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(54) **Title:** COMPREHENSIVE INTERFERENCE TREATMENT FOR ICP-MS ANALYSIS

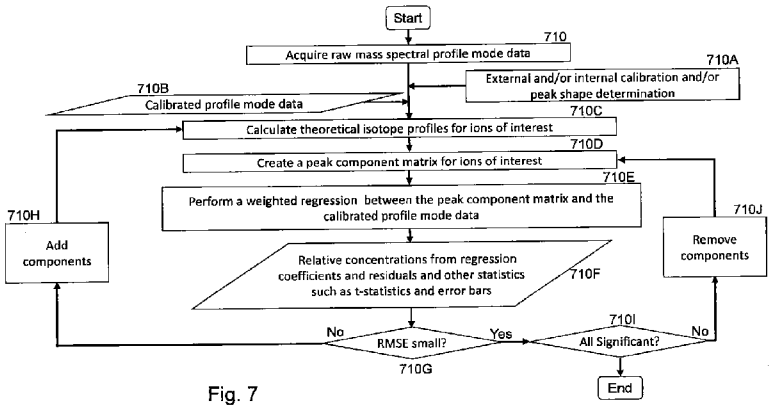


Fig. 7

(57) **Abstract:** A method for elucidating spectral data or compensating for interferences in analysis of mass spectral data includes steps of acquiring profile mode mass spectral data; considering possible forms of analytes and/or interferences within the mass spectral range; calculating a theoretical mass spectral response or measuring an actual mass spectral response for a corresponding form of analyte and/or interference; forming a peak component matrix including one mass spectral response for each corresponding form of analytes and/or interferences; performing regression analysis involving the peak component matrix and the acquired mass spectral data; and reporting regression coefficients or associated mass spectral signals as representing the concentrations or mass spectral signals of the corresponding forms of analytes and/or interferences. A mass spectrometer system operating (Fig. 7) in accordance with the method. A medium having computer code thereon for use by a computer with the mass spectrometer, for operating the mass spectrometer.

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COMPREHENSIVE INTERFERENCE TREATMENT FOR ICP-MS ANALYSIS

Cross-Reference to Related Applications

This application is related to the following patent applications:

United States Serial No. 10/689,313 filed on 20 October, 2003, granted as United States Patent No. 6,983,213, and International Patent PCT/US2004/034618 filed on 20 October, 2004 which claims priority therefrom and designates the United States of America as an elected state.

United States Provisional patent applications 60/466,010; 60/466,011 and 60/466,012 all filed on 28 April, 2003, and International Patent Applications PCT/US2004/013096 and PCT/US2004/013097 both filed on 28 April, 2004 and designating the United States of America as an elected state (US patents 7,577,538 and 7,529,629, respectively).

U.S. provisional application Ser. Nos. 60/623,114 filed on 28 Oct., 2004; 60/670,182 filed on 11 Apr., 2005, and 60/685,129 filed on 29 May, 2005 and International Patent Applications PCT/US2005/039186 filed on 28 October, 2005 and designating the United States of America as an elected state.

U.S. provisional patent application 61/057,804 filed on May 30, 2008 and United States Serial No. 12/475,548 filed on 31 May, 2009.

The entire teachings of these patent applications are hereby incorporated herein by reference, in their entireties, for all purposes.

BACKGROUND

Field of the Disclosure

The present invention relates to improvements and applications with respect to the teachings of the above referenced patent applications in the field of general mass spectrometry including inductively coupled plasma mass spectrometry (ICP-MS).

Background Art

The background art has been described in the above referenced patent applications dealing with mass spectrometry in general including the general purpose system illustrated in Fig. 1.

According to Wikipedia:

(http://en.wikipedia.Org/wiki/Inductively_coupled_plasma_mass_spectrometry#777Inductively_coupled_plasma), an inductively coupled plasma is a plasma that contains a sufficient concentration of ions and electrons to make the gas electrically conductive. The plasmas used in spectrochemical analysis are essentially electrically neutral, with each positive charge on an ion balanced by a free electron. In these plasmas the positive ions are almost all singly charged and there are few negative ions, so there are nearly equal amounts of ions and electrons in each unit volume of plasma.

An inductively coupled plasma (ICP) is sustained in a torch that consists of three concentric tubes, usually made of quartz. The end of this torch is placed inside an induction coil supplied with a radio-frequency electric current. A flow of argon gas (usually 14 to 18 liters per minute) is introduced between the two outermost tubes of the torch and an electric spark is applied for a short time to introduce free electrons into the gas stream. These electrons interact with the radio-frequency magnetic field of the induction coil and are accelerated first in one direction, then the other, as the field changes at high frequency (usually 27.12 million cycles per second). The accelerated electrons collide with argon atoms, and sometimes a collision causes an argon atom to part with one of its electrons. The released electron is in turn

accelerated by the rapidly changing magnetic field. The process continues until the rate of release of new electrons in collisions is balanced by the rate of recombination of electrons with argon ions (atoms that have lost an electron). This produces a 'fireball' that consists mostly of argon atoms with a rather small fraction of free electrons and argon ions. The temperature of the plasma is very high, of the order of 10,000 K.

The ICP can be retained in the quartz torch because the flow of gas between the two outermost tubes keeps the plasma away from the walls of the torch. A second flow of argon (around 1 liter per minute) is usually introduced between the central tube and the intermediate tube to keep the plasma away from the end of the central tube. A third flow (again usually around 1 liter per minute) of gas is introduced into the central tube of the torch. This gas flow passes through the centre of the plasma, where it forms a channel that is cooler than the surrounding plasma but still much hotter than a chemical flame. Samples to be analyzed are introduced into this central channel, usually as a mist of liquid formed by passing the liquid sample into a nebulizer.

As a droplet of nebulized sample enters the central channel of the ICP, it evaporates and any solids that were dissolved in the liquid vaporize and then break down into atoms. At the temperatures prevailing in the plasma a significant proportion of the atoms of many chemical elements are ionized, each atom losing its most loosely bound electron to form a singly charged ion.

For coupling to mass spectrometry, the ions from the plasma are extracted through a series of cones into a mass spectrometer, usually a unit mass resolution quadrupole MS.

Due to the high energy and temperature breakdown of samples inside the ICP source, typically only ion species of smaller masses (m/z) would be observed in the mass spectrometer in the range between m/z 1-350, which covers all known 118 elements in its +1 charge state in the most recent periodic table. Combined with the high sensitivity and generality of a mass spectrometer as detector, ICP-MS has become the

most versatile analytical technique in terms of its elemental coverage. On the other hand, this also means that there would be serious mass spectral interferences in samples containing multiple elements or other complex matrices as all mass spectral information would be compressed in such a narrow mass spectral range of only about 350Da. In addition to mutual mass spectral interferences among elements such as Hg, Bi, and Pb in the mass spectral range of 194-212 Da, there are also interferences formed inside the ICP source via chemical reactions with the torch gas Ar, the oxygen/nitrogen from the air background, and other elements from the sample (including the solvent) itself such as S, CI, Ca, Na etc. These singly charged or multiply charged ion species involving more than one atom are called polyatomic ions. For example, the polyatomic ion, ArO+, formed between the carrier gas and oxygen inside ICP torch, would interfere with Fe around m/z 56. Since there is typically no front end separation available and that most of the polyatomic ions are formed inside the ICP source itself, two types of prior art have been used to deal with the interference problem in ICP-MS.

One way to counter the mass spectral interference problem is to replace the lower-cost and easier-to-maintain quadrupole MS with a more expensive and higher resolution MS systems such as double focusing magnetic-electrostatic sector systems with both single and multiple collector, as well as time of flight (TOF) systems (both axial and orthogonal accelerators have been used) so as to mass spectrally separate various interferences before quantitation or identification. Since these higher resolution MS systems come with reduced sensitivity due to the loss of ion signals through higher resolution ion optics (in the case of magnetic sector instrument) or much increased maintenance and/or capital budget (in the case of TOF), the use of higher resolution MS in ICP-MS has been quite limited. Instead, another lower-cost and easier-to-maintain alternative approach has been developed and widely adopted commercially, which helps to eliminate or reduce mass spectral interferences through the use of a collision or reaction cell with an otherwise conventional and lower cost quadrupole MS.

According to Wikipedia:

(http://en.wikipedia.org/wiki/Collision/reaction_cell), there are several different commercial implementations of the collision or reaction cell technology:

(1) Dynamic reaction cell: The collision reaction cell known by the trade name dynamic reaction cell was introduced by Perkin-Elmer on their Elan DRC (followed by Elan DRC II and Elan DRC-e) instrument. The dynamic reaction cell is a chamber placed before the traditional quadrupole chamber of an ICP-MS device, for eliminating isobaric interferences. The chamber has a quadrupole and can be filled-up with reaction (or collision) gases (ammonia, methane, oxygen or hydrogen), with one gas type at a time or a mixture of two of them, which reacts with the introduced sample, eliminating some of the interference.

The DRC is characterized by the following paramters, that can be modified: RPq (the corresponding q parameter from the Mathieu equation), RPa (the corresponding a parameter from the Mathieu equation), which refer to the voltage applied to the quadrupole rods and the gas flow of the reaction gas.

Ammonia gas is the best solution for the majority of interferences, but it is far for being the perfect gas. Sometimes, for specific isotopes, other gas must be used for better results or even mathematical correction, if no gas offers a satisfactory advantage.

(2) Collisional reaction interface (CRI): The proprietary collisional reaction interface (CRI) used in the Bruker ICP-MS destroying interfering ions. These ions are removed by injecting a collisional gas (He), or a reactive gas (H2), or a mixture of the two, directly into the plasma as it flows through the skimmer cone and/or the sampler cone. Supplying the reactive/collisional gas into the tip of the skimmer cone and/or into the tip of the sampler cone induces extra collisions and reactions that destroy polyatomic ions in the passing plasma.

Axial field technology: Axial field technology (AFT) is a patented improvement of DRC made by Perkin-Elmer, which consists in two supplementary rods placed in the DRC cell, smaller than normal quadrupole's rods, with the purpose of "pushing" the ions faster to the exit by generating a supplementary electric potential, minimizing the time needed for the gas to be in the DRC and improving analysis speed. The supplementary potential of the AFT rods does not contribute significantly to the global energy, but drastically improve ion passage time.

(3) Collision cell technology with kinetic energy discrimination: Thermo Scientific's XSeries2 instrument utilizes a collision/reaction cell for interference removal, consisting of a non-consumable hexapole and chicane ion deflector, which takes the ion beam off-axis and leads to low instrument backgrounds of <0.5 integrated counts per second (icps) at vacant masses such as 5 and 220. This hexapole is inherently part of the Thermo lens system and is present in the ion path, regardless of the use of the collision cell. The collision/reaction gas mixtures can be 1% NH3 in He, 7% H2 in He and 100% H2, where the NH3 and H2 are reactive gasses and the He is a collisional gas. The 3rd generation cell utilizes kinetic energy discrimination, which employes running the quadrupole bias slightly less negative (more positive) than the hexopole bias. Polyatomic ions generated within the plasma can have larger atomic radii than analyte ions of similar mass, i.e. the interferent NaAr+ (mass 63) is larger than the analyte Cu+ (mass 63). Thus, when using a collisional/reactive gas mixture, these larger species undergo more collisions/reactions in the cell, in which they lose increasingly more energy, and are then excluded from the quadrupole mass filter by the kinetic energy barrier.

