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Simultaneous Quantification of Eight Biogenic Amine Compounds in Tuna by Matrix Solid-Phase Dispersion followed by HPLC−Orbitrap Mass Spectrometry

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ABSTRACT: A method for the extraction of agmatine, cadaverine, histamine, phenyethylamine, putrescine, tryptamine, tyramine, and urocanic acid from canned tuna and frozen tuna loin matrices by matrix solid-phase dispersion, followed by separation and quantification of these compounds by ultrahigh-performance hydrophilic interaction chromatography (UHPLC-HILIC) with orbitrap mass spectrometric detection, is described. Tuna samples are dispersed in a CN-silica sorbent and eluted with a mixture of aqueous ammonium formate buffer and acetonitrile. Separation and detection are carried out on an Agilent 1200 high-performance liquid chromatograph coupled to a Thermo Exactive orbitrap mass spectrometer, and metformin is used as the internal standard. Spike recoveries are determined across a range of 20−100 ppm for each compound, and the method is validated with respect to linearity, reproducibility, accuracy, and limits of quantitation and detection. The method is demonstrated to be suitable for use in quantifying these target compounds in the studied matrices.

KEYWORDS: biogenic amine, matrix solid-phase dispersion, LC-MS, orbitrap, tuna

INTRODUCTION

An important issue in the quality and safety of seafood products is the formation of biogenic amine compounds through the process of bacterial decarboxylation of amino acids during decomposition.[1] The most well-studied of these compounds is histamine, which is known to cause an illness commonly referred to as scombroid poisoning.[2] Currently this is the only biogenic amine compound for which the U.S. Food and Drug Administration has an established limit, and it is considered to be the primary chemical indicator of decomposition.[3] Other biogenic amine compounds are known to arise from similar decomposition pathways, however, and may also potentially contribute to health effects of consuming decomposed seafood products, especially in cases when histamine is not present in the product.[4]

The current regulatory strategy is to first analyze suspect samples by organoleptic (sensory) analysis and confirm failed samples by a chemical test for histamine involving fluorescence spectroscopy.[5] Histamine, however, is not directly detectable by sensory analysis, which creates potential difficulties with correlating the results of these two tests in a meaningful way. A preferable technique would be to use a chemical analysis that produces a more comprehensive look at the profile of biogenic amine compounds present in samples which fail sensory analysis and, particularly, those which have resulted in illness in order to better understand the chemical factors of the decomposition process and potentially establish a more meaningful regulatory strategy.

The current work involves a single-extract, multiresidue approach to this issue. The selected panel of target analytes includes agmatine (AGM), cadaverine (CAD), histamine (HIS), phenylethylamine (PEA), putrescine (PUT), tryptamine (TRP), tyramine (TYR), and urocanic acid (UCA). Chemical analyses for the majority of these biogenic amines are not routinely performed by most food safety laboratories as their role in food poisoning is still not completely clear but, nevertheless, they were commonly found in decomposed seafood.[4] These amines were extracted from canned and frozen raw tuna samples utilizing a simple and rapid matrix solid-phase dispersion (MSPD) technique that requires no additional cleanup, with the use of metformin (MET) as an internal standard. MSPD is a robust analytical technique patented for the isolation of components from biological specimens[6] and has been previously applied to extraction of a variety of analytes from such difficult sample matrices as seafood,[7] meat,[8] and milk.[9] It has also been previously explored for the extraction of biogenic amines from other difficult matrices such as cheese,[10] poultry,[11] and tomatoes.[12]

Biogenic amine compounds in these sample extracts were separated by means of ultraperformance hydrophilic interaction liquid chromatography (UHPLC-HILIC) and detected by a Thermo Exactive orbitrap mass spectrometer. Preliminary method development work on the instrument analysis portion, prior to the development of the MSPD extraction study from food matrices, was recently reported in an FDA/ORA Laboratory Information Bulletin (LIB).[13] This streamlined approach eliminates the need for derivatization, which has been the traditional option for liquid or gas chromatographic analysis of many of these compounds, including in our laboratory,[14−20] and provides an alternative to the ion chromatographic technique previously used without derivatization for a similar compound set.[21] Using the

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Exactive instrument also allows the identification of these compounds based on accurate mass and thus eliminates the need to form MS^n fragments, which would not be practical for most of these compounds given their extremely small mass in relation to useful mass ranges for mass spectrometry.

