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Quantitation of Antiviral Drugs in Chicken Samples by Ultra-High Performance Liquid Chromatography Tandem Triple Quadrupole Mass Spectrometry with Triggered MRM for Unprecedented Confirmation

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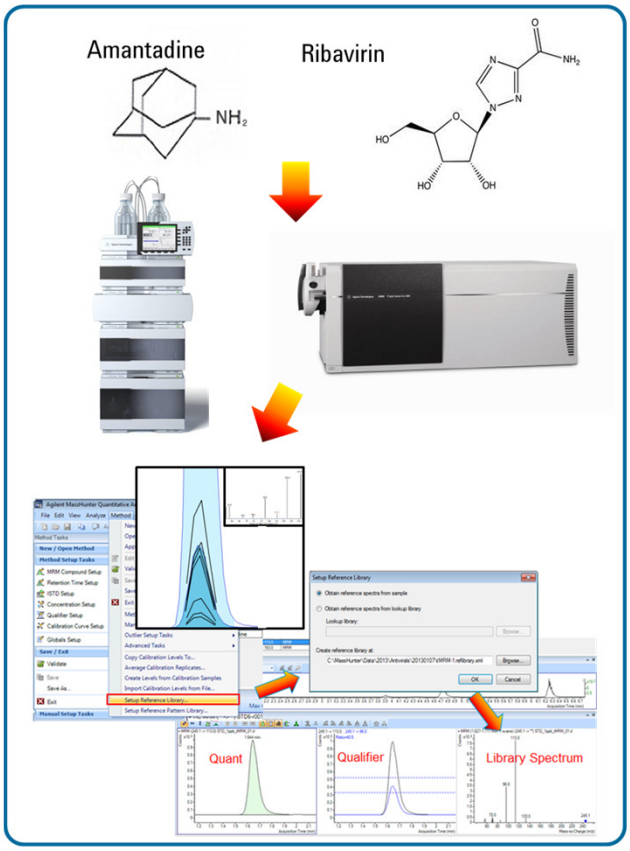
Introduction

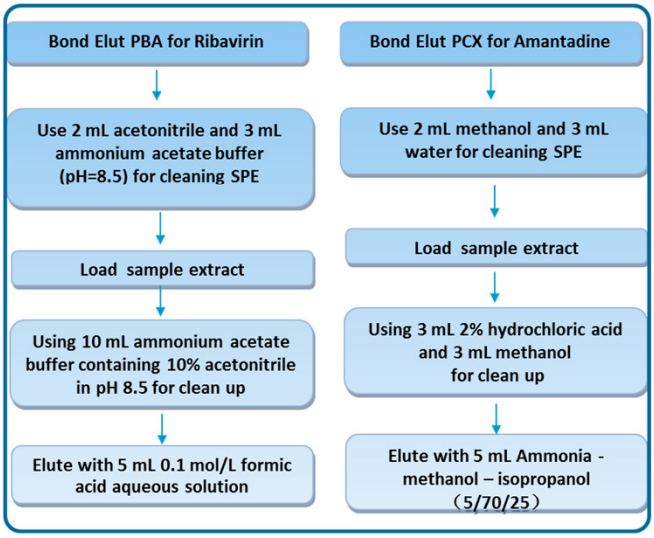
Antiviral drugs are an important class of illegal chemicals in food safety, so it is crucial to develop robust methods for analyzing antiviral drugs in complex food matrices. In this study, an ultra-sensitive and rapid approach for the determination of two essential antiviral drugs (ribavirin and amantadine) in chicken samples was developed. Due to the complexity of food matrices, false positive results of veterinary drugs in food products are a major concern when employing an LC/QQQ technique. Two MRM transitions cannot ensure reliable confirmation for most cases, especially for trace analysis in the complex food matrices. A recently developed technique, triggered MRM (tMRM) can effectively avoid false positive results by acquiring data for additional confirmatory ions and using that data for reference library matching. With the use of an optimized collision energy and dwell time for each MRM, tMRM is very sensitive and generates high quality, reproducible spectra even at trace concentrations. Our work demonstrated the novel application of the new tMRM acquisition mode for the confirmation of antiviral drugs in chicken samples, effectively eliminating the false positive results.



Experimental

Sample Extraction

Chicken samples were extracted following an SPE protocol using Agilent Bond Elut PBA (500 mg 6mL, P/N 12102105) and PCX (60 mg 3 mL, P/N 12108603). 2 g homogenized chicken samples were extracted with 5 mL mixture of acetonitrile and 1% trichloroacetic acid (7:3, v/v) twice, and the mixture was shaken and centrifuged (10000 rpm for 10 min). The extract was finally cleaned-up by dispersive SPE (PBA and PCX).



