Simultaneous Analysis of Chloramphenicol and Tetracycline Antibiotics in Food Samples Using the SCIEX Triple Quad™ 3500 System

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Overview

Utilizing liquid chromatography with tandem mass spectrometry (LC-MS/MS) to analyze for antibiotic residues in a food samples offers many benefits to routine food testing labs, including the ability to screen for many compounds at once, the selectivity to meet regulatory guidelines, and the sensitivity to reduce sample preparation time to get to results faster. The SCIEX Triple Quad 3500 System enables labs performing antibiotic testing in foods to upgrade to LC-MS/MS and capitalize on its many benefits, at an affordable price.

Here we present a method using QuEChERS extraction (for the analysis of milk, meat and shrimp samples) with Phenomenex roQ kits and dilute-and-shoot (for honey samples), separation using a Kinetex Biphenyl 2.6u (50 x 2.1mm) column, and the SCIEX Triple Quad 3500 System for the detection of Chloramphenicol and Tetracyclines. The mass spectrometer was operated in highly selective and sensitive Multiple Reaction Monitoring (MRM) mode. Limits of detection (LOD) met regulatory limits. Compound identification and quantitation was achieved by monitoring two or three MRM transitions for each analyte. The MRM ratio was automatically evaluated in the MultiQuant™ Software.

Introduction

Antibiotics are widely used as growth promoting agents and therapeutics against microbial infections. The presence of antibiotics in food of animal origin is of concern due to the potential of increasing bacterial resistance and to hypersensitivity for some individuals. Tolerance limits and maximum residue limits (MRL) have been established around the world and agencies monitor the food supply to ensure that antibiotic residue concentrations do not exceed these levels.

LC-MS/MS based methods for single-residue and single-class residues are used to monitor veterinary drugs in food. Recently multi-class multi-residue methods have been introduced to further increase monitoring efficiency.[1-3]

Generic extraction procedures[4-5], ultra high performance LC systems combined with core-shell particles columns, providing good resolution and excellent peak shape, made it possible to detect a variety of antibiotics in a single method. The LC-MS/MS system is typically used in MRM mode because of its excellent sensitivity, selectivity, and speed.

The SCIEX Triple Quad 3500 System takes the best features of the API 3200™ System and enhances them with modern engineering and electronics. The proven design of Turbo V™ source and Curtain Gas™ interface provide exceptional robustness and ruggedness. The advanced eQ™ electronics and the curved LINAC® collision cell were designed for ultra-fast speed of MRM detection and fast polarity switching for comprehensive multi-component analysis.

A triple quadrupole based method for the quantitation of Chloramphenicol and three selected tetracyclines was developed using selective Multiple Reaction Monitoring (MRM) with the *Scheduled* MRM Algorithm activated. The ratio of quantifier and qualifier transition was used for compound identification. Sensitivity of detection met existing regulatory requirements, such as Codex Alimentarius' Maximum Residue Limits (MRL) of 200 µg/kg (tissue) and 100 µg/L (milk) for tetracyclines, the MRL

of 50 µg/kg set by Chinese government, and the Minimum Required Performance Limit (MRPL) for Chloramphenicol set by the European Union of 0.3 µg/kg.[6-8]

The method was successfully applied to the analysis of store-bought milk, meat, shrimp, and honey samples.

