



Metabolic Profiling of Biofluids by Laser Ablation Electrospray Ionization Mass Spectrometry (LAESI-MS)

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Introduction

Laser Ablation Electrospray Ionization mass spectrometry (LAESI-MS) can be used for the direct analysis of aqueous samples. Laser ablation of water-rich targets in the mid-infrared region (2940 nm) occurs due to the strong absorption band of the water at this wavelength as a result of the OH vibrations. Minimal pre-treatment of the sample is required, and the analysis can be performed at atmospheric pressure.

Direct analysis of biological tissues and fluids by LAESI-MS permits identification and characterization of a variety of biomolecules, including metabolites, lipids, phospholipids, peptides, and proteins. Profiling of these analytes in biofluids represents a method for assessment of a patient's health status and can be indicative of pathology as a tool for clinical diagnostics.

Presented here is an assessment of human biofluids by LAESI-MS for identification and characterization of the biomolecules present in these aqueous samples, which include serum, urine, and breast milk. Each biofluid was analyzed directly and after solid phase extraction for desalting to generate metabolic profiles. Method development was performed for each sample to improve sensitivity and signal-to-noise by optimizing the LAESI source parameters, including laser power and frequency. After the initial molecular fingerprint, the analytes were characterized by tandem mass spectrometry for identification, classification, and tabulation.

Methods

Breast milk, serum, and urine were analyzed by LAESI-MS. Sample preparation for the breast milk involved simple dilution (1:10) in water. For milk fat analysis, whole milk was allowed to separate, and the milk fat layer was dissolved in 50% methanol, 0.1% acetic acid (1:4). Human serum samples were depleted of albumin and IgG with the ProteaPrep Albumin and IgG Depletion Sample Prep Kit. After lyophilization, the samples (depleted and albumin fraction) were redissolved in 50% methanol, 0.1% acetic acid to 2.5 times the original volume of whole serum. Urine samples were analyzed directly by LAESI-MS without any sample preparation.

LAESI was performed with an integrated platform designed by Protea Biosciences, Inc. For the LAESI-MS analysis, 20 µL of sample, as described above, was pipetted upon a glass depression slide. The glass slide was placed upon an aluminum sample tray that was maintained at 10°C by a Peltier cooling stage in the LAESI unit. The samples were ablated with 750-820 µJ of energy from the mid-infrared laser (2940 nm) at a repetition rate of 10 Hz with the period of sample collection lasting approximately 1 min. ESI was performed using 50% methanol, 0.1% acetic acid or 0.1% formic acid at 0.5 µL/min flow rate and a voltage of 3.5 kV. For negative ion mode, a buffer (pH 10.5) containing 50% methanol, 0.1% ammonium hydroxide was used. Mass detection was performed by a Thermo LTQ-XL mass spectrometer. For tandem MS analysis, data was collected for 4 min during which the 10 most abundant ions were selected for CID. Background ESI signal was subtracted prior to compound identification, which was aided by database searching (Human Metabolome Database), Protein Pilot search engine, and literature searches.