Octopole reaction system: Another implementation of this type of interference removal is an octopole (instead of a quadrupole) collision cell, implemented by Agilent's 7500 series. The octopole reaction system (ORS) uses only helium or hydrogen and the volume of the cell is smaller than that of a DRC. The small molecules of helium and hydrogen collide with the large, unwanted polyatomic ions formed in the plasma and break them up into other ions that can be separated in the quadrupole mass analyzer. However, unlike the DRC, the ORS system is based only on collision reactions and not on chemical reactions.

While these hardware solutions involving reaction or collision cells have enjoyed great commercial success, there are quite a few fundamental limitations to this approach, including:

(1) Higher cost of instrument, maintenance, and operation involving the collision or reaction cell with the collision or reaction gas.

(2) Different analytes or different sample matrices typically leads to different level and types of interferences, requiring different collision or reaction gas, flow rate, or energy. This not only increases the method development time but also significantly increases the time it takes to analyze multiple different elements in a single sample with reduced sample throughput in order to allow for the changes to take effect and/or enough equilibrium time from one set of condition to another, not to mention that a concentration standard curve will need to be built under each set of conditions.

(3) The collision or reaction or the mere need for ions to pass through an extra cell can lead to significant ion loss. Inside a collision or reaction cell, while the polyatomic ion species are broken apart, the analyte ions are also knocked out of their normal trajectories leading to significant ion or signal loss. In addition, the ion transmission efficiency is typically less than 100% through the cell, due to the presence of this extra component inside the mass spectrometer. All these factors lead to significant loss in analyte

sensitivity, eroding a key advantage of ICP-MS over ICP-OES (with optical emission spectrometer as detector) which comes at a much lower cost.

(4) The reaction or collision may not reach 100% completion and therefore there would always be residual interferences even with the use of a cell.

(5) Not all polyatomic ions can be broken down or reacted away with the cell technology using available collision or reaction gases.

(6) The mutual interferences among co-existing elements themselves that do not involve polyatomic ion species typically could not be addressed through reaction or collision cells. This includes, for example, the interference in the mass spectral range of m/z 194 to 210 among Hg, Bi, and Pb.

SUMMARY OF THE DISCLOSURE

The present disclosure is directed to the following improvements, which are applied to inductively coupled plasma mass spectrometry (ICP-MS) where the ionization source is the inductively coupled plasma (ICP):

1. A new search method and apparatus for molecular formula determination with applications from metabolite identification to peptide or protein identification by taking advantage of multiple observable isotopes and the profile mode mass spectral data available.

2. The use of this search method and/or apparatus in the deconvolution of overlapped isotopes or clusters for the purpose of identifying and quantifying the ions involved.

3. A novel method and apparatus for unbiased and accurate quantitation of all relevant components in isotope labeling experiment through the use of a mass spectrometer by incorporating the knowledge of the naturally

occurring and labeled isotopes and the profile mode mass spectral data available.

Each of these aspects will be described below along with preliminary results to demonstrate their utilities. For the purpose of easily describing the inventions made, no distinction will be made between the neutral and the ion form of a molecule or its fragments with the understanding that, for example, it typically requires an added proton to convert a neutral into the corresponding positive ion in order to measure it in a mass spectrometer.

Contrary to conventional wisdom, when proper calibration of a profile mode spectrum (not a centroiding or stick spectrum), as described herein, is used, the method in accordance with the invention allows for the highly accurate quantitation of a mixture of ions where their monoisotopic masses differ by as little as a small fraction of IDa with a conventional mass spectrometer of unit mass resolution.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a block diagram of an analysis system in accordance with the invention, including a mass spectrometer.

Fig. 2A is a table of exact isotope distribution for a polyatom CSi.

Fig. 2B is the simulated mass spectral profile mode data for CSi on a unit mass resolution system.

Fig. 2C is the simulated mass spectral profile mode data for CSi M+l isotope cluster on very high resolution system.

Fig. 2D is the simulated mass spectral profile mode data for CSi M+2 isotope cluster on the same high resolution system.

Fig. 3 is the theoretical isotope profile mode data at unit mass resolution for two candidate matches of As and adjacent interference whose isotope profile overlaps the isotope profile of As.

Fig. 4 is a flow chart for the highly selective compound identification process disclosed herein.

Fig. 5A is a simulated raw mass spectral profile mode data for the As.

Fig. 5B is an externally calibrated version of mass spectral profile mode data for As.

Figs. 6A, 6C, and 6E are calibrated data measured on a unit mass resolution system and the fitted version with AS, As+Ar2, and As+Ar2+ArCl, respectively.

Figs. 6B, 6D, and 6F are the corresponding fitting residuals of FIG. 6A, 6C, and 6E.

Fig. 7 is a flow chart for the unbiased quantitative analysis process disclosed herein.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring to Fig. 1, there is shown a block diagram of an analysis system 10, that may be used to analyze atoms or polyatoms, as noted above, incorporating features of the present invention. Although the present invention will be described with reference to the embodiments shown in the drawings, it should be understood that the present invention can be embodied in many alternate forms of embodiments. In addition, any suitable types of components could be used.

Analysis system 10 has a sample preparation portion 12, a mass spectrometer portion 14, a data analysis system 16, and a computer system 18. The sample preparation portion 12 may include a sample introduction unit 20, of the type that introduces a sample containing metal elements of interest to system 10, such as the iCAP Q ICP-MS, manufactured by Thermo Electron Corporation of Waltham, MA, USA. The sample preparation portion 12 may optionally also include an analyte separation unit 22, which is used to perform a preliminary separation of analytes, such as the metal elements to be analyzed by system 10. Analyte separation unit 22 may be any one of a liquid chromatography system.

The mass spectrometer portion 14 may be a conventional mass spectrometer and may be any one available, but is preferably one of MALDI-TOF, quadrupole MS, ion trap MS, qTOF, TOF/TOF, or FTICR-MS. If it has a MALDI or electrospray ionization ion source or inductively coupled plasma, such ion source may also provide for sample input to the mass spectrometer portion 14. In general, mass spectrometer

portion 14 may include an ion source 24, a mass analyzer 26 for separating ions generated by ion source 24 by mass to charge ratio, an ion detector portion 28 for detecting the ions from mass analyzer 26, and a vacuum system 30 for maintaining a sufficient vacuum for mass spectrometer portion 14 to operate efficiently. If mass spectrometer portion 14 is an ion mobility spectrometer, generally no vacuum system is needed.

The data analysis system 16 includes a data acquisition portion 32, which may include one or a series of analog to digital converters (not shown) for converting signals from ion detector portion 28 into digital data This digital data is provided to a real time data processing portion 34, which process the digital data through operations such as summing and/or averaging. A post processing portion 36 may be used to do additional processing of the data from real time data processing portion 34, including library searches, data storage and data reporting.

Computer system 18 provides control of sample preparation portion 12, mass spectrometer portion 14, and data analysis system 16, in the manner described below. Computer system 18 may have a conventional computer monitor 40 to allow for the entry of data on appropriate screen displays, and for the display of the results of the analyses performed. Computer system 18 may be based on any appropriate personal computer, operating for example with a Windows® or UNIX® operating system, or any other appropriate operating system. Computer system 18 will typically have a hard drive 42, on which the operating system and the program for performing the data analysis described below is stored. A drive 44 for accepting a CD or floppy disk is used to load the program in accordance with the invention on to computer system 18. The program for controlling sample preparation portion 12 and mass spectrometer portion 14 will typically be downloaded as firmware for these portions of system 10. Data analysis system 16 may be a program written to implement the processing steps discussed below, in any of several programming languages such as C++, JAVA or Visual Basic.

Mass Spectral Fitting for Molecular Search

Mass spectrometry with highly accurate ion mass measurement offers a quick and unique way for the determination of elemental compositions or molecular formulae, which can offer great insights for the ions under the measurement, ranging from unknown metabolite identification to DNA or protein identification or sequencing.

The conventional approach for molecular formula determination starts with high mass accuracy determination of a mass spectral peak of interest and searches for all possible formula within a given mass error window (typically measured as parts per million or ppm), for example, +/-5ppm from the determined mass. Since all elements in the periodic table have their exact masses carefully measured for the lowest isotope, the elemental composition or molecular search algorithm amounts to the following optimization:

$$\min_{p,n_i} \left| m - \sum_{i=1}^{p} n_i m_i \right| \qquad \text{Equation 1}$$

where m is the measured accurate monoisotopic mass for the ion of interest, n_i is the number of elements for the i-th element, and m_i is the lowest exact mass among all isotopes of this i-th element. This optimization problem can typically be solved through integer programming, which can be drastically sped-up through the introduction of such constraints as the lowest possible and the highest possible number of each element n and the maximal number of elements p. Other constraints may include the existence of rings, double bonds, or a limited selection of possible elements (for example, a typical small molecule drug may contain only C, H, N, O, S, P, CI etc.).

This approach works well under the following conditions:

1. The mass spectrometer is of high resolution, typically a quadruple time-of-flight (qTOF) system or FTMS, allowing for the monoisotopic peak of an

ion to be baseline-resolved from its other isotopes in order to achieve high mass accuracy and facilitate the compound identification.

2. High signal to noise in the measurement of the monoisotopic peak.
3. The monoisotopic peak is pure and free from any interfering ions or isobaric interferences.
4. The molecule being searched is generally a small molecule with molecular weight less than lOOODa where the only pure isotope peak is the monoisotopic peak which is typically the most abundant peak.
5. A sufficiently symmetrical peak shape, available after extensive tuning of the instrument involving even hand-tuning of specific voltages for reliable mass determination.
6. A reliable and unbiased algorithm for mass determination.

It has been pointed out in the patent applications referenced above that high mass accuracy is available on even unit mass resolution systems where the monoisotopic peak is not baseline-resolved from other isotope peaks and it is possible to determine the accurate mass of the monoisotopic peak in the presence of interfering isotope peaks. In spite of all the benefits of the mass spectral instrument calibration and peak analysis disclosed in the applications referenced above, it should be noted that the process of mass (and area) determination from a continuum mass spectral response is one of deconvolution that is prone to error propagation and noise amplification. This becomes particularly problematic for M+l or M+2 isotope peaks where there are many individual isotopes located very close in masses to each other.

