

# **Rozwój nowych technologii a możliwości oznaczania niedoborów witamin z grupy B**

dr inż. Jakub Nawął

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Instytut Matki i Dziecka



**Fundusze Europejskie**  
Wiedza Edukacja Rozwój



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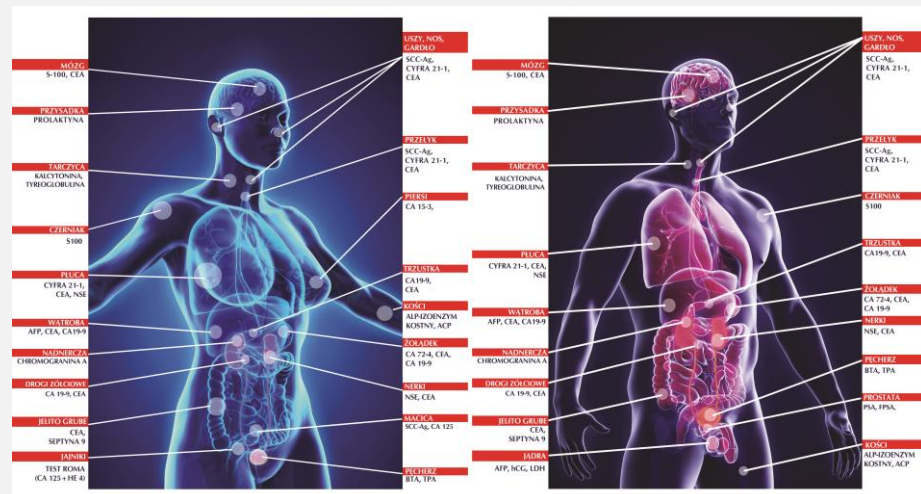


# Diagnostyka medyczna

... to **nauka o rozpoznawaniu chorób.**

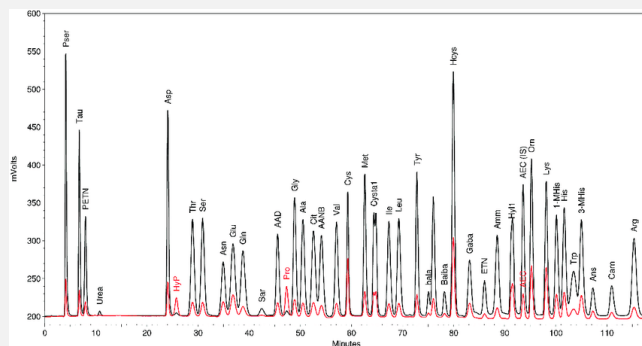
Diagnoza jest wynikiem:

1. badania podmiotowego
2. badania przedmiotowego
3. badania dodatkowego
  - badania fragmentu tkanek, krwi, wydzielin i wydaliny
  - badania obrazowe
  - badania endoskopowe

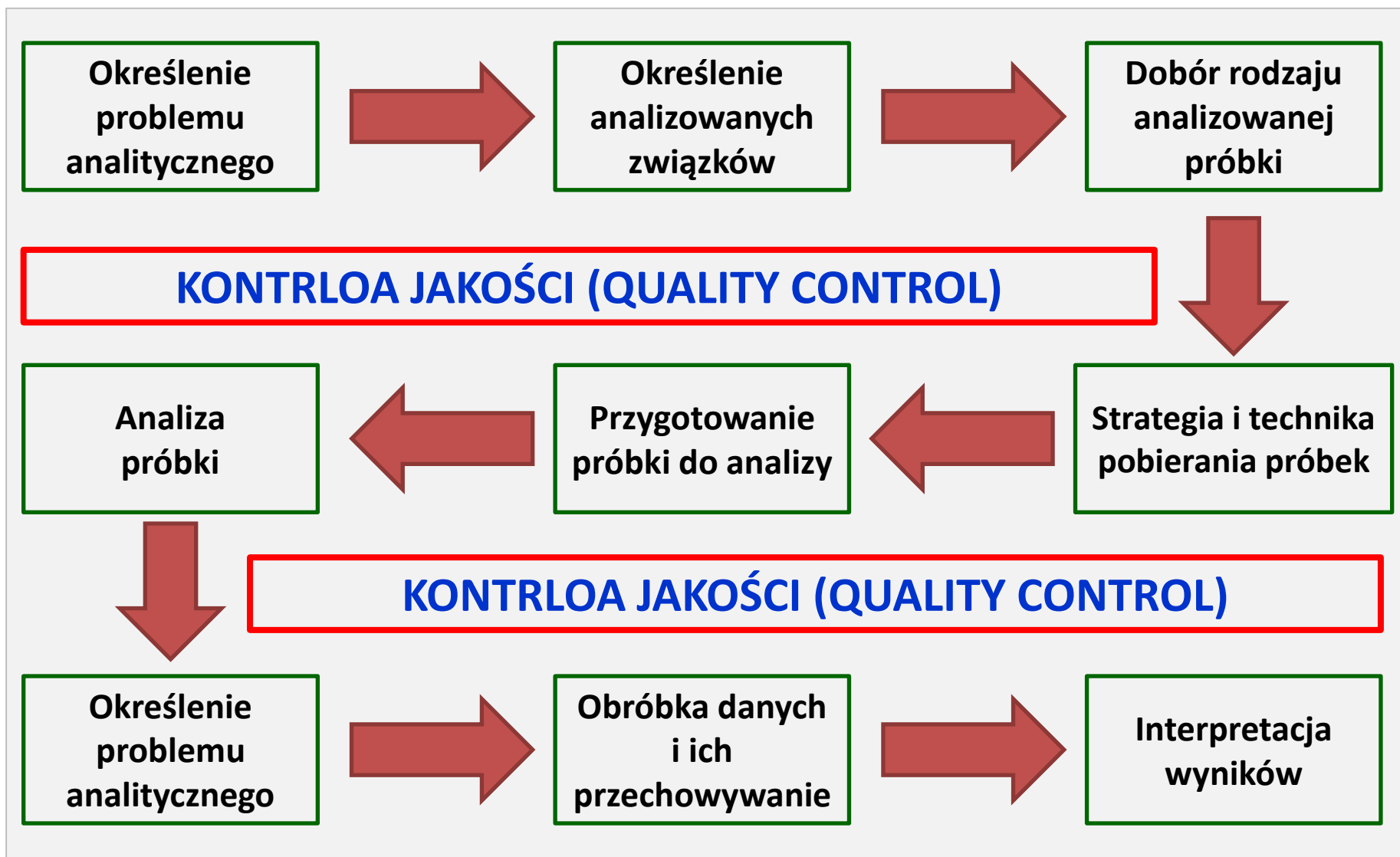


# Specyfika diagnostyki laboratoryjnej

- Brak bezpośredniego kontaktu z pacjentem
- Operowanie na materiale pobranym od pacjenta
- Bardzo duża ilość wykonywanych badań
- Wykonywania analiz zarówno jakościowych jak i ilościowych
- Czas trwania analizy bardzo często musi być krótki
- Bardzo dobra komunikacja na drodze lekarz – biochemik
- Opracowanie nowych metod analitycznych, udoskonalanie istniejących, serwisowanie aparatury analitycznej
- kontrola jakości

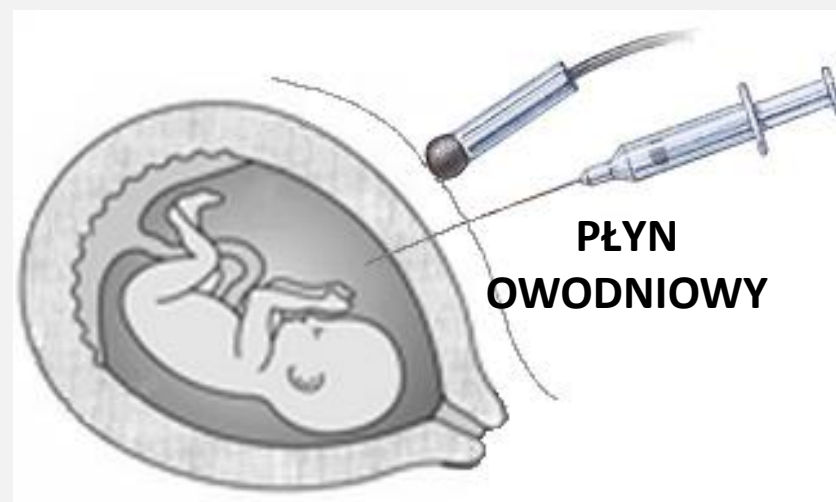


# Proces analityczny

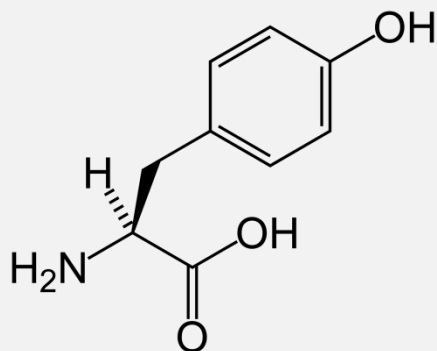


# Problem analityczny

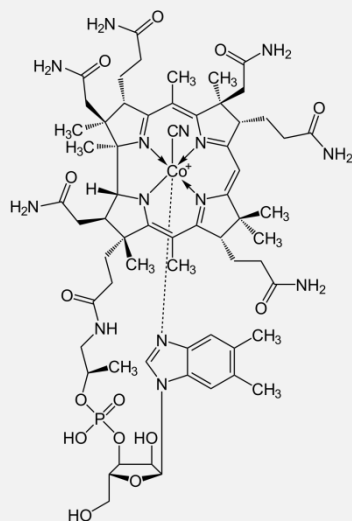
## Rodzaj badanego materiału oraz cel badania



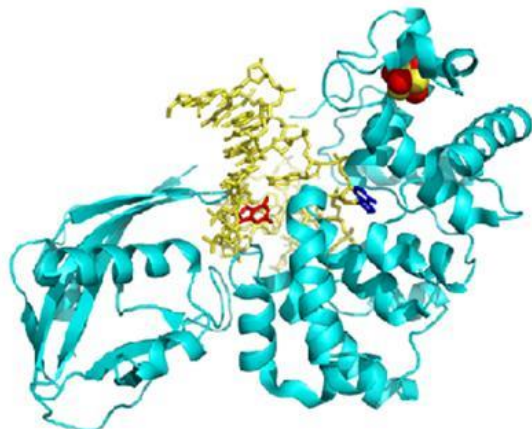
## Określenie badanych związków



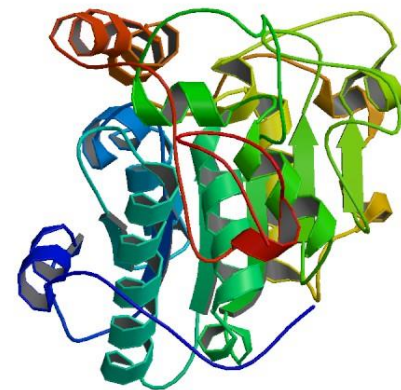
## Amino kwasy



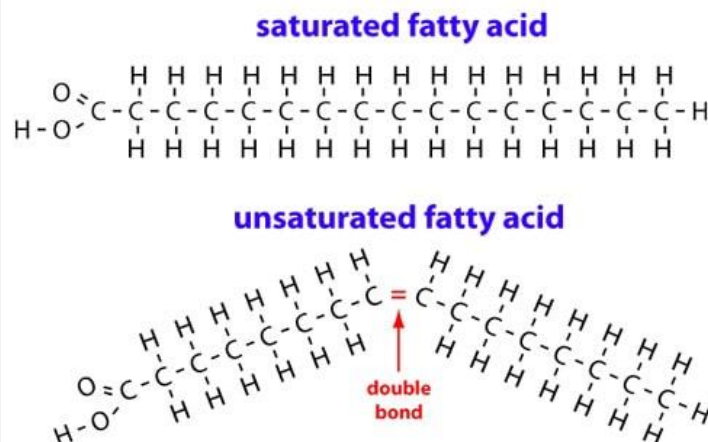
## Witaminy



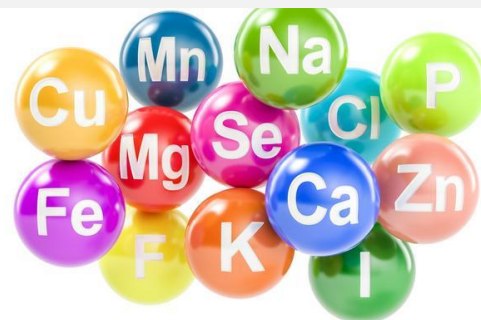
# Białka



## Enzyme



## Kwasy tłuszczowe

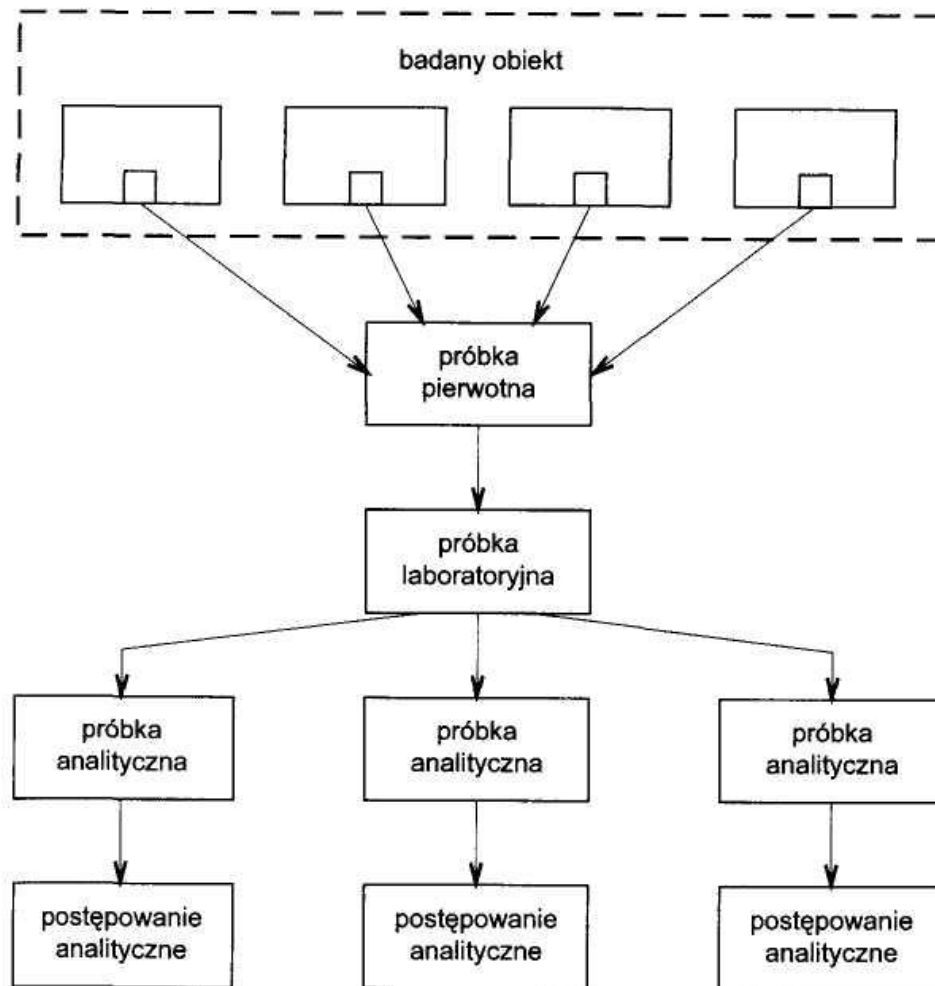


## Makro i mikroelementy

# Przygotowanie próbek do analizy

| Cel                                                                                                                                                   | Sposób realizacji                                                                       |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| Homogeniczność pobranej próbki                                                                                                                        | Mielenie, mieszanie, analiza sitowa                                                     |
| Stabilność próbki na etapie przechowywania lub transportu                                                                                             | Konserwacja chemiczna, termiczna lub liofilizacja                                       |
| Usunięcie interferentów z matrycy próbki                                                                                                              | odpylanie, usuwanie, zawiesiny, osuszanie, odtlenienie, usuwanie składników reaktywnych |
| Przekształcenie analitów w pochodne w celu:<br>1. Ułatwienia izolacji<br>2. Rozdzielenie składników mieszaniny<br>3. Analizy ilościowej i jakościowej | Reakcje derywatyzacji                                                                   |
| Zmiana matryc próbki pod daną technikę analityczną                                                                                                    | Odparowanie do sucha, ekstrakcja, sorpcja                                               |
| Zwiększenie stężenia substancji śladowych                                                                                                             | Techniki wzbogacania i zateżniania próbek                                               |

# Przygotowanie próbek do analizy



# Przygotowanie próbek do analizy

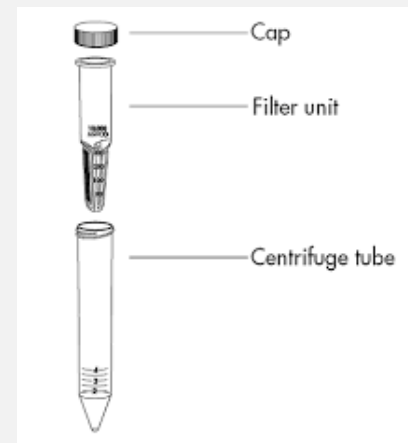
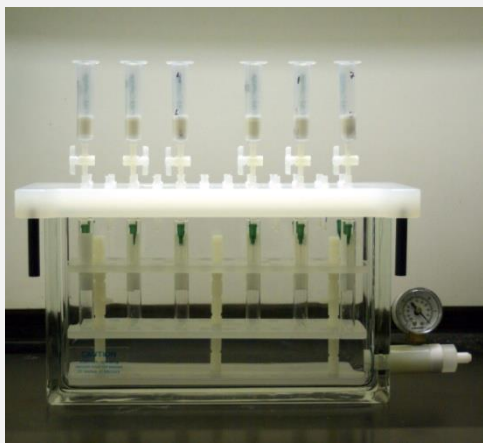
Najczęstsze problemy spotykane na etapie przygotowania próbek:

1. Czas przygotowania próbek
2. Zbyt niskie odzyski
3. Obecność zanieczyszczeń
4. Powtarzalność
5. Koszt



# Przygotowanie próbek do analizy

Techniki stosowane na etapie przygotowania próbek

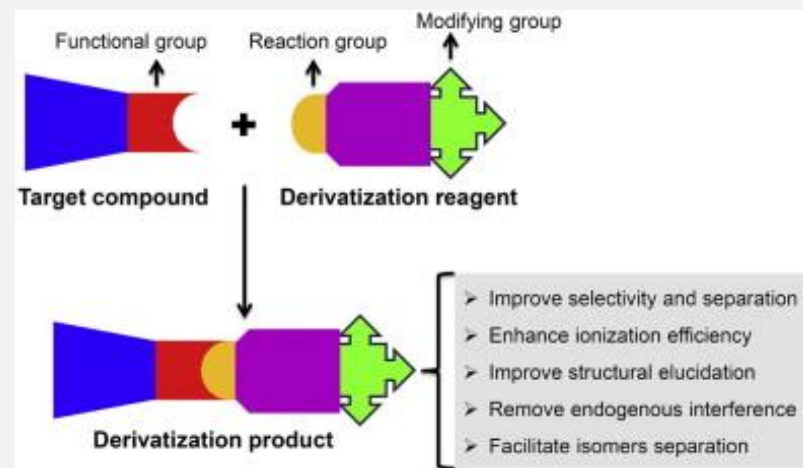
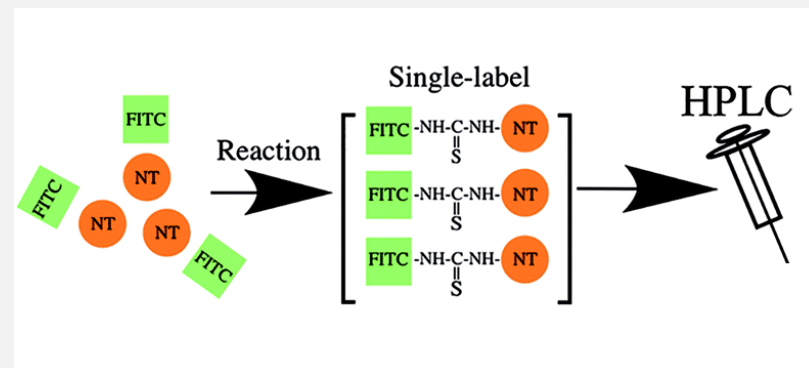


# Przygotowanie próbek do analizy

Derywatywacja to proces polegający na przeprowadzeniu analizowanych związków umożliwiając ich oznaczanie z wykorzystaniem danej metody analitycznej.

Derywatyzację stosuje się w celu:

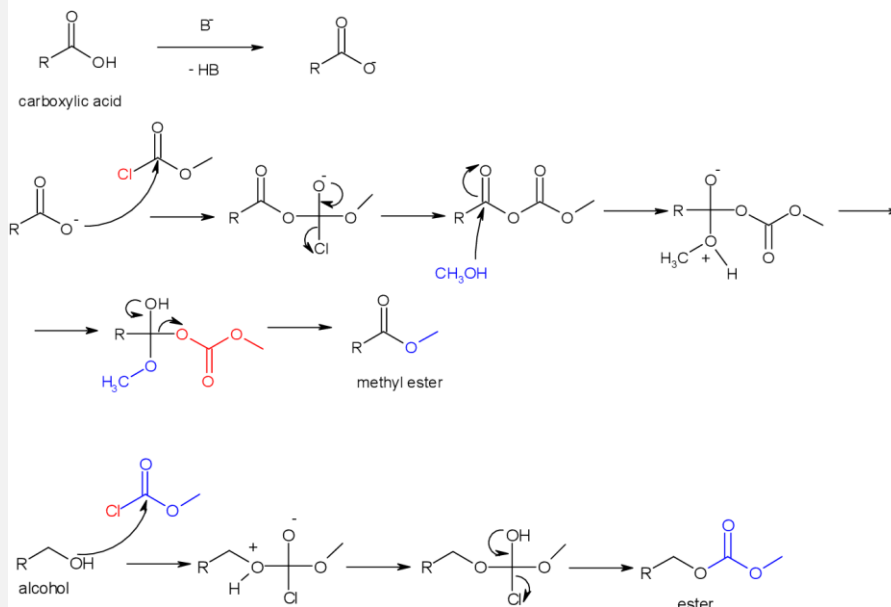
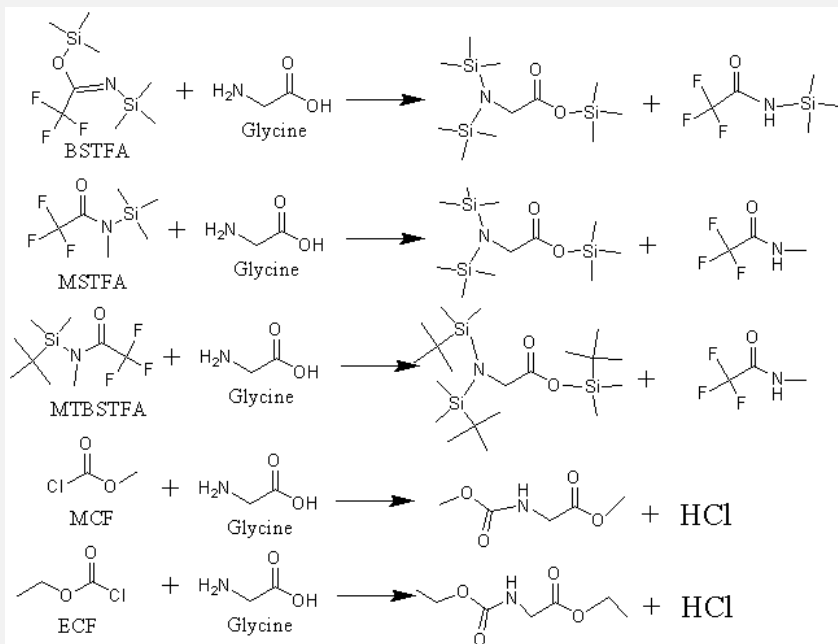
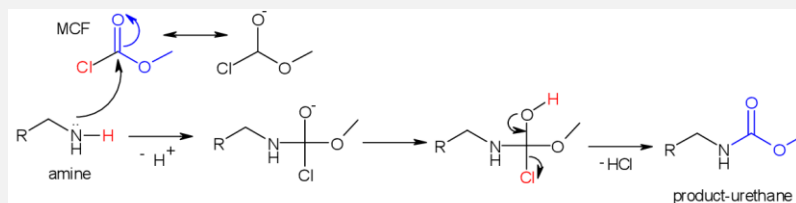
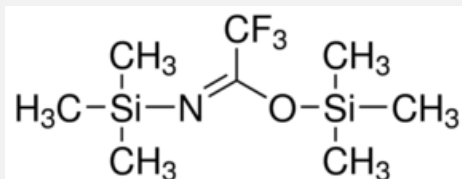
- zwiększenia lub zmniejszenia lotności analitów
- zwiększenia stabilności termicznej analitów
- zwiększenie czułości lub specyficzności oznaczenia przez wprowadzenie odpowiednich grup funkcyjnych
- zmianę polarności analitu, przez zablokowanie grup funkcyjnych
- uzyskanie barwnego produktu reakcji bądź zmiany barwy analitu, co umożliwia zastosowanie prostych metod kolorymetrycznych
- specyficznego zatrzymania analitu na warstwie nośnika pokrytego reagentem (wzbogacenia)



# Przygotowanie próbek do analizy

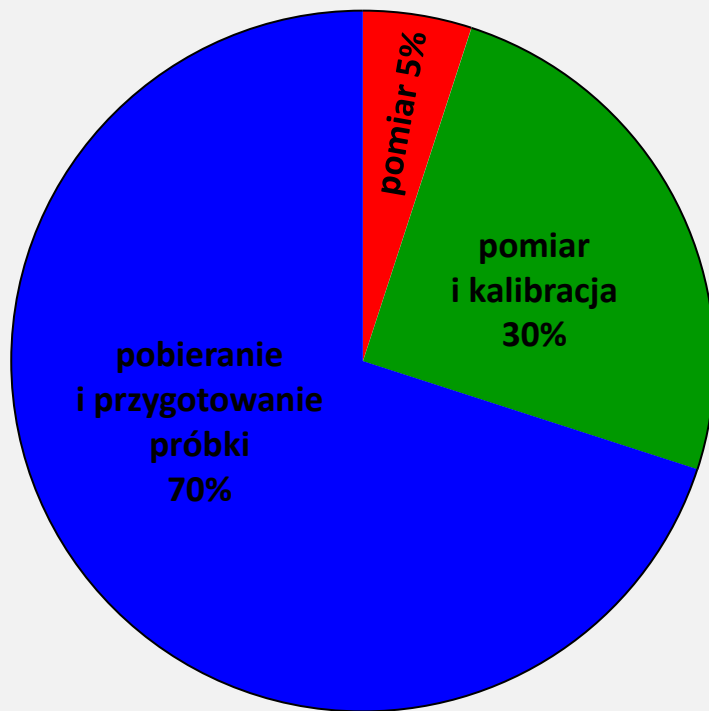
Odczynnik derywatyzujący powinien zawierać dwie grupy funkcyjne, z których jedna jest reaktywna i łączy się z analitem oraz sygnałowa, która umożliwi detekcję i zwiększa czułość analizy. Derywatyzacja opiera się na następujących reakcjach:

