The Exploration of Separation Method Scaling Up by SepaFlash™ Reversed-Phase Cartridges

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Chromatography Application Note AN008

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Introduction

As a separation technology in which high purity compounds could be obtained, preparative liquid chromatography (LC) has been developed rapidly in recent years. Compared with analytical LC, the purpose of running preparative LC is not to identify the components in the sample mixture but to separate and collect each of the components. In this case, these points should be considered including the purity of the collected components, recovery, operating time, solvent consumption, etc. There are two kinds of preparative LC, high pressure preparative LC and medium/low pressure preparative LC. The advantages of high pressure preparative LC are high separation efficiency as well as high purity and recovery for the sample. Meanwhile there are several disadvantages in high pressure preparative LC including low sample loading capacity, complex and expensive instruments, etc. As a comparison, medium/low pressure preparative LC has the following advantages including rapid separation procedure, high sample loading capacity, simple instruments and low cost in method development, etc. Therefore it has been more and more applied in pharmaceutical synthesis, natural product extraction, fine chemical and other fields in recent years.

For the synthesis staff focusing on small molecule drugs, they often deal with highly polar or highly lipophilic samples which are not suitable for normal phase separation due to the excessively strong interaction between the samples and the silica stationary phase in the normal phase (NP) column. In contrast, reversed-phase (RP) separation mode could meet the separation requirements of such samples. Therefore, RP separation mode has been more and more widely used. In this application note, the method scaling up in RP separation was explored. The establishment of RP separation method is usually based on the results of analytical HPLC. By adjusting the separation conditions, the separation method was transferred to RP flash columns (4 g, 12 g or 25 g sized) for small scale sample separation. With the separation method further optimized, the method was transferred to larger sized flash columns to achieve the purpose of purifying a large number of samples.

Experimental Section

In the application note, the SepaFlash™ Bonded Series flash cartridges from Santai Technologies were used for the exploration of scaling up in RP separation method. The cartridges were pre-packed with spherical silica which is bonded with C18. The diameter of the silica is 20 – 45 μm and the pore size is 100 Å. The specifications of this product series are shown in Table 1.

Column Size	Cartridge Length (mm)	Cartridge ID (mm)	Flow Rate (mL/min)	Sample Size
4g	105.5	12.4	5-15	5.4mg-108mg
12g	124.5	21.2	10-25	20mg-0.4g
25g	172.7	21.3	10-25	33mg-0.66g
40g	176.0	26.7	15-30	48mg-0.96g
80g	246.8	30.9	20-50	105mg-2.1g
120g	264.6	36.2	30-60	155mg-3.1g
220g	203.7	60.1	40-80	300mg-6.0g
330g	275.0	60.4	40-80	420mg-8.4g

Table 1. SepaFlash™ Bonded Series C18 flash cartridges. Packing materials: High efficiency spherical silica boned with C18, 20 - 45 μm, 100 Å.

Sample

A standard mixture was used for the preliminary study of the experimental parameters in separation method scaling up. Component A (0.1 g), component B (0.4 g) and component C (7.5 g) was dissolved in 100 mL methanol and fully mixed. The chemical structure of each component in the mixture was shown in Figure 1.

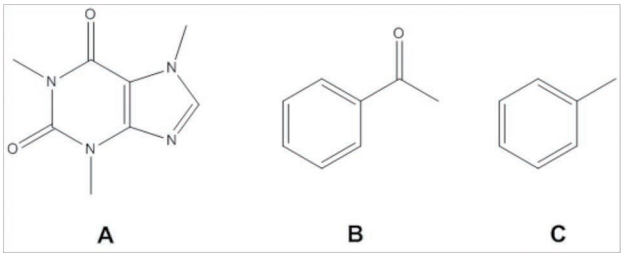

Figure 1. The chemical structure of each component in the mixture.

Sample Loading Capacity and the Choice of the Flash Cartridges

The scaling up of the separation method is to magnify the load of the sample to be purified. Larger sample load requires larger flash cartridges. In

general, the sample loading capacity for the RP flash cartridges is about 0.5% - 1% of the pre-packed material weight. For some easily separated samples, the loading capacity could exceed the limitation. Therefore, the sample load should be adjusted according to the sample properties in specific situation.

