The Universal method was designed as "one for all" solution for the analysis of highly abundant, complex samples as well as low abundant low complexity ones. The method is a basic data dependent acquisition method which generates a MS1 scan to shortlist precursors for subsequent fragmentation. The MS1 scan is conducted in the Orbitrap, while the fragment masses are scanned in the ion trap following CID fragmentation. This setup is the simplest scan/fragmentation combination in the instrument and requires the least handling and transfer of ions, due to the isolation happening in the quadrupole and the fragmentation happening in the high pressure cell of the instrument. Due to the layout of the instrument, this scan combination is the fastest possible. Instead of defining a TopN duty cycle in which the MS1 scan is followed by up to N MS2 scans, the Universal method uses a TopSpeed cycle, in which the MS1 scan is set at a chosen interval and the number of MS2 scans varies, depending on the injection times and number of selected precursors. Injection times and number of precursors are determined by the previous full scan.

However, in the Universal method the automatic gain control is set to a value that allows good ion statistics for peptide identification, but the ion injection time is left at a high maximal value (250ms). This way, the ion trap can scan longer on fragments of low abundant precursor ions. As high abundant precursors are prioritized, the dutycycle usually starts with some quick MS2 scans, followed by longer MS2 scans on lower abundant precursors. The latter increases the chance to generate a MS2 spectrum of a low abundant precursor due to improved ion statistics. The scan parameters are listed below in the method transcript. We also supplied the actual method files generated in Tune 2.0.1258.14. However, the Universal method is available as a template in the method generator and has been described by Eluik et al. (42).

Universal
Method Summary

Method Duration (min): **103**

Ion Source
Ion Source Type: **NSI**
Spray Voltage: **Static**
Positive Ion (V): **2000.00**
Negative Ion (V): **600.00**

Positive Ion

Positive Ion	
Time (min)	Voltage (V)

Negative Ion

Negative Ion	
Time (min)	Voltage (V)

Sweep Gas (Arb): **0**

Ion Transfer Tube Temp (°C): **305**

MS

Pressure Mode: **Standard**
Default Charge State: **2**
Internal Mass Calibration: **True**
Note: Enable EASY-IC in each Orbitrap scan as desired
Internal Mass Calibration: **EASY-IC**

Start Time (min): **0**
End Time (min): **103**
Cycle Time (sec): **3**

Master Scan:

MS OT

Detector Type: **Orbitrap**
Orbitrap Resolution: **120000**
Mass Range: **Normal**
Use Quadrupole Isolation: **True**
Scan Range (m/z): **400-1500**
RF Lens (%): **30**
AGC Target: **4.0e5**
Maximum Injection Time (ms): **50**
Microscans: **1**
Data Type: **Profile**
Polarity: **Positive**
Source Fragmentation: **Disabled**
Use EASY-IC: **True**

Filters:

Monoisotopic Precursor Selection (MIPS)

Monoisotopic Peak Determination:

: **Peptide**
Relax Restrictions when too few Precursors are Found: **True**
Exclude undetermined Charge States: **True**

Charge State

Include charge state(s): **2-7**
Include undetermined charge states: **False**
Include charge states 25 and higher: **False**

Dynamic Exclusion

Exclude after n times: **1**
Exclusion duration (s): **60**
Mass Tolerance: **ppm**
Low: **10.00**
High: **10.00**
Exclude Isotopes: **True**
Perform dependent scan on single charge state per precursor only: **False**

Intensity Threshold

Intensity Threshold: **5.0e3**

Decision:

Decisions

        Data dependent mode: **Top Speed**

        Precursor Priority: **Most Intense**

        Number of Scan Event Types: **1**

Scan Event Type 1:

Condition

          No Condition 0

Scan Event Type 1:

Scan:

ddMS^2 IT CID

        MS^n Level: **2**

        Isolation Mode: **Quadrupole**

        Use Isolation m/z Offset: **False**

        Activation Type: **CID**

        CID Collision Energy (%):   **35**

        Activation Q: **0.25**

        Multistage Activation: **False**

        Detector Type: **Ion Trap**

        Scan Range Mode: **Auto: m/z Normal**

        Ion Trap Scan Rate: **Rapid**

        AGC Target: **4.0e3**

        Inject Ions for All Available Parallelizable Time: **True**

        Maximum Injection Time (ms):   **250**

        Microscans: **1**

        Data Type: **Centroid**