

STABILITY OF EXTEMPORANEOUS PEDIATRIC ORAL LIQUIDS COMPOUNDED FROM TABLETS AND DRUG SUBSTANCE: CASE OF PROPRANOLOL AND THEOPHYLLINE

MONIKA MUŚKO^{1*} and MAŁGORZATA SZNITOWSKA²

¹Department of Pharmaceutical Technology, Medical University of Białystok,
2C Mickiewicza St., 15-222 Białystok, Poland

²Department of Pharmaceutical Technology, Medical University of Gdansk,
107 Gen. J. Hallera St., 80-416 Gdańsk, Poland

Abstract: The stability of theophylline (T) and propranolol hydrochloride (P) in extemporaneously compounded oral suspensions (25 mg/mL or 50 mg/mL for T and 2 mg/mL or 5 mg/mL for P) were studied. Suspension with P and T were prepared with bulk substance or tablets using three different suspending vehicles: Ora-Sweet® (M1), modified Ora-Sweet® (M2) and simple syrup with glycerol and sorbitol (M3). Each suspension was stored for 35 days in a dark place at 25°C and 4°C. The results demonstrated that the prepared suspensions with P either from tablets or from a substance were stable in all three studied vehicles (more than 95% of initial concentration remaining). However, it is recommended that storage at 4°C of suspensions prepared with M2 should be avoided because of crystallization of the buffer substances. Extemporaneous suspensions with T in an appropriate pediatric concentrations of the drug were not obtained because the problem of fast crystallization of T was not eliminated.

Keywords: theophylline, propranolol hydrochloride, stability, extemporaneous compounding, suspensions, syrups

Problems in therapy of pediatric patients have been focused for last years. Many drugs are routinely prescribed by physicians although they are not approved by registration agencies for use in infants or children. To reduce this problem much attention in European legislation is already paid to development of suitable medicines for children taking into consideration appropriate routes of administration, dosage form, excipients, taste/palatability and delivery devices (1). Liquid orals are easiest to administer for patients who cannot swallow or have swallowing difficulty of solid dosage forms. Since many drugs are sold as tablets, therefore, modification of the commercial forms is necessary. This modification at home consists in crushing, cutting into smaller segments, removing the content from the capsules and finally mixing with food or liquids. In pharmacies extemporaneous pediatric preparations are also made, however, the final formulation depends on the geographical region: in Poland, Finland or Italy pharmacists prepare powders in capsules (starch or gelatin) or in sachets, while for example in USA,

Sweden, Portugal or Spain more frequently the syrups and suspensions are prepared. In the latter practice commercial suspending vehicles/syrups are very helpful (2, 3). The liquid preparations are ready for administration, however, their stability may be problematic and the pharmacist has to take responsibility to determine proper storage conditions, including the beyond-use date.

Each extemporaneously prepared dosage form is unique in its physical and pharmaceutical properties and the pharmacist must carefully ensure compatibility and stability of the used ingredients. Extemporaneous oral liquid dosage forms with active substances may be prepared according to a published formulary (e.g., in Portugal) or instructions in some other publications. Sometimes the formulation is developed locally and the drug is classified as a *special product* (e.g., in the UK) (4, 5). However, in many countries availability of bulk drug substances for compounding practice is very limited and commercial drug products (tablets or content of capsules)

* Corresponding author: e-mail: monikam@umb.edu.pl

are used instead. This means that excipients present in these products are also introduced to the compounded formulation, what can result in unexpected physicochemical interactions. This makes the stability issue more complex and, for formulations prepared from tablets or capsules, any published data considering particular drug products and suspending media are very helpful.

For oral liquids in European formulary practice the universal, commercial suspending vehicles are used (for example: Ora[®]-products). They consist of suitable preservatives (methylparaben, potassium sorbate) and flavors. These vehicles allow to spare time for preparing formulation, mask unpleasant taste and provide conditions which make the preparation more stable. However, not always commercial multicomponent suspending media are compatible with the drug or components of the used tablets or capsules. For that reason other simple media can be also proposed.

