

# Automated Sample Preparation/Concentration of Biological Samples Prior to Analysis via MALDI-TOF Mass Spectroscopy

Application Note 222

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## Introduction

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Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) is used to obtain fast and accurate determinations of molecular mass. MALDI-TOF MS is a popular qualitative tool based on its high sensitivity, rapid analysis time and extensive mass range. Non-volatile and thermally labile molecules, such as protein and peptides, can also be analyzed via MALDI-TOF MS.

Applications of MALDI-TOF MS for proteins and peptides in biological fluids are urgently needed by researchers in the expanding area of proteomics. Being able to couple a sample preparation procedure prior to MALDI spotting of such biological samples would improve the analysis of the analyte by decreasing the background and signal suppression, as well as the resolution of the MS signal. The use of pipette tips that contain a bed of chromatographic media in the tips (or a similar phase coated on the inside walls of the tips) would allow the sample cleanup and concentration of the analytes, essentially mimicking a solid phase extraction column prior to MALDI-TOF MS. The sample can be prepared in as little as a few microliters and spotted directly onto a MALDI plate for analysis.

Biomolecules covering a molecular weight range and present in biological fluids will be evaluated under the sample preparation procedures and analyzed via MALDI-TOF MS. Results will be presented for the analysis of the proteins and peptides. The practical limitations associated with automating the sample preparation procedure and MALDI spotting also will be presented. The data will pertain to the spotting of samples after sample cleanup, as well as direct spotting via a HPLC onto a MALDI plate.

## Materials & Methods

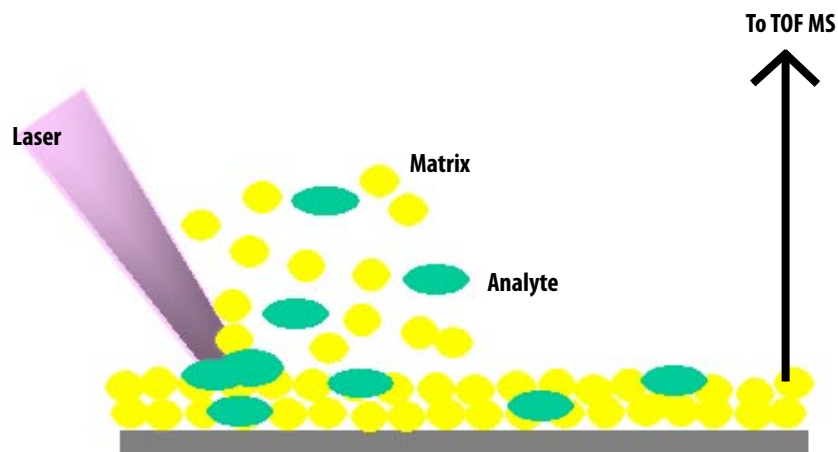
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### **Matrix-Assisted Laser Desorption Ionization (MALDI)**

MALDI is a method that allows for the vaporization and ionization of non-volatile biological samples from a solid phase directly into a gas phase. The sample (analyte) is suspended, or dissolved, in a matrix (usually in a 1000x molar excess). Matrices are small organic compounds that are co-crystallized with the analyte. The presence of a matrix seems to protect the analyte from degradation, resulting in the detection of intact molecules as large as 1 million Da.

In the MALDI process, a laser beam serves as the desorption and ionization source. The matrix absorbs the laser light energy, causing part of the illuminated substrate to vaporize. The matrix plume carries some of the analyte into the vacuum with it, which aids the sample ionization process. The matrix molecules absorb most of the incident laser energy minimizing sample damage and

ion fragmentation. Once the sample molecules are vaporized and ionized, they are transferred electrostatically into a time-of-flight mass spectrometer (TOF MS). In the TOF MS, the molecules are separated from the matrix ions. The molecules are then individually detected based on their mass-to-charge ( $m/z$ ) ratios and then analyzed.



