Application Note: 30116

High Resolution Multiple Ion Detection (MID)

Data Acquisition for Target Compound Analysis using the DFS High Resolution Mass Spectrometer

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Key Words

- DFS
- GC-HRMS
- Mass Accuracy
- MID
- POPs

Introduction

Target compound analysis e.g. for polychlorinated dioxins & furans (PCDD/F), pesticides, persistent organic pollutants (POPs) or performance enhancing steroids are typically performed by monitoring the compound specific ionsat the expected retention time for each analyte. The Thermo Scientific DFS High Resolution GC/MS benefits from a unique technical feature referred to as the *lock-plus-cali mass technique* for performing multiple ion detection (MID) analyses. The *lock-plus-cali mass technique* provides ease of use, combined with a maximum quantitative precision and certainty in analyte confirmation.

This technical note describes the DFS mode of MID operation using a *lock-plus-cali mass technique* in detail and gives valuable hints for the setup of rugged routine methods.

Inherent Mass Calibration

The DFS GC-HRMS is delivered with a magnet mass calibration provided by the factory. This mass calibration is stable for a long time and valid for immediate MID use.

The basic equation for sector mass spectrometers

$$m/z = c \bullet B^2/V$$

with c = instrument constant,
B = magnetic field strength and
V = acceleration voltage

shows that mass calibrations are feasible either at constant acceleration voltage by calibrating the magnetic field strength or vice versa.

For MID data acquisition, the fixed magnet setting with a variable acceleration voltage is used. The mass calibration is following a special procedure during the data acquisition as described here.

For MID data acquisition, the DFS system uses a unique mass calibration. This mass calibration is performed during MID analysis in every scan, just before monitoring the target compound intensities. The *lock-plus- cali mass technique* provides optimum mass accuracy for all chromatographic situations. The scan-to-scan mass calibration provides the highest confidence for the acquired analytical data.

The mass calibration process is performed in the background without being noticed by the operator and provides a versatile tool for rugged and matrix independent acquisition methods. In particular it provides superior stability especially for high sample throughput with extended runtimes. Consequently, no separate or dedicated mass calibrations for MID runs are required and have to be maintained when using the DFS GC-HRMS. In addition, the instrument mass resolution is monitored constantly and documented within all MID data files.

A reference compound continuously infused from the reference inlet system into the ion source during sample analysis. Typically perfluoro-tributylamine (FC43) is used[1] as reference compound in GC-HRMS for dioxin analysis. Other reference compounds may be used to suit the experimental conditions or the analyst preference.

The exact ion masses of the reference compound are used in the MID acquisition windows for internal calibration. For best performance, two ions of the reference substance are individually selected for each MID window; one mass which is close, but below the analyte target mass, and the other which is slightly above the analyte target masses. Although both reference masses are used for the inherent scan calibration, it became common practice to name the lower reference mass the *"lock mass"* and the upper reference mass the *"calibration mass"*.

During the MID scan, the mass spectrometer is parking the magnet at the start of each MID window and then consecutively performing the mass calibration using the lock and calibration masses followed by the acquisition of target and internal standard mass intensities.

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Lock-plus-Cali Mass Technique

Parking the Magnet

At the start of each MID retention time window, the DFS magnet is automatically set to one mass (Da) below the lowest mass found in the MID descriptor. The magnet is parked and remains with this setting throughout the entire MID window. The analyzer then jumps from between ions (lock, cali and target compound ions) using a series of fast electrical jumps in accelerating voltage.

Scan Inherent Calibration

The lock mass (L) is scanned in a small mass window starting below the mass peak by slowly decreasing the ion source acceleration voltage (see Figure 1: ①). The mass resolution of the lock mass peak is calculated and written to the data file.

Using the lock mass setting, a second reference mass is used for building the MID mass calibration. This is the unique feature of the DFS scan technique in MID. The calibration mass is checked by an electrical jump. A fine adjustment of the electrical calibration is made based on this measurement (see Figure 1: ③). The electrical "jump" (see Figure 1: ②) is very fast and takes only a few milliseconds. The dwell times for the sufficiently intense reference ions are very short.

The resulting electrical calibration is used for subsequent MID data acquisition.

