

Comparison of Performance Characteristics of a New Silica-C18 Micropipette Tip with Similar, Commercially Available Tips

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Abstract

During the last decade, Micropipette tips have emerged and have seen rapid development as a new analytical tool for sample preparation. Micropipette tips are a miniature form of solid phase extraction (SPE) cartridges, and function to reduce the sample volumes required for SPE. Therefore, 1-10 μL volumes can be utilized which are required for small-volume sample preparation in the proteomics and biotechnology fields.

In this micropipette technique a sorbent is packed and/or attached to the inner walls of the pipette tip with an adhesive (or other chemistries) at the working end of the tips. The same analytical principles used in SPE apply for the sample preparation with micropipette tips. Micropipette tips are used to handle samples volumes as low as 1.0 μL .



Abstract (contd.)

Samples are desalted or concentrated using these tips and analyzed by ESI-MS, MALDI-TOF-MS, LS-MS, HPLC and mass spectrometry/chromatography techniques.

In this poster we will show the results of our studies with the new Minitips™ C18 and compare with other micropipette tips in terms of reproducibility, recovery and capacity of biomolecules.



Introduction

- When preparing small volume samples with micropipettes, variables such as reproducibility, micropipette tip draw and delivery speeds, binding capacity, recovery, and chemical compatibility are augmented.
- The experiments performed for this study focus on the above-mentioned variables, using several commercially-available silica-C18 micropipette tips.
 - Reproducibility data for substance P recovery have been generated.
 - Draw speed and delivery speed data have been generated using a solution of acetonitrile:water (70:30).
 - Binding capacity data have been generated using substance P, Bradykinin Fragments 1-7, β -Amyloid Protein and Insulin Chain B Oxidized.
 - Chemical compatibility data have been generated using a series of organic solvents, and aqueous acids and bases.



Experimental Results and Discussion

The experimental approach utilized to evaluate the micropipette tips for substance P recovery and/or tip capacity are presented below.

1. Attach a tip to a 10 μ L pipette.
2. Pre-wet with 70% ACN/0.1%TFA three times (3x10 μ L)
3. Wash with 0.1% TFA twice (2x10 μ L)
4. Bind 10X with standard solution (10x draw and 10x delivery).
5. Wash with 0.1% TFA twice (2x10 μ L).
6. Elute with 3x10 μ L 70% acetonitrile in a HPLC vial.
7. Add 20 μ L 0.1%TFA in vial in step 7. These are the samples for the recovery tests.
8. Analyze all samples using HPLC or MALDI-TOF.

HPLC Method used for these analyses:

Parameters	Description	Gradient	
		Time (min)	%B
Instrument	HPLC instrument		
Buffer A	0.1% TFA in water	0.00	25
Buffer B	0.08% TFA in acetonitrile	2.00	25
Flow Rate	1.0 mL/min.	7.00	70
Wavelength	215 nm	9.00	70
Column	Discovery BIO Wide Pore C18 (5 cm x 4.6 mm) 5 μ m	9.10	25
Injection Volume	10 μ L	12.00	25
Temperature	25 $^{\circ}$ C		



Experimental Results and Discussion (contd.)

Four sets of reproducibility data, obtained at various times over a 1-year period for substance P, are presented in Charts 1-5. Comparisons are made to Competitor X in Charts 1-5, Competitors V and Z in Chart 3, Competitor W in Chart 4 and Competitor V in Chart 5.

Chart 1

%Recovery of Substance P

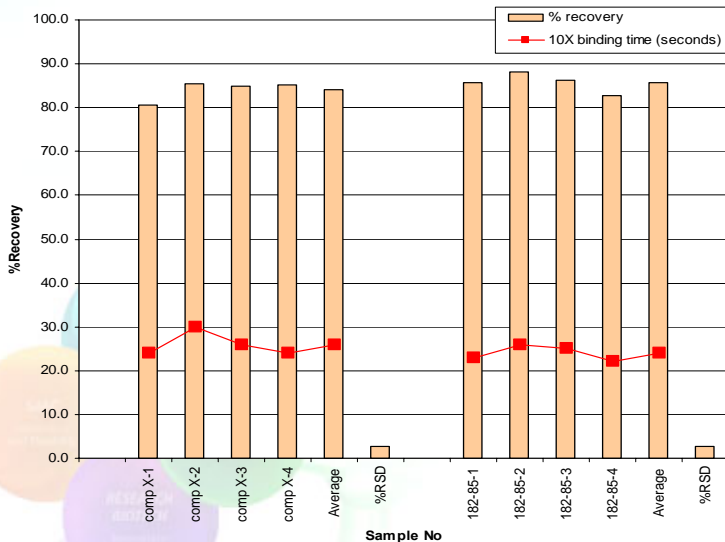
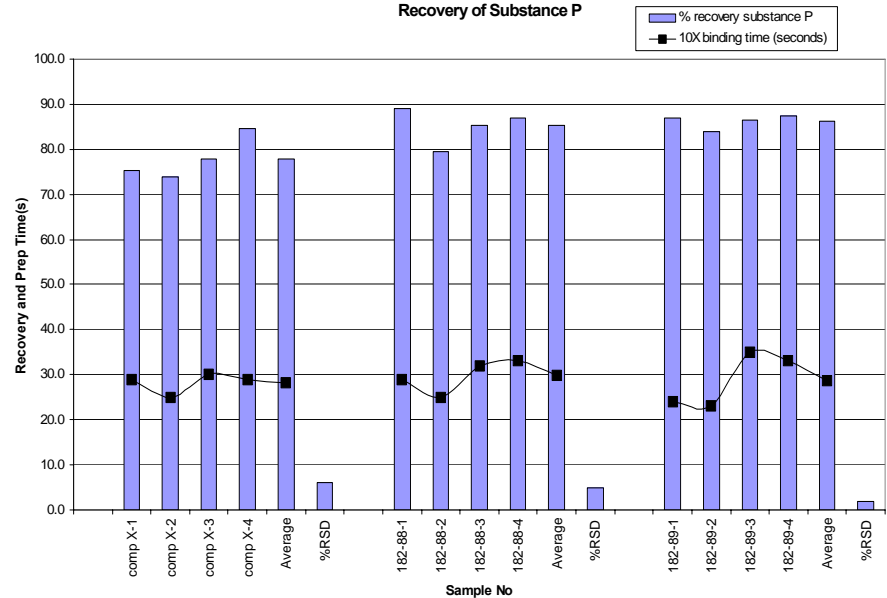