Experimental Parameters and Results Discussion

The flash cartridges used in the experiments were 25 g and 80 g sized. As a start, we choose a 25 g SepaFlash™ Bonded Series C18 cartridge for the purification of the sample mixture of 1.2 mL. The experimental settings were listed in Table 2 and the flash chromatogram was shown in Figure 2. When the sample load was 96 mg, which was approximately 0.4% of the pre-packed material weight for the 25 g cartridge, the chromatogram showed that all the components in the sample mixture were completed separated at the baseline level. Based on this result, we used an 80 g cartridge to purify 4 mL sample mixture, only the flow rate was adjusted and meanwhile the elution gradient was kept without modification. The flash chromatogram was shown in Figure 3. Comparing Figure 2 and Figure 3, it could be concluded that excellent reproducibility and reliability was achieved by keeping the ratio of sample load for different cartridges, suggesting predictable performance for RP separation method scale-up.

Instrument	SepaBean™ machine	
Flash cartridge	25 g SepaFlash™ Bonded Series C18 cartridge (20-45μm, 100Å, Order #: SW-5222-025-SP)	80 g SepaFlash™ Bonded Series C18 cartridge (20-45μm, 100Å, Order #: SW-5222-080-SP)
Flow rate	25 mL/min	35 mL/min
Sample load	1.2 mL (96 mg)	4.0 mL (320 mg)
Wavelength	254 nm (detection), 280 nm (monitoring)	
Mobile phase	Solvent A: Water Solvent B: Methanol	
Gradient	Column volume (CV)	Solvent B (%)
	0	25
	6	90
	8	90

Table 2. The experimental setup for flash separation.

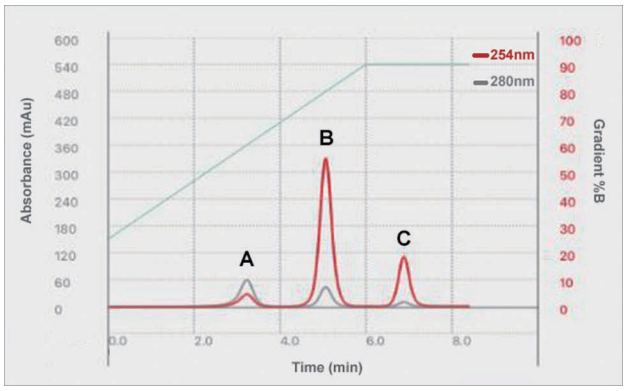

Figure 2. The flash chromatogram of 1.2 mL sample mixture on a 25 g flash cartridge.

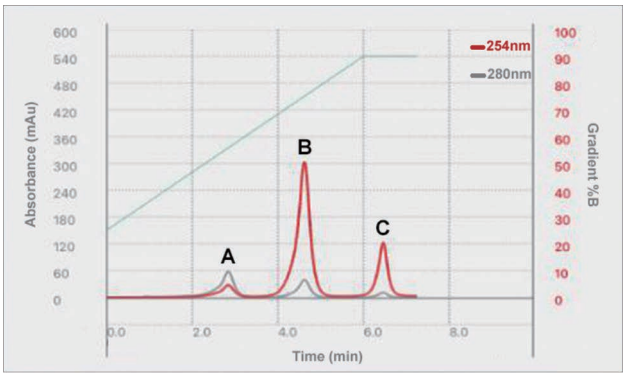

Figure 3. The flash chromatogram of 4.0 mL sample mixture on an 80 g flash cartridge.

In conclusion, when we are dealing with the purification for a large amount of samples, a small sized flash cartridge could be used as a start for separation method exploration of a small amount of sample. In the following step, the optimized separation method could be directly applied to a larger sized cartridge for larger sample load. In this way a large amount of samples could be rapidly purified with improved efficiency, reducing both time and solvent consumption.

Method Validation

To verify the actual effect of separation method scaling up by RP flash cartridges, the final product of a synthetic reaction was used as the sample. The chemical structure of the sample was shown in Figure 4. It should be noticed that the sample is high in polarity, which requires RP flash cartridges for purification. Furthermore, the target product should be separated and purified from impurities since synthetic reaction will always generate unwanted by-products. Finally, the purity of the target product should be more than 95% for most of the time.

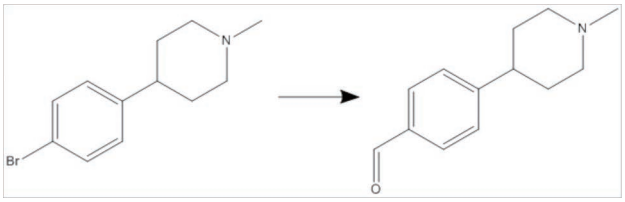

Figure 4. The final step of a synthetic reaction. Right part shows the target product.

