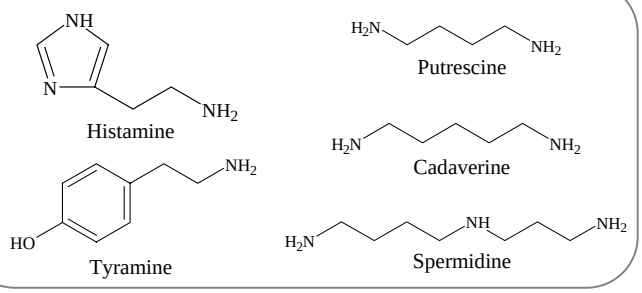
Analysis of Putrefactive Non-Volatile Amines in Food by Pre-column HPLC

This note describes a determination method for non-volatile putrefactive amines in food using pre-column derivatization.

Putrefactive amines in food are produced by microorganisms. Many of the biogenic amines are known to be responsible for food-poisoning. To determine these amines, HPLC systems coupled with pre-column or post-column derivatization methods are frequently used.

Based on the inspection guideline by Japan Food Hygiene Association, pre-column derivatization method using DNS-Cl (5-dimethylaminonaphthalene-1-sulfonyl chloride) was adopted in this note. The putrefactive amines were successfully fluorescently derivatized, and the calibration curves showed excellent linearity. （K.Suzuki）

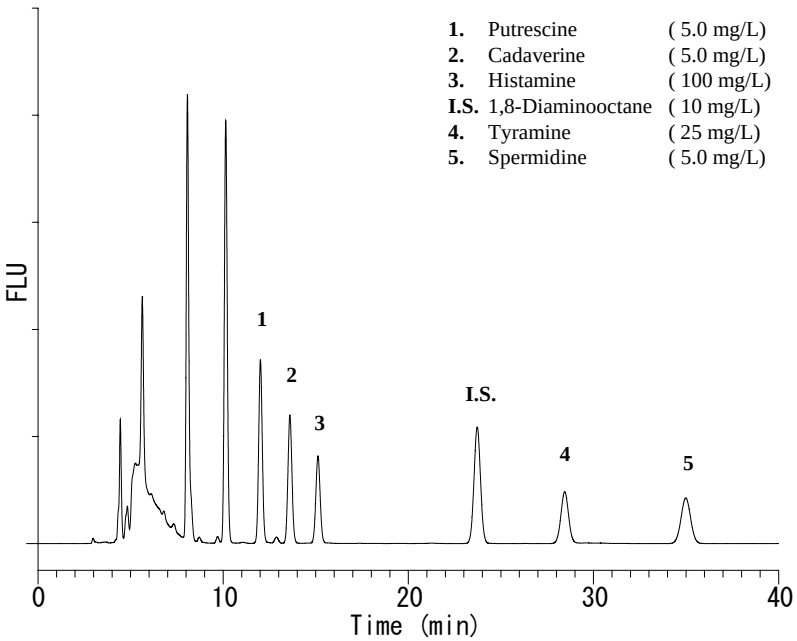
Chemical structures



Structures are created using Chemistry 4-D Draw which is provided by ChemInnovayion Software, Inc.

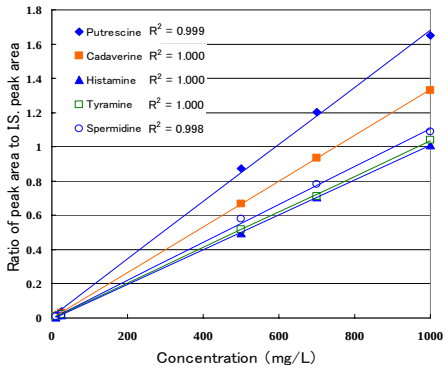
A chromatogram obtained from standard solution

Standard solution derivatized by DNS-Cl was analyzed.



Conditions

・ Column	Inertsil ODS-SP (5 μm, 250 x 4.6 mm I.D.) Cat.No. 5020-02746
・ Guard column	Cartridge Guard Column E ODS-SP (5 μm, 10 x 4.0 mm I.D.) Cat.No. 5020-08520
・ Eluent	A) CH_3CN B) H_2O A/B= 65/35, v/v , 1.0 mL/min
・ Column Temp.	40 ℃
・ Detection	FL Ex 325 nm, Em 525 nm
・ Injection Vol.	10 μL



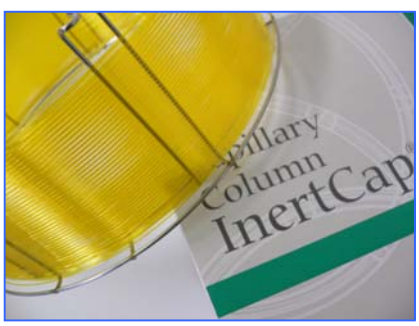
The calibration curves for the putrefactive amines

