CE-LIF

442 nm or 410* nm

Analysis of 5 key Amino Acids and Catecholamines in 1 µL Microdialysate (single run)

Determination in a twelve minutes single run of Gaba, Aspartate, Glutamate, Norepinephrine and Dopamine from a 1µL of microdialysate sample by Capillary Electrophoresis with Laser Induced Fluorescence Detection

Limit of Detection*:
nanoMolar LOD or lower

** Estimated for a S/N of 3*

Preliminary acknowledgment:

We are extremely grateful to Mr. Serge Gobaille, "Laboratoire de Microtechniques Neurochimiques appliquées à l'animal vivant", IFR Neurosciences, Strasbourg France, for implementing the present method and applying it to the analysis of real microdialysates, but also for sharing the present data with Picometrics and so strongly collaborating to the constant improvement of the solutions that we promote.

Introduction

This application note describes a unique method to simultaneously detect 5 key amino acids and catecholamines in microdialysates by coupling Capillary Electrophoresis (CE) to LIF detection. This technique provides neuroscientists with a unique analytical solution for the analysis of microdialysates.

We describe the separation and the calibration results using standards to validate the method and then describe data collected from real microdialysate samples from various rat brain areas. The data from real samples is presented on the back of this note.

The method lets you get the maximum data from your costly microdialysates

The CE-LIF quantification method described herein allows the determination of five essential parameters in a single neuro-pharmaceutical assay. It is no longer necessary to perform a number of runs to determine these five parameters.

In addition, it should be noted that the protocol requires less than 1 µL of microdialysate, so that a number of additional tests can be performed to provide other biological data (for example ACH can be separately determined).

Since a smaller sample is required, the analyst can obtain higher temporal resolution in the fraction collection process, and allows for more highly resolved kinetic studies.

The method is easy and readily automated

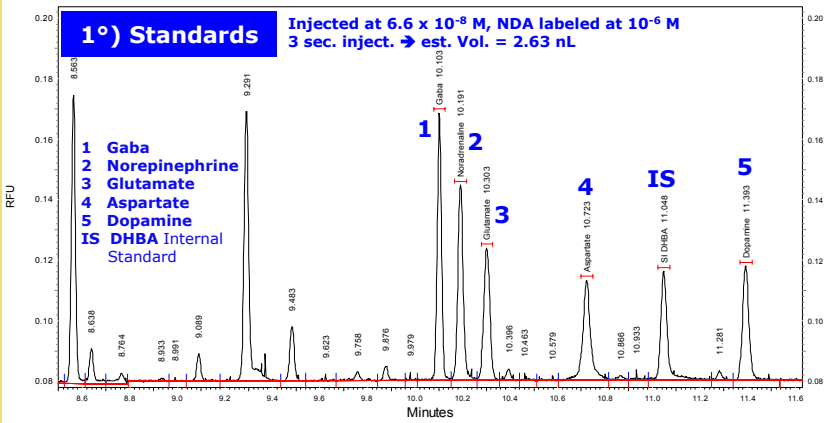
There are two steps in the method, derivatization with NDA and the CE separation, which lasts within less than 12 minutes. The reproducibility of the method (derivatization & analysis) is excellent and its linearity as well as shown.

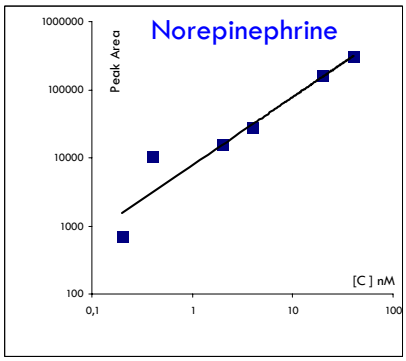
Analysis of real samples

The 2 examples shown on the reverse from hippocampus and pineal gland microdialysates illustrate the efficiency of the CE-LIF approach and all the possible benefits for the neuroscientists. In comparison such determination of the extra cellular Dopamine released in the hippocampus, and the analysis of Norepinephrine in pineal gland microdialysates would have be very difficult with conventional HPLC and Electrochemical detection for example.

1°) Standards

Injected at 6.6 x 10^{-8} M, NDA labeled at 10^{-6} M
3 sec. inject. → est. Vol. = 2.63 nL

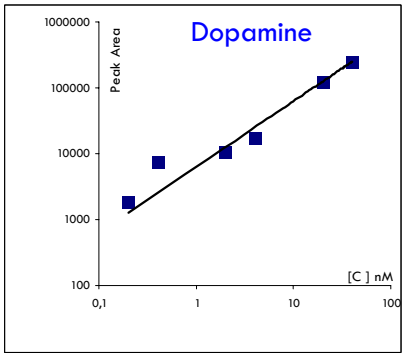




Calibrations

Calibration
Area = 7770.3 x [C] + 1558.5
R2 = 0.9988

Calibration
Area = 6244.3 x [C] + 500
R2 = 0.9977



Calibration data are shown here for Dopamine and Norepinephrine only but have been obtained for all 5 compounds.

