

BIOGENIC AMINES



Biogenic amines (or polyamines) mainly include histamine, putrescence, cadaverine, spermidine and spermine. These are low-molecular, organic bases that are produced and metabolised during the normal metabolism of humans, animals, plants and micro-organisms. However, biogenic amines show a detrimental effect on the organism when taken up in large quantities. Their presence in food indicates unwanted microbial activity and, consequently, can be taken as an indicator for the degree of microbiological contamination.

In order to detect traces of biogenic amines, PICKERING offers a complete method (PINNACLE PCX and application kit) for food and beverages. The kit for biogenic amines contains, in addition to the column, reagents, diluents, eluents and a standard.

Description of the Method

The separation of biogenic amines is performed on a special potassium ion-exchange column that significantly simplifies sample preparation due to its high selectivity and robustness.

Biogenic amines are derivatized in a one-step reaction using *o*-phthaldialdehyde (OPA) and Thiofluor[®] (2-mercaptoethanol derivative) to a fluorescing isoindole derivative.

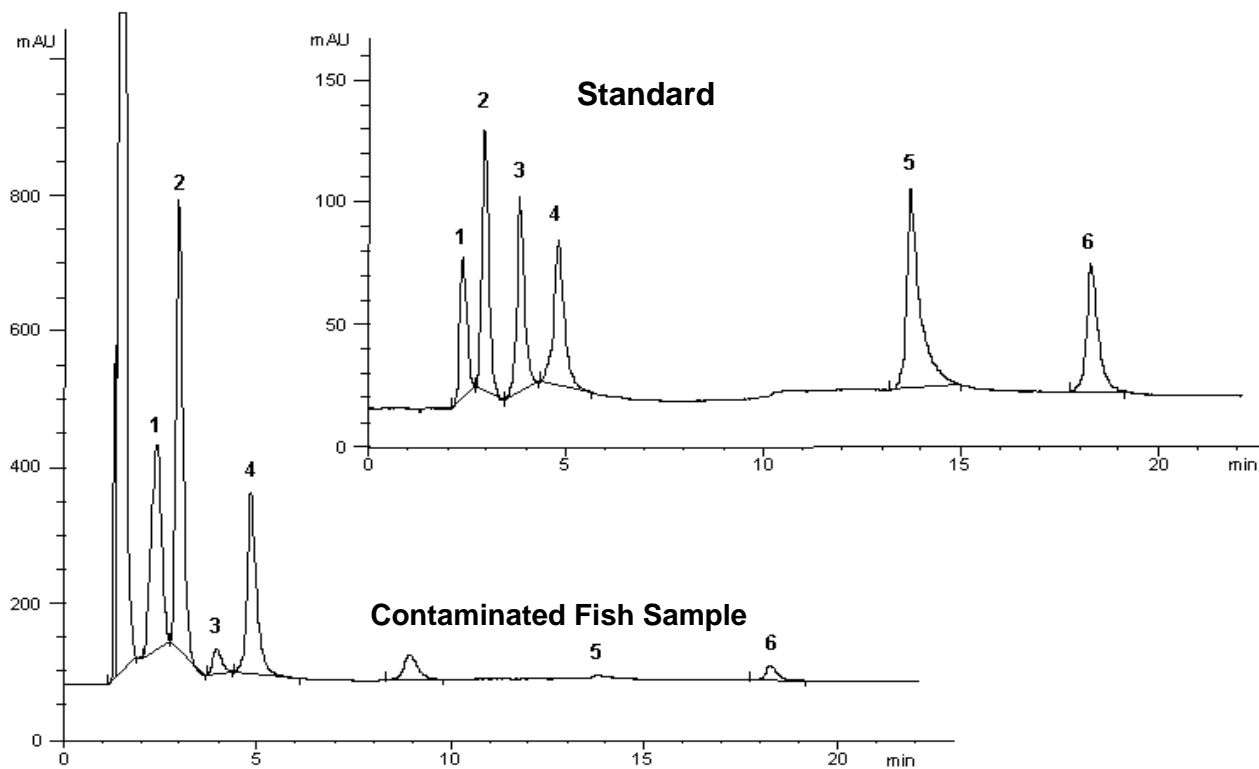
In the literature, different methods for biogenic analysis can be found describing post-column derivatization with different types of columns and mobile solvents. Of course, the PINNACLE PCX can be used in connection with these methods. Please ask for advice!

