The quantitation of each of the equine estrogens separated in this fashion was accomplished by using the relative response ratios of each estrogen as compared to the internal standard, ethinyl estradiol. For such determinations, the response ratio and linearity of response were determined by injection of six aliquots of each individual steroid at levels bracketing the anticipated quantity in the mixture with a constant amount of internal standard. For all such measurements, the correlation between peak area ratios and concentrations was 0.998 or better.

Extraction of the water soluble sodium sulfate salts of the equine estrogens required the addition of sodium chloride to suppress emulsion formation. The methylene chloride extraction effectively removed lipid soluble formulation excipients as evidenced by a chromatogram that was essentially devoid of extraneous peaks other than those of the equine estrogens. Subsequent hydrolysis of the sulfate conjugates with 2000 U of sulfatase enzyme had earlier been reported (5) as sufficient for hydrolysis of 1 mg of the conjugates, even in the presence of small amounts of phosphates.

The quantitative assay data obtained from the analysis of two aliquots of a vaginal cream formulation is given in Table I. The quantities of estrone and equilin are within the limits specified for conjugated estrogens in the United States Pharmacopeia (3). The total potency determined indicates that the product was 99.9% of labeled claim.

In summary, this method represents the first quantitative procedure for the total analysis of the steroid composition in an estrogen vaginal cream formulation.

REFERENCES

(1) R. Roman, C. A. Yates, J. F. Miller, and W. J. A. VandenHeuvel, *Can. J. Pharm. Sci.*, **10**, 8 (1974).

(2) R. N. Dickerson, R. Bressler, C. D. Christian, and H. W. Hermann, *Clin. Pharmacol. Ther.*, **26**, 502 (1979).

(3) "The United States Pharmacopeia," 20th rev., Mack Publishing, Easton, Pa., 1980.

(4) R. Johnson, R. Masserano, R. Haring, B. Kho, and G. Schilling, *J. Pharm. Sci.*, **64**, 1007 (1975).

(5) K. M. McErlane and N. M. Curran, *ibid.*, **66**, 523 (1977).

(6) G. K. Pillai and K. M. McErlane, *ibid.*, **70**, 1072 (1981).

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Effect of Some Formulation Adjuncts on the Stability of Benzoyl Peroxide

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Abstract □ The stability of benzoyl peroxide in polyethylene glycol ointment base and some liquid vehicles (acetone, ethanol, propylene glycol, and their mixtures) was studied. Some solutions also contained an additional ingredient (acetanilide, benzoic acid, chlorhydroxyquinoline, and hydroxyquinoline) as a possible stabilizer. Benzoyl peroxide decomposed very fast (first-order K value 0.028 day^{-1} at 24°) in polyethylene glycol ointment base. At 50°, the potency of benzoyl peroxide in polyethylene glycol ointment base decreased to <1% in 5 days. Decomposition in solutions is complex. Considering acetone as a standard vehicle, ethanol improved the stability of benzoyl peroxide and propylene glycol had an adverse effect on the stability. Of the stabilizers studied, only chlorhydroxyquinoline improved the stability.

Keyphrases □ Benzoyl peroxide—stability, effect of some formulation adjuncts □ Formulations—benzoyl peroxide, effect of some formulation adjuncts on stability □ Keratolytics—benzoyl peroxide, effects of some formulation adjuncts on stability

Benzoyl peroxide formulations are used extensively for the treatment of acne; however the literature on the stability of benzoyl peroxide is scarce. Gruber *et al.* (1) studied the stability of some commercial lotions containing I but the formulations' excipients were not reported. According to that report, benzoyl peroxide usually decomposes to benzoic acid and carbon dioxide.

The stability of benzoyl peroxide in pharmaceutical gels was reported by Bollinger *et al.* (2). These authors recommended the use of sodium hydroxide over triethanolamine as a neutralizing agent. Moreover, gels containing some acetone were reported to be very stable (even at higher temperature) *versus* ethanol which had an adverse effect on the stability of benzoyl peroxide. These studies used a selective titrimetric analysis method (3).

A stability-indicating assay method using high-pressure liquid chromatography (HPLC) was reported recently (4). The report questioned the accuracy of the titrimetric method (3) which is also official in the USP (5). The latest USP–NF edition (6) recommends the same titrimetric method of analysis for both hydrous benzoyl peroxide and its lotion.

The purpose of these investigations was to study the effect of some formulation adjuncts, polyethylene glycol ointment USP (7), some liquid vehicles, and some possible stabilizers, on the stability of benzoyl peroxide.

EXPERIMENTAL

Chemicals and Reagents—All the chemicals and reagents were either USP, NF, or ACS grade and were used without further purification. Benzoyl peroxide granules[1] and hydrous benzoyl peroxide granules[2] were used as received.

