Supplementary Material

Advancing Carbohydrate Quantification in MALDI Mass Spectrometry by Rapidly Freeze-Drying Droplet (RFDD) Method

Yu-Cheng Wu, [1] Xin-Wen Zhang,[1] Yi-Ching Huang,[1] I-Chung Lu[1*]

[1]Department of Chemistry, National Chung Hsing University, Taichung City 40227, Taiwan

* Corresponding author. E-mail: iclu@nchu.edu.tw

Contents:

1. Chemicals and materials.

The MALDI matrices, 2,5-Dihydroxybenzoic acid (2,5-DHB, 98%); and the analyte, Sucrose (99.5%), D-(+)-glucose (99.5%), Dextran, Pullulan, Human Serum, and Sodium Chloride (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). D-Glucose-$^{13}C_6$ was purchased from Merck KGaA (Darmstadt, Germany). Bradykinin acetate salt (98%) was purchased from Alfa-Aesar (Heysham, UK). Artificial urine was purchased by PICKERING (Mountain View, CA, USA). Acetonitrile (ACN) was purchased from Avantor (Radnor, PA, USA). Water was purified using a purification system (PORETECH, Ultrapure water system, Taiwan). All organic solvents were HPLC grade. The chemicals were used without further purification and pretreatment before experiments.

2. General preparation of MALDI sample

The stock solution of 2,5-DHB was dissolved in ACN/H_2O (3:1 v/v), and the concentration was 2M. The stock solutions of analytes were all dissolved in deionized water, including glucose (0.1~10 mM), sucrose (1~ 10 mM), dextran (10 μM), pullulan (10 μM), Bradykinin (10 μM). Then, the analyte solution was mixed with 2M NaCl aqueous solution (v/v = 99/1) to increase and ensure uniform sodium ion content in the environment for comparative ion signal in various sample preparation methods. The analyte solution was mixed with matrix solution (v/v = 1/1) before applying the Dried-Droplet (DD), Two-layer (TL), and Rapidly Freeze-Drying Droplet (RFDD) process.

In the quantitative analysis for the calibration curves, the various amounts of glucose, sucrose, glucose, and D-(+)-glucose were spiked into the solvents and real samples before mixing with the matrix solution. The urine samples were diluted 250 times with deionized water before mixing them with a matrix solution and further analysis. The serum samples (5 uL) were mixed with 3-fold acetonitrile (15 uL) for protein precipitation. After centrifuging at 6000 rpm for 10 min, the supernatant was collected (5 uL) for further sample preparation.

Dried-Droplet

The mixture of 1 μL solution in a vial was deposited onto the stainless-steel MALDI plate (MTP 384 target) and dried at atmospheric pressure at room temperature before being transferred to a mass spectrometer for mass analysis.

Two Layer

The TL sample was prepared through two steps. The matrix solution (0.5 μL) was applied and air-dried on the MALDI sample plate, and then the analyte solution (0.5 μL) was deposited onto the matrix crystal.

3. Experimental parameter of MALDI-TOF

Mass spectra were collected by an UltrafleXtreme MALDI TOF/TOF mass spectrometer from Bruker Daltonics. The instrument was controlled by Bruker's flexControl data collection software and was equipped with an Nd: YAG laser ($\lambda = 355$ nm). MS measurements were carried out in reflectron mode using acceleration voltages of 20.00 and 17.60 kV (ion sources 1 and 2, respectively). The lens voltage was 7.00 kV. The reflector voltages 1 and 2 were 21.10 and 10.85 kV, respectively. To achieve a high signal-to-noise ratio (SNR), each spectrum integrated at least 200 individual laser shots.

