Performance Features of 2 µm Superficially Porous Particle Columns

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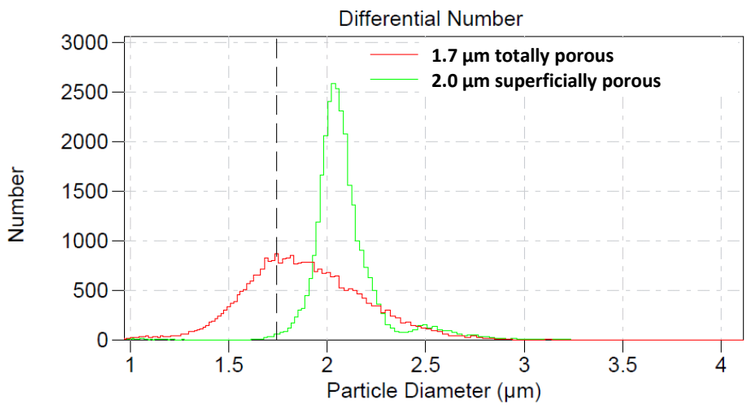
Introduction

Superficially porous particles (SPP) in the 2.5 - 2.7 µm range provide nearly the same efficiency and resolution of sub-2 µm totally porous particles (TPP), but at one-half to one-third of the operating pressure. The performance advantage of SPP over TPP shown by these 2.5 – 2.7 µm SPP has led to the introduction of smaller diameter SPP as a natural extension of this technology. The 40 to 50% efficiency advantage of SPP over TPP continues to be demonstrated as particle size is reduced to 2.0 µm. The characteristics of these 2.0 µm SPP are described and studies comparing some present sub-2 µm SPP and TPP commercial columns for efficiency and pressure are presented. High speed reversed-phase application examples include explosives, anticoagulants, and steroids. High throughput enzymatic analyses using HILIC have been applied to analysis of both crude tissue extracts and purified enzyme preparations.

An Alternative to Sub-2µm: 2µm SPP

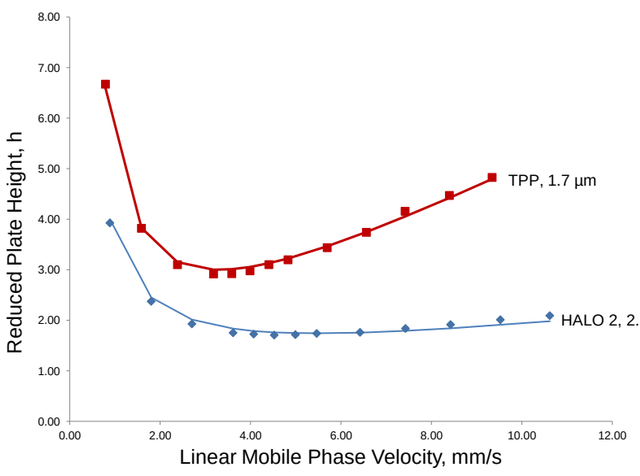
- 2µm SPP keeps pressure within a comfort zone and retains most of advantages of sub-2µm columns
  - Higher efficiencies than sub-2µm TPP columns
  - Lower pressure than sub-2µm columns (TPP or SPP)
  - Short columns exhibit the high efficiencies wanted for fast method development
- Minimizes disadvantages of sub-2µm columns
  - Greater efficiencies than sub-2µm TPP with lower pressure requirements
  - Similar efficiencies as sub-2µm SPP with lower pressure requirements
  - Uses 1-micron frits that are less prone to plugging
  - Reduced frictional heating

Particle Size Distributions



The particle size distribution is much narrower for the SPP compared to the TPP.