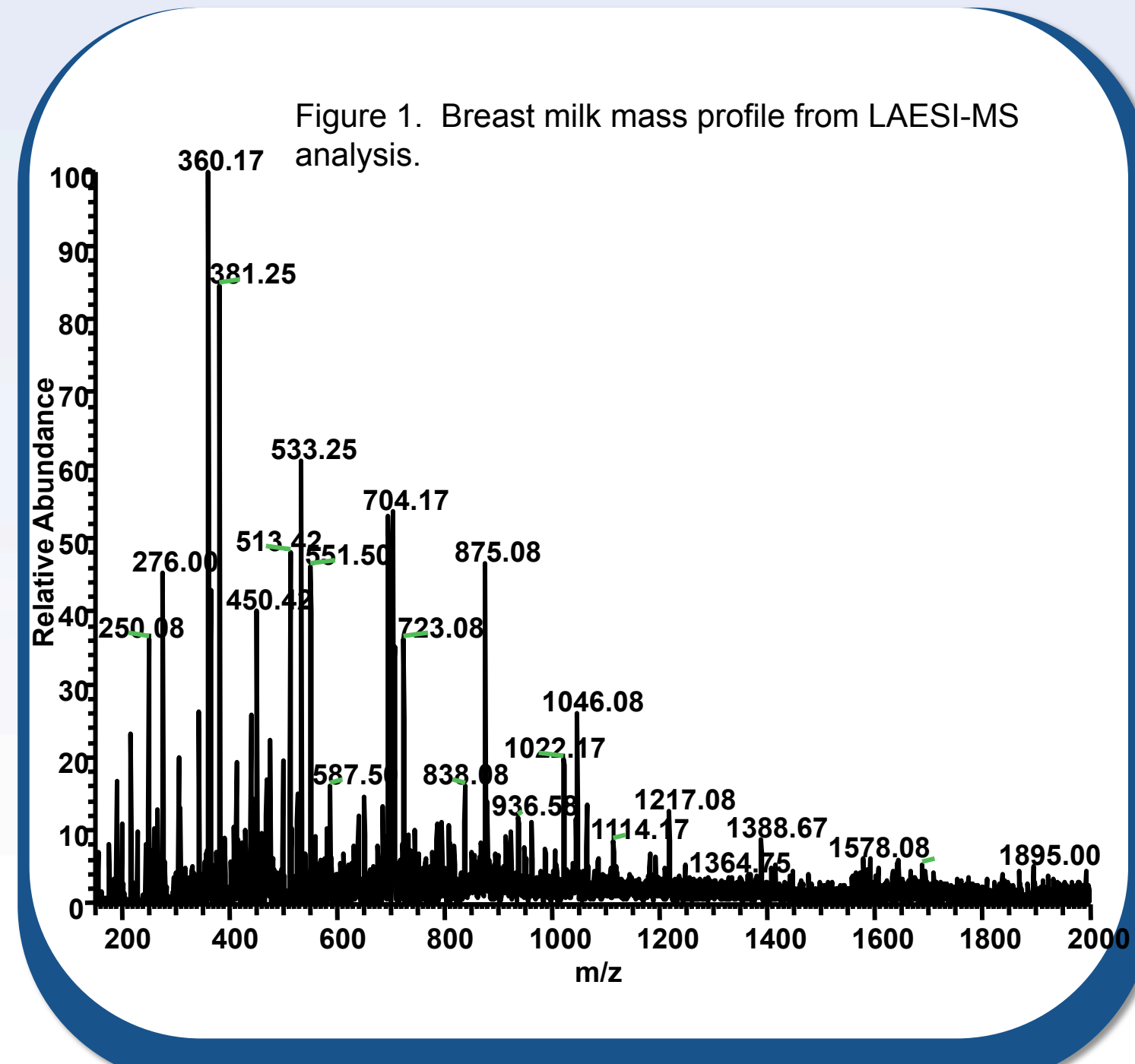


Figure 1. Breast milk mass profile from LAESI-MS analysis.

Table 1. Compounds identified through LAESI-MS analysis of human breast milk.

Compound	m/z
Carbohydrates	
Lactose + H ⁺	343.21
Lactose + H ₂ O	360.14
Lactose + K ⁺	381.23
2 Lactose + Na ⁺	707.16
2 Lactose + K ⁺	723.04
3 Lactose + H ₂ O	1043.96
Lactose + formate	387.30
2 Lactose + formate	729.05
3 Lactose + formate	1071.97
Lipids	
18-carbon fatty acid	279.37
Peptides from β-casein	
LAQPAVLPVPQPEIMEVPKA	1113.94
QPAVLPVPQPEIMEVPKA	1021.76
VKHEDQQQGEDEHQDK	651.03
YPVTQPLAPVH (deamidated Q)	612.27
ELLNPTHQ	533.47
QIYPTQPLAPVHNP	838.02
QPAVLPVPQPEIMEVPKAK (deamidated at Q1 and Q10)	725.24

