IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Application Of Hplc-Pda Technique And Molecular Docking For Evaluation Of Leaching, Interaction And Toxicity Of Tri-Octyl-Trimellitate In Iv Infusion Set

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Abstract **:** Tri-Octyl-Trimellitate (TOTM) is a plasticizer belongs to trimellitate family, has been widely used in the manufacturing of IV infusion sets. A novel HPLC-PDA method for the simultaneous estimation of TOTM and labetalol directly in the PVC IV sets used to assess the leachability and evaluating whether any interaction is possible with labetalol. The TOTM toxicity was predicted by performing molecular docking study.

Limited data regarding leachability of TOTM from IV infusion set and its interaction with labetalol which is infused remains the matter of concern to this research work for clinical safety. TOTM and labetalol mixture was separated using stationary phase C_{18} column and mobile phase Methanol: Acetonitrile 80:20 v/v under isocratic elution mode with a 1 ml/min flow. They were detected at 237 nm. The R_t for labetalol and TOTM was 3.9 ± 0.1 min and 15.4 ± 0.3 min respectively. The developed method was validated according to ICH-Q2 (R1) guidelines. The linearity of TOTM was found to be within the range of 1-6mcg/ml. The LOD and LOQ for TOTM was 0.2mcg/ml and 0.6mcg/ml respectively. Interaday and intraday precision values were within the limit of < 2% RSD. The developed HPLC-PDA method was applied for the tube study. Leaching study conducted in tube reveals that tube doesn't contain TOTM, whereas, Di-Butyl-Phthalate (DBP) was leached out during study period. Interaction study discloses that the concentration of labetalol was not affected significantly during the interaction study period, when it was present with TOTM. Molecular docking study divulge that TOTM and its metabolites have the **potential to cause toxic effects in humans. Except for THR** and **SHBG** receptors, metabolites of TOTM such as DOTM and MOTM have shown **higher binding affinity for PPAR-γ (A-chain), GCR,** and **AR, GCR,** respectively.

Key words **: Plasticizer, interaction, HPLC , Docking**

I. INTRODUCTION

Medical devices have emerged as a key aspect in the healthcare system of any developing healthcare perceptive nation. According to World Health Organization a medical device can be any instrument, apparatus, implement, machine, appliance, implant and reagent for *in vivo* (or) *in vitro* use, software material (or) other similar (or) related article intended by the manufacturer to be used alone (or) in combination for a medical purpose [1]. PVC is used for a range of Medical devices, such as oxygen masks, surgical gloves, IV bags, infusion sets, catheters, feeding tubes, respiratory tubes, drug delivery devices, etc. However, virgin PVC is a rigid and unstable material. The **flexibility of PVC can be obtained with the help of plasticizers.** Plasticizers provide durability, elasticity and flexibility to polymeric products. The content **of plasticizer can be up to 40–50%** of the final weight of PVC [2].

Leachability refers to the process by which substances are dissolved or released from material in which it is present. In medical devices, leaching occurs under normal storage conditions, or it may occur when temperature gets altered and the duration of use is longer, when chemicals from delivery devices or packaging migrate into and potentially impact the safety or efficacy of a drug substance. Leachable compounds may cause toxicity to those who are exposed to them. Phthalate Esters, or PEs, have dominated the plasticizer market since the 1970s. The most often utilized of these plasticizers is Di-Ethyl-Hexyl-Phthalate (DEHP). Due to the chemical structure of PVC, DEHP is not chemically bound to the polymer and it is known to be released with time and use of medical devices. Because of toxic effects of DEHP plasticizer and restriction on the usage in Medical devices by various countries force the Medical device manufacturers to find out alternative plasticizers for DEHP [3].

