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Review Article

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ENDOXIFEN CITRATE: A REVIEW ON ANALYTICAL METHOD AND VALIDATION

Gokulapriya A.[1*] and Vetrichelvan T.[2]

M. Pharm Student[1], HOD cum Dean Research[2]

Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur-603319.

Affiliated to The Dr. M. G. R. Medical University Chennai-600032, Tamil Nadu, India.

***Corresponding Author: Gokulapriya A.**

M. Pharm Student, Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur-603319.

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ABSTRACT

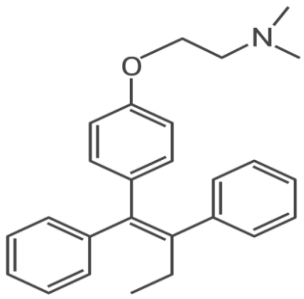
Endoxifen citrate is the anticancer drug, under the development for the treatment of estrogen receptor positive breast cancer and treatment of mania in bipolar disorder. It is a potent active metabolite of tamoxifen, has gained significant attention in recent years due its enhanced anti-estrogenic activity and therapeutic potential in hormone responsive breast cancer. Unlike, its parent compound, endoxifen exhibits a direct mechanism of action by binding with high affinity to estrogen receptors, leading to effective inhibition of estrogen mediated cell proliferation. This review provides a comprehensive overview of endoxifen citrate, encompassing its chemical structure, Pharmacokinetic, Pharmacodynamics and mechanism of action. The Clinical and Pharmaceutical analysis of this drug requires effective analytical procedures for mechanism of action and quality control and Pharmacodynamics and Pharmacokinetic studies as well as stability study. The literature from numerous journals pertaining to Pharmaceutical analysis and Pharmacology has been thoroughly reviewed, and instrumental analytical methods that were developed and used for determining the presence of a drug alone in bulk drugs, formulations, and biological fluids have been examined. This review covers the most recent many analytical methods including 2D NMR, chromatographic method including LC-MS, UPLC-MS and HPLC were reported.

KEYWORDS: Endoxifen, development, anticancer, analytical method, 2D-NMR, LC-MS, UPLC-MS, HPLC.

INTRODUCTION

Endoxifen citrate was first identified as a Tamoxifen metabolite in 1988. Tamoxifen is a selective estrogen receptor modulator (SERM). This means that it exhibits estrogen-blocking capabilities in some tissues, whereas in others, it acts more like an estrogen. In the breast, tamoxifen mainly exhibits antiestrogenic effects. Tamoxifen was originally developed as an oral contraceptive, but it was unsuccessful in this capacity. In the 1960s and 1970s, tamoxifen was shown to have anticancer effects. It was developed as a treatment for women with estrogen receptor–positive breast cancer, with the idea that blocking this receptor could promote anticancer effects. The US Food and Drug Administration (FDA) first approved tamoxifen in 1977 for metastatic breast cancer. However, it was the subsequent three decades of clinical trial research that led to the critical observations that the administration of tamoxifen after surgery reduced breast cancer recurrence and mortality and, most importantly, prolonged survival. During that time, studies identified the estrogen receptor as the critical predictive biomarker and defined the importance of duration (first five years, and then ten years). Later, studies demonstrated the benefit of tamoxifen for the adjuvant treatment of ductal carcinoma in situ, as well as in the prevention setting for women at increased risk of developing breast cancer. Tamoxifen is also approved for the treatment of female breast cancer.

STRUCTURE OF TAMOXIFEN



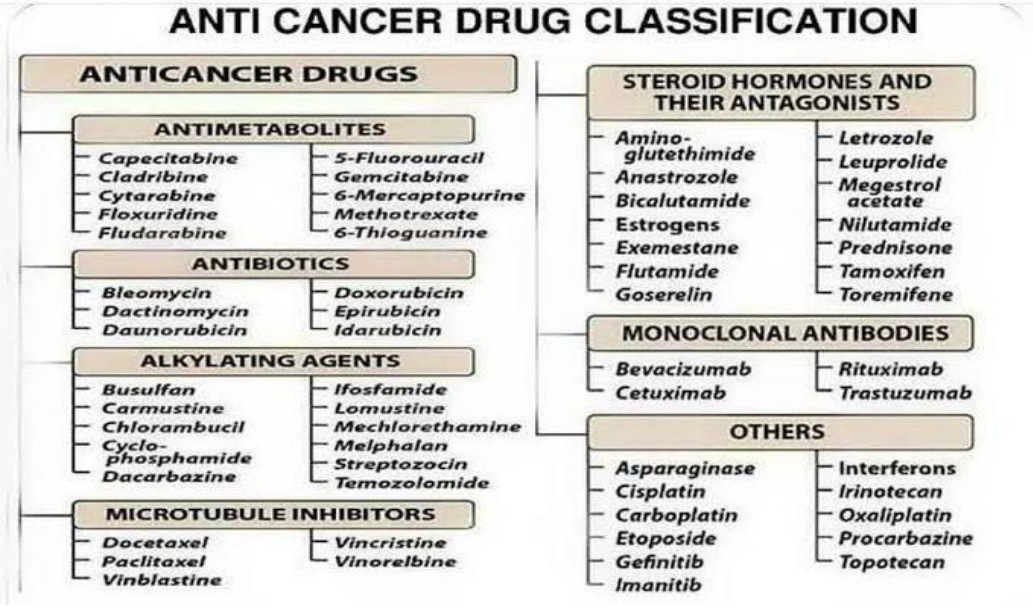
The chemical structure of endoxifen has been known for many years, Pharmaceutical companies expressed concern that there was inadequate protection for intellectual property. I was part of a team of investigators at the Mayo Clinic who entered into discussions with the National Cancer Institute (NCI) that aimed to develop a public-private partnership to investigate if endoxifen had antitumor activity and could be used as a drug. To

