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Methods for the Determination of Stable Isotopes of Carbon and Nitrogen Directly in Valine, Proline, Glutamine, and Glutamic Acid

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Abstract

Amino acids are very important compounds for the body and are involved in important functions that keep us healthy. Amino acids are essential components such as valine, proline, glutamine and glutamic acid. They can be synthesized either naturally or artificially. To examine the metabolism and regulate the synthesis process, compounds labeled with nitrogen or carbon isotopes need to be used. These isotopic compounds allow for more extensive research and enable studies that would otherwise be impossible. However, their use is dependent on the availability of simple, efficient methods for isotopic analysis. Currently, the determination of the atomic fraction of carbon and nitrogen isotopes is only possible through their conversion into molecular nitrogen or carbon monoxide or carbon dioxide. This leads to the loss of information about isotopic enrichment in specific centers of the molecule. This article explores a new direct approach to determining the atomic fraction of carbon and nitrogen isotopes in the isotope-modified or identical centers of these compounds. This method eliminates the transfer process and dilution due to nitrogen and carbon impurities. It is now possible to simultaneously determine the atomic fraction of nitrogen and carbon isotopes in the research substance. This method can be applied to amino acids, making it an effective tool for proposing new research methods. Several articles [1] [2] [3] have proposed similar methods for organic compounds and amino acids.

Keywords

Valine, Proline, Glutamine, Glutamic Acid, Mass Spectrometer, Mass Spectrum, Ion Current, Intensity, Mass Lines, Molecular and Fragment Ions

1. Introduction

Amino acids are organic compounds in which the molecule is both carboxylic

and amino functional groups. These compounds are important in biochemistry because amino acids are the basic building blocks of proteins, where linear polymeric chains of amino acids are linked by peptide bonds. The sequence of amino acids in a protein is determined by the sequence of genes encoded in the genetic code [4]. Amino acids play crucial roles in regulating various functions of the body. They help to stabilize mood and concentration, as well as control aggression, attention, sleep, and sexual activity. Through carbon or nitrogen labeled amino acids can be studied their kinetics and metabolism in the body. As soon as you get there, proteins break down into amino acids, and then separated amino acids are used for the body's necessary proteins and to synthesize enzymes. When we talk about amino acids, we are mainly referring to alpha-amino acids. The term "alpha" is used because the amino and carboxyl groups are connected to the alpha carbon of the carbon chain. However, there are different types of amino acids in nature, such as beta amino acids, in which the carboxyl and amino groups are connected to different carbons in the carbon chain. Within the human body, there are up to 20 different types of amino acids present. All alpha amino acids, except glycine, have four different substituents on the alpha carbon and are therefore chiral molecules. Amino acids can be categorized into two groups: replaceable and non-replaceable [5]. The body can produce replaceable amino acids, while non-replaceable amino acids are obtained through a protein-rich diet. The main elements of amino acids are carbon, hydrogen, oxygen and nitrogen. Isotopes of any of these elements can be used in biochemical studies as bio-markers to study their metabolism in the body and also to control the synthesis process [6]. Ways of determination directly from organic compounds carbon-13 and nitrogen-15 and some other ones compounds are given in these articles [7] [8] [9]. This article discusses techniques for analyzing isotopes in valine, proline, glutamine, and glutamic acids. These methods allow for the direct determination of the atomic fraction of nitrogen and carbon isotopes in these amino acids, regardless of whether they are located in the same or different nitrogen and carbon centers. Measurements were performed on an isotope mass spectrometer МИ-1201. Based on the study of the mass spectra of these amino acids, the ionic currents were determined. This allows us to determine the atomic fraction of carbon and nitrogen isotopes present in these compounds.

2. Experimental

Valine - In the valine molecule there are two branches, due to which the molecule is easily fragmented and therefore the molecular peak is not fixed.

