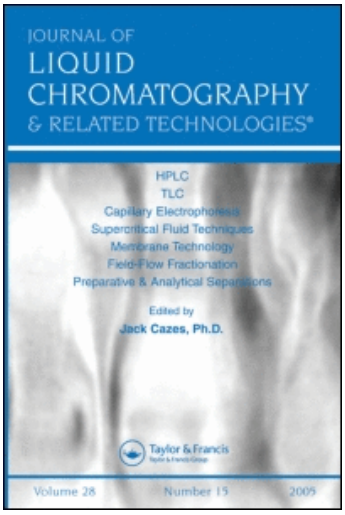

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Theodore A. Tyler[a]; Robert R. Shrago[a]
[a] Herbert V. Shuster Inc., Massachusetts

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DETERMINATION OF NIACIN IN CEREAL SAMPLES BY HPLC

Theodore A. Tyler and Robert R. Shrago
Herbert V. Shuster Inc.
5 Hayward St.
Quincy, Massachusetts 02171

ABSTRACT

A liquid chromatographic method for the analysis of niacin in cereal products is presented. The sample is extracted in the presence of $Ca(OH)_2$, concentrated and purified on an anion exchange resin, and further cleaned up by oxidation with $KMnO_4$. Final quantitation is by HPLC with ultraviolet detection at 254 nm.

Using a μBondapak C_{18} column, a mobile phase consisting of 5% methanol in water containing 0.005 M tetrabutyl ammonium phosphate, and a flow rate of 2.0 ml/min., niacin was found to have a retention time of about 7 minutes.

INTRODUCTION

Niacin in food samples is officially determined either by the microbiological method (1) or by the chemical method (2). The microbiological method requires maintaining a stock of viable microorganisms and the assay requires an incubation period of 3 days. The chemical method requires the use of CNBr and is subject to several interferences (3). Variations of these methods (3) meet with similar difficulties.

Since most modern laboratories now have HPLC capability, a liquid chromatographic alternative to the chemical or the microbiological method should find widespread acceptance.

METHOD

Apparatus

The high pressure liquid chromatograph consisted of a Waters Associates (Milford, MA) 6000A pump, a U6K injector, a μBondapak C_{18} column, and a Model 440 absorbance detector operating at 254 nm.

Waters Associates C_{18} Sep-Pak cartridges were used to clarify the samples before final filtration through 0.45 micrometer filters.

Open-column chromatography tubes were Kontes (Vineland, NJ) Chromaflex Column K-420150, 200 mm x 6 mm O.D. with a 25 ml capacity top reservoir.

Reagents and Solutions

All chemicals were reagent quality (Fisher Scientific Co., Pittsburgh, PA) and all solvents were HPLC quality (Burdick and Jackson, Muskegon, MI).

The mobile phase consisted of 50 ml of methanol plus one vial of Waters PIC-A reagent, brought to 1000 ml with water and filtered through a 0.45 micrometer filter.

The niacin standard solution was prepared by diluting 10 ml of a solution containing 50 mg niacin/ 100 ml to 1000 ml with water. The $Ca(OH)_2$ suspension was prepared by combining 50 grams of $Ca(OH)_2$ with 500 ml of water. The anion exchange resin was Bio-Rad AG1-X8, 100-200 mesh, in the acetate form (Bio-Rad Laboratories, Richmond, CA). The 5% acetic acid was prepared by diluting 50 ml of glacial acetic acid to 1000 ml with water. The 1% $KMnO_4$ was prepared by dissolving 1.00 grams of $KMnO_4$ in water and then diluting to 100 ml with water.

