

# 農学部 GC-MS・NMR測定室



「MS NMR」で検索

<http://www.agr.hokudai.ac.jp/ms-nmr/>

質量分析計(飛行時間型)



質量分析計(磁場型)

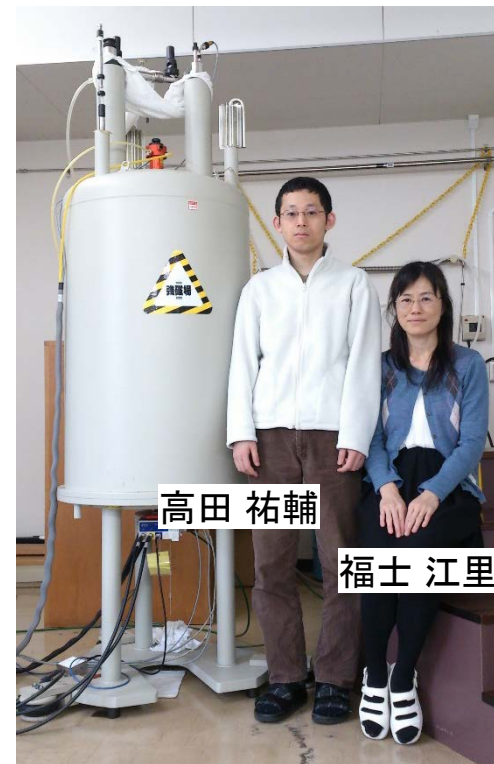


N379室

図書室の上  
情報処理室の向かい  
窓なし金属ドア

平日8:30-17:00

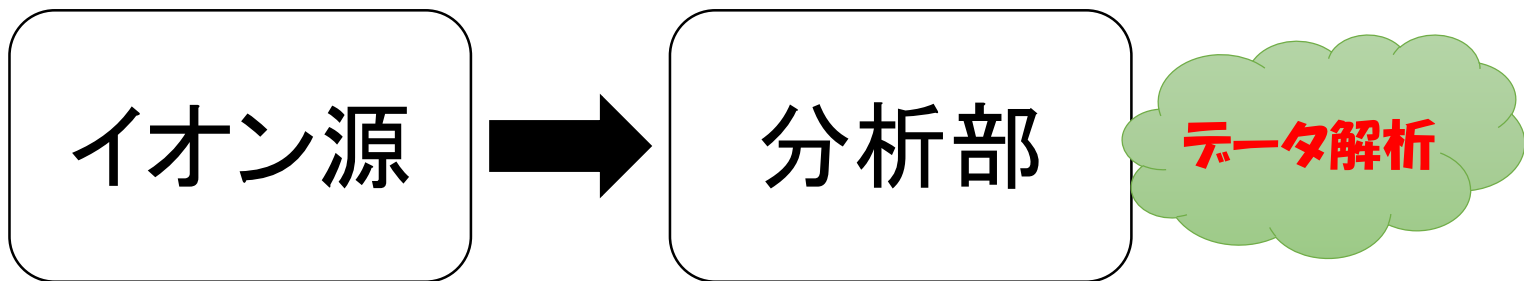
NMR



高田 祐輔

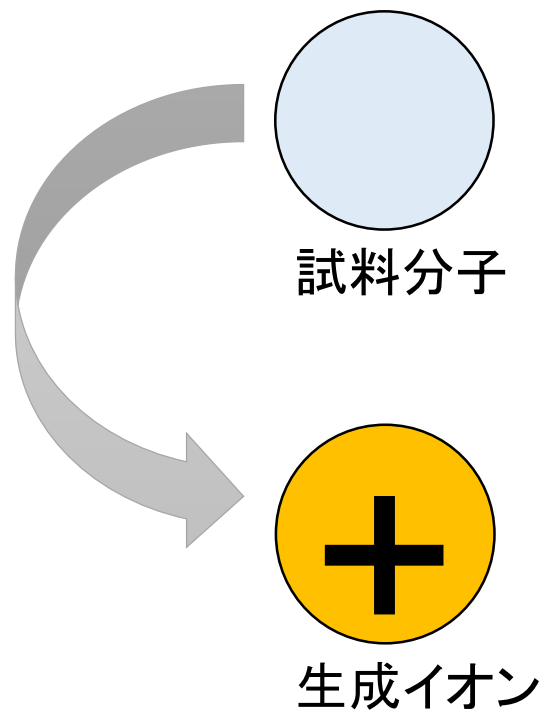
福士 江里

# 質量分析計 (Mass Spectrometer)



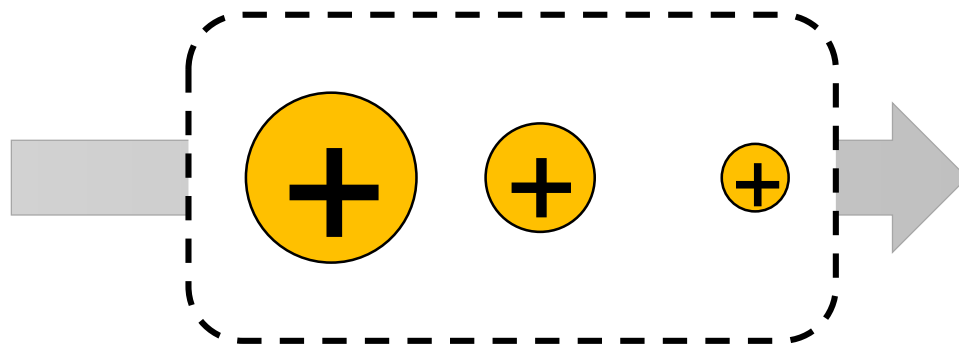
分子をイオンにする

イオンの質量を求める

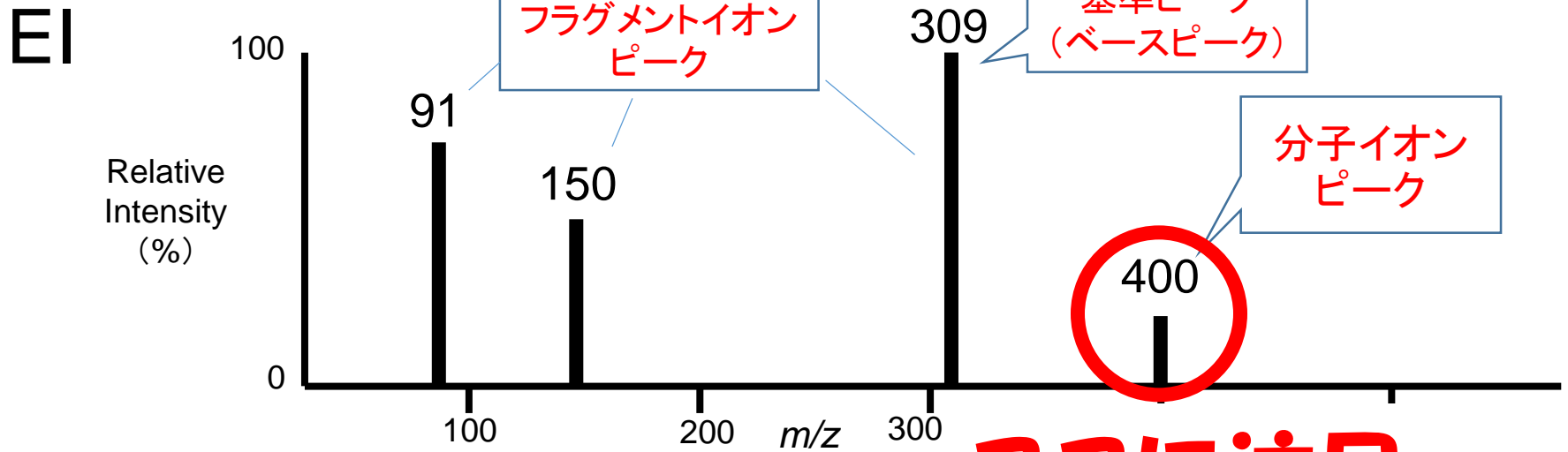


元の分子の質量

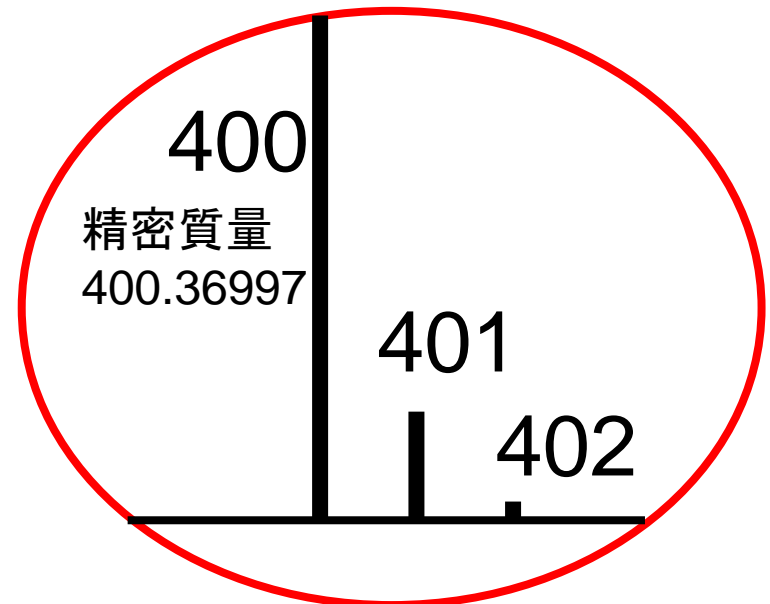
↓  
分子式



# マススペクトル (Mass Spectrum)



ここに注目



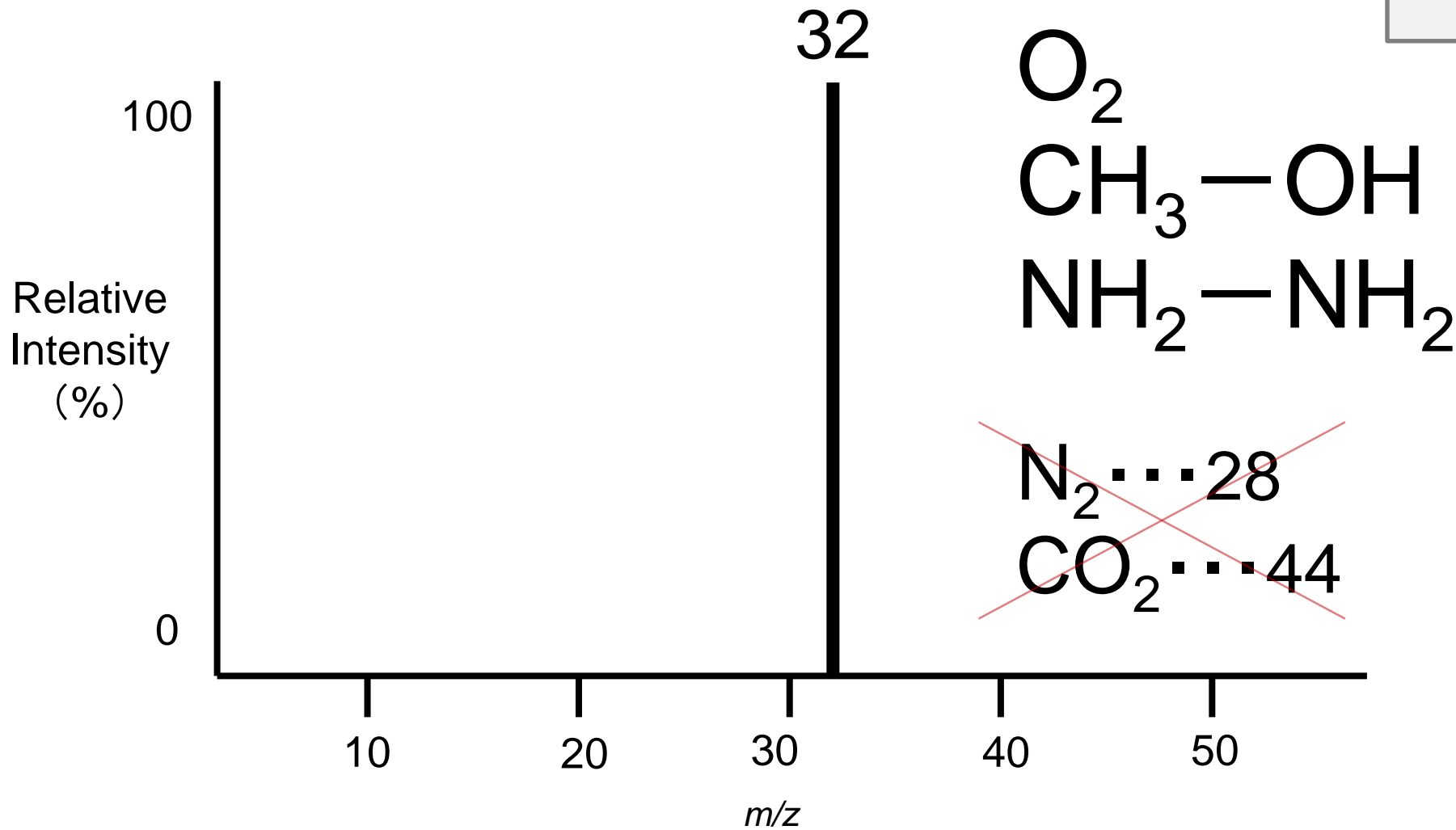
