Analytical Methods

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Journal:	*Analytical Methods*
Manuscript ID	AY-ART-05-2016-001297
Article Type:	Paper
Date Submitted by the Author:	03-May-2016
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A new atomic absorption-based method development for indirect determination of histamine in fish samples, dairy products, and alcoholic beverages by flame AAS

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Abstract

A new and sensitive analytical method for the indirect determination of histamine in fish samples, dairy products, and alcoholic beverages by flame atomic absorption spectrometry (FAAS) was developed. The method is based on the complex formation of histamine with Fe(III) and 2′,4′,5′,7′-tetrabromofluorescein (eosin) at pH 4.5, and then extraction into the micellar phase of polyethylene glycol dodecyl ether (Brij 35). In this study, ultrasonic assisted cloud point extraction (UA-CPE) procedure was used for separation/preconcentration of histamine from the related sample matrices. The optimal conditions were established, and a good preconcentration was achieved using 25 μmol L^{-1} Fe(III), 5 μmol L^{-1} eosin, 10 mmol L^{-1} Brij 35 and 0.7 % (w/v) Na_2SO_4, pH 4.5 citrate buffer, equilibrium temperature of 45 ^oC, incubation time of 20 min and ultrasonic effect of 40 kHz at 300 watt. At optimal conditions, a detection limit of 0.25 μg L^{-1} with a sensitivity improvement of 143-fold in linear working range of 0.8–170 μg L^{-1} was obtained. The proposed method was evaluated for the analysis of some food samples and received a good recovery with the standard addition assay. In addition, the validity of the method was tested by intra- and inter-day precision studies and recovery experiments, obtaining satisfactory results. To our knowledge, this method is also the first study for indirect determination of histamine in the selected samples using UA-CPE coupled to FAAS.

Keyword: Eosin, Fe(III), Indirect Determination, Histamine, Food, Preconcentration, Atomic-Based Method

1. Introductıon

Histamine is known as a biogenic amine, which is low molecular mass and possesses biological activity for its complex physiological role in human body.[1] The compound is essential in humans owing to its important role in functioning of physiological processes, such as neurotransmission, allergic reactions, microcirculation regulation.[2]

In particular, the histamine easily consists in fermented foods and beverages. This formation can be explained as follows. It is thought that histidine is transformed to histamine during fermentation in response to the decarboxylation reaction of lactic acid bacteria.[3] The consumption of vegetables, fermented foods and certain fish species containing a large amount of histamine has been implicated in causing allergy-like food poisoning known as scombroid poisoning.[4]

Low amounts of histamine in real samples are normally present and are not considered as a serious health risk for human. Increased levels of histamine in the real samples are most often related to inappropriate food processing or storage, and might be a good indicator of hygienic food quality.[1]

The any food sample is unsuitable for consumption when the histamine amount exceeds 100 μg g^{-1}.[5] When consumed above this amount, it can cause to adverse health effects such as respiratory disorders, abdominal cramps, vomiting, diarrhea and itching of the skin.[6] Moreover, the monitoring of histamine has now been globally accepted for safety confirmation of fish and seafood samples.[7] For all these reasons, a simple, inexpensive and

rapid analytical method is now required for the accurate and reliable monitoring of histamine in the related samples all over the world.

Various instrumental methods including flow injection analysis (FIA),[8] capillary zone electrophoresis (CZE) with fluorescence detection,[9] ion-exchange chromatography with conductivity detection,[10] spectrophotometry,[11] amperometric biosensor,[12] nuclear magnetic resonance (NMR),[13] tandem mass spectrometry (MS/MS),[14] capillary electrophoresis coupled to laser-induced fluorescence detection,[15] flow injection-capillary electrophoresis-mass spectrometry (FI-CE/MS),[16] high performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS),[17] thin-layer chromatography (TLC)/densitometry,[18] cyclic voltammetry (CV),[19] ion mobility spectrometry,[20] optical waveguide light mode spectroscopy (OWLS)-based immunosensor, [21] ELISA and HPLC with fluorimetric detection [22] have been proposed for separation, preconcentration and determination of the histamine from various real samples. As seen from the above methods, there is no study indirectly for the determination of histamine until now. By considering this lack, flame atomic absorption spectrometry (FAAS) was used for the indirect determination of histamine in this study. Flame AAS is easy to use, high precision, inexpensive, well established and well known detection technique with high sample throughput, which is widely used for the determination of trace metals. In order to achieve indirect analysis using FAAS, the analyte must linearly be associated to a metal signal. In this sense, a method was proposed by our research group for the indirect analysis of sulfite in food matrices using FAAS.[23]

However, the determination of analyte at trace level by FAAS is limited not only by insufficient sensitivity, but also by matrix interference especially in the selected samples.[24] In order to overcome these disadvantages, preconcentration procedure is needed to achieve accurate and reliable results at trace levels prior to determination of analyte by FAAS.[25] The preconcentration procedures including liquid–liquid extraction (LLE) and co-precipitation

suffer from limitations, such as significant chemical additives, solvent losses, complex equipment, large secondary wastes, unsatisfactory preconcentration factors and high time consumption, that limit their applications.[26] A new trend in analytical chemistry is miniaturization of preconcentration techniques to reduce the consumption of reagents and containing of green chemistry properties.[27]

In this study, ultrasonic assisted cloud point extraction (UA-CPE) procedure containing the above features was used for preconcentration of histamine from the related matrix prior to determination by FAAS. The UA-CPE is widely used because of its simplicity, effectively, fast, inexpensive, low consumption of reagents and the ability to combine with different detection techniques whether in on-line or off-line mode. In addition, surfactants are used to separate hydrophobic complexes of analyte from the aqueous phase under ultrasonic effect. The surfactants circumvents the use of volatile organic solvents which have a negative impact on the environment and human health.[28]

In this research, we proposed a simple UA-CPE procedure for the preconcentration and indirect determination of histamine in presence of Fe(III) using 2′,4′,5′,7′-tetrabromofluorescein (eosin) as complexing agent and polyethylene glycol dodecyl ether (Brij 35) as extracting agent by FAAS. As improved extraction procedure, the UA-CPE has not been widely applied especially for analysis of histamine by FAAS.