Extraction

Sufficient sample was weighed into a 200 ml volumetric flask to contain not more than 0.20 milligrams of niacin. Approximately 80 ml of water and 10 ml of well shaken $Ca(OH)_2$ suspension were added and the mixture was heated on a steambath for 30 minutes. After swirling to disperse the sample, the flask was capped with aluminium foil and autoclaved for 30 minutes at 15 psi. After cooling to room temperature, the sample was brought to volume with water and transferred to a 200 ml centrifuge bottle. The bottle was cooled overnight in a refrigerator and the following morning the cold solution was centrifuged for five minutes at 2000 rpm. The supernatant was carefully decanted into a 250 ml flask and capped prior to use.

Open-Column Chromatography

A 30 mm column of AG1-X8 anion exchange resin was formed between two plugs of cotton in a 25 ml Chromaflex column. The column was washed with 25 ml of 5% acetic acid followed by 10 ml of water. Two successive 25 ml aliquots of sample extract were pipetted onto the column. When the level of sample extract reached the surface of the packing, 20 ml of water were pipetted onto the column. When the level of water reached the surface of the packing, a 50 ml erlenmeyer flask was placed under the column and the niacin was eluted from the column with 10 ml of 5% acetic acid followed by 5 ml of water.

Permanganate Oxidation

The erlenmeyer flask was transferred to a shallow boiling water bath and the solution was brought to a gentle simmer. One ml of 1% aqueous $KMnO_4$ was added, and the solution was immediately swirled and removed from the bath. After cooling to room temperature, the solution was quantitatively transferred to a 25 ml volumetric flask and brought to volume with water.

Preparation of the Standard

A 10 ml aliquot of the 5 μg/ml niacin solution was pipetted onto a 30 mm anion exchange column, and using the previously outlined open-column chromato-

graphy technique, the niacin was eluted from the column with 10 ml of 5% acetic acid followed by 10 ml of water. The eluate was collected directly in a 25 ml volumetric flask and was brought to volume with water.

Determination

A portion of each sample solution and standard solution was passed through a C_{18} Sep-Pak and a 0.45 micrometer filter. Twenty-five µl of the eluate were injected into the liquid chromatograph at 0.005 AUFS and the peak height corresponding to the niacin was measured.

Calculation

$$\frac{\text{mg Niacin}}{\text{100 grams}} = \frac{20}{SW} \times \frac{H(sam)}{H(std)}$$

where H(sam) = peak height of niacin for the sample

H(std) = peak height of niacin for the standard

SW = sample weight in grams

RESULTS AND DISCUSSION

Retention of Niacin on C_{18}

Initial results using 5% acetic acid in water as the mobile phase yielded a k' of 0.18 for niacin. Since more retention is required to separate niacin from the rapid eluters in a food sample, the PIC-A system was tried. The retention at various methanol concentrations is shown in Table 1.

TABLE 1

%CH_3OH	t_R(min)	k'
0	8.0	4.0
5	6.9	3.3
9	6.2	2.9
17	3.5	1.2

Chromatography of Crude Niacin Extract

A semolina sample was digested with $Ca(OH)_2$ and a portion of the extract was passed through a C_{18} Sep-Pak and through a 0.45 micrometer filter. Using 9% CH_3OH in water with PIC-A as the mobile phase, the LC chromatogram resulted in a well-defined niacin peak, partially resolved from nearby peaks, as shown in Figure 1.

Chromatography of Cleaned-Up Niacin Extract

Additional clean-up was provided by ion-exchange chromatography, which gave approximately 98% recovery of the standard, and by permanganate oxidation, which gave no loss of standard. The slight loss of niacin on the ion-exchange column is partially compensated for by passing both sample and standard through identical columns. The LC chromatograms for a semolina sample and for a bread sample are shown in Figure 2.

