

## Brief communication (Original)

# Physical, chemical, and microbiological stability of extemporaneous furosemide suspensions

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**Background:** Furosemide is a potent diuretic used in treatment of oedematous states associated with cardiac, renal, and hepatic failure and the treatment of hypertension. In Thailand, no liquid formulation of furosemide is commercially available for pediatric administration and for adult who cannot swallow furosemide tablets.

**Objective:** Prepare extemporaneous furosemide suspensions from commercial furosemide tablets using two compounded suspending vehicles, and determine the physical, chemical, and microbiological stability of these preparations.

**Methods:** Two formulations of extemporaneous furosemide suspensions were prepared from commercially available furosemide 40-mg tablets using two compounded suspending vehicles. The final concentration of furosemide in each formulation was 2 mg/mL. Three samples of each formulation were stored in glass bottles protected from light, and kept at three controlled temperature,  $4\pm 2^\circ\text{C}$ , room temperature ( $30\pm 2^\circ\text{C}$ ), and  $45^\circ\text{C}$ . A sample was removed from each bottle immediately after preparation and at 7, 14, 30, 45, and 60 days. The stability-indicating high-performance liquid chromatography was used to analyze for furosemide. The pH was measured. The physical and microbiological properties of these formulations were evaluated after storage for two months. The stability of furosemide suspensions was determined by calculating the percentage of the initial concentration remaining on each test day. Stability was defined as retention of at least 90% of the initial concentration.

**Results:** At least 93% of the initial furosemide concentration remained in both compounded furosemide suspensions for up to 60 days. There were no substantial changes in the appearance (color and consistency) or odor of both formulations. The pH values of both formulations kept at  $4\pm 2^\circ\text{C}$  increased slightly, while the pH values of both formulations kept at  $45\pm 2^\circ\text{C}$  decreased significantly compared with the initial pH value of both formulations. Both formulations maintained microbiologic stability for 60 days.

**Conclusion:** Extemporaneously compounded furosemide suspensions, 2 mg/mL, were stable for at least 60 days when stored in glass bottles protected from light at three controlled temperatures. These compounded furosemide suspensions are better suited for administration to children and adults who cannot swallow furosemide tablets. They may provide an alternative in situations where the marketed suspension is unavailable.

**Keywords:** Chemical, extemporaneous, furosemide, microbiological, physical, stability, suspension

Furosemide, 4-chloro-N-furfuryl-5-sulfamoyl-anthranilic acid, is a potent diuretic used in treatment of oedematous states associated with cardiac, renal, and hepatic failure and the treatment of hypertension [1]. It is a white to slightly yellow crystalline powder, odorless, practically insoluble in water, sparingly soluble in ethanol, and soluble in acetone.

In many countries, furosemide is commercially available as tablets, injection, and oral solutions. In Thailand, it is available as 40-mg and 500-mg tablets, and as 5 mg/mL and 10 mg/mL injection. It is not available commercially in an oral liquid dosage form. An oral liquid preparation would be advantageous for patients, especially pediatric and adult patients, who are unable to swallow tablets or who must receive medications by nasogastric or gastrostomy tube [2]. It would allow for more accurate dosing for patients, especially children, who may require doses that cannot easily be provided with 40-mg tablet [3].

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Tablets and capsules are generally unsuitable for administration to children aged less than four years old. The suitable strength of tablet might not be available for use in older children. The range of commercially available pediatric oral liquids and tablets is limited due to the low market value perceived by the industrial product developers and manufacturers.

In this study, we formulated an extemporaneous oral suspension of furosemide from commercially available 40-mg furosemide tablets. Two compounded-suspending vehicles by mean of a procedure simple enough to allow the suspension to be reproduced extemporaneously in hospital pharmacy were used. The short-term physical, chemical, and microbiological stability of these formulations over 60-days period were determined.

