3-Hydroxykynurenine

Introduction

3-Hydroxykynurenine (3-HK or 3-HKyn) is a metabolite in the kynurenine pathway, the major route of tryptophan degradation in mammals. 3-HK is a potential endogenous neurotoxin whose increased levels have been described in several neurodegenerative disorders [1]. The ALEXYS Neurotransmitter Analyzer is a versatile system for the trace analysis of various neurotransmitters, like catecholamines, serotonin, acetylcholine, GABA, Glu and other amino acids [2-4]. This note shows the proof of principle for the analysis of 3-HK using the ALEXYS Neurotransmitter analyzer.

Fig. 1. ALEXYS Analyzer

Method

The ALEXYS Neurotransmitter Analyzer (Fig. 1) consists of a P6.1L pump with integrated degasser, DECADE Elite electrochemical detector, AS 110 autosampler and Clarity data acquisition software. The LC-ECD conditions are listed in Table 1. Separation of 3-HK was achieved using an Acquity UPLC HSS T3, 1x50 mm 1.8 µm C18 column in combination with an acidic mobile phase (pH 3.2) with ion-pairing agent (OSA) and 2% acetonitrile as organic modifier. After separation, 3-HK was detected in DC mode at a potential of 0.5 Volt versus Ag/AgCl on a glassy carbon working electrode using a DECADE Elite detector in combination with a SenCell wall-jet electrochemical flow cell [5]. An example chromatogram of a 5 µL injection of 10 nM 3-HK standard in 0.1 M perchloric acid is shown in Fig. 2, together with a blank injection. 3-HK is eluting within 10 minutes and could be detected down to a (calculated) detection limit of 0.4 nM.
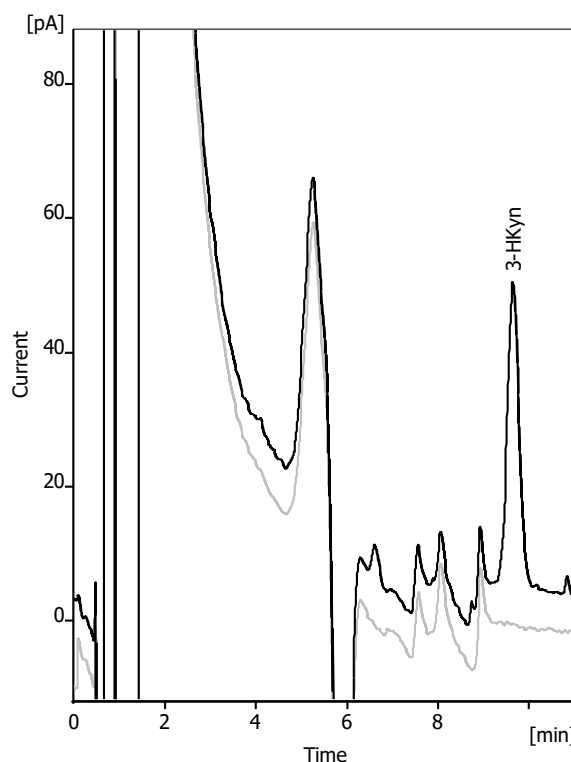

Fig. 2. Overlay of chromatogram overlay of 10 nM 3-HKyn in 0.1 M perchloric acid (black) and a blank (grey). Peak SN ratio of 3-HKyn is 75.

Table 1. LC-ECD conditions

UHPLC	ALEXYS Neurotransmitter Analyzer
Column	Acquity UPLC HSS T3, 1x50 mm 1.8 µm
Pre-column filter	Acquity in-line filter kit (Waters)
Mobile phase	100 mM phosphoric acid, 0.27 mM EDTA.Na$_2$, set to pH 3.2 with 50% NaOH solution, 2% acetonitrile, 1 g/L octane sulfonic acid sodium salt
Flow rate	50 µL/mL
Temperature	35 °C for separation and detection
Backpressure	About 100 bar
V$_{injection}$	5 µL
Injection method	Full loop fill
Flow cell	SenCell™ with 2 mm GC working electrode and saltbridge reference electrode, AST setting 2
Ecell	0.5 V vs. salt bridge reference electrode
Range	5 nA/V
ADF	0.05 Hz
I-cell	About 1 nA

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References

1. S. Okuda et al., 3-Hydroxykynurenine, an endogenous oxidative stress generator, causes neuronal cell death with apoptotic features and region selectivity, *J Neurochem.,* 70 (1)(1998), 299-307

2. Antec Scientific, ALEXYS Neurotransmitter Analyzer for Monoamines and their Acidic Metabolites, Application note, 213.028

3. Antec Scientific, ALEXYS Neurotransmitter Analyzer for GABA & Glutamate, Application note, 213.020

4. Antec Scientific, ALEXYS Neurotransmitter Analyzer for Acetylcholine and Choline, Application note, 213.023

5. H. Louw, H.J. Brouwer, N. Reinhoud, Electrochemical flowcell, (2016) US patent 9310330

Ordering information

ALEXYS Neurotransmitter Analyzer for analysis of 3-HK	
180.0091UW	ALEXYS Neurotransmitters SCC base
116.4120	SenCell with 2 mm GC WE and sb REF
250.1165*	Acquity UPLC in-line filter kit + 6 frits (205000343)
250.1160*	Acquity UPLC C18 HSS T3, 1x50 mm 1.8 µm (186003535)

*) Columns are products of Waters Corporation (Milford, USA). The Waters part numbers are given between parenthesis for reordering purposes.

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