Stability Test in Extemporaneous Preparations: Furosemide Syrup, Spironolactone Suspension and Hydrochlorothiazide Suspension

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ABSTRACT

Objective: This study aimed to evaluated the stability of 3 extemporaneous oral liquid formulations such as 2 mg/ml syrup of furosemide, 2 mg/ml suspension of spironolactone and 10 mg/ml suspension of hydrochlorothiazide in storage conditions with container lid closure.

Methods: Three batches of 3 extemporaneous formulations were prepared. Each batch was stored in light-resistant containers of 60 ml with child-resistant caps. All of these preparations were stored in at the refrigerator (5 ± 3ºC). The physical, chemical and microbiological stability was evaluated for 1 year, 2 months and 1 month for furosemide syrup, spironolactone suspension and hydrochlorothiazide suspension, respectively.

Results: At least 90 percent of the initial furosemide concentration remained after 360 days in furosemide syrup. Spironolactone and Hydrochlorothiazide suspension declined to lower than 90 percent in 60 days and 30 days, respectively. There were no detectable changes in color, odor, pH and the microbiological tests were negative in all preparations.

Conclusion: In light-resistant containers and 5 ± 3ºC condition, furosemide syrup, spironolactone and hydrochlorothiazide suspension were stable for 360, 60 and 30 days, respectively. These results should be introduced into the hospital pharmacy to set the new expiration date of these 3 preparations which are commonly used in the pediatric patient.The original expiry date of the medicine was for 30 days after production. This study found that the drug lasted longer, so the expiry date of the drug should be longer.

Keywords: Stability, furosemide syrup, spironolactone suspension, hydrochlorothiazide suspension, extemporaneous preparation

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INTRODUCTION

Drugs used in pediatrics are often not available in suitable dosage forms, so liquid orals are easiest to administer for patients who cannot swallow or have swallowing difficulty of solid dosage forms including the pediatric patients. Modification of the solid dosage form is necessary. The solid dosage form which is commonly used in the pediatric or adult patient who cannot swallow is diuretic such as furosemide, spironolactone, and hydrochlorothiazide, so the extemporaneous preparation was prepared by pharmacists.

Furosomide, spironolactone and hydrochlorothiazide are potent diuretics used in treatment of oedematous states associated with cardiac, renal, and hepatic failure and the treatment of hypertension. Furosemide is commercially available as tablets, and injection. Furosemide is available in 40 mg and 500 mg tablets for oral adminstration, and 20 mg/2 ml and 250 mg/25 ml solution for injection in Siriraj Hospital. Spironolactone is available in 25 mg and 100 mg tablets, and hydrochlorothiazide is available in 25 mg and 50 mg tablets.[1,2] At Siriraj Hospital, 2 mg/ml syrup of furosemide, 2 mg/ml suspension of spironolactone and 10 mg/ml suspension of hydrochlorothiazide were available, but their stability may be problematic and the pharmacist has to determine the expiration date and the

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appropriate storage conditions of the extemporaneous preparations. After review of the literature, we found that most extemporaneous suspensions were stable for 30 or 60 or 90 days.[3-14] For example, furosemide liquid formulation was stable for 30 or 60 days in different diluents[9] Oral liquid furosemide repackaged in either polypropylene oral syringes or glass vials can be stored at 4ºC and 25ºC, respectively, for 180 days with less than 10% loss of potency.[15] The expiration date of prepared medicine in Siriraj Hospital has been set at the expiration of 30 days from the date of manufacture to reduce the risk that the medicine is not effective in the treatment and patients receiving deteriorated drugs. We are without information on the stability from pharmaceutical support. The expiration date of the medicine may last longer days or shorter days than that. In this study, we evaluated the physical, chemical, and microbiological stability of furosemide, spironolactone, and hydrochlorothiazide liquid formulations under refrigerated condition (5 ± 3ºC). We prepared 3 batches for all preparation for the study to compare the difference of batches and 5 samples per batch to measure the stability.[16,17]

MATERIALS AND METHODS

Three batches of 3 extemporaneous formulations were prepared. Each batch was stored in light-resistant containers (Polyethylene terephthalate bottle) of 60 ml with child-resistant caps. All of preparations were stored in at the refrigerator (5 ± 3ºC). The physical, chemical and microbiological stability was evaluated for 1 year, 2 months and 1 month of furosemide syrup, spironolactone suspension and hydrochlorothiazide suspension, respectively.