The mass spectrum was taken on an isotope mass spectrometer MI-1201 (**Figure 1**). Fragment ions formed by the elimination of water and hydroxyl groups are fixed as traces. The most probable process in the fragmentation of a molecular ion in the spectrum is decarboxylation. At this time ions, whose m/z = 71; 72; 73, are formed, and fragmentation by removal of the methyl group is less likely. A rearrangement process also occurs in the molecular ion with the migration of the hydrogen atom

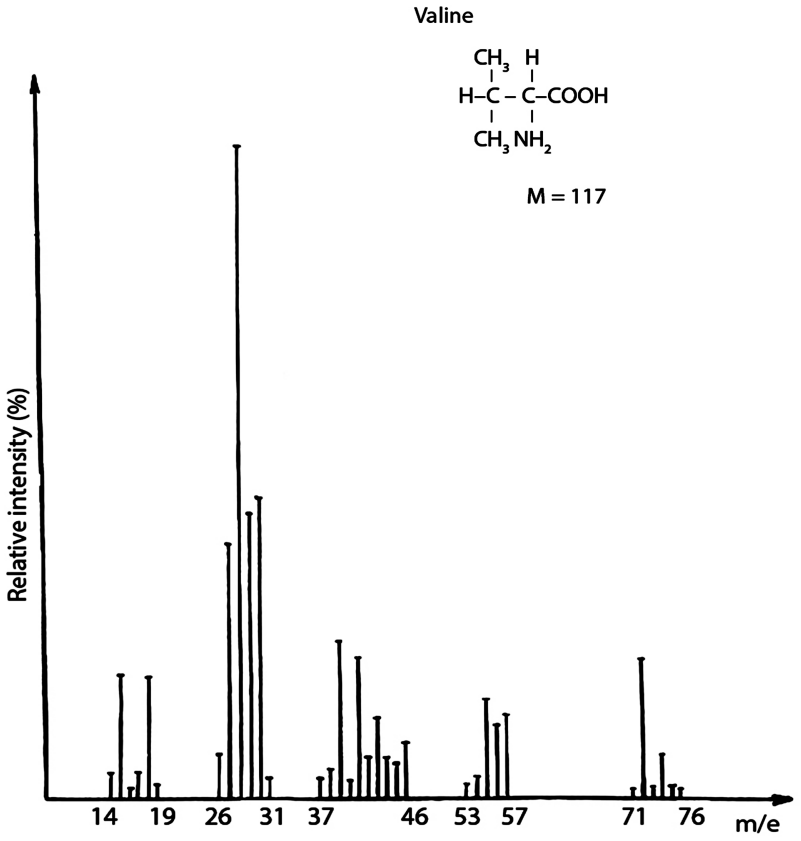

Figure 1. The mass spectrum of valine.

and ions m/z = 74; 75; 76 are formed. Ions with m/z = 28; 29; 43 are also seen in its spectrum. Which are undesirable for the determination of the atomic fraction. As research has shown, valine is relatively "inconvenient" for direct analysis. Specter analysis showed that carbon isotope analysis can be performed in valine modified with a carboxyl group or a carbon chain, with peak intensities at m/z = 74; 75; 76. In this case, we have to consider two processes, the elimination of the carboxyl group and the migration of hydrogen from the chain to the carboxyl group. In this case, the system of equations [5] used to calculate the atomic fraction of the carbon-13 isotope will be reduced to the following equation:

$$I_{74}y^2 - \left(I_{75} - 0.01620I_{74}\right)y + \left(I_{76} - 0.01634I_{75} - 0.00384I_{74}\right) = 0 \quad (1)$$

where I_{74} I_{75} I_{76} are the intensities of those peaks with m/z = 74; 75; 76. Accordingly, numerical coefficients take into account the natural distribution of isotopes $y = Xc^{12}/Xc^{13}$. The atomic fraction of the carbon-13 isotope is calculated by the formula

$$Xc^{13} = y/\left(y+1\right) \quad (2)$$

An approximate calculation may be made for m/z = 74; 75 mass lines, with the formula:

$$Xc^{13} = \left(I_{75} - 0.01620I_{74}\right)/\left(0.98380I_{74} + I_{75}\right) \quad (3)$$

However, neglecting the hydrogen migration process increases the measurement error. Table 1 shows the measurement results of carbon-13 isotope atomic fraction in natural valine, calculated by Formulas (1) and (3).

As can be seen from the table, accurate measurements can be made only by deriving the quadratic equation. By analyzing the Formula (3) and the spectrum, according to the change in the intensities of the ion currents, it is possible to determine the root of the equation when such a problem arises.

