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Sampling and Analytical Method for Workplace Monitoring of Aspartame in Air

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Aspartame™ (L-aspartyl-L-phenylalanine methyl ester; Nutrasweet®; Nutrasweet Company, Chicago, Illinois) is a dipeptide methyl ester that imparts a sweet taste sensation. It has been approved for use in the United States since 1981. In the course of a National Institute for Occupational Safety and Health (NIOSH) study to examine potential worker health effects at a food plant in the U.S., a method of sampling aspartame in air, and its analysis, was developed. A walk-through survey of the abovementioned plant identified potential aspartame exposures to employees during weighing, blending, and packaging of dry dessert mixes. Potential analytical interferences included sodium citrate, ascorbic and fumaric acids, gelatin, maltodextrin, and mannitol.

The collection system, using portable high flow (1–5 L/min) air pumps, is suitable for personal and area air sampling in industrial settings. Samples were collected on 1.0 micron pore size, 37-ml diameter polytetrafluoroethylene (PTFE) filters with a polyethylene backing. Analysis was by high-pressure liquid chromatography.

Laboratory experiments and field testing demonstrated excellent recovery of aspartame from PTFE filters. It was also found that aspartame does not migrate or decompose on the filter during sampling or when stored at ambient temperature for one month. No special precautions are necessary for either sample collection or transportation to the analytical laboratory.

No analytical interferences were found from food additives which were collected along with aspartame during actual field sampling. The effect of humidity extremes on sampling and subsequent measurement was not evaluated. With minor modifications, this method should be applicable to sampling most dipeptides in air. **Albrecht, W.N.; Burr, G.A.; Neumeister, C.E.: Sampling and Analytical Method for Workplace Monitoring of Aspartame in Air. Appl. Ind. Hyg. 4:217–221; 1989.**

Introduction

Several dipeptides (two covalently-bonded amino acids) possess properties which impart a taste sensation indistinguishable from salt or sugar. For example, ornithyltaurine tastes as salty as sodium chloride but is not, as yet, commercially available.[1] Aspartame™ (L-aspartyl-L-phenylalanine methyl ester), better known by the tradename Nutrasweet®, is approximately 180 times as sweet as sucrose and has been approved for use in this country since 1981.[2] Previously, health hazards from the ingestion of aspartame have centered on risks to phenylketonurics (individuals who do not properly metabolize phenylalanine) and the potential for aspartame to increase the level of excitatory neurostransmitters in the brain. The former has been satisfactorily resolved by requiring a warning label to appear on all products containing aspartame. The latter remains a point of controversy.[3,4] An individual has presumptively demonstrated an immune response (urticaria) after ingestion.[5]

Workers engaged in the manufacture or blending of aspartame are conceivably exposed, by inhalation, to doses many times greater than the general public if adequate engineering controls are not in place. The possible role of aspartame in the genesis of occupational asthma is as yet unclear even though studies have shown that it was not a direct mast cell or basophil secretogogue *in vitro,* or *in vivo* as assessed by skin testing.[6] In addition, during acute incubation, aspartame did not affect IgE-mediated histamine release from mast cells. Inconsistencies remain, however, since aspartame, or its diketopiperazine derivative (DKP, a spontaneous decomposition product, approximately 2% by weight in the final aspartame product) can presumably act as an antigen, and DKP has not yet been specifically examined for antigenic properties.[5]

In the course of evaluating potential health hazards to workers engaged in the blending of aspartame at a food plant that packages dry, sugar-free dessert products, and in anticipation of evaluating other dipeptide exposures in the future, researchers from the National Institute for Occupational Safely and Health (NIOSH) developed a sampling and analytical method for aspartame in air. This method, a modification of an existing high-performance liquid chromatography (HPLC) assay of aspartame and its precursors and decomposition products, only addresses aspartame.[7] A walk-through survey of the abovementioned food plant identified potential aspartame exposure to employees during

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weighing, blending, and packaging of dry dessert mixes. Potential analytical interferences included sodium citrate, ascorbic and fumaric acids, gelatin, maltodextrin, and mannitol.

Materials and Methods

L-aspartyl-L-phenylalanine methyl ester (99.9% pure; molecular weight 294.3; melting point 248°–250°C), was purchased from the Aldrich Chemical Company (Milwaukee, Wisconsin) for use in all experiments and as standards for the calibration curve. The sampling medium used was a Millipore "Fluoropore" polytetrafluoroethylene (PTFE) filter. These filters have a polyethylene backing, a pore size of 1.0 micron, and a diameter of 37 mm. PTFE filters are extremely inert and can accommodate a maximum flow rate of 18 L/min without appreciable pressure drop which could affect the performance of the air sampling pump. The entire sampler, consisting of a filter and a cellulose backup pad, is placed within a two-piece plastic cassette.