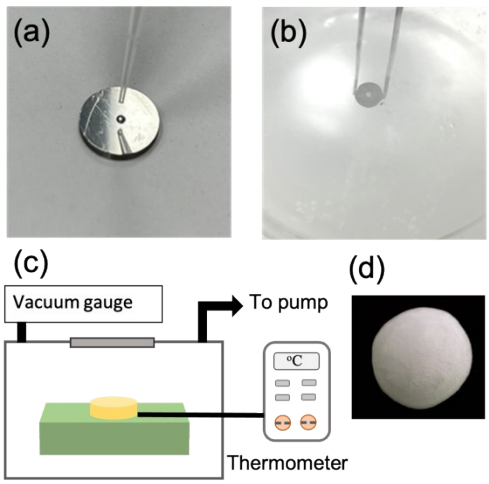

Fig. S1 RFDD sample preparation process of RFDD (a) the homemade stainless-steel sample plate with a 10 mm diameter, (b) immersion of the sample plate in liquid nitrogen, (c) the scheme of the vacuum chamber for sublimation with pressure and temperature monitoring, and (d) a photograph of the MALDI sample prepared by RFDD using 2,5-DHB as the matrix.

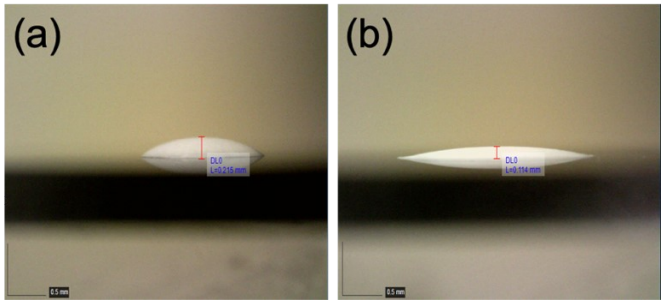

Fig. S2 Microscopic images of sucrose mixed with 2,5-DHB matrix using the RFDD method. The stainless-steel sample plate was treated (a) without and (b) with Ar plasma before dropping the sample solution.

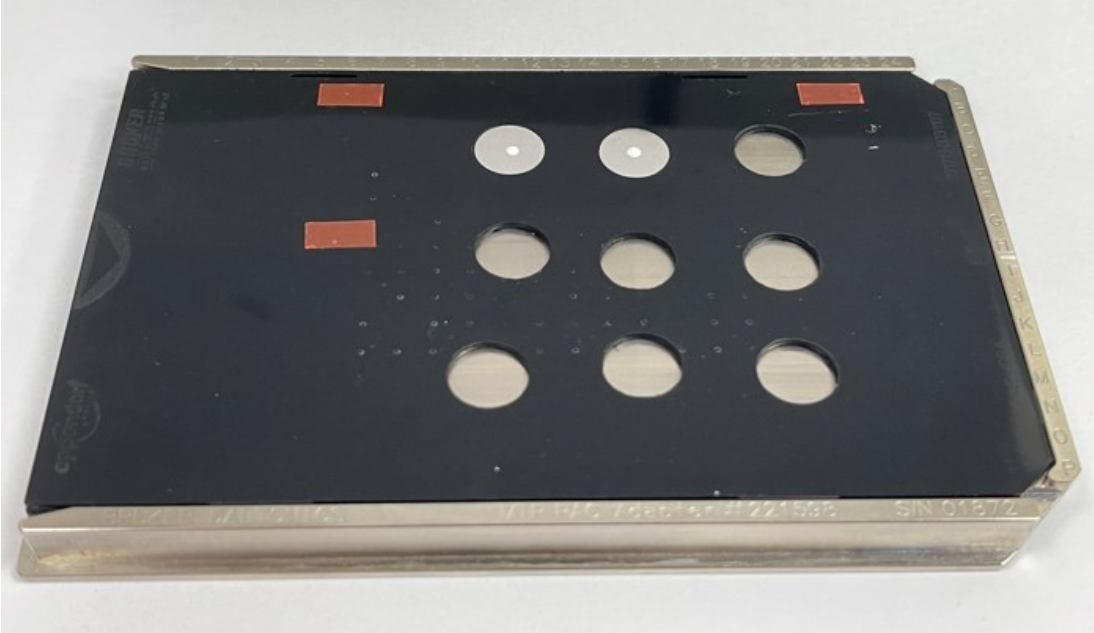

Fig. S3 The photo of the MALDI sample plate ((Prespotted AnchorChip PAC 384 HCCA) with self-drilled holes. The homemade stainless-steel sample plates (diameter 10 mm; thickness 1 mm) were installed on the first row. The white spots were the MADLI sample prepared by the RFDD method.

