DEVELOPMENT AND VALIDATION OF A MULTICLASS METHOD FOR OVER 60 ANTIBIOTICS IN EGGS BY LC-HRMS/MS

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Keywords: Eggs, antibiotics, liquid-chromatography high resolution mass-spectrometry (LC-HR-MS/MS), validation study

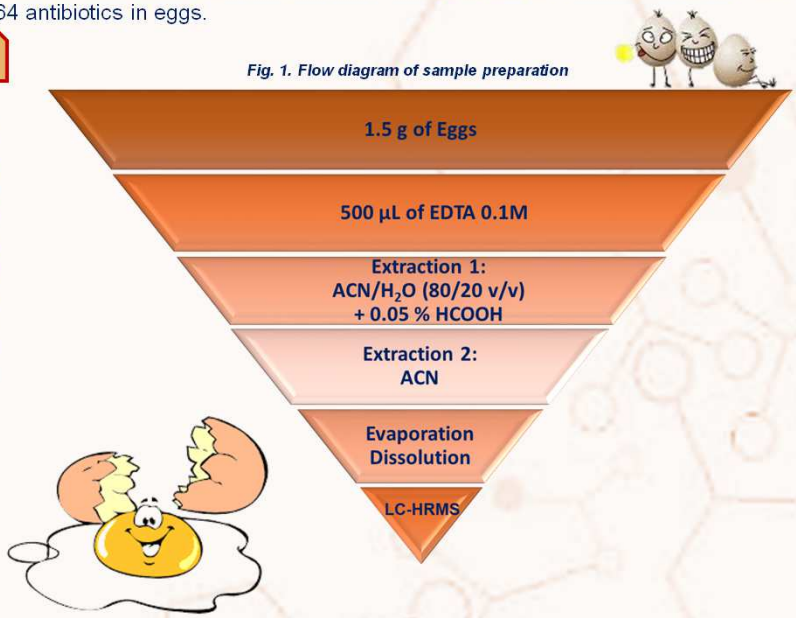
Introduction

The European Union requires the member states to implement yearly monitoring plans to assess the presence of antibiotic residues in food. This surveillance is mainly aimed to verify the sample compliance with the Maximum Residue Limits fixed in Regulation 37/2010 [1] or possible uses for purposes or under conditions other than those laid down in European legislation. Some years ago, our group developed multi-class methods for the simultaneous determination of over 60 antibiotics in meat and milk applying liquid chromatography coupled to high resolution mass spectrometry (LC-Q-Orbitrap) [2,3]. The aim of this work was to extend these procedures to other food matrices collected within the Italian Residue Control Plan, developing and validating a multi-class method to screen and confirm 64 antibiotics in eggs.

Experimental

The sample preparation was that proposed for meat by Moretti et al. (2016) [2] with slight modifications (Figure 1). The analytical determination was carried out using a Thermo Ultimate 3000 Ultra High Performance Liquid Chromatography system coupled with a Thermo high resolution Q-Exactive mass spectrometer (Thermo Scientific, San Jose, CA, USA). The instrumental analysis was performed in positive ionization mode (ESI+) using FullMS-ddMS^2 as acquisition mode. The method was validated according to Commission Decision 2002/657/EC [4] in the range 3.3 µg kg^{-1} -3333 µg kg^{-1} performing four replicates (n=4) at each validation level repeated on three separate occasions [4,5]. Seven concentrations were tested for a total of 84 experiments.

Fig. 1. Flow diagram of sample preparation



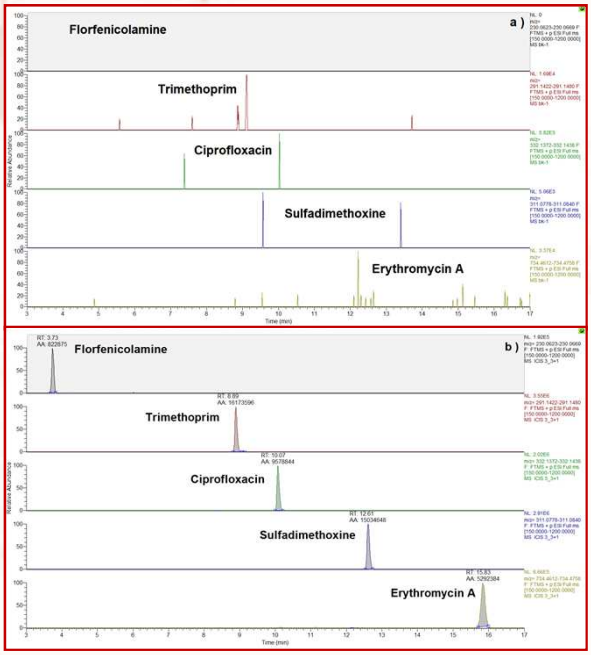


Fig. 2. Chromatograms of a blank (a) and a spiked egg sample (b) at 3.3 µg kg-1

Results and discussion

Recovery factors were in the range 70-100 % for the majority of analytes, whereas coefficients of variation (CV%) evaluated both in repeatability and intra-laboratory reproducibility conditions were lower than 15 %. The limits of detection (LODs) and quantification (LOQs) were 3.3 µg kg^{-1} and 10 µg kg^{-1}, respectively. The only exception was cefacetrile, which was detected starting from 100 µg kg^{-1}. In Figure 2 the LC-HRMS chromatograms of five representative antibiotics in eggs sample spiked at 3.3 µg kg^{-1} and a blank sample are shown. The procedure demonstrated suitable performance characteristics in terms of selectivity, linearity, precision, recovery, decision limits (CCαs), detection capabilities (CCβs), LODs and LOQs. Since several antibiotics are not authorized in laying hens [1], the achievement of LODs lower than 10 µg kg^{-1} is fundamental to control abuses in farm. Finally, starting from similar procedures developed in other matrices [2,3], the optimization time has been very short (about two weeks) confirming one of the characteristics of the multiclass approach, that is its flexibility.

Conclusions

Although the advantages of multiclass methods are well-known, they are fully exploitable only if all the matrices included in the official control plans are analysed with this kind of methods enabling the complete elimination of single-class procedures with different sample preparation protocols and detection techniques. For this purpose, experiments are in progress to further extend this approach also to other food involved in food safety monitoring programmes for antibiotic residues (e.g. honey).

Acknowledgements

The authors gratefully acknowledge financial support from the Italian Health Ministry (Project code: IZSUM RC0032019 "Multiclass method for antibiotic residues in food. Closing the circle: eggs and honey")

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