Quad-Z 215 with Disposable Tips: Implementing the Use of ZipTips® for the Purification of Biological Samples

Application Note 201

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Introduction

A ZipTip® is a 10-µL pipette tip with approximately a 0.6-µL bed of chromatography media fixed at its end (no dead volume). It is ideal for concentrating and purifying peptides, proteins, or oligonucleotides in seconds—prior to mass spectrometry, HPLC, capillary electrophoresis, or other analytical techniques. The ZipTip can be used manually with either a single- or multi-channel pipettor or with an automated liquid handling/sample preparation instrument, like the Quad-Z 215 with Disposable Tips.

The process implemented with the ZipTips is synonymous with generic protocols for SPE (solid phase extraction). The ZipTip media is conditioned prior to sample binding, washed, and eluted in as little as 1–5 µL of compatible solvent prior to direct analysis. The purpose of this application is to automate sample cleanup using the ZipTips on the Quad-Z 215 equipped with tip holder B (10 µL tip, DL 10).

Materials & Methods

Chemicals and Reagents

Millipore Corp. ZipTip®, C-18 (Cat No. ZTC18S096)

HPLC-grade acetonitrile

NANOpure® water

Spectro-grade trifluoroacetic acid

Sigma-Aldrich HPLC peptide standard mixture (Cat No. H2016)

Human plasma

Instruments and Accessories

Gilson Quad-Z 215 Liquid Handler, equipped with: 175-mm arm and tip holder B

Gilson 444 QuadDilutor, equipped with: four 250-µL syringes and 1.5-mL transfer tubing

Gilson Tower Pack™ pipette tip refill system (empty) and universal reload box

Gilson Code 235 Rack for the Tower Pack

Gilson Code 205H Rack

Gilson Code 201 Rack

Nunc™ 384-well microplate; 96-well, shallow, round bottomed microplate

Microliter Analytical Supplies 96-well, deep, square bottomed, 2-mL, polypropylene microplate

Eppendorf® PCR plate; 96-well, V-bottomed microplate

Analysis Instrumentation

Gilson 215 Injector equipped with 175-mm arm, micro-septum-piercing probe, 100-µL dilutor syringe, and 1.1-mL transfer tubing

Gilson 819 Injection Module with 10-µL loop (center loop injection)

