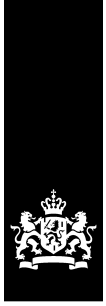

Food and Consumer Product Safety Authority
Ministry of Economic Affairs, Agriculture and Innovation

CHEK Proficiency study 467

Histamine in mackerel

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1. Introduction

Proficiency study number 467 as directed by the CHEK working group concerned the investigation of the histamine content of two mackerel samples. To get an impression of the performance of the quantitative determination of histamine in mackerel 20 laboratories were invited to join this proficiency study.

2. Time-table

Distribution of samples	31 January 2011
Deadline for the production of results	4 March 2011
Final report	17 March 2011

3. Sample preparation

Mackerel samples A and B uniform level
9.84 kg of smoked mackerel was first diminished and minced for 30 minutes using a Stephan mixing/mincer machine. 1.57 g histamine.2HCl (dissolved in 150 mL water) was added and minced for 25 minutes using a planet mincer, yielding 95 mg/kg histamine. The bulk sample was divided in approximately 50 sub-samples of about 100 g, coded A and B and prepared and packed in sterilised plastic containers.

The heterogeneity was checked by the Food and Consumer Product Safety Authority, Region East by randomly selecting samples for the determination of the histamine content (see table II). The average histamine content was 97 mg/kg. Random chosen values for samples A and B from table II were added to the list of results by the organiser of this study.

Samples were stored in a freezer until shipment. To each of the participants two samples coded A and B were sent. Participants were instructed to thaw the samples and mix them to a homogeneous sample before investigation.

4. Statistical results

The results of the laboratories and the Z-scores are given in table I. Figures I to III give graphical presentations of the results. One-way analysis of variance (ANOVA), preceded by checking for normality and outlier checking of the results (Cochran/Grubbs) is the statistical procedure for obtaining the estimates of within-laboratory and between laboratory variability.

The performance of a determination is assessed as follows:

satisfactory = 0-2 x maximum allowable RSD_R
questionable = >2-3 x maximum allowable RSD_R
unsatisfactory= >3 x maximum allowable RSD_R

The value of a certain parameter, resulting from a single analysis, above which a sample is regarded as rejectable with a probability of 95%, is expressed as x_{max}.

4.1. Histamine in mackerel uniform level

4.1.1. Histamine in mackerel samples A and B uniform level

20 labs: Results of all laboratories.
19 labs: Results of laboratory 11 are rejected by the Cochran outlier test.

	20 labs	**19 labs**	**Unit**
Average	95	95	mg/kg
Repeatability standard deviation (s_r')	3.2	2.1	mg/kg
Repeatability rel. standard deviation (RSD_r')	3.4	2.2	%
Reproducibility standard deviation (s_R')	10.6	10.6	mg/kg
Reproducibility rel. standard deviation (RSD_R')	11.1	11.2	%
Horwitz acceptable value for (RSDR')	8.1	8.1	%
Repeatability r'	9.1	5.9	mg/kg
Reproducibility R'	29.6	29.7	mg/kg
x_{max} value at level 100 mg/kg		118	mg/kg

4.1.2. Summary histamine in mackerel uniform level

The results of this proficiency study and earlier performed studies are summarised in the table below.

Prof. study	**Number of labs**	**Average A-B mg/kg**	**RSD_R %**	**Horwitz RSD_R %**	**x_{max}/standard mg/kg**
467	19	95	11.2	8.1	118/100
444	23	128	8.7	7.7	114/100
418	26	123.0	9.3	7.8	115/100
391	16	95.3	7.3	8.1	112/100

4.1.3. Conclusions histamine in mackerel samples A and B uniform level

- The performance of the determination of histamine in mackerel is **satisfactory** (see 4).
- The results for sample A and B are drawn from a normal distribution.
- The results of laboratory 11 are marked as outliers by the Cochran test indicating bad repeatability and are rejected.
- No Grubbs outliers are found in the dataset of sample A and B.

5. Method of analysis

Lab 1 Extraction of a test portion with 0.4 M perchloric acid solution after addition of 1,8-diamino-octane as internal standard. Injection of derivatives in a HPLC system using UV detection.
HPLC conditions:
Column : 250 x 4.6 mm Shiseido C18, 5 μm.
Mobile phase : acetonitrile/15 mM phosphate buffer (75:25).
Detection : UV 254 nm.

