



## 1. Semi-Prep conditions

**Device:** puriFlash® 450 (or now puriFlash® 5.050)

**Solvents:** A: Water

B: Methanol

**HPLC Column:** Uptisphere® Strategy™ RP 150x10.0mm, 10µm (US10RP-150/100)

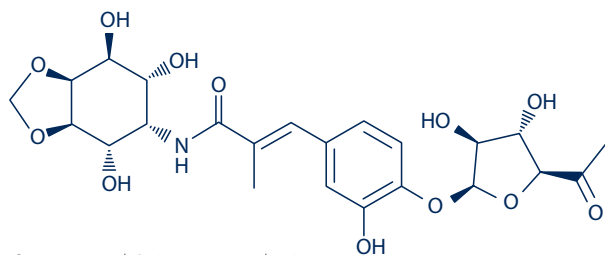
**Flow rate:** 2mL/min

**Detection:** UV 254nm (blue), UV Scan 240-600nm (orange)

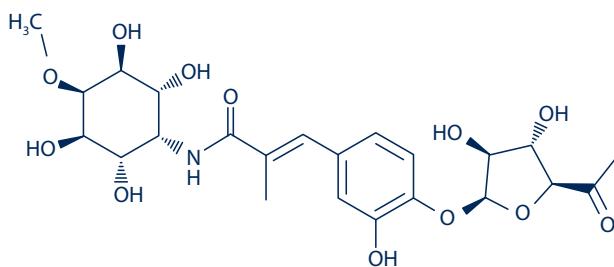
**Elution conditions:**

t (min)	A (%)	B (%)
00:00	90	10
02:00	90	10
42:00	0	100

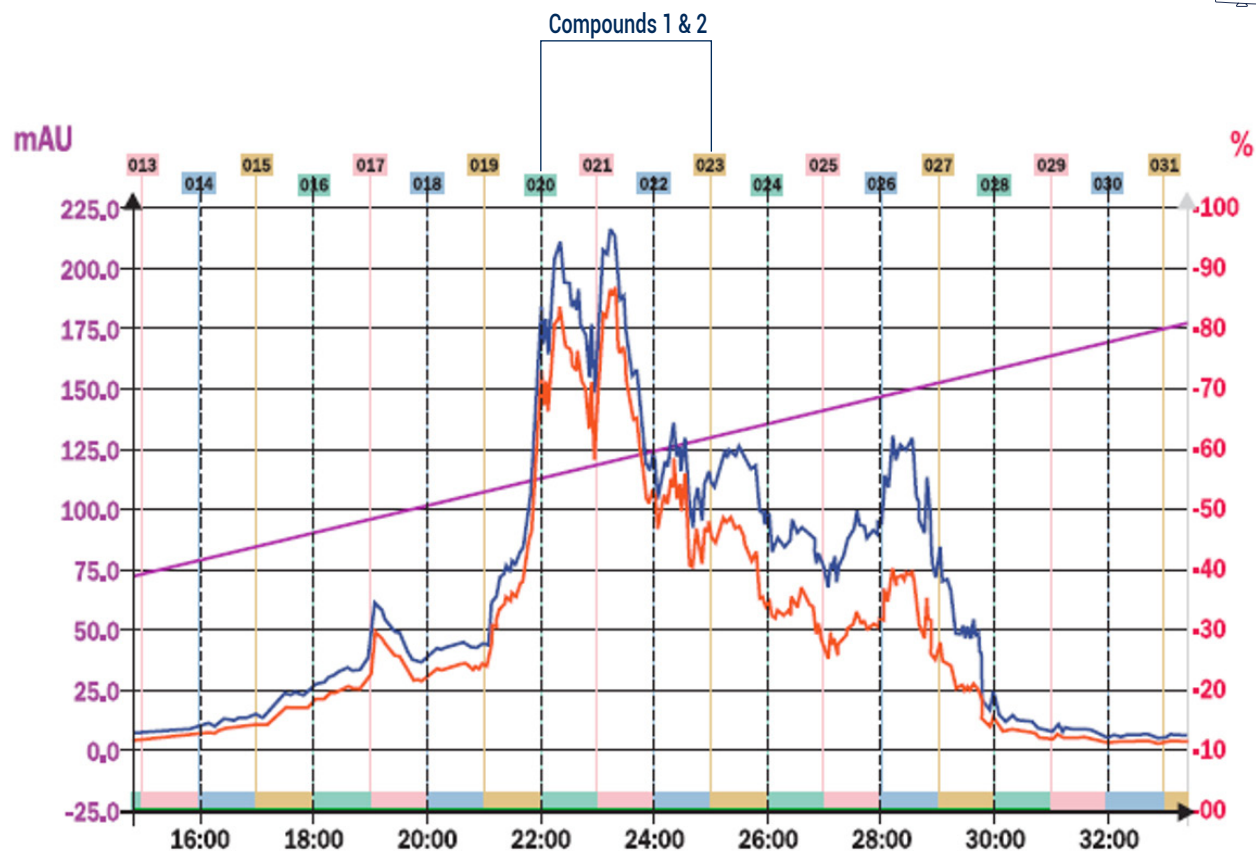
Compounds of interest: Compounds 1 & 2



Compound 1: Hygromycin A



Compound 2: Methoxyhygromycin A



## 2. Flash conditions

**Device:** puriFlash® 450 (or now puriFlash® 5.050)

**Solvents:** A: Water

B: Methanol

C: EtOAc

**Column:** PF-50C18HP-F0004

**Flow rate:** 7mL/min

**Injection mode:** Liquid injection

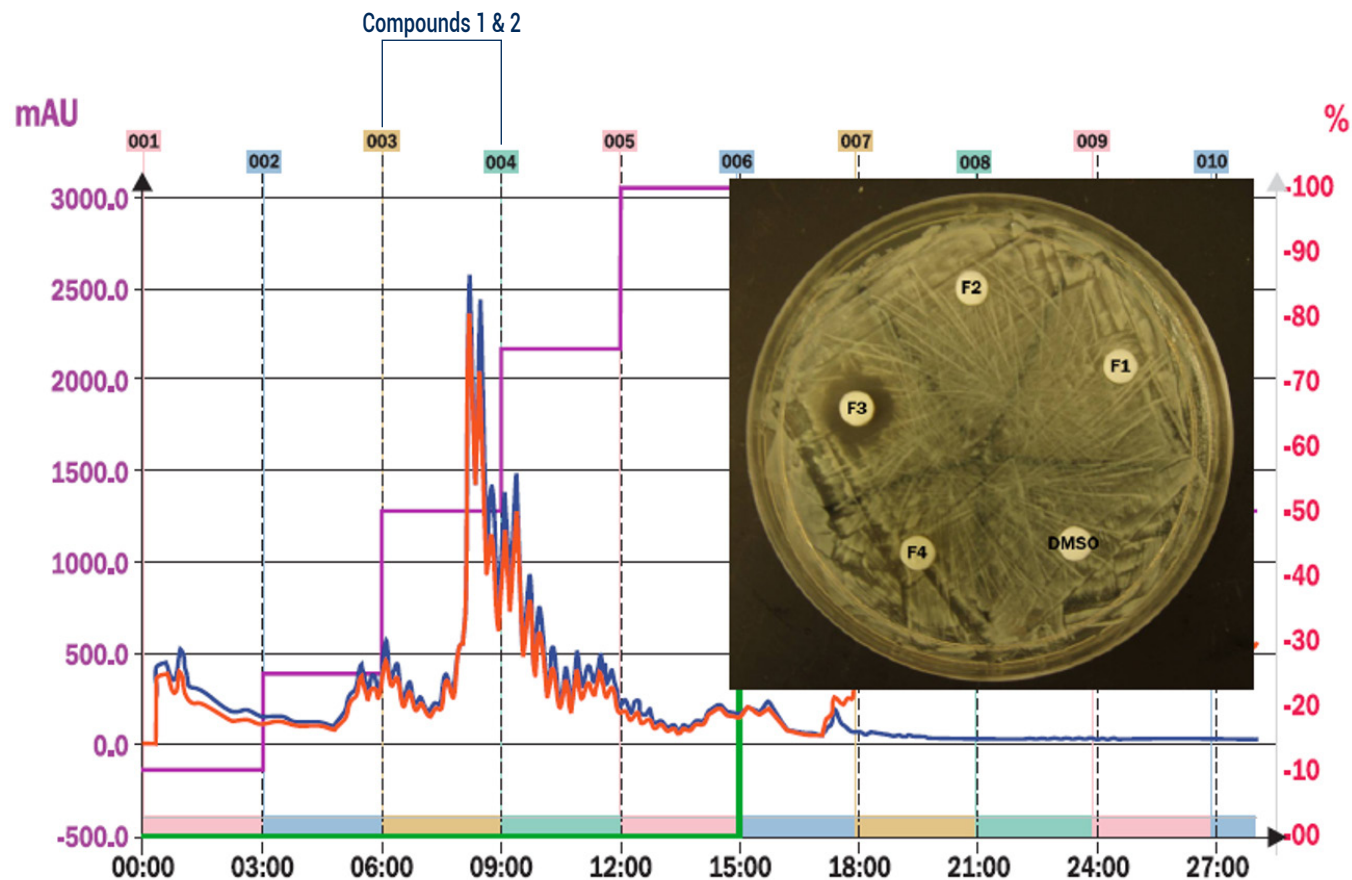
**Concentration:** 17mg/mL in Water/Methanol 90/10

**Detection:** UV 254nm (blue),

UV Scan 230-550nm (orange)

**Elution conditions:**

t (min)	A (%)	B (%)	C (%)
00:00	90	10	0
03:00	90	10	0
03:03	75	25	0
06:00	75	25	0
06:03	50	50	0
09:00	50	50	0
09:03	25	75	0
12:00	25	75	0
12:03	0	100	0
15:00	0	100	0
15:03	0	50	50
18:00	0	50	50



### To achieve this purification:

#### You will need

- puriFlash® 5.050  
[Discover it](#) [Add to card](#)
- Uptisphere® Strategy™ column US10RP-150/100  
[Discover it](#) [Add to card](#)
- puriFlash® column PF-50C18HP-F0004  
[Discover it](#) [Add to card](#)

#### We highly recommend

- Tubes 13x100mm  
 BH3901 [Add to card](#)
- Ballasting for 1/8" tubing - 5 units  
 DZ7360 [Add to card](#)
- Trolley  
 AYHF20 [Add to card](#)