Fig. 2A shows the theoretical isotope distribution for a polyatom CSi, where the monoisotope (39.9764Da) is the most abundant and composed of a single isotope. All other isotope peaks are weaker and composed of multiple individual isotopes that are about IDa from the monoisotope and easily separated from the monoisotope on a unit mass resolution system as shown in Fig. 2B. The separation of the 2 most abundant isotopes (41.9732 and 41.9793 Da) under the M+2 peak, however, would have required high resolving power, a feature only available on the high resolution systems where some sensitivity may have to be compromised.

Fig. 2D shows the simulated mass spectrum for the M+2 isotopes at such high resolving power that the two most abundant isotopes can be visually observed. For the M+1 peak as shown in Fig. 2C, however, the two isotopes at 40.9759 and 40.9797Da are no longer separated even at the same high resolution, resulting in some ambiguity in peak picking or centroiding which can adversely impact quantitative ICP-MS analysis based on peak picking results alone. The profile data in Fig. 2C, on the other hand, contains all relevant information about the M+1 isotope cluster of this polyatom and provides a unique signature on which a quantitative mixture analysis may be based.

Based on the comprehensive mass spectral calibration disclosed in United States Patent No. 6,983,213, the peak analysis can still be performed on peaks with unresolved isobaric interferences to arrive at a unique accurate mass for the isotope clusters. Since the peak shape function has been converted into a symmetrical function after the calibration transformation, this unique accurate mass is in fact a weighted average of all the isotopes included in the cluster with their relative abundances as weights, i.e., a mathematically defined centroid. With the centroids for all isotope clusters clearly defined and calculated, one can in theory perform a molecular formula search based on the actual observed centroids and the theoretical centroids calculated from the corresponding isotope distributions given the elemental compositions. One may even incorporate the apparent peak areas for the identified peaks as weights into the subsequent searches and scoring based on centroid masses to reflect the relative abundances of these isotope clusters.

The match between observed centroid and theoretical centroid masses can be performed through a weighted least squares regression which will automatically provide some measurement for the goodness-of-fit or probability for the molecular formula assignment or library hit. The statistics and assignment of probabilities, however, become less rigorous or elegant or diagnostic due to the loss in information content during the peak analysis process where all unresolved isotopes are effectively binned together.

The details of a more preferred embodiment will now be presented that utilizes the full mass spectral information available for molecular formula or library search,

search diagnostics, quantitative mixture analysis, and statistical measures, all without the peak centroding step.

While accurate mass of the monoisotopic peak is a very important piece of information for an ion, its other isotopes and the pattern in which they overlap provide crucial additional information about a particular ion, which when properly utilized, can further enhance the discrimination between this and other candidate atoms or polyatoms of even very similar monoisotopic masses. Fig. 3 shows the mass spectra of one ion with its monoisotopic mass within 10 mDa of that of the ion of interest, but with very dissimilar spectral patterns due to the differences in their elemental compositions. To quantify the ion of interest (As) through isotope pattern analysis, all possible interferences need to be considered such as Ar_2^+ in this case.

This embodiment described herein:

1. Takes advantage of the isotope patterns available for each atom or polyatom generated by the ion source of ICP-MS as additional information to discriminate among the many atoms or polyatoms of very similar monoisotopic masses.
2. Avoids using peak picking and centroiding as the only means of quantitative ICP-MS analysis and thus avoids an extra step of data processing where errors may occur and random noises may be amplified.
3. Makes possible the quantitative ICP-MS analysis through continuum profile data by the use of comprehensive and total mass spectral calibration disclosed in United States Patent No. 6,983,213. The comprehensive mass spectral calibration allows for an exact match of the mass as well as the peak shape functions.
4. This comprehensive mass spectral calibration enables interference correction without use of high resolution mass spectrometers or collision cells on even unit mass resolution mass spectrometers, a unique feature generally thought of as being reserved for higher resolution systems.
5. Quantitative ICP-MS analysis can now be performed without identifying the monoisotope peak, which may be quite weak or even un-observable.

Furthermore, this analysis can also be performed using any section of the isotope clusters that may contain many individual isotopes without physically separating them. It may even be possible to use a single isotope cluster, for example, the M+2 cluster from Fig. 2B, for a quantitative ICP-MS analysis, especially when other clusters have poor signal to noise, encounters nonlinearity, or have significant overlaps from interferences.

The specific steps are similar to what was disclosed in the PCT/US2004/013096 filed on 04 April, 2004 entitled "COMPUTATIONAL METHOD AND SYSTEM FOR MASS SPECTRAL ANALYSIS" and are described along with an example below:

1. Acquire raw mass spectral data in the profile mode with many points across a mass spectral peak. This raw mass spectral data may or may not have internal standard or standards included. Fig. 5A shows a section of such a simulated raw mass spectral data corresponding to As. This step is shown as 410 in the flowchart of Fig. 4.
2. Apply a total calibration filtering matrix developed earlier as part of an external or instrument calibration to the raw mass spectral data collected to correct for any mass axis error and transform the mass spectral peak shape function into a known peak shape function, preferably a mathematically definable symmetrical function. Fig. 5B shows the same section in Fig. 5A after external calibration. This step is shown as 410B in the flowchart of Fig. 4 where the calibration from 410A of Fig. 4 is applied.
3. If at least one internal standard is available, perform a new calibration using the at least one standard as the standard in a separate new instrument calibration (internal calibration). Apply this newly determined internal calibration to the externally calibrated data to obtain externally and internally calibration data. If only the actual peak shape functions are determined without the comprehensive calibration either separately or while acquiring the raw profile mode mass spectral data (410A in Fig. 4, both step 2 and this step can be optionally by-passed with all subsequent

data processing applied to raw profile mode data instead of calibrated profile mode data.

4. In quantitative ICP-MS analysis corresponding to the fully calibrated mass spectral data shown in Fig. 5B, one typically goes through a peak picking process, preferably one disclosed in the section starting from line 8 on page 32 of United States Patent Application Serial No. 10/689,313 or PCT/US2004/034618 filed on 20 October, 2004 (section starting from line 14 of page 34), to generate a peak list containing peak mass locations as well as integrated peak areas. Either target peak shape functions or actual peak shape functions may be used for peak analysis, depending on whether a calibration step 410B in Fig. 4 has been performed. The monoisotopic peak mass thus calculated from the data trace in Fig. 5B is 74.931 IDa. .

5. Instead of relying on monoisotopic mass for molecular formula determination, this aspect of the invention calculates the theoretical mass spectral isotope profile for each of the candidate atom or polyatom identified and compare its theoretical mass spectral profile with that of the actual isotope profile as acquired or after the calibration (external and/or internal calibration, step 410B in Fig. 4). This calculation involves calculating the theoretical isotope distribution followed by convolution with either the target peak shape functions or actual peak shape functions, all defined in the comprehensive calibration process disclosed in the United States Patent Application Serial No. 10/689,313 or PCT/US2004/034618 filed on 20 October, 2004. The actual mass spectral peak shape after calibration where applicable will be transformed to the target peak shape function.

6. Form a different peak component matrix P (page 32 of United States Patent Application Serial No. 10/689,313 and page 34 in PCT/US2004/034618 filed on 20 October, 2004) for each of the candidate atoms by including any linear or nonlinear functions as possible baseline components and the corresponding theoretical isotope profile as the sole peak component. If a subset of the atoms or polyatoms are known, the theoretical isotope profiles of these known atoms or polyatoms can be

calculated directly without elemental composition or library search and included in the peak component matrix to be treated just like baseline components. If there is evidence that the mass spectrum may have shifted after the most recent calibration (either external or internal), one may add one more component, the 1st derivative of the actually measured or calibrated isotope profile, into the matrix P to account for any additional mass axis shift. This step is illustrated as 4101 in Fig. 4.

7. A Weighted Multiple Linear Regression (WMLR, equation 6 on page 34 of United States Patent Application Serial No. 10/689,313 and equation 6 on page 35 of PCT/US2004/034618 filed on 20 October, 2004) is now performed between the acquired raw or calibrated isotope profile (for example, Fig. 5B) and each peak component matrix using the inverse of the peak intensity variance w (page 34 of United States Patent Application Serial No. 10/689,313 and page 35 in PCT/US2004/034618 filed on 20 October, 2004) as weights. A fitting error (Root-Mean Squared Error or RMSE) or t-value is calculated from each regression (pages 35 and 39 of United States Patent Application Serial No. 10/689,313 and on pages 36 and 39 in PCT/US2004/034618 filed on 20 October, 2004). This step is illustrated as 410J in Fig. 4.

This aspect of the invention eliminates intermediate and error-prone steps for quantitative analysis of mixture of ions, yielding more reliable results by taking into consideration of all the isotopes available, their relative abundances, and their differing masses. For most of metal elements and their polyatomic products generated by the ion source of ICP-MS, this profile-based quanitative analysis offers significant advantages even though the monoisotopic peak is likely to be the most abundant for these atoms or polyatoms. For the elements whose monoisotopic peak is typically not the most abundant if observable at all and the instrument resolution width (FWHM) typically increases on mass spectrometers such as TOF or FTMS while the isotope distribution becomes more complex, making peak analysis and exact mass determination even more difficult and subject to even larger error. This is where this new aspect of the invention may make an even bigger difference by avoiding

peak analysis altogether and by taking into consideration other more significant isotopic peaks.

The critical role that comprehensive mass spectral calibration plays in this novel search process will become apparent to one skilled in the art due to its intrinsic capability of making mass spectral peak shapes known, analytically calculatable, or even uniform across a full mass spectral range. It should nonetheless be pointed out that as long as the peak shape function is known, even just in numerical form, this novel searching algorithm can be used through proper replication schemes such as shifting or interpolation. Moreover, if the instrument has been tuned well enough to have its peak shape function resemble a mathematically definable peak shape, this novel searching algorithm can also be used to yield some useful, if not best attainable, results.

Another aspect of this invention is that the fitting residual can be used as a good indicator of whether the mass spectral peak segment contains a single atom or a polyatom or a linear combination of multiple atoms or multiple polyatoms of very similar masses. Fig. 6A shows a simulated mass spectral isotope profile measured on a unit mass resolution instrument after the comprehensive calibration (solid line) and the fitted As (exact monoisotopic mass 74.921 0Da) theoretical profile (dashed) with residual given in Fig. 6B. Fig. 6C shows the fit with both As and Ar_2^+ (fitting residual shown in Fig. 6D). When fitting with As, Ar_2^+, and ArCl, the fitting is much improved (Fig. 6E) with the residual much reduced (Fig. 6F). As a result of WMLR from step 410J in Fig. 4, the regression coefficients represent the relative contribution of each ion into the combined mass spectral profile data, providing quantitative information about the ions involved in addition to qualitative identification information.

