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MOLECULAR DOCKING-BASED PREDICTIONS OF ESTROGEN RECEPTOR-ALPHA BINDING AFFINITY OF COMMON PARABENS AND CORRELATION WITH HPLC-DETERMINED PLASMA CONCENTRATIONS

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ABSTRACT
Endocrine disrupting properties have raised numerous concerns, and they have been investigated for their interactions with the estrogen receptor and their presence in biological systems. The present study was designed to assess the estrogen receptor alpha (ERα)-binding potential of four widely used Parabens (PBs) (Methylparaben (MePB), ethylparaben (EtPB), Propylparaben (PrPB), and Butylparaben (BuPB)) by molecular docking and to compare the docked result with in vivo plasma exposure data obtained from a validated HPLC method. Ligand structures were retrieved from the PubChem data base and then upload into SeamDock for docking with the human ERα ligand-binding domain (PDB ID:1A52) using the AutoDock Vina scoring algorithm through the SeamDock platform. Docking affinities and interaction profiles were used to assess protein-ligand interactions. Simultaneously, the HPLC method was validated for chromatographic performance and plasma quantification of PBs. Based on the molecular docking results, PrPB showed the highest binding affinity towards the ERα, followed y EtPB, BuPB, and MePB with binding energies of -5.5, -5.2, -5.1, and -3.7 kcal/mol, respectively. Multiple hydrogen-bonding and hydrophobic interactions were found to be important in the stabilization of the receptor-protein interaction by employing the protein-ligand interaction analysis. The HPLC method showed excellent analytical performance with the recovery of 98.83% to 99.85%, theoretical plate numbers of 8219 and 8989, and correlation coefficient values of 0.9927-0.9992, which indicated good performance for the analysis of PBs. Overall, the analytical and computational results suggest that structural differences in PBs greatly affect the binding affinity of ERα. PrPB was found to have the highest predicted estrogen receptor-binding potential among the compounds investigated. The combination of HPLC with molecular docking is a useful platform for assessing potential endocrine disrupting effects of common parabens and can be used as a tool for future exposure risk assessment.

KEYWORDS: Parabens, Estrogen receptor alpha (ERα), Molecular docking, HPLC, Endocrine disruption, AutoDock Vina, Exposure assessment.

INTRODUCTION

PBs are a family of antimicrobial activity alkyl esters of p-hydroxybenzoic acid (PABA) that have been used for many decades in cosmetics, pharmaceuticals, food, and personal care products. They are used widely known for their broad-spectrum antimicrobial activity, chemical stability and low production cost. Exposure to PBs has been nearly impossible to avoid for humans due to their extensive use in industry and for commercial products. Biomonitoring studies repeatedly found parabens in human blood, urine, breast milk, placenta and other bodily tissues, which have shown that the compound is present systematically across various populations and places globally[1,2]. The increased detection frequency of PBs has raised important concerns regarding their long-term safety and potential effects on human health. Parabens have been under a lot of scientific scrutiny in the past 20 years, due to their categorization as emerging endocrine disrupting chemicals (EDCs). They can disrupt their own hormone signaling pathways and can cause negative impacts at moderate levels. In the past PBs were thought to be safe preservatives, but increasing toxicological data indicates that certain members of this chemical family mimic endogenous estrogen and trigger estrogen responsive pathways. These findings have raised concerns about their possible role in reproductive abnormalities, developmental dysfunctions, hormone-dependent cancers, and metabolic dysregulations[3,4].

ERα is one of the molecular targets linked with endocrine disruption, and its function in the regulation of reproductive biology, differentiation, cell proliferation, and gene transcription. A ligand-activated transcription factor, Erα regulates the biological activities of endogenous estrogens by binding the hormone. Notably, the estrogenic potency of parabens

seems to be dependent on the length of the alkyl chain, indicating that their structure plays a significant difference in receptor binding and biological activity[5,6]. Recent studies using ERα dimerization and transcriptional activation assays found that parabens such as methylparaben (MePB), ethylparaben (EtPB), propylparaben (PrPB), and Butylparaben (BuPB) can elicit estrogen receptor-mediated responses, with the higher molecular weights showing more estrogenic activity than the lower molecular weights[5]. Along with toxicological studies, level of human exposure has been simultaneously identified in analytical studies. MePB, EtPB, PrPB, and BuPB are frequently detected in human biological samples in several biomonitoring programs and clinical studies. Although both PrPB and BuPB are more estrogenic than MePB, they are generally found in lower concentrations in human samples, which are often present in a broad range of consumer products[2,7]. It illustrates a significant issue in endocrine risk assessment, since a compound that is biologically most active is not necessarily the one which is most exposed in human. Thus, the magnitude of the endocrine-disrupting risk should be evaluated on a basis of measured biological levels of EDCs, and receptor binding potency.

In recent years, molecular docking has become a useful computational tool to study ligand-receptor interactions and to predict their binding affinity to biological targets. The advantage of molecular docking over experimental screening is the ability to obtain quick snapshots of binding poses, interactions and structure-activity relationships. Molecular docking has been successfully used in previous studies to access the estrogenic activity of PBs and other environmental contaminants. The computational studies also showed that increasing the length of the alkyl chain will improve the hydrophobic interactions in the ERα ligand-binding domain, which could lead to higher receptor binding and estrogenic activity[4,8]. However, the majority of scientific publications have addressed on only either computational prediction or measurements of biological activity, rather than combining quantitative exposure data and analytical measurements. Despite considerable progress in understanding the endocrine-disrupting properties of PBs, there is still a significant knowledge gap remains. Studies that do examine both receptor binding potential and human exposure separately, and only a few studies combine these two measurements in one risk-based approach. The association between the predicted estrogen receptor affinity and the experimentally measured levels of PBs in human plasma has been less thoroughly investigated. This is necessary since endocrine risk is related not only to the biologic potency of a chemical, but also to the amount of human exposure. Thus, the potential of ECDs combined with validated analytical measurements and computational receptor-binding analyses may yield a more realistic assessment for endocrine-disrupting potential than individual method[2,4,7].

The main purpose of the present study was to use molecular docking analysis to assess the interaction of four widely used PBs, namely MePB, EtPB, PrPB, and BuPB with human estrogen receptor alpha by molecular docking analysis. The binding affinities and the molecular interaction profiles of the selected PBs were calculated using the crystal structure of the ERα ligand binding domain (PDB ID: 1A52). Additionally, the docking outcomes have been correlated with plasma concentrations determined from HPLC to explore exposure-receptor binding potency relationships. This study combines analytical exposure data with molecular docking predictions and thus supplies a holistic view of the potential for individual PBs to contribute to the estrogenic load and exposure-potency based assessment of endocrine risk.

MATERIALS & METHODOLOGY

Study Design:

In the present study, four commonly used parabens (MePB, EtPB, PrPB, and BuPB) were subjected to molecular docking analysis to assess their potential binding affinity to ERα due to their estrogenic effects (Figure 1). Three-dimensional ligand structures were obtained from the PubChem database and were docked with the Human estrogen receptor alpha ligand binding domain (PDB ID: 1A52). Docking affinity scores and interaction profiles were used to choose the best binding conformations. The receptor-binding activity was then predicted and correlated with the plasma concentration of these drugs determined by using a validated HPLC method. The integrated assessment was carried out to explore the systemic exposure and the predicted estrogenic potential of the compounds selected for the PBs.

