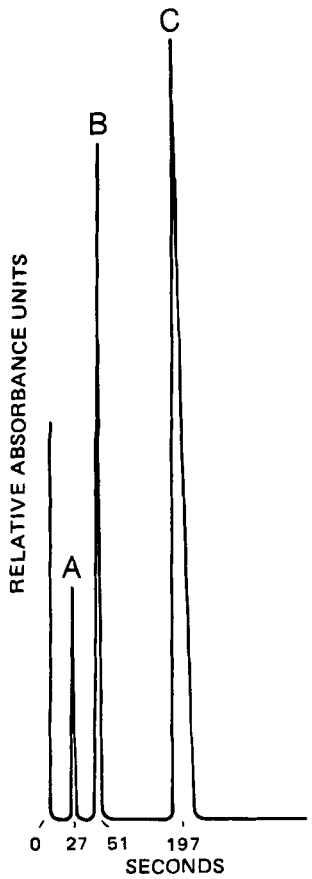

Figure 1—*High-pressure liquid chromatogram for benzoyl peroxide and analogous compounds. Key: A, benzoic acid; B, benzaldehyde; and C, benzoyl peroxide.*

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).

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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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kg/day was associated with toxicity (4). In adults, peak plasma concentrations of 20–40 μg/ml are reached within 2 hr after oral administration of a 2-g dose, and the drug has a biological half-life of 1.5–3.5 hr (1).

BACKGROUND

An increase in susceptibility to erythropoietic depression was associated with a decrease in chloramphenicol clearance from the blood (5). This effect is especially significant in patients with hepatic impairment, because toxicity occurred more frequently in patients with hepatic cirrhosis (5). Therefore, measurements of biological half-lives or steady-state concentrations of chloramphenicol may permit dosage adjustment to prevent serious toxicity (6). The variable bioavailability of different chloramphenicol preparations (7) also can complicate the dosage regimen required for optimal chemotherapy.

General assays for chloramphenicol were reviewed recently (8, 9). Among the methods for biological specimens, GLC procedures (10, 11) probably are superior in specificity and sensitivity. However, the GLC method using electron-capture detection (10) showed nonlinearity of response toward the methylsilyl derivatives in the range of concentrations encountered clinically. The GLC method using flame-ionization detection (11) required multiple solvent extractions and evaporations to separate chloramphenicol from the biological matrixes and also silylation to make the compounds suitable for chromatography. It used 0.5 ml of plasma or serum and more than 1 hr for the assay of each sample (11).

This report describes a high-pressure liquid chromatographic (HPLC) method for the determination of chloramphenicol in plasma.

EXPERIMENTAL

Apparatus—A high-pressure liquid chromatograph[1] equipped with an injector[2], a reversed-phase column[3], and a spectrophotometric detector attached to an attenuator[4] was used for separation and detection of chloramphenicol.

Reagents—Chloramphenicol[5], alcohol USP, acetonitrile[6], and phosphoric acid[7] were used.

Sample Preparation—Aliquots of plasma samples, 0.2 ml (or less), were vortex mixed with 0.5 ml (or 2.5 volumes) of acetonitrile in 13 × 100-mm culture tubes for about 10 sec and then centrifuged at 2000 rpm for 1 min. The supernates were poured into another set of culture tubes, 20-μl aliquots of which were chromatographed. The supernates could also be injected without transfer into new tubes.

Plasma samples for chloramphenicol analyses were obtained from a human subject after oral dosing of 250 mg of chloramphenicol and from an albino rabbit after intravenous injection of 20 mg of chloramphenicol/kg. Pooled human plasma samples supplemented with chloramphenicol in concentrations up to 50 μg/ml also were analyzed to establish calibration curves.

Chromatographic Conditions—The mobile phase consisted of ethanol–dilute phosphoric acid, prepared by mixing 2 volumes of alcohol USP (95% ethanol) and 8 volumes of 0.05% phosphoric acid (pH 2.5 ± 0.01). The ethanol and dilute phosphoric acid were separately filtered through membrane filters[8] before mixing. The flow rate of the mobile phase was 1.5 ml/min.

The column effluent was monitored by UV absorption at 280 nm. The chromatograms were recorded on a potentiometric recorder[9], and peak height measurements were used for quantitation. For all separations, the column was maintained at a constant 30° temperature in an oven.