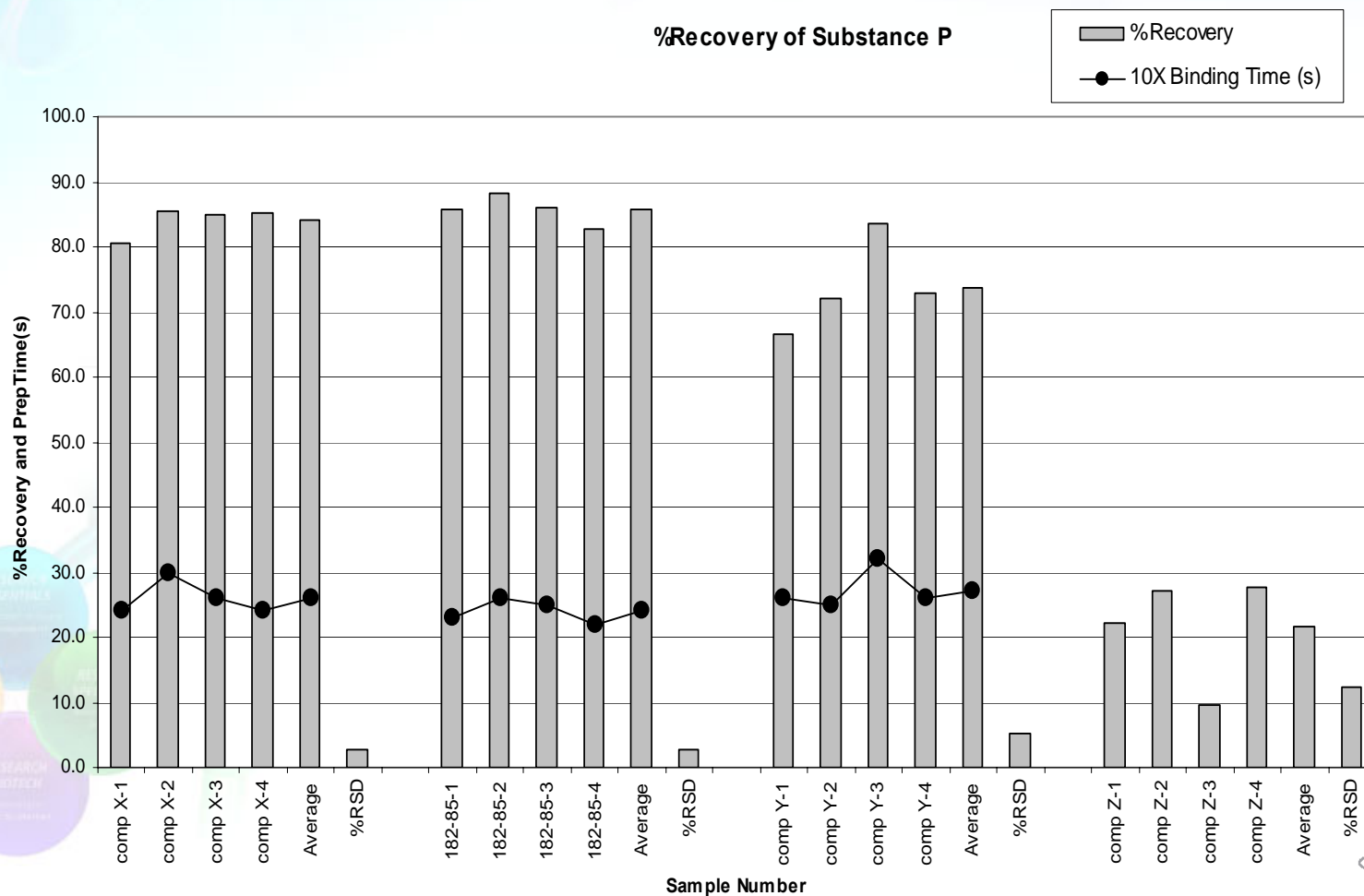
Chart 2

Recovery of Substance P



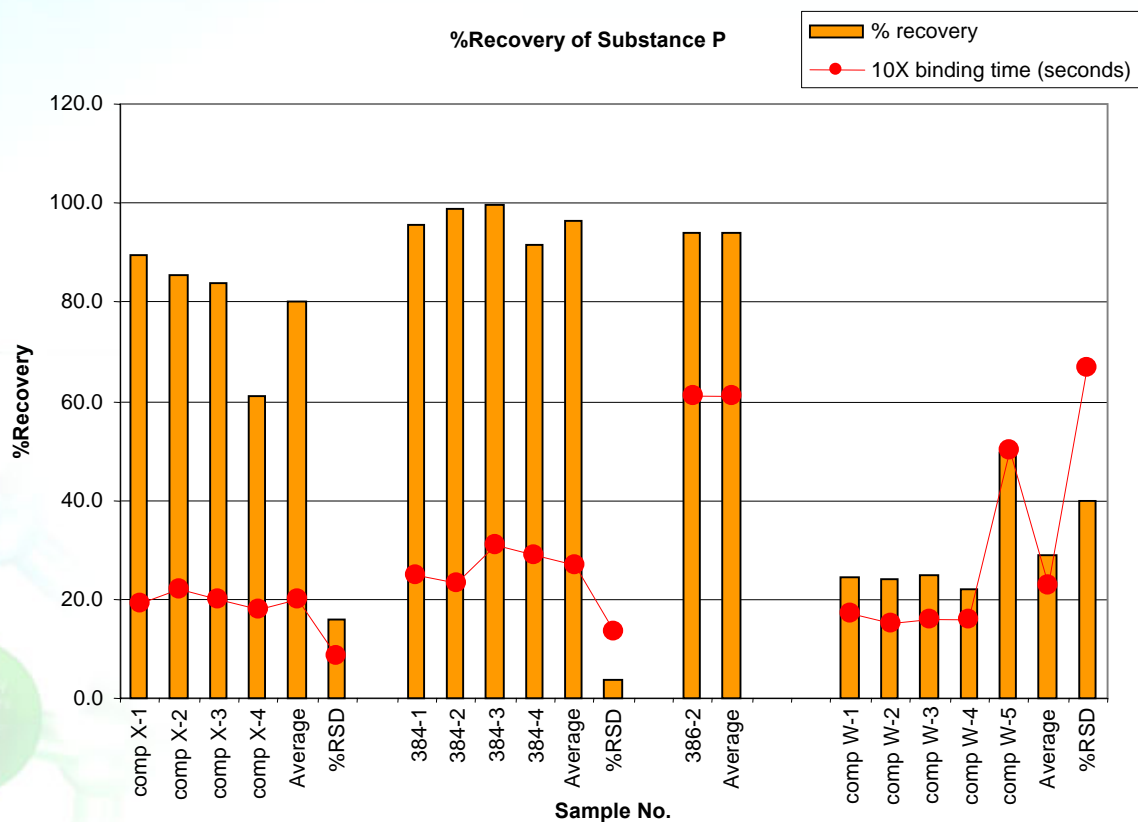
Experimental Results and Discussion (contd.)

