

Control and optimization of humidity levels for optimal in-situ tryptic digestion and MALDI MSI of peptides

Paulina Kret¹, Rachel S. Pryce², Nidia Lauzon³, Anna Bodzon-Kulakowska¹, Pierre Chaurand²

INTRODUCTION

MALDI mass spectrometry imaging (MSI) of peptides is a powerful technique for determining the distribution and relative abundance of related proteins in tissue sections. For fresh-frozen tissues, the sample preparation procedure follows a well-defined protocol: tissue sectioning, section washing, homogeneous trypsin deposition, incubation (trypsin digestion), homogeneous matrix deposition and MALDI MSI analysis of the resulting peptides. This complex protocol is tedious to optimize due to the number of steps, leading to difficulty in achieving consistent and reproducible digestion results. In this research, section washing and digestion conditions were carefully evaluated and optimized. In particular, the humidity within the digestion chamber used was carefully controlled using saturated salt solutions and monitored using a humidity sensor. By optimizing some aspects of the sample preparation protocol, consistent, high-quality digestion results were achieved.

METHODS

The tissues were sectioned at 12 μm and thaw-mounted on ITO-coated glass slides, then washed to remove lipids and salts. The slides were digested with trypsin and incubated for 18 hours at 37°C. After incubation, CHCA matrix was spray deposited to generate a 0.1667 mg/mm^2 layer. Imaging was performed using a Bruker ultrafleXtreme MALDI-TOF/TOF MS at a spatial resolution of 50 μm .

