

Solid Phase Extraction/Liquid Chromatography Method for the Determination of Niacin in Commercial Flour Products

Denis E. LaCroix^{1,2} and Wayne R. Wolf^{1,3}

Cereal Chem. 84(2):116–118

The Food Composition Laboratory has previously reported on successful methods to determine niacin (or nicotinamide) in a variety of food matrices. Various problems previously encountered in applying these approaches to determination of niacin in enriched commercial flour products have now been overcome.

We had previously employed solid phase extraction (SPE) following a standard acid digestion procedure (Methods 944.13, 960.46; AOAC 2000) as a sample clean-up step before analysis of infant formula and processed cereal samples for nicotinic acid by liquid chromatography (LC) (LaCroix et al 2001, 2002a,b). This SPE/LC method was then successfully applied for determination of niacin in wheat flour reference materials but was unsuccessful for commercial all-purpose wheat flour due to the co-elution of excessive endogenous 260-nm absorbing LC peaks occurring for this matrix (LaCroix 1999).

Since nicotinamide is the main form of niacin added to formulated or enriched products, Woollard and Indyk (2002) have developed a trichloroacetic acid (TCA) extraction of nicotinamide in milk products before LC analysis, eliminating the acid digestion step. We have investigated the application of Woollard's TCA/LC method with modifications for the successful quantitative analysis of processed enriched cereal products for nicotinamide (LaCroix et al 2005) but were unable to utilize this method for enriched commercial wheat flour due to the presence of excessive fine particles that were not removed by 0.25- μ m filtration. Therefore, we have reexamined our earlier acid digestion SPE/LC method to determine niacin in the enriched commercial flours as nicotinic acid.

As described in earlier reports (LaCroix et al 2002b, 2005), use of a multiwavelength photodiode array detector and analysis of the subsequent spectral scans allows definitive visual information of the presence of one or more compounds under an LC peak, allowing confirmation of the purity of the chromatographic peak.

MATERIALS AND METHODS

Samples

It is our practice to routinely include available reference materials from commercially available sources in the process of validating development of new analytical methods to assess the accuracy of the procedure in a similar matrix. For this study, RM-8436 durum wheat, RM-8437 hard red spring, and RM-8438 soft winter wheat were obtained from the National Institute of Standards and

Technology (NIST). An in-house reference flour (RM-4B) was obtained courtesy of Medallion Laboratories, Minneapolis, MN. Samples representing a variety of commercial flour matrices were purchased from local supermarkets. A total of eight samples including four manufacturers' brands of all-purpose wheat flour, one brand each of rice flour, corn meal flour, pancake mix, and all-purpose baking mix products were chosen. The flours were reground in a blender and stored in plastic bags with no further storage precautions taken.

Sample Extraction for LC Analysis

Niacin was extracted from the flour matrix following the acid digestion/solid phase extraction (SPE) method of LaCroix (2001, 2002a). The dry flour powders were accurately weighed to give a level of 100–200 μ g of nicotinic acid per analysis, based on the declared label value. The weighed dry flour powders were digested by autoclaving at 121°C for 45 min in 2.5*N* H₂SO₄ (ACS grade, Aldrich Chemical Company, St. Louis, MO), which converts the enriched nicotinamide to nicotinic acid, followed by SPE using a cation-exchange ArSCX-SPE column. This digestion will also free endogenous niacin bound to protein to give a total niacin value. The nicotinic acid is eluted from the SPE column using a 0.5*M* sodium acetate-acetic acid buffer (ACS grade, Aldrich) of pH 5.6. Nicotinic acid standard is prepared in the same manner as the flour products.

Liquid Chromatographic Instrumentation

A ThermoSeparation Products (TSP) liquid chromatography system, equipped with a photodiode array detector (PDA) and ChromQuest software (ThermoQuest Manual 1998) was used. Nicotinic acid was separated from endogenous 260-nm peaks using a mobile phase of sodium acetate-acetic acid buffer at pH 4.0–4.2. Mobile phase flow rate was 1.3 mL/min (3,100 psi) with a sample injection size of 100 μ L. A poly(styrene-divinylbenzene)trimethyl ammonium (Alltech Anion/R) anion-exchange column (250 mm \times 4.1 mm, particle size 10 μ m) (Alltech Associates, Deerfield, IL) was used. Nicotinic acid is determined at the peak maximum of 260 nm.

Spectral Analysis

The spectral analysis feature of ChromQuest software (LaCroix et al 2002b) was used to determine the purity of the HPLC analyte chromatographic peak by comparison to the standard nicotinic acid chromatographic peak, including visual examination of a three-dimensional configurational analysis (absorbance vs. wavelength of a spectral scan from 220 to 300 nm at the peak retention time).