Results

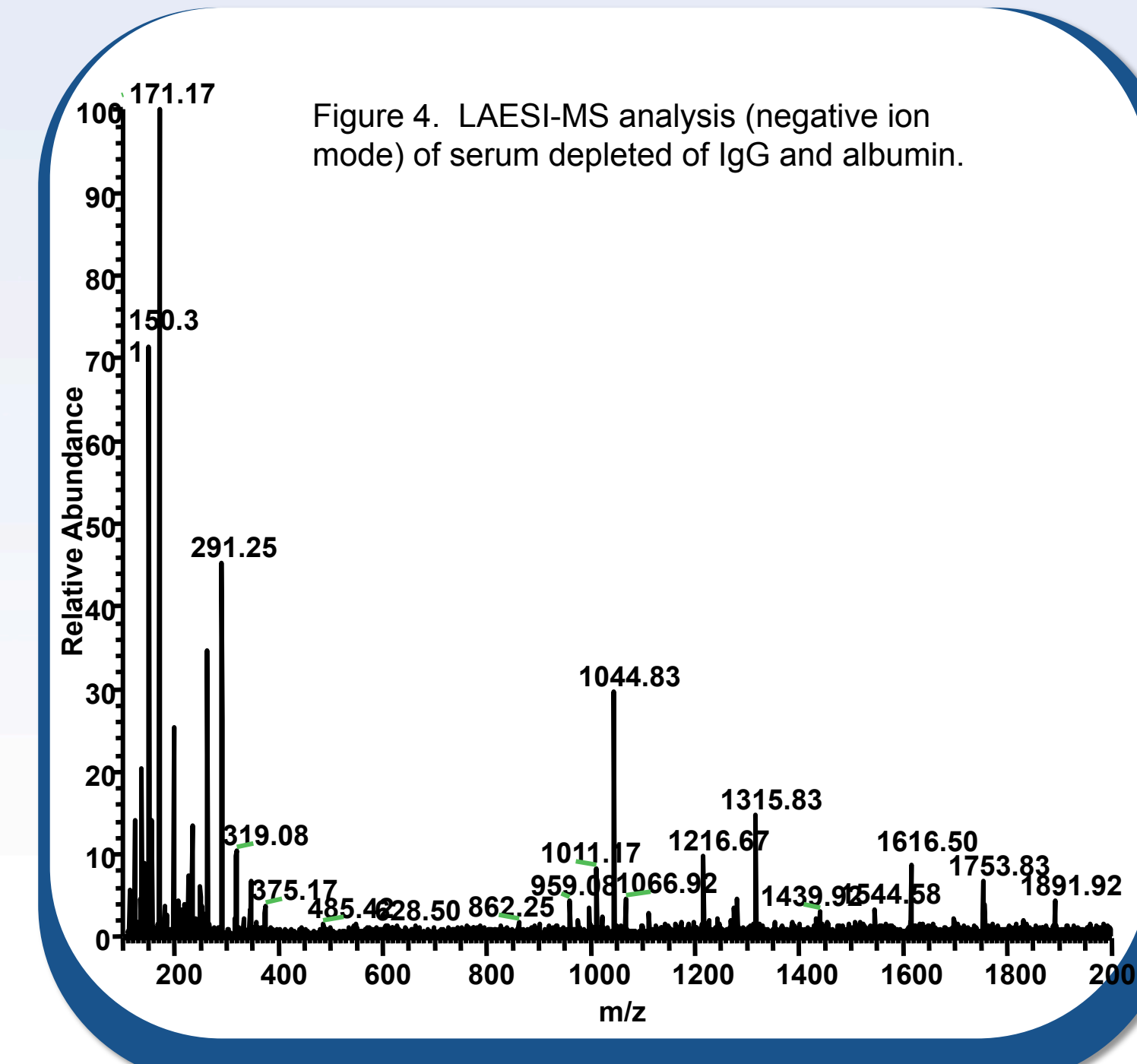


Figure 4. LAESI-MS analysis (negative ion mode) of serum depleted of IgG and albumin.

