

THE NUTRASWEET COMPANY
Nutrasweet Applications Technology Analytical Method
Method # NAM87-026

DETERMINATION OF ASPARTAME AND DKP IN INSTANT
AND QUICK COOKED OATMEAL

ISSUED: OCTOBER 1987

SUPERSEDES: NEW


AUTHOR


APPROVED BY

SCOPE:

This method is provided for the determination of aspartame and diketopiperazine in dry or cooked instant and quick-cooking oatmeal.

APPARATUS:

1. HPLC system consisting of suitable pump, injector, variable UV detector, strip chart recorder and/or A/D converter and integrator.
2. Laboratory centrifuge.
3. Mechanical shaker.
4. Nalgene centrifuge cups, 250-ml.
5. Ultrasonic bath and a vacuum source for degassing.

REAGENTS:

1. Methanol, HPLC Grade.
2. Phosphoric Acid, ACS Grade.
3. Sodium Phosphate Monobasic, ACS Grade.
4. Acetonitrile, HPLC Grade.
5. Milli-Q water or equivalent.
6. Sodium Sulfate, Anhydrous, ACS Grade.

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PREPARATION OF 0.0125 M SODIUM PHOSPHATE BUFFER:

1. Weigh 9.3 grams of sodium phosphate monobasic and transfer to a 6 liter flask. Add 5400 ml Milli-Q water and stir to dissolve the salt.
2. Adjust the pH to 3.5 with phosphoric acid.

PREPARATION OF 65:35 ACETONITRILE:PHOSPHATE BUFFER SOLUTION:

1. Add together in a 1 liter flask, 325 ml acetonitrile and 175 ml sodium phosphate buffer.
2. Stir until well mixed.

PREPARATION OF MOBILE PHASE:

1. In a 4 liter flask, mix together, 3600 ml sodium phosphate buffer and 400 ml acetonitrile. Stir until well mixed.
2. Filter through a 0.45 micron membrane (such as Millipore HAWP-025-00). Degas by sonification under vacuum or by helium purge.

PREPARATION OF EXTRACTION SOLVENT:

1. Weigh and transfer into a 4 liter flask, 10.00 Na_2SO_4 . Add 1000 ml of Milli-Q water and stir until salt is dissolved.
2. Add 1500 ml of methanol. Stir to mix well.

PREPARATION OF APM STANDARDS:

1. Accurately weigh, to the nearest 0.01 mg, 10 mg, 15 mg, and 25 mg amounts of Aspartame Working Standard into separate 100-ml volumetric flasks. Label the flasks APMSTD-1, APMSTD-2 and APMSTD-3, respectively.
2. Dissolve the aspartame in 15 ml of the 65:35, acetonitrile: phosphate buffer solution. Q.S. each flask with phosphate buffer and mix well.
3. Filter each standard solution through a 0.45 micron membrane (such as a Millex-HA filter) into WISP vials.

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PREPARATION OF DKP STANDARDS:

1. Accurately weigh, to the nearest 0.01 mg, 20 mg DKP Reference Standard into a 100-ml volumetric flask.
2. Dissolve the DKP in 15 ml of the 65:35, acetonitrile: phosphate buffer solution. Q.S. the flask with phosphate buffer and mix well. This is the Stock Solution.
3. Pipet 5-, 10-, and 20-ml aliquots of the Stock Solution into separate 50-ml volumetric flasks. Q.S. each flask with mobile phase and mix well.
4. Filter each standard solution through a 0.45 micron membrane into WISP vials.

SAMPLE PREPARATION:

1. To determine the amount of APM in uncooked instant or quick-cooking oatmeal, weigh in duplicate, to the nearest 0.01 g, 10.00 g of the product into a 250-ml Nalgene centrifuge bottle. Skip steps 2 through 4 and proceed with the sample extraction in step 5.
2. To determine the APM content in cooked oatmeal weigh, in duplicate, 30.00 g of the uncooked product into a tared bowl. Record the gross and tare weights.
3. Add 160 ml of rapidly boiling Milli-Q water and stir the samples for no longer than 2 minutes. Let stand for the desired period of time. Immediately place the bowls in an ice water bath and stir until cooled to between 65° and 70° F. Reweigh the samples and record the weights.
4. Weigh, in duplicate, 50.00 g of the cooked oatmeal into tared 250-ml Nalgene centrifuge bottles.
5. Pipet into each sample 100.00 ml of the extraction solvent. Shake the samples using a mechanical shaker for 60 minutes. Centrifuge at 3500 rpm for 10 minutes.
6. Filter an aliquot through a 0.45 micron chemically resistant membrane (Millex FH Filters or Gelman Acrodisc-CR Filters) into WISP vials. Label each vial.

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7. Prepare a spiked placebo sample as a control using oatmeal which does not contain APM. Weigh either 10.00g of uncooked or 50.00 g of cooked oatmeal into a 250-ml Nalgene centrifuge bottle. Add a known amount of APM equivalent to the expected ppm level of APM. Proceed with the sample extraction in steps 5 and 6.

