Fast screening methods to detect antibiotic residues in food samples

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Antibiotic residues in edible animal products are of great concern to regulatory agencies and consumers, so reliable screening methods for rapid, selective and sensitive detection of these residues are necessary to ensure food safety.

In recent years, great efforts have been made to simplify the treatment of solid food samples and also to introduce high-throughput methods, so different screening methods have been developed.

This review presents a general overview on the progress of the three most important screening approaches to detect antibiotic residues in food samples (i.e. immunoassays, microbiological tests and biosensors). Their main advantages are short analysis time, high sensitivity and selectivity for immunoassays, simplicity and low cost for microbiological tests, and automation and the possibility of *in situ* analysis for biosensors. Moreover, it is important to note a great increase in the number of commercial kits.

Regarding the detection mode employed, the lowest limits of detection were achieved using microbiological inhibition tests and immunoassays with time-resolved fluorometry detection, so antibiotic residues at levels lower than the maximum residue limits established by legislation were detected.

Keywords: Antibiotic residue; Biosensor; Commercial kit; Food; Immunoassay; Maximum residue limit; Microbiological inhibition test; Microbiological test; Screening method; Time-resolved fluorometry detection

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1. Introduction

Antibiotics are drugs of natural, semi-synthetic or synthetic origin [1]. They have been used increasingly for treatment of bacterial diseases in humans and animals. In addition, they are important in animal husbandry because they significantly enhance growth when added to animal feed, although European Union (EU) legislation has forbidden this practice since 2006 [2].

Several antibiotic families are used in veterinary medicine:
- β-lactams (penicillins and cephalosporins);
- tetracyclines;
- chloramphenicols;
- macrolides;
- spectinomycin;
- lincosamide;
- sulphonamides;
- nitrofuranes;
- nitroimidazoles;
- trimethoprim;
- polymyxins;
- quinolones; and,
- macrocyclics (ansamycins, glycopeptides and aminoglycosides).

Macrocyclics are characterized by their high selectivity as they are chiral selectors [3].

The extensive use of antibiotics has triggered the development of bacterial resistance [4], which, in recent years, has become an international concern [5]. Much attention has been paid to food-producing animals as potential source of antibiotic-resistant bacteria in humans. As a result, there is increasing pressure on laboratories responsible for ensuring the safety of food for human consumption. Legislation regarding the control of antibiotic residues in live animals and animal products is given in Council Directive 96/23/EC [6], and maximum residue limits (MRLs) have been established for different matrices, so fast, sensitive methodologies to determine antibiotic residues in food samples are critical in food-safety control laboratories. In this field, Kantiani et al. (2009) presented a review on the analytical methods developed for penicillin and cephalosporin residues in milk and feed samples [7].

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In general, analytical methods for monitoring antibiotic residues can be classified in two groups (i.e. confirmatory and screening). Confirmatory methods are mostly based on liquid chromatography (LC) coupled to mass spectrometry (MS) [8,9] to quantify analyte concentrations. Some papers are devoted to detect antibiotics by means of LC with UV detection [10], and other methods based on capillary electrophoresis (CE) are also reported in the literature [11]. However, all these are time-consuming, expensive, and require complex laboratory equipment and trained personnel. Also, they require tedious sample-preparation procedures based on solid-phase extraction (SPE) and multi-step clean-up [12].

Screening methods can detect an analyte or a family at the level of interest, and usually provide semi-quantitative results [13,14]. The ideal characteristics of a screening method are low rate of false-positive samples, high throughput, ease of use, short analysis time, good selectivity and low cost.

In general, the reported methods (from the Web of Knowledge database) are grouped depending on the type of analytical technique applied, and the corresponding percentages for each one are depicted in Fig. 1. As can be seen, LC-MS is the most employed analytical method (38%), followed by LC-UV (18%) and ELISA (18%). However, the development of other screening methods (12%) and biosensors (8%) is increasing considerably.

Some interesting reviews have been published focusing on the role of LC-MS within the determination of antibiotics in food [15–17]. However, only Blasco et al. [17] included some information on biological methods (e.g., immunoassays and biosensors).

In this article, we provide an overview of recently developed bioanalytical screening methods for antibiotic-residue detection in food. Most of them are based on microbiological assays [18], immunochemistry [19] and, more recently, biosensors [20]. In general, these methods are fast and can be carried out *in situ*.

We also focus on analytical methods based on commercial kit tests that allow fast, sensitive detection of antibiotic residues in food samples with minimum sample treatment, so this work fills a gap in the theme of antibiotic-residue determination that was not covered in previous ones.

2. Antibiotics and food

2.1. Analytes and samples

The screening methods were applied to determine different antibiotics in several food samples. The most important classification of antibiotics divides them into bactericides (that kill bacteria) and bacteriostatics (that prevent bacteria from dividing). However, they can be classified into different groups, according to the chemical structure. As we can see in Fig. 2, seven antibiotic families have been employed as veterinary drugs in recent years, sulfonamides and fluoroquinolones being the most used (information obtained from the Web of Knowledge database).

As a consequence of the extensive use of antibiotics in food-producing animals, edible animal products must be analyzed for regulatory control. Table 1 summarizes the antibiotic families assayed in this kind of sample. As can be seen, milk is the most studied matrix [20,21,14,22–25], and sulfonamides, fluoroquinolones, phenicols, aminoglycosides and β-lactams are the most analyzed antibiotic families in this matrix.

Screening methods were also applied to determine tetracycline, sulfonamide, nitrofurane, phenicol and fluoroquinolone residues in fish samples [26–32], as well as fluoroquinolone residues in eggs [26].

Other matrices exhaustively studied were meat and various tissues from different animals. Several published

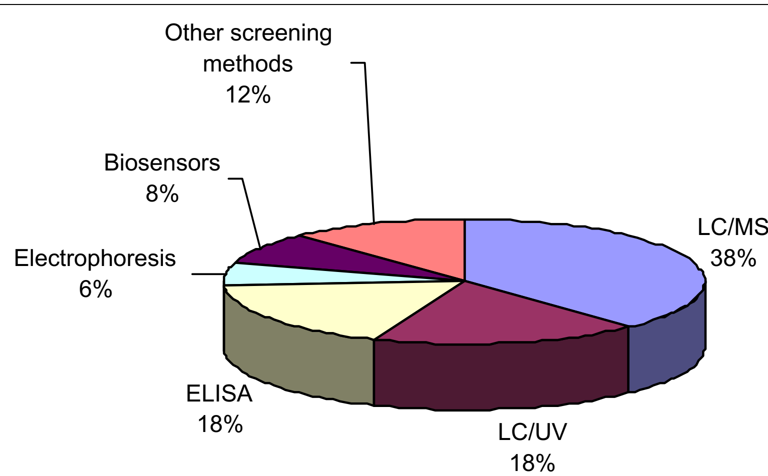

Figure 1. Distribution of the analytical methods used for antibiotics determination in food.