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Physicochemical and Microbiological Stability of Ursodiol Oral Compounded Suspensions

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OBJECTIVE In the United States, ursodiol is commercially available as solid dosage forms, which represents a problem for children who cannot swallow capsules or tablets. There is a lack of an age-appropriate formulation for ursodiol, and the extemporaneous preparation of an oral suspension with an extended beyond-use-date (BUD) may represent a good therapeutic alternative for the pediatric population. However, all pharmacists need validated stability studies to prepare oral liquids with high quality and safety.

METHODS Oral compounded suspensions for ursodiol 20 to 60 mg/mL were prepared by adding the contents of ursodiol 300-mg commercial capsules (Actavis, KVK Tech, and Mylan) to a proprietary oral suspending vehicle. The BUD of the oral compounded suspensions was determined by using a valid, stability-indicating analytical method. The physical characterization consisted of observing all samples for appearance and color, and testing for pH. Microbiological stability testing followed the United States Pharmacopeia (USP) Chapter 51: Antimicrobial Effectiveness Testing.

RESULTS The ursodiol oral compounded suspensions exhibited a homogeneous white color and the pH did not change significantly. The potency of the oral suspensions remained within ±10% of the specifications. Considering the microbiological characterization, there was no growth of challenge microorganisms throughout the study for all samples.

CONCLUSION This study demonstrates that ursodiol (Actavis, KVK Tech, and Mylan) is physically, chemically, and microbiologically stable in the oral suspending vehicle at room temperature for up to 6 months.

ABBREVIATIONS APIs, active pharmaceutical ingredients; ATCC, American Type Culture Collection BUD, beyond-use-date; PCCA, Professional Compounding Centers of America; UPLC, ultra performance liquid chromatography; USP, United States Pharmacopeia

KEYWORDS extemporaneous preparations; oral vehicle; pharmaceutical compounding; ursodiol suspensions

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Introduction

Ursodiol (ursodeoxycholic acid) is a naturally occurring bile acid that promotes the dissolution of gallstones rich in cholesterol.[1] It is frequently prescribed in children with cholestasis and cystic fibrosis. Typical dosing in children ranges from 5 to 15 mg/kg, depending on therapeutic indication and age. A standard dose for cystic fibrosis in children is 10 to 15 mg/kg/day administered twice daily.[2] In the United States, ursodiol is commercially available as solid dosage forms (200–500 mg),[3] which represents a problem for children who cannot swallow capsules or tablets, and also for the caregivers who have to adjust the dosage strength to meet the individual patient needs. This can often result in cutting tablets or opening capsules to get the required dose.[4]

There is a lack of an age-appropriate formulation for ursodiol, and the extemporaneous preparation of an oral suspension with an extended beyond-use-date (BUD) may represent a good therapeutic alternative for the pediatric population. Oral suspensions may be compounded to include alternative excipients and active pharmaceutical ingredients (APIs) in dosage strengths specifically adapted for children. Despite its advantages, there are complex aspects in terms of formulation and stability of oral compounded suspensions that need to be accounted for, including solubility; uniformity; taste masking; and physical, chemical, and microbiological stability.[5] Oral compounded suspensions may be rapidly prepared by using ready-made vehicles, also adapted for children, which facilitate the extemporaneous preparation in the pharmacy and make the oral administration more pleasant. Ready-made vehicles are particularly important in a hospital setting, considering the particular and often critical health conditions of hospitalized patients who are likely

to need customized medications in a timely manner.[6] Hospital pharmacists require standard operating procedures and stability-indicating studies to rapidly prepare and dispense oral suspensions with the appropriate quality and safety.

