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Review

The Application of Molecularly Imprinted Polymers in Forensic Toxicology: Issues and Perspectives

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Abstract: Molecularly imprinted polymers (MIPs) are synthetic receptors designed to selectively bind specific molecules, mimicking natural antibody–antigen interactions. Produced through polymerization around a target molecule (template), MIPs create imprints that confer high specificity and binding affinity upon template removal. Initially developed in the 1970s with organic polymers, MIPs now play critical roles in separation sciences, catalysis, drug delivery, and sensor technology. In forensic science, MIPs offer potential for sample preparation, pre-concentration, and analyte detection, especially with complex biological and non-biological matrices. They exhibit superior stability under extreme conditions, enabling their use in challenging forensic contexts such as detecting new psychoactive substances or trace explosives. Despite advantages like reusability and high selectivity, MIPs face limitations in forensic analysis due to their complex synthesis, potential template leakage, and non-specific binding. Moreover, the lack of standardized protocols limits their mainstream adoption, as forensic applications require validated, reproducible methods. This review systematically assesses MIPs in forensic toxicology, focusing on their current capabilities, limitations, and potential for broader integration into forensic workflows. Future research should address standardization and evaluate MIPs' effectiveness in diverse forensic applications to realize their full potential.

Keywords: forensic toxicology; molecular imprinting technology (MIT); molecularly imprinted polymers (MIPs); biosensors

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Citation: Mohamed, S.; Santelli, S.; Giorgetti, A.; Pelletti, G.; Pirani, F.; Fais, P.; Pascali, J.P. The Application of Molecularly Imprinted Polymers in Forensic Toxicology: Issues and Perspectives. *Chemosensors* **2024**, *12*, 279. https://doi.org/10.3390/chemosensors12120279

Received: 6 November 2024
Revised: 13 December 2024
Accepted: 17 December 2024
Published: 23 December 2024

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1. Introduction

Molecularly imprinted polymers (MIPs) are synthetic receptors engineered to selectively bind a target molecule, functioning as artificial analogs of natural antibody–antigen systems. Their production involves the use of a template molecule (the analyte of interest), functional monomers, and cross-linkers. During the polymerization process, a solid polymer block is formed, embedding imprints of the template. These imprints are structurally complementary to the original template, allowing MIPs to bind the target with high specificity and affinity once the template is removed [1]. The concept of molecular imprinting dates back to the early 20th century. However, significant advancements in the field were made in the 1970s when organic polymers replaced silica gel as the primary solid substrate. This shift accelerated research, enabling a more in-depth exploration of template–monomer interactions. Today, non-covalent imprinting has become the predominant technique for synthesizing modern imprinted polymers [2]. MIPs are highly versatile and customizable, which has led to their application in various fields. In separation sciences and purification, they are widely used to enhance analytical efficiency in techniques such as liquid chromatography, capillary electrochromatography, capillary electrophoresis, and solid-phase extraction [3]. Additionally, MIPs can serve as catalyst [1,4] components in drug delivery systems and elements in monitoring devices [5]. In recent years, MIPs have

gained prominence, particularly when integrated with transduction platforms, leading to the development of biosensors used in clinical diagnostics, environmental monitoring, and food safety analysis [6–8]. MIPs offer several advantages over natural recognition systems like antibody–antigen interactions, most notably their higher stability across varying environmental conditions such as extreme temperatures and pH levels [7]. Since their inception, MIPs have garnered significant interest in forensic science due to their high selectivity, sensitivity, and cost-effectiveness. These characteristics of MIPs are of interest for analytical forensic toxicologists, who constantly face new challenges in the identification of new psychoactive substances in complex biological material. The introduction of new molecules into this illicit market requires toxicologists to adopt increasingly novel methods for sample purification and enrichment, and the use of traditional antibodies to screen for analytes may not be competitive anymore. Moreover, their stability and reusability make them attractive for sample preparation, pre-concentration, and the detection of a range of forensic analytes, including drugs, fire debris residues, explosives, and other critical substances [9]. Despite their promise, the integration of MIPs into mainstream forensic analysis remains in its early stages and represents an underexperienced methodology. The forensic field presents unique challenges, including the detection of trace-level analytes across a broad spectrum, which requires further focused research to evaluate the effectiveness of MIPs compared to existing forensic techniques. Moreover, studies should explore how MIPs can be seamlessly incorporated into current forensic workflows. The objective of our research is to provide a comprehensive, systematic review of the impact of MIPs in forensic toxicology, focusing on both biological and non-biological substrates. We aim to assess the current capabilities of MIPs, identify areas for improvement, and explore their potential for broader adoption within forensic practices.

