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(57) **Abrégé/Abstract:**

A method of mass spectrometry comprising analysing ions with a mass analyser; searching mass data or a mass spectrum for ions having a decimal mass or mass to charge ratio component that falls within a decimal mass or mass to charge ratio window; selecting ions of potential interest for further analysis by virtue of the Fact that the decimal mass or mass to charge ratio components of the ions falls within the decimal mass or mass to charge ratio window, wherein a profile of the decimal mass or mass to charge ratio window varies as a function of ΔM , wherein ΔM is the difference in the accurate mass or mass to charge ratio between that of a first substance or ion and a second substance or ion; and providing an output based at least in part on said searching.



Abstract

A method of mass spectrometry comprising analysing ions with a mass analyser; searching mass data or a mass spectrum for ions having a decimal mass or mass to charge ratio component that falls within a decimal mass or mass to charge ratio window; selecting ions of potential interest for further analysis by virtue of the fact that the decimal mass or mass to charge ratio components of the ions falls within the decimal mass or mass to charge ratio window, wherein a profile of the decimal mass or mass to charge ratio window varies as a function of ΔM , wherein ΔM is the difference in the accurate mass or mass to charge ratio between that of a first substance or ion and a second substance or ion; and providing an output based at least in part on said searching.

MASS SPECTROMETER

The present invention relates to a mass spectrometer and
5 a method of mass spectrometry.

In drug metabolism studies metabolites of interest
cannot usually be predicted. This is because the formation of
metabolites may be determined by novel enzymatic reactions and
by factors which are difficult to predict in advance such as
10 bio-availability.

At present in order to detect and identify metabolites
it is known to separate out the many different components
present in a complex biological matrix using liquid
chromatography (LC or HPLC). The mass or mass to charge ratio
15 of the components eluting from the liquid chromatograph is
then measured using mass spectrometry (MS).

It is usually necessary to make many measurements using
LC-MS (wherein parent ions eluting from a liquid chromatograph
are mass analysed) and LC-MS-MS (wherein specific parent ions
20 eluting from a liquid chromatograph are fragmented and the
fragment products are mass analysed) often in both positive
and negative ionisation modes. The exact accurate mass or
mass to charge ratio of the components eluting from the liquid
chromatograph is normally determined since this enables many
25 of the large number of endogenous peaks present in different
biological matrices such as bile, plasma, faeces and urine to
be discounted.

Ions which are determined as having a mass to charge
ratio which indicates that they may relate to a metabolite of
30 interest are then fragmented in a collision cell. The
resulting fragment products are then mass analysed enabling
the structure of each possible metabolite to be predicted.

The conventional approach is, however, relatively time
consuming since it is necessary to interrogate all of the mass
35 spectral data to look for potential metabolites of interest.
It is then necessary to arrange for all ions which are
considered likely to relate to metabolites of interest then to
be separately fragmented so that the structure of potential
metabolites of interest can then be determined.

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It will be appreciated that the process of searching mass spectra relating to a complex mixture, identifying potential ions which may relate to metabolites of interest, selecting certain ions to be fragmented, fragmenting the ions of interest and then mass analysing the fragment products can be relatively time consuming.

Within the pharmaceutical and biotechnology industries it is particularly important to be able to analyse samples quickly and accurately. This has led to automated methods wherein the major peaks present in a mass spectrum are automatically selected for analysis by MS/MS (wherein specific parent ions are selected for fragmentation). This allows the user to acquire parent ion mass spectra and several MS/MS spectra from a single HPLC injection. It is known for to automatically select most intense peaks (i.e. ions) in a parent ion mass spectrum for subsequent analysis by MS/MS. Some conventional systems allow a few filters to be defined to make this process slightly more efficient. For example, ions having certain masses or mass to charge ratios may be entered into a data system so that they are automatically excluded from consideration. These masses or mass to charge ratios may, for example, correspond to the masses or mass to charge ratios of solvent peaks which are known to be present, or the masses or mass to charge ratios of components which have already been analysed.

An advantage of the conventional automated mode of data acquisition is that a fair degree of data may be acquired from a single HPLC injection. However, a disadvantage of the conventional approach is that only those peaks which have an intensity which exceeds a pre-defined intensity threshold are normally selected for subsequent MS/MS analysis (i.e. fragmentation analysis). Importantly, if a large number of intense peaks are present or observed at any one particular time, then some of these peaks may simply fail to be selected for MS/MS analysis due to there being insufficient time to record all the separate MS/MS spectra within the relatively short duration of an observed chromatography peak.

Another particular problem with the conventional approach is that since the mass or mass to charge ratios of

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potential metabolites is not generally known in advance, then time can be wasted analysing a large number of peaks all or many of which subsequently turn out to be of little or no interest. This can also mean that actual peaks of potential interest which could have been analysed if only they had been recognised fail to be analysed at all because the mass spectrometer is busy analysing other ions.

It is therefore desired to provide an improved method of mass spectrometry and in particular to improve upon the current approach of searching for metabolites of interest.

Schmidt F et al: "Iterative data analysis is the key for exhaustive analysis of peptide mass fingerprints from proteins separated by two-dimensional electrophoresis", Journal of the American Society for Mass Spectrometry, Elsevier Science Inc., New York, NY, US, vol. 14, no. 9, September 2003 (2003-09), pages 943-956, XP004453138, ISSN: 1044-0305, discloses a

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method of identifying proteins using the knowledge that the first decimal place of a peptide mass is related to the first few digits of the overall peptide mass.

Karty, J.A., Ireland, M.M.E., Brun, Y.V., Reilly, J.P.: "Artifacts and unassigned masses encountered in peptide mass mapping", Journal of Chromatography B, vol. 782, 2002, pages 363-383, XP002399179, discloses a method a discarding non-peptides if their decimal masses fall outside of a predetermined band of decimal masses.

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According to a first embodiment of the present invention there is provided a method of mass spectrometry comprising:

analysing ions with a mass analyser;

searching mass data or a mass spectrum for ions having a decimal mass or mass to charge ratio component that falls within a decimal mass or mass to charge ratio window;

selecting ions of potential interest for further analysis by virtue of the fact that the decimal mass or mass to charge ratio component of the ions falls within the decimal mass or mass to charge ratio window, wherein a profile of the decimal mass or mass to charge ratio window varies as a function of ΔM , wherein ΔM is the difference in an accurate mass or mass to charge ratio between that of a first substance or ion and a second substance or ion; and

providing an output based at least in part on said searching.

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In an aspect of the first embodiment of the invention the first substance or ion comprises or relates to a pharmaceutical compound, drug or active component.

In an aspect of the first embodiment of the invention the second substance or ion comprises or relates to a metabolite or derivative of the first substance or ion.

In an aspect of the first embodiment of the invention selecting ions of potential interest comprises filtering out, attenuating, removing, or reducing the significance of ions having a decimal mass or mass to charge ratio component which falls outside of the decimal mass or mass to charge ratio window.

In an aspect of the first embodiment of the invention the profile of the decimal mass or mass to charge ratio window varies as a function of ΔM in a symmetrical or asymmetrical manner.

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According to a second embodiment of the invention there is provided a method of mass spectrometry comprising:

analysing ions with a mass analyser;

searching mass data or a mass spectrum for ions having a decimal mass or mass to charge ratio component that falls within a decimal mass or mass to charge ratio window;

filtering out, removing, attenuating, or reducing the significance of ions having a decimal mass or mass to charge ratio component which falls outside of the decimal mass or mass to charge ratio window; wherein a profile of the decimal mass or mass to charge ration window varies as a function of ΔM , wherein ΔM is the difference in the accurate mass or mass to charge ratio between that of a first substance or ion and a second substance or ion; and

providing an output based at least in part on said searching.

An aspect of the second embodiment of the invention further comprises fragmenting ions, resulting in fragment product ions having a decimal mass or mass to charge ratio component falling within the decimal mass or mass to charge ratio window.

An aspect of the second embodiment of the invention further comprises mass analyzing the fragment product ions to obtain a mass spectrum of the fragment product ions.

In an aspect of the second embodiment of the invention the first substance or ion comprises or relates to a pharmaceutical compound, drug or active component.

In an aspect of the second embodiment of the invention the second substance or ion comprises or relates to a metabolite or derivative of the first substance or ion.

In an aspect of the second embodiment of the invention the profile of the decimal mass or mass to charge ratio window varies as a function of ΔM in a symmetrical or asymmetrical manner.

