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Determination of Carbon and Nitrogen Isotope Fractions in Asparagine, Aspartic Acid, Threonine and Methionine

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Abstract

The nomenclature for compounds that are modified with isotopes is growing every day. Compounds can be modified with isotopes either individually, in a functional group or groups, or completely with all atomic centers of the element. This diversity of isotope-modified compounds increases the range of researches that can be studied using them. Compounds modified with isotopes of carbon-13 or nitrogen-15 can be converted into carbon monoxide, carbon dioxide and molecular nitrogen. Currently, only the average value of carbon-13 or nitrogen-15 isotopes can be determined. However, by directly determining the atomic share of these isotopes in organic compounds modified with isotopes, information about the isotopic centers of the element can be obtained. The atomic fraction of an element is defined as a single carbon or nitrogen isotope-modified center or centers, or all centers that are isotope-modified with that element at the same time. Carbon-13 or nitrogen-15 isotopes' atomic fraction can be determined molecularly or with fragment ions of different elemental content, or both. This makes the method self-verifying, increasing the accuracy and reliability of the results obtained. Amino acids, such as asparagine, aspartic acid, methionine, and threonine, are essential for the human body. This proposed method of isotopic analysis will increase the possibilities for scientific research using these compounds.

Keywords

Asparagine, Aspartic Acid, Threonine, Methionine, Mass Spectrometer, Isotopic Analysis, Atomic Share

1. Introduction

The use of isotope-modified compounds significantly increases the area of their

application. With their help, it is possible to conduct such studies that were impossible without the use of isotope-modified compounds. Their use depends to a significant extent on the existence of cheap and simple methods. Amino acids are very important organic compounds. Among them, asparagine, aspartic acid, methionine and threonine are important biological products. These compounds are critical for protein synthesis and play a multifaceted role in maintaining the structure and function of body tissues and organs.

Asparagine and aspartic acid are essential for a variety of biological processes, including protein structure, neurotransmitter synthesis, and metabolic processes. Asparagine is important for stabilizing protein structure. It participates in acid-base reactions and is important for maintaining acid-base balance. It participates in the urea cycle and contributes to the production of energy and the destruction of excess nitrogen. Asparagine and aspartic acid are extremely important for the precise structure of proteins [1] [2] [3].

Threonine is a basic building block for proteins. It is an important component of collagen. Threonine is involved in the production of antibodies and proteins related to the immune system, which protects the body from infectious diseases. It participates in the transmission of signals in the brain and spinal cord, and the protection of the central nervous system. It also helps in various metabolic processes, maintenance and growth of muscle tissue, and detoxifies the liver. It is not produced by the human organism. People eat it from poultry, meat, milk, and nuts.

Methionine is an essential amino acid. The human body cannot synthesize it, but takes it from food. Methionine is an essential substance for protein synthesis. A coenzyme is formed from methionine in the human body, through which further methylation takes place, *i.e.* the methyl group is transferred to DNA, proteins and lipids. The content of the sulfur atom determines the synthesis of glutathione, thereby deoxygenating the human body and protecting cells from oxidative stress. Methionine frees the liver from heavy metals and harmful substances.

These amino acids have many other functions in the human body. Therefore, carbon-13 and nitrogen-15 isotope-modified compounds will give us more detailed information about them.

The rapid growth of the nomenclature of isotope-modified compounds has greatly increased their use in scientific research. Organic compounds modified with carbon and nitrogen elements included in organic compounds are especially important. For their successful use in research, it is necessary to use simple, accurate and informative methods of isotope analysis.

Amino acids are very important organic compounds for the human body. Direct measurement of carbon-13 and nitrogen-15 isotopes in asparagine, aspartic acid, methionine and threonine. Information about modified elements in individual groups and the determination of carbon and nitrogen isotopic content in them significantly expands the possibility of research. Determining the isotopic

content using ions with different mass numbers increases the accuracy and reliability of the measurement. Determination of the isotopic content in the existing carbon dioxide and in them is done only by determining their average value [4].

Methods for determining the atomic fraction of carbon and nitrogen isotopes directly in asparagine, aspartic acid, methionine and threonine are not known [5]. The authors proposed a general approach to the possibility of directly determining the atomic fraction of carbon isotopes in organic compounds [6]. The possibility of determining the atomic fraction of nitrogen isotopes is discussed in the article. Based on a detailed study of the mass spectrum of each organic compound, it is determined by the mass fraction of molecular or fragmented ions and the processes that need to be taken into account, after which, based on the principle of the given general system, a system of equations is created, which is reduced to one equation.

