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CAPILLARY GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC DETERMINATION OF HISTAMINE IN TUNA FISH CAUSING SCOMBROID POISONING

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SUMMARY

A documented case of "scombroid poisoning" is reported by confirming the presence of high histamine levels in fresh tuna fish tissues by gas chromatography–mass spectrometry. Histamine is confirmed by *in situ* trimethylsilylation of the tissue extract on a 12-m SP-2100 fused-silica capillary column connected directly to a mass spectrometer ion source operated in the positive ion chemical ionization mode using methane as reactant gas. The trimethylsilyl derivative of histamine is readily resolved by the high-resolution fused-silica capillary column and confirmed in the complex tissue extract by this very simple but specific procedure.

INTRODUCTION

Histamine toxicity from fish products, clinically called "scombroid poisoning", generally involves the ingestion of scombroid fish from the families Scomberesocidea and Scombridae[1]. Scombroid fish include saury, tuna, bonito, seerfish, butterfly kingfish and mackerel. These fish usually contain free histidine in their muscle tissue[2] that under certain conditions can be decarboxylated by some bacteria to produce high levels of histamine. This can occur with improperly refrigerated fresh fish[3]. Human consumption of both "fresh" and processed fish having significant levels of histamine has resulted in systemic histamine toxicity causing clinical illness. Controversy still remains whether histamine alone produces scombroid toxicity, but it is generally agreed that a distinct correlation exists between high histamine levels in tissues of ingested fish and clinical toxicity[1,3].

A review of the literature reveals that fluorometry has evolved as a major tool

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for histamine determination in fish[4]. A sequence of improved extraction and detection methods began with Sager and Horwitz[5] and continued with Ota[6], Kawabata *et al.*[7] and Lerke and Bell[8]. Recently Staruszkiewicz *et al.*[4] optimized the fluorometric method using a rapid ion-exchange cleanup step to minimize interference by endogenous materials.

Although gas chromatographic (GC) analysis of standard histamine was reported by Fales and Pisano in 1962[9] and later as a heptafluorobutyric acid anhydride (HFBA) derivative by Navert[10], there are no reports of GC or GC–mass spectrometric (MS) analysis for histamine from complex matrices such as tuna fish. An improved method for detection and quantification of histamine in fish tissue could substantially improve the ability to detect and verify cases of scombroid poisoning[1]. We report a new analytical method for the analysis of histamine in tuna fish based upon trimethylsilylation using a high-resolution fused-silica capillary column interfaced directly to a mass spectrometer operated in the positive ion chemical ionization (PCI) mode.

EXPERIMENTAL

Materials

Histamine and histidine standards were purchased from Aldrich (Milwaukee, WI, U.S.A.) and used without further purification. N,O-Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) was purchased from Pierce (Rockford, IL, U.S.A.) and diluted 10:1 in reagent-grade ethyl acetate. All other solvents were reagent grade or better and used without further purification.

Apparatus

The GC–MS system was a Hewlett-Packard 5985B (Hewlett-Packard, Palo Alto, CA, U.S.A.) equipped with a 12 m × 0.24 mm I.D. SP-2100 fused-silica high-resolution capillary column (Hewlett-Packard, Avondale, PA, U.S.A.). The exit of the fused-silica capillary was positioned at the entrance to the MS ion source by "snaking" the flexible capillary through a 1/16-in. stainless-steel transfer line tubing positioned in the GC–MS interface oven. A vacuum seal around the capillary column was provided by a 0.4 mm I.D. graphite ferrule (LC Co., Schaumburg, IL, U.S.A.) placed in a symmetrical 1/16-in. stainless-steel Swagelok union (Crawford Fitting, Solon, OH, U.S.A.; Cat. No. 100-6) attached to the stainless-steel transfer line tubing in the GC oven.

The standard Hewlett-Packard capillary column injector was operated in the splitless mode at 200°C. All GC conditions consisted of an initial oven temperature of 100°C with temperature programming at 20°C/min 1 min post injection to a maximum oven temperature of 250°C. The GC–MS transfer line oven was maintained at 275°C and the MS ion source temperature at 200°C. The mass spectrometer was operated in the PCI mode using methane reagent gas at 0.5 Torr ion source pressure. The electron energy was set at 230 eV with an emission current of 300 μA.

