PRACTICE REPORTS

STABILITY OF EXTEMPORANEOUSLY PREPARED SPIRONOLACTONE SUSPENSIONS IN SAUDI HOSPITALS

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تم دراسة ثبات دواء سبيرونولاكتون في المستعلقات المحضرة معمليا بواسطة خمسة مستشفيات سعودية . وقد خزنت المستعلقات عند درجة حرارة ٤ م في قوارير من زجاج الكهرمان . وقد تم فحص المستعلقات بصريا على فترات زمنية تبلغ خمسة أسابيع لرصد التغيرات في اللون والرائحة ، وتكون الرغوة ، والترسيب ، والتفل والخواص الريولوجية (علم الجريان) . وقد قدرت محتويات العينات من السبيرونولاكتون بواسطة طريقة محسنة لكروماتوغرافيا السوائل عالية الكفاءة . كما تم اختبار المستعلقات لمعرفة التغيرات في الأس الهيدروجيني (درجة الحموضة) ، والحجم الجسيمي بالإضافة إلى فحص النمو الميكروبي . وقد كان متوسط تراكيز السبيرونولاكتون في جميع المستعلقات خلال الأسبوعين الأولين من التخزين أكبر من ٩٠% من التراكيز المبدئية ، ما عدا مستعلق واحد. وبعد ثلاثين يوما فقط بقي مستعلقان منها (D,C) ثابتان بأكثر من ٩٠% من تراكيز السبيرونولاكتون المبدئية. بينما بلغت تراكيز السبيرونولاكتون حد ٩٠% في العينة C لمدة لا تقل عن 35 يوما. وبقي الأس الهيدروجيني والحجم الجسيمي للمستعلقات دون تغيير طوال فترة الاختبار ودون تغير في المظهر المادي. وقد تبين أن مستعلقين للسبيرونولاكتون (5 مغ/مل) لهما فترة الصلاحية (30 يوما) المذكورة على بطاقة الدواء.

The stability of spironolactone in suspensions prepared extemporaneously by five Saudi hospitals was studied. The suspensions were stored at 4°C in amber glass bottles. At intervals up to five weeks, the suspensions were visually inspected for color and odor change, foaming, precipitation, sedimentation and rheological properties. Samples were assayed for spironolactone content by high-performance liquid chromatography (HPLC) method. Also, the suspensions were tested for changes in pH, particle size, as well as for microbial growth. During the first 14 days of storage, mean spironolactone concentrations in all suspensions were >90% of the initial concentrations, except one individual suspension. After 30 days only two suspensions remained stable, with >90% of the initial spironolactone concentration. Whereas, mean spironolactone concentrations in only one sample met the 90% cut off for at least 35 days. Suspensions pH, and particle size remained unchanged throughout the period of test and there were no changes in physical appearance. Also counts of bacteria and fungi remained within acceptable limits within 35 days. Only two spironolactone suspensions (5 mg/ml) were found to fulfil the expiry date (30 days) stated in the labels.

Keywords: Spironolactone, extemporaneous suspensions, storage, high-performance liquid chromatography, stability.

Introduction

Spironolactone, 7α-Acetylthio-3-oxo-17α-pregn-4-ene-21,17β-carbolactone, is used extensively as a

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potassium sparing diuretic. The adult dosage is ranging from 25 to 400 mg daily. The initial dose of spironolactone for children is 3.0 mg/Kg body-weight daily, in divided doses. The drug is commercially available as film-coated tablets of 25, 50 and 100 mg, but not in liquid or suspension dosage form. However, pharmacists are routinely asked to prepare such oral liquid or suspension forms for pediatric and elderly patients who can't swallow solid dosage forms, as well as those who can only take oral medication via a nasogastric tube. This involves preparation of the oral liquid from commercially available dosage form or from pure drug powder.

