

# Optimization of Selective Molecularly imprinted Solid-Phase extraction for the determination of Venlafaxine by HPLC-DAD

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**Abstract**— In this work a venlafaxine selective molecularly imprinted polymer was applied as a selective adsorbent for venlafaxine extraction from water samples. The workflow combines validated HPLC-DAD quantification after molecularly imprinted solid-phase extraction of venlafaxine. The influence of the presence of other pharmaceuticals on the extraction and determination of venlafaxine was examined, especially desvenlafaxine, which has a structure similar to venlafaxine. The efficiency and selectivity of the molecularly imprinted solid-phase extraction were compared with the results of the solid-phase extraction procedure using a commercially available sorbent (Strata X). Demonstrating excellent recovery and pronounced selectivity, the molecularly imprinted polymer tailored for venlafaxine outperformed the commercial Strata X sorbent. This confirms the significant advantage of the molecularly imprinted structure for high affinity and specific recognition and retention of venlafaxine. This integrated approach offers a precise, selective, and reliable tool for selective determination of venlafaxine in water samples.

**Keywords**— Molecularly Imprinted Polymer, Pharmaceuticals, Solid-Phase Extraction, High-Performance Liquid Chromatography

## I. INTRODUCTION

The growing awareness of pharmaceutical compounds and their metabolites in the environment, particularly in aquatic systems, emphasizes the global need for effective detection, monitoring, and removal strategies [1]. Among emerging contaminants, antidepressants, especially venlafaxine (VEN) and its active metabolite desvenlafaxine (DV), stand out, as they are frequently detected in wastewater due to their widespread use and potential environmental impact [2]. Antibiotics, such as ciprofloxacin (CIP), enrofloxacin (ENRO), sulfamethoxazole (SMX), trimethoprim (TMP), and tetracycline (TC), also pose a significant challenge, contributing to antimicrobial resistance and ecological risks [1-6]. Hydroxychloroquine (HCQ), an immunosuppressive drug, has gained attention due to its increased use during the COVID-19 pandemic, raising concerns about environmental release [7].

Due to the very low concentrations of these compounds, the complexity of environmental matrices, and structural similarities among analytes, selective and efficient sample preparation is essential. Molecularly imprinted polymers (MIPs) have emerged as a highly promising group of synthetic

materials to address these challenges. They feature polymer matrices with cavities specifically tailored to the shape and functional groups of the target molecule. Compared to conventional materials, MIPs offer high selectivity, chemical and thermal stability, robustness, and low synthesis costs, making them increasingly valuable in separation techniques, particularly molecularly imprinted solid-phase extraction (MISPE) [8].

Given the complexity of environmental and biological matrices and the need for selective and sensitive analysis, MIPs represent a promising and rapidly developing approach for the selective monitoring of pharmaceuticals, which is why their application is being explored for compounds such as VEN. This work builds on recent findings to further investigate and optimize MIP-based analysis, with particular attention to VEN as a priority target.

## II. MATERIALS AND METHODS

### A. Materials

#### Chemicals and reagents

Acetonitrile (ACN) (HPLC grade) was purchased from Fisher Scientific (Loughborough, UK). Ethanol (EtOH) (96%, p.a.) was obtained from KEFO (Sisak, Croatia). Methanol (MeOH) (HPLC grade) was supplied by Avantor Performance Materials Poland S.A. (Gliwice, Poland). Acetic acid (HAc) (99.8%, p.a.) was purchased from Lach-ner (Neratovice, Czech Republic). Dimethyl sulfoxide (DMSO) (p.a.) was obtained from Grammol (Zagreb, Croatia). Methacrylic acid (>99%) and ethylene glycol dimethacrylate (> 97.0 %) was supplied by Tokyo Chemical Industry (Tokyo, Japan). 2,2'-azobisisobutyronitrile (AIBN, 98%) was purchased from Sigma-Aldrich (Buchs, Switzerland). Ultrapure water was prepared using a Milli-Q purification system (Millipore, Burlington, MA, USA).

#### Analytical standards

High-purity (>98%) analytical standards of venlafaxine (CAS No. 99300-78-4) and desvenlafaxine (CAS No. 93413-62-8) were obtained from Tokyo Chemical Industry (Tokyo, Japan). Hydroxychloroquine sulfate (≥98%, CAS No. 747-36-4) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ciprofloxacin (CAS No. 85721-33-1), enrofloxacin (CAS No.