Through alpha cleavage during fragmentation, ions with m/z = 72; 73 are formed. But the presence of ions with m/z = 71 in the spectrum indicates the elimination of hydrogen from this fragment ion. which must be taken into account when determining the atomic fraction of carbon-13 and nitrogen-15 isotopes. Taking these two processes into account, the system of equations [6] will be reduced to the following equation:

$$10I_{71}y^2-\left(4I_{72}-0.02058I_{71}\right)y+\left(I_{73}-0.00514I_{72}-0.03513I_{71}\right)=0 \quad (4)$$

It is possible to determine approximately the atomic fraction of carbon-13 with m/z = 72; 73 mass lines, at the same time of need as described above.

$$Xc^{13}=\left(I_{73}-0.00514I_{72}\right)/\left(I_{73}+3.99486I_{72}\right) \quad (5)$$

But we have a relatively large error.

In order to simultaneously determine the atomic fraction of the carbon-13 isotope, both in the carboxylic and main chain carbon centers, the following system of equations must be solved:

$$\begin{cases}\left(y'+y''\right)^2 I_{74}-\left(I_{75}-0.00516I_{74}\right)\left(y'+y''\right)-I_{74}y'y'' \\ \quad +\left(I_{76}-0.00516I_{75}-0.00405I_{74}\right)=0 \\ 0.00516\left(y''\right)^2 I_{74}-\left(0.00516I_{75}-0.00003I_{74}\right)\left(y'y''\right) \\ \quad -0.00501I_{74}y'y''+\left(I_{77}-0.00408I_{75}\right)=0\end{cases} \quad (6)$$

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

	Equation (1)	Equation (3)
1	1.12	1.14
2	1.12	1.20
3	1.13	1.25
4	1.11	1.25
5	1.13	1.32
6	1.13	1.18
7	1.12	1.39
8	1.12	1.22
9	1.13	1.29
10	1.12	1.15
	1.12 ± 0.01	1.20 ± 0.01

Ions with m/z = 74; 75; 76 mass lines are also used to determine the atomic fraction of nitrogen-15 isotope in valine. In this case $y = N^{15}/N^{14}$ is determined by the equation:

$$I_{74}y^2 - \left(I_{75} - 0.02389I_{74}\right)y + \left(I_{76} - 0.02389I_{75} - 0.00368I_{74}\right) = 0 \quad (7)$$

In contrast to Equation (7), we can approximately calculate with the peaks of ions whose m/z = 74; 75

$$Xn^{15} = \left(I_{75} - 0.02374I_{74}\right)/\left(0.97626I_{74} + I_{75}\right) \quad (8)$$

The atomic fraction of nitrogen can also be calculated by ions with m/z = 71; 72; 73 mass lines. In this case, the system will be reduced to the following equation:

$$I_{71}y_2 - \left(I_{72} - 0.04630I_{71}\right)y + \left(I_{73} - 0.04630I_{72} - 0.00210I_{71}\right) \quad (9)$$

For an approximate definition, the formula has the following form:

$$Xn^{15} = \left(I_{73} - 0.04630I_{72}\right)/\left(0.95370I_{72} + I_{73}\right) \quad (10)$$

The atomic fraction of the nitrogen-15 isotope is calculated with simplified formulas in case the difficulty of root selection is created.

The results of the determination of the atomic fraction of the nitrogen-15 isotope in valine of natural content are given in Table 2.

Proline - is a cyclic amino acid, so it is a relatively stable compound, the spectrum of which is shown in Figure 2, taken on an isotope mass spectrometer MI-1201.

In the mass spectrum of proline, molecular ions are observed only as traces. During the ionization of the molecule, the charge is mainly localized on the nitrogen atom that is part of the nucleus, the fragment ion m/z = 70 will be observed in the spectrum, and during further fragmentation, the most intense are

Table 2. The atomic fraction of the nitrogen-15 isotope is determined by the equation.

	Equation (7)	Equation (8)
1	0.36	0.40
2	0.34	0.41
3	0.36	0.37
4	0.37	0.29
5	0.35	0.35
6	0.38	0.31
7	0.36	0.39
8	0.36	0.41
9	0.37	0.38
10	0.36	0.39
	0.36 ± 0.01	0.37 ± 0.01

