# Bulk Substance Analysis Using DART Source Coupled to LTQ XL Ion Trap and Q Exactive Focus Mass Spectrometers

Marta Kozak, Mari Prieto Conaway, Kristine Van Natta
Thermo Fisher Scientific, San Jose, CA

Application Note 626

## Key Words
DART, bulk substance, forensic, Ion Trap, Orbitrap

## Goal
To describe an analytical workflow for bulk substance analysis using a DART® (Direct Analysis in Real Time) ion source coupled to a Thermo Scientific™ LTQ XL™ ion trap mass spectrometer and a Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap mass spectrometer.

## Introduction
Forensic laboratories perform bulk substance identification to support investigations in cases involving possession, trafficking, and manufacturing of illicit drugs. A variety of analytical techniques can be used, but mass spectrometry provides methodologies that produce rapid and confident results.

We evaluated two analytical workflows using a DART® (Direct Analysis in Real Time) ion source (IonSense, Saugus, USA) coupled to a LTQ XL ion trap mass spectrometer and a Q Exactive Focus hybrid quadrupole-Orbitrap mass spectrometer (Figures 1 and 2). Pharmaceutical pills were analyzed to demonstrate method performance.


Figure 1. DART source coupled to LTQ ion trap mass spectrometer.


Figure 2. DART source coupled to Q Exactive Focus mass spectrometer.

## Experimental
### Sample Preparation
Pills were ground and mixed well. A small amount of powder was deposited on a QuickStrip™ sample card. Figure 3 shows the DART QuickStrip direct injection source, which allows automated analysis of 18 samples.

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Figure 3. DART QuickStrip direct injection source.

### DART Method

DART source settings (run temperature and run speed) were optimized using an automated procedure that comes with the DART software (Figure 4).


Figure 4. DART source settings.

### Mass Spectrometry Method

#### LTQ XL method

The LTQ XL ion trap method consisted of data-dependent experiments collecting MS2 and MS3 spectra for targeted compounds specified in the method inclusion list in both positive and negative ionization modes. The method had ten scan events: seven in positive ionization mode and three in negative ionization mode (Figure 5). There were more positive ionization scan events because there are more positively ionized compounds. The MS2 and MS3 spectra were collected for the three most abundant target compounds in positive ionization mode and for the single most abundant compound in negative ionization mode. Data were acquired for one minute as each sample moved through the DART source.

#### Q Exactive Focus method

The Q Exactive Focus MS method utilized a data-dependent experiment collecting MS2 spectra for targeted compounds specified in the method inclusion list in both positive and negative ionization modes. MS2 spectra were collected for two of the most abundant target compounds in both positive and negative ionization modes (Figure 6). Full-scan data were collected with a resolution of 70K (FWMH), and MS2 spectra were collected with resolution of 17.5K (FWMH). Data were acquired for one minute as each sample moved through the DART source.

### Data Processing

#### LTQ XL data processing

Data were processed with Thermo Scientific™ ToxID™ software. Compounds were identified and confirmed with precursor *m/z*, MS2 and MS3 spectra. ToxID software used the most abundant scan spectra for the library search. NIST software was used to store and search MS2 and MS3 spectral libraries.

#### Q Exactive Focus data processing

Data were processed using Thermo Scientific™ ToxFinder™ software. Compounds were identified based on accurate *m/z*, isotopic pattern, and high-resolution MS2 spectra. NIST software was used to store and search the MS2 spectral library.

## Results and Discussion

### LTQ XL Results

Data collected for a pharmaceutical pill in which diazepam was identified are presented in Figure 7. The data show chronograms collected for the TIC, triggered MS2 and MS3 scans, as well as the corresponding spectra from the MS2 and MS3 scans.

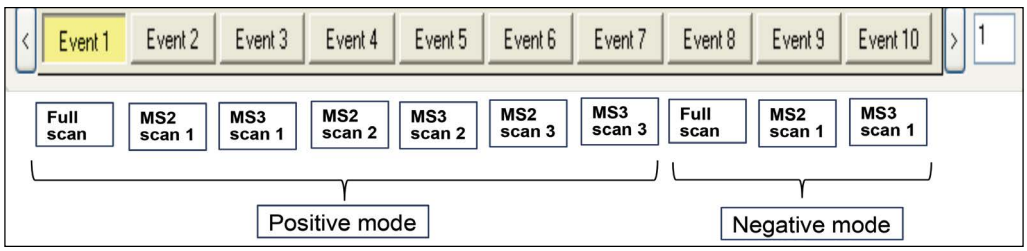



Figure 5. LTQ method scan events: A positive ion full-scan event is followed by MS2 (and associated MS3) scan events for the three most abundant compounds from a targeted list. This is repeated in negative ionization mode with the single most abundant targeted compound.

