

COUPLED ACHIRAL/CHIRAL: DETERMINING DRUG ENANTIOMERS IN SERUM

Analytes: Warfarin, I; and internal standard benoxaprofen, II.

Sample Matrix: Human serum

Sample Size: 100 microliters

Column 1: Achiral, ISRP, 5-micron GFF, 5 cm x 4.6 mm I.D.; ambient temperature.

Regis Product Number: 731450

Column 2: Chiral, bovine serum albumin (BSA), 10-micron, dimensions not stated; 30° C.

Mobile Phase: Composition:

X/Y, Buffer/1-Propanol.

Buffer: 0.2 M sodium phosphate, pH 7.5.

* 3mM trichloroacetic acid modifier.

	ISRP	BSA
X/Y	99/1	97*/3

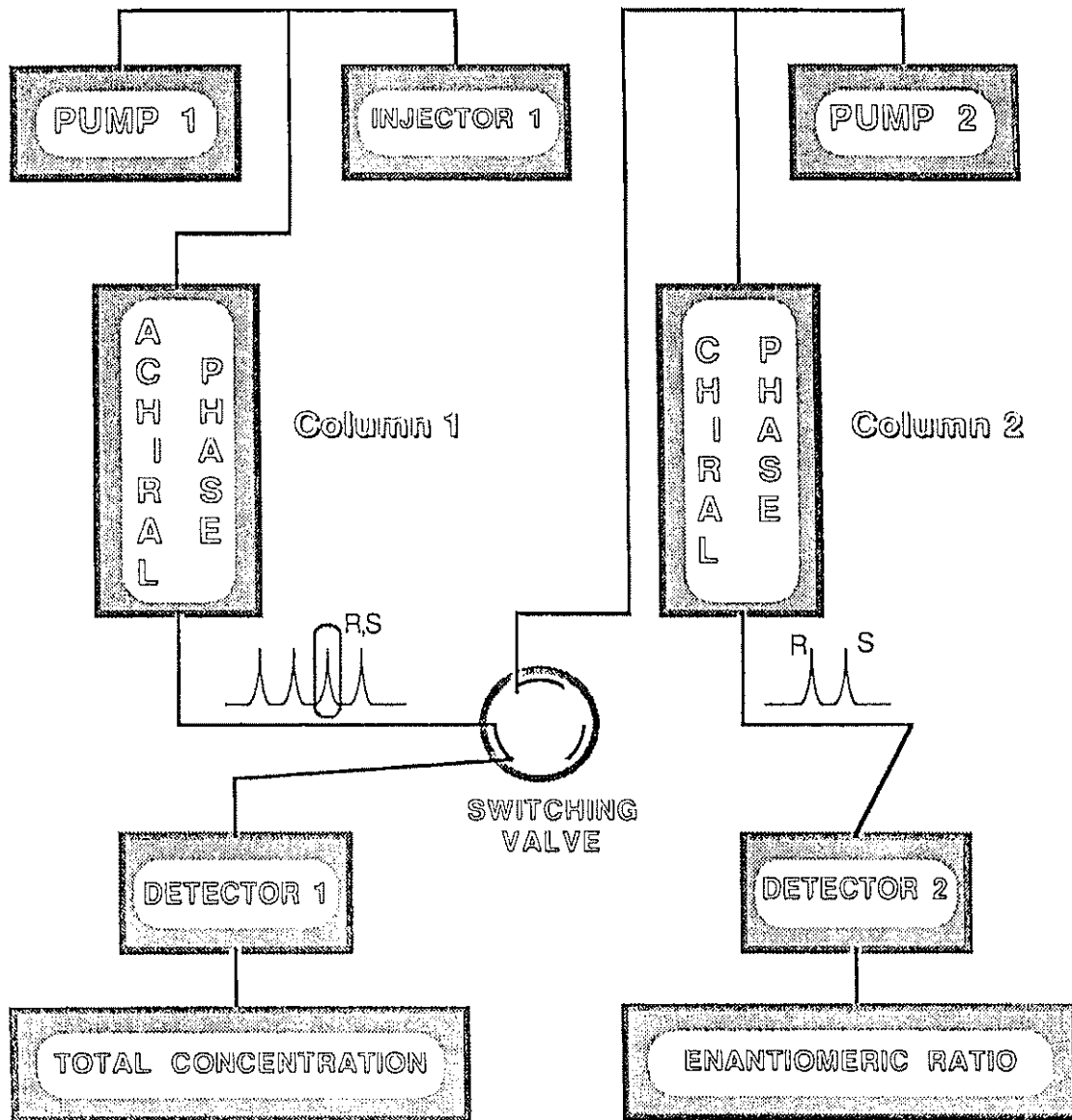
Flow Rate: 1.0 mL/min

Detection: Fluorescence, Exc 310 nm, Em 370 nm

Discussion: Serum, first spiked with an internal standard, was prepared for injection into the chromatographic system (Fig. 1). This system included a achiral ISRP column, into which was injected the prepared serum; and a chiral column, into which was injected the ISRP-cleaned chiral drug peak. Also in Figure 1, chromatographic diagrams illustrate ISRP--->chiral peak selection and transfer.

The model drug was warfarin; the internal standard, benoxaprofen. In Figure 2 are shown representattive chromatograms from injections into the ISRP column. In Figure 3 are shown chromatograms of warfarin enantiomers that were eluted from the chiral column.

