

NOTE NO. 39 - August 30 1993

ON-LINE ISRP CLEANING, REDISCOVERED

Dr. Thomas Pinkerton invented and developed not only the first example of a restricted access medium (RAM), the internal surface reversed phase (ISRP), but also, at the same time, 1985, the "elegant procedure" (1) of "on-line ISRP cleaning" (2). This procedure, "rediscovered independently" (1) in 1990 by Haginaka (3) and Matlin et al (4) and in 1992 by Pompon et al (1), is the subject of this first RAM Application Note, a continuation of the Pinkerton ISRP Application Notes.

In the diagram of Figure 1 is presented valving suitable for on-line ISRP cleaning by coupled columns. (This diagram, originally differently labeled, was first published earlier in this series (5).

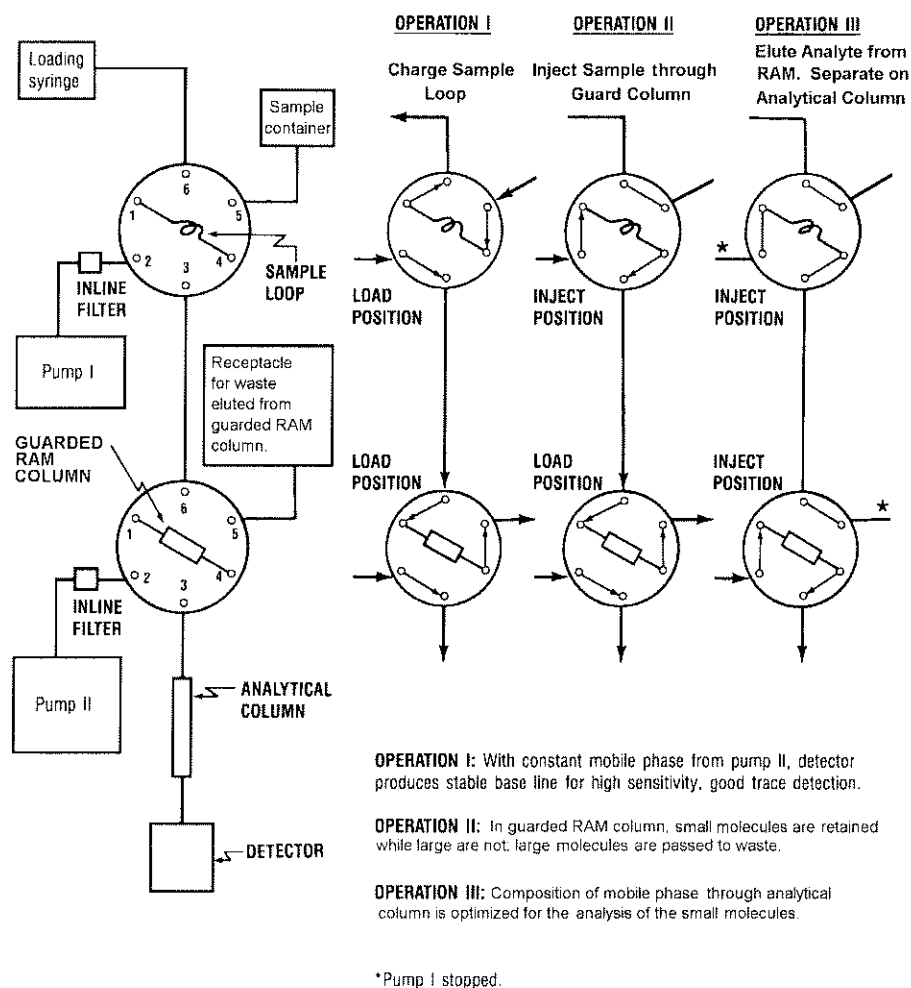


Figure 1. In one continuous operation, this procedure can clean up the sample, secure adequate detectability of the analyte, and determine its concentration.

In this procedure, the prefiltered but otherwise untreated sample is injected directly into a RAM column. In the RAM column the smaller molecules are retained while most of the larger molecules are not, and are passed to waste. Next, the cleaned effluent of the RAM column is redirected to a second column--often octadecylsilyl--wherein the analytes, here strongly retained, are concentrated. This trace concentration on the second column allows sample volume--and thus analyte detectability--to be increased to any desired level. Finally, the composition of the mobile phase is changed

so as to elute from the second column the analyte peaks, now satisfactorily clean and detectable.

