Reversed Phase Purification of Natural Products

Reversed-phase chromatography facilitates the isolation of milligram to multi-gram quantities of polar compounds from naturally occurring materials or from synthetic reaction products. Combining advanced detection features such as λ-All with ELSD detection methods greatly enhances the use of flash chromatography in the purification of natural products.

Introduction

Natural products are compounds produced by living organisms and usually have pharmacological or biological activity. These molecules have provided the source of inspiration for many FDA-approved drugs and still continue to be significant for drug discovery.

The feed materials for these natural products can be flowers, leaves, bark, root systems, fruit, or other natural substances. Active Pharmaceutical Ingredients (APIs) may be present in small (0.3% to 3%) quantities[4]. Procedures for extraction vary widely and are dependent on the matrix as well as the compound of interest ranging from cold pressing to extraction to distillation and steam distillation methods.

The ease with which the API can be isolated depends on its structure, stability and concentration in the matrix. For example, Alexander Fleming recognized the antibiotic properties of penicillin but this and other natural products were not initially considered clinically useful until new extraction procedures including flash purification were developed.

Since then, flash chromatography[1] has become the method of choice for purification of natural products. Reversed-phase chromatography [2,3] using pre-packed flash cartridges is a development that facilitates the isolation of milligram to multi-gram quantities of polar compounds from naturally occurring materials or from synthetic reaction products.

Results and Discussion

In our study we looked at four classes of natural products: extracts from commercially available tobacco, spinach, commercial food sweeteners and cyclodextrins (page 2-3).

Detection of Natural Products

Flash chromatography systems are typically equipped with an ultraviolet (UV) or UV-vis detector. Natural products do not always absorb energy in the UV or visible region of the spectrum, so they may be missed by these light absorption techniques. They may be visualized by TLC after reacting with a visualization reagent . However, a far better solution is the use of a detector designed to capture signals from weakly UV absorbing molecules such as the Biotage® Evaporative Light Scattering Detector (ELSD-1080). The detector vaporizes a portion of the eluant and is able to detect the particles left, making it universal in its application.

Figure 1: In this study, extract from commercial tobacco was analyzed along with spinach, food sweeteners and cyclodextrins.
Photo: Wikimedia commons

Biotage®

Cyclodextrins

The ELSD-1080 detector enables fractionation of compound classes with little or no UV or visible light absorption, for example lipids, terpenes, steroids and carbohydrates such as cyclodextrins, thus expanding the use of the Biotage® Isolera™ flash purification systems (Figure 2).

Run Conditions	
Column	C18 5 µm, 150 x 4.6 mm, (such as Biotage® Resolux™)
Eluent A	Water
Eluent B	Acetonitrile
Gradient	50–95% B in 5 min
Flow Rate	1.0 mL/min
Load	20 µL
Detector	Biotage ELSD (neb=30 °C, evap=50 °C, gas=1.0 SLM)

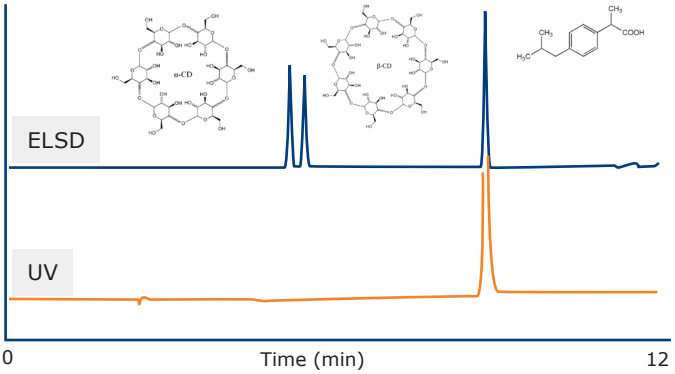


Figure 2: The ELSD revealed the true composition of a mixture of cyclodextrin and ibuprofen due to its sensitivity to compounds that possess weak or no UV chromophores.

Sweeteners

In another example, a reversed-phase mixture using commercially available Equal® (Aspartame®, dextrose, maltodextrin) was separated into its components (Figure 3).