Experimental

- Store-bought food samples (milk, meat, shrimp) were extracted following the protocol of the European standard method 15662[5] using the Phenomenex roQ QuEChERS kit buffer-salt mix and the dSPE kit (#KS0-8913) containing 150 mg $MgSO_4$, 25 mg PSA, and 25 mg C18
- QuEChERS extracts were diluted 10 times with water to minimize possible matrix effects
- Honey samples were diluted with 5 times water and injected directly
- The injection volume was set to either 10 or 50 µL, depending on targeted LOQ
- LC separation was achieved using a Phenomenex Kinetex Biphenyl 2.6u (50 x 2.1mm) column and a fast gradient of water and acetonitrile with 0.1% formic acid at a flow rate of 0.5 mL/min (see Table 1 for the gradient profile)
- The SCIEX Triple Quad 3500 System was operated with Turbo V source and Electrospray Ionization (ESI) probe set to 500°C
- Two MRM transitions were monitored for Chloramphenicol and three transitions were monitored for each tetracycline (Table 2)
- The *Scheduled* MRM Algorithm was activated to achieve best data quality
- Fast polarity switching of 50 msec was used. The IS voltage was to -4000 V and +5000 V, respectively
- MultiQuant Software 3.0 was used for quantitative and qualitative data processing

Table 1. Gradient conditions used for the separation

Step	Time (min)	A (%)	B (%)
0	0.0	80	20
2	4.0	5	95
3	7.0	5	95
4	7.1	80	20
5	10.0	80	20

Table 2. MRM transitions and retention times (RT) used for the detection of Chloramphenicol and tetracyclines

Compound	Polarity	RT (min)	Q1 (amu)	Q3 (amu)
Chloramphenicol 1	negative	1.32	321	152
Chloramphenicol 2	negative	1.32	321	257
Chlortetracycline 1	positive	1.30	479	444
Chlortetracycline 2	positive	1.30	479	462
Chlortetracycline 3	positive	1.30	479	154
Oxytetracycline 1	positive	0.57	461	426
Oxytetracycline 2	positive	0.57	461	444
Oxytetracycline 3	positive	0.57	461	201
Tetracycline 1	positive	0.76	445	410
Tetracycline 2	positive	0.76	445	427
Tetracycline 3	positive	0.76	445	154

Results and Discussion

Sensitivity, Reproducibility, Linearity and Accuracy

The LC-MS/MS chromatogram of a 10 ng/mL solvent standard is shown in Figure 1 highlighting the excellent separation and peak shape achieved using the Phenomenex Kinetex Biphenyl with a fast gradient of water and acetonitrile containing 0.1% formic acid. Fast polarity switching was required to detect all compounds in a single method since Chloramphenicol (negative polarity) and Chlortetracycline (positive polarity) are not chromatographically separated by this method.

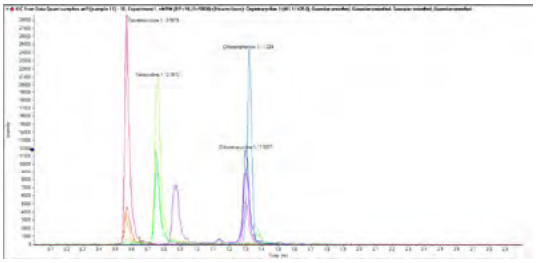

Figure 1. LC separation and detection in MRM mode of three tetracyclines and Chloramphenicol at 10 ng/mL

Figures 2 and 3 show the achieved sensitivity for all targeted antibiotics. Tetracyclines can be easily quantified at the target MRL using a small injection volume of 10 µL reducing the matrix

load for the mass spectrometer to increase robustness and to reduce potential ion suppression.

However, Chloramphenicol sometimes requires a larger injection volume to match the target MRPL while still allowing sufficient dilution to minimize potential matrix effects. In these cases, 50 µL injection volumes were utilized.

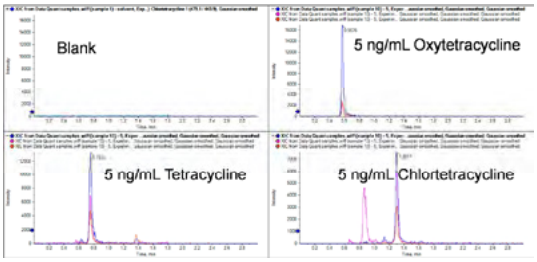

Figure 2. Sensitivity of a 5 ng/mL standard of tetracyclines (injection volume of 10 µL)

