of the experiment (approximately 24 hr).

Based on these results, one could hypothesize that one daily application would be sufficient for the optimal therapeutic effect, provided the formulation is not washed or rubbed off by the patient. The results also show that the formulation with 0.1% diflorasone diacetate offers no therapeutic advantage over the 0.05% formulation. Recent clinical studies comparing the therapeutic benefit of diflorasone diacetate 0.05 and 0.1% ointments (28) support this hypothesis.

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High-Pressure Liquid Chromatographic Assay of Benzoyl Peroxide in Dermatological Gels and Lotions

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Abstract □ A high-pressure liquid chromatographic method for the assay of benzoyl peroxide in dermatological preparations is described. Degradation products such as benzoic acid and perbenzoic acid do not interfere. The method is simple, precise, accurate, and stability indicating.

Keyphrases □ Benzoyl peroxide—high-pressure liquid chromatographic analysis in pharmaceutical preparations □ High-pressure liquid chromatography—analysis, benzoyl peroxide in pharmaceutical preparations □ Keratolytics—benzoyl peroxide, high-pressure liquid chromatographic analysis in pharmaceutical preparations

Benzoyl peroxide is inherently a very reactive compound, and its chemical stability has been studied extensively. Depending on the experimental conditions (temperature, pH, solvent, *etc.*), the degradation of benzoyl peroxide may lead to benzoic acid, biphenyl, phenyl benzoate, benzene, and carbon dioxide. In alcoholic solutions, the degradation products were carbon dioxide, benzoic acid, and alcohol esters of benzoic acid (1). In pharmaceutical lotions, benzoic acid was the significant product (2).

BACKGROUND

The existing stability assays for benzoyl peroxide in pharmaceutical preparations have used spectrophotometric, iodometric, polarographic, and TLC techniques (3–6). Controversial claims of superiority of one method over the others have been made. Gruber and Klein (4) reported that the polarographic method is superior to the spectrophotometric method which, in turn, is much better than the iodometric method. Simmons *et al.* (6) compared the iodometric method with a combination TLC–spectrophotometric method and reported good agreement in assay results, suggesting that the iodometric method is stability indicating.

Daly *et al.* (5) reported "fair agreement" between the assay results by the iodometric method and a combination TLC–iodometric method. Furthermore, the results were reported to be lower than the corresponding assay results by the iodometric and spectrophotometric methods of Gruber and Klein (4). From these observations, Daly *et al.* (5) concluded that the described iodometric method is a stability-indicating procedure. This iodometric procedure has been accepted as the USP method for benzoyl peroxide in lotions (7).

In evaluating the stability-indicating nature of the methods, one or both of the following criteria were used:

1. The results obtained by the method should agree with those obtained by a separate and inherently stability-indicating "reference" method (*e.g.*, chromatographic methods).
2. The results obtained by the method should be lower than those obtained by other methods.

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In applying the first criterion, it is essential that the chosen reference method be truly stability indicating. Simmons *et al.* (6) and Daly *et al.* (5) chose TLC as the reference. However, certain TLC conditions (6) do not appear to be capable of separating benzoyl peroxide from its polar degradation products (*e.g.*, benzoic acid). The more polar degradation products also would migrate with benzoyl peroxide and appear as one spot at the solvent front. Hence, the TLC method of Simmons *et al.* (6) is not truly stability indicating and the conclusions reached regarding its reliability (6) are subject to question.

The second criterion was applied by Gruber and Klein (4), who concluded that the iodometric method is not a good stability-indicating method. Similar reasoning by Daly *et al.* (5) led them to conclude that their iodometric method is better than the iodometric and the spectrophotometric methods of Gruber and Klein. Although Daly *et al.* (5) reported fair agreement between the results by the iodometric method and the reference TLC method, the reported results indicate that the agreement was good only for four of seven cream and lotion samples.

