[APPLICATION NOTE]

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DETERMINATION OF CHLORAMPHENICOL USING THE ACQUITY UPLC AND THE QUATTRO PREMIER XE IN ES NEGATIVE ION MODE MS/MS

Antonietta Gledhill1, Gordon Kearney[1], John Hopkins[1], M. Lynne Cantley[2], Paul B. Young[2], S. Armstrong Hewitt[2]
[1]Waters Corporation, Manchester, UK; [2]DARDNI, Belfast, Northern Ireland, UK

INTRODUCTION

Chloramphenicol (CAP), shown in Figure 1, is an inexpensive, broad spectrum antibiotic, which has very effective antibacterial properties. It was isolated from the soil bacterium *Streptomyces venezuelae* in 1947 but, unlike many antibiotics derived from bacteria and fungi, it is readily synthesised and inexpensive to produce. Due to CAP's low cost and high availability, the antibiotic has been used to treat food-producing animals, which is of some concern since it is reportedly a cause of the potentially fatal blood condition idiosyncratic aplastic anemia. Additionally, hypersensitivity to the drug affects around one in thirty thousand people, regardless of the dosage.[1] Due to the various adverse effects associated with the use of CAP in the treatment of infections, its use in humans is restricted to cases where safer antibiotics have proved ineffective and the benefits of the drug outweigh the risks associated with toxicity.

In the EU, no Maximum Residue Limit (MRL) has been set for CAP in animal derived food and it is listed in Annex IV of EU Council regulation 2377/90/EEC. It has been banned from use in food-producing animals since 1994. EU Decision 2003/181/EC, sets a Minimum Required Performance Limit (MRPL) of 0.3 ppb for CAP.

There is a requirement to achieve the lowest possible levels of quantification and confirmation. Since its ban in 1994, CAP has been reported to be found in food like shrimp, honey, and chicken.[2,3]

This paper describes an extraction from chicken and a LC/MS/MS method for the quantification and confirmation of CAP in chicken.

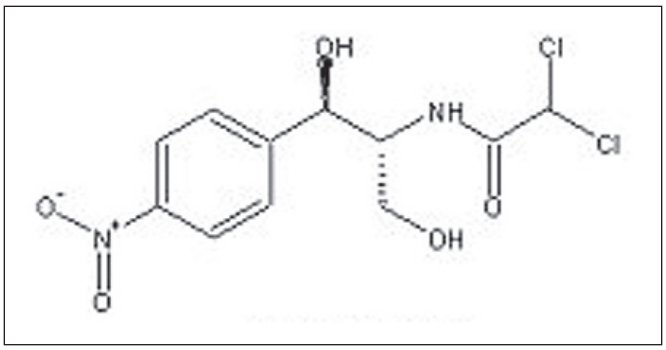


Figure 1. Structure of Choramphenicol.

METHODS

Extraction of CAP from Chicken

- Weigh minced tissue (3 ± 0.1 g) into 30 mL screw capped glass boiling tube.
- Add 200 µL of ISTD, D_5-CAP to the tubes and let stand for 10 min.
- Add 4 mL PBS and homogenise for 1 min.
- Add 1 mL of sodium chloride solution and mix for 15 seconds.
- Add 4 mL acetonitrile, vortex and sonicate for 10 min. Centrifuge at 2,200 rpm for 20 min.
- Transfer supernatant to a clean 35 mL screw capped tube and add 10 mL water.
- Add 10 mL hexane and shake gently for 30 seconds.
- Centrifuge the tubes at 2,200 rpm for 20 min. and discard the upper hexane layer. Add 8 mL of ethyl acetate.
- Mix by inversion of tube for 1 min. and centrifuge tubes at 2,200 rpm for 20 min.
- Transfer organic solvent layer (upper layer) to 9 mL clean tubes and evaporate to dryness at 65 °C under nitrogen.
- Reconstitute residue in 5 mL water, then carry out SPE using a C_{18} cartridge.

- Evaporate the eluant at 65 °C under a stream of nitrogen and reconstitute sample residue in 100 µL of 50% methanol and transfer to micro vials for analysis.
- Reconstitute standards in 200 µL of 50% methanol prior to LC analysis.

LC Method

System:	Waters® ACQUITY UPLC®	
Mobile phase A:	Water	
Mobile phase B:	Methanol	
Column:	ACQUITY® BEH C_{18} 2.1 x 50 mm, 1.7 µm	
Column temp:	55 °C	
Flow rate:	0.5 mL/min	
Injection volume:	10 µL	
Gradient:		
Time 0.00 min:	95% A	5% B
Time 0.40 min:	95% A	5% B
Time 1.00 min:	0% A	100% B
Time 1.50 min:	0% A	100% B
Time 1.55 min:	95% A	5% B
Time 3.00 min:	95% A	5% B

MS Method

System: Waters Micromass® Quattro Premier™ XE in electrospray mode with negative polarity

MRM transitions along with the cone voltages and collision energies are listed in Table 1. For CAP, two transitions were chosen, one for quantification (bold type) and another as confirmation (regular type), in accordance with European guidelines.[4] The transitions were optimized with argon as the collision gas. D_5-CAP was used as the internal standard for the method. Since the calibration standards were prepared in mobile phase solvents, a comparison of peak areas between calibration and recovery experiments allowed an estimation of matrix suppression effects to be made.

Software

Data were acquired with Waters MassLynx™ software and processed with Waters TargetLynx™ Application Manager.