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According to a third embodiment of the invention there is provided a method of mass spectrometry comprising:

analysing ions with a mass analyser;

obtaining a parent ion mass spectrum of a sample containing one or more metabolites of interest;

searching for ions of potential interest by searching the parent ion mass spectrum for mass peaks which meet a criteria that a decimal mass or mass to charge ratio component of a corresponding ion is within a decimal mass or mass to charge ratio window about a decimal mass or mass to charge ratio component of the parent ion;

selecting ions of potential interest for further analysis by virtue of the fact that the respective decimal mass or mass to charge ratio components of the ions falls within the decimal mass or mass to charge ratio window, wherein a size of the decimal mass or mass to charge ratio window varies as a function of a difference in an accurate

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mass or mass to charge ratio between that of the parent ion and ions being searched for; and

providing an output based at least in part on said searching.

An aspect of the third embodiment of the invention further comprises selecting the ions of potential interest for further analysis comprises fragmenting the ions of potential interest to form fragment product ions.

An aspect of the third embodiment of the invention further comprises mass analyzing the fragment product ions to obtain a mass spectrum of the fragment product ions.

An aspect of the third embodiment of the invention further comprises selecting ions of potential interest comprises filtering out, attenuating, removing, or reducing the significance of ions having a decimal part of an accurate mass or mass to charge ratio which falls outside of the decimal mass or mass to charge ratio window.

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In an aspect of the third embodiment of the invention the ions of potential interest comprise one or more metabolites of the parent ion.

In an aspect of the third embodiment of the invention the profile of the decimal mass or mass to charge ratio window varies in a symmetrical or asymmetrical manner.

Potentially only drug related metabolite peaks are selected for subsequent analysis by MS/MS and all or at least a majority of the endogenous peaks are effectively ignored from further consideration. The preferred embodiments therefore aid in the process of searching for and mass analysing ions relating to metabolites of interest. The preferred embodiment also enables metabolites of interest to be selected for further analysis by, for example, fragmenting them within the inherent short timescales of liquid chromatography.

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The preferred embodiment, in effect, filters out or substantially removes from consideration a number of possible precursor ions for subsequent analysis by MS/MS in drug

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metabolism studies by selecting only those ions which have a mass or mass to charge ratio wherein the decimal part of the mass or mass to charge ratio falls within a pre-defined and preferably relatively narrow decimal mass or mass to charge ratio window.

Various embodiments of the present invention will now be described, by way of example only, and with reference to the accompanying drawings in which:

Fig. 1 shows the structure and exact mass of a parent drug called Midazolam and the structure and exact mass of a hydroxylated metabolite of Midazolam;

Fig. 2 indicates the upper and lower limits of a decimal mass or mass to charge ratio window according to the preferred embodiment which is applied to the decimal mass or mass to charge ratio value of ions when searching mass spectral data or a mass spectrum for metabolites of a parent drug;

Fig. 3 shows a parent ion mass spectrum of Midazolam;

Fig. 4 shows a parent ion mass spectrum of a hydroxylated metabolite of Midazolam; and

Fig. 5A shows the structure and exact masses of Ketotifen and Verapamil and the structure and exact masses of a metabolite of Ketotifen and Verapamil, and Fig. 5B shows the structure and exact mass of Indinavir and the structure and exact mass of a metabolite of Indinavir.

In metabolism studies the elemental composition of a parent drug is usually generally well known and hence it is possible to calculate the theoretical exact mass or mass to charge ratio of the parent drug. An example of a pharmaceutical drug and a related metabolite which may be recognised (and hence selected for further analysis) according to the preferred embodiment is shown in Fig. 1. Fig. 1 shows the elemental composition of a parent drug called Midazolam ($C_{18}H_{13}ClF_3N_3$) which has a monoisotopic protonated mass of 326.0860 Da. A common metabolic route for the drug is the addition of oxygen. Accordingly, if an oxygen is added to Midazolam then the mass will be increased by +15.9949 Da so that the monoisotopic mass of the new compound (i.e. the hydroxylated metabolite of Midazolam) will be 342.0809 Da.

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The structure of the hydroxylated metabolite of Midazolam is also shown in Fig 1. It is to be noted that the difference in the decimal part of the accurate mass of the parent drug Midazolam and its hydroxylated metabolite is only
5 0.0860-0.0809 = 0.0051 Da (i.e. a mass deficiency of only 5.1 mDa). It is apparent therefore that there is only a very small difference in the decimal mass component of the parent drug and the corresponding metabolite even though the total or absolute mass of the parent and metabolite differ by nearly 16
10 Da.

In mass spectrometry an ion may be assigned either an integer nominal mass or mass to charge ratio (e.g. 326 in the case of Midazolam) or an accurate or exact mass or mass to charge ratio (e.g. 326.0860 in the case of Midazolam).
15 Accurate or exact masses or mass to charge ratios can be considered as comprising an integer component or value and a decimal component or value. This largely stems from the fact that all the elements (with the exception of Carbon) have approximately but not exactly integer masses. In the
20 international scale for atomic masses the most abundant isotope of carbon is assigned an exact atomic mass of 12.0000 Dalton (Da). On this scale, the accurate atomic masses of the most abundant isotopes of the most abundant elements in biological systems are Hydrogen (H) 1.0078 Da, Nitrogen (N)
25 14.0031 Da and Oxygen (O) 15.9949 Da.

Accurate or exact (i.e. non-integer) masses or mass to charge ratios can be represented as an integer nominal mass or mass to charge ratio value or component together with a corresponding mass sufficiency or deficiency value or
30 component. The mass sufficiency or deficiency may be considered to represent the deviation from an integer value and may be expressed in milli-dalton (mDa). For example, Hydrogen (H) can be expressed as having an integer nominal mass of 1 and a mass sufficiency of 7.8 mDa, Nitrogen (N) can
35 be expressed as having an integer nominal mass of 14 and a mass sufficiency of 3.1 mDa and Oxygen (O) can be expressed as having an integer nominal mass of 16 and a mass deficiency of 5.1 mDa.

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In a similar manner, the mass or mass to charge ratio of an ion of an organic molecule can be assigned an integer nominal mass or mass to charge ratio together with a corresponding mass sufficiency or deficiency from that integer value.

When considering the mass or mass to charge ratio of ions or compounds according to the preferred embodiment, the method of ionisation is also preferably taken into consideration as this allows the ionic elemental composition to be determined and hence also the ionic mass or mass to charge ratio to be calculated. For example, if a solution is ionised by Electrospray ionisation then the analyte molecules may be protonated to form positively charged ions.

From knowledge of the theoretical accurate mass or mass to charge ratio of these ions it is possible, according to the preferred embodiment, to make certain predictions concerning the accurate mass or mass to charge ratio of possible or potential metabolites of interest. This in turn allows a better prediction of peaks that are likely to be metabolites of interest and thus potential metabolites can be searched for, recognised and then passed or selected for further analysis such as structural analysis by MS/MS.

Metabolites are the result of bio-transformations to a parent drug. An aspect of the preferred embodiment is the recognition and exploitation of the fact that the mass sufficiency or mass deficiency of a potential metabolite of interest will be substantially similar to the mass sufficiency or mass deficiency of the corresponding parent drug.

An aspect of the preferred embodiment is the recognition that the potential similarity between the mass sufficiency or mass deficiency of the parent ion and potential metabolites can be used to search more strategically for potential metabolites of interest. In particular, the preferred embodiment searches for metabolites on the basis that the decimal part of the accurate or exact mass or mass to charge ratio of a parent drug will be substantially similar to the decimal part of the accurate or exact mass or mass to charge ratio of a metabolite of the parent drug.

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According to the preferred embodiment the decimal part of the accurate mass or mass to charge ratio of a precursor ion of a parent drug is calculated. A decimal mass or mass to charge ratio window is then preferably set about the precise decimal mass or mass to charge ratio of the parent drug. According to the preferred embodiment an upper limit and a lower limit to the decimal mass window may be set. However, according to other embodiments only an upper limit or only a lower limit to the decimal mass window may be set. According to an embodiment, the upper and lower limits may have the same magnitude or width, or alternatively the upper and lower limits may differ in magnitude or width.