Gilson 155 UV/VIS Dual-wavelength Detector with analytical flow cell

Gilson UniPoint™ LC System Software, version 4.0, Microsoft® Windows® 2000

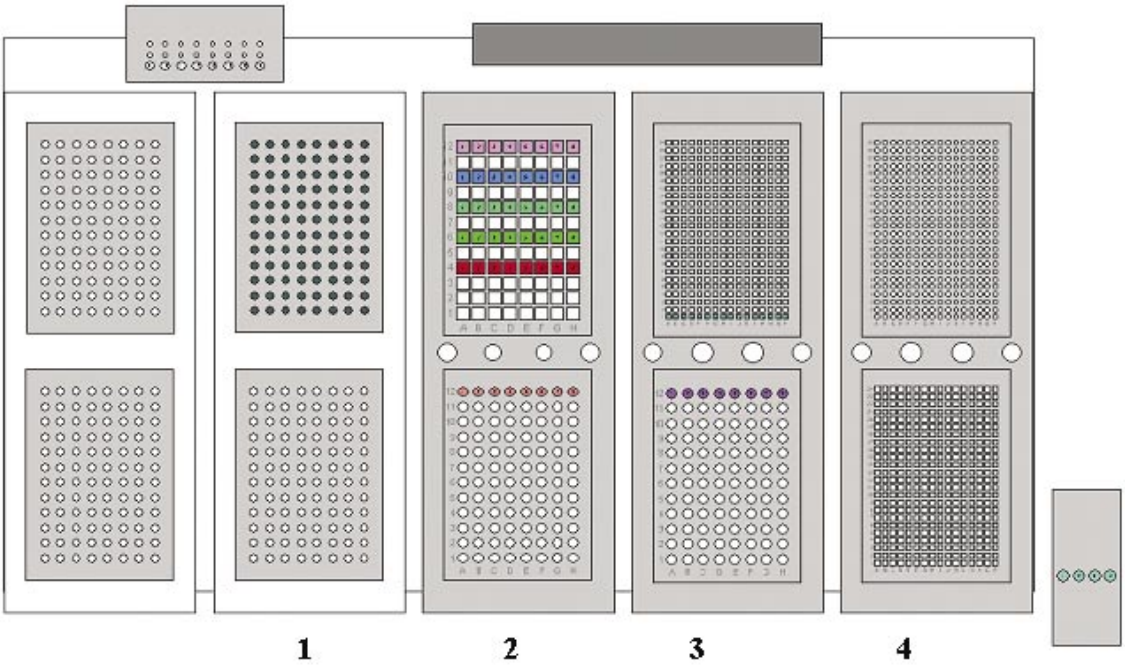
Description of the Procedure

The following stock solutions were prepared for the sample preparation procedure:

1) Wetting Solution: 50% acetonitrile (ACN) in NANOpure water
2) Equilibration Solution: 0.1% trifluoroacetic acid (TFA) in NANOpure water
3) Sample Preparation: final concentration of TFA should be between 0.1–1.0% at a pH of <4
4) Wash Solution: 0.1% TFA in NANOpure water
5) Elution Solution: 50% ACN in 0.1% TFA

The peptide standard was dissolved in NANOpure water (0.1% TFA) at a concentration of 1.5 µg/µL and used as the *ZipTip Standard*. The peptide standard was also dissolved directly in human plasma and used as the *ZipTip Biological Standard*.

Figure 1: 735 Tray File Representing the ZipTip Layout Employed



In Figure 1, rack (1) represents the positioning of the ZipTips; rack (2) shows the use of a deep-well, square bottomed microplate in the back position and the location of the 4 or 5 sample preparation solutions used in the ZipTip procedure. The order of the solutions starting from the back is as follows: Wetting Solution, Equilibration Solution, Wash Solution, Wash 2 Solution, and Elution Solution. The shallow, round bottomed microplate in the front position is used as a waste location for the above solvents, excluding the Elution Solution. Rack (3) holds a Nunc 384-well plate in the back position for the samples and an Eppendorf V-bottomed, 96-well microplate for the collected eluent. Rack (4) could hold

MALDI plates so that the eluent could be directly transferred to the MS plate for analysis.

The sample preparation procedure is as follows:

1) **Dispense with Tips Task**: Aspirate 5 µL of Wetting Solution and dispense into the Waste Location at a rate of 1 mL/min. (for both the aspirate and dispense); repeat twice.
2) **Dispense with Tips Task**: Aspirate 5 µL of Equilibration Solution and dispense into the Waste Location at a rate of 1 mL/min. (for both the aspirate and dispense); repeat twice.
3) **Mix with Tips**: Aspirate and dispense 10 µL of sample from the sample location at a rate of 1 mL/min.; repeat 5 times to ensure proper binding.
4) **Dispense with Tips Task**: Aspirate 5 µL of Wash Solution and dispense into the Waste Location at a rate of 1 mL/min. (for both the aspirate and dispense); repeat twice.
5) **Dispense with Tips Task**: Aspirate 5 µL of Elution Solution and dispense into the Result Location at a rate of 1 mL/min. (for both the aspirate and dispense); repeat three times. On the last dispense, increase the rate to 5 mL/min.

