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Determination of a small amount of niacin in foodstuffs by high-performance liquid chromatography

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ABSTRACT

A high-performance liquid chromatographic method for the determination of a small amount of niacin in foodstuffs is described. This method is based on hydrolysis of sample, extraction, clean-up (anion-exchange and cation-exchange columns) and separation from interferences by chromatography on an amino phase column. The detection limit of niacin in foodstuff is about 0.01 mg per 100 g. The results of analysis of foodstuffs by this method are in good agreement with those obtained by the microbiological method.

INTRODUCTION

Determination of niacin in foodstuffs is generally performed by the chemical method [1] or the microbiological method [2]. In these methods, the sample is treated with strong acid or alkali to free niacin before quantitation because niacin is usually bonded with nucleotides or proteins in its natural form. Therefore many hydrolysis interferences are contained in the extracted solution and some separation procedures are needed before colorimetric measurement.

Today, the microbiological method is generally better than the above chemical method and therefore is widely accepted as the official method. There are two microbiological methods. One method is quantitation by titration after a 72 h incubation period. The other method is analysis by turbidimetric measurement after 16–22 h incubation. These procedures are very time-consuming, and culture of test organisms and their handling are troublesome. This method is also not able to determine levels of niacin below 0.1 mg per 100 g. High-performance liquid chromatography (HPLC) has been suggested to offer a good approach to the precise determination of niacin in foodstuffs [3–6] at the mg per 100 g level. However, in the case of many foodstuffs, notably those containing a small amount, niacin cannot be completely separated from interferences and therefore a good result with high sensitivity cannot be obtained. This situation is the same as in the Association of Official Analytical Chemists chemical method.

The purpose of this paper is to develop a simple, efficient and highly sensitive method for determination of a small amount of niacin in foodstuffs by means of hydrolysis of sample, clean-up (anion-exchange column and disposable cation cartridge column) and liquid chromatographic determination.

EXPERIMENTAL

Apparatus

The HPCL system (Waters, Division of Millipore, Milford, MA, USA) consisted of a Model 590 pump, a U6K injector, a Model 490 UV–VIS detec-

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tor and a Model 840 data system. The column was a 250 mm × 4.6 mm I.D. Asahipak NH2P-50 column, 5 μm particle size, preceded by an NH2P 50 mm × 4.6 mm I.D. guard column supplied by Asahi Kasei (Tokyo, Japan). The chromatographic column used for clean-up was a 150 mm × 27 mm I.D. glass column, equipped with a stopcock.

Reagents

Standard niacin was purchased from Wako (Osaka, Japan). The AGI-X8 anion-exchange resin (acetate$^-$, 100–200 mesh) was purchased from Bio-Rad (Tokyo, Japan) and the Toyopack IC-SP M cartridge column was purchased from Toso (Tokyo, Japan). This column is a cation-exchange gel and its exchange capacity is 0.4 mequiv. Acetonitrile used was HPLC grade. All other chemicals and solvents were reagent grade and were used without purification.

Chromatographic conditions

The mobile phase consisted of acetonitrile–water (60:40, v/v) containing 0.075 *M* sodium acetate. The flow-rate was 0.5 ml/min. Separation was carried out at ambient temperature. The eluate was monitored by UV–VIS detection at a wavelength of 261 nm.

Procedure

Extraction and hydrolysis. The sample (5–10 g) was accurately weighed into a 100-ml beaker. A 4-ml aliquot of 40% sodium hydroxide solution and approximately the same volume of water were added and the beaker was covered with a watch-glass. The mixture was heated on a steam bath for 30 min. After cooling to room temperature, the solution was neutralized with 25% hydrochloric acid and the same volume of methanol added. This solution was filtered through Toyo No. 5 filter paper into a 100-ml volumetric flask. The residues were rinsed with 50% methanol and passed into the same flask. Then the flask was filled up to 100 ml with 50% methanol. A 30-ml aliquot of this solution was transferred into a 200-ml round-bottom flask and methanol was added. The solution was evaporated to dryness using a rotary evaporator. The residues were taken up in 20 ml of water.