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93106-60-6), sulfamethoxazole (CAS No. 723-46-6), trimethoprim (CAS No. 738-70-5), and tetracycline (CAS No. 60-54-8), all with a purity >98%, were supplied by Sigma-Aldrich (St. Louis, MO, USA).

Stock standard solutions of individual pharmaceuticals (Table I.) were prepared at concentration of 100 mg/L in MilliQ water. From these solutions, working solutions of individual pharmaceuticals as well as pharmaceutical mixtures were prepared by dilution of stock solutions with MilliQ water.

TABLE I:  
PROPERTIES OF SELECTED PHARMACEUTICALS

Compound	Group of pharmaceuticals	Molecular formula	Water solubility [9] (mg/mL)
VEN	Antidepressant	C <sub>17</sub> H <sub>27</sub> NO <sub>2</sub>	572
DV	Antidepressant	C <sub>16</sub> H <sub>25</sub> NO <sub>2</sub>	1.4
HCQ	Antimalarial, Immunosuppressive	C <sub>18</sub> H <sub>26</sub> ClN <sub>3</sub> O	0.0261
TMP	Antibiotic	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O <sub>3</sub>	<1
TC	Antibiotic	C <sub>22</sub> H <sub>24</sub> N <sub>2</sub> O <sub>8</sub>	0.231
CIP	Antibiotic	C <sub>17</sub> H <sub>18</sub> FN <sub>3</sub> O <sub>3</sub>	<1
ENRO	Antibiotic	C <sub>19</sub> H <sub>22</sub> FN <sub>3</sub> O <sub>3</sub>	>0.0539
SMX	Antibiotic	C <sub>9</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S	>0.038

### B. Solid-phase extraction

Solid-phase extraction (SPE) of pharmaceuticals from aqueous samples was optimized using commercial column Strata X (Phenomenex). Analyte recovery was investigated with variations in sample volume (50, 100, 150 mL), eluent volume (5, 10, 15), pH (4, 7, 10) and solvent type (MeOH or EtOH). The results obtained allow the selection of optimal conditions for maximum recovery of VEN.

The columns were conditioned with MeOH and MilliQ water (at different pH), and then a sample of varying volumes was passed through the column at the flow of 4 mL/min under vacuum. The column was then dried for 5 min, and the analytes were eluted with varying volumes of solvent (5–15 mL) to dryness. The resulting extracts were then rotary evaporated at 40 °C and reconstituted in 1 mL of 1:1 ACN/MilliQ water. Finally, the samples were analyzed by HPLC-DAD.

### C. Synthesis of molecularly imprinted polymer and non-imprinted polymer

The MIP was prepared by polymerizing a mixture of VEN and methacrylic acid as monomers, ethylene glycol dimethacrylate as crosslinker, 2,2'-azobisisobutyronitrile as initiator, and DMSO as porogen, at 60 °C for 24 hours. The resulting polymer was ground and subjected to Soxhlet extraction to remove template (VEN). In parallel, the non-imprinted polymer (NIP) was prepared using the same procedure, but without the addition of VEN.

#### D. Molecularly imprinted polymers solid-phase extraction

The sorbents (MIP or NIP) were packed into SPE columns containing a frit insert. The columns were then placed on a

solid-phase extraction manifold (Supelco Visiprep™ 24). Before sample loading, the sorbent was sequentially conditioned with 5 mL of MeOH and then with 5 mL of MilliQ water.

Following conditioning, various samples, including individual solutions of VEN and DV, a mixture of pharmaceuticals, and a blank sample, were passed through the columns. The sample volume for each run was held constant at 100 mL, with a controlled flow rate of 4 mL/min. Once the entire sample volume had passed through, the sorbent bed was dried under vacuum for 5 minutes to remove residual sample matrix components.

The retained analytes were subsequently eluted by passing the appropriate elution solvent through the column until the sorbent bed was completely dry. The extracts were then rotary evaporated at 25 °C and reconstituted in 1 mL of 1:1 ACN/MilliQ water. Extracts were then transferred to vials for subsequent analysis by HPLC-DAD.

The entire extraction procedure was performed in parallel using the NIP to verify the selectivity of the MIP.

