Antibiotics Detection in Food Products

Subjects: Food Science & Technology

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Overuse of antibiotics leads to their circulation in the food chain due to unmanaged discharge. These circulating antibiotics and their residues are a major cause of antimicrobial resistance (AMR), so comprehensive and multifaceted measures aligning with the One Health approach are crucial to curb the emergence and dissemination of antibiotic resistance through the food chain. Different chromatographic techniques and capillary electrophoresis (CE) are being widely used for the separation and detection of antibiotics and their residues from food samples.

antibiotics solid-phase extraction chromatography capillary electrophoresis surface-enhanced Raman scattering biosensors

1. Introduction

Antibiotics are life-saving drugs, which have made a remarkable revolution in the medical sector in the twentieth century, but many antibiotics are now “endangered species” due to the global emergence of antibiotic resistance. These miracle drugs have substantially improved the life expectancy and health of humans and animals by combating a wide range of infectious diseases [1]. Most of the antibiotics are natural products that are secreted primarily by *Streptomyces* spp. [2]. Most commonly prescribed antibiotics in clinics are glycopeptides, polyethers, sulfonamides, tetracyclines, aminoglycosides, β-lactams, fluoroquinolones, and macrolides [3]. Among the several classes of antibiotics, most of them are classified based on their chemical structure, action mechanism, action spectrum, and route of administration [4]. **Figure 1** depicts the various sources of how antibiotics reach up to our table [5][6]. Similarly, the structures of different classes of antibiotics are shown in **Figure 2**, and the examples of antibiotics belonging to the different classes are summarized in **Table 1**. Misuse and overuse of antibiotics in farming and subsequent contamination of the surrounding environment have been significantly linked with the emergence and spread of antimicrobial resistance (AMR). This increase also marks adverse effects on the food chain. A wide array of antibiotics, such as aminoglycosides, β-lactams, fluoroquinolones, and sulphonamides, etc., are reported as possible environmental pollutants by the World Health Organization; these are public health threats [7]. The emergence of antibiotic pollution has led to potential toxic effects on microorganisms, plants, animals, and ultimately humans [4]. When antibiotics are widely used, antibiotic-resistant bacteria evolved due to genetic or mutational alterations, which are also considered a new type of contaminant in the environment [7][8]. The uncontrolled use of antibiotics has made their presence almost everywhere in the environment, including water resources and soils. The wastewater and other biological wastes from farming soils, hospitals, and pharmaceutical industries may contain traces of antibiotics that could mix with water resources if discharged without proper

treatment [9]. Hospital wastes are considered a breeding spot of antibiotic-resistant bacteria (ARB) [10]. ARB strains can survive and multiply even in harsh conditions compared to wild strains [11].

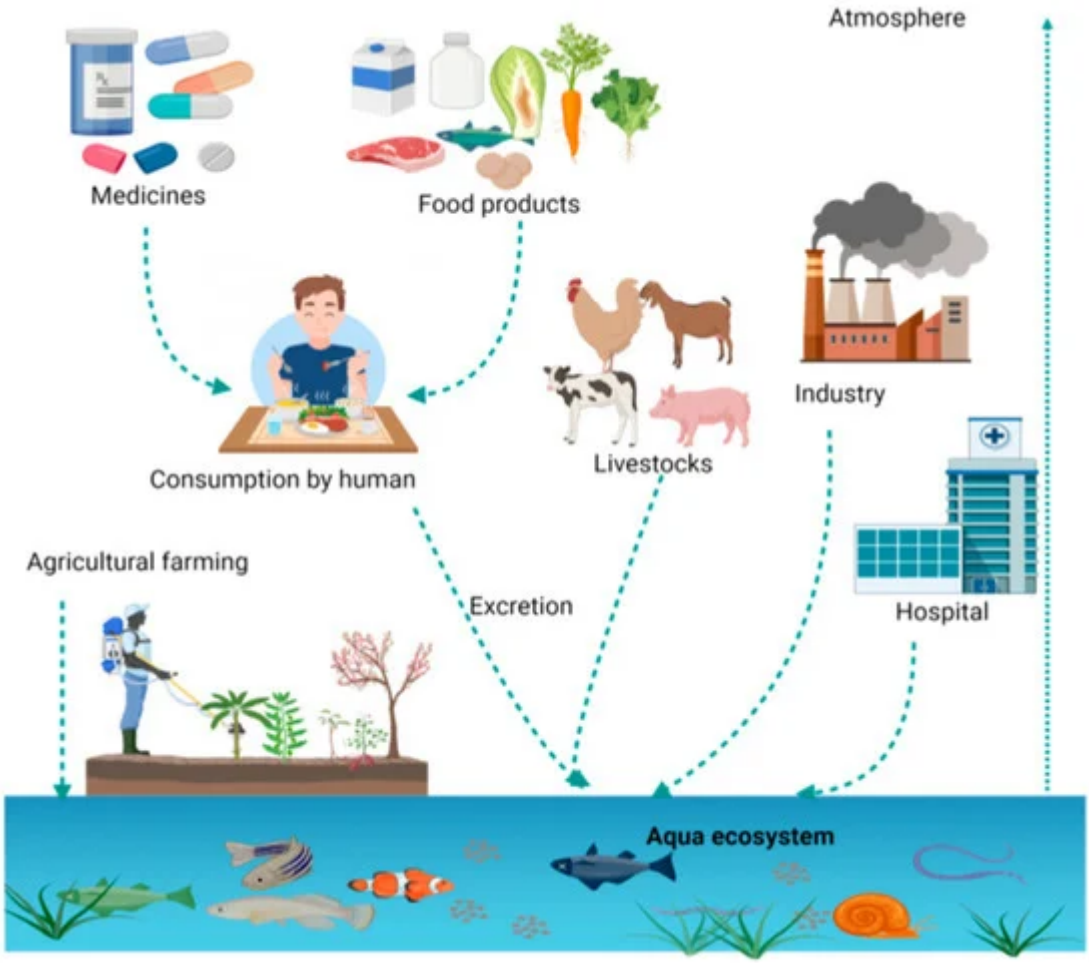
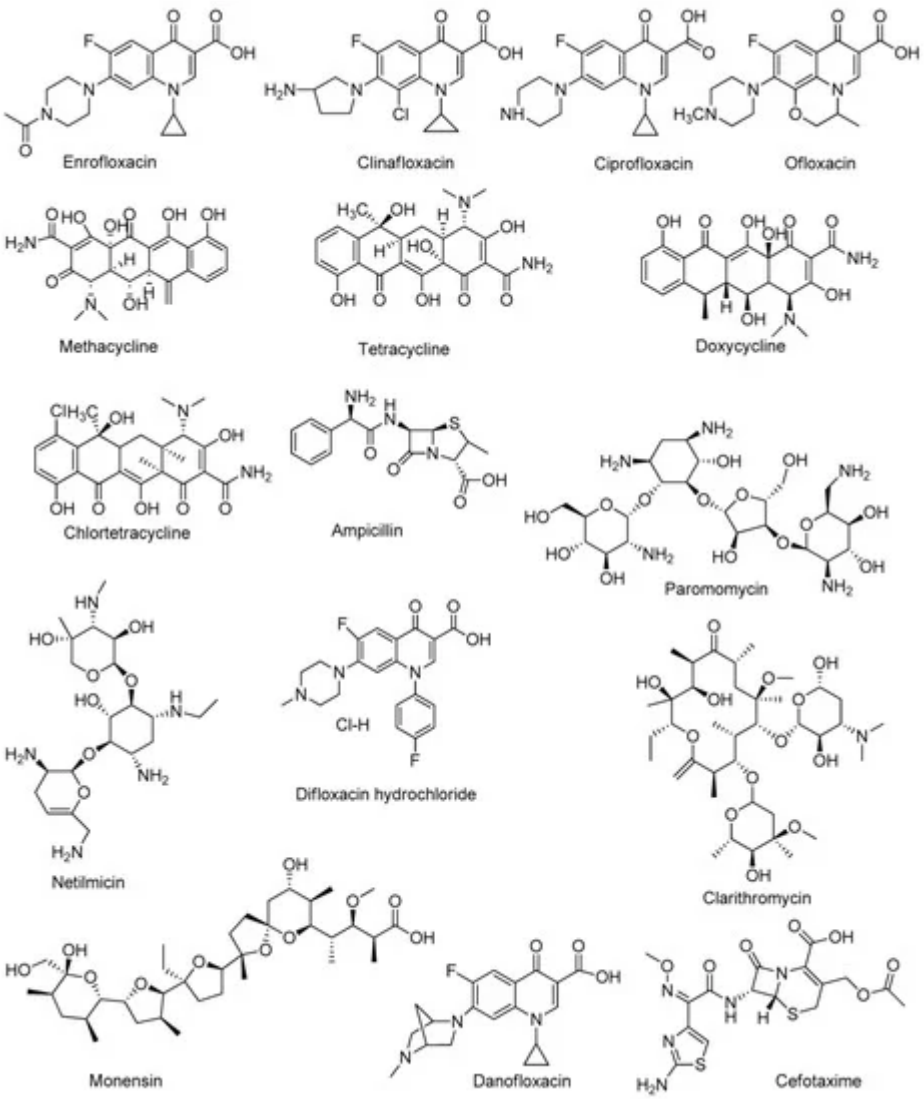


Figure 1. Pathways for antibiotic residues into the environment.


Enrofloxacin
Clinafloxacin
Ciprofloxacin
Ofloxacin
Methacycline
Tetracycline
Doxycycline
Chlortetracycline
Ampicillin
Paromomycin
Netilmicin
Difloxacin hydrochloride
Clarithromycin
Monensin
Danofloxacin
Cefotaxime

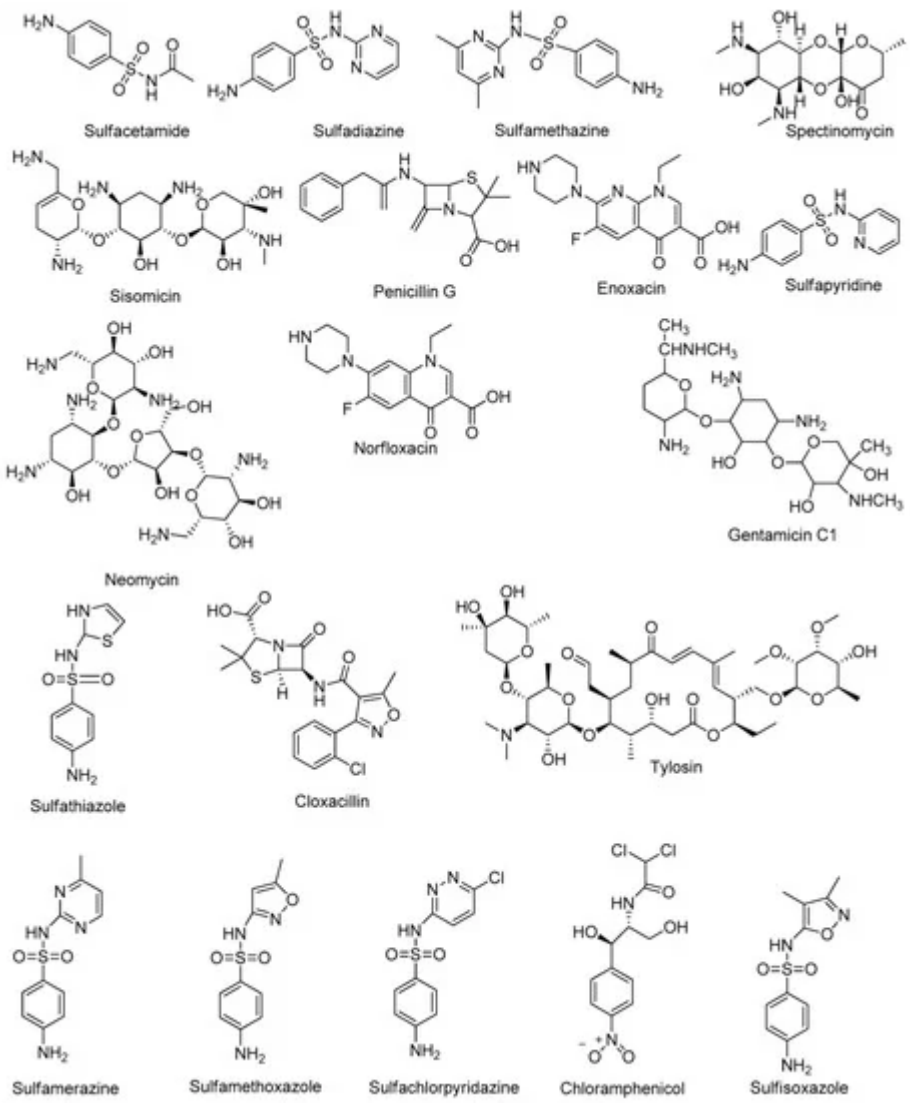


Figure 2. Structures of some antibiotics isolated from food products.

Table 1. Major classes of antibiotics.

Class	Examples	References
Glycopeptides	Vancomycin, Teicoplanin, Telavancin, Oritavancin, Dalbavancin	[12]
Sulfonamides	Sulfacetamide, Sulfadiazine, Sulfathiazole, Sulfapyridine, Sulfamerazine, Sulfamethazine, Sulfamethoxazole, Sulfasoxazole, Sulfachloropyridazine	[13]
Tetracyclines	Tetracycline, Oxytetracycline, Doxycycline, Chlorotetracycline, Methacycline	[13]

Class	Examples	References
Aminoglycosides	Amikacin, Paramomycin, Dihydrostreptomycin, Hygromycin, Kanamycin, Netilmycin, Spectinomycin, Sisomycin, Streptomycin, Tobramycin, Gentamicin, Neomycin	[13]
B—Lactams	Amoxicillin, Ampicillin, Cloxacillin, Penicillin G	[13]
Macrolides	Erythromycin, Clarithromycin, Tylosin	[13]
Fluoroquinolones	Lomefloxacin, Ciprofloxacin, Enroflaxacin, Danofloxacin, Difloxacin hydrochloride, Clinafloxacin	[12][13]
Polyethers	Lasalocid, Salinomycin, Monensin, Narasin, Nigericin	[14]

esulting in
osis, liver problems, pneumonia, and even carcinogenicity in humans due to antibiotic-resistant bacteria [15][16]. To protect public health, maximum residue limits (MRLs) of antibiotics and other pharmacologically active substances in foodstuffs of animal origin were established by the European Commission [17][18]. In this connection, there have been significant efforts in the development of robust analytical methods with a combination of techniques such as High-Performance Liquid Chromatography (HPLC) coupled with Mass Spectrometry (MS), and Liquid Chromatography coupled with tandem mass spectrometry (LC-MS/MS) to monitor antibiotic residues in food products. Antibiotic residues may be present in food products, and it is often difficult to detect because residues are found to be associated with matrices, which interfere with their analysis [19]. In essence, a small portion of the initial antibiotics is likely to be bioactive, which challenges extraction of antibiotic residues as well as detecting and quantifying them [20]. Old-fashioned screening techniques, such as paper-based devices as µPAD, are simple and affordable, but their sensitivity is not enough to capture antibiotic traces in food samples. Therefore, this need has led to the research and development of highly sensitive and selective analytical tools for the detection and quantification of antibiotic residues in complex matrices [21][22].

The food consumed comprises nutrients including, however not limited to, lipids, proteins, and carbohydrates that form a complex food matrix. This matrix may interfere with the signals obtained for analysis; thus, the preconcentration step is carried out to minimize matrix interferences and to increase the sensitivity of detection [23]. The sample pretreatment is a challenging process since many antibiotics are thermally unstable and have no chromophore (e.g., aminoglycosides) [24]. Before instrumental analysis, different preconcentration/extraction processes, such as liquid-liquid extraction (LLE), SPE, liquid-liquid microextraction (LLME), solid-phase microextraction (SPME), and magnetic solid-phase extraction (MSPE), are used to isolate antibiotic residues from different samples [25]. SPE is also widely used for preconcentration and cleaning up steps since it is safer, easily operated, and efficient [26][27].

2. Extraction of Antibiotics from Food Samples

The selection of suitable pretreatment methods is important due to the complexity of matrices and the low concentration of antibiotic residues in food samples. Solid-phase extraction (SPE) is the most extensively used pretreatment approach for the isolation and preconcentration of trace contaminants in complex samples (**Figure 3**). This is due to its convenience of use, minimal organic solvent consumption, and high enrichment factor [28]. The activation of the sorbent, percolation/sorption of the analyte in the sample matrix into the sorbent, removal of matrix interferences, and elution and concentration of the analyte with an appropriate technique are the key steps for a simple SPE procedure. However, the mechanism of extraction can vary depending on the nature of the sorbent used, and choosing an appropriate method of SPE for each application is vital [29]. The analysis of numerous classes of compounds using SPE format has used cartridges, different columns as classical SPE sorbents; however, the use of novel sorbent-based materials, such as metal-organic frameworks (MOFs), carbon nanotubes (CNTs), graphene oxide (GO), and molecularly imprinted polymers (MIPs) have brought about very good extraction recoveries with smaller amounts of sorbents, which are discussed below.

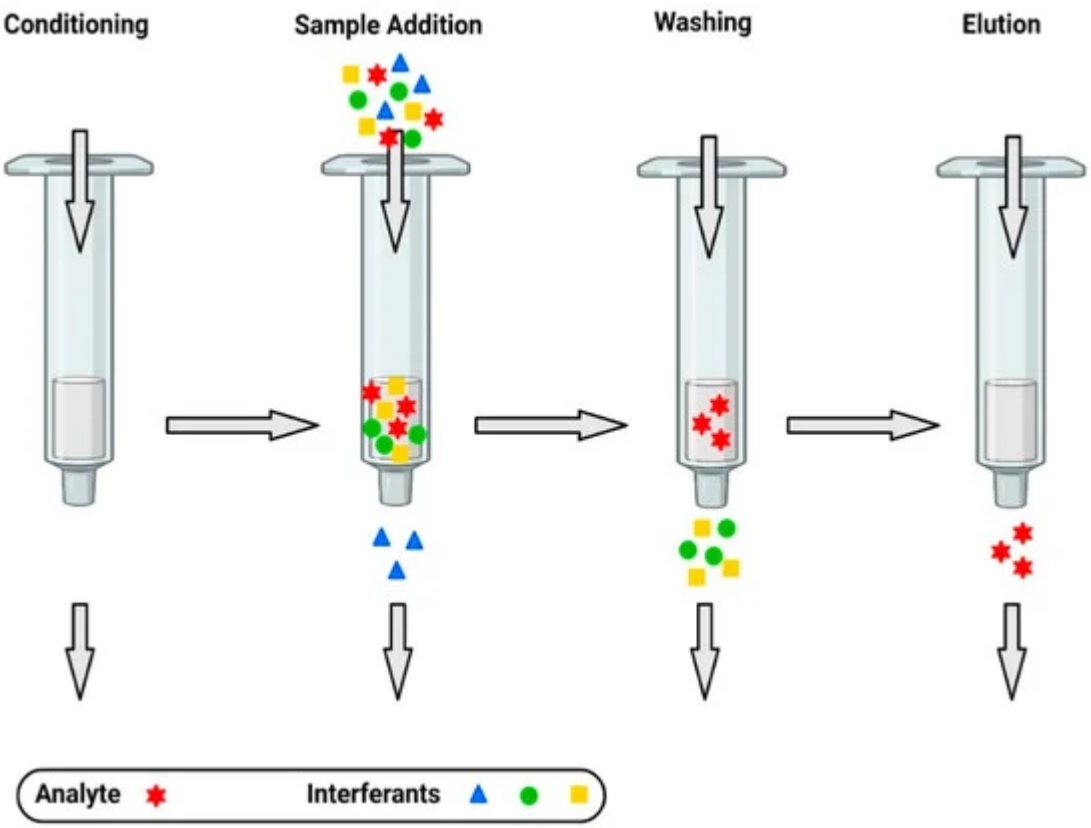


Figure 3. Typical four steps of SPE procedure.

3. Separation and Detection of Antibiotics

3.1. Chromatography

Previously, paper chromatography and thin-layer chromatography were fundamental tools for the separation, identification, and quality control of antibiotics. Later advancements of modern technology such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry(LC-MS), Ultra-High Performance Liquid Chromatography (UHPLC), and others have revolutionized the separation and determination of different antibiotics in food and other biological samples [30][31][32]. Liquid chromatography coupled with mass spectrometry is being widely used in the detection of antibiotics [33][34]. However, the high cost of a mass spectrometer limits its use in small laboratories for routine analysis. As a result of which, the LC system coupled with other detectors, mainly ultraviolet (UV), diode array detector (DAD), and fluorescence detector (FLD), are considered as potential alternatives [34][35][36][37]. For antibiotics lacking a chromophore, an evaporative light scattering detector (ELSD) is being used [38]. Such detectors are used in the detection of polypeptide antibiotics, which showed better separation, narrower peaks, and little peak tailing as compared to the traditional C18 column [39].

3.2. Capillary Electrophoresis

The chromatographic techniques require excess solvent, more time for sample preparation, and different types of stationary phases, which make the techniques more cumbersome in laboratories with limited resources [40][41]. Capillary electrophoresis (CE) is cost-effective, is simple to operate, consumes fewer reagents, and provides high separation efficiency; this technique was widely employed in the separation and detection of antibiotics in a wide range of food samples. The performance of CE can be improved by various means, such as changing buffer type, pH, voltage, mode of the CE, etc. [42][43]. Similarly, various preconcentration techniques were employed to enhance the sensitivity in CE. Apart from these, injection techniques were modified to optimize the CE [44].

CE optimization can be achieved through maintaining buffer type, pH, and voltage [45]. Buffer composition not only enhances the separation but also improves the electrophoretic characteristics of the target analytes; when electrolytes are mixed with phosphate and borate, complex formation between borate anions (tetrahydroxyborate) and tetracyclines occurs, resulting in the modification of electrophoretic characteristics and thereby separation of tetracyclines [46]. With an increase in the buffer concentration under the combined influence of the electro-osmotic flow and the electrophoretic force, the separation of the sulfonamides in the milk sample was improved. However, with the increase in the buffer concentration above optimum concentration, friction between the buffer and the inner capillary wall increased. As a result, the temperature of the column increased, resulting in the broadening of the peaks and reduction in the capillary lifetime [46][47]. It was found that the migration rate and chemiluminescence signals of sulfonamides were affected by the concentration of sodium borate buffer, and the signal intensity was found to be inversely proportional to the buffer concentration [48]. **Figure 4** depicts the synthesis of PEG@MoS2 and its application in the determination of eight sulfonamides in milk samples by dispersive solid-phase extraction (DSPE)-Capillary zone electrophoresis (CZE).

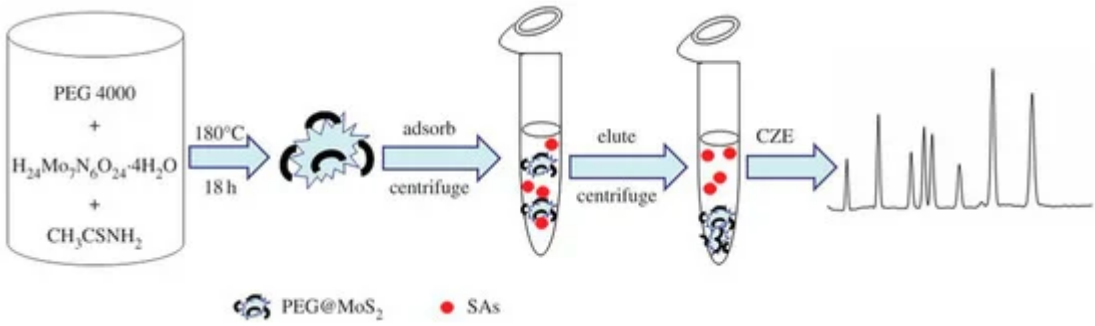


Figure 4. Schematic illustration of detection of eight sulfonamides by using DSPE-CZE [47] (Reproduced with permission of the publisher).

In CE, the pH value of the running buffer plays an important role in the separation of analytes. In the separation of tetracyclines and quinolones, a basic condition was maintained using ammonium acetate and ammonium carbonate buffers at pH 9.0 as the basic condition was found to be suitable for effective separation of these antibiotics [45]. It was also demonstrated that with an increase in pH, the separation between sulfadiazine and sulfachlorpyridazine first increased and then decreased, with the optimum pH for separation being 7.26 [47]. Similarly, the pH of the running buffer at about 9.5 was found to provide good separation of sulfamethazine, sulfadiazine, and sulfathiazole [48].

The effect of pH was significant in the separation of five macrolides from a milk sample; when the pH is less than 6.5, erythromycin and clarithromycin somewhat overlap. However, when the pH is greater than 8.0, tylosin almost completely overlaps the negative solvent peak, making it difficult to detect [49]. The baseline separation of macrolides was accomplished when the pH was between 6.5 and 7.5; in addition, the shortest migration time and the best peak shape were achieved at pH 7 [49]. Similarly, the applied voltage is another factor that affects the separation of analytes in CE; for example, adequate separation of fluoroquinolones was observed at an applied voltage of 20 kV [50]. It should be noted that the voltage applied on a certain range is better for the separation because higher voltage increases Joule heating, which represses the capillary efficiency and viscosity of the running buffer [50].

Detectors are a key component in CE, and an efficient combination of the separation capillary and a detector is required for the optimal detection of separated analytes. For effective detection of analytes using CE, the capillary was combined with various detectors such as a mass spectrometer, UV detector, light-emitting diode, etc. [45][47]. The CZE coupled with ion trap mass analyzer (for MS/MS analysis) was developed as an alternative to LC-MS/MS for the separation and detection of twelve benzimidazoles in meat samples [51]. Employing MS/MS as a detection system improves the selectivity and sensitivity of CE analysis [51]. Similarly, graphene quantum dots (GQDs) were used to enhance the sensitivity of CE with fluorescence detectors for the quantitative determination of ofloxacin in milk samples [50]. Sensitivity was determined in the presence and absence of GQDs, and a significant enhancement in sensitivity was observed when GQDs are injected into the capillary before sample loading [50]. Interestingly, it was observed that the GQDs enhance the photoluminescence of hydrophobic antibiotics, such as lomefloxacin, norfloxacin, and ofloxacin, an aqueous medium [50]. Moreover, a low-cost microchip CE system

containing an electrophoresis chip and fluorescence detector was constructed for the determination of ciprofloxacin in milk samples [52]. Apart from these, CZE using online chemiluminescence (CL) detector and CE-UV detector were used for the detection of the sulfonamides and fluoroquinolones, respectively, in food samples [48][53]. **Figure 5** depicts the schematic representation of CZE with an online chemiluminescence detector.

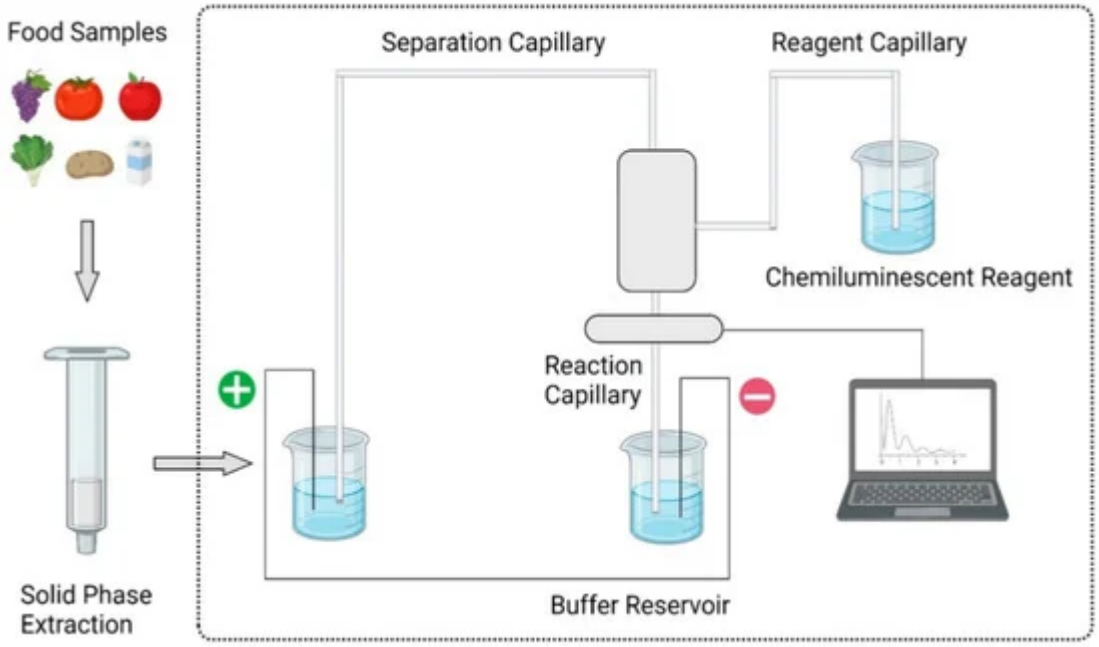


Figure 5. Schematic representation of CZE with chemiluminescence detection.

3.3. Immunological Methods

Chromatographic and CE-based methods are specific, accurate, and can be used for simultaneous determination of multiple antibiotics; however, these methods are limited by high instrumental cost and long and complicated procedures due to which on-site detection of antibiotics is not possible. Immunological assays, on the other hand, are simple, highly selective, rapid, and cost-effective; thus, they can be used for the on-site detection of antibiotics [53]. Enzyme-linked immunosorbent assay (ELISA), indirect complete ELISA (ic-ELISA), fluorescence polarization immunoassay (FPIA), immunochromatographic assay (ICA), etc., are various immunological techniques currently in use [54][55][56][57]. The principle of the immunoassays for the detection of antibiotics is shown in **Figure 6**.

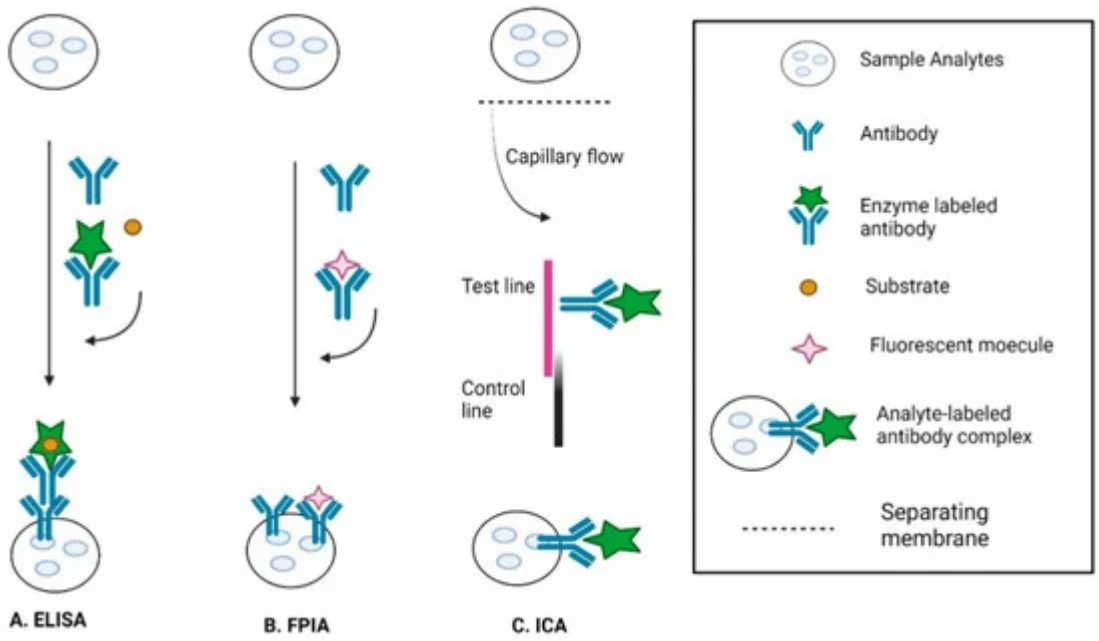


Figure 6. Schematic representation for detection of antibiotics by immunoassay methods.

The antibody is the most important factor for the development of efficient immunological assays. Immunoglobulin G (IgG)-derived antibodies from rabbit and mouse was traditionally used for the development of immunoassays. Recently, there were several studies in the use of immunoglobulin Y (IgY), a chicken egg yolk antibody, as a superior alternative to IgG. Liang et al. compared under parallel conditions to know the sensitivity, specificity, and matrix effects in the detection of sulfamethazine in milk samples and found that IgY can be an alternative to IgG for the detection of antibiotic residues in food products [58]. Similarly, Li et al. evaluated immunoglobulin Y (IgY) using FPIA and ic-ELISA to detect gentamicins/kanamycin and found that the LOD and IC_{50} values for the ic-ELISA are better than FPIA, which indicated the suitability of ic-ELISA over FPIA for detecting antibiotics in animal-derived samples [55].

Moreover, the use of monoclonal antibodies (mAbs) has widened the scope of the immunological assays; Li et al. developed an ultra-high sensitive ic-ELISA based on the broad-specificity mAbs for simultaneous detection of five antibacterial synergists (trimethoprim, diaveridine, brodimoprim, ormetoprim, and baquiloprim) in chicken and milk samples [55]. The traditional ELISA requires a time-consuming multi-step separation process, and to overcome this limitation, fluorescence polarization immunoassay (FPIA) was developed, which obviates the need for a separation step [59].

A simple fluoroimmunoassay was developed to detect tetracyclines, in which the receptor of tetracyclines, Tet repressor protein (TetR), was directionally mutated to produce two mutants that exhibited high affinity and high sensitivity for the detection of tetracyclines; the mutants were combined with fluorescence-labeled tracers for simultaneous detection of nine tetracyclines in egg samples [60]. The assay process of the Tet repressor protein (TetR) mutant-based fluoroimmunoassay for the detection of antibiotics is shown in **Figure 7**.

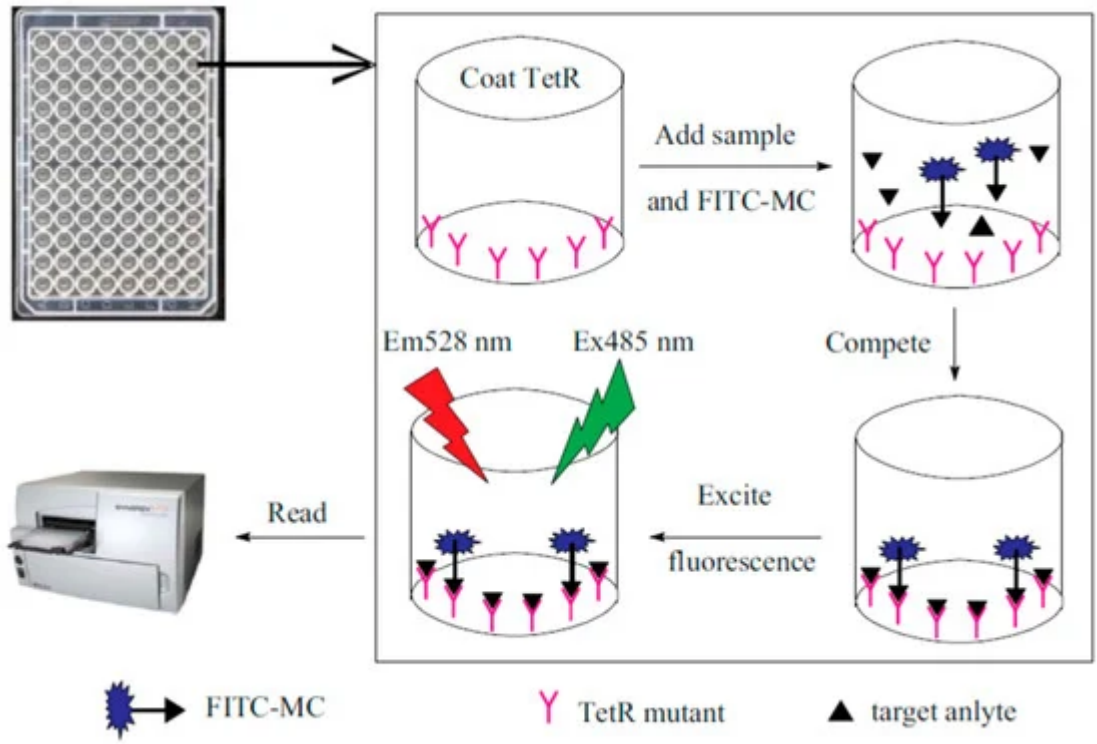


Figure 7. Schematic of the assay process of the Tet repressor protein (TetR) mutant based fluoroimmunoassay for the detection of antibiotics (Reproduced with permission of the publisher) [60].

3.4. Surface-Enhanced Raman Spectroscopy (SERS)-Based Methods

SERS is a new technique developed based on Raman spectroscopy; this technique has benefits of high detection speed, low detection cost, and simple operation, and the high statistical binding of target analytes to active sites or "hot spots" of noble metal nanostructures is crucial for enhanced detection sensitivity [61]. The core components of a SERS-based method are a target molecule, a metal nanostructure, and electromagnetic radiation [62]. Previously, metal or metal oxide film layers were deposited on the surface to enhance the Raman signal to detect antibiotics. Recently, the majority of SERS-based sensor substrates were modified with metal nanoparticles [63], primarily gold or silver colloids. For instance, ciprofloxacin was physically adsorbed on a self-assembling gold nanofilm for quantitative detection, and SERS was utilized to detect it in fish [64]. A schematic of the pretreatment of fish samples followed by SERS detection on the gold nanofilm is shown in **Figure 8**. Jiang et al. assembled silver nanoparticles on a glass substrate to prepare SERS substrate to detect benzylpenicillin sodium [65].

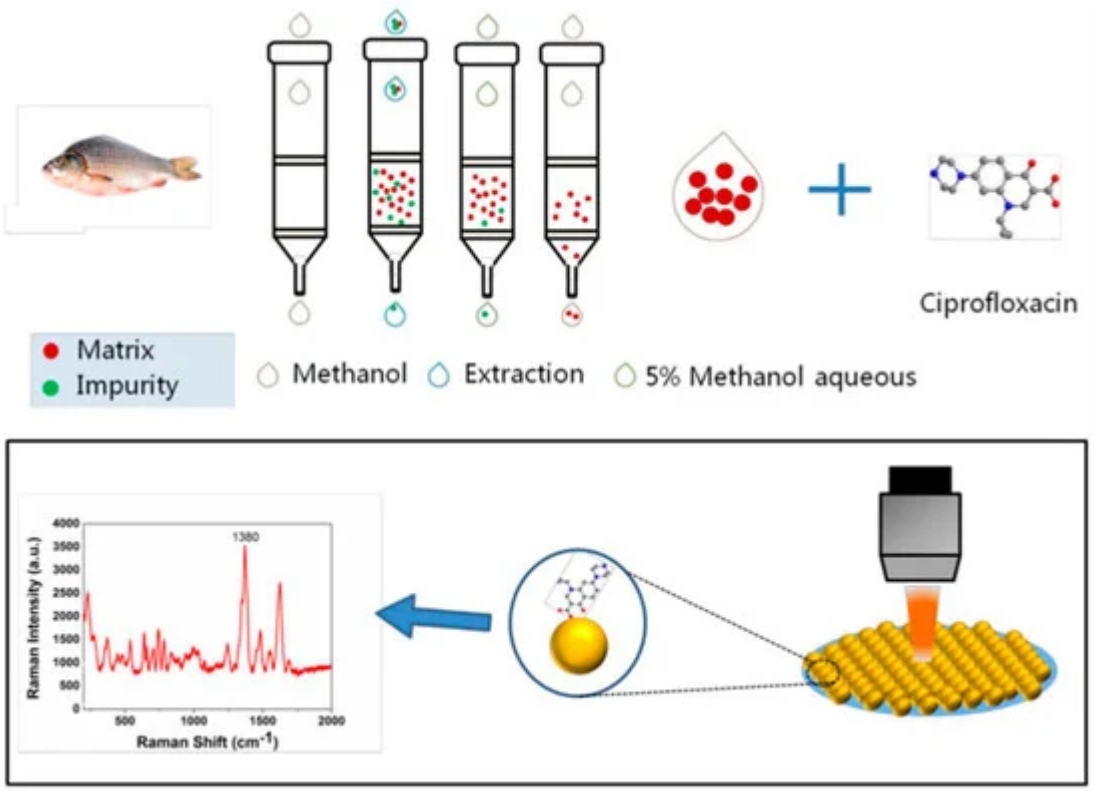


Figure 8. Schematic of the pretreatment of fish samples with SERS detection of ciprofloxacin on a self-assembled gold nanofilm (Reproduced with permission of the publisher) [64].

3.5. Biosensors

A biosensor is a functional integrated device that combines a biological recognition element or bioreceptor for generating signals that vary as a function of the concentration of analytes present in a sample [66]. Biosensors emerged as an innovative alternative tool for rapid, sensitive, and on-site screening of antibiotic residues in food products [22]. Currently, fluorescent, electrochemical, colorimetric, surface plasmon resonance (SPR), and quartz crystal microbalance (QCM) biosensor techniques are most commonly used for the screening of antibiotic residues [67][68]. Fluorescent biosensors are powerful analytical tools for the detection of antibiotics present in different matrices due to their inherent advantages, such as high sensitivity, high selectivity, operation convenience, rapid hybridization kinetics, and ease of automation [69]. Fluorescent biosensors based on GO and AuNPs nanomaterial-based quenchers are used for antibiotics detection. Tan et al. designed a GO hydrogel-based fluorescence aptasensor with ssDNA as the recognition element for oxytetracycline detection, and the limit of quantitation (LOQ) of 25 µg/L was achieved [70]. In other studies, an aptamer-based fluorescent biosensor was developed to detect chloramphenicol using MNPs and UCNPs [71][72]. Similarly, Yue et al. reported sensitive fluorescent biosensors based on UCNPs for the detection of kanamycin [73]. The schematic representation of biosensor-based detection of antibiotics is shown in **Figure 9**.

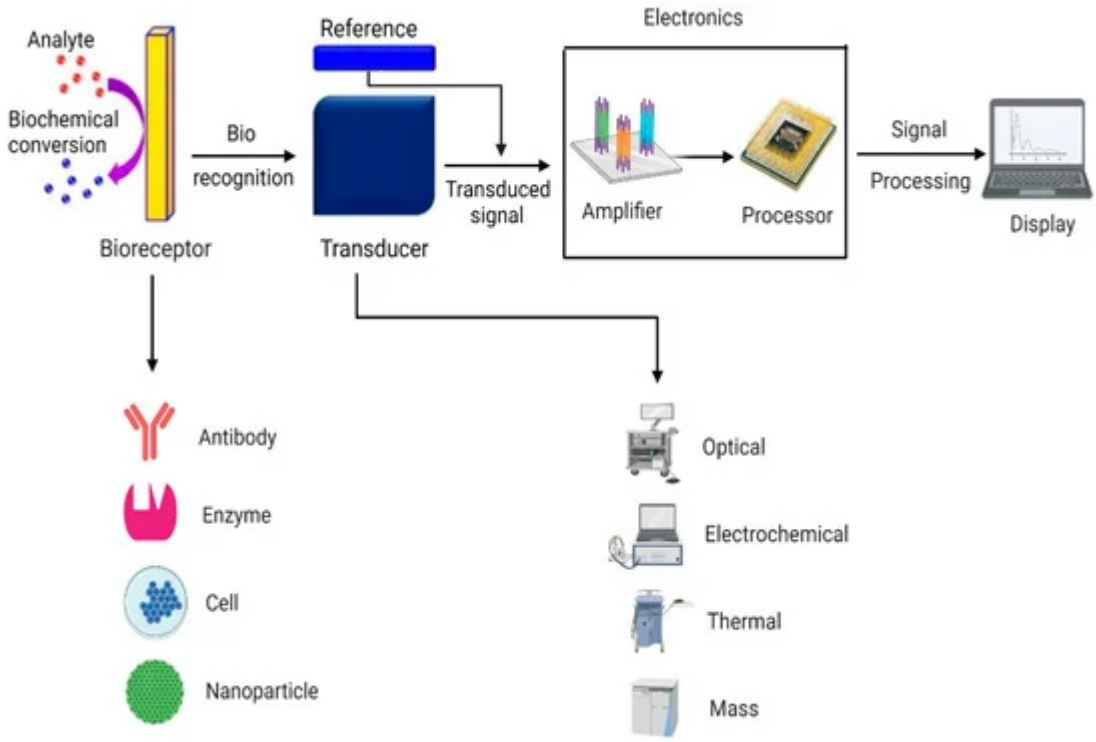


Figure 9. Biosensor-based detection of antibiotics.

References

1. Chandler, C.I.R. Current Accounts of Antimicrobial Resistance: Stabilisation, Individualisation and Antibiotics as Infrastructure. Palgrave Commun. 2019, 5, 53.
2. Angelakis, E.; Merhej, V.; Raoult, D. Related Actions of Probiotics and Antibiotics on Gut Microbiota and Weight Modification. Lancet Infect. Dis. 2013, 13, 889–899.
3. Menkem, Z.E.; Ngangom, B.L.; Tamunjoh, S.S.A.; Boyom, F.F. Antibiotic Residues in Food Animals: Public Health Concern. Acta Ecol. Sin. 2019, 39, 411–415.
4. Gothwal, R.; Shashidhar, T. Antibiotic Pollution in the Environment: A Review: Antibiotic Pollution in the Environment. Clean Soil Air Water 2015, 43, 479–489.
5. Ji, K.; Kho, Y.; Park, C.; Paek, D.; Ryu, P.; Paek, D.; Kim, M.; Kim, P.; Choi, K. Influence of Water and Food Consumption on Inadvertent Antibiotics Intake among General Population. Environ. Res. 2010, 110, 641–649.
6. Santos, L.; Ramos, F. Analytical Strategies for the Detection and Quantification of Antibiotic Residues in Aquaculturefishes: A Review. Trends Food Sci. Technol. 2016, 52.
7. Joshi, A.; Kim, K.-H. Recent Advances in Nanomaterial-Based Electrochemical Detection of Antibiotics: Challenges and Future Perspectives. Biosens. Bioelectron. 2020, 153, 112046.

8. Bueno, I.; Williams-Nguyen, J.; Hwang, H.; Sargeant, J.M.; Nault, A.J.; Singer, R.S. Systematic Review: Impact of Point Sources on Antibiotic-Resistant Bacteria in the Natural Environment. Zoonoses Public Health 2018, 65, e162–e184.
9. Rozman, U.; Duh, D.; Cimerman, M.; Turk, S.Š. Hospital Wastewater Effluent: Hot Spot for Antibiotic Resistant Bacteria. J. Water Sanit. Hyg. Dev. 2020, 10, 171–178.
10. Petrovich, M.L.; Zilberman, A.; Kaplan, A.; Eliraz, G.R.; Wang, Y.; Langenfeld, K.; Duhaime, M.; Wigginton, K.; Poretsky, R.; Avisar, D.; et al. Microbial and Viral Communities and Their Antibiotic Resistance Genes Throughout a Hospital Wastewater Treatment System. Front. Microbiol. 2020, 11, 153.
11. Kumar, A.; Pal, D. Antibiotic Resistance and Wastewater: Correlation, Impact and Critical Human Health Challenges. J. Environ. Chem. Eng. 2018, 6, 52–58.
12. Hutchings, M.I.; Truman, A.W.; Wilkinson, B. Antibiotics: Past, Present and Future. Curr. Opin. Microbiol. 2019, 51, 72–80.
13. Coates, A.R.; Halls, G.; Hu, Y. Novel Classes of Antibiotics or More of the Same?: New Antibiotic Classes Are Urgently Needed. Br. J. Pharmacol. 2011, 163, 184–194.
14. Ha, J.; Song, G.; Ai, L.-F.; Li, J.-C. Determination of Six Polyether Antibiotic Residues in Foods of Animal Origin by Solid Phase Extraction Combined with Liquid Chromatography-Tandem Mass Spectrometry. J. Chromatogr. B 2016, 1017–1018, 187–194.
15. Chen, J.; Ying, G.-G.; Deng, W.-J. Antibiotic Residues in Food: Extraction, Analysis, and Human Health Concerns. J. Agric. Food Chem. 2019, 67, 7569–7586.
16. Phillips, I. Does the Use of Antibiotics in Food Animals Pose a Risk to Human Health? A Critical Review of Published Data. J. Antimicrob. Chemother. 2003, 53, 28–52.
17. Kneebone, J.; Tsang, P.C.W.; Towson, D.H. Rapid Antibiotic Screening Tests Detect Antibiotic Residues in Powdered Milk Products. J. Dairy Sci. 2010, 93, 3961–3964.
18. Jammoul, A.; El Darra, N. Evaluation of Antibiotics Residues in Chicken Meat Samples in Lebanon. Antibiotics 2019, 8, 69.
19. Farouk, F.; Azzazy, H.M.E.; Niessen, W.M.A. Challenges in the Determination of Aminoglycoside Antibiotics, a Review. Anal. Chim. Acta 2015, 890, 21–43.
20. Larsson, D.G.J. Antibiotics in the Environment. Upsala J. Med. Sci. 2014, 119, 108–112.
21. Majdinasab, M.; Mishra, R.K.; Tang, X.; Marty, J.L. Detection of Antibiotics in Food: New Achievements in the Development of Biosensors. TrAC Trends Anal. Chem. 2020, 127, 115883.
22. Khan, M.Z.H. Recent Biosensors for Detection of Antibiotics in Animal Derived Food. Crit. Rev. Anal. Chem. 2020, 1–11.

23. Colombo, R.; Papetti, A. Advances in the Analysis of Veterinary Drug Residues in Food Matrices by Capillary Electrophoresis Techniques. Molecules 2019, 24, 4617.
24. Li, W.; Dai, X.; Pu, E.; Bian, H.; Chen, Z.; Zhang, X.; Guo, Z.; Li, P.; Li, H.; Yong, Y.; et al. HLB-MCX-Based Solid-Phase Extraction Combined with Liquid Chromatography–Tandem Mass Spectrometry for the Simultaneous Determination of Four Agricultural Antibiotics (Kasugamycin, Validamycin A, Ningnanmycin, and Polyoxin B) Residues in Plant-Origin Foods. J. Agric. Food Chem. 2020, 68, 14025–14037.
25. Di, S.; Yu, J.; Chen, P.; Zhu, G.; Zhu, S. Net-like Mesoporous Carbon Nanocomposites for Magnetic Solid-Phase Extraction of Sulfonamides Prior to Their Quantitation by UPLC-HRMS. Microchim. Acta 2020, 187, 112.
26. Alampanos, V.; Samanidou, V.; Papadoyannis, I. Trends in Sample Preparation for the HPLC Determination of Penicillins in Biofluids. J. Appl. Bioanal. 2019, 5, 9–17.
27. Sun, Y.; Zhao, J.; Liang, L. Recent Development of Antibiotic Detection in Food and Environment: The Combination of Sensors and Nanomaterials. Microchim. Acta 2021, 188, 21.
28. Xu, G.; Zhang, B.; Wang, X.; Li, N.; Zhao, Y.; Liu, L.; Lin, J.-M.; Zhao, R.-S. Porous Covalent Organonitridic Frameworks for Solid-Phase Extraction of Sulfonamide Antibiotics. Mikrochim. Acta 2018, 186, 26.
29. Pérez-Rodríguez, M.; Pellerano, R.G.; Pezza, L.; Pezza, H.R. An Overview of the Main Foodstuff Sample Preparation Technologies for Tetracycline Residue Determination. Talanta 2018, 182, 1–21.
30. Li, T.; Wang, C.; Xu, Z.; Chakraborty, A. A Coupled Method of On-Line Solid Phase Extraction with the UHPLC–MS/MS for Detection of Sulfonamides Antibiotics Residues in Aquaculture. Chemosphere 2020, 254, 126765.
31. Xu, G.; Dong, X.; Hou, L.; Wang, X.; Liu, L.; Ma, H.; Zhao, R.-S. Room-Temperature Synthesis of Flower-Shaped Covalent Organic Frameworks for Solid-Phase Extraction of Quinolone Antibiotics. Anal. Chim. Acta 2020, 1126, 82–90.
32. Betina, V. Bioautography in Paper and Thin-Layer Chromatography and Its Scope in the Antibiotic Field. J. Chromatogr. A 1973, 78, 41–51.
33. Zhao, Y.; Wu, R.; Yu, H.; Li, J.; Liu, L.; Wang, S.; Chen, X.; Chan, T.-W.D. Magnetic Solid-Phase Extraction of Sulfonamide Antibiotics in Water and Animal-Derived Food Samples Using Core-Shell Magnetite and Molybdenum Disulfide Nanocomposite Adsorbent. J. Chromatogr. A 2020, 1610, 460543.
34. Song, X.; Zhou, T.; Li, J.; Su, Y.; Xie, J.; He, L. Determination of Macrolide Antibiotics Residues in Pork Using Molecularly Imprinted Dispersive Solid-Phase Extraction Coupled with LC-MS/MS. J. Sep. Sci. 2018, 41, 1138–1148.

35. Armentano, A.; Summa, S.; Lo Magro, S.; Palermo, C.; Nardiello, D.; Centonze, D.; Muscarella, M. Rapid Method for the Quantification of 13 Sulphonamides in Milk by Conventional High-Performance Liquid Chromatography with Diode Array Ultraviolet Detection Using a Column Packed with Core-Shell Particles. J. Chromatogr. A 2018, 1531, 46–52.
36. Hui, W.; Li, Q.; Ma, H.; Wu, M.; Feng, K.; Zhu, H.; Yang, P.; Li, J.; Chen, C.; Yan, K. Rapid Screening for 15 Sulfonamide Residues in Foods of Animal Origin by High-Performance Liquid Chromatography–UV Method. J. Chromatogr. Sci. 2018, 56, 636–643.
37. Łukaszewicz, P.; Białk-Bielińska, A.; Dołżonek, J.; Kumirska, J.; Caban, M.; Stepnowski, P. A New Approach for the Extraction of Tetracyclines from Soil Matrices: Application of the Microwave-Extraction Technique. Anal. Bioanal. Chem. 2018, 410, 1697–1707.
38. Wang, Z.; Song, X.; Zhou, T.; Bian, K.; Zhang, F.; He, L.; Liu, Q. Simultaneous Determination of Ten Macrolides Drugs in Feeds by High Performance Liquid Chromatography with Evaporation Light Scattering Detection. RSC Adv. 2015, 5, 1491–1499.
39. Song, X.; Xie, J.; Zhang, M.; Zhang, Y.; Li, J.; Huang, Q.; He, L. Simultaneous Determination of Eight Cyclopolypeptide Antibiotics in Feed by High Performance Liquid Chromatography Coupled with Evaporation Light Scattering Detection. J. Chromatogr. B 2018, 1076, 103–109.
40. Lu, Z.; Deng, F.; He, R.; Tan, L.; Luo, X.; Pan, X.; Yang, Z. A Pass-through Solid-Phase Extraction Clean-up Method for the Determination of 11 Quinolone Antibiotics in Chicken Meat and Egg Samples Using Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry. Microchem. J. 2019, 151, 104213.
41. Yu, H.; Jia, Y.; Wu, R.; Chen, X.; Chan, T.-W.D. Determination of Fluoroquinolones in Food Samples by Magnetic Solid-Phase Extraction Based on a Magnetic Molecular Sieve Nanocomposite Prior to High-Performance Liquid Chromatography and Tandem Mass Spectrometry. Anal. Bioanal. Chem. 2019, 411, 2817–2826.
42. He, T.; Xu, Z.; Ren, J. Pressure-Assisted Electrokinetic Injection Stacking for Seven Typical Antibiotics in Waters to Achieve Mg/L Level Analysis by Capillary Electrophoresis with UV Detection. Microchem. J. 2019, 146, 1295–1300.
43. Moreno-González, D.; Krulišová, M.; Gámiz-Gracia, L.; García-Campaña, A.M. Determination of Tetracyclines in Human Urine Samples by Capillary Electrophoresis in Combination with Field Amplified Sample Injection. Electrophoresis 2018, 39, 608–615.
44. Ferreira, T.A.; Flores-Aguilar, J.F.; Santos, E.M.; Rodriguez, J.A.; Ibarra, I.S. New Poly(Ionic Liquid) Based Fiber for Determination of Oxytetracycline in Milk Samples by Application of SPME-CE Technique. Molecules 2019, 24, 430.
45. Moreno-González, D.; Hamed, A.M.; Gilbert-López, B.; Gámiz-Gracia, L.; García-Campaña, A.M. Evaluation of a Multiresidue Capillary Electrophoresis-Quadrupole-Time-of-Flight Mass

Spectrometry Method for the Determination of Antibiotics in Milk Samples. J. Chromatogr. A 2017, 1510, 100–107.

46. Díaz-Quiroz, C.A.; Francisco Hernández-Chávez, J.; Ulloa-Mercado, G.; Gortáres-Moroyoqui, P.; Martínez-Macías, R.; Meza-Escalante, E.; Serrano-Palacios, D. Simultaneous Quantification of Antibiotics in Wastewater from Pig Farms by Capillary Electrophoresis. J. Chromatogr. B 2018, 1092, 386–393.
47. An, J.; Wang, X.; Ming, M.; Li, J.; Ye, N. Determination of Sulfonamides in Milk by Capillary Electrophoresis with as a Dispersive Solid-Phase Extraction Sorbent. R. Soc. Open Sci. 2018, 5, 172104.
48. Dai, T.; Duan, J.; Li, X.; Xu, X.; Shi, H.; Kang, W. Determination of Sulfonamide Residues in Food by Capillary Zone Electrophoresis with On-Line Chemiluminescence Detection Based on an Ag(III) Complex. Int. J. Mol. Sci. 2017, 18, 1286.
49. Hong, Y.; Guo, X.; Chen, G.; Zhou, J.; Zou, X.; Liao, X.; Hou, T. Determination of Five Macrolide Antibiotic Residues in Milk by Micellar Electrokinetic Capillary Chromatography with Field Amplified Sample Stacking. J. Food Saf. 2018, 38.
50. Lahouidak, S.; Soriano, M.L.; Salghi, R.; Zougagh, M.; Ríos, Á. Graphene Quantum Dots for Enhancement of Fluorimetric Detection Coupled to Capillary Electrophoresis for Detection of Ofloxacin. Electrophoresis 2019, 40, 2336–2341.
51. Tejada-Casado, C.; Moreno-González, D.; Lara, F.J.; García-Campaña, A.M.; del Olmo-Iruela, M. Determination of Benzimidazoles in Meat Samples by Capillary Zone Electrophoresis Tandem Mass Spectrometry Following Dispersive Liquid–Liquid Microextraction. J. Chromatogr. A 2017, 1490, 212–219.
52. Bosma, R.; Devasagayam, J.; Singh, A.; Collier, C.M. Microchip Capillary Electrophoresis Dairy Device Using Fluorescence Spectroscopy for Detection of Ciprofloxacin in Milk Samples. Sci. Rep. 2020, 10, 1–8.
53. Tian, Z.; Gao, J.; Qin, W. Determination of Fluoroquinolones in Milk by Ionic Liquid-Mediated Two Phase Extraction Followed by Capillary Electrophoresis Analysis. Madr. J. Anal. Sci. Instrum. 2018, 3, 62–67.
54. Kergaravat, S.V.; Nagel, O.G.; Althaus, R.L.; Hernández, S.R. Magneto Immunofluorescence Assay for Quinolone Detection in Bovine Milk. Food Anal. Methods 2020, 13, 1539–1547.
55. Li, C.; Zhang, Y.; Eremin, S.A.; Yakup, O.; Yao, G.; Zhang, X. Detection of Kanamycin and Gentamicin Residues in Animal-Derived Food Using IgY Antibody Based Ic-ELISA and FPIA. Food Chem. 2017, 227, 48–54.
56. Li, H.; Ma, S.; Zhang, X.; Li, C.; Dong, B.; Mujtaba, M.G.; Wei, Y.; Liang, X.; Yu, X.; Wen, K.; et al. Generic Hapten Synthesis, Broad-Specificity Monoclonal Antibodies Preparation, and

Ultrasensitive ELISA for Five Antibacterial Synergists in Chicken and Milk. J. Agric. Food Chem. 2018, 66, 11170–11179.

57. Wang, Z.; Hu, S.; Bao, H.; Xing, K.; Liu, J.; Xia, J.; Lai, W.H.; Peng, J. Immunochromatographic Assay Based on Time-Resolved Fluorescent Nanobeads for the Rapid Detection of Sulfamethazine in Egg, Honey, and Pork. J. Sci. Food Agric. 2020.
58. Liang, X.; Sheng, Y.; Yu, W.; Zhao, S.; Shan, H.; Zhang, Q.; Wang, Z. Comparison of Chicken IgY and Mammalian IgG in Three Immunoassays for Detection of Sulfamethazine in Milk. Food Anal. Methods 2018, 11, 3452–3463.
59. Shen, X.; Chen, J.; Lv, S.; Sun, X.; Dzantiev, B.B.; Eremin, S.A.; Zherdev, A.V.; Xu, J.; Sun, Y.; Lei, H. Fluorescence Polarization Immunoassay for Determination of Enrofloxacin in Pork Liver and Chicken. Molecules 2019, 24, 4462.
60. Wang, G.; Xia, W.Q.; Liu, J.X.; Wang, J.P.; Liu, J. Directional Evolution of TetR Protein and Development of a Fluoroimmunoassay for Screening of Tetracyclines in Egg. Microchem. J. 2019, 150, 104184.
61. Liu, H.; Sun, Y.; Jin, Z.; Yang, L.; Liu, J. Capillarity-Constructed Reversible Hot Spots for Molecular Trapping inside Silver Nanorod Arrays Light up Ultrahigh SERS Enhancement. Chem. Sci. 2013, 4, 3490–3496.
62. Jiang, Y.; Sun, D.-W.; Pu, H.; Wei, Q. Surface Enhanced Raman Spectroscopy (SERS): A Novel Reliable Technique for Rapid Detection of Common Harmful Chemical Residues. Trends Food Sci. Technol. 2018, 75, 10–22.
63. Wang, Q.; Zhao, W.-M. Optical Methods of Antibiotic Residues Detections: A Comprehensive Review. Sens. Actuators B Chem. 2018, 269, 238–256.
64. Zhang, Y.; Teng, Y.; Qin, Y.; Ren, Z.; Wang, Z. Determination of Ciprofloxacin in Fish by Surface-Enhanced Raman Scattering Using a Liquid-Liquid Self-Assembled Gold Nanofilm. Anal. Lett. 2020, 53, 660–670.
65. Jiang, X.; Qin, X.; Yin, D.; Gong, M.; Yang, L.; Zhao, B.; Ruan, W. Rapid Monitoring of Benzylpenicillin Sodium Using Raman and Surface Enhanced Raman Spectroscopy. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 2015, 140, 474–478.
66. Bhalla, N.; Jolly, P.; Formisano, N.; Estrela, P. Introduction to Biosensors. Essays Biochem. 2016, 60, 1–8.
67. Mungroo, N.; Neethirajan, S. Biosensors for the Detection of Antibiotics in Poultry Industry—A Review. Biosensors 2014, 4, 472–493.
68. Dixon, M.C. Quartz Crystal Microbalance with Dissipation Monitoring: Enabling Real-Time Characterization of Biological Materials and Their Interactions. J. Biomol. Tech. JBT 2008, 19,

151–158.

69. Shen, Y.; Xu, L.; Li, Y. Biosensors for Rapid Detection of Salmonella in Food: A Review. Compr. Rev. Food Sci. Food Saf. 2021, 20, 149–197.
70. Tan, B.; Zhao, H.; Du, L.; Gan, X.; Quan, X. A Versatile Fluorescent Biosensor Based on Target-Responsive Graphene Oxide Hydrogel for Antibiotic Detection. Biosens. Bioelectron. 2016, 83, 267–273.
71. Gaudin, V. Advances in Biosensor Development for the Screening of Antibiotic Residues in Food Products of Animal Origin-A Comprehensive Review. Biosens. Bioelectron. 2017, 90, 363–377.
72. Wu, S.; Zhang, H.; Shi, Z.; Duan, N.; Fang, C.; Dai, S.; Wang, Z. Aptamer-Based Fluorescence Biosensor for Chloramphenicol Determination Using Upconversion Nanoparticles. Food Control 2015, 50, 597–604.
73. Yue, F.; Li, F.; Kong, Q.; Guo, Y.; Sun, X. Recent Advances in Aptamer-Based Sensors for Aminoglycoside Antibiotics Detection and Their Applications. Sci. Total Environ. 2021, 762, 143129.