Chart 3



Experimental Results and Discussion (contd.)

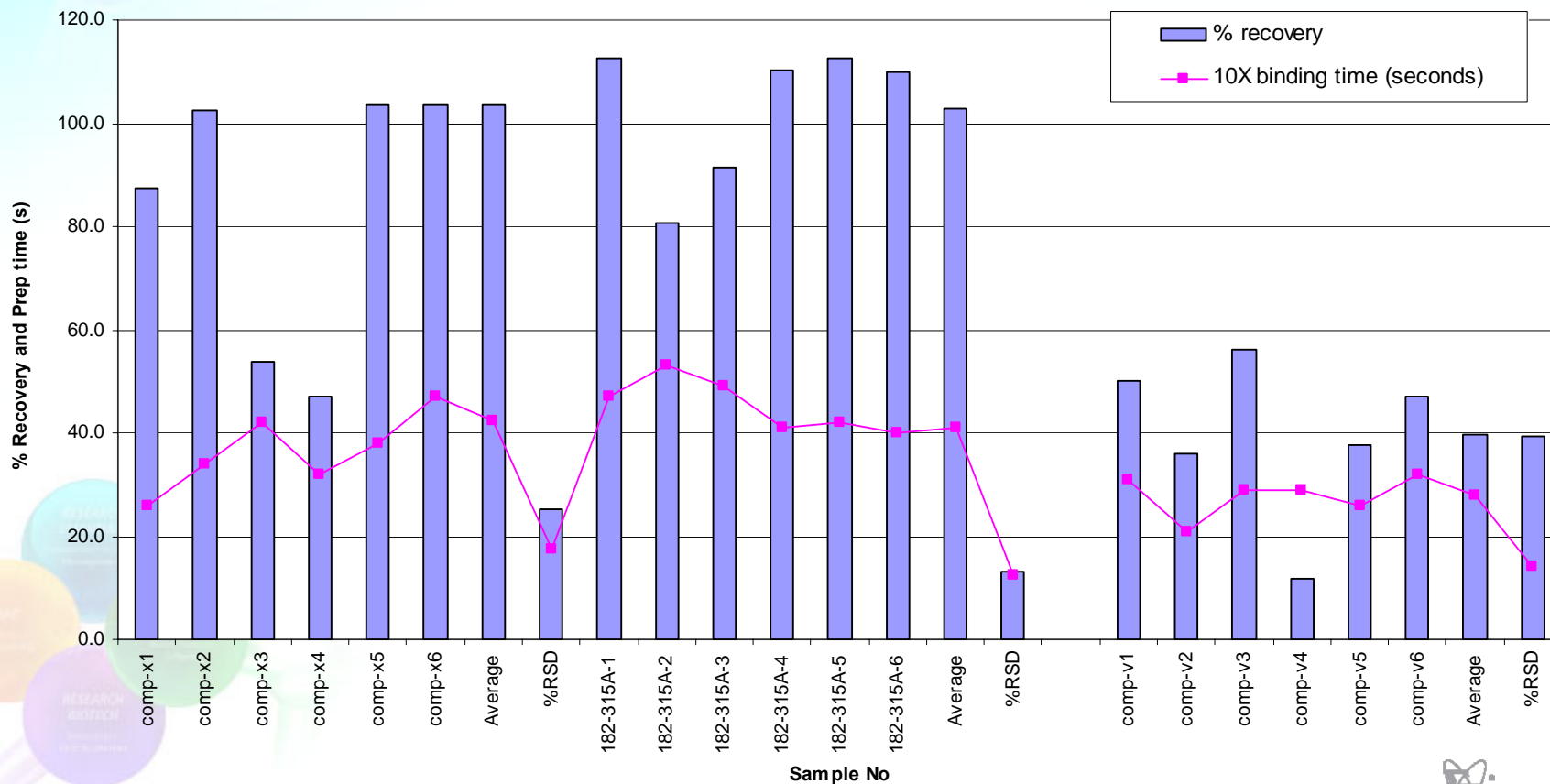
Chart 4



Experimental Results and Discussion (contd.)

Chart 5

Comparison of MiniTipC18 and Competitors X and V



Experimental Results and Discussion (contd.)

Additional reproducibility data are presented in Charts 6 and 7.

