therapy with atenolol, such as diuretics, might cause additional chromatographic peaks and/or interferences. These studies are currently underway.

The analysis of atenolol in urine involves the addition of one step to the analytical procedure: an initial sample dilution. Chromatograms (Fig. 2) of the urine extract were free of interfering peaks and had the same precision and accuracy as comparable concentrations in plasma.

The method was used in the analysis of 768 plasma and 384 urine samples in a pharmacokinetic study over 4 months. A plot of the plasma concentration *versus* time following a 100-mg dose to one volunteer is shown in Fig. 3. Over the 4 months, standard curves were prepared daily. When the same column was used, the day-to-day change in the slope of the standard curve never exceeded 7.2%; however, when new columns were installed, changes up to 15% were observed.

REFERENCES

(1) A. M. Barrett, J. Carter, J. D. Fitzgerald, R. Hull, and D. Lecount, *Br. J. Pharmacol.*, **48**, 340P (1973).

(2) J. Conway and M. Reeves, *Br. Heart J.*, **34**, 963 (1973).

(3) R. Hainsworth, F. Karim, and J. B. Stoker, *Br. J. Pharmacol.*, **48**, 342P (1973).

(4) J. D. Harry, M. F. Knapp, and R. J. Linden, *ibid.*, **48**, 340P (1973).

(5) F. J. Conway, J. D. Fitzgerald, J. McAnish, D. J. Rowlands, and W. T. Simpson, *Br. J. Clin. Pharmacol.*, **3**, 267 (1976).

(6) M. G. Myers, G. R. J. Lewis, J. Steiner, and C. T. Dollery, *Clin. Pharmacol. Ther.*, **19**, 502 (1976).

(7) L. Hansson, H. Alberg, S. Jameson, B. Karlberg, and R. Malmcrona, *Acta Med. Scand.*, **194**, 549 (1973).

(8) J. Sassard, N. Pozet, M. Vincent, and P. Y. Zech, *N. Engl. J. Med.*, **294**, 787 (1976).

(9) A. Amery, L. Billiet, J. V. Joossens, J. Meekers, T. Reybrouk, and W. VanMiechem, *Acta Clin. Belg.*, **28**, 358 (1973).

(10) J. O. Malbica and K. R. Monson, *J. Pharm. Sci.*, **64**, 1992 (1975).

(11) B. Scales and P. B. Copsey, *J. Pharm. Pharmacol.*, **27**, 430 (1975).

(12) C. M. Kaye, *Br. J. Clin. Pharmacol.*, **1**, 84 (1974).

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Benzoyl Peroxide Assay Using High-Pressure Liquid Chromatography

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Received August 1, 1977, from the *G. S. Herbert Laboratories, Allergan Pharmaceuticals, Irvine, CA 92713.* Accepted for publication November 9, 1977.

Abstract □ A highly specific assay for benzoyl peroxide was developed using high-pressure liquid chromatography. A concentration curve was recorded from 0.1 to 3.0 μg with a correlation coefficient of 0.9969. The standard deviation for 10 individual analyses of a benzoyl peroxide preparation was ±0.547 (1.80% *RSD*).

Keyphrases □ Benzoyl peroxide—high-pressure liquid chromatographic analysis in bulk drug and commercial preparations □ High-pressure liquid chromatography—analysis, benzoyl peroxide in bulk drug and commercial preparations □ Keratolytic agents—benzoyl peroxide, high-pressure liquid chromatographic analysis in bulk drug and commercial preparations

Benzoyl peroxide is widely used in topical preparations for the treatment of acne. The methods currently available for benzoyl peroxide include titrimetry (1), spectrophotometry (2), and polarography (2). Only polarography is specific for benzoyl peroxide.

The visible absorption curves for benzoyl peroxide and analogous compounds, such as benzaldehyde and benzoic acid, obtained with the spectrophotometric method described in the USP (3) show marked similarity. If benzoyl peroxide is mixed with any of these analogous compounds, an accurate determination of benzoyl peroxide is impossible.

The sodium thiosulfate titration of liberated iodine is a back-titration, which is a limitation in itself. It also does not distinguish the difference between benzoyl peroxide and its chief degradation products, benzoic acid and benzaldehyde, because of the addition of iodine to aromatic double bonds.

The high-pressure liquid chromatographic (HPLC) method described in this paper is specific and provides accurate determinations of benzoyl peroxide, benzoic acid, and benzaldehyde, all with a single injection (Fig. 1).

EXPERIMENTAL

Apparatus—Absorbance measurements were recorded with a spectrophotometer[1] with matched 1-cm path length quartz cells[2]. A high-pressure liquid chromatograph[3] with a UV-visible detector[3] was used with an integrator[3] to separate and quantitate benzoyl peroxide.

Instrument Settings—All separations were run on an octadecylsilane 10-μm reversed-phase column utilizing isocratic elution (1.2 ml/min) with acetonitrile–water (50:50). A detector sensitivity of 0.16 aufs and a chart speed of 20 cm/hr were used.

Reagents—Acetonitrile[4] and deionized water were purified by filtration through a 0.22-μm solvent inert filter.