The HPLC system used for measurement consisted of a Waters model 720B autosampler, two 6000A pumps, a 760 system controller, and C_{18} Radial Compression Column in a Radial Compression Module, with a Kratos Spectroflow 783 Programmable Absorbance Detector set at 220 nanometers (nm). A Hewlett-Packard Model 3357 Laboratory Automation System completed the system. The isocratic mobile phase was 60 percent eluent A: 2.062 g 1-heptanesulfonic acid sodium salt (Fisher Chemical Co., Cincinnati, Ohio) and 0.45 g monobasic potassium phosphate (Aldrich Chemical Co., Milwaukee, Wisconsin) in 1.0 L distilled water (purged, degassed, and pH adjusted to 2.5 with dilute phosphoric acid); and 40 percent eluent B: 2.062 g of 1-heptanesulfonic acid sodium salt in 1.0 L of 3:2 acetonitrile-water (distilled water purged, degassed, and pH adusted to 3.0 with dilute phosphoric acid). The flow rate was 1.0 ml/min with an injection volume of 25 µL.

Since personal as well as area air samples were to be collected, the sampling system was tested using SKC Model 224 Universal sampling pumps calibrated at 2.5 L/min. Similar high-flow air pumps, however, would be adequate for this sampling method. A total sampling volume of 1000 L was tested.

A calibration curve ranging from 0.5 to 463 µg/ml of aspartame was prepared. A typical calibration curve with 95 percent confidence limits is shown in Figure 1. Stock solution was made by dissolving 0.05 g of aspartame in 10 ml of extraction solution (eluent B). Serial dilution of this stock with additional eluent B was used to prepare the calibration standards.

For sample preparation, the PTFE filters were removed from the cassettes in the laboratory and placed into 20 ml vials; the back-up pads were discarded. Two ml of extraction solution (eluent B) were added to each vial.

Since known masses of aspartame were required to evaluate extraction efficiency and sample stability, standard working solutions were prepared by dissolving known amounts of aspartame in methanol. Four concentration levels of aspartame, over the range of two orders of magnitude, were used in these evaluations: 435.4, 217.7, 43.5, and 4.35 µg per filter. Twenty-seven filters, containing these known amounts of aspartame, were prepared and analyzed, in triplicate, to evaluate overall sample recovery. The standard solution was applied, via syringe, to the PTFE filters and the methanol allowed to evaporate.

Both static and dynamic extraction efficiency for aspartame on PTFE filters was determined. Static extraction efficiency was measured by spiking three filters at each of the four concentration levels, allowing the methanol to evaporate, and then extracting the filters with eluent B. Dynamic extraction stability was measured by drawing 1000 L of room air through 12 identically spiked filters to ascertain if migration or decomposition of aspartame occurred during simulated sampling and then proceeding as with the measurement of static efficiency. The storage stability of aspartame was measured by spiking each of three filters with 43.5 µg of aspartame, then placing the closed cassettes on a laboratory benchtop, at ambient temperature (23.3°C), for one month without drawing air through them.

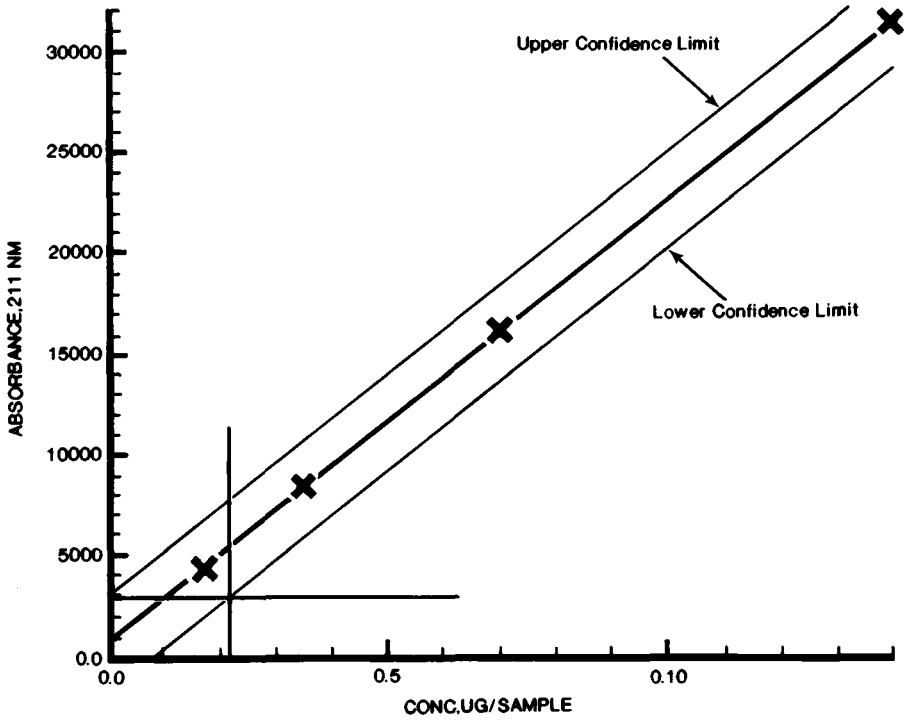

FIGURE 1. Calibration curve with 95% confidence limits.

