the apparatus comprising:

an ion source for generating a stream of parent ions;

a quadrupole ion guide, for receiving the stream of parent ions and provided with a buffer gas, for collision-induced dissociation between the parent ions and the buffer gas;

means for generating a radio frequency signal in the quadrupole ion guide, for guiding ions through the quadrupole ion guide, said generating means being connected to the quadrupole ion guide;

means for generating an excitation signal connected to the quadrupole ion guide for causing resonance excitation of the parent ions, thereby causing enhanced collision-induced dissociation between the parent ions and the buffer gas, generating fragment ions; and

a mass analyzer connected to the quadrupole ion guide, for receiving parent and fragment ions and for analyzing the ion spectrum.

While it is preferred to use a quadrupole device in the present invention, it is also envisaged that the invention could be applied to a variety of multipole instruments, such as a hexapole or octopole device. In these devices, the secular frequency of an ion depends on its position, so that the mass resolution and selectivity would not be as high. However, for some applications, the selectivity available in other multipole devices might be sufficient, and hence both the method and apparatus of the present invention could be implemented using a variety of multipole devices.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

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 in which:

Figure 1 shows schematically a first embodiment of an apparatus in accordance with the present invention;

Figure 2 shows schematically a second embodiment of an apparatus in accordance with the present invention;

Figures 3a and 3b show graphs of a spectrum of ion count against mass for a sample on different scales;

Figures 4a and 4b show graphs of a spectrum of the same sample as for Figure 3, after fragmentation and also on different scales;

Figure 5 shows a graph similar to Figure 4a, operating in a different mode; and

Figure 6 shows the spectrum of Figure 4a, after subtraction of the spectrum of Figure 3a.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Figure 1 shows a first embodiment of an apparatus generally designated by the reference 10. The apparatus 10 includes an electrospray ion source 12. A gas curtain stage 14 is used to evaporate charged droplets by means of hot dry nitrogen 16. A heated capillary 18 introduces the gas-ion mixture to a vacuum chamber 22 which is the first stage of an interface between atmospheric pressure and high vacuum.

The chamber 22 is pumped through the line or connection 24, so the pressure in chamber 22 is usually about 2.4 Torr. A focusing electrode 20 helps to separate ions from the buffer gas and to direct these ions toward a skimmer 28. The skimmer 28 separates the first chamber 22 of the interface from the second chamber 26. A connection 30 is provided for the next pump and pressure at this stage is about 0.1 Torr. The quadrupole ion guide 32 is provided in the chamber 26 in known manner.

A third stage 36 of the interface is separated from the second stage by a wall 34 with a small orifice for ions to pass through. Grid electrodes 37 focus ions to an entrance aperture 40 of a time of flight (TOF) analyzer.

Within the TOF analyzer chamber an acceleration column 42 is located. It is constructed from an array of electrodes. In known manner, ions first fill an accumulation-extraction region, during an accumulation period, in which no potential difference is applied across electrodes at the bottom of the TOF. Then, voltage to the bottom plate is pulsed in order to extract or to drive ions into the acceleration column. The repetitive process of accumulation-extraction permits analysis of a continuous ion beam, without dramatic losses. As indicated at 44 ions after acceleration pass into the main TOF chamber 46.

An ion mirror 48 consisting of an electrode array generates a field to reverse the motion of the ions as indicated in 50, and also improves the TOF spectral quality due to the so called "time focusing effect". The ions are collected at detector 52, and their time of flight from the bottom of the acceleration column 42 to this plane is measured, to give an indication of the mass-to-charge ratio of the ions.

Now, in accordance with the present invention, the quadrupole ion guide 32 in the second chamber is operated to cause collision induced dissociation of the ions of interest. In this respect, among many multipole designs available, the quadrupole ion guide has a unique feature. Ions having stable trajectories in a perfect quadrupole field oscillate around the central axis of the quadrupole with a so-called fundamental or secular frequency determined by their m/z ratio and the parameters of the RF field applied to the quadrupole. The fundamental frequency for each ion is independent of the initial coordinates and velocity of the ion.

Now, the present inventors have realized that if an appropriate additional field oscillating at the fundamental frequency (or its multiples) is applied to the quadrupole ion guide 32, then this field can cause resonance excitation of ions with the particular m/z ratio. Such a field is given by:

(1) $f = f_{fund} \pm n^* f_{RF}$ (n = 0, ±1, ±2 etc)

Alternatively, other periodic changes in quadrupole parameters, such as the RF amplitude could provide similar resonance excitation. Moreover, it is expected that excitation at several different preset frequencies could cause a number of different ions with different m/z ratios to be excited.

As a result of such excitation, the average velocity of the selected ions will be increased. Such resonance excitation is known and has been proposed for use in isotope separation, by selectively exciting the m/z of one isotope in order to cause it to be removed from the quadrupole by striking the rods, thus causing the ion beam to be enriched in the preferred isotope (Dawson, Quadrupole mass spectrometry and its application). Similar resonance excitation methods have been used for ion detection and collision-induced dissociation in a 3D (three dimensional) quadrupole ion trap.

