(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 December 2024 (19.12.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/257037 A1

(51) International Patent Classification:
H01J 49/00 (2006.01)

(21) International Application Number:
PCT/IB2024/055837

(22) International Filing Date:
14 June 2024 (14.06.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/508,185 14 June 2023 (14.06.2023) US

(71) Applicant: **DH TECHNOLOGIES DEVELOPMENT PTE. LTD.** [SG/SG]; 33 Marsiling Industrial Estate Road 3, #04-06, Singapore 739256 (SG).

(72) Inventor: **DUCHOSLAV, Eva**; c/o Sciex, 71 Four Valley Drive, Concord, Ontario L4K 4V8 (CA).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ,

(54) Title: FRAGMENT TYPE DRIVEN SPECTRAL PEAK

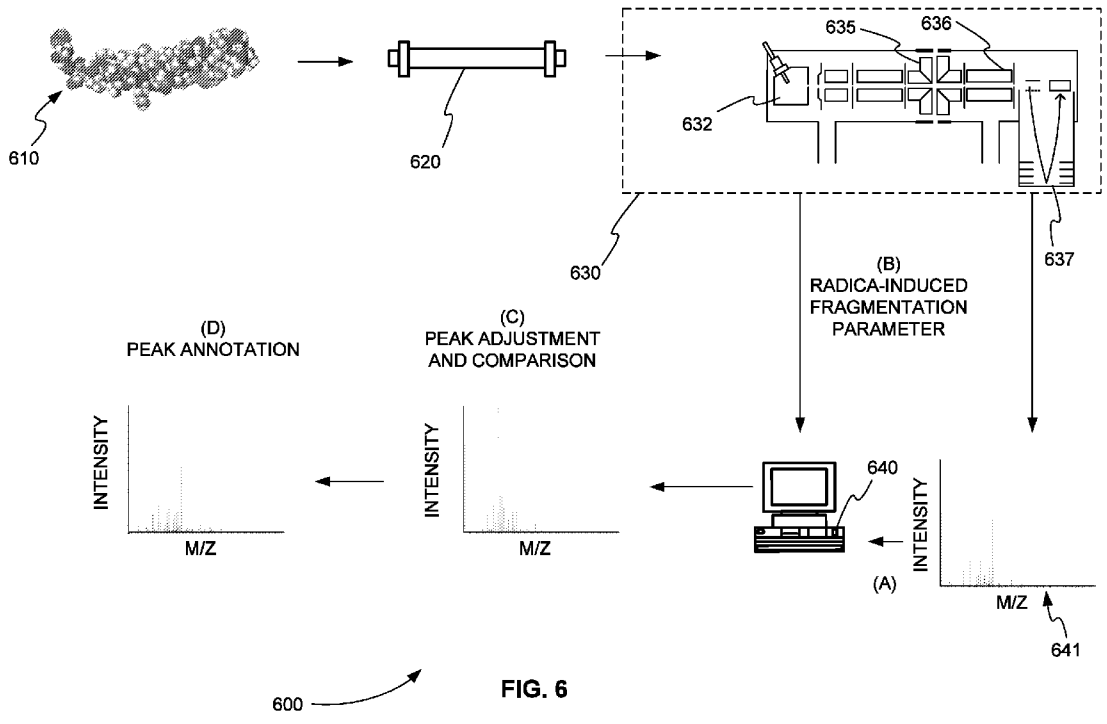

FIG. 6

(57) Abstract: A method for annotating product ions of a spectrum is disclosed. A product ion mass spectrum is received. A parameter that indicates the mass spectrum was produced using a radical-induced dissociation method is received. At least one charge state mass-to-charge ratio (m/z) adjustment based on the parameter is performed for at least one product ion peak of the mass spectrum and the adjustment is compared to one or more other product ion peaks of the mass spectrum. The at least one product ion peak is annotated based on the comparison. The charge state adjustment includes the loss of an electron or the loss or gain of a hydrogen atom. The radical-induced dissociation method includes electron-based dissociation (ExD), ultraviolet photodissociation (UVPD), or infrared photodissociation (IRMPD).

[Continued on next page]

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RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

FRAGMENT TYPE DRIVEN SPECTRAL PEAK

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/508,185 filed on June 14, 2023, the contents of which are incorporated herein in their entirety.

INTRODUCTION

[0002] The teachings herein relate to a method for annotating product ion peaks of a mass spectrum. More particularly, the teachings herein relate to systems and methods for annotating product ions based on the type of fragmentation used.

[0003] The systems and methods herein can be performed in conjunction with a processor, controller, or computer system, such as the computer system of Figure 1.

Current Spectral Peak Annotation

[0004] An accurate understanding of fragment ion types is needed when characterizing structural features from mass spectrometry data. These structural features include specifically, for example, the chain double bond position in endogenous lipids or ionizable lipids of lipid nanoparticles (LNP) where the structure information is encoded in low signal to noise (S/N) mass spectrometry data. In general, characterization of such features is needed for the confident and accurate identification of compounds or compound structure.

[0005] Increasingly, information about the fragmentation method used is an important component in understanding the fragment ion types found. For example, Ducati *et al.*, Improved metabolite characterization by liquid chromatography - Tandem mass spectrometry through electron impact type fragments from adduct ions,

Analytica Chimica Acta 1150 (2021) (hereinafter the "Ducati Paper"), have reported that compared to collision-induced dissociation (CID), electron-induced dissociation (EID) "resulted in additional specific and informative fragments." The Ducati Paper highlights additional fragment types produced by a radical-induced dissociation method.

[0006] Unfortunately, current spectral peak annotation methods that determine charge state and monoisotopic peaks do not consider additional information such as the fragmentation method or the width of the precursor ion mass filter when annotating product ion peaks. As a result, conventional peak annotation methods often include inaccurate fragment ion understanding that complicates compound structure elucidation and confirmation.

[0007] As a result, additional systems and methods are needed for annotating product ions of a spectrum that include an accurate understanding of fragment ion types.

LC-MS and LC-MS/MS Background

[0008] Mass spectrometry (MS) is an analytical technique for the detection and quantitation of chemical compounds based on the analysis of mass-to-charge ratios (m/z) of ions formed from those compounds. The combination of mass spectrometry (MS) and liquid chromatography (LC) is an important analytical tool for the identification and quantitation of compounds within a mixture. Generally, in liquid chromatography, a fluid sample under analysis is passed through a column filled with a chemically-treated solid adsorbent material (typically in the form of small solid particles, e.g., silica). Due to slightly different interactions of components of the mixture with the solid adsorbent material (typically referred to as the stationary phase), the different components can have different transit

(elution) times through the packed column, resulting in separation of the various components.

[0009] Note that for singly charged species, the terms "mass" and "m/z" are used interchangeably herein. One of ordinary skill in the art understands that a mass can be found from an m/z by multiplying the m/z by the charge. Similarly, the m/z can be found from a mass by dividing the mass by the charge.

[0010] In LC-MS, the effluent exiting the LC column can be continuously subjected to MS analysis. The data from this analysis can be processed to generate an extracted ion chromatogram (XIC), which can depict detected ion intensity (a measure of the number of detected ions of one or more particular analytes) as a function of retention time.

[0011] In MS analysis, an MS or precursor ion scan is performed at each interval of the separation for a mass range that includes the precursor ion. An MS scan includes the selection of a precursor ion or precursor ion range and mass analysis of the precursor ion or precursor ion range.

[0012] In some cases, the LC effluent can be subjected to tandem mass spectrometry (or mass spectrometry/mass spectrometry MS/MS) for the identification of product ions corresponding to the peaks in the XIC. For example, the precursor ions can be selected based on their mass/charge ratio to be subjected to subsequent stages of mass analysis. For example, the selected precursor ions can be fragmented (e.g., via collision-induced dissociation), and the fragmented ions (product ions) can be analyzed via a subsequent stage of mass spectrometry.

Fragmentation Techniques Background

[0013] Electron-based dissociation (ExD), ultraviolet photodissociation (UVPD), infrared photodissociation (IRMPD), and collision-induced dissociation (CID) are often used as fragmentation techniques for tandem mass spectrometry (MS/MS). CID is the most conventional technique for dissociation in tandem mass spectrometers. CID, in-source fragmentation, blackbody infrared radiative dissociation and IRMPD are examples of thermal-dissociation methods in this description. Thermal-dissociation methods included herein are non-radical dissociation methods that do not involve the use of radical formation in the dissociation process.

[0014] ExD can include, but is not limited to, electron-induced dissociation (EID), electron impact excitation in organics (EIEIO), electron capture dissociation (ECD), or electron transfer dissociation (ETD). Radical-induced dissociation methods, mentioned herein, include ExD, UVPD, electron detachment dissociation (EDD), plasma electron detachment dissociation (pEDD), and electron photodetachment dissociation (EPD).

