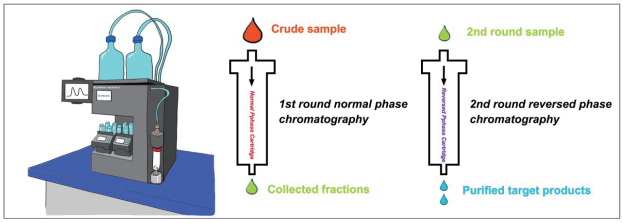
The Application of Orthogonal Chromatography for the Purification of Synthetic Pharmaceutical Intermediates

a santai science company
SANTAI TECHNOLOGIES quality since 2004

Chromatography Application Note
AN024

Meiyuan Qian, Bo Xu
Application R&D Center



Introduction

Normal phase chromatography and reversed phase chromatography are two separation modes commonly used in flash chromatography and they are widely used in the separation and purification of various organic synthetic products. In normal phase chromatography, with the combination of polar stationary phase (such as bare silica, alumina, silica bonded with diol, amino or cyano groups) and non-polar mobile phase (such as n-hexane), the sample molecules are separated according to their different polarities. Normal phase chromatography is also called adsorption chromatography since its separation basis is adsorption effect. As a comparison, in reversed phase chromatography the sample was separated with the elution by polar stationary phase (such as acetonitrile, methanol) flowing through non-polar stationary phase (such as silica bonded with C18 groups). These two separation modes are based on different separation mechanisms. Therefore they can be called orthogonal chromatography when they are combined used, bringing higher resolution and better separation performance for complex samples.

In this post, SepaFlash™ Standard Series silica cartridges as well as SepaFlash™ Bonded Series C18 cartridges were combined used for the purification of a synthetic pharmaceutical intermediate. As a result, the sample was purified and the target product which could meet the purity requirement was obtained, suggesting a good method for the rapid purification of these complex samples.

Experimental Section

The sample was provided by a company focusing on new drug research and development. Firstly, a small amount of the sample was dissolved in methanol for purity analysis by HPLC. As shown in Figure 1, the purity of the raw sample was about 66%. One of the impurities had quite similar retention time with the target product, proposing higher requirements on the separation procedure.

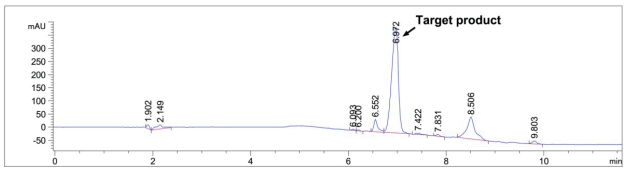


Figure 1. The chromatogram of raw sample by HPLC analysis.

Due to the high polarity of the sample, normal phase chromatography was considered to be the separation method as the first trial. To prepare the sample solution, 3 g of the raw sample was dissolved in methanol. Then the sample was absorbed onto 6 g of silica (the same type with the one packed in cartridge which was used in flash purification). After the organic solvent was removed by rotary evaporation, the sample was poured into an empty 12 g iLOK™ cartridge for solid sample loading (order number: SD-0000-012). The experimental setup of flash chromatography for the sample was listed in Table 1. And the flash chromatogram of the sample by normal phase chromatography was shown in Figure 2.

Instrument	SepaBean™ machine T
Cartridges	80 g SepaFlash™ Standard Series silica cartridge (irregular silica, 40 - 63 μm, 60 Å, Order number: S-5101-0080)
Wavelength	220nm (detection), 254 nm (monitoring)
Mobile phase	Solvent: ethyl acetate
Flow rate	50 mL/min
Sample loading	3 g of raw sample
Gradient	Isocratic elution

Table 1. The experimental setup for normal phase flash chromatography.

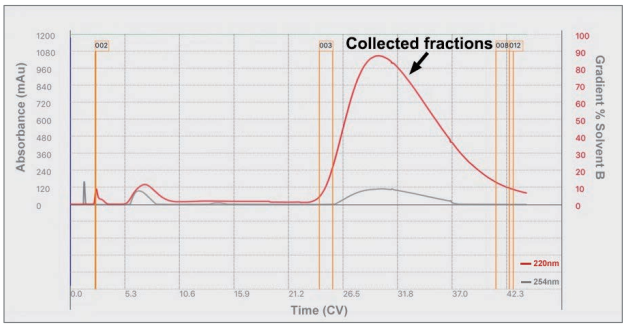


Figure 2. The flash chromatogram of the raw sample by a normal phase cartridge.

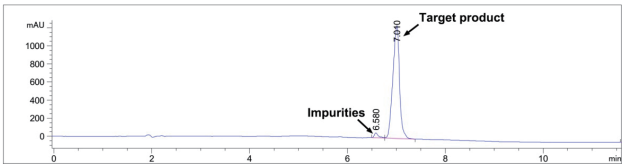


Figure 3. The HPLC chromatogram of the collected fractions from normal phase flash purification.

The collected fractions from normal phase flash purification were analyzed by HPLC. As shown in Figure 3, the purity of the collected fractions was about 92% and could not meet the requirement of 95% or higher in purity. As can be seen from Figure 3, the target product was not completely purified from the impurities which have approximate retention time with the target product. Considering the orthogonality between reversed phase chromatography and normal phase chromatography, additional resolution may be provided when hyphenating reversed phase separation with normal phase separation. Therefore the collected fractions from normal phase separation were rotary evaporated to remove the solvents. Then 300 mg of the sample was dissolved in 2.5 mL methanol with additional several drops of DMSO for complete sample dissolution. After that the liquid sample was loaded onto a C18 reversed phase flash cartridge by an injector. The experimental setup of flash chromatography for the sample was listed in Table 2. And the flash chromatogram of the sample by reversed phase chromatography was shown in Figure 4.