## Materials and methods

### Materials

Furosemide, analytical grade (Sigma-Aldrich, St. Louis, USA), furosemide tablets (FURETIC 40-mg tablet, Siam Pharmaceutical Co, Bangkok, Thailand), methanol, HPLC grade (Fluka, New York, USA), sodium hydroxide (Fluka, New York, USA), potassium dihydrogen phosphate (Fluka, New York, USA), citric acid (Fluka, New York, USA), Sabouraud dextrose agar (Difco, Kansas, USA), soybean-casein digest agar (Difco, Kansas, USA), and MacConkey agar (Difco, Kansas, USA). The other chemicals were USP or BP grade.

### Preparation of furosemide (2 mg/mL) suspensions

Two formulations, formulation 1 and formulation 2, of furosemide suspensions in the concentration of 2 mg/ml were prepared using compounded suspending vehicle 1 and compounded suspending vehicle 2, respectively. Compounded suspending vehicle 1 consisted of xanthan gum 0.25 g, glycerin 10 mL, syrup USP 50 mL, parabens concentrate 1 mL, and purified water to 100 mL. Compounded suspending vehicle 2 consisted of sodium carboxymethylcellulose 0.1 g, glycerin 5 mL, sorbitol solution 20 mL, syrup USP 50 mL, parabens concentrate 1 mL, and purified water to 100 mL. To prepare the suspensions, five furosemide 40-mg tablets were crushed and ground to a fine powder using a mortar and pestle. Then, a small amount of compounded suspending vehicle was added and levigated into a smooth paste. The 75 mL of compounded suspending vehicle was added and mixed.

The mixture was transferred to a graduate cylinder. The remainder of the compounded suspending vehicle was added by repeatedly rinsing the mortar and pestle with small amounts to make a final volume of 100 mL. Nine identical samples were prepared and filled in glass bottles. The three bottles each were stored either at three controlled temperature,  $4\pm2^{\circ}\text{C}$ , room temperature ( $30\pm2^{\circ}\text{C}$ ), and  $45^{\circ}\text{C}$  in the absence of light.

### Chemical stability study

The suspension was vigorously shaken by hand for 30 seconds. Then, from each bottle, a 100  $\mu\text{L}$  sample was drawn from approximate center of the remaining liquid and transferred into a 10-mL volumetric flask. Then, 2 mL of 0.1 N NaOH was added and sonicated for 10 minutes. The mixture was diluted with 50% methanol to 10 mL and mixed for one minute. The mixture was centrifuged at 4,000 rpm for 10 minutes. The supernatant was assayed for furosemide in duplicate by high performance liquid chromatography immediately after preparation and after 7, 14, 30, 45, and 60 days. The apparent pH was determined initially and at 30 and 60 days using a pH meter. The suspensions were examined at each sampling time for any change in appearance or odor.

### High performance liquid chromatographic (HPLC) analysis

The HPLC technique was used for analysis of furosemide concentration in the preparation. The technique was modified based on the method from Mills et al. [4]. The reverse phase HPLC (Waters, Milford, USA) in Waters C18 column (10  $\mu\text{m}$ , 3.9x300 mm ID) was used. The mobile phase consisted of potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ): methanol (70:30) and citric acid solution to adjust pH to 5.5. The flow rate was 1 mL/minute. Detection wavelength was 235 nm. Twenty L of each sample was assayed in duplicate. The drug concentrations were calculated by comparing peak area of the samples with a standard curve.

### Preparation of standard solution and standard curve

On each day of sample analysis, a 50  $\mu\text{g/mL}$  stock solution of analytical grade furosemide was prepared by accurately weighing furosemide 5 mg, dissolving this drug in 20 mL of 0.1 N NaOH, sonicating for 10 minutes, and diluting to 100 mL with 50% methanol.

Standard samples of furosemide were prepared by diluting appropriate volumes of the stock solution with 50% methanol to obtain concentrations of 10, 15, 20, 25, and 30 µg/mL. The standard curve ( $n=3$ ) was constructed on each day by plotting the peak area of furosemide against the furosemide concentration and was used for calculating the drug concentration of the sample. The standard curve was linear ( $r^2 > 0.999$ ) over the working range of concentrations. Three furosemide concentrations at 10, 20, and 30 µg/mL were assayed in triplicate. The inter-day and intra-day coefficient of variation for furosemide assay were 1.03% and 1.22%, respectively.

