Waters
Lab Highlights
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"HOW MUCH CAN I LOAD ON A SEP-PAK® CARTRIDGE?"

The question is often asked, "How much can I load onto a SEP-PAK^R cartridge?" And the answer is given, "Oh, about 2 mg." or, "Oh, about 100 mg." or, "Oh, you can put on 100 ml easily."

Unfortunately, all of these hypothetical answers may be correct since the original question doesn't have an "absolute" answer for all situations. The answer depends upon the capacity factor(s) of the component(s) on the SEP-PAK^R cartridge and concentration of the component(s).

For the purpose of example, refer to the Figure A below with the assumption that we have two compounds which were loaded onto a SEP-PAK^R cartridge and Peaks 1 and 2 have a k' of 10 and 30. This means that if a continuous stream of liquid containing Components 1 and 2 were pumped across a SEP-PAK^R cartridge, a frontal chromatogram would result, as shown in Figure B below, as it would on any LC column.

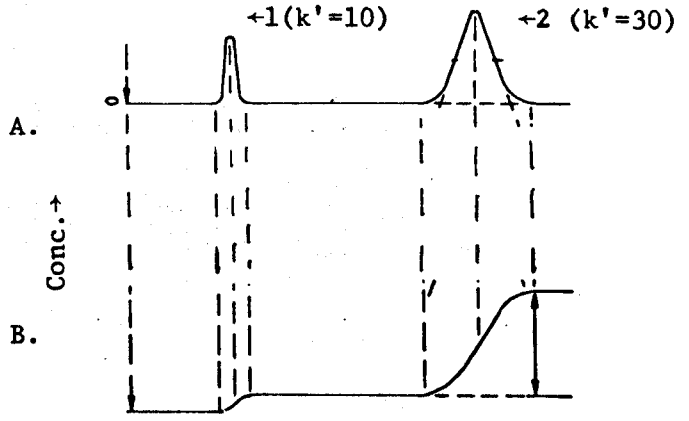

Figure 1: Comparison of analytical (differential) chromatograms and the frontal (integral) chromatogram.

The Implications: If we were doing a trace enrichment, the quality of the results would vary depending upon how much volume of sample was passed through the SEP-PAK^R cartridge. Let's assume our example is on a SEP-PAK^R cartridge which has a column volume of 1.0 ml. SEP-PAK^R cartridges have a nominal void volume of 1.0 ml for silica and 0.5 ml for C_{18}. For this example we will assume a narrow band.

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Let's look at Table I and II to understand the "loadability" and "recovery."

TABLE 1

Amount of Compound Retained on SEP-PAK® Cartridge (conc. 1 X 10^9 g/ml)

Compound 1

ml. "pumped" into a SEP-PAK	Amount Retained, g	Maximum Recovery after elution with strong solvent
5	5 X 10^{-9}	100%
9	9 X 10^{-9}	100%
10	9 X 10^{-9}	90%
20	9 X 10^{-9}	45%
30	9 X 10^{-9}	30%
50	9 X 10^{-9}	18%

TABLE II

Compound 2

Amount of Compound Retained on SEP-PAK® Cartridge (conc. 1 X 10^6 g/ml)

ml. "pumped" into a SEP-PAK	Amount Retained, g	Maximum Recovery after elution with strong solvent
5	5 X 10^{-6}	100%
20	20 X 10^{-6}	100%
29	29 X 10^{-6}	100%
30	29 X 10^{-6}	97%
50	29 X 10^{-6}	58%

For Compound 1, because the k'=10, we can "pump" up to 11 ml of sample onto a SEP-PAK^R cartridge and obtain 100% recovery. If we load a larger sample, for instance 20 ml, the recovery is only 50%. The total amount we can retain is 9 X 10^{-9} g.

For Compound 2, because the k'=30, we can "pump" up to 29 ml of sample and obtain 100% recovery. If we load a 30 ml volume, the recovery will only be 97%. The total amount which can be retained is 2.9 X 10^{-5}g.

The Real World. Obviously the larger the k', the better the capacity of the SEP-PAK^R cartridge. (The ideal is k'= ∞.) However, compounds may have finite k' values, perhaps a value of 100 or 500; therefore, there is a finite volume which can be loaded onto a SEP-PAK^R cartridge until a "breakthrough" occurs.

Since SEP-PAK^R cartridges are used in trace analysis, mass overload is unlikely. However, it is important to realize that mass overloading can occur on a SEP-PAK^R cartridge, just the same as it can on our analytical column. Overloading will cause the analytical peak (or frontal breakthrough) to occur at earlier retention times. Therefore, when a SEP-PAK^R cartridge is to be used, if there is any concern about recovery or loading, the analyst must test recovery and breakthrough. Choose the packing and solvent to give optimum retention. As in analytical LC, retention (capacity) of SEP-PAK^R cartridges depends on sample volume (and concentration, solvent "strength", and packing "strength."