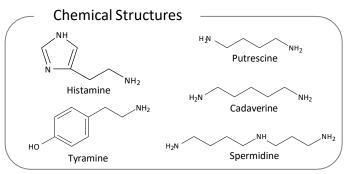
Analysis of Putrefactive Non-Volatile Amines in Food by Pre-column HPLC

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

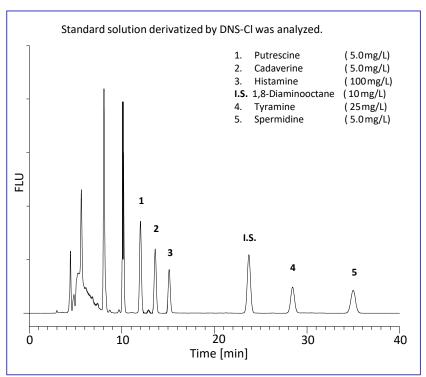
Putrefactive amines in food are produced by microorganisms. Many of the biogenic amines are known to be responsible for food-poisoning. To determine these amines, HPLC systems coupled with pre-column or post-column derivatization methods are frequently used.

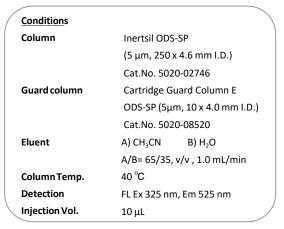
Based on the inspection guideline by Japan Food Hygiene Association, pre-column derivatization method using DNS-Cl (5-dimethylaminonaphthalene-1-sulfonyl chloride) was adopted in this note. The putrefactive amines were successfully fluorescently derivatized, and the calibration curves showed excellent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

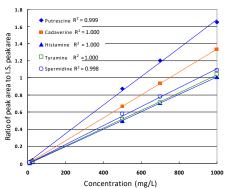


Structures are created using Chemistry 4-D Draw which is provided by ChemInnovayion Software, Inc.

A Chromatogram Obtained from Standard Solution





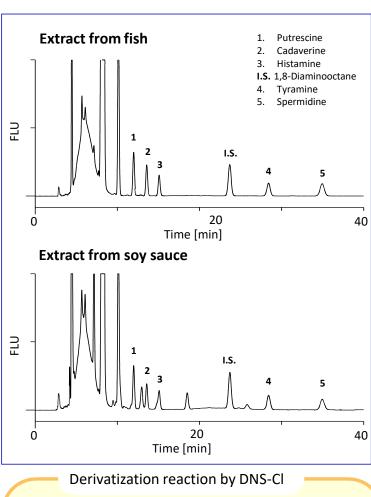


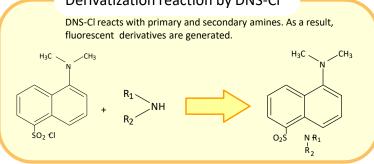
The calibration curves for the putrefactive amines



Examples of Sample Pretreatment Fish samples Soy sauce samples Sample Sample - 10 g — 10 g **Extraction** Extraction 20% trichloroacetic acid 20% trichloroacetic acid aqueous solution 10 mL water 150 mL aqueous solution 10 mL water 150 mL homogenize for 10 min make up to 200 mL make up to 200 mL withwater with water wait for 30 min wait for 30 min filtration filtration Crude sample **Crude sample** Crude sample 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 °C 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**







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