Transition	Precursor ion m/z	Product ion m/z	Dwell time (s)	Cone voltage (V)	Collision energy (eV)
Quantification	321	152	0.04	25	18
1st Confirmatory	321	257	0.04	25	12
IS (D_5-CAP)	326	157	0.04	25	18

Table 1. MRM transition parameters for CAP and D5-CAP in negative mode electrospray.

RESULTS AND DISCUSSION

The absolute sensitivity for the ACQUITY UPLC/Quattro Premier XE is shown in Figure 2, with a 0.01 pg/µL standard giving a signal-to-noise ratio of approximately 45:1.

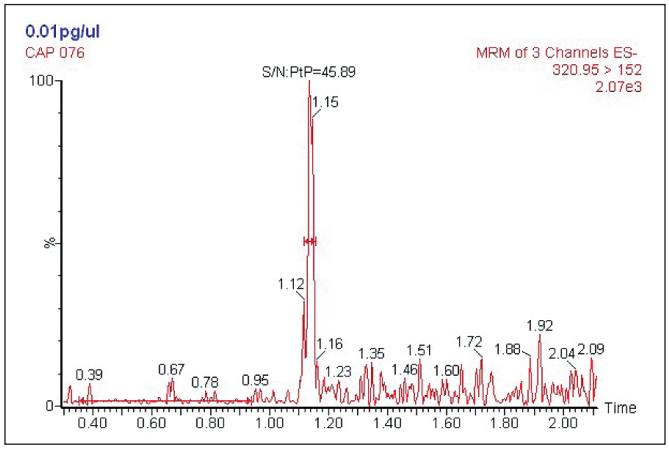


Figure 2. Signal-to-noise ratio of solvent standard (0.01pg/µL) on the ACQUITY UPLC/Quattro Premier XE.

The three MRM transitions used for CAP and D5-CAP are shown in Figure 3 at a concentration of 0.3 µg/kg. For the data acquired using the ACQUITY UPLC/Quattro Premier XE, calibration was performed using solvent standards and matrix-spiked chicken samples were analyzed.

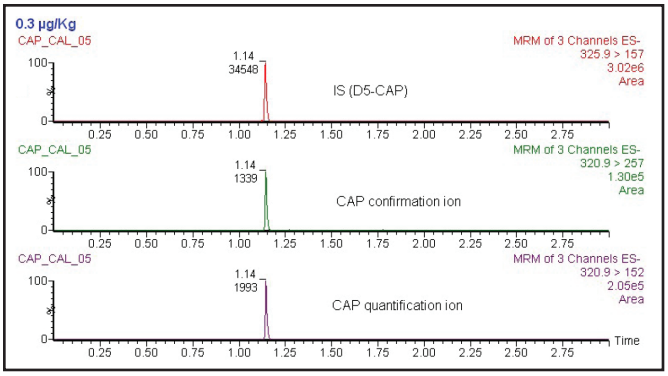


Figure 3. Sensitivity of 0.3 µg/kg CAP and D5-CAP using ACQUITY UPLC/ Quattro Premier XE.

Figures 4 and 5 illustrate the typical linearity and repeatability that is obtained by using the extraction method discussed with the ACQUITY UPLC/Quattro Premier XE.

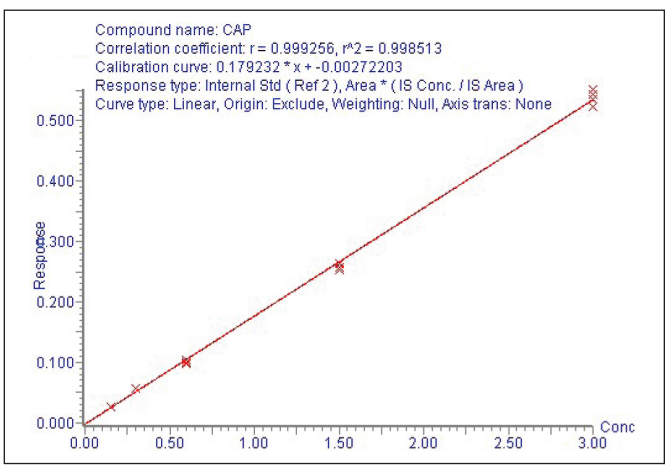
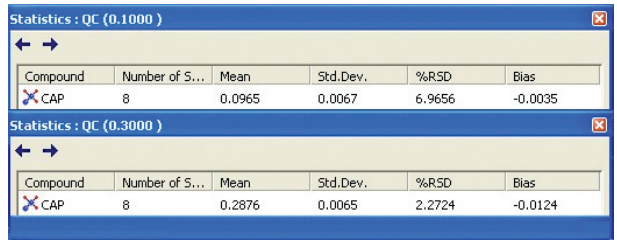


Figure 4. Calibration curve for CAP in solvent standards in negative electrospray mode using the ACQUITY UPLC/Quattro Premier XE.



Statistics : QC (0.1000)

Compound	Number of S...	Mean	Std.Dev.	%RSD	Bias
CAP	8	0.0965	0.0067	6.9656	-0.0035

Statistics : QC (0.3000)

Compound	Number of S...	Mean	Std.Dev.	%RSD	Bias
CAP	8	0.2876	0.0065	2.2724	-0.0124

Figure 5. Repeatability of chicken spiked at concentrations of 0.1 µg/kg and 0.3 µg/kg.

CONCLUSION

A rapid method for the determination and quantification of chloramphenicol in chicken has been described. The Waters ACQUITY UPLC/Quattro Premier XE provides a sensitive, selective, and reproducible analysis method. The limits of determination achieved are below that required by legislation for any country in the European Union.

References

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com