The chemical stability of spironolactone in solution and suspension dosage forms has been evaluated in various studies using different vehicles, including simple syrup (1), cherry syrup (2), or other commercially available vehicles (3,4). Spironolactone was found to be stable for 164 days at 24°C when prepared from pure drug powder using simple syrup containing 10% alcohol and preservatives (5), and for four weeks at 5°C and 30°C when compounded in cherry syrup with preservatives (2). It was also stable in a suspension containing syrup, caboxymethylcellulose and purified water for three months when stored in amber glass bottles at 4°C and 22°C (3). Another study conducted at four temperatures (5, 30, 50 and 60 °C) showed that spironolactone in suspension was stable for a period of three months (6). Furthermore, spironolactone was prepared in clear liquid containing alcohol, propylene glycol, polyethylene glycol 400 and was found stable for 93 days at 40°C (7,8). The effect of pH and ionic strength on the stability of spironolactone liquid dosage form was also studied (9).

This study was performed in response to inquiries about stability of extemporaneously prepared spironolactone suspension dosage forms. The objective of the present work was to investigate the physical and chemical stability, as well as the microbial limit test of liquid formulations prepared from commercially available tablets in five hospitals present in Riyadh city of Saudi Arabia.

Methods

Collection and testing of suspensions

Five major national hospitals in Riyadh city, capital of the Kingdom of Saudi Arabia were requested to prepare spironolactone suspensions according to physician's order. 500 ml of each sample was supplied in amber bottles and labeled "shake well and refrigerate. Stable for 30 days".

Immediately after receipt of suspensions (day 0) microbial testing was performed according to the United States Pharmacopoeia 23, employing the multiple tube method (10). The suspensions were then stored at 4°C. On days 0, 7, 14, 21, 28, and 35, the bottles were brought to room temperature and the liquids were visually inspected for color and odor changes, foaming and precipitation. The bottles were then thoroughly shaken (30 inversions), and the pH of the samples were determined on each of the above-mentioned days with a pH meter (Metrohm 744 pH meter, Metrohm Co., Switzerland). Particle size distributions in each sample were determined microscopically (total count greater than 500 particles) on days 0, 14 and 35.

High-performance liquid chromatographic (HPLC) assay

The HPLC system consisted of an LC-10AT pump (Shimadzu, Kyoto, Japan) at a flow rate of 1.8 ml/min during analysis. A variable-wavelength UV detector (Waters M-480, Millipore, Milford, MA, USA) set at 254 nm was used along with a Rheodyne injector (Supelco, Bellefonte, PA, USA) fitted with 50 µl loop, a Waters-746 data module integrator, and a solvent waste container. Chromatographic separation was accomplished using a Waters reverse-phase µBondapak C_{18}, 3.9 x 300 mm stainless steel analytical column, with 10 µm particle-size. The mobile phase consisted of HPLC grade mixture of methanol-water (63 : 37% v/v). The mixture was filtered through a 0.22-µm membrane filter (Type GVWP, Millipore, Bedford, MA, USA) under vacuum, then degassed by flushing with nitrogen for 5 min prior to use to get the best reproducibility. The injection volume was 50 µl, and the assay was performed at room temperature under subdued light. The chromatograms were recorded and integrated at a speed of 0.25 cm/min. The internal standard was gliclazide (Servier Research and Development, UK) in methanol (0.5 mg/ml), kept at 4°C.

Immediately after receipt of samples, the concentration of spironolactone in the suspensions was determined with a simple HPLC assay. Samples from each bottle of suspension were assayed in duplicate on study days 0, 7, 14, 21, 28,

and 35. After thorough shaking, the duplicate, 5-ml were diluted to 100 ml with methanol, well shaken and filtered through a 0.20-µm Millex-HV nylon syringe operated filter unit (Millipore Corporation, MA, USA). A 100 µl of the clear filtrate was transferred to a 1.5-eppendorf tube, containing 30 µl of the internal standard, volume completed to 1ml with the mobile phase, vortex-mixed for 30 seconds and 50 µl was then injected into the HPLC column.

A 0.5 mg/ml stock solution of spironolactone (Sigma Chemicals Co., MO, USA) in methanol, kept wrapped in aluminum foil at –20°C, was used to prepare the spironolactone standard solutions. The assay was validated before samples were assayed. A 6-point standard curve was constructed for each run on validation and study days. The spironolactone assay was validated over the concentration range 10-30 µg/ml. On the study day, volumes of 30, 36, 48, 60, 75 and 90 µl of spironolactone stock solution were each added to a 1.5-ml eppendorf tube. The volume was made up to 1.5 ml with the mobile phase, vortex-mixed for 30 seconds, to provide spironolactone standards of 10, 12, 16, 20, 25 and 30 µg/ml. For calibration curve plot, a 30-µl aliquot of internal standard stock solution was transferred to each of six 1.5 eppendorf tubes and volume completed to 1 ml with each of the above-mentioned prepared spironolactone standard solutions, vortex-mixed for 30 seconds and 50 µl of each concentration was injected in duplicate directly into the HPLC column. Validation of the assay was repeated over the above-mentioned range of concentrations before study began.