Statistical Analysis

The nicotinic acid data for reference materials and commercial products was examined for outliers using Dixon's outlier criteria (Dixon and Massey 1957). Method performance characteristics were examined by the method of standard additions (MOSA) (Mishalanie 1996).

¹ Food Composition Laboratory (FCL), Beltsville Human Nutrition Research Center (BHNRC), United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Building 161, Beltsville, MD.

² Retired, FCL, USDA.

³ Corresponding author. Phone 301-504-8927. Fax: 301-504-8314. E-mail: wayne.wolf@ars.usda.gov

RESULTS AND DISCUSSION

Figure 1 is a representative liquid chromatogram of the reference material and corresponding commercial wheat flour samples used in this study. Modification of the previous (LaCroix 1999) LC protocol by using a poly(styrene-divinylbenzene)trimethyl ammonium anion-exchange (Alltech Anion/R) LC column and by increasing the flow rate to 1.3 mL/min results in the complete LC separation of nicotinic acid eluting at ≈ 35 min of retention time from the interfering, earlier eluting, endogenous 260-nm absorbing peaks.

A typical MOSA plot is shown in Fig. 2, along with a corresponding nicotinic acid standard curve. Also shown is the identity curve that is obtained by subtraction of the peak area of the endogenous niacin in the unspiked sample from the peak areas of the spiked samples in the MOSA. In the absence of significant bias errors, the identity curve should match the dose response curve of the niacin standard, which is true within analytical error for these samples (Mishalanie 1996). The MOSA curve also shows linearity over the analysis range ($R^2 > 0.99$). Data from the MOSA curves can also be used to assess analyte recovery over the range of standardization. A comparison of the slope of the MOSA curve to the standard curve represents recovery of the added

analyte (i.e., for Fig. 2, $4586/5050 = 91\%$ recovery). Because each of the samples was determined with a separate MOSA curve, recovery was assessed for each sample, with an overall recovery of 95% of added niacin being observed.

Using this SPE/LC method, the nicotinic acid content of the four reference flours and eight commercial flour products are listed in Tables I and II, respectively. Niacin values obtained with this method were in agreement with assigned values for the four reference materials. Seven of the commercial flour products were near to or had higher niacin content than what was listed on the package label. These higher levels are not surprising because food manufacturers commonly provide these increased nutrient levels in foods to ensure shelf-life compliance with USFDA nutritional labeling requirements (USFDA 1993). The acid digestion procedure used in this method frees any protein-bound niacin and provides a value of total niacin, not just added niacin enrichment. Only one product (pancake flour) had a niacin value significantly lower than the declared label value. The %RSD for these commercial samples were in the vicinity of those values obtained for the reference flours, again with the exception of the one commercial flour product with the lower niacin value.

Spectral analysis data of the LC nicotinic acid peak obtained from the commercial flour products closely mimic the data of the reference flours. The similarity indices (>0.98) and peak purity indices (>0.91) approached the ideal value of unity for all samples, with the exception of one sample (the all-purpose baking mix) that had a slightly asymmetrical peak ($PPI < 0.79$).

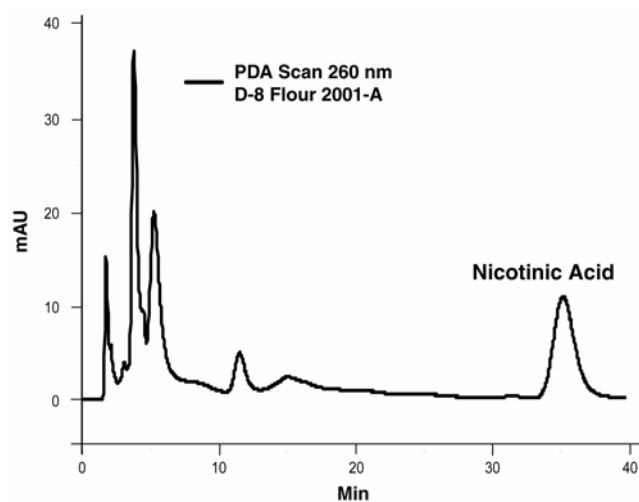


Fig. 1. Liquid chromatogram of a typical flour sample.

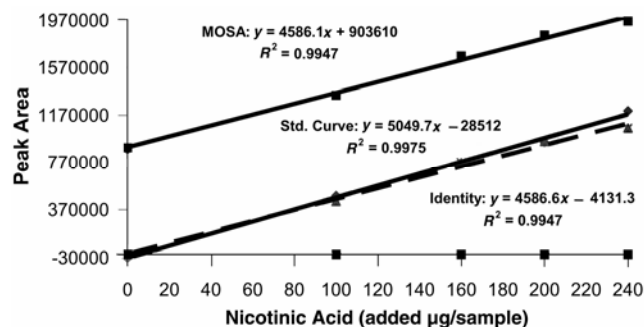


Fig. 2. Method of standard additions (MOSA), identity, and standard curves for a typical flour analysis.