Lab 2 Extraction of a test portion with 0.6 M perchloric acid solution. Filtration and injection of derivatives in a HPLC system using post-column derivatisation with OPA and fluorescence detection.
HPLC conditions:
Column : 250 x 4.6 mm C18, 5 μm.
Mobile phase A : acetic acid/sodium acetate/sodium heptane sulphonate, pH 5.2.
Mobile phase B : acetic acid/sodium acetate/sodium heptane sulphonate, pH 4.5.
Mobile phase C : acetonitrile.
Gradient : t_0 80% A - 13.2% B - 6.8% C.
t 45% A - 36.3% B - 18.7% C.
Detection : fluorimetric, excitation: 359 nm & emission: 445 nm.

Lab 3 Extraction of a test portion with 6% perchloric acid solution. Filtration and injection of derivatives in a HPLC system using post-column derivatisation with OPA and fluorescence detection.
HPLC conditions:
Column : 250 x 4.6 mm Inertsil ODS-2, 5 μm.
Mobile phase : 0.01 M phosphate buffer pH 3/acetonitrile (875:125).
Detection : fluorimetric, excitation: 340 nm & emission: 455 nm.

Lab 4 Extraction of a test portion with 5% trichloroacetic acid solution. Injection of derivatives in a HPLC system using fluorescence detection.
HPLC conditions:
Column : 3.0 x 50 mm X-Terra MS C18, 3.5 μm.
Mobile phase A : 0.1 M sodium acetate buffer.
Mobile phase B : 0.2 M sodium acetate buffer.
Mobile phase C : methanol.
Gradient : t_0 90% A - 10% B - 0% C.
t_8 65% A - 35% B - 0% C.
t_{12} 24% A - 50% B - 26% C.
t_{13} 90% A - 10% B - 0% C.
Detection : fluorimetric, excitation: 338 nm & emission: 448 nm.

Lab 5 Extraction of a test portion with 0.6 M perchloric acid solution after addition of diaminohexane as internal standard. Injection of derivatives in a HPLC system using fluorescence detection.
HPLC conditions:
Column : 125 x 3 mm Nucleodur 100-3 C18 with guard column.
Mobile phase A : solution + 8.03 g sodium acetate and 2.16 g octane sulphonic acid sodium salt.
Mobile phase B : solution + 12.7 g sodium acetate and 2.16 g octane sulphonic acid sodium salt.
Gradient : t_0 65% A - 35% B.
t_{17} 55% A - 45% B.
Detection : fluorimetric, excitation: 366 nm & emission: 450 nm.

Lab 6 Derivatisation of an aliquot of the test portion with OPA and spectrofluorphotometrically determination of the histamine content.

Lab 7 Clean up of an aliquot of the test portion using a cation exchange column (Amberlite), derivatisation with OPA and spectrofluorphotometrically determination of the histamine content (emission 450 nm).

Lab 8 Extraction of a test portion with perchloric acid solution. Filtration and injection of derivatives in a HPLC system using post-column derivatisation with OPA and fluorescence detection.
HPLC conditions:
Column : Thermo Hypurity C18.
Mobile phase A : 0.1 M acetic acid/0.01 M sodium 1-heptane sulphonate, pH 5.2.
Mobile phase B : 0.12 M acetic acid/0.01 M sodium 1-heptane sulphonate, pH 4.5/acetonitrile, pH 4.5.
Gradient : t_{30} 65% A - 35% B.
t_{31} 45% A - 55% B, hold 3 min.
t_{35} 85% A - 15% B.
Detection : fluorimetric, excitation: 340 nm & emission: 445 nm.

Lab 9 Extraction of a test portion with 0.6 M perchloric acid solution. Injection of derivatives in a HPLC system using post-column derivatisation with OPA and fluorescence detection.
HPLC conditions:
Column : Hypersil ODS2 with guard column.
Mobile phase : methanol/octane sulphonic acid/acetic acid, pH 4.5.
Detection : fluorimetric, excitation: 365 nm & emission: 418 nm.

Lab 10 Derivatisation of an aliquot of the test portion with dansylchloride. Injection of derivatives in a HPLC system using UV detection.
HPLC conditions:
Column : 250 x 4 mm ODS, 5 μm.
Mobile phase A : phosphate buffer/acetonitrile (10:1).
Mobile phase B : phosphate buffer/acetonitrile (20:13).
Gradient : t_0 85% A - 15% B.
$t_{9.5}$ 5% A - 95% B, hold 20.5 min.
t_{31} 85% A - 15% B.
Detection : UV 254 nm.