RESULTS AND DISCUSSION

Figure 1 shows the chromatograms of chloramphenicol in the plasma of a human subject and of a rabbit receiving this antibiotic. Chromatograms of a human plasma blank before and after addition of chloramphenicol are also shown. Notably, all three chromatograms are quite similar. Chloramphenicol has a retention time of 6 min under the chromatographic conditions.

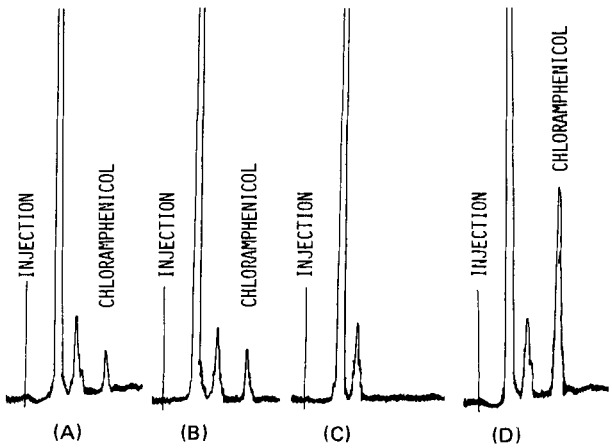

Figure 1—*Chromatograms of chloramphenicol from plasma of a human 1.5 hr after a 250-mg oral dose (A), a rabbit 0.5 hr after 20 mg/kg iv (B), human plasma blank (C), and human plasma supplemented with 35 μg of chloramphenicol/ml (D).*

Plasma samples from rabbits, healthy human subjects, and patients treated with theophylline, procainamide, propranolol, and gentamicin showed no interference with the assay. Plasma samples spiked with salicylates, acetaminophen, phenobarbital, phenytoin, ampicillin, and sulfisoxazole also did not show interference. No peaks that could be assigned to metabolites were observed in chromatograms from plasma drug samples of the human subject and the rabbit.

The major metabolites, the 3-glucuronide and deacylated form of chloramphenicol (1), are more polar than the parent drug. Under the reversed-phase separation conditions, they would probably be eluted early from the column and not interfere with the assay. These metabolites, however, were not investigated in this study.

The linearity of a typical standard curve is summarized in Table I. The response factors (peak height/concentration) were quite constant for all concentrations, even though no internal standard was used. The reproducibility is indicated by a coefficient of variation of 2.24% on replicate analyses of six 20-μg/ml samples. The resolution of separation of the column was good for a prolonged period (longer than 1 year), with occasional cleansing with methanol or dimethyl sulfoxide.

To test the potential use of this HPLC procedure in clinical drug monitoring, plasma samples collected from the rabbit at different times after intravenous chloramphenicol administration were analyzed. Figure 2 shows that the plasma antibiotic concentrations declined rapidly, with a half-life of about 33 min. This biological half-life of chloramphenicol in the rabbit is much shorter than that of 1.5–3.5 hr reported in humans (1).

HPLC has been used for the detection of chloramphenicol and some of its synthetic intermediates in manufacturing (12). However, the application of HPLC to the assay of chloramphenicol in biological specimens was not reported. The proposed method uses acetonitrile to precipitate plasma proteins to obtain a clear supernate after centrifugation. This sample preparation makes the assay fast, simple, and subject to less potential errors. It takes only about 8 min to complete. Since only 20-μl aliquots of the supernate were required for injection, small volumes (10–20 μl) of plasma would be sufficient. Thus, the assay would be particularly valuable for pediatric monitoring.

Table I—Linearity of Calibration Curve of Chloramphenicol

Concentration in Plasma, μg/ml	Peak Height, cm	Response Factor[a]
2.5	0.40	0.160
5.0	0.78	0.156
10.0	1.56	0.156
20.0	3.13[b]	0.157
30.0	4.59	0.153
40.0	6.13	0.153
50.0	7.70	0.154

[a] Response factor = peak height/concentration. [b] Average of six replicate analyses; *SD* = 0.07 cm.

[1] Model 601, Perkin-Elmer Corp., Norwalk, Conn.
[2] Model SVOV-6-IX, Glenco Scientific Co., Houston, Tex.
[3] HC ODS Sil-X-1, Perkin-Elmer Corp., Norwalk, Conn.
[4] Model LC-55, Perkin-Elmer Corp., Norwalk, Conn.
[5] Sigma Chemical Co., St. Louis, Mo.
[6] Burdick & Jackson Laboratory, Muskegon, Mich.
[7] Fisher Scientific Co., Fair Lawn, N.J.
[8] Type HA, 0.45 μm for water; type FH, 0.5 μm for ethanol. Millipore Corp., Bedford, Mass.
[9] Perkin-Elmer Corp., Norwalk, Conn.

