The Application of SepaFlash™ HILIC ARG Cartridge for the Purification of Strong Polar Thiazide Compound

a santai science company
SANTAI TECHNOLOGIES quality since 2004

Chromatography Application Note AN026

Run Xiao, Rui Huang, Bo Xu
Application R&D Center

Introduction

Thiazides are a class of heterocyclic molecules containing nitrogen and sulfur. Thiazide drugs belong to diuretic drugs and are commonly used to treat hypertension and edema which are caused by heart failure, liver failure or renal failure, etc. Thiazide diuretics reduce the risk of death, stroke, heart disease and heart failure induced by hypertension [1]. Thiazide drugs were first discovered and developed by Merck in 1950s. The first thiazide drug, chlorothiazide, was launched in 1958 and sold under the trade name Diuril [2]. In most countries, thiazides are the cheapest antihypertensive drugs [3]. The mechanism by which thiazide drugs lower blood pressure in the long term is not fully understood. When administered acutely, thiazides lower blood pressure by causing diuresis, decreased plasma levels, and decreased cardiac output. However, after long-term administration, thiazides cause a decrease in blood pressure by reducing peripheral resistance (i.e., vasodilation) [4, 5]. Despite the low toxicity of thiazides, overdose or long-term administration may still cause dose-dependent pharmacological adverse effects, including ion disorders, hyperuricemia, hyperglycemia, etc.

In this post, the sample molecule contained a thiazide parent structure which led to poor separation by normal phase silica cartridge or regular C18 reversed phase cartridge. With the research and development by application engineers from Santai Technologies, a SepaFlash™ HILIC ARG cartridge combined with the preparative flash chromatography system SepaBean™ machine were successfully applied for the separation and purification of the sample. The target product meeting the purity requirement was obtained, suggesting an efficient and feasible method for the purification of these compounds.

Experimental Section

The sample used in the experiment was a lead compound from a pharmaceutical research and development company. The sample was an alkaline and polar compound containing thiazide parent structure (as shown in Figure 1). However it has low solubility in commonly used solvents including methanol, water, etc. The purity of the raw sample was about 81% as analyzed by HPLC.

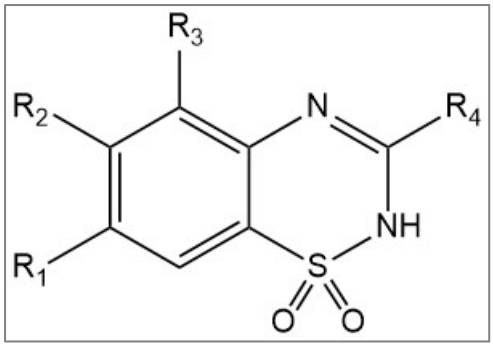

Figure 1. The chemical structure of the sample molecule.

Due to the high polarity meanwhile low solubility in normal phase solvents of the sample molecule, normal phase separation mode was excluded first. Next, reversed phase separation mode by using a C18-bonded silica cartridge was considered for the sample purification. To increase the sample solubility in water, small amount of trifluoroacetic acid (TFA) was added into water to make the sample molecule forming a salt and then dissolved in water. Appropriate amount of the sample was loaded with an injector and then injected into the flash cartridge. The experimental setup of flash chromatography for the sample was listed in Table 1.

Instrument	SepaBean™ machine T			
Cartridges	12 g SepaFlash™ Bonded Series C18 cartridge (spherical silica, 20 - 45 μm, 100Å, Order number: SW-5222-012-SP)		120 g SepaFlash™ HILIC ARG cartridge (spherical silica, 20 - 45 μm, 100Å, Order number: SW-5622-120-SP)	
Wavelength	220 nm, 254 nm			
Mobile phase	Solvent A: water (0.1%TFA) Solvent B: acetonitrile (0.1%TFA)			
Flow rate	15 mL/min		50 mL/min	
Sample loading	100 mg		500 mg	
Gradient	Time (min)	Solvent B (%)	Time (min)	Solvent B (%)
	0	10	0	70
	10.0	90	35.0	0

Table 1. The experimental setup for flash chromatography.

Results and Discussion

The flash chromatogram of the sample by the regular C18 reversed phase cartridge was shown in Figure 2. As shown in Figure 2, the sample has poor retention on the regular C18 cartridge and directly eluted out from the cartridge with the mobile phase due to its high polarity. Therefore the sample was not effectively purified from the impurities.

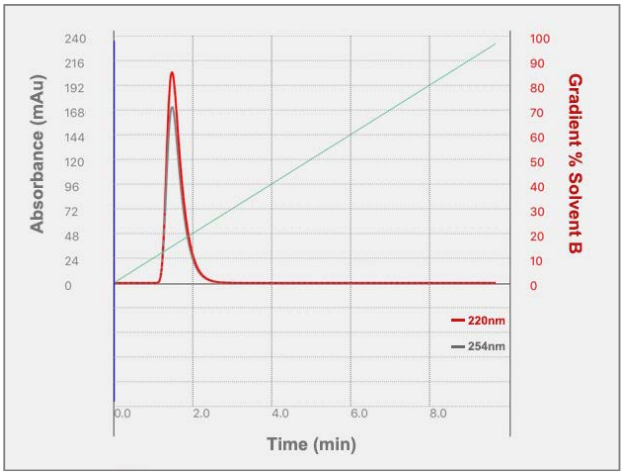

Figure 2. The flash chromatogram of the sample by a regular C18 reversed phase cartridge.