Lab 11 Extraction of a test portion with water. Derivatisation of an aliquot of the extract with n-hydroxysuccinimidobiotin followed by enzyme immunoassay (EIA).
EIA conditions:
Microtiter plate : coated with n-acylhistamine.
Assay principle : competitive.
Detection enzyme : horseradish peroxidase.
Detection : 450 nm.

Lab 12 Extraction of a test portion with toluene after addition of diaminopropane as internal standard. Injection of derivatives in a HPLC system using diode array detection.
HPLC conditions:
Column : 4.6 mm C18, 5 μm with guard column.
Mobile phase A : water.
Mobile phase B : acetonitrile.
Gradient : t_0 40% A - 60% B.
t_6 25% A - 75% B, hold 7 min.
t_{20} 5% A - 95% B.
$t_{20.01}$ 40% A - 60% B, hold 10 min.

Lab 13 Extraction of a test portion with 10% trichloroacetic acid solution. Filtration and injection of derivatives in a HPLC system using post-column derivatisation with OPA and fluorescence detection.
HPLC conditions:
Column : 150 x 3.9 mm Novapak C18, 4 μm with guard column.
Mobile phase A : 0.1 M sodium acetate, pH 4.5.
Mobile phase B : 0.2 M sodium acetate/acetonitrile (10:3), pH 4.5.
Mobile phase C : methanol.
Gradient : t_1 75% A - 25% B - 0% C.
t_{20} 35% A - 65% B - 0% C.
t_{25} 0% A - 40% B - 60% C.
t_{30} 75% A - 25% B - 0% C, hold 5 min.
Detection : fluorimetric, excitation: 365 nm & emission: 418 nm.

Lab 14 Extraction of a test portion with 0.4 M perchloric acid solution. Injection of derivatives in a HPLC system using diode array detection.
HPLC conditions:
Column : 125 x 4 mm Lichrospher 60RP-select B, 5 μm with guard column.
Mobile phase A : 0.1 M ammonium acetate buffer.
Mobile phase B : acetonitrile.
Gradient : t_0 60% A - 40% B.
t_{10} 25% A - 75% B.
t_{12} 10% A - 90% B.
t_{16} 60% A - 40% B, hold 3 min.
Detection : diode array 254 nm.

Lab 15 Extraction of a test portion with perchloric acid solution. Addition of biguanide as internal standard. Injection of derivatives in a HPLC system using UV detection.
HPLC conditions:
Column : Luna RP C18 with guard column.
Mobile phase A : acetonitrile.
Mobile phase B : phosphate buffer/methanol.
Detection : diode array 214 nm.

Lab 16 Spectrofluorphotometrically determination of the histamine content.

Lab 17 Extraction of a test portion with perchloric acid solution Filtration and injection of derivatives in a HPLC system using post-column derivatisation with OPA and fluorescence detection.
HPLC conditions:
Column : 250 x 4 mm Hypersil ODS C18.
Mobile phase A : 0.1 M sodium acetate/10 mM heptane sulphonic acid, pH 5.2.
Mobile phase B : acetonitrile/0.1 M sodium acetate + 10 mM heptane sulphonic acid (34:66), pH 4.5.
Gradient : t_0 82% A - 18% B.
Detection : fluorimetric, excitation: 340 nm & emission: 445 nm.

Lab 18 Extraction of a test portion with 5% trichloroacetic acid solution after addition of 1,7-diaminoheptane as internal standard. Filtration and derivatisation of an aliquot of the filtrate with dansylchloride. Injection of derivatives in a HPLC system using diode array detection.
HPLC conditions:
Column : 250 x 4.6 mm Lichrospher with guard column.
Mobile phase A : 0.02 M acetic acid/methanol/THF (90:6:4).
Mobile phase B : 0.02 M acetic acid/methanol/THF (10:70:20).
Gradient : t_0 40% A - 60% B, hold 5 min.
t_5 26% A - 74% B, hold 9 min.
t_{14} 0% A - 100% B, hold 6 min.
Detection : diode array 252 and 344 nm.

Lab 19 Derivatisation of an aliquot of the test portion with dansylchloride after addition of diaminoheptane as internal standard. Injection of derivatives in a HPLC system using diode array detection.
HPLC conditions:
Column : C18.
Mobile phase : ammonium acetate buffer/acetonitrile.
Detection : diode array 254 nm.