Factors affecting the efficiency of MISPE of VEN (type of elution solvent (MeOH, EtOH, ACN, MeOH+5% HAc and MeOH+10% HAc) and mass of sorbent) were optimized regarding the recovery and selectivity in pharmaceuticals mixture. VEN recovery and selectivity of MIP were compared with the results obtained on the commercial sorbent Strata X.

#### E. HPLC with DAD detection

Standard solutions of VEN, DV, pharmaceutical mixtures (VEN, DV, HCQ, CIP, ENRO, SMX, TMP, TC), a blank (MilliQ water) and the obtained extracts were analyzed by HPLC-DAD. The analyses were performed on a high-performance liquid chromatograph Nexera LC-40 HPLC System (Shimadzu) with a Kinetex C18 column (150 × 4.6 mm, 5 μm, 100 Å; Phenomenex). The mobile phase was composed of ACN (B) with 1% formic acid and MilliQ water with 1% formic acid (A), using gradient elution (Table II). All pharmaceuticals were detected at a wavelength of 278 nm, except for hydroxychloroquine, which was detected at 340 nm.

TABLE II:  
GRADIENT ELUTION

A(%)	B(%)	t (min)
89	11	0.00
89	11	13.50
83	17	15.00
83	17	25.00
89	11	28.00

## III. RESULTS AND DISCUSSION

### A. HPLC-DAD method validation

HPLC-DAD method was validated through assessment of method performance characteristics: linearity, trueness and repeatability. The validation of the HPLC-DAD method for the determination of eight pharmaceuticals (CIP, ENRO, HCQ, SMX, TMP, VEN, DV, TC) demonstrated satisfactory

analytical performance, Table III. Selectivity was confirmed by comparing the chromatogram of pharmaceutical mixture standard with the chromatogram of blank sample (Fig. 1).

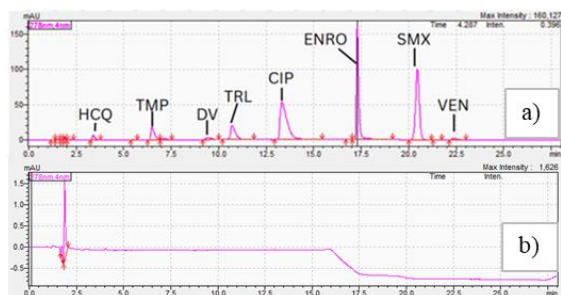


Fig. 1. Chromatogram of a standard solution of a pharmaceutical mixture of 10 mg/L and chromatogram of blank sample

Linearity was achieved within the concentration range of 0.05–10 mg/L for all analytes ( $R^2 > 0.99$ ). Repeatability was acceptable ( $RSD < 10\%$ ) for all pharmaceuticals investigated. Trueness was confirmed at 9 mg/L for all analytes. Detection limits ranged from 0.01 mg/L (SMX) to 0.43 mg/L (CIP), and quantification limits from 0.05 mg/L (SMX and TMP) to 1.29 mg/L (CIP). The working range was confirmed for all pharmaceuticals, from the respective quantification limits up to 10 mg/L.

TABLE III:  
PERFORMANCE CHARACTERISTICS OF THE HPLC-DAD METHOD

Analyte	$R^2$	Sensitivity (slope)	Precision RSD % (2 / 9 mg/L)	Trueness (%) (9 mg/L)	LOQ (mg/L)	LOD (mg/L)
CIP	0.9979	141988	4.4 / 1.9	97.2	1.29	0.43
ENRO	0.9915	137976	2.5 / 1.7	91.4	0.63	0.21
HCQ	0.9985	47445	1.3 / 0.6	102.9	0.99	0.33
SMX	0.9992	69542	0.2 / 0.2	98.7	0.05	0.01
TMP	0.9993	22422	0.2 / 0.2	98.5	0.05	0.02
VEN	0.9997	4485	1.0 / 0.5	98.0	0.15	0.05
DV	0.9994	5242	0.4 / 0.3	100.0	0.16	0.05
TC	0.9987	38602	7.4 / 2.3	93.3	0.06	0.02

### B. Solid-Phase Extraction

The SPE procedure using Strata-X SPE cartridge was evaluated for VEN in the presence of other pharmaceuticals (CIP, ENRO, HCQ, SMX, TMP, DV, TC) under varying experimental conditions: sample volume of 50, 100 and 150 mL, eluent (MeOH) volume of 5, 10 and 15 mL, and pH value of 4, 7 and 10.