Apparatus—The HPLC[3] was equipped with a multiple wavelength detector[4], a recorder[5], and a digital integrator[6].

Column—A nonpolar column[7] (30 cm × 4-cm i.d.) was used.

Chromatographic Conditions—The mobile phase contained 60% (v/v) of acetonitrile and 0.8% (v/v) of glacial acetic acid in water. The flow rate was 2.5 ml/min and the temperature was ambient. The sensitivity was set at 0.04 (254 nm) and the chart speed was 30.5 cm/hr.

Preparation of Ointments for Stability Studies—A 2.0% (w/w)

[1] Aldrich Chemical Co., Milwaukee, Wis.

[2] Pennwalt Corp., Buffalo, N.Y.; generously supplied by Alcon Laboratories, Fort Worth, Texas.

[3] Model ALC 202 equipped with a U6K universal injector, Waters Associates, Milford, Mass.

[4] Spectroflow monitor SF770, Schoeffel Instrument Corp., Westwood, N.J.

[5] Omniscribe 5213-12, Houston Instruments, Austin, Texas.

[6] Autolab minigrator, Spectra-Physics, Santa Clara, Calif.

[7] μBondapak C_{18} (catalog no. 27324), Waters Assoc., Milford, Mass.

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Table I—Solutions Prepared For Stability Studies

Solution Number	Concentration of Benzoyl Peroxide	Vehicle, % v/v	Other Ingredient
1	0.1	Acetone	—
2	0.1	Acetone 75 Ethanol 25	—
3	0.1	Acetone 50 Ethanol 50	—
4	0.1	Acetone 25 Ethanol 75	—
5	0.1	Acetone 75 Propylene glycol 25	—
6	0.1	Acetone 50 Propylene glycol 50	—
7	0.1	Acetone 25 Propylene glycol 75	—
8	0.1	Acetone 50 Ethanol 50	0.01% of 8-hydroxyquinoline
9	0.1	Acetone 25 Propylene glycol 75	0.01% of 8-hydroxyquinoline
10	0.1	Acetone 25 Propylene glycol 75	0.01% of acetanilide
11	0.1	Acetone 25 Propylene glycol 75	0.01% of benzoic acid
12	1.0	Acetone 50 Propylene glycol 50	—
13	1.0	Acetone 50 Propylene glycol 50	0.1% of 8-hydroxyquinoline sulfate
14	1.0	Acetone 50 Propylene glycol 50	0.1% of 5-chloro-8-hydroxyquinoline

benzoyl peroxide ointment was prepared by mixing 612.0 mg of benzoyl peroxide granules with 6 ml of acetone and then incorporating this mixture with enough polyethylene glycol ointment USP (7) to make 30 g of ointment. Separate lots of ointments containing 0.01% (w/w) of acetanilide or benzoic acid in addition to 2% (w/w) of benzoyl peroxide were also prepared. After the initial assays, the ointments were divided into two portions and transferred to 30-g opal ointment jars[8]. One portion was stored at 50°; the other portion at room temperature (24°).

Preparation of Solutions for Stability Studies—Solutions containing 0.1% of benzoyl peroxide in various vehicles (acetone, ethanol, propylene glycol, or mixtures of two) were prepared using a simple solution method. Benzoyl peroxide granules were always dissolved in acetone. Some of the solutions also contained an additional ingredient (Table I). After the initial assays, the solutions were transferred to amber-colored, 120-ml glass bottles[8] and stored at 24°.

Another set of solutions containing 1.0% of benzoyl peroxide were prepared from hydrous benzoyl peroxide granules. The vehicle and additional ingredients added are listed in Table I. These solutions were also stored in amber-colored glass bottles at 24°.

Assay Procedure—The HPLC analysis used is similar to one reported previously (4). The internal standard was hydroxyprogesterone caproate. The stock solutions of benzoyl peroxide and the internal standard in methanol (1.0 mg/ml each) were prepared fresh daily. The standard solution was prepared by mixing 2.0 ml of the stock solution of benzoyl peroxide with 4.0 ml of the internal standard stock solution and then bringing the volume to 50.0 ml with methanol.

Preparation of Assay Solutions from Ointments—An appropriate quantity of the ointment containing 2.0 mg of benzoyl peroxide was weighed accurately and dissolved in 30 ml of methanol. A 4.0-ml portion of the internal standard stock solution was added and the mixture brought to volume (50.0 ml) with methanol.

[8] Brockway Glass Co., Brockway, Pa.

