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Full Length Research Article

INSIGHTS INTO FOLIC ACID MIXTURES COMPOUNDED WITH COMMERCIALLY AVAILABLE VITAMIN SYRUPS

*[1]Anslem F., [1]Oloyede R.B., [1]Kassim A.A., [1]Bashir A., [1]Yahaya Z.S.

[1]Department of Pharmaceutical and Medicinal Chemistry, Kaduna State University, Kaduna, Nigeria
[2]Department of Pharmaceutics and Industrial Pharmacy, Kaduna State University, Kaduna, Nigeria

*Corresponding Author Email Address: rukayyat01@gmail.com

ABSTRACT
Stability assessments are crucial in determining the shelf-life and quality of compounded pharmaceutical products. Folic acid, an essential B-vitamin, is widely used to prevent and treat folate deficiency-related conditions. However, because of patient-specific demands or customised dose requirements, compounded folic acid syrups are frequently used in pediatric patients. This study aims to examine the stability of compounded folic acid mixtures prepared with some commercially available vitamin syrups. Three commercially available vitamin syrups were utilized as compounding vehicles for folic acid tablets, ensuring a representative sample of products commonly used in compounding practices in hospitals when conventional compounding vehicles are not available. The compounded folic acid mixtures were stored under ambient (21- 25 ºC) and refrigerated conditions (2-8 ºC), to simulate real-life scenarios, and tested using parameters such as, organoleptic characteristics, sedimentation rate, pH, microbial growth and drug content determination at day 7, 14 and 30. The extemporaneous preparations of the compounded folic acid in vitamin syrups generally maintained their physical characteristics over the 30-day study period. However, there was either decrease in folic acid concentration or microbial contamination of the preparations at day 7. Therefore, folic acid compounded in vitamin syrups are generally stable for less than seven days. The data obtained will aid healthcare professionals and compounding pharmacies in decision making concerning the choice of vehicle, compounding practices and strategies to ensure the preparation of wholesome extemporaneous products.

Keywords: Compounding, Extemporaneous, Stability, Folic acid, Vitamin syrups

INTRODUCTION

The stability of an oral suspension is important in ensuring the efficacy and safety of the drug. A suspension should not only have its active ingredient at effective concentrations throughout the period of the dosage regimen but should also be physically stable and free from chemical and microbial impurities/ contamination (Praneta *et al*., 2019). The chemical and microbial stability of a pharmaceutical mixture is of great importance because it becomes less effective and safe due to degradation of active pharmaceutical ingredients (API), production of toxic substances and contamination with microorganisms that can cause infections, which can be hazardous to human health, especially in children who are the major consumers of extemporaneous (compounded) mixtures (Matovu *et al*., 2023). Drugs are compounded as suspensions in order to meet the needs of special patients such as pediatric, geriatric and tube-fed patients (Cutaia *et al*., 2018; Allen, 2019). Studies have reported variations in pH, viscosity, particle size distribution, API content and microbial contamination of extemporaneous preparations, and suggested that such preparations were suitable for use only for a specified period of time, thus leading to the introduction of beyond-use-dates (BUD), after which the preparation cannot be consumed (Alfred-Ugbenbo *et al*., 2017; Silva *et al*., 2020; Soares Rodrigues Costa *et al*., 2020). However, the bioavailability and palatability of such preparations can still not be proven, even though they have been found to be physically, chemically or microbiologically stable, until they are supported by evidenced based data (Belayneh and Tessema, 2021). In some hospitals in Nigeria, medicines formulated as solid dosage forms may be compounded into liquid mixtures or suspensions using commercial vitamin syrups in the pharmacy. This method of compounding is gaining popularity due to: convenience of using the fixed pre-determined volume of the vitamin syrups, the unavailability of unmedicated oral vehicles and materials for compounding (Orubu *et al*., 2021). However, it is important that the drug and vehicle utilized for compounding be compatible to produce a product that maintains its efficacy and safety (Praneta *et al*., 2019). Excipients present in tablet formulations and vitamin syrups can change the pH, viscosity, and other characteristics of the dispersion medium in which they are compounded, which can lead to emergence of undesirable products and/or decrease in API content, thus compromising the safety and efficacy of the extemporaneous mixture. (Alfred-Ugbenbo *et al*., 2017). Therefore, it is important to study the stability of commonly compounded suspensions in order to determine their BUD in commonly used commercially available vitamin syrups. The aim of this research is to examine the stability of compounded folic acid mixtures prepared with some commercially available vitamin syrups, which are commonly used in Nigerian hospitals.

