ELSEVIER

International Journal of Pharmaceutics 197 (2000) 153–160

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Investigation of the chemical stability of an erythromycin–tretinoin lotion by the use of an optimization system

M. Brisaert *, M. Gabriëls, J. Plaizier-Vercammen

Laboratory of Pharmaceutical Technology and Physical Pharmacy, Pharmaceutical Institute, Free University of Brussels, Laarbeeklaan 103, B-1090 Brussels, Belgium

Received 28 August 1999; received in revised form 26 November 1999; accepted 8 December 1999

Abstract

A combination of 2% erythromycin and 0.05% tretinoin in an alcohol–isopropanol lotion was prepared. Two parameters were investigated for their influence on the stability of erythromycin and/or tretinoin, namely pH and the concentration of butylhydroxytoluene (BHT) as antioxidant. To investigate these two parameters, an optimization technique was used with two factors (pH and concentration of BHT) at two levels. Accelerated stability analysis was performed at 45°C in the dark to exclude isomerization of tretinoin. To analyse erythromycin and tretinoin in the combination preparation, a TLC method, previously developed in the laboratory, was used. The degradation of erythromycin seemed to be much faster than the tretinoin degradation. Optimal stability is shown in the pH range of 8.2–8.6 for erythromycin and 7.2–8.2 for tretinoin while the concentration of BHT had no significant influence.

Keywords: Erythromycin–tretinoin topical solution; Chemical stability; Influence of pH and concentration antioxidant; Composite rotative design

1. Introduction

The combination of the antibiotic erythromycin and the comedolytic agent tretinoin in a solution for the dermatological treatment of acne, seemed to be very interesting since the modes of action of these two compounds differ completely. In a study performed by Mills and Kligman (1978), erythromycin and tretinoin were applied separately to avoid the possibility of incompatibility. In an open multicentre study (Korting and Braun-Falco, 1989), the efficacy and tolerability of a fixed combination of tretinoin (0.025%) and erythromycin (4%) in a hydrogel were evaluated. The results of this study showed a good therapeutic response. Moreover, side-effects occurred far less frequently as compared to tretinoin alone. In the previous studies, no data were given about the

* Corresponding author. Tel.: +32-2-4774592; fax: +32-2-4774735.

E-mail address: apobrimy@yahoo.com (M. Brisaert)

0378-5173/00/$ - see front matter
PII: S0378-5173(99)00462-7

stability of the two products in the preparations. Since it is known that erythromycin is not very stable, we found it interesting, to investigate the chemical stability of erythromycin and tretinoin in a combination preparation.

Erythromycin is a drug, which is very unstable in solution, especially when the solution contains water (Brisaert et al., 1994). Therefore, water should not be added to the preparations. It is replaced by isopropanol. The degradation of erythromycin is also very pH dependent (Brisaert et al., 1996) leading to the development of different degradation products.

Tretinoin on the other hand is a drug which is very susceptible to photo-degradation (Elbaum, 1988) but we will not consider this type of degradation in this study. Tretinoin also is easily oxidizable and thermally unstable. To diminish this type of degradation, an antioxidant will be added to the preparations. Butylhydroxytoluene (BHT) seemed to be the most suitable antioxidant to prevent the oxidation of tretinoin (Rosing et al., 1989).

To investigate the influence on the chemical stability of erythromycin and/or tretinoin of these two important parameters, namely the pH and the addition of BHT, and to select the most stable formulation, an optimization technique was used. The procedure of optimization consists of preparing a series of formulations, varying the concentrations of the formulation ingredients in some systematic manner. In this study, the pH and the concentration of BHT will be changed. In this way, the formulations are evaluated according to one or more attributes, in our case the stability of erythromycin and tretinoin. Based on the results of these tests, a particular formulation may be predicted to be optimal (Bolton, 1990).

2. Experimental methods

2.1. Materials

Erythromycin (Alpha-Pharma, Zwevegem, Belgium) and tretinoin (BASF, Ludwigshafen, Germany) were used as active compounds. Butylhydroxytoluene (Alpha-Pharma, Zwevegem, Belgium) was added for its antioxidative properties and denatured ethanol and isopropanol (Fravers Lab., Gent, Belgium) were used as solvents. Hydrochloric acid (Merck, Darmstadt, Germany) and Sodium Hydroxide (Merck, Darmstadt, Germany) both in a concentration of 0.5 M, were used to adjust the pH of the test solutions.

