A Rapid, Easily Used Test Kit To Determine Histamine Concentrations in Fish

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BACKGROUND

Recently the U.S. Food and Drug Administration announced it was improving its histamine policy in the revised Compliance Policy Guide 7108.24 Decomposition and Histamine "Raw, Frozen Tuna and Mahi-Mahi; Canned Tuna; and Related Species" (Fed. Reg. v. 60/149, August 3, 1995; pp. 39754-39756). In summary the FDA:

> lowered the Defect Action Level (DAL) to 50 ppm for decomposition.
>
> eliminated the requirement that findings of < 200 ppm had to be confirmed by organoleptic tests [i.e., histamine determination alone is sufficient].
>
> application of the revised DAL applies to raw and frozen tuna and mahi-mahi; and furthermore, on a case-by-case basis histamine levels ≥ 50 ppm to < 500 ppm may be used as evidence of decomposition in other species.
>
> the Action Level (AL) of 500 ppm will apply to species of fish that have been implicated in histamine poisoning outbreaks.

The FDA notice calls our attention to the fact that "...nonvolatile spoilage compounds such as histamine remain in the product (the fish) and can be determined reliably by chemical analysis." The most commonly used method for histamine determination is AOAC Official Method 977.13--the fluorometric method, which received Final Action approval in 1987. This method has three phases:

> First - extraction of histamine from it matrix (fish). This phase consists of a methanol extraction of histamine from (blended) fish followed by heating (60°C) and filtrations (Fig. 1).
>
> Second - purification of the histamine from the "extract." This phase involves ion exchange chromatography whereby histamine passes through the column but interferences, such as histidine and other free amino acids are retained.

Third - detection of histamine in the column effluent. This phase involves adjusting the sample to alkaline pH, reaction with OPT (o-phthalic dicarboxaldehyde a.k.a. OPA), sample neutralization followed by fluorescent detection (λ ex = 350 nm and λ em = 444 nm) (Fig. 2).

Quantitative histamine concentrations are determined by comparing sample fluorescence values to a standard curve generated daily or more often. Standards are prepared in a fashion that they are subjected only to the detection phase of the method.

THE NEW "OLD" ASSAY

Our assay utilizes an AOAC approved chemical method (spectrophotometric) in a new, user-friendly format. The first phase, the extraction procedure is identical to the fluorometric AOAC method.

The second phase uses an anionic exchange resin to remove interfering substances only in a batch-wise fashion by means of a simple filtration device. The filtrate is pH adjusted by means of dilution and a 100 μL aliquot is added to a detector cup. Then, captured histamine is reacted with diazotized p-nitroaniline (previously activated by reacting sodium nitrite in a crushable ampoule within a tube of p-nitroaniline). The reflectance value of the reaction product (diazo dye), which is reddish colored, is measured in a simple reflectometer, the AgriMeter (Fig. 3, 4).

Table 1 shows the reflectance ranges for important levels of histamine including the Defect Action Level of 50 ppm.

Figure 5 shows a standard curve with error bars derived from values like those in Table 1.

Figure 6 is a "scattergram" comparing the fluorometric AOAC method values for 85 samples of canned and fresh frozen tuna to the ranges of the Alert™ for Histamine test with these same samples. Please note the single high sample (ca. 53 ppm) in the > 5 < 19 range. Upon examination, that particular sample was found to have a high salt content.

Figure 7 shows the effects of salt on the Alert assay; i.e. at > 2.0 % (w/w) salt a standard curve is changed markedly. However, canned tuna should have ≤ 1.0% salt, a specification that is determined separately in Good Manufacturing Practices (QA) procedures.

Table 2 lists histamine levels in nine samples of fresh/frozen mahi-mahi as determined by the AOAC fluorometric method and the Alert for Histamine test both of which used standard curves. The classification column was the conclusion drawn by the technologist conducting the test. It should be noted that extractions with 75% methanol were used.

ACKNOWLEDGMENTS

We acknowledge with thanks the interests of Drs. Walter Staruszkiewicz, Patricia Rogers and George Hoskin of the FDA's Office of Seafood. In addition, we would like to acknowledge the support of the tuna canning industry which supplied samples of fresh/frozen and canned tuna for the evaluation of the Alert for Histamine test.

Fig. 1

AOAC Method of Extraction

Homogenize Sample (AOAC 937.07)

↓

Extract 10 g in
50 ml 100% MeOH in
Blender 2 min.