In a 3D trap, ions are shared for a selected time period, which allows them to be excited and then fragmented after an appropriate time interval. In the present invention, ions are fragmented as they pass through the quadrupole, without trapping them. Since the ions spend only a limited time in the quadrupole, it had previously been thought that they would not have sufficient time to be excited and fragmented before reaching the end of the quadrupole, without striking the rods. Similarly, ion traps are operated at a pressure of about 1 millitorr or less of helium and this gives no indication as to whether ions could be selectively excited and caused to fragment at a pressure such as 100 mTorr, since the higher pressure acts to damp the radial ion motion. Additionally, the "resolution" (actually a window of about 100 Daltons wide, as shown in Figure 4a), the high pressure, and the efficiency of fragmentation at this high pressure, could not at all have been derived from the prior art.

Also, the differences between 3D ion trap and quadrupole ion guide are in electrode configurations and in working regimes. An ion trap is a storage type of mass spectrometer; ions are first accumulated, then processed and then detected. A 3D quadrupole field in an ion trap acts in all 3 dimensions and focuses ions toward the center of the trap. A quadrupole ion guide or 2D quadrupole is usually a flow device. It provides a constant flow of ions from the entrance to the exit. A 2D field acts in 2 dimensions orthogonal to the quadrupole axis and focuses ions toward the axis of the quadrupole.

The Finnigan patent is an exception in the field of 2D quadrupoles. There, the inventors propose to use it in a storage mode closing both ends by the means of higher DC potentials applied to elements at the ends of the main quadrupole. In contrast, in the present invention the excitation method is used in the flow mode. Also the Finnigan patent proposes the use of radial ejection of the ions to be detected. The patent suggests resonance excitation and extraction through a slit in one rod of the quadrupole, which is similar to the detection methods implemented in 3D ion traps. That means the beam of extracted ions will have broad space and velocity distributions. Thus it will be hard to manage this beam, to introduce it into another analyzing device, for instance TOF or ICR mass spectrometer, and to obtain the best resolution that the latter device is capable of. In our case, extraction is in the axial direction which gives a beam of high quality that can be easily introduced into another device, the TOF mass analyzer in Fig. 1.

Here, the excited ions acquire high kinetic energies and collision-induced dissociation is more likely to take place. Resulting fragmented ions usually have m/z ratios different from the parent ions so that they are not subject to the resonance excitation. In effect, these fragment ions cool and become focused onto the axis of the quadrupole 32.

Thus, the method of the present invention enables ions to be selected for fragmentation by proper choice of the excitation frequency, i.e. selecting the ions on the basis of their m/z ratios. This is somewhat analogous to the selection of an ion in an upstream quadrupole mass filter for fragmentation in a separate collision cell. Here, the two steps of selection and collision are accomplished in a single quadrupole, without the addition of any other apparatus apart from extra signal generation or modulation equipment. As such, the apparatus should be able to provide much higher sensitivity, since there are no losses at selection and intermediate stages.

As noted above, any suitable form of excitation can be provided. More particularly, there are three preferred modes of excitation, which are described separately below: an excitation signal at its own frequency added to the quadrupole field; amplitude modulation of the main RF quadrupole field at the excitation frequency; and phase or frequency modulation of the main RF signal for the quadrupole at the excitation frequency. The provision of this additional excitation signal can be readily provided using known equipment. This is shown schematically in Figure 1, where 60 indicates conventional equipment for providing RF and DC excitation to the quadrupole ion guide 32, and 62 indicates additional circuitry or equipment for providing the additional excitation signal required by the present invention.

Addition of an excitation signal to the conventional quadrupole RF signal is represented by the following equation:

$$(2) \qquad U(t) = U_{RF}{*}\sin(\Omega{*}t) + U_{RF}\Delta_m{*}\sin(\omega{*}t),$$

where $U(t)$ = quadrupole potential,

U_{RF} = main RF wave amplitude,

Ω = main RF frequency,

Δ_m= excitation factor,

ω = excitation frequency.

Alternatively, for amplitude modulation the signal applied to the quadrupole ion guide is represented by the following equation:

$$(3) \qquad U(t) = U_{RF}*\sin(\Omega*t)*(1+\Delta_{exc}*\sin(\omega*t)),$$

where Δ_{exc} = modulation factor.