2. Materials and Methods

2.1. Literature Search and Selection Process

A systematic search of three electronic databases (i.e., PubMed, Web of Science, and Science Direct) was conducted until September 2024, considering forensic applications in biological and non-biological matrices separately. The following search strategy was applied, respectively, for biological and non-biological matrices:

- Non-biological matrices (molecularly imprinted polymers) AND (inorganic matrices) OR (explosives);
- Biological matrices: (gunshot OR gunshot wound OR gunshot injur* OR ballistic forensic) AND (radiolog* OR imaging).

Duplicates were manually excluded.
The following eligibility criteria were applied to the retrieved papers:

a. English language.
b. Relevance to the topic (i.e., application of MIPs in forensic science).

The exclusion criteria were

c. A time range of 10 years was considered for this review, and papers published before 2014 were excluded.

Records were screened by two sets of authors, one dedicated to biological matrices and one to non-biological matrices, applying the abovementioned inclusion and exclusion criteria. The first screening was broad and inclusive, on the basis of titles and abstracts. Then, the reports of included records were sought, excluding unretrievable records from further selection. Then, the retrieved articles were re-analyzed using the same inclusion and exclusion criteria with a more rigorous and selective process by reading the full text. The references of the retrieved manuscripts were additionally screened and cross-referenced for further relevant literature, and duplicates were removed manually. If a set of authors identified a paper that they excluded based on the analyzed matrix but that could potentially meet the criteria of the other set of authors, they passed it along to them. In the

case of discrepancies in opinions among the authors, a third, more experienced author was consulted (Figure 1).