explore this idea, we first performed experiments in laboratory animals to determine the Pharmacokinetics of endoxifen, and we determined that endoxifen exhibited excellent bioavailability when administered orally to rats and dogs. This led the NCI to develop a formulation called Z-endoxifen, which was tested in preclinical toxicology studies and eventually in patients. Ongoing studies are aiming to determine whether endoxifen has activity in patients who cannot tolerate treatment with tamoxifen or who have predicted or confirmed low concentrations of endoxifen (e.g., those who are poor metabolizers of CYP2D6). One regulatory path forward is for Z-endoxifen to be studied and eventually approved for this specific group of patients (ie, those with low concentrations of endoxifen). This group will include women who poorly metabolize CYP2D6, but there are other reasons that concentrations of endoxifen might be low. For example, some drugs, including several antidepressants, inhibit CYP2D6 enzyme activity. In that situation, patients who would have previously been advised to discontinue drugs that inhibit CYP2D6 enzyme activity could remain on them while being treated with endoxifen instead of tamoxifen.

Endoxifen citrate may hold the greatest promise, however, in premenopausal women. In premenopausal women with early-stage, estrogen receptor–positive breast cancer, an updated analysis of the International Breast Cancer Study Group 24–02 study demonstrated that strategies that more deeply suppress estrogen (e.g., ovarian function suppression in addition to tamoxifen or an aromatase inhibitor) are superior to tamoxifen monotherapy. Although this latter approach is likely the most effective, it is associated with substantial short-term and potential long-term side effects. Therefore, endoxifen may be an effective but more tolerable approach to targeting the estrogen receptor in premenopausal women with early-stage breast cancer.[1]

CLASSIFICATION

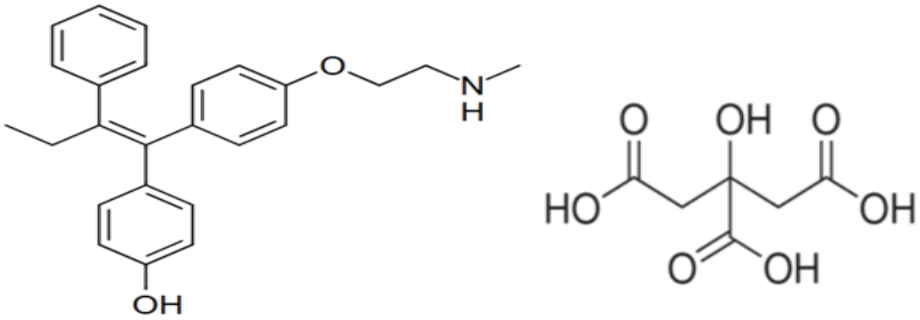
Anticancer drugs, also known as antineoplastic drugs, are classified in a number of ways including.



CHEMISTRY OF ENDOXIFEN CITRATE

Chemical name of endoxifen citrate, known as 4-hydroxy-N-desmethyltamoxifen citrate, is a nonsteroidal selective estrogen receptor modulator (SERM) of the triphenylethylene group as well as a protein kinase C (PKC) inhibitor.

