

Histamine

BioSystems

human - centred biotech

Food & Beverage analysis



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Analyte of interest

Histamine is a biogenic amine present in human body. It plays an important role in many physiological and pathological processes such as local responses of the immune system.

Apart from the endogenous production, histamine is introduced to the organism from exogenous sources by ingestion of some types of food, such as fish and fishery products, meat, cheese and fermented foods, where histamine can be present in a high concentration.



Importance of its analysis

Fresh fish contains amino acid L-histidine which can be converted into histamine by bacteria after fish being caught, and this can occur immediately due to inadequate storage conditions or improper handling of fish. Therefore, biogenic amines like histamine are considered as **indicators of fish spoilage**.

In regards to this matter, the interest and importance of analyzing histamine in fish relies on the risk on human health. Although, histamine is quickly inactivated in healthy individuals thanks to Diamine Oxidase (DAO) or histamine N-methyltransferase (HNMT) enzymes, even in them, **histamine intoxication** or **scombroid poisoning** can occur.

Moreover, some individuals have high hypersensitivity for histamine which is called **histamine intolerance**, due to a decrease or inhibition of DAO and HNMT activity, and can easily have several health problems when eating fish with high histamine content.



Related legislation

Histamine is the only biogenic amine with regulatory limits in fish set around the world. The analytical method specified by Food and Drug Administration (FDA) for determination of histamine levels in seafood is AOAC Official Method 977.13, a fluorometric analysis. However, in the European Union and some other countries high-performance liquid chromatography (HPLC) is the recommended method according to Regulation (EC) No. 2073/2005.

Food category	European Union ¹	USA ²
Fishery products from fish species associated with a high amount of histidine*	100 mg/Kg	50 mg/Kg
Fishery products which have undergone enzyme maturation treatment in brine, manufactured from fish species associated with a high amount of histidine*	200 mg/Kg	-

* Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae y Scombresosidae

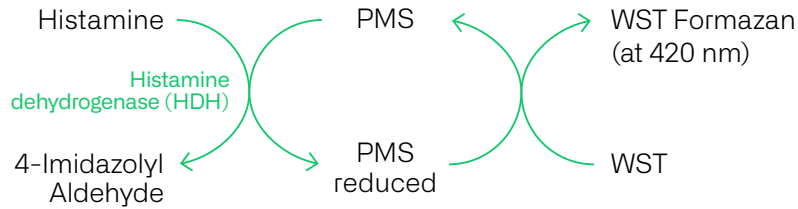
¹ Regulation (EC) No 2073/2005

² Fish and Fishery Products Hazards and Controls; June 2022 Edition (FDA)



Principle of the method

The enzyme histamine dehydrogenase (HDH) catalyzes the oxidation of histamine to 4-imidazolylaldehyde in the presence of 1-methoxy-5-methylphenazinium methyl sulfate (PMS) an electron mediator and a water soluble tetrazolium salt (WST). When WST is reduced, the corresponding formazan dye is formed and can be measured at 420 nm. The increase of absorbance is proportional to the histamine concentration.



Histamine kit for automated procedure, certified as AOAC Performance Tested MethodSM #072001.

Performance characteristics

Strengths:

- AOAC certification (Performance Tested Method No. 072001)
- Liquid reagents and calibrators included ready to use.
- Reagent stability up to 24 months at 2 – 8°C.
- Dedicated reagents, automated in BioSystems Y15 analyser (375 tests /kit).
- Validated in different matrices.
- Simple and validated extraction procedure without using harmful solvents.
- Final sample extract is stable for at least 1 day at 15-25°C, 7 days at 2-8°C or 3 months at -20°C.
- High Sensitivity.
- Ranges fit with current legislation.
- Multiparametric system with no competitor available.
- Other analysis in fish and crustacean with the same system available (sulfite, ascorbic acid, phosphate).
- More economic and easier to use system compared to current methodologies.
- Histamine Spike Solution (ref. 12891) to get internal controls.

Weaknesses:

- 20 minutes incubation in a boiling water bath.

