Stability of warfarin sodium flavoured preservative-free oral liquid formulations

Joël Schlatter,¹ Salvatore Cisternino^{1,2}

¹Pharmacie Clinique, Hôpital Universitaire Necker - Enfants Malades, Paris, France
²Faculté de Pharmacie, UMR-S 1144, Université Paris Descartes, Paris, France

Correspondence to

Dr Joël Schlatter, Pharmacie Clinique, Hôpital Universitaire Necker - Enfants Malades, Paris, France; joel.schlatter@aphp.fr

Received 6 April 2017
 Revised 22 May 2017
 Accepted 5 June 2017
 Published Online First 13 July 2017

EAHP Statement 3:
 Production and
 Compounding.

ABSTRACT

Objectives This study was designed to assess the physicochemical stability of warfarin sodium liquid formulations stored at room temperature and under refrigeration.

Methods Formulations of warfarin sodium 1mg/mL were prepared from either powder or tablets dispersed in mixture containing deionised water, glycerin, sodium phosphate and strawberry alcohol-free concentrate flavour and stored in amber glass bottles at 25°C and 4°C. Stability indicating high-performance liquid chromatography assay procedure was used to analyse the concentration of the drug at the time of preparation and on days 7, 14, 21, 30, 45, 60 and 90. Changes in colour, taste and pH, as well as visual evidence of precipitation, were assessed for physicochemical instability.

Results Between day 0 and day 90, the pH of the powder and tablet formulations stored at 25°C decreased by -1.40 units and -1.16 units, respectively. There were minor changes in pH for both formulations stored at 4°C. The formulation from powder stored at 25°C and 4°C maintained at least 90% of the initial warfarin concentration for 30 days and 45 days, respectively. The formulation from tablets stored at 25°C and 4°C maintained at least 90% of initial warfarin concentration for 21 days and 30 days, respectively.

Conclusion Warfarin sodium in flavoured preservative-free formulation from powder is recommended and stable in amber glass bottles for at least 30 days stored at 25°C and 45 days stored at 4°C.

INTRODUCTION

Warfarin is the most powerful antithrombotic agent prescribed in infants and children used for prophylaxis after Fontan surgery, mechanical prosthetic valves, Kawasaki disease with large aneurysms, dilated cardiomyopathy and idiopathic pulmonary arterial hypertension.^{1,2} Warfarin by inhibiting the vitamin K-dependent coagulation factors synthesis (factors II, VII, IX, X, the anticoagulant proteins C and S) reduces the regeneration of vitamin K from vitamin K epoxide in the vitamin K cycle through inhibition of vitamin K epoxide reductase, a multiprotein enzyme complex.^{3,4} Warfarin is commercially available as tablet and in some countries as an oral suspension. Since it is widely used in paediatric patients, it has been widely dispensed as extemporaneously compounded suspensions to treat infants unable to swallow tablets or the insertion of nasogastric tube. A literature survey yielded one stability study of warfarin sodium suspensions.⁵ Stability of warfarin sodium mixtures at 1mg/mL formulated from tablets or warfarin sodium

clathrate pure powder and both using the additive compound hydroxybenzoate sodium Australian Pharmaceutical Formulary (APF) as preservative was demonstrated for 28 days at controlled room temperature (22±2°C). Preliminary studies with several vehicles known as Ora-Plus, Ora-Sweet and Inorpha caused immediate warfarin clumping and were therefore discarded from our evaluation. Indeed, the percentage of the warfarin sodium initial concentration 1 hour after mixing with these vehicles remained at 45.7% in Ora-Plus and Ora-Sweet mixture (1:1) and 37.6% in Inorpha. We then developed alternative vehicles using limited ingredients and parabens free. The purpose of this study was to design warfarin sodium oral liquid formulations that allow for the optimal stability conditions and minimise the number of excipients. The formulations did not contain these ingredients with deleterious effect such as parabens, alcohol or polysorbates.