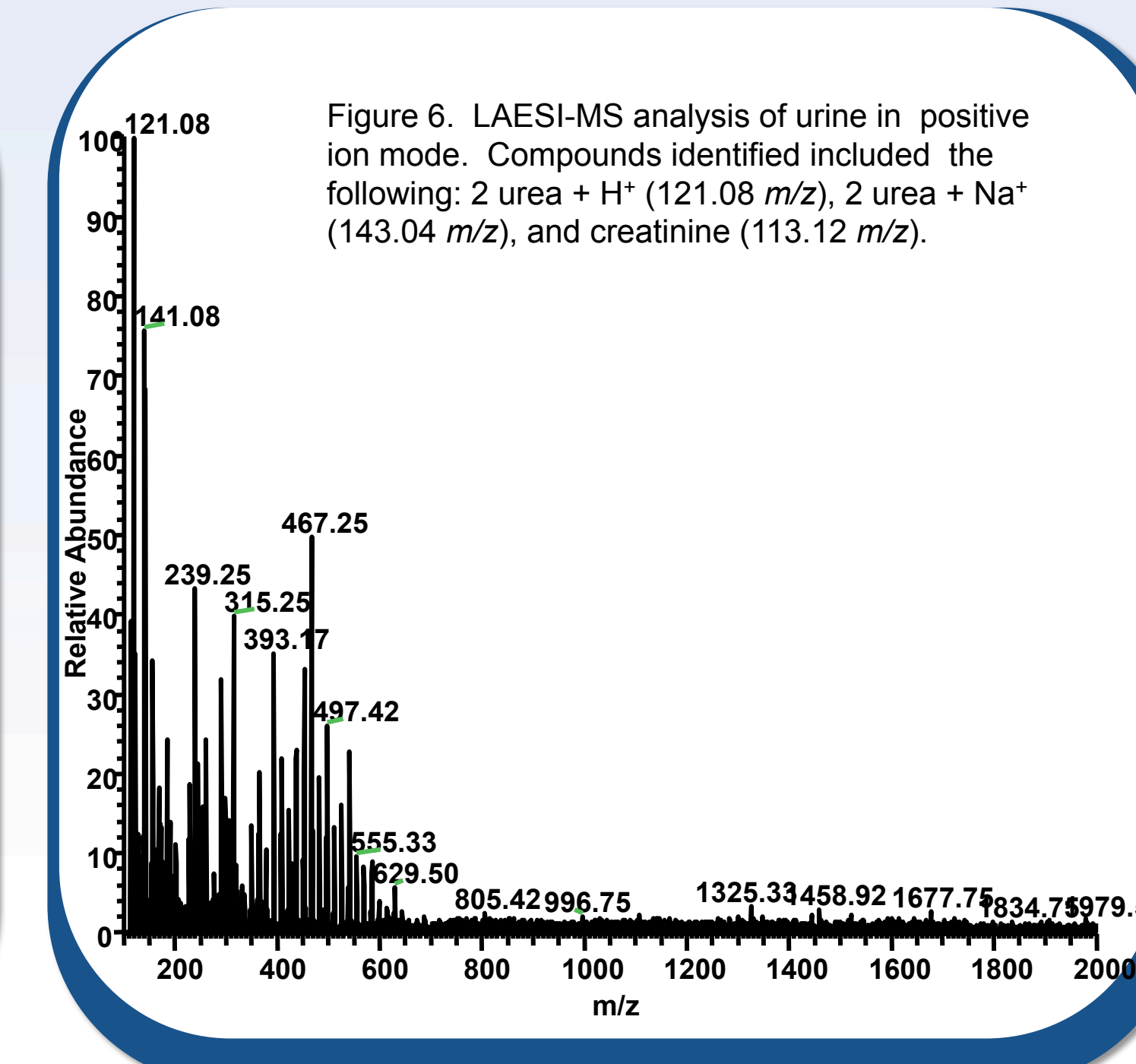


Figure 6. LAESI-MS analysis of urine in positive ion mode. Compounds identified included the following: 2 urea + H⁺ (121.08 m/z), 2 urea + Na⁺ (143.04 m/z), and creatinine (113.12 m/z).

