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Design of Potentiometric Sensor Based on Molecularly-imprinted Polymer for Direct Detection of Levofloxacin in Plasma for Point-of-Care Applications

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Abstract

Background: With the goal of "personalizing" the patient's dosage regimen, the concentration of the drug is measured in biological fluids as a part of therapeutic drug monitoring (TDM). A reproducible and disposable molecularly imprinted polymer (MIP)-based potentiometric sensor was constructed as a TDM analysis platform for levofloxacin (LVF) assay in spiked plasma samples and pharmaceutical formulation.

Materials and methods: In order to obtain high selectivity for the drug, a host guest interaction technique was employed, using methacrylic acid (MAA) as a functional monomer and ethylene glycol dimethacrylate (EGDMA) as a cross linker in the presence of the template, LVF. Fourier transform infrared (FT-IR) spectroscopy was used for polymer structural characterization.

Results: the sensor responded quickly within 3-5 seconds in the pH range (3.0 – 6.0). Within the concentration range of (1×10^{-5} - 1×10^{-2} M), the potential profile showed a linear relationship with limit of detection (LOD) of 7.41×10^{-6} M. Significantly, the selectivity towards LVF was promoted using the MIP modified sensor. The sensor was successfully employed for LVF assay in spiked plasma samples and pharmaceutical dosage form without any interference from any common additives or excipients. Using analytical eco-scale and Green Analytical Procedure Index (GAPI) techniques, the suggested method's greenness was assessed, and it showed outstanding green analysis.

Conclusion: the obtained results showed that the developed MIP-based sensor is selective, simple, easily handled, and rapid for LVF assay in pure form, spiked plasma samples, and tablets with good selectivity, accuracy, and precision.

Keywords: Molecularly-imprinted polymer; Ion-selective electrodes; Therapeutic drug monitoring; Levofloxacin; plasma samples.

1. Introduction

Therapeutic drug monitoring (TDM) stands for drug dosage individualization; in which certain drugs are measured at predetermined intervals with subsequent dose adjustment to keep a constant concentration in patients' blood or plasma. TDM is primarily used to monitor medications with limited therapeutic index, noticeable pharmacokinetic variations, challenging in monitoring their intended concentrations, or a history of both therapeutic and serious side effects [1,2]. Immunosuppressants (mycophenolate, and tacrolimus), antineoplastic drugs (methotrexate), drugs for respiratory system diseases (caffeine, and theophylline), cardioactive medications (quinidine, and digoxin), antipsychotics (clozapine), mood stabilizers (nortriptyline, and lithium), and anticonvulsant drugs (valproic acid, phenytoin, clonazepam, carbamazepine, primidone, phenobarbital) are among the targeted medications [3]. Bacterial infections that resist antibiotic treatment kill about 700,000 individuals worldwide each year; by 2050, that number may reach 10 million [4]. Therefore, TDM for certain antibiotics e.g. quinolones, is of great importance to avoid the serious side effects or emergence of bacterial resistance in case of high or low dose, respectively [2,5].

The fluoroquinolone antibacterial drug, LVF (Figure 1) is the S-isomer of ofloxacin. LVF inhibits the DNA gyrase enzyme (bacterial topoisomerase II), which prevents the bacterial cells from growth. Gram-negative aerobic cocci and bacilli are extremely susceptible to fluoroquinolones. Oral, topical, and intravenous LVF formulations are used to treat specific bacterial infections, including hospital and community-acquired pneumonia, tuberculosis, complicated skin infections, urinary tract infections, acute bacterial sinusitis, and bacterial conjunctivitis [6,7]. In certain conditions like bronchitis, acute sinusitis, and uncomplicated urinary tract infections, the side effects of fluoroquinolones exceed their benefits [8]. Therefore, assay of LVF in biological fluids and pharmaceutical dosage forms is essential.

