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Review Article

Tamoxifen: An Investigative Review for Nano Dosage Forms and Hyphenated Techniques

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ABSTRACT
Tamoxifen (TAM), a non-steroidal antiestrogen, has revolutionized female breast cancer treatment. The mechanism involves is to compete with 17β-estradiol (E2) at the receptor site and blocking the promotional role of E2 in breast cancer. Tam is a pioneering medicine because of its widespread usage in breast cancer treatment and chemoprevention, as well as research into novel selective estrogen receptor modulators (SERMs). In the vast majority of patients, TAM is cost-effective, life-saving, and free of significant adverse effects. In this context, this review presents several nanoformulations for selectively delivered lower dosages of TAM to breast tumors. Various TAM-containing nanosystems have been effectively created to deliver TAM to particular molecular targets with decreasing harmful effects. Following that, there is a summary of chromatographic techniques to quantify the drug in human biological samples. The quantification of the drug in different biological matrices is the major importance to assist the control of quality, efficacy, and safety.

Keywords: Tamoxifen, Breast cancer, Chromatographic techniques, Nanotechnology, Targeted therapy.

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INTRODUCTION

Beatson discovered in the late 1800s that advanced breast cancer in premenopausal women was sometimes amenable to oophorectomy. As a result, it was hypothesized that inhibiting estrogen could slow the growth of an estrogen-dependent tumor. Endocrine treatments such as adrenalectomy and hypophysectomy were employed in the treatment of advanced breast cancer in the 20th century, but there was an obvious need for a non-surgical approach to this disease management. The efficacy of early treatments including estrogens and androgens was restricted by toxicity. MER-25 (Ethamoxytriphetol) and Clomiphene, two anti-estrogens, were developed in the 1950s (Figure 1). Walpole and colleagues produced a series of triaryl ethylene compounds in the 1960s that had alkyl replacements for chlorine in the clomiphene molecule, primarily for the treatment of hormone-dependent tumors. Animal studies at the time suggested that clomiphene and related chemicals caused cataracts in mice by accumulating desmosterol, a cholesterol precursor.[1]

ICI 46474 was synthesized and purified, and it was discovered that the trans isomer was largelyanti-estrogenic, whilst the cis-isomer was pure estrogen. It would be the first when a pharmacological agent's isomeric forms were shown to have different pharmacology. Breast cancer is the most prevalent malignancy among women in the Western world. The introduction of Tamoxifen (TAM), an antiestrogen that inhibits estrogen binding to estrogen receptors, reduced the need for these surgical operations. Oophorectomy, hypophysectomy, or adrenalectomy can cause breast cancer to regress since it is estrogen-dependent. TAM was first approved by the Food and Drug Administration (FDA) in 1977 for the treatment of advanced breast cancer in women, and then again a few years later for the adjuvant treatment of early breast cancer.[2]

The overwhelming evidence suggests that estrogen exposure is a significant factor in breast cancer risk. The conversion of estrogen to genotoxic, carcinogenic metabolites and the stimulating of tissue development are two mechanisms of estrogen-induced carcinogenesis in the breast. These processes work together to initiate, promote, and develop carcinogenesis. Understanding the processes by which estrogen causes cancer will lead to the discovery of predictors of breast cancer susceptibility as well as novel targets for preventive and therapeutic intervention.[3] Classification of drugs based on the type of target. Drugs can be directed towards variety of targets.[4]

DRUG PROFILE OF TAMOXIFEN

TAM, a triphenylethylene derivative, is a Selective estrogen receptor modulator (SERM) that has become the therapy of choice for women with hormone-responsive breast cancer (both ER and/or progesterone positive) at all stages. It was first used as an antineoplastic in 1971 and is still the world's most commonly prescribed chemotherapy.[5]

In the early 1970s, the anti-estrogen TAM was introduced, marking a watershed moment in the treatment of breast cancer. TAM is still effective in high-risk pre- and postmenopausal women, not just for early and advanced breast cancer, but also for Ductal carcinoma *in situ* (DCIS) and breast cancer chemoprevention, more than 30 years later. TAM suppresses cancer cell development by competitively inhibiting estrogen receptors. TAM, on the other hand, stops estrogen-receptor-negative breast cancer cells from growing. This suggests that other mechanisms aren't relevant to estrogen receptor mediation. TAM has also been discovered to inhibit protein kinase C without interfering with the enzyme's active site. TAM has been shown to cause liver damage by interfering with mitochondrial activity in many studies.[1,2]

