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Study of the stability of erythromycin in a hydrophilic creme basis by liquid chromatography

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Abstract

The stability of the macrolide antibiotic erythromycin, incorporated at a 2% m/m concentration in a hydrophilic creme basis containing 2% m/m of chlorocresol, was monitored over a period of 2 months using liquid chromatography as the analytical method. Extracts of the creme were analysed using wide-pore poly(styrene–divinylbenzene) PLRP-S 1000 Å as the stationary phase and a mixture of 2-methyl-2-propanol–acetonitrile–potassium phosphate buffer (pH 11.0; 0.02 M)–water (165:30:50:755, v/v/v/v) as the mobile phase. The method showed good selectivity towards chlorocresol, erythromycin A, its related substances and degradation products. As the pH of the creme containing erythromycin was 8.6, alkaline degradation products were expected to be formed. The presence of pseudoerythromycin A enol ether was observed after storage of the creme for 1 week at a temperature of 25°C. After 1 month the content of erythromycin was still more than 95%.

Keywords: Erythromycin; Stability; Hydrophilic creme; Liquid chromatography

1. Introduction

Erythromycin is a macrolide antibiotic frequently applied in a creme or gel basis for the treatment of acne. Glaxoderm® (Glaxo) is a hydrophilic creme basis which is available to pharmacies and into which erythromycin, or other drugs, can be incorporated. It is therefore important to know the stability of erythromycin in this formulation. Such preparations on prescription are not sterile and do not carry an official expiry date and are intended for immediate use during not longer than 1 month. Therefore it was considered sufficient to follow the stability over a period of 2 months. In Belgium general content limits of 95–105% are used for preparations. In weakly acidic aqueous conditions erythromycin enol ether is the major degradation product while in weakly basic conditions pseudoerythromycin enol ether is formed [1–3].

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2. Experimental

2.1. Method of analysis—liquid chromatography (LC)

2.1.1. Equipment

A Milton Roy Minipump (Laboratory Data Control, Riviera Beach, FL), a Marathon autosampler (Spark Holland, Emmen, Netherlands), a Merck Hitachi L-4000 variable UV detector (Darmstadt, Germany) set at 215 nm and a Hewlett-Packard integrator model 3396 (Avondale, PA)

2.1.2. Chromatographic conditions

- Stationary phase: poly(styrene–divinylbenzene), PLRP-S 8 μm, 1000 Å (25 cm × 4.6 mm i.d.) from Polymer Labs (Church Stretton, Shropshire, UK).
- Mobile phase: 2-methyl-2-propanol–acetonitrile–potassium phosphate (pH 11.0; 0.2 M)–water (165:30:50:755, v/v/v/v).
- Flow rate: 2.0 ml min^{-1}.
- Column temperature: 70°C (water bath).
- Injection volume: 100 μl.

2.2. Preparation of Glaxoderm® containing 2% m/m erythromycin

30.0 g Of Glaxoderm® were weighed and a part of it was thoroughly mixed with 0.60 g of a commercial sample of erythromycin on a glass plate. The rest of the creme was added and mixed in order to obtain a homogeneous creme. Two different cremes were prepared (creme A and creme B).

2.3. Preparation of samples for analysis

30 ml Hexane and 20.0 ml of a mixture of equal volumes of methanol and water were added to 3.00 g of creme. The mixture was shaken vigorously in order to obtain a homogeneous emulsion, which was transferred into a centrifugation tube. After centrifugation to crack the emulsion, an aliquot of the aqueous layer was analysed immediately. Freshly prepared samples and reference solutions were always used. The recovery and repeatability of this extraction procedure was determined on 3 different samples of 3.00 g.

