Stability of Spironolactone in an Extemporaneous Preparation: Effect of Storage Temperature and Packaging Materials

ความคงตัวของสไปโรโนแลคโตนในผลิตภัณฑ์ยาเตรียมพิเศษเฉพาะราย: ผลของอุณหภูมิการเก็บรักษาและชนิดของวัสดุบรรจุภัณฑ์

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Abstract

The objectives of this study were to study physical, chemical and microbiological stability of the extemporaneous spironolactone suspensions. An extemporaneous spironolactone suspension was prepared from commercially available spironolactone 25-mg tablets using compounded suspending vehicles. The final concentration of spironolactone formulation was 2 mg/mL. Samples were separately filled in HDPE, PET and amber glass bottles then stored in refrigerator at 5±3°C and room temperature (30±3° C). A sample was collected from each bottle immediately after preparation and after 7, 14, 30, 60, and 90 days. High performance liquid chromatography was used for quantitative analysis of spironolactone in each sample. The chemical stability of spironolactone in suspensions was determined by calculating the percentage of the remaining spironolactone on each sampling interval. The acceptable stability was defined as retention of at least 90% of the initial spironolactone concentration. The physical and microbiological stabilities were intermittently evaluated. At least 90% of the initial

spironolactone concentration remained in the compounding suspensions for up to 30 days regardless of packaging material. There were no substantial changes in the appearance (color and consistency) of the samples stored at 5±3°C and room temperature (30±3°C). The pH values of the samples kept at room temperature decreased significantly compared to the initial pH value. The suspension maintained microbiologic stability for 30 days. In conclusion, utilizing the studied suspending vehicle for the compounding of 2.0 mg/mL spironolactone extemporaneous suspension afforded a stable preparation for a month long storage at room temperature regardless the filling bottle material.

Keywords : Spironolactone, extemporaneous compounding, stability, microbiological

บทคัดย่อ

วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาความคงตัวทางกายภาพ ทางเคมีและการเกิดเชื้อจุลชีพของยาเตรียมสไปโรโนแลคโตนความเข้มข้น 2 มิลลิกรัมต่อมิลลิลิตรที่เตรียมสำหรับผู้ป่วยเฉพาะรายในโรงพยาบาล โดยบรรจุยาเตรียมในขวดต่างชนิดกัน 3 ประเภท

ยาเตรียมสไปโรโนแลคโตนสำหรับผู้ป่วยเฉพาะรายเตรียมได้จากยาเม็ดสไปโรโนแลคโตน ขนาด 25 มิลลิกรัมผสมกับน้ำกระสายยาให้มีความเข้มข้นของตัวยาสำคัญเท่ากับ 2 มิลลิกรัมต่อมิลลิลิตร นำยาที่เตรียมได้บรรจุใส่ขวด HDPE, PET และขวดแก้วสีชา เก็บไว้ในตู้เย็นที่อุณหภูมิ (5±3 องศาเซลเซียส) และอุณหภูมิห้อง (30±3 องศาเซลเซียส) นำตัวอย่างยาจากขวดต่าง ๆ มาวิเคราะห์หาปริมาณตัวยาสไปโรโนแลคโตนเมื่อเตรียมเสร็จใหม่และที่เวลา 7, 14, 30, 60 และ 90 วัน โดยใช้เทคนิคโครมาโตกราฟฟี่สมรรถนะสูง ความคงตัวทางเคมีของยาสไปโรโนแลคโตนในตำรับได้จากการคำนวณร้อยละของยาที่เหลืออยู่ที่เวลาต่าง ๆ ร้อยละของยาสไปโรโนแลคโตนต้องมีค่ามากกว่า 90 ของปริมาณยาตั้งต้นจึงจะจัดว่ายาเตรียมนั้นมีความคงตัว ทำการประเมินความคงตัวทางกายภาพและเชื้อจุลชีพเป็นระยะ

ผลจากการศึกษา พบว่าปริมาณตัวยาสไปโรโนแลคโตนยังคงเหลืออยู่ในตำรับอย่างน้อยร้อยละ 90 ของปริมาณยาตั้งต้น เมื่อเวลาผ่านไป 30 วัน โดยไม่ขึ้นกับชนิดของภาชนะบรรจุ ลักษณะของยาเตรียม เช่นสีและการกระจายตัวของผงยาของตัวอย่างที่เก็บไว้ในตู้เย็นและที่อุณหภูมิห้องไม่มีการเปลี่ยนแปลง ในขณะที่ค่าความเป็นกรด-ด่างของตำรับที่เก็บไว้ที่อุณหภูมิห้องมีค่าลดลงเรื่อย ๆ จากค่าตั้งต้น ไม่พบเชื้อจุลชีพในตำรับตลอดระยะเวลา 30 วัน