**Figure 1.** Simplified diagram of the MALDI process.

### System Components

#### Quad-Z 215 with ZipTips

Gilson Quad-Z 215, equipped with: Millipore Corp. ZipTip® (C-18)

Gilson 444 QuadDilutor, equipped with: four 250- $\mu$ L syringes and 1.5-mL volume transfer tubing

Gilson 735 Sampler Software, version 5.2 or higher

#### HPLC System with MALDI Fraction Collection

Gilson 350 Micro Pumps (gradient system, "splitless" flow), equipped with: Nano Mixer (300 nL–50  $\mu$ L/min)

Gilson 155 UV/VIS Dual-wavelength Detector, equipped with: capillary flow cell (35 nL x 8 mm)

Gilson 223 Fraction Collector, equipped with: 56-mm arm with spring-loaded probe, 0.003" ID x 90  $\mu$ m capillary PEEK tubing, Upchurch Mixing Tee and rack to hold a Bruker AnchorChip™ MALDI plate

Gilson 402 Syringe Pump, equipped with: matrix valve and 250- $\mu$ L syringe

SGE Chromatography Products ProteCol™-C18 Capillary LC Column (300  $\mu$ m x 50 mm, 3  $\mu$ m, 1/16")

Gilson UniPoint™ LC System Software, 5.1 or higher, 506C interface

Bruker Biflex III Mass Spec, equipped with: XACO 4.04 acquisition software, Xmass 5.1 data processing software and AutoXecute 5.0

### Application of the Matrix and Samples to the MALDI Plate: General Overview

- A matrix stock solution of  $\alpha$ -cyano-4-hydroxycinnamic acid was prepared (5 mg/mL solution in 60:40 water/ACN).
- The 223 Fraction Collector equipped with mixing tee and spring loaded probe was employed to spot the fractions onto the Bruker MALDI plate.
- The working solutions were mixed together to achieve the following final concentration:
  - « 1.5  $\mu$ g/ $\mu$ L solution of the peptide standard either in water (0.1% TFA) or in human plasma was used in the cleanup/concentration (ZipTip) procedure and then analyzed by analytical HPLC with fraction collection directly onto the MALDI plate.

## Control and Data Handling

The Quad-Z 215 automated the sample preparation/concentration (ZipTip) prior to analysis with control by 735 Sampler Software. The results were analyzed by HPLC and fractions by MALDI-TOF MS.

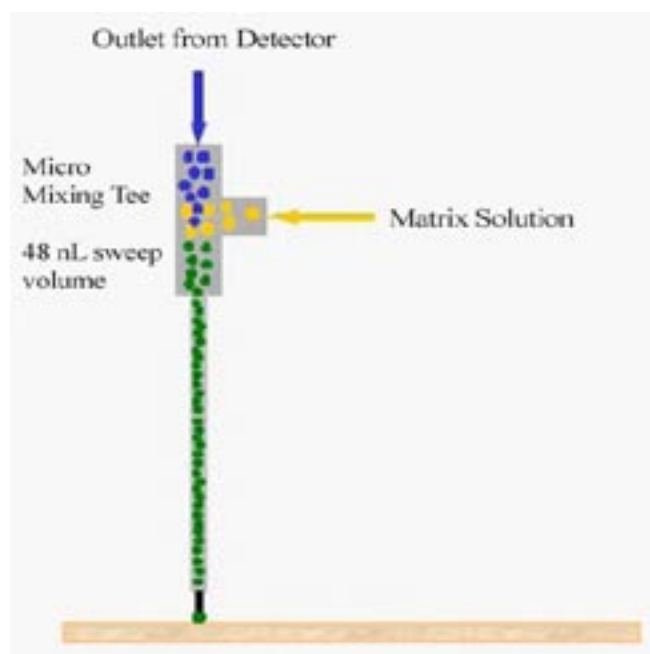
The HPLC system was controlled by UniPoint Software, and fractions were collected onto the Bruker MALDI plate after concentration and clean up via the Quad-Z 215 employing the ZipTips. UniPoint Software allows for multiple collection windows and volume per fraction spot onto the MALDI plate based on time; all are set within the method and are available as an option to the user.