Finally, for the third possibility, frequency or phase modulation of the radio frequency excitation signal is represented by the following equation:

$$(4) \qquad U(t) = U_{RF}*\sin(\Omega*t + \Delta_{exc}*(\Omega/\omega)*\sin(\omega*t)).$$

Reference will now be made to Figures 3-6, which show spectra obtained using the method of the present invention. A peptide, substance P, was used to generate ions in the apparatus of Figure 1. In the first test, the RF potential was 690 volts at a frequency of 1.93 MHz, as given by the following equation:

$$(5) \qquad U(t) = 690<V>*\sin(2*\pi*1930000*t<s>).$$

The results are shown in Figure 3. In the test, both doubly charged ions and singly charged ions were observed. A significant peak of doubly charged ions at m/z=674 m/z was observed as indicated at 70. This is shown in more detail in the insert Figure 3b, showing, on an enlarged scale, the spectrum in the range 600-800 m/z.

The second test was run with the same peptide, and with the same base signal for the RF field. An additional component was added to this field having a potential of 9 volts and a frequency of 231 kHz, the total signal being represented by the following equation:

$$(6) \qquad U(t) = 690<V>*\sin(2*\pi*1930000*t<s>) + 9<V>*\sin(2*\pi*231000*t(s)).$$

As shown in Figure 4a, it was found that the doubly charged ions were excited so that energetic collisions of the ions with buffer gas took place, causing ion fragmentation. The buffer gas was nitrogen. As a consequence, there is a dip in the spectrum from approximately 600 to just above 700 m/z, as best shown in the insert 4b, and intense peaks of the fragments were observed. Correspondingly, the fragmented ions give greater concentrations in other parts of the spectrum.

In fact, it has been found that the best results can be obtained if spectra accumulation/subtraction is done on line, i.e. spectrum with and without excitation recorded alternately. By this means, slow variations in ion intensity will not effect the resulting subtracted spectra.

To better show the effect of this excitation fragmentation, Figure 6 shows the spectra of Figure 4a with the spectra Figure 3a subtracted. Figure 6 has been marked with standard notation to show the various fragments identified in this spectrum.

Figure 5 shows another alternative excitation regime, following equation 2 above, i.e. amplitude modulation. Again, the same voltage and frequency were used for the base RF signal, with the amplitude of the signal being subjected to sinusoidal fluctuations to a maximum of 17%, again at a frequency of 231 kHz, as given by the following equation:

$$(7) \quad U(t) = 690<V>*\sin(2*\pi*1930000*t<s>) +(1 + 0.17*\sin(2*\pi*231000*t(s)).$$

It can be seen that the spectrum obtained in Figure 5 is very similar to that obtained in Figure 4a, although with slight variation and the distribution of the different fragments. The dip in the spectrum is shown at 78 and fragments at 79. It will be appreciated that depending upon the substance under investigation and other characteristics, an appropriate excitation regime can be selected, to give optimum results.

It has been found that the effect of excitation becomes noticeable in spectra only when a certain level of superimposed voltage is reached. This threshold is determined by the balance of excitation dissipation forces averaged over the period of the excitation frequency. As a result, the dissipation forces can be measured, giving the values of ion mobility and collisional cross-section.

It is expected that the method of the present invention, providing selective CID, will provide higher sensitivity as compared to conventional standard tandem MS-MS. In a standard MS-MS technique or experiment, the transmission of ions through the mass filter selecting the parent ion can be as low as 10%, so only a small fraction of the potentially available ion beam can possibly give rise to fragment ions. In contrast, with the technique of the present invention, all parent ions are available for fragmentation. It will be appreciated that these sorts of analysis techniques are often used in situations where only a very small amount of a sample is available. For example, in certain scientific or biological studies, only very small amounts of samples are available. These type of spectrometers are also often used in criminal investigations, concerning drugs, explosives and the like, and again often only a trace or small amount of a sample is available. Hence, it is highly desirable to have an instrument with a high sensitivity.

A further advantage is that the apparatus of the present invention only requires one mass analyzer, either a quadrupole, or a time of flight device, the latter being shown in Figure 1, instead of the two or more mass analyzers required for standard MS-MS instruments.

Reference will now be made to Figure 2, which shows an alternative or second embodiment of an apparatus in accordance with the present invention, generally indicated by the reference 80. This apparatus 80 has an ion source 82, and a first mass analyzer or quadrupole 84. In known manner, this includes an entrance skimmer plate 85 and a quadrupole rod set 86. This would be operated at a pressure as low as in a conventional quadrupole mass filter. Pressure here could vary from 10^{-4} Torr down to a higher vacuum. It will depend on the operating parameters, mainly dimensions, and would operate purely to select ions with an m/z of desired interest. These ions would then be passed into second quadrupole, generally indicated by the reference 88, with a rod set 89. Then, like the quadrupole set or guide 32 of the first embodiment, this would be operated at an elevated pressure of, for example, 10^{-4} Torr to 1 Torr, but again this will depend on the operating parameters, mainly dimensions. A signal in accordance with one of the equations above would be applied, to effect excitation of a desired ion, fragmentation etc. The fragmented ions would then be passed through to a final mass analyzer 90, which could be any suitable analyzer such as a quadrupole or time of flight mass spectrometer.