The purpose of the current study was to propose composition of stable extemporaneous pediatric oral liquids with propranolol hydrochloride or theophylline, compounded from bulk drug substance or from tablets. The tablets used for compounding the syrups were products available on Polish market. Three suspending vehicles were

studied. While considering therapeutic dosage range in the pediatric group of the age from 0 to 12 years, two concentrations of liquids were necessary to make possible dosage in volumes suitable for swallowing by children in the whole age range (suitable volume for children under 5 years is below 5 mL and under 10 years – below 10 mL). To ensure this, syrups with propranolol HCl were prepared in concentrations: 2 mg/mL and 5 mg/mL, while for theophylline the concentrations were 25 mg/mL, and 50 mg/mL. The stability of the preparations was determined during 35 days of storage at 25°C and 4°C.

Propranolol hydrochloride (P) is a white odorless crystalline powder with a bitter taste. The solubility in water is approximately 50 mg/mL. The pH of 1% aqueous solution is 5–6, however, the maximum stability of propranolol HCl was reported around pH 3.0, while at alkaline pH the substance undergoes rapid decomposition (9). P has well documented safety in therapy of cardiac diseases in children. As a non-selective β -blocker is commonly used in the treatment of hypertension and ventricular or supraventricular tachycardia. Recent studies have found P to be a novel drug for treatment of hemangiomas in pediatric patients. Because of the variable bioavailability of P, the therapeutic dose

Table 1. Composition of liquid vehicles used in the study [% w/w].

Ingredients	M 1 (Ora-Sweet [®])	M 2	M 3	M 4
Sucrose	53.96	54.0	54.0	2% Methylcellulose solution
Glycerol	4.84	5.0	5.0	
Sorbitol	5.51	4.0	4.0	
Sodium phosphate monobasic	0.58	–	–	
Sodium phosphate dibasic	–	0.12	–	
Citric acid	0.06	0.25	–	
Potassium sorbate	0.08	0.1	–	
Methylparaben	0.03	0.1	–	
Flavor	1.24	–	–	
Water	to 100.0	to 100.0	to 100.0	
pH	4.2	4.2	7.3	6.0
Viscosity [mPas]	72	50	62	100

should be individualized and the average dosage is 0.5–4.0 mg/kg/24 h to maximum 5–10 mg/kg/24 h (10–14).

Because of good solubility of P in water (50 mg/mL) it would be easy to achieve extemporaneous syrups with the drug completely dissolved, however, solution-type preparations were obtained only when a bulk substance was used. Because excipients present in tablets are mostly insoluble in water, therefore, the extemporaneous suspensions from the P tablets were obtained, however, the sediment was formed only by the excipients from tablets.

Theophylline (T) is a white, odorless crystalline powder with a bitter taste and occurs in anhydrous or monohydrate form. It is slightly soluble in water (8.3 mg/mL). The pH of aqueous solutions is 3.5–6 and due to pK_a of 8.8 its solubility increases with increasing pH (6, 7). As an antiasthmatic drug T is used in children in daily therapeutic doses: 5–10 mg/kg b.w. Commercially available tablets or capsules with theophylline are in doses intended for adults (100, 300 mg) and often are in extended-release form. A special care should be paid to the modified-release formulations since their splitting or crushing in order to receive smaller doses is not allowed (8).

EXPERIMENTAL

Chemicals and reagents

Theophylline anhydrous (T) was purchased from Sigma-Aldrich (Steinheim, Germany), propranolol hydrochloride (P) was kindly donated by Polfa Warszawa SA, Poland. Tablets used to prepare liquids were: Theovent 100® (theophylline 100 mg, tablet mass 177 mg, GlaxoSmithKline) and Propranolol WZF® (propranolol hydrochloride 40 mg, tablet mass 249 mg, Polfa Warszawa).

Ora-Sweet® was purchased from Paddock Laboratories (Minneapolis, USA). Media M2–M4 were prepared from chemicals, which were of analytical grade and their composition is presented in Table 1.

The following chemicals were used in HPLC analysis and were of HPLC-grade: acetonitrile (POCH, Gliwice, Poland), methanol (J.T.Baker, Deventer, Netherlands), ortho-phosphoric acid (Fluka, Steinheim, Switzerland) and triethylamine (Fisher Scientific, Loughborough, United Kingdom).

In a spectrophotometric analysis of T methanol (Chempur, Piekary Slaskie, Poland) of analytical grade was used.