CHROMATOGRAPHIC CONDITIONS:

Column: uBondapak C-18, 30 cm. x 3.9 mm i.d. equipped with a uBondapak C-18 Guard-Pak precolumn.

Mobile Phase: 10% Acetonitrile/90% 0.0125 M NaH_2PO_4 adjusted to pH 3.5 with phosphoric acid.

Flow Rate: 2.0 ml/minute.

Injection Volume: 20 uL (microliters).

Detection: UV, 210 nm, 0.1 AUFS.

Pressure: 2000 psig.

Temperature: ambient.

Approximate Run Time: 17 minutes.

SAMPLE ANALYSIS:

1. System suitability check: Make three 20 uL injections of the APM Std-2. The relative standard deviation of the peak response and retention times should be less than 3%.
2. Make duplicate injections of each APM std, DKP std, and sample.

CALCULATIONS:

PART I. APM

1. Calculate the concentration of APM in each APM standard solution using the following formula:

$$\text{Conc. APM, mg/ml} = \frac{W \times F}{100}$$

Where, W = Weight of Aspartame Working Standard in each APM standard, mg.

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F = Correction for Aspartame Standard Purity. .

2. For the cooked oatmeal, calculate the total solvent volume, M, per sample. For uncooked oatmeal the total solvent volume, M, is equal to the amount of extraction solvent added.

$$\text{Total solvent volume, ml, M} = [(B-A)/B] \times C + D$$

Where, A = Wt. of dry, uncooked oatmeal, g.

B = Total wt. of cooked oatmeal, g.

C = Wt. of cooked oatmeal in extraction sample, g.

D = Amount of extraction solvent, ml.

Note: Calculation assumes the weight of 1 ml water = 1 g.

3. Record the peak area for each APM Standard injection and calculate the average peak area for set of duplicate injections.

3. Plot the average peak area versus the corresponding APM standard concentration. Using least squares or regression analysis, calculate the slope and y-intercept of the APM standard curve.

4. Record the APM peak area for each sample injection and calculate the average peak area for each set of duplicate injections. Subtract the area of any placebo interference.

5. Determine the APM concentration in mg/ml (Cx) for each sample using the slope and y-intercept of the standard curve.

6. Calculate the APM concentration in ppm for the uncooked samples using equation 6A. Calculate the APM, ppm, for the cooked samples using equation 6B.

$$6A. \quad \text{APM, ppm} = \frac{(Cx) \times M \times 1000}{W_s}$$

$$6B. \quad \text{APM, ppm} = \frac{(Cx) \times M \times B \times 1000}{W_s \times A}$$

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Where: Cx = Concentration of APM in the sample, mg/ml.

Ws = Wt. of extracted sample, g.

A = Wt. of dry, uncooked oatmeal, g.

B = Total wt. of cooked oatmeal, g.

M = Total solvent volume, ml.

7. Calculate the theoretical APM, mg/ml, in the spiked control sample.

$$\text{Theo. APM, mg/ml} = \frac{\text{mg APM Added}}{M}$$

Where, M = Total solvent volume, ml.

8. Calculate the % APM recovery from the spiked control sample.

$$\% \text{ APM Recovery} = \frac{\text{Actual APM, mg/ml}}{\text{Theo. APM, mg/ml}} \times 100$$

9. The % APM recovery from the control sample should be between 93.0% and 99.0%. If not, repeat the analysis. (If desired, the APM concentration in the samples may be corrected for the control sample recovery by dividing the APM, ppm, by the decimal value of the control recovery.)

PART II. DKP

1. Calculate the concentration of DKP in each DKP Std.

2. Record the peak areas of DKP in each DKP Std injection and calculate the average peak areas for each set of duplicate injections.

3. Plot the average peak areas of the DKP standards versus the corresponding standard concentrations. Using least squares or regression analysis, calculate the slope and y-intercept of the DKP standard curve.

4. Record the DKP peak area for each sample injection and calculate the average peak areas for each set of duplicate injections. Subtract the area of any placebo interferences.

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5. Determine the DKP concentration (Cy) for each sample using the slope and y-intercept of the standard curve.

6. Calculate the DKP concentration in ppm for each uncooked sample using equation 6A. Calculate the DKP, ppm, in the cooked oatmeal using equation 6B.

$$6A. \text{ Conc. DKP, ppm} = \frac{(Cy) \times M}{Ws} \times 1000$$

$$6B. \text{ Conc. DKP, ppm} = \frac{(Cy) \times M}{Ws} \times \frac{B}{A} \times 1000$$

Where: Cy = Concentration of DKP in the sample, mg/ml.

Ws = Wt. of extracted sample, g.

B = Total wt. of cooked oatmeal, g.

A = Wt. of dry, uncooked oatmeal, g.

