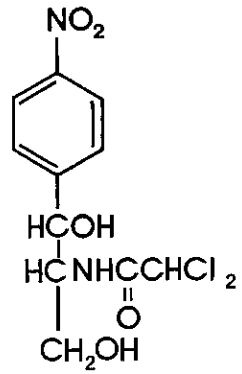
CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Chloramphenicol

Chloramphenicol, a broad-spectrum antibiotic, was originally derived from a Venezuelan strain of *Streptomyces*, and is partly responsible for bringing typhoid fever under control since the end of World War II. More than fifty million people have been treated with this drug in the past four decades. Many analogs of chloramphenicol have been synthesized, leading to the observation that the nitro group in the para position on the phenyl ring is essential to the antibacterial activity of this drug.



Measurement of plasma chloramphenicol after dosing is essential for several reasons. High plasma concentrations may be responsible for such toxic side effects as aplastic anemia and reversible bone-marrow suppression. The drug is primarily inactivated in the liver by glucuronidation and cleavage of the amide bond, but these processes are highly dependent on the individual metabolism of the patient. Poor correlation exists between the prescribed dosage and the resultant plasma concentration. Most susceptible bacteria demonstrate inhibition by plasma levels in the 5-15 mg/L range.

Several procedures have been developed for the assay of plasma chloramphenicol, including gas chromatography, fluorescence, colorimetry, polarography, and enzymatic assays. More recently, modern liquid chromatographic techniques have proved popular. These are usually based on reverse phase separations followed by ultraviolet detection at 254 or 280 nm [1-3]. Since therapeutic concentrations are high, sample preparation is fairly simple, often involving simply mixing equal volumes of plasma and diluent. The latter often contains a solvent (such as CH_3CN) and an internal standard. A portion of the resulting spun-down, diluted mixture is directly injected.

Since chloramphenicol is easily reduced at a mercury film electrode, liquid chromatography/electrochemistry serves as a useful alternative to LCUV schemes. The electrochemical reaction proceeds as follows:

$$R\text{-}NO_2 + 4e^- + 4H^+ \rightarrow RNHOH + H_2O$$

As there are very few reducible compounds found in blood naturally, the reductive LCEC assay should be highly selective for exogenous drugs such as chloramphenicol.

Proposed Method

Deproteinized plasma supernatant is extracted with ether using Clin-Elut® extraction tubes. The ether is evaporated, the residue dissolved in methanol/H_2O, and the extract then injected into the LC.

Procedure

1. Add 50 µL 4 M $HClO_4$ to 1 mL of Plasma. Centrifuge 5 min at 1000 x g.

2. Load 300 µL of the supernatant into a Clin-Elut® extraction tube (300 µL capacity, p/n CE 1000 M, from Analytichem International Inc., Harbor City, CA).

3. Wait 2 minutes.

4. Elute the chloramphenicol with 3 mL of diethyl ether. Collect the eluent.

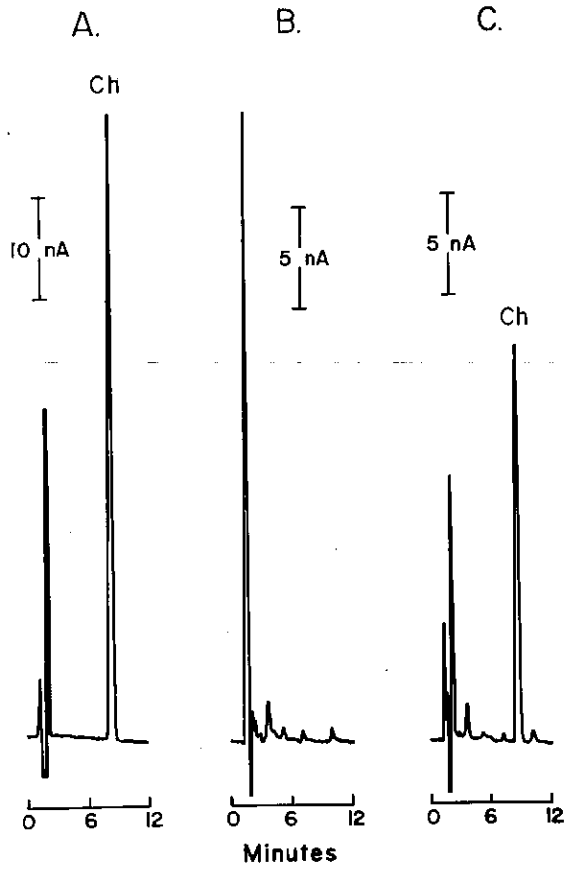


Figure 1. (A) Chromatogram of 295 pmole chloramphenicol. (B) Unspiked drug-free plasma blank from healthy subject. (C) Same plasma spiked with 0.7 µg/mL chloramphenicol.

5. Evaporate the ether to dryness under nitrogen at 40-45°C and redissolve the residue in 300 µL of 20% ethanol/H_2O mixture.

6. Inject 50 µL of the mixture into the LC.

Chromatographic Conditions

Liquid Chromatograph: LC-304 or LC-154 (BAS) modified for mobile phase degassing for reductive LCEC.[4] The more modern BAS 200 instrument includes all the features needed.

Mobile Phase: 0.020 M monochloroacetic acid, 0.001 M Na_2EDTA, 0.0146M sodium acetate (92%) and n-propanol (8%). Degassing was accomplished by refluxing.

Stationary Phase: Biophase ODS 5µm (p/n 6017, BAS).

Flow rate: 2 mL/min

Detector: Hg/Au (TL-6A), -0.85 V vs. Ag/AgCl.

References

1. R.L. Thies and L.J. Fischer, Clin Chem., 24(1978) 778.

2. G.W. Peng, M.A.F. Gadalla, and W.L. Chiou, J. Pharm. Sci., 67(1978) 1036-1038.

3. S.H. Petersdorf, V.A. Raisys, and K.E. Opheim, Clin. Chem., 25(1979) 1300-1302.

4. LC-4A Manual, Bioanalytical Systems Inc., West Lafayette, IN 1980, section 6.