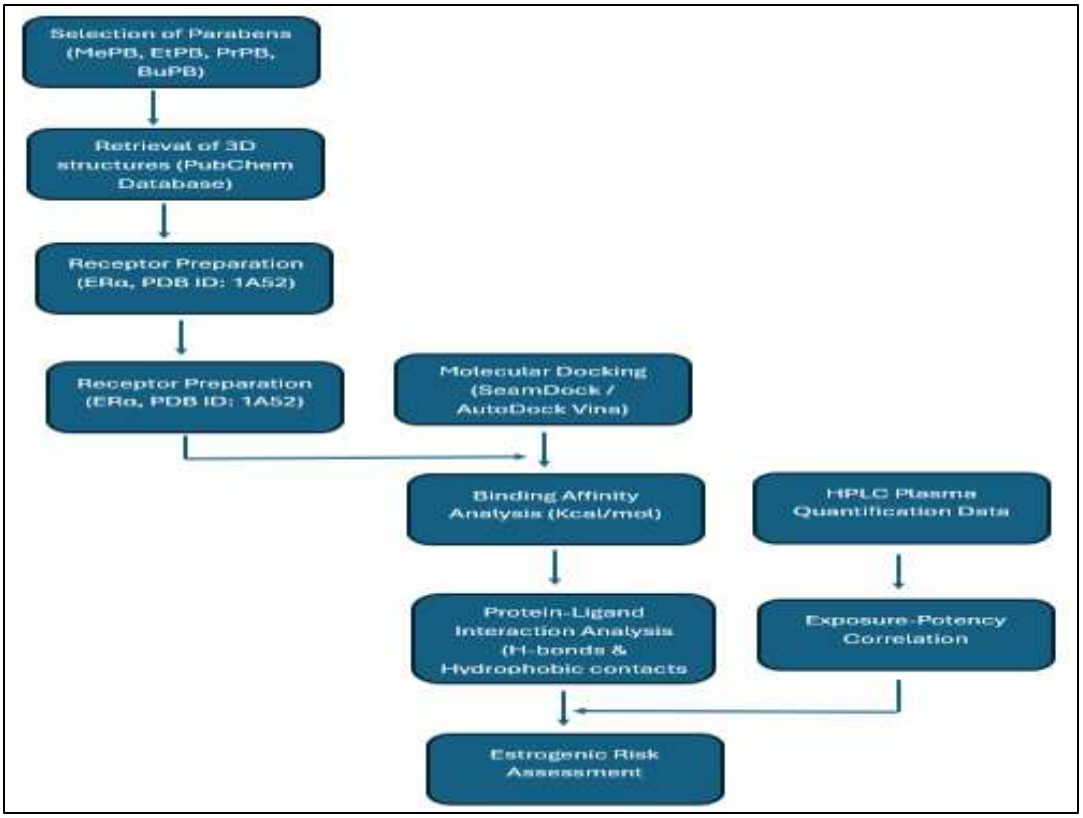


Figure 1: Overall Workflow of the study

Selection of Paraben Compounds:

The PBs selected for the current study are all used as antimicrobial preservatives in pharmaceutical products, cosmetics, personal care products, and in food formulations. The most common compounds of this family, paraben, are regularly found in human biological samples, signifying exposure to the whole population. Earlier studies have also shown that the parabens have different estrogenic properties, qualifying them for further analyses of structure-dependent interactions with ERα. Hence, the four parabens (MePB, EtPB, PrPB, and BuPB) were chosen to study the correlation between receptor-binding ability, and plasma levels following oral administration[11,12,9].

Compound	Hydrogen Bond Donor Count	Hydrogen Bond Acceptor Count	Exact Mass	Monoisotropic Mass	Complexity	Heavy Atom Count	Reference
MePB	1	3	152.047344113 Da	152.047344113 Da	136	11	Computed by Cactvs 3.4.8.24 (PubChem release 2025.09.15)
EtPB	1	3	166.062994177 Da	166.062994177 Da	148	12	Computed by Cactvs 3.4.8.24 (PubChem release 2025.09.15)
PrPB	1	3	180.078644241 Da	180.078644241 Da	160	13	Computed by Cactvs 3.4.8.24 (PubChem release 2025.09.15)
BuPB	1	3	194.094294304 Da	194.094294304 Da	171	14	Computed by Cactvs 3.4.8.24 (PubChem release 2025.09.15)

Table 1: Chemical Properties of the selected parabens (PBs)

Compound	Description	Color	Odor	Taste	Boiling Point	Melting Point	Solubility
MePB	Dry Powder; Other Solid; Liquid; Wet Solid; Other Solid	White needles	Odorless or has faint characteristic odor	Slight burning taste	270.5 °C	125.2 °C	In water, 2.50X10+3 mg/L at 25 °C

EtPB	Liquid; Other Solid	Small, colorless crystals or powder at room temperature	Odorless	-	297-298 °C	117 °C	In water, 8.85X10+2 mg/L at 25 °C
PrPB	Liquid; Other Solid; Dry Powder	White crystals	Odorless or has faint odor	-	271 °F at 1 mmHg	96.1 °C	In water, 5.00X10+2 mg/L at 25 °C, Soluble in ethane, ethyl ether; slightly soluble in chloroform
BuPB	Colorless solid; Insoluble in water (207 mg/L at 20 deg C)	Small, colorless crystals or powder	Odorless	-	156-157 °C	68.5 °C	Insoluble in water; soluble in ether, acetone and propylene glycol, slightly soluble in oils.

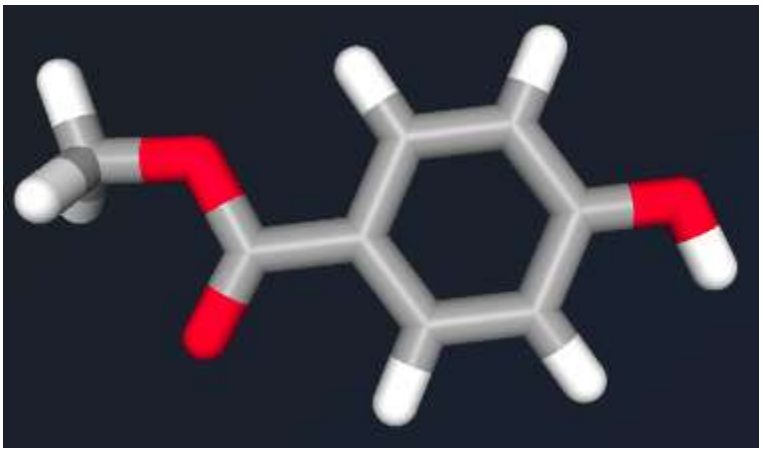
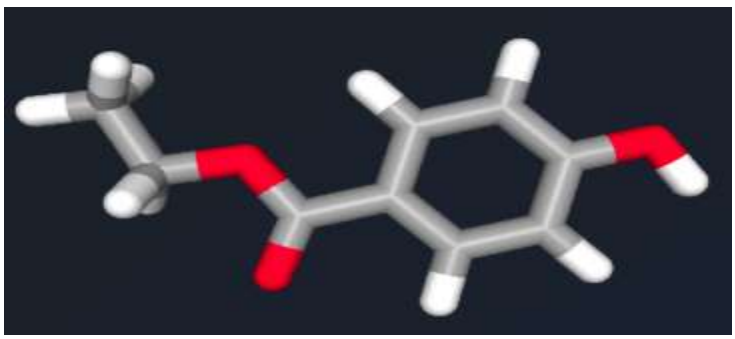
Table 2: Physical Properties of the selected parabens (PBs)

Ligand Preparation:

MePB (CID 7456), EtPB (CID8434), PrPB (CID 7175), and BuPB (CID 7184) were downloaded from the PubChem Compound Database in Structure Data File (SDF) format. The compounds selected were chosen because of their broad use as preservatives and because of their previously reported estrogenicity. The downloaded structures were checked or structural integrity and then converted to docking compatible formats before molecular docking. Hydrogen atoms and bond information were retained to ensure chemical identity of each ligand during structure preparation. The synthesized ligands were then uploaded to the SeamDock platform for molecular docking studies with human ERα ligand-binding domain[3,10,8].