The solvents which were used to prepare the mobile phase for the TLC analysis were acetonitrile (Carlo Erba Reagenti, Milano, Italy), acetic acid 96% (w/w), dichloromethane, methanol, ammonia 25% (w/w) (Merck, Darmstadt, Germany) and water purified through a Millipore purification system. For the preparation of the dipping reagents of the TLC-method, acetic acid 96% (w/w), 4-methoxybenzaldehyde (= anisaldehyde), sulfuric acid 98% (w/w), chloroform (Merck, Darmstadt, Germany), denatured ethanol and water were used.

2.2. TLC method

To analyse the concentration of erythromycin and tretinoin in the combination preparation, a TLC method followed by spectrodensitometry, previously developed in our laboratory (Gabriëls et al., 1999), was used.

To analyse tretinoin, a reversed phase method was preferred using RP-18 F 254 S plates from Merck (Darmstadt, Germany) as stationary phase and a combination of acetonitrile, water and acetic acid in a ratio of 50:25:1 (v/v) as mobile phase. The dipping reagent for tretinoin consisted of ethanol, water, acetic acid, sulfuric acid, anisaldehyde (50:40:10:2:1) (v/v) and the detection wavelength for densitometry was set at 520 nm.

A normal phase method, using Silica Gel 60 F (with gypsum) 254 plates from Merck (Darmstadt, Germany), was chosen to perform the quantitative analysis of erythromycin. As a mobile phase, dichloromethane, methanol and ammonia (60:6:1) (v/v) were used and ethanol, chloroform, acetic acid, sulfuric acid, anisaldehyde (30:60:10:2:1) (v/v) were the components of the dipping reagent. Spectrodensitometry was performed at a wavelength of 565 nm.

Under these circumstances, a selective analytical method was developed for either tretinoin and erythromycin without interference of BHT or the degradation products of tretinoin or erythromycin. The precision of the method has been investigated by analyzing five spots of an erythromycin and a tretinoin solution. The variation coefficient of the determination of each analyte was consistently $< 5\%$. The limit of detection was 1 μg for erythromycin and 0.5 μg for tretinoin.

2.3. Optimization technique

A composite rotative design served as optimization technique. This design consisted of a 2^2 factorial design with two factors (pH and conc. BHT) at two levels (high ($+1$) level and low (-1) level) plus extra-points. The extra points include a center point (all factors at 0 level) and 2×2 extra-design points, appropriately chosen to maintain orthogonality of the design (levels $+1.414$ and -1.414).

In this way, the different levels of the two factors were determined.

In literature, optimal pH values for the stability of erythromycin vary from 7 to 8.5 (Pluta and Morgan, 1986). We defined pH 6.5 as the extreme low level (-1.414) and pH 8.6 as the extreme high level ($+1.414$). Hereafter, the other levels were calculated and they are shown in Table 1.

The concentration of BHT used commonly in tretinoin preparations is about 40 mg/100 ml (Rosing et al., 1989) but we would also like to see the effect of omission of BHT. Therefore 0 mg BHT was defined as the extreme low level (-1.414) and 50 mg/100 ml BHT as the extreme high level ($+1.414$). The other, calculated, levels are shown in Table 1.

The composite design with two factors requires 3^2 formulations: four formulations combining $+1$ and -1 levels, four formulations combining the extreme points ($+/-1.414$) of 1 factor and the centerpoint (0) of the other factor and finally 1 point with all factors at 0 level. The centerpoint was prepared in duplicate to make an estimation of the experimental error.

2.4. Preparation of the formulations

Commonly used concentrations in anti-acne preparations vary from 2 to 4 g erythromycin per 100 ml and 0.05 g tretinoin per 100 ml. We decided to prepare formulations containing 2 g erythromycin and 0.05 g tretinoin in 100 ml of lotion which consisted of 35% (v/v) ethanol and 65% (v/v) isopropanol.

