New OPTI-PAK® Capillary Trap Cartridge Design Improvement

Optimize Technologies OPTI-PAK® capillary trap cartridges are one of the most functional and efficient means of sample processing available to the mass spectrometrist. Recently, a change in the design of the OPTI-PAK® has improved upon its effectiveness as a trapping column.

INTRODUCTION

Trap cartridges are small packed beds that utilize the adsorptive properties of analytes to effectively concentrate dilute or large samples, desalt, remove detergents, and more. Unlike off-line procedures that require significant sample handling and potential sample loss, Optimize trap cartridges have the advantage of being able to be used in a completely on-line environment.
On-line clean up not only increases recovery, but is also amenable to automation for unattended use and higher throughput.

Traps work on the principle of "digital chromatography." This is the most apt description because there are typically only two states of the analyte: bound or unbound. This on/off behavior is exploited by either trapping the analyte and letting sample matrix and contaminants go to waste; or by trapping the undesirable components and allowing analytes to pass unimpeded. The discontinuous interaction of the analyte in a trap cartridge prevents dilution and dispersion that may occur in larger beds with a more distributive partitioning effect.

OPTI-PAK® capillary trap cartridges are specially designed for use in low volume applications. Previously, this meant bed volumes in the range of 0.25 - 1.0 µL. However, in response to the trend of increasingly lower flow rates, and customer requests, we have now expanded the available bed volume range to span 0.12 – 5.0 µL.

The OPTI-PAK® has also undergone a beneficial design change, relocating the packed bed from the stem head to the stem itself. A packed stem, enabled by a proprietary method of frit placement, places the packed bed directly into the flowpath, eliminating all excess swept volume (See Figure 1). In addition, the move of the packed bed has also changed the bed dimensions leading to increased efficacy.

In addition to the design changes listed above, the OPTI-PAK® also incorporates the auto-adjusting stem, another proprietary design of Optimize Technologies, that ensures a zero-dead-volume connection no matter what the tube stop depth. These design elements combine with some of the best packing materials in the industry **(custom packing is also available)** to deliver a trap column that is both easy to use, and extremely effective.

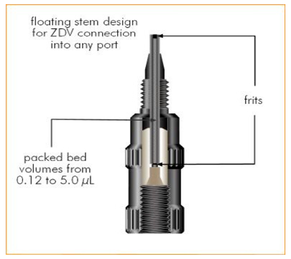

Figure 1 Diagram of new OPTI-PAK® design with packed bed directly in stem tube.

The remainder of this poster will show some of the preliminary results we have obtained with the newly designed OPTI-PAK® in comparison to the older design.

METHODS

Sample Preparation
BSA and Carboxypeptidase A standards were obtained from Michrom BioResources, Inc. (Auburn, CA). Each were brought to a concentration of 1 pm/µL with Solvent A (95% H2O / 5% ACN / 0.1% Formic Acid).

Chromatography Conditions
Chromatographic separations were performed with a Finnigan Surveyor MS Pump, flow split with a Valco HPLC Tee, coupled to a Finnigan AS300 Autosampler fitted with a 20 µL loading loop (see plumbing diagram, Figure 2). Samples were loaded with Solvent A at 10 µL/ minute onto an Optimize Technologies OPTI-PAK® Capillary Trap out of line with the analytical column. Two different styles of OPTI-PAK were used in the testing: the updated OPTI-PAK® that has the packed bed placed within the stem, and the former OPTI-PAK® design with the packed bed in the stem head (See Figure 1). A volume of 15 µL was injected at a concentration of 1 pmol/µL, for a total protein content of 15 pmol/injection.

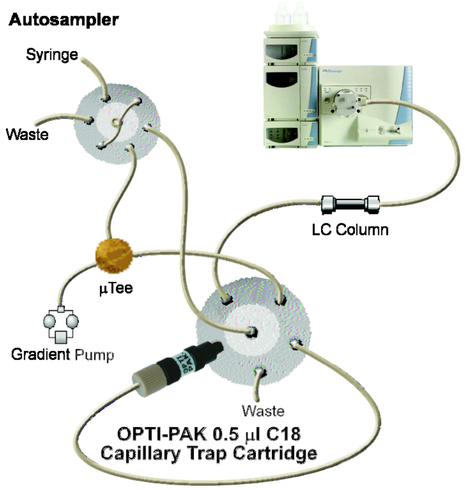

Figure 2 Plumbing and experimental set-up.

