**Messungen für WEB (Fullscan/MS², positiver/negativer Ionenmodus, profile/centroid)**

* Proben aus Enzymansätzen, Nachweis von Methylierungen

**Probenvorbereitung**

* alle Proben wurden 1:3 verdünnt und zentrifugiert
* jeweils Fullscan sowie MS2- und HCD-Experimente

|  |  |  |
| --- | --- | --- |
| **Probe** | **Positiver Modus** | **Negativer Modus** |
| WEB346\_A\_3 | MS2(303) [45%]  MS2(317) [45%] %, HCD [100%] | - |
| WEB346\_E\_3 |
| WEB346\_A\_4 | MS2(317) [45%] %, HCD (317) [100%] | - |
| WEB346\_B\_4 |
| WEB346\_F\_4 |
| WEB346\_E\_5 | MS2(271) [45%]  MS2(285) [45%], HCD (285)[100%] | - |
| WEB346\_A\_6 | MS2(287) [45%]  MS2(301) [45%], HCD (301) [100%] | - |
| WEB346\_D\_6 |
| WEB346\_H\_6 |
| WEB346\_A\_7 | MS2(301) [45%]  MS2(315) [45%], HCD (315) [100%] | - |
| WEB346\_D\_7 |
| WEB346\_E\_7 |
| WEB346\_C\_8 | - |
| WEB346\_E\_8 |
| WEB346\_H\_9 | MS2(165) [45%]  MS2(179) [45%] | MS2(163) [30%]  MS2(177) [30%] |
| WEB346\_D\_10 |
| WEB346\_D\_11 |
| WEB346\_G\_11 |
| WEB346\_H\_12 | MS2(181) [45%]  MS2(195) [45%] | MS2(179) [30%]  MS2(193) [30%] |
| WEB346\_G\_13 | MS2(195) [45%]  MS2(209) [45%] | MS2(193) [30%]  MS2(207) [30%] |
| WEB346\_E\_14 |
| WEB346\_G\_14 |
| WEB346\_E\_15 | MS2(287) [45%]  MS2(301) [45%], HCD (301) [75%] | - |
| WEB346\_F\_15 |
| WEB346\_B\_16 | MS2(303) [45%]  MS2(317) [45%], HCD (317) [75%]  MS2(331) [45%], HCD (331) [75%] | - |
| WEB346\_E\_16 |
| WEB346\_F\_16 |
| WEB346\_B\_17 | MS2(319) [45%]  MS2(333) [45%], HCD (333) [75%]  MS2(347) [45%], HCD (347) [75%]  MS2(361) [45%], HCD (361) [75%] | - |
| WEB346\_D\_17 |
| WEB346\_E\_17 |

**Standardsubstanzen**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Probe** | **Verbindung** | **Elementar-**  **zusammenensetzung/** | **positiv** | **negativ** |
| WEB346\_S\_1 | **Naringenin** | C15H12O5 | MS2(273) [45%]  MS2(287) [45%], HCD [75%] | **-** |
| WEB346\_S\_2 | **Eriodictyol** | C15H12O6 | MS2(289) [45%]  MS2(303) [45%], HCD [75%] | **-** |
| WEB346\_S\_3 | **Hesperetin** | C16H14O6 | MS2(303) [45%]  MS2(317) [45%] %  HCD (317) [75%] | **-** |
| WEB346\_S\_4 | **Homoeridictyol** | C16H14O6 | **-** |
| WEB346\_S\_5 | **Apigenin** | C15H10O5 | MS2(285) [45%]  MS2(271) [45%] %  HCD [75%] | **-** |
| WEB346\_S\_6 | **Luteolin** | C15H10O6 | MS2(287) [45%]  MS2(301) [45%] %  HCD [75%] | **-** |
| WEB346\_S\_7 | **Diosmetin** | C16H12O6 | MS2(301) [45%]  MS2(315) [45%] HCD [75%] | **-** |
| WEB346\_S\_8 | **Chrysoeriol** | C16H12O6 | **-** |
| WEB346\_S\_9 | **p-Cumarsäure** | C9H8O3 | **-** | MS2(163) [30%]  MS2(177) [30%] |
| WEB346\_S\_10 | **m-Cumarsäure** | C9H8O3 | **-** | MS2(163) [30%]  MS2(177) [30%] |
| WEB346\_S\_11 | **o-Cumarsäure** | C9H8O3 | **-** | MS2(163) [30%]  MS2(177) [30%] |
| WEB346\_S\_12 | **Kaffeesäure** | C9H8O4 | **-** | MS2(179) [30%]  MS2(193) [30%] |
| WEB346\_S\_13 | **Ferulasäure** | C10H10O4 | **-** | MS2(193) [30%]  MS2(207) [30%] |
| WEB346\_S\_14 | **Iso-Feruläsäure** | C10H10O4 | **-** | MS2(193) [30%]  MS2(207) [30%] |
| WEB346\_S\_15 | **Kaempferol** | C15H10O6 | MS2(287) [45%]  MS2(301) [45%] %, HCD [75%] | **-** |
| WEB346\_S\_16 | **Quercetin** | C15H10O7 | MS2(303) [45%]  MS2(317) [45%] %, HCD [75%]  MS2(331) | **-** |
| WEB346\_S\_17 | **Myricetin** | C15H10O8 | MS2(319) [45%]  MS2(333) [45%] %, HCD [75%]  347  MS2(361) | **-** |

