Supplementary materials

Molecularly Imprinted Polymer Filter Modified with Natural Polyphenols for Extraction of Levothyroxine from Biological Samples

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Materials and solutions

Levothyroxine, with a purity level exceeding 98%, was procured from Sigma-Aldrich (Germany). Furthermore, the HPLC grade solvents, including acetonitrile, water, ethanol, and methanol were acquired from Merck (Darmstadt, Germany). Sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium permanganate ($KMnO_4$), methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), azobisisobutyronitrile (AIBN), polyvinyl pyrrolidone (PVP), lysine and a formaldehyde solution (~30% in water) were also secured from Merck (Darmstadt, Germany).

The stock solution of levothyroxine, featuring a concentration of 100 mg/L, was carefully prepared with ethanol. Following this, a series of working standard solutions were generated by executing precise dilutions of the initial stock solution with deionized water.

Instrumentation

The HPLC system (Shimadzu, Japan) used for the separation and analysis of levothyroxine is equipped with a UV detector (model SPD-10Avp) operating at a wavelength of 225 nm, a dual solvent pump (model LC-10Avp), and an injection valve (model EIG 001). The chromatographic

separation is facilitated by a KNAUER column (4.6 mm × 150 mm, 5 μm particle size; Eurospher 100-5 C8). The mobile phase, a 50:50 (v/v) mixture of acetonitrile and water, flows steadily at a rate of 0.5 mL/min. An S1122 solvent delivery system from Sykam (Germany) used for passing the solutions of analyte from filters. FT-IR spectroscopy, conducted with a Bruker-vertex 70 model, identified significant functional groups within the MIP structure. X-ray diffraction using a PHILIPS PW1730 diffractometer evaluated the crystallinity of the MIP by examining the obtained diffraction patterns. Additionally, FE-SEM, using a TESCAN MIRA II instrument, captured detailed images of the surface morphology, while EDS analysis provided the elemental composition of MIP.

Figure caption:

Fig. S1. Pareto plot of CCD (X_1: pH; X_2: [NaCl] (%w/v); X_3: Desorption time (min); L: linear; Q: qudratic).

Fig. S2. Desirability functions diagrams of CCD (X_1: pH; X_2: [NaCl] (%w/v); X_3: Desorption time (min)).

Fig. S3. HPLC-UV chromatograms of **(A)** levothyroxine standard solution, **(B)** urine blank, **(C)** serum blank and **(D)** spiked serum sample with 100 µg/L of levothyroxine after modified MIP filtration.

Fig. S4. Reusability data for modified MIP filter.

Fig. S5. Pictogram obtained from BAGI tool.

