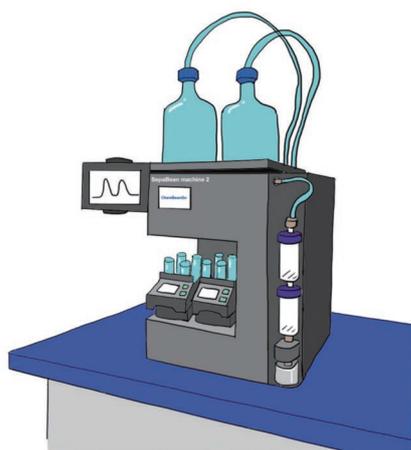


# The Improvement of the Resolution by Column Stacking and Its Application in the Compound Purification

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## Abstract

In liquid chromatography theory, resolution ( $R_s$ ) is a key parameter that determines the ability of a chromatography column to separate samples. The higher the Resolution, the easier it is to achieve baseline separation of the two different components in the sample. Resolution depends on column efficiency (usually expressed as the theoretical plate number,  $N$ ), selectivity ( $\alpha$ ) and retention factor (or capacity factor,  $k$ ). The equation to calculate resolution can be seen in Equation 1.

$$R_s = \frac{\sqrt{N}}{4} \frac{(\alpha-1)}{\alpha} \frac{k}{(k+1)}$$

### Equation 1. Resolution equation.

According to Equation 1, resolution as a function of column efficiency, selectivity or retention factor can be visualized in Figure 1.

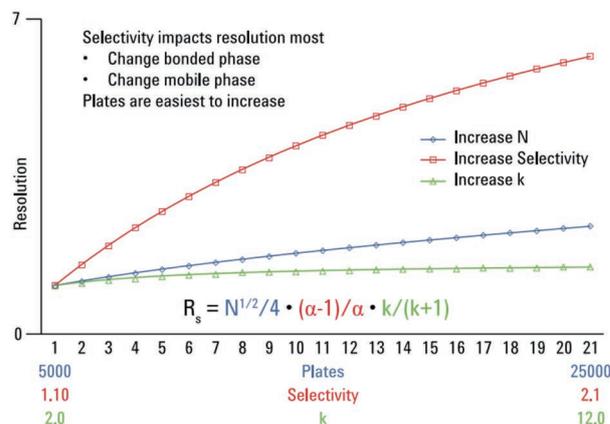


Figure 1. Resolution as a function of column efficiency, selectivity or retention factor<sup>[1]</sup>.

As can be seen from Figure 1, higher column efficiency (plate number) will bring better resolution. When other conditions remain unchanged, doubling plate number will increase the resolution by about 40%. In practical operation, plate number can be increased in two ways to improve resolution: 1) use long separation column or stack multiple columns in series; 2) use the separation column packed with smaller sized particles. The disadvantage of the former is that the separation time is prolonged, and the latter has the drawback of requiring the liquid chromatography system with higher pressure limit.

Selectivity refers to the ability of a stationary phase/mobile phase to separate two compounds. Selectivity is mainly related to factors such as stationary phase, mobile phase composition and column temperature. Also selectivity is closely related to retention factor. Among these factors, stationary phase and mobile phase composition have more influence on selectivity. Take the most commonly used separation mode, reversed phase (RP), as an example. On RP columns (including C18, C8, C4, etc), the compounds are separated based on their different partition coefficients between stationary phase and mobile phase. If there are significant differences in the water solubility as well as the partition coefficients in alkane/water system for two compounds, then these compounds can be separated by using a RP column.

For the capacity factor  $k$ , the resolution increases with the increase of the capacity factor, and this effect is very obvious when the  $k$  value is low. However, when the  $k$  value is greater than 10, the

effect on the resolution increase by the increase in  $k$  value is no longer significant. In general, increasing the density of the bonded phase will increase the  $k$  value. Besides, changing the type of bonded phase will also change the  $k$  value. For example, in RP chromatography, with the length increasing of the carbon chain which is bonded to the supporting matrix, the  $k$  value will increase accordingly.

As shown in Figure 1, improving selectivity is the best way to effectively improve resolution. However, in practice, improving selectivity refers to comparing different stationary phases, exploring different mobile phase systems, and so on. These optimization methods often require a lot of time and resources to achieve effective improvement on selectivity. In contrast, improving column efficiency is much simpler. Just simply stacking multiple columns in series can effectively improve the resolution. Due to its simple operation, this is a rather feasible and applicable way for the improvement of resolution. In this post, we will introduce the improvement of the resolution by column stacking and its application in the sample purification.

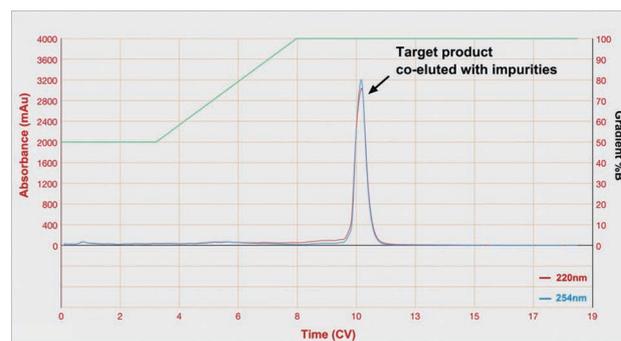
## Experimental Section

The sample is from a company focusing on new material research and development. For its weak polarity, RP flash cartridge is used for sample purification. The sample was dissolved in a proper solvent and loaded on the flash preparative liquid chromatography system. The experimental setup of the flash purification is listed in Table 1.

