

# Simultaneous Determination of Propranolol Hydrochloride and Sodium Benzoate in Oral Liquid Preparations by HPLC

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**Abstract** A simple, selective and sensitive HPLC–UV method for quantification of propranolol hydrochloride and sodium benzoate in oral liquid preparations was developed and fully validated. Separation was performed by Supelco Discovery® C18 (25 cm × 4.6 mm, particles 5 µm) column. UV/VIS absorbance detector was set at wavelength 230 nm. Column oven was conditioned to 25 °C. Mobile phase was prepared by dissolving 1.6 g of sodium dodecyl sulphate and 0.31 g of tetrabutylammonium dihydrogen phosphate in 450 mL of ultrapure water; 1 mL of sulphuric acid (95–97 %) and 550 mL of acetonitrile were added. Sodium hydroxide solution (2.1 M) was used for adjusting pH to value 3.3 (±0.05). Retention times of sodium benzoate, propranolol hydrochloride and butylparaben (internal standard) were 2.2, 3.3 and 4.1 min, respectively. Newly developed method is suitable for simultaneous determination of propranolol hydrochloride and sodium benzoate in oral liquid preparations which are used for therapy of haemangiomas in paediatric patients. Method

has been applied for stability testing of extemporaneous paediatric oral formulations containing propranolol hydrochloride.

**Keywords** HPLC · Propranolol hydrochloride · Sodium benzoate · Paediatric oral formulations

## Introduction

Infantile haemangiomas (IHs) are the most common soft-tissue tumours of infancy. Corticosteroids are currently the main treatment for complicated IHs, with interferon or vincristine as second- or third-line treatment. Because of the partial efficacy and side effects of these drugs, new treatments are needed [1]. Propranolol (PRO) is a non-selective beta blocker. Its main indication has changed from therapy of cardiovascular diseases (such as hypertension) to therapy of IHs in paediatric patients during last few years. Propranolol administered orally at 2–3 mg kg<sup>-1</sup> per day has a consistent, rapid, therapeutic effect, leading to considerable shortening of the natural course of IHs, with good clinical tolerance [1]. Sodium benzoate (BEN) is used as an antimicrobial preservative in cosmetics, foods, and pharmaceuticals. BEN is used to prolong microbial stability in preparations dedicated for children over 3 years. There is no registered medicinal product containing propranolol available in the Czech Republic [2]. Paediatric formulations have many specificities. The most important are the ability to dose variable amount of active substance according to the weight of the children and dosage form has to be easy to swallow [3, 4]. The most suitable way is using oral liquid extemporaneous preparations with antimicrobial agent for older children and with no antimicrobial agent for infants. The cooperation of

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Department of Pharmaceutical Technology (Hradec Králové) and Hospital Pharmacy of General Hospital in Motol (Prague) led to development of ten versions of oral liquid preparations with PRO as an active substance and BEN as a preservative. Determination of PRO by means of HPLC has been already mentioned in literature [5–8], but simultaneous determination of PRO and BEN in various matrices of liquid dosage forms has not been published yet. The aim of this study was to develop and validate selective, simple and rapid HPLC method for the determination of active substance propranolol hydrochloride and antimicrobial agent sodium benzoate in newly developed paediatric oral preparations.

## Materials and Methods

### Materials and Reagents

Propranolol hydrochloride (Sigma-Aldrich, Steinheim, Germany) and sodium benzoate (Dr. Kulich Pharma, Hradec Králové, Czech Republic) were used as standards. Labetalol hydrochloride (Sigma-Aldrich, Steinheim, Germany), acebutolol hydrochloride (Sigma-Aldrich, Steinheim, Germany), pindolol (Sigma-Aldrich, Steinheim, Germany), metoprolol tartrate (Sigma-Aldrich, Steinheim, Germany), methylparaben (Fluka, Buchs, Germany), ethylparaben (Fluka, Buchs, Germany), propylparaben (Fluka, Buchs, Germany) and butylparaben (BP) (Fluka, Buchs, Germany) were used as internal standards. Sodium dodecyl sulphate (Sigma-Aldrich, Steinheim, Germany), tetrabutylammonium dihydrogen phosphate (Sigma-Aldrich, Steinheim, Germany), sulphuric acid (Sigma-Aldrich, Steinheim, Germany), sodium hydroxide (Penta, Chrudim, Czech Republic), acetonitrile (ACN) gradient grade (Sigma-Aldrich, Steinheim, Germany) and methanol (MeOH) gradient grade (Sigma-Aldrich, Steinheim, Germany) were used for the preparation of the mobile phase. Water for sample and mobile phase preparation was purified by Milli-Q Integral 15 system with 0.22 µm output filter. 0.45 µm Nylon membrane filters were used for mobile phase filtration (Merck Millipore, Billerica, USA). 0.45 µm Nylon membrane filters (Fisher Scientific, Pardubice, Czech Republic) and 2 mL syringes (Chirana T. Injecta, Stará Tůra, Slovak Republic) were used for filtration of the solutions before injection. Micropipette Transferpette 1,000 µL (Brand, Wertheim, Germany) was used. Formulations ST1–ST10 (incl. blank solutions) that contain propranolol hydrochloride (2 or 8 mg mL<sup>-1</sup>), sodium benzoate (0.5 mg mL<sup>-1</sup>) and excipients (e.g. water for injection, sirupus simplex, sodium saccharin, citrate–phosphate buffer pH 3 and citric acid) were obtained from Hospital Pharmacy of General Hospital in Motol, Prague, Czech Republic.

### Instrumentation and Chromatographic Conditions

Chromatographic analysis was performed on integral HPLC system Shimadzu LC-2010C (Shimadzu, Kyoto, Japan). Supelco Discovery<sup>®</sup> C18 (25 cm × 4.6 mm, particles 5 µm) column was used. Dual absorbance UV/VIS detector was set at wavelength 230 nm. Isocratic elution with flow 1.8 mL min<sup>-1</sup> was used. Column temperature was conditioned to 25 °C. Injection volume was 5 µL and time of analysis was 5 min.

### Preparation of Mobile Phase (~1 L)

1.6 g of sodium dodecyl sulphate and 0.31 g of tetrabutylammonium dihydrogen phosphate were dissolved in 450 mL of ultrapure water, 1 mL of sulphuric acid (95–97 %) and 550 mL of acetonitrile were added. Sodium hydroxide solution (2.1 M) was used for adjusting pH to value 3.3 (±0.05). Mobile phase was filtered through nylon membrane filter (0.45 µm) by using Millipore Glass Filter Holder and degassed in ultrasonic bath. Mobile phase was stored in the refrigerator in closed glass bottles.

### Preparation of Stock, Standard, Sample and Blank Solutions

Preparation of stock, standard, sample and blank solutions is described in Table 1.

## Results and Discussion

### Method Development

Composition of mobile phase, column type and flow rate used in monograph Propranolol hydrochloride (Related substances) in European Pharmacopoeia 7.0 [9] provided total separation of PRO and BEN. Pharmacopoeia uses mobile phase with two ion-pair reagents, sodium dodecyl sulphate (forms ion pairs with cations–propranolol cations) and tetrabutylammonium dihydrogen phosphate (forms ion pairs with anions–benzoate anions). It was observed that addition of sulphuric acid according to the Pharmacopoeia method [9] helps in dissolving sodium dodecyl sulphate in water and prevents the formation of bubbles in mobile phase caused by this surfactant. Optimization steps in changing mobile phase pH and water component:ACN ratio were performed to find out the best separation performance. Mobile phase pH (at water component:ACN = 45:55) was tested in the range of 3.3 ± 0.2 and it was observed that even little changes in pH led to undesirable increase in retention time of analytes (up to 112 % relatively). Water

**Table 1** Sample, standard and blank solutions preparation

Composition and process	Stock solution of standards	Stock solution of IS	Standard solution	Sample solution 1	Sample solution 2	Blank solution 1	Blank solution 2
Propranolol hydrochloride (PRO)	~40.00 mg	–	–	–	–	–	–
Sodium benzoate (BEN)	~10.00 mg	–	–	–	–	–	–
Butylparaben (BP)	–	~1200.00 mg	–	–	–	–	–
Stock solution of standards	–	–	1.000 mL	–	–	–	–
Stock solution of internal standard	–	–	1.000 mL	1.000 mL	1.000 mL	–	–
Oral preparation (PRO 2 mg mL <sup>-1</sup> )	–	–	–	1.000 mL	–	–	–
Oral preparation (PRO 8 mg mL <sup>-1</sup> )	–	–	–	–	0.500 mL	–	–
Placebo of preparation (PRO 2 mg mL <sup>-1</sup> )	–	–	–	–	–	1.000 mL	–
Placebo of preparation (PRO 8 mg mL <sup>-1</sup> )	–	–	–	–	–	–	0.500 mL
Dissolvent water:ACN	50:50 (v/v)	50:50 (v/v)	50:50 (v/v)	50:50 (v/v)	50:50 (v/v)	50:50 (v/v)	50:50 (v/v)
Total volume	20.00 mL	100.00 mL	25.00 mL	25.00 mL	25.00 mL	25.00 mL	25.00 mL
Membrane filtration 0.45 µm	–	–	Yes	Yes	Yes	Yes	Yes
Injection to the column	–	–	Yes (5 µL)	Yes (5 µL)	Yes (5 µL)	Yes (5 µL)	Yes (5 µL)

component:ACN ratio (at optimal pH 3.3) was tested from values 35:65–55:45. Increasing of water component amount led to prolongation of retention times and 55:45 ratio did not ensure total separation of PRO and BP. Mobile phase with increased proportion of ACN than original (45:55) might be used to shorten analysis time, but it also causes significant decrease of BEN and PRO peaks resolution, and reduces method robustness. Simple mobile phases containing only water:MeOH = 20:80–30:70 or water:ACN = 40:60–60:40 were also tested, but peak shapes of analytes and resolution were not acceptable. Various injection volumes (1–10 µL) were tested and concentrations of analytes and internal standard (IS) both in standard and sample solutions were adjusted to ensure suitable tailing factor, sufficient response (absorbance) and similar absorbance level of determined analytes. Labetalol, acebutolol, pindolol, metoprolol, methylparaben, ethylparaben, propylparaben and BP were tested as the possible ISs. BP was chosen as IS, because the other mentioned substances were not totally separated from substances of pharmaceutical formulation or were eluted with insufficient tailing factor. Wavelength of UV/VIS absorbance detector was tested at 200–300 nm range. PRO maximum absorbance was at wavelength 214 nm, BEN at 224 nm and BP at 256 nm. It was observed that measuring at wavelength lower than 220 nm raises significantly baseline noise. Wavelength was set to 230 nm according to good sensitivity of PRO and BEN and minimum baseline noise. All optimal conditions, mobile phase composition and preparation of solutions are mentioned in “Instrumentation and Chromatographic Conditions”, “Preparation of Mobile Phase (~1 L)”, and Preparation of Stock, Standard, Sample and Blank Solutions.

### Sample Preparation Development

Simple method “dilute and shoot” was used for sample preparation. Water:ACN = 50:50 (v/v) solution was chosen to ensure good solubility of tested compounds. 50:50 = water:ACN ratio is also similar to the water component:ACN ratio used in mobile phase to avoid worsening of peak shapes. It was observed that insufficient dilution (e.g. 10) led to recovery of determined substances significantly over 100 %. Dilution 25 (i.e. 1.000 mL of preparation was diluted into 25 mL of mixture water:ACN = 50:50 v/v) contributed to elimination of matrix effects and recovery of all formulations resulted in range of 100 ± 2 %. Sample solution was prepared by the same way as standard solution. Concentration of PRO, BEN and BP was selected to ensure the same concentration level both in sample and standard solution.

### Method Validation

Presented method was validated according to ICH Q2(R1) [10] guideline. System suitability test (repeatability of retention times and areas, number of theoretical plates, resolution, tailing factor), precision, linearity, accuracy, selectivity and robustness were evaluated during method validation. Validation results are summarized in Tables 2 and 3.

### System Suitability Test (SST)

SST was performed on standard solution which was injected into the column six times. Presented values are arithmetic means of six injections.

**Table 2** Validation parameters of formulations ST1–ST5

Parameter	Formulations										Criteria
	ST1		ST2		ST3		ST4		ST5		
SST	PRO	BEN	PRO	BEN	PRO	BEN	PRO	BEN	PRO	BEN	
Repeatability $t_R$ RSD (%) <sup>a</sup>	0.16	0.24	0.16	0.24	0.16	0.24	0.16	0.24	0.16	0.24	$X < 1 \%$
Repeatability area RSD (%) <sup>a</sup>	0.09	0.08	0.09	0.08	0.09	0.08	0.09	0.08	0.09	0.08	$X < 1 \%$
Theoretical plates <sup>a</sup>	8,441	6,408	8,441	6,408	8,441	6,408	8,441	6,408	8,441	6,408	$N > 1,500$
Resolution <sup>a</sup>	8.82	–	8.82	–	8.82	–	8.82	–	8.82	–	$R_{ij} > 1.5$
Tailing factor <sup>a</sup>	1.19	1.23	1.19	1.23	1.19	1.23	1.19	1.23	1.19	1.23	$T = 0.8–1.5$
Validation											
Precision RSD (%) <sup>b</sup>	0.20	–	0.44	0.59	0.28	0.39	0.25	0.16	0.21	0.19	$X < 5 \%$
Linearity (correlation coefficient) <sup>c</sup>	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	$R > 0.9990$
Accuracy recovery (%) <sup>b</sup>	100.49	–	101.49	101.11	99.42	98.14	100.24	100.15	99.55	99.09	$X = 100 \pm 5 \%$
Accuracy RSD (%) <sup>b</sup>	0.24	–	0.80	0.78	0.76	0.78	0.55	0.64	0.30	0.29	$X < 5 \%$
Selectivity	No interference		No interference		No interference		No interference		No interference		No interference

PRO propranolol hydrochloride, BEN sodium benzoate, STx formulations with various excipients

<sup>a</sup> Six injections

<sup>b</sup> Six samples, three injections of each sample

<sup>c</sup> At 50, 75, 100, 135, 170, 200 % levels

**Table 3** Validation parameters of formulations ST6–ST10

Parameter	Formulations										Criteria
	ST6		ST7		ST8		ST9		ST10		
SST	PRO	BEN	PRO	BEN	PRO	BEN	PRO	BEN	PRO	BEN	
Repeatability $t_R$ RSD (%) <sup>a</sup>	0.16	0.24	0.16	0.24	0.16	0.24	0.16	0.24	0.16	0.24	$X < 1 \%$
Repeatability area RSD (%) <sup>a</sup>	0.09	0.08	0.09	0.08	0.09	0.08	0.09	0.08	0.09	0.08	$X < 1 \%$
Theoretical plates <sup>a</sup>	8,441	6,408	8,441	6,408	8,441	6,408	8,441	6,408	8,441	6,408	$N > 1,500$
Resolution <sup>a</sup>	8.82	–	8.82	–	8.82	–	8.82	–	8.82	–	$R_{ij} > 1.5$
Tailing factor <sup>a</sup>	1.19	1.23	1.19	1.23	1.19	1.23	1.19	1.23	1.19	1.23	$T = 0.8–1.5$
Validation											
Precision RSD (%) <sup>b</sup>	0.16	0.18	0.49	0.41	0.63	0.67	0.34	0.18	0.33	–	$X < 5 \%$
Linearity (correlation coefficient) <sup>c</sup>	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	0.9997	$R > 0.9990$
Accuracy recovery (%) <sup>b</sup>	99.99	99.73	99.49	99.16	100.20	100.11	100.11	100.02	99.51	–	$X = 100 \pm 5 \%$
Accuracy RSD (%) <sup>b</sup>	0.28	0.29	0.42	0.37	0.23	0.22	0.37	0.36	0.23	–	$X < 5 \%$
Selectivity	No interference		No interference		No interference		No interference		No interference		No interference

PRO propranolol hydrochloride, BEN sodium benzoate, STx formulations with various excipients

<sup>a</sup> Six injections

<sup>b</sup> Six samples, three injections of each sample

<sup>c</sup> At 50, 75, 100, 135, 170, 200 % levels

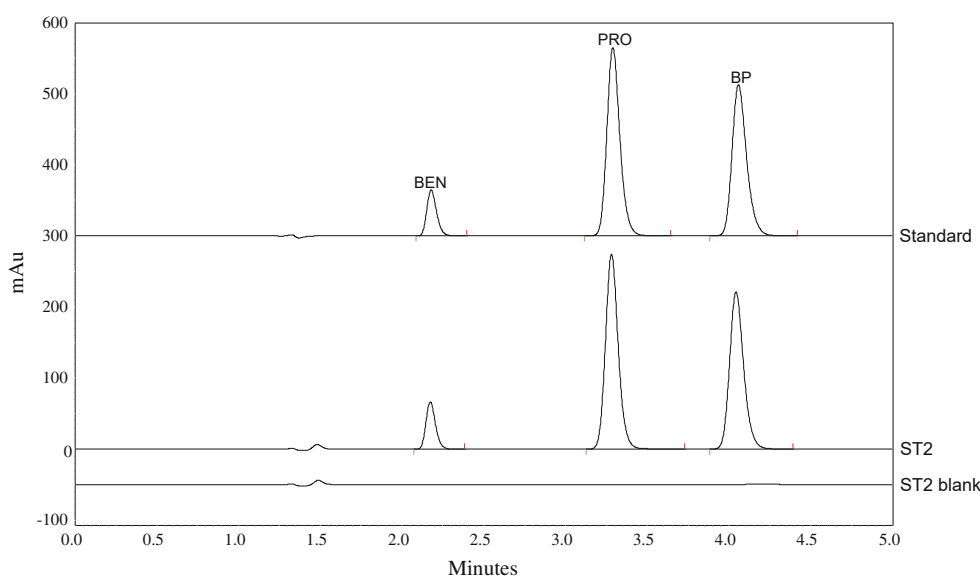
### Precision

Six sample solutions were prepared from each of ten preparations. Each sample was injected three times. Final results are presented as relative standard deviations (RSD) of BEN/BP and PRO/BP ratios.

