The Pharma Innovation Journal 2023; 12(5): 251-256

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(5): 251-256

www.thepharmajournal.com
Received: 19-04-2023
Accepted: 23-05-2023

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Impact of chromatographic techniques in determining folic acid: UV spectrometry and TLC chromatography

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Abstract
Folic acid, or vitamin B9, is one of the crucial vitamins. The body requires folic acid in a smaller amount, even though it plays an important role during pregnancy. This led to fortification in cereals and grains and folic acid supplementation was started. Several methods are available to determine folic acid in food samples and in drugs. UV spectrometry and TLC are very simple and convenient to use; there is no need for a skilled person. In UV spectrometry, folic acid standards were observed with phosphate buffer saline and water. The wavelength detected for phosphate buffer saline was 281nm and for water, 280nm. The fruits were also analyzed, apples and black dried grapes both fruits formed good chromatograms at 280nm and 281 nm, respectively. In Thin layer chromatography, folic acid standards with different combinations of solvents and chemicals formed bands, but in the majority, TLC formed smudges. UV and TLC support the synthesized form of folic acid, not the naturally occurring forms of folate. Phosphate buffer and water are good options for extracting, but there are still many more separation techniques needed.

Keywords: Chromatographic techniques, determining folic acid, UV spectrometry, TLC chromatography

1. Introduction
Folic acid is one of the water-soluble vitamins. Folic acid is an oxidized form of folate and contains only one residue. Folates are naturally present in fruits and vegetables; contain glutamic acid, pterin ring and para-aminobenzoic group. The water-soluble nature impacts the body's ability to observe folates or folic acid. The human body needs folic acid in lower concentrations, but once the body does not get enough of this nutrient, it causes deficiency during pregnancy. This cannot be synthesized in the body, so through diet, it should be consumed. The deficiency has major effects on the growth and development of a baby. Folic acid supplements and folic acid fortification through foods can prevent the deficiency. The World Health Organisation and Food and Drug Administration ruled for synthetic forms of folic acid fortified through cereals and grain supplements. Several different techniques were used to determine folic acid at a quantitative level. The different chromatographic techniques are high performance liquid chromatography [1, 2], liquid chromatography-mass spectrometry (electron spinning ionization positive and negative) [3, 4], voltammetry (Differential plus) [5, 6], biosensor [7], fluorescent paper-based sensor [8], electroanalytical sensor [9], inductively coupled plasma-optical emission spectrometry [10]. These techniques are sensitive in nature; selectivity spectrophotometry requires trained personnel to operate; the capital cost is high; and the procedure and sample treatment are time-consuming.
Ultraviolet spectrometry is one of the most simple, useful, and convenient methods for both qualitative and quantitative methods. This work focuses on the basic principals of Beer-Lambert's law. This will be widely used in biochemistry, mainly for determining species and biochemical processes. It also helps quantify the bacterial cell count and the amount of DNA and protein. The wide application of UV spectrometry used in pharmacopoeias. UV absorption covers the wavelengths 100-400 nm. There are three bands in UV: UVA (315-400 nm) long wave, applications coatings and adhesives. UVB (280-315 nm) medium wave applications are mainly disinfections. UVC (100-280 nm) near wave applications are mainly used as mercury lamps to inactivate microorganisms, and it have strong germicidal effects [11]. The advantages of using UV over the other techniques are higher sensitivity, a wide range of concentrations, linearity, use in gradient elution and a small quantity of sample [12, 13]. UV is used as a detector in high-performance liquid chromatography. Several studies have been done using UV to determine the folic acid in multivitamin drugs and folic acid supplements.

Thin layer chromatography is one of the affinity-based methods of chromatography. This is the simplest, fastest and easiest method compared to other chromatography techniques. This technique is used in both quantitative and qualitative analysis to separate and isolate the mixtures that are non-volatile.

This consists of

1. **Stationary phase:** A strip or plate coated with silica gel. This phase adsorbent should not be soluble in solvent and should not interact.
2. **Mobile phase:** Developing agent where selection of solvents is based on the separation of the adsorbent.

