Review

Recent Advances in Solid-Phase Extraction (SPE) Based on Molecularly Imprinted Polymers (MIPs) for Analysis of Hormones

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Abstract: Steroid hormones are active substances that are necessary in the normal functioning of all physiological activities in the body, such as sexual characteristics, metabolism, and mood control. They are also widely used as exogenous chemicals in medical and pharmaceutical applications as treatments and at times growth promoters in animal farming. The vast application of steroid hormones has resulted in them being found in different matrices, such as food, environmental, and biological samples. The presence of hormones in such matrices means that they can easily come into contact with humans and animals as exogenous compounds, resulting in abnormal concentrations that can lead to endocrine disruption. This makes their determination in different matrices a vital part of pollutant management and control. Although advances in analytical instruments are constant, it has been determined that these instruments still require some sample preparation steps to be able to determine the occurrence of pollutants in the complex matrices in which they occur. Advances are still being made in sample preparation to ensure easier, selective, and sensitive analysis of complex matrices. Molecularly imprinted polymers (MIPs) have been termed as advanced solid-phase (SPE) materials for the selective extraction and preconcentration of hormones in complex matrices. This review explores the preparation and application of MIPs for the determination of steroid hormones in different sample types.

Keywords: sample preparation; endocrine disruptors; steroid hormones; chromatography; molecularly imprinted polymers

1. Introduction

Exogenous chemicals with the ability to interfere with the normal function of hormones are known as endocrine disrupting compounds (EDCs) [1]. The effects of EDCs include reduced fertility and elevated chances of cancer [2]. One of the most active and potent group of EDCs in the environment is hormones [3]. Hormones that are naturally found in mammals are called endogenous hormones, while foreign hormones are classified as exogenous hormones and can be either natural or synthetic [4]. Steroids are the most active endocrine hormones found in the environment [1].

Animals produce hormones in endocrine glands, organs, and tissues. These hormones control a vast amount of activities, including essential ones, such as the regulation of cell activity and mood control [5]. Hormones can be broadly classified as steroids and nonsteroids [6]. Structurally, all steroid hormones contain a characteristic arrangement

of four cycloalkane rings [7]. Steroids are further classified into mineralocorticoids (e.g., aldosterone), glucocorticoids (e.g., hydrocortisone), androgens (e.g., testosterone (TST)), estrogens (e.g., β-estradiol (E2)), and progesterone (PRO) [5]. Their chemical structures are shown in Figure 1.
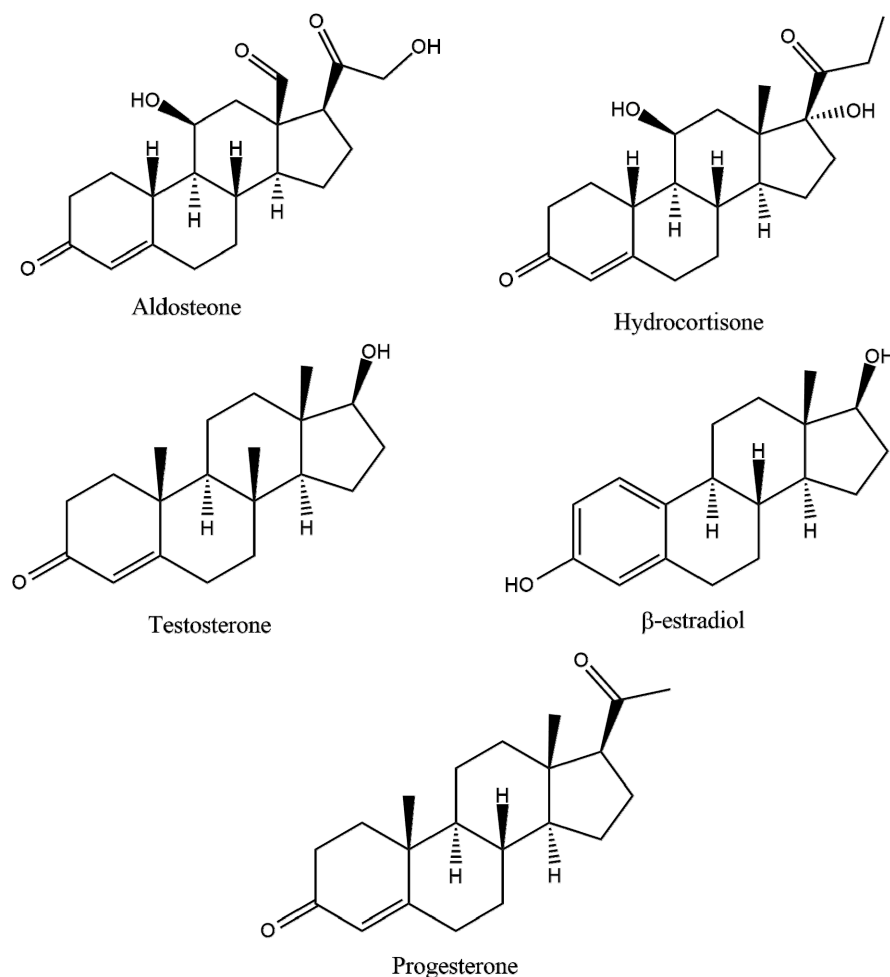

Figure 1. Chemical structures of hormone group representatives.

Human and animal excretion often contain natural and exogenous variation of hormones [8]. This is due to the fact that while steroids such as estrogens, mineralocorticoids (aldosterone), glucocorticoids, androgens, and progesterones can be secreted in the adrenal cortex [9], there are still exogenous hormones used as contraceptives [10], medicines [11], and growth promoters [12]. As a result, hormones can end up in the environment via wastewater treatment plant effluent discharge into receiving water bodies [13].

Hormonal compounds in the water can pose a health risk, such as reproductive disorders, feminization, masculinization, infertility, and cancer [2]. Water is a good medium for carrying and distributing polar and semipolar compounds [13]. As a result, incomplete removal of hormone pollutants in wastewater treatment plants means effluent discharge into nearby rivers will introduce hormonal pollution into water systems [14]. Table 1 shows the concentration levels of hormones in water and other matrices in different countries. As can be seen, while mostly prevalent in water, hormones can still be detected in soil, food, and even humans. Unusual exposure to hormonal compounds can result in endocrine disruption that can have negative effects, such as abnormal cell growth of hormone-regulated tissues [15]. This can lead to neoplasia, hyperplasia, and even cancer [15].

