Niacin (Vitamin B_3) - A review of analytical methods for use in food

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Niacin (Vitamin B3) - A review of analytical methods for use in food

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Abstract

Niacin, a water-soluble vitamin, is an essential nutrient which is also known as vitamin B_3 or vitamin PP. It exists as nicotinic acid and nicotinamide which have equal biological activity and can be synthesised from tryptophan. The terms niacin, nicotinamide, and vitamin B_3 are often used interchangeably to refer to any member of this family of compounds. Niacin is directly or indirectly involved in many metabolic functions including the digestive system, skin, and nerves. It is also important for converting food to energy. Mild niacin deficiency can slow metabolism, and cause headaches and other minor symptoms, but severe deficiency causes the disease pellagra, characterised by diarrhoea, dermatitis and other skin disorders, dementia, inflammation of the mouth and tongue, and other symptoms which can be fatal if left untreated. As most niacin is obtained from tryptophan in proteins, deficiency is rarely seen in developed countries but can be seen in developing countries or in conditions of poverty, malnutrition or chronic alcoholism. Supplementation is therefore largely unnecessary in developed countries but a wide range of vitamin supplements containing nicotinamide are available and certain food types are often fortified with nicotinamide and other vitamins. Analysis is primarily required to confirm label declarations in fortified foods, feeds or supplements. A number of chemical or microbiological assay techniques have been used for many years. HPLC methods using UV detection are available, although sensitivity and selectivity can be a problem. The use of mass spectrometric (MS) detection following HPLC has been used in more recent methods. Official methods are listed. LGC has extensive experience of the determination of B-group vitamins using an in-house microbiological assay procedure, although this technique is no longer supported. A commercial test kit method has been used on a semi-routine basis, largely for determination of niacin in infant formulae. A method using aqueous extraction followed by ion-pair HPLC with UV detection has also been used for analysis of vitamin premixes but is only suitable for this purpose. Deuterated niacin compounds are commercially available for MS procedures for the determination of niacin. Further work may be required in this area.

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Introduction

Niacin, a water-soluble vitamin, is an essential nutrient which is also known as vitamin B_3 or vitamin PP. It exists as nicotinic acid and nicotinamide which have equal biological activity and can also be synthesised from tryptophan (1mg niacin is equivalent to 60mg tryptophan). Nicotinamide is sometimes referred to as niacinamide. The terms niacin, nicotinamide and vitamin B_3 are often used interchangeably to refer to any member of this family of compounds.

Niacin is a precursor to the two forms of nicotinamide adenine dinucleotide (NAD+ and NADH) and nicotinamide adenine dinucleotide phosphate (NADP+and NADPH), which play essential metabolic roles in living cells. It is important for converting food to energy.

Mild niacin deficiency can slow metabolism, and cause minor symptoms, but severe can be fatal if left untreated. Niacin deficiency also causes a condition called “black tongue” in cats and dogs.

Niacin deficiency is rarely seen in developed countries but can be seen where protein balance is compromised, such as areas where people eat large quantities of maize as a staple food since niacin in corn is biologically unavailable unless the corn is first treated with alkali before consumption.

Although, supplementation is largely unnecessary in developed countries, a wide range of nicotinamide-containing supplements are available and breakfast cereals, infant formula and other foods are often fortified.

This report summarises a review of analytical methods for the determination of niacin (vitamin B_3) in food. Mention of a commercial analytical product in this report does not imply endorsement by the Government Chemist.

Dietary sources of vitamin B_3

Few unfortified foods have significant levels of free niacin. Most of the requirement for niacin in humans and animals is met by biosynthesis from tryptophan present in food proteins. Both nicotinic acid and nicotinamide can be used for fortification although the latter can cause side-effects such as flushing and is toxic to the liver at higher concentrations. Nicotinamide is therefore usually used for supplementation.

Foods containing protein are good niacin sources and include milk, eggs, meat, vegetables even though the free niacin content is very low. Fortified cereal grains are important sources in some countries, including the UK, where flour is fortified by law to restore processing losses.

