The Application of SepaFlash® Strong Cation Exchange Chromatography Columns in the Purification of Alkaline Compounds

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Introduction

Ion exchange chromatography (IEC) is a chromatographic method commonly used to separate and purify the compounds which are presented in ionic form in solution. According to the different charge states of exchangeable ions, IEC can be divided into two types, cation exchange chromatography and anion exchange chromatography. In cation exchange chromatography, acidic groups are bonded to the surface of the separation media. For example, sulfonic acid ($-SO_3H$) is a commonly used group in strong cation exchange (SCX), which dissociates H^+ and the negatively charged group $-SO_3^-$ can thus adsorb other cations in the solution. In anion exchange chromatography, alkaline groups are bonded to the surface of the separation media. For instance, quaternary amine ($-NR_3OH$, where R is hydrocarbon group) is usually used in strong anion exchange (SAX), which dissociates OH^- and the positively charged group $-N^+R_3$ can adsorb other anions in the solution, resulting in anion exchange effect.

In flash chromatography, the commonly used separation media for ion exchange is silica gel matrix where ion exchange groups are bonded to its surface. The most commonly used ion exchange modes in flash chromatography are SCX (usually sulfonic acid group) and SAX (usually quaternary amine group). In the synthesis of pharmaceutical intermediates, many nitrogen-containing heterocyclic compounds are usually alkaline in solution, including imidazole, pyridine, quinolone, piperidine and etc. In addition, as natural products, alkaloids are a class of alkaline organic compounds containing nitrogen, which have attracted the attention of researchers for their remarkable biological activities and thus have broad application prospects in drug development. For the separation and purification of these alkaline compounds, ion exchange chromatography is an alternative option in addition to conventional normal phase and reversed phase chromatography. In this post, a mixture of a synthetic compound and another standard was used as the sample to explore the application of SCX columns in the purification of alkaline compounds.

Experimental Section

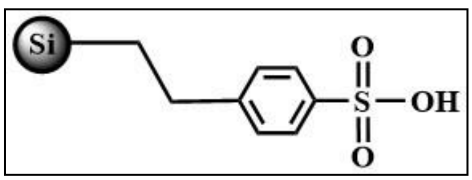

Figure 1. The schematic diagram of the stationary phase bonded to the surface of SCX separation media.

In this post, a SCX column pre-packed with sulfonic acid bonded silica was used (as shown in Figure 1). A mixture of Chromone and a nitrogen-containing heterocyclic compound was used as the sample to be purified (as shown in Figure 2). The mixture was dissolved in methanol and loaded onto the flash cartridge by an injector. The experimental setup of the flash purification is listed in the Table 1.

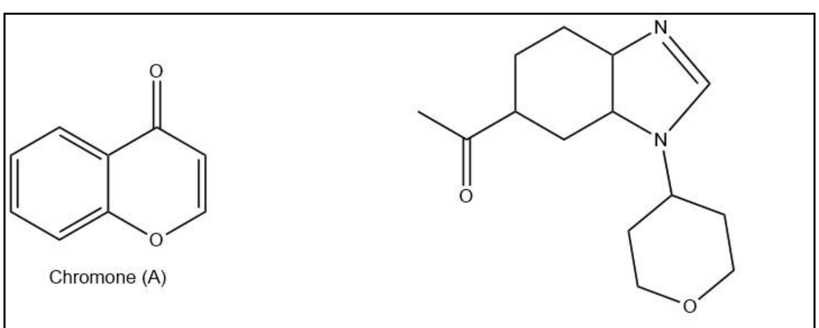

Figure 2. The chemical structure of the two components in the sample mixture.

Instrument	**SepaBean™ machine 2**	
Cartridges	4 g SepaFlash® Standard Series flash cartridge (irregular silica, 40 - 63 μm, 60 Å, Order number: S-5101-0004)	4 g SepaFlash® Bonded Series SCX flash cartridge (irregular silica, 40 - 63 μm, 60 Å, Order number：SW-5701-004-IR)
Wavelength	254 nm (detection), 280 nm (monitoring)	
Mobile phase	Solvent A：DCM Solvent B：Methanol	
Flow rate	15 mL/min	
Sample loading	30 mg (a mixture of Component A and Component B)	
Gradient	Time (CV)	Solvent B (%)
	0	0
	2	0
	19	20
	20	20

Table 1. The experimental setup for flash purification.