The solvent mixture was prepared previously and also a stock solution of 50 mg/100 ml BHT was prepared in the same ethanol–isopropanol mixture. First, 2 g erythromycin was weighed into an amber recipient, thereafter, 0.05 g tretinoin and an appropriate quantity of BHT stock solution was added. Then, the recipient was filled with the solvent mixture and the pH was adjusted with NaOH 0.5 M or with HCl 0.5 M. Finally the lotion was brought to 100 ml with the solvent mixture. The pH and the concentration of BHT of each of the ten preparations are shown in Table 2. The pH values mentioned in this study are apparent pH values because of the lack of water in the formulations.

The formulations were prepared at random and after preparation, the 100 ml lotion was divided into ten amber flasks for storage so that we could use another flask for each analysis to exclude contamination of the formulation. Amber flasks were used to exclude irradiation, which could cause photo-degradation of tretinoin. Immediately after each preparation, the formulation was analysed quantitatively to determine the correct

Table 1
Experimental values for all levels and factors

Level	−1.414	−1	0	+1	+1.414
pH	6.5	6.81	7.55	8.29	8.60
BHT (mg/100ml)	0.00	7.32	25.00	42.68	50.00

Table 2
Composition and stability of the lotions

Form. no.	x_1	x_2	pH	BHT mg/100 ml	Erythromycin (20 days)	Tretinoin (250 days)
1	−1	−1	6.81	7.32	38.5%	45.8%
2	+1	−1	8.29	7.32	80.8%	59.5%
3	−1	+1	6.81	42.68	24.7%	51.9%
4	+1	+1	8.29	42.68	83.8%	55.2%
5	−1.414	0	6.50	25.00	15.7%	51.7%
6	+1.414	0	8.60	25.00	97.0%	64.1%
7	0	−1.414	7.55	0.00	77.0%	66.7%
8	0	+1.414	7.55	50.00	74.9%	64.8%
9	0	0	7.55	25.00	73.1%	73.6%
10	0	0	7.55	25.00	78.6%	74.6%

starting concentration of erythromycin and tretinoin. Thereafter, the formulations were stored in the dark, to exclude irradiation, at 45°C to accelerate the degradation of the compounds. At determined time intervals, the formulations were analysed quantitatively after appropriate dilution and the concentrations were calculated from a calibration line, which was measured each day of analysis for erythromycin and for tretinoin. The concentration of erythromycin and tretinoin was any time expressed as percentage of the concentration analysed immediately after the preparation.

3. Results and discussion

3.1. Degradation of erythromycin

The degradation of erythromycin was followed over 5 weeks. A typical plot of the degradation of erythromycin is shown in Fig. 1. After 5 weeks at 45°C, erythromycin was almost completely degraded in some preparations whereas the other preparations still contained more than 50% of the initial concentration. As a response for the design (y value), the concentration erythromycin, which was left after 20 days of storage, was chosen because this value was situated in the middle of the degradation plots. This allowed some variation in the responses while the less stable preparations were not completely degraded yet.

Comparing the responses, shown in Table 2, and the composition of the preparations, we can conclude that the pH has a large influence on the stability of erythromycin. The preparations with extreme low pH (no. 5) or low pH (no. 1 and 3) are most susceptible to degradation

On the other hand, when only the concentration of BHT varies while the pH is kept constant, the stability of erythromycin is rather similar and the influence of this factor is not clear.

3.2. Degradation of tretinoin

Within the 5 weeks of stability analysis of erythromycin, little or any degradation of tretinoin was seen. These findings correspond with the results of a previous study where tretinoin was stable in most of the investigated preparations over 3 months (Brisaert et al., 1995). Therefore,

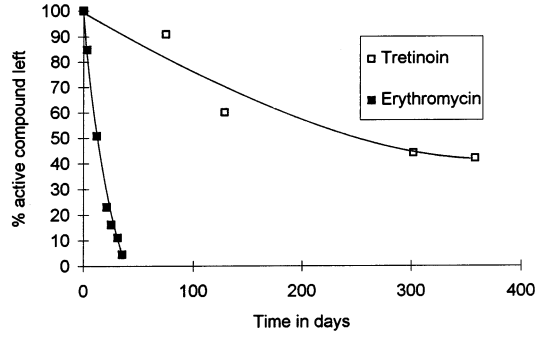

Fig. 1. Degradation plots of erythromycin and tretinoin together in one formulation.