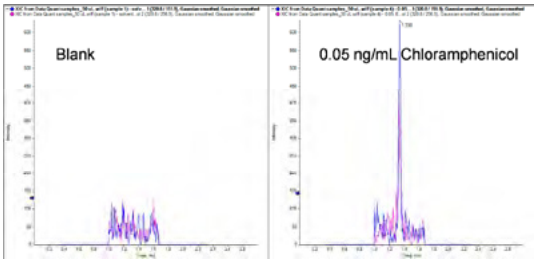

Figure 3. LOQ for Chloramphenicol of less than 0.05 ng/mL with an injection volume of 50 µL, allowing 10x dilution of matrix extracts

Calibration lines are shown in Figure 4, over the range of 0.05 to 100 ng/mL for Chloramphenicol and 0.1 to 100 ng/mL for tetracyclines, respectively, with a coefficient of regression > 0.997.

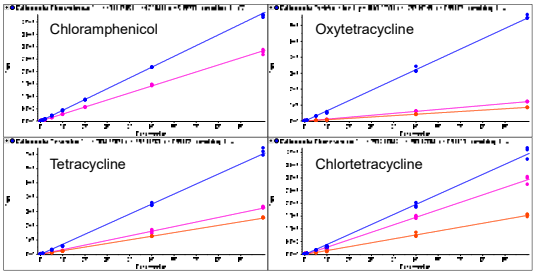

Figure 4. Calibration lines for all 4 compounds analyzed in this study

Accuracies for all calibration standards were between 80 and 120%, and repeatability was found to be better than 5% CV and 10% at the LOQ (n=3).

The achieved method performance allowed diluting sample extracts by a factor of 10 to reduce possible matrix effects. The additional use of isotope labeled internal standards is recommended to compensate matrix effects.

Findings in Food Samples

Figures 5 and 6 show matrix samples tested negative for Chloramphenicol and tetracyclines. The honey sample had a trace contamination with Chloramphenicol below the LOQ of 0.05 ng/mL (0.25 µg/kg in matrix after accounting for the 5x dilution during sample preparation).

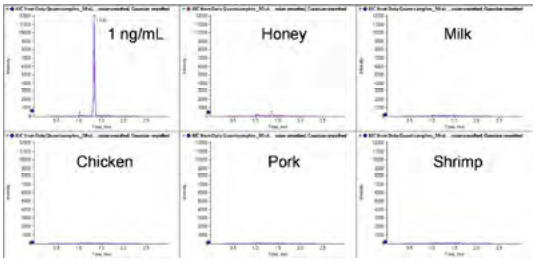

Figure 5. Blank matrices tested for Chloramphenicol (50 µL injection), the honey sample had a trace contamination with Chloramphenicol below the LOQ of 0.05 ng/mL (0.25 µg/kg in matrix after 5x dilution)

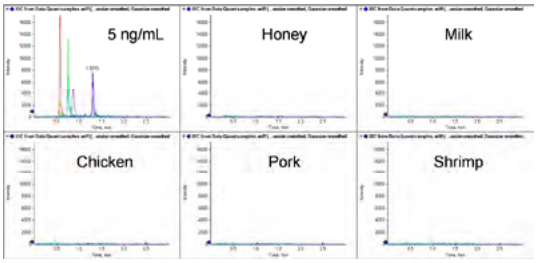

Figure 6. Blank matrices tested for tetracyclines (10 µL injection)

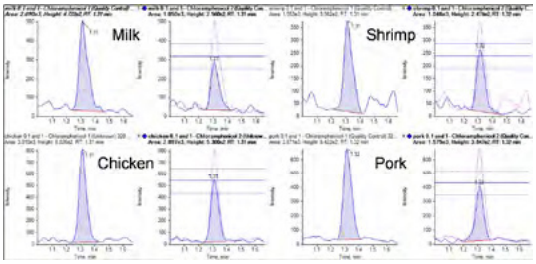

Figure 7. Different food extracts spiked with Chloramphenicol at 0.1 µg/kg (50 µL injection), the MRM ratio tolerances are displayed in the peak review window

