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FORMULATION AND STABILITY STUDY OF THE EXTEMPORANEOUS ORAL SOLUTIONS OF CARDIOLOGIC DRUGS FOR PERSONALISED THERAPY OF NEWBORNS

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OBJECTIVES

The commercially available medicinal products do not completely cover the wide spectra of preparations particularly when targeted to the pediatric patients. Under such circumstance, the pharmacist needs to compound the preparation extemporaneously. In the case of neonates, an oral solution is the best dosage form from both the application and the correct dose points of view.

This study deals with the formulation and stability testing of the extemporaneous aqueous solutions of three cardiologic drugs: propranolol, sotalol and furosemide, directed to newborns.

Propranolol hydrochloride (PCL)

is a non-cardio selective beta blocker administered in therapy of cardiovascular diseases, particularly Fallot tetralogy and hypertension; the relatively new indication includes infantile hemangioma *(Léauté-Labrèze 2008)*. PCL is orally administered from newborns to school children at an initial dose of 2 to 3 mg/kg daily in two or three divided doses.

Sotalol hydrochloride (SCL)

is an antiarrhythmic beta-blocker, highly effective and well tolerated in the treatment of ventricular and supraventricular tachycardia in children. It is recommended to administer sotalol to children in initial oral dose 1 mg/kg twice daily, increased as necessary every 3-4 days to max. 4 mg/kg twice daily. *(Läer 2005)*.

Furosemide (FSM)

represents a traditional diuretic widely used in paediatric patients inthe treatment of hypertension and oedema associated with heart failure. Usually, the oral dose for neonates is 0.5 to 2 mg per kilogram of weight every 12 to 24 hours, in two or three divided doses from 1 month, increasing the dose in older children.

Formulation study

While both PCL and SCL are water soluble substances, FSM is practically insoluble in water, however, it dissolves in solutions of alkali hydroxides (pH > 8.0). To avoid the use of ethanol or sodium hydroxide, freshly prepared aqueous solution of **disodium hydrogen phosphate dodecahydrate (DNaHP)** was used.
The proposed solutions are targeted to neonates **(NEO)**, therefore, they should be preservative free. The microbiological stability is provided due to aseptic technique and final sterilization of the product. The influence of the sterilisation method on the stability of a drug was investigated; stability limit of maximum **5%degradation** of the drug content was the basic criterion.

METHODS

Preparation of solutions (Hospital Pharmacy of University Hospital Motol)
The solutions of **propranolol hydrochloride (PCL) 2 mg/mL** and **sotalol hydrochloride (SCL)5 mg/ml** were prepared by dissolution of the substance in **water for injection (WFI)**.
The solution of furosemide (FSM)2 mg/mL was prepared as fast as possible (protection from the light degradation) by dissolution of FSM in approximately 20 mL of freshly prepared **7.5% (w/w) of disodium hydrogen phosphate dodecahydrate (DNaHP)** solution and then filled up to a total volume of 100.0 ml with WFI.
The solutions were prepared under aseptic conditions and sterilised either using a bacteria retentive filter (0.22 µm, *Samples BF*) or autoclaved (121°C, 15 minutes, *Samples A*).

