Evaluation of Variable Data Independent Acquisition (vDIA) Approach for Non-target Screening of Veterinary Drugs in Animal Feed

Zhe Zhou, Zheng Jiang; Thermo Fisher Scientific, Shanghai, China

ABSTRACT

Purpose: To evaluate the effect of variable Data Independent Acquisition (vDIA) for non-target screening over the classical data dependent MS^2 ($ddMS^2$) and All Ion Fragmentation (AIF).

Methods: Animal feed samples spiked with veterinary drugs were analyzed with different scan modes for comparison.

Results: The comprehensive information acquired by vDIA mode generated more efficient screening and confirmation results while the result of targeted quantitation was also gained at the same time, which suggested good performance for both qualitative and quantitative analysis with vDIA mode.

INTRODUCTION

Due to the high specificity, sensitivity, and accuracy, the wide use of high resolution accurate mass (HRAM) mass spectrometers in residue analysis leads to numerous new options. Full scan with data dependent MS^2 ($ddMS^2$) acquisition has proved to be a practical way for target and non-target screening and confirmation. However, MS^2 triggering relies on the intensity of parent ions and thus might cause the possibility of triggering missing for low concentrate compounds or co-elution peaks. AIF is another strategy for non-target screening with all co-eluted precursor ions to be fragmented, but the selectivity will be sacrificed due to no selection of parent ions. Herein vDIA on Thermo Scientific™ Q Exactive™ Focus Hybrid Quadrupole-Orbitrap mass spectrometer was tested using 150 veterinary drugs spiked in animal feed matrix samples. By applying vDIA, the full scan mass range is divided into several narrower ranges which enhance the dynamic range for the fragment scans and also result in higher selectivity and sensitivity of the characteristic fragments needed for compound confirmation. At the same time, all options for suspect screening or even unknown screening remain fully available as in AIF.

MATERIALS AND METHODS

Sample Preparation

150 veterinary drug standards (50 ppb for each single compound) were mixed and diluted with methanol-extracted blank matrix of mixed piglet feed into 50 ppt, 500 ppt, 5 ppb, and 25 ppb.

Liquid Chromatography

Thermo Scientific™ UltiMate™ 3000 Binary RSLC system was used as HPLC system. Thermo Scientific™ Accucore™ C8 (100 × 2.1 mm, 2.6 µm) column was used for separation. Mobile phases: 0.01% formic acid in water (A) and 0.01% formic acid in acetonitrile (B), gradient elution was processed as Table 1.

TABLE 1. Gradient elution program.

Time (min)	A	B
0	95	5
15	5	95
17	5	95
18	95	5
20	95	5

Mass Spectrometry

Q Exactive Focus quadrupole-Orbitrap mass spectrometer (Figure1).

Ionization Condition: Electrospray (ESI) mode, heat temperature 350° C; capillary temperature 325° C; spray voltage 3.2 kV (Positive) and 2.8 kV (Negative); sheath gas flow 40 arb; auxiliary gas flow 10 arb, collision energy 20, 30, 40.

$ddMS^2$ Mode: Full scan at the resolution of 70,000 FWHM and the scan range of 100–1000 *m/z*, while inclusion-list-based data dependent MS^2 at the resolution of 17,500 FWHM. Isolation window 1.5amu. Top1. Dynamic exclusion 8s.

AIF Mode: Full scan at the resolution of 70,000 FWHM and the scan range of 100–1000 *m/z*, while AIF MS^2 at the resolution of 17,500 FWHM and the scan range of 100–1000.

vDIA Mode: Full scan at the resolution of 70,000 FWHM while data independent MS^2 at the resolution of 17,500. Isolation windows and scan ranges were set according to Figure 2.

FIGURE 1. Q Exactive Focus mass spectrometer with Ultimate 3000 LC.

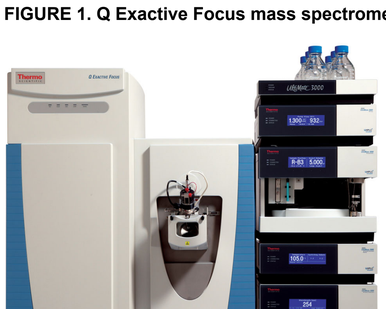
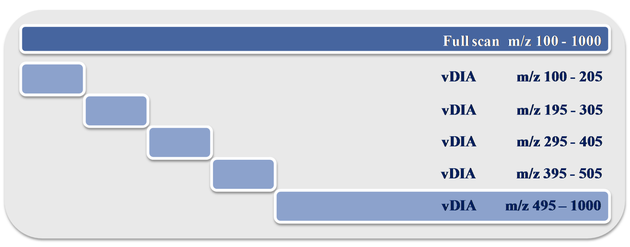


FIGURE 2. Isolation windows in vDIA mode.



