

## THE APPLICATION OF BIOANALYTICAL METHOD OF TAMOXIFEN AND ITS ACTIVE METABOLITES FOR THERAPEUTIC DRUG MONITORING IN BREAST CANCER PATIENTS: A REVIEW

MUHAMMAD IKHSAN<sup>1</sup>, YAHDIANA HARAHAP<sup>1,2\*</sup>

<sup>1</sup>Faculty of Military Pharmacy, Republic of Indonesia Defense University, Bogor, West Java, Indonesia. <sup>2</sup>Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia

\*Corresponding author: Yahdiana Harahap; \*Email: yahdiana03@yahoo.com

Received: 23 Nov 2023, Revised and Accepted: 27 Mar 2024

### ABSTRACT

Breast cancer is the most common cancer around the world and in Indonesia. The most widely used agent for breast cancer treatment is tamoxifen, with a fixed dose of 20 mg per day. Tamoxifen is metabolized by cytochrome P450 3A4 (CYP3A4) and 2D6 (CYP2D6) to endoxifen and 4-hydroxytamoxifen, which have 30-to 100-fold more potent antiestrogenic activity than tamoxifen. High variations of CYP3A4 and CYP2D6 genes can lead to interpatient variability in its metabolites concentration. The dose can be increased to 40 or 60 mg per day based on individual needs. Therapeutic drug monitoring (TDM) is required to measure the concentration of tamoxifen and its metabolites to decide the individualized dose. The measurement of drug levels should use a sensitive, selective, accurate, precise, and reliable bioanalytical method. Various bioanalytical methods have been developed in several matrices: urine, scalp hair, serum, plasma, dried blood spot (DBS), and volumetric absorptive microsampling (VAMS) samples, with different sample preparations, and frequently using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The bioanalytical method of tamoxifen and its metabolites in the DBS sample was more suitable in the TDM application due to the low invasive sampling technique, more stable sample, and rapid sample preparation. Therefore, it is more time- and cost-efficient than the other methods.

**Keywords:** Bioanalytical method, Breast cancer, Endoxifen, Tamoxifen, Therapeutic drug monitoring

© 2024 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<https://creativecommons.org/licenses/by/4.0/>)  
DOI: <https://dx.doi.org/10.22159/ijap.2024v16i3.49957> Journal homepage: <https://innovareacademics.in/journals/index.php/ijap>

### INTRODUCTION

Breast cancer is the most prevalent cancer around the world and in Indonesia. According to the Global Cancer Observatory (Globocan) 2020 database, there were 2.26 million new breast cancer cases and 684.99 thousand breast cancer-related deaths around the world. In Indonesia, there were 65.85 thousand new cases of breast cancer and 22.43 thousand people died of breast cancer. During the last five years, the number of new breast cancer cases was 7.79 million cases around the world and 201.14 thousand cases in Indonesia [1, 2].

Breast cancer is caused by many factors, which are genetic and non-genetic factors such as obesity, physical inactivity, alcohol consumption, short breastfeeding periods, unhealthy diet, and pollution exposure [3]. Surgery, radiation, chemotherapy, immunotherapy, endocrine therapy, and targeted therapy are currently used to treat breast cancer [3-5]. The best kind of treatment for breast cancer is endocrine therapy since estrogen binding to the estrogen receptor (ER) on cancer cells accounts for about 70% of occurrences. Endocrine therapy directly blocks the actions of estrogen at ER and inhibits estrogen-dependent growth of cancer cells [6]. Tamoxifen has been the most widely used in the treatment of endocrine therapy for breast cancer in the last 40 y [6-8]. Cytochrome P450 3A4 (CYP3A4) and 2D6 (CYP2D6) convert tamoxifen to endoxifen and 4-hydroxytamoxifen. Both metabolites have been shown to have antiestrogenic activity that is 30-to 100-fold higher than tamoxifen itself. Highly variable CYP3A4 and CYP2D6 genes can lead to interpatient heterogeneity in metabolite concentrations [6].

Tamoxifen is currently administered to all patients as a fixed dose of 20 mg tamoxifen per day for the treatment of breast cancer. The fixed-dose frequently results in a lower concentration of tamoxifen and its metabolites than the minimum effective concentration. The fixed dose also may potentially result in a higher concentration than the threshold of toxic level. The more rational therapy should use individualized doses to achieve a high degree of accuracy in the drug concentration. The most direct approach to individualizing tamoxifen dose is therapeutic drug monitoring (TDM) which

quantifies the levels of tamoxifen and its metabolites. Depending on the measured drug concentrations, the dose can be adjusted for each patient [6, 9]. The recommended threshold concentration of endoxifen is 5.97 ng/ml in serum [10, 11] and 3.3 ng/ml in dried blood spot samples [12]. The dose can be increased to 40 or 60 mg per day when the measured level is lower than the threshold level [13].

