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Use of column switching for the determination of niacinamide in compound feed

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SUMMARY

A high-performance liquid chromatographic method is described for the determination of niacinamide in compound feed. The niacinamide is extracted with 0.2 *M* hydrochloric acid in order to suppress the hydrolysis of the niacinamide to nicotinic acid. The chromatography is carried out with the aid of column switching. An RP-18 column is used for the preseparation, and the analytical separation takes place in a cation-exchange column. With the proposed method, the limit of determination is about 2 ppm of niacinamide.

INTRODUCTION

The provision of amino acids and vitamins, now mostly produced synthetically by large-scale industrial processes, is an important factor in achieving high levels of productivity in intensive livestock husbandry.

One vitamin that is acquiring growing importance is niacin (vitamin B_3). Niacinamide, similarly to nicotinic acid, participates in the form of coenzymes NAD and NADP in all living cells in vital metabolic reactions. A deficiency of niacinamide or nicotinic acid in animal nutrition has adverse effects; *e.g.*, poultry it leads to disorders in the development of feathers, reduced laying activity, decreased hatchability and to delays in growth. With pigs, the deficiency manifests itself in the development of rough skin and dermatitis at the ears. Cases of intestinal inflammation can also occur.

The requirement for niacin is partly met through the particular feed diet. Independent of this, however, niacinamide/nicotinic acid is generally supplemented to ensure that the requirement is met. As the rate of supplementation is only a few parts per million, it is necessary to have an efficient method for determining these low levels reliably and quickly.

The quantification of niacinamide/nicotinic acid is usually carried out spectrophotometrically[1–4], by thin-layer chromatography[5,6] or by high-performance liquid chromatography (HPLC)[7,8]. Of these, HPLC is certainly the most efficient. However, the polar structure of niacinamide/nicotinic acid results in most HPLC processes

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being based on ion-pair chromatography[9–14]. Because, in our experience, good retention time stability is not always achieved in ion-pair chromatography, reversed-phase HPLC without ion-pair reagents was employed for the quantification of niacinamide in compound feed. A requirement was that costly preparation of the samples should be avoided.

EXPERIMENTAL

Equipment

The HPLC system consisted of a Hewlett-Packard 1090 liquid chromatograph fitted with a column-switching mechanism and a (Spectra-Physics SP 8450) UV detector. The evaluation was carried out by means of a Spectra-Physics SP 4270 computer–integrator which was connected to a Labnet laboratory data station. The measurements were made at 264 nm at a flow-rate of 1.5 ml/min and at 50°C. The following columns were obtained from Macherey, Nagel & Co. (Düren, F.R.G.): precolumn, RP-18, 5 μm (17 × 4.6 mm I.D.); column A, RP-18, 5 μm (250 × 4.6 mm I.D.); and column B, Nucleosil 5 SA cation-exchange column (250 × 4.6 mm I.D.). The retention time of niacinamide on column A was 13.7 min and the total analysis time was *ca.* 32 min.

The column switching system is presented in Fig. 1 and proceeds as follows: column switching, 0 min CS = 1, 12.9 min CS = 0, 14.5 min CS = 1 and 26 min