According to a preferred embodiment a precursor or parent ion mass spectrum of a sample believed to contain one or more metabolites of interest is preferably obtained. The parent ion mass spectrum is then preferably automatically searched for some or all mass peaks which meet the criteria that the decimal part of the accurate mass or mass to charge ratio of an ion must be very close to the decimal mass part of the accurate mass or mass to charge ratio of the known parent compound or ion. According to the preferred embodiment ions of potential interest (which preferably relate to one or more metabolites of the parent compound) are recognised, identified or otherwise selected for further analysis by virtue of the fact that the decimal mass or mass to charge ratio of the ion is determined as falling within a relatively narrow band or range of masses or mass to charge ratios about the decimal mass or mass to charge ratio of the parent compound or ion.

The characteristics of the decimal mass or mass to charge ratio window which is preferably used in the process of searching for metabolites of interest will now be described in more detail with reference to Fig. 2.

Fig. 2 indicates the width of a decimal mass or mass to charge ratio window which may be used or applied according to the preferred embodiment. The width of the decimal mass or mass to charge ratio window (in mDa) is shown as a function of the difference in the absolute mass (in Da) or mass to charge ratio between that of the parent ion or compound and ions or compounds being searched for which may include metabolite ions

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or compounds. The difference in absolute mass or mass to charge ratio between the parent compound or ion and the ions or compounds being searched for, which may include metabolite ions or compounds of interest, may be referred to as ΔM .

5 Similarly, the upper and lower limits of the decimal mass or mass to charge ratio window may be referred to as having a value δm .

By way of example, if the absolute difference in mass or mass to charge ratio between the parent ion and a potential
10 ion of interest is 10 Da then according to the embodiment shown in Fig. 2 a decimal mass or mass to charge ratio window having an upper limit + 20 mDa greater than the precise decimal mass or mass to charge ratio of the parent ion and a lower limit 20 mDa below the precise decimal mass or mass to
15 charge ratio of the parent ion may be set.

According to the preferred embodiment, the upper and lower limits of the decimal mass or mass to charge ratio window vary as a function of the absolute difference ΔM in the mass or mass to charge ratio of the parent ion to that of a
20 possible metabolite ion. Therefore, as also shown in Fig. 2, if the absolute difference in mass or mass to charge ratio between the parent ion and a potential ion of interest is say 100 Da, then according to the embodiment shown and described with reference to Fig. 2 the upper and lower limits of the
25 decimal mass or mass to charge ratio window are asymmetric. According to the particular embodiment shown in Fig. 2 the mass or mass to charge ratio window has an upper limit + 92 mDa greater than the precise decimal mass or mass to charge ratio of the parent ion and a lower limit only 50 mDa lesser
30 than the precise decimal mass or mass to charge ratio of the parent ion.

In general terms and as shown in Fig. 2, when the difference ΔM in mass or mass to charge ratio between the parent ion or compound and the metabolite ion or compound of
35 interest is relatively small (e.g. $\pm 0-30$ Da) then the size of the upper and lower limits of the decimal mass or mass to charge ratio window according to the preferred embodiment may also be relatively small (e.g. in the region of 20-30 mDa). However, as the absolute difference ΔM in the mass or mass to

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charge ratio between the parent ion or compound and a possible metabolite ion or compound of interest increases, then so the size of the upper and lower limits of the decimal mass or mass to charge ratio window also preferably increases.

- 5 According to the embodiment shown in Fig. 2, when searching for metabolites of interest wherein the mass or mass to charge ratio difference ΔM (i.e. the mass or mass to charge ratio of the parent ion or compound minus the mass or mass to charge ratio of the metabolite ion or compound) is in the
- 10 range -40 to 20 Da, then the upper limit of the decimal mass or mass to charge ratio window is preferably set to a constant value of 20 mDa. If the mass or mass to charge ratio difference between the parent ion or compound and the metabolite ion or compound of interest is > 20 Da, then the
- 15 upper limit of the decimal mass or mass to charge ratio window preferably increases at a rate of +0.09% times ΔM above 20 Da (i.e. when ΔM is +100, then the upper limit of the decimal mass window or mass to charge ratio is preferably set at 20 mDa + 0.09%*(100 Da - 20 Da) = 20 mDa + 0.072 Da = 92 mDa).
- 20 If the mass or mass to charge ratio difference between the parent ion or compound and the metabolite ion or compound of interest is < -40 Da, then the upper limit of the decimal mass or mass to charge ratio window preferably increases at a lesser rate of 0.05% times ΔM below -40 Da (i.e. when ΔM is -
- 25 100, then the upper limit of the decimal mass or mass to charge ratio window is set at 20 mDa + 0.05%*(100 Da - 40 Da) = 20 mDa + 0.030 Da = 50 mDa).

- Similarly, when searching for metabolites of interest wherein the mass or mass to charge ratio difference ΔM between
- 30 the parent ion or compound and the metabolite ion or compound is in the range -20 to 40 Da, then the lower limit of the decimal mass or mass to charge ratio window is preferably set to a constant value of - 20 mDa. If the mass or mass to charge ratio difference between the parent ion or compound and the metabolite ion or compound of interest is > 40 Da, then
- 35 the lower limit of the decimal mass or mass to charge ratio window preferably increases negatively at a rate of -0.05% times ΔM above 40 Da (i.e. when ΔM is +100, then the lower limit of the decimal mass or mass to charge ratio window is

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preferably set at $-20 \text{ mDa} - 0.05\% \times (100 \text{ Da} - 40 \text{ Da}) = -20 \text{ mDa} - 0.030 \text{ Da} = -50 \text{ mDa}$). If the mass or mass to charge ratio difference between the parent ion or compound and the metabolite ion or compound of interest is $< -20 \text{ Da}$, then the lower limit of the decimal mass or mass to charge ratio window preferably increases negatively at a rate of -0.09% times ΔM below -20 Da (i.e. when ΔM is -100 , then the lower limit of the decimal mass or mass to charge ratio window is set at $-20 \text{ mDa} - 0.09\% \times (100 \text{ Da} - 20 \text{ Da}) = -20 \text{ mDa} - 0.072 \text{ Da} = -92 \text{ mDa}$).

10 It will be appreciated that each different parent drug will have a specific known mass or mass to charge ratio. The approach according to the preferred embodiment assumes that metabolites of the parent drug will have a similar structure to that of the parent drug and that the decimal part of the accurate mass or mass to charge ratio of each metabolite will be similar to the decimal part of the accurate mass or mass to charge ratio of the parent drug.

Ions which according to the preferred embodiment are determined as having an accurate mass or mass to charge ratio with a decimal part which falls within the decimal mass or mass to charge ratio window as determined by the preferred embodiment are then preferably selected for further analysis by, for example, MS/MS. For example, a mass filter such as a quadrupole mass filter may be used to select specific ions which are considered to be potentially metabolite ions of interest having a specific mass to charge ratio to be onwardly transmitted to a collision or fragmentation cell. The ions are then fragmented within the collision or fragmentation cell and the resulting fragment product ions are mass analysed.

30 The preferred embodiment enables a large number of endogenous ion peaks that would otherwise have been selected for analysis by MS/MS according to the conventional approach to be automatically eliminated from consideration. This is particularly advantageous and as a result the preferred embodiment relates to a significantly improved method of recognising potential metabolites.

The decimal mass or mass to charge ratio window within which the decimal part of the accurate mass or mass to charge ratio of a metabolite should fall may be defined prior to

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proceeding with LC-MS and/or LC-MS-MS experiments. The value or size of the decimal mass or mass to charge ratio window may be set to accommodate the mass errors likely to occur during an experimental run. The value or size may also be set according to the elemental composition of the parent drug. For example, if the parent drug does not contain elements other than carbon, hydrogen, nitrogen, oxygen and fluorine, then the upper and/or lower limits of the decimal mass or mass to charge ratio window may be set to a lower (smaller) value than if the parent drug contains any or all of the elements phosphorous, sulphur and chlorine. This is because phosphorous, sulphur and chlorine all have larger mass deficiencies than carbon, hydrogen, nitrogen, oxygen and fluorine.

The greater the mass or mass to charge ratio difference between that of the parent drug and that of the metabolite, then the more atoms which are likely to be involved in the bio-transformation. Accordingly, if several atoms are considered to be involved in the bio-transformation then greater allowance should preferably be made for the change in the decimal part of the accurate mass or mass to charge ratio. In other words, as the difference in the absolute mass or mass to charge ratio between that of parent drug and of the metabolite increases, then preferably the width or size of the decimal mass or mass to charge ratio window or the upper and/or lower limits of the decimal mass or mass to charge ratio window should also increase since the metabolite is likely to have a greater mass deficiency or sufficiency.

