

Review

# Fluorescence Polarization Assays for Organic Compounds in Food Safety

Liliya I. Mukhametova<sup>1</sup>, Sergei A. Eremin<sup>1,\*</sup><sup>1</sup>Chemical Department, Lomonosov Moscow State University, 119991 Moscow, Russia\*Correspondence: [eremin\\_serгей@hotmail.com](mailto:eremin_serгей@hotmail.com) (Sergei Eremin)

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## Abstract

Elevated concentrations of toxic organic compounds observed in food products pose serious dangers to human health. Both natural and artificial pollutants can cause food contamination. The stages of food production, packaging, transportation, and storage can also largely cause the appearance of undesirable substances in food products. The health consequences of ingesting food containing toxic contaminants range from mild gastroenteritis to deaths resulting from dysfunctional internal organs and neurological syndromes. The World Health Organization (WHO) sets recommendations for the content of such chemicals in food, including a minimum allowable concentration considered safe for human consumption. However, the control of food products from chemical pollutants is necessary. Moreover, fast, sensitive, and inexpensive methods are needed to detect them at the point of need. Currently, immune analysis methods are most widely used to determine pollutants in food. The development of fluorescence polarization immunoassay (FPIA) methods in a competitive format is a powerful and modern tool for detecting organic molecules in various matrices, thereby making FPIA methods useful for food safety applications. Due to the availability of portable devices for measuring the fluorescence polarization signal, FPIA methods can be used at the point of need. The variety of fluorescent labels and recognizing elements (receptors, monoclonal and polyclonal antibodies, and nanobodies) permits fluorescence polarization (FP) assays to detect significantly lower limits of organic substances. The FP assay is a homogeneous, fast, and quantitative method. The development of various formats of FP assays makes them promising in determining food pollutants. This review summarizes publications on FP analyses for detecting organic contaminants (pesticides, hormones, toxins, antibiotics, and other pharmaceuticals) in food products during 2018–2023. Further, it demonstrates the prospects for using this method to determine pollutants at the point of need and for detecting high molecular weight substances, fungi, and bacterial infections during food safety inspections.

**Keywords:** fluorescence polarization; food contamination monitoring; nanotechnology; point-of-need sensors; pesticides; mycotoxins; antibiotics; phthalates

## 1. Introduction

Organic contaminants are dangerous chemicals that can cause severe harm to human health and disrupt the Earth's ecosystem. Ensuring food safety and quality is an urgent public health concern [1]. Food safety directly affects the lives and health of people and represents the main problem in modern life. Food contamination can be caused by natural environmental pollutants and those artificially introduced by humans [2]. Organic pollutants in food, water, air, and soil are mainly from industrial wastes, food additives, natural poisons, pesticides, pharmaceuticals used in agriculture and medicine, and substances generated during food preparation and packaging [1–3]. Pesticides and other agrochemicals are important tools for farmers to reduce crop losses and increase yields. However, their use must be strictly regulated and implemented correctly to avoid threats to human health and the environment. Veterinary drugs are used to combat animal diseases and improve production. They can get into food products and pose a danger to human health. Pesticides and veterinary drugs (for example, antibiotics) can get into

food products during the growth of plants and animals; mycotoxins—secondary metabolites of some fungi—can contaminate both human and animal food during storage. Mycotoxins are hazardous to the health and productivity of humans and animals, meaning they should be closely monitored and controlled. Another possible way for toxic organic compounds to get into food is during its storage and packaging. Currently, the widespread use of plastic tableware and packaging has led to the discovery of compounds such as phthalates and bisphenol A. These compounds cause oncological diseases, suppress reproductive functions, disrupt the endocrine system (EDS), delay brain development in children, and promote diabetes, obesity, and cardiovascular diseases. People are exposed to harmful organic substances primarily through food, water, and air. Thus, food pollutants can cause severe illnesses, from acute gastroenteritis to oncological diseases [1]. According to reports by the World Health Organization (WHO), about two million people die annually worldwide from diseases associated with food contamination [3]. Thus, the WHO repeatedly emphasizes that food products must be non-toxic and safe. Food production companies must use approved



safety standards, such as Food Safety System Certification 22000 (FSSC22000) and Hazard Analysis and Critical Control Point (HACCE). However, the consumer may still encounter food products containing harmful organic substances. Therefore, products need to be checked to detect the presence of undesirable organic substances. Some reviews have previously analyzed the detection of antibiotics [4,5] and the chemical organic compounds found in food [6–9] and water [10,11].

Various modern technologies are currently used to detect low molecular weight organic substances in food products. Therefore, the development of highly sensitive and selective analytical methods required to determine the contaminants in food at low concentrations is of great importance for ensuring food quality and safety. Presently, there are numerous analytical techniques for detecting biological and chemical pollutants in food products. Traditional methods for determining biological pollutants are either culture-based, for example, bacterial counting [12], immunoanalysis (ELISA), or immunochromatographic (ICA) [5,7,9], alongside fluorescence polarization (FP) assays, and DNA-based methods, including polymerase chain reaction (PCR) [13]. Instrumental methods, such as liquid chromatography (LC) or gas chromatography (GC) and mass spectrometry (MS), are often used to discover chemical pollutants [14,15]. Although these instrumental methods are highly sensitive and reliable, they require longer analysis times, sample pre-processing, and a highly qualified specialist and are expensive and complicated tools; these requirements significantly complicate their more extensive uses. Therefore, the food industry needs to develop alternative methods with fast responses, reliable specificity, and sensitivity.

Alternative methods include using biological sensors, which are rapid, specific, selective, sensitive, and efficient tools for monitoring food contamination [16,17]. Since 1852, when Stokes coined the term fluorescence, many phenomena and properties of fluorescent materials have been applied to detect and analyze chemicals and biomolecules [18]. Biosensor systems use a variety of fluorescence mechanisms: Fluorescence polarization (FP) [19,20], fluorescence resonance energy transfer (FRET) [21], fluorescence intensity (FI) endpoint measurement [20], fluorescence lifetime imaging microscopy [18], and time-resolved fluorescence immunoassay [22]. These systems demonstrated outstanding performance and can rapidly determine the different analytes. Among them, fluorescence polarization is the most promising method for analyzing the small organic molecules found in food products. Homogeneous mix-and-read FP assays deserve special attention due to them providing several key advantages, such as the possibility of automation, the use of portable equipment, the high stability of reagents, the ease of experiment, and most importantly, the fact that they do not require the use of radioactive substances. Since the 1980s, when the first commercial device for FP investigation (TDx® analyzer (Abbott Diagnostics, Maidenhead, UK) was released, FP meth-

ods have undergone extensive development. The creation of microplate fluorimeters that possess the ability to measure FP contributed to the miniaturization of analysis in microplates with simultaneous measurements of 96, 384, and 1536 reactions, while also being used in clinical diagnostics and imaging, drug development, research of protein/protein interactions, high-throughput screening, and determining nanoparticles sizes [23]. In addition, the release of the portable devices Sentry-100, 200, and 300 by Ellie made it possible to determine low-molecular-weight analytes remotely (<https://ellielab.com/sentry-300/>). Nowadays, the fluorescence polarization assay is a modern tool for detecting organic compounds in food and the environment [19,24–27]. The achievements of the FP method in antibiotic detection [28], mycotoxins [29,30], and pesticides [31] have already been described in previously published reviews.

FP analyses have made significant progress due to the creation of new promising materials and labeling technologies and the development of nanotechnology. This review briefly describes the basic principles of the fluorescence polarization method and the latest achievements of FP analyses in the rapid detection of low-molecular-weight organic compounds in food and environmental objects. Particular emphasis has been placed on the achievements of the FP analysis of plasticizers, veterinary drugs, mycotoxins, pesticides, and drugs. The fluorescent dyes, recognition elements used in the FP assay, and various analysis formats have also been considered. Strategies for multiplex detection of organic pollutants and using new materials to enhance the FP signal are described. In addition, prospects for using FP in the detection of high-molecular-weight organic compounds, viruses, and protease inhibitors are presented.