The advantage of this second embodiment is that, to give greater selectivity, certain ions can, effectively, be filtered out in the first mass analyzer 84. Then, just desired ions are excited in the second quadrupole 88. It will be recognized that the selectivity of the technique of the present invention is not perfect, and this second technique can ensure prior removal of ions that could cause interference with or degradation of a signal.

It will also be appreciated that the present invention can be applied in order to extend standard triple quad or quadrupole time of flight (Q-TOF or QqTOF) instruments to MS-MS-MS or even MS^n instruments. For MS-MS-MS, this means selecting a parent ion in Q1 (the first MS selection) in the normal way, accelerating and introducing the ions into the buffer gas Q2 at energies of tens of eV, using the described invention to selectively excite one of the fragments in Q3, and analyzing the resulting spectrum in Q2 (or in the last MS, which could be a TOF). The subtraction methods above could be used to separate the "fragments of the fragment" from the fragments of the original parent. In any case, this can be termed MS-MS-MS, since it provides a fragment spectrum of a fragment. MS-MS-MS-MS would carry this idea further, and provide two excitations, one tuned to fragment of the fragment, and the other to a "fragment of the fragment of the fragment" etc. Subtraction methods (i.e. excitation on/off methods) would be used to deconvolute or analyze, as detailed above. In effect, the present invention enables a number of steps to be carried out in a single stage, which, in a conventional instrument, would require two or more MS stages. This avoids the problems of multiple stages and loss of sample between stages.

It will be appreciated that various modifications are possible within the scope and spirit of the present invention. Thus, while the above equations suggest a single additional frequency applied to the base excitation frequency, it is possible that several additional frequencies could be used for excitation. This would enable a number of different ions to be excited simultaneously. The additional frequencies could be used either to excite additional parent ions, or to excite fragment ions that it is known will be generated by the excitation caused by the first frequency applied. In other words, the first frequency can be selected to cause excitation of a desired ion. Knowing that this will generate certain fragments, for example, fragments 72, 73 or 74 in Figure 4a, a second additional frequency can simultaneously be applied, selected for fragments 74, for example. This in turn will then give secondary fragments. It will be appreciated that this compounding effect can be applied as many times as desired, so that, in effect, one can have $(MS)^n$ carried out in a single collisional quadrupole. Again, this can lead to high efficiencies, since inevitably transfer of ions from one quadrupole to another leads to loss of ions and loss of signal.

WE CLAIM:

1. A method of analyzing a substance, the method comprising the steps of:

(1) ionizing the substance to generate a stream of ions;

(2) supplying the stream of ions to a quadrupole ion guide;

(3) providing a buffer gas in the ion guide;

(4) applying a radio frequency field by the quadrupole ion guide to maintain desired ions in a stable trajectory through the ion guide;

(5) in addition to the radio frequency field applied in step (4), applying a periodic change to the ion guide to cause resonance excitation of ions having a selected m/z ratio whereby the selected ions acquire increased kinetic energies resulting in enhanced collision-induced dissociation with the buffer gas; and

(6) analyzing the ion spectrum after fragmentation.

2. A method as claimed in claim 1, which comprises subjecting the selected ions to resonance excitation by one of: application of an additional field in the quadrupole; amplitude modulation of the radio frequency field applied by the quadrupole; frequency modulation of the radio frequency field applied by the quadrupole; and periodic variation in the quadrupole radius, the resonance excitation being at a frequency different from the frequency of the radio frequency field.

3. A method as claimed in claim 2, which includes selecting the resonance excitation to be at the fundamental frequency of the ion of interest.

4. A method as claimed in claim 1, which includes applying at least one additional excitation field in the quadrupole which additional excitation field is selected to cause resonance excitation of one of an additionally selected parent ion and a fragment ion.

5. A method as claimed in claim 4, which comprises applying the additional excitation by one of a field generated by the quadrupole ion guide and a field generated by additional electrodes.

6. An apparatus, for analyzing a substance by resonance excitation of selected ions and selective collision-induced dissociation, the apparatus comprising:

an ion source for generating a stream of parent ions;

a quadrupole ion guide, for receiving the stream of parent ions and provided with a buffer gas, for collision-induced dissociation between the parent ions and the buffer gas;

means for generating a radio frequency signal in the quadrupole ion guide, for guiding ions through the quadrupole ion guide, said generating means being connected to the quadrupole ion guide;

means for generating an excitation signal connected to the quadrupole ion guide for causing resonance excitation of the parent ions, thereby causing enhanced collision-induced dissociation between the parent ions and the buffer gas, generating fragment ions; and

a mass analyzer connected to the quadrupole ion guide, for receiving parent and fragment ions and for analyzing the ion spectrum.

