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Comparison of microbiological and HPLC – fluorescence detection methods for determination of niacin in fortified food products

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Abstract

A comparison has been made between results obtained by a recently published HPLC method, involving post-column reaction and fluorescence detection, with a microbiological assay using *Lactobacillus plantarum*. The HPLC method includes a modified acid hydrolysis extraction step (0.1 M HCl) and yields niacin values from fortified foods somewhat lower than by the microbiological assay. The most significant differences were observed for the cereal-based products. These differences arise principally from the lack of specificity of *L. plantarum* and from the stronger acid hydrolysis extraction conditions (1 M HCl) of the microbiological assay, which probably releases a part of the non-bioavailable niacin. Moreover by HPLC, excellent recoveries of added nicotinic acid and nicotinamide (95–100%) were obtained and better precision (RSDr = 0.3–0.8%) was observed than from the micobiological assay.

Keywords: Niacin; HPLC; Fluorimetric; Microbiological assay; Fortified foods

1. Introduction

Niacin or vitamin PP (B3) is the generic descriptor for two vitamers; nicotinic acid and nicotinamide, which are both based on a pyridine ring bound to a carboxylic group or respectively, to a carboxamide group. The amide form, which is normally used for enrichment, has the same activity as nicotinic acid. This vitamin is normally very stable.

Niacin occurs naturally in a wide variety of foods (Ball, 1998; Friedrich, 1988). In mature cereal grains, it exists largely as chemically bound forms of nicotinic acid that are nutritionally unavailable. Indeed, nicotinic acid in grains is bound to macromolecules, which consist of polysaccharides and peptides or glycopeptides. However, niacin in other non-cereal plant foods is present mainly as free nicotinic acid.

The terms "total" and "free" niacin occurring in foods are defined by the extraction conditions employed for the analysis (Ball, 1998). In general, total niacin refers to the amount of niacin extracted by autoclaving the sample with alkali or 0.5 or 1 M acid while free niacin refers to the amount of niacin extracted by extraction with 0.1 M acid. However, alkaline extraction can also release unavailable niacin in cereals. Nevertheless, not only the extraction conditions have a considerable influence on the results, but also the specificity of the mode of detection.

Niacin can be determined by colorimetric (AOAC, 2000), microbiological (AACC, 2000; AOAC, 2000) and HPLC methods (Chase, Landen, Soliman & Eitenmiller, 1993; Krishnan, Mahmud & Matthees, 1999; LaCroix, Wolf & Vanderslice, 1999; Lahély, 1998; Lahély, Bergaentzlé & Hasselmann, 1999; Mawatari, Iinuma & Watanabe, 1991; Tyler & Genzale, 1990; Vidal-Valverde & Reche, 1991; Van Niekerk, Smit, Strydom & Armbruster, 1984). The AOAC colorimetric method (AOAC, 2000) is based on the König reaction, in which pyridine derivatives are reacted with cyanogen

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bromide and an aromatic amine, sulphanilic acid. However, this method lacks specificity, because all 3-pyridine derivatives react and requires the use of the highly toxic reagent cyanogen bromide.

Microbiological assay can be used for the determination of free niacin using selective microorganisms such as *Lactobacillus casei* or *Leuconostoc mesenteroides* subsp. *mesenteroides* (Ball, 1998) or for total niacin, using *Lactobacillus plantarum* (ATCC 8014), which responds to nicotinic acid, nicotinamide, nicotinuric acid (an inactive metabolite) and NAD, but not to tryptophan. This latter microorganism is also able to utilise bound nicotinic acid, present in cereals, to a considerable extent (Ball, 1998). It is specified in official methods (AACC, 2000; AOAC, 2000) for the determination of the total niacin activity in food. The AOAC and AACC extraction procedures involve autoclaving the sample at 121–123°C for 30 min with 0.5 M H_2SO_4.

HPLC determination of niacin has generally been carried out with ion-pairing, reversed-phase chromatography with UV detection. The application of this technique to food products often requires complex clean-up procedures, like cartridge extractions and column-switching (Chase et al., 1993; LaCroix et al., 1999; Tyler & Genzale, 1990; Van Niekerk et al., 1984; Vidal-Valverde & Reche, 1991).