MATERIAL AND METHODS

Preparation of warfarin suspension and solution

The method below was used to prepare a 1 mg/mL suspension from commercially available tablets and a 1 mg/mL solution from powder. One litre of mixture was prepared in deionised water (lot 13KEP071; Versylene, Fresenius Kabi, Sèvres, France) containing of 10 g sodium phosphate (lot 15398103; Inresa Pharma, Bartenheim, France), 5 mL strawberry alcohol-free concentrate flavour (lot 14311210, Inresa Pharma) and mixed to yield a clear dispersion. The strawberry alcohol-free flavour is obtained from pharmaceutical laboratory and certified safe to be eating. To prepare the 1 mg/mL warfarin sodium suspension, 20 Coumadine 5 mg tablets (lot 163329; Bristol-Myers-Squibb, Rueil-Malmaison, France) were crushed in a mortar to a fine powder and mixed with glycerin (lot 15036903, Inresa Pharma) (4 mL) to form a homogeneous paste. The previous mixture was then added to the mortar with constant trituration to wash out any tablet powder residue and transferred to the 100 mL graduated flask. The suspension was made to volume with the mixture (table 1). To prepare the 1 mg/mL warfarin sodium solution, 108.7 mg warfarin sodium clathrate (lot 4006WARI, Inresa Pharma) (92% warfarin sodium and 8% 2-propanol, warfarin sodium clathrate 1.10 mg is equivalent to 1.0 mg of warfarin sodium) were accurately weighed and mixed with glycerin (4 mL) until forming a smooth paste. The same procedure above was used to obtain the final solution (table 1). Three independent batches of each formulation were prepared and placed in 125 mL amber glass bottles (Cooper, Melun, France) with

To cite: Schlatter J, Cisternino S. *Eur J Hosp Pharm* 2018;**25**:e98–e101.

Table 1 Formulations of the warfarin sodium 1 mg/mL preparations

Ingredients	Quantity
Coumadine 5 mg tablets	20
Warfarin sodium clathrate powder	108.7 mg
Glycerin	4 mL
Sodium phosphate	1 g
Strawberry alcohol-free concentrate flavour	0.5 mL
Sterile water for irrigation to	100 mL

universal bottle adaptor (Baxter, Guyancourt, France). A total of six bottles were conditioned for each formulation. Three bottles of each formulation were stored at room temperature (22°C–25°C), and three bottles were stored under refrigeration (4°C–8°C). A sample (50 µL) was withdrawn from each bottle immediately after preparation and at 7, 14, 30, 45, 60 and 90 days. Prior to sampling, bottles of tablet formulation were vigorously shaken (10 s). At each time point, all bottles were examined for obvious changes in homogeneity, colour and odour. Warfarin sodium concentration was determined by a previous validated stability-indicating high-performance liquid chromatography (HPLC) method.⁶ The pH of the contents of each bottle was determined using a pH metre (SevenExcellence; Mettler Toledo, Viroflay, France), which was calibrated at the beginning of each time point using commercially available standards (buffer standard solutions at pH 4.01 and pH 7.00, lots 1Y113A and 1Y113D, respectively; Mettler Toledo, Viroflay, France).

Standard solution and standard curve

A stock solution of warfarin sodium 1 mg/mL was prepared by dissolving 100 mg of certified reference material warfarin sodium powder (lot LRAA2743; Sigma-Aldrich, St Quentin Fallavier, France) in 100 mL of deionised water. Aliquots from this solution were suitable diluted with mobile phase to get the working standard solutions of warfarin sodium in the concentration range of 10–100 µg/mL. Six-point calibration curve (10, 20, 40, 50, 75 and 100 µg/mL) was prepared at the beginning of each run to ensure no carryover from one run to the next. The calibration curve was generated by the least-squares regression of the peak area of warfarin sodium and the concentration of each warfarin sodium standard. The precision of the assay was evaluated by intraday and interday validation methods. Intraday validation was determined by running low, medium and high concentrations (15, 30 and 80 µg/mL, n=6) throughout a single day, whereas interday variability was determined by running the same concentrations as those used for intraday validation in triplicate daily for 4 days. The accuracy of the assay method was evaluated at different levels (20%, 50%, 80%, 100% and 120%), and the mean recovery of warfarin of the target concentration (50 µg/mL) was calculated. Acceptable limits of coefficients of variation were defined a priori as less than 5%, and acceptable limits for accuracy were defined as greater than 95%. The system suitability data was performed and monitored. A 50 µL sample was transferred into a 3 mL tube and mobile phase (950 µL) was then added to a drug final concentration of 50 µg/mL and the content was transferred into a 2 mL vial for HPLC analysis.