Compound	PubChem ID	Molecular Formula	Molecular Weight	CCDC No.	Link
Methylparaben	7456	$C_8H_8O_3$	152.15 g/mol	617627	DOI:10.5517/ccnqpgh
Ethylparaben	8434	$C_9H_{10}O_3$	166.17 g/mol	7125078	-
Propylparaben	7175	$C_{10}H_{12}O_3$	180.20 g/mol	765227	DOI:10.5517/cctp8rh
Butylparaben	7184	$C_{11}H_{14}O_3$	194.23 g/mol	933598	DOI:10.5517/cc10bh2x

Table 3: Identification Details of the selected parabens (PBs) from PubChem

Compound	3D Conformer
Methylparaben	
Ethylparaben	

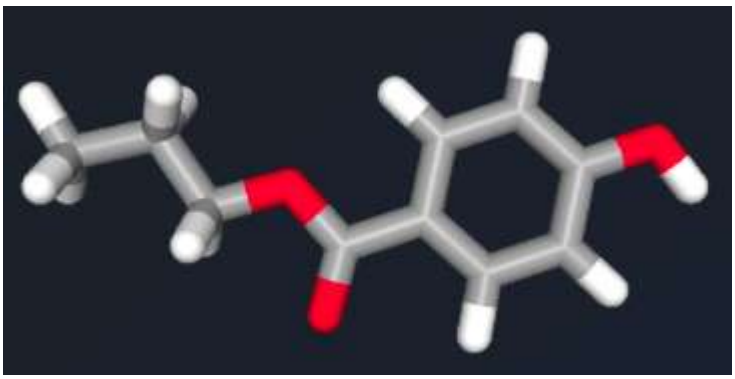
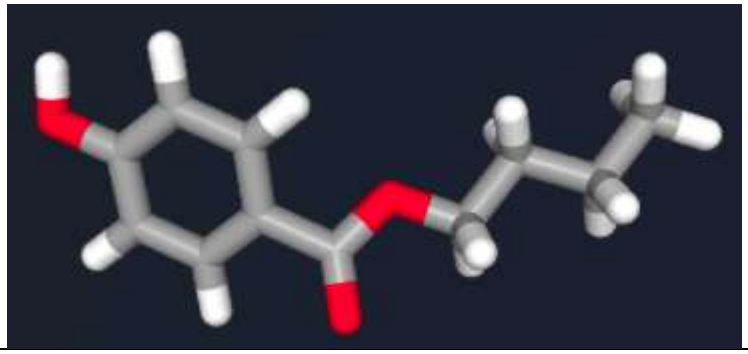
Propylparaben	
Butylparaben	

Figure 2: 3D conformers of MePB, EtPB, PrPB, and BuPB obtained from the PubChem Database

Receptor Preparation:
The crystal structure of human estrogen receptor alpha complexed with estradiol was obtained from the protein data bank (PDB ID: 1A52). The receptor structure was determined by X-ray diffraction at 2.80 Å. The receptor was selected due to the presence of its native ligand-binding pocket that is structurally complimentary to the estradiol molecule, which allows investigation of the possible interactivity of parabens with the estrogen receptor binding site. The receptor structure was manually uploaded to the SeamDock platform to be inspected for structure integrity before molecular docking. The docking region was selected around the estradiol binding pocket to accommodate this co-crystallized molecule for targeted docking simulations. The receptor structure was then fixed, and all the docking calculations were performed under the same conditions using selected parabens[4,14,15].

Classification	Receptor
Organism(s)	Hom sapiens
Expression System	Escherichia coli
Mutation(s)	No (0)
Chains	A, B
Sequence Length	258
Global Symmetry	Cyclic-C2
Global Stoichiometry	Homo 2-mer – A2
Gene Names	ESR
UniProtKB	P03372
Method	X-Ray Diffraction
Resolution	2.80 Å
R-Value Free	0.274 (Depositor), 0.281 (DCC)
R-Value Work	0.223 (Depositor), 0.235 (DCC)
R-Value Observed	0.223 (Depositor)

Table 4: Structural characteristics of estrogen receptor alpha (ERα) (PDB ID: 1A52)

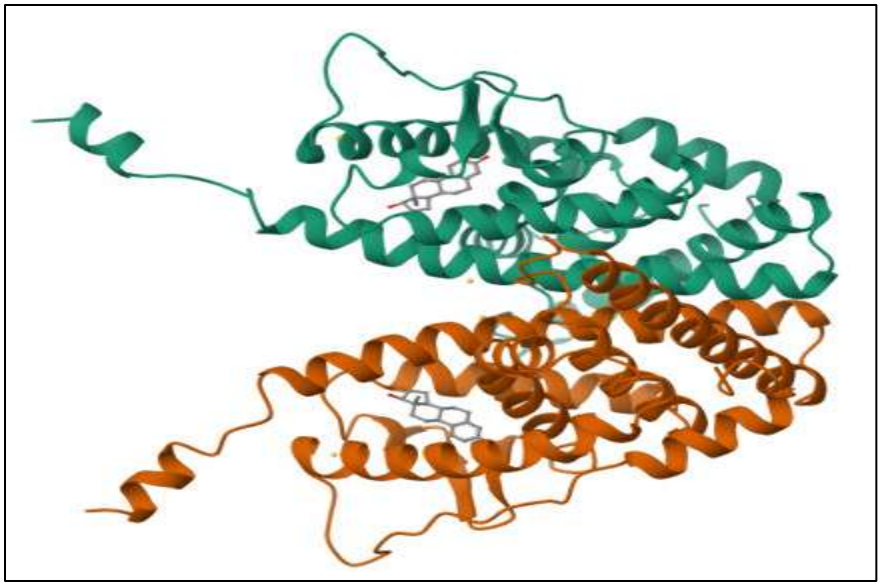


Figure 3: 3D structure of human estrogen receptor alpha ligand-binding domain complexed with estradiol

Molecular Docking Procedure:

To assess their binding affinities, the human ERα ligand-binding domain was used in molecular docking analysis. The interaction profile of the docked ligand and the amino acid residues in the ERα binding pocket was analyzed using the integrated interaction analysis tools of the SeamDock platform. AutoDock Vina is a popular tool for structure-based drug design because of its fast conformational search algorithm and capacity for accurate prediction of binding poses. The optimized ligand structures were docked one by one in the active site of ERα (PDB ID: 1A52). The docking region was defined by the co-crystallized cavity of the estradiol binding site to allow targeted interaction analysis of the native ligand binding site. To ensure consistency and direct comparison of binding affinities among the selected parabens, identical docking parameters were used for all of them. For each ligand, ten docking poses were generated and ranked according to their predicted binding energies (kcal/mol). The docking poses that showed the lowest binding energy was believed to represent the most favorable binding conformation and was chosen to undergo further interaction analysis. The binding affinity values derived from the highest scoring poses were then used to rank the selected parabens based on their ERα-binding potential.

Parameter	**Value**
Docking Platform	SeamDock
Docking Engine	AutoDock Vina
Receptor	ERα (PDB ID: 1A52)
Ligands	MePB, EtPB, PrPB, and BuPB
Number of docking poses	10
Scoring Unit	Kcal/mol
Binding Site	Estradiol-binding pocket

Table 5: Molecular Docking parameters used during the study

Chemicals and Reagents:

The reference standards of MePB, EtPB, PrPB, and BuPB were purchased from Indian Pharmacopoeia Commission (IPC), India. HPLC-grade acetonitrile used from Merck (Darmstadt, Germany) and mobile phase and sample processing was performed using deionized water from a Milli-Q water purification system. Chemicals and reagents used during this study were all analytical grade and used as supplied.

Plasma Sample Collection:

Peripheral venous blood samples were collected using sterile vacutainer tubes containing an anticoagulant (EDTA – Ethylene Diamine Tetrachloride Acetic acid). The blood samples were centrifuges immediately after collection for 10 min at 4,000 rpm to separate plasma from cellular components. The retrieve plasma layer was carefully placed in clean polypropylene tubes and refrigerated before analysis. Multiple freeze-thaw cycles were avoided to maintain sample integrity throughout the study[13,18,21].

Stock and Working Standard Solutions:
Primary stock solutions of 10 ppm for each preservative (MePB, EtPB, PrPB, and BuPB) were prepared by dissolving 10 mg of each reference standard in 10 ml of diluent. A mixed working standard solution was prepared by transferring appropriate aliquots of each stock solution into a volumetric flask and diluting to volume with diluent. The mixed working solution was then serially diluted to develop the calibration standards in the required range for quantitative analysis. All prepared standard solutions were stored under refrigerated conditions and allowed to reach room temperature before use[15,17,19].

Sample Preparation Procedure:
Plasma samples were left to warm to room temperature and mixed will before analysis. An aliquot of plasma was placed in a centrifuge tube, and sample preparation was carried out using the previously optimized protocol. After processing, the sample was passed through a 0.22 µm membrane syringe filter prior to chromatographic analysis. The filtrate obtained was placed in HPLC vials for quantitative determination of MePB, EtPB, PrPB, and BuPB.

