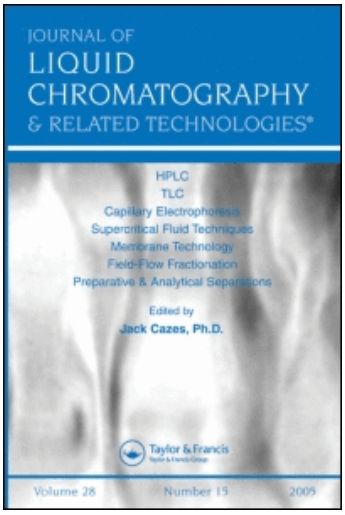


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# One Step Assay for Serum Chloramphenicol by HPLC

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# ONE STEP ASSAY FOR SERUM CHLORAMPHENICOL BY HPLC

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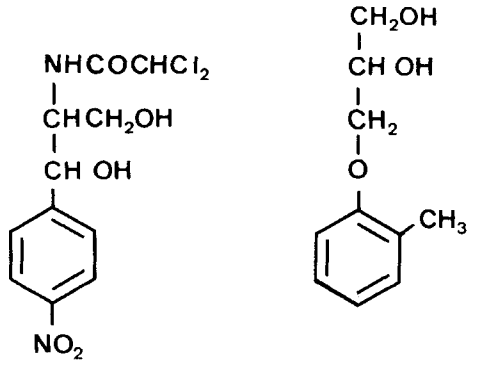
Because of the ability of chloramphenicol to provoke concentration-related adverse effects, it is recommended that regular blood estimations should be made during treatment of all patients(1). Analysis of chloramphenicol by HPLC has particular advantages to offer such as low sample volume requirement and good specificity.

Sample preparation techniques appear to be of two main types: (a) multi-step procedures involving sample extraction of chloramphenicol into diethyl ether (2,3), methylene chloride (4) or ethyl acetate (5), or (b) those involving direct sample protein precipitation by acetonitrile (6-8) or methanol (9). Since extraction methods are more time-consuming than the simpler direct precipitation techniques and since acetonitrile is possibly more toxic and certainly more expensive than methanol, the latter reagent was selected. However a previously described technique which employed this approach did not incorporate an internal standard and used a methanol to sample volume ratio which was too low. It therefore required an additional filtration step in order to obtain a particle-free solution (9). By increasing the methanol to sample volume ratio and including mephenesin as internal standard, a simpler one-step assay was produced. The structures and




CHLORAMPHENICOL λmax 278nm

MEPHENESIN λmax 277nm

FIGURE 1 Structure of chloramphenicol and the mephenesin internal standard



peak absorbances of chloramphenicol and the mephenesin internal standard are given in figure 1.

The equipment, from Waters Associates; included a Model 6000A solvent delivery system, a U6K injector and a Model 450 variable wavelength detector. The column used was a C18 5 um Radial-Pak™ cartridge for use with a Waters Radial Compression Module. The pump flow rate is set at 2.0 ml/min, and the column effluent is monitored at 278 nm using a sensitivity range of 0 to 0.02A full scale.

A 30 mg/l standard solution is prepared in chloramphenicol-free serum. 200 ul of serum, standard or control is mixed with 1.0 ml of internal standard solution (mephenesin, 30 mg/l in methanol). After mixing and centrifugation, 25 ul of clear supernate is injected on to the column.

Figure 2 shows a typical chromatogram of a patient's serum. Concentration and peak height ratio are linearly related to at least 50 mg/l. Analytical recovery of chloramphenicol added to drug-free serum ranged from 96.5 to 101.25%. Precision was acceptable (within and between batch CVs at 20 mg/l, 2.9 and 3.7% respectively.) Comparison

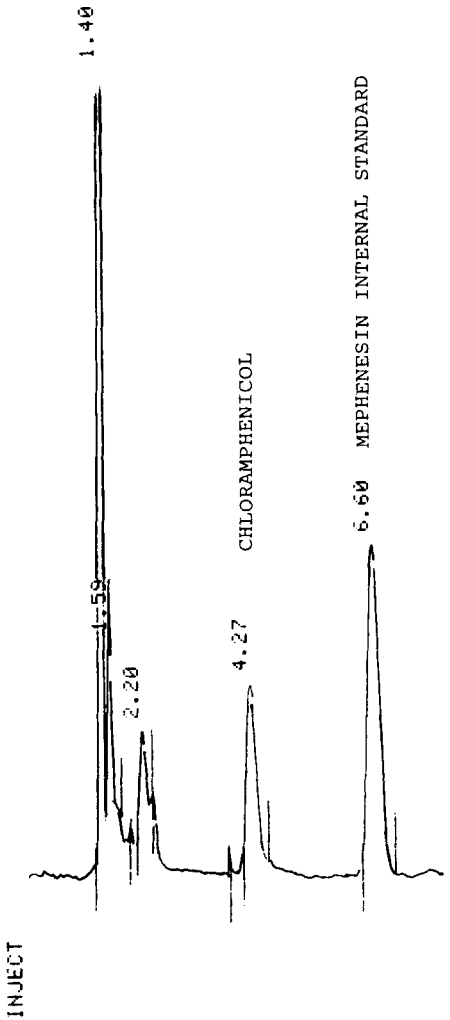



FIGURE 2 A typical chromatogram from a methanol precipitated serum sample

of values by the present method with the target values for the American Association for Clinical Chemistry therapeutic drug monitoring control over sixteen months, by least squares linear regression analysis showed, slope = 1.089, intercept ■ 0.273, r = 0.998. There was no observable interference in these samples; in any of various commercial control sera which contain a wide variety of other drugs; or in any patients samples.

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