STRUCTURE OF ENDOXIFEN CITRATE



Trade name: ZONALTA
Route of Administration: By mouth
IUPAC Name: 4-[(E)-1-[4-[2-(methylamino) ethoxy] phenyl]-2-phenylbut-1-enyl] phenol citrate.[2]

CHEMICAL AND PHYSICAL PROPERTIES

Chemical Formula: $C_{32}H_{37}NO_8$
Molecular weight: 563.65g/mol
Solubility: Endoxifen is soluble in organic solvents such as ethanol, DMSO, water and dimethyl formamide (DMF). Endoxifen is sparingly soluble in aqueous buffers.[3]

MEDICINAL USES

BIPOLAR DISORDER

Endoxifen is used to treat manic or mixed episodes associated with bipolar I disorder in India.[4] It has been found that the endoxifen improves manic symptoms as well as mixed episode symptoms of patients with bipolar I disorder and has been considered an effective and well-tolerated treatment for this condition.[5]

Bipolar disorder is associated with overactive protein kinase C (PKC) intracellular signa ling.[6] To date, there have been three phases of clinical trials. And, in the phase III trials, endoxifen reduced the total Young Mania Rating Scale (YMRS) score from 33.1 to 17.8. A significant ($p < 0.001$) improvement in Montgomery–Åsberg Depression Rating Scale (MADRS) score was observed for endoxifen (4.8 to 2.5). The endoxifen is well-tolerated by the subjects as depicted in the changes in Clinical Global Impression-Severity of Illness scores.[7]

SIDE EFFECTS

The most prevalent side effects for endoxifen include headache, vomiting, insomnia. Other side effects were: gastritis, epigastric discomfort, diarrhea, restlessness, somnolence, etc. Some of the adverse events reported with other therapies for the management of manic episodes of bipolar I disorder were not observed during the clinical development program of endoxifen like reduction in platelet count, change in blood thyroid-stimulating hormone levels. There were no deaths, serious or significant adverse events during the conduct of trials. Overall, endoxifen was found to be well-tolerated and safe in patients of bipolar I disorder with acute manic episodes with or without mixed features.[7] An important caveat here is that the trial was of very short duration (only three weeks). The long-term safety of Endoxifen has not been established among patients with bipolar disorder.

PHARMACODYNAMICS

Selective estrogen receptor modulator

Endoxifen is a selective estrogen receptor modulator (SERM)
with estrogenic and antiestrogenic actions. In the first study to evaluate the pharmacology of endoxifen, it showed 25% of the affinity of estradiol for the estrogen receptor (ER) while afimoxifene had 35% of the affinity of estradiol for the ER. The antiestrogenic actions of endoxifen and afimoxifene in this study were very similar.[8] In another study, the affinity of endoxifen for the ERα was 12.1% and its affinity for the ERβ was 4.75% relative to estradiol. For comparison, afimoxifene had relative binding affinities for the ERα and ERβ of 19.0% and 21.5% compared to estradiol, respectively.[9] In yet another investigation, both endoxifen and afimoxifene had 181% of the affinity of estradiol for the ER whereas tamoxifen had 2.8% and *N*-desmethyltamoxifen had 2.4%.[10]