Lab 20 Extraction an test portion with methanol, purification on ion exchange resin and spectrofluorphotometrically determination of the histamine content.

6. Remarks from participants

Lab 13 Results were adjusted for 99.71% recovery.

Lab 20 Method of analysis is in accordance with AOAC method 977.13. Procedure is accredited since 2004.

7. List of invited participants

Austria
Austrian Agency for Health and Food Safety - ILMU Wien, Vienna.

Belgium
Scientific Institute of Public Health - Toxic and Pharmacological Residues, Brussels.
LFSAL, Liege.

Czech, Republic
Czech Agricultural and Food Inspection Authority, Prague.

Denmark
Fødevareregion Øst, Ringsted.

France
ISHA, Longjumeau.
SGS Multilab, Saint Etienne du Rouvray.

French Polynesia
Institut Louis Malarde – LASEA, Papeete-Tahiti.

Germany
Chemisches Untersuchungsamt der Stadt Hagen.
Landesbetrieb Hessisches Landeslabor, Gießen.
Institut für Hygiene und Umwelt, Hamburg.
Landeslabor Schleswig-Holstein, Neumünster.
Labor Diagnostika Nord GmbH & Co KG, Nordhorn.

Greece
General Chemical State Laboratory - Division of Pireas.

Ireland
Public Analyst's Laboratory, Cork.
Public Analyst's Laboratory, Galway.
Public Analyst's Laboratory, Dublin.

The Netherlands
The Food and Consumer Product Safety Authority, Zutphen.

Norway
Trondheim commune, Trondheim.

Spain
Centro Analítico de Inspección y Control de Calidad de Comercio Exterior, Madrid.

8. Explanation of graphical presentations

Z-score
As a criterion for evaluation of the performance of an individual laboratory a so-called Z-score is used. The Z-score is given by the following equation:

$$Z = \frac{x - \mu}{s}$$

Where:
x = an (average) laboratory result.
μ = the average result of all laboratories (calculated exclusive outliers).
s = an assigned precision standard or fixed target value.

As a fixed target value the maximum allowable standard deviation is used at a given concentration-level (according to Horwitz). The Z-score for an individual laboratory can be compared with those of previous proficiency studies to determine whether the laboratory performance has improved. Because Z is standardised, it is comparable for all analytes, testmaterials and analytical methods.

In general, an absolute value of Z greater than three suggests poor performance in terms of accuracy. When overall performance in a specific interlaboratory test is graded as "good", values of $|Z| < 1$ would be very common and values of $|Z| > 3$ would be very rare. It is possible to classify these scores:

Satisfactory	=	$1 > \|Z\| \leq 2$
Questionable	=	$2 < \|Z\| < 3$
Unsatisfactory	=	$\|Z\| \geq 3$

Saw-tooth plot
The results of the samples are presented in a so-called saw-tooth plot. In this figure individual results of two (nearly) identical individual results are plotted. The average of the sample, the 2s- and 3s-intervals of the target values and the 2s- and 3s-intervals after removal of outliers are also included (group s). For split-level samples 2s- and 3s-intervals are presented for each sample.

Youden-plot
Based on Youden statistics the calculated variance is split in a variance caused by systematic and random errors of the individual laboratories. It is necessary that the samples are similar. Generally the points form an elliptical pattern with the major axis of the ellipse running diagonally at an angle of 45° to the X-axis. The lengths of the perpendiculars drawn from the points to the 45° line are directly related to the random errors. Systematic errors will be presented along the 45° line. The perpendiculars intersect the 45° line at various distances from the point through which the 45° line was drawn. These distances are directly related to the systematic errors.

9. Tables and graphical presentations

Table I; Proficiency study 467, Histamine in mackerel [mg/kg]

Lab		Sample A	Sample B	Z-score	Samples/ year
1		81	80	-1.91	10
2		101	100	0.70	50
3		98	100	0.50	-
4		86	87	-1.13	60
5		81	79	-1.98	1300
6		108	105	1.48	330
7		100	99	0.57	250
8		110	110	1.94	200
9		82	83	-1.65	50-100
10		116	113	2.52	0
11	**(1)**	**109**	**93**	0.76	-
12		99	103	0.76	0
13		97	92	-0.09	150
14		96	95	0.04	100
15		98	101	0.57	600
16		83	80	-1.78	10
17		100	93	0.17	100
18		101	102	0.83	50
19		96	101	0.44	100
20		80	80	-1.98	1

(1) = Cochran outlier (2) = Grubbs outlier (3) = Results removed by hand

Calculations of the Z-scores is based on the replicates of the sample (95 mg/kg) and the assigned standard deviation (7.7 mg/kg) calculated from the Horwitz equation.

