Method modifications to FDA method LIB4306 allow for the determination of chloramphenicol residues in food grade enzyme powders by SPE-LC-ESI-MS/MS

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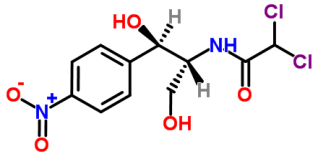
Introduction

The fermentation process associated with the manufacture of food grade enzyme powders was recently determined to support the colonization of bacteria. To control this issue, manufactures following different international regulatory procedures have found the addition of familiar antibiotic compounds to manufacturing processes benficial. This becomes an issue of public health as the concentration of antibiotic residues in food grade materials promotes unknown exposure to the global community. Continued interest in the biomonitoring of these compounds have inspired a number of method development strategies; however, classic methods are labor intensive and require multi-step time consuming efforts. The FDA has recently mandated a method for the determination of chloramphenicol in shellfish. This study evaluates the feasibility of method modifications to incorporate enzyme powder matrices into this method. Linearity was determined over a range of 0.1 – 5 ng/mL (r^2 > 0.990). Relative recovery was determined >80%. The typical repeatability (%RSD) for n=7 replicates <20%. Strategies to control solids prior to SPE load will be presented as they were determined critical in method performance. Method robustness was determined by evaluating multiple lots and multiple variants of enzyme powder origin. The key variable is the nature of the excipients as high level sugars of different sources need to be controlled to minimize the ESI suppression.

Analytes of Interest



Chloramphenicol
MW = 323.1 g/mole
pKa = 11.03 , log P = 1.1

Figure 1: Structure of the analytes of interest

Experimental Procedure

Workflow

An overview of the sample workflow is given in Figure 2. Enzyme powder samples were weighed and loaded into a single use container. The sample was exposed to ethyl acetate:ammonium hydroxide (98:2) for an optimized time of 10 min. The sample was then centrifuged and filtered to control the solids prior to SPE loading. Optimized SPE parameters are given in Table 1. After SPE cleanup, the recovered extracts were evapoarted to dryness using a Biotage TurboVap LV (Temp = 50° ; N_2 psi = 10-15). Samples were reconstituted with HPLC grade water and analyzed by LC-ESI-MS/MS.

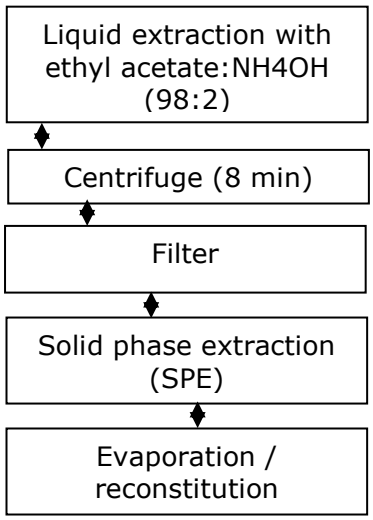


Figure 2: *Sample preparation workflow*

Reagents

HPLC grade water was obtained via a Biopure water system, LC/MSMS grade methanol, and acetonitrile were purchased from Sigma Aldrich. Ethyl Acetate, ammonium hydroxide, and acetic acid were EMD (Billerica, MA) products purchased from VWR. Chloramphenicol and Chloramphenicol D-5 standards were obtained from Sigma-Aldrich (St. Louis, MO), and Crescent Chemical Co. (Islandia, NY) respectively. The enzyme powders were obtained from multiple vendors and will be reported anonymous for this study.

Solid Phase Extraction for analyte enrichment: ISOLUTE C18 Solid Phase Extraction Columns, 500 mg / 6 mL . Extracts were evaporated using a Biotage TurboVap LV.

Table 1. *Optimized ISOLUTE C18 Procedure for analyte enrichment*

Step	Details
Sample	Syringe filtered (0.45um)
Column Condition/Equilibration	3mL Methanol, 3mL Water
Sample Load	10mL
Interference Wash 1	____
Interference Wash 2	____
Analyte Elution	Methanol

HPLC Conditions

Instrument: Shimadzu LC30 Liquid Handling System (Shimadzu, Kyoto, Japan).
Column: Phenomenex Kinetex C18 column (2.1 mm x 100mm, 2.6 µm).
Mobile Phase: The mobile phase A and B were HPLC grade water and Acetonitrile respectively
Flow Rate: 0.2 mL/min
Gradient: Water, Acetonitrile
Injection Volume: 30 µL
Temperature: 35° C

Mass Spectrometry

Instrument: Shimadzu LCMS 8030 triple quadrapole mass spectrometer equipped with an electrospray ionization source operated in negative ion mode. Detection of the target analytes was optimized using a Shimadzu LCMS 8030 triple quadrapole mass spectrometer equipped with an electrospray ionization source operated in negative ion mode. The m/z 321 Q1 assignment corresponds to the pseudomolecular ion [M-H]- was selected. CAP transitions were defined as: 257, 194, 152, 121 and CAP D5 transitions were defined as: 262, 199, 157, 126, and 35.

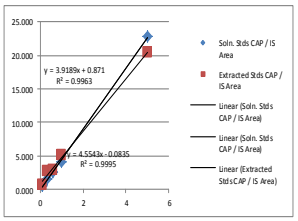
Reference for default instrument conditions prior to optimization:
ttp://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm113314.htm

Results

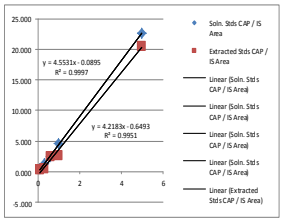
A summary of method performace is detailed in Table 2. Matrix suppression was determined over a concentration range of interest.A set of unprocessed solution standards were prepared for comparison with a set of fortified enzyme podwers extracted in parallell. Suppression was determined by comparing slopes. The results are given in Figure 3. The experiment was repeated with 3 different enzyme powders for robustness. Suppression was determined to be < 20% for all matrices.

Table 2: *Method performance determined*

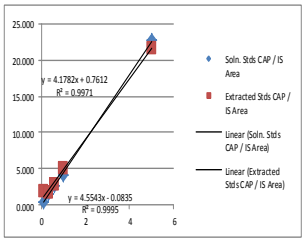
Specification	Candidate method performance
[2]reference range defined in LIB4306 (ppb)	0.1 – 5.0
Candidate Method LOQ (ppb)	0.3
Linearity 0.1 – 5 (ppb)	r^2 =0.9998
%Recovery Concentration level 1 (0.3 ppb)	94.3 %
%Recovery Concentration level 2 (0.6 ppb)	103.6 %
Method repeatability (%RSD, n= 7)	< 5



Enzyme 1 = lactase



Enzyme 2= protease



Enzyme 3 = glucoamylase

Figure 3: *Analyte suppression determined across the concentrations range of interest for 3 enzyme matrices.*

Conclusions

- The ISOLUTE C18 cartridge format was demonstrated as a viable option for residue measurements over a relevant concentration range in food safety laboratory applications.
- The additional filter step prior to the SPE load was key in controlling solids to ensure rugged method performance when applied to batch analysis

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