

3-hydroxy kynurenine (3-HK)

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 Neurotransmitter Analyzer

Method

The ALEXYS Neurotransmitter Analyzer (figure 1) consists of a P6.1L pump with integrated degasser, DECADE Elite electrochemical detector, AS 110 autosampler and Clarity data acquisition software. The LC-EC 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 figure 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.

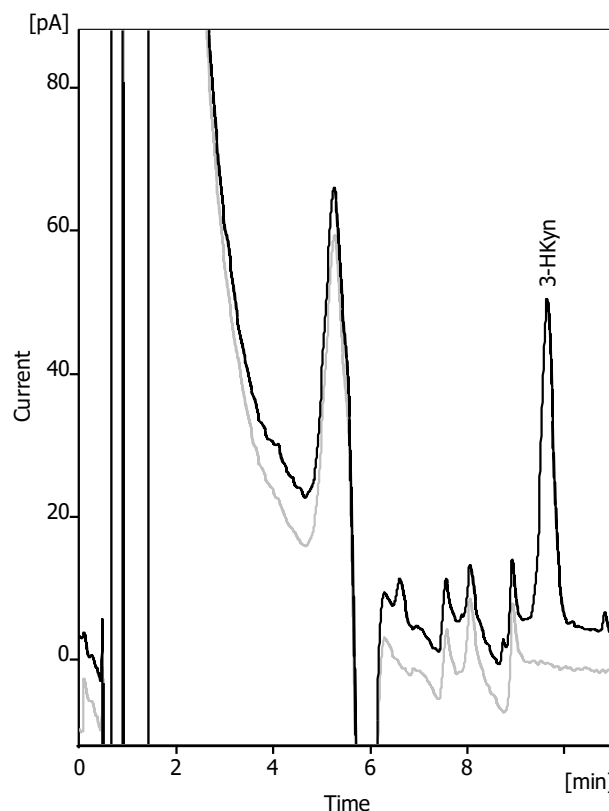
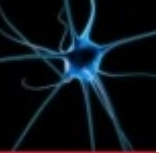


Fig. 2. Overlay of chromatogram overlay of 10 nM 3-HKyn in 0.1 M perchloric acid (black) and a blank (grey). Peak ratio of 3-HKyn is 75.

Table 1. LC-EC conditions

HPLC	ALEXYS Neurotransmitter Analyzer
Column	Acquity UPLC HSS T3, 1x50 mm 1.8 μm
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 $\mu\text{L}/\text{min}$
Backpressure	About 100 bar
Injection	5 μL (flushed loop fill)
Temperature	35°C for separation & detection
Flow cell	2 mm GC SenCell saltbridge (sb), AST 2
E-cell	0.5 V vs sb
I-cell	about 250 nA
ADF	0.05 Hz
Range	5 nA/V



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, Histamine, LNAAs and other Amino Acids, 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

180.0091W	ALEXYS Neurotransmitter Analyzer, 1 channel
191.0055UL	AS 110 autosampler UHPLC cool 6p
116.4120	SenCell with 2 mm GC WE and sb REF
250.1160*	Acquity UPLC C18 HSS T3, 1x50 mm 1.8 µm (186003535)
250.1165*	Acquity UHPLC in-line filter kit + 6 frits (205000343)

*) Columns are products of Waters Corporation (Milford, USA), between parenthesis the Waters pn's are shown for reference.

For research purpose only. The shown information in this communication is solely to demonstrate the applicability of the ALEXYS system. The actual performance may be affected by factors beyond Antec's control. Optimization of the method may be necessary for analysis of real samples. Specifications mentioned in this application note are subject to change without further notice.

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