จากผลการวิจัยพบว่า น้ำกระสายยาที่ใช้ในการเตรียมยาน้ำสไปโรโนแลคโตนสำหรับผู้ป่วยเฉพาะรายความเข้มข้น 2.0 มิลลิกรัม/มิลลิลิตร ทำให้ยาเตรียมมีอายุหนึ่งเดือนเมื่อเก็บไว้ที่อุณหภูมิห้อง โดยไม่ขึ้นกับชนิดของขวดที่บรรจุยาเตรียม

คำสำคัญ : สไปโรโนแลคโตน ยาเตรียมพิเศษเฉพาะราย ความคงตัว จุลชีพ

Introduction

Spironolactone is a competitive aldosterone antagonist, acting at the distal convoluting renal tubule, thus increasing sodium and water excretion and reducing potassium elimination. It is a potassium-sparing diuretic type antihypertensive. It may also reverse aldosterone-induced cardiac fibrosis and improves morbidity and survival of patients with congestive heart failure. It is used in neonates, infants and children with congestive heart failure secondary to congenital heart disease[1]. The recommended dose of spironolactone in neonates is 1 to 3 mg/kg/day in the treatment of congestive heart failure, whilst spironolactone is merely available as film-coated 25-mg and 100-mg tablets. In some countries, it is not available commercially in an oral liquid dosage form. Therefore, compounding of oral suspensions is routinely done in pharmacies to accommodate neonatal and pediatric use. The extemporaneous preparation has to be modified by pharmacists to make them appropriate for administration and it must remain stable and efficacious during the course of its use. The United States Pharmacopeia (USP) has stipulated beyond-use-dating (BUD) for oral and topical compounded preparations. The purpose of BUD is to ensure patients for the maximum therapeutic benefit of extemporaneous products. Studies should be conducted to examine stability of the extemporaneously prepared products regarding dosage form, containers and storage condition.

Hospitalized extemporaneous suspensions are typically prepared from commercially available tablets. Crushing of tablets by mortar and pestle is a critical point in powder preparation. The powder is subsequently mixed with compounding vehicle rendering homogeneity of the mixture. However, the uniformity of content and on loss of drug substance during its dispensing are a concerning issue of this preparation, especially

when practically insoluble drugs are formulated. To facilitate drug uniformity, most of extemporaneous suspensions contain complex vehicle with Newtonian flow properties. Although, a number of studies had described the physicochemical and microbiological stability at various storage temperatures of spironolactone extemp-oraneous preparations, yet none investi-gated the influence of packaging material on stability of spironolactone extemp-oraneous preparations.

In this study, an extemporaneous oral suspension of spironolactone prepared from commercially available 25-mg spironolactone tablets was formulated using simple suspending vehicle. The extemporaneous suspensions were kept in three different material types of 60-mL bottle and stored in different storage temperatures (in refrigerator compared with in ambient temperature) which are the general storage temperature for keeping the in-used drug at home. The organoleptic, chemical, and microbiological stability of the formulations over 90-days period were determined.

Objectives

Materials and Methods

Materials Spironolactone and hydrocortisone 21-acetate, analytical grade (Sigma-Aldrich, St. Louis, USA), 25-mg spironolactone tablets, methanol, HPLC grade (Fluka, New York, USA), Sabouraud dextrose agar (Oxoid Ltd., Hampshire, England), and Tryptone soya agar (Oxoid Ltd., Hampshire, England). The other chemicals were USP or BP grade.

Methods

Suspension preparation The extemporaneous oral suspension was prepared using 25-mg spironolactone tablets. Four tablets were grounded to a fine powder in a mortar then filled it in bottles. Compounded suspending vehicle consisted of sodium carboxymethylcellu-lose 0.05 g, glycerin 7.5 mL, simple syrup 30 mL, paraben concentrate 0.5 mL, and purified water to 50 mL. The bottles containing crushed spironolactone tablets or compounding vehicle were prescribed separately. The extemporaneous suspension was prepared by transferring the compounding vehicle into the bottle, which was previously filled with crushed tablets, making a final volume of 50

mL suspension. The studies extemporaneous suspensions were also prepared in the same way as a prescribed sample. The material types of bottles, HDPE, amber PET and amber glass, influenced on stability of spironolactone suspension were studied.