Tri-Octyl-Trimellitate (TOTM) is one of the alternative plasticizers (Fig-1) from the trimellitate family among the available alternatives to DEHP plasticizer. In a repeated dose toxicity on animals, TOTM plasticizer shows peroxisome proliferation similar in pattern to those produced by DEHP and there is no proper evidence on reproductive toxicity, chronic toxicity, carcinogenicity. According to Globally Harmonized System (GHS) TOTM is classified as H361 (72.29%) which means it is suspected of damaging fertility or reproductive toxicity. According to Comparative Toxicogenomics Database (CTD), TOTM have top gene interaction with ESR1 **(Estrogen Receptor-1**), ESR2 **(Estrogen Receptor-2),** AR (Androgen Receptor), NR112 (gene product belongs to the nuclear receptor superfamily), THRB (Thyroid hormone receptor beta) and it may have linked that TOTM may have a possibility of causing more than 80 diseases [4, 5].

A large number of plastic medical devices are removed from the market due to the interaction between drugs and medical devices. Researchers found that lipophilic characteristics of drugs and the duration of medication administered via plastic medical devices could be one of the reasons for drugs and medical devices interaction. The study of neonatal exposure to phthalate and alternative plasticizers due to the parenteral nutrition [6], leaching and exposure of phthalates from medical devices [7], determination of TOTM from PVC tube [8] and exposure of hospitalized pregnant women to plasticizers [9] underlines the possibility of leachability of plasticizers from medical devices when it used along with lipophilic substances.

The lipophilic nature of TOTM is a matter of concern in this context. Similar to DEHP plasticizer, possibility of TOTM leaching occur when it is used with any lipophilic substances or drugs. Still, there is scant data on the exposure limit of TOTM plasticizer in high-risk populations such as pregnant women and unborn children who are in intensive care units. In India, labetalol (Fig-2) is a drug having lipophilic nature that is widely used in ICUs to treat preeclampsia. Labetalol is given until the blood pressure returns to normal, which means it can be administered to patients for a long duration. So there is a possibility of leaching of TOTM plasticizer due to the duration of administration and the lipophilic nature of labetalol.

This study aims to describe a High Performance Liquid Chromatography-Photo Diode Array (HPLC-PDA) method for the simultaneous estimation of TOTM and labetalol for the evaluation of their interaction and lechability in IV tubes. Molecular docking study was carried out to evaluate the toxicity of TOTM.