Clearly, the existing methods for benzoyl peroxide are either stability indicating only when applied to certain formulations or only partially stability indicating.

This paper describes the development of a new high-pressure liquid chromatographic (HPLC) method for benzoyl peroxide and compares its performance with that of the USP iodometric method (7). The HPLC method is inherently stability indicating and is simple, accurate, and precise.

EXPERIMENTAL

Apparatus—Column 1 was a stainless steel column (50 cm long, 2.6 mm i.d.) dry packed with macroparticulate (37–50-μm range) bonded octadecylsilane material[1]. The HPLC unit[2] had a syringe-type pump, a septum injector, and a 254-nm absorbance detector.

Stability samples were analyzed using Column 2, a commercial column[3] (30 cm long, 3.9 mm i.d.) prepacked with microparticulate (10 μm) bonded octadecylsilane material. The HPLC unit[4] used with this column had a low volume positive displacement pump[5], a universal injector[6], and a 254-nm absorbance detector[7]. This change from Column 1 to Column 2, with a corresponding change in HPLC units, became necessary when the former column did not provide adequate separation of benzoic acid (a degradation product) from the solvent front.

Reagents—Hydrous benzoyl peroxide[8] (~70% benzoyl peroxide) was used after assaying within the week by the USP procedure (8). Perbenzoic acid was synthesized by the procedure described by Brown (9). Ethyl benzoate[9] was purified by distillation and used as the internal standard. All other reagents used were ACS grade. Water used for preparing HPLC eluents and solutions was distilled from an all-glass still[10].

Samples—The method was developed utilizing samples of two products: a gel formulation containing 5% benzoyl peroxide, polyethylene glycol lauryl ether, carboxypolymethylene, diisopropanolamine, ethylenediaminetetraacetic acid, and water; and a lotion containing 4% benzoyl peroxide, sodium octoxynol-3-sulfonate, water, dioctyl sodium sulfosuccinate, sodium lauryl sulfoacetate, magnesium aluminum silicate, methylcellulose, and ethylenediaminetetraacetic acid.

Control blanks were produced in the same manner as the commercial products, except that the benzoyl peroxide was not added. Synthetic samples of the gel and lotion formulations were made by adding an exactly known aliquot of freshly prepared benzoyl peroxide solution in a suitable solvent (acetone or acetonitrile) to the corresponding control blank. Each synthetic sample was made independently from the control blank and benzoyl peroxide stock solution, and the entire sample was assayed by the chosen method.

Synthetic samples prepared as described are close approximations to commercial products but have an exactly known amount of benzoyl peroxide; the only difference was that benzoyl peroxide was added as a solution. To study the effect of relative amounts of control blank and benzoyl peroxide, some synthetic samples with various ratios of benzoyl peroxide to control blank also were prepared by a similar procedure.

[1] Bondapak C_{18}/Corasil, Waters Associates.
[2] Model 1220, Perkin-Elmer.
[3] μBondapak C_{18}, Waters Associates.
[4] Model ALC/GPC 204, Waters Associates.
[5] Model 6000A, Waters Associates.
[6] Model U6K, Waters Associates.
[7] Model 440, Waters Associates.
[8] Aztec Chemicals.
[9] Eastman Chemicals.
[10] F1-Still 4, Barnstead.