The articles discuss the possibility of determining the atomic shares of carbon and nitrogen in certain organic compounds using selected mass lines [6]-[12]. The authors present the mass spectra of asparagine, aspartic acid, threonine and methionine, which were obtained using an isotope mass spectrometer МИ-1201. The study focuses on the elemental content of the thiolate mass line, and the mass lines used to determine the atomic share of carbon-13 and nitrogen-15. Depending on the mass spectrum, molecular and fragment ions can be used to determine these quantities using different mass lines. When multiple methods are used, the results can be verified, which increases the accuracy and reliability of the method.

2. Experimental

Asparagine molecule contains two amino groups, so it has a different type of fragmentation.

Obtaining a mass spectrum involves bombarding the sample with electrons using an isotope mass spectrometer МИ-1201, as shown in **Figure 1**.

Asparagine, unlike most amino acids, does not have a fixed molecular peak due to the presence of functional groups in the molecule. The spectrum shows a strong peak $(\text{M-COOH})^+$ with a mass-to-charge ratio of m/z = 87. The most likely process of molecule fragmentation is the breaking of the β-bond, which leads to the formation of the fragment ion C with m/z = 44 (NH_2-C = 0) and a charged amino group. If the charge is on the second amino group, then a fragment $(NH_2\text{-CH-}CH_2)^+$ with m/z = 43 is obtained instead. Additionally, the spectrum shows the elimination of water and the removal of the hydroxyl radical from the molecule, which leads to the formation of the fragment ion C with m/z = 114, 115. Subsequent β-bond breaking results in ions with m/z = 70

$$(O=C-\underset{NH_2}{\overset{C}{|}}-CH_2)^+$$

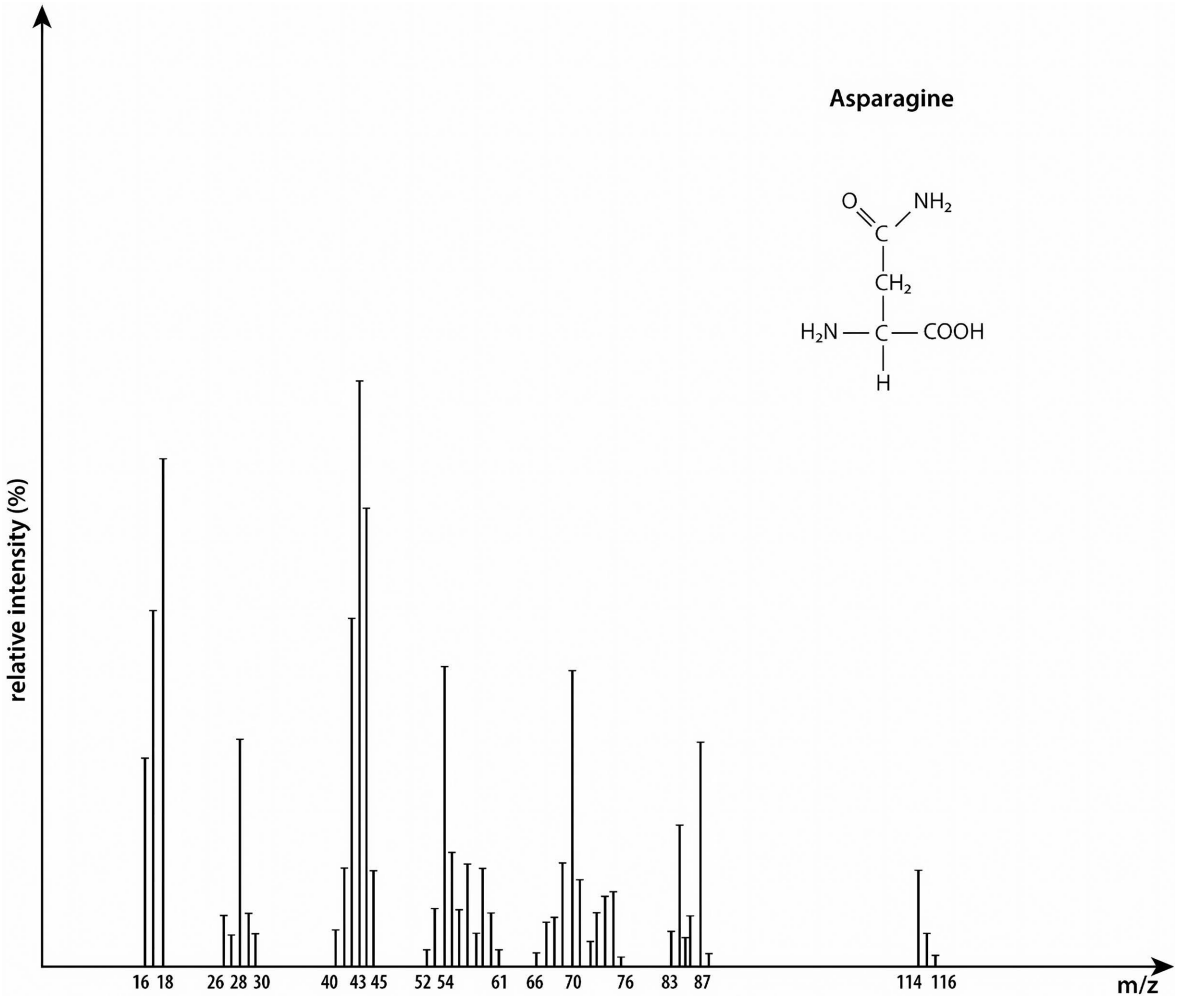

Figure 1. The mass spectrum of asparagine.

