Automated Preparation of MALDI-TOF Plates via a Spring-Loaded Probe

Application Note 203

Joan Stevens, PhD, Luke Roenneburg, Alan Hamstra, and Kevin Fawcett (Gilson, Inc.)

Introduction

Matrix Assisted Laser Desorption/Ionization (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 spare 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 finally analyzed.

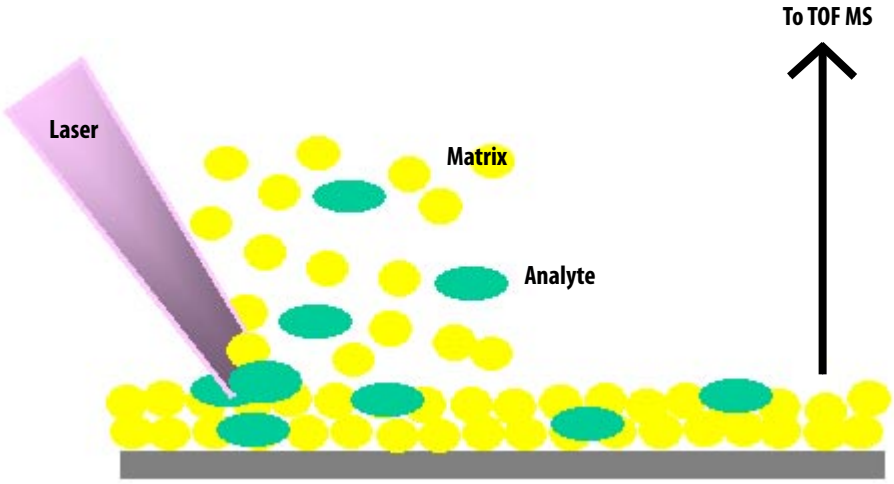


Figure 1: Simplified Diagram of the MALDI Process

Instruments and Accessories

Gilson Micro 215 Liquid Handler, equipped with: 175-mm arm and spring-loaded probe (for MALDI spotting) or micro-septum-piercing probe (for sample injection), 100-µL dilutor syringe, 841 Micro Injection Module with 1-µL internal loop, and Code 201 and Code 205 racks to hold microplates

Gilson 321 Pump, equipped with: H1 (15 mL) pump head, 811C low-volume dynamic mixer (65 µL), and graduated micro-splitter valve with stainless steel needle (Upchurch Scientific)

Gilson 155 UV/VIS Dual-wavelength Detector, equipped with: capillary flow cell (35 nL x 8 mm, 0.01 AUFS, 210 nm)

Gilson 223 Liquid Handler, equipped with: 56-mm arm and spring-loaded probe, 1/32" OD x 0.004"ID PEEK capillary tubing, and modified rack to hold a Bruker AnchorChip™ MALDI plate

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

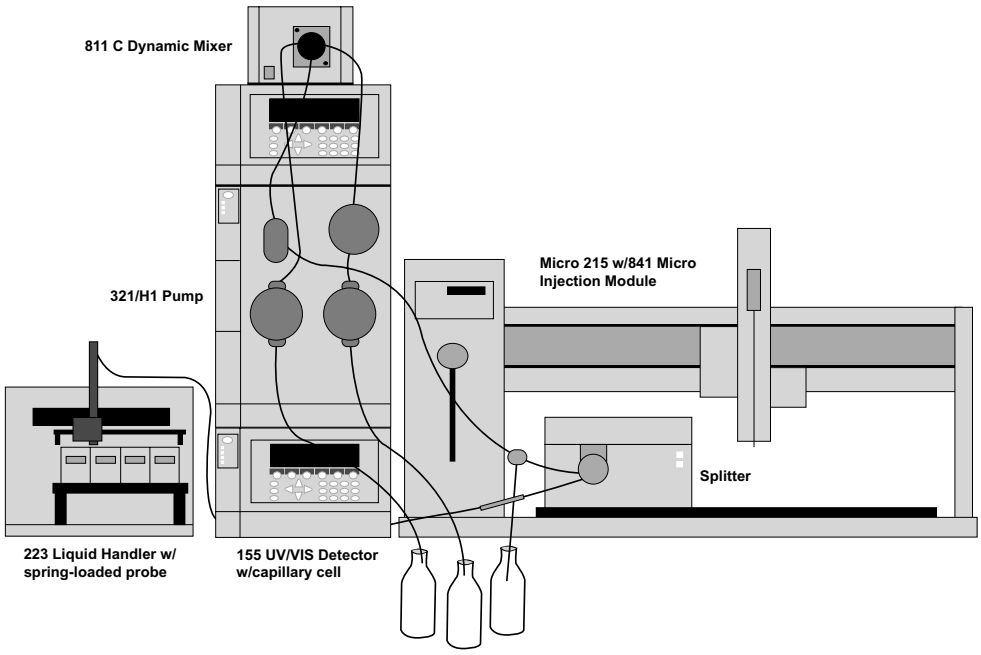
Gilson UniPoint™ System Software, version 3.3