The analysis and quantification of hormones in water matrices has predominantly been done using chromatography, namely gas chromatography, due to efficient separation

and successful identification [16]. However, the biggest limitation of gas chromatography determination is the need for derivatization and conversion [17]. These manipulations can result in loss of analyte [17]. This has driven a surge in liquid chromatography (LC) methods that do not require chemical pretreatment for hormonal analysis and quantification [18]. LC methods often lack the GC specificity in complex matrices and thus require sample preparation in order to ensure accurate quantification [19].

Sample preparation allows for the preconcentration/isolation of ultratrace pollutants [20]. A vast number of sample preparation methods can be distinguished as being either liquid–liquid or solid-phase extraction [20]. Liquid–liquid extraction uses an organic solvent as the extracting phase [21,22]. In contrast, solid-phase extraction, as the name suggests, uses a solid phase (adsorbent material) to extract substances [23]. Solid-phase extraction (SPE) has been one of the most commonly used sample preparation techniques since its development in the 1980s [23]. Its advantages are based on its simplicity, selectivity, high enrichment factors, ease of automation, and use of different adsorbents [24,25]. Molecularly imprinted polymers have been termed as advanced adsorbent materials for SPE [20,24].

Molecular imprinting was first reported by [26] in a bid to generate artificial receptors. The imprinting process is done by polymerizing a functional monomer in the presence of a template molecule [27]. During polymerization, there is formation of a functional monomer–template complex [28]. This results in a 3D polymer network where a template is trapped. The template can then be removed by washing, thereby leaving cavities within the polymer network that are complementary to the size, shape, and molecular interaction of the template [27].

This review aims to investigate the use of molecularly imprinted polymers in the determination of hormones in different matrices, namely water, food, and biological samples. In addition, the most commonly used MIP synthesis methods, such as precipitation polymerization, and bulk and surface imprinting, are briefly discussed.

2. Global Concentration Levels of Hormones

The increasing concentrations of steroid hormone residues in various matrices in the environment is proof that even with the bans that are currently in place, their increased application is still predominant (Table 1).

Table 1. Global hormone concentration levels.

Country	Sample Type	Type of Hormone	Conc. Level	Ref.
Malaysia	Wastewater	17-β-E2 and 17-α-EE2	0.02–93.9 ng/L	[29]
South Africa	Wastewater	17-β-E2	15–2000 ng/L	[9]
Brazil	River water	17-β-E2	14.9 μg/L	[30]
China	River and wastewater	E1 and E3	2.1–360 ng/L	[31]
France	Butter	Medroxyprogesterone	4.1 μg/kg	[32]
Portugal	Wastewater	17-β-E2	0.085 μg/L	[33]
Romania	Wastewater	E3 and ethynylestradiol (EE)	2.6–4.7 μg/L	[34]
Poland	Ground water	E1	309 ng/L	[35]
China	Soil	PRO, androstenedione, TST, and 17α-E2	0.06–1/20 μg/kg	[36]
Hungary	River water	E2, coprostanol, cholesterol, stigmasterol, and β-stosterol	0.322–488 μg/L	[37]
China	River water	PRO, boldenone, and norgestrel	8.22–66.2 ng/L	[38]
China	River and surface water	E1, E3, and bisphenol A (BPA)	1.0–690 ng/L	[39]
Switzerland	Milk	E1, PRO, hydroprogesterone, cortisone, 4-androstenedione, and E2	10–342 ng/kg	[40]
Brazil	Surface water	E1, 17β-E2l, PRO, and 17α-EE2	0–5.84 μg/L	[41]
South Africa	Wastewater and river water	PRO, E1, E2, and E3	0–7133 ng/L	[42]
Serbia	Surface and wastewater	Cholesterol, coprostanol, campesterol, stigmasterol, β-sitosterol, and sitostanol	12–4808 ng/L	[43]
Chile	Human urine	TST, PRO, and E2	0.20–21.23 ng/L	[44]

In general, global studies have shown that these hormones are frequently detected in waste and surface waters [9,29,32–34,36–42]. The detection of steroid hormones in the environment has been reported to differ from country to country (Table 1). In some cases, their concentrations within the same country also differ depending on the regions or provinces. For example, in South Africa, maximum concentrations for estradiol of 7133 ng/L were detected in wastewaters from Gauteng Province [42], while concentrations of up to 2000 ng/L were reported in KwaZulu Natal Province for estrone (E1), 17-β-estradiol (E2), estriol (E3), 17-α-ethinylestradiol (17-α-EE2), androgens, and progestogens (PRO) [9]. Olatunji et al. [45] reported concentrations ranging from 600 to 45,500 ng/L for E2 and E3 in surface water around animal farms in Western Cape. The presence of steroid hormones in various environmental samples around the world are shown in Table 1. Maximum concentrations of up to 14.1 µg/L for E1, E2, E3, 17α-EE2, PRO, and testosterone (TST) were reported in Brazil [30,41], while Beldean-Galea et al. [34] reported steroid hormone concentrations ranging from 2.6 to 47 µg/L in Romanian water matrices. Other significantly high concentrations of steroid hormones were observed in Hungary (0.33–488 µg/L) [37] and Serbia (12–4808 ng/L) [43]. From these studies, it can be noted that wastewater treatment plants (WWTPs) are the main source of pollution of water by steroid hormones. This is because effluent from wastewater plants is often released into nearby rivers. Additionally, WWTPs are not able to completely remove most emerging pollutants, including hormones, during the treatment process [46]. In other instances, such as the study by Olatunji and coworkers [45], farm waste, wash water discharge, and incorrect disposal of unused products were found to be responsible for steroid hormones in surface water around farm lands. Table 2 also shows that steroid hormones have been detected in a variety of sample types. These include surface [39], ground [35], and wastewater [29] as well a food [32], soil [36], and human excreta [44]. This not only increases the chance of pollution but also means that exposure of these potentially endocrine disruptors is increased.

Table 2. Summary of application of MIPs for the determination of hormones in water samples.