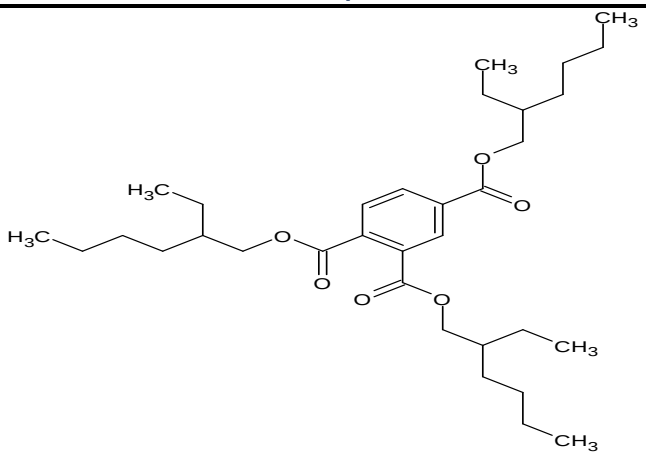


Fig-1 Structure of TOTM

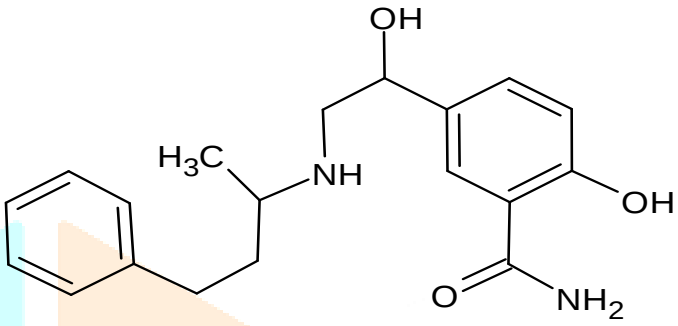


Fig-2 Structure of labetalol

II. MATERIALS AND METHODS

2.1 Chemicals and Solvents

HPLC grade water, HPLC grade Acetonitrile, HPLC grade Methanol, HPLC grade Isopropyl alcohol, Distilled water. All the solvents were supplied by Sigma-Aldrich chemicals Pvt. Ltd, Maharashtra, India, Qualigens Fine Chemicals Pvt.Ltd., Mumbai, India, CDH Pvt. Ltd, New Delhi, India, SDFCL Pvt,Ltd, LOBA chemie Pvt.Ltd, Mumbai, India, HiMedia laboratories, Thane (W), India. Tri-Octyl-Trimellitate (TOTM) was obtained from Sigma-Aldrich chemicals Pvt. Ltd, Maharashtra, India. Lablol (Labetalol injection I.P) was obtained from Sri Ramakrishna hospital pharmacy, Coimbatore, Tamilnadu, India.

2.2 Materials

RMS vented infusion set, manufactured by Romsons group private limited, Intrafix safeset, manufactured by B|Braun was obtained from Sri Ramakrishna hospital pharmacy, Coimbatore, Tamilnadu, India.

2.3 Preparation of standard solutions

2.3.1 Plasticizer

A quantity of about 0.1 ml (100 mg/ml) **of TOTM** was pipetted out and diluted in 10 ml of methanol to give **10 mg/ml.** Further dilution to a 100 µg/ml solution was prepared by diluting 0.1 ml of 10 mg/ml in a 10 ml volumetric flask and make up the volume with methanol. Then the required dilutions were made using methanol.

2.3.2 Labetalol

A quantity of about 0.2 ml **of labetalol** parenteral formulation was pipetted out of a 4 ml vial containing 5 mg/ml and diluted in 10 ml of methanol to give **100** µg/ml**.** Further dilutions were carried out using methanol.

2.3.3 Preparation of calibration and validation standard

During the three days of validation process the calibration and validation standards were prepared for each day.

2.4 Instrumentation

The analysis was carried out using Shimadzu Prominence UFLC (Shimadzu), Kyoto, Japan equipped with LC-20AD Pump, SPD M20A Diode array Detector, DGU-20A3, Degasser, SIL- 20AC Autosampler, CTO-10AS vp Column oven with RP-C_{18} column, shim pack solar -4..6 × 250mm HSS (Shimadzu cooperation, Kyoto, Japan), LC solution chromatographic software, version-3.41.324), was used for the separation of plasticizer and labetalol. For the selection wavelength, Jasco V-730 UV spectrophotometer (Jasco corporation, Tokyo, Japan) was used. For the preparation of mobile phase Leelasonic ultrasonic cleaner (Leelasonic Mumbai, India) and Vacuum pump – Gelman sciences, (Pall Pharmalab filtration, Pvt.Ltd, Mumbai, India) were used. The mobile phase was methanol: acetonitrile (80:20% v/v). In an optimized condition the mobile phase was delivered under isocratic elution at a flow rate of 1 ml/min.

2.5 Method validation

The developed method was validated using the TOTM standard. The ICH-Q2 (R2) guidelines were followed for the validation [10]. The specificity was assessed by a blank injection. The linearity of the response of the developed method was obtained by injecting standard TOTM at a concentration range of 1-6 µg/ml. Linear regression was performed by plotting the peak area against the concentration. Interday and intraday precision were evaluated using three determinations of two TOTM standard concentrations. The repeatability was evaluated by six determinations of a single TOTM standard. The standard deviation of the y-intercept of the regression line was used to determine the LOD and LOQ.

2.6 Evaluation of labetalol interaction with TOTM

Samples were prepared in a volumetric flask and kept at room temperature. Both individual samples and mixture of labetalol 25 µg/ml and TOTM 500 µg/ml were withdrawn every hour and analysed. The study period for the interaction study was fixed for 4 hrs.