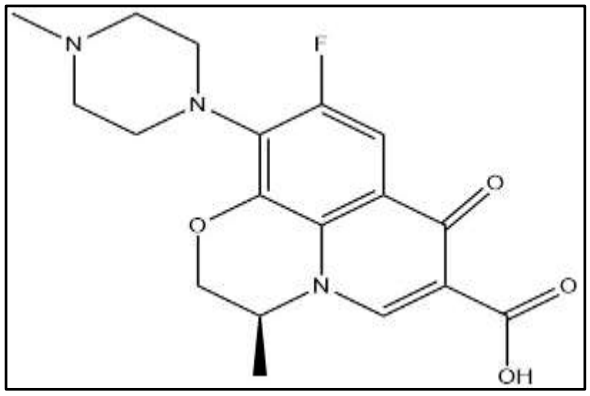


Figure 1: *Chemical structure of LVF.*

Molecularly imprinted polymers (MIPs) are one group of the biomimetic receptors that can recognize the target template with enormously high affinity and selectivity. The MIPs have the potential to provide the biological receptors' specificity and selectivity along with the overt benefits of affordability, durability and low cost [9,10].

MIP-based sensors rely on the monomers self-assemble around the template to recognize the imprinting species. Following the template removal, the polymer develops 3D vacant cavities that are complementary to the template in terms of size, shape, and functionality. Because of this molecular memory, the MIP can selectively identify and rebind the template [11]. The macro-porous network of the polymer may have one or more points in which the template and the monomers interact. The advantage of these several bonds is that they possess high recognition of the ligand that mimic the biological interactions. Moreover, because

of the multiple interactions or points of contact between the analyte and the polymer, the MIP-based sensors' selectivity is higher when compared to the non-molecularly imprinted polymer (NIP)-based ones [12].

The literature survey revealed that there are several analytical methods have been reported for LVF assay include spectroscopy [13–18], LC [19–26], CE [27], and electrochemistry [28–37]. Electrochemical methods have been proven to be sensitive and reliable for the assay of various drugs in biological fluids and pharmaceutical formulations [38]. Ion selective potentiometric sensors have a variety of applications due to its excellent advantages like high sensitivity, high selectivity, and being quick and easy to use [39]. Electrochemical techniques are excellent choices for point-of-care (POC) monitoring and dose adjustment due to their low price and ease of miniaturization [4].

The main aim of this study is to develop a portable and disposable MIP-based ion-selective electrode using polyvinyl chloride (PVC) ion-sensing membrane to increase the selectivity towards LVF assay by the constructed polymer. FT-IR spectroscopy was utilized to characterize the chemical structure of the synthesized MIP. The sensor performance was assessed in accordance with IUPAC recommendations and evaluated for its ability to detect the LVF in spiked plasma samples and pharmaceutical dosage form. The impact of the developed approach on the environment was taken into consideration. Two greenness evaluation metrics namely; Analytical eco-scale and Green Analytical Procedure Index (GAPI) were utilized to evaluate the effectiveness and safety of the proposed methodology.

2. Experimental

2.1. Instrument

Jenway (Model 3510, England) potentiometer was utilized for all potentiometric and pH measurements. The reference electrode utilized was Ag/AgCl double-junction type. A UV/Visible spectrophotometer (Jasco, Japan), magnetic stirrer (Hungarian), centrifuge (model K241), and a sonicator (model WUC-A06H) were also included. For FT-IR spectrum measurement, a single-beam high energy ceramic light source FT-IR instrument (IRaffinity-1, Shimadzu, Kyoto, Japan) was utilized.

2.2. Materials and chemicals

2.2.1. Pure samples

LVF, with 99.6% certified purity, was gifted from Amoun pharmaceutical company, Al Obour city, Egypt.

