Waters Lab Highlights

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Determination of the Enantiomers of an Anti-arrhythmic Drug in Human Plasma and Urine

Many concerns regarding enantioselective action and disposition of racemic drugs have been raised in recent years. Regulatory scientists have recently questioned the relevance of pharmacological, pharmacokinetic, and therapeutic drug monitoring data obtained from assays which do not discriminate between enantiomers, particularly for highly potent drugs. In the future, the U.S. Food and Drug Administration will require that such studies be conducted on each enantiomer as well as the racemate.

Flecainide (Figure 1) is a new antiarrhythmic drug, which exhibits potent blocking effects on cardiac sodium channels. The drug, the first of its class released in North America, is administered as a racemate. In vivo studies have reported the two enantiomers to be equally active in mouse and dog models, but until recently no reports of analytical methods have dealt with the pharmacokinetics of flecainide enantiomers in animals or humans.

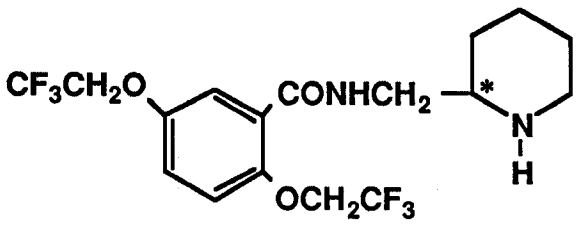

Figure 1. Structure of the antiarrhythmic drug flecainide. The asymmetric center is indicated by *.

Earlier this year, researchers at the University of Alberta reported a stereospecific HPLC method for the determination of flecainide acetate in human plasma and urine[1]. Their objective was to develop an assay which could be applied to the investigation of the pharmacokinetics of flecainide enantiomers in patients. Pre-column derivatization of the drug with 1-[(4-nitrophenyl)-sulfonyl]-L-prolyl chloride (NPSPC) resulted in formation of diastereomers which were separated by reverse phase liquid chromatography on a μ-Bondapak™ C18 column (3.9 X 300 mm). Figure 2 shows the chromatography of spiked plasma, a patient's plasma, and blank plasma. The blank shows no interfering peaks in the analytical region of the chromatogram. Interday accuracy and precision of the method are shown in Table 1.

Tom Tarvin

Waters
Division of MILLIPORE 34 Maple Street Milford, MA 01757 508-478-2000

Table 1. Intraday Accuracy and Precision

Concentration, ng/ml			Error, %		CV, %	
Theoretical	Measured					
	R	S	R	S	R	S
500	492.2	480.8	-1.6	-3.8	3.7	2.8
375	354.2	355.2	-5.5	-5.3	5.6	1.1
250	235.9	234.3	-5.6	-6.3	8.5	9.8
125	122.1	116.2	-2.3	-7.0	3.6	9.2
50	44.4	42.7	-11.2	-14.6	4.9	4.4

n = 4

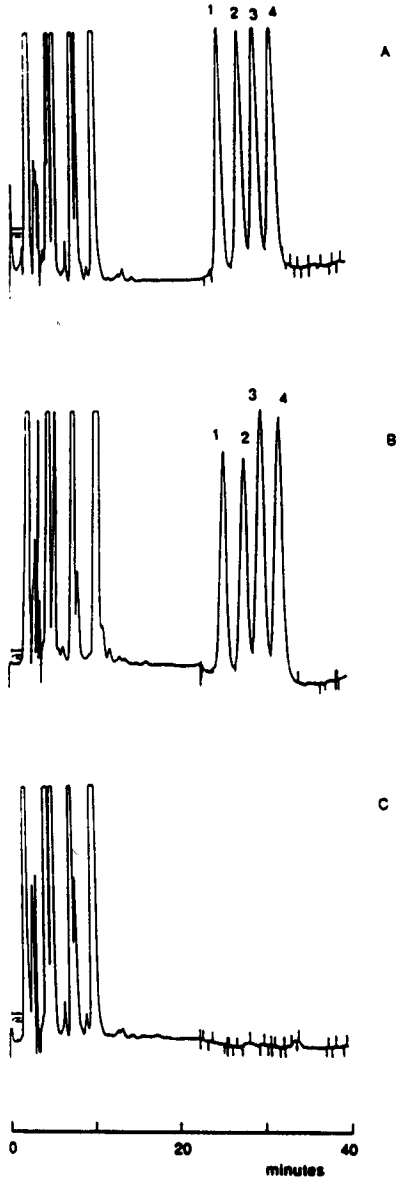

Figure 2. Chromatograms of (A) blank plasma spiked with 750 ng of racemic flecainide and internal standard; (B) plasma sample taken from a patient 5 hours after oral administration of a 100 mg dose of racemic flecainide; and (C) blank plasma. Peaks 1 and 2 are the internal standard diastereomers, and peaks 3 and 4 are the diastereomers of R- and S-flecainide, respectively.

The HPLC system consisted of a Waters Model 590 pump, a Model 710B Autosampler, and a Model 481 Variable Wavelength Detector. The lowest quantifiable concentration was found to be 50 ng/ ml. Flecainide is naturally fluorescent, but quenching occurred after derivatization, resulting in inadequate sensitivity with fluorescence detection.

The mobile phase consisted of water: acetonitrile: TEA (55:45:0.2). The flow rate was 1 ml/min, and the detection wavelength was 280 nm.

Reference

1. Alessi-Severini, S., Jamali, F., Pasutto, F. M., Coutts, R. T., and Gulamhusein, S., *J. Pharm. Sci.* **257**(79) (1990) 257-260.

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