Stability evaluation

For all extemporaneous preparations we evaluated the stability by using 3 batches to compare the difference of batches and 5 samples per batch to measure the stability, at the above mentioned temperature and conditions.

Chemicals and reagents

Furosemide powder (Siam Pharmaceutical Co. Ltd., Bangkok), tablets containing 25 mg of spironolactone (Berlin Pharmaceutical Industry, Bangkok) and hydrochlorothiazide tablet 50 mg (Government Pharmaceutical Organization, Bangkok), furosemide syrup 2 mg/ml, spironolactone suspensions 2 mg/ml and hydrochlorothiazide suspension 10 mg/ml (Pharmaceutical Production, Siriraj Hospital) were used. Ultra pure water, methanol (HPLC grade), ammonium formate, potassium dihydrogen ortrophosphate, acetonitrile AR, grade, and formic acid (Darmstadt, Germany), were also used. All unspecified chemicals and reagents were reagent grade or equivalent (Merck, Darmstadt, Germany).

Chemical stability study

Preparation of standard solution and standard curve

On each day of sample analysis, a 1,000 µg/mL stock solution of analytical grade furosemide, spironolactone and hydrochlorothiazide was prepared by accurately weighing furosemide 10 mg by dissolving this drug in 20 mL of 50% methanol/mobile phase, spironolactone 25 mg tablet by dissolving this drug in 5 mL of methanol and diluting with 70% acetonitlile/mobile phase and hydrochlorothiazide tablet 50 mg by dissolving this drug in 5 mL of methanol: ultra pure water (45:55 V/V) sonicating for 10 minutes, and diluting with 50% methanol. Standard samples were prepared by diluting appropriate volumes of the stock solution with 50% methanol to obtain concentrations of 10, 25, 50, 100, 250 and 500 µg/mL. The standard curve (n=3) was constructed on each day by plotting the peak area against the concentration and was used for calculating the drug concentration of the sample. The standard curve was linear over the working range of concentrations. Three concentrations at 10, 25, and 250 µg/mL were assayed in triplicate for LOD and LOQ for repeatability and relative error.

Analytical method

The ultra performance liquid chromatography (UPLC) technique was used for analysis of furosemide, spironolactone and hydrochlorothiazide concentration in the preparation.

For furosemide, the reverse phase UPLC (Waters, Milford, USA) in Waters ACQUITY UPLC HILIC 2.1x10 mm. x 1.7 µm. was used. Column temperature was 30ºC with Mobile phase A: 0.1M HCOONH4 (pH 3.8 formic acid) B, and acetonitrile, flow rate 0.25 ml/min with detector: PDA at 235 nm and resolution 1.2 nm using injection 0.1 µl.

The furosemide syrup 2 mg/ml was vigorously shaken by hand for 30 seconds. Then, from each bottle, a 100 µL sample was drawn from approximate center of the remaining liquid and transferred into a 1-mL centrifuge tube. Then, 900 µl of 50% methanol / mobile phase was added and mixed for 5 minutes. The mixture was centrifuged at 2,500 rpm for 5 minutes. The supernatant was assayed for furosemide in triplicate by ultraperformance liquid chromatography immediately after preparation and after 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 270, 300, 330 and 360 days. This study used a sample of 250 bottles.

For spironolactone, the reverse phase UPLC (Waters, Milford, USA) in Waters ACQUITY UPLC HILIC 2.1x10 mm. x 1.7 µm. was used. Column temperature was 60ºC with mobile phase A: 0.1M $HCOONH_4$ (pH 3.8 formic acid) B and acetonitrile, flow rate 0.5 ml/min with detector PDA at 242 nm, resolution 1.2 nm using injection 0.1 µl.

