LC/MS Application Note

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Analysis Of Chloramphenicol by Negative Ion Electrospray LC/MS/MS

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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.

Instrumentation

- Varian ProStar 430 AutoSampler
- Varian ProStar 210 Isocratic Solvent Delivery Modules
- Varian 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.

HPLC Conditions

Column	3 µm, 50 mm x 2 mm ID Polaris C18-A (Varian Part Number: 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	50µL			
Sample Solvent	methanol:water at 1:1 (v/v)			

MS Parameters

Collision Gas	2.1 mTorr Argon
API Drying Gas	20 psi at 300 °C
API Nebulizing Gas	50 psi
Scan Time	1 sec
SIM Width	0.7 amu
Needle	-4400V
Shield	-300V
Capillary	-40V
Detector	2000V

Scan Parameters

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
CAP	321	152	20
	321	257	8

Results and Discussion

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.

Conclusion

The choice of either product ion for quantitation is especially important when complex matrices interfere with one of the product ions. The Varian 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 substance in food imports/ exports to improve food safety throughout the world.

Reference

1. B.K. Neuhaus, J.A. Hurlbut. and W. Hammack "LC/MS/MS Analysis of Chloramphenicol in Shrimp." U.S. FDA Laboratory Information Bulletin No. 4290, *Volume 18, No. 9, September 2002.* http://vm.cfsan.fda.gov/~frf/lib4290.html

* pg/g=ppt, fg/µL=ppt, and pg/µL=ppb

These data represent typical results.
For further information, contact your local Varian Sales Office.

Standard Calibration Curves

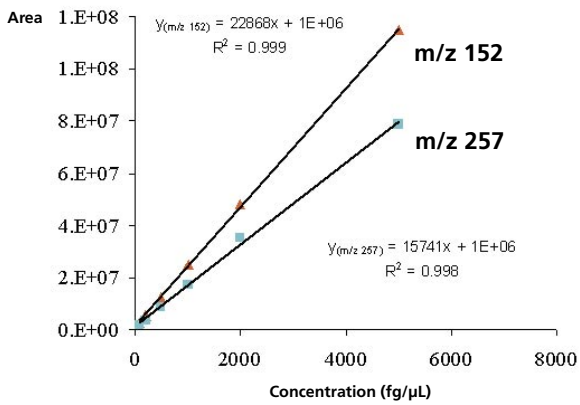

Figure 1. The linearity was excellent for both product ions with a concentration range of 0.1 pg/µL to 5 pg/µL.

Precision Curves

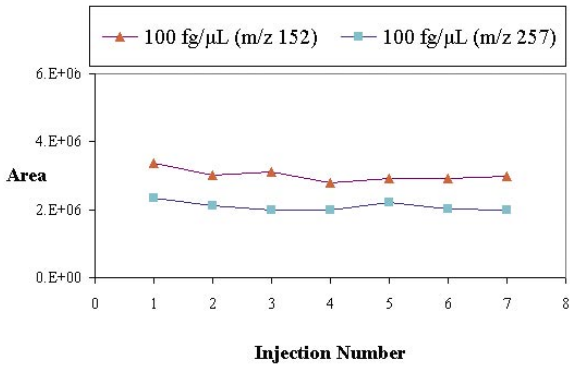

Figure 2. The RSD for both product ions is ~6% at the level of 100 fg/µL.

SRM Chromatograms of CAP

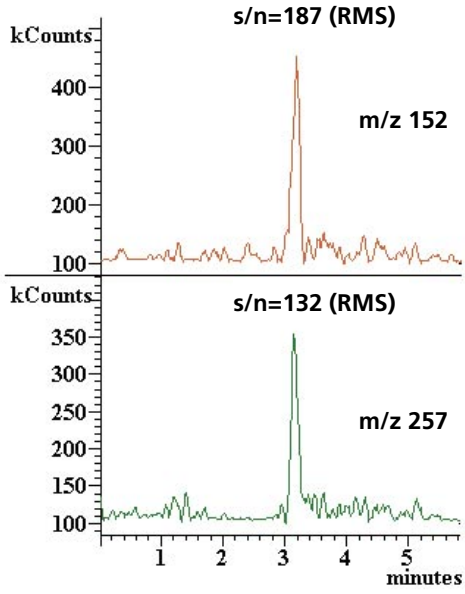

Figure 3. Above are smooth chromatograms for each product ion at a concentration of 100 fg/µL.