## 2. Bases Principals of Fluorescence Polarization

The principles of the fluorescence polarization methods have been described in sufficient detail previously [19,32]. Here, we will briefly review the important aspects. Perrin elucidated the theoretical foundations of FP in 1926, establishing the phenomenon that the emission of a small fluorescent molecule excited by plane-polarized light is depolarized due to rotational diffusion during the fluorescence lifetime [32,33]. Perrin derived Eqn. 1, which describes the relationship between the FP value and the molecule volume (1):

$$\frac{1}{FP} - \frac{1}{3} = \left( \frac{1}{FP_0} - \frac{1}{3} \right) \left( 1 + \frac{RT}{\eta V} \tau \right) \quad (1)$$

where FP is the detectable FP, R is the universal gas constant, T is the temperature,  $\eta$  is the viscosity of the solution,  $FP_0$  is the internal FP, V is the volume of the molecule, and  $\tau$  is the fluorescence lifetime of the excited fluorophore. Thus, Eqn. 1 demonstrates that the observed FP hinges on

the volume of the fluorescent molecules at a constant temperature and viscosity. Therefore, FP over a specific molecular weight range, where the volume of a molecule is proportional to the degree of FP, can be used to detect biological processes that are accompanied by a change in the molecular weight of a substance, for example, during binding or enzymatic degradation of a substrate.

Currently, various studies use the FP measurement to show the changes in the size of molecules during their interaction. A schematic diagram of FP signal measurement is shown in Fig. 1. Polarized light is produced by a light source of a given wavelength, which subsequently passes through a polarizing filter, where the vertically polarized light is elected. The fluorescence molecules are excited by polarized light, which then passes through polarizing filters to produce vertically and horizontally polarized light [34–37]. To detect FP, both the parallel ( $I_{\parallel}$ ) and perpendicular ( $I_{\perp}$ ) lights are measured. There are usually two detectors for reading the intensity of the fluorescence value or one detector for double reading through a polarizing filter that can be rotated  $90^{\circ}$ .

These measurements can be applied to quantify FP or fluorescence anisotropy (A), both of which are now widely used. Eqn. 2 shows the FP and A calculations, which are used to calculate the difference between the parallel ( $I_{\parallel}$ ) and perpendicular ( $I_{\perp}$ ) fluorescence intensities via normalization to the total intensity of the emitting light:

$$FP = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} ; A = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \quad (2)$$

Notably, the value of A does not provide important additional information, yet both functions apply. In some experiments, anisotropy is preferable since anisotropy decomposition into its component values seems to be simpler than for FP. In addition, the total fluorescence intensity calculated as the denominator in the equation is useful for labeling fluorescent molecules.

It is convenient to investigate the antigen/antibody interaction by FPIA should their masses differ significantly in comparison. It is preferable to synthesize fluorescently labeled small molecule derivatives whose masses do not exceed 1500 Da. Fluorescein derivatives have been used traditionally as fluorescent labels, although there are currently a large number of fluorescent labels with different fluorescent properties that have appeared on the market. Such antigen conjugates with fluorescent labels are called tracers. In short, if a fluorescently labeled low-molecular-weight substance is present in solution, then due to its small molecular volume, the polarization value is small. However, after binding to a macromolecule (for example, an antibody), the complex has a larger volume, which leads to a slowdown in its movement and an increase in the fluorescence polarization signal (Fig. 2).

Thus, in practice, FP can be used to measure variations in the fluorophore rotational diffusion rate, as shown

in Fig. 2. Moreover, FP measurements can provide information on the size and shape of the fluorophore and the fluorophore molecular complex with another molecule. This forms the basis for quantifying the proportion of fluorophore that is bound to a macromolecule (Fig. 2). Antibody assays called FP immunoassays (FPIAs) were first introduced in the 1960s and have since been widely applied to advance various fluorescence polarization assays. Currently, antibodies, receptors, enzymes, and proteins are all used as recognition reagents.

### 3. Application of FP Analysis in Determining Organic Pollutants in Foods

Technological, industrial, and agricultural progress has led to an increase in the number and variation of compounds circulating in the environment that are alien to humans and animals. Thus, significant changes have occurred in the composition and quality of food products. The intensive application of pesticides, mineral fertilizers, medicines, surfactants, and many other bioactive substances has led to dangerous medical and environmental problems. Hence, food quality control requires simple and rapid methods to determine the main contaminants, the amounts of which can be measured in their thousands, while the concentrations vary from insignificant to very high—tens to hundreds of times higher than the maximum permissible concentrations (MPCs). To monitor the environment and consumer products effectively, large numbers of samples must be analyzed. Therefore, the following basic requirements are imposed on modern analytical methods: Low detection limits (high sensitivity), high accuracy and reproducibility, ease of implementation, short duration of the technique, and a low cost of analysis; the facility must be able to determine large numbers of samples simultaneously. In addition, there is often a need to identify pollutants at the point of need.

Fluorescence polarization analysis is a traditional method for detecting small molecules. The organic contaminants most commonly found in foods are primarily low molecular weight substances (pesticides, hormones, veterinary drugs, and plasticizers). Classical FPIA was first proposed by Dundlicker [38] and was formed in the competitive binding of specific antibodies to a free and fluorescently marked antigen.

Currently, investigations in food safety and the environment using FP analysis have expanded. Over the past decades, reviews have been published on the use of FP methods in detecting pesticides [31], mycotoxins [29], and other bioactive substances [26] in food and the environment. Table 1 (Ref. [16,39–100]) summarizes the previous publications on the application of FP assays in the detection of chemical pollutants for food and environmental safety during 2018–2023. The names of analyzed substances, fluorophores, recognition elements, and limits of detection (LOD) are grouped in Table 1. Following our detailed lit-

