Variable Data-Independent Acquisition (vDIA) Delivers High Selectivity and Sensitivity in Combined Targeted and Untargeted Analyses for Small Molecules

Olaf Scheibner[1], Markus Kellmann[1], Charles Yang[2], Maciej Bromirski[1],
[1]Thermo Fisher Scientific, Bremen, Germany; [2]Thermo Fisher Scientific, San Jose, CA, USA

Technical Note 64283

Key Words
Q Exactive Focus, Orbitrap, veterinary drugs, small molecule HRAM quantitation, small molecule HRAM screening, UHPLC, vDIA

Goal
Forty-four multi-class veterinary drugs of known concentrations were employed to demonstrate how the use of a generic variable data-independent acquisition (vDIA) method with wide MS/MS precursor isolation windows achieves sensitivity and selectivity comparable to data-dependent MS^2 acquisition (using narrow isolation windows) in quantitative and qualitative small molecule applications. In addition, a full record of MS and MS/MS data for the measured sample fit for non-targeted and unknown screening purposes is delivered.

Introduction
Untargeted screening approaches need data acquisition methods that gather as much MS and MS/MS information from a sample as possible, regardless of the nature of the sample or the primary analytic purpose. To date, combinations of full-scan measurements and wide-range fragmentation techniques like all-ion fragmentation (AIF) are commonly used methods in this approach. These scan modes fragment all ions in a single fragmentation event without precursor ion isolation and detect all fragment ions in a single mixed spectrum. As a result, they suffer from limitations in sensitivity, selectivity, and dynamic range compared to data-dependent acquisition methods where detected precursors are isolated with narrow isolation windows prior to fragmentation and detection.

In this technical note, a new high-resolution, accurate-mass (HRAM) scan mode, termed variable data-independent acquisition (vDIA), which is available on Thermo Scientific™ Orbitrap™-based instrumentation for screening and quantitation of known and unknown samples, is described and compared to standard data-dependent acquisition (DDA) methods. vDIA may utilize up to eight isolation windows ranging from 50 to 800 Da, covering the entire mass range of the full preceding scan. Typically, smaller windows are used for lower mass regions to increase dynamic range and therefore sensitivity; larger windows cover higher mass regions to improve the duty cycle. In a typical vDIA acquisition setup, five isolation windows are set, covering the entire mass range of the preceding full scan. Figure 1 diagrams a representative vDIA method setup. Figure 1B shows an alternative setup, covering a wider mass range. Up to eight isolation windows can be used to span the full-scan mass range. However, earlier studies indicate that five fragmentation windows represent an optimal compromise between sensitivity and selectivity on the one hand and scan speed on the other.[1]

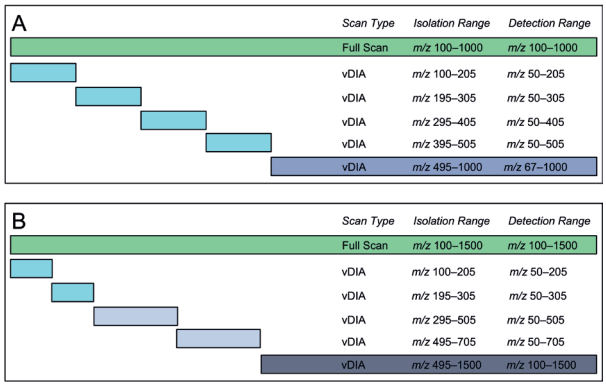

Figure 1. Examples for vDIA setup with five fragment scan windows, covering a mass range from *m/z* 100 to 1000 (A) and from *m/z* 100 to 1500 (B).

Experimental
Material
Forty-four multiclass veterinary drug residues, listed in Table 1, were analyzed using a single standardized chromatographic and mass spectrometric method in extracts of muscle, kidney, milk, and plasma. For absolute quantification, standard samples with known concentrations of all 44 veterinary drug residues covering eight calibration points (from 100 pg/mL (ppt) to 500 ng/mL (ppb)) were prepared. For evaluation of the method, spiked matrix samples (muscle and kidney for antibiotics, milk for avermectins, and plasma for nitroimidazoles) were analyzed by HRAM LC-MS/MS.

vDIA method is not available in the United States of America.

Thermo SCIENTIFIC

Table 1. Components used for evaluation of the vDIA approach.