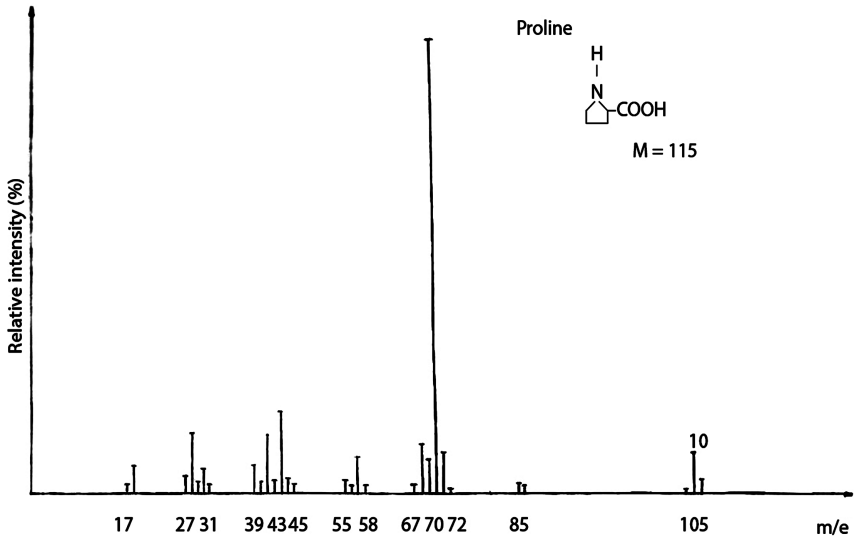

Figure 2. The mass spectrum of proline.

the ions obtained as a result of decarboxylation. The rest of the ions in the spectrum are obtained as a result of cycle decomposition and are relatively less intense. Therefore, when analyzing the atomic fraction of the carbon-13 isotope in the carbon centers of the cycle in proline, we must take into account both the decarboxylation process and hydrogen removal. The atomic fraction of the carbon-13 isotope can be determined by the ion peaks with m/z = 69, 70, and 71 in the following equation:

$$10I_{69}y^2 - \left(4I_{70} - 0.01939I_{70}\right)y + \left(I_{71} - 0.00485I_{70} - 0.01880I_{69}\right) = 0 \qquad (11)$$

In this case, I_{69}; I_{70}; I_{71} are the intensities of those peaks whose m/z = 69; 70; 71 and the atomic fraction of carbon-13 is calculated by the Formula (2).

Ions with m/z = 70; 71, a rough calculation can be made using the following formula:

$$Xc^{13} = \left(I_{71} - 0.00486\right)/\left(I_{71} + 3.99514I_{70}\right) \qquad (12)$$

where $I_{70.71}$ are the intensities of the peaks and the numerical coefficient takes into account the natural distribution of heavy isotopes included in the molecule.

In the mass spectrum of proline, it is impossible to determine the atomic fraction of the carbon-13 isotope in the carbon center of the carboxyl group by direct mass spectrometric analysis.

The results of measuring the atomic fraction of carbon-13 isotope in proline of natural content, calculated by Formulas (12) and (2) are given in **Table 3**.

As it is clear from the table, to get accurate results, we should use a quadratic equation that takes into account two processes during fragmentation.

Theoretically, it is possible to determine the atomic fraction of the carbon-13 isotope in proline with all centers equally enriched, with mass lines 114, 115, 116, using the following equation:

$$15I_{114}y - \left(5I_{115} - 0.0304I_{114}\right)y + \left(I_{116} - 0.00604I_{115} - 0.00185I_{114}\right) = 0 \qquad (13)$$

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

	Equation (12)	Equation (2)
1	1.11	1.17
2	1.12	1.24
3	1.12	1.28
4	1.11	1.25
5	1.12	1.19
6	1.11	1.21
7	1.14	1.18
8	1.12	1.22
9	1.12	1.24
10	1.13	1.18
	1.12 ± 0.01	1.20 ± 0.01

When proline is isotope modified with nitrogen-15 isotope. The atomic fraction, as in the case of carbon-13, is calculated taking into account the mentioned two processes, and the equation has the following form:

$$I_{69}y^2-\left(I_{70}-0.04600I_{69}\right)y+\left(I_{71}-0.04600I_{70}-0.00206I_{69}\right)=0 \quad (14)$$

To make approximate measurements, the following formula can be used:

$$Xn^{15}=\left(I_{71}-0.04600I_{70}\right)/\left(I_{70}+0.95400I_{71}\right) \quad (15)$$

Table 4 presents the results obtained by equation (15).

Glutamine - The mass spectrum of glutamine is shown in **Figure 3**, the spectrum was taken on an isotope mass spectrometer MI-1201.