The use of fluorescence detection (Krishnen et al., 1999; Mawatari et al., 1991) increases the specificity and the sensitivity of the detection mode, but requires a post-column derivatisation because niacin is not naturally fluorescent. Krishnen et al. (1999) described a HPLC post-column derivatisation system using cyanogen bromide and *p*-aminophenol for the fluorescence determination of niacin in cereals. However, this method involves the exposure to toxic chemicals and the installation of a post-column pump. Overestimation of niacin was observed in one sample, in spite of the high specificity of the mode of detection. Mawatari et al. (1991) proposed a post-column UV irradiation in presence of hydrogen peroxide and copper (II), both reagents directly added in the HPLC mobile phase, and fluorescence detection for the detection of nicotinamide and nicotinic acid in human serum. This method was then succesfully applied to food matrixes (Lahély 1998; Lahély et al., 1999).

For the fluoresence method, Lahély (1998; Lahély et al., 1999) proposed an acid hydrolysis (0.1 M HCl in a water bath at 100°C for 1 h) followed by an alkaline hydrolysis of the filtered acid hydrolysate (0.8 M NaOH, autoclaved at 120°C for 1 h). This two-step sample treatment was reported to release only the bioavailable forms of niacin and to convert all vitamers to nicotinic acid. Moreover, the application of this method to beef liver and yeast gave comparable niacin values to those when simulating gastric digestion conditions.

The aim of the present study was to test the approach of Lahély (1998; Lahély et al., 1999) which seems to be promising for routine use. Niacin was determined by HPLC on a range of fortified food products and the results obtained were compared with those from the microbiological assay.

2. Materials and methods

2.1. Reagents and chemicals

Clara-diastase and taka-diastase (40 U/mg) were purchassed from Fluka Chemie (Buchs, Switzerland). Taka-diastase Pfalz-Bauer was from Chemie Brunschwig (Basel, Switzerland). Nicotinic acid and nicotinamide were obtained from Sigma (division of Fluka Chemie, Buchs, Switzerland). All other chemicals and solvents were AR grade or HPLC grade. Sodium hydroxide, potassium dihydrogen phosphate, copper (II) sulphate pentahydrate, hydrogen peroxide (30%), hydrochloric acid (37%) and acetonitrile were purchased from Merck (Dietikon, Switzerland). HPLC-grade water was purified in a Milli-Q system (Millipore, Beldford, MA, USA)

Standard reference material VMA 195 was obtained from the American Association of Cereal Chemists (St. Paul, MN, USA) and the SRM 1846 (infant formula) was supplied by the National Institute of Standards & Technology (NIST; Gaithersburg, MD, USA).

2.2. Standard preparation

Nicotinic acid and nicotinamide stock solution (1 mg/ml) was prepared by dissolving 100 mg of nicotinic acid and 100 mg of nicotinamide in 100 ml water. This solution was stored for a maximum of 1 week at 4°C.

A working standard solution (50 μg/ml of nicotinic acid and nicotinamide, respectively) was prepared by pipetting 5 ml of the above nicotinic acid and nicotinamide stock solution into a 100-ml volumetric flask, and diluting to the mark with water. From this solution, further dilutions were performed in order to obtain standard solutions of, respectively, 1, 2.5 and 5 μg/ml of nicotinic acid and nicotinamide. These solutions were prepared fresh daily.

2.3. Equipment

All experiments were carried out using a HPLC system from Merck Hitachi (Dietikon, Switzerland) equipped with a Model L-7100 gradient pump, a L-7200 autosampler, a L-7480 fluorescence detector, a D-7000 HPLC System Manager software and a Inertsil ODS 3 column (5 μm, 250×4.6 mm I.D.) from GL Sciences Inc. (supplied by Alltech, Deerfield (IL), USA). For post-column UV irradiation, a polytetrafluoroethylene (PTFE) tube of 12 m×0.5 mm I.D. was wound around a black

light tube (300–400 nm, 18 W, 230 V; model TL-D18 W/08 from Philips, Elevite SA, Gland, Switzerland) and connected between the column and the fluorescence detector.

2.4. Sample preparation

The whole laboratory sample was homogenised by mixing or grinding. For milk- and cereal-based products, a test portion of 50 g was weighed into a 250-ml beaker and 100 g of water at 40°C was added. If the suspension was too viscous more water was used, for example 150 g. The suspension was mixed until it was homogeneous with either a glass rod or a laboratory mixer. Test portions of 6–15 g (corresponding to 2–5 g of dry sample) were used for the analysis. For breakfast cereals, the whole laboratory sample was homogenised by grinding with a mill. Ground sample portions of 2–5 g were used for the analysis. For liquid clinical nutrition products, the whole laboratory sample was homogenised by gentle mixing using a magnetic stirrer and sample portions of 5 g were used for the analysis.