The advantages of TLC are that it is more convenient, a wide range of reagents are used to give visual detection, the procedure can be performed independently, the sample number will be higher, it requires a smaller amount of sample, the plates are disposable and flexibility in the choice of both stationary and mobile phases. In this study both UV and TLC were used to determine folic acid.

2. Materials and Methods

2.1 Sample collection: Fruit samples were bought from the local market. The standard HPLC graded folic acid, ammonia, propanol, ethanol, monosodium phosphate, acetonitrile, sodium hydroxide, petroleum ether and benzene were purchased from Sigma Aldrich.

2.2 Sample preparation for UV: Fruits like apple, wood apple, pomegranate, grapes, orange, dried black grapes, grapes (blue and green color) and papaya were peeled, homogenized and added to one set of 10 ml of phosphate buffer saline and another set of 10 ml of water. The samples were centrifuged at 4500 rpm for 10 minutes. The supernatant solution was filtered and used for UV detection.

2.3 Standard preparation for both UV and TLC: 1000 ppm concentrations were prepared for folic acid. The TLC concentration was diluted with 0.1 N NaOH. In UV spectrometry, folic acid is diluted with Phosphate buffer saline and water. The standard folic acid concentration was 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 µl.

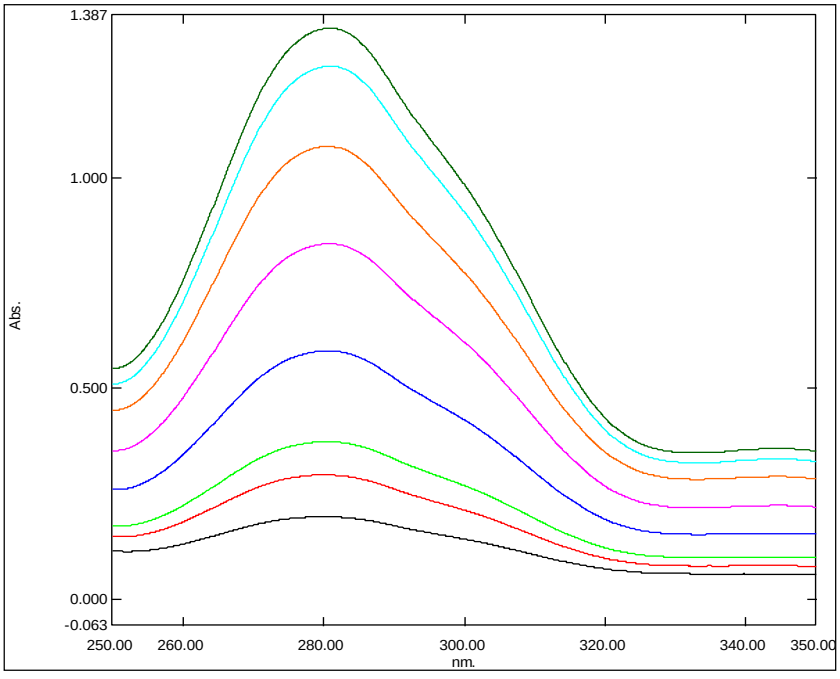
2.4 Solvents and chemical preparations for TLC: There were five different solvent combinations used.

1. **Ammonia:** Propanol: Ethanol (20:20:20 v/v),
2. **Monosodium Phosphate:** Acetonitrile (50:50, 80:20, 20:80, 70:30, 30:70),
3. **Ethanol:** propanol (60:40, 40:60), Sodium hydroxide with folic acid and methanol.
4. **Petroleum ether:** Benzene: Methanol.