The molecular ion rearrangement process results in the migration of a hydrogen atom to the amine group, leading to fragmentation and the formation of ions with $m/z = 88$.

The atomic fraction of carbon and nitrogen isotopes can be determined by analyzing the intensities of fragmented ions with $m/z = 114, 115, 116$. However, it is necessary to remove dehydration and hydroxyl radicals for accurate results.

When asparagine is isotope-modified with all four carbon centers, then the system of equations reduces to the equation:

$$10I_{114}y^2 - \left(4I_{115} - 0.0364I_{114}\right)y + \left(I_{116} - 0.0091I_{115} - 0.00403I_{114}\right) = 0 \quad (1)$$

where

$$y = Xc^{13} / Xc^{12} \quad (2)$$

The formula for calculating the atomic fraction of the carbon-13 isotope is as follows:

$$Xc^{13} = \frac{y}{y+1} \quad (3)$$

To identify isotope-modified *n* asparagine, we consider only the *α*-carbon or carboxyl group carbon. We also account for dehydration and removal of the hydroxyl group from the molecular ion. By using ions with mass lines of m/z = 114, 115, and 116, we can simplify the system of equations to a single equation:

$$I_{114}y^2 - \left(I_{115} - 0.04272I_{114}\right)y + \left(I_{116} - 0.04272I_{115} - 0.00298I_{114}\right) = 0 \quad (4)$$

Similarly, the atomic fraction of the carbon-13 isotope is always calculated using formula (3).

If the carbon chain modifies asparagine isotopically, the equation will take a specific form:

$$3I_{114}y^2 - \left(2I_{115} - 0.06302I_{114}\right)y + \left(I_{116} - 0.03151I_{115} - 0.00347I_{114}\right) = 0 \quad (5)$$

It is possible to determine the amount of the carbon-13 isotope in a sample by using fragment ions with m/z = 86, 87, and 88. However, these fragment ions cannot contain carboxyl carbon and can only be used when asparagine is modified with all carbon centers. In such cases, the system of equations can be simplified to a single equation.

$$6I_{86}y^2 - \left(3I_{87} - 0.02577I_{86}\right)y + \left(I_{88} - 0.00874I_{87} - 0.00200I_{86}\right) = 0 \quad (6)$$

Asparagine is a molecule that can be modified with an isotope of nitrogen to create nitrogen-15. This is important because we can calculate the amount of nitrogen-15 present by measuring the fragment ions with a m/z = 114, 115, 116. We consider two processes—dehydration and removal of the hydroxyl group—which can be represented by the following equation system:

$$3I_{114}y^2 - \left(2I_{115} - 0.09319I_{114}\right)y + \left(I_{116} - 0.04674I_{115} - 0.00491I_{114}\right) = 0 \quad (7)$$

in this case

$$y = Xn^{15}/Xn^{14} \quad (8)$$

and

$$Xn^{15} = \frac{y}{y+1} \quad (9)$$

If asparagine is isotope modified with nitrogen-15 isotope with only one nitrogen center, in this case, the system reduces to the following equation

$$I_{114}y^2 - \left(I_{115} - 0.05026I_{114}\right)y + \left(I_{116} - 0.05026I_{115} - 0.00507I_{114}\right) = 0 \quad (10)$$

It is interesting to determine the atomic share of the nitrogen-15 isotope through the fragment with m/z = 86, 87, 88. In asparagine modified by both atomic centers, the system of equations is reduced to the following equation

$$3I_{86}y^2 - \left(2I_{87} - 0.07004I_{86}\right)y + \left(I_{88} - 0.03502I_{87} - 0.00125I_{86}\right) = 0 \quad (11)$$

And when it is modified with one nitrogen center, the equation has the form

$$I_{86}y^2 - \left(I_{87} - 0.03868I_{86}\right)y + \left(I_{88} - 0.03868I_{87} - 0.00111I_{86}\right) = 0 \quad (12)$$

The atomic fraction of carbon-13 isotope is calculated by formula (3).

The results of measuring the atomic fraction of carbon-13 isotope in asparagine of natural content, calculated by formulas (1) and (6) are given in **Table 1**.

Table 1. Atomic fraction of carbon-13 isotope determined by equations.