Run Conditions	
Solvent A	Deionized water
Solvent B	Methanol
Flow rate	12 mL/min
Equilibration	5 CV at 50 mL/min
Gradient	0% B for 1 CV 0 to 100% B in 5 CV 100% B for 1 CV
Injection	0.5 mL
Detection	UV1: 200, UV2: 205, ELSD, EVAP 40, NEB 40, GAS 2.5, LED 50, PMT 1, Smth 50
Threshold	50 mAu
Collection Mode	Collect all

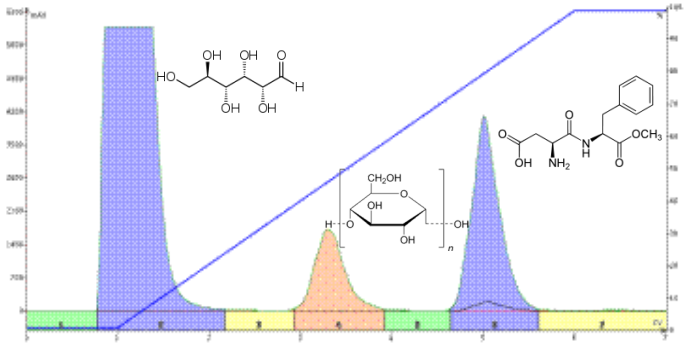


Figure 3: 1 packet (1 g) of Equal® was dissolved in 10 mL of water and separated using a reversed phase gradient with a pre-packed Biotage® SNAP KP-C18-HS cartridge.

Tobacco Extract

Tobacco extract contains a lot of compounds, and its composition is subject of interest to a wide variety of researchers. In this example, commercially available tobacco was extracted and purified by reversed-phase flash chromatography using a 12 g Biotage® SNAP KP-C18-HS flash cartridge (Figure 4).

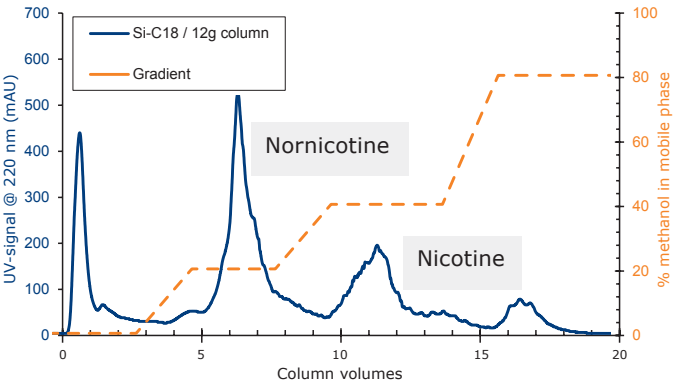


Figure 4: Chromatogram of crude tobacco extract. 1 g of commercially available tobacco was extracted with 10 mL of 20 mM ammonium acetate, pH 6.8 for 24 hours. The mixture was centrifuged to remove solids and purified by reversed phase flash chromatography using a pre-packed 12 g SNAP KP-C18-HS column. The identity of all peaks was not confirmed, but the major constituents (nornicotine, anabasine, anatabine, nicotine and flavorings) confirmed.

Spinach Extract

In this example, extract from spinach was purified using a 25 g Biotage® SNAP Ultra cartridge on a Biotage® Isolera™ Four system (Figure 5).

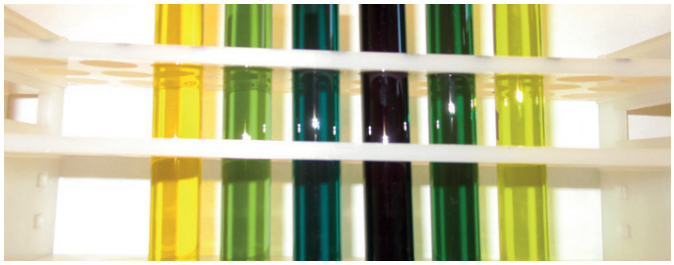


Figure 6: Fractions from the spinach extract.

Run Conditions	
System	Biotage Isolera Four
Cartridge	Biotage SNAP KP-SIL 25 g
Detector	UV-vis
Solvent A	Heptane
Solvent B	EtOAc
Equilibration	10% B for 3 CV at 50 mL/min
Gradient	10% B for 1 CV 10–100% B in 10 CV 100% B for 2 CV
Flow Rate	25 mL/min
Load	1 mL heptane solution
Detection	λ-All (200–800 nm)
Monitor λ	430 nm, 459 nm
Threshold	60 mAU