Method	HDH/ PMS / WST
Analysis mode	Differential bireagent
Measurement interval	10 – 200 mg/Kg. For samples higher than 200 mg/kg an automatic predilution can be performed
Wavelength	420 nm
Stability	Until expiry date marked in the label at 2-8°C (manufactured with 24 months)
Precision	RSD, of <10%
Recovery	80 – 110%
Sample type	Raw, water-canned and oil-canned tuna, raw and oil-canned sardines, semi-preserved anchovy fillets and raw salmon.
Interferences	Agmatine interferes (7%). Other biogenic amines do not interfere.

Marketing – Market

Justification:

Histamine analysis is mandatory in order to guarantee food quality and safety. Current methods are manual, complex and generally require hazardous solvents for the extraction procedure. We present an innovative and automated method, a unique system in the market, that simplifies histamine quantification using liquid and ready to use reagents together with an automatic random access analyser.

Target market:

Fish and fishery industries.
Specialized laboratories in the fish sector.

Methodologies:

Different quantitative methods have been assessed for analyzing histamine, including HPLC, fluorometry, ELISA, colorimetric reagents and biosensors.

Price:

The main advantage of working with an automated system is the minimal use of reagents, resulting in a higher number of tests per kit. In this way price is optimized.

Marketing – Commercial Comparison

Histamine	BioSystems	Neogen Veratox	LDN	Prognosis
Commercial name	Y15 Histamine Enzymatic	Veratox for Histamine	HistaSure ELISA- Fast Track	Bio-Shield Histamine
Principle of the method	Histamine Dehydrogenase with ready-to-use liquid reagents automated in Y15 instrument or manually with Y350 spectrophotometer	ELISA. Free histamine in the samples and standards is allowed to compete with enzyme-labeled histamine (conjugate) for the antibody binding sites in the microtiter well.	ELISA. Acylated histamine and solid-phase bound histamine compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum-peroxidase complexes are removed by washing.	Competitive Quantitative ELISA test. The wells of microtiter strips are coated with HA specific antibodies
Wavelength	420 nm	650 nm	450 nm	450 nm
Price	Medium-Low	Medium-High	Medium	Medium
Shelf-life reagents	24 months	6 months	24 months	12 months
Number of tests/kit	375 (automatic)	48 wells	48 wells	48/96 wells
LOQ	10 mg/kg	2,5 mg/kg	1,27 mg/kg	2,5 mg/kg
Range of measurement	10-200 mg/mg for fresh fish, canned fish and salted fish samples	2,5-40 mg/kg fresh, canned, or pouched tuna, packed in either oil or water	1,27-300 mg/kg	2,5-200 mg/kg
AOAC validated matrices	Raw, oil and water-canned tuna; Raw and oil-canned sardines; Semi-preserved anchovy filets; Raw salmon	Fresh, canned, or pouched tuna, packed in either oil or water	Fresh, frozen yellowfin tuna, canned tuna-chunk light in water, fresh/frozen mahimahi, canned sardines in oil and fish meal	Fresh or thawed frozen raw fish, canned fish, fish meal
Use of organic solvent	No, water	No, water	No, water	No, water
User friendly	Easy	Medium	Medium	Medium
Extraction time	30'	12'	Fish samples: 7' Fishmeal: 12'	5'
Interferences	Agmatine 7%	L-anserine, L-carnosine, L-histidine <2.5 mg/kg apparent histamine	3-methylhistamine 0.44 %, tyramine 0.69 %, cadaverine 0.4 %	Not specified
Assay time	Y15: 1st result after 10', then after 48s (72 samples/hour)	20'	25'	20'
Portability	Y15: no; Y350: yes	Plate reader, non portable	Plate reader, non portable	Plate reader, non portable
Validation	Yes, 2020 (PTM#072001)	Yes, 2007 (PTM# 070703)	Yes, 2018 (PTM# 021402)	No, TÜV Nord validation