### Linearity

Calibration curve was created by six points which cover the concentration range of PRO from 0.04 to 0.16 mg mL<sup>-1</sup> and of BEN from 0.01 to 0.04 mg mL<sup>-1</sup>. Linear regression was used for processing of calibration data. Correlation

**Fig. 1** Chromatogram of standard solution (BEN 0.02 mg mL<sup>-1</sup>, PRO 0.08 mg mL<sup>-1</sup>, BP 0.48 mg mL<sup>-1</sup>), sample solution and its blank solution (ST2: 1.000 mL of formulation ST2 and 1.000 mL of stock solution of internal standard BP in 25 mL; ST2 blank: 1.000 mL of placebo ST2 in 25 mL); injection volume 5  $\mu$ L, mobile phase flow 1.8 mL min<sup>-1</sup>, wavelength 230 nm, 25 °C



coefficient of linearity was 0.9997 for PRO and 0.9997 for BEN which means good correlation between peak areas and concentrations.

#### Accuracy

Solutions for injection were prepared by using placebo and stock solution of standards instead of oral preparation. Six solutions were prepared from each of ten preparations. Each solution was injected into the column three times. Accuracy is presented as a recovery parameter with relative standard deviations.

#### Selectivity

Selectivity was observed by comparing chromatograms of sample solutions, standard solution and blank solutions. It is obvious that active substance PRO, antimicrobial agent BEN and internal standard BP are all completely separated both in standard solution and in sample solution (Fig. 1). No interference was found (Fig. 1).

#### Robustness

Various pH and composition of mobile phase were tested. It is possible to use mobile phase with pH range from 3.1 to 3.5 without remarkable changes of accuracy (98.60–100.24 %). It is possible to use water component:ACN ratio from 35:65 to 50:50 (v/v) without remarkable changes of accuracy (99.59–100.00 %). Last tested mobile phase ratio 55:45 (v/v) is not suitable because peaks of PRO and BP were not completely separated to baseline. Stability of standard solution was tested at room temperature and at 4 °C after 24, 48 and 72 h from preparation. Accuracy range was 99.67–100.50 %.

#### Conclusion

Optimal chromatographic conditions cover using Supelco Discovery<sup>®</sup> C18 (25 cm  $\times$  4.6 mm, particles 5  $\mu$ m) column, isocratic elution mode with flow rate 1.8 mL min<sup>-1</sup>. Mobile phase contains ion-pair reagents sodium dodecyl sulphate and tetrabutylammonium dihydrogen phosphate, water component:ACN = 45:55 and pH is adjusted to 3.3. Dual absorbance UV/VIS detector was used for detection and was set at wavelength 230 nm. Column temperature was conditioned to 25 °C. Injection volume was 5  $\mu$ L. Method is fast with total analysis time of 5 min. Sample preparation is simple “dilute and shoot” with using of internal standard BP. Validation results show that newly developed HPLC method is selective, precise and accurate (Tables 2, 3) and is suitable for identification and quantification of liquid preparations containing PRO and BEN. Presented method has been already used for stability testing of ten variants of paediatric oral preparations and is suitable for evaluating content of PRO and BEN in these preparations.

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#### References

1. Sans V, Dumas de la Roque E, Berge J, Grenier N, Boralevi F, Mazereeuw-Hautier J, Lipsker D, Dupuis E, Ezzedine K, Vergnes P, Taïeb A, Léauté-Labrèze C (2009) Propranolol for Severe Infantile Hemangiomas: follow-up Report. *Pediatrics* 124(3): e423–e431

2. State Institute for Drug Control (2013) Medicinal products database [cit. 2013-03-10]. <http://www.sukl.eu/modules/medication/search.php>
3. Standing JF, Tuleu C (2005) Paediatric formulations—Getting to the heart of the problem. *Int J Pharm* 300(1–2):56–66. doi: [10.1016/j.ijpharm.2005.05.006](https://doi.org/10.1016/j.ijpharm.2005.05.006) ISSN 0378-5173
4. Nunn T, Williams J (2005) Formulation of medicines for children. *Br J Clin Pharmacol* 59:674–676. doi: [10.1111/j.1365-2125.2005.02410.x](https://doi.org/10.1111/j.1365-2125.2005.02410.x)
5. Venkatesh G, Ramanathan S, Mansor SM, Nair NK, Sattar MA, Croft SL, Navaratnam V (2007) Development and validation of RP-HPLC-UV method for simultaneous determination of buparvaquone, atenolol, propranolol, quinidine and verapamil: A tool for the standardization of rat in situ intestinal permeability studies. *J Pharm Biomed Anal* 43(4):1546–1551. doi: [10.1016/j.jpba.2006.11.013](https://doi.org/10.1016/j.jpba.2006.11.013) ISSN 0731-7085
6. El-Saharty YS (2003) Simultaneous high-performance liquid chromatographic assay of furosemide and propranolol HCl and its application in a pharmacokinetic study. *J Pharm Biomed Anal* 33(4):699–709. doi: [10.1016/S0731-7085\(03\)00229-2](https://doi.org/10.1016/S0731-7085(03)00229-2) ISSN 0731-7085
7. Modamio P, Lastra CF, Montejo O, Marifio EL (1996) Development and validation of liquid chromatography methods for the quantitation of propranolol, metoprolol, atenolol and bisoprolol: application in solution stability studies. *Int J Pharm* 130(1): 137–140. doi: [10.1016/0378-5173\(95\)04257-1](https://doi.org/10.1016/0378-5173(95)04257-1) ISSN 0378-5173
8. Ververs FFT, Schaefer HG, Lefevre JF, Lopez LM, Derendorf H (1990) Simultaneous assay of propranolol, diltiazem and metabolites of diltiazem in human plasma by liquid chromatography. *J Pharm Biomed Anal* 8(6):535–539. doi: [10.1016/0731-7085\(90\)80064-V](https://doi.org/10.1016/0731-7085(90)80064-V) ISSN 0731-7085
9. Directorate for the Quality of Medicine & HealthCare of the Council of Europe (2010) *European Pharmacopoeia (7th edn.)* Strasbourg, pp 2811–2812, ISBN 978-92-871-6700-2
10. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (2005) *Validation of Analytical Procedures: Text and Methodology Q2(R1). Step 4 version* ([http://www.ich.org/fileadmin/Public\\_Web\\_Site/ICH\\_Products/Guidelines/Quality/Q2\\_R1/Step4/Q2\\_R1\\_Guideline.pdf](http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf))

## ORIGINAL ARTICLE

# Pediatric oral solutions with propranolol hydrochloride for extemporaneous compounding: the formulation and stability study

## Pediatrické perorální roztoky s propranolol-hydrochloridem pro magistraliter přípravu: formulace a hodnocení stability

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### Summary

The aim of this study is to formulate an extemporaneous pediatric oral solution of propranolol hydrochloride (PRO) 2 mg/ml for the therapy of infantile haemangioma or hypertension in a target age group of 1 month to school children and to evaluate its stability. A citric acid solution and/or a citrate-phosphate buffer solution, respectively, were used as the vehicles to achieve pH value of about 3, optimal for the stability of PRO. In order to mask the bitter taste of PRO, simple syrup was used as the sweetener. All solutions were stored in tightly closed brown glass bottles at  $5 \pm 3$  °C and/or  $25 \pm 3$  °C, respectively. The validated HPLC method was used to evaluate the concentration of PRO and a preservative, sodium benzoate, at time intervals of 0–180 days. All preparations were stable at both storage temperatures with pH values in the range of 2.8–3.2. According to pharmacopoeial requirements, the efficacy of sodium benzoate 0.05 % w/v was proved (Ph.Eur., 5.1.3). The preparation formulated with the citrate-phosphate buffer, in our experience, had better palatability than that formulated with the citric acid solution.

**Keywords:** propranolol hydrochloride • pediatric preparation • extemporaneous preparation • solution • stability testing • HPLC

### Souhrn

Cílem práce je formulace pediatrického perorálního přípravku s propranolol-hydrochloridem (PRO) 2 mg/ml pro magistraliter přípravu, určeného k terapii infantilního hemangiomu nebo hypertenze u cílové skupiny dětí od 1 měsíce do školního věku, a hodnocení jeho stability. K dosažení pH okolo 3, optimálního pro PRO, byl jako vehikulum využito roztok kyseliny citronové nebo citráto-fosfátový pufr. K maskování hořké chuti PRO byl použit prostý sirup. Všechny roztoky byly uchovávány v dobře uzavřené hnědé lékovce při  $5 \pm 3$  °C a/nebo  $25 \pm 3$  °C. V časových intervalech 0–180 dní byla hodnocena koncentrace PRO a protimikrobní látky, benzoanu sodného, validovanou HPLC metodou. Všechny přípravky byly stabilní při obou teplotách s hodnotou pH v rozmezí 2,8–3,2. V souladu s požadavky lékopisu byla prokázána účinnost protimikrobní látky, benzoanu sodného (Ph. Eur., 5.1.3). Podle našich zkušeností má přípravek s citráto-fosfátovým pufrům lepší chuť než přípravek s kyselinou citronovou.

**Klíčová slova:** propranolol-hydrochlorid • pediatrický přípravek • magistraliter přípravek • roztok • testování stability • HPLC

### Introduction

Propranolol hydrochloride (PRO) is a non-cardio selective beta blocker. It is usually administered in the form of tablets or capsules in therapy of cardiovascular diseases, to control symptoms of hyperthyroidism, the prophylaxis of migraine, and many other indications<sup>1</sup>. A successful treatment of infantile hemangioma has been observed recently; PRO 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<sup>1–3</sup>.

A liquid preparation is the best dosage form for paediatric patients as young children are simply unable to swallow conventionally sized tablets or capsules. Unfortunately, no pediatric oral liquid dosage form is on the market until now.

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Under these circumstances, the pharmacist needs to compound such a preparation extemporaneously. When formulating a pediatric preparation in a hospital pharmacy, the pharmacist should attend to the stability of the active pharmaceutical substance for a labelled time period, the suitability and safety of excipients for children in the indicated target age groups, and expected duration of treatment<sup>4, 5</sup>. A simple way of preparing an oral liquid preparation is to crush commercial tablets to make a fine powder and mix it with a suitable vehicle.

Many empirical formulations prepared that way have been published for PRO<sup>6–8</sup>. Unfortunately, some authors of the earlier publications have used excipients which are not suitable for paediatric patients; a commercial suspending vehicle consisting of ethanol 1%, saccharin 0.05%, and cherry-flavoured 33% polyethylene glycol 8000 base, is an example<sup>7</sup>. The lack of valid stability data is the second common disadvantage of earlier publications.

This study was focused on the formulation of an extemporaneous solution containing PRO 2 mg/ml, suitable for therapy of infantile hemangioma in a target group of children from 1 month to approximately 6 years for hospital and/or home care. The stability of PRO was evaluated under two different conditions of storage within a shelf life of 180 days using high performance liquid chromatography (HPLC).

## Experimental part

### Materials

Citric acid monohydrate, sodium phosphate dibasic dodecahydrate, sodium benzoate (SB), and propranolol hydrochloride (PRO) of pharmaceutical quality were used. Simple sucrose syrup (64% w/w) was obtained from Fagron (Czech Republic). Water for injection (WFI) was used throughout the study as the solvent in the preparation of the vehicles and solutions.

### Analytical reagents

The following reagents of analytical grade were used: acetonitrile, sulphuric acid ( $\geq 95$ – $97\%$ ), and sodium dodecyl sulphate ( $\geq 98.5\%$ ) (all obtained from Sigma-Aldrich, Germany), butylparaben and tetrabutylammonium

dihydrogenphosphate ( $\geq 97.0\%$ ) (both from Fluka, Germany), and sodium hydroxide (Penta, Czech Republic).

### Methods

#### Compounding of buffer solution

To prepare a citrate-phosphate buffer solution of pH 3 (CPB), 1.67 g of citric acid and 1.47 g of dibasic sodium phosphate were dissolved in WFI and made up to 100.0 ml of a solution with WFI. The stock solution was stored in a tightly closed brown glass bottle, protected from light, and refrigerated ( $5 \pm 3$  °C).

#### Compounding of solutions of PRO

The composition of all prepared solutions **F1–F3** is shown in Table 1.

The **F1** solution of PRO 2 mg/ml was prepared by dissolution of 0.20 g of the substance and 0.05 g of sodium benzoate in an appropriate volume of CPB, then filled with buffer solution up to 50 ml and made up to the total volume of 100.0 ml with Simple Sucrose Syrup.

In the formulation **F2**, 0.2 g of propranolol hydrochloride, 0.05 g of sodium benzoate, and 0.2 g of citric acid were dissolved in an appropriate volume of WFI, made up to 50 ml with WFI and then filled up to a total volume of 100.0 ml with Simple Sucrose Syrup.

The solution **F3** was prepared by dissolution of 0.20 g of propranolol hydrochloride and 0.05 g of citric acid that way as the previous one. This solution was preservative-free.

#### Measurement of pH

The pH value was measured under stabilized conditions using a pH meter (pH 212 Microprocessor pH Meter, Hanna instruments, Germany) with a combined pH electrode. The pH meter was calibrated at pH 4.01 and 7.00 at 20 °C using standard buffer solutions (WTW, Germany). The results obtained at the time intervals chosen in the stability study are presented in Table 2.

