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A rapid method for sensitive profiling of folates from plant leaf by ultra-performance liquid chromatography coupled to tandem quadrupole mass spectrometer

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ABSTRACT

Previous published methods for the analysis of folates are time consuming because of lengthy sample extraction, clean-up and total running time. This study details the development and validation of a rapid, sensitive and robust method that combines a simple extraction step with ultra-performance liquid chromatography coupled to tandem quadrupole mass spectrometry. Here, we reported application of a tandem quadrupole mass spectrometer to analyze maximum seven vitamers of folate from plant origin. The analytical performance was evaluated by linearity, sensitivity, precision, recovery test and analysis of certified reference materials. The limit of detection and limit of quantification ranged between 0.003 and 0.021 μg/100 g FW and between 0.011 and 0.041 μg/100 g FW, respectively; the recovery and precession ranged from 71.27 to 99. 01% and from 1.7 to 7.8% RSD, respectively, depending upon folate vitamers. This newly developed and validated method is rapid (a chromatographic run time of 5 min), easy to be performed (no laborious and time consuming clean-up) and can be used to simultaneously analyze seven vitamers of folate from plant sources.

1. Introduction

Folates, the generic term for a group of water-soluble vitamin have attained great nutritional importance. Chemically, folates molecules are comprised of three parts: pteridine, *p*-aminobenzoate, and a glutamyl chain (from1 to 14 links) (Fig. 1) [1]. They participate in one-carbon transfer reactions in the biosynthesis of purine and pyrimidine as well as amino acid inter-conversions [2]. However, intake of this group of vitamins from natural food sources is considered to be below the dietary recommendations for human [3,4]. Low levels of plasma folate concentrations are associated with a number of impairments, including development of neural tube defects (NTDs) and other congenital defects, macrocytic anaemia, cardiovascular disease, and certain types of cancer [5,6]. Folate deficiency, characterized by a folate intake below the recommendations (<400 μg per day) [4], is an important type of micronutrient deficiency nearly all over the world. Humans and animals cannot synthesize folates by themselves, so plant food is a main source of this important group of vitamin. However, the naturally occurring folate content in most plant foods usually is quite low. Therefore, mandatory food crop fortification programs with folic acid are a common practice in many countries [7]. Another alternative is to increase the natural folate level in food crops either by plant breeding or by metabolic engineering. This strategy recently became an increasingly important topic in the area of plant and human nutrition [8]. However, the availability of an accurate method for analysis of various folate vitamers in food from the plant origin is a challenging issue for the researchers in plant and human nutrition. This is because of plant samples contain very minute amount of folate and complex food matrix.

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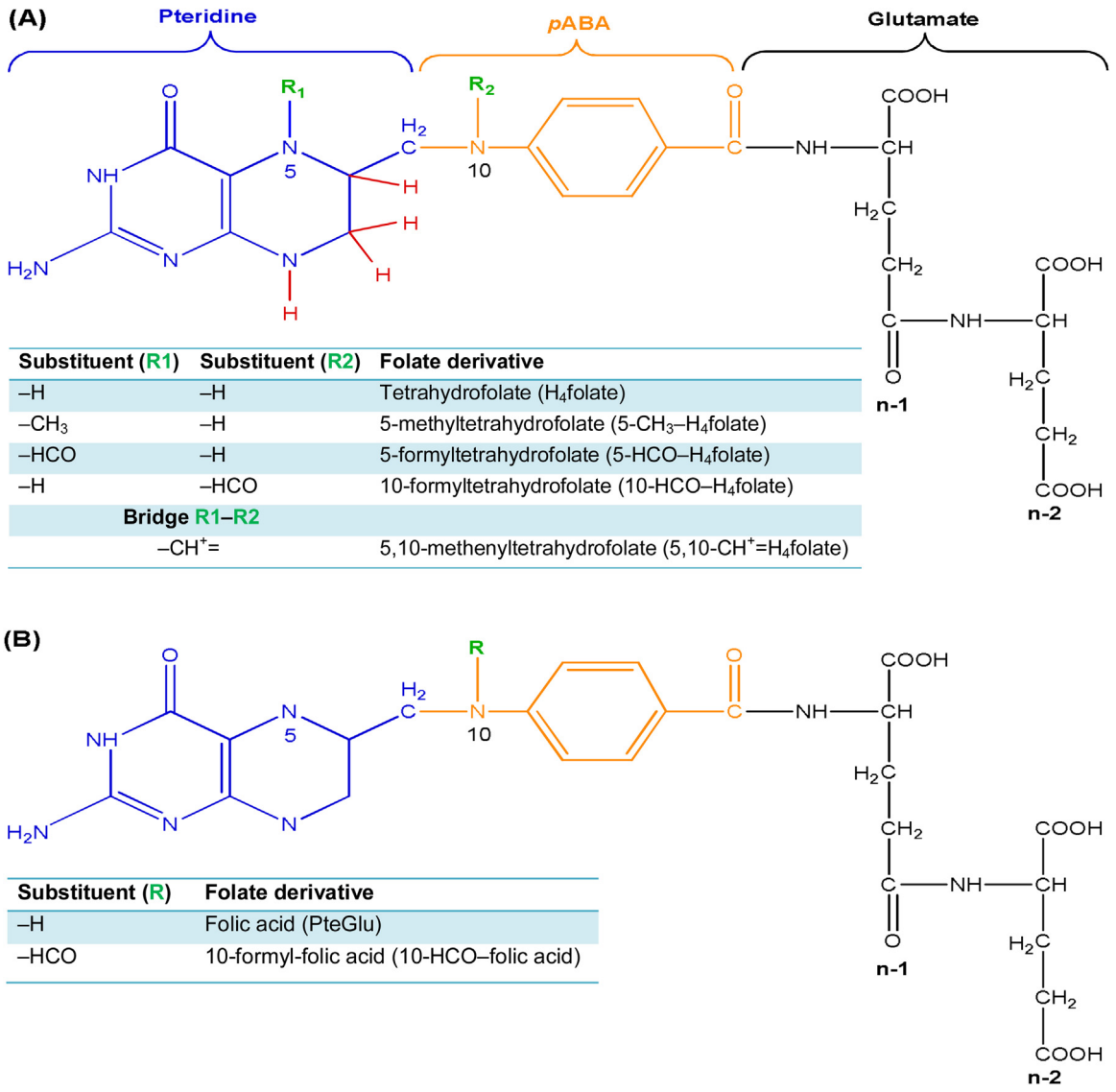