Extraction procedure for tuna fish

Toxic tuna fish was obtained from a patient who experienced scombroid poisoning. Control fresh tuna was obtained from a local market. Both fish were pan fried by the patient and frozen. Ten grams each of control and toxic tuna fish were

blended separately in 30 ml methanol on a Waring blender (Waring, New Hartford, CT, U.S.A.) for 30 sec. Each sample was transferred to a 23 mm × 148 mm glass test tube and homogenized on a Brinkmann Polytron homogenizer (Brinkmann, Westbury, NY, U.S.A.) at full speed for 2 min. These tubes were centrifuged at 1500 *g* and the supernatent transferred to clean test tubes for storage. After adjusting the volume to 20 ml, aliquots (100 μl) of each methanolic tuna fish extract were transferred to 16 mm × 125 mm glass test tubes and concentrated to dryness under a gentle stream of nitrogen at 65°C. The residues were taken up in 2 ml of ethyl acetate and 1 μl of this solution analyzed by *in situ* trimethylsilylation GC–MS by co-injecting 1 μl of a 10:1 dilution of BSTFA in ethyl acetate. The PCI GC–MS conditions used readily reveal the presence of the $(M + 1)^+$ ions for histamine or histidine present in these extracts. No additional sample cleanup was performed subsequent to methanol extraction of the fish tissue.

RESULTS AND DISCUSSION

A series of GC–MS experiments were carried out to determine the preferred method. Initially, electron impact ionization (EI) GC–MS on a 1 m × 2 mm I.D. glass column packed with 3% OV-101 was performed with 50 ng injected levels of standard histamine and histidine. After obtaining disappointing results analysing these materials neat, derivatization methods were tried. Methylation with trimethylanilinium hydroxide (TMAH) and trimethylsilylation with BSTFA revealed that the latter method was clearly preferred. However, molecular ion abundances with EI were very weak or absent at trace levels with fragmentation dominated by β-cleavage of the side chain nitrogen atom. In addition, packed-column GC–MS of the fish extracts under temperature programming conditions produced unresolved interfering components at the retention times for histamine.

Capillary GC–MS of the trimethylsilyl (TMS) derivatives of histamine and histidine under PCI conditions proved to be the method of choice. The high-resolution capillary column provided sharp, well resolved chromatographic peaks, the $(M + 1)^+$ ions for the TMS derivatives for both histamine and histidine were abundant, and the minimum detectable limit (MDL) for histamine was 1 ng injected in the full-scan MS mode. Spiked fish tissue homogenates produced standard curves linear to 5 mg histamine per gram of fish tissue with the above dilution. Negative ion chemical ionization (NCI) capillary GC–MS experiments did not provide molecular weight information and were therefore discounted.

The PCI capillary GC–MS extracted ion current profiles (EICP)[11] for a mixture of standard histamine and histidine TMS derivatives provided the mass spectra shown in Fig. 1. The molecular weights for the histamine di-TMS derivative and the histidine tri-TMS derivative are observed as the $(M + 1)^+$ ions at m/z 256 and m/z 372 respectively. The diagnostically useful $(M + 29)^+$ and $(M + 41)^+$ (ref. 12) ions are also present providing added verification for the molecular weights of 255 and 371 for the di-TMS and tri-TMS derivatives for histamine and histidine respectively. The well known loss of a methyl group from TMS derivatives[13] and other logical neutral losses supporting the structural identity of each derivative are also readily apparent.