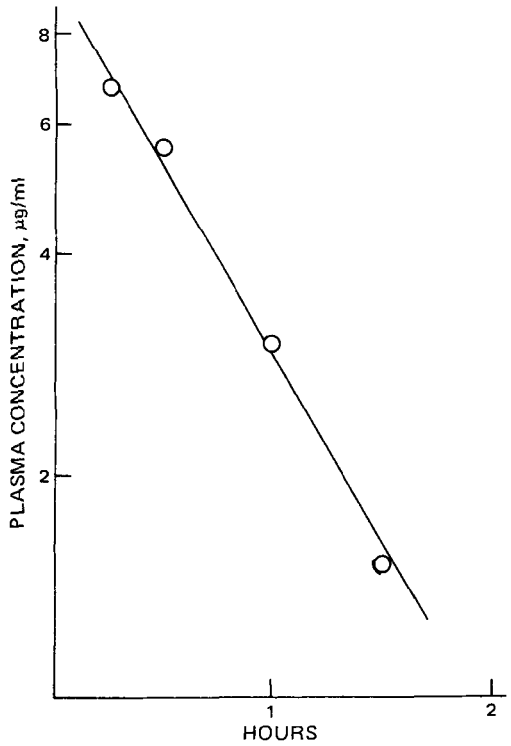

Figure 2—*Plasma concentration profile of chloramphenicol in a rabbit after 20 mg/kg iv.*

The low limit of accurate quantitative measurement of chloramphenicol was about 2.5 μg/ml in plasma when the detector sensitivity was set at 0.004 absorbance unit for full-scale deflection by use of the attenuator. Chloramphenicol at the 0.5-μg/ml level could be detected by the enhancement of the sensitivity to 0.0029 absorbance unit for full-scale deflection. Sample preparation by solvent extraction can improve the low limit of accurate measurement greatly. Sensitivity by solvent extraction depends on the sample size and the volume fraction of the reconstituted solution of the extract injected. For example, by extracting 0.2 ml of plasma with 2.5 ml of ethyl acetate and injecting 20 μl of the 50-μl reconstituted solution of residue from a 2.0-ml aliquot of the organic extract, the low limit of accurate quantitation could be as low as 0.5 μg/ml.

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Perfluorooctyl Bromide Concentration in Plasma and Tissues of Beagle Dogs

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Received August 22, 1977, from the *Department of Drug Metabolism and Disposition, Sterling-Winthrop Research Institute, Rensselaer, NY 12144.* Accepted for publication November 10, 1977.

Abstract □ Plasma levels were determined frequently after single doses of perfluorooctyl bromide were administered to beagle dogs at doses of either 30.8 g/kg po or 3.9 g/kg intratracheally. The apparent first-order half-life during the terminal elimination phase was about 1 day after oral medication and about 7 days after intratracheal administration. Analysis of tissues revealed the highest concentrations of the compound in abdominal fat of dogs autopsied 4 weeks later.

Keyphrases □ Perfluorooctyl bromide—tissue distribution in dogs after oral and intratracheal administration □ Distribution, tissue—perfluorooctyl bromide in dogs after oral and intratracheal administration □ Fluorocarbons—perfluorooctyl bromide, tissue distribution in dogs after oral and intratracheal administration

Liquid fluorocarbon studies provided the stimulus for the development of radiopaque perfluorocarbon compounds (1). The most effective compound tested was perfluorooctyl bromide (I), $C_8F_{17}Br$ (2). Toxicity and efficacy studies indicated a low toxicity and satisfactory radiographic density (3). Concentrated oil-in-water emulsions were found useful for bronchographic examinations in humans and animals (4). The use of I as a diagnostic contrast medium for gastroenterography of laboratory animals was reported (5). Recently, I was reported as a potential antiobesity agent (6).

Some data on the disposition of I after intratracheal administration to rats, dogs, and human subjects were reported (7); the sensitivity of the method did not allow quantitation of I in blood. This report describes studies on the plasma and tissue I concentrations in beagle dogs after oral and intratracheal administrations.

EXPERIMENTAL

Animal Procedure—Following an overnight fast, two groups of beagle dogs of both sexes (Tables I and II) received either a 30.8-g/kg po dose

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