The output from the detector is introduced into to the matrix mixing tee at the top. The Matrix Solution is introduced at a constant flow via a 402 Syringe Pump to the mixing tee from the side.

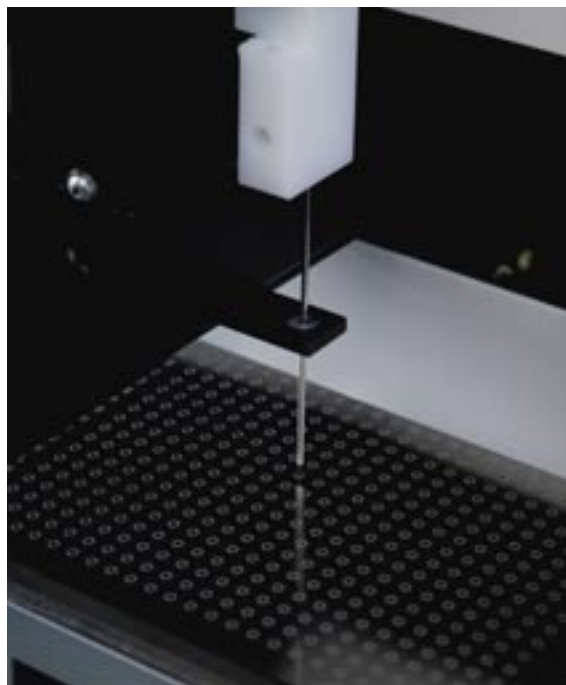
The ratio of mobile phase-to-matrix solution is an adjustable parameter within the software. The length of tubing is minimized between the detector and the spring-loaded probe; there is a 3.2- $\mu$ L delay from the output of the detector to the tip of the spring-loaded probe.

|    | Time | Device(s)                                    | Command   |
|----|------|--|---|
| 1  | 0.01 | Pump 1 / Pump 2                              | 5 (ul/min): 99% Pump 1, 0% Pump 2                     |
| 2  | 0.02 | Fraction Collector                           | Move to Rinse RINSE_SITE1, 11                         |
| 3  | 0.04 | MATRIX PUMP                                  | Home Syringe  |
| 4  | 0.10 | 235 total loop injection w/ rinse above seal | <start> SAMPLE, 1                                     |
| 5  | 0.34 | System Controller                            | Synchronize   |
| 6  | 0.35 | Detector 10                                  | Autocore Channels                                     |
| 7  | 0.36 | Data Channels                                | Start Chromatogram Channels                           |
| 8  | 0.50 | Pump 1 / Pump 2                              | 5 (ul/min): 90% Pump 1, 10% Pump 2                    |
| 9  | 0.55 | Matrix Addition                              | <start> MATRIX_VOLUME, MATRIX_RATE                    |
| 10 | 0.57 | Fraction Collector                           | Set Fraction Site FC_SITE                             |
| 11 | 0.59 | Fraction Collector                           | Set Collection, Travel and Touch Off Depths 26, 26, 0 |
| 12 | 0.61 | Fraction Collector                           | Set Time per Tube FC_TIME                             |
| 13 | 0.70 | Fraction Collector                           | Start Collection                                      |
| 14 | 3.30 | Fraction Collector                           | Wait for Start Next Collection Window WICK_PLATE2     |
| 15 | 4.20 | Fraction Collector                           | Start Next Collection Window                          |
| 16 | 5.00 | Fraction Collector                           | Stop Collection                                       |
| 17 | 6.02 | Fraction Collector                           | Move to Rinse RINSE_SITE1, 11                         |

**Figure 2.** UniPoint Control Method for the Capillary HPLC System. Fractions were collected directly onto the MALDI plate.



**Figure 3.** Spring-loaded probe with mixing tee.



**Photo 1.** Collection of fractions directly onto the MALDI plate with a spring-loaded probe on the 223 Fraction Collector.