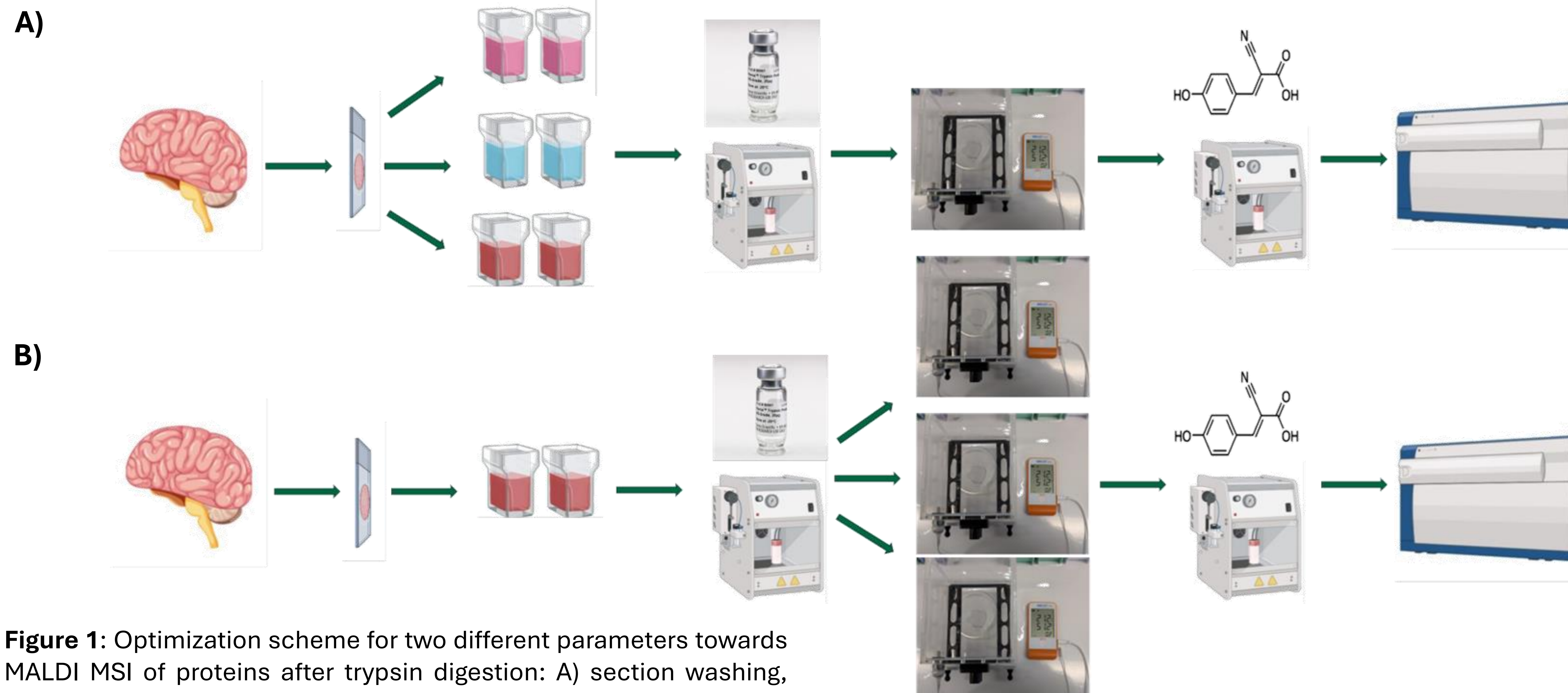


Figure 1: Optimization scheme for two different parameters towards MALDI MSI of proteins after trypsin digestion: A) section washing, and B) incubation conditions.

RESULTS

Wash	Protocol
EtOH (1)	30s 70% EtOH, 30s 100% EtOH
MeOH (1)	30s 70% MeOH, 30s 100% MeOH
Carnoy's (2)	30s 70% EtOH, 30s 100% EtOH, 2min Carnoy's solution, 30s 100% EtOH, 30s H ₂ O, 30s 100% EtOH

(1) Seeley EH et al., *J Am Soc Mass Spectrom*, **19**, 1069-1077 (2008).
 (2) Gessel M. et al., *J Mass Spectrom*, **50**, 1288-1293 (2015).

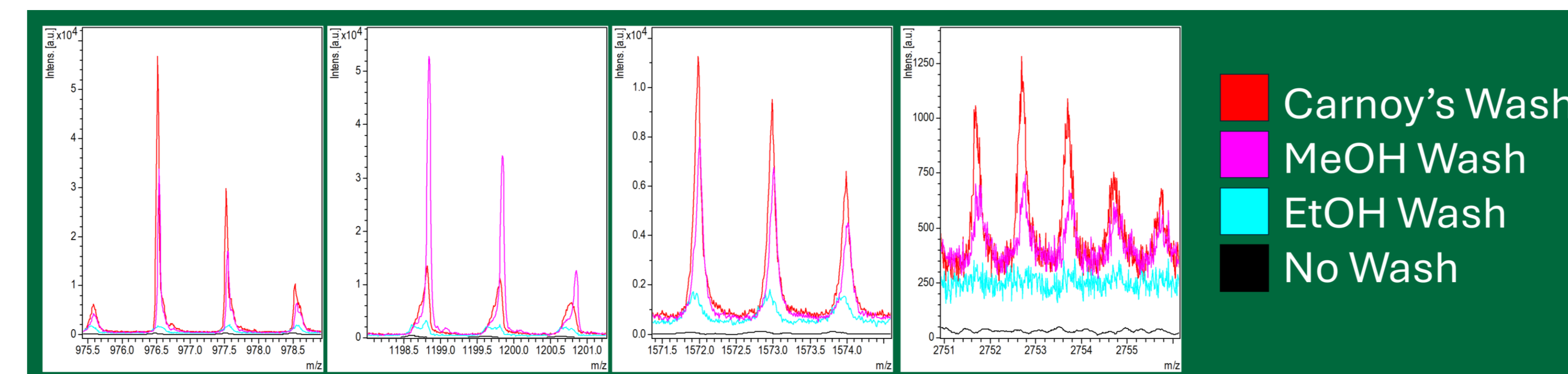


Figure 2: MALDI MS signal intensities of selected peptides after on-tissue trypsin digestion of brain homogenate sections. Each spectrum shows the sum of 5000 laser shots acquired randomly across the section. Incubation was performed with water as solvent for 18 hours at 37°C.