↓

Heat Sample to 60^0C
in H_2O Bath, Incubate
15 Min.

↓

Cool to 25^0 and
Dilute to 100 ml

↓

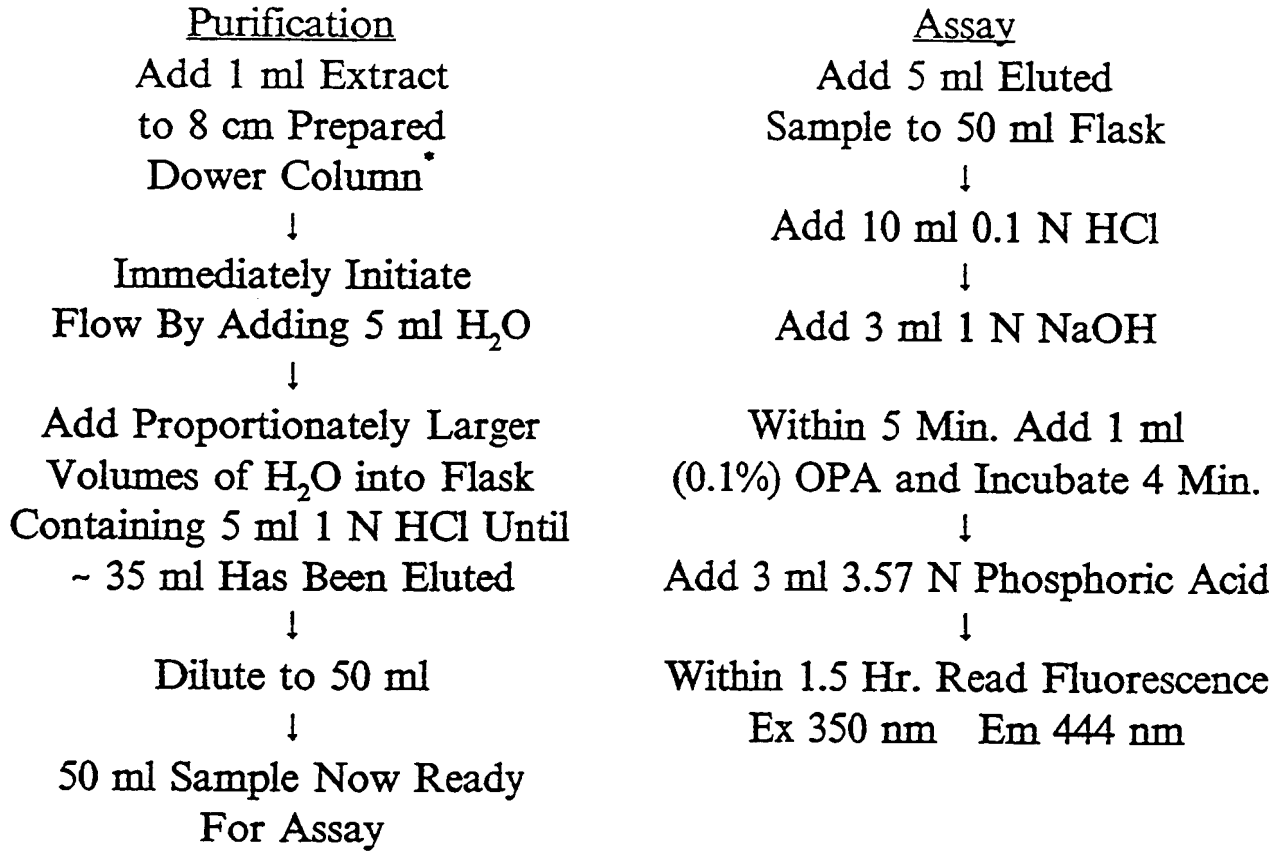
Filter Through Folded
Filter Paper

↓

Sample Now Ready
For Purification

Fig. 2

AOAC Fluorometric Method



*Prepare columns before test by (preparing weekly):
1. treating with 1N NaOH for approximately 1 hour (15 ml/g resin);
2. washing extensively;
3. transferring resin to columns 8 cm in height;
4. washing column with 10 ml of H_2O prior to purification.