The decision to add components into the peak component matrix P is made at step 410L in Fig. 4, typically based on statistical measures from the regression such as F-test or t-test. The step of adding one or more components into the peak component matrix for mixture analysis and identification in a mixture is illustrated as 410M in Fig. 4. When previously added components become statistically insignificant with the addition of other components, a decision can be made to remove one or more components at step 410N based on similar significance test such as F-test or t-test. The step of deleting one or more components from the peak component matrix is

illustrated as 410O in Fig. 4. The added or removed components mentioned above may also include baseline component or components or the 1st derivative terms mentioned above.

Quantitation of Ions with Interfering Isotopes

In mass spectral experiments involving isotope labeling such as ICAT™ or iTRAQ™ (both marketed by Applied Biosystems, Foster City, CA) for quantitation or isotope tracing for metabolism study, there are typically overlapping isotope patterns between the labeled and un-labeled ion or fragments or among the differently labeled ion or fragments. A good example is the isobaric tags used in iTRAQ™ (WO 20004/070352 A2) where digested peptides from different samples may be labeled with a different reporter tag (with mass of 114.1, 115.1, 116.1, or 117.1), which is attached to a corresponding balance tag of 31, 30, 29, or 28 such that the combined tag has the same nominal mass, allowing for peptides from different samples to be tagged differently with the same combined mass. When different samples are mixed, combined, and separated through chromatography prior to mass spectral analysis, the same peptide from different samples would be tagged with tags of the same combined mass, giving the peptide of different tags the same apparent mass in MS analysis where one MS/MS will be performed to break apart the differently tagged peptide ion into a reporter tag, balance tag, peptide and its fragments during the MS/MS fragmentation. Each reporter tag would now have different mass of 114.1, 115.1, 116.1, or 117.1, the signal intensity of each corresponding to the amount of this peptide in a particular sample before the mixing and combining.

In the 4x multiplexed experiment where four samples are tagged and combined, one expects to observe all four reporters at the 4 masses in MS/MS analysis, the relative intensities of these reporters would indicate the relative amount of the peptide in each of the four samples. Since these tags are only 1 mass unit apart from each other, their isotope patterns would overlap, especially on a lower resolution system such as ABI/Sciex QTRAP.

Another example involves drug metabolism resulting from the dehydrogenation of the parent drug or its fragment where a combined isotope profile from the ion before

and after dehydrogenation will be observed. The combined isotope profile is a linear combination of two individual isotope profiles only 2Da apart from each other with significant overlaps. It is desirable to measure the relative concentration of the dehydrogenated metabolite to that of the parent drug or drug fragment in order to assess the extent of this particular metabolic process.

Another example involves mass spectral measurement of a mixture of "cold" and "hot" samples where the "cold" sample refers to an unlabeled sample and "hot" sample refers to a (radio) labeled sample such as C^{14}-labeled sample, resulting in an observed mass spectral response composed of two mutually overlapping isotope profiles. Due to the high chemical and ionization similarity between the unlabeled and labeled ion, they each serve as a great internal reference to the other when quantitative information is sought after in an analysis. It is therefore highly desirable to quantify the relative concentrations of the unlabeled and labeled ion with overlapping isotope profiles.

In this aspect of the invention, a novel and unbiased approach will be taken to quantify each of the ions measured in an overlapping mass spectral range regardless of the m/z separation between or among them, even at unit mass resolution.

The steps involved are:

1. Acquire raw profile mode data containing all labeled tags and their isotopes in a mass spectral range. This step is illustrated as 710 in Fig. 7.
2. If applicable, develop an instrument calibration and/or obtain the peak shape functions suitable for the mode of MS operation such as full MS or MS/MS using the comprehensive calibration disclosed in United States Patent No. 6,983,213 and United States Patent No. 7,348,553, which was filed as application serial number 11/261,440, based on United States provisional patent application serial number 60/623,114 filed 28 October, 2004. This step is illustrated as 710A in Fig. 7.
3. If applicable, apply the instrument calibration on the above acquired data to obtain the externally calibrated data and the corresponding weights (pages 29-31 of United States Patent Application Serial No. 10/689,313

and pages 28-32 in PCT/US2004/034618 filed on 20 October, 2004). If necessary, apply internal calibration to the externally calibrated data and obtain the corresponding new weights (pages 29-31 of United States Patent Application Serial No. 10/689,313 and pages 28-32 in PCT/US2004/034618 filed on 20 October, 2004). This step is illustrated as 710B in Fig. 7.

4. Calculate the theoretical isotope distributions for all ions of interest including native or labeled ions or any known background ions of relevance in this mass range based on their molecular compositions. This step is illustrated as 710C in Fig. 7.
5. Convolute the theoretical isotope distributions with the target peak shape function specified during instrument calibration or the actual peak shape functions to obtain theoretical isotope profiles for all ions selected. This step is also illustrated as 710C in Fig. 7.
6. Construct a peak component matrix P (page 32 of United States Patent Application Serial No. 10/689,313 and page 34 of PCT/US2004/034618 filed on 20 October, 2004) to include any linear or nonlinear functions as baseline components and all theoretical isotope profiles calculated above as peak components. This step is illustrated as 710D in Fig. 7.
7. Perform a regression analysis (equation 6 on page 34 of United States Patent Application Serial No. 10/689,313 and equation 6 on page 35 in PCT/US2004/034618 filed on 20 October, 2004) between the calibrated or raw profile mode data and the peak component matrix P. This step is illustrated as 710E in Fig. 7.
8. Report the regression coefficients as representing the relative concentrations for each of the components including the baseline and ion components. These relative concentrations are free from each other's interference and thus unbiased due to the regression analysis performed. These concentration estimates are also more accurate due to the comprehensive calibration applied and the noise filtering as part of the calibration process when applied. Other well established statistics such as

t-values, p-values, and residuals can also be reported. This step is illustrated as 710F in Fig. 7.

When no calibration is available, one may omit steps 2 & 3 and consider a generally accepted peak shape function, either mathematically defined or numerically derived from the measurement of standard ions, as the peak shape function for the convolution operation in step 5. In this case or in the case of external calibration without further internal calibration, there may be significant mass shift between the theoretically calculated isotope profiles (in peak component matrix P) and the actually measured or externally calibrated mass spectral profile data. One may consider adding a first derivative of the measured or externally calibrated mass spectral profile data into the peak component matrix P in step 6 to compensate for this shift without incurring much computational expense.

Sometimes one may have started with too many components including baseline components in the peak component matrix P and find at the end (710G in Fig. 7) that some of the components may be statistically insignificant (7101 in Fig. 7) and therefore should be removed (710J in Fig. 7) prior to another round of regression analysis. These insignificant components may include baseline components or 1st derivative components mentioned above.

At other times one may find that not enough components have been included due to the large residual (RMSE, 710G in Fig. 7) and may proceed to add more components (710H in Fig. 7) for inclusion into the peak component matrix prior to another round of regression analysis.

In both the mass spectral fitting for molecular search and the quantitation of ions with overlapping isotopes, it is conceptually possible to perform a peak analysis involving centroiding prior to the regression step. Centroding is a very typical approach according to prior art from commercially available systems. As mentioned above, the centroiding process prone to error due to the deconvolution nature of the operation. In addition, it destroys information from closely located isotopes. Furthermore, it

reduces the degrees of freedom for the peak component matrix P and limits the number of ions that can be searched or quantified. For example, on a unit mass resolution system with mass spectral data covering 4.5Da mass range of a isotope profile (such as 74.0-78.5Da mass range for element As and its possible interference in Fig. 5A-5B), typically 4 peaks may be identified as results of peak centroiding, limiting the quantitative or qualitative search power of the centroid data to at most 4 co-existing ions whereas the profile mode quantitation presented above can handle at up to 40 co-existing ions. This profile mode analysis, when performed in the preferred embodiment with the comprehensive mass spectral calibration, can further enhance the analytical capacity from 4 ions to possibly 100-400 co-existing ions in such a narrow mass window due to the high mass accuracy (down to 5-10mDa mass error) achievable on even unit mass resolution systems.

Application to the Spectral Elucidation or Compensation of Mutually Interfering Signals in Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

A new approach to interference treatment will now be described, which can be used either in combination with or instead of the reaction or collision cell technology. Reciting fitting equations from the cross-referenced United States Provisional patent applications 60/466,010 and 60/466,011 all filed on 28 April, 2003, and International Patent Applications PCT/US2004/013096 filed on 28 April, 2004 and designating the United States of America as an elected state (US patents 7,577,538), a linear relationship typically exists between the measured mass spectral data and its contribution components,

$$r = Kc + e \qquad \text{Equation 1}$$

where r is a (n x 1) matrix of the acquired mass spectral data sampled at n number of m/z data points, c is a (p x 1) matrix of the regression coefficients, which are representative of the relative concentrations of the p components in the sample, and K is an (n x p) matrix composed of mass spectral responses for the p components (called

peak component matrix), and e (n x 1) is the mass spectral error. Note in this notation, each baseline component is also considered as an "analyte" or interference with the corresponding regression coefficients representing the contribution of this baseline component to the total measured mass spectral data r.

If the peak component matrix K can be established either experimentally or calculated theoretically, with the acquired mass spectral data r, the regression coefficients c can be numerically calculated using any of the available matrix or non-matrix computational routines well established in the art, along with the fitting residual e, which can be used to compute relevant fitting statistics for statistical inference on the significance or the lack thereof for any of the components included in the peak component matrix K. Components can be either added or removed according to Fig. 7, using any relevant statistic metric thus derived or other user input parameters.

The construction of the peak component matrix K will now be described.

A baseline component in the peak component matrix can be measured experimentally in a separate data acquisition using the matrix-matched blank sample, in which case the baseline component (a mass spectral data array the same dimension as the r) would be arranged as a column in the peak component matrix K in above equation 1 and the corresponding regression coefficient (an element in c) would represent the relative change in mass spectral intensities between the blank when the baseline component is measured and unknown sample when the mass spectral data r is acquired. It should be noted that more than one baseline components can be accommodated into the peak component matrix K resulting in more than one regression coefficients. Alternatively, some or all of the baseline columns in K can be calculated, e.g., as a flat line (zeroth order) or a tilted line (1^{st} order) or any nonlinear form such as quadratic or exponential.

In the organic or biological mass spectrometry application described previously, where the number of possible organic or biological compounds can be in the tens of thousands or even millions, it is neither practical nor efficient to consider all possible

compounds in a single analysis, even though it is theoretically possible. Fortunately for ICP-MS, due to the unique nature of ICP-MS measurements, it is both practical and efficient to list and accommodate all possible analytes in the peak component matrix K, namely, all 118 known elements in the current periodic table. These 118 elements would only take up 118 columns in K, leaving enough degrees of freedoms for the subsequent regression analysis. Another novel and interesting aspect of ICP-MS analysis is that some of these elements may not be stable or their pure standards hard to come by, making it difficult to individually measure them experimentally before putting them into corresponding columns in K. In this case, their well known discrete theoretical isotope distribution/abundances can be used as inputs to calculate their truly pure mass spectral responses (to go into columns in matrix K), using a known peak shape function, a target peak shape function from mass spectral calibration involving peak shape function or an approximated peak shape function, all as described above. Depending on the sources of the sample (e.g., a meteorite rock from outer space), some or all elements may not have naturally occurring isotope abundances typically observed on earth, in which case their actual isotope abundances established through isotope ration mass spectrometry (IRMS) may be used instead. Due to the use of all available isotopes from all elements, a peak component matrix K thus constructed of all the known 118 elements is likely to be very well conditioned for the subsequent regression analysis.