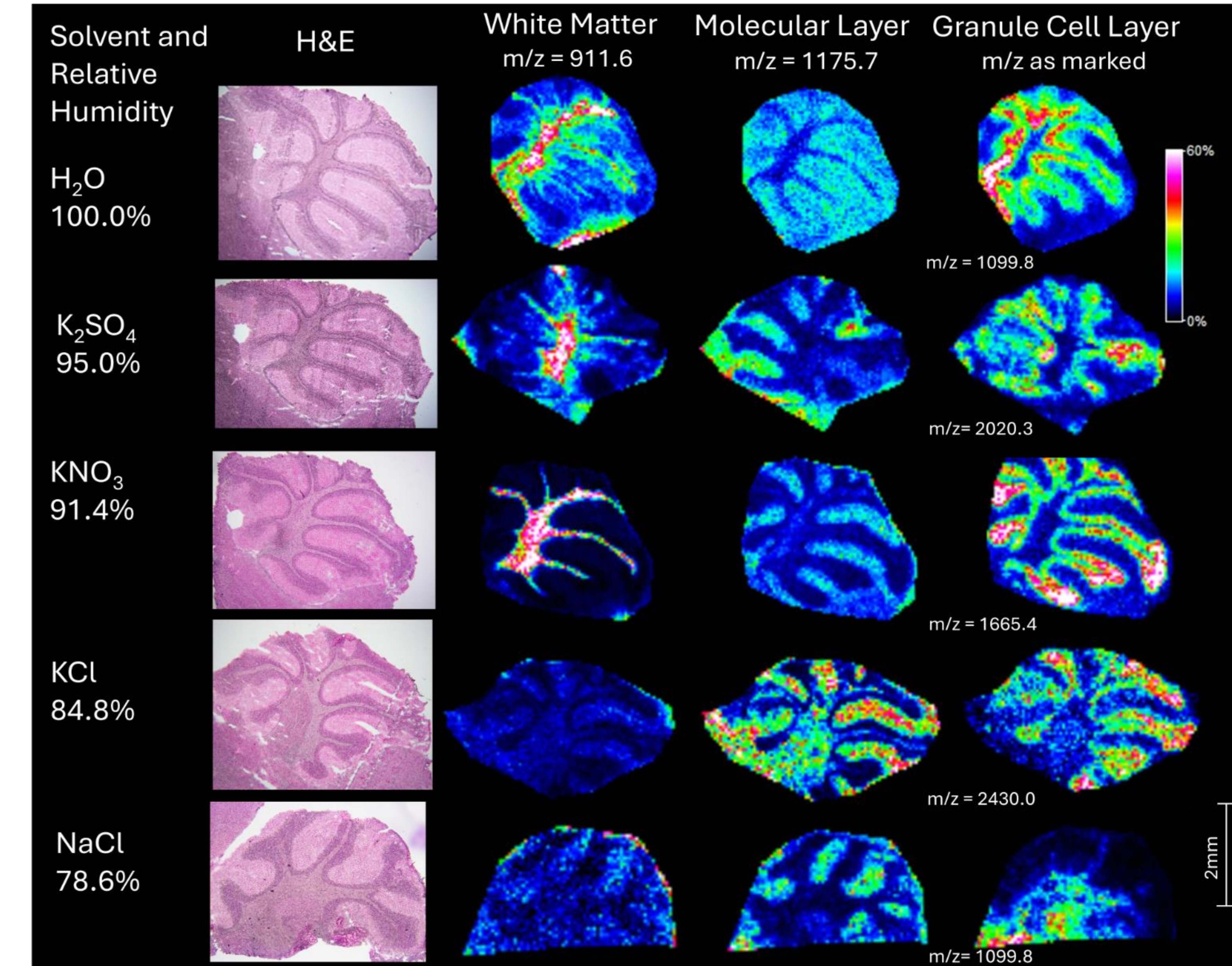


Figure 3: MALDI MSI distribution of selected tryptic peptides from a mouse brain section after trypsin digestion incubation with different salt solutions. Incubations were performed for 18 hours at 37°C. The average measured humidity of each condition is listed. Spatial resolution: 50 μm .

CONCLUSIONS

The best wash tested was determined to be the Carnoy's procedure, which gave consistent, high-quality MALDI MS results post trypsin digestion (Figure 2).

At humidity levels of 96% and above, any increase in sensitivity was offset by a notable increase in peptide delocalization. Saturated KNO₃ with a relative humidity of 91%, was determined to be the best incubation solution for optimal trypsin digestion giving high peptide signal yields while minimizing delocalization allowing to maintain an accurate relationship with histology (Figure 3).

ACKNOWLEDGMENTS

¹ Department of Analytical Chemistry and Biochemistry, AGH University of Krakow, Krakow, Poland. ² Department of Chemistry, Université de Montréal, ³ McGill University, Montreal, QC, Canada. This research was supported by the program "Excellence initiative-research university" for the AGH University of Krakow (no. 13561) and was partially financed from the subsidy no. 16.16.160.557 from the Polish Ministry of Science and Higher Education. The incubation chamber used was a kind gift from the Martina Marchetti-Deschmann laboratory, Vienna University of Technology, Austria.

contact email: pkret@agh.edu.pl