

## The Concept of a Resolution Mixture in Developing and Validating Process Chromatographic Methods

### Definition

One of the most useful concepts in developing a process chromatographic method is that of a "resolution mixture." Briefly defined, a resolution mixture for a preparative separation contains the target molecule and early and late contaminants—all at approximately the same concentration. It is produced by pooling early and late side-fractions adjacent to the large peak of target product (Fig. 1).

In most cases the chemical identity of the contaminants is not known at the outset. In the original chromatogram, some contaminant peaks may be invisible because they are obscured by the much larger product peak. Reducing the concentration of target product is helpful because hidden peaks and true resolution of the chromatographic run can be observed much more clearly when all peaks are of similar size.

### Purpose

A resolution mixture can be used early in method development to screen parameters such as solvent, buffer and pH, column type and manufacturer in order to optimize the preparative separation. Using a resolution mixture dramatically simplifies the screening of operating parameters and their effects on resolution. With the crude material alone, method optimization requires collecting and analyzing fractions to compare different mobile phases and columns. Using a resolution mixture requires only visual inspection of chromatograms, and resolution can be calculated automatically by an automated data system.

### Additional Benefits in Development

Purified peaks from side fractions in the resolution mixture can also be used for chemical identification of contaminants by MS, NMR, etc. Early identification of contaminants gives valuable insight for process development and makes method development a more educated process. Knowing the differences between a contaminant and the target molecule can point to the most efficient separation technique. For example, knowing that the nearest contaminant in a reversed-phase separation is a deamidation product suggests using ion exchange prior to the reversed-phase step, even for a simple synthetic peptide separation. Knowing the identity of a contaminant can sometimes even suggest changes in fermentation or synthetic conditions to eliminate or reduce the contaminant at the source.

### Continuing Impact on Process Quality

When documenting production methods, SOPs written with resolution requirements and instructions for adjusting conditions to achieve the required resolution are inherently robust. The resolution mixture provides a functional test for system suitability and lot qualification for components (packing, buffers, etc.) in a validated method. It can be useful for troubleshooting. It permits flexibility in system specification, allowing adjustments to performance in order to meet specified requirements and avoiding single-source specifications for components. The SOP becomes a working document, allowing continuing investigation of secondary parameters and improvement in consistency and effectiveness of the method as experience is gained. And, the resolution mixture can be useful for developing and validating analytical methods as well.

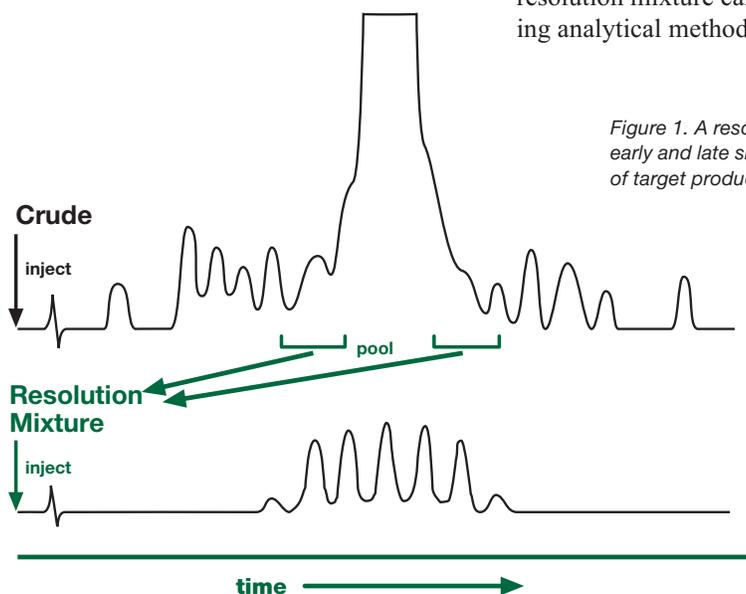


Figure 1. A resolution mixture is produced by pooling early and late side-fractions adjacent to the large peak of target product.

## Vydac Products for Process Chromatography

### 100 Kg+ Quantities Available

Vydac has a long history supplying over-100-kg orders of process separation media to pharmaceutical GMP facilities. In addition to large scale pre-packed columns, Vydac provides bulk material and shares expertise on packing the Prochrom Dynamic Axial Compression™ column, the SepTech Annular Expansion™ column, and other systems designed to be packed on-site.

### Chemically Identical Adsorbents

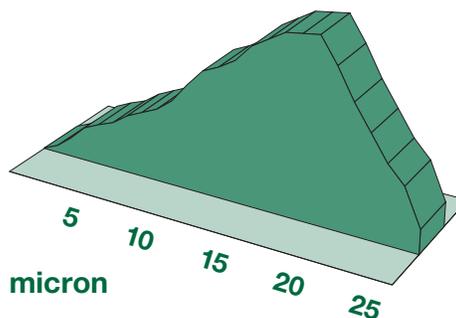
The manufacturing process for Vydac 300 angstrom pore diameter TP silica yields particle sizes from less than five to over twenty microns. Size fractionation yields five and ten micron silica for research scale separations and 10-15, 15-20 and 20-30 micron silica for larger scale chromatography. Vydac process silicas are virtually the same (except for particle size) as research scale silica.

Because they are chemically identical to Vydac's analytical adsorbents, Vydac process chromatography products are ideal for developing high resolution purification procedures for biopharmaceuticals. Research scale columns can be used to develop purification procedures in the laboratory for pre-clinical samples with the assurance that when clinical trials begin the purification procedure can be easily scaled up to 2 inch (50 mm) or 4 inch (100 mm) diameter columns to purify gram quantities of peptide. After PLA approval, Vydac Process Chromatography products can continue in the product stream in columns of 4 inch or larger or as bulk material in specialized column configurations to ensure long-term delivery of quality biopharmaceutical products.



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### Unique Synthetic Silica for Purity and Reproducibility

Reproducibility is important, and with silica-based packings control of the substrate is essential. Much attention has focused on silica surface and pore characteristics. But chemical impurities are also problematic.

Concern about chemical purity first led Vydac to pioneer synthetic silica processes, as opposed to silica sol methods used by other manufacturers. Silica sols are alkaline suspensions produced from naturally occurring silica. In addition to silicon and oxygen, they contain sodium ions and other impurities. Silica gels are produced when sols are acidified with sulfuric acid, leaving traces of sulfate and ion-exchange character.

In the late 1970s, Vydac began making silica in a new way, starting with purified organic silicates. Perfection of this technology led to chromatographic adsorbents of exceptional performance. The Vydac silica-based adsorbents available today employ silica manufactured by this unique synthetic process, providing assurance of superior purity and reproducibility to Vydac preparative and process customers.

### Vydac High-Purity Adsorbents

- Available in C4, C8, C18, and diphenyl reversed phases.
- Choose from small particles for analytical columns or large particles for process applications.
- Vydac routinely supplies 100 kg orders to pharmaceutical GMP facilities.

**To place an order or obtain a quotation for Vydac preparative and process products, call The Nest Group 800.347.6378 your Vydac distributor.**