**UHPLC-Methode:**

* + Säule A (Ventil 1):1,9 µm; 50 x 2,1 mm; Hypersil GOLD; Thermo Scientific
  + Säulentemp.: 30°C
  + Schleife: 10 µL
  + Injekt. Vol.: 2 µL
  + Injekt. Meth.: Partial Injection with Needle Overfill
  + PDA, λ =190 bis 400 nm; 280 nm
  + Laufmittel: A1 = H2O, 0.2 % CHOOH

B1 = ACN, 0.2 % CHOOH

* + Gradient:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Time | Flow [mL/min] | A1 | B1 |
| 1 | 0.00 | 0.150 | 95 | 5 |
| 2 | 2.50 | 0.150 | 95 | 5 |
| 3 | 12.50 | 0.150 | 0 | 100 |
| 4 | 15.50 | 0.150 | 0 | 100 |
| 5 | 16.50 | 0.150 | 95 | 5 |
| 6 | 26.50 | 0.150 | 95 | 5 |

Methode: WEB\_pMS2/HCD\_Masse Edukt,Produkt; WEB\_pMS2\_Masse Edukt,Produkt

# UHPLC/ESI high-resolution mass spectrometry

The positive and negative ion high-resolution ESI and collision induced dissociation (CID) MSn spectra were obtained

The positive and negative ion high resolution ESI and collision induced dissociation (CID) MSn spectra as well as higher energy collision induced MS/MS spectra were obtained from an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a HESI electrospray ion source (positive spray voltage 4.5 kV, negative spray voltage 3.5 kV, capillary temperature 275 °C, source heater temperature 250 oC, FTMS resolution 30.000). Nitrogen was used as sheath and auxiliary gas. The MS system is coupled with an ultra-high performance liquid chromatography (UHPLC) system (Dionex UltiMate 3000, Thermo Fisher Scientific), equipped with a RP‑C18 column (particle size 1.9 µm, pore size 175 Å, 50 x 2.1 mm ID, Hypersil GOLD, Thermo Fisher Scientific, column temperature: 30°C), and a photodiode array detector (190-400 nm, Thermofisher Scientific). For the UHPLC a gradient system was used starting from H2O:CH3CN 95:5 (each of them containing 0.2% formic acid) raised to 0:100 within 10 min and then hold on 0:100 for further 3 min, flow rate 150 μl·min-1.

The CID mass spectra (buffer gas: helium) were recorded using normalized collision energies (NCE) of …-..% and for the HCD mass spectra NCE between …-…(see Supporting Information).

The instrument was externally calibrated for positive ion mode by the Pierce® LTQ Velos ESI positive ion calibration solution (product number 88323, Thermofisher Scientific, Rockford, IL, 61105 USA) and for negative ion mode by the Pierce® LTQ Velos ESI negative ion calibration solution (product number 88324, Thermofisher Scientific, Rockford, IL, 61105 USA). The data were evaluated by the Xcalibur software 2.7 (Thermo Fisher Scientific).