Instrumentation and Chromatographic Conditions:
The chromatographic analysis was performed by using HPLC system (ASRCTL/2017/733, Adarsh Scientific Research Center & Testing Lab Pvt. Ltd., Panvel, India) and C18 Inertsil analytical HPLC column (250 mm x 4.6 mm, 5 µm) with diode array detector set at 254 nm and was performed briefly. Isocratic separation with a water-acetonitrile mobile phase (50:50, v/v) and flow rate of 1.0 ml min^{-1}. The injection volume was kept at 10 µl and the total chromatographic run time was set to 20 min[17,18].

Method Validation:
The HPLC method used in the present work has been developed and validated according to the ICH Q2(R1) and IPC analytical validation guidelines. Validation parameters were peak symmetry, plate numbers, Recovery %, asymmetry, and linearity (correlation coefficient). The method was developed with good chromatographic performance and repeatability for the simultaneous quantification of four parabens in plasma samples. The validated procedure then was applied to quantify plasma and to compare the results of this plasma quantification with the ERα-binding affinity data provided by molecular docking[17,18].

Correlation of Plasma Exposure and Receptor-Binding Affinity:
The plasma concentration of the selected parabens measured by HPLC were plotted against the molecular docking-derived affinity values for ERα to assess the possible estrogenic load. Plasma concentration was considered as a measure of systemic exposure, whereas docking affinity was considered a indicator of the predicted receptor-binding potential of each paraben. The identified parabens were ranked separately based on the HPLC-measured plasma concentrations and molecular docking affinity separately. Ranking was performed by highest to lowest exposure for plasma concentrations and strongest to weakest binding affinity (by the magnitude of the negative binding energy values) for docking affinities. These rankings were then contrasted to see if compounds with greater systemic exposure also showed greater receptor-binding affinity towards ERα. A comparative exposure/potency assessment framework was used to determine which parabens are likely to have a major contribution to cause a substantial estrogenic burden[19,20,21].

RESULTS & DISCUSSION

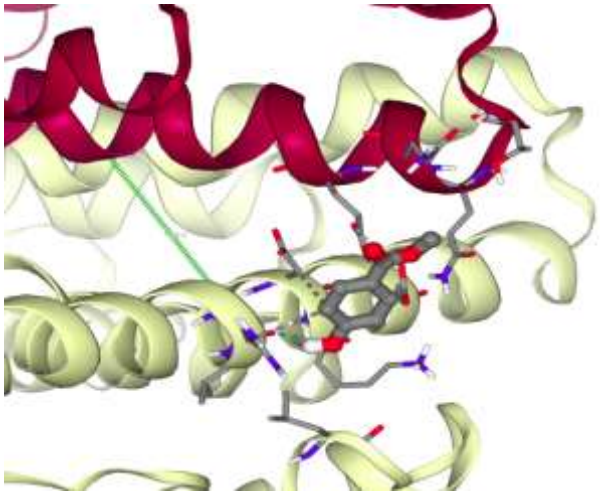
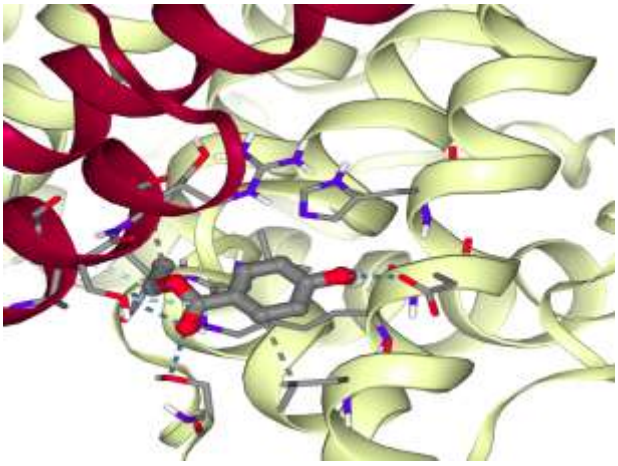
Molecular Docking Analysis:
The binding affinity of four parabens was successfully assessed towards the ligand-binding domain of human ERα using molecular docking analysis (Table 6). The calculated binding affinities showed significant differences between the tested PBs, suggesting that structural changes affected the receptor-binding behavior. The docking scores were in the range of -3.4 to -5.5 kcal/mol (Table 7). Based on their predicted ERα binding affinities, PrPB showed the highest binding affinity score of -5.5 kcal/mol. EtPB and BuPB had similar binding affinities of -5.2 and -5.1 kcal/mol, respectively. In contrast, MePB displayed the weakest interaction with a binding affinity of -3.4 kcal/mol. The results indicate that PrPB has the highest predicted ERα-binding potential of the PBs studied, with lower binding energy values indicating better ligand-receptor complex formation. The observed affinity trend was:[7,6]

PrPB > EtPB > BuPB > MePB … (1)

The stronger receptor interaction exhibited by PrPB could be attributed to its good fit of the alkyl side chain into the hydrophobic binding pocket of ERα. However, the shorter alkyl chain found in MePB could hinder the ability to form hydrophobic interactions and therefore affect binding stability. These findings suggest that the binding affinity of the receptor and the estrogen-mimicking activity of PB analogues may be sensitive to relatively small structural changes[12,13,14].

IUPAC Name	InChl	InChlKey	Smiles	ChEMBL ID	DrugBank ID
methyl 4-hydroxybenzoate	InChI=1S/C8H8O3/c1-11-8(10)6-2-4-7(9)5-3-6/h2-5,9H,1H3	LXCFILQKKLGQFO-UHFFFAOYSA-N	COC(=O)C1=CC=C(C=C1)O	CHEMBL325372	DB14212
ethyl 4-hydroxybenzoate	InChI=1S/C9H10O3/c1-2-12-9(11)7-3-5-8(10)6-4-7/h3-6,10H,2H2,1H3	NUVBSKCKDOMJSU-UHFFFAOYSA-N	CCOC(=O)C1=CC=C(C=C1)O	CHEMBL15841	DB13628
propyl 4-hydroxybenzoate	InChI=1S/C10H12O3/c1-2-7-13-10(12)8-3-5-9(11)6-4-8/h3-6,11H,2,7H2,1H3	QELSKZZBTMNZEB-UHFFFAOYSA-N	CCCOC(=O)C1=CC=C(C=C1)O	CHEBI:32063	DB14177
butyl 4-hydroxybenzoate	InChI=1S/C11H14O3/c1-2-3-8-14-11(13)9-4-6-10(12)7-5-9/h4-7,12H,2-3,8H2,1H3	QFOHBWFCKVYLES-UHFFFAOYSA-N	CCCCOC(=O)C1=CC=C(C=C1)O	CHEMBL459008	DB14084

Table 6: Compound Descriptors of the selected parabens from PubChem

Compound	PubChem ID	Binding Affinity (kcal/mol)	Rank	Binding-pose
MePB	7456	-3.4	4	
EtPB	8434	-5.2	2	