- sililowanie
- alkilowanie
- acylowanie
- estryfikacja

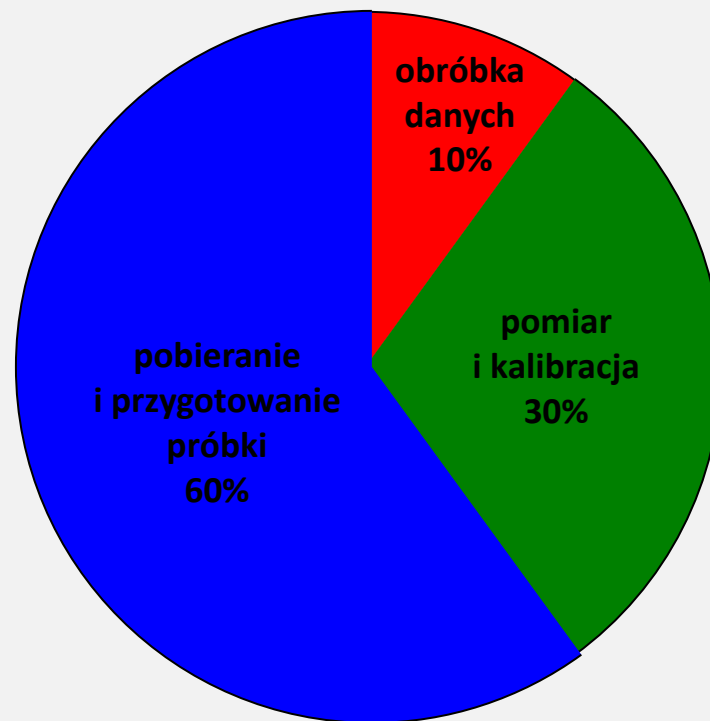


# Przygotowanie próbek do analizy

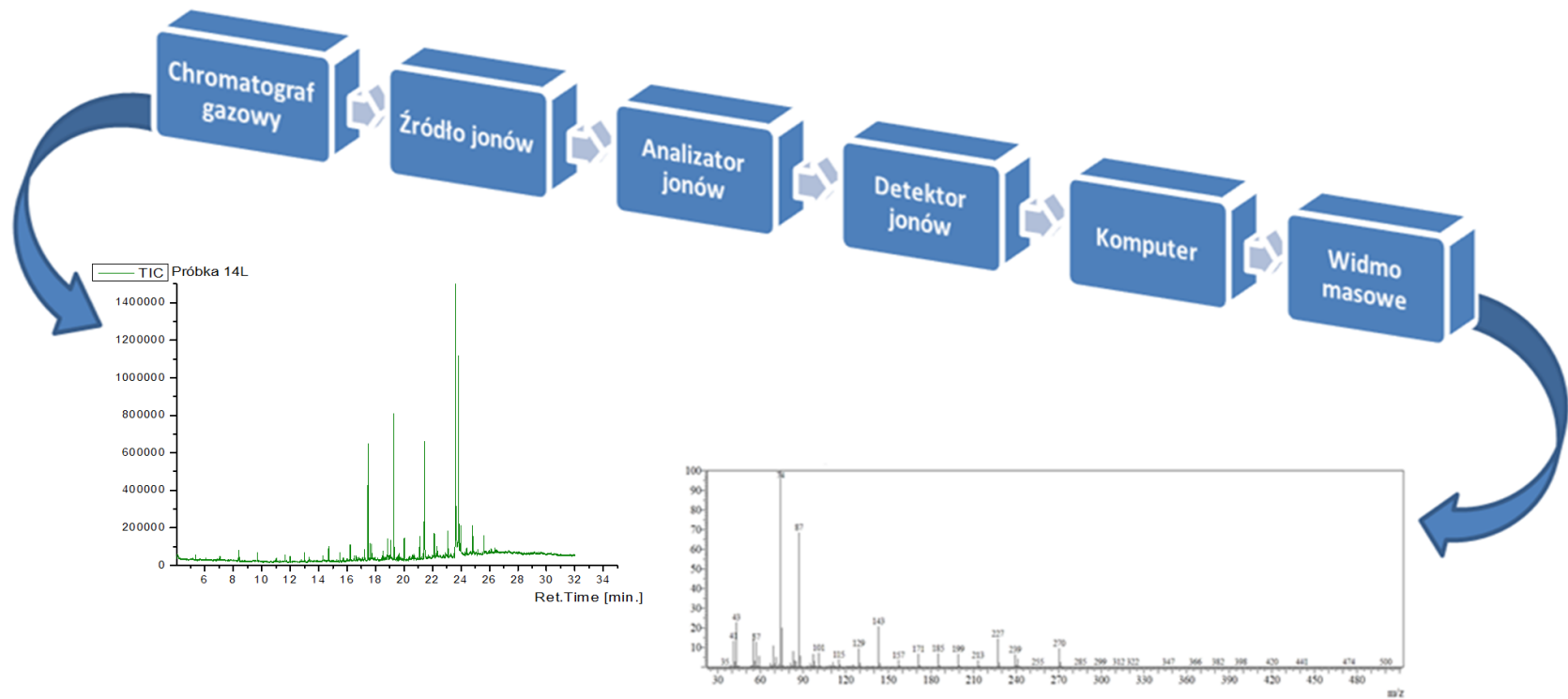
Potrzebny czas



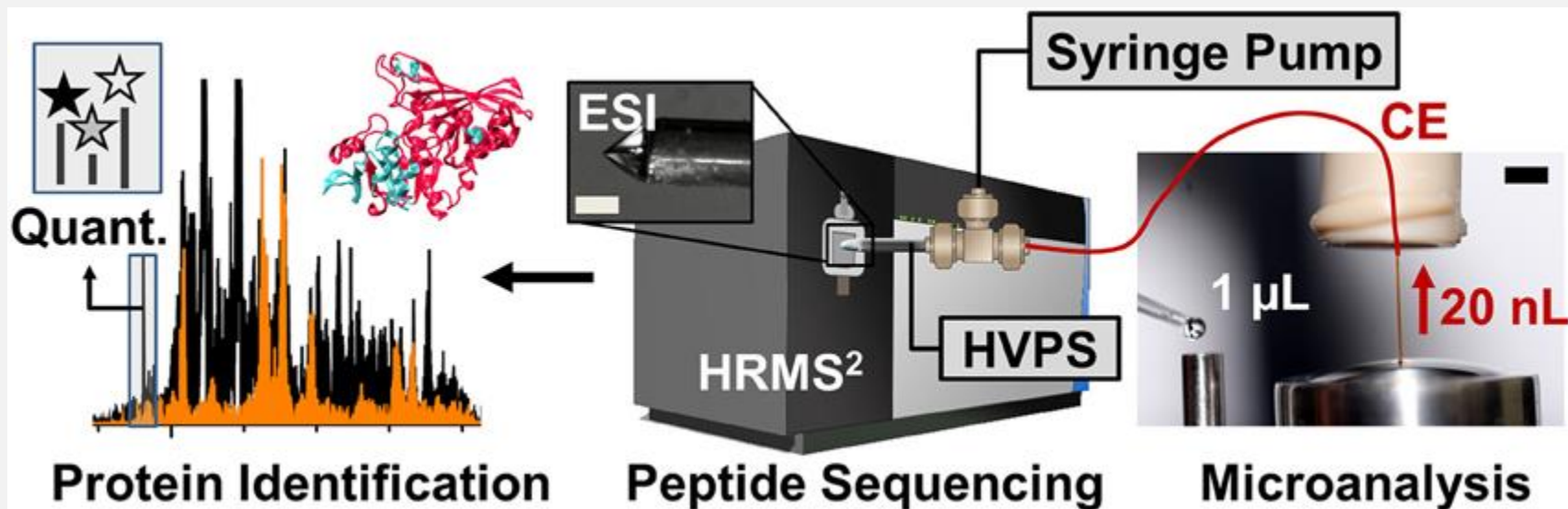
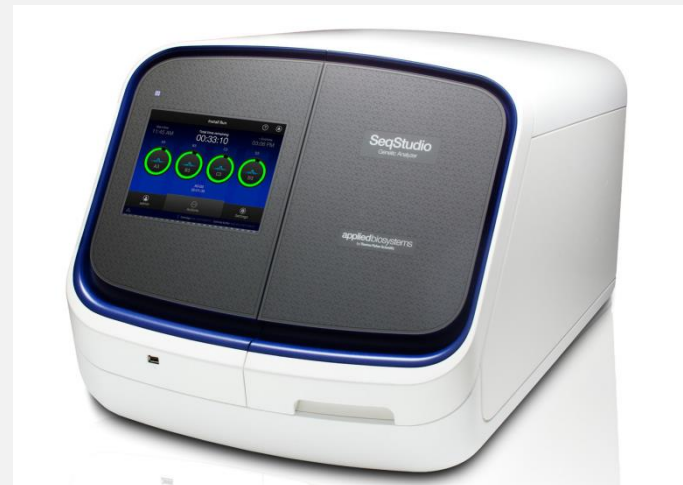
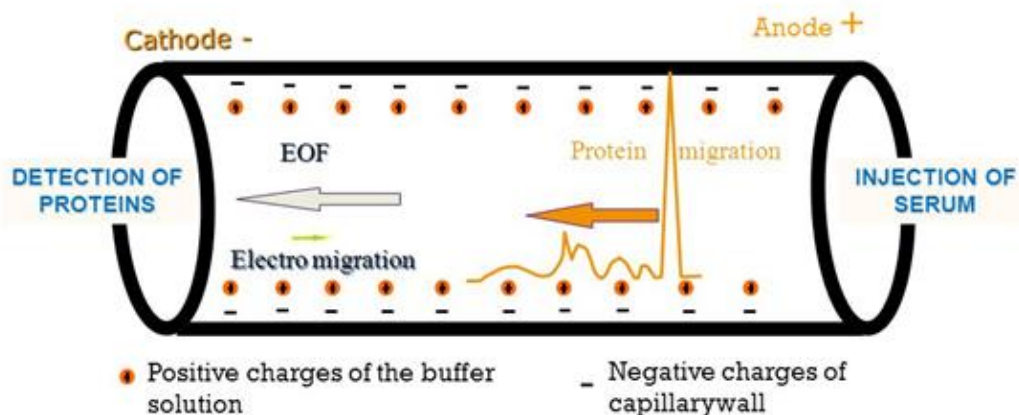
Źródła błędów



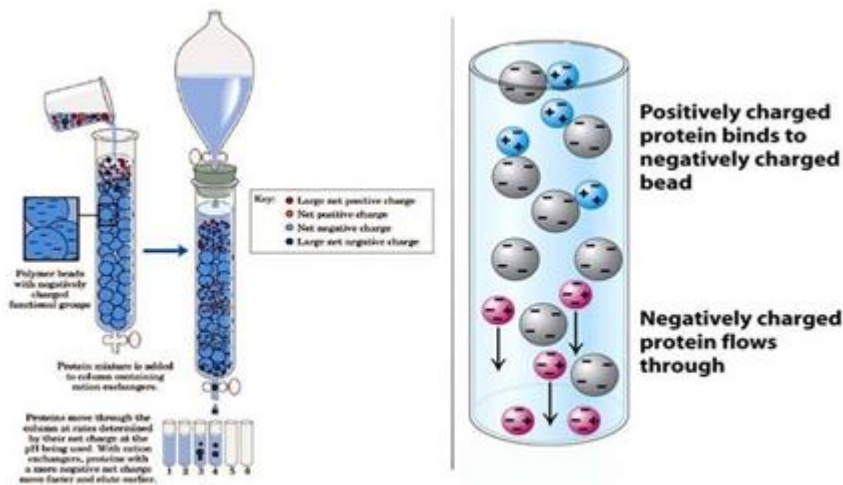
# Analiza próbek



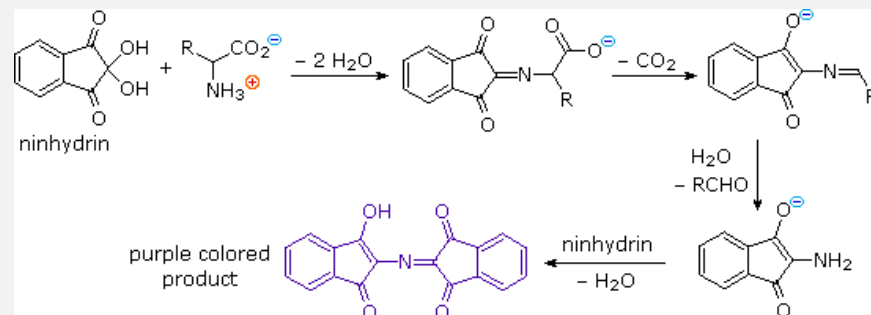
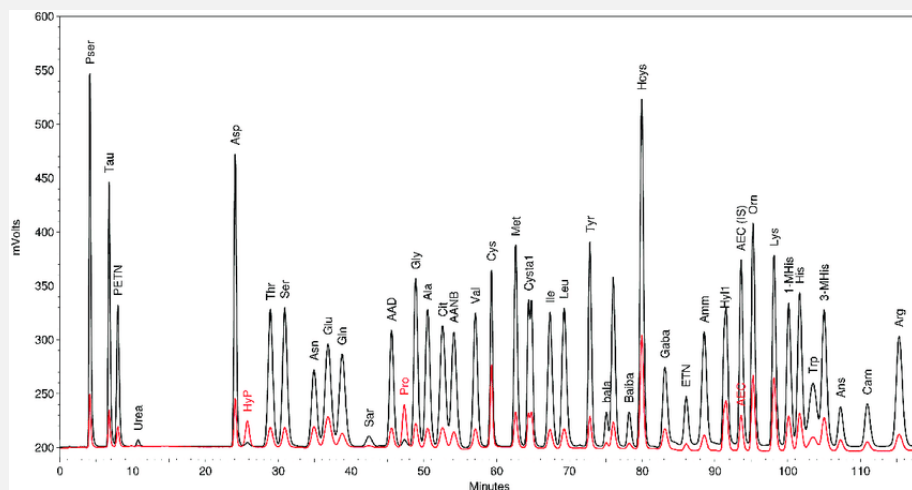
# Analiza próbek – Układ wprowadzania próbki



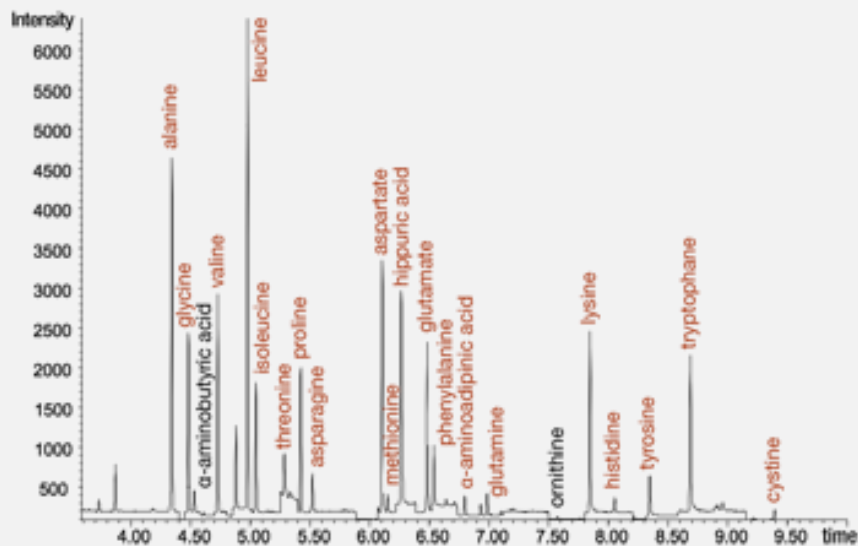
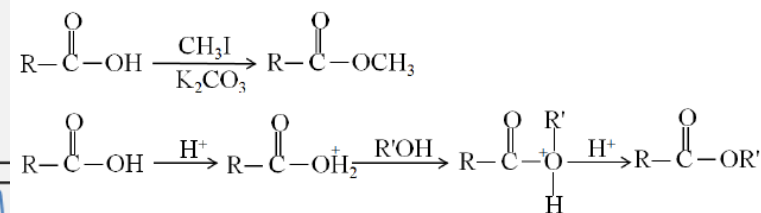
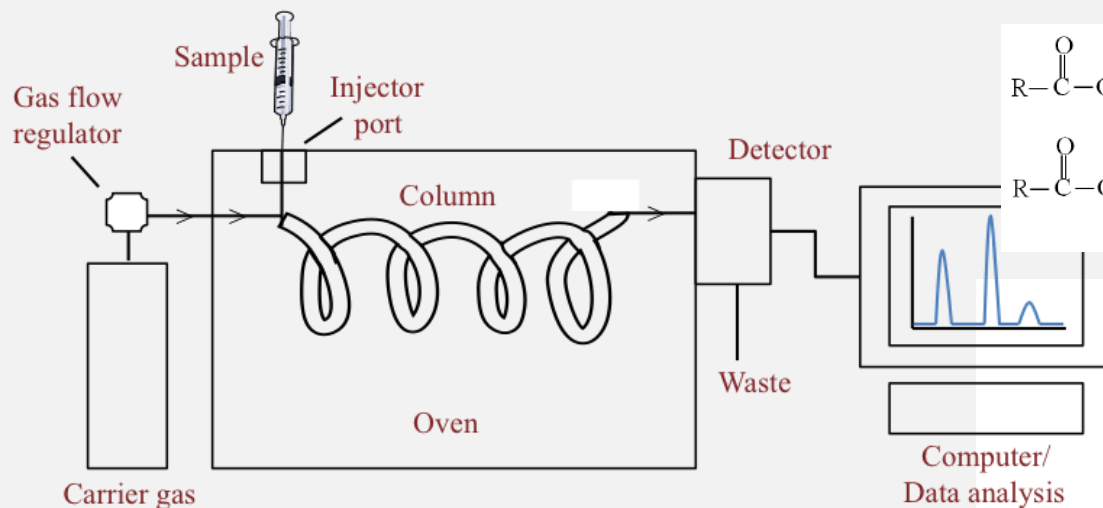
## Analiza próbek – Układ wprowadzania próbki



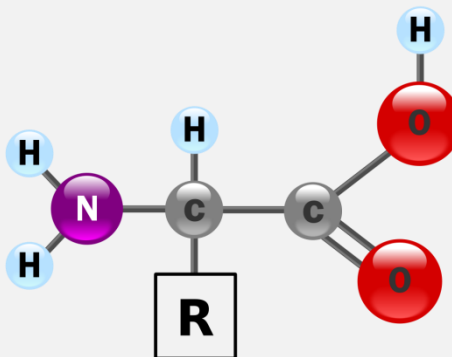
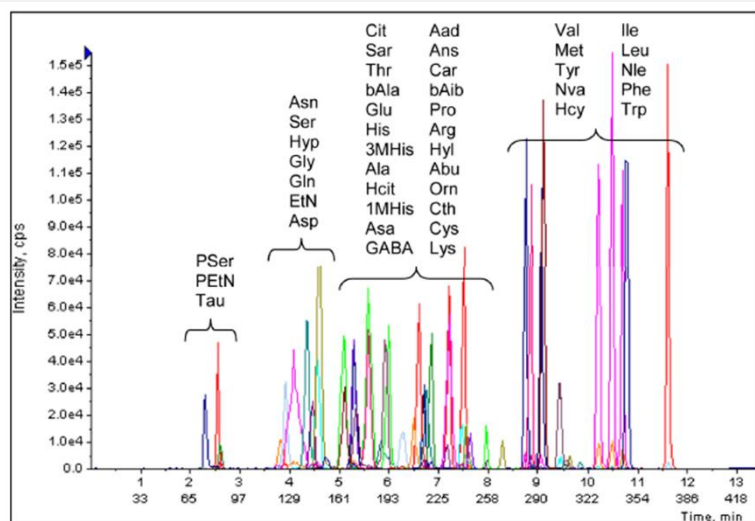
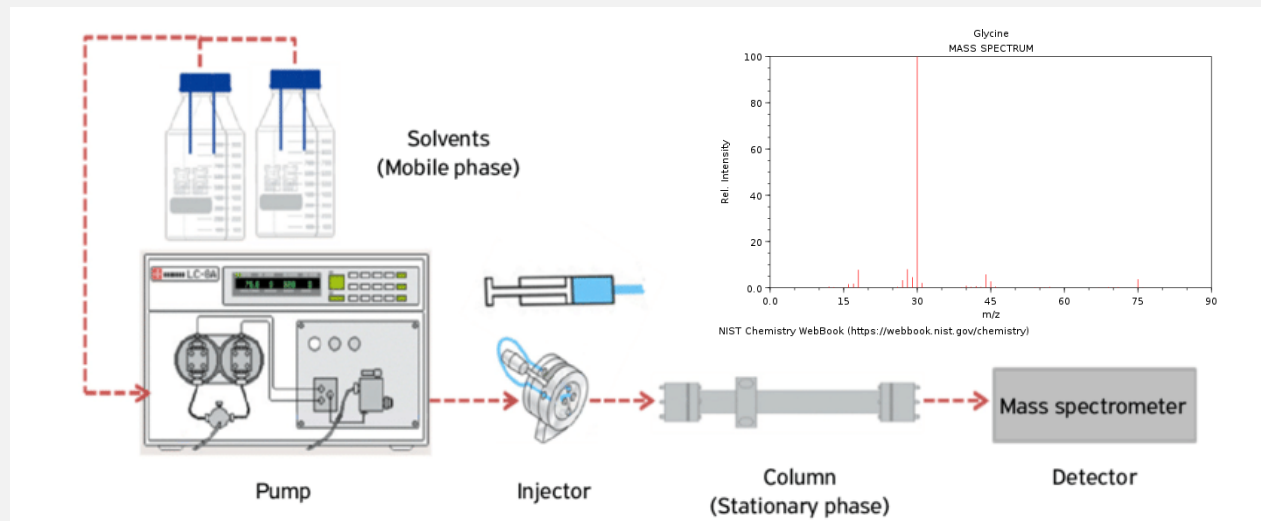
[www.technologyinscience.blogspot.com](http://www.technologyinscience.blogspot.com)



# Analiza próbek – Układ wprowadzania próbki



# Analiza próbek – Układ wprowadzania próbki



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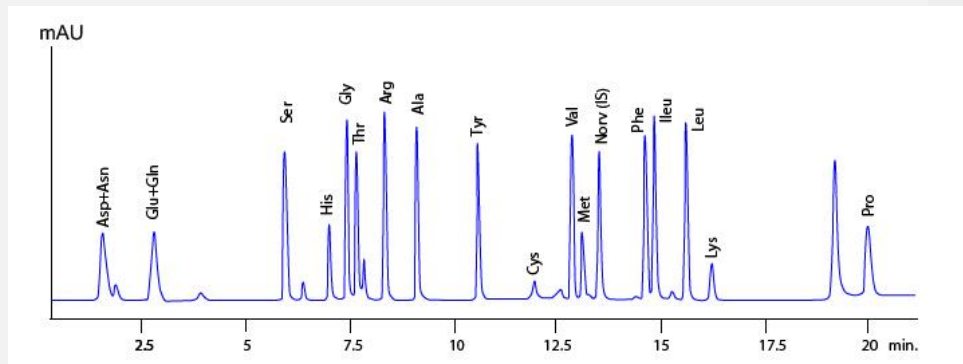
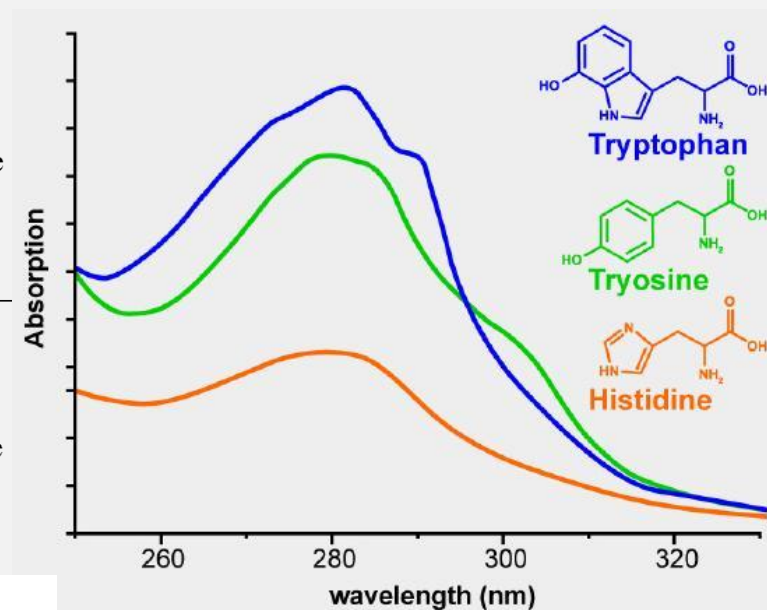
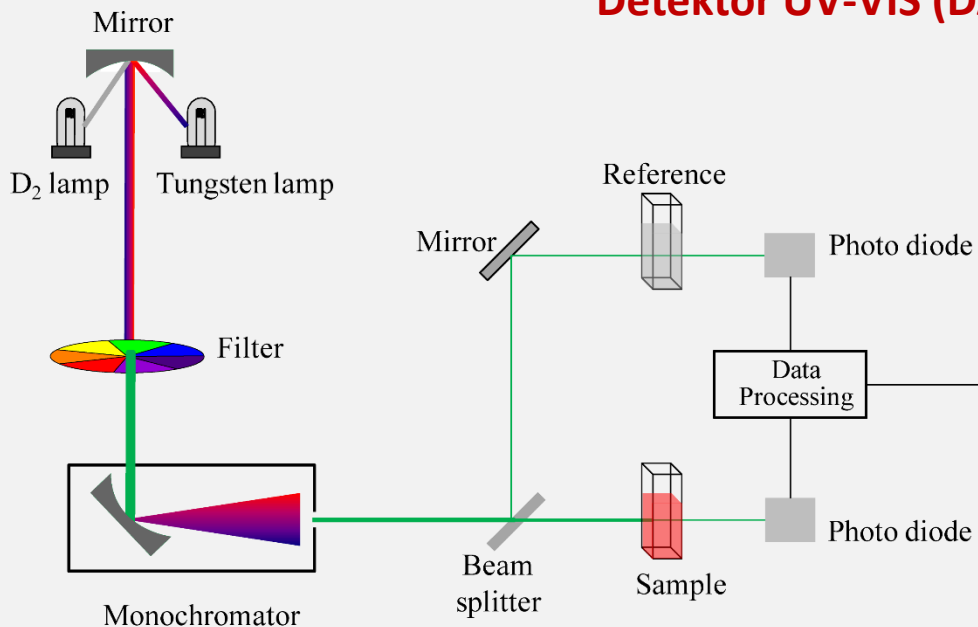
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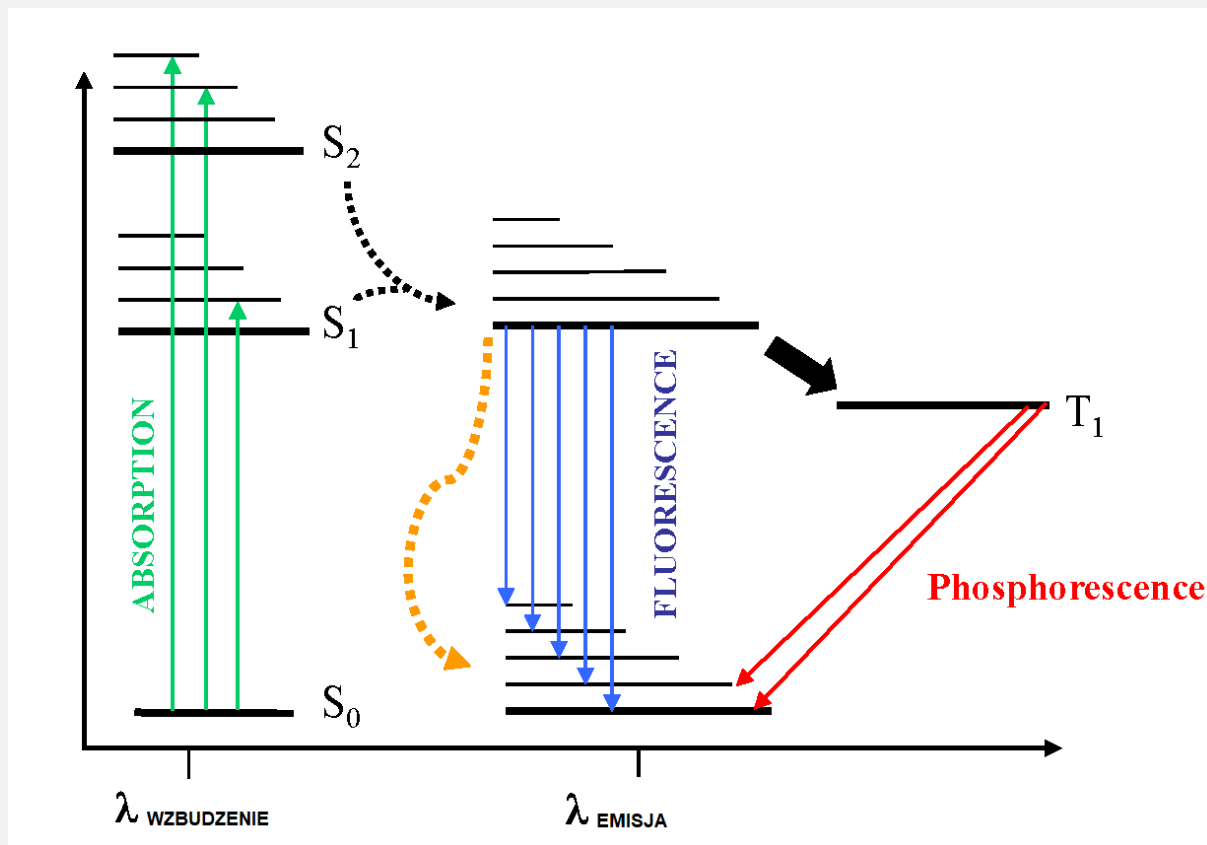
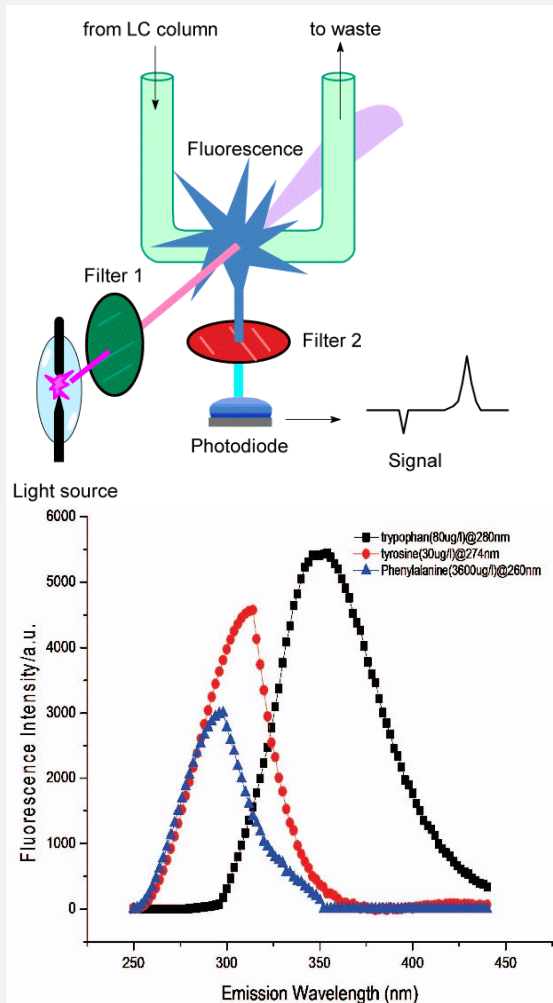
# Analiza próbek – Detektory