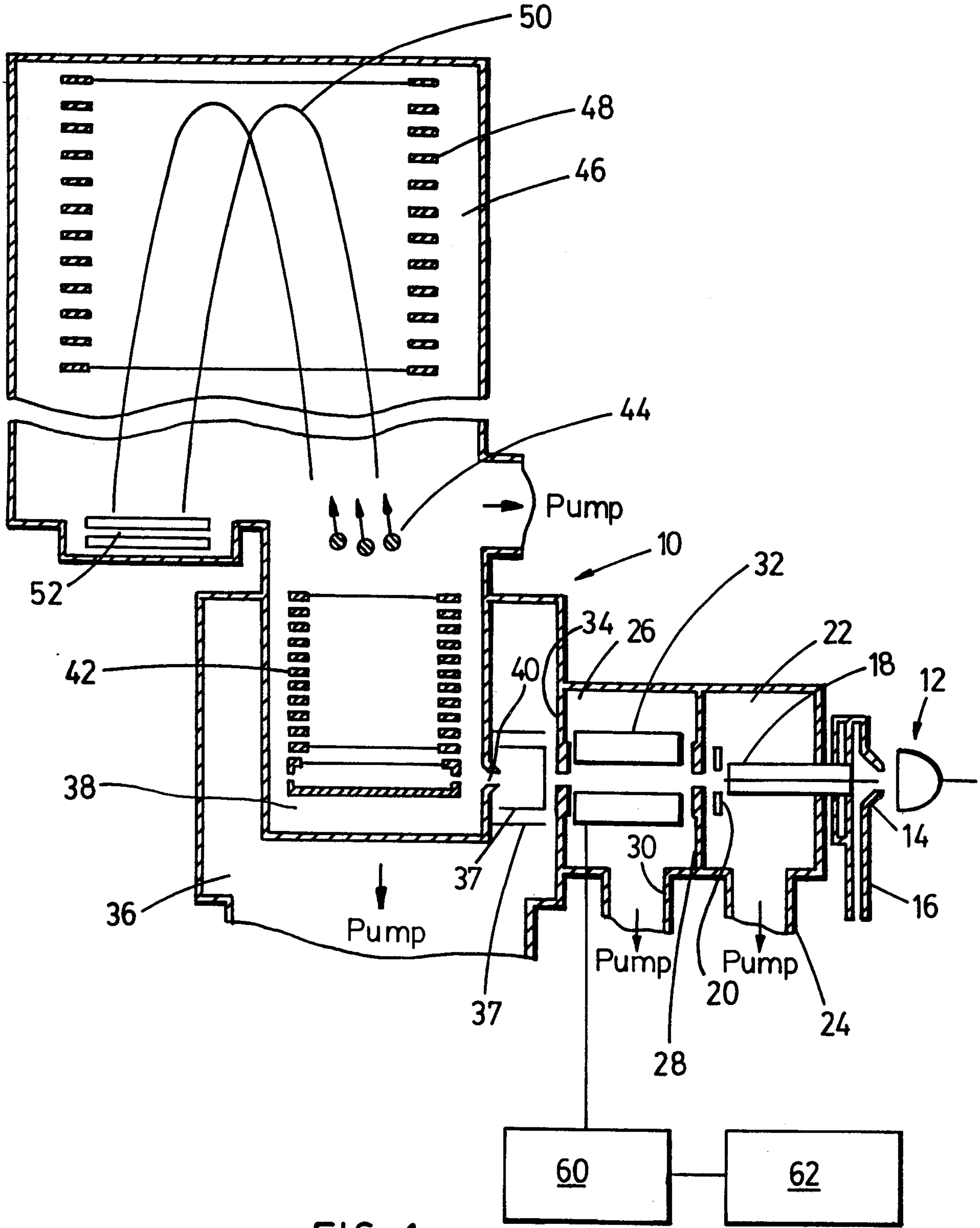
7. An apparatus as claimed in claim 6, wherein the means for generating the excitation signal comprises one of: means for generating an additional signal for addition to the radio frequency signal; means for providing amplitude modulation of the radio frequency signal; and means for providing frequency modulation of the radio frequency signal.

8. An apparatus as claimed in claim 6, wherein the mass analyzer comprises a time-of-flight mass analyzer.

9. An apparatus as claimed in claim 6, wherein the ion source

comprises an electrospray ionization source.

10. An apparatus as claimed in claim 6, which includes an additional quadrupole analyzer provided between the ion source and the quadrupole ion guide, for selecting parent ions of interest, for transmission through to the quadrupole ion guide.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48
46
44
Pump
10
32
52
34
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22
18
12
42
40
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37
36
Pump
Pump
Pump
16
37
20
24
28
60
62

