

Mass-Spectrometric Method of Measurement of Isotopic Content of Nitrogen in Organic Compounds

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Abstract

Nitrogen-15 isotope-modified compounds are widely used in medicine, pharmacology, agriculture and various fields of science and their nomenclature is gradually increasing. Their widespread use depends on the availability of inexpensive and simple isotope analysis methods. The present article is an attempt to determine the nitrogen-15 isotope content directly in organic compounds without their conversion. The general principle of possibility of determination of the isotopes of nitrogen directly in organic compounds is proposed. Based on the study of mass-spectra of Carbamide Carbonyldiamide, isocyanic acid and nitrobenzene the mass peaks are selected, by which it is possible to determine the atomic fraction of the isotopes of nitrogen. The respective formulas are proposed.

Keywords

Atomic Fraction, Isotope, Nitrogen, Molecular Ions, Mass Spectrometer, Mass Spectrum, Monoisotopic Forms, Carbamide Carbonyldiamide (Urea), Isocyanic Acid, Nitrobenzene, Isotope Analysis, Isotope-Modified Compound, Nitrogen Center, Monoisotopic Form, Two Different Nitrogen Centers

1. Introduction

Compounds labeled with stable isotopes of light elements are widely used in various fields of science. At the same time, their applicable scope is gradually expanding. Use of these compounds allows for solving problems that could not be solved without their use. The increase in the use of stable isotopes was due to the increase in the nomenclature of these compounds. At the same time, the number of isotopically labeled compounds increases day by day.

The use of labeled compounds, to a large extent, depends on the existence of accurate, reliable, express and inexpensive methods of determining the atomic fraction of the labeled element in them. Until recently, these compounds are being converted into compounds “convenient” for mass spectrometric analysis. Compounds labeled with isotopes of nitrogen are being transformed into molecular nitrogen or nitrogen oxide [1]. But meantime a relatively large amount of expensive organic compounds is consumed; the conversion process is added that reduces rapidness and accuracy. During the conversion process, it is possible to distort the true value of the isotopic concentration not only by the atmospheric nitrogen, but also by nitrogen-containing impurities existing in the compound. In some cases, conversion is impossible due to the small quantity of synthesized compounds.

We have proposed general mass spectrometric methods for the determination of isotopic content of hydrogen [2], carbon [3], oxygen [4] and boron [5], directly in the organic compounds to be analyzed.

2. Experimental

This paper discusses the mass spectrometric method of determining the isotopic content of nitrogen.

The fragmentation of organic compounds during ionization by electron bombardment is the main difficulty in determining the isotopic content of nitrogen in them, because the monoisotopic forms of molecular and various types of fragmentary ions with equal mass-to-charge ratio are registered as a single mass peak. It should also be noted that the protonated, molecular, and fragmentary ions may occur due to the secondary processes taking place in the ionization chamber. The formation of isobaric fragments is mainly due to hydrogen abstraction, protonation, or hydrogen migration processes. The mass spectra of synthesized organic compounds and the mechanisms of their fragmentation are known. There are spectra banks of these compounds, but the use of these spectra for isotopic analysis requires additional study because small probabilistic processes that are not considered in the study of spectra, make it impossible to process the isotopic content of the elements directly in these compounds.

To address this superposition, we have proposed a mathematical model for solving a problem. We derive a system of equations. At this point, we take into account that bond breaking is equally probable for different isotopes of elements, and that the magnitude of the ionic currents is proportional to the isotopic concentration of the elements in it. When compiling the system, we consider only the number of nitrogen and hydrogen atoms in the compound. The presence of isotopes of other elements does not change the form of the system of equations. They are accounted for by the natural distribution through numerical coefficients for a given specific compound.