Comparison of LC and Microbiological Methods

The semolina sample assayed at 8.0 mg niacin/100g by the microbiological method and at 8.4 mg/100g by

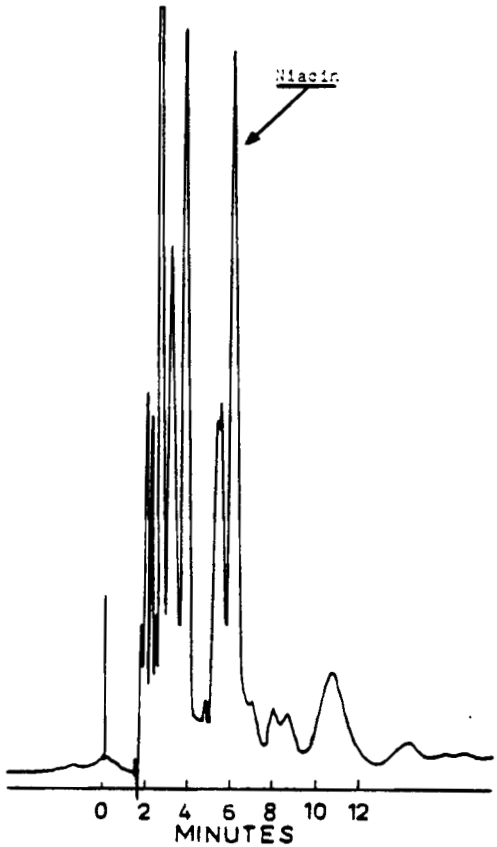

Figure 1: Partial resolution of niacin in crude semolina extract. Flow rate = 2.0 ml/minute. Mobile Phase = 9% methanol in water with PIC-A.

the LC method. The average error from the mean was 0.5 mg/100g for both methods.

The bread sample assayed at 4.1 mg niacin/100g by the microbiological method and at 4.23 mg niacin/100g by the LC method. The microbiological method had an average error of 0.3 mg/100g and the LC method had an average error of 0.10 mg/100g.

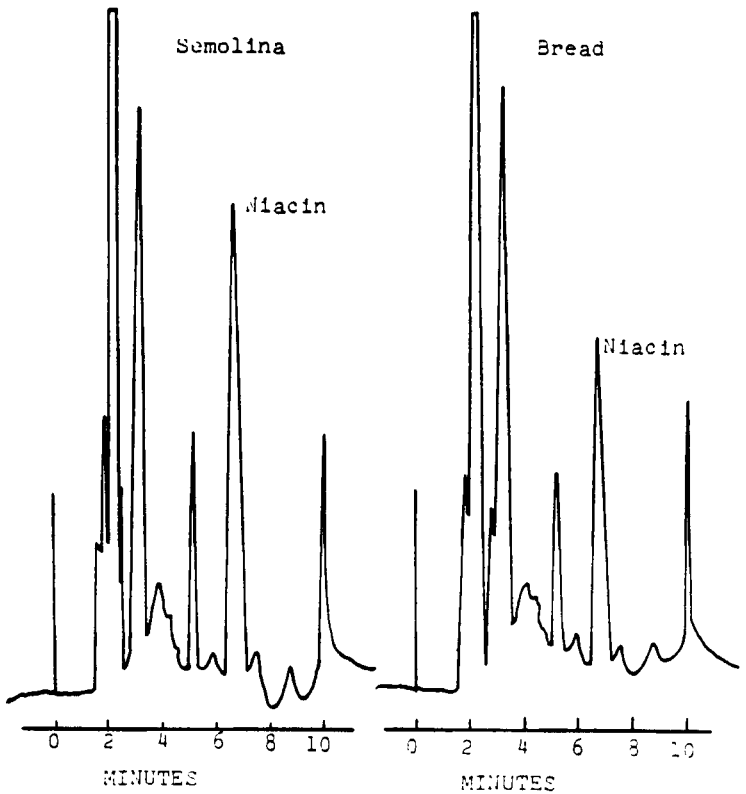

Figure 2: Niacin in semolina and bread samples after ion-exchange and permanganate clean-up. Flow rate = 2.0 ml/minute. Mobile phase = 5% CH_3OH in water with PIC-A.

Conclusion

Refinement of the techniques of sample extraction, purification, and clean-up will soon enable liquid chromatographic methods to replace many assays that are now being done by more laborious methods.

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