Procedure for the preparation of liquids

Liquids with P were prepared in two pediatric doses (2 mg/mL and 5 mg/mL) from drug substances (PS 2, PS 5) or tablets (PT 2, PT 5). Liquids with T were prepared either from drug substance (TS 25, TS 50) or from tablets (TT 25, TT 50) at drug concentration 25 mg/mL or 50 mg/mL. The suspensions (TS, TT, PT) were compounded in a mortar, by adding the media (M1–M4) to the drug substance or to powdered tablets. Because of the reported best stability for P at around pH 3.0, all liquids with P were adjusted to this value using citric acid solution (0.01 mol/L). Each formulation was stored for 35 days in amber bottles, in a dark place at 25°C and 4°C.

Additional preparations were made for T in order to study the effect of compounding technique on the physical stability of liquids.

To eliminate assumption that the intensive grinding of T substance or mass tablet with media in a mortar affected the drug crystallization in the resulting syrups, various preparation techniques were used. The T substance or powdered tablet mass was dissolved/dispersed directly in the vehicle using a magnetic stirrer, without making a concentrate in a mortar. Then, the mixture was transferred to the bottle, the appropriate amount of a vehicle was added and the mixture was shaken for at least 2 min.

Analysis

For all preparations the pH and viscosity (rotational viscometer Viscotester 6 Plus, Thermo Haake, Karlsruhe, Germany) were tested and microscopic (Motic BA400 biological microscope, Moticon, Germany), visual (color) and odor observations, as well as taste evaluation were performed during stability studies.

The T assay was performed spectrophotometrically (Hitachi U-1800 spectrophotometer). The assay for P was performed using the HPLC system consisting of an Agilent Technologies 1200 chromatograph, equipped with a binary pump, thermostat, degasser and diode array detector.

Analysis of propranolol hydrochloride

The stability-indicated HPLC assay was developed by modification based on the previously reported methods (15–18). An isocratic separation was carried out on an Eclipse XDB-C18 column (particle size 5 μ m, 4.6 \times 150 mm). The mobile phase was composed of methanol/acetonitrile/phosphate buffer (0.064 mol/L, pH 3.0) 20 : 15 : 65 (v/v/v) with 0.2% (w/v) of triethylamine and with

