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Note

Simultaneous determination of chloramphenicol and chloramphenicol succinate in plasma using high-performance liquid chromatography

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Chloramphenicol (I) is a drug of choise in the treatment of serious or life-threatening infections resulting from ampicillin-resistant *Haemophilus influenzae* [1], particularly in infants and children. Because of the potential toxicity of chloramphenicol, however, the need to monitor plasma levels of the drug is clearly indicated [2—4]. The sodium salt of chloramphenicol-3-monosuccinate (II), an ester prodrug of I, is soluble in water and is used clinically for intravenous administration. This ester is primarily hydrolyzed in the liver [5, 6] to I and the rate of hydrolysis may play an important role in accumulation and toxicity of chloramphenicol. The analytical work described herein was undertaken to devise a procedure for the simultaneous measurement of I and II in plasma.

Chloramphenicol succinate exists in solution as an equilibrium mixture of II and chloramphenicol-1-monosuccinate (III) [7]. A high-performance liquid chromatographic (HPLC) method for I, II and III has recently been published [8] and involves initial precipitation of plasma proteins with trichloroacetic acid followed by direct injection of the supernatant. This procedure, which is similar to another recently pubished method [9], however, can affect column life adversely due to the build-up of solids on the HPLC column. One of the other three published HPLC procedures for I and II does not provide adequate information about accuracy and precision [10] while the second requires elevated column temperature [11] and the third [12] affords poor absolute recoveries of I and II. Furthermore, all of the above noted methods (except the one in ref. 9) use internal standards that are structurally dissimilar to I and II.

There is some evidence in the literature that thiamphenicol (IV) is useful as an internal standard; however, the one method employing it only permits esti-

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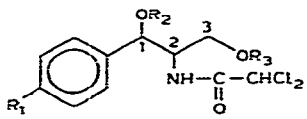
mation of I [13]. The presently reported procedure incorporates advantages of all the published methods and possesses requisite accuracy and precision [14] for reliable determinations of therapeutically relevant concentrations of I and II (i.e. as a summation of II and III) in plasma from pediatric patients. Moreover, the chromatographic interference by five drugs commonly co-prescribed with I and/or II has been tested and found to be negligible.



I; $R_1 = NO_2$, R_2 and $R_3 = H$
II; $R_1 = NO_2$, $R_2 = H$, $R_3 = COCH_2CH_2CO_2H$
III; $R_1 = NO_2$, $R_2 = COCH_2CH_2CO_2H$, $R_3 = H$
IV; $R_1 = SO_2CH_3$, R_2 and $R_3 = H$

MATERIALS AND METHODS

HPLC

The HPLC system used throughout consisted of a Waters Model 6000A pump and U6K injector, Tracor 970A variable-wavelength UV—Vis detector and Altex integrator (Model C-RIA). Analyses were performed with a Waters Assoc. (Milford, MA, U.S.A.) μBondapak C_{18} column (30 cm × 4 mm I.D., 10 μm particle size). The mobile phase was 20% acetonitrile in 0.05 *M* sodium acetate buffer adjusted to pH 5.3. The buffer was filtered through 0.2-μm Whatman GF/F glass fibre filters and the mobile phase was degassed by ultrasonication. The flow-rate was set at 1.5 ml/min. The monochromator was adjusted to 278 nm.

Materials

The solvents used in the HPLC separation were distilled-in-glass grade. All other chemicals were reagent-grade or better. Thiamphenicol, chloramphenicol and chloramphenicol-3-monosuccinate were obtained from Warner Lambert (Ann Arbor, MI, U.S.A.). These reference standards were found to be homogeneous by HPLC and were used without further purification.

Plasma extraction

All the glassware used was silylated with 2% trimethylchlorosilane in toluene, washed with acetone and finally rinsed with distilled deionized water. Blank plasma was obtained from a blood bank and was spiked with I and II to produce final concentrations as indicated below.

A 100-μl portion of 1 *M* sodium acetate buffer (pH 4.6), 50 μl of standard or patient plasma, and 50 μl of thiamphenicol solution (1 mg/ml) in water, were added to a 12-ml screw-capped culture tube and vortexed for 5 sec. One ml of ethyl acetate was added, the tube was capped and vortexed for 10 sec at maximum speed using a Vortex Genie (Scientific Products, McGaw Park, IL, U.S.A.). The samples were centrifuged at 1000 *g* for 10 min, the ethyl acetate layer was separated and evaporated to dryness at ambient temperature with air

using a Brinkmann SC/27R Sample Concentrator (Brinkmann Instruments, Westbury, NY, U.S.A.). The sides of the concentrating tubes were washed with 0.2-ml portions of ethyl acetate during the process and the residues were reconstituted with 100-μl portions of mobile phase and injected.

Extraction recovery

The completeness of extraction of I, II, and IV from plasma using ethyl acetate was examined. Six plasma samples having 10 μg/ml of I and II and 50 μg/ml of IV were extracted as described in the extraction procedure. The peak areas were compared to those resulting from direct injection of standard solutions of I, II and IV which were prepared in mobile phase.

Plasma standard curve

Separate plasma samples containing 2, 5, 10, 20, 30 and 50 μg/ml concentrations of I and II were extracted and submitted to HPLC as indicated above. Each concentration was analyzed in triplicate on each day. Peak area ratios (drug/internal standard) were regressed against the plasma concentrations of I and II. Chloramphenicol succinate was estimated as total succinate by adding the areas under the peaks for chloramphenicol-1-monosuccinate and chloramphenicol-3-monosuccinate.