## Detektor UV-VIS (DAD)



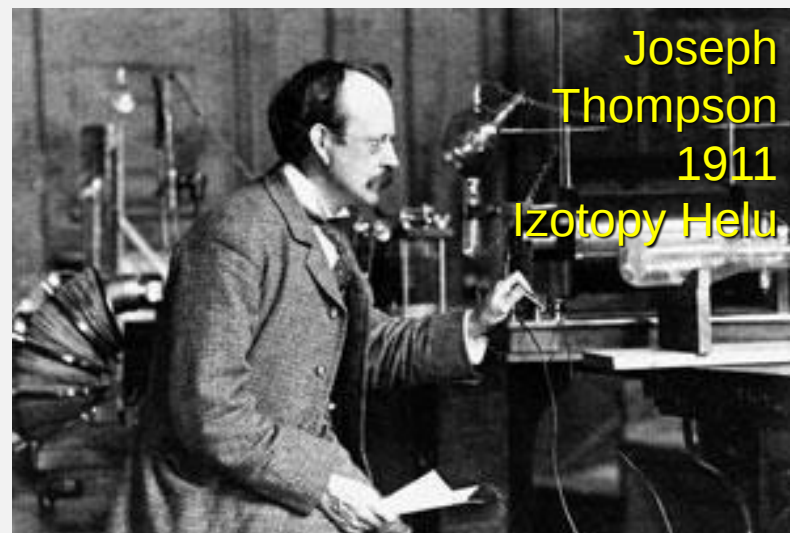
# Analiza próbek – Detektory

## Detektor fluorescencyjny

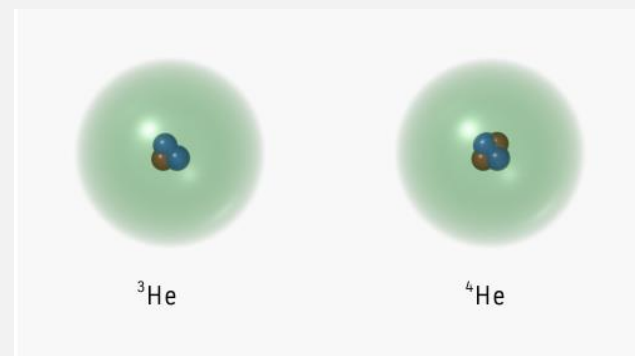


# Analiza próbek – Detektory

## Detektor mas



Komputer



# Analiza próbek – Detektory

## Detektor mas

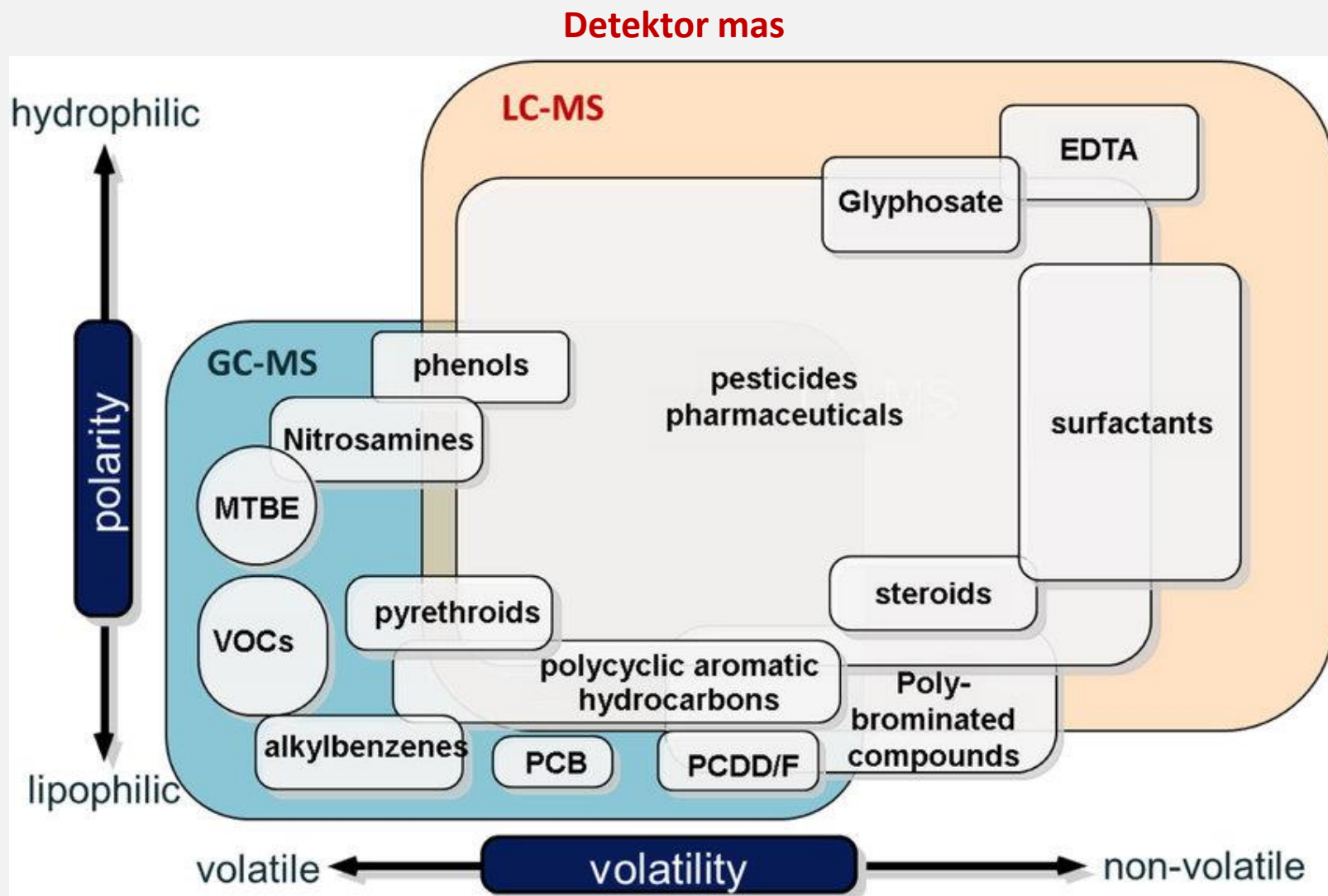


**Chromatograf gazowy  
z detektorem mas (GC-MS)**



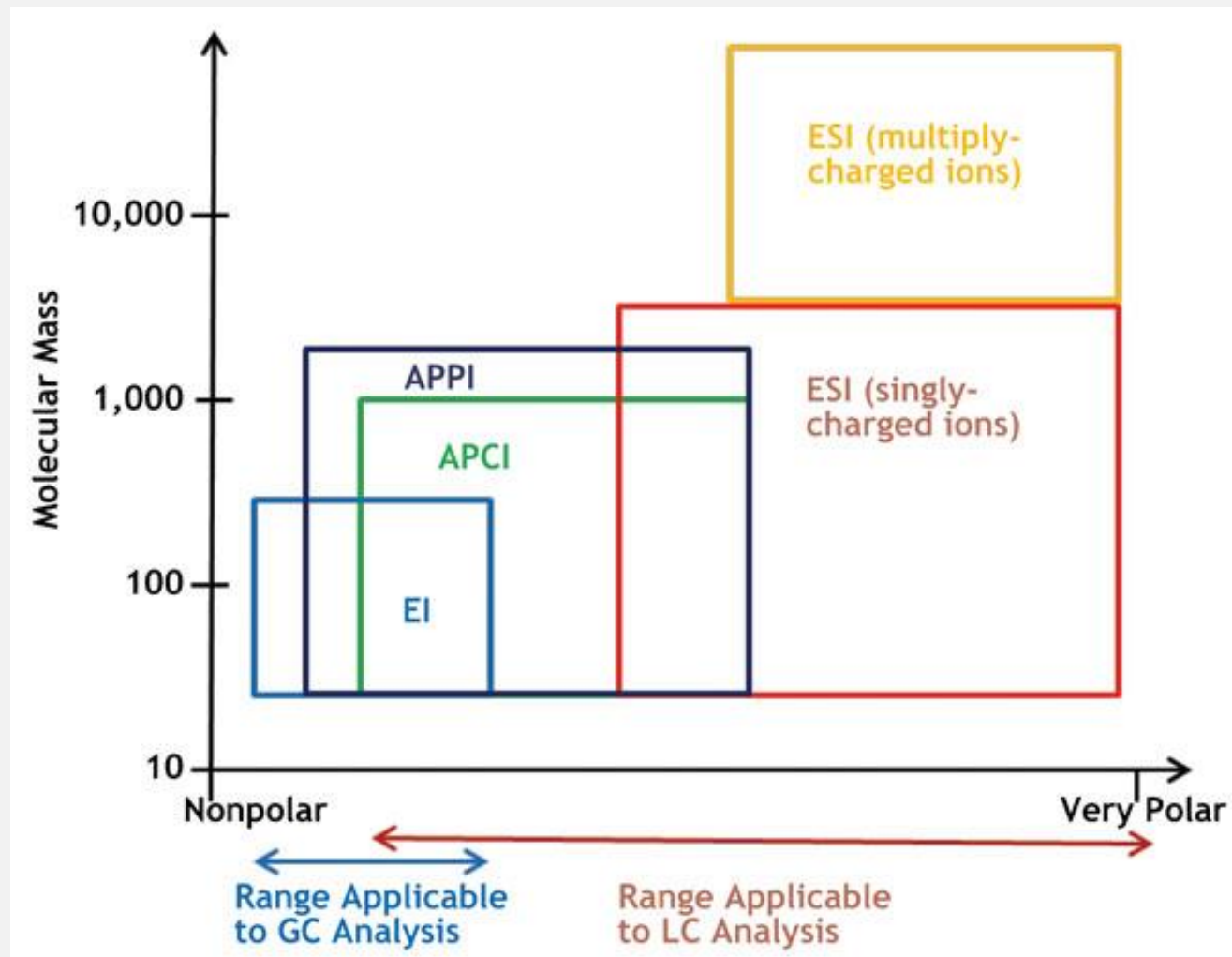
**Chromatograf ciekowy  
z detektorem mas (LC-MS)**

# Analiza próbek – Detektory



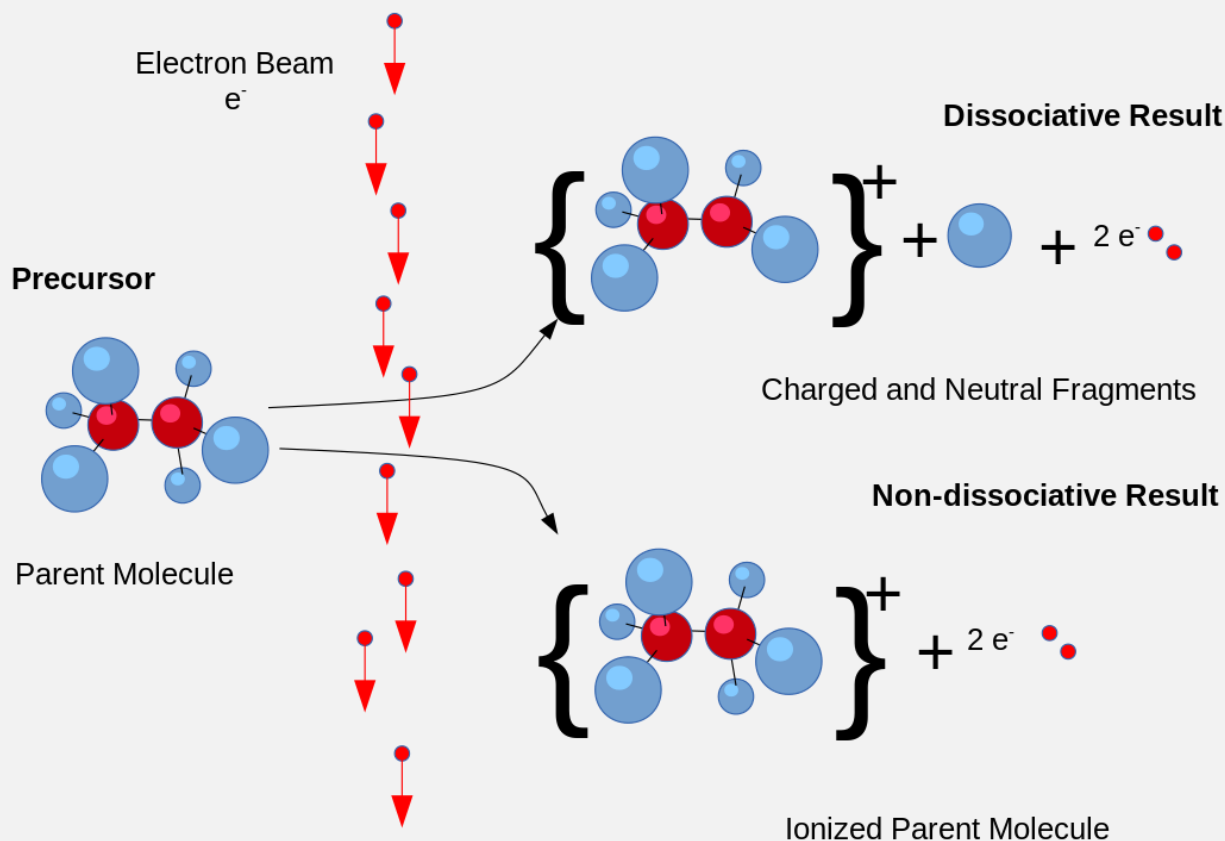
# Analiza próbek – Detektory

## Detektor mas – Źródła jonów

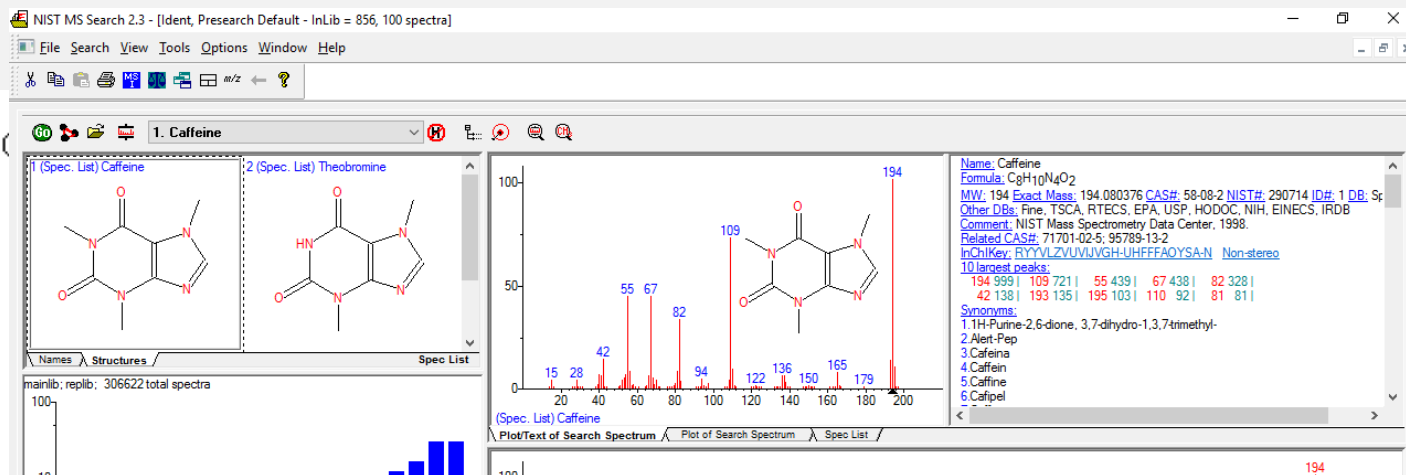


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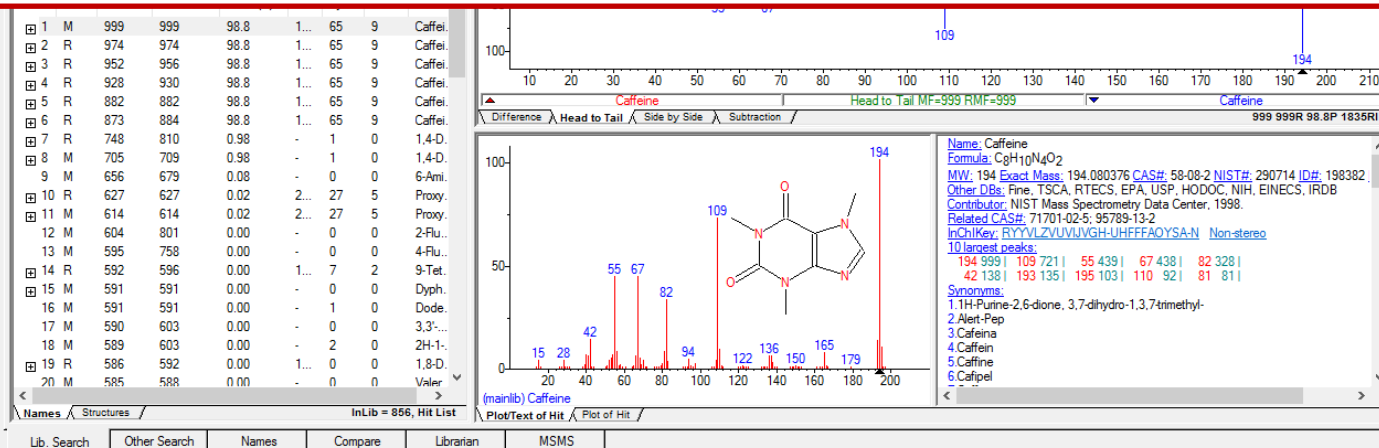
## Detektor mas – Jonizacja strumieniem elektronów



# Analiza próbek – Detektory

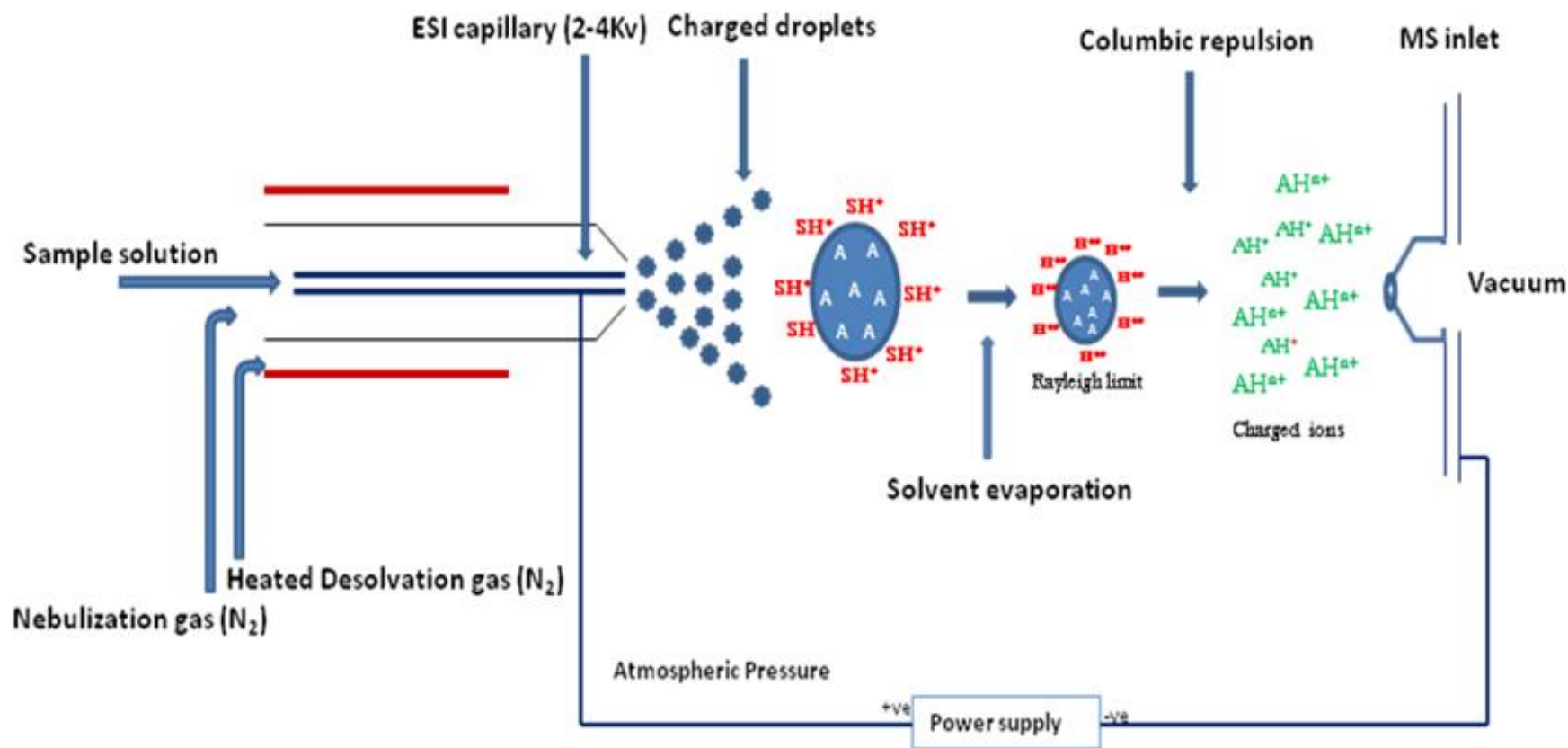


2017 r. – 267 376 związków w bazie



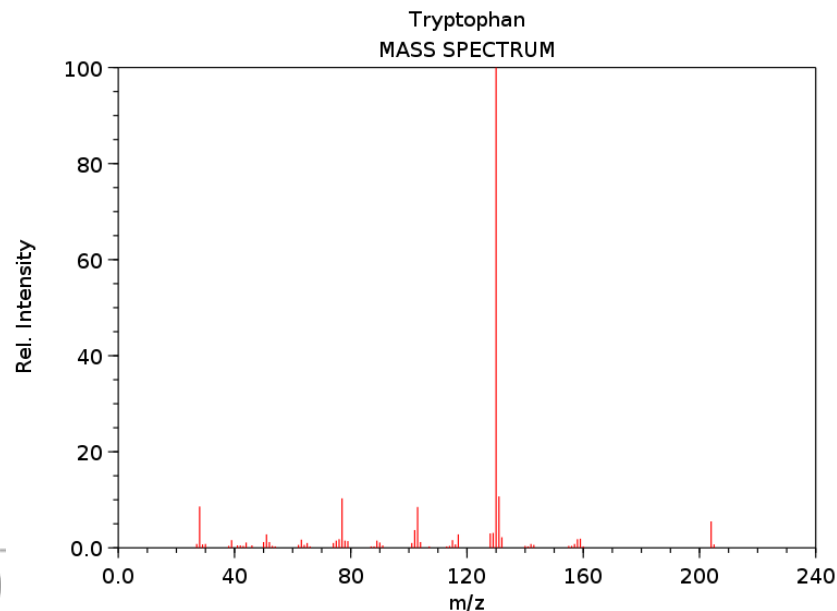
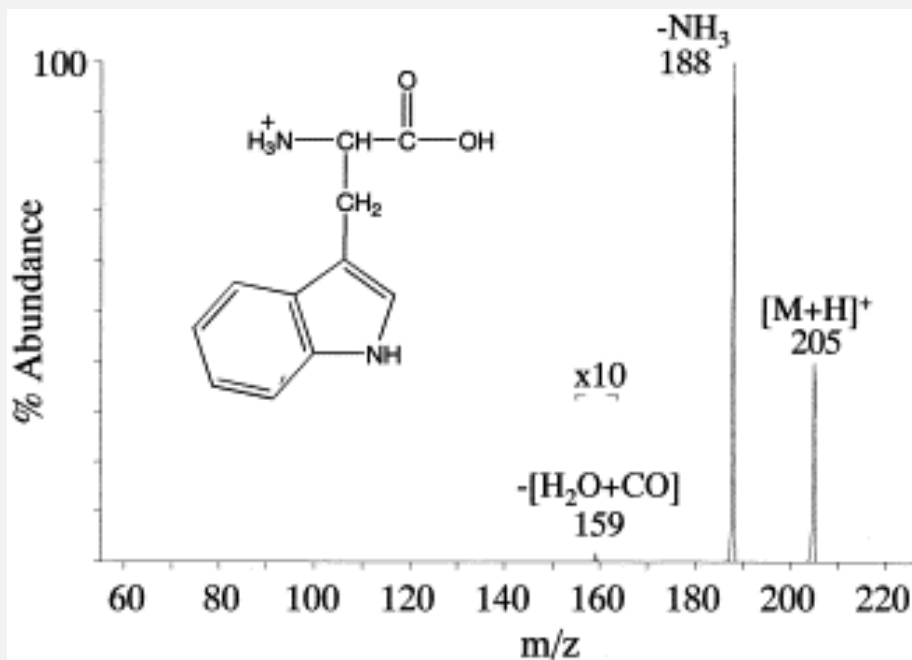
# Analiza próbek – Detektory

## Detektor mas – Jonizacja przez elektrorozpylanie



# Analiza próbek – Detektory

## Detektor mas – Źródła jonów



NIST Chemistry WebBook (<https://webbook.nist.gov/chemistry>)

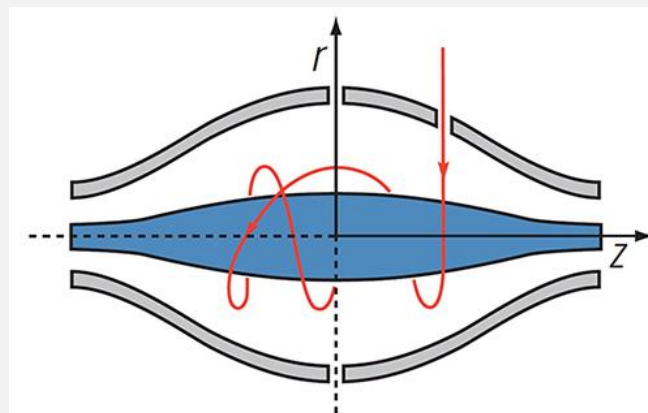
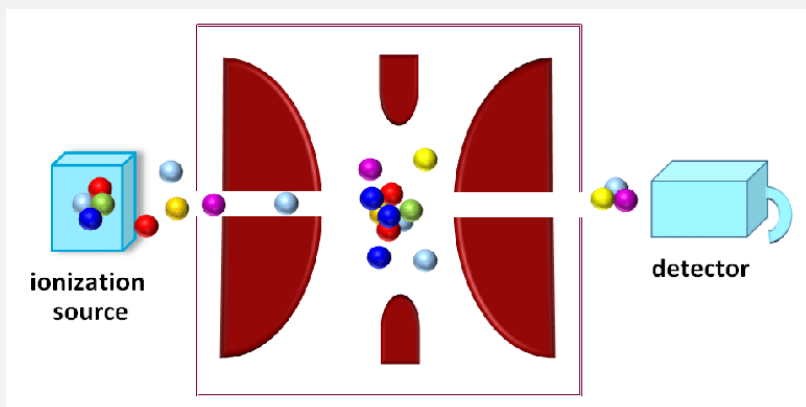
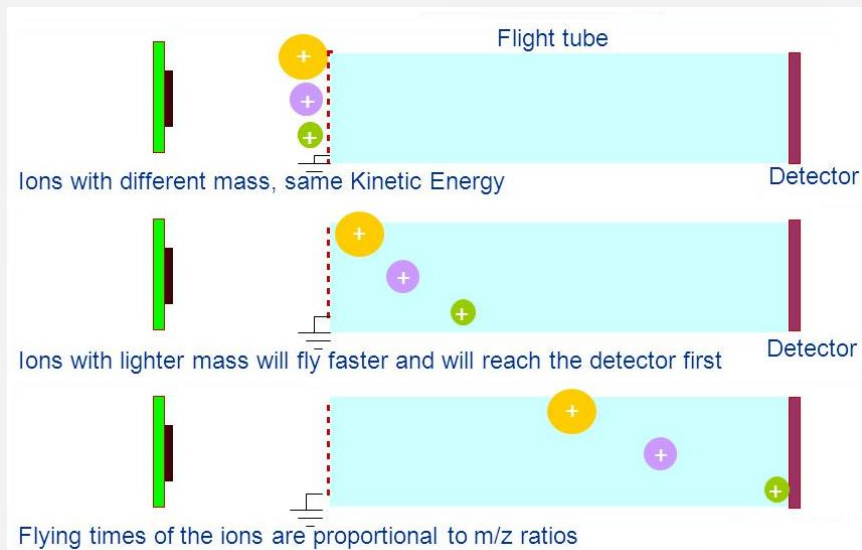
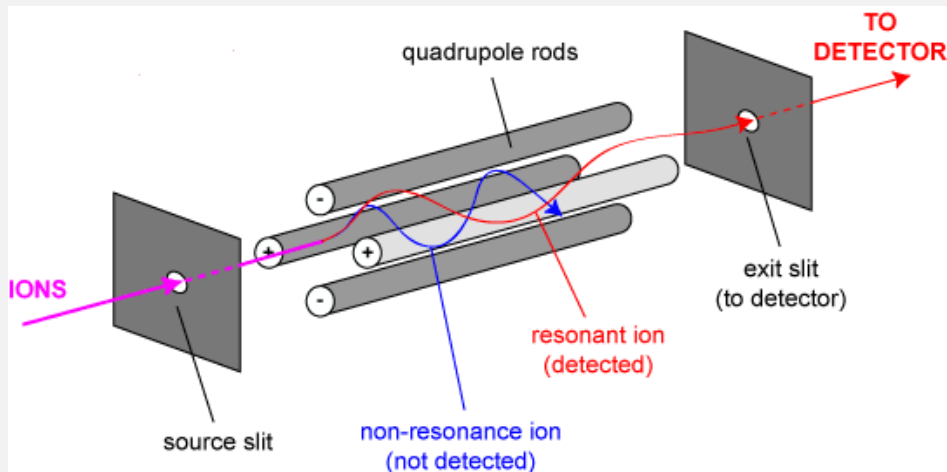
ESI

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EI

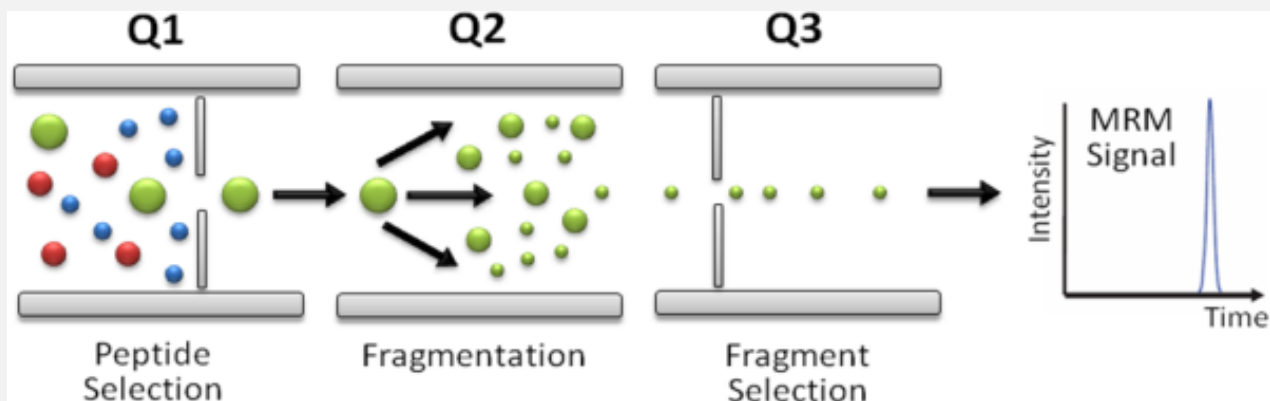
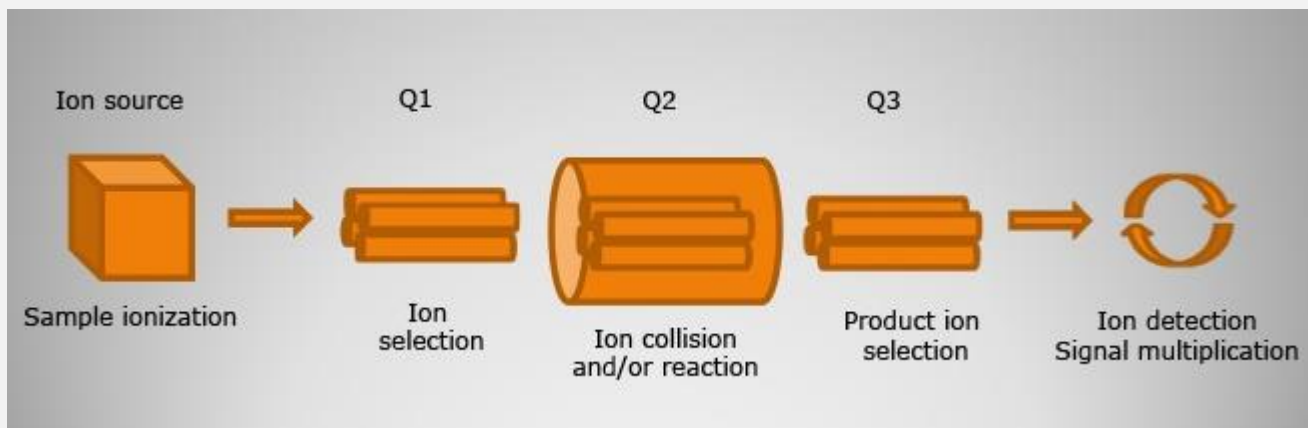
# Analiza próbek – Detektory

## Detektor mas - Analizatory



# Analiza próbek – Detektory

## Tandemowa spektrometria mas

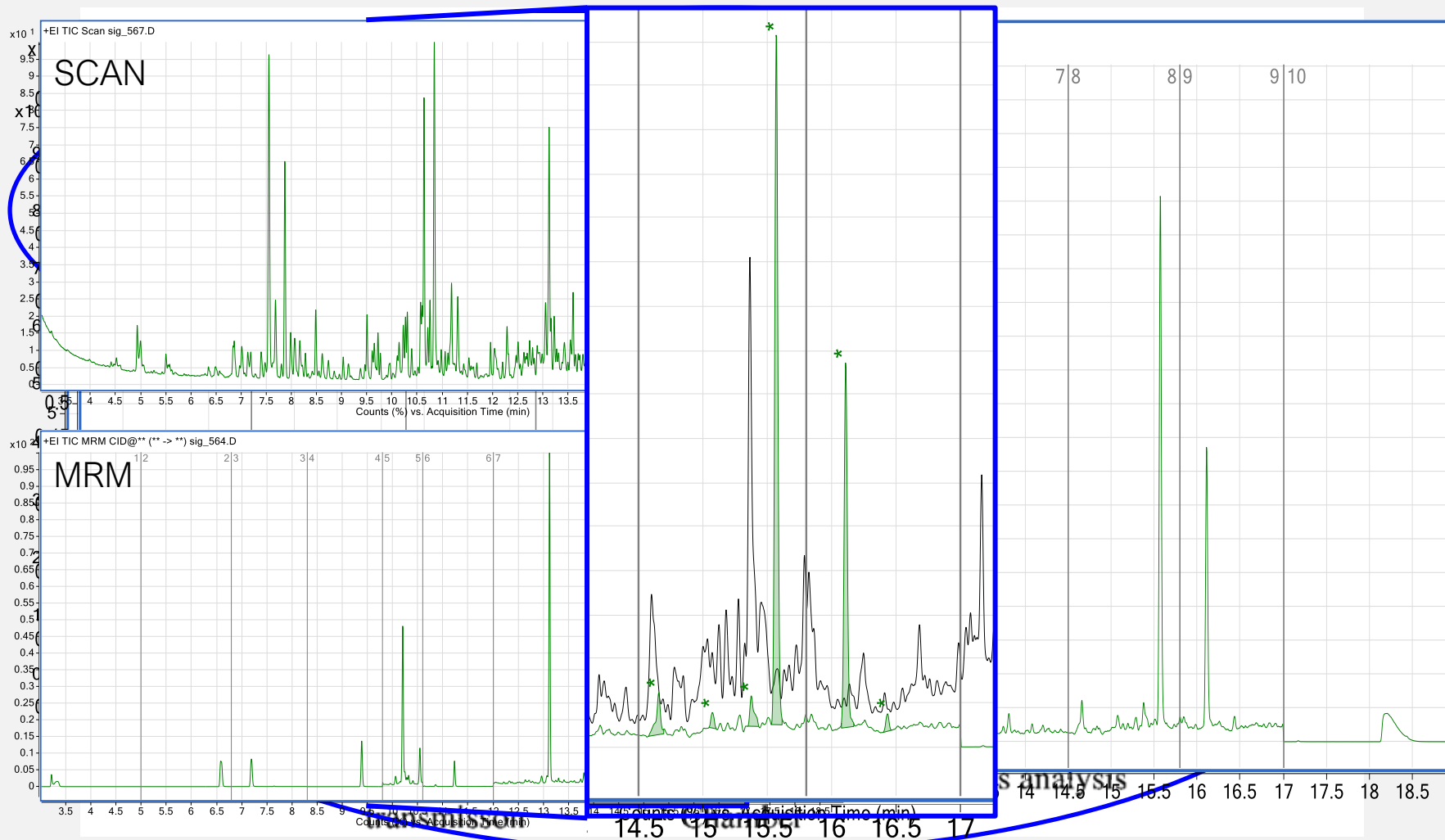


Większa czułość !!!