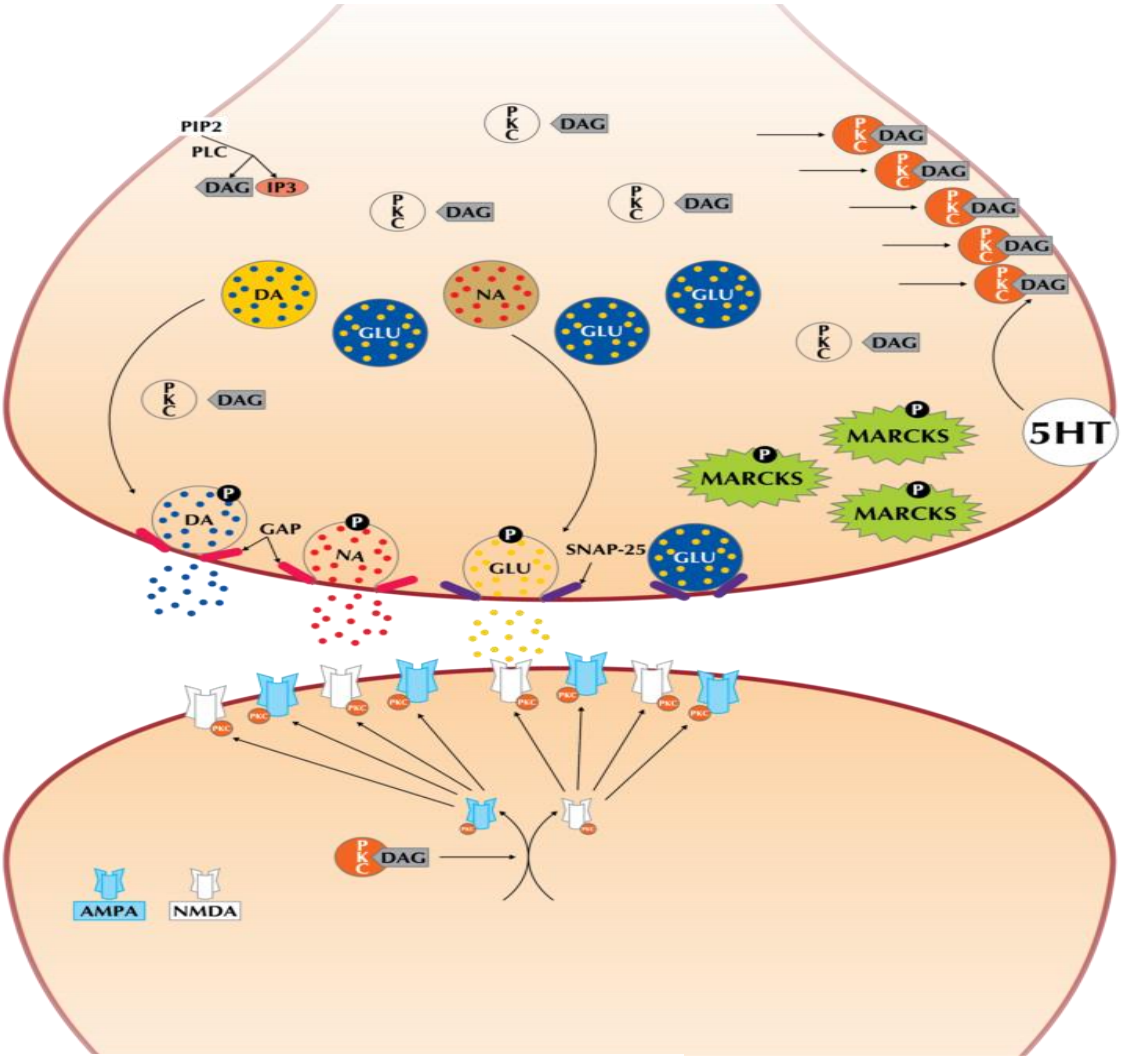
Protein kinase C inhibition

The exact mechanism by which endoxifen exerts its therapeutic effects has not been established in bipolar I disorder. However, the efficacy of endoxifen could be mediated through protein kinase C (PKC). The PKC represents a family of enzymes highly enriched in the brain, where it plays a major role in regulating both pre- and post-synaptic aspects of neurotransmission. Excessive activation of PKC results in symptoms related to bipolar disorder. The PKC signalling pathway is a target for the actions of two structurally dissimilar antimanic agents – lithium and valproate.

Endoxifen exhibits 4-fold higher potency in inhibiting PKC activity compared to tamoxifen in preclinical studies and is not dependent on the isozyme cytochrome P450 2D6 (CYP2D6) for action on the target tissues.[11]

PHARMACOKINETICS

Orally administered endoxifen is rapidly absorbed and systemically available. The time to peak (Tmax) is between 4.5 and 6 hours after oral administration. It is not metabolized by cytochrome P450 enzymes. The half-life (t½) life of endoxifen is 52.1 to 58.1 hours.[12]



5HT: 5-hydroxytryptamine;
IP3: Inositol 1,4,5-triphosphate;
PIP2: phosphatidylinositol-4,5-bisphosphate;
GAP: Growth-associated protein of 43 kDa;
SNAP-25: Synaptosomal-associated protein 25;
PIP2: phosphatidylinositol-4,5-bisphosphate;
NMDA: N-methyl-D-aspartate;
NA: Noradrenaline;
NMDA: N-methyl-D-aspartate;
PLC: Phospholipase-C;
PKC: Protein Kinase C;
GLU: Glutamate;
DAG: Diacetylglycerol;
DA: Dopamine;
MARCKS: Myristoylated alanine-rich C-kinase substrate;
AMPA: Alpa-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid;
Orally administered ZONALTA is rapidly absorbed and systemically available.

The time to peak (T_{max}) is between 4.5 and 6 hours after oral administration.	ZONALTA is not metabolized by Cytochrome P450 enzymes.	Half-life ($t_{½}$): 52.1–58.1 hours

Predose PK samples were collected. The treatment was started by orally administering investigational medications from Day 0 onwards. Four mL blood for the Day 0 sample and 2 mL blood samples on Day 4, Day 7, Day 14, and Day 20 were collected from patients just before dosing the investigational medications at the clinic/hospital site. The last sample of 2 mL was collected on Day 49 when the patient visited the site for posttreatment safety follow-up. A total of six samples were collected per patient for PK assessment.

The blood samples were processed to separate plasma and blood cells. The plasma samples were then frozen at –20°C until use. Plasma samples of patients were analysed using a validated LC-MS/MS method for endoxifen at the bioanalytical facility laboratories. The concentration data were tabulated using WinNonlin Professional Software v. 5.3.

