Waters
Lab Highlights
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RAPID, HIGH SENSITIVITY ASSAY FOR A NEW ANTIARRHYTHMIC DRUG USING PRE-COLUMN DERIVATIZATION

Mexiletine [1-(2,6-dimethylphenoxy)-2-aminopropane], a new oral antiarrhythmic drug, is currently undergoing clinical trials in North America. Previously reported liquid chromatographic methods employed either direct UV measurement or derivatization followed by UV or fluorescence detection. The former lack sensitivity, while the latter are time-consuming or require complex hardware. Mssrs. Grech-Belanger, Turgeon, and Gilbert (1), Laval University (Quebec) have reported a new technique based on derivatization with fluorescamine, followed by reversed-phase LC with fluorescence detection.

Figure 1 shows chromatograms obtained from 1 ml aliquots of control serum and serum containing 165 ng/ml obtained 8 hr after administration of a 200 mg dose (1 ml of internal standard solution was added prior to extraction). The method is linear over the range 100-500 ng/ml, and coefficients of variation for repetitive analysis of spiked serum (10-400 ng/ml) were <3.80% and <5.78% for within-day and day-to-day variation, respectively. The minimum measurable concentration in serum was 1 ng/ml.

The authors concluded that this new assay for mexiletine is "...faster and more sensitive than other HPLC assays for mexiletine."

Column:	μBONDAPAK™ C_{18}, 3.9 X 300 mm
Mobile Phase:	CH_3CN:H_2O:PIC B-5:CH_3COOH, 65:33.6:0.4:1
Flow Rate:	1 ml/min
Chart Speed:	0.62 cm/min
Internal Standard:	Methylmexiletine Hydrochloride (Boehringer-Ingleheim, Canada Ltd.)

FOR INVESTIGATIONAL USE ONLY.
THE PERFORMANCE CHARACTERISTIC FOR THIS PROCEDURE HAS NOT BEEN ESTABLISHED.

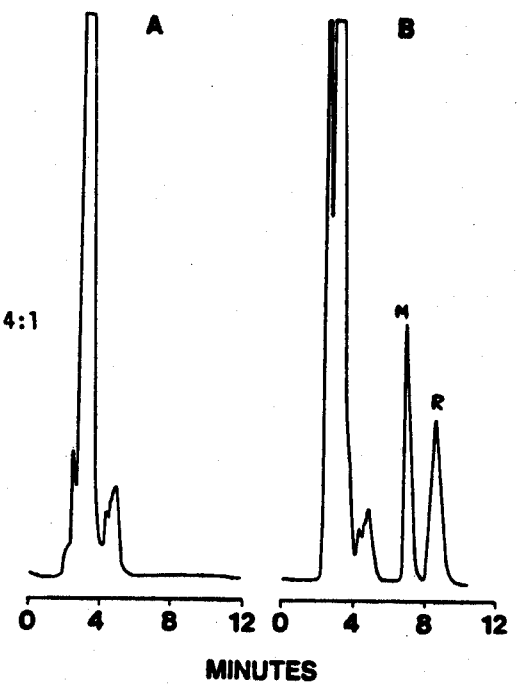

Figure 1. Typical chromatogram of (A) pre-dose serum and (B) post-dose serum of a subject who received a 200-mg dose of mexiletine hydrochloride. The calculated concentration of mexiletine (M) is 165 ng/ml and the amount of internal standard (R) added was 250 ng.

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EXTRACTION AND DERIVATIZATION

A 1.0-ml aliquot of serum or plasma was rendered alkaline (pH 12-13) with 1 ml of 4N NaOH. One milliliter of an aqueous solution of the internal standard (250 ng/ml) was then added and the mixture was extracted twice with 6 ml of diethyl ether. The combined ethereal extracts were evaporated to dryness after addition of 25 µl of a 0.1 M solution of hydrochloric acid in acetone. The residue was reconstituted in 50 µl of borate buffer (pH 8.2, 0.02 M) by vortex mixing; while still on the mixer, 50 µl of a fluorescamine solution in acetone (0.25 mg/ml) was added and the resulting solution was mixed vigorously for 30 sec. Mobile phase (50 µl) was then added and 75 µl of the resulting solution was injected onto the LC.

1. O. Grech-Belanger, J. Turgeon, and M. Gilbert, J. Chrom. Sci., 22,p 490-491 (1984).