Sample Preparation Procedure for

Insects
Ethanol / Formic Acid Extraction Modified for Insects (EtOH-FA-I)

General Information

This protocol for insects, provided by CVUA Freibrug, is based on the sample preparation procedure using ethanol/formic acid by Bruker Daltonik GmbH [1] with modification for sample quantity, homogenization and purification. It was used to create and validate a reference database for insects, which will be continuously expanded.

A selection of reference spectra (msp) and single spectra using this protocol is available for exchange on the MALDI-UP homepage (https://maldi-up@ua-bw.de).

Field of Application

Insects: whole, ground, processed, in natural form, raw, cooked, frozen or freeze-dried

Chemicals and Material

- 1.5 ml reaction tubes
- Pipettes and tips for volumes from 1 – 1000 µl
- Silica beads (0.1 mm resp. 0.5 mm diameter)
- Micropestle, compatible with the tubes used
- Bead mill (optional)
- Benchtop centrifuge
- HCCA matrix solution (please see »Tipps and Recommendation«, page 2)
- Ethanol absolute
- 70 % Formic acid
- Ultrapure water
- Acetonitrile (ACN)

References

[1] Bruker Daltonik GmbH. Ethanol/formic acid extraction sample preparation procedure.

Insects:

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Extraction Procedure

- Transfer up to 5 mg of sample material into a clean reaction tube
- Pipet 100 µl water into the tube and homogenize / grind the sample using a micropestle
- Add 200 µl water and mix thoroughly (if the suspension is jellylike, start again by using less sample material)
- Add 900 µl ethanol into the tube and mix thoroughly
- Centrifuge at 18,400 rcf for 2 minutes and decant the supernatant
- Wash the pellet by adding 300 µl water to the tube and mix thoroughly
- Centrifuge at 18,400 rcf for 2 minutes, decant the supernatant or remove it from the pellet by using a pipet
- Dry the pellet for some minutes to increase the extraction efficiency
- Add between 50 – 200 µl 70 % formic acid to the pellet and mix thoroughly by pipetting
- Add silica beads and mix thoroughly for about 1 minute (alternatively use a bead mill to disrupt the cells, e.g. at frequency of 50 Hz for 2 minutes)
- Add acetonitrile in the same amount as formic acid and mix for about 1 minute by pipetting (if the suspension is jellylike, add 70% formic acid and acetonitrile in equal volumes until the suspension is liquid)
- Centrifuge at 18,400 rcf for 2 minutes
- Pipet 1 µl supernatant onto a target sample spot (we recommend spotting the supernatant in duplicate or triplicate)
- As soon as the sample spot has dried, overlay the sample with 1 µl HCCA matrix solution (to prevent oxidation reactions which might cause peak shifts)
- Allow the sample spot to air dry before analysis ➔ MALDI measurement

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