Novel 3-D Sample Plate using Monolithic Capture Media in Collimated-Hole Structures for Interfacing High Capacity Separations with MALDI-TOF

Poster Number TP 060

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Overview/Introduction:

-Novel MALDI targets are being developed that use collimated hole structures (CHS) combined with monolithic chromatography media to enable capture & concentration of sample and serve as a direct interface between the mass spectrometer and different separation schemes -HPLC, electrophoresis and tissue imaging-

-Plate construction, sample deposition (LC) and sample elution are presented

-Results of capacity and LC interface at 15 and 50 μL/min separation speeds are presented

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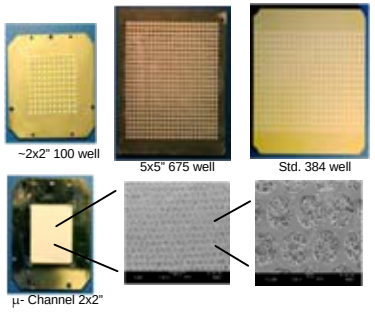

Figure 1: CHS plates: Row 1: 100, 675 and 384 wells plate of 1.5, 3 and 10 mm thickness respectively constructed in metal and plastic designed for modes of discrete sample deposition such as LC or robotic and hand spotting.

Row two shows glass μ-channel CHS plate (25μm holes) designed for interface with gel and tissue applications.

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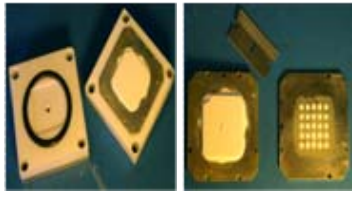

Figure 2: Plate Construction:

-styrene/divinylbenzene based polymers for reversed phase capture media of protein/peptides -monomer solutions are injected into tetrafluoroethylene molds

-polymerization is thermally initiated

-Excess polymer is shaved to a level coincident with the plate substrate (metal, plastic, glass). -ratio and constitution of dilution solvent(porogens) are used to tailor pore-size/flow properties -Sample application/analysis occur on the polymer surface.

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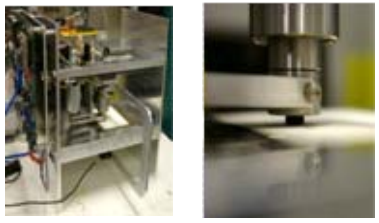

Figure 3: Prototype LC deposition System

-X Y control of solvent capillary

-Tip seals with plate surface

-column pressure forces column effluent through discrete locations on CHS plates.

4

Figure 4: Prototype Elution System

-uses pressure on reservoir of eluent residing on one side of the plate to force the liquid through all holes simultaneously.

-enclosed chamber with exhaust fan on opposite side of plate enhances solvent evaporation and matrix crystallization.

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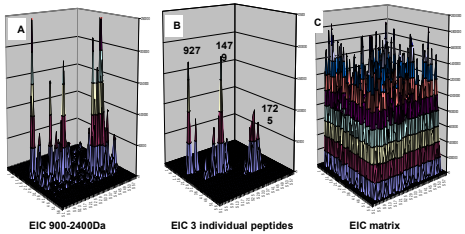

Figure 5 LC Results:

-3D plots of signal intensity as function of surface location from 1pmol BSA digest LC separation *Conditions: 0.5 mm column ID C-18 (eksigent), 15uL/min, 5-45%ACN gradient (50min), dwell time 30s/spot 10x10 CHS plate (Fig. 1 top left)*

-Frame A: extracted ion chromatogram (EIC) all peptides from 900-2400 Da.

-Frame B: EIC of three individual peptides (927,1479,1725 Da)

-Frame C: EIC of 379Da ACCH matrix dimer.

Figure demonstrates the ability of the CHS plates to capture/concentrate sample without loss of chromatographic resolution and uniform sample elution.

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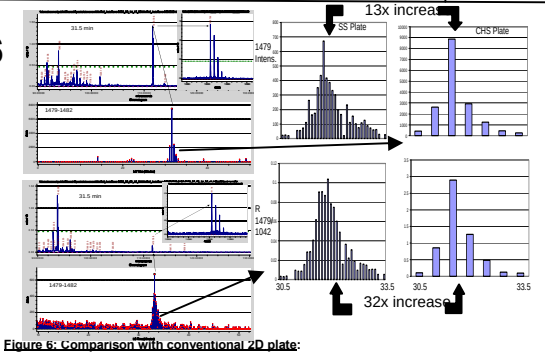

Figure 6: Comparison with conventional 2D plate:

-Identical 1hr separation of 1pmol BSA at 15uL/min flow spotted at 5s intervals on a 2D surface and 20s intervals on a CHS plate.

