

NOTE NO. 49 - Dec 15 1994

DIRECT INJECTION DETERMINATION OF THE FREE WARFARIN ENANTIOMER

Reviewed: Shibukawa, A.; Nagan, M.; Kuroda, Y.; Nakagawa, T. Anal.Chem. **1990**, 62, 712-716 (Ref. 9 herein).

Introduction

Chiral molecules that are enantiomers are mirror images of each other. When used as drugs, enantiomers differ from each other in pharmacological activity, side effects, hepatic metabolism, and state of equilibrium binding to blood plasma proteins.

A drug molecule that interacts closely with a protein is said to be bound; in equilibrium with the bound molecules are the free. To reach its site of action--to pass through a cell membrane, for instance--a given drug molecule must be free. Thus drug efficacies are affected by blood protein binding as well as chirality.

Often, a chiral drug is administered as a racemate, that is, an equal mixture of two enantiomers--determinations of the blood concentrations of which we have reviewed (1,2). However, the blood contains not only the two enantiomers, but also the free and bound forms of each enantiomer. Only the free forms are effective. Needed to be determined--preferably in one analytical operation--are the blood concentrations of each free enantiomer. Such a method is reviewed herein. We proceed.

Drugs interact much faster with protein than with a chromatographic stationary phase (3,4). Consequently, from an adequately large injection of serum into an ISRP column, both the free and the protein-bound forms of a given drug can be determined (3-5). This was first pointed out in 1987 in Japan by ISRP-inventor T. S. Pinkerton (3)--see also Note 32 (5). It was applied in Japan by A. Shibukawa in 1988 (6), 1989 (7), and 1990 (8).

As now to be reviewed, in 1990 Shibukawa et al. combined free-bound and chiral separations to determine in blood the free enantiomer concentrations of racemate-administered warfarin (9), as follows: With protein-drug equilibrium carefully maintained, the protein and protein-bound drug are first separated from the free drug by passage through an ISRP column. Subsequent passage through a chiral column then separates the enantiomers of the free drug from each other. Their concentrations are then measured.

Experimental

Analytes: Warfarin.

Sample Matrix: Human serum albumin (HSA)

Column 1: Achiral, ISRP, 5-micron GFF, 15 cm x 4.6 mm I.D.; 37° C.

Regis Product Number: 731451

Column 2: Chiral, (AGP), 5-micron, 10 cm x 4.0 mm I.D.; 37° C.

Regis Product Number: 732200

Sample volume: Function of HSA concentration, as follows:

HSA content, micromoles

	550	300	100
Sample volume, μL	40	60	300

Sample throughput: four per hour

Mobile Phase:

Composition: X/Y, Buffer/Acetonitrile.

Buffer: potassium phosphate,
pH 7.4*, ionic strength (I) 0.17*

* The authors emphasize the strong dependence of pH and ionic strength on X/Y.

	ISRP	AGP
X/Y	100/0	89/11
ph	7.4*	7.0*
Ionic strength I	0.17*	0.02*
Flow rate, mL/min	0.5	1.0
Sample volume,	40	90
Detection: UV, nm	308	210

Procedures and Results

The apparatus is diagramed in Figure 1. (For more on the nature, rationale, and use of the apparatus, see (8).) From loop F, the human serum albumin (HSA)/warfarin (HSA/Wf) sample to be analyzed is injected into the ISRP mobile phase, wherein the drug-protein equilibrium remains undisturbed--a necessary condition for this analysis. The injected sample is carried into ISRP column G.

Consonant with ISRP column function, the injected proteins pass unretained through the ISRP column, in so doing carrying the protein-bound drug out of the ISRP column. Meanwhile, however, the free drug is retained within the ISRP particles. Once the drug retention volume that is characteristic of these conditions has been swept out, the free drug begins to emerge from the column.

For a drug of free fraction no higher than this one, increasing to 40 microliters the volume injected into the ISRP column generates a plateau. Under this plateau the free drug concentration is stable and can be sampled repeatedly. In the analysis, the plateau is sampled just once--into loop J for the subsequent chiral separation and detector L for the measurements of the free enantiomer concentrations (Figure 2).

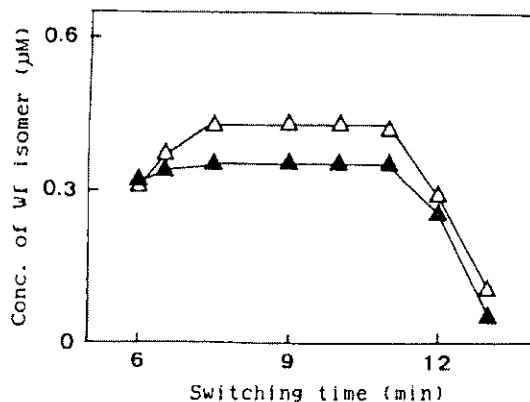
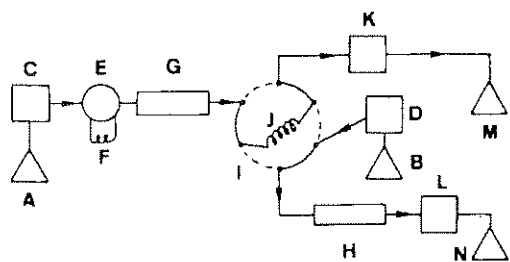


Figure 1. Schematic diagrams of the present on-line HPLC system: (A) mobile phase for

Figure 2. Relation between column switching time and the concentration of R (Δ) and S (\bullet)

HPFA; (B) mobile phase for chiral separation; (C,D) pump; (E) sample injector; (F) injector loop; (G) column for HPFA; (H) column for chiral separation; (I) six-way switching valve; (J) loop; (K,L) detector; (M,N) waste.

Wf isomers in the heart-cut portion.
Figures 1. and 2, and the corresponding captions, reproduced from ref. 9 with permission of Analytical Chemistry.

References:

- (1) Chu, Y.; Wainer, I. W. RAM Note No. 45, Regis Technologies, Morton Grove, IL 60053, June 1, 1994.
- (2) Chu, Y.; Wainer, I. W. Pharm. Res. **1988**, 5, 680-683.
- (3) Pinkerton, T. C.; Plenary lecture presented to the 30th Annual Symposium on Chromatography, Kyoto, Japan, Jan. 27, 1987.
- (4) Pinkerton, T. C.; Miller, T. D.; Janis, L. J. Anal. Chem. **1989**, 61, 1171-1174.
- (5) Pinkerton, T. C. RAM Note No. 32, Regis Technologies, Morton Grove, IL 60053, June 15, 1988.
- (6) Shibukawa, A.; Nakagawa, T.; Miyake, M.; Tanaka, H. Chem. Pharm. Bull. **1988**, 36, 1988.
- (7) Shibukawa, A.; Nakagawa, T.; Nishimura, N; Miyake, M.; Tanaka, H. Chem. Pharm. Bull. **1989**, 37, 702-706.
- (8) Shibukawa, A.; Nishimura, N; Nomura, K.; Kuroda, Y.; Nakagawa, T. Chem. Pharm. Bull. **1990**, 38, 443-447.
- (9) Shibukawa, A.; Nagan, M.; Kuroda, Y.; Nakagawa, T. Anal. Chem. **1990**, 62, 712-716.

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