Tandem Mass Spectrometry or MS/MS Background

[0015] Tandem mass spectrometry or MS/MS involves ionization of one or more compounds of interest from a sample, selection of one or more precursor ions of the one or more compounds, fragmentation of the one or more precursor ions into product ions, and mass analysis of the product ions.

[0016] Tandem mass spectrometry can provide both qualitative and quantitative information. The product ion spectrum can be used to identify a molecule of

interest. The intensity of one or more product ions can be used to quantitate the amount of the compound present in a sample.

[0017] A large number of different types of experimental methods or workflows can be performed using a tandem mass spectrometer. These workflows can include, but are not limited to, targeted acquisition, information dependent acquisition (IDA) or data dependent acquisition (DDA), and data independent acquisition (DIA).

[0018] In a targeted acquisition method, one or more transitions of a precursor ion to a product ion are predefined for a compound of interest. As a sample is being introduced into the tandem mass spectrometer, the one or more transitions are interrogated during each time period or cycle of a plurality of time periods or cycles. In other words, the mass spectrometer selects and fragments the precursor ion of each transition and performs a targeted mass analysis for the product ion of the transition. As a result, a chromatogram (the variation of the intensity with retention time) is produced for each transition. Targeted acquisition methods include, but are not limited to, multiple reaction monitoring (MRM) and selected reaction monitoring (SRM).

[0019] MRM experiments are typically performed using "low resolution" instruments that include, but are not limited to, triple quadrupole (QqQ) or quadrupole linear ion trap (QqLIT) devices. With the advent of "high resolution" instruments, there was a desire to collect MS and MS/MS using workflows that are similar to QqQ/QqLIT systems. High-resolution instruments include, but are not limited to, quadrupole time-of-flight (QqTOF) or orbitrap devices. These high-resolution instruments also provide new functionality.

[0020] MRM on QqQ/QqLIT systems is the standard mass spectrometric technique of choice for targeted quantification in all application areas, due to its ability to

provide the highest specificity and sensitivity for the detection of specific components in complex mixtures. However, the speed and sensitivity of today's accurate mass systems have enabled a new quantification strategy with similar performance characteristics. In this strategy (termed MRM high resolution (MRM-HR) or parallel reaction monitoring (PRM)), looped MS/MS spectra are collected at high-resolution with short accumulation times, and then fragment ions (product ions) are extracted post-acquisition to generate MRM-like peaks for integration and quantification. With instrumentation like the TRIPLETOF® Systems of AB SCIEX™, this targeted technique is sensitive and fast enough to enable quantitative performance similar to higher-end triple quadrupole instruments, with full fragmentation data measured at high resolution and high mass accuracy.

[0021] In other words, in methods such as MRM-HR, a high-resolution precursor ion mass spectrum is obtained, one or more precursor ions are selected and fragmented, and a high-resolution full product ion spectrum is obtained for each selected precursor ion. A full product ion spectrum is collected for each selected precursor ion but a product ion mass of interest can be specified and everything other than the mass window of the product ion mass of interest can be discarded.

[0022] In an IDA (or DDA) method, a user can specify criteria for collecting mass spectra of product ions while a sample is being introduced into the tandem mass spectrometer. For example, in an IDA method a precursor ion or mass spectrometry (MS) survey scan is performed to generate a precursor ion peak list. The user can select criteria to filter the peak list for a subset of the precursor ions on the peak list. The survey scan and peak list are periodically refreshed or updated, and MS/MS is then performed on each precursor ion of the subset of

precursor ions. A product ion spectrum is produced for each precursor ion. MS/MS is repeatedly performed on the precursor ions of the subset of precursor ions as the sample is being introduced into the tandem mass spectrometer.

[0023] In proteomics and many other applications, however, the complexity and dynamic range of compounds is very large. This poses challenges for traditional targeted and IDA methods, requiring very high-speed MS/MS acquisition to deeply interrogate the sample in order to both identify and quantify a broad range of analytes.

[0024] As a result, DIA methods, the third broad category of tandem mass spectrometry, were developed. These DIA methods have been used to increase the reproducibility and comprehensiveness of data collection from complex samples. DIA methods can also be called non-specific fragmentation methods. In a DIA method the actions of the tandem mass spectrometer are not varied among MS/MS scans based on data acquired in a previous precursor or survey scan. Instead, a precursor ion mass range is selected. A precursor ion mass selection window is then stepped across the precursor ion mass range. All precursor ions in the precursor ion mass selection window are fragmented and all of the product ions of all of the precursor ions in the precursor ion mass selection window are mass analyzed.

[0025] The precursor ion mass selection window used to scan the mass range can be narrow so that the likelihood of multiple precursors within the window is small. This type of DIA method is called, for example, MS/MS^{ALL}. In an MS/MS^{ALL} method, a precursor ion mass selection window of about 1 Da is scanned or stepped across an entire mass range. A product ion spectrum is produced for each 1 Da precursor mass window. The time it takes to analyze or scan the entire mass

range once is referred to as one scan cycle. Scanning a narrow precursor ion mass selection window across a wide precursor ion mass range during each cycle, however, can take a long time and is not practical for some instruments and experiments.

[0026] As a result, a larger precursor ion mass selection window, or selection window with a greater width, is stepped across the entire precursor mass range. This type of DIA method is called, for example, SWATH acquisition. In a SWATH acquisition, the precursor ion mass selection window stepped across the precursor mass range in each cycle may have a width of 5-25 Da, or even larger. Like the MS/MS^{ALL} method, all of the precursor ions in each precursor ion mass selection window are fragmented, and all of the product ions of all of the precursor ions in each mass selection window are mass analyzed.

[0027] However, identifying compounds of interest in a sample analyzed using SWATH acquisition, for example, can be difficult. It can be difficult because either there is no precursor ion information provided with a precursor ion mass selection window to help determine the precursor ion that produces each product ion, or the precursor ion information provided is from a mass spectrometry (MS) observation that has a low sensitivity. In addition, because there is little or no specific precursor ion information provided with a precursor ion mass selection window, it is also difficult to determine if a product ion is convolved with or includes contributions from multiple precursor ions within the precursor ion mass selection window.

[0028] As a result, a method of scanning the precursor ion mass selection windows in SWATH acquisition, called scanning SWATH, was developed. Essentially, in scanning SWATH, a precursor ion mass selection window is scanned across a

mass range so that successive windows have large areas of overlap and small areas of non-overlap. This scanning makes the resulting product ions a function of the scanned precursor ion mass selection windows. This additional information, in turn, can be used to identify the one or more precursor ions responsible for each product ion.

SUMMARY

[0029] According to embodiments disclosed herein, a system, method, and computer program product are disclosed for annotating product ions of a spectrum.

[0030] In one general aspect, a method for annotating product ions of a spectrum can include: (a) receiving a product ion mass spectrum; (b) receiving a parameter that indicates the mass spectrum was produced using a radical-induced dissociation method; (c) performing at least one charge state mass-to-charge ratio (m/z) adjustment based on the parameter for at least one product ion peak of the mass spectrum and comparing the adjustment to one or more other product ion peaks of the mass spectrum; and (d) annotating the at least one product ion peak based on the comparison.

[0031] In some embodiments, the adjustment can include multiplying the m/z of the least one product ion peak by an absolute charge, subtracting one or more masses of an electron from the product, and dividing the difference by a charge adjusted for the number subtracted electron masses.

[0032] In some embodiments, the charge can be 2 or 3.

[0033] In some embodiments, the adjustment can include adding or subtracting an m/z of a hydrogen atom.

[0034] In some embodiments, the method can further include, if the adjustment is equal to an m/z of at least one other product ion peak of the one or more other product ion peaks within a first m/z tolerance value, annotating the at least one other product ion peak in the mass spectrum as having an electron loss.

[0035] In some embodiments, the method can further include, if a singly charged precursor ion was mass filtered to produce the spectrum, before step (c), annotating each peak of the spectrum as a monoisotopic peak.

[0036] In some embodiments, the method can further include performing steps (c)-(d) on each product ion peak of the spectrum.

[0037] In some embodiments, the method can further include, after performing steps (c)-(d) on each product ion peak of the mass spectrum, analyzing each product ion peak of the mass spectrum for isotopic peaks and annotating each isotopic peak found as an isotopic peak.

[0038] In some embodiments, the method can further include transforming annotated peaks into neutral mass peaks.

[0039] In some embodiments, the method can further include receiving a parameter that indicates an energy value that was used by the radical-induced dissociation method to produce the spectrum.

[0040] In some embodiments, the method can further include producing a peak list that includes an electron loss annotation for one or more peaks of the mass spectrum.

[0041] In some embodiments, the radical-induced dissociation method can include electron-based dissociation (ExD).

[0042] In some embodiments, the radical-induced dissociation method can include ultraviolet photodissociation (UVPD) or infrared photodissociation (IRMPD).