Efficiency Comparison: HALO 2 vs. Sub-2µm TPP



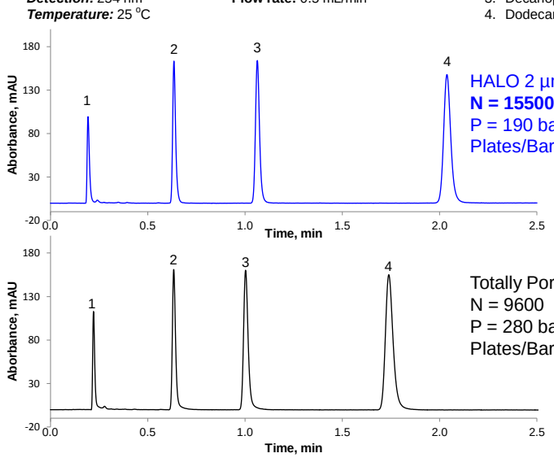
The curve for HALO 2 remains flat over a wide range of linear velocities allowing operation at increased flow rate with minimal loss of efficiency.

Chromatographic Comparison: HALO 2 vs. Sub-2µm TPP

Columns: 2.1 x 50 mm
Instrument: Shimadzu Nexera
Injection Volume: 0.2 µL
Detection: 254 nm
Temperature: 25 °C

Mobile Phase A: water
Mobile Phase B: acetonitrile
Ratio A/B: 15/85
Flow rate: 0.5 mL/min

Peak Identities:
1. Uracil
2. Pyrene
3. Decanophenone
4. Dodecanophenone



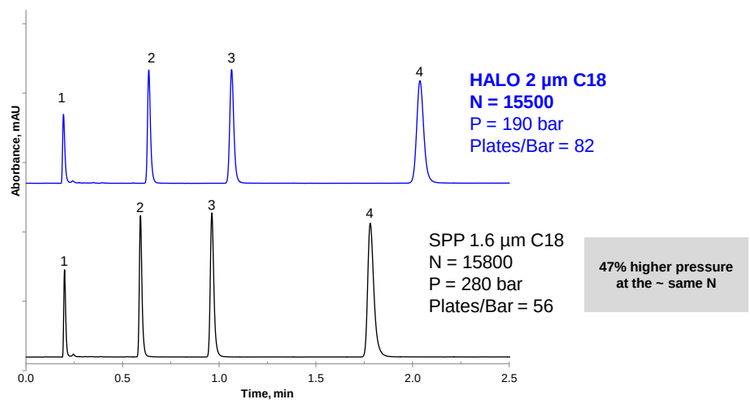
32% lower pressure with 38% higher N

HALO 2 vs. Competitor Solid-Core Sub-2µm

Columns: 2.1 x 50 mm
Instrument: Shimadzu Nexera
Injection Volume: 0.2 µL
Detection: 254 nm
Temperature: 25 °C

Mobile Phase A: water
Mobile Phase B: acetonitrile
Ratio A/B: 15/85
Flow rate: 0.5 mL/min

Peak Identities:
1. Uracil
2. Pyrene
3. Decanophenone
4. Dodecanophenone



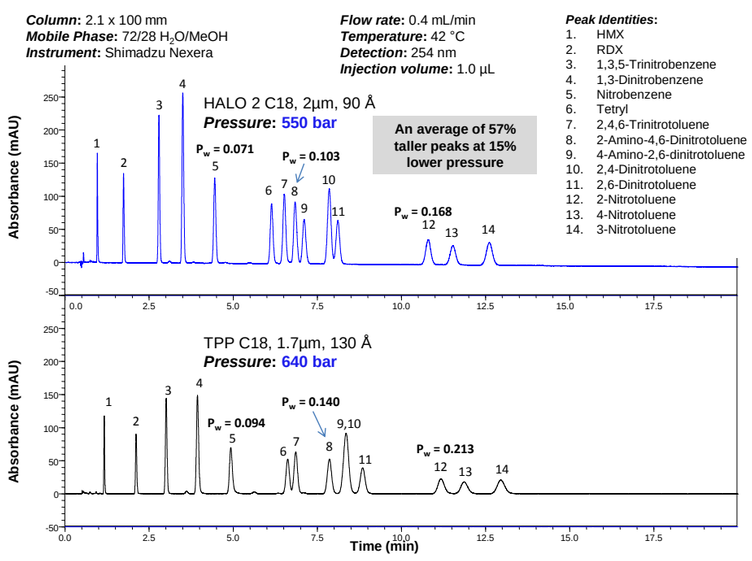
47% higher pressure at the ~ same N

Explosives Separation : HALO 2 vs. Sub-2µm TPP

Column: 2.1 x 100 mm
Mobile Phase: 72/28 H_2O/MeOH
Instrument: Shimadzu Nexera

Flow rate: 0.4 mL/min
Temperature: 42 °C
Detection: 254 nm
Injection volume: 1.0 µL

