SepaFlash Large: Purification Products for Samples up to Hundreds of Grams

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Chromatography Application Note AN005

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Introduction

With the continuous development of medium/low pressure preparative chromatography instruments, more and more researchers tend to use automatic instruments to separate and purify samples owing to its great convenience and efficiency. However, due to the limitation of instrument configuration or other factors, researchers usually handle with small amounts of sample (less than 10g) by the instrument. When handling with dozens or even hundreds of grams of samples, column packing, eluting and collecting by manual methods are always employed by researchers (as shown in Figure 1).

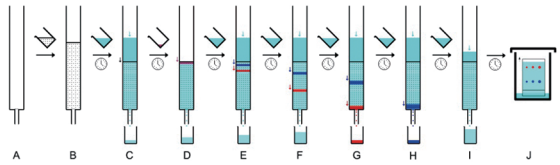

Figure 1. Schematic diagram of traditional manual column chromatography. A–B: Column packing. C: Column equilibrium. D. Sample loading. E-F: Elution. G-H: Fraction collection. I: Column cleaning. J: Detection by TLC.

To improve the working efficiency of the researchers, SepaFlash Large purification cartridge series are developed. The maximum packing size is up to 3kg of silica gel, making the large cartridge able to purify hundreds of grams of crude samples in single run. In this application note, an 800g-sized SepaFlash large cartridge pre-packed with ultra-pure silica gel (Product number: S-5101-0800) combined with a preparative flash chromatography system was utilized for the purification of 100g sample. The results showed higher product yield as well as lower time and solvent consumption comparing with manual method, suggesting a better method for large amount sample purification.

Experimental

Cartridge installation and equilibrium

An 800g SepaFlash Standard Series flash cartridge (Order number: S-5101-0800) was used in the separation experiment. The inlet and outlet for the mobile phase were properly connected with tubes according to the Guidelines on Santai Adaptor Kit for 800g and 1600g Flash Columns. Afterwards n-hexane was used to equilibrate the cartridge until the silica gels packed in the cartridge were completely wetted.

Solid sample loading

A 100g of sample mixture (as shown in Figure 2) was dissolved in ethyl acetate and absorbed onto 150g of silica gel of 100-200 meshes. Ethyl acetate was removed by vacuum and the absorbed sample placed in a 220 iLOK empty cartridge for sample loading (as shown in Figure 3). Sample was then eluted automatically by a preparative flash chromatography system according to the parameters as shown in Table 1.

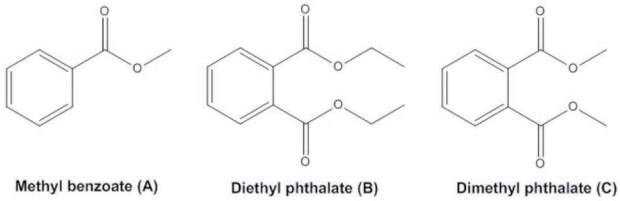

Figure 2. The chemical structure of Sample A, B and C.

Figure 3. The experimental setup for automatic flash chromatography.

Table 1. The experimental parameters for automatic flash chromatography

Instrument	A preparative flash chromatography system	
Cartridges	800g SepaFlash™ Standard Series flash cartridge (UltraPure irregular silica, 40-63μm, 60Å, Order number: S-5101-0800)	
Wavelength	254 nm (detection), 280 nm (monitoring)	
Sample	100g mixture of Sample A (5.0g), B (6.0g) and C (89.0g)	
Mobile phase	Solvent A: N-hexane Solvent B: Ethyl acetate	
Flow rate	120 mL/min	
Gradient	Solvent B (%)	Time (min)
	0	0
	0	3
	7	4
	10	13
	12	14
	25	52
	25	56

Manual column chromatography

1000 grams of silica gel (irregular silica, particle size: 40-63 μm, pore size: 60 Å) were slowly poured into a measuring glass with 1500 ml of mixed solvent (n-hexane and ethyl acetate, V/V = 95:5). The mixture was stirred evenly and then slowly poured into a glass column with gently tapping on the column body. When the solvent level reached the top of the silica gel, the sample mixture was then slowly added into the glass column. After pouring 50 grams of quartz sand for cushioning, then the column was rinsed by n-hexane and ethyl acetate (V/V = 95:5) for 20 minutes. The polarity of the mobile phase was gradually increased until the sample mixture was completely eluted.