UHPLC/MS/MS Parameters

LC Conditions

Agilent UHPLC 1290 System		
Column	Zorbax SB-Aq C18 , 2.1 x 100 mm, 1.8 µm	
Mobile phase	A=Water (5 mM ammonium formate and 0.1% formic acid) B=Acetonitrile	
Flow rate	0.3 mL/min	
Gradient program	Time (min)	B(%)
	0.0	0
	2.0	0
	3.0	15
	4.0	15
	6.0	50
	7.0	60
	7.5	98
	10.0	98
Oven temp	35°C	

MS Conditions

Agilent 6460 QQQ LC/MS	
Ion source	AJS ESI
Polarity	Positive mode
Ion spray voltage	3000V
Dry gas temp	325°C
Dry gas flow	8 L/min
Nebulizer pressure	40 psi
Sheath gas flow	12 L/min
Sheath gas temp	300°C
Acquisition mode	tMRM

Results and Discussion

A UHPLC/MS/MS method has been developed for the analysis of ribavirin and amantadine in chicken samples. The resulting chromatogram is shown in Figure1.

UHPLC Separation and Triggered MRM Analysis

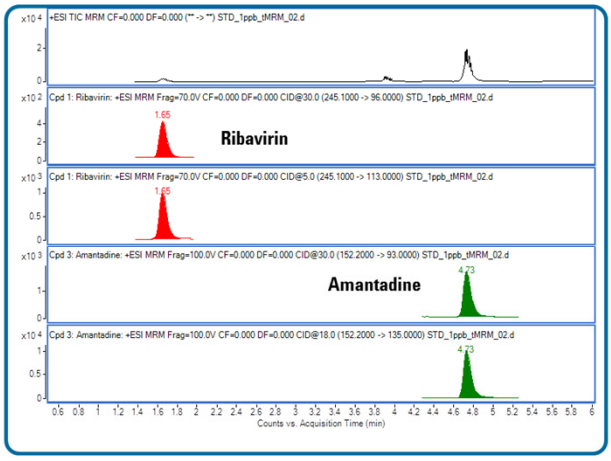


Figure 1: UHPLC/MS/MS Chromatogram of a spiked chicken sample acquired with triggered MRM

Because of the strong polar nature of ribavirin, weak retention in the reversed phase C18 column is observed. Starting the gradient at 100% aqueous phase with the Agilent Zorbax SB-Aq column effectively separates ribavirin from the chicken matrix interference. The resulting chromatogram is shown in Figure 2.

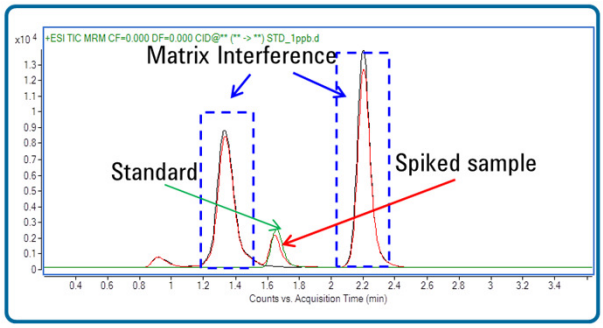


Figure 2: UHPLC system with Zorbax SB-Aq column for separating ribavirin from sample disruptors

For the ribavirin and amantadine acquisition, tMRM enabled the acquisition of a total of 9 MRM transitions per compound. The compounds in this analysis utilized two primary transitions and up to seven secondary transitions per compound. Figure 3 shows tMRM conditions and Figure 4 shows overall EIC chromatograms for a quality control standard of ribavirin included in this method at the minimum reporting level (MRL) .

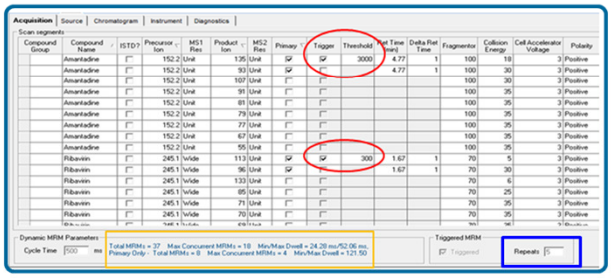


Figure 3: Agilent MassHunter acquisition software allows a dynamic MRM method to be easily changed to a tMRM method by selecting the Triggered MRM Enabled box and specifying the number of repeats.

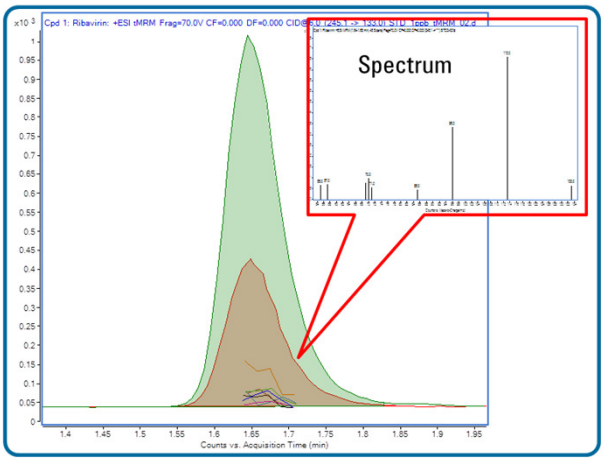


Figure 4: Primary transitions (integrated), triggered MRM transitions (5 repeats), and resulting MRM spectrum for ribavirin