Glutamine contains two amino groups and one carboxyl group. Due to the presence of three functional groups, the molecule is easily fragmented and molecular ions are not observed in the mass spectrum, the most intense peak of ions with m/z = 84, which is formed by the migration of the hydrogen atom in the molecular ion to one of the amine groups, and at the same time the carboxyl group and ammonia are removed. Hydroxyl radical is also removed from the carboxyl group and ions with m/z = 129 are formed.

It is appropriate to use isotopic analysis for ions with m/z values of 128, 129, and 130. However, it is important to consider the elimination of the hydroxyl group and water from the molecular ion. As a result, the system of equations will be reduced to a single equation.

When glutamine is isotopically modified at all carbons, it is appropriate to use isotopic analysis for ions with m/z values of 128, 129, and 130. At this point, we have to take into account the elimination of the hydroxyl group and water from the molecular ion. In this case, the system of equations will be reduced to the following equation:

$$15I_{128}y^2-\left(5I_{129}-0.04625I_{128}\right)y+\left(I_{130}-0.00940I_{129}-0.00403I_{128}\right)=0 \quad (16)$$

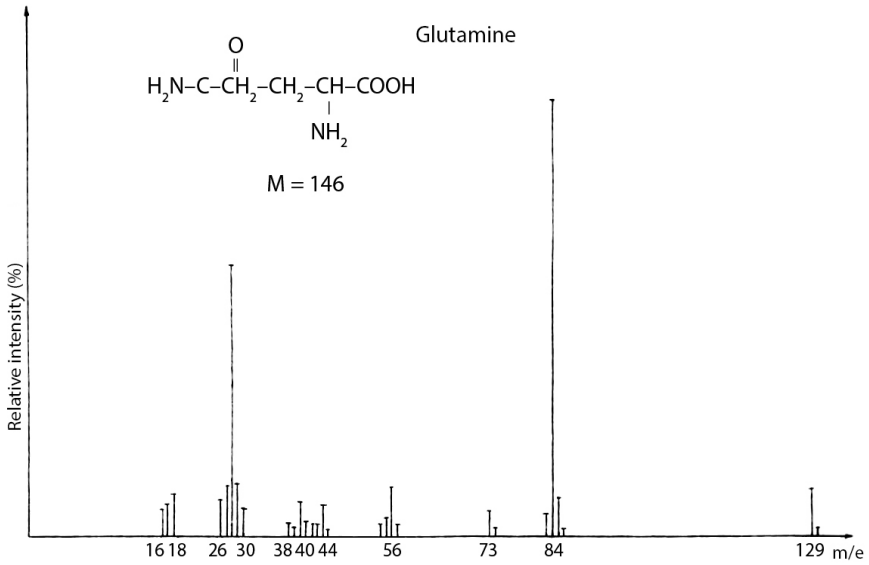

Figure 3. The mass spectrum of glutamine.

Table 4. Results of nitrogen-15 isotope measurements in naturally occurring proline.

	Equation (15)	Equation (16)
1	0.37	0.35
2	0.35	0.38
3	0.36	0.39
4	0.36	0.40
5	0.34	0.40
6	0.37	0.38
7	0.36	0.37
8	0.37	0.35
9	0.36	0.39
10	0.35	0.37
	0.36 ± 0.01	0.38 ± 0.01

In the case of approximate analysis, we can use the formula:

$$Xc^{13} = \left(I_{130} - 0.00940 I_{129}\right) / \left(4.99060 I_{129} + I_{130}\right) \quad (17)$$

Table 5 shows the results of the analysis of glutamine of natural content with all carbons, calculated by Formulas (16) and (17):

When glutamine is modified only by the carbon of the carboxyl group, then we similarly take into account the dehydration and elimination of the hydroxyl group, and the atomic fraction of the carbon-13 isotope is calculated by the formula:

$$I_{128} y^2 - \left(I_{129} - 0.05422 I_{128}\right) y + \left(I_{130} - 0.05422 I_{129} - 0.00234 I_{128}\right) = 0 \quad (18)$$

Table 5. Atomic fraction of carbon-13 isotope determined by equations.