2.5. Extraction of niacin

2.5.1. Acid hydrolysis

Two to 5 g of dry sample or 5 g of liquid sample or 6–15 g of sample suspension (corresponding to 2–5 g of dry sample) were weighed into a 250-ml flat-bottomed flask. Seventy millilitres 0.1 M HCl were added and the suspension was heated in a water bath at 100°C for 1 h under magnetic stirring. After cooling to room temperature, the pH of the hydrolysate was adjusted to 4.5–4.6 with NaOH solution (5 M then 1 M). The hydrolysate was then quantitatively transferred into a 100- or 200-ml volumetric flask, made up to the volume with water and finally filtered through a folded quantitative filter paper. An aliquot was filtered through a 0.45-μm membrane filter.

2.5.2. Alkaline hydrolysis

Fifty millilitres of the filtrate obtained above (Section 2.5.1) was transferred quantitatively into a 250-ml conical flask and 10 ml of 5 M NaOH were added. After being autoclaved for 1 h at 120°C, the alkaline solution was cooled to room temperature in a bath of tap water. The pH of the hydrolysate was then adjusted to 4.5–4.6 with HCl solution (5 M then 1 M). The hydrolysate was finally transferred into a 100-ml volumetric flask and made up to volume with water. An aliquot was filtered through a 0.45-μm membrane filter.

2.5.3. Enzymatic digestion

2.5.3.1. Enzymatic digestion before acid hydrolysis. Fifteen grams of sample suspension (corresponding to 5 g of dry sample) were weighed into a 250-ml flat-bottomed flask. About 60 ml of water and 150 mg of Taka-diastase or Clara-diastase were added and the suspension was heated in an oven at 45°C for 30 min. Seven millilitres of 1 M HCl were then added. Acid hydrolysis for 1 h or 2 h, pH adjustment and filtration were then performed as described under Section 2.5.1.

2.5.3.2. Enzymatic digestion after acid hydrolysis. After the acid hydrolysis of the sample suspension and pH adjustment to 4.5–4.6, as described in Section 2.5.1, about 150 mg of Clara-diastase were added to the hydrolysate and incubated at 45°C for 1 h. The hydrolysate was then transferred into a 150-ml volumetric flask, made up to the mark with water and finally filtered through a folded quantitative filter paper. An aliquot was filtered through a 0.45-μm membrane filter.

2.6. HPLC analysis

Mobile phase A was prepared by dissolving 9.54 g of potassium dihydrogen phosphate in about 800 ml water, by adding 7.6 ml of 30% hydrogen peroxide and 1 ml of 5.10^{-3} M copper (II) sulphate solution and by diluting to 1 l with water. Mobile phase B (washing eluent) was prepared by mixing 10% (v/v) acetonitrile in eluent A. These mobile phases were protected from light by wrapping the bottles with aluminium foil, were degassed for about 10 min under a helium stream just before use and not stored for more than 24 h.

HPLC separation was performed at room temperature using the mobile phase A at a flow rate of 1 ml/min. In order to avoid clogging of the column with sample solutions, which can influence the post-column reaction, a column-washing step with mobile phase B was added at the end of each run as follows:0 min (A); 33.5 min (A); 34.0 min (B); 36.0 min (B); 36.5 min (A); 51.0 min (A). This washing step increased the chromatographic run time to 51 min but markedly improved the repeatability of the nicotinic acid and niacinamide responses. The fluorescence detector operated at an excitation wavelength of 322 nm and at an emission wavelength of 380 nm. The injection volume was 30 μl. Nicotinamide and nicotinic acid were identified by their retention times and peak area measurements were used. Results were expressed as niacin by summing the concentrations of nicotininic acid and nicotinamide.

2.7. Microbiological assay

A microbiological assay using *Lactobacillus plantarum* (ATCC 8014) was used, similar in principle to the AOAC method 944.13. The major difference is in the extraction conditions, since products were hydrolysed by autoclaving at 120°C for 15 min (i.e. about 35 min in total with overall operation sequence from heating up to cooling down up to 100°C) with 1 M HCl instead of 0.5 M H_2SO_4 at 121°C for 30 min.

3. Results and discussion

3.1. Optimisation of extraction conditions for HPLC method

Several extraction methods were investigated.

3.1.1. Acid/alkaline hydrolysis

Since alkaline hydrolysis is known to release non-bioavailable niacin from fortified food products, a two-step extraction method of Lahély et al. (1999; i.e. acid hydrolysis with removal of non-bioavailable insoluble forms by filtration followed by alkaline hydrolysis) was investigated. The acid hydrolysate was adjusted to 4.5–4.6 to make the filtration easier.