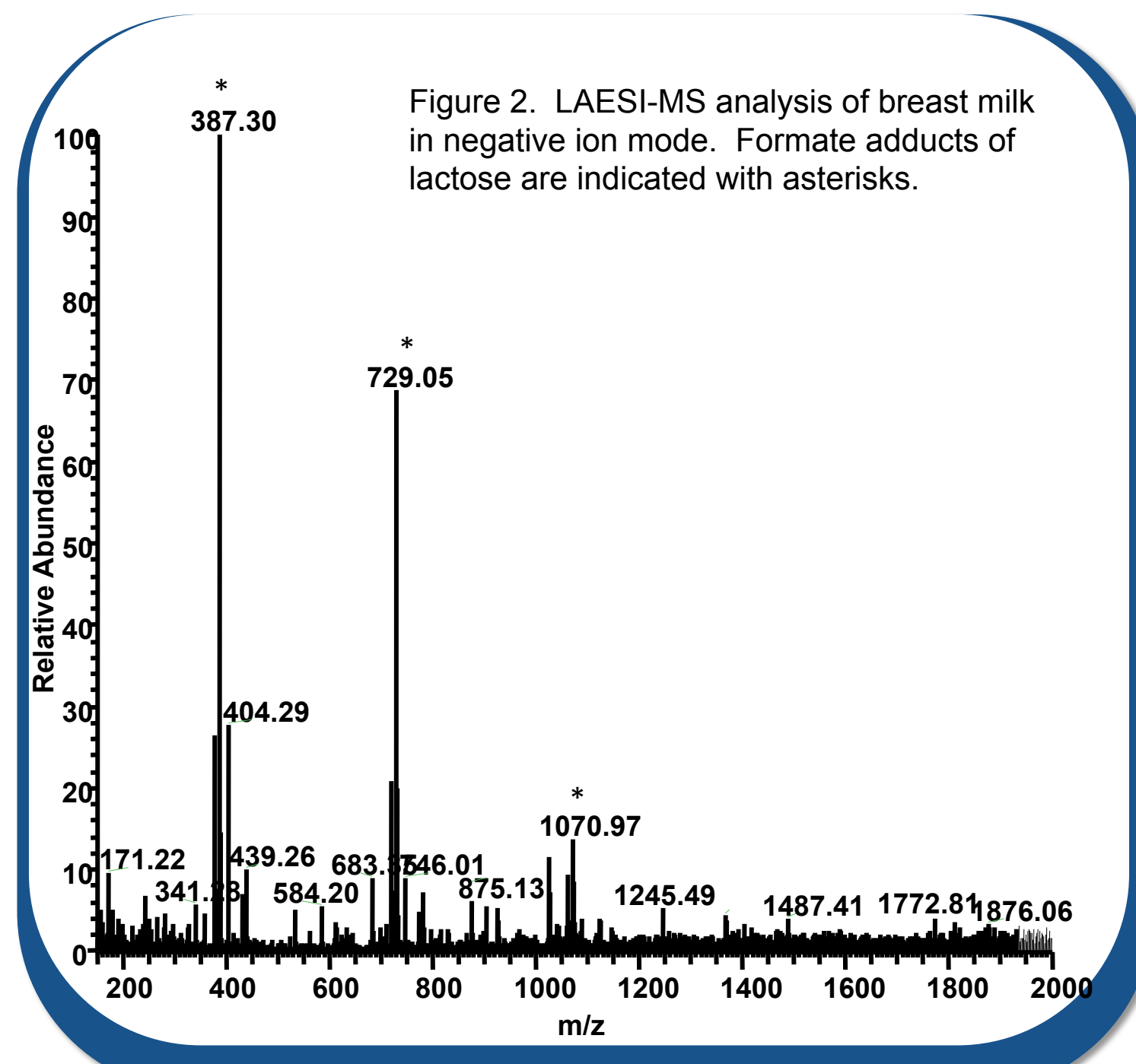


Figure 2. LAESI-MS analysis of breast milk in negative ion mode. Formate adducts of lactose are indicated with asterisks.