Fig. 3

ALERT® HISTAMINE ASSAY

<u>Purification and Dilution</u>

↓

Add 1 mL Filtered Extract to 0.5 g Dowex AG 1x8-OH, Mix ~ 15 Sec. 100-200 Mesh

↓

Using Serum Separator Push Extract Through Filter Separating From Dowex

↓

Transfer 0.5 mL Eluate to Clean Test Tube

↓

Add 0.20 mL Neutralization Buffer (0.05 M Phosphate 6.5 pH)

↓

Sample Now Ready For Testing

<u>Assay</u>

Add 0.1 mL Diluted Sample to Detector

↓

Squeeze on 2 Drops of Wash Buffer (0.05 M Phosphate 6.5 pH)

↓

Squeeze on 2 Drops of Recently Prepared Diazo Reagent[1]

↓

Squeeze on 1 Drop of 10% Carbonate 3.5% Borate Buffer

↓

Read Color with AgriMeter™

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[1]The diazo reagent is made by reacting p-nitroaniline (0.1 g/100 mL 0.1 N HCl) with $NaNo_2$ (4 g/100 mL H_2O). This is done in a dropper bottle by crushing a glass ampoule containing the $NaNO_2$; allowing it to mix with the solution of p-nitroaniline. This activated reagent is immediately ready for use and is stable for up to 8 hours.