2.7 Evaluation of the leachability of TOTM in IV tubes

Selected IV sets were cut into 35 cm lengths for the leachability study. The IV tubes were filled with different matrices such as normal saline with labetalol 100 µg/ml and methanol only. The study timing was framed as 6 hours. The IV tubes were kept at room temperature and for every hour sample was taken for analysis.

2.8 Tube extraction

Based on the literature [11] chloroform 100 ml was used as an extraction solvent to confirm whether the TOTM plasticizer is used in IV tubes. The tubes were cut into small pieces and refluxed with chloroform. After the completion of the extraction, chloroform was evaporated and the residues were reconstituted with methanol prior to the injection into HPLC.

2.9 In silico study

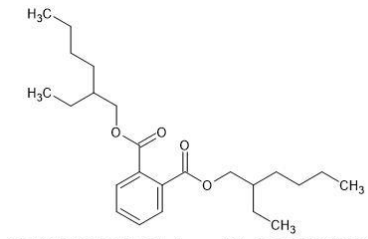
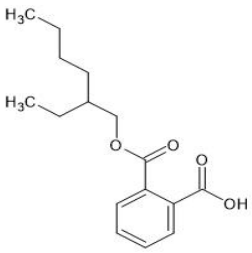
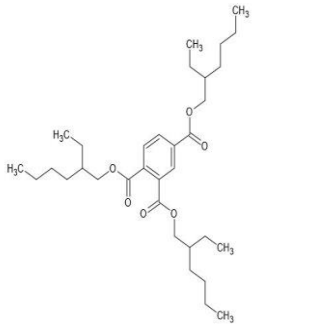
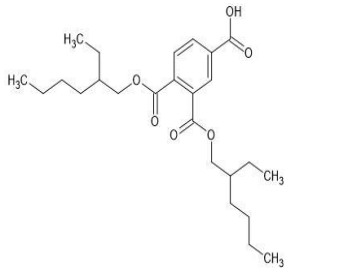
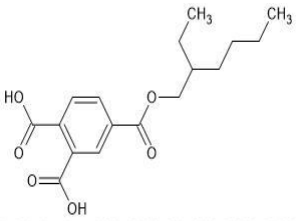
2.9.1 Protein preparation

The database protein data bank (PDB: https://www.rcsb.org) was explored to obtain the crystal structure complex of human AR (PDB ID: 2AM9) co-complexed with natural ligand testosterone at 1.64Å resolution [12], human SHBG (PDB ID: 1D2S) at 1.55 Å resolution co-complexed with dihydrotestosterone [13], human TR-α (PDB ID: 2H79) in complex with the native ligand T3 at a very at 1.87 Å resolution [14], PPAR- α (PDB ID: 1I7G) & PPAR-γ (PDB ID: 1I7I) ligand binding domain with tesaglitazar at resolution of 2.20Å [15] and human GR (PDB ID: 4UDC) with dexamethasone at resolution of 250 Å [16]. The crystal structure was prepared for docking analysis using Biovia Discovery studio visualizer 2021. Briefly the crystal structure of the above receptors was prepared by removing the water molecules, ligands present in the structure, add H-atoms. Then the prepared targets are converted to Autodock file AutodockVina 1.5.7 (Autodock tools). The natural ligand and ligands present in the crystal structure selected for the generation of grid boxes.

2.9.2 Ligand preparation

The 2-Dimensional structure of DEHP (Di-Ethyl-Hexyl-Phthalate), MEHP (Mono-Ethyl-Hexyl-Phthalate), TOTM (Tri-Octyl-Trimellitate), DOTM (Di-Octyl-Trimellitate), MOTM (Mono-Octyl-Trimellitate), (Table-1) were drawn using ChemSketch and this was followed by conversion of 2-Dimensional structure into 3-Dimensional structure using Avogadro software. Then the prepared ligands are converted to Autodock file by AutodockVina 1.5.7.