HPLC method

The stability-indicating HPLC method developed by Bhat *et al* was adapted for the warfarin sodium assay.⁶ The quantitation of the drug was performed using a reversed-phase HPLC system (Thermo-Fischer Dionex Ultimate 3000) comprising an SRD-3200 integrated solvent and degasser, a HPG-3200SD

Figure 1 Typical chromatograms of warfarin sodium formulation prepared from powder (A) and tablets (B).

quaternary pump, an WPS-3000TSL refrigerated autosampler, a TCC-3000 column compartment and a MWD-3000 multiple wavelength photodiode array detector. The flow rate was 1 mL/min, and the injection volume was 25 µL. The separation was performed on a C₁₈ 4.6×150 mm column (Nova-Pak; Waters, Guyancourt, France) maintained at ambient temperature. An isocratic elution of 4 min was performed using a mobile phase consisted of acetonitrile (Lichrosolv; Merck, Fontenay-sous-Bois, France) and phosphate buffer pH 5 (70:30, by volume). The ultraviolet detector wavelength was set at 220 nm. The stability-indicating capacity of the assay was re-evaluated in our laboratory. Degradation of warfarin sodium was forced by mixing 1 mL separate samples of each formulation type with 100 µL of 1 N hydrochloric acid (VWR International, Fontenay-sous-Bois, France), 1 N sodium hydroxide (Cooper, Melun, France) or 3% hydrogen peroxide (Gifrer Barbezat, Decines, France) in a Vortex mixer and heated to 60°C for 2 hours. The samples were cooled to room temperature, and the pH was corrected to 7. The samples were diluted with mobile phase to reach a target warfarin sodium concentration of 50 µg/mL and injected into the HPLC system. The chromatograms obtained were compared with a chromatogram obtained from the calibration curve.

Data analysis

The stability of warfarin sodium was determined by calculating the percentage of the initial concentration remaining at each time point. The percentage of warfarin sodium remaining at the end of the study period was calculated from the concentration

Figure 2 Chromatograms of warfarin sodium after acid hydrolysis (1N HCl, 2 hours, 60°C) (A), base hydrolysis (1N NaOH, 2 hours, 60°C) (B) and oxidative degradation (3% H₂O₂, 2 hours, 60°C) (C).

Table 3 pH determination at each day assay

Formulation	Mean±SD	pH (deviation between actual pH and initial pH)							
		Day 0	Day 7	Day 14	Day 21	Day 30	Day 45	Day 60	Day 90
Powder	8.63±0.01								
25°C		7.96 (−0.67)	7.84 (−0.79)	7.78 (−0.85)	7.68 (−0.95)	7.37 (−1.26)	7.31 (−1.32)	7.23 (−1.40)	
4°C		8.50 (−0.13)	8.43 (−0.20)	8.36 (−0.27)	8.09 (−0.54)	7.91 (−0.72)	7.86 (−0.77)	7.76 (−0.87)	
Tablets	8.12±0.01								
25°C		7.73 (−0.39)	7.56 (−0.56)	7.49 (−0.63)	7.37 (−0.75)	7.18 (−0.94)	7.09 (−1.03)	6.96 (−1.16)	
4°C		8.03 (−0.09)	8.00 (−0.12)	7.94 (−0.18)	7.83 (−0.29)	7.71 (−0.41)	7.68 (−0.44)	7.57 (−0.55)	