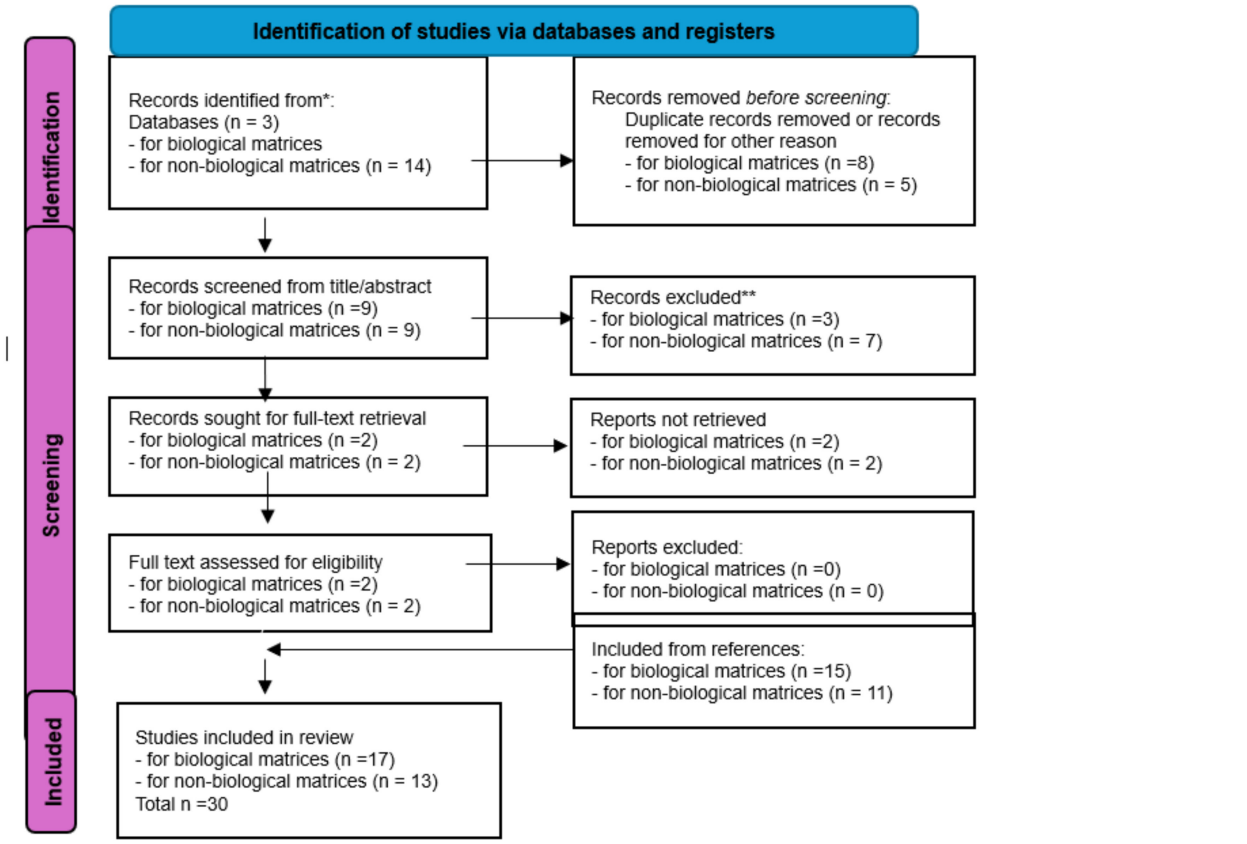

Figure 1. PRISMA 2020 flow diagram for new systematic reviews, which included searches of databases and registers only. Source: [10] This work is licensed under CC BY 4.0. To view a copy of this license, visit https://creativecommons.org/licenses/by/4.0/ (accessed on 17 September 2024). * Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers). ** If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

2.2. Data Extraction

Two separate Excel databases were created, each focusing on a different topic: biological matrices and non-biological matrices. These databases included relevant articles and reported publication details such as author, year, and journal. From each paper, specific pre-defined data were extracted, including the analyte of interest, the matrix or surface analyzed, the type of molecularly imprinted polymer (MIP) used, the instrumentation employed for forensic analysis, and key analytical parameters such as the limit of detection (LOD) and lower limit of quantification (LLOQ). For studies involving biological matrices, the instrumentation was further classified into pre-defined categories: liquid chromatography (LC) or gas chromatography (GC) coupled with mass spectrometry (MS), tandem mass spectrometry (MS/MS), or other techniques.

3. Results

3.1. Biological Matrices

A bibliographic review focusing on the application of molecularly imprinted polymers (MIPs) in the analysis of psychotropic drugs, including cocaine, amphetamines, cannabinoids, opiates, benzodiazepine, and new psychoactive substances (NPSs), with particular attention paid to their application in biological samples, was conducted by studying papers published over the past decade. A total of 16 studies were selected, all applying MIPs in the field of forensic toxicology. Most of these methods employed ethylene dimethacrylate (EDMA) or ethylene glycol dimethacrylate (EGDMA) as cross-linkers, with methacrylic acid (MAA) or methyl methacrylate (MMA) as the monomer (details in Table 1).

Table 1. Forensic toxicological MIP application on biological matrices.

Analytes	Matrix	MIP	Technique	LOD (ng/mL)	LLOQ or Lower Concentration Point (ng/mL)	References
Cocaine and metabolites	Urine	EDMA, DVB, AIBN	HPLC-MS/MS	0.049–0.50	0.081–0.83	[11]
Cocaine	Oral fluid	free radical polymerization	PS-MS	0.27	1	[12]
Cocaine	Oral fluid	MAA, EDMA, AIBN	IMS	18	60	[13]
Amphetamine-type drugs	Urine	MPEA-MIPs SPE	LC-MS/MS	0.05–0.29	0.16–0.98	[14]
Cannabinoids	Plasma/urine	EDMA, DVB	LC-MS	plasma 0.11–0.15 urine 0.14–0.17	0.36	[15]
Cannabinoids	Urine/oral fluid	AM and EDMA	LC-MS-MS	0.75/0.5	1/0.75	[16]
Morphine	Plasma/urine	magnetic molecularly imprinted polymer	UHPLC with diode array detection	0.03	0.08	[17]
Synthetic cathinones	Urine/blood	AFFINILUTE MIP-Amphetamine SPE cartridge	LC-MS/MS	0.015–0.15	10	[18]
Synthetic cathinone 4-MDMC	Urine	MAA, EGDMA	HPLC-UV	ND	ND	[19]
Synthetic cathinone 4-MMC	Urine	Fe_3O_4@SiO_2@Au-MIPs	HPLC-UV	ND	ND	[20]
Amphetamine and cathinone derivates	Oral fluid	MAA, EDMA, AIBN	UHPLC-MS/MS and IMS	0.03–1.3 10–80	329	[21]
Oxazepam bromazepam alprazolam	Urine	MAA, EGDMA	LC-UV and LC-MS/MS	15–55 0.003–0.33	50–290 0.01–1.1	[22]
Diazepam oxazepam temazepam nordiazepam bromazepam tetrazepam	Urine	MAA, EGDMA	HPLC-DAD	13.5–21.1	44.5–69.3	[23]
Arylcyclohexylamine	Oral fluid	MAA, EDMA, AIBN	IMS	15	50	[24]
Oxycodone	Plasma	MAA, EDMA, AIBN	LC-UV	1.24	3.76	[25]
Ethylphenidate	Oral fluid	MAA, EGDMA, AIBN	IMS	20	66	[26]
Methadone	Plasma	PE	LC-MS/MS	1	5	[27]