MATERIALS AND METHODS

Solvents and Chemicals. Acetonitrile (LC-MS grade) and formic acid (highly purified grade) were obtained from Fisher Scientific (Pittsburgh, PA). Ammonium formate (97%) was obtained from Spectrum (Gardena, CA). Putrescine (1,4-diaminobutane) (99%), cadaverine dihydrochloride (98%), histamine dihydrochloride (99%), and metformin (1,1-dimethyl biguanide) (97%) were obtained from Aldrich (Milwaukee, WI). Tryptamine hydrochloride (99%) and tyramine (99%) were obtained from Sigma-Aldrich (St. Louis, MO). Agmatine sulfate (99%) was obtained from Fluka (Buchs, Switzerland). Urocanic acid (99%) was obtained from Acros (Geel, Belgium). Bulk Bondesil 40 μm CN-U sorbent was obtained from Varian (Palo Alto, CA). High-purity deionized water was produced by a Direct-Q purification system from Millipore (Billerica, MA). Ammonium formate buffer is 50 mM ammonium formate in high-purity deionized water, pH 3.00, with formic acid. Elution buffer is 20:80 (v/v) ammonium formate buffer in acetonitrile.

Equipment. A Denver model 250 pH-meter (Denver Instrument, Gottingen, Germany) was used for pH measurement. Extraction tubes are 6 mL polypropylene tubes with 6 mL/1.2 cm polypropylene frits obtained from Restek (Bellefonte, PA). A 24-port vacuum manifold, model 210124, was obtained from Alltech Associates, Inc. (Deerfield, IL). Syringe filters were Titan-2 17 mm, 0.45 μm, nylon, obtained from Sun Sri (Rockwood, TN). Other general equipment used includes a food processor, an ultrasonic bath, polypropylene conical centrifuge tubes (15 mL), HPLC vials and caps, glass screw-top vials, and a 5 mL polypropylene syringe.

Standard Preparation. Approximately 10 mg free base equivalent of each target compound and internal standard was dissolved and diluted to 10.0 mL in elution buffer to create primary stock standards. Volumes of 1.00 mL of UCA, PEA, TYR, TRP, and AGM primary stock standards and 1.50 mL of HIS, PUT, and CAD primary stock standards were combined and diluted to 10.0 mL in elution buffer to create the mixed stock standard. Metformin primary stock standard was diluted 1:20 (v/v) in elution buffer to create the diluted internal standard. Mixed stock standard was diluted in elution buffer to create five standards with known concentrations of approximately 0.2, 0.4, 0.8, 1.6, and 2.0 μg/mL of UCA/PEA/TYR/TRP/AGM and 0.3, 0.6, 1.2, 2.4, and 3.0 μg/mL of HIS/PUT/CAD. Diluted internal standard was added to each preparation to give constant MET concentrations of 0.5 μg/mL in each. Spiking solutions were prepared by mixing primary stock standards of each target compound and diluting in elution buffer to create concentrations of 20, 40, 60, 80, and 100 μg/mL of each compound. Metformin primary stock standard was added to each spiking solution to give constant concentrations of 25 μg/mL in each. An extraction internal standard solution, for addition to unspiked samples, was created by diluting MET primary stock standard in elution buffer to a concentration of 25 μg/mL.

Each standard solution was stored at −35 °C when not in use. Primary stock standards were kept for up to 1 month; all others were prepared on the day of the extraction run. Each linear calibration standard was filtered by syringe filtration prior to analysis.

Sample Preparation. Bulk MSPD sorbent was washed with 2 mL each of hexane, isopropanol, and acetonitrile per gram via vacuum filtration as previously described[9] and dried in a 105 °C oven prior to use. Canned chunk light tuna in water (opened, drained of excess water) or whole steak of previously frozen tuna loin (thawed) was transferred to a small food processor and processed until it became a uniform, homogeneous paste. This was stored at −4 °C in a vacuum-sealed bag when not in use and thawed/mixed prior to extraction.