Inert Family

“Inertsil” High Performance LC Column

“InertCap” GC Capillary Column

“InertSep” Solid Phase Extraction for pretreatment of analysis



Examples of sample pretreatment

Fish samples

Sample
- 10g

Extraction
- 20% trichloroacetic acid aqueous solution 10 mL
- water 150 mL
- homogenize for 10 min
- make up to 200 mL with water
- wait for 30 min
- filtration

Crude sample

Soy sauce samples

Sample
- 10g

Extraction
- 20% trichloroacetic acid aqueous solution 10 mL
- water 150 mL
- make up to 200 mL with water
- wait for 30 min
- filtration

Crude sample

Crude sample
- 5 mL
- 0.1M sodium 1-octanesulfonate aqueous solution 5 mL

SPE cartridge C18 ※
- water 20 mL
- elute with 10 mL of 60% methanol aqueous solution

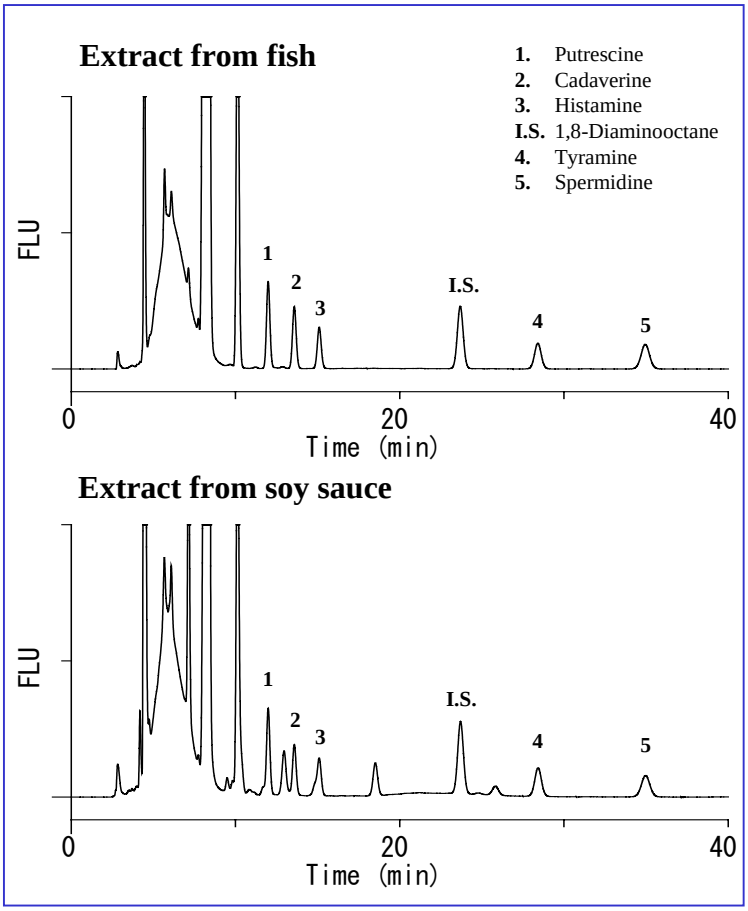
Eluate
- evaporate and concentrate to 1 mL
- internal standard solution 0.50 mL （20 mg/L 1,8-diaminoocatne）
- sodium carbonate anhydrous 0.2g
- 2% DNS-Cl acetone solution 2 mL
- Heat at 45 ℃ for 2 h
- 10% proline aqueous solution 0.5 mL
- shaking
- wait for 10 min
- toluene 5 mL
- shaking

Liquid-liquid extraction
- toluene layer
- evaporate to dryness

Residue
- acetonitrile 1.0 mL

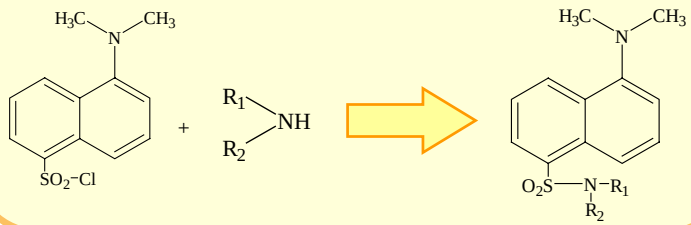
HPLC-FL

※Before loading the sample on SPE cartridge, conditioning was carried out with 10 mL of methanol followed by 10 mL of water.



Derivatization reaction by DNS-Cl

DNS-Cl reacts with primary and secondary amines. As a result, fluorescent derivatives are generated.



Contact us

GL Sciences, Inc. Japan

22-1 Nishishinjuku 6-chome, Shinjuku-ku, Tokyo, 163-1130 Japan

TEL: +81-3 (5323)6620 FAX: +81-3 (5323)6621

GL Sciences, Inc. USA

4733 Torrance Blvd. Ste 255, Torrance, CA 90503

Tel: (310)265-4424 FAX (310)265-4425

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