Improving Flash Purification of Chemically Related Pyrazines

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Four pyrazine derivatives are successfully separated using Biotage® SNAP Ultra flash chromatography technology.

Pyrazines are a class of organic molecules often used to provide flavor to foods. They are typically synthesized but some are found in fruits and vegetables, e.g. grapes, bell peppers, peas, asparagus, beetroot, tobacco, and roasted foods[1,2]. Pyrazine's heterocyclic chemistry can yield some challenges to their purification due to the various separation kinetics between the compound and silica.

The purification is typically performed using normal-phase flash chromatography with silica as the stationary phase and a binary mixture of a hydrocarbon solvent (hexane, heptane, etc.) and ethyl acetate as the mobile phase. This solution works for most applications but often standard flash silica does not provide enough separation or resolution and product yield and purity are compromised.

While most chromatographic silica has a nominal 500 m^2/g surface area with a porosity of 60Å, this may not always be adequate for challenging sample mixtures.

In this application we compare the separating power of Biotage® HP-Sphere™ silica with >700 m^2/g of surface (used in Biotage® SNAP Ultra flash chromatography cartridges) to a cartridge packed with standard surface area silica.

Results and Discussion

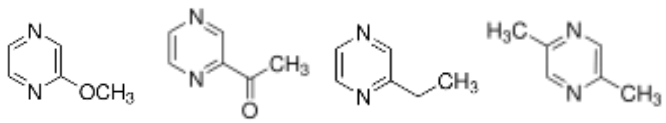

Figure 1: The four compounds separated. From left to right: 2-methoxy pyrazine; 2-acetyl pyrazine; 2-ethyl pyrazine; 2,5-dimethyl pyrazine.

The four pyrazine compounds (Figure 1) have differing functional groups and varying affinities for silica. The TLC data predicts a separation of the four compounds but with the high Rf values and low ΔCV values (selectivity) the resolution between peaks is expected to be quite low, limiting sample load.

Compound	Rf	CV	ΔCV
2-methoxy pyrazine	0.89	1.12	
2-acetyl pyrazine	0.73	1.37	0.25
2-ethyl pyrazine	0.60	1.67	0.30
2,5-dimethyl pyrazine	0.42	2.38	0.71

However, it is logical to assume that silica with more surface area will increase compound retention and loading capacity. The comparison of the standard surface area cartridge (Biotage® SNAP HP-Sil) and the high surface area Biotage SNAP Ultra cartridge separation clearly shows the benefits of using a 700 m^2/g+ silica for challenging sample mixtures such as these pyrazines, Figure 2.

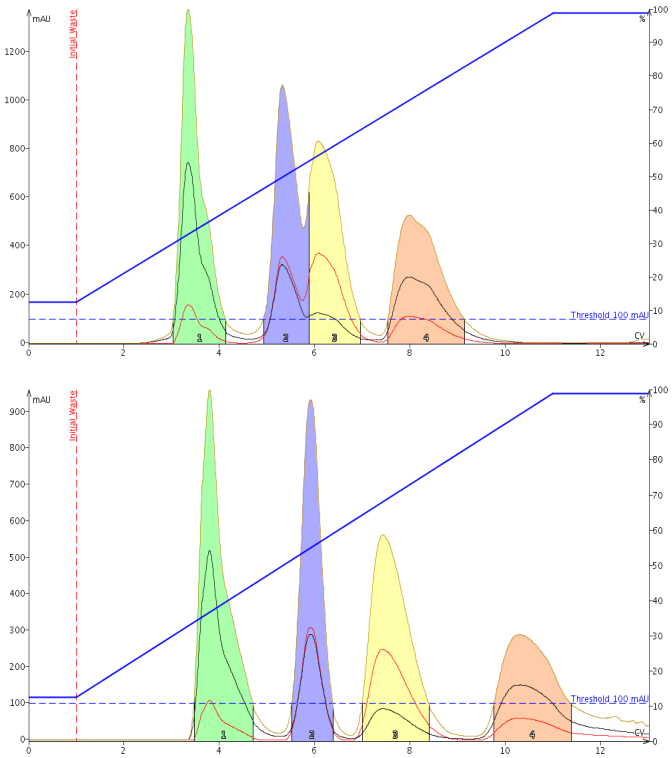

Figure 2: Top – the Biotage® SNAP HP-Sil cartridge (500 m^2/g surface area) is unable to fully separate all of the pyrazine compounds. Bottom – all four pyrazine compounds are completely separated using a Biotage® SNAP Ultra packed with HP-Sphere silica (>700 m^2/g of surface area) as predicted by TLC.

Conclusion

The separation and purification of chemically similar pyrazine compounds using traditional 500 m^2/g surface area silica has been shown to be improved by using Biotage® SNAP Ultra flash cartridges, containing 700 m^2/g silica.

Experimental

Components

1. 2-methoxy pyrazine
2. 2-acetyl pyrazine
3. 2-ethyl pyrazine
4. 2,5-dimethyl pyrazine

Sample preparation

Dissolve 50 mg of each compound in 1 mL of acetone for a total sample concentration of 200 mg/mL

Method development

TLC at 50% Ethyl acetate in Hexane

Compound	Rf	
2-methoxy pyrazine	0.89	
2-acetyl pyrazine	0.73	
2-ethyl pyrazine	0.60	
2,5-dimethyl pyrazine	0.42	

Equipment

Flash system:	Isolera Spektra Four equipped with a 200–400 nm UV detector
Flash cartridges:	Biotage® SNAP Ultra 10 g Biotage® SNAP HP-Sil 10 g
Flow-rate:	30 mL/min
Solvents:	A: Hexane B: Ethyl acetate
Equilibration:	12% B at 50 mL/min for 3 CV
Gradient:	12% B for 1 CV 12% to 100% B in 10 CV 100% B for 2 CV
Detection:	λ-All with baseline correction, 200–400 nm
Threshold:	100 mAU
Sample load:	20 mg
TLC:	Biotage® KP-SIL 10 x 10 cm

Cartridge comparison

	Biotage® SNAP HP-Sil	Biotage® SNAP Ultra
Size	10g	10g
Surface area (m^2/g)	500	>700
Pore diameter (Å)	60	50
Particle size (µm)	25	25

References

1. Foodreference.com
2. Tobaccodocuments.org

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