1.25 g of the final product was taken as the sample and dissolved in 10 mL methanol with fully mixture. The experimental settings of the flash purification were listed in Table 3. And the flash chromatograms were shown in Figure 5 and Figure 6.

Instrument	SepaBean™ machine	
Flash cartridge	25 g SepaFlash™ Bonded Series C18 cartridge (20-45μm, 100Å, Order #: SW-5222-025-SP)	120 g SepaFlash™ Bonded Series C18 cartridge (20-45μm, 100Å, Order #: SW-5222-120-SP)
Flow rate	25 mL/min	40 mL/min
Sample load	1.3 mL (162 mg)	8.0 mL (1.0g)
Wavelength	220 nm (detection), 254 nm (monitoring)	
Mobile phase	Solvent A: water Solvent B: methanol	
Gradient	Time (min)	Solvent B (%)
	0	10
	20	40
	33	40
	35	46
	50	46
	70	60
	72	90
	85	90

Table 3. The experimental setup for flash purification.

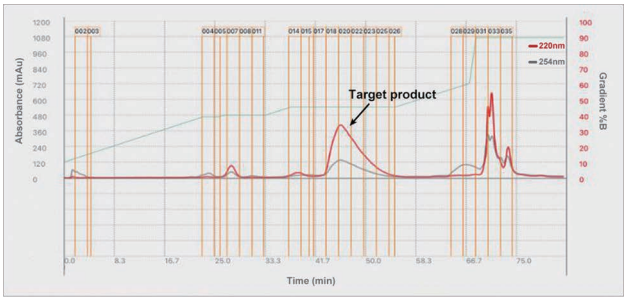

Figure 5. The flash chromatogram of the synthetic product on a 25 g flash cartridge.

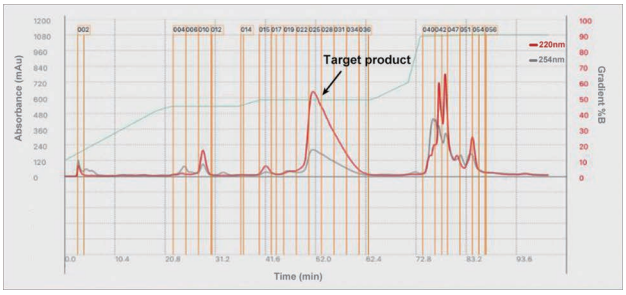

Figure 6. The flash chromatogram of the synthetic product on a 120 g flash cartridge.

As shown in Figure 5 and Figure 6, during the separation method scaling up for actual sample, only flow rate should be adjusted in accordance with specific sized cartridge and good performance could be achieved without any changes to other parameters. Furthermore, the retention time and peak shape of the target product and other impurities shows high consistency in the chromatogram, indicating a producible and reliable separation method scaling up. The purity of the target product is >95% by HPLC analysis, meeting the requirements to purity of the target product.

Conclusion

In the application note, the sample load of a synthetic product is scaled up from 162 mg to 1.0 g (6x fold scale-up) and the related flash cartridge size is also changed from 25 g to 120 g. In this case the sample was successfully separated and purified with good resolution and reproducibility. This result shows a general method for scaling up of high polar sample separation and purification by the SepaBean™ machine combined with the SepaFlash™ RP cartridges. The whole procedure is simple and convenient, suggesting an efficient solution for research staff when dealing with related projects.

About SepaFlash™ Bonded Series C18 cartridges

There are a series of the SepaFlash™ Bonded Series C18 cartridges with different specifications from Santai Technology (as shown in Table 4).

Item Number	Weight of Pre-packed Materials	Flow Rate (mL/min)	Max. Pressure (psi/bar)
SW-5222-004-SP	5.4 g	5-15	400/27.5
SW-5222-012-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	20-50	350/24.0
SW-5222-120-SP	155 g	30-60	300/20.7
SW-5222-220-SP	300 g	40-80	300/20.7
SW-5222-330-SP	420 g	40-80	250/17.2

Table 4. SepaFlash™ Bonded Series C18 cartridges. Packing materials: High efficiency spherical silica bonded with C18, 20 - 45 µm, 100 Å.

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