The main parameters influencing complex formation and extraction such as the sample pH, metals type and concentrations, chelating agent concentration, surfactants type and concentration, sample volume, salt effects, ultrasonic conditions, and extraction time were investigated and optimized in detail. The validity of proposed method was checked by intra- and inter-day precision studies and recovering experiments. To the best of our knowledge, the proposed method is for the first time used for indirect determination of histamine in fishes, dairy products and alcoholic beverages.

2. Experimental

2.1. Apparatus

The indirect determination of histamine were performed on the apparatus from the Shimadzu AAS-6300 model (Tokyo, Japan) flame atomic absorption spectrometer equipped with a deuterium background correction and hollow cathode lamp of iron as the radiation source. The instrumental operating conditions of the FAAS spectrometer for iron were wavelength, 248.3 nm, lamp current, 12 mA, slit width, 0.2 nm and burner height 7.0 mm. The absorbance measurements were carried out using an air/acetylene flame at flow rates of 18 and 2.2 L min^{-1}.

To ensure cloud point temperature (CPT), CPT of nonionic surfactants in aqueous solutions was carried out in an ultrasonic bath (UCS-10 model, Seoul, Korea) with a good temperature control within 1.0 ^{o}C. The Universal Hettich Centrifuge (London, England) with 50-mL calibrated tubes was used to accelerate the phase separation assays. A refrigerator was used to keep the chemicals and real samples fresh and cool till the analysis. The pH adjustments in the aqueous solutions were measured with Selecta 2001 (Sartorius Docu-model, North America) glass- calomel electrode pH meter.

2.2. Chemicals and reagents

Ultra-pure water (18.2 MΩ cm) was produced by a commercial mixed-bed ion-exchange purification system Labconco (Kansas City, USA) fed with distilled water. All chemicals were of analytical grade purchased from Merck Company (Darmstadt, Germany) and Sigma (St. Louis, MO. USA). A 1000 mg L^{-1} of Fe(III) stock solution was prepared by dissolving 0.18 g of $FeCl_3$ supplied from Sigma in water. A stock solution 0.03 mol L^{-1} of histamine was prepared by dissolving 5.52 g of its dihydrochloride in water and diluting to 1.0 L.

The working solutions were prepared daily by serial dilutions of the stock solutions. The non-ionic surfactant, a 1.0×10^{-3} mol L^{-1} of Brij 35 solution was prepared with a mixture of the water and ethanol from Merck. A 2.0×10^{-4} mol L^{-1} of eosin was prepared by dissolving appropriate amount of reagent (Sigma) in 50.0 mL of 95 % ethanol and diluting to 100.0 mL with the water in a volumetric flask. Store at room temperature in dark. The pH of the model solutions was adjusted to pH 3–6 with citrate buffer solution. Before starting the experiment, all glassware and plastic bottles used were cleaned by rinsing with the water, soaking with 10 % (v/v) HNO_3 solution and dilute HCl (1.0 mol L^{-1}) for one day and then rinsing five times with the water.

2.3. Recommended procedure

For UA-CPE, an aliquot of the solution containing in the concentration range of 0.8–170 µg $L^{-1}$of histamine was transferred into a centrifuge tube of 50 mL containing 0.3 mL of pH 4.5 citrate buffer, 25 µmol L^{-1} Fe(III) and 5 µmol L^{-1} eosin as chelating agent. In order to obtain a hydrophobic stable complex containing iron, the solution was then allowed to stand for 10 min at room temperature. At the end of this time, a 10 mmol L^{-1} Brij 35 and 0.7 % (w/v) Na_2SO_4 solution were added and made up to the mark with the water. To perform the cloud event of nonionic surfactant, the solution was left in an ultrasonic bath (40 kHz) for 10 min at 45 ^{o}C.

In order to accelerate separation of two phases, the cloudy solution was then centrifuged for 5 min at 4000 rpm. After being cooled into a refrigerator during 5 min, the surfactant rich phase became a viscous gel at the bottom of tube, and the phase was separated by inverting of the tube from the aqueous phase. The phase obtained in this process results is very viscous and low volume for analysis. Thus, it was completed up to 1.0 mL using HNO_3 in methanol (1.0 mol L^{-1}) solution.

Finally, histamine contents of calibration solutions and samples were determined by making either direct calculation from the calibration curve obtained by FAAS depending on the change in iron signal or using standard additions approach. To determine signals resulting from the used reagents, a blank solution was also run under the same experimental conditions in parallel without adding any histamine.

2.4. Pre-treatments for samples

Different fish samples (anchovies, canned tuna, canned sardines, and canned mackerels), dairy products (goat cheese, sheep's cheese, mild cream, ricotta, whole milk and strawberry milk), and alcoholic beverages (three beer, two white and two red wine), which have been investigated in order to assess the applicability of the proposed method, were acquired from local markets in Sivas, Turkey. Depending on the complexity of the sample matrix, extraction and pre-treatment steps can be necessary prior to indirect determination of histamine.

In sample preparation for dairy products, firstly, 20 g of all cheese samples were ground prior extraction using an electric blender. The samples were weighed directly in a centrifuge tube of 50 mL. In order to provide extraction efficiency of histamine from cheese, 25 ml of solvent, including 5.0 mL of methanol, 2 mL of 5 0 % (v/v) H_2O_2 , 10 mL trichloroacetic acid (TCA, 5.0 %, w/v) and 3 mL of 3.0 mol L^{-1} HCl solution were added to the samples. In order to perform ultrasonic extraction, the resulting mixture was placed in an ultrasonic bath, and subjected to ultrasonic effect for 10 min at 60 ^{o}C.[1] The resulting solution was left to cool down, and then was centrifuged at 4000 rpm for 15 min, filtered using 0.45 μm filter paper.