Compound	Compound
Abamectin*	Marbofloxacine
Amoxicillin	Metronidazole
Ampicillin	Metronidazole-OH
Cefalexin	Moxidectin
Cefalonium	Nafcillin
Cefaperazone	Oxacillin
Cefapirim	Penicillin G
Cefquinome	Penicillin V
Chlorotetracycline	Ronidazol
Ciprofloxacin	Sarafloxacine
Cloxacillin	Sulfadiazine
Danofloxacin	Sulfadimethoxin
Dapsone	Sulfadimidin / Sulfamethazine
Difloxacin	Sulfadoxin
Dimetridazol	Sulfamerazin
Doramectin*	Sulfamethoxazole
Doxycyclin	Sulfamethoxypyridazine
Enrofloxacin	Sulfathiazole
Eprinomectin	Tetracycline
Erythromycine	Thiamphenicol
Flumequine	Trimethoprim
Ipronidazol-OH	Tylosine

Liquid Chromatography Method

A generic LC method was run on a Thermo Scientific™ UltiMate™ 3000 XRS Quaternary Rapid Separation LC (RSLC) and used for all samples:

Column	Thermo Scientific™ Accucore™ C18 aQ 100 x 2.1 mm, 2.6 µm particle size (p/n 17326-102130)
Mobile phase A	Water + 0.1% formic acid
Mobile phase B	Acetonitrile + 0.1% formic acid
Gradient	6 min gradient from 5% B to 95% B
Flow rate	300 µL/min
Total chromatographic cycle	15 min

vDIA method is not available in the United States of America.

Mass Spectrometry Method

A generic full scan with wide-isolation variable data-independent acquisition (FS-vDIA) MS/MS method on a Thermo Scientific™ Q Exactive™ Focus MS system was used for all samples:

Full Scan	
Resolution setting	70,000 (FWHM) at *m/z* 200
Mass range (*m/z*)	100-1000
vDIA	
Resolution setting	17,500 (FWHM) at *m/z* 200
Isolation windows (*m/z*)	100–205, 195–305, 295–405, 395–505, 495–1000
Spray voltage	4.4 kV
Sheath gas	30.0 arb.
Aux gas	5.0 arb.
Capillary temp.	250 °C
Heater temp.	300 °C
RF-lens level	50
HCD collision energy	35 eV

Data Processing

Data processing was performed using Thermo Scientific™ TraceFinder™ processing software version 3.2. For generation of extracted ion chromatograms, an extraction window of 5 ppm was used. For non-targeted screening, a built-in component and fragment *m/z* values database was used together with a spectral library, consisting of 1500 components each.

Results and Discussion

In the vDIA approach, a full scan is followed by two to eight wide-isolation fragmentation scans, which together cover the same isolation range as the preceding full scan. Figure 1 shows two examples of a vDIA method with five fragmentation scans. For each the applied isolation and scan ranges are shown. Example A covers a mass range from *m/z* 100 to 1000 and was used in this study. Example B shows an alternative setup, covering a scan range from *m/z* 100–1500 using the same number of scan windows. Earlier work has shown that the selectivity of the method increases as the size of the isolation ranges for the fragment scans is decreased, resulting in a higher number of scan ranges for the same mass range covered.[1] Since the overall cycle time for this setup increases with the number of fragmentation scan ranges, the same work showed that a setup with five fragmentation windows maintains enough scan speed to be suitable for fast chromatography. The setup used in this study resulted in an overall cycle time of 650 ms.

The vDIA approach described above bridges the gap between full scan-data-dependent MS^2 (FS-ddMS^2) experiments and full-range fragmentation scan modes such as AIF. FS-ddMS^2 experiments, where MS^2 scans are performed on targets of interest (present on an inclusion list) upon their detection in the full scan, are known to be very selective and sensitive with respect to the fragment ion information obtained. Retrospective FS-ddMS^2 data analysis for additional compounds of interest, however, is limited to full-scan quantitation by accurate mass without confirmation of identity by MS/MS. To maintain scan

speed, this method has to work with dynamic exclusion, so only one survey MS² scan is triggered per compound. As a result, no elution profiles can be extracted for the confirming fragment ions.

Full-range fragmentation experiments like AIF, where fragments from all species present in the full scan are detected in a single MS² scan, have the advantage of collecting all possible full scan and MS/MS information for the sample. Thus, they are fully suitable for retrospective data analysis. However, dynamic range, selectivity, and achieved detection limits are limited as the number of ions fragmented per species is lower due to the combined nature of the analyses.