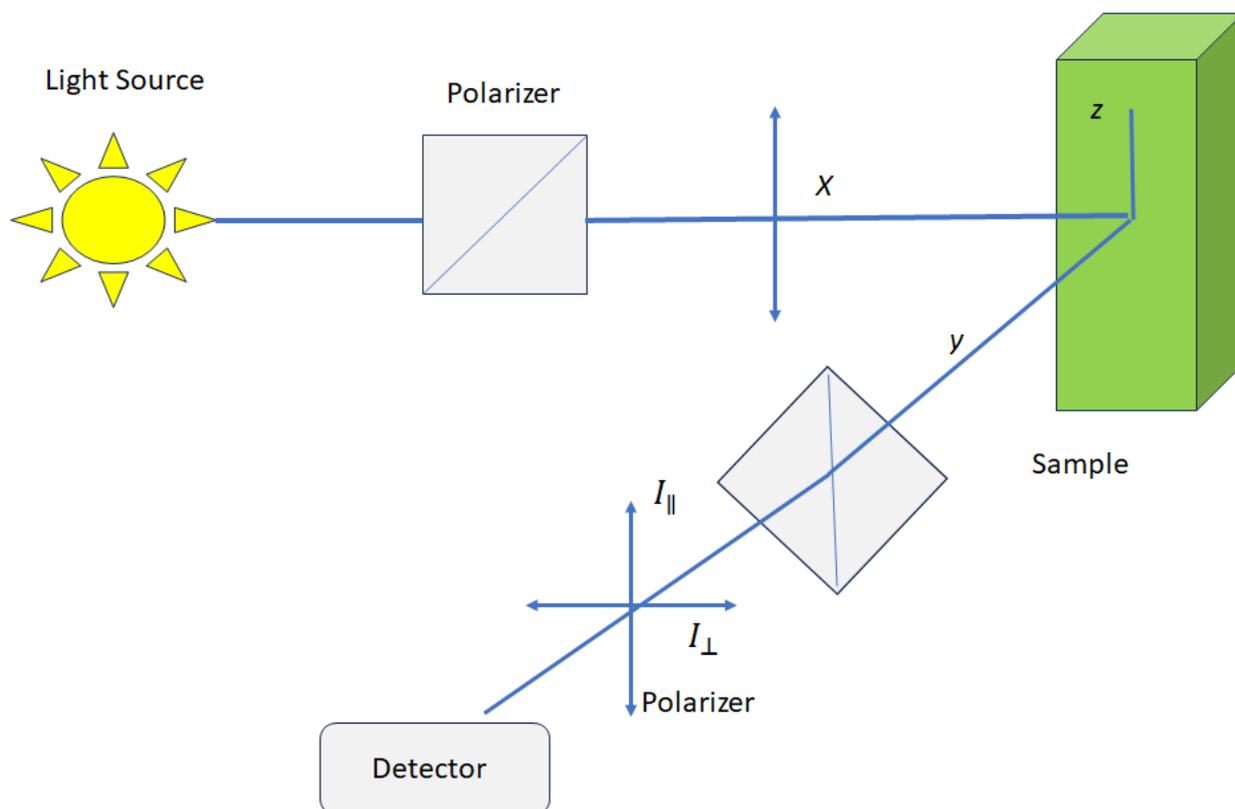


Fig. 1. Scheme for measuring the fluorescence polarization signal.

erature investigation, it was shown that FP assays are designed to detect herbicides, pesticides, veterinary drugs, mycotoxins, endocrine-disrupting chemicals (EDCs), toxins, medicines, and other organic substances encountered during the production or packaging of food products. It should be noted that there has been an increase in the detection of EDCs in food and water in recent years [10,11]. The content of harmful organic substances in the water is relatively low. It is known that the sensitivity of the FPIA method is somewhat inferior to ELISA; however, the development of new technologies and the appearance of new recognition elements (receptors, aptamers, and nanobodies) allow the FP assay to compete with other analytical platforms successfully. FP techniques can be used in both the laboratory and at the point of need. The advantages of using the FP assay include the possibility of automation, simple sample preparation, short analysis time, and the ability to simultaneously determine up to 1536 samples, making it popular for analyzing food products.

#### 4. Reagents for FP Analysis

The process of developing a sensitive FP assay, as a rule, consists of the following stages: Synthesizing conjugates of a low molecular substance with a fluorescent label (tracer), selecting a recognition reagent (antibodies, receptors, enzymes, or aptamers), and optimizing the analy-

sis conditions (for example, choice of buffer system) [26]. Since the FP assay consists of the antigen/antibody interaction reaction, the main task involves obtaining antibodies with high specificities for the antigens, including the choice of hapten, carrier protein, and animal immunization regimen. The present development of hybridoma technologies makes it possible to obtain highly specific monoclonal antibodies in almost any quantity. However, the antibodies can be used as recognition elements, as can the proteins, enzymes, and aptamers. An important challenge in developing FPIA methods remains the synthesis and purification of fluorescently labeled antigens (tracers) with an antibody specificity comparable to the native antigen [26]. Currently, fluorescent labels with various functional groups and linkers are widely available and allow for solving any problem during conjugate synthesis. In addition, these commercial fluorescent tags have different excitation and emission wavelengths, which permit the development of multidetection methods for organic compounds. Producing modern nanomaterials and their use in immunoassays also contributes to the progress of highly sensitive methods for determining contaminants in food products.

##### 4.1. Tracers for FP Analysis

The conjugate of a low molecular weight analyte with a fluorescent label (tracer) plays a vital role in the sensi-

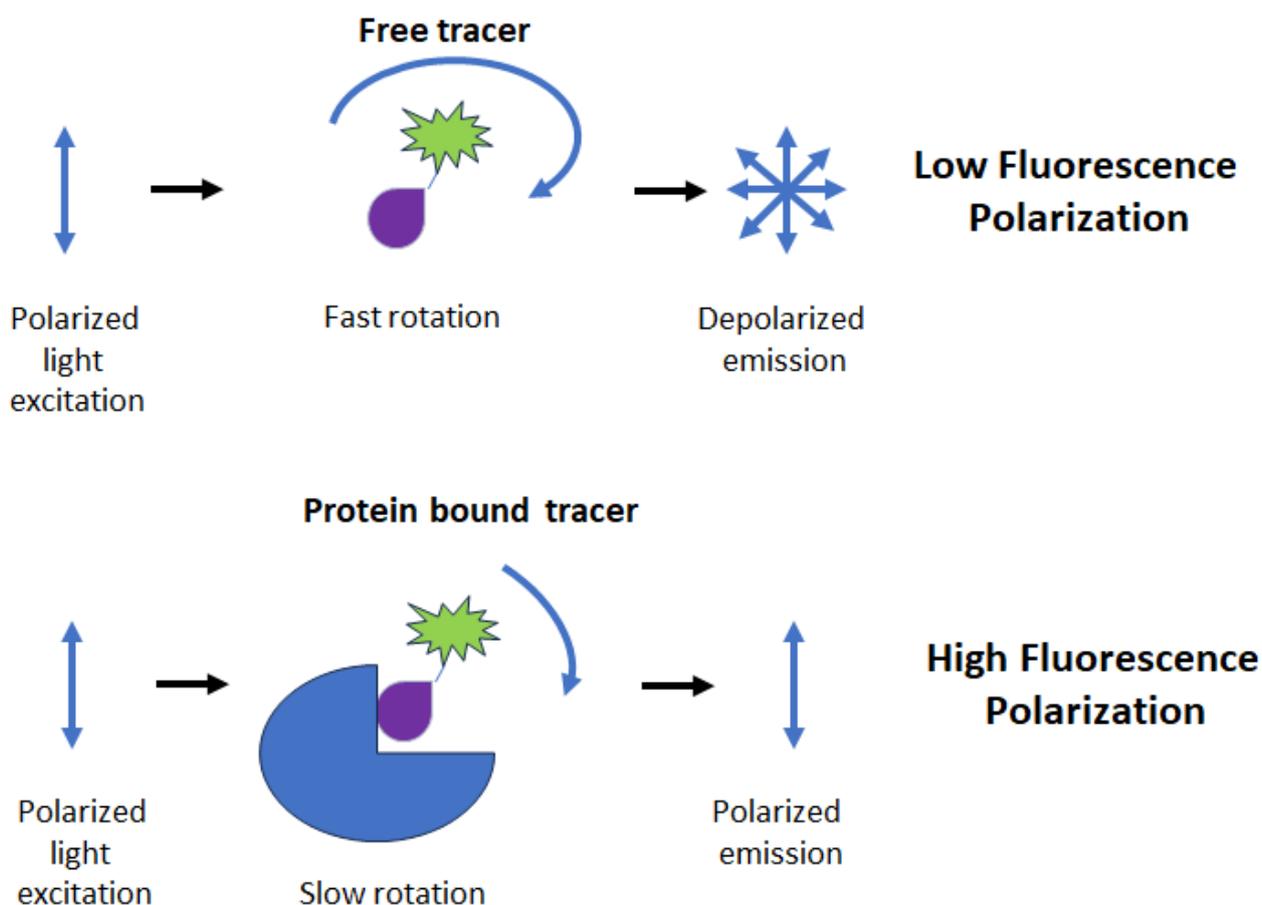


Fig. 2. The principle for changes in the FP signal upon binding fluorescently labeled antigens to large molecules.

tivity, accuracy, and stability of FP analysis. To develop a sensitive FPIA, one of the recognizing components must be conjugated with a fluorescent dye. As a rule, the analyte or another molecule structurally similar to the target analyte is selected for conjugation with a fluorescent dye. The tracer usually has three groups—a low molecular weight antigen, a fluorescent label, and the linker between the antigen and the fluorophore.