TABLE I
Nicotinic Acid Values Obtained for Reference Flours

Reference Flour	Reference Value ($\mu\text{g/g}$)	Analyzed Value Average ($\mu\text{g/g}$)	SD	% RSD	% Assigned Value	n
RM-8436 durum wheat	93.2 ^a	89.9	7.6	8.5	96.4	20
RM-8437 hard red spring wheat	39.4 ^a	35.2	3.4	9.7	89.3	33
RM-8438 soft winter wheat	39.2 ^a	35.5	3.9	10.9	90.5	23
RM-4B hard wheat	61.6 ^b \pm 4.6	66.1	7.9	12.0	107.2	5

^a Literature value (Tanner 1988).

^b Analyzed value (Medallion Laboratories, Minneapolis, MN).

TABLE II
Nicotinic Acid Values Obtained for Commercial Flour Products^a

Commercial Flour	Label Value ($\mu\text{g/g}$)	Analyzed Value Average ($\mu\text{g/g}$)	SD	% RSD	% of Label Value	n
All-purpose flour #1	53.3	65.8	4.7	7.1	123.5	13
All-purpose flour #2	53.3	55.3	2.3	4.2	103.7	16
All-purpose flour #3	51.6	83.4	2.9	3.5	162.7	14
All-purpose flour #4	53.3	57.9	4.5	7.8	108.7	16
Rice #1	45.7	42.2	8.0	18.9	92.4	11
Corn meal	40.0	51.1	6.3	12.4	127.8	17
Pancake mix	36.4	21.7	4.8	21.9	59.6	17
All-purpose baking mix	40.0	35.7	4.1	11.5	89.0	9

^a Niacin value on cereal box label based Code of Federal Regulations (CFR) 1.01(9) at 20 mg/day recommended daily allowance (RDA).

Visualization of 3D configurational spectra clearly showed that the LC peak contained only nicotinic acid, free from the interference by endogenous 260-nm absorbing materials.

CONCLUSIONS

An acid digestion/solid phase extraction of nicotinic acid followed by anion-exchange LC was evaluated for analysis of niacin, as nicotinic acid, content of commercial flour products. The inclusion of reference flours with certified niacin content as an integral part of the verification and validation of the method gives the user confidence in the data quality obtained. Obtained nicotinic values were close to the assigned values of the reference products and, in general, somewhat above the declared values of the commercial flour products.

LITERATURE CITED

AOAC. 2000. Official Methods of Analysis, 17th Ed. Methods 944.13, 960.46. AOAC Int.: Gaithersburg, MD.
Dixon, W. J., and Massey, Jr., F. J. 1957. Introduction to Statistical Analysis. McGraw-Hill: New York.
LaCroix, D. E., and Wolf, W. R. 2001. Determination of niacin in infant formula by solid-phase extraction and anion-exchange liquid chromatography. PVM:2000. J. AOAC. Int. 84:789-804.

LaCroix, D. E., Wolf, W. R., and Vanderslice, J. T. 1999. Determination of niacin and wheat flour by anion-exchange liquid chromatography with solid-phase extraction cleanup. J. AOAC Int. 82:128-132.
LaCroix, D. E., Wolf, W. R., and Chase, Jr., G. W. 2002a. Determination of niacin in infant formula by solid-phase extraction/liquid chromatography: Peer-verified method performance—Interlaboratory validation. J. AOAC Int. 85:654-664.
LaCroix, D. E., Wolf, W. R., and Hindsley, T. H. 2002b. Evaluation of niacin LC methods by diode-array/spectral analysis. Anal. Lett. 35:2187-2198.
LaCroix, D. E., Wolf, W. R., and Kwansa, A. 2005. Rapid TCA extraction/LC method for the determination of nicotinamide in commercial cereals. Cereal Chem. 82:277-281.
Mishalanie, E. A. 1996. Intralaboratory Analytical Method Validation, Short Course Manual. AOAC Int.: Gaithersburg, MD.
Tanner, J. T., Angyal, G., Smith, J. S., Weaver, C., Bueno, M., Wolf, W. R., and Ihnat, M. 1988. Survey of selected materials for use as an organic nutrient standard. Frezenius Z. Anal. Chem. 332:701-703.
ThermoQuest. 1998. Chromatography System for Windows NT and Thermo LC Users Guide. Manual A009651. ThermoQuest: San Jose, CA.
USFDA. 1993. Nutrition Labeling Manual: A Guide for Developing and Using Databases. United States Food and Drug Administration: Washington, DC.
Woollard, D. C., and Indyk, H. E. 2002. Rapid determination of thiamine, riboflavin, pyridoxine, and nicotinamide in infant formulas by liquid chromatography. J. AOAC Int. 85:945-951.

[Received November 16, 2005. Accepted September 7, 2006.]