Table 3
Regression data for erythromycin

Regression coefficient		Value of coefficient		sig T	Significance	
B_0		−1386.54		0.018388	+	
b_1		358.3616		0.019436	+	
b_2		−2.15959		0.381797		
b_{11}		−21.8473		0.025083	+	
b_{22}		−1.000723		0.547825		
b_{12}		0.321022		0.317577		
Form. no.	Y exper.	Y pred.	Residuals	(Residuals)^2		
1	38.5	40.5	−2.0	4.00		
2	80.8	86.1	−5.3	28.09		
3	24.7	28.7	−4.0	16.00		
4	83.8	91.1	−7.3	53.29		
5	15.7	13.4	2.3	5.29		
6	97.0	90.1	6.9	47.61		
7	77.0	73.7	3.3	10.89		
8	74.9	68.9	6.0	36.00		
9	73.1	75.8	−2.7	7.29		
10	78.6	75.8	2.8	7.84		
			SS resid	216.30	SS pure	15.13
			d.f.	9−6=3	d.f.	2−1=1
			MS resid	72.10	MS pure	15.13
			F calc	4.77	F 3,1	216

the stability of tretinoin was followed during a period of 1 year as shown in Fig. 1. Here again, a value situated in the middle of the degradation curve, namely the concentration tretinoin left after 250 days of storage, was chosen as response value for the design. The responses for the different solutions, given in Table 2, only vary between 45 and 75%. This means that the influence of the different parameters is much smaller than for erythromycin.

3.3. Optimization

The purpose of using an optimization technique is to see if the responses, in our case the stability, can be described with a regression model as a function of the chosen factors. Following regression model was tested: $y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2$ with: y, estimated response; b_0, mean response; b_{1-12}, regression coefficients and x_1 and x_2, respectively, factor 1 and 2. The coefficients of this model were calculated with the Excel computer program, using the experimental stability values as responses. The calculated coefficients for the stability of erythromycin and tretinoin are given in Tables 3 and 4, respectively. For both stabilities, only x_1 and x_1^2 are significant terms, with a confidence interval of 95%, while the terms x_2, x_2^2 and x_1x_2 are not significant, as shown in Tables 3 and 4. This means that only the pH (x_1) seems to play a significant role in the stability of both erythromycin and tretinoin and not the concentration of BHT (x_2). Moreover there is no interaction between these two factors since the term x_1x_2 is not significant in the model. To see if the proposed polynomial equation describes the results very good, the predicted responses and the experimental responses were compared with an F-test. Since the experimental F-values, as shown in Tables 3 and 4, are smaller than the theoretical F-values with a confidence interval of 95%, we can conclude that the predicted responses correspond very well with the experimental responses. In that way, the proposed model can be used to predict the stability of erythromycin and tretinoin.

Table 4
Regression data for tretinoin

Regression coefficient		Value of coefficient		sig T	Significance	
b_0		−1082.24		0.024460	+	
b_1		292.187		0.022462	+	
b_2		2.512195		0.251452		
b_{11}		−18.6354		0.025049	+	
b_{22}		−0.02036		0.096260		
b_{12}		−0.19873		0.454027		
Form. no.	Y exper.	Y pred.	Residuals	(Residuals)^2		
1	45.8	50.7	−4.9	24.01		
2	59.5	64.5	−5.0	25.00		
3	51.9	55.7	−3.8	14.44		
4	55.2	59.1	−3.9	15.21		
5	51.7	47.4	4.3	18.49		
6	64.1	59.6	4.5	20.25		
7	66.7	61.5	5.2	27.04		
8	64.8	61.2	3.6	12.96		
9	73.6	74.1	−0.5	0.25		
10	74.6	74.1	0.5	0.25		
			SS resid	157.90	SS pure	0.50
			d.f.	9−6=3	d.f.	2−1=1
			MS resid	52.63	MS pure	0.5
			F calc	105.26	F 3,1	216

Therefore three-dimensional plots and contour plots are constructed where the predicted stability is plotted as a function of pH and of concentration of BHT, as shown respectively in Figs. 2 and 3 for erythromycin and in Figs. 4 and 5 for tretinoin.