[0043] In another general aspect, a computer program product can include a non-transitory tangible computer-readable storage medium whose contents cause a processor to perform a method for annotating product ions of a spectrum. The method can include: providing a system including one or more distinct software modules, wherein the one or more distinct software modules include an input module and an analysis module; (a) receiving a product ion mass spectrum using the input module; (b) receiving a parameter that indicates the spectrum was produced using a radical-induced dissociation method using the input module; (c) performing at least one charge state mass-to-charge ratio (m/z) adjustment based on the parameter for at least one product ion peak of the mass spectrum and comparing the adjustment to one or more other product ion peaks of the mass spectrum using the analysis module; and (d) annotating the at least one product ion peak based on the comparison using the analysis module.

[0044] In another general aspect, a system for annotating product ions of a spectrum can include one or more processors that: (a) receive a product ion mass spectrum, (b) receive a parameter that indicates the spectrum was produced using a radical-induced dissociation method, (c) perform at least one charge state mass-to-charge ratio (m/z) adjustment based on the parameter for at least one product ion peak of the mass spectrum and comparing the adjustment to one or more other product ion peaks of the mass spectrum, and (d) annotating the at least one product ion peak based on the comparison.

[0045] These and other features of the applicant's teachings are set forth herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] The skilled artisan will understand that the drawings, described below, are for illustration purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

[0047] Figure 1 is a block diagram that illustrates a computer system, upon which embodiments of the present teachings may be implemented.

[0048] Figure 2 is an exemplary plot of a product ion spectrum showing a doubly charged peak corresponding to a singly charged precursor ion peak, in accordance with various embodiments.

[0049] Figure 3 is an exemplary plot of traces from spectra showing a gain in hydrogen, in accordance with various embodiments.

[0050] Figure 4 is an exemplary plot traces from spectra showing a loss in hydrogen, in accordance with various embodiments.

[0051] Figure 5 is an exemplary diagram of a product ion annotation, in accordance with various embodiments.

[0052] Figure 6 is an exemplary schematic diagram of a system for annotating product ions of a spectrum, in accordance with various embodiments.

[0053] Figure 7 is an exemplary flowchart showing a method for annotating product ions of a spectrum, in accordance with various embodiments.

[0054] Figure 8 is an exemplary schematic diagram of a system that includes one or more distinct software modules and that performs a method for annotating product ions of a spectrum, in accordance with various embodiments.

[0055] Before one or more embodiments of the present teachings are described in detail, one skilled in the art will appreciate that the present teachings are not limited in their application to the details of construction, the arrangements of components,

and the arrangement of steps set forth in the following detailed description or illustrated in the drawings. Also, it is to be understood that the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting.

DESCRIPTION OF VARIOUS EMBODIMENTS

COMPUTER-IMPLEMENTED SYSTEM

[0056] Figure 1 is a block diagram that illustrates a computer system 100, upon which embodiments of the present teachings may be implemented. Computer system 100 includes a bus 102 or other communication mechanism for communicating information, and a processor 104 coupled with bus 102 for processing information. Computer system 100 also includes a memory 106, which can be a random-access memory (RAM) or other dynamic storage device, coupled to bus 102 for storing instructions to be executed by processor 104. Memory 106 also may be used for storing temporary variables or other intermediate information during execution of instructions to be executed by processor 104. Computer system 100 further includes a read only memory (ROM) 108 or other static storage device coupled to bus 102 for storing static information and instructions for processor 104. A storage device 110, such as a magnetic disk or optical disk, is provided and coupled to bus 102 for storing information and instructions.

[0057] Computer system 100 may be coupled via bus 102 to a display 112, such as a cathode ray tube (CRT) or liquid crystal display (LCD), for displaying information to a computer user. An input device 114, including alphanumeric and other keys, is coupled to bus 102 for communicating information and command selections to processor 104. Another type of user input device is cursor control

116, such as a mouse, a trackball or cursor direction keys for communicating direction information and command selections to processor 104 and for controlling cursor movement on display 112.

[0058] A computer system 100 can perform the present teachings. Consistent with certain implementations of the present teachings, results are provided by computer system 100 in response to processor 104 executing one or more sequences of one or more instructions contained in memory 106. Such instructions may be read into memory 106 from another computer-readable medium, such as storage device 110. Execution of the sequences of instructions contained in memory 106 causes processor 104 to perform the process described herein.

[0059] Alternatively, hard-wired circuitry may be used in place of or in combination with software instructions to implement the present teachings. For example, the present teachings may also be implemented with programmable artificial intelligence (AI) chips with only the encoder neural network programmed – to allow for performance and decreased cost. Thus, implementations of the present teachings are not limited to any specific combination of hardware circuitry and software.

[0060] The term "computer-readable medium" or "computer program product" as used herein refers to any media that participates in providing instructions to processor 104 for execution. The terms "computer-readable medium" and "computer program product" are used interchangeably throughout this written description. Such a medium may take many forms, including but not limited to, non-volatile media and volatile media. Non-volatile media includes, for example, optical or magnetic disks, such as storage device 110. Volatile media includes dynamic memory, such as memory 106.

[0061] Common forms of computer-readable media include, for example, a floppy disk, a flexible disk, hard disk, magnetic tape, or any other magnetic medium, a CD-ROM, digital video disc (DVD), a Blu-ray Disc, any other optical medium, a thumb drive, a memory card, a RAM, PROM, and EPROM, a FLASH-EPROM, any other memory chip or cartridge, or any other tangible medium from which a computer can read.

[0062] Various forms of computer readable media may be involved in carrying one or more sequences of one or more instructions to processor 104 for execution. For example, the instructions may initially be carried on the magnetic disk of a remote computer. The remote computer can load the instructions into its dynamic memory and send the instructions over a telephone line using a modem. A modem local to computer system 100 can receive the data on the telephone line and use an infra-red transmitter to convert the data to an infra-red signal. An infra-red detector coupled to bus 102 can receive the data carried in the infra-red signal and place the data on bus 102. Bus 102 carries the data to memory 106, from which processor 104 retrieves and executes the instructions. The instructions received by memory 106 may optionally be stored on storage device 110 either before or after execution by processor 104.

[0063] In accordance with various embodiments, instructions configured to be executed by a processor to perform a method are stored on a computer-readable medium. The computer-readable medium can be a device that stores digital information. The computer-readable medium is accessed by a processor suitable for executing instructions configured to be executed.

[0064] The following descriptions of various implementations of the present teachings have been presented for purposes of illustration and description. It is not

exhaustive and does not limit the present teachings to the precise form disclosed. Modifications and variations are possible in light of the above teachings or may be acquired from practicing of the present teachings. Additionally, the described implementation includes software but the present teachings may be implemented as a combination of hardware and software or in hardware alone. The present teachings may be implemented with both object-oriented and non-object-oriented programming systems.

ANNOTATION BASED ON FRAGMENTATION TYPE

[0065] As described above, an accurate understanding of fragment ion types is needed when characterizing structural features from spectrometry data, especially the data of low signal to noise (S/N). Increasingly, information about the fragmentation method used is an important component in understanding the fragment ion types found. Unfortunately, current spectral peak annotation methods that determine charge state and monoisotopic peaks do not consider additional information such as the fragmentation method or the width of the precursor ion mass filter when annotating product ion peaks.

[0066] As a result, additional systems and methods are needed for annotating product ions of a spectrum that include an accurate understanding of fragment ion types.

[0067] In various embodiments, product ion peaks are annotated with respect to their potential relationships that are given by the fragmentation technique used. This annotation enables accurate assignment of the fragment spectral peak properties, such as charge state, isotope index, and fragment ion type. It also leads to an accurate determination of fragment-neutral masses and compositions that can be used in a proposed molecular structure or a confirmation of a structure. This

method helps improve understanding of the MS/MS data by recognizing the relationships between fragment peaks (based on accurate m/z differences) and grouping complementary fragment peaks before qualitative analysis.

[0068] In various embodiments, this method aids in the accurate annotation of product ions in radical-driven fragmentation data that yield charged fragments as "open shell" molecules by loss of an electron. Annotation of isotopic peaks is also improved, producing an accurate set of monoisotopic peaks.

[0069] In various embodiments, knowledge of fragmentation type (class) is used to determine which ion molecule fragment ion types are in the MS/MS data. The types are then annotated. The resulting annotation allows the information to be combined into "a z=0 reconstructed small molecule MSMS" or transformed to a different data format that serves as an entry point for structure elucidation. This method gives a more accurate answer with respect to the relative evidence to support an identified compound.

[0070] In various embodiments, the method also uses knowledge of the precursor ion peaks in the precursor ion mass filter window to determine whether or not isotopic peaks are in the product ion spectrum. It also determines if isotope patterns are expected to be skewed and the likely maximum charge state of the product ions.

[0071] In various embodiments, as precursor ion type is considered, product ion peaks of non-protonated precursor ions are consolidated, especially those from alkali metal precursors.