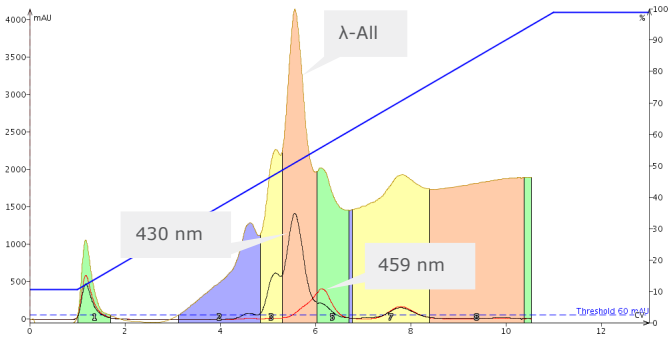
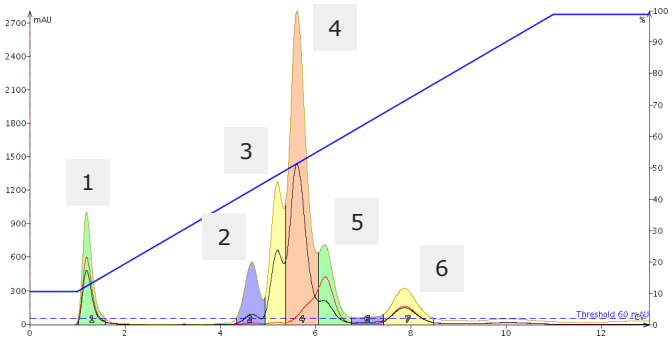


Figure 5: Spinach extract chromatograms without baseline correction (top) and with (bottom), using λ-All.



Peak legend (bottom): 1: ß-Carotene, 2: Phytophyll, 3: Phytophyll, 4: Chlorophyll A, 5: Chlorophyll B, 6. Lutein.

Flash Chromatography Method Selection Guide by Application

Product	Examples	Normal-Phase Silica	Reversed-Phase Silica	Amino Phase Silica
Alkaloids	Cocaine, morphine, nicotine, quinine	•	•	•
Amino acids			•	
Analgesics	Aspirin, acetaminophen, ibuprofen	•	•	
Aromatics		•	•	•
Basic drugs			•	•
Carbohydrates	Sugars		•	•
Flavanoids			•	
Glycosides			•	•
Lipids	Phospholipids	•	•	
Natural Products	Terpenes, saponins, polyphenols	•	•	
(Oligo)nucleotides			•	
Peptides (< 2k MW)		•	•	
Steroids		•	•	
Tannins			•	
Vitamins	Tocopherols (Vitamin E), retinol (Vitamin A), Vitamin D, Vitamin K	•	•	•

Table 1: Scope of reversed phased flash purification for a number of different natural product and compound types.

Conclusion

Biotage reversed phase KP-C18-HS SNAP cartridges have been shown in a number of applications to be effective for the purification of natural products. The methodology is robust and may be applied generically to new extraction purification runs.

References

1. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* 1978, 43(14), 2923–2925
2. Molnar, I.; Horvarth, C. *Clin.Chem.*, 1976, 22 1497.
3. Howard, G. A.; Martin, A. J. P. The separation of the C12-C18 fatty acids by reversed-phase partition chromatography. *Biochem. J.* 1950, 46, 532.
4. *Industrial Scale Natural Products Extraction*; Bart, H-J.; Pilz, S., Eds. 1st edition. Wiley-VCH: Weinheim, 2011.

Ordering Information

Product	Quantity	Part Number	Approximate Sample Load (g)
Biotage® SNAP KP-C18-HS cartridges for reversed phase separation			
SNAP 12 g KP-C18-HS cartridge	2/case	FSL0-1118-0012	0.005–0.120
SNAP 30 g, KP-C18-HS cartridge	2/case	FSL0-1118-0030	0.01–0.30
SNAP 60 g KP-C18-HS cartridge	2/case	FSL0-1118-0060	0.02–0.60
SNAP 120 g KP-C18-HS cartridge	2/case	FSL0-1118-0120	0.04–1.20
SNAP 400 g KP-C18-HS cartridge	1/case	FSL0-1118-0400	0.1–4.0
SNAP 950 g KP-C18-HS cartridge	1/case	FSL0-1118-0950	0.2–9.0
SNAP 1850 g KP-C18-HS cartridge	1/case	FSL0-1118-1850	0.4–18.0
Biotage® SNAP KP-C18-HS Samplets™ for reversed phase separation			
SNAP 1 g KP-C18-HS samplet cartridges	20/case	SAS-1118-0012	max 0.12
SNAP 3 g KP-C18-HS samplet cartridges	20/case	SAS-1118-0030	max 0.30
SNAP 12 g KP-C18-HS samplet cartridges	20/case	SAS-1118-0120	max 1.20
SNAP 40 g KP-C18-HS samplet cartridges	6/case	SAS-1118-0400	max 4.0

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