The general system of equations for calculation of atomic fractions of nitrogen isotopes in organic compounds, uniformly isotopically modified for every nitro-

gen center, has the following form:

$$\left\{ \begin{array}{l} I_{M-p} = K\alpha_0(1-x)^n \\ I_{M-p+1} = K\left[\alpha_1(1-x)^n + n\alpha_0(1-x)^{n-1}X\right] \\ I_{M-p+2} = K\left[\alpha_2(1-x)^n + n\alpha_1(1-x)^{n-1}X + \frac{n(n-1)}{2}\alpha_0(1-x)^{n-2}X^2\right] \\ \vdots \\ I_{M+p} = K\left[\alpha_p(1-x)^n + n\alpha_{p-1}(1-x)^{n-1}X + \dots + \alpha_{p-n}X^n\right] \\ I_{M+p+1} = K\left[n\alpha_p(1-x)^{n-1}X + \frac{n\cdot n-1}{2}\alpha_{p-1}(1-x)^{n-2}X^2 + \dots + \alpha_{p-n+1}X^n\right] \\ \vdots \\ I_{M+p+n} = K\alpha_pX^n \end{array} \right. \quad (1)$$

where x is the atomic fraction of nitrogen-15, $I_{M-p}, I_{M-p+1}, \dots, I_{M+p+n}$ are the intensities of those peaks, which $m/z = M-p, p-n+1, \dots, I_{M+p+n}$; M is the mass of ion, z —charge, k is the coefficient of proportionality between probability of origination of various types of ions and their intensities; $\alpha_0, \alpha_1, \dots, \alpha_p$ are the probabilities of origination of ions with the given gross-formula, n — is the number of nitrogen atoms, p — the number of hydrogen atoms.

The system of equations consists of $p + 2$ unknown quantities. We choose various equations according to the value of atomic fraction of the isotope nitrogen-15. If the atomic fraction of the isotope nitrogen-15 is less than 50%, we choose the first $p + 2$ equations. In such case the system of equations is reduced to the equation:

$$\begin{aligned} & (-1)^{p+1} \frac{(n+p)!}{(p+1)!(n-1)!} I_{M-p} Y^{p+1} + (-1)^p \frac{(p+n-1)!}{p!(n-2)!} I_{M-p+1} Y^p \\ & + (-1)^{p-1} \frac{(p+n-2)!}{(p-1)!(n-3)!} I_{M+2} Y^{p-1} + \dots + (-1)n I_{M+p} Y + I_{M+p+1} = 0 \end{aligned} \quad (2)$$

where,

$$Y = \frac{{}^{15}X}{{}^{14}X} \quad (3)$$

is the ratio of values of atomic fractions of the isotopes nitrogen-15 and nitrogen-14.

The atomic fraction of the isotope nitrogen-15 in percent is calculated by the formula:

$$X_{15} \% = \frac{Y}{Y+1} \% \quad (4)$$

If the atomic fraction of the isotope nitrogen-15 is more than 50%, then we take the last $m + 2$ equations; then the system of equations is reduced to the equation:

$$\begin{aligned} & (-1)^{p+1} \frac{(p+n)!}{(p+1)!(n-1)!} I_{M+p+n} Y_1^{p+1} + (-1)^p \frac{(p+n-1)!}{p!(n-2)!} I_{M+p+n-1} Y_1^p \\ & + (-1)^{p-1} \frac{(p+n-2)!}{(p-1)!(n-3)!} I_{M+p+n-2} Y_1^{p-1} + \dots + (-1)n I_{M+p} Y_1 + I_{M+p-1} = 0 \end{aligned} \quad (5)$$

where

$$Y_1 = \frac{{}^{14}X}{{}^{15}X}$$

is the ratio of values of atomic fractions of the isotopes nitrogen-14 and nitrogen-15. The atomic fraction of the isotope nitrogen-15 in this case is calculated by the formula:

$$X = \frac{1}{1 + Y_1} \quad (6)$$

If an organic compound is isotopically modified only by one isotope center, then the system is written in the form:

$$\begin{cases} I_{M-p} = K\alpha_0(1-x) \\ I_{M-p+1} = K[\alpha_1(1-x) + \alpha_0x] \\ I_{M-p+2} = K[\alpha_2(1-x) + \alpha_1x] \\ \vdots \\ I_M = K[\alpha_p(1-x) + k\alpha_{p-1}x] \\ I_{M+1} = K\alpha_p x \end{cases} \quad (7)$$