VEN showed robust recovery with low variability within the range from 88% to 103% at all tested experimental conditions. As VEN exists predominantly in cationic form within the tested pH range, pH value did not significantly influence the recovery. The highest recoveries for VEN were obtained with 100 mL sample volume and 10 mL eluent volume, while lower sample volumes (50 mL) or higher eluent volumes (15 mL) resulted in slightly lower recoveries. VEN and SMX showed almost 100% recoveries, while the other pharmaceuticals (HCQ, TMP, DV, TC, CIP and ENRO) had significantly lower recovery values (Fig. 2). The method showed good reproducibility ( $RSD$  3.53%), confirming the reliable performance of the extraction procedure for VEN.

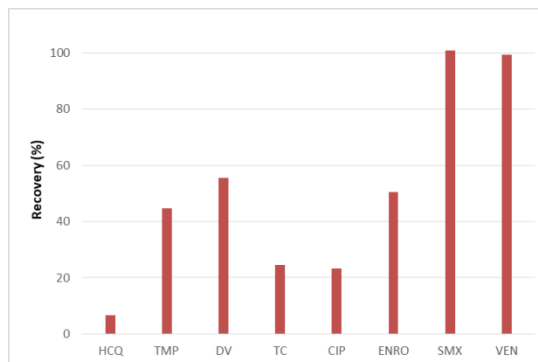


Fig. 2. Recoveries of pharmaceuticals on Strata-X SPE cartridge, 100 mL sample volume, 10 mL MeOH volume

### C. Molecularly imprinted solid-phase extraction

The first step of MISPE optimization was to select the most suitable elution solvent (sorbent mass was 60 mg). Three solvents were tested: MeOH, EtOH and ACN. As shown in Fig. 3., MeOH gave the highest recovery of VEN on MIP and at the same time slightly lower on NIP. EtOH and ACN showed higher recovery on NIP, especially ACN. MeOH, as a polar protic solvent effectively breaks the interactions between VEN and MIP and enables efficient elution. Therefore, MeOH was selected as the optimal solvent for elution from MIP. Under the same experimental conditions, the elution of DV was tested to assess the selectivity of MIP towards VEN. Significantly lower recoveries were obtained for DV compared to VEN, with similar values observed on both MIP and NIP for all three tested solvents. Due to hydrogen bond formation with the solvent, the hydroxyl group in DV likely causes a poorer fit into the MIP cavities, resulting in reduced binding affinity. When MeOH was used as the elution solvent for the pharmaceutical mixture, lower recovery of VEN on MIP was observed due to competitive binding of other pharmaceuticals with similar structures.

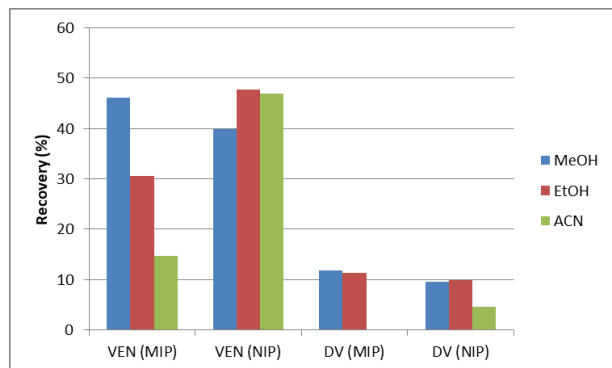


Fig. 3. Recoveries of VEN and DV using 60 mg of MIP

In the next optimization step, VEN was eluted with a mixture of MeOH and HAC to reduce strong interactions between the MIP and the analyte. Concentrations of 5% and 10% HAC were tested. The addition of 5% HAC almost doubled the recovery of VEN on the MIP with a lower recovery on the NIP. Increasing the concentration of HAC to 10% led to a slight decrease in recovery. It was concluded that HAC protonates the analyte and breaks hydrogen bonds, thereby improving elution. MeOH with 5% HAC was selected as the optimal elution solvent, achieving VEN recovery of 88% (Fig. 4.).