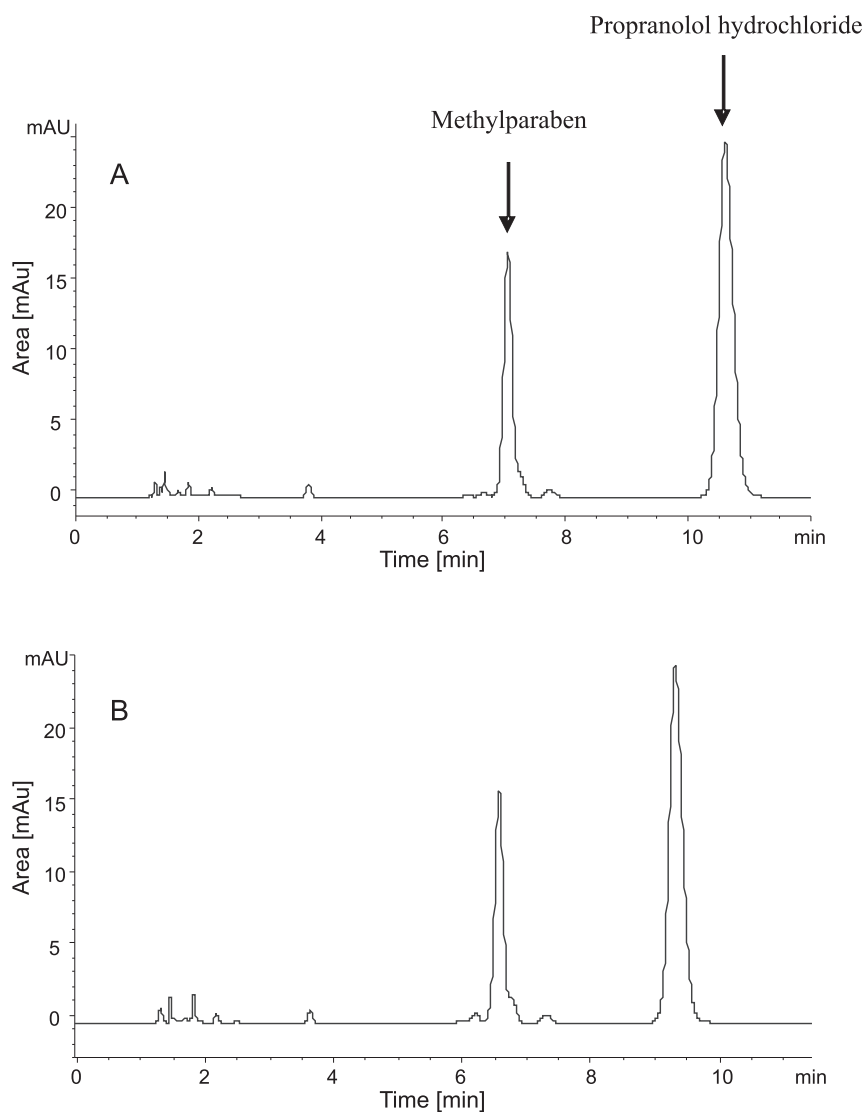


Figure 1. Representative chromatograms of propranolol hydrochloride (2 mg/mL) in a syrup prepared from tablets with M1 vehicle: after preparation (A) and after 35 days of storage at 25°C (B).

the pH adjusted to 3.0. The flow rate was 1 mL/min. The samples of each liquid (1.0 mL) were diluted (to 100 mL) with methanol. A 20 μ L aliquots were injected and analyzed with the UV detection at 291 nm. Chromatographic retention time of propranolol was about 11 min. It was ensured that other components of the preparations did not interfere with the peak of P on a chromatogram (Fig. 1). Concentrations of P were determined from the calibration curve obtained for standard solutions.

Analysis of theophylline

Samples from each compounded suspension were withdrawn (1.0 mL) and diluted (to 25.0 mL) with methanol. The solution was filtered through a filter paper and 100 μ L was diluted to 10.0 mL with methanol. The samples were assayed in triplicate at an analytical wavelength 273 nm. Methanol was used as a reference solution. Concentrations of T in each sample were determined from the calibration curve (19).

RESULTS AND DISCUSSION

Propranolol hydrochloride

Extemporaneous liquids with P were prepared using three different media: commercial syrup – Ora-Sweet® (M1) and compounded media M2 and M3. Medium M2 was prepared according to the US monograph *Vehicle for oral solution* and M3 medium was a sucrose syrup (54% w/w) with sorbitol and glycerol (Table 1) (20). The vehicles M1 and M2 contain a mixture of methylparaben and potassium sorbate as preservatives. Neither detectable changes in color or odor and taste, nor visual microbial growth were observed in any formulation prepared with P, stored at 25°C or 4°C, in spite of the fact that possibility of fungal growth was reported if P was compounded with media without preservatives (9). Although we did not observe any microbial growth in a preservative-free M3 medium, however, according to the USP guidelines only 14 days expiry date can be proposed when a compounded liquid does not contain preservatives.

Each formulation with P prepared with M1, M2 or M3 had the same sweet taste but with various intensity. However, slight bitter aftertaste in all P formulations was detected either. Liquids prepared with M1 were the most palatable because of the cit-

rus-berry sweet flavor coming from Ora-Sweet®. The palatability of P liquids was determined by an adult person in a *swill and spit* test (21). Samples of P liquid were tested by swilling in the mouth for 30 s without swallowing and then were spat out. The mouth was washed by mineral water after each tested sample. Taking into consideration that the sense of taste and smell in children is different from that of adults, the palatability of P liquids was conducted only as a screening test.

As a hydrochloride salt, P demonstrates good solubility in water (see above). Precipitation of the active substance was not observed during the study period in syrups prepared from the substance dissolved in M1 and M3 and stored at 4°C or 25°C. Because of good solubility of P in water the sediment in formulations prepared from tablets was formed only by the insoluble tablet's excipients (tablets of Propranolol 40 are white and uncoated, consisting of lactose monohydrate, sucrose, starch, talc, magnesium stearate and Povidone K25). The sediment was downy and easily re-suspendable after shaking, thus homogeneity of the formulation was not problematic, which is very important for accurate dosing. The microscopic analysis revealed no P crystals but other particles, e.g., starch grains, with size not exceeding 20 µm, were not growing during the study period.