-Left frames shows the EIC and max-intensity spectrum of 1479Da peptide from each run (CHS top:2D bottom).

-Right frames are blow-ups of the EIC region of interest.

-Right top: raw signal intensity 13x increase in intensity

-Right bottom: signal intensity normalized against a 1042Da internal standard (matrix addition)

demonstrates 32x increase in signal intensity

Based dwell time and spot dimensions a 36x increase in signal was calculated therefore the above results indicate near 100% sample recovery.

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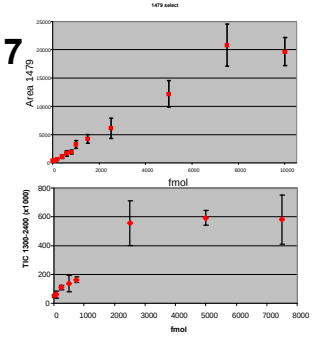

10fmol-10pmol dilution series different sample Conc. loaded in identical volumes

10fmol-7.5pmol conc. series identical sample loaded in different volumes

Figure 7: Capacity study:

-plots from different loading schemes aimed at determining capacity of CHS plate with ~5μL void volume.

-Plot A: dilution series of different conc. loaded in some volume as not overwhelm the void volume of well shows ~ 8pmol load capacity limited primarily by analyte/matrix ratio/ ionization efficiency

-Plot B Same sample different load volume --void volume exceeded-- Limited by binding capacity of CHS wells ~2.5 pmol of digested protein.

-Binding capacity will vary with well dimensions

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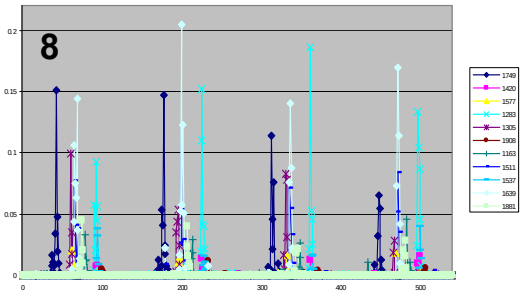

Figure 8: Four Identical, 25 min, 50μL/min LC separation on single CHS plate *Conditions: 50x1mm, 200A, C-18 5μm, Higgins Anal., 0-45% ACN (25 min) 50μL/min flow, dwell time/spot 10s*

-overlay of EIC from 12 mid-intensity range peptides plotted as ratio against1042internalstandardand show and average relative standard deviation of 22%.

-5pmol of BSA digest run in series and spotted on a single 675 well CHS plate (Fig. 1 top middle)

-separations conditions represent a 50-100X increase in flow rate/sample load and capture over conventional LC-MALDI schemes performed on 2D plates.

-relative large sample load, high speed, reproducible separation afforded by CHS plates.

Conclusions:

-Novel 3-dimensional MALDI target that captures and concentrates peptide/protein sample and serves as a direct interface between separation and mass spectrometer

-Plates allow for higher load capacity (potentially 100s μg to mg quantity) and higher flow (100s μL- mL/min) separations that should enable dramatic increase in the detection levels of lower concentration proteins/peptides in complex proteomic mixtures.

-Plates are designed to be reusable with current prototypes easily withstanding 25-50 analyses (like typical LC columns).

-Presentation focuses on the reversed phase LC-MALDI interface because of its near ubiquitous capture of peptide material; however, plates focused on affinity capture (ie. glyco-capture) and studies focused on direct interface of Gel and tissue sample preparation schemes with MALDI are underway.

Acknowledgement:

Recognition and thanks is deserved for the entire Virgin Instruments staff for significant contributions to this project; Christina Vestal, Kevin Hayden, Roger Voyer, Joseph Fitzpatrick, Joseph Valentine "*as in Valentine's Day*", George Mills, Steve Gabeler, Matthew Gabeler and Mark Dahl.

Funding:

This work was supported by SBIR Grant GM079833