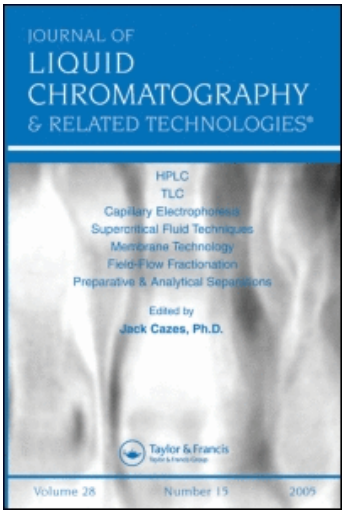

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A Review of HPLC Methods for the Determination of Selected Biogenic Amines in Foods

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A REVIEW OF HPLC METHODS FOR THE DETERMINATION OF SELECTED BIOGENIC AMINES IN FOODS

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ABSTRACT

This manuscript summarizes HPLC methodologies used for the determination of selected biogenic amines in foods. It includes methods of extraction of these compounds, methods used to eliminate potential interferring compounds and the HPLC determinant step.

INTRODUCTION

The biogenic amine class encompasses a substantial number of diverse structural elements.Individual members of this class can either be aromatic or aliphatic. The purpose of this review is not to provide a treatise on all members of

Table 1

Precursor Amino Acid & Corresponding Biogenic Amine

Precursor Amino Acid	Biogenic Amine
Tyrosine	Tyramine
Tryptophan	Tryptamine
Phenylalanine	Phenylethylamine
Histidine	Histamine

this diverse class but to provide an overview on the use of HPLC techniques to accomplish this assay in foodstuff. This review will restrict itself to a select few members of this class that are outlined in Table 1. Since these compounds are deamination products of selected amino acids, the precursor amino acid and resulting biogenic amine are provided.

These compounds are also called the "pressor amines" and the ingestion of certain members of this class by patients taking monoaminoxidase (MAO) inhibitors can cause significant side effects,so the interest in biogenic amines in foodstuffs is evident. There are several excellent references on the chemical and physiological characteristics of the compounds(1-6) and it is beyond the scope of this review to adequately cover this subject area. Rather it will focus on analytical methodology using HPLC to accomplish this determination.

Prior to the introduction of HPLC, methods in use for this assay involved the use of other techniques such as paper chromatography,thin layer chromatography,gas chromatography

and spectrofluorometry(7-11). Many of these techniques are still currently used but HPLC has replaced them in many cases.

Extraction

Prior to any final determinant step the compound of interest must first be extracted from the matrix. In the case of liquids,no extraction is usually necessary but in the case of solid foods,extraction sometimes followed by other forms of sample preparation is necessary. This portion of the manuscript will outline some extraction protocols used in the HPLC determination of biogenic amines.

The most prevalent method used to initially extract the biogenic amine is solvent extraction with acid. The acid is used to insure that all the amines are dissolved. Three kinds of acid have been used in various methods and include HCl, HClO4 and TCA . After the initial extraction, additional steps are used to further isolate the compounds. Table 2 provides the extracting solution composition and matrix for a number of these determinations and includes not only those acidic solutions used but also some alternatively used solvent systems.

As can be seen in Table 2 the most prevelant extracting solvent is an acidic medium. In some cases HPLC was not used for the final step but the information is provided for completeness and the use of this solvent would probably result in an extract suitable for further analysis by HPLC.

Table 2

Summary of Solvents Used to Extract Biogenic Amines from Foods.

