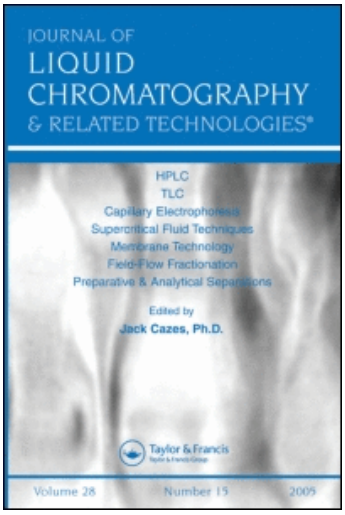

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Determination of Serum Chloramphenicol by High Performance Liquid Chromatography

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DETERMINATION OF SERUM CHLORAMPHENICOL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

by

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ABSTRACT

A simple single extraction procedure for the analysis of serum chloramphenicol levels by high performance liquid chromatography (HPLC) is described. Serum is mixed with buffer and extracted with ether, which is then evaporated. The residue is dissolved in the eluting solvent and analyzed on a reverse-phase column. The eluting solvent is methanol/distilled water (50/50, V/V) and the effluent is monitored at 280 nm. Serum samples as small as 50 µl can be used.

INTRODUCTION

High performance liquid chromatography (HPLC) is establishing itself as a clinical tool for the quantitation of drugs in biological fluids. The simplicity of sample preparation and the fact that, in many cases, the drug can be analyzed without synthesizing a derivative makes HPLC a rapid method of analyses. Chloramphenicol, while it is an effective antibiotic, does have several serious side effects (1). Thus, in those patients receiving the drug there is a necessity to monitor its concentration in the serum. The procedure described here can be carried out in less than one hour and is capable of measuring less than 1 µg of chloramphenicol per sample.

MATERIALS AND METHODS

Instrumentation

The chromatograph was a Model 110 pump and a Model 7120 Rheodyne sample injector. (The Anspec Co., Ann Arbor, Michigan). A Model UA-5 dual beam multiple wavelength dector, with 5 µl cells (Instrument Specialties Co., Lincoln, NB.), was used at a sensitivity setting of 0.10 and a wavelength of 280 nm. The column was a prepacked Partisil PXS 10/25 ODS-2 (Whatman, Clifton, NJ). A 10" recorder (Linear Instruments, Irvine, Ca.) was used at a 100 mv setting.

Reagents

Diethylether and methanol-analytical grade.

Mephenesin (3-(2-methylphenoxy)-propane-1, 2-diol) and chloramphinicol-obtained from Sigma Chemical Co., St. Louis, MO. Both were pure based on HPLC.

Bromocresol green-prepared by dissolving 0.1 g in 250 ml of distilled water containing 1.43 ml of 0.1N NaOH.

Tris buffer-prepared by dissolving 12.11 g of Tris (hydroxymethyl) aminomethane in 1 liter distilled water and adjusting the pH to 10.4 with 0.8N NaOH.

Internal Standard-prepared by dissolving mephenesin in methanol to a concentration of 0.2 mg/ml.

Mobile phase-methanol/distilled water (50/50, V/V), degassed under vacuum.

Procedure

The extraction procedure is a modification of that suggested by Thies and Fischer (2). The experimental sample (200 µl) is placed in a 5 ml Mini-Vial (The Anspec Co. Inc., Ann Arbor, MI). The mephenesin internal standard (20 ug) and 100 µl of Bromocresol green solution are added. The addition of Bromocresol green makes it easier

to define the aqueous phase and does not interfer with the assay. The Tris buffer (1 ml) is added and the contents of each Mini-Vial mixed, followed by the addition of 2 ml of diethyl ether. Each Mini-Vial is then shaken vigorously for 1 minute and centrifuged at 3000 rpm for 3 minutes. Following centrifugation the lower, blue, aqueous phase is aspirated away. This is accomplished using a disposable Pasteur pipette attached to rubber tubing, which can be squeezed to stop aspiration. We routinely let a small amount of the upper ether phase get into the pipette to ensure complete removal of the aqueous phase. Since the calculations are based on peak height ratios to the mephenesin internal standard this slight loss of the ether phase is of no consequence. The remaining ether phase is then evaporated at 35°C under a stream of nitrogen. The residue is dissolved in 25 µl of mobile phase and duplicate 10 µl aliquots are injected into the chromatograph. The mobile phase of methanol/distilled water (50/50, V/V) is delivered at a flow rate of 2 ml/min.