2.2.2. Samples for application

Floxabact® tablets (manufactured by Eva Pharma for Pharmaceutical Industries in Egypt, batch number 2110538) was obtained from the local market. Each tablet is labeled to contain 500 mg LVF. Samples of human plasma were acquired from the Holding Company of Biological Products and Vaccines (VACSERA), in Giza, Egypt, with approval.

2.2.3. Chemicals

Analytical-grade chemicals and solvents and bi-distilled water were used throughout the work. Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), α,α'-azobis-isobutyronitrile (AIBN), tetrahydrofuran (THF), potassium tetrakis 4-cholorophenyl boron (K-TCPB), PVC (high molecular weight), and potassium chloride (KCl) were purchased from (Sigma-Aldrich, Germany). Calcium nitrate (Oxford laboratory reagent, Egypt), sodium chloride,

and magnesium sulphate heptahydrate (Piochem laboratory reagent, Egypt) were included. Dioctylphathalate (DOP) and dimethyl sulfoxide (DMSO) were obtained from (Fluka, Germany). Glacial acetic acid and sodium acetate were purchased from El-NASR Pharmaceutical Chemical Co., Cairo, Egypt to prepare (1×10^{-2} M) acetate buffer (pH 4.1), (by dissolving 0.24 gm of sodium acetate and 0.7 mL of glacial acetic acid into 1000 mL of bi-distilled water).

2.3. Synthesis of LVF-MIP and NIP

MIP has been prepared, using MAA as a functional monomer, EGDMA as a crosslinker, and AIBN as an initiator, in a similar manner to the reported method in the literature, but with some changes [40]. In a thick-walled glass tube, 1 mmol of the template, LVF and 4 mmol mL of MAA were mixed into 10 mL DMSO and sonicated for 30 minutes, followed by the addition of 20 mmol EGDMA and 10 mg of AIBN. The tube content was purged for 20 minutes with nitrogen gas, mixed well and then placed into oil bath with a constant temperature of 60 °C to start the heat induced polymerization process for 24 hours. The obtained MIP was grounded with a mortar and pestle. To extract the LVF template and the unreacted monomers from the MIP, washing with a mixture of methanol: glacial acetic acid (6: 4, v/v) was employed. By monitoring the UV absorbance at 295 nm, the complete removal of the template was verified. Finally, the polymer was dried in the oven at 60 °C. In the same manner as MIP, the NIP was prepared, but without the addition of LVF.

2.4. Sensors fabrication

In two separate glass Petri dishes, 188.3 mg of PVC powder, 400 mg of DOP plasticizer, 1.70 mg K-TCPB, 10 mg of either imprinted or nonimprinted polymers, and 6 mL THF were homogenized and remained at room temperature until dryness. A cork borer was used to cut small discs, and THF was used to glue them at the ends of the tygon tubes. Equal volumes of (1×10^{-3} M) LVF and (1×10^{-3} M) KCl were used as the inner filling solutions. The electrodes were prepared for use by soaking overnight into (1×10^{-3} M) of the drug solution.

2.5. Sensors calibration

Standard solutions of LVF in a concentration range of (1×10^{-7} M - 1×10^{-2} M) were prepared in (1×10^{-2} M) acetate buffer (pH 4.1). In conjugation with the reference electrode, the prepared electrodes were separately dipped into the drug solutions, and the potential was acquired by the potentiometer. By plotting the potential values as a function of the logarithm of the drug concentrations, the calibration curves were constructed.

2.6. Sensors selectivity

Ions that are prevalent in many biological fluids (Na^+, K^+, Ca^{2+}, and Mg^{2+}) were used for testing the selectivity of the constructed MIP electrode towards LVF. To determine the biased selectivity coefficients ($K_{LVF,Int}^{Pot}$), the separate solution method was followed [41]. The same linearity concentration range (1×10^{-5} - 1×10^{-2}) was used to measure the potential of the drug and the interfering ions in accordance with IUPAC guidelines [42], utilizing the equation below:

$$log\ K_{LVF,Int}^{Pot} = \frac{E_{Int} - E_{LVF}}{S} + (1 - \frac{Z_{LVF}}{Z_{Int}}\ log\ a_{LVF})$$

Where, E_{Int} and E_{LVF} are the potentials of the similar concentration of the interferants and

Levofloxacin, respectively. Z_{LVF} is the charge of Levofloxacin and Z_{Int} is the charge of the interferants, S is the calibration curve slope, and a_{LVF} is the Levofloxacin concentration.