Peak Identities:
1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. Tetryl
7. 2,4,6-Trinitrotoluene
8. 2-Amino-4,6-Dinitrotoluene
9. 4-Amino-2,6-dinitrotoluene
10. 2,4-Dinitrotoluene
11. 2,6-Dinitrotoluene
12. 2-Nitrotoluene
13. 4-Nitrotoluene
14. 3-Nitrotoluene



Ballistic Separation of Anticoagulants

Column: 2.1 x 30 mm HALO 2 C18, 2µm, 90 Å
Mobile Phase A: 20 mM formic acid
Mobile Phase B: 50/50 ACN/MeOH
Gradient:

Time	%B
0 – 0.06	20
0.06 – 1.06	20 – 75%

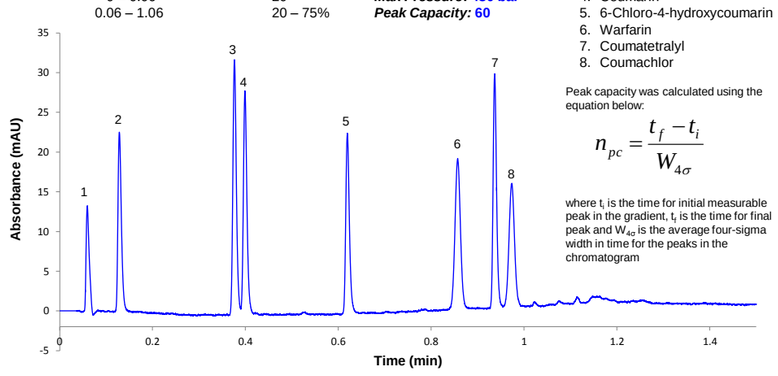
Flow rate: 1.1 mL/min
Temperature: 45 ºC
Detection: 254 nm
Injection: 0.2 µL
Max Pressure: 430 bar
Peak Capacity: 60

Peak Identities :
1. Uracil
2. 6,7-Dihydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 6-Chloro-4-hydroxycoumarin
6. Warfarin
7. Coumatetralyl
8. Coumachlor

Peak capacity was calculated using the equation below:

$$n_{pc} = \frac{t_f - t_i}{W_{4\sigma}}$$

where t_i is the time for initial measurable peak in the gradient, t_f is the time for final peak and $W_{4\sigma}$ is the average four-sigma width in time for the peaks in the chromatogram



A rapid separation of anticoagulants with high peak capacity in under a minute is achieved due to the high efficiency of a short HALO 2 C18 column.

Carbonyl-DNPH High Resolution Separation

Column: 2.1 x 150 mm, HALO 2 C18, 2µm, 90 Å
Mobile Phase A: H_2O
Mobile Phase B: 80/20 ACN/THF
Gradient:

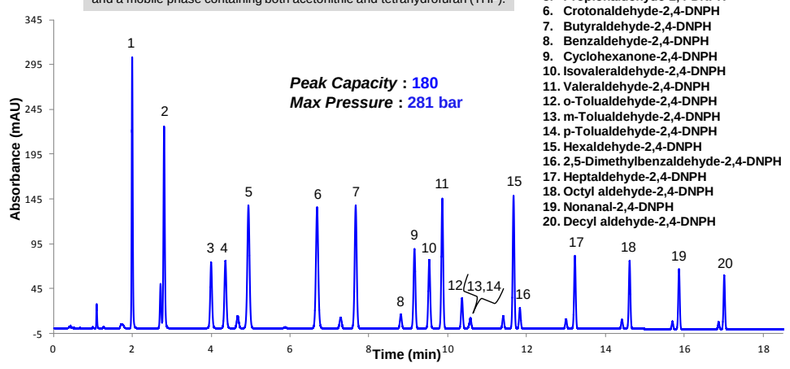
Time	%B
0 – 5 min	45
5 – 20 min	45 – 90

Flow rate: 0.5 mL/min
Temperature: 30 °C
Detection: 360 nm
Injection volume: 1.0 µL