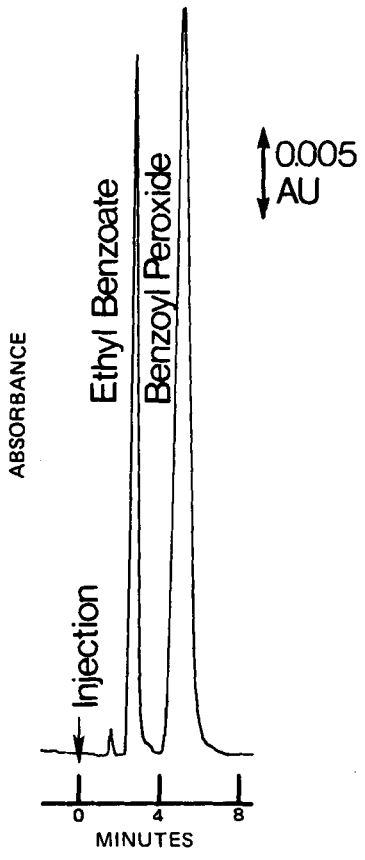

Figure 1—*HPLC separation of ethyl benzoate and benzoyl peroxide using Column 1, 50% acetonitrile in water, and a flow rate of 1 ml/min.*

HPLC Method—A sample containing about 40 mg of benzoyl peroxide was shaken with 50 ml of acetonitrile until the sample was dislodged from the sides and dispersed in solution. The sample solution was then sonicated for 5 min and clarified by centrifugation. The solution for HPLC analysis was made by mixing 10 ml of the clear centrifugate with 5 ml of ethyl benzoate (~18 mg) solution in acetonitrile and then diluting to 25 ml with acetonitrile. Unless otherwise mentioned, the following HPLC parameters were used: eluent, 50% acetonitrile in water; flow rate, 1 ml/min; optical density setting, 0.05 unit; recorder, 10 mv full scale; and chart speed, 5 mm/min.

USP Iodometric Titration Method (5)—Benzoyl peroxide in the sample was extracted into acetone, phenyl sulfide was added, and the solution was titrated iodometrically.

RESULTS AND DISCUSSION

HPLC Method Development—Preliminary TLC studies of benzoyl peroxide formulations showed that the excipients of the formulations are more polar than benzoyl peroxide. Based on this observation, a reversed-phase column was an obvious choice because it permitted the elution of excipients before benzoyl peroxide. Furthermore, dermatological creams and lotions are generally soluble in polar solvents (*e.g.*, methanol and acetonitrile), and these solvents are compatible with the packing of the reversed-phase column. Sample preparation prior to HPLC analysis is simply dissolution and filtration. Acetonitrile was selected as the solvent for sample dissolution because the samples dissolved easier in acetonitrile than in methanol and because benzoyl peroxide is much more soluble in acetonitrile.

The separation of benzoyl peroxide on Column 1 was studied with different acetonitrile–water mixtures as eluents. A reasonably symmetrical (gaussian) benzoyl peroxide peak was observed at a retention time of about 6 min using 50% acetonitrile in water as the eluent at a flow rate of 1 ml/min. With the same HPLC parameters, ethyl, butyl, and phenyl benzoates were evaluated as possible internal standards. Phenyl and butyl benzoates gave peaks that were not fully resolved from the benzoyl peroxide peak. Ethyl benzoate was fully resolved and was chosen as the internal standard. A typical chromatogram of the benzoyl peroxide and ethyl benzoate mixture is shown in Fig. 1.

Table I—HPLC Determination of Benzoyl Peroxide in Synthetic Gel Samples [a]

Synthetic Sample: Control Blank, g	Synthetic Sample: Benzoyl Peroxide, mg	Benzoyl Peroxide Found, mg	Recovery, %
2	71.6	71.1, 71.1, 71.4	99.3, 99.3, 99.7
2	95.5	95.3, 94.8, 93.4	99.8, 99.3, 97.8
2	119.5	121.8, 120.3, 119.1	101.9, 100.7, 99.7

[a] Benzoyl peroxide was added to the control blank as its solution in acetonitrile. A sample containing 2 g (control blank) and 100 mg of benzoyl peroxide approximates the normal gel containing 5% benzoyl peroxide.