	Equation (1)	Equation (6)
1	1.10	1.11
2	1.14	1.13
3	1.11	1.10
4	1.13	1.12
5	1.12	1.11
6	1.10	1.13
7	1.14	1.12
8	1.13	1.11
9	1.12	1.10
10	1.11	1.12
	1.12 ± 0.02	1.12 ± 0.02

Aspartic acid is the simplest representative of amino dicarboxylic acids Mass spectrum of aspartic acid taken with isotope mass spectrometer МИ-1201 is given in **Figure 2**.

A molecular ion is not observed in the mass spectrum of aspartic acid, which is due to the presence of two carboxyl groups in the molecule. Most likely, the removal of the carboxyl group from the molecule resulted in the formation of ions with $m/z = 88$. Parallel to this process, α-cleavage is observed with hydrogen migration to the amine group, resulting in ions with $m/z = 89$. Also charge ions, whose $m/z = 87$ after the removal of the hydrogen atom after the removal of the carboxyl group.

A peak of low intensity is also observed, arising from the elimination of water from the molecular ion and the cleavage of the hydroxyl radical. These fragment ions are also unstable and dissociate into fragments with m/z = 70 and 71. α-cleavage, on the other hand, fragment ions with $m/z = 69$ and 74.

Analysis of the spectrum shows that isotopic analysis can be performed using the ion peaks of the mas-spectrum with m/z = 87, 88, 89, 90. That is, we need to consider three processes: removal of the carboxyl group, removal of hydrogen from this fragment ion, and migration of hydrogen from the carboxyl group to the amine group.

When the asparagine molecule is isotope modified with all carbon centers, then the system of equations reduces to the following equation

$$\begin{aligned} &10I_{87}y^3-\left(6I_{88}-0.03264I_{87}\right)y^2+\left(3I_{89}-0.01632I_{88}-0.01218I_{87}\right)y \\ &-\left(I_{90}-0.00544I_{89}-0.00406I_{88}-0.000037I_{87}\right)=0 \end{aligned} \quad (13)$$

where $y = X_{12}/X_{13}$, $Xc^{13} = y/1 + y$.

If the asparagine molecule is modified by carboxyl groups, then the system of equations is reduced to the equation

$$\begin{aligned} &I_{87}y^3-\left(I_{88}-0.02785I_{87}\right)y^2+\left(I_{89}-0.02785I_{88}-0.00356I_{87}\right)y \\ &-\left(I_{90}-0.002785I_{89}-0.00356I_{88}-0.00016I_{87}\right)=0 \end{aligned} \quad (14)$$