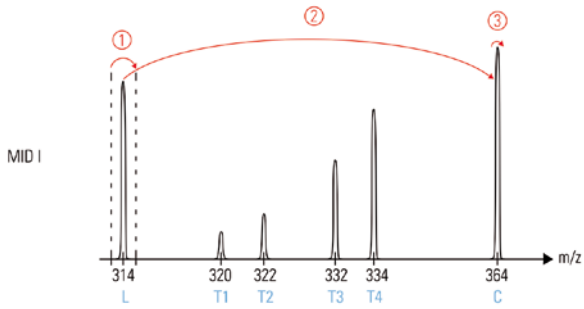

Figure 1: DFS mass detection scheme in MID calibration. The red arrows show the sequence of measurement in the mass calibration steps (The magnet is "locked" in this example at ***m/z*** 313).

①	Magnet locking and "lock mass" sweep, mass calibration and resolution determination	
②	Electrical jump to calibration mass	
③	Calibration mass sweep and mass calibration	
L:	from FC43	lock mass (L), ***m/z*** 313.983364
C:	from FC43	calibration mass (C), ***m/z*** 363.980170
T1, T2:	from native TCDD	analyte target masses ***m/z*** 319.895992, 321.893042
T3, T4	from ^{13}C-TCDD	internal standard masses ***m/z*** 331.936250, 333.933300

Data Acquisition

With the updated and exact mass calibration settings, the analyzer now sets the acceleration voltage to the masses of the target ions. The intensity of each ion is measured based on a preset dwell time (see Figure 2: ④). The dwell times to measure the analyte target ion intensities are significantly longer than the lock or calibration mass ions dwell times. This is done to achieve the optimum detection sensitivity for each analyte ion (see Figure 5). The exact positioning on the top of the target ion mass peak allows for higher dwell times, significantly increased sensitivity, and higher S/N values compared to sweep scan techniques still used in HRMS systems from other vendors. It is important to note that the lock-plus-cali mass technique extends the dynamic range of the DFS significantly into the lower concentration range.

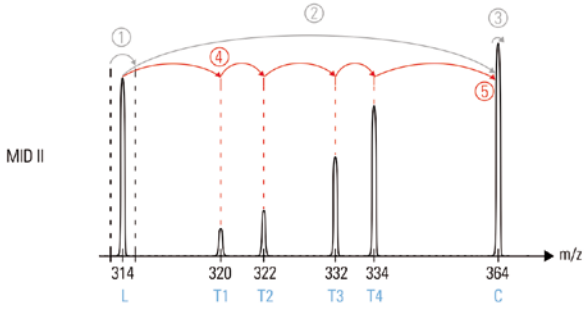

Figure 2: DFS mass detection scheme in MID data acquisition. The red arrows show the sequence of measurement in the target compound and in-ternal standard data acquisition (legend for the mass scale see in Figure 1).

④	Consecutive electrical jumps to target and internal standard masses
⑤	Electrical jump to calibration mass, mass calibration

Benefits of the DFS Lock-plus-Cali Mass Technique

The unique *lock-plus-cali mass calibration technique* provides extremely stable conditions for data acquisitions of long sequences even over days e.g. over the weekend including the performance documentation for quality control.

The electrical jumps of the acceleration voltage are very fast, and provide an excellent instrument duty cycle. Any outside influences from incidental background ions, long term drift or minute electronics fluctuations are taken care of by the lock-plus-cali mass calibration procedure and do not influence the result.

Both, the lock and cali masses are monitored in parallel during the run providing an excellent confirmation of system stability for highest data certainty. Together with the constant resolution monitoring, this unique technique provides the maximum traceability for unsurpassed safety in MID data analysis. During every sample acquisition resolution is calculated for all MID windows and documented within the results file of every sample, for complete confidence in instrument performance. See Figure 4.

