

## Continuous flow injection

### *Electrospray*

High-resolution electrospray mass spectra (HR-ESI-MS) were recorded on a *timsTOF Pro* TIMS-QTOF-MS instrument (*Bruker Daltonics GmbH*, Bremen, Germany). The samples were dissolved in (e.g. MeOH) at a concentration of ca. 50  $\mu\text{g ml}^{-1}$  and analyzed via continuous flow injection (2  $\mu\text{L min}^{-1}$ ). The mass spectrometer was operated in the positive (or negative) electrospray ionization mode at 4'000 V (-4'000 V) capillary voltage and -500 V (500 V) endplate offset with a N<sub>2</sub> nebulizer pressure of 0.4 bar and a dry gas flow of 4 l min<sup>-1</sup> at 180°C. Mass spectra were acquired in a mass range from  $m/z$  50 to 2'000 at ca. 20'000 resolution ( $m/z$  622) and at 1.0 Hz rate. The mass analyzer was calibrated between  $m/z$  118 and 2'721 using an *Agilent* ESI-L low concentration tuning mix solution (*Agilent*, USA) at a resolution of 20'000 giving a mass accuracy below 2 ppm. All solvent used were purchased in best LC-MS quality.

### *APCI*

High-resolution APCI mass spectra (HR-APCI-MS) were recorded on a *timsTOF Pro* TIMS-QTOF-MS instrument (*Bruker Daltonics GmbH*, Bremen, Germany). The samples were dissolved in (e.g. MeOH, CH<sub>2</sub>Cl<sub>2</sub>) at a concentration of ca. 100  $\mu\text{g ml}^{-1}$  and analyzed via continuous flow injection at 103  $\mu\text{L min}^{-1}$  using a tee (3  $\mu\text{L min}^{-1}$  sample solution, 100  $\mu\text{L min}^{-1}$  mobile phase consisting of MeOH/CH<sub>2</sub>Cl<sub>2</sub> 1:1 + 0.1% HCOOOH). The mass spectrometer was operated in the positive (or negative) ionization mode at 4'000 V (-4'000 V) capillary voltage and -500 V (500 V) endplate offset with a APCI heater temperature of 300°C, N<sub>2</sub> nebulizer pressure of 0.2 bar, and a dry gas flow of 4 l min<sup>-1</sup> at 200°C. Mass spectra were acquired in a mass range from  $m/z$  50 to 2'000 at ca. 20'000 resolution ( $m/z$  622) and at 1.0 Hz rate. The mass analyzer was calibrated between  $m/z$  121 and 2'721 using an 1:20 diluted *Sigma-Aldrich* APCI/APPI calibrant solution (*Agilent*, USA) at a resolution of 20'000 giving a mass accuracy below 2 ppm. All solvent used were purchased in best LC-MS quality.

## LC-MS

Samples (1 µl injection) were analyzed with a *Vanquish™ Horizon UHPLC* System (*Thermo Fisher Scientific*, Waltham, USA) connected to a *Vanquish eλ* detector and a *timsTOF Pro* TIMS-QTOF high-resolution mass spectrometer (*Bruker Daltonics*, Bremen, Germany). Separation was performed with an *Acquity BEH C18* HPLC column (1.7 µm particle size, 2x100 mm, *Waters*) kept at 30 °C.\* The mobile phase consisted of A: H<sub>2</sub>O + 0.1% HCOOH and B: CH<sub>3</sub>CN + 0.1% HCOOH. A linear gradient was run from 5 to 98% B within 5 min followed by flushing with 98% B for 1 min at a flow rate of 400 µl min<sup>-1</sup>. UV spectra were recorded between 200 and 600 nm at a 1.2 nm resolution and at 20 points s<sup>-1</sup>. The mass spectrometer was operated in the positive (negative) electrospray ionization mode at 4'000 V (-4'000 V) capillary voltage and -500 V (500 V) endplate offset with a N<sub>2</sub> nebulizer pressure of 2.8 bar and dry gas flow of 8 l min<sup>-1</sup> at 220°C. Spectra were acquired in the mass range from *m/z* 50 to 2'000 at ca. 20'000 resolution (*m/z* 622) and at a 1.5 Hz rate. The mass analyzer was calibrated at the beginning of each LC run between *m/z* 158 and 1'450 using a 10 mM solution of sodium formate that was injected using a 6-port-valve with a 20 µL loop at a resolution of 20'000 giving a mass accuracy below 2 ppm.