Table II; Heterogeneity of samples A and B for histamine in mackerel [mg/kg]

Sample no	1st analysis	2nd analysis
1	98	98
2	99	96
3	95	91
4	96	96
5	96	96
6	96	99
7	98	98
8	100	97
9	98	95
10	96	96
average	97	96
t-test		
$t_{samples}$	1.46	
$t_{critical.95\%}$	2.26	
Heterogeneity		
S_s	1.1	
S_a	1.6	
σ	7.8	
S_s/σ	0.15	

Based on the t-test there is no significant difference between the averages of 1st and the 2nd analysis for histamine samples A and B. The ratio of the sampling standard deviation and the target value σ is lower then the recommended value of 0.3. Samples A and B are regarded as sufficient homogeneous for the purpose of the proficiency study.

S_a = analytical standard deviation.
S_s = sampling standard deviation.
σ = target value calculated from the Horwitz equation or assigned precision standard.

Graphical presentations histamine in mackerel

Figure Ia; Saw-tooth plot histamine in mackerel, calculated with target s.

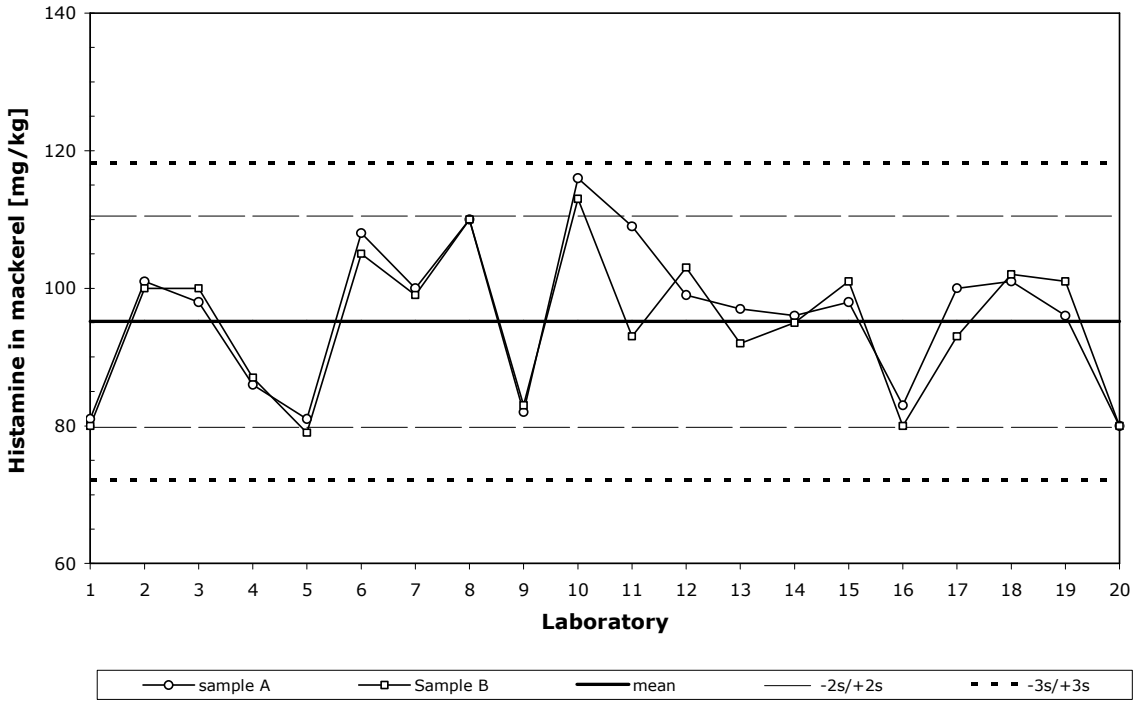

Figure Ib; Saw-tooth plot histamine in mackerel, calculated with group s (exclusive laboratory 11.