The need for this alkaline treatment was evaluated by the analysis of sample extracts after acid hydrolysis and after the two-step acid/alkaline hydrolyses, respectively. These food products were also systematically spiked with a standard solution of nicotinic acid and nicotinamide at concentrations corresponding to their declared values. The results obtained for a range of food products are compared in Table 1.

Alkaline hydrolysis, which totally converts the NAD, NADP (nicotinamide adenine dinucleotide phosphate) and nicotinamide into nicotinic acid (Lahély, 1998) simplifies the chromatographic interpretation of the results since only one peak is measured. The analysis time is also lower. However, lower or comparable concentrations of niacin in the food products and slightly lower recoveries of spikes were obtained after this treatment. The alkaline hydrolysis of such foods did not seem to release significantly more niacin. In addition, several components co-eluted with the nicotinic acid peak, and could not be completely resolved on the Inertsil ODS 3 column (Fig. 1).

Another alkaline treatment with calcium hydroxide, as proposed by Van Niekerk et al. (1984), was not tested in the present study, since it is less effective for the conversion of NAD and NADP into nicotinic acid than sodium hydroxide (Lahély, 1998).

3.1.2. Enzymatic and acid hydrolyses

To release total bioavailable niacin from cereal-based foods, acid hydrolysis followed by enzymatic digestion has often been proposed in the literature (Krishnan et al.,1999; Van Niekerk et al., 1984; Vidal-Valverde & Reche, 1991). The effect of three different amylolytic preparations (Taka-diastases from two different suppliers and a Clara-diastase) were compared in the present study. Three cereal-based products (two fortified with niacin and one not) were studied. Samples were initially treated with one of the enzyme preparations to clarify the suspensions and to obtain more homogenous extracts for the acid hydrolysis. However, enzymatic digestion after acid hydrolysis was also tested to check for the possibility of better release of naturally occurring niacin from the non-fortified cereal-based dietetic

Table 1
Comparison of results for niacin in various fortified food products by HPLC after acid hydrolysis or acid followed by alkaline hydrolyses

Type of food	Sample	Declared value (mg/100 g)	Acid hydrolysis		Acid + alkaline hydrolysis	
			Niacin[a] (mg/100 g)	Recovery[b] NA/NM (%)	Niacin[a] (mg/100 g)	Recovery[b] NA + NM (%)
Infant formulae	A	4.1	5.46/5.49	96/95	4.42/4.44	63
	Nestlé reference product	6.51	6.28/6.23	102/nd [c]	5.75/5.94	82
	C	5.1	6.44/6.46	103/101	4.91/4.62	72
	D (ready to feed)	0.9	1.38/1.33	99/95	1.38/1.32	93
Cereal products	Breakfast cereal A	31	47.0/46.6	99/98	44.3/44.2	92
	Breakfast cereal B	15.3	18.5/18.6	107/99	16.5/16.4	89
	Breakfast cereal C	24.1	25.4/25.3	109/89	26.4/25.6	100
	Breakfast cereal D	16.8	19.4/19.9	102/nd	20.8/17.4	106
	Infant cereals and fruits A	8	9.10/8.97	nd	7.49/7.77	nd
	Infant cereals and fruits B	4	4.80/4.75	94/100	4.10/4.04	78
Chocolate drink and clinical nutrition products	Chocolate drink powder	19	21.1/20.5	102/103	16.2/15.5	88
	Clinical nutrition product A	5.17	6.32/5.78	nd	5.98/6.12	nd
	Clinical nutrition product B	2.63	3.14/3.13	99/97	2.95/3.02	97
	Clinical nutrition product C	2.64	3.75/3.81	97/94	3.69/3.62	96
	Clinical nutrition product D	1.97	2.37/2.42	88/97	2.46/2.53	94
	Clinical nutrition product E	1.54	1.91/1.93	nd	1.62/1.44	nd
	Clinical nutrition product F	1.61	1.90/1.93	100/102	1.77/1.79	95

[a] Niacin values are reported in duplicate.
[b] Recoveries are calculated from single spiked samples with a standard solution of nicotinic acid (NA) and nicotinamide (NM).
[c] Not determined.

product. The results obtained are summarised in Table 2.

Concentrations of niacin determined after enzymatic-acid and acid-enzymatic treatments were very similar to those obtained after acid hydrolysis alone. Moreover, a longer acid hydrolysis time (i.e. 2 h instead of 1 h) did not release significantly more niacin. Acid hydrolysis (0.1 M HCl for 1h at 100°C) seems sufficient to release niacin from cereal- and starch-based foods.