For the selective extraction of cocaine and its metabolites—benzoylecgonine (BEC), ecgonine methylester (EME), and cocaethylene (CE)—from human urine, Sánchez-González et al. [11] proposed a micro-solid-phase extraction (μ-SPE) method using an MIP as the adsorbent. Quantification was performed via high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS), achieving detection limits (LOD) between 0.049 ng/mL and 0.5 ng/mL. The method was applied to seven urine samples from poly-drug abusers.

In another study, Tavares et al. [12] developed an MIP membrane used in a paper spray MS source for cocaine analysis in oral fluid. This method achieved a limit of quantification (LOQ) of 1 ng/mL and required no sample preparation, minimal solvent use, and an analysis time of less than 30 s. Similarly, Sorribes-Soriano et al. [13] designed an MIP-based solid-phase extraction (SPE) method for cocaine determination in oral fluid, using ion mobility spectrometry (IMS) as a potential alternative to immunoassay techniques. Their method yielded an LOD of 18 ng/mL and an LOQ of 60 ng/mL and was applied to 19 saliva samples, demonstrating its comparability with reference methods.

Xiong et al. [14] developed an MIP-SPE method for the determination of amphetamine-type stimulants (ATSs) in urine, followed by LC-MS/MS analysis. Their approach achieved good linearity (1.0–50.0 ng/mL), low matrix effects (85–112%), and an LOD of 0.05 ng/mL, with the method being validated on spiked urine samples. The novelty of this study lies in the use of dummy MIPs synthesized via reversible addition–fragmentation chain transfer polymerization (RAFT), showing great potential for ATS detection.

Two studies focused on cannabinoids. Sánchez-González et al. [15] created an MIP that is selective for Δ9-THC and its metabolites, offering a simple, cost-effective extraction method suitable for toxicological analysis. Their μ-SPE device could be reused at least 19 times, making it a promising alternative for detecting cannabis abuse. Its sensitivity and selectivity proved suitable for forensic applications. Cela-Pérez et al. [16] developed an MIP-SPE method followed by LC-MS/MS for detecting Δ9-THC, THC-COOH, CBN, and CBD in urine and oral fluid, with linear ranges from 1 ng/mL to 500 ng/mL in urine and 0.75 ng/mL to 500 ng/mL in oral fluid. Magnetic molecularly imprinted polymers were developed by Rahnmani and colleagues for the selective extraction of morphine in urine and plasma by using a core–shell methodology. Briefly, particles of Fe_3O_4NP were obtained from $FeCl_2$*$4H_2O$ and $FeCl_3$*$6H_2O$ and magnetically collected from the solution. Morphine was used in its free base form. To form the template–monomer complex, morphine, MAA (methacrylic acid), and Fe_3O_4/SiO_2-NH_2 were mixed together. Acetonitrile, EGDMA (ethylene glycol dimethacrylate), and AIBN (2, 2-azobisisobutyronitrile) were added to prepare the prepolymerizing mixture with continuous stirring to complete the polymerization reaction to form MMIPs (morphine molecularly imprinted polymers). The authors concluded that under the experimental conditions, the synthesized MIPs showed strong magnetic properties, high adsorption capacity, good selectivity, cost-effectiveness, and fast adsorption kinetics for morphine extraction.

Murakami et al. [18] proposed an MIP-SPE method for detecting synthetic cathinones (SCs) in whole blood and urine, while Chen et al. [18] evaluated the extraction of 4-methylmethcathinone (4-MMC) from spiked urine samples using an MIP synthesized through non-covalent polymerization. In another study, Fe_3O_4@SiO_2@Au (FSA) MIPs were prepared for the selective determination of 4-MMC in human urine [20], showing excellent enrichment and recovery. This method is expected to extend to the separation of other SCs. The methodology employed a four-step synthesis procedure to prepare $Fe_3O_4SiO_2$Au MIPs from Fe_3O_4NP produced by a solvothermal reaction, the preparation of NH_2-modified Fe_3O_4@SiO_2, and the subsequent inclusion of Au to prepare the Fe_3O_4@SiO_2@Au nanospheres. The synthesis of the Fe_3O_4@SiO_2@Au MIPs was carried out according to the existing literature by adding MAA and the template (4MMC).

