$C_{14}H_{18}N_2O_5$ MW: 294.34 CAS: 22839-47-0 RTECS: WM3407000

METHOD: 5031, Issue 2 **EVALUATION: PARTIAL** **Issue 1: 15 August 1990**
Issue 2: 15 August 1994

OSHA : no standard
NIOSH: no standard
ACGIH: no standard

PROPERTIES: solid; MP 248-250 °C

SYNONYMS: L-aspartyl-L-phenylalanine methyl ester; 3-amino-N-(alpha-carboxyphenethyl)-succinamic acid N-methyl ester

SAMPLING		MEASUREMENT	
SAMPLER:	FILTER (1-µm, 37-mm PTFE)	**TECHNIQUE:**	HPLC, UV DETECTION
FLOW RATE:	1 to 3 L/min	**ANALYTE:**	Aspartame
VOL-MIN:	70 L @ 0.1 mg/m^3	**EXTRACTION:**	2 mL of eluent B.
-MAX:	1200 L	**INJECTION:**	25 µL
SHIPMENT:	routine	**COLUMN:**	C_{18}, 10-µm particle size
SAMPLE STABILITY:	stable at least 30 days at 20 °C [1]	**DETECTOR:**	UV, 220 nm
FIELD BLANKS:	2 to 10 field blanks per set	**MOBILE PHASE:**	60% eluent A/40% eluent B, isocratic
MEDIA BLANKS:	at least 3 per sample set	**CALIBRATION:**	standard solutions of Aspartame in eluent B
ACCURACY		**RANGE:**	7 to 800 µg per sample [1]
RANGE STUDIED:	not studied	**ESTIMATED LOD:**	2 µg per sample [1]
BIAS:	not determined	**PRECISION ($\hat{S}_r$):**	0.026 @ 4.4 to 435 µg per sample [1]
OVERALL PRECISION ($\hat{S}_{rT}$): not determined			
ACCURACY:	not determined		

APPLICABILITY: The working range is 0.07 to 8 mg/m^3 for a 100-L air sample. The method has been used to analyze personal and area air samples during a health hazard evaluation at a commercial food packaging plant. [1]

INTERFERENCES: Food additives such as flavoring (artificial flavors), stabilizers (ascorbic acid) and food colors (FD&C yellow) collected along with Aspartame sampling, did not affect the analysis. [1]

OTHER METHODS: An HPLC method for the analysis of Aspartame in bulk has been reported. [2]

REAGENTS:

1. Aspartame,™ 96% pure.
2. Water, deionized.
3. Acetonitrile, distilled in glass.
4. Methanol, distilled in glass.
5. 1-Heptanesulfonic acid sodium salt.
6. Monobasic potassium phosphate.
7. Phosphoric acid, reagent grade.
8. Eluent A: Add 2.062 g 1-heptanesulfonic acid sodium salt and 0.45 g monobasic potassium phosphate to 1.0 L deionized water and adjust pH to 2.5 with dilute phosphoric acid.*
9. Eluent B: Add 2.062 g 1-heptanesulfonic acid sodium salt to 1.0 L of 3:2 (v/v) acetonitrile-water, and adjust pH to 3.0 with dilute phosphoric acid.
10. Calibration stock solution: 5 mg/mL. Add 0.05 g Aspartame and dilute with 10 mL eluent B.

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: 1-µm, 37-mm PTFE filter (Millipore FALP or equivalent) with a cellulose backup pad in a 37-mm polystyrene 2-piece cassette.
2. Personal sampling pump capable of 1 to 3 L/min, with flexible connecting tubing.
3. High-performance liquid chromatograph (HPLC) with UV detector, 220-nm, integrator and C_{18} column (page 5031-1).
4. Syringe filters, 13-mm: 0.45 µm, PTFE.
5. Syringe, 10-mL, disposable.
6. Vials, glass, scintillation, 20-mL with PTFE-lined caps.
7. Syringes, microliter, readable to 0.1 µL.
8. Pipets, various sizes.
9. Tweezers.
10. Ultrasonic water bath.
11. pH meter.