Not only all currently known 118 elements could be accommodated into the peak component matrix K, the typical interferences from the ICP source can also be added, including possible polyatomic ions such as Ar_2^+, ArO^+, each element with one oxygen added etc. These polyatomic ions can even have a range of charges even though singly charged positive ion is most likely the case for ICP-MS. Though it is in theory possible to measure these possible interferences to be arranged into columns in K matrix, some of these interferences may not be stable at all to be isolated and measured alone, making theoretical calculations of them more desirable and preferred. The user may optionally elect to consider some but not other forms of the interference based on the experimental conditions such as the type of sample, sample matrix, ICP source condition such as power level, carrier gas or collision or reaction gas involved.

With the peak component matrix K constructed and the regression coefficients estimated from Equation 1, the separated signal contribution from each component, free from baseline or mutual or other interferences ions including possible baselines, elements of interests (analytes), and interferences (polyatomic ions, for example) can be calculated as

$$r_i = c_i k_i \qquad \text{Equation 2}$$

where r_i (n x 1) refers to the net mass spectral signal for component i, c_i refers to the regression coefficients or relative concentration for this same component, and k_i (n x 1) is taken from the ith column from the peak component matrix K. Some of these components can be grouped and combined to reflect the total baseline signal if there are more than one baseline components or total Ar-containing polyatomic ion signal etc.

The separated mass spectral signal r_i or its corresponding regression coefficient c_i can be used along with similar terms obtained after the same processing via Equation2 1 and 2 of other concentration standard samples to establish a calibration curve which relates the separated mass spectral signal or its regression coefficient to the known/given concentration. This calibration curve could then be applied to determine the unknown concentrations of an unknown sample through its separated mass spectral signal or corresponding regression coefficient, after a similar processing of the unknown sample through Equations 1 and 2.

In ICP-MS analysis, there are typically internal concentration standards of fixed or known concentrations at various m/z values added to or co-introduced along with each concentration standard or unknown sample so as to monitor and compensate for any mass spectral intensity variations such as those from the ICP source fluctuation. Proper interpolation is typically carried out to arrive at a compensation or normalization factor for any given m/z value of interest. Rather than using the raw mass spectral signal as acquired for intensity normalization or compensation, the

separated mass spectral signal r_i or its corresponding regression coefficient c_i can be used instead so as to eliminate any contamination of internal standard signals by interferences or even the analyte of interest, making the selection of suitable internal standards much more easier.

In ICP-MS, it is much preferred or often a required for the end user to know something about the sample to be analyzed before proceeding to the actual measurement, so that proper method could be selected or method development be carried out to arrive at a reasonable set of measurement conditions including the selection of the right type, amount, and the energy of the collision or reaction cell etc. This can be a cumbersome, tedious, and time consuming process before actual useful results could be obtained. With the new approach just described, however, all this can be eliminated completely through the use of an all-inclusive peak component matrix K so that any unknown sample would be analyzed for all currently possible 118 elements in the presence of all conceivable associated interferences. This is uniquely possible or practical for elemental analysis such as ICP-MS where the possibilities are either enumerable or so limited to be practical, due to the limited number of feasible elements from the current periodic table and the limited mass spectral range for elemental analysis, typically within m/z 300 Da. This provides an effective and novel approach to fully elucidate and compensate for any co-exiting elements or their respective polyatomic ion species, without the use of either collision or reaction cell. This approach can also be used in combination with the collision or reaction cell, to elucidate and compensate for these challenging interferences that are difficult or even impossible to be fully rid of through hardware. The regression coefficients obtained from Equation 1 can be used in any statistical significance test to determine if and how much of a particular element exists, thus leading to an ultimate elemental analysis system capable of delivering on-the-spot qualitative and quantitative analysis for any sample without any method development.

It should be noted that the mass spectral data r from Equation 1 does not have to be continuously sampled or even equally spaced profile mode data from the mass spectrometer covering the complete mass spectral range (m/z values). It can have

holes or gaps to avoid very intense signals (from Ar_2 in the ICP source, for example) that are saturating the detector. It can also just be a segment of mass spectral range or a combination of a few segments, e.g., set up to monitor and analyze a particular set of analytes of interest for given applications.

As described in previous sections for organic or biological mass spectrometry, the new approach here described here are best carried out with mass spectral data after the mass spectral calibration involving peak shape function determination or calibration. This is to ensure that the peak shape function between the left side of above Equation 1 match that of the peak component matrix on the right side, in order to achieve accuracy in subsequent regression analysis and statistical estimation. The calibration of mass spectral data can be carried out using mass spectral calibration standards typically involving a few pure elements located across the mass spectral range of interest, e.g., a calibration mixture of Be (m/z 9)/Rh (m/z 103), Tb (m/z 159), and Bi (m/z 209), measured under the same ICP-MS conditions as the concentration standard or unknown sample (including internal standard), i.e., under the same ICP source power, temperature, carrier gas flow rate, mass spectrometer scan range/rate, settle/dwell time etc. Though uncalibrated mass spectral data can also be used to some effect, the error term from Equation 1 above would be larger due to the error in m/z calibration or peak shape mismatch between r and those components in K, leading to sub-optimal results.

The advantage of using the separated mass spectral signal r_i or its corresponding regression coefficient c_i after processing through Equations 1 and 2 are that the results would be free from mutual or other interferences in the unknown sample or even in the standard (both the internal and concentration standard) samples. The standard samples would not even be mass spectrally pure or free of interferences. For example, for a blank sample which is supposed to contain pure water, often times other elemental signals are observed due to the high sensitivity of ICP-MS. These signals may come from solvents used, the container involved, the purification device, etc. Likewise, sample introduction or preparation system contamination can create additional or unexpected elemental signals through carry-over. This new software

based approach of interference treatment addresses all the issues associated with the hardware based reaction or collision cell approaches without the loss of ion signal and thus preserving the high sensitivity of ICP-MS.

Although the description above contains many specifics, these should not be construed as limiting the scope of the invention but as merely providing illustrations of some feasible embodiments of this invention. For example, in the descriptions given above, linear equations and linear regressions are used for illustrative purpose but the same approach can be utilized to nonlinear equations with the nonlinear regressions for cases where such nonlinear relationship is preferred. Likewise, although the calibrated profile mode mass spectral data are preferred, it is possible to use the same methodology for un-calibrated data or centroid data with typically inferior results. As another example, if a different form of ionization source is capable of turning elemental species into ions for mass spectral analysis, the same approach can be applied to the same or similar effects.

Thus the scope of the invention should be determined by the appended claims and their legal equivalents, rather than by the examples given. Although the present invention has been described with reference to the embodiments shown in the drawings, it should be understood that the present invention can be embodied in many alternate forms of embodiments. In addition, any suitable size, shape or type of elements or materials could be used. Accordingly, the present invention is intended to embrace all such alternatives, modifications and variances which fall within the scope of the appended claims.

CLAIMS

What is claimed is:

1. A method for analyzing spectral data or compensating for interferences, in elemental analysis of data acquired by a mass spectrometer, comprising:

acquiring profile mode mass spectral data including a plurality of points across a mass spectral range;

considering possible forms of analytes and/or interferences within said mass spectral range;

calculating a theoretical mass spectral response or measuring an actual mass spectral response for a corresponding form of analyte and/or interference;

forming a peak component matrix including one of said mass spectral response or previously measured mass spectral response for each corresponding form of analytes and/or interferences;

performing regression analysis involving the peak component matrix and the acquired mass spectral data; and

reporting regression coefficients or associated mass spectral signals as representing the concentrations or mass spectral signals of the corresponding forms of analytes and/or interferences.

2. The method of claim 1, further comprising:

adding candidate ions or components into an existing peak component matrix to form an augmented peak component matrix; and

performing further regression analysis involving the augmented peak component matrix and the acquired mass spectral data, if a fitting error is significantly larger than a predetermined amount as deemed appropriate for the level of noise in data.

3. The method of claim 1, further comprising:

eliminating candidate ions or components; and

performing further regression analysis involving the acquired mass spectral data, if an ion or component is deemed to be statistically insignificant.

4. The method of claim 1, wherein the mass spectral data are acquired from an ICP-MS (or inductively coupled plasma mass spectrometry or spectrometer) instrument.

5. The method of claim 1, wherein the mass spectral data are acquired for a standard with known or fixed concentration.

6. The method of claim 1, wherein the mass spectral data are acquired for an unknown sample for which concentration or identity of analytes or interferences is to be estimated or determined.

7. The method of claim 1, wherein the mass spectral data are acquired for an internal standard used to monitor or compensate for variation in the regression coefficient or mass spectral signal.

8. The method of claim 1, wherein the regression coefficients or mass spectral signals are related to the concentrations of analytes or interferences through a calibration curve.

9. The method of claim 8, wherein the calibration curve is one of linear and nonlinear form.

10. The method of claim 9, wherein the calibration curve is established through a set of standards with known concentrations.

11. The method of claim 8, wherein the calibration curve is used to determine the concentrations of analytes or interferences.

12. The method of claim 1, wherein one or more regression coefficients or mass spectral signals are used as inputs to determine the presence or absence of a corresponding analyte or interference.

13. The method of claim 1, wherein one or more regression coefficients or mass spectral signals are used as inputs to determine or estimate concentration of a analyte or interference.

14. The method of claim 7, wherein plural regression coefficients or mass spectral signals for plural internal standards are obtained across the mass spectral range covering possible unknown samples.

15. The method of claim 14, wherein an interpolation of linear or nonlinear form is performed on the regression coefficients or mass spectral signals to estimate a regression coefficient or mass spectral signal at any given m/z value.

16. The method of claim 15, wherein estimated regression coefficient or mass spectral signal at a given m/z value is used as input to compensate for variation in regression coefficient or mass spectral signal for an analyte or interference at that given m/z value.

17. The method of claim 16, wherein the regression coefficient or mass spectral signal after said compensation is used as input for quantitative analysis including the estimation or determination of concentration for an analyte or interference.

18. The method of claim 1, further comprising performing a mass spectral calibration involving at least one of m/z and peak shape using at least one of internal calibration standard and external calibration standard.

19. The method of claim 1, wherein the mass spectral data are acquired in raw profile mode with a plurality of data points across a mass spectral peak.

20. The method of claim 1, wherein the mass spectral data have been processed into centroid data.

21. The method of claim 18, wherein the actual peak shape function is transformed to a mathematically definable function through said mass spectral calibration.