In this case the system of equations is reduced to the equation:

$$\begin{aligned} & (-1)^{p+1} I_{M-p} Y^{p+1} + (-1)^p I_{M-p+1} Y^p + (-1)^{p-1} I_{M-p+2} Y^{p-1} + \dots \\ & + (-1) I_M Y + I_{M+1} = 0 \end{aligned} \quad (8)$$

In the case, if a compound is isotopically modified by not one, but several centers, the system of equations will take the form:

$$\begin{cases} I_{M-p} = K\alpha_0(1-x)^q \\ I_{M-p+1} = K[\alpha_1(1-x)^q + \alpha_0q(1-x)^{q-1}X] \\ I_{M-p+1} = K[\alpha_2(1-x)^q + \alpha_1q(1-x)^{q-1}X + \alpha_0\frac{q(q-1)}{2}(1-x)^{q-2}X^2] \\ \vdots \\ I_M = K[\alpha_p(1-x)^q + \alpha_{p-1}q(1-x)^{q-1}X + \dots + \alpha_{p-q}X^q] \\ I_{M+1} = K[\alpha_p(1-x)^{q-1}X + \alpha_{p-1}\frac{q(q-1)}{2}(1-x)^{q-2}X^2 + \dots + \alpha_{p-q+1}X^2] \\ \vdots \\ I_{M+q-1} = K[\alpha_{p-q}(1-X)X^{q-1} + \alpha_{p-1}X^q] \\ I_{M+q} = K\alpha_p X^q \end{cases} \quad (9)$$

where q is the quantity of isotopically modified nitrogen centers that is reduced to the equation:

$$\begin{aligned} & (-1)^{p+1} \frac{(p+q)!}{(p+1)!(q-1)!} I_{M-p} Y^{p+1} + (-1)^p \frac{(p+q-1)!}{p!(q-2)!} I_{M-p+1} Y^p \\ & + (-1)^{p-1} \frac{(p+q-2)!}{(p-1)!(q-3)!} I_{M-p+2} Y^{p-1} + \dots + (-1)^q I_M Y + I_{M+1} = 0 \end{aligned} \quad (10)$$

The value of the isotope nitrogen-15 in the non-modified centers is taken into account by means of numerical coefficients, according to a natural distribution.

The given equations allow to determine the atomic fractions of nitrogen isotopes, directly in an organic compound. However in each specific case it is necessary to study the mass spectrum of the compound and determine the processes that are to be taken into account during formation of the system of equations. For stable molecules abstraction of two or more hydrogens is less probable process, so it is not necessary to take them into consideration. In this case in the system of equations the number of equations and the unknown quantities decrease, respectively the degree of equation decreases. It is also necessary to take into account the process of protonation in polar molecules, at determination of the isotope content, while in non-polar molecules it is of such low probability that the damage caused by the process is one order less than the measuring inaccuracy.

For the given specific compound, using this mathematical model it is possible to determine content of nitrogen isotopes even on fragmentary ions that increases the accuracy and reliability of the method.

3. Results and Discussion

Based on the aforesaid the methods of determination of atomic fractions of nitrogen isotopes directly in Carbamide Carbonyldiamide, Isocyanic acid and Nitrobenzene have been proposed. The mass spectrum of Carbamide Carbonyldiamide, recorded on the mass spectrometer MI-1201, is given on **Figure 1**.

As is well known The positive charge on the ion obtained by electron bombardment of Carbamide Carbonyldiamide is predominantly observed on one of the amino groups. This determines the form of the spectrum. The maximum in the spectrum is the molecular ion with $m/z = 60$. Fragmentation of the molecular ion by the abstraction of hydrogen will be virtually unnoticed. The mass peak 16, *i.e.* the peak, corresponding to the ionic current NH_2^+ , is intensive. The mass peak $m/z = 44$, *i.e.* the peak corresponding to the ionic current $(\text{NH}_2\text{-CO})^+$ is