Sedimentation rate

The sedimentation of suspended spironolactone tablet particles was studied by placing 10 ml of spironolactone suspension from the same batch (Manually shaken for one minute) directly into each of five borosilicate glass tubes with polypropylene screw caps (13 mm in diameter x 100 mm in length) and visually observing the settling process. The sedimentation time that the first visible preciptation was noted on the bottom of the tube and precipitate (height in mm) level after one minute, five minutes, 10 minutes, one hour and six hours were determined.

Rheolology of spironolactone suspensions

The rheological properties of all collected samples were compared. Viscosity measurments were made using a Brookfield (Model DV-II + Version 3.0) digital viscometer at 6 rev/minute and number 1 spindle at 20±1 °C. Readings were taken after a stabilizing period of several minutes. Viscosties measured (cp) were apparent viscosties.

Analysis of data

The average ratios of peak areas for spironolactone to peak areas of the internal standard were plotted against the spironolactone concentration range of 10-30 µg/ml. The slope, intercept and Pearson correlation coefficients (r^2) were determined by the method of weighted least squares (linear model) and was used to calculate the spironolactone concentration in the suspension samples. The amount of spironolactone per ml of suspension was determined from the following equation:

$$Q = [R/A + B] \text{ X dilution factor}$$

Where Q is the mg spironolactone/ml of suspension, R is the peak area ratio (drug/internal standard), A is the slope of the calibration curve and B is the y-intercept.

The reproducibility of the assay method was assessed by assaying replicate spironolactone samples (n=8) at three concentrations (14, 22 and 28 µg/ml) each day for three consecutive days, for intra- and interday precision studies by calculating the average peak area ratio response and the % relative standard deviation. All results were expressed as mean ± SD.

Significant drug loss was defined as a ≥10% decrease from the initial drug concentration. Linear regression analysis to obtain lines of best fit and correlation coefficients for HPLC standard curves were determined by the method of weighted least squares (linear model).

Results and Discussion

Following the chromatographic conditions described above, the internal standard and spironolactone were well separated and their retention times (T_R) were 5.88 and 7.84 min, respectively (Fig. 1A). For both compounds sharp and symmetrical peaks were obtained with good base line resolution and minimal tailing, thus