Optimal pH values for the stability of erythromycin in our alcohol-isopropanol lotion seem to be situated between pH 8.2 and 8.6. Also, in an earlier study performed in our laboratory (Brisaert et al., 1996) as well as in the study of Steffansen and Bundgaard (1989), it was seen that the stability of erythromycin increased with increasing pH value of the solutions. Vandenbossche et al. (1991), on the other hand, revealed that adjusting the pH of emulsions containing erythromycin to pH 8.5 had a deleterious effect on the stability of erythromycin, but one has to be reminded that the behavior of erythromycin in emulsions is certainly not the same as in solutions.

For the stability of tretinoin, an optimal pH interval between 7.2 and 8.2 should be chosen.

4. Conclusion

The stability of an alcoholic erythromycin–tretinoin lotion has been investigated by the use of a composite rotative design. For erythromycin and for tretinoin, a model was constructed that

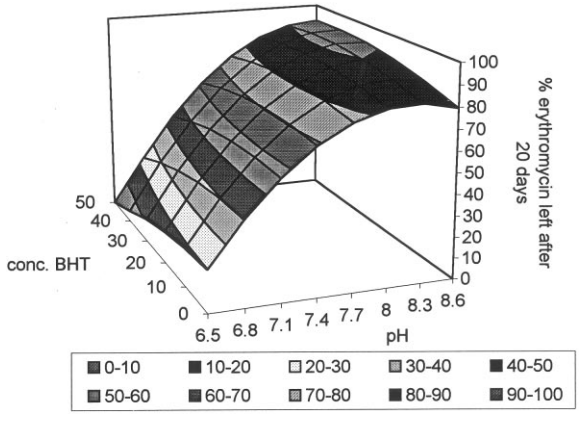

Fig. 2. Three-dimensional plot of the predicted stability of erythromycin as a function of pH and concentration of BHT.

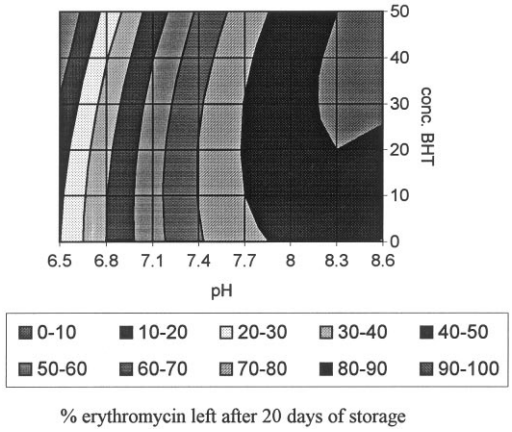

Fig. 3. Contour plot of the predicted stability of erythromycin as a function of pH and concentration of BHT.

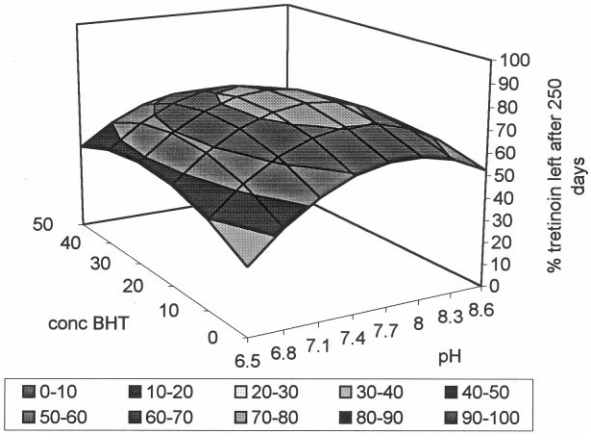

Fig. 4. Three-dimensional plot of the predicted stability of tretinoin as a function of pH and concentration of BHT.

described the response (stability) as a function of two factors, namely pH and concentration of BHT. So the stability of erythromycin and tretinoin could be predicted as a function of pH and of concentration of BHT. An area of optimum stability was marked out for both products. The concentration of BHT played no significant role neither for the stability of erythromycin nor for tretinoin but the pH was very important. Erythromycin, which was far less stable than tretinoin, showed optimum stability between pH 8.2 and 8.6. The optimum stability of tretinoin occurrs between pH 7.2 and 8.2. This means that