Results and Discussion

Firstly, the mixture sample was separated on a regular normal phase flash cartridge. As shown in Figure 3, the two components in the sample were eluted from the flash cartridge one after another. Next, a SCX flash cartridge was utilized for the purification of the sample. As shown in Figure 4, the alkaline Component B in the sample was totally retained on the SCX cartridge, while another neutral Component A (Chromone) was rapidly eluted from the cartridge.

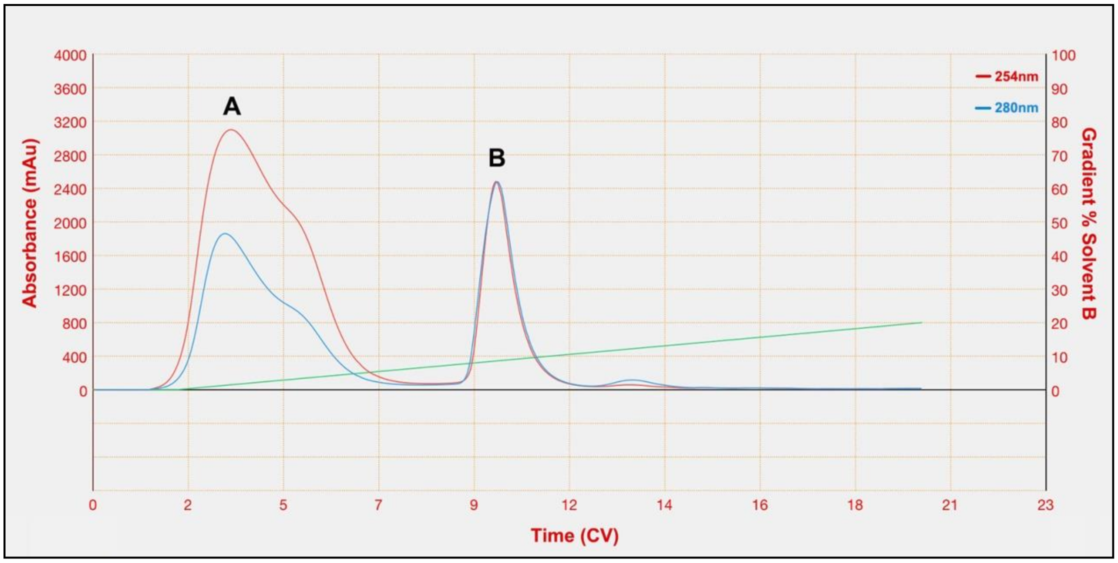

Figure 3. The flash chromatogram of the sample on a regular normal phase cartridge.

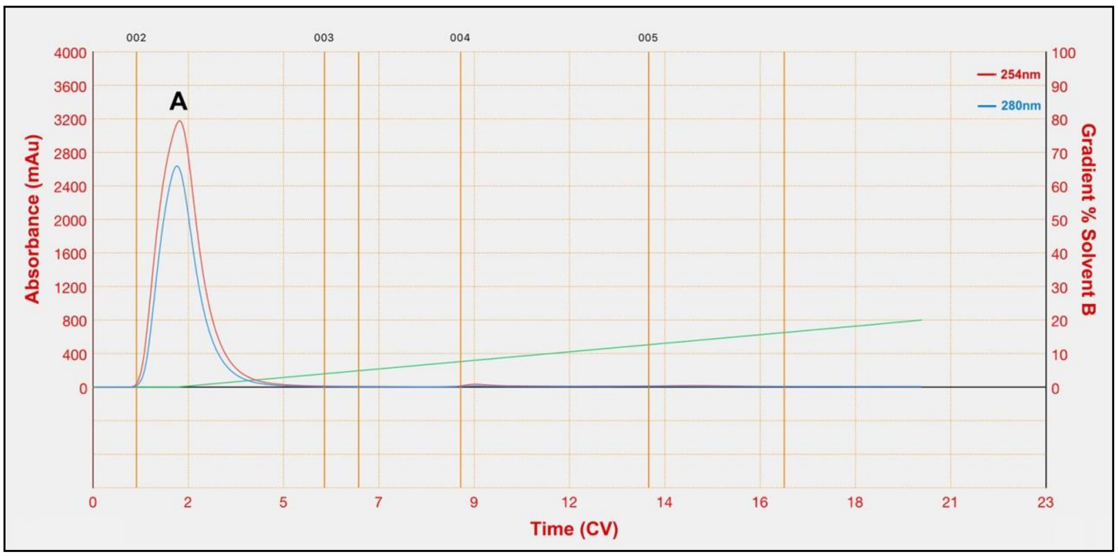

Figure 4. The flash chromatogram of the sample on a SCX cartridge.