The vDIA experiment combines the advantages of both approaches by using smaller isolation windows for the fragmentation scans while still fragmenting all precursors from the preceding full scan. With this, a complete record of MS and MS/MS data is kept, so all compounds of interest can be processed even in retrospective manner and full elution profiles of all confirming ions for all components can be extracted.

To evaluate the power of the new vDIA approach, animal product samples (pig muscle, pig plasma, and cow milk), spiked with 44 veterinary drugs (Table 1), were measured with the FS-vDIA method described. The spike levels were 5 µg/kg for antibiotics in pig muscle matrix, 1 µg/kg for nitroimidazoles in pig plasma matrix, and 1 µg/kg for avermectins in cow milk matrix. As shown in Figure 2, the FS-vDIA method shows very good selectivity at low concentration levels, all analyte ions are confirmed with multiple fragment ions matching both with high mass accuracy as well as retention time of elution profiles. In contrast to data-dependent MS² methods, full elution profiles of all fragments are available, providing an additional option for data quality control since confirmation results can easily be checked for false positive hits on random spikes or elevated background signals.

vDIA method is not available in the United States of America.

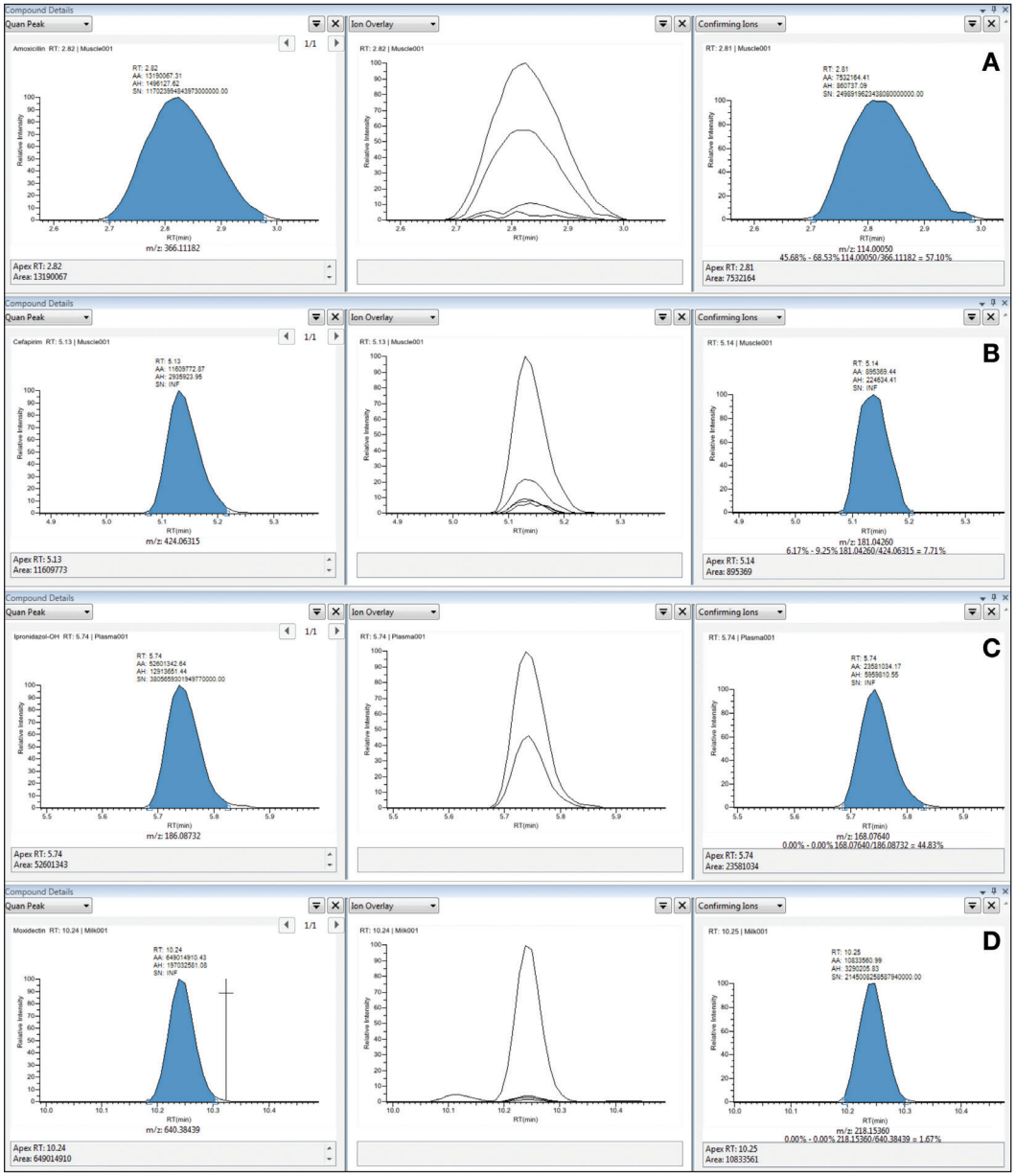

