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Short communication

Simultaneous Determination of Orotic Acid and Folic Acid in Pure forms and in Pharmaceuticals Formulations by HPLC

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Abstract

Studies were carried out to develop a simple, rapid and accurate HPLC method for the simultaneous determination of Orotic Acid and Folic Acid in pharmaceutical formulation. The separation was done on ODS column by the application of isocratic reversed-phase liquid chromatographic technique. Mobile phase consisted of 800ml phosphate buffer pH 7.2 and 70ml methanol. The method was successfully used for the determination of these drugs in the presence of additives and excipients, which were normally encountered in pharmaceutical formulations. The proposed method of analysis was applied for the individual analysis of both these constituents in their pure forms and found equally effective.

Introduction

Orotic acid is also know as 1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidinecarboxylic acid, Uracil-6-carboxylic acid, Whey factor, Animal galactose factor, Oropur, Orotyl, Oroturic and Vitamin B_{13} [1]. It is primarily used for metabolization of folic acid and Vitamin B_{12}.The best natural sources of orotic acid are whey and root vegetables. Its determination is useful in delineating the cause of hyperammonemia. Nutritional studies in humans and animals revealed that orotic acid has a sparing effect on Vitamin B_{12}, meaning that supplemental orotic acid can partially compensate for B_{12} deficiency.

Folic acid is also know as N-(p-(((2-amino – 4 – hydroxyl – 6 - pteridinyl) methyl) amino) benzoyl)-l-glutamic acid, l-pteroylglutamic acid, Folacin, Folate, Pteglu, Pteroyl-l-glutamic acid, Vitamin M, PGA, liver lactobacillus casei factor, Foliamin, Folipac, Folsaure, Folsan, Foluite, Incafolic, Millafol, Folettes, and Folsav [2]. It is necessary for cell development; for the metabolism of specific biochemical reactions in the body, such as the conversion of homocysteine to methionine; and for the metabolism of specific anticonvulsant drugs. The best food sources of folic acid are liver, kidney, dry bean, asparagus, mushrooms, broccoli & collards. Other good sources include spinch, peanut, lima beans, cabbage, sweet corn, chard, turnip greens, lettuce, milk and whole wheat products [3]. A deficiency of folate increases the risk of neural tube defects (NTDs), as well as contributing to hyperhomo-cystinemia, a condition associated with increased cardiovascular disease and neural tube defects [4]. The risk of neural tube defects (NTDs) is decreased in women who take folic acid during the periconceptional period [5].

Several methods have been reported for determination of Folic acid like stable isotope dilution assay (SIDA) [6], radioassay [7], and for orotic acid such as Reverse phase liquid chromatography [8], GC/MS [9], Capillary zone electrophoresis [10, 11]. Here an attempt is made to determine orotic and folic acid simultaneously in multivitamin preparation (containing Vitamin B_{12}, Folic acid and Orotic acid) by reverse phase liquid chromatography. Method was found selective and accurate for quantitation of folic and orotic acid in the presence of drug additives. The proposed method was also applied for the individual analysis of these drugs in their pure forms and found equally effective.

Experimental

Instrumentation

An isocratic liquid chromatographic system consisted of a Shimadzu (Japan) LC-10AS pump, a Rheodyne (USA) Model 7725 syringe loading sample injector equipped with a 20µl sample loop, a reversed-phase ODS column(Shim pack (M)) and a Shimadzu (Japan) SPD-10A UV detector fitted with 8µl flow cell was used during the study. Instrumental settings were a flow rate of 1ml min^{-1}, an ambient column temperature, and a detector wavelength of 277nm.

Reagents and Solutions

All reagents were of analytical reagent grade / HPLC Grade and doubly distilled, deionized water was used throughout the experiment. Sodium perchlorate (BDH), Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, potassium hydroxide, sodium hydroxide and HPLC grade methanol used were from (Riedel-dehaen). A 0.45 micron filter paper, 0.2 micron syringe filter and filtration assembly used were made by Sartorious.

Mobile phase

The mobile phase consisted of 800ml phosphate buffer (pH 7.2) and 70ml methanol. The phosphate buffer was prepared by dissolving 0.938% Sodium perchlorate and 0.075% Potassium dihydrogen phosphate in distilled water. The pH of the buffer was adjusted to 7.2 with 0.56% Potassium hydroxide solution. The mobile was filtered through 0.45-µm filter.

Solvents

Solvent "A"

0.57% Dipotassium hydrogen phosphate was prepared in distilled water.

Solvent "B"

0.4% Sodium hydroxide was prepared in distilled water.