If the target product to be collected is the alkaline Component B, the following purification strategy could be considered.

- Load the sample on the SCX cartridge to capture the alkaline target product on the stationary phase, while other neutral or acidic impurities will be eluted to waste by the mobile phase.
- By monitoring the chromatogram, when the first step is completed, switch the mobile phase to 5% ammonia in methanol solution to elute the captured alkaline target product. In this case, the SCX cartridge works in a capture-release manner.

Let's consider another scenario that the target product to be collected is the neutral

Component A. The following purification strategies can be adopted.

- As shown in Figure 5, stack a SCX cartridge with another normal phase cartridge.
- Load the sample on the SCX cartridge to capture the alkaline impurities in the sample by ion exchange interaction.
- Elute the sample to the following normal phase cartridge for a further separation of the neutral components. The target product is then purified and collected.

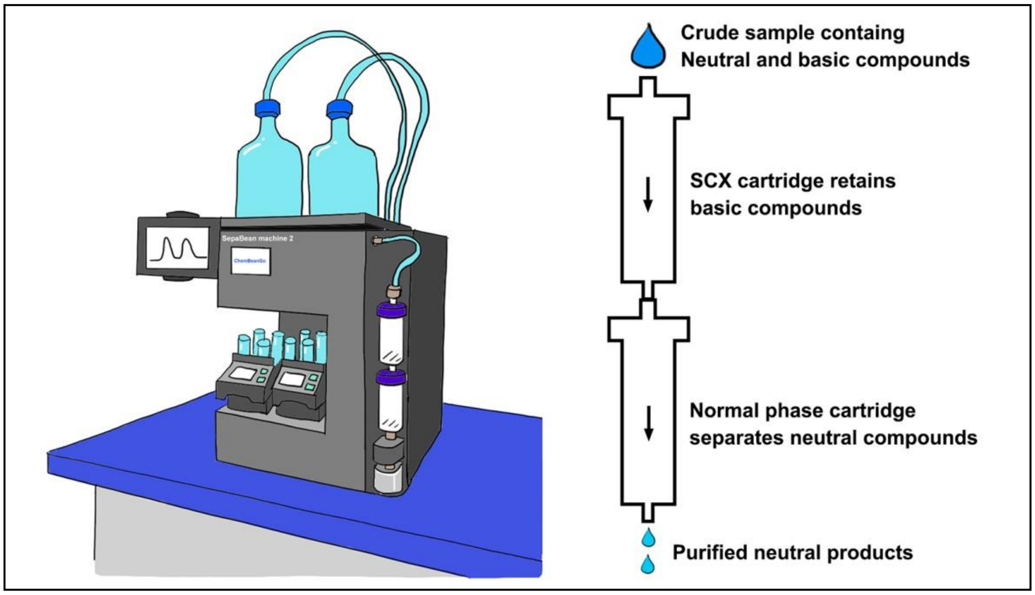

Figure 5. The purification strategies of column stacking.

In conclusion, alkaline or neutral sample could be rapidly purified by SCX cartridge combined with normal phase cartridge utilizing different purification strategies. Furthermore, for the purification of alkaline compounds in the capture-release manner, fast switching between mobile phases of different compositions can be easily achieved with the built-in feature of quaternary solvent lines in the next generation flash preparative liquid chromatography system, SepaBean^{TM} machine 2, thereby improving work efficiency.

About the SepaFlash® Bonded Series SCX flash cartridges

There are a series of the SepaFlash® Bonded Series SCX 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-5701-004-IR	5.9 g	10-20	400/27.5
SW-5701-012-IR	23 g	15-30	400/27.5
SW-5701-025-IR	38 g	15-30	400/27.5
SW-5701-040-IR	55 g	20-40	400/27.5
SW-5701-080-IR	122 g	30-60	350/24.0
SW-5701-120-IR	180 g	40-80	300/20.7
SW-5701-220-IR	340 g	50-100	300/20.7
SW-5701-330-IR	475 g	50-100	250/17.2

Table 2. SepaFlash® Bonded Series SCX flash cartridges. Packing materials: Ultra-pure irregular SCX-bonded silica, 40 - 63 µm, 60 Å.

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

www.velocityscientific.com.au