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Note

# High-performance liquid chromatographic micro-assay for chloramphenicol in human blood plasma and cerebrospinal fluid

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Chloramphenicol (CAP) is an effective antibiotic whose use may be expected to increase [1]. The drug is associated with serious toxic side effects [2]. Some of these side effects and the therapeutic efficacy seem to be related to the circulating concentrations of the drug, and it is desirable, therefore, to monitor its concentration in biological fluids during therapy [3, 4].

Several different methods have been used in the analysis of chloramphenicol in biological fluids [5, 6], but high-performance liquid chromatography (HPLC) promises to be the method of choice [1]. However, the published HPLC procedures for the determination of CAP [1, 5, 7, 8], with one exception [7], do not use an internal standard, and rely instead on accurate volume transfers and on comparing the peak height of CAP to those of authentic standards for quantification. This is clearly a severe disadvantage in a high-volume routine clinical laboratory. A relatively large sample [0.5 ml] of plasma or serum is required in two of the published methods [1, 5], a serious limitation in pediatric work. The published method [7] which does use an internal standard also suffers from some disadvantages; the procedure involves extraction of the drug with an organic solvent followed by evaporation of the latter. The authors caution against heating during the evaporation, although no reason is given for this limitation. A variable-wavelength detector at 278 nm is used in the assay, and the procedure is lengthy.

The purpose of this communication is to describe a simple and rapid HPLC method for the determination of chloramphenicol in small-volume samples.

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## EXPERIMENTAL

### *Chemicals*

CAP was obtained from Calbiochem (Los Angeles, Calif., U.S.A.). *threo*-2-Amino-1-(*p*-nitrophenyl)-1,3-propanediol and N-(β-hydroxy-*p*-nitrophenethyl)-acetamide were purchased from the ABC Library of Rare Chemicals, Aldrich (Milwaukee, Wisc., U.S.A.). Gentamicin sulfate and ampicillin were obtained from Sigma (St. Louis, Mo., U.S.A.). Chloramphenicol sodium succinate (Parke, Davis & Co., Ann Arbor, Mich., U.S.A.) and penicillin G (Squibb, Princeton, N.J., U.S.A.) were provided by the pharmacy of the University of Colorado Medical Center.

An aqueous standard solution of CAP was prepared containing 30 μg/ml CAP. Methanol and acetonitrile, glass-distilled grade, were obtained from Burdick & Jackson Labs., Muskegon, Mich., U.S.A. Ammonium phosphate, monobasic, was Baker-Analyzed reagent grade.

### *HPLC conditions*

A Waters Assoc. HPLC system consisting of a Model U6K injector, a Model 6000A pump, and a Model 440 detector (254 nm) was used. The mobile phase was 20% methanol in water, 0.01 *M* in monobasic ammonium phosphate adjusted to pH 3 with concentrated hydrochloric acid. The mobile phase was pumped at 2.5 ml/min. The column was a Waters Assoc. μBondapak $C_{18}$, particle size 10 μm, 25 cm × 4 mm I.D. The retention times were: CAP, 8.8 min; internal standard, 5.2 min.

### *Assay procedure*

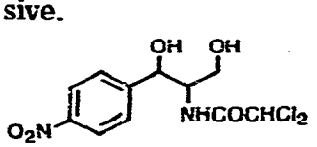
Aliquots of the standard, plasma, serum or cerebrospinal fluid, 50 μl, were placed in polypropylene 400-μl microcentrifuge tubes. Acetonitrile, 50 μl, containing 5 μg of the internal standard was added, the mixture was vortex mixed, and then centrifuged at 1500 *g* for 1 min. The supernatant (20—30 μl) was injected into the HPLC instrument with the detector set at 0.01 a.u.f.s.

### *Calculations*

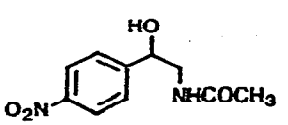
The concentration of CAP in the sample was obtained by comparing the CAP:internal standard peak height ratio from the sample to that of the 30 μg/ml aqueous CAP standard carried through the procedure.

## RESULTS AND DISCUSSION

After evaluating several candidates, N-(β-hydroxy-*p*-nitrophenethyl)acetamide (I) was selected to serve as internal standard in the assay. Compound I is closely related in chemical structure to CAP, has a suitable retention time under the chromatographic conditions used, is readily available, and is inexpensive.




