The Application of SepaBean™ Machine in the Field of Organic Optoelectronic Materials

a santai science company
SANTAI TECHNOLOGIES quality since 2004

Chromatography Application Note
AN010

Meiyuan Qian, Bo Xu
Application R&D Center

Recently, a request to purify very polar and low soluble sample from a pharmaceutical company was received by the purification engineer in Santai Technologies. According to the sample properties, normal phase separation was firstly utilized. However, the result was poor due to the strong retention of the sample on the normal phase flash cartridge, making the sample hard to be eluted out. Next, reversed-phase separation mode was tried out. 50 mg of the sample was completely dissolved in 5 mL acetonitrile/methanol/water/DMF (V : V : V : V = 23 : 23 : 2 : 2) with heating and ultrasonic. Afterwards the elution gradient was set according to the HPLC analysis results of the sample. The experimental setup of the flash purification for the sample was listed in Table 1.

Instrument	SepaBean™ machine	
Flash cartridge	25 g SepaFlash™ Bonded Series C18 cartridge (40 - 60 μm, 120 Å, Order number: SW-5231-025-SP)	
Wavelength	220 nm (detection), 254 nm (monitoring)	
Mobile phase	Solvent A: Water Solvent B: Acetonitrile	
Flow rate	20 ml/min	
Sample load	5 mL (50 mg)	
Gradient	Time (min)	Solvent B (%)
	0	5
	20	35
	23	35
	26	40
	30	40
	43	60
	45	90
	50	90

Table 1. The experimental setup for flash purification of small amount sample.

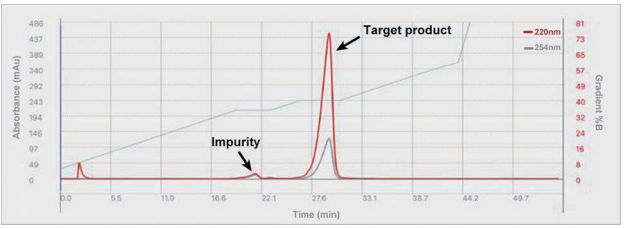

Figure 1. The flash chromatogram of small amount sample.

The flash chromatogram of the small amount sample was shown in Figure 1. According the results of preliminary separation experiment, the target product could be effectively separated from the impurities and the demand of collecting both the target product and the impurity could be met. Next, scale-up separation experiment was performed. 1 g of the sample was completely dissolved in 50 mL acetonitrile/methanol/water/DMF (V : V : V : V = 23 : 23 : 2 : 2) with heating and ultrasonic. The experimental setup of the flash purification was listed in Table 2.

Instrument	SepaBean™ machine	
Flash cartridge	330 g SepaFlash™ Bonded Series C18 cartridge (spherical silica, 40 - 60 μm, 120 Å, Order number: SW-5231-330-SP)	
Wavelength	220 nm (detection), 254 nm (monitoring)	
Mobile phase	Solvent A: Water Solvent B: Acetonitrile	
Flow rate	55 ml/min	
Sample load	50 mL (1 g)	
Gradient	Time (min)	Solvent B (%)
	0	10
	2	12
	10	12
	23	27
	36	27
	38	30
	41	30
	55	50
	62	50
	64	90
	70	90

Table 2. The experimental setup for scale-up flash purification.

Figure 2. The chromatogram of scale-up flash purification.

As show in Figure 2, the scale-up separation for the sample was not satisfying due to the solvent effect. Since DMF would interfere with the detection of the sample due to its strong absorbance in the UV band, therefore high ratio of methanol and acetonitrile was used in the preparation of the sample. In this condition, volume overload occurred when large volume of the sample was loaded, resulting a large amount of the sample eluted quickly with the elution solvents without enough retention as well as separation. Furthermore, the effective bed height of the 330 g flash cartridge did not increase proportionally to the 25 g flash cartridge, thus the column efficiency of the 330 g flash cartridge was lower than the 25 g flash cartridge. This is another reason for the failure to effectively separate the target product from the impurities.

Based on the discussion above, it was impossible to complete the task in one step. Multiple injections and separations for small amount of sample dissolved in DMF were performed in the following experiments. 600 mg of the sample was completely dissolved in 6 mL DMF with heating and ultrasonic. The experimental setup of the flash purification was listed in Table 3.

Instrument	SepaBean™ machine	
Flash cartridge	120 g SepaFlash™ Bonded Series C18 cartridge (spherical silica, 40 - 60 μm, 120 Å, Order number: SW-5231-120-SP)	
Wavelength	220 nm (detection), 254 nm (monitoring)	
Mobile phase	Solvent A: Water Solvent B: Acetonitrile	
Flow rate	40 ml/min	
Sample load	6 mL (600 mg)	
Gradient	Time (min)	Solvent B (%)
	0	10
	15	26
	25	26
	50	50
	52	90
	62	90

Table 3. The experimental setup for improved scale-up flash purification.