#### **Microbiological stability**

The total aerobic bacterial count was performed by using the standard plate method. Ten mL of sample was diluted with pH 7.2 phosphate buffer saline containing polysorbate 20, 4.0% to obtain 1:10 dilution. The sample was further diluted with the same diluent so that one mL will be expected to contain 30 and 300 colonies. One mL of the final dilution was pipetted onto each of two sterile Petri dishes and promptly added 20 mL of soybean-casein digest agar medium. The Petri dishes were incubated at 35°C for 48 hours. The plates were examined for growth. The colonies were counted and expressed as the average for the two plates in the terms of number of colonies per mL of the sample.

The total yeast and mold count was made using the plate method under the total aerobic bacterial count. We also used the same amount of Sabouraud dextrose agar medium, instead of soybean-casein digest medium and we incubated the Petri dishes at 25°C for five days.

For detection of *E. coli*, the samples were added with polysorbate 20, 4% to neutralize the preservative. One mL of the sample was added into each of two sterile Petri dishes. MacConkey agar medium 20 mL was added to each plate. The plates were incubated at 35°C for 48 hours. If no brick-red colonies were found, the samples meet the requirements of the test for absence of *E. coli*, even though there might be some surrounding zone of precipitated bile.

#### **Data analysis**

The initial concentration of furosemide was defined as 100% and sample concentrations were expressed as a percentage of the initial concentration remaining. The stability of the drug was defined as

not less than 90% of initial drug concentration remaining in the preparation. The significance of any difference between initial and final pH values was evaluated by a Student's t-test.

## **Results**

#### **Physical stability**

The 2 mg/mL of furosemide suspensions were formulated using commercially available tablets. The resulting preparations are readily dispersible suspension. There was no detectable change in physical characteristics such as color, odor, and no visible microbiological growth in any sample during the two months of storage at three controlled temperatures.

#### **Chemical stability**

The percentage of initial concentration remaining of furosemide from Formulation 1 and Formulation 2 was shown **Figures 1** and **2**, respectively.

The percentage of furosemide remaining in Formulation 1 after storage at  $4 \pm 2^\circ\text{C}$ , room temperature ( $30 \pm 2^\circ\text{C}$ ),  $45^\circ\text{C}$  for 60 days was  $99.23 \pm 1.12\%$ ,  $96.75 \pm 1.55\%$ , and  $93.13 \pm 1.86\%$ , respectively, while in Formulation 2, was  $99.52 \pm 1.45\%$ ,  $98.34 \pm 1.34\%$ , and  $96.12 \pm 1.97\%$ , respectively.

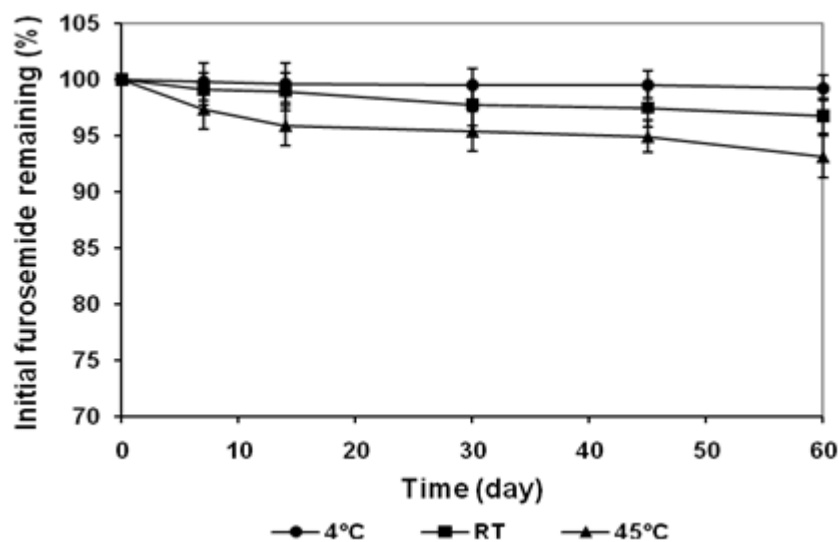
The pH value of each formulation after storage for two months was showed in **Table 1**. The initial apparent pH values of furosemide suspension in Formulation 1 and Formulation 2 were  $5.29 \pm 0.03$ , and  $5.28 \pm 0.04$ , respectively.

