firmly established from both previous studies of sorption in the absence of air (1, 2) as well as the present study in which the moderating effects of diffusion are evident.

APPENDIX: GLOSSARY[3]

a' radius of sphere bounded by outside radius of vapor diffusion layer
c molar gas concentration; also subscript denoting "chamber"
C^* dimensionless concentration variable
k_h mass transport coefficient associated with heat transport-controlled aspect of sorption
k_m mass transport coefficient associated with mass transport-controlled aspect of sorption
P_i pressure of inert gas
P_T total pressure
P^* dimensionless pressure variable
RH_s unknown relative humidity at the sample surface associated with conditions of balanced heat and mass transport in which the two processes control sorption to a similar degree
RH^* dimensionless relative humidity variable
W_{sp} sorption rate per unit surface area
W_m sorption rate associated with mass transport control
W_{mh} sorption rate associated with the combined control of both heat and mass transport
x_w mole fraction water vapor
δ vapor diffusion layer thickness

[3] Refer to the first paper in this series (1) for the majority of symbol identification. Listed here are only those symbols introduced in the present paper.

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Quantitative Determination of Benzoyl Peroxide by High-Performance Liquid Chromatography and Comparison to the Iodometric Method

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Abstract □ A selective high-performance liquid chromatographic (HPLC) procedure for the quantitative determination of benzoyl peroxide in pharmaceutical dosage forms is described. Benzoyl peroxide was dissolved or extracted in the presence of an internal standard, acenaphthylene. The specificity of the stability-indicating HPLC and iodometric procedures are presented for benzoyl peroxide.

Keyphrases □ Benzoyl peroxide—degradation products, stability-indicating high-performance liquid chromatography, comparison with iodometric procedures □ High-performance liquid chromatography—stability indicating, benzoyl peroxide and its degradation products, commercial formulations, comparison with iodometric procedures □ Degradation products—benzoyl peroxide, stability-indicating high-performance liquid chromatography in commercial formulations.

Benzoyl peroxide (dibenzoyl peroxide), active against acne-causing bacteria, is widely used in pharmaceutical preparations as an antibacterial and keratolytic agent (1). Analytical methods currently available include spectrophotometry (2), polarography (2), TLC (3), titrimetry (4), and high-performance liquid chromatography (HPLC) (5, 6).

BACKGROUND

Benzoyl peroxide is a chemically reactive molecule which readily decomposes in various solvents (7) to give compounds such as biphenyl, phenyl benzoate, benzoic acid, benzene, 4-biphenylcarboxylic acid, homophthalic acid, homoterephthalic acid, and carbon dioxide (8). Daley *et al.* reported a selective titrimetric procedure (9), modifying the conventionally utilized iodometric method. They proposed the addition of phenyl sulfide prior to the titration to eliminate potential interferences caused by the presence of hydroperoxide impurities such as perbenzoic acid. This iodometric procedure has been accepted as the USP method for analysis of benzoyl peroxide lotion (10). Oliveri-Vigh and Hainsworth proposed an HPLC procedure that is selective in the presence of benzoic acid and benzaldehyde (5). Burton *et al.* proposed a similar HPLC procedure specific for analysis of benzoyl peroxide in gels and lotions in the presence of benzoic acid and perbenzoic acid (6); this procedure has been adopted as the USP method for analysis of benzoyl peroxide gel (11).

Analyses of benzoyl peroxide using Burton *et al.*'s procedure or the compendial HPLC procedure (6, 11) may give less accurate results, because this method depends on the use of an homogeneous reference standard. The aforementioned analytical procedures utilize aqueous benzoyl peroxide as the reference material to prepare the standard solutions. Aqueous benzoyl peroxide (70% benzoyl peroxide) is a heterogenous mixture which is nonuniform in its water content, typically varying

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by a few percent from sample to sample. The specification in the pharmacopeia for aqueous benzoyl peroxide provides a range of not less than 65% and not more than 82% of $C_{14}H_{10}O_4$ (12).

Standard preparation in the proposed HPLC procedure has been designed to eliminate the inaccuracies associated with the use of a nonhomogeneous reference material like aqueous benzoyl peroxide. The HPLC procedure described in this paper is specific, provides the direct and accurate determination needed to evaluate the stability of pharmaceutical products containing benzoyl peroxide, and has the added advantages of detecting and quantifying degradation products.