It has been proven to reduce disease recurrence and mortality rates by up to 50% and 30%, respectively. Inter-individual variability in TAM response is substantial, which may be related in part to differences in

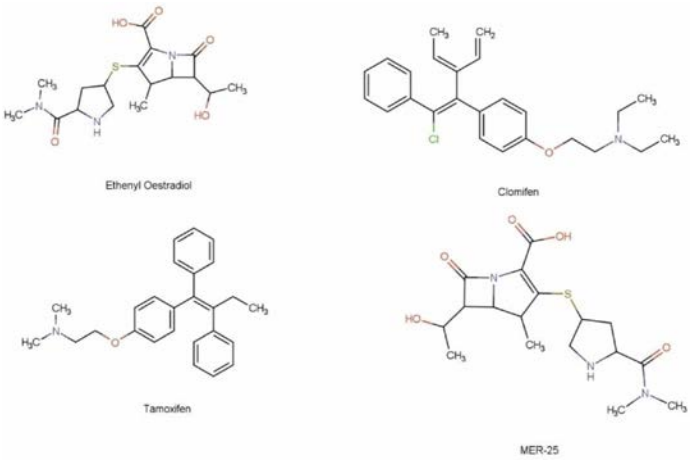

Figure 1: Molecular Structure of Oestrogen (Ethenyl Oestradiol, Clomifen) and Antioestrogen (Tamoxifen, MER-25).

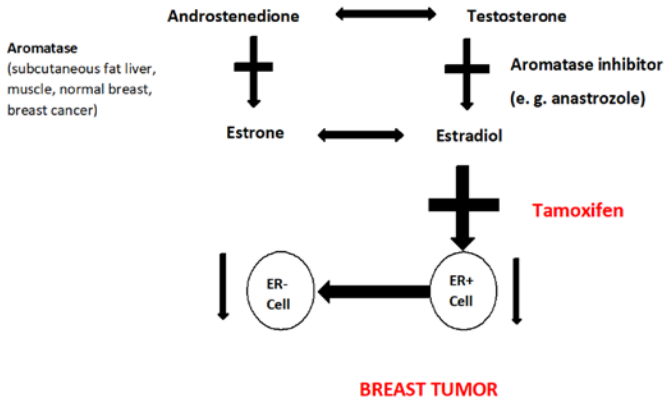

Figure 2: Mechanism of action of Tamoxifen.[7]

the drug's metabolism *in vivo*. The most typical side effect of TAM is hot flashes, which affect up to 80% of women.[6]

Mechanism

TAM has a twofold mode of action; first, it competes with 17-estradiol (E2) at the receptor site-blocking E2's development of breast cancer, secondly, it binds DNS following metabolic activation, initiating carcinogenesis (Figure 2).[7]

Physicochemical Characteristics

TAM is (Z)-2- [4-(1,2-diphenylbut- 1-enyl) phenoxy]-N, N-dimethyle-thanolamine (ICI 46,474). In most countries, the medicine is offered as the monocitrate salt under the brand name 'Nolvadex.' $C_{26}H_{29}NO'C_6H_{807}$ is the chemical formula, and the molecular mass is 563.6.[8] TAM is a white or almost white crystalline powder that is practically insoluble in water (log P of 5.9).[9] In the biopharmaceutics classification system (BCS), TAM is classified as a class II molecule with low solubility and favorable permeability characteristics, indicating that it has a low oral bioavailability.[10]

Pharmacokinetics and Pharmacodynamics

The pharmacokinetics of TAM in humans is still unknown. Maximum plasma concentrations of the parent medication and the dimethyl metabolite are attained within several hours after a single oral dose. Chronic administration, on the other hand, takes 3 to 4 weeks to reach steady-state concentrations. TAM is highly plasma protein bound at therapeutic concentrations. It is extensively metabolized in the liver; the main metabolic route is N-demethylation, followed by sidechain deamination to the primary alcohol. The percentage of the dosage eliminated in urine as an unaltered medication is minimal. The major route of elimination is biliary excretion; elimination appears to be biphasic, with an early phase lasting 7 to 14 hr and a terminal phase lasting about 7 days.