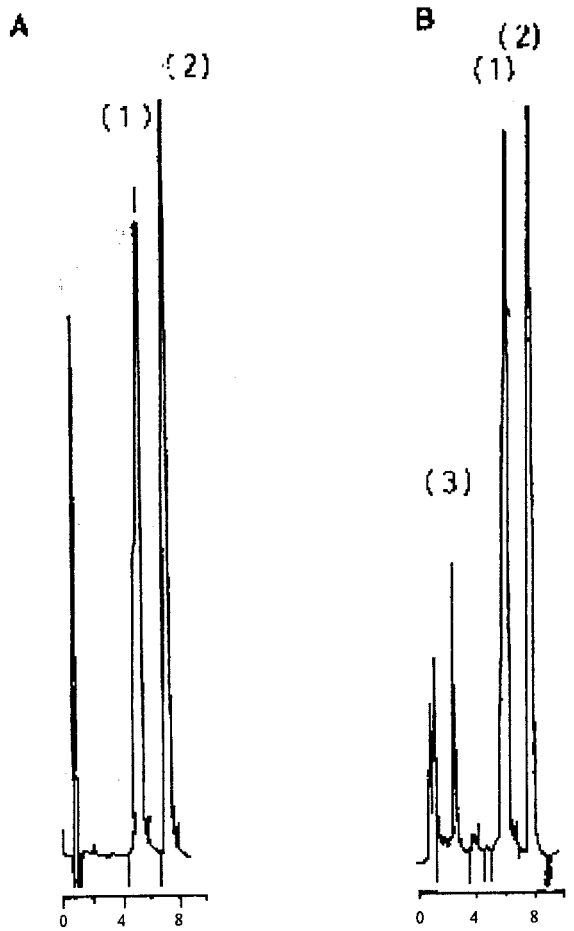

Figure 1: Chromatograms of spironolactone and gliclazide internal standard as freshly prepared (A) and after exposure to a strong acid and light (B). Retention times for unidentified degradation product, the internal standard, and spironolactone were 3, 5.9 and 7.8 minutes, respectively. Peak 1 = gliclazide, peak 2 = spironolactone, peak 3 = unidentified degradation.

facilitating the accurate measurement of the peak area ratio. No interfering peaks were found in the chromatogram (Fig. 1B) due to excipients, or other formulation additives or any apparent spironolactone decomposition products. The retention time of the unidentified spironolactone degradation product is 3.0 minutes and, thus, will not interfere with spironolactone or the internal standard. The HPLC method was shown to be accurate and precise, and evaluated by the calculation of individual standard values obtained from the regression line of the standard curve run on each day. The plots were linear (mean r^2, 0.9997, n=8) and the regression analysis of the data gave the slope and intercept as : $y = 0.0664 (\pm 0.0003)x + 0.0004$, where y is the peak-area ratio of spironolactone to the internal standard and x is the spironolactone concentration, added over a concentration range of 10 to 30 μg/ml.

Table 1 summarizes the intra- and interday precision of the present method. The within-day precision showed a coefficient of variation (C.V.) of 1.27 to 3.39 % and a day-to-day precision of 1.66 to 4.82 %.

Table 2 summarizes the HPLC-assay results of the drug in five suspensions (obtained from five major different Saudi hospitals), in addition to results of recoveries after storage at 4°C for five weeks. During the first 14 days of storage, mean spironolactone concentrations remained above 90% of the initial concentration in all suspensions except one (suspension A, Table 2). No evidence of degradation products was observed in the suspensions throughout the study period. After 28 days, only two suspensions (C & D) remained stable, with more than 90% of the initial spironolactone concentrations. By day 35, spironolactone levels had decreased to <90% of the initial concentrations in samples assay, except suspension C which retained approximately 95% of the initial spironolactone concentration. The pH values remained essentially unchanged throughout the 35 study days from the values on day 0 (Table 2). No appreciable change in color or odor was observed for any of the suspensions tested. Some of the preparations were pourable (B, C, D and E) while others were viscid and difficult to pour (A). Some suspensions showed slight aggregation of particles on storage, but re-suspended on shaking. All suspensions (except A) passed the microbial limit test, and found to be free from contamination with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* species, throughout the study period. Particle size distribution are shown in Figure 2. Although the frequency distributions have the same mode, two suspensions (A & D) have much longer tails of large particles. These large particles, although relatively few in number may contribute greatly to the weight of spironolactone. This is made clear in Figure 3, where the fractional weight undersize is related to particle diameter. For suspension E 99% of spironolactone exist as particle of diameter less than 15 μm, compared with only 92%, 89%, 79% and 77% for suspensions D, C, B and A, respectively. This variation might be due to difference in the degree of tablet grinding during preparation of these suspensions.

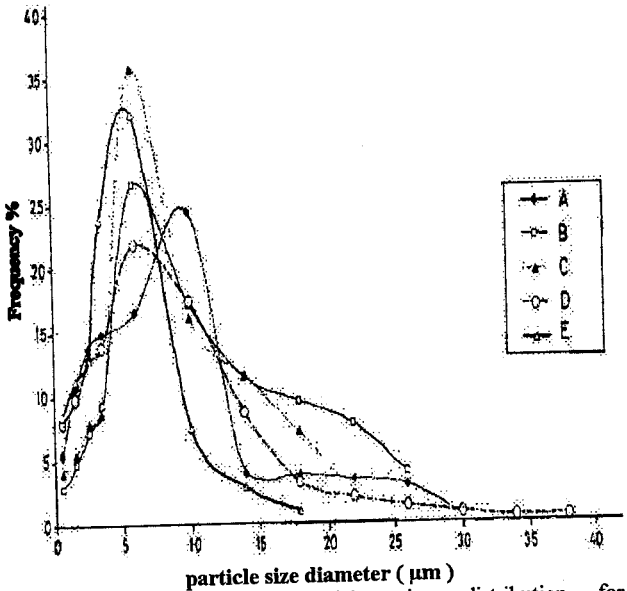

Figure 2: Differential particle size distribution for spironolactone suspensions extemporaneously prepared by five hospitals.