Matrix	Extracting Solution	Cmpd of Interest	Reference
Dairy Products (yogurt, cheese, infant formula)	CH3OH	tyramine	13
Beer	no sample preparation		14
Various foods	water	Histamine tyramine phenylethylamine	15
Cocoa	0.1NHClO4	tyramine	16
Cheese & chocolate	0.07M tri-sodium citrate followed by 0.3MTCA	tyramine histamine phenylethylamine tryptamine	18
Chocolate	0.1NHClO4	tyramine tryptamine phenylethylamine serotinn	19
Chicken	0.6M HClO4	tyramine tryptamine phenylethylamine histamine	20

Table 2 Continued

Matrix	Extracting Solution	Cmpd of Interest	Reference
Sausage	IN HClO4	tyramine tryptamine phenylethylamime histamine	21
Soy Sauce	HCl	tyramine tryptamine phenylethylamine histamine	22
Cheese	5% TCA	tyramine tryptamine phenylethylamine histamine	23
Fish products	10% TCA	histamine tyramine	24
Cheese	0.1NHCl	histamine tryptamine tyramine	7

Thin Layer & Paper Chromatography Methods

Table 3 provides summary information on selected paper and thin layer chromatography (TLC) methods.

ISOLATION

In some few instances the acidic extract could be used with other further treatment but in most cases further sample

Table 3

Selected Thin Layer and Paper Chromatography Systems Used for the Determination of Biogenic Amines

Separation Mode: TLC

Support:Silica Gel

Developing
Solution: CHCl3:CH3OH:NH4OH(12:7:1)

Visualization: 0.2% NBC-Cl(7-chloro-4-nitrobenzofuran)

Compounds: tyramine,histamine,tryptamine

Reference: 7,10

Separation Mode:Paper Chromatography

Support: Whatman #1

Developing
Solution(s): n-Butanol:acetic acid:H2O(4:1:5)
Isopropanol: NH4OH:H2O(8:1:1)

Visualization: Diazotized sulfanilic acid
Diazolized p-nitroaniline
ninhydrin

Compounds(s): Tyramine + other phenolic amines

Reference:11

Separation Mode: Paper Chromatography

Support: Cellulose

Developing
Solution: Butanol:Acetic Acid:H2O (12:5:3)

Visualization: 0.1% alpha-nitrous-beta-naphtholin
95% ethanol + 3M nitric acid
containing sodium nitrite

Reference:12

treatment was necessary to eliminate potential interferring compounds. This sample treatment can be divided into two categories: solvent extraction and solid phase extraction(SPE) or column cleanup. In the case of dairy products (13) the amine was extracted from the products with warm CH3OH(60 degrees C),cooled with tap water,brought to volume with additional methanol and filtered prior to analysis. This is an extremely simple procedure and was found very suitable. In this case no additional cleanup steps were necessary prior to HPLC analysis. In another method (18) cheese and chocolate were initially extracted with 0.7M trisodium citrate and a portion of this extract mixed with 0.6M TCA and centrifuged at 10,000 g and 4 degrees C. The pellet was extracted with additional TCA and further centrifuged. The resulting TCA extracts were combined,filtered and the final volume adjusted with water prior to HPLC analysis.

Fish and saurkraut were initially extracted with water. A portion of this extract was mixed with an equal portion of TCA and centrifuged and filtered for analysis.

In other cases some form of additional solvent extractions or column cleanup was used. Literature indicates that many of the methods used to extract and isolate biogenic amines from foods are variations of methods used in clinical laboratories.

After initial extraction with acid, several schemes have been used to further purify the amine containing

fractions. In two studies using a final TLC step (7,10), after the samples are centrifuged, the aqueous layer is removed and adjusted to pH 10 with solid NaCO3 and then saturated with excess NaCl. Butanol was then added to this mixture and the resulting solution was vortexed several times. The resulting solution was then centrifuged again at 12,000 g and the butanol layer removed for quantitation. While this procedure had a TLC determinant step it is not unreasonable to assume that this sample preparation might also be suitable for use in an HPLC method. An alternative to this methodology has been used by several authors (16,19).In these cases, the acidic extract was adjusted to pH 10.3 with NH4OH and again saturated with NaCl. The resulting solution was extracted with several portions of ethyl acetate: acetone (2:1),dried with Na2SO4 and the solvent was removed prior to analysis with a stream of nitrogen. The residue was then suitable for HPLC or TLC analysis.