Chart 6

%Recovery of Substance P

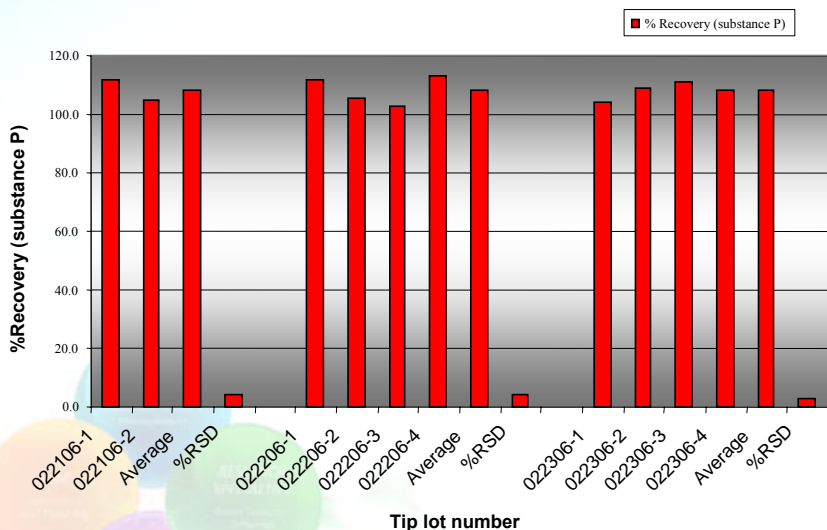
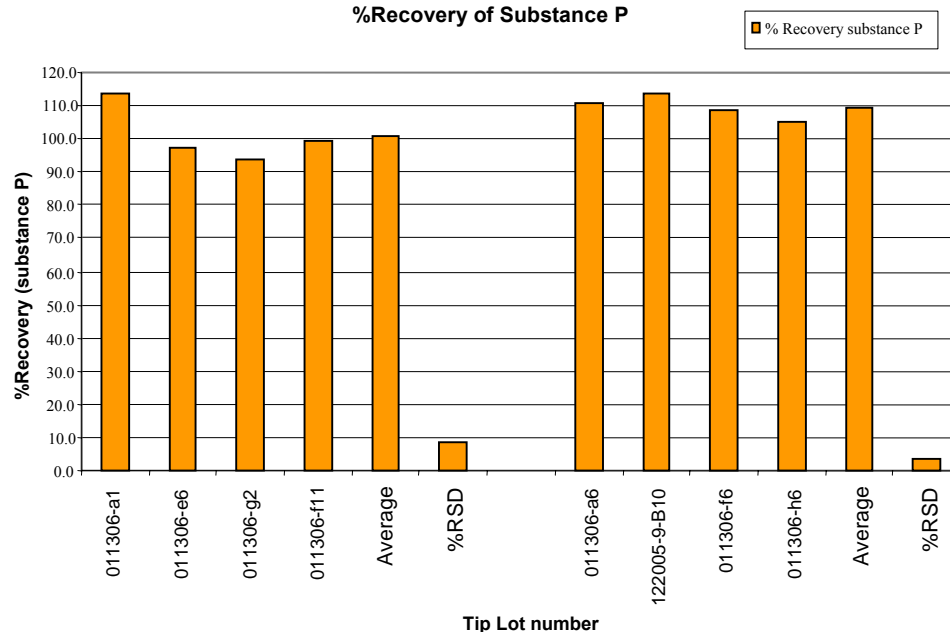


Chart 7

%Recovery of Substance P

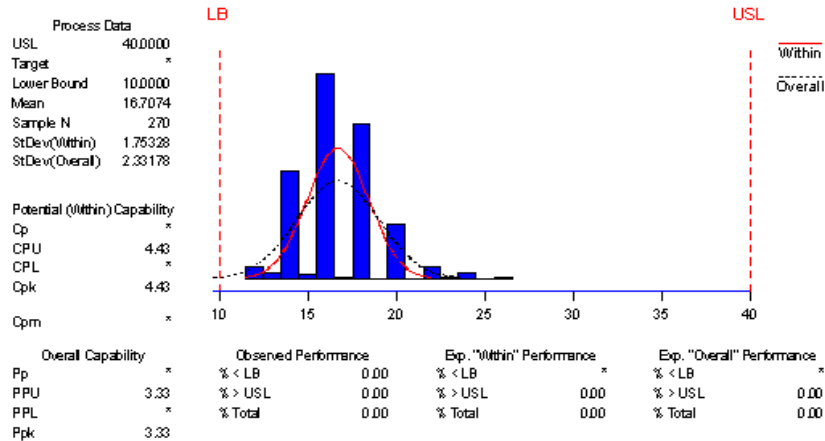


Experimental Results and Discussion (contd.)

The draw speed (i.e., 10X binding time) and delivery speed data are presented below in Graphs 1, 2 and 3.