Mniejsze tło !!!

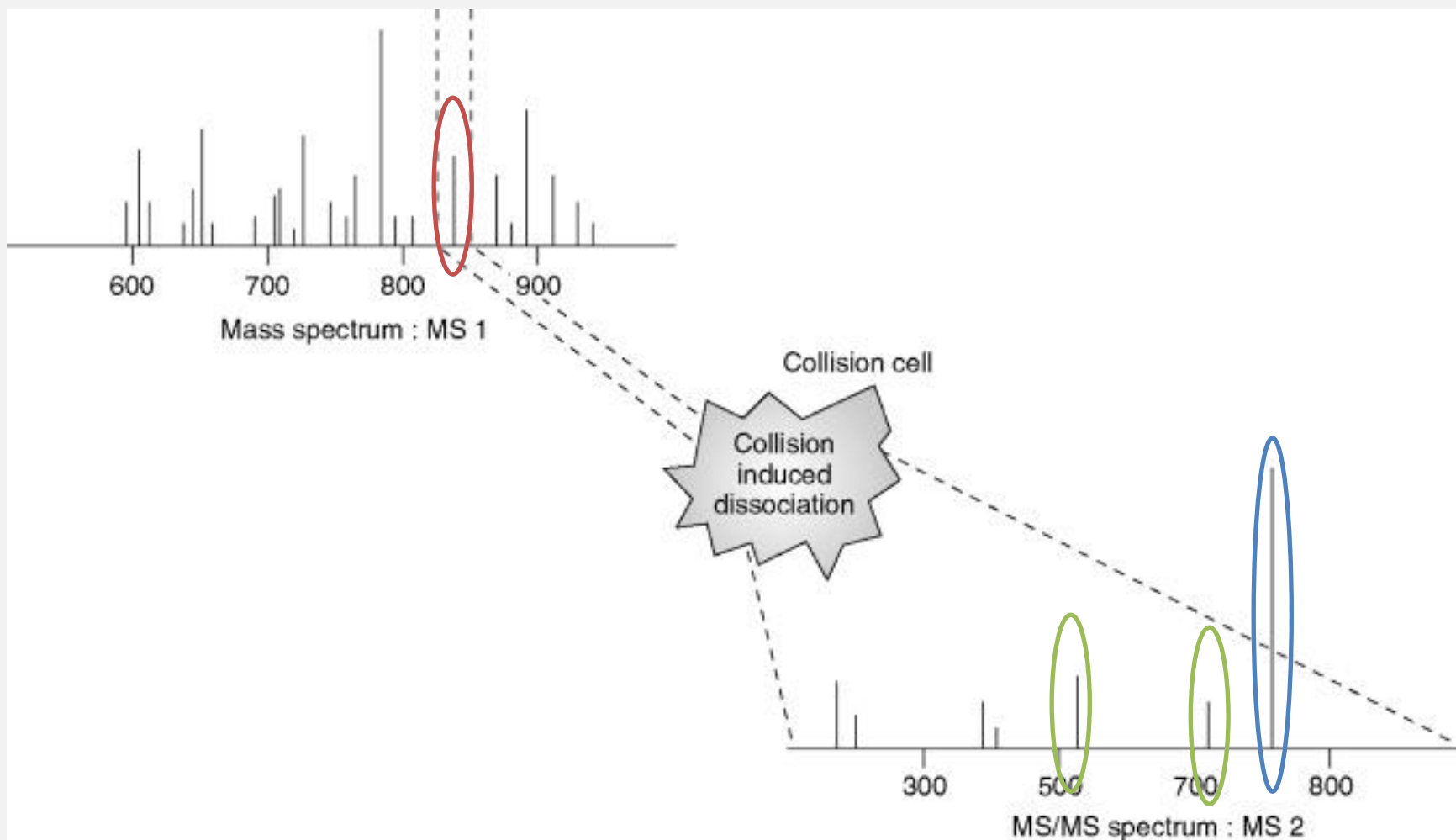
# Analiza próbek – Detektory

## Tandemowa spektrometria mas



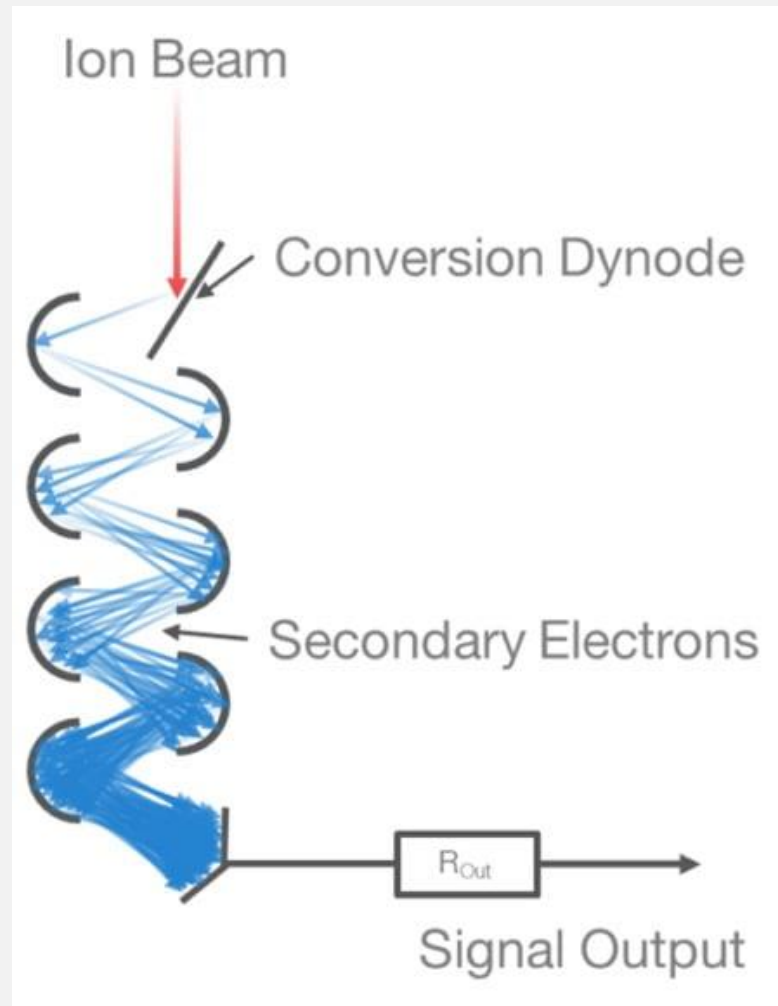
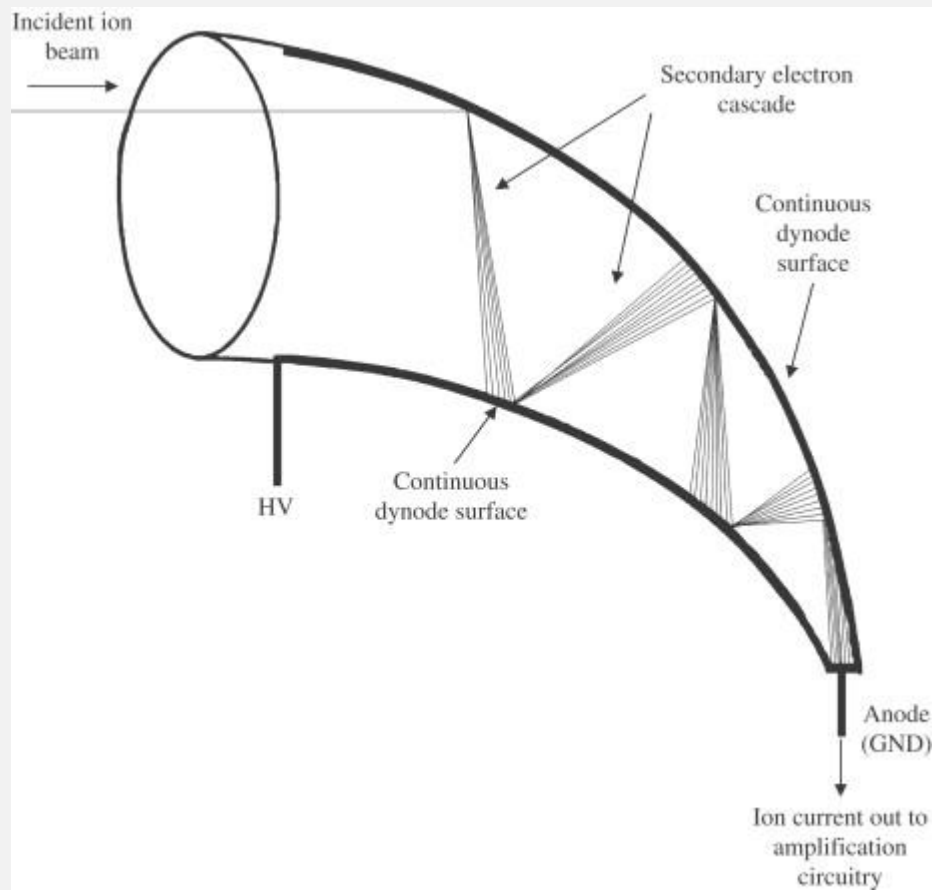
# Analiza próbek – Detektory

## Tandemowa spektrometria mas

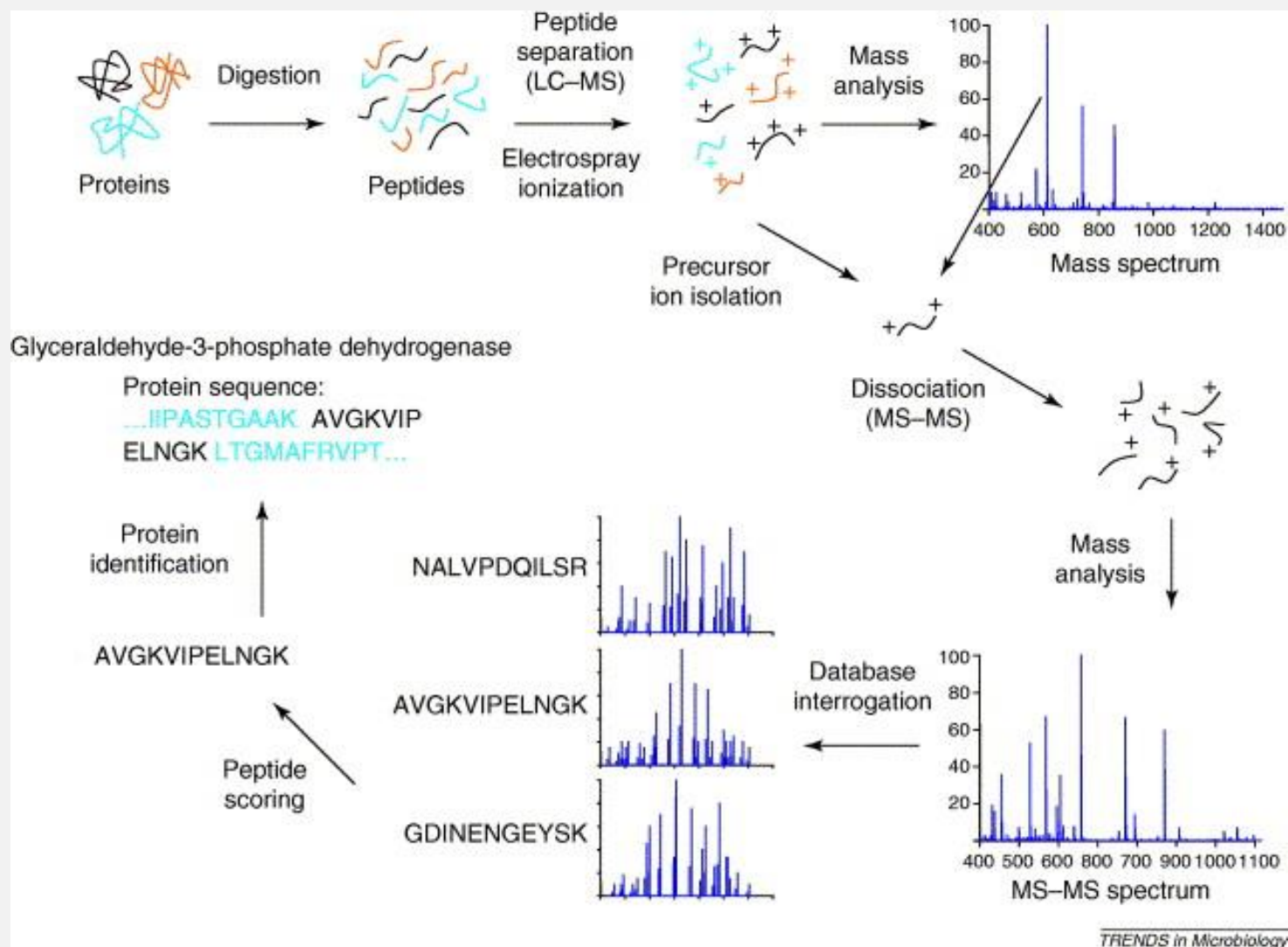


# Analiza próbek – Detektory

## Detektor mas - detektor

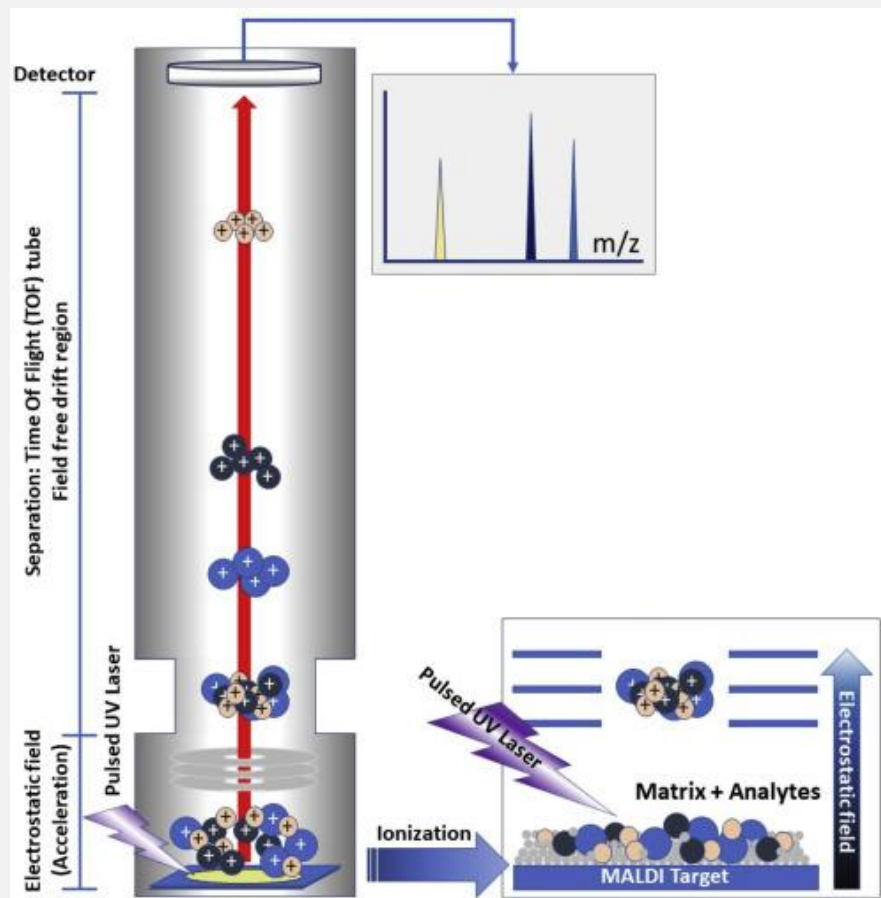


# Wysokorozdzielcza spektrometria mas

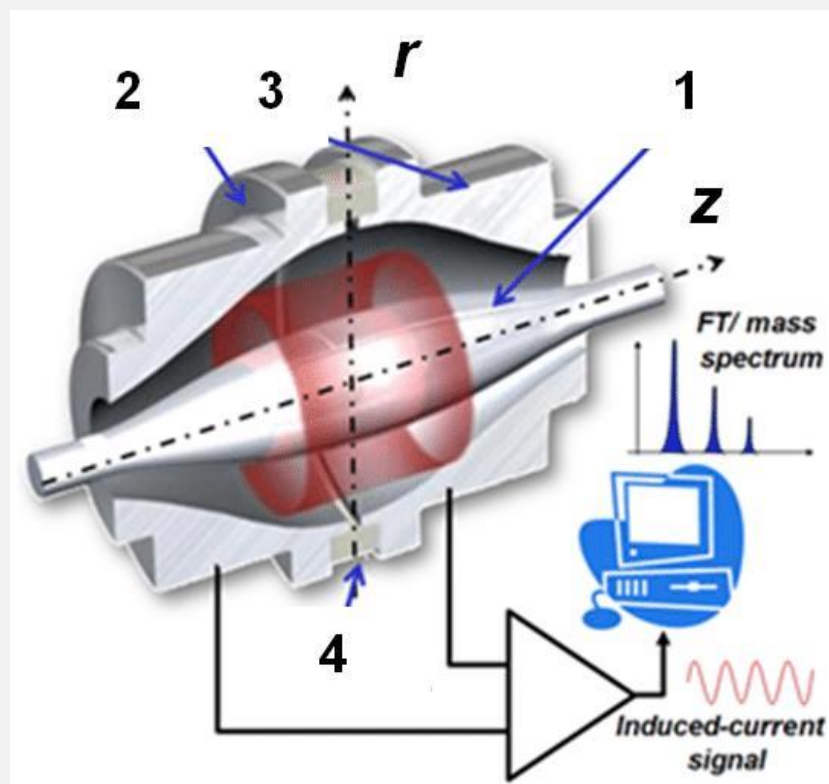


# Wysokorozdzielcza spektrometria mas

## TOF



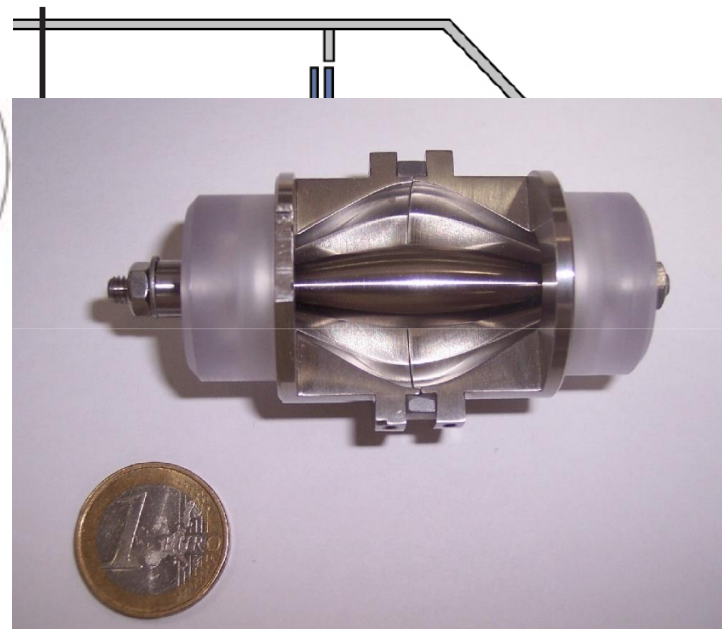
## ORBITRAP



# Wysokorozdzielcza spektrometria mas



drupole  
s Filter



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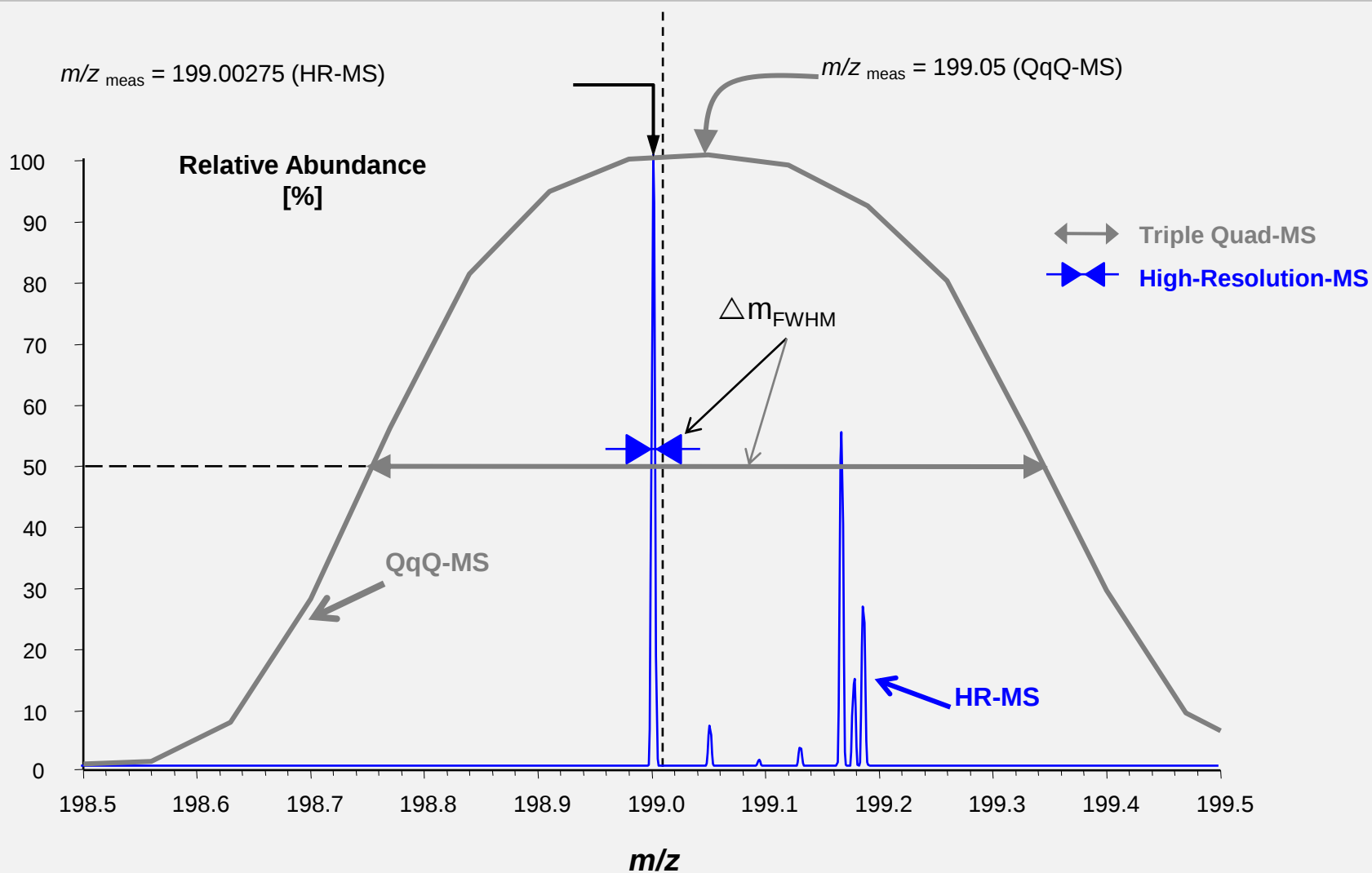


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# Wysokorozdzielcza spektrometria mas



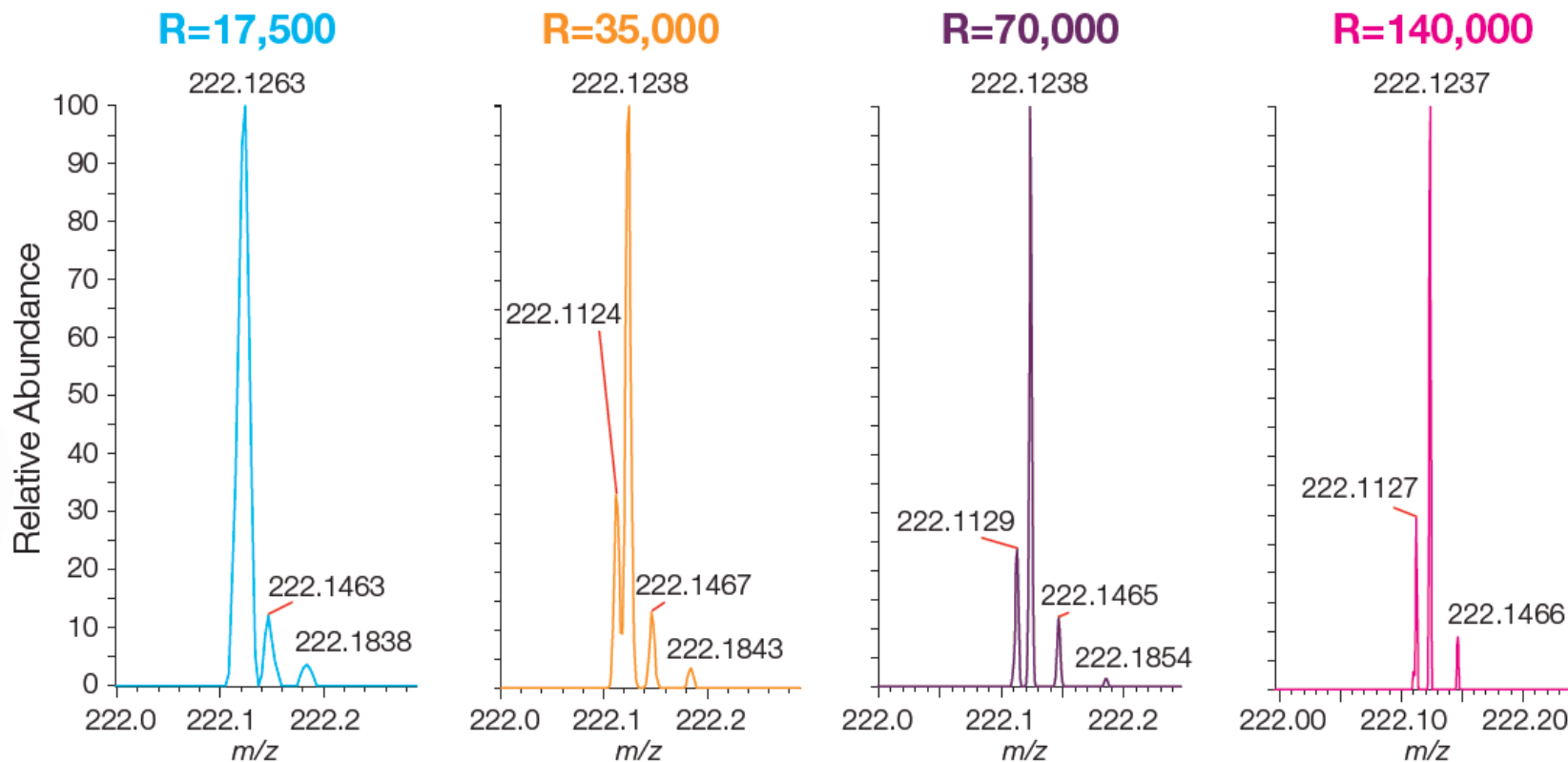
# Wysokorozdzielcza spektrometria mas

Carbofuran  
Formetamate

$C_{12}H_{15}NO_3$   
 $C_{11}H_{15}N_3O_2$

$M+H^+ = 222.1125$

$M+H^+ = 222.1237$



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Rzeczpospolita  
Polska

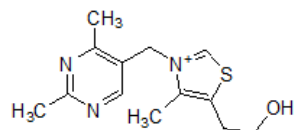


Instytut  
Matki i Dziecka

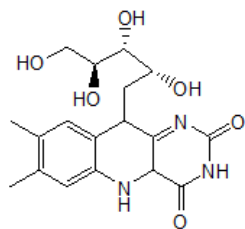
Unia Europejska  
Europejski Fundusz Społeczny