While solvent extraction has been used successfully to isolate amine containing fractions of foodstuffs, a larger number of methods use a column cleanup procedure consisting of an alumina column or a cation exchange resin. The use of these column types is a direct transfer from the clinical laboratory where alumina was successfully used for the isolation of catecholamines, which are a group of closely related compounds. The adsorption on alumina is very specific and allows for a substantial degree of purification of the extract. The

phenomena is optimal above pH 8.4 (25) so the acidic extracts must have a pH adjustment to use this procedure. This procedure is routinely applied to the determination of catecholamines in biological samples and has been applied to foodstuffs in some instances. The amine compounds are then desorbed through the use of an appropriate acid. The acid washed alumina can either be in a small packed column or loose granules and both geometries have been used. After the pH of the extract has been adjusted to 8.4 or higher the compounds are adsorbed onto acid-washed alumina. While the examples in the literature have used "hand-made " or lab manufactured columns, it is reasonable to expect that the current generation of commercial SPE (Solid Phase Extraction) columns should prove most useful.

The second column clean-up procedure involved the use of a cation exchange resin and has also been used in a substantial number of methods for the determination of catecholamines in samples of serum and urine. Several authors (15,26) have reported the use of this method for food analysis. In one instance the acidic extract was adjusted to pH 6.5 with HCl and passed through a weak cation exchange resin (Bio-Rex 70) which had been washed successively with H2O, HCl, NaOH adjusted to pH 6.5 with HCL and finally treated with sodium phosphate buffer at pH 6.5. After the extract was placed on the column it was washed with distilled water and the amine fraction eluted with HCl (26). Another method of this type has a pH adjustment to 7.0 with NaOH and then adsorption onto

another weak cation exchange resin (Zerolit-236 Na+). The column was washed with water and amines removed through the use of HCl.(15)

Due to the large amount of activity in the determination of the catecholamine there has been substantial activity in their isolation using some novel SPE packings such as Phenyl Boronic Acid (27) which form reversible complexes. It is not unreasonable to assume that the commercially available SPE columns would be excellent for this assay and that phases such as PBA would not provide some interesting selectivity for sample cleanup of amines in foods.

HPLC ASSAYS

There have been a substantial number of HPLC techniques used to separate and quantify biogenic amines in foodstuffs. A large number of methods that were used to analyze for these and other related compounds in the clinical laboratory. Many of the solvent and detection systems used in clinical assays would be appropriate for the food analyst.

LC With UV Detection

The most straightforward way to accomplish the assay couples a reversed phase column,the appropriate mobile phase and UV detection. Three different wavelengths have been used in various assays: 254, 210 and 280 nm. Koehler and Eitenmiller (17) utilized a uBondapak C-18 column with a mobile phase

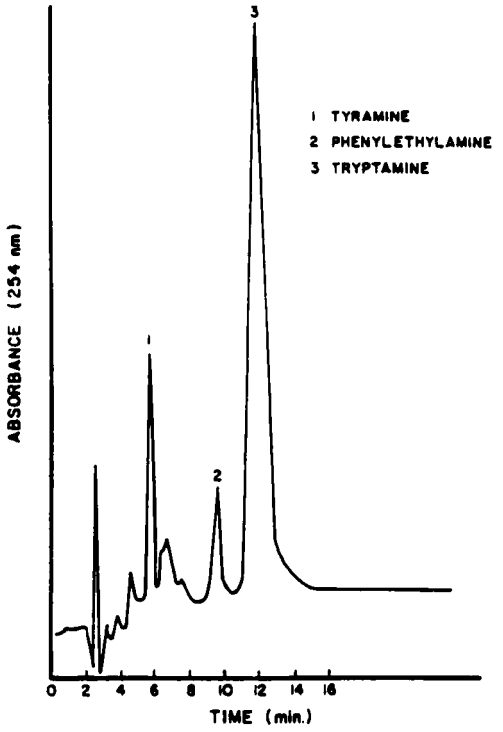

Figure 1

Separation of Biogenic Amines in Cheese (17)
(Used With Permission)

consisting of 35/65 (U/V) water/methanol containing .005m 1-heptane sulfonic acid as a counter ion with detection at 254 nm for the determination of tyramine ,phenylethylamine and tryptamine in sausage,cheese and chocolate. Figure 1 provides an illustration of the separation of a cheese extract.