▪ **精密質量→分子式**

▪ 同位体パターン

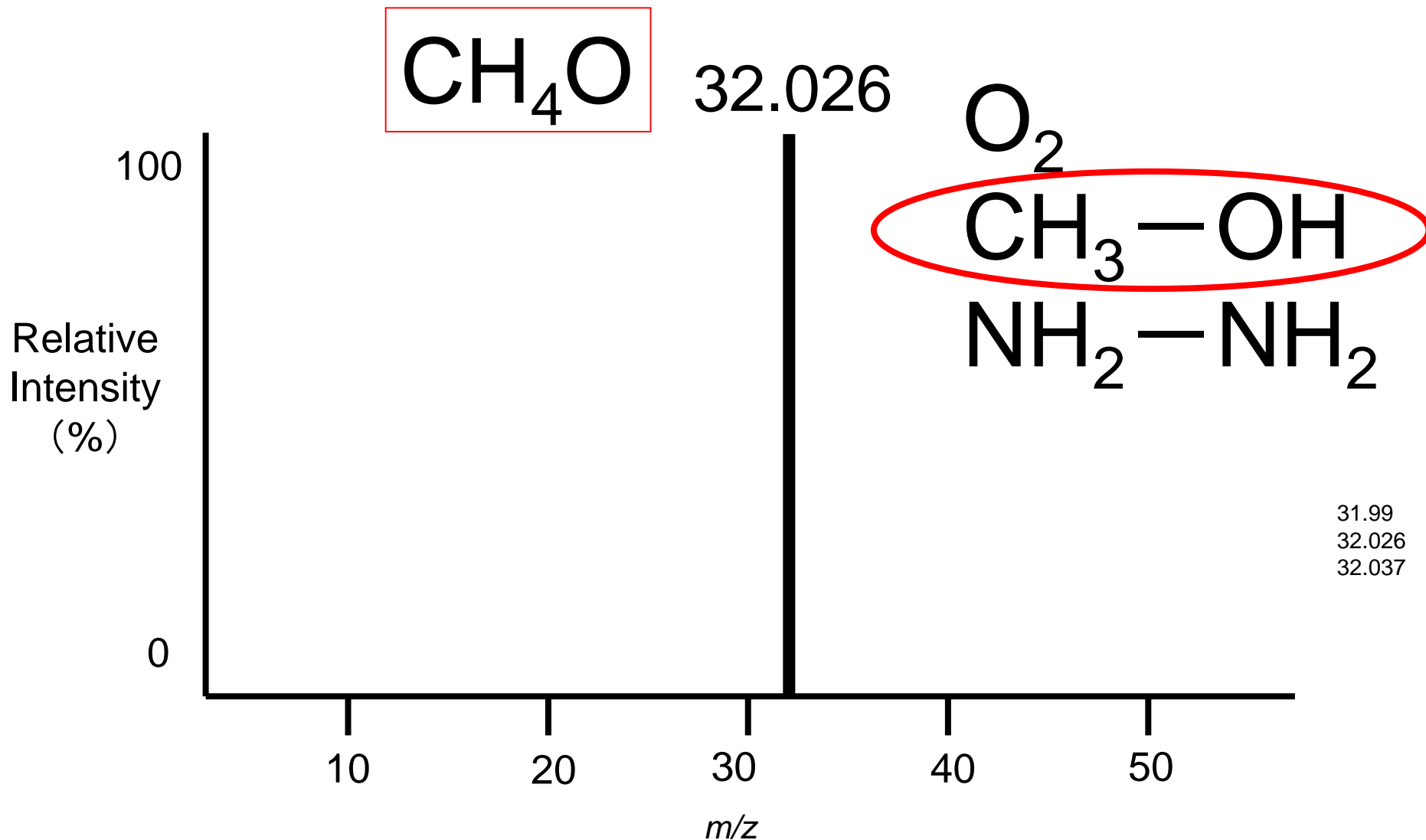
▪ 水素不足指標

# マススペクトル (Mass Spectrum)

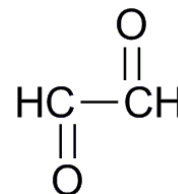
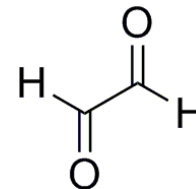
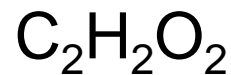
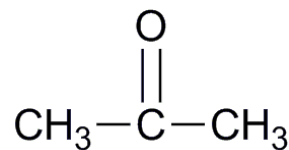
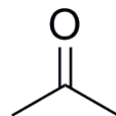
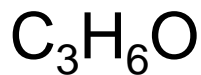
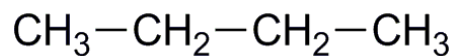
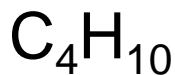
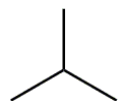
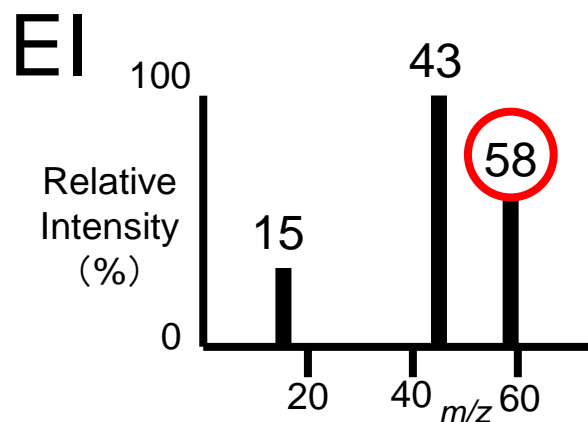
C:12  
H: 1  
N:14  
O:16