HPLC method
Analytical reagents (PCL)
The following reagents of analytical grade were used in HPLC methods: acetonitrile, methanol, tetrahydrofuran, sulphuric acid (≥ 95 - 97%), and sodium dodecyl sulphate (≥ 98.5%) (all obtained from Sigma-Aldrich, Germany), ethylparaben, butylparaben,sodium dihydrogen phosphate and tetrabutylammonium dihydrogen phosphate (≥ 97.0%) (both from Fluka, Germany),phosphoric acid, formic acid (Merck, Germany), and sodium hydroxide (Penta, Czech Republic).
Instrumentation and analytical conditions (PCL)
A stability indicating HPLC assay was developed using ethylparaben and butylparaben as internal standards. The HPLC system consisted of a Shimadzu LC-2010C (CLASS-VP Software, Shimadzu, Japan) with Dual λ Absorbance UV Detector. Separation was achieved using a Supelco Discovery® C18 column (25 cm x 4.6 mm x 5 µm), Ascentis Express C18 (100 x 4.6 mm x 2.7 µm) and Supelco Discovery® C18 (150 x 4.6 mm x 5 µm) (all Supelco, USA). The isocratic flow rates were 1.8 ml/min, 1.3 ml/min, and 1.5 ml/min, respectively, and the UV detector was set at a wavelength of 230 nm (PCL), 237 nm (SCL) and 270 nm (FSM), respectively.
The mobile phases consisted of buffers mixed with organic solvents (methanol, acetonitrile); the isocratic as well as the gradient (SCL) elution were used.
The mobile phase solutions were filtrated through a 0.45 µm filter (Glass Microfiber Filters, Whatman, UK) and then sonicated for the few minutes (Sonorex Digitec, Bandelin, Germany) before HPLC analysis.
The HPLC methods were successfully and completely validated by following Q2(R1) ICH guideline (1997). System suitability parameters (n = 6) and validation data are summarized in **Table 1. Details** of the methods are referred to in the articles: Zahálka et al 2013, Matysová et al 2015, Zahálka et al 2017.

Table 1: Validation of HPLC methods

System suitability parameters	PCL	SCL	FSM	Criteria
Repeatability t_R RSD (%)	0.16	0.00	0.26	X < 1 %
Repeatability Area RSD (%)	0.09	0.22	0.16	X < 1 %
Theoretical Plates	8441	1181	2499	-
Resolution	8.82	-	1.57	R_{ij} > 1.5
Tailing factor	1.19	1.10	1.18	T = 0.8–1.5
Validation criteria	**PCL**	**SCL**	**FSM**	
Precision RSD (%)[a]	0.16	0.31	3.55	X < 5 %
Linearity (R)	0.9997	0.9995	0.9990	R ≥ 0.9990
Accuracy Recovery (%)[a]	99.99	99.91	103.48	X = 100 ± 5 %
Accuracy RSD (%)[a]	0.28	0.83	0.61	X < 5 %
Selectivity	No interference			No interference

a - six samples, three injections of each sample; t_R – retention time, SST – System suitability test

Stability study
Two batches of the preparation were prepared, each batch was divided into **two separate** samples which were filled into the closed bottles (Sample BF) or into an autoclave bottle (Sample A). Number of containers was chosen considering to open new container at each stability time point in order to avoid the contamination of samples.
The preparations were stored at room temperature (25 ± 3°C), protected from the light.
The concentration of drugs (PCL, SCL, FSM) was evaluated at **the beginning** of the stability assay (at time of compounding, $\mathbf{t_0}$, a content of 100 %), **after the sterilization in an autoclave** ($\mathbf{t_{0A}}$) and thereafter at time intervals of **7 – 14 – 30 days**. The value of pH vas estimated (pH 212 Microprocessor pH Meter, Hanna instruments, Germany), as well.
Each sample was measured in triplicate; the average values of the remaining percentage content (n = 6) of drug with the relative standard deviations (RSD, %) in brackets are summarized.

RESULTS AND DISCUSSION

The average content of drug in mg/mL in the samples sterilised using bacterial filtration (BF) and/or autoclave (A), respectively, are summarized in **Tables 2 – 4** (relative standard deviations RSD in % in brackets). The limit ± 5% of the initial drug concentration is shown in **Figures**. In **Table 5**, pH values of drug solutions are listed.

