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Note

Simultaneous determination of niacin and niacinamide in meats by high-performance liquid chromatography

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Niacin and niacinamide belong to the vitamin B group. They are widely distributed in living cells and exist mainly in bound forms as part of nicotinamide adenine dinucleotide. They also occur in free form and are used as colour fixatives to develop and maintain a fresh colour in meats[1]. However, because high intakes of niacin from treated meats cause hyperaemia symptoms in humans, *i.e.*, red, flushed, itchy skin and headaches, such uses of niacin and niacinamide have been prohibited in Japan since 1982. Hence, the levels of niacin and niacinamide in meats are of concern to health authorities.

The conventional method for the assay of total niacin and niacinamide involves spectrophotometry after hydrolysis and derivatization (König reaction)[2]. However, this method suffers from several drawbacks: niacin and niacinamide are not distinguished, sample workup is tedious and the reagents used are noxious and unstable. Since the advent of high-performance liquid chromatography (HPLC) in the 1970s, in a number of studies this technique has been applied for the determination of niacin and/or niacinamide in pharmaceuticals[3], biological fluids[4] and foodstuffs[5–7]. Whereas the determination of niacin or niacinamide in food samples was achievable using different chromatographic conditions[6,7], their simultaneous determination by HPLC was difficult owing to the different polarities of the two vitamins and/or accompanying interfering substances in the sample extract.

The aim of this study was to introduce a convenient and reliable method for the determination of niacin and niacinamide. This paper deals with the simple extraction of meats and suitable chromatographic conditions for the simultaneous detection of niacin and niacinamide.

EXPERIMENTAL

Apparatus and conditions

The HPLC apparatus consisted of a Model 3A liquid chromatograph (Shimadzu, Japan) with a 20-μl loop injector (Rheodyne Model 7125), a Shimadzu UV detector set

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at 260 nm and a data processor (Shimadzu Model C-R3A). The chromatographic separation was carried out using a 25 cm × 4.6 mm I.D. cation-exchange column (Partisil SCX, 10 μm) (Sumitomo, Japan). The flow-rate of the mobile phase (50 m*M* phosphate buffer, pH 3.0) was 1.0 ml/min. Separations were performed at ambient temperature (25°C).

Reagents

Niacin and niacinamide were obtained from Wako (Osaka, Japan). They were of the highest purity available and were not further purified. Ultrapure HPLC water was generated by a Milli-RO4 coupled to a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.). Stock solutions of niacin and niacinamide (1 mg/ml each) were prepared in ultrapure water. A mixed working standard solution was prepared by diluting the stock solution to give 5–50 μg/ml of each vitamin in ultrapure water. This solution was prepared freshly before use.

Procedure

A 5-g meat sample was weighed accurately into a 50-ml test-tube and about 30 ml of deionized water were added. The sample was homogenized using a Polytron blender (Model PT 20; Brinkmann, Westbury, NY, U.S.A.) for 1 min at maximum speed. The solution was boiled gently for 10 min, cooled, diluted to 50 ml with deionized water, allowed to stand briefly and the supernatant liquid filtered through 0.45-μm filter. The filtrate was subjected to HPLC analysis as described below.

HPLC analysis

A portion (10 μl) of the filtrate was injected into the HPLC system and the eluent was detected at 260 nm. The peak heights of niacin and niacinamide were measured and the concentration of each vitamin in the sample was determined from a calibration graph obtained with a working standard.

RESULTS AND DISCUSSION

Conditions for HPLC analysis

Takatsuki *et al.*[6] reported that reversed-phase chromatography is convenient for the routine determination of niacin and niacinamide in meats. However, different

TABLE I

EFFECT OF pH OF PHOSPHATE BUFFER ON THE RETENTION TIMES OF NIACIN, NIACINAMIDE, ASCORBIC ACID AND SORBIC ACID

*pH**	*Retention time (min)*			
	Niacin	*Niacinamide*	*Sorbic acid*	*Ascorbic acid*
6.0	3.66	7.02	3.80	2.92
5.0	4.26	7.22	4.77	3.05
4.0	4.69	8.26	5.70	3.09
3.0	5.49	10.3	7.30	3.22

* The pH of 50 m*M* phosphate buffer was adjusted with 0.1 *M* phosphoric acid.

chromatographic conditions are needed for the determination of each vitamin in the method reported. Hence the simultaneous detection of both vitamins was impossible by reversed-phase chromatography. We therefore attempted to make use of cation-exchange chromatography[8] for their simultaneous detection.