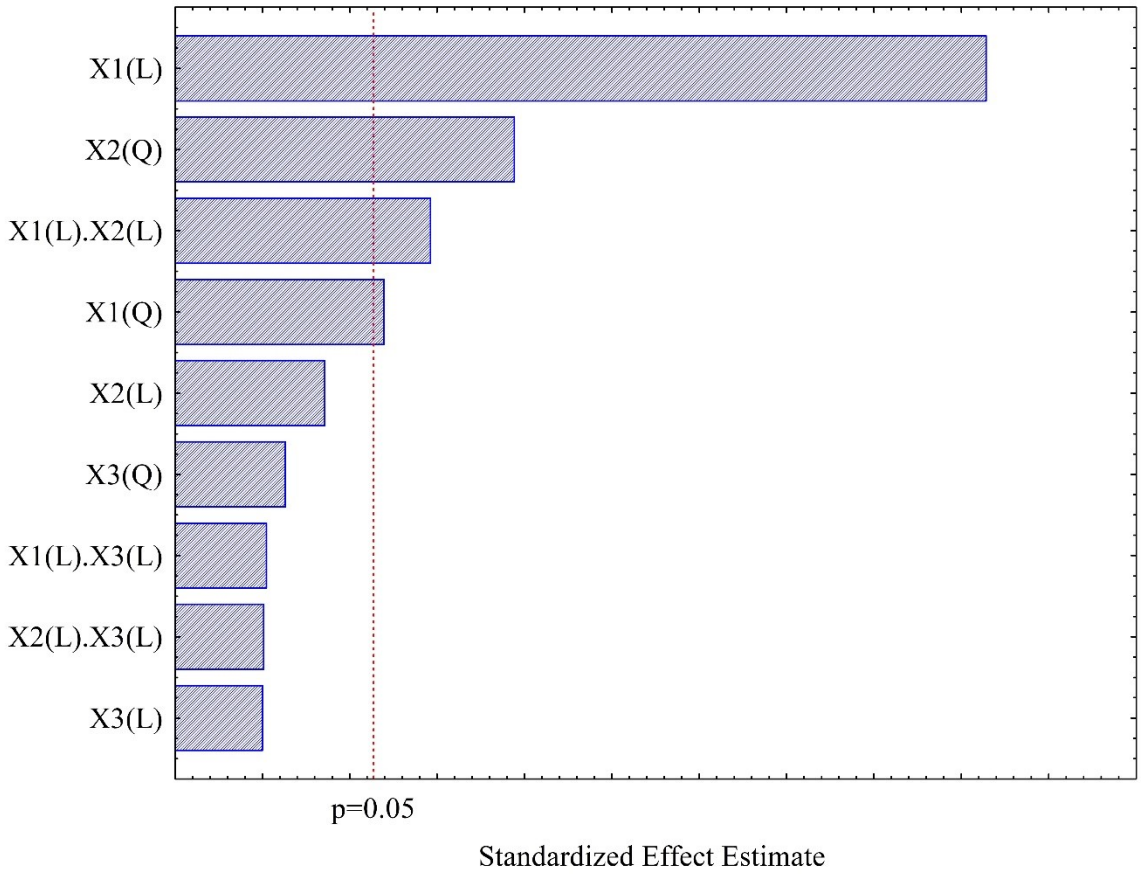
X1(L)
X2(Q)
X1(L).X2(L)
X1(Q)
X2(L)
X3(Q)
X1(L).X3(L)
X2(L).X3(L)
X3(L)
p=0.05
Standardized Effect Estimate