[0072] In various embodiments, an annotation workflow includes one or more of the following steps. MS/MS XY data, precursor information (*e.g.*, width of precursor ion mass filter window), and a fragmentation type data are received. Precursor information can include, but is not limited to, m/z, charge, and ion type including

isotope index. Fragmentation type can optionally include experimental parameters, such as collision energy (CE) or kinetic energy (kE) values.

[0073] A peak list is obtained from the MS/MS spectrum. The peak list can include, but is not limited to, values for m/z, height or area, and peak width at half height. In various embodiments, any charge state is assumed for the peaks (from 1 to the precursor ion charge state and its elevation).

[0074] A list of possible fragment types in the spectrum is collated. These fragment types can include, but are not limited to, default (gain or loss of proton(s)), mix of protons with any alkali metals based on precursor ion charge, isotopes (if isotope passing through precursor ion mass filter), loss of electron (charge state elevation) – for radical driven dissociation, loss or gain of hydrogen - radical driven dissociation.

[0075] Initially, all fragment types are assumed to be of the default type. Then, considering selected fragment charge states, isotope peak relationships are identified. The charge state is assigned to the fragments that are accompanied by isotopic peaks in the MS/MS.

[0076] Starting from the most intense peak, for each peak in each charge state, each peak is inspected for the possibility of a relationship to other peaks. The relationships between peaks and the respective mass errors are tracked.

[0077] Any competing relationships are resolved based on associated mass errors. For example, m/z 153.0898 is related to m/z 154.0978 (-0.26mDa) and not to m/z 154.0882 (9.418mDa).

[0078] After the relationships are established, the related fragments are grouped based on the common neutral mass. A group representative is then used in further analysis.

Charge State Adjustment

[0079] In various embodiments, relationships to other peaks investigated include the loss of an electron (charge state elevation) – for radical-driven dissociation and the loss or gain of hydrogen – for radical-driven dissociation.

Loss of an Electron

[0080] Figure 2 is an exemplary plot 200 of a product ion spectrum showing a doubly charged peak corresponding to a singly charged precursor ion peak, in accordance with various embodiments. In plot 200, doubly charged peak 210 is located at 308.3025 m/z and corresponds to singly charged precursor ion peak 220 located at 616.6050 m/z.

[0081] In various embodiments, the following formula is used to find a peak with y m/z value that is related to a peak of x m/z value and z charge state:

[0082] $y = (x*|z|-n* m_e)/(z+n)$

[0083] where y = putative m/z of the related peak assuming n electron loss(es) and m_e = electron mass, 0.00055 Da.

[0084] For example, in plot 200, the peak of x m/z value and z charge state is peak 220 with an x m/z value of 616.6050 m/z and z charge state of 1. The related peak y m/z value is then found by applying the above formula:

[0085] $y = (616.6050-1*0.00055)/(1+1)= 308.3025$.

[0086] Thus, peak 210, which is located at 308.3025 m/z, is likely a related peak with one lost electron, or a doubly charged version of peak 220.

Loss or Gain of Hydrogen

[0087] Figure 3 is an exemplary plot 300 of traces from spectra showing a gain in hydrogen, in accordance with various embodiments. The traces in plot 300 represent coeluting compounds (lighter and heavier) that differ by a double bond (2 hydrogen atoms). Intensities are shown in plot 300 relative to the lighter

compound, represented by peak 312. Trace 310 represents precursor ion intensities (TOF MS) of the pure lighter compound. Peak 311 at m/z 781 corresponds to the first isotope of the lighter compound shown by peak 312. Trace 320 corresponds to the precursor ion intensities (TOF MS) of a mixture of the compounds. Trace 330 represents product ion intensities (ExD (radical-driven) MS/MS) of the heavier compound.

[0088] Peak 311 of trace 310 has an m/z distance of 1.003 from peak 312 of trace 310. Peak 321 of trace 320 has an m/z distance of 1.009 from peak 312 of trace 310. The target m/z distance of a hydrogen difference is 1.0078, for example. In various embodiments, a tolerance of ~ 0.002 m/z is used to accommodate for measurement error.

[0089] Figure 4 is an exemplary plot 400 of traces from spectra showing a loss in hydrogen, in accordance with various embodiments. The traces in plot 400 also represent coeluting compounds (lighter and heavier) that differ by a double bond (2 hydrogen atoms). However, intensities are shown in plot 400 relative to the heavier compound, represented by peak 423. Trace 410 represents precursor ion intensities (TOF MS) of the pure lighter compound. Trace 420 corresponds to the precursor ion intensities (TOF MS) of a mixture of the compounds. Trace 430 trace represents product ion intensities (ExD (radical-driven) MS/MS) of the heavier compound. Peak 431 of trace 430 now shows a hydrogen radical loss relative to peak 423.

[0090] Peak 411 of trace 410 has an m/z distance of 1.012 from peak 432 of trace 430. Peak 431 of trace 430 has an m/z distance of 1.006 from peak 432 of trace 430.

[0091] Figures 3 and 4 show how measurable differences in delta m/z are used to distinguish between an isotope peak and a hydrogen loss in the case of mixed spectra.

[0092] Figure 5 is an exemplary diagram 500 of a product ion annotation, in accordance with various embodiments. As described above, a peak list is obtained from the MS/MS spectrum.

System for annotating product ions of a spectrum

[0093] Figure 6 is an exemplary schematic diagram 600 of a system for annotating product ions of a spectrum, in accordance with various embodiments. The system includes processor 640. Processor 640 represents just one of one or more processors that can be used in various embodiments. In other words, various embodiments are not limited to performing the steps described herein with a single processor. Processor 640 can be, but is not limited to, a controller, a computer, a microprocessor, the computer system of Figure 1, or any device capable of analyzing data. Processor 640 can also be any device capable of sending and receiving control signals and data.

[0094] In step (A), processor 640 receives a product ion mass spectrum 641. In step (B), processor 640 receives a parameter that indicates mass spectrum 641 was produced using a radical-induced dissociation method. In step (C), processor 640 performs at least one charge state mass-to-charge ratio (m/z) adjustment based on the parameter for at least one product ion peak of mass spectrum 641 and compares the adjustment to one or more other product ion peaks of mass spectrum 641. In step (D), processor 640 annotates the at least one product ion peak based on the comparison.

[0095] In various embodiments, the adjustment in step (C) includes multiplying the m/z of the least one product ion peak by an absolute charge, subtracting one or more masses of an electron from the product, and dividing the difference by a charge adjusted for the number subtracted electron masses. The charge is, for example, 2 or 3.

[0096] In various embodiments, the adjustment in step (C) includes adding or subtracting an m/z of a hydrogen atom.

[0097] In various embodiments, if the adjustment in step (C) is equal to an m/z of at least one other product ion peak of the one or more other product ion peaks within a first m/z tolerance value, annotating the at least one other product ion peak in the mass spectrum as having an electron loss.

[0098] In various embodiments, if a singly charged precursor ion was mass filtered to produce the spectrum, before step (C), processor 640 further annotates each peak of the spectrum as a monoisotopic peak.

[0099] In various embodiments, after performing steps (C)-(D) on each product ion peak of the mass spectrum, processor 640 further analyzes each product ion peak of the mass spectrum for isotopic peaks and annotates each isotopic peak found as an isotopic peak.

[00100] In various embodiments, processor 640 further transforms annotated peaks into neutral mass peaks.

[00101] In various embodiments, processor 640 further produces a peak list that includes an electron loss annotation for one or more peaks of the mass spectrum.

[00102] In various embodiments, the radical-induced dissociation method includes electron-based dissociation (ExD).

[00103] In various embodiments, the radical-induced dissociation method includes ultraviolet photodissociation (UVPD) or infrared photodissociation (IRMPD).

[00104] In various embodiments, the radical-induced dissociation method includes a plasma EDD method.

[00105] In various embodiments, the radical-induced dissociation method includes a beam-type negative electron-transfer dissociation (ETD) method.

[00106] In various embodiments, the radical-induced dissociation method includes any UVPD, EPD, ECD, ETD, EDD, pEDD, or EED method.

[00107] In various embodiments, the system of Figure 6 further includes mass spectrometer 630. Ion source device 632 of mass spectrometer 630 ionizes a compound 610, producing an ion beam. Ion source device 632 is controlled by processor 640, for example. Ion source device 632 is shown as a component of mass spectrometer 630. In various alternative embodiments, ion source device 632 is a separate device. Ion source device 632 can be, but is not limited to, an electrospray ion source (ESI) device or a chemical ionization (CI) source device such as an atmospheric pressure chemical ionization source (APCI) device or an atmospheric pressure photoionization (APPI) source device.