Approximately 0.5 g of tuna composite was transferred into a glass mortar. A 0.50 mL aliquot of either extraction internal standard solution (for unspiked samples) or spiking solution corresponding to the desired spike level was added via volumetric glass pipet, and the sample was ground with the glass pestle to disperse and homogenize the solution. Washed and dried bulk MSPD sorbent (2.0 g) was added, and the sample was lightly ground in the mortar and pestle, for approximately 2 min, until a light orange-brown, homogeneous, free-flowing dry powder was observed with no adhesion to the sides of the mortar or pestle.

The powder was then transferred to an extraction tube and placed on the vacuum manifold. A 4 mL aliquot of elution buffer was added to the tube and allowed to flow by gravity into a glass vial. When elution was complete, vacuum (approximately 130 mmHg) was applied to the chamber to remove the remaining liquid. A second 4 mL aliquot of either pure ammonium formate buffer (original method) or elution buffer (revised method) was then added to the extraction tube, and this process was repeated.

The collected eluate was transferred to a 25 mL volumetric flask and diluted to volume with either acetonitrile (original method) or elution buffer (revised method) and mixed thoroughly. This solution was then filtered by syringe filtration into an autosampler vial for analysis.

HPLC-MS Conditions. An Agilent 1200 series HPLC outfitted with high-performance autosampler and binary pump was used with a Waters Acquity UPLC BEH HILIC (2.1 mm × 150 mm, 1.7 μm) column and a Thermo Exactive orbitrap mass spectrometer for detection. Instrumental conditions include an injection volume of 5.0 μL, a column temperature of 30 °C, and a flow rate of 0.750 mL/min. Mobile phase A was ammonium formate buffer, and mobile phase B was acetonitrile. Solvent gradient conditions were as follows: 8.0% A from 0 to 2.5 min, linear ramp to 16.0% at 3 min, hold until 12 min, then return via linear ramp to 8.0% at 12.5 min. Total run time was 18 min.

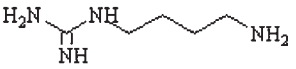
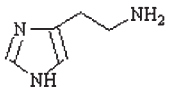
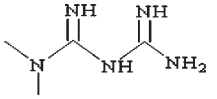
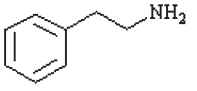
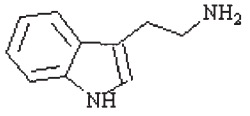
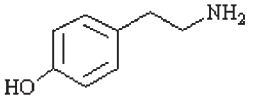
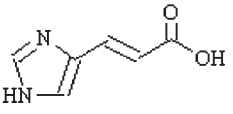
Detection was carried out in positive ion atmospheric pressure chemical ionization (PI-APCI) mode. APCI probe position was A,0,0. Ultrahigh-purity nitrogen (99.999%) was used as the sheath and auxiliary gas. A scan range of m/z 50.0−250.0 was used in medium-resolution (10,000) mode with a balanced (1,000,000) AGC target and maximum inject time of 50 ms. The vaporizer temperature was 350 °C. Sheath and auxiliary gas flows were 60 and 20 units, respectively. Capillary, tube lens, and skimmer voltages were 30.00, 60.00, and 16.00 V, respectively. Tuning and optimization were performed using a direct injection of putrescine standard (1 μg/mL) standard and a 0.750 mL/min HPLC flow at 16% mobile phase A and 84% mobile phase B. Additional identification data were collected by a second scan operation utilizing the instrument's high-collisional dissociation (HCD) feature with the same settings described above and 10 eV collision energy. Carrier gas was ultrahigh-purity nitrogen.

Method Validation Design. Five replicate analyses of unspiked samples were made, in addition to five replicates of samples spiked with each of the five spiking solutions as described above, resulting in levels of 20, 40, 60, 80, and 100 ppm of each compound, for a total sample population of $n = 30$. Calibration standards were injected at the beginning of each run of samples, and calculations were performed on the basis of the linear regression of the standard curve. Calculation of residues in spiked samples is based on the ratio of the area of the extracted ion chromatogram of the accurate mass of each analyte (Table 1) with a mass tolerance of 15.0 ppm to that of the internal standard. The percent recoveries of each compound were calculated at each level, and the average and relative standard deviation (RSD) of these recoveries are evaluated. Limits of quantitation and detection and linear regression of standard curves are also evaluated for each target analyte.