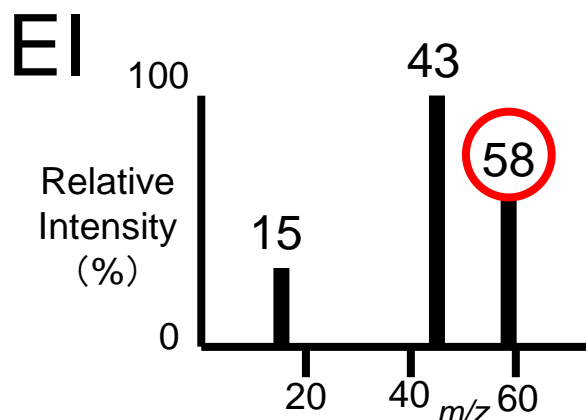
# マススペクトル (Mass Spectrum)



# 分子式 (Molecular Formula) の決定



# 精密質量 (Exact Mass)



測定値 58.0415

$C_4H_{10}$  58.0783

★  $C_3H_6O$  58.0419

$C_2H_2O_2$  58.0055

$^{12}C$  12.00000

$^1H$  1.00783

$^{16}O$  15.99491

$$12.00000 \times 3 + 1.00783 \times 6 + 15.99491 = 58.04189$$



# 同位体 (Isotope)

	同位体精密質量	存在比
$^{12}\text{C}$	<u>12.0000</u>	98.9%
$^{13}\text{C}$	13.0034	1.1%

$$12.0000 \times 0.989 + 13.0034 \times 0.011 = 12.0110$$

精密質量計算に使うのは  
最も軽い同位体の質量

原子量

出番なし

※原子量を足して  
いったら分子量

# 同位体 (Isotope)

	同位体精密質量	存在比
${}^1\text{H}$	<u>1.0078</u>	99.99%
${}^2\text{H}$	2.0141	0.01%

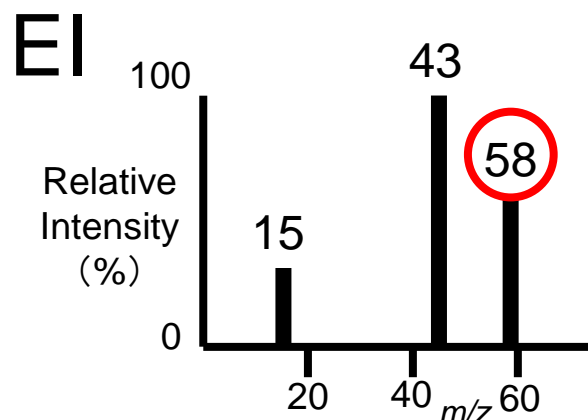
$$1.0078 \times 0.9999 + 2.0141 \times 0.0001 = 1.0079$$

精密質量計算に使うのは  
最も軽い同位体の質量

原子量

出番なし

# 分子式 (Molecular Formula) の決定



測定値 58.0415

$C_4H_{10}$  58.0783

★  $C_3H_6O$  58.0419

$C_2H_2O_2$  58.0055

$^{12}C$  12.00000

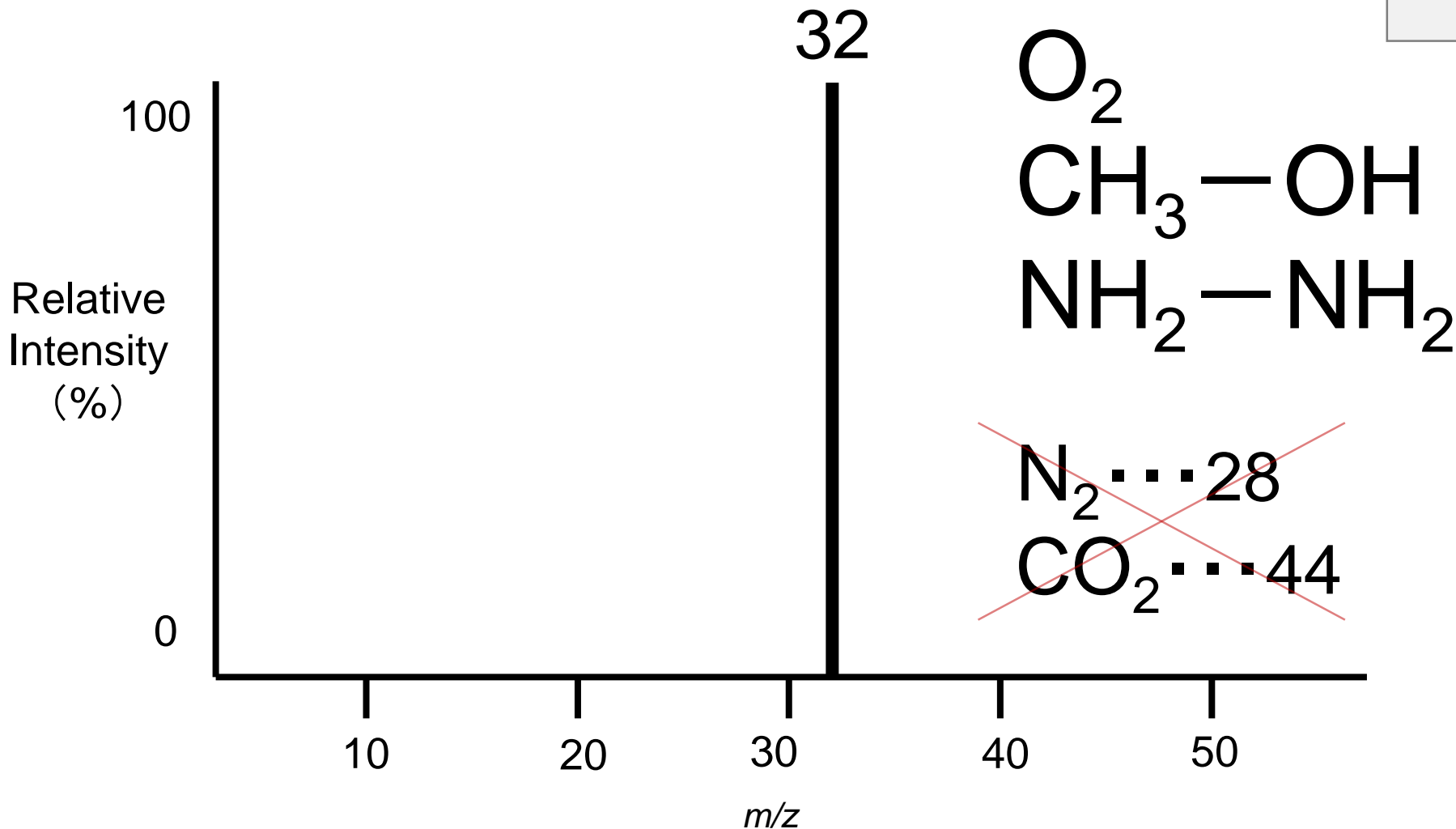
$^1H$  1.00783

$^{16}O$  15.99491

$$12.00000 \times 3 + 1.00783 \times 6 + 15.99491 = 58.04189$$

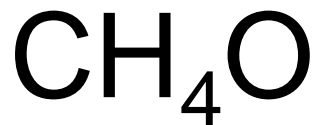
# 分子式 (Molecular Formula) の決定

C:12  
H: 1  
N:14  
O:16

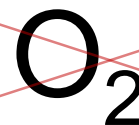


# 分子式 (Molecular Formula) の決定

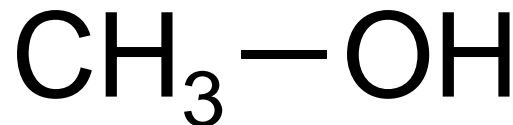
C:12.00000  
H: 1.00783  
N:14.00307  
O:15.99491