[00108] Mass spectrometer 630 selects and fragments compound 610 and mass analyzes product ions of nucleic acid 610 from the ion beam. Mass spectrometer 630 further includes CID device 636, radical-induced dissociation device 635, and mass analyzer 637. Mass spectrometer 630 produces first spectrum 641 using radical-induced dissociation device 635, for example.

[00109] In Figure 6, mass analyzer 637 is shown as a time-of-flight (TOF) device. One of ordinary skill in the art can appreciate that mass analyzer 637 can be any type of

mass analyzer including, but not limited to, a quadrupole, an ion trap, an orbitrap, or Fourier transform ion cyclotron resonance (FT-ICR) device.

[00110] In various embodiments, the system of Figure 6 further includes a separation device 620 that separates compound 610 from a sample. As shown in Figure 6, additional device 620 is an LC device. In various alternative embodiments, additional device 620 can be, but is not limited to, a gas chromatography (GC) device, capillary electrophoresis (CE) device, or an ion mobility spectrometry (IMS) device.

Method for annotating product ions of a spectrum

[00111] Figure 7 is an exemplary flowchart showing a method 700 for annotating product ions of a spectrum, in accordance with various embodiments.

[00112] In step 710 of method 700, a product ion mass spectrum is received.

[00113] In step 720, a parameter that indicates the mass spectrum was produced using a radical-induced dissociation method is received.

[00114] In step 730, at least one charge state mass-to-charge ratio (m/z) adjustment based on the parameter is performed for at least one product ion peak of the mass spectrum and the adjustment is compared to one or more other product ion peaks of the mass spectrum.

[00115] In step 740, the at least one product ion peak is annotated based on the comparison.

Computer program product for annotating product ions of a spectrum

[00116] In various embodiments, a computer program product includes a non-transitory tangible computer-readable storage medium whose contents include a program

with instructions being executed on a processor so as to perform a method for annotating product ions of a spectrum. This method is performed by a system that includes one or more distinct software modules.

[00117] Figure 8 is an exemplary schematic diagram of a system 800 that includes one or more distinct software modules and that performs a method for annotating product ions of a spectrum, in accordance with various embodiments. System 800 includes input module 810 and analysis module 820.

[00118] In step (A), input module 810 receives a product ion mass spectrum.

[00119] In step (B), input module 810 receives a parameter that indicates the spectrum was produced using a radical-induced dissociation method.

[00120] In step (C), analysis module 820 performs at least one charge state mass-to-charge ratio (m/z) adjustment based on the parameter for at least one product ion peak of the mass spectrum and compares the adjustment to one or more other product ion peaks of the mass spectrum.

[00121] In step (D), analysis module 820 annotates the at least one product ion peak based on the comparison.

[00122] While the present teachings are described in conjunction with various embodiments, it is not intended that the present teachings be limited to such embodiments. On the contrary, the present teachings encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

[00123] Further, in describing various embodiments, the specification may have presented a method and/or process as a particular sequence of steps. However, to the extent that the method or process does not rely on the particular order of steps set forth herein, the method or process should not be limited to the particular sequence of

steps described. As one of ordinary skill in the art would appreciate, other sequences of steps may be possible. Therefore, the particular order of the steps set forth in the specification should not be construed as limitations on the claims. In addition, the claims directed to the method and/or process should not be limited to the performance of their steps in the order written, and one skilled in the art can readily appreciate that the sequences may be varied and still remain within the spirit and scope of the various embodiments.

WHAT IS CLAIMED IS:

1. A method for annotating product ions of a spectrum, comprising:

(a) receiving a product ion mass spectrum;

(b) receiving a parameter that indicates the mass spectrum was produced using a radical-induced dissociation method;

(c) performing at least one charge state mass-to-charge ratio (m/z) adjustment based on the parameter for at least one product ion peak of the mass spectrum and comparing the adjustment to one or more other product ion peaks of the mass spectrum; and

(d) annotating the at least one product ion peak based on the comparison.

2. The method of claim 1, wherein the adjustment comprises multiplying the m/z of the least one product ion peak by an absolute charge, subtracting one or more masses of an electron from the product, and dividing the difference by a charge adjusted for the number subtracted electron masses.

3. The method of claim 2, wherein the charge comprises 2 or 3.

4. The method of claim 1, wherein the adjustment comprises adding or subtracting an m/z of a hydrogen atom.

5. The method of any one of claims 1 to 4, further comprising, if the adjustment is equal to an m/z of at least one other product ion peak of the one or more other product ion peaks within a first m/z tolerance value, annotating the at least one other product ion peak in the mass spectrum as having an electron loss.

6. The method of any one of claims 1 to 4, further comprising, if a singly charged precursor ion was mass filtered to produce the spectrum, before step (c), annotating each peak of the spectrum as a monoisotopic peak.

7. The method of any one of claims 1 to 4, further comprising, performing steps (c)-(d) on each product ion peak of the spectrum.

8. The method of any one of claims 1 to 4, further comprising after performing steps (c)-(d) on each product ion peak of the mass spectrum, analyzing each product ion peak of the mass spectrum for isotopic peaks and annotating each isotopic peak found as an isotopic peak.

9. The method of any one of claims 1 to 4, further comprising transforming annotated peaks into neutral mass peaks.

10. The method of any one of claims 1 to 4, further comprising receiving a parameter that indicates an energy value that was used by the radical-induced dissociation method to produce the spectrum.

11. The method of any one of claims 1 to 4, further comprising producing a peak list that includes an electron loss annotation for one or more peaks of the mass spectrum.

12. The method of any one of claims 1 to 4, wherein the radical-induced dissociation method comprises electron-based dissociation (ExD).

13. The method of any one of claims 1 to 4, wherein the radical-induced dissociation method comprises ultraviolet photodissociation (UVPD) or infrared photodissociation (IRMPD).

14. A computer program product, comprising a non-transitory tangible computer-readable storage medium whose contents cause a processor to perform a method for annotating product ions of a spectrum, the method comprising:

providing a system, wherein the system comprises one or more distinct software modules, and wherein the distinct software modules comprise an input module and an analysis module;

(a) receiving a product ion mass spectrum using the input module;

(b) receiving a parameter that indicates the spectrum was produced using a radical-induced dissociation method using the input module;

(c) performing at least one charge state mass-to-charge ratio (m/z) adjustment based on the parameter for at least one product ion peak of the mass spectrum and comparing the adjustment to one or more other product ion peaks of the mass spectrum using the analysis module; and

(d) annotating the at least one product ion peak based on the comparison using the analysis module.