FIG. 1

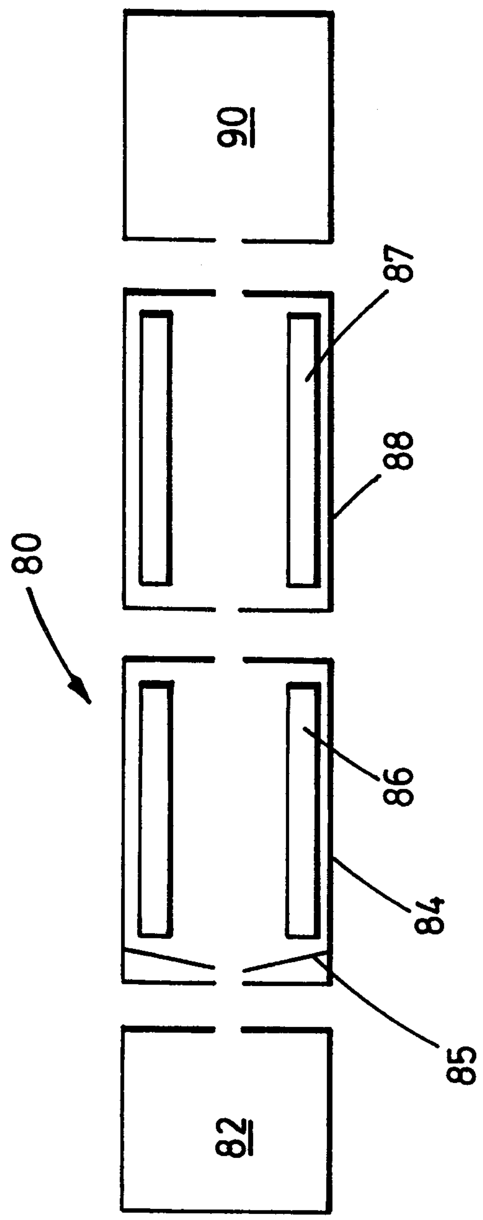


FIG. 2

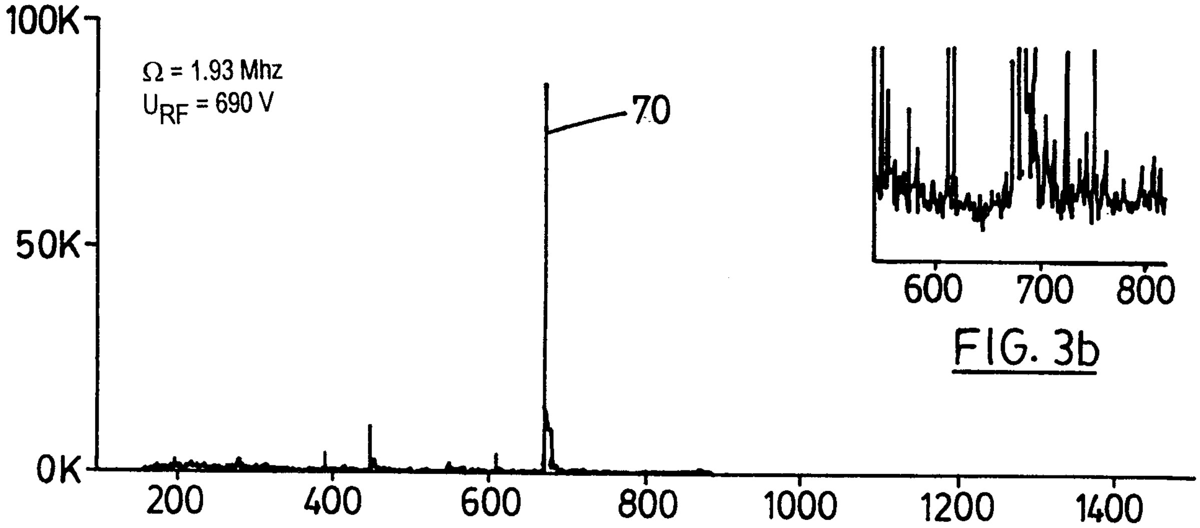