Fig. S1

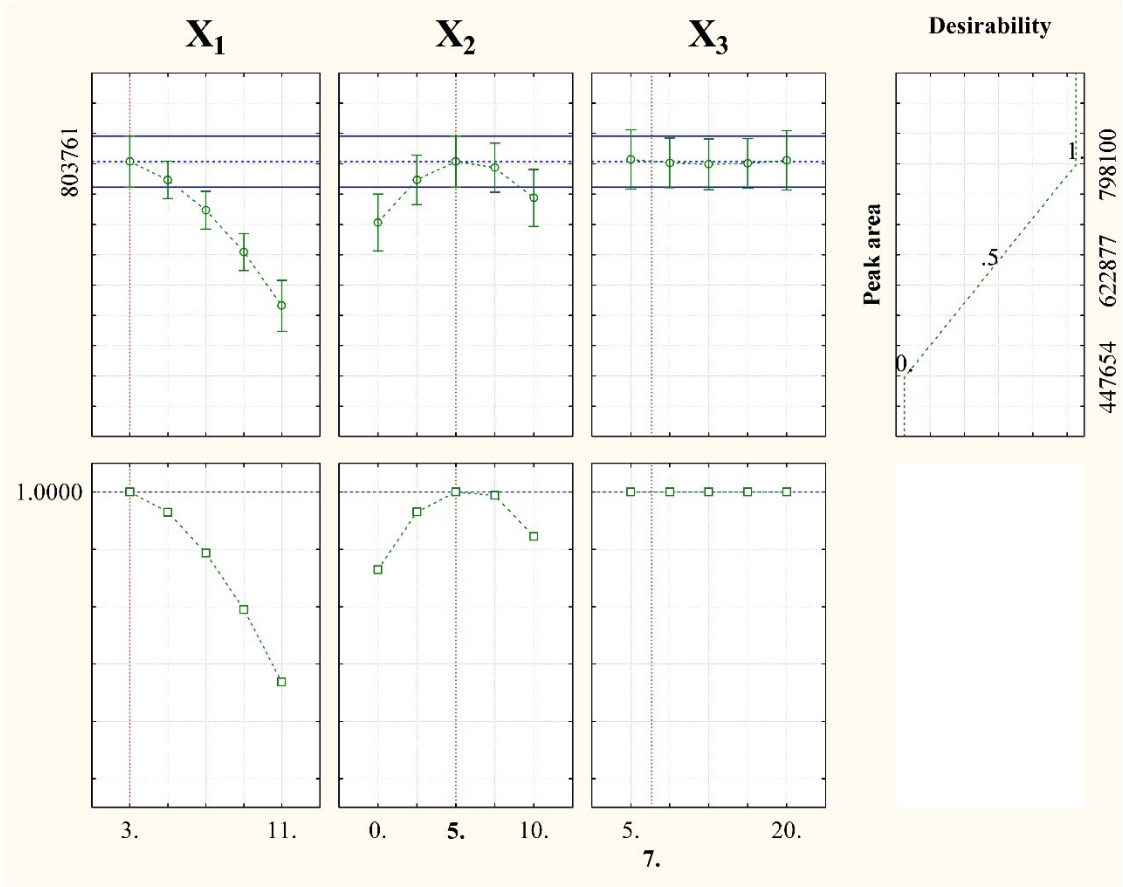
X1
X2
X3
Desirability
803761
1.0000
Peak area
798100
622877
447654
1.
.5
0.
3.
11.
0.
5.
10.
5.
20.
7.