According to the preferred embodiment allowance may be made for the fact that the maximum change in mass sufficiency that may have occurred in the bio-transformation may be different to the maximum change in mass deficiency which may have occurred. Accordingly, an asymmetric decimal mass or mass to charge ratio window may be used similar, for example, to the asymmetric decimal mass or mass to charge ratio window shown and described in relation to the embodiment depicted in Fig. 2.

According to other less preferred embodiments a simple symmetrical decimal mass or mass to charge ratio window may be

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used. For example, for mass or mass to charge ratio differences ΔM between that of parent drug and ions of interest of up to ± 20 Da, a decimal mass or mass to charge ratio window having upper and lower limits of ± 20 mDa may be used. If the mass or mass to charge ratio difference between that of the parent drug and the ions of interest is < -20 Da or > 20 Da then the upper and lower limits of the decimal mass or mass to charge ratio window may increase at a rate of 0.1% for mass or mass to charge ratio differences < -20 Da or > 20 Da.

In the general case, the decimal mass or mass to charge ratio window may have multiple values of decimal mass or mass to charge ratio difference δm for a mass or mass to charge ratio difference ΔM between that of the parent drug ions of interest. The values of δm and ΔM may preferably be defined independently for each polarity of δm and ΔM .

According to the preferred embodiment, the mass spectrometer is preferably capable of recording parent ion mass spectra and fragment ion mass spectra from selected precursor or parent ions that are induced to fragment. The mass spectrometer may, for example, comprise a magnetic sector, a Time of Flight, an orthogonal Time of Flight, a quadrupole mass filter, a 3D quadrupole ion trap, a linear quadrupole ion trap or an FT-ICR mass analyser, or any combination thereof.

According to a particularly preferred embodiment, the mass spectrometer may comprise either a magnetic sector, a Time of Flight, an orthogonal Time of Flight or an FT-ICR mass analyser.

The mass spectrometer may according to an embodiment be arranged to default to the acquisition of full parent ion mass spectra unless and until a mass peak is detected wherein the decimal part of the accurate mass or mass to charge ratio of the detected ion falls within a preferably pre-defined decimal mass or mass to charge ratio window. Once such a mass peak is detected then the mass spectrometer and related control software may then preferably switch the instrument so that parent ions having a specific decimal mass or mass to charge ratio or interest are selected and transmitted by a mass

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filter whilst other ions having decimal masses or mass to charge ratios falling outside the decimal mass or mass to charge ratio window are preferably substantially attenuated or lost to the system. Selected parent ions of interest are then preferably passed to a fragmentation or collision cell which preferably comprises an ion guide and a collision gas maintained at a pressure preferably $> 10^{-3}$ mbar. The ions are preferably accelerated into the collision or fragmentation cell at energies such that upon colliding with the collision gas present in the collision or fragmentation cell, the ions are preferably caused to fragment into fragment product ions. The fragment product ions are then preferably mass analysed and a full mass spectrum of the fragment product ions is then preferably obtained.

Although the size of the decimal mass or mass to charge ratio window is preferably pre-defined, according to other less preferred embodiments the size of the decimal mass or mass to charge ratio window may be altered in response to experimental data or on the basis of another parameter. According to an embodiment, for example, a first experimental run may be performed wherein a decimal mass or mass to charge ratio window having a first profile or size as a function of ΔM may be applied and then in a second subsequent experimental run a decimal mass or mass to charge ratio window having a second different profile or size as a function of ΔM may be applied.

According to an embodiment control software may select or determine other parameters including the optimum fragmentation collision energy appropriate for a selected precursor or parent ion.

An important advantage of the preferred embodiment is that it enables more useful MS/MS spectra to be acquired within the limited timescale of a single LC-MS experiment. This reduces the time taken to get the required data. Another important advantage of the preferred embodiment is that the preferred method facilitates the detection of low level metabolites that might otherwise be missed, if the conventional approach were adopted, due to the presence of a large number of relatively intense endogenous mass peaks.

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With reference to the example of Midazolam, Fig. 3 shows a parent ion mass spectrum of the drug Midazolam as recorded using a hybrid quadrupole Time of Flight mass spectrometer. The measured mass to charge ratio for the major isotope was
5 determined as being 326.0872 (cf. a theoretical value of 326.0860). Fig. 4 shows a parent ion mass spectrum of the hydroxylated metabolite of Midazolam as recorded using the same hybrid quadrupole Time of Flight mass spectrometer. The
10 measured mass to charge ratio for the major isotope was determined as being 342.0822 (cf. a theoretical value of 342.0809). From the experimental data, the difference in the decimal part of the accurately determined mass to charge ratio of the parent drug and the decimal part of the accurately
15 determined mass to charge ratio of the hydroxylated metabolite was $0.0872 - 0.0822 = 0.0050$ Da i.e. a mass deficiency of only 5 mDa.

From the experimental data shown in Figs. 3 and 4 it will be appreciated that more generally, potential metabolites of Midazolam including the hydroxylated metabolite of
20 Midazolam could be searched for, located and then be selected for further consideration and analysis (preferably by MS-MS). This can be achieved by searching parent ion mass spectral data for mass peaks which may have potentially quite different absolute mass to charge ratios but wherein the difference in
25 the decimal mass or mass to charge ratio of the parent drug and the ion in question is, for example, less than 10 mDa.

The method according to the preferred embodiment provides an effective way of being able to detect efficiently mass peaks likely to be (or at least include) metabolites of
30 interest with no (or relatively few) ions relating to endogenous components also being analysed. The preferred method therefore advantageously effectively filters out or removes from further consideration numerous endogenous mass peaks which would otherwise have been included for
35 consideration according to the conventional techniques.

The preferred embodiment advantageously enables a mass spectrometer to switch to record the fragment ion spectrum of ions which are likely to relate to metabolites of interest within the time scales during which a typical liquid

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chromatography mass peak is observed without wasting time analysing a large number of ions which turn out not to be metabolites of interest.

5 According to an embodiment an intelligent exact mass deficiency algorithm may be used together with in silico metabolite prediction to predetermine DDA experiments for metabolism studies preferably using a hybrid quadrupole Time of Flight mass spectrometer.

10 One of the main problems when carrying out DDA (data dependant experiments) is that a considerable amount of time may be spent performing DDA experiments on ions that turn out not be of interest. As a result, important putative metabolites can easily be missed.

15 According to an embodiment specific metabolites may be predicted in advance by computer and an appropriate exact decimal mass or mass to charge ratio data filter window may be set. According to the embodiment the metabolites from a given new chemical entity or a standard compound are therefore predicted and then searched for. Once the metabolites have
20 been predicted, an exact decimal mass window may be set so as to only switch to perform a DDA experiment when ions having decimal masses or mass to charge ratios within the set decimal mass or mass to charge ratio window (which may, for example, have an upper and/or lower limit of 10-20 mDa) are observed as
25 being present.

According to an embodiment potentially unknown metabolites may be discovered. A user may, for example, select or set an exact decimal mass or mass to charge ratio window to detect metabolites already predicted on the basis of
30 their exact decimal mass or mass to charge ratio so that MS/MS experiments maybe carried out. In addition to this, an exact mass deficiency based upon the exact mass or mass to charge ratio of the parent compound can be determined. This particular data filter may be considered more specific than
35 the data filter according to the previously described embodiment since there may be cases where not all of the metabolites will be predicted. Therefore, metabolites which are not predicted will be detected in the DDA experiments with an exact mass or mass to charge ratio data filter.

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An exact mass or mass to charge ratio deficiency filter may operate in the following mode. An exact mass or mass to charge ratio deficiency filter based upon the decimal places of the mass or mass to charge ratio of the parent drug under analysis may be used. According to this embodiment a post processing filter may be used that allows the removal of unexpected metabolite entries in a MetaboLynx browser which do not agree with user-defined criteria. The use of this filter can dramatically reduce the number of false entries in an unexpected metabolite table by filtering out the vast majority of matrix-related entries which may share the same nominal mass as potential metabolites. This allows users to use low threshold values during data processing so that very low metabolite levels are identified without going through the tedious task of manually excluding false positives. The filter is preferably an accurate and specific filter since it is based on exact mass and mass deficiencies which are specific to each parent drug of interest.