15. A system for annotating product ions of a spectrum, comprising:

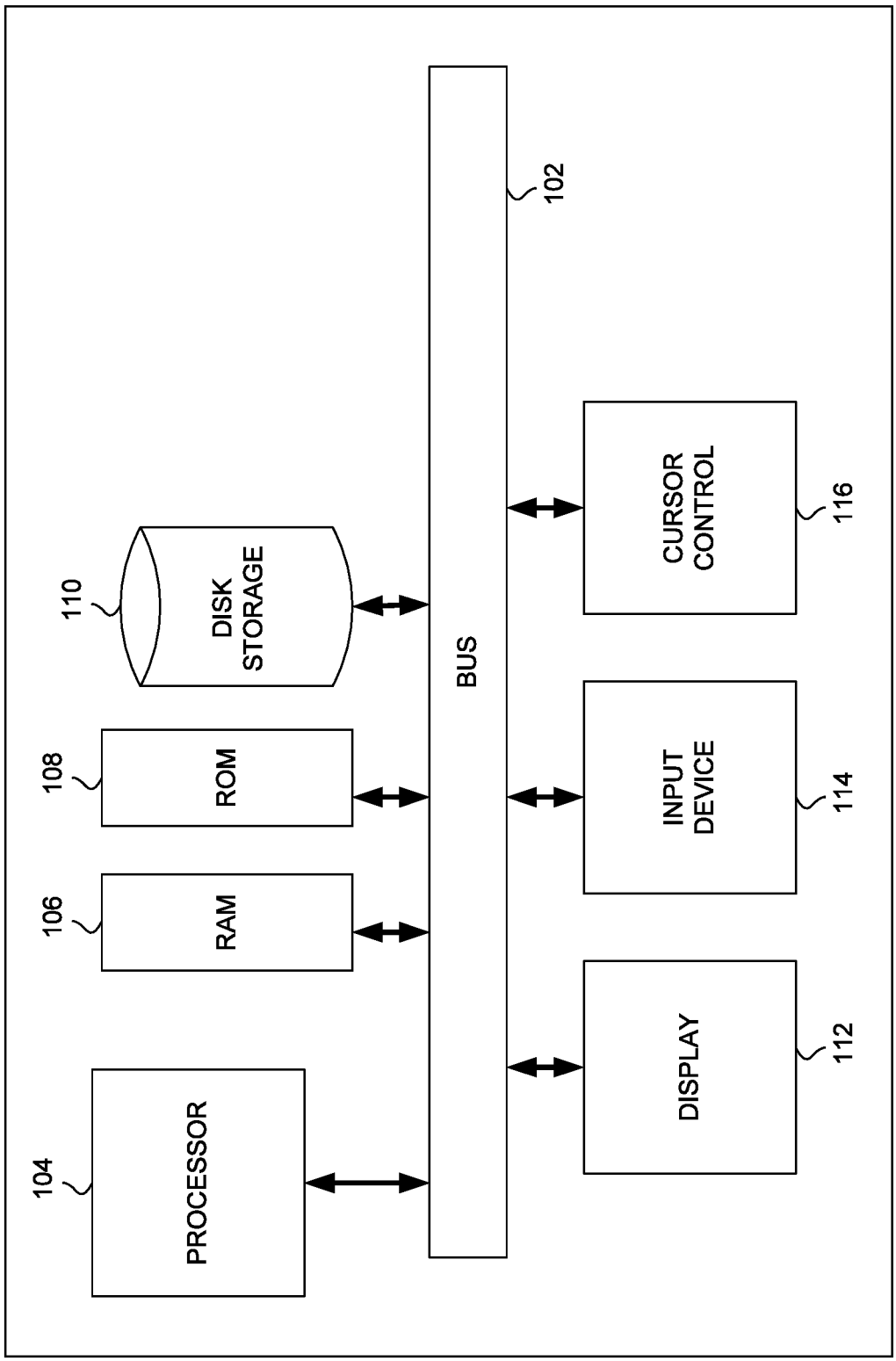
one or more processors that

(a) receive a product ion mass spectrum,

(b) receive a parameter that indicates the spectrum was produced using a radical-induced dissociation method,

(c) perform at least one charge state mass-to-charge ratio (m/z) adjustment based on the parameter for at least one product ion peak of the mass spectrum and comparing the adjustment to one or more other product ion peaks of the mass spectrum, and

(d) annotating the at least one product ion peak based on the comparison.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DISK STORAGE
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BUS
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DISPLAY
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INPUT DEVICE
114
CURSOR CONTROL
116
100

FIG. 1

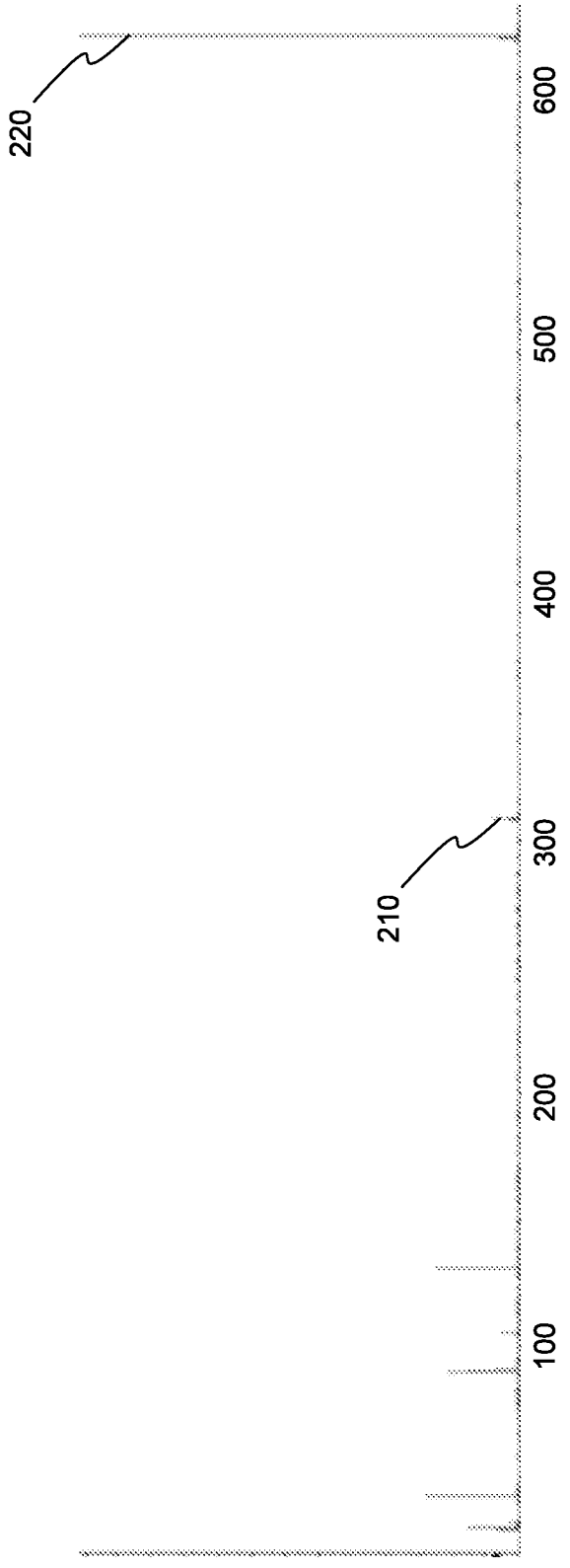
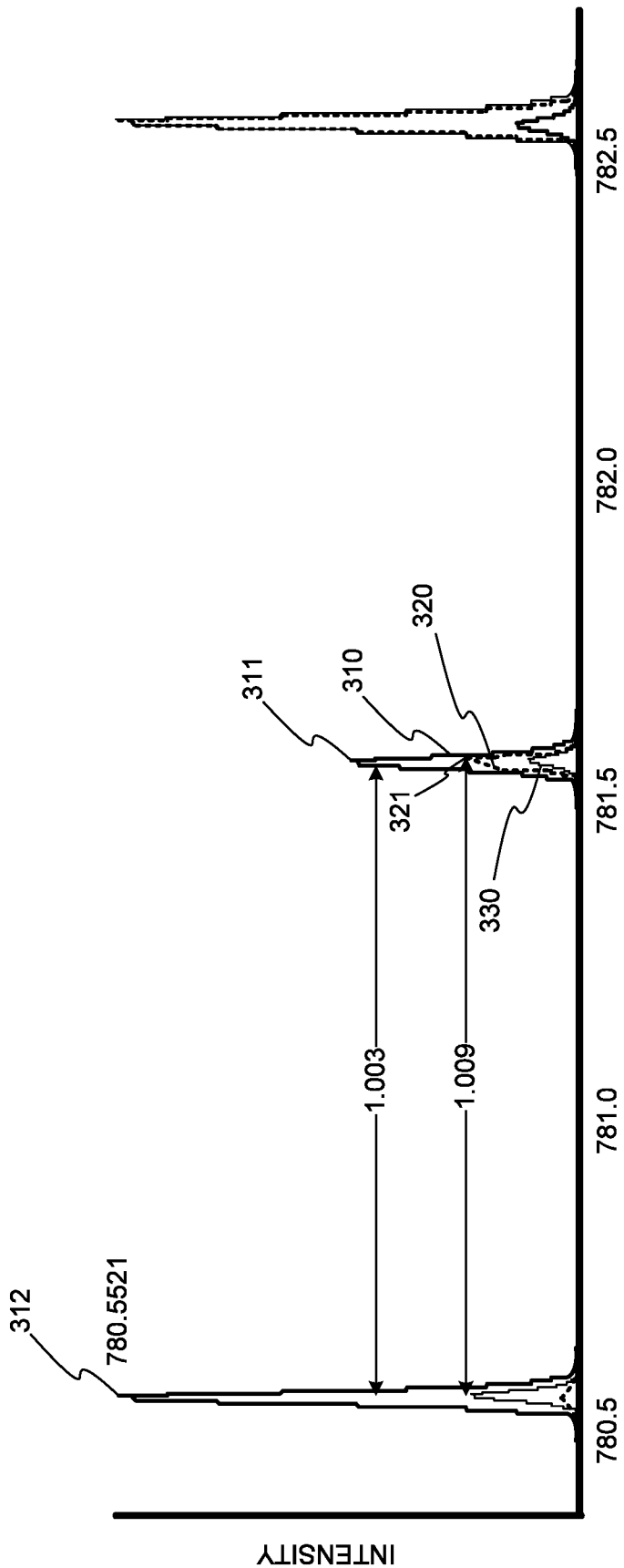