TABLE I. Extraction and Stability Studies for Aspartame

Sample	Amount Spiked in μg/filter[A]	Amount Recovered in μg/filter	% Recovery	% RSD[B]
F1	435.4	445.8	102.3	
F2	435.4	434.6	99.8	
F3	435.4	439.0	100.8	
AVG(F)	435.4	439.8	101.0	1.3
A1	435.4	438.3	100.7	
A2	435.4	443.1	101.8	
A3	435.4	433.2	99.5	
AVG(A)	435.4	438.2	100.6	1.2
AVG(F + A)	435.4	439.0	100.8	1.1
F4	217.7	221.6	101.8	
F5	217.7	221.7	101.8	
F6	217.7	214.9	98.7	
AVG(F)	217.7	219.4	100.8	1.8
A4	217.7	221.5	101.7	
A5	217.7	220.6	101.3	
A6	217.7	212.2	97.4	
AVG(A)	217.7	218.1	100.2	2.4
AVG(F + A)	217.7	218.8	100.5	1.9
F7	43.5	44.3	101.8	
F8	43.5	47.5	109.2	
F9	43.5	43.4	99.8	
AVG(F)	43.5	45.1	103.6	4.8
A7	43.5	41.1	94.5	
A8	43.5	43.6	100.2	
A9	43.5	42.9	98.6	
AVG(A)	43.5	42.5	97.7	3.1
ST1	43.5	42.9	98.6	
ST2	43.5	44.7	102.8	
ST3	43.5	45.2	103.9	
AVG(ST)	43.5	44.3	101.8	2.7
AVG(F + A + ST)	43.5	44.0	101.1	4.1
F10	4.4	4.3	97.7	
F11	4.4	4.5	102.3	
F12	4.4	4.4	100.0	
AVG(F)	4.4	4.4	100.0	2.3
A10	4.4	4.3	97.7	
A11	4.4	4.4	100.0	
A12	4.4	4.4	100.0	
AVG(A)	4.4	4.4	100.0	1.3
AVG(F + A)	4.4	4.4	100.0	1.7

Comments:
F—Static extraction efficiency samples (no air drawn through the spiked filter).
A—Dynamic extraction efficiency samples (1000 L of air drawn through each spiked filter).
ST—30-day passive stability test of pure aspartame on filter.

[A]Micrograms of aspartame per filter.
[B]Relative standard deviation.

Results and Discussion

Regression analysis of the calibration curve data indicated a limit of detection (LOD) of 2 μg per filter with a limit of quantitation (LOQ) of 7 μg per filter. The results of the static and dynamic stability at four filter loading levels are presented in Table I.

For the 12 filters prepared for the static extraction efficiency experiment, the mean percent recovery over 4 filter loading levels was 99.5 percent ± 2.1 percent with a range of 94.5 –101.8 percent. The dynamic extraction efficiency experiment was found to be 101.3 percent ± 2.9 percent over the range of 97.7 –109.2 percent. The three filters prepared at 43.5 μg/filter and evaluated for storage stability after one month showed a mean of 101.8 percent ± 2.7 percent with a range 98.6 –103.9 percent.

To compute the overall extraction efficiency, the data obtained were normalized to complete recovery, and from the analysis of 27 spiked samples, an average recovery of 1.005, with a relative standard deviation (S_R) of 0.026, was obtained (overall precision criteria stipulates that pooled S_R should be less than 0.105).[7] The range was 1.092 to 0.945.

Sixty-six personal and area air samples were collected using this method during a health hazard evaluation of aspartame exposure at a commercial food packaging plant. A walk-through survey of the facility identified potential aspartame exposures to employees during weighing, blending, and packaging of dry, sugar-free dessert mixes. After sampling, the filters were transported to the analytical laboratory with no special precautions.