Fig. S2

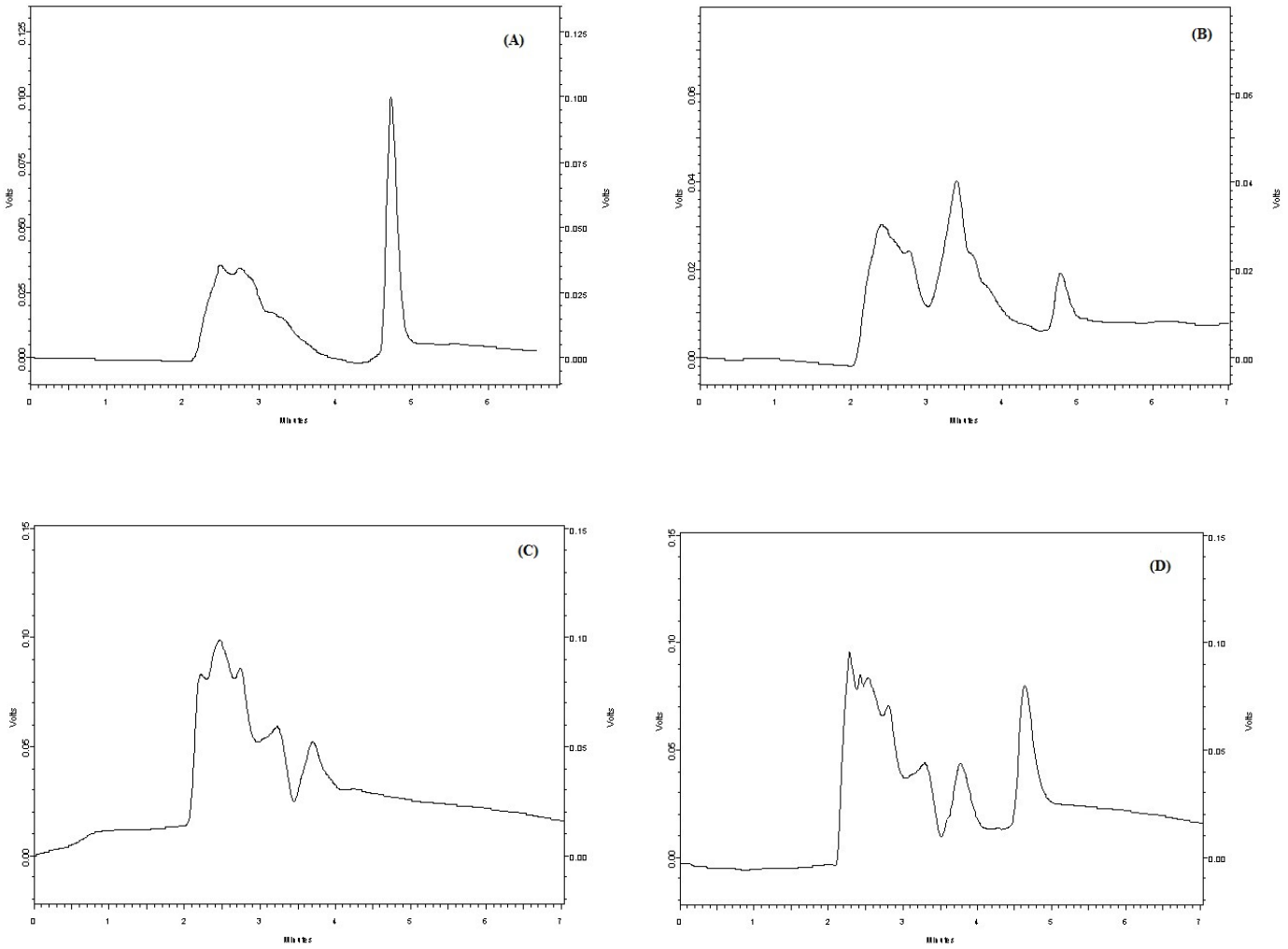

Fig. S3

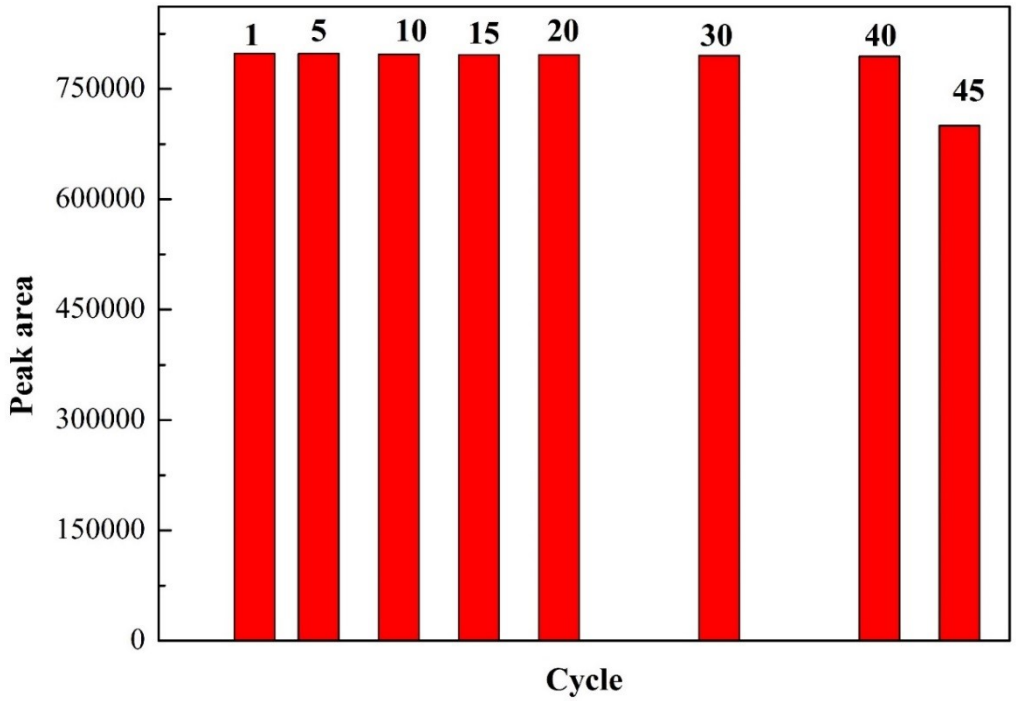
1
5
10
15
20
30
40
45
750000
600000
450000
300000
150000
0
Peak area
Cycle