Data Analysis

Data was processed by automatic processing using Thermo Scientific™ TraceFinder™ software which comprises several basic confirmatory criteria: accurate mass of parent ion in full scan, isotope pattern of parent ion in full scan, compound-specific fragment ions, retention time, and so on.

RESULTS

Evaluation of Separation Conditions

With regard to the large number of target compounds, careful attention should be paid to the evaluation of the separation system. 150 veterinary drugs were separated evenly under the optimized LC condition, but there was still a large number of overlapping chromatographic peaks that can be observed from 1 min to 11 min (Figure 3).

FIGURE 3. Scattergram demonstrating elution of target analytes within the chromatographic run.

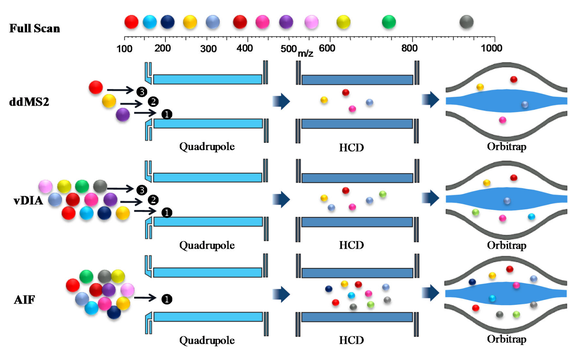
Comparison of Three MS Scan Modes

The full scan–$ddMS^2$ method with the inclusion list of target analytes has been proved to be a practical way for target and non-target screening and confirmation. This scan mode comprised the full scan of parent ions followed by successive isolation of ions present on the list of targeted compounds.

The full scan-AIF is another strategy for non-target screening, all co-eluted precursor ions will be passed through quadrupole and be fragmented. The selectivity will be sacrificed due to no selection of parent ions and the sensitivity will be restricted due to the lower ratio of target product ions in detector.

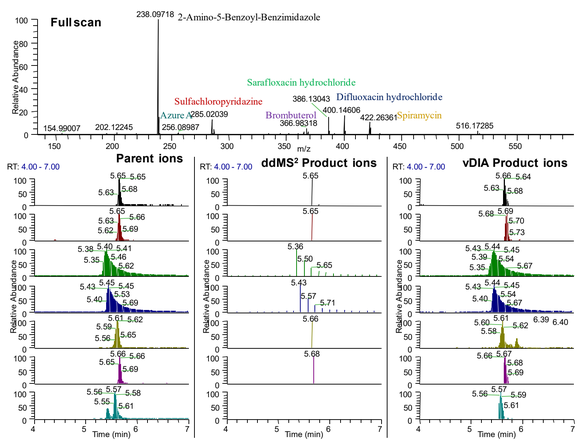
The full scan-vDIA is a new scan mode on the Q Exactive Focus mass spectrometer, the full scan mass range is divided into variable smaller ranges which enhances the dynamic range for the fragment scans, resulting in higher selectivity and sensitivity of the significant fragments needed for compound confirmation. Figure 4 shows a schematic of $ddMS^2$, vDIA, and AIF scan modes.

FIGURE 4. Schematic of ddMS2, vDIA and AIF scan mode.



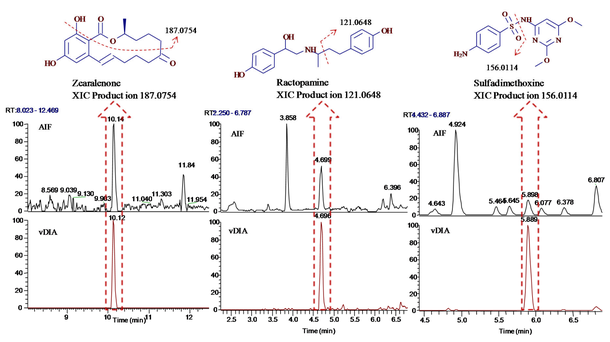
Since the full scan–$ddMS^2$ method triggers MS/MS scan according to the intensity of parent ions in pre-full scan, the lower intensity compounds will be interfered by the higher ones and thus causes the possibility of triggering missing among co-elution peaks. There were at least seven compounds eluted at 5.6 min and as a result of intensive co-elution, Azure A was not triggered by the $ddMS^2$ method. Instead, all the product ions of seven compounds were acquired by the vDIA method and the selectivity of product ions is even slightly better than parent ions such as Azure A. Figure 5 shows a comparison of product ions acquired by $ddMS^2$ and vDIA in the case of co-elution peaks.

FIGURE 5. Comparison of product ions acquired by ddMS2 and vDIA in the case of co-elution peaks.



Compared with AIF, the vDIA method narrows isolation windows and thus reduces much of the background interference caused by no selection of parent ions. Figure 6 shows the XICs of product ions for Zearalenone, Ractopamine, and Sulfadimethoxine as an example. The background noise and interference peaks were significantly visible in AIF XICs which became much cleaner in vDIA XICs.