Fig. 4

HISTAMINE CAPTURE AND DETECTION

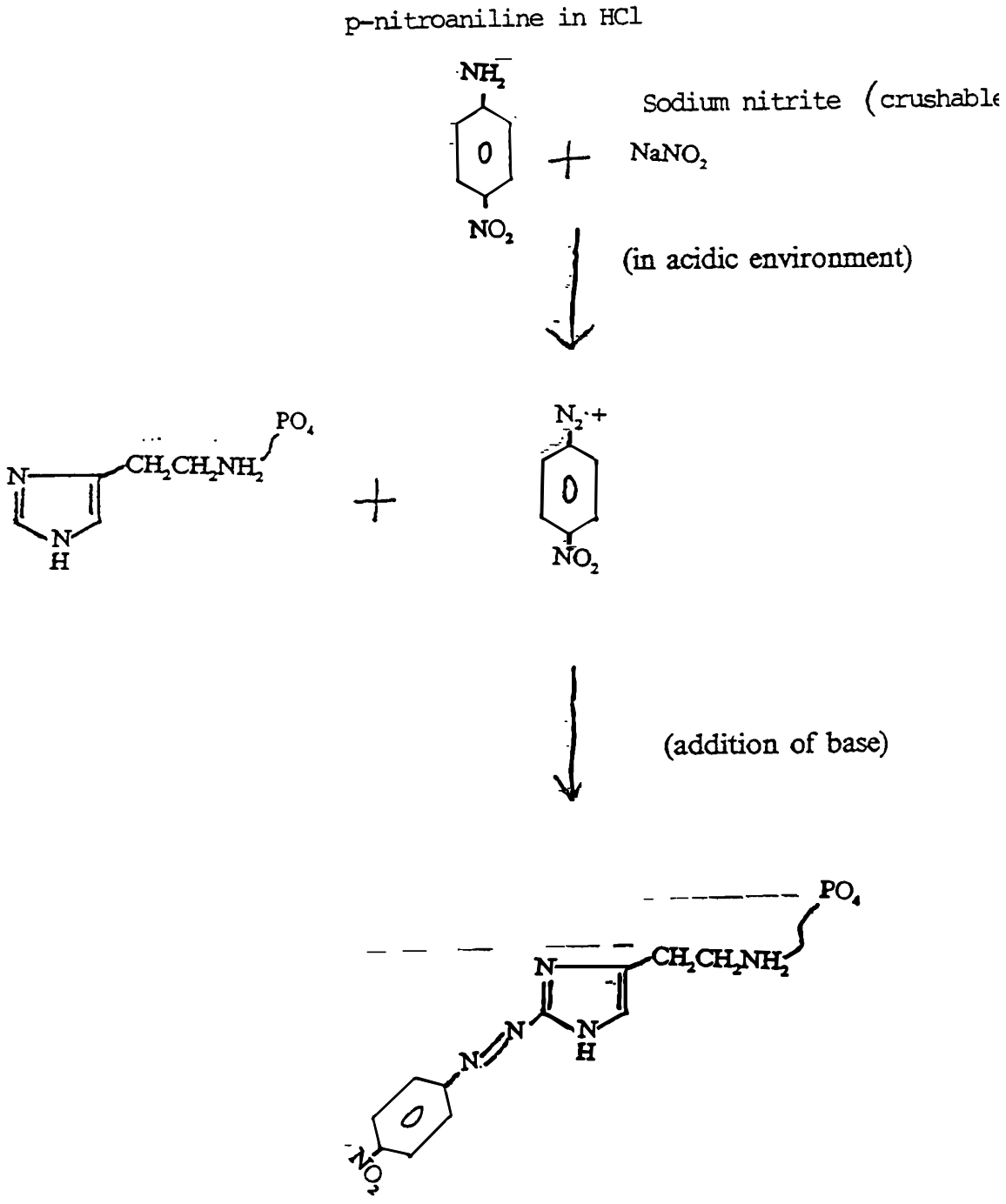


Table 1

Critical Control Levels of the Alert® for Histamine Test

Histamine Level (μg/mL)	Reflectance (Arbitrary Units) X ± 2 S.D.[1]	Range
0	515 ± 40	475 - 555
5	465 ± 40	425 - 505
20	365 ± 20	345 - 385
50	225 ± 35	190 - 260

[1]Critical control levels were established using 10 runs/meter and 3 meters (n = 30).