Marketing – Commercial Comparison

Histamine	BioSystems	Biolan	R-Biopharm	Bio Scientific	Prognosis RT	Fluorometry	HPLC
Commercial name	Y15 Histamine Enzymatic	BioFish 3000	Ridascreen Histamine Enzymatic	MaxSignal® Histamine Enzymatic Assay	Symmetric Histamine	-	-
Principle of the method	Histamine Dehydrogenase with ready-to-use liquid reagents automated in Y15 instrument or manually with Y350 spectrophotometer	Electrochemical reader together with disposable gold electrodes modified with a specific enzyme that oxidizes histamine molecules. The electric signal is measured by potentiometry.	Microtiter plate (coated with an electron carrier and a dye) and ready-to-use reagents, including histamine dehydrogenase	It measures the chemical reduction of a dye molecule using a histamine-specific enzyme. The color change is related to the amount of histamine in the sample	Immuno-chromatography assay where the target analyte, HA, binds to histamine specific antibodies conjugated to colloidal gold. Lateral flow in dip stick format.	Metanol extraction, use of ionic-exchange columns and fluorometric detection of derivatives	High Performance Liquid Chromatography
Wavelength	420 nm	-	450 nm	450 nm	-	-	-
Price	Medium-Low	Medium	Medium-High	Medium	Medium-Low	Medium	High
Shelf-life reagents	24 months	5 months	Medium	24 months	12 months	Non Applicable	Non Applicable
Number of tests/kit	375 (automatic)	Up to 100 tests (BT FHIS100)	96 wells	96 wells	30 test sticks	Manual method	Depends on column
LOQ	10 mg/kg	5 ppm	Fresh/canned fish: 2 mg/kg; Fresh fish with ascorbic acid: 3 mg/kg; Fish meal: 10 mg/kg	Fresh or frozen fish: 3 mg/kg; Fish meal: 30 mg/kg (fish meal not AOAC validated)	8 mg/kg	Aproximately 5-10 mg/kg	Approximately 5-10 mg/kg
Range of measurement	10-200 mg/mg for fresh fish, canned fish and salted fish samples	5-50 mg/kg raw fish, 10-100 mg/kg canned fish, 100-1000 mg/kg fish meal, 300-3000 mg/kg fish meal	Fresh-canned fish: 2-10 mg/kg; Fish with ascorbic acid: 2,62-131 mg/kg; Fish meal: 10-500 mg/kg	3-72 mg/kg	8-300 mg/kg	Depending on the instrument	Depending on the instrument
AOAC validated matrices	Raw, oil and water-canned tuna; Raw and oil-canned sardines; Semi-preserved anchovy filets; Raw salmon	Raw fish (tuna, sardine, anchovy, mackerel); canned and cooked fish (tuna), preserved, salted anchovy, fish meal	Fresh tuna, mackerel and herring Fresh and canned tuna in sunflower oil, yellowfin tuna and pilchard	Fresh/frozen tuna, canned tuna, pouched tuna, and frozen mahi mahi samples	No, AOAC Validation but validated in Fresh or thawed frozen: tuna, tuna related species, anchovy, bonito, bluefish, cod, mackerel, mahi mahi, marlin, sardines.	All kind of fish and fishery products	All kind of fish and fishery products
Use of organic solvent	No, water	No, water	No, water	Yes, specific reagent for fish samples and methanol for fish meal	No, water	Yes, methanol	Yes, TCA (Trichloroacetic acid). Also Perchloric acid (HClO4) can be used
User friendly	Easy	Medium	Medium	Medium	Easy	Complex	Complex
Extraction time	30'	2-15'	30'	15' seafood; 20' fish meal	5'	Aproximately 40' depending on the protocol	15'-1h depending on protocol
Interferences	Agmatine 7%	Agmatine	Agmatine: 0,75% at 10 g/kg; Ascorbic acid: more than 250 mg/kg in fish	Agmatine 4.1% Putrescine 0.9%	Not specified	No	No
Assay time	Y15: 1st result after 10', then after 48s (72 samples/hour)	2'	13'	5'	3'	Medium, it depends on fluorometer	15'-40' depending on protocol
Portability	Y15: no; Y350: yes	Portable, digital reader. It needs daily calibration	Plate reader, non portable	Plate reader, non portable	Yes, small reader	No	No
Validation	Yes, 2020 (PTM#072001)	Yes 2017 (PTM#051604), renewed for changes in the method in 2023	Yes, 2019 (PTM#031901)	Yes, 2019 (PTM#051701)	No	AOAC Official Method 977.13	Official in EU (Regulation (EC) No 2073/2005)

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