\* check for effectively used chromatographic conditions (column and solvent system).

## LC-MS/MS

Samples (1 µl injection) were analyzed with a *Vanquish™ Horizon UHPLC* System (*Thermo Fisher Scientific*, Waltham, USA) connected to a *Vanquish eλ* detector and a *timsTOF Pro* TIMS-QTOF high-resolution mass spectrometer (*Bruker Daltonics*, Bremen, Germany). Separation was performed with an *Acquity BEH C18* HPLC column (1.7 µm particle size, 2x100 mm, *Waters*) kept at 30 °C.\* The mobile phase consisted of A: H<sub>2</sub>O + 0.1% HCOOH and B: CH<sub>3</sub>CN + 0.1% HCOOH. A linear gradient was run from 5 to 98% B within 5 min followed by flushing with 98% B for 1 min at a flow rate of 400 µl min<sup>-1</sup>. UV spectra were recorded between 200 and 600 nm at a 1.2 nm resolution and at 20 points s<sup>-1</sup>. The mass spectrometer was operated in the positive (negative) electrospray ionization mode at 4'000 V (-4'000 V) capillary voltage and -500 V (500 V) endplate offset with a N<sub>2</sub> nebulizer pressure of 2.8 bar and dry gas flow of 8 l min<sup>-1</sup> at 220°C. Spectra were acquired in the mass range from *m/z* 50 to 2'000 at 20'000 resolution (*m/z* 500 full width at half maximum) and at a 1.5 Hz rate. The mass analyzer was calibrated at the beginning of each LC run between *m/z* 158 and 1'450 using a 10 mM solution of sodium formate that was injected using a 6-port-valve with a 20 µL loop at a resolution of ca. 25'000 (*m/z* 622) giving a mass accuracy below 2 ppm. MS/MS spectra were acquired at a collision energy of e.g. 35 eV with an 3 *m/z* isolation width in a mass range from *m/z* 50 to 600 at a 2.0 Hz rate. N<sub>2</sub> was used as a collision gas.

\* check for effectively used chromatographic conditions (column and solvent system).

## IM-MS

High-resolution electrospray mass spectra including ion mobility data (HR-ESI-IM-MS) were recorded on a *timsTOF Pro* TIMS-QTOF-MS instrument (*Bruker Daltonics GmbH*, Bremen,

Germany). The samples were dissolved in (e.g. MeOH) at a concentration of ca. 50  $\mu\text{g ml}^{-1}$  and analyzed via continuous flow injection (2  $\mu\text{L min}^{-1}$ ). The mass spectrometer was operated in the positive (or negative) electrospray ionization mode at 4'000 V (-4'000 V) capillary voltage and -500 V (500 V) endplate offset with a  $\text{N}_2$  nebulizer pressure of 0.4 bar and a dry gas flow of 4  $\text{l min}^{-1}$  at 180°C. Mass spectra were acquired in a mass range from  $m/z$  50 to 2'000. TIMS inverse reduced mobility values ( $\text{K}_0^{-1}$ ) were recorded in a range of 0.45 – 1.2  $\text{K}_0^{-1}$  with an ion charge control of 5.00 million and a ramp time of 250 ms resulting in a 3.92 Hz rate and a resolution of 80.  $\text{N}_2$  was used as a drift gas. Mobility and  $m/z$  measurements were both calibrated between  $m/z$  118 and 2'721 using an *Agilent* ESI-L low concentration tuning mix solution (*Agilent*, USA) at a  $m/z$  resolution of 40'000 and a mobility resolution of 80 giving a mass accuracy below 2 ppm and a mobility deviation below 0.2  $\text{\AA}^2$ . All solvent used were purchased in best LC-MS quality.