Example chromatograms of different food samples spiked with antibiotics are presented in Figures 7 and 8. Compound identification was based on the criteria of directive 2002/657/EC[9] (retention time tolerance of ± 2.5% and maximum tolerances for ion ratios of ± 20 to 50% depending on the ratio). All quantitative and qualitative results were automatically calculated in MultiQuant Software (Figure 6).[10]

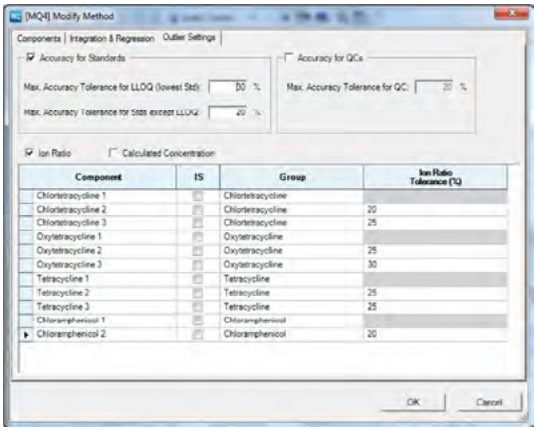

Figure 6. MRM ratio tolerances setup in the method editor of MultiQuant Software

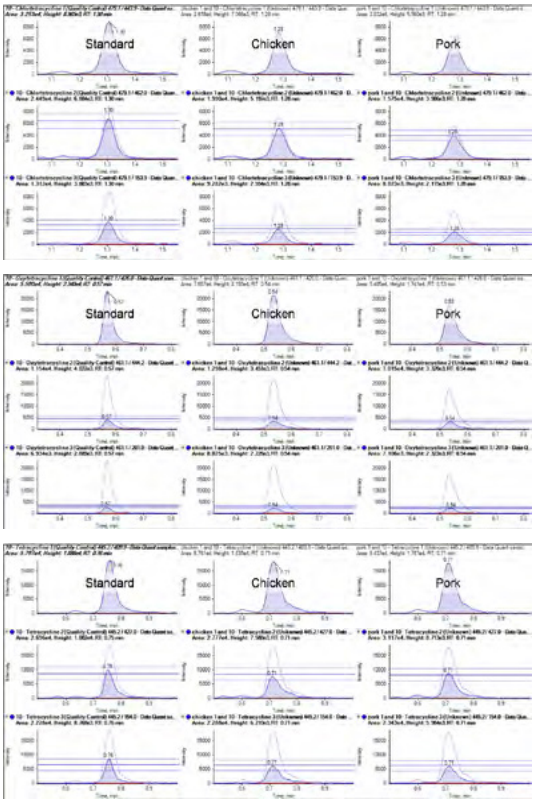

Figure 8. Side-by-side peak review of a standard injection (left) and spiked meat extracts (middle and right) with automatic calculation of MRM ratios, the MRM ratio tolerances are displayed in the peak review window

Summary

A new LC-MS/MS method for the identification and quantitation of antibiotics was developed and successfully applied to different food samples, including honey, milk, shrimp and meat.

The method consists of QuEChERS extraction followed by dilution to minimize possible ion suppression and a dilute and shoot approach for honey. The SCIEX Triple Quad 3500 System operated in MRM mode and utilizing the *Scheduled* MRM Algorithm was used for detection. Limits of detection (LOD) met regulatory requirements. Two to three MRM transitions were monitored for each analyte and the ratio of quantifier and qualifier transition was used for identification. Data processing was performed in MultiQuant Software. Identification criteria of directive 2002/657/EC were used for identification.

Acknowledgement

The author thanks Cheryl Stephenson (Eurofins Central Analytical Laboratories US) New Orleans, LA for providing standards.

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Publication number: 10641514-01

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