The fluorophore selection should consider their spectral properties. The fluorescence lifetime ( $\tau$ ) [72]; the high quantum yield, especially for high sensitivity analyses, which require a low tracer concentration; the rigid geometry of the excited state, which could maintain the stability of fluorophores in various environments and during storage, and the constancy of emissions between the free and related conditions; the fluorophore must have reactive groups and be easily subject to conjugation with the analyte or its analogs; the fluorophore should have minimum nonspecific interaction with other components of the FPIA system.

Previous reviews have presented the properties of fluorophores in more detail [19,26,72]. Zhang *et al.* [26] conducted a study on fluorophores applied in FP assays for high-throughput drug screening, which provided detailed

information on commonly used fluorophores. The results showed that fluorescein dyes were used preferentially, such as fluorescein isothiocyanate (FITC), carboxyfluorescein (FAM), 4,6-dichlorotriazinylaminofluorescein (DTAF), glycylaminofluorescein (GAF), fluoresceinamine (FA), 4'-(aminomethyl) fluorescein (4'-AMF), aminoacetamidofluoresceinamine (AF), etc. The popularity of fluorescein dyes can be explained by their spectral properties (high quantum yield of 92% and a fluorescent lifetime of 4.05 ns), chemical stability, and availability of reagents with various reactive groups (sulfhydryls, amino, and carboxyl). Furthermore, they are relatively inexpensive and commercially available. Moreover, most FP readers have filters to measure the fluorescein fluorescence. In addition to fluorescein, FPIA uses rhodamine [101–103] and relatively new dyes based on boron compounds: BODIPY [104,105]. Indeed, BODIPY dyes have longer excited-state lifetimes than fluorescein and rhodamine dyes, making their FP sensitive to binding interactions over a more extensive molecular weight range [19,26,104,105]. In addition to using dyes, such as Alexa Fluor [106–108] and OregonGreen [108].

New chromophores based on an N-condensed tricyclic heterocycle are being developed in addition to classical

**Table 1. Summary of FP assay for screening organic molecules (2018–2023).**

Analyte	Fluorophore	Recognition element	Limit of detection	Ref.
<b>Endocrine-disrupting chemicals</b>				
Bisphenol A	Tetramethylrhodamine	Aptamer	0.5 $\mu\text{mol/L}$	[39]
Bisphenol A and analogs	Fluorescein	Receptor	210–10,300 ng/mL	[40]
Bisphenol A	Fluorescein	MAB	1.0 $\mu\text{g/L}$	[41]
Diisobutyl phthalate	Fluorescein	PAb	0.82 ng/mL	[42]
Dibutyl phthalate	Fluorescein	PAb	0.35 $\mu\text{g/mL}$	[43]
PCB-77	Silver nanoparticles	Aptamer	5 pg/L	[44]
Progesterone	Fluorescein	MAB	*	[45]
Dehydroepiandrosterone	Fluorescein	PAb	1.1 ng/mL	[46]
Glucocorticoids— Dexamethasone	Fluorescein	MAB	1.00 ng/mL	[47]
<b>Veterinary drugs</b>				
Ciprofloxacin	Near infrared fluorescence dye	MAB	1 ppb	[48]
Enrofloxacin	Fluorescein	PAb	1.7 ng/mL	[49]
4,4-Dinitrocarbanilide	Fluorescein	MAB	5.7 ng/mL	[50]
Sulfonamides	Fluorescein	Dihydropteroate synthase	Detection range: 2.0–38.5 ng/g	[51]
Sulfonamides	Fluorescein	MAB	100 mg/L	[52]
Sulfathiazole	Fluorescein	PAb	3 ng/mL	[53]
35 Sulfonamides	Fluorescein	Dihydropteroate synthase	*	[54]
Chloramphenicol	SYBR Green I	Aptamer	0.06 nM	[55]
Erythromycin	Fluorescein	MAB	14.08 $\mu\text{g/L}$	[56]
Vancomycin	Fluorescein	MAB	*	[57]
Gentamicin	Fluorescein	MAB	*	[58]
10 Aminoglycosides	Fluorescein	Ribosomal Protein S12	Range of 5.25–30.25 ng/g	[59]
Gentamicin, streptomycin, and amikacin	Carbon Quantum Dots	PAb	20, 10, and 3 ng/mL	[60]
10 Tetracyclines	Fluorescein	TetR protein	Range of 0.4–1.5 ng/mL	[61]
Tobramycin	Atto655	MAB	*	[62]
Ractopamine	Fluorescein	PAb	0.56 ng/mL	[63]
Polymyxins	Fluorescein	MAB	1.4 $\mu\text{g/L}$	[64]
<b>Mycotoxins</b>				
Aflatoxin B1	Fluorescein	Aptamer	0.05 nM	[65]
Aflatoxin B1	Fluorescein	MAB	0.8 ppb	[66]
Aflatoxin B1	Tetramethylrhodamine	Aptamer	125 pmol/L	[67]
Aflatoxin B1 and ATP	Fluorescein	Aptamer	25 pM and 1 $\mu\text{M}$	[68]
AFB1, OTA, ATP	Fluorescein	Aptamer	60 pM	[69]
Aflatoxin M1	Tetramethylrhodamine	MAB	3.8 ng/L	[70]
Aflatoxins and zearalenones	Fluorescein	MAB	4.98 mg/kg	[71]
Deoxynivalenol	Fluorescein	MAB	*	[72]
Fumonisin FB1 and FB2	Fluorescein	MAB	441 and 344 $\mu\text{g/kg}$	[73]
Ochratoxin A	Fluorescein	Aptamer	2.3 $\mu\text{g/kg}$	[74]
Ochratoxin A	Lissamine rhodamine B	Aptamer	2.5 nM	[75]
Ochratoxin A	Fluorescein	MAB	*	[76]
Ochratoxin A	Tetramethylrhodamine	Aptamer	2.4 nM	[77]

**Table 1. Continued**

Analyte	Fluorophore	Recognition element	Limit of detection	Ref.
T-2 and HT-2 toxins	Fluorescein	MAB	10 µg/kg	[78]
T-2 toxin	Fluorescein	Aptamer	0.03 nM	[79]
Tenuazonic Acid	Fluorescein	PAb	0.13 µg/mL	[80]
Zearalenone	Fluorescent	Aptamer	0.004 ng/mL	[81]
Mycotoxin	Fluorescein	MAB	*	[82]
Pesticides				
Benzothiostrubin	Fluorescein	MAB	19.7 ng/mL	[83]
Imidacloprid	Fluorescein	MAB	1.7 µg/L	[84]
Thiabendazole and tetraconazole	Fluorescein	MAB	20 and 200 µg/kg	[85]
Organophosphorus pesticides	Fluorescein	Acetylthiocholine	0.01 ng/mL	[86]
Propisochlor	Fluorescein	PAb	8.4 ng/mL	[87]
3-phenoxybenzoic acid	Fluorescein	Nanobodies	IC <sub>50</sub> — 20.2 ng/mL	[88]
Thiacloprid	Fluorescein	MAB	2.43 ng/mL	[89]
Toxins and drugs				
Amantadine	Fluorescein	MAB	0.9 µg/kg	[90]
Amantadine and ribavirin	AF647 and Fluorescein	MAB	AMD 1.7 µg/kg RVB 1.0 µg/kg	[91]
Amitriptyline	Fluorescein	PAb	(7–8) × 10 <sup>-11</sup> M	[92]
Carbamazepine	*	*	*	[93]
Capsaicinoids	Fluorescein	MAB	1.56 ng/mL	[94]
Diclofenac	Fluorescein	MAB	2.0 µg/L	[95]
Domoic acid	Fluorescein	MAB	*	[16]
Glycocolic acid	Fluorescein	PAb	9 ng/mL	[96]
Okadaic acid	Fluorescein	MAB	0.08 ng/mL	[97]
Mycophenolic acid	Near-infrared fluorescent dye	MAB	0.8 ng/mL	[98]
Nicotinic acid	Fluorescein	PAb	1.1 µg/mL	[99]
Niclosamide	FITC	Receptors	*	[100]

\*—information was not described in articles. FP, fluorescence polarization; PCB-77, polychlorinated biphenyls; MAB, monoclonal; PAb, polyclonal; FITC, fluorescein isothiocyanate; AFB1, aflatoxin B1; OTA, ochratoxin A; ATP, adenosine triphosphate.

dyes. The central core of this prototype molecule, which is a “hard” monomethine cyanine dye, is isoelectric with the pyridine-based BODIPY analogs. The development of dyes, fluorophores, or dual-emission fluorescent probes with an excitation region in the green region of the spectrum could begin with this molecule [109].