MATERIALS AND METHOD

Materials

Folic acid tablets (Emzor; Batch no.- 2865X; Expiry date- June, 2021), vitamin C syrups (Emzor; Batch no.- L453Z; Expiry date- May, 2023), vitamin B Complex syrups (Emzor; Batch no.- L599Y; Expiry date- June, 2022), multivitamin syrups (Emzor; Batch no.- L329Z; Expiry date- April, 2023), phosphate buffer 0.1M, distilled water, Eosin-methylene blue (EMB) agar (Alpha Biosciences), Sabouraud dextrose agar (Alpha Biosciences), Nutrient Broth, pH meter (Hanna instruments, USA), UV spectrophotometer (UV 752, PEC Medical, USA)

Methods

Preparation of compounded folic acid mixtures

Different batches of folic acid mixtures (1mg/ml) were made using different vehicles (vitamin C syrup, vitamin B complex syrup, and

multivitamin syrup). In each case, 20 tablets containing 5mg of folic acid were pulverized in a washed and dried mortar into a fine powder, then 20 ml of the vehicle was added and mixed using a pestle to form a smooth paste. An additional 20 ml of the vehicle was added and mixed to form a slurry of pourable consistency. The slurry was transferred into a pre-calibrated, labeled amber bottle, in which the vitamin syrups were previously stored. The mortar was rinsed with more vehicle and added to the bottle. The remaining vehicle was added to make up the volume (100 ml) and then shaken vigorously for 2 min.

Sedimentation test
A volume equivalent to 50 ml of the compounded batches was measured, placed in a volumetric flask, covered and kept for 30 days and after the 30-day period, the sample was shaken vigorously and allowed to stand for 5 minutes (Alfred-Ugbenbo *et al*., 2017). The volume of sedimentation was observed and recorded.

Organoleptic test
The colour, smell, taste and/ or evolution of gas of each batch was examined on day 0, 7, 14 and 30.

pH Test
The pH of the batches was determined on day 0, 7, 14 and 30.

Assay of compounded folic acid syrup

The assay was carried out using the method described by Musa *et al*., (2022). A quantity (1 ml) of each batch was made up to 10 ml with freshly prepared phosphate buffer. It was then shaken using a rotary shaker for 20 minutes, and centrifuged for 5 minutes. A quantity (0.9 ml) of the clear solution obtained after centrifugation was taken and made up to 25 ml with phosphate buffer. The resulting solution was assayed using a UV spectrophotometer at 282.5 nm. The results were observed and recorded for each of the samples on day 0, 7, 14 and 30.

Microbial Analysis
Preliminary microbial analysis was carried out on the stored compounded folic acid mixtures. The batches were examined for the presence of gram-negative bacteria (e.g. *E. coli*) using Eosin-methylene blue (EMB) agar; dermatophytes and yeast species using the Sabouraud dextrose agar (SDA). The plates were prepared using the pour plate method. A quantity of the compounded folic acid syrup was sterilized and added to the pre-prepared sterilized nutrient broth and a cotton bud swab was dipped into the mix of nutrient broth and sample and the swab was streaked on the already set agar in the petri dish. The EMB agar were inverted and incubated at 35 °C for 24 hours and the samples in SDA agar were also inverted and incubated at 25 °C for 24hrs. Presence of growth was observed after one day. The procedure was repeated on each of the stored samples on day 0, 7, 14 and 30 and results were observed and recorded (Tortorello, 1999; Weagant and Feng, 2002; USP, 2009).

RESULTS
Sedimentation tests of the compounded folic acid mixtures showed that there was minimal to no sedimentation after dispersal on day 30 (Table 1).

Table 1: Sedimentation test of compounded folic acid mixtures

Vehicle (Syrups)	Volume of sample (ml)	Volume of sediment (ml)
Vitamin C	50.0	0.1
Vitamin B complex	50.0	0.0
Multivitamin	50.0	0.0

Organoleptic tests (Table 2) of the compounded folic acid mixtures using normal human perception of taste, smell, colour and sight showed that there were no physical changes in the mixtures after the 30-day period. However, there was evolution of gas in mixtures compounded in vitamin B complex and multivitamin syrups from day 14 and 30 respectively.