### **Application of the Quad-Z 215 with ZipTips Prior to Analytical HPLC Analysis**

#### **Description of the Procedure**

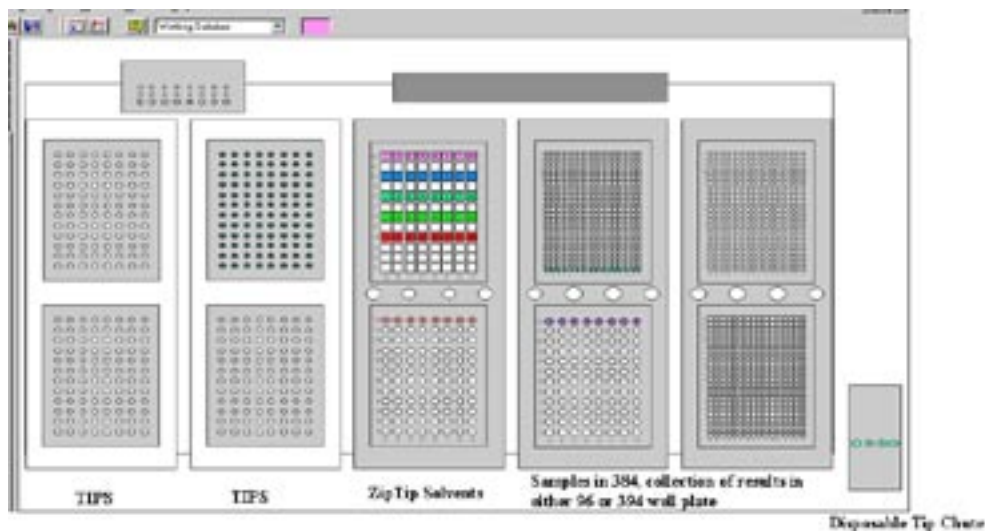
The following stock solutions were prepared for the sample preparation procedure:

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

#### **Actual Sample Cleanup/Concentration Procedure**

- 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 aspiration and dispense task. 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 aspiration and dispense task. Repeat twice.
- 3) Mix with Tips Task: Aspirate and dispense 10 µL sample from the Sample Location five times to ensure proper binding at a rate of 1 mL/min.
- 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 aspiration and dispense task. 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 aspiration and dispense task. Repeat three times. On the last dispense, increase the rate to 5 mL/min.