Analytical method Spironolactone content was determined after preparation and throughout the stability study, using a modification of a HPLC technique[2,3]. A Thermo Fisher Scientific liquid chromato-graphy system (CA, USA) equipped with a Luna C-18 column (250x4.6 mm i.d., Ø 5 mm, CA, USA) and analyzed with Chomquest program were used. The internal standard, hydrocortisone 21-acetate, was added into each analyzed sample. The mobile phase consisted of methanol:water (60:40) delivered at a flow-rate of 1.0 mL/min. It was filtered through a 0.45 μm membrane and degassed by a sonication prior to use. Triplicate samples (0.1 mL) were collected after shaking the suspension, added 0.1 mL internal standard then made to volume of 10 mL with methanolic solution (80% v/v). Samples were then sonicated for 1 minute, filtered through a 0.45 μm membrane and the 10 μL filtrate was injected into the column. The UV-Vis detector operated at 254 nm. The analytical technique was validated for linearity, precision, accuracy and the limits of detection of spirono-lactone and canrenone (degraded sub-stance).

Validation of analytical method

Linearity. The alcoholic solution of spironolactone was accurately prepared in a concentration range of 0.5–8 μg/mL. Hydrocortisone 21-acetate as an internal standard was added into the solution to make a concentration of 40 μg/mL. The solution was injected in triplicate to HPLC column. The calibration curves relating the integrated area under the peak to the corresponding concentrations of spirono-lactone were constructed.

Accuracy. An accuracy study was performed by adding known amounts of spironolactone to suspension samples. The actual and measured concentrations were compared. Recovery of the method was evaluated at three different concentration levels (corresponding to 75, 100 and 125% of test preparation concentration). For each concentration level, three sets were prepared.

Precision. The precision of the assay method was evaluated in terms of repeatability by performing six indepen-dent assays of spironolactone test samples and calculating the percent relative standard deviation (% RSD) of the assay (intra-day). Intermediate precision of the method was checked by performing the same procedure on a different day (inter-day) under the same experimental conditions.

Chemical stability. Chemical stability was evaluated for 3 months during which suspensions were protected from light and stored at 5 ± 3ºC and 30 ± 3ºC. Samples were collected at days 0, 7, 14, 30, 60 and 90. The preparation is considered stable if physical characteristics have not changed and spironolactone concentration has remained above 90% of the original concentration.

Microbial stability. Microbiological quality of spironolactone suspensions stored at 30±3ºC was assessed at days 7 and 30 according to the European Pharmacopoeia (PhEur) monograph 5.1.4. Tryptone soya agar and Sabouraud-dextrose agar were used as culture mediums for bacteria and fungi, respectively. Pour plate method was used to test for total viable aerobic count. The samples from each container of the extemporaneous spironolactone suspension were prepared by using a 1 in 10 dilution technique. Add 1 mL of the samples and 15–20 mL of a liquefied agar medium into the dishes for the cultivation of the bacteria or fungi. Incubate the plates for 5 days at 30-35º C for bacteria or 20–25ºC for fungi. Take the arithmetic average of the counts and calculate the number of colony–forming unit for evaluation.

Results and Discussion

Analysis of spironolactone in extemporaneous suspension. The chroma-tographic separation of spironolactone and hydrocortisone 21-acetate, internal standard, was shown in **Figure 1**. The retention time of spironolactone and hydrocortisone 21-acetate was 12.8 minutes. and 10.9 minutes, respectively. The main degraded substance, canrenone, of which peak was very small, could be magnifiably observed at 16.33 minutes. Linear relationships were obtained by plotting the spironolactone concentrations against peak area ratio between spironolactone and internal standard, hydrocortisone 21-acetate (**Figure 2**). The corresponding concentration ranges and other parameters for linearity were listed in Table 1.