In sample preparation for fishes, the samples were analyzed immediately after opening. Experimental steps were carried out as follows: (1) The samples were sequentially homogenized in an electric blender, divided into several samples of 10 g, and were placed in

50 mL centrifuge tube. (2) The volume of samples was then completed to 50 mL using TCA (5.0 %, w/v). (3) In order to perform ultrasonic extraction, the resulting mixture was further assisted by ultrasonication for 20 min at 40 ^{o}C, left to cool down, and followed by centrifugation for 10 min at 4000 rpm. (4) The resulting solutions were filtered using 0.45 μm filter paper. (5) The final volume was completed to 100 mL with the water prior to analysis.[29]

In sample preparation for alcoholic beverages, initially, 1.0 mL of 0.5 % (v/v) 1-octanol solution as antifoam was added to 10 mL of the wine and beer samples to prevent foaming, and they were degassed for 2 min using an ultrasonic bath. Later, a 0.5 g of polyvinylpyrrolidone (PVP) was added to the samples in 50 mL centrifuge tube. The resulting mixtures were placed in an ultrasonic bath under sonication effect (with ultrasound frequency of 40 kHz at 300 W) for 10 min at 30 ^{o}C, and then were centrifuged for 10 min at 4000 rpm. The resulting solutions were finally filtered through 0.45 μm filter paper, then diluted 2-times with the water.[30]

Each sample was processed in triplicate. 2.5 mL of the all samples prepared was applied for indirect determination of histamine using the proposed method. Data processing and all statistical calculations (ANOVA) were performed using computer program Excel 2010 (Microsoft Office®). The resulted data of three independent replicates were expressed as means ± SD. Differences were statistically considered significant at values of $P < 0.05$.

3. Results and discussion

In this study, the recoveries of histamine were evaluated to determine the optimal parameters affecting the preconcentration step. For this purpose, preliminary investigations were carried out in various analytical variables (pH, metals type and concentrations, chelating agent concentration, surfactants type and concentration, sample volume, salt effects, and ultrasonic

conditions) in order to estimate their influence on recovery % of the histamine. The recovery % was calculated by the following equation (Eq. (1)):

$$Recovery\ \% = \frac{C_x V_x - C_y V_y}{C_x V_x}\ 100 \qquad (Eq.(1))$$

Where C_x symbolizes the concentration of histamine in the initial sample volume V_x, and C_y, symbolizes the concentration of histamine in the surfactant rich phase of volume V_y.

3.1. Optimization of the variables affecting complex formation and extraction efficiency

The pH value of the solution is an important factor that affects the redox and/or complexation behavior of the histamine, and may strongly influence the formation of hydrophobic ternary complex as follows:

$Fe(OH)^{2+} + HCitrate^{2-} \rightarrow Fe(OH)Citrate^{-}$ at pHs ≤ 3.5 (1)

$Fe(OH)_3 + HCitrate^{2-} \rightarrow Fe(OH)_2Citrate^{2-}$ at pH range of 4.0-7.0 (2)

$Fe(OH)_2Citrate^{2-} + HHis^{+} \rightarrow Fe(OH)(His)Citrate^{-} + H_2O$, binary complex formation in presence of histamine at pH 4.5 (3)

$Fe(OH)(His)Citrate^{-} + HL^{-}$ (Eosin) $\rightarrow Fe(His)L + HCitrate^{2-} + H_2O$, ternary complex formation in presence of eosin at pH 4.5 (4)

Given the importance of the pH in the interactions between chemical species, the effect of the pH on the percent recovery was investigated in the range of 3.0 to 11.0 in a model solutions containing 10 μg L^{-1} of histamine using different buffer solutions. The pH of the model solutions was adjusted to pH 3–6 with citrate buffer solution, pH 6-8 with K_2HPO_4/KH_2PO_4 buffer solution and pH 8-11 with Britton-Robinson buffer solution.

From the results, the best recovery of histamine was obtained with citrate buffer solution. Figure 1 presents the recovery rate of histamine. The pH change from 3.0 to 4.5 causes to higher recovery, so as to give a peak at pH 4.5 while the pH increase from 4.5 to 6.0 causes to lower recovery of histamine. Hence, a pH value of 4.5 was chosen as the optimal to perform further experiments. In addition, the precision (as RSD %) in working pHs as a measure of signal fluctuation for each pH were in range of 1.2-1.9 %.

From the comprehensive literature review results, [31-33] eosin, which is an anionic xanthene dye with pK_a values of 2.02 and 3.80,[34] was selected as the complexing agent. Effect of concentrations of eosin on the recovery of histamine was investigated in range of 0.1-10 μmol L^{-1} in presence of Fe(III). As shown in Figure 2, by increase in the eosin concentration from 0.1 to 5.0 μmol L^{-1}, the recovery of histamine gradually increased. At higher concentrations, there was no significant change. Hence, a concentration of 5.0 μmol L^{-1} was chosen as the optimal one to perform further experiments. In addition, the precision as RSD % in working concentrations were in range of 1.7-2.0 %.

In order to ensure indirect determination of histamine by FAAS, firstly, signal change of any metal in presence of histamine should be utilized. For this purpose, preliminary experiments were carried out with metal ions including Fe(III), Cu(II) and Sn(IV) at isomolar concentrations. As a result of studies, the stable maximum signal was obtained with Fe. This behavior is an indicator of highly selective and sensitive affinity of Fe(III) to citrate (log β: 19.8 in pH range of 2.5-6.5) and histamine including eosin, so as to give especially pH dependent stable complexes with citrate as a component of buffer in aqueous micellar media.[35-37] Also, it was observed that Fe^{3+} ions gave a stable complex with chlorphenamine having a similar structure to histamine at pH 6.0 (log β: 3.6 at ratio of 1:1).[38]

Thus, the effect of concentration of Fe(III) ions on the recovery of histamine was investigated in range of 1-60 μmol L^{-1}. The results are shown in Figure 3. In the Eosin-

Fe(III)-Brij 35 system in presence of histamine, when Fe(III) concentration is in the range 1-25 μmol L^{-1}, the recovery of histamine reached to maximum value at 25 μmol L^{-1} and then remained constant. Hence, Fe(III) concentration of 25 μmol L^{-1} was chosen as the optimal to perform further experiments. In addition, the precision as RSD % in working concentrations were in range of 2.2-2.5 %.