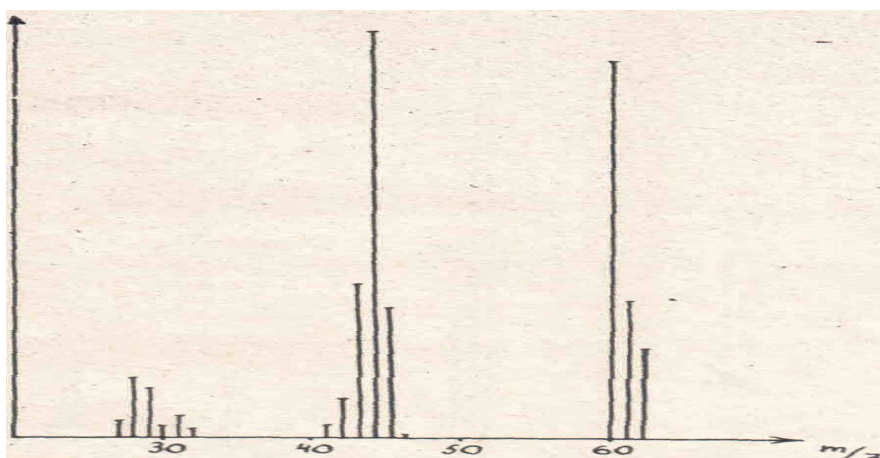


Figure 1. The mass spectrum of Carbamide Carbonyldiamide.

also intensive. It is unlikely that a positive charge will be localized on the oxygen atom, so $m/z = 28$ (ionic current CO^+) is of much lower intensity. Based on the spectrum analysis, we have a nitrogen element on the following mass peaks, $m/z = 60$ (molecular), $m/z = 44$ (fragmentary ions $(\text{NH}_2\text{-CO})^+$) and $m/z = 16$ $(\text{NH}_2)^+$. Nevertheless, on the mass peak $m/z = 44$ the background superposition of the ions CO_2^+ is probable, as well as the superposition of some substances in the analyzed sample that increases the error. A background superposition occurs on the mass peak $m/z = 16$, which is obtained by fragmentation of carbon dioxide, molecular oxygen, and water. Therefore, we use only molecular ions to determine the atomic fraction of nitrogen isotopes.

The molecule of Carbamide Carbonyldiamide is symmetric in respect to amino groups, therefore the nitrogen isotopes are uniformly distributed in both nitrogen centers.

The atomic fraction of the isotope nitrogen-15 in percent is expressed by the formula:

$$X^{15}\text{N}\% = \frac{1}{1 + 2 \frac{I_{60}}{I_{61} - 0.01217I_{60}}} \cdot 100\% \quad (11)$$

whereas

$$X^{14}\text{N}\% = \frac{2 \frac{I_{60}}{I_{61} - 0.01217I_{60}}}{1 + 2 \frac{I_{60}}{I_{61} - 0.01217I_{60}}} \cdot 100\% \quad (12)$$

where I_{60} , I_{61} are the intensities of the peaks of mass numbers $m/z = 60, 61$; $X^{15}\text{N}$ and $X^{14}\text{N}$ are the atomic fractions of the isotopes nitrogen-15 and nitrogen-14. The numeric coefficient takes into account the natural distribution of heavy isotopes of carbon, oxygen and hydrogen in the molecule. Using this approach we have conducted the isotope analysis of the samples, in which the atomic fractions of the isotope nitrogen-15 was 20.0%; 56.1% and 61.7%. The results of measurements carried out directly in the sample of urea were 20.2%; 56.0% and 61.7%, *i.e.* within the accuracy of measurements agree with the data of certificate.

The mass spectrum of Isocyanic acid, recorded on the isotope mass spectrometer MI-1201B, is given on **Figure 2**.

The molecular peak in the spectrum is sufficiently intensive and amounts to 18% of the maximal peak. The maximal peak of the spectrum is $m/z = 41$ that is obtained by localization of charge on the atom, followed by hydrogen migration on amino group and elimination of carbon dioxide, *i.e.* the ionic fragment $(\text{CH}_2 = \text{C} = \text{NH})^+$ is formed. The peak with the mass number $m/z = 68$, *i.e.* the ionic fragment $(\text{M-OH})^+$, is also intensive in the spectrum, where the abstraction of oxygen atom occurs with low probability after migration of hydrogen. Abstraction of hydrogen atoms from molecular ions was not registered in the spectrum, but the ion-molecular reaction is observed, during which the hydrogen atom