Hormones	Matrix	Analytical Techniques	Polymerization Method	LOD (µg/L)	%Recovery	Reference
17β-E2	Lake, river water, effluent	HPLC	One-spot solvothermal reaction	0.04	88.3–99	[47]
E1, E3, 17β-E2, 17α-EE2, trans-androsterone, TST, and PRO	Wastewater	GC × GC-TOFMS and LC-MS	Bulk polymerization	-	-	[48]
Diethylstilbestrol (DES), E1, and E3	River, lake and tap water	HPLC–UV	Semicovalent polymerization	10–16	96–98	[49]
E3 and 17β-E2	Wastewater	LC–Q-TOFMS	-	-	-	[50]
E2, E3, and EE2	Tap, drinking, river water	HPLC–FLD	Surface polymerization	2.5–5.8	72–102	[51]
E2, E3, and DES	Lake and river water	HPLC–UV	Surface polymerization and sol–gel method	0.08–0.27	85–95	[52]
E1, E2, E3, DES, and EE2	River water	LC–MS	-	0.0045–0.0098	47–104	[53]
E1, E2, and DES	Lake and river water	HPLC–DAD	-	0.3–1.5	75–93	[54]
E1, E2, E3, and EE2	Wastewater	HPLC	-	1.96–2.76	81–113	[55]
17α-E2, 17β-E2, E1, hexestrol (HEX), 17α-EE2, DES, dienestrol (DS), zearalenone (ZEN), α-zearalanol (α-ZAL), and β-zearalanol (β-ZAL)	Mineral water and wastewater	HPLC–DAD	-	0.01–0.44	65–101	[56]
17β-E2, E1, and E3	Water	HPLC–DAD/ECD	Bulk polymerization	0.07–10.99	74–82	[57]
E2, EE, DES, ethisterone (ES), and E1	River water	HPLC–UV	Surface polymerization	0.1 to 0.26 mmol/L	50–96	[58]
estrogen dienestrol (DIS)	Seawater	HPLC–DAD	Surface polymerization	0.16	87.3–96.4	[59]

GC × GC–TOFMS = comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry, LC–MS = liquid chromatography–mass spectroscopy, HPLC–PDA = high-performance liquid chromatography–photodiode array, UV = ultraviolet spectrophotometry, LC–Q-TOFMS = liquid chromatography–quad time-of-flight mass spectroscopy, HPLC–DAD = high-performance liquid chromatography–diode array detector, ECD = electrochemical detector, HPLC–FLD = high-performance chromatography–fluorescence detection.

3. Molecularly Imprinted Polymer Synthesis Methods

Molecularly imprinted polymers (MIPs) offer a synthetic route to developing tailorable stationary phases, which are particularly useful in solid-phase extraction [60]. MIPs are synthetic polymers that can rebind a target molecule even in the presence of interferences [61], and they are made by copolymerizing functional monomers in the presence of a template molecule or substitute and a cross-linker [62]. The general preparative method for molecularly imprinted polymers is presented in Figure 2. The functional monomer is chosen based on its affinity for the template, which may be based on chemical properties as well as molecular modelling routines [63]; subsequent polymerization produces a three-dimensional polymer network with binding sites that are ideally complementary to the template in scale, shape, and functionalities [61].
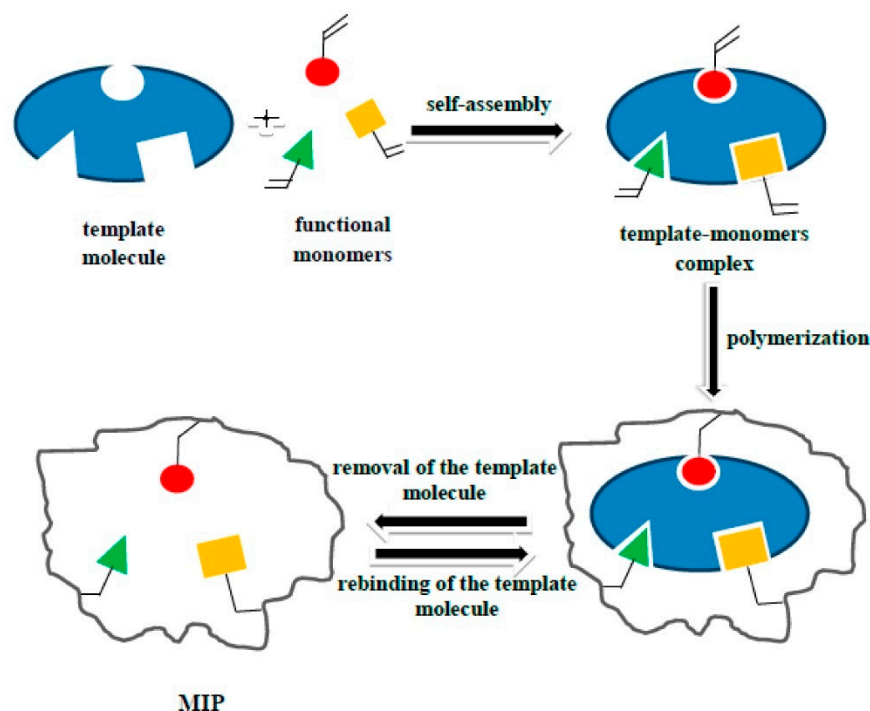

Figure 2. General scheme for molecular imprinting (figure adapted from [64]).

The type of functional monomer has a significant impact on the properties of the MIP being synthesized. The relationship between the functional monomer and the prototype should be either covalent or noncovalent [65]. In 2016, Figueiredo and coworkers identified methylacrylic acid, acrylic acid, and 4 vinylpyridine as the most common functional monomers used in imprinting [65]. The use of methylacrylic acid, acrylic acid, and 4 vinylpyridine, particularly for hormone application, could be due to high favorability of the resulting MIPs [63]. The stability of the functional monomer–template complex, the ability to form hydrogen bonds, and the absence of polymerizable groups are all important factors in MIP synthesis. The crosslinker, which influences MIP morphology, is the final component required for MIP synthesis. It also helps to keep the binding cavities of MIPs stable. The reviews by Spivak and Vasapollo and coworkers [66,67] highlight cross-linkers that are compliant with MIPs.

In general, three separate MIP synthesis approaches have been reported in the literature: covalent, semicovalent, and noncovalent synthesis [61]. The covalent method involves forming prepolymerization complexes in which the template is covalently bound to the monomers and then removing the template through chemical cleavage [68]. The covalent synthesis results in a homogeneous binding site population and limited nonspecific binding sites due to the high stability of the monomer–template interaction [67]. However,

reversible cleavage of the complex under mild conditions is difficult to achieve because of covalent monomer–template interactions [69].