Gilson 735 Sampler Software, version 5.2

Gilson 506C System Interface Module

Intel® Pentium® 4 Processor, >2 GHz, 512 MB RAM, 80 GB hard drive

Figure 2: Capillary HPLC System with MALDI-FC



Description of the Procedure

1) A stock solution of α-cyano-4-hydroxycinnamic acid (matrix) was prepared by dissolving 10 mg in 1 mL of 50% ACN in 0.05% TFA solution (10 mg/mL solution).
2) The Micro 215 with a spring-loaded probe (1/32" OD x 0.020" ID PEEK) was used to spot 0.5 µL of the matrix onto the Bruker AnchorChip, 384-well plate and allowed to dry.
3) A stock solution of bradykinin, angiotensin II, and ACTH at a concentration of 100 pmol/µL was used for the testing of the HPLC and FC.
4) The solutions were mixed together to achieve the following final concentrations:
   a) 33 pmol/µL of bradykinin, angiotensin II, and ACTH
   b) 15 pmol/µL of bradykinin, angiotensin II, and ACTH
5) The mass spectrometer used was a Bruker Biflex III, XACO 4.04 acquisition software, Xmass 5.1 data processing software, and AutoXecute 5.0.

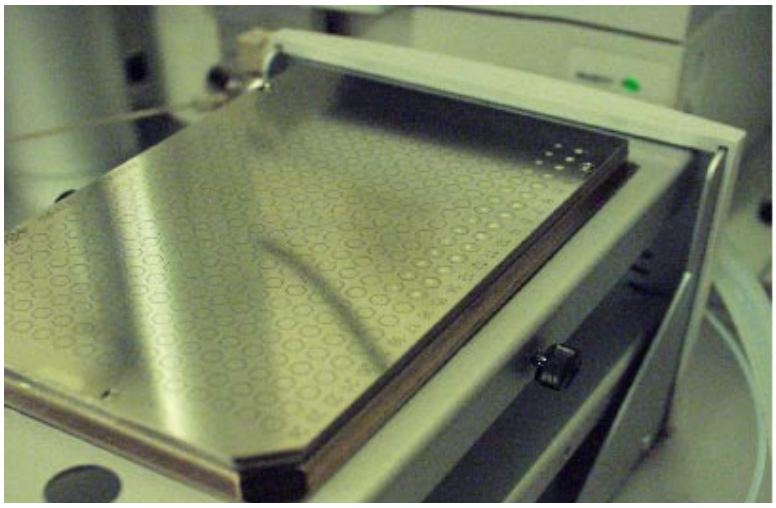


Photo 1: Bruker AnchorChip™ Plate with Matrix Spotted via the Spring-Loaded Probe (1 and 0.5 µL, respectively)