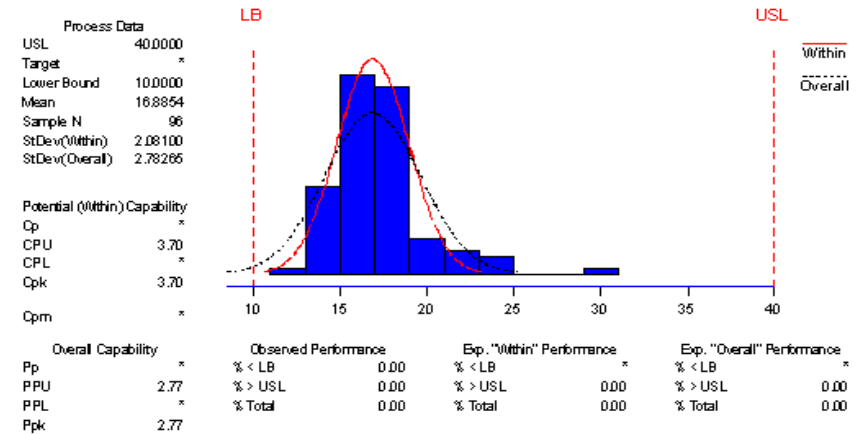
Graph 1

Process Capability Analysis for Flow speed



Graph 2

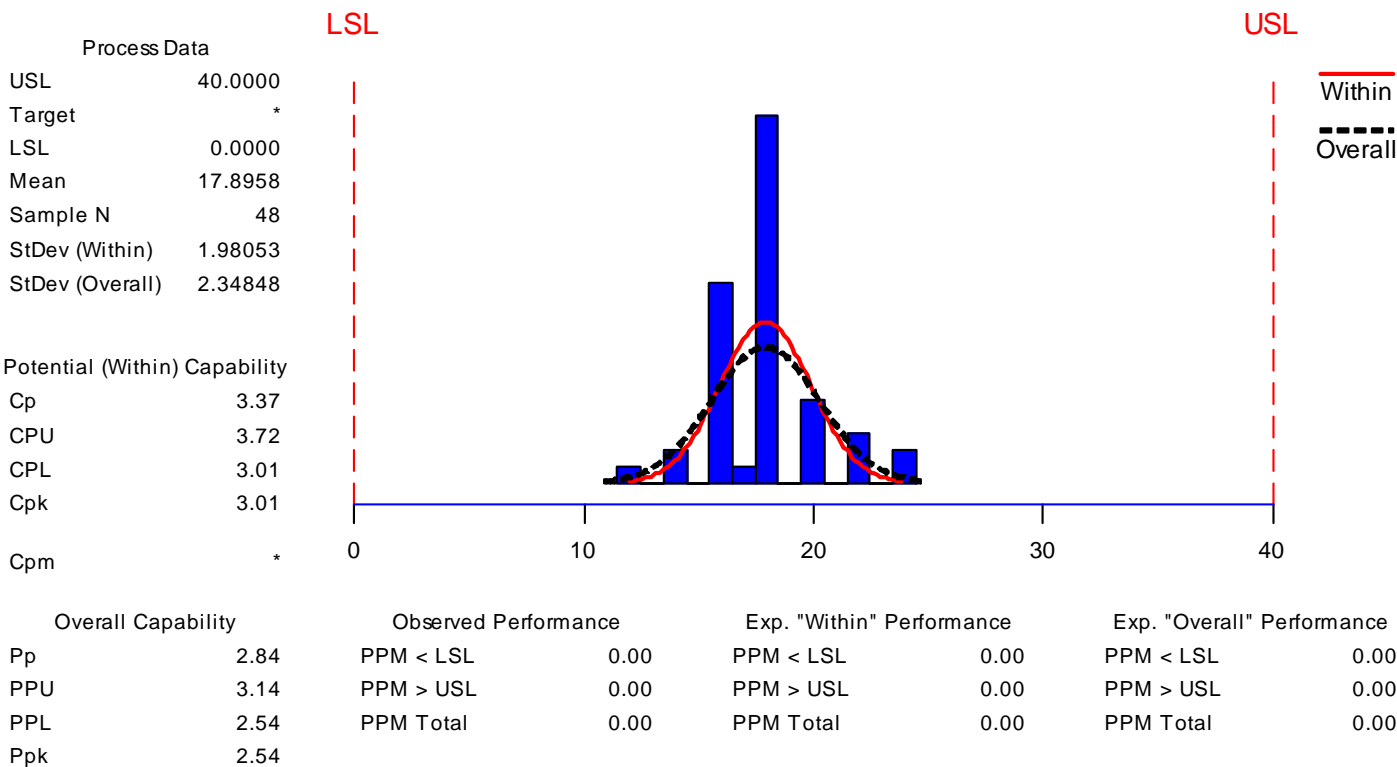
Process Capability Analysis for Speed (s)



Experimental Results and Discussion (contd.)

Graph 3 (draw speed continued)

Process Capability Analysis for flow rate



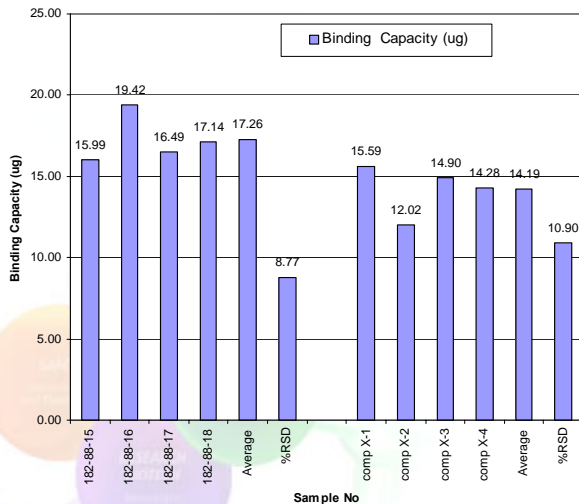
Experimental Results and Discussion (contd.)

Binding Capacity Data

The binding capacity data for Insulin Chain B Oxidized are presented below in Chart 8, and the calibration curve data for Insulin are presented in Graphs 4 and 5.

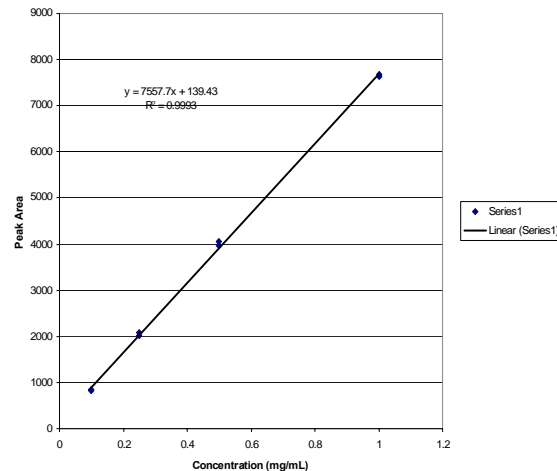
Chart 8

Total Binding Capacity of Insulin (ug)



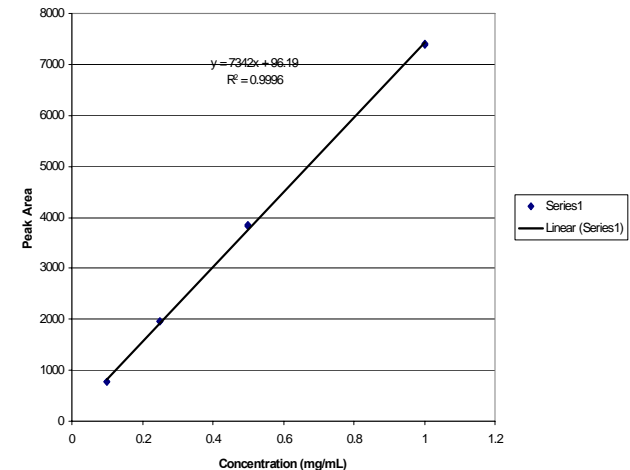
Graph 4

Insulin Standard Samples Series B
(Without Organic present)



Graph 5

Insulin Standard Samples Series A
(With Organic Present)