The area under the curve (AUC) was estimated using Excel with the linear trapezoidal method. For a given time interval ($t_1 – t_2$), the AUC were calculated with the following equation.

$$AUC=1/2 \times(C1-C2) \times(t2-t1)$$

Where C is the plasma concentration of endoxifen at a given time (t) of each patient. In this case, $C_1 = 0$, $t_1 = 0$. The dose–response relationship between mean YMRS score (change from baseline) and mean corresponding AUC at a certain time point was also analysed using Excel and a through linear regression.

CONCOMITANT MEDICATIONS

All psychotropic medications except benzodiazepines (lorazepam/diazepam only) were discontinued at least two days before randomization. Benzodiazepines (lorazepam/diazepam only) (up to 5 mg/day, preferably in divided doses) were allowed as adjunctive medication as needed at the discretion of the investigator from two days prior to randomization but not beyond the first ten days of investigational medicinal product dosing. Benzodiazepines were avoided within 12th of scheduled mania ratings. The usage of two benzodiazepines was permitted to reduce undue excitement by using these adjuvants in an appropriate manner while avoiding efficacy or safety overlap with the endoxifen or divalproex.

EFFICACY AND SAFETY ASSESSMENTS

All psychiatrists participating in this double-blind trial at different sites were well trained and had experience in using Diagnostic & Statistical Manual of Mental Disorders. All baseline scores were recorded by due administration of all defined scales. Efficacy and safety assessments were done based on evaluation parameters on Days 4, 7, 14, and 21 (end of treatment). Safety blood Clinical Global Impression of Improvement (CGI-I) was collected on Day 21 / discharge day from hospital. The primary end point was defined as the proportion of responders in each arm on Day 21 based on change in the Young Mania Rating Scale (YMRS) and total score (≥50% decrease from baseline). Secondary end points were mean change from baseline to the end of treatment in the Montgomery–Åsberg Depression Rating Scale (MADRS) and total score, Clinical Global Impressions–Severity of Illness Scale (CGI-S) score, and Columbia–Suicide Severity Rating Scale (C-SSRS) score.

SAFETY AND TOLERABILITY

The safety analysis included all eighty-four patients who received at least one dose of the study medication. A total of sixty-three adverse events (AEs) were reported by twenty-seven patients during the conduct of this study. Out of sixty-three, fifty-nine were mild, two were moderate, and two were severe in intensity. In all, thirty-three AEs were reported by 41.38% (n = 12) of twenty patients in the divalproex arm (active raw material) and nineteen AEs were reported by 29.63% (n = 8) of twenty-seven patients in the endoxifen 4 mg arm and eleven AEs were reported by 25.00% (n = 07) of twenty-eight patients in the endoxifen 8 mg arm. There were two patients who left the study in the 4 mg/day group due to adverse effect, three patients withdrew consent, and one patient was lost to follow-up. No patients left the study in the 8 mg/day endoxifen or in the divalproex arm. There were no deaths, other significant AEs, or serious AEs reported during the conduct of the study. Overall, endoxifen was well tolerated.

The most common AEs reported in the trial were psychiatric in nature. Insomnia and nausea were the most common AEs in the divalproex arm. Headache was reported in two patients at endoxifen 4 mg, three patients at endoxifen 8 mg, and three patients in the divalproex 1,000 mg arm. Gastrointestinal disorders like dyspepsia, nausea, and vomiting were found in four, one, and seven patients in the endoxifen 4 mg, endoxifen 8 mg, and divalproex arm respectively. Only two AEs were found to be severe in the 4 mg endoxifen group and one AE was moderate in the divalproex group; all other AEs were mild in nature. The two severe AEs observed in the 4 mg endoxifen group were related to delusions in patients. Based on the above results, it is concluded that endoxifen 4 mg and 8 mg was well tolerated and safe as compared with divalproex.[13]

ANALYTICAL METHODS

Validation is an applied approach to verify that a method is suitable to function as a quality control tool. The objective of any analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal. An analytical method consists of the techniques, method, procedure and protocol. Analytical method validation includes the determination of accuracy, precision, LOD, LOQ, linearity and range. The results from method validation can be used to moderator the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories. The main objective of this review article is to guide the young researchers to improve the quality of analytical method development and validation process.[14]

It is crucial for ensuring the efficacy and safety of medicinal products by assessing their overall purity during storage, distribution, and use. Validated procedures establish the quality relationship between the examined substance and the one initially evaluated

pharmaceutically, toxicologically, and pharmacologically. Sound quality control relies on validated analytical methods, essential for manufacturing, government control laboratories, or pharmacopoeia inclusion. Key validation characteristics include specificity, linearity, range, accuracy, precision, detection limit, quantitation limit, and robustness. These parameters ensure that the analytical method produces reliable data fit for its intended purpose.