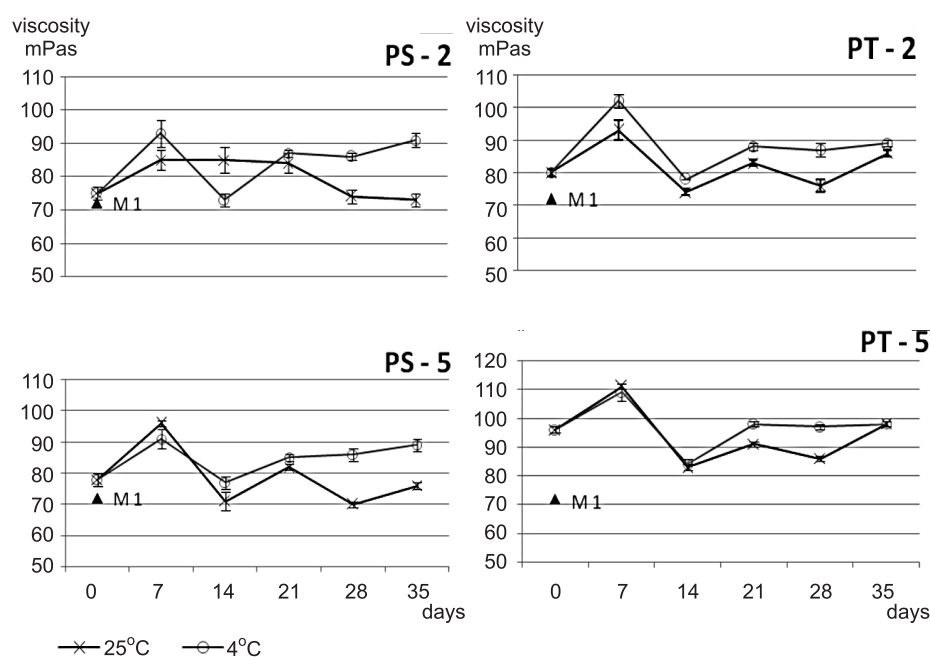


Figure 2. Change in viscosity [mPas] of liquids with propranolol hydrochloride (PS2 and PT2 – 2 mg/mL and PS5 and PT5 – 5 mg/mL) prepared with M1 vehicle from the substance (PS) or from tablets (PT) and stored for 35 days at 25°C and 4°C (mean ± S.D.; n = 3).

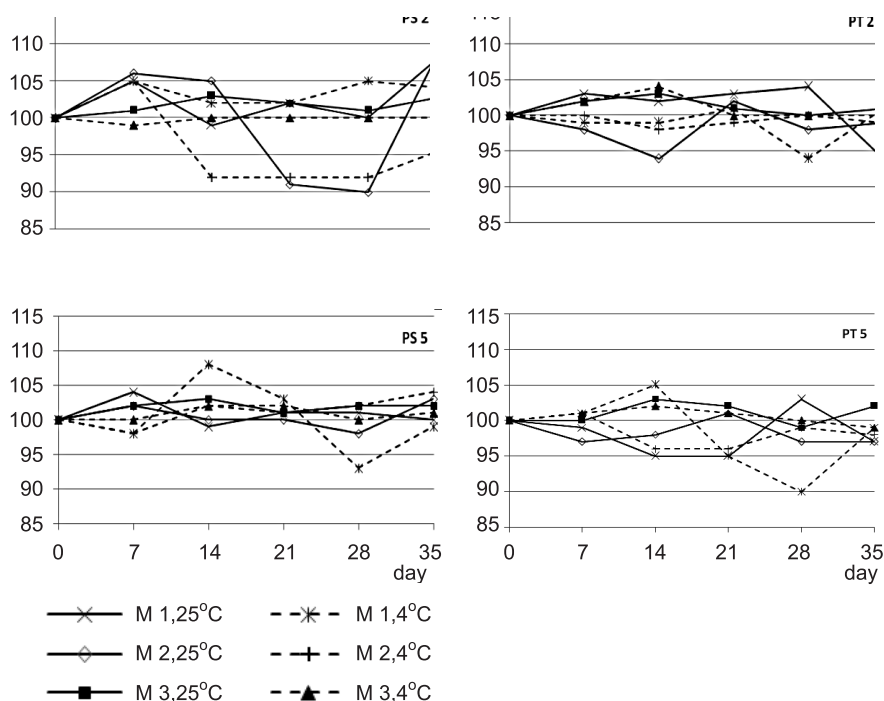


Figure 3. Stability of propranolol hydrochloride in oral liquids (PS2 and PT2 – 2 mg/mL and PS5 and PT5 – 5 mg/mL) prepared with M1, M2 or M3 media from the substance (PS) or from tablets (PT), stored for 35 days at 25°C and 4°C (mean values, $n = 3$).