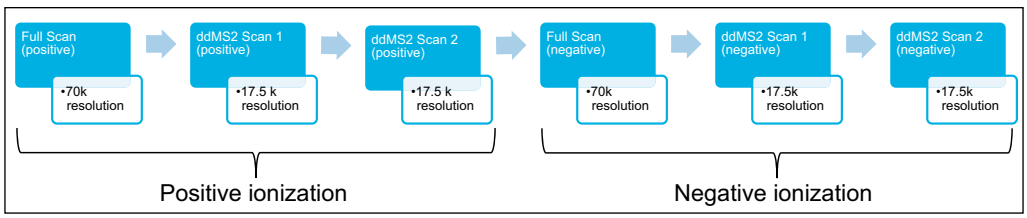



Figure 6. Q Exactive Focus MS method scan events: After a full-scan event in positive ionization mode, two ddMS2 scan events are triggered for the two most abundant masses from a targeted inclusion list. This is then repeated in negative ionization mode.

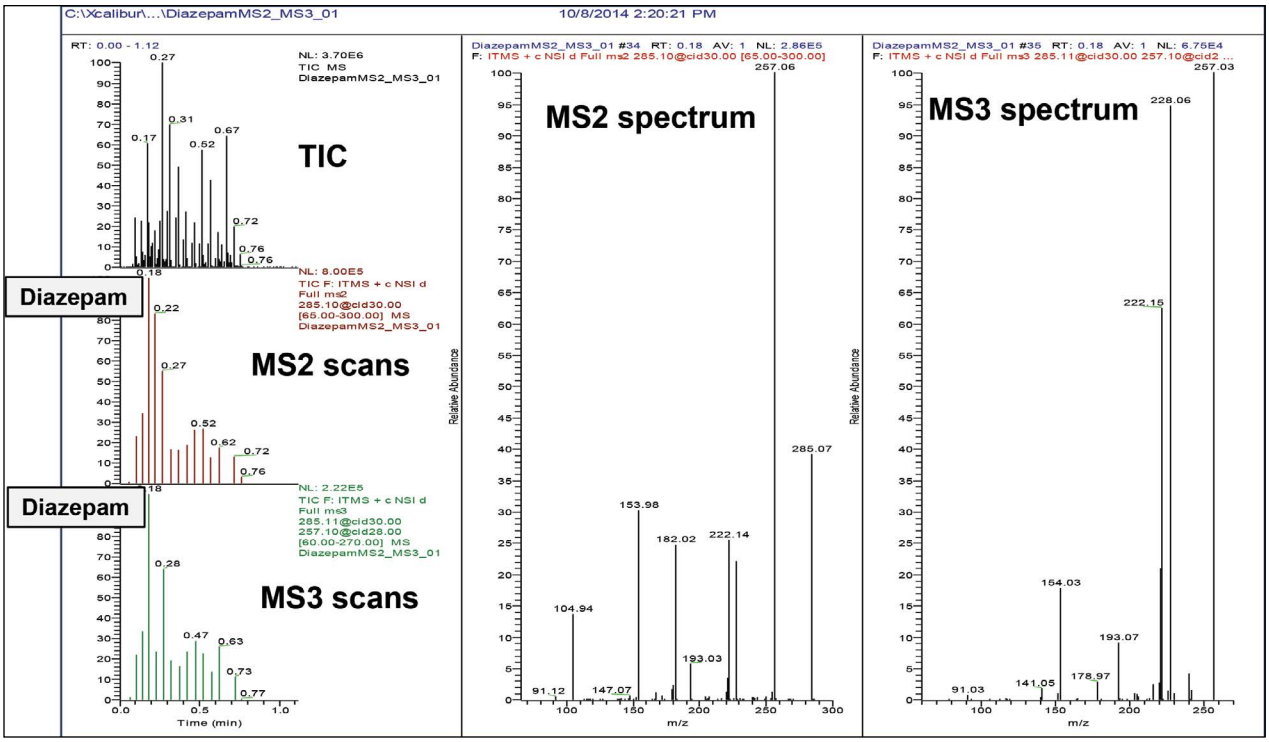


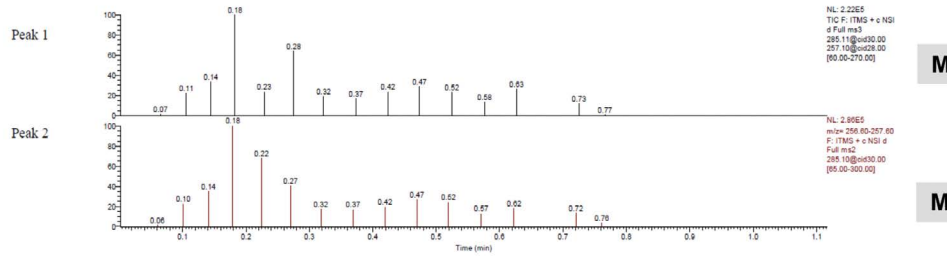

Figure 7. Data collected for diazepam in a pharmaceutical pill. The first column shows chronograms collected for the TIC, and triggered MS2 and MS3 scans for diazepam. The next two columns show representative MS2 and MS3 spectra collected for diazepam.

Figure 8 presents results of ToxID software automated compound identification in the summary report. Diazepam in the pill was identified by MS2 and MS3 spectra with search index (SI) and reverse search indexes (RSI) above 800 on scale of 1000. Collection of MS3 spectra allows for identification of compounds that may not produce specific MS2 spectra due to the presence of interfering compounds with the same nominal masses.

## Summary Report

Raw File Name: C:\Xcalibur\data\DART\PowderSamples\DiazepamMS2_MS3_01.raw