FIG. 3b

FIG. 3a

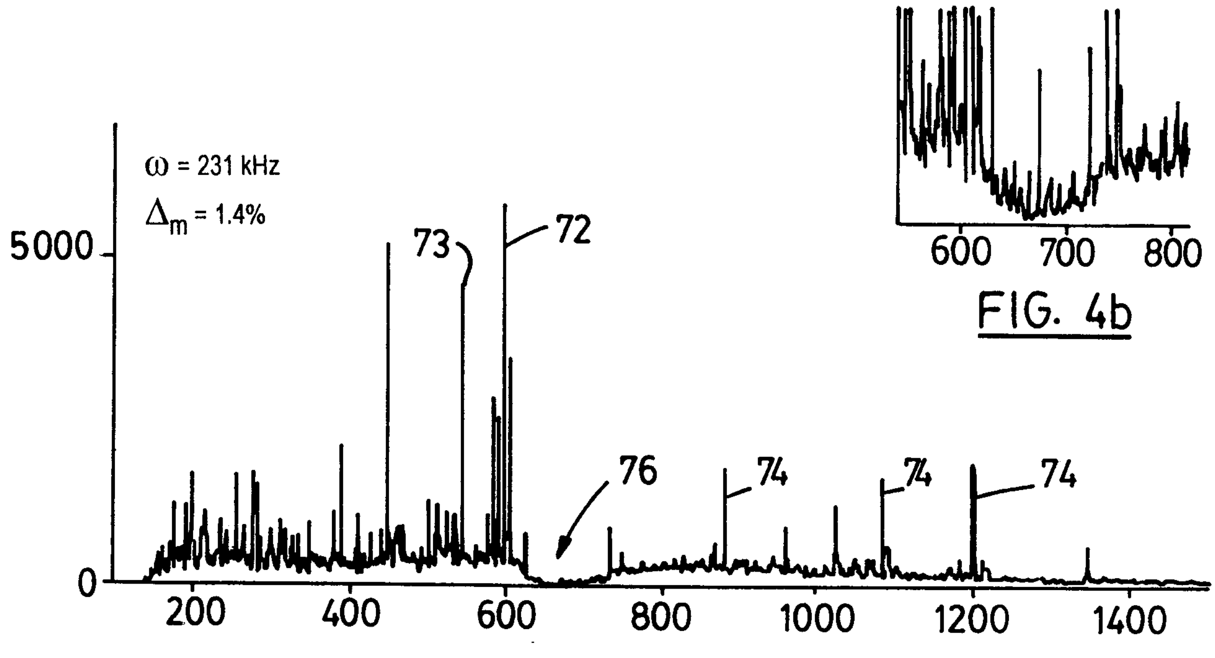
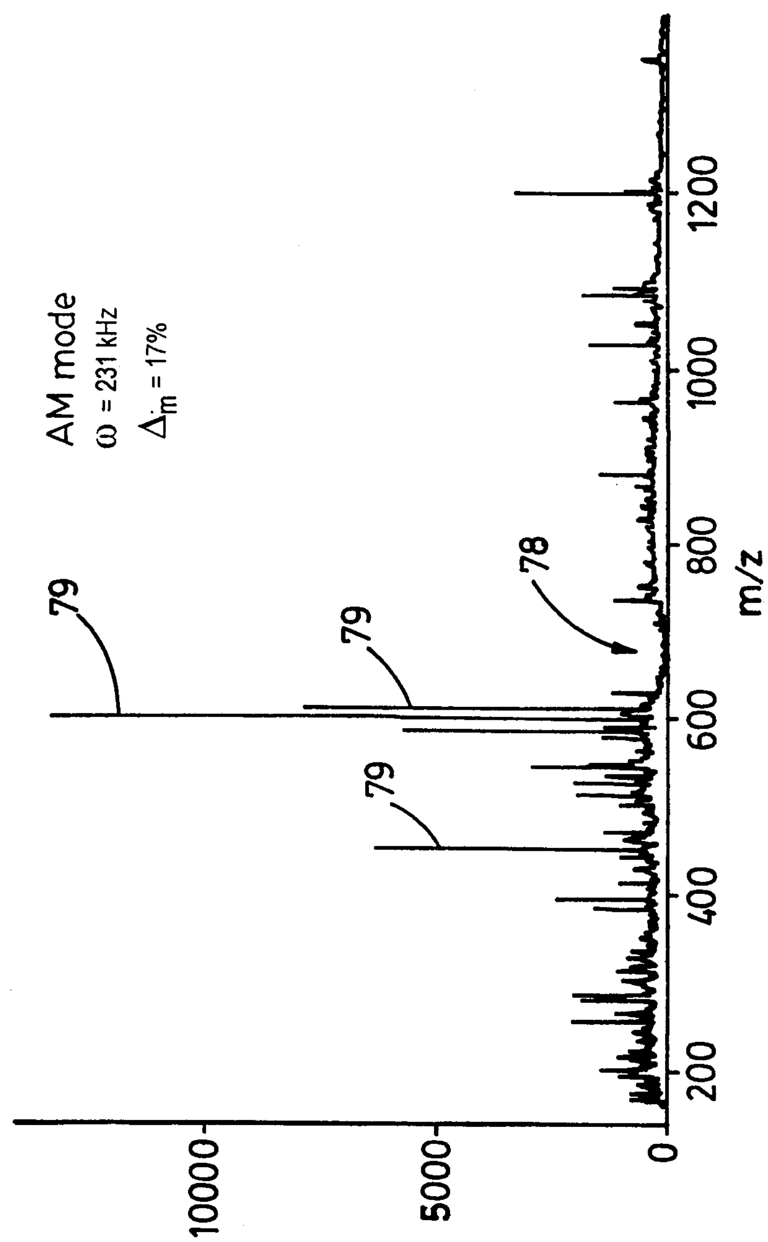