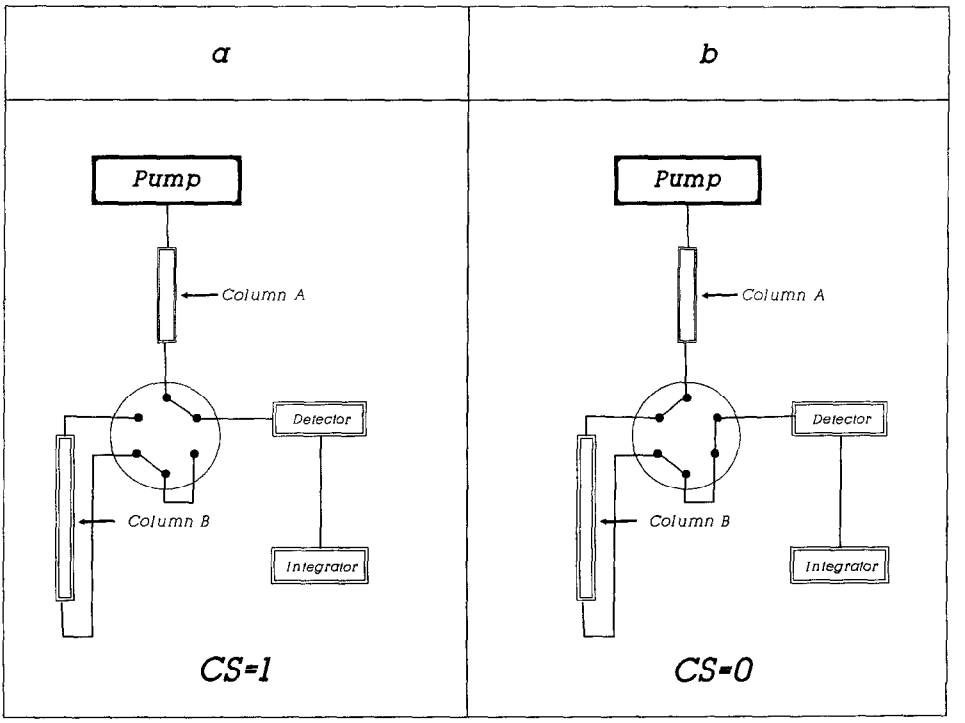

Fig. 1. Flow diagram for the determination of niacinamide. For explanations, see Experimental. (a) Separation only on column A (equal to CS = 1), (b) separation on columns A and B (equal to CS = 0).

CS = 0 (CS = 1, via precolumn and column A; CS = 0, via precolumn, column A and column B); and eluent, 0 min 0.01 *M* KH_2PO_4, 17 min 0.01 *M* KH_2PO_4–acetonitrile (60:40) and 20 min 0.01 *M* KH_2PO_4. The time parameters of the column switching must be checked from time to time and corrected if necessary. The column switching times given above apply for a retention time of niacinamide of 13.7 min on column A.

Materials and solvents

All substances were obtained from Merck (Darmstadt, F.R.G.). The compound feeds used for the feeding of poultry and pigs were obtained from various producers. The eluent was a 0.01 *M* aqueous solution of potassium dihydrogenphosphate (pH 4.80). Before use, it was degassed ultrasonically.

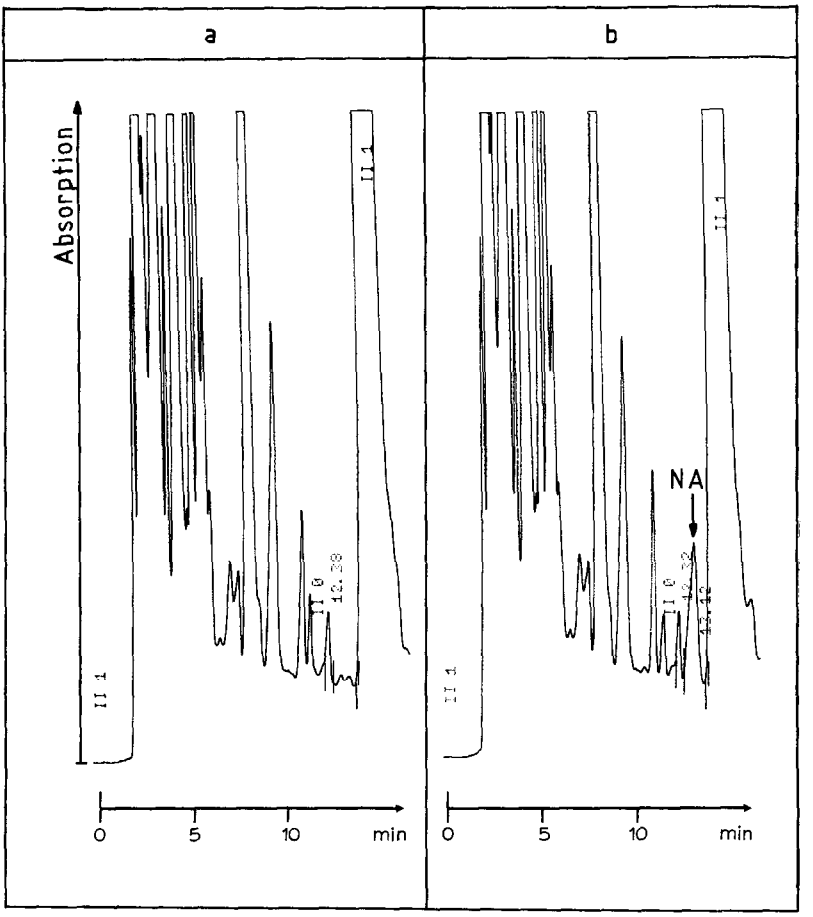

Fig. 2. HPLC of niacinamide (NA) without column switching. (a) Compound feed without niacinamide; (b) compound feed supplemented with 10 ppm of niacinamide. Eluent: 0.01 *M* KH_2PO_4.

Reagent solutions

Solutions of known concentrations of niacinamide were prepared with doubly distilled water, using these calibration solutions, a calibration graph was then plotted, and was linear in the range 5–50 ppm.

Preparation of samples

A 2-g amount of finely ground (0.25 mm) compound feed, weighed exactly, was mixed with 10.0 ml of 0.2 *M* hydrochloric acid and stirred for 10 min. The mixture was centrifuged and filtered through a 0.5-μm membrane filter and the filtrate was used for the HPLC measurement.