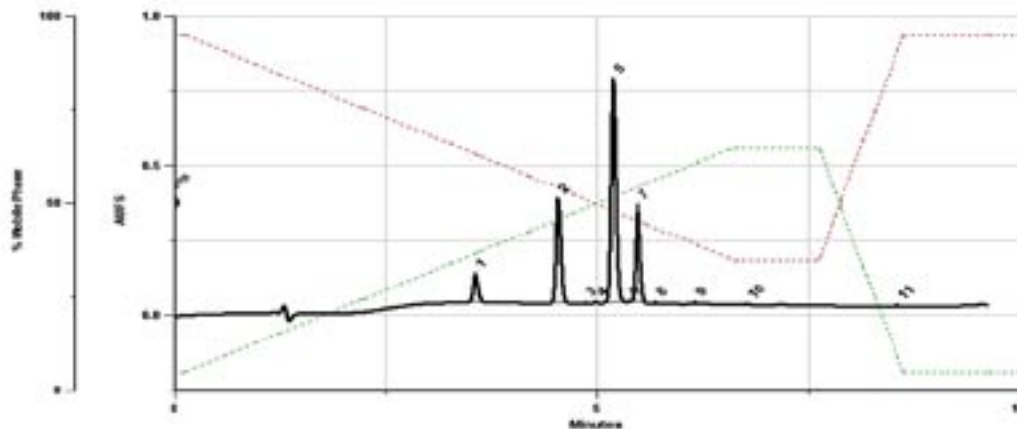


**Figure 4.** 735 Tray File for ZipTips application employed on the Quad-Z 215.

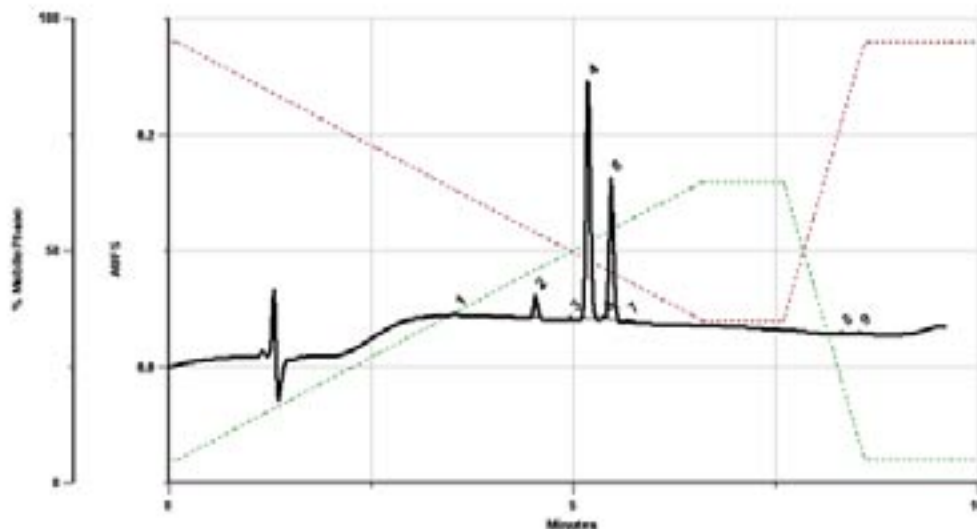
## Results

| Volumetric Accuracy and Precision of Spring-Loaded Probe |                          |     |     |     |
|--|--------------------------|-----|-----|-----|
|  | Volume ( $\mu\text{L}$ ) |     |     |     |
|  | 0.5                      | 1.0 | 2.0 | 5.0 |
| STD (%)  | .2                       | .5  | .7  | .9  |
| CV (%)   | 3.2                      | 3.7 | 3.0 | 1.6 |

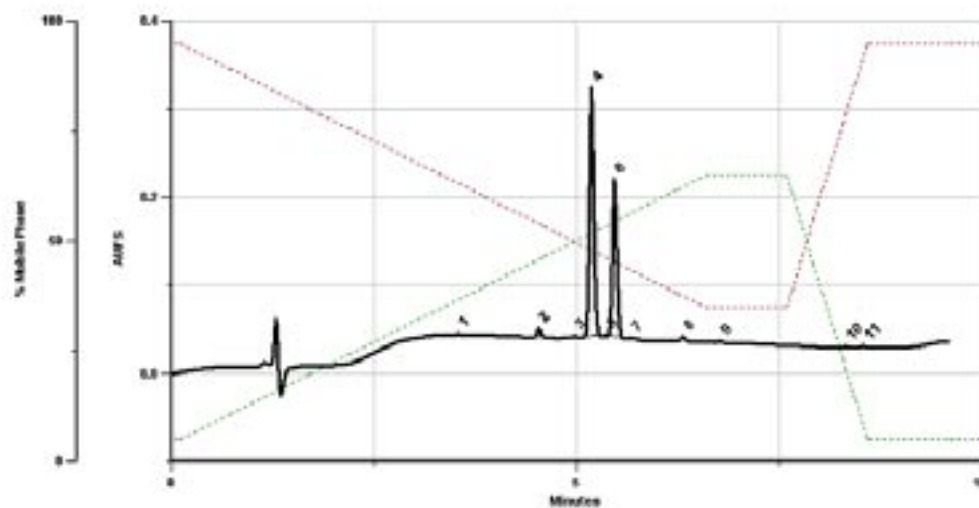
**Table 1.** Volumetric accuracy and precision for the spring-loaded probe on the Gilson 223 Fraction Collector.