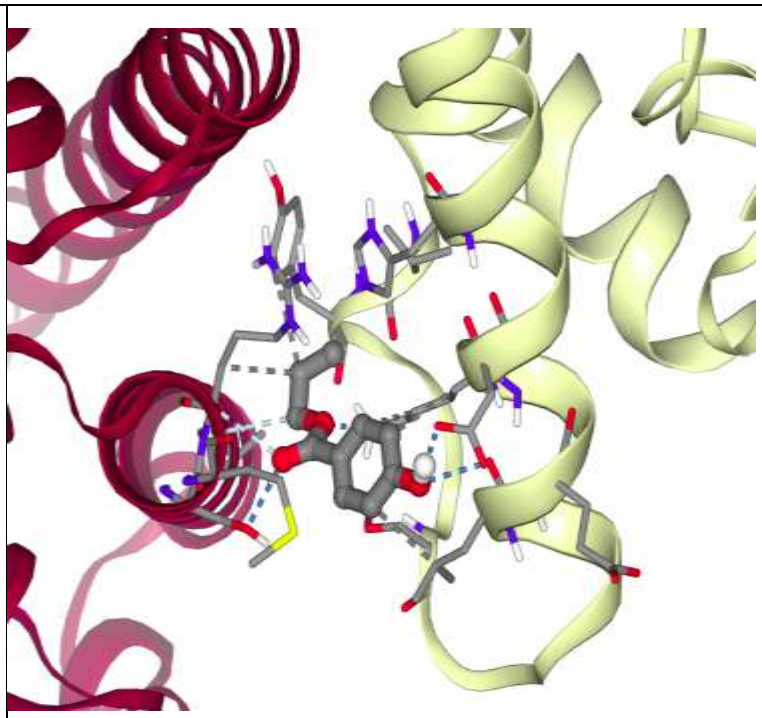
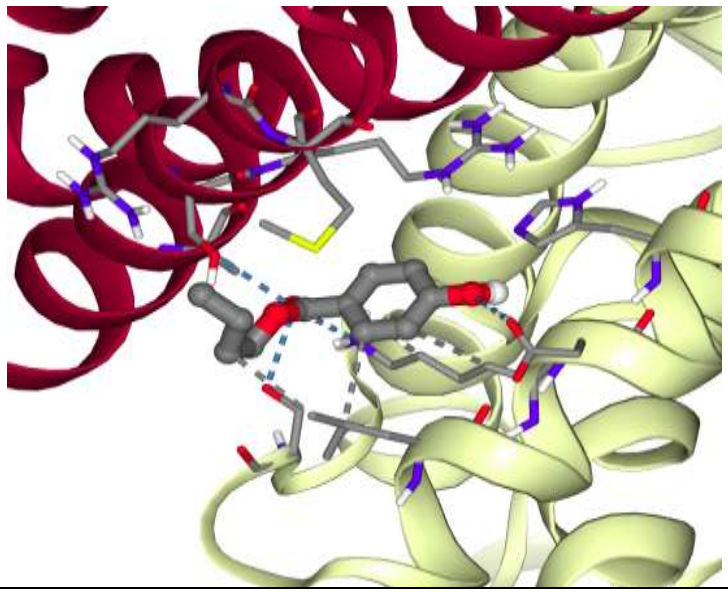
PrPB	7175	-5.5	1	
BuPB	7184	-5.1	3	

Figure 4: Molecular docking-derived binding affinities of selected Pbs against ERα

Protein-Ligand Interaction:

Among the investigated compounds, PrPB showed significant binding affinity (-5.5 kcal/mol) and multiple stabilization interaction with essential amino acid residues of ERα. The hydrogen bond interactions were observed with Ser433, Leu469, Asp473 and Lys472 and the hydrophobic interaction with Arg434, Tyr459, Leu469 and Lys472. In addition to multiple conventional hydrogen bonds, the PrPB-ERα complex had hydrophobic interactions that helped stabilize the complex and may account for its high docking score compared to the other PBs that were investigated. EtPB showed a binding affinity of −5.2 kcal/mol and established multiple hydrogen bond interactions with Asp473, Ser463, and Ser433. Leu469 and Lys472 were found to be hydrophobic interactions, these interaction pattern indicates that ethylparaben can form a stable interaction network in the receptor cavity that facilitates favorable interaction with ERα. Similarly, BuPB showed high binding affinity of −5.1 kcal/mol and had several hydrogen bond interactions with Ser463, Asp473, Lys472, and Ser433. Two additional hydrophobic interactions with Leu469 and Lys472 stabilized the ligand in the binding pocket. BuPB has a longer alkyl side chain than PrPB, but their binding affinity is a little lower, indicating that the longer chain might create steric constraints within the receptor cavity, that would create a

less favorable binding orientation. MePB had the lowest docking affinity and interaction profile of the PBs analyzed. The formed ligand made only hydrogen bond interactions with Arg477 and Ser433 and made only hydrophobic interactions with Asp480, Arg434 and Leu469. Additionally, a number of weak hydrogen bond interactions were found involving Gln498, Glu502 and Ala430. The binding affinity of methylparaben is comparatively low when compared to the longer chain paraben analogues and may be due to the lower number of stabilizing interactions.[21,19,17]
The comparison of interaction patterns showed the following residues involved in ligand recognition and stabilization of the complex repeatedly: Ser433, Leu469, Lys472, and Asp473. Thus the residues in these cell walls seem to contribute significantly to the ability of the ERα ligand-binding cavity to accommodate PB molecules. The progressive improvement in receptor-binding affinity from MePB, PrPB, and EtPB increased with the length of the side chain, indicating that larger hydrophobic interactions occur within the binding pocket. The slightly weaker binding affinity for BuPB than for PrPB suggests, that receptor binding is not just a function of hydrophobicity, but also of a steric fit between the bound ligand and the receptor cavity.[16,15]

Compound	Binding Affinity (kcal/mol)	Hydrophobic Contact		Hydrogen Bond		Weak Hydrogen Bond	
		Ligand atom	Receptor	Ligand atom	Receptor	Ligand atom	Receptor
MePB	-3.4	C4	R477(B) CB	O2	R477(B) O	C8	Q498(A) OE1
		C4	D480(B) CB	-	-	C7	E502(A) OE1
EtPB	-5.2	C9	R434(A) CB	O3	S433(A) OG	C5	A430(A) O
		C2	L469(B) CG	O3	S463(B) OG	C4	S433(A) OG
		C2	K472(B) CD	O2	D473(B) OD1	-	-
		-	-	O3	S463(B) OG		
				O3	S433(A) OG		
				O1	S433(A) OG		
PrPB	-5.5	C1	R434(A) CB	O2	S433(A) OG	C2	A430(A) O
		C1	Y459(B) CD1	O3	L469(B) O	O2	R434(A) CA
		C8	L469(B) CG	O3	D473(B) OD1	-	-
		C9	K472(B) CB	O2	S433(A) OG		
		C7	K472(B) CD	O1	K472(B) NZ		
BuPB	-5.1	C8	L469(B) CG	O2	S433(A) OG	-	-
		C1	L469(B) CD2	O2	S463(B) OG		
		C10	K472(B) CB	O3	D473(B) OD1		
		C5	K472(B) CD	O2	K472(B) NZ		
		-	-	O2	S463(B) OG		
				O2	S433(A) OG		

Table 8: Protein-Ligand interaction profiles of PBs docked with ERα

HPLC Plasma Quantification of Parabens:
The validated HPLC method exhibited satisfactory chromatographic performance for simultaneous determination of selected four PBs. Based on the optimized chromatographic conditions, all four PBs were separated with good resolution and were well resolved with retention times varying from 3.75 to 9.91 min (Table 9). As expected, an increase in the retention time was seen from MePB to BuPB that corresponds to the increasing hydrophobicity of the PB molecules. The peak areas obtained in the chromatograms showed that, all the analytes were detected efficiently in the selected analytical range. EtPB and MePB showed the highest values of the relative area percentage (26.93% and 26.81%, respectively), followed closely by PrPB (22.91%) and BuPB (23.33%) that exhibited the lower values of the relative area percentage.[17,18] The comparable peak area distribution indicates good chromatographic separation and consistent response from the detector for the compounds explored. Peak symmetry is an important parameter for assessing chromatographic performance. The asymmetry values were in the range of 1.73975-1.85906 for investigated PBs, which are satisfactory, as they do not show any considerable tailing. The level of asymmetry of the chromatographic peak observed in each compound is recorded. The peak of EtPB was the most symmetric, and BuPB showed a slightly higher asymmetry value, possibly due to greater interaction with the stationary phase by the longer, more hydrophobic alkyl chain. Conformity of the chromatographic system was also further validated using theoretical plate analysis.[16,14] The calculated plate numbers were in the range from 8219-8989 for the PBs, which showed high

column efficiency and satisfactory chromatographic resolution. The increase in plate number was generally observed with an increase in retention time, indicating a stable chromatographic performance during the analysis. Excellent extraction and analytical accuracy were obtained as the recovery values of all investigated PBs were higher than 98%. The recovery percentage value obtained for every analyte varied from 98.83-99.85% for EtPB and BuPB, respectively, allowing for a minimal loss of these analytes during the sampling process, thus demonstrating the reliability of the analytical procedure for quantitative determination of these analytes in plasma samples. The method was evaluated for linearity by performing correlation coefficient (R2) analysis on the calibration curve. Excellent linear responses were observed for all parabens, ranging from $R^2 = 0.9927$-$R^2 = 0.9992$. PrPB showed the highest degree of linearity ($R^2 = 0.9992$), followed by BuPB ($R^2 = 0.9963$), EtPB ($R^2 = 0.9950$), and MePB ($R^2 = 0.9927$). These values show a high level of correlation between analyte concentration and detector response, and the method is suitable for quantitative plasma analysis. Based on overall results of the chromatographic analysis, the developed HPLC method proves the separation, quantification and recovery of the selected PBs in plasma matrix.[17,18,22] The recovery was high with excellent linearity, acceptable peak symmetry, and high column efficiency, which would allow the use of the analytical method for exposure assessment and subsequent comparison with the ERα-binding affinity data obtained by molecular docking.