For routine daily analysis of plasma samples we use two control serum samples (a high and a low) prepared by adding chloramphenicol to pooled samples of serum previously found to be free of the drug or any interfering ether soluble substances. The high level sample containing 55 mg/L has given a peak height ratio (chloramphenicol/mephenesin) of 4.49 ± 0.31 (S.D., n = 25) and the low level sample containing 13.6 mg/L has given a peak height ratio of 1.03 ± 0.02 (S.D., n = 25). We have found no change in these ratios over a 2 month period with storage at 5°C.

RESULTS AND DISCUSSION

Typical HPLC chromatograms obtained by our procedure are shown in Figure 1. As can be seen good separation is achieved between

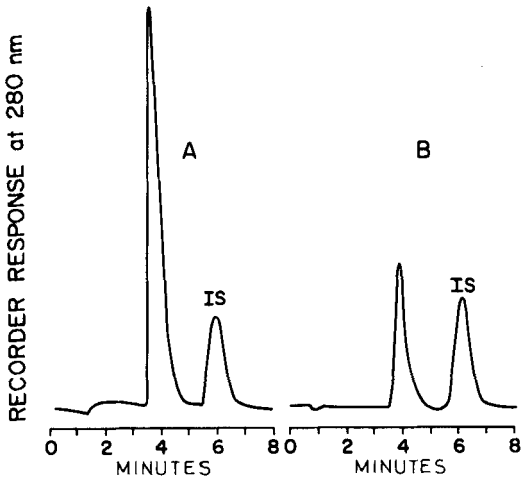

FIGURE 1

Typical chromatograms showing the separation of chloramphenicol and mephenesin internal standard (IS). The chromatogram in A is from a 55 mg/L prepared control serum. Chromatogram B was obtained from the serum of a patient under drug therapy.

chloramphenicol (elution time 4 minutes) and mephenesin (elution time 6.5 minutes). Figure B is a typical serum sample and shows the absence of any interfering substances. For comparison, the level of chloramphenicol in the sample in Figure 1B was calculated to be 15.7 mg/L.

Figure 2 is a typical standard curve and shows the linearity obtained by our procedure. One can extend the sensitivity into the ng level by adjusting the detector sensitivity range or changing the recorder mv range. However, the range of the standard curve given in Figure 2 is adequate for routine clinical samples.

We have assessed the recovery of chloramphenicol by comparing peak height ratios of standard solutions of chloramphenicol and

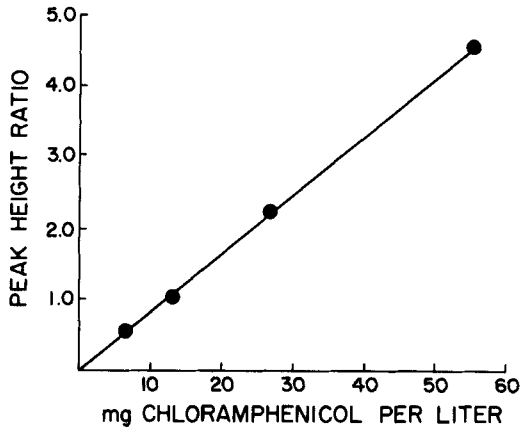

FIGURE 2

Plot showing the linearity of peak height ratio of chloramphenicol to mephenesin versus the concentration of chloramphenicol.

mephenesin in methanol/water (50/50, V/V) injected directly into the chromatograph to peak height ratios obtained on the same samples following the use of our procedure. The recoveries were 96.9 ± 0.2 (S.D., n = 6) and 101.2 ± 0.1 (S.D., n = 6) for samples of 10 mg/L and 50 mg/L respectively.

The assay described here can conveniently be scaled down to as little as 50 µl of sample, thus making it quite applicable to pediatric patients.

Others (2) have shown that the buffer used in this assay does not release chloramphenicol from its glucuronide conjugate which would result in erroneously high values. These same workers have also shown that no interfering metabolites of chloramphenicol are formed.

Other methods of assay for chloramphenicol have been published. These include microbiological (3,4) colorimetric (5,6) enzymatic (7),

fluorometric (8) and gas chromatographic (9). However, these methods either lack specificity or are time consuming.

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