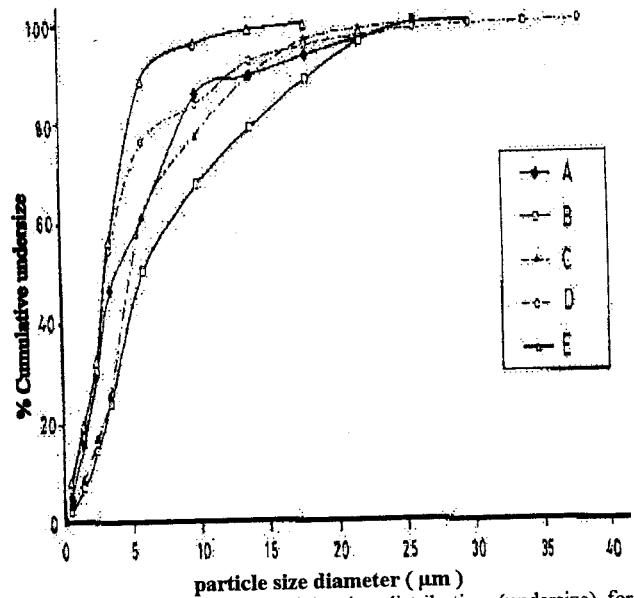

Figure 3: Cumulative particle size distribution (undersize) for spironolactone suspensions extemporaneously prepared by five hospitals.

Table 1: Within-day and day-to-day accuracy and precision of spironolactone in solutions.

Added Concentration (μg/ml)	Within-day			Day-toDay		
	Measured concentration (μg/ml)	Accuracy (%)	Precision (%)	Measured concentration (μg/ml)	Accuracy (%)	Precision (%)
14.0	13.93 ± 0.359 n = 8	- 0.41	2.58	13.359 ± 0.644 n = 15	- 4.58	4.82
22.0	22.912 ± 0.776 n = 8	4.15	3.39	21.775 ± 1.085 n = 15	- 1.02	4.59
28.0	27.766 ± 0.353 n = 8	-0.84	1.27	27.436 ± 0.457 n = 15	- 2.01	1.66

Table 2: Stability of spironolactone in five extemporaneously prepared suspensions, after storage at 4°C.

Conc. / Hospital	Reported Conc. (mg/ml) (in label)	Actual Initial Conc. (mg/g)[a]	% Initial concentration Remaining[a]					Initial pH[b]
			After 7 days	After 14 days	After 21 days	After 30 days	After 35 days	
A	5 mg/ml	5.56 ± 0.02	92.6 ± 1.8	88.2 ± 2.1	86.7 ± 0.8	81.3 ± 4.3	< 80.0	6.84
B	5 mg/ml	4.41 ± 0.01	98.7 ± 0.9	92.5 ± 1.4	90.1 ± 0.75	87.4 ± 1.9	82.6 ± 3.3	5.85
C	10 mg/ml	8.53 ± 0.03	99.2 ± 3.1	97.6 ± 2.4	97.6 ± 0.5	96.8 ± 1.1	95.7 ± 1..1	4.20
D	5 mg/ml	4.20 ± 0.02	101.0 ± 2.4	100.0 ± 4.3	94.9 ± 2.4	91.7 ± 3.8	89.7 ± 3.5	5.31
E	5 mg/ml	4.98 ± 0.01	97.2 ± 1.6	93.4 ± 1.0	92.5 ± 2.8	88.4 ± 1.4	83.8 ± 2.0	5.87

[a] Values represent mean ± S.D. of duplicate determinations for three suspension preparations from each hospital (n = 6).

[b] Values represent means of duplicate determinations for three suspension preparations from each hospital (n = 6). pH values on remaining study days were essentially unchanged from the values on day 0.