The semicovalent approach is an immediate solution to the covalent approach restriction. In this approach, the monomer–template complex rebinding is based on noncovalent interactions, thereby making reversible cleavage simple [40].

Noncovalent imprinting allows the use of weak interactions and is regarded as a more versatile option [69]. The interactions responsible for the self-assembly between the template and monomer include hydrogen bonding, π–π bonding, and electrostatic and hydrophobic interactions [70]. The success of imprinting is characterized by high-affinity binding sites and is said to be dependent on the choice of the functional monomer [71]. Chen and colleagues [71] discussed in detail the different synthesis methods for MIPs. Hence, only precipitation, bulk, and surface imprinted polymerization will be covered in this review.

3.1. Precipitation Polymerization

One preparative phase is needed for precipitation polymerization. This method of polymerization yields uniform and spherical particles (diameters usually less than 1 µm), but it necessitates a significant amount of template [72]. Precipitation polymerization is a surfactant-free process that involves polymerizing monomers in dilute solutions (without overlap or coalescence) and removing the resulting polymer particles from the solution [73]. Entropic precipitation of nanogel (seed) particles, followed by continuous capture of oligomers from solution, is the most common way for particles to develop. The general scheme for precipitation polymerization in shown in Figure 3. In contrast to bulk polymerization, this form of polymerization necessitates a significant amount of solvent [74]. It should also be noted that many variables, such as the polarity of the solvent, reaction temperature, and stirring speed, affect the size of the particles obtained, so reaction conditions should be carefully regulated [75].
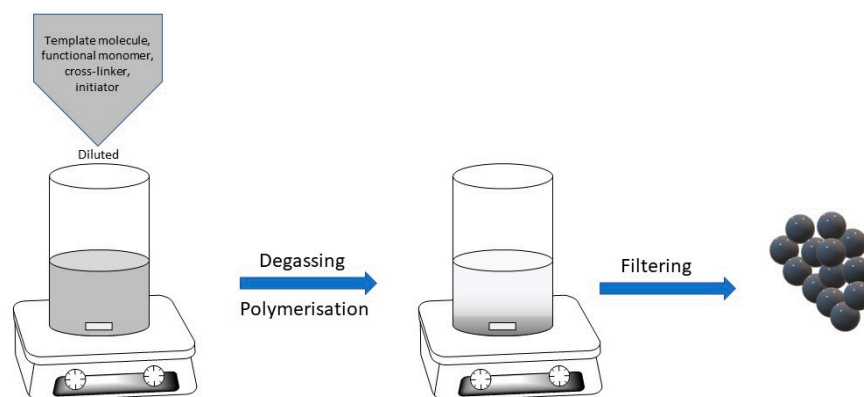

Figure 3. General synthetic scheme for polymerization.

3.2. Bulk Imprinting

Bulk imprinting involves imprinting a template molecule in its entirety in a polymer matrix and then fully removing it from the molecularly imprinted substance after polymerization [76]. The bulk polymer is mechanically crushed, and the particles formed are then fractionated in the next step to form small particles from these bulk polymers. For relatively smaller molecules, bulk imprinting is preferred. Due to the ease of adsorption and release of the template molecule, reversible binding can be carried out, which provides the potential for several rounds of reuse [77].

In sensor applications, using a whole polymer as a prototype has certain advantages over other methods [73]. Because the template protein (and the target at the same time) is fully imprinted, the template structure would be very similar to the target structure. However, the approach has certain disadvantages when dealing with larger structures,

such as proteins, living cells, and microorganisms. Maintaining the conformational stability of a protein during the polymerization process is difficult [4,78,79]. Furthermore, because of the size of the template, large imprinted sites may be attractive to smaller polypeptides, resulting in cross-reactivity and decreased selectivity [78]. Because of the thick morphology of bulk imprints, large template molecules are embedded too deeply in the matrices, limiting or preventing target molecules from binding to the sites. Low accessibility causes significantly longer response times, drift issues, and poor regeneration A more drastic situation is that removing the target molecule from the MIP is difficult. This will result in hindered binding or, in the worst-case scenario, no binding at all. Alternative imprinting methods, such as surface imprinting, have been designed to address these limitations [79,80].

3.3. Surface Imprinting

Surface imprinting is a useful technique for depositing a thin layer of polymeric material on a variety of substrates, including carbon nanotubes (CNTs), Fe_3O_4, TiO_2, and SiO_2. More accessible adsorption sites, rapid mass transfer, fast binding kinetics, and high selectivity are all provided by this layer of MIPs with imprinted cavities on the surface of the particles [72,81]. A general scheme of the synthesis of surface imprinting on a silica nanoparticle core is presented in Figure 4. Although bulk imprinting with these methods is the most popular synthesis technique, more recently, so-called surface imprinting methods have revealed distinct advantages. Such advantages include minimal material waste, enhanced access to binding sites exclusively on the particle's surface, tailorability of bead size from micro to nanoscale, access to sophisticated core–shell configurations (e.g., inorganic silica core particle with nanothin MIP shell), and reduced mass transfer limitations, leading to rapid binding kinetics [79,81,82].
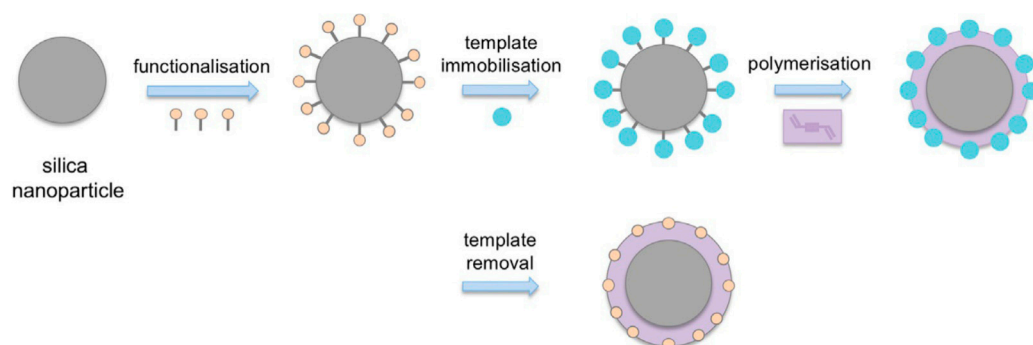

Figure 4. General preparation scheme for surface imprinting on a nanoparticle (adopted from Riedel and Mizaikoff 2019 [79] with permission).