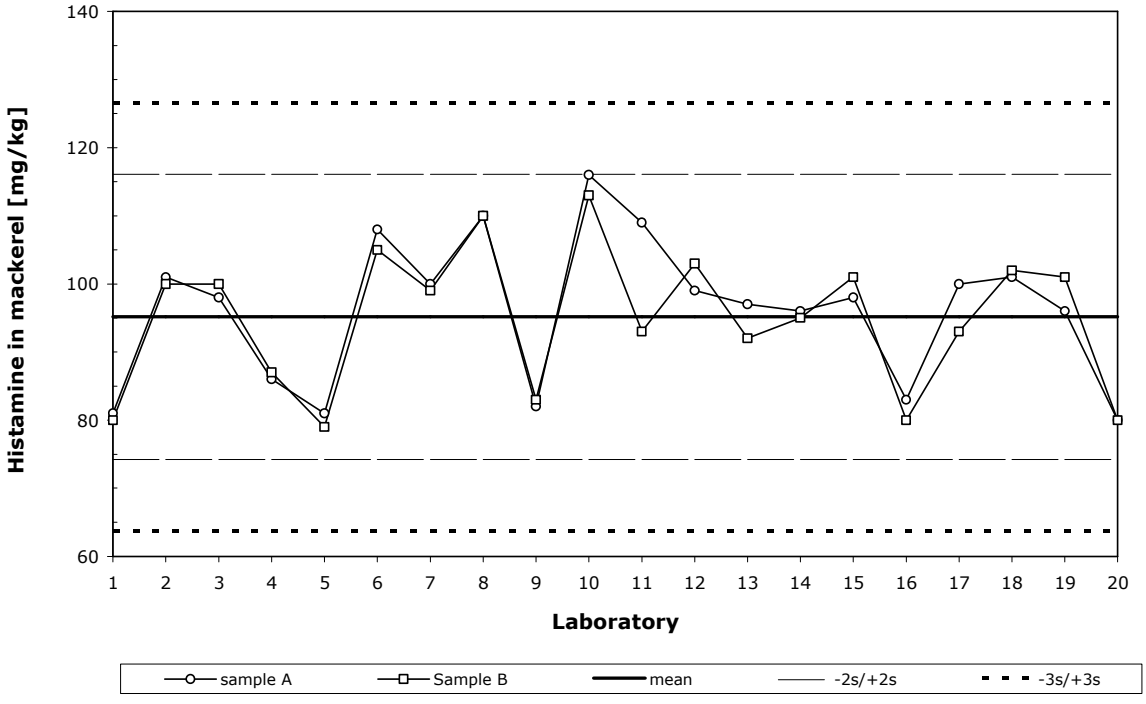

Figure II; Youden plot histamine in mackerel.

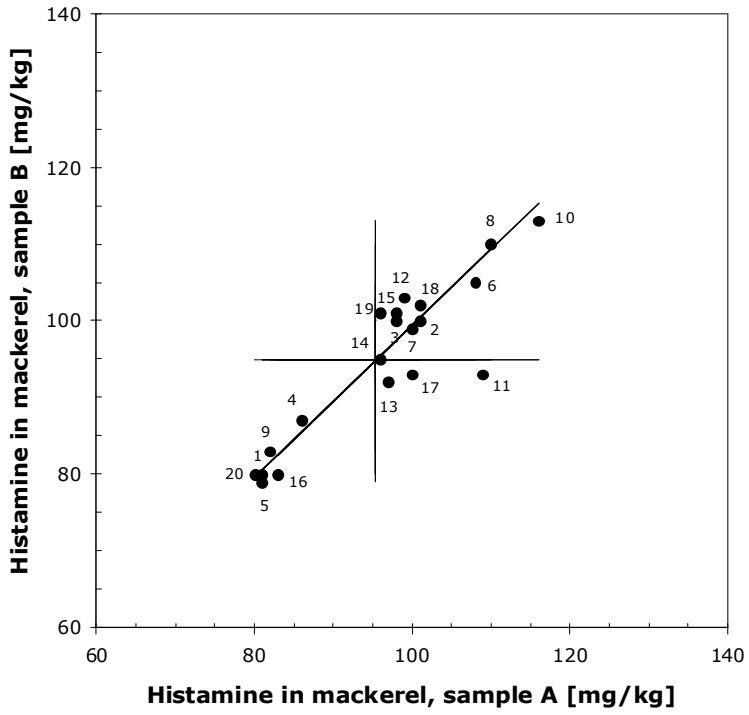

Figure III; Z-score histamine in mackerel.