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Figure 1
Chromatogram of APM Standard

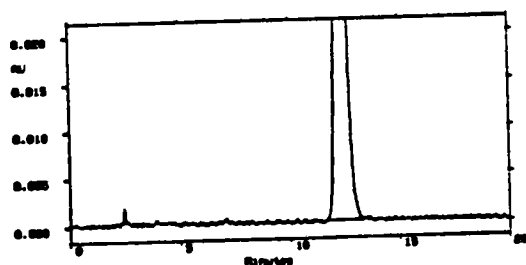
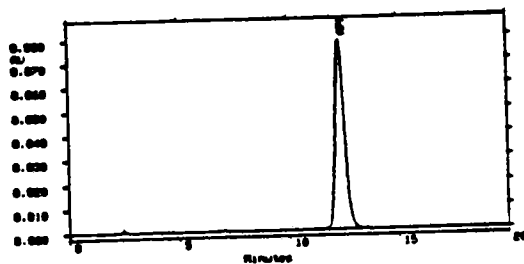
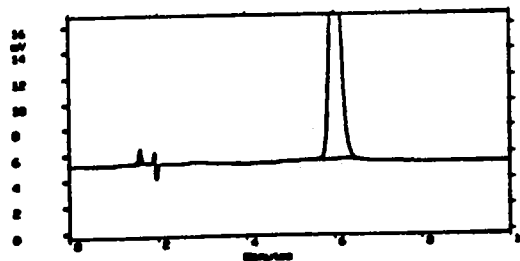
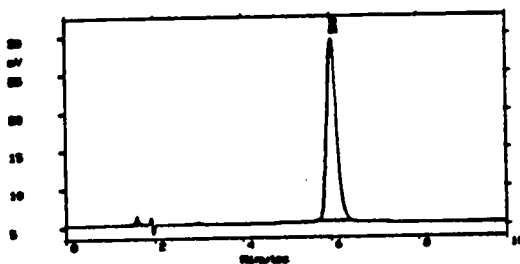


Figure 2
Chromatogram of DKP Standard



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Figure 3
Chromatogram of Instant Oatmeal

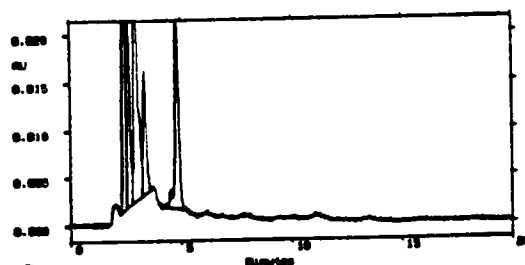
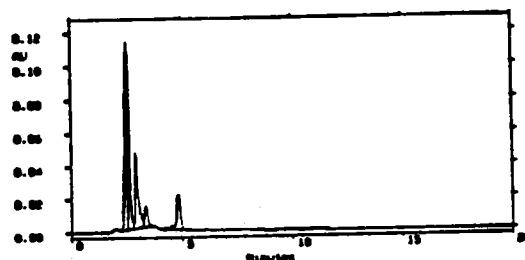
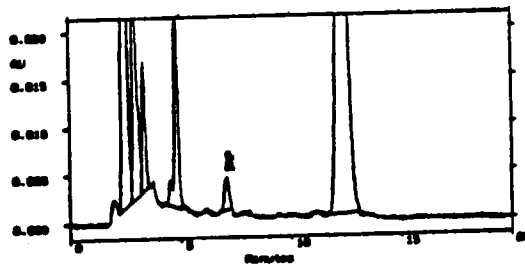
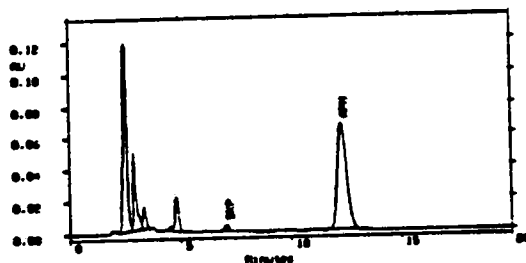


Figure 4
Chromatogram of Oatmeal Containing APM



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Method Validation Summary

APM

Precision

The within run precision of the method determined using cooked oatmeal spiked with APM standard in the range of 660 to 2450 ppm APM cake was 3.2% (N=6, 1 day/1 analyst).

The between run precision of the method using the spiked placebos above was 3.2%.

Accuracy

The average percent recovery of the method determined from the spiked placebo recoveries, was 96.9% (N=6). The method is linear in the range of 0.05 mg/ml to 0.27 mg/ml.

Specificity

There is no interference between the APM, DKP, and other chromatographic responses.

Sensitivity

An increase of 1 ug of APM concentration will give a corresponding increase of 1.07×10^7 area counts. The sensitivity is considered satisfactory for this method.

References

N.B. Ref. 144, p.114 to 138, J. Harrison.

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Method Validation Summary

DKP

Precision

The total precision for the method determined using cooked oatmeal spiked with DKP standard in the range of 90 to 180 ppm DKP (equivalent to 5% to 10% of the theoretical APM level) was 0.95% (N=6, 1 day/1 analyst).

Accuracy

The average percent recovery of the method determined from the spiked placebo recoveries, was 95.4% (N=6, 2 days/1 analyst).

The method is linear in the range of 0.01 to 0.08 mg/ml.

Specificity

Refer to the specificity statement given for APM.

Sensitivity

An increase of 1 μ g of DKP concentration will give an increase 1.4×10^7 area counts. This is considered satisfactory for this method.

References

N.B. Ref. 144, p.123 to 125, J. Harrison.