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Application of a liquid chromatography tandem mass spectrometry method to the analysis of water-soluble vitamins in Italian pasta

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Abstract

A sensitive and selective liquid chromatography tandem mass spectrometry (LC–MS/MS) method for the determination of several water-soluble vitamins, namely vitamins B_1, B_2, B_6 (pyridoxine, pyridoxal, and pyridoxamine), and PP (nicotinamide and nicotinic acid), pantothenic acid, and folic acid was developed and validated. The analytes were characterized by means of their electrospray (ESI) and atmospheric pressure chemical ionization (APCI) mass spectra. In general, the positive ion spectra were 100- to 1000-fold more intense than the corresponding negative ion ones. Chromatography of water-soluble vitamins was obtained by using a reversed-phase C16 Amide (15 cm, 5 μm) column and a mobile phase made of ammonium formate buffer (20 mM, pH 3.75)/methanol under gradient elution conditions. Linearity of the MS response was observed over three to four orders for both ESI and APCI, and limits of detection were in the low μg/l range for both the ionization techniques. In particular, the sensitivity of ESI was about two- to five-fold higher for all vitamins except PP vitamers, for which APCI produced a better response. Precision calculated at two concentration levels (0.05 and 1.0 mg/l) was within 0.2–7.4% for all intra- and inter-day determinations and for all analytes. The LC–ESI-MS/MS method was applied to the quantitative analysis of the natural content of vitamins in typical Italian pasta samples, as well as in fortified pasta samples produced for the US market.

Keywords: Water-soluble vitamins; Pyridoxine; Pyridoxal; Pyridoxamine; Nicotinamide; Nicotinic acid; Pantothenic acid; Folic acid; LC–MS/MS; Italian pasta; Fortified food

1. Introduction

Vitamins are minor but essential constituents of food. Although, the vitamin requirement of the body is usually adequately supplied by a balanced diet, significant subgroups in most European populations are still subjected to the risks associated with low micronutrient intakes [1]. Addition of vitamins and minerals to foods may be useful to address this risk. The water-soluble vitamin group includes diverse compounds [2] with respect to structure, molecular weight, chemical properties, and biological activity: thiamine (vitamin B_1), riboflavin (vitamin B_2), pantothenic acid (vitamin B_5), vitamin B_6 vitamers (pyridoxal, pyridoxine, and pyridoxamine), cyanocobalamin (vitamin B_{12}), L-ascorbic acid and L-dehydroascorbic acid (vitamin C), niacin (nicotinic acid) and its amide (nicotinamide, vitamin PP), folic acid, and biotin. In addition, other biologically active compounds have been recognized as pseudo-vitamins. The rapid and reliable analysis of vitamins in foods represents a relevant objective for food manufacturers. In fact, the customers' demand for nutritional information about food composition is constantly increasing and, on the other hand, a number of fortified foods are nowadays commercially available. Since

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there is a relatively low margin of safety between adequate levels of intake and maximum safe intake, the levels that can be added safely to foods need to be properly estimated in order to avoid unacceptably high intakes in people consuming large amounts of food. Hence, the amount of vitamins added to food should respect the limits set by the governmental authorities, for example, in Europe a nutritional label has to report the amount of vitamins when they reach at least the level of 15% of the European commission recommended daily intake (ECRDA) [3]. The EC has recently published a draft proposal [4,5] for a harmonized regulatory framework on the voluntary addition of vitamins and minerals to foods in EU. Furthermore, there are specific limits set in the United States for different classes of products and severe controls are made by the Food and Drug Administration to verify the conformity of imported food to the standard of the US market.

