

STABILITY OF EXTEMPORANEOUS ENALAPRIL MALEATE SUSPENSIONS FOR PEDIATRIC USE PREPARED FROM COMMERCIALY AVAILABLE TABLETS

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Abstract: In this paper, the stability of enalapril maleate in oral formulations prepared from commercially available tablets was investigated. Extemporaneously compounded, 0.1 mg/mL and 1.0 mg/mL, oral suspensions of enalapril maleate in sugar-containing and sugar-free vehicles were stored in the absence of light at 4° and 25°C for 30 days. Enalapril maleate stability was quantified after 7, 14, 21, and 30 days using HPLC method. Viscosities and pH of prepared suspensions were measured on each study day and no appreciable changes from the initial pH and initial viscosities occurred in any of the samples both at 25° and 4°C. It was shown that all the formulations retain minimum 98% of the initial enalapril maleate concentration after 30 days of storage at 25° and 4°C and they may provide an option in situations where the marketed suspension is unavailable.

Keywords: stability, enalapril maleate, oral suspension, storage, pediatric dosage form, high-performance liquid chromatography

Enalapril maleate is a pro-drug without direct biological activity which is rapidly absorbed after oral administration and de-esterified *in vivo* to its active metabolite enalaprilat, a potent angiotensin-converting enzyme inhibitor (1, 2). Enalapril maleate is widely used in pediatric cardiology – in the treatment of essential and renovascular hypertension and in congestive heart failure. The daily dose of enalapril maleate in children is in the range of 0.2-1.0 mg/kg (3-6). Enalapril maleate is currently available as 5 mg, 10 mg and 20 mg tablets and there are no commercially available suspensions for oral administration that can be used to treat younger children who are unable to swallow capsules or tablets.

The most frequent manner of prescription of enalapril maleate for infants is grounding the commercially available dosage form (tablets, capsules) into a fine powder and then packing to the capsules. Recommendations for administration in young patients involve opening capsules and mixing the powder into the liquid foods. However, accuracy of measurement and ease of administration can be problematic. The powder has an unpleasant taste and mixing them into liquid food before administration does not mask the bitter taste.

In the absence of a ready-made product, a frequent approach by pharmacists is to prepare an oral liquid from tablets or capsules. Compounded oral suspensions should be proposed as the most appropriate formulation when the tablets or capsules are prescribed for small children. During preparing suspensions from tablets or capsules one should consider a lot of important problems including: solubility and stability of the active substance, pH and composition of the suspending medium, and antimicrobial protection. The main problems appearing during preparation the formulations include achieving dose uniformity and chemical and physical stability (7). It is important that the drug should be stable in the vehicle for the proposed duration of storage and administration of the product. The characteristics of the vehicles (pH, taste, viscosity, stability, presence of preservatives) determine its suitability and compatibility for use with the drug (8).

The purpose of this study was to prepare sugar-containing and sugar-free extemporaneous oral liquid dosage form of enalapril maleate from commercially available tablets and to determine the physical and chemical stability of these products for up to 30 days.

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EXPERIMENTAL

Materials

Enalapril maleate tablets (Enareal, 20 mg) were purchased from Polpharma S.A. (Starogard Gdański, Poland) and enalapril maleate standard was from Farmhispania (Barcelona, Spain). Methanol and hydrochloric acid were HPLC grade and purchased from Merck (Darmstadt, Germany). Citric acid was from Chempur (Piekary Śląskie, Poland) and methyl hydroxybenzoate was from Caelo Caesar & Loretz (Hilden, Germany). Hydroxyethylcellulose (Natrosol HR) was purchased from A.C.E.F. s.p.a. (Piacenza, Italy). Raspberry syrup was from Gemi (Karczew, Poland) and 85% orthophosphoric acid solution was from Fluka (Buchs, Switzerland). Water for HPLC was distilled and passed through a reverse osmosis system Milli-Q Reagent Water System (Billerica, MA, USA).