The non-ionic surfactant, Brij 35 was selected to form micelle centers in aqueous solutions because of its low toxicological properties and cost. Also, Brij 35 was successfully used in indirect determination of histamine by square wave stripping voltammetry [39] and as micellar phase in determination of other biogenic aminoacids including histamine by non-ionic micellar electrokinetic chromatography with laser-induced fluorescence.[40-41] The concentration of the non-ionic surfactant affects not only the recovery, but also the volume of surfactant-rich phase. The effect of its concentrations on the recovery of histamine was carefully investigated in the range of 1–30 mmol L^{-1}.

As shown in Figure 4, it was found that the recovery of histamine increased rapidly with the increasing Brij 35 concentrations from 1 to 10 mmol L^{-1}, and after that remarkably decreased with the further increasing of the concentration of Brij 35. Hence, Brij 35 concentration of 10 mmol L^{-1} was chosen as the optimal to perform further experiments. In addition, the precision as RSD % in working concentrations were in range of 1.8-2.3 %.

The cloud point of micellar solutions can be controlled by addition of inorganic salts solution. In addition, ionic strength improves the phase separation due to the breakage of hydrogen bond between molecules. To investigate these effects, the effect of ionic strength on the recovery of histamine was tested by adding different concentrations of Na_2SO_4 in range of 0.01–1.5 % (w/v). The results obtained are shown in Figure 5. It was observed that the recovery of histamine increased rapidly with the addition of Na_2SO_4 within 0.01–0.7 % (w/v), and at higher values had no significant change. Hence, Na_2SO_4 concentration of 0.7 % (w/v)

was chosen as the optimal one to perform further experiments. In addition, the precision as RSD % in working concentrations were in range of 1.9-2.5 %.

The sample volume is the one of the analytical parameters for obtaining high preconcentration factor, due to be the low amounts of histamine in the real samples. To evaluate the effect of sample volume, seven different volumes (5, 10, 15, 20, 30, 40 and 50 mL) of sample solutions were investigated by using models under optimum conditions. As can be seen in Figure 6, the recovery of the histamine remained stable up to 30 mL sample volume. Above this volume, the recovery of the histamine decreased. To achieve high preconcentration factor, sample volume of 10 mL was chosen as the optimal one to perform further experiments. In addition, the precision as RSD % in working volumes were in range of 1.6-2.5 %.

3.2. Optimization of the ultrasonic bath conditions

In this study, ultrasonic bath was used, instead of the conventional heating and incubation under ultrasonic effect. The ultrasound extraction time (UET) plays an important role in the preconcentration process, and defined as interval time between additions of the nonionic surfactant before starting the centrifugation. The ultrasonic effect can accelerate the interaction between hydrophobic complex and micelles, and consequently the complex containing analyte could be easily extracted into the surfactant-rich phase. The effect of UET on the recovery of histamine was investigated in the range of 2-30 min under ultrasonic effect (300 W, 40 kHz). As can be seen in Figure 6, the best recovery of histamine could be obtained since 10 min and longer extraction times did not significantly improve the recovery. Hence, the UET of 10 min was chosen as the optimal to perform further experiments. In addition, the precision as RSD % in working interval was in range of 1.9-2.3 %.

When the preconcentration procedure was processed at equilibration temperature of the surfactant, the quantitative recovery of histamine was achieved. If the temperature of the experimental medium is lower than the cloud point, the phase separation is difficult to be formed, but the formed complexes can reversibly be dissociated at higher temperatures. For these reasons, it is important to determine the optimum temperature of the ultrasonic bath. The effect of optimum temperature on the recovery of histamine was investigated in the range of 30-70 ^{o}C.

As can be seen in Figure 7, an increase in the recovery from 30 ^{o}C to 45 ^{o}C, followed by a decrease as the temperature increased. Hence, the optimum temperature of 45 ^{o}C was chosen as the optimal one to perform further experiments. In addition, the precision as RSD % in working temperatures was in range of 1.7-2.2 %. To optimize centrifugation conditions, centrifugation rate (in range of 500–4000 rpm) and time (in range of 2–25 min) were also investigated under optimal conditions. It was found that time of 5 min at 4000 rpm was enough for quantitative recovery of histamine.

3.3. Analytical performance

The analytical features of the proposed method, including detection limit, quantification limit, correlation coefficient, linear range, calibration equation, recovery rate, and precision, preconcentration and sensitivity enhancement factors were determined to evaluate quality method performance under optimized condition. The figures of merit of the proposed method are represented in Table 1. The regression equation established for five replicate measurements of each point was highly linear with a correlation coefficient (r) of 0.9920 in the concentration range of 0.8–170 μg L^{-1}. The limits of detection (LOD) and quantification (LOQ) were evaluated using the blank signals and their standard deviations, and calculated based on the signal at intercept and three and ten times the standard deviation about regression of the calibration curve, respectively.[42] The LOD and LOQ were 0.25 μg L^{-1} and 0.83 μg L^{-1}

respectively. The precision (as % RSD) as a result of five replicate measurements of 5 and 10 μg L^{-1} was ranged from 1.9 % to 4.8 %. The sensitivity enhancement factor expressed as the ratio of the final concentration of the histamine in the surfactant-rich phase to its concentration in the initial solution or ratio of slopes of the calibration curves with and without preconcentration was 143.

3.4. Study of interferences

The proposed method was developed using the standard model solutions. Therefore, the effect of foreign ions, which can be potentially found in real sample, should be investigated. Moreover, the foreign ions can be interact to form stable chelating complexes with eosin, Fe(III) and/or histamine at pH 4.5. The effect of foreign ions on the recovery of histamine were evaluated in presence of histamine according to the following systematic. 50 μg L^{-1} histamine at different interfering to analyte ratios in a centrifuge tube of 50 mL were subjected to the UA-CPE procedure.

The results of the investigated ions, including tolerance limits and recovery rates are shown in Table 2. An ion was considered as interfering when it caused a variation in the absorbance of the analyte greater than 5.0 %. The recovery rate is calculated as the ratio of signal of each experiment including the foreign ion plus histamine, and the corresponding experiment performed only with histamine, and was found to be in range of 93.1-102.9 % with a standard deviation in range of 1.2-2.2 %.