FIG. 4b

FIG. 4a


AM mode
ω = 231 kHz
Δ_m = 17%
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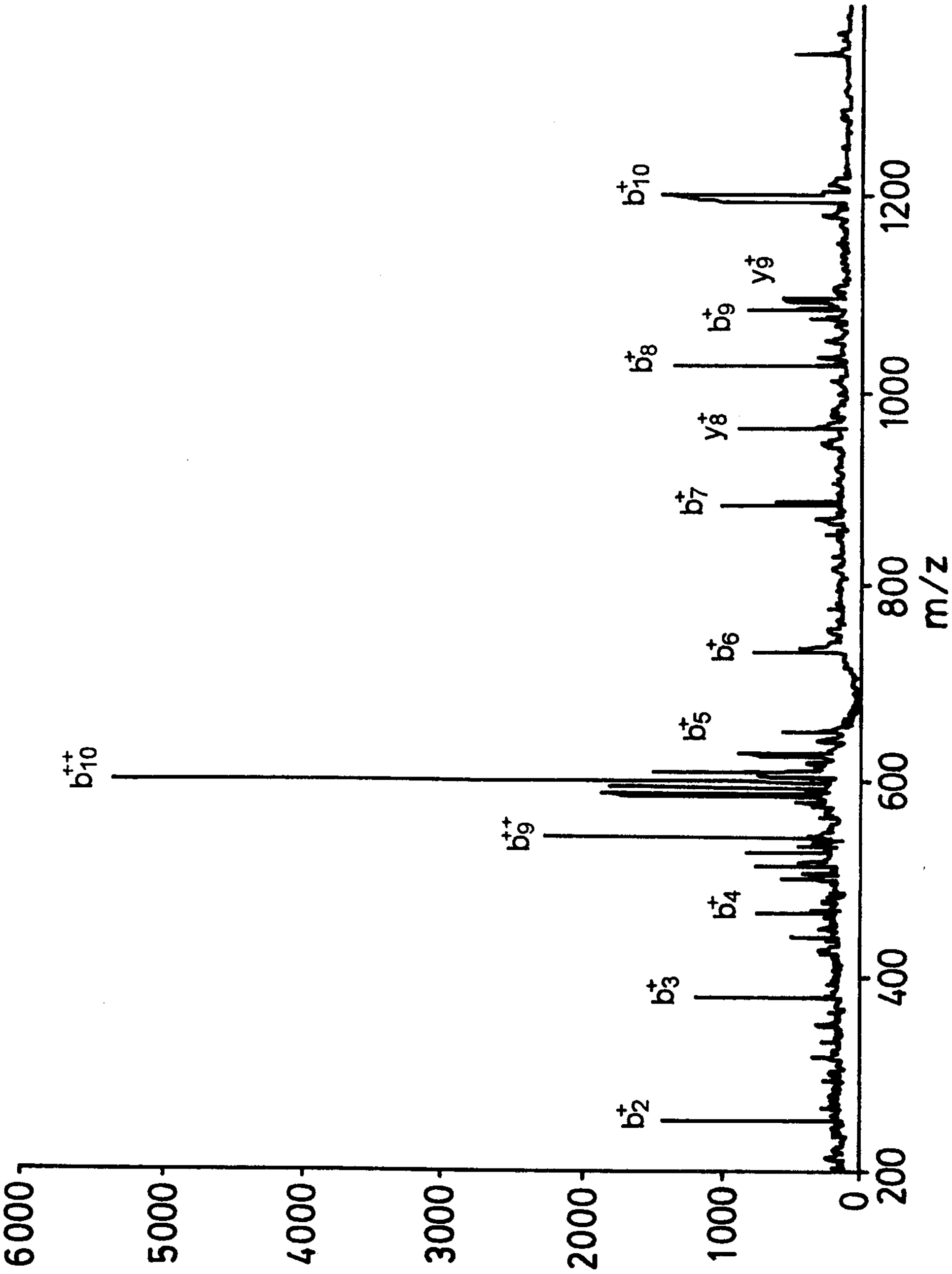
79
79
78
0
5000
10000
200
400
600
800
1000
1200
m/z

FIG. 5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5000
4000
3000
2000
1000
0
200
400
600
800
1000
1200
m/z
b2+
b3+
b4+
b9++
b10++
b5+
b6+
b7+
y8+
b8+
b9+
y9+
b10+

FIG. 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/01098

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 H01J49/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 H01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 529 885 A (MDS HEALTH GROUP LTD) 3 March 1993	1-3,6,7, 9
A	see page 8, line 56 - page 9, line 5 see page 9, line 52 - page 9, line 54; figures 1,4	4,5
A	GB 2 301 705 A (BRUKER FRANZEN ANALYTIK GMBH) 11 December 1996 see page 1, line 13 - page 1, line 25	1-3,6,7, 9
A	US 5 576 540 A (JOLLIFFE CHARLES L) 19 November 1996 see column 9, line 34 - column 9, line 65	1-3,6,7, 9
A	US 5 672 870 A (FLORY CURT A ET AL) 30 September 1997 see figure 5	6,7

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 March 1999	23/03/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Zuccatti, S

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INTERNATIONAL SEARCH REPORT

Int   .tional Application No
PCT/CA 98/01098

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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