Ingles,Tindale and Galimore (28) reported the use of a Lichrosorb Si-60 column with a mobile phase consisting of

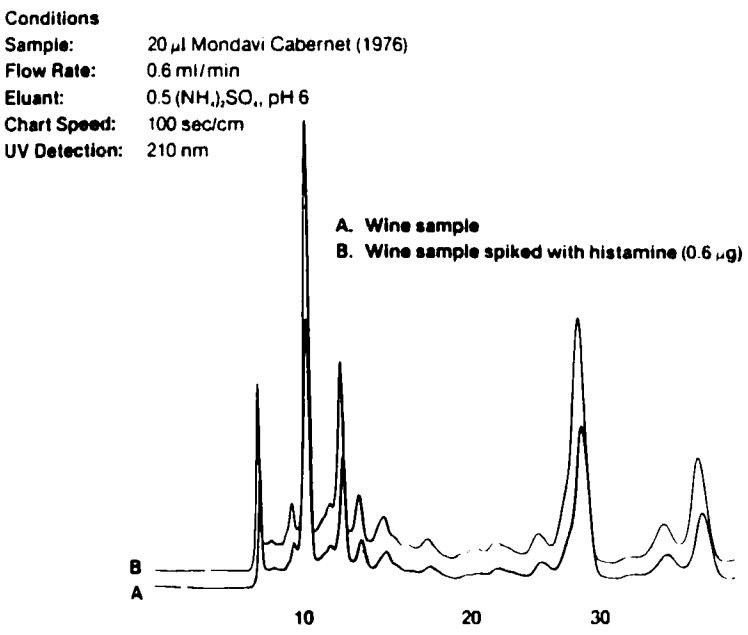

Figure 2

Chromatogram of Histamine in Wine (26)
(Used With Permission)

40:20:1 (V/V/V) hexane/2-propanol/ammonia with detection at 280 for the determination of tyramine in chocolate. This system was found not to be suitable for the determination of histamine. Figure 2 illustrates the separation of histamine in wine using ion exclusion chromatography with UV detection at 210 nm.

While they have not been used to analyze food extracts several manuscripts have been published outlining the HPLC analysis of biogenic amines using silica columns with acidic

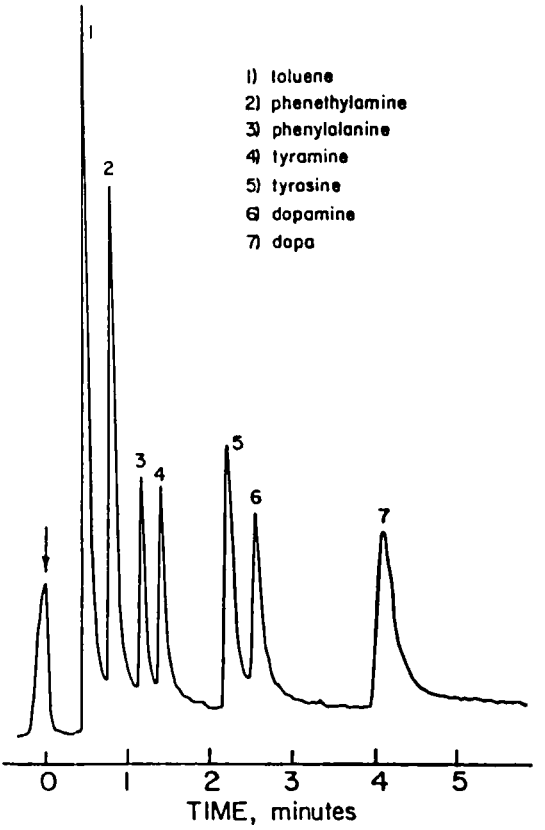

Figure 3

Separation of Biogenic Amines & Precursor Amino Acids (29)
(Used With Permission)