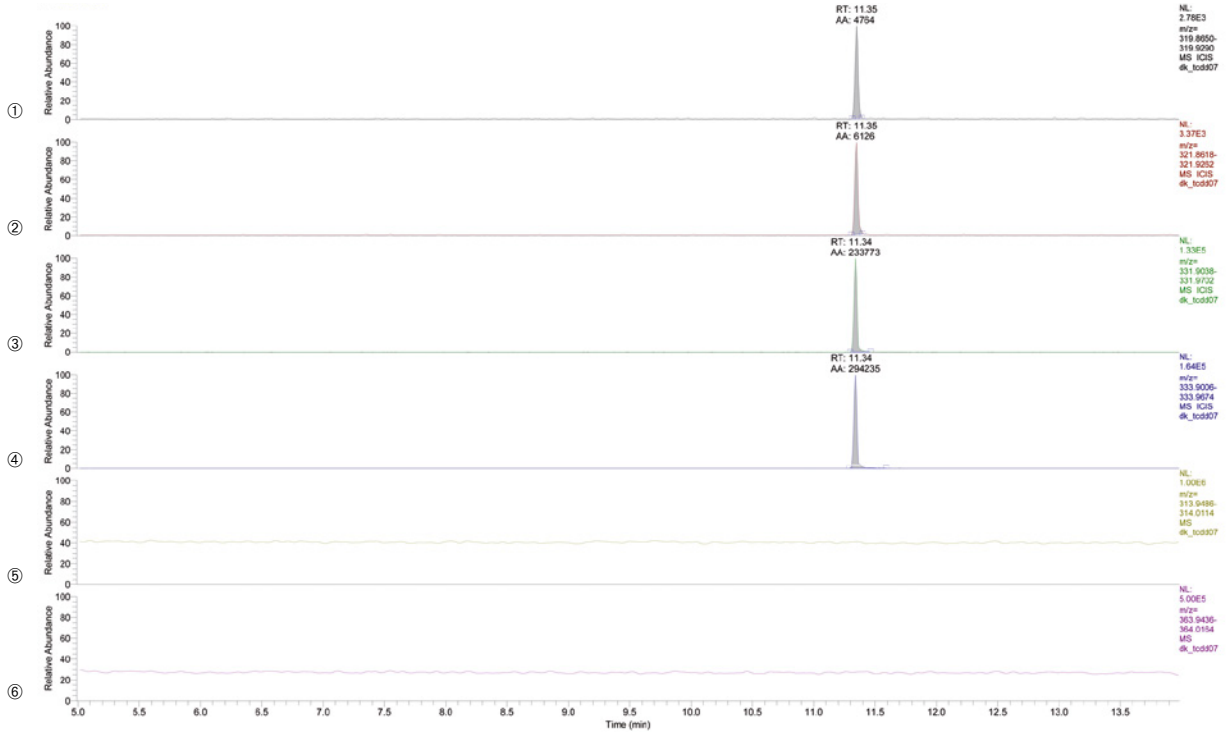

Figure 3: Resulting mass chromatograms of a TCDD standard solution at 100 fg/µL (DB-5MS 60 m x 0.25 µm x 0.1 µm);
① Ratio mass of 2,3,7,8-tcdd (native) ***m/z*** 319.8960; ② Quan mass of 2,3,7,8-tcdd (native) m/z 321.8930; ③ Ratio mass of 2,3,7,8-$^{13}C_{12}$-TCDD(ISTD) ***m/z*** 331.9362
④ Quan mass of 2,3,7,8-$^{13}C_{12}$-TCDD (ISTD) ***m/z*** 333.9333; ⑤ Lock mass of FC43 ***m/z*** 313.983364; ⑥ Cali mass of FC43 ***m/z*** 363.980170

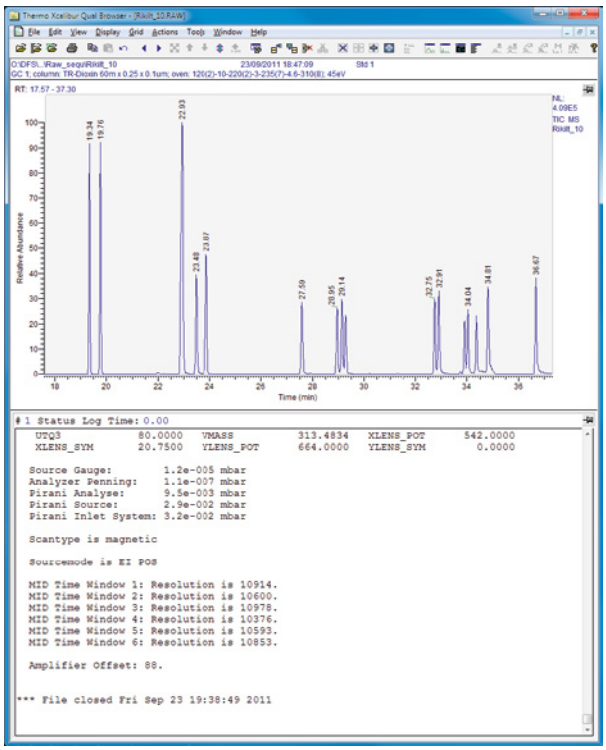

Figure 4: Xcalibur data file; showing status log with resolution reported for every MID in every data file.

