IASMA, E Mach Fondation Metabolomic platform:

Practical trials GC-MS/MS

Practical trials for GC-MS/MS are designed to test the performances of the analytical system in conditions which mimics real experimental ones.
The configuration of the instrument selected for practical trials have to be **identical** to the proposed one.
Samples will be provided by IASMA well in advance in order to allow the definition of the best experimental conditions. Experimental condition can be freely optimized by the applicationist in order to maximize the performances of the analytical system. Practical trials should be completed before the meeting with the IASMA commission.

After the practical trials a one day meeting with the IASMA researchers will be organized at the trial site. The meeting agenda will cover:

> Practical demonstration of the proposed analytical system (hardware + software).
> Presentation and discussion of the practical trial results.
> Definition of the data which have to be included in the "Practical Trial Report"
> We will try also a SPME analysis (fiber and sample will be provided by the IASMA scientists)

Experimental protocols
Objective: to evaluate the MS/MS functionalities of the instrument,
To evaluate the procedure necessary for MRM optimisation
To evaluate the sensitivity of the instrument (limit of detection and quantification), the linearity range, the dynamic range, the stability of the signal (signal intensity, precision/accuracy of detected mass)
To evaluate post acquisition data treatment software utilities: multi analyte quantitation, construction of calibration curves, quantitation of real samples.

Material: 1 vial with a concentrated solution (mixture of volatile standard in ethanol Mix F1) that should be used a mother solution for dilutions to standards in order to construct calibration curves for the analytes. Furthermore two vials with real sample in matrix (wine extract in pentane/dichloromethane 2:1 v/v) in 2 different concentration are provided W1 and W2.
Quantitative analysis of the target analytes should be performed in these samples.
Mode: gradient separation on a PEG wax capillary column and acquisition of chromatographic and spectral data (MS and MS/MS). Sequential injections to check dynamic range, linearity and detection limit of standards.

- Identification potential: Two unknown compounds X and Y are included in the sample Mix F1. These should be identified based on the injection and the use of spectral libraries. Next they should be quantified along with the rest of the sample components
- Dynamic range: injection of different dilutions of the sample Mix F1 over at least 6 order or magnitude
- Linearity: Injections of ca 8 serial dilutions of the calibration standards. If necessary repeated injections of outliers and repeated injections close to the limit of detection. The trial report has to include calibration curves and peak areas and in the report Table and Figure should be given.
- Detection limit: the detection limit for each standard has to be estimated in MS/MS mode and indicated in terms of injected pg. To check the reliability of the estimated detection limit

the report must include the chromatograms for each standard at the lowest detectable concentration.

Solvents and materials to be provided:

Examples of GC run conditions: Separation on a polar poly(ethylene) glycol **Wax** fused-silica capillary column (30 m, 0.25 mm ID, 0.25 μm film thickness). Oven temperature program: 4 min at 50°C; up to 200°C (8°C/min, held 1 min) and then to 230°C (15°C/min, held 2 min). Helium as carrier gas with a flow rate of 1.2 mL min^{-1}. Transfer line temperature set to 250°C. Splitless injection: 250° C, splitless time 0.5 min
1.5ul injection.
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Composition of **Mix F1**

Compound	MW	Mix F1 mg/L	transitions
methyl anthranilate	151	116	151>119, 151>92
ethyl anthranilate	165	111	165>137, 165>119, 165>92
X	135	56	135>120, 135>92
rose oxide	154	84	154>139
geraniol	154	84	154>139, 154>121, 154>97
terpinen-4-ol	154	89	154>139, 154>125
geranyl acetate	196	88	136>121, 136>93
3-mercapto-hexanol	134	42	134>101, 134>67, 134>57
3-mercapto-hexanol acetate	176	48	116>101, 116>88, 116>87
Y	152	49	152>137, 152>124

MRM conditions
Q2 Gas pressure 1.5
Scan width 0.2
Scan time 0.1
Emission current 50 uA

Contact person for technical questions regarding the lab trial.
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