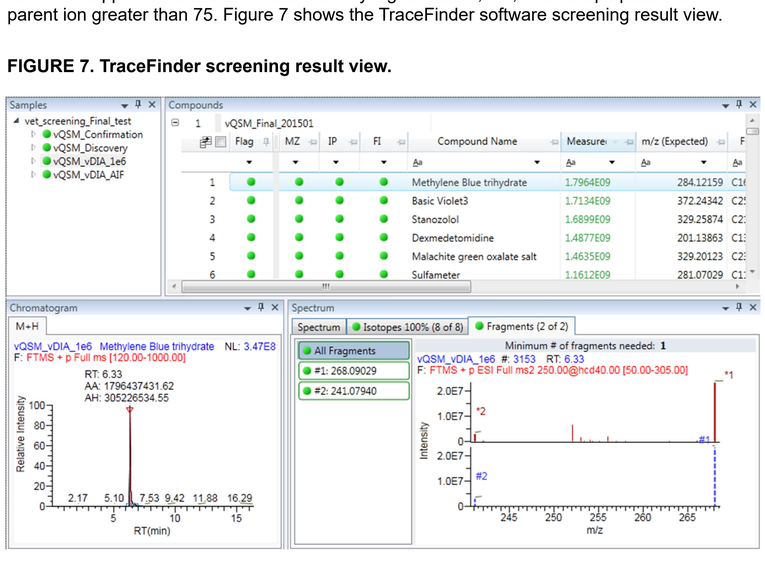
FIGURE 6. Background interference exist in AIF filtered out by vDIA.



Screening Result

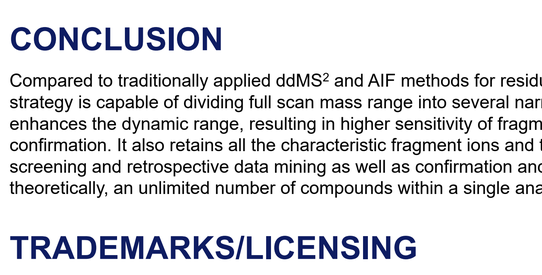
vDIA data was processed by the TraceFinder software described before. The criteria for identification of target analytes and further data evaluation are peak detection of parent ion within theoretical accurate mass ±5 ppm extraction window, at least one characteristic fragment ion within 5 ppm mass tolerance and intensity higher than 5,000, and isotope pattern score of parent ion greater than 75. Figure 7 shows the TraceFinder software screening result view.

FIGURE 7. TraceFinder screening result view.



The result found that the ratio of spiked veterinary drugs confirmed by vDIA strategy can reach up to 96 percent in this case, the number of which turned out to be more than that confirmed under $ddMS^2$ with inclusion list. Also, vDIA approach showed more clean product ion spectra than AIF and allowed approximately 85 percent of compounds to be detected and confirmed below the concentration of 5 ppb, which means vDIA can achieve a more specific, sensitive, and comprehensive screening result. Figure 8 is a histogram that demonstrates the sensitivity of target analytes.

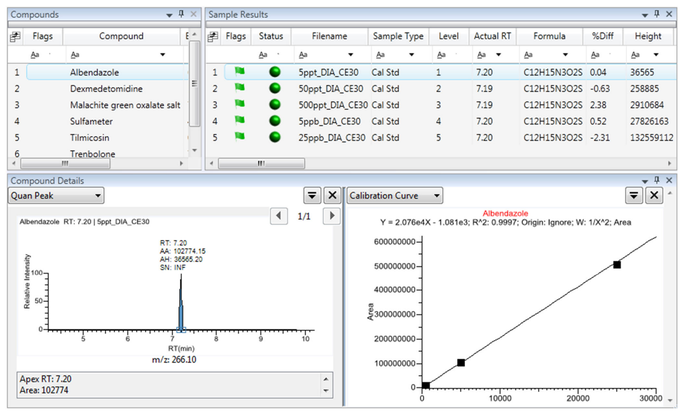
FIGURE 8. Histogram demonstrating the sensitivity of target analytes.



Quantitative Result

Due to the division of full scan range and HRAM MS^2 data, vDIA mode could achieve a highly sensitive and wide linear range quantitative result as demonstrated in Figure 9, and at the same time, all options for quantitation remain available in this strategy.

FIGURE 9. TraceFinder quantitative result view.



CONCLUSION

Compared to traditionally applied $ddMS^2$ and AIF methods for residue screening, the vDIA strategy is capable of dividing full scan mass range into several narrower ranges which enhances the dynamic range, resulting in higher sensitivity of fragment ions needed for confirmation. It also retains all the characteristic fragment ions and thus allows for non-target screening and retrospective data mining as well as confirmation and quantitation of, theoretically, an unlimited number of compounds within a single analytical run.

TRADEMARKS/LICENSING