Video Article

Simplified LC/MS/MS Bioanalytical Method Development with Radar Technology - ADVERTISEMENT

Robert S. Plumb[1]

[1]Pharmaceutical Business Operations, Waters Corporation

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Abstract

The development of a bioanalytical assay requires the development of a MRM method as well as a chromatography method, which requires positioning of the analyte peak of interest away from the endogenous background signal. The Xevo™ TQ-S tandem quadrupole mass spectrometer with RADAR™ Technology allows the simultaneous collection of full scan and MS/MS MRM data, vastly simplifying the method development process.

For a bioanalysis method to be effective it must be transferable, reproducible, and robust. The development of a reliable LC/MS/MS bioanalysis assay involves the thorough optimization of sample preparation, MS detection, and chromatography conditions. This can often be a time-consuming process, requiring the analyte peaks(s) be resolved from endogenous matrix components that cause ion suppression and assay irreproducibility. This often requires multiple analytical runs to acquire the necessary MRM, product ion, and full scan data.

Described here is the use of Xevo™ TQ-S Mass Spectrometer with RADAR™ Technology - a novel dual-scan data collection capability to simplify bioanalysis method development. The Xevo TQ-S employs a novel collision cell design which allows for the simultaneous collection of full scan MS and MRM data.

Video Link

The video component of this article can be found at http://www.jove.com/video/2267/

Protocol

I.Simplified LC/MS/MS Bioanalytical Method Development With RADAR Technology

1. To demonstrate simplified LC/MS/MS bioanalytical method development with RADAR technology, chromatography was performed on a Waters ACQUITY UPLC® System, using an ACQUITY UPLC Bridged Ethyl Hybrid C18 column.
   LC Conditions:
   ACQUITY UPLC BEH C18 column 2.1 x 50 mm, 1.7 µm
   Mobile Phase:
   1. 0.1% formic acid or 0.1% aqueous ammonium hydroxide
   2. Methanol or acetonitrile

   Gradient: 5-95% organic over 0-2 minutes at a flow rate of 600 µL/min.
2. The analytes were eluted at a flow rate of 600 µL/min with a 5 to 95% organic gradient over 2 minutes, where 0.1% aqueous formic acid or 0.1% aqueous ammonium hydroxide was exchanged for methanol or acetonitrile.
3. Mass spectrometry detection was performed on a Waters Xevo™ TQ-S Mass Spectrometer operated in a positive ion electrospray MRM mode, with simultaneous acquisition of full scan data.
   MS Conditions:
   Positive ion electrospray:
   MRM: 315 > 129
   Full scan MS: 50-500 m/z at 5000 amu/sec.
   Product ion scan: Precursors of m/z = 184 from 200-600
4. To illustrate the use of the RADAR technology, the common H2 receptor antagonist ranitidine hydrochloride was spiked into rat plasma, precipitated with acetonitrile (2:1), and analyzed using a UPLC/MS/MS system.

II.Representative Results Bioanalysis Using RADAR™ Technology

Chromatograms obtained using a conventional acidic aqueous buffer and acetonitrile organic modifier gradient were compared with chromatograms obtained using a basic aqueous buffer (Fig. 1). In the LC/MS/MS analysis of ranitidine hydrochloride in rat plasma, the RADAR

MRM data shows that with an acidic aqueous modifier, ranitidine is unretained, eluting with the void of the chromatographic system. However, when a basic aqueous modifier is employed, the compound is retained, eluting at 0.9 min. The full scan and parents of m/z = 184 data show that with both the acidic and basic separation, the analyte is resolved from phospholipid and other endogenous materials in the matrix.

The use of a methanol organic modifier with a basic aqueous buffer increased the analyte retention to 1.30 min. The analyte signal response was also increased by a factor of 4 (Fig. 2). However, with a simple 5-95% basic-methanol gradient, the full scan data revealed that the analyte peak co-eluted with the endogenous material in the sample. This co-elution could cause ion suppression and assay irreproducibility.

To resolve the analyte peak from the endogenous material, the gradient steepness was adjusted. The Xevo TQ-S RADAR functionality was used to quickly select the best LC conditions with the best throughput. The final chromatographic conditions were a gradient of 15-60% methanol over 2 minutes with a 95% organic wash from 2-2.5 minutes (Fig. 3). The analyte peak is well resolved from the endogenous material in the sample as seen from the full scan trace, in green. This approach can also be used during sample analysis to check for drug-related metabolites, co-administered therapies, and variations in matrix which could affect the veracity of the results.