Table I shows the effect of the pH of the phosphate buffer on the retention times of niacin and niacinamide. Retention times of ascorbic acid and sorbic acid, which are commonly used additives in meat products, are also given in Table I.

Within the examined pH range (3–6), the lower the pH of the buffer the longer were the retention times of niacin and niacinamide, and also those of ascorbic acid and sorbic acid. In general, the best separation of the two vitamins from ascorbic acid and sorbic acid was achieved when 50 m*M* phosphate buffer (pH 3.0) was used.

Next, the effect of the concentration of the buffer (pH 3.0) on the retention times of the four compounds was evaluated in the range 10–100 m*M*. As was expected from the separation principle using ion-exchange column materials, the retention times of all four comoounds decreased with increasing concentration of the buffer. A concentration of 50 m*M* was a compromise between the speed of analysis and the best separation of the compounds.

Fig. 1 shows a typical chromatogram obtained for the four compounds under the

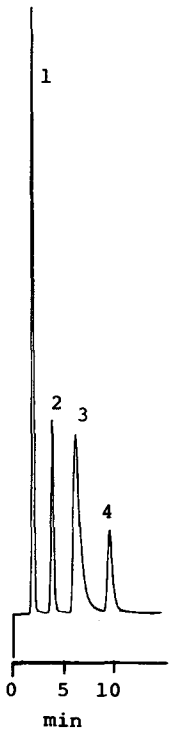

Fig. 1. Chromatogram of two vitamins and two additives. Conditions: Partisil SCX column (25 cm × 4.6 mm I.D.); mobile phase, phosphate buffer (50 m*M*, pH 3.0); flow-rate, 1 ml/min; detection, UV at 260 nm. Peaks: 1 = ascorbic acid; 2 = niacin; 3 = sorbic acid; 4 = niacinamide. Concentration of each compound, 2 μg/ml.

TABLE II

AMOUNTS OF NIACIN AND NIACINAMIDE PRESENT IN MEAT SAMPLES AND RECOVERIES

Recoveries were determined in triplicate. N.D. = Not detected.

Sample	*Niacin*				*Niacinamide*			
	Amount present (μg/g)	*Amount added (μg/g)*	*Amount recovered (μg/g)*	*Recovery (%)*	*Amount present (μg/g)*	*Amount added (μg/g)*	*Amount recovered (μg/g)*	*Recovery (%)*
Beef	N.D.	50	48.8	97.6	18.7	50	68.9	100.4
Pork	N.D.	50	48.2	96.4	32.1	50	81.5	98.8

conditions described under Experimental. Niacin and niacinamide were well separated from each other and from ascorbic acid and sorbic acid.

Linearity, repeatability and detection limit

Under the described chromatographic conditions, a rectilinear chromatographic response was demonstrated over at least a ten-fold range of concentration (5–50 μg/ml) for niacin and niacinamide. The relative standard deviations obtained for niacin and