Table II—HPLC Determination of Benzoyl Peroxide in Synthetic Lotion Samples [a]

Synthetic Sample: Control Blank, g	Synthetic Sample: Benzoyl Peroxide, mg	Benzoyl Peroxide Found, mg	Recovery, %
0.9	48.3	48.5, 48.6, 48.9	100.4, 100.6, 101.2
1.2	48.3	48.9, 48.2, 48.4	101.2, 99.8, 100.2
1.5	48.3	47.9, 47.4, 47.7	99.2, 98.1, 98.7

[a] Benzoyl peroxide was added to the control blank as its solution in acetonitrile. A sample containing 1.2 g (control blank) and 48 mg of benzoyl peroxide approximates the normal lotion containing 4% benzoyl peroxide.

Likely degradation products of benzoyl peroxide are benzoic acid (2) and perbenzoic acid (5). With the same HPLC system as that used to obtain Fig. 1, benzoic acid gave a peak at the solvent front, thereby causing no interference in the benzoyl peroxide assay. Since it is desirable to determine benzoic acid also, particularly in stability studies, the more efficient Column 2 was tested to effect the separation of benzoic acid from the solvent front. Adequate separation was observed with 50% acetonitrile in water as the eluent at a flow rate of 1 ml/min. Under the same HPLC conditions, the separation of ethyl benzoate and benzoyl peroxide was also adequate, suggesting that both benzoyl peroxide and benzoic acid may be determined by a single HPLC method using Column 2. A typical chromatogram of a mixture of the three compounds is shown in Fig. 2. The use of Column 1 permits only the determination of benzoyl peroxide and not benzoic acid.

The HPLC characteristics of perbenzoic acid were studied using both columns. When 50% acetonitrile in water was the eluent, perbenzoic acid eluted with the solvent front in both cases. With Column 2 and 20% acetonitrile in water as the eluent, perbenzoic acid could be separated from both the solvent front and benzoic acid. A typical chromatogram of the benzoic acid and perbenzoic acid mixture is shown in Fig. 3. When the development of an HPLC method to include the determination of perbenzoic acid was investigated, it was found that perbenzoic acid degraded significantly to benzoic acid in dilute solutions.

In refining and optimizing the HPLC method for the benzoyl peroxide assay, commercial samples of benzoyl peroxide gel and lotion were used. The conditions for extraction of benzoyl peroxide into solution (dispersion, sonication, and centrifugation) were selected such that the percentage of benzoyl peroxide found in these samples by the HPLC method was maximum and further increases in dispersion time or sonication time had no effect on the assay values.

Analysis of the control blanks for the benzoyl peroxide gel and lotion by the proposed HPLC method showed no chromatographic peaks at the retention times for benzoyl peroxide and ethyl benzoate (internal standard). Thus, the control blanks caused no direct interference in the benzoyl peroxide assay with either column.

The results obtained in the analysis of nine synthetic samples representing benzoyl peroxide gel (control blank to benzoyl peroxide ratio altered in some cases) with Column 1 are shown in Table I. The method yielded accurate results (average recovery of 99.7%), and the control blank to benzoyl peroxide ratio had no noticeable effect on the recovery values. Similar data obtained with nine synthetic lotion samples (Table II) suggest that the method gave accurate results for the lotion also. Again, the ratio of control blank to benzoyl peroxide had no noticeable effect on the recovery values.