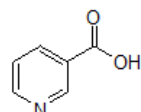
# Witaminy z grupy B



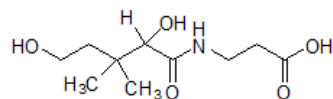
Vit B<sub>1</sub>



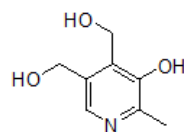
Vit B<sub>2</sub>



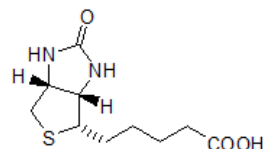
Vit B<sub>3</sub>



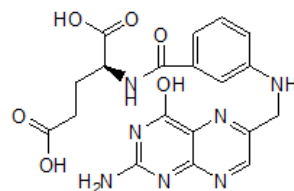
Vit B<sub>5</sub>



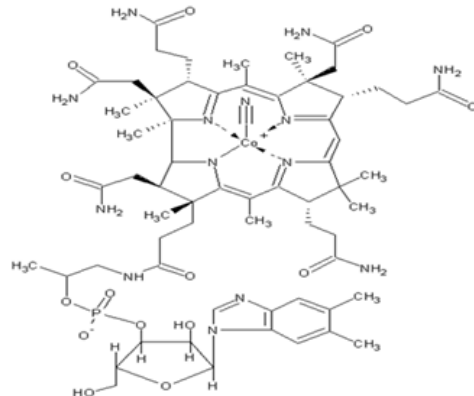
Vit B<sub>6</sub>



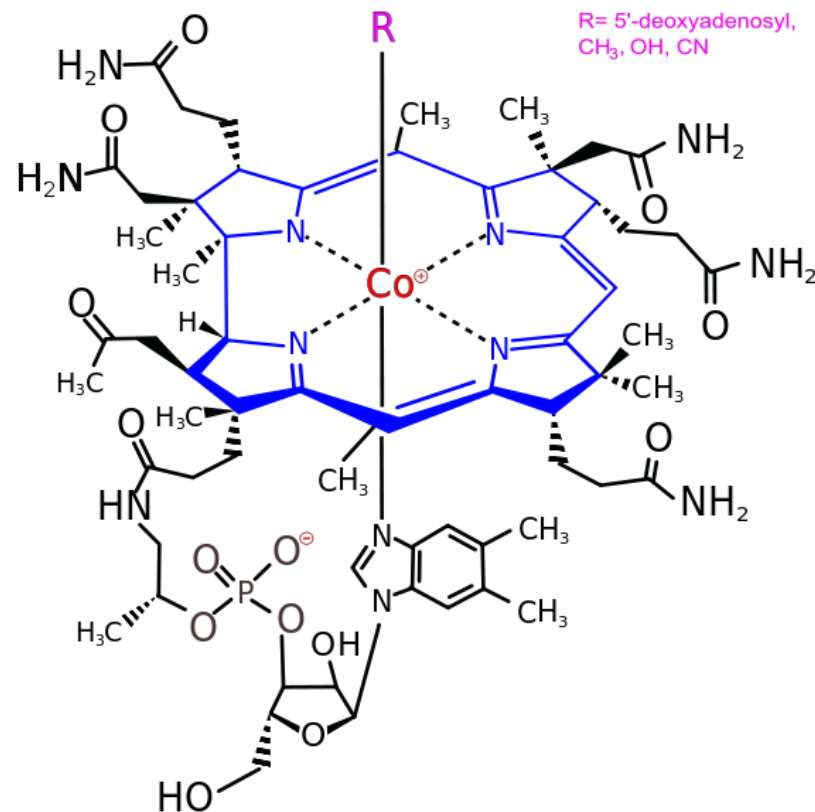
Vit B<sub>7</sub>



Vit B<sub>9</sub>

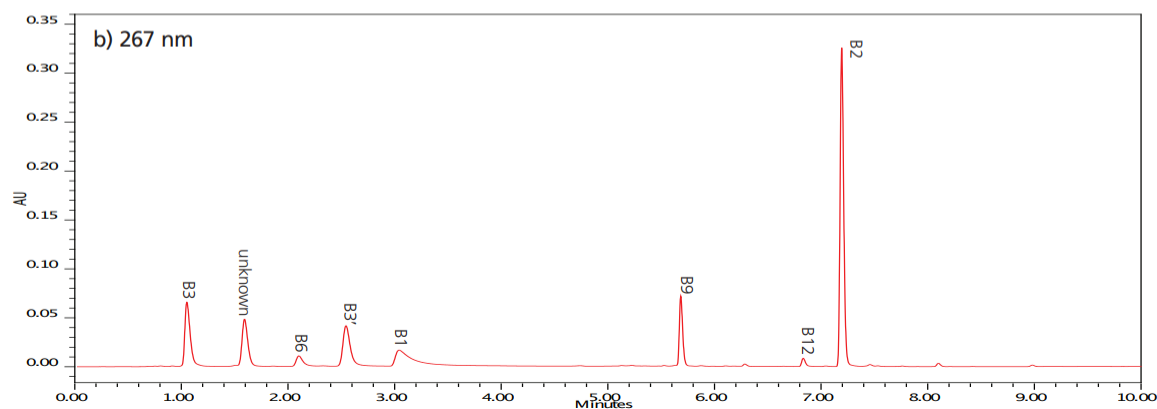
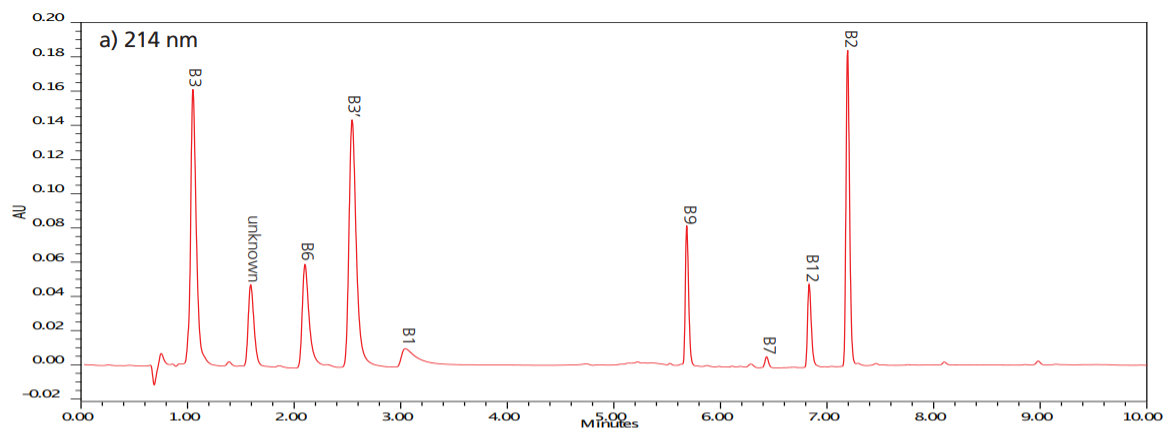


Vit B<sub>12</sub>



# Oznaczanie witamin z grupy B

## HPLC-PDA



The Qualitative and Quantitative Analysis of Water-Soluble B Vitamins by HPLC-PDA in Various Multivitamin Tablets, Andrew P. Zdziebło Wilhad M. Reuter, PerkinElmer, Inc. USA  
<https://pepolska.pl/wp-content/uploads/2020/03/NOTA-HPLC-Analiza-jako%C5%9Bciowa-i-ilo%C5%9Bciowa-witamin-B-w-tabletkach-multiwitaminowych-rozpuszczalnych-w-wodzie-technik%C4%85-HPLC.pdf>

### HPLC Conditions

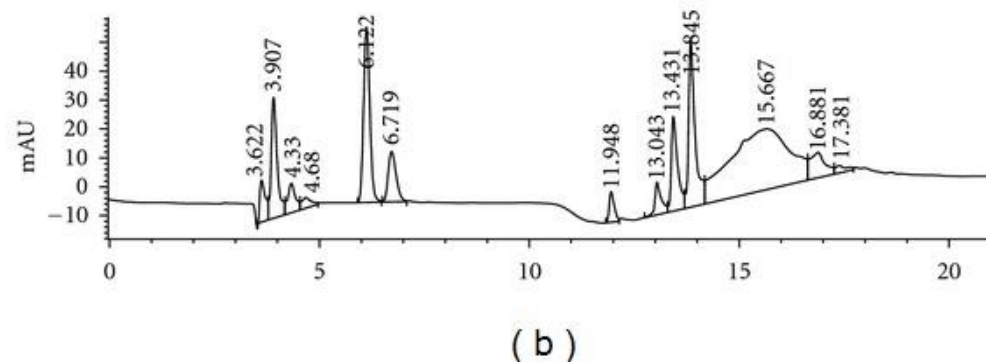
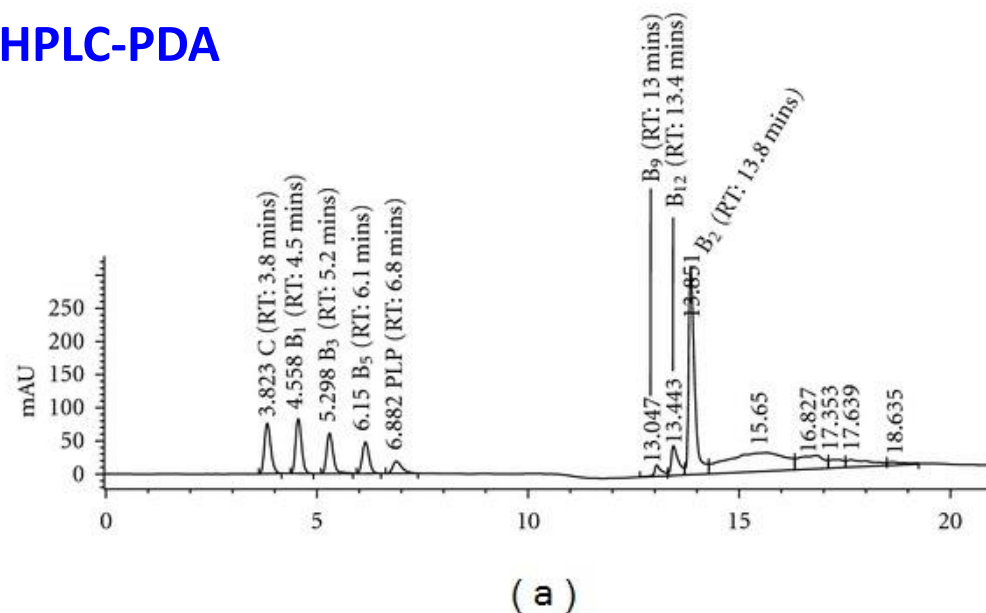
| Column:               | PerkinElmer Brownlee™ SPP C18 2.7 μm,<br>3.0 x 100 mm (Part# N9308410)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |      |        |      |     |     |       |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|--------|------|-----|-----|-------|----|-------|---|--|------|--------|-----|-----|-----|--|---|------|------|--------|-----|-----|-----|---|---|------|------|-------|------|-----|-----|---|---|-------|------|-------|------|-----|-----|---|---|-------|------|--------|-----|-----|-----|---|
| Mobile Phase:         | Mobile Phase A: 5 mM ammonium formate (NH <sub>4</sub><br>formate) buffer; pH 4.8 – 4.9<br>Mobile Phase B: Acetonitrile (ACN)<br>Solvent program: <table><tr><th></th><th>Time</th><th>Flow</th><th>%A</th><th>%B</th><th>%C</th><th>%D</th><th>Curve</th></tr><tr><td>1</td><td></td><td>0.60</td><td>100.00</td><td>0.0</td><td>0.0</td><td>0.0</td><td></td></tr><tr><td>2</td><td>2.00</td><td>0.60</td><td>100.00</td><td>0.0</td><td>0.0</td><td>0.0</td><td>6</td></tr><tr><td>3</td><td>8.00</td><td>0.60</td><td>70.00</td><td>30.0</td><td>0.0</td><td>0.0</td><td>6</td></tr><tr><td>4</td><td>10.00</td><td>0.60</td><td>70.00</td><td>30.0</td><td>0.0</td><td>0.0</td><td>6</td></tr><tr><td>5</td><td>10.01</td><td>0.60</td><td>100.00</td><td>0.0</td><td>0.0</td><td>0.0</td><td>6</td></tr></table> |      | Time   | Flow | %A  | %B  | %C    | %D | Curve | 1 |  | 0.60 | 100.00 | 0.0 | 0.0 | 0.0 |  | 2 | 2.00 | 0.60 | 100.00 | 0.0 | 0.0 | 0.0 | 6 | 3 | 8.00 | 0.60 | 70.00 | 30.0 | 0.0 | 0.0 | 6 | 4 | 10.00 | 0.60 | 70.00 | 30.0 | 0.0 | 0.0 | 6 | 5 | 10.01 | 0.60 | 100.00 | 0.0 | 0.0 | 0.0 | 6 |
|                       | Time                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Flow | %A     | %B   | %C  | %D  | Curve |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
| 1                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 0.60 | 100.00 | 0.0  | 0.0 | 0.0 |       |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
| 2                     | 2.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 0.60 | 100.00 | 0.0  | 0.0 | 0.0 | 6     |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
| 3                     | 8.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 0.60 | 70.00  | 30.0 | 0.0 | 0.0 | 6     |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
| 4                     | 10.00                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.60 | 70.00  | 30.0 | 0.0 | 0.0 | 6     |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
| 5                     | 10.01                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 0.60 | 100.00 | 0.0  | 0.0 | 0.0 | 6     |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
| Analysis Time:        | 10 min.; 6-minute injection delay time between injections                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |      |        |      |     |     |       |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
| Flow Rate:            | 0.6 mL/min. (max. pressure: ~2300 psi/~153 bar)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |      |        |      |     |     |       |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
| Oven Temp.:           | 40 °C                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |      |        |      |     |     |       |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
| Detection:            | Altus A-10 PDA;<br>Wavelengths used for calibration/quantitation:<br>214 nm: for B3, B3', B6, B7, B9, and B12<br>267 nm: for vitamins B1 and B2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |      |        |      |     |     |       |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
| Injection Volume:     | 3 μL                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |      |        |      |     |     |       |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |
| Sampling (Data) Rate: | 10 pts./sec                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |      |        |      |     |     |       |    |       |   |  |      |        |     |     |     |  |   |      |      |        |     |     |     |   |   |      |      |       |      |     |     |   |   |       |      |       |      |     |     |   |   |       |      |        |     |     |     |   |

# Oznaczanie witamin z grupy B

| Vitamins (nm)                                                    | Peak area [mAU] | % RSD |
|------------------------------------------------------------------|-----------------|-------|
| 200 $\mu$ L pure acetonitrile + solid phase extraction           |                 |       |
| C (230)                                                          | 150.92          | 2.6   |
| B <sub>1</sub> (270)                                             | 62.60           | 1.7   |
| B <sub>2</sub> (265)                                             | 366.16          | 10.3  |
| B <sub>5</sub> (266)                                             | 27.14           | 0.4   |
| B <sub>6</sub> (257)                                             | 110.28          | 2.4   |
| B <sub>9</sub> (280)                                             | 69.13           | 2.0   |
| B <sub>12</sub> (230)                                            | 179.13          | 5.2   |
| L/L extraction_aqueous phase                                     |                 |       |
| C (230)                                                          | 197.91          | 4.7   |
| B <sub>1</sub> (270)                                             | 64.10           | 2.9   |
| B <sub>2</sub> (265)                                             | 216.31          | 6.0   |
| B <sub>5</sub> (266)                                             | 14.83           | 0.2   |
| B <sub>6</sub> (257)                                             | 145.07          | 9.4   |
| B <sub>9</sub> (280)                                             | 112.93          | 0.9   |
| B <sub>12</sub> (230)                                            | 132.36          | 2.6   |
| 600 $\mu$ L ethanol : methanol (95 : 5) + solid-phase extraction |                 |       |
| C (230)                                                          | 169.14          | 2.8   |
| B <sub>1</sub> (270)                                             | 87.75           | 2.3   |
| B <sub>2</sub> (265)                                             | 302.29          | 1.9   |
| B <sub>5</sub> (266)                                             | 45.52           | 0.7   |
| B <sub>6</sub> (257)                                             | 60.97           | 1.0   |
| B <sub>9</sub> (280)                                             | 285.33          | 5.4   |
| B <sub>12</sub> (230)                                            | 111.74          | 1.0   |

| Vitamin                 | Limit of detection [ng $\mu$ L <sup>-1</sup> ] at given UV ( $\lambda$ , nm) | Regression line                            | Regression coefficient |
|-------------------------|------------------------------------------------------------------------------|--------------------------------------------|------------------------|
| C                       | 0.5 ng $\mu$ L <sup>-1</sup> (230 nm)                                        | $y = 0.657(\pm 0.003)x + 127.1(\pm 0.002)$ | $R^2 = 0.994$          |
| B <sub>1</sub>          | 1 ng $\mu$ L <sup>-1</sup> (270 nm)                                          | $y = 8.789(\pm 0.002)x + 16.31(\pm 0.004)$ | $R^2 = 0.996$          |
| B <sub>2</sub>          | 0.5 ng $\mu$ L <sup>-1</sup> (265 nm)                                        | $y = 301.7(\pm 0.005)x + 467.3(\pm 0.002)$ | $R^2 = 0.996$          |
| B <sub>5</sub>          | 1 ng $\mu$ L <sup>-1</sup> (266 nm)                                          | $y = 1.052(\pm 0.008)x + 66.43(\pm 0.003)$ | $R^2 = 0.979$          |
| B <sub>6</sub><br>(PLP) | 0.5 ng $\mu$ L <sup>-1</sup> (257 nm)                                        | $y = 7.451(\pm 0.003)x + 12.81(\pm 0.004)$ | $R^2 = 0.997$          |
| B <sub>9</sub>          | 1 ng $\mu$ L <sup>-1</sup> (280 nm)                                          | $y = 246.1(\pm 0.002)x + 54.31(\pm 0.005)$ | $R^2 = 0.999$          |
| B <sub>12</sub>         | 2 ng $\mu$ L <sup>-1</sup> (230 nm)                                          | $y = 132.9(\pm 0.007)x + 209.6(\pm 0.008)$ | $R^2 = 0.995$          |

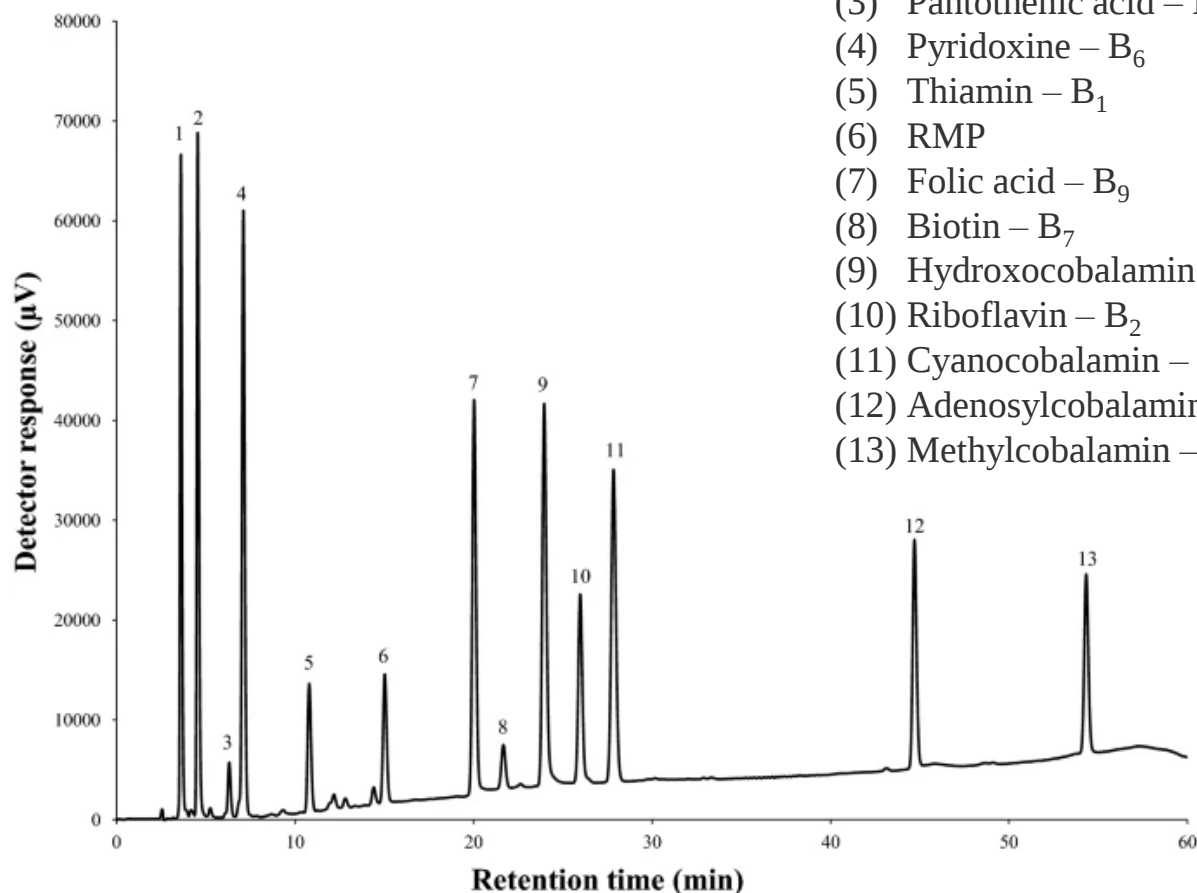
## HPLC-PDA



M. Grotzkyj Giorgi, K. Howland. C. Martin, A.B. Bonner, A Novel HPLC Method for the Concurrent Analysis and Quantitation of Seven Water-Soluble Vitamins in Biological Fluids (Plasma and Urine): A Validation Study and Application, *Scientific World Journal*, 2012; 2012: 359721.

# Oznaczanie witamin z grupy B

HPLC-UV

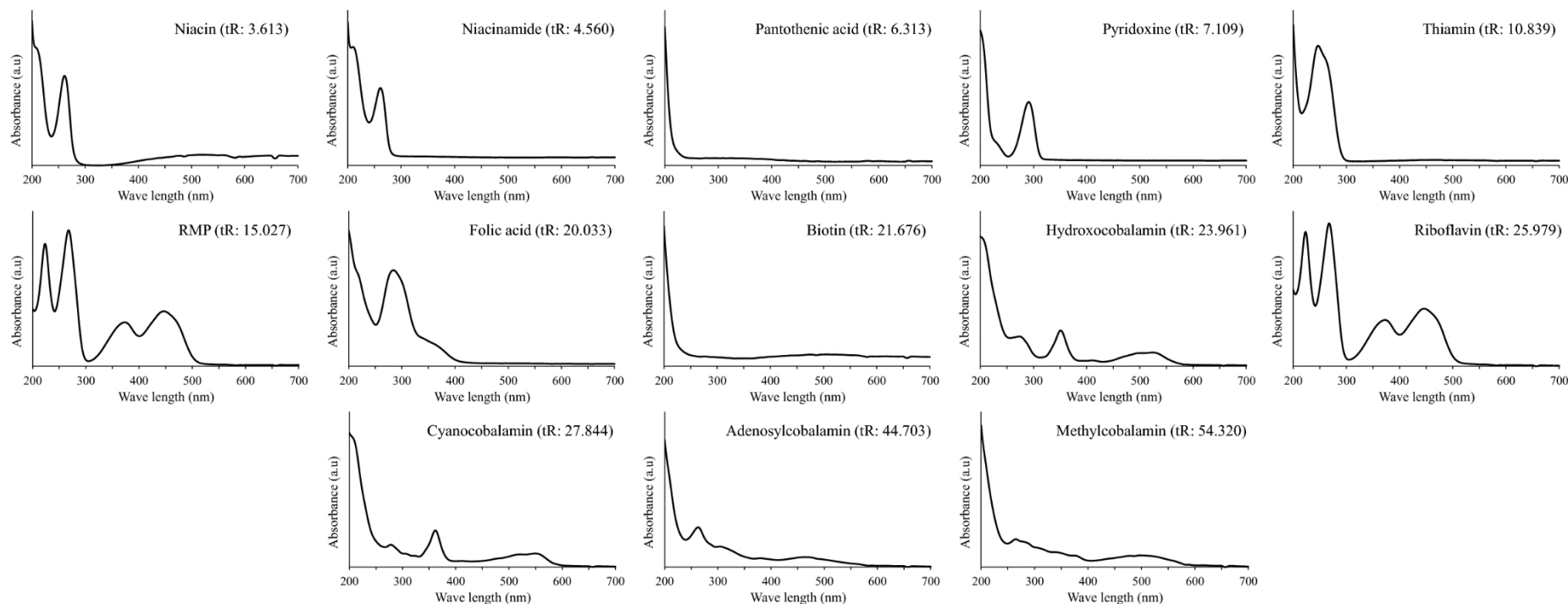


- (1) Niacin – B<sub>3</sub>
- (2) Niacinamide – B<sub>3</sub>
- (3) Pantothenic acid – B<sub>5</sub>
- (4) Pyridoxine – B<sub>6</sub>
- (5) Thiamin – B<sub>1</sub>
- (6) RMP
- (7) Folic acid – B<sub>9</sub>
- (8) Biotin – B<sub>7</sub>
- (9) Hydroxocobalamin – B<sub>12a</sub>
- (10) Riboflavin – B<sub>2</sub>
- (11) Cyanocobalamin – B<sub>12</sub>
- (12) Adenosylcobalamin – koenzym B<sub>12</sub>
- (13) Methylcobalamin – B<sub>12</sub>

K. Saski, H. Hatate, R. Tanaka, Determination of 13 Vitamin B and the Related Compounds Using HPLC with UV Detection and Application to Food Supplements, *Chromatographia*, volume 83, pages839–851(2020)

# Oznaczanie witamin z grupy B

## HPLC-UV



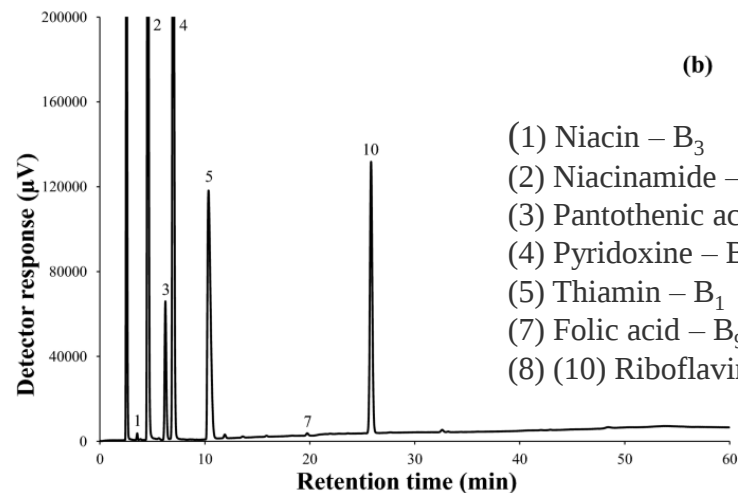
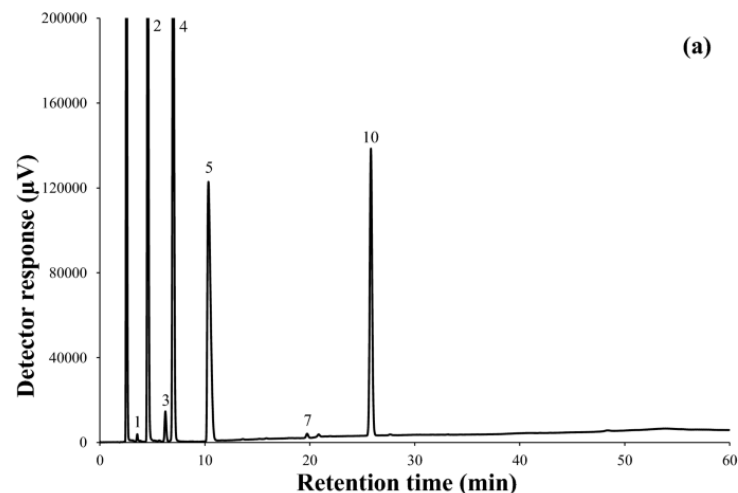
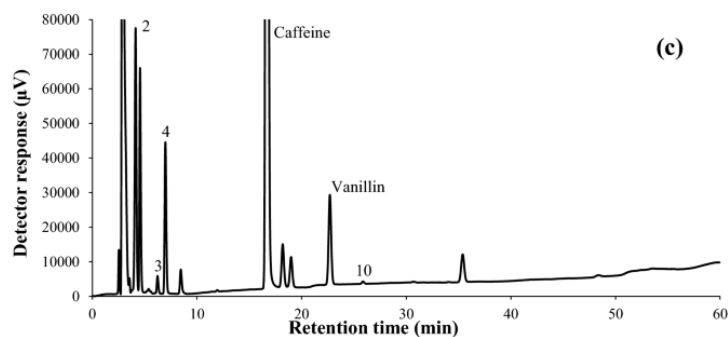
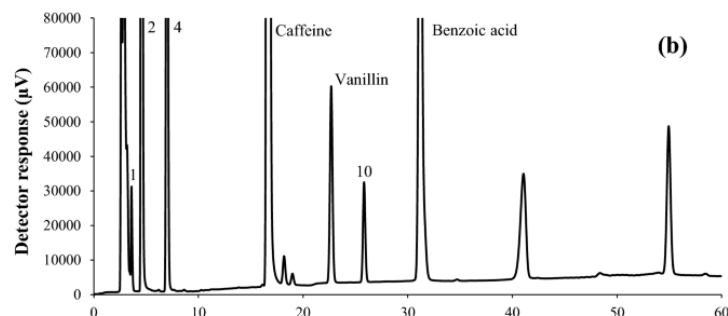
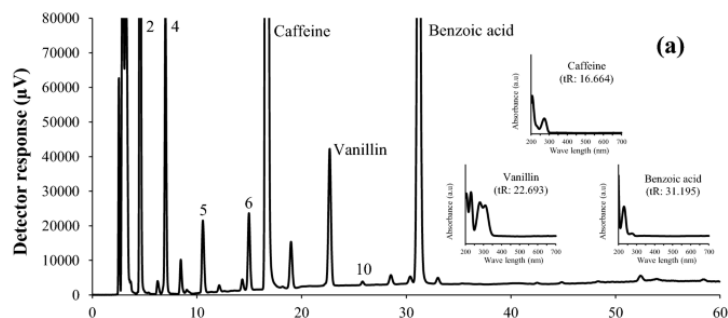
K. Saski, H. Hatate, R. Tanaka, Determination of 13 Vitamin B and the Related Compounds Using HPLC with UV Detection and Application to Food Supplements, *Chromatographia*, volume 83, pages839–851(2020)

# Oznaczanie witamin z grupy B

Napoje energetyczne

HPLC-UV

Multiwitaminy



- (1) Niacin – B<sub>3</sub>
- (2) Niacinamide – B<sub>3</sub>
- (3) Pantothenic acid – B<sub>5</sub>
- (4) Pyridoxine – B<sub>6</sub>
- (5) Thiamin – B<sub>1</sub>
- (7) Folic acid – B<sub>9</sub>
- (8) (10) Riboflavin – B<sub>2</sub>

K. Saski, H. Hatate, R. Tanaka, Determination of 13 Vitamin B and the Related Compounds Using HPLC with UV Detection and Application to Food Supplements, *Chromatographia*, volume 83, pages839–851(2020)

# Oznaczanie witamin z grupy B

## HPLC-UV

| Czas [min]        | A [%] | B [%] |
|-------------------|-------|-------|
| 0                 | 80    | 20    |
| 5                 | 80    | 20    |
| 50                | 20    | 80    |
| 80                | 20    | 80    |
| Przepływ [ml/min] | 1     |       |

**A** : 5 mmol L<sup>-1</sup> sodium hexanesulfonic acid, 20 mmol L<sup>-1</sup> phosphoric acid, 16 mmol L<sup>-1</sup> triethylamine; pH 3.0