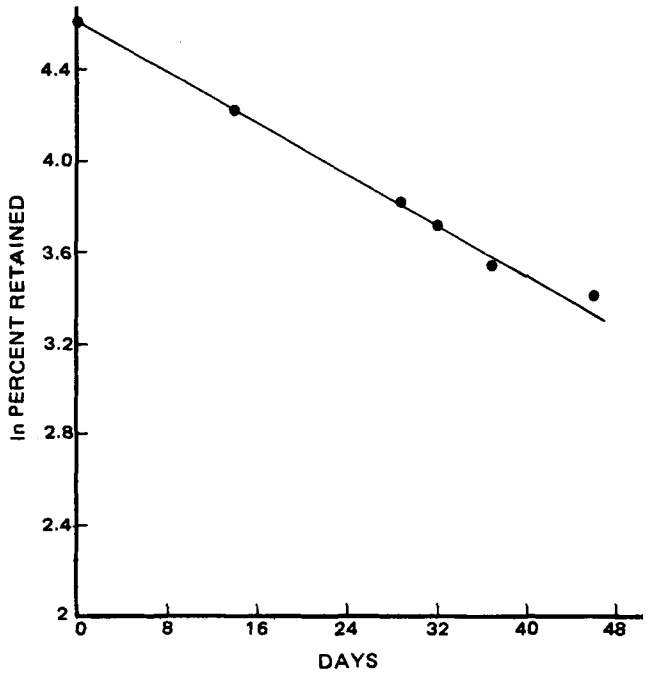

Figure 1—*First-order plot of decomposition (at 24°) of benzoyl peroxide in polyethylene glycol ointment base USP. The results of ointments containing an additional ingredient (acetanilide or benzoic acid) were similar.*

All the solutions studied were diluted with methanol to a concentration (based on the label claim) of 40.0 μg/ml of benzoyl peroxide. Before final dilution, enough of the internal standard stock solution was added to contain 80.0 μg/ml in the final solution.

Injections—A 20.0-μl aliquot of the assay solution was injected into the chromatograph at the conditions described. For comparison, an identical volume of the standard solution was injected after the assay eluted.

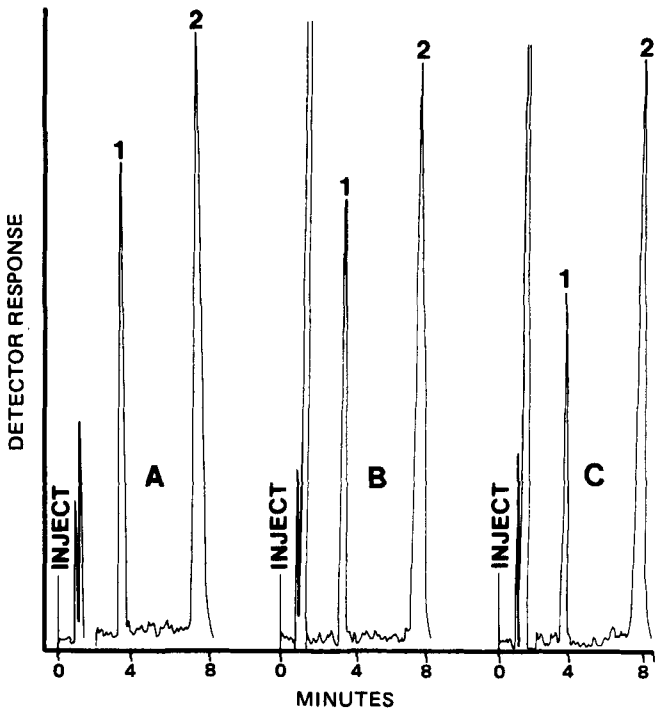

Figure 2—*Sample chromatograms. Peaks 1 and 2 are from benzoyl peroxide and hydroxyprogesterone caproate (internal standard), respectively. The long peak (out of scale) after injection in chromatograms B and C is from acetone. Chromatogram A is from a standard solution (see text) and B and C from 97-days-old solutions (Numbers 4 and 7, Table I), respectively.*

Table II—Assay Results of Solutions [a]

Solution Number[b]	Percent Retained					
	0 day	10 days	46 days	50 days	60 days	97 days
1	101.3	101.4	97.7	—	—	85.2
2	101.8	100.8	98.2	—	—	85.6
3	101.3	101.1	100.2	—	—	89.5
4	102.2	101.8	100.7	—	—	90.9
5	101.1	98.3	96.5	—	—	82.8
6	102.0	98.3	94.2	—	—	81.7
7	100.9	95.6	90.3	—	—	74.7
8	100.0	100.8	99.2	—	—	86.4
9	99.8	97.7	93.3	—	—	72.6
10	100.6	98.2	92.5	—	—	73.2
11	100.6	99.1	95.1	—	—	76.8
12	101.2	97.7	—	81.8	76.9	—
13	100.7	100.1	—	83.1[c]	78.3[c]	—
14	100.1	98.7	—	94.2[c]	87.1[c]	—

[a] For results of ointments stored at 24°, see Fig. 1. Almost all benzoyl peroxide decomposed in 5 days in ointments stored at 50°. [b] For composition of the solutions, see Table 1. [c] These solutions had discolored to light yellow due to oxidation of hydroxyquinoline/chlorhydroxyquinoline.