Figure 2. Selectivity of selected component in matrix; A: ampicillin in pig muscle at 5 µg/kg; B: sulfadiazin in pig kidney at 5 µg/kg; C: ronidazol in pig plasma at 1 µg/kg; D: moxidextin in cow milk at 1 µg/kg.

In addition, a dilution series of a standard mixture of 44 veterinary drugs was analyzed to evaluate the linearity of calibration. The range of concentration levels was set from 0.01 µg/kg to 500 µg/kg. Linear calibration curves were obtained over the entire calibration range for all 44 compounds. Figure 3 shows the calibration curves of ampicillin, cefalonium, dimetridazol, and metronidazole as examples using the vDIA method.

Analytical samples are often used for both known screening as well as unknown screening purposes. To conduct a wide-range unknown screening, the data was processed against a 1500 component accurate-mass database and spectral library contained fully within TraceFinder software. In addition to the known spiked components, 30 components could be identified and confirmed by means of accurate mass, isotope pattern match, fragment ion match, and library search identification. As an example, cortisol (hydrocortisone) was identified with all four methods of identification and confirmation. Figure 4 shows the screening result in TraceFinder software for a plasma sample.

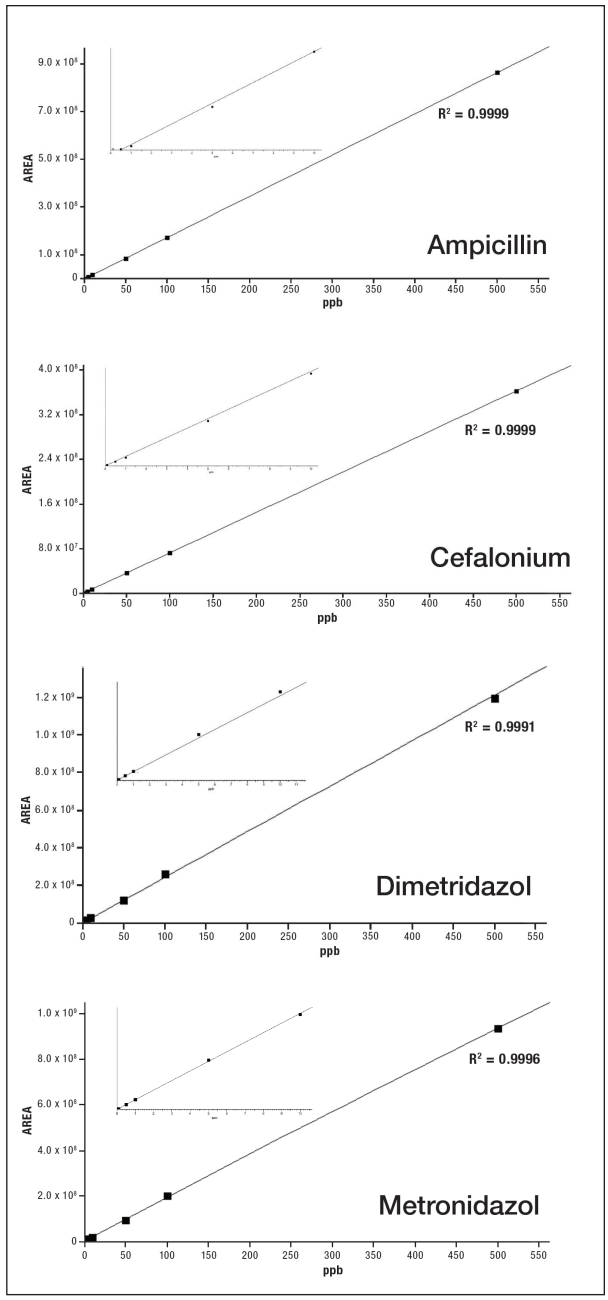

Figure 3. Selected examples of linearity for different compounds in the calibration range of 0.01–500 µg/kg.

vDIA method is not available in the United States of America.

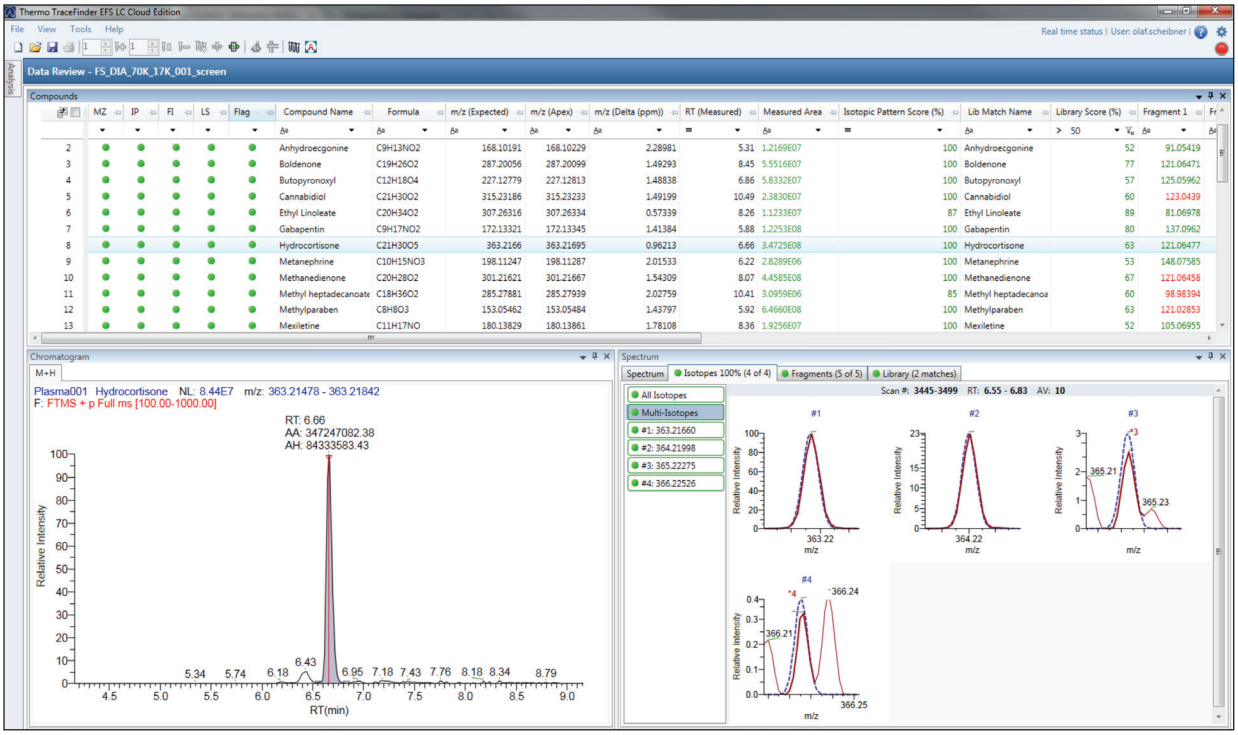

Figure 4. Identification of cortisol (hydrocortisone) in a pig meat sample with all four stages of confirmation.

Conclusion

The vDIA scan mode on the Q Exactive Focus MS serves as a generic acquisition method for diverse analytes, delivering a complete data record of the measured samples. It is perfectly suited to acquiring data for quantitative purposes while additionally offering uncompromised performance for non-targeted and unknown screening applications. Overcoming the limitations of full-range fragmentation techniques, vDIA provides selectivity and sensitivity comparable to data-dependent MS^2 measurements while generating a complete record of full scan and fragmentation data for each of the measured samples.

References

1. Vogler, B., Master's Thesis, Institute of Organic Chemistry, University of Zürich, Zürich, Switzerland, 2013, 76.

vDIA method is not available in the United States of America.