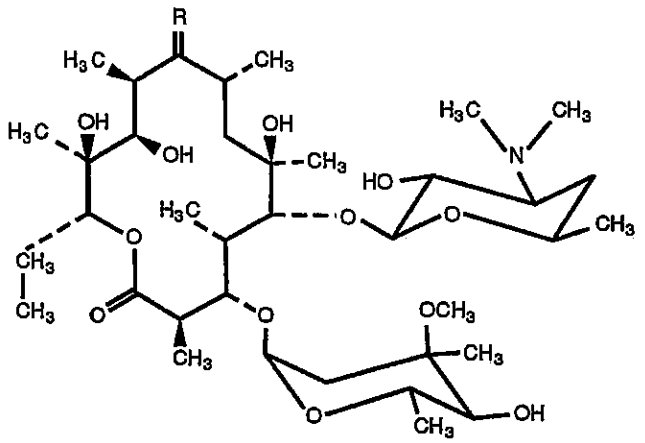
CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Erythromycin in Neonatal Serum

Purpose
Determination of erythromycin in neonatal serum.



[I] R = O
[II] R = N-OCH_2-$OCH_2CH_2OCH_3$

Figure 1. Structures of erythromycin [I] and roxithromycin [II].

Erythromycin is a macrolide antibiotic frequently used for penicillin-resistant bacterial and mycoplasmic infections. In particular, it is being tested for the treatment of chronic lung disease of premature neonates, which may be caused by *Ureaplasma urealyticum* [1].

Sample sizes for these patients range from 10 to 100 µL. Therefore, it was necessary to develop a highly sensitive assay [2]. Moreover, since other antibiotics are commonly co-administered, the assay had to be highly selective.

Although not ideal electrophores, the tertiary amino groups often present in macrolide antibiotics are capable of anodic oxidation under sufficiently basic pH conditions [3]. Other antibiotics in this class that contain an aliphatic tertiary amine on the desosamine ring (clarithromycin, roxithromycin, azithromycin, dirithromycin) should have similar electrochemical properties.

Existing Method
LCUV, and LCEC at more neutral pH. Neither has the sensitivity required for the small samples obtained from neonates.

Conditions
System: BAS-200 Liquid Chromatograph
Detector: Dual-series, glassy carbon
Potential: Upstream: +1500 mV; Downstream: +875 mV (both *vs.* Ag/AgCl)
Column: Chromegabond Gamma RP1, 150 x 4.6 mm, 5 µm particle size (ES Industries, Marlton NJ)
Mobile Phase: 160 mL methanol, 60 mL *n*-propanol, 780 mL 0.016 M potassium phosphate/0.04 M sodium perchlorate (pH 11).
Flow Rate: 1 mL/min
Temperature: Ambient
Detection Limit: 32 ng/mL
Linear Range: 0.03 - 6 µg/mL

Sample Preparation
To 100 µL samples, add 50 µL roxithromycin (12 µg/mL, Internal Standard) and vortex. Add 200 µL 0.06 M Na_2CO_3 and vortex. Add 1 mL methyl-*t*-butyl ether. Vortex, centrifuge, and transfer the ether layer to a new tube. Evaporate to dryness and reconstitute in 63 µL of mobile phase which has been adjusted to pH 8. Inject 55 µL.

Notes
Only the downstream electrode was monitored. Setting a high potential on the upstream electrode stabilized the baseline obtained from the downstream electrode.

F2 and F3 are chromatograms of serum from a patient, shortly after an initial dose and 24 hours after the final dose.

Pharmacokinetic data are presented in F4 and F5.

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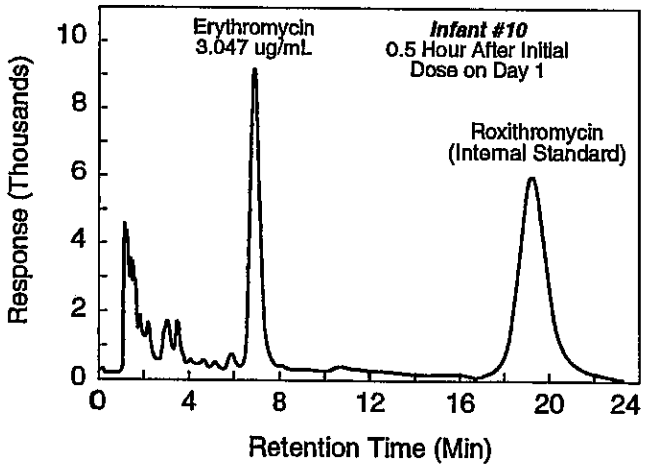

Figure 2. Sample from one patient shortly after the initial dose.

