

Comparison of histamine quantification by HPLC and enzymatic method in fish

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Introduction

ISO 19343 (Detection and quantification of histamine in fish and fishery products-HPLC method) describes a method for quantification of histamine in fish and fish products using HPLC after perchloric acid extraction and dansyl chloride pre-column derivatization. Within the informative Annex B of this standard, results for tuna, herring and mackerel from a collaborative test (9 participants) are presented (table 1).

Since results for tuna are excellent, recoveries for mackerel were quite acceptable between 67 and 73 % while recoveries for herring were unacceptable low (27 to 44 %). These results were confirmed using an official German HPLC method (§ 64-LFVG, L10.00/5; see also Annex B of ISO 19343). We would like to present results using the RIDASCREEN® Histamine (enzymatic) test kit for quantification of histamine in mackerel and herring.

Table 1: Data from collaborative HPLC test cited in Annex B of ISO 19343 regarding histamine measurement in tuna, herring and mackerel.

	Spiked histamine concentration		
	25 mg/kg	100 mg/kg	220 mg/kg
Tuna			
Recovery (%)	96	93	91
RSD _R (%)	31	12	9
Herring			
Recovery (%)	44	27	41
RSD _R (%)	28	4	9
Mackerel			
Recovery (%)	73	70	67
RSD _R (%)	15	9	10

Material & methods

Homogenized samples from fresh herring and mackerel were spiked at several levels and incubated for one hour at room temperature before extracted with boiling water. The enzymatic reaction converts

histamine to imidoacetaldehyde. With a dye a colored product is measured at 450 nm. The linear range is from 0.4 mg/l up to 20 mg/l histamine corresponding to 2 mg/kg up to 100 mg/kg in a fish sample.

Picture 1: RIDASCREEN® Histamin (enzymatic, Art. No. R1605)



Results & discussion

Recoveries for mackerel ranged from 75 -93 % for spiked histamine concentration between 3.0 mg/kg and 60 mg/kg (table 2). It should be noted that the mackerel sample showed a background contamination of about 4.35 mg/kg histamine which was subtracted

from the values obtained for spiked samples. Therefore, it goes without saying that the samples spiked at 3.0 mg/kg showed a somewhat higher CV of about 11 %. Without subtracting the mean blank value the CV is at 3.7 % (not shown).

Table 2: Spiking experiment (precision profile) of fresh mackerel with a background contamination of 4.35 mg/kg spiked with histamine levels between 3 mg/kg and 60.4 mg/kg with 6 replicates per level.

Rep.	Fresh mackerel [mg/kg], blank corrected											
	0 (blank)	3.0 mg/kg	Rec. %	15.1 mg/kg	Rec. %	30.2 mg/kg	Rec. %	45.3 mg/kg	Rec. %	60.4 mg/kg	Rec. %	
1	4.78	2.06	68	14.3	95	25.3	84	39.7	88	55.2	91	
2	4.30	2.28	75	14.4	96	25.9	86	41.2	91	54.6	90	
3	3.61	2.01	67	14.0	92	25.2	83	41.0	90	54.2	90	
4	4.35	2.70	89	14.0	93	25.6	85	39.5	87	55.7	92	
5	4.78	2.22	74	13.8	91	25.9	86	39.5	87	55.5	92	
6	4.30	2.28	75	13.5	90	26.0	86	39.8	88	54.8	91	
Mean	4.35	2.26	75	14.0	93	25.6	85	40.1	89	55.0	91	
SD	0.43	0.24		0.32		0.34		0.78		0.56		
CV (%)	9.9	10.8		2.3		1.3		1.9		1.0		

In case of herring, recoveries ranged from 85 - 92 % using the same spike levels as in ISO 19343 (25, 100, and 220 mg/kg). Precision of extraction is in both cases at or

below 3 % for concentrations higher than 15 mg/kg. The blank herring sample showed values below LoD.

Table 3: Spiking experiment of fresh herring with histamine levels of 25, 100 and 200 mg/kg; 5 replicates per level.

Rep.	Fresh herring [mg/kg], not blank corrected					
	25 mg/kg	Rec. %	100 mg/kg	Rec. %	220 mg/kg	Rec. %
1	21.6	86	87.1	87	191.5	87
2	21.3	85	85.2	85	201.3	92
3	21.1	84	90.4	90	208.4	95
4	21.6	86	89.2	89	206.4	94
5	21.0	84	86.7	87	202.6	92
Mean	21.3	85	87.7	88	202.0	92
SD	0.3		2.0		6.5	
CV (%)	1.3		2.3		3.2	

This clearly demonstrates that the enzymatic determination is as good as or even better than the HPLC method described in

ISO 19343. Results have a higher precision, the assay is easier to use and contains no toxic reagents. Overall, it is faster than any HPLC.

References
ISO 19343, Microbiology of the food chain – Detection and quantification of histamine in fish and fishery products – HPLC method (under publication)