Kinetics of niacinamide hydrolysis

The sample was prepared as described above. In addition to 0.2 *M* hydrochloric acid, 0.1 *M* hydrochloric acid and doubly distilled water were used. After filtration, the pH and the niacinamide content of the solution were determined as a function of time.

RESULTS AND DISCUSSION

A compound feed for animal nutrition is a very complex matrix when regarded analytically. It is built up from mostly vegetable raw materials with minerals, amino acids and vitamins added. Consequently, a large number of peaks must be identified even when detection is carried out at 264 nm (Fig. 2).

If one wants to conduct the analysis without ion-pair reagents, it is not possible to maintain a retention time of niacinamide of appreciably more than 10 min with RP-18 phases. Although this is possible with cation-exchange columns, the same problem arises with regard to the number of peaks as with RP-18 columns. As a complicated and therefore time- and cost-intensive preparation of samples was to be avoided, column switching was considered[15,16].

Our positive experience with column switching[17] was continued in the determination of niacinamide. Fig. 2a shows the chromatogram of a compound feed without addition of niacinamide, and Fig. 2b shows that of a compound feed with 10 ppm of niacinamide added. Without the zero diet, however, it is not possible to be certain that the niacinamide peak is free from interferences.

By using column switching, it is now possible to obtain the niacinamide peak virtually free from interferences. Here, preseparation is carried out on an RP-18 column with 0.01 *M* potassium dihydrogenphosphate solution. Then, by means of column switching, the niacinamide fraction is applied to the second column and chromatographed there, also with 0.01 *M* potassium dihydrogenphosphate solution. The second column contains a cation exchanger, which makes it possible to separate the niacinamide cleanly from possible interfering substances present (Fig. 3a and b).

In conjunction with a more extensive study, various compound feeds were analysed for their niacinamide contents. The compound feeds to which no niacinamide was added proved to be virtually interference-free in the niacinamide region. Some vegetable and animal raw materials were likewise studied for their free niacinamide contents and the results are given in Table I.

The reproducibility of the extraction and recovery of niacinamide were determi-

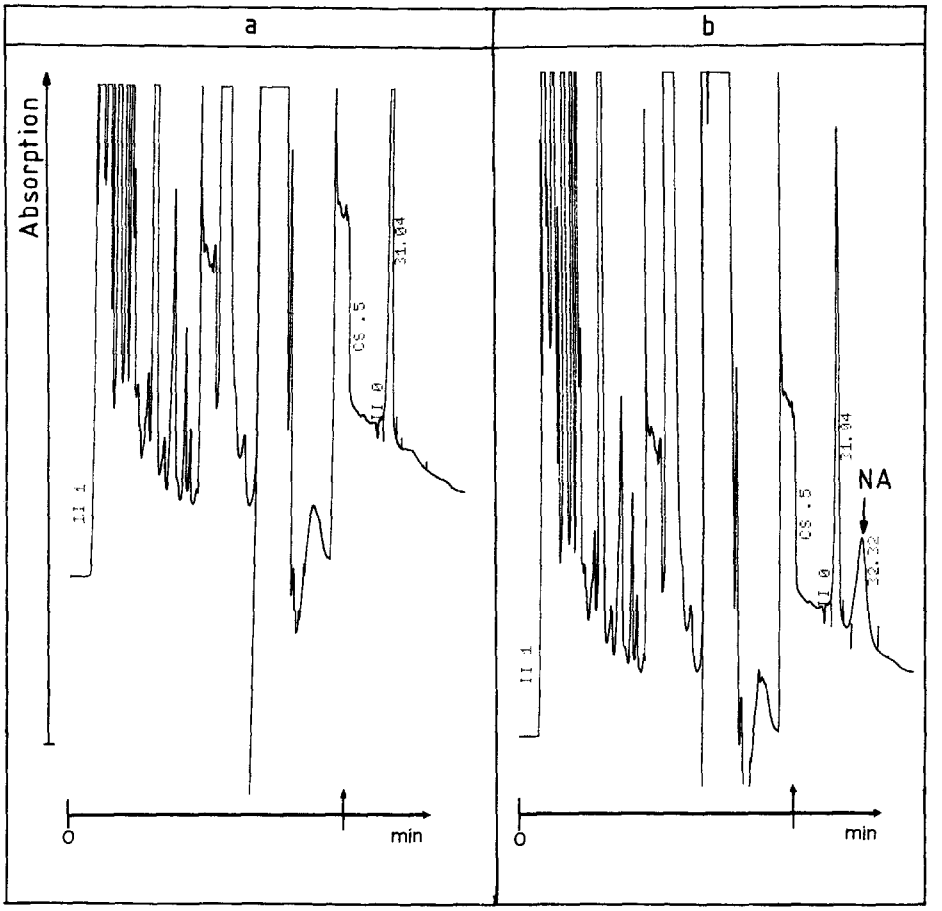

Fig. 3. HPLC of niacinamide (NA) with column switching. (a) Compound feed without niacinamide; (b) compound feed supplemented with 10 ppm of niacinamide. Conditions as described under Experimental. The arrows indicate a change in chart speed from 0.25 to 0.50 cm/min.