Clean-up. A 9-g portion of anion-exchange resin was packed in a glass tube (mentioned in the Experimental section) and washed with 50 ml of water. Aliquots of 20 ml of sample extracts were added to the column and passed through at a flow-rate of about 1 ml/min. After being washed with 50 ml of water, the niacin was eluted from the column with 50 ml of 13% acetic acid into a 50-ml volumetric flask. A 30-ml aliquot of the above fractionated solution was transferred in a 200-ml round-bottom flask, 50 ml of methanol was added and the solution was evaporated to dryness using a rotary evaporator. The residues were taken up in 5 ml of water. This solution was passed through a Toyopak cation cartridge column which was previously conditioned with 4 ml of 1 *M* hydrochloric acid and rinsed with 10 ml of water. Then niacin was eluted from cartridge with 15 ml of 1 *M* hydrochloric acid. This fraction was collected in a 100-ml round-bottom flask. After adding methanol, the solution was evaporatred to dryness with a rotary evaporator until free from chloride odor. Then the residues were taken up in 2 ml of water accurately.

Analysis. This solution was passed through a membrane filter (0.45 μm) and 5–20 μl of sample solution were injected into the chromatograph. The eluate was monitored by UV–VIS detection at a wavelength of 261 nm. A standard stock solution was prepared by weighing out 10.0 mg of niacin, dissolving it in water and diluting it to 100 ml accurately. The working standards used for the normalization as well as for the fortification of recovery samples were prepared by pipetting the stock solution.

RESULTS AND DISCUSSION

Extraction

Samples of foodstuff may be of many kinds, for example with high fat or protein contents and liquid or solid, etc. It is generally very difficult to extract niacin from samples without interferences. In this study, we attempted to find a general procedure that can be used for many kinds of sample and the following one is given as an example. Samples were hydrolyzed in the presence of alkali, neutralized with acid, and the same volume of methanol was added to the neutralized solution. After hydrolysis, filtration from the solution can be performed effectively, especially for high-protein or carbohydrate-rich samples, when methanol is added to the neutralized solution.

Elution from AGI-X8 anion-exchange column

If the methanolic solution is applied to the top of AGI-X8 column, niacin cannot be sufficiently retained on the column unless the column has previously been conditioned with 50% methanol solution for equilibration. In this experiment, the sample solution was evaporated to dryness by a rotary evaporator and residues were taken up in 20 ml of water. The solution so obtained was applied to the top of column.

Fig. 1 shows the elution pattern of niacin from AGI-X8 with the eluate of 13% acetic acid. Of the niacian, 89% was eluted with the first 20 ml and 98% with 50 ml. In this case, since no salt existed, niacin could not be eluted from the AGI-X8 column with 50 ml of acetic acid. Therefore elution in the above experiment was carried out int he presence of a 0.4 *M* sodium chloride solution, about the same amount as in the practical samples.

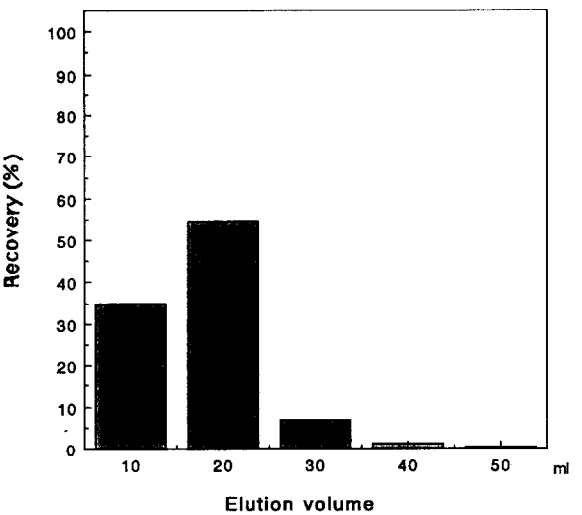

Fig. 1. Elution pattern of niacin from an AG1-X8 column. Eluent, 13% acetic acid; niacin, 100 μg.

Additional clean-up with an IC-SP M cation-exchange column.

It is difficult to determine a small amount of niacin with high sensitivity by utilizing only a one-step clean-up with an anion-exchange column. It is effective to carry out additional clean-up with a cation-exchange column with which the pyridine nucleus in a niacin molecule interacts.

