

## Amino acids and catecholamines analysis

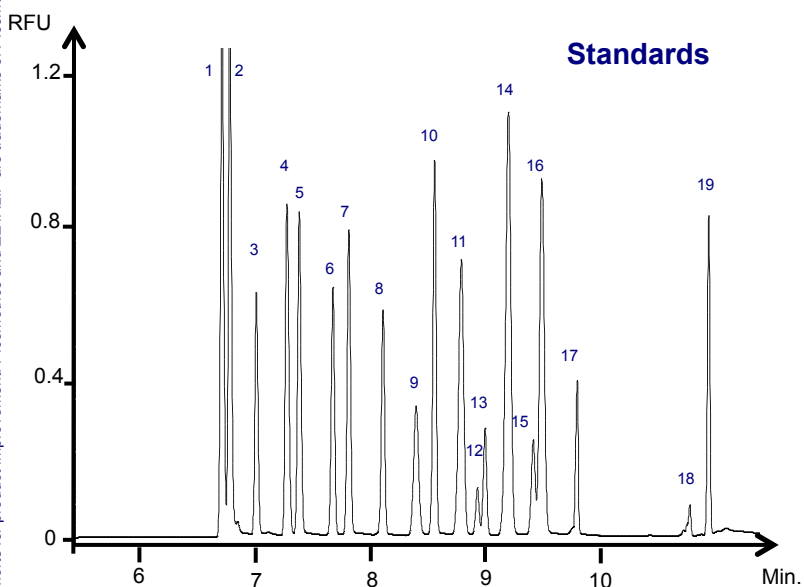


### Application note Ref : AN 2.002-V2

We show in this study the comparison between CE/L(ed)IF and capillary LC/L(ed)IF analysis of 20 primary amino acids and catecholamines labelled with NDA. It permits to do the best compromise for the neuroscientist needs.

We used NDA (naphthalene-2,3-dicarboxyaldehyde) because it perfectly allows derivatizing primary amines containing molecules at concentrations as low as 100 picomolar making this method THE labeling method of choice for easy and sensitive detection.

### CE-L(ed)IF analysis

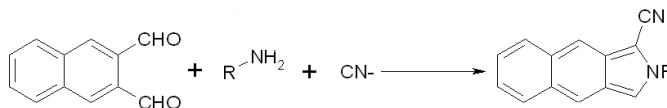


- |                       |                |                   |
|-----------------------|----------------|-------------------|
| 1 Glutamine/Threonine | 7 Asparagine   | 13 Norepinephrine |
| 2 Histidine/Serine    | 8 Gaba         | 14 Leucine        |
| 3 Tyrosine            | 9 Valine       | 15 Glutamate      |
| 4 Alanine             | 10 Methionine  | 16 Aspartate      |
| 5 Glycine             | 11 Tryptophane | 17 Dopamine       |
| 6 Phenylalanine       | 12 Isoleucine  | 18 Lysine         |
|                       |                | 19 Arginine       |

**Instruments:** Capillary electrophoresis: Agilent Technologies CE7100 Detector: Picometrics ZETALIF LED with LED 450nm/30nm

**Sample:** Standards : 10<sup>-7</sup>M (labeled at 10<sup>-6</sup>M and diluted 10 times prior to injection)

**Labeling:** NDA is a fluorogenic dye which fluoresces weakly in its native form but has a good fluorescent yield when reacted with CN<sup>-</sup> and a primary amine. Such derivatives are excitable at 450 nm using a LED.