RESULTS AND DISCUSSION

Separation Conditions. The current work employs a combination of HILIC, which offers much in the way of separation power for small, polar molecules,[22] and ultrahigh-performance

Table 1. Target Compounds and Relevant Data

Analyte	Abbreviation	Formula	Accurate Mass (M+H)	Structure
Agmatine	(AGM)	$C_5H_{14}N$	131.1291	
Cadaverine	(CAD)	$C_5H_{14}N_2$	103.1230	
Histamine	(HIS)	$C_5H_9N_3$	112.0869	
Metformin	(MET)	$C_4H_{11}N_5$	130.1087	
Phenylethylamine	(PEA)	$C_8H_{11}N$	122.0964	
Putrescine	(PUT)	$C_4H_{12}N_2$	89.1073	
Tryptamine	(TRP)	$C_{10}H_{12}N_2$	161.1073	
Tyramine	(TYR)	$C_8H_{11}NO$	138.0913	
Urocanic Acid	(UCA)	$C_6H_6N_2O_2$	139.0502	

liquid chromatography (UHPLC) to further enhance the separation efficiency.[23]

Gianotti et al. applied HILIC methodology to a similar set of compounds and a different detection instrument [24] without the need to focus on chromatographic separation. Using the Exactive instrument, the mass accuracy for these compounds is adequate to ensure that extracted ion chromatograms do not contribute to one another; however, no enhanced fragmentation techniques are available to distinguish fragment ions of one coeluting compound from those of another, as would be the case with typical tandem MS. It is therefore desirable to achieve at least a minimum level of chromatographic separation sufficient to allow a mass spectrum to be obtained from the apex of each peak without influence from adjacent peaks. In addition, separation of chromatographic peaks allows the usage of retention time as an additional identification mode, which is useful in regulatory analysis.

As many of the studied compounds are very structurally similar, chromatographic separation is difficult and has previously been achieved by use of derivatization,[14−20] which enhances both detection capability and separation. In the current work, an adequate level of separation was achieved by utilizing the UHPLC HILIC column and optimizing chromatographic conditions for separation (Figure 1).

Development of the chromatographic method began with conditions similar to those of Gianotti et al.,[24] with pH 4 buffer and a standard analytical HILIC column (Waters Atlantis 2.1 mm × 150 mm, 5 μm packing). Significant modifications to chromatographic conditions were then made to optimize the separation of all eight target compounds.

The effect of pH was observed by analyzing mixtures with mobile phase A at pH 3.0, 4.0, and 5.0. None of the target compounds was retained with the pH 5.0 buffer, which appears to be outside the operational chromatographic range. No appreciable chromatographic effect was observed in most of the compounds between pH 3.0 and 4.0; however, UCA, being an acid, was significantly affected. The optimal placement of UCA with respect to the other target compounds was observed at pH 3.0, which was therefore employed as the selected pH value to enhance overall separation of the early eluting compounds.

We were unable to optimize the method using the standard HPLC column to achieve adequate separation between PUT and CAD or between TRP, PEA, and TYR, respectively, even with very slow gradients and run times of up to 40 min. Taking advantage of the ultrapressure chromatography feature of the instrument system, a UPLC BEH-HILIC column (2.1 × 100 mm) was then selected for further development. Separation

