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L-tyrosine and 3-nitro-L-tyrosine

Introduction

Nitric oxide (NO) is produced in the endothelial cells and neurons by nitric oxide synthetase and plays an important role in humans under many physiological and pathological conditions. It is known to function as an endothelium derived vascular relaxing factor or to be involved in the signal transduction in the brain. Recently NO itself or an oxidant derived from NO were proposed to be cytotoxic. NO contains an unpaired electron that can combine with free radicals such as superoxide (O_2^-) and NO and O_2^- produce a strong oxidant, peroxynitrite ($ONOO^-$) in vivo. $ONOO^-$ is supposed to be involved in several process that lead to oxidative stress and chronic ischemic injury of the brain. Therefore a sensitive detection method is required for this unstable molecule in human material. Peroxynitrite has been reported to react with L-tyrosine to produce 3-nitro-L-tyrosine (NO_2-Tyr), which appears to be a suitable marker for ONOO- mediated tissue damage. In this application NO_2-Tyr standards are determined with a detection limit of 0.5 nmol/L.

Method

Amperometric detection at 600 mV in combination with a reactor potential of -850 mV (Fig. 2) results in the best detection sensitivity for NO_2-Tyr standards. Increasing the detection potential to 1000 mV results in an improved signal for tyrosine.

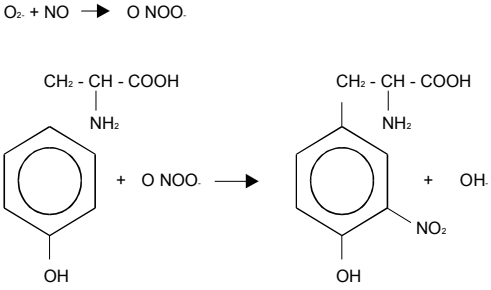


Fig. 1 Synthesis of 3-nitro-L-tyrosine from L-tyrosine and peroxynitrite.

LC-EC conditions

Column	C18, 50 x 1 mm, 5 µm
Flow rate	0.05 ml/min
Mobile phase	H_3PO_4 50 mM, citric acid 50 mM, pH=3.1 with KOH, 40 mg/l EDTA, 100 mg/l octane sulphonic acid (OSA), 5% methanol
Sample	10 µl injection
Temperature	30 °C
E-reactor	-850 mV (vs. HyREF)
E-cell	600 mV (vs. Ag/AgCl sat'd)*

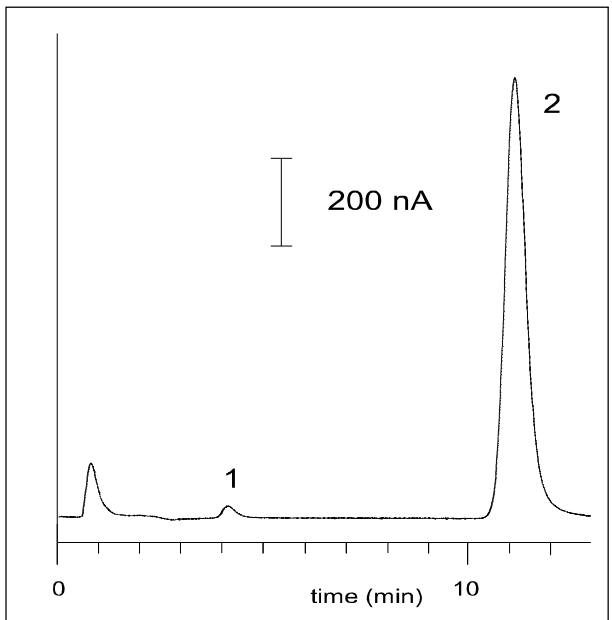


Fig. 2 Analysis of 6.5 µmol/l L-tyrosine (1) and 4.3 µmol/l 3-nitro-L-tyrosine (2) standards using a working potential of 600 mV in combination with a reactor potential of -850 mV.

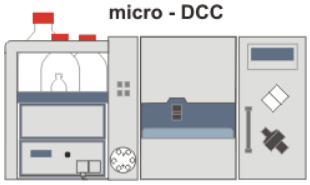
Reference

1. 1. W. Maruyama, Y. Hashizume, K. Matsubara and M. Naoi, J. Chromatogr. B, 676 (1996) 153-158.

Configuration suggestions & comment

For the analysis of Nitrotyrosine in microdialysates we recommend the VT03 flow cell with ISAAC*. This cell not only has an excellent reproducibility but also the best possible detection sensitivity. Microbore HPLC is the method of choice because of the small sample volume and better conversion rate in a Reactor cell.

Part no.	Description
180.0072	ALEXYS 100 Nitrotyrosine
250.1097	ALB-105 column, 50 x 1.0mm, 3um C18



*Note that 2 mM KCl must be added to the mobile phase when using a cell with ISAAC REF, also cell potential must be adapted to 410 mV.