The purpose of this study was to determine the physiochemical and microbiological stability of extemporaneously prepared ursodiol oral compounded suspensions 20 to 60 mg/mL in SuspendIt (PCCA [Professional Compounding Centers of America], Houston, TX), for a period of 6 months. SuspendIt is a ready-made, proprietary, oral suspending vehicle used in pediatrics that includes the following ingredients: water, amorphophallus konjac root powder, monk fruit extract (natural sweetener), xanthan gum, potassium sorbate, sodium benzoate, citric acid, and disodium EDTA. This suspending vehicle has special thixotropic properties; it thickens upon standing to minimize settling of particles and becomes fluid upon shaking to allow convenient administration. It is also sugar-free, paraben-free, dye-free and gluten-free, all of which are advantageous in pediatrics.[7] There are many stability-indicating studies in the literature demonstrating extended BUDs of APIs in this suspending vehicle, most recently azathioprine 10 to 50 mg/mL,[8] pyrimethamine 2 mg/mL,[9] and amitriptyline hydrochloride 1 to 5 mg/mL.[10] Pramar et al[11] previously studied the stability of ursodiol (powder) 50 to 100 mg/mL in this suspending vehicle and determined a BUD of 181 days for the oral compounded suspensions stored at both 5°C and 25°C. However, bulk powder APIs are not always readily available in all settings because of the sourcing complexities. For this reason, the sources of ursodiol chosen for this study were the commercial capsules of ursodiol 300 mg from the pharmaceutical companies Actavis (Parsippany, New Jersey), KVK Tech (Newtown, Pennsylvania), and Mylan (Canonsburg, Pennsylvania).

The aim of this study is to provide all pharmacists with a standardized formula for ursodiol, indicated in pediatrics, which can be rapidly prepared extemporaneously by using commercially available products, and that can be used for a prolonged time owing to an extended BUD.

Materials and Methods

Extemporaneous Preparation. Oral compounded suspensions for ursodiol 20 mg/mL and 60 mg/mL were prepared extemporaneously by adding the contents of ursodiol 300-mg commercial capsules (Actavis, KVK Tech, and Mylan) to the oral suspending vehicle according to the general standardized formula and method of preparation detailed in Table 1. A batch of 495 mL was prepared for each strength and for each commercial source of ursodiol, which resulted in a total of 6 different batches. The oral compounded suspensions were evenly distributed into 6 prescription oval amber plastic bottles and stored in an environmentally controlled chamber (model No. 3940, ThermoScientific, Waltham, Massachusetts at a relative humidity of 60% ± 5% and a temperature of 25°C ± 2°C (also referred to as controlled room temperature). The samples were stored for the study period of 6 months.

Physical and Chemical Stability. The physical and chemical stability of the samples were evaluated on days 0 (baseline), 14, 30, 59, 90, and 181 from the extemporaneous preparation. At each predetermined time point, a study sample of each strength and each commercial source (Actavis, KVK Tech, Mylan) was

Table 1. Standardized Formula and Method of Preparation for Ursodiol 20 mg/mL and Ursodiol 60 mg/mL Oral Compounded Suspensions

Rx		495 mL	
Ursodiol 20 mg/mL oral compounded suspension		Ursodiol 60 mg/mL oral compounded suspension	
Ursodiol 300 mg commercial capsules	33	Ursodiol 300 mg commercial capsules	99
Oral suspending vehicle (SuspendIt)	q.s. 495 mL	Oral suspending vehicle (SuspendIt)	q.s. 495 mL

1. Add a SpinBar to a glass beaker calibrated to 495 mL.
2. Empty the contents of the ursodiol capsules to a mortar and pestle.
3. Add approximately 50 mL of the oral vehicle to the mortar and pestle and mix well to make a smooth paste.
4. Add approximately 90 mL of the oral vehicle to the mortar and pestle in portions while mixing and transfer completely to the glass beaker.
5. Rinse the mortar and pestle with approximately 50 mL of the oral vehicle and add to the glass beaker.
6. Add the oral vehicle up to the final volume of 495 mL and mix well.
7. Store in an air-tight, light-resistant container.

withdrawn from the storage chamber at controlled room temperature, shaken vigorously, and tested for physiochemical and microbiological stability.