Sorribes-Soriano et al. also developed an MIP for methamphetamine screening in oral fluid samples [21], targeting amphetamines, cathinones, and 2C-family drugs. After SPE pretreatment, 32 NPSs and amphetamine-related substances were analyzed using

IMS and ultra-high-performance liquid chromatography–mass spectrometry (UHPLC-MS), with LODs ranging from 10 ng/mL to 80 ng/mL (IMS) and 0.03 ng/mL to 1.3 ng/mL (UHPLC-MS). This method was applied to spiked samples.

Among benzodiazepines, Varenne et al. [22] focused on the selective extraction of oxazepam from urine samples, obtaining an LLOQ of 0.01–1.1 ng/mL depending on the instrumentation used. Similarly, Gil Tejedor et al. [23] synthesized a new MIP for extracting six benzodiazepines from urine, with LOD values ranging from 13.5 ng/mL to 21.1 ng/mL. Their method was successfully applied to a real case of benzodiazepine use.

In the case of arylcyclohexylamines, an MIP was synthesized for the selective extraction of 3-hydroxyphencyclidine (3-OH PCP) from oral fluid [24]. Adjustments to the washing solvent allowed for both class-selective and more specific extractions of 3-OH PCP. The method was validated on spiked oral fluid samples and analyzed using IMS.

Khanlari et al. [25] developed an MIP-SPE method for determining oxycodone residues in human plasma, achieving an LLOQ of 3.76 ng/mL.

A novel MIP sol–gel tablet was prepared for the micro-solid-phase extraction of methadone from plasma samples [26], capable of performing more than 20 extractions. Additionally, one study explored the determination of ethylphenidate, a central nervous system stimulant, in oral fluid using MIP extraction and IMS, highlighting the novelty of also using MIPs for this class of substances of interest for anti-doping controls [27]. The applicability for real cases still remained to be verified at this point.

3.2. Non-Biological Matrices

On the base of the abovementioned criteria, we selected a total of 13 articles published in the last 10 years, most of which (n = 9, 69.23%) focused on the detection of TNT [28–35]. Other molecules of interest were RDX (n = 3, 23.08%) [29,36,37], HMX (n = 1, 7.69%), and CL-20 (n = 1, 7.69%) [37], DNT (n = 2, 15.38%) [35,38], TATP (n = 1, 7.69%) [39], TNP (n = 1, 7.69%) [40]. As shown in Table 2, three articles considered more than one molecule. All the articles included were experimental studies, and MIPs were synthetized for a specific purpose. For their synthesis, in half of the cases (n = 7, 53.85%), the original molecule was used as the template, whereas in the other studies, dummies or analogs were employed. As observed for biological matrices, most of the methods employed EDMA or EGDMA as cross-linkers and MAA as a monomer. Leibl et al. [29] used dopamine as a functional monomer since it was identified as the best molecule for that purpose and its electro-polymerization with trimesic acid or Kemp's acid as dummy templates for, respectively, TNT and RDX was easily achieved by cyclic voltammetry. Experiments were mainly conducted on prepared mixtures and solutions to assess the characteristics of the synthetized MIPs. Some studies tried to apply their method in real samples. The most screened real matrix was water (tap water, sea water, river water, industrial wastewater, and surface water) [28–33,39,40]. Another real matrix analyzed was soil [36,39,40]. All the studies that applied MIPs in water samples showed good results. In particular, Alizadeh et al. demonstrated the reliability of their method in real samples even if no specifications were made about quality assessment [28]. Another study by Alizadeh et al. [30] showed satisfactory results for RDX analyses in tap water samples, with no significant differences with the reference method usually used. Guo et al. [32] and Lu et al. [35] applied their method in different water samples spiked with TNT and TNT plus 2,4-DNT, respectively. Both studies obtained good results: Guo et al. found recovery values ranging between 96.70% and 102.17%, suggesting potential further uses of MIPs for environmental applications. Lu et al. obtained values for the adsorption and recovery of TNT ranging between 88.57% and 103.98%, while the adsorption of 2,4-DNT was 60%, and they observed the complete removal of TNT and DNT from the water samples. Similarly, Xu et al. [39] reported results in real samples (soil and water) that were in agreement with those obtained with other validated methods (HPLC), and, in another further study, satisfactory recovery values of 88.6–95.7% were obtained [34]. Regarding the practical advantages of using MIPs, all the studies reported them to be a low-cost analytical method. Moreover, Alizadeh et al. [28] reported on the good durability