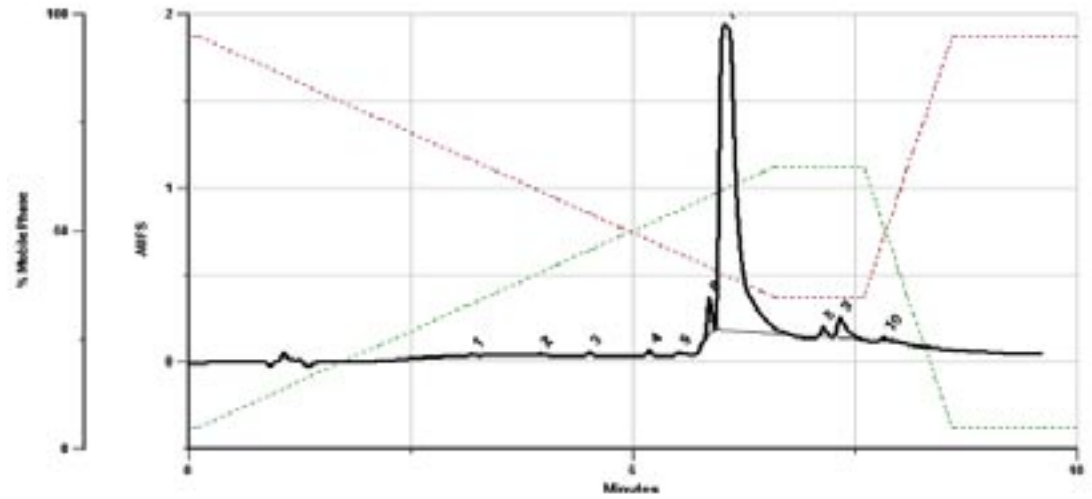
**Figure 5.** Chromatogram for the peptide standard in water with 0.1% TFA standards. 5- $\mu\text{L}$  injection of the stock solution (1.5  $\mu\text{g}/\mu\text{L}$ ). The peptide standard is a mixture of Val-Tyr-Val, methionine enkephalin, angiotensin II and leucine enkephalin.



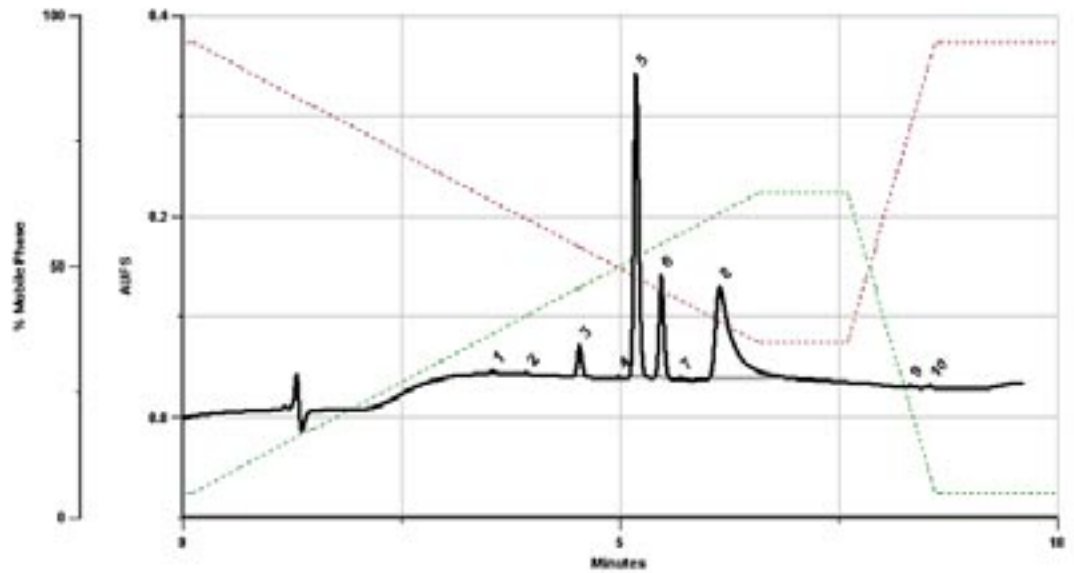
**Figure 6.** Chromatogram representing the results from the Manual ZipTip method. The ZipTip was placed onto a P-10 tip and the procedure (as noted above) was performed on a series of 10- $\mu$ L peptide standards in water (0.1% TFA), 5- $\mu$ L eluant injection.