The spironolactone suspensions 2 mg/ml was vigorously shaken by hand for 30 seconds. Then, from each bottle, a 100 µL sample was drawn from approximate center of the remaining liquid and transferred into a 1-mL centrifuge tube. Then, 900 µl of 70% Acetonitrile/ mobile phase was added and sonicated for 5 minutes. The mixture was centrifuged at 14,000 rpm for 5 minutes. The supernatant was assayed for spironolactone in triplicate by ultraperformance liquid chromatography immediately after preparation and after 8, 15, 22, 30, 36, 53, 60 and 67 days. This study used a sample of 135 bottles.

For hydrochlorothiazide, the reverse phase UPLC (Waters, Milford, USA) in Waters ACQUITY UPLC BEH C18, 2.1x10 mm. x 1.7 µm. was used. Column temperature was 35ºC with mobile phase A: 0.1M Potassium dihydrogen ortrophosphate (pH 3.8 formic acid) B and acetonitrile, flow rate 0.3 ml/min with detector: PDA at 273 nm, resolution 1.2 nm, using injection 0.1 µl.

The hydrochlorothiazide suspension 10 mg/ml was vigorously shaken by hand for 30 seconds. Then, from each bottle, a 100 µL sample was drawn from approximate center of the remaining liquid and transferred into a 1-mL centrifuge tube. Then, 900 µl of 0.01% HCl / Methanol 900 µl was added and sonicated for 5 minutes. The mixture was centrifuged at 14,000 rpm for 5 minutes. The supernatant was assayed for hydrochlorothiazide in triplicate by ultraperformance liquid chromatography immediately after preparation and after 7, 14, 21, 24, 27, 30, 32 and 34 days. This study used a sample of 135 bottles.

The samples were examined at each sampling time for any change in appearance or order.

Physical stability

For all preparations the pH and viscosity were tested and visual (color) observations were performed during stability. Physical stability was defined as the absence of either visible color or appearance changes.

Microbiological stability

The samples were subjected to microbiological evaluation. The criteria is the attributes of non-sterile pharmaceutical products which is set as total microbial count below 200 CFU/ml, total and absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella spp.*[18] Microbacterial examination was performed by the Department of Biology.

Data analysis

The initial concentration of all extemporaneous preparations was defined as 100%. We measured the concentration of active ingredients by UPLC as the scheduled time until the final volume or the percentage of active ingredient was less than 90% of initial drug concentration.[19]

RESULTS

Three batches of 3 extemporaneous preparations were prepared to compare the differences of each batch and 5 samples per batch. All of the solutions were kept in a refrigerator at 2-8°C. The criteria of this study was the concentration of active ingredients that was not less than 90% until the final volume and we determined from the minimum time duration that the active ingredient concentrations remained greater than 90% for 3 batches and 5 samples per batch.

Physical stability

The 2 mg/ml of furosemide syrup, spironolactone suspension 2 mg/ml and hydrochlorothiazide suspension 10 mg/ml were formulated using commercially available powder and tablets. The resulting preparations were readily dispersible syrup. There was no detectable change in physical characteristics such as color, odor, and no visible microbiological growth in any sample during the 360, 67 and 34 days of storage at controlled temperatures show in Table 1, 2 and 3, respectively.

Chemical stability

The percentage of initial concentration remaining of furosemide has been shown in Fig 1A. and UPLC chromatogram of the drug analyzed shown in Fig 1B. The percentage of furosemide remaining in syrup after storage at 5±3°C for 360 days was 99.49±0.22%, (Linearity (R^2) = 0.9996, LOQ = 0.05 ppm, LOD = 0.015 ppm, % relative error = 1, repeatability (%CV) = 0.0058 - 3.35). All batches were running out of volume in 360 days.

For spironolactone suspension 2 mg/ml, the concentration of spironolactone of the second and the third batches declined to lower than 90% in 67 days, but the

TABLE 1. Results of physical and microbiological tests. (Product not enabled).