TAM is a trans-isomer of a triphenylethylene derivative that is taken orally in the form of citrate salt. TAM has a wide range of pharmacological qualities, acting as an estrogen antagonist, partial or full agonist, or both, depending on the target tissue and species. In premenopausal patients, plasma estrogen levels are higher. TAM has a mild estrogen-like effect in postmenopausal individuals, resulting in lower levels of circulating gonadotrophin and prolactin and higher levels of serum gestation zone protein and sex estrogen globulin.[11] TAM is official in IP 2010,[12] BP 2013,[13] USP 27 2004.[14]

SUMMARY OF HYPHENATED TECHNIQUES OF TAMOXIFEN

Because all phases of the process need accurate and dependable analytical techniques, pharmaceutical analysis is one of the most essential components of drug development. The hyphenated technique was created by combining a separation technique with an on-line spectroscopic detection system. Table 1 denotes the summary of hyphenated techniques of tamoxifen.

NANO DRUG DELIVERY SYSTEM OF TAMOXIFEN

TAM is a selective estrogen modulator and a hydrophobic anticancer agent. TAM resistance is a key hurdle to endocrine therapy and a significant barrier to cancer treatment. Nanotechnology is a promising tumor-specific drug delivery method as well as a co-delivery method for many anticancer medicines. TAM sensitivity and toxicity to the human body could be improved with nanotechnology-based drug carriers.[29]

Nanoparticulate drug delivery systems are being studied for a variety of reasons, including their compact size, controlled drug release capability, targeting ability, improved therapeutic efficacy, and reduced toxicity. As a result, Solid Lipid Nanoparticles have recently attracted a lot of attention as a potential drug delivery vehicle.[30]

The revolution in innovative drug delivery technologies is being driven by continuing improvements in medication pharmacological and therapeutic characteristics. To meet this developing need, a diverse range of therapeutic nanocarriers has been studied extensively. Delivery systems can be employed for TAM carriers, such as hydrogel, microemulsion, nanostructured lipid carriers, nanosuspensions-based gel, nanospheres, nanosponges, etc.

Swarnakar *et al*. formulated TAM-loaded liquid crystalline nanoparticles (TAM-LCNPs) to enhance the recent treatment's oral bioavailability and safety. The dilution-through-hydrotrope technique was utilized to make hexagonal Glyceryl monooleate-based TAMLCNPs (GLCNPs) and Phytantriol-based TAMLCNPs (PLCNPs) for oral administration. It can be determined through detailed optimization, *in vitro*, and *in vivo* evaluations that the TAM-LCNPs formulations developed in the present study have a much higher relative bioavailability, which leads to improved tumor regression with less hepatotoxicity. When comparing the two LCNP formulations, it was discovered that TAM-PLCNPs performed better in terms of illness recovery.[31] Chawla and team have developed and characterized nanoparticle formulation using poly(o-caprolactone) (PCL). The nanoparticles were indicating

Table 1: Summary of hyphenated techniques of tamoxifen.

Method	Matrices	Wavelength	Column	Chromatographic conditions	Ref
RP-HPLC	Human Urine	240nm	Zorbax Eclipse XDB-C_8	ACN and AcOH-NH_3 Buffer (pH4, 20mM) (45/55, %v/v) Flow rate-1mg/ml	15
HPLC	Human plasma	260 nm 375 nm	Agilent Extend C_{18}	Methanol-1% triethylamine aqueous solution(pH11) (82:18, % v/v) Flow rate-1mg/ml)	16
HPLC	Human plasma and liver tissue	280nm	BDS-Hypersil column (250×4.6 nm ID)	Methanol/0.5M ammonium acetate (75:25%v/v) Flow rate-1mg/ml	17
HPLC	Human Blood	320nm	(Spherisorb C_{18} CNRP)	K_3PO_4(20mM/L): acetonitrile, (65:35%v/v) Flow rate-1mg/ml	18
HPLC	Human plasma	200-380nm	Hypersil Gold®C_{18} Column	Triethyl ammonium phosphate buffer (5Mm pH3.3): Acetonitrile, (57:43 %v/v) Flow rate-1mg/ml	19
RP-HPLC	Human plasma	256 nm	Acquity C_{18} Column	Acetonitrile and phosphate buffer (PH3.5) (52:48%v/v) Flow rate-0.7mg/ml	20
HPLC	Human Plasma	256-380nm	Cyano Column	Acetonitrile 20Mm and potassium phosphate buffer (pH3) (35:65, %v/v) Flow rate-1mg/ml	21
Micellar liquid chromatography	Human Plasma	260-380nm	Kromasil 5 C_{18} Column	0.15 M SDS and (7%v/v) n-butanol (buffered at pH 3) Flow rate-1.5mg/ml	22
RP-UPLC	Rat Plasma	--	Acquity UPLC BEH C_{18} Column	0.1 % formic acid in acetonitrile and 0.1 % formic acid in water (80:20 %v/v) Flow rate-0.4mg/ml	23
TLC	Dissolution media	258 nm	Silica gel 60 F_{254}	Toluene: methanol: glacial acetic acid 57:38:5(%v/v)	24 25
TLC	Patients' serum	241 nm	Silica gel F_{254}	Butanol: acetic acid: water (6:0.5:0.5, %v/v)/v)	26
UPLC -MS/MS	Patients' serum	--	Acquity UPLC® BEH C_{18} column	Aqueous ammonium format (0.2mM) and acetonitrile Flow rate-0.3mg/ml	27
UHPLC -MS-MS	Dried blood spot sample	--	Acquity C_{18} Column	Formic acid 0.1%pH 2.7 and acetonitrile plus 0.1% formic acid Flow rate-0.4mg/ml	28