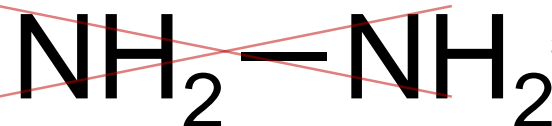
32.026



31.99



32.026



32.037

Relative Intensity (%)

100

0

10

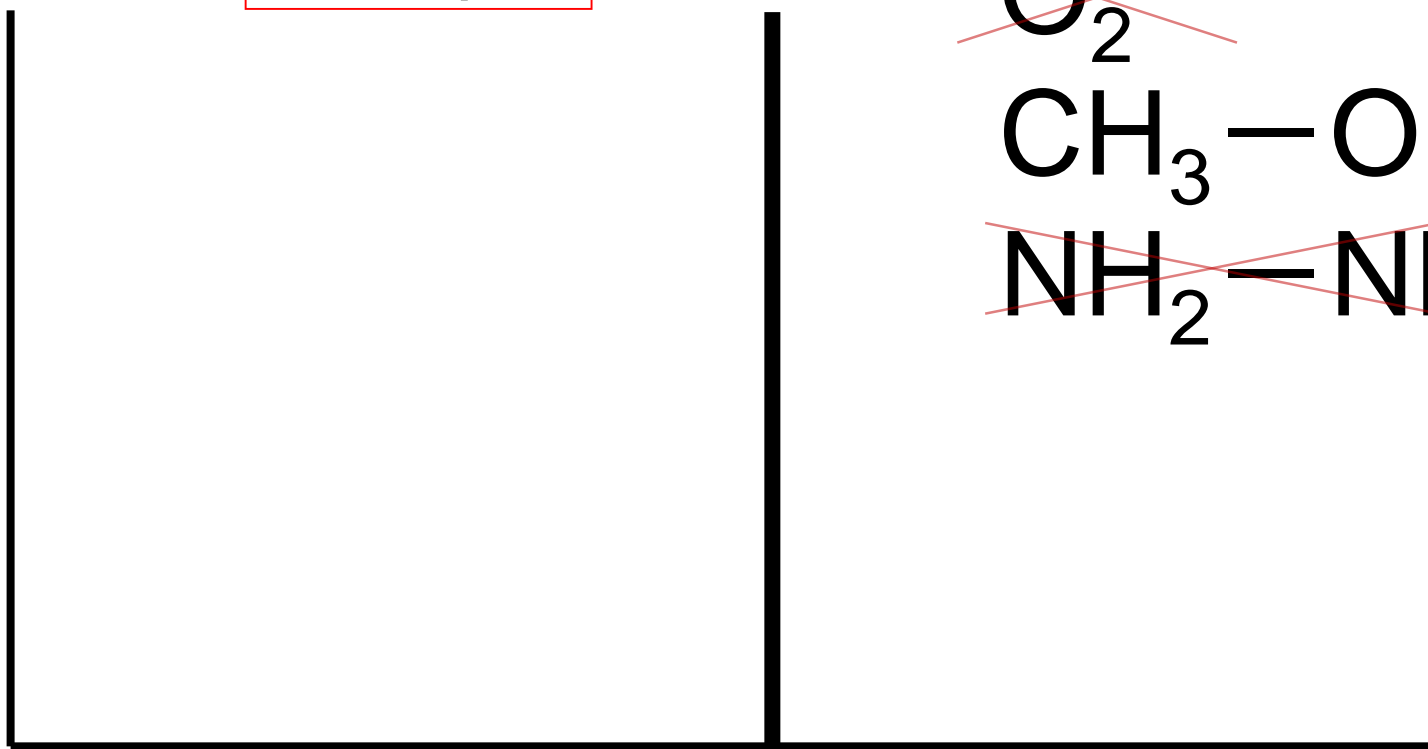
20

30

40

50

*m/z*



# ChemDraw精密質量計算

The screenshot shows the ChemDraw Ultra software interface. The 'View' menu is open, and the 'Show Analysis Window' option is selected. A blue arrow points from this menu item to the 'Analysis' window. The 'Analysis' window displays the following information:

- Formula:  $C_3H_6O$
- Exact Mass: 58.0419
- Mol. Wt.: 58.0791
- m/z: 58.0419 (100.0%)  
59.0452 (3.2%)
- Elem. Anal.: C, 62.04; H, 10.41; O, 27.55

A 'Paste' button is located at the bottom of the window. The chemical structure of acetone ( $CH_3COCH_3$ ) is shown on the grid. A mass spectrum plot is partially visible at the bottom right, showing a base peak at m/z 58 and a smaller peak at m/z 59.

分子量...出番なし

同位体  
ピーク

# ChemCalc精密質量計算と組成推定

ChemCalc



EPFL  
ÉCOLE POLYTECHNIQUE  
FÉDÉRALE DE LAUSANNE

Institute of chemical sciences and engineering ISIC

Welcome to ChemCalc Peptides Molecular Formula finder Web service Contact us

Follow @chemcalc

## Your Molecular Formula

C3H6O

List of allowed groups

FWHM : 0.01

Resolution:

5804

Apply Gaussian:

Submit

Reference version: 2013

FWHM =  $\Delta M$   
(Peak Full Width at Half-Maximum)

Resolution =  $M / \Delta M$

C3H6O

MF: C3H6O

MW: 58.079257

EM: 58.04186

Unsat.: 1

## Welcome to ChemCalc

If you are using our tools please don't forget to cite us

ChemCalc: a building block for tomorrow's chemical infrastructure  
*Journal of Chemical Information and Modeling* 2013. DOI: 10.1021

Cheat poster: Get HELP on how to use chemcalc !

### What would you like to do ?

- Calculate molecular formula, molecular mass / molecular weight, elemental analysis and plots the isotopic distribution
- Find molecular formula from an accurate mass
- Calculate peptide fragmentation
- Integrate the tools in your website

Accurate mass experimental result: 58.0419

Optional: Filter the result based on the molecular formula

MF range: Allowed Molecular Formula range in the format Xn-mYo-p (ex. C1-10H1-15S2):

C0-100 H0-100 N0-0 O0-10

You may also use groups in the definition of the range: HAla0-10Gly0-10Pro0-10OH

You may even define your own group like: [C2H4]0-4[Ala]0-2

Example: Natural amino acids

NEW: Enter the charge ! You may now fix a charge in the molecule (even multi-charge)

Example: Doubled charged

Unsaturation: The number of unsaturations can be taken into account if only the following elements are present: C, Cl, Br, I. It is defined as  $(2n_c - n_h - n_{cl} - n_{br} - n_i - n_{ox} + n_w) / 2 + 1$ .

Limit the results by unsaturations:

Unsaturation allowed from: 0 to 100

Allow only integer unsaturation values:

Mass range: 0.05

Reference values version: 2013

Search MF

Results:

Color by difference:  $\leq -0.0010$   $\leq -0.01$   $\leq -1.0$

Number of results: 3. Brute force iterations: 1160. Real iterations: 71.

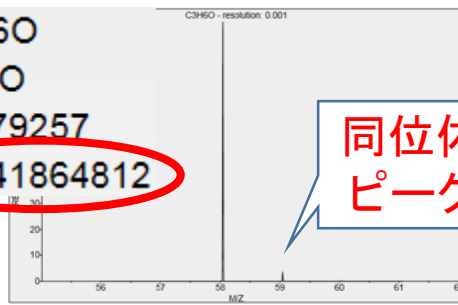
MF	Monoisotopic mass	PPM	mDa	unsaturation
1 C3H6O	58.042	0.606	-0.035	1
2 C4H10	58.078	625.885	36.35	0
3 C2H2O2	58.005	627.884	-36.421	2

Your request: C3H6O

Molecular formula: C<sub>3</sub>H<sub>6</sub>O

Molecular weight: 58.079257

Monoisotopic mass: 58.041864812



同位体  
ピーク

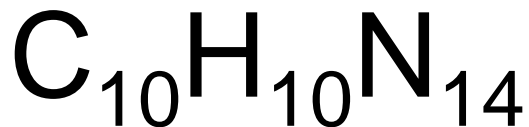
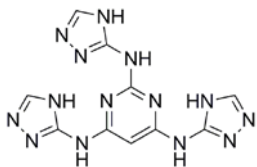
- 精密質量→分子式

- 同位体パターン

- 水素不足指標

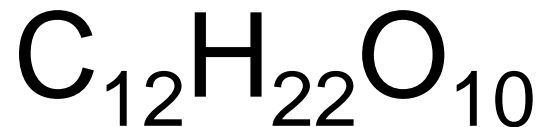
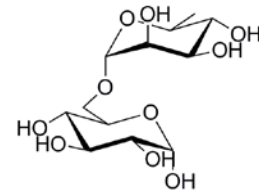