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Instruments:
Detector: Picometrics ZETALIF detector
Laser: HeCd laser, 442 nm, 33 mW

** For NDA applications Picometrics now recommend a 410 nm laser diode which is suitable for this application.*

Capillary Electrophoresis: The Agilent 3DCE and the Beckman PACE MDQ have been used in the development of this method.

Samples:
Standards in solution or real microdialysates (only 1 µL required). 10^{-3} M standards stock solutions are kept frozen in HCl 0.1 M. DHBA used as internal standard.

Labeling:
Derivatization agent: Naphthalene Dicarboxaldehyde (NDA). (1 µL sample + 2 µL Labeling buffer**), diluted 5 times in sterilized and 0.22 µ filtered H_20
Incubation ≥ 5min

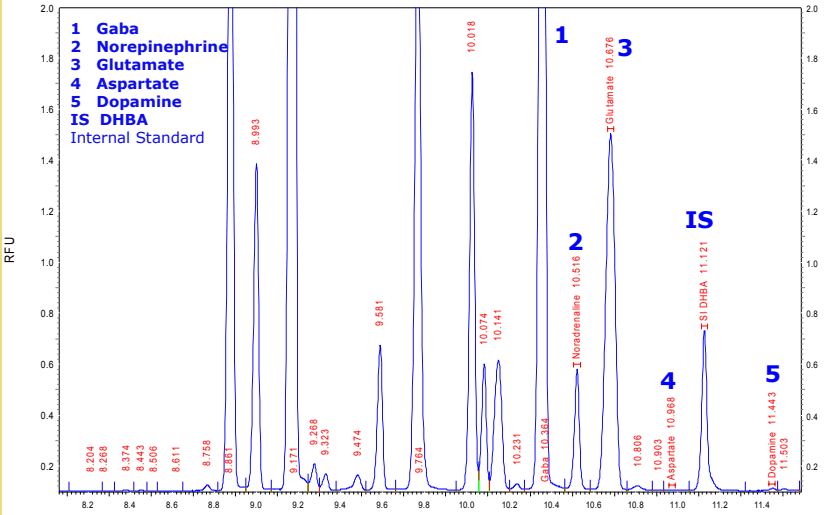
**labeling buffer: Borate/KCN/NDA = 10/2.5/2 (V/V/V)
Borate: Boric Acid 182 mM & Tetraborate 157.6 mM
KCN: 43 mM in H_20
NDA: 5 mM in methanol

Methods:
Capillary: 50 µm ID, 65 cm length (50 cm eff. length), washed with NaOH 1%
Injections: done under 0.5 psi; inj. times and volumes specified on each figure
Working temp.: 20°C, samples 4°C
Buffer: Lithium Tetraborate 22.5 mM + LiDS 20 mM, pH 9.2
CE Conditions: 16 kV / 14.8 µA for 7 min, then 30 kV / 30.7 µA for 5 min.

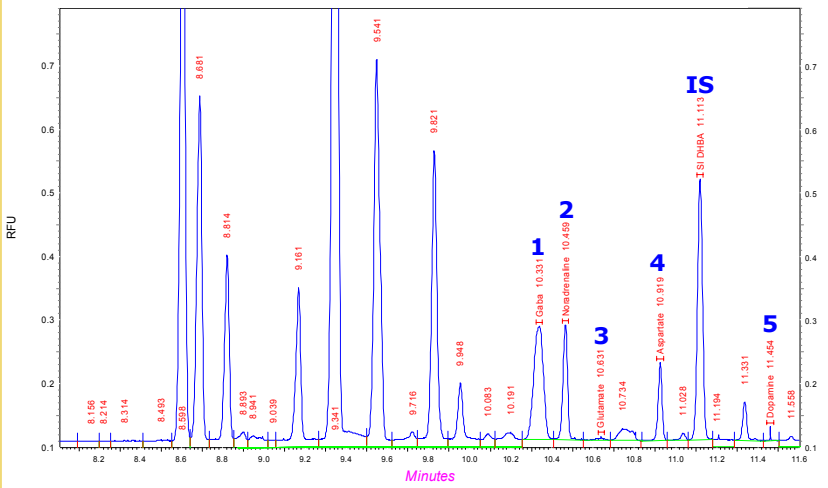
Microdialysis conditions
Perfusion liquid: NaCl 145 mM; KCl 2.7 mM; $MgCl_2$ 1 mM; $CaCl_2$ 1.2 mM; NaH_2PO_4 2.33 mM; pH 7.4 .
Probe length: 2 mm for Hippocampus assay and 1 mm for Pineal gland assay
20 kD membrane polycarbonate/polyether copolymer
Perfusion flow rate: 1 µL /min.

2°) Analysis of Real Microdialysates — 10 sec. inject. ➔ est. volume 8.78 nL

A) Hippocampus microdialysate from awake rat



B) Pineal gland microdialysate from awake rat



SPECIAL ACKNOWLEDGMENTS
The separation method used in this work has been developed by the Picometrics application lab. We would like to thank Prof. F. Couderc, IMRCP, Paul Sabatier Uty Toulouse (France) for advising Picometrics on analytical issues.

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