The Application of SepaFlash® Strong Anion Exchange Chromatography Columns in the Purification of Acidic Compounds

Chromatography Application Note
AN020

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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 (-SO3H) is a commonly used group in strong cation exchange (SCX), which dissociates H+ and the negatively charged group -SO3- 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 (-NR3OH, where R is hydrocarbon group) is usually used in strong anion exchange (SAX), which dissociates OH- and the positively charged group -N+R3 can adsorb other anions in the solution, resulting in anion exchange effect.

Among natural products, flavonoids have attracted the attention of researchers because of their role in the prevention and treatment of cardiovascular diseases. Since the flavonoid molecules are acidic due to the presence of phenolic hydroxyl groups, ion exchange chromatography is an alternative option in addition to conventional normal phase or reversed phase chromatography for the separation and purification of these acidic compounds. 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 previously published application note with the title “The Application of SepaFlash® Strong Cation Exchange Chromatography Columns in the Purification of Alkaline Compounds” by Santai Technologies, SCX columns were employed for the purification of alkaline compounds. In this post, a mixture of neutral and acidic standards was used as the sample to explore the application of SAX columns in the purification of acidic compounds.

Experimental Section

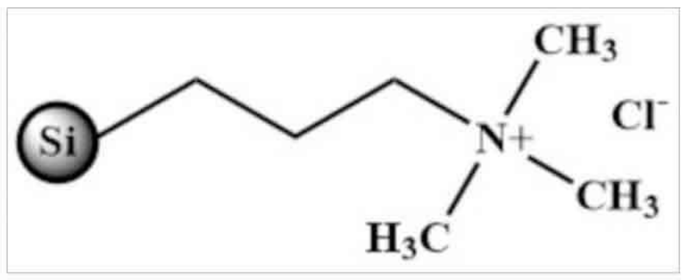

Figure 1. The schematic diagram of the stationary phase bonded to the surface of SAX separation media.

In this post, a SAX column pre-packed with quaternary amine bonded silica was used (as shown in Figure 1). A mixture of Chromone and 2,4-dihydroxybenzoic acid 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.

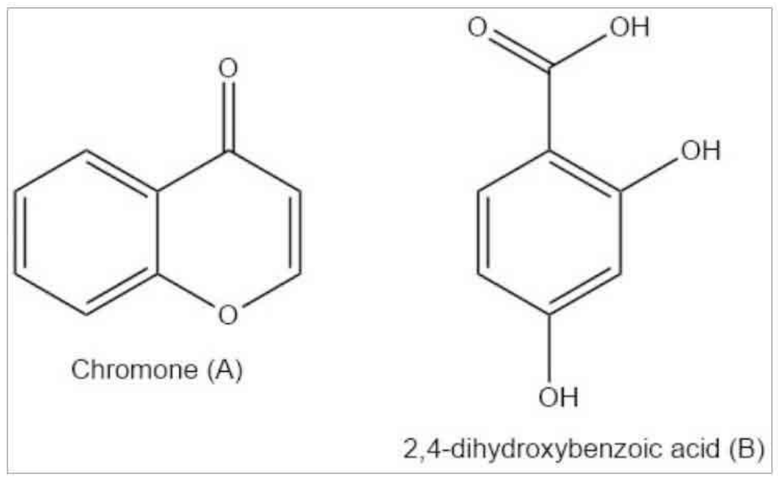

Figure 2. The chemical structure of the two components in the sample mixture.