#### **Microbiological stability**

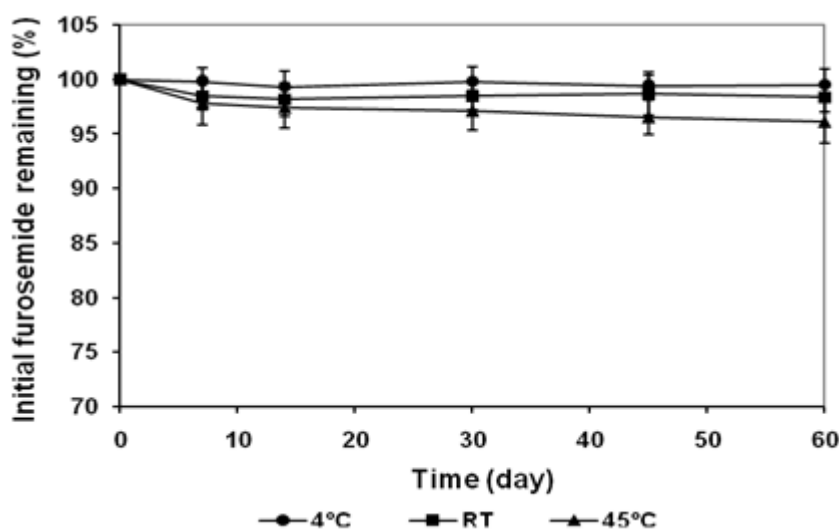
The total of bacterial, yeast and mold count of all samples was less than 10 colonies per g (cfu/g) of the sample. The suspected colony of *E. coli* was not found in MacConkey agar medium of any samples.

## **Discussion**

In many countries, furosemide oral solution is licensed as 20 mg/5 mL, 40 mg/5 mL and 50 mg/5 mL. In addition, furosemide sugar-free oral solution is one of cardiovascular drugs that commonly used in UK, but all strengths of these preparations contain ethanol and propylene glycol. Propylene glycol is considered less toxic than other glycols, but is estimated to be one-third as intoxicating as ethanol and its administration in significant volume was associated with adverse effects on the central nervous system [5], especially in neonates and children [6].



**Figure 1.** The percentage of initial concentration remaining of furosemide, Formulation 1, after storage at 4°C, room temperature and 45°C.



**Figure 2.** The percentage of initial concentration remaining of furosemide, Formulation 2, after storage at 4°C, room temperature and 45°C

**Table 1.** pH of extemporaneous furosemide suspensions after storage at 4°C, room temperature and 45°C

	Temperature	pH		
		0 day	30 day	60 day
Formulation 1	4°C		5.67±0.03	5.86±0.04
	RT	5.29±0.03	5.23±0.02	5.25±0.03
	45°C		4.79±0.03	4.53±0.03
Formulation 2	4°C		5.65±0.02	5.75±0.02
	RT	5.28±0.04	5.31±0.02	5.25±0.02
	45°C		5.00±0.04	4.62±0.03

