Application Note
Reference AN 0559-01

UPLC™-LIF or **442 nm 410 nm**

Amino Acids

Separation of primary aminoacids in 4 minutes by Waters® ACQUITY UPLC™ System and Laser Induced Fluorescence Detection

Limit of Detection: ***femtomol***

Introduction:
The recent development of new column phases and innovative LC instrumentation leads to the development of extremely rapid separations. As the speed of the separation is increased, the peak becomes sharper and the use of a conventional fluorescence detector becomes significantly more difficult. This difficulty arises because the size of the cell becomes significant with respect to the peak volume and the resolution is severely reduced. An additional concern with the use of **U**ltra **H**igh **P**ressure **L**iquid **C**hromatography is that the design of the flow cell of the detector has to be compatible with the relatively high pressure at the outlet of the column.

In this application note presents our very first data up to the separation of 17 amino acids (all the common amino acids, except Pro and Lys) labeled with naphthalene-2,3-dicarboxyaldehyde (NDA) using a Waters® ACQUITY UPLC™ System (Waters Corp., Milford MA) with a 1 or 2 mm micro-column and detection with a ZetaLIF Laser Induced fluorescence detector (Picometrics SA, France). The linear velocity in the capillary is optimized and subnanomolar LODs are reached.

This is the fastest LC separations of amino acids using fluorescence detection at such low concentrations, opening the way to high throughput amino acids analyses in areas ranging from the foodstuff industry to neuropharmacology.

Instruments:
Waters® ACQUITY Ultra Performance LC™ System

Picometrics ZETALIF Evolution detector.
Diode Laser: 410 nm, 15 mW

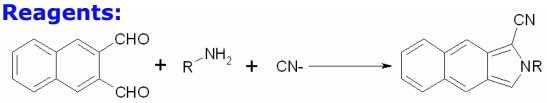
Sample:
Standards in solution. Proline was not added (secondary amine; cannot be labelled by NDA. Lysine which can be labelled by two NDA molecules and is not excitable at 410nm (Exc.max = 462nm) is not present either, and Tyrosine is not seen in this run. or real microdialysates

Methods:
Flowrate: as indicated in the figures T= 35°C
Column: (BEH C18) 1 or 2 mm x 150mm
Capillary: 150 µm ID

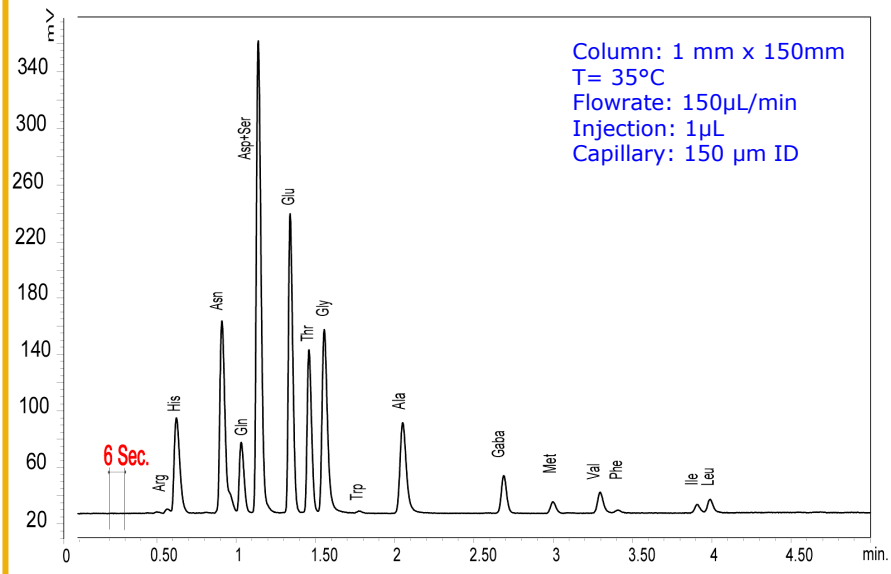
T (min)	0	4	5
Phase A%	70	40	70
Phase B%	30	60	30

Gradient= Mix of : .Sol. A = Sodium Citrate 5mM, pH 3.2
.Sol. B = Acetonitrile

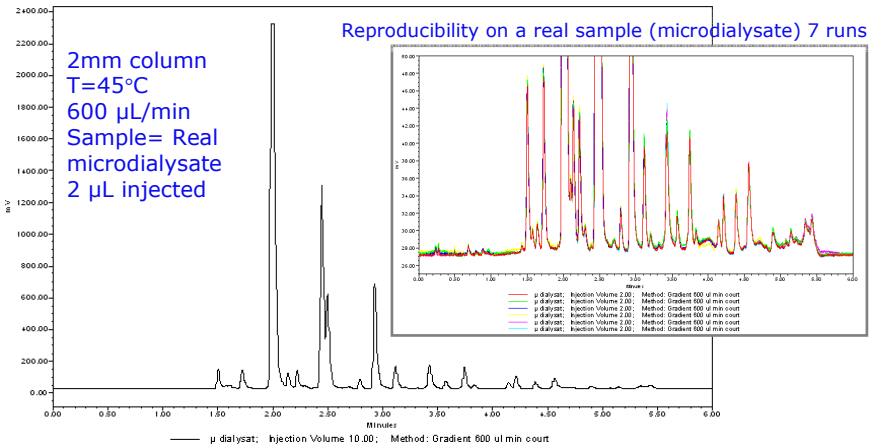
Reagents:



Standards labeled at 10^{-7} M each with NDA.



Example: Microdialysate injected on a 2 mm Column



NDA is a fluorogenic dye which fluoresces weakly in its native form but has a good fluorescent yield when reacted with CN- and a primary amine. Such derivatives are excitable at 410 nm or 442 nm using, respectively, diode or HeCd lasers. Derivatization procedure according to Picometrics TN002

picometrics

Source: Picometrics application lab. 12/2005.

more on ***www.picometrics.com***

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