TABLE I

FREE NIACINAMIDE CONTENT IN VARIOUS RAW MATERIALS

Sample	*Niacinamide content from extraction with 0.2 M HCl (ppm)*	
Blood meal	2– 3[a]	(n = 2)
Corn	–[a]	(n = 2)
Soybean meal	6– 8	(n = 2)
Fish meal	19–72	(n = 5)
Meat meal	3–36	(n = 5)

[a] <0.5 ppm.

TABLE II

RECOVERY OF SUPPLEMENTED NIACINAMIDE IN COMPOUND FEED

Rate of supplementation (ppm)	*Content from extraction with 0.2 M HCl (ppm)*	*Recovery (%)*
5	5.0	100
	4.9	98
10	10.2	102
	10.1	101
15	15.1	100.7
	14.7	98

ned. The study of the extraction reproducibility was carried out with a sample that contained 10 ppm of niacinamide. The coefficient of variation was 0.86% (n = 10). The recovery was determined for the addition of 5, 10 and 15 ppm niacinamide (Table II).

With the sample preparation described, the limit of determination was about 2 ppm and the detection limit about 0.5 ppm. Both limits can be lowered, especially by changing the range adjustment of the UV detector. However, this is not relevant for the analysis of feedstuffs because niacinamide is supplemented at levels of about 20 ppm upwards.

A parameter of essential importance for the determination of niacinamide in feedstuffs is the pH of the test solution. In extensive studies, the hydrolysis of the niacinamide to nicotinic acid in the test solution was analysed at various pH values and the results are shown in Fig. 4. It can be clearly seen that a reduction in pH is accompanied by an increase in the stability of the niacinamide in the test solution.

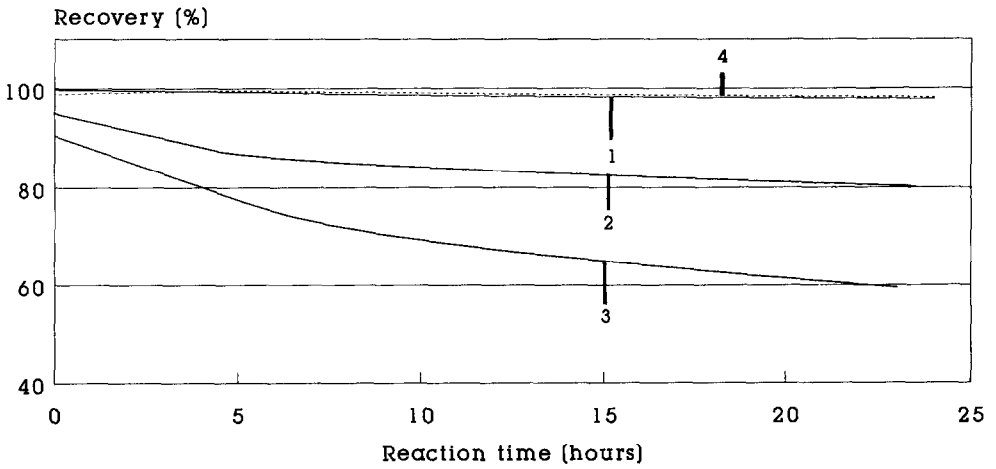

Fig. 4. Hydrolysis of niacinamide. Compound feed (CF) supplemented with 30 ppm of niacinamide (NA). (1) NA in water; (2) CF + NA in 0.1 *M* HCl; (3) CF + NA in water; (4) CF + NA in 0.2 *M* HCl. Actual pH values: (1) 5.5; (2) 5.3; (3) 3.5; (4) 2.1.

However, it is not only the pH, but also the composition of the test solution that is of decisive importance for the hydrolysis of niacinamide.

Niacinamide in water at pH 5.2 shows no notable decomposition even after 24 h. A compound feed sample supplemented with niacinamide, however, was found to contain only about 60% of niacinamide after 24 h. As the feed acts as a buffer, it is therefore advisable to suppress the hydrolysis by lowering the pH of the test solution. As a result, it is possible to analyse several samples overnight with an autosampler without the risk of hydrolysis of niacinamide occurring.

It is not possible to determine nicotinic acid by this procedure, because it co-elutes with a number of other feed components. A separation could not be achieved by column switching, so that one cannot determine both forms of the vitamin with a single injection. However, using this procedure, improved stability of the retention times over ion-pair chromatography could be achieved.

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