Physical Stability Testing. The physical characterization of the oral compounded suspensions consisted of visually inspecting the samples, withdrawn from the storage chamber at controlled room temperature, for appearance and color, and testing for pH. The procedure to assess the appearance and color of the samples was to transfer about 5 mL of sample to a clear test tube after vigorously shaking the bottle. Sample was visually inspected against a white background. Any visually detectable characteristics was added to the description of the appearance. The pH was measured with a Horiba LaquaTwin pH meter (Kyoto, Japan). The pH meter was calibrated at pH 4.0 and 7.0 with certified pH buffer solutions before each use.

Chemical Stability Testing. The chemical characterization of the oral compounded suspensions consisted of assay testing using a stability-indicating ultra performance liquid chromatography (UPLC) developed and validated specifically for the quantitation of ursodiol in this formula. A Waters (Milford, Massachusetts) Acquity UPLC H-class system was equipped with a quaternary solvent manager, a sample manager with a flow-through needle, a column heater, and a photodiode array detector. A Waters Acquity UPLC CSH C18 column (130Å, 1.7 µm, 2.1 mm × 75 mm) was heated to 45°C per the method used. Mobile phases consisted of 0.1% trifluoroacetic acid in purified water (A), acetonitrile (B), and purified water (C), with a flow rate of 0.5 mL/min. The elution of the mobile phases is displayed in the supplemental material (Supplemental Table S1). Each injection had an injection volume of 2 µL and a run time of 8 minutes. The wavelength was set at 210 nm.

At each predetermined time point (0, 14, 30, 59, 90, and 181 days from the extemporaneous preparation), 0.5 mL of each oral compounded suspension was withdrawn from the storage chamber at controlled room temperature and transferred to a 50-mL conical centrifuge tube used for sample extraction. A volume of 9.5 mL and 29.5 mL of diluent (methanol) was added to the conical tube for the ursodiol 20 mg/mL and 60 mg/mL oral compounded suspensions, respectively. Samples were well mixed with a vortex mixer for 30 seconds, sonicated for 2 minutes, and centrifuged at 6000 rpm for 10 minutes. The supernatant was transferred to an UPLC vial for injection.

Method Validation. The UPLC method was validated for linearity and range, accuracy, precision (repeatability and intermediate), solution stability, robustness, system suitability, and specificity. The characteristics and acceptance criteria of the method validation testing are displayed in Supplemental Tables S2 and S3.

A linear relationship was evaluated across the range of the analytical procedure. Ursodiol reference standard solutions were prepared at 5 concentrations as 50%, 75%, 100%, 125%, and 150% of 1-mg/mL target concentration.

Accuracy was carried out by spiking the analyte in blank matrices. Spiked samples were prepared in triplicate at 3 concentrations over a range of 80% to 200% of the target concentration. Spiked samples were analyzed by using the analytical method.

Intermediate precision (within-laboratory variation) was performed on 2 different UPLC systems with 2 different lots of columns and on the same instrument on different days. The assay results were evaluated at 3 concentration levels (50%, 100%, and 150%). Spiked samples at each concentration were prepared in triplicate and assayed. Repeatability was analyzed by 6 replicate injections of ursodiol reference standard at target concentration.

The stability of ursodiol reference standard and sample preparation solutions in UPLC vials at room temperature was evaluated for 1 day.

The robustness study was done by ensuring sufficient separation between the ursodiol peak and the adjacent peak after making small changes in flow rate (±3%), mobile phase composition (±2%), and temperature (±2%) to the optimized method parameters.

System suitability tests are an integral part of an analytical method. These tests are used to verify that the chromatographic system is adequate for the intended analysis. Ursodiol reference standard solution at 100% of target level was prepared and injected 6 times.

Stress tests were performed to determine the specificity of the UPLC method for detection of degradation products during storage of the ursodiol oral compounded suspensions. The effects of acidic and basic conditions, as well as heat and oxidation, on the stability of ursodiol were studied. Ursodiol suspension samples were treated with 0.2M hydrochloric acid and 0.2M sodium hydroxide and the resulting preparations were then incubated at 60°C for 3 days to forcibly degrade the ursodiol under acidic and basic conditions. Samples were treated with 6% hydrogen peroxide (H_2O_2) as oxidative agent then incubated at 40°C for 3 days. Samples were incubated at 80°C for 13 days for heat-stressed testing. These samples were analyzed in order to observe for any interference to the ursodiol peak from degradant peaks on chromatograms. Results were expressed in percentage of degradation for acid, base, oxidation, and heat conditions (Supplemental Table S2).