mobile phase. Persson and Karger first (29) reported the use of a silica gel support,a stationary phase consisting of mixtures of HClO4 and NaClO4 and a variety of mobile phases ranging from butanol/hexane to mixtures of ethyl acetate /tributyl/phosphate and hexane. Figure 3 provides an illustration of this

separation indicating several biogenic amines and their precursor amino acids with detection at 280nm. Svendsen and Greibrokk (30) published further studies in this area and explored the effect of various acids, pH, ionic strength and various organic modifiers on this mechanism.

While UV detection is the most straight-forward of the detection schemes for the determination of biogenic amines it suffers from a lack of sensitivity and sometimes necessitates the use of extensive sample cleanup protocols. A large number of compounds that could be extracted with the biogenic amines of interested would interfere with the final determination using UV detection.

LC With Direct Fluorescence Detection

A second direct detection system is native fluorescence. The native fluorescene of these compounds can be attributed to the backbone of the amine (31). An excitation wavelength of 280-285 nm has been used for native fluorescence detection with various emission wavelengths. Lower limits range from 2 to 70 ng depending on the compound of interest. Work in our laboratory utilized this technique to determine biogenic amines in extracts of chocolate (19). The use of native fluorescence affords a substantial increase in sensitivity over UV detection schemes; from a practical viewpoint allows for less sample to be utilized to accomplish the determination and a potentially less rigorous sample cleanup.

LC With Electrochemical Detection

By far the most widely used of the direct detection modes for the determination of biogenic amines involves the use of electrochemical detection. The catechol compounds are oxidizable at a graphic electrode to generate a orthoquinone 2 protons and 2 electrons (32). The modern electrochemical detector measures this oxidation as the anodic current. There are a large number of the reports on the use of this technique to measure various catecholamines in samples of plasma,urine and tisssue but few in the area of biogenic amines in foods. Kengherez and Kissinger reported the determinaton of tyramine from cocoa using electrochemical detection.(16) The separation was accomplished using a C18 column with a mobile phase consisting of ammonium acetate,methanol and sodium octyl sulfate. The applied potential for the detector was + 0.95 volts vs SCE. Another manuscript outlined the separation of twenty biogenic amines and their derivatives through the use of a column switching technique and on-line fluorometric and electrochemical detectors (33). While this separation was not applied to foodstuffs it would seen appropriate for this assay.

Recently Medford (45) reported on the use of a microbore HPLC technique for the determination of biogenic amines. The column used was laboratory packed with dimensions of 1.2 mm I.D. x 10 cm. It was packed with 3um C-18 resin. The

solvent system used consisted of 0.2M NaH2PO4,33mg/liter sodium octyl sulfate,50 mg/liter EDTA,pH 3.2 at a flow rate of 180 ul/min. Electrochemical detection with a glassy carbon electrode was used. While it was not used for the determination of biogenic amines per se in foodstuffs, it should be suitable for this assay. Figure 4 illustrates the use of electrochemical detection in the separation of some of these compounds with serotonin being peak 12. The other peaks represent other monamine standard compounds.

LC With Derivative Formation

Following the direct modes of detection of selected biogenic amines, the various derivatives that can be formed must be outlined. As in previous cases, there is not a large volume of data on the use of these derivatives for the determination of biogenic amines in foodstuffs but all could be applied to this assay.These derivatives can be divided into several categories including UV and fluorescent derivatives and pre and postcolumn derivatives.