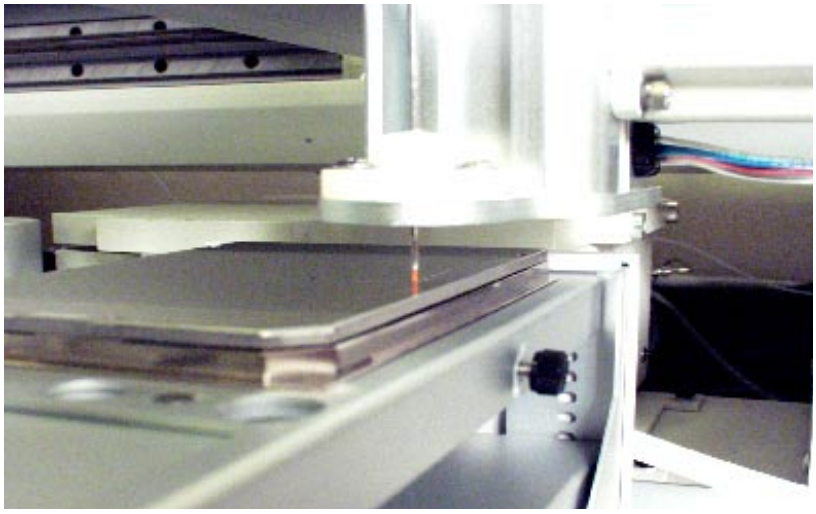


Photo 2: Side View of the Spring-Loaded Probe (on the Micro 215) Spotting the MALDI Plate

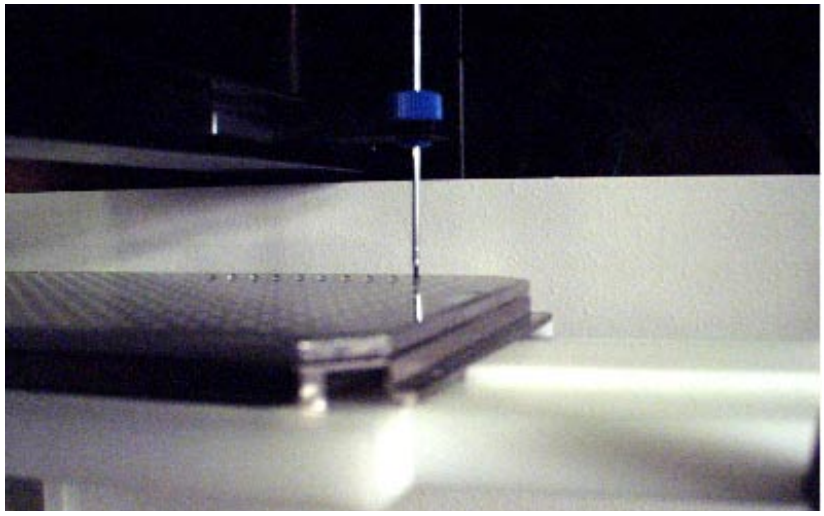
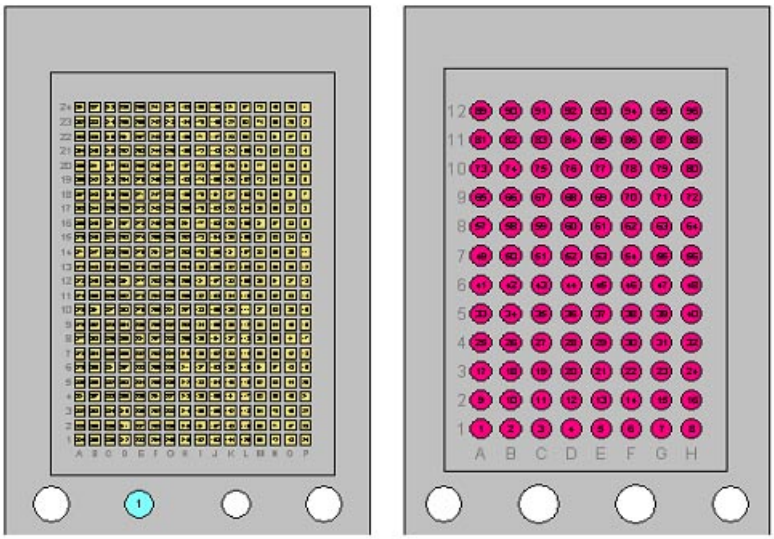
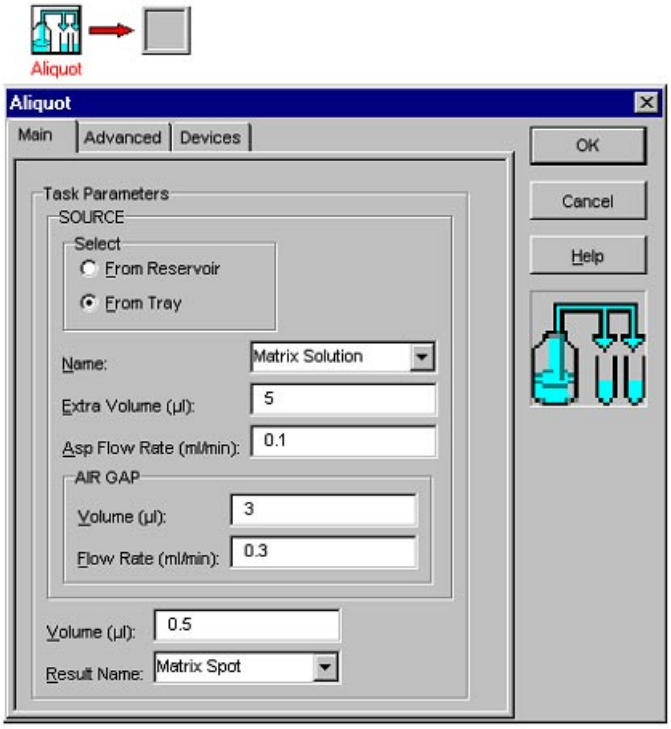