Table 2: The average content of propranolol-hydrochloride (mg/mL) in NEO solutions 2 mg/ml *(% RSD)*

Day	0 (100 %)	0A	7	14	30
PCL BF	2.088 (1.72)	-	2.093 (0.36)	2.113 (0.29)	2.091 (0.33)
PCL A	2.048 (0.99)	2.075 (2.60)	2.019 (1.50)	1.940 (1.42)	1.855 (0.45)

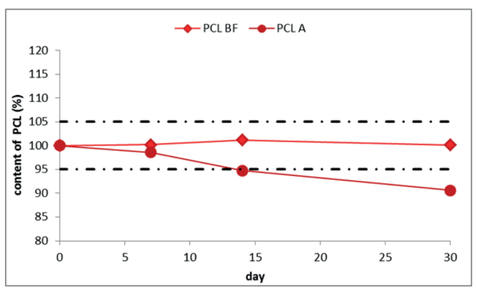

Table 3: The average content of sotalol hydrochloride (mg/mL) in NEO solutions 5 mg/mL *(% RSD)*

Day	0 (100 %)	0A	7	14	30
SCL BF	5.221 (1.74)	-	5.241 (1.70)	5.134 (1.29)	5.164 (1.17)
SCL A	5.170 (1.65)	5.143 (2.22)	5.157 (1.30)	5.134 (1.49)	5.142 (1.55)

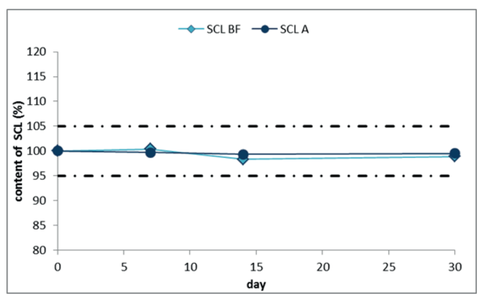

Table 4: The average content of furosemide (mg/mL) in NEO solutions 2 mg/mL *(% RSD)*

Day	0 (100 %)	0A	7	14	30
FSM BF	2.157 (2.94)		2.212 (0.68)	2.213 (1.46)	2.190 (0.83)
FSM A	2.192 (0.97)	2.199 (0.76)	2.212 (2.64)	2.236 (1.62)	2.222 (1.42)

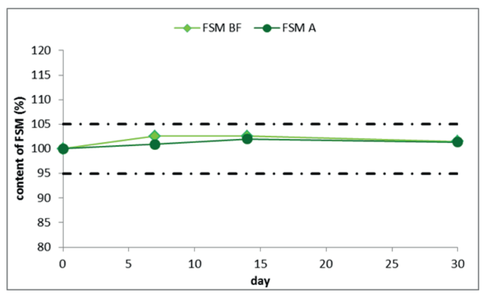

Table 5: pH values of NEO solutions

Day	0	0A	7	14	30
PCL BF	5.94	-	6.13	6.24	6.36
PCL A	5.41	5.62	5.72	4.79	3.89
SCL BF	5.44	-	5.43	5.52	5.50
SCL A	5.44	5.43	5.42	5.50	5.51
FSM BF	7.65	-	7.67	7.61	7.66
FSM A	7.66	5.65	7.67	7.61	7.68

CONCLUSIONS

The preparation of FSM solution using disodium hydrogen phosphate is easy and fast as DNaHP is easier to manipulate and weigh than sodium hydroxide. Moreover, faster preparation serves better light protection of a drug.
The concentration of the aqueous solutions of SOT and FSM was unchanged after autoclaving; the pH value remained in the range of 5.42 -5.51 for SCL and 7.65 – 7.68 for FSM, respectively, within the time period of 30 days of storage at room temperature.
Although the PCL solution sterilised using BF was stable at room temperature having pH value in a range of 5.94 -6.36, the preparation complied to the main criterion of maximum drug concentration decrease 5 % only for less than two weeks. The decrease in PCL concentration was associated with the decrease in pH value. In order to increase stability during autoclaving, the buffering with citric acid before sterilisation is probably necessary. This would be verified in the following study.

ACKNOWLEDGEMENTS

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