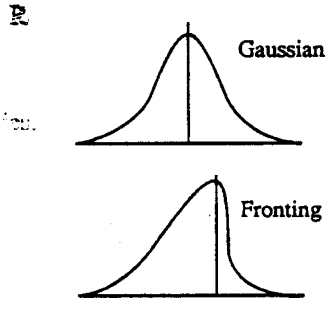
Hints for the Capillary Chromatographer

Sample Capacity and Column Overload

What is column overload?

Column overload occurs when the amount of sample injected exceeds the column's capacity for that component. Overload is normally observed as a fronting, non-gaussian peak shape (Figure 1). A column's capacity is a function of several parameters including the columns internal diameter (ID), its film thickness (df), the solubility of the compound in the columns stationary phase, and capacity factor (k).

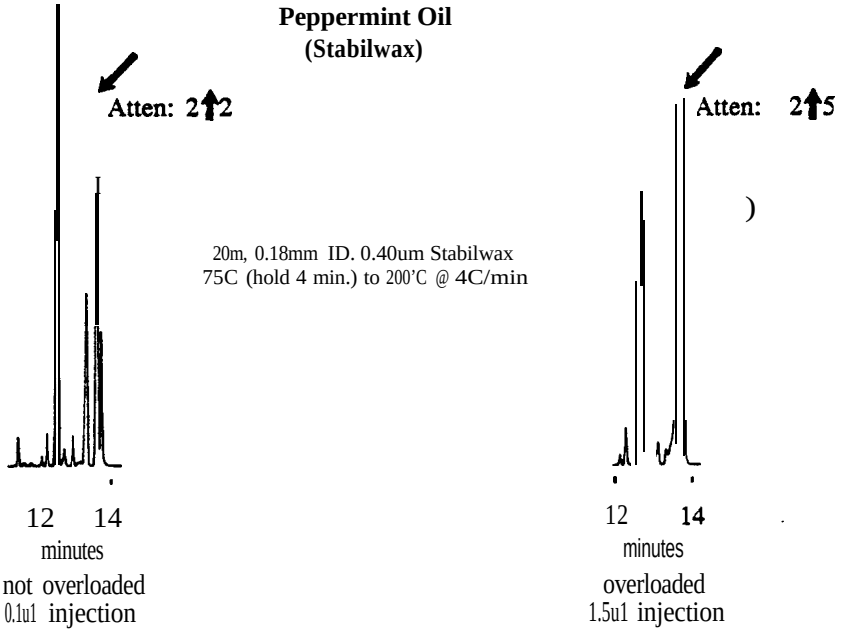
Figure 1 · Normal Gaussian vs. Overloaded Fronting Peak Shapes



Why is **it important not to exceed a column's capacity?**

Capillary columns have much lower sample capacities than packed columns, therefore, it is extremely important to optimize the amount of sample injected. When sample capacity is exceeded, peak symmetry is lost and resolution is affected. Because the peak shape will be *much broader, resolution between two closely eluting peaks can be lost. Figure 2 shows the loss of peak symmetry and resolution in the analysis of peppermint oil on a Stabilwax column. In the first chromatogram, 0.1 ul of neat peppermint oil was injected. At these low concentrations, very good resolution between the menthyl acetate, neo-menthol,

Figure 2 - Minimize the amount of sample injected to maximize resolution.



b-caryophyllene, and terpinene-4-ol is obtained. In the second chromatogram, 1.5ul of neat peppermint oil was injected. Because the sample concentration exceeded the column's capacity, a significant loss in resolution occurred.

How can overload be prevented?

Two choices are available to prevent overload:

- ▼ reduce the sample concentration reaching the column
- ▼ choose a column and run conditions that will allow greater sample capacity

To reduce the sample concentration reaching the column, the sample components can be diluted by increasing the split ratio, diluting with additional solvent, or by introducing a smaller amount.

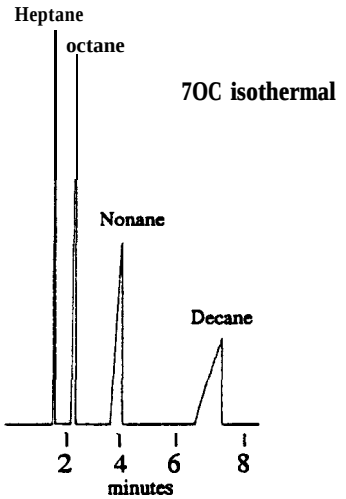
How does column ID affect sample capacity?