SPECIAL PRECAUTIONS: Phosphoric acid is extremely corrosive. Work with care in a hood using protective gloves.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Remove the front and rear plugs immediately before sampling. Attach sampler to personal sampling pump with flexible tubing.
3. Sample at an accurately controlled flow rate between 1 and 3 L/min for a sample volume of 70 to 1200 L.
4. At the completion of sampling, replace both plugs and pack the sampler securely for shipment. Include appropriate number of blanks in the shipment.

SAMPLE PREPARATION:

5. Remove the PTFE filter with tweezers and transfer to a 20-mL scintillation vial. Discard the back-up pad. Add 2.0 mL eluent B.
6. Agitate the samples for 1 hour in an ultrasonic water bath.
7. Filter the sample solution using a disposable syringe fitted with a 0.45-µm PTFE filter into a clean vial.

CALIBRATION AND QUALITY CONTROL:

8. Calibrate daily with at least six working standards.
   a. Add known amounts of calibration stock solutions to eluent B, using serial dilutions as needed to obtain concentrations in the range of 1 to 400 µg /mL.
   b. Analyze with samples and blanks (steps 11 through 13).

c. Prepare a calibration graph (peak area vs. mass of Aspartame, µg per sample).

9. Determine the recovery at least once for each lot of filters used for sampling in the range of interest. Prepare six filters at each of three levels plus three media blanks.
   a. Add known amounts of Aspartame in methanol to the filters.
   b. Cover filters. Allow to stand overnight for solvent evaporation.
   c. Prepare samples (step 5 through 7), and analyze (step 11 through 13) together with standards.
   d. Determine recovery (R). Construct a graph of R vs. µg Aspartame recovered.
10. Analyze three quality control blind spikes and three analyst spikes to ensure that the calibration graph and recovery graph are in control.

MEASUREMENT:

11. Set the liquid chromatograph to manufacturer's recommendations and parameters given on page 5031-1.
12. Inject sample aliquot manually or with autosampler.
13. Measure peak areas.

CALCULATIONS:

14. Determine the mass, µg (corrected for recovery) of Aspartame found on the sample filter (W) and the average blank filter (B).
15. Calculate concentration, C, of Aspartame in the air volume sampled, V (L):

$$C = \frac{W - B}{V}, \text{ mg/m}^3.$$

EVALUATION OF METHOD:

A calibration curve constructed from nine standards over the range 0.5 to 463 µg/mL Aspartame in solution was linear and indicated a limit of detection (LOD) of 2 µg per filter and a limit of quantitation (LOQ) of 7 µg per filter. Test samples were prepared by spiking filters with known amounts of Aspartame from a solution in methanol at levels between 4.4 µg per filter and 434 µg per filter. At each level, three filters were subjected to each of the following studies (Table 1):

a. To evaluate extraction efficiency, the filters were analyzed immediately.
b. To assess storage stability, spiked filters were stored for 1 month prior to analysis.
c. To investigate the stability of the material on the filter during sampling, spiked filters were attached to a sampling pump and 1000 L of laboratory air was drawn through them at 2.5 L/min before analysis.

TABLE 1: Average Percent Recovery (n=3) of Aspartame

Spiked Amount (µg) (n=3)	Extraction Efficiency (%RSD)	Sampling Stability (%RSD)	Storage Stability @ 30 days (%RSD)
4.4	100 (2.3)	100 (1.3)	-
43.5	103 (4.8)	98 (3.1)	102 (2.7)
217	101 (1.8)	100 (2.4)	-
435	101 (1.3)	101 (1.3)	-

REFERENCES:

[1] Albrecht, W., G. Burr, and C. Neumeister. Sampling and Analytical Method for Workplace Monitoring of Aspartame in Air., App. Ind. Hyg., 4:9 (1989).
[2] Verzella, G., F. Bagnasco, and A. Mangia. Ion-Pair High-Performance Liquid Chromatographic Analysis of Aspartame and Related Products., J. Chromatogr., 349, 83 (1985).

METHOD WRITTEN BY:

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