Phyllis Elkins *et al*

Title: Characterization of the isomeric configuration and impurities of (Z)-endoxifen by 2D NMR, high resolution LC–MS, and quantitative HPLC analysis

This paper describes confirmation of the configuration of the active (Z)-isomer through 2D NMR experiments, including NOE (ROESY) to establish spatial proton–proton correlations, and identification of the major impurity as the (E)-isomer in endoxifen drug substance by HPLC/HRMS (HPLC/MS-TOF). Stability of NMR solutions was confirmed by HPLC/UV analysis. For pre-clinical studies, a reverse-phase HPLC–UV method, with methanol/water mobile phases containing 10 mM ammonium formate at pH 4.3, was developed and validated for the accurate quantitation and impurity profiling of drug substance and drug product. The retention time was found to be 12 mins. Validation included demonstration of linearity, method precision, accuracy, and specificity in the presence of impurities, excipients (for the drug product), and degradation products. Ruggedness and reproducibility of the method were confirmed by collaborative studies between two independent laboratories. The method is being applied for quality control of the API and oral drug product. Kinetic parameters of Z- to E isomerization were also delineated in drug substance and aqueous formulation, showing conversion at temperatures above 25 °C.[15]

Joel M Reid *et al*

Title: Development and Validation of a Liquid Chromatography-Mass Spectrometry Assay for Quantification of Z- and E-Isomers of Endoxifen and its Metabolites in Plasma from Women with Estrogen Receptor Positive Breast Cancer

To quantify ENDX and its metabolites in patient plasma samples, we have developed and validated a rapid, sensitive, and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantitative determination of the E- and Z isomers of ENDX (0.5-500 ng/ml) and the ENDX metabolites nor endoxifen (1-500 and 0.5-500 ng/ml E and Z, respectfully), ENDX catechol (3.075-307.5 and 1.92-192 ng/ml E and Z, respectfully), 4'-hydroxy ENDX (0.33-166.5 and 0.33-333.5 ng/ml E and Z, respectfully), ENDX methoxy catechol (0.3-300 and 0.2-200 ng/ml E and Z, respectfully), and ENDX glucuronide (2- 200 and 3-300 ng/ml E and Z, respectfully) in human plasma. Chromatographic separation was accomplished on a HSS T3 precolumn attached to an Poro shell 120 EC-C18 analytical column using 0.1% formic acid/water and 0.1% formic acid/methanol as eluents followed by MS/MS detection. The analytical run time was 6.5 min. Standard curves were linear ($R2 \geq 0.98$) over the concentration ranges. The intra- and inter-day precision and accuracy, determined at high-, middle-, and low-quality control concentrations for all analytes, were within the acceptable range of 85% and 115%. The average percent recoveries were all above 90%. The method was successfully applied to clinical plasma samples from a Phase I study of daily oral Z-ENDX.[16]

M Van Nuland *et al*

Title: Development and validation of an UPLC-MS/MS method for the therapeutic drug monitoring of oral anti-hormonal drugs in oncology

A liquid chromatography-mass spectrometry assay was developed and validated for simultaneous quantification of anti-hormonal compounds abiraterone, anastrozole, bicalutamide, Δ (4)-abiraterone (D4A), N-desmethyl enzalutamide, enzalutamide, Z-endoxifen, exemestane and letrozole for the purpose of therapeutic drug monitoring (TDM). Plasma samples were prepared with protein precipitation. Analyses were performed with a triple quadrupole mass spectrometer operating in the positive and negative ion-mode. The validated assay ranges from 2 to 200 ng/mL for abiraterone, 0.2–20 ng/mL for D4A, 10–200 ng/mL for anastrozole and letrozole, 1–20 ng/ mL for Z-endoxifen, 1.88–37.5 ng/mL for exemestane and 1500–30,000 ng/mL for enzalutamide, N-desmethyl enzalutamide and bicalutamide. Due to low sensitivity for exemestane, the final extract of exemestane patient samples should be concentrated prior to injection and a larger sample volume should be prepared for exemestane patient samples and QC samples to obtain adequate sensitivity. Furthermore, we observed a batch-dependent stability for abiraterone in plasma at room temperature and therefore samples should be shipped on ice. This newly validated method has been successfully applied for routine TDM of anti-hormonal drugs in cancer patients.[17]

Sven de Krou *et al*

Title: Fast and Adequate Liquid Chromatography–Tandem Mass Spectrometric Determination of Z-endoxifen Serum Levels for Therapeutic Drug Monitoring