The time for complete sedimentation of tablet particles was long: the longest in M3 (28 days) while the earliest separation was observed in M1 and M2 (7 days). In M2 preparations stored at 4°C after one week, the crystallization was observed, however, it was found that high concentration of citric buffer caused the problem. To eliminate assumption that P crystallized, the vehicle M2 without the active drug with pH value adjusted to 3.0, was stored at 4°C and it was found that similar crystallization occurred after 7 days. Qualitative inorganic analysis also indicated that the citric ions formed crystals.

The pH is a very important parameter for physical and chemical stability of the liquid formulations. The pH of all liquids with P was adjusted to 3.0 and there were no significant changes in pH during storage for 35 days at 4°C or 25°C.

Liquids were also evaluated for the change of viscosity. After combining drug substance or powdered tablets with the vehicle the increase of viscosity was recorded. Considering the high ratio of excipients to drug substance in P tablets (5 : 1), the effect of excipients on viscosity was minor, resulting in similar viscosity of PS and PT syrups. Significant growth of the viscosity was observed

after 7 days, what was generally independent of the storage conditions and the vehicle used, although in the PS 2 and PS 5 prepared with M3 such effect was not recorded. The viscosity changes in liquids prepared with M1 either from the substance or tablets were similar, demonstrating an initial increase after 7 days with further decrease during the following days in such a manner that after 14 days this parameter was similar or lower than the initial value (Fig. 2). The slight growth of the viscosity in P liquids prepared from tablets using M2 was noted during the study period at 4°C and 25°C. The highest increase was observed in PT 2 and PT 5 stored at 4°C. The viscosity measured on 35th day was 65 mPas and 62 mPas, respectively, while initial values were 50 mPas and 55 mPas, respectively. In syrups prepared with M2 from the P substance the viscosity remained unchanged. Any changes in viscosity did not result in any application difficulty and were not seen visually either.

Good chemical stability of P in suspensions (1 mg/mL) prepared from tablets during storage for 120 days at ambient and refrigerated temperatures was documented by Henry et al. (22). However, later stability studies demonstrated numerous physi-

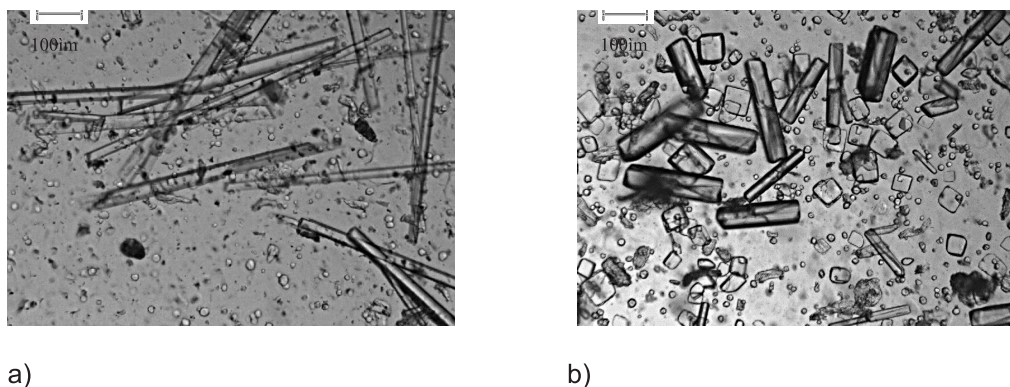


Figure 4. Crystals of theophylline in liquids at concentration of 25 mg/mL, prepared from tablets with M3 (a) or M4 (b) suspending vehicle: after 7 days of storage at 25°C.

cal and visual changes of P formulations (1–5 mg/mL) in various vehicles after 12 weeks of storage at 4°C and 30°C (9). In the current study, the results show that P in oral liquids prepared in M1, M2 and M3 media, either from the bulk substance or from tablets, was stable for at least 35 days at 25°C and 4°C as demonstrated in Figure 3. The acceptable limit 95–105% of the initial concentration was easily achieved in the majority of the investigated formulations. However, the slight exceptions were observed, since the HPLC determination of P showed that after 4 weeks at 4°C and after 5 weeks at 25°C the per cent of the P remaining in PT 2 prepared with M1 was smaller than 95% but not lower than 90% of the initial concentration. In liquids PS 2 prepared with M2 the similar changes were observed either. These deviations from the acceptable concentration limit can not be associated with the analytical error since reproducibility of the method was proved.

