

NOTE NO. 27 - February 2 1988

**"...ORGANIC SOLVENT ACCELERATES THE RATE OF RELEASE OF THE DRUG FROM PLASMA PROTEINS."**

**Discussion:**

This quotation comes from the seminal paper of Nakagawa et al in J. Chromatog. 420 (1987) 297-311 (the full title and authorship are given in Application Note No. 26). The authors were commenting on their study of the ISRP determination of cefpiramide in human plasma. The study was summarized in their Table II, the data from which we present below in 3 parts. In these, column efficiency, precision, and drug recovery are presented as functions of mobile phase composition. First we consider column efficiency.

**Column Efficiency as a Function of Mobile Phase Composition**

Mobile Phase	HETP (microns) Plasma	HETP (microns) Aqueous
Buffer, 0.05 M	470	200
Buffer, 0.10 M	350	170
2.5% isopropanol	190	110
2.5% acetonitrile	190	100
2.5% tetrahydrofuran	140	118

This table shows that even a 2.5% concentration of any of the common admissible organic modifiers (we now know that methanol is also admissible) suffices to double the column efficiency that would be obtained with a straight aqueous mobile phase.

**Analytical Precision as a Function of Mobile Phase Composition**

Mobile Phase	C.V. (n=5)(%) Plasma	C.V. (n=5)(%) Aqueous
Buffer, 0.05 M	1.64	1.29
Buffer, 0.10 M	7.22	0.78
2.5% isopropanol	0.53	0.41
2.5% acetonitrile	0.90	0.24
2.5% tetrahydrofuran	1.28	0.44

**Discussion (continued):** Not only is column efficiency strikingly improved by some organic modifier in the mobile phase, but also, precision. This, however, does not necessarily follow; sharper peaks are not necessarily more precisely measured. It must be that--as the authors indicate--some more basic cause is involved, namely, an improved release of the cefpiramide from the proteins. That improvement is manifested as release that is faster--which shows up as sharper

peaks--and also as release that is more reliable and more uniform--which shows up as greater precision.

### Drug Recovery as a Function of Mobile Phase Composition

Mobile Phase	% Recovery from human plasma
Buffer, 0.05 M	71.4
Buffer, 0.10 M	84.7
2.5% isopropanol	99.5
2.5% acetonitrile	96.5
2.5% tetrahydrofuran	100.5

That ISRP methods can actually determine the whole drug, even though the drug is tightly bound to protein, has always been viewed skeptically by many. Here, the authors show that at least for cefpiramide a small concentration of organic solvent causes drug recovery to become substantially complete. They also show this for probenecid and lidocaine, given 5 or 10% solvent. There is little reason to think the result does not have general significance.

The basic cause that underlies the sharper peaks, the greater reproducibility, and the essentially complete drug recovery is the improved drug release that reflects the presence of organic modifier. The authors suggest (p. 306) that the small concentration of organic modifier is causing a "cleavage of protein binding...by the partial denaturation of the protein at the particular (binding) site...without precipitation of the plasma proteins."

It is a conclusion of profound usefulness for ISRP determinations, and the authors deserve much credit for having made it clear and stated it so specifically.

J.A. Perry, Regis Chemical Company,  
8210 Austin Avenue, Morton Grove, IL 60053