FIG. 2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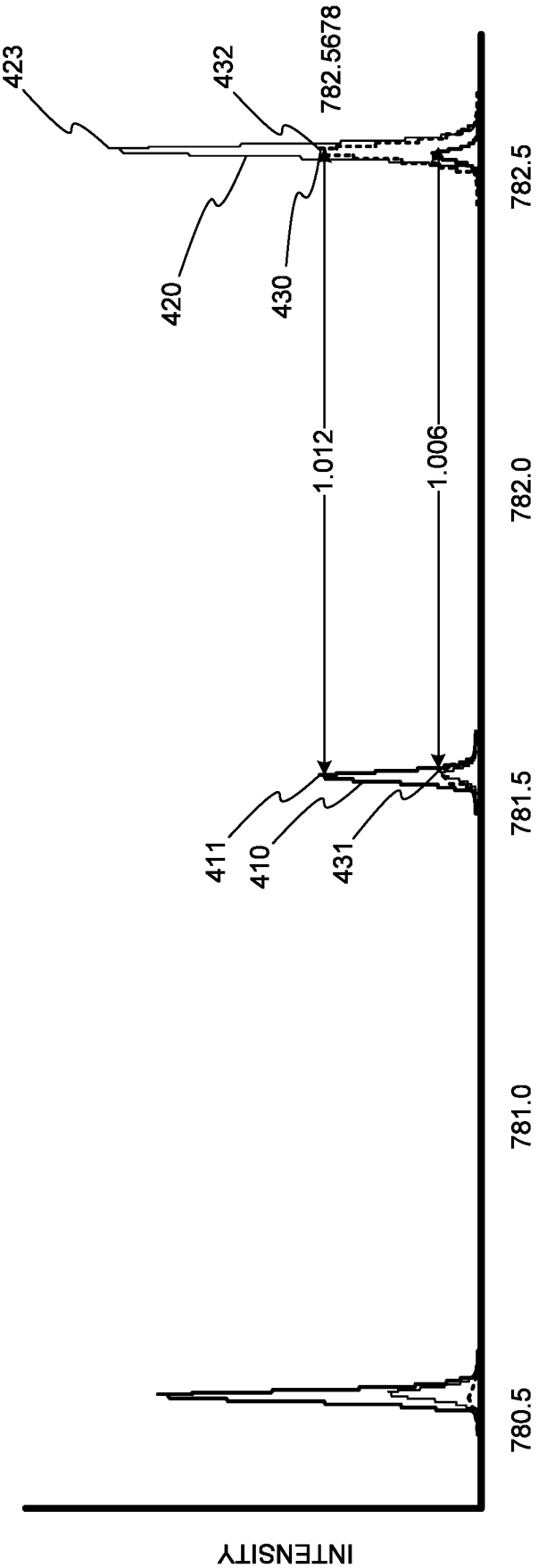
310
320
300

FIG. 3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INTENSITY
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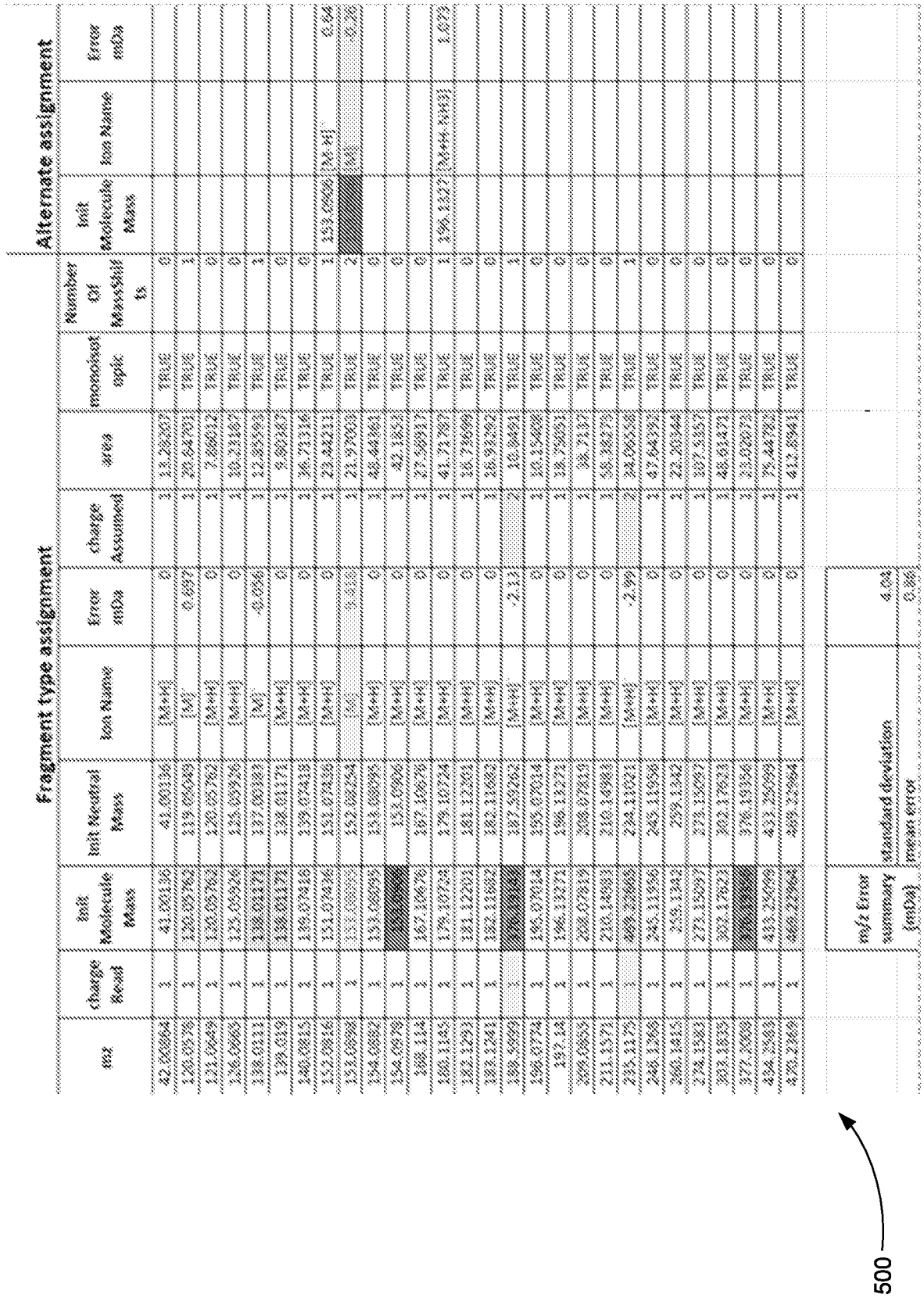
FIG. 4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Fragment type assignment
Alternate assignment
m/z
charge Read
Init Molecule Mass
Init Neutral Mass
Ion Name
Error mDa
Charge Assumed
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monoisot opic
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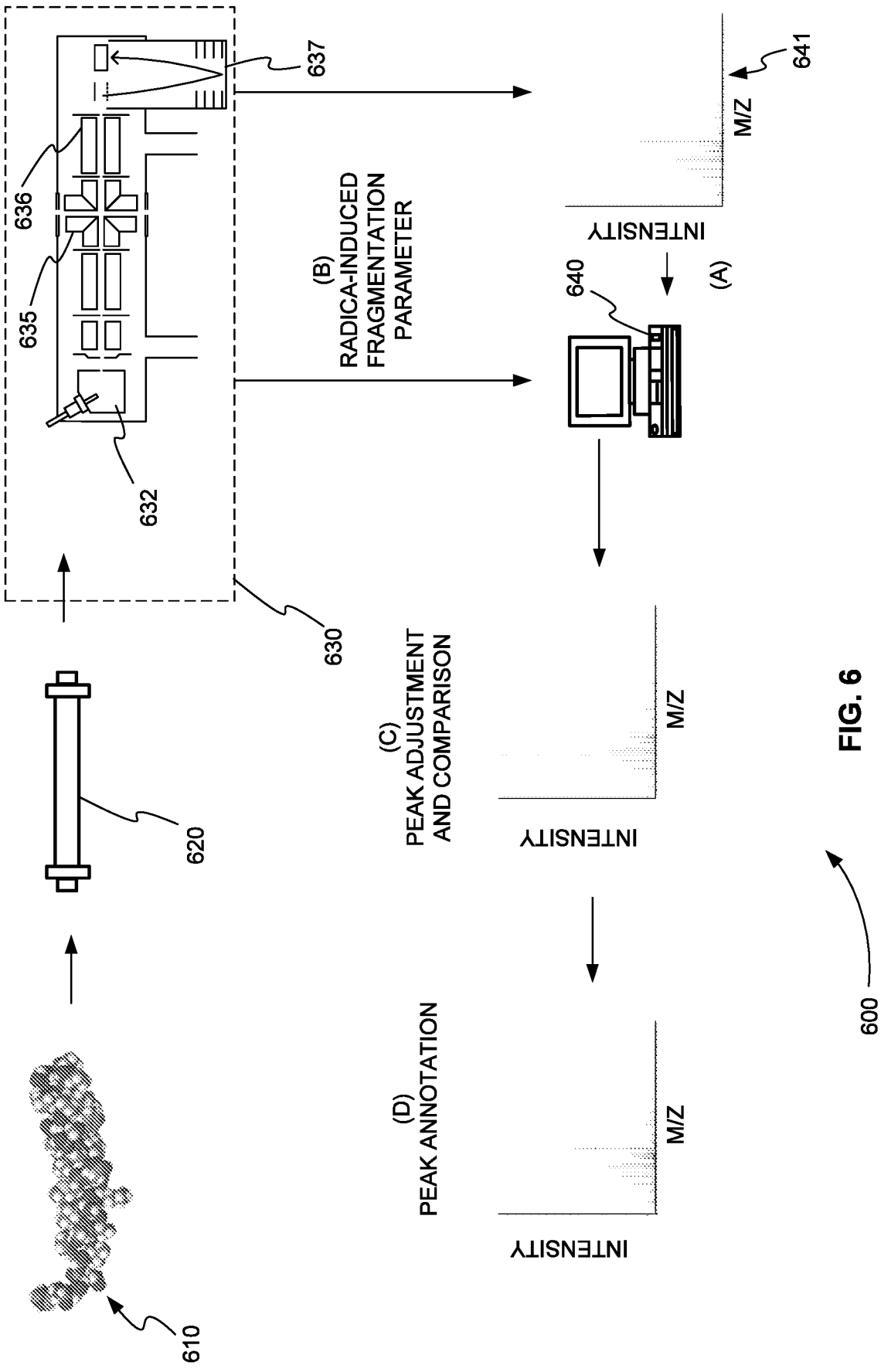
FIG. 5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620
630
632
635
636
637
(B)
RADICA-INDUCED
FRAGMENTATION
PARAMETER
640
(A)
641
INTENSITY
M/Z
(C)
PEAK ADJUSTMENT
AND COMPARISON
INTENSITY
M/Z
(D)
PEAK ANNOTATION
INTENSITY
M/Z
600