# 同位体パターン (Isotope Pattern)



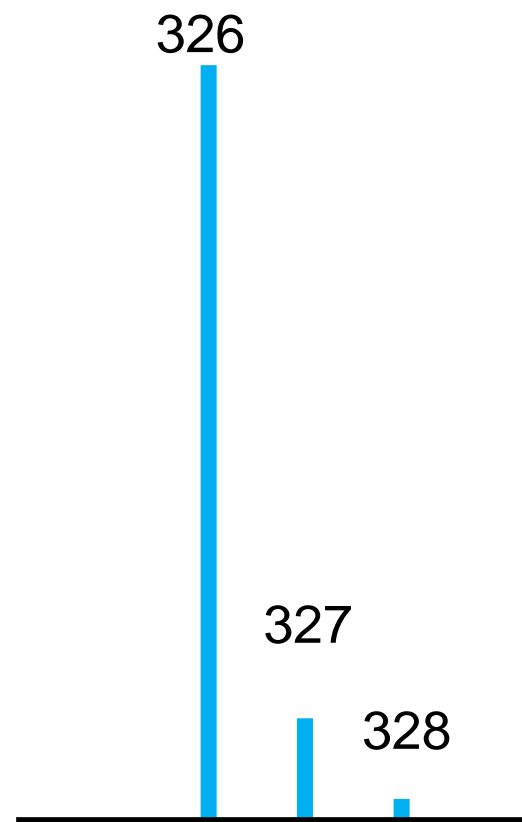
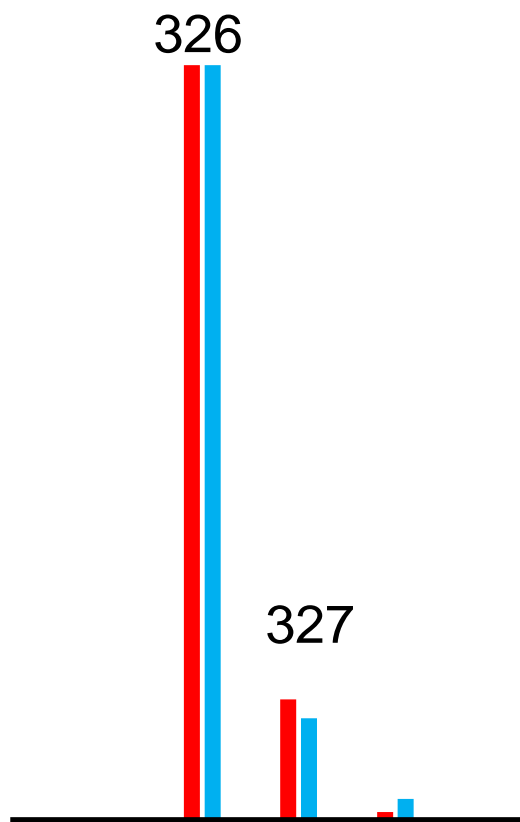
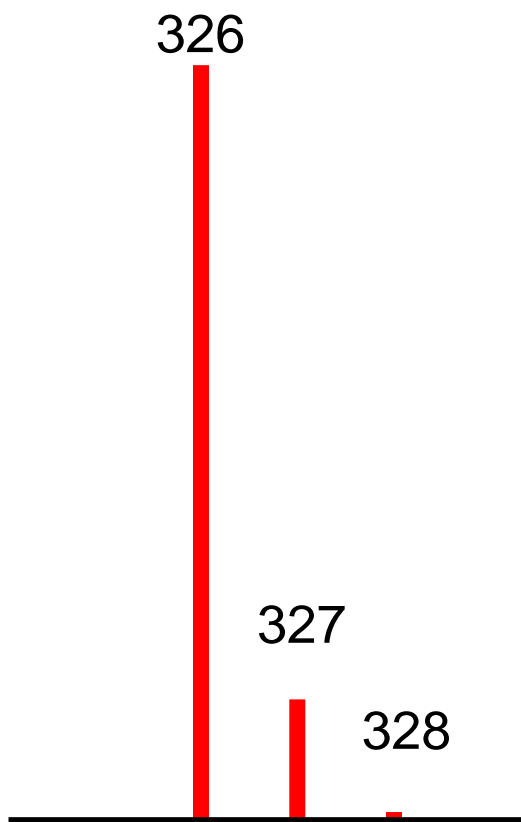
Exact Mass: 326.12129

m/z: 326 (100.0%), 327 (16.1%), 328 (1.2%)



Exact Mass: 326.12130

m/z: 326 (100.0%), 327 (13.6%), 328 (2.9%)



# 同位体 (Isotope)

	同位体精密質量	存在比
$^{12}\text{C}$	<u>12.0000</u>	98.9%
$^{13}\text{C}$	13.0034	1.1%

$$12.0000 \times 0.989 + 13.0034 \times 0.011 = 12.0110$$

精密質量計算に使うのは  
最も軽い同位体の質量

原子量

出番なし

# 同位体 (Isotope)

	同位体精密質量	存在比
${}^1\text{H}$	<u>1.0078</u>	99.99%
${}^2\text{H}$	2.0141	0.01%

$$1.0078 \times 0.9999 + 2.0141 \times 0.0001 = 1.0079$$

精密質量計算に使うのは  
最も軽い同位体の質量

原子量

出番なし

モノアイソトピック  
ピーク

# 同位体パターン (Isotope Pattern)





30

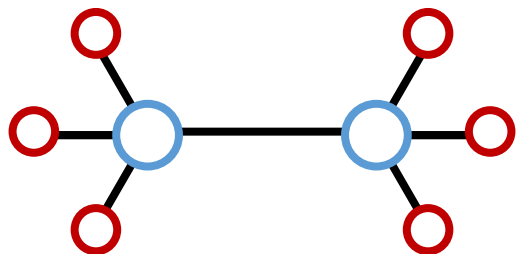
同位体  
ピーク

31

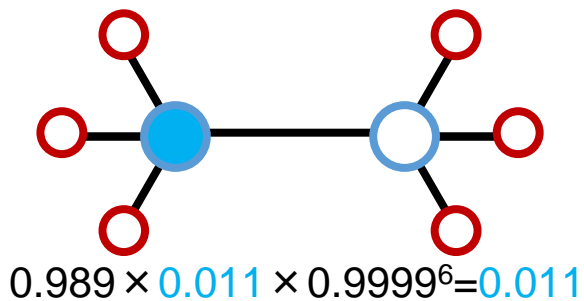
(32)

1 : 0.02 : 0.0001

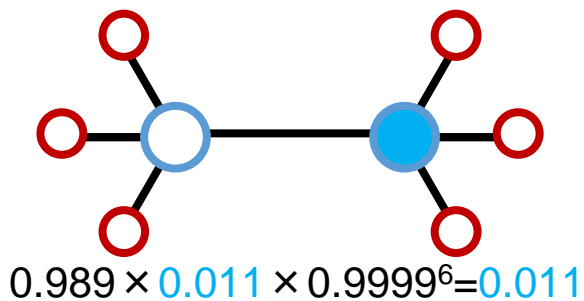
-   $^{12}\text{C}$  98.9%
-   $^{13}\text{C}$  1.1%
-   $^1\text{H}$  99.99%
-   $^2\text{H}$  0.01%



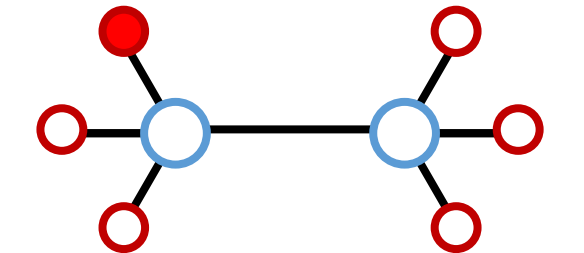
$$0.989^2 \times 0.9999^6 = 0.978$$



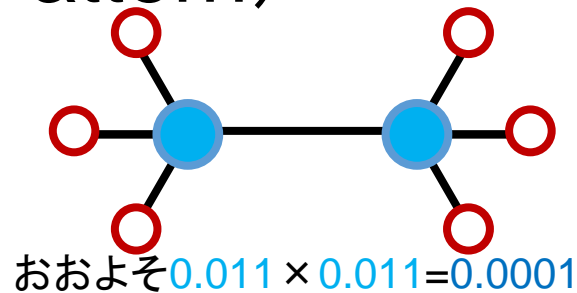
$$0.989 \times 0.011 \times 0.9999^6 = 0.011$$



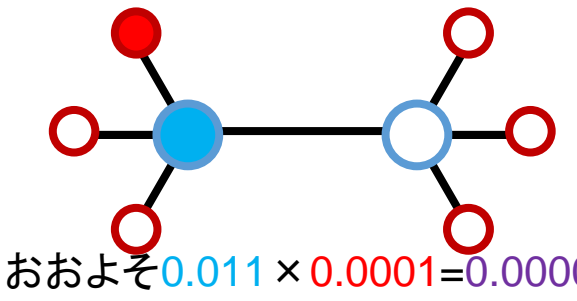
$$0.989 \times 0.011 \times 0.9999^6 = 0.011$$