Instrument	SepaBean® machine T			
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 SAX flash cartridge (irregular silica, 40 - 63 μm, 60 Å, Order number: SW-5001-004-IR)	
Wavelength	254 nm (detection), 280 nm (monitoring)			
Mobile phase	Solvent A: N-hexane Solvent B: Ethyl acetate			
Flow rate	30 mL/min		20 mL/min	
Sample loading	20 mg (a mixture of Component A and Component B)			
Gradient	Time (CV)	Solvent B (%)	Time (CV)	Solvent B (%)
	0	0	0	0
	1.7	12	14.0	100
	3.7	12	/	/
	16.0	100	/	/
	18.0	100	/	/

Results and Discussion

Firstly, the sample mixture was separated by a normal phase flash cartridge pre-packed with regular silica. As show in Figure 3, the two components in the sample were eluted from the cartridge one after another. Next, a SAX flash cartridge was utilized for the purification of the sample. As show in Figure 4, the acidic Component B was completely retained on the SAX cartridge. The neutral Component A was gradually eluted from the cartridge with the elution of the mobile phase.

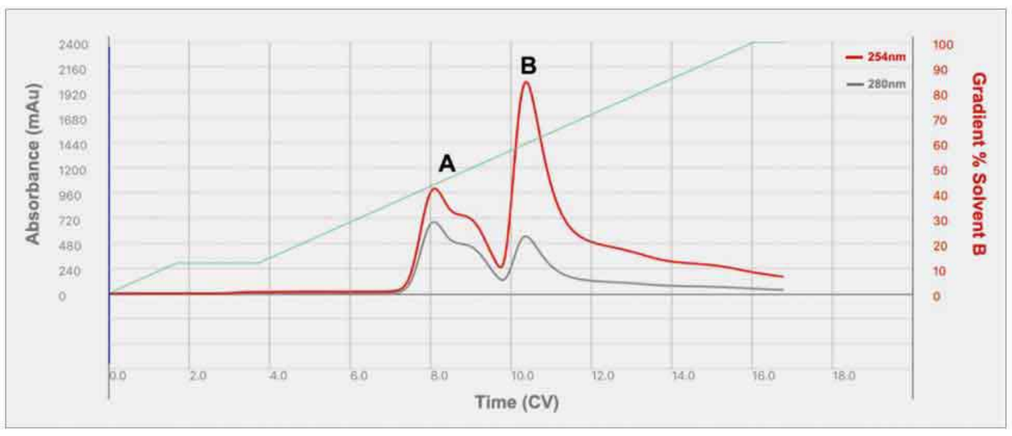

Figure 3. The flash chromatogram of the sample on a regular normal phase cartridge.

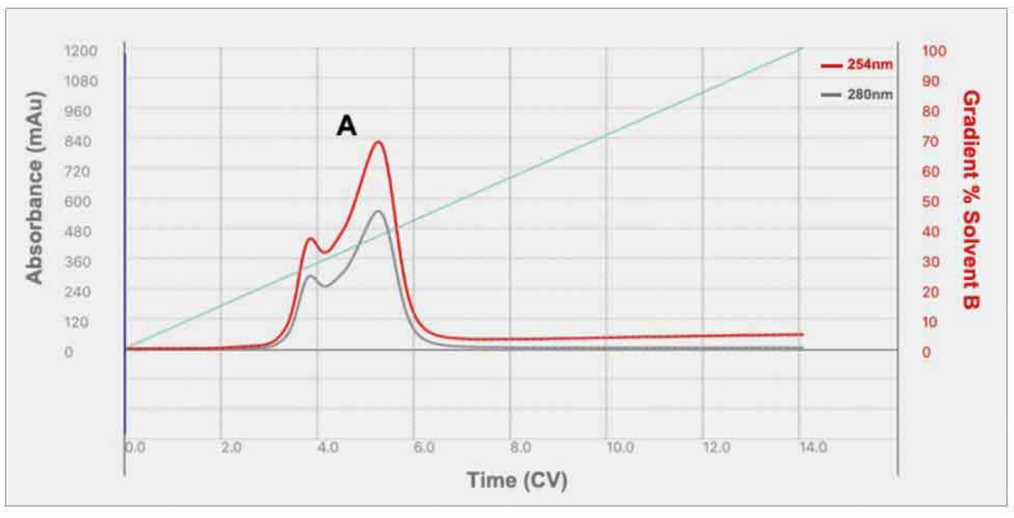

Figure 4. The flash chromatogram of the sample on a SAX cartridge.