FIG. 6

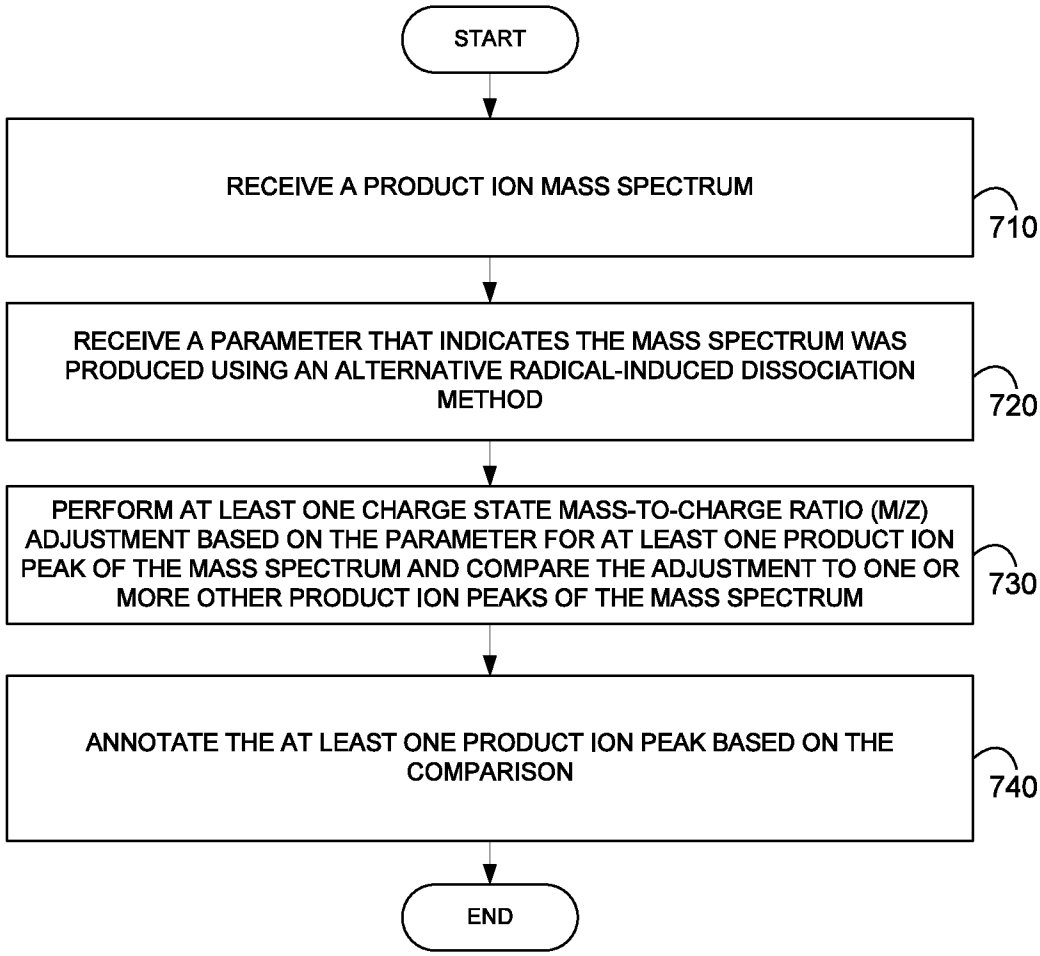
START
RECEIVE A PRODUCT ION MASS SPECTRUM
710
RECEIVE A PARAMETER THAT INDICATES THE MASS SPECTRUM WAS PRODUCED USING AN ALTERNATIVE RADICAL-INDUCED DISSOCIATION METHOD
720
PERFORM AT LEAST ONE CHARGE STATE MASS-TO-CHARGE RATIO (M/Z) ADJUSTMENT BASED ON THE PARAMETER FOR AT LEAST ONE PRODUCT ION PEAK OF THE MASS SPECTRUM AND COMPARE THE ADJUSTMENT TO ONE OR MORE OTHER PRODUCT ION PEAKS OF THE MASS SPECTRUM
730
ANNOTATE THE AT LEAST ONE PRODUCT ION PEAK BASED ON THE COMPARISON
740
END
700

FIG. 7

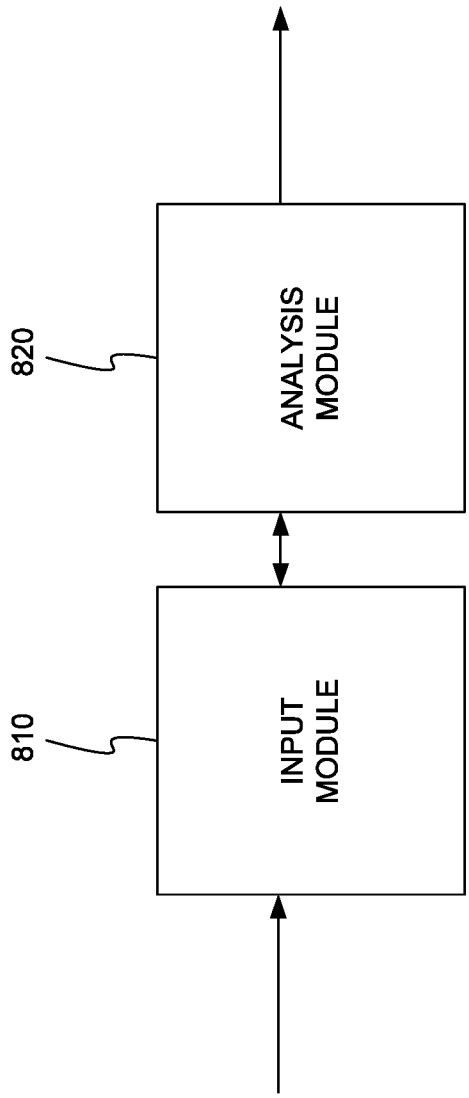

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/055837

A. CLASSIFICATION OF SUBJECT MATTER
INV. H01J49/00
ADD.

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)
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 practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NARDIELLO DONATELLA ET AL: "Strategies in protein sequencing and characterization: Multi-enzyme digestion coupled with alternate CID/ETD tandem mass spectrometry", ANALYTICA CHIMICA ACTA, ELSEVIER, AMSTERDAM, NL, vol. 854, 4 November 2014 (2014-11-04), pages 106-117, XP029107038, ISSN: 0003-2670, DOI: 10.1016/J.ACA.2014.10.053 figure 2 section "2.4 Data analysis and database search" ----- -/--	1-15

[x] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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
20 September 2024	10/10/2024

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, Fax: (+31-70) 340-3016	Cornelussen, Ronald

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/055837

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MATTHIAS. MANN ET AL: "Interpreting mass spectra of multiply charged ions", ANALYTICAL CHEMISTRY, vol. 61, no. 15, 1 August 1989 (1989-08-01), pages 1702-1708, XP055032591, ISSN: 0003-2700, DOI: 10.1021/ac00190a023 section "II. Averaging Algorithm"	1-15
Y	Anonymous: "Interpreting Electrospray Mass Spectra", , 18 July 2005 (2005-07-18), pages 1-2, XP093206292, Retrieved from the Internet: URL:https://www.ionsource.com/tutorial/spectut/spec5.htm the whole document	1-15

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