The development of a stable oral solution of captopril for paediatric patients

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ABSTRACT
Study objectives: Many major drugs are not available in paediatric form. The aim of this study was to develop a stable liquid solution of captopril for oral paediatric use allowing individualised dosage and easy administration to newborn and young patients.
Methods: A specific HPLC-UV method was developed. In a pilot study, a number of formulations described in the literature as affording one-month stability were examined. In the proper long-term study, the formulation that gave the best results was then prepared in large batches and its stability monitored for two years at 5°C and room temperature, and for one year at 40°C.
Results: Most formulations described in the literature were found wanting in our pilot study. A simple solution of the drug (1 mg/mL) in purified water (European Pharmacopeia) containing 0.1% disodium edetate (EDTA-Na) as preservative proved chemically and microbiologically stable at 5°C and room temperature for two years.
Conclusion: The proposed in-house formulation fulfils stringent criteria of purity and stability and is fully acceptable for administration to newborn and young patients.

KEYWORDS
Captopril, formulation, pharmaceutical technology, chemical stability, microbial stability, high-performance liquid chromatography (HPLC)

INTRODUCTION
According to recent studies, a significant proportion of medicines prescribed to hospitalised newborns and children do not exist as paediatric formulations [1, 2]. This does not imply that the prescribed drug is ineffective or unsafe in such patients, but more simply, that manufacturers have no incentive to develop such a form.

This situation presents hospital pharmacists with a clear challenge. Given the impossibility of administering tablets or capsules to newborns or infants, a liquid formulation has to be developed whose stability must be optimised and shelf-life determined. This paper describes such a study involving the oral formulation of captopril.

Captopril (Figure 1) is a well-known inhibitor of angiotensin-converting enzyme (ACE) frequently used to treat arterial hypertension and congestive cardiac failure in adults and children [3-5]. A comprehensive study carried out in 1997 in 53 French hospitals has shown that the most frequently prescribed paediatric drugs were (in decreasing order): diphenamid, captopril, fludrocortisone, ranitidine, spironolactone and ursodeoxycholic acid [6]. Initial doses of captopril administered to premature neonates and newborns range from 0.01 to 0.1 mg/kg every eight to 24 hours. The dose has then to be titrated up to a maximum of 0.5 mg/kg/dose given every six to 24 hours [3].

Whereas the hydrolytic cleavage of captopril is negligible
under pharmaceutically relevant conditions, its oxidative
dimerisation to a disulphide is a significant pharmaceutical
problem. The reaction is catalysed by metal ions and its rate
depends on pH and oxygen concentrations. Thus, the oxi-
dation of captopril is lowest at pH 4, and is markedly slowed
down by chelating agents, antioxidants, high concentrations
of the drug, and a small and nitrogen-saturated headspace
[7-11].

A number of studies have investigated the stability of cap-
topril in boiled tap water, distilled water, sterile water, dilut-
ed syrup containing 2% methylcellulose, and in the oral
vehicles Ora-Sweet, Ora-Sweet SF and Ora-Plus. EDTA-
Na and ascorbic acid were also used as additives. The
stability of captopril in these preparations was highly vari-
able and depended on the quality of the raw materials
[12-24]. In a one-month study, the most stable sam-
ple of captopril was obtained with a pH 3.2 solution con-
taining EDTA-Na and stored at 5°C in flasks of brown
glass [20].

The objective of our study was to obtain a captopril solution
suitable for oral administration to newborns and young chil-
dren and showing very good chemical and microbiological
stability. We have already reported some preliminary results
[25].

MATERIALS AND METHODS

Chemicals

Water complying with European Pharmacopeia (Pharm
Eur) standards, obtained by reverse osmosis, was used [26].
Captopril Pharm Eur (produced by BUFA BV
Pharmaceutical Products) was supplied by Dynapharm
(Meyrin, Switzerland). Ascorbic acid Pharm Eur was pur-
chased from Hänseler (Herisau, Switzerland) and EDTA-Na
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Materials and methods

Analytical methods

The concentration of captopril was measured by HPLC as
described in the literature [12, 15, 16, 19]. A Varian automa-
ted HPLC system with StarStation software was used consist-
ing of a 9012 pump, a Prostar 410 autosampler, an incorpo-
rated column oven (temperature range from room tempera-
ture to 60°C, accuracy: ± 1°C), a 9065 diode-array (DAD)
detector and a computer. The stationary phase was a
Hamilton PRP-1 analytical column (150 x 4.1 mm, 5 μm par-
ticle size, 100 Å pore size) heated to 50°C. The mobile
phase was a 77:23 v/v mixture of phosphoric acid 0.01 M
and acetonitrile. Samples were diluted 1:10 with phosphoric
acid 0.01 M, and the injection volume was 20 μL. Flow rate
was 1.0 mL/min, and measurements were made at 205 nm.
The retention time of captopril was three minutes.