Comparing Figure 3 and Figure 4, the Component A has inconsistent peak shape on the two different flash cartridges. To confirm whether the elution peak is corresponding to the component, we can utilize the full wavelength scanning feature which is built into the control software of SepaBean® machine. Open the experimental data of the two separations, drag to indicator line on the time axis (CV) in the chromatogram to the highest point and the second highest point of the elution peak corresponding to the Component A, and the full wavelength spectrum of these two points will be automatically shown below the chromatogram (as shown in Figure 5 and Figure 6). Comparing the full wavelength spectrum data of these two separations, the Component A has consistent absorption spectrum in two experiments. For the reason of the Component A has inconsistent peak shape on two different flash cartridges, it is speculated that there is specific impurity in the Component A which has different retention on the normal phase cartridge and the SAX cartridge. Therefore, the eluting sequence is different for the Component A and the impurity on these two flash cartridges, resulting inconsistent peak shape on the chromatograms.

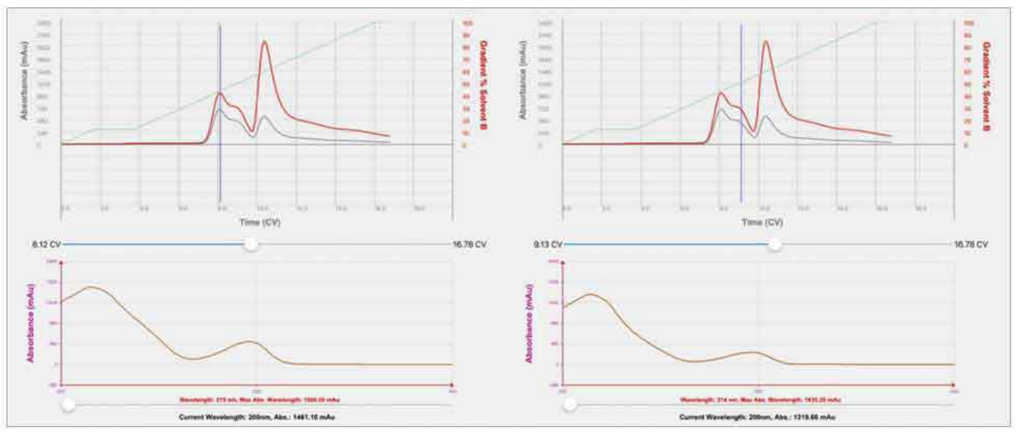

Figure 5. The full wavelength spectrum of the Component A and the impurity separated by normal phase cartridge.

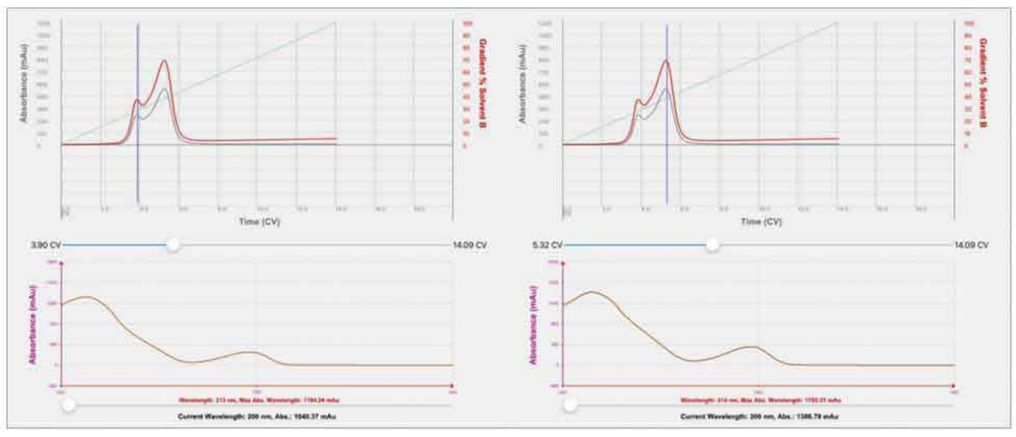

Figure 6. The full wavelength spectrum of the Component A and the impurity separated by SAX cartridge.