an acceptable solvent displacement process with an acetone/water mixture. Nanoparticle characterization included Differential scanning calorimetry (DSC), zeta potential studies, particle size analysis, and scanning electron microscopy. After 1 hr, the nanoparticles were discovered in the perinuclear area. According to the findings, nanoparticle formulations of selective ER modulators, such as TAM, might provide improved therapeutic advancement by delivering the drug near the ER.[32] Martinez and his co-workers produced TAM-loaded thiolate alginate-albumin nanoparticles. Coacervation was used to make nanoparticles based on disulfide link reduced bovine serum albumin and thiolate alginate (alginate-cysteine conjugate) that were loaded with TAM. By freezing the systems before loading, the TAM load into the nanoparticles was controlled (4–6 lg/mg NP). Between 2 and 25 hr, the maximum TAM emission (45–52%) occurred.[33] Memisoglu-Bilensoy *et al.* formulated nanoparticles like nanospheres and nano capsules from β-CDC6 according to the nanoprecipitation technique. The release profiles of nano capsules and nanospheres were similar; however, the release profile of TAM citrate-loaded nanosphere formulation was substantially faster than that of TAM citrate-loaded nano capsules. The transcription efficiency of TAM citrate-loaded nanoparticles was tested against MELN cells, and drug-loaded nanospheres and nanoparticles did not suppress E2-mediated luciferase gene expression in the presence