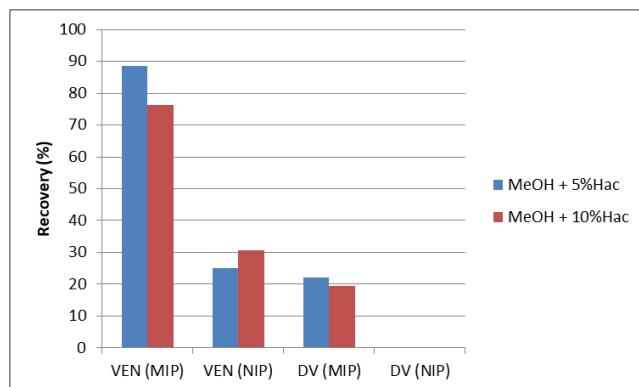


Fig. 4. Recoveries of VEN and DV on 60 mg MIP and elution with MeOH + HAC

The decrease in recoveries of the other pharmaceuticals confirms the higher selectivity of the MIP towards VEN. The largest differences between the MIP and the NIP were observed for VEN suggesting specific binding while the other compounds were bound by non-specific interactions. Taking into account the higher selectivity and recovery with the addition of 5% acid, MeOH with 5% HAC was chosen as the optimal solvent for further optimization (Fig. 5.).

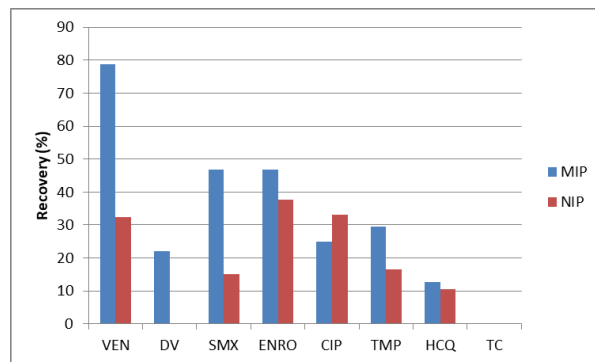


Fig. 5. Recoveries of pharmaceuticals on 60 mg MIP and elution with MeOH + 5% HAC

The effect of the sorbent mass (60, 100 and 150 mg) on the extraction efficiency was examined. Increasing the mass from 60 mg to 100 mg achieved almost 100% recovery of VEN on MIP, while a further increase to 150 mg resulted in a decrease in efficiency and equalization of recovery on MIP and NIP. Although higher recovery was also recorded on NIP at 100 mg, it was still lower than on MIP. Increasing the mass increases the surface area of the sorbent and the number of binding sites, this improves selective binding. The optimal sorbent mass was determined to be 100 mg (Fig. 6.).

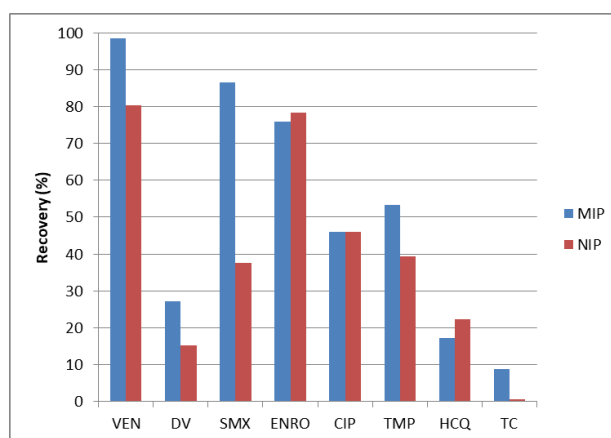


Fig. 6. Recoveries of pharmaceuticals on 100 mg MIP sorbent and elution with MeOH + 5% HAC

Comparing the results obtained on MIP sorbent and commercial Strata X cartridge, it is clearly seen that the key difference in the extraction of VEN is related to the selectivity mechanism. Strata X cartridges are based on a mixed mechanism that combines hydrophobic interactions and ion exchange. Such an approach allows for high recovery of VEN, close to 100% under optimal conditions, but with simultaneous co-extraction of other pharmaceuticals. As a result, the extract is often burdened with interferences, which reduces selectivity and makes subsequent analysis difficult.