Sample: DNPH Derivatized Carbonyl Compounds

Using modified EPA methods 8315 and 554, a high resolution separation of the sample components is achieved by the use of a HALO 2 column and a mobile phase containing both acetonitrile and tetrahydrofuran (THF).

Peak Identities:
1. Formaldehyde-2,4-DNPH
2. Acetaldehyde-2,4-DNPH
3. Acetone-2,4-DNPH
4. Acrolein-2,4-DNPH
5. Propionaldehyde-2,4-DNPH
6. Crotonaldehyde-2,4-DNPH
7. Butyraldehyde-2,4-DNPH
8. Benzaldehyde-2,4-DNPH
9. Cyclohexanone-2,4-DNPH
10. Isovaleraldehyde-2,4-DNPH
11. Valeraldehyde-2,4-DNPH
12. o-Tolualdehyde-2,4-DNPH
13. m-Tolualdehyde-2,4-DNPH
14. p-Tolualdehyde-2,4-DNPH
15. Hexaldehyde-2,4-DNPH
16. 2,5-Dimethylbenzaldehyde-2,4-DNPH
17. Heptaldehyde-2,4-DNPH
18. Octyl aldehyde-2,4-DNPH
19. Nonanal-2,4-DNPH
20. Decyl aldehyde-2,4-DNPH



Steroids Separation: HALO 2 PFP

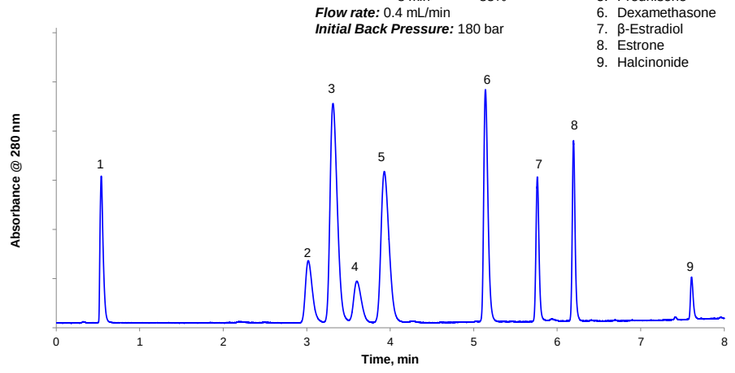
Column: 3 x 50 mm HALO 2 PFP
Instrument: Shimadzu Prominence UFLC XR
Injection Volume: 2 µL
Detection: 280 nm
Temperature: 35 C

Mobile Phase A: water
Mobile Phase B: methanol
Gradient:

Time	% B
0 min	47%
3 min	47%
8 min	88%

Flow rate: 0.4 mL/min
Initial Back Pressure: 180 bar

Peak Identities:
1. Uracil
2. Hydrocortisone
3. Prednisolone
4. Cortisone
5. Prednisone
6. Dexamethasone
7. β-Estradiol
8. Estrone
9. Halcinonide



HALO 2 PFP has unique selectivity for the separation of closely related steroids.