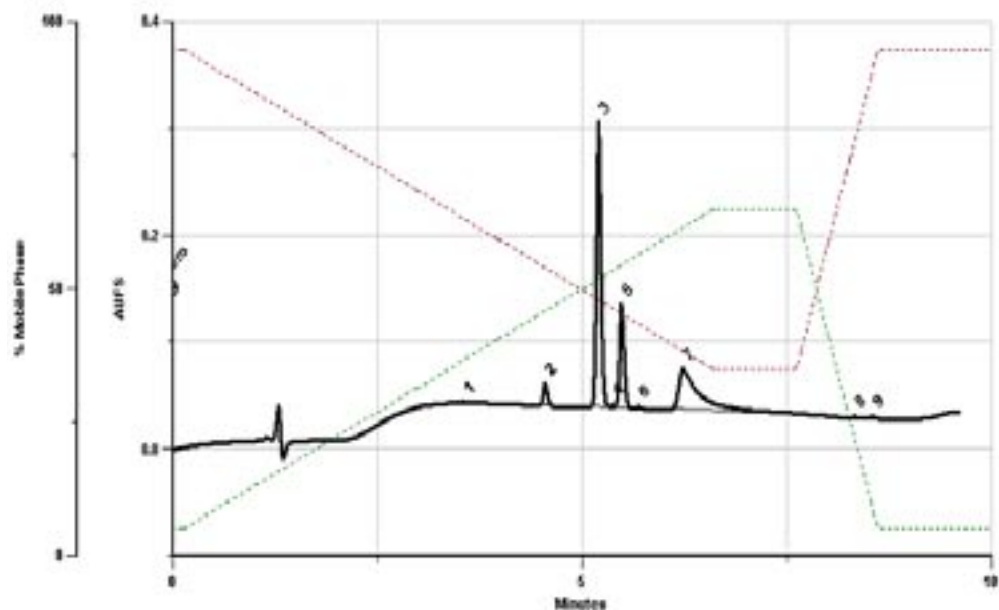
**Figure 7.** Chromatogram representing the results from the Quad-Z with ZipTip method. The ZipTip procedure was automated on the Quad-Z 215 for a series of 10- $\mu$ L peptide standards in water (0.1% TFA), 5- $\mu$ L eluant injection.



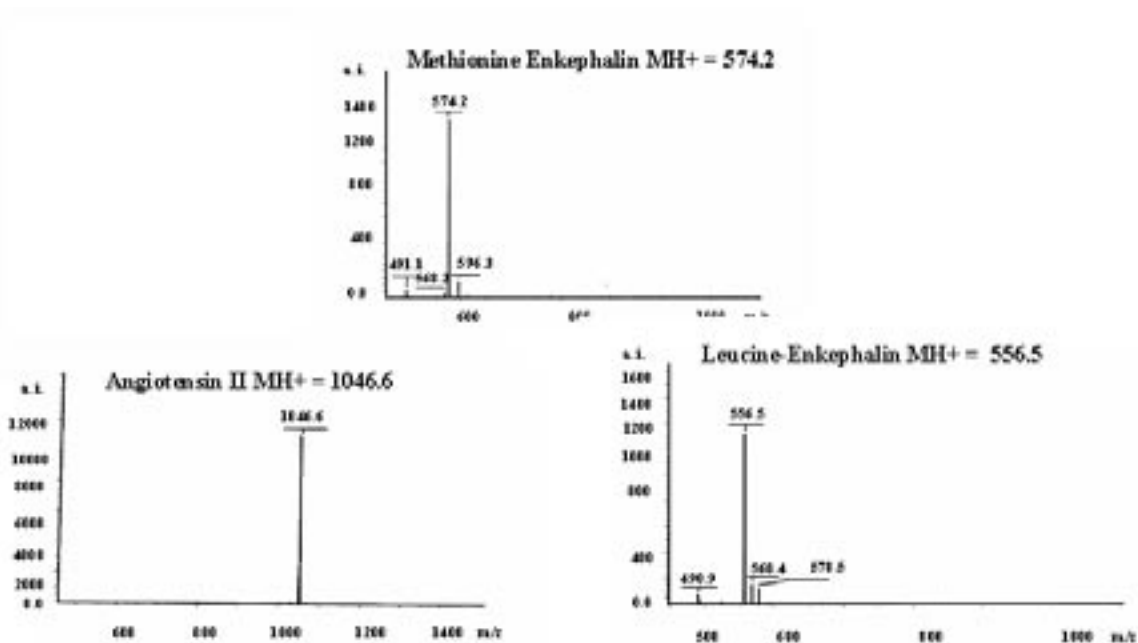
**Figure 8.** Chromatogram representing the results with Human Plasma. A 5- $\mu$ L sample of human plasma was injected onto the HPLC system to determine background absorbance.



