Application Brief

Food Testing

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Analysis of Ribavirin and Amantadine in Chicken and Eggs Using SPE with LC/MS/MS

Authors

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Abstract

This study developed and validated a method for the quantitative analysis of ribavirin and amantadine in chicken and eggs. The method used SPE coupled with LC/MS/MS. Sample extraction used 5% trichloroacetic acid followed by cleanup with Agilent Bond Elut PBA/PCX SPE cartridges. The method provides a reliable solution with good recoveries and reproducibility for the simultaneous determination of ribavirin and amantadine.

Experimental

Instrument method

The samples were run on an Agilent 1260 Infinity II LC system coupled to an Agilent G6470 triple quadrupole LC/MS system equipped with an Agilent Jet Stream electrospray ion source. Agilent MassHunter workstation software was used for data acquisition and analysis.

HPLC conditions

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 SB-Aq, 100 × 2.1 mm, 2.7 µm (p/n 685775-914)
Column Temperature	35 °C
Injection Volume	10 µL
Mobile Phase	A) 0.5 mM ammonium acetate solution with 0.1% formic acid B) ACN
Gradient	Time (min) %A %B 0 100 0 2.0 100 0 4.0 88 12 5.0 88 12 5.1 2 98 9.0 2 98 9.1 100 0

MS conditions

Parameter	Value
Gas Temperature	250 °C
Gas Flow	7 L/min
Nebulizer	35 psi
Data Acquisition	MRM as in Table 1.

Sample extraction

First, accurately weigh 2 g of sample into a 50 mL centrifuge tube, add 40 µL of internal standard (1 ppm ribavirin-$^{13}C_5$, 200 ppb amantadine-D_{15}) followed by 5 mL of 0.5 mM ammonium acetate (pH 4.8) and 40 µL of acid phosphatase (0.25 U/µL). Vortex for 30 seconds, then shake for 2 hours at 37 °C in the dark for enzymatic hydrolysis. Once finished, add 4 mL of 5% trichloroacetic acid, and vortex for 30 seconds. Centrifuge at 10,000 rpm for 10 minutes. Transfer the supernatant into a new centrifuge tube, and adjust the pH to 8.5 with 5% ammonium hydroxide. Compensate the volume to 10 mL with water, and centrifuge again to transfer 5 mL of supernatant for SPE cleanup.

Figure 1 shows the SPE procedure.

Table 1. Target analytes MRM conditions.

Analyte	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Fragmentor (V)	CE (V)
Ribavirin-$^{13}C_5$	250.1	113	70	5
Amantadine-D_{15}	167.2	150.1	100	20
Ribavirin	245.1	113	70	5
		96	70	30
Amantadine	152.2	135	100	18
		93	100	30

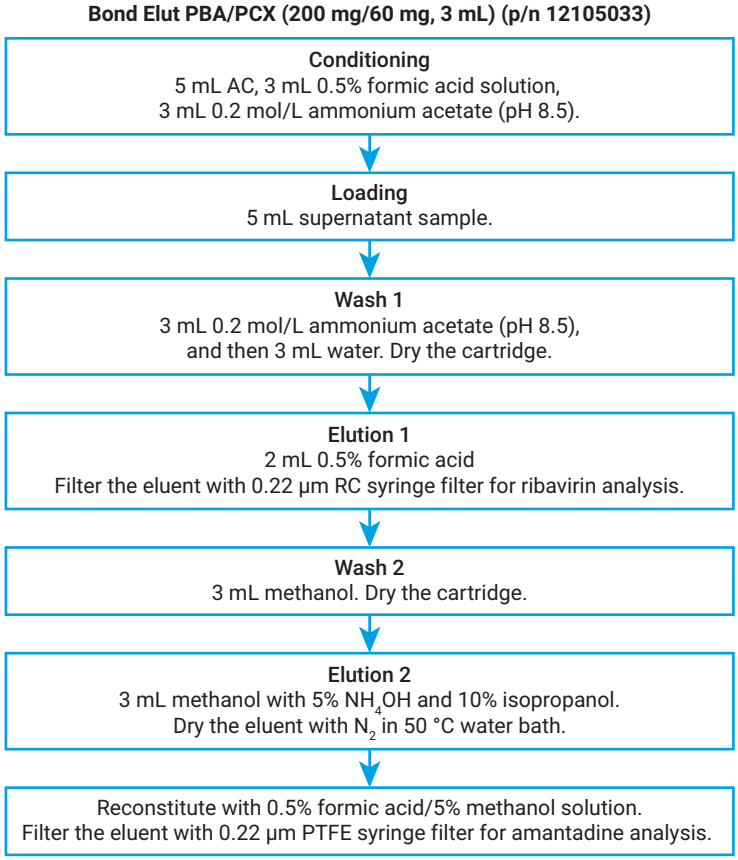

Figure 1. Sample preparation workflow chart.

Results and discussion

The method delivers good linearity for both ribavirin and amantadine (Table 2 and Figures 2 through 4). In the spiking levels of 1.5 to 15 µg/kg for ribavirin and 0.3 to 3 µg/kg for amantadine, the recoveries were 94 to 110% and 85 to 112% , respectively. The RSD was <4 and the limits of quantitation were 0.08 µg/kg and 0.03 µg/kg, respectively.

Table 2. Method recovery and RSDs.