Accuracy and precision of assay

Spiked plasma samples (3, 7.5, 15, 25, 45 μg/ml each of I and II) were extracted and analyzed in replicates of six, as described above. Using the standard curve constructed on the day of analysis, concentrations of I and II were calculated. The means and standard deviations of these values were calculated.

Analysis of patient plasma samples

Three pediatric patients with symptoms of meningitis were given II by intravenous infusion and four blood samples were drawn from each patient within a 6-h interval after the infusions were completed. Plasma was separated and frozen (−4°C) until analyzed. Samples were only drawn from patients for whom parental consent was obtained using a protocol and consent procedure approved by the University of Texas at Austin Institutional Review Board.

RESULTS AND DISCUSSION

A chromatogram resulting from the HPLC analysis of a pediatric plasma sample obtained following intravenous infusion of chloramphenicol-3-monosuccinate is depicted in Fig. 1. The HPLC system described herein provided good resolution of the internal standard, thiamphenicol, chloramphenicol and its succinate ester which exists as an equilibrium mixture of isomers [7]. No interferences were observed for the drugs or internal standard in blank plasma extracts.

The absolute recovery of IV from plasma using ethyl acetate as an extraction solvent was 93.4 ± 5.4% (n=6) while I and II were recovered to the extent of 85.7 ± 6.4% (n=6) and 87.4 ± 6.0% (n=6), respectively. Calibration curves for I

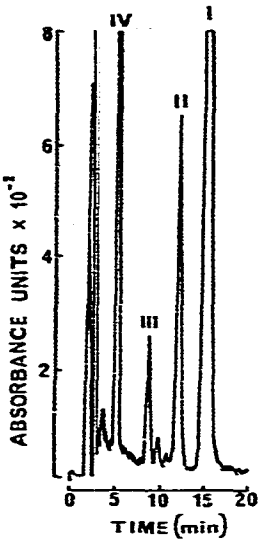


Fig. 1. HPLC chromatogram of ethyl acetate extract of pediatric patient sample obtained after intravenous infusion of chloramphenicol-3-monosuccinate and spiked with internal standard. Development is on a 30 cm x 4 mm I.D. μBondapak C_{18} column eluted at 1.5 ml/min with 20% acetonitrile in 0.05 *M* acetate buffer (pH 5.3). UV detector set at 278 nm. Peaks: I = chloramphenicol; II = chloramphenicol-3-monosuccinate; III = chloramphenicol-1-monosuccinate; IV = thiamphenicol, internal standard.

and II were consistently linear ($r \geqslant 0.999$) over the concentration range of 2—50 μg/ml.

The results of replicate analyses of spiked plasma samples are given in Table I. The concentration values determined were very close to the known concentrations, thus, indicating a high degree of accuracy. Precision values were measured by the calculated relative standard deviations and were within an acceptable range. Minimum detectable plasma concentrations were about 0.5 μg/ml for I and 1.0 μg/ml for II (signal-to-noise ratio = 5). More acceptable lower limits for the procedure are 2 μg/ml for II and 1 μg/ml for I (R.S.D. $\leqslant \pm$ 10%). Attempts to measure levels of I below 1.0 μg/ml and II below 2.0 μg/ml resulted in larger relative standard deviations ($\geqslant \pm$ 20%).

TABLE I

ACCURACY AND PRECISION OF HPLC ASSAY FOR CHLORAMPHENICOL AND CHLORAMPHENICOL SUCCINATE IN PLASMA

Concentration of chloramphenicol and chloramphenicol succinate prepared (μg/ml)	Recovery (% ± S.D.)*	
	Chloramphenicol	Chloramphenicol succinate**
3.0	106.7 (3.9)	102.4 (3.9)
7.5	101.7 (1.5)	100.2 (2.2)
15.0	103.5 (1.8)	102.9 (2.9)
25.0	100.9 (2.1)	99.0 (2.0)
45.0	99.9 (1.2)	100.7 (1.6)

* n = 6.

** Chloramphenicol succinate measured as summation of peaks for chloramphenicol-1-monosuccinate and chloramphenicol-3-monosuccinate.

TABLE II

HALF-LIVES OF CHLORAMPHENICOL AND CHLORAMPHENICOL SUCCINATE IN PEDIATRIC PATIENTS AS DETERMINED BY HPLC

Patient No.	Age (months)	Half-life of elimination (h^{-1})	
		Chloramphenicol	Chloramphenicol monosuccinate
1	11	7.5	0.4
2	20	5.3	0.2
3	35	2.9	0.6

Three pediatric patients were administered II by intravenous infusion and blood samples were drawn at different times. Resulting plasma samples were analyzed for I and II. The half-lives of these compounds as determined from the patient data are presented in Table II. The calculated half-lives are comparable to those reported previously in the literature [15, 16].

A selection of drugs that are most commonly co-administered with chloramphenicol in pediatric populations were tested for potential interference in the developed assay. The drugs tested were aspirin, acetaminophen, ampicillin, gentamicin and phenobarbital, all at therapeutically relevant concentrations. None of the additional drugs showed peaks which would interfere with chloramphenicol, its succinate, and the internal standard, thiamphenicol.

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