of MIPs, which seem to be effective even after prolonged storage for 6 months, and Lu et al. [34] reported on the good stability of the whole tested sensor after three years of storage. Other studies [34,37] also reported that MIPs can be used for more cycles of analyses with the same results or with a slight decrease in effectiveness, demonstrating good reusability proprieties. All the studies reviewed were in agreement regarding the specificity and sensitivity of MIPs, which showed excellent results. MIPs showed good specificity for the target molecule, which was recognized and bound even in the presence of other confounding molecules or analogs in the same solution [30,32–34,37]. Moreover, Leibl et al. [29] reported that by selecting the functional monomer during MIPs' synthesis, it is possible to optimize the affinity of the MIP film toward the target molecule. The sensitivity was found to be very good, and the LODs obtained in all the studies confirmed the potential usability of MIPs in real-life applications, even for a small concentration of the molecule of interest.

Table 2. Forensic toxicological MIP application on non-biological matrices.

Analytes	Sample	MIP	Instrumentation	LOD	LLOQ or Lower Concentration Point	References
TNT	Tap and sea water	MAA, TNT, EGDMA, AIBN	Carbon paste electrode	0.5 nM	1.0 nM	[28]
TNT and/or RDX	Aqueous solution	Trimesic acid (TMA) and Kemp's triacid as structural analogs, dopamine as functional monomer	Gold electrode (working electrode), DRIREF-2 Ag/AgCl (WPI) (reference electrode), platinum coil (as a counter electrode)	50–100 pM	50–100 pM	[29]
TNT	Soil and water	Amino CDs as functional monomers	MIP carbon dot fluorescence sensor ing	17 nM	50 nM	[30]
TNT	Aqueous solution	TNT as template, MAA as functional monomer, DVB as cross-linker, and AIBN as the radicalic initiator	Plastic optical fibers (POFs)	2.4×10^{-6}–7.2×10^{-7} M	ND	[31]
TNT	Tap and river water	TNT as template molecule; both micro-porous metal–organic frameworks (MMOFs) as conductive films; gold electrodes; intermediate monolayer of p-aminothiophenol (PATP)	MIP-modified gold electrodes	0.04 fM	44 nM	[32]
TNT	Soil and water	3-aminopropyl triethoxy silane (APTES) as functional monomer and tetraethyl orthosilicate (TEOS) as cross-linker in the presence of green semiconductor quantum dots	MIP-coated quantum dot fluorescence sensoring	15 nM	50 nM	[33]
TNT	TNT methanol/water solution	TNT as template molecule; acrylamide (AM); methylmethacrylate (MMA)	Molecularly imprinted colloidal particles photonic crystal (PhC) sensing	3×10^{-4} mM	0.3 mM	[34]

Table 2. *Cont.*

Analytes	Sample	MIP	Instrumentation	LOD	LLOQ or Lower Concentration Point	References
TNT/DNT	Industrial wastewater and surface water	TNT and DNT as templates	Ion mobility spectrometry	TNT: 0.1 ppm DNT: 0.05 ppm	TNT: 0.5 ppm DNT: 0.1 ppm	[35]
RDX	Tap water	Methacrylic acid as functional monomer, addition of EGDMA (crosslinking agent) and AIBN	Glassy carbon electrode	4.5×10^{-6} mg/L	0.2 nM	[36]
HMX, RDX, TNT, CL-20	Simulated post-blast samples prepared from motor oil and vacuum pump oil.	Silca nanospheres as a sacrificial matrix, acrylamide as a functional monomer, ethylene glycol dimethacrylate as a cross-linker	HPLC-DAD	Compound / Vacuum pump oil (µmol/L) / Motor oil (µmol/L) HMX / 0.16 / 0.28 RDX / 0.33 / 0.36 TNT / 0.13 / 0.19 CL-20 / 0.19 / 0.32	Compound / Vacuum pump oil (µmol/L) / Motor oil (µmol/L) HMX / 0.5 / 1 RDX / 1 / 1 TNT / 0.5 / 0.5 CL-20 / 0.5 / 1	[37]
DNT	Water	MA as functional monomer, EGDMA as the crosslinking agent, AIBN as the initiator	Amino-functionalized carbon dots	0.28 ppm	1 ppm	[38]
TATP	Standard solutions of PETN, TNT, RDX, and HMX and then TATP	TATP as template, pyrrole as functional monomer	MIP-modified glassy carbon electrode	26.9 µg/L	81.6 µg/L	[39]
TNP	TNP solution	NH2-S4 as functional monomer, CLM as crosslinking monomer	Imprinted polymer recognition unit crosslinked to fluorophore trasducer	0.2 ng/L	0.2 ng/L	[40]