EXPERIMENTAL

Apparatus and Reagents—A high-performance liquid chromatograph[1] equipped with an automatic sampler system[2], a reverse-phase column[3], and a variable-wavelength spectrophotometric detector[4] interfaced to an electronic integrator[5] was used. Aqueous benzoyl peroxide[6], acenaphthylene[7], homophthalic acid[7], 4-biphenylcarboxylic acid[7], benzaldehyde[7], phenyl benzoate[7], biphenyl[7], *o*-terphenyl[7], *p*-terphenyl[7], benzoic acid[8], benzene[8], acetone[8], UV-grade methanol and acetonitrile[9], reagent-grade potassium iodide and sodium thiosulfate[10], and phenyl sulfide[11] were used as received.

HPLC Assay—*Chromatographic Conditions*—The mobile phase consisted of methanol–water (75:25, v/v) filtered through a 0.45-μm membrane filter[12]. The flow rate of the mobile phase was set at 1.0 ml/min. The injection volume for the standard and sample preparations was maintained at 20 μl, and the column effluent was monitored by UV absorption at 238 nm (maximum absorbance for benzoyl peroxide).

Procedure—A stock solution of internal standard was prepared by dissolving acenaphthylene in acetonitrile at a concentration of 2.0 mg/ml. A reference standard stock solution was prepared by dissolving ~360 mg of aqueous benzoyl peroxide in 100 ml of acetonitrile. The potency of the reference standard stock solution was determined by pipetting a 10.0-ml aliquot and measuring the amount of benzoyl peroxide by the compendial iodometric procedure described under the monograph of benzoyl peroxide lotion (12), except that acetonitrile was substituted for acetone. A 20.0-ml aliquot of the reference standard stock solution was diluted to 100 ml with acetonitrile for the HPLC standard stock solution. A standard pair solution of benzoyl peroxide (0.05 mg/ml) and acenaphthylene (0.20 mg/ml) was prepared by mixing 10.0 ml of the internal standard stock solution and 10.0 ml of the HPLC standard stock solution, and diluting to 100 ml with acetonitrile.

Sample solutions were made by accurately weighing a sample equivalent to 50 mg of benzoyl peroxide into a 100-ml volumetric flask, sonicating for 15 min in 70 ml of acetonitrile, and then diluting to volume with acetonitrile. Sample pair solutions were prepared by mixing 10.0 ml of the internal standard solution and 10.0 ml of the sample solution in a 100 ml-volumetric flask and diluting this to volume with acetonitrile. All solutions were filtered through 0.45-μm membrane filter[13].

Compendial Titrimetric Assay—A reference standard stock solution was prepared by dissolving 250 mg of benzoyl peroxide in 100.0 ml of acetone. Aliquots (10.0 ml) of the reference standard stock solution were assayed in the presence of postulated degradation compounds for specificity evaluation, and in the presence of placebo ingredients for synthetic recovery studies for the lotion and wash formulations of benzoyl peroxide. Reference standard solutions and benzoyl peroxide formulations (cream, lotion, gel, and wash) were assayed by the pharmacopeial procedure described under the monograph for benzoyl peroxide lotion.

RESULTS AND DISCUSSIONS

Assay Selectivity—*HPLC Procedure*—A stability-indicating method must be able to discriminate between degradation products and the active ingredient. Preferably, a stability-indicating procedure should also quantitatively demonstrate the inverse relationship between the decrease in the active ingredient and increase in the degradation products. To show that a method is stability-indicating, routes of degradation products may be postulated and tested in the assay system for interference. The specificity of the HPLC system has been demonstrated (Fig. 1), and the retention times of the postulated degradation compounds are listed in Table I. Analyses of the synthetic placebos for benzoyl peroxide lotion and wash formulations by the proposed HPLC method showed no chromatographic peaks at the retention times of benzoyl peroxide and acenaphthylene (internal standard). A typical chromatogram of the standard pair mixture is shown in Fig. 2.

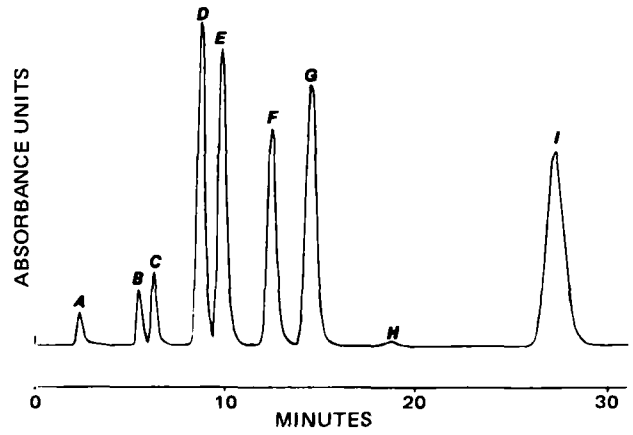

Figure 1—*High-performance liquid chromatogram of benzoyl peroxide and its decomposition products. Key: (A,B) degradation products of benzoyl peroxide in methanol; (C) benzene; (D) phenyl benzoate; (E) benzoyl peroxide; (F) acenaphthylene (internal standard); (G) biphenyl; (H) impurity from acenaphthylene; (I) o-terphenyl.*