3.1.3. Extraction conditions adopted for HPLC method

As shown in Sections 3.1.1. and 3.1.2. the acid hydrolysis (0.1 M, 1 h, 100°C) conditions yielded slighly better recoveries for niacin in a range of fortified foods to those from the two-step (acid-alkaline or acid-enzymatic) methods. Thus, the one-step acid hydrolysis was adopted for the final HPLC procedure and was used in the following validation studies and for comparison with results from the microbiological assay. Typical chromatograms obtained after acid hydrolysis are shown in Fig. 2. The clean chromatograms show a freedom from interfering components.

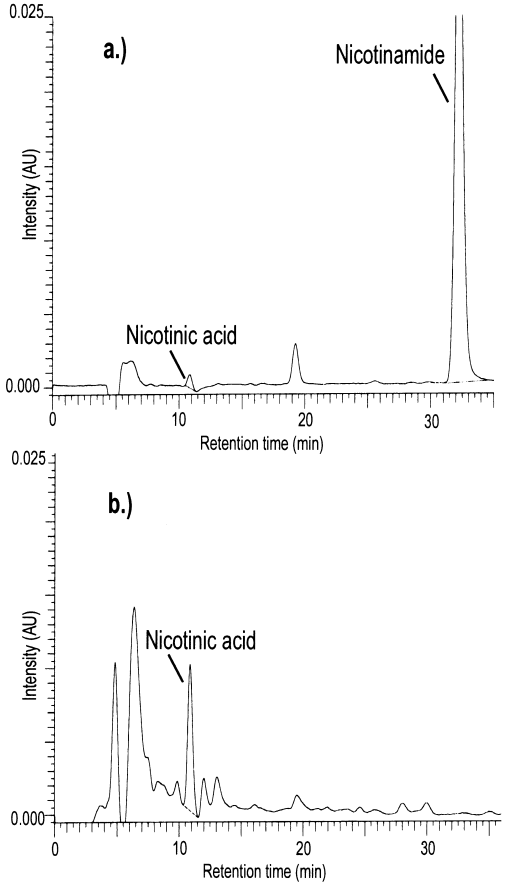

Fig. 1. HPLC chromatographic separation of nicotinic acid and nicotinamide in an infant formula: (a) after acid hydrolysis alone and (b) after acid and alkaline hydrolyses which totally convert nicotinamide into nicotinic acid (see concentrations in Table 1, infant formula A sample).

3.2. Validation of the HPLC-fluorescence method

3.2.1. Linearity and detection limits

Detector responses for nicotinic acid and nicotinamide, were evaluated with multi-level standards (nine concentration levels in the range from 0.25 to 100 μg/ml) and found to be linear for both vitamers with $r^2 \geqslant 0.9995$.

Detection limits of about 5 ng/ml for nicotinic acid and nicotinamide were determined by peak height measurements corresponding to about three times the baseline noise. Limits of quantification of 0.10 mg/100g were estimated as 10 times the limits of detection for 5 g of sample diluted in 100 ml. These values were of the same order of magnitude when using the microbiological assay.

3.2.2. Recoveries of nicotinic acid and nicotinamide spikes in foods

Accuracy of the HPLC method was determined by spiking three products, milk-based infant formula, breakfast cereal and infant cereal, with a standard solution of nicotinic acid and nicotinamide at concentrations of about 0.5, 1 and 2 times the amounts present in these foods. Concentrations of nicotinamide determined in these food products were about 10 times higher than the concentrations of nicotinic acid. Recoveries of the spikes in the range of 90–134% for

Table 2
Comparison of results for the quantification of niacin in cereal- and starch-based foods after various acid and enzymatic digestion procedures

Sample	Sample treatment	Niacin[a] (mg/100 g)
Infant cereals A	Acid hydrolysis 1 h	8.98/8.92
	Acid hydrolysis 2 h	9.22/9.11
	Taka-diastase (Fluka) digestion + acid hydrolysis 2 h	8.84/8.80
Infant cereals B	Acid hydrolysis 1 h	11.3/11.2
	Acid hydrolysis 2 h	11.2/11.2
	Taka-diastase (Fluka) digestion + acid hydrolysis 2 h	11.1/11.1
	Clara-diastase digestion + acid hydrolysis 2 h	10.8/10.8
Infant cereals C	Acid hydrolysis 1 h (non-fortified with niacin)	0.90/0.89
	Acid hydrolysis 2 h	1.05/0.73
	Taka-diastase (Fluka) digestion + acid hydrolysis 2 h	1.22/1.01
	Taka-diastase (Pfalz-Bauer) digestion + acid hydrolysis 2 h	1.08/0.65
	Clara-diastase digestion + acid hydrolysis 2 h	1.08/0.77
	Acid hydrolysis 1 h + Clara-diastase digestion	1.09/1.04