Reported by Puhlmann et al (6), the chromatograms of Figures 2 and 3 illustrate the advantages to be gained by this approach. For Figure 2, only a 25-cm ISRP column was used; for Figure 3, after the sample was cleaned up by the ISRP column, the creatinine peak was passed to an ODS column, from which it was gradient eluted. The authors reported that the procedure showed "good long-term stability, simple sample handling without pretreatment, high selectivity, a broad linearity (0.3-30 mg/dL creatinine), good reproducibility (interassay coefficient of variation less than 3%), and high recovery (97-100% relative to values obtained with gas chromatography-mass spectrometry)" (6).

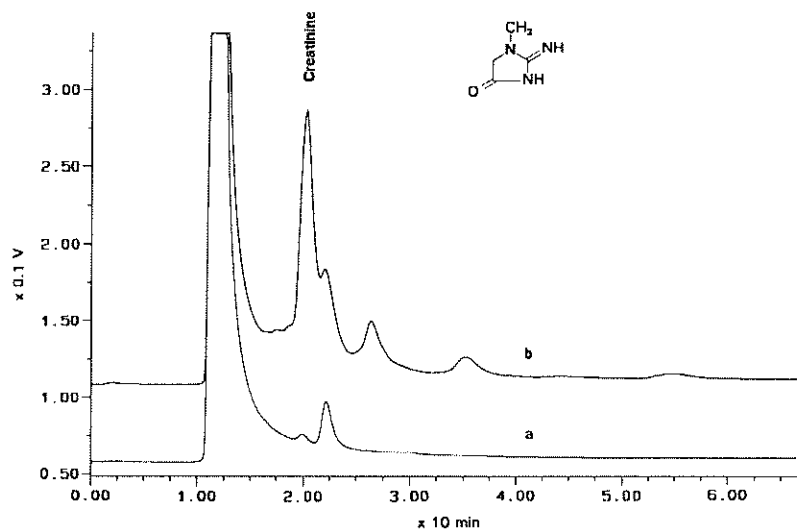


Figure 2. Chromatograms of serum from an ISRP column alone: a. normal serum; b. serum from a dialysis patient. The structure shown is that of creatinine. (Reproduced with permission from reference 6. Copyright Elsevier Publishing Company.)

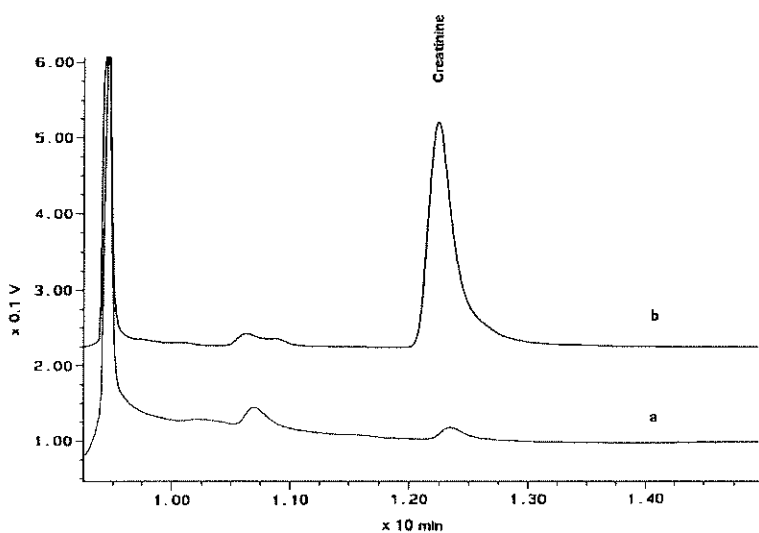


Figure 3. Chromatograms similar to those of Figure 2, but obtained from an ISRP column used for cleanup followed by gradient elution from an ODS column, for further separation and analyte concentration. (Reproduced with permission from reference 6. Copyright Elsevier Publishing Company.)

(2) Hagestam, I. H.; Pinkerton, T. C. *Anal. Chem.* **1985**, *57*, 1757-1763.

(3) Haginaka, J. J. *Chromatogr.* **1990**, *529*, 455-461

(4) Matlin, S. A.; Thomas, C.; Vince, P. M. *J. Liq. Chromatogr.* **1990**, *13*, 2253-2260.

(5) Szczerba, T. J.; Perry, J. A. Pinkerton Application Note No. 14a, August 23, 1986. Regis Chemical Company, Morton Grove, Illinois 60053.

(6) Puhlmann, A.; Duelffer, T.; Kobold, U. *J. Chromatogr.* **1992**, *581*, 129-133.

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