Introduction

Erythromycin is a macrolide antibiotic that is commonly used in topical applications for the treatment of acne. It is often used in solutions containing 2-4% erythromycin and has a particular effect on the inflamed lesions and not on the comedons [1].

Erythromycin is insoluble in water and soluble in alcohol and diluted hydrochloric acid. The antibiotic is thermally unstable especially in solutions containing water. Furthermore, the stability of erythromycin is pH dependent. Optimal pH values found in literature are between pH=7 and 8 [2-3]. The degradation pathway is dependent on the pH. In a slightly acidic medium, erythromycin A (EA), the major component of erythromycin, is converted to erythromycin A enol ether (EAEN). In a strongly acidic medium, EA is converted to EAEN and anhydro erythromycin A (AEA). At a pH of about 7, EA is transformed to pseudo-erythromycin A enol ether (ps-EAEN) and this transformation is even more important at a pH > 8, while the degradation to EAEN and AEA is less important in this pH area. EA is the only active component and all the degradation products are inactive.

Sometimes a combination of erythromycin and zinc acetate is used for the treatment of acne [4]. From clinical studies, it was shown that erythromycin combined with zinc acetate is more efficient than erythromycin alone. On the other hand, the addition of zinc acetate to the erythromycin lotion can also influence the stability of erythromycin. Amer [2] concluded that zinc had a stabilizing effect on erythromycin stability but he suggested that it could lower the biological activity of the antibiotic by the formation of chelates.

From an earlier report [5], it was clear that the decomposition of erythromycin was much faster in aqueous medium than in pure alcoholic medium. Thus regarding to the stability of the product, one could consider a pure alcoholic erythromycin lotion. Unfortunately, a pure alcoholic solution is very irritating to the skin. A possible alternative is using dimethyl isosorbide as co-solvent instead of water. Dimethyl isosorbide is a water and oil soluble, colorless, non-greasy solvent and it has skin penetrating, pleasant feel and spreading properties. It is stable over a pH range of 2-10 and it is an excellent solvent for many products [6].

In this investigation three parameters that may influence the erythromycin stability are studied: the pH, the zinc acetate concentration and the alcohol/dimethyl isosorbide concentration.

Experimental methods

Materials

Erythromycin (Alpha-Pharma, Zwevegem, Belgium) and zinc acetate (Merck, Darmstadt, Germany) were used as active compounds, denaturated alcohol and
dimethyl isosorbide (ICI-surfactants, Everberg, Belgium) as solvents, Neutrol TE (BASF, Brussels, Belgium) and hydrochloric acid (Merck, Darmstadt, Germany) were used to adjust the pH-values. The mobile phase for HPLC consisted of acetonitrile (Carlo Erba, Milano, Italy), tetramethylammoniumhydroxide (TMA) 25 % w/w (Janssen Chimica, Geel, Belgium), monobasic ammoniumphosphate (Merck, Darmstadt, Germany), phosphoric acid (UCB, Drogenbos, Belgium) and milli-Q water. The buffer solution used for dilutions was prepared with monobasic potassiumphosphate (UCB, Drogenbos, Belgium) and sodium hydroxide (UCB, Leuven, Belgium).

HPLC-method
The HPLC-method used was based on the investigational work of Cachet [7-8] at with some corrections were made for our conditions. A Hewlett Packard 1084 B liquid chromatograph with a variable UV detector was used.

Mobile phase: 40 % acetonitrile, 5 % 0.2 M ammoniumphosphate buffer pH = 4, 20 % 0.2 M TMA solution pH = 4 and 35 % water.

Stationary phase: Lichrocart (125-4) column filled with Lichrospher 100 RP-18, 5 μm.

Flow: 0.5 ml/min; Column temperature: 35 °C; Wavelength: 215 nm; Injection volume: 20 μl. All solutions, containing 4 % w/v erythromycin, were diluted 40 times with 0.2 M potassium phosphate buffer solution pH = 7.45 before injection.

Under these conditions, the migration of erythromycin A, the only detectable component of erythromycin, was good, and sufficient separation between EA and his degradation products was obtained as showed in Figure 1.

Linearity, reproducibility and selectivity of the HPLC method under the given conditions were defined. Linearity between the peak height and the concentration of EA was observed in a concentration range of 0-0.1 % (w/v). The within-day reproducibility of the system, expressed in terms of relative standard deviation (RSD), was less than 3 %.

The day-to-day reproducibility, expressed as RSD, was less than 5 %. The HPLC method was also validated for the selectivity between EA and its degradation products by changing the mobile phase composition.

Optimization technique.
We used a factorial analysis with three factors at two levels: a high level (+1) and a low level (-1). This means that we needed $2^3 = 8$ experimental points for factorial analysis [9].

For the composite rotative design, two extreme points (1 at high level and 1 at low level) were added for each factor [10]. Thus with three factors we got six extreme points each at a level of 1.682 ($\pm 1.42$) in the positive or negative sense. Factor 1: pH The investigated pH-area was between 6 and 7.6. We could not reach higher pH values because of solubility problems. We defined pH=6 as extreme low level (-1.682) and pH=7.6 as extreme high level (+1.682). These values are of course apparent pH values because it is impossible to measure in a simple way a correct pH value in absence of water.

Factor 2: zinc-concentration The use of zinc acetate as Zn-salt was based on different existing acne-preparations containing Zn-acetate in concentrations from 1.0 % to 1.2 % (4). We defined 1.0 % as low level (-1) and 1.2 % as high level (+1).

Factor 3: concentration alcohol/DMI The concentration alcohol/dimethyl isosorbide (DMI) was varied from 50/50 (0) to 35/65 (-1.682) and 65/35 (+1.682). Thus high level of factor 3 implies a high concentration of alcohol and less DMI.

The values of all factors at every level are shown in Table 1.

For factorial analysis, eight solutions were prepared combining -1 and +1 levels. For the composite design, additionally six solutions were prepared combining 0 and +/- 1.682 levels. A solution at the center point was prepared in duplicate with all factors at zero level. So we obtained 16 solutions that were stored at 45 °C to accelerate the degradation of erythromycin. The composition of the 16 solutions is given in Table 2 and they all contain 4 % w/v erythromycin. At specified time intervals these solutions were analyzed by HPLC to measure erythromycin concentration.

Stability analysis using Arrhenius equation
Depending on the results of the stability tests at 45 °C, the two most stable solutions were selected. These solutions were stored at 25 and 37 °C and analyzed at specified time intervals with HPLC.

Results and discussion
Degradation kinetics
Figure 2 shows a plot of the typical breakdown of erythromycin. The degradation of erythromycin fol-