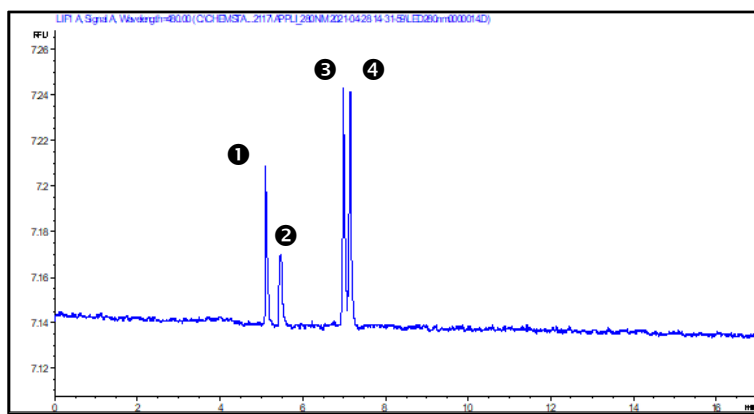


Analysis of Tryptophan / Tyrosine / HIAA / HMMA in Native Fluorescence

Application Note: AN 2.007

Tryptophan and Tyrosine, which are aromatic molecules, are amino acids that have a major role in detecting proteins in native fluorescence. 5-HIAA and Mandelic Acid (HMMA) are bigger molecules. 5-HIAA is a muscle stimulant and the primary metabolite of serotonin, a hormone derived from the amino acid tryptophan. HMMA is part of the family of fruit acids, also called alpha-hydroxy acid (AHA) which is used in the treatment of infections or in cosmetics for its ability to treat a number of skin imperfections such as wrinkles, pigment spots or acne. This application note shows the separation of these four molecules with a 275 nm LED induced fluorescence detector.

Method of Separation



- 1 Tryptophan 1.10^{-7} M
- 2 Tyrosine 1.10^{-6} M
- 3 5-Hydroxyindole-3-acetic acid (HIAA) 1.10^{-7} M
- 4 DL 4-Hydroxy-3-Methoxy Mandelic Acid (HMMA) 1.10^{-6} M

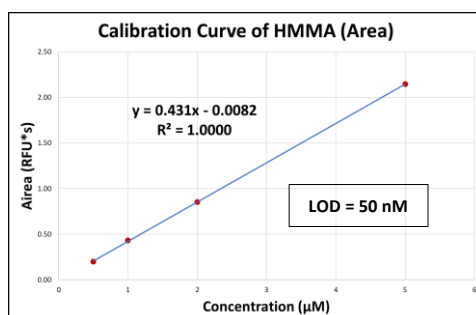
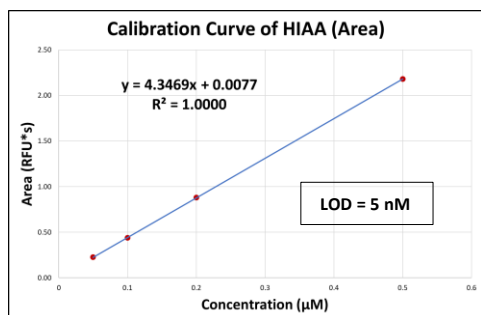
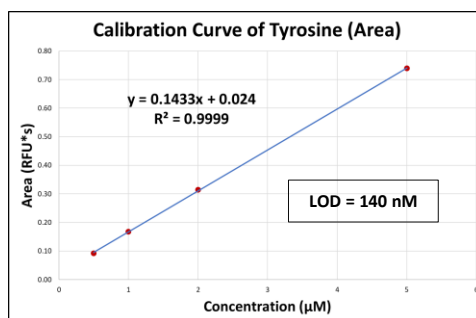
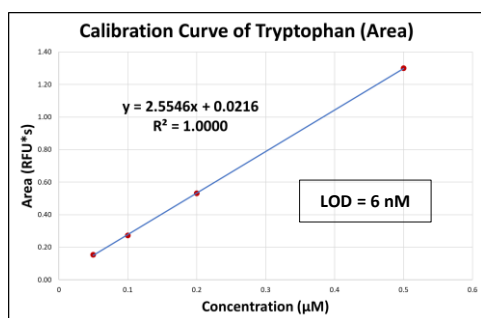
Key features of the method:

- Hydrodynamic injection of sample : 50 mBar during 10 s.
- Separation: 16 kV during 17 min.
- Temperature: 25 ° C
- Buffer: CAPS 10 mM, Sodium Tetraborate 15 mM
- No labelling of analytes

Instruments:

- Capillary Electrophoresis: Agilent Technologies 7100 CE
- Detector: ZETALIF LED 275
- Fused Silica Capillary: 75 μ m ID
Total Length = 65 cm / Effective Length = 44 cm

Limit of Detection (LOD)



Molecules	Minimum Concentration Tested (nM)	S/N Ratio	LOD (nM)
Tryptophan	50	23.6	6
Tyrosine	500	10.7	140
HIAA	50	30.7	5
HMMA	500	28.1	50

Conclusion:

The 275 nm LED offers a sensitivity comparable to the 266 nm laser for the detection of molecules in native fluorescence. Unlike the pulsed laser, it makes it possible to obtain linear calibration curves.