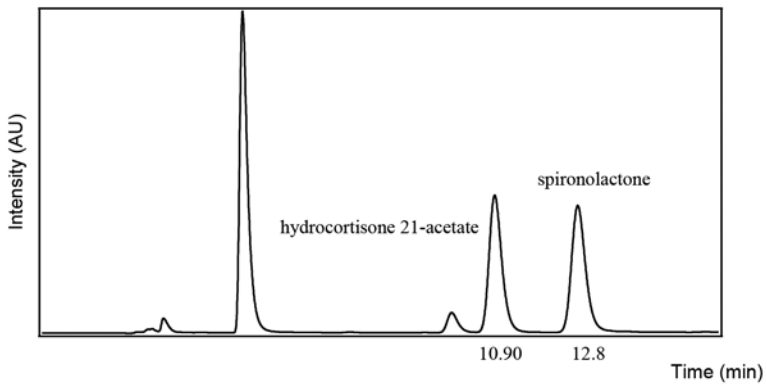

Figure 1. The representative HPLC chromatogram of spironolactone and hvdrocortisone 21-acetate

Table 1. Result of linearity for the determination of spironolactone in extemporaneous suspension

Parameters	HPLC Method
Beer's Law Range	**0.5 – 8 µg/mL**
Slope	220022
Intercept	0.0054
r^2	0.9998

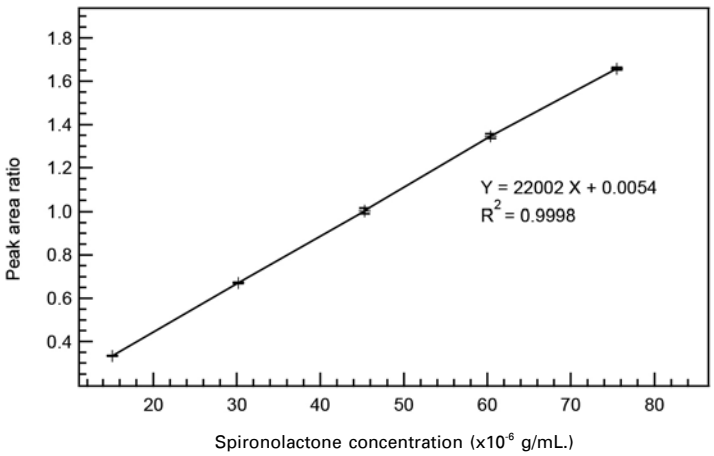

Figure 2. Sprionolactone standard curve : area is displayed on the ordinate and concentration in g/ml. on the abscissa

Table 2. Evaluation data of precision study

Set	Spironolactone (% Assay)	
	Intraday	Interday
1	106.5618	101.7705
2	106.2377	97.9867
3	104.3142	100.1282
4	108.3014	100.0158
5	107.5835	100.9844
6	108.7259	99.7612
Mean	106.9541	100.1078
SD.	1.6116	1.2776
% RSD	1.5068	1.2762

Table 3. Evaluation data of accuracy study

	% Level	Theoretical Concentration (mg/mL)	Observed Concentration (mg/mL)	% Recovery	% RSD
Spironolactone	75	0.0153	0.0150	100.1835	2.1836
	100	0.0204	0.0200	99.8874	1.7244
	125	0.0255	0.0250	98.1763	0.4737

Results in Table 1-3 indicated satisfactory linearity, accuracy and precision of the proposed methods and proved to be adequate for the simul-taneous determination of spironolactone in suspesion. The drug recovery was in the range of 98-102%. The % RSD of precision study was not more than 2%, indicated that the developed method was precised[4].

Chemical stability. The stability indicating HPLC technique proved to be suitable for the simultaneous determina-tion of spironolactone and its main degradation product (canrenone). The extemporaneous suspension prepared from crushed 25-mg spironolactone tablets was rather viscous and homogeneous since it was formulated in

sodium CMC 0.1% solution. The preparation was sufficiently homogenous to permit the removal of the accurate dose after consistent shaking. This could be implied by a narrow range of standard deviation (% SD) from each spironolactone assay. As previous study showed that the cellulose containing suspending vehicles could retain the suspended particles such that prepara-tions were taken homogeneously. The preparation remained unchanged in appearance at tested temperatures throughout the stability study.[5] As depicted in Table 4, no significant drug losses were observed up to 90-day storage at 5±3°C, whilst over 90% of the drug still remained stable in suspension kept at 30±3°C for 30 days. Meanwhile, the pH values of samples were slightly shifted to lower pH value (Table 5).

Stability data indicated that spironolactone was chemically stable in the studied compounding vehicle, allowing a fairly long beyond-use date. Considering the usual acceptance limits of 90 to 110% for the nominal quantity of active ingredients in extemporaneous prepara-tions,[6] a 30-day beyond-use date at ambient temperature could be established for this formulation regardless of material types of the studied bottles. Meanwhile, samples kept in PET, HDPE and amber glass bottles and stored in refrigeration (5±3°C) still remained stable over 90-day period. The microbial contamination was successfully prevented by the selected preservatives.