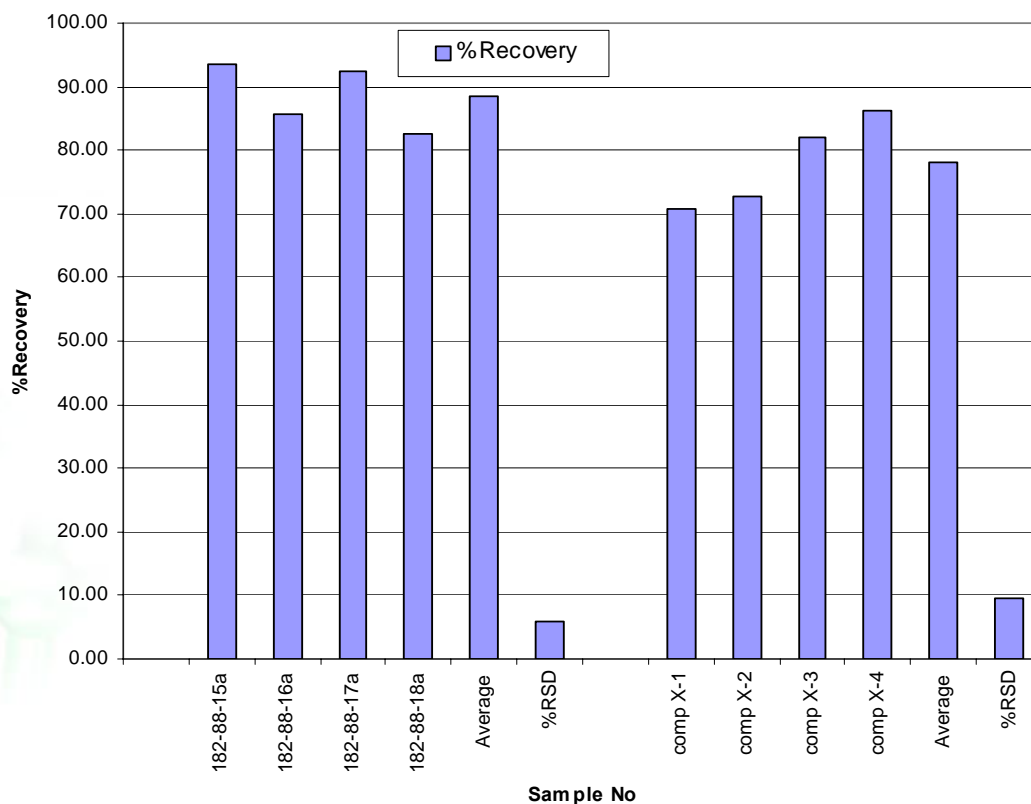


Experimental Results and Discussion (contd.)

The recovery data for Insulin are presented below in Chart 9.

Chart 9

%Recovery of Insulin

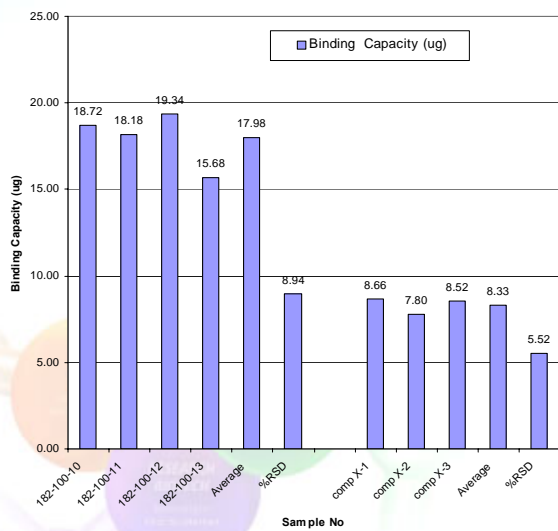


Experimental Results and Discussion (contd.)

The binding capacity data for β -Amyloid Protein are presented below in Chart 10, and the calibration curve data are presented below in Graphs 6 and 7.

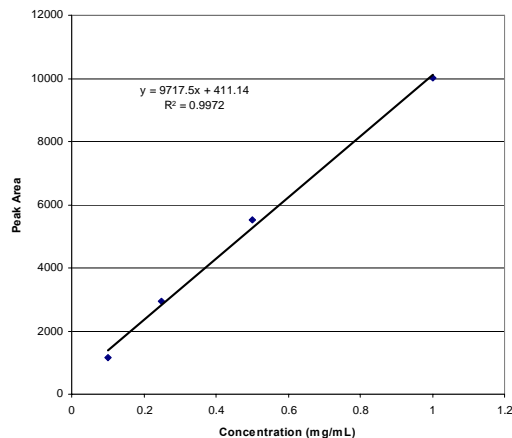
Chart 10

Binding Capacity of β -Amyloid Protein (ug)



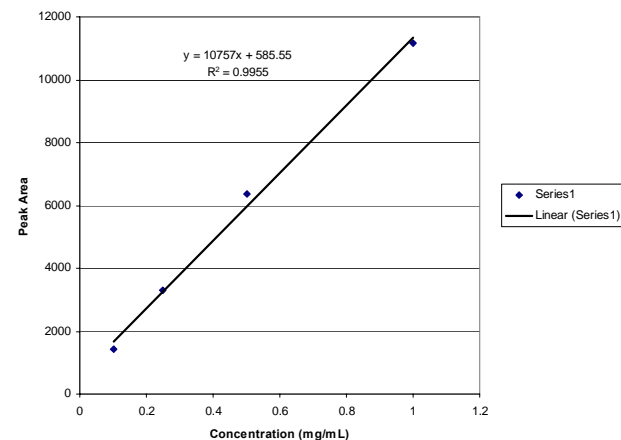
Graph 6

Beta-Amyloid Standard Samples Series B
(Without Organic present)



Graph 7

Beta-Amyloid Standard Samples Series A
(With Organic Present)