Config File Name: C:\Xcalibur\data\DART\PowderSamples\Meth...\ConfigFile_MS2_MS3_Test.csv
Sample Name: Laboratory:
Acquistion Start Time: October 08,2014 2:20:21 PM

Screening Conditions: Based on MS2 and MS3 scans. m/z window (amu): 0.50, RT window (min): 0.50, MS2 Search libraries: Tox_Library_LXQ,MS3 Search Libraries: tox_library_lxq_MS3,Do not use full MS scan to confirm




| Peak Number | Compound Name | Code | MS Order | SI | RSI | m/z | Expected RT | Actual RT | Intensity | Library Name |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Diazepam | P | 3 | 911 | 912 | 285.10 | 0.30 | 0.18 | 221526 | tox_library_lxq_MS3 |
| 2 | *Diazepam | P | 2 | 818 | 818 | 285.10 | 0.30 | 0.18 | 286020 | Tox_Library_LXQ |

Figure 8. ToxID summary report for analysis of diazepam.

## Q Exactive Focus Results

Figure 9 presents the data collected for a pharmaceutical pill in which acetaminophen (positively and negatively ionized) and codeine (positively ionized) were identified. It shows a chronogram of TIC, chronograms of targeted compounds reconstructed from full scan data with *m/z* accuracy of 5 ppm, chronograms of data-dependent MS2 scans, and resulting MS2 spectra.

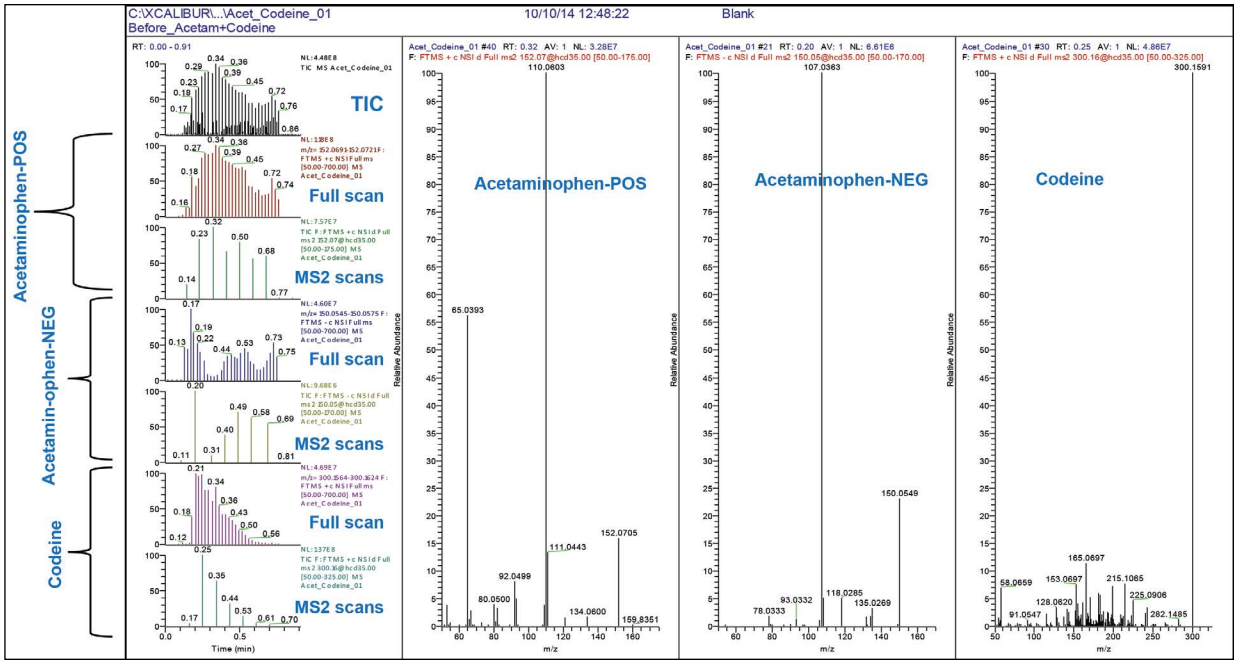



Figure 9. Data collected for acetaminophen and codeine in a pharmaceutical pill. The first column shows chronograms of TIC, full scan of targeted compound reconstructed with 5 ppm mass accuracy, and the data-dependent MS2 scans. The remaining columns show resulting MS2 spectra.

This technique does not distinguish between compounds with the same exact masses, e.g. codeine and hydrocodone. However, library search results strongly favored codeine over hydrocodone with a probability of 98%, and thus codeine was confirmed.

Figure 10 presents results for acetaminophen identification obtained with ToxFinder software.

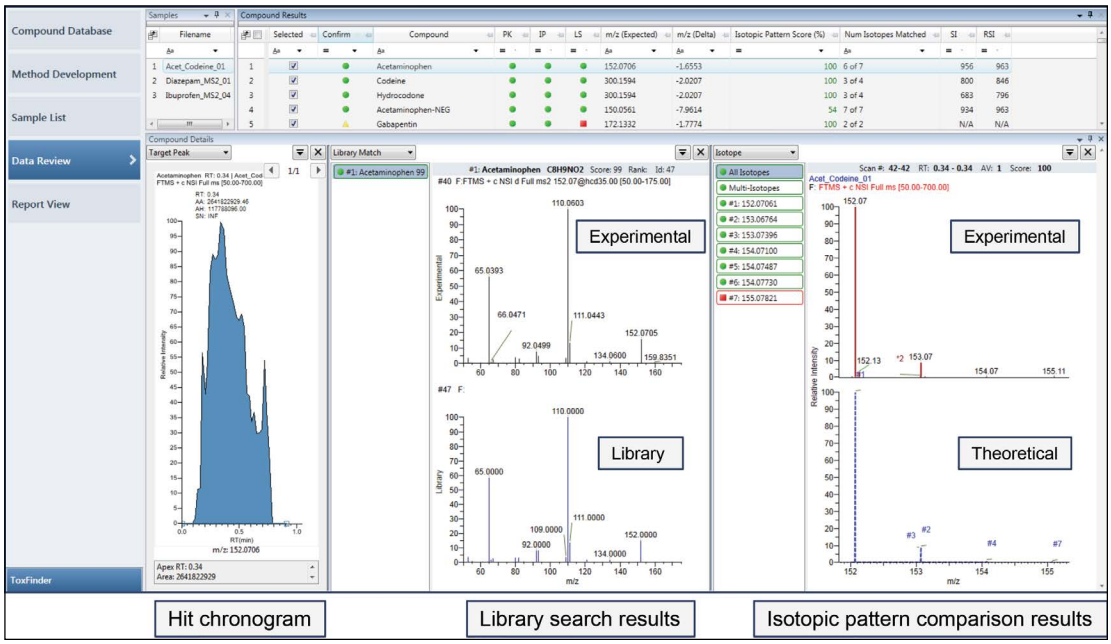



Figure 10. ToxFinder software, Data Review page.

## Conclusion

The DART source coupled to the LTQ ion trap mass spectrometer provides an analytical workflow for bulk substance analysis characterized by:

- Rapid results
- Confident identification
- High specificity with MS3 spectra
- Easy to use system
- Affordable platform

The DART source coupled to the Q Exactive Focus mass spectrometer provides an analytical workflow for bulk substance analysis characterized by:

- Rapid results
- Confident identification
- High specificity with ultra-high-resolution data
- Retrospective data analysis
- Unknown identification
- Easy to use system
- Affordable Orbitrap mass spectrometer

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