Matrix	Analytes	Spiking Level (µg/kg)	Recovery (%)	RSD% (n = 3)
Chicken	Ribavirin	1.5	108.50	2.26
		5	94.67	3.26
		15	94.90	2.68
	Amantadine	0.3	94.71	2.94
		1	86.66	2.19
		3	92.12	3.75
Egg	Ribavirin	1.5	96.21	2.50
		5	94.26	1.78
		15	96.73	0.09
	Amantadine	0.3	105.14	3.47
		1	96.78	1.06
		3	111.90	2.64

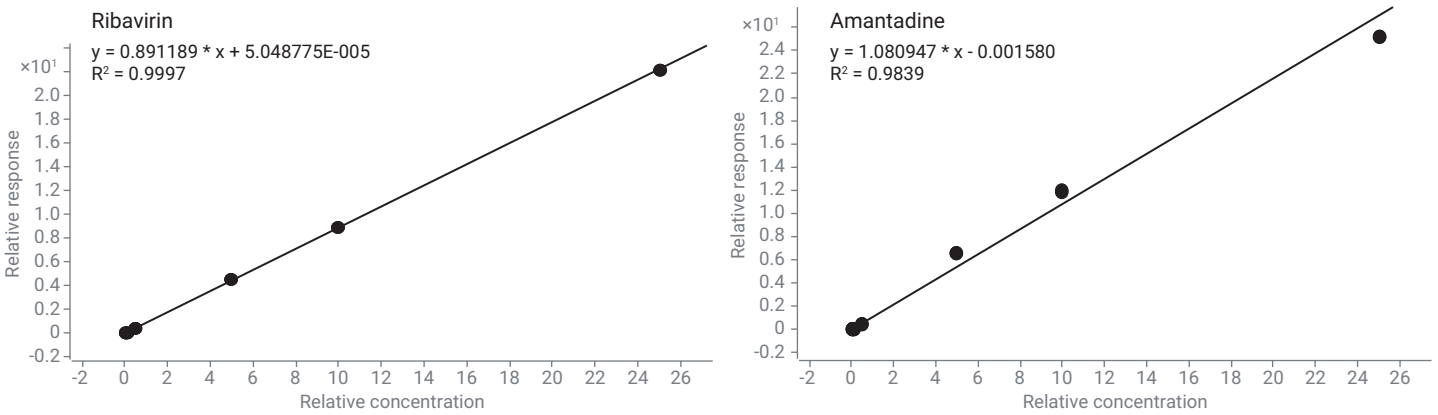

Figure 2. Calibration curves of ribavirin and amantadine.

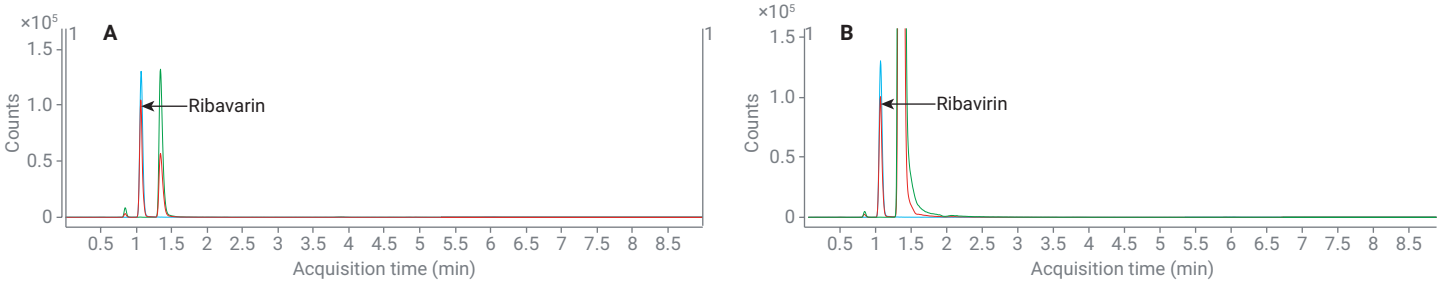

Figure 3. MRM chromatograms of ribavirin (A: chicken; B: egg) for neat standard (blue), prespiked sample (red), and matrix blank (green).

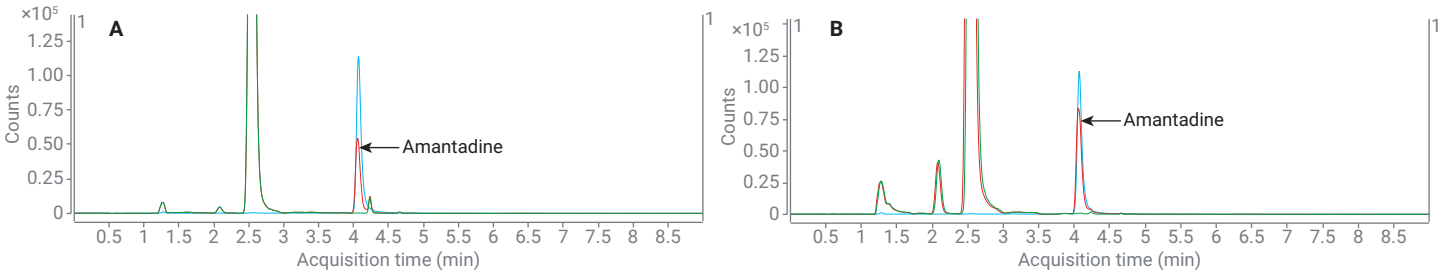

Figure 4. MRM chromatograms of amantadine (A: chicken; B: egg) for neat standard (blue), prespiked sample (red), and matrix blank (green).

Conclusion

Bond Elut PBA/PCX, a silica-based sorbent with phenylboronic acid functionality and polymer-based cation exchanger with mixed mode sorbent, provides good recoveries for this method. The Bond Elut SPE with two extraction mechanisms delivered a reliable solution, excellent recoveries, and reproducibility for both ribavirin and amantadine in both chicken and egg.

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