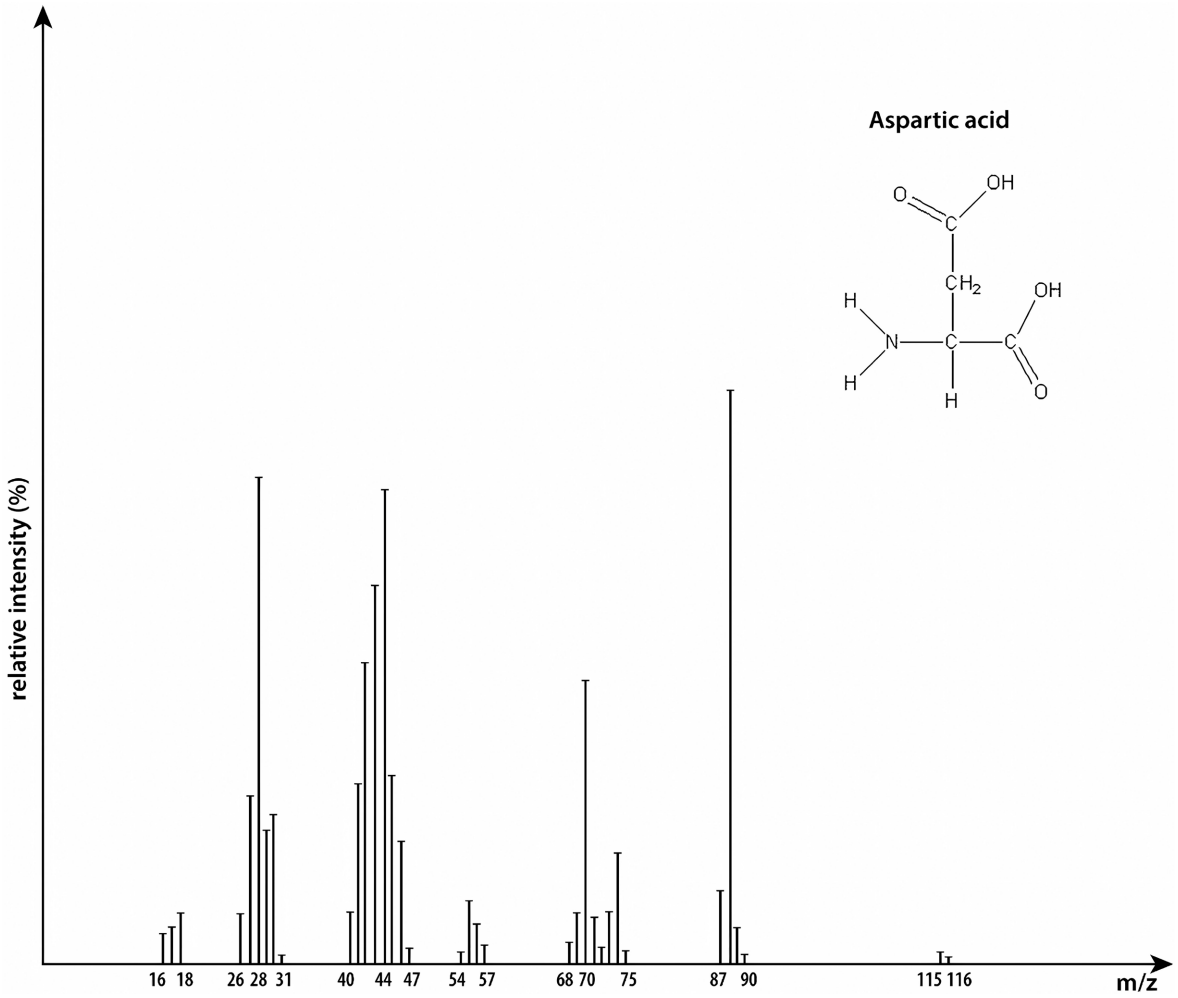

Figure 2. The mass spectrum of aspartic acid.

And in the case when the carbon of the aspartic acid chain is isotope-modified, the system of equations will be reduced to the equation

$$\begin{aligned} &4I_{87}y^3-\left(3I_{88}-0.04994I_{87}\right)y^2+\left(2I_{89}-0.00333I_{88}-0.0077I_{87}\right)y\\ &-\left(I_{90}-0.01665I_{89}-0.00378I_{88}-0.00007I_{87}\right)=0 \end{aligned} \tag{15}$$

The atomic fraction of nitrogen-15 isotope in aspartic acid modified with nitrogen isotope is also calculated by the equation

$$\begin{aligned} &I_{87}y^3-\left(I_{88}-0.03539I_{87}\right)y^2+\left(I_{89}-0.03539I_{88}-0.00327I_{87}\right)y\\ &-\left(I_{90}-0.03539I_{89}-0.00327I_{88}-0.00014I_{87}\right)=0 \end{aligned} \tag{16}$$

The results of measuring the atomic fraction of carbon-13 isotope in aspartic acid of natural content, calculated by formulas (13) and (16) are given in Table 2.

Threonine or α-amino β-hydroxy acid. Mass spectrum of threonine is shown in Figure 3.

The molecule being discussed contains functional amino, hydroxyl, and carboxyl groups. This makes the molecular peak easily fragmented and not fixed in

Table 2. Atomic fraction of carbon-13 isotope determined by equations.

	Equation (13)	Equation (16)
1	1.11	1.12
2	1.13	1.13
3	1.14	1.13
4	1.13	1.12
5	1.12	1.11
6	1.11	1.11
7	1.12	1.12
8	1.14	1.13
9	1.12	1.11
10	1.11	1.12
	1.12 ± 0.01	1.12 ± 0.01