**B** : mobile phase A: acetonitrile, 75:25 (v/v)

Kolumna: **CAPCELL PAK C18 SG120** (250 nm × 4.6 mm, 5-μm particle size)

| Sample              | Vitamin found Drinks: (μg mL <sup>-1</sup> ) Pills: (mg g <sup>-1</sup> ) |                  |               |               |                         |             |             |                        |
|---------------------|---------------------------------------------------------------------------|------------------|---------------|---------------|-------------------------|-------------|-------------|------------------------|
|                     | Niacin <sup>a</sup>                                                       | Pantothenic acid | Pyridoxine    | Thiamin       | Riboflavin <sup>b</sup> | Folic acid  | Biotin      | Cobalamin <sup>c</sup> |
| Energy drink 1      | 228.65 ± 0.69                                                             | ND               | 58.07 ± 0.58  | 61.51 ± 0.33  | 59.71 ± 0.18            | ND          | ND          | ND                     |
|                     | (200) <sup>d</sup>                                                        | (nd)             | (50)          | (50)          | (50)                    | (nd)        | (nd)        | (nd)                   |
| Energy drink 2      | 573.15 ± 0.94                                                             | ND               | 213.66 ± 0.36 | ND            | 46.26 ± 0.16            | ND          | ND          | ND                     |
|                     | (500)                                                                     | (nd)             | (200)         | (nd)          | (40)                    | (nd)        | (nd)        | (nd)                   |
| Energy drink 3      | 41.29 ± 0.70                                                              | 42.47 ± 0.71     | 29.56 ± 0.34  | ND            | 1.09 ± 0.01             | ND          | ND          | 0.03 ± 0.01            |
|                     | (30)                                                                      | (20)             | (20)          | (nd)          | (0.9)                   | (nd)        | (nd)        | (0.02)                 |
| Multivitamin pill 1 | 53.36 ± 0.53                                                              | 28.04 ± 0.37     | 57.98 ± 0.66  | 130.06 ± 1.52 | 55.00 ± 0.60            | 0.52 ± 0.02 | 0.09 ± 0.02 | 0.07 ± 0.03            |
|                     | (44)                                                                      | (22)             | (40)          | (100)         | (48)                    | (0.8)       | (0.18)      | (0.08)                 |
| Multivitamin pill 2 | 87.67 ± 1.18                                                              | 124.79 ± 1.71    | 76.74 ± 1.15  | 117.43 ± 1.78 | 50.58 ± 0.78            | 0.28 ± 0.01 | 0.08 ± 0.01 | 0.10 ± 0.01            |
|                     | (100)                                                                     | (100)            | (75)          | (100)         | (75)                    | (0.5)       | (0.125)     | (0.05)                 |

K. Saski, H. Hatate, R. Tanaka, Determination of 13 Vitamin B and the Related Compounds Using HPLC with UV Detection and Application to Food Supplements, *Chromatographia*, volume 83, pages839–851(2020)

# Oznaczanie witamin z grupy B

## Witamina B<sub>12</sub> – LC-MS/MS

| (a) LC condition   |                                      |            |               |
|--------------------|--------------------------------------|------------|---------------|
| Parameter          | Condition                            |            |               |
| Column             | UG120V C18 1.5×250 mm 5 µm, Shiseido |            |               |
| Detector           | MS/MS                                |            |               |
| Mobile phase       | A: 20 mM ammonium formate in water   | Time (min) | Solvent A (%) |
|                    | B: Acetonitrile                      |            | Solvent B (%) |
|                    | ※ Gradient mode                      | 0          | 95            |
|                    |                                      | 5          | 95            |
|                    |                                      | 10         | 80            |
|                    |                                      | 14         | 80            |
|                    |                                      | 15         | 20            |
|                    |                                      | 35         | 20            |
|                    |                                      | 36         | 95            |
|                    |                                      | 40         | 95            |
| Flow rate          | 0.2 mL/min                           |            |               |
| Column temperature | 35°C                                 |            |               |
| Run time           | 40 min                               |            |               |
| Injection volume   | 10 µL                                |            |               |

J-H. Lee, J-H Shin, J-M Park, H-J Kim, J-H Ahn, B-M Kwak, J-M Kim, Analytical Determination of Vitamin B12 Content in Infant and Toddler Milk Formulas by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS), *Korean J Food Sci Anim Resour.*, 2015; 35(6): 765–771

# Oznaczanie witamin z grupy B

## Witamina B<sub>12</sub> – LC-MS/MS

### (b) MS/MS condition

| Parameter          | Condition                          |
|--------------------|------------------------------------|
| Ion source         | ESI (Electro spray ionization)     |
| Polarity           | Positive                           |
| Nebulizer gas      | N <sub>2</sub>                     |
| Nebulizer pressure | 50 psi                             |
| Gas flow           | 10 L/min                           |
| Ion spray voltage  | 5000 V                             |
| Source temp.       | 350°C                              |
| Resolution         | Q1(unit) Q3(unit)                  |
| Scan mode          | MRM (Multiple reaction monitoring) |

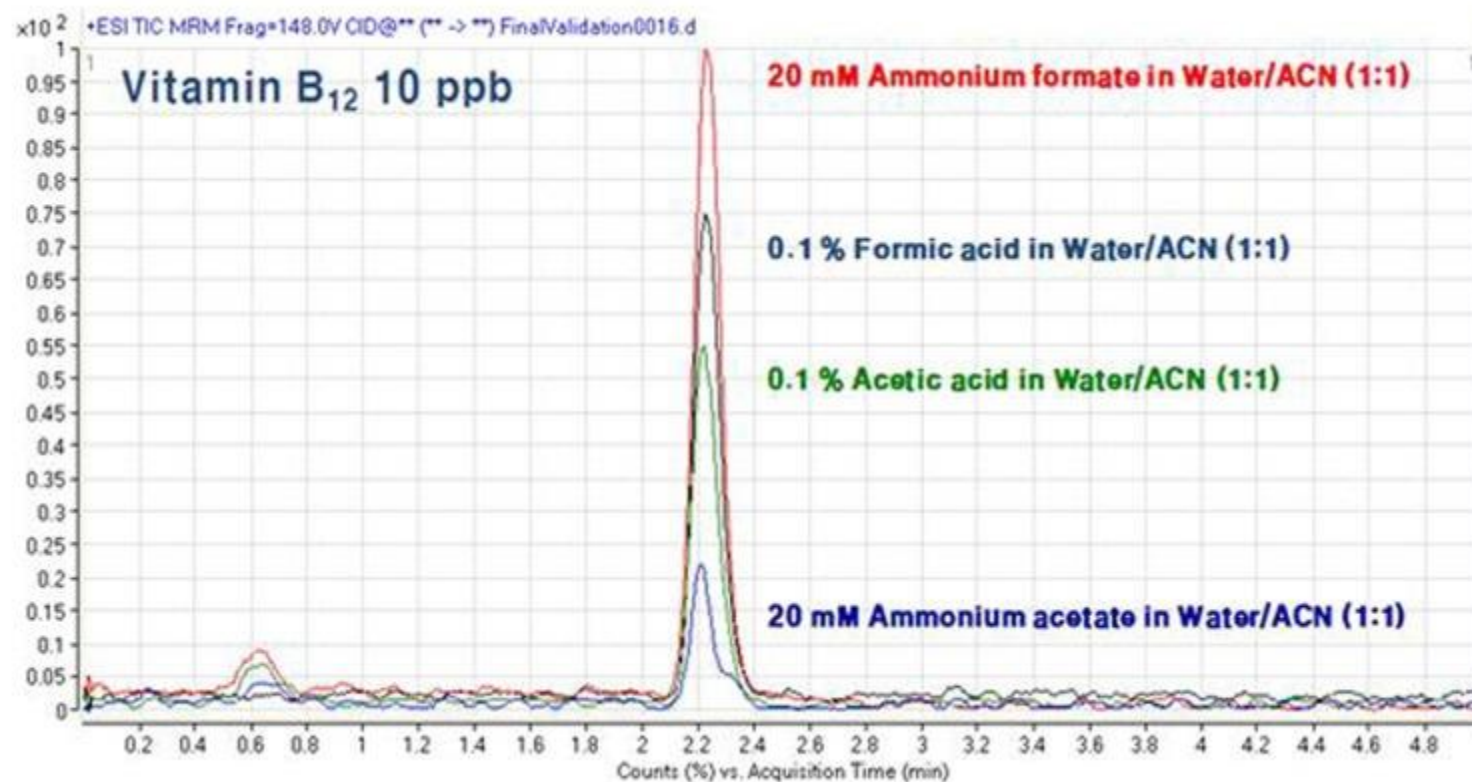
### MRM condition

| Retention time (min) | Compound       | Precursor ion (m/z) | Product ion (m/z) | Dwell (ms) | Fragmentor (V) | Collision energy (V) |
|----------------------|----------------|---------------------|-------------------|------------|----------------|----------------------|
| 13.34                | Cyanocobalamin | 678                 | 147 Quantitative  | 200        | 158            | 40                   |
|                      |                |                     | 359 Quantitative  | 200        | 158            | 24                   |

J-H. Lee, J-H Shin, J-M Park, H-J Kim, J-H Ahn, B-M Kwak, J-M Kim, Analytical Determination of Vitamin B12 Content in Infant and Toddler Milk Formulas by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS), *Korean J Food Sci Anim Resour.*, 2015; 35(6): 765–771

# Oznaczanie witamin z grupy B

## Witamina B<sub>12</sub> – LC-MS/MS



J-H. Lee, J-H Shin, J-M Park, H-J Kim, J-H Ahn, B-M Kwak, J-M Kim, Analytical Determination of Vitamin B12 Content in Infant and Toddler Milk Formulas by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS), *Korean J Food Sci Anim Resour.*, 2015; 35(6): 765–771



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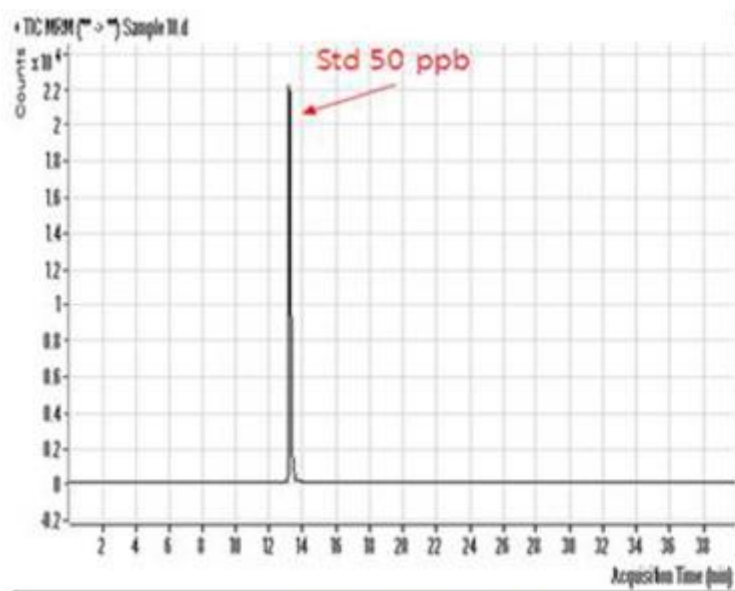
Instytut  
Matki i Dziecka

Unia Europejska  
Europejski Fundusz Społeczny

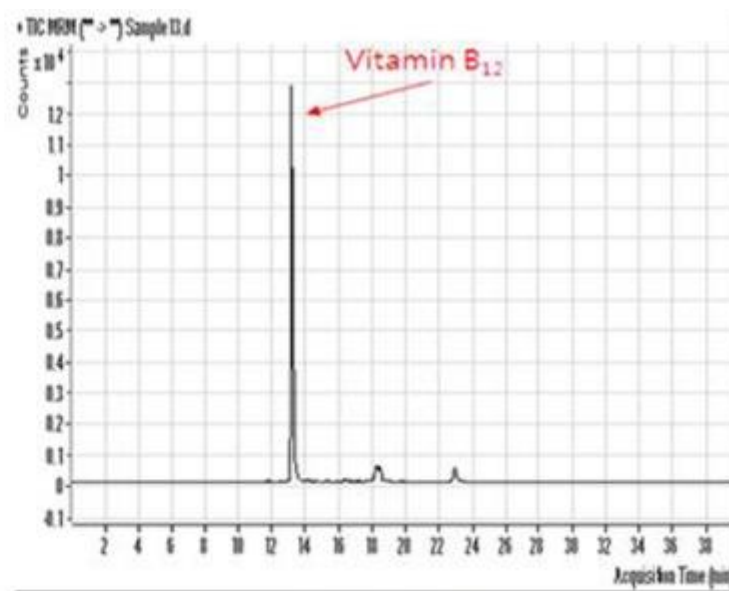


# Oznaczanie witamin z grupy B

## Witamina B<sub>12</sub> – LC-MS/MS



Vitamin B<sub>12</sub> Standard



SRM 1849a

Odzysk – około 40%

|                   |           |                   |                        |
|-------------------|-----------|-------------------|------------------------|
| LOD <sup>2)</sup> | 0.03 µg/L | r <sup>2</sup>    | 0.9986                 |
| LOQ <sup>3)</sup> | 0.10 µg/L | Linear Regression | y = 2737.71x - 5508.48 |
| MDL <sup>4)</sup> | 0.20 µg/L | Range             | 1–100 µg/L             |

J-H. Lee, J-H Shin, J-M Park, H-J Kim, J-H Ahn, B-M Kwak, J-M Kim, Analytical Determination of Vitamin B12 Content in Infant and Toddler Milk Formulas by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS), *Korean J Food Sci Anim Resour.*, 2015; 35(6): 765–771

# Oznaczanie witamin z grupy B

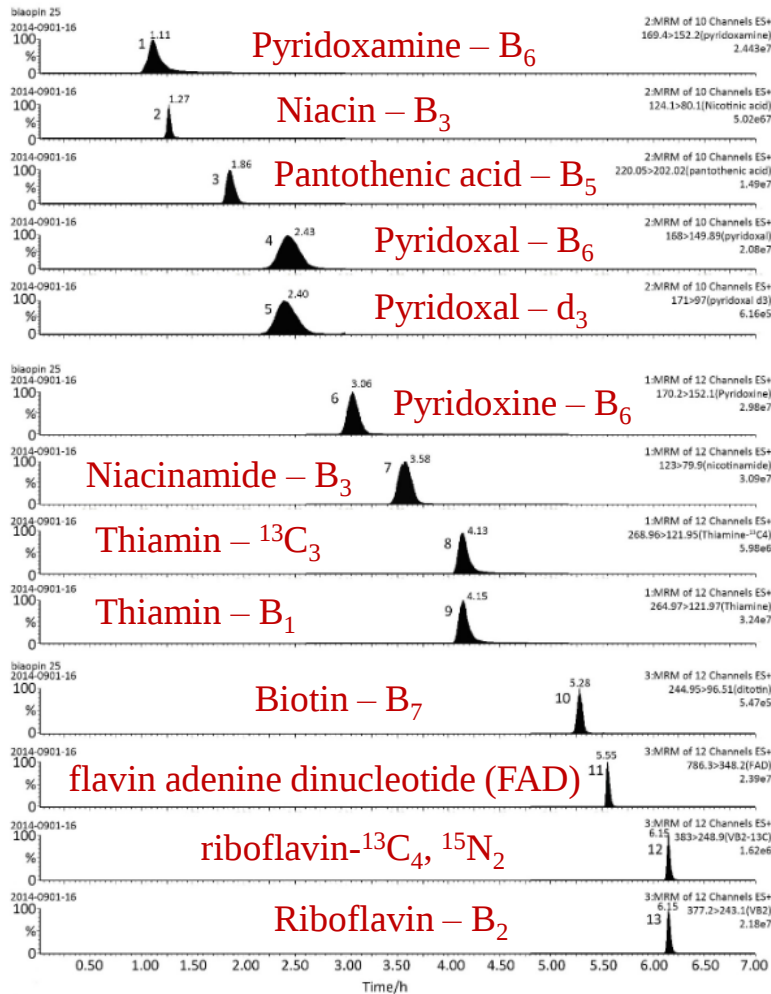
## Witaminy B – LC-MS/MS

| Analyte name                                                            | Collecting Function | Retention Time (min) | Parent Ion (m/z) | Daughter Ion (m/z) | Dwell Time (s) | Cone Voltage (V) | Collision Energy (eV) |
|-------------------------------------------------------------------------|---------------------|----------------------|------------------|--------------------|----------------|------------------|-----------------------|
| Pyridoxamine                                                            | 1                   | 1.11                 | 169.4            | 134.0              | 0.04           | 25.0             | 25.0                  |
|                                                                         |                     |                      |                  | 152.2 <sup>a</sup> | 0.04           | 25.0             | 18.0                  |
| Nicotinic acid                                                          | 1                   | 1.27                 | 123.4            | 77.0               | 0.06           | 35.0             | 20.0                  |
|                                                                         |                     |                      |                  | 79.7 <sup>a</sup>  | 0.06           | 35.0             | 20.0                  |
| Pantothenic acid                                                        | 1                   | 1.86                 | 220.1            | 89.5 <sup>a</sup>  | 0.06           | 22.0             | 13.0                  |
|                                                                         |                     |                      |                  | 202.0              | 0.06           | 22.0             | 8.0                   |
| Pyridoxal-d <sub>3</sub>                                                | 1                   | 2.40                 | 171.0            | 97.0               | 0.18           | 45.0             | 20.0                  |
|                                                                         |                     |                      |                  | 153.0 <sup>a</sup> | 0.18           | 45.0             | 12.0                  |
| Pyridoxal                                                               | 1                   | 2.43                 | 168.0            | 93.9               | 0.18           | 18.0             | 25.0                  |
|                                                                         |                     |                      |                  | 149.9 <sup>a</sup> | 0.18           | 18.0             | 12.0                  |
| Pyridoxine                                                              | 2                   | 3.06                 | 170.1            | 133.9              | 0.06           | 25.0             | 20.0                  |
|                                                                         |                     |                      |                  | 151.7 <sup>a</sup> | 0.06           | 25.0             | 15.0                  |
| Nicotinamide                                                            | 2                   | 3.58                 | 123.0            | 53.0               | 0.06           | 32.0             | 25.0                  |
|                                                                         |                     |                      |                  | 79.9 <sup>a</sup>  | 0.06           | 32.0             | 20.0                  |
| Thiamin                                                                 | 2                   | 4.15                 | 265.0            | 121.3 <sup>a</sup> | 0.06           | 20.0             | 15.0                  |
|                                                                         |                     |                      |                  | 143.4              | 0.06           | 20.0             | 15.0                  |
| Thiamin- <sup>13</sup> C <sub>3</sub>                                   | 2                   | 4.15                 | 268.9            | 121.6 <sup>a</sup> | 0.06           | 19.0             | 21.0                  |
|                                                                         |                     |                      |                  | 147.3              | 0.06           | 19.0             | 22.0                  |
| Biotin                                                                  | 3                   | 5.28                 | 244.9            | 96.5 <sup>a</sup>  | 0.02           | 30.0             | 35.0                  |
|                                                                         |                     |                      |                  | 226.6              | 0.02           | 30.0             | 25.0                  |
| flavin adenine dinucleotide (FAD)                                       | 3                   | 5.55                 | 786.3            | 348.2 <sup>a</sup> | 0.02           | 25.0             | 20.0                  |
|                                                                         |                     |                      |                  | 439.1              | 0.02           | 25.0             | 20.0                  |
| Riboflavin                                                              | 3                   | 6.15                 | 377.2            | 172.1              | 0.02           | 25.0             | 35.0                  |
|                                                                         |                     |                      |                  | 243.1 <sup>a</sup> | 0.02           | 25.0             | 25.0                  |
| Riboflavin- <sup>13</sup> C <sub>4</sub> , <sup>15</sup> N <sub>2</sub> | 3                   | 6.15                 | 383.0            | 174.6              | 0.02           | 40.0             | 48.0                  |
|                                                                         |                     |                      |                  | 248.9 <sup>a</sup> | 0.02           | 40.0             | 34.0                  |

R. X. Nan, Y. S. An, Y. Z. Yu, Y. Z. Guang, S. Bing, R. Y. Ping, Z. Jing, Application of UPLC-MS/MS Method for Analyzing B-vitamins in Human Milk, Biomed Environ Sci, 2015; 28(10): 738-750

# Oznaczanie witamin z grupy B

## Witaminy B – LC-MS/MS



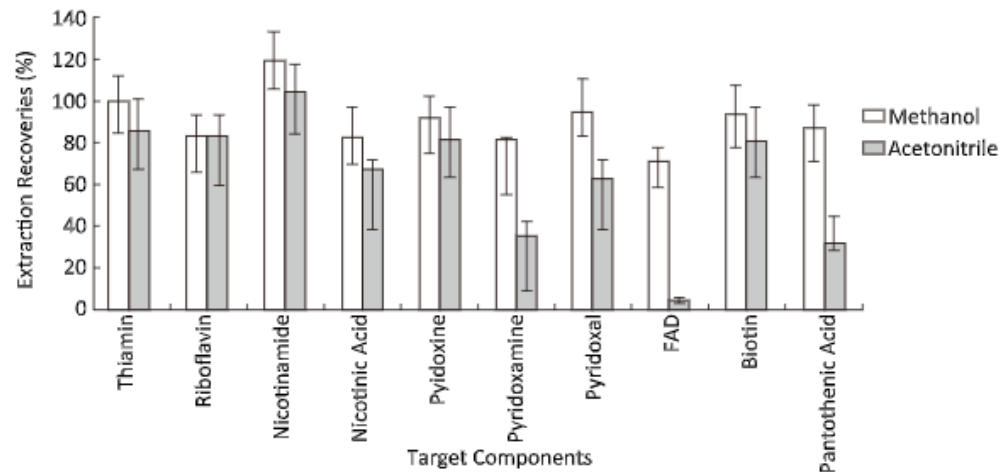
A : Acetonitryl

B : 2.5 mmol/L mrówczan amonu w wodzie

Kolumna: **Waters UPLC HSS T3** (2.1x100 mm, 1.8 μm)

Gradient: 0-2 min 1%A, 2-4 min 1-5%A, 4-5 min 5-20% A, 5-6 min 20-25%, 6-7 min 25-30%A, 7-7.1 min 30-95%A, 7.1-9 min 95%A, 9-9.1 min 95-1%A, 9.1-11 min 1%A,

Przepływ 0.35 mL/min



R. X. Nan, Y. S. An, Y. Z. Yu, Y. Z. Guang, S. Bing, R. Y. Ping, Z. Jing, Application of UPLC-MS/MS Method for Analyzing B-vitamins in Human Milk, Biomed Environ Sci, 2015; 28(10): 738-750

# Oznaczanie witamin z grupy B

## Witaminy B – LC-MS/MS

| Analyte          | Coefficient | LOD 1 (µg/L) | LOQ 1 (µg/L) | LOQ 2 (µg/L) | Reference Data (µg/L)      |
|------------------|-------------|--------------|--------------|--------------|----------------------------|
| Thiamin          | 0.997       | 0.05         | 0.25         | 0.01         | 2-221 <sup>[10]</sup>      |
| Riboflavin       | 0.996       | 0.20         | 1.00         | 0.03         | 0-845 <sup>[10]</sup>      |
| Nicotinamide     | 0.995       | 0.10         | 0.50         | 0.10         | 2-3179 <sup>[10]</sup>     |
| Nicotinic acid   | 0.990       | 0.50         | 2.00         | 0.20         |                            |
| Pyridoxine       | 0.999       | 0.10         | 0.50         | 0.06         |                            |
| Pyridoxamine     | 0.991       | 1.50         | 5.00         | 0.04         | 6-692 <sup>[10]</sup>      |
| Pyridoxal        | 0.999       | 0.20         | 0.70         | 0.03         |                            |
| FAD              | 0.993       | 0.20         | 1.00         | 0.05         | 29-818 <sup>[10]</sup>     |
| Biotin           | 0.994       | 1.00         | 3.00         | 0.40         | 2.8-5.9 <sup>[9]</sup>     |
| Pantothenic acid | 0.997       | 0.50         | 1.00         | 0.50         | 360-2900 <sup>[9,16]</sup> |

R. X. Nan, Y. S. An, Y. Z. Yu, Y. Z. Guang, S. Bing, R. Y. Ping, Z. Jing, Application of UPLC-MS/MS Method for Analyzing B-vitamins in Human Milk, Biomed Environ Sci, 2015; 28(10): 738-750

# Oznaczanie witamin z grupy B

## Witaminy B – LC-MS/MS

| Analyte          | Theoretical Concentrations of NIST (µg/L) | Determined Data of NIST (µg/L) | Accuracy of NIST (%) | Inter-day RSD (%) (n=36) | Mean of Pooled milk (µg/L) | Accuracy of Pooled Milk (%) | Inter-day RSD (%) (n=36) |
|------------------|-------------------------------------------|--------------------------------|----------------------|--------------------------|----------------------------|-----------------------------|--------------------------|
| Thiamin          | 125.7<br>(106.1-145.3)                    | 135.8±12.1                     | 108.0                | 8.9                      | 33.2                       | 84.9-118.9                  | 9.8                      |
| Riboflavin       | 203.7<br>(193.3-214.1)                    | 208.4±13.2                     | 102.3                | 6.4                      | 64.3                       | 85.2-114.2                  | 10.3                     |
| Nicotinamide     | 1090<br>(890-1290)                        | 1137.6±117.3                   | 104.4                | 10.3                     | 220.8                      | 85.7-113.2                  | 9.2                      |
| Pyridoxine       | 134.6<br>(116-153.2)                      | 139.3±9.1                      | 103.5                | 6.5                      | 3.3                        | 80.3-119.7                  | 12.2                     |
| Biotin           | 19.9<br>(17.3-22.5)                       | 19.6±1.3                       | 98.4                 | 6.6                      |                            |                             |                          |
| Pantothenic acid | 682<br>(644-720)                          | 700.9±58.4                     | 102.8                | 8.3                      | 910.7                      | 82.8-120.6                  | 14.5                     |
| Pyridoxal        |                                           |                                |                      |                          | 62.6                       | 81.0-111.5                  | 8.7                      |
| Nicotinic acid   |                                           |                                |                      |                          | 183.3                      | 85.4-115.9                  | 11.4                     |
| FAD              |                                           |                                |                      |                          | 205.1                      | 84.2-118.0                  | 9.9                      |

R. X. Nan, Y. S. An, Y. Z. Yu, Y. Z. Guang, S. Bing, R. Y. Ping, Z. Jing, Application of UPLC-MS/MS Method for Analyzing B-vitamins in Human Milk, Biomed Environ Sci, 2015; 28(10): 738-750

# Oznaczanie witamin z grupy B

## Witaminy B – LC-MS/MS

| Analyte          | Theoretical Concentrations of NIST (µg/L) | Determined Data of NIST (µg/L) | Accuracy of NIST (%) | Inter-day RSD (%) (n=36) | Mean of Pooled milk (µg/L) | Accuracy of Pooled Milk (%) | Inter-day RSD (%) (n=36) |
|------------------|-------------------------------------------|--------------------------------|----------------------|--------------------------|----------------------------|-----------------------------|--------------------------|
| Thiamin          | 125.7<br>(106.1-145.3)                    | 135.8±12.1                     | 108.0                | 8.9                      | 33.2                       | 84.9-118.9                  | 9.8                      |
| Riboflavin       | 203.7<br>(193.3-214.1)                    | 208.4±13.2                     | 102.3                | 6.4                      | 64.3                       | 85.2-114.2                  | 10.3                     |
| Nicotinamide     | 1090<br>(890-1290)                        | 1137.6±117.3                   | 104.4                | 10.3                     | 220.8                      | 85.7-113.2                  | 9.2                      |
| Pyridoxine       | 134.6<br>(116-153.2)                      | 139.3±9.1                      | 103.5                | 6.5                      | 3.3                        | 80.3-119.7                  | 12.2                     |
| Biotin           | 19.9<br>(17.3-22.5)                       | 19.6±1.3                       | 98.4                 | 6.6                      |                            |                             |                          |
| Pantothenic acid | 682<br>(644-720)                          | 700.9±58.4                     | 102.8                | 8.3                      | 910.7                      | 82.8-120.6                  | 14.5                     |
| Pyridoxal        |                                           |                                |                      |                          | 62.6                       | 81.0-111.5                  | 8.7                      |
| Nicotinic acid   |                                           |                                |                      |                          | 183.3                      | 85.4-115.9                  | 11.4                     |
| FAD              |                                           |                                |                      |                          | 205.1                      | 84.2-118.0                  | 9.9                      |

R. X. Nan, Y. S. An, Y. Z. Yu, Y. Z. Guang, S. Bing, R. Y. Ping, Z. Jing, Application of UPLC-MS/MS Method for Analyzing B-vitamins in Human Milk, Biomed Environ Sci, 2015; 28(10): 738-750

# Oznaczanie witamin z grupy B

## Witaminy B – LC-MS/MS

|                  |                                                                       |            |                    |       |      |       |
|------------------|-----------------------------------------------------------------------|------------|--------------------|-------|------|-------|
| Column           | PerkinElmer Altus UPLC® BEH C18, 1.7 µm, 2.1 x 50-mm (Part# N2972000) |            |                    |       |      |       |
| Mobile Phase     | Solvent A: 5 mM ammonium formate, pH to 4.9 with formic acid          |            |                    |       |      |       |
|                  | Solvent B: Acetonitrile (ACN)                                         |            |                    |       |      |       |
|                  |                                                                       | Time (min) | Flow rate (mL/min) | %A    | %B   | Curve |
|                  | 1                                                                     | Initial    | 0.5                | 100.0 | 0.0  |       |
|                  | 2                                                                     | 1.60       | 0.5                | 100.0 | 0.0  | 6     |
|                  | 3                                                                     | 4.50       | 0.5                | 70.0  | 30.0 | 6     |
|                  | 4                                                                     | 5.00       | 0.5                | 50.0  | 50.0 | 6     |
|                  | 5                                                                     | 6.00       | 0.5                | 50.0  | 50.0 | 6     |
| 6                | 6.05                                                                  | 0.5        | 100.0              | 0.0   | 6    |       |
| Analysis Time    | 6 min; re-equilibration time: 4 min                                   |            |                    |       |      |       |
| Pressure         | 6900 psi/460 bar (maximum)                                            |            |                    |       |      |       |
| Oven Temp.       | 40 °C                                                                 |            |                    |       |      |       |
| Injection Volume | 3 µL                                                                  |            |                    |       |      |       |

W. M. Reuter, S. Reddy, A. Dalmia, Analysis of Water-Soluble Vitamins in Infant Formula by UHPLC-MS/MS, PerkinElmer, Inc. Shelton, CT

|                       |                |           |         |                 |        |
|-----------------------|----------------|-----------|---------|-----------------|--------|
| Ionization Mode:      | ESI - positive |           |         |                 |        |
| Drying Gas (Nitrogen) | 75             | HSID Temp | 320 °C; | Electrospray V1 | 5000 V |