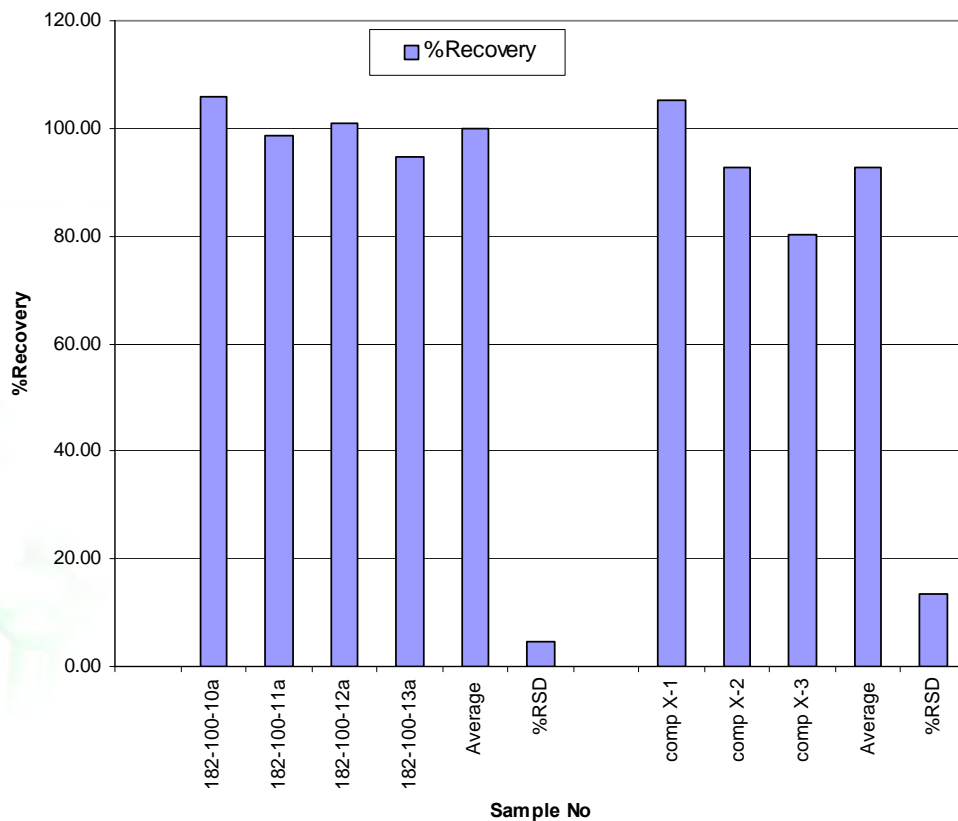


Experimental Results and Discussion (contd.)

The recovery data for β -Amyloid are presented below in Chart 11.

Chart 11

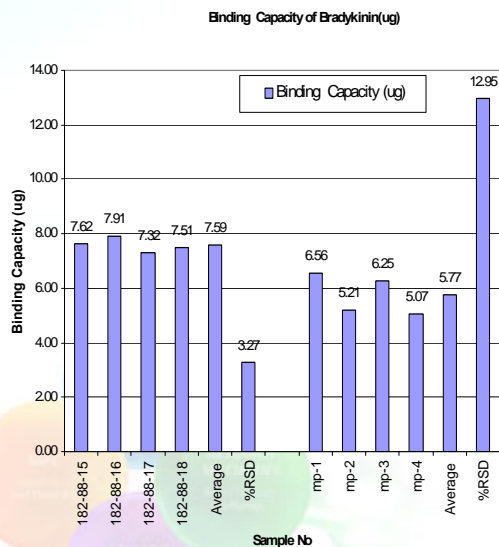
%Recovery of Beta-Amyloid Protein



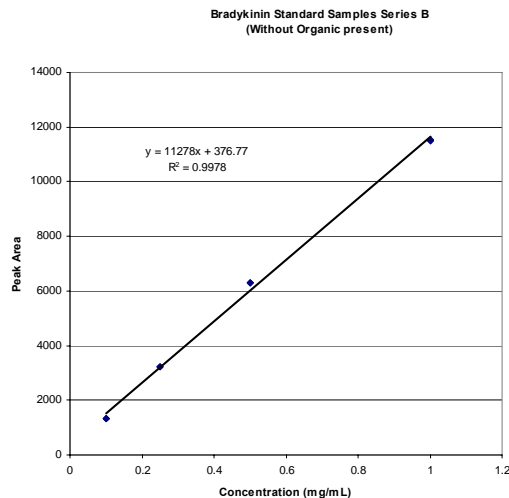
Experimental Results and Discussion (contd.)

The binding capacity data for Bradykinin Fragments 1-7 are presented below in Chart 12 and the calibration curve data are presented below in Graphs 8 and 9.