Lot	Detail	Day 0	Day 15	Day 30	Day 45	Day 60	Day 90	Day 120	Day 180	Day 270	Day 360
1	Analysis bacteria	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	pH	6	6	6	6	6	6	6	6	6	6
	Redispersibility	-	-	-	-	-	-	-	-	-	-
	Color	0	0	0	0	0	0	0	0	0	0
2	Analysis bacteria	✓	✓	✓	-	-	-	✓	-	-	✓
	pH	6	6	6	-	-	-	6	6	6	6
	Redispersibility	-	-	-	-	-	-	-	-	-	-
	Color	0	0	0	-	-	-	0	0	0	0
3	Analysis bacteria	✓	✓	✓	-	-	-	✓	-	-	✓
	pH	6	6	6	-	-	-	6	6	6	6
	Redispersibility	-	-	-	-	-	-	-	-	-	-
	Color	0	0	0	-	-	-	0	0	0	0

Note: Analysis bacteria: ✓: Total Count 0-200 CFU/ml, ✗: Total Count >200 CFU/ml
- Redispersibility: 1: Shake 1-10 times. 2: Shake 1-20 times.
- Color : -1: Light, 0: do not change color, +1: darker, - is no analysis.

TABLE 2. Results of physical and microbiological tests. (Product not enabled).

Lot	Detail	Day 0	Day 15	Day 30	Day 45	Day 60	Day 80	Day 90
	Analysis bacteria	✓	✓	✓	✓	✓	✓	✓
1	pH	7	7	7	7	7	7	7
	Redispersibility	1	1	1	1	1	1	1
	Color	0	0	0	0	0	0	0
	Analysis bacteria	✓	✓	-	✓	✓	✓	✓
2	pH	7	7	-	7	7	7	7
	Redispersibility	1	1	-	1	1	1	2
	Color	0	0	-	0	0	0	0
	Analysis bacteria	✓	✓	-	✓	✓	-	✓
3	pH	7	7	-	7	7	-	7
	Redispersibility	1	1	-	1	1	-	1
	Color	0	0	-	0	0	-	0

Note: Analysis bacteria: ✓: Total Count 0-200 CFU/ml, ✗: Total Count >200 CFU/ml
- Redispersibility: 1: Shake 1-10 times. 2: Shake 1-20 times.
- Color: -1: Light, 0: do not change color, +1: darker, - is no analysis.

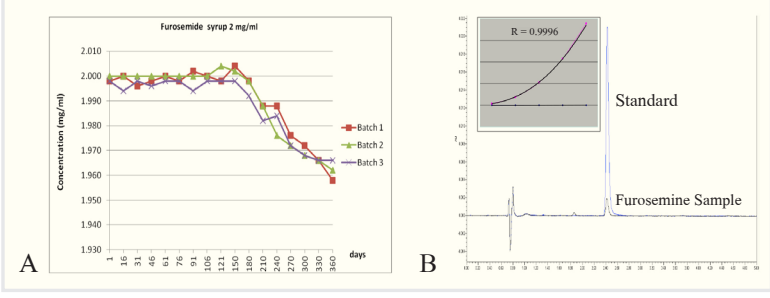

Fig 1A. Change in concentration of furosemide in furosemide syrup 2 mg/ml when stores for 360 days at 2-8°C. **Fig 1B.** UPLC chromatogram of the drug analyzed.

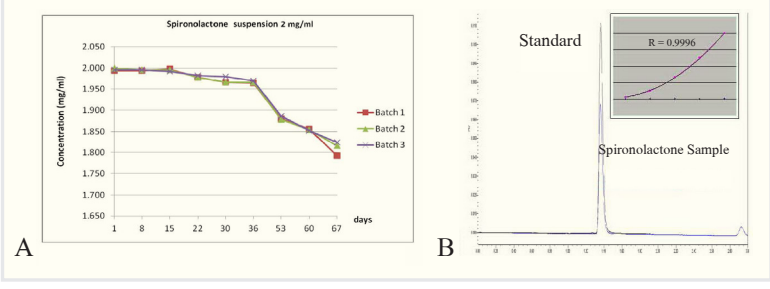

Fig 2A. Change in concentration of spironolactone in spironolactone suspension 2 mg/ml when stores for 67 days at 2-8°C. **Fig 2B.** UPLC chromatogram of the drug analyzed.