22. The method of claim 18, wherein the mass spectral calibration involves plural calibration standards located at varying m/z values.

23. The method of claim 1, wherein possible forms of analytes or interferences are the elements of the periodic table.

24. The method of claim 1, wherein possible forms of analytes or interferences are some combinations of elements of the periodic table.

25. The method of claim 24, wherein the combination of elements involves reaction products with one or more of Ar, C, Ca, Ag, **O,** N, Na, CI, and S.

26. The method of claim 25, wherein the combination of elements involves any of Ar, C, Ca, Ag, **O,** N, Na, CI, and S resulting in mass spectral signals within the acquired mass spectral range.

27. The method of claim 1, wherein the calculation or measurement of mass spectral response or data includes all isotopes of elements or combinations of elements found in the periodic table.

28. The method of claim 27, wherein the isotopes and their corresponding abundances are based on those from naturally occurring materials or samples.

29. The method of claim 27, wherein the isotopes and their corresponding abundances have been modified from their naturally occurring values to reflect the actual isotopes and their abundances.

30. The method of claim 1, wherein the regression is a linear regression with regression coefficients proportional to the relative concentrations of analytes or interferences.

31. The method of claim 1, wherein the regression is a weighted regression.

32. The methed of claim 31, wherein the weight used in the weighted regression is inversely related to the variance of signal or signal at a given m/z location.

33. The method of claim 1, wherein the calculation of mass spectral response involves calculated or given discrete isotope distribution and one of measured peak shape function, actual peak shape function, calculated peak shape function, or mathematically defined peak shape function.

34. The method of claim 33, wherein the discrete isotope distribution is calculated from at least one of the elemental isotope distribution and elemental compositions.

35. The method of claim 1, wherein the mass spectral data or response is one or a combination of continuously sampled profile mode data, centroid data, or a continuously sampled profile mode data with gaps or holes along a m/z axis.

36. The method of claim 35, wherein the gaps or holes are created to avoid the signal saturation or interferences from other analytes or interferences.

37. The method of claim 1, wherein the possible forms of analytes or interferences are generated by an exhaustive list of all possible combinations of elements from the periodic table for a given mass spectral range.

38. The method of claim 37, wherein the list of all possible combinations is refined by user inputs of at least one of possible elements and their relative concentrations.

39. The method of claim 1, wherein the possible forms of analytes or interferences are generated by taking into consideration user inputs.

40. The method of claim 1, wherein the peak component matrix includes baseline components.

41. The method of claim 40, wherein a baseline component includes at least one of linear or nonlinear components.

42. The method of claim 1, wherein the peak component matrix includes derivatives of mass spectral data or response.

43. The method of claim 1, wherein calculating the mass spectral response comprises convoluting the theoretical isotope distribution with one of the target peak shape functions, actual peak shape functions, and approximated peak shape functions.

44. The method of claim 1, wherein a regression statistic is calculated as one of t-value, p-value, F-statistic, and residuals.

45. The method of claim 44, wherein the regression statistic is used to decide on the statistical significance of a given form of analyte or interference.

46. The method of claim 1, further comprising:

calculating the actual mass spectral peak shape function as part of a calibration process; and

using the calculated actual mass peak shape function to create the mass spectral response for inclusion in a peak component matrix.

47. The method of claim 1, further comprising:

approximating the actual mass spectral peak shape function as part of an instrument tuning process; and

using the approximated actual mass peak shape function to create the mass spectral response for inclusion in a peak component matrix.

48. The method of claim 1, wherein forming a peak component matrix comprises including the measured or calculated mass spectral responses of any already identified background ions into said peak component matrix.

49. The method of claim 1, wherein separating the mass spectral signal of one component from that of another component eliminates the need for collision or reaction cell for inductively coupled plasma mass spectrometry (ICP-MS).

50. The method of claim 1, wherein separating the mass spectral signal of one component from that of another component avoids the loss of signal due to the use of collision or reaction cell for inductively coupled plasma mass spectrometry (ICP-MS).

51. The method of claim 1, wherein elemental analysis is performed in addition to the use of a collision or reaction cell for inductively coupled plasma mass spectrometry (ICP-MS).

52. The method of claim 1, wherein the peak component matrix contains all conceivable components for a given application enabling on-the-spot qualitative and quantitative analysis without inductively coupled plasma mass spectrometry (ICP-MS) method development.

53. A mass spectrometer system operated in accordance with any one of the methods of claims 1 to 52.

54. A non-transitory computer readable medium having computer readable program instructions readable by the computer for causing a mass spectrometer system to perform in accordance with the methods of at least one of any one of claims 1 to 52.

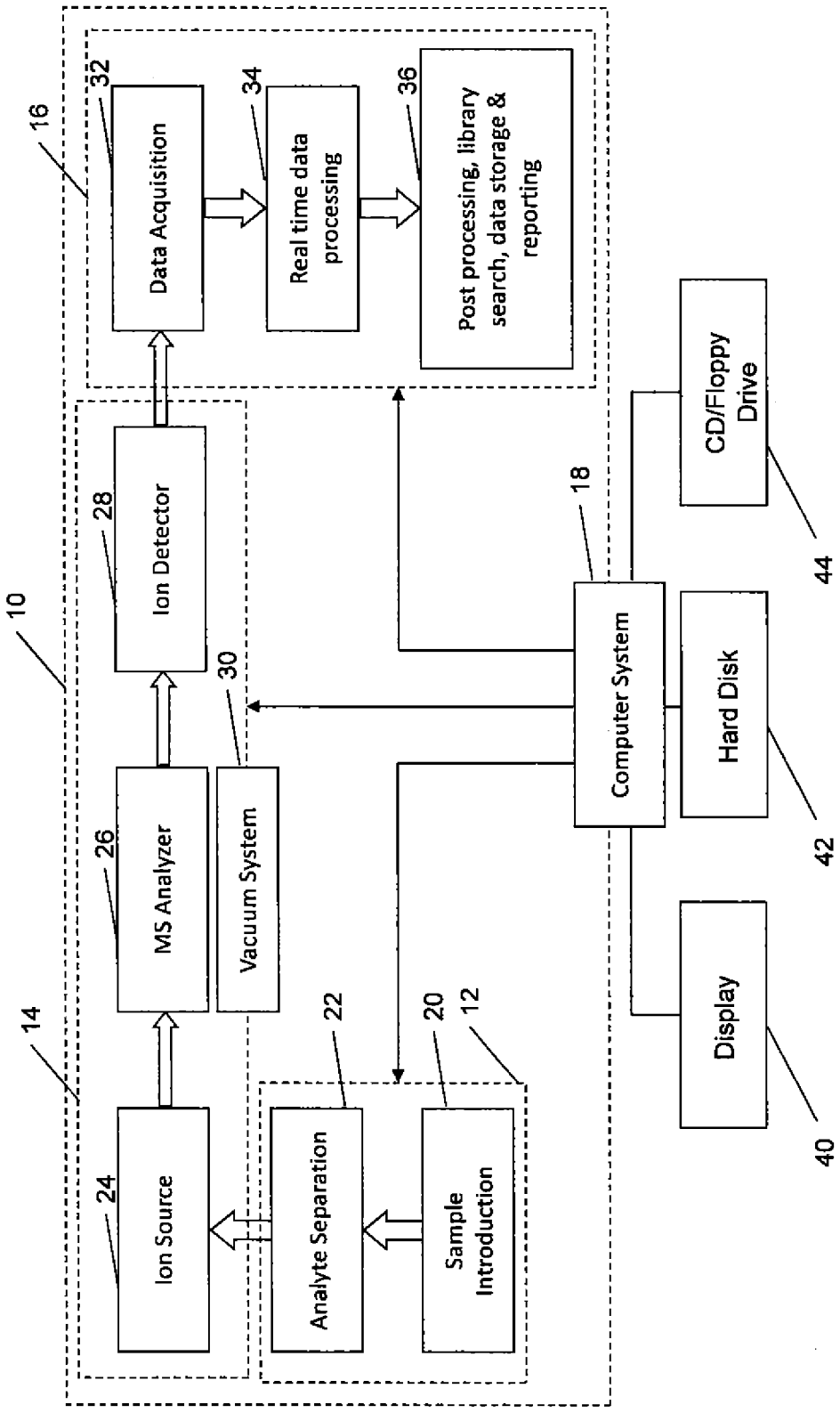

16
32
Data Acquisition
34
Real time data processing
36
Post processing, library search, data storage & reporting
10
28
Ion Detector
30
26
MS Analyzer
Vacuum System
14
24
Ion Source
22
Analyte Separation
20
12
Sample Introduction
18
Computer System
CD/Floppy Drive
44
Hard Disk
42
Display
40

Fig. 1

Fig. 2A

Mass	Abundance
39.9764	91.11%
40.9759	4.68%
40.9797	3.12%
41.9732	1.01%
41.9793	0.05%
42.9766	0.03%