Compendial Titrimetric Procedure—The compendial iodometric procedure involves a sodium thiosulfate titration of liberated iodine, resulting from the oxidation of potassium iodide by benzoyl peroxide. Phenyl sulfide is added prior to the introduction of potassium iodide to prevent the liberation of free iodine by the reduction of hydroperoxide impurities such as perbenzoic acid. To determine the influence of adding phenyl sulfide, benzoyl peroxide solution was tested with varying quantities of phenyl sulfide, and a linear relationship was observed between the decrease in recovery and amount of phenyl sulfide added. The regression equation (n = 7) for the volume of titrant consumed (y) for 18 mg of benzoyl peroxide and the amount of phenyl sulfide added (x, expressed in milliliters) was $y = 21.3867 + (-0.9134)(x)$. The coefficient of determination was 0.995, $CV = 0.25\%$, and at the 95% confidence interval the slope and intercept were -0.9134 ± 0.074 and 21.387 ± 0.078, respectively. To challenge the specificity of this method, benzoyl peroxide samples were analyzed in the presence of postulated degradation products; the data (Table II) demonstrate specificity of the iodometric procedure.

Statistical Evaluation—*HPLC Procedure*—Quantitation was based on the benzoyl peroxide–internal standard peak ratio. With these ratios, the linearity between detector response at 238 nm and the amount of active ingredient injected was established for concentrations between 13.5 and 76.4 μg of benzoyl peroxide. The regression equation (n = 9) for the benzoyl peroxide–internal standard peak area ratio (y) and the

Table I—Location of the Degradation Compounds of Benzoyl Peroxide as a Function of Retention Time

Compounds	Retention Time, min
Homophthalic acid	2.20
Benzoic acid	2.76
4-Biphenylcarboxylic acid	3.38
Phenol	3.65
Benzaldehyde	4.12
Benzene	6.00
Phenyl benzoate	8.46
Benzoyl peroxide (active)	9.46
Acenaphthylene (internal standard)	11.96
Biphenyl	13.87
o-Terphenyl	25.82
p-Terphenyl	55.94

[1] Model 6000 Solvent Delivery System, Waters Associates, Milford, Mass.
[2] Intelligence Sampler System 710A, Waters Associates, Milford, Mass.
[3] μBondapak C_{18}, 3.9-mm i.d. × 30-cm, Waters Associates, Milford, Mass.
[4] Model 450 Variable-Wavelength Absorbance Detector, Waters Associates, Milford, Mass.
[5] Model 3385 A, Hewlett-Packard, Paramus, N.J.
[6] Penn Walt Corp., Rochester, N.Y.
[7] Aldrich Chemical Co., Inc., Metuchen, N.J.
[8] J. T. Baker Chemical Co., Phillipsburg, N.J.
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[10] Fisher Scientific Co., Fair Lawn, N.J.
[11] Eastman Kodak Co., Rochester, N.Y.; Pfaltz and Bauer, Stamford, Conn.
[12] Type OE-67, Schleicher and Schuell, Inc., Keene, N.H.
[13] Type RC-55, Schleicher and Schuell, Inc., Keene, N.H.

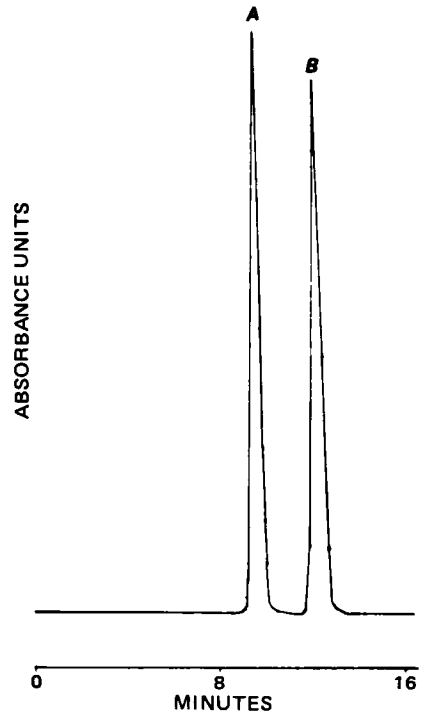

Figure 2—*High-performance liquid chromatogram of benzoyl peroxide. Key: (A) Benzoyl peroxide; (B) acenaphthylene (internal standard).*