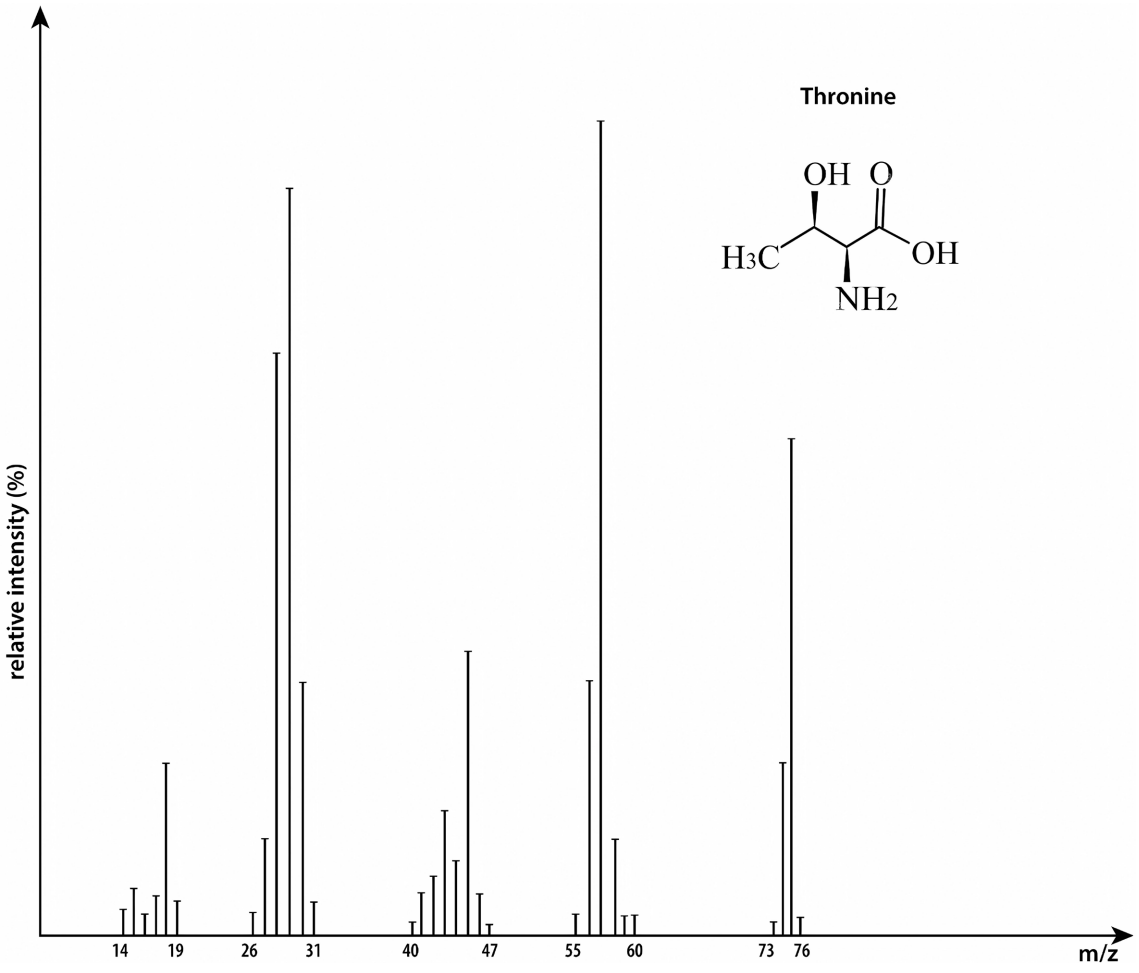

Figure 3. The mass spectrum of threonine.

the spectrum (2). During ionization, the charge is mostly on the amine groups, so we will mainly observe amine decomposition fragments in the spectrum. The

peak $m/z = 74, 75$ is intense, and these fragment ions are formed by breaking the α-bond, resulting in $(C_3ONH_8)^+$ or $(M\text{-}COOH)^+$ ions. On these mass lines, we also see ions formed by breaking the β-bond, which are superimposed with the previous ions. In the spectrum, we will observe the maximum fragment ion peaks with $m/z = 57$ $(NH_2 = CH\text{-}C = 0)^+$, which is obtained by further decomposition of the fragment obtained by the removal of the hydroxyl radical.

Localization of the charge on the carboxyl group is a small probability process, that's why the fragment ions with $m/z = 45$ are of relatively low intensity. Hydrogen migration from the carboxyl group will also be observed and fragment ions with $m/z = 30$ $(CH\text{-}NH_3)^+$ and $m/z = 43$ $(CH\text{-}CH\text{-}NH_3)$ will be formed.

It should be noted that fragment ions with $m/z = 75, 57$ are partially formed through hydrogen migration to amine groups. Fragment ions with $m/z = 29$ $(NH_2\text{-}CH)^+$ are intense, which once again confirms the charge localization on the amine group.

According to the mass spectrum analysis, it is not possible to directly determine the atomic fraction of carbon-13 and nitrogen-15 in threonine due to the presence of intense mass lines. The mass lines are overlapped by fragment ions $(C_3ONH_8)^+$ and $(C_2O_2NH_4)^+$ at $m/z = 74, 75$, and the isotopic form of $(C_2O_2NH_5)^+$ ion and $(C_2O_2NH_5)^+$ fragment ions at $m/z = 75$.

Ions of different elemental content are superimposed on fragmented ions with $m/z = 57, 58, 59$. Fragment ions have different elemental contents. The isotopic form of fragment ions $C_2ONH_3^+$, $C_3OH_6^+$, and $C_2ONH_5^+$ and superimposition of fragments caused by hydrogen removal. Therefore, determining the atomic fraction of carbon-13 or nitrogen-15 isotopes in threonine is impossible at this stage.

Direct determination of threonine is possible only via high resolution mass spectra or by converting molecular nitrogen into carbon monoxide or carbon dioxide.

Methionine is an amino acid containing a sulfur atom.

Methionine molecule contains two functional groups, amino and carboxylic. Due to the presence of a sulfur atom in the molecule, it is relatively stable which is evident from **Figure 4**.