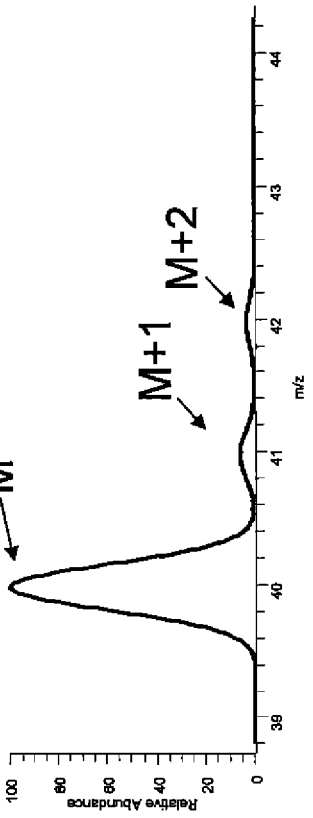


Fig. 2B

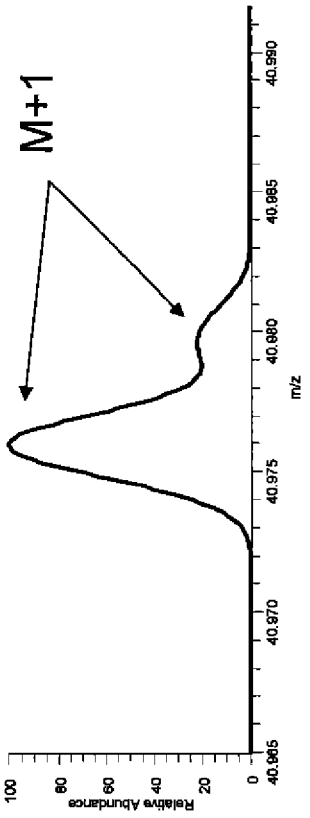


Fig. 2C

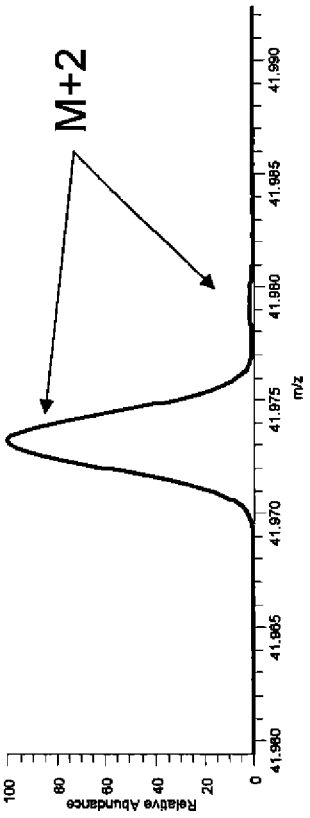


Fig. 2D

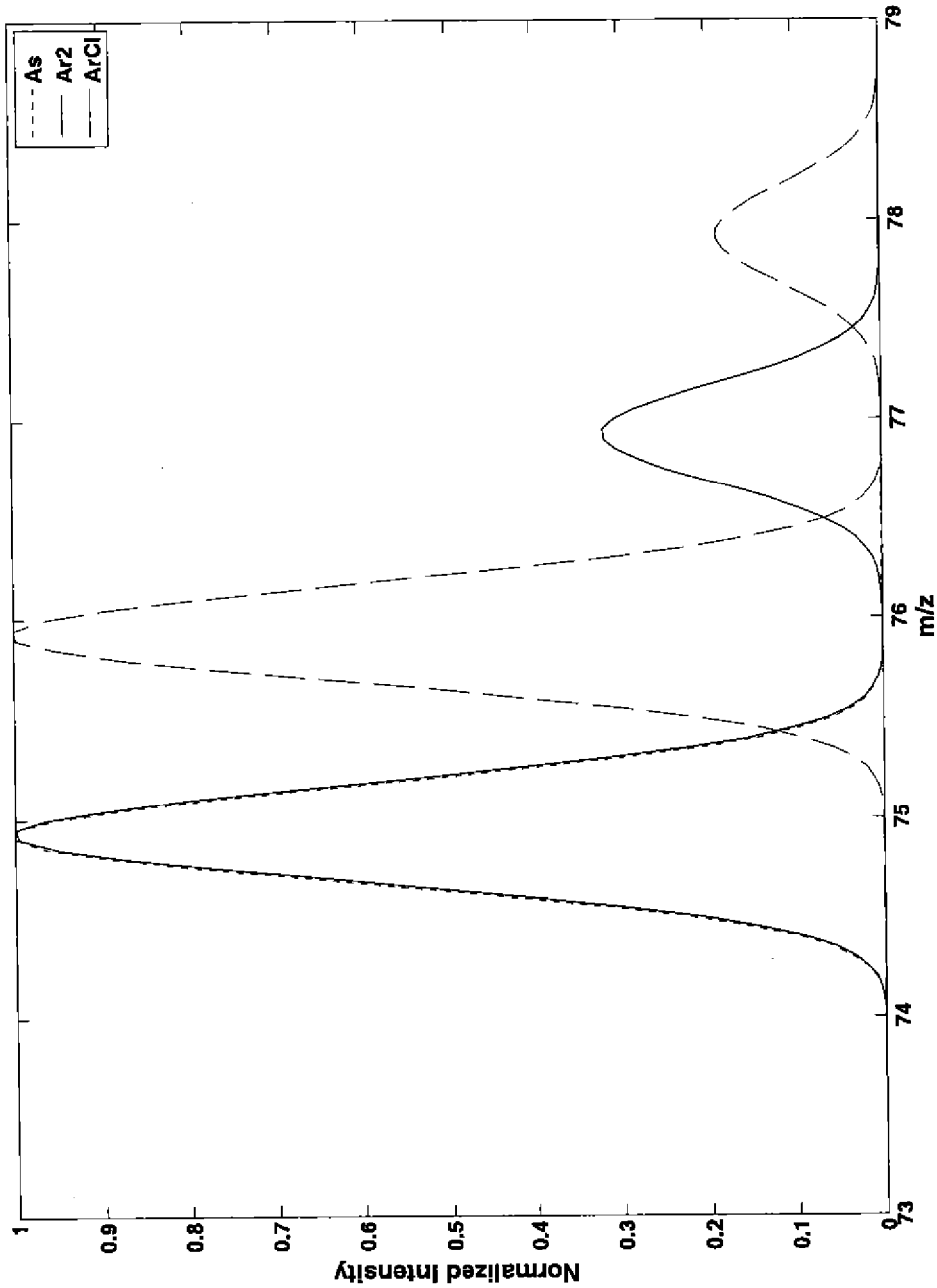

As
Ar2
ArCl
Normalized Intensity
m/z
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
73
74
75
76
77
78
79

Fig. 3

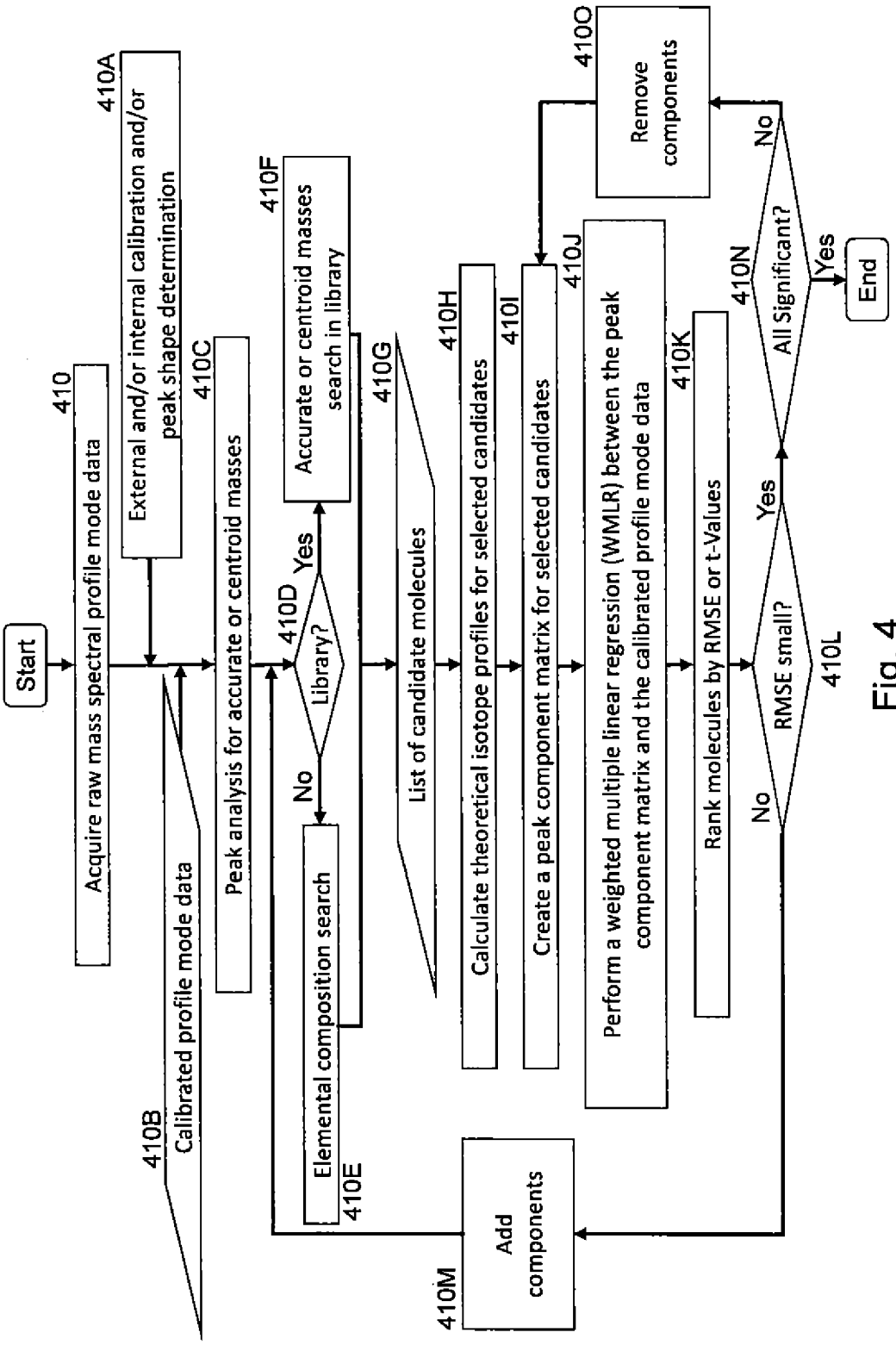

Start
410
Acquire raw mass spectral profile mode data
410A
External and/or internal calibration and/or peak shape determination
410B
Calibrated profile mode data
410C
Peak analysis for accurate or centroid masses
410D
Library?
Yes
No
410E
Elemental composition search
410F
Accurate or centroid masses search in library
410G
List of candidate molecules
410H
Calculate theoretical isotope profiles for selected candidates
410I
Create a peak component matrix for selected candidates
410J
Perform a weighted multiple linear regression (WMLR) between the peak component matrix and the calibrated profile mode data
410K
Rank molecules by RMSE or t-Values
410L
RMSE small?
Yes
No
410M
Add components
410N
All Significant?
Yes
No
410O
Remove components
End

Fig. 4

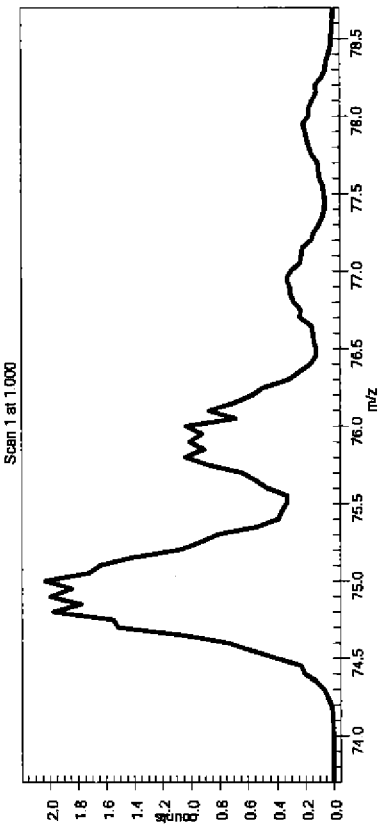

Scan 1 at 1.000
Abund
m/z
74.0
74.5
75.0
75.5
76.0
76.5
77.0
77.5
78.0
78.5
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0