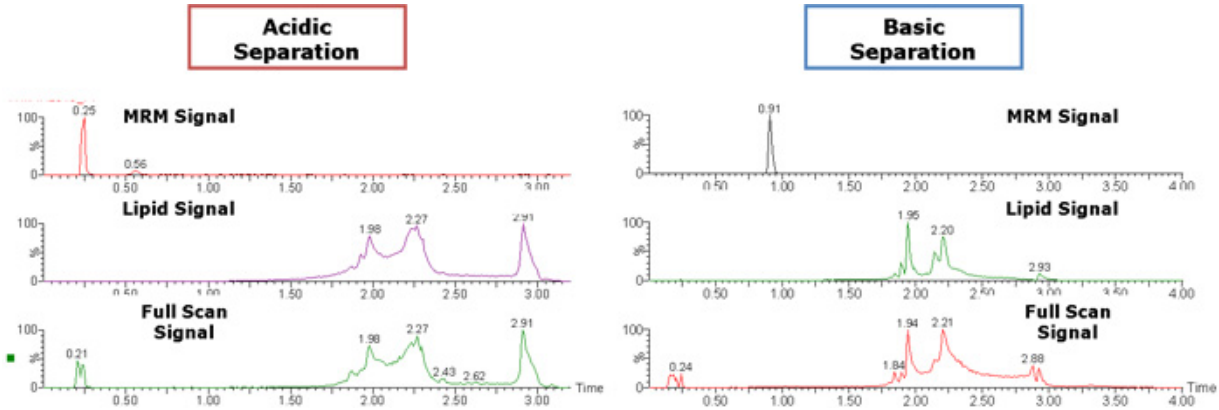

Figure 1: Chromatograms obtained using a conventional acidic aqueous buffer and acetonitrile organic modifier gradient compared with chromatograms obtained using a basic aqueous buffer. With an acidic aqueous modifier, ranitidine is unretained, eluting with the void. When a basic aqueous modifier is employed, the compound is retained, eluting at 0.9 min. The full scan and parents of m/z = 184 data show that with both the acidic and basic separation, the analyte is resolved from phospholipid and other endogenous materials in the matrix.

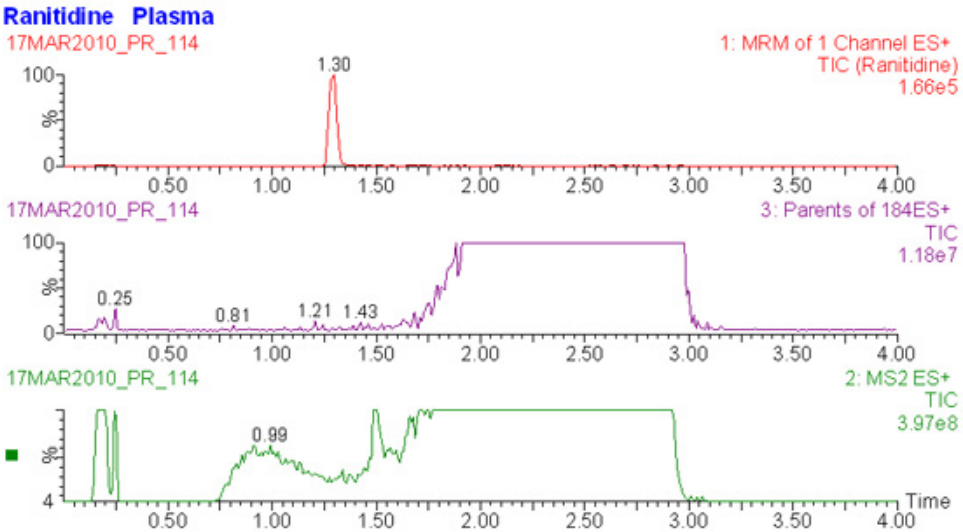

Figure 2: The use of a methanol organic modifier with a basic aqueous buffer increased the ranitidine hydrochloride retention to 1.30 min. The analyte signal response was also increased by a factor of 4. However, the full scan data reveals the analyte peak co-eluted with the endogenous material in the sample when a simple 5-95% basic-methanol gradient is employed.

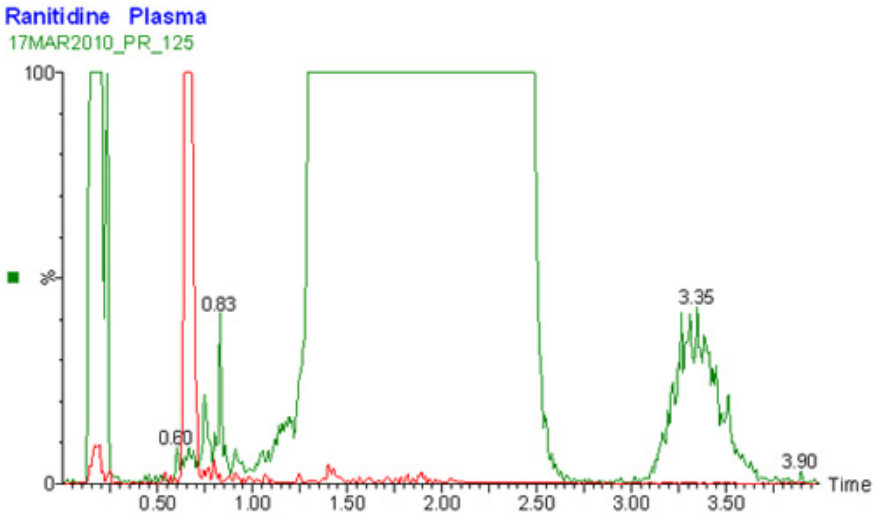

Figure 3: The final chromatographic conditions for ranitidine hydrochloride included a gradient of 15-60% methanol over 2 minutes with a 95% organic wash from 2-2.5 minutes. The analyte peak is well resolved from the endogenous material in the sample as seen from the full scan trace, in green.

Discussion

Bioanalytical method development is significantly simplified using Xevo TQ-S RADAR technology. The ability to simultaneously acquire full scan data as well as MS/MS and MRM data allows the endogenous sample matrix to be monitored at the same time as the analyte peak, facilitating:

- Faster method development
- Elimination of the need for repeat injections to obtain background signal
- Matrix monitoring during sample analysis
- Easy troubleshooting
- Detection of *in vivo* metabolites during the pre-clinical and clinical development

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