The capillary column total ion current profiles (TICP)[11] shown in Fig. 2 were obtained from *in situ* trimethylsilylation of diluted tissue extracts of control tuna

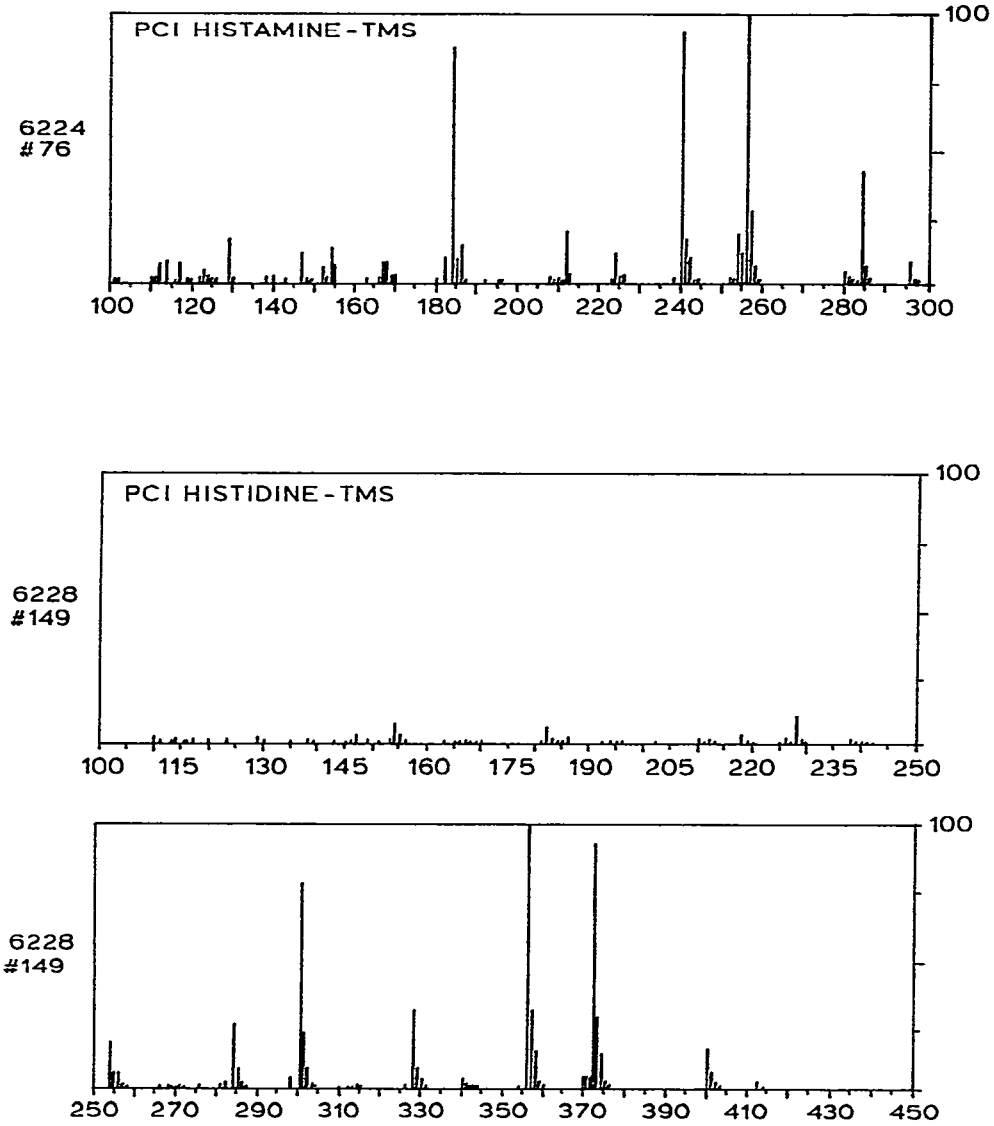

Fig. 1. PCI mass spectra of authentic histamine di-TMS derivative (upper panel) and histidine tri-TMS derivative (lower two panels) obtained by fused silica capillary GC–MS using methane as reagent gas.

(upper) and toxic tuna (lower) in the PCI GC–MS mode using methane as CI reagent gas. The other chromatographic peaks are unidentified components of the control tuna extract.

