

Rapid Monitoring of Chloramphenicol by HPLC

Therapeutic testing for chloramphenicol in infants and children is critical in assuring safe levels in blood. There is a narrow margin between therapeutic and toxic ranges, and quick, convenient testing is a necessity. SUPELCOSM LC-8-DB HPLC columns can perform efficiently and accurately.

Key Words:

- antibiotic
- chloramphenicol
- HPLC
- therapeutic drug monitoring

Chloramphenicol is a broad spectrum antibiotic frequently used to treat infants and children for ampicillin-resistant *H. influenza* infections. The therapeutic level of chloramphenicol in blood is 10-20mg/liter. Sustained levels above 25mg/liter have produced anemia, while levels above 50mg/liter have been associated with cardiovascular collapse. The narrow margin between therapeutic and toxic ranges of this drug necessitates that it be monitored accurately. Variations in chloramphenicol metabolism (in the livers of newborns, for instance) may be significant.

Chloramphenicol analyses for therapeutic monitoring may be conveniently and rapidly carried out by HPLC, using a 5cm SUPELCOSM LC-8-DB column. Chloramphenicol is usually administered as chloramphenicol monosuccinate (Figure A). The monosuccinate persists in the blood stream for a short time and may account for 30% of the drug that is excreted in the urine. Consequently, a liquid chromatographic method for analyzing chloramphenicol should also resolve chloramphenicol monosuccinate well (1). The monosuccinate, the active drug, and the internal standard suggested by Gal *et al.* (2) are readily separated in about seven minutes on a 5cm x 4.6mm SUPELCOSM LC-8-DB column (Figure B). When sufficient time has elapsed between drug administration and sampling, the monosuccinate is no longer in the blood and the chromatography can be completed in three to four minutes.

Chloramphenicol can be extracted from plasma (50µL) with methylene chloride:isopropanol, 95:5 v/v (2.0mL). The required 50µL plasma sample is quite reasonable for monitoring the drug in newborns and infants. After mixing (vortex) and centrifugation, the solvent layer is removed and evaporated in a clean tub. The residue is then resuspended in 0.2mL of mobile phase (3). A 50µL aliquot of the resuspended material provided an appropriate amount of the drug for analysis.

Under the conditions used in Figure B, the drug phenacetin coelutes with chloramphenicol, and timethoprim interferes with the internal standard. Many other compounds that might be coadministered with chloramphenicol are eluted before the inter-

Figure A. Structures of Chloramphenicol and Related Compounds

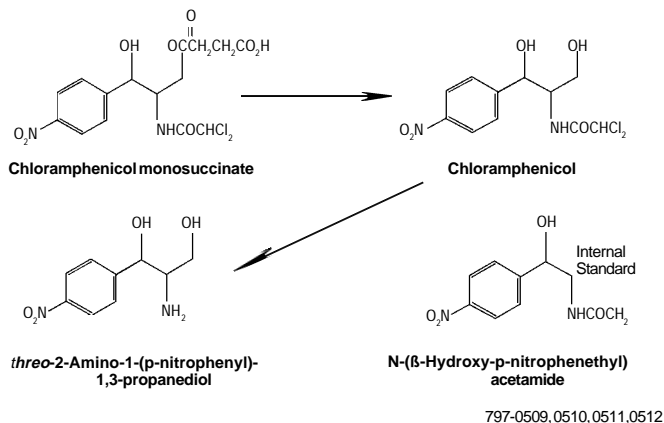
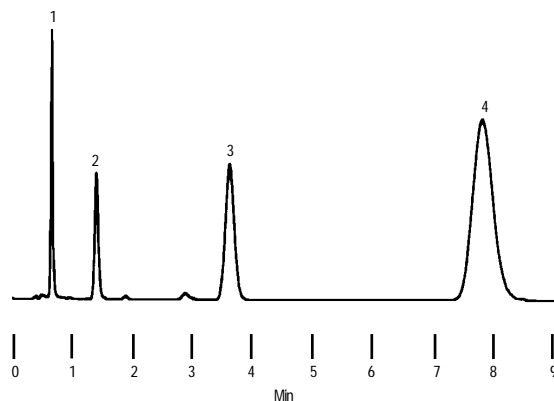