By establishing binding sites near to and/or at the substrate level, surface imprinting improves the interaction between the template molecule and MIP, ensuring effective mass transfer. For imprinting larger molecules, surface imprinting is an especially promising technique [79]. Surface imprinting also has the advantage of requiring less template during the polymerization process than traditional bulk imprinting. Several imprinting methods have been investigated, including lithographic imprinting [83], dispersed-phase polymerization [84], and grafting via core–shell imprinting [85]. Additionally, electrochemical imprinting is another form of surface imprinting.

In electrochemical polymerization, the solution used contains the template, solvent, functional monomer, and supporting electrolyte [86,87]. There is no use of a core nanoparticle as in traditional surface imprinting polymerization. Electropolymerization is divided into potentiodynamic, potentiostatic, and galvanostatic electropolymerization. The resulting polymers may be neutral or charged due to the movement of solvated counter ion into or out of the film upcharging and discharging during film growth [86]. MIPs prepared using electropolymerization can easily adhere to transducer surfaces, the preparation is rapid,

and the film thickness can be controlled [87,88]. This makes them good candidates for sensor applications. The most attractive feature of molecular imprinting using electropolymerization is the complete removal of the template molecule by overoxidation [64,89,90].

4. Sorbent-Based MIP Applications

4.1. Water Samples

Lu and Xu [89] conducted a study investigating the concentrations of E1, 17β-estradiol (E2), and estriol (E3) in tap, river, and lake water samples. In their work, they explored the use of E1-imprinted $Fe_3O_4@SiO_2@mSiO_2$ (MM–MIPs) as an adsorbent DSPE for selective preconcentration and specific recognition of E1, E2, and E3. The MM–MIPs sorbent presented high adsorption capacity, high extraction efficiency, and fast mass transfer for the target analytes. The MM–MIP–SPE combined with HPLC–PDA showed relatively good analytical characteristics. The recoveries ranged from 85 to 95%, and the precision of the method was less than 6%. Low detection limits ranging from 0.09 to 0.4 $\mu g\ L^{-1}$ and high enrichment factor of 1700 were obtained. The use of magnetic MIP was also recently reported by Guc and Schroeder [64], MIPs and magnetic MIPs prepared using bulk polymerization and core–shell method procedures, respectively, were used as adsorbents for selective extraction and preconcentration of E1 and E2 from water samples. The quantification of the analytes in environmental samples was achieved using electrospray ionization mass spectrometry (ESI–MS) and flowing atmospheric pressure after glow mass spectrometry (FAPA–MS). The results obtained revealed that FAPA–MS (LODs = 0.135 $\mu g\ L^{-1}$) was more sensitive that ESI–MS (LODs ranging from 13.6 to 27 $\mu g\ L^{-1}$). The combination of MIP/magnetic MIP–SPE method (methodology scheme in Figure 5) with FAPA–MS resulted in accurate quantification of trace amounts of E1 and E2 in water, and the concentrations were found to be 0.271–0.273 $\mu g\ L^{-1}$. These methods combine the features of MIPs, which ensures the production of selective cavities on the surface of the iron oxide core. This provides magnetic properties to the MIPs, thus facilitating easy removal and reuse. The recognition sites located on the surface of each magnetic MIP particle result in increased selectivity and sensitivity.
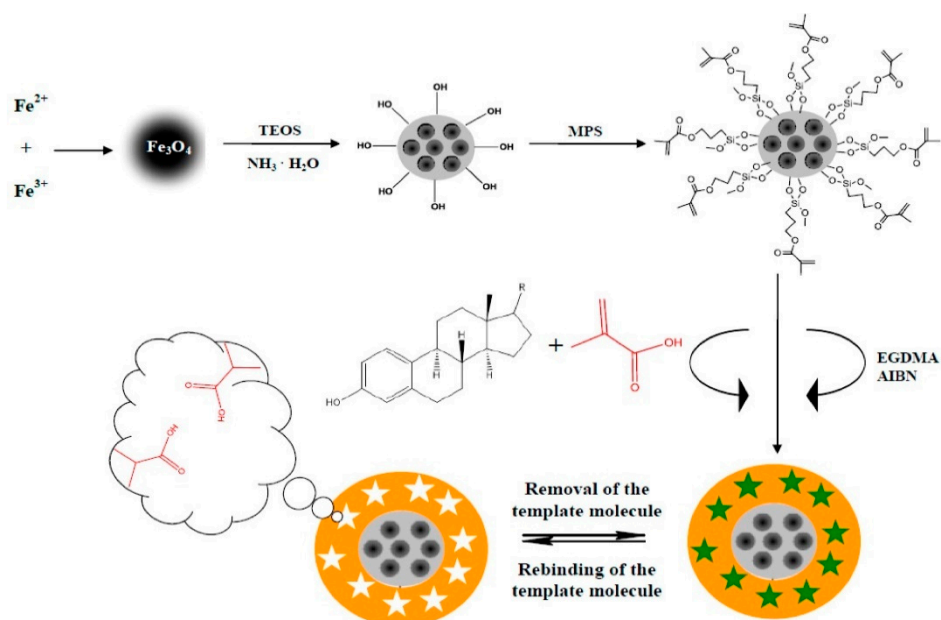

Figure 5. Magnetic MIP–SPE of steroid hormones reported by Guc and Schroeder [64].

In another study, the MIP–SPE method was coupled with high-performance liquid chromatography coupled with diode array detection (HPLC–PDA) for selective determination of E1, E2, E3, 17-α-EE2, PRO, and TST in water [91]. The method proved to be suitable for quantification of steroid hormones in water because it had relatively low LODs in the

range of 0.0182–0.0898 µg/mL. In addition, high recoveries (79–101%) and low matrix effects (<20%) suggested that the developed MIP–SPE method could be used for selective identification and quantification of the target analytes [91].

Guedes-Alonso et al. [90] reported the synthesis and application of MIP as an adsorbent for SPE of estrogens from wastewater collected from a veterinary hospital and a wastewater treatment plant. The developed method demonstrated adequate LOD (0.180.45 ng/mL), acceptable recoveries (>60%), and high precision (<RSD 10%). The coupling of MIP–SPE with UPLC enabled accurate quantification of the analytes at concentrations ranging from 1.35 to 2.57 ng/mL.