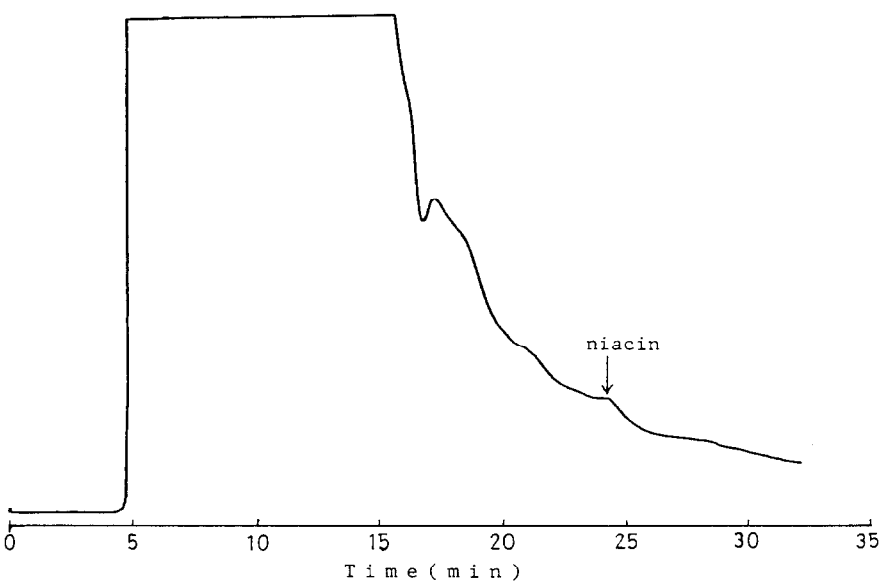

Fig. 2. Chromatogram of niacin in strawberry jam (niacin content: 0.07 mg per 100 g), after clean-up by anion-exchange resin only. Column: Hitachi gel 3014N, 300 mm × 4 mm; column temperature, 40°C; mobile phase, 0.1 *M* ammonium chloride + 0.025 *M* potassium dihydrogenphosphate + 0.0125 *M* dipotassium hydrogenphosphate, butyl alcohol (97:3); flow-rate, 0.6 ml/min.

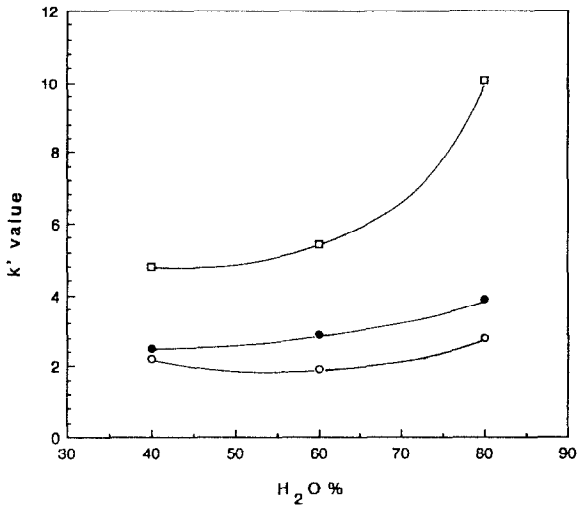

Fig. 3. Variation of k' value with mobile phase composition. (□) 0.02 M, (●) 0.05 M and (○) 0.075 M sodium acetate.

HPLC separation of niacin

Several types of column were tried to separate niacin from interferences in foodstuff. Niacin was eluted very rapidly at near-void volume in the C_{18} stationary phase using methanol–water or acetonitrile–water mixtures as the mobile phase, because in this case niacin interacts weakly with the octadecyl group in the stationary phase. By using an ion-exchange column (Hitachi gel, 3014N) niacin can be interferences with phosphate buffer solution as eluent. Fig. 2 shows a typical chromatogram obtained from strawberry jam (niacin content: 0.07 mg per 100 g) after clean-up by anion exchange resin only. However, in the case of the carbohydrate-rich sample, niacin is hindered by the impurities arising from the hydrolysis process and cannot be separated from interferences. For this reason, the detection limit of niacin is only about 0.3 mg per 100 g. However, this column may be used for determination of a small amount of niacin in foodstuffs by adding more clean-up steps. On the other hand, niacin strongly interacts with amino groups and may be separated by an amino phase column. Niacin is not eluted using an acetonitrile–water mixture only as the mobile phase but can be eluted quickly by increasing the salt concentration in the mobile phase. Fig. 3 shows a few examples of the variation of capacity factor, k', with the mobile phase composition. Niacin is eluted faster with increasing salt concentration.