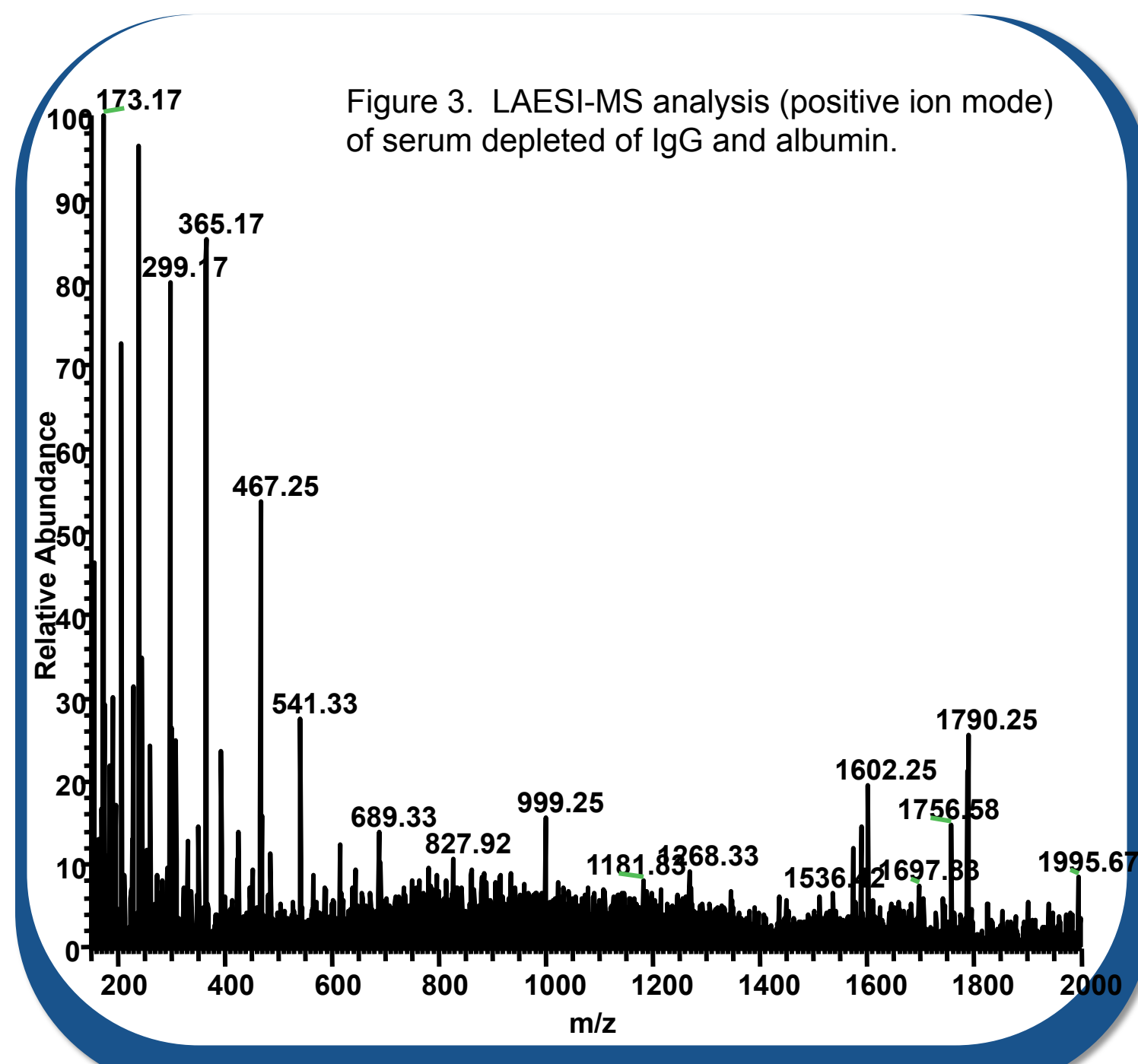


Figure 3. LAESI-MS analysis (positive ion mode) of serum depleted of IgG and albumin.