Less accurate techniques using only one lock mass position require a separate pre-run electrical mass calibration and do not allow the scan inherent correction of the mass position which may arise due to long term drifts of the analyzer during data acquisition.

As a consequence, with one mass lock techniques, the mass jumps have been less precise with increasing run times. Deviations from the peak top position when acquiring data at the peak slope result in less sensitivity, less reproducibility and poor isotope ratio confirmation.

The unique *lock-plus-cali mass technique* available with the DFS GC-HRMS has proven to be superior in achieving lower LOQs and higher S/N values than any other acquisition method used in high resolution MID before.

Figure 3 shows the typical chromatogram display of a dioxin analysis with the TCDD target masses as well as the ^{13}C internal standard masses. In addition the continuously monitored FC43 lock and cali masses are displayed as constant mass traces. Both traces are of valuable diagnostic use and uniquely confirm the correct measurement of the target compounds.

Setup of the MID Descriptor

The MID descriptor within the data acquisition page contains all the information required by the DFS mass analyzer for continued automated analysis. Included in each descriptor is the retention time information for switching between different target ions, the exact mass calibration, the target masses to be acquired, and the corresponding dwell times.

Up to 50 selected masses can be acquired by each MID window; up to 50 MID retention time windows can be programmed during a single GC run.

To set the retention time window, a sample chromatogram is used as a template. The sample chromatogram is displayed in the Thermo Scientific Xcalibur MID editor as a total ion chromatogram to facilitate the window setting, as shown in Figure 5. The sample chromatogram is used to optimize the GC component separation and set the MID windows before data analysis, and usually consists of a higher concentration standard mix.

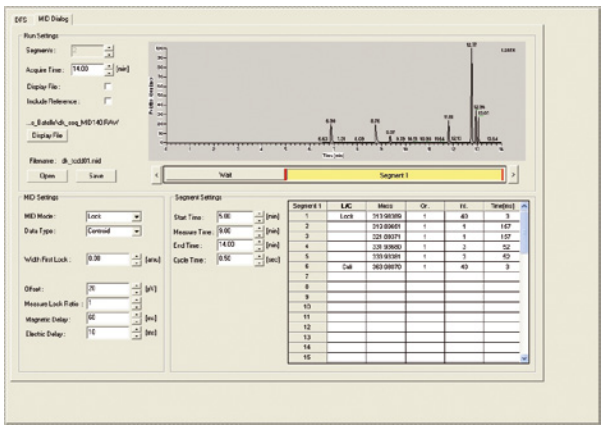

Figure 5: MID editor with sample chromatogram (top) and target mass list and duty cycles for the highlighted retention time window (bottom right).

MID Cycle Time

To provide a representative and reproducible GC peak integration, the total MID cycle time on the chromatographic time scale should allow for the acquisition of 8 to 10 data points over a chromatographic peak. This requirement also complies with the EPA 1613 method for dioxins. The cycle time has a direct influence on the available measurement time for each ion and the dwell time. If the MID cycle time is too short, the sensitivity of the instrument is compromized, too high values lead to a poor GC peak definition.

Dwell Times

The MID descriptor offers a convenient way to adjust the dwell times for targets and standards. Using the total cycle time for a given MID window, the dwell times for the selected masses in the displayed MID window are automatically calculated by the data system. MID windows may use different total cycle times for optimum layout.

The individual dwell times for each target mass, as well as the lock and calibration masses, are automatically set to expected intensities, which are controlled by using the intensity value (column “Int.”). This higher level parameter allows for the relative adjustment of the measurement time in each of the MID cycles.

Example: Typical dwell times for analyte target ions used in the dioxin/furan MID setup are set with intensity = 1 to > 100 ms for the native compounds and with intensity = 5 up to 50 ms for the internal standards. The lock and calibration masses of the reference compound are typically adjusted by intensity = 40 to less than 5 ms dwell time due to their higher intensity.

When finished, all MID windows and MID descriptors are saved for use in the sample analysis sequence.

Documentation of MID Settings

All Xcalibur™ data files contain the measurement conditions used during data acquisition for the GC, autosampler and mass spectrometer. For each MID window, the measured resolution of the lock mass is provided for continued quality control.

Also, additional instrument status information is provided for quality control purposes.

References

[1] Perfluorotributylamine (PFTBA, FC43) Reference Table, Thermo Fisher Scientific Data Sheet PS30040_E.

[2] Polychlorinated Dibenzodioxins and -furans, Thermo Fisher Scientific Data Sheet PS30042_E.

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AN30116_E 12/11G