This mechanism of bonded-phase chromatography is very complex, but in this case the retention behavior may be affected by the interaction of oxygen in the carbonyl group of a niacin molecule with the hydrogen of the amino group in the stationary phase. In the practical analysis, acetonitrile–water (60:40, v/v) containing 0.075 M sodium acetate was used as the mobile phase to avoid precipitation of salt. A typical chromatogram of niacin obtained from strawberry jam (the same sample as used in Fig. 2) after clean-up is shown in Fig. 4.

Quantitation of niacin

The calibration curve for niacin was linear over the concentration range 10–100 ng. The minimum detectable amount of niacin was found to be approximately 1 ng.

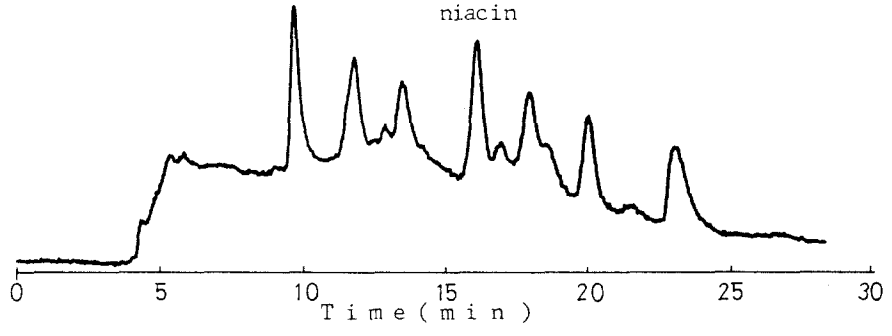

Fig. 4. Chromatogram of niacin extracted from strawberry jam (niacin content: 0.07 mg per 100 g) after clean-up.

TABLE I

RECOVERY AND REPRODUCIBILITY TEST

Experiment No.	Recovery (μg)	Recovery (%)
Fortification level of 60 μg per 10 g		
1	58.4	97.3
2	56.0	93.3
3	57.1	95.2
4	55.5	92.5
5	56.6	94.3
Mean	56.7	94.5
S.D.	1.1	1.9
Fortification level of 5 μg per 10 g		
1	4.90	98.0
2	4.25	85.0
3	4.50	90.0
4	4.46	89.2
5	4.60	92.0
Mean	4.54	90.8
S.D.	0.24	4.7

The recovery and reproducibility of the method were measured with vinegar samples fortified with a standard niacin. Niacin was added at a concentration of 60 and 5 μg per 10 g in five experiments and subjected to analysis as described in the Experimental section. The standard deviation of this method was found to be 1.9 and 4.7%, respectively (Table I).

Comparison of the HPLC method with the microbiological method

Niacin in a several kinds of vinegar and jam was determined using the HPLC method mentioned above (Table II). Niacin in the same sample was also determined by the microbiological method. The titration technique was used in the determination because this procedure is more precise than turbidimetric measurement. But it is not easy to determine correctly a small amount of niacin in these samples using the microbiological method. The results obtained by the HPLC method were in reasonable agreement with the microbiological data.

TABLE II

DETERMINATION OF NIACIN IN FOODSTUFFS

Sample	Concentration (mg per 100 g)	
	HPLC	Microbiological method
Rice vinegar	0.13	0.1
Grain vinegar	0.02	Trace
Strawberry jam	0.07	0.1
Orange jam	0.07	0.1
Apple jam	0.02	Not detected

CONCLUSION

The microbiological method has been widely used but it is difficult to determine correctly a small amount of niacin in foodstuffs. The HPLC method described here has been used for the analysis of niacin at concentrations as low as 0.1 mg per 100 g or less in foodstuffs simply and with high sensitivity. In this study, most of work was carried out using vinegar or jam as samples, but other foodstuffs can be handled similarly. The method described here can be adopted as a routine method.

REFERENCES

1 *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, DC, 15th ed., 1990, Section 961.14.
2 *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, DC, 15th ed., 1990, Section 960.46.
3 T. A. Tyler and R. R. Shrago, *J. Liq. Chromatogr.*, 3 (1980) 26.
4 P. J. Niekerk, S. C. C. Smit, E. S. P. Strydom and G. Armbruster, *J. Agric. Food Chem.*, 32 (1984) 304.
5 K. Takatsuki, S. Suzuki, M. Sato, K. Sakai and J. Ushizawa, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 698.
6 T. Hamano, Y. Mitsuhashi, N. Aoki, S. Yamamoto and Y. Oji, *J. Chromatogr.*, 457 (1988) 403.