4. Discussion

In our review, we focused on the application of MIPs in forensic toxicology by considering biological and non-biological analyses. In the literature, MIPs have a wide range of applications, and an average of 500 papers were published each year between 2015 and 2024. However, the search term *MIPs and forensic analysis* retrieved less than ten papers/year in the same period. This observation may deserve some consideration when evaluating the possibility of including MIPs in forensics. MIPs have mainly been adopted for sample purification in biological specimens such as urine, plasma, blood, and oral fluid, and solid-phase extraction (SPE) has been applied in the past. In the case of non-biological analytes, MIPs have been used to prepare chemosensors to allow for simultaneous detection in the matrix, with the main target being explosives. MIPs may offer several advantages over traditional extraction/purification methods, making them a superior choice in sample preparation in complex matrices. The results obtained demonstrate excellent sensitivity, reaching levels in the nanograms per milliliter (ng/mL) or parts per billion (ppb) range. This high sensitivity enables this method's applicability to both biological specimens and non-biological samples. Within this context, MIPs offer a viable alternative to traditional sample pretreatment methods commonly employed in forensic toxicology. Furthermore, the ability to seamlessly perform analyses using liquid chromatography coupled with mass spectrometry (LC-MS) following sample purification facilitates rapid adaptation to routine activities.

However, one of the most notable and unique advantages of MIPs is their exceptional selectivity.

MIPs are designed with tailor-made binding sites that are complementary to the target molecule in terms of shape, size, and functional groups. This allows MIPs to selectively

recognize and bind specific analytes, even in complex matrices, reducing the chances of interference from other compounds. In contrast, solid-phase extraction methods (SPE) typically rely on general interactions like polarity or ionic charge [41], which may lead to lower specificity. They reflect a customizable design by adapting to almost any type of molecule, from low molecular weights to higher molecular weights. For example, this customizable design feature is not available with standard SPE materials, which are typically limited to broad categories of molecules. In this context, MIPs offer the flexibility to create extraction methods tailored to specific analytical needs. They also fit the Green Chemistry Principle of preventing waste since they are reusable [42]. MIPs are generally robust and can withstand harsh chemical and physical conditions, allowing for them to be used multiple times without a significant loss in binding capacity. This reusability also reduces the overall cost of the extraction process, as MIPs do not need to be replaced after every extraction, unlike many SPE cartridges, which are often single use. While the initial cost of developing MIPs can be higher than purchasing off-the-shelf SPE cartridges, their longevity and high reusability result in a more cost-effective solution over time. Moreover, the reduction in sample preparation time due to the high selectivity of MIPs leads to a more efficient workflow, further decreasing operational costs. On the one hand, MIPs offer several advantages over traditional sample purification steps; on the other hand, they also present certain limitations, particularly when applied to forensic analysis. One of the main drawbacks that limit the adoption of MIPs in forensic analysis remains the design process, which limits their applications to a few templates of molecules both in biological and non-biological fields. The preparation of highly selective MIPs includes complex and time-consuming steps that necessitate optimization for each specific reaction. These processes demand specialized skills, posing significant challenges for the direct development of MIPs within forensic laboratories. Consequently, external expertise is often required, limiting the practical application and integration of MIP technology in forensic settings. This dependency not only increases the overall time and costs required but also restricts the adaptability of MIPs for on-site forensic analyses. Addressing these limitations through streamlined methodologies or automation could greatly enhance the accessibility and utility of MIPs in forensic science, where time is often critical. Thus, the development of an MIP for a new target molecule may not be feasible in most forensic casework.

Moreover, the phenomenon of "template leakage", arising from the incomplete removal of the template molecule from the polymer matrix during synthesis, can lead to residual template contamination. This incomplete extraction may pose a significant and uncontrollable issue, particularly in forensic applications.