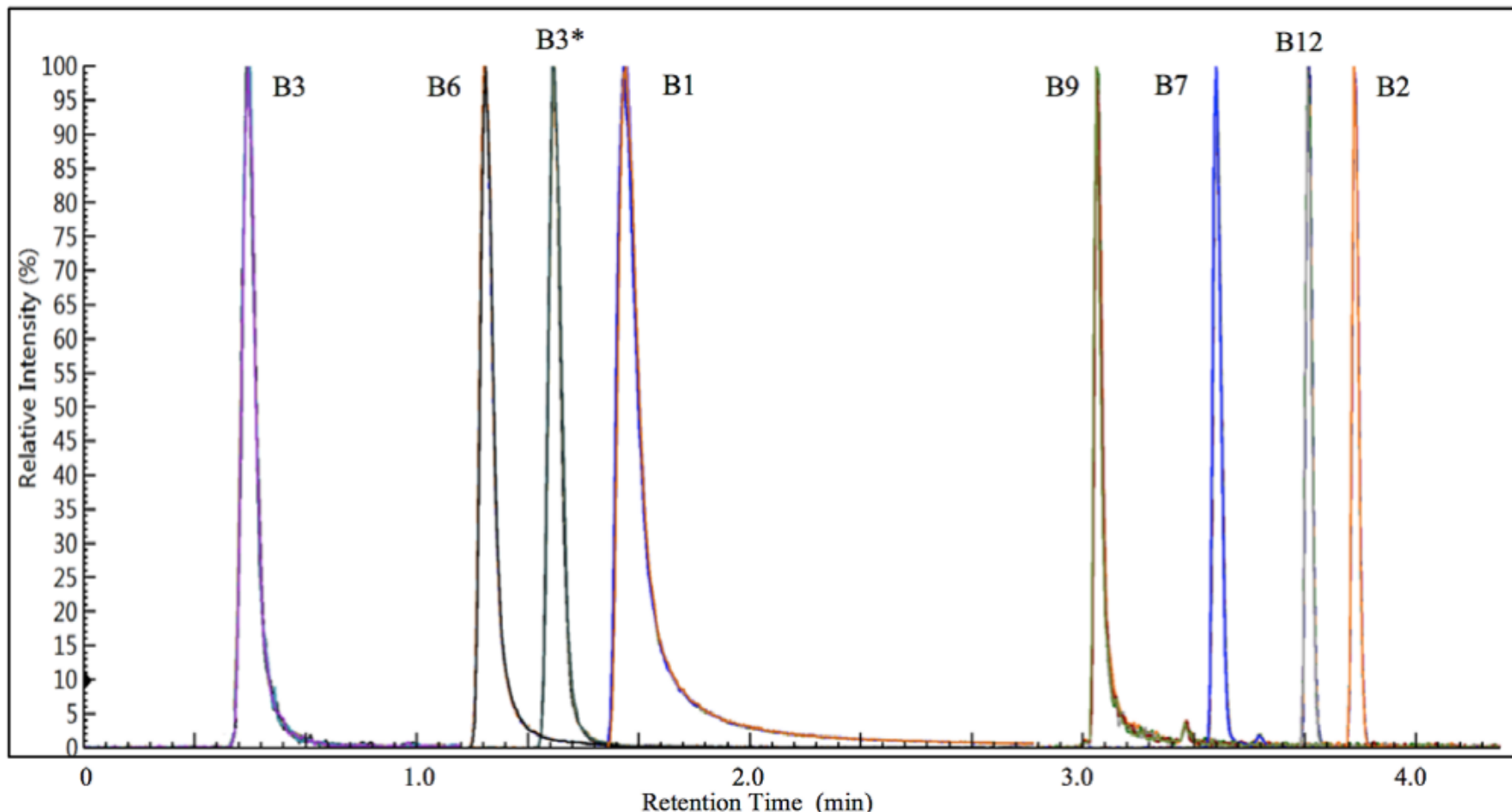
| Exper. Group 1 (0.20 – 0.85 min) | MRM Transitions (amu) |               |    |       |                   |
|----------------------------------|-----------------------|---------------|----|-------|-------------------|
|                                  | Quantifier Ion        | Qualifier Ion | EV | CE(V) | Dwell Time (msec) |
| B3 (niacin)                      | 124.3/80.2            | 124.3/53.2    | 55 | -30   | 100               |

| Exper. Group 2 (0.7 – 2.5 min)                                                                          | MRM Transitions (amu) |               |    |       |                   |
|---------------------------------------------------------------------------------------------------------|-----------------------|---------------|----|-------|-------------------|
|                                                                                                         | Quantifier Ion        | Qualifier Ion | EV | CE(V) | Dwell Time (msec) |
| B1 (thiamine)                                                                                           | 265.0/121.7           | 265.0/144.0   | 20 | -25   | 50                |
| B1' (thiamine; <sup>13</sup> C <sub>4</sub> H <sub>8</sub> N <sub>4</sub> O <sub>5</sub> <sup>+</sup> ) | 269.0/122.0           |               | 20 | -25   | 50                |
| B3* (niacinamide)                                                                                       | 123.3/80.2            | 123.3/53.2    | 25 | -30   | 50                |
| B6 (pyridoxine)                                                                                         | 170.3/133.7           | 170.3/105.8   | 22 | -28   | 50                |

| Exper. Group 3 (3.0 – 4.4 min)                                                                                                             | MRM Transitions (amu) |               |    |       |                   |
|--------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|---------------|----|-------|-------------------|
|                                                                                                                                            | Quantifier Ion        | Qualifier Ion | EV | CE(V) | Dwell Time (msec) |
| B7 (biotin)                                                                                                                                | 245.0/96.4            | 245.0/104.5   | 24 | -45   | 35                |
| B7' (biotin; C <sub>10</sub> D <sub>2</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub> S)                                               | 247.0/98.7            |               | 24 | -45   | 35                |
| B9 (folic acid)                                                                                                                            | 442.1/295.2           | 442.1/176.0   | 21 | -22   | 35                |
| B12 (cyanocobalamin):                                                                                                                      | 678.9/147.2           | 678.9/399.2   | 23 | -45   | 35                |
| B2 (riboflavin)                                                                                                                            | 377.0/172.3           | 377.0/198.2   | 35 | -48   | 35                |
| B2' (riboflavin; <sup>13</sup> C <sub>4</sub> C <sub>13</sub> H <sub>20</sub> <sup>15</sup> N <sub>2</sub> N <sub>2</sub> O <sub>6</sub> ) | 383.0/175.4           |               | 35 | -48   | 35                |

# Oznaczanie witamin z grupy B

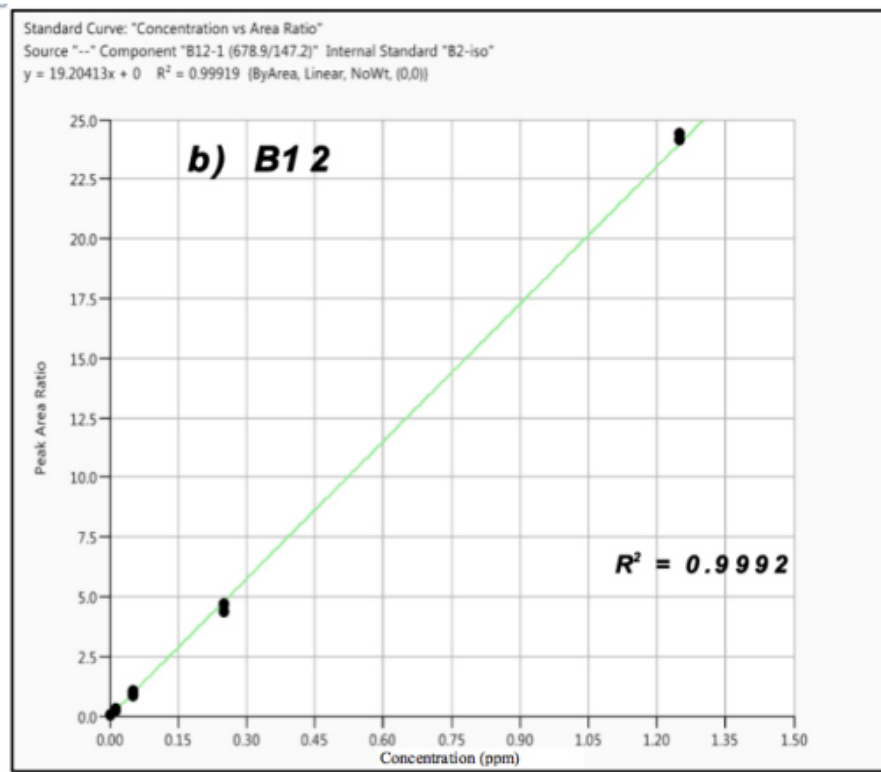
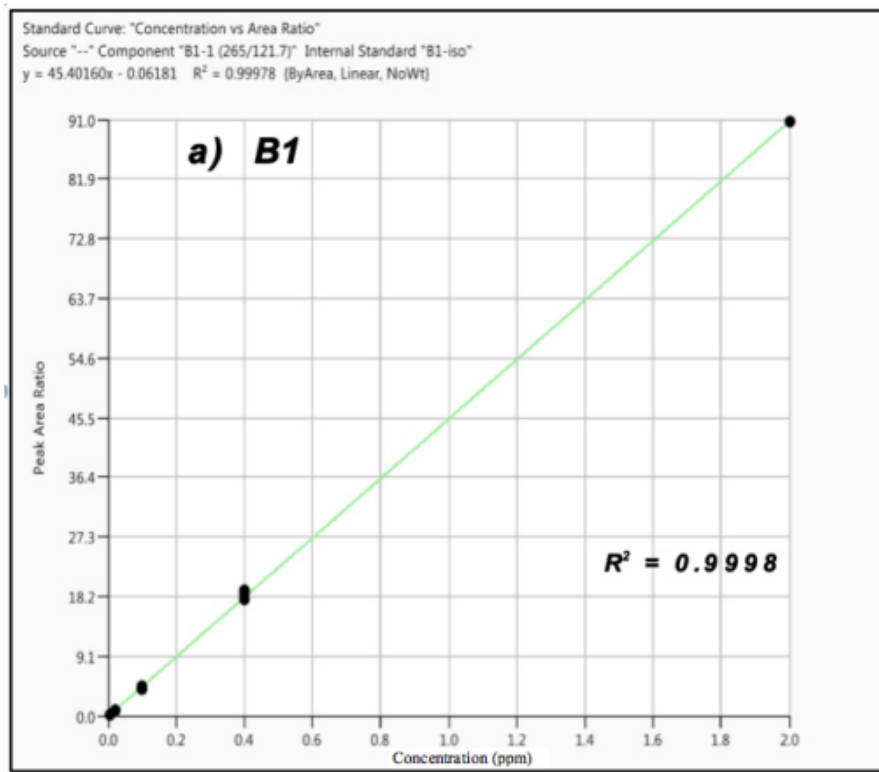
## Witaminy B – LC-MS/MS



W. M. Reuter, S. Reddy, A. Dalmia, Analysis of Water-Soluble Vitamins in Infant Formula by UHPLC-MS/MS, PerkinElmer, Inc. Shelton, CT

# Oznaczanie witamin z grupy B

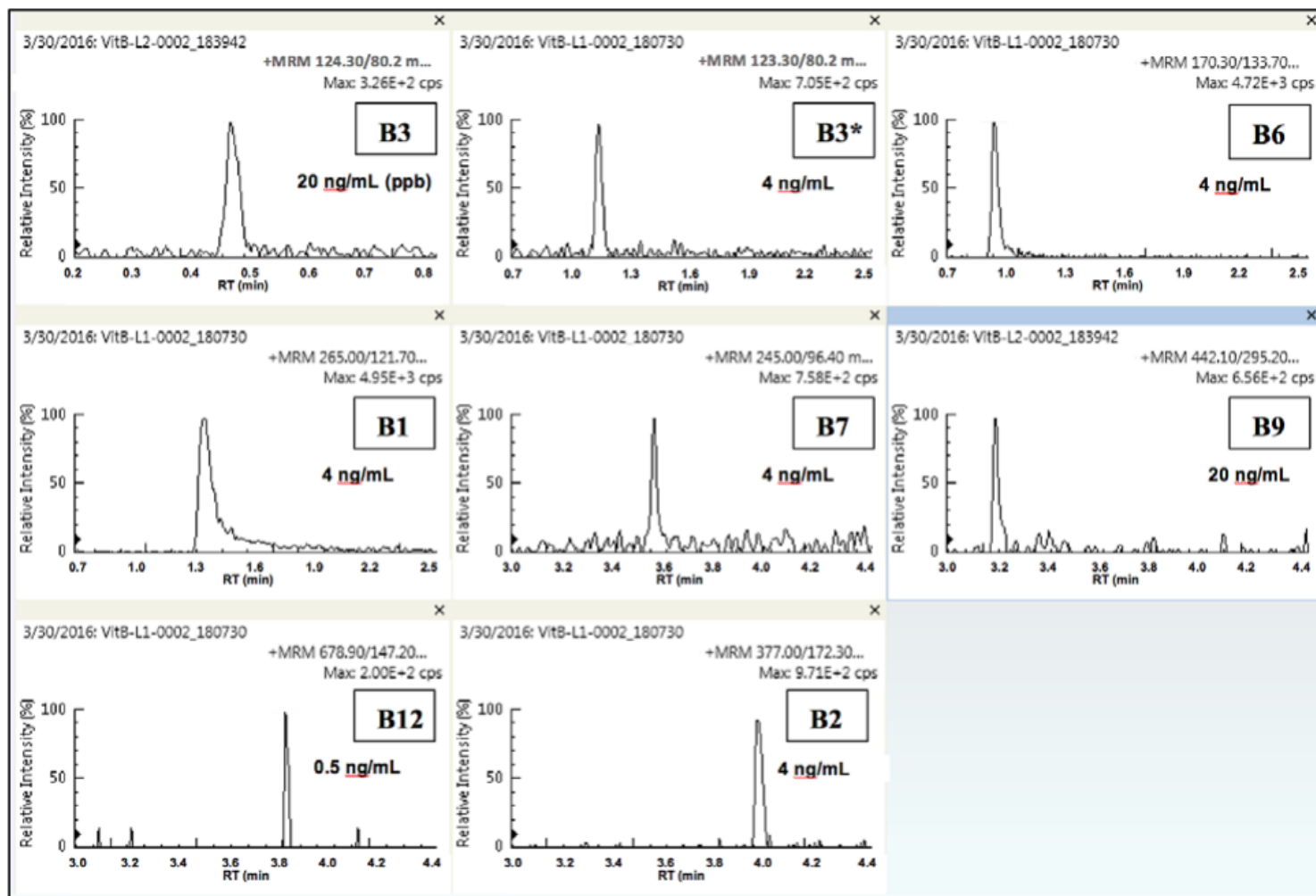
## Witaminy B – LC-MS/MS



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# Oznaczanie witamin z grupy B

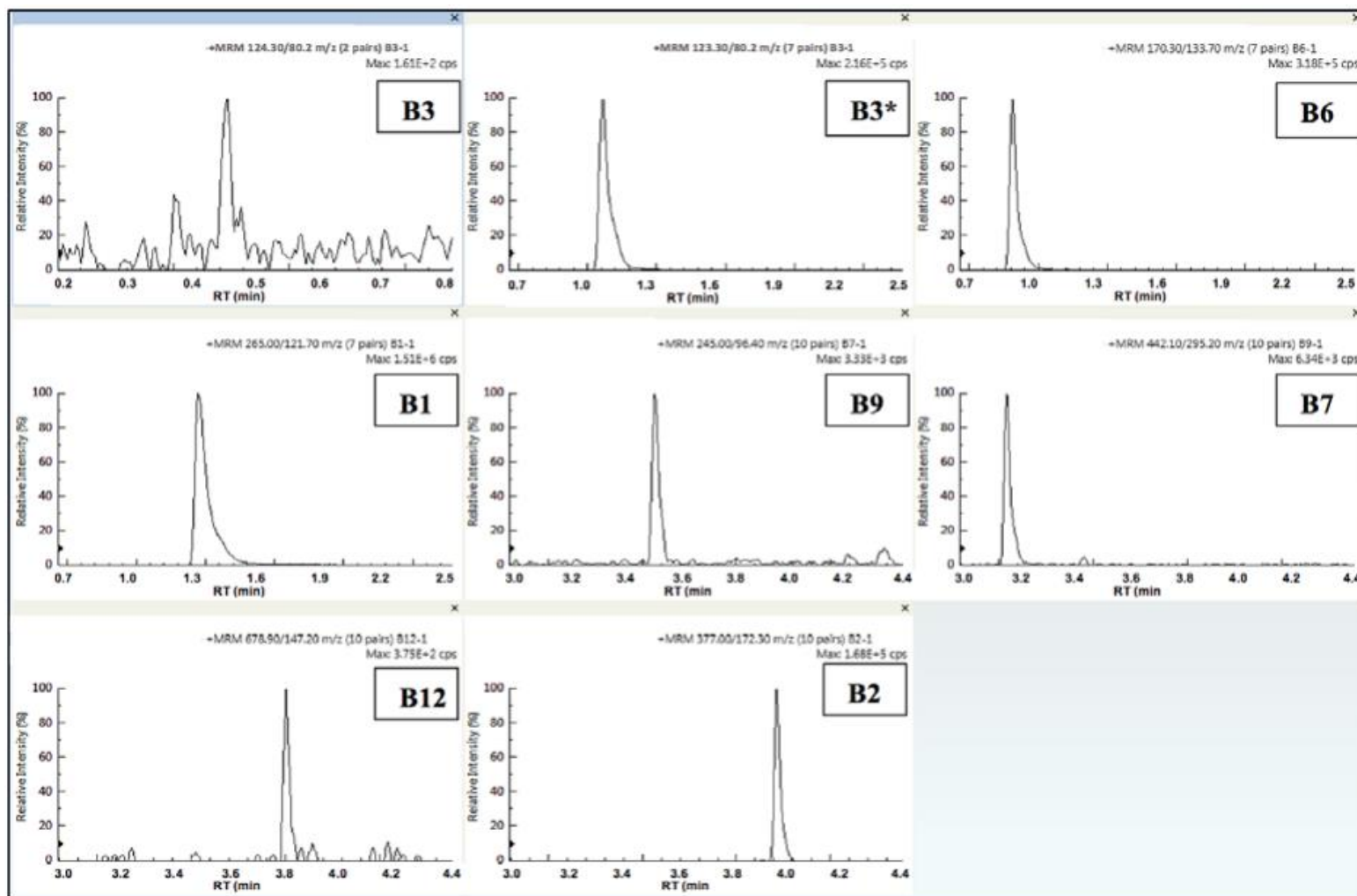
## Witaminy B – LC-MS/MS



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# Oznaczanie witamin z grupy B

## Witaminy B – LC-MS/MS

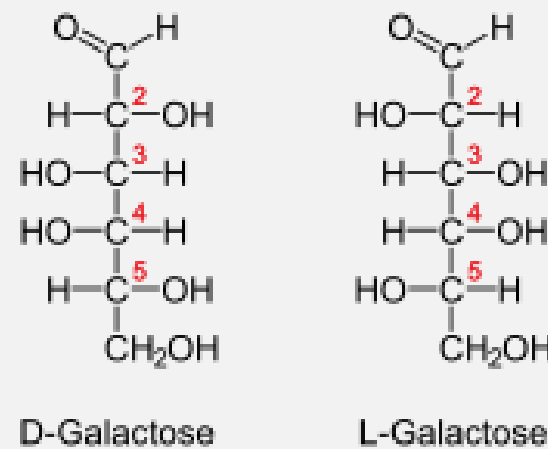
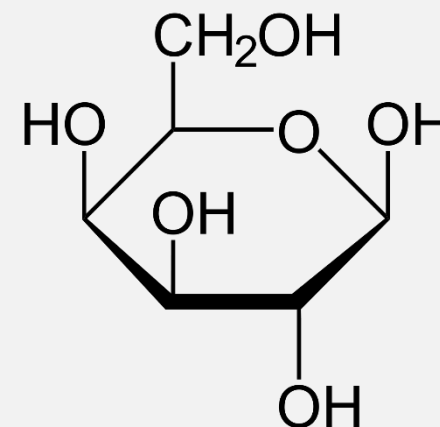


W. M. Reuter, S. Reddy, A. Dalmia, Analysis of Water-Soluble Vitamins in Infant Formula by UHPLC-MS/MS, PerkinElmer, Inc. Shelton, CT

# Opracowanie nowej metody

## Oznaczanie Galaktozy-1-fosforanu

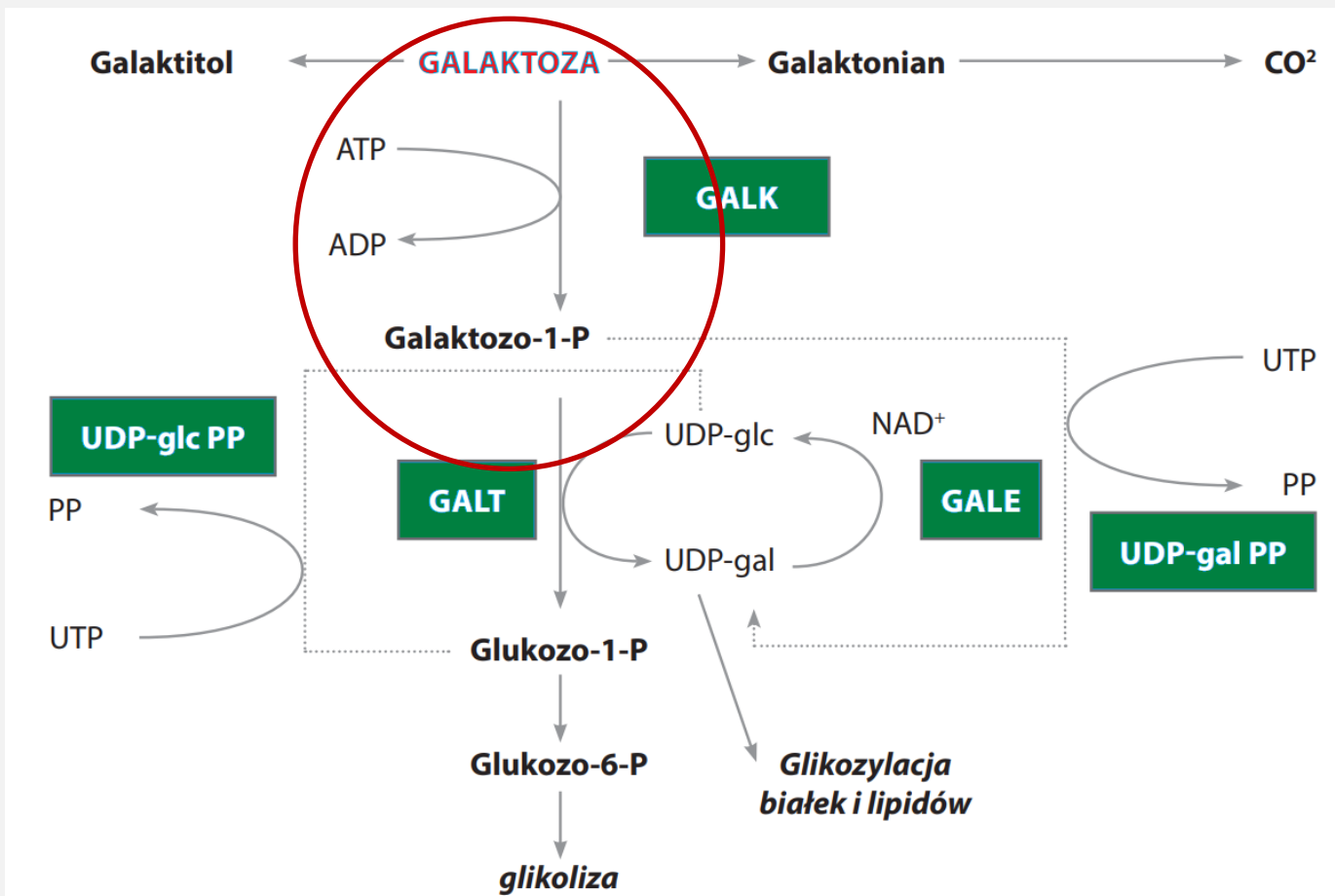
- ✓ **Galaktozemia** to wrodzona choroba metaboliczna zaliczana do zaburzeń procesu przemian węglowodanów, która wynika z braku zdolności organizmu do metabolizmu **galaktozy**.
- ✓ **Kumulacja toksycznych metabolitów** gromadzonych powyżej bloku enzymatycznego oraz powstających na drodze alternatywnych przemiany galaktozy może prowadzić do uszkodzenia wątroby, nerek, soczewki oka i układu nerwowego.
- ✓ Dziedziczenie: autosomalne recesywne.
- ✓ Główne źródło: laktoza, kazeina



# Opracowanie nowej metody

## Określenie problemu analitycznego

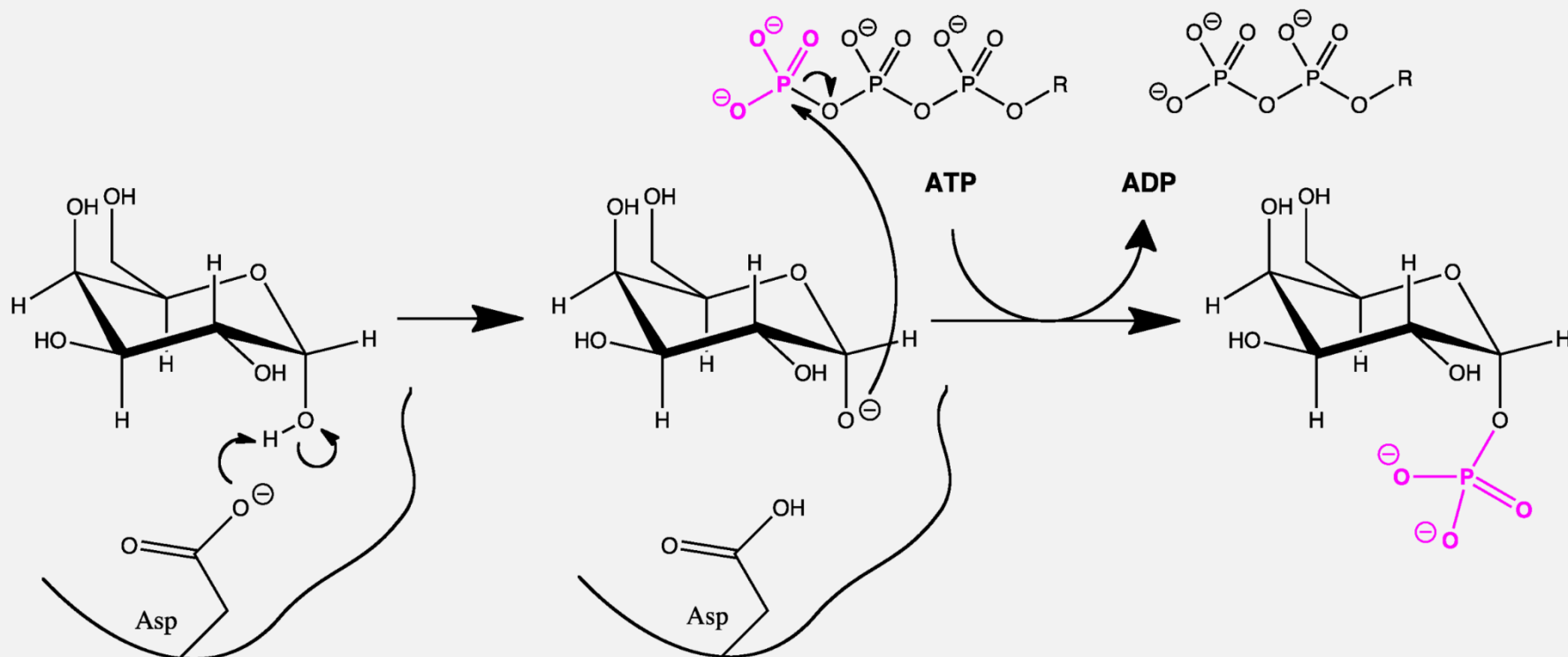
## Szlak metaboliczny Leloir



# Opracowanie nowej metody

## Określenie problemu analitycznego

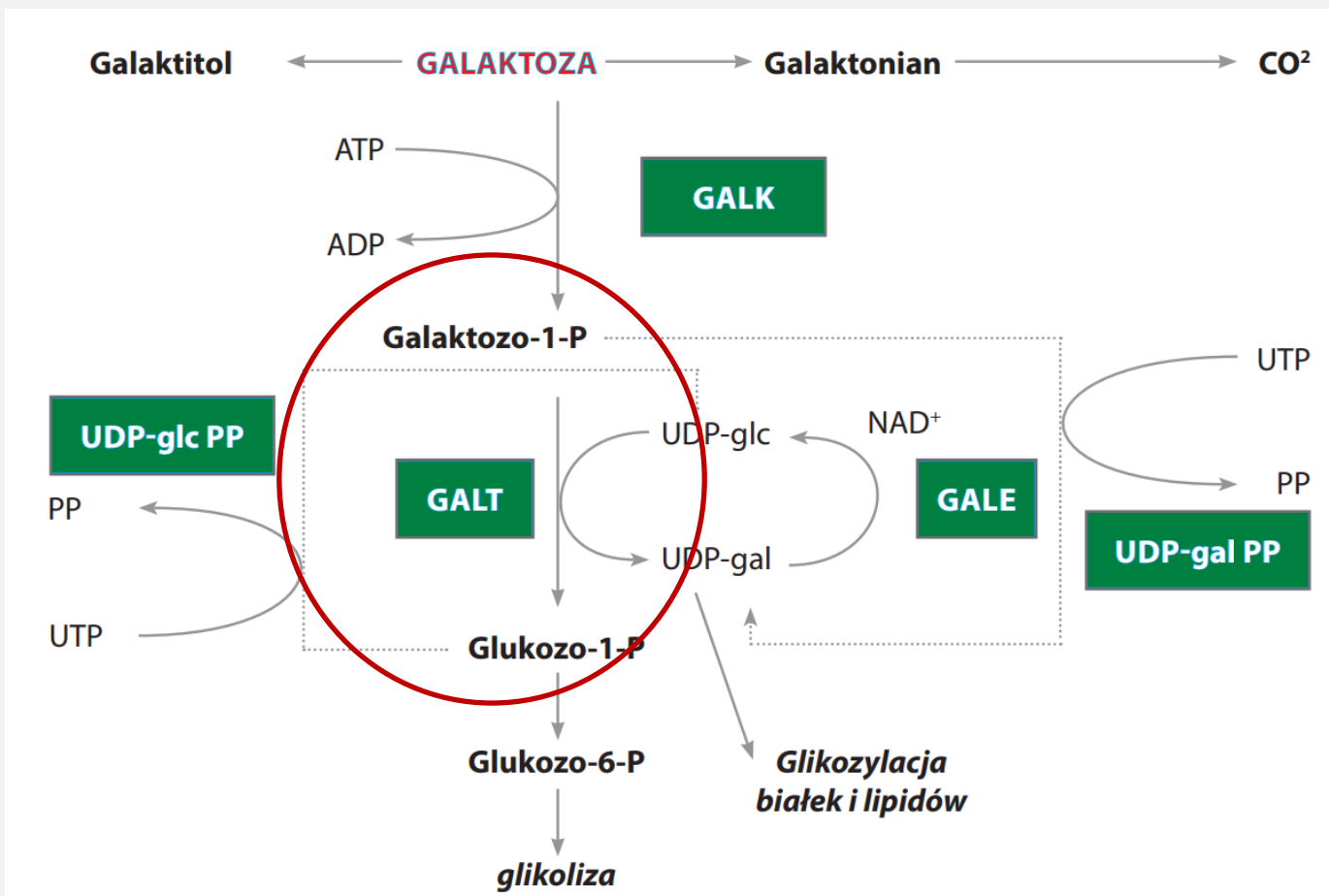
**Galaktokinaza (GALK)** – to enzym fosfotransferaz, który katalizuje fosforylację galaktozy do galaktozy-1-fosforanu przy wykorzystaniu jednej cząsteczki ATP



# Opracowanie nowej metody

## Określenie problemu analitycznego

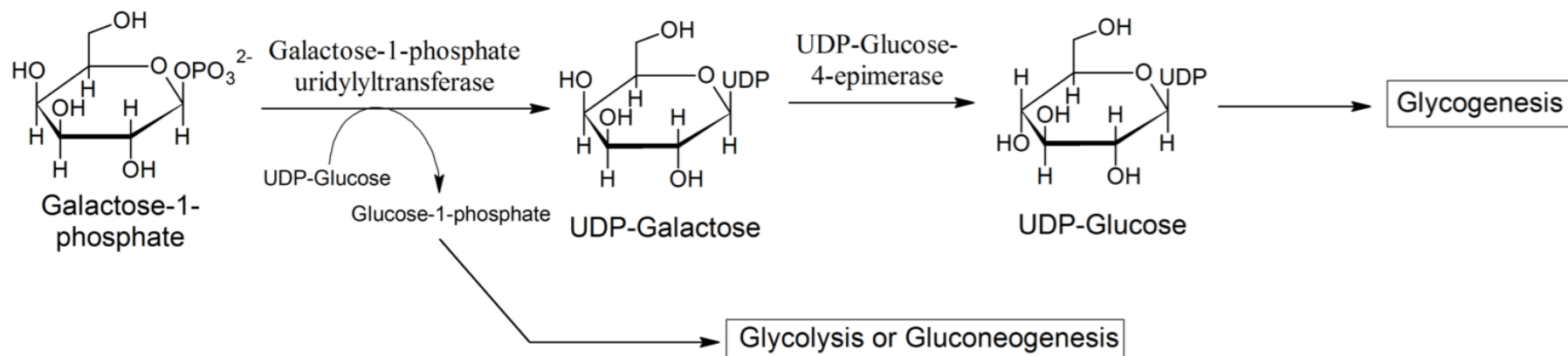
## Szlak metaboliczny Leloir



# Opracowanie nowej metody

## Określenie problemu analitycznego

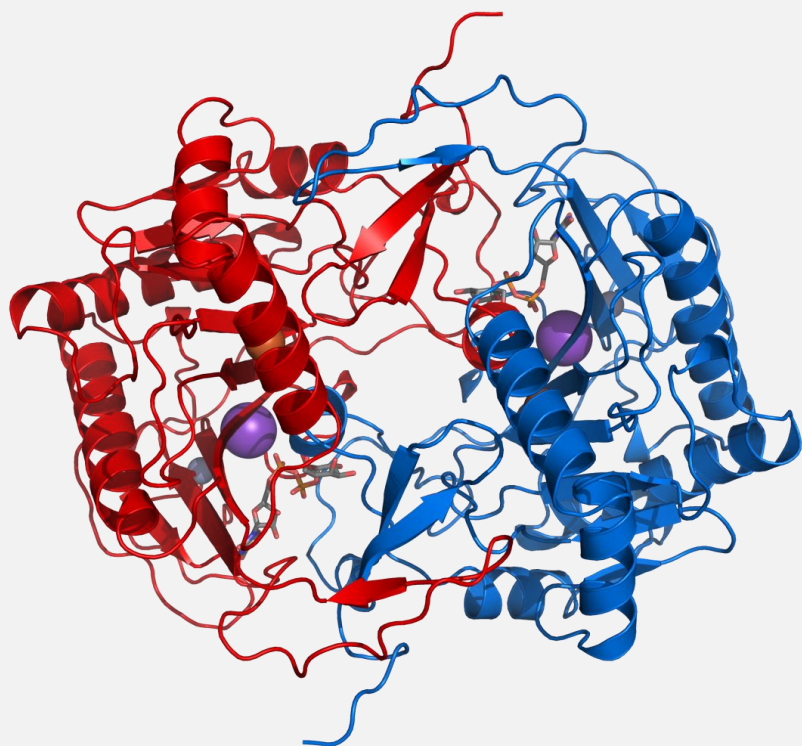
Urydylotransferaza galaktozo-1-fosforanowa (GALT) – to enzym, który katalizuje przemianę galaktozy-1-fosforanu do urydynodifosforan galaktozy.