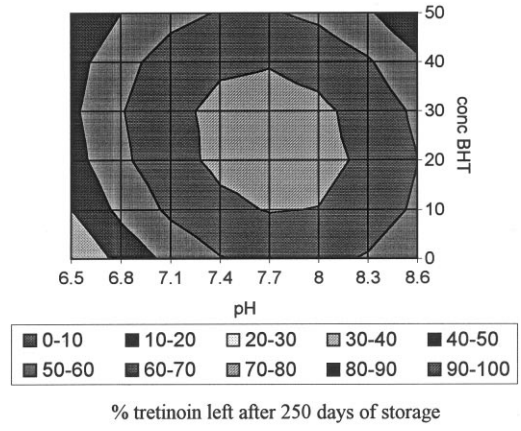

Fig. 5. Contour plot of the predicted stability of tretinoin as a function of pH and concentration of BHT.

the areas of optimal stability of the two products do not match. When we should superpose the two contour plots, we should not find a common area of maximum stability of the two. But since tretinoin is much more stable than erythromycin, we will choose the optimal area of erythromycin as criterion for the preparation of an optimal formulation containing erythromycin and tretinoin, meaning a pH of about 8.3. In this optimal formulation, erythromycin is stable during 24 days and tretinoin during 65 days at 45°C. However we have to be aware that tretinoin will be dissociated at a pH of 8.3 ($pK_a = 6$), which can possibly cause problems for the therapeutic activity of the product. We do not know if tretinoin still has comedolytic properties in its dissociated form. Another problem can be that a dermatological preparation with a pH of 8.3 can irritate the skin, which is not desirable. These can be objects for further investigation in this direction.

References

Bolton, S., 1990. Pharmaceutical Statistics, Practical and Clinical Applications, second ed. Dekker, New York.

Brisaert, M., Van Acker, T., Plaizier-Vercammen, J.A., 1994. Onderzoek naar de chemische stabiliteit van erythromycine base in dermatologische lotions. Farmaceutisch Tijdschrift voor België 71, 2–5.

Brisaert, M., Everaerts, I., Plaizier-Vercammen, J.A., 1995. Chemical stability of tretinoin in dermatological preparations. Pharmaceutica Acta Helvetiae 70, 161–166.

Brisaert, M., Heylen, M., Plaizier-Vercammen, J.A., 1996. Investigation on the chemical stability of erythromycin in solutions using an optimization system. Pharm. World Sci. 18, 182–186.

Elbaum, D.J., 1988. Comparison of the stability of topical isotretinoin and topical tretinoin and their efficacy in acne. J. Am.. Acad. Dermatol. 19, 486–491.

Gabriëls, M., Brisaert, M., Plaizier-Vercammen, J., 1999. Densitometric thin layer chromatographic analysis of tretinoin and erythromycin in lotions for topical use in acne treatment. Eur. J. Pharm. Biopharm. 48, 53–58.

Korting, H.C., Braun-Falco, O., 1989. Efficacy and tolerability of combined topical treatment of acne vulgaris with tretinoin and erythromycin in general practice. Drugs Expl. Clin. Res. XV (9), 447–451.

Mills, O.H., Kligman, A.M., 1978. Treatment of acne vulgaris with topically applied erythromycin and tretinoin. Acta Derm. Venereol. 58, 555–557.

Pluta, P.L., Morgan, P.K., 1986. Stability of erythromycin in intravenous admixtures. Am. J. Hosp. Pharm. 43, 2732–2738.

Rosing, H., Elferink, F., Van de Vaart, F.J., 1989. The influence of different antioxidants on the stability of tretinoin in a cream. Proceedings of the 49th International Congress of Pharmaceutical Sciences of F.I.P. in Munchen, Germany, 242.

Steffansen, B., Bundgaard, H., 1989. Erythromycin prodrugs: kinetics of hydrolysis of erythromycin and various erythromycin 2′-esters in aqueous solution and human plasma. Int. J. Pharm. 56, 159–168.

Vandenbossche, G.M.R., Vanhaecke, E., De Muynck, C., Remon, J.P., 1991. Stability of topical erythromycin formulations. Int. J. Pharm. 67, 195–199.