

NOTE NO. 29 - March 15 1989

## SEPARATION OF 4-AMINOPYRIDINE AND 3,4-DIAMINOPYRIDINE

**Analytes:** 4-Aminopyridine (2  $\mu\text{g/ml}$ ), 3,4-Diaminopyridine (2  $\mu\text{g/ml}$ )

**Sample Matrix:** Human serum

**Sample Size:** 20 microliters

**Cleanup column:** 5 micron GFF ISRP column, 5 cm x 4.6 mm ID

**Regis Product Number:** 731450

**Analytical column:** 3 micron 300 angstrom Rexchrom ODS, 5 cm x 4.6 mm ID

**Mobile Phase:** Cleanup column: 0.005 M S12 Ion Pair Cocktail (pH 6.0)

Analytical column: 75% 0.005M S12 Ion Pair Cocktail (pH 2.9), 25% Methanol

**Procedure:** Refer to Application Note Nos. 14 and 14a for procedure and system setup. The sample is injected onto the ISRP column, which retains analytes while flow is continued for 2.5 minutes and the serum-containing effluent is sent to waste without passing through the detector. The ISRP column is then switched in-line with the 5 cm analytical ODS column; by the second mobile phase analytes are eluted from the ISRP column, separated by the analytical ODS column, and detected.

**Detection:** 275 nm, 0.02 AUFS

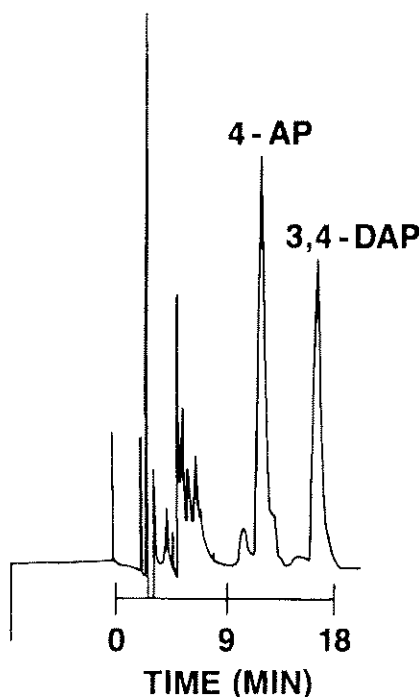
**Flow Rate:** Cleanup column, 1.0 ml/min

Analytical column, 1.5 ml/min

**Note:** Sample filtered through 0.2  $\mu\text{m}$  membrane prior to injection

**Background:** We thank Dr. Howard Cooper of Miles Laboratories, Elkhart, Indiana for permission to adapt his ISRP-ODS sequence to our purposes.

For previous separations of these analytes, see D. Lamiabile; Millart, H. J. *Chromatog.* **1983**, 272, 221-225.



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