#### Instrumentation and analytical conditions

A stability indicating HPLC assay was developed for PRO and sodium benzoate, using butylparaben as an internal standard. The HPLC system consisted of a Shimadzu LC-2010C (CLASS-VP Software, Shimadzu, Japan) with a Dual – Absorbance UV Detector. Separation was achieved using a Supelco Discovery<sup>®</sup> C18 column

Table 1. Composition of the evaluated propranolol hydrochloride solutions

Composition	F1	F2	F3
PRO	0.20 g	0.20 g	0.20 g
Citric acid	–	0.20 g	0.05
CPB	50 ml	–	–
Sodium benzoate	0.05 g	0.05 g	–
Simple syrup	to 100 ml	50 ml	50 ml
WFI	–	to 100 ml	to 100 ml
Taste	sweet&sour	sweet slightly bitter	sweet slightly bitter



Table 2. The results of pH measurement during the stability study at room temperature (room) and/or in a refrigerator (cold)

Time (days)	F1		F2		F3	
	Room	Cold	Room	Cold	Room	Cold
t <sub>0</sub>	3.14	3.14	2.89	2.89	2.87	2.88
t <sub>1</sub>	3.14	3.16	2.89	2.90	2.86	2.88
t <sub>3</sub>	3.15	3.14	2.90	2.88	2.87	2.87
t <sub>7</sub>	3.16	3.15	2.90	2.90	2.89	2.89
t <sub>14</sub>	3.15	3.15	2.90	2.87	2.92	2.89
t <sub>30</sub>	3.16	3.16	2.91	2.90	2.86	2.87
t <sub>60</sub>	3.13	3.13	2.88	2.87	–	–
t <sub>90</sub>	3.08	3.11	2.82	2.84	–	–
t <sub>120</sub>	3.09	3.08	2.82	2.82	–	–
t <sub>180</sub>	3.12	3.13	2.89	2.90	–	–

Table 3. System suitability parameters of HPLC method for determination of propranolol hydrochloride (PRO) and sodium benzoate (SB)

System suitability parameters	F1		F2		F3	
	PRO	SB	PRO	SB	PRO	SB
Repeatability t <sub>R</sub> RSD (%)	0.16	0.24	0.16	0.24	0.16	–
Repeatability Area	0.09	0.08	0.09	0.08	0.09	–
Theoretical Plates	8441	6408	8441	6408	8441	–
Resolution	8.82	–	8.82	–	8.82	–
Tailing factor	1.19	1.23	1.19	1.23	1.19	–

Table 4. Validation data of HPLC method for determination of propranolol hydrochloride (PRO) and sodium benzoate (SB)

Validation criteria	F1		F2		F3	
	PRO	SB	PRO	SB	PRO	SB
Precision RSD (%) <sup>a</sup>	0.44	0.59	0.21	0.19	0.33	–
Linearity (R) <sup>b</sup>	0.9997	0.9997	0.9997	0.9997	0.9997	–
Accuracy Recovery (%) <sup>a</sup>	101.49	101.11	99.55	99.09	99.51	–
Accuracy RSD (%) <sup>a</sup>	0.80	0.78	0.30	0.29	0.23	–
Selectivity	No interference		No interference		No interference	

<sup>a</sup> six samples, three injections of each sample

<sup>b</sup> at 50, 75, 100, 135, 170, 200 % levels

Table 5. The percentage content of propranolol hydrochloride during the stability study at room temperature (room) and/or in a refrigerator (cold). RSD (%) in brackets

Time (days)	F1		F2		F3	
	Room	Cold	Room	Cold	Room	Cold
t <sub>0</sub>	100.00 (0.34)	100.00 (0.68)	100.00 (0.11)	100.00 (0.49)	100.00 (0.40)	100.00 (0.40)
t <sub>1</sub>	98.82 (0.06)	99.01 (0.86)	100.17 (0.14)	98.65 (1.03)	100.17 (0.27)	100.34 (0.07)
t <sub>3</sub>	100.60 (0.14)	100.14 (0.18)	103.24 (0.08)	101.13 (2.06)	100.39 (0.30)	100.14 (0.31)
t <sub>7</sub>	99.57 (0.16)	100.15 (0.09)	99.94 (0.35)	101.23 (0.65)	99.87 (0.23)	100.37 (0.13)
t <sub>14</sub>	101.99 (0.16)	100.25 (0.45)	101.89 (0.46)	100.83 (0.77)	100.97 (0.11)	101.30 (0.15)
t <sub>30</sub>	102.31 (0.13)	102.51 (0.39)	102.96 (0.75)	102.47 (0.23)	99.87 (0.18)	99.80 (0.09)
t <sub>60</sub>	99.14 (0.51)	98.20 (0.11)	98.96 (0.24)	97.87 (0.04)	–	–
t <sub>90</sub>	100.40 (0.07)	100.77 (0.41)	100.79 (0.14)	100.34 (0.26)	–	–
t <sub>120</sub>	101.18 (0.34)	100.91 (0.04)	102.32 (0.62)	101.09 (0.50)	–	–
t <sub>180</sub>	101.82 (0.14)	100.86 (0.17)	101.71 (0.28)	101.63 (0.09)	–	–

(25 cm × 4.6 mm × 5 μm) (Supelco, USA). The isocratic flow rate was 1.8 ml/min and the UV detector was set at a wavelength of 230 nm.

The mobile phase consisted of 1.6 g of sodium dodecyl sulphate, 0.31 g tetrabutylammonium dihydrogenphosphate, 1 ml of sulphuric acid, 450 ml of HPLC grade water, and

550 ml of acetonitrile, and was adjusted to the pH value of 3.3 using sodium hydroxide solution. The mobile phase solution was filtrated through a 0.45 μm filter (Glass Microfiber Filters, Whatman, UK) and then was sonicated for a few minutes (Sonorex Digitec, Bandelin, Germany) before HPLC analysis.

Table 6. The percentage content of sodium benzoate during the stability study at room temperature (room) and/or in a refrigerator (cold). RSD (%) in brackets

Time (days)	F1		F2	
	Room	Cold	Room	Cold
t <sub>0</sub>	100.00 (0.37)	100.00 (0.74)	100.00 (0.21)	100.00 (0.47)
t <sub>1</sub>	98.15 (0.65)	97.67 (1.15)	97.52 (0.26)	97.40 (0.30)
t <sub>3</sub>	99.91 (0.60)	99.14 (0.59)	99.55 (0.19)	99.83 (0.91)
t <sub>7</sub>	99.42 (0.23)	99.71 (0.35)	99.35 (0.18)	99.76 (0.23)
t <sub>14</sub>	100.82 (0.19)	99.46 (0.14)	100.48 (0.21)	99.43 (0.14)
t <sub>30</sub>	102.76 (0.13)	102.89 (0.17)	102.96 (0.60)	102.69 (0.19)
t <sub>60</sub>	98.54 (0.51)	97.67 (0.10)	98.42 (0.28)	97.47 (0.10)
t <sub>90</sub>	99.83 (0.16)	100.40 (0.33)	100.00 (0.16)	99.42 (0.27)
t <sub>120</sub>	99.48 (0.35)	99.28 (0.64)	99.89 (0.52)	99.02 (0.25)
t <sub>180</sub>	101.08 (0.23)	99.71 (0.19)	100.37 (0.08)	100.17 (0.10)

The HPLC method for the analysis of the proposed oral solution was successfully and completely validated by following the Q2(R1) ICH guideline (1997). System suitability parameters (n = 6) and validation data are summarized in Tables 3 and/or 4, respectively.

#### Stability study

The batch of the preparation was divided into two separate samples and stored in a tightly closed brown glass bottle at room temperature (25 ± 3 °C) and in a refrigerator (5 ± 3 °C). The concentration of propranolol hydrochloride and the preservative, sodium benzoate, in the preparations **F1** and **F2** were evaluated at the beginning of the stability assay (t<sub>0</sub>, a content of 100 %) and thereafter at time intervals of 1 – 3 – 7 – 14 – 30 – 60 – 90 – 120 – 180 days. The concentration of propranolol hydrochloride in solution **F3** was evaluated the same way but only at the time interval up to 30 days. Stability limit of maximum 5% degradation of the drug and the preservative contents were the basic criteria.

Each sample was measured in triplicate. The average values of the percentage content (n = 6) of propranolol hydrochloride with relative standard deviations (RSD, %) in brackets are summarized in Table 5. Similarly, the results for sodium benzoate are shown in Table 6.

## Results and Discussion

In an aqueous vehicle, PRO has good solubility (50 mg/mL). Solutions are stable at about pH 2.8 – 4 with the best at pH 3<sup>9)</sup>. A disadvantage of PRO is a bitter taste leading to the necessity of the addition of a sweetener.

In this study, three formulations of PRO solution were compounded (Table 1). The citric acid and/or the citrate-phosphate buffer solution, respectively, were used as the vehicles to achieve pH value of about 3. Generally, a multi-dose preparation needs an addition of a preservative. Since there are some references indicating possible incompatibility between PRO and parabens resulting in the degradation of the parabens<sup>6)</sup>,

sodium benzoate was used as an alternative<sup>8, 10)</sup> assuming the use in a children target group of 1 month and older (the formulations **F1** and **F2**). Simple Sucrose Syrup is added to improve palatability of the solutions. The preparation **F3** was formulated preservative-free assuming the use for neonates below 1 month.

According to the analytical procedures validation ICH guidelines (Q2(R1)), the HPLC method was completely validated. In Tables 3 and 4, system suitability parameters (n = 6) and validation data are presented.

All solutions were stored in tightly closed brown glass bottles at 5 ± 3 °C and/or 25 ± 3 °C, respectively. At time intervals mentioned in the experimental section, samples were withdrawn and used to estimate pH value and the content of PRO and SB (preserved preparations **F1** and **F2**). The results in Table 2 show good consistency in pH value during the stability study. This is important particularly in the case of the preserved solutions **F1** and **F2** as sodium benzoate has an alkaline effect on pH value, which might lead to degradation of PRO<sup>9)</sup>.

The percentage content of PRO and SB content estimated using HPLC during the stability study at room temperature and/or refrigerator are summarized in Table 5 and/or Table 6, respectively. As **F3** did not contain sodium benzoate, only the results for **F1** and **F2** are shown in Table 6. In all cases, the concentration of drug and/or preservative, respectively, was within recommended limits of ± 5% of the initial concentration at the beginning of the stability assay (t<sub>0</sub>)<sup>11)</sup>. Based on the results, the estimated shelf-life<sup>12)</sup> of 180 days was proved at both temperatures of storage for **F1** and **F2** formulations when stored in a tightly closed brown glass bottle.

## Conclusions

The aim of the study was to find an optimal vehicle for paediatric oral solution of PRO and to verify its stability at two temperatures of storage. The proposed oral aqueous solutions **F1** and **F2** for extemporaneous compounding

were stable at room temperature and/or refrigerator for 180 days. In accordance with the European Pharmacopoeia (Ph.Eur. 7.0, 5.1.3 Efficacy of antimicrobial preservation), the efficacy of the antimicrobial preservative, sodium benzoate 0.05 % w/v, was demonstrated by an accredited laboratory. A labelled shelf-life of 3 months, storage in a refrigerator at  $5 \pm 3$  °C, and protection from light can be recommended. The formulation **F1** consisting of citrate-phosphate buffer mixed with sugar syrup we considered better than **F2** for a sweet and sour taste, particularly in the therapy of older children. Formulation **F3** represents the composition formulated with a minimal content of excipients and is preservative-free. It must, therefore, be prepared under aseptic conditions. It can be expected for use in the therapy of neonates under supervision of a caregiver. A labelled shelf-life of 7 days can be recommended for extemporaneous compounding in real-life situations if stored in a refrigerator at  $5 \pm 3$  °C. To protect from microbial contamination and to allow easy administration, preparations should be packaged in a glass container with a screw cap suitable for administration using a syringe for oral use.

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#### References

1. Propranolol hydrochloride. In Sweetman S. C. ed. Martindale: The Complete Drug Reference, 37<sup>th</sup> Ed. London: Pharmaceutical Press 2011; 989–990.
2. Léauté-Labrèze C., Dumas de la Roque E., Hubiche T., Boralevi F., Thambo J. B., Taïeb A. Propranolol for severe hemangiomas of infancy. *N Engl J Med* 2008; 358, 2649–2651.
3. Bagazgoitia L., Torrelo A., Gutiérrez J. C. L., Hernández-Martín A., Luna P., Gutiérrez M., Baño A., Tamariz A., Larralde M., Alvarez R., Pardo N., Baselga E. Propranolol for infantile hemangiomas. *Ped Dermatol* 2011; 28, 108–114.
4. **European Pharmacopoeia Commission.** Quality Guideline On The Pharmaceutical Development Of Medicines For Paediatric Use. Strassbourg, 2010. EMEA/CHMP/PEG/194810/2005: *Reflection Paper: Formulations of choice for the paediatric population*, 45 s., date accessed 25.11.2012 ([http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2009/09/WC500003782.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003782.pdf))
5. Resolution on quality and safety assurance requirements for medicinal products prepared in pharmacies for the special needs of patients. 2011. date accessed 25.11.2012 <https://wcd.coe.int/ViewDoc.jsp?id=1734101>
6. Brown G. C., Kayes J. B. The stability of suspensions prepared extemporaneously from solid oral dosage forms. *J Clin Pharm* 1976; 1, 29–37.
7. Henry D. W., Repta A. J., Smith F. M., White S. J. Stability of propranolol hydrochloride suspension compounded from tablets. *Am J Hosp Pharm* 1986; 43, 1492–1495.
8. Ahmed G. H., Steward P. J., Tucker I. G. The stability of extemporaneous paediatric formulations of propranolol hydrochloride. *Aust J Hosp Pharm* 1988; 18, 312–318.
9. Propranolol hydrochloride. In: Trissel L. A. ed *Stability of compounded Formulations*, 4<sup>th</sup> Ed, American Pharmacists Association: Washington 2009; 478–481.
10. Gupta V., Stewart K. R. Stability of propranolol hydrochloride suspension and solution compounded from injection or tablet. *Am J Hosp Pharm* 1987; 44, 360–361.
11. Bardin C., Astier A., Vulto A., Sewell G., Vigneron J., Trittler R., Daouphars M., Paul M., Trojniak M., Pinguet F. Guidelines for the practical stability studies of anticancer drugs: a European consensus conference. *Eur J Hosp Pharm* 2012; 19, 278–285.
12. Capen R., Christopher D., Forenzo P., Ireland C., Liu O., Lyapustina S., O'Neill J., Patterson N., Quinlan M., Sandell D., Schwenke J., Stroup W., Tougas T. On the shelf life of pharmaceutical products. *AAPS Pharm Sci Tech* 2012; 23 June; DOI: 10.1208/s12249-012-9815-2.

## Research Article

# Development of a Gradient HPLC Method for the Simultaneous Determination of Sotalol and Sorbate in Oral Liquid Preparations Using Solid Core Stationary Phase

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A selective and sensitive gradient HPLC-UV method for quantification of sotalol hydrochloride and potassium sorbate in five types of oral liquid preparations was developed and fully validated. The separation of an active substance sotalol hydrochloride, potassium sorbate (antimicrobial agent), and other substances (for taste and smell correction, etc.) was performed using an Ascentis Express C18 (100 × 4.6 mm, particles 2.7 μm) solid core HPLC column. Linear gradient elution mode with a flow rate of 1.3 mL min<sup>-1</sup> was used, and the injection volume was 5 μL. The UV/Vis absorbance detector was set to a wavelength of 237 nm, and the column oven was conditioned at 25°C. A sodium dihydrogen phosphate dihydrate solution (pH 2.5; 17.7 mM) was used as the mobile phase buffer. The total analysis time was 4.5 min (+2.5 min for reequilibration). The method was successfully employed in a stability evaluation of the developed formulations, which are now already being used in the therapy of arrhythmias in pediatric patients; the method is also suitable for general quality control, that is, not only just for extemporaneous preparations containing the mentioned substances.

## 1. Introduction

Sotalol (SOT) is a Class III antiarrhythmic agent that prolongs the QT interval and exhibits beta-adrenergic blocking properties. SOT has been widely used in the management of atrial arrhythmias for several decades including patients in the pediatric age group and those with congenital heart disease. In pediatric patients, SOT has proven efficacy in suppressing supraventricular arrhythmias and maintaining a sinus rhythm with recurrence-free intervals of >80% and has also been used in the management of ventricular arrhythmias with more modest efficacy [1].

Potassium sorbate (SORB) is an antimicrobial preservative with antibacterial and antifungal properties and is used in pharmaceuticals, foods, enteral preparations, and cosmetics.

In general, SORB is used at concentrations of 0.1–0.2% in oral and topical formulations. Potassium sorbate is used in approximately twice as many pharmaceutical formulations as sorbic acid due to its greater solubility and stability in water. As with sorbic acid, potassium sorbate exhibits minimal antibacterial properties in formulations with pH values higher than 6 [2].