In the words of the authors, the analytical system "is sensitive and accurate, does not require extensive precolumn manipulations, and can be automated for use in large-scale clinical studies" (1).



$$[R\text{-ISOMER}] = [\text{TOTAL}] \cdot R/S$$

$$[S\text{-ISOMER}] = [\text{TOTAL}] - [R]$$

Figure 1. The chromatographic coupled-column system. In the diagram, RS indicates the racemate peak, which was isolated and transferred from the ISRP column 1 to the BSA column 2.; R, the separated R enantiomer peak; and S, the S. (Reproduced from ref. 1 by permission of Pharmaceutical Research.)

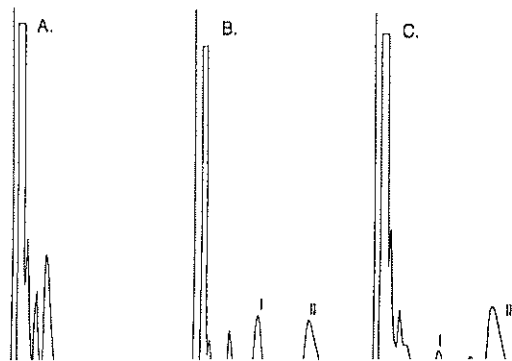


Figure 2. Chromatograms from the ISRP column: A, prepared but blank serum; B, serum spiked with 10 microgram/mL warfarin racemate I plus benoxaprofen internal standard II; C, serum, clinical sample. (Reproduced from ref. 1 by permission of Pharmaceutical Research.)

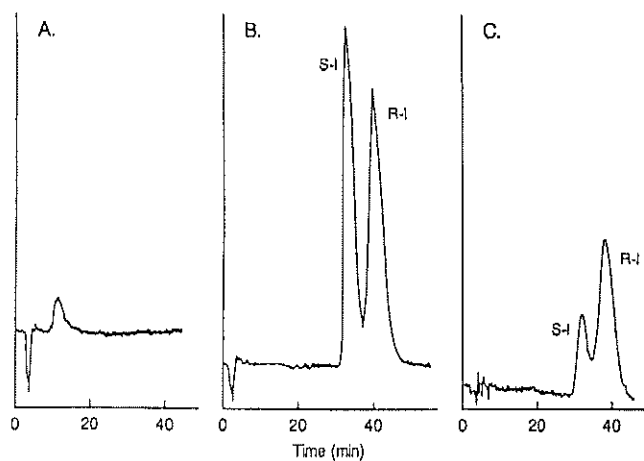


Figure 3. Chromatograms from the BSA chiral column: A, prepared but blank serum; B, serum spiked with 5 microgram/mL warfarin racemate; C, serum, clinical sample. (Reproduced from ref. 1 by permission of Pharmaceutical Research.)

References: (1) Chu, Y.; Wainer, I. W. THE MEASUREMENT OF WARFARIN ENANTIOMERS IN SERUM USING COUPLED ACHIRAL/CHIRAL, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC). *Pharm. Res.* 1988, 5, 680-683.

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