Photo 3: Fraction Collection onto the MALDI Plate with the Spring-Loaded Probe (on the 223 Liquid Handler)

Control and Data Handling

Both the Micro 215 Liquid Handler used to spot the MALDI plate and the 223 Liquid Handler used for spotting the fractions directly onto the plate employed 735 Sampler Software for control. The HPLC system was controlled via UniPoint™ System Software and ran simultaneously with the 735 Sampler Software as the fractions were collected.





Figures 3 & 4: 735 Tray File and Method for the MALDI Spotting of the Matrix

Spotting of the matrix on the Micro 215 was accomplished by a simple aliquot task. The 384-well MALDI plate was completed in 18 minutes.

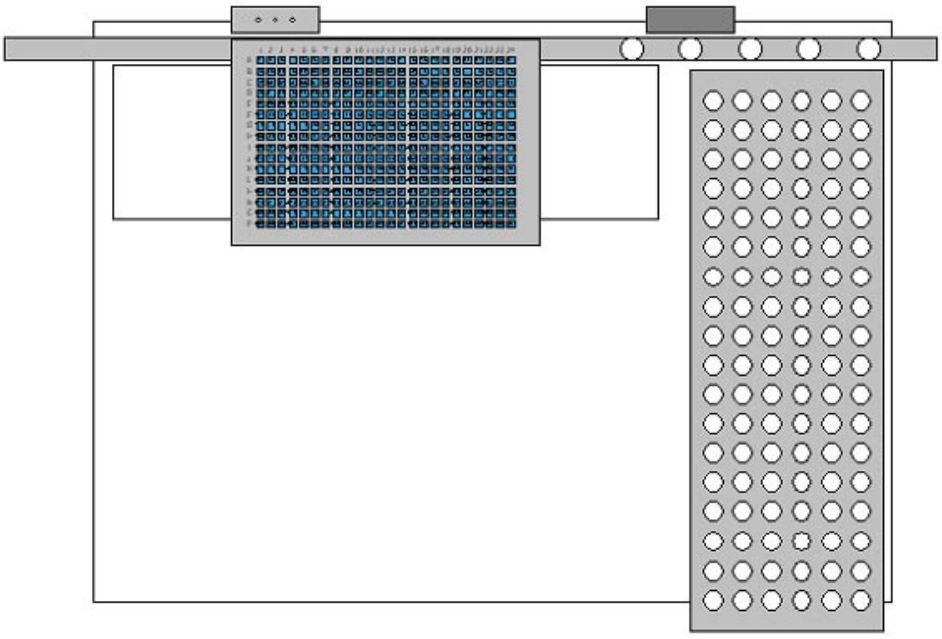
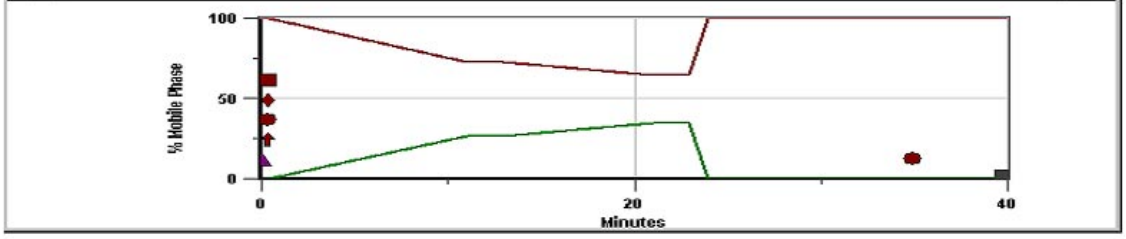