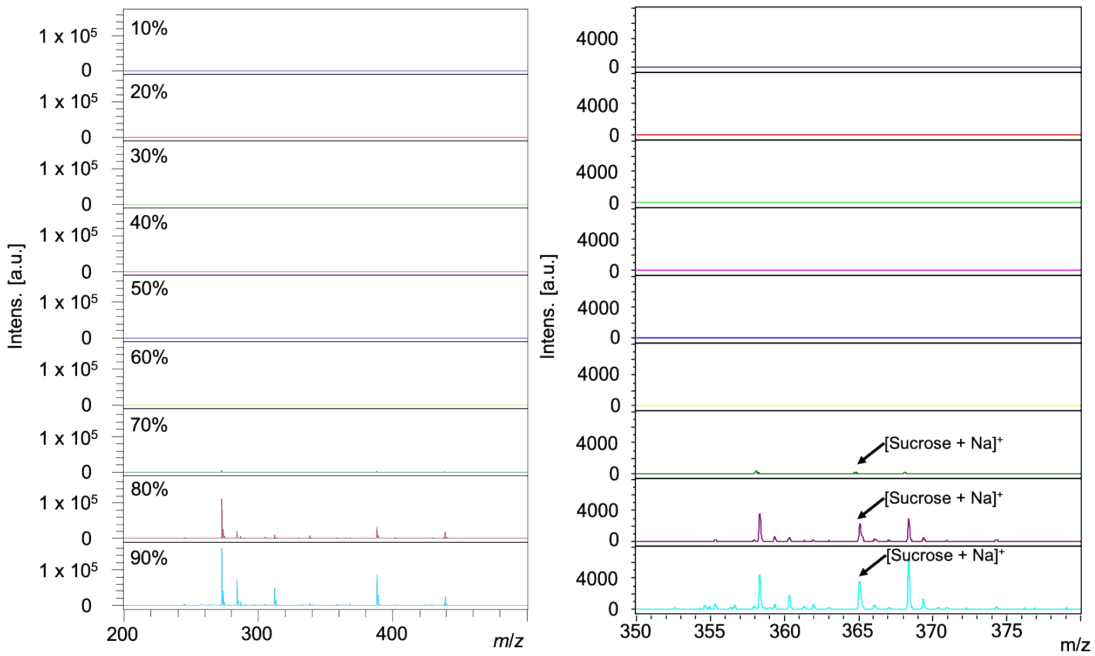

Fig. S4 MALDI-TOF MS spectrum of sucrose (50 nmol/μL) in DI water using 2,5-DHB as the matrix, prepared by the DD method at various relative laser energy levels (10-90%) is presented in full (left) and an enlarged scale (right). Each spectrum represents 100 laser shots at 20 randomly selected sampling positions.