The most popular types of derivatives are the fluorescent compounds; one of the most widely used is orthophthaldehyde ,OPA (34). The OPA reacts with the primary amine function of the biogenic amine forming a fluorescent derivative. An example of this technique can be seen in Figure 5 which illustrates the separation of numerous biogenic amines (35). This derivative formation can occur either pre-column or

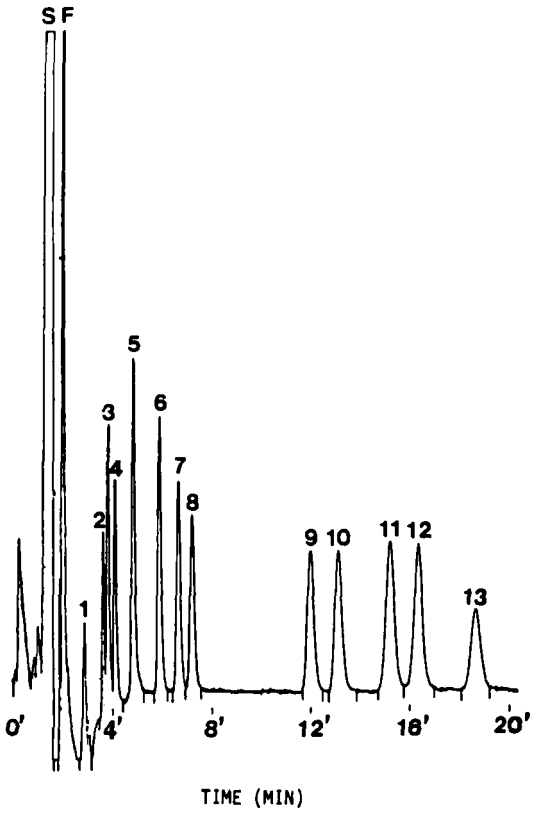

Figure 4

Monoamine Standard Compounds With Electrochemical Detection (46)
(Used With Permission)

post-column. There are distinct reasons for each mode of derivatization that will not be discussed in this manuscript. The formation of these fluroscent derivatives enhances sensitivity and selectivity to these assays. Other derivatives that have been used include ninhydrin,dansyl chloride,