There are no registered medicinal products containing sotalol suitable for administration in pediatric patients and available in the European Union (EU) member states and selected non-EU countries (Supplement) (see Supplementary Material available online at <http://dx.doi.org/10.1155/2015/806736>) [3]. Pediatric formulations have many specific characteristics. The most important one is the ability to administer dosages of an active substance in variable and precise

amounts according to the actual weight of a child. In addition, the dosage form has to be easily swallowed [4, 5]. When no appropriate dosage form is commercially available, the most suitable alternative is the use of oral liquid extemporaneous preparations with an antimicrobial agent for older children and with no antimicrobial agent for infants. The cooperation between the Department of Pharmaceutical Technology (Charles University, Faculty of Pharmacy, Hradec Kralove) and the Hospital Pharmacy (University Hospital in Motol, Prague) has led to the development of five versions of oral liquid preparations with sotalol hydrochloride as the active substance and potassium sorbate as the preservative. The HPLC determination of sotalol has been previously reported [6–10], but the simultaneous determination of sotalol and sorbate in various matrices (e.g., sirupus simplex~sucrose syrup, saccharine, and citric acid) in a liquid dosage has not been previously published. The aim of this study was to develop and validate a selective and rapid method using standard HPLC system for the determination of sotalol hydrochloride (i.e., the active substance) and potassium sorbate (i.e., the antimicrobial agent) and their separation from other present substances in newly developed pediatric oral preparations and its application for stability study. In order to achieve total separation of sotalol, sorbate, and other analytes that possess different chromatographic properties at the lowest possible analysis time at standard HPLC system, modern solid core columns and gradient elution were adopted during method development.

Columns of solid core particles exhibit unusual chromatographic efficiency. Presumably, this is due to the ability to form very homogeneous packed beds as a result of an extremely narrow particle size distribution and higher particle density. Solid core particles exhibit highly improved mass transfer (kinetic) effects because of the thin porous shell surrounding a solid core, allowing solutes to rapidly diffuse in and out of the porous structure containing the stationary phase for interaction. Columns of the solid core particles (2.7  $\mu\text{m}$ ) exhibit theoretical plates nearly comparable to those of sub-2-micron totally porous particles, but with much reduced pressure requirements and thus it is possible to use them at standard HPLC systems [11].

## 2. Materials and Methods

**2.1. Materials and Chemicals.** Sotalol hydrochloride (Fagron, Olomouc, Czech Republic) and potassium sorbate (Dr. Kulich Pharma, Hradec Kralove, Czech Republic) were used as the standards. Ethylparaben (Sigma-Aldrich, Steinheim, Germany) was used as an internal standard (IS). Sodium dihydrogen phosphate dihydrate (Sigma-Aldrich, Steinheim, Germany), orthophosphoric acid (Merck, Darmstadt, Germany), acetonitrile (ACN) gradient grade (Sigma-Aldrich, Steinheim, Germany), methanol (MeOH) gradient grade (Sigma-Aldrich, Steinheim, Germany), and tetrahydrofuran (THF) Chromasolv (Sigma-Aldrich, Steinheim, Germany) were used to prepare the mobile phase. Water for the sample and mobile phase preparation was purified by Milli-Q Integral 15 system with 0.22  $\mu\text{m}$  output filter. Nylon membrane filters

TABLE 1: Linear gradient.

$T$ [min]	% A (buffer)	% B (ACN)
0.00	90	10
4.00	40	60
4.49	40	60
4.50	90	10
7.00	90	10

(0.20  $\mu\text{m}$ ) were used for mobile phase filtration (Albet, Dassel, Germany). Nylon membrane filters (0.22  $\mu\text{m}$ ) (Vitrum, Prague, Czech Republic) and 2 mL syringes (Chirana T. Injecta, Stara Tura, Slovak Republic) were used to filter the samples. A 1000  $\mu\text{L}$  Transferpette micropipette (Brand, Wertheim, Germany) was used. Formulations F1–F5 (and respective blank solutions), which contain sotalol hydrochloride (5 mg mL<sup>-1</sup>), potassium sorbate (1 mg mL<sup>-1</sup>), and excipients (e.g., water for injection, sirupus simplex, citric acid, disodium hydrogen phosphate dodecahydrate, and sodium saccharine), were obtained as extemporaneous preparations from the Hospital Pharmacy at the University Hospital in Motol, Prague, Czech Republic.

**2.2. Instrumentation and Chromatographic Conditions.** The chromatographic analysis was performed on an integral system Shimadzu LC-2010C (Shimadzu, Kyoto, Japan). The following chromatographic columns were tested during method development: Ascentis Express C18 (150  $\times$  4.6 mm, particles 2.7  $\mu\text{m}$ ), Ascentis Express C18 (100  $\times$  4.6 mm, particles 2.7  $\mu\text{m}$ ), and Ascentis Express Phenyl-Hexyl (100  $\times$  4.6 mm, particles 5  $\mu\text{m}$ ). An Ascentis Express C18 (100  $\times$  4.6 mm, particles 2.7  $\mu\text{m}$ ) column was finally chosen for the method validation and stability testing. The dual absorbance UV/Vis detector was set to a wavelength of 237 nm. Linear gradient elution (Table 1) with a flow rate of 1.3 mL min<sup>-1</sup> was used. A column oven was conditioned at 25°C. The injection volume was 5  $\mu\text{L}$  and analysis time was 4.5 minutes (7 minutes with reequilibration time incl.).

**2.3. Preparation of Buffer Component of Mobile Phase (Approximately 1L).** 2.76 g of sodium dihydrogen phosphate dihydrate was dissolved in 1L of ultrapure water. An orthophosphoric acid solution (6%) was used to adjust the pH to 2.5 ( $\pm 0.05$ ). The mobile phase buffer was filtered through a nylon membrane filter (0.20  $\mu\text{m}$ ) using a Millipore glass filter holder. The mobile phase buffer was used immediately after preparation or stored in the refrigerator in closed borosilicate glass bottles for a maximum of 24 hours.

**2.4. Preparation of Stock, IS, Standard, Sample, and Blank Solutions.** The preparation of the stock, IS, standard, sample, and blank solutions is described in Table 2.

## 3. Results and Discussion

**3.1. Method Development.** The initial chromatographic conditions and mobile phase composition were chosen to be

TABLE 2: Stock, IS, standard, sample, and blank solutions preparation.

Composition and process	Stock solution of standards	Stock solution of IS	Standard solution	Sample solution	Blank solution
Sotalol hydrochloride	~100.00 mg	—	—	—	—
Potassium sorbate	~20.00 mg	—	—	—	—
Ethylparaben	—	~100.00 mg	—	—	—
Stock solution of standards	—	—	1.000 mL	—	—
Stock solution of IS	—	—	1.000 mL	1.000 mL	—
Oral preparation (SOT 5 mg mL <sup>-1</sup> )	—	—	—	1.000 mL	—
Placebo of preparation (neither SOT nor SORB)	—	—	—	—	1.000 mL
Dissolvent ACN : water	50 : 50 (v/v)	50 : 50 (v/v)	30 : 70 (v/v)	30 : 70 (v/v)	30 : 70 (v/v)
Total volume	20.00 mL	50.00 mL	25.00 mL	25.00 mL	25.00 mL
Membrane filtration 0.22 $\mu$ m	—	—	Yes	Yes	Yes
Injection to the column	—	—	Yes (5 $\mu$ L)	Yes (5 $\mu$ L)	Yes (5 $\mu$ L)

similar to those used by Delamoye et al. for the separation of thirteen  $\beta$ -blockers [12]. C18 stationary phase column with solid core 2.7  $\mu$ m particles, 4.6 mm i.d., and 100 mm length was initially tested. A mobile phase consisting of sodium dihydrogen phosphate dihydrate (pH 3.8; 17.7 mM)-ACN (65 : 35, v/v) did not provide separation of the sotalol peak from the dead volume peak. An increase in the phosphate buffer (pH 3.8; 17.7 mM) component led to a desirable increase in the sotalol retention. Phosphate buffer (pH 3.8; 17.7 mM)-ACN (80 : 20 and 85 : 15, v/v) only provided partial separation of sotalol from the dead volume peak. Phosphate buffer (pH 3.8; 17.7 mM)-ACN (90 : 10, v/v) was sufficient for proper sotalol retention. The addition of THF was tested to observe possible positive effects on peak shape. Unfortunately, THF addition did not provide any advantages and led to a rapid increase in the baseline noise and drift. The use of MeOH instead of acetonitrile also led to a less stable baseline as well as an undesirable increase in the system back pressure. Avoiding the phosphate buffer and using only ACN-water mobile phases caused unacceptable peak fronting and tailing. Therefore, the buffer is necessary for maintaining good peak shapes and separation. Isocratic elution with the phosphate buffer (pH 3.8; 17.7 mM)-ACN (90 : 10, v/v) mobile phase cannot be used due to a significant increase in the analysis time caused by different retention properties of sotalol (base) and sorbate (acid). Under acidic conditions sotalol is in ionized form and thus it is not well retained on the stationary phase; opposite sorbate is in nonionized form and it is therefore significantly retained on the column. Different gradient curve profiles were tested, and a linear gradient was chosen because it resulted in the lowest baseline drift. A terminal gradient concentration of ACN was tested up to 70%, but a maximum usable concentration of 60% was required to maintain a straight baseline. Gradient elution with initial phosphate buffer (pH 3.8; 17.7 mM)-ACN (from 90 : 10 to 40 : 60, v/v) could be used for the separation of sotalol and sorbate. Unfortunately, these conditions cannot be used for analysis of preparations containing the artificial sweetener saccharine (SACC) due to its coelution with

the sotalol peak. An increase in the temperature up to 60°C or the use of an Ascentis Express Phenyl-Hexyl column did not provide any favorable changes in the selectivity and using of Ascentis Express C18 (150  $\times$  4.6 mm, 2.7  $\mu$ m particles) also did not provide sufficient SOT-SACC separation. An elevated temperature resulted even in a decreased resolution of the SOT and SACC peaks. Several buffer pH values (4.6; 3.8; 3.0; 2.5; and 2.0) were tested (Figure 1). Using 4.6 or 3.8 pH buffers caused coelution of SOT/SACC, pH 3.0 buffer provided reasonable separation of the SOT/SACC peaks (resolution = 1.44), and finally the 2.5 buffer led to complete separation of the mentioned analytes to the baseline (resolution > 1.5). The pH 2.0 buffer also provided total SOT/SACC separation but it is not recommended due to an expected decrease in the column lifetime. These experimental results correspond to the theoretical useful pH range of phosphate buffer which is 2.1–3.1 [13]. Methylparaben, ethylparaben, propylparaben, butylparaben, paracetamol, and salicylic acid were tested as possible internal standards (IS). Paracetamol was coeluted with the dead volume peak, methylparaben and salicylic acid were not sufficiently separated from the sorbate peak, and propylparaben with butylparaben was eluted with unfavorable long retention times. Ethylparaben was finally chosen as the IS because it is stable in solution, inexpensive, and well separated from all of the analytes in the oral preparations. In addition, ethylparaben exhibits good UV absorption in UV. Various concentrations of the sample solution and injection volumes were tested to ensure a suitable tailing factor and sufficient response (absorbance). The UV spectra of sotalol and sorbate were obtained with a UV/Vis DAD spectrophotometer, and the tested wavelengths of the UV/Vis absorbance HPLC detector ranged from 200 to 300 nm. Finally, the wavelength was set to 237 nm to ensure good sensitivity, as well as low baseline noise.

**3.2. Sample Preparation Development.** The simple method known as “dilute and shoot” was used for sample preparation. The pharmaceutical preparation was diluted 25 times (i.e., 1.000 mL of the preparation with 1.000 mL of the IS stock

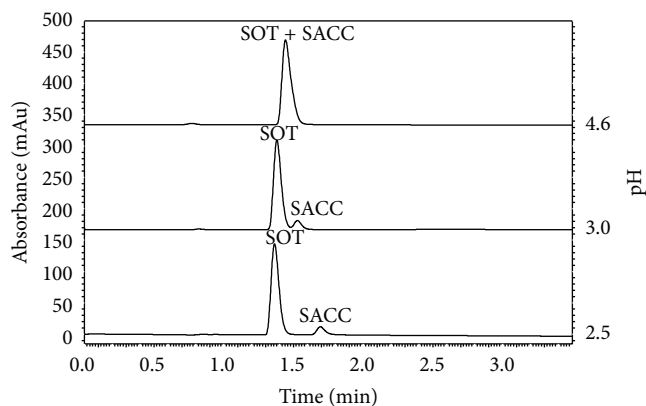


FIGURE 1: Significance of mobile phase buffer pH controlling in SOT/SACC separation; pharmaceutical formulation F4 (1.000 mL of pharmaceutical preparation diluted to 25.00 mL); injection volume 5  $\mu$ L; mobile phase flow 1.3 mL  $\text{min}^{-1}$ ; linear gradient (ACN: 10% to 60% in 4 minutes); UV/Vis detector wavelength 237 nm; column oven 25°C.

solution was diluted to 25.00 mL with a mixture of ACN-water (30:70, v/v) to avoid previously reported matrix effects of the liquid pharmaceutical formulations [14]. The standard solution was prepared in the same way as the sample solution using a stock solution of the standards. The concentration of SOT, SORB, and EP was selected to ensure the same concentration level in the sample and standard solutions. An increase in the ACN component (e.g., to 50%) led to rapid deterioration of the peak shapes, especially significant fronting of the SOT and SACC peaks. Therefore, an ACN concentration higher than 30% is not practical.

**3.3. Method Validation.** The method was validated according to ICH Q2 (R1) guidelines [15]. The system suitability (i.e., repeatability of retention times and areas, number of theoretical plates, resolution, and tailing factor), precision, linearity, accuracy, selectivity, and robustness were evaluated during method validation (Table 3). The parameters accuracy, precision, and selectivity were performed and evaluated for all five pharmaceutical formulations.

**3.3.1. System Suitability Test (SST).** SST was performed on a standard solution that was injected into the column six times. The reported values are arithmetic means of six injections.

**3.3.2. Precision.** Six sample solutions were prepared from each of the five preparations. Each sample was injected three times. The final results are reported as relative standard deviations (R.S.D.) of the SOT/EP and SORB/EP ratios of the peak areas.

**3.3.3. Linearity.** A calibration curve was created using 6 points that covered the concentration range of sotalol hydrochloride from 0.1  $\text{mg mL}^{-1}$  to 0.4  $\text{mg mL}^{-1}$  and potassium sorbate from 0.02  $\text{mg mL}^{-1}$  to 0.08  $\text{mg mL}^{-1}$ . Linear regression was used to process the calibration data. The correlation

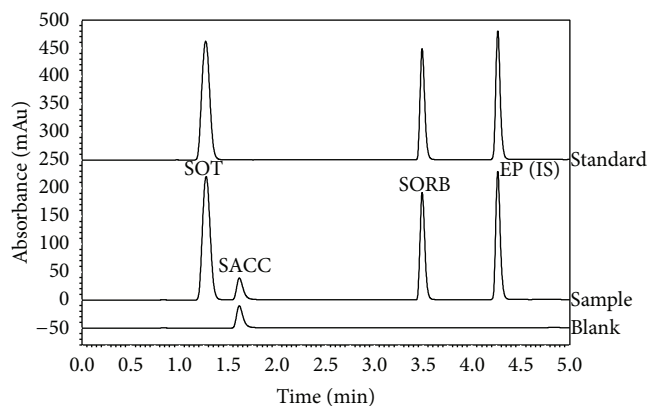


FIGURE 2: Chromatograms of the standard solution (SOT 0.2  $\text{mg mL}^{-1}$ , SORB 0.04  $\text{mg mL}^{-1}$ , and EP 0.08  $\text{mg mL}^{-1}$ ), sample solution (1.000 mL of pharmaceutical preparation and 1.000 mL of stock solution of internal standard EP diluted to 25.00 mL), and blank solution (1.000 mL of placebo diluted to 25.00 mL); injection volume 5  $\mu$ L; mobile phase flow 1.3 mL  $\text{min}^{-1}$ ; linear gradient (ACN: 10% to 60% in 4 minutes); UV/Vis detector wavelength 237 nm; column oven 25°C.

coefficients of linearity were 0.9995 for sotalol hydrochloride and 0.9995 for potassium sorbate, which indicate good correlation between the peak areas and the range of concentrations studied.

**3.3.4. Accuracy.** The solutions for injection were prepared using a placebo and stock solution of standards instead of the oral preparation. Six solutions were prepared from each of the five preparations. Each solution was injected onto the column three times. Accuracy is reported as a parameter recovery with relative standard deviations.