	Equation (16)	Equation (17)
1	1.12	1.20
2	1.13	1.21
3	1.13	1.21
4	1.12	1.28
5	1.14	1.14
6	1.12	1.23
7	1.12	1.18
8	1.13	1.19
9	1.11	1.28
10	1.12	1.22
	1.12 ± 0.01	1.20 ± 0.01

The simplified formula has the following form:

$$Xc^{13} = \left(I_{130} - 0.005422I_{129}\right)/\left(0.99458I_{129} + I_{130}\right) \quad (19)$$

When glutamic acid is modified only by chain carbons, then the system reduces to the following equation:

$$10I_{128}y^2 - \left(4I_{129} - 0.08183I_{128}\right)y + \left(I_{130} - 0.02060I_{129} - 0.00380I_{128}\right) = 0 \quad (20)$$

Calculating the atomic fraction of carbon in glutamine is also possible with m/z = 84; 85; 86 mass lines. When glutamine is isotopic modified by the carbon centers of the chain, the system of equations will take the following form:

$$10I_{84}y^2 - \left(4I_{85} - 0.001969I_{84}\right)y + \left(I_{86} - 0.00507I_{85} - 0.00203I_{84}\right) = 0 \quad (21)$$

And for approximate calculations:

$$Xc^{13} = \left(I_{86} - 0.00492I_{85}\right)/\left(3.99508I_{85} + I_{86}\right) \quad (22)$$

When glutamine is modified with both functional groups, the atomic fraction of carbon-13 is calculated along the same mass lines with the following equation:

$$3I_{128}y^2 - \left(2I_{129}y - 0.08602I_{128}\right)y + \left(I_{130} - 0.04301I_{129} - 0.00296I_{128}\right) = 0 \quad (23)$$

When glutamine is isotopically modified with both nitrogen centers equally, then the nitrogen-15 isotope calculation system reduces to:

$$3I_{128}y^2 - \left(2I_{129} - 0.11618I_{128}\right)y + \left(I_{130} - 0.05809I_{129} - 0.00209I_{128}\right) = 0 \quad (24)$$

The following formula can be used for approximate calculations:

$$Xn^{15} = \left(I_{130} - 0.0509I_{129}\right)/\left(0.94191I_{129} + I_{130}\right) \quad (25)$$

When glutamine is modified with only one nitrogen center, a system of isotope's atomic fraction calculation reduces to the following equation:

$$I_{128}Y^2 - \left(I_{129} - 0.06176I_{128}\right)y + \left(I_{130} - 0.06176I_{129} - 0.00186I_{128}\right) = 0 \quad (26)$$

The following formula can be used for approximate calculations:

$$Xn^{15} = \left(I_{130} - 0.06161 I_{129}\right) / \left(0.93839 I_{129} + I_{130}\right) \quad (27)$$

The atomic fraction of the nitrogen-15 isotope can be calculated using the ions with m/z = 84; 85; 86. In this case, the system of equations will be reduced to the equation:

$$I_{84} y^2 - \left(I_{85} - 0.04622 I_{84}\right) y + \left(I_{86} - 0.04622 I_{85} - 0.04395 I_{84}\right) = 0 \quad (28)$$

Because of the high intensity, it is best to perform basic calculations using the m/z = 83; 84; 85 mass lines. Table 6 shows the atomic fraction of nitrogen-15 isotope in the natural isotopic content of glutamine calculated by Formulas (24) and (28).

Glutamic Acid - Figure 4 shows the mass spectrum of glutamic acid

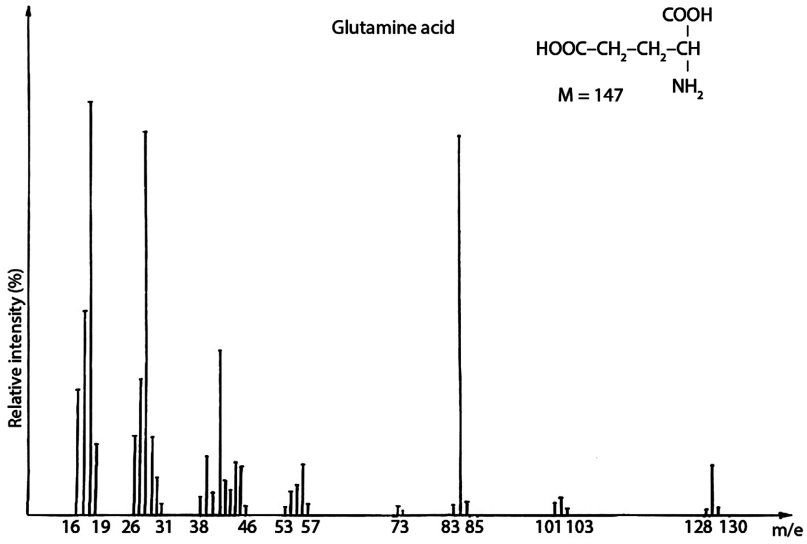

Figure 4. The mass spectrum of glutamine acid.