Microbiological Stability Testing. Non-sterile aqueous dosage forms may be exposed to the growth of microorganisms inadvertently introduced during or after the extemporaneous preparation. As such, it is common to add antimicrobial preservatives to inhibit the growth of potential microorganisms. The microbiological attributes of the non-sterile ursodiol oral compounded suspensions were established at baseline, as well

as on days 90 and 181. Microbiological stability testing followed the United States Pharmacopoeia (USP) Chapter 51: Antimicrobial Effectiveness Testing, which uses cultures of the following challenge microorganisms: *Aspergillus brasiliensis* (American Type Culture Collection, ATCC No. 16404), *Candida albicans* (ATCC No. 10231), *Escherichia coli* (ATCC No. 8739), *Pseudomonas aeruginosa* (ATCC No. 9027), and *Staphylococcus aureus* (ATCC No. 6538). For oral products other than antacids, made with aqueous bases or vehicles (category 3), the following criteria apply: bacteria, no less than 1.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days; yeasts and molds, no increase from the initial calculated count at 14 days and 28 days.[12]

Results

Considering the physical characterization, the ursodiol 20-mg/mL and 60-mg/mL oral compounded suspensions presented a homogeneous, white color at every time point of the 6-month study. The pH values of the suspensions did not change significantly throughout the study period, as follows: 5.00 to 5.26 (Actavis ursodiol capsules), 5.00 to 5.24 (KVK Tech ursodiol capsules), and 5.10 to 5.50 (Mylan ursodiol capsules) (Table 2).

With regard to the microbiological characterization, there was no growth of challenge microorganisms throughout the study for all samples, as shown in Table 3. These results complied with microbiological stability requirements established in USP Chapter 51: Antimicrobial Effectiveness Testing.

Considering the chemical characterization, the mean concentration of ursodiol in the oral compounded suspensions did not change significantly throughout the 6 months of the study. For the ursodiol 20-mg/mL oral compounded suspensions, the mean concentration values ranged from 18.7 to 20.2 mg/mL (Actavis ursodiol capsules), 19.8 to 20.3 mg/mL (KVK Tech ursodiol capsules), and 19.5 to 21.2 mg/mL (Mylan ursodiol capsules). Similarly, for the ursodiol 60-mg/mL oral compounded suspensions, the mean concentration values ranged from 57.3 to 60.1 mg/mL (Actavis ursodiol capsules), 57.2 to 59.9 mg/mL (KVK Tech ursodiol capsules), and 59.4 to 61.4 mg/mL (Mylan ursodiol capsules). As such, the potency of all the oral suspensions remained within ±10% of the specification throughout the study, as displayed in Figures 1 and 2. The UPLC method validation demonstrated that the assay testing is linear, precise, accurate, robust, and suitable, as well as stability indicating. The specificity study showed that there was no chromatographic interference to the ursodiol peak and no indication of the detected peak being impure. The results of the method validation testing are displayed in Supplemental Tables S1 and S2.

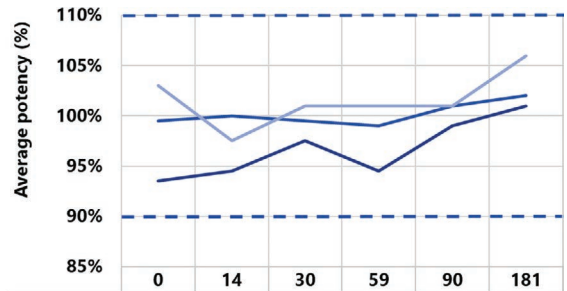
Table 2. pH Values for Ursodiol 20 mg/mL and 60 mg/mL Oral Compounded Suspensions (Actavis, KVK Tech, and Mylan) per Time Point