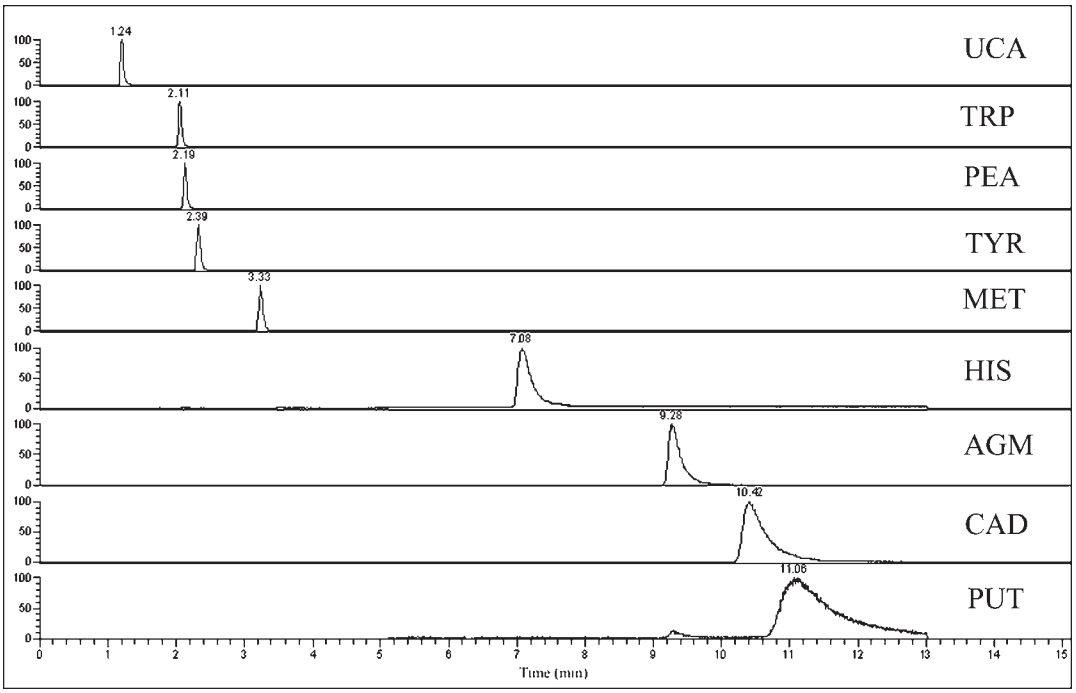


Figure 1. Typical set of extracted ion chromatograms from a sample extract injection. Accurate mass of each compound is monitored with a window of 15.0 ppm.

capability and run times for this column were significantly better than those of the standard column; however, separation of PUT and CAD was still inadequate. Finally, a longer version of the UPLC column (2.1 mm × 150 mm) was considered in order to provide additional separation power. This eventually allowed for adequate separation of all eight compounds. The longer column required a lower flow rate to stay within the maximum recommended pressure of the high-performance Agilent 1200 high-performance binary pump (600 bar). Adjustment of the flow rate to 0.75 mL/min resulted in maximum pressures in the 500 bar range. The lower flow rate and the longer column do result in a somewhat longer run time; however, the total time (with equilibration) of 18 min is sufficiently short to allow for a relatively rapid analysis, comparable to existing methodology.

Use of an appropriate internal standard helps to ensure that the extraction generates accurate and reliable results. Dimethyl biguanide, also known as metformin, has previously been used as an internal standard in the quantitation of similar target compounds.[25] It was chosen for its structural similarity to the target compounds and its retention time, which is in the appropriate range and well resolved from other compounds.

Detection Conditions. Initial experimentation with this method utilized electrospray ionization (ESI), which is indicated due to the high polarity of the target compounds. We observed, however, that use of ESI could not achieve sufficient linearity even within a very small concentration range. The observed correlation coefficient (R^2) values were as low as 0.95 for some of the compounds. The situation was greatly improved when we switched to APCI. Use of the APCI probe led to much more linear data, likely due to the very low mass of the target compounds, despite their high polarity.

Whereas it is typically recommended[26,27] to perform quantitation with the orbitrap in a high-resolution mode (100,000 resolution), this setting results in a scan rate of 1 scan/s. This is problematic with respect to quantitation when the instrument is coupled to the UHPLC separation because many of the peaks, particularly those of the early-eluting compounds, are very narrow with peak widths on the order of about 3 s. This results in a number of scans per peak that is insufficient to perform adequate quantitation. Using the lower resolution setting (10,000), the scan rate becomes 10 scans/s, which allows for quantitation of even these narrow peaks.

The typical trade-off with lower resolution scanning is a loss of specificity; however, with the relatively small number of well-resolved target analytes and their extremely small m/z values, this issue is not of great concern. For very small m/z values (<300), a resolution of 10,000 is generally considered to result in unambiguous identification,[28] resulting in a negligible potential for interference.

Identification data from the HCD scans added negligible additional information as may be expected of such low-mass compounds; this scanning event could be omitted in future applications of the method to increase the scan rate of full scan data. Comparing both the accurate mass spectral information (Figure 2) and retention time to those obtained from a reference standard will allow for positive identification of these compounds in an unknown sample.