Inspection of the EICP (not shown) for the $(M + 1)^+$ ion for the TMS derivative of histamine (m/z 256) in Fig. 2 suggests that the chromatographic component centered at retention time 8.2 min is the sought after histamine component. The PCI mass spectrum for this component was identical in all respects to the mass spectrum

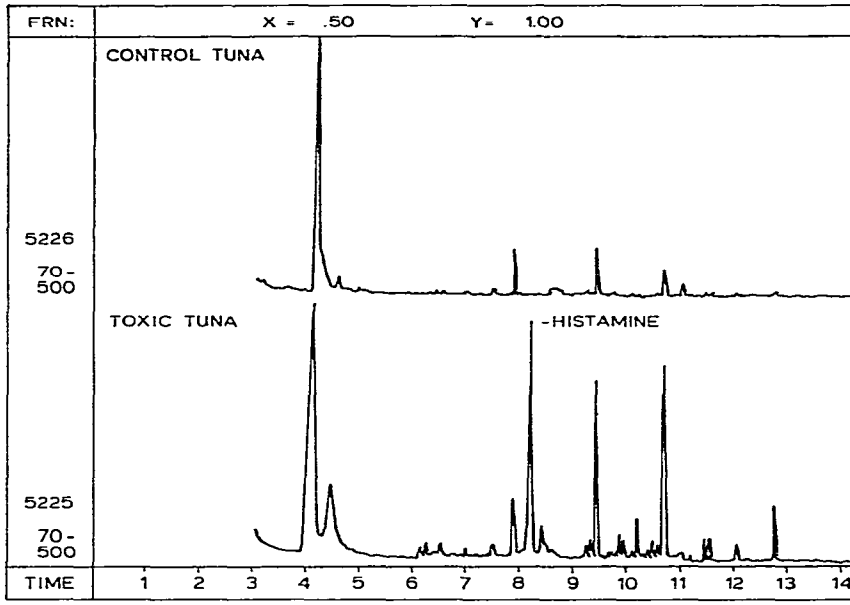

Fig. 2. Total ion current profiles from PCI fused silica capillary GC–MS analysis of control tuna fish (upper) and toxic tuna fish (lower). The component with retention time 8.2 min in the TICP of toxic tuna fish is the di-TMS derivative of histamine.

of the authentic histamine TMS derivative shown in Fig. 1. Thus PCI capillary GC–MS analysis of a one-step methanolic tuna fish extract provides unequivocal identification of histamine in this case of scombroid tuna poisoning.

The capillary GC–MS technique described here has the advantage that the use of shorter columns makes possible a considerable reduction of the analysis time, and at the same time the separation of the components is superior to that obtainable with packed columns. With the 12-m capillary column used in this work all fish extract components had eluted within 15 min.

The sensitivity of the procedure is sufficient for the detection of 10^{-9} g levels of histamine in fish extracts, but this clearly is not necessary since this material is usually present at much higher levels in scombroid poisonings. We prefer the splitless injection mode for routine determinations although the high levels of histamine found in scombroid poisonings would ordinarily preclude this injection technique. Appropriate dilution of the extract easily avoids overloading the capillary column.

The high-resolution chromatographic capability of the capillary columns coupled with MS specificity, an extremely simple tissue extraction procedure, and short analysis time provides an improved method for unequivocal histamine determination. Quantitative determination of histamine is readily accomplished by the method of standard additions of histamine to the control tuna homogenate. The high-resolution chromatography capability minimizes interfering additive effects that are often present in complex matrices.

The significance of capillary GC–MS analysis of scombroid tuna fish lies in the

possibility of detecting and identifying the elusive "saurine" component(s) that have been sought after for so long[1,3]. It has been suggested that trace levels of this unknown substance may act with histamine in a synergistic manner to cause the dramatic clinical effects produced from scombroid poisoning[3]. Histamine is the only substance discovered that correlates with scombroid toxicity. This, however, may be due to inadequate sensitivity and/or specificity afforded by analytical techniques reported thus far. High-resolution capillary GC–MS possesses superior resolving power that may allow detection and identification of additional unknown components. Preliminary results indicate some distinct differences in the chemical composition of control *versus* toxic tuna extracts. The results of our continuing investigation will be reported subsequently.

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