

An Advanced Tool for Method Development in Complex Matrices Using Xevo TQD with RADAR for the Analysis of Chloramphenicol in Chicken

GOAL

To successfully quantify chloramphenicol in chicken, while also evaluating matrix interferences using RADAR™ Technology.

BACKGROUND

Chloramphenicol, an effective broad spectrum antibiotic, is widely used in medicinal and veterinary practices. Its use in humans is restricted due to the potential for harmful effects. It is reportedly a cause of a potentially fatal blood condition called idiosyncratic aplastic anemia, and hypersensitivity to the drug affects around one in 30,000 thousand people, irrespective of dosage. It is also anticipated to be carcinogenic. As a consequence, chloramphenicol is not approved for use in food-producing animals. However, due to its wide availability and low cost, it is used to prevent bacterial infections in aquaculture, apiculture, and poultry farming. Chloramphenicol levels in animal products are strictly monitored. In Europe, the minimum required performance limit (MRPL) for chloramphenicol is 0.3 µg/kg in any food of animal origin, and similar limits have been adopted in other countries, including the U.S.A.

Development of analytical methods for the detection of contaminants in food is often challenging due to the complexity of the matrix.

Xevo® TQD with RADAR functionality has the capability of acquiring MRMs and full scan data in the same run, resulting in faster method development.

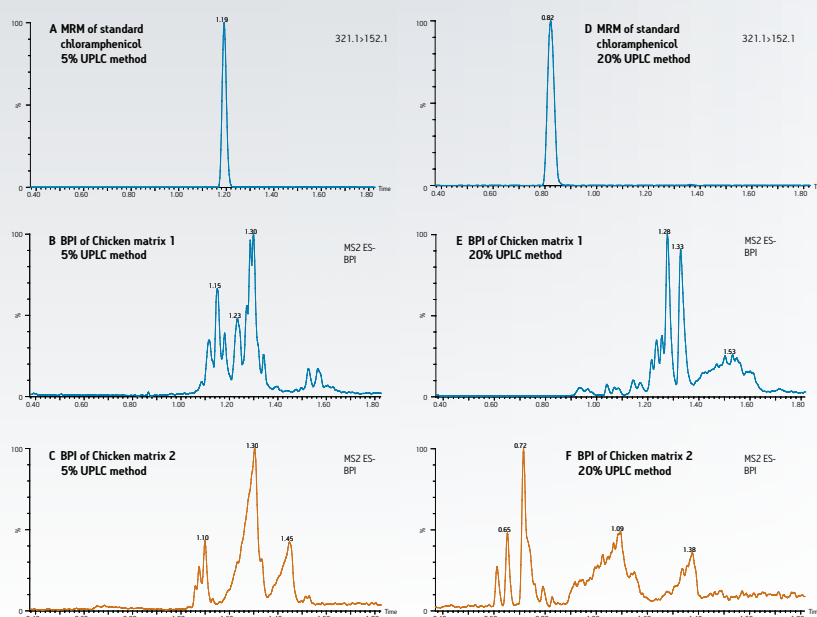


Figure 1. MRM chromatograms of chloramphenicol standard and base peak intensity (BPI) chromatograms of two different blank chicken extracts using two different UPLC methods. A-C: starting conditions using 5% organic solvent; D-F: starting conditions using 20% organic solvent.

In LC/MS/MS co-eluting matrix components may compete with the analyte of interest during the ionization process, which can lead to suppression or enhancement of the analyte signal. It is therefore necessary to characterize these potential matrix effects during method development and eliminate or minimize their impact on the quantification of the analyte. Minimizing the matrix components also helps to ensure method robustness. The ability to monitor matrix interferences by simultaneously monitoring full scan background data during quantitative MS/MS method development represents an important advance in instrument design. The Waters® Xevo TQD employing RADAR functionality provides an innovative platform to observe MRM (multiple reaction monitoring) data and full scan background data simultaneously for faster, more robust method development.

THE SOLUTION

The Xevo TQD coupled with an ACQUITY UPLC® System was used for the routine quantitative analysis of chloramphenicol at trace levels in chicken muscle. Prior to analysis, the prepared chicken muscle extract was spiked with chloramphenicol at 0.3 ppb (0.3 µg/kg). The Xevo TQD was operated in RADAR mode. This allowed for the simultaneous acquisition of MRMs and full scan data without any compromise in the MRM data quality or accuracy. During the method development process, this acquisition mode

enabled informed decisions to be made during optimization of the chromatographic separation. Figure 1 shows the chromatograms for the chloramphenicol MRM, along with the full scan MS base peak ion (BPI) chromatogram for two different chicken muscle samples. The chromatograms shown in Figure 1A-C were generated using an LC gradient method that started with 5% organic solvent. The chromatograms shown in Figure 1D-F were generated with an LC gradient method that started with 20% organic solvent. From these data, it can be seen that when starting at 5% organic solvent, chloramphenicol elutes in a region of the chromatogram that shows a high level of matrix co-elution. By modifying the method to start at 20% organic, chloramphenicol elutes earlier (0.82 min) in a region with less potential matrix interference. The acquisition of full scan background information also shows the vast difference between two different chicken samples. Both samples were prepared from organic chicken breast muscle; however, the matrix interference is very different in the two samples, as observed from the RADAR BPI chromatograms. This information can be used to make informative decisions during the method development process. When used during routine operation, it offers the ability to proactively identify when compositional changes in the matrix may potentially impact the analysis.

SUMMARY

This technology brief demonstrates the utility of the Xevo TQD for simultaneous acquisition of full scan background data with MRMs without any compromise in quantitative performance. Using the RADAR data, matrix interferences can be monitored and informed decisions made, leading to faster and more rugged method development.

The Xevo TQD is a reliable, reproducible, and accessible solution for routine quantitative and qualitative trace analysis. The use of RADAR provides information-rich analysis while providing MRM data for quantification.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