Table 3: Sedimentation of extemproneously prepared spironolactone suspensions

Hospital	Time Precipitate Appeared on Test Tube Bottom (min)	Level of Precipitate (mm)			% of Precipitate appears after 10 min
		10 min	60 min	6 Hrs	
A	8	2.0		50	4.0%
B	25	UD*	UD**		
C	8	0.50		12	4.2%
D	10	0.50		2.5	20.0%
E	10	1.50		10.5	14.30%

UD* = unable to determine because of the turbidity of the suspension
UD**= unable to determine because of separation in two layers

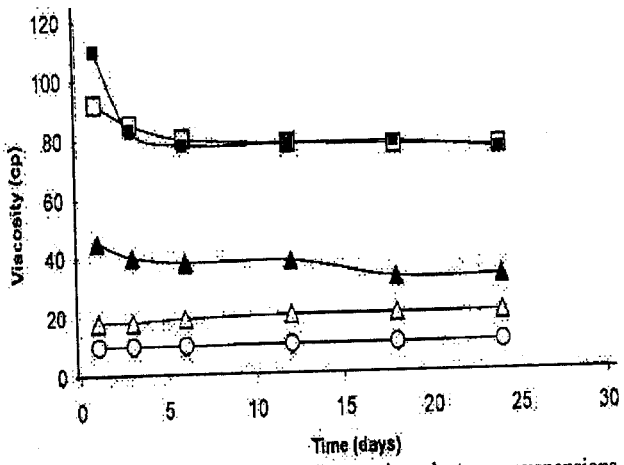

Figure 4: Viscosities of five spironolactone suspensions determined at 20 ±1°C using a brookfield viscometer: solid square represents suspension A, open square represents C, solid triangle represents B, open triangle E, and open circle represents D.

In the settling experiments, the mean time for sediments to first appear on the test tube bottom was 8, 25, 8, 10 and 10 minutes for suspentions A, B, C, D and E, respectively. Approximately, 4%, 4.2%, 20% and 14.5% of the total sediment level (mean 50, 12, 2.5 and 10.5 mm) for suspensions A, C, D and E were obsererved 10 minutes after shaking, except suspension B unable to be determined due to turbidity (Table 3). These findings may suggest that the particles of spironolactone are suspended for a reasonable time after shaking to ensure that the prescribed dose is measured.

Apparent viscosities are shown in Figure 4. Suspensions A, B and C showed decrease in their viscosities after day 3. The reasons are not clear but it might be due to depolymerization. It is also clear from the figure that suspensions A and C have higher initial viscosities compared with the others.

The results of the study also, revealed that pH of the suspensions play a major role on the stability of spironolactone. Since the from the figure that suspensions A and C have optimum pH for stability of spironolactone appears to be approximately 4.5, accordingly only two suspensions (C&D) which fulfill this requirement remained stable for four weeks. This finding agrees with the studies which reported four weeks stability in extemporaneously compounded oral suspensions of cherry syrup (pH 4.5) with spironolactone concentrations of 2.5, 5 and 10 mg/ml (9). In contrast those suspensions having pH of more than 5.0 were only stable for up to 3 weeks.

Some individual suspensions are rather thick and viscous (suspension A). This may be attributed to either interaction with a filler or some other ingredient or may be due to sub-optimal preparation technique. However, this finding is consistent with a previous study (11), which suggests the following points when preparing spironolactone suspensions:

1. Tablets should be pulverized to the finest particle size using a Wedgewood-type mortar.
2. If glycerin is used in preparing the suspension, utilize the smallest amount possible, or replace by water.
3. Add the vehicle or syrup mixture in geometric proportions.
4. Finally, the suspension should be homogenized using a homogenizer, to get the best particle size uniformity.

Commentary and Conclusion

Compounding of brand or generic name dosage forms into suspension for oral use involves many considerations. The pharmacist's responsibilities in preparing such products should include factors such as: dose uniformity, a conveniently measurable

volume containing the usual dose, reasonable stability (chemical, physical and microbial), acceptable bioavailability of the active ingredients and reasonable palatability.

Pharmaceutical manufacturers generally, do not disclose the various ingredients. This lack of information can result in problems regarding stability and therapeutic efficacy for the compounder who use those dosage forms to prepare extemporaneous preparations. For example a drug which is compounded from tablet into extemporaneous suspension may be unstable because of the interaction of the vehicle with unspecified tablet constituents.