Extraction Method. Initial exploration of the potential for the use of MSPD techniques to extract these compounds from seafood samples involved a simple matrix of sorbents (C18, CN, C8, and C2, each on 40 μm silica particles) and solvents (hexane, methylene chloride, acetonitrile, methanol, and 0.1% formic acid in water) to roughly gauge the extractability of each compound from standard solutions. The C18 and CN sorbents appeared to perform best, with polar or aqueous solvents, with CN showing slightly better performance. Finally, a technique utilizing CN sorbent, heavily modified from a previously published method[10] to fit the instrumental method and analyte list, was explored.

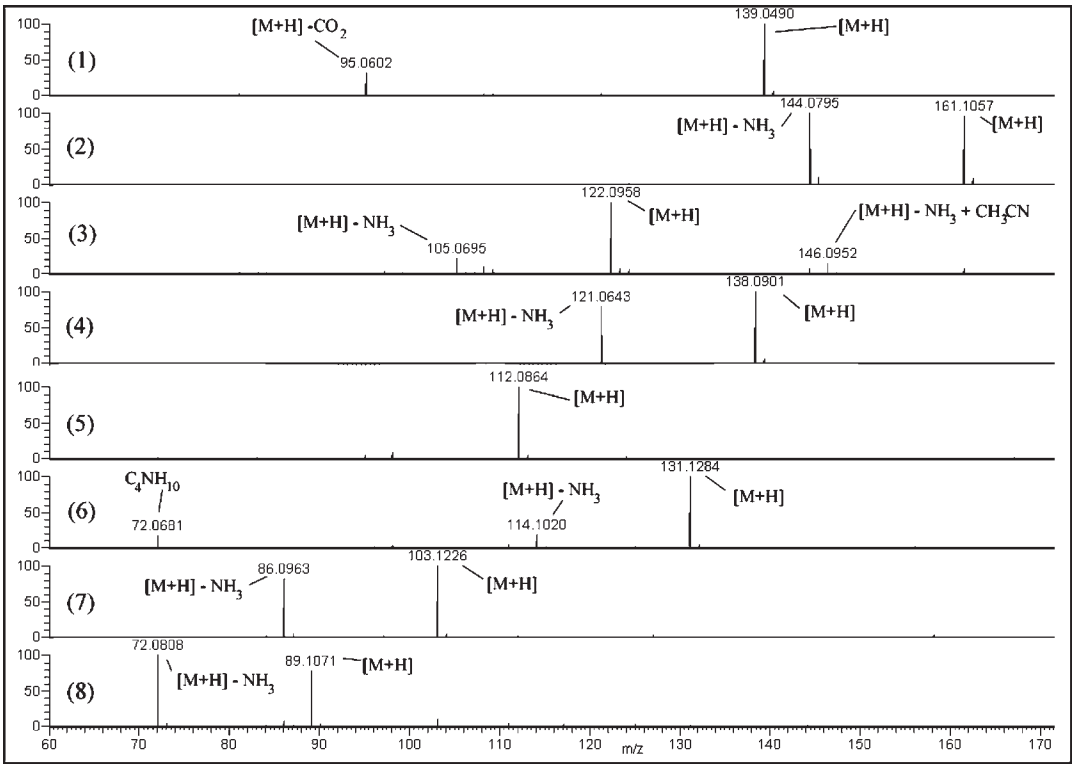


Figure 2. Set of mass spectra from sample extracts of (1) UCA, (2) TRP, (3) PEA, (4) TYR, (5) HIS, (6) AGM, (7) CAD, and (8) PUT. Proposed formula assignments for major observed ions are given.

Because HILIC is sensitive to changes in the injection solvent, it is desirable to achieve a final extraction composition that comes as close to the mobile phase composition as is practical while maintaining the solubility and extractability of each target analyte. To achieve this, the samples were eluted with aliquots of elution buffer followed by pure aqueous buffer and then diluted in pure acetonitrile such that the final aqueous concentration is approximately 20% by volume. This technique worked quite well for the canned tuna sample (Table 2); however, for the frozen loin sample, the recoveries of PUT and CAD in initial tests fell into the 40−60% range. The apparent cause of this is salting-out of analytes in the final dilution. Upon the addition of acetonitrile to the clear eluate, a thick cloudy haze was observed. This persisted even after a full hour in an ultrasonic bath. The haze was observed in the canned tuna samples as well, but was much less intense. Injections of unfiltered samples of frozen loin extract showed a large filtration effect on the PUT and CAD recoveries, whereas this effect had been previously observed as negligible in both canned tuna extracts and neat standards, and hence these were apparently being salted-out after the acetonitrile addition, likely due to the slightly different chemistry of the frozen sample.