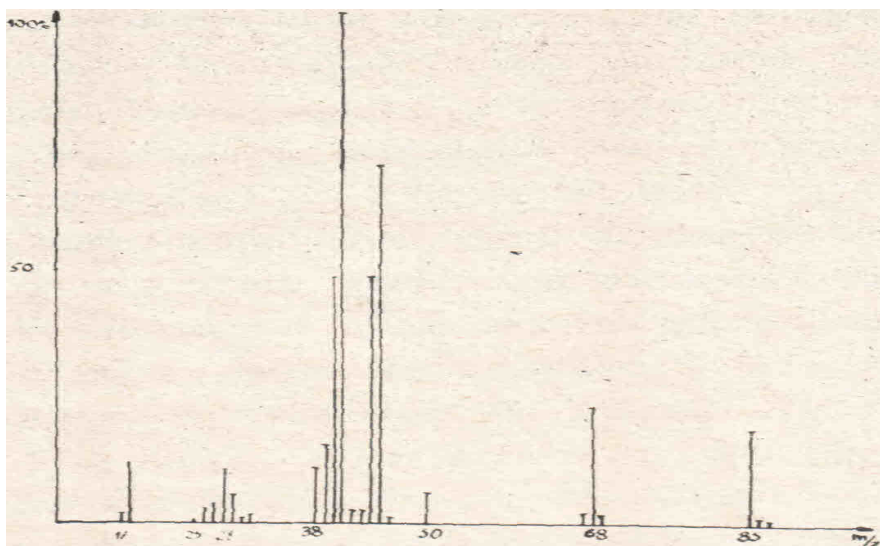


Figure 2. The mass spectrum of Isocyanic acid.

addition, or protonation and formation of $(M+H)^+$ takes place. Despite the low probability of the process, it is important to take it into account, if the atomic fraction of the isotope nitrogen-15 is less than 2%. From the maximal peak with the mass number $m/z = 41$, from this fragmentary ion the atoms of hydrogen are intensively abstracted, as a result for determination of the atomic fraction of nitrogen-15 by these mass peaks it is necessary to solve the high order equations that is undesirable.

Proceeding from the aforesaid, the atomic fraction of the nitrogen isotopes must be determined by means of the intensities of the ionic currents of the ions M^+ and $(M-OH)^+$.

At determination of the atomic fraction of the isotope nitrogen-15 by the peaks of ions $(M-OH)^+$ the low probable process must be taken into account—elimination of water and fragmentation by abstraction of oxygen. By taking into consideration these processes, the system of equations will be reduced to the cubic equation of the following form:

$$I_{67}Y^3 - (I_{68} - 0.03443I_{67})Y^2 + (I_{69} - 0.03443I_{68} - 0.00127I_{68})Y - (I_{70} - 0.03443I_{69} - 0.00116I_{68} - 0.00012I_{67}) = 0 \quad (13)$$

where

$$Y = {}^{15}X/{}^{14}X \quad (14)$$

whereas the fraction of nitrogen-15 and nitrogen-14 is calculated by the formula:

$$X^{15}N\% = \frac{Y}{Y+1} \cdot 100\% \quad (15)$$

$$X^{14}N\% = \frac{1}{Y+1} \cdot 100\% \quad (16)$$

A determination of atomic fraction of the isotope nitrogen-15 by means of molecular ions is far easier, if $X^{15}N\% > 5\%$, then

$$X^{15}N\% = \frac{1}{1 + \frac{I_{85}}{I_{86} - 0.03480I_{85}}} \cdot 100\% \quad (17)$$

whereas

$$X^{14}N\% = \frac{\frac{I_{85}}{I_{86} - 0.03480I_{85}}}{1 + \frac{I_{85}}{I_{86} - 0.03480I_{85}}} \cdot 100\% \quad (18)$$

whereas, when $X^{15}N\% < 3\%$, then the protonation process is to be taken into account. The system of equations will be reduced to the equation:

$$I_{85}Y^2 - 2(I_{86} - 0.03477I_{85})Y + (I_{87} - 0.03492I_{86} - 0.00325I_{85}) = 0 \quad (19)$$

The conducted measurements have demonstrated that the process of evaporation of the sample is unstable, as a result the accuracy of the measurement is rather low. Therefore tungsten powder is to be added to the sample in proportion 1:1. It slows and stabilizes the evaporation process. After that the spectrum is stable and the accuracy of measurement significantly increases.