The forced degradation of captopril in solution (1 mg/mL)
showed the HPLC assay to be stability-indicating and
allowed its validation according to the guide published by the
Société Française des Sciences et Techniques Pharma-
aceutiques [27]. The five distinct conditions for forced degra-
dation were as follows:

1. Heat degradation of the unbuffered solution of captopril at
100 ± 1°C for one hour.
2. Acidification of the captopril solution with the same vol-
ume of a hydrochloric acid (HCl) 5N solution, then stor-
ing for one hour at room temperature. This acidic solution
was neutralised with sodium hydroxide (NaOH) 2N
before analysis.
3. Alkalinisation of the captopril solution with the same vol-
ume of a NaOH 5N solution, then storing for one hour at
room temperature. This alkaline solution was neutralised
with HCl 2N before analysis.
4. Mixing of the captopril solution with the same volume of a
sodium peroxide (H2O2) 0.3% solution, then storing for
one hour at room temperature.
5. Exposure of the captopril solution to daylight for a total of
30 hours.

In the chromatograms obtained from these tests, captopril
was always well separated from its degradation products
(see later). External standard curves were produced for
each assay using five dilutions of the solution (0.06-0.14
mg/mL).

Other tests

Microbiological tests were carried out at the beginning, after
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Analytical validation data
The linearity coefficient of determination was $r^2$ 0.999 and the relative standard deviation (percentage of RSD) of response factor (peak area/concentration) was < 2%. The relative standard deviation (percentage of RSD) of intraday and interday variation was 1.58 and 2.02%, respectively.

Pilot stability study
The 10 formulations examined in the month-long pilot study and the stability results are shown in Table 1. After one month, no breakdown product was seen in the control formulation (No.1, captopril 1 mg/mL and EDTA-Na 0.1% in water). The stability of captopril in the nine other formulations already described in the literature was consistent with published results. These results showed that captopril solutions prepared by dissolving commercial tablets [19-21, 24] had a limited stability despite the addition of ascorbate and/or EDTA.

RESULTS AND DISCUSSION

HPLC method
The chromatograms obtained after forced degradation by heat, acid, base, oxidising agent and daylight showed an excellent separation between the peak of captopril (retention time three minutes) and those of the breakdown products (Figure 2). For example, H$_2$O$_2$ degradation produced two peaks (retention times 4.1 and 6.3 minutes) completely separated from that of captopril. Under these stress conditions there was a distinct peak separation between the drug and its degradation products as shown by the standard deviation (SD) of the absorbance spectra. In all cases, the SD of the average purity parameter of all peaks was lower than 1nm (for example: SD = 0.344 for H$_2$O$_2$ degradation). Moreover, in the best and worst correlations, the similarity factor was 0.9995 and 0.996 respectively, and the dissimilarity factor was 0.031 and 0.087 respectively.
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Table 1: Pilot study of 1 mg/mL captopril solutions stored for one month in 10 mL brown flasks

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. 1</td>
</tr>
<tr>
<td>Source of captopril: Powder</td>
<td>+</td>
</tr>
<tr>
<td>Tablets</td>
<td>-</td>
</tr>
<tr>
<td>Solvent: Purified water</td>
<td>+</td>
</tr>
<tr>
<td>Ora-Plus / Ora-Sweet (1:1)</td>
<td>-</td>
</tr>
<tr>
<td>Antioxidant: EDTA-Na (1 mg/mL)</td>
<td>+</td>
</tr>
<tr>
<td>Ascorbate (5 mg/mL)</td>
<td>-</td>
</tr>
<tr>
<td>pH at time 0</td>
<td>3.35</td>
</tr>
<tr>
<td>pH after one month:</td>
<td></td>
</tr>
<tr>
<td>At room temperature</td>
<td>3.26</td>
</tr>
<tr>
<td>At 5 ± 3°C</td>
<td>3.33</td>
</tr>
<tr>
<td>% captopril remaining after one month (± SD, n = 3):</td>
<td></td>
</tr>
<tr>
<td>At room temperature</td>
<td>100.3 ± 0.55</td>
</tr>
<tr>
<td>At 5 ± 3°C</td>
<td>99.7 ± 110</td>
</tr>
</tbody>
</table>

