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TECHNOLOGICAL ADVANTAGES OF METHODS FOR THE SIMULTANEOUS DETECTION OF SEVERAL CLASSES OF ANTIBIOTIC RESIDUES IN CHICKEN MEAT

Elena Narcisa POGURSCHI[1], Dana Cătălina POPA[1], Tomiţa DRAGOTOIU[1], Andreea Ioana RUSU[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Vasile Goldis Western University of Arad, Department of Pharmaceutical Sciences, 86[th]Liviu Rebreanu Str., 310045, Arad, Romania

Corresponding author email: rusu.andreea@uvvg.ro

Abstract

Antibiotics are routinely used to treat and prevent infections in humans as well as in animals. Excessive use of antibiotics in animals, especially in poultry, accumulates residues in the meat and organs beyond legal limits. A large number of classes and types of antibiotics often used in the poultry sector raises problems in quantitative detection methodologies. These techniques must be economical, comply with the requirements of the international standard for maximum residue limits detection, and be sensitive, reproducible, and reliable. Given the complexity of the requirements that such a methodology must meet, the study presents the technological advances of modern technology such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), Ultra-High Performance Liquid Chromatography (UHPLC) in the simultaneous detection of several antibiotic residues belonging to different classes. The paper aims to facilitate the simultaneous detection of as many antibiotic residues in chicken meat in the spirit of the One Health concept.

Key words*: antibiotic residues, chicken meat, detection, multiclass.*

INTRODUCTION

The greatest discovery of the last century (Clardy et al., 2009), antibiotics, has become a major public health concern. The reason for this concern is the antimicrobial resistance that humans can develop. Antimicrobial resistance is regarded as a critical One Health issue (Robinson et al., 2016). Antimicrobial resistance involves three distinct elements: man, animal and the environment, elements that are interconnected and cannot exist without each other. The One Health concept agreed that there is a close connection between the three elements and that the approach to antimicrobial resistance must be multidisciplinary (O'Neill, 2015; Holmes et al., 2016). Although antimicrobial resistance is a naturally occurring phenomenon, the excessive and inappropriate use of antibiotics in both human and veterinary medicine amplifies the phenomenon and negatively affects the health systems already severely affected by the Covid 19 crisis. Any intervention to reduce the consumption of antibiotics is beneficial for overcoming the phenomenon of antimicrobial resistance. An effective approach to estimating the occurrence of antimicrobial resistance is to closely monitor sales of antimicrobial substances in veterinary medicine products. The latest report in this regard (EMA, 2021) expresses sales of antimicrobials in veterinary medicinal products (VMPs) in mg/PCU where PCU represents the population correction unit. 31.1% of the overall sales of antimicrobials in 31 countries in 2020, (in mg/PCU), were represented by penicillins, 26.7% by tetracyclines and 9.9% by sulfonamides (9.9%). In Romania, in 2020, 173.7 tons of VMPs were sold for food-producing animals, which means 57, 8 mg/PCU. Tetracyclines, penniclines and sulfonamides had the highest percentage in the sales structure. These antibiotics are on the list of the most common classes of antibiotics prescribed in human medicine (Menkem et al., 2019). Poultry accounted for 15% of the PCU in 31 countries, including Romania. Chicken meat is a relatively inexpensive source of protein, consumed in all

religions and parts of the world. Chicken meat is preferred by children and the elderly, the most vulnerable categories of the population.

Several studies (Al-Ghamdi et al., 2000; Er et al., 2013; Ezenduka, 2019; Baghani et al., 2019; Widjastuti et al., 2022) have reported the presence of many categories of antibiotics in chicken meat.

Taking into account all these aspects, more accurate analysis of several antibiotics in chicken meat is required.

To protect human health and prevent adverse effects from antibiotics in animal products, maximum residue limits (MRLs) of antibiotics and other drugs were established by the European Commission.

Maximum Residues Limits for chicken meat are presented in Table 1.

Table 1. Maximum residue limits (MRL) in chicken meat

Antibiotic	Muscle	Fat*	Liver	Kidney
Amoxicillin	50	50	50	50
Ampicillin	50	50	50	50
Avilamycin	50	100	300	200
Benzylpenicillin	50	50	50	50
Chlortetracycline	100	-	300	600
Cloxacillin	300	300	300	300
Colistin	150	150	150	200
Danofloxacin	100	50	200	200
Dicloxacillin	300	300	300	300
Difloxacin	300	400	1900	600
Doxycycline	100	300	300	600
Enrofloxacin	100	100	200	300
Erythromycin	200	200	200	200
Florfenicol	100	200	2500	750
Flumequine	400	250	800	1000
Kanamycin	100	100	600	2500
Lasalocid	20	100	100	50
Lincomycin	100	50	500	1500
Neomycin	500	500	500	5000
Oxacillin	300	300	300	300
Paromomycin	500	-	1500	1500
Penicillin	25	25	25	25
Sarafloxacin	-	10	100	-
Spectinomycin	300	500	1000	5000
Spiramycin	200	300	400	-
Sulfonamides	100	100	100	100
Tetracycline	100	-	300	600
Thiamphenicol	50	50	50	50
Tiamulin	100	100	1000	-
Tilmicosin	75	75	1000	250
Tylosin	100	100	100	100
Tylvalosin	-	50	50	-