2.7. pH effect

Investigation was performed on how the pH (2.0 – 12.0) of the LVF test solutions (1×10^{-4} M and 1×10^{-3} M) affected the MIP electrode potentials. The reference electrode was immersed with the developed MIP-based electrode along with the glass pH electrode in the buffered solutions. The obtained potential was noted at each pH value.

2.8. Practical assessment of the developed Electrode

Various concentrations of LVF were determined with the constructed MIP- based electrode in spiked plasma samples and pharmaceutical dosage form without any sample pretreatments. Plasma samples were spiked with different concentrations of LVF to prepare (1×10^{-5} and 1×10^{-4} M) solutions. For dosage form assay, Five Floxabact® tablets (500 mg LVF) were weighed, ground, and (1×10^{-3} M) LVF solution was prepared in acetate buffer (pH 4.1). The MIP sensor and the reference electrode were dipped into the liquids and the potential was recorded. Recovery % was calculated from the calibration curve and statistical analysis was carried out, to make sure that the findings were precise and repeatable.

3. Results and discussion

3.1. FT-IR analysis

FT-IR spectroscopy was utilized to study the imprinting process. The FT-IR spectrum of MIP beads (Figure 2) shows a broad band at 3441 cm^{-1} that corresponds to the stretching vibrations of O-H group found in MAA. In addition, the peaks of -C=O and -C-O- vibrations caused by the EGDMA is the characteristic sharp peaks at 1732.08 cm^{-1} and 1261.45 cm^{-1}, respectively. Moreover, the characteristic vibrating bands appeared at 1388.75 cm^{-1}, 1458.18 cm^{-1}, 2958.80 cm^{-1}, and 2989.66 cm^{-1} represented the aliphatic C-H in MAA and EGDMA. The results indicate the successful polymerization process and formation of MIP beads.

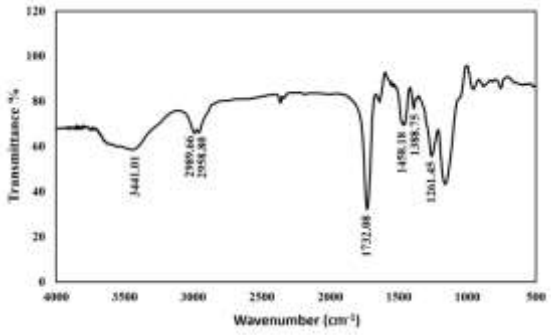


Figure 2: *Fourier Transform-Infrared (FT-IR) spectrum for the molecular imprinted polymer beads.*

3.2. Analytical features of the sensor

The linearity range was used to carry out the calibration curves for both MIP and NIP sensors. The MIP-based sensor had a linear relationship between the readings of the potential and various LVF concentrations (1×10^{-5} - 1×10^{-2} M) (Figure 3), while the NIP-based sensor did not show linear response in all the working concentration range. In comparison to the NIP electrode, the MIP sensor displayed a higher response for LVF with a near-Nernstian response of 52.1 mV/decade (Table 1), which revealed that the polymer's suited cavities served as the sole basis for LVF selective recognition.