Therefore, the molecular ions in the mass spectrum are relatively intense. In the mass spectrum, the peak of ions with $m/z = 61$ is the most intense which is obtained by breaking the β-bond $(CH_3\text{-}S\text{-}CH_2)^+$. The charge is localized on the sulfur atom. Water is eliminated intensively and the hydroxyl radical is removed, forming ions with $m/z = 131, 132$. From this fragment, a fragment ion with $m/z = 116$ is obtained by removing the radical of the further method (C_4ONH_7). It is quite likely that the carboxyl group is removed and ions with $m/z = 104$, M-COOH, are formed. Ions with $m/z = 101, 106, 107$ fragment ions will also be observed.

In the mass spectrum of methionine, the type of amine decomposition is weakly expressed. Therefore, fragment ions with $m/z = 74$ are of lower intensity

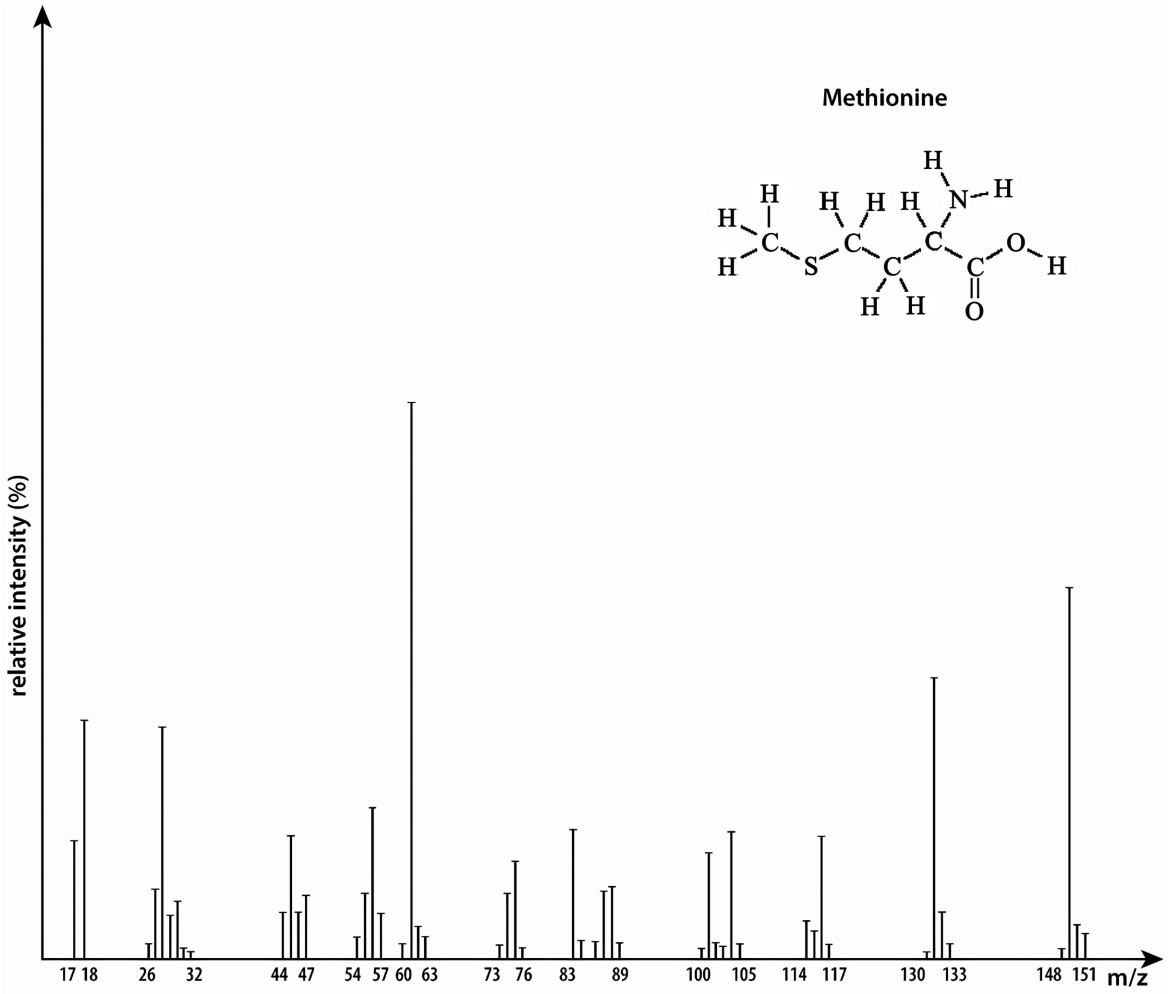

Figure 4. The mass spectrum of methionine.

than fragment ions with m/z = 75, Ions will also be observed in the spectrum NH_2-CH^+ (m/z = 57), $COOH^+$ m/z = 45 and

$$CH_2-\underset{NH_2}{\overset{CH}{|}}-COOH^+,\ m/z = 88.$$

Carbon-13 isotope atoms in all carbon-centered modified methionine can be determined directly through molecular ions, as well as fragment ions formed by dehydration of molecular ions and cleavage of the hydroxyl radical. Ion peaks with m/z = 131, 132 are formed, as well as fragment ions with m/z = 61, 62. It is best to understand the atomic fraction of the carbon-13 isotope through molecular ions. At this time, together with the molecular ions, we must take into account hydrogen dissociation. In this case, the system of equations will be reduced to the equation:

$$15I_{148}y^2-\left(5I_{149}-0.07002I_{148}\right)y+\left(I_{150}-0.01404I_{149}-0.04837I_{148}\right)=0 \quad (17)$$

The atomic fraction of carbon-13 isotope is calculated by formula (3).

