



## Analysis of Biogenic Amines

### Food and Physiological Samples

**H**istamine 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.

#### 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 5 minutes. The clear supernatant is filtered (0.45 µm Nylon) and placed in an autosampler vial.

#### METHOD

##### Analytical Conditions

COLUMN: ALKION™ cation-exchange, K<sup>+</sup> form,  
4 x 150 mm, Catalog No. 9410917  
ALKION™ Guard column, 3 x 20 mm,  
Catalog No. 9493020

TEMPERATURE: 40 °C

FLOW RATE: 0.8 mL/min

MOBILE PHASE: K600, K563, K130

##### Post-column Conditions

POST-COLUMN SYSTEM: Pinnacle PCX or Vector PCX

REACTOR VOLUME: 0.15 mL

TEMPERATURE: 45 °C

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

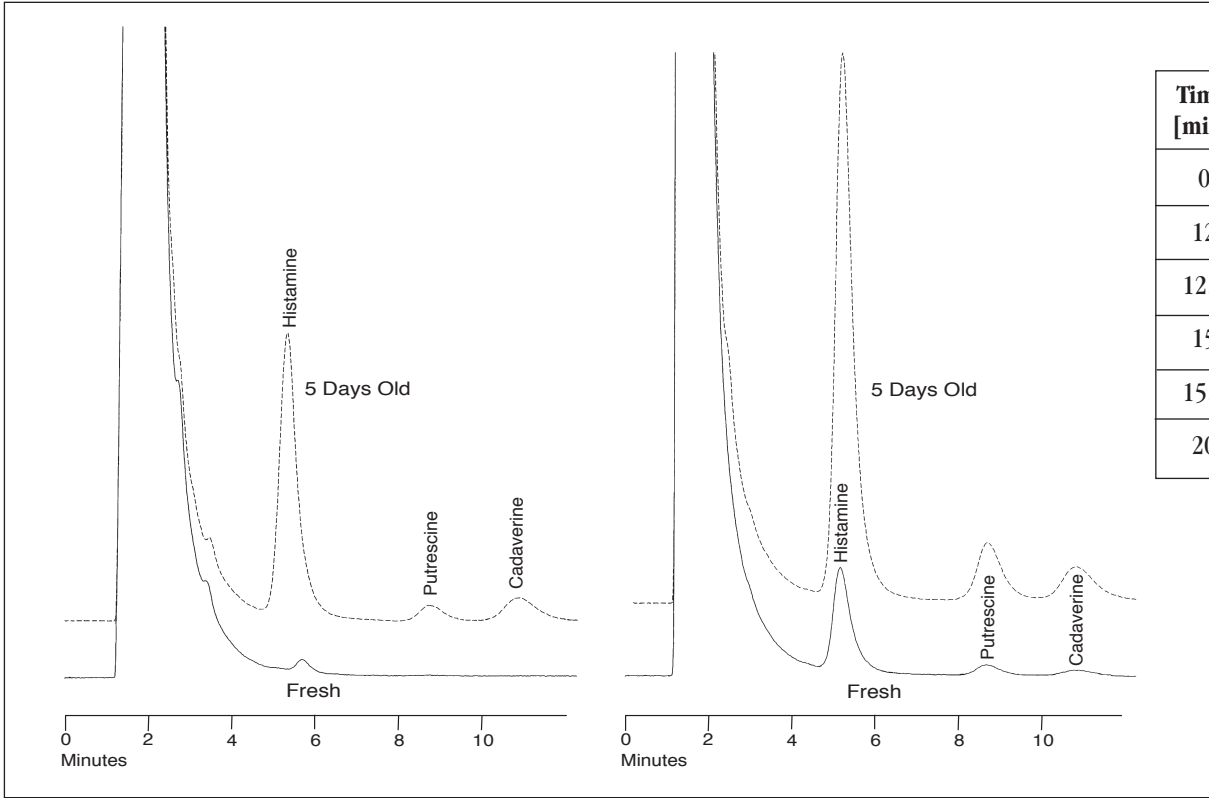
FLOW RATE: 0.3 mL/min

DETECTION: Fluorometer

λ<sub>ex</sub>: 330 nm

λ<sub>em</sub>: 465 nm

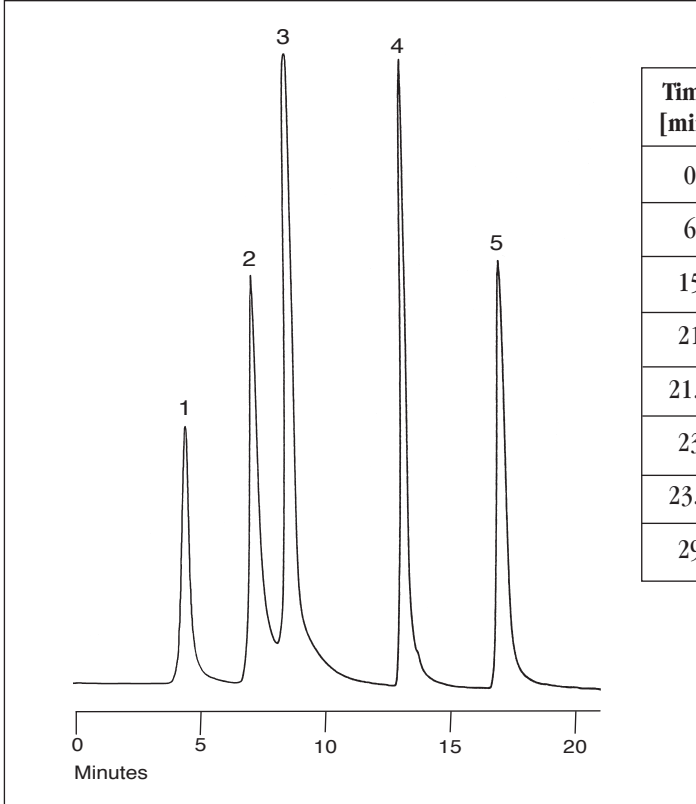
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Time [min]	K600 [%]	K130 [%]
0	100	0
12	100	0
12.1	0	100
15	0	100
15.1	100	0
20	100	0

**Mahi-Mahi**

**Ahi Tuna Fillet**



Time [min]	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

- 1 Histamine
- 2 Putrescine
- 3 Cadaverine
- 4 Spermidine
- 5 Spermine

**Gradient Separation of Polyamine Mixture**