A valve switch placed the trap in-line with the analytical column, Vydac Everest™ 0.1 x 150 mm C18 5 µm 300A. The gradient was run through the trap eluting the peptides onto the analytical column. The gradient was run from 0-60% Solvent B (95% ACN / 5% H2O / 0.1% Formic Acid) over 60 minutes at a flow rate of 0.5 µL/minute.

Electrospray MS Conditions
All MS spectra were acquired on a Finnigan LCQ Advantage with a standard orthogonal ESI source and low flow metal needle in general MS positive ion mode.

Results and Discussion
The spectra from BSA and Carboxypeptidase A using both the previous and new design of the OPTI-PAK® may be seen in Figures 3 and 4, respectively.

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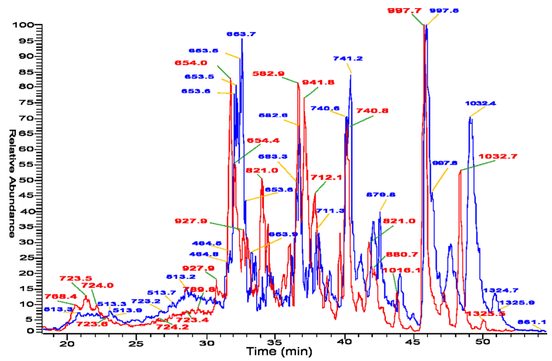

Figure 3 Overlay of BSA test digest using the improved (red) and former (blue) OPTI-PAK® design.

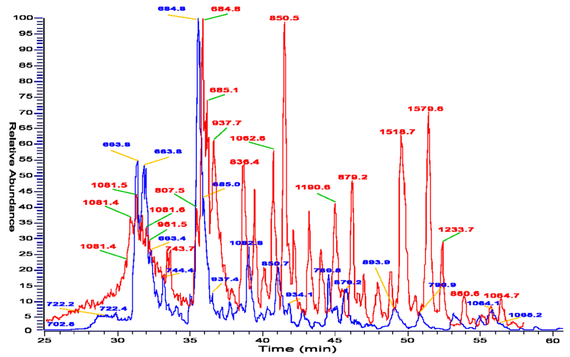

Figure 4 Overlay of Carboxypeptidase A test digest using the improved (red) and former (blue) OPTI-PAK® design.

In the case of BSA, it can be seen by a comparison of the two spectra that the new OPTI-PAK® resulted in sharper peaks with less broadening. Some regions of the spectra have also had additional peaks teased out, which may have been swamped out by the larger peaks using the previous OPTI-PAK® design. Despite these differences, sequence coverage remained fairly constant between the two designs of the OPTI-PAK®.

Much greater differences were observed in the analysis of Carboxypeptidase A. Once again there is a decrease in peak broadening seen. There is also an observed increase in the number of identifiable peaks. This increase is attributable to both an increase in peak intensity and in the total number of peaks resolved. The overall effect of these changes resulted in a 2-3 fold increase in sequence coverage of Carboxypeptidase A.

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In a limited study such as this, it is difficult to incontrovertibly ascribe the differences observed solely to the improved OPTI-PAK® design. However, certain preliminary connections can be made. The most significant change in the OPTI-PAK® was eliminating what was once an empty stem connecting the packed bed to the flowpath. By moving the packed bed into the stem, two things were accomplished: 1.) the amount of swept volume in the trap was brought to an absolute minimum and 2.) the bed dimensions were changed from one of short and wide to long and narrow.

Decreasing the amount of swept volume results in less overall volume in the system, in this case about 0.33 µL. At the flow rates used in this experiment, 0.5 µL, this volume will not affect peak broadness greatly. However, nanoflow applications would see a great improvement in the peak broadening and dispersion affects with the new OPTI-PAK®. The longer bed may also aid in LC/MS analysis by improving the OPTI-PAK's trapping capacity, preventing analytes from passing through the trap to waste during the loading phase.

CONCLUSIONS

The new design of the OPTI-PAK® has improved upon the original by increasing peak shape and resolution, and in turn, unknown identification. While these claims are not yet wholly substantiated, this study as well as anecdotal accounts provide strong support. Further studies need to and will be performed to test these claims and to guide future improvements on the OPTI-PAK® Capillary Trap design.

ACKNOWLEDGEMENT

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