Formulations preparation

Tablets were ground to fine powder and combined with a hydroxyethylcellulose 0.5% solution (formulation **A**) or 1:10 mixture of raspberry syrup and hydroxyethylcellulose 0.5% solution (formulation **B**). Raspberry syrup masked the unpleasant taste of enalapril maleate powder. The solubility of enalapril maleate in water is 25 mg/mL at room temperature (9). Enalapril maleate has pK_a values of 3.0 and 5.4. The maximum stability of enalapril maleate is at a pH of ~ 3 (10), and therefore the pH value of prepared formulations was adjusted to 3.0 using cit-

ric acid (1 M). Initial enalapril maleate concentrations in suspension were: 0.1 mg/mL and 1.0 mg/mL. All suspensions contained methyl hydroxybenzoate 0.2% as a preservative. The product was filled in separated 200-mL orange glass bottles and stored at $4^\circ \pm 2^\circ\text{C}$ and at $23^\circ - 25^\circ\text{C}$, in the absence of light. Before removing samples, the containers were agitated to ensure uniform resuspension of insoluble materials.

Instrumentation and chromatographic conditions

The HPLC system consisted of an Agilent Technologies 1200 series instruments equipped with a G1312A binary pump, a G1316A thermostat, a G1379B degasser and a G1315B diode array detector (Agilent, Waldbronn, Germany). Data collection and analysis were performed using Chemstation 6.0 software (Agilent, Waldbronn, Germany). The experimental method was modified according to that previously reported (11-13). Separation was achieved on a C18 Spherisorb column 4.6×250 mm ODS2, 5 μm (Waters, Dublin, Ireland). Mobile phase was methanol/water (5:95, v/v) (pH 2.5), the flow rate was 1.0 mL/min and UV detection was performed at a wavelength of 215 nm. pH of the mobile phase was adjusted to 2.5 by using 10% orthophosphoric acid solution. The column temperature was maintained at 25°C . For injection into the HPLC system 50 μL of sample was used. All reagents used for analysis were of HPLC grade.

Calibration standards of 1.0 mg/mL stock solution of enalapril maleate (standard powder) in hydrochloric acid (0.1 M) were prepared.

Table 1. Stability of enalapril maleate 0.1 mg/mL and 1.0 mg/mL in sugar-free (formulation **A**) and sugar-containing (formulation **B**) vehicles stored at 25°C and 4°C .

Formulation/ Temperature	Initial ^a concentration (mg/mL)	% Initial concentration remaining ^a			
		Day 7	Day 14	Day 21	Day 30
A					
25°C	0.1 ± 0.02	99.6 ± 0.3	99.2 ± 0.6	99.3 ± 0.4	98.2 ± 0.9
4°C	0.1 ± 0.02	99.4 ± 0.4	99.4 ± 0.4	99.3 ± 1.2	99.1 ± 1.0
25°C	1.0 ± 0.04	99.4 ± 0.8	101.2 ± 0.7	98.3 ± 1.0	98.4 ± 0.8
4°C	1.0 ± 0.04	98.9 ± 1.1	99.4 ± 0.3	98.5 ± 0.8	98.0 ± 0.9
B					
25°C	0.1 ± 0.01	99.5 ± 0.4	99.6 ± 0.4	99.3 ± 0.2	99.4 ± 0.4
4°C	0.1 ± 0.01	99.7 ± 0.9	99.3 ± 0.5	98.4 ± 0.5	98.3 ± 0.5
25°C	1.0 ± 0.03	99.2 ± 0.5	99.4 ± 0.6	99.1 ± 1.1	99.1 ± 0.6
4°C	1.0 ± 0.03	99.4 ± 0.4	99.4 ± 0.6	99.1 ± 0.7	98.2 ± 0.8

^a The mean values ± S.D. from three independent experiments done in duplicate are presented