Table 2: Long-term stability study of formulation No. 1

<table>
<thead>
<tr>
<th>Time</th>
<th>Storage temperature</th>
<th>Percentage of initial concentration ± SD, n = 9 (lower and upper 95% confidence limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 ± 3°C</td>
<td>22 ± 2°C * (room temperature)</td>
</tr>
<tr>
<td>Day 0</td>
<td>100.0 ± 0.94</td>
<td>100.0 ± 0.94</td>
</tr>
<tr>
<td>7 days</td>
<td>100.6 ± 0.87</td>
<td>101.4 ± 0.73</td>
</tr>
<tr>
<td>14 days</td>
<td>100.1 ± 0.56</td>
<td>98.9 ± 0.80</td>
</tr>
<tr>
<td>21 days</td>
<td>98.0 ± 0.77</td>
<td>97.6 ± 0.64</td>
</tr>
<tr>
<td>1 month</td>
<td>97.5 ± 1.20</td>
<td>97.9 ± 0.61</td>
</tr>
<tr>
<td>2 months</td>
<td>103.4 ± 1.02</td>
<td>102.6 ± 0.61</td>
</tr>
<tr>
<td>3 months</td>
<td>98.0 ± 0.32</td>
<td>98.8 ± 0.33</td>
</tr>
<tr>
<td>6 months</td>
<td>98.5 ± 0.22</td>
<td>99.3 ± 0.37</td>
</tr>
<tr>
<td>8 months</td>
<td>1004 ± 2.50</td>
<td>1014 ± 0.72</td>
</tr>
<tr>
<td>12 months</td>
<td>1027 ± 2.47</td>
<td>1032 ± 0.92</td>
</tr>
<tr>
<td>18 months</td>
<td>1046 ± 0.32</td>
<td>1036 ± 0.86</td>
</tr>
<tr>
<td>24 months</td>
<td>1014 ± 0.56</td>
<td>1006 ± 0.76</td>
</tr>
</tbody>
</table>

Key: *: ICH recommendation is 25 ± 2°C; #: initial concentration: 1.01 (± 0.01) mg/mL; ND = not determined

Na. This may be explained by the presence in the tablets of enough metal ions to catalyse oxidation. The solutions prepared with Ora-Sweet and Ora-Plus also showed a limited stability with formation of a yellow colour within three weeks.

Long-term study

Based on the results of the pilot study, formulation No. 1 was selected and examined further in a long-term stability study. Organoleptic observations (visual and olfactory) did not reveal any noticeable change over the entire storage time. The pH in all preparations (3.33 ± 0.01) remained constant throughout. This value is considered to be in the optimal range for captopril conservation [9, 19]. Furthermore, slightly acidic pH values such as these are well tolerated orally. For example, the Ora-Sweet and Ora-Plus preparations have pH values in the range 4.0 to 4.5.

Captopril concentrations remained remarkably stable over the entire study at 5°C and at room temperature (Table 2). At 40°C, a marginal drop was seen such that the concentration had decreased by a few per cent to 95.8 ± 0.68% after 12 months.

No microbial growth (aerobic, anaerobic or fungal) was detected during the study in any of the samples stored at 5°C, room temperature and 40°C. A number of factors may explain this favourable outcome, namely:
1. The microbiological purity of the water used
2. The acidity of the solution
3. The known bacteriostatic effect of EDTA-Na [30, 31]

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These results demonstrate that captopril can be prepared in oral solutions stable for two years or more when stored at room temperature or at 5°C. Three characteristics of the proposed formulation are worth mentioning:

1. It appears important to use captopril in powder form rather than dissolved from tablets liable to liberate compounds (e.g. metal ions) that accelerate the oxidation of the drug in solution
2. The solvent used was purified water containing neither microbes nor metal ions
3. The addition of EDTA-Na inactivates traces of metal ions released by the container

When comparing various captopril solutions, Lye et al. [20] found that EDTA-Na 0.1% and methylcellulose 2% afforded a good stability at one month. We did not add methylcellulose to our preparations because preliminary tests discounted its value and even suggested the possible liberation of traces of metal ions [20].

The duration of stability was determined with solutions kept in well-closed glass bottles that were opened 11 times to remove samples. In clinical practice, the duration of stability has been set at one month after the first opening, as based on systematic tests (data not shown) in which the flasks were opened six days per week for one month.

Safety of EDTA in paediatric patients
EDTA-Na is widely used in topical, oral and parenteral pharmaceutical formulations. It is also extensively used in cosmetics and foods. The usual concentrations employed in pharmaceutical formulations are in the range 0.005-0.1% w/v [32]. To the best of our knowledge, no data or guidelines have been published regarding accepted doses in paediatric patients. The WHO (World Health Organization) has set an estimated acceptable daily intake for disodium edetate in foodstuffs at up to 2.5 mg/kg body-weight [33]. Our 1 mg/mL captopril solution is stabilised with 1 mg/mL EDTA-Na. The usual titrate dose of captopril administered to premature neonates and newborns being 0.5 mg/kg/dose, young patients administered captopril 0.5 mg/kg/dose given every six to 24 hours will receive up to a maximum of 2 mg EDTA-Na per day, which is below the WHO limit of 2.5 mg/day tolerated in foods. It should also be noted that for infants who weigh 12 kg or more, administration of marketed captopril preparations (usually dosed at 12.5, 25.0 and 50.0 mg) is recommended.

CONCLUSION
The liquid formulation developed and validated at our hospital offers an outstanding chemical and microbiological stability (at least two years at room temperature). This can be explained by using purified water as solvent and mastering the major factors favouring captopril oxidation. The determining role of EDTA-Na is worth stressing, because it combines cation-chelating and bacteriostatic properties. In summary, we propose an oral formulation of captopril for paediatric use which can be prepared with ease by qualified professionals, is stable for at least two years at room temperature, and allows individualised dosage and easy administration, even to newborn patients.
REFERENCES