Currently, nanoparticles are increasingly being used in analytical chemistry. Nanoparticles with dimensions close to the electron wavelength (1–10 nm) are called quantum dots (QDs). Their absorption wavelength, or fluorescence, could be easily varied by changing their size. QDs have unusual properties (high photostability, light absorption in a wide range of the spectrum, and long fluorescence lifetime) and are used actively as fluorescent dyes. Unlike classical QDs composed of metal chalcogenides, carbon quantum dots (CQDs) have high hydrophilicity and low toxicity and can be obtained easily [110]. Currently, QDs have proven themselves to be efficient fluorescent labels in immunochromatography [111,112], microfluidic [113], and ELISA [114,115]. The immunosensor predicated on

carbon quantum dots with dual-emission labeled aptamers for dibutyl phthalate detection in food samples is described [116]. The possibility of using CQDs as a label in FPIA was demonstrated when determining aminoglycoside antibiotics. The LODs for amikacin, streptomycin, and gentamicin were 3, 10, and 20 ng/mL, respectively. The FPIA was tested to determine aminoglycosides in dairy products [60]. A dopamine biosensor was created because of the strong photostability of the QDs in the second near-infrared window (NIR-II) and their low level of substrate spontaneous interference. A streptavidin biosensor with fluorescence anisotropy probe (DFAP-SAB) is based on NIR-II QDs and combined with streptavidin signal amplification to measure dopamine in human serum quickly and painlessly. With a detection limit of 11.2 nM, the detection signal exhibits good linearity from 50 to 3000 nM. It has been shown that employing NIR-II QDs creates the possibility of using biosensors for complicated samples. The design of the streptavidin signal magnification apparatus presents a novel opportunity for detecting small molecules [117].

The important component of the fluorescent conjugate is the spacer between the hapten and fluorescent labels. The chemistry of the linker between the antigen and the fluorophore can influence the FP signal. Tracers with short linkers can minimize the propeller effect, although a linker that is too short may affect the binding affinity of the tracer to the antibody [26]. As shown in preceding reports, the spacer length between the hapten and fluorescein directly influences the FPIA sensitivity [63,68,72,91,118,119]. Thus, to minimize the steric hindrance and maximize the ability of the antibody to recognize the tracer through its inherent affinity, a spacer should typically be placed between the dye and the hapten. Quenching by fluorescein can also be lessened by the spacer between it and the hapten [120]. The chemical structure tracers for ribavirin determination with various spacer lengths are shown in Fig. 3 (Ref. [91]).

However, as described in the next investigation, conjugating a fluorescent label with a longer spacer promoted a more sensitive FPIA analysis. For example, to maximize the sensitivity of FPIA pesticides, fluorescein-labeled derivatives with different linkers (2-, 4-, and 6-carbon) were synthesized. The analysis showed that the fluorescein thiocarbamyl hexamethylenediamine-labeled conjugate with a six-carbon linker had better sensitivity for determining some organic compounds in FPIA [121]. Chun *et al.* [122] also reported that the six-carbon spacer tracer produced the most sensitive FPIA when screening for zearalenone. However, the length of the spacer can influence the sensitivity of the FP assay either positively or negatively; meanwhile, it may also depend on the high molecular weight antibody recognition element.

#### 4.2. The Recognition Elements of FP Assays

Recognition elements are an essential component of any immunochemical assay. Recognition elements in FP analyses are responsible for identifying target substances and improving the analytical performance of the method. Currently, many known recognition elements have high specificity, stability, sensitivity, and cost-effectiveness. FPIA predominantly uses classical monoclonal and polyclonal antibodies, egg yolk antibodies (IgY), recombinant antibodies, molecularly imprinted polymers (MIPs), aptamers, enzymes, and receptors.

##### 4.2.1. Polyclonal and Monoclonal Antibodies

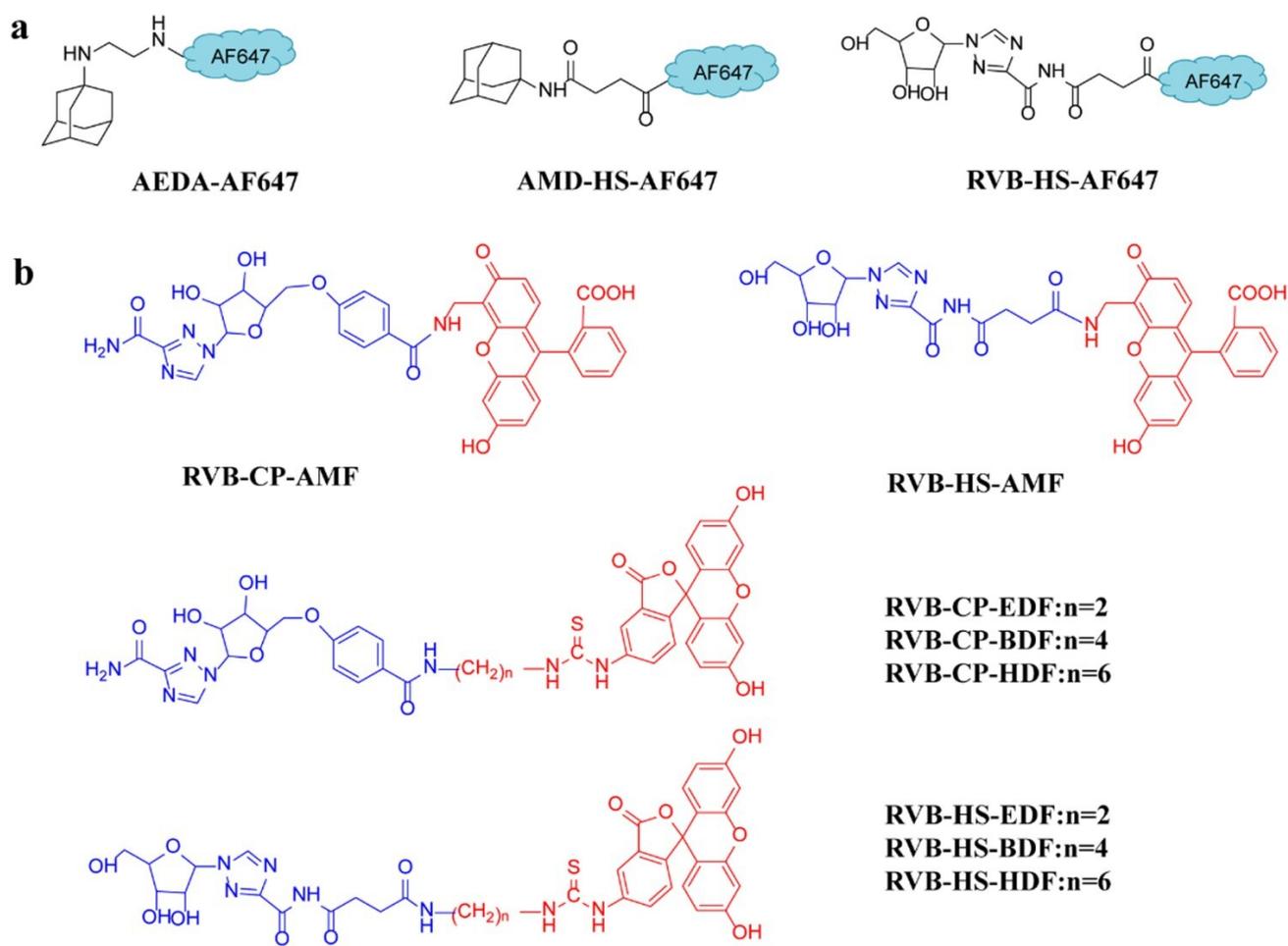
Traditionally, antibodies are used as recognition reagents in FPIA: Polyclonal (PAb) [42,43,63] and monoclonal (MAb) [88,89]. They provide different opportunities for determining target antigens due to differences in specificity and affinity. Polyclonal antibodies consist of biochemically distinct paratopes and antigen-binding sites, which provide affinity by binding to the target antigen. Moreover, they are advantageous for research purposes, including their high affinity for the antigen and even minor changes in the target antigen. Due to their biophysical va-

riety to the target antigen, PABs often demonstrate higher sensitivity than their monoclonal counterparts in diagnostic assays. However, the ability to use polyclonal antibodies on a large scale is limited because of the inability to reproduce these proteins on an industrial scale.