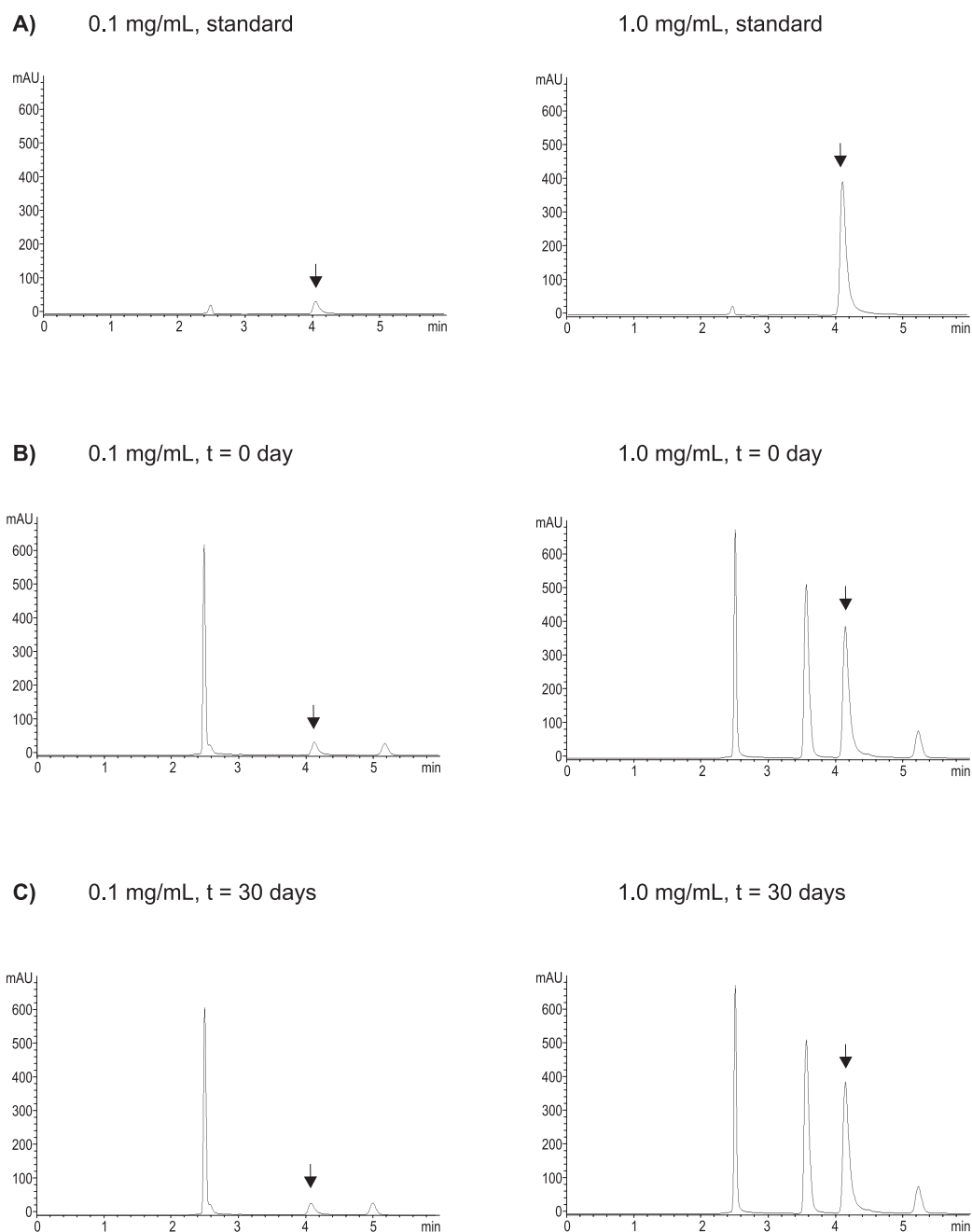


Figure 1. The representative chromatograms of enalapril maleate 0.1 mg/mL and 1.0 mg/mL in formulation B: standards (A), at time 0 (B) and stored for 30 days at 25°C (C). Enalapril maleate retention time = 4.120 min. Arrows indicate enalapril peaks.

Calibrations standard solutions at five levels were prepared by diluting the stock solution for the analytical range of 0.005-0.1 mg/mL. Volumes of 50 μ L of this standards were injected to obtain calibration curve. Standard curve was produced by the software included in the HPLC system and it was linear with the r^2 values greater than 0.999.