Calculations—Since the ratios of peak heights were directly related to concentrations, the results were calculated using the equation:

$$100 \times \frac{R_{pha}}{R_{phs}} = \text{percent of the label claim} \qquad \text{(Eq. 1)}$$

where R_{pha} is the ratio of the peak heights of benzoyl peroxide and hydroxyprogesterone caproate of the assay solution and R_{phs} is that of the standard solution.

After the initial assays, the ointments and solutions were reassayed after appropriate time intervals.

RESULTS AND DISCUSSION

The results (Fig. 1, and footnote *a* in Table II) indicate that benzoyl peroxide decomposes quickly when mixed with polyethylene glycol ointment base. The K value at room temperature was 0.028/day. It has been postulated (2) that OH groups in the vehicle had an adverse effect on the stability of benzoyl peroxide. At 50°, almost all of the benzoyl peroxide in polyethylene glycol ointment base decomposed in 5 days. The addition of acetanilide (a commonly used stabilizer for hydrogen peroxide) and benzoic acid (a major decomposition product of benzoyl peroxide) did not improve the stability of benzoyl peroxide. The lag period, which was prominent in solutions, almost disappeared in 2% (w/w) ointment of benzoyl peroxide in polyethylene glycol base.

After the lag period, the solutions decomposed slowly without following any particular order of reaction. However, the following observations might be made from the data (Table I, and Figs. 1 and 2):

1. Ethanol improves the stability of benzoyl peroxide when substituted for acetone (Solutions 3 and 4 *versus* 1, Table II).
2. Propylene glycol hastens the degradation when substituted for acetone (solutions 5–7 *versus* 1, Table II). An attempt to substitute glycerin for acetone (50% v/v) was unsuccessful. There was a problem preparing a single homogeneous phase.
3. The addition of acetanilide, benzoic acid, and hydroxyquinoline did not improve the stability (Solutions 9–11 *versus* 7, Table II). 8-Hydroxyquinoline (oxine) has been reported (8) to improve the stability of hydrogen peroxide in certain pharmaceutical gels.
4. Chlorhydroxyquinoline improved the stability of benzoyl peroxide (solution 14 *versus* 12–13, Table II). This is probably the reason why commercial formulations contain this compound (9).
5. The decomposition of benzoyl peroxide appears to be concentration dependent (solution 6 *versus* 12, Table II). Solution 12 was prepared from hydrous benzoyl peroxide (71%) *versus* Solution 6 which was prepared from benzoyl peroxide (98%). Another solution similar to 12 was prepared using benzoyl peroxide (98%) and this solution was slightly more stable than Solution 12. All commercial formulations are prepared from hydrous benzoyl peroxide to prevent explosion.

In brief, the decomposition of benzoyl peroxide is complex and susceptible to many factors, some of them still unknown. Furthermore, the benefits of adding stabilizer(s) are themselves controversial (2). It has been suggested (2) that addition of chelating agents (citric acid or edetate disodium) might have an adverse effect on the stability of benzoyl peroxide. Yet some commercial products (9) add these chelating agents to improve the stability.

The effect of small traces of impurities in benzoyl peroxide, the vehicles, and additives is still not well understood. The stability of benzoyl peroxide needs to be thoroughly investigated. Further work is in progress in this laboratory.

REFERENCES

(1) M. Gruber, R. Klein, and M. Foxx, *J. Pharm. Sci.*, **58,** 566 (1969).

(2) J. N. Bollinger, D. Lewis, and V. M. Mendez, *ibid.*, **66,** 718 (1977).

(3) R. E. Daly, J. J. Lomner, and L. Chafetz, *ibid.*, **64,** 1999 (1975).

(4) F. W. Burton, R. R. Gade, and W. L. McKenzie, *ibid.*, **68,** 280 (1979).

(5) "The United States Pharmacopeia," 19th rev., Mack Publishing, Easton, Pa., 1975, p. 51.

(6) "The USP-NF," 1980 ed., United States Pharmacopeial Convention, Rockville, Md., 1980, pp. 75–76.

(7) *Ibid.*, p. 1246.

(8) A. I. El Assasy, A. A. Elbary, and Y. H. Hanza, *Cosmet. Toilet.*, **91,** 54 (1976).

(9) "Physicians Desk Reference," 34th rev., Medical Economics, Oradell, N.J., 1980, p. 818.

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