To alleviate this, the method was revised to use two aliquots of the elution buffer, and the final dilution was also done with elution buffer. This results in the same final aqueous concentration (20% v/v) but maintains equal composition between the eluate and the diluent, which eliminates the salting-out of the analytes. This was readily observed in the final extract, which was much clearer in appearance, and the recoveries of PUT and CAD were much higher using this technique, with no influence from filtration.

The original method was used in the validation of the canned tuna sample, the revised method was then used for the frozen loin sample, and a followup was done with the canned sample in which one test at each spiking level was performed to verify that the revised method would produce comparable recovery results (Table 2).

Method Validation. Urocanic acid was the only residue detected in either of the unspiked sample sets. It was detected in both the canned and the frozen loin samples and was quantified by low-end extrapolation of the standard curve. Average values of 10.28 and 11.25 ppm were calculated in this way for the canned sample and the frozen loin sample, respectively. These values are subtracted from each sample's respective spike values for UCA.

Spike recovery results (Table 2) ranged from 83.4 to 112.5%, with both of these extremes arising from UCA, which is most likely due to natural variation in the amount of UCA present in the tissue, generating anomalous results. Ranges for all of the compounds, however, were quite good for the intended purpose of the method. Relative standard deviation (RSD) results ranged from 0.5 to 9.1% (Table 2), within the expected range of this type of method and, again, with UCA representing the highest variability due to the natural occurrence. Comparison of the original and revised methods in the canned tuna sample demonstrates that the performance was very similar for these two methods, and the canned sample compared nicely with the frozen tuna loin sample with respect to the revised method, indicating that this method is more robust in terms of sample type. The results of the spike recovery data demonstrate that the method has an acceptable level of accuracy and reproducibility.

Linearity in terms of least-squares correlation coefficient (R^2) was calculated for each compound's calibration curve for each of the three analytical runs for which revised method data were collected (Table 3). These values were all above 0.995, and most frequently above 0.999.

Table 2. Recovery Results and Statistics

parameter	spike level				
	20 ppm	40 ppm	60 ppm	80 ppm	100 ppm
Canned Tuna					
urocanic acid					
average recovery (%) [original method]	83.4	95.9	95.7	112.5	105.6
recovery RSD (%) [original method]	2.7	6.6	3.4	3.2	9.1
recovery (%) [revised method]	87.1	85.2	102.8	102.5	105.5
tryptamine					
average recovery (%) [original method]	97.0	105.5	97.4	104.1	100.9
recovery RSD (%) [original method]	2.5	8.6	5.3	6.1	10.4
recovery (%) [revised method]	93.1	93.5	101.0	96.8	91.0
phenylethylamine					
average recovery (%) [original method]	84.8	97.7	94.3	99.7	99.5
recovery RSD (%) [original method]	4.4	7.3	3.3	9.1	9.0
recovery (%) [revised method]	97.8	92.1	91.0	90.8	101.4
tyramine					
average recovery (%) [original method]	90.3	101.1	97.7	103.7	104.6
recovery RSD (%) [original method]	3.0	8.1	3.8	4.0	8.4
recovery (%) [revised method]	102.2	97.4	101.0	88.9	107.6
histamine					
average recovery (%) [original method]	92.4	90.2	86.5	93.3	109.1
recovery RSD (%) [original method]	0.9	3.9	3.9	2.5	0.6
recovery (%) [Revised method]	96.9	93.9	98.7	99.9	97.6
agmatine					
average recovery (%) [original method]	108.5	105.3	108.0	104.7	107.5
recovery RSD (%) [original method]	1.2	0.6	2.9	4.7	1.7
recovery (%) [revised method]	96.9	82.8	100.2	94.4	99.2
cadaverine					
average recovery (%) [original method]	85.3	92.2	86.4	86.1	97.5
recovery RSD (%) [original method]	2.1	4.2	2.7	5.0	8.2
recovery (%) [revised method]	103.3	95.4	99.9	98.0	103.9
putrescine					
average recovery (%) [original method]	85.2	96.1	95.8	102.7	96.3
recovery RSD (%) [original method]	2.2	4.7	0.5	6.0	3.9
recovery (%) [revised method]	105.1	107.6	101.8	99.4	103.6
Tuna Loin [Revised Method]					
urocanic acid					
average recovery (%)	86.0	98.2	101.3	100.2	103.6
recovery RSD (%)	4.4	1.6	1.6	2.8	4.2
tryptamine					
average recovery (%)	88.3	96.6	102.1	94.1	99.1
recovery RSD (%)	4.0	5.3	4.1	3.7	4.8
phenylethylamine					
average recovery (%)	100.3	95.3	96.8	87.4	96.7
recovery RSD (%)	2.5	2.8	4.0	5.9	3.3
tyramine					
average recovery (%)	93.2	99.0	99.6	93.3	97.6
recovery RSD (%)	6.3	4.9	5.7	6.1	5.8
histamine					
average recovery (%)	84.3	96.6	85.2	91.0	101.1
recovery RSD (%)	6.5	4.2	0.9	1.3	4.1
agmatine					
average recovery (%)	95.1	82.4	98.4	85.2	98.6