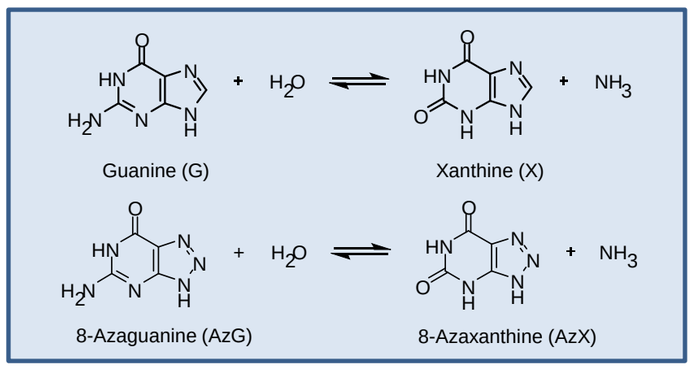
Rapid 2 µm Halo Penta-HILIC Analysis of Bovine Guanine Deaminase

Guanine deaminase, also variously known as “nedasin “ or “cypin”, catalyzes the purine catabolic commitment step from guanine, through xanthine, to the elimination product, uric acid. In mammals, the enzyme is predominantly cytoplasmic as a homodimer, with catalytic domains for the Zn^{+2}-dependent hydrolytic deamination of guanine to xanthine plus ammonia.

- Structure: c. 50 kDa subunits with sequences that vary at internal and terminal sites, due to exon selection
- Interactions: tubulin, snapin, and post-synaptic domain protein 95 (PSD-95, via PZD binding motifs at the C-termini)
- Sequence variants occur mostly at the protein binding domains, although minor variants lack the catalytic site
- In mammalian brain high levels are in telencephalic brain regions, low in white matter and cerebellum
- Moderate levels in liver and certain other specialized organs
- Low levels in plasma/serum; altered by liver dysfunction
- Actual role of guanine deaminase in specialized organ metabolism and synaptic physiology is uncertain
- Previous tissue homogenate enzyme assays are complex, using coupled enzyme/cofactor indirect readout
- Spectrophotometric high throughput assay has uncertain specificity and requires guanine as substrate near the limit of aqueous solution solubility

To measure tissue enzyme levels, follow purification processes, and assay enzyme kinetics and inhibitors, LC/UV and LC/MS methods are needed. Our assay measures deamination of guanine to xanthine, using rapid HILIC separation. Crude homogenates use 8-azaguanine as substrate due to the presence of endogenous xanthine; product 8-azaxanthine reflects enzyme activity without correction for background. HTP assay and kinetic analysis with partially purified or purified enzyme measures the direct conversion of guanine to xanthine.

Reactions Catalyzed by Guanine Deaminase



Assay Conditions and LC Analysis

Enzymatic Reaction:
- Temp: 25 °C
- Substrate: 0.2 mM guanine (HTP) or 0.4 mM AzG (tissue)
- Buffer: 0.1 M Bicine-HCl, pH 7.8
- Internal standard: 0.5 µM 1-methylguanine (tissue)
  0.5 µM 7-methylguanine (HTP)
- Stop Solution: 1% HOAc/99% Acetonitrile with IS
- Workflow:
  - Incubate homogenate (10%) or purified enzyme in 10-100 µL of substrate mix
  - 9 volumes Stop Solution, ice bath 5 minutes
  - 10 minute centrifuge (16,000 x G)
  - Direct injection of supernatant on LC

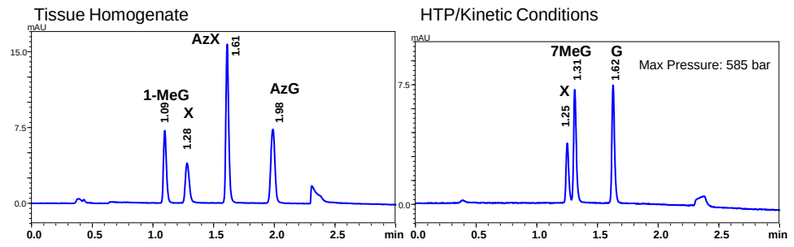
LC Conditions:
- 3.0 x 75 mm 2.0 µm particle HALO 2 Penta-HILIC
- Shimadzu Nexera with SPD-30A DAD at 270 nm (10 nm BW)
- 35 °C, 0.8 mL/min initial flow rate; 2-20 µL injection
- Eluents: A – 0.1 M NH_4OAc (pH 6.5); B – AcN
- During equilibration, flow rate increased to 1.5 mL/min