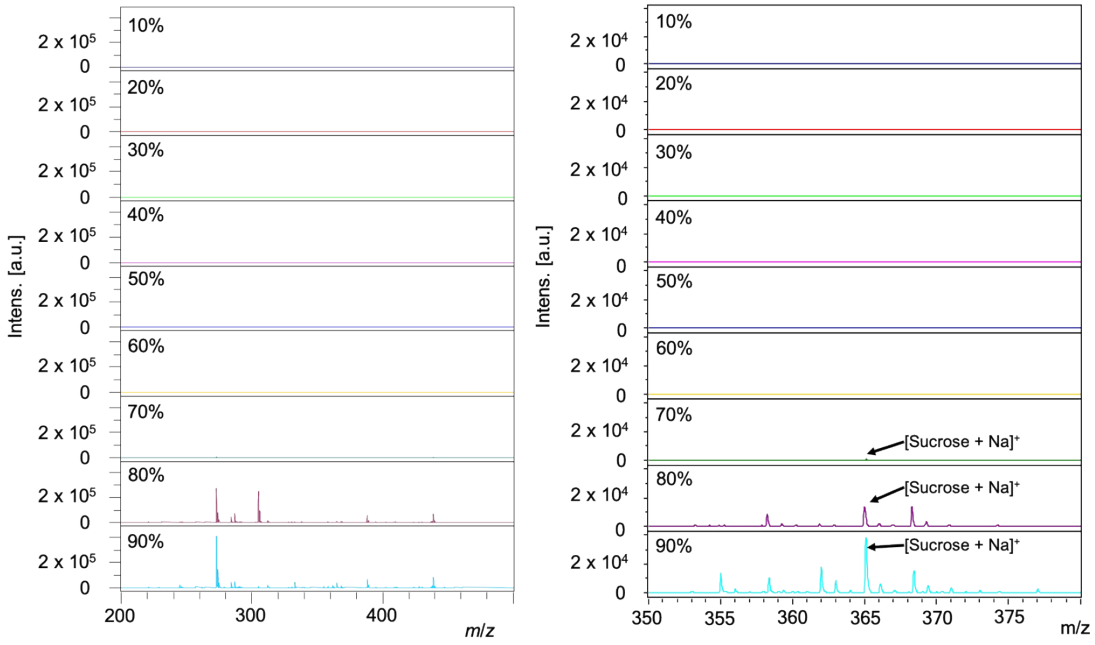

Fig. S5 MALDI-TOF MS spectrum of sucrose (50 nmol/μL) in DI water using 2,5-DHB as the matrix prepared by the DD method at various relative laser energy levels (10-90%) is presented in full (left) and an enlarged scale (right). Each spectrum represents 100 laser shots at 20 specific sampling positions on the crystal.