Substituent (R1)	Substituent (R2)	Folate derivative
–H	–H	Tetrahydrofolate (H_4folate)
$–CH_3$	–H	5-methyltetrahydrofolate (5-CH_3–H_4folate)
–HCO	–H	5-formyltetrahydrofolate (5-HCO–H_4folate)
–H	–HCO	10-formyltetrahydrofolate (10-HCO–H_4folate)
Bridge R1–R2		
$–CH^+=$		5,10-methenyltetrahydrofolate (5,10-CH^+=H_4folate)

Substituent (R)	Folate derivative
–H	Folic acid (PteGlu)
–HCO	10-formyl-folic acid (10-HCO–folic acid)

Fig. 1. Chemical structure of seven folates vitamers separated from the sample of plant origin. (A) Partially oxidized pteridine ring, and (B) Fully oxidized pteridine ring indicated in red color. Possible substitutions at the N5 and/or N10 positions by different C1 unite indicated in green color. Pterin, pABA, and glutamate moieties are depicted in blue, orange and black color, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Theoretically, more than 150 different vitamers of folates exist [9] although less than 50 are probably present in animal and plant tissue [10]. Most of the published chromatographic methods can only determine two to three vitamers, i.e. folic acid (PteGlu), tetrahydrofolate (H_4folate), and/or 5-methyltetrahydrofolate (5-CH_3-H_4folate), which is the main bottleneck when low folate amounts have to be analyzed. Therefore, it is important to develop a novel method that can determine more folate vitamers to obtain precise data of total folates in foods from plant origin [11]. Due to the large number of structural analogs, their low stability, and very low concentration in plant samples, folate analysis is an analytical challenge for the analytical food chemist [10]. The accuracy and sensitivity of folate analysis is highly dependent on the extraction method, the enzymatic deconjugation and detection techniques applied [12]. Additionally, folates are very sensitive to light, oxidants and pH of the medium. All these factors can significantly affect the accuracy of analysis. Furthermore, depending on the pH of the extraction buffer, heat treatment can affect the stability of folates and also causes non-enzymatic inter conversion [13]. Moreover, some protein and carbohydrate rich food require additional tri-enzyme treatment (α-amylase, protease and deconjugase) for the complete extraction of folates trapped in complex protein or carbohydrate structures [14]. This tri-enzyme digestion procedure is particularly effective for cereal-based and milk-based products. However, till now minimal information is available regarding the use of tri-enzyme digestion in analyzing foods of plant origin.

In the last 60 years, the most commonly used technique for quantitative analysis of food folates has been the microbiological assay (MA) that provides only a total folate content [15], associated with several drawbacks, for instance, it lacks information on specficity and vitamer distribution. Therefore, there is increasing application of chromatography based methods. Liquid chromatography (LC) [16] and Gas chromatography (GC) [17] are currently available for analysis of folate in both biological and food samples. The bottleneck of the GC method, similar to MA method, is the lack of capability to differentiate different folate vitamers in the sample. MA and GC method reduce their applicability because information of folate vitamers composition is important, as the different vitamers differ in their bioavailability [18]. In contrast to this, LC methods enable the determination of individual folate forms. In