**3.3.5. Selectivity.** The selectivity was determined by comparing the chromatograms of sample solutions, standard solution, and blank solutions. Figure 2 shows that sotalol hydrochloride (i.e., the active substance), potassium sorbate (i.e., antimicrobial agent), and ethylparaben (i.e., internal standard) are all completely separated from each other and from the saccharine peak both in the standard solution and in the sample solution. No interference was observed.

**3.3.6. Robustness.** Various buffer pH values and compositions of the mobile phase were tested. A mobile phase buffer with a pH ranging from 2.3 to 2.7 was used without remarkable changes in the accuracy (98.99–100.37%). A sodium dihydrogen phosphate dihydrate (pH 2.5; 17.7 mM)-ACN initial gradient ratio ranging from 92:8 to 89:11 (v/v) was used without remarkable changes in the accuracy (97.42–100.70%). However, the 88:12 ratio led to higher fluctuations in the retention times, and, therefore, this ratio is not recommended. All of the tested ratios ensured complete separation to the baseline for all of these compounds. The stability of the standard solution was tested at room temperature without light protection and at  $5 \pm 3^\circ\text{C}$  light protected 24, 48, and 72 hours after its preparation. The accuracy of the peak areas for

TABLE 3: Validation parameters of formulations F1–F5.

	F1		F2		F3		F4		F5		Criterion
	SOT	SORB	SOT	SORB	SOT	SORB	SOT	SORB	SOT	SORB	
Repeatability $t_R$ (% R.S.D.) <sup>a</sup>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	$X < 1\%$
Repeatability area (% R.S.D.) <sup>a</sup>	0.22	0.57	0.22	0.57	0.22	0.57	0.22	0.57	0.22	0.57	$X < 1\%$
Theoretical plates per meter	11,810	282,650	11,810	282,650	11,810	282,650	11,810	282,650	11,810	282,650	—
Resolution <sup>a</sup>	—	18.39	—	18.39	—	18.39	—	18.39	—	18.39	$R_{ij} > 1.5$
Tailing factor <sup>a</sup>	1.10	1.23	1.10	1.23	1.10	1.23	1.10	1.23	1.10	1.23	$T = 0.8–1.5$
Precision (% R.S.D.) <sup>b</sup>	0.43	—	0.16	—	0.49	0.98	0.26	1.51	0.21	1.11	$X < 5\%$
Linearity (correlation coefficient) <sup>c</sup>	0.9995	0.9995	0.9995	0.9995	0.9995	0.9995	0.9995	0.9995	0.9995	0.9995	$R > 0.9990$
Accuracy recovery (%) <sup>b</sup>	101.09	—	99.57	—	99.59	98.26	99.93	98.70	99.35	98.57	$X = 100 \pm 5\%$
Accuracy (% R.S.D.) <sup>b</sup>	0.58	—	0.85	—	1.44	2.27	0.71	2.07	0.57	1.14	$X < 5\%$
Selectivity	No interference		No interference		No interference		No interference		No interference		No interference

<sup>a</sup>Six injections.

<sup>b</sup>Six samples, three injections of each sample.

<sup>c</sup>At 50, 75, 100, 134, 166, and 200% concentration levels.

SOT: sotalol hydrochloride.

SORB: potassium sorbate.

Fx: formulations with various excipients.

% R.S.D.: relative standard deviation in %.

both storage conditions during the entire 72 hours was 99.32–100.62%.

## 4. Conclusions

The optimal chromatographic conditions for separation of an active substance sotalol hydrochloride, potassium sorbate, and other substances were achieved on an Ascentis Express C18 (100 × 4.6 mm, particles 2.7 μm) solid core particles column and with a linear gradient elution at a flow rate of 1.3 mL min<sup>-1</sup>, using pH 2.5 phosphate buffer-ACN mixture (ACN~10–60%) as mobile phase and detection set to a wavelength of 237 nm. The method is rapid with a total analysis time of 4.5 minutes (+2.5 minutes of reequilibration). The sample preparation is a simple “dilute and shoot” method using an internal standard (ethylparaben). All measured parameters of the validation demonstrate the suitability of this new HPLC method for the analysis of oral liquid pharmaceutical preparations containing the above substances. The method was successfully employed in a stability evaluation of the four developed formulations with different composition, which are now already being used in the therapy of arrhythmias in pediatric patients. The method is also suitable for general quality control, that is, not only just for extemporaneous preparations containing the mentioned substances.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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## References

- [1] L. Harris and K. Nair, “Arrhythmia management: advances and new perspectives in pharmacotherapy in congenital heart disease,” *Progress in Pediatric Cardiology*, vol. 34, no. 2, pp. 69–73, 2012.
- [2] R. C. Rowe, P. J. Sheskey, and M. E. Quinn, *Handbook of Pharmaceutical Excipients*, 6th edition, 2009.
- [3] National competent authorities for human medicines, Medicinal products databases, available online.
- [4] J. F. Standing and C. Tuleu, “Paediatric formulations—getting to the heart of the problem,” *International Journal of Pharmaceutics*, vol. 300, no. 1–2, pp. 56–66, 2005.
- [5] T. Nunn and J. Williams, “Formulation of medicines for children,” *British Journal of Clinical Pharmacology*, vol. 59, no. 6, pp. 674–676, 2005.
- [6] R. G. Morris, “Improved liquid chromatographic fluorescence method for estimation of plasma sotalol concentrations,” *Therapeutic Drug Monitoring*, vol. 11, no. 1, pp. 63–66, 1989.



- [7] M. J. Bartek, M. Vekshteyn, M. P. Boarman, and D. G. Gallo, "Liquid chromatographic determination of sotalol in plasma and urine employing solid-phase extraction and fluorescence detection," *Journal of Chromatography*, vol. 421, no. 2, pp. 309–318, 1987.
- [8] I. Baranowska and A. Wilczek, "Simultaneous RP-HPLC determination of sotalol, metoprolol, alpha-hydroxymetoprolol, paracetamol and its glucuronide and sulfate metabolites in human urine," *Analytical Sciences*, vol. 25, no. 6, pp. 769–772, 2009.
- [9] S. Laer, I. Wauer, and H. Scholz, "Small blood volumes from children for quantitative sotalol determination using high-performance liquid chromatography," *Journal of Chromatography B: Biomedical Sciences and Applications*, vol. 753, no. 2, pp. 421–425, 2001.
- [10] B. Lemmer, T. Ohm, and H. Winkler, "Determination of the beta-adrenoceptor blocking drug sotalol in plasma and tissues of the rat by high-performance liquid chromatography with ultraviolet detection," *Journal of Chromatography B: Biomedical Sciences and Applications*, vol. 309, no. 1, pp. 187–192, 1984.
- [11] J. J. DeStefano, T. J. Langlois, and J. J. Kirkland, "Characteristics of superficially-porous silica particles for fast HPLC: some performance comparisons with sub-2- $\mu\text{m}$  particles," *Journal of Chromatographic Science*, vol. 46, no. 3, pp. 254–260, 2008.
- [12] M. Delamoye, C. Duverneuil, F. Paraire, P. de Mazancourt, and J.-C. Alvarez, "Simultaneous determination of thirteen  $\beta$ -blockers and one metabolite by gradient high-performance liquid chromatography with photodiode-array UV detection," *Forensic Science International*, vol. 141, no. 1, pp. 23–31, 2004.
- [13] A. N. Heyrman and A. R. Henry, "Importance of controlling mobile phase pH in reversed phase HPLC," *Keystone Technical Bulletin TB 99-06*, 1999.
- [14] L. Zahalka, L. Matysova, Z. sklubalova, S. Klovrzova, and P. Solich, "Simultaneous determination of propranolol hydrochloride and sodium benzoate in oral liquid preparations by HPLC," *Chromatographia*, vol. 76, no. 21-22, pp. 1553–1558, 2013.
- [15] ICH Expert Working Group, *Validation of Analytical Procedures: Text and Methodology Q2(R1)*, 2005, [http://www.ich.org/fileadmin/Public\\_Web\\_Site/ICH\\_Products/Guidelines/Quality/Q2\\_R1/Step4/Q2\\_R1\\_Guideline.pdf](http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf).

# Extemporaneous sotalol hydrochloride oral solutions for use in paediatric cardiology: formulation and stability study

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## ABSTRACT

**Objectives** Sotalol hydrochloride (SOT) is an antiarrhythmic  $\beta$ -blocker which is highly effective for the treatment of supraventricular tachycardia in children. However, a licensed paediatric dosage form with sotalol is not currently available in Europe. The aim of this work was to formulate paediatric oral solutions with SOT 5 mg/mL for extemporaneous preparation in a hospital pharmacy with the lowest possible amount of excipients and to determine their stability.

**Methods** Three aqueous solutions were formulated. One preparation without any additives for neonates and two preparations for children from 1 month of age were compounded using citric acid to stabilise the pH value, potassium sorbate 0.1% w/v as a preservative, and simple syrup or sodium saccharin as a sweetener. The samples were stored at room temperature and in a refrigerator, respectively, and the content of SOT and potassium sorbate was determined simultaneously using a validated high performance liquid chromatography method at different time points over 180 days.

**Results** At least 95% of the initial sotalol concentration remained throughout the 180-day study period in all three preparations at both temperatures. The content of potassium sorbate decreased by 17% with sodium saccharin stored at room temperature.

**Conclusions** The three proposed oral aqueous solutions of SOT for neonates and infants were stable for 180 days. Storage in a refrigerator is preferred, particularly with sodium saccharin. The additive-free solution of SOT can be autoclaved to ensure microbiological stability and used particularly for neonates and in emergency situations.

## INTRODUCTION

Sotalol hydrochloride (SOT) is an anti-arrhythmic  $\beta$ -blocker which is well tolerated and highly effective for the treatment of ventricular and supraventricular tachycardia in children.<sup>1</sup> The British National Formulary recommends sotalol should be administered to children in an initial oral dose of 1 mg/kg twice daily, increased as necessary every 3–4 days to a maximum of 4 mg/kg twice daily.<sup>2</sup> Recently, age-specific dosage guidelines for sotalol were developed by Læer *et al*<sup>3</sup> to ensure safe and effective anti-arrhythmic therapy in children, especially neonates and infants.

Sotalol is commercially available in tablet dosage forms for adults in four strengths: 80, 120, 160 and 240 mg.<sup>1</sup> However, the lack of marketed low-dose

paediatric products means extemporaneous preparation is often necessary. Extemporaneous preparations for paediatric use must be formulated in accordance with the guidelines of the European Medicines Agency.<sup>4 5</sup> Compounding should be restricted to an approved institution, for example, a hospital pharmacy.

In general, there are three basic approaches to the pharmacy preparation of paediatric dosage forms.

1. *The preparation of capsules from licensed tablets or from the active substance* is time-consuming for pharmacists and inconvenient for caregivers. As a small child is unable to swallow capsules, they should be opened and mixed with baby food or a beverage before administration. The advantage of this method is relatively good chemical and microbiological stability without the need to add preservatives.
2. *The preparation of a suspension from licensed tablets or a solution from licensed injection* is a simple way to prepare an oral liquid preparation. Commercial tablets should be crushed to a fine powder and mixed with a suitable vehicle; commercial injections could be diluted with water. Excipients improving stability and palatability should be added. However, the stability of the final product is not ensured due to the presence of other excipients in licensed medicines and their potential interactions with vehicles. Above all, there is a high risk of an inaccurate dose in the case of suspensions and drugs with a narrow therapeutic range, particularly in children.<sup>6</sup>
3. *The preparation of an aqueous oral solution from the active substance* is the best method if the active ingredient is of the required pharmacopoeial quality and soluble in water.

In all these circumstances, the pharmacist should pay attention to the stability of the active pharmaceutical substance for the labelled time period, excipient safety and tolerability, particularly for very young children, and expected duration of treatment.<sup>7</sup> Special attention must be given to formulations for neonates to whom no preservatives, antioxidants or hyperosmotic solutions should be administered.<sup>5</sup>

Regarding the paediatric use of SOT, some suspensions prepared from commercial tablets are referred to in the literature as being stable for a maximum of 90 days.<sup>8–11</sup> The presence of many different additives in tablets as well as in commercial vehicles (ORA-Sweet, ORA-Plus), sedimentation



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and possible dose inaccuracy make suspensions a less suitable dosage form for infants.

The aim of our research was to formulate extemporaneous paediatric solutions of SOT 5 mg/mL for two different paediatric groups: neonates to 1 month of age (without any additives) and infants (with the lowest possible amount of excipients) and to evaluate their stability under two different conditions of storage (refrigerated and room temperature) throughout the 180-day study period. In the unpreserved solution, the influence of autoclaving on the stability of SOT was also investigated. High performance liquid chromatography (HPLC) was used to simultaneously estimate the concentrations of SOT and potassium sorbate (PS) in the preserved preparations.

## MATERIALS AND METHODS

### Materials

SOT, PS, citric acid monohydrate, sodium saccharin and simple syrup (64% w/w, preservative-free) of pharmaceutical quality were used. Water for injection (WFI) was used throughout the study as a solvent.

### Methods

#### Sample preparation

Sotalol samples were carefully prepared in University Hospital Motol in Prague.

Solution S1 5 mg/mL was prepared by dissolving 0.50 g of SOT in WFI and made up to 100 mL under aseptic conditions. One sample (S1<sub>aut</sub>) was filled into a infusion glass bottle, stoppered and crimped, and sterilised in a laboratory autoclave at 121°C for 20 min.

Solution S2 5 mg/mL was made by dissolving 0.50 g of SOT, 0.10 g of PS and 0.08 g of citric acid in an appropriate amount of WFI. Then, 20 g of simple sucrose syrup (64% w/w) was added and the solution was made up to 100 mL (ie, 105 g) with WFI.

Solution S3 5 mg/mL was prepared by dissolving 0.50 g of SOT, 0.10 g of PS, 0.08 g of citric acid and 0.10 g of sodium saccharin in WFI and made up to 100 mL.

The composition of solutions S1, S2 and S3 is shown in [table 1](#).

#### Measurement of density, osmolality and pH value

The density of the preparations was measured at 20±0.1°C using a DMA 4100M density meter (Anton Paar, Austria). The

osmolality of the solutions was measured using an automatic semi-micro osmometer (Knauer, Germany) calibrated in accordance with Ph. Eur. 8.0 (2.2.35. Osmolality). Density and osmolality were measured five times in each formulation.

pH was measured under stabilised conditions using a pH metre (pH 212 meter, Hanna instruments, Germany) with a combined pH electrode. Samples were measured at 7, 14, 30, 60, 90, 120, 150 and 180 days.

#### Instrumentation and analytical conditions

A stability-indicating HPLC assay was developed for simultaneous determination of SOT and PS by Matysova *et al.*<sup>12</sup> Briefly, determination of SOT and PS was performed on an HPLC system with an absorbance UV detector. Separation was achieved using an Ascentis Express C18 (100×4.6 mm, particles 2.7 µm; Supelco, USA) column. Linear gradient elution was used.

#### Stability method and sample analysis

All preparations (S1, S2 and S3) were prepared in duplicate with the same composition. Each solution was divided into four amber glass bottles (50 mL). Samples were stored at room temperature (25±2°C) or in a refrigerator (5±3°C) and protected from light; that is, two samples from each batch were stored at each of the experimental conditions (n=4).

The concentration of SOT in all preparations and of the preservative, PS, in preparations S2 and S3 was evaluated at the beginning of the stability assay (t<sub>0</sub>, an initial content of 100%) and at the time points mentioned above. Each sample was measured in triplicate.

Samples of solution S1<sub>aut</sub> were stored in an autoclave bottle under the same storage conditions as above. The concentration of SOT was evaluated before sterilisation in an autoclave, after sterilisation (t<sub>0</sub>) and then at 7, 14 and 30 days.

#### Data analysis

At each time point, the percentage of the actual initial concentration remaining was calculated for sotalol and PS (n=4). Stability was defined as the retention of at least 95% of the initial concentration of sotalol and 90% of PS.