[a] Niacin values are reported in duplicate.

nicotinic acid and in the range of 94–98% for nicotinamide were obtained. Further investigations by spiking samples with nicotinamide alone showed that about 2% of nicotinamide were converted into nicotinic acid during the analytical procedure. As spikes of nicotinamide were 10 times more concentrated than spikes of nicotinic acid, this explains the higher recoveries obtained for nicotinic acid. By expressing recoveries as the sum of both vitamers, recoveries in the range of 95–100% were obtained.

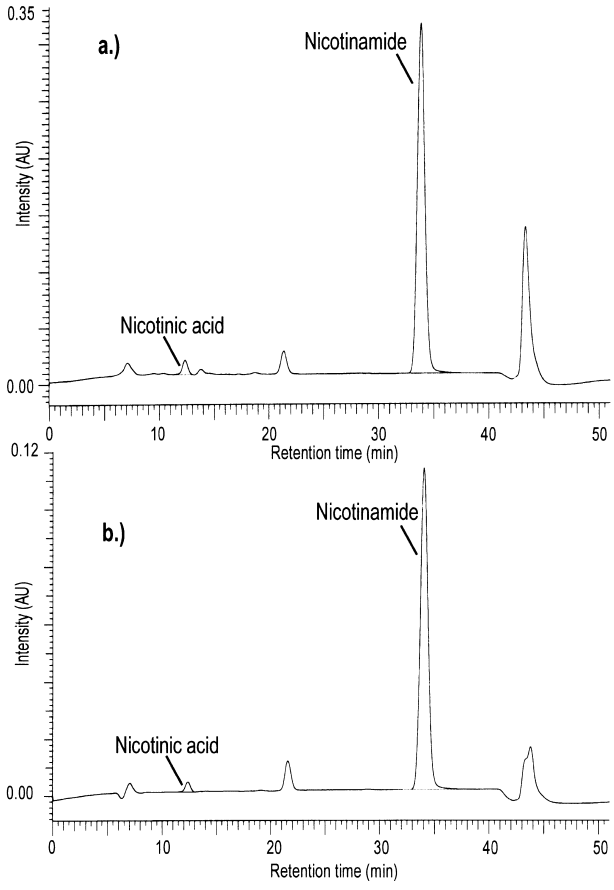

Fig. 2. HPLC chromatographic separation of nicotinic acid and nicotinamide: (a) of cereal-based reference material VMA and (b) of the clinical nutrition product A. (see concentrations in Table 4).

3.2.3. Comparison of the precision of the HPLC-fluorescence and microbiological methods

The repeatability and the day-to-day reproducibility or "within-laboratory reproducibility" of the HPLC-fluorescence method were evaluated (Table 3) by analysing nicotinic acid and nicotinamide in a milk-based infant formula, a breakfast cereal and an infant cereal in duplicate, over 5 days, in the same laboratory using the same equipment. For a comparison of methods, the same protocol on the same homogenised samples was carried out in parallel using the microbiological assay. A statistical test (*t*-test) showed that the results obtained by the HPLC and microbiological methods for niacin are significantly different (bias were different from zero at 5% significance level).

When expressing the sum of nicotinic acid and nicotinamide results as niacin, repeatabilities (RSD_r) from 0.3 to 0.8% and reproducibilities (RSD_R) from 0.9 to 2.7% were obtained (Table 3). For the microbiological method, higher values with RSD_r in the range of 2.6–3.1% and RSD_R in the range of 5.7–6.8% were observed.