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Table 2. Continued

parameter	spike level 20 ppm	40 ppm	60 ppm	80 ppm	100 ppm
recovery RSD (%)	2.8	3.9	1.0	2.1	0.8
cadaverine					
average recovery (%)	98.0	92.2	94.5	95.1	100.1
recovery RSD (%)	0.8	4.8	1.4	0.9	3.2
putrescine					
average recovery (%)	99.1	94.1	94.8	95.4	100.8
recovery RSD (%)	4.4	2.3	1.8	2.2	2.1

Table 3. Additional Validation Data Derived from Revised Method Data

parameter	UCA	TRP	PEA	TYR	HIS	AGM	CAD	PUT
Canned Tuna[a]								
LOQ (ppm)	0.0680	0.115	0.0940	0.272	5.22	2.17	1.53	8.61
LOD (ppm)	0.0204	0.0345	0.0282	0.0817	1.57	0.650	0.459	2.58
Frozen Loin[b]								
LOQ (ppm)	0.0657	0.114	0.0791	0.348	5.47	1.66	1.47	1.61
LOD (ppm)	0.0197	0.0343	0.0237	0.104	1.64	0.498	0.442	0.483
Linearity (R^2)[c]								
spike level 1−2	0.9965	0.9951	0.9952	0.9986	0.9963	0.9962	0.9983	0.9979
spike level 3−4	0.9995	0.9995	0.9997	0.9968	0.9986	0.9952	0.9987	0.9973
spike level 5	0.9987	0.9989	0.9995	0.9968	0.9978	0.9976	0.9993	0.9986

[a] Calculated from the spike level 1 revised method injection. [b] Calculated from the first spike level 1 injection. [c] Applies to both frozen and canned samples, as these were run concurrently. Spike levels 1−2 and 3−4 were run with the same standard curve.

Limits of quantitation and detection (LOQ and LOD) were calculated for each compound for each sample on the basis of the signal-to-noise ratio (s/n) of each peak in a level 1 spike injection (Table 3). These are calculated on the basis of s/n = 10 for LOQ and s/n = 3 for LOD. Although these values varied greatly among the eight compounds, due to much greater sensitivity of the early-eluting compounds, the observed values are adequate for analysis of these compounds in the expected ranges of spoiled seafood products.[29−34]

In the current study, a novel method for the quantitation of eight biogenic amine compounds was developed utilizing UHPLC-HILIC for separation coupled with orbitrap mass spectrometry for detection. The method was validated with respect to linearity, repeatability, and limits of quantitation for each of the eight compounds studied. Its practical application in selected food matrices, that is, canned/cooked and raw tuna, has also been preliminarily demonstrated. The work described in this paper suggests that this method can be potentially useful to quantitatively analyze each of these compounds and serves as a promising alternative to existing methodology for biogenic amine determination. Further matrix extension study will be needed to evaluate the robustness of this method in various food matrices for analysis of multiple biogenic amines.

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DISCLOSURE

The views presented in this paper are those of the authors and do not necessarily represent those of the U.S. Food and Drug Administration. No official support or endorsement of this paper by the Food and Drug Administration is intended or should be inferred. Mention of brand or firm name does not constitute an endorsement by the U.S. FDA over others of a similar nature not mentioned.

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