Gradient Profile

Time	Component	Action	Parameter
0.00			
1.90	Pumps	Pump B Conc.	80
1.91	Pumps	Total Flow	0.8
2.10	Pumps	Total Flow	1.5
2.11	Pumps	Pump B Conc.	40
3.10	Pumps	Pump B Conc.	40
3.20	Pumps	Pump B Conc.	90
6.00	Pumps	Total Flow	1.5
6.20	Pumps	Total Flow	0.8
6.50	Controller	Stop	

Assay Features

Resolution of Standard Purines

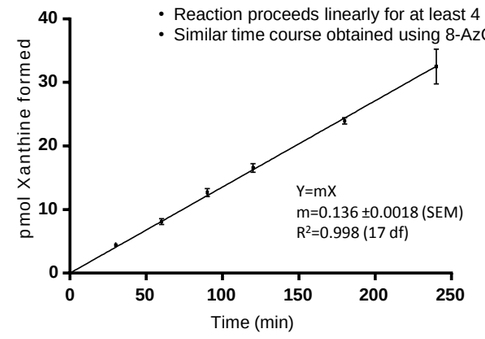


- Analysis time for this HILIC method is 6.5 minutes run to run, with complete re-equilibration of column

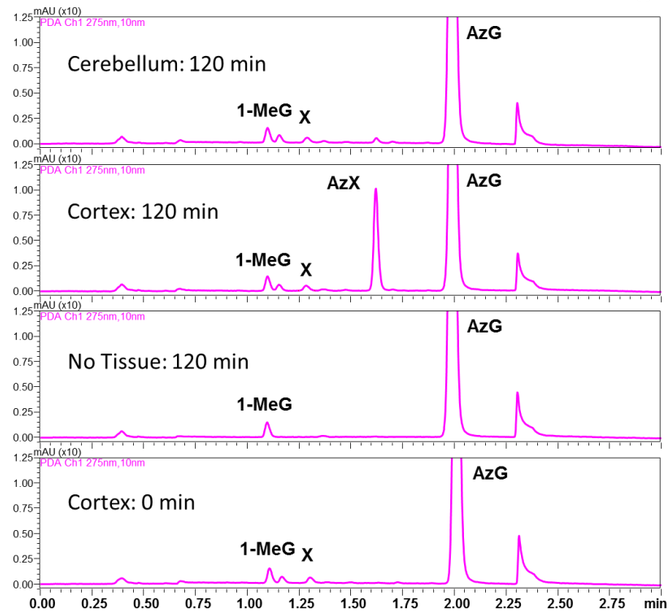
Time Course of Guanine Deamination by Partially Purified Bovine Cortex Enzyme (HTP Conditions)

25°C; 0.1M Bicine (pH 7.8); 200 µM Guanine; 4 µg protein/rx

- Reaction proceeds linearly for at least 4 hours
- Similar time course obtained using 8-AzG as substrate



Guanine Deaminase in Brain Tissue Homogenates



- In tissue homogenates, endogenous xanthine is a significant background for the enzyme assay
- Neocortex and cerebellum tissue homogenates samples exhibit endogenous X (and other unknown detected molecules)
- Use of 8-AzaG as substrate yields 8-AzaX as a product, well resolved from endogenous X and other compounds
- Neocortex shows 8-AzaX formed by guanine deaminase – no significant interferences from tissues at AzX or IS elution
- Cerebellum exhibits little guanine deaminase activity, as expected

Conclusions

- **Sub-2µm SPP are not needed for most routine applications. In fact, they present challenges in laboratory environments, i.e. higher back pressure.**
- **HALO 2 columns are a good compromise of speed and efficiency with superior advantages for complex samples.**
- **HILIC method permits highly selective resolution of these polar analytes.**
- **Highly efficient 2 µm HILIC allows rapid and selective assay of this important purine catabolic enzyme.**

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