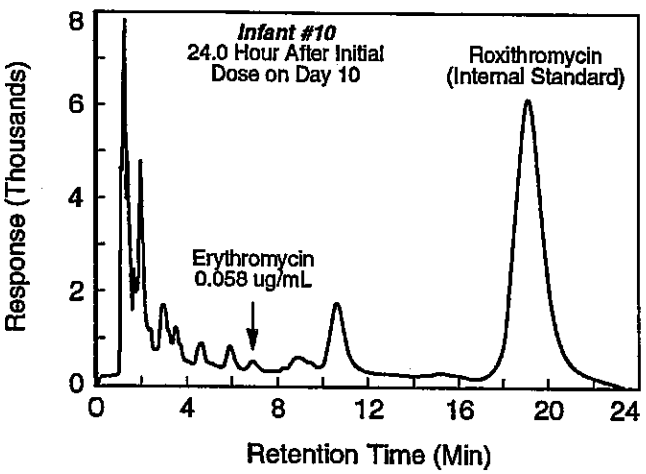

Figure 3. Sample from the same patient a day after the final dose.

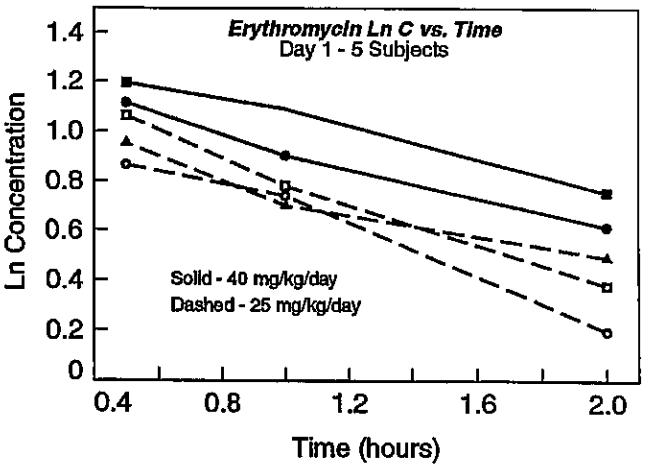

Figure 4. Two-hour pharmacokinetic data.

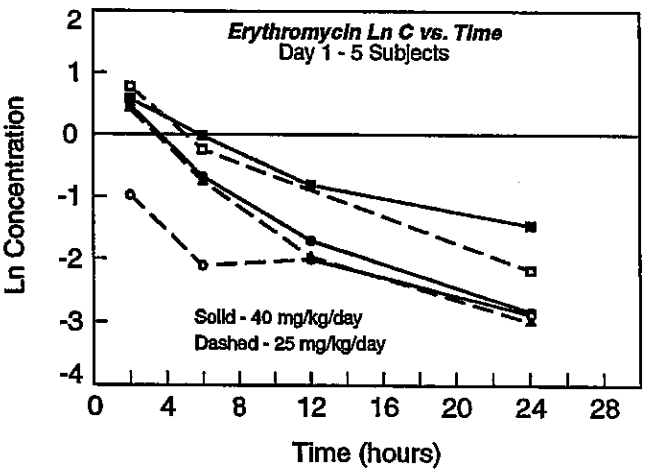

Figure 5. Twenty-four hour pharmacokinetic data.

References

1. *G.H. Cassell, K.B. Waites and D.T. Crouse, Clinics in Perinatology 18 (1991): 241-262.*
2. *R.E. Shoup, S. Hessong, K.B. Waites and D.W. Knuth, poster session presented at the American Association of Pharmaceutical Scientists 6th Annual Meeting, Washington. D.C., 1991.*
3. *G.S. Duthu, J. Liquid Chromatogr. 7 (1984): 1023.*