3.5. Method validation and analysis of real samples

We have not a certified reference material (CRM) for the histamine, therefore the accuracy (as % recovery) and precision (as % RSD) of the method were evaluated using standard addition method and intra-day/inter-day repeated studies. At the optimized reagent conditions, the intra-day/inter-day studies was evaluated by analyzing three real samples which were

prepared just according to the "pre-treatments for samples". This study was carried out as follows: The method was repeated five times on the same day to evaluate intra-day variability, and was repeated on five consecutive days to determine inter-day variability. The results are summarized in Table 3. It was found that a good agreement was obtained between the intra-day and inter-day for the amounts of histamine.

In addition, the intra-day precision as RSD % ranged from 1.9 to 4.2 % while the inter-day precision as RSD ranged from 2.3 to 4.8 %. In addition, the standard addition method for determination of recovery of histamine from spiked samples were carried out in five replicates of fishes, dairy products and alcoholic beverages containing 5 μg L^{-1} and 20 μg L^{-1} of histamine. The recovery values for histamine were in the range of 95.3–102.9 %. These values were quantitative, and it shows that the method can be applied for the preconcentration of histamine in the selected samples.

After evaluating the validity parameters, this method was applied to the determination of histamine in fishes, dairy products and alcoholic beverages, and the results are tabulated in Table 4. The each sample was conducted and analyzed in five replicates. From the results, the recoveries from spiked solutions at levels of 5 and 20 μg L^{-1} are quantitatively varied in the range of 97.3-102.6 % with RSDs of 1.9-3.8 % for fish samples, in the range of 95.3-103.4 % with RSDs of 2.2-3.9 % for dairy products, and in the range of 96.7-103.9 % with RSDs of 1.7-3.1 % for alcoholic beverages.

The lowest amount of histamine in fish, dairy products and alcoholic beverages was found in anchovies as 7.8±0.2 μg L^{-1}, in mild cream as 0.9±0.02 μg L^{-1} and in white wine as 5.1±0.1 μg L^{-1}, respectively. Finally, these results demonstrate the applicability of the proposed method for histamine evaluation in the selected samples. The recovery rates and RSDs of histamine, which is added to the samples, demonstrates the efficiency of the method.

3.6. Comparison with other methods

According to other analytical methods in literature, the advantages of the proposed method are explained by using a comparison table. The comparison of the method is discussed in Table 5 in terms of analytical capabilities, including linear working range, intra-day and inter-day precision RSDs %, recovery %, and limit of detection. The proposed method can also give lower detection limit and good RSDs % with a wide linear range when compared with previously published analytical methods.

The determination of histamine by the proposed method is obviously advantageous as compared to the traditional treatment processing (LLE and co-precipitation), related to pollution with toxic organic solvents that harmful to humans and the environment. One of the most important advantages of the method is the first study to detect indirectly histamine using FAAS. The proposed method can be an alternative to expensive techniques like ICP-MS, and chromatographic and electrophoretic methods with UV and/or fluorescence detection in terms of equipment used.

4. Conclusions

In this study, a new, rapid and green ultrasonic assisted cloud point extraction (UA-CPE) procedure was first time launch for the indirect determination of histamine in some foods and wines using flame atomic absorption spectrometry (FAAS). The method was adequate in terms of sensitivity to reach food and beverage guideline values, a LOD of 0.25 μg L^{-1}; precise with RSD lower than 4.8 %; recovery % in range of 95.3-103.9 %; and a high sensitivity enhancement factor of 143.

The proposed method is fast, sensitive, low cost, less time-consuming, eco-friendly, as well as easy-to-use approach to sample preparation. Validation of the method was carried out developing recovery and repeatability experiments for the real samples (fish, wine and milk)

obtaining acceptable results. Matrix effects were reasonably tolerable using complexing agents like EDTA and cation-exchange resin like Amberlite IR-120 before pre concentration when necessary. These results clearly showed that the current approach was considerably alternative for the determination of histamine in relatively complicated matrices.

Compliance with Ethics Requirements

Authors have no financial relationship with the organization that sponsored the research.

Conflict of Interest

Authors declare that he has no conflict of interest.

Ethical Approval

This article does not contain any studies with human or animal subjects.

Informed consent

On behalf of other authors, informed consent was obtained from all individual participants included in the study

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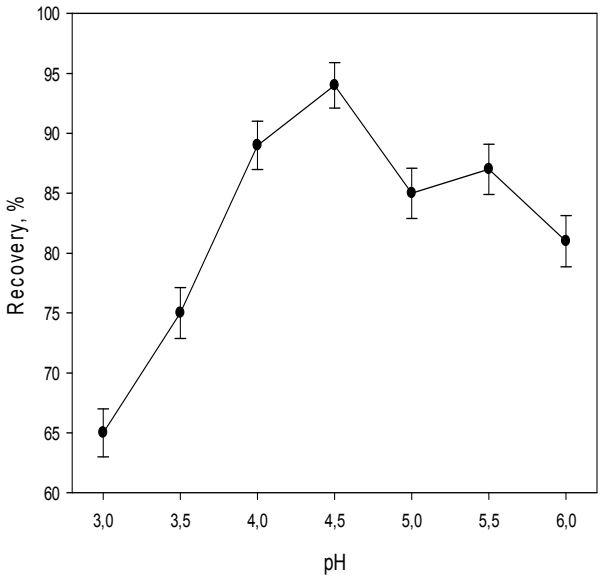

Figure 1. Effect of pH. UA-CPE conditions: eosin, 5 μmol L^{-1}; Fe(III), 25 μmol L^{-1}; Brij 35, 10 mmol L^{-1}; Na_2SO_4, 0.7 % (w/v), sample volume, 10 mL; ultrasound extraction time, 10 min; equilibration temperature, 45 ^{o}C; dilution solvent, acidic methanol of 1.0 mL. The error bars for average ± standard deviation of three trials.