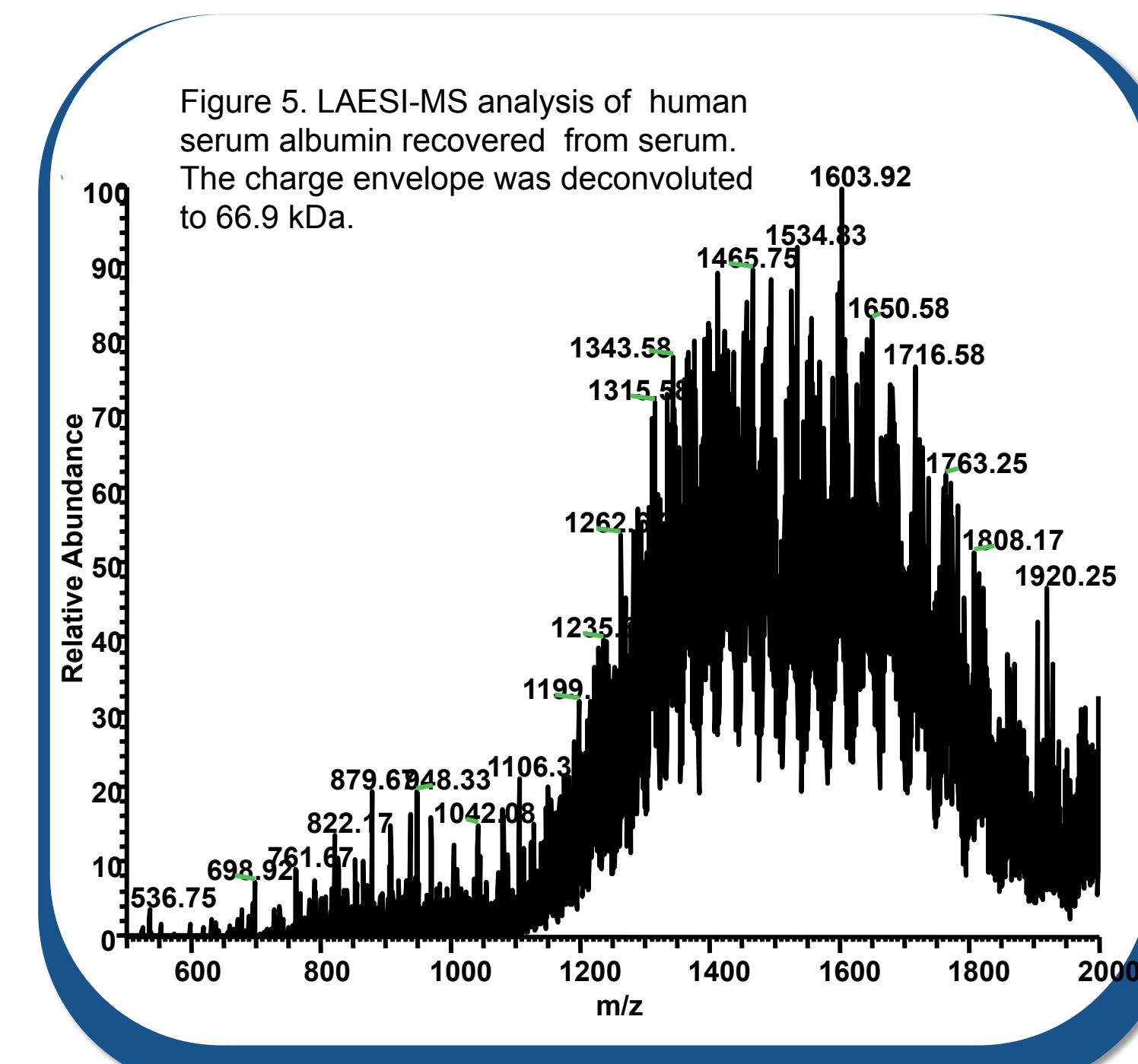


Figure 5. LAESI-MS analysis of human serum albumin recovered from serum. The charge envelope was deconvoluted to 66.9 kDa.