Table -1 Two dimensional representation of plasticizers and its metabolites.

DEHP  CCC(CCCC)COC(=O)c1ccccc1C(=O)OCC(CC)CCCC	MEHP  O=C(O)c1ccccc1C(=O)OCC(CC)CCCC	TOTM  CCC(CCCC)COC(=O)c1cc(C(=O)OCC(CC)CCCC)c(cc1)C(=O)OCC(CC)CCCC
DOTM  O=C(O)c1cc(C(=O)OCC(CC)CCCC)c(cc1)C(=O)OCC(CC)CCCC	MOTM  OC(=O)c1cc(ccc1C(=O)O)C(=O)OCC(CC)CCCC	

2.9.3 Molecular docking analysis

A computational ligand-target docking approach was used to analyse structural complexes of the selected targets with selected plasticizers and its metabolites. Taking DEHP and its metabolite MEHP as standard. The molecular docking studies were carried out using Autodock tools (ADT), which is a free graphic user interface (GUI) for the AutodockVina 1.5.7 program. AutodockVina with standard protocol from https://autodock.scripps.edu/ was used to dock the compounds against the active site of proteins. The grid box was constructed using natural ligands and ligands x, y, z directions in the target.

III. Result and discussion

3.1 Method development

A HPLC-PDA method was developed for the simultaneous estimation of TOTM and labetalol with a targeting resolution of >2. The Fig- 3 represents the resulting chromatogram from the optimized HPLC conditions. The retention times for labetalol and TOTM were found to be 3.9 ± 0.1 min and 15.4 ± 0.3 min at 237 nm, respectively.

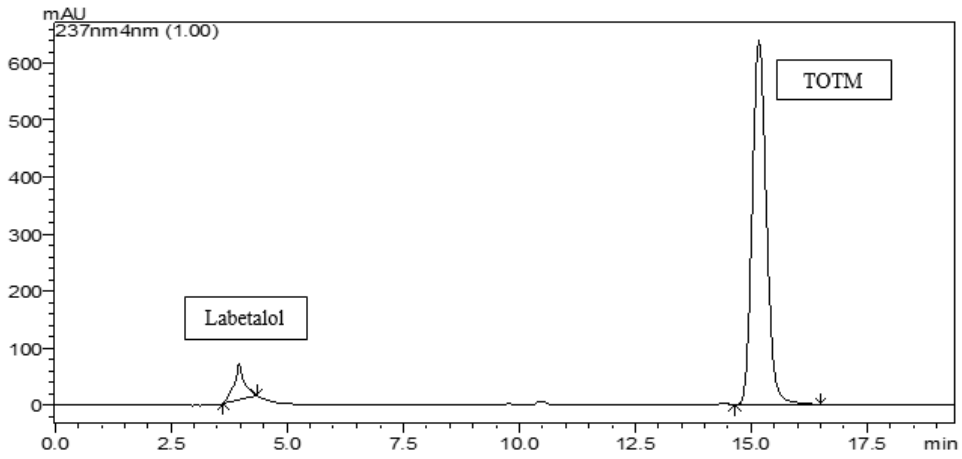


Fig-3 Chromatogram obtained for labetalol and TOTM in Methanol: Acetonitrile-(80:20 v/v)

3.2 Method validation

There is no additional peak or interference appeared in the blank injection, which was injected for the assessment of specificity. The peak of labetalol and TOTM was spectrally pure and the peak purity for each was found to be 0.999. The correlation coefficient value was found to be 0.995 for TOTM. The calibration graph and regression data for a TOTM concentration of 1-6 µg/ml is given in Fig-4. The results of intraday, interday and repeatability were within the limits (RSD <2).