If the target product to be collected is the neutral Component A, the purification task can easily be completed by directly using the SAX cartridge for elution after sample loading. On the other hand, if the target product to be collected is the acidic Component B, the capture-release manner could be adopted with only a slight adjustment in the experimental steps: when the sample was loaded on the SAX cartridge and the neutral Component A was completely eluted out with normal phase organic solvents, switch the mobile phase to methanol solution containing 5% acetic acid. The acetate ions in the mobile phase will compete with the Component B for binding to the quaternary amine ion groups on the stationary phase of SAX cartridge, thereby eluting the Component B from the cartridge to obtain the target product. The chromatogram of the sample separated in ion exchange mode was shown in Figure 7.

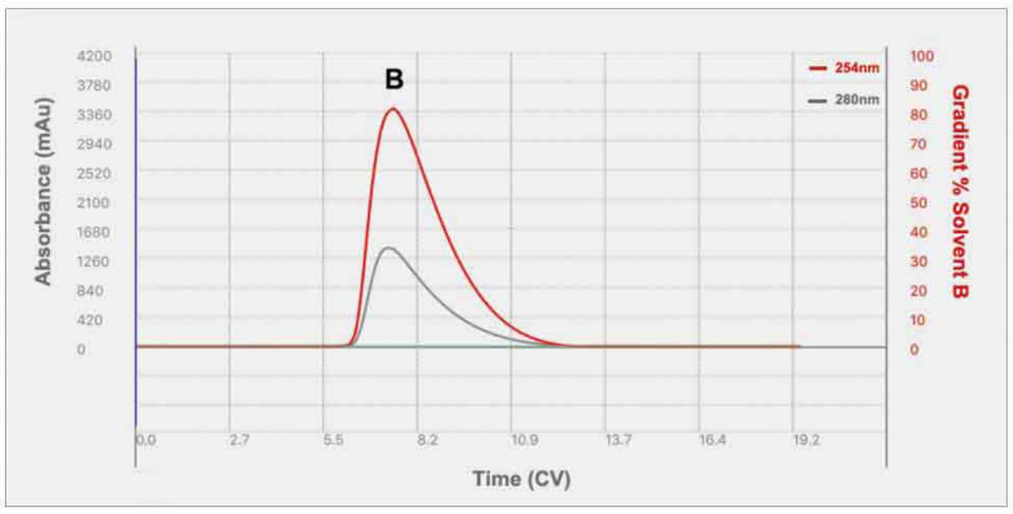

Figure 7. The flash chromatogram of the Component B eluted in ion exchange mode on a SAX cartridge.

In conclusion, acidic or neutral sample could be rapidly purified by SAX cartridge combined with normal phase cartridge utilizing different purification strategies. Furthermore, with the help of full wavelength scanning feature built into the control software of SepaBean® machine, the characteristic absorption spectrum of the eluted fractions could be easily compared and confirmed, helping researchers quickly determine the composition and purity of the eluted fractions and thus improving work efficiency.

About the SepaFlash® Bonded Series SAX flash cartridges

There are a series of the SepaFlash® Bonded Series SAX 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-5001-004-IR	5.9 g	10-20	400/27.5
SW-5001-012-IR	23 g	15-30	400/27.5
SW-5001-025-IR	38 g	15-30	400/27.5
SW-5001-040-IR	55 g	20-40	400/27.5
SW-5001-080-IR	122 g	30-60	350/24.0
SW-5001-120-IR	180 g	40-80	300/20.7
SW-5001-220-IR	340 g	50-100	300/20.7
SW-5001-330-IR	475 g	50-100	250/17.2

Table 2. SepaFlash® Bonded Series SAX flash cartridges. Packing materials: Ultra-pure irregular SAX-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: http://www.santaitech.com/en/index.php .

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