Patients receiving adjuvant tamoxifen treatment with endoxifen levels below the threshold of 5.9 ng/mL may have an increased risk of breast cancer recurrence. Several factors, such as genetic polymorphisms, drug interactions, and (non)adherence, lead to large interpatient variability in endoxifen exposure, resulting in a substantial number of patients showing subtherapeutic levels. As genotyping and phenotyping are not able to adequately predict endoxifen exposure, therapeutic drug monitoring (TDM) seems to be the best approach for tailored tamoxifen therapy. Methods: To support TDM services, a rapid and sensitive high performance liquid chromatography–tandem mass spectrometry assay for the quantification of endoxifen in

human serum was developed and validated. Validation was performed according to the latest US FDA and EMA guidelines on bioanalytical method validation. Results: The successfully validated serum assay quantifies endoxifen with a linear regression calibration model (weighted 1/x2) in the concentration range from 1.00 to 25.0 ng/mL. The assay was vali dated with an inaccuracy of 67.7% and an imprecision of #3.9%, obtained with an IS normalized matrix factor of 0.925 and a signal to-noise ratio of .66. Conclusions: All validation parameters fulfilled their acceptance criteria, and the developed assay is now successfully being used to support TDM services. Thus far, 32.7% of the more than 500 determined endoxifen serum levels were below the threshold of 5.9 ng/Ml.[18]

Enrique Ochoa Aranda *et al*

Title: Development of a methodology to quantify tamoxifen and endoxifen in breast cancer patients by micellar liquid chromatography and validation according to the ICH guidelines

A simple micellar liquid chromatographic procedure is described to determine tamoxifen and endoxifen in plasma. For the analysis, tamoxifen and endoxifen solutions were diluted in water and UV-irradiated for 20 min to form the photo-cycled derivative with a phenanthrene core which shows intense fluorescence. Samples were then directly injected, thus avoiding long extraction and experimental procedures. The resolution from the matrix was performed using a mobile phase containing 0.15molL−1 SDS–7% n-butanol at pH 3, running at 1.5mLmin−1 through a C18 column at 40 ◦C. Detection was carried out by fluorescence, and the excitation and emission wavelengths were 260 and 380nm, respectively. The chromatographic analysis time was 20 min. The analytical methodology was validated following the International Conference on Harmonisation of Technical Requirements for Registration of pharmaceuticals for human use (ICH) guidelines. The response of the drugs in plasma was linear in the 0.5–15 gmL−1 range, with r2 >0.99.[19]

Nynke GL Jager *et al*

Title: Determination of tamoxifen and endoxifen in dried blood spots using LC–MS/MS and the effect of coated DBS cards on recovery and matrix effects

Background: We developed an HPLC-MS/MS method to quantify tamoxifen (2.5–250 ng/ml) and its metabolite (Z)-endoxifen (0.5–50 ng/ml) in dried blood spots. Results: Extraction recovery of both analytes from Whatman DMPK-A cards was 100% and consistent over time, however, recovery of (Z)-endoxifen from Whatman 903 cards was incomplete and increased upon storage. When SDS, a constituent of the DMPK-A coating, was present during the extraction, recovery improved. The method using DMPK-A cards was validated using bioanalytical guidelines. Additionally, influence of haematocrit (0.29–0.48 L/L), spot volume (20–50µl) and homogeneity was within limits and both analytes were stable in DBS for at least 4 months. Conclusion: The method for the quantification of tamoxifen and (Z)-endoxifen in DBS collected on DMPK-A cards was successfully validated.[20]

Marina Venzon Antunes *et al*

Title: Sensitive HPLC–PDA determination of tamoxifen and its metabolites N-desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen in human plasma

A highly sensitive HPLC–UV method for the simultaneous determination of tamoxifen, N desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen in human plasma samples was developed and validated. The method employs a two-step liquid–liquid extraction and a reversed phase separation on a Hypersil Gold® C18 column (150 mm × 4.6 mm, 5 m) with isocratic elution. Mobile phase was a mixture of triethylammonium phosphate buffer 5 mM pH 3.3 and acetonitrile (57:43, v/v). Total analytical run time was 16 min. The retention time were found to be endoxifen 5.2 mins, 4-hydroxytamoxifen 5.8 mins and N-desmethyltamoxifen 12 mins. Precision assays showed CV % lower than 10.53% and accuracy in the range of 93.0–104.2%. The lower limits of quantification (0.75–8.5 ng ml−1) are adequate to measure clinically relevant concentrations in plasma samples. The method was successfully applied to 110 clinical plasma samples. Median plasma levels and interquartile range were: tamoxifen 55.77 ng ml−1(38.42–83.69 ng ml−1), N-desmethyltamoxifen 124.83 ng ml−1(86.81–204.80 ng ml−1), 4 hydroxytamoxifen 1.09 ng ml−1(0.76–1.53 ng ml−1) and endoxifen 6.18 ng ml−1(4.17–8.22 ng ml−1). The procedure has adequate analytical performance and can be employed in therapeutic drug monitoring of tamoxifen or pharmacokinetics studies.[21]