Examples of "template leakage" during the preparation of MIPs have been discussed in various studies. One example is the synthesis of MIPs for dye removal, where incomplete extraction of the template molecules from the polymer matrix was a challenge. This residual template can subsequently leach out, particularly when the MIP is exposed to the target analyte or other solvents, leading to contamination and reduced specificity. Such challenges are often attributed to insufficient optimization of the extraction process or overly strong interactions between the template and polymer [43,44]. To mitigate template leakage, methods like optimizing the solvent and conditions for template removal or using alternative approaches, such as sacrificial templates, have been explored. These strategies aim to ensure that the cavities retain their selective binding properties without residual template interference. In forensic analysis, this leakage can contaminate the unknown sample, leading to false positives or inaccurate results. This is especially problematic in cases where the target molecule is present at trace levels, as even a small amount of contamination can significantly affect the outcome. Approaches to solving leakage problems include the use of dummy templates for surface imprinting [45]. To date, the dummy imprinting strategy using a template structural analog (auxiliary template) has produced satisfactory outcomes [46]. Moreover, the dummy template represents an effective solution in cases when the original template is a dangerous or unstable material or is very expensive. In the field of forensic science, TNT was replaced with trinitrophenl (TNP) as

a dummy template by Xu and colleagues in 2013 [47]. Specificity may also represent a problem in complex matrices, as although they are designed to have high selectivity, they can still exhibit non-specific binding. To address non-specific binding in MIPs, researchers employ strategies aimed at enhancing specificity while minimizing unwanted interactions, particularly in complex matrices like biological fluids or environmental samples. The most promising approach is incorporating nanomaterials, such as nanoparticles or carbon nanotubes, which can enhance the precision of binding by creating more uniform and selective binding sites, as in the case of the MDPV biosensor [48,49]. Matrices such as blood and urine may contain a wide variety of compounds, and MIPs might interact with non-target substances, reducing the accuracy and reliability of the analysis. MIPs are often custom-made for specific applications, and off-the-shelf options are limited. In forensic science, where validated and standardized methods are essential, the need for custom MIP development can be a drawback. The lack of commercially available, widely tested MIPs may slow down the adoption of this technology in routine forensic laboratories. In addition, forensic analysis requires highly standardized and validated methods to ensure that the results are reproducible and legally defensible. Forensic toxicology needs strict requirements such as reproducibility, accuracy, and precision [50]. To achieve this, analytical techniques used in forensic science are rigorously standardized and validated across laboratories, ensuring that the methods produce consistent results regardless of who performs the analysis or where it is conducted. This level of standardization is crucial because any variability in procedures or outcomes could cast doubt on the integrity of the forensic evidence, potentially leading to challenges in court. Molecularly imprinted polymers (MIPs), while offering great potential for the selective recognition and detection of specific molecules, face significant hurdles in forensics due to the lack of widely accepted, standardized protocols for their use both in biological and non-biological fields. Since MIPs are still a relatively novel technology, there is no robust or established framework for their application in forensic analyses, unlike more traditional purification techniques such as liquid–liquid extraction and SPE, which have well-documented protocols and long histories of validation [51]. This absence of standardization may result in inconsistent performance across different laboratories. Factors such as the polymer synthesis method, the choice of monomers, the imprinting process, and the removal of the template molecule can vary significantly, leading to different binding affinities and selectivity and sensitivity values in different MIP batches. Without quality control procedures, these variations may produce different results for the same sample, raising concerns about the reproducibility and reliability of forensic evidence obtained using MIPs. MIPs are still a relatively novel technology in the field of forensics, and the lack of standardized protocols for their use can be a major obstacle. In conclusion, while MIPs show promise for forensic analysis due to their selectivity, their limitations—such as template leakage, non-specific binding, high development costs, and lack of standardization—pose significant challenges for routine forensic applications.

Author Contributions: Conceptualization, J.P.P. and P.F.; methodology, S.M. and S.S.; software, A.G.; validation, A.G. and F.P.; formal analysis, A.G. and S.S.; data curation, J.P.P.; writing—original draft preparation, S.M., A.G., G.P. and S.S.; writing—review and editing, JPP; visualization, PF and GP. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AM	Acrylamide
AIBN	azobisisobutyronitrile
CL-20LC-MS	liquid chromatography coupled with mass spectrometry
DNT	2,4-dinitrotoluene
DVB	divinylbenzene
EDMA	ethylene dimethacrylate
EGDMA	ethylene glycol dimethacrylate
GC	gas chromatography
HMX	cyclotetramethylene-tetranitramine
IMS	ion mobility spectrometry
LC	liquid chromatography
LLOQ	lower limit of quantification
LOD	limit of detection
MAA	methacrylic acid
MIPs	molecularly imprinted polymers
MMA	methyl methacrylate
MPEA	N-methylphenylethylamine
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NPS	new psychoactive substance
PE	polyethylene
RDX	Royal Demolition eXplosive
SPE	solid-phase extraction
TATP	triacetone triperoxide
THC	tetrahydrocannabinol
TNP	trinitrophenol
TNT	trinitrotoluene

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