Analyte	Retention Time (min)	Area	Area %	Asymmetry	Theoretical Plates (USP)	Recovery %	Correlation Coefficient
MePB	3.75	1386438	26.811	1.79375	8219	99.47	**0.9927**
EtPB	6.12	1393031	26.938	1.73975	8744	98.83	**0.995**
PrPB	8.24	1184863	22.913	1.76489	8969	98.95	**0.9992**
BuPB	9.91	1206831	23.338	1.85906	8989	99.85	**0.9963**

Table 9: Chromatographic performance and analytical characteristics of the investigated parabens

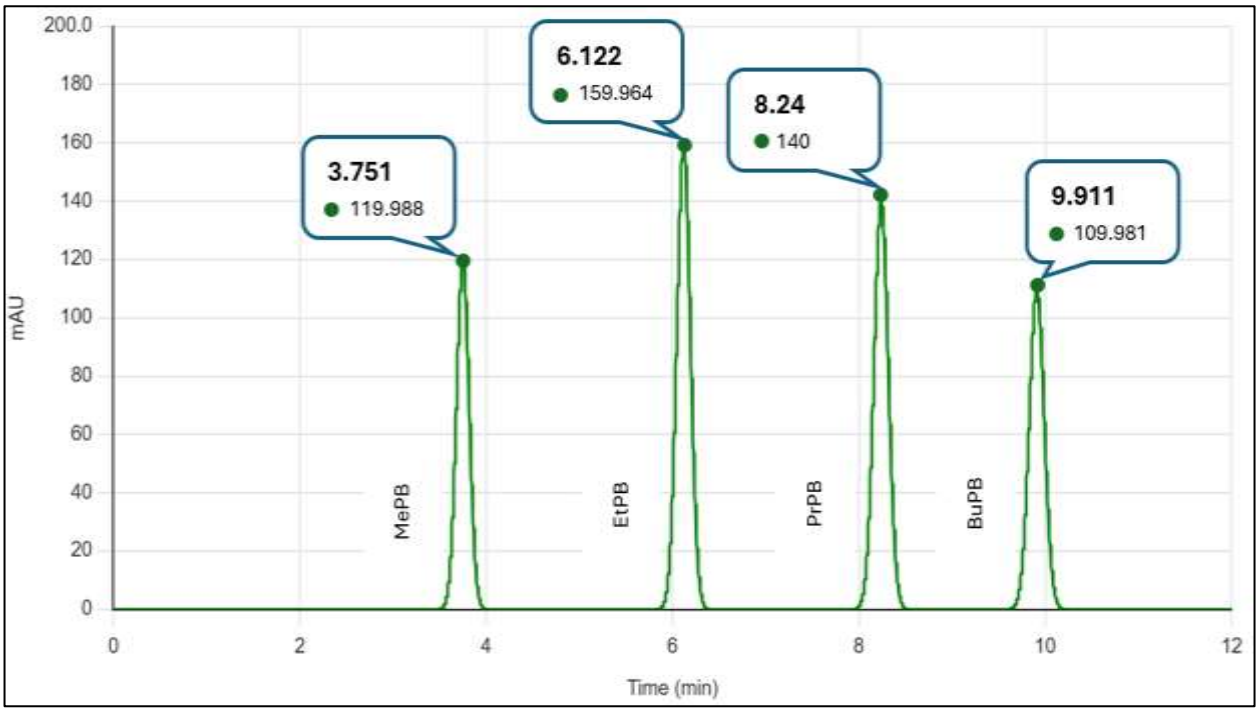


Figure 5: Representative HPLC chromatogram showing the separation of MePB, EtPB, PrPB, and BuPB under the optimized chromatographic conditions

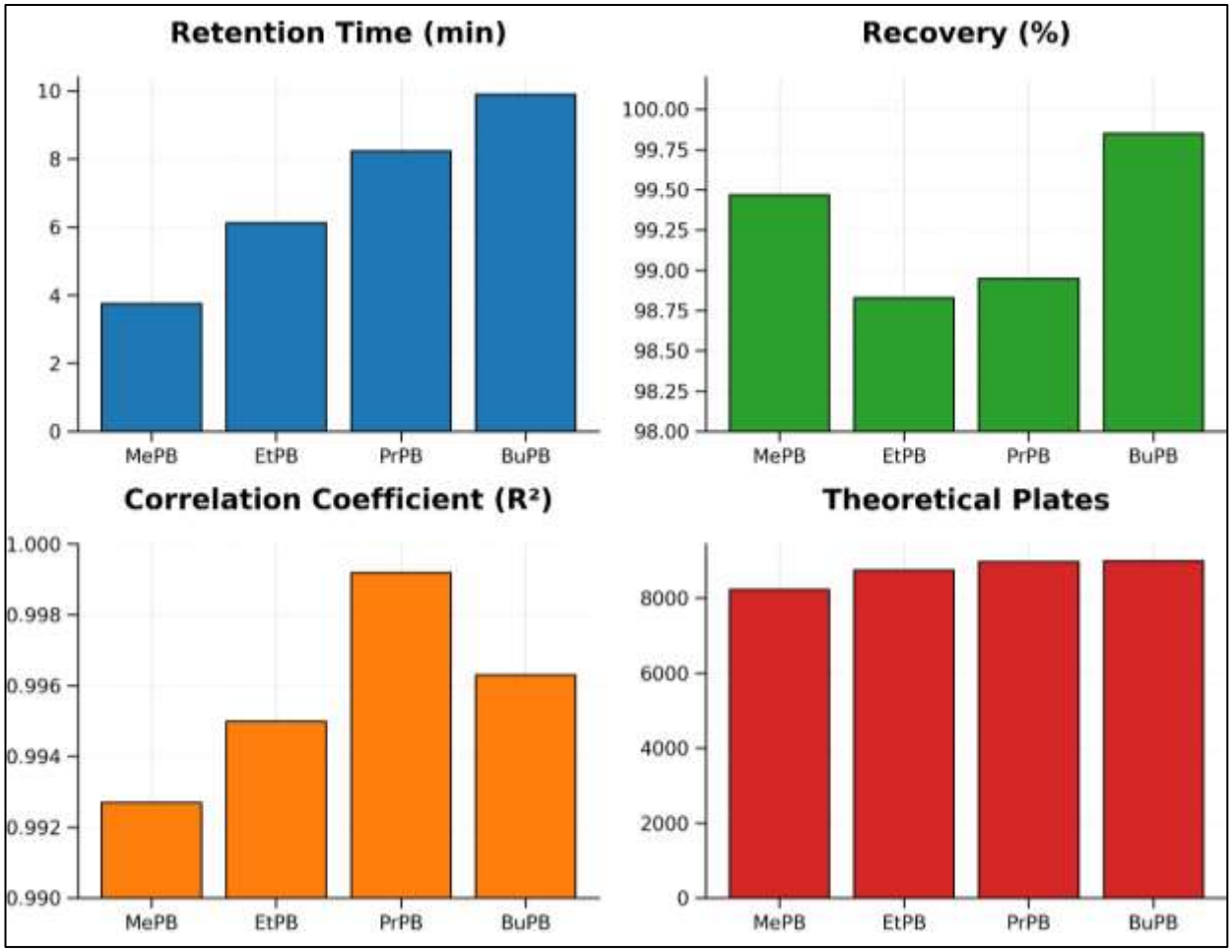


Figure 6: Analytical performance characteristics of selected parabens illustrates the retention time, recovery percentage, correlation coefficient (R^2), and theoretical plate count of the investigated analytes

Correlation Between HPLC and Molecular Docking Analysis:

The estrogenic activity of the PBs is influenced not only by their presence in biological fluids but also depends on their ability to bind to ERα, a member of the estrogen receptor family. Thus, an integrated assessment based on chromatographic analysis and molecular docking studies was executed to gain a better understanding of the comparative estrogenic activity of selected four PBs. HPLC method exhibited acceptable chromatographic performance for all parabens studied, achieving >98% and correlation coefficient (R^2) > 0.99, indicating that the method was reliable and suitable for quantitative determination. The retention times were found to be increasing in order of addition from MePB (3.75 min) to BuPB (9.91 min) which corresponds to the increased hydrophobicity of the longer side chains. Molecular docking analysis showed that the investigated parabens exhibited the binding-affinity to the ERα binding domain that were distinct[13,15,19]. The consistently highest predicted binding affinity of -5.5 kcal/mol was obtained for PrPB and a binding affinity of -5.2 kcal/mol was found for BuPB and a binding affinity of -5.2 kcal/mol was obtained for EtPB among the molecules studied. In contrast, MePB showed the weakest interaction with a binding affinity of -3.7 kcal/mol. The docking energies calculated for the PBs are more negative, indicating more thermodynamic stability of their respective receptor-ligand complexes. The observed trend suggests that the extension of the alkyl side chain increases the hydrophobic interaction of PBs with the ERα. The longer-chain PBs can form stronger contacts to the hydrophobic and more stabilizing interactions in the receptor cavity, providing better binding affinity. BuPB is slightly less hydrophobic than PrPB, so the affinity for the receptor is not just dependent on hydrophobicity but also on how well the ligand fits the receptor's binding pocket[22,17,20].

Combining the chromatographic results with the computational results, PrPB had the greatest predicted estrogen receptor-binding potential though it did not have the longest alkyl chain. The result of this study demonstrates molecular orientation and receptor compatibility as important parameters in the binding of the ligands. In addition, the highly analytical recoveries for all the PBs studied suggest that the results for exposure-related parameters can be reliably combined with computational predictions for comparative assessment of endocrine risk. The overall collective HPLC and molecular docking results indicate that all the studied PBs have the potential to bind to ERα, but there are

variations in terms of the magnitude of those interactions. Based on comparative assessment of plasma exposure and ERα-binding affinity the selected PBs are followed in the order[15,8,5]:

PrPB > EtPB > BuPB > MePB … (2)

The results indicate that the side chain of the alkyl ester may undergo structural changes that affect the receptor recognition and binding properties. Therefore, PBs with more potent ERα-binding properties could have a higher risk of contributing to ER-mediated biological effects and should be investigated further toxicological and epidemiologically[17,18].

Analyte	Retention Time (min)	Recovery %	Binding Affinity (kcal/mol)	Binding Rank
MePB	3.75	99.47	-3.4	4
EtPB	6.12	98.83	-5.2	2
PrPB	8.24	98.95	-5.5	1
BuPB	9.91	99.85	-5.1	3

Table 10: Comparative assessment of plasma exposure and ERα-binding affinity of selected PBs

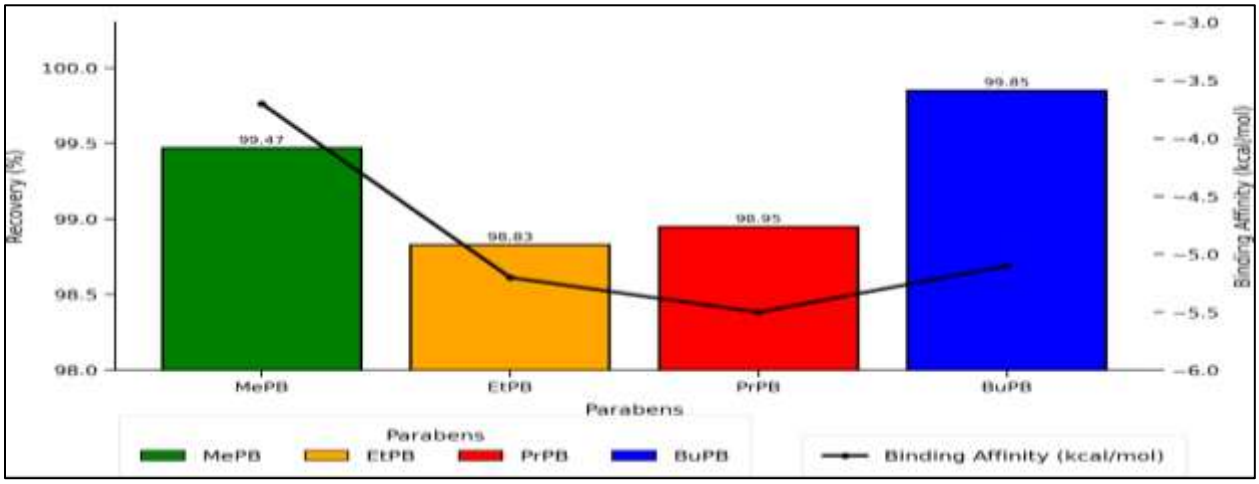


Figure 7: Correlation between HPLC analytical performance and molecular docking-derived ERα-binding affinity of MePB, EtPB, PrPB, and BuPB.

Statistical Interpretation

Analytical performance of the parabens investigated were found to have low inter-compound variances, which means that the chromatographic system was reproducible. In the analysis of plasma, recovery values exceeding 98% with correlation coefficients close to 1($R^2 > 0.99$) are an indication of robustness, accuracy, and reliability of the validated HPLC method for plasma quantification. The overall analytical performance meets the commonly accepted bioanalytical validation criteria and is suitable for comparative exposure-potency assessment of the exposure data generated.

CONCLUSION

Considering the critical unfilled niche in Pb risk assessment, the present study combined HPLC-based analytical evaluation and molecular docking ERα analysis. While many studies have documented their detection in biological matrices, few studies have integrated exposure-related analytical information with receptor-binding studies in a single framework to assess their estrogenic potential. The current study was therefore conducted to link the analytical confirmation of common PBs with predicted ERα molecular interactions, which would give a more comprehensive view of their possible endocrine-disrupting activity. All investigated PBs interacted with the ERα ligand-binding domain as shown in molecular docking analysis. PrPB had the highest binding affinity of (-5.5 kcal/mol), followed by EtPB (-5.2 kcal/mol), BuPB (-5.1 kcal/mol), and MePB (-3.7 kcal/mol) among the selected compounds[4,5]. The interaction analysis showed hydrogen bonding and hydrophobic interactions were also important for receptor-ligand complexes. The results indicate that structural differences in the alkyl side chain significantly affect receptor recognition and that receptor recognition may differ for paraben analogues. The analytical part of the study showed satisfactory chromatographic performance with excellent recoveries, acceptable peak symmetry, good linearity, and high chromatographic efficiency for MePB, EtPB, PrPB, and BuPB. The validated HPLC method is therefore a reliable tool for future biomonitoring studies on plasma samples and PBs exposure assessment[7,9,3].

One of the major contributions of this effort is the development of an exposure/potency assessment strategy where the chromatographic detection and receptor binding predictions are evaluated together. The integrated approach is more informative than either analytical quantification or molecular docking alone and could better inform the interpretation of the endocrine-disrupting potential of widely used preservatives. The results showed that the receptor-binding affinity is not only related to molecular size but also depends on structural compatibility of the ligand within the receptor-binding cavity[11,15,19]. However, a few limitations should be mentioned, the molecular docking results do not measure biological activity in physiology but are computational predictions. Moreover, this current study is limited to ERα interactions only and has not examined receptor activation, modulation of gene expression or downstream signaling pathways. Hence, the predicted binding affinity is simply an indicator of potential receptor interaction and should not be seen as an absolute reflection of the estrogenic potency[21,22].

This study could be extended in the future by analyzing the quantitative plasma biomonitoring data from larger population and by confirming the computed results by experimental models in vitro and in vivo. Further receptor activation assay, molecular dynamics simulation, binding free energy (BF) calculation and multi-receptor endocrine screening experiments would further elucidate the mechanism of endocrine disruption caused by PBs. Combining exposure assessment, computational toxicology, and biological validation will ultimately help to better assess the potential health risk of chronic PB exposure. In conclusion, this study shows that HPLC based analytical assessment combined with molecular docking analysis was a useful and practical approach for studying the endocrine disrupting potential of PBs. The integrated methodology can be expanded to other merging contaminants (ECs) and other endocrine-disrupting chemicals (EDCs) for future toxicological risk assessment and regulatory decision-making processes[7,9,10,16].