Results

The ZipTip sample prep application was run in parallel, manual (P-10), and automated (Quad-Z 215 with Disposable Tips). The following chromatograms are representations of the results achieved with the ZipTips:

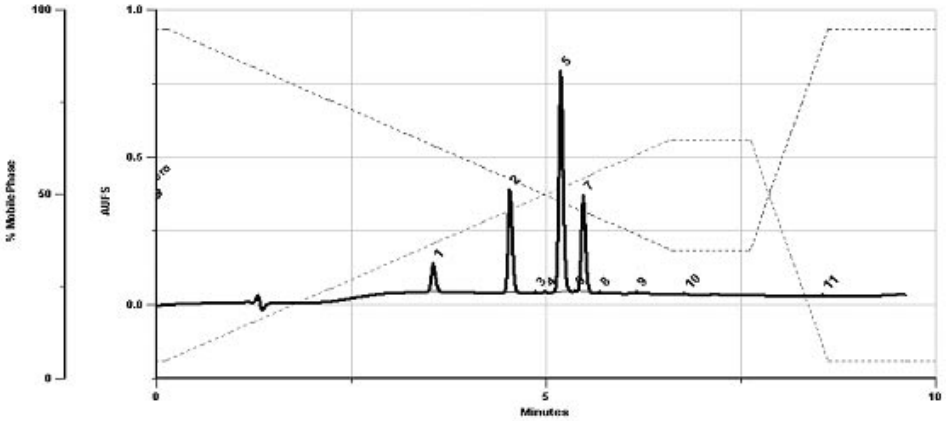


Figure 2: Chromatogram for the Peptide Standard in Water with 0.1% TFA

5-uL injection of the stock solution (1.5 µg/µL). The peptide standard is a mixture of Val-Tyr-Val, methionine enkephalin, leucine enkephalin, and angiotensin II.

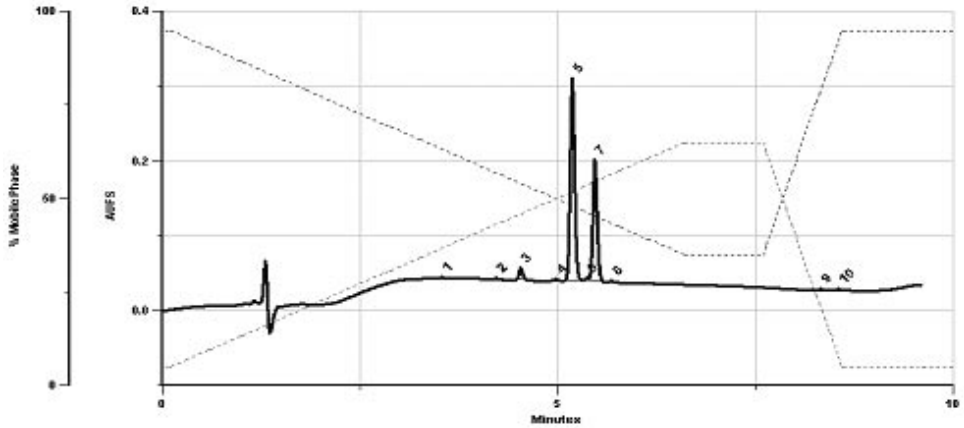


Figure 3: Chromatogram Representing the Results from the Manual ZipTip Method

The ZipTip was placed onto a P-10, and the procedure as noted above was performed on a series of 10-µL peptide standards in water (0.1% TFA); 5-µL eluent injection.