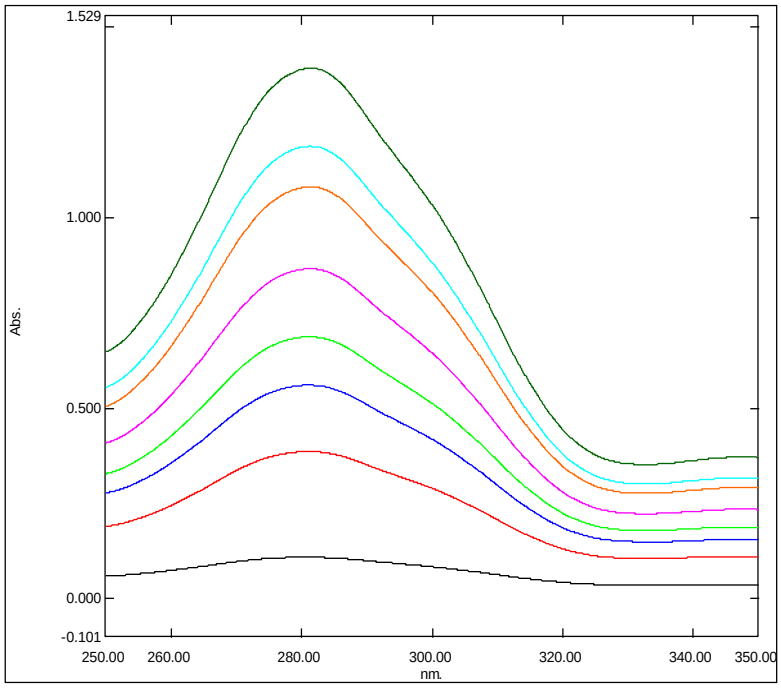
2.5 TLC preparation: The silica gel was activated by keeping it in hot air at 105 °C for 5 minutes. The silica gel was labelled and samples were placed near one end of the stationary phase. After the samples were dried. The stationary phase end is placed in a mobile phase containing two or more solvents, inside a closed chamber. Once the stationary phase reaches another end or an appropriate distance, it is removed and dried. The zones were observed in the UV light source chamber.

3. Results and Discussion

3.1 UV spectrometry: Folic acid standard run with phosphate buffer saline and water. Folic acid is naturally water soluble in nature. The wavelength observed for folic acid in phosphate buffer saline was 281nm and for water was 280nm. The chromatogram for the phosphate buffer saline and water was shown in Figure 1.0, with a linearity of R^2 values of 0.995 and 0.9809. The fruit sample was diluted with both water and phosphate buffer saline. Fruits with phosphate buffer saline extract showed positive results, but only dried black grapes and apple fruits formed chromatograms near 280 nm. The chromatogram formed from fruits with both phosphate buffer saline folic acid standard and water, folic acid standard is represented in Figure 2.0. The UV spectrometry studies were carried out at several pharmacies. The multivitamin drugs and folic acid supplements used to determine folic acid by using UV spectrometry [13, 14].

