

NOTE NO. 33 - July 15 1989

## MINIMUM CRITERIA FOR ISRP SOLUTE DETERMINATION

**Analyte:** 2% low-fat milk

**Flow Rate:** 0.6 ml/min

**Detection:** 254 nm

**Sample Size:** 10 microliters

**Column:** 5 micron GFF ISRP, 15 cm x 4.6 mm ID

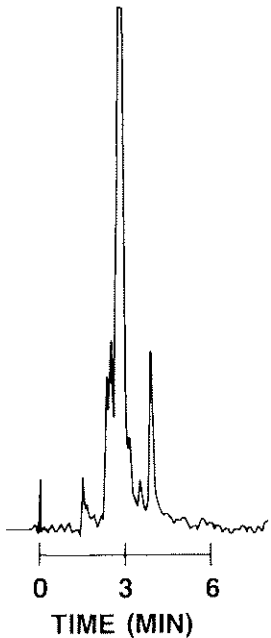
**Regis Product Number:** 731451

**Mobile Phase:** 0.1M  $\text{KH}_2\text{PO}_4$  (pH 6.8)

**Discussion:** The chromatogram presented here shows that 0.2 micron-filtered milk can easily and successfully be injected into an ISRP column, and that the column segregates the proteins in the milk from whatever smaller molecules may be also present.

So, can an ISRP column be used to determine an Antibiotic in milk? (Similarly, can an ISRP column be used to determine a given drug in serum or urine?)

For an ISRP column to be useable for such an analysis, the substance sought must show certain characteristics in the ISRP analysis. The characteristics are these:



**Adequate retention:** The substance must be retained long enough to be separated from the protein peak. As a rule, the substance must have a capacity factor of 2.4 to 2.5, that is, take at least 2.4 to 2.5 times as long to go through the column as an unretained component does. For example, if the mobile phase flow rate is 0.5 ml/min, an unretained component takes about 166 seconds to go through a 25-cm ISRP column; under those conditions, an adequately retained substance would have to take at least 398 seconds. Theophylline, for instance, has a capacity factor of 2.4, and just barely meets these requirements.

**Adequate detectability:** The substance must be detectable at the concentration in which it exists in the material to be analyzed. Normally for detection by UV absorption of drugs in serum, this minimum concentration is 0.1 mg/ml of serum--depending on the drug and the analytical technique, this figure can change by a factor of 100.

**Adequate availability:** The substance to be analyzed must have a name, and enough of it must be available to enable the analysis of it to be set up and calibrated.

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