Results and Discussion

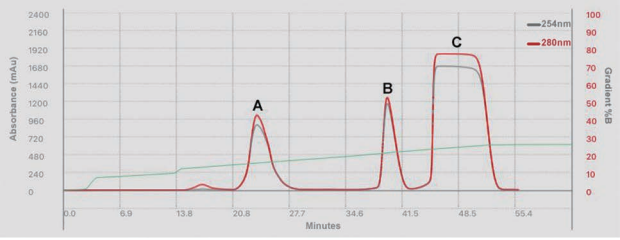

Figure 4. The chromatogram of the sample mixture in an 800g SepaFlash Standard Series flash cartridge.

As shown in Figure 4, the large amount of the sample mixture (about 100g) was successfully separated and purified in a single run. Combining the results from manual column chromatography, the separating results were summarized in Table 2 and illustrated in Figure 5 and Figure 6.

Table 2. The comparison of the separating results.

	Time consumption	Solvent consumption	Amount and yield for Sample A	Amount and yield for Sample B	Amount and yield for Sample C
Automatic flash chromatography	0.9 h	6.6 L	4.93g (98.6%)	5.85g (97.5%)	86.5g (97.2%)
Manual column chromatography	6 h	20 L	4.67g (93.4 %)	4.12g (68.7%)	69.4g (78.0%)

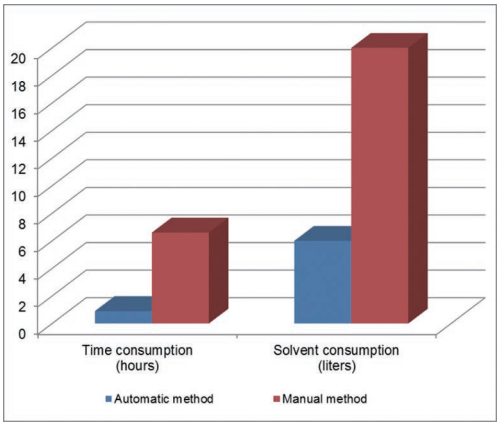

Figure 5. The comparison of the time and solvent consumption between automatic and manual methods.

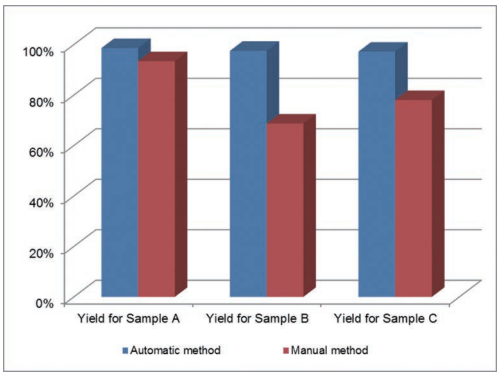

Figure 6. The comparison of the yield for Sample A, B and C between automatic and manual methods.

Comparing with manual packed columns, the SepaFlash cartridge pre-packed by automated instrument has higher product yield as well as lower time and solvent consumption. In traditional manual separation by glass column, much time was spent during the procedure from glass column packing to manual fraction collection then frequently fraction confirmation by thin layer chromatography (TLC). In contrast, about 85% of the purification time could be saved with the help of commercial flash cartridge combined with automated flash chromatography system. Furthermore, the programmable gradient elution not only reduces the solvent consumption (6.6 L V.S. 20 L, about 67% of the solvent was saved) but also greatly decreases the solvent amount in the collected fractions, which in result reduces post treatment time (e.g., solvent removal by rotary evaporation). Moreover, due to the automatic column packing technology, the pre-packed flash cartridge has higher column efficiency than manually packed glass column, resulting in higher yield for purification products.
In conclusion, comparing with traditional manual column chromatography, SepaFlash Large purification cartridge combined with a preparative flash chromatography system offered a better method for large amount sample purification.

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