of E2. It is self-evident that making nanospheres and nano capsules directly from pre-formed TAM: -CDC6 inclusion complexes increases entrapment by a factor of two.[34] Jain *et al*. were prepared, optimized, and characterized more oral absorbable nanoparticles (NP) of TAM with Quercetin (QT). The indicated combination in NP form exhibited greater cell cytotoxicity and antitumor tumor effectiveness than the free drug combination. TAM-QT-NPs had increased cytotoxicity, cellular uptake, and nuclear co-localization in MCF-7 cells, indicating that the formulation was more efficient. *In vivo* pharmacokinetics showed a 5-fold and 3-fold boost in oral bioavailability when compared to free TAM citrate and free QT, respectively.[35] Viveka *et al*. looked into the possibilities of a smart pH-responsive drug delivery system (DDS) based on Chitosan (CH) Nanoparticles (NPs) in enabling more intelligent controlled release and improving TAM chemotherapy efficiency. TAM was loaded onto CH-nanoparticles by forming complexes, and at pH 4.0 and 6.0, TAM was released from the DDS considerably faster than at pH 7.4. This is an advantageous property for tumor-targeted medication delivery. MTT-assay, AO/EtBr, and Hoechst nuclear staining all showed that TAM-loaded CH nanoparticles improved anticancer efficacy significantly. RT-PCR was also used to investigate the putative signaling route. TAM-loaded CH nanoparticles, for example, were shown to increase intracellular TAM concentration and anticancer efficiency in human breast cancer MCF-7 cells by inducing apoptosis in a caspase-dependent manner, indicating that drug-loaded nanoparticles could act as an efficient DDS importing TAM into target cancer cells.[36] Shenoy and his group conducted to assess and compare the biodistribution profile of TAM when given intravenously (i.v.) as a simple solution or when encapsulated in polymeric nanoparticulate formulations, with or without surface stabilizing agents. Physical adsorption of a cross-linked polymer with a central hydrophobic binding unit and hydrophilic side chains to biodegradable polymeric nanoparticles loaded with a hydrophobic anti-cancer medication successfully surface-modified them. These nanoparticles, which had a mean size of 150 to 250 nm, demonstrated tumor-selective biodistribution and circulation times that might be used in clinical trials. The nanoparticles are predicted to release the encapsulated medicine by diffusion and biodegradation once they have collected within the tumor mass.[37] By using the nanoprecipitation approach, TAM citrate-loaded h-CDC6 nanospheres and nano capsules of appropriate particle size developed by the Imran and his co-workers. The drug was contained within the matrix/membrane in the cyclodextrin cavity or entangled in aliphatic chains, according to particle size, zeta potential, and *in vitro* performance. It is feasible to avoid the burst effect and extend the nanoparticle release profile to 6 hr this way.h-CDC6 nanospheres loaded with TAM citrate and Nano capsules have a high level of cytotoxicity against cancer cells.[38] The influence of surfactants and their concentrations was studied utilizing an emulsion/solvent evaporation approach to create PLA nanoparticles containing TAM. PLA NP were excellent TAM carriers with the potential to be utilized in cancer therapy.[39] Brigger *et al*. aimed at encapsulating TAM in long-circulating poly (MePEGcyanoacrylate-co-hexadecyl cyanoacrylate) 1:4 nanospheres. TAM-loaded poly (MePEGcyanoacrylate-co-hexadecyl cyanoacrylate) nanospheres were effectively produced and evaluated in terms of hydrophobicity/hydrophilicity using a principal component analysis model made up of near-infrared spectra. When TAM was contained in nanospheres, it still had a transcription. *Ex vivo* tests indicated an inhibitory effect. TAM, on the other hand, was essentially contained at the surface of the nanoparticles, resulting in considerable and fast drug release, according to zeta potential and *in vitro* release.[40] TAM-loaded magnetite/poly (L-lactic acid) composite nanoparticles (TMCN), were developed by Hu *et al*. The aim of the current work was to investigate the synthesis and characterization of TAM-loaded magnetite/poly (L-lactic acid) composite nanoparticles (TMCN) as well as their anti-cancer activity in MCF-7 breast cancer cells *in vitro*. Within 4 hr, MCF-7 cancer cells undergo structural alterations, and after four days, 80% of the cells were no longer viable. These findings suggest that the TMCN has a lot of potential as a carrier for TAM delivery.[41]

TAM citrate Self-nano emulsifying drug delivery systems (SNEDDS) were prepared by Yosra and his team. The morphology of the particles was revealed using transmission electron microscopy. The drug release from the chosen formulation was also much higher than that from other SNEDDS and drug suspension. Our findings suggested that including a bioactive surfactant in an optimized nano-sized SNEDDS system could improve oral efficacy.[42] Batool *et al*. was developed TAM loaded SNEDDS which were characterized for their drug release, surface chemistry, biocompatibility, permeation enhancement, and antitumor activity. The mucoadhesive self nanoemulsifying drug delivery using Hyaluronic Acid TAM was released over a longer period using the SNEDDS. According to the data, the TAM-PAP-HA-ss-LCA SNEDDS formulation appears to be a beneficial system in terms of mucopermeation, cellular absorption, nontoxicity, and increased anti-cancer activity.[43]

Meng and his team developed a temperature-sensitive phase-change hydrogel for TAM (TAM-Gel). At normal temperature, TAM-Gel changes from a liquid to a hydrogel. TAM's slow-release or anticancer effects was improved by injecting this Gel into the tumor. TAM-Gel, but not TAM-Sol, greatly reduced the uptake of radionuclide probes (18F-fluorestradiol) by cells in rats' livers and the intrahepatic development of MCF-7 cells in rats' livers once it was supplied via intratumoral injection. A unique slow-release mechanism was developed to improve the long-term release of TAM in breast cancer tissues, and it was found to have a long-term anticancer effect.[44] Bhatia *et al*. developed lecithin organogels (LOs) are semi-solid systems with immobilized organic liquid phase in a 3-D network of self-assembled gelators). The research effectively illustrated the application to manufacture a PLO-based topical carrier with the essential properties. The optimized LO was discovered to be very stable, easy to apply, and biocompatible. The insights of the study can be used to build LO systems for different medications, making topical delivery safer and more successful.[45]

