Application note

Neuroscience

Histamine

Introduction

Histamine is a biogenic amine, consisting of an imidazole ring attached to an ethylamine chain (Fig. 1). It is involved in may (patho-)physiological processes, like inflammation, allergic reactions, gastric acid secretion, and neurotransmission [1].

Histamine is also known as scombroid (or scombrotoxin) a foodborne poison found in marine fish. Levels of histamine rapidly increase by bacterial degradation in dead fish in case it hasn't been stored cold enough: too much intake results in food poisoning with symptoms very much like those of food allergy. For consumer safety, a histamine limit of 5 mg/100 g fish has been set by the FDA [2].

This note highlights the analysis of histamine based on automated in-needle OPA-sulfite pre-column derivatization using the system and method principles that are described in detail in reference [3]. The chromatograms were provided by the Univ. of Utrecht, where a study was conducted with RBL-2H3 cells (mast cell model) to study allergic reactions.

Method

RBL-2H3 cells (mast cell model) were used for a study at the University of Utrecht. The cells were exposed to DNP-BSA allergen and the release of histamine into the surrounding cell culture was measured. Samples were deproteinized with perchloric acid, followed by centrifugation. The supernatant was collected and the pH was adjusted to pH > 8 using a sodium hydroxide solution to assure efficient pre-

Fig. 1. ALEXYS Analyzer

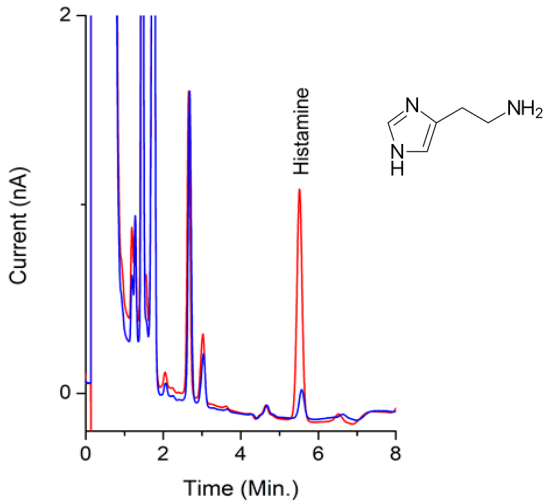

Fig. 2. Analysis of the histamine release after an allergen trigger. Chromatogram overlay from solution with RBL-2H3 (mast cell model) before exposure (blue trace) and after exposure to DNP-BSA allergen (red trace). Conditions according to Table 1. Chromatogram courtesy of Mrs. Gerdien Korte-Bouws, Department of Pharmaceutical Sciences, division of Pharmacology, University of Utrecht, The Netherlands.

Table 1. Recommended LC-ECD conditions

UHPLC	ALEXYS Analyzer
Column	Acquity UPLC HSS T3 1.0 x 50 mm, 1.8 µm (Waters)
Pre-column filter	Acquity in-line filter kit (Waters)
Mobile phase A (separation)	50 mmol/L phosphoric acid, 50 mmol/L citric acid and 0.1 mmol/L EDTA.Na_2, set to pH 6.0 with NaOH solution, 1% acetonitrile and 2% methanol
Mobile phase B (post-separation)	Mobile phase A, but with 50% acetonitrile
Flow rate	200 µL/mL: 100% A during separation, 12% A 88% B during 2-min post-separation flush
Temperature	40 °C for separation and detection
$V_{injection}$	1.5 µL
Injection method	Automated in-needle derivatization program using 10 µL sample and 1 µL reagent, followed by full loop fill
OPA-sulfite reagent	125 mM OPA, 125 mM sodium sulfite, 90 mM sodium borate buffer pH 10.4
Needle wash solution	20 % methanol in 0.1 M acetic acid
Flow cell*	SenCell™ with 2 mm GC working electrode and saltbridge reference electrode, AST setting 1
Ecell*	850 mV
Range	5 nA
ADF	0.5 Hz

*) The original work was done with a (now obsolete) 0.7 mm GC electrode vs ISAAC (Ecell 0.7 V/8 mmol/L chloride).

column derivatization with OPA. For use of this specific type of sample with high amounts of various (competing) amino acids, the strength of the derivatization reagent was increased relative to the original protocol [3], and the histamine standards were prepared in 0.01N NaOH. The samples were filtered over a 4 mm diameter 0.2 µm syringe filter before analysis.

The ALEXYS system is a versatile UHPLC-ECD platform that is dedicated to analyses that require sensitivity and small sample. The recommended LC-ECD settings and conditions for analysis of histamine are summarized in Table 1. The method showed a linear response (corr. coeff >0.999) in the range up to at least 2.5 µM histamine and a detection limit of about 10 nM. For identification purpose, other amino acids and related compounds were analyzed under the same LC conditions.

Results

Two chromatograms of histamine analysis in cell culture extracts are given in Fig. 2. The histamine response is clearly visible and showed an almost 8-fold increase in response after the allergen trigger. The chromatogram in Fig. 3 shows the elution order of other amino acids and related compounds using the same method.

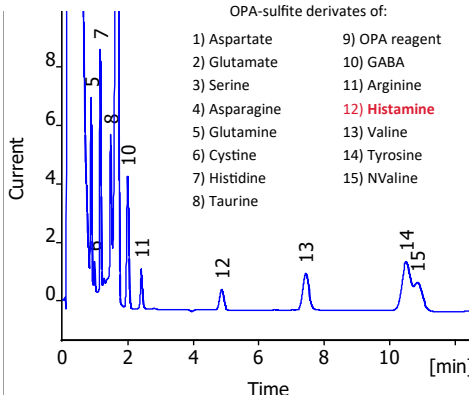

Fig. 3. Analysis of a standard mix of various amino acids and related compounds analyzed under the same conditions as the chromatograms shown in Fig. 1. Chromatogram courtesy of Mrs. Gerdien Korte-Bouws, Department of Pharmaceutical Sciences, division of Pharmacology, University of Utrecht, The Netherlands.

Ordering information

180.0091UW	ALEXYS Neurotransmitters SCC base
180.0504W	Add-on parts for (2-pump) HPG option
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). The Waters part numbers are given between parenthesis for reordering purposes.

Conclusion

This note demonstrates the sensitive analysis of histamine with the versatile ALEXYS Analyzer. The method, based on automated OPA/sulfite pre-column derivatization, offers a user -friendly and fast solution for the analysis of histamine and other amino acids.

References

1. Tiligada, Ekaterini, and Madeleine Ennis. "Histamine pharmacology: from Sir Henry Dale to the 21st century." British Journal of Pharmacology 177.3 (2020): 469-489.
2. U.S. Food and Drug Administration. CPG Sec. 540.525. Decomposition and histamine raw, frozen tuna and mahi-mahi; canned tuna; and related species. (2005) Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-540525-decomposition-and-histamine-raw-frozen-tuna-and-mahi-mahi-canned-tuna-and-related. Accessed 27 May 2020.
3. Antec Scientific, ALEXYS Neurotransmitter Analyzer for GABA and glutamate and other amino acids, Application note, 213.020

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