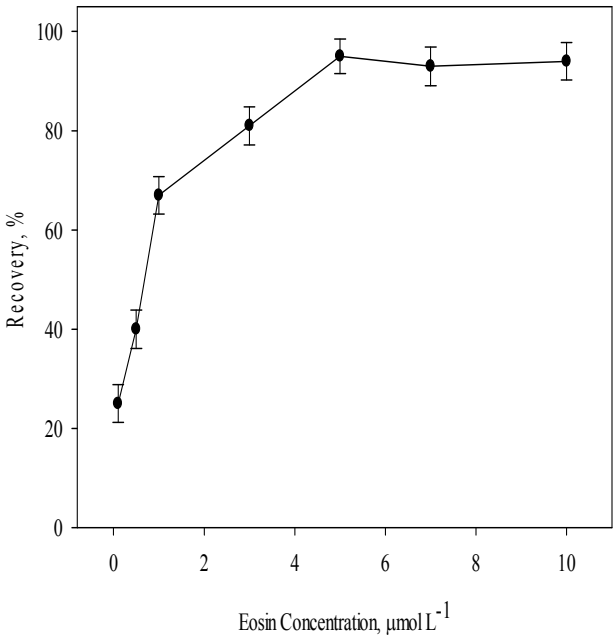

Figure 2. Effect of eosin concentration. UA-CPE conditions: pH 4.5; Fe(III), 25 μmol L^{-1}; Brij 35, 10 mmol L^{-1}; Na_2SO_4, 0.7 % (w/v), sample volume, 10 mL; ultrasound extraction time, 10 min; equilibration temperature, 45 ^{o}C; dilution solvent, acidic methanol of 1.0 mL. The error bars for average ± standard deviation of three trials.

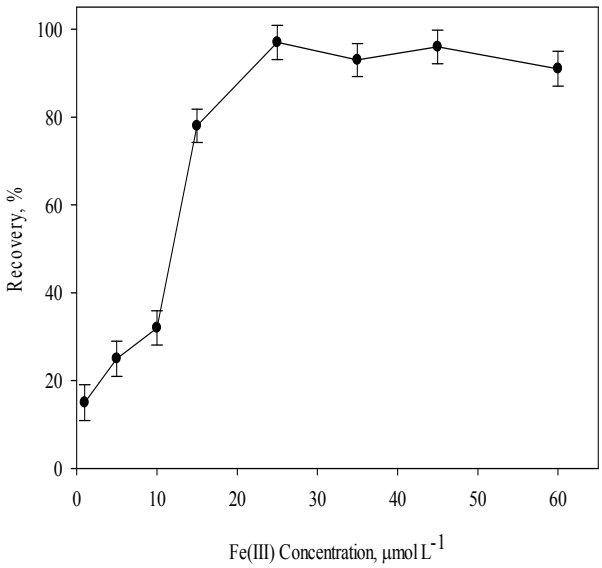

Figure 3. Effect of Fe(III) concentration. UA-CPE conditions: pH 4.5; eosin, 5 µmol L^{-1}; Brij 35, 10 mmol L^{-1}; Na_2SO_4, 0.7 % (w/v), sample volume, 10 mL; ultrasound extraction time, 10 min; equilibration temperature, 45 ^{o}C; dilution solvent, acidic methanol of 1.0 mL. The error bars for average ± standard deviation of three trials.

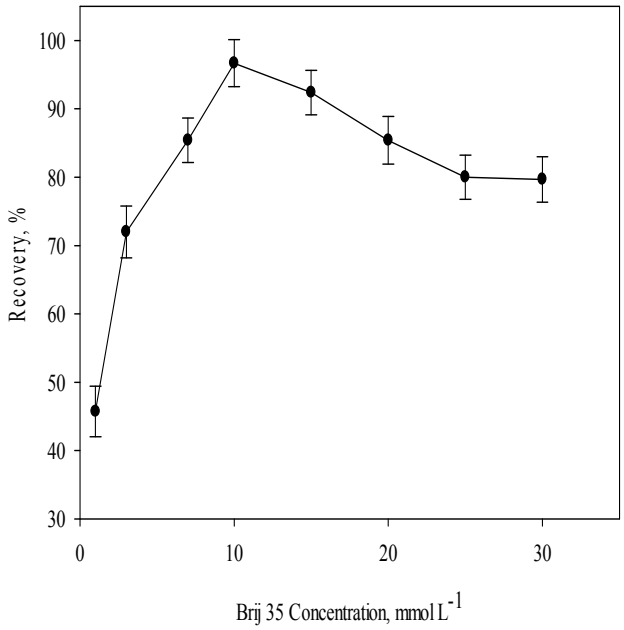

Figure 4. Effect of Brij 35 concentration. UA-CPE conditions: pH 4.5; eosin, 5 µmol L^{-1}; Fe(III), 25 µmol L^{-1}; Na_2SO_4, 0.7 % (w/v), sample volume, 10 mL; ultrasound extraction time, 10 min; equilibration temperature, 45 ^{o}C; dilution solvent, acidic methanol 1.0 mL. The error bars for average ± one standard deviation of three trials.

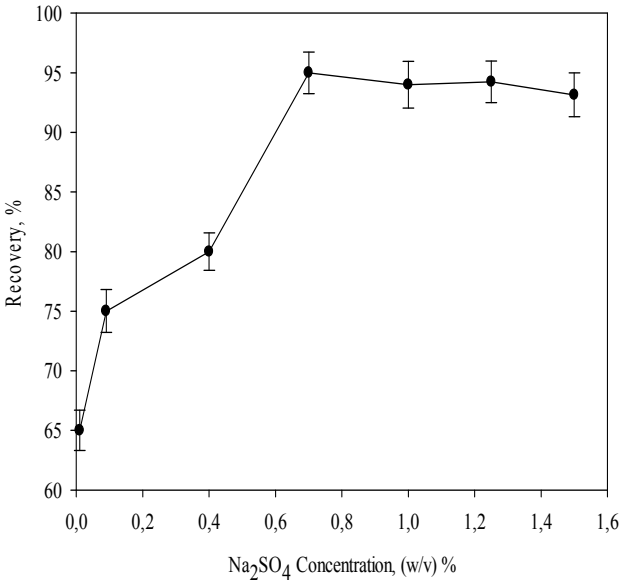

Figure 5. Effect of Na_2SO_4 concentration. UA-CPE conditions: pH 4.5; eosin, 5 μmol L^{-1}; Fe(III), 25 μmol L^{-1}; Brij 35, 10 mmol L^{-1}; sample volume, 10 mL; ultrasound extraction time, 10 min; equilibration temperature, 45 ^{o}C; dilution solvent, acidic methanol of 1.0 mL. The error bars for average ± standard deviation of three trials.