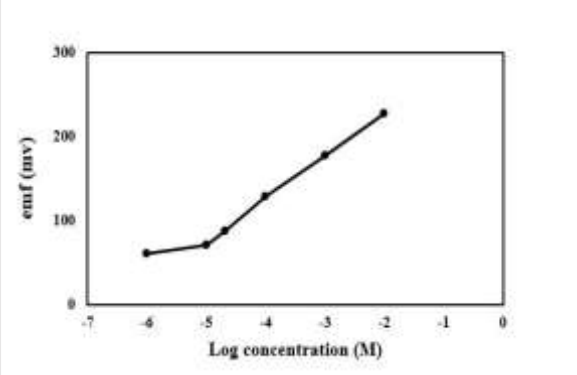


Figure 3: *The Potential profile in mV as a function of log concentration of LVF established by the optimized MIP-based electrode in various LVF concentrations.*

Parameters	Acetate buffer
Concentration range (Molar)	1×10^{-5} - 1×10^{-2}
LOD (Molar)	7.41×10^{-6}
Accuracy (mean ± SD)	100.63 ± 0.98
Repeatability Precision (±RSD)	1.263
Intermediate Precision (±RSD)	0.748
Slope	52.1
Intercept	333.6
Correlation coefficient (r)	0.9995
pH range	3 - 6
Response time (sec.)	3-5 sec.
Sensor lifetime (weeks)	8 weeks

Table 1: *Validation parameters of the modified electrode utilized for LVF determination.*

3.3. pH effect

LVF test solutions (1×10^{-4} M and 1×10^{-3} M) were used to study the effect of pH on the electrode potential for MIP sensor. As displayed in (Figure 4), a stable potential was observed by the MIP electrode throughout the pH range of (3.0 – 6.0). This shows that the electrode is suitable for use in this pH range with a high sensitivity. pH 4.1, which is selected for measurements in this study, was reported as the optimum pH for LVF potentiometric assay due to the existence of the highest ratio of the monocationic form of LVF to other forms [37].

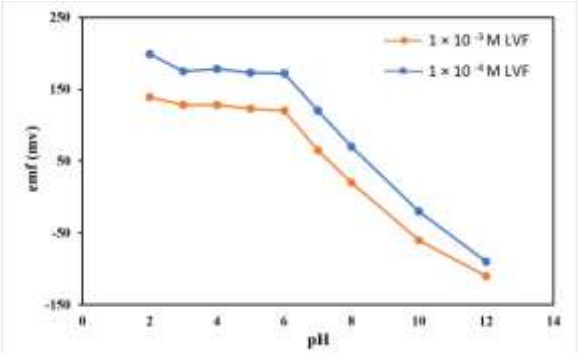


Figure 4: *The influence of various pH ranges on the response of the MIP electrode.*

3.4. The electrode selectivity

Avoiding false results during TDM is of great importance. Hence, one of the main and most significant characteristics of the ion selective electrodes is selectivity. The formation of novel synthetic materials with selective binding has been realized by the molecular imprinting process. Due to the positioning of the monomer's functional groups around the template, the MIP exhibits a high selectivity for the imprinted molecule after its removal. Various interfering ions (Na^+, K^+, Ca^{2+}, and Mg^{2+}), which are exist in biological fluids and pharmaceutical formulations, were used to compare the selectivity of the NIP- and MIP-based electrodes, following the separate solution method [41].

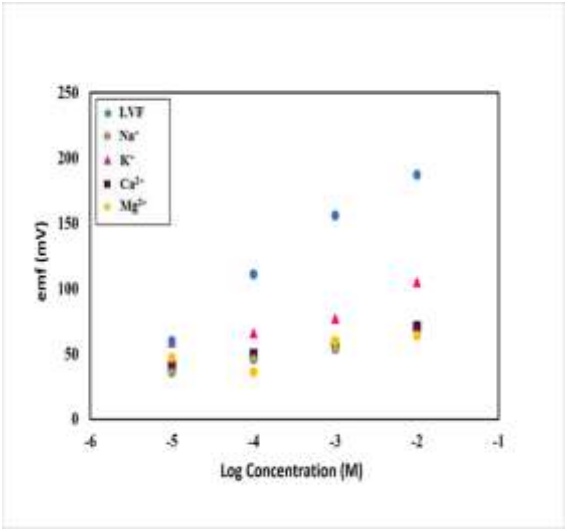
As shown in (Table 2), the selectivity of the developed sensor towards LVF was dramatically improved by utilizing the MIP modified electrode in comparison to the NIP one. When using the NIP-based sensor, the potassium ion interferes most with $log\ K_{LVF,Int}^{Pot}\ of\ (-1.83)$. However, the $log\ K_{LVF,Int}^{Pot}$ value for the K^+ ion and the other ions were improved by the developed MIP-based sensor, with significant improvement of Mg^{2+} and Ca^{2+} selectivity coefficients. The outcomes showed that even in the presence of other interfering

