Analysis of chloramphenicol by negative ion electrospray LC/MS/MS

Application Note

Environmental

Authors

Agilent Technologies, Inc.

Introduction

Chloramphenicol (CAP) is a potent, broad-spectrum antibiotic and potential carcinogen used in humans only at therapeutic doses for treatment of serious infections. Its use in meat-producing animals, food producing insects, aquaculture, and animal-feed products has been banned in the United States, Canada and the European Union. However, the illegal use of CAP remains a possibility due to its broad activity, ready availability, and low cost.

CAP can cause an irreversible illness called aplastic anemia. The incidence rate of aplastic anemia is one out of every 25,000 to 40,000 people. Onset may occur weeks or months after ending treatment with CAP. A very low concentration of CAP may be enough to trigger the illness and the safe level of dosage has not been identified.

Due to known effects of CAP and the recent discovery of CAP in imported food, the U.S. Food and Drug Administration (FDA) and other government agencies throughout the world have increased sampling and surveillance of imported shrimp, crawfish, honey, royal jelly, feed, and milk products for the presence CAP. In addition, the U.S. FDA has developed an LC/MS/MS method to determine CAP in shrimp. 1 The detection limit for the method is 80 pg/g* of shrimp, corresponding to 800 fg/µL of the solution injected into the LC/MS.

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Instrumentation

- Agilent ProStar 430 AutoSampler
- Agilent ProStar 210 Isocratic Solvent Delivery Modules
- Agilent 1200L LC/MS equipped with ESI source

Materials and Reagents

- Chloramphenicol (Part no. C1919) from Sigma-Aldrich Corp. (St. Louis, Missouri, USA).
- All other chemicals are reagent grade or HPLC grade.

Conditions

Column	: Agilent Polaris C18-A, 2 x 50 mm id, 3 µm (Agilent Part no. A2001050X020)			
Mixer	: 50 µL static mixer			
Solvent A	: water			
Solvent B	: methanol			
LC Program	: Time (min:sec)	%A	%B	Flow (mL/min)
	0:00	75	25	0.2
	1:00	5	95	
	2:00	5	95	
	2:21	75	25	
	6:00	75	25	0.2
Injection Volume	: 50 µL			
Sample Solvent	: methanol:water at 1:1 (v/v)			

MS Parameters

Collision Gas	: 2.1 m Torr Argon		
API Drying Gas	: 20 psi at 300 °C		
API Nebulizing Gas	: 50 psi		
Scan Time	: 1 s		
SIM Width	: 0.7 amu total		
Needle	: -4400 V		
Shield	: -300 V		
Capillary	: -40 V		
Detector	: 2000 V		
Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
CAP	321	152	20
	321	257	8

Results

The LC method used a 6 minute run cycle with CAP giving a retention time of 3.1 minutes with a peak width at half height of 0.12 minutes. Both product ions can be quantitatively analyzed at the level of 100 fg/µL in solution (Figure 1). The linearity of the detector response was found to be excellent (Figure 2). The R^2 for 321 >152 and 321 >257 are 0.999 and 0.998, respectively. In addition, good precision was found at these low levels with RSD (relative standard deviations) of -6% (Figure 3).

The limit of detection (LOD) is estimated to be <30 fg/µL in solution corresponding to a LOD of <3 pg/g in shrimp according to the U.S. FDA method.

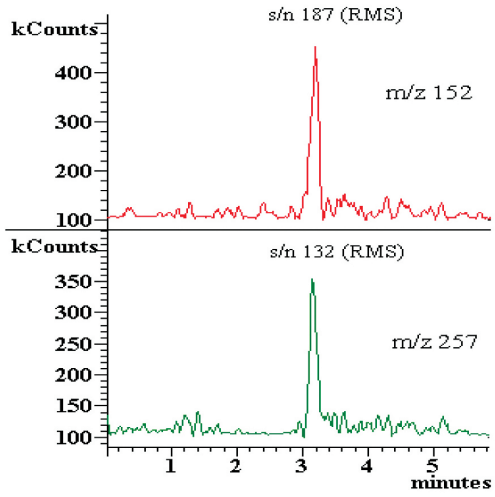

Figure 1. MRM Chromatograms of CAP (100 fg/JJL)

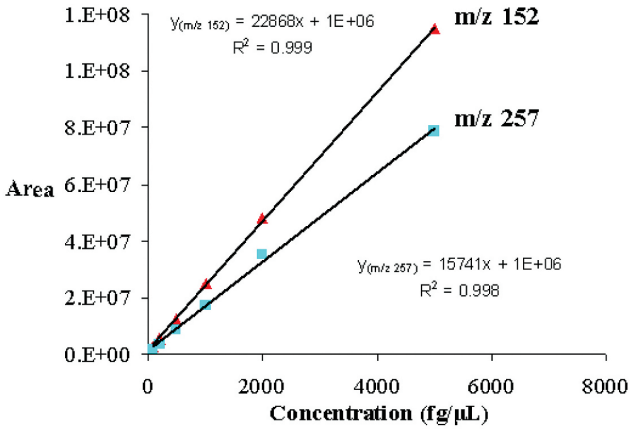

Figure 2. Standard Calibration Curves

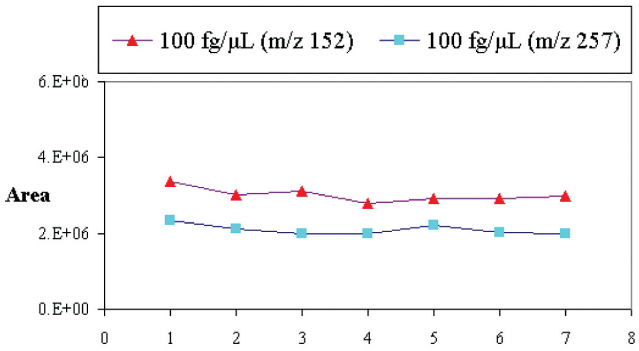

Figure 3. Precision Curves

Conclusion

The choice of either product ion for quantitation is especially important when complex matrices interfere with one of the product ions. The Agilent 1200L LC/MS/MS system demonstrates excellent sensitivity, reproducibility and performance for the analyses of chloramphenicol and is able to assist in the detection of potentially harmful substances in food imports/exports to improve food safety throughout the world.

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