Procedure—Samples were prepared by taking aliquots of a benzoyl peroxide preparation containing approximately 30.0 mg of benzoyl peroxide and stirring in 75.0 ml of acetonitrile for 5 min. The samples were then filtered through a prewetted 0.22-μm solvent inert filter into a 100-ml volumetric flask and diluted to volume with acetonitrile. Samples were injected *via* a 10.0-μl fixed loop injector, and the UV absorption at 254 nm was used for detection and quantitation.

The concentration curve was prepared by accurately weighing 1.0, 2.0, 5.0, 10.0, 20.0, and 30.0 mg of benzoyl peroxide into 100-ml volumetric flasks and diluting to volume with acetonitrile.

[1] Beckman.
[2] Markson.
[3] Spectra Physics.
[4] Burdick & Jackson.

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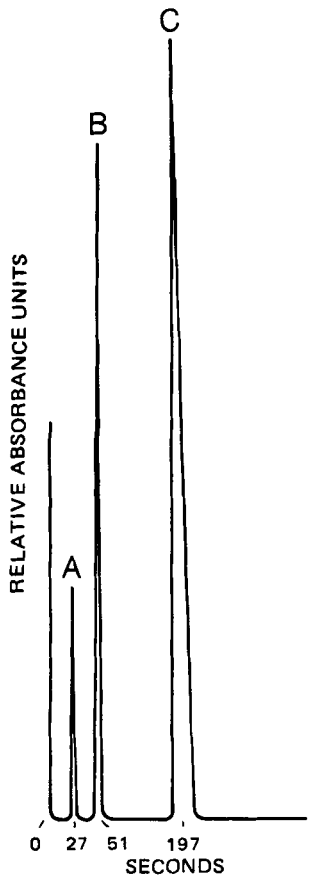

Figure 1—*High-pressure liquid chromatogram for benzoyl peroxide and analogous compounds. Key: A, benzoic acid; B, benzaldehyde; and C, benzoyl peroxide.*

RESULTS

A correlation coefficient of 0.9969 was obtained for the concentration curve, which was linear over the range of 0.1–3.0 μg, when peak areas were plotted *versus* concentration.

Table I—Marketed Product Survey

Product	Benzoyl Peroxide Claimed, %	Benzoyl Peroxide Found by HPLC, %	Benzoic Acid/ Benzaldehyde, %	Product Age, Months
A	5.0	5.15	0.07	6
B	10.0	10.11	0.14	6
C	10.0	10.93	—	3
D	5.0	4.35	0.57	3
E	10.0	8.58	0.12	3
F	10.0	9.27	0.35	3

Reproducibility of the method was checked by taking aliquots of a benzoyl peroxide preparation, equivalent to 30.0 mg of benzoyl peroxide, and working the sample up as described (resulting concentration of 30.0 mg/100 ml). The average value for 10 runs was 30.45 mg of benzoyl peroxide with a standard deviation of ±0.5468 (±1.80% *RSD*). The precision ($ts/\sqrt{N}$ for nine degrees of freedom at the 95% confidence level) was ±1.237 (±4.05% *RSD*).

CONCLUSION

The HPLC method is specific and reproducible and offers greater efficiency over current methods. Once the chromatograph is primed, 20 samples can be run in 1 hr, including a determination for all three compounds per sample. Suitable modifications of the procedure can make it applicable to other preparations of benzoyl peroxide (Table I).

REFERENCES

(1) D. H. Wheeler, *J. Am. Oil Chem. Soc.*, **25**, 144 (1948).
(2) M. P. Gruber and R. W. Klein, *J. Pharm. Sci.*, **56**, 1505 (1967).
(3) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975.

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Rapid and Micro High-Pressure Liquid Chromatographic Determination of Chloramphenicol in Plasma

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Received July 25, 1977, from the *Clinical Pharmacokinetics Laboratory and the Department of Pharmacy, University of Illinois, Chicago, IL 60612.* Accepted for publication November 3, 1977.

Abstract □ A high-pressure liquid chromatographic method was developed for chloramphenicol in plasma. Plasma samples were deproteinized with 2.5 volumes of acetonitrile, and the supernates were chromatographed on a reversed-phase column, using acidified ethanol–water as the mobile phase and UV spectrophotometry for detection. The sensitivity for accurate quantitation of chloramphenicol was about 2.5 μg/ml in plasma, and concentrations as low as 0.5 μg/ml could be detected. Only about 8 min is needed for each sample. This method is specific, rapid, and sufficiently sensitive and may be useful for clinical monitoring.

Keyphrases □ Chloramphenicol—high-pressure liquid chormatographic analysis in plasma □ High-pressure liquid chromatography—analysis, chloramphenicol in plasma □ Antibacterial–antirickettsials—chloramphenicol, high-pressure liquid chromatographic analysis in plasma

Chloramphenicol is effective against several Gram-negative bacteria and exhibits antirickettsial activity (1). Unfortunately, this antibiotic also depresses the erythropoietic elements of bone marrow and causes prolonged morbidity and high fatality of aplastic anemia (2).

A plasma concentration of 5–10 μg/ml was reported to be clinically effective for veterinary use in dogs (3). Similar data for humans are lacking. In humans, the activity as well as toxicity appears to be dose related (1, 2). For example, 25–30 mg/kg/day was judged to be adequate and 50 mg/

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