The measurement has been carried out on the sample, in which the atomic fraction of the isotope nitrogen-15 according to the certificate was 69.8%.

The mass spectrum of Nitrobenzene is sufficiently specific, the mass spectrum, recorded on the isotope mass spectrometer is given on **Figure 3**. The maximal peak of the mass spectrum is (M-46), which $m/z = 47$, i.e. the ion $(C_6H_7)^+$, from which the fragmentary ions of the spectrum are obtained. Only the sufficiently intensive molecular ion contains nitrogen.

Proceeding from the aforesaid, the atomic fraction of nitrogen isotopes in Nitrobenzene is determined by the formula:

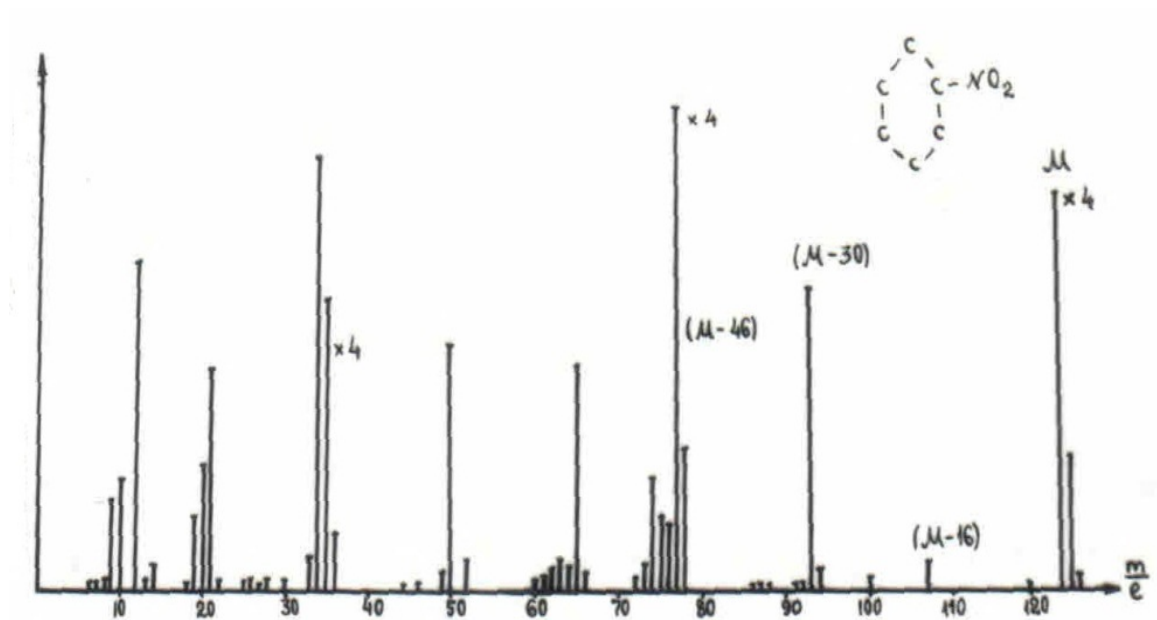


Figure 3. The mass spectrum of Nitrobenzene.

$$X^{15}N\% = \frac{1}{1 + \frac{I_{123}}{I_{124} - 0.06871I_{123}}} \cdot 100\% \quad (20)$$

$$X^{14}N\% = \frac{\frac{I_{123}}{I_{124} - 0.06871I_{123}}}{1 + \frac{I_{123}}{I_{124} - 0.06871I_{123}}} \cdot 100\% \quad (21)$$

The atomic fractions of the isotope nitrogen-15 has been measured by this method in the samples, in which according to the certificate, it was 64.5% and 89.5%, that corresponds to the results of the measurement 64.6% and 89.6%.

4. Conclusion

The given methods meet requirements to the isotope analysis and are successfully used for determination of the atomic fraction of nitrogen in Carbamide Carbonyldiamide, Isocyanic acid and Nitrobenzene isotopically modified by nitrogen.

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Conflicts of Interest

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

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