Table 6. Atomic fraction of nitrogen-15 isotope determined by equations.

	Equation (24)	Equation (28)
1	0.35	0.38
2	0.36	0.35
3	0.36	0.40
4	0.35	0.39
5	0.36	0.38
6	0.37	0.40
7	0.35	0.38
8	0.35	0.38
9	0.36	0.39
10	0.37	0.39
	0.36 ± 0.01	0.38 ± 0.01

In glutamic acid, alpha cleavage is unlikely and the peak ion m/z = 102 is of low intensity. The most important event is the dehydration of the molecule, which results formation of ions with m/z = 129 and 130. Subsequently, the carboxyl group is removed, and ions with m/z = 84 and 85 are formed. The spectrum shows a peak with m/z = 83, which means hydrogen was removed. This compound, like other amino acids, does not undergo amine-type decomposition. Therefore, the peaks of ions with m/z = 73, 74, 57, and 30 are very low in intensity. The spectrum will also observe ions with m/z = 56 $(CH\text{-}CH2\text{-}CH\text{-}NH2)^{+}$ and ions with m/z = 41 $(CH2\text{-}CH2\text{-}CH, CO\text{-}CH)^{+}$.

In all carbon-modified glutamic acids, direct isotopic analysis is preferably performed on the ion peaks formed by the elimination of water and hydroxyl groups from the molecular ion. In this case, the system of equations will be reduced to the following equation:

$$15I_{129}y^2-\left(5I_{130}-0.05885I_{129}\right)y+\left(I_{131}-0.00596I_{130}-0.00002I_{129}\right) \quad (29)$$

where $y = Xc^{12}/Xc^{13}$, I are the intensities of the corresponding peaks. Numerical coefficients take into account the natural distribution of nitrogen, oxygen and hydrogen. The atomic fraction of the carbon-13 isotope is calculated by the formula:

$$Xc^{13} = y/\left(1+y\right) \quad (30)$$

As the analysis of the spectrum showed, it is better to calculate the atomic fraction of carbon and nitrogen isotopes with the peaks of ions with m/z = 83; 84; 85 with mass lines. The system of equations in this case is reduced to the following equation:

$$10I_{83}y^2-\left(4I_{84}-0.01968I_{83}\right)y+\left(I_{85}-0.00492I_{84}-0.00218I_{83}\right)=0 \quad (31)$$

When selecting a root, the approximate formula for the determination of the carbon-13 isotope can be used:

$$Xc^{13}=\left(I_{85}-0.00492I_{84}\right)/\left(3.99508I_{84}+I_{85}\right) \quad (32)$$

When glutamic acid is isotopically modified with the carbon of the carboxyl group, then the system of equations reduces to the following equation:

$$I_{83}y^2-\left(I_{84}-0.03839I_{83}\right)y+\left(I_{85}-0.03853I_{84}-0.00111I_{83}\right)=0 \quad (33)$$

And the approximate (auxiliary) formula has the following form:

$$Xc^{13}=\left(I_{85}-0.03853I_{84}\right)/\left(0.96147I_{84}+I_{85}\right) \quad (34)$$

When glutamic acid is enriched with chain carbons, the system of equations reduces to the following equation:

$$6I_{83}y^2-\left(3I_{84}-0.04839I_{83}\right)y+\left(I_{85}-0.01613I_{84}-0.00183I_{83}\right)=0 \quad (34)$$

It is also possible to:

$$6I_{129}y^2-\left(3I_{130}-0.16932I_{129}\right)y+\left(I_{131}-0.02837I_{130}-0.00559I_{129}\right)=0 \quad (35)$$

The atomic fraction of the carbon-13 isotope in glutamic acid enriched with a carboxylic group or any carbon in the chain is calculated by the following for-

mula:

$$I_{83}y^2-\left(I_{84}-0.03853I_{83}\right)y-\left(I_{85}-0.03853I_{84}-0.00073I_{83}\right)=0 \quad (36)$$

or

$$3I_{128}y^2-\left(2I_{130}-0.15798I_{129}\right)y-\left(I_{131}-0.03957I_{130}-0.00526I_{128}\right)=0 \quad (37)$$