Fig . S4

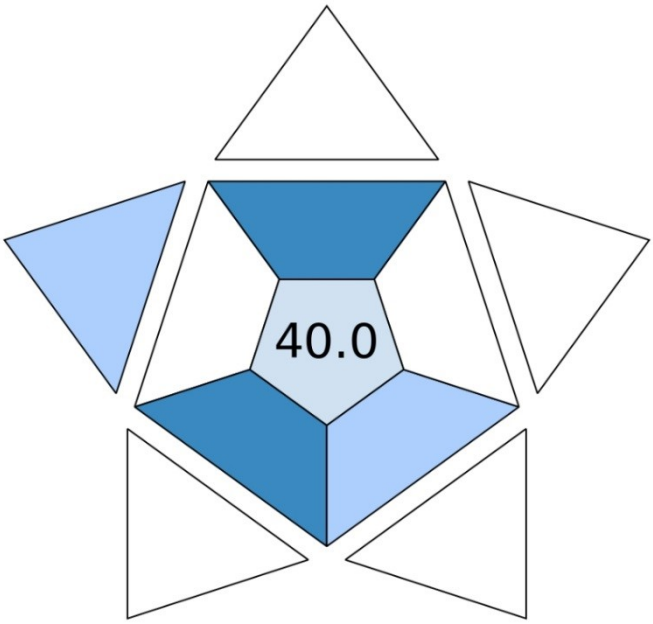

Fig. S5

Table S1 Results of ANOVA study obtained by CCD.

	Statistical parameters				
Factors	Sum of square	Degree of freedom	Mean of square	F-value	p-Value
X_1 (L)[a]	1.40×10^{11}	1	1.40×10^{11}	6862.075	0.007685
X_1 (Q)[b]	3.96×10^{9}	1	3.96×10^{9}	194.094	0.045617
X_2 (L)	1.03×10^{9}	1	1.03×10^{9}	50.852	0.088696
X_2 (Q)	1.69×10^{10}	1	1.69×10^{10}	831.274	0.022072
X_3 (L)	5.56×10^{3}	1	5.56×10^{3}	0.000	0.989489
X_3 (Q)	1.38×10^{8}	1	1.38×10^{8}	6.779	0.233458
X_1 (L)×X_2 (L)	7.56×10^{9}	1	7.56×10^{9}	370.072	0.033063
X_1 (L)×X_3 (L)	4.78×10^{6}	1	4.78×10^{6}	0.234	0.713052
X_2 (L)×X_3 (L)	5.54×10^{5}	1	5.54×10^{5}	0.027	0.896010
LOF	1.72×10^{8}	5	3.44×10^{7}	1.685	0.524164
Pure Error	2.04×10^{7}	1	2.04×10^{7}		
Total SS	1.86×10^{11}	15			
R^2	0.9989				
R^2 adjusted	0.9974				

[a] L (linear); [b] Q (quadratic)

Table S2 Comparison of modified MIP filter/HPLC-UV method with other methods for determination of levothyroxine.

Parameter	Ref. [34] (Shah, R. B., et al.2008)	Ref. [35] (Rohit,Dutt, et al.2011)	Ref. [36] (Permana, Andi Dian, et al.2021)	Ref. [37] (AlJamal,Marwa.et al.2023)	This method
Instrument	HPLC-UV	UPLC-MS/MS	HPLC-UV	Ion Exchange HPLC	HPLC-UV
Method	Direct injection without complex extraction	Protein precipitation and LLE	Protein precipitation	Dissolution in solvent and filtration	Modified MIP filter
Liner range	2-20 μg/mL	5.01-300.13 μg/L	0.5-1000 μg/L	3.5-200 μg/mL	1-1000 μg/L
LOD	1 μg/mL	4.9 μg/L	0.44 μg/L	0.953 μg/mL	0.36 (μg/L)
R^2	<0.99	≤0.98	≤0.998	<0.999	0.9999
RSD %	<10%	0.73-8.28	8.21-9.99	>2	<6.1
Recovery %	95.0-105.0	49.7-55.5	80.0-85.0	98.0-102.0	98.3-107.7
Sample	Levothyroxine dosage form	Human serum	Plasma from rats	Levothyroxine dosage form	Human urine and blood serum