APPLICATION NOTE

Chromatograms

Chromatogram of a Standard and a Contaminated Sample (BAI = 17)

Signal	Compound	Standard [ng abs.]	Sample [ng abs.]
1	Tyramine	100	240
2	Histamine	100	220
3	Putrescine	200	70
4	Cadaverine	200	430
5	Spermine	100	< 10
6	Spermidine	200	30



APPLICATION NOTE

HPLC Conditions and Derivatization Parameters

HPLC	
Operation Mode	Ternary gradient
Eluent	K600 eluent and K563 eluent (potassium hydrogenphosphate buffer / isopropanol) K130 regenerant (aqueous potassium hydroxide solution / isopropanol)
Degassing	Helium- or vacuum degassed
HPLC Column	ALKION [®] ion-exchange column; K ⁺ -form; 4x 150mm
Column Oven	40°C
Flow Rate	0,8 mL/min
Injection Volume	Up to 100 µL
Post-Column Derivatization	
Pinnacle PCX	Single-pump; 150 µL reactor
Reactor Volume	0,150 mL
Reactor Temperature	45 °C
Reagent	Solution of <i>o</i> -phthalaldehyde (OPA) and Thiofluor [®] (2-mercaptoethanol derivative) in OD104 diluent (sodium borate buffer, pH-value 10.4).
Reagent Flow	0.3 mL/min
Detection	
Detection Type	Fluorescence detection
Excitation Wavelength	330 nm
Emission Wavelength	465 nm
Flowcell	Analytic; pressure stable up to 7 bar

Caution: Extreme pH-range!

APPLICATION NOTE

As a consequence of the alkaline regenerant (pH 13), components made from Vespel may not be present in the HPLC system, instead these must be exchanged for components made from pH-inert materials (Tefzel or PEEK). For advice, please contact your LC-representative or consult your technical manual.

An inert version (Titanium, PEEK) of the HPLC system is not necessary, a piston seal wash for the pump heads, however, is recommended.

To avoid corrosion of the system and contamination of the ion-exchange column with metal ions, passivating is recommended; this is especially valid for older systems. For advice, please contact your LC-representative or consult your technical manual.

Autosampler

To achieve reproducible retention times in biogenic amine analysis with ion-exchange columns, it is important to keep to exact time intervals between injections. Should only a manual injector be available, then the injection times should be controlled with a laboratory timer.

Gradient Program

METHOD: ALKION[®] ion-exchange column for biogenic amines

Flow rate: 0.8 mL/min; Column temperature: 40°C

Step	Time [min]	Interval [min]	K600 [%]	K563 [%]	K130 [%]	
0	0	0	100	0	0	Injection
1	6	6	100	0	0	Isocratic
2	15	9	0	100	0	Linear gradient
3	21	6	0	100	0	Isocratic
4	21 - 23	2	0	0	100	Step change + rinsing
5	23	5 - 9	100	0	0	Re-equilibration

APPLICATION NOTE

Literature

- 1) M. J. Walters., *J. Assoc. Off. Anal. Chem.* **1984**, 67 (6), 1040 – 1043.
- 2) M. L. Tracy, M. V. Pickering, T. VerHulst, *Food Testing and Analysis* **1995**, 1 (3), 48-50.
- 3) M. L. Izquierdo-Pulido, M. C. Vidal-Carou, A. Marine-Font, *J. Assoc. Off. Anal. Chem.* **1993**, 1027 – 1032.
- 4) L. Simon-Sarkadi, W. H. Holzapfel, *Z. Lebensm. Unters. Forsch.* **1994**, 230 – 233.