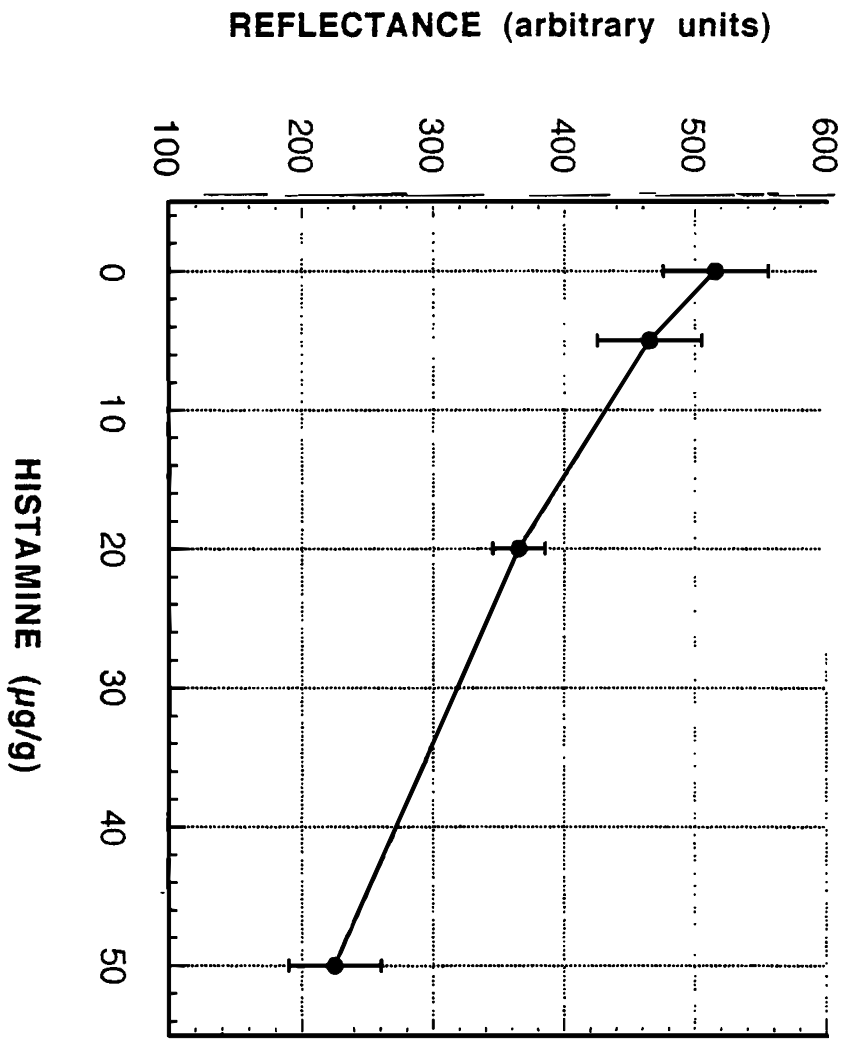
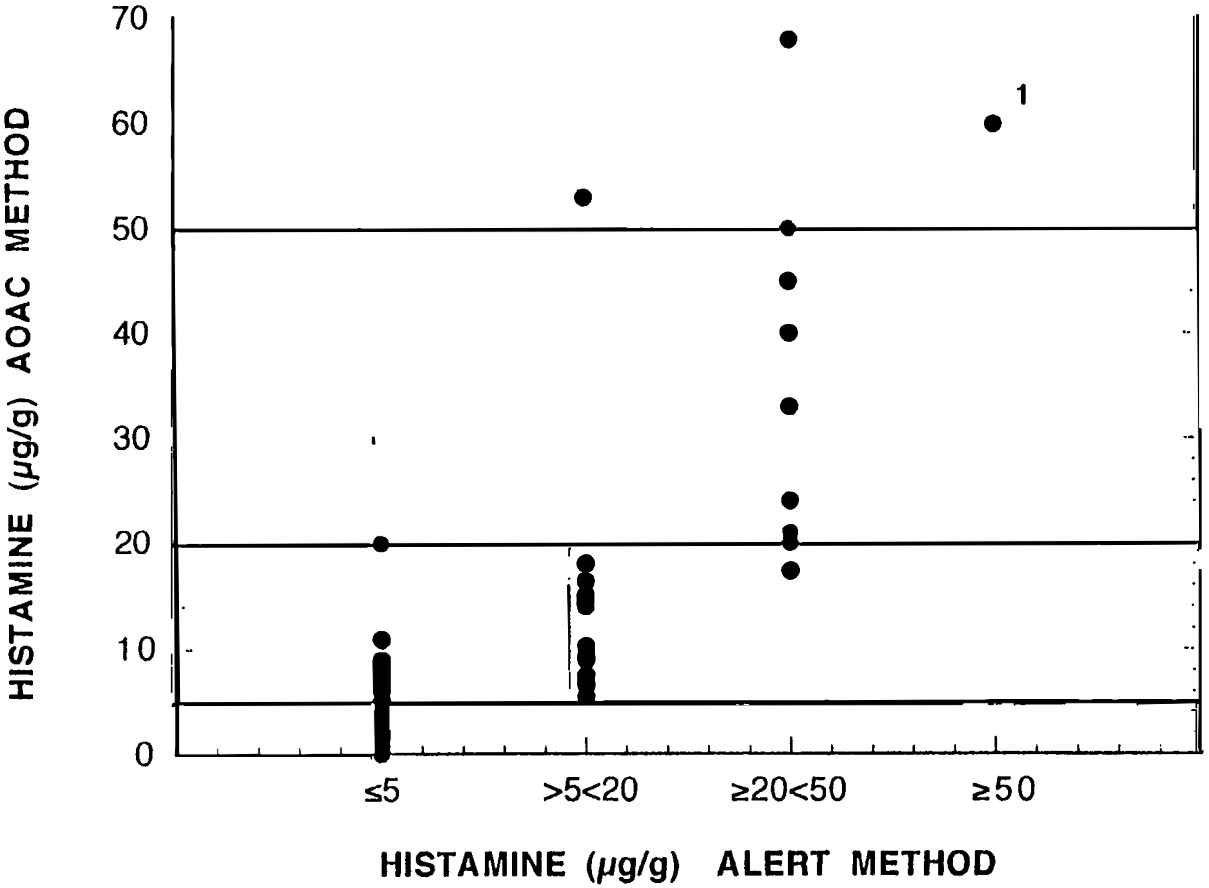

Critical Control Levels of the Alert® for Histamine Test
REFLECTANCE (arbitrary units)
100
200
300
400
500
600
0
10
20
30
40
50
HISTAMINE (µg/g)

Fig. 5

Fig. 6

Comparison of the Alert for Histamine Test and the AOAC Fluorometric Method in Canned and Fresh Tuna



[1]Seven additional samples fell into this range that exceeded 70 μg/g.