Results and Discussion

This established quantitative method demonstrates excellent recovery (above 80% for both analytes) in chicken samples, good linearity (R>0.999 for two analytes as shown in Figure 5), good inter-day reproducibility (Area RSD=2.9% and n=4 for ribavirin at 100pg/ml; Area RSD=1.5% and n=4 for amantadine at 100pg/ml), and excellent sensitivity (LOQ=5 pg/ml for ribavirin; and LOQ=10 pg/ml for amantadine in chicken matrix).

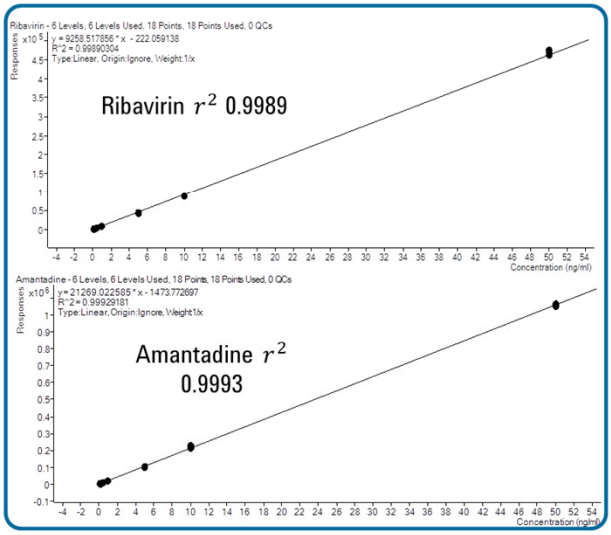


Figure 5: Calibration curve for ribavirin and amantadine from 0.1ppb to 50ppb

Sample preparation and matrix effects

Two different SPE systems were tested: PBA and PCX. Because ribavirin remains neutral at pH 2, it does not interact with PCX cartridge and elutes during application of the extract and the washing procedure. Amantadine is positively charged at pH 2 and therefore is retained. PCX cartridge is considered suitable for the clean-up of amantadine.

The PBA material has multiple interaction mechanisms. The strongest is the covalent binding at high pH with cis-diol groups as present in ribavirin.

During method development, recovery and matrix effects were investigated. Recovery was calculated by dividing the signal of a blank chicken sample with addition of the compounds before sample clean-up (matrix-matched standards) by the signal of a blank chicken sample with addition of an equivalent amount of the compounds, based upon 100% recovery, after sample clean-up(matrix-matched recovery standard). After sample clean-up by SPE, no obvious matrix effect was found (matrix effects ≤10%).

Confidence of library matching score

Figure 6 shows the accurate library search results for the endogenous chicken compound with the same retention time, quantifier ions and qualifier ions as ribavirin, which easily resulted in the false positive. By the library matching, the endogenous compound was excluded with low library matching score of 71.0. Meanwhile the ribavirin in the matrix demonstrated high matching score with reliable confirmation. and we were able to confidently reject the native compound as ribavirin.

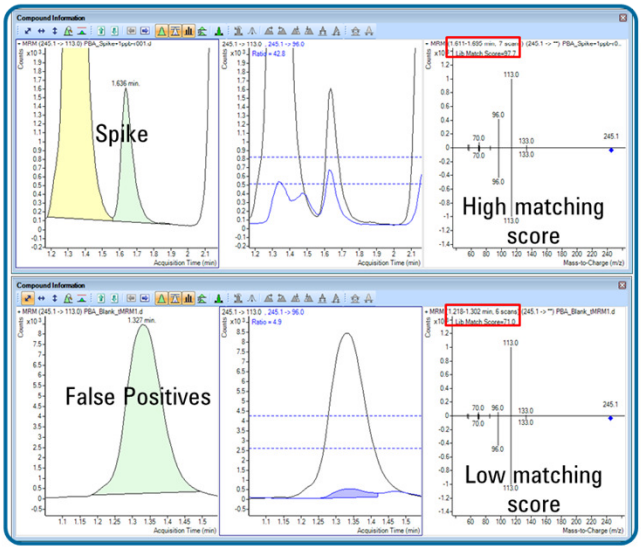


Figure 6: Library matching results for identifying endogenous compound and ribavirin in the chicken sample to avoid the positive identification

Conclusions

The analyses of ribavirin and amantadine in chicken extracts with tMRM acquisition achieved accurate quantitative analysis with the confidence of library matching in the single run. Ribavirin was well distinguished from the co-eluting endogenous compounds, and the false positives were avoided by the library matching. tMRM acquisition is robust to provide quantitative and qualitative results on a single instrument, in a single injection.