Time Points (days)	Ursodiol 20 mg/mL			Ursodiol 60 mg/mL		
	Actavis	KVK Tech	Mylan	Actavis	KVK Tech	Mylan
0	5.09	5.15	5.22	5.22	5.21	5.42
14	5.18	5.19	5.25	5.26	5.24	5.50
30	5.09	5.17	5.14	5.16	5.13	5.38
59	5.20	5.20	5.18	5.19	5.19	5.48
90	5.00	5.00	5.10	5.10	5.10	5.30
181	5.17	5.17	5.23	5.17	5.16	5.17

Table 3. Microbiological Stability Testing for Ursodiol 20 mg/mL and 60 mg/mL Oral Compounded Suspensions (Actavis, KVK Tech, and Mylan) at 0, 90, and 181 Days

Challenge Microorganisms	Ursodiol 20 mg/mL			Ursodiol 60 mg/mL		
	Actavis	KVK Tech	Mylan	Actavis	KVK Tech	Mylan
Aspergillus brasiliensis	Pass	Pass	Pass	Pass	Pass	Pass
Candida albicans	Pass	Pass	Pass	Pass	Pass	Pass
Escherichia coli	Pass	Pass	Pass	Pass	Pass	Pass
Pseudomonas aeruginosa	Pass	Pass	Pass	Pass	Pass	Pass
Staphylococcus aureus	Pass	Pass	Pass	Pass	Pass	Pass

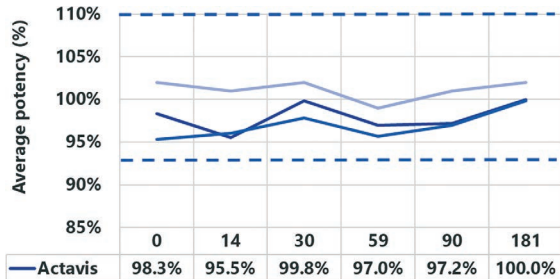
Figure 1. Average percent potency of the ursodiol oral compounded suspension 20 mg/mL over 6 months from extemporaneous preparation. Dashed lines represent the lower and upper limits, corresponding to 90% and 110% of the labelled concentration, respectively.



	0	14	30	59	90	181
Actavis	93.5%	94.5%	97.5%	94.5%	99.0%	101.0%
KVK Tech	99.5%	100.0%	99.5%	99.0%	101.0%	102.0%
Mylan	103.0%	97.5%	101.0%	101.0%	101.0%	106.0%

Days from extemporaneous preparation

Figure 2. Average percent potency of the ursodiol oral compounded suspension 60 mg/mL over 6 months from extemporaneous preparation. Dashed lines represent the lower and upper limits, corresponding to 90% and 110% of the labelled concentration, respectively.



	0	14	30	59	90	181
Actavis	98.3%	95.5%	99.8%	97.0%	97.2%	100.0%
KVK Tech	95.3%	96.0%	97.8%	95.7%	97.0%	99.8%
Mylan	102.0%	101.0%	102.0%	99.0%	101.0%	102.0%

Days from extemporaneous preparation

Discussion

It is hypothesized that the physical stability of the oral compounded suspensions was supported by the buffer system contained in the suspending vehicle, which may have contributed to a relatively constant pH. The pH value can affect the effectiveness of preservatives and the rate of growth of microorganisms. Microbial growth is optimal at a pH range of 6 to 8, and the growth rates of microorganisms are expected to decline outside this range. Therefore, the pH values of the ursodiol oral compounded suspensions were unfavorable for microorganism growth. It is also assumed that the preservative system in the suspending vehicle is likely to have protected the oral compounded suspensions from microbial contamination. The preservatives in the suspending vehicle are potassium sorbate and sodium benzoate, which are broad spectrum preservatives, potentially able to inhibit microorganism growth in the oral compounded suspensions.