As the column ID increases so does sample capacity. Table 1 shows typical column capacity for several different diameter columns. Figure 3 compares sample capacity on 0.25 and 0.53mm ID columns. Four hydrocarbons Oleptane, octane, nonane, and decane) were analyzed at a concentration of 1000ng on

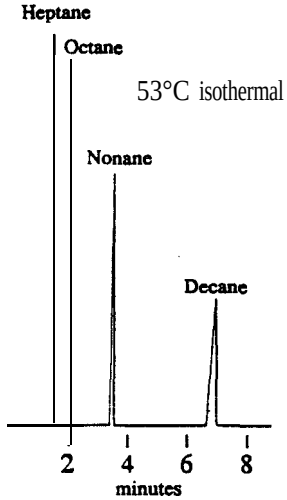
Table 1

Column ID	0.18mm	0.25mm	0.32mm	0.53mm
Sample Capacity	<50ng	50-100ng	400-500ng	1000-2000ng

Figure 3 - Increase sample capacity by increasing column ID.



15m, 0.25mm ID, 0.25um Rtx-1 (cat.10120)



15m, 0.53mm ID. 0.25um Rtx-1 (cat.# 10122)

both a 0.25mm ID and 0.53mm ID column. The 0.25mm ID column exhibits overload and severe peak fronting for nonane and decane. In comparison, the 0.53mm ID column shows symmetrical peak shapes for nonane and only slight overload for decane. This illustrates the effect of increasing sample capacity by increasing column ID.

How does column film thickness affect sample capacity?

Increasing the column's stationary phase film thickness also increases sample capacity. Figure 4 shows this effect. Again, we show the same series of hydrocarbons at the 1000ng concentrations on 30 meter, 0.25mm ID, 0.25um and1.0um Rtx-I columns. On the 0.25um column, the nonane peak shows some overload and the decane peak shows severe fronting. By increasing the film thickness to 1.0urn, the peak symmetry of nonane is restored and the decane peak shows only slight fronting.

How does solubility affect sample capacity?

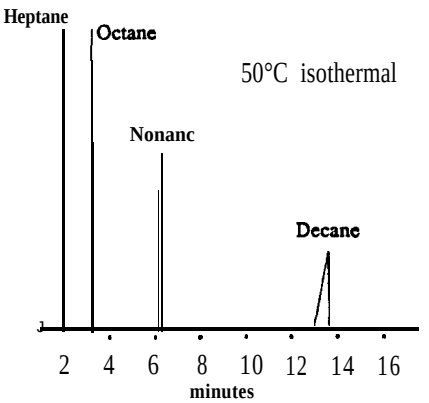
The solubihty of a sample component in the column's stationary phase also has an effect on sample capacity. The more soluble a component is in the stationary phase, the greater the column capacity for the solute. For example, a polar compound will have greater solubility in a polar stationary phase than in a non-polar stationary phase. For environmental analysts, this phenomenon is very common when analyzing acid andbase-neutral extracts on a non-polar, 5% diphenyl stationary phase. Benzoic acid, a polar compound, always exhibits very poor peak symmetry, demonstrating overload on this non-polar stationary phase. Even though it is at the same concentration, it is less soluble in this phase than the other priority pollutants and exceeds the column's capacity at a much lower concentration.

How does component retention affect sample capacity?

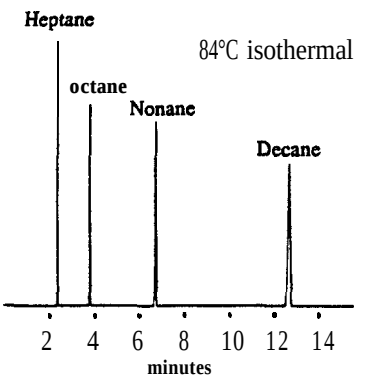
Sample capacity is also affected by how long the component remains in the stationary phase. The capacity factor or k gives us an indication of how long the component remains on the stationary phase. The longer a sample component remains in the stationary phase, the greater the chance for overload. The capacity for a component can be increased by selecting run conditions that will create lower *k* values by causing the component to elute faster from the column (faster flow rates or faster temperature programming).

When choosing a column, the analyst must keep in mind the range of component concentrations. By optimizing column ID and film thickness, and by matching the solubilities of sample components with the stationary phase, samples can be analyzed without overload. Also, by optimizing run conditions, the ***k*** value for components can be minimized resulting in better sample capacity.

Figure 4 - Increasing the stationary phase film thickness increases column sample capacity.



30m, 0.25mm ID, 0.25um Rtx-1 (cat.#10123)



30m. 0.25mm 1.0um Rtx-1 (cat.# 10153)

If there's a topic you'd like to see covered in Hints for the Capillary Chromatographer, write to:

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