

膠錠保健食品中葉酸檢驗方法之探討

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食品藥物管理署研究檢驗組

摘 要

參照衛生福利部食品藥物管理署(下稱食藥署)之「膠囊與錠狀食品中水溶性維生素之檢驗方法」,以酸性液萃取葉酸進行檢測,發現於部分產品中葉酸含量偏低。為瞭解pH值對檢測膠錠食品中葉酸含量之差異,將葉酸標準原液分別調製成酸性(pH 2.5)與鹼性(pH 9.0)之2系列標準溶液,經液相層析儀分析結果,所得之標準曲線之線性關係均良好。此外,5件市售錠狀產品以鹼性液萃取所得葉酸之檢測值為其標示值之89-108%;而於酸性液萃取之檢測結果,3件含葉酸之綜合型維生素產品之檢測值分別為其標示值之112、83及22%,另2件以葉酸為單一成分之產品,其檢測值均低於方法定量極限(0.025 mg/g),顯示葉酸經鹼性液萃取分析之回收率較佳,而於酸性液萃取環境下,產品中抗氧化成分之存在,具有保護葉酸安定性之效果。當檢體為僅含葉酸之膠錠食品,或對產品中葉酸含量檢驗結果有疑義時,應使用鹼性液配製標準溶液及進行檢體之萃取,以確保葉酸檢驗結果之正確性。上開說明已加註於修正之檢驗方法中並於110年10月1日公開於食藥署網站供大眾參考。

關鍵詞：膠錠保健食品、葉酸、pH值、液相層析儀

食藥署建議方法「膠囊與錠狀食品中水溶性維生素之檢驗方法」⁽¹⁾係將葉酸標準品配製於鹼性溶液中,並以酸液萃取8項水溶性維生素進行HPLC檢測,即以pH 9.0磷酸緩衝溶液配製葉酸標準原液(Stock solution),再使用pH 2.5磷酸緩衝溶液配製標準溶液(Working solution),並以pH 2.5磷酸緩衝溶液作為樣品萃取液調製檢液(Sample solution)。該法雖與2009年Chen等人⁽²⁾檢測綜合維他命時,以pH 9.0磷酸緩衝溶液配製葉酸標準溶液,再以pH 2.0磷酸緩衝溶液同時萃取樣品中多種維生素之檢驗流程雷同,惟以此檢驗方法⁽¹⁾檢測僅含葉酸成分之錠狀產品時,發現葉酸含量低於定量極限(0.025 mg/g),故進一步檢視該方法之

檢驗流程。基於葉酸在鹼性下安定、酸性下易降解之特性,且為瞭解含葉酸產品中檢驗葉酸含量受酸性或鹼性分析條件之影響情況,並考量檢液與標準品配製溶液之一致性,參照上述方法⁽¹⁾,分別對葉酸標準溶液及萃取液之酸鹼性影響進行探討。

本研究分別以pH 2.5磷酸緩衝溶液(下稱酸性液)與pH 9.0磷酸緩衝溶液(下稱鹼性液),將葉酸標準原液調製成0.1-11 $\mu\text{g/mL}$ 之7種濃度之酸性或鹼性標準溶液,經液相層析儀分析,所得標準曲線之決定係數(R^2)分別為0.9995及0.9998,顯示葉酸之酸性標準溶液或鹼性之標準溶液,均能呈現良好線性關係,與文獻中以鹼性溶液或去離子水配製之葉酸標準溶液之標

準曲線之決定係數均大於0.9995 (表一)，皆具有良好線性關係之結果相同⁽²⁻⁶⁾。另於相同濃度下，則以鹼性稀釋液經液相層析分析所得葉酸之波峰面積較高(圖一)。

至於pH值對檢體中葉酸萃取之影響，發現經鹼性液萃取之5件檢體，其葉酸之檢測值為標示值之89-108%，顯示鹼性液皆可完全萃取此產品中葉酸成分且準確定量；經酸性液萃取時，其中3件含葉酸成分之綜合型維生素檢體(S3-S5)中，葉酸之檢測值分別為標示值之112、83及22%；另2件(S1, S2)僅含葉酸之檢體中葉酸檢測值均低於定量極限(表二)。

對於部分含葉酸檢體之葉酸含量分析結果偏低之情形，推測可能與葉酸本身之化學特性及產品組成分有關。依據5件檢體之成分標示，其中2件檢體(S1, S2)除賦形劑外僅有葉酸成分；而3件綜合型維生素檢體中有2件(S3, S4)含維生素C與番茄紅素等，1件(S5)含維生素B群與肌醇但不含有維生素C成分。雖本次