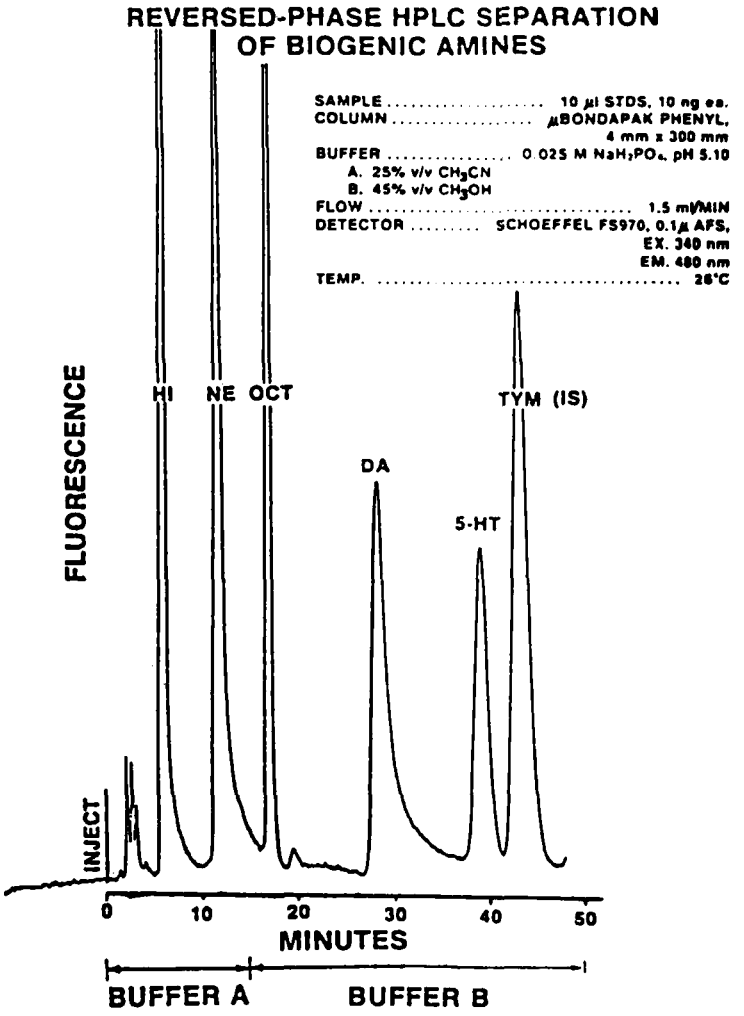

Figure 5
Reversed Phase HPLC Separation of Biogenic Amines
With Direct Fluorescence Detection (35)
(Used With Permission)

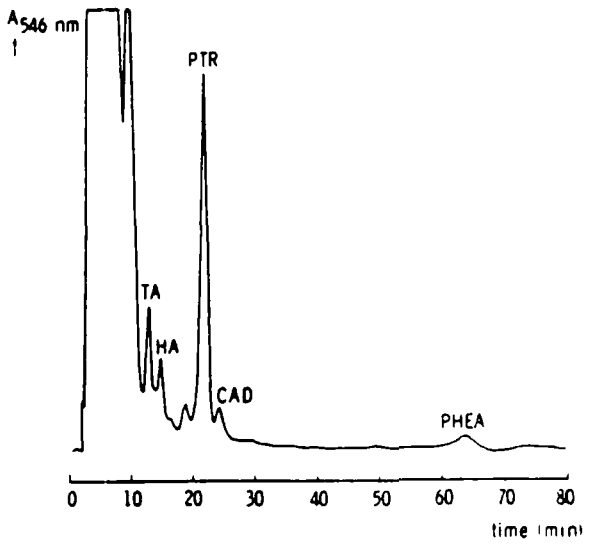

Figure 6
Chromatogram of Sauerkraut Extract Indicating
Presence of Tyramine(TA), Histamine (HA)
Other Amines (18)
(Used With Permission)

fluorescamine and recently 9-fluorenylmethyl chloroformate (18,36,37,38). Potential UV/VIS derivatives include N-Succinyl -p-nitrophenyl acetate(SNPA),Dabsyl Chloride and 4-N,N -dimethylaminoazobenzenthio-4-isocyanate (DABITC). (40,41,42,43)

Additionally some analysts have utilized unique reaction mechanisms; the work of Joosten and Olieman(18) used reversed phase ion pair chromatography with a ninhydrin -containing eluent which eliminates the need for a post column reagent pump. Fig 6 is a chromatogram of a sauerkraut extract

Table 4

Derivatives for the Detection of Biogenic Amines

Derivative	Detection	Reference
OPA (Orthophthadlehyde)	Flourescence	34,35
DABITC (4-N,N-dimethylaminoazo benzene-4-isothiocyanate)	Visible	42,43
Dabsyl Chloride (4-N,N-dimethylaminoazo benzene-4-sulfonylchloride	Visible	41
Dansyl Chloride (5-N,N-dimethylamino naphthalene-1-sulfonylchloride)	Fluorescence Visible	37
Ninhydrin	Fluorescence	18
PITC (Phenyl isothiocyanate)	UV	34
SNPA (N-succinyl-p-nitrophenyl acetate)	UV	40
Fluorescamine	Fluorescence	38
9-Fluorenylmethyl chloroformate	Fluorescence	39

which contained tyramine and histamine and other amines not included in this review. Kiba,Mizano and Furusawa (44) provided information on the determination of catecholamines using catalytic photometric detection that could be applied to other related compounds . Table 4 provides a summary of some of the various derivatives that have been used or potentially could be used to accomplish the assay for the biogenic amines.

Summary

This overview has provided a summary of some of the various procedures used to isolated select biogenic amines from food including clean-up protocols and final HPLC determinant steps. As was indicated in the introduction it will not provide a treatise on this class or even provide information on all of the potential biogenic amines in food but does provide a review of the current technology and a starting place for interested researchers.

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