

Amino acids and catecholamines analysis

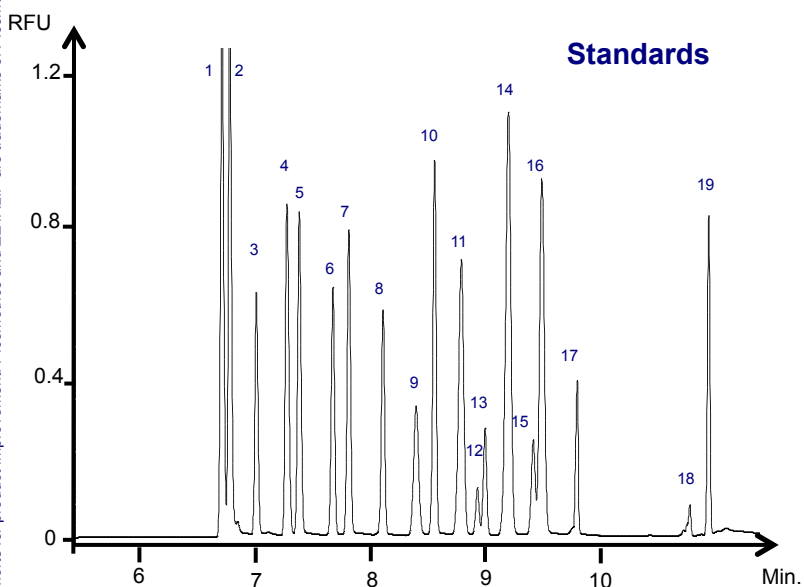


Application note Ref : AN 2.002-V2

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

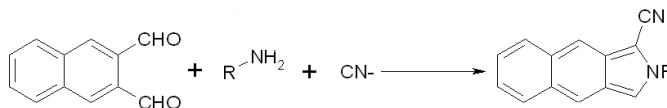


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|-----------------------|----------------|-------------------|
| 1 Glutamine/Threonine | 7 Asparagine | 13 Norepinephrine |
| 2 Histidine/Serine | 8 Gaba | 14 Leucine |
| 3 Tyrosine | 9 Valine | 15 Glutamate |
| 4 Alanine | 10 Methionine | 16 Aspartate |
| 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 : 10⁻⁷M (labeled at 10⁻⁶M 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⁻ and a primary amine. Such derivatives are excitable at 450 nm using a LED.

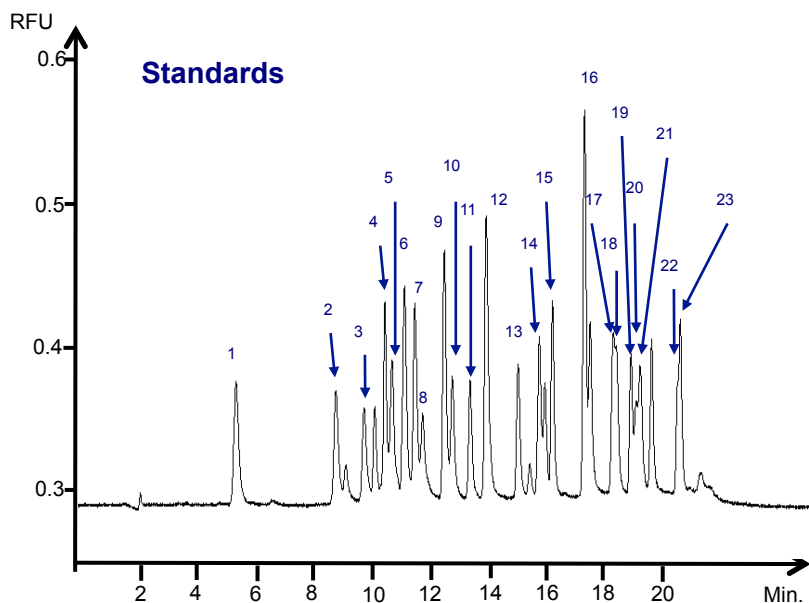


Method:

- 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 ⁻¹⁰ mol/l
LOD Gaba	8,4.10 ⁻¹⁰ 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 μ L/min,
Injection volume: 1 μ L,
Column: Inertsil ODS3, 3 μ m
150mm x 300 μ m GROM
Run time: 22 minutes

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|--------------|--------------|-------------------|------------------|
| 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
Run Time	22 min
Injection volume	1 μ L
LOD Glutamate	$5,5 \cdot 10^{-10}$ mol/l
LOD Gaba	$2,4 \cdot 10^{-10}$ 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 μ 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.