ions, the MIP-based electrode exhibited high selectivity for LVF.

Interfering ion (1×10^{-3} M)	$log\ K^{Pot}_{LVF,Int}$	
	NIP	MIP
Na^+	-2.39	-2.80
K^+	-1.85	-2.55
Ca^{2+}	-3.80	-5.01
Mg^{2+}	-3.75	-5.20

Table 2: *Selectivity coefficients of the modified electrode towards the interfering ions.*

The selectivity of both NIP- and MIP-based electrodes was assessed under real life measurement circumstances. The electrodes were subjected to various concentrations of the ions that interfere (Figure 5). The interfering ions did not affect the measurements after the MIP-based electrode was subjected to LVF because the electrode reacts to the interfering ions in a non-Nernstian manner. This might be because the lipophilic membrane makes it challenging to exchange these hydrophilic ions after being exposed to the hydrophobic LVF, confirming that there is not any interference between the biologically relevant ions and the MIP-based electrode response.



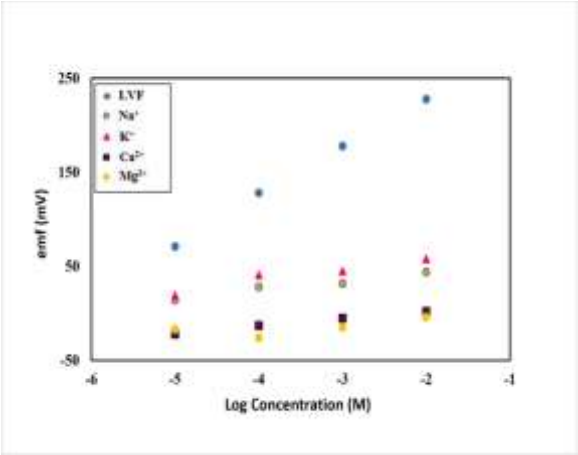


Figure 5: *Calibration curves obtained for LVF and some relevant concentrations of interfering ions using (a) NIP-based electrode and (b) MIP-based electrode. The curves show a Nernstian response towards LVF due to its relative lipophilicity. Measurements were performed in (1×10^{-2} M) acetate buffer (pH = 4.1).*

3.5. Method validation

Method validation was accomplished in accordance with the IUPAC guidelines [43].

3.5.1. Linearity and range

Plotting the potential values versus negative logarithmic LVF concentrations in molarity was done to construct the calibration curve under the outlined experimental conditions for the method (Figure 3). The regression curve was revealed to be linear over the concentration range of (1×10^{-5} - 1×10^{-2} M). The linear regression equation was:

$$E = -52.1 \log [C] + 333.6$$

where, E is the difference in potential and [C] is the molar concentration of LVF. (Table 1) shows the linearity range, intercept, slope, and correlation coefficient for the calibration data.

3.5.2. Limit of detection (LOD)

To determine the LOD of LVF in accordance with the recommendations of IUPAC [43], extrapolated lines of the calibration curve was done to estimate the LVF concentration correlated to the

extrapolation intersect. It was found to be (7.41×10^{-6} M) (Table 1).

