

Performance Features of 2 μm Superficially Porous Particle Columns

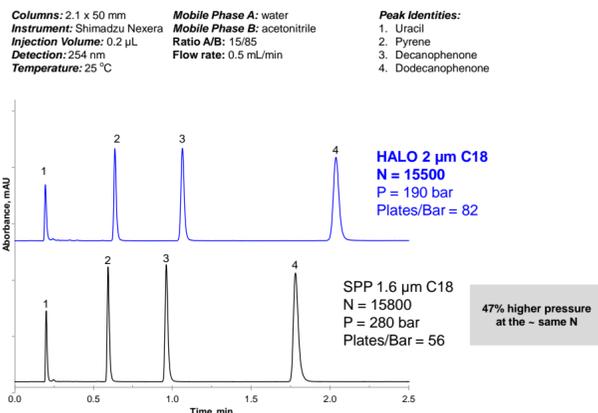
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Introduction

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.

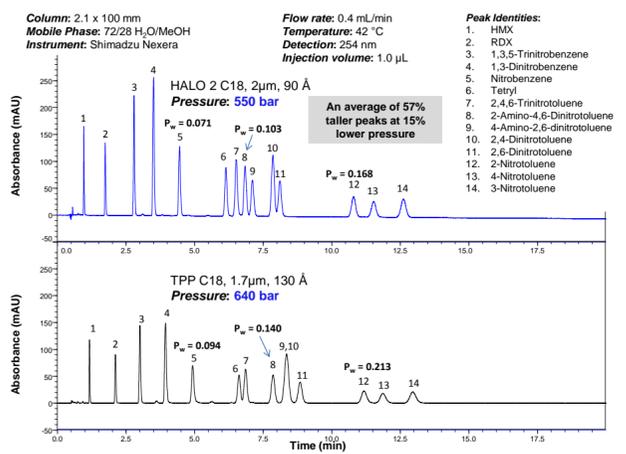
HALO 2 vs. Competitor Solid-Core Sub-2μm



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

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



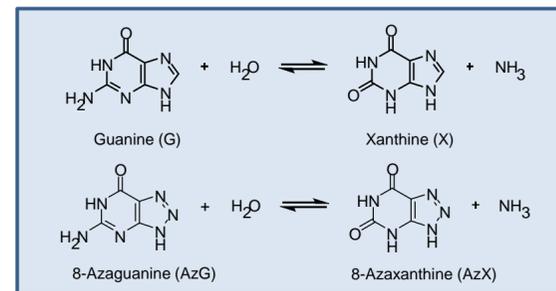
Rapid 2 μm Halo Penta-HILIC Analysis of Bovine Guanine Deaminase

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 Zn²⁺-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



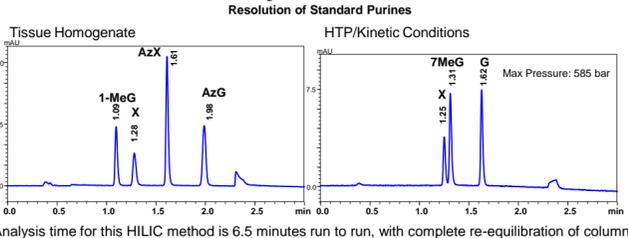
Assay Conditions and LC Analysis

- 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) or 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 NH₄OAc (pH 6.5); B - AcN
 - During equilibration, flow rate increased to 1.5 mL/min

Gradient Profile

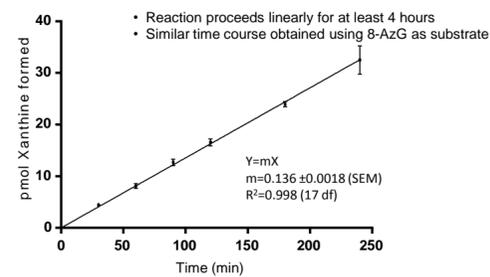
Time	Component	Action	Parameter
0.00			
1.90	Pumps	Pump B Conc.	80
1.91	Pumps	Total Flow	0.8
2.10	Pumps	Total Flow	1.5
2.11	Pumps	Pump B Conc.	40
3.10	Pumps	Pump B Conc.	40
3.20	Pumps	Pump B Conc.	90
6.00	Pumps	Total Flow	1.5
6.20	Pumps	Total Flow	0.8
6.50	Controller	Stop	

Assay Features

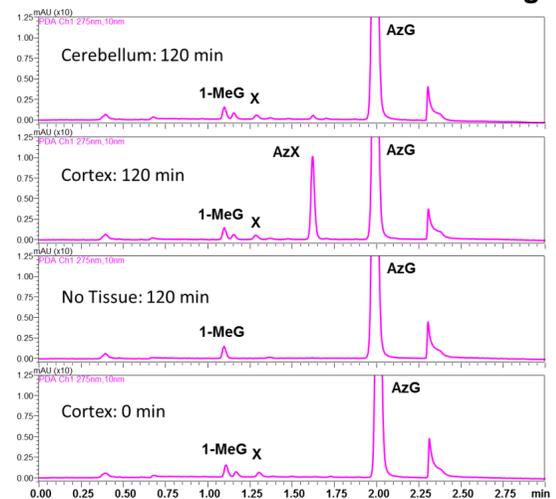


Time Course of Guanine Deamination by Partially Purified Bovine Cortex Enzyme (HTP Conditions)

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



Guanine Deaminase in Brain Tissue Homogenates



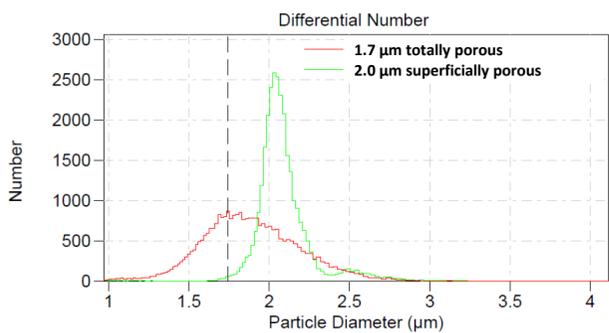
- In tissue homogenates, endogenous xanthine is a significant background for the enzyme assay
- Neocortex and cerebellum tissue homogenates samples exhibit endogenous X (and other unknown detected molecules)
- Use of 8-AzG as substrate yields 8-AzAX as a product, well resolved from endogenous X and other 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.