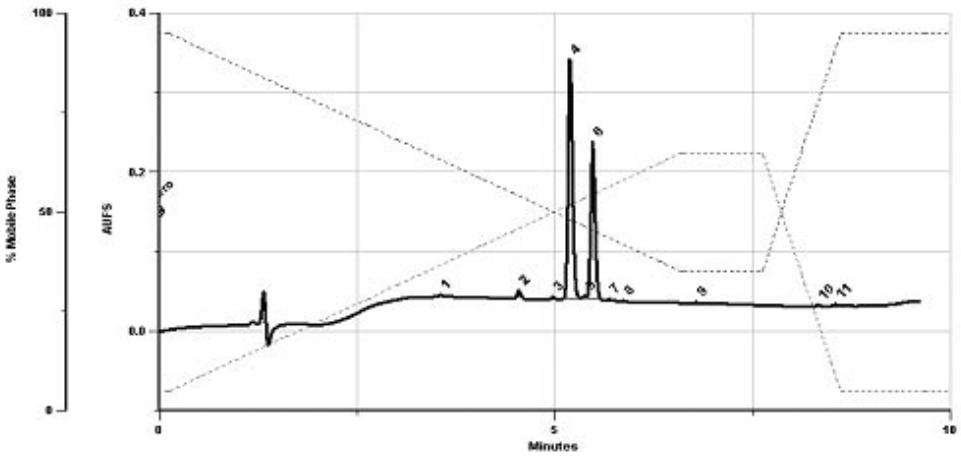


Figure 4: Chromatogram Representing the Results from the Quad-Z 215 with ZipTips Method
The ZipTip procedure was automated on the Quad-Z 215 with "B" tips for a series of 10-µL peptide standards in water (0.1% TFA); 5-µL eluent injection.

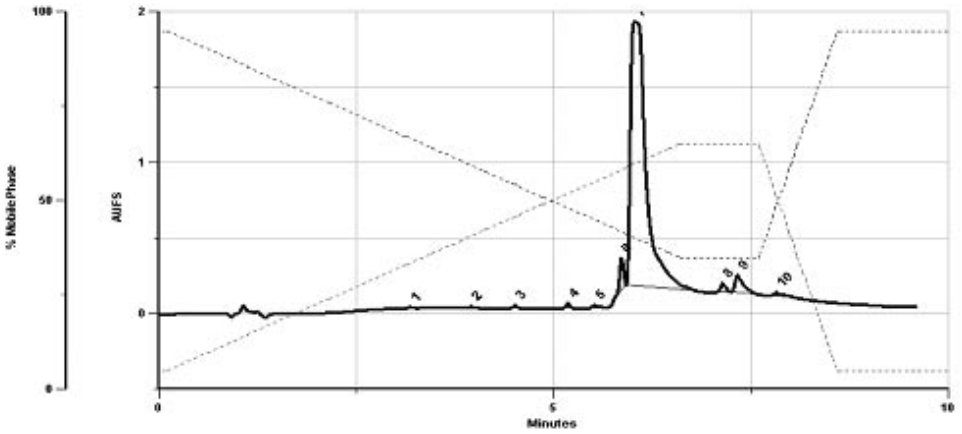


Figure 5: Chromatogram of Human Plasma
A 5-µL sample of human plasma was injected onto the HPLC system to determine background absorbance.

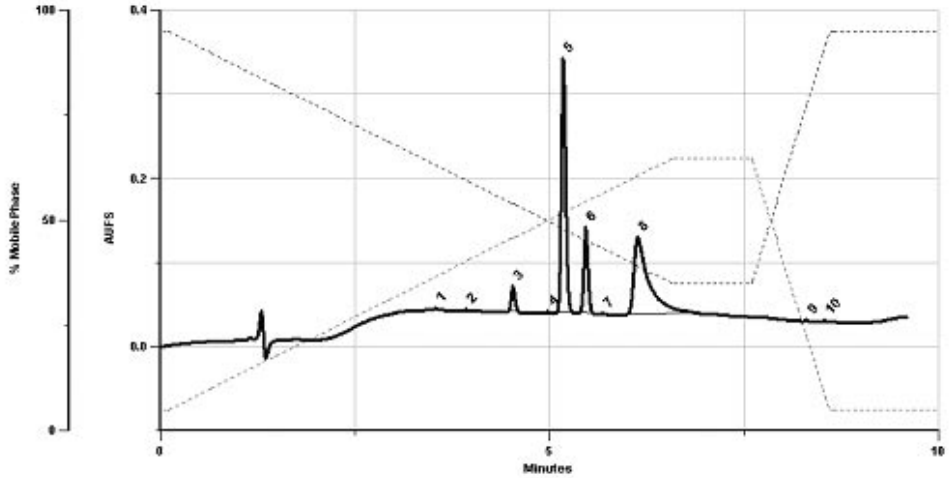


Figure 6: Chromatogram of Plasma Spiked with Peptide Standard, Manual ZipTip Method
The ZipTip was applied to the tip of a P-10, and the above procedure was performed. 5 µL of the eluent was then injected onto the HPLC.