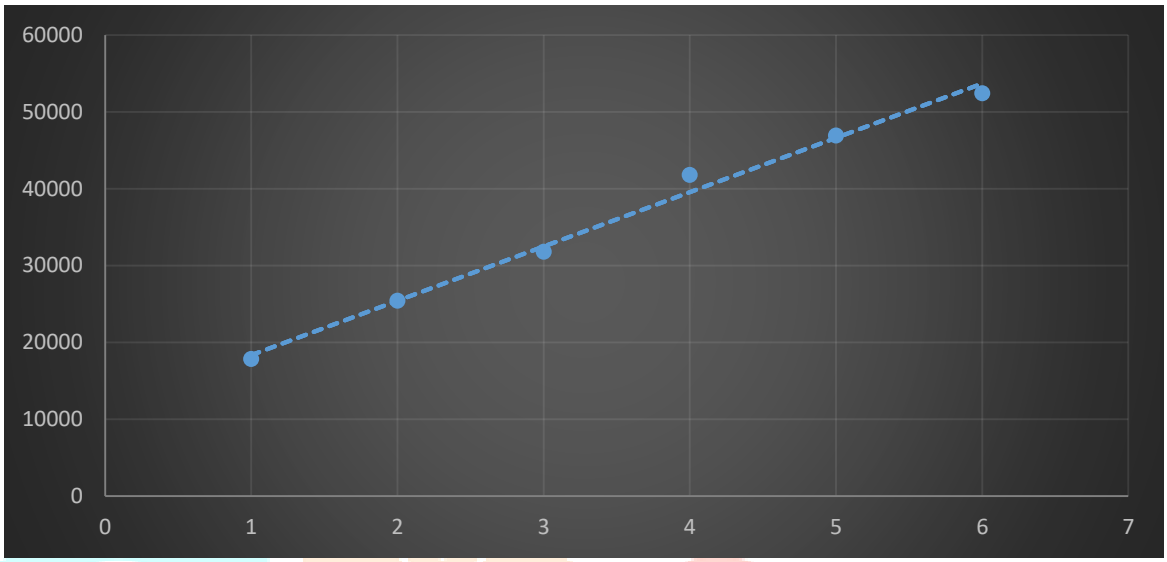


Fig-3 Calibration graph for TOTM (1-6 µg/ml)

3.3 Evaluation of labetalol interaction with TOTM

The peak area obtained for individual samples of labetalol, TOTM at different time intervals was presented in Table-2 and 3. The peak area obtained for labetalol was not altered significantly when it presents along with TOTM. The values were very much comparable with individual standard labetalol peak areas. It indicates that the concentration and efficacy of labetalol may not be affected even when it was present with TOTM. Hence, it was found that there is no interaction between labetalol and TOTM.

Table-2 Peak area obtained for labetalol 25 µg/ml at different time intervals.

Time interval (hr)	Peak area	%RSD
0	22168	0.8
1	22332	
2	22378	
3	22427	
4	22693	

Table-3 Peak area obtained for TOTM 500 µg/ml at different time intervals.

Time interval (hr)	Peak area	%RSD
0	8650554	0.2
1	8650677	
2	8680487	
3	8690648	
4	8640793	

3.4 In silico study

According to published literature [2] on the toxicity assessment of DEHP, secondary metabolites of DEHP, such as MEHP, cause adverse events in humans. So for the binding affinity (kcal/mol) calculation in the in silico study, DEHP and MEHP was taken as standard. In view of the toxic effects of TOTM and its metabolites DOTM and MOTM on various targets, it was done to predict potential adverse effects. There are different metabolites available for TOTM plasticizer among the other metabolites, DOTM and MOTM, selected for the *in silico* study. The objective of this *in silico* study was to characterize the structure-based interactions of TOTM and its metabolites. Binding energy between standards (DEHP and MEHP) and samples (DOTM and MOTM) with selected targets was calculated. The values of binding energy are given in the Table-10. An amino acid interaction study was also done with the help of Biovia Discovery Studio, to identify common amino acid residues shared between the common DEHP and selected plasticizers and their metabolites represented in Fig-4-5. The percentage of residue common with DEHP was calculated and tabulated (Table 4-6).