Instrument	SepaBean™ machine T	
Cartridges	120 g SepaFlash™ Bonded Series C18 cartridge (spherical silica, 20 - 45 μm, 100Å, Order number: SW-5222-120-SP)	
Wavelength	220nm (detection), 254 nm (monitoring)	
Mobile phase	Solvent A: water Solvent B: methanol	
Flow rate	30 mL/min	
Sample loading	2.5 mL (300 mg)	
Gradient	Time (CV)	Solvent B (%)
	0	60
	8	80
	12	80
	12.5	100
	13.5	100

Table 2. The experimental setup for reversed phase flash chromatography.

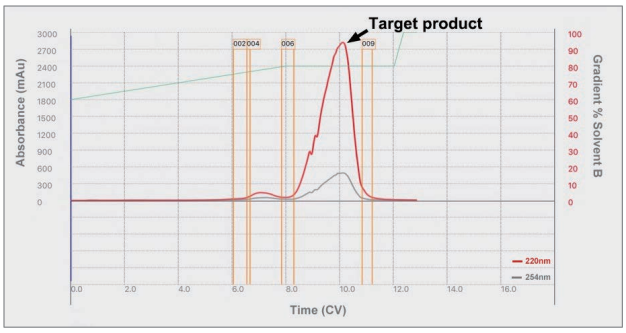


Figure 4. The flash chromatogram of the collected fractions from normal phase separation by a reversed phase cartridge.

The collected fractions from reversed phase separation were analyzed by HPLC (data not shown) and the results indicated that the purity was about 98%. With the purity meeting the requirement, the product could be used in the next step research and development.

Conclusion

With the combination of normal phase chromatography and reversed phase chromatography, the resolution of the flash purification can be effectively improved. This strategy is especially suitable for the purification of some complex samples which could not be purified by single separation mode, either normal phase or reversed phase. Meanwhile, flash chromatography has great advantages in both instrument and column cost when compared with preparative HPLC, suggesting a cost-effective purification solution for researchers.

About the SepaFlash™ Standard Series and Bonded Series C18 flash cartridges

There are a series of the SepaFlash™ Standard Series and Bonded Series C18 flash cartridges with different specifications from Santai Technology (as shown in Table 3 and Table 4).

Item Number	Column Size	Sample Size	Flow Rate (mL/min)	Max.Pressure (psi/bar)
S-5101-0004	4 g	4 mg - 0.4 g	15-40	300/20.7
S-5101-0012	12 g	12 mg - 1.2 g	30-60	300/20.7
S-5101-0025	25 g	25 mg - 2.5 g	30-60	300/20.7
S-5101-0040	40 g	40 mg - 4.0 g	40-70	300/20.7
S-5101-0080	80 g	80 mg - 8.0 g	50-100	200/13.8
S-5101-0120	120 g	120 mg - 12 g	60-150	200/13.8
S-5101-0220	220 g	220 mg - 22 g	80-220	150/10.3
S-5101-0330	330 g	330 mg - 33 g	80-220	150/10.3
S-5101-0800	800 g	800 mg - 80 g	100-300	100/6.9
S-5101-1600	1600 g	1.6 g - 160 g	200-500	100/6.9
S-5101-3000	3000 g	3.0 g - 300 g	200-500	100/6.9

Table 3. SepaFlash™ Standard Series flash cartridges. Packing materials: UltraPure irregular silica, 40 - 63 µm, 60 Å.

Item Number	Column Size	Sample Size	Flow Rate (mL/min)	Max.Pressure (psi/bar)
SW-5222-004-SP	5.4 g	5.4 mg - 108 mg	5-15	400/27.5
SW-5222-012-SP	20 g	20 mg - 0.40 g	10-25	400/27.5
SW-5222-025-SP	33 g	33 mg - 0.66 g	10-25	400/27.5
SW-5222-040-SP	48 g	48 mg - 0.96 g	15-30	400/27.5
SW-5222-080-SP	105 g	105 mg - 2.1 g	25-50	350/24.0
SW-5222-120-SP	155 g	155 mg - 3.1 g	30-60	300/20.7
SW-5222-220-SP	300 g	300 mg - 6.0 g	40-80	300/20.7
SW-5222-330-SP	420 g	420 mg - 8.4 g	40-80	250/17.2
S-5101-0800	800 g	800 mg - 80 g	100-300	100/6.9
S-5101-1600	1600 g	1.6 g - 160 g	200-500	100/6.9
S-5101-3000	3000 g	3.0 g - 300 g	200-500	100/6.9

Table 4. SepaFlash™ Bonded Series C18 flash cartridges. Packing materials: High-capacity spherical C18, 20 - 45 µm, 100 Å.

For further information on detailed specifications of SepaBean™ machine, or the ordering information on SepaFlash™ series flash cartridges, please visit our website:
http://en.santaitech.com/.

Santai Technologies Inc.
Address: No. 78 Qingyang Road, Xinbei District, Changzhou, Jiangsu Province, China
Website: www.santaitech.com

a santai science company
SANTAI™ TECHNOLOGIES quality since 2004

CHINA
Tel.: +86 (519) 8515 0175
Fax: +86 (519) 8515 3561
Email: info@santaitech.com
Website: www.santaitech.com

CANADA
Tel: +1 418-580-0437
Order mail: ca_order@santaitech.com
Support mail: ca_support@santaitech.com

INDIA
Tel: +91 937-181-2696
Order mail: in_order@santaitech.com
Support mail: in_support@santaitech.com