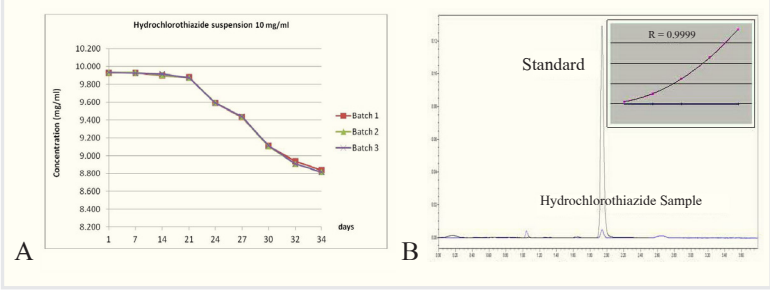

Fig 3A. Change in concentration of hydrochlorothiazide in hydrochlorothiazide suspension 10 mg/ml when stores for 34 days at 2-8°C. **Fig 3B.** UPLC chromatogram of the drug analyzed.

TABLE 3. Results of physical and microbiological tests. (Product not enabled).

Lot	Detail	Day 0	Day 15	Day 30	Day 50
1	Analysis bacteria	✓	✓	✓	✓
	pH	6	6	6	6
	Redispersibility	1	1	1	1
	Color	0	0	0	0
2	Analysis bacteria	✓	✓	✓	✓
	pH	6	6	6	6
	Redispersibility	1	1	1	1
	Color	0	0	0	0
3	Analysis bacteria	✓	✓	✓	✓
	pH	6	6	6	6
	Redispersibility	1	1	1	1
	Color	0	0	0	0

Note - Analysis bacteria: ✓: Total Count 0-200 CFU/ml, ✗: Total Count >200 CFU/ml
- Redispersibility: 1: Shake 1-10 times. 2: Shake 1-20 times.
- Color : -1: Light, 0: do not change color, +1: darker, - is no analysis.

first batch declined to lower than 90% in 60 days so we determined spironolactone suspension 2 mg/ml, preserved its active ingredient for 60 days (Fig 2A). The percentage of spironolactone remaining in suspension after storage at 5±3°C for 67 days was 89.00±0.99%. UPLC chromatogram of the drug analyzed was linear (R^2) = 0.9996, LOQ = 0.051 ppm, LOD = 0.017 ppm, %relative error = 1, and repeatability (%CV) = 0.144 - 11.568. (Fig 2B).

After 30 days, the concentration of hydrochlorothiazide degraded to lower than 9.00 mg/ml in all batches of hydrochlorothiazide suspension (Fig 3A).

The percentage of hydrochlorothiazide remaining in suspension after storage at 5±3°C for 34 days was 89.02±1.48%. UPLC chromatogram of the drug analyzed was linear (R^2) = 0.9999, LOQ = 0.006 ppm, LOD = 0.002 ppm, %relative error = 0.76, repeatability (% CV) = 0.0052 - 0.892 (Fig 3B).

The pH value of each formulation after storage for 360, 67 and 34 days has been shown in Table 1, 2 and 3, respectively.

Microbiological stability

The total of bacterial, yeast and mold count of all samples was less than 200 colonies per ml (cfu/ml) of the sample. The suspected colony of *E. coli* was not found in all samples.

DISCUSSION

All samples were prepared to measure the percentage of active ingredients at the schedule time until the concentration of active ingredients was less than 90% as indicated by the results.

The number of days from these results can only be used for the practical use of the patient who uses three extemporaneous preparations in the PET package that consists of active ingredients and the specific formulated diluents in the formula. Other similar formulas such as the formula that contains other substances were not included in this study, so their practical usage time in the hospital or by the patient who uses them at homecshould be conservatively estimated.

The period of time for effective use of all preparation was shown as the number of days after opening the container which should not be longer than the period time periods explained above, otherwise the potency of drugs that we need to use for treatment decrease which leads to their disadvantage to use.