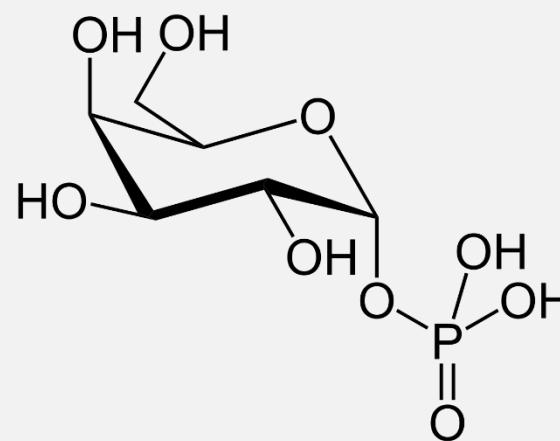
**Klasyczna galaktozemia jest spowodowana mutacją genu GALT, który koduje enzym urydylotransferazę galaktozo-1-fosforanową.**

# Opracowanie nowej metody

## Określenie problemu analitycznego



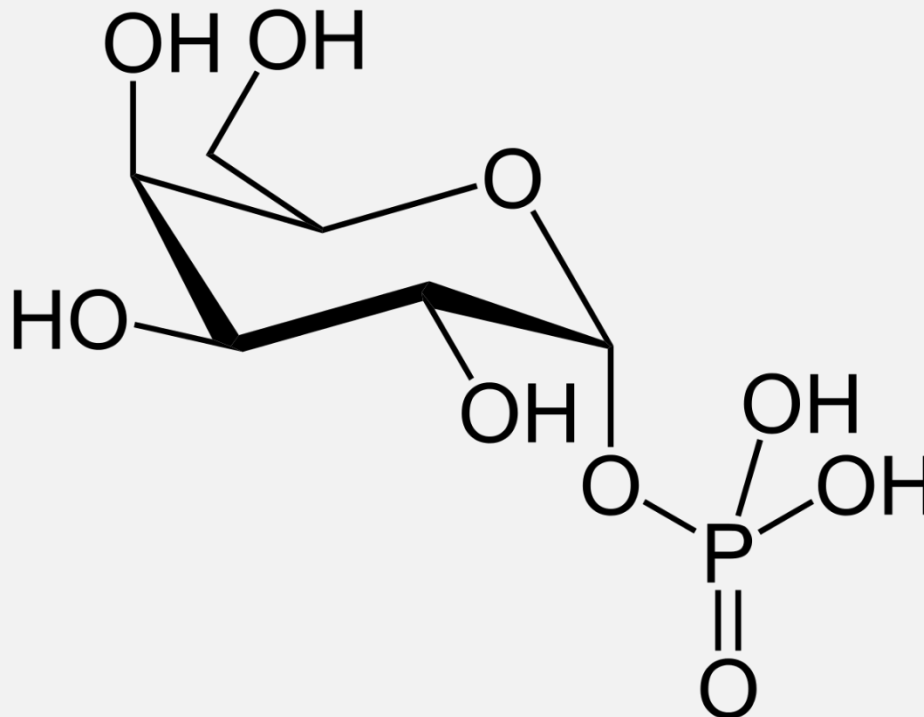
GALT



Produkty szlaku metabolicznego

# Opracowanie nowej metody

## Określenie problemu analitycznego



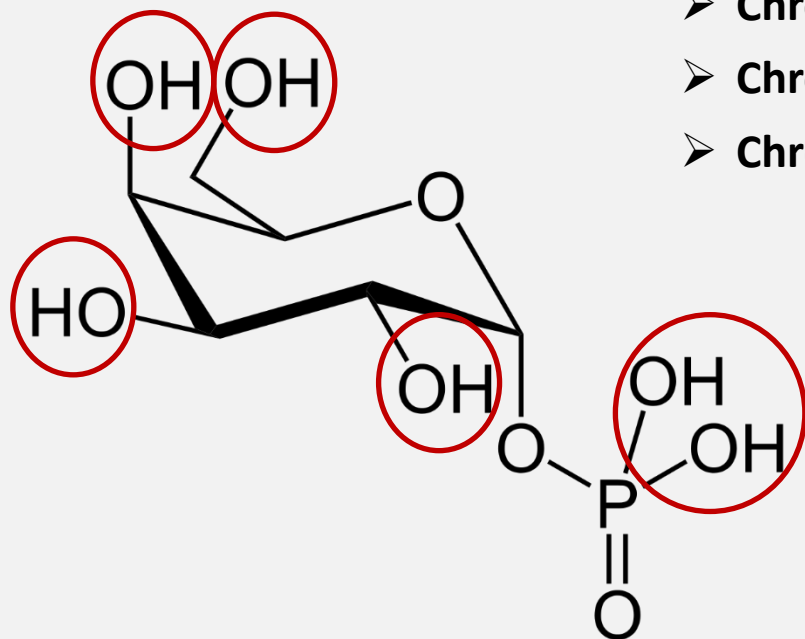
**Możliwość oznaczania galaktozy-1-fosforanu na  
poziomie stężenia poniżej 10  $\mu\text{moli/L}$**

# Opracowanie nowej metody

## Możliwości analityczne laboratorium

Dostępna aparatura analityczna:

- Chromatograf gazowy z detektorem mas
- Chromatograf gazowy z detektorem FID
- Chromatograf ciekowy z detektorem DAD
- Chromatograf ciekowy z detektorem fluorescencyjny
- Chromatograf ciekowy z detektorem mas



Derywatyzacja ?

Tak / Nie

# Opracowanie nowej metody

## Rodzaj badanego materiały



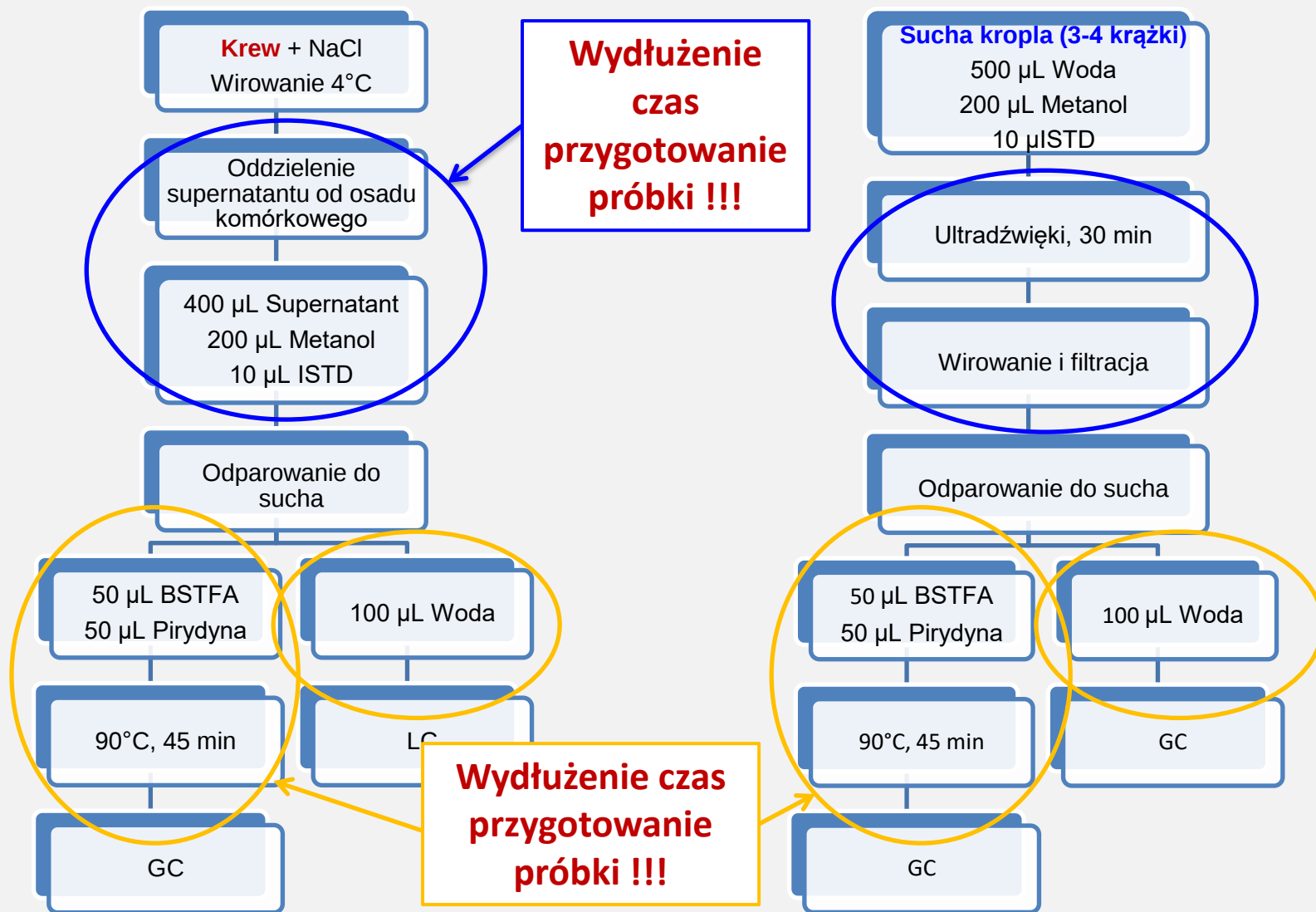
**IIIIIIII**  
Tu nakleić kod paskowy  
Nie dotykać powierzchni krążków!  
Nie używać bibuły uszkodzonych!

**Dane matki**  
Pesel: \_\_\_\_\_  
Nazwisko: \_\_\_\_\_  
Imię: \_\_\_\_\_

**Dane dziecka**  
Płeć: \_\_\_\_\_  
Data urodz.: \_\_\_\_\_ Godz.: \_\_\_\_\_  
Data pobr.: \_\_\_\_\_ Godz.: \_\_\_\_\_  
Ciężar: \_\_\_\_\_ Hbd: \_\_\_\_\_  
Apgar: \_\_\_\_\_ Transfuzja? data: \_\_\_\_\_  
Antybiotyki? \_\_\_\_\_  
Kamienie: Piers ☐ Butelka ☐ Porzeczka ☐  
Adres do kontaktu  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
Telefon: \_\_\_\_\_  
Pobrał: \_\_\_\_\_

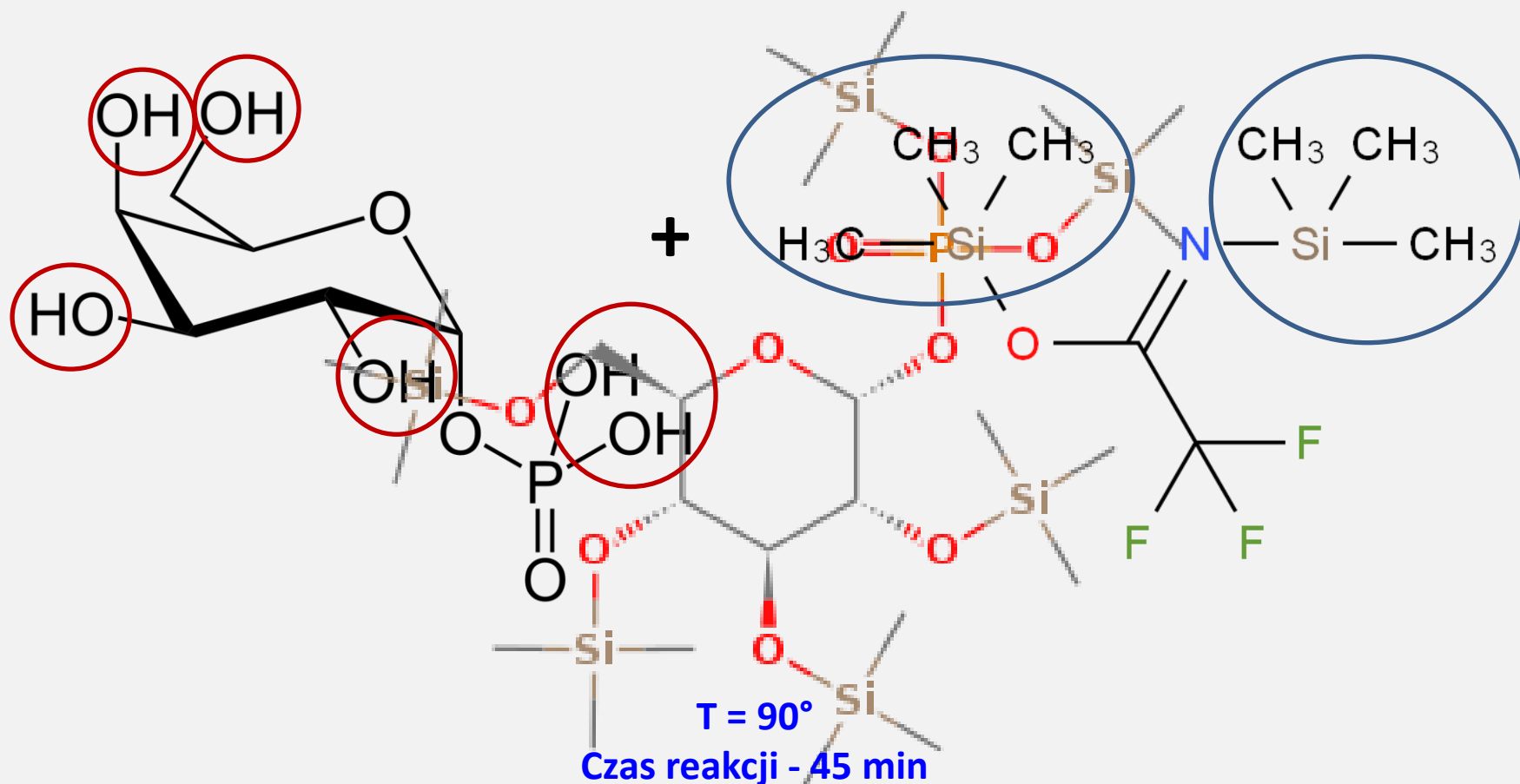
    
 2019-09-30

# Opracowanie nowej metody



# Opracowanie nowej metody

## Reakcja derywatywacji dla GC



# Opracowanie nowej metody

## Parametry metody

Method type: Acquisition - Timed Use timed acquisition methods to acquire timed SRM or SIM data.

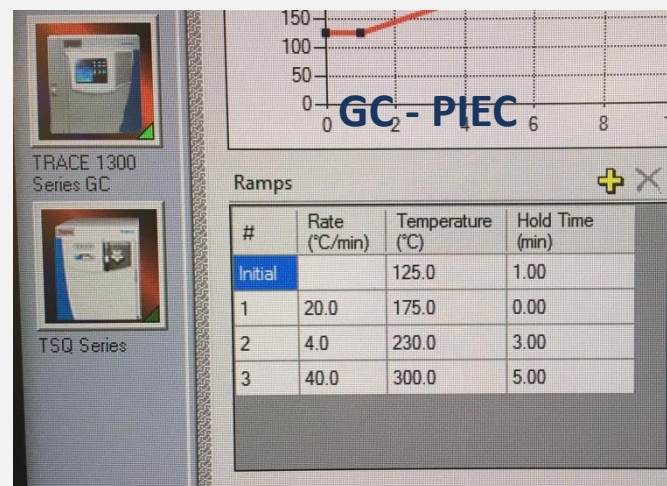
MS transfer line temp.: 300 °C Ionization mode: EI  
 Ion source temp.: 250 °C CI gas type: Methane  
 Acquisition threshold: 100 CI gas flow: 1.00 mL/min

**MS**

Scans

Scan type: SRM Link to external file

| Name              | RT    | Ion Polarity | Window (min) | Pre-width (min) | Post-width (min) | Mass  | Product Mass | Collision Energy |
|-------------------|-------|--------------|--------------|-----------------|------------------|-------|--------------|------------------|
| Galactose_13C...  | 9.60  | Positive     | 0.40         | 0.5             | 0.5              | 147.1 | 73           | 15               |
| Galactose_13C...  | 9.60  | Positive     | 0.40         | 0.5             | 0.5              | 205.1 | 73.1         | 15               |
| Galactose_13C...  | 9.60  | Positive     | 0.40         | 0.5             | 0.5              | 218.2 | 73.1         | 15               |
| Galactose_TMS...  | 9.61  | Positive     | 0.50         | 0.5             | 0.5              | 147.1 | 73.1         | 15               |
| Galactose_TMS...  | 9.61  | Positive     | 0.50         | 0.5             | 0.5              | 204.1 | 73           | 25               |
| Galactose_TMS...  | 9.61  | Positive     | 0.50         | 0.5             | 0.5              | 217.1 | 73           | 15               |
| Galactose_Phos... | 17.13 | Positive     | 0.50         | 0.5             | 0.5              | 147.1 | 73.1         | 15               |
| Galactose_Phos... | 17.13 | Positive     | 0.50         | 0.5             | 0.5              | 205.1 | 73.1         | 15               |
| Galactose_Phos... | 17.13 | Positive     | 0.50         | 0.5             | 0.5              | 299.1 | 73.1         | 20               |
| Galactose_Phos... | 17.14 | Positive     | 0.50         | 0.5             | 0.5              | 147.1 | 73.1         | 15               |
| Galactose_Phos... | 17.14 | Positive     | 0.50         | 0.5             | 0.5              | 204.1 | 73.1         | 15               |
| Galactose_Phos... | 17.14 | Positive     | 0.50         | 0.5             | 0.5              | 217.1 | 73           | 15               |



Autosampler Method

**AUTOSAMPLER**

Sampling

Sample volume (uL): 1.00  
 Plunger strokes: 8  
 Viscous sample: No  
 Sampling depth in vial: Bottom

Injection

Pre-injection  
 Solvent: A  
 Cycles: 3

Sample  
 Rinses: 2

Injection  
 Injection depth: Standard  
 Pre-inj dwell time (sec): 0.00  
 Post-inj dwell time (sec): 0.50

Post-injection  
 Solvent: B  
 Cycles: 3

Status: The autosampler is in stand-by.

Oven S/SL (front) Run Table

S/SL mode: GC - DOZOWNIK

Inlet  
 Temperature: 280 °C  
 Split flow: 200.0 mL/min  
 Split ratio: 200.0  
 Splitless time: 2.00 min

Surge  
 Surge pressure: 10.00 kPa  
 Surge duration: 0.00 min

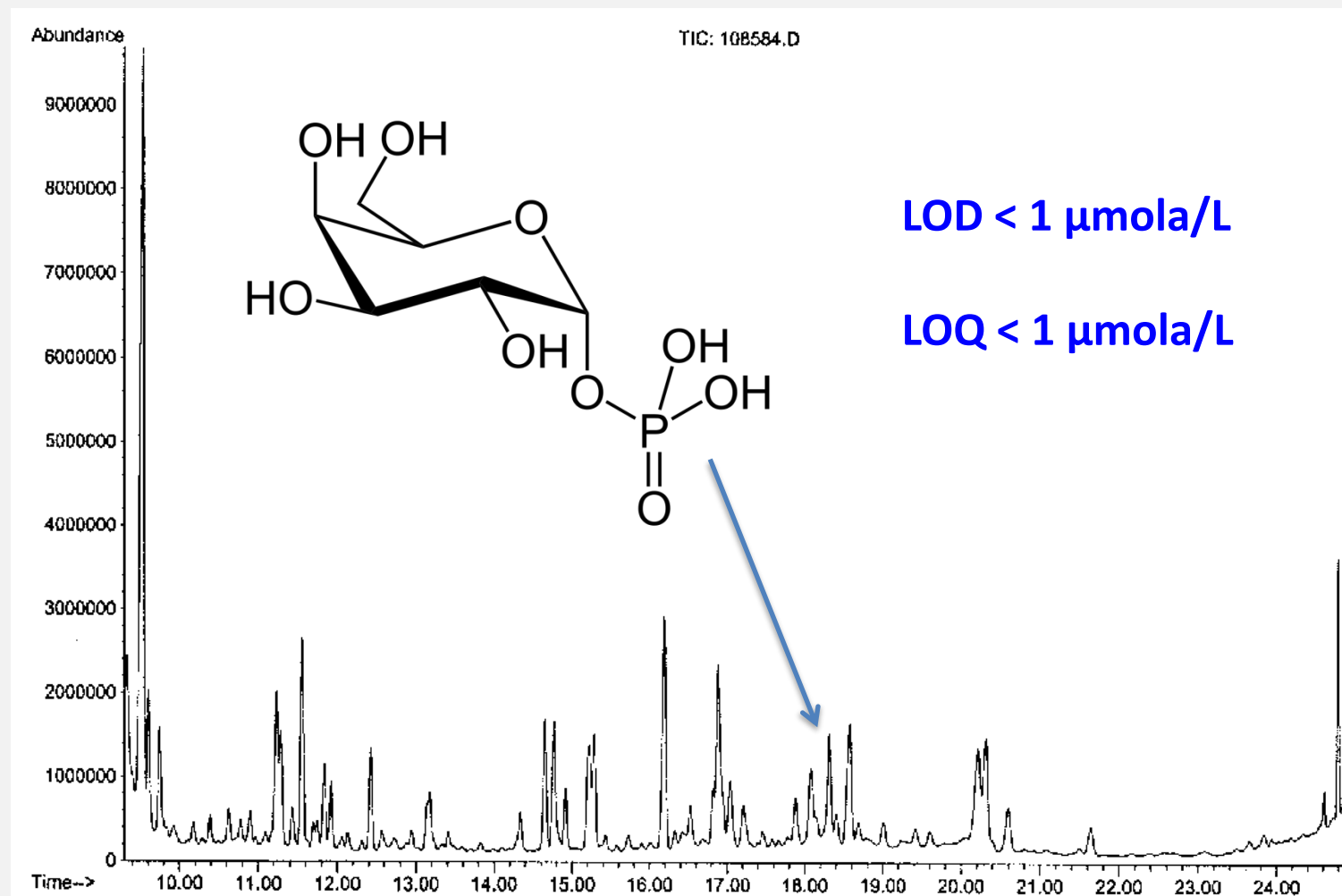
Septum purge  
 Purge flow: 20.0 mL/min  
 Constant septum purge: ☒  
 Stop purge for: 0.00 min

Carrier flow  
 Flow: 1.000 mL/min

Carrier options  
 Vacuum compensation: ☒  
 Carrier gas saver: ☒  
 Gas saver flow: 5.0 mL/min  
 Gas saver time: 1.00 min

# Opracowanie nowej metody

## Oznaczenie



# Opracowanie nowej metody

**Walidacja** jest potwierdzeniem przez zbadanie i przedstawienie obiektywnego dowodu, że zostały spełnione szczegółowe wymagania dotyczące konkretnie zamierzonego zastosowania (wg PN-EN ISO/IEC 17025:2005).

## Walidacja obejmuje:

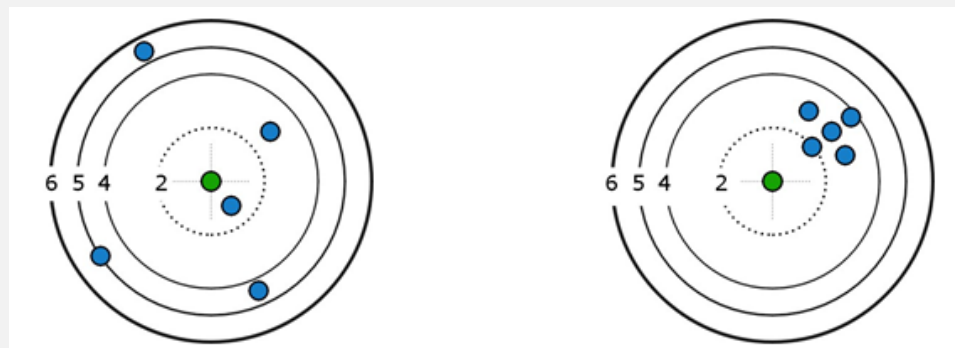
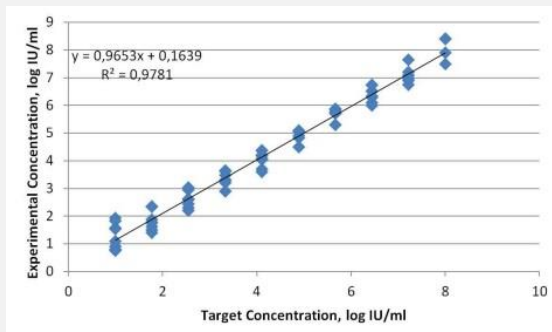
- metody nieznormalizowane,
- opracowane lub rozwijane przez laboratorium,
- metody znormalizowane, stosowane poza zakresem normalizacji,
- w praktyce, wszystkie metody stosowane w danym laboratorium.

# Opracowanie nowej metody

## Walidacja

Podczas procesu walidacji należy określić następujące **cechy charakterystyczne metod analitycznych**:

1. dokładność,
2. precyzję,
3. powtarzalność,
4. odtwarzalność,
5. niepewność pomiaru,
6. granicę wykrywalności,
7. specyficzność i selektywność,
8. liniowość,
9. zakres pomiarowy.

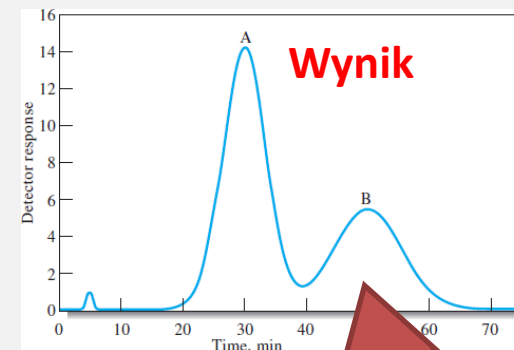


# Implementacja metody

**Pacjenci**



**Laboratorium  
biochemiczne**



**Próbki**

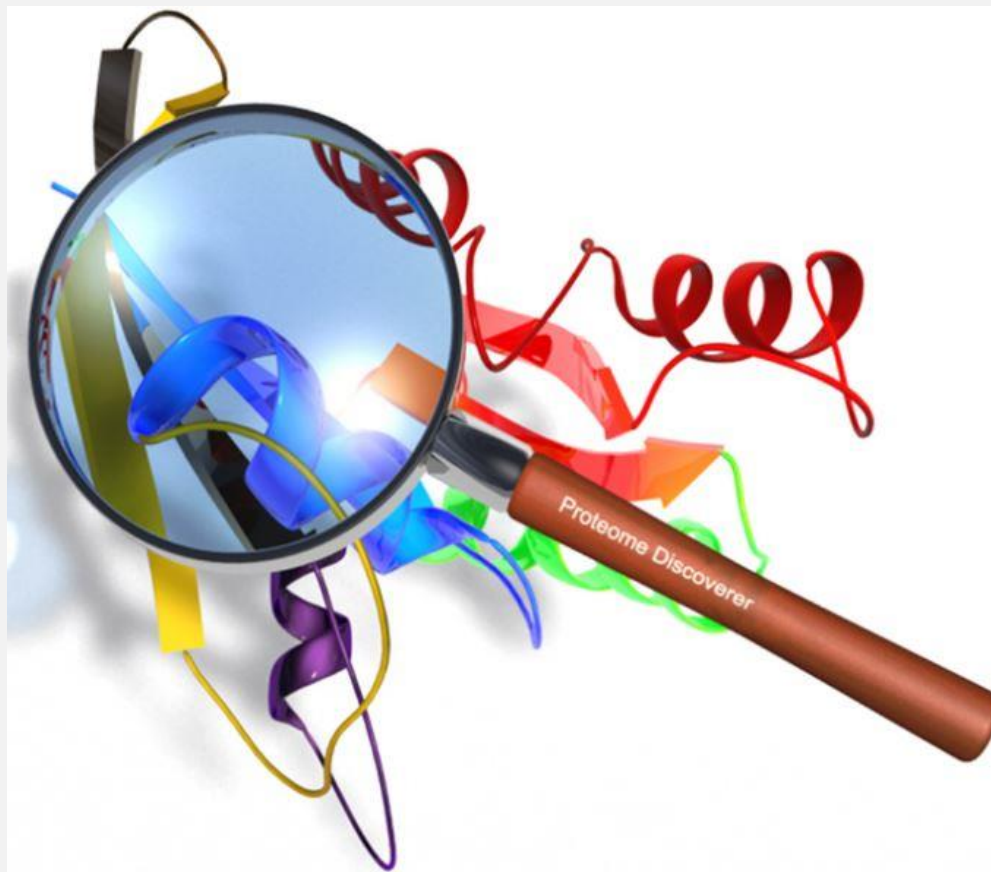


**Przygotowanie próbki**



**Analiza**





**DZIĘKUJE ZA UWAGĘ**