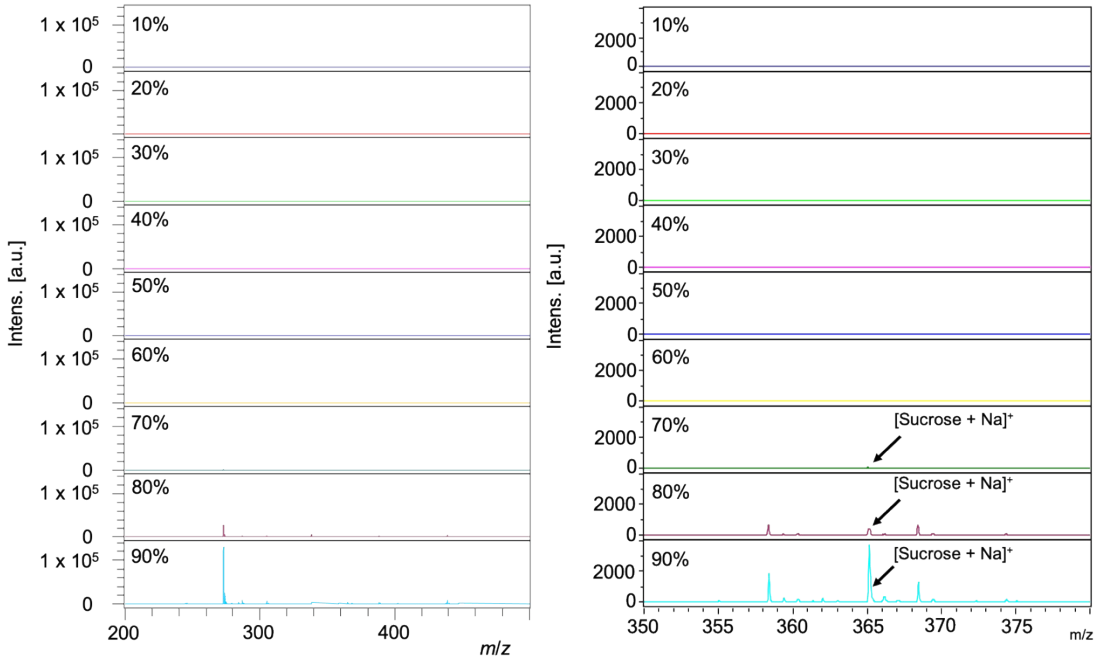

Fig. S6 MALDI-TOF MS spectrum of sucrose (50 nmol/μL) in DI water using 2,5-DHB as the matrix prepared by the TL method at various relative laser energy levels (10-90%) is presented in full (left) and an enlarged scale (right). Each spectrum represents 100 laser shots at 20 randomly selected sampling positions.

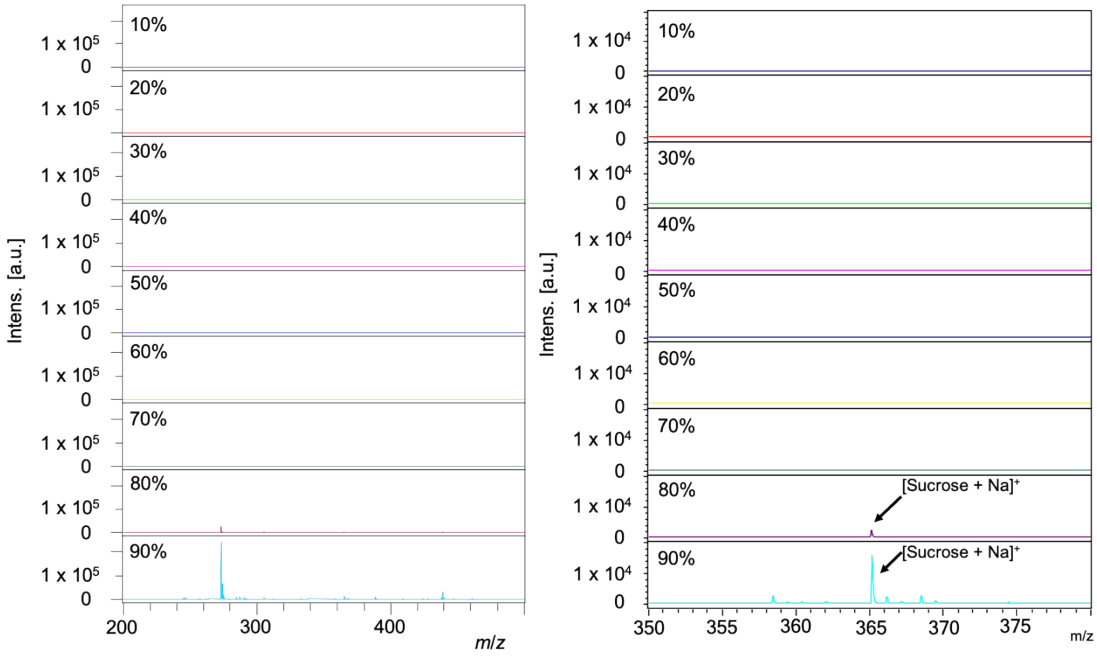

Fig. S7 MALDI-TOF MS spectrum of sucrose (50 nmol/μL) in DI water using 2,5-DHB as the matrix prepared by the TL method at various relative laser energy levels (10-90%) is presented in full (left) and an enlarged scale (right). Each spectrum represents 100 laser shots at 20 specific sampling positions on the crystal.