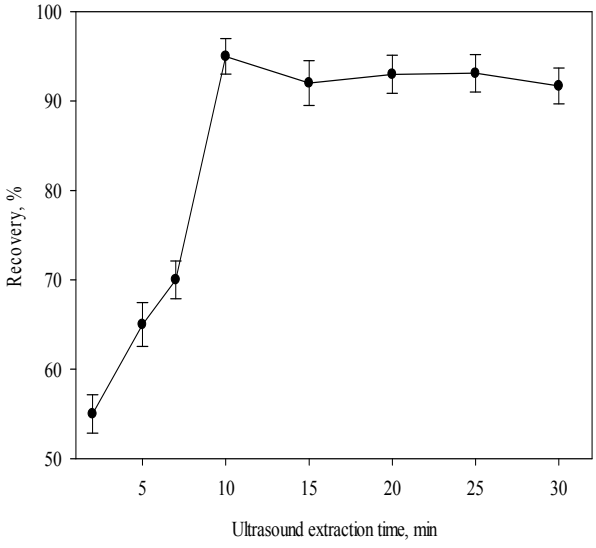

Figure 6. Effect of ultrasound extraction time. UA-CPE conditions: pH 4.5; eosin, 5 μmol L^{-1}; Fe(III), 25 μmol L^{-1}; Brij 35, 10 mmol L^{-1}; Na_2SO_4, 0.7 % (w/v), sample volume, 10 mL; equilibration temperature, 45 ^{o}C; dilution solvent, acidic methanol of 1.0 mL. The error bars for average ± standard deviation of three trials.

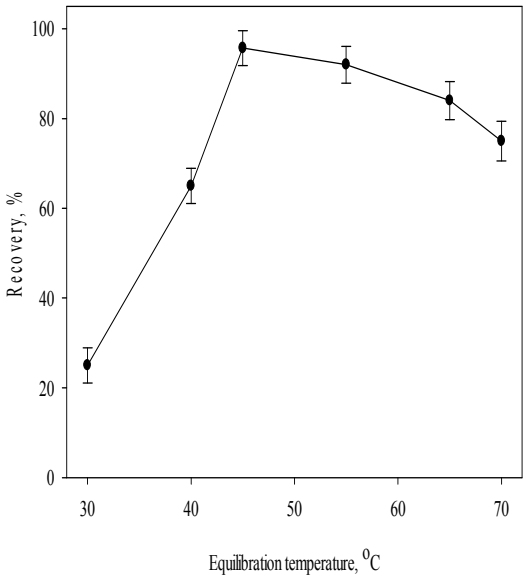

Figure 7. Effect of equilibration temperature. UA-CPE conditions: pH 4.5; eosin, 5 μmol L^{-1}; Fe(III), 25 μmol L^{-1}; Brij 35, 10 mmol L^{-1}; Na_2SO_4, 0.7 % (w/v), sample volume, 10 mL; ultrasound extraction time, 10 min; dilution solvent, acidic methanol of 1.0 mL. The error bars for average ± standard deviation of three trials''.

Table 1. Analytical features of the proposed method

Quantitative analysis	After UA-CPE	Before UA-CPE
Regression equation	$A=(1.6\pm0.1)\times10^{-2}C_{histamine}+(3.5\pm1.3)\times10^{-2}$	$A= 1.12\times10^{-4}\ C_{histamine}+0.176$
Correlation coefficient, r	0.9920	0.9850
Linear working range, µg L^{-1}	0.8–170	75-500
Limit of detection, µg L^{-1}	0.25	22.5
Limit of quantification, µg L^{-1}	0.83	75
Reproducibility (RSD, %)	1.9-4.2	-
Repeatability (RSD, %)	2.3-4.8	-
Recoveries (%) in the spiked samples	95.3-103.9	-
Preconcentration factor	50	-
Sensitivity enhancement factor	143	-

Table 2. Effect of potentially interfering ions and their tolerance limits in the determination and the recovery of 50 μg L^{-1} of histamine

Interference ions	Added as	Tolerance ratio	*Recovery %
Cr^{2+}	$Cr(NO_3)_2$	1500	97.3±1.8
Ni^{2+}	$Ni(NO_3)_2$	1250	98.1±2.2
SO_4^{2-}	Na_2SO_4	750	101.4±1.7
Ca^{2+}	$CaCl_2$	1000	95.8±1.4
K^+	KCl	1000	96.6±1.6
F^-	NaF	750	94.3±1.9
Quercetin	-	100	102.9±2.0
Pb^{2+}	$Pb(NO_3)_2$	250	94.7±1.5
Mg^{2+}	$Mg(NO_3)_2$	500	95.5±1.5
Tartrazine	-	150	96.0±1.9
NH_4^+	NH_4NO_3	400	96.4±1.6
Fe^{2+}	$Fe(NO_3)_2$	100	95.9±1.8
Co^{2+}	$Co(NO_3)_2$	200	97.2±1.7
Hemotoxylin	-	75	93.1±1.3
Azure B	-	150	93.9±1.2
Carmine	-	300	94.8±1.5

*The results were expressed as means ±standard deviation.

Table 3. The results of the inter-day and intra-day study to assess the validation of the proposed method.