Figure 5: 735 Tray File for Fraction Collection onto the MALDI Plate

Only one MALDI plate was spotted for this application. A custom rack is available that holds four MALDI plates for fraction collection. Fractions were collected at 0.07 min. intervals, 10 µL delay.

Control Method [maldi_25.gct]

	Time	Device(s)	Command
1	0.00	Aqueous / Organic	.2(ml/min): 100% Aqueous, 0% Organic
2	0.05	215 Micro total loop injection w/ rinse above seal	<start> AIRGAP_VOLUME, SAMPLE, LOOP_VOLUME
3	0.35	System Controller	Synchronize
4	0.36	Data Channels	Start Chromatogram Channels
5	0.37	Start FC	Pulse
6	0.38	Detector 17	Autozero Channels
7	0.50	Aqueous / Organic	.2(ml/min): 100% Aqueous, 0% Organic
8	11.00	Aqueous / Organic	.2(ml/min): 73% Aqueous, 27% Organic
9	13.00	Aqueous / Organic	.2(ml/min): 73% Aqueous, 27% Organic
10	21.00	Aqueous / Organic	.2(ml/min): 65% Aqueous, 35% Organic
11	23.00	Aqueous / Organic	.2(ml/min): 65% Aqueous, 35% Organic
12	24.00	Aqueous / Organic	.2(ml/min): 100% Aqueous, 0% Organic
	35.00	Aqueous / Organic	.2(ml/min): 100% Aqueous, 0% Organic



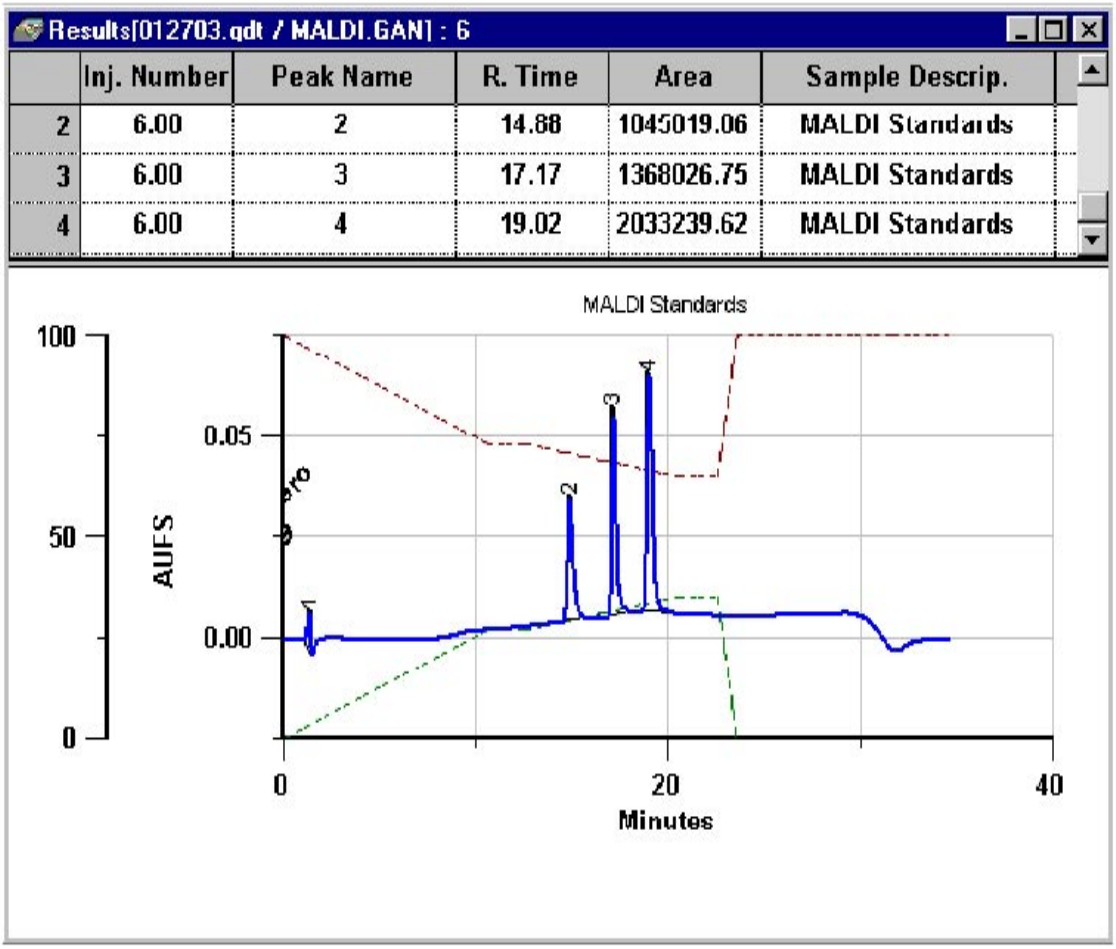
The system was run at 0.2 mL/min. with the splitter set to achieve a flow rate of 6 µL/min. through the capillary column.

