



Tyrosine, Tyramine and Octopamine

Introduction

Neurotransmitters of insects share some overlap with those of mammals, but there are also specific differences. For example, the function of noradrenaline in mammals seems to be substituted by octopamine in insects. The biosynthesis of octopamine involves tyrosine and tyramine [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 some typical insect neurotransmitters using the ALEXYS Neurotransmitter analyzer.

Method

The ALEXYS Neurotransmitter Analyzer (Fig. 1) consists of a P6.1L pump with integrated degasser, DECADE Elite electrochemical detector, AS6.1L autosampler and Clarity data acquisition software. The LC-ECD conditions are listed in Table 1. After separation on a C18 UPLC column, the neurotransmitters were detected in DC mode 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 insect neurotransmitters is shown in Fig. 2. The detection limit is around 0.5 nM for a 5 μ L injection.



Fig. 1. ALEXYS Neurotransmitter Analyzer

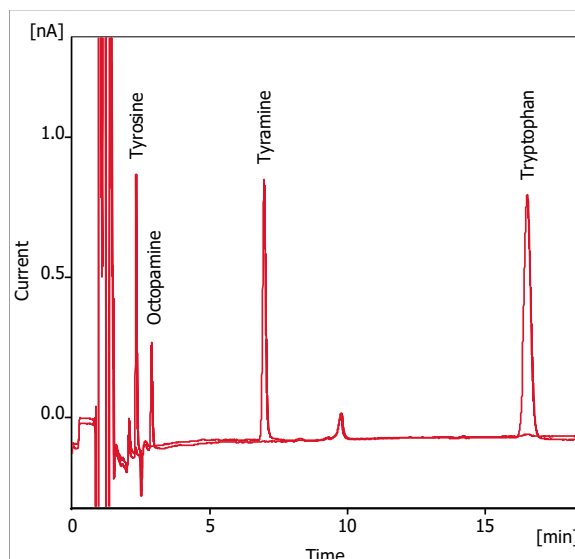


Fig. 2. Chromatograms of 100 nmol/L standards in Ringers solution with 10 mmol/L acetic acid, 2 μ L injection. Conditions as in Table 1.

Table 1. Recommended LC-ECD conditions

UHPLC	ALEXYS Neurotransmitter Analyzer
Column	Acquity UPLC BEH C18, 1.7 μ m, 1x100 mm (Waters)
Pre-column filter	Acquity in-line filter kit (Waters)
Mobile phase	100 mM phosphoric acid, 100 mM citric acid, 0.1 mM EDTA.Na ₂ set to pH 3.0, 600 mg/L octanesulfonic acid sodium salt, 8% acetonitrile Refresh at least every 3 days.
Flow rate	50 μ L/mL
Temperature	37 $^{\circ}$ C for separation and detection
Backpressure	About 265 bar
V _{injection}	2 μ L (up to 5 μ L max)
Injection method	Dedicated user program with minimal sample use
Needle wash	Water (refresh weekly)
Pump piston wash	15% isopropanol in water (refresh weekly)
Flow cell*	SenCell™ with 2 mm GC working electrode and saltbridge reference electrode, AST setting 1
Ecell	1.0 V vs. salt bridge reference electrode
Range	10 nA/V
ADF	0.5 Hz
I-cell	About 2 nA

*) The original work was done with an ISAAC reference electrode (Ecell 0.85 V/8 mmol/L chloride).



References

1. Farooqui, T. (2012). Review of octopamine in insect nervous systems. *Open access insect physiology*, 4, 1.
2. Antec Scientific, Monoamines and acidic metabolites, Application note, 213.028
3. Antec Scientific, GABA and glutamate, Application note, 213.020
4. Antec Scientific, Acetylcholine and choline, Application note, 213.023
5. H. Louw, H.J. Brouwer, N. Reinhoud, Electrochemical flow cell, (2016) US patent 9310330

Ordering information

ALEXYS Neurotransmitter Analyzer	
180.0091UA	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)
250.1163*	Acquity UPLC BEH C18, 1.7 μ m, 1 x 100 mm (186002346)

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

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