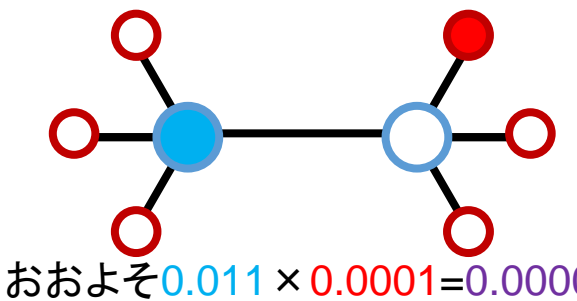
$$0.989^2 \times 0.9999^5 \times 0.0001 = 0.0001$$



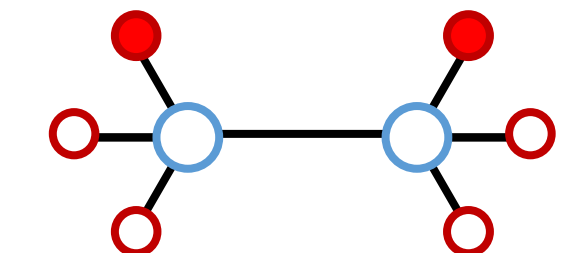
$$\text{おおよそ } 0.011 \times 0.011 = 0.0001$$



$$\text{おおよそ } 0.011 \times 0.0001 = 0.000001$$



$$\text{おおよそ } 0.011 \times 0.0001 = 0.000001$$

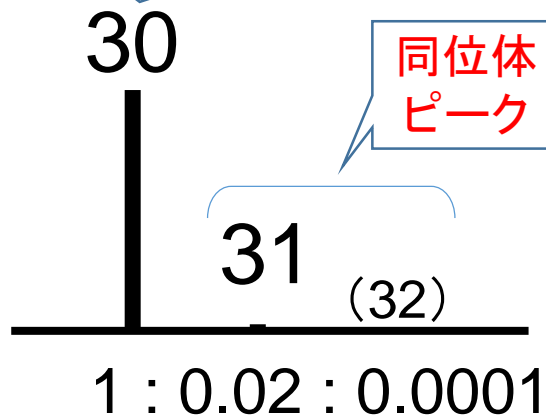


$$\text{おおよそ } 0.0001 \times 0.0001 = 0.00000001$$

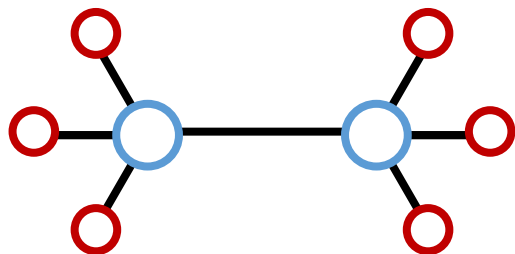
モノアイソトピック  
ピーク

# 同位体パターン (Isotope Pattern)

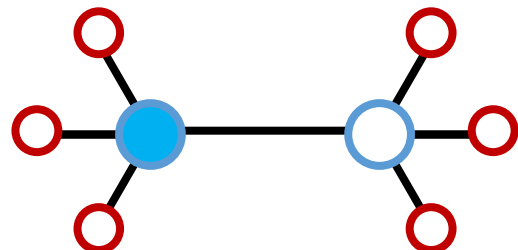
同位体  
ピーク



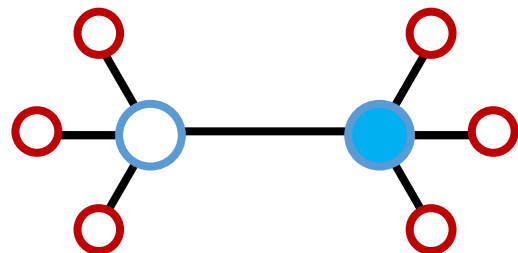
	$^{12}\text{C}$	98.9%
	$^{13}\text{C}$	1.1%
	$^1\text{H}$	99.99%
	$^2\text{H}$	0.01%



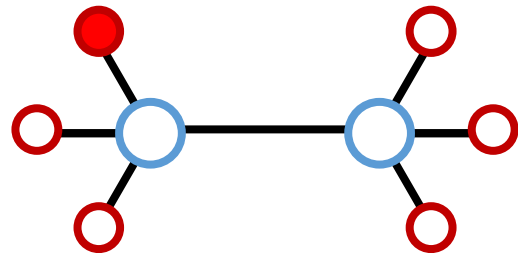
$$0.989^2 \times 0.9999^6 = 0.978$$



$$0.989 \times 0.011 \times 0.9999^6 = 0.011$$

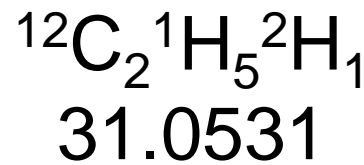
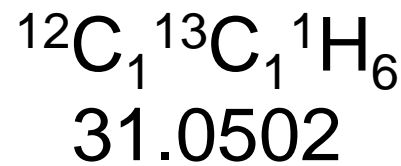
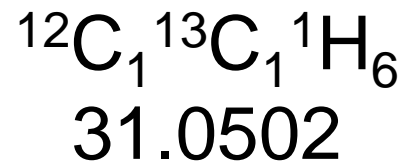


$$0.989 \times 0.011 \times 0.9999^6 = 0.011$$



$$0.989^2 \times 0.9999^5 \times 0.0001 = 0.0001$$

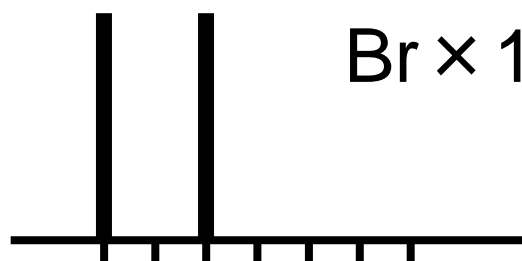
**同位体ピークは  
純粋ではない**  
精密質量の計算には使わない



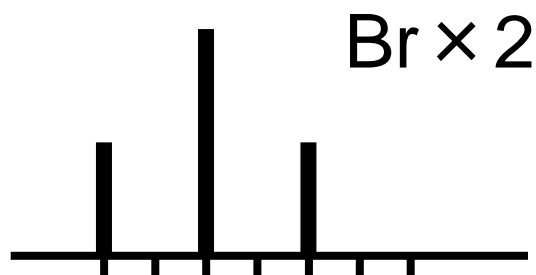
# 同位体パターン (Isotope Pattern)

$^{79}\text{Br}$      $^{81}\text{Br}$   
51%    49%

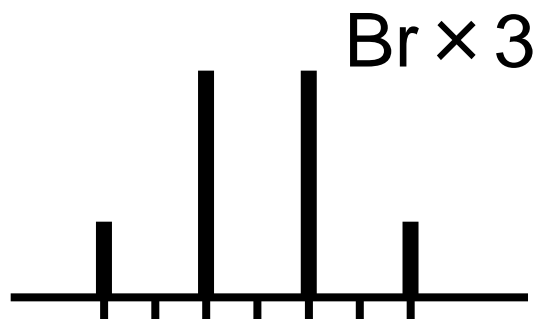
1 : 1



1 : 1



1:2:1



1:3:3:1

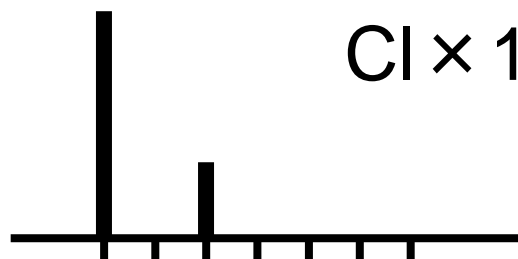
パスカルの  
三角形

1	1				
1	2	1			
1	3	3	1		
1	4	6	4	1	
1	5	10	10	5	1

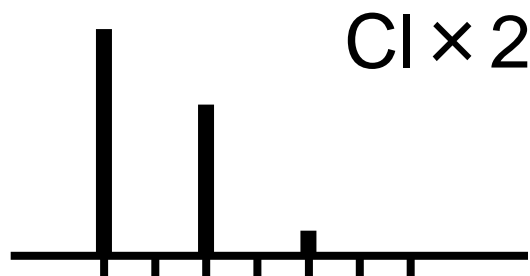
# 同位体パターン (Isotope Pattern)