The consolidated summary of the application of MIPs as adsorbent for the extraction and preconcentration of different types of steroid hormones is presented in Table 2. As can be seen, the use of MIPs as selective adsorbents allows the use of conventional techniques such as HPLC–UV (DAD) or HPLC–FLD for simultaneous determination of hormones in environmental matrices. Furthermore, it can be noted that acceptable to low detection limits (0.04–1.5 µg/L) were obtained using HPLC–DAD/FLD [47,49,50,52–56]. The introduction of MIPs to various analytical techniques has brought much improvement in terms of selectivity and sensitivity. This can be attributed to the properties of MIPs such as the cavities formed, which are complementary to the template's structural signature (size, shape, and functional group positioning) [64]. The studies conducted by [55,56,58,59,92] examined different steroid hormones, such as E2, beta, and alpha-zearalonol. The application of MIPs as a sorbent in SPE (Table 2) shows that low limits of detection for instruments such as HLPC can be achieved using MIPs.

4.2. Food Samples

Residues of steroid hormones have been detected in various food matrices, especially in milk samples (Table 3). This might be due to the use of steroid hormones as growth promoters. For instance, Tang and colleagues [93] reported the dummy molecularly imprinted polymer microspheres (DMIPMS) as adsorbent for extraction and preconcentration of natural and synthetic estrogens E1, 17β-E2, E3, EE2, DS, DES, and HEX in milk samples. The microspheres were synthesized via Pickering emulsion polymerization, and genistein (GEN) was employed as a dummy template molecule. The FTIR analysis confirmed that the DMIPMS were successfully prepared as all the expected functional groups were observed. The developed method was found to be selective toward the selected seven estrogens, and their quantification was conducted using HPLC–MS/MS. The DMIP–SPE coupled with HPLC–MS/MS displayed excellent linearity with LODs in the range of 0.10–0.35 µg L^{-1}. The recoveries after spiking the milk samples at three levels were between 88.9 and 102.3%. These results suggested that DMIPMS-based SPE could be used for monitoring of trace estrogens in food samples such as milk.

Table 3. Summary of application of MIPs for the determination of hormones in food samples.

Hormones	Food Type	Analytical Technique	Polymerization Method	LOD µg/L	%Recovery	Refs
E2	Goat milk	HPLC–PDA	Bulk polymerization	4.81	76–90	[94]
PRO, TST, β-sitosterol, cholesterol, and campesterol	White meat, egg yolks, and vegetables	HPLC	-	0.003–0.005	97–101	[95]
E2	Milk powder	UV	Surface polymerization	9.533	84	[96]
E2	Milk	HPLC–UV	Surface polymerization	0.01	89–92	[97]
E2	Beef	HPLC–PDA	Surface initiated atom transfer radical polymerization	0.25	97–99	[98]

Table 3. *Cont.*

Hormones	Food Type	Analytical Technique	Polymerization Method	LOD µg/L	%Recovery	Refs
E1, E2, and E3	Milk	HPLC	Surface polymerization	-	81.6–91.6	[99]
E1, E3, and EE2	Fish and shrimp	HPLC–UV	Multiple copolymerization	0.98–2.39	80–94	[92]
E1, E2, E3. and DES	Milk powder	HPLC–UV	surface polymerization	1.5–5.5 ng/g	81–95	[100]
E2 and E3	Milk tablets	HPLC–UV	Surface polymerization	1.49–1.83	89.1–93.5	[101]
DES	Pork and chicken	HPLC–UV	Surface polymerization	0.28–0.47	83–99	[102]
E2	Milk	HPLC–FLD	-	0.006	95–107	[103]

HPLC–PDA = high-performance liquid chromatography–photodiode array, UV = ultraviolet spectrophotometry, HPLC–FLD = high-performance chromatography–fluorescence detection.

