Automatic spraying with TM sprayer for imaging proteins with 2,5-cDHA

Equipment and Materials:

Equipment

- Petri dish (glass, Fisher, 100mm x 15mm),
- TM sprayer from HTX
- Indium oxide coated glass slide(ITO)
- Metal target

Chemicals

- Acetic acid (AcOH), trifluoroacetic acid (TFA), acetonitrile
- (E)-4-(2,5-dihydroxyphenyl)but-3-en-2-one (2,5-cDHA)
- MilliQ water
- Carnoy's fluid: 6 ethanol, 3 chloroform, 1 acetic acid

Procedure

1. Tissue preparation prior to spraying.
   The tissue, sectioned and placed on plate (ITO slides, metal target, etc.), is dried under ambient conditions for 10 minutes and stored in a slides mailer box (Electron microscope science, 71548-01) sealed with parafilm at -80°C. When ready for analysis, the slides box was placed into a vacuum desiccator under room temperature for 30 minutes to allow the slides to reach room temperature while preventing water condensation on the sections that could cause delocalization of analytes.
2. Washing conditions for detection of proteins: 70% ethanol (30s), 100% ethanol (30s), Carnoy's fluid (2min), 100% ethanol (30s), 40% ethanol (30s), 100% ethanol (30s).
3. Matrix solutions:
   solution (A) for seeding: 30 mg of 2,5-cDHA is dissolved in 5 mL ethyl acetate and 5 mL of toluene.

solution (B) for extraction: 90 mg 2,5-cDHA is dissolved in 3 mL ACN, 7 mL H_2O, 100 µL TFA and 50 µL ammonium hydroxide.

4. Spraying with TM sprayer:
    1) Nozzle temperature is set at 95°C
    2) Inject the TM sprayer loop with 5mL of ethyl acetate/toluene (1:1) and prime the TM sprayer with ethyl acetate/toluene (1:1) for 2 min, at 0.5 mL/min using, then change the flow rate to 0.05 mL/min
    3) Seeding spraying

       Solution A is sprayed on the slide tissue sections with the TM Sprayer™ using the following conditions: LC solvent as ethyl acetate/toluene (1:1), 95°C nozzle temperature, 600 mm/min nozzle velocity, 1.5 mm track spacing, 0.05 mL/min flow rate, and 4 passes.
    4) Inject the TM sprayer loop with 5 mL of ACN and then 5 mL of 30% ACN and prime the TM sprayer with 30% ACN for 2 min at 0.5 mL/min. then change the flow rate to 0.05 mL/min
    5) Extraction spraying

       Solution B is sprayed on the slide from step 3 using the following conditions: LC solvent 30% ACN, 95°C nozzle temperature, 1100 mm/min nozzle velocity, 1.5 mm track spacing, 0.05 mL/min flow rate and 8 passes.

Note:

1. The flow rate is critical for spraying 2,5-cDHA, too slow a rate will result a very wet surface and could cause delocalization of analytes and too fast a rate will result poor extraction of proteins.
2. The resulting coating from this spraying procedure is about 0.1 mg/cm^2.
3. A washing protocol is extremely critical to obtain high quality spectra.
4. The seeding procedure dictates the size of crystals and homogeneity of the coating.

Reference:

Yang J, Norris JL, Caprioli R. Novel vacuum stable ketone-based matrices for high spatial resolution MALDI imaging mass spectrometry. J Mass Spectrom. 2018;53:1005–1012. https://doi.org/10.1002/jms.4277