Monoclonal antibodies are highly specific proteins that recognize a single epitope of an antigen. The specificity of the antibody is detected by the relative affinity between its binding domain and the target antigen in the presence of other molecules. Additionally, the sensitivity of the antibody is an important parameter that determines its applicability in the assay. High-sensitivity antibodies are well-suited for detecting low antigen concentrations in immunoassays. FPIA could use PABs or MABs to determine the target antigen in solution [123]. For example, polyclonal antibodies are useful in immunoassays that require a highly sensitive test to detect specific assay groups. To determine the concentration of an already known antigen, it is advisable to use monoclonal antibodies, which usually demonstrate high specificity in the analysis and low cross-reactivity with compounds structurally similar to antigens [88,89,97,124].

##### 4.2.2. Usage of Aptamers and Nanomaterials for Signal Amplification

Aptamers are yet another illustration of an immunoassay recognition element. Nucleic acid aptamers are extremely useful affinity ligands because they can selectively bind to relevant targets, such as proteins, tiny molecules, metal ions, and cells [125]. Creating aptamers and altering them with desired functional groups, such as fluorophores or other nanomaterials, is simple. Therefore, aptamers are very convenient to use in FP analyses. It has been previously shown [126] that FP is primarily used to measure aptamer binding to large protein molecules. This method could only be used to analyze aptamer-target interactions in which the aptamer was conjugated with a fluorescent dye, and the target was noticeably larger than the aptamer. To date, numerous aptamers that specifically bind to small molecules have been identified [127]. Thus, the use of FP assays has increased due to recent developments in the field of aptamers. Aptamers are promising additions to FP analyses due to the possession of many significant characteristics. Fluorophores can be used to label aptamers in specific locations accurately. However, the aptamer structures can change based on whether target binding occurs. This can lead to a change in the aptamer polarization, which has been fluorophore-labeled because of the shape change by the aptamer. The aptamers can form duplexes with complementary sequences, although these can also be dislodged by the target binding to the aptamers [125] since this modifies the molecular volume and anisotropy of fluorophore-labeled oligonucleotides. These unique properties allow the development of new strategies for using aptamers in FP assays, which are unattainable when using antibodies. Based on such interactions, fluorophore-labeled aptamers can be



**Fig. 3. The tracer structures for detecting ribavirin (RVB).** Hapten is blue, and fluorescein is red [91]. (a) Long-wavelength tracers of MWFPIA detection for AMD and RVB. (b) Tracers of FPIA for RVB detection.

directly used to detect targets. Such interactions precisely regulate changes in the anisotropy of fluorophores associated with aptamers.

However, the sensitivity of these approaches is relatively low since the molecular weights of widely distributed molecules are too small to cause noticeable changes in the FP or FA values [128]. Thus, the overall strategy, including signal amplification design, will be a very valuable addition to this area. More recently, the sensitivity of the FA/FP analyses has been significantly improved through some developed approaches that signal amplification [129].

Proteins or antibodies, which have larger molecular weights or volumes than aptamers, can enhance the FA or FP signal [130]. Using this approach, the mass-enhancing probe consists of an aptameric domain, which is aimed at the target molecule, and an aptameric domain that enhances the molecular weight of the booster protein. An increase in the molecular weight of the probe/target complex can lead to a noticeable increase in the FA value.

Further, a method for determining ochratoxin A (OTA) was developed using an approach based on the increase in size difference between the bound and unbound

fluorophores. A fluorescence polarization OTA assay with competitive binding of the fluorophore-labeled and free OTA to aptamer-based receptors has been developed. Here, the aptamer is included in the complexes with protein modules and used as a receptor, while the anchor protein modules are used as enhancers. The OTA detection limit in wine was 1.1  $\mu\text{g}/\text{kg}$ , and the recovery rates ranged from 83% to 113%. The study shows that the proposed strategy effectively improved the sensitivity of FP-based aptamer assays. [130].

Detection of aflatoxin B1 (AFB1) in the presence of a specific aptamer to this toxin and adenosine triphosphate (ATP) was conducted by applying an antibody connected with complementary DNA at the 3' end and a FAM-marked aptamer at the 5' end. Hybridization of the aptamer to the cDNA induced a FAM label close to the large antibody, which hampered the FAM rotation and provided a high fluorescence anisotropy signal. When an analyte is added, it binds to the aptamer probe and inhibits the aptamer-cDNA duplex, which decreases the fluorescence anisotropy signal. This method reaches detection limits of 25 pM for aflatoxin B1 and 1  $\mu\text{M}$  for adenosine triphosphate [68].

The use of nanomaterials for fluorescence polarization signal amplification offers a significant advantage compared to proteins. Unlike proteins, nanomaterials have high thermal stability and are simple to synthesize and modify chemically, while the FA or FP signal change is more significantly elaborated. Recently, to improve the measurement sensitivity, a number of nanomaterials, such as gold nanoparticles, carbon nanomaterials, graphene oxide, metal-organic frameworks, silicon nanoparticles, QDs, etc., have been applied to the FA and FP sensor systems [65].

The subsequent investigation proposed a cheap, highly sensitive FP method to determine aflatoxin B1 by applying aptamer sensors grounded in graphene oxide (GO). The FAM-marked aptamer bound to the GO surface, forming an aptamer/GO macromolecular complex with a high FP signal. The addition of AFB1 to the system led to aptamers dissociating from the GO surface and binding to AFB1 due to their high specificity following the formation of the aptamer/AFB1 complexes. Subsequently, significant alterations in molecular weights were observed, resulting from the GO/aptamer complex dissociations and the formation of the new aptamer/AFB1 complexes, which led to significant changes in the FP value. The limit of detection (LOD) for aflatoxin was 0.05 nM. The aflatoxin recovery test for rice samples ranged from 89.2% to 112%. Thus, this method has demonstrated high sensitivity, cost-effectiveness, and future prospects for its application [65].

Notably, using large proteins, such as streptavidin, as an effective signal amplifier for detecting small organic molecules significantly improves the analytical performance of the method [69]. The authors applied the aptamer assay using streptavidin-based anchor protein modules and IgG as an amplifier of the FP signal [130]. The FP signal was doubly enhanced by streptavidin and IgG. Therefore, this method can be applied for 15 minutes to determine low levels of ochratoxin A in wine. The response and sensitivity of the FP assay are significantly enhanced by protein. However, there are limitations associated with using protein enhancers in FP assays, namely their variability and specificity for certain conditions, for example, to ensure the stability of biomolecules [131]. MnO<sub>2</sub> nanosheets, a type of two-dimensional nanomaterial, can be used to enhance the FP signal. These nanosheets are excellent nanocompensators, catalysts, and adsorbents [22–24], which can use physical sorption to effectively adsorb ssDNA [25], thereby allowing them to act as FP amplifiers [86].