Each parent drug is comprised of a specific number of elements (C, H, N, O etc.). Depending upon the number of each one of the elements mentioned, the decimal mass or mass to charge ratio of the drug will be very specific. For example, with reference to Fig. 5A, Verapamil contains the following elements: C₂₇ H₃₈ N₂ O₄. This equates to a monoisotopic protonated mass of 455.2910 Da. If an alkyl group is taken away (N-dealkylation, a common metabolic route) and a glucuronide is added, then the mass is shifted by precisely + 162.0164 Da. The metabolite therefore has a monoisotopic mass of 617.3074 Da. The decimal mass difference between Verapamil and its N-dealkylated metabolite corresponds with an exact mass deficiency of $0.3074 - 0.2910 = 0.0164$ Da (16.4 mDa). Therefore, if a decimal mass or mass to charge ratio window of around 20 mDa were used then it would be possible to detect its N-dealkylated glucuronidated metabolite. Prior knowledge of the metabolites of Verapamil may not be necessary if some or all of the following assumptions are made: (i) all metabolites will have decimal masses or mass to charge ratios within 250 mDa of the decimal mass or mass to charge ratio of the corresponding parent; (ii) the metabolites of interest

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will, in general, have a decimal mass or mass to charge ratio within 100 mDa of the parent if there are no major cleavages leading to much smaller fragments (e.g. the largest phase II biotransformation, glutathione conjugation, will lead to a mass defect difference of 68 mDa compared to the parent drug); and (iii) most metabolites will fall within a 180 mDa decimal mass or mass to charge ratio window of the parent compound even if certain cleavages take place in the structure to yield smaller fragments.

10 Figs. 5A and 5B show a metabolite of Ketotifen, Verapamil and Indinavir and include cleavages. The maximum decimal mass or mass to charge ratio deficiency is in the case of Indinavir (Fig. 5B) wherein the metabolite has a decimal mass or mass to charge ratio which is 167.7 mDa different from
15 the decimal mass or mass to charge ratio of the parent compound. Mass deficiency shifts are very specific for each metabolite and parent drug.

The various embodiments of the present invention may be implemented not only on hybrid quadrupole orthogonal Time of
20 Flight instruments as according to the preferred embodiment, but also using nominal mass instruments such as triple quadrupoles, linear and 3D ion traps and exact mass instruments such as MALDI/Quadrupole Time of Flight and FTMS.

CLAIMS:

1. A method of mass spectrometry comprising:
analysing ions with a mass analyser;
searching mass data or a mass spectrum for ions having a decimal mass or mass to charge ratio component that falls within a decimal mass or mass to charge ratio window;
selecting ions of potential interest for further analysis by virtue of the fact that the decimal mass or mass to charge ratio component of the ions falls within the decimal mass or mass to charge ratio window, wherein a profile of the decimal mass or mass to charge ratio window varies as a function of ΔM , wherein ΔM is the difference in an accurate mass or mass to charge ratio between that of a first substance or ion and a second substance or ion; and
providing an output based at least in part on said searching.
2. The method of claim 1, wherein the first substance or ion comprises or relates to a pharmaceutical compound, drug or active component.
3. The method of claim 1 or 2, wherein the second substance or ion comprises or relates to a metabolite or derivative of the first substance or ion.
4. The method of any one of claims 1-3, wherein selecting ions of potential interest comprises filtering out, attenuating, removing, or reducing the significance of ions having a decimal mass or mass to charge ratio component which falls outside of the decimal mass or mass to charge ratio window.
5. The method of any one of claims 1-4, wherein the profile of the decimal mass or mass to charge ratio window varies as a function of ΔM in a symmetrical or asymmetrical manner.

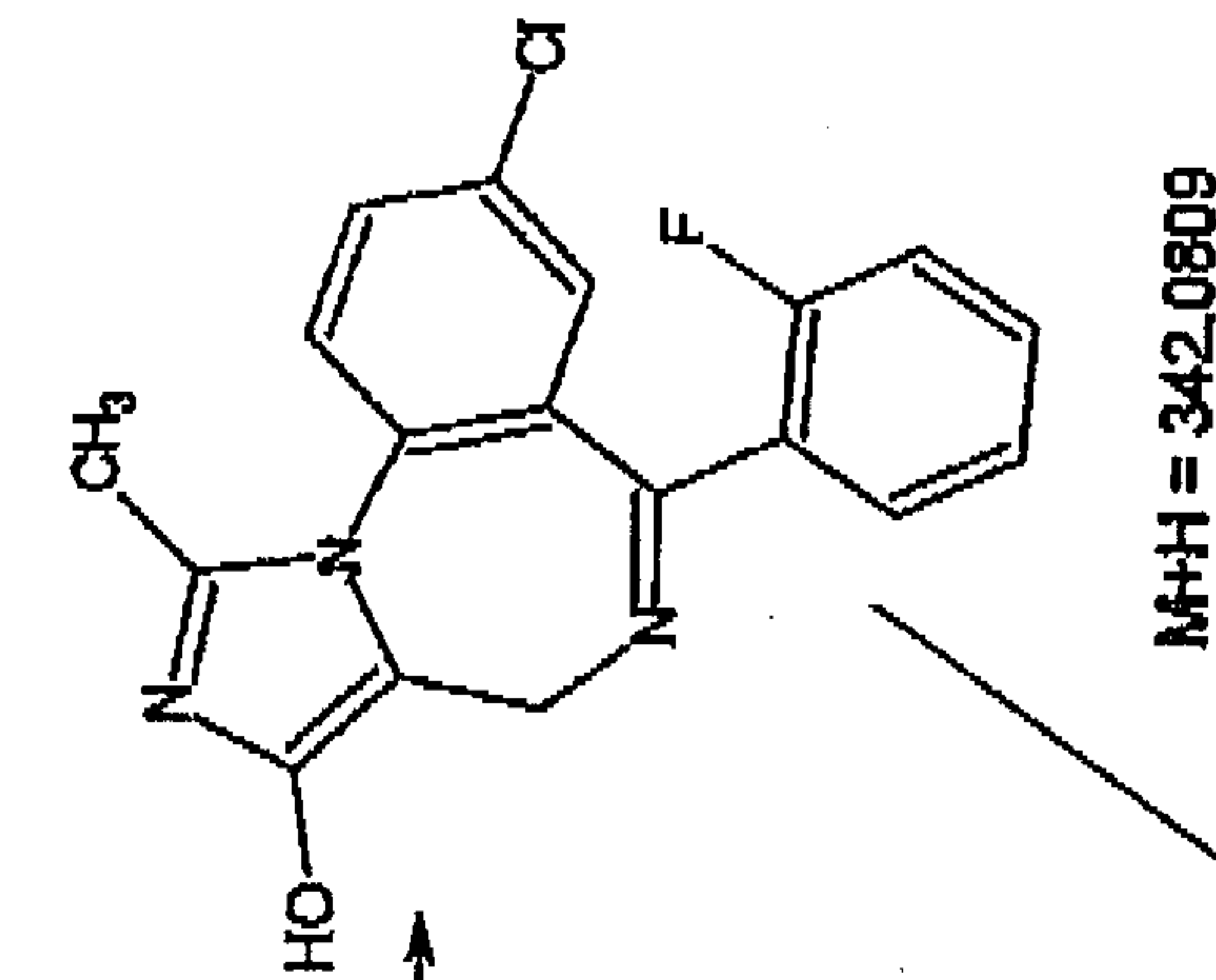
6. A method of mass spectrometry comprising:
 - analysing ions with a mass analyser;
 - searching mass data or a mass spectrum for ions having a decimal mass or mass to charge ratio component that falls within a decimal mass or mass to charge ratio window;
 - filtering out, removing, attenuating, or reducing the significance of ions having a decimal mass or mass to charge ratio component which falls outside of the decimal mass or mass to charge ratio window; wherein a profile of the decimal mass or mass to charge ratio window varies as a function of ΔM , wherein ΔM is the difference in the accurate mass or mass to charge ratio between that of a first substance or ion and a second substance or ion; and
 - providing an output based at least in part on said searching.
7. The method of claim 6, further comprising fragmenting ions having a decimal mass or mass to charge ratio component falling within the decimal mass or mass to charge ratio window.
8. The method of claim 7, further comprising mass analyzing the fragment product ions to obtain a mass spectrum of the fragment product ions.
9. The method of claim 6, 7 or 8, wherein the first substance or ion comprises or relates to a pharmaceutical compound, drug or active component.
10. The method of any one of claims 6-9, wherein the second substance or ion comprises or relates to a metabolite or derivative of the first substance or ion.
11. The method of any one of claims 6-10, wherein the profile of the decimal mass or mass to charge ratio window varies as a function of ΔM in a symmetrical or asymmetrical manner.