If glutamic acid is unevenly enriched with chain and carboxyl function groups carbon centers, then it is necessary to determine the intensities of the peaks with m/z = 130, 131, 132 and the system of equations will be reduced to the following type of equation:

$$\begin{cases}\left(2y'+3y''\right)^2 I_{129}-\left(y'^2+6y'y''+3y''^2\right)I_{129}-\left(I_{130}-0.005985I_{129}\right)\\ \quad *\left(2y'+3y''\right)+\left(I_{131}-0.005985I_{130}-0.00609I_{129}\right)=0\\ \left(y'^2+6y'y+3y''^2\right)\left(2y'+3y\right)I_{129}-\left(3y'^2y+6y'y^2+y''^3\right)I_{129}\\ \quad -\left(y'^2+3y^2+6y'y''\right)I_{130}-\left(0.005985I_{130}-0.00003I_{129}\right)\\ \quad *\left(2y'+3y''\right)+\left(I_{132}-0.00612I_{130}\right)=0\end{cases} \quad (38)$$

$y'$is the ratio of carbon-13 isotope to carbon-12 isotope in the carboxyl group and y''in the carbon centers of the chain.

It is also possible to calculate m/z = 84; 85; 86; 87 with mass lines:

$$\begin{cases}\left(y'+3y''\right)^2 I_{84}-\left(3y'y''+y''^2\right)I_{84}-\left(I_{85}-0.005085I_{84}\right)\left(y'+3y''\right)\\ \quad +\left(I_{86}-0.005085I_{85}-0.06201I_{84}\right)=0\\ \left(3y'y+3y^2\right)\left(y'+3y\right)I_{84}-\left(3y'y+3y^2\right)I_{85}+0.00509\left(y'+3y\right)^2 I_{84}\\ \quad -\left(3y'y''^2+y''^3\right)I_{84}-\left(0.005085I_{85}-0.00003I_{84}\right)\left(y'+3y''\right)\\ \quad +\left(I_{87}-0.00204I_{85}\right)=0\end{cases} \quad (39)$$

The atomic fraction of carbon-13 isotope in glutamic acid is calculated by Formulas (29) and (31) and the results are given in Table 7.

Table 7. Atomic fraction of carbon-13 isotope in glutamic acid.

	Equation (29)	Equation (31)
1	1.13	1.21
2	1.12	1.21
3	1.13	1.21
4	1.11	1.27
5	1.12	1.18
6	1.12	1.22
7	1.12	1.20
8	1.13	1.19
9	1.12	1.25
10	1.14	1.22
	1.12 ± 0.01	1.20 ± 0.01

To calculate the atomic fraction of the nitrogen-15 isotope, we can use m/z = 83; 84; 85 mass lines

$$I_{83}y^2-\left(I_{84}-0.04608I_{83}\right)y+\left(I_{85}-0.04608I_{84}-0.00074I_{83}\right)=0 \quad (40)$$

And the auxiliary formula has the following form:

$$Xn^{15}=\left(I_{85}-0.04608I_{84}\right)/\left(0.95392I_{84}+I_{85}\right) \quad (41)$$

The isotopic content of nitrogen and carbon can be determined by m/z = 129; 130; 131 using mass lines. In this case, the system reduces to the following equation:

$$I_{129}y^2-\left(I_{130}-0.05832I_{129}\right)y+\left(I_{131}-0.05832I_{130}-0.00412I_{129}\right)=0 \quad (42)$$

3. Results and Discussion

With mass spectrums, it's possible to directly determine the atomic shares of carbon-13 and nitrogen-15 isotopes in compounds, whether they are completely modified or just modified with a certain center. These methods are self-verifying, as the results can be verified simultaneously using different mass lines. The higher order of the equation depends on the concentration, and it's unnecessary to consider the processes that cause the result to change within the given error margin. Therefore, when determining the atomic fraction of these elements, relatively low-quality equations can be used to determine the isotopic ratio with the necessary precision for absolute measurement.

4. Conclusion

It is possible to directly determine the carbon-13 and nitrogen-15 atomic shares in valine, proline, glutamine, and glutamic acid compounds. The accuracy of the absolute measurements obtained can be checked using other mass lines and is considered quite acceptable.

Conflicts of Interest

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

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