Binding affinity values and **percentages of amino acid residues common with DEHP** show that the selected plasticizer TOTM and its metabolites have the **potential to cause toxic effects in humans. Except for THR** and **SHBG** receptors, TOTM shows **higher binding affinity than the standards.** Metabolites of TOTM such as DOTM and MOTM show **higher binding affinity for PPAR-γ (A-chain), GCR,** and **AR, GCR,** respectively, than the standard, which indicates metabolites of TOTM have the potential to cause adverse effects in humans.

Table-4 Docking score (kcal/mol) for plasticizers and its metabolites.

PLASTICIZER		AR	THR	SHBG	PPAR-α	PPAR-γ (A)	PPAR-γ (B)	GCR
STANDARD	DEHP	-4.1	-8.5	-8.2	-8.0	-7.6	-8.4	-5.9
	MEHP	-7.2	-7.5	-7.3	-71	-7.9	-7.6	-7.0
SAMPLE	TOTM	-4.4	-4.2	-7.2	-8.4	-7.9	-8.4	-7.7
	DOTM	-3.3	-8.0	-7.5	-7.9	-8.5	-8.3	-6.9
	MOTM	-7.4	-8.2	-7.6	-7.7	-7.8	-7.8	-7.1

Table-11 Number of interacting residues, number and percentage of residues common with DEHP in Androgen receptor.

S.No	Ligand	Number of interacting AR residue	Number of interacting residue common with DEHP
1	TOTM	16	8 (73%)
2	DOTM	14	9 (82%)
3	MOTM	10	3 (27%)
4	DEHP	11	11 (100%)

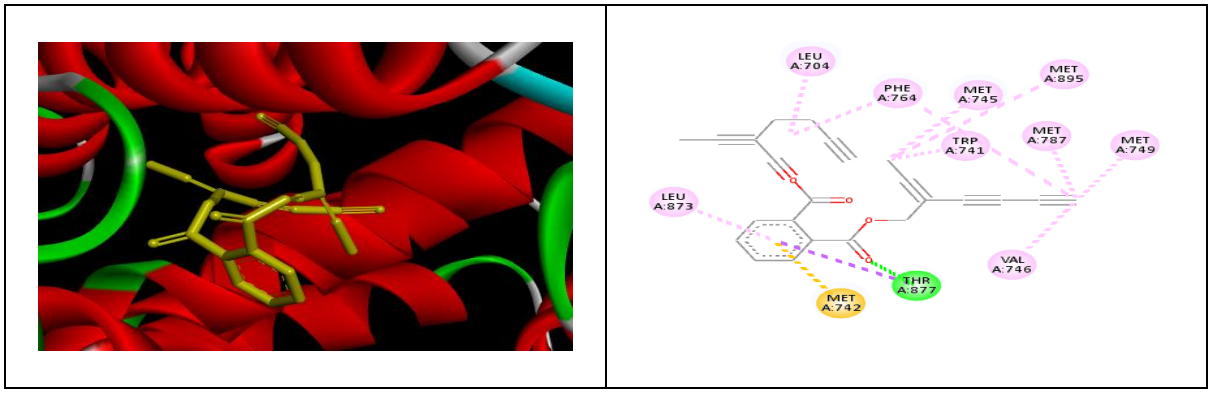


Fig-4 Ribbon diagram representing the docking pose of human Androgen receptor (AR) with DEHP (left panel) Interaction of DEHP with amino acid residues in the binding pocket of AR (right panel)..