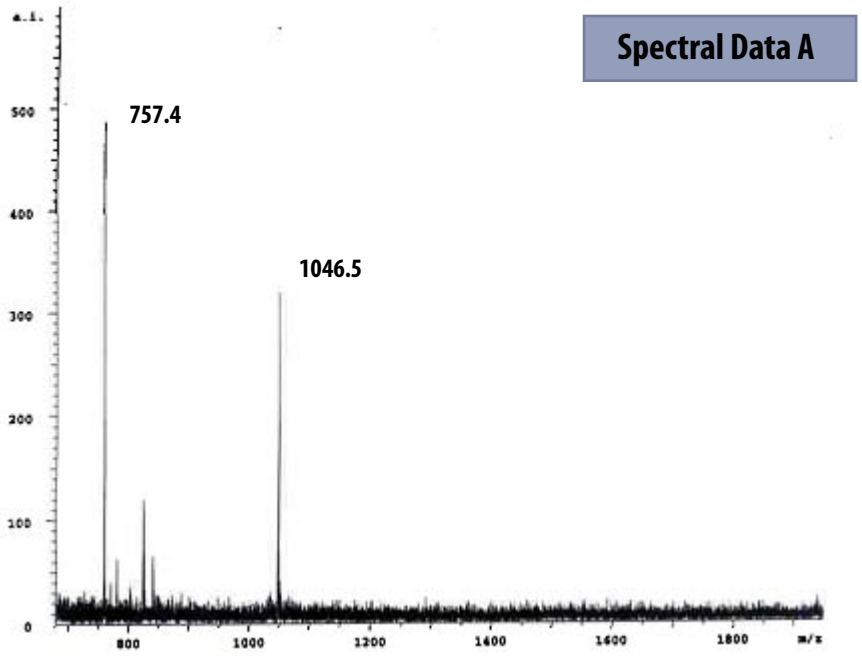
Table 1: Volumetric Accuracy and Precision for the Micro 215 Using the Spring-Loaded Probe

