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Development and Validation of an optimized HPLC-UV analytical method for the quantification of 5-Methyltetrahydrofolate (Active Folate) in dietary Supplements: Application to commercial tablets and capsules

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ABSTRACT

5-MTHF is the active form of folic acid, and it is provided as a dietary supplement in the market. Therefore, in this study we provide a new, simple and economic HPLC-UV analysis method to quantify 5-MTHF in dietary supplement. A new HPLC-UV method was developed and validated for the quantification of 5-methyltetrahydrofolate in dietary supplements. The method was optimized in terms of chromatographic conditions, including the mobile phase composition, chromatographic column, and the sample preparation diluent using ascorbic acid. A combination of ammonium acetate buffer with methanol (90:10) was the optimal mobile phase, achieving a retention time of approximately 11.4 min on C18 column with good column performance (N = 6786, Asymmetry = 1.03). The method was validated for selectivity, linearity, precision, accuracy, and robustness. The calibration curve was linear ($r^2 > 0.999$) over the concentration range of 1.20 to 4.80 μg/mL. Precision was demonstrated with RSD% values ranging from 1.38 % to 2.90 % for intra-day precision and 0.96 % to 2.61 % for inter-day precision. Accuracy was confirmed with recovery values between 96.5 % and 110.6 % for spiked samples. To demonstrate the method's applicability, ten brands of 5-MTHF dietary supplements were collected from local pharmacies. Assay results showed significant variability, with four brands having contents below the acceptable range (90–110 % of labeled contents) and two brands exceeding this range. The stability of 5-MTHF in these products appeared to be formula-dependent, emphasizing the need for regulatory revisions to mandate stability testing for dietary supplements.

1. Introduction

Folate, also known as vitamin B9, is a crucial water-soluble vitamin involved in various bodily functions, including DNA synthesis, RNA transcription, methionine synthesis from homocysteine, and numerous cellular metabolic reactions [1]. It plays a particularly critical role in fetal development, as sufficient folate intake during pregnancy reduces the risk of neural tube defects in babies [2]. However, the human body cannot synthesize folate; therefore, dietary sources and supplementation are essential [2].

Green leafy vegetables, fruits, brewer's yeast, animal liver, and supplements are all sources of folate. Given the challenges of consistently obtaining adequate folate from food alone, supplementation with 5-Methyltetrahydrofolate (5-MTHF, Fig. 1), the predominant active form of folate, is often recommended. Unlike folic acid, the synthetic form of folate, 5-MTHF requires no conversion within the body for utilization [2]. This bypasses the folate metabolism pathway, which can be beneficial for individuals with MTHFR polymorphisms that impair folate conversion [3]. As 5-MTHF is directly absorbed and exerts its biological activity, supplementation is crucial to maintain optimal folate levels in such individuals. Therefore, for external supplementation, 5-MTHF is strongly recommended over folic acid (FA) [2,4]. 5-MTHF is commercially available in two main forms: di-glucosamine salt and calcium salt (Fig. 1 and Table S1).

The quantitation of 5-MTHF has been reported in various matrices, primarily using HPLC separation techniques. LC-MS has been employed for biological samples [3,5–7] and food samples [8,9], in the other hand, LC-MS had expensive analysis procedure and require highly trained

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