Fig. 5A

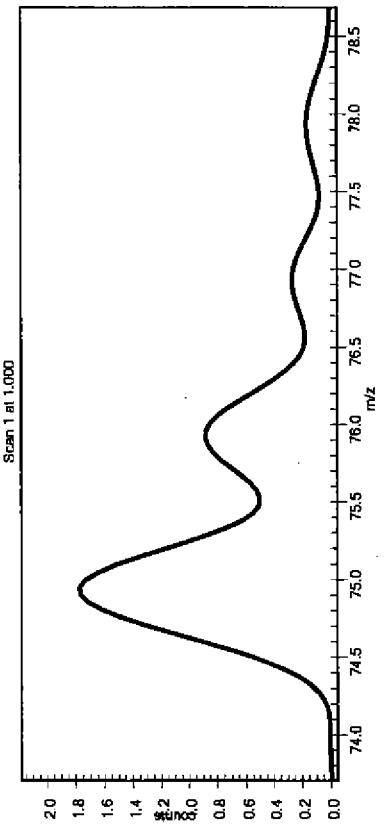

Scan 1 at 1.000
Abund
m/z
74.0
74.5
75.0
75.5
76.0
76.5
77.0
77.5
78.0
78.5
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0

Fig. 5B

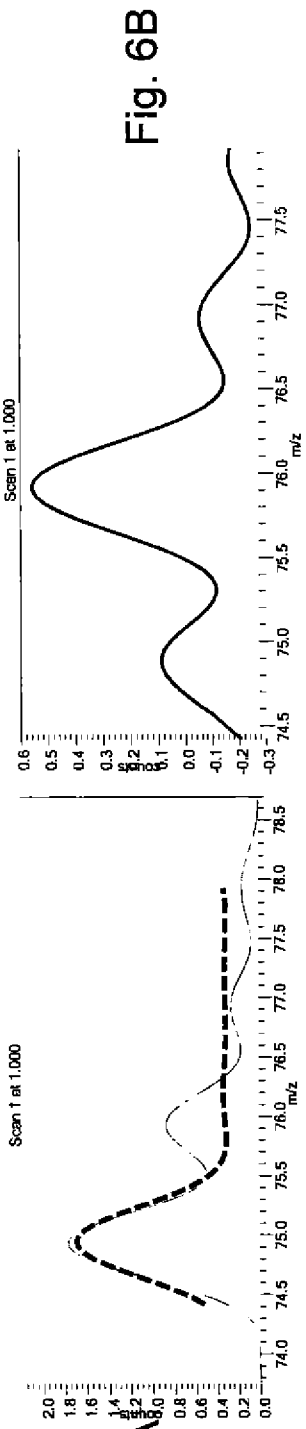


Fig. 6A

Fig. 6B

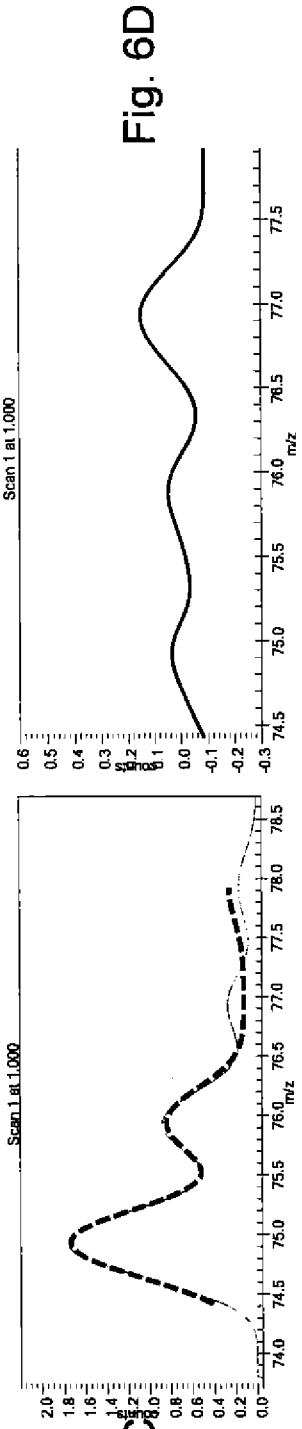


Fig. 6C

Fig. 6D

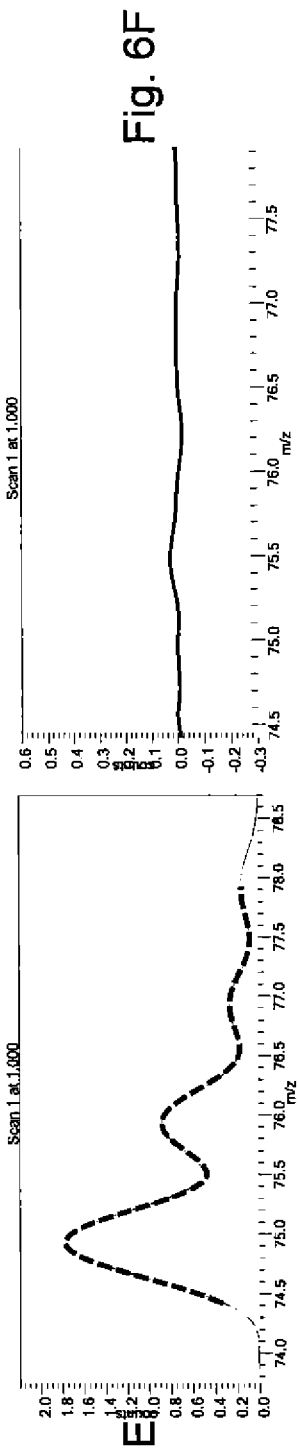


Fig. 6E

Fig. 6F

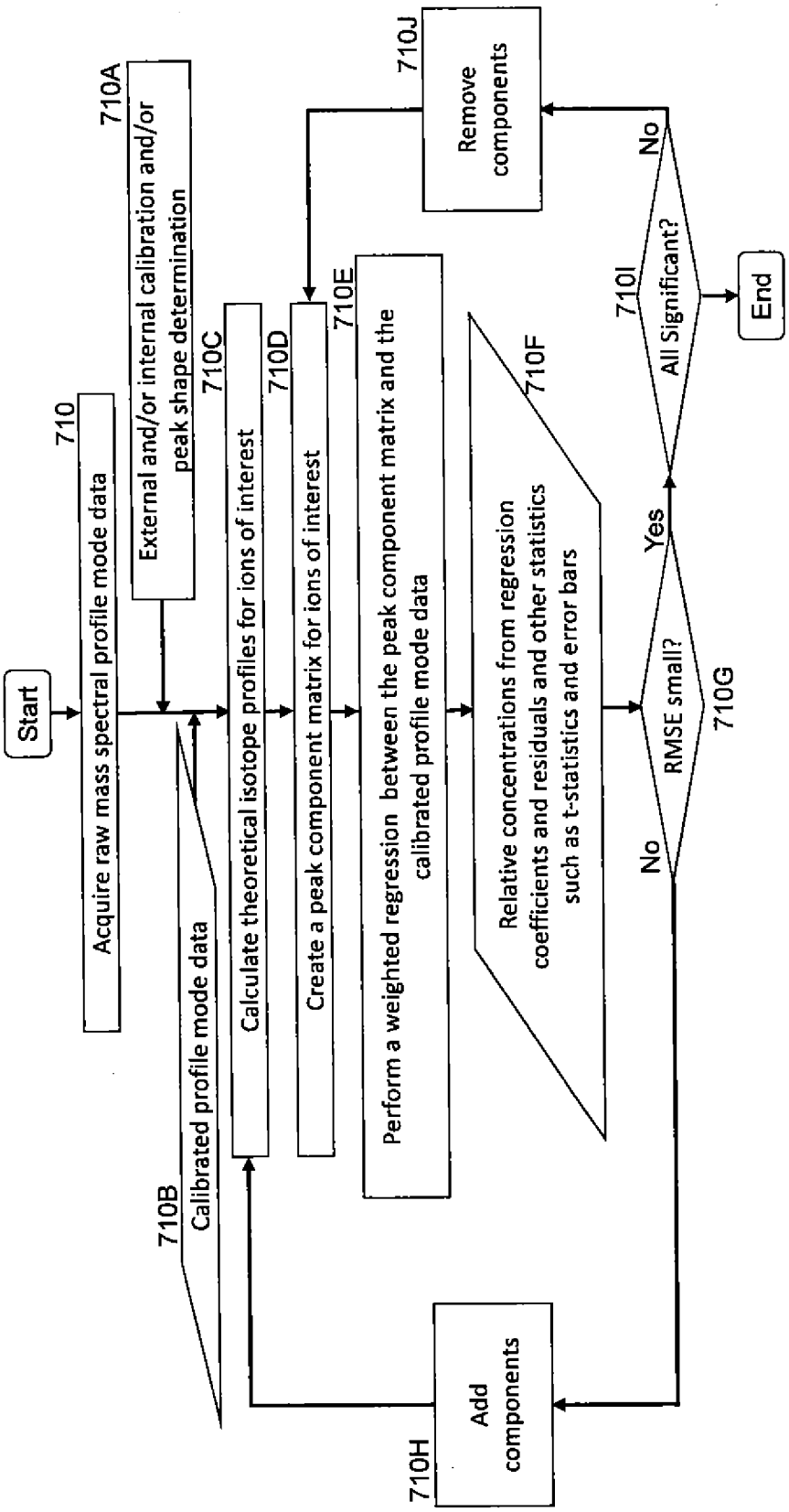

Start
710
Acquire raw mass spectral profile mode data
710A
External and/or internal calibration and/or peak shape determination
710B
Calibrated profile mode data
710C
Calculate theoretical isotope profiles for ions of interest
710D
Create a peak component matrix for ions of interest
710E
Perform a weighted regression between the peak component matrix and the calibrated profile mode data
710F
Relative concentrations from regression coefficients and residuals and other statistics such as t-statistics and error bars
710G
RMSE small?
Yes
No
710H
Add components
710I
All Significant?
No
Yes
710J
Remove components
End

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2013/020663

A. CLASSIFICATION OF SUBJECT MATTER

G01N 30/86 (2006.01)
B01D 59/44 (2006.01)

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)

B01D 59/00, 59/44, H01J 49/00, 49/26, 49/34, 49/40, G06F 19/00, GOIN 30/72, 30/86

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 practicable, search terms used)

PatSearch (RUPTO internal), Esp@cenet, PAJ, USPTO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document; with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/0255258 A1 (YONGDONG WANG et al.) 16.11.2006, abstract, claims 19-34	1-54
X	US 2008/00520 11 A1 (YONGDONG WANG et al.) 28.02.2008, abstract, claims 1-32	1-54
X	US 2006/0169883 A1 (YOUGDONG WANG et al.) 03.08.2006, abstract, claims 1-16	1
A	RU 2332748 C1 (BAKLANOV ALEXEI VASILIEVICH et al.) 27.08.2008, abstract	1-54

☐ **Further documents are listed in the continuation of Box C.** ☐ See patent family 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; tire 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
20 March 2013 (20.03.2013)	04 April 2013 (04.04.2013)
Name and mailing address of the ISA/ FIPS Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37	Authorized officer E. Iritsky Telephone No. (499) 240-25-91