## RESULTS

[Table 1](#) shows the composition and the properties: the average of five measurements of density and osmolality, the relative SD of which was less than 1%, and the taste of the prepared solutions. In our opinion, both solutions formulated with a sweetener tasted sweet, while solution S3 containing sodium saccharin had a slightly bitter aftertaste. [Table 1](#) also gives the pH values measured at the stability study time points. The pH of the aqueous solution of sotalol S1 without additives varied between 5.43 and 5.87; the average pH value of 4.15 in the buffered solutions with preservative (S2, S3) remained practically unchanged throughout the stability study.

In [figure 1](#), the HPLC chromatograms of sample S1<sub>aut</sub> before (A) and after (B) autoclaving are compared. The lack of change in the retention time of sotalol demonstrated that autoclaving did not influence SOT stability. The concentration of sotalol before and after autoclaving was unchanged at 5.17±0.11 mg/mL and therefore taken to be equal to the initial value (t<sub>0</sub>).

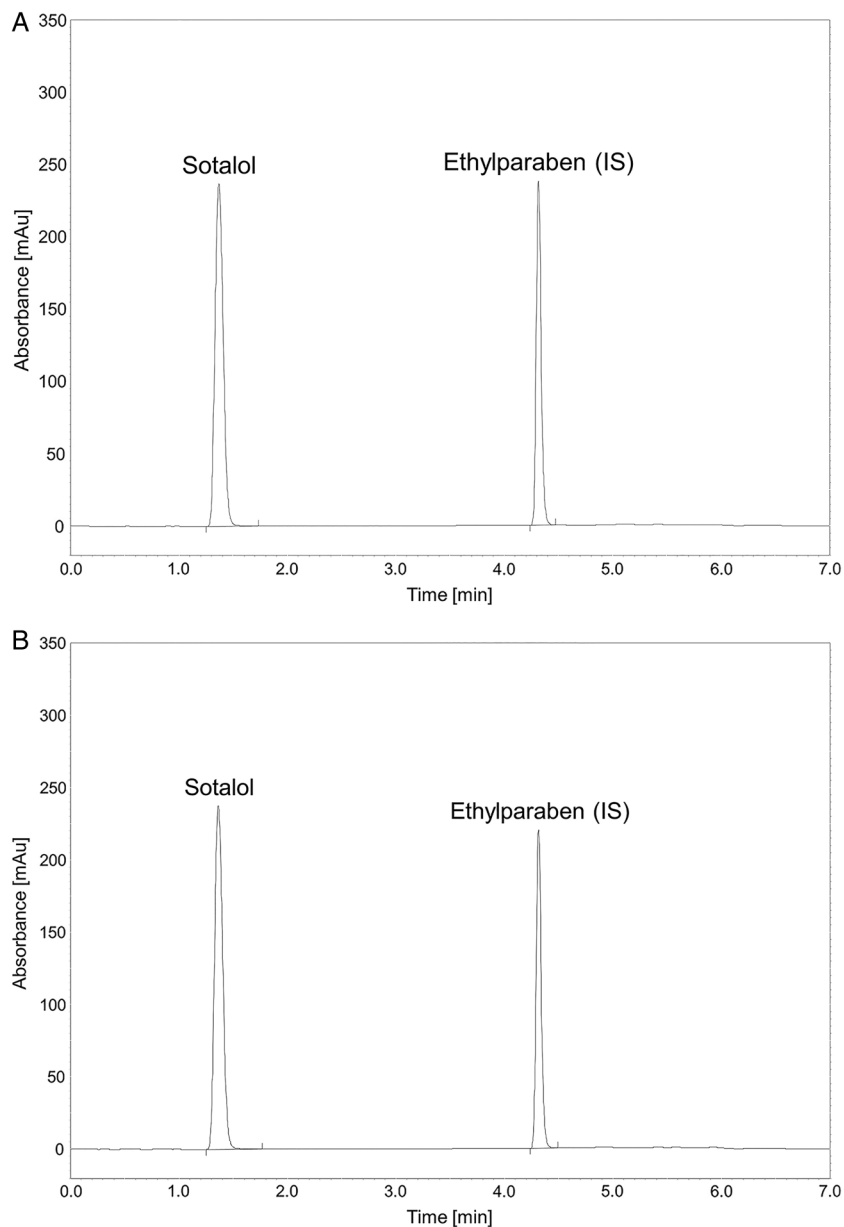
[Table 2](#) shows the percentage±SD of the initial concentration of SOT in solutions S1, S2 and S3 (n=4) stored under various conditions as mentioned above. The first row gives the amount of SOT in milligrams per millilitre at the beginning of the study (t<sub>0</sub>=100%). SOT demonstrated good stability in the

**Table 1** Composition and properties of sotalol hydrochloride solutions

	S1 (g)	S2 (g)	S3 (g)
Sotalol hydrochloride	0.50	0.50	0.50
Citric acid	–	0.08	0.08
Potassium sorbate	–	0.10	0.10
Simple syrup	–	20.0	–
Sodium saccharin	–	–	0.10
Water for injection to	100.0 mL	100.0 mL (=105.0 g)	100.0 mL
Density* (g/mL)	0.9997	1.0500	1.0008
Osmolality (mOsmol/kg)	49	497	60
pH	5.43–5.87	4.16–4.19	4.14–4.19
Taste	Slightly bitter	Sweet	Sweet, slightly bitter

\*At 20±0.1°C.

**Figure 1** (A) High performance liquid chromatography chromatogram of sotalol hydrochloride in sample S1<sub>aut</sub> before sterilisation. (B) HPLC chromatogram of sotalol hydrochloride in sample S1<sub>aut</sub> after sterilisation.



preparations, with final content being within  $\pm 5\%$  of the initial concentration after 180 days of storage at cold or room temperature. Chromatograms showed no evidence of degradation products throughout 6-month stability study.

The results for PS are presented in [table 3](#). The remaining percentage content of PS was within  $\pm 5\%$  of the initial PS concentration for solutions S2 and S3 stored in a refrigerator for 180 days. At room temperature, the percentage of PS declined

**Table 2** The percentage content of sotalol hydrochloride during the stability study at cold and room temperature\*

Time point (day)	Cold ( $5\pm 3^{\circ}\text{C}$ )			Room ( $25\pm 2^{\circ}\text{C}$ )		
	S1	S2	S3	S1	S2	S3
0 (100%)	5.17 $\pm$ 0.11 mg/mL	5.19 $\pm$ 0.03 mg/mL	5.19 $\pm$ 0.05 mg/mL	5.17 $\pm$ 0.11 mg/mL	5.19 $\pm$ 0.03 mg/mL	5.19 $\pm$ 0.05 mg/mL
7	101.10 $\pm$ 1.37	99.03 $\pm$ 0.93	100.89 $\pm$ 0.88	100.37 $\pm$ 0.89	99.52 $\pm$ 0.74	100.19 $\pm$ 0.93
14	96.72 $\pm$ 0.48	99.58 $\pm$ 1.29	98.48 $\pm$ 0.23	98.32 $\pm$ 0.73	100.22 $\pm$ 0.69	98.12 $\pm$ 0.34
30	100.65 $\pm$ 0.66	100.55 $\pm$ 1.16	101.20 $\pm$ 0.33	98.91 $\pm$ 1.01	99.69 $\pm$ 1.18	99.0 $\pm$ 0.39
60	98.41 $\pm$ 0.32	98.75 $\pm$ 1.12	99.36 $\pm$ 0.80	98.78 $\pm$ 0.59	99.29 $\pm$ 0.69	98.79 $\pm$ 0.84
90	98.75 $\pm$ 0.28	99.02 $\pm$ 0.89	98.84 $\pm$ 0.63	99.04 $\pm$ 0.30	99.54 $\pm$ 0.29	99.27 $\pm$ 0.40
120	98.58 $\pm$ 0.97	98.99 $\pm$ 0.66	99.22 $\pm$ 0.56	98.39 $\pm$ 0.60	98.89 $\pm$ 0.39	98.27 $\pm$ 0.92
150	97.33 $\pm$ 0.67	99.17 $\pm$ 0.83	98.62 $\pm$ 0.74	97.84 $\pm$ 0.34	99.23 $\pm$ 0.70	98.27 $\pm$ 0.25
180	99.29 $\pm$ 0.83	98.85 $\pm$ 0.91	101.14 $\pm$ 0.91	100.07 $\pm$ 0.52	99.27 $\pm$ 0.81	98.97 $\pm$ 1.06

\*Mean $\pm$ SD of determinations for four samples (n=4).

**Table 3** The percentage content of potassium sorbate during the stability study at cold and room temperature\*

Time point (day)	Cold (5±3°C)		Room (25±2°C)	
	S2	S3	S2	S3
0 (100%)	1.03±0.02 mg/mL	1.04±0.01 mg/mL	1.03±0.02 mg/mL	1.04±0.01 mg/mL
7	98.65±1.99	102.55±1.90	99.34±1.82	101.42±1.92
14	99.04±2.02	99.49±1.46	99.39±0.81	98.24±1.92
30	99.17±2.25	99.94±1.64	99.56±0.84	97.69±1.38
60	98.20±1.31	98.54±2.45	97.45±0.99	95.34±0.89
90	97.46±1.65	98.83±0.89	97.99±0.51	94.28±0.67
120	98.53±1.42	98.09±1.58	96.13±0.68	89.94±1.34
150	97.95±0.58	99.34±1.73	94.34±0.79	86.76±1.51
180	98.37±0.32	98.78±1.19	92.60±0.75	83.42±0.75

\*Mean±SD of determinations for four samples (n=4).

slowly, remaining within ±5% for 60 days, within ±10% for 90 days, and then decreasing further, particularly for S3. Nevertheless, no detectable changes in colour, odour or taste were observed in any formulation.

## DISCUSSION

Approximately 14 000 capsules containing 5–30 mg of SOT were prepared in the hospital pharmacy of the University Hospital Motol in Prague in 2014 for paediatric patients. In cooperation with the children's heart centre at the same hospital, the aim of this work was to replace the preparation of SOT-containing capsules with extemporaneous 5 mg/mL oral solutions which would cover most paediatric needs in the hospital.

Oral paediatric solutions provide many benefits including easy and faster preparation in a hospital pharmacy and more flexible and accurate dosing. Unfortunately, aqueous solutions often have less stability and a short shelf-life, so preservatives must generally be added to multi-dose preparations. In addition, the pharmacist is responsible for the selection of suitable excipients safe for children in the targeted age groups. Adequate palatability also plays an important role in patient acceptability, with flavours or sweeteners often added to improve taste.<sup>7</sup>

SOT is a white powder, freely soluble in water and chemically stable at pH 4–5.<sup>9–13</sup> PS is believed to be a safe antimicrobial preservative, is freely soluble in water and is generally used at 0.1–0.2% concentration in oral formulations.<sup>14</sup> PS (in the form sorbic acid) displays highest antimicrobial efficacy at pH 4–5, the same pH as sotalol.<sup>15</sup> Because SOT has a slightly bitter taste, sucrose syrup and/or sodium saccharin were used to improve the palatability of the S2 and S3 preparations, respectively.

A simple aqueous solution of SOT without any additives is proposed for neonates. Microbiological stability is ensured by the aseptic technique and final sterilisation of the product. Bacteria retention using a 0.22 µm membrane filter, sterilisation after compounding in an autoclave or a combination of both are the most common sterilisation methods employed in hospital pharmacies. The stability of solution S1 is documented in [table 2](#). The effect of sterilisation in an autoclave at 121°C for 20 min on the concentration of SOT is shown in [figure 1](#), where the HPLC sample chromatograms are compared before (A) and after (B) autoclaving. As can be seen, autoclaving did not influence the retention time of sotalol. The concentration of sotalol (5.17±0.11 mg/mL) before autoclaving was the same as that after autoclaving and therefore considered to be the initial value (t<sub>0</sub>). The percentage content of SOT remained within ±5% of

the initial concentration during 30 days of storage at both cold and room temperature.

Solutions S2 and S3 were formulated with an antimicrobial agent and are proposed for children above 1 month of age. The results in [table 2](#) document the good stability of SOT in all preparations tested. As can be seen in [table 3](#), the concentration of PS remained within ±5% of its initial concentration for solutions S2 and S3 stored in a refrigerator for 6 months. However, the percentage content of PS decreased slowly at room temperature, declining finally below 90% of the original concentration after 90 days. This was noted particularly for solution S3.

## CONCLUSIONS

Three aqueous oral solutions of SOT 5 mg/mL for antiarrhythmic therapy in children were formulated for extemporaneous preparation in a hospital pharmacy. Validated HPLC analysis demonstrated that the concentration of SOT in the formulations was in accordance with the criterion that at least 95% of the initial content should remain during storage at cold or room temperature throughout the 180-day study period.

The used excipients ensured stable pH and a more pleasant taste, while the preservative afforded sufficient antimicrobial stability in solutions S2 and S3 targeted at children aged 1 month and over. Storage in a refrigerator is preferred, and the solutions

## Key messages

### What is already known on this subject

- ▶ Most sotalol hydrochloride preparations on the market are not suitable for small children.
- ▶ Good stability of sotalol hydrochloride in an aqueous preparation has been shown.
- ▶ Extemporaneous preparation of stable pharmaceutical products in pharmacies is essential if marketed paediatric products are lacking.

### What this study adds

- ▶ Three oral aqueous solutions of sotalol hydrochloride for neonates and infants were formulated and stability for 180 days was demonstrated in a validated high performance liquid chromatography assay.
- ▶ Autoclaving had no effect on the stability of an additive-free aqueous solution of sotalol hydrochloride which can be used in particular for neonates and in emergency situations.

were stable for 180 days under this condition. Preparations should be stored in a brown glass container with a screw cap suitable for use with a graduated pipette for accurate oral dosing. The efficacy of PS 0.1% w/v in formulation S2, which is a better candidate for microbial contamination due to the content of sucrose syrup, was demonstrated by an accredited laboratory (Ph. Eur., 5.1.3 Efficacy of antimicrobial preservation).

In formulation S1<sub>auts</sub> no effect of autoclaving on the stability of SOT was observed; the solution was stable for 30 days regardless of storage conditions. Although this preservative-free solution is particularly targeted at neonates, it could be prepared in advance in the pharmacy and stored until needed.

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## REFERENCES

- Sweetman SC, ed. *Martindale: the complete drug reference*. London: Pharmaceutical Press, 2011.
- Paediatric Formulary Committee. *British National Formulary for Children 2012–2013*. London: Pharmaceutical Press, 2012.
- Läer S, Elshoff JP, Meibohm B, et al. Development of a safe and effective pediatric dosing regimen for sotalol based on population pharmacokinetics and pharmacodynamics in children with supraventricular tachycardia. *J Am Coll Cardiol* 2005;46:1322–30.
- Draft guideline on pharmaceutical development of medicines for paediatric use. European Medicines Agency. [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2011/06/WC500107908.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/06/WC500107908.pdf) (accessed 5 Apr 2015).
- EMA/536810/2008 Guideline on the investigation of medicinal products in the term and preterm neonate. 2010. [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2009/09/WC500003750.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003750.pdf) (accessed 5 Apr 2015).
- Hurtado J, Moffett BS. Pediatric oral formulations: a continual challenge. *Int J Pharm Compound* 2007;11:17–19.
- Tuleu C, Breikreutz J. Educational paper: Formulation-related issues in pediatric clinical pharmacology. *Eur J Pediatr* 2013;172:717–20.
- Nahata MC, Pai VB, eds. *Pediatric drug formulations*. 6th edn. Cincinnati: Harvey Whitney Books, 2011.
- Dupuis LL, James G, Bacola G. Stability of a sotalol hydrochloride oral liquid formulation. *Can J Hosp Pharm* 1988;41:121–3.
- Nahata MC, Morosco RS. Stability of a sotalol in two liquid formulations at two temperatures. *Ann Pharmacother* 2003;37:506–9.
- Sidhom MB, Rivera N, Almoazen N, et al. Stability of sotalol hydrochloride in extemporaneously prepared oral suspension formulations. *Int J Pharm Compound* 2005;9:402–6.
- Matysova L, Zahalkova O, Klovrcova S, et al. Development of a gradient HPLC method for the simultaneous determination of sotalol and sorbate in oral liquid preparations using solid core stationary phase. *J Anal Methods Chem* 2015;2015:806736.
- Trissel LA, ed. *Trissel's stability of compounded formulations*. Washington: American Pharmacists Association, 2009.
- Rowe RC, Sheskey PJ, Owen SC, eds. *Handbook of pharmaceutical excipients*. London: Pharmaceutical Press, 2006.
- Denyer SP, Baird RM, eds. *Guide to microbiological control in pharmaceuticals and medical devices*. 2nd edn. New York: CRC Press, Taylor & Francis, 2006.