Table 4 Stability of warfarin 1 mg/mL liquid formulations stored at 25°C and 4°C

Formulation	Mean±SD initial drug concentration (mg/mL)	% Initial concentration remaining						
		Day 7	Day 14	Day 21	Day 30	Day 45	Day 60	Day 90
Powder	1.045±0.004							
25°C		99.9±1.7	96.2±1.6	95.0±1.3	93.0±1.2	90.3±1.5	88.3±1.0	87.3±1.2
4°C		99.4±1.9	99.0±1.3	96.4±1.4	94.5±1.3	93.7±1.4	90.7±1.6	89.7±1.4
Tablets	1.002±0.003							
25°C		96.6±1.2	94.2±1.4	91.7±1.2	89.4±1.8	88.5±1.0	86.2±1.3	82.8±1.5
4°C		96.1±1.3	95.6±1.3	90.8±1.6	91.5±1.2	89.3±1.3	87.0±1.4	85.3±1.8

measured on the last day (ie, day 90), as determined by linear regression and the concentration observed on day 0, per the formula: (concentration at day 90/concentration on day 0) × 100%. A new calibration curve was run on each day of the study.

Table 2 Forced degradation studies

Stress condition	% degradation	Retention time of degraded products
Acid hydrolysis (1N HCl, 2 hours, 60°C)	95.2	1.44 min
Base hydrolysis (1N NaOH, 2 hours, 60°C)	23.5	1.31 min
Oxidative stress (3% H ₂ O ₂ , 2 hours, 60°C)	46.4	1.35 and 1.54 min
Thermal stress (60°C, 4 days)	10.0	–
Direct sunlight exposure (4 days)	0	–

Stability was defined as the retention of at least 90% of the initial warfarin sodium concentration.

RESULTS

The linear calibration plot for above method was obtained over the calibration range 10–100 µg/mL (20%–200% of warfarin sodium, nominal concentration 50 µg/mL), and the correlation coefficient obtained was greater than 0.999. The highest intra-day and interday coefficients of variation for both formulations were 1.22% and 0.53% at 15 µg/mL, 0.95% and 1.96% at 30 µg/mL, and 0.53% and 1.73% at 80 µg/mL, respectively. Percentage of recovery for both formulations was found to be 97.3–102.3 with % RSD ranges 0.18–2.04. The retention times of warfarin were 2.44 min and 2.45 min for powder and tablets

formulations (figure 1), respectively. Warfarin sodium was found to degrade greatly by acid hydrolysis and more slightly with base hydrolysis or peroxide stress conditions (table 2). Stress condition by either direct sunlight or high temperature was associated with less 10% of degradation (table 2). The assay of warfarin sodium was unaffected by the presence of its degradation products and thus confirms the stability-indicating capacity of the method (figure 2). No suspension odour or colour evolution was noted during the study period except for the formulation from powder stored at 25°C with the formation of turbid at day 45. The mean pH of the two formulations using powder or tablets was initially 8.63 and 8.12, respectively. Throughout the study period, notable decrease by −1.40 units and −1.16 pH units was observed for the warfarin solutions prepared from powder and tablet, respectively, when stored at 25°C (table 3). No visible microbial growth was detected. The formulation from powder stored at 25°C and 4°C maintained at least 90% of the initial warfarin sodium concentration for 30 days and 45 days, respectively (table 4). The formulation from tablets stored at 25°C and 4°C maintained at least 90% of initial warfarin sodium concentration for 21 days and 30 days, respectively (table 4). No degradation products appeared on chromatograms during the study period.

DISCUSSION

The literature search yielded one stability study of warfarin mixtures 1 mg/mL formulated using tablets or warfarin sodium clathrate powder with 28 days of stability.⁵ However, parabens were used as preservative and warfarin sodium powder was not pharmaceutical grade.⁵ Our study investigates the stability of the pharmaceutical grade warfarin sodium in flavoured preservative-free mixtures with tablets and powder during longer time. During the preparation of the warfarin sodium formulation from powder, it was noted that solution was easier to prepare and to obtain a clear mixture. However, the homogenisation of the formulation from tablets was more difficult and the apparent mixture was unclear due to insolubility excipients. Sodium phosphate was included in all preparations to ensure the solubility of warfarin that is slightly soluble in solutions with a pH below 8.⁵ There were minor changes in pH for both formulations stored at 4°C. During the stability study, some variability was observed as calculated pH of formulations stored at 25°C fluctuated above 1 unit. This study showed the stability of warfarin sodium formulations was less than in the study of Sharley. Formulations evaluated in this study were compounded using a few ingredients and free from preservatives. The lack of microbiological evaluation in this study could be a limitation in stability data. However, the high pH of the formulations can affect the rate of growth of microbes. In general, microbial growth is optimal between pH 6 and 8 and declines significantly outside this range.⁷ In addition, the most used parabens preservatives degraded in solution at pH 7.5 and above.⁸ In alkaline solutions, parabens are hydrolysed to p-hydroxybenzoic acid and the corresponding alcohol.⁹ To compensate for chemical instability of the preservative system, excess parabens need to include in formulations with a high risks of adverse effects.