CONCLUSION

The concentration was quantified by UPLC method for 3 batches and 5 samples per batch. The results showed that furosemide syrup was prepared in PET package size of 60 ml of our institution. The furosemide syrup ran out of volume in 360 days. The spironolactone and hydrochlorothiazide concentrations declined to lower than 90% in 60 and 30 days respectively. We can be confident that three extemporaneous preparations in our institution preserved their potency within 360 days for furosemide, 60 days for spironolactone and 30 days for hydrochlorthiazide after production. The original expiry date of these medicines was 30 days after manufacture. This study found that these drugs laste longer than their expiry date of the drug which should be extended.

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REFERRENCES

1. Sinclair R, Wewerinke M, Jolley D. Treatment of female pattern hair loss with oral antiandrogens. Br J Dermatol. 2005 Mar;152(3):466-73.
2. Lacy CF, Armstrong LL, Goldman MP, Lance LL. Drug Information Handbook International with Canadian and International Drug Monographs. 19th ed. Ohio: Lexi-Comp, 2010. p. 2161
3. Alexander KS Rph Phd, K S Vangala SS, White DB Phd, Dollimore D. The Formulatiion development and stability of spironolactone suspension. Int J Pharm Compd. 1997 May-June;1(3):195-9.
4. Mathur LK, Wickman A. Stability of extemporaneously compounded spironolactone suspensions. Am J Hosp Pharm. 1989 Oct;46(10):2040-2.
5. Nahata MC, Morosco RS, Hipple TF. Stability of spironolactone in an extemporaneously prepared suspension at two temperatures. Ann Pharmacother. 1993 Oct;27(10):1198-9.
6. Allen LV Jr, Erickson MA 3rd. Stability of ketoconazole, metolazone, metronidazole, procainamide hydrochloride, and spironolactone in extemporaneously compounded oral liquids. Am J Health Syst Pharm. 1996 Sep 1;53(17):2073-8.
7. Das Gupta V, Gibbs CW Jr, Ghanekar AG. Stability of pediatric liquid dosage forms of ethacrynic acid, indomethacin, methyldopate hydrochloride, prednisone and spironolactone. Am J Hosp Pharm. 1978 Nov;35(11):1382-5.
8. Lin YM, Chiang YC, Kuo YT, Ho MY, Chen HY. Outcomes of Pharmacy Interventions on pediatric medication prescribing patterns in Taiwan. J Exper Clin Med. 2010;2:173-80.

9. Noppawinyoowong C, Srisangchun J, Sirisangtragul C. Stability of furosemide in extemporaneously prepared oral solutions. Srinakarin Med J. 2001;16(Suppl):347.
10. Allen LV Jr, Erickson MA 3rd. Stability of labetalol hydrochloride, metoprolol tartrate, verapamil hydrochloride, and spironolactone with hydrochlorothiazide in extemporaneously compounded oral liquids. Am J Health Syst Pharm. 1996 Oct 1;53(19):2304-9.
11. Fajolle V, Dujols C, Darbord JC, Brion F, Rieutord A. Oral suspensions of spironolactone, hydrochlorothiazide and captopril: microbiological stability study and clinical use review. J Pharm Clin. 2005;24(1):23-29.
12. HK A. Handbook of Pharmaceutical Excipients: American Pharmaceutical Association; 2000. p. 79-90.
13. The United State Pharmacopoeia 24 TNF, Asian edition, United States Pharmacopeial Convention. Inc., Rockville, MD, 1999. p.58-60.
14. Guideline on stability testing: stability testing of existing active substances and related finished products CQ, European Agency for the Evaluation of Medicinal Products (2003).
15. Christensen JM, Lee RY, Parrott KA. Stability of three oral liquid drug products repackaged in unit dose containers. Am J Hosp Pharm. 1983 Apr;40(4):612-5.
16. Prakongpan S. Stability of medicine. Pharmaceutics Department, Mahidol University, Bangkok; 1997.
17. Sinchaipanich N, Ithimongkol B, Maitreevej A. Method for study the stability of medicine. The Pharmaceutical Association of Thailand, Bangkok; 1999.
18. The United State Pharmacopoeia 24, The National Formulary 19, Asian edition, United States Pharmacopeial Convention. Inc., Rockville, MD, 1999.
19. National committee for drug, List of Hospital Formu¬lary A.D. in National List of Essential Drugs. 2006. p. 65.