ORIGINAL ARTICLE

**FUROSEMIDE ETHANOL-FREE ORAL SOLUTIONS FOR PAEDIATRIC USE –  
FORMULATION, HPLC METHOD, AND STABILITY STUDY**

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## **KEY MESSAGES**

### *What is already known about this subject*

- Ethanol is widely used in registered furosemide oral preparations to improve its solubility. However, ethanol is not a suitable excipient for preparations intended for use in paediatrics.
- If marketed paediatric product is not available, extemporaneous preparation of a stable pharmaceutical product in a pharmacy has an essential role in the therapy of children.
- The stability of furosemide in disodium hydrogen phosphate dodecahydrate aqueous solution in the presence of methylparaben is not known.

### **What this study adds**

- Two developed formulations of furosemide ethanol-free oral solution targeted for infants were proposed for easy extemporaneous compounding in pharmacies. Stability for 270 days under room storage temperature was demonstrated by HPLC analytical assay and pH measurement.
- The preparation containing disodium hydrogen phosphate dodecahydrate to reach the alkaline pH necessary for FUR dissolution in water is easier to prepare in routine practice and has a more pleasant taste than that one prepared with sodium hydroxide.
- The preparations proposed offer personalisation of child therapy reflecting the actual need.



## **ABSTRACT**

**Objectives.** Oral liquid solutions of diuretic active ingredient furosemide (**FUR**) marketed across Europe do not comply with the recent requirements for paediatric preparation due to their ethanol content and, moreover, in some countries only tablet or injection dosage forms of furosemide are available.

**Methods.** Our work presents two developed formulations of furosemide ethanol-free paediatric oral solutions 2 mg mL<sup>-1</sup> for easy extemporaneous compounding in a pharmacy. Furosemide solubility avoiding the use of ethanol was achieved by using sodium hydroxide (formulation F1) or disodium hydrogen phosphate dodecahydrate (formulation F2). The preparations were stored at 25 ± 3°C or at 40 ± 0.5°C, protected from light. For FUR and preservative, methylparaben (MP), a stability assay was conducted by high performance liquid chromatography validated method and pH stability.

**Results.** The remaining furosemide concentration was higher than 90 % of the initial concentration after 270 days in both formulations at both storage conditions, 25°C and 40°C. The concentration of methylparaben decreased significantly in the formulation F2 stored at 40°C.

**Conclusions.** Both formulations were stable when stored at room temperature for up to 9 months; the formulation F1 was stable even at 40°C. Methylparaben used as an antimicrobial agent fully satisfied the recommended criteria for preservative efficacy in oral preparations according to Ph. Eur. 9.0 (5.1.3).

**KEY WORDS:** furosemide, extemporaneous preparation, oral solution, ethanol-free, stability, HPLC

## INTRODUCTION

Furosemide (FUR) is a traditional diuretic widely used in adults and in paediatric patients; it is generally administered intravenously or orally. FUR is used in the treatment of hypertension and oedema associated with heart failure including pulmonary oedema.[1] Usually, the oral dose for neonates is 0.5 to 2 mg per kilogram of weight every 12 to 24 hours, for children aged from 1 month to 12 years the same dose 2–3 times daily is used, and for children 12 years and above 20 to 40 mg daily is administered. In resistant oedema, the higher dose can be permitted.[1]

However, the registered tablets contain at least 40 mg of FUR in one tablet. To achieve the required paediatric dose, it is necessary to crush commercially available tablets, mix the powder with a filler, and prepare capsules extemporaneously in a pharmacy. Afterwards, the capsule has to be opened prior to use and mixed with baby food or liquid before administration. In the Hospital Pharmacy Motol in Prague, the usually prepared dose for therapy of paediatric patients is 3 to 5 mg per capsule in agreement with the doctor's prescription.

A liquid preparation represents a better way with the advantages of more flexible dosing, improved patient as well as caregiver compliance, and, moreover, it is also easier for compounding in a pharmacy.[2-4] Registered oral liquid preparations containing FUR cannot generally be recommended for administration in children because of the high-concentration ethanol (EtOH) vehicle used. As examples: Frusol 20mg/5ml Oral Solution (Rosemont Pharmaceuticals Ltd; registered in UK) contains 10 % EtOH, Impugan 10 mg/ml oral drops (Actavis Group hf.; registered in Sweden) contains 9.8 % EtOH, and finally LasixR liquid 10 mg/ml (Sanofi-Aventis Deutschland GmbH, Germany) contains

even 11.9 % EtOH [5-7]. Using ethanol as the excipient in paediatric drugs does not comply with the general requirements for paediatric preparations[1] and it is considered unsuitable for use in paediatric patients by paediatric drug committees, drug agencies, and papers.[8-13]

One, although not optimal, way of preparing furosemide oral solutions in a pharmacy is simply diluting a commercially available registered aqueous injection of FUR with water. The absence of preservatives and the unpleasant taste of the active ingredient are limiting factors for use in oral multi-dose liquid preparations. If the active pharmaceutical ingredient (API) is available on the market and it is freely soluble in water, the preparation of an aqueous solution could be considered as the best way for extemporaneous compounding in the pharmacy. However, lower stability of API and excipients could occur in water solution and a shorter shelf life of the aqueous preparation over the capsules is expected. Therefore, a stability assay of each drug composition should be conducted prior to administering the preparation to the patients. FUR occurs as a white to slightly yellow, odourless, light sensitive, crystalline powder with a  $pK_a$  value of 3.9. It is sparingly soluble in ethanol, freely soluble in solutions of alkali hydroxides ( $pH > 8.0$ ) but, unfortunately, practically insoluble in water or dilute acids.[14]

The aim of our work was to formulate extemporaneous paediatric ethanol-free solutions of FUR ( $2 \text{ mg mL}^{-1}$ ) with a suitable solubility of FUR in the aqueous vehicle as well as an acceptable taste for use in paediatric cardiology and to evaluate their stability under two different storage conditions during a 9-month study period. A high performance liquid chromatography (HPLC) method was developed, validated, and used to determine the concentration of furosemide and the antimicrobial agent methylparaben (MP) throughout

the stability period as well. The main criterion of stability was defined as the retention of at least 90 % of initial concentration of furosemide and at least 80 % of initial concentration of methylparaben.

## **MATERIALS AND METHODS**

### **Materials and chemicals**

Furosemide (FUR), methylparaben (MP), disodium hydrogen phosphate dodecahydrate, and sodium hydroxide were obtained from Fagron, Czech Republic; sodium saccharine was obtained from Dr. Kulich Pharma, Czech Republic. Water for injection (WFI) was used for the preparation of the extemporaneous oral solutions and their blank solutions; it was obtained from the Hospital Pharmacy of the University Hospital in Motol, Prague, Czech Republic.

In an analytical study, the following substances were used for preparing the mobile phase and samples: methanol (MeOH) CHROMASOLV<sup>®</sup> gradient grade, acetonitrile (ACN) CHROMASOLV<sup>®</sup> gradient grade, formic acid 95 % and triethylamine (TEA) 99.5 % were obtained from Sigma-Aldrich, Czech Republic; and 18 M $\Omega$ .cm ultrapure water from Milli-Q<sup>®</sup> Integral water purification system with 0.22  $\mu$ m Millipak<sup>®</sup> output filter (Millipore, USA).

### **Methods**

#### ***Sample preparation***

2 mg mL<sup>-1</sup> furosemide solutions F1 and F2 were prepared from the furosemide substance and excipients (Table 1).

Formulation 1 (F1) was prepared by dissolving FUR in approximately 2.4 mL of 1 % w/v sodium hydroxide solution (60°C, freshly prepared from NaOH and WFI). Sodium

saccharine and 50 mL of 0.2 % w/v MP solution (prepared by dissolving MP in WFI at 100°C and cooled down) were added and the solution was made up by adding WFI to the final volume of 100.0 mL and transferred to a 100 mL amber glass vial with a syringe adapter.

In formulation 2 (F2), FUR was dissolved in approximately 20 mL of disodium hydrogen phosphate dodecahydrate solution freshly prepared from 1.5 g of disodium hydrogen phosphate dodecahydrate and WFI. Sodium saccharine and 50 mL of 0.2 % w/v MP solution (prepared by dissolving MP in WFI at 100°C and cooled down) were added and the solution was made up by adding WFI to the final volume of 100.0 mL and transferred to a 100 mL amber glass vial with a syringe adapter.

### ***Instrumentation and analytical assay***

#### ***Liquid chromatography***

A HPLC method for the determination of active pharmaceutical ingredient FUR and the antimicrobial preservative MP in presence of FUR impurity A (mentioned in the European Pharmacopoeia)[15] and pharmaceutical excipients used was developed and validated. Integral HPLC system Shimadzu LC-2010C (SW Class VP, ver. 6.13; Shimadzu Corp.) with a octadecyl (C18) silica gel HPLC column (Supelco Discovery<sup>®</sup> HS C18, 150 x 4.6 mm, 5 µm; Sigma-Aldrich) was used for the chromatographic analysis. The mobile phase consisted of the buffer (1000 mL of Milli-Q<sup>®</sup> water, 250 mL of formic acid, and 750 µL of triethylamine; adjusted to the pH value of 5.75) and acetonitrile in the ratio 65: 35 (v/v); the mobile phase was filtered by 0.45 µm nylon membrane filter before use. The isocratic flow rate was 1.5 mL/min and the dual absorbance UV detector was set at a wavelength of

270 nm. Chromatograms of standard solution and selected formulation (injection volume 5  $\mu\text{L}$ ) are shown in Figure 1, and method validation results are presented in Table 2.

#### *Reference standard solution preparation*

A standard solution was prepared by dissolving the active substance and impurity A in methanol. The final concentrations of the reference standards were 50  $\mu\text{g mL}^{-1}$  of furosemide and 10  $\mu\text{g mL}^{-1}$  of impurity A.

#### *Sample preparation*

An accurately weighed portion of pharmaceutical formulation corresponding to 2.5 mg of furosemide (about 1.25 g) was transferred into a 50 ml volumetric flask and methanol was added to 50.00 mL. The solution was mixed and after filtration (0.45  $\mu\text{m}$ -pore filter) was injected into the column and analysed by HPLC.

#### *Method validation*

The method was validated according to ICH Q2 (R1) guidelines. [16] The system suitability (i.e., repeatability of retention times and areas, number of theoretical plates, resolution, tailing factor), precision, linearity, accuracy, selectivity and robustness were evaluated during method validation (Table 2). The parameters of accuracy, precision, and selectivity were performed and evaluated for both pharmaceutical formulations.

#### *System suitability test (SST)*

SST was performed on a standard solution that was injected into the column six times. The reported values are the arithmetic means of six injections.

#### *Precision*

Six sample solutions were prepared from each of the preparations. Each sample was injected three times. The final results are reported as relative standard deviations (R.S.D.) of the FUR and MP peak areas.

#### *Linearity*

A calibration curve was created using 6 points that covered the concentration range of furosemide from 0.02 mg mL<sup>-1</sup> to 0.8 mg mL<sup>-1</sup> and methylparaben from 0.01 mg mL<sup>-1</sup> to 0.04 mg mL<sup>-1</sup>. Linear regression was used to process the calibration data.

#### *Accuracy*

The solutions for injection were prepared using a placebo and stock solution of standards instead of the oral preparation. Six solutions were prepared from both preparations. Each solution was injected into the column three times. Accuracy is reported as a parameter recovery with relative standard deviations.

#### *Selectivity*

The selectivity was determined by comparing the chromatograms of sample solution, and standard solution. Figure 1 shows that furosemide (i.e., the active substance), methylparaben (i.e., the antimicrobial agent) and the impurity A are all completely separated from each other and from the saccharine peak in the standard solution as well as in the sample solution. No interference was observed.

#### *Robustness*

Various buffer pH values and compositions of the mobile phase were tested. A mobile phase buffer with a pH 5.6 was used without a remarkable change in the accuracy (98.50 %). The mobile phases from ratio 55:45 (buffer:acetonitrile) are not recommended, because the peaks of furosemide and impurity A are not separated. The stability of the standard

solution was tested at room temperature without light protection and at  $5\pm 3$  °C light protected 24, 48 and 72 hours after its preparation. The accuracy of the peak areas for storage at room temperature without light protection was higher than 1 %, and therefore the storage at  $5\pm 3$  °C light protected condition is recommended.

### ***Stability assay and sample analysis***

Two batches were prepared for each out of two formulations and each batch solution was divided into four 100-mL amber glass bottles; two of them for storage at room temperature ( $25 \pm 3^\circ\text{C}$ ), and two for storage at  $40 \pm 0.5^\circ\text{C}$  (i.e.  $n = 4$  in each of the experimental conditions). The samples were protected from light.

The concentration of FUR and MP in the samples was estimated at the beginning of the stability study ( $c_0$  = day of solution preparation, an initial content of 100 %) and then at the time intervals of 7, 30, 90, 180, and 270 days. Each sample was measured in triplicate.

### ***Measurement of pH value***

The pH value was measured under stabilized conditions using a pH meter (pH 212 Microprocessor pH Meter, Hanna instruments, Germany) with a combined pH electrode. Each sample was measured at the time intervals mentioned above.

### ***Efficacy of antimicrobial preservation***

The test of the antimicrobial activity of the preservative methylparaben 0.1 % w/v (Ph.Eur., 5.1.3) which consists of challenging the preparation with a prescribed inoculum of microorganisms was carried out with the accredited laboratory (ITEST plus, Hradec Kralove, Czech Republic).

### ***Data analysis***



At each time interval, the percentage of the actual initial concentration remaining was calculated for FUR and MP (n = 4). Stability was defined as the retention of at least 90 % and/or 80 % of initial concentration of furosemide and/or methylparaben, respectively.

## RESULTS

The compositions of the preparations F1 and F2 are shown in Table 1. Both formulations contained saccharine sodium 0.1 % w/v as a taste modifier. They were prepared as quickly as possible in order to prevent decomposition of FUR by light.

In Figure 1, the HPLC chromatogram showing the separation of standard solution  $50 \mu\text{g mL}^{-1}$  of furosemide and formulation F2 is illustrated; the results of method validation are summarized in Table 2.

In Tables 3 and 4, the mean value of percentage concentration  $\pm$  SD of the initial furosemide and the antimicrobial agent methylparaben, respectively, in preparations F1 and F2 (n = 4) are shown for the stability time points and conditions mentioned in the Methods section. The amount of FUR and MP in milligrams per millilitre at the beginning of the study ( $c_0 = 100 \%$ ) is listed in the first row.

As illustrated in Table 3, the FUR concentration remaining was higher than 91 % after 270 days in both formulations F1 and F2 stored at both storage conditions (25°C and 40°C). The remaining MP concentration was higher than 80 % after 270 days in both formulations stored at 25°C as well as in the formulation F1 stored even at 40°C as shown in Table 4. In all cases, the chromatograms showed no evidence of product degradation throughout the 9-month stability study. No detectable changes in colour, odour or taste were observed in either furosemide formulations.

In contrast, a significant decrease in MP concentration in the formulation F2 stored at 40°C was observed. The percentage of MP remained within  $\pm 11\%$  for 30 days, decreasing to

approximately 70 % of the initial content after 90 days. At the end of the stability study (270 days), only approximately 40 % of MP was found (Table 4). Nevertheless, no apparent changes in colour, odour or taste were observed.

The value of pH for formulations F1 and F2 under conditions of the stability testing mentioned above was measured. The pH 6.6 and 7.5 for F1 and F2, respectively, remained practically unchanged throughout the storage at room temperature as well as for F2 at 40°C; in the preparation F1, the pH value slightly declined to 6.1 after 270 days when stored at 40°C.