**Figure 9.** Chromatogram of human plasma spiked with peptide standard—Manual ZipTip method. The ZipTip was applied to a P-10 tip and the procedure (as noted above) was performed. 5  $\mu$ L of the eluant was then injected onto the HPLC.



**Figure 10.** Chromatogram of human plasma spiked with the peptide standard after automated sample preparation with the 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- $\mu$ L injection of the eluant produced the chromatogram shown here.



**Figure 11.** Resulting spectrum from the collected fractions. The MS spectra from the fractions collected directly onto the MALDI plate. A spectra was not obtained for Val-Try-Val ( $MH^+ = 379$ ) because of background interference.

## Conclusion

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The 350 Micro Pumps and 233 Fraction Collector equipped with spring-loaded probe and matrix mixing tee work well for the fraction collection onto the MALDI plates for analysis via MALDI-TOF MS. The volumetric accuracy and precision data for the 223 Fraction Collector show its capabilities at spotting MALDI plates in conjunction with the 402 matrix delivery system. The smaller footprint of the 223 offers a solution for fraction collection directly onto the MALDI plate at a fraction of the cost of larger MALDI spotters.

The Quad-Z 215 with ZipTips offers an automated solution to the sample preparation/concentration of samples prior to TOF MS or HPLC analysis. Gilson's 735 Sample Software offers user-friendly control for the Quad-Z 215 instrument, while still allowing customization of racks, trays and tasks to accommodate the needs of the researcher.

Automated MALDI plate preparations are becoming necessary with the increase in popularity of MALDI-TOF. Spotting directly onto a MALDI plate with fractions from a capillary HPLC system is advantageous, because it negates the need for transferring fractions collected in a 384-well plate onto the MALDI plate.

The focus of this application was to present an alternative to the larger, more expensive MALDI spotters. The 223 Fraction Collector's capability of collecting the fractions on the MALDI plate offers an attractive option via the spring-loaded probe. The spring-loaded probe is essential to ensure that a touch off of the solution onto the plate is achieved without damaging the surface of the plate. It should be noted that the sample-to-matrix ratio on a MALDI plate is very important in MALDI-TOF and will directly affect results

In capillary HPLC, it is crucial to minimize dead/delay volumes in the system, especially when collecting fractions, as there is only 3.2  $\mu\text{L}$  of delay volume within the collection system.

Gilson's Quad-Z 215 with disposable tips is capable of automating the sample preparation procedure with the use of ZipTips®. (The procedure took less than 5 minutes to complete four samples.) The chromatograms show an excellent correlation between the manual (P-10) and automated (Quad-Z 215 with Disposable Tips) results. The Quad-Z 215 produced a better recovery (11–18%) of the peptide mixture in 0.1% TFA than the manual method.

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