Stability examination (Enalapril maleate analysis)

Enalapril maleate stability was quantified after 7, 14, 21, and 30 days. Ten mL samples of each formulations were centrifuged at 3800 rpm for 20 min to sediment insoluble excipients. Aliquots of supernatant were diluted 1:10 with hydrochloric acid (0.1

Table 2. pH of enalapril maleate 0.1 mg/mL and 1.0 mg/mL in sugar-free (formulation A) and sugar-containing (formulation B) vehicles stored at 25°C and 4°C.

Formulation/ Temperature	Concentration (mg/mL)	pH ^a	
		Day 0	Day 30
A			
25°C	0.1	3.00 ± 0.03	3.02 ± 0.05
4°C	0.1	3.00 ± 0.04	2.98 ± 0.02
25°C	1.0	3.05 ± 0.02	3.10 ± 0.02
4°C	1.0	3.05 ± 0.02	3.10 ± 0.03
B			
25°C	0.1	3.05 ± 0.01	3.09 ± 0.02
4°C	0.1	3.05 ± 0.03	3.04 ± 0.04
25°C	1.0	3.04 ± 0.03	3.10 ± 0.01
4°C	1.0	3.04 ± 0.02	3.03 ± 0.03

^a The mean values ± S.D. from three independent experiments done in duplicate are presented

Table 3. Viscosity [mPa·s] of enalapril maleate 0.1 mg/mL and 1.0 mg/mL in sugar-free (formulation A) and sugar-containing (formulation B) vehicles stored at 25°C and 4°C.

Formulation/ Temperature	Concentration (mg/mL)	Viscosity (mPa·s) ^{aa}	
		Day 0	Day 30
A			
25°C	0.1	109 ± 2.0	106 ± 2.0
4°C	0.1	109 ± 2.0	110 ± 3.0
25°C	1.0	115 ± 3.0	113 ± 2.0
4°C	1.0	115 ± 3.0	118 ± 3.0
B			
25°C	0.1	103 ± 3.0	100 ± 2.0
4°C	0.1	103 ± 3.0	103 ± 3.0
25°C	1.0	110 ± 1.0	106 ± 3.0
4°C	1.0	110 ± 1.0	114 ± 2.0

^a The mean values ± S.D. from three independent experiments done in duplicate are presented

M), filtered through a 0.45 µm CA membrane filter (Witko, Łódź, Poland) and injected into the HPLC system. Replicate injections were made for all samples.

The stability of enalapril maleate in all formulations was determined by calculating the percentage of the initial concentration remaining at each time interval. Stability was defined as the retention of not less than 90% of the initial concentration (14).

pH examination

The apparent pH was determined initially (day 0) and on each study day. Examination was conducted with pH-meter CP-401 (Elmetron, Zabrze, Poland). pH of each sample was measured six times and then the results were averaged.

Viscosity determination

To determine if storage period at 4°C and 25°C had an effect on viscosity of enalapril maleate suspensions, they were subjected to shear stress in a rotational viscometer Viscotester 6 Plus (Thermo Haake, Karlsruhe, Germany). Viscosity, which is the inverse of fluidity, was measured in units of milliPascal × seconds (mPa·s).

Viscosity measurements of 15 mL aliquots of each suspension were taken initially (day 0) and on each study day. Samples were subjected to a constant shear rate 100 min⁻¹ for 60 s. Preliminary experiments had shown that viscosity became constant for preparations analyzed in this study 60 s into the run. Therefore, viscosities reported here are those recorded at 60 s. Viscosity of the suspensions

was measured six times and the results were averaged.

Statistical analysis

The results were analyzed by analysis of variance (ANOVA) and multiple comparison were done to check statistical significance. The data were expressed as the mean value for 3 independent assays in duplicate \pm S.D. The statistical significance between means was verified by Sheffe's comparison test accepting $p < 0.05$ as significant.