3.5.3. Accuracy

The accuracy of the developed method was assessed by applying the previously outlined method for the determination of three different concentrations of LVF (1×10^{-5}, 1×10^{-4}, and 1×10^{-3} M) in triplicate. As shown in (Table 1), the percentage recovery values demonstrated the accuracy of the applied method.

3.5.4. Precision

For estimation of the intra- and inter-day precision of the applied method, triplicate assay of three different concentrations of LVF (1×10^{-5}, 1×10^{-4}, and 1×10^{-3} M) were measured on the same day and on three successive days, respectively. The relative standard deviation percentage (%RSD) was calculated, and its low values, i.e. < 2%, represent a repeatable and reproducible method (Table 1).

3.6. Analysis of LVF in spiked plasma samples and Floxabact® tablets

The quantitative analysis of LVF in spiked plasma samples and pharmaceutical formulation was done using the developed sensor. As shown in (Table 3), the obtained findings demonstrated good recoveries with low %RSD values which revealed that the suggested method could accurately and precisely determine the LVF in biological fluids and pharmaceutical dosage forms without any interference.

Table 3: *Average recovery values of three determinations of LVF in spiked plasma samples and pharmaceutical formulation using the modified sensor.eviation*

Samples	LVF concentration (M)	R% ± RSD *
Plasma	1×10^{-4}	98.50 ± 0.28
	1×10^{-5}	101.83 ± 0.44
Tablets	1×10^{-3}	98.91 ± 0.65

* R%: recovery%, RSD: relative standard d

3.7 *Adherence of the proposed sensor to the World Health Organization (WHO) "ASSURED" criteria*

The WHO [44] "ASSURED" criteria for the ideal POC devices were almost met by the developed sensor, which is as follows: (a) cost-effective, PVC was used for construction of the sensor. The PVC-based membranes is an affordable choice as a substrate; (b) sensitive: with a LOD of (7.41×10^{-6} M), the sensor was able to reduce the incorrect findings; (c) specific: the selectivity towards LVF was dramatically improved by the proposed sensor. It was able to quantify LVF in various matrices without sample pretreatment as well as when various interfering ions were present; (d) user-friendly: people lacking training can easily use the sensor for POC detection of LVF; (e) rapid: The sensor responds quickly and steadily in 3-5 seconds. (f) affordable equipment: only a minimal quantity of mobile equipment is needed for analysis, unlike other widely used methods that need sophisticated equipment; (g) delivery is easy for final consumers; (h) environmentally-friendly: less harmful wastes along with green impact on the environment.

3.8 Assessing the greenness of the proposed method

Green chemistry refers to a way of thought that focuses on conducting activities in accordance with the ideas of sustainable development. It is the use of a wide range of concepts that reduces the environmental impact of both chemical processes and products. Finding the proper method to assess an analytical procedure's greenness is crucial because numerous factors have to be

taken into consideration [45]. In this study, Analytical eco-scale [46] and GAPI [47] metrics were utilized to evaluate the greenness of the developed method. These scales consider several aspects of the analytical process.

Parameters	Penalty points	
Reagents		
Deionized water	0	
Glacial acetic acid	4	
Instrument		
Energy (≤ 0.1 kWh per sample)	0	
Occupational hazards (analytical process hermetization)	0	
Waste (1-10 mL, no treatment)	6	
Total Penalty points		∑10
Analytical eco-scale total score [a, b]	Excellent green analysis	90

[a] *Analytical Eco-Scale total score = 100 - total penalty points.*
[b] *If the score is > 75, it represents excellent green analysis. If the score is > 50, it represents acceptable green analysis. If the score is < 50. It represents inadequate green analysis.*
Table 4: *The analytical eco-scale tool used to evaluate the greenness of the developed method.*

The analytical eco-scale is regarded as a semiquantitative tool of analysis. Amount of reagent, occupational hazard, energy used, and waste production are the four main analytical method parameters that acquire penalty points when they deviate from the ideal green perspective. They are then subtracted from 100 to obtain the analytical eco-scale score. A study is considered to be ideal if it has an eco-scale value of 100. It is deemed excellent if it exceeds 75, acceptable if it reaches more than 50, and insufficient if it does not. As shown in (Table 4), only 10 penalty points were assigned by the developed method demonstrating excellent green analysis.