The study emphasizes the need for the availability of drug in optimal dosage forms for administration to pediatric and elderly patients or those who cannot swallow tablets, which should be stable enough during the administration period specified in the container label. This also emphasizes the great need to conduct stability studies of the drug in such formulations.

The present study suggests that other suspensions made extemporaneously from tablets might exhibit similar trends. Also, the results obtained from this work can be extended to other formulations made extemporaneously from tablets.

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References

1. Nahata MC, Morosco RS and Hipple TF. Stability of spironolactone in an extemporaneously prepared suspension at two temperatures. The Annals of pharmacotherapy. 1993; 27:1198-1199.
2. Mathur LK and Wickman A. Stability of extemporaneously compounded spironolactone suspensions. Am.J.Hosp.Pharm. 1989; 46:2040-2042.
3. Allen LV and Erickson MA. Stability of labetalol hydrochloride, metoprolol tartarate, verapamil hydrochloride, and spironolactone with hydrochlorothiazide in extemporaneously compounded oral liquids. Am.J.Health-syst.Pharm. 1996; 53(19):2304-2309.
4. Allen LV and Erickson MA. Stability of ketoconazole, metolazone, metronidazole, procainamide hydrochloride and spironolactone in extemporaneously compounded oral liquids. Am J Health-Syst Pharm 1996; 53(17):2073-2078.
5. Das Gupta V, Gibbs CW Jr, and Ghanekar AG. Stability of pediatric liquid dosage forms of ethacrynic acid, indomethacin, methyldopa hydrochloride, prednisone and spironolactone. Am.J.Hosp.Pharm.1978; 35(11):1382-1385.
6. Alexander KS, Vangala SSK, White DB and Dollimore D. The formulation development and stability of spironolactone suspension. Int J Pharm Compound 1997; 1(3):195-199.
7. Pramar Y, Das Gupta V. and Bethea C. Development of a stable oral liquid dosage of spironolactone. J Clin Pharmacy and Therapeutics 1992; 17:245-248.
8. Mcknight DL and Emmott N. Preliminary formulation and stability of a spironolactone oral suspension for paediatric use. Aust J Hosp Pharm 1997; 27(5):390-394.
9. Pramar Y and Gupta VD. "Preformulation studies of spironolactone: Effect of pH, Two buffer species, Ionic strength, and temperature on stability". J Pharm Sci 1991; 80(6):551-553.
10. The United States Pharmacopeia XXIII/ The National Formulary XVIII, United States Pharmacopeial Convention, Inc. Rockville, MD, 1995, p.1435.
11. James E Dice. Excessively thick extemporaneous spironolactone suspension. Am J Hosp Pharm 1989; 46:486.

Appendix

Formulations of Extemporaneously Compounded Spironolactone Suspensions

Suspension (A)	**(5 mg/ml)**	
	Spironolactone	500 mg (5 tabs x 100 mg)
	Methylcellulose 2%	30 ml
	Dextrose 70% Q.S.	100 ml
Suspension (B)	**(5 mg/ml)**	
	Spironolactone	500 mg (5 tabs x 100 mg)
	Methylcellulose 2%	30 ml
	Syrup B.P. Q.S.	100 ml
Suspension (C)	**(10 mg/ml)**	
	Spironolactone	1,000 mg (100 tabsx100 mg)
	Carboxymethyl cellulose 4%	30 ml
	Diluent Flavor*	500 ml
	Sterile Water Q.S.	1000 ml
Suspension (D)	**(5 mg/ml)**	
	Spironolactone	500 mg
	Sodium benzoate	100 mg
	Ethanol	10%
	Simple syrup Q.S. ad	100 ml
Suspension (E)	**(5 mg/ml)**	
	Spironolactone	500 mg (5 tabs x 100 mg)
	Distilled Water	5 ml
	M.S.Vehicle** Q.S. ad	100 ml

***Diluent flavor contains:** cherry flavored glycol in aqueos base with preservative.

****MS Vehicle (Methylcellulose syrup) contains:** 1% methylcellulose solution (methylcellulose, sodium benzoate in sterile water) and simple syrup.