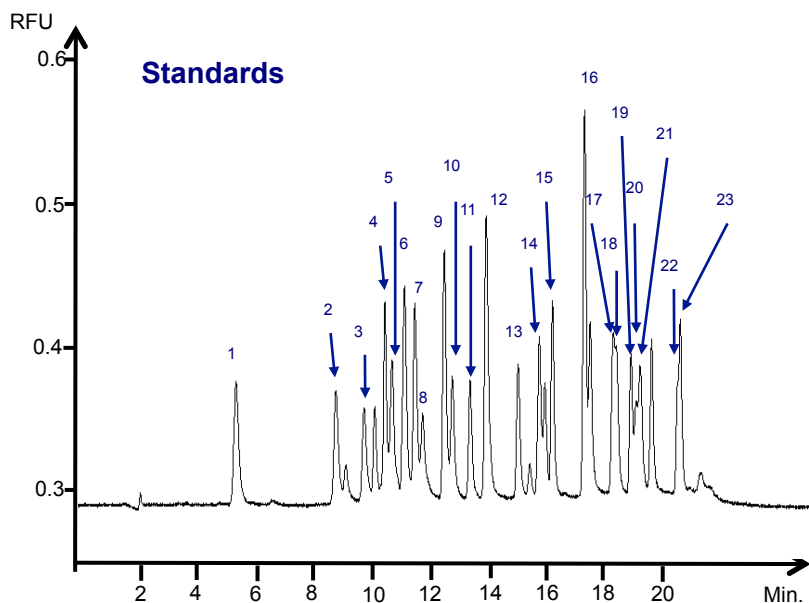


**Method:**

- Capillary: 65 cm x 50 µm ID
- Run time: 16 minutes
- Injection: 10 seconds at 50mbar

	CE-L(ed)IF
Run Time	16 min
Injection volume	32nl
LOD Glutamate	8.8.10 <sup>-10</sup> mol/l
LOD Gaba	8,4.10 <sup>-10</sup> mol/l

## Capil.LC-L(ed)IF analysis



**Instruments:** Capillary HPLC: Ultimate LC-Packing system; Detector: Picometrics ZETALIF LED with LED 450nm/30nm

**Sample:** Standards : Amino acids at  $10^{-8}$  M

**Labeling:** Amino acids are labeled with NDA

**Method:** Mobile Phase: A:Sodium citrate buffer 5mM pH=3,2 B :Acetonitrile  
Flow rate: 5  $\mu$ L/min,  
Injection volume: 1  $\mu$ L,  
Column: Inertsil ODS3, 3 $\mu$ m  
150mm x 300 $\mu$ m GROM  
Run time: 22 minutes

- |              |              |                   |                  |
|--------------|--------------|-------------------|------------------|
| 1 Histidine  | 7 Serine     | 13 Tyrosine       | 19 Dopamine      |
| 2 Arginine   | 8 Aspartate  | 14 Alanine        | 20 Valine        |
| 3 Asparagine | 9 Taurine    | 15 Norepinephrine | 21 Phenylalanine |
| 4 histamine  | 10 Glutamate | 16 GABA           | 22 Isoleucine    |
| 5 Glutamine  | 11 Threonine | 17 Methionine     | 23 Leucine       |
| 6 Citrulline | 12 Glycine   | 18 Tryptophane    |                  |

Capil.LC-L(ed)IF	
<b>Run Time</b>	22 min
<b>Injection volume</b>	1 $\mu$ L
<b>LOD Glutamate</b>	$5,5 \cdot 10^{-10}$ mol/l
<b>LOD Gaba</b>	$2,4 \cdot 10^{-10}$ mol/l

### Conclusion:

This application allows the simultaneous detection of Amino acids and catecholamines with capil.LC-L(ed)IF and CE-L(ed)IF. These methods provide the neuroscientist with one of the most powerful tool for sensitivity in analysis of microdialysates. In CE-L(ed)IF, the volume of injection of sample is 1  $\mu$ L, neurochemist can increase the number of tests on the same sample to provide other analytical data with complementary methods.

### References:

- [i] Siri N, Lacroix M, Garrigues JC, Poinot V, Couderc F. Electrophoresis. 2006, 27, 4446-55
- [ii] Poinot V, Rodat A, Gavard P, Feuerer B, Couderc F. Electrophoresis, 2008, 29, 207-223
- [iii] Lacroix M, Garrigues JC, Couderc F. J Am Soc Mass Spectrom. 2007, 18, 1706-13.