Table 2 Organoleptic tests of compounded folic acid mixtures

Vehicle (Syrup)	Storage time	Storage temperature (**ºC**)	Change in taste	Change in smell	Change in colour	Evolution of gas
Vitamin C	Day 0	NA	-	-	-	-
	Day 7	2- 8	-	-	-	-
		21- 25	-	-	-	-
	Day 14	2- 8	-	-	-	-
		21- 25	-	-	-	-
	Day 30	2- 8	-	-	-	-
		21- 25	-	-	-	-
Vitamin B complex	Day 0	NA	-	-	-	-
	Day 7	2- 8	-	-	-	-
		21- 25	-	-	-	-
	Day 14	2- 8	-	-	-	-
		21- 25	-	-	-	+
	Day 30	2- 8	-	-	-	-
		21- 25	-	-	-	+
Multivitamin	Day 0	NA	-	-	-	-
	Day 7	2- 8	-	-	-	-
		21- 25	-	-	-	-

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	Day 14	2- 8	-	-	-	-
		21- 25	-	-	-	-
	Day 30	2- 8	-	-	-	-
		21- 25	-	-	-	+

Key: No change (-), Change (+)
There were slight decreases in the pH of compounded folic acid mixtures after the 30-day period as shown in Table 3.

Table 3 pH of compounded folic acid mixtures

	Storage temperature (ºC)	pH Day 0	Day 7	Day 14	Day 30
Vehicle (Syrups)					
Vitamin C	2- 8	3.1	3.0	2.5	2.7
	21- 25		3.3	3.0	2.6
Vitamin B complex	2- 8	5.4	5.4	5.3	4.8
	21- 25		5.4	5.3	4.8
Multivitamin	2- 8	4.1	4.0	3.6	3.5
	21- 25		4.0	3.6	3.5

Quantitative determination of folic acid in the compounded mixtures (Table 4) showed that there was a decrease in concentration over time except for the mixtures compounded in vitamin C syrup.

Table 4 Assay of compounded folic acid mixtures

	Storage temperature (ºC)	Percentage recovery (%) Day 0	Day 7	Day 14	Day 30
Vehicle (Syrups)					
Vitamin C	2- 8	99.9	99.9	99.9	99.9
	21- 25		99.9	99.9	99.9
Vitamin B complex	2- 8	59.0	37.0	37.0	ND
	21- 25		59.0	ND	ND
Multivitamin	2- 8	99.2	99.2	13.5	ND
	21- 25		4.3	ND	ND

Key: ND-Not detectable

Gram-negative bacteria test of the compounded folic acid mixtures showed no growth after the 30-day period (Table 5).

Table 5 Eosin-methylene blue (EMB) test for detection of Gram-negative bacteria (*E. coli)* in compounded folic acid mixtures

Vehicle (Syrup)	Storage time	Storage temperature (ºC)	Growth	Number of colonies
Vitamin C	Day 0	NA	-	-
	Day 7	2- 8	-	-
		21- 25	-	-
	Day 14	2- 8	-	-
		21- 25	-	-
	Day 30	2- 8	-	-
		21- 25	-	-
Vitamin B complex	Day 0	NA	-	-
	Day 7	2- 8	-	-
		21- 25	-	-
	Day 14	2- 8	-	-
		21- 25	-	-
	Day 30	2- 8	-	-
		21- 25	-	-
Multivitamin	Day 0	NA	-	-
	Day 7	2- 8	-	-
		21- 25	-	-
	Day 14	2- 8	-	-
		21- 25	-	-
	Day 30	2- 8	-	-

		21- 25	-	-

Key: No growth (-), Growth (+)

Total combined yeast test of the compounded folic acid mixtures showed that folic acid syrups compounded in vitamin C and multivitamin showed growth of yeast at day 30, when stored at room temperature, while folic acid syrups compounded with vitamin B complex showed growth of yeast as early as the 7th day when stored at room temperature (Table 6).

Table 6 Total combined yeast test of compounded folic acid mixtures

Vehicle (Syrup)	Storage time	Storage temperature (ºC)	Growth	Number of colonies
Vitamin C	Day 0	NA	-	-
	Day 7	2- 8	-	-
		21- 25	-	-
	Day 14	2- 8	-	-
		21- 25	-	-
	Day 30	2- 8	-	-
		21- 25	+	3
Vitamin B complex	Day 0	NA	-	-
	Day 7	2- 8	-	-
		21- 25	+	1
	Day 14	2- 8	-	-
		21- 25	+	2
	Day 30	2- 8	+	1
		21- 25	+	2
Multivitamin	Day 0	NA	-	-
	Day 7	2- 8	-	-
		21- 25	-	-
	Day 14	2- 8	-	-
		21- 25	-	-
	Day 30	2- 8	-	-
		21- 25	+	2

KEY: No growth (-), Growth (+)