$^{35}\text{Cl}$      $^{37}\text{Cl}$   
76%    24%

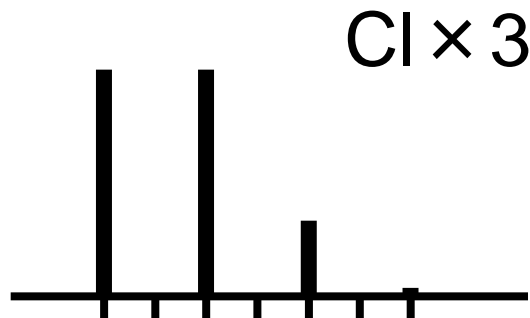
3 : 1



3 : 1



9:6:1



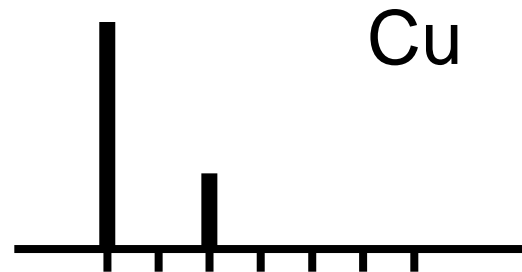
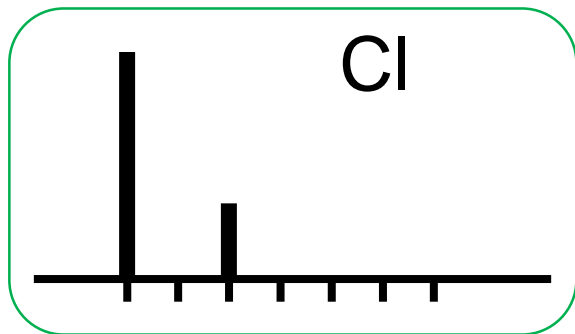
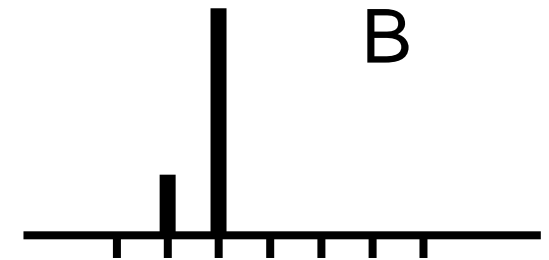
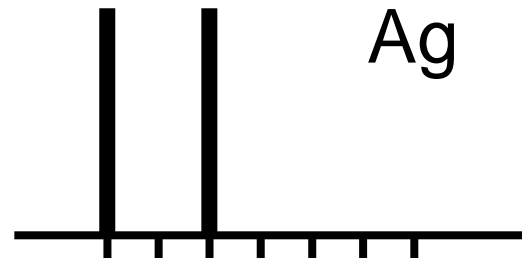
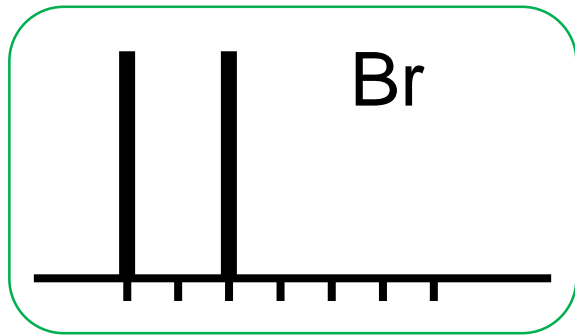
27:27:9:1

パスカルの  
三角形

3 1  
×3 ×1 ×3 ×1  
9 6 1

27 27 9 1

# 同位体パターン(Isotope Pattern)





- 精密質量→分子式

- 同位体パターン

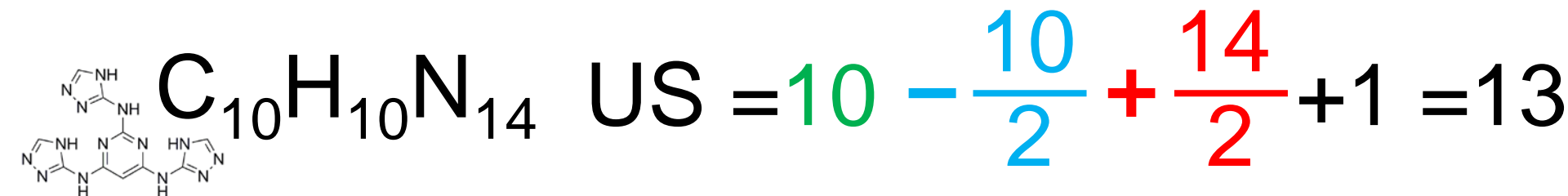
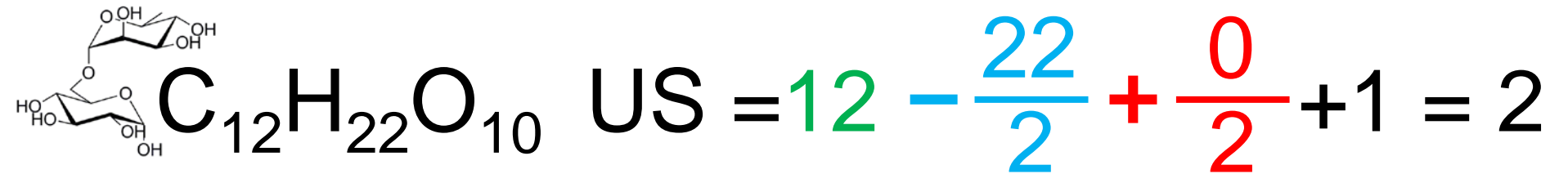
- **水素不足指標**

# 水素不足指標 (Index of Hydrogen Deficiency)

通称:不飽和度 (Unsaturation Degree)、UあるいはUS

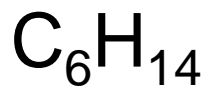
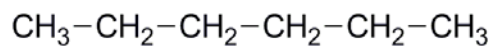
$$US = \overset{C}{\text{炭素数}} - \frac{\overset{H}{\text{水素数}}}{2} + \frac{\overset{N}{\text{窒素数}}}{2} + 1$$

飽和の鎖状分子にするために切断しなければならない結合の本数  
環(+1)、二重結合(+1)、三重結合(+2)の個数の和になる

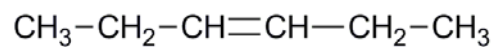
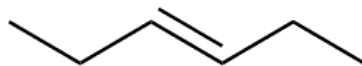


# 水素不足指標

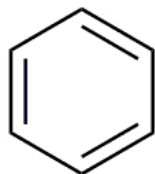
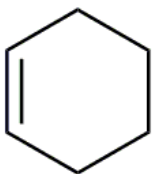
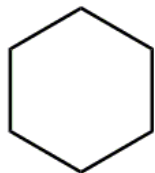
$$US = \overset{C}{\text{炭素数}} - \frac{\overset{H}{\text{水素数}}}{2} + \frac{\overset{N}{\text{窒素数}}}{2} + 1$$



$$US = 6 - \frac{14}{2} + \frac{0}{2} + 1 = 0$$

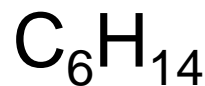
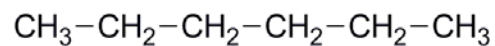
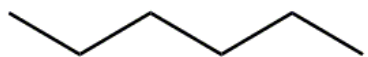


$$US = \quad - \frac{\quad}{2} + \frac{\quad}{2} + 1 =$$

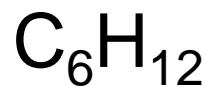
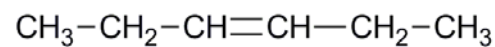
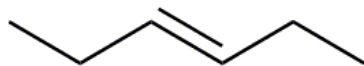


# 水素不足指標

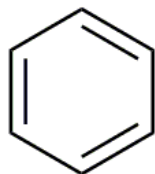
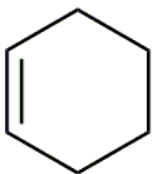
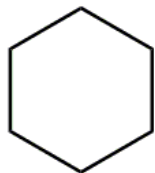
$$US = \overset{C}{\text{炭素数}} - \frac{\overset{H}{\text{水素数}}}{2} + \frac{\overset{N}{\text{窒素数}}}{2} + 1$$



$$US = 6 - \frac{14}{2} + \frac{0}{2} + 1 = 0$$

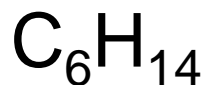
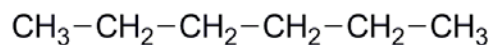
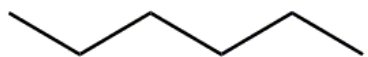


$$US = 6 - \frac{12}{2} + \frac{0}{2} + 1 = 1$$

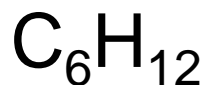
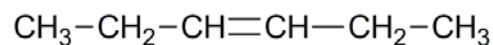
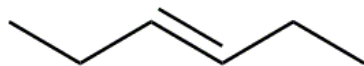


# 水素不足指標

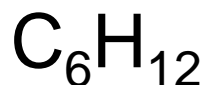
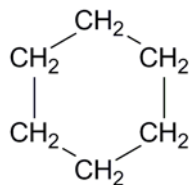
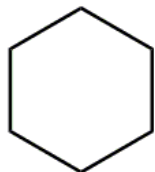
$$US = \overset{C}{\text{炭素数}} - \frac{\overset{H}{\text{水素数}}}{2} + \frac{\overset{N}{\text{窒素数}}}{2} + 1$$