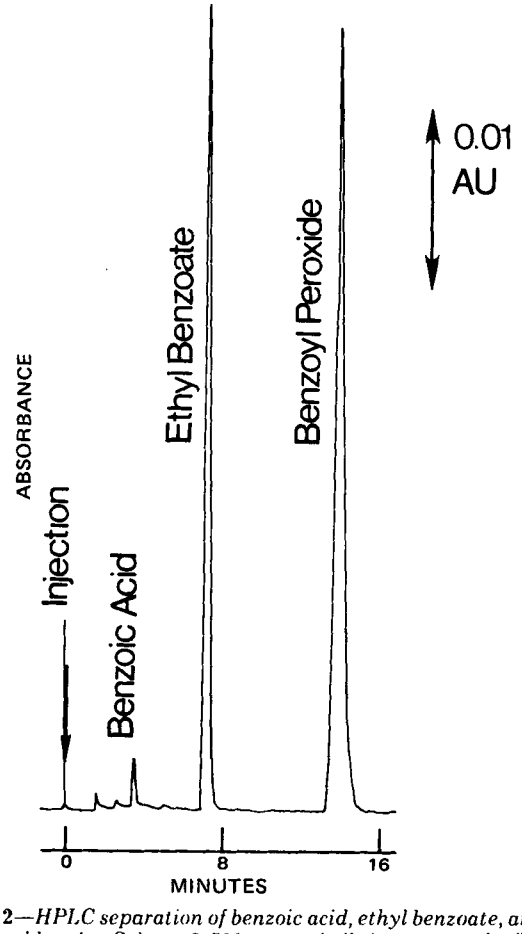

Figure 2—*HPLC separation of benzoic acid, ethyl benzoate, and benzoyl peroxide using Column 2, 50% acetonitrile in water, and a flow rate of 1 ml/min.*

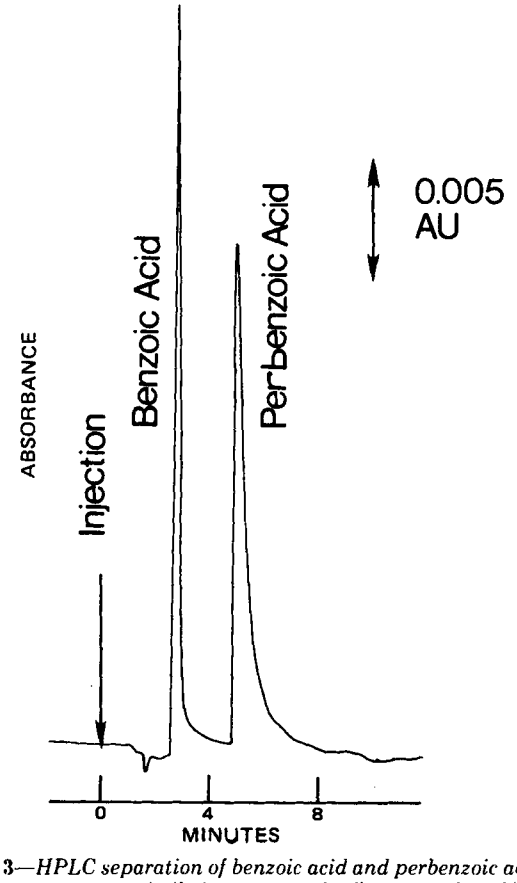

Figure 3—*HPLC separation of benzoic acid and perbenzoic acid using Column 2, 20% acetonitrile in water, and a flow rate of 1 ml/min.*

Table III—Benzoyl Peroxide Assay of Stability Samples of Benzoyl Peroxide Gel by HPLC and USP Methods

Stability Parameters	HPLC Method			USP Iodometric Method[a]	
	Benzoyl Peroxide, %	Percent Initial	Benzoic Acid, %	Benzoyl Peroxide, %	Percent Initial
Initial	5.77	—	—	5.39	—
60°, 2 days	5.64	97.7	0.20	5.38	99.8
60°, 4 days	5.48	95.0	0.27	5.20	96.5
60°, 7 days	5.34	92.5	0.44	5.00	92.8
60°, 9 days	5.30	91.9	0.48	4.90	90.9

[a] Recommended method for benzoyl peroxide lotions (5).

Nine analyses of a product sample[11] of gel containing 10% benzoyl peroxide by the HPLC method with Column 1 gave results with a relative standard deviation of 0.82%. Similarly, three analyses of the gel containing 5% benzoyl peroxide gave a relative standard deviation of 1.2%. Analysis of the lotion samples containing 4% benzoyl peroxide led to assay values with a relative standard deviation of 0.67%.