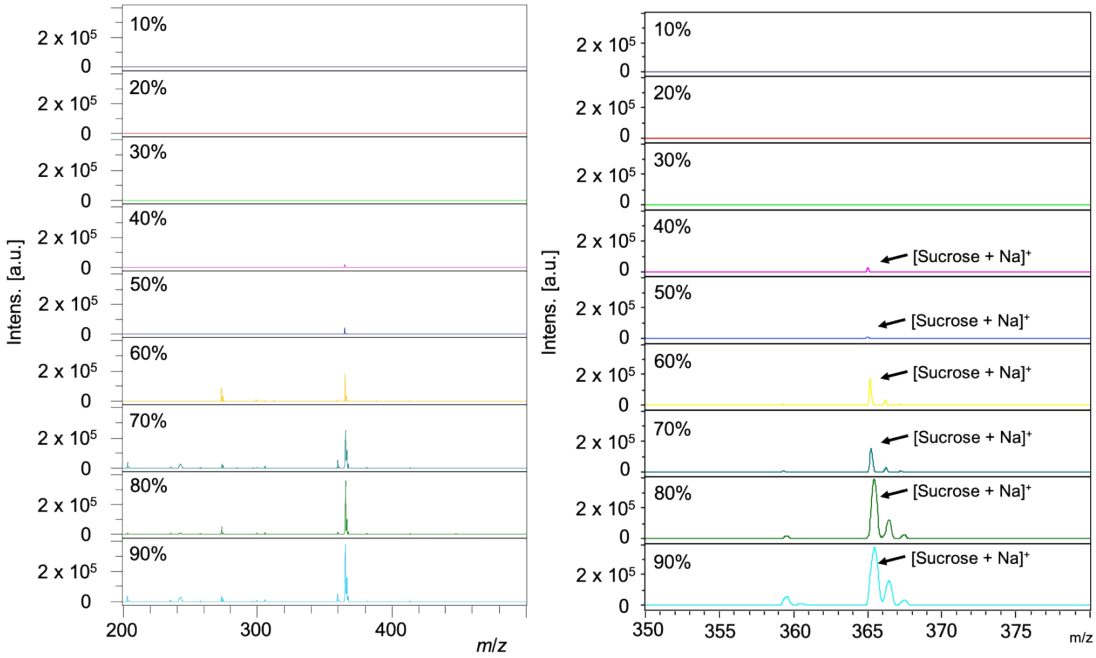

Fig. S8 MALDI-TOF MS spectrum of sucrose (50 nmol/μL) in DI water using 2,5-DHB as the matrix prepared by the RFDD method at various relative laser energy levels (10-90%) is presented in full (left) and an enlarged scale (right). Each spectrum represents 100 laser shots at 20 randomly selected sampling positions.

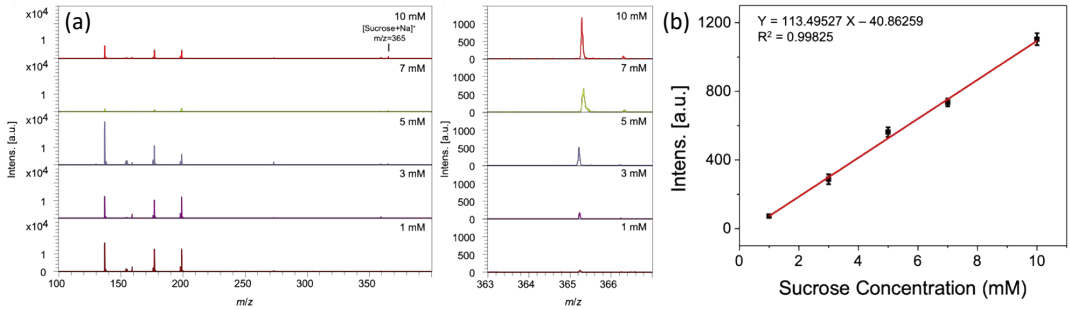

Fig. S9 (a) MALDI-TOF MS spectrum of doped sucrose (1 mM - 10 mM) in DI water using the RFDD preparation method with the addition of 0.02 M NaCl and 2,5-DHB as the matrix. The mass spectrum on the right shows an enlarged range of the [sucrose + $Na]^+$ (m/z 365.30). (b) The calibration curve used for the quantitative determination was obtained by plotting the ion intensity and glucose concentration. Each data point reflects the average of 200 laser shots in 10 sampling points (N=10).