Standard stock solutions

Standard solutions were prepared by dissolving the drugs to the desired concentrations.

Folic Acid

Folic acid standard was accurately weighed to obtain the concentration of 1mg $ml^{-1}$and dissolved with solvent "A" up to volume in a 100-mL volumetric flask.

Orotic Acid

Orotic acid standard was accurately weighed to obtain the concentration of 1mg $ml^{-1}$and dissolved with solvent "B" up to volume in a 100-mL volumetric flask.

Standard mixture solution

A standard mixture solution was prepared from these stock solutions by mixing the required volumes each stock solution and dissolved with solvent "A" up to volume in a 25-mL volumetric flask. The solution was filtered through a 0.45-µm filter before injection into the HPLC system.

Sample preparations

The syrup solution was homogenized by shaking and diluted with solvent "A" to required final concentration. The solution was filtered through a 0.45-µm filter before injection into the HPLC system.

Results and Discussion

Selectivity

Selectivity was assessed by the chromatograms obtained from samples and placebo. Possible interferences resulting from substances present in the medicaments were not observed. A typical HPLC chromatogram of the standard mixture is shown in Figure 1. The two peaks were well separated from each other. The retention time difference between them is 7.3 minutes approximately.

Linearity

The linearity graphs were obtained by plotting the peak area against the concentration of drugs (Figure 2 Orotic acid & Figure 3. Folic acid). In the simultaneous determination, the calibration graphs were found to be linear in the mentioned concentrations (the correlation coefficients are shown in Table 1).

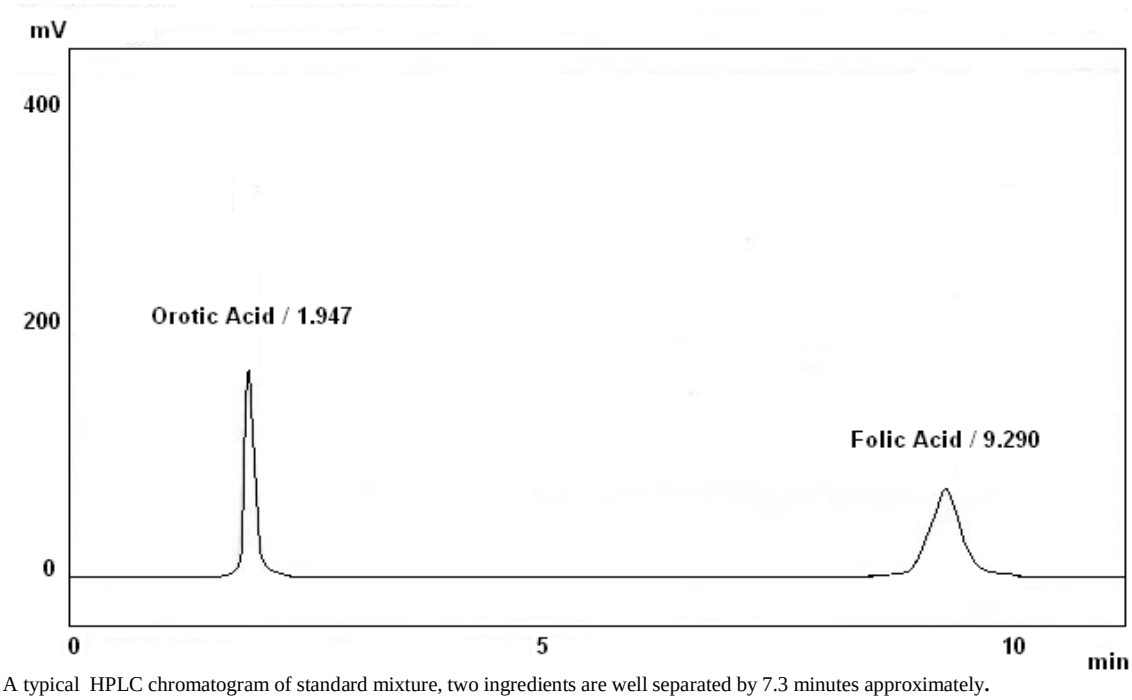

Fig. 1. A typical HPLC chromatogram of standard mixture, two ingredients are well separated by 7.3 minutes approximately.