RESULTS AND DISCUSSION

A large number of drugs are prepared extemporaneously by pharmacists as oral liquid dosage forms. Pediatric oral formulations can be quite scientifically challenging to develop and the prerequisites for both measurable dosage form to administer based upon body-weight, and also taste-masking are two of the challenges unique for pediatric oral formulations. Extemporaneous compounding of this type usually involves preparing an oral liquid from a commercially available dosage form. It is important that the drug must be stable in the vehicle for the proposed duration of storage and administration of the product. Each vehicle possesses unique physico-chemical characteristics that determine its suitability for use with various drugs. These characteristics include pH, viscosity, taste, appearance, presence of preservatives, and stability. There are several studies concerning the stability of enalapril maleate, which were conducted in USA (15, 16) and other countries (17). In these examinations, the stability of enalapril maleate in deionized water, citrate buffer solution and carboxymethylcellulose as a suspending agent was studied. In our work the stability of enalapril maleate in oral suspensions made from tablets of Polish producer was examined. All the suspensions contained methyl hydroxybenzoate 0.2% as a preservative, hydroxyethylcellulose as the suspending agent and citric acid as the pH adjusting agent.

The stability of enalapril maleate in concentration of 0.1 mg/mL and 1.0 mg/mL in oral suspensions was studied by modified HPLC method (11-13). The results obtained in this work confirmed that enalapril maleate was stable in suspensions stored both at 25°C and 4°C. All suspensions showed only 1% – 2% degradation after 30 days of storage, which fulfilled the USP criterion (14). Figure 1 exhibits representative chromatograms obtained from samples of formulation **B** stored for 30 days at 25°C. Refrigerated and stored at room temperature formulations **A** and formulations **B** retained more than

98% of their initial enalapril maleate concentrations. There were no evident changes in enalapril maleate stability evoked by sugar (Table 1). Enalapril maleate stability was also examined under stressed conditions (autoclaved three times at $121 \pm 2^\circ\text{C}$) and no additional peaks of degradation products was observed.

It was shown that storage conditions did not influence the suspensions pH in significant way. No appreciable change from the initial pH (3.0) occurred in any of the samples both at 25°C and 4°C (Table 2). The pH examination of all formulations stored at 25° and 4°C showed only less than 0.1 pH unit change throughout the study. The data in Table 2 showed that enalapril maleate pH is stable after one month storage.

Viscosity was measured in order to determine if the temperature of storage had an effect on the viscosity. Refrigerated suspensions demonstrated higher viscosity than suspensions stored at 25°C but prolonged refrigeration did not increase the suspensions' viscosities in significant manner. It was likewise noted that addition of the raspberry syrup (formulation **B**) slightly lowered the viscosity of enalapril maleate suspensions in concentrate of 0.1 and 1.0 mg/mL stored either at 25°C or at 4°C (Table 3). Viscosity of the analyzed enalapril maleate suspensions was in the range of viscosities of the commercially available pediatric oral suspensions.

The tablet suspension in water would be expected to readily support microbial growth, especially at room temperature during in-use conditions, therefore 0.2% methyl hydroxybenzoate as compatible with the drugs preservative was added (17). No colonies or other evidence of bacterial or fungal growth were detected for any of the formulations tested. There was also no detectable change in color, odor, and taste in any sample. However, in the absence of microbiological data, the shelf-life given to extemporaneous products containing preservatives is usually 30 days – the time period in which the formulations were tested.

The obtained data suggest that these formulations may provide an option in situations where the marketed suspension is unavailable.

CONCLUSION

Extemporaneously compounded enalapril maleate 0.1 mg/mL and 1.0 mg/mL oral suspensions, in sugar-containing and sugar-free vehicles, were stable for at least 30 days when stored at 4° and 25°C. The viscosities and pH of all tested formula-

tions did not significantly change over 30 days. All the formulations retain minimum 98% of the initial enalapril maleate concentration after 30 days of storage at 4° and 25°C and may provide flexible and convenient dosage forms for pediatric patients.

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Received: 13. 10. 2008