CAP

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While CAP has an absorption maximum at 278 nm [9], the 254-nm fixed-wavelength detector in use in our laboratory was found suitable for the assay. It is also advantageous when using the same HPLC instrument for the analysis of several different drugs to use the same mobile phase if possible. This is because re-equilibration may take a relatively long time when mobile phases are changed. Therefore, the mobile phase selected for the determination of CAP is the same used in the analysis of other drugs (e.g., theophylline and acetaminophen) by HPLC in our laboratory.

In the procedure 50 $\mu$l of sample is treated with 50 $\mu$l of acetonitrile containing the internal standard. After brief centrifugation to separate the precipitated proteins, an aliquot of the supernatant is injected into the HPLC system.

Fig. 1a shows the chromatogram of the internal standard and authentic chloramphenicol. Plasma samples from individuals not exposed to the drug were carried through the procedure with the slight modification that the acetonitrile added to the sample did not contain the internal standard. Chromatograms obtained in this fashion (Fig. 1b) showed no interference from endogenous compounds. Samples from patients on chloramphenicol therapy were also examined in the above manner, i.e., without internal standard, in order to ascertain that no metabolite of chloramphenicol interfered with the chromatography of the internal standard. No such interference was found. Fig. 1c shows a typical chromatogram obtained upon analysis of the plasma of a patient on chloramphenicol therapy. The procedure is suitable for the analysis of the drug in blood plasma, serum, and cerebrospinal fluid.

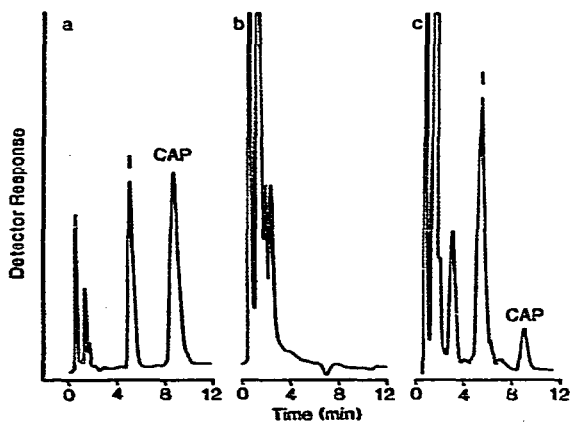


Fig. 1. (a) Chromatogram of authentic CAP and compound I. (b) Typical chromatogram obtained upon analysis of human plasma sample from an individual not exposed to CAP. Acetonitrile not containing internal standard was used to precipitate proteins. (c) Chromatogram obtained upon analysis of plasma from patient on CAP therapy. Concentration of CAP, 4.0 $\mu$g/ml. See Experimental for assay and chromatographic conditions.

Potential interference from several substances was examined. Chloramphenicol succinate, a frequently used dosage form of CAP, had a retention ca. 10 min longer than that of CAP and thus does not interfere. The N-deacylated derivative of CAP, *threo*-2-amino-1-(*p*-nitrophenyl)-1,3-propanediol, a major metabolite of CAP in man [9], had a retention time of 2 min, and therefore

does not interfere. The other major metabolite of CAP, a glucuronide conjugate [9], was not investigated, but in view of its polar nature, this conjugate would be expected to have a very short retention time under the reversed-phase conditions used. In addition, the plasma concentrations of such a metabolite may be very low. At any rate, no interference from metabolites was observed, as noted above.

Potential interference from other drugs was also investigated. Gentamicin, ampicillin, penicillin, theophylline, acetaminophen and salicylate were studied and were found not to interfere. Since some patients on CAP are sometimes administered anticonvulsant drugs for seizure disorders [10, 11] we also examined this class of drugs. Phenobarbital, phenytoin, primidone and cabamazepine were studied and were found not to interfere.

The assay procedure was developed for, and is linear in, the 1—60 μg/ml CAP concentration range. For routine use of the assay a single-point aqueous standard was found suitable.

The small sample size makes this procedure highly suitable for pediatric samples. The assay is rapid, and a sample can be carried through the procedure in 15 min.

Reproducibility was examined using nine replicate samples of pooled human plasma containing 10 μg/ml CAP. The mean value found was 9.0 μg/ml, with a coefficient of variation of 6.8%.

In conclusion, a rapid and simple microassay for CAP has been presented. The procedure uses the common 254-nm detector in an HPLC system under conditions also suitable for the determination of theophylline and acetaminophen.

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