2.4. Stability study

Creme A and creme B, prepared as above, were stored in closed containers in an incubator at 25°C. The content of erythromycin A and the formation of degradation products was determined, using the described LC method, immediately after the preparation of the creme (T_0), after 1 week (T_7), 2 weeks (T_{14}), 1 month (T_{30}) and 2 months (T_{60}). Each time, two extractions per creme were carried out. A freshly prepared solution of the erythromycin sample used to prepare cremes A and B containing 3.0 mg ml^{-1} in methanol–water (1:1) was used as a reference solution. The content of the degradation products pseudoerythromycin A enol ether (psEAEN) and erythromycin A enol ether (EAEN) was calculated using conversion factors of 15 and 11, respectively, as the specific absorbance at 215 nm of these substances is 15 and 11 times higher than that of erythromycin A.

3. Results and discussion

3.1. Selectivity of the analytical method

In the European Pharmacopoeia, erythromycin is assayed using liquid chromatography [4]. This method has been described in the literature [5,6] and is now proposed for use in the US Pharmacopeia [7]. Chlorocresol, a component of Glaxoderm® interferes with this LC method, because it is eluted together with the main component erythromycin A. Attempts to remove chlorocresol by extraction were unsuccessful and therefore the LC method was modified in order to obtain separation of chlorocresol from the major components of erythromycin and the degradation products of erythromycin. The modification consisted in an increase of the pH of the buffer solution in the mobile phase from 9.0 to 11.0, which made the chlorocresol elute in the very beginning of the chromatogram. Contrary to silica gel based re-

versed phases, such a high pH can be used with poly(styrene–divinylbenzene) stationary phases, which are very stable at high pH.

Fig. 1 shows a typical chromatogram obtained at T_0. The pH of the extract was 8.6. Erythromycin A, the main component of erythromycin, is well separated from chlorocresol and the related erythromycins C (EC), E (EE) and B (EB), which are present in the bulk material. The position of the degradation products pseudoerythromycin A hemiketal (psEAHK), the hydrolysis product of erythromycin A (EAHP), anhydroerythromycin A (AEA), pseudoerythromycin A enol ether (psEAEN) and erythromycin A enol ether (EAEN) is indicated on the chromatogram. The structures of these substances are described elsewhere [1–3].

Fig. 2 shows a chromatogram of a blank sample of Glaxoderm®. The pH of the blank extract was about 5. Only the peak of chlorocresol is detected. Erythromycin F (EF), a minor component of erythromycin, which may be present in bulk erythromycin, is not separated from chlorocresol. However, EF is not a degradation product of erythromycin A. Erythromycin *N*-oxide, an oxidative degradation product, is not separated from chlorocresol either. However, since the preparation is stored in a closed container oxidative degradation is not relevant, unless oxidative agents such as benzoylperoxide are present. Benzoylperoxide is also used in the treatment of acne. There is no interference of other components of the creme basis with the detection of EA, its main related substances and potential degradation products.

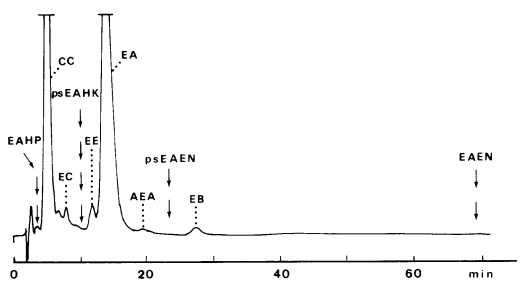

Fig. 1. Typical chromatogram of an extract of the creme at T_0.EAHP, erythromycin hydrolysis product; CC, chlorocresol; EC, erythromycin C; psEAHK, pseudoerythromycin A hemiketal; EE, erythromycin E; EA, erythromycin A; AEA, anhydroerythromycin A; psEAEN, pseudoerythromycin A enol ether; EB, erythromycin B; EAEN, erythromycin A enol ether.

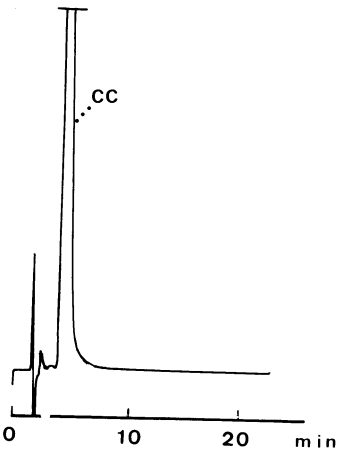

Fig. 2. Typical chromatogram of an extract from the blank creme Glaxoderm®.