Further, a novel biosensor using decahedral silver nanoparticles (Ag<sub>10</sub>NPs) amplified FP was demonstrated to diagnose polychlorinated biphenyls (PCB-77). The aptamer-modified Ag<sub>10</sub>NPs were used as an analytical probe; PCB-77 could be detected with high sensitivity and selectivity in these conditions. The detection range was 0.02–390 ng/L, and the LOD was 5 pg/L. The FP aptasensor can effectively detect PCB-77 in ambient water and has good application prospects [39].

#### 4.2.3. Nanobodies

Nanobodies or single-domain antibodies (scFvs) are small ( $\approx 15$  kDa;  $\approx 4$  nm  $\times$  2.5 nm  $\times$  3 nm) and a recombinant antibody form that contains only heavy chains. They have become a good alternative to conventional IgG. Nanobodies have only one variable domain, and their affinity and specificity for the target analyte compare favorably with traditional IgGs [132,133]. Their small size, high conformational stability, and ease of modification and preparation encourage their use in various immunoassay formats [133–138]; however, the use of nanobodies in fluorescence polarization immunoassays varies. The FP assay is a sensitive analytical method for detecting the molecular weight; thus, there is no doubt that the nanobody molecule size is a concern. However, there are also known techniques (FPIA) that are based on the application of the scFvs for aflatoxin B1 [132], fumonisin Bs (FBs) [88], tetrabromobisphenol A [139], and 3-phenoxybenzoic acid [88]. However, using nanobodies in the FPIA diagnosis of small molecules is insufficient, yet fluorescently labeled nanobodies can be applied to detect high-molecular-weight organic pollutants or large molecules.

#### 4.2.4. Application Receptors, Proteins, and Enzymes

Compared to antibodies, the use of receptors in the FP analysis has a number of advantages. Firstly, the receptor is usually the target of one class of preparates and can recognize every ligand. The receptor can be obtained using a simple method, which takes only a few days. The receptor has low costs, and its affinity for ligands can be increased by site-directed mutagenesis [91]. Thus, screening methods that use receptors are promising, especially for multiformat analyses. As a result, various receptors have been applied as recognition elements to develop immunoassays for determining veterinary drugs and hormones. Receptor investigations are widely used to detect multiple antibiotics and other medicinal residues [140]. PPARs have been confirmed as phthalate esters (PAE) receptors, while PAE toxicity is mediated by their binding to PPARs [141]. The broad spectrum of PPARs is advantageous because it promotes the detection of the entire group of PAEs. A competitive binding-based FP assay was developed to determine the PAEs in Chinese liquors quantitatively. A sensitive assay was developed by coupling the soluble receptor protein mPPAR $\alpha$ -LBD with the tracer C4-BODIPY-C9 [105]. Many drugs have been designed to specifically bind to receptors or enzymes, the inhibition or activation of which leads to a therapeutic effect. The same approach is used when analyzing a drug in food samples or physiological body fluids. For example, this approach was used previously to determine the anthelmintic drug nicodamid (NIC). It is known to bind to estrogen receptors (ERs) and estrogen-related receptors (ERRs). The binding potency of NIC with receptors (ER $\alpha$ , ER $\beta$ , and ERR $\gamma$ ) was determined by fluorescence competitive binding assays, which demonstrate an IC<sub>50</sub> (the con-

centration of NIC needed to displace 50% of the probe from the receptor) of  $90 \pm 4.1$  nM,  $10 \pm 1.7$  nM, and  $0.59 \pm 0.07$  nM, respectively [100].

Enzymes are also used in FP analyses, for example, in determining organophosphorus (Ops) pesticides. The OPs can suppress the activity of acetylcholinesterase (AChE), thereby limiting the reaction of acetylthiocholine (ATCh) hydrolysis to obtain thiocholine (TCh) by AChE. TCh can promote the decomposition of  $\text{MnO}_2$  nanosheets into manganese ions. This method can increase the FP sensitivity, with a LOD for diazinon of 0.01 ng/mL, and it is also applicable to determining the other four OPs in water samples [86]. Wang *et al.* [142] applied dihydropteroate synthase for the multidetection of sulphonamides by FP. This FP analysis was characterized as simple and rapid. Moreover, this method determined about 29 sulfonamides with high sensitivity ( $\text{IC}_{50}$  was less than 100 ng/mL). He *et al.* [51,54] used dihydropteroate synthase to detect 31 and 35 sulfonamides. The result indicated that using the mutant dihydropteroate synthase increased the SA affinity. This method was used in large-scale pork samples for the multidetection of a residue in 35 SAs. The work [59] describes the use of ribosomal protein S12 in the multiscreening of 10 aminoglycosides in pork muscle. This detection method was easier and faster than the others. Thus, the variety of recognition elements in the FP analysis makes it possible to develop methods for diagnosing organic molecules with a low LOD and high specificity and sensitivity.

## 5. Multiwavelength FP Assays

Food products often may contain various residues of organic pollutants simultaneously, for example, antibiotics, pesticides, or mycotoxins. Therefore, analyses need to be created that can simultaneously determine numerous pollutants in foods. An example of simultaneously determining toxins in grain has been proposed previously [78]. T-2 and NT-2 toxins and their main modified forms (T-2 glucoside and NT-2 glucoside) can occur in grains and grain-based products. Indeed, 12 MABs and four tracers were obtained and tested for their rapid determination by the FPIA method, which applied an NT-2-specific antibody that had demonstrated high sensitivity ( $\text{IC}_{50} = 2.0$  ng/mL) and high cross-reactivity (100% to T-2 toxin and 80% to T-2 and NT-2 glucosides). The advantage of using this method was its ease of use and rapid determination (about 15 minutes). However, this method is limited in that it is difficult to determine the content of individual toxins, although the method works well from the point of view of contamination detection.

Multi-assay immunoassays are becoming increasingly popular because of their rapid turnaround times, minimal sample requirements, and low costs for detection in each assay. Homogeneous and high-throughput multi-wavelength FPIAs for multiplex drug detection have been described in several studies [51,52,54,59,61].

Sulfonamides (SAs) and antibacterial synergists (ASGs) were simultaneously detected in food products using a multi-analysis through homogeneous and high-throughput screening dual-wavelength fluorescence photoimmunization analyses [52]. Dual-color tracers were synthesized to perform highly sensitive and specific FPIA using various fluorescent dyes and linker lengths combined with particular monoclonal antibodies (MABs). The combination of SADMPM-HDF tracers with the longest linker, MAb 10E6 for SA, and tracer HaptenA-DSCA linking MAb 9C9 for ASG was chosen to develop a dual-wavelength FPIA with high sensitivity:  $\text{IC}_{50}$  values for 23 SAs below 100  $\mu\text{g/L}$  and five ASGs below 50  $\mu\text{g/L}$ . The duration of the subsequent analysis, including sample pretreatment, was a maximum of 15 minutes. An example of the multi-wave determination of toxins, deoxynivalenol (DON), T-2 toxin, and fumonisin B1 (FB1), is provided in [143], which used different dyes to identify each individual toxin. Three mycotoxins were detected simultaneously using FPIA multidetection tracers and specific monoclonal antibodies. Under optimal conditions, the LOD for DON, T-2 toxin, and FB1 in this method was 242.0  $\mu\text{g/kg}$ , 17.8  $\mu\text{g/kg}$ , and 331.5  $\mu\text{g/kg}$ , respectively. This level of sensitivity was adequate to match the established action levels by the EU for each of these three pollutants in corn.

As discussed above, receptors and proteins make suitable recognition reagents in developing multi-assays capable of simultaneously detecting dozens of medicines [54,59,61]. The recognition element of the mutant dihydropteroate synthase was used to detect 35 SAs in pork. Due to the application of a tracer containing two fluorophore molecules instead of one, the sensitivities were increased by 2.8–8.6 fold ( $\text{LODs} = 0.03\text{--}1.16$  ng/mL) compared to the usual fluorescent tracer.