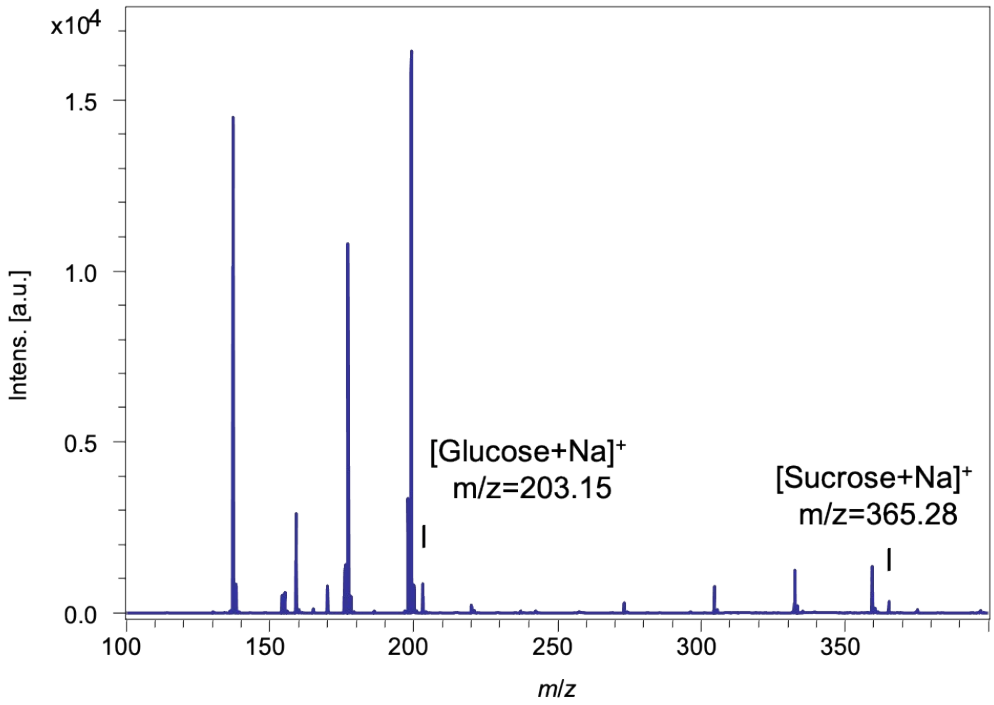

Fig. S10 MALDI-TOF MS spectrum of Red Bull using the RFDD preparation method, supplemented with 0.02 M NaCl and 2,5-DHB as the matrix. The Red Bull sample underwent a 100-fold dilution with DI water before applying the RFDD method without additional pretreatment.

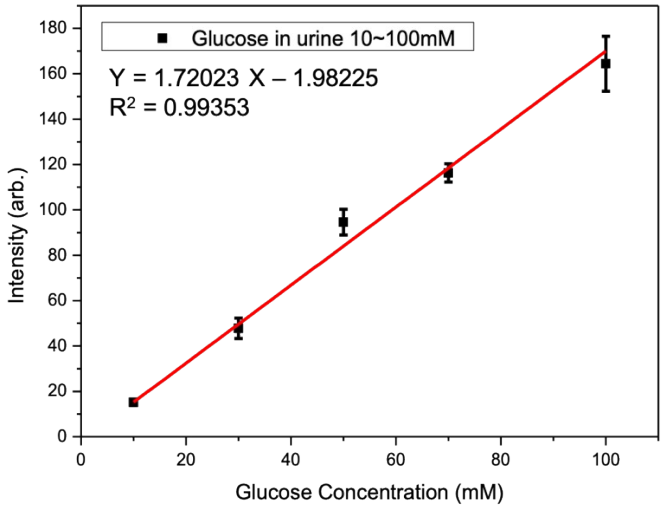

Fig. S11 The calibration curve used for the quantitative determination was obtained by plotting the ion intensity and the concentration of doped glucose (10-100 mM) in human urine. The urine sample underwent a 500-fold dilution with DI water before applying the RFDD method without additional pretreatment. Each data point reflects the average of 200 laser shots in 10 sampling points (N=10).