The determination of vitamins in food represents a complex analytical problem for several reasons: (i) due to their different chemical structures and properties, it is extremely difficult to develop a "general" method suitable for the simultaneous determination of several vitamins in foods; (ii) foods themselves are very complex matrixes; (iii) of which vitamins are only micro-constituents; and (iv) in the case of fortified food, the natural vitamin content should be accurately known in order to make additions in compliance with the terms set by the laws. Different liquid chromatographic methods have been used for the separation of water-soluble vitamins [6,7], including reversed-phase (RP) chromatography with phosphate or acetate buffers, ion-suppressed RP chromatography (folic acid), ion-pair and ion-exchange chromatography (vitamin PP). UV detection is applied in most cases, although fluorescence detection (FLD) is a useful alternative for those vitamins that are naturally fluorescent (B_2 and B_6) or become fluorescent after derivatization (B_1). Besides the recent proposal of new approaches for the detection of vitamins using matrix assisted laser desorption ionization (MALDI) [8] or surface plasmon resonance (SPR) [9] techniques, the development of thermospray and electrospray interfaces for liquid chromatography-mass spectrometry (LC–MS) offered significant advantages of increased sensitivity and the ability to analyze highly polar compounds such as water-soluble vitamins [10]. Despite the potential of this coupled technique, up to date only few studies have been published in this field [11–15]. A limiting factor remains the extraction of the analytes from the matrix. On the other hand, in the case of water-soluble vitamins the application of a technique like LC–MS, which is sensitive and selective itself, could allow a considerable simplification of the pre-analytical procedure. The aim of this work was to develop a fast, sensitive, and reliable method, suitable for the quantitative analysis of most water-soluble vitamins, i.e. the B-group vitamins, niacin, pantothenic acid, and folic acid, in typical Italian pasta samples as well as in fortified pasta samples produced for the US market.

2. Experimental

2.1. Chemicals and reagents

Vitamin B_1 hydrochloride and vitamin B_2 were obtained from Fluka (Buchs, Switzerland); nicotinamide, folic acid, pantothenic acid hemicalcium salt, and vitamin B_6 vitamers, pyridoxal, pyridoxine, and pyridoxamine were purchased from Sigma (Milan, Italy); nicotinic acid was from Carlo Erba (Milan, Italy). All chemicals were of analytical-reagent grade (purity > 98%) and were used without further purification. HPLC-grade water, methanol and acetonitrile were supplied by Lab-Scan (Dublin, Ireland). Solvents were filtered on 0.2-μm membranes (Millipore, Bedford, MA, USA) and degassed before the use. Analytical-grade formic acid (85%), glacial acetic acid (99%), hydrochloric acid (37%), anhydrous citric acid, ammonium formate, ammonium acetate, ammonium hydroxide (30%), sodium phosphate ($Na_2HPO_4{\cdot}12H_20$), sodium acetate, sodium sulfate, and sodium ascorbate were supplied by Carlo Erba. α-Amilase from Aspergillus oryzae was obtained from Sigma and papaine from Merck (Darmstadt, Germany).

2.2. Liquid chromatography with fluorescence and UV detection

LC analyses were performed by using a Waters 510 dual pump liquid chromatograph equipped with a Waters 712 Wisp autosampler (Waters, Milan, Italy). Vitamins were separated on a Supelcosil C18 column (25 cm × 4.6 mm, 5 μm, Supelco, Bellefonte, PA, USA) using different mobile phases [16,17]: (i) an isocratic 60/40 (v/v) methanol/sodium acetate buffer (0.05 M, pH 4.5) mixture for vitamins B_1 and B_2; (ii) a mixture containing sodium acetate buffer 0.2 M, acetic acid 0.9 M, and sodium 1-heptanesulfonate monohydrate 5 mM for vitamin PP; (iii) an isocratic 85/15 (v/v) mixture of (A) sodium acetate (20 mM) and sodium sulfate (20 mM), adjusted to pH 5.3 with acetic acid and (B) 640 ml of eluent A + 360 ml of acetonitrile for folic acid. Detection was performed using a Waters 470 scanning fluorescence detector for vitamins B_1 after derivatization to thiochrome (λ_{ex} = 366 nm, λ_{em} = 435), and B_2 (λ_{ex} = 422 nm, λ_{em} = 522), or a Jasco 870 UV/VIS detector (Jasco Inc. Easton, USA) for vitamin PP (λ = 260 nm) and folic acid (λ = 280 nm).

2.3. Liquid chromatography–mass spectrometry

LC–MS/MS analyses were carried out on a PE-Sciex API 365 triple-quadrupole mass spectrometer (Sciex, Thornhill, Canada) equipped with an atmospheric pressure ionization (API) source, an ionspray interface for pneumatically assisted electrospray (ESI) and a heated nebulizer (HN) interface for atmospheric pressure chemical ionization (APCI). The liquid chromatographic system consisted of a Perkin-Elmer series 200 dual solvent delivery system (Norwalk, CT, USA) equipped with an ASPEC XL Autosampler (Gilson,