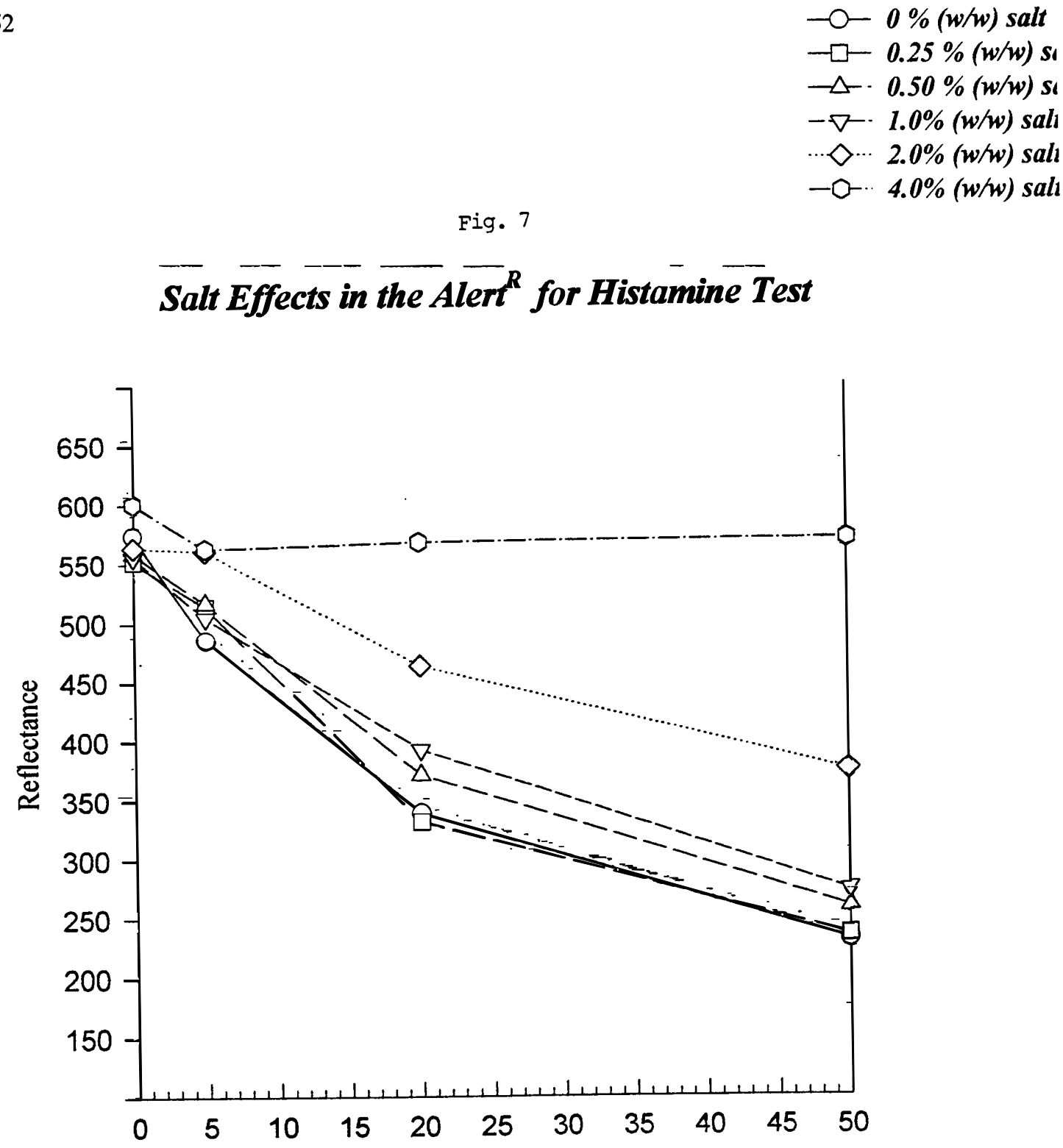

0 % (w/w) salt
0.25 % (w/w) s
0.50 % (w/w) s
1.0% (w/w) sal
2.0% (w/w) sal
4.0% (w/w) sal
Salt Effects in the Alert^R for Histamine Test
Reflectance
650
600
550
500
450
400
350
300
250
200
150
0
5
10
15
20
25
30
35
40
45
50
Histamine ppm (µg/g)

Fig. 7

Table 2

Detection of Histamine Levels in Mahi Mahi using AOAC Fluorometric Method and the Alert® for Histamine Test: Prepared by FDA Office of Seafood

Fish #[1]	AOAC Fluorometric Method	Alert for Histamine[2]	Classification
1	24	37	P
6	106	> 50	F
7	1466	Hi (> 750)	Toxic
10	2	5	P
14	1138	Hi	Toxic
16	10	13	P
17	191	> 50	F
22	162	81	F
22-2	148	175	F

[1]Extracted in 75% MeOH.

[2]Levels determined using a standard curve in the Alert for Histamine Format.