Although the precision and accuracy data of the HPLC method were collected using Column 1, the method was as good or better with Column 2. Five analyses of synthetic benzoyl peroxide gel samples using Column 2 gave recoveries of 99.9, 99.5, 99.1, 100.7, and 99.7% (average of 99.8%). Similarly, five analyses of the gel containing 5% benzoyl peroxide gave results with a relative standard deviation of 0.65%.

Application to Stability Studies—Samples of benzoyl peroxide gel were force degraded at 60°. Samples were taken at different time intervals and assayed by the HPLC method with Column 2. For comparison, the samples were also assayed by the USP iodometric method for benzoyl peroxide lotions (Table III).

Both the HPLC and the USP iodometric methods were stability indicating (Table III). Judging from the percent initial values of benzoyl peroxide obtained by both methods, the HPLC method showed only slightly higher degradation of samples. Excellent agreement between the amount of benzoyl peroxide degraded and the amount of benzoic acid formed was obtained with the HPLC method, which suggests that benzoic acid is the only, or the major, degradation product of benzoyl peroxide under the experimental conditions.

Comparison of the HPLC and USP iodometric methods (Table III) showed that the results (percent benzoyl peroxide) obtained by the USP method were consistently lower. To explain this difference, the accuracy of the USP method was evaluated using synthetic benzoyl peroxide gel samples. Analysis of three samples gave recovery values of 90.9, 90.1, and 86.6%. Evidently, the USP iodometric method for benzoyl peroxide lotion is not suitable for the benzoyl peroxide gel tested. Although the USP method appeared to be stability indicating, as judged from the percent initial values, its poor accuracy makes its use questionable for this gel.

Assay of five synthetic samples of benzoyl peroxide lotion by the USP method gave an average recovery value of 99.8%. Similar assay of five commercial benzoyl peroxide lotion samples gave a relative standard deviation of 0.83%. These results showed that the USP iodometric method for benzoyl peroxide lotions was accurate and precise. Limited comparative assay data of benzoyl peroxide lotion samples by the USP iodometric method and the HPLC method suggest that both methods are stability indicating when applied to lotions.

In summary, the HPLC method described is stability indicating for both gel and lotion formulations of benzoyl peroxide products; furthermore, the most likely degradation products or impurities, benzoic acid and perbenzoic acid, do not interfere in the assay. The method may be used with either type of octadecylsilane column. The more efficient Column 2, however, permits the simultaneous determination of benzoic acid.

[11] Similar composition to 5% benzoyl peroxide gel.

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Identification and Synthesis of a Methylated Catechol Metabolite of Glutethimide Isolated from Biological Fluids of Overdose Victims

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Received May 30, 1978, from the *Analytical Toxicology Laboratory, College of Pharmacy, University of Florida, Gainesville, FL 32610.* Accepted for publication August 3, 1978.

Abstract □ Urine samples from victims severely intoxicated by glutethimide were hydrolyzed enzymatically. TLC, GLC, and mass spectral analyses revealed a methylated catechol metabolite of the parent drug. Two synthetic pathways are described for the preparation of 2-ethyl-2-(3-methoxy-4-hydroxyphenyl)glutarimide and 2-ethyl-2-(3-hydroxy-4-methoxyphenyl)glutarimide. Comparisons of GLC and mass spectral data to a compound isolated from the body fluids of glutethimide overdose victims conclusively identified a new 3-methoxy-4-hydroxyphenyl metabolite of glutethimide in humans.

Keyphrases □ Glutethimide—metabolites identified in human urine, synthesized □ Metabolites—of glutethimide, identified in human urine, synthesized □ Sedatives—glutethimide, metabolites identified in human urine, synthesized

Since the introduction of glutethimide, (±)-2-ethyl-2-phenylglutarimide, in 1954 (1), it has been the subject of considerable study. Originally assumed to be a nonbarbiturate and sedative–hypnotic with few side effects

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