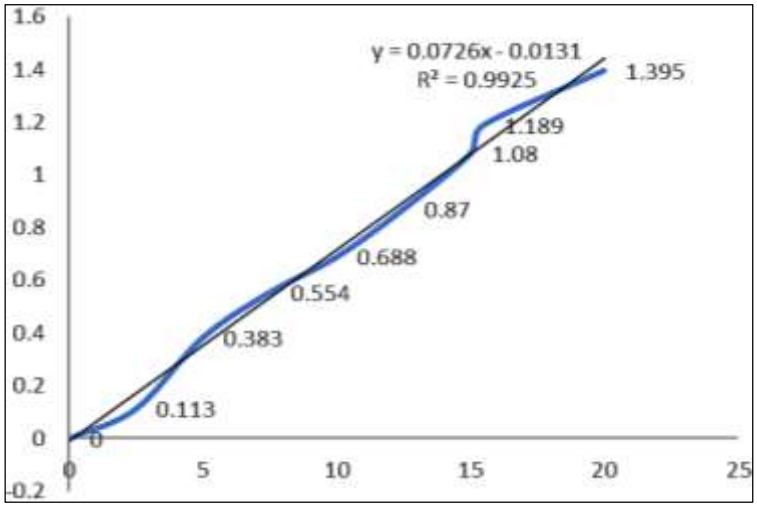


a) Water

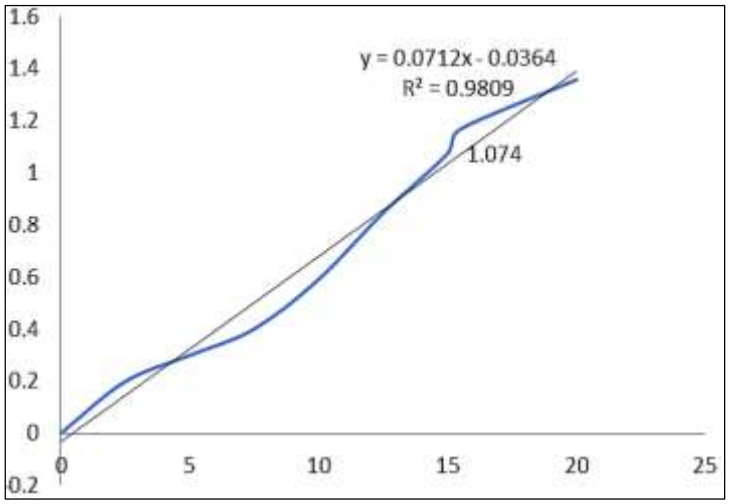


B) Phosphate buffer saline

Linearity for folic acid with Phosphate buffer saline and water.