Values expressed in µg/kg

* The fat MRL relates to 'skin and fat in natural proportions'

Source: COMMISSION REGULATION (EU) No 37/2010

The data in Table 1 show that the determinations should be emphasized on muscle, where MRL values are equal or lower than those of kidney, liver, or fat. This paper aims to investigate the advantages of chromatographic techniques and critically discuss their current limitations in the context in which the methods must be validated according to the European Commission Directive 2002/657/EC.

MATERIALS AND METHODS

Information about the simultaneous detection of several classes of antibiotic residues in chicken meat was obtained from a literature search of electronic databases such as Science Direct, Google Scholar, Pub Med, and Scopus. Only articles published in English were included in the study, which may be considered a minor limitation of the present study. The keywords used were: methods, simultaneous detection, antibiotics, and chicken meat. As expected in the databases consulted, a relatively small number of articles discussing the proposed topic were found, respectively 61 articles. Of these articles, only those that were intended for the determination of antibiotic residues in chicken meat samples in one analytical run have been studied. The rest of them were excluded.

RESULTS AND DISCUSSIONS

Confirmatory analysis methods of antibiotics in food matrices are expensive. For the quantitative determination of antibiotics to be performed as economically as possible and as accurately as possible, new methods of confirmation have been successfully developed and validated. The chromatographic systems coupled to different types of detectors and mass spectrometry is the technique that responds best to these requirements. Chromatography is defined as a physical separation method based on different interactions of the specimen compounds with the mobile phase and with the stationary phase as the compounds travel through a support medium. Chromatographic methods also have disadvantages, such as the cost of technical training, laboratory infrastructure, trained personnel, or the cost of equipment implementation.

One method is more economical and can be used for routine analyses in the laboratory when several classes of antibiotics in the same matrix can be detected simultaneously.

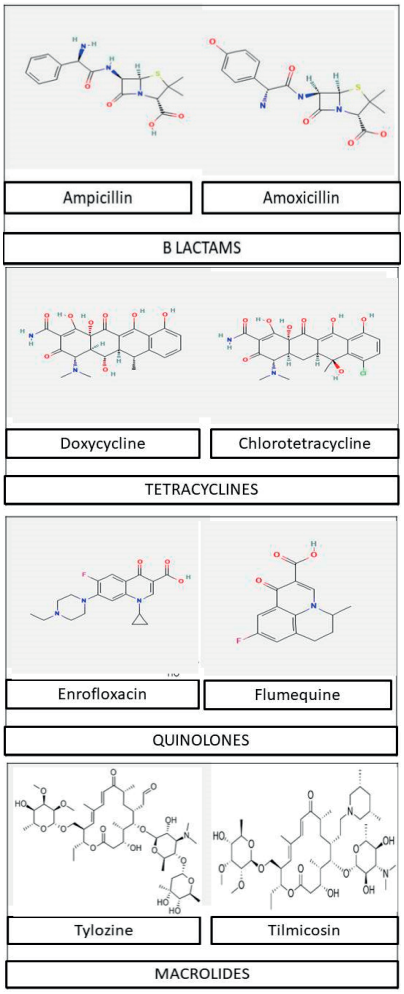

Figure 1. Chemical structure of some common antibiotics used in human and poultry

All these aspects required efforts from specialists in the successful validation of methods for the quantitative determination of antibiotics in chicken meat. The proteins, lipids and other nutrients in the meat make it a complex food matrix. The complexity of this matrix as well as the low level of antibiotic residues leads to the need for pre-determination techniques in themselves, techniques that isolate and concentrate the traces of antibiotics in the meat

When choosing the most efficient method, maximum attention should be paid to the following aspects: chromatographic column and mobile phase selection. Song et al., 2018, used the Kinetex Biphenyl column for the simultaneous determination of eight cyclopolypeptide antibiotics. An Inertsil ODS-3 highly inert and reliable column with high reproducibility and efficiency was used by Hui et al., 2018, for screening 15 sulfonamide residues in different meat types. Using a Waters Symmetry Shield C18 (150 × 4.6 mm^2, 5 µm) column, Mishra et al., in 2020 successfully validated a method of simultaneous and rapid detection of 20 antibiotics in chicken tissues. Neutral alumina, and C18 SPE columns were successfully used to detect 11 quinolone antibiotics in chicken meat by Lu et al., 2019. For the separation of polypeptide antibiotics, the most effective separation effect and the highest response was obtained when was used a Poroshell 120 SB-C18 column (Liu et al., 2019). 30 antibiotics belonging to four different classes were determined by Jammoul & El Darra (2019) using the C18 analytical column (Zorbax 2.1 mm inner diameter I.D × 150 mm length, 3.5 µm particle size). Analyzing the latest data from the literature, eleven analysts were separately using a C8 column (150 mm 3 mm 4 m) in Brazil by Barros et al., 2021. Hypersil BDS-C18 (3 µm, 100 mm × 4 mm) column was used by Lakew et al. (2022) to determine seven classes of antibiotics from chicken meat. The chromatographic column or stationary phase is one of the main parts of the chromatographic system. In modern chromatography, the evolution of the columns was based mainly on the reduction of the internal diameter and the size of the filler particle, which has resulted in an increase in selectivity and an improvement in the resolution of the chromatograms (Snyder et al., 2010).

The mobile phase influences the detection in chromatography, for this reason, its choice is extremely important when determining antibiotic residues by chromatographic methods. The mobile phase can be gas, gel, or liquid but in veterinary drugs determination in one analytical run, the last, (liquid) proved to be most efficient when coupled with mass spectrometry (MS). The most common mobile phases used in the separation of different antibiotics are methanol, acetonitrile, and formic acid. Compare to

the others methanol is cheaper. Lai et al. (2020) showed other advantages of methanol, not only the price.
Methanol increases the solubility of analysts which improves their detection.
In the separation of several classes of antibiotics (quinolones, nitroimidazoles, pleuromutilins, and β-lactams) the roles and efficacy of methanol and acetonitrile have been demonstrated. Acetonitrile provided better peak shape and methanol provided better separations (Wang et al., 2016). When formic acid was added to the mobile phase it was demonstrated that this increases the acidity and improves the ionization efficiency in HPLC–MS/MS.
To improve the ionization efficiency and signal strength in LC-MS / MS, Mishra et al. (2020) used two additives, respectively ammonium formate and ammonium acetate. These additives also generated high chromatographic resolution.
Chromatographic methods that can simultaneously detect several classes of antibiotics are shown in Table 2.

Table 2. Efficient chromatographic methods for the detection of antibiotics in one analytical run

Chromatographic methods		
High-Performance Liquid Chromatography (HPLC)	Liquid Chromatography-Mass Spectrometry (LC-MS)	Ultra-High Performance Liquid Chromatography (UHPLC)

The major challenge during the detection of antibiotics using chromatographic techniques is to detect as many classes as possible.
A comparison of the analytical performance of the methods used for the determination of several classes of antibiotics in real samples is presented in Table 3.

Table 3. Comparison of the analytical performance of the methods used for antibiotics detection

Method	Reference	Antibiotics (n)	Matrix
LC- MS/MS	Yamaguchi et al., 2015	n = 28/multi classes	pork, chicken, beef meat
HPLC-UV	Hui et al., 2018	n = 15/sulfonamides	pork, beef, mutton tissues
HPLC-ELSD	Song et al., 2018	n = 8/cyclopolypeptide	feed
LC-MS	Jammoul & El Darra, 2019	n = 30/multi classes	chicken tissues
HPLC-MS/MS	Liu et al., 2019	n = 4/poplypeptide	infant formula powder
UHPLC-MS	Lu et al., 2019	n = 11/ quinolone	chicken meat, egg
LC-MS/MS	Mishra et al., in 2020	n = 20/multi classes	food, soil
LC-MS/MS	Barros et al., 2021	n = 11/multi classes	chicken meat, liver
LC-UV	Lakew et al., 2022	n = 7/multi classes	chicken tissues

CONCLUSIONS

The chemists and researchers who determine antibiotics in the food matrix are currently facing a situation where there is a requirement to detect more and more compounds at lower and lower concentrations. The challenge is even greater given that degradation products and their metabolites often need to be determined. Chromatographic techniques are commonly used for the detection of antibiotic residues in food samples for a long period.
In the last decade, these methods have been greatly improved by coupling detectors such as detectors, mainly ultraviolet (UV), diode array detector (DAD), and fluorescence detector (FLD) or coupled with mass spectrometry (MS). Among these methods, LC-MS / MS become the gold standard for one-run analysis of antibiotic multi-class in food matrix.

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