A total amount of 6.5 g sample was purified using multiple injections with the same experimental settings as listed in Table 3. The flash chromatograms of two injections were shown in Figure 3. As shown in Figure 3, the sample was effectively separated from the impurities with good reproducibility. As shown in Figure 4, the analytical HPLC indicated that the purity of the target product is 98.4% and the yield is 89.4%. For the impurities, the purity is 88.1% and the yield is 74.8%.

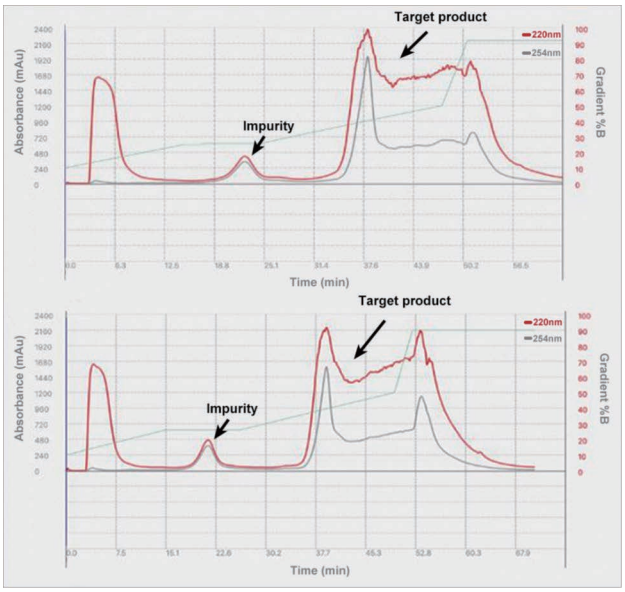

Figure 3. The chromatogram of improved scale-up flash purification.

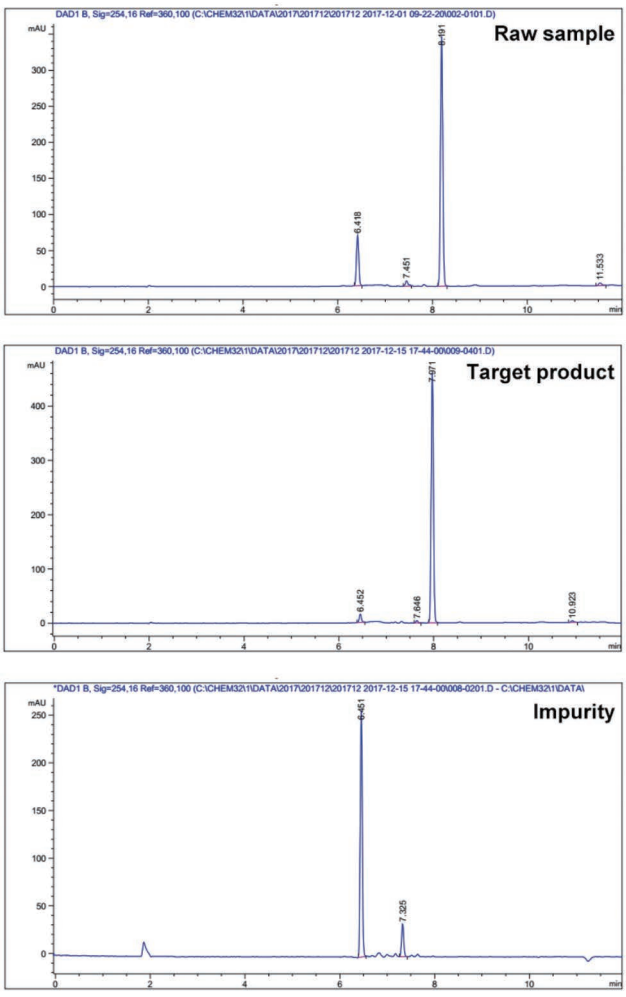

Figure 4. The analytical HPLC chromatogram of the raw sample, the target product and the impurities.

In conclusion, for the purification of such samples that are very polar and difficult to dissolve in water, a feasible way is to dissolve the sample in small amount of organic solvents following with multiple injections on the flash cartridges. The SepaBean™ machine from Santai Technologies combined with SepaFlash™ series reversed-phase flash cartridges could offer fast and efficient solutions to help researchers easily complete various types of sample purification tasks.
For further information on detailed specifications of SepaBean™ machine, or the ordering information on SepaFlash™ series flash cartridges, please visit our ChemBeanGo online store: https://store.chembeango.com/.

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

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