A multi-wavelength FPIA (MWFPIA) for rapid detection of antiviral preparations (amantadine (AMD) and ribavirin (RVB)) contemporaneously was shown. The MWFPIA could determine AMD and RVB concentrations simultaneously within 1 minute. Tracers for determining the AMD and RVB concentrations using fluorescent labels with different properties were obtained, and the method for determining the two preparates was optimized. The LODs for AMD and RVB were 1.7 and 1.0  $\mu\text{g/kg}$ , respectively, in chicken and 17.6 and 10.4  $\mu\text{g/L}$ , respectively, in human serum. This work demonstrated that a MWFPIA could be applied to determine antiviral preparates quickly, with great accuracy and at a high capacity [91].

## 6. Perspectives of the FP Assay

FP analysis is simple, fast, homogeneous, cheap, and does not require extended sample preparation. These advantages make it the preferred method for detecting contaminants at the point of need. The requirement to diagnose residues of harmful organic pollutants in food products, animal feeds, and environmental objects at the point of need

stimulates the development of portable and easy-to-use instruments for measuring fluorescence polarization signals. Currently, a portable fluorescence polarization imaging analyzer has been developed to measure the FP signal. Moreover, FP proposes ease of use and fast processing, making it useful in determining molecular interactions. A unique FP measurement system that uses a liquid crystal (LC) layer and an image sensor has been developed [144]. The system is based on the principle of synchronous detection between the LC-level switching speed and the sampling frequency of the CCD. The FP system can simultaneously detect multiple samples; however, the measurement accuracy is lower than for the conventional FP machine. By using a newly developed high transmittance LCD with improved timing, the performance of the system was significantly improved [145]. In addition, the cost of such a device was significantly reduced by using a cheap CCD matrix and an LED as the excitation source. The ability to determine simultaneous FP immunoassays for several prostaglandin E2 samples has been demonstrated [144,145], while this device can also be used for high-throughput screening. Simultaneous FPIA analysis of 96 mycotoxin samples was conducted using a microdevice of appropriate design with an FP analyzer [38]. Moreover, FPIA was performed on more than 500 samples to highlight the potential for high-throughput analysis.

Another approach to miniaturizing and analyzing at the point of need is to use smartphones as the detection equipment [146]. An apparatus for measuring the FP signal was manufactured using a compact 3D printed holder and a built-in smartphone camera to measure fluorescence polarization signal changes accurately. Special software was developed to calculate the average intensity in the regions of interest and the degree of polarization. The system was validated by measuring the polarization of the dye molecules dissolved in solutions of various viscosities. As an example of biomolecule sensing using the developed system, a competitive FP immunoassay for prostaglandin E2 was demonstrated with a LOD of 1.57 ng/mL. The smartphone-based FP analysis platform can also be implemented to detect toxins, disease biomarkers, and pathogens.

Another cell phone-based bioanalysis platform was presented that can be used to perform fluorescence assays [146]. The system consists of a tablet, a polarizer, a smartphone (used as a camera), and a box that permits reading in the dark. Subsequently, collagenase assay results were obtained using this device. The lowest collagenase concentration was 3.75  $\mu\text{g/mL}$ , and the total concentration in the sample was 0.938  $\mu\text{g}$ , which is comparable to concentrations obtained using a microplate reader. The lowest amount of trypsin was 930 pg; this concentration is comparable to the low LOD for this assay of 400 pg obtained in the microplate reader. The device is suitable for diagnosing clinically significant diseases, including arthritis, cystic fibrosis, and acute pancreatitis.

Most FP techniques used to determine small molecule analytes are competitive assays, in which the analyte and

a fluorescently labeled analyte competitively bind to antibodies, proteins, or enzymes [147]. The use of new recognition reagents, aptamers, can significantly expand the number of substances determined by the FP assay, for example, determining adenosine triphosphate concentrations [148–150] in nutritional supplements for athletes. The production of monoclonal antibodies against galactomannans makes it possible to detect fungal contamination of food products [151]. The use of the non-competitive FP format allows the development of analytical methods to determine large molecules. For example, a method for antibody detection was developed using fluorescently labeled o-polysaccharide derived from the membrane of *Brucella abortus* [152]. FP analysis can be used to determine antibodies against immunoglobulin E [153], antibodies against the influenza A virus [154], SARS-CoV-2 antibodies [155], SARS-CoV-2 receptor binding domain [156], H5 avian influenza virus [157], bacteria [158], mRNA [159,160], and exosome quantification [161]. Using fluorescently labeled Fab antibody fragments, a non-competitive FPIA was developed to quantify the C-reactive protein [162]. The use of miniature camel single-domain antibodies (“nanobodies”) with a size of 12–15 kDa ( $2 \times 4$  nm) may be promising in the development of FPIA detection methods for proteins. Due to its small molecular weight, fluorescently labeled nanobodies can be used as a recognition reagent, which was previously performed to develop the human IgG [163] and lactoferrin [164] assays.

Thus, fluorescence polarization-based assays are promising for identifying low molecular weight organic contaminants in foods, high molecular weight proteins, and even bacterial contamination.

## 7. Conclusions

Food safety is a major concern worldwide, with contaminated food causing many illnesses and deaths globally, particularly in developing countries. Low molecular weight organic substances (pesticides, mycotoxins, veterinary drugs, antibiotics, and hormones) are often found in food products and environmental objects. In recent years, a lot of attention has been paid to identifying phthalic acid esters, which cause serious endocrine disruptions, especially in children and adolescents. Therefore, developing simple, fast, and accessible methods for analyzing such food contaminants is relevant to ensuring the safety and quality of food products.

The FP assay is a simple and fast method that does not require duration and complicated sample preparation, similar to instrumental analysis methods. The use of specific recognition reagents (monoclonal and polyclonal antibodies, aptamers, proteins, and receptors) in a homogeneous FP assay facilitates the selective determination of contaminants without complex and multistep operations, making it preferable to ELISA. FP analysis is a mix-and-measure method that only takes a few minutes to perform. Due to

these advantages, the competitive format of FP analysis is widely used to determine low molecular weight substances. Over the recent decades, significant progress has been made in FP analysis due to developments in producing new nanomaterials, dyes, and technologies for preparing various conjugates. This review presents the current state-of-the-art methods, recognition elements used in FP analysis, multi-assay applications, and challenges associated with performing FP assays to detect chemical contaminants. In addition, the fundamental possibility of extending FP analysis to detect high-molecular-weight organic pollutants, antibodies, and bacteria has been demonstrated. FP assays can use antibodies as recognition reagents as well as receptors, proteins, enzymes, and aptamers, which provide excellent sensitivity and selectivity for organic pollutants and allow the development of assays that are both highly specific for determining a specific pollutant and a whole group of structurally similar reagents.

The development of new instruments for measuring the FP signal also facilitates the spread of this method for diagnostic purposes in the study of protein/protein interactions, particle size determination, and high-throughput screening. In recent years, both microplate readers capable of simultaneously detecting up to 1536 samples and portable devices for detecting biological food contaminants based on fluorescence polarization have been developed. Such devices are mobile, easy to use, economical, and allow food to be checked for contaminants in real-time at the point of need. However, some characteristics, such as size, cost, reproducibility, and accuracy, should be prioritized for future projects. This new information and significant commentary will be critical in further developing the fluorescence polarization method in the future.

### Author Contributions

Conceptualization, LM and SE; methodology, SE.; investigation, LM; resources, SE; data curation, SE; writing—original draft preparation, LM and SE; writing—review and editing, LM and SE; visualization, LM; supervision, LM and SE; project administration, SE; funding acquisition, SE. Both authors have read and agreed to the published version of the manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

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### Conflict of Interest

The authors declare no conflict of interest.

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