Cosco *et al*. studied the impact of lipid composition on the physicochemical and technical aspects of a multidrug carrier (MDC) containing both Gemcitabine (GEM) and TAM, as well as its antitumoral effectiveness *in vitro* on several breast cancer cell lines. DPPC/Chol/DSPE-mPEG2000 (6:3:1 molar ratio, formulation A), DPPC/Chol/DOTAP (6:3:1 molar ratio, formulation B), and DPPC/Chol/DPPG (6:3:1 molar ratio, formulation C) were the three liposomal formulations created Using the TLE approach, colloidal systems were developed. Evidence of GEM and TAM loaded into a PEGylated liposomal carrier having a greater antitumoral effect than the free single forms could open up new approaches for breast cancer treatment.[46] liposome formulations of TAM and raloxifene were developed by N.B. Mutlu Ağardan and his team with penetration enhancers dimethyl-β-cyclodextrin (DM-β-CD) or sodium taurocholate (NaTC). Liposomes were proven to be a better formulation for increasing TAM oral absorption, especially when combined with dimethyl-CD. TAM with dimethyl-CD treatment of tumor-bearing rats the tumor area was reduced by 92.5 percent when liposome formulations were used. This represented a 50% therapeutic efficacy.[47] The creation of a stable Nanostructured lipid carrier (NLC) technology as a TAM carrier is reported by Rasedeea *et al*. TAM-NLC appears to be a potential drug delivery system for breast cancer therapy, according to the findings. TAM-NLC was tested *in vitro* against human and animal mammary cancer cell lines.[48] Torne and his coworkers prepared cyclodextrin nanosponges. The goal of this project was to create TAM-loaded-cyclodextrin nanosponges for oral administration. The three varieties of TAM-loaded -cyclodextrin nanosponges were made by altering

the molar ratios of -cyclodextrin to carbonyl diimidazole as a cross linker, which was 1:2, 1:4, and 1:8. Freeze drying was used to create the TAM nanosponge complex (TNC), which has a particle size of 400–600nm. The complexation of TAM with cyclodextrin nanosponge was validated by DSC, Fourier transformed infrared spectroscopy, and X-ray powder diffraction. After gastric intubation, the AUC and C_{max} of the TNC formulation were 1.44 and 1.38 times higher than the standard medication, respectively.[49] Several approaches, including pre-milling, magnetic stirring, and high-pressure homogenization nano-forms, have been used to create very stable TAM nanosuspensions. Surfactants like Tween 80® (Polysorbate 80) and the stabilizer Pluronic F-68® (polaxamer188) formulations to stabilize the suspension. Because of their exceptionally small size and zeta potential of 8.06 mV, which is well within the needed range of +30 to 30 mV for nanoparticles suspended in nanosuspension, this nanosuspension was claimed to be a very promising intravenous solution for resistance to TAM therapy.[50]

Kumar *et al*. produced phospholipid-based mixed micelles to investigate the biocompatible carrier'spotential. The method used was the self-assembled technique. The TAM-nanocarrier that has beencreated can deliver TAM to cancer cells safely and effectively while also being compatible with bloodand skin. The specific anticipated goals are an increase in skin bioavailability and long-termmedication release from skin layers using biocompatible nanocarriers.[51]

Chowdhury used HME technology to generate a stable solid dispersion of TAM and RES. In comparison to the simple drug suspension, our formulation showed improved *in vitro* release. In comparison to TAM suspension, the new formulation revealed higher TAM systemic exposure.[52]

CONCLUSION

TAM is a pioneering medicine because of its widespread usage in breast cancer treatment and chemoprevention, as well as research into novel selective estrogen receptor modulators. Compared with other anticancer drugs TAM is of class II drug which shows less solubility and favorable permeability, which can be further improved by a nano carrier system. This review includes various literatures for nano dosage forms of TAM and all hyphenated techniques for the quantification of TAM in various biological matrices. Based on this critical literature review, it was concluded that the researchers could readily adapt or alter the published procedures for achieving their future goals.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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