CONCLUSION

Warfarin oral liquid formulations 1 mg/mL can be prepared from either tablets or powder with various chemically stability.

Our study demonstrate a better stability for warfarin sodium 1 mg/mL solution prepared from the pharmaceutical grade in flavoured preservative-free formulation for at least 30 days at 25°C or 45 days at 4°C. Due to absence of preservative in studied formulations, the preferential use of the formulation stored at 4°C and prepared from the powder is recommended with a maximum expiry date of 1 month. The palatability of the formulations seemed acceptable by children with a pleasant and slightly sweet taste.

What this paper adds

What is already known on this subject

- Published data on the stability of warfarin mixtures are limited.
- Stability of warfarin sodium mixtures at 1 mg/mL formulated from tablets or powder and using preservatives was demonstrated for 28 days at controlled room temperature.

What this study adds

- This study proposes an alternative formulation using limited ingredients and parabens free.
- Warfarin sodium 1 mg/mL preservative-free oral liquid prepared from commercially available tablets stored at 25°C and 4°C maintained at least 90% of initial warfarin sodium concentration for 21 days and 30 days, respectively.
- Warfarin sodium 1 mg/mL preservative-free oral liquid prepared from pharmaceutical grade powder stored at 25°C and 4°C maintained at least 90% of the initial warfarin sodium concentration for 30 days and 45 days, respectively.

Contributors JS conceived and initiated the study. SC contributed to refinement of the study protocol and approved the final manuscript.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

© European Association of Hospital Pharmacists (unless otherwise stated in the text of the article) 2018. All rights reserved. No commercial use is permitted unless otherwise expressly granted.

REFERENCES

- 1 Marek E, Momper JD, Hines RN, *et al.* Prediction of warfarin dose in pediatric patients: an evaluation of the predictive performance of several models. *J Pediatr Pharmacol Ther* 2016;21:224–32.
- 2 Jain S, Vaidyanathan B. Oral anticoagulants in pediatric cardiac practice: a systematic review of the literature. *Ann Pediatr Cardiol* 2010;3:31–4.
- 3 Incampo F, Carrieri C, Galasso R, *et al.* Effect of warfarin treatment on thrombin activatable fibrinolysis inhibitor (TAFI) activation and TAFI-mediated inhibition of fibrinolysis. *J Thromb Haemost* 2013;11:315–24.
- 4 Czogalla KJ, Biswas A, Höning K, *et al.* Warfarin and vitamin K compete for binding to Phe55 in human VKOR. *Nat Struct Mol Biol* 2017;24:77–85.
- 5 Sharley NA, Yu AMC, Williams DB. Stability of Mixtures formulated from Warfarin Tablets or Powder. *Journal of Pharmacy Practice and Research* 2007;37:95–7.
- 6 Bhat PV, Manisha Phoujdar SS, *et al.* Stability indicating RP-HPLC method development and validation for estimation of warfarin sodium clathrate in bulk and pharmaceutical dosage form. *World J Pharm Pharmaceut Sci* 2015;4:1366–76.
- 7 Elder DP, Crowley PJ. Antimicrobial preservatives part two: choosing a preservative. *Am Pharm Rev* 2012. <http://www.americanpharmaceuticalreview.com/Featured-Articles/38885-Antimicrobial-Preservatives-Part-Two-Choosing-a-Preservative> January (accessed May 2015).
- 8 Blaug SM, Grant DE. Kinetics of degradation of the Parabens. *J Soc Cosmetic Chemistry* 1974;25:495–506.
- 9 Błędzka D, Gromadzińska J, Wąsowicz W, Parabens WW. Parabens from environmental studies to human health. *Environ Int* 2014;67:27–42.