Figure B. Chloramphenicol

Column: SUPELCOSM LC-8-DB, 5cm x 4.6mm, 5µm particles (Cat. No. 58344) w/ SupelguardSM guard column (Cat. No. 59553)
Temp.: ambient
Mobile Phase: acetonitrile:methanol:20mM H₃PO₄ (pH to 3.0 with KOH) containing 0.02% triethylamine (pH 3.3), (5:17:78, v/v/v)
Flow Rate: 2mL/min
Det.: 254nm UV

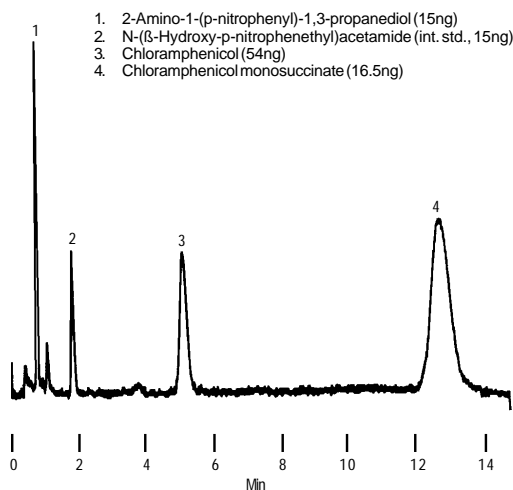
1. 2-Amino-1-(p-nitrophenyl)-1,3-propanediol (150ng)
2. N-(β-Hydroxy-p-nitrophenethyl)acetamide (int. std., 150ng)
3. Chloramphenicol (540ng)
4. Chloramphenicol monosuccinate (165ng)



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Figure C. Mobile Phase Adjusted to Detect Major Chloramphenicol Metabolite

Column: SUPELCOSIL LC-8-DB, 5cm x 4.6mm, 5µm particles (Cat. No. 58344) w/ Supelguard guard column (Cat. No. 59553)
 Temp.: ambient
 Mobile Phase: acetonitrile:methanol:20mM H₃PO₄ (6:20:74, v/v/v)
 Flow Rate: 2mL/min
 Det.: 254nm UV



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nal standard, including acetaminophen, N-acetyl-procainamide, β-OH-theophylline, caffeine, diphylline, diiril, hydrochlorothiazide, metronidazole, paraxanthine, theobromine, and theophylline. Three drugs are eluted between the internal standard and chloramphenicol: sulfamethoxazole, sulfisoxazole, and quinidine. Dihydroquinidine, papaverine, and furosemide are eluted slightly after the mono-succinate, while chlordiazepoxide and carbamazepine are eluted a few minutes later. None of approximately 50 other tested compounds, including barbiturates anticonvulsants, antiarrhythmics, antidepressants, and antibiotics interfered with the analysis.

The major chloramphenicol metabolite, 2-amino-1-(p-nitrophenyl)-1,3-propanediol (3), is briefly retained in this mobile phase. To reliably quantify this metabolite, we recommend adjusting the mobile phase composition to 5% acetonitrile, 17% methanol, and 78% buffer. This prolongs retention time by approximately 15%, thus avoiding baseline instability at t₀ (Figure C).

HPLC provides a simple, convenient method for analyzing the antibiotic chloramphenicol, its administered form, a major metabolite, and an internal standard, without interference from most other drugs. With a 5cm SUPELCOSIL LC-8-DB column, the analysis is rapid and economical.

We offer chloramphenicol-related compounds (except chloramphenicol monosuccinate) as standard solutions, with concentrations adjusted to partially reflect response factors and provide a relatively uniform chromatogram.

Ordering Information:

Description	Cat. No.
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5cm x 4.6mm	58344
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25cm x 4.6mm	58354
Supelguard LC-8-DB guard column kit (packed 2cm x 4.6mm cartridge column, column holder, hardware to connect to 1/16" OD tubing)	59553
Supelguard LC-8-DB guard columns (pk. of 2)	59563
Chloramphenicol standards	
1000mg, neat	442513
1mg/mL in methanol, 1mL solution	C9028

□ DB – Deactivated for basic compounds

References

- Nahata, M.C. and D.A. Powell, J. Chromatogr., **223**: 247-251 (1981).
 - Gal, J., P.D. Marcell, and C.M. Tarascio, J. Chromatogr., **181**: 123-126 (1980).
 - Glazko, A.J., W.A. Dill, and M.C. Rebstock, J. Biol. Chem., **83**:179 (1950).
- References not available from Supelco.

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