There are few reported problems at normal fortification levels of niacin, but ingestion of very high doses, particularly nicotinic acid, can cause toxicity.

Stability

Nicotinic acid and nicotinamide have similar pyridine-based structures which are readily interconvertible. Nicotinamide has higher water solubility than nicotinic acid. Both compounds are stable in the dry form and in solution, and are the most stable of the water soluble vitamins. Activity is not affected by heat, light, acid, alkali or oxidation.

Analytical requirements

Nutritional status is determined by analysis of plasma or serum concentrations of various niacin metabolites. Since deficiency is rare, the need for analysis of foods is low. Analysis is primarily required to confirm label declarations in fortified foods, feeds or supplements since unfortified foods contain little free niacin.

Extraction

Because niacin is so stable, acid, alkali or enzymatic hydrolysis can be used to extract niacin from foods. These processes release niacin from the bound forms such as NAD and NADP whilst simultaneously breaking down the food matrix. It should be noted that the niacin measured by some processes may not be bioavailable to humans; therefore the extraction process must be carefully considered if the data is to be used for nutritional intake purposes.

Choice of extraction protocol needs to consider the food to be analysed and the type of niacin to be determined. If only free or added niacin is required, a simple aqueous extraction can be used for some matrices. However, acid hydrolysis is often used, but there may be some conversion of nicotinic acid to nicotinamide. Alkaline hydrolysis is required to determine total niacin and converts all of the nicotinamide or other forms to nicotinic acid.

The products of enzymatic hydrolysis will depend on the enzymes used. It is important to be sure that the extraction procedure will release all of the niacin required in a form that the subsequent method will be able to quantify. In addition, the bioavailability of the measured forms must be considered for nutritional purposes.

It should be noted that many existing methods claim to be suitable for a range of foods but give extraction procedures which are not universally applicable.

Analytical methods

A number of chemical or microbiological assay techniques have been used for many years. HPLC methods using UV detection are available, although sensitivity and selectivity can be a problem. The use of mass spectrometric (MS) detection following HPLC has been used in more recent methods.

Niacin (nicotinic acid and nicotinamide) can be determined colorimetrically using the König reaction with cyanogen bromide. The derivatives are coupled to aromatic amines to form coloured compounds which, under controlled conditions, are proportional to the niacin content.

Microbiological assay using Lactobacillus plantarum has been widely used for many years for many of the water–soluble vitamins including niacin. It relies on the specific requirement for niacin by Lactobacillus plantarum to enable their growth in a supporting medium. Under appropriate conditions, the amount of growth is proportional to the amount of niacin in the test extract. Such assays can be considered routine but require overnight incubation, can be subject to contamination and require considerable laboratory set-up and maintenance time.

The development of commercial test kits for the analysis of water-soluble vitamins in foods using microbiological assay (e.g. VitaFast® kits) has simplified these procedures, although careful use is still required and the extraction procedures given in the kit may need further validation depending on the samples to be analysed.

Official methods

The following official methods are available:

USP/BP:

Determination of nicotinic acid and nicotinamide in pure substance, injectable solutions and oil or water soluble vitamins tablets and capsules – spectrophotometric and HPLC methods with specific scope.

AOAC:

The AOAC procedures are based on colourimetric or microbiological assay techniques:
AOAC 944.13 Niacin and niacinamide in vitamin preparations; Microbiological assay

AOAC 961.14 Niacin and niacinamide in drugs, food and feeds; Colourimetric

AOAC 975.41 Niacin and niacinamide in cereal products; Colourimetric

AOAC 981.16 Niacin and niacinamide in food, drugs and feeds; Colorimetric

AOAC 968.32 Niacinamide in multivitamin preparations; Spectrophotometric

AOAC 985.34 Niacin and niacinamide in ready to feed, milk-based infant formula; Microbiological assay

AACC:

Microbiological and colourimetric assays for cereal products – similar to AOAC

ISO/CEN/BS:

BS EN 15652:2009 - Foodstuffs. Determination of niacin by HPLC

Niacin vitamers are extracted from food by an acid (option A), enzymatic (option B) or acid/alkaline (option C) treatment and quantified by HPLC with a fluorimetric detection after a post-column derivatisation with UV irradiation. For option A and option B, niacin is determined as the sum of nicotinamide and nicotinic acid. Niacin is expressed as nicotinic acid after correction of the molecular weights. For option C, niacin is determined and expressed as nicotinic acid. The alkaline treatment transforms all nicotinamide into nicotinic acid. Validation is principally on dietetic foods.