Chemicals and Columns

Post-Column Derivatization Unit

Order Number	Description
1153-1012	PINNACLE PCX; single-pump, 150 µL reactor

Biogenic Amines Kit

Order Number	Description
0352-0040	Application kit 'Biogenic Amines' consists of:
9410917	ALKION [®] , Cation-exchange column, K ⁺ -form, 4 x 150 mm
9493020	Guard column for ALKION [®] column, 3 x 20 mm
O120	o-Phthalaldehyde (OPA), „Chromatographic Grade“, 5 g
OD104	OPA Diluent, 4 x 950 ml
3700-2000	Thiofluor [®] , „Chromatographic Grade™“, 10 g
K600	Potassium phosphat buffer, 4 x 950 ml
K563	Potassium phosphat buffer, 4 x 950 ml
K130	Potassium column regenerant, 950 ml

Cation-Exchange Columns for Biogenic Amines Analysis

Each biogenic amines column is tested by applying a biogenic amines calibration standard and running it according to a specific gradient elution protocol. After a column passes this rigorous QC test, it receives a serial number and is packaged with its test chromatogram.

Order Number	Description
9410917	ALKION [®] , Cation-exchange column K ⁺ -form, 4 x 150 mm
9493020	Guard column for ALKION [®] column, 3 x 20 mm

APPLICATION NOTE

o-Phthalaldehyde Reagent

Primary amines such as biogenic amines form highly fluorescent compounds when reacted with o-phthalaldehyde (OPA) and a mercaptan under basic conditions. At a pH >9 and ambient temperature, reaction is generally complete within 1 - 30 seconds. The products of this reaction, 1-alkyl-2-alkylthio-substituted isoindoles, exhibit optimal excitation at 330 nm and maximal emission at 465 nm.

For an oxygen-sensitive reagent like OPA to remain stable for days instead of hours, it is important to start with the purest and most stable ingredients available, and to store and use the reagent under anaerobic conditions.

Using the chemicals described below, a long-lasting (up to ten days) OPA reagent for post-column derivatization of primary amines can be prepared. Each chemical is accompanied by clear instructions for formulating the reagent in your laboratory within minutes.

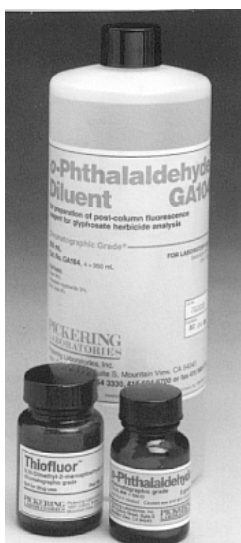
For the preparation of o-phthalaldehyde reagent PICKERING offers:

- 1) Ultra-pure, crystalline OPA
- 2) Borate diluent; free of heavy metals, particulates and amines
- 3) Thiofluor; crystalline substitute for 2-mercaptoethanol

o-Phthalaldehyde

Order Number	Description
O120	o-Phthalaldehyde (OPA), „Chromatographic Grade™“, 5 g

o-Phthalaldehyde Diluent



For the analysis of biogenic amines Pickering offers a sodium borate buffer with pH 10.4 capable to buffer acidic eluents (pH 2)

Order Number	Description
OD104	OPA diluent, „Chromatographic Grade™“, 4 x 950 mL

APPLICATION NOTE

Thiofluor®

Pickering's Thiofluor, a solid, nearly odorless nucleophile, is a superior substitute for 2-mercaptoethanol in the preparation of OPA reagents. It forms a more stable and longer-lasting fluorophore with OPA than does 2-mercaptoethanol, while possessing the same fluorescence properties.

Unlike the volatile 2-mercaptoethanol, Thiofluor will not migrate through the gas manifold and regulator of the OPA reagent pressurization system.

Order Number	Description
3700-2000	Thiofluor®, "Chromatographic Grade™" 10 g

Potassium Phosphate Buffer

- 1) Filtered, ready to use
- 2) Free from fluorescing impurities
- 3) Cost-effective: long shelf life; can be used to the last ml
- 4) Consistent elution profiles, bottle to bottle, lot to lot

These buffers are used for the elution of biogenic amines according to the method developed by Pickering.

Order Number	Description
K600	Potassium phosphat buffer, 4 x 950 mL
K563	Potassium phosphat buffer, 4 x 950 mL

Column Regenerant for the Analysis of Biogenic Amines

- 1) Removes strongly bonded matrix interferences from the column
- 2) Free from fluorescent impurities
- 3) Filtered, ready to use
- 4) Consistent elution profiles, bottle to bottle, lot to lot

Order Number	Description
K130	Potassium column regenerant, 950 mL