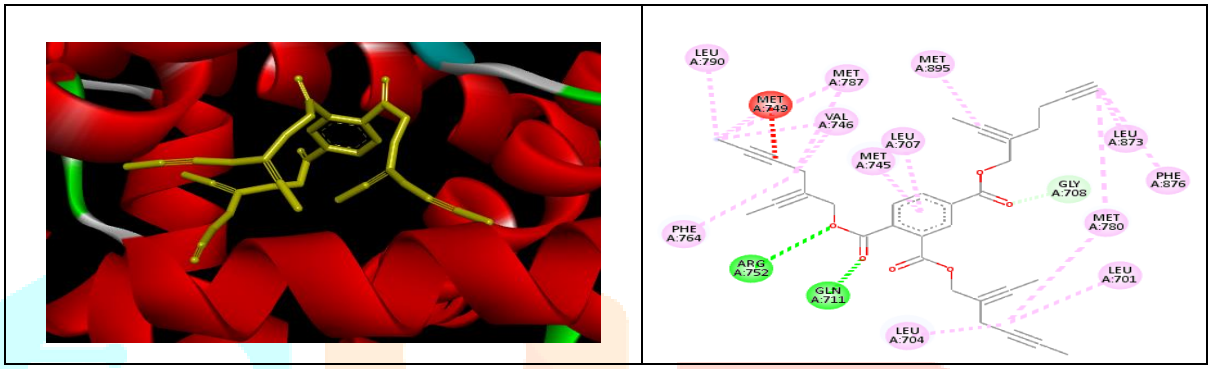


Fig-5 Ribbon diagram representing the docking pose of human Androgen receptor (AR) with TOTM (left panel). Interaction of TOTM with amino acid residues in the binding pocket of AR (right panel).

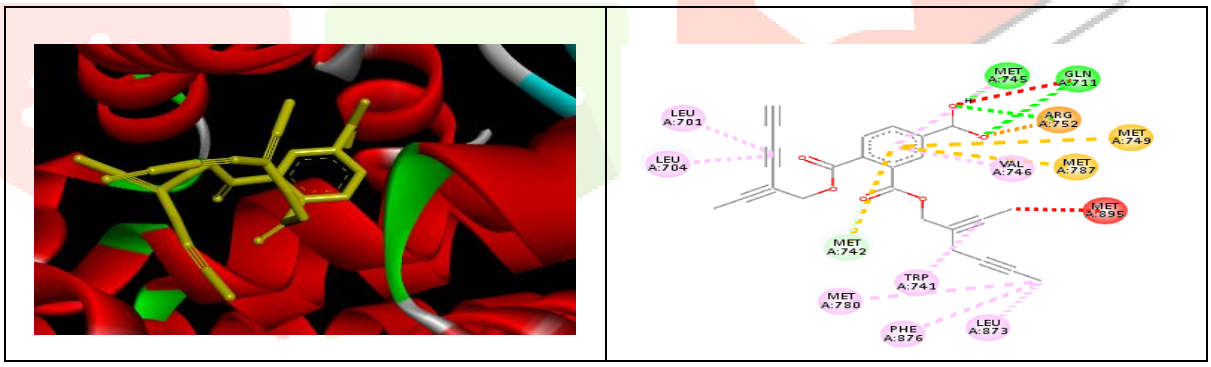


Fig-6 Ribbon diagram representing the docking pose of human Androgen receptor (AR) with DOTM (left panel). Interaction of DOTM with amino acid residues in the binding pocket of AR (right panel).

IV. Conclusion

A HPLC-PDA method was developed and validated for the simultaneous determination of TOTM and labetalol and applied to evaluate the leachability from the PVC IV infusion set. The results of the leachability study and tube extraction unveils that the TOTM plasticizer is not used in the selected IV sets. Evaluation of labetalol interaction with TOTM shows that there is no significant alteration in the concentration of labetalol when it is present with TOTM, so the therapeutic efficacy of labetalol may not be affected. The toxicity prediction of plasticizer by a molecular docking approach with selected targets underlines the potential of TOTM plasticizer to cause toxic effects in humans. The developed method will serve as an important tool for the migration assessment of TOTM plasticizer from medical devices. The results of the interaction and toxicity prediction in this study would contribute immensely to the scientific and clinical research society for the safety of pregnant woman who could possibly treated with labetalol .

Acknowledgements : Authors are thankful to the principal and SNR son's charitable trust, for providing complete facilities required for the work.

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