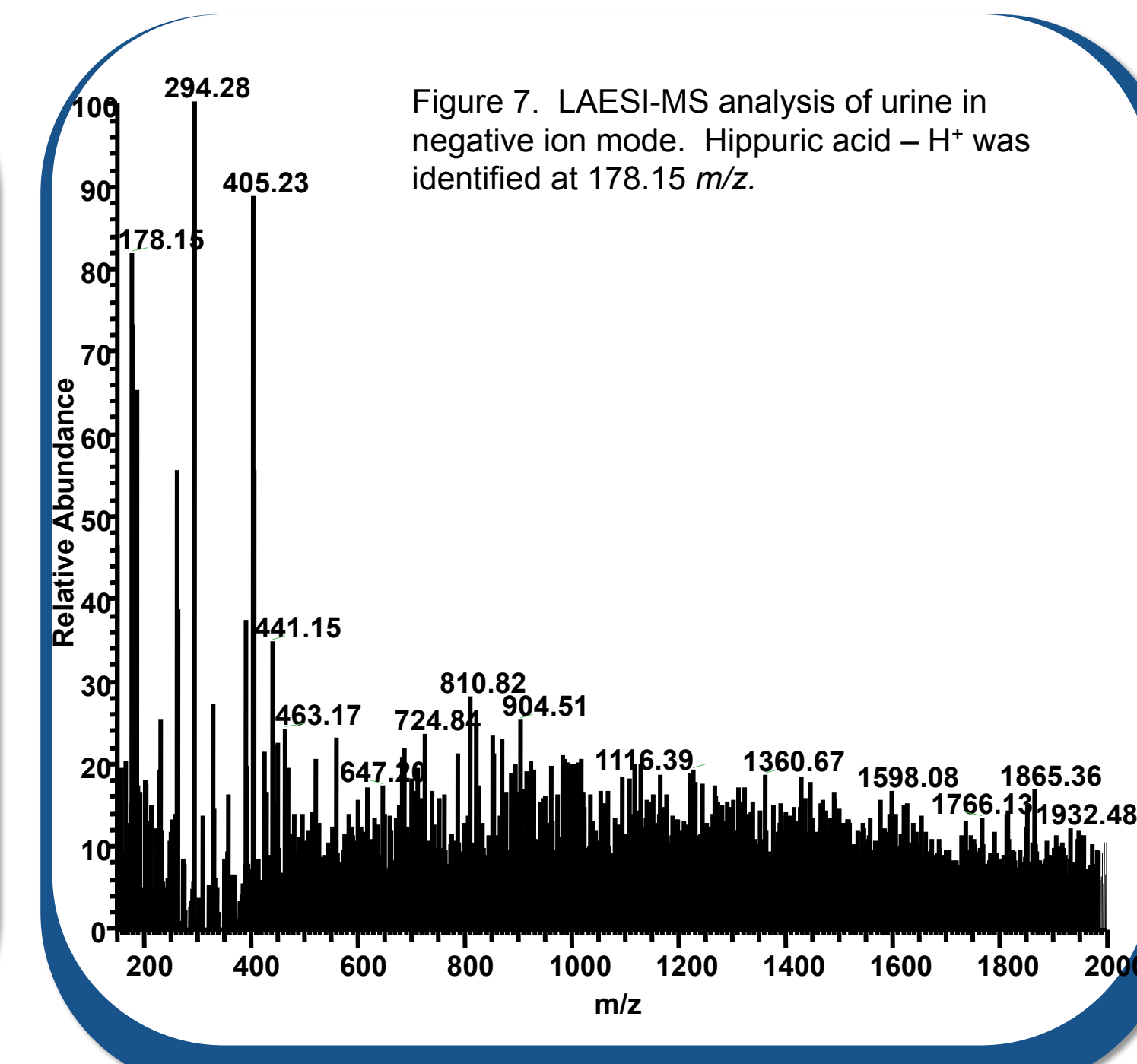


Figure 7. LAESI-MS analysis of urine in negative ion mode. Hippuric acid - H⁺ was identified at 178.15 m/z.

Discussion

LAESI-MS was performed upon human breast milk, serum, and urine. Identified in the breast milk were several salt adducts of lactose, an 18-carbon fatty acid (stearic, oleic, linoleic, or linolenic), and 7 peptides from β-casein (26% protein coverage at 95% confidence). Identified in the urine were urea adducts, creatinine, and hippuric acid. Human serum albumin was recovered from serum, analyzed by LAESI-MS, and deconvoluted to 66.9 kDa. Validation of these results and further biofluid analyses are ongoing. Future analyses will utilize an MS instrument with higher resolution and greater mass accuracy to aid in yielding more and higher confidence compound identifications.

Conclusions

- ❖ The LAESI-MS platform produced complex mass fingerprints of biofluids, including human breast milk, serum, and urine.
- ❖ Minimal sample preparation was required for analysis of human biofluids.
- ❖ LAESI-MS enabled identification of carbohydrates, fatty acids, and peptides from human breast milk. Several metabolites were identified in the urine samples.
- ❖ The LAESI platform is amenable to rapid human biofluid analysis without requiring extensive sample preparation.



Figure 8. The DP-1000 LAESI source.

Acknowledgments

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