In contrast, MIP sorbent was developed with the aim of achieving higher selectivity. During their synthesis, cavities complementary to the shape and functional groups of VEN are created, thus achieving a "lock and key" mechanism. Such a structure allows for high affinity for the target molecule and at the same time reduces the retention of other compounds. In

conclusion, while Strata X provides high efficiency but low selectivity, MIP sorbents represent a more advanced solution as they enable targeted separation of VEN.

#### IV. CONCLUSION

An HPLC-DAD method was successfully developed and validated for eight pharmaceuticals (CIP, ENRO, HCQ, SMX, TMP, VEN, DV, TC), with high selectivity, a wide linear range (0.05–10 mg/L) and good precision confirmed, thus laying a solid foundation for further analyses. Optimization of sample preparation by SPE was carried out by systematically investigating key factors, including sample volume, eluent volume and pH, which enabled achieving high recovery rates for all target analytes, including VEN, and defining optimal conditions for an efficient extraction process. To increase the selectivity of the analytical process, a MIP was prepared, which showed significantly superior selectivity and high recovery of VEN (88% at the optimal MeOH/5% HAc eluent and 100 mg sorbent) compared to the NIP and the commercial Strata X sorbent. This confirmed the ability of the MIP to selectively isolate targeted analyte from a complex matrix, effectively minimizing interferences. In conclusion, the developed MIP proved to be a highly selective and efficient material for the isolation of VEN from complex environmental samples.

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#### REFERENCES

- [1] B. Tegegne, L. Chimuka, B. S. Chandravanshi, F. Zewge, "Molecularly imprinted polymer for adsorption of venlafaxine, albendazole, ciprofloxacin and norfloxacin in aqueous environment," *Separation Science and Technology*, vol. 56, pp. 2217-2231, Oct. 2021. Available: <https://doi.org/10.1080/01496395.2020.1819323>
- [2] M. A. Bajaber, A. H. Kamel, "All-solid state potentiometric sensors for desvenlafaxine detection using biomimetic imprinted polymers as recognition receptors," *Polymers*, vol. 14, pp. 4814, Nov. 2022. Available: <https://www.mdpi.com/2073-4360/14/22/4814>
- [3] S. Sharma, R. Tomar, R. D. Kaushik, "Core-shell molecularly imprinted polymers on magnetic yeast for the removal of sulfamethoxazole from water," *Polymers*, vol. 12, pp. 1385, Jun. 2020. Available: <https://doi.org/10.3390/polym12061385>
- [4] J. A. Sarabia-Sainz, J. C. Encinas-Encinas, A. Gallegos-Tabanico, J. Jimenez-Canale, S. G. Hernandez-Leon, "Development of an electrochemical sensor conjugated with molecularly imprinted polymers for the detection of enrofloxacin," *Chemosensors*, vol. 10, pp. 448, Oct. 2022. Available: <https://doi.org/10.3390/chemosensors10110448>
- [5] Y. Qing, Q. Peng, Y. Gao, Y. Zhao, F. Li, "Highly efficient removal of tetracycline by using molecularly imprinted polymer-based materials: A review," *Journal of Hazardous Materials*, vol. 469, pp. 133887, May 2024. Available: <https://doi.org/10.1016/j.jhazmat.2024.133887>
- [6] J. Zou, W. Wang, S. Xu, J. Fan, Q. Guo, "Facile synthesis of magnetic molecularly imprinted polymers for the selective extraction and determination of trimethoprim in environmental water samples," *Talanta*, vol. 281, pp. 126229, Aug. 2024. Available: <https://doi.org/10.1016/j.talanta.2024.126229>
- [7] National Library of Medicine, "Hydroxychloroquine for COVID-19 Post-exposure Prophylaxis (PEP)," *ClinicalTrials.gov*,

- Available: <https://www.clinicaltrials.gov/study/NCT04328961>
- [8] M. G. Metwally, A. H. Benhawry, R. M. Khalifa, R. M. El Nashar, M. Trojanowicz, "Application of Molecularly Imprinted Polymers in the Analysis of Waters and Wastewaters," *Molecules*, vol. 26, 6515, Oct. 2021. Available: <https://doi.org/10.3390/molecules26216515>
  - [9] National Center for Biotechnology Information, "PubChem", Retrieved October 14, 2025. Available: <https://pubchem.ncbi.nlm.nih.gov/>