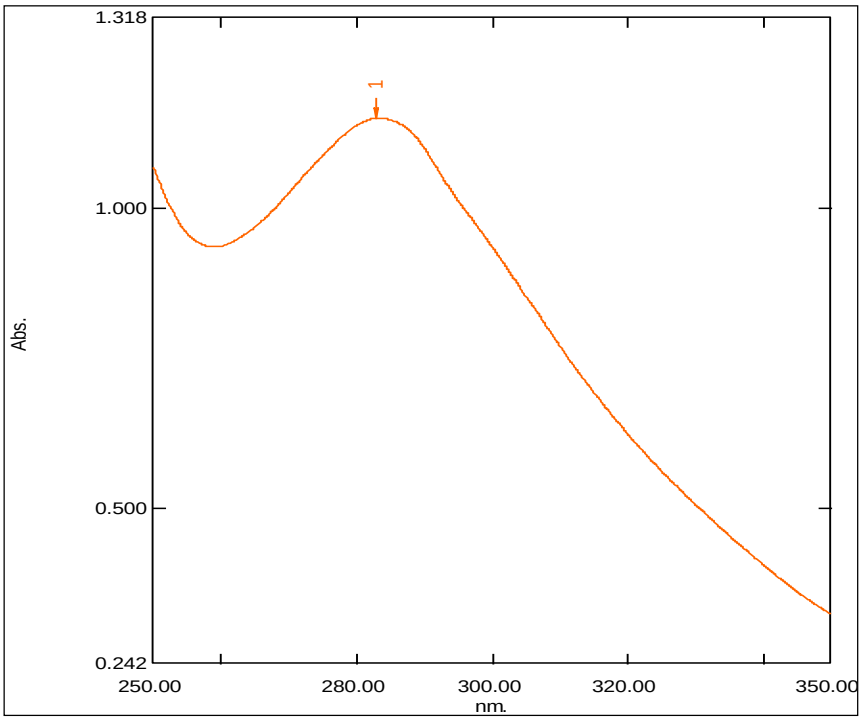


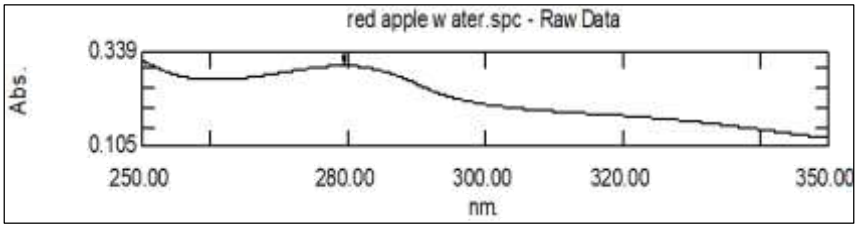
a) Phosphate buffer saline



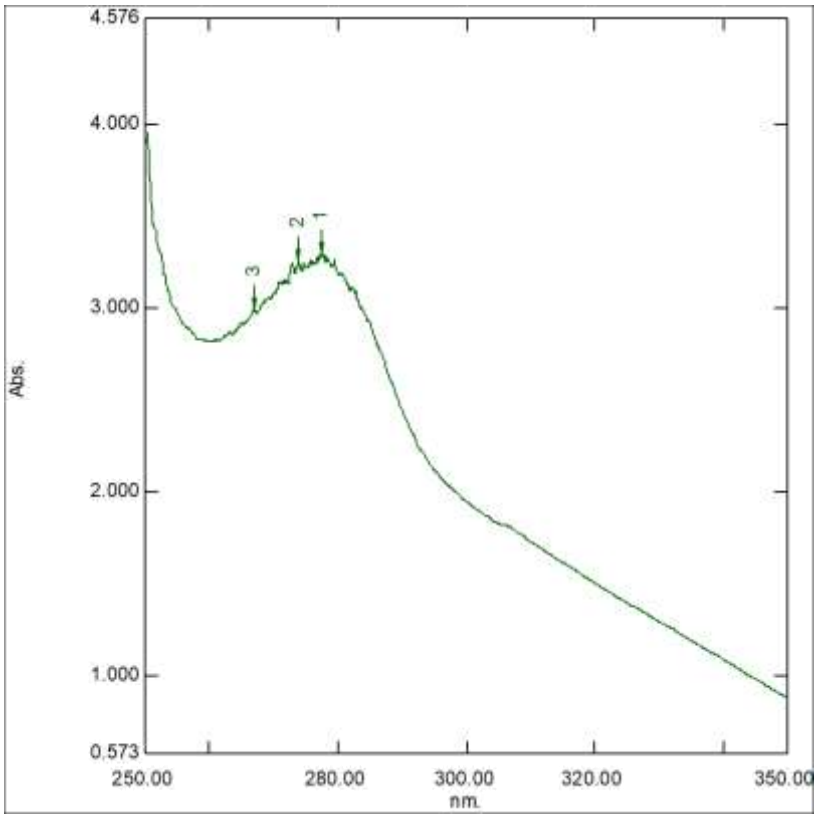
b) Water

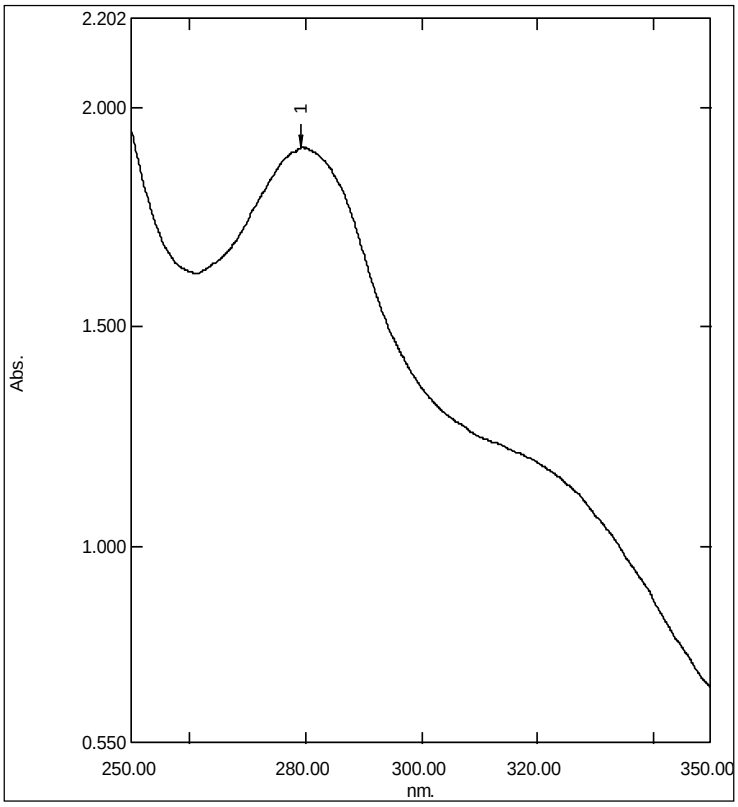
Fruits sample with water at 280nm chromatogram:



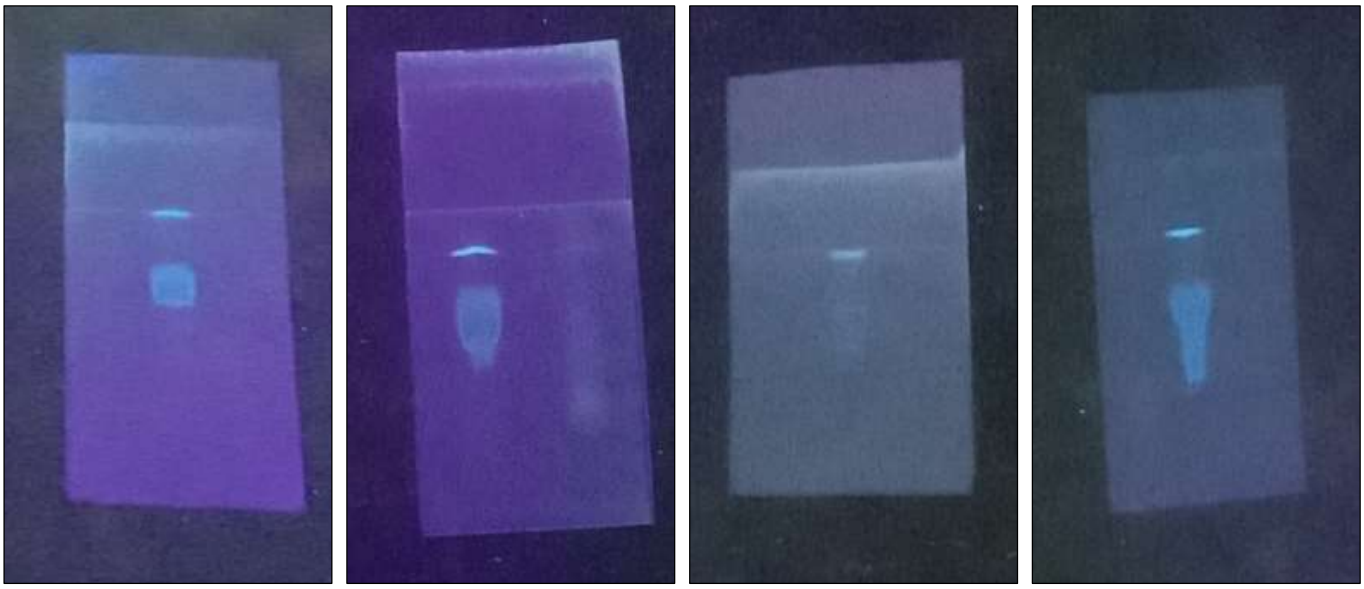


Fruits sample with Phosphate buffer saline at 281nm chromatogram





Thin layer chromatography under short and long UV wavelength



Ethanol: Propanol (60:40) Ethanol: Propanol (60:40) Methanol:0.1 N NaOH Methanol

Fig 1: Folic acid standard with Phosphate buffer saline and Water at 281nm and 280nm

3.2 Thin layer chromatography: In a thin layer, the first combination of ammonia: propanol: ethanol formed smudged lines, and monosodium phosphate: acetonitrile with different concentration levels formed straight lines of smudging. There was a band observed in ethanol: propanol (60:40), but (40:60) combination also formed bands but smudging also appeared. Folic acid diluted with 0.1 N NaOH and methanol formed good bands as compared to the ethanol and propanol combination. In the third combination, four different combinations were done. In that NaOH with folic acid, only folic acid, methanol with NaOH and folic acid and the final combination was methanol with folic acid. In this combination, methanol: folic acid: 0.1 N NaOH formed a good band compared to the other three combinations. The last combination was petroleum ether: benzene: methanol, which formed smudged.

4. Conclusion

The UV spectrometry and TLC methods are simple and easy compared to other techniques. UV spectrometry will suit the synthesized form of folic acid that was observed but this process is not suitable for the natural form of folate. There is

still scope to extract the natural form of folate. In TLC, several solvents and chemicals were used, but only two combinations formed bands with slight smudging. This will help further studies to use UV and TLC to determine the natural form of folate present in fruits.

5. Acknowledgement

The authors would like to express gratitude to the NIFTEM, Thanjavur, Tamil Nadu, India. Formerly (Indian Institute of Food Processing Technology) for constant support and facility.

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