When methionine is isotope-modified only with the carbon of the carboxyl

group, then the system of equations reduces to the equation:

$$I_{148}y^2-\left(I_{149}-0.05886I_{148}\right)y+\left(I_{150}-0.5886I_{149}-0.04648I_{148}\right)=0 \quad (18)$$

When the system of equations of methionine isotope modified chain with carbon-13 isotope is reduced to the face

$$10I_{148}y^2-\left(4I_{149}-0.10036I_{148}\right)y+\left(I_{150}-0.02524I_{149}-0.061797I_{148}\right)=0 \quad (19)$$

The system for calculating the atomic fraction of nitrogen isotopes in methionine modified with the nitrogen-15 isotope is reduced to the equation

$$I_{148}y^2-\left(I_{149}-0.06639I_{148}\right)y+\left(I_{150}-0.06639I_{149}-0.06180I_{148}\right)=0 \quad (20)$$

$$I_{148}y^2-\left(I_{149}-0.05886I_{148}\right)y+\left(I_{150}-0.05886I_{149}-0.04652I_{148}\right)=0 \quad (21)$$

The isotopic content of carbon and nitrogen can be determined similarly for m/z = 129, 130, 131 with mass lines. This fragment ion contains both molecular carbon and nitrogen atoms. These fragments, as mentioned above, are formed by dehydration of the molecule and cleavage of the hydroxyl radical.

When methionine is isotope-modified at all carbon atoms, then the system of equations reduces to the equation:

$$15I_{129}y^2-\left(5I_{130}-0.06610I_{129}\right)y+\left(I_{131}-0.01322I_{130}-0.04634I_{129}\right)=0 \quad (22)$$

In threonine modified by the carbon centers of the methionine chain, by the equation

$$10I_{129}y^2-\left(4I_{130}-0.09708I_{129}\right)y+\left(I_{131}-0.02442I_{130}-0.04607I_{129}\right)=0 \quad (23)$$

and in methionine isotope modified with the carbon center of the carboxylic radical

$$I_{129}y^2-\left(I_{130}-0.05803I_{129}\right)y+\left(I_{131}-0.05803I_{130}-0.04449I_{129}\right)=0 \quad (24)$$

The following equation determines the isotope ratio of nitrogen in methionine modified with a nitrogen atom

$$I_{129}y^2-\left(I_{130}-0.06924I_{129}\right)y+\left(I_{131}-0.06924I_{130}-0.04348I_{129}\right)=0 \quad (25)$$

In methionine modified by chain carbon centers, the ratio of carbon isotopes can be determined by the equation

$$3I_{60}y^2-\left(2I_{61}-0.01748I_{60}\right)y+\left(I_{62}-0.00877I_{61}-0.04435I_{60}\right)=0 \quad (26)$$

The results of measuring the atomic fraction of carbon-13 isotope in methionine of natural content, calculated by formulas (17), (22) and (26) are given in Table 3.

Table 3. Atomic fraction of carbon-13 isotope determined by equations.

	Equation (17)	Equation (22)	Equation (26)
1	1.08	1.13	1.12
2	1.09	1.12	1.13
3	1.10	1.10	1.10

Continued

4	1.15	1.12	1.15
5	1.13	1.14	1.10
6	1.14	1.09	1.12
7	1.10	1.10	1.13
8	1.12	1.11	1.13
9	1.10	1.12	1.12
10	1.12	1.13	1.10
	1.11 ± 0.02	1.12 ± 0.01	1.12 ± 0.01

3. Conclusion

To determine the atomic percentage of carbon-13 and nitrogen-15 isotopes present in asparagine, aspartic acid, threonine, and methionine that have been modified with carbon and nitrogen, the mass spectra of these compounds were studied in detail. Based on the analysis, the mass lines were determined through which the fraction of atoms of carbon-13 and nitrogen-15 isotopes could be calculated, along with the processes that must be taken into account. With the given equations, it is possible to determine the atomic percentage of these elements in asparagine, asparagine derivatives, and methionine, both in separate functional groups and in isotopically modified compounds with several carbon centers or all centers. It is not possible to directly analyze the threonine mass spectrum for isotopes.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

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