Marina Ari~ no Martin *et al*

Title: Photodegradation of (E)- and (Z)-Endoxifen in water by ultraviolet light: Efficiency, kinetics, by-products, and toxicity assessment

This research investigates ultraviolet (UV) radiation (253.7 nm) application to degrade (E)- and (Z)-endoxifen in water and wastewater and photo-transformation by-products (PBPs) and their toxicity. The effects of light intensity, pH and initial concentrations of (E)- and (Z)-endoxifen on the photodegradation rate were examined. Endoxifen in water was eliminated 99.1% after 35 s of irradiation (light dose of 598.5 mJ cm2). Light intensity and initial concentrations of (E)- and (Z)-endoxifen exhibited positive trends with the photodegradation rates while pH had no effect. Photodegradation of (E)- and (Z) endoxifen in water resulted in three PBPs. Toxicity assessments through modelling of the identified PBPs suggest higher toxicity than the parent compounds. Photodegradation of (E)- and (Z)-endoxifen in wastewater at light doses used for disinfection in WWTPs (16, 30 and 97 mJ cm2) resulted in reductions of (E)- and (Z)-endoxifen from 30 to 71%. Two of the three PBPs observed in the experiments with water were detected in the wastewater experiments. Therefore, toxic compounds are potentially generated at WWTPs by UV

disinfection if (E)- and (Z)-endoxifen are present in treated wastewater.[22]

Marina Ari˜ no Martin *et al*
Title: Photolytic fate of (E)- and (Z)-endoxifen in water and treated wastewater exposed to sunlight
Endoxifen has been recently detected in the final effluent of municipal wastewater treatment plants. The antiestrogenic activity of endoxifen could bring negative effects to aquatic life if released to the water environment. This study elucidated the fate and susceptibility of (E)- and (Z)-endoxifen (2 µg mL 1, 1:1 wt ratio between the two easily interchangeable isomers) in wastewater and receiving surface water to sunlight. Photo-transformation by-products (PBPs) and their toxicity were determined. Sunlight reduced at least 83 % of endoxifen concentration in wastewater samples, whereas in surface water samples, 60 % of endoxifen was photodegraded after 180 min of the irradiation. In ultrapure water samples spiked with endoxifen, PBPs were mainly generated via con-rotatory 6 π-photo cyclization, followed by oxidative aromatization. These PBPs underwent secondary reactions leading to a series of PBPs with different molecular weights. Eight PBPs were identified and the toxicity analysis via the Toxicity Estimation Software Tool revealed that seven of these PBPs are more toxic than endoxifen itself. This is likely due to the formation of poly-aromatic core in the PBPs due to exposure to sunlight. Therefore, highly toxic PBPs may be generated if endoxifen is present in water and wastewater exposed to sunlight. The presence, fates and ac tivities of these PBPs in surface water especially at locations close to treated wastewater discharge points should be investigated.[23]

Author	Method	Solvent/mobile phase
Joel M. Reid	LC - TMS	A-4mmol/L ammonium formate at pH 3.5 and B-ACN
Sarah A. Buhrow	LC-MS/MS	0.1% formic acid/water and 0.1% formic acid/methanol
Phyllis Elkins	2D NMR, high resolution LC–MS, and quantitative HPLC analysis	methanol/water with ammonium formate at pH 4.3
M. Van Nuland	UPLC-MS/MS	A-0.1% formic acid in water B-ACN and Methanol
Nynke GL Jager	HPLC-MS/MS	1)Methanol: water 2)ACN: water 3)Methanol: ACN
Marina Arino Martin	UHPLC-MS/MS	Water
Marina Arino Martin	UHPLC-MS/MS	Water and waste water
Marina Venzon Antunes	HPLC–PDA	triethylammonium phosphate buffer 5 mM pH 3.3 and acetonitrile (57:43, v/v)
nrique Ochoa Aranda	Micellar Liquid Chromatography	0.15mol/L SDS-7% n-butanol at pH 3

CONCLUSION

The present review contains maximum information related to analytical method development and validation of endoxifen by LC-MS/MS, UPLC-MS/MS and drug profile. The present review is advantageous to researchers in this area engaged in analysis of endoxifen.

AUTHORS CONTRIBUTIONS: All the authors have contributed equally.

CONFLICT OF INTERESTS: Declared none.

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