Method description for publication of mass spectrometry analysis carried out in UCL Chemistry Mass Spectrometry Facility

MALDI-TOF analysis

MALDI mass spectra were acquired in UCL Chemistry Mass Spectrometry Facility using a Waters MALDI-TOF micro MX (Waters, UK). The instrument operated in positive reflectron mode using the mass range of *m/z* 500 to 5,000 with 100 shots/spectrum. Samples were prepared by 1:1 and 1:2 dilution with matrix (α-cyano-4-hydroxy-cinnamic acid in water-acetonitrile (2:8, v/v), 0.5% formic acid. Three µL of the resulting sample was spotted onto the MALDI target plate and then irradiated with a pulsed N_2 laser 337nm, with delayed extraction (500 nsec) and an accelerating voltage of 200 V, pulse 2,500 and detector 2,000.

Accurate mass measurements using the LCT Premier Q-TOF

Mass measurements were performed in UCL Chemistry Mass Spectrometry Facility using a Waters 2720 autosampler connected to the LCT Premier XE Q-TOF mass spectrometer. Five µL of the sample was injected through the autosampler into the QTOF using a six-valve loop. The analysis time was 2 min. The mass spectrometer operated in positive and negative ionisation ESI with full MS scan. The mass range was from *m/z* 100 to 2,000. The LCT Premier XE mass spectrometer was operated in W-mode with the following parameters:- the source capillary was 2,500 V, sample cone 30 V, desolvation temperature 400°C, source temperature 80°C, cone gas flow 50, and desolvation gas flow 450 L/hr. The Q-TOF was calibrated with sulfadimethyhoxidne $[M]^+$ with *m/z* value of 311.0814 and leucine encephalin $[M]^+$, *m/z* value of 556.2771.

UPLC-MS analysis

Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC-MS) analyses were carried out on an Acquity UPLC-SQD system consisting of a 515 pump, 2525 mixer and 1998 UV detector set at 254 nM (Waters, UK). The UPLC system was connected to a Micromass ZQ mass spectrometer, which scanned the *m/z* range from 100 to 2000. Ten µL of the sample was injected on a C_4 column, 1.9 µM pore size, 2.1 mm x 150 mm (Thermo Scientific, UK). The flow rate was 0.6 mL/min. Mobile phases were (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The gradient was employed as follows:- 5% (B) for 0.5 min following a gradual increase to 95% (B) over 4.5 min and return to 5% (B) in 30 sec and held for 1 min at 5% (B).

Accurate mass measurements using an ASAP-HESI ionisation connected to the Q Exactive Plus mass spectrometer

ASAP-HESI mass spectra were acquired in UCL Chemistry Mass Spectrometry Facility using an ASAP probe integrated into the HESI ion source. The generated gas phase ions were measured using the Exactive Plus mass spectrometer. HESI parameters were the auxiliary gas to 5 and the sheath and sweep gases to 0 and the auxiliary gas heater to 400℃ and typical operating temperatures were between 100°C and 450°C but higher temperatures are needed for some compounds and lower more appropriate for others. The discharge voltage was set to between 3.5 and 4.0 kV to give a stable background ion signal would depend on the structure of analyte.

Liquid Chromatography – Mass Spectrometry using the Vanquisher LC connected to the Q Exactive Plus mass spectrometer

The Vanquisher LC system connected to the Orbitrap Q Exactive mass spectrometer (ThermoScientific, UK) was utilised for LC-MS analysis. The samples were diluted in 1 mL of methanol or dichloromethane. Two µL of the sample was injected on a C_4 column, 1.9 µM pore size, 2.1 mm x 150 mm (Thermo Scientific, UK). Mobile phases were (A) water, 0.1 % formic acid and (B) acetonitrile, 0.1% formic acid. The gradient was as follows:- 5% B was for 1 min and after linear increase to 95% B over 4 min, which followed by change to 5%B in 0.1 min and left at 5%B for another 0.9 min. The total LC-MS analysis time was 6 min and the flow rate was 0.3 mL/min. The HESI ion source parameters were:- spray voltage 3500, capillary temperature 320˚C, sheath gas 25, auxiliary gas 10 and probe heater 310˚C. The mass spectrometer operated in both positive and negative ionisation modes. The mass range was *m/z* 100 to 3,000 Da. The instrument was externally calibrated with using caffeine, MRFA (MET-ARG-PHE-ALA) and Ultramark 1621.

LC-MS analysis of bio-conjugates using the Agilent 6510 QTOF LC-MS instrument

The samples were analysed on the Agilent 6510 QTOF LC-MS system in UCL Chemistry Mass Spectrometry Facility. Ten µL of each sample (around 0.2 mg/mL of protein) was injected onto a PLRP-S, 1000A, 8 µM, 150 mm x 2.1 mm column, which was maintained at 60 °C. The separation was achieved using mobile phase A (water with 0.1% formic acid) and B (acetonitrile, with 0.1% formic acid) using a gradient elution at the flow rate 0.3 mL/min. The column effluent was continuously electrosprayed into capillary ESI source of the Agilent 6510 QTOF mass spectrometer. ESI mass spectra were acquired in positive electrospray ionisation (ESI) mode at the *m/z* range 1,000−3,200 in profile mode. The raw data was converted to zero charge mass spectra using maximum entropy deconvolution algorithm within the MassHunter software version B.07.00.

Accurate mass measurement using MAT900 XP magnetic sector mass spectrometer

Mass spectrometry analyses were performed at UCL Chemistry Mass Spectrometry Facility using a Finnigan MAT 900 XP double focusing hybrid (EBqQ) mass spectrometer (Bremen, Germany) with a direct insertion probe. The magnet was scanned from *m/z* 100 -1000 at 5 s/decade. Gas phase ions were generated in EI-volume and detected by a PATRIC (positron and time resolved ion counter) scanning array detector. The instrument resolution was 10,000. The accurate mass determination was performed by the peak-matching method.

GC-MS analysis

GC-MS analysis was performed on a Thermo Scientific GC system connected to Thermo Scientific Autosampler and coupled with an ISQ single quadrupole mass spectrometer with electron impact (EI) ionisation volume. GC separation was performed on a TR-5MS, 30m x 0.25mm x 0.25µm column from Thermo Scientific, Part Number 260F047P. Helium was the carrier gas with a flow rate 1.2 mL/min. One µL of sample was injected in splitless mode at 200°C. The initial temperature of the oven was 50°C and ramped with a rate of 40°C per minute until achieving 260°C. The temperature was held at 260°C for 3 minutes. Mass spectrometric parameters were set with EI ionisation energy of 70 eV, ion source temperature of 230°C, and MS quadrupole temperature of 230°C.