TDM of tamoxifen requires a sensitive, selective, accurate, precise, and reliable bioanalytical method. Various bioanalytical methods of tamoxifen and its metabolites have been developed. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the most often used analytical instrument due to being more sensitive and selective in determining very low analyte concentrations than other instruments such as gas chromatography-tandem mass spectrometry (GC-MS) and high-performance liquid chromatography with ultraviolet detector (HPLC-UV) [8, 10-12, 14-19, 20-24]. Many biological matrices have been used, such as urine [19], scalp hair [23], serum [20], plasma [8, 16, 17, 24], dried blood spot (DBS) [10-12, 18], and volumetric absorptive microsampling (VAMS) [25]. The sample preparations were also different: protein precipitation, liquid-liquid extraction, and solid-phase extraction [8, 10-12, 14-19, 20-24].

This article reviews bioanalytical methods of tamoxifen and its metabolites and their application for TDM in breast cancer patients to reach individualized medicine. The literature was collected from online databases such as Google Scholar, PubMed, ScienceDirect, and Directory of Open Access Journals. The data used in the review were gathered from English-language articles published between 2008 and 2023.

### Breast cancer

Breast cancer is a malignant tumor that develops as a result of DNA damage and gene mutation in breast cells such as ductal, lobular, and both. The development and occurrence of breast cancer are caused by a variety of factors, including genetic and non-genetic factors (table 1) [3, 26-30].

**Table 1: The primary factors responsible for the occurrence and development of breast cancer**

Factors	Detail	References
Genetic	The woman born to mother with breast cancer	[3, 29]
Reproductive	The woman with premature menarche, late menopause, nulliparity, short breastfeeding period, high testosterone level	[3, 26]
Lifestyle-related	Obesity, smoking, alcohol consumption, physical inactivity, unhealthy diet	[3, 27-29]
Environmental	Exposure to pollution and exogenous estrogen	[3, 29]

Breast cancer patients can be treated locally and systemically. The local treatments include surgery and radiotherapy. The systemic therapies are chemotherapy, immunotherapy, endocrine therapy, and targeted therapy [3-5, 31]. Cancer cell proliferation is associated with abnormal expression of estrogen receptor (ER) and human epidermal growth factor 2 (HER2) [32]. Approximately 70% of breast cancer cases were caused by abnormal expression of ER. Therefore, endocrine therapy is the most widely used treatment for breast cancer [33-35].

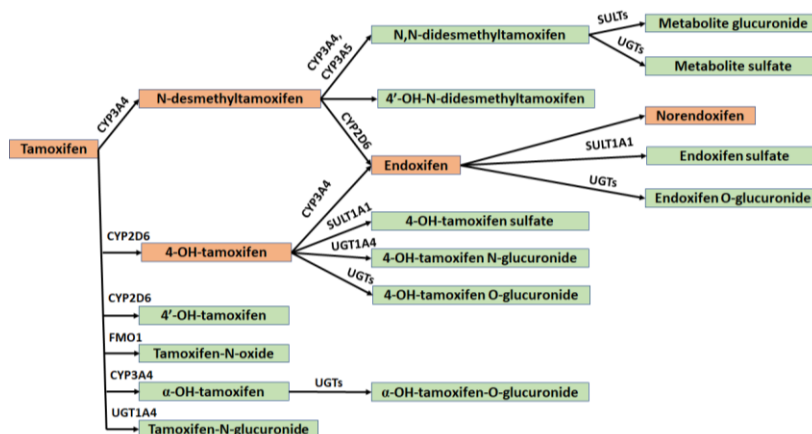
### Tamoxifen

Tamoxifen is a triphenylethylene derivative that acts as a selective estrogen receptor modulator (SERM). It antagonizes the effect of estrogen on breast tissue and reproductive organs. During the last 40 y, tamoxifen has been the most frequently used endocrine agent in breast cancer treatment because it can reduce recurrence and

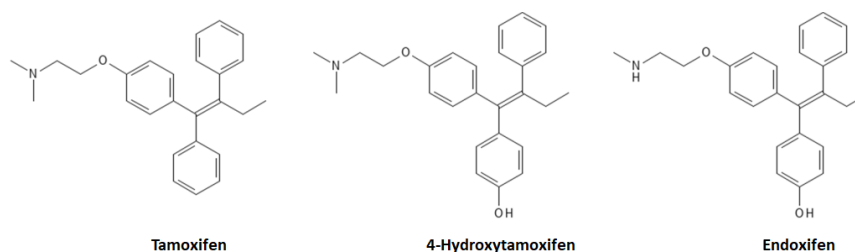
mortality rates after years of treatment [33-35]. On the other hand, tamoxifen has many side effects, especially above toxic levels, such as diarrhea, anorexia, nausea, vomiting [36], stroke, uterine cancer, and cataract [33].

### Tamoxifen pharmacokinetics

Tamoxifen is rapidly metabolized into a variety of metabolites in the liver (fig. 1). Almost 90% of tamoxifen is demethylated by CYP3A4 to N-desmethyltamoxifen during phase I metabolism, and then CYP2D6 oxidizes it to 4-hydroxy-N-desmethyltamoxifen (endoxifen). The minor route of tamoxifen metabolism is the hydroxylation reaction of tamoxifen to 4-hydroxytamoxifen. The active metabolites of tamoxifen are endoxifen and 4-hydroxytamoxifen, which have antiestrogenic action that is 30-100 times greater than tamoxifen itself [6-8, 33, 34, 37]. The structure of tamoxifen