

Histamine and other polyamines such as Putrescine, Cadaverine, Spermidine and Spermine are products of decomposition. The level of histamine, in particular, has been used as a regulatory guideline for degree of decomposition in fish. Also, Histamine and Tyramine levels are of interest to producers of wine and cheese.

The polyamines, Spermidine, Spermine and Putrescine are elevated in neoplastic and damaged, e.g., burned tissue. In fact, the level of circulating and urinary polyamines is an indicator of total body damage. The levels can be monitored for the effect of diet and other modalities on recovery.

This method employs ion-exchange chromatography so, after extraction, centrifugation/filtration are the only sample preparations necessary.

### Method

#### Analytical Conditions

**Analytical Column:** ALKION™ cation-exchange, K<sup>+</sup> form, 4 x 150 mm, Catalog No. 9410917

**Guard Column:** ALKION™ Guard column, 3 x 20 mm, Catalog No. 9493020

**Column Temperature:** 45 °C

**Flow Rate:** 0.8 mL/min

**Mobile Phase:** K600, K563, K130

#### Extraction Procedure

Weigh 10 g of sample into a small glass blender cup (any fortifications should be added at this time). Add 50 mL of extraction solution, (80% HPLC Methanol and 20% 0.1N HCl) and homogenize for two minutes. Centrifuge for five minutes. Mix equal portions of the supernatant with the mobile phase (K600). Allow to coagulate at -4 °C for 15 minutes, then centrifuge for five minutes. The clear supernatant is filtered (0.45 µm Nylon) and placed in an autosampler vial.

#### Post-Column Conditions

**Post-Column System:** Onyx PCX, Pinnacle PCX or Vector PCX

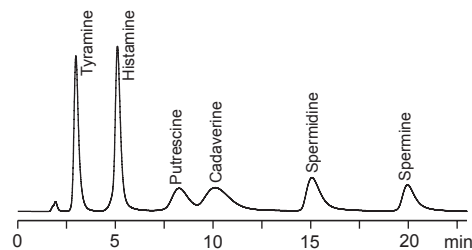
**Reactor Volume:** 0.15 mL

**Reactor Temperature:** 45 °C

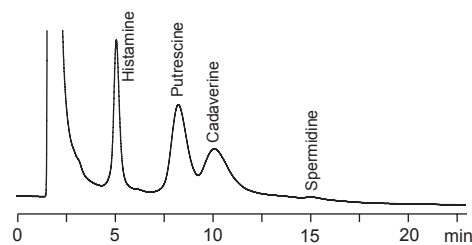
**Reagent:** 300 mg of OPA, 2 g of Thiofluor™, 3 mL of 30% Brij® 35 in 950 mL of OD104

**Flow Rate:** 0.3 mL/min

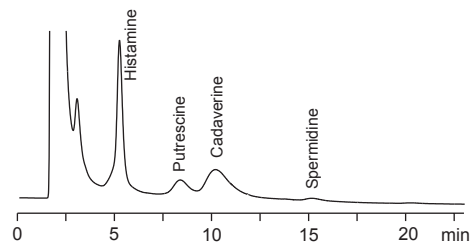
**Detection:** Fluorometer  
 $\lambda_{ex}$ : 330 nm,  $\lambda_{em}$ : 465 nm



Chromatogram of calibration standard, 100 µM



Chromatogram of fish sample



Chromatogram of fish sauce sample

Time	Conditions		
	K600 %	K563 %	K130 %
0	100	0	0
6	100	0	0
15	0	100	0
21	0	100	0
21.1	0	0	100
23	0	0	100
23.1	100	0	0
29	100	0	0

Flow Rate: 0.8 mL/min,  
Column Temp.: 45 °C,  
Injection Volume: 10 mL