## **DISCUSSION**

Furosemide is an active compound traditionally used in paediatric cardiology. In paediatrics, oral liquid preparations, particularly solutions, are the best dosage forms for flexible and accurate dosing and compliance of the patients. However, there is no commercially available liquid preparation that follows the latest recommendations on safety of paediatric drugs in terms of excipients used. Furosemide is practically insoluble in water, which is the main complication when preparing aqueous solutions. To increase the solubility of FUR in water, ethanol is often used in commercial preparations. [5-7, 17]. Unfortunately, preparations containing ethanol cannot be recommended for use in paediatric age group patients. The formation of FUR sodium salt by adjusting the alkaline pH is another method of making FUR soluble in water. In general, an aqueous solution of sodium hydroxide is used to achieve this. A furosemide injection solution whose pH value is approximately 9 is the example. In pharmacy, the commercially available aqueous injection can be simply diluted by WFI to achieve the paediatric suitable FUR concentration, e.g. 2 mg mL<sup>-1</sup>. Apart from the mentioned high pH value, moreover, such

an extemporaneously prepared oral solution has an unpleasant taste due to the presence of sodium hydroxide.

According to Pharmacopoeial requirements, multi-dose liquid preparations must be protected from microbial contamination by an addition of a suitable preservative.[15]

Unfortunately, widely used preservatives such as sodium benzoate or potassium sorbate show practically no antibacterial activity in the alkaline pH value. On the other hand, the paraben group of preservatives is effective over a wide pH range of 4–8 having a broad spectrum of antimicrobial activity. The activity of the parabens increases with increasing chain length of the alkyl moiety, but solubility decreases. [18] However, the reproductive toxicity of parabens appears to increase with increasing length of the alkyl chain, and there are specific data showing adverse reproductive effects in male rats of propyl and butyl parabens. In view of this and the fact that propyl and butyl parabens were not included in the acceptable daily intake (ADI) group for parabens, the World Health Organization committee concluded that the propyl and butyl paraben specifications for their use as a food additive should be withdrawn. In contrast to propyl and butyl parabens, neither methylparaben nor ethylparaben showed any effects on male reproductive organs, sperm parameters or sex hormones in juvenile rats. [19] Therefore, and also due to better solubility, methylparaben was finally chosen as a preservative.

To improve palatability of the oral solution, the addition of a suitable sweetener is usually necessary. Sucrose is often used in most paediatric liquid preparations and it was also tested during furosemide formulation development. Unfortunately, we observed two main disadvantages. The first one, the decrease in the pH value to approximately 6 leading to the risk of furosemide decomposition and/or precipitation. [14] The second, the change in

the solution colour to yellow or light brown during storage. The stability of sucrose containing solutions was determined by HPLC to only 90 days at room temperature (data not shown in this article). Finally, sodium saccharine 0.1 % w/v was used in both formulations presented in this work (F1, F2) due to its better stability.

### ***Developed paediatric formulations***

Two preparations of FUR (Table 1) were formulated for extemporaneous preparation in a hospital or community pharmacy. The composition F1 was prepared by dissolving FUR in an appropriate volume of 1 % sodium hydroxide solution similarly to the large scale manufacturing of FUR injections. The accurate added volume of hydroxide solution was determined by observing the dissolution visually. The final pH value of F1 preparation was 6.6. The preparation is similar to the simple dilution of parenteral injection; of course, the content of hydroxide makes its taste unpleasant for oral administration in children and a sweetener (sodium saccharine 0.1 % w/v) was therefore used to improve palatability.

In the formulation F2, the alkaline pH necessary for FUR dissolution in water was reached by the addition of disodium hydrogen phosphate dodecahydrate which was successfully used also in the previous paediatric propranolol and sotalol liquid formulations. [20-22] An appropriate amount of disodium hydrogen phosphate dodecahydrate was adjusted experimentally during the preparation development. In our experience, this formulation possesses more pleasant taste properties compared to the F1 formulation.

In the stability study, two batches of the formulated FUR aqueous solutions F1 and F2 were prepared in the Motol hospital pharmacy and stored in tightly closed amber glass bottles at  $25 \pm 3^{\circ}\text{C}$  and  $40 \pm 0.5^{\circ}\text{C}$ . The concentration of the FUR and the preservative MP was estimated throughout the time interval of 0 – 270 days using HPLC method. The content

of the furosemide in mg per mL at time of preparation was considered to be the actual initial one ( $c_0 = 100\%$ ). As can be seen in Table 3, the FUR percentage content remained within the targeted limit of the initial concentration in both formulations throughout the 270-day storage period at room temperature. Both preparations had suitable pH for maintaining FUR solubility.

The concentration of methylparaben remained within  $\pm 20\%$  of the initial concentration for both solutions stored at room temperature as well as at  $40^\circ\text{C}$  for F1 for 9 months. On the contrary, a significant decrease in concentration was observed for methylparaben in formulation F2 stored at  $40^\circ\text{C}$ , probably due to its decomposition. As can be seen in Table 4, the targeted remaining concentration  $\geq 80\%$  was maintained only up to 30 days.

Preparations F1 and F2 should be packaged in a brown glass container to protect from light. A screw cap suitable for use with a graduated pipette for oral use to achieve accurate dosing is recommended.

## **CONCLUSIONS**

Two aqueous, ethanol-free oral solutions containing furosemide in the concentration  $2\text{ mg mL}^{-1}$  were developed in accordance with the recent requirements of the safety of paediatric drugs. The preparations formulated for easy extemporaneous compounding in a pharmacy are suitable for the oedema therapy of various origins as well as hypertension in paediatric age groups above 1 month of age. The excipients used ensured stable pH, antimicrobial stability, and pleasant taste. A 9-month stability study performed by validated HPLC analysis demonstrated that the concentration of FUR in both F1 and F2 formulations was in accordance with the criterion that at least  $90\%$  of the initial content should remain during storage at  $25^\circ\text{C}$  or  $40^\circ\text{C}$ . Nevertheless, preparation F1 having a worse, slightly burning

taste caused by the presence of sodium hydroxide, although a sweetener sodium saccharine 0.1 % w/v was added, is less preferable when compared to F2 containing disodium hydrogen phosphate dodecahydrate. Moreover, sodium hydroxide is a highly caustic base which readily absorbs moisture and carbon dioxide from the air. This makes its manipulation problematic and the routine preparation of its solution quite inconvenient in a pharmacy. On the other hand, the preparation F2 has a more pleasant taste and is easier to prepare in a pharmacy as disodium hydrogen phosphate is easier to manipulate and weigh than sodium hydroxide. Formulation F2 therefore represents a compromise between good FUR solubility in water, taste acceptance in paediatric patients, and fast compounding procedure. For long stability at room temperature, the stock F2 solution could be prepared in advance in the pharmacy and be available until needed. Methylparaben 0.1 % w/v in preparation F2 stored at room temperature fully satisfied the recommended criteria for preservative efficacy in oral preparations according to Ph. Eur. 9.0 (5.1.3 Efficacy of antimicrobial preservation).

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#### **REFERENCES**

1. Sweetman SC. *Martindale: The Complete Drug Reference*, 37<sup>th</sup> Ed., Pharmaceutical Press, 2011. Furosemide, 1421-1425.
2. Nunn T, Williams J. Formulation of medicines for children. *British J Clin Pharmacol* 2005;59(6):674-76. doi: 10.1111/j.1365-2125.2005.02410.x

3. Standing JF, Tuleu C. Paediatric formulations - Getting to the heart of the problem. *Int J Pharm* 2005;300(1-2):56-66. doi: 10.1016/j.ijpharm.2005.05.006
4. Cram A, Breitzkreutz J, Desset-Brethes S, Nunn T, Tuleu C. Challenges of developing palatable oral paediatric formulations. *Int J Pharm* 2009;365(1-2):1-3. doi: 10.1016/j.ijpharm.2008.09.015
5. Frusol 20mg/5ml Oral Solution. <https://www.medicines.org.uk/emc/medicine/16559> (accessed 13 May 2017).
6. Impugan 10 mg/ml orala droppar, lösning <https://www.fass.se/LIF/product?userType=2&nplId=19880429000038> (accessed 13 May 2017).
7. Lasix® liquidum [https://www.medikamente-per-klick.de/images/ecommerce/04/66/04661307\\_2011-08\\_de\\_o.pdf](https://www.medikamente-per-klick.de/images/ecommerce/04/66/04661307_2011-08_de_o.pdf) (accessed 13 May 2017).
8. Drugs Com. Ethanol in liquid preparations intended for children. *Pediatrics* 1984;73(3):405-07. <http://pediatrics.aappublications.org/content/pediatrics/73/3/405.full.pdf> (accessed 13 May 2017)
9. Salunke S, Giacoia G, Tuleu C. The STEP (Safety and Toxicity of Excipients for Paediatrics) database. Part 1-A need assessment study. *Int J Pharm* 2012;435(2):101-11. doi: 10.1016/j.ijpharm.2012.05.004
10. Salunke S, Brandys B, Giacoia G, Tuleu C. The STEP (Safety and Toxicity of Excipients for Paediatrics) database: Part 2-The pilot version. *Int J Pharm* 2013;457(1):310-22. doi: 10.1016/j.ijpharm.2013.09.013

11. Fiocchi A, Riva E, Giovannini M. Ethanol in medicines and other products intended for children: Commentary on a medical paradox. *Nutr Res* 1999;19(3):373-79. doi: [http://dx.doi.org/10.1016/S0271-5317\(99\)00004-4](http://dx.doi.org/10.1016/S0271-5317(99)00004-4)
12. Draft guideline on pharmaceutical development of medicines for paediatric use. European Medicines Agency.  
[http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2011/06/WC500107908.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/06/WC500107908.pdf) (accessed 13 May 2017)
13. EMA/ HMPC/85114/2008. Reflection paper on ethanol content in herbal medicinal products<sup>1</sup> and traditional herbal medicinal products used in children. 2010.  
[http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2010/02/WC500070331.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/02/WC500070331.pdf). (accessed 13 May 2017).
14. Troy, DB. ed. *Remington: The science and practice of pharmacy*. 21<sup>th</sup> Ed. Philadelphia: Lippincott Williams & Wilkins 2005;1430.
15. EDQM. *European Pharmacopoeia*. 9th Edition ed, 2016.
16. ICH Expert Working Group (2005) *Validation of Analytical Procedures: Text and Methodology Q2(R1)*.  
[http://www.ich.org/fileadmin/Public\\_Web\\_Site/ICH\\_Products/Guidelines/Quality/Q2\\_R1/Step4/Q2\\_R1\\_Guideline.pdf](http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf) (accessed 13 May 2017).
17. Ghanekar AG, Das Gupta V, Gibbs CW, Jr. Stability of Furosemide in Aqueous Systems. *J Pharm Sci* 1978;67(6):808-11. doi: 10.1002/jps.2600670621
18. Rowe RC. *Handbook of Pharmaceutical Excipients*. 6<sup>th</sup> Ed. London: Pharmaceutical Press 2009.



19. WHO Technical Report Series. Evaluation of Certain Food Additives and Contaminants. 2007.

[http://apps.who.int/iris/bitstream/10665/43592/1/WHO\\_TRS\\_940\\_eng.pdf](http://apps.who.int/iris/bitstream/10665/43592/1/WHO_TRS_940_eng.pdf). (accessed 13 May 2017)

20. Zahálka L, Matysová L, Šklubalová Z, Klovrzová S, Solich P. Simultaneous determination of propranolol hydrochloride and sodium benzoate in oral liquid preparations by HPLC. *Chromatographia* 2013;76(21-22):1553-58. doi: 10.1007/s10337-013-2457-z

21. Klovrzová S, Zahálka L, Matysová L, Horák P, Šklubalová Z. Pediatric oral solutions with propranolol hydrochloride for extemporaneous compounding: the formulation and stability study. *Cesk Slov Farm* 2013;62(1):35-39.

22. Matysová L, Zahálková O, Klovrzová S, Šklubalová Z, Solich P, Zahálka L. Development of a gradient HPLC method for the simultaneous determination of sotalol and sorbate in oral liquid preparations using solid core stationary phase. *J Anal Meth Chem* 2015;2015:6. doi: 10.1155/2015/806736

## FIGURE LEGEND

**Figure 1.** Liquid chromatography separation of standard  $50 \mu\text{g mL}^{-1}$  solution of furosemide and formulation F2 ( $2 \text{ mg mL}^{-1}$  of furosemide)

SACC (saccharine), IMP A (FUR impurity A), FUR (furosemide), MP (methylparaben).

**Table 1.** Composition of formulations

	<b>F1</b>	<b>F2</b>
Furosemide	0.2 g	0.2 g
Methylparaben	0.1 g	0.1 g
Sodium hydroxide	0.024 g	–
Disodium hydrogen phosphate dodecahydrate	–	1.5 g
Saccharine sodium	0.1 g	0.1 g
Water for injections	to 100.0 mL	to 100.0 mL

**Table 2.** Validation results of pharmaceutical formulation F1 and F2

	F1		F2		Criterion
	FUR	MP	FUR	MP	
Repeatability tR (%R.S.D.) <sup>a</sup>	0.26	0.12	0.26	0.12	X < 1 %
Repeatability Area (%R.S.D.) <sup>a</sup>	0.16	0.32	0.16	0.32	X < 1 %
Number of theoretical plates	2 499	7 892	2 499	7 892	-
Resolution <sup>a</sup>	1.57	12.49	1.57	12.49	R <sub>ij</sub> > 1.5
Tailing factor <sup>a</sup>	1.18	1.09	1.18	1.09	T = 0.8–1.5
Precision (%R.S.D.) <sup>b</sup>	3.55	3.54	2.13	1.52	X < 5 %
Linearity (Correlation coefficient) <sup>c</sup>	0.9990	1.0000	0.9990	1.0000	R ≥ 0.9990
Accuracy Recovery (%) <sup>b</sup>	103.48	104.35	100.83	102.56	X = 100 ± 5 %
Accuracy (%R.S.D.) <sup>b</sup>	0.61	0.36	1.75	1.84	X < 5 %
Selectivity	No interference		No interference		No interference

*a**six injections**b**six samples, three injections of each sample**c**at 40, 50, 80, 100, 120 and 150 % concentration levels**FUR**furosemide**MP**methylparaben**FUR X**formulations with various excipients**%R.S.D.**Relative Standard Deviation in %*

**Table 3.** Stability of furosemide in formulations F1 and F2 stored at 25 °C and at 40 °C.\*

<b>Time (days)</b>	<b>25 ± 3°C</b>		<b>40 ± 0.5°C</b>	
	<b>F1</b>	<b>F2</b>	<b>F1</b>	<b>F2</b>
0 (100 %)	2.15 ± 0.02 mg/mL	2.12 ± 0.05 mg/mL	2.15 ± 0.02 mg/mL	2.12 ± 0.05 mg/mL
7	102.33 ± 1.39	98.14 ± 2.10	99.06 ± 2.35	92.89 ± 2.62
30	98.60 ± 1.30	97.67 ± 0.12	100.00 ± 1.50	94.34 ± 1.77
90	98.14 ± 1.48	96.28 ± 0.78	98.58 ± 0.99	94.81 ± 1.27
180	99.07 ± 0.61	91.63 ± 0.66	100.00 ± 0.61	95.75 ± 1.05
270	93.95 ± 0.56	92.56 ± 1.05	95.28 ± 2.33	91.98 ± 0.50

\*Mean ± SD of determinations for four samples (n = 4)

**Table 4.** Stability of methylparaben in formulations F1 and F2 stored at 25 °C and at 40°C.\*

<b>Time (days)</b>	<b>25 ± 3°C</b>		<b>40 ± 0.5°C</b>	
	<b>F1</b>	<b>F2</b>	<b>F1</b>	<b>F2</b>
0 (100 %)	1.03 ± 0.03 mg/mL	1.04 ± 0.02 mg/mL	1.03 ± 0.03 mg/mL	1.04 ± 0.02 mg/mL
7	100.00 ± 0.15	98.06 ± 2.80	95.14 ± 2.75	89.52 ± 2.26
30	99.03 ± 1.91	98.06 ± 0.25	97.08 ± 0.81	83.50 ± 1.50
90	99.03 ± 1.06	96.11 ± 0.47	95.14 ± 0.80	69.90 ± 1.37
180	98.06 ± 1.41	92.29 ± 2.00	90.29 ± 0.59	52.43 ± 1.06
270	97.12 ± 1.10	91.98 ± 0.30	89.42 ± 2.21	41.75 ± 0.53

\*Mean ± SD of determinations for four samples (n = 4)