12. A method of mass spectrometry comprising:
 - analysing ions with a mass analyser;
 - obtaining a parent ion mass spectrum of a sample containing one or more metabolites of interest;
 - searching for ions of potential interest by searching the parent ion mass spectrum for mass peaks which meet a criteria that a decimal mass or mass to charge ratio component of a corresponding ion is within a decimal mass or mass to charge ratio window about a decimal mass or mass to charge ratio component of the parent ion;
 - selecting ions of potential interest for further analysis by virtue of the fact that the respective decimal mass or mass to charge ratio components of the ions falls within the decimal mass or mass to charge ratio window, wherein a size of the decimal mass or mass to charge ratio window varies as a function of a difference in an accurate mass or mass to charge ratio between that of the parent ion and ions being searched for; and
 - providing an output based at least in part on said searching.
13. The method of claim 12, wherein selecting the ions of potential interest for further analysis comprises fragmenting the ions of potential interest to form fragment product ions.
14. The method of claim 13, further comprising mass analyzing the fragment product ions to obtain a mass spectrum of the fragment product ions.
15. The method of any one of claims 12-14, wherein selecting ions of potential interest comprises filtering out, attenuating, removing, or reducing the significance of ions having a decimal part of an accurate mass or mass to charge ratio which falls outside of the decimal mass or mass to charge ratio window.
16. The method of claim 12, wherein the ions of potential interest comprise one or more metabolites of the parent ion.

17. The method of any one of claims 12-16, wherein the profile of the decimal mass or mass to charge ratio window varies in a symmetrical or asymmetrical manner.

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Midazolam Hydroxylated Metabolite

 $M+H = 342.0809$ $+ 15.9949$

Midazolam Parent Drug

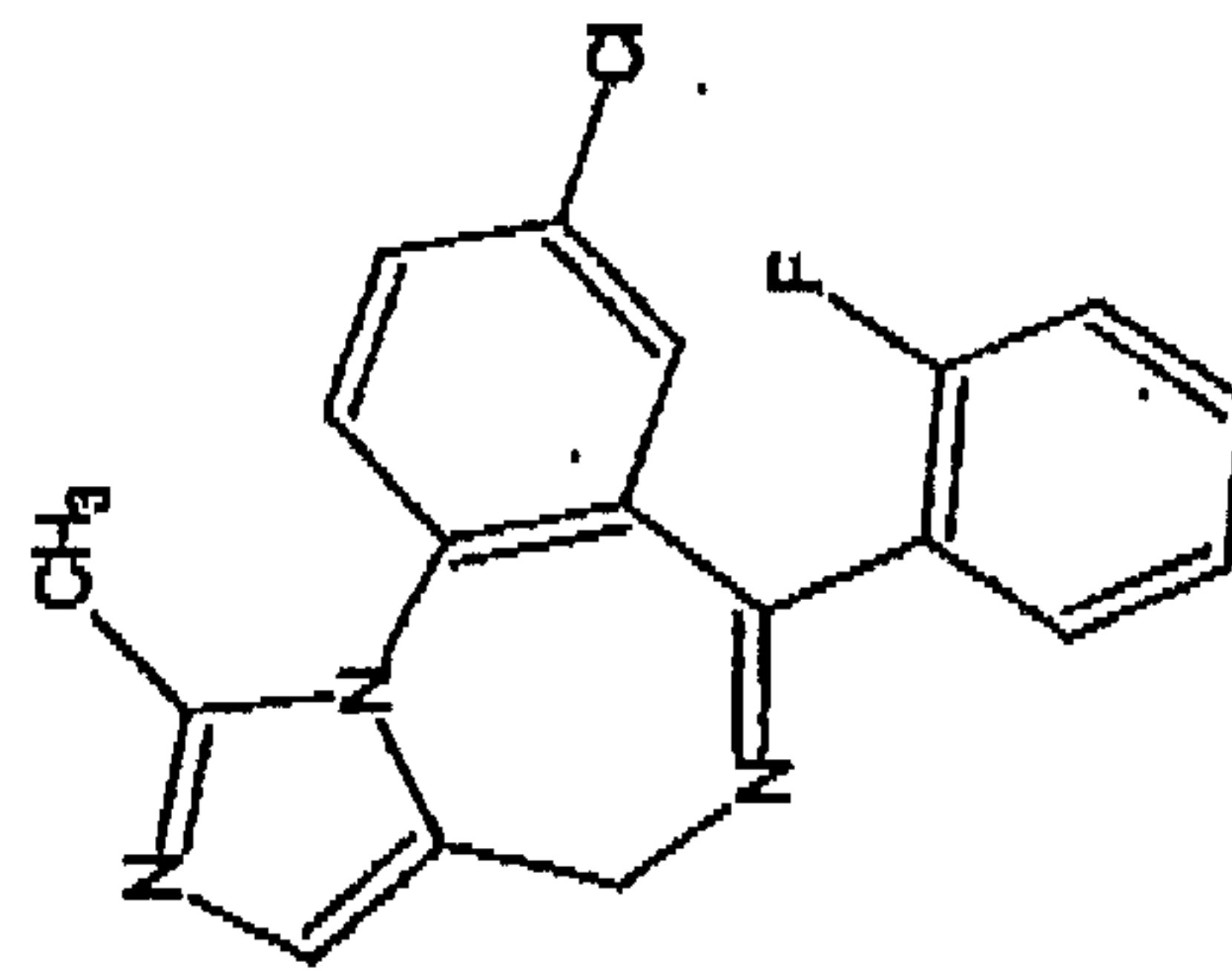
 $M+H = 326.0860$ $\text{Mass deficiency} = 0.0860 - 0.0809 = 0.0051 \text{ Da}$

FIG. 1

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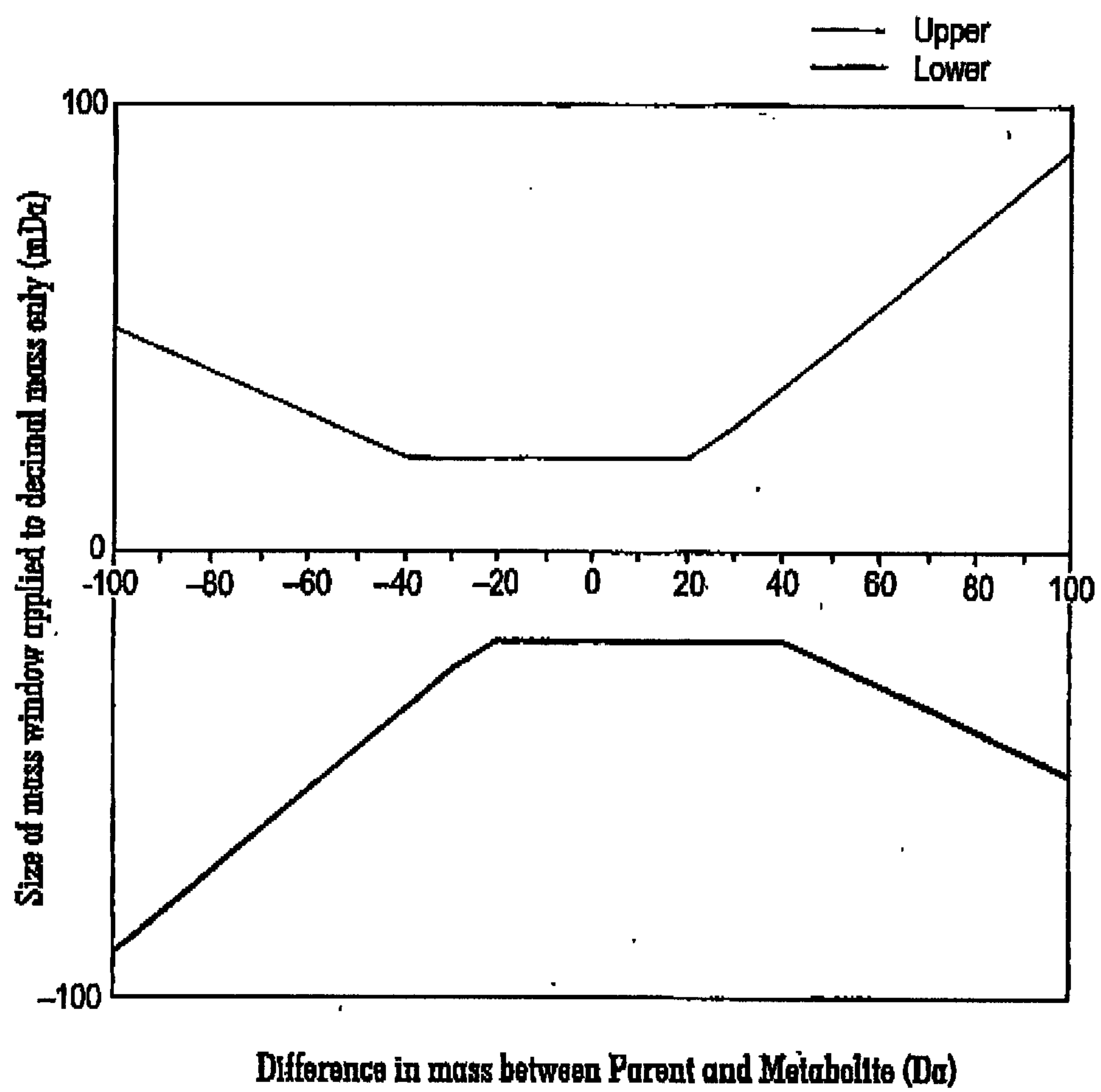