Recommended by USP the beyond-use date for extemporaneous oral aqueous liquid dosage forms, i.e., 14 days, was achieved for the preparations stored at cold temperature. Current study has shown that P is stable for 35 days at 25°C or 4°C in liquids prepared with Ora-Sweet® and M3 vehicles using either tablets or bulk substance. Even if no microbial or fungal growth was observed in the non-preserved M3 medium, it is not sufficient evidence for microbiological stability and storage time longer than 14 days should be avoided. Due to crystallization of the citric buffer in refrigerated formulations prepared with M2 stability for 35 days can be only achieved when they are stored at 25°C what is not favorable for microbiological stability and for this reason shorter beyond-use date should be proposed.

Theophylline

No detectable changes in color or odor and taste were observed in the suspensions prepared with T during 35 days storage at 25°C or 4°C. Even in suspensions prepared with the medium without preservatives (M3) no microbial growth was noted. Because of high concentrations of T, very strong bitter aftertaste was experienced in all liquids regardless of the sweet media flavor.

Due to low solubility of T in the prepared syrups the drug occurred as suspension. However, T was partially dissolved in the aqueous phase and after one week of storage, both at 25°C and 4°C, in all media the fast crystallization of this fraction occurred. The formed crystal sediment was difficult to disperse by manual shaking. Microscopic analysis demonstrated crystals in size 60–200 µm or bigger. Therefore, stable extemporaneous suspensions in the proposed doses (25 mg/mL and 50 mg/mL) were not obtained, regardless of the starting material (substance or tablets). In order to avoid re-crystallization methycellulose viscous solution was used as a suspending medium (M4), however, this was not helpful, although crystallization appeared later (after 2 days). Figure 1 shows crystals of T in suspensions prepared from tablets using M3 and M4 media. The size of the T crystals exceeds 200 µm, but their shape is different, depending on a vehicle. When the pH of the suspension (25 mg/mL) was increased to 8.0 crystallization of T from the supersaturated solution occurred immediately after preparation.

To recognize in which concentration crystallization does not occur, T was introduced to media at lower concentrations, i.e., 2 mg/mL, 5 mg/mL and 10 mg/mL. All liquids were developed with two various techniques of preparation, both from tablets or

a bulk substance. The crystallization of T was not observed only if the concentration was 2 mg/mL. However, in this case administered volume of T liquid ensuring therapeutic dose would be too large, e.g., for 1 year child 50 mL vs. 4 mL if the concentration would be 25 mg/mL.

Results of the current study showed that pH of liquids prepared either from the substance or from tablets remained unchanged and was 6.4 and 6.7–7.0, respectively. The initial viscosity values for TS 25, TS 50, TT 25 and TT 50 liquids prepared with M3 were 71, 94, 84 and 97 mPas, respectively. These values were higher than observed for P liquids prepared with the same vehicle, what resulted from larger quantity of the added drug substance and tablet mass. However, despite of T crystallization, viscosity of the TS 25 remained unchanged during the study period at both temperatures. The highest increase of viscosity (up to 150 mPas within 7 days) was observed in TT 50 formulation.

Johnson et al. (8) evaluated stability of T in non-alcoholic liquids containing the drug at low concentration (5 mg/mL) and they demonstrated good stability of T at least for 90 days in mixtures (1 : 1) of Ora-Sweet® and Ora-Plus® or Ora-Sweet® SF and Ora-Plus®. Determination of T in liquids showed that only in the TS 25 and TS 50 prepared with M3 the initial concentration remained unchanged (100% and 101%, respectively, after 4 weeks of storage at 25°C). In the other liquids after 2 weeks at both temperatures, the percent of the determined concentration was even higher than 105% of the initial value, what can be related to the crystallization problems.

These data demonstrated that stable liquids with T, prepared from tablets or substance, with drug concentration 25 mg/mL or 50 mg/mL were not obtained because of the fast crystallization of T. Besides, bitter taste of the drug is another serious problem that makes the compounding of liquid aqueous preparations with T impossible. Thus, powders prepared from a substance or from tablets, mixed before administration with a food is recommended practice in pediatric care.