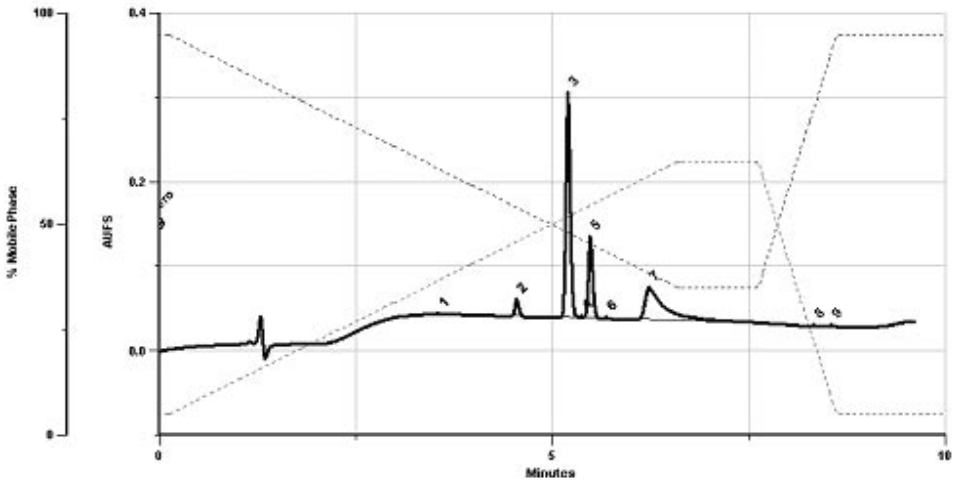


Figure 7: Chromatogram of the Plasma Spiked with the Peptide Standard after Automated Sample Preparation with ZipTips on the Quad-Z 215

A plasma sample was spiked with the peptide standard and then subjected to the ZipTip sample preparation procedure on the Quad-Z 215. A 5-µL injection of the eluent produced the above chromatogram.

The chromatograms presented above show that the Quad-Z 215 with Disposable Tips ("B" tip option) is capable of automating the sample preparation procedure employing the ZipTips. The graphs show an excellent correlation between the manual (P-10) and automated (Quad-Z 215 with Disposable Tips) results. In fact, the Quad Z-215 produced a better recovery of the peptide mixture in 0.1% TFA than the manual method, on the order of 11–18% greater recovery. The Quad-Z 215 showed a slightly lower recovery for the plasma peptide mixture when compared to the manual ZipTip procedure (4–11%). The procedure takes less than 9 minutes to complete for four samples if the initial sample is placed in a 384-well microplate

Conclusion

Although the automated technique employing the Quad-Z 215 with the plasma sample showed slightly lower recovery, optimization is available through varying the aspirate/dispense rates and the amount of time the sample is in contact with the chromatography media. The amount of time required (<9 minutes) for 4 samples via the Quad-Z 215 is a result of the samples being in a 384-well format. Thus, the Quad-Z 215 could only access one sample at a time (less than 9 mm spacing). If the samples were in a 96-well format, or spaced every other well in the 384-well microplate, it would have taken less than 4 minutes to process 4 samples—or less than 60 seconds per sample.

The use of the ZipTips on the Quad-Z 215 offers an excellent method for automating sample preparation prior to analysis—at a fraction of the cost when compared to a larger automated workstation. The Quad-Z 215 is capable of automating the ZipTip procedure, as well as completing numerous liquid handling techniques—with or without tips—under varying plate formats. The task-driven 735 Sampler Software, with drag-and-drop capabilities, allows the user to easily formulate and change methods and then store them under unique configurations. The Quad-Z 215 with Disposable Tips lends itself nicely to the automated requirements of many sample preparation methods.

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