3.2. Quantitative analysis

The linearity of the method was checked by analysing solutions of erythromycin in methanol–water (1:1) with a content corresponding to 80, 90, 100 and 110% of that expected to be found after preparation of a creme sample. Three analyses of each solution were performed. The following regression line was obtained $y = 2.289x - 9.863$ with y the peak area and x the percentage (w/v) content of each solution, $r = 0.997$ and the S.E. of estimate $S_{y,x} = 2.047$. The intraday repeatability of the LC method (R.S.D. on the mean of three independent assays) of the analytical method was 0.9%. The interday repeatability of the LC method (R.S.D., three independent assays on three days) was 0.4%. These experiments were carried out with standard solutions which were treated in the same way as the samples. The mean recovery, determined on 3 extractions of the same creme, was 101.6% with R.S.D. = 0.5%.

Table 1
Content (% m/m) of erythromycin A expressed as a percentage of the label content at different times

Creme	Time				
	T_0	T_7	T_{14}	T_{30}	T_{60}
A	102.8	100.1	98.8	96.6	96.9
	99.8	100.6	99.0	99.8	94.0
B	100.0	99.2	99.3	98.5	94.9
	99.2	101.0	99.4	96.3	91.1
Mean	100.5	100.2	99.1	97.8	94.2
R.S.D.	1.6	0.8	0.3	1.7	2.4

Table 2
Content (% m/m) of pseudoerythromycin A enol ether at different times

Creme	Time				
	T_0	T_7	T_{14}	T_{30}	T_{60}
A	<0.05	0.45	1.1	2.3	4.9
B	<0.05	0.45	1.2	2.3	5.4

3.3. Stability study

Solutions of erythromycin in a methanol–water mixture at pH 8–9 have been described to be stable at room temperature for several hours [3]. This was confirmed by the fact that the reference solutions used did not show any sign of decomposition over the period of use in a series of analysis. Samples and reference solutions were injected alternately. This also indicates the absence of on-column degradation. Table 1 gives the content of erythromycin A, expressed as a percentage of the label content, at times T_0, T_7, T_{14}, T_{30} and T_{60}. The content of erythromycin A decreased slightly over the period examined. After 1 month, the content of erythromycin A was still more than 95%. As the pH of the methanol–water extract of the creme containing erythromycin was 8.6, alkaline degradation products could be expected to be formed during storage. EAHP and psEAHK were not detected in significant amounts. After 1 week, the presence of psEAEN was observed. Table 2 reports the content of psEAEN formed at times T_7, T_{14}, T_{30} and T_{60} in the two cremes. Fig. 3 shows a typical chromatogram obtained at T_{30}. At T_{30} and T_{60}, a small amount of EAEN was also detected (0.3 and 0.8% respectively). The formation of these impurities explains the decrease in EA. The mass balance is between 99 and 101%.

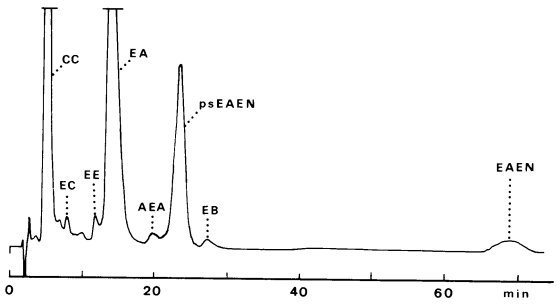

Fig. 3. Typical chromatogram obtained after storage of the creme for 30 days at 25°C. See Fig. 1 for abbreviations.

4. Conclusion

After 1 month at 25°C, the content is still well above 95% of its original value. Erythromycin when incorporated in a pharmacy in the Glaxoderm® creme basis can be considered to be stable for at least one month.

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