Instrument	SepaBean™ machine 2	
Cartridges	12 g SepaFlash™ Bonded Series C18 RP flash cartridge (spherical silica, 15 $\mu$ m, 100 Å, Order number: SW-5223-012-SP)	
Wavelength	220 nm (detection), 254 nm (monitoring)	
Mobile phase	Solvent A: Water Solvent B: Methanol	
Flow rate	30 mL/min	
Eluting gradient	Time (CV)	Solvent B (%)
	0.0	50
	3.5	50
	8.0	100
	18.5	100

**Table 1. The experimental setup for flash purification.**

As a start, single flash cartridge was used for the purification of the sample and the flash chromatogram was shown in Figure 2. As shown in Figure 2, the target product and the impurities were not separated at all during the elution procedure, thus appeared as a single elution peak in the chromatogram. Therefore, the resolution of single flash cartridge is far from enough for this sample.



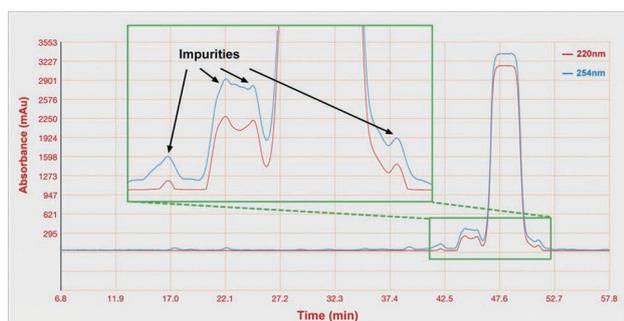
**Figure 2. The flash chromatogram of the sample on single flash cartridge.**

Next, we tried to stack two flash cartridges with the same specifications in series for the sample purification. The flash chromatogram was shown in Figure 3.



**Figure 3. The flash chromatogram of the sample on two stacking flash cartridges.**

As shown in Figure 3, some of the impurities in the sample were separated from the target product, however with low resolution. To obtain the target product with the purity meeting the requirements, the sample loss will be quite large under this condition. Therefore, we tried to stack three flash cartridges of the same specifications in series to further improve the resolution in the following experiments. The flash chromatogram was shown in Figure 4.



**Figure 4. The flash chromatogram of the sample on three stacking flash cartridges.**

As shown in Figure 4, the target product and the impurities were further separated. The cross sections of the elution peaks were greatly improved. The target product was collected under this condition. Further analysis suggested that the purity of the target product can meet the requirement (>99%). Moreover, the yield of the target product was also satisfying (~80%), indicating the sample loss during the purification procedure was lowered.

## Results and Discussion

Stacking multiple columns in series can effectively improve the resolution. However, as the number of the columns used in series increases, the back pressure of the system pipeline also rises significantly. Taking the separation conditions in this post as an example, the system pressure was about 160 ~ 180 psi when using single flash cartridge packed with 15  $\mu\text{m}$  particle sized media. When two stacking flash cartridges of the same specifications was used, the system pressure went up to 240 ~ 260 psi. And the system pressure could rise to about 350 -380 psi while using three stacking flash cartridges of the same specifications. General speaking, most medium or low pressure preparative liquid chromatography systems have a pressure limit of 200 psi. In order to protect the system pumps or other aspects, the system will give an indication of overpressure warning when the system pressure reaches ~180 psi during separation experiments. In case the system pressure continues to rise, the flow rate will be lowered by the system in order to protect the system pumps.

To resolve the above mentioned problems, Santai Technologies has launched new generation flash preparative liquid chromatography system, SepaBean™ machine 2, in which the system pressure limit reaches up to 500 psi. Combining SepaBean™ machine 2 with the SepaFlash™ series spin-welded flash cartridges which have the pressure limit of 400 psi, flash purification of complex samples will become a simple and quick routine operation.

## References

1. The LC Hand Book: Guide to LC Columns and Method Development. Agilent Technologies.

## About the SepaFlash™ Bonded Series C18 RP flash cartridges

There are a series of the SepaFlash™ Bonded Series C18 RP flash cartridges with different specifications from Santai Technology (as shown in Table 2).

Item Number	Column Size	Flow Rate (mL/min)	Max. Pressure (psi/bar)
SW-5223-004-SP	5.4 g	5-15	400/27.5
SW-5223-012-SP	20 g	10-25	400/27.5
SW-5222-025-SP	33 g	10-25	400/27.5
SW-5222-040-SP	48 g	15-30	400/27.5
SW-5222-080-SP	105 g	25-50	350/24.0
SW-5222-120-SP	155 g	30-60	300/20.7
SW-5222-220-SP	290 g	40-80	300/20.7
SW-5222-330-SP	420 g	40-80	250/17.2

**Table 2. SepaFlash™ Bonded Series C18 RP flash cartridges. Packing materials: High-efficiency spherical C18-bonded silica, 15  $\mu\text{m}$ , 100 Å.**

For further information on detailed specifications of SepaBean™ machine 2, or the ordering information on SepaFlash™ series flash cartridges, please visit our website:

<http://www.santaitech.com/en/index.php> .

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