In the present experiment, both formulations showed the physical stability throughout the two months of storage. Chemical stability was defined as not less than 90% of initial concentration remaining in the preparation. The percentage of furosemide remaining in both formulations after storage at three controlled temperatures was more than 90% as shown in **Figures 1** and **2**. These indicate that furosemide in the two compounded suspending vehicles is chemically stable under condition studied for 60 days. The final pH values of both formulations kept at room temperature were not significantly different ( $p > 0.05$ ) compared with the initial pH values. The pH values of both formulations kept at 4°C for two months were higher than the initial pH values. Based on pH rate profile of furosemide presented by Connors et al. [7], the furosemide might be more stable at higher pH value, and thus the increasing in pH values of both formulations during storage might not affect on furosemide stability. In contrast, the final pH values of both formulations kept at 45°C were significantly lower than the initial pH value ( $p < 0.05$ ). Both formulations kept at 45°C showed maximum furosemide degradation (about 7% and 4% decreasing of initial concentration remaining of furosemide from Formulation 1 and Formulation 2, respectively). This might probably be due to the acid-catalyzed hydrolysis in aqueous medium, which lead to the degradation product of furosemide, 4-chloro-5-sulfamoyl-anthranilic acid [8] that occurred and resulted in the decline of pH of final product. Therefore, the pH of formulation had impact on the chemical stability of furosemide.

Many factors reduce the effectiveness of the preservative including use of contaminated materials, chemical degradation, binding of preservative to suspending agents or tablet excipients, incorrect storage or unhygienic use of the final product [9]. Microbial growth in an oral liquid may cause foul odor, turbidity and adversely effect palatability and appearance. High titres of microorganisms may be hazardous to health especially in very young or immunocompromised patients. By-products of microbial metabolism may cause a change in the pH of the preparation and reduce the chemical stability or solubility of the drug. According to the United States Pharmacopeia 30/National Formulary 25 [10], acceptance criteria for non-sterile pharmaceutical products in the category of aqueous preparations for oral use based upon the total bacterial count and total

combined yeasts and mold count are not more than  $10^3$  cfu/g and 10 cfu/g of sample, respectively and *E. coli* must not be found in 1 g of the sample. Therefore, from the microbial stability test, both formulations meet the requirement of the test for total bacterial, yeast and mold count and for the absence of *E. coli*.

In conclusion, extemporaneous furosemide 2 mg/mL suspensions prepared from commercial tablets are stable for at least 60 days when stored at  $4 \pm 2^\circ\text{C}$ , room temperature ( $30 \pm 2^\circ\text{C}$ ) and 45°C, protected from light. These preparations may be an alternative to the administration of tablets for the pediatric patients or those who are unable to swallow solid dosage forms.

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### References

1. Murray MD, Haag KM, Black P.K, Hall SD, Brater DC. Variable furosemide absorption and poor predictability of response in elderly patients. *Pharmacotherapy*. 1997; 17:98-106.
2. Johnson CE, Wagner DS, Bussard WE. Stability of dolasetron in two liquid vehicles. *Am J Health Syst Pharm*. 2003; 60:2242-4.
3. Johnson CE, Price J, Hession JM. Stability of norfloxacin in an extemporaneously prepared oral liquid. *Am J Health Syst Pharm*. 2001; 58:577-9.
4. Mills CD, Whitworth C, Rybak LP, Henley CM. [Qualification of furosemide from serum and tissue using high-performance liquid chromatography](#). *J Chromatogr B*. 1997; 701:65-70.
5. [Arulanantham K, Genel M. Central nervous system toxicity associated with ingestion of propylene glycol](#). *J Pediatr*. 1978; 93:515-16.
6. Standing JF, Tuleu C. Paediatric formulations - getting to the heart of the problem. *Int J Pharm*. 2005; 300: 56-66.
7. Connors KA, Amidon GL, Stella VJ. Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists. 2nd ed. New York: John Wiley & Sons, 1986; p. 474-7.
8. Bundgaard H, Norgaard T, Nielson NM. Photodegradation and hydrolysis of furosemide and furosemide esters in aqueous solutions. *Int J Pharm*.

- 1988;42:217-24.
9. Long PF. Microbiological tests on oral pediatric medicines - requirements for an improved Pharmacopoeia monograph. *Ann Pharmacother.* 2006; 40:158-9.
  10. The United States Pharmacopeia 30 / National Formulary 25. Asian edition. Rockville: The United States Pharmaceutical Convention Inc, 2007; p. 1, 83-9, 585-8.