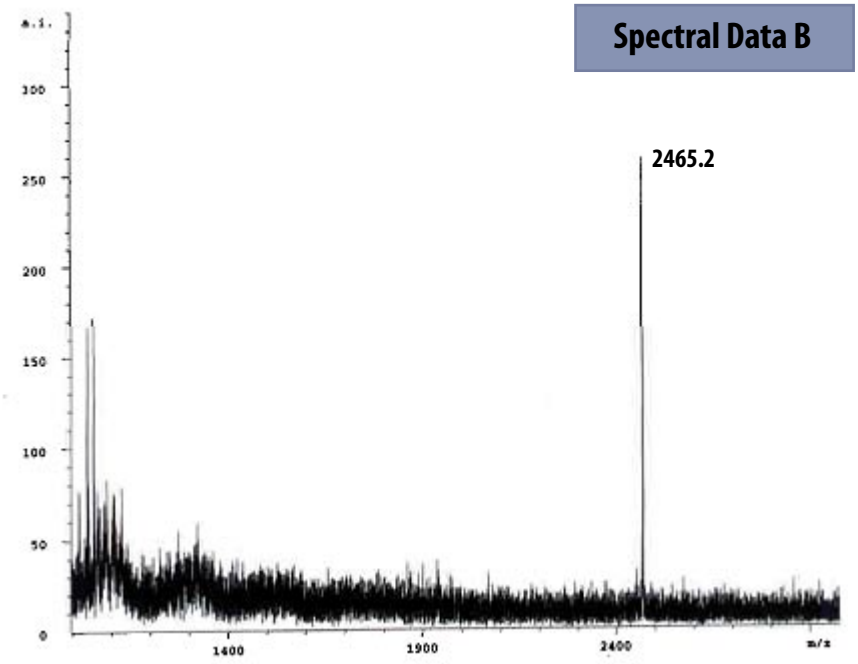
	Volumes (µL)			
	0.5	1.0	2.0	5.0
STD (%)	.2	.5	.7	.9
CV (%)	3.2	3.7	3.0	1.6

Graph 1: Chromatogram of the MALDI Standards

A 1-µL injection (internal loop) of the 15 pmol/µL peptide mix (bradykinin, angiotensin II, and ACTH, respectively).







Graphs 8 & 9: Results from the Bruker Biflex III Mass Spec for the Collected Fractions

The above spectral data represents the fractions collected (at 0.07 min. intervals) onto the MALDI plate by the 223: bradykinin 757.4 and angiotensin II 1046.5 (A); ACTH 2465.2 (B) $(M+H)^+$. The co-elution peptides of bradykinin and angiotensin II suggest band broadening between the detector and the fraction collector.

Summary of Results

Although the Micro 215 is a single-probe instrument, the data associated with volumetric accuracy and precision show its capabilities at spotting MALDI plates. The Micro 215 can also be used to collect fractions directly onto the MALDI plate. The smaller footprint of the 223 offers another solution for fraction collection directly onto the MALDI plate at a fraction of the cost of larger MALDI spotters. 735 Sampler Software offers a user-friendly control over the instruments, while still allowing customization of rack, tray, and tasks to accommodate the required needs of the researcher.

Conclusion

Automated MALDI plate preparations are becoming necessary with the increase in the popularity of MALDI-TOF MS. 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 384-well plates onto the MALDI plate. The focus of this application was to present an alternative to the larger, more expensive MALDI spotters. Both the Micro 215 and 223 Liquid Handlers can be used to spot the matrix and the fractions on the MALDI plate, offering two viable options for automated MALDI spotting. The key behind these automated instruments is the use of a spring-loaded probe. The spring-loaded probe is essential so that a touch off of the solution onto the plate is achieved without damaging the surface of the plate. In capillary HPLC, it is crucial to minimize dead/delay volumes in the system, especially when collecting fractions. Continuing studies are addressing this issue to optimize these factors in the system. It should be noted that the ratio of the sample-to-matrix on a MALDI plate is very important in MALDI-TOF MS and will directly affect results. Although this study did not incorporate this concept, it will be evaluated in future experiments.

Gilson, Inc. World Headquarters
3000 W. Beltline Hwy., P.O. Box 620027, Middleton, WI 53562-0027 USA
Telephone: (1) 800-445-7661 or (1) 608-836-1551• Fax: (1) 608-831-4451

Gilson S.A.S.
19, avenue des Entrepreneurs, BP 145, F-95400 VILLIERS LE BEL France
Telephone: (33-1) 34 29 50 00 • Fax: (33-1) 34 29 50 20

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sales@gilson.com, service@gilson.com, training@gilson.com