DISCUSSION

Sedimentation tests conducted on the compounded batches showed that the formulations maintained their physical stability over the 30-day period. The batches formulated using vitamin C syrup exhibited little sedimentation, while no sedimentation was observed in the batches made using vitamin B-complex and multivitamin syrup indicating that the formulations possessed controlled flocculation and satisfactory sedimentation characteristics. An ideal suspension will stay suspended for a long time, and resuspension will only require a small amount of shaking (Naveed *et al*., 2017). However, a formulation with poor sedimentation characteristics will contain a dispersed phase that sediment too quickly before a consistent dose can be withdrawn (Yahaya *et al*., 2023). There was slight discharge of gas in the batch formulated using vitamin B complex and multivitamin syrups on the 14th and 30th day respectively. Evolution of gas in a suspension can be due to formation of oxygen or carbon dioxide, which may be due to presence of gas-producing bacteria or occurrence of a chemical reaction (Silva *et al*., 2020; Qu *et al*., 2022). The initial pH of the folic acid syrups compounded in the three vitamin syrups were within the pH range of 3-6 reported by Bamise *et al*. (2014) as being the optimal pH range of stability of liquid formulations. A gradual increase in acidity was observed over the 30-day period, but it was only the batch compounded with vitamin C that showed a decrease in pH lower than the required lower pH limit of 3. Absorption of most drugs is facilitated in an acidic medium, thus it is important to maintain a slight acidic pH of liquid formulations (Bamise *et al*., 2014). The drug content of the mixtures prepared with vitamin B complex and multivitamin decreased over time, ultimately indicating instability in these mediums just before the 7th day. However, the assay results of the batches formulated with vitamin C syrup, showed no change in the amount of active ingredient over the 30-day period. There is a possibility of the occurrence of a drug-drug chemical interaction between folic acid and vitamin C, which led to the formation of degradation product(s), reflected as elevated folic acid concentrations in the assay results. This is consistent with a previous study carried out on the degradation study of metronidazole by UV spectroscopy, where elevated metronidazole concentration was an indication of formation of degradation products (Naveed *et al*., 2014). Microbial studies of the folic acid mixtures compounded in the three vitamin syrups, showed that there was no contamination with gram negative bacteria over the 30-day period. This is expected as gram negative bacteria do not fare well in pH less than 4 (Suehr *et al*., 2020). Folic acid mixtures compounded in vitamin C and multivitamin showed growth of yeast only at the 30th day, when stored at room temperature. However, folic acid syrups compounded with vitamin B complex showed growth of yeast as early as the 7th day when stored at room temperature. It has been reported that, most yeast cells thrive in acidic medium especially at pH 5.5, and at room temperatures of 25 – 30 ºC (Salas-Navarrete *et al*., 2023). The pH of the formulations compounded with vitamin B complex syrup had an average pH of 5 which explains why yeast cells were viable as early as the 7th day.

Compounded formulations are regarded as being chemically, physically and microbiologically stable when they contain ≥ 90 %

of the original amount of the active ingredient, there is no apparent change in physical property and there is no microbial growth (Belayneh and Tessema, 2021). Alfred-Ugbenbo *et al*. (2017) and Belayneh and Tessema (2021) also reported that most extemporaneous formulations were stable at ICH recommended storage conditions and duration, but only when compounded in Syrup USP or other standardized compounding vehicles. It is important to state that none of the previously reported studies used vitamin syrups in preparation of extemporaneous formulations. Results from our study has not fulfilled all of the stated conditions of stability stipulated by Belayneh and Tessema (2021), therefore the compounding of folic acid in these vitamin syrups should be discouraged. It is recommended that clinical pharmacists develop stable and palatable suspending vehicles, using locally available and cheap materials, for use in the hospital setting, which will also the guarantee the stability of extemporaneous preparations over an appreciable period of time, similar to what was reported in a previous research work, where an oral suspending agent was developed and used to produce a palatable and sugar-free formula as a compounding vehicle used in hospitals, which maintained the physical characteristics and potency of the fluconazole 50 mg/ml for a considerably longer period of time (Kendice *et al*., 2018).

Conclusion and Recommendations

The results of our investigations revealed that there were notable microbial and chemical changes in the compounded mixtures, and these changes raise concerns regarding the stability, efficacy, and safety of the compounded mixtures over time. None of the three vitamin syrups used as vehicles for compounding had optimum stability during the first post-storage test at day 7. Hence, it is recommended to reconsider the compounding of folic acid in these vitamin syrups due to their compromised stability. Further research is necessary to identify the underlying causes of these changes in order to explore possible strategies to enhance stability and to develop cheap and palatable compounding vehicles which will guarantee the stability of extemporaneous preparations over an appreciable period of time.

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