3.2.4. Comparison of HPLC results with microbiological assay

The HPLC-fluorescence method and the microbiological assay were applied to a range of products. The latter method uses stronger conditions for acid hydrolysis (HCl 1 M, for 15 min at 120°C). Table 4 presents the declared amounts of niacin and the values obtained by the HPLC-fluorescence and the microbiological methods. It can be seen that, the microbiological values were often higher than those obtained by HPLC. Microbiological results were between 0 and

Table 3
Precision parameters for the analysis of niacin by HPLC and microbiological assay

Statistical parameters	HPLC-fluorescence			Microbiological assay		
	Infant formula A	Breakfast cereal E	Infant cereals D	Infant formula A	Breakfast cereal E	Infant cereals D
Mean [a] (mg/100 g)	5.48	17.7	10.8	6.70	21.5	16.5
S.D. (mean)[b]	0.03	0.4	0.3	0.42	1.4	0.9
$S.D._r$[c]	0.05	0.06	0.05	0.21	0.17	0.43
Repeatability limit[d]	0.14	0.17	0.14	0.58	0.47	1.19
RSD_r (%)	0.8	0.3	0.5	3.1	3	2.6
$S.D._R$[e]	0.05	0.43	0.29	0.45	0.51	0.83
Reproducibility limit[d]	0.14	1.19	0.80	1.25	1.41	2.30
RSD_R (%)	0.9	2.4	2.7	6.7	6.8	5.7

[a] Results are the mean of duplicate determinations over 5 days in the same laboratory.
[b] Standard deviation of the mean of duplicate.
[c] Standard deviation of repeatability.
[d] At 95% confidence level.
[e] Standard deviation of reproducibility.

21% higher for infant formulae and clinical nutrition products, but the largest differences (up to 77%) were observed for cereal-based products.

To check the accuracy of the HPLC and microbiological methods, two standard reference materials, an infant formula 1846 and a fortified cereal VMA 195, were analysed. Results by HPLC were in good accordance with the certified values. For the VMA 195 sample, higher values, out of the certified range of concentration, were however obtained by microbiological assay.

A further study was made to check the influence of acid hydrolysis conditions on niacin values obtained by microbiological assay (Table 5) for cereal-based products and one milk-based product. For the cereal-based products the niacin values were somewhat higher with 1.0 M HCl/120°C than with 0.1 M HCl/100°C. In both cases the values obtained by microbiological assay were considerably higher than those found by the HPLC procedure.

The higher values obtained by the microbiological method, when the acid hydrolysis conditions become more drastic, are certainly due to a more important release of unavailable niacin. However, the higher concentrations of niacin obtained by microbiological assay with respect to those obtained by HPLC, even when

Table 4
Comparison of results for niacin by HPLC and microbiological assay

Type of food	Sample	Declared value	Niacin (mg/100 g)				Relative difference[b] (%)
			HPLC		Microbiological assay		
			n	Mean[a]	*n*	Mean[a]	
Infant formulae	SRM 1846 infant formula	6.33±0.76[c]	6	6.18	1	5.45	13
	B	6.51	8	6.28	2	7.08	−11
	C	5.1	4	6.49	2	7.49	−13
	D (ready to feed)	0.9	2	1.31	2	1.35	−3
	E	4.8	2	5.64	3	6.42	−12
Cereal products	Breakfast cereal A	31	2	46.8	2	45.0	4
	Breakfast cereal D	16.8	2	19.6	2	20.6	−5
	Infant cereals D	8	10	10.8	10	16.5	−34
	Infant cereals and fruits C	4	4	4.82	2	8.15	−41
	Infant cereals C	nf[d]	4	0.90	2	3.90	−77
	VMA 195	13.9–20.1[e]	2	18.3	3	29.9	−39
Chocolate drink & clinical nutrition products	Chocolate drink powder	19	2	20.8	4	22.8	−9
	Clinical nutrition product A	5.17	2	6.05	2	7.67	−21
	Clinical nutrition product B	2.63	2	3.13	2	3.53	−11
	Clinical nutrition product C	2.64	2	3.79	2	4.05	−6

[a] Results are the mean of *n* replicates.
[b] Difference between values obtained by the HPLC and microbiological methods, relatively to the microbiological method.
[c] Certified niacin value and uncertainty in NIST 1846 infant formula reference sample.
[d] Non-fortified in niacin.
[e] Certified range of niacin in VMA 195 reference sample.

Table 5
Comparison of results for niacin in several food products hydrolysed with 0.1 M or 1.0 M HCl prior to microbiological assay

Sample	Declared value	Niacin (mg/100 g)		
		HPLC	Microbiological assay	
		0.1 M HCl 1 h at 100°C	0.1 M HCl[a] 1 h at 100°C	1 M HCl 15 min at 120°C
Infant cereals C	nf[b]	0.90	3.20	3.9
Infant cereals and fruits C	4	4.82	7.10	8.15
VMA 195	13.9–20.1	18.3	20.8	29.9
Infant formula F	13	16.4	19.1	18.6

[a] Niacin values are the mean of six determinations.
[b] Non-fortified in niacin.

identical conditions of hydrolysis were used (0.1 M HCl, 100°C, 1 h), provide further evidence that the assay with *L. plantarum* is not completely specific for the bioavailable forms of niacin.