Study	Added	Canned tuna fish			Strawberry flavored milk			Red wine		
		*Found	RSD %	Recovery %	*Found	RSD %	Recovery %	*Found	RSD %	Recovery %
Inter-day	-	25.3±0.5	1.9	-	7.4±0.1	2.5	-	17.8±0.5	2.9	-
	5	29.0±0.7	2.5	95.7	11.7±0.4	3.7	94.5	21.9±0.9	3.9	96.2
	20	43.6±1.2	2.8	96.3	26.3±1.1	4.2	95.9	36.7±1.5	4.1	97.5
Intra-day	-	23.9±0.7	3.0	-	7.3±0.2	2.3	-	19.1±0.7	3.5	-
	5	28.1±1.0	3.6	97.4	11.8±0.4	3.1	95.6	23.3±0.9	3.9	95.5
	20	43.0±1.7	4.1	97.8	26.4±1.0	3.6	97.0	37.7±1.8	4.8	96.3

*The results were expressed as means ±standard deviation.

Table 4. The analysis results of histamine extraction from the real samples

Sample	Spiked ($\mu g\ L^{-1}$)	*Found ($\mu g\ L^{-1}$)	Recovery (%)	RSD (%)
Fish samples				
Anchovies	-	7.8±0.2	-	2.4
	5	12.5±0.3	97.3	2.5
	20	27.2±0.7	97.7	2.7
Canned tuna	-	25.5±0.5	-	1.9
	5	29.8±0.7	97.5	2.3
	20	44.6±1.4	98.1	3.1
Canned sardines	-	35.8±1.0	-	2.8
	5	39.9±1.3	97.9	3.3
	20	55.0±1.9	98.6	3.4
Canned mackerels	-	19.7±0.5	-	2.9
	5	25.3±0.9	102.6	3.5
	20	40.2±1.5	101.3	3.8
Dairy products				
Goat cheese	-	2.2±0.05	-	2.2
	5	6.9±0.2	96.0	2.4
	20	21.6±0.6	97.3	2.9
Sheep's cheese	-	4.1±0.1	-	2.6
	5	8.7±0.2	95.3	2.7
	20	23.1±0.7	95.8	2.9
Mild cream	-	0.9±0.02	-	2.5
	5	5.7±0.2	96.6	2.8
	20	20.5±0.7	97.9	3.4
Ricotta	-	1.6±0.04	-	2.9
	5	6.8±0.2	103.4	3.1
	20	22.0±0.7	102.1	3.4
Whole milk	-	11.3±0.3	-	3.0
	5	15.5±0.5	95.5	3.5
	20	30.0±1.1	96.0	3.9
Strawberry flavored milk	-	7.4±0.2	-	2.7
	5	12.5±0.4	101.2	3.0
	20	27.6±0.9	100.9	3.3
Alcoholic beverages				
Beer containing 4.5%(v/v) alcohol	-	17.6±0.3	-	1.9
	5	21.9±0.5	97.0	2.4
	20	36.7±1.0	97.8	2.8
Beer containing 5%(v/v) alcohol	-	20.5±0.5	-	2.2
	5	29.5±0.8	96.7	2.7
	20	39.4±1.2	97.3	3.1
Beer containing 7.5% (v/v)alcohol	-	31.3±0.6	-	1.8
	5	37.1±0.7	102.1	1.9
	20	52.0±1.1	101.4	2.2
White wine-1	-	5.1±0.1	-	2.0
	5	9.8±0.2	97.3	2.5
	20	24.6±0.7	98.2	2.7
	-	9.7±0.2	-	1.7

White wine-2	5	14.2±0.3	96.7	1.9
	20	29.3±0.7	98.5	2.4
	-	45.8±0.8	-	1.8
Red wine-1	5	52.7±1.0	103.9	2.0
	20	67.4±1.6	102.5	2.3
	-	36.9±0.8	-	2.2
Red wine-2	5	42.9±1.0	102.4	2.5
	20	58.0±1.5	101.8	2.6

*The results were expressed as means ±standard deviation.

Table 5. Comparison of the proposed method with the other preconcentration methods reported in literature

Preconcentration method	Detection method	Linear range	Limit detection	RSD %	Recovery %	References
Electrolytic accumulation at E_p: -420 mV	SWSV	1-8, 30-90 nmol L^{-1}	0.3, 10 nmol L^{-1}	5-8	-	39
Nonionic MEKC	Laser induced fluorescence	6-1000 nmol L^{-1}	0.42-1.26 nmol L^{-1}	< 3.0	93-104	40
Nonionic MEKC	Laser induced fluorescence	6-1000 nmol L^{-1}	1.02 nmol L^{-1}	1.2-3.1	99.3-102	41
Ni-FGCE	Electrocatalytic	0.5-110 mg L^{-1}	0.11 mg L^{-1}	3.71	-	43
[c]ITP–CZE	Photometric	-	0.35 mg L^{-1}	2.60	92.10	44
HPLC	UV-detection	5-70 mg L^{-1}	0.7 mg L^{-1}	4.3	98-99	45
CZE	ESI-MS	-	40 µg L^{-1}	0.56	90-115	46
HPLC	Colorimetric	-	0.01 mg g^{-1}	2.61 - 9.63	>91	47
Ultrasonic extraction	Chronopotentiometry	2-100 mg L^{-1}	0.27 mg L^{-1}	1.73-6.83	92.71-104.8	48
HPLC	Fluorescence and UV–Vis	5-100 mg L^{-1}	1.5 mg L^{-1}	1.35	>55	49
UA-CPE	FAAS	0.8-170 µg L^{-1}	0.25 µg L^{-1}	1.9-4.8	95.3-103.9	The current study

[c]ITP–CZE: on-line combination of capillary isotachophoresis–capillary zone electrophoresis, Ni-FGCE: Nickel-Film Glassy Carbon Electrode, UA-CPE: ultrasonic assisted cloud point extraction, FAAS: flame atomic absorption spectrometry; CZE; capillary zone electrophoresis; ESI-MS: electrospray ionization-mass spectrometry; HPLC: High-performance liquid chromatography;UA-CPE: ultrasonic assisted cloud point extraction; FAAS: flame atomic absorption spectrometry; SWSV: square wave stripping voltammetry; MEKC: micellar electrokinetic chromatography.