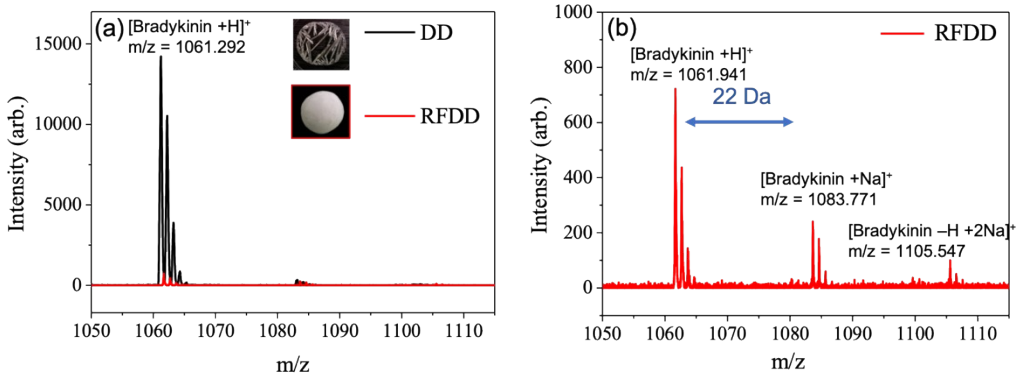

Fig. S12 MALDI-TOF MS spectrum of bradykinin (10 pmol/μL) using the DD and RFDD preparation methods, respectively. (b) An enlarged view of the RFDD-prepared bradykinin spectrum highlights a notable increase in the proportion of sodium adducts, $[A+Na]^+$(m/z=1083.77), and $[A-H+2Na]^+$ (m/z=1105.55), in comparison to the DD-prepared sample.

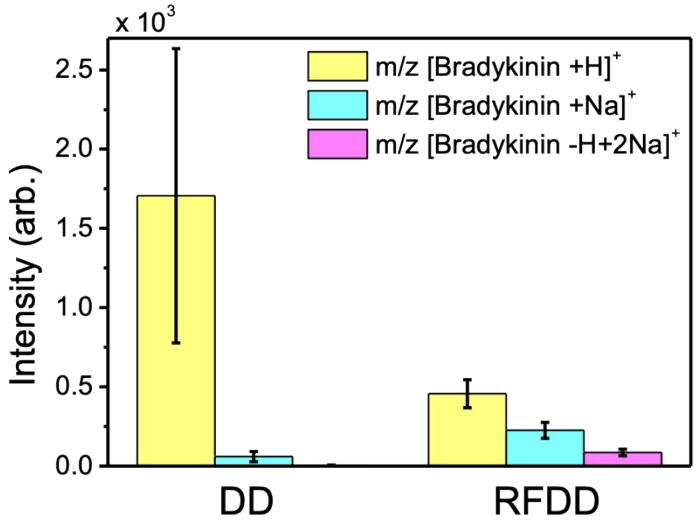

Fig. S13 Signal intensities of bradykinin (10 pmol/μL) obtained from MALDI-MS using the DD and RFDD preparation methods, respectively. The signal intensity reflects the average of 200 laser shots in 10 different sampling points (N=10). Yellow bar: $[A+H]^+$(m/z=1061.94); cyan bar: $[A+Na]^+$(m/z=1083.77); and pink bar: $[A-H+2Na]^+$(m/z=1105.55).