Other studies reported similar results. Van Niekerk et al. (1984) compared acid-enzyme hydrolyses with alkaline hydrolysis for both analytical techniques. Lower results were obtained by HPLC with an acid extraction (0.1 M H_2SO_4, autoclaved at 1 bar pressure, 1 h), but comparable results were obtained for the alkaline extracts. Using stronger acid hydrolysis, Chase et al. (1993) reported that results obtained by microbiological assay averaged 11% higher than the HPLC results. These results seem to confirm that a non-negligible part of niacin in acid extracts is bound and available to microorganisms but is not detected by HPLC.

4. Conclusions

The proposed HPLC method, involving extraction of bioavailable niacin with 0.1 M HCl, reversed-phase chromatography, with post-column derivatisation and fluorescence detection, is a suitable method for determining niacin in cereal and milk-based products. It is a very specific and selective method. This HPLC-fluorescence technique quantifies nicotinic acid and nicotinamide free of interferences and is consequently an improvement over the less specific HPLC methods with UV detection, which often require more complex sample clean-up.

The HPLC method yields niacin values in foods somewhat lower than by the microbiological assay. The most significant differences were observed for the cereal-based products. These differences arise principally from the lack of specificity of *L. plantarum* used in the microbiological assay and from the stronger acid hydrolysis extraction conditions, which certainly also release a part of the non-bioavailable niacin. Moreover, good accuracy and better precision were observed by HPLC. The HPLC values obtained are certainly closer to the real bioavailable concentrations of niacin in food than the microbiological values.

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References

AACC (2000). In *Niacin-microbiological method AACC Methods 86-51 and 86-50A (reapproved 3 November 1999)* (10th ed.). St Paul, MN: American Association of Cereal Chemists Inc.

AOAC (2000). Methods 985.34, 944.13, 968.32 and 975.41. In W. Horwitz, *Official methods of analysis* (17th ed.). Gaithersburg, MD: Association of Official Analytical Chemists International.

Ball, G. F. M. (1998). Niacin and tryptophan. In *Bioavailability and analysis of vitamins in foods* (pp. 319–359). London: Chapman & Hall.

Chase, G. W., Landen, W. O., Soliman, A. G., & Eitenmiller, R. R. (1993). Liquid chromatographic analysis of niacin in fortified food products. *Journal of the Association of Official Analytical Chemists International, 76*(2), 390–393.

Friedrich, W. (1988). Niacin: nicotinic acid, nicotinamide, NAD(P). In *Vitamins* (pp. 473–542). Berlin: Walter de Gruyter.

Krishnan, P. G., Mahmud, I., & Matthees, D. P. (1999). Postcolumn fluorimetric HPLC procedure for determination of niacin content of cereals. *Cereal Chemistry, 76*(4), 512–518.

LaCroix, D. E., Wolf, W. R., & Vanderslice, J. T. (1999). Determination of niacin in infant formula and wheat flour by anion-exchange liquid chromatography with solid-phase extraction cleanup. *Journal of the Association of Official Analytical Chemists International, 82*(1), 128–133.

Lahély, S. (1998). *Dosage de vitamines du groupe B (niacine, acide pantothénique, biotine, et folates) dans les aliments après isolement chromatographique*. Thèse de doctorat, Université de Strasbourg, France.

Lahély, S., Bergaentzlé, M., & Hasselmann, C. (1999). Fluorimetric determination of niacin in foods by high-performance liquid chromatography with post-column derivatization. *Food Chemistry, 65*, 129–133.

Mawatari, K. I., Iinuma, F., & Watanabe, M. (1991). Determination of nicotinic acid and nicotinamide in human serum by high-performance liquid chromatography with postcolumn ultraviolet-irradation and fluorescence detection. *Analytical Sciences, 7*, 733–736.

Tyler, T. A., & Genzale, J. A. (1990). Liquid chromatographic determination of total niacin in beef, semolina, and cottage cheese. *Journal of the Association of Official Analytical Chemists, 73*(3), 467–469.

Van Niekerk, P. J., Smit, C. C. S., Strydom, E. S. P., & Armbruster, G. (1984). Comparison of a high-performance liquid chromatographic and microbiological method for the determination of niacin in foods. *Journal of Agricultural and Food Chemistry, 32*, 304–307.

Vidal-Valverde, C., & Reche, A. (1991). Determination of available niacin in legumes and meat by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 39*, 116–121.