REFERENCES

1. Golden R, Gandy J, Vollmer G. A review of the endocrine activity of parabens and implications for potential risks to human health. Crit Rev Toxicol. 2005 Jun;35(5):435-58. doi: 10.1080/10408440490920104. https://pubmed.ncbi.nlm.nih.gov/16097138/

2. Mitra, P., Chatterjee, S., Paul, N. et al. An Overview of Endocrine Disrupting Chemical Paraben and Search for An Alternative – A Review. Proc Zool Soc **74**, 479–493 (2021). https://doi.org/10.1007/s12595-021-00418-x

3. Liang J, Liu QS, Ren Z, et al. Studying paraben-induced estrogen receptor- and steroid hormone-related endocrine disruption effects via multi-level approaches. The Science of the Total Environment. 2023 Apr;869:161793. DOI: 10.1016/j.scitotenv.2023.161793. PMID: 36702264. https://doi.org/10.1016/j.scitotenv.2023.161793

4. Kim JY, Park Y, Lee SH, Park EJ, Lee HJ. Comparative study on estrogen receptor alpha dimerization and transcriptional activity of parabens. Toxicol Res. 2023 Oct 5;40(1):153-161. doi: 10.1007/s43188-023-00212-1. https://pubmed.ncbi.nlm.nih.gov/38223674/

5. Karpuzoglu E, Holladay SD, Gogal RM Jr. Parabens: potential impact of low-affinity estrogen receptor binding chemicals on human health. J Toxicol Environ Health B Crit Rev. 2013;16(5):321-35. doi: 10.1080/10937404.2013.809252. https://pubmed.ncbi.nlm.nih.gov/23909435/

6. Boberg J, Taxvig C, Christiansen S, Hass U. Possible endocrine disrupting effects of parabens and their metabolites. Reprod Toxicol. 2010 Sep;30(2):301-12. doi: 10.1016/j.reprotox.2010.03.011. https://pubmed.ncbi.nlm.nih.gov/20381602/

7. Bergquist BL, Jefferson KG, Kintz HN, Barber AE, Yeagley AA. Disconnecting the Estrogen Receptor Binding Properties and Antimicrobial Properties of Parabens through 3,5-Substitution. ACS Med Chem Lett. 2017 Dec 15;9(1):51-55. doi: 10.1021/acsmedchemlett.7b00431. https://pmc.ncbi.nlm.nih.gov/articles/PMC5767889/

8. Hu P, Chen X, Whitener RJ, Boder ET, Jones JO, Porollo A, Chen J, Zhao L. Effects of parabens on adipocyte differentiation. Toxicol Sci. 2013 Jan;131(1):56-70. doi: 10.1093/toxsci/kfs262. https://pubmed.ncbi.nlm.nih.gov/22956630/

9. Kang HS, Kyung MS, Ko A, et al. Urinary concentrations of parabens and their association with demographic factors: A population-based cross-sectional study. Environmental Research. 2016 Apr;146:245-251. DOI: 10.1016/j.envres.2015.12.032. https://doi.org/10.1016/j.envres.2015.12.032

10. Özcan S, Levent S, Can NÖ, Kozanli M. A Novel HPLC Method for Simultaneous Determination of Methyl, Ethyl, n-propyl, Isopropyl, n-butyl, Isobutyl and Benzyl Paraben in Pharmaceuticals and Cosmetics. Comb Chem High Throughput Screen. 2021;24(3):352-365. doi: 10.2174/1386207323999200728121657. https://pubmed.ncbi.nlm.nih.gov/32723231/

11. Yang X, Liu Z, Sun F, You T, Feng H, Yao J, Liao Y, Liang W, Chen C, Qiu J. Associations of phenols, parabens, and phthalate compounds related to personal care products with osteoarthritis: evidence from population-based and network toxicology studies. Environ Int. 2025 Oct;204:109796. doi: 10.1016/j.envint.2025.109796. https://pubmed.ncbi.nlm.nih.gov/40986981/

12. Ding K, Kong X, Wang J, Lu L, Zhou W, Zhan T, Zhang C, Zhuang S. Side Chains of Parabens Modulate Antiandrogenic Activity: In Vitro and Molecular Docking Studies. Environ Sci Technol. 2017 Jun 6;51(11):6452-6460. doi: 10.1021/acs.est.7b00951.
https://pubmed.ncbi.nlm.nih.gov/28466639/
13. Mostafavi ES, Asoodeh A, Chamani J. New insights on the binding of butyl-paraben to trypsin: experimental and computational approaches. J Biomol Struct Dyn. 2023 Dec;41(20):10302-10314.
doi: 10.1080/07391102.2022.2154268. https://pubmed.ncbi.nlm.nih.gov/36510660/
14. Nasrollahi, Sara. (2023). Determination of parabens in different samples using green analytical chemistry approaches since 2015. TrAC Trends in Analytical Chemistry. 166. 10.1016/j.trac.2023.117163. https://www.researchgate.net/publication/372078993_Determination_of_parabens_in_different_samples_using_green_analytical_chemistry_approaches_since_2015
15. Parla, A., Zormpa, E., Paloumpis, N., Kabir, A., Furton, K. G., Roje, Ž., Samanidou, V., Vinković Vrček, I., & Panderi, I. (2021). Determination of Intact Parabens in the Human Plasma of Cancer and Non-Cancer Patients Using a Validated Fabric Phase Sorptive Extraction Reversed-Phase Liquid Chromatography Method with UV Detection. Molecules, 26(6), 1526. https://doi.org/10.3390/molecules26061526
16. BenchChem. [optimization of mobile phase for better separation of parabens in HPLC]. BenchChem, [2026]. [Online PDF]. Available at: [https://www.benchchem.com/product/b1676471/docs#optimization-of-mobile-phase-for better-separation-of-parabens-in-hplc]
17. Nandkumar Govindrao Patil, Vishwambhar Shinde, Anil H. Gore, Mrudula Wadekar, Ajinkya Mandake. Optimizing Chromatographic Techniques for Comprehensive Paraben Analysis to Enhance Safety in Consumer Products. Journal of Polymer & Composites. 2025; 13(06):228-243. Available from:
https://journals.stmjournals.com/jopc/article=2025/view=226034
18. Nandkumar Patil, Sourabh Mohite and Vishwambhar Shinde. A systematic review of HPLC-based analytical approaches for decoding parabens. International Journal of Chemical Studies. 2026; 14(5): 96-107.
https://www.doi.org/10.22271/chemi.2026.v14.i5a.12707
19. Sun L, Yu T, Guo J, Zhang Z, Hu Y, Xiao X, Sun Y, Xiao H, Li J, Zhu D, Sai L, Li J. The estrogenicity of methylparaben and ethylparaben at doses close to the acceptable daily intake in immature Sprague-Dawley rats. Sci Rep. 2016 Apr 28;6:25173. doi: 10.1038/srep25173. https://pubmed.ncbi.nlm.nih.gov/27121550/
20. Rastogi SC, Schouten A, de Kruijf N, Weijland JW. Contents of methyl-, ethyl-, propyl-, butyl- and benzylparaben in cosmetic products. Contact Dermatitis. 1995 Jan;32(1):28-30. doi: 10.1111/j.1600-0536.1995.tb00836.x. https://pubmed.ncbi.nlm.nih.gov/7720367/
21. Ocaña-González JA, Villar-Navarro M, Ramos-Payán M, Fernández-Torres R, Bello-López MA. New developments in the extraction and determination of parabens in cosmetics and environmental samples. A review. Anal Chim Acta. 2015 Feb 9;858:1-15. doi: 10.1016/j.aca.2014.07.002. https://pubmed.ncbi.nlm.nih.gov/25597796/
22. Piao C, Chen L, Wang Y. A review of the extraction and chromatographic determination methods for the analysis of parabens. J Chromatogr B Analyt Technol Biomed Life Sci. 2014 Oct 15;969:139-48.
doi: 10.1016/j.jchromb.2014.08.015. https://pubmed.ncbi.nlm.nih.gov/25173495/