Table - 1. Result of linearity study

Ingredients		Concentration (mg ml^{-1})					Correlation Coefficient
		0.005	*0.01*	*0.015*	*0.02*	*0.025*	
Peak Area	**Orotic Acid**	176932	355069	530647	707815	893305	0.99998
	Folic Acid	140947	287994	429090	574608	720198	0.99995

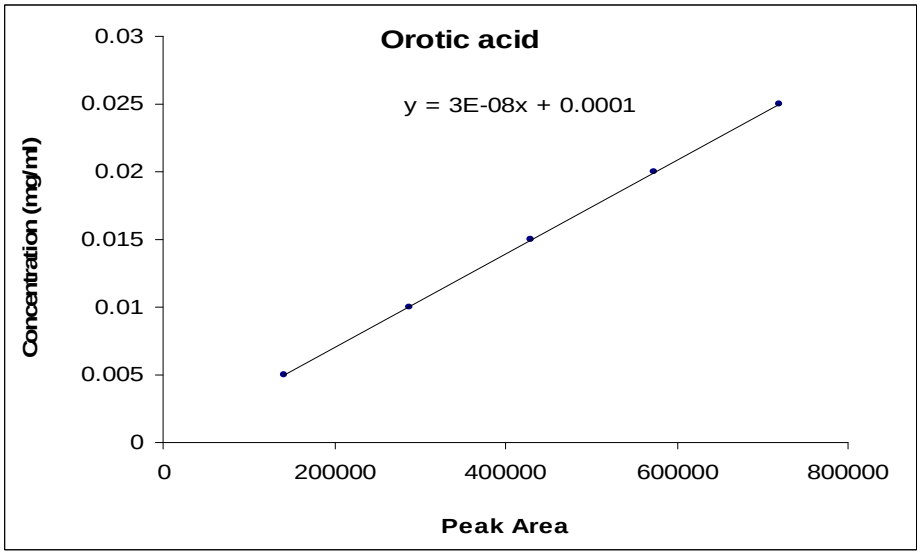

Fig 2. Linearity study of Orotic acid was carried out between peak area concentrations (mg ml^{-1}).

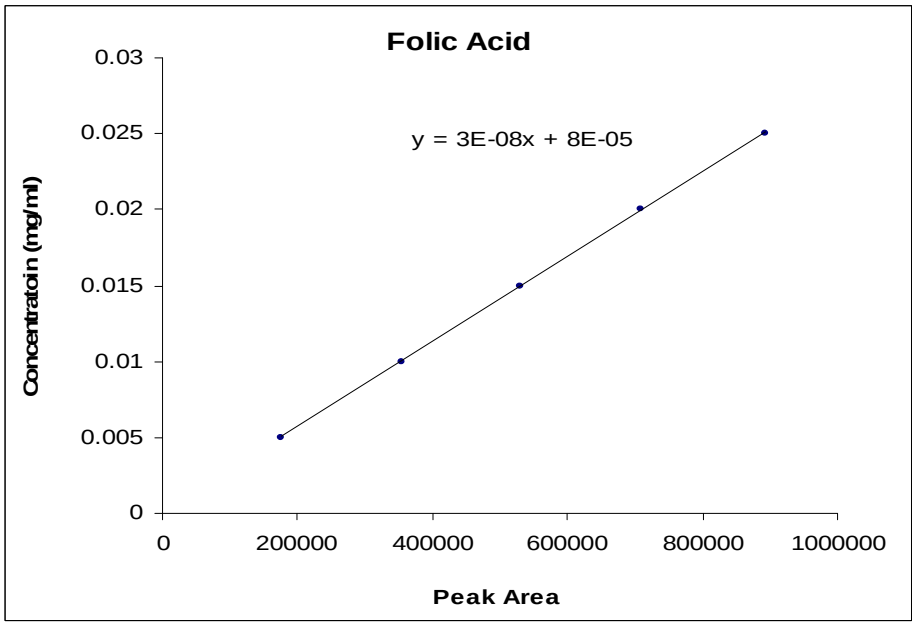

Fig. 3. Linearity study for Folic acid between Peak areas and concentration (mg ml^{-1}**)**

Recovery/ Accuracy

Recovery tests were performed by adding a known amount of each drug in syrup. Mean recoveries obtained during the spiking experiments were in the range of 98.31 to 99.72% for Orotic acid and 97.96 to 99.89 % for folic acid respectively, and these can be considered to be good recoveries.

Conclusion

As Orotic and folic acid are from vitamin family, and may occur in multivitamin dosage forms.

Methods for their individual testing were available, but time consuming when investigating qualitatively and quantitatively. Using this method one can determine both folic and Orotic acid simultaneously precisely and accurately. This method can be used for the routine analysis of the pharmaceutical dosage form in quality control and R&D laboratories for products of similar type and composition.

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