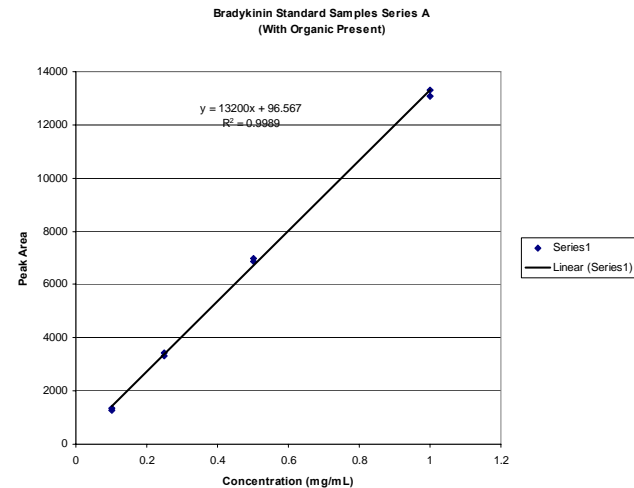
Chart 12



Graph 8



Graph 9

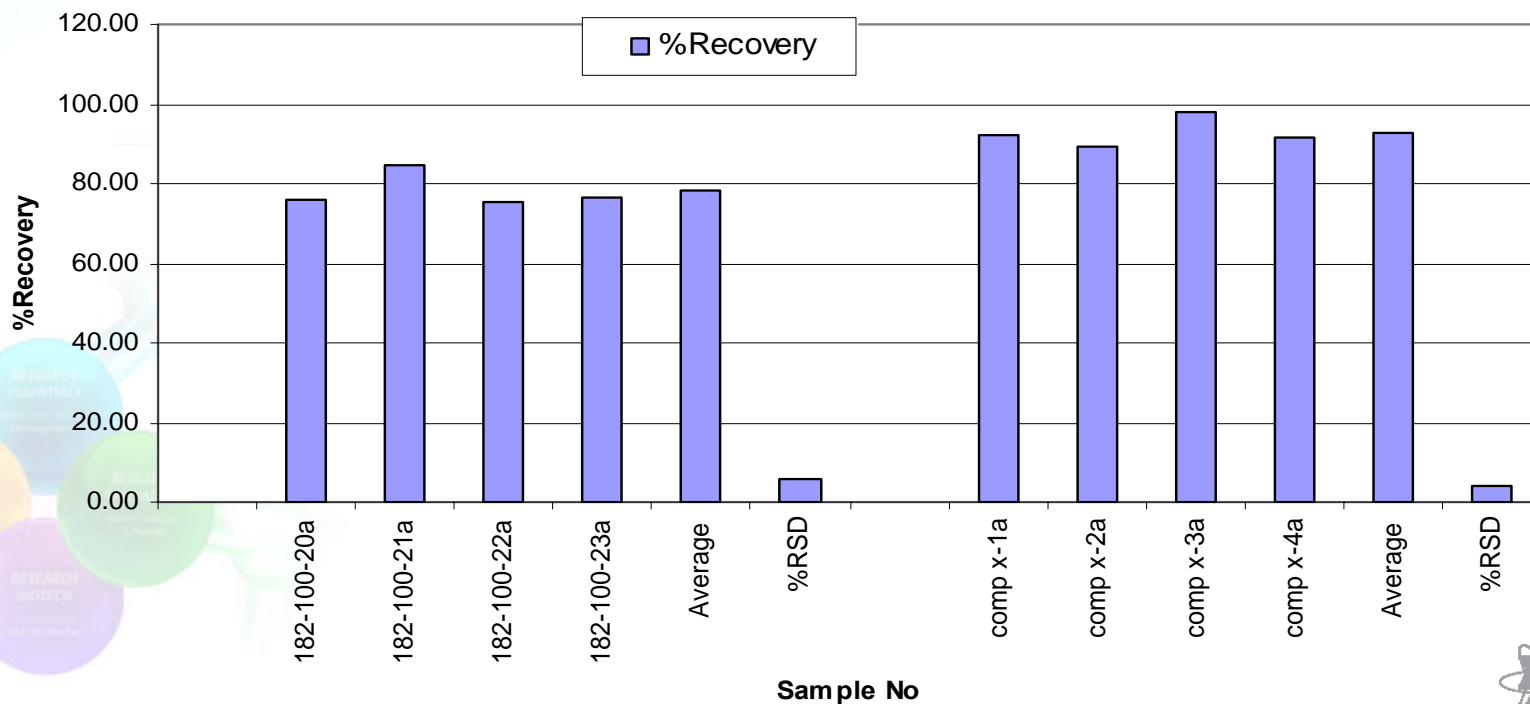


Experimental Results and Discussion (contd.)

The recovery data for Bradykinin are presented below in Chart 13.

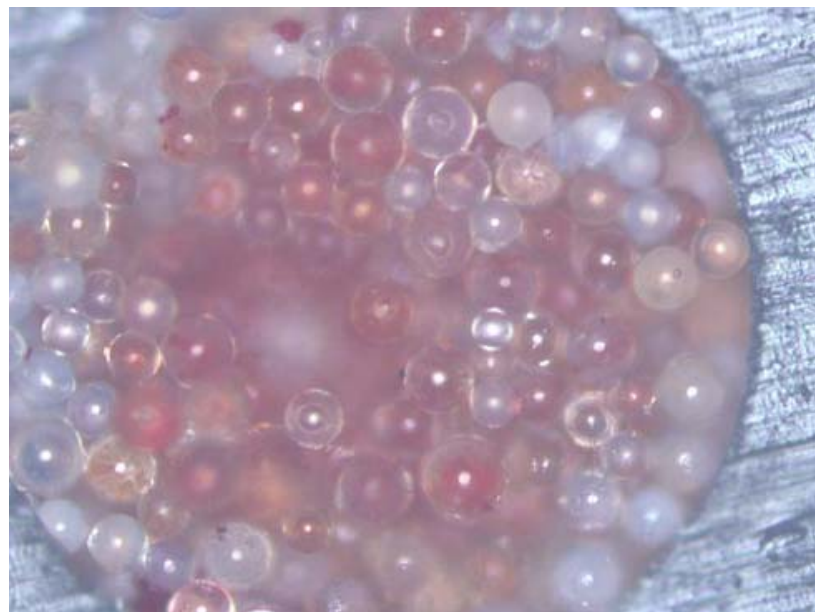
Chart 13

%Recovery of Bradykinin



Experimental Results and Discussion (contd.)

Light microscope photos are of the MiniTips™ C18 tips are presented below in Photos 1 and 2.



Experimental Results and Discussion (contd.)

The chemical compatibility data are presented below in Table 1.

Table 1

solvent/chemical	compatible (yes/no)	solvent/chemical	compatible (yes/no)
Acetic acid	yes	Hydrochloric acid (1%)	yes
Acetone	yes	Isopropyl alcohol	yes
Acetonitrile	yes	Mercaptoethanol	yes
Ammonium hydroxide (28%)	yes	Methyl alcohol	yes
Benzene	yes	Methyl ethyl ketone	yes
Benzyl alcohol	yes	Nitric acid (1%)	yes
Butyl alcohol	yes	Nitric acid (concentrated)	no
Carbontetrachloride	yes	o-Xylene	yes
Chloroform	yes	Phenol (0.5%)	yes
Dichloromethane	yes	Phosphoric acid (concentrated)	yes
Diethanolamine	yes	Sodium hydroxide (1 M)	yes
Dimethyl formamide	yes	Sulfuric acid (1%)	yes
Ethyl alcohol (200 proof)	yes	Tetrahydrofuran	yes
Formic acid (96%)	yes	Toluene	yes
Guanidine HCl (6 M)	yes	Trifluoroacetic acid (10%)	yes
Hydrochloric acid (concentrated)	no	Urea	yes



Conclusions

The data obtained from this study indicate the following:

- The substance P recovery data indicate that the MiniTips™ C18 micropipette tips performed more effectively compared to Competitors V, W, X, Y and Z.
- The substance P reproducibility data for the MiniTips™ C18 micropipette tips appear effective, and show an improvement over Competitors V, W, X, Y and Z.
- For the four biomolecules chosen, the MiniTips™ C18 micropipette tips possess an improved superior capacity compared to the chosen competitors.
- The proprietary, patented adhesive process for preparing the MiniTips™ C18 micropipette tips enables the 50 µm silica-C18 particles to function effectively for high capacity and trace-level sample preparation processes.



References

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3. L.R. Snyder, J.L. Glajch, J.J. Kirkland, *Practical HPLC Method Development*, John Wiley & Sons, New York, 1988.
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