Getting the best results from your expression CMS™ – Direct Injection (Flow Injection Analysis).

Direct Injection System Requirements:

- expression CMS fitted with ESI or APCI ion source
- ACC360 Pump for isocratic flow (or other suitable LC-type pump capable of pumping 100 µL/min at 1000 psi)
- HPLC grade spray solvent (see below)
- Guard Column (such as Phenomenex AJ-4286 guards in KJ0-4282 holder)

Sample Preparation:

1. Solids and oils. Pre-weigh 0.5 – 1.5 mg of sample into suitable vial. Dissolve the sample in 0.5 – 1.5 mL of methanol or water to give 1 mg/mL stock solution. Dilute the stock solution 20 µL into 1 mL of the mobile phase to give 20 µg/mL in the mobile phase.
2. Reaction solutions. Transfer 2 – 3 µL of solution (use a capillary micro-pipette) to a suitable vial and dilute to 1 mL with the appropriate spray solvent.

Suggested spray solvent: For direct injection: 80/20 Methanol/Water with 0.1% Formic Acid. Alternatively 0.5 g/L (6.5 mM) ammonium acetate may be used as a buffer. Acetonitrile should be avoided for direct injection if possible as it can result in the formation of *m/z* +41 adducts which complicate the spectrum. There is no advantage in using Acetonitrile for direct injection as there is no separation and no UV measurement.

A guard column, such as Phenomenex Security Guard, is highly recommended for direct injection. This will help separate the salts from your sample before they get to the CMS and will help to protect the CMS from contamination.

3. Normal Phase Flash Fractions. For this we recommend dilution 100:1 with a mobile phase that is miscible with normal phase solvents and aqueous solvents - 80/20 Isopropyl /Water with 0.1% Formic Acid.

All mobile phase solvents MUST be HPLC grade.

Analysis:

Set the pump flow to 100 µL/min using a spray solvent, as described above. Set up a suitable analysis method in the Mass Express software and inject at least 10 µL of your sample into the manual injection port on the front of the expression CMS – this is fitted with a 5 µL loop so you will be injecting 100 ng of your sample – and press the 'run' button in Mass Express. Increasing concentration beyond this level will not improve results (see guidelines below).

Electrospray Mass Spectrometry Guidelines

- Samples work best if they contain an ionizable (can be protonated or deprotonated) functional group (-NH_2, CO_2H, SO_3H, Ph-OH).
- Samples may also ionize if they form adducts with ammonium acetate, ammonium formate, sodium or potassium.
- Solvents that are compatible with aqueous ESI conditions include methanol, acetonitrile and isopropanol.
- Non-aqueous solvents include chloroform and methylene chloride.
- Sample concentration above 20 µg/mL increase the formation of cluster ions and may cause mass-to-charge intensity ratios to be unreliable.
- Samples must be free of non-volatile additives such as EDTA, phosphate buffers, SDS, Triton X-100 (or massively diluted).
- Additives and Buffers compatible with ESI include acetic acid, ammonium acetate, ammonium formate, formic acid and trifluoroacetic acid at 0.1% or less concentration.

Notes on Sample Concentration

- Electrospray full-scan mass spectra typically requires sample concentration from 1 – 20 µg/mL (1-20 ng/µL) This is 5 to 100 pMol/µL or **10 – 200 ng per 10 µL injection.**
- ESI response becomes non-linear at high sample concentration.
- Increasing sample concentration >20 µg/mL does not improve results.
    - And it causes the mass spectrometer to get dirty and lose performance
- Concentrated samples increase the formation of dimer and cluster ions.
- If a molecule doesn't ionize in ESI mode, change the sample pH or ionization mode (polarity, APCI) rather than increasing the sample concentration.
- Problems caused by high sample concentration:
    - Incorrect isotope ratios due to saturation of data system
    - Unreliable abundance ratios caused by saturation of main peak or non linearity of response
    - 'negative' chromatographic peaks in the total ion chromatogram/ e.g. peaks 'go down instead of up'
    - Sample carryover due to contamination in injection port and valve and MS capillary.
    - Plugging of HPLC tubing/sprayer capillary due to poor solubility.