Table II—Recovery of I[a] in the Presence of Postulated Degradation Products

Degradation Product	Amount Added[b], mg	Benzoyl Peroxide Found, mg	Recovery, %
Homophthalic acid	3.7	25.5	98.5
Benzoic acid	2.5	25.5	98.5
4-Biphenylcarboxylic acid	4.0	25.6	99.2
Phenol	1.9	25.5	98.5
Benzene	0.3	26.0	100.4
Phenyl benzoate	3.9	26.2	101.2
Biphenyl	3.3	26.2	101.2
o-Terphenyl	4.7	26.0	100.4
p-Terphenyl	4.7	26.2	101.2

[a] Each aliquot contained 25.9 mg. [b] Equivalent to ~20% decomposition assuming a 1:1 stoichiometry.

amount of benzoyl peroxide injected (x, expressed as μg/ml) was $y = (1.104 \times 10^{-3}) + (1.953 \times 10^{-2})(x)$. The coefficient of determination was 0.9999, $CV = 0.74\%$, and at the 95% confidence interval the slope and intercept were 0.0195 ± 0.0002 and 0.0011 ± 0.0072, respectively. The response factor ratios, defined as the area ratio (acenaphthylene/benzoyl peroxide) times the concentration ratio (benzoyl peroxide/acenaphthalene) were quite constant for all concentrations. Precision was demonstrated by a relative standard deviation of 0.4% for 16 replicate injections. The results of the analyses of mixtures consisting of the addition of active ingredient to wash and lotion placebo mixtures demonstrate the accuracy of the proposed method with average recoveries of 99.5 and 99.7%, respectively.

Compendial Titrimetric Procedure—Linearity was obtained by the iodometric procedure between 2.0 and 81.4 mg of benzoyl peroxide. The regression equation ($n = 11$) for the volume of titrant consumed (y) and the amount of benzoyl peroxide (x, expressed in milligrams) were $y = (9.151 \times 10^{-2}) + 0.8107x$. The coefficient of determination was 0.9999, $CV = 0.73\%$, and at the 95% confidence interval the slope and intercept were 0.8107 ± 0.0055 and 0.915 ± 0.2422, respectively. Precision of this USP titration method was demonstrated by a relative standard deviation of 0.3% for seven replicate analyses. The analytical results of active and placebo mixtures for wash and lotion formulations demonstrate the accuracy of the iodometric procedure with average recoveries of 99.8 and 100.2%, respectively.

Table III—HPLC and USP Iodometric Analyses of Commercial Formulations of Lotion, Cream, Wash, and Gel

Formulation	Dosage Claimed, mg of I/g	HPLC, mg of I/g	USP Iodometric, mg of I/g
Lotion	100	105.6	106.2
	100	100.5	103.3
	100	107.9	105.3
	50	48.4	50.1
	50	58.0	56.6
	50	45.3	45.8
Cream	100	110.0	109.0
	100	98.5	95.1
Wash	100	103.3	103.0
	100	108.0	107.4
	50	57.0	55.1
	40	41.7	41.2
Gel	50	54.8	54.7

CONCLUSIONS

In five determinations of aqueous benzoyl peroxide using the USP iodometric determination, the benzoyl peroxide ranged from 66.9 to 73.5% ($CV = 4.25\%$) indicating the nonhomogenous distribution of water in the benzoyl peroxide raw material. Therefore, the utilization of aqueous benzoyl peroxide as the reference standard in HPLC analysis affects the analytical performance based on the homogeneity of the material. Standard preparation in the aforementioned HPLC procedure has been designed to compensate for the variability of water content in aqueous benzoyl peroxide.

The experimental data shown in Table III were obtained on randomly selected samples of benzoyl peroxide lotion, wash, cream, and gel formulations. Each sample was analyzed by the HPLC and the USP iodometric procedures. The results from both methods are comparable, and the procedures are precise, accurate, and specific for determination of benzoyl peroxide in commercial formulations. The HPLC procedure presented in this paper is stability indicating, capable of detecting and quantifying the decomposition products of benzoyl peroxide, and adopts a new technique for the standard preparation to improve the precision, accuracy, and reproducibility of the analysis for benzoyl peroxide formulations.

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