A restricted access media–MIP (RAM–MIP) was reported by Wang and coworkers [93] for selective extraction of 17β-E2 from milk samples. RAM–MIP was prepared via the surface imprinting method whereby monodisperse crosslinked poly(glycidyl methacrylate-co-ethylene glycol dimethacrylate) microspheres were used as the carrier and acryloyl chloride-modified β-cyclodextrin as the hydrophilic functional monomer. The resultant adsorbent was found to have high adsorption affinity toward E2. The RAM–MIP–DSPE coupled with HPLC–PDA was used for analysis of E2 in milk samples. The method showed promising analytical performance, such as high recoveries (up to 95%), high precision (<4%), and relatively high sensitivity (LOD = 2.1 µg/L). In another study, Zhu and colleagues [104] reported the synthesis of zipper-like on/off switchable magnetic molecularly imprinted microspheres (SM–MIMs) as an adsorbent for the analysis of E2 in milk samples (methodology of the scheme is presented in Figure 6). The SM–MIMs was prepared by surface polymerization of acrylamide (AAm) and 2-acrylamide-2-methyl propanesulfonic acid (AMPS). It was observed that the adsorption of E2 to the adsorbent was temperature dependent. The authors reported that this phenomenon was due to the interactions between two polymers (poly(AAm) (PAAm) and poly(AMPS) (PAMPS)), which had on/off switchable property to temperature. The SM–MIM–SPE combined with HPLC–PDA displayed attractive analytical performance, such as high selectivity, low LOD (2.5 µg/L), and high recoveries (79–90%), of E2 in complex matrices. Table 3 also shows that the use of MIPs allows researchers to use simple and inexpensive detection techniques, such as ultraviolet spectrophotometry (UV) [96] and high-performance liquid chromatography with UV detectors [95,97,100,103] and achieve low limits of detection. This demonstrates that the use of MIPs can reduce the dependency on GC analysis.
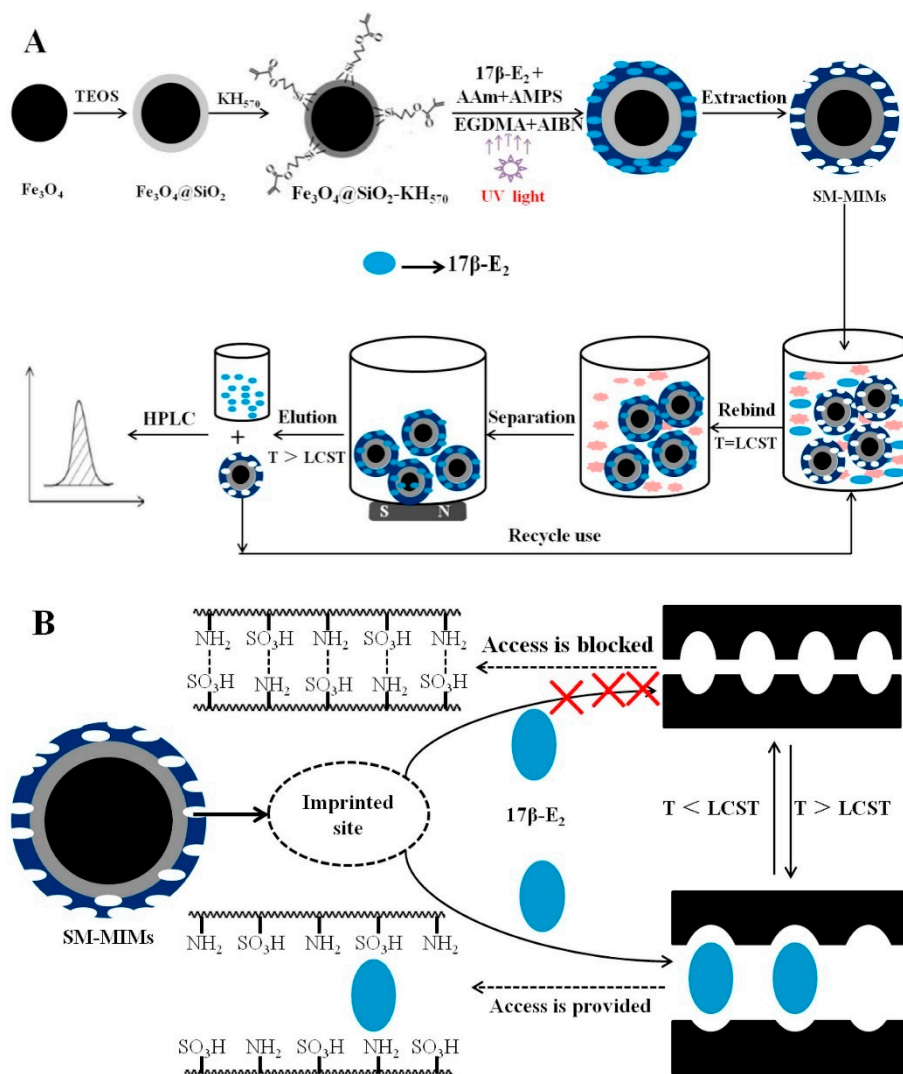
Figure 6. Zipper-like on/off switchable magnetic molecularly imprinted microspheres used by Zhu and colleagues [104] for the solid-phase extraction of steroid hormones in milk samples. (**A**) Represent the preparation of molecularly imprinted microspheres and (**B**) on/off-switchable recognition mechanisms of molecularly imprinted microspheres.

4.3. Biological and Other Complex Samples

Qiu et al. [105] investigated the levels of anabolic steroids, such as androsterone, stanolone, androstenedione and methyltestosterone, in human urine. This was achieved using MIP-coated SPME fibers combined with GC–MS. In their study, a testosterone MIP was prepared using thermal radical copolymerization of MAA and trimethylolpropanetrimethacrylate (TRIM). The developed method presented promising analytical figures of merit. The LOQs, precision, linearity, and recoveries were in the range of 0.01–0.08 ng/mL, 4–15%, 0.02–1 ng/mL, and 87–108%, respectively. The authors reported enrichment factors, i.e., the enrichment of the chromatographic peaks between these results suggested that the developed MIP-coated SPME/GC–MS was suitable for rapid extraction and determination of trace anabolic steroids in biological matrices.

A selective extraction and determination of PRO hormones from biological matrices such as urine and blood samples were reported by Nezhadali et al. [106]. The extraction and preconcentration of target analytes were achieved using a polypyrrole MIP (prepared via bulk polymerization) as adsorbent followed by GC–FID quantification. Under optimized conditions, the LOD, LOQ, and recoveries were 0.63, 1.9 ng/mL, and 86–101%, respectively.

The method was successfully applied for the determination of the target analyte in urine and blood.

Du et al. [107] reported the preparation of dexamethasone-imprinted polymers (DEXA-MIP) via surface molecular imprinting. The synthesis and application summary can be seen in Figure 7. The surface molecular imprinting was achieved using a method called reversible addition–fragmentation chain transfer polymerization on the surface of magnetic nanoparticles. The prepared MIP was used as a magnetic adsorbent for SPE of DEXA from skincare cosmetic samples prior to HPLC–PDA determination. The developed method displayed relatively good accuracy (93–97.6%), high precision (RSD < 3%), and low LODs (0.05 µg/mL). Furthermore, the developed MIP–SPE/HPLC method possessed attractive features, such as specific molecular recognition, high adsorption affinity and selectivity, and simplicity, and it was considered as a good candidate for monitoring trace concentration of DEXA in various complex matrices.
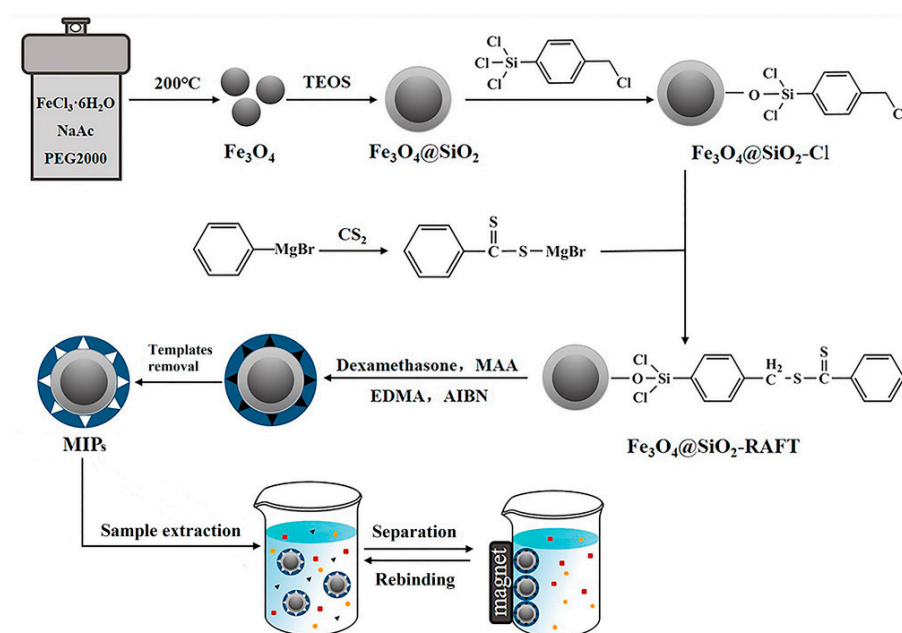

Figure 7. Surface-imprinted magnetic polymers used by Du and coworkers [107] for the extraction and determination of dexamethasone.