FIG. 2

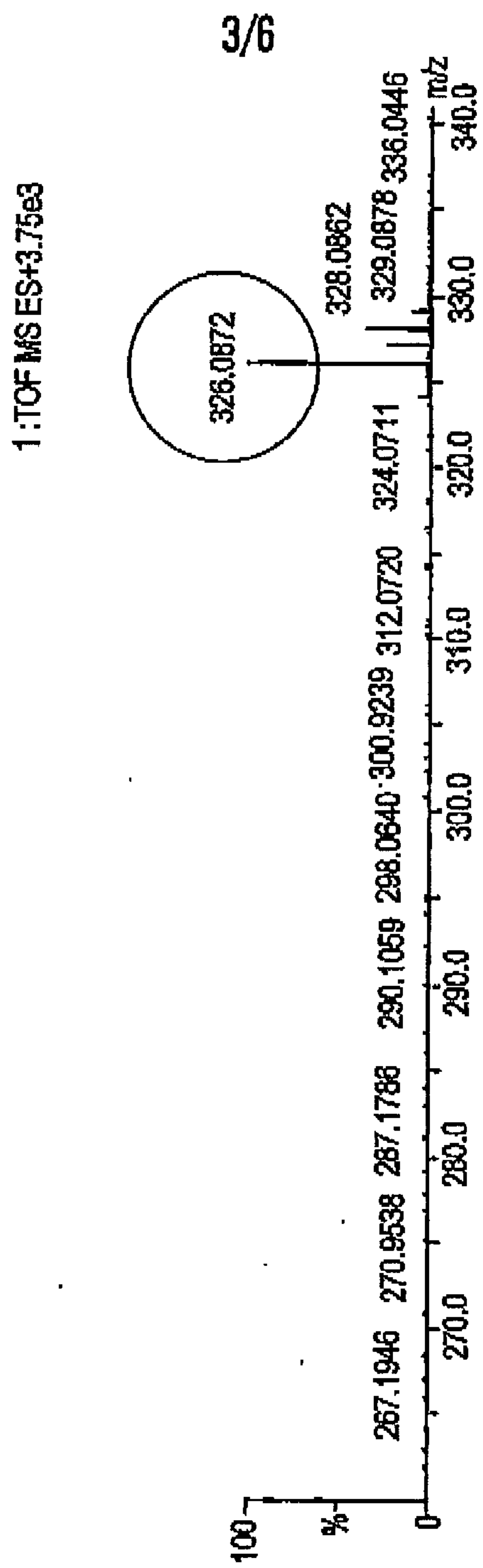


FIG. 3

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1: TOF MS ES+2.19e3

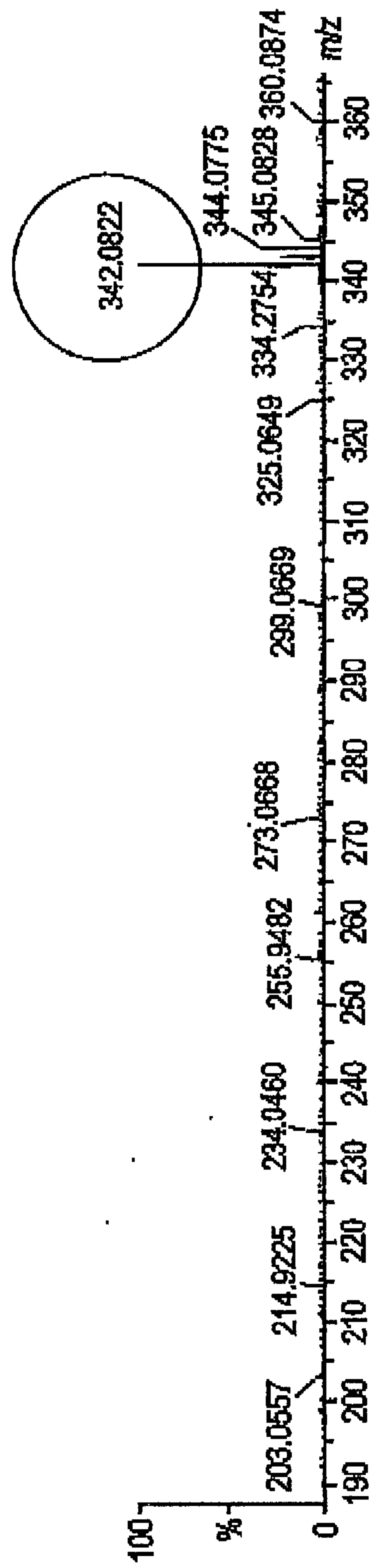


FIG. 4

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