Other methods:

Microbiological assay: This technique is still widely used for determination of niacin. Methods have been applied to many different foods and/or feeds for the determination of total niacin in foods at natural or added concentrations. Methods require experience of microbiological assay techniques and significant in-house set-up and maintenance and are not well suited to one-off analysis unless a commercial test kit is used.

VitaFast® test kit: 96 well microtitre plate-based microbiological assay. These are produced by R-Biopharm and provide a relatively simple means of applying the microbiological assay without the need for in-house maintenance of organisms etc. The niacin test kit is described for the determination of niacin in food, feed and pharmaceutical products. However, although extraction conditions are given in the kit instructions care is required to select a suitable extraction protocol for different food types. Additional validation may be required.

HPLC or LC-MS methods:

There are a number of published procedures using HPLC with UV or fluorescence detection and more recent procedures using MS detection.

Methods for niacin have been reviewed by Eitenmiller et al: Vitamin analysis for the health and food sciences 2nd Ed; Eitenmiller et al; 2007 CRC press. Relevant published procedures include the following:

Table 1: Instrumental methods for niacin

Analyte / Matrix	**Technique**	**Reference**
Niacin in various foods	Ion-exchange LC-UV	1
Niacin in various foods	CE (& HPLC)	2
Niacin in various foods (acid extraction)	CE (& HPLC)	3
Niacin in various foods (CEN procedure)	HPLC- post column derivatisation - Fluorescence	4
Niacin and metabolites in human plasma	LC-MS	5
Water soluble vitamins in pasta	LC/MS/MS	6
Niacin in various foods	LC-IDMS	7

Methods used at LGC

LGC has extensive experience of the determination of B-group vitamins using an in-house microbiological assay procedure, although this technique is no longer supported. The Vita-Fast® test kit is used on a semi-routine basis, largely for determination of infant formulae.

A method using aqueous extraction followed by ion-pair HPLC with UV detection has also been used routinely for analysis of vitamin premixes, but is only suitable for this purpose.

Deuterated niacin compounds are commercially available for MS procedures for the determination of niacin. Further work may be required in this area.

Conclusions

Niacin, a water-soluble vitamin, is an essential nutrient which is also known as vitamin B3 or vitamin PP. It exists as nicotinic acid and nicotinamide which have equal biological activity and can also be synthesised from tryptophan. Niacin is directly or indirectly involved in many metabolic functions including the digestive system, skin, and nerves. It is also important for converting food to energy. A wide range of vitamin supplements containing nicotinamide are available and certain food types are often fortified with nicotinamide and other vitamins. Analysis is primarily required to confirm label declarations in fortified foods, feeds or supplements since unfortified foods contain little free niacin. A number of chemical, or microbiological assay techniques have been used for many years. HPLC methods using UV detection are available, although sensitivity and selectivity can be a problem. The use of mass spectrometric detection following HPLC has been used in more recent methods. Official methods are listed above. Microbiological assay has been the most commonly used assay technique for foods and has been used for the majority of available food nutrition datasets. However such techniques are expensive to support in the absence of a minimum level of use. A commercial test kit method is available that has been used successfully although care is required to select a suitable extraction protocol for different food types. Additional validation may be required. A method using aqueous extraction followed by ion-pair HPLC with UV detection is also available for analysis of vitamin premixes but is only suitable for this purpose. Deuterated niacin compounds are commercially available for MS procedures for the determination of niacin.

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