A study by Xu et al. [54] reported the application of dual-template MIP–SBSE combined with HPLC–DAD for analysis of E1, E2, and DES in plastic samples. The developed methods were found to be suitable for selective determination of steroid hormones in complex matrices with recoveries ranging from 78 to 97%. In cases where analytes are different structurally, MIPs are not suitable for analysis of target analytes in complex real samples, such as cosmetics [108]. To overcome these shortcomings, dual-template MIPs are used. For instance, Liu et al. [108] conducted a study investigating the presence of glucocorticoids in cosmetics samples. In their study, they explored the use of novel dual-template magnetic MIP as an adsorbent for SPE. The magnetic MIP was synthesized using the surface polymerization method, and hydrocortisone and DEXA were used as templates. The prepared dual-template magnetic MIP had high affinity toward target analytes, and it was used for extraction and enrichment of hydrocortisone and DEXA in cosmetic products. The magnetic MIP–SPE/HPLC method displayed satisfactory recoveries ranging 86.8–107.5% as well as good precision (RSD <3%). Other applications of MIP based SPE for preconcentration and extraction of hormones prior HPLC analysis are presented in Table 4. As seen in this Table, SPE based MIP enabled accurate quantification of various steroid hormones from urine and serum us-ing less sensitive such as HPLC-UV. In comparison with UHPLC-MS/MS, HPLC-UV had high detection limits (25-92µg/L).

Table 4. Summary of application of MIPs for the determination of hormones in biological samples.

Hormones	Matrix	Analytical Techniques	Polymerization Method	LOD µg/L	%Recovery	Refs
E1 and E3	Urine	HPLC–UV	-	25–32	70–80	[109]
EE2 and E2	Urine	HPLC–UV	-	76–92	96–99	[110]
E1, 17α- α-E2, β-E2, E3, EE2, DES, BPA, bisphenol S (BPS), 4-n-octylphenol (OP), 4-n-coumestrol (COU), genistein (GEN), and enterolactone (ENT)	Maternal serum, cord serum, and urine	UHPLC–MS/MS	-	0.01–0.7	>100	[111]
PRO and TST	Human urine	HPLC–DAD	Bulk polymerization	0.47	>80	[111]

HPLC–UV = high-performance liquid chromatography with ultraviolet, UHPLC–MS/MS = ultrahigh-performance liquid chromatography coupled with tandem mass spectroscopy, DAD = diode array detector.

The benefits of using MIPs in solid-phase extraction include improvement of the recognition selectivity, simplicity, flexibility, and detection sensitivity of the extraction process and the solventless nature associated with solid-phase extraction [112,113] MIPs in solid-phase extraction provides an important tool for chemo/bioanalysis in complex matrices and benefits from distinguished advantages, such as easy operation, high throughput, low cost, high selectivity, and durability [114]. However, there are also disadvantages, such as the lack of compatibility between the solvent needed to desorb analytes from the MIP and the mobile phase used (typical drawback of online MISPE protocols) [115].

5. MIP Challenges

Though inexpensive and easy to scale up after calibration of a particular setup, MIP synthesis needs to be highly customized to the desired target(s). This can be a challenging task as there is no universal preparation protocol that ensures adequate selectivity and the MIP technology is not easily transferable among different applications [116,117]. The removal of the template after successful imprinting is important for the steps following it, such as assessing binding capability and nonspecific adsorption on nonimprinted polymers [118]. Because different templates and complementary functional monomers interact differently, techniques for template removal and assessment of binding capability and nonspecific adsorption on nonimprinted polymers are often inconsistent [116]. For example, different polymers necessitate different solvent strength for template removal. Acrylic acid polymers are more resistant than self-polymerizing PDA MIPs [119]. Although their stability is also advantageous in bioapplications because they can be sterilized and reused, the verification of template elimination in is often inconsistent [116]. While some studies have evaluated template removal using separation methods such as HPLC, which is evaluated indirectly as a residual of analyte in the sample that is not bound by MIP, others have not found any precise technique for confirming template removal [117]. Additionally, MIPs do not produce any signal to show analyte binding onto the polymer, so they must be used in conjunction with a suitable detection method [73]. MIP surfaces are often formed over a functional NP, such as one with optical properties. Even though fluorescence and luminescence methods are becoming more common, the problem of determining the optimal penetration depth of light emission for in vivo applications has yet to be solved. When used for whole-body optical imaging, however, the signal may be insufficient [73,120].

6. Conclusions

Growing interest in the use of selective adsorbents for extraction of environmental pollutants, especially those with endocrine-disrupting properties, has resulted in the development of MIP-based SPE methods. The number of publications reporting the use of MIP-based SPE in environmental analysis suggest that MIP-based sorbents remain one of the main factors in the field of sample preparation. This review summarized the

recent application of MIP-based methods for selective extraction and preconcentration of steroid hormones as well as sample clean-up of complex matrices, such as soil, food, and biological samples. Previous studies have revealed that the use of MIP-based SPE methods enable accurate quantification of steroid hormones in water, food, biological, and other complex samples. Furthermore, the literature shows that precipitation, bulk, and surface imprinting are the most frequently used methods for the preparation of MIPs. However, the absence of universal synthesis protocols that do not require method tuning and do not have inconsistent template molecule removal after synthesis or nonspecific adsorption on the polymer has become a major challenge associated with the preparation and application of MIPs. Researchers have developed novel hybrid MIP-based sorbents to address these challenges. More recently, the use of sensors is likely to be part of the next generation of analysis methods, especially MIP-based sensors. This is because the synthesis methods used for the preparation of MIP sensors, such as electropolymerization, also addresses one of the most important challenges of MIP synthesis, which is related to the removal of the template. Finally, the use of sensors would reduce costs associated with analysis as they eliminate the need for sophisticated instrumentation. The use of MIPs as selective adsorbents for steroid hormone detection could still be the driver of the next set of innovations.

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