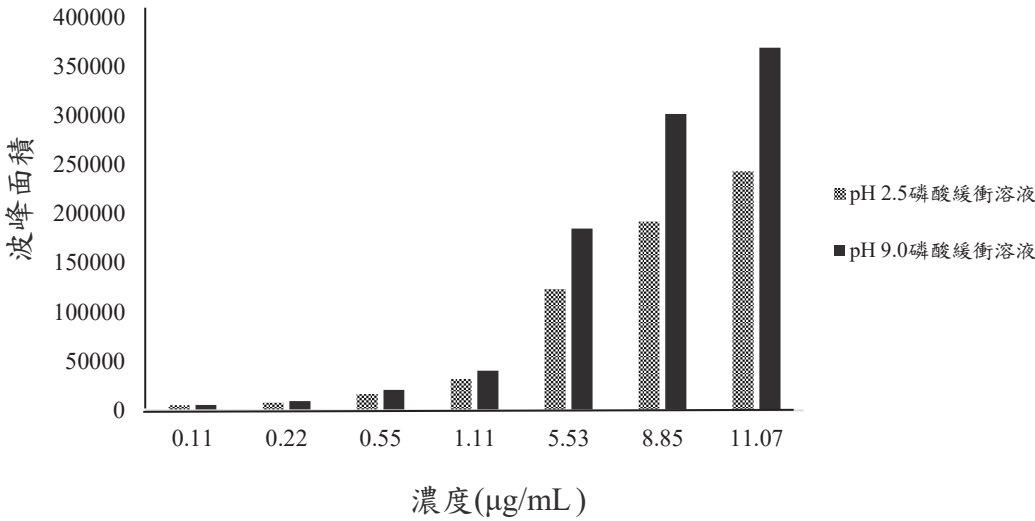
抽測之3件綜合型維生素檢體含有維生素C、維生素E、番茄紅素、肌醇、類黃酮等具抗氧化性質之成分，可作為抗氧化劑，但其抗氧化能力以及在各產品組成之比例皆會影響對葉酸之保護程度，導致1件產品(S5)之葉酸於酸性萃取液之萃取效果不佳。相較於文獻中綜合維他命產品之葉酸回收率介於88-100%之間，顯示不論是以酸性液、中性去離子水或是鹼性液對於綜合維他命產品中葉酸之檢測皆有良好回收率。另有文獻⁽⁷⁾提及維生素C可作為抗氧化劑，對葉酸具有保護作用，故即使在酸性之萃取環境下仍可確保葉酸檢驗結果之準確性。

綜上結果，當檢體為僅含葉酸之膠錠食品，或對產品中葉酸含量檢驗結果有疑義時，應使用鹼性液配製標準溶液及進行檢體之萃取，以確保葉酸檢驗結果之正確性。上開說明已加註於修正之建議檢驗方法⁽⁸⁾，並於110年10月1日公開於食藥署網站供各界參考使用。

表一、保健食品中水溶性維生素之葉酸成分分析方法彙整

產品成分 流程	出處	TFDA ⁽⁸⁾	Chen等人 ⁽²⁾	Jin等人 ⁽³⁾	Kucukkolbasi 等人 ⁽⁴⁾	Thermo Fisher Scientific公司 ⁽⁵⁾	Thermo Fisher Scientific公司 ⁽⁶⁾
		葉酸	綜合維他命	綜合維他命	綜合維他命	綜合維他命	綜合維他命
標準溶液配製 溶液		磷酸緩衝溶液 (pH 2.5)	磷酸緩衝溶液 (pH 9.0)	氨水溶液	去離子水	氫氧化鉀溶液	碳酸氫鉀溶液
樣品萃取溶液		磷酸緩衝溶液 (pH 9.0)					
		磷酸緩衝溶液 (pH 2.5)	磷酸緩衝溶液 (pH 2.5)	氨水溶液	0.01%三氟乙 酸:甲醇溶液 (80:20,v/v)	氫氧化鉀溶液	去離子水
		磷酸緩衝溶液 (pH 9.0)					
標準曲線之決定 係數(R ²)		0.9995 0.9998	0.9997	1.0000	0.9999	0.9996	1.0000
回收率(%)		N.D. ^a 112 N.D. ^a 83	96.2	98.8	88.3 87.4	100	100
		97 108 112 89					

^a N.D.：低於定量極限(0.025 mg/g)



圖一、以pH 2.5及pH 9.0磷酸緩衝溶液配製不同濃度葉酸標準溶液之波峰面積比較

表二、市售錠狀產品中葉酸含量之檢測結果

編號	產品成分	磷酸緩衝溶液	檢測值 (µg/錠)	標示值 (µg/錠)	檢測值/標示值 (%)
S1	僅含葉酸	pH 2.5	N.D. ^a	500	—
		pH 9.0	485		
S2	僅含葉酸	pH 2.5	N.D. ^a	800	—
		pH 9.0	835		
S3	綜合型維生素(含維生素C)	pH 2.5	447	400	112
		pH 9.0	433		
S4	綜合型維生素(含維生素C)	pH 2.5	332	400	83
		pH 9.0	354		
S5	綜合型維生素(未含維生素C)	pH 2.5	22	100	22
		pH 9.0	96		

^a N.D. : 低於定量極限(0.025 mg/g)

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Investigation of the Analytical Method for Folic Acid Quantification in Functional Foods in Capsule or Tablet Form

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ABSTRACT

Some products showed lower folic acid content than labeled using the acidic extraction method (Method of Test for Water-Soluble Vitamins in Foods in Capsules or Tablets Forms) published by the TFDA, therefore, the effect of pH on the detection of folic acid content in foods in capsule or tablet form was studied. The linear regressions of the folic acid standard curve were good using high performance liquid chromatography (HPLC), regardless of the standard stock solution was diluted with acidic (pH 2.5) or alkaline solution (pH 9.0). Five products labeled with folic acid were investigated. With alkaline extraction, the ratios of the amounts of folic acid detected to those labeled in 5 samples in tablet form were in the range of 89-108%; with acidic extraction, the ratios of the amounts of folic acid detected to those labeled in 3 samples labeled as multivitamin tablets were 112%, 83% and 22%, respectively, while the amounts of folic acid detected in the other 2 samples labeled as single folic acid ingredient tablets were lower than the limit of quantification (0.025 mg/g). It was indicated that recoveries of folic acid by the alkaline extraction solution were higher than those by the acidic extraction solution. However, the presence of antioxidant components in the samples could protect the stability of folic acid in the acidic extraction solution. The alkaline solution should be used as the diluent for the standard solution and the extraction solvent of the sample to ensure the accuracy of the results if the sample contains only folic acid ingredient or there is any doubt about the analytical results of folic acid in the product. The above description has been annotated in the amended recommended method which was published on the TFDA website on October 1, 2021.

Key words: functional foods in capsule or tablet form, folic acid, pH value, high performance liquid chromatography