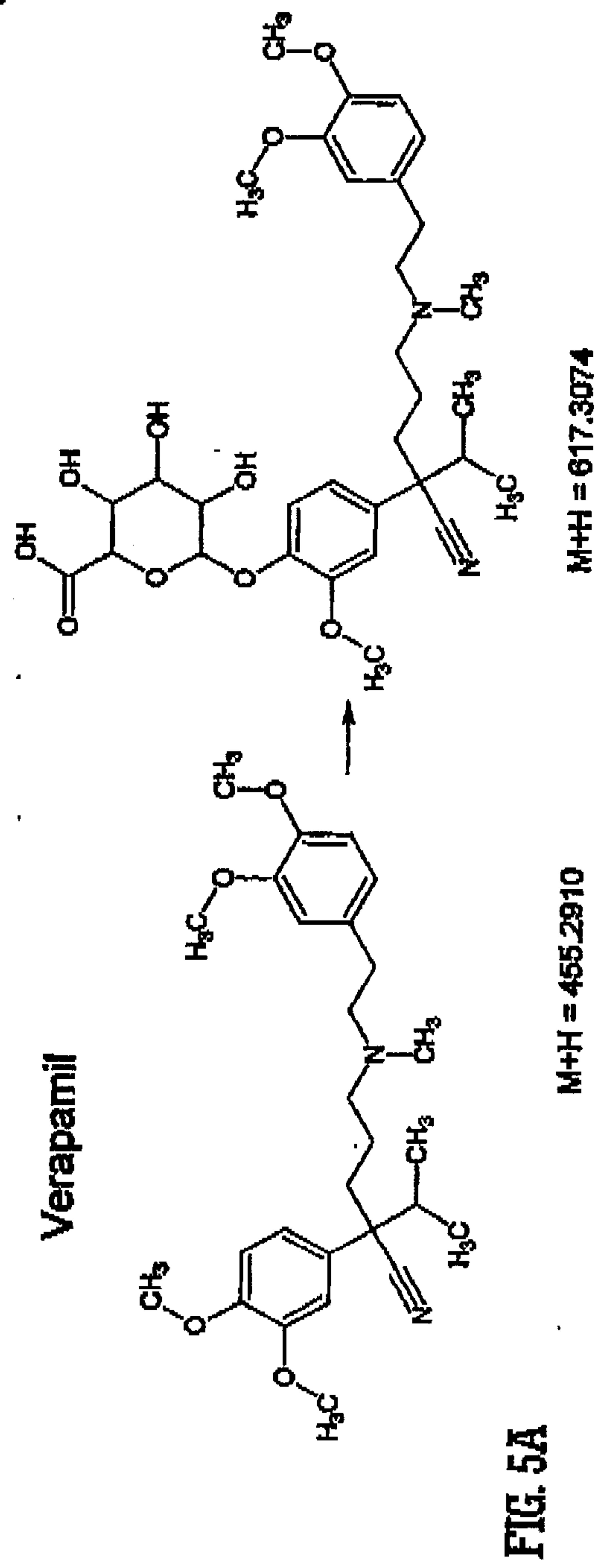
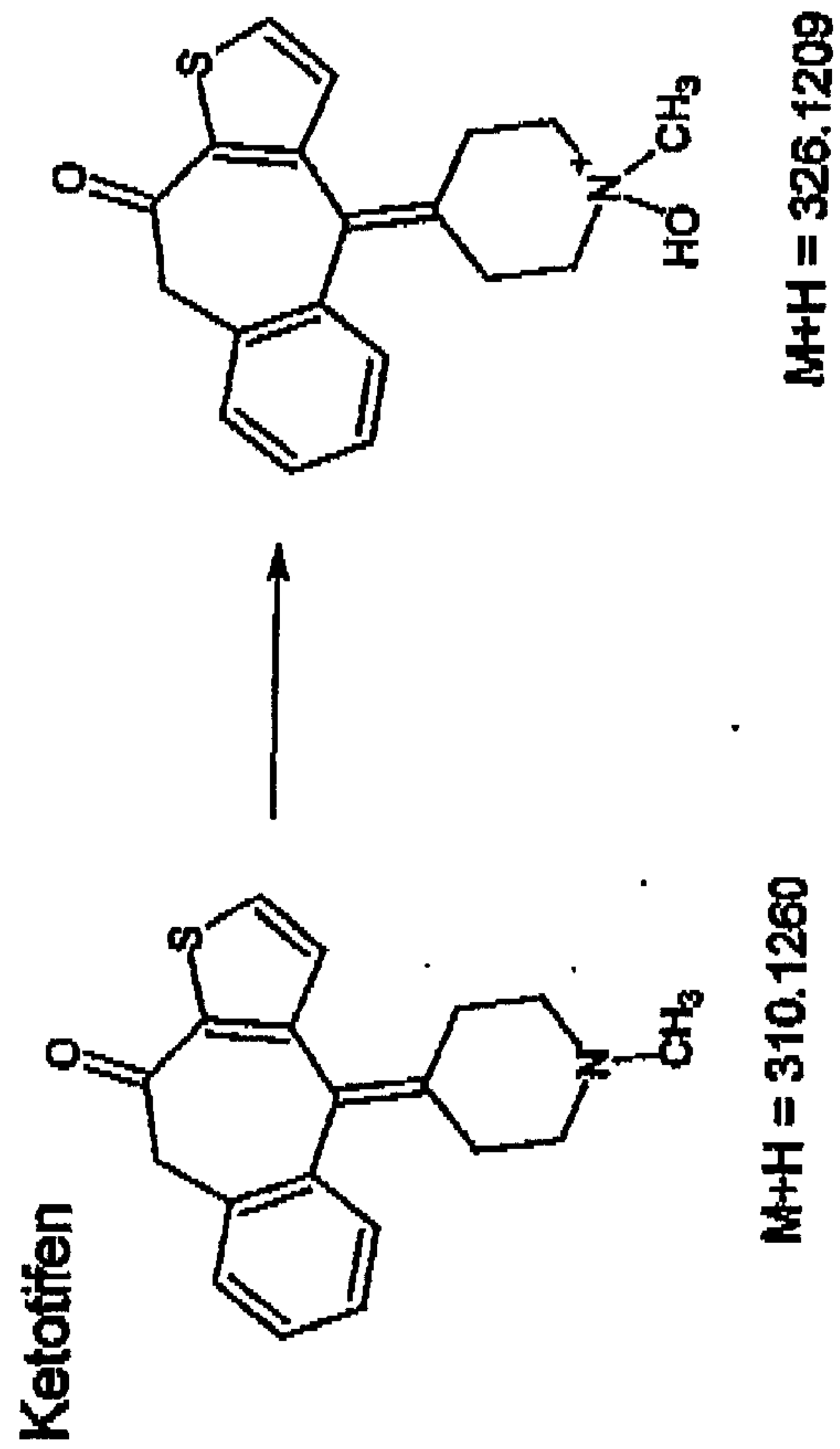


FIG. 5A

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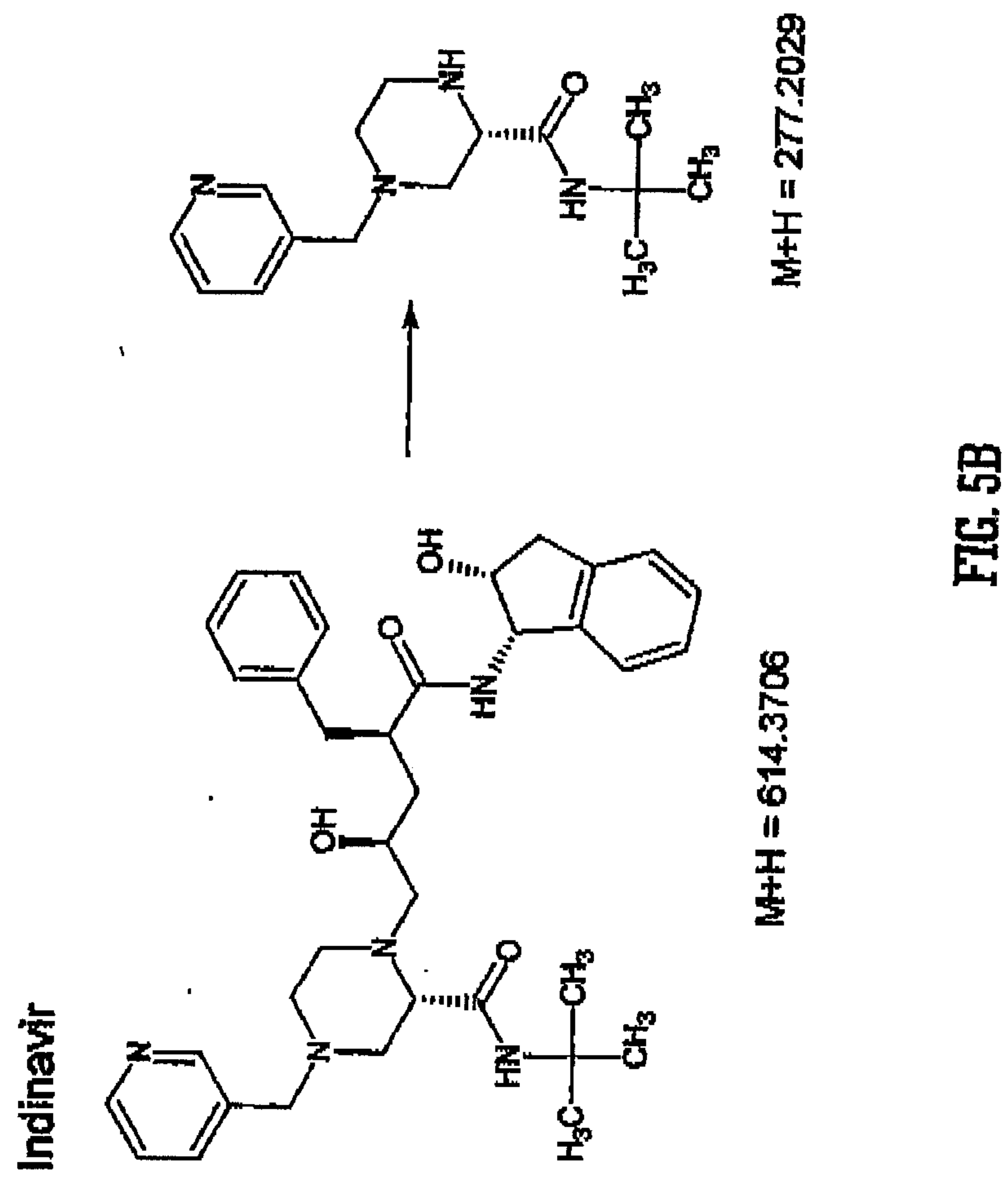


FIG. 5B