As is known to all, strong polar stationary phase was used in hydrophilic interaction chromatography (HILIC). The water ratio in the mobile phase is gradually increased during the elution process, making it suitable for the separation of samples with higher polarity. The SepaFlash™ HILIC ARG cartridges from Santai Technologies were pre-packed with spherical silica bonded with highly polar Arginine groups (as shown in Figure 3), which have sufficient retention for hydrophilic samples.

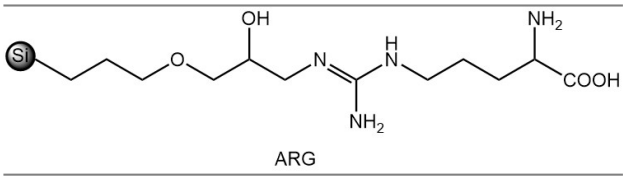

Figure 3. The schematic diagram of the stationary phase bonded to the surface of ARG separation media.

The sample was injected into a HILIC ARG cartridge and the corresponding flash chromatogram was shown in Figure 4. As shown in Figure 4, the sample was well retained on the stationary phase of HILIC ARG cartridge in the HILIC separation mode. The target product was effectively separated from the impurities in the crude sample. The collected fractions were lyophilized and further analyzed by HPLC. The purity of the purified product was 95.3% (as shown in Figure 5) and could be utilized for next step research and development.

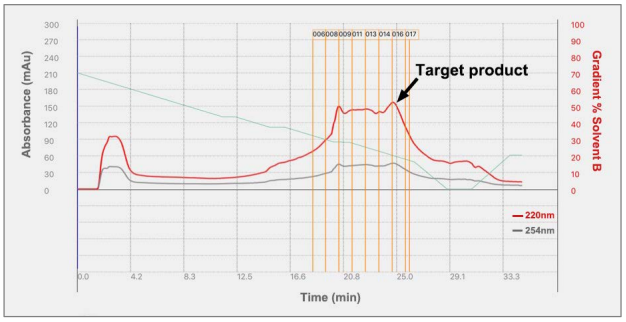

Figure 4. The flash chromatogram of the sample by a HILIC ARG cartridge.

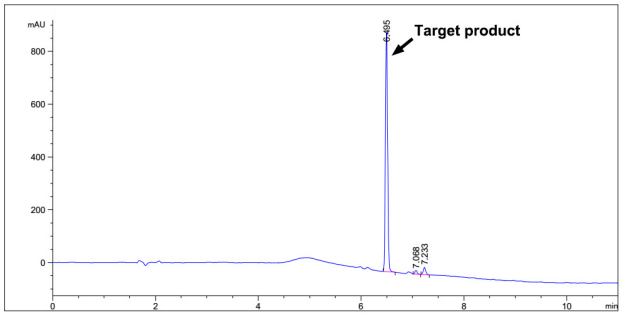

Figure 5. The HPLC chromatogram of the purified product.

In conclusion, for strong polar thiazide compounds, SepaFlash™ HILIC ARG cartridges combined with the flash chromatography system SepaBean™ machine could offer an efficient and feasible solution for the purification of these samples.

About SepaFlash™ HILIC ARG flash cartridges

There are a series of the SepaFlash™ HILIC ARG flash cartridges with different specifications from Santai Technologies (as shown in Table 2).

Item Number	Column Size	Sample Size	Max.Pressure (psi/bar)
SW-5622-004-SP	5.4 g	5.4 mg – 108 mg	400/27.5
SW-5622-012-SP	20 g	20 mg – 0.40 g	400/27.5
SW-5622-025-SP	33 g	33g – 0.66 g	400/27.5
SW-5622-040-SP	48 g	48 mg – 0.96 g	400/27.5
SW-5622-080-SP	105 g	105 mg – 2.1 g	350/24.0
SW-5622-120-SP	155 g	155 mg – 3.1 g	300/20.7
SW-5622-220-SP	300 g	300 mg – 6.0 g	300/20.7
SW-5622-330-SP	420 g	420 mg – 8.4 g	250/17.2
SW-5622-330-SP	420 g	420 mg – 8.4 g	250/17.2

Table 2. SepaFlash™ HILIC ARG flash cartridges. Packing materials: High-efficiency spherical ARG-bonded silica, 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://www.santaitech.com/index/.

References

1.Wright JM, Musini VM (2009). Wright, James M, ed. "First-line drugs for hypertension". ***Cochrane Database Syst Rev.*** 8 (3): CD001841.

2.Beyer KH (1993). "Chlorothiazide. How the thiazides evolved as antihypertensive therapy". ***Hypertension.*** 22 (3): 388–91.

3.Whitworth JA, World Health Organization, International Society of Hypertension Writing Group (November 2003). "2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension". ***J Hypertens.*** 21 (11): 1983–92.

4.Hughes AD (2004). "How do thiazide and thiazide-like diuretics lower blood pressure?". ***J Renin Angiotensin Aldosterone Syst.*** 5 (4): 155–60.

5.Zhu Z, Zhu S, Liu D, Cao T, Wang L, Tepel M (2005). "Thiazide-like diuretics attenuate agonist-induced vasoconstriction by calcium desensitisation linked to Rho kinase". ***Hypertension.*** 45 (2): 233–9.

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