The whole analytical method was covered by GAPI metric. It uses five pentagrams to represent every analysis step, including collection of sample, transport, preparation stage, preservation, and storage. It also includes pentagrams for the reagents and the equipment used, as well as the volume of waste produced and its treatment. Each pentagram includes three color-specific codes (red, yellow, or green) to indicate the low, medium, or high environmental impact of every step of analysis. (Figure 6) shows the greenness assessment profile of the developed method using the GAPI metric which confirms the suggested platform's green analysis.

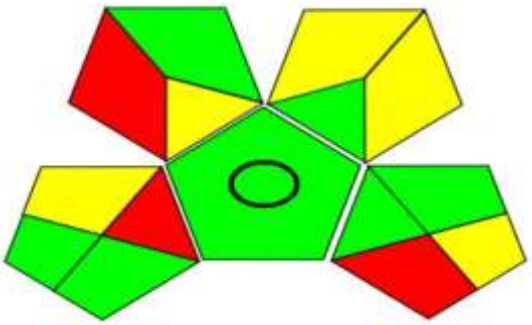


Figure 6: *Assessment of greenness of the analytical method by GAPI metric.3.8 Statistical analysis*

The results obtained from the developed method and the reported method [23] were compared statistically regarding the t-test and F-ratio. As shown in (Table 5), the theoretical values of t-test and F-ratio were more than the calculated ones, showing that there was not any significant difference between the methods at a 95% confidence .

Parameter	Developed method	Reference method[23] **
Mean	98.91	99.90
± SD	0.64	1.06
Variance	0.410	1.124
n	5	5
***t*-test** (2.31)*	1.79	-
F-ratio (6.39)*	2.74	-

**The values in parenthesis are the corresponding theoretical values for t and F at P = 0.05.*

*** Reported method: RP-HPLC; C_{18} column was used for separation, at ambient temperature using MeOH: water (70: 30, v/v) as the mobile phase at a flow rate of 1 mL/min and UV detection at 294 nm.*

Table 5: *Statistical comparison between the results obtained by the developed method for determination of LVF in pharmaceutical formulation and those obtained by the reference method.*

4. Conclusion

An eco-friendly, innovative, and selective MIP-based sensor was developed for the potentiometric assay of LVF in spiked plasma samples and pharmaceutical dosage form. The fabricated electrode was able to detect the drug in complex matrices without any interferences. The sensor selectivity was based on the molecularly imprinting polymerization technique utilizing MAA and EGDMA as the functional monomer and the cross linker, respectively. The strong selectivity of the modified sensor was consistent with the polymer's high affinity for LVF. A linear response was seen over the concentration range of (1×10^{-5} - 1×10^{-2} M). The obtained findings showed that the developed approach is relevant for the regular assessment of the drug in the laboratories of quality control without being hampered by other additives.

Conflicts of interest statement

All authors confirm that there are no conflicts of interest to declare.

Funding

There is no funding to declare.

CRediT authorship contribution statement

Amr M. Mahmoud: research idea conceptualization; supervised the study; data analysis; manuscript revision and editing.

Hanaa Saleh: research idea conceptualization; supervised the study; data analysis; manuscript revision and editing.

Michael Gamal Fawzy: research idea conceptualization; supervised the study; data analysis; manuscript revision and editing.

Alaa Reda: conducted the laboratory work, data curation and presentation and Writing—original draft.

Eman A. Bahgat: research idea conceptualization; supervised the study; data analysis; manuscript revision and editing.

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