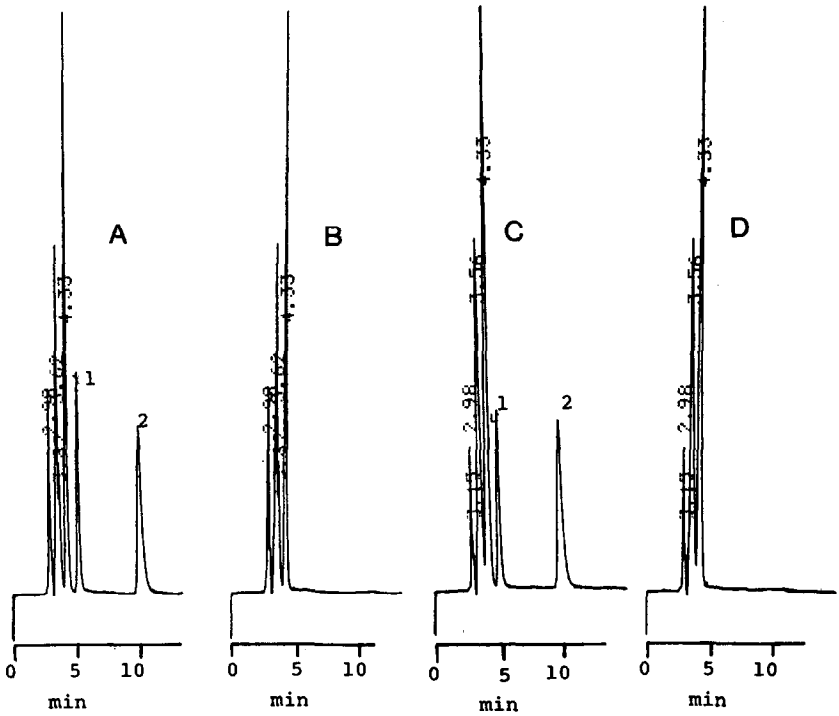

Fig. 2. Typical chromatograms obtained with samples of beef and pork using the proposed procedure. Conditions as in Fig. 1. (A) Extract from beef spiked with standards; (B) extract from blank beef; (C) extract from pork spiked with standards; (D) extract from blank pork. Peaks: 1 = niacin; 2 = niacinamide. Spiked levels of niacin and niacinamide, 50 μg/g each.

niacinamide based on four replicate analyses were 1.57 and 0.98%, respectively. The minimum detectable amounts of niacin and niacinamide determined at a signal-to-noise ratio of 3:1 were 2 and 4 ng per 10-μl injection, respectively.

Recovery studies and comparison with spectrophotometric analysis

Recovery studies were performed using beef and pork. To the weighed samples (5 g) in a 50-ml test-tube, 0.25 ml of each standard solution (1 mg/ml in water) of niacin and niacinamide was added and subjected to analysis as described under Experimental. In this way, potential losses occurring during the sample treatment procedure were assessed, together with interferences occurring during the chromatographic separation. The results are given in Table II and indicate that satisfactory recoveries were achieved for the samples tested.

Fig. 2A and C show typical chromatograms obtained with samples of beef and pork spiked with a standard mixture of niacin and niacinamide. Comparison of the chromatograms of blank samples (Fig. 2B and D) with those of the spiked samples clearly indicates that the system did not suffer from interferences from accompanying substances in the sample extracts.

Table III shows a comparison of the results for beef and pork using the proposed method and a standard spectrophotometric method[2]. The latter method gives the sum of the niacin and niacinamide contents. The agreement of the two methods was poor, and this situation persisted when the analysis was repeated several times. The recovery data given in Table II suggest that the lack of agreement between the two analytical procedures may be attributed to deficiencies in the spectrophotometric procedure. These deficiencies include such factors as a low specificity and the hydrolysis procedure for separating free niacin from complex molecules. In comparison, the proposed chromatographic method is rapid and precise.

Typical results obtained when using the proposed method for the routine examination of commercial beef and pork were as follows: none detected to 8.7 μg/g of niacin and none detected to 47.5 μg/g of niacinamide for twenty samples of beef; and none detected to 9.7 μg/g of niacin and none detected to 50.3 μg/g of niacinamide for

TABLE III

COMPARISON OF CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF NIACIN AND NIACINAMIDE

N.D. = Not detected.

Sample	*Chromatographic method*		*Spectrophotometric method: total niacin**
	Niacin (μg/g)	*Niacinamide (μg/g)*	*(μg/g)*
Beef	N.D.	18.7	26.7
	N.D.	28.6	33.7
	8.3	40.6	52.3
Pork	N.D.	25.7	41.8
	N.D.	32.1	43.7
	7.9	38.3	63.9

* Sum of niacin and niacinamide.

ten samples of pork. These values were considered to be within the naturally occurring levels compared with the values reported in the literature[7].

In conclusion, the sumultaneous determination of free niacin and ciacinamide in meats can be reliably performed by HPLC using a cation-exchange column and successfully applied to the routine inspection of such samples.

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