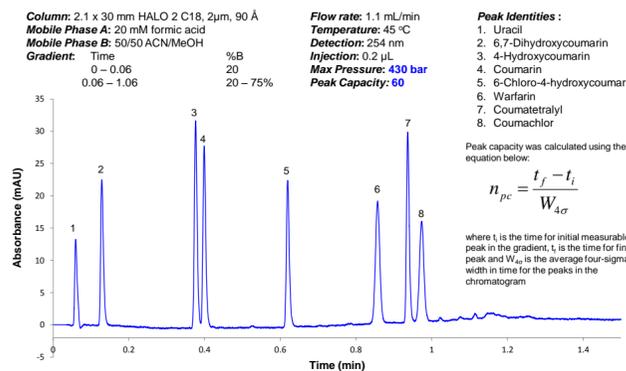
Acknowledgements: Robert Moran, Benjamin Libert, and William Johnson for advice and technical assistance. Supported in part by NIH Grant GM093747 (BEB).

Particle Size Distributions



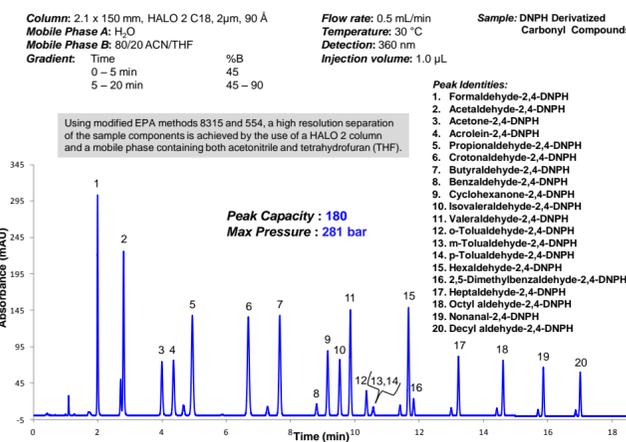
The particle size distribution is much narrower for the SPP compared to the TPP.

Ballistic Separation of Anticoagulants

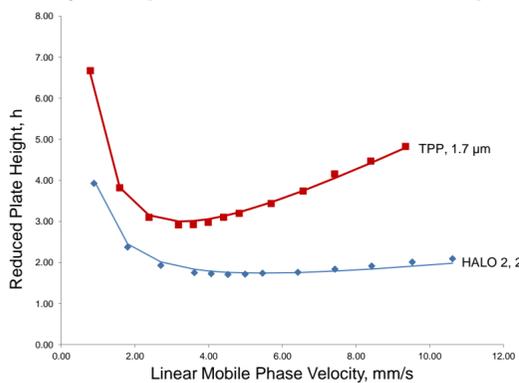


A rapid separation of anticoagulants with high peak capacity in under a minute is achieved due to the high efficiency of a short HALO 2 C18 column.

Carbonyl-DNPH High Resolution Separation

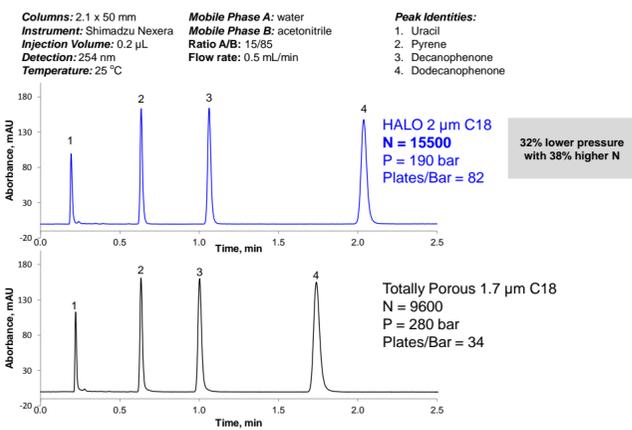


Efficiency Comparison: HALO 2 vs. Sub-2μm TPP

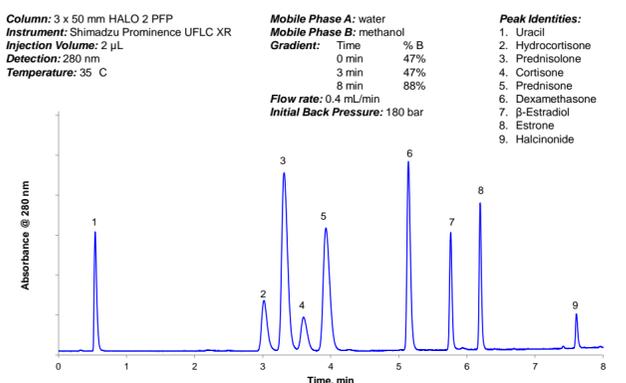


The curve for HALO 2 remains flat over a wide range of linear velocities allowing operation at increased flow rate with minimal loss of efficiency.

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



Steroids Separation: HALO 2 PFP



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