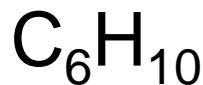
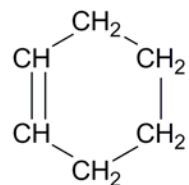
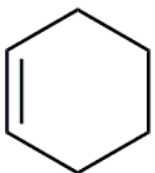
$$US = 6 - \frac{14}{2} + \frac{0}{2} + 1 = 0$$



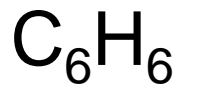
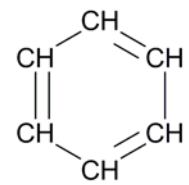
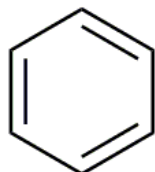
$$US = 6 - \frac{12}{2} + \frac{0}{2} + 1 = 1$$



$$US = 6 - \frac{12}{2} + \frac{0}{2} + 1 = 1$$



$$US = 6 - \frac{10}{2} + \frac{0}{2} + 1 = 2$$

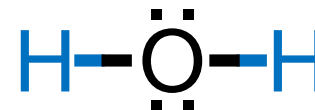
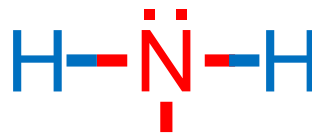
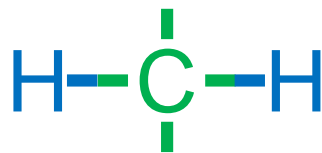


$$US = 6 - \frac{6}{2} + \frac{0}{2} + 1 = 4$$

# 水素不足指標

$$US = \overset{C}{\text{炭素数}} - \frac{H}{2} + \frac{N}{2} + 1$$

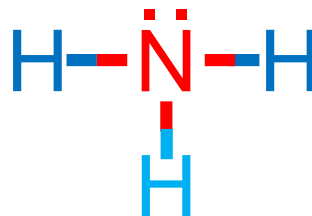
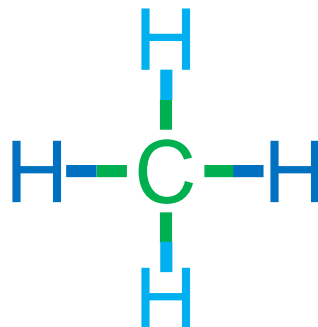
飽和・・・もう水素を付加させることができない  
可能な限り水素を付けた構造



↓ 水素分子  
1個

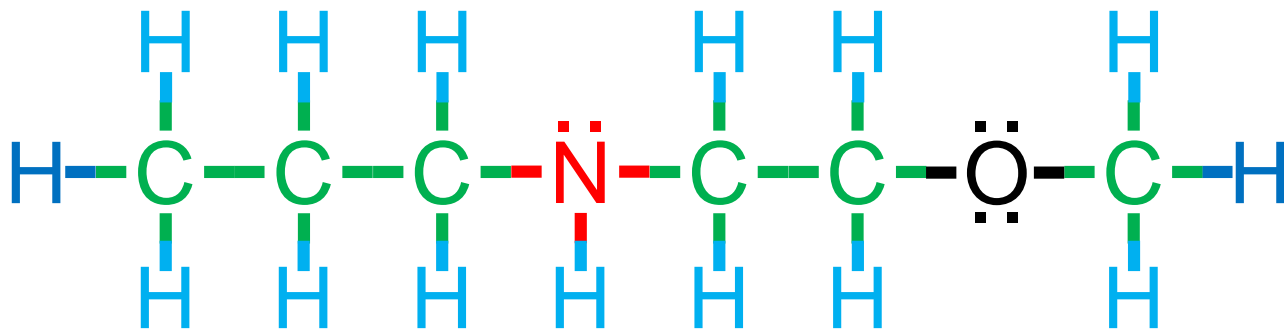
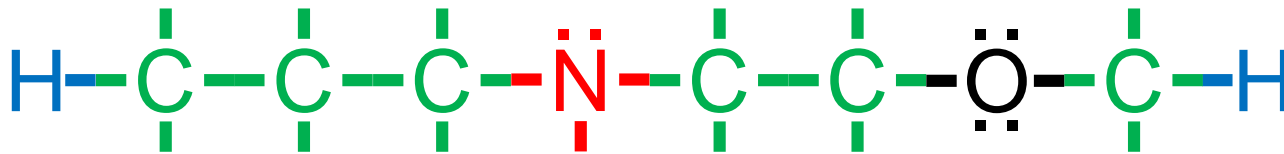
↓ 水素分子  
0.5個

↓ 水素分子  
0個



# 水素不足指標

$$US = \overset{C}{\text{炭素数}} - \frac{H}{2} + \frac{N}{2} + 1$$



0以上の整数

飽和した構造に必要な水素数

— 実際の水素数

両隣と繋がるのに使う2本

$$US = \frac{(\text{手の数}-2) \times \text{個数} + (\text{手の数}-2) \times \text{個数} + (\text{手の数}-2) \times \text{個数} + \dots + 2\text{-水素数}}{2}$$

$$= \frac{(4-2) \times \text{個数} + (3-2) \times \text{個数} + (2-2) \times \text{個数} + (1-2) \times \text{個数} + \dots + 2\text{-水素数}}{2}$$

水素原子数 → 水素分子数

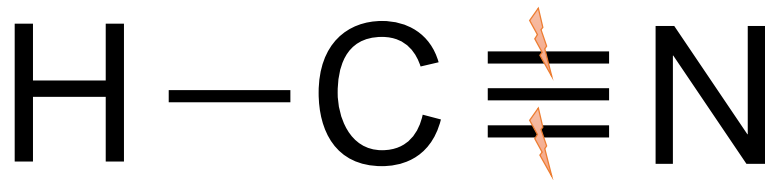
# 水素不足指標 (Index of Hydrogen Deficiency)

通称:不飽和度 (Unsaturation Degree)、UあるいはUS

$$US = \text{炭素数} - \frac{\text{水素数}}{2} + \frac{\text{窒素数}}{2} + 1$$

飽和の鎖状分子にするために切断しなければならない結合の本数  
環(+1)、二重結合(+1)、三重結合(+2)の個数の和になる

困ったら...



$$\text{C}_1\text{H}_1\text{N}_1 \quad US = 1 - \frac{1}{2} + \frac{1}{2} + 1 = 2$$



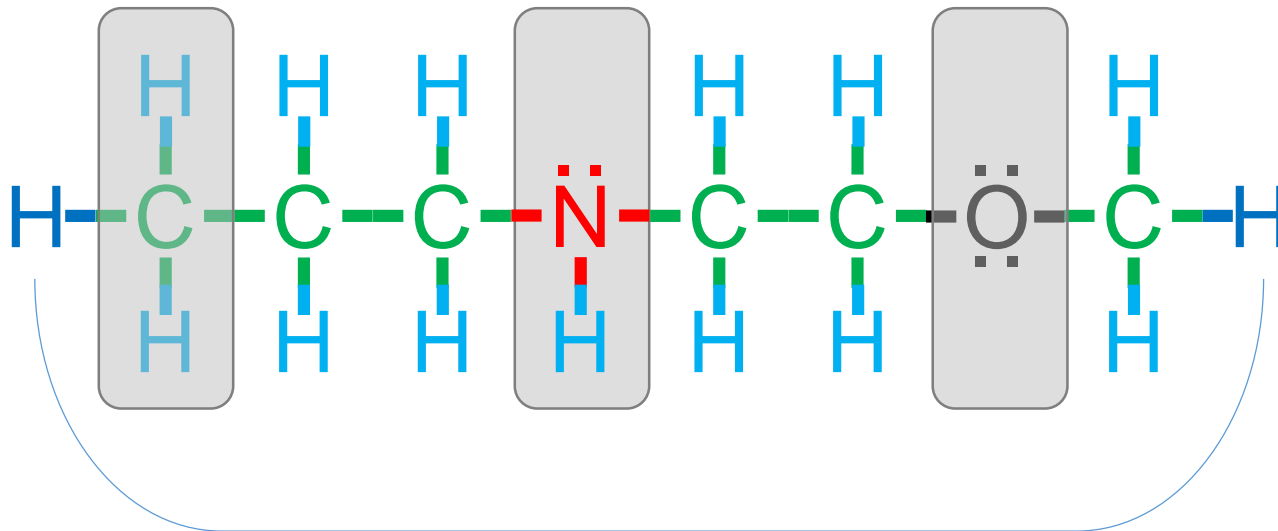
# 窒素ルール (Nitrogen Rule)

窒素が**奇数**個なら分子の質量は**奇数**  
窒素が**偶数**個なら分子の質量は**偶数**  
0を含む

$$12+2 \\ =14$$

$$14+1 \\ =15$$

$$16+0 \\ =16$$



$$\text{両端 } 1+1=2$$

## ▪ 精密質量→分子式

小数点以下4桁程度まで精密に測定した値と同位体の精密質量から計算した値を照合する

## ▪ 同位体パターン

元素によって同位体の存在比が異なるためピーク強度比から含有元素を推定する

## ▪ 水素不足指標

計算結果が0以上の整数にならないものは通常のものでないため候補から除外する

