Amino Acids separation by UPLC and LIF detection after AccQ Tag labelling

Application note Ref : AN 061

This is a fast method to separate Amino Acids and quantitate them with a good sensitivity. Amino Acids were derivatizated with Waters® AccQ. Tag™ Reagent Kit and the Waters AccQ Tag method was applied. The LIF detection with Zetalif Detector from Picometrics provides a good sensitivity up to 2.10^{-8} mol/L (2.10^{-14} moles).

Figure 1 shows the separation we obtained with the optimal parameters :

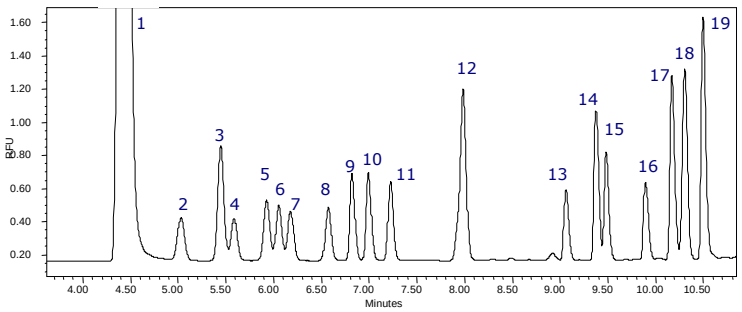
⇒ **High speed method : 11 minutes for a run**

⇒ **Limit of detection :**
Glutamate : $1,2.10^{-7}$ mol/L ($1,2.10^{-13}$ moles)
Aspartate : $1,1.10^{-7}$ mol/L ($1,1.10^{-13}$ moles)
Valine : $3,3.10^{-8}$ mol/L ($3,3.10^{-14}$ moles)
(LOD calculated for a S/N ratio of 3)

⇒ **Sample injection of 1 µl only**
(sample solution diluted to 1/10 during derivatization)

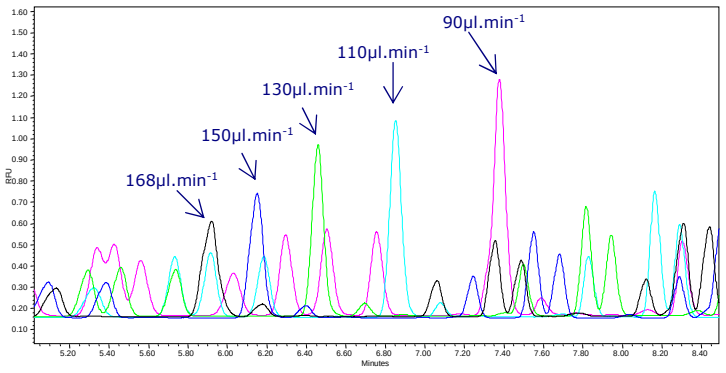
Figure 2 shows how the flow rate influence the sensitivity.

Figure 1 : Separation Obtained at 90 µl.min^{-1}



1-AccQ
2-Aspartate
3-Serine + asparagine
4-Glutamate
5-Glycine
6-Histidine
7-Glutamine
8-NH3
9-Arginine
10-Threonine
11-Alanine
12-GABA + Proline
13-Tyrosine
14-Valine
15-Methionine
16-Lysine
17-Isoleucine
18-Leucine
19-Phenylalanine

Figure 2 : Infuence of the Flow rate on sensitivity



Samples:
Standards : Amino Acids at 10^{-5} M after derivatization

Instruments:
Waters® ACQUITY Ultra Performance LC™ System
Picometrics ZETALIF™ Evolution detector.
Laser: 325 nm, 15 mW

Methods:
Flowrate: 90 µl/min
T= 40°C
Column: Acquity UPLC BEH C18 1.7µm 1.0x50mm
Phases : A : 96% H_2O 4% AccQ Tag buffer
B : 40% H_2O 60% Acetonitrile

For complete experimental details, please contact Picometrics

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