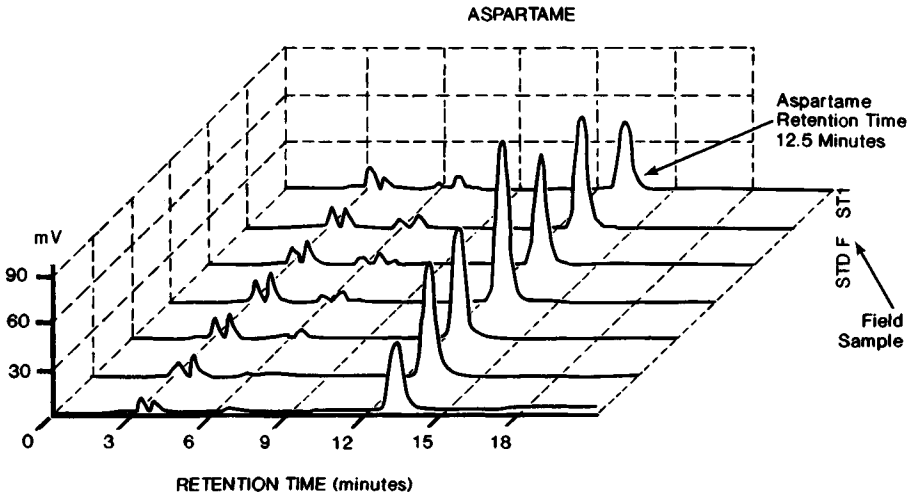

FIGURE 2. Typical chromatogram—field sample, aspartame in air.

As shown in stability studies, pure aspartame samples may be stored for up to one month with no migration or decomposition of the collected aspartame.

TABLE II. Aspartame Sample Results: Full-shift Personal Samples

Operation	Day 1 (μg/m³)	Day 2 (μg/m³)
Weigh-out	132	151
	40	26
Blender	102	55
Packing Operator	100	70
	7	4
Packing Helper	20	5
	2	ND

TABLE III. Aspartame Sample Results: Short-term Personal Samples

Operation	μg/m³	Comments
Weight-out	133	Only
	147	sugar free
	432	batches
Blending	140	Only
	213	sugar free
	153	blends
	197	
Other Plant Areas	ND	Sugar batches

TABLE IV. Aspartame Sample Results: High Volume Area Air Samples

Area	Day 1 (μg/m³)	Day 2 (μg/m³)
Weight-out Room	12	24
Blending Room	15	35
	3	5

Flow rate 8.7 to 11 L/min.

In the first of two field studies at this plant, fumaric acid, sodium citrate, and ascorbic acid, additional ingredients used at the food plant, were collected along with aspartame on the sample filters. These three compounds were chromatographically separated from aspartame in the laboratory. Retention times for fumaric acid, sodium citrate, and ascorbic acid were approximately 2 minutes, compared to 12.5 minutes for aspartame. Thus, recovery of aspartame under these chromatographic conditions was not affected. A copy of a typical field sample chromatogram is shown as Figure 2. At the onset of these field sample analyses, the following method modifications were made:

1. The extraction volume was increased from 2 to 4 ml due to the large amount of material on the PTFE filters.
2. An increase in sample response of aspartame was obtained by using an absorbance maximum of 211 nm for quantitation instead of 220 nm.

Analyses of samples from the second field study showed a LOD of 2 μg/filter and LOQ of 5 μg/filter. The lower LOQ is attributed to the smaller signal-to-noise ratio exhibited by the HPLC during the analysis. All samples from this determination were run in duplicate and agreement between the two was within 6 percent. Tables II, III, and IV summarize the results of air sampling for aspartame conducted in this plant study.

Conclusions

This study demonstrated excellent recovery of aspartame from PTFE filters. It was found that aspartame does not migrate or decompose on the filter during sampling or when stored at ambient temperature for one month. This method complies with the portion of the NIOSH standards completion criteria requiring greater than or equal to 90 percent recovery of sample from media.[8] No special precautions are necessary for either sample collection or transportation to the analytical laboratory.

The collection system, using portable high flow (1–5 L/min) air pumps, is suitable for personal and area air sampling in industrial settings. Food additives, such as flavorings ("artificial flavors"), stabilizers (ascorbic acid), and food colors (e.g., FD&C yellow #5), which were incorporated into the various dessert products and collected along with aspartame during actual field sampling, were shown not to affect the analysis. The effect of humidity extremes on sampling and subsequent analysis was not evaluated.

Recommendations

The manufacture of biologically active peptides is a rapidly emerging segment of industry. Their proliferation will continue as new discoveries are made and applications found. Since these peptides are, by nature, "active compounds," a clear and distinct potential hazard exists whenever occupational exposure exists.

This sampling and analytical method is applicable for such an emgerging industry. Using this procedure, aspartame may now be specifically measured instead of collecting total or respirable dust samples. With minor modifications, this method should be applicable to sampling most biologically active peptides in air.

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