CONCLUSIONS

Compounded extemporaneous oral liquids with P may provide flexible and convenient dosage forms for pediatric patients using both tablets and bulk powder. Oral pediatric liquids with P, prepared from tablets or from a substance (at concentrations of 2 mg/mL and 5 mg/mL) in Ora-Sweet® (M1) or in a simple syrup with sorbitol and glycerol (M3), are

stable for at least 35 days when stored either at 25°C or 4°C. When unpreserved vehicles/syrups are used for compounded oral liquids, the short-term storage condition must be considered. Due to better palatability commercial Ora-Sweet® vehicle is a better choice. On the other hand, M2 vehicle crystallizes when the preparations are refrigerated. Although during storage, the increase of viscosity in the preparations compounded with M1 and M2 media were observed, it does not present an application problem. Regardless of the pH being unchanged, crystallization process of theophylline in all investigated media remained an unsolved problem what makes impossible compounding of T liquids in the required concentrations of 25 mg/mL or 50 mg/mL.

REFERENCES

1. Beverley D.G., Haywood A.: *J. Pharm. Pharmaceut. Sci.* 9, 398 (2006).
2. Brion F., Nunn A.J., Rieutord A.: *Acta Paediatr.* 92, 486 (2003).
3. Hempenstall J., Tuleu C.: *Int. J. Pharm.* 379, 143 (2009).
4. Carvalho M., Tuleu C., Taylor K.M.G.: *Int. J. Pharm. Comp.* 12, 8 (2008).
5. Carvalho M.: *Int. J. Pharm. Comp.* 9, 449 (2005).
6. Trissel L.A.: *Stability of compounded formulations*, p. 249, Am. Pharm. Assoc., Washington 1996.
7. www.drugbank.ca
8. Johnson C.E., Van De Koppel S., Myers E.: *Am. J. Health Syst. Pharm.* 62, 2518 (2005).
9. Trissel L.A.: *Stability of compounded formulations*, p. 224, Am. Pharm. Assoc., Washington 1996.
10. Zimmermann A.P., Wiegand S., Werner J.A., Eivazi B.: *Int. J. Pediatr. Otorhinolaryngol.* 74, 338 (2010).
11. Boyce J., Dodge-Palomba S.: *J. Am. Soc. Register. Nurs.* 35, 22 (2010).
12. Sharp S., Thomas C., Rosenberg L., Rosenberg M., Meyer W.: *J. Trauma* 68, 193 (2010).
13. Seikaly M.G.: *Curr. Opin. Pediatr.* 19, 170 (2007).
14. Polakowska E., Polakowski M.: *Wiad. Lek.* 59, 5 (2006).
15. Modamio P., Lastra C.F., Montejo O., Mariño E.L.: *Int. J. Pharm.* 130, 137 (1996).
16. Basci N.E., Temizer A., Bozkurt A., Isimer A.: *J. Pharm. Biomed. Anal.* 18, 745 (1998).
17. Modamio P., Lastra C.F., Mariño E.L.: *J. Pharm. Biomed. Anal.* 17, 507 (1998).

18. El-Saharty Y.S.: J. Pharm. Biomed. Anal. 33, 699 (2003).
19. The Polish Pharmacopoeia VI, p. 252, The Polish Pharmaceutical Society, Warszawa 2002.
20. United States Pharmacopoeia 32, National Formulary 27, United States Pharmacopoeia Convention, Rockville 2009.
21. Wróblewska M., Winnicka K.: Farm. Pol. 67, 66 (2011).
22. Henry D.W., Repta A.J., Smith F.M., White S.J.: Am. J. Health. Syst. 43, 1492 (1986).
23. Bartolomei M., Bertocchi P., Ramusino M.C., Signoretti E.C.: Thermochem. Acta 321, 43 (1998).
24. Bartolomei M., Bertocchi P., Ramusino M.C., Santucci N., Valvo L.: J. Pharm. Biomed. Anal. 21, 299 (1999).
25. Uwai K., Tani M., Ohtake Y., Abe S., Maruko A., Chiba T., Hamaya Y., Ohkubo Y., Takeshita M.: Life Sci. 78, 357 (2005).

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