According to the pre-formulation study which reported that spironolactone has been known to undergo degradation with an apparent first-order rate. Its activation energy has been reported to be 18.9 kcal/mole (79 kJ/mole).[7] Considering the structure of spironolactone, the presence of a lactone (cyclic ester) and a thioester bond suggested an instability area when it exposed to water.[8] Spironolactone undergoes two main degradation pathways, which are thioacetylation to canrenone and deacetylation to 7-thiospironolactone. The presence of acid or base could expedite a hydrolytic reaction. The pH rate profile of spironolactone is V shape. In this case, optimum stability of active pharmaceutical ingredient in aqueous preparation can be obtained at specific pH range presenting in the pH rate profile. Spironolactone is less stable in alkaline conditions with an optimum stability at pH around 5.0. Therefore, an increasing or decreasing in pH values of formulations from 5.0 during storage might affect spironolactone stability. The studied

compounding vehicle primarily had slightly high pH value (pH ~ 6.1) thus, the freshly prepared spironolactone suspension showed fairly higher pH value **(Table 5).** This possibly activated hydrolysis of spironolactone. The increase in pH of extemporaneous preparation might be due to filler, calcium sulfate, containing in spironolactone tablets. The degradation product, canrenone, was detected in samples stored at 30±3°C given that less than 90% of initial drug concentration in all samples at the sixty day analysis point.

Microbial stability. No visible microbiological growth was detected in all studied samples. The total of bacterial, yeast and mold count in all samples after one month storage was less than 20 colonies at 10^1 CFU of the sample. These results were complied with the acceptance criteria for microbiological quality according to the European Pharmacopoeia. The suspected colony of *E. coli* was not found in Tryptone soya agar medium of any samples. It was implied that the preservatives were active and effective during 30-days at the temperatures tested.

Table 4. Percentage remaining of spironolactone in extemporaneous suspension

Time (days)	Amount of Spironolactone HCl (%)					
	HDPE Bottle		PET Bottle		Amber Glass Bottle	
	5±3°C	30±3°C	5±3°C	30±3°C	5±3°C	30±3°C
0	115.6±0.119		97.5±0.136		101.2±0.137	
7	100.2±0.127	105.3±0.037	107.7±0.084	101.0±0.105	101.3±0.044	99.4±0.010
14	101.2±0.097	91.9±0.015	93.5±0.052	97.5±0.029	102.9±0.111	92.6±0.053
30	104.3±0.037	101.1±0.040	105.9±0.048	95.0±0.020	105.8±0.076	94.9±0.070
60	99.1±0.022	89.9±0.207	97.5±0.087	80.6±0.199	111.6±0.124	79.8±0.125
90	103.0±0.039	65.8±0.020	104.8±0.048	66.9±0.088	111.8±0.094	72.2±0.124

Table 5. pH of spironolactone extemporaneous suspension

Time (days)	pH of Spironolactone HCl Suspension		
	HDPE Bottle	PET Bottle	Amber Glass Bottle
0	6.63 ± 0.02	6.54 ± 0.03	6.54 ± 0.03
7	6.66 ± 0.02	6.62 ± 0.03	6.66 ± 0.02
14	6.62 ± 0.01	6.60 ± 0.01	6.64 ± 0.04
21	6.59 ± 0.01	6.57 ± 0.01	6.59 ± 0.02
35	6.52 ± 0.01	6.48 ± 0.01	6.50 ± 0.02
42	6.48 ± 0.01	6.45 ± 0.01	6.47 ± 0.03
49	6.45 ± 0.01	6.43 ± 0.01	6.43 ± 0.01
56	6.43 ± 0.02	6.43 ± 0.01	6.42 ± 0.02
63	6.42 ± 0.02	6.42 ± 0.01	6.41 ± 0.02
70	6.31 ± 0.01	6.32 ± 0.01	6.31 ± 0.01
77	6.29 ± 0.02	6.27 ± 0.01	6.28 ± 0.02
90	6.28 ± 0.02	6.24 ± 0.02	6.26 ± 0.02

Conclusion

According to the United States Pharmacopeia,[9] the beyond-use date in the absence of stability information for water containing oral formulation is not later than 14 days when stored at controlled temperature. However, stability studies still need to be conducted for other hospital extemporaneous products to ensure patients for the maximum therapeutic benefit. In conclusion, the extemporaneous suspension of spironolactone could be successfully prepared using CMC-based compounding vehicle providing a 30-day beyond-use date at ambient temperature (30±3°C).

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