The physical, chemical, and microbiological results obtained in this study[13] are consistent with the observations of Pramar et al,[11] who also attributed a BUD of 6 months to ursodiol (powder) 50 to 100 mg/mL in the suspending vehicle, stored at both 5°C and 25°C.

Conclusions

Oral compounded suspensions may be rapidly prepared, allow dosing flexibility, and are easy to administer. However, all pharmacists need validated stability studies to prepare oral liquids with high quality and safety. A palatable, sugar-free formula was developed for ursodiol 20 to 60 mg/mL in an oral suspending vehicle to facilitate the extemporaneous preparation in all settings. This study demonstrates that ursodiol (Actavis, KVK Tech, and Mylan) is physically, chemically, and microbiologically stable in the suspending vehicle at room temperature for up to 6 months. As such, all pharmacists may prepare ursodiol oral compounded suspensions in advance owing to its prolonged stability, ensuring that pediatric patients access an optimized formula in a timely manner.

Article Information

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Disclosure. The authors Kendice Ip, Courtaney Davis, A. J. Day, Craig Urwin, and Maria Carvalho are affiliated with Professional Compounding Centers of America (PCCA), the manufacturer of the proprietary oral suspending vehicle discussed in this study. The authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. Medicines Complete. Ursodeoxycholic acid. In: Brayfield A, Cadart C, eds. *Martindale: The Complete Drug Reference*. London: Pharmaceutical Press; 2023. Updated May 18, 2023. Accessed May 18, 2023. http://www.medicinescomplete.com/
2. Paediatric Formulary Committee. Ursodeoxycholic acid. In: *British National Formulary for Children (BNFc) 2022-2023*. Pharmaceutical Press; 2022.
3. Monograph: ursodeoxycholic acid, ursodiol. In: *Clinical Pharmacology*. Tampa, FL: Elsevier Inc; 2023.
4. De Carvalho R, João M. *Extemporaneously Compounded Oral Medicines in European Hospital Pharmacies* [doctoral thesis]. London, UK: University College London; 2013.
5. Belayneh A, Tessema ZA. Systematic review of the stability of extemporaneous pediatric oral formulations. *ScientificWorldJournal*. 2021;2021:8523091.
6. Carvalho M, Taylor K, Tuleu C. Why do we need hospital pharmacy preparation? *Eur J Hosp Pharm*. 2012;19(5):467–468.
7. Professional Compounding Centers of America (PCCA). SUSPENDIT (30-4825). Accessed January 29, 2024. https://www.pccarx.com/products/PCCASUSPENDIT%C2%AE/30-4825/PROPRIETARYBASES
8. Pramar YV, Mandal TK, Bostanian LA, et al. Physiochemical and microbiological stability of azathioprine suspensions in PCCA Base, SuspendIt. *Int J Pharm Compd*. 2023;27(4):330–339.
9. Pramar YV, Mandal TK, Bostanian LA, et al. Physicochemical and microbiological stability of pyrimethamine in paraben-free PCCA Base, SuspendIt. *Int J Pharm Compd*. 2023;27(2):160–168.
10. Pramar YV, Mandal TK, Bostanian LA, et al. Physicochemical and microbiological stability of amitriptyline hydrochloride oral liquid dosage forms in PCCA Base, SuspendIt. *Int J Pharm Compd*. 2022;26(4):342–351.
11. Pramar YV, Mandal TK, Bostanian LA, et al. Stability of compounded ursodiol suspensions in PCCA Base, SuspendIt. *Int J Pharm Compd*. 2019;23(1):70–76.
12. United States Pharmacopeial Convention, Inc. Antimicrobial effectiveness testing. In: *United States Pharmacopeia 43-National Formulary 38*. Rockville, MD: United States Pharmacopeial Convention, Inc; 2023:6449.
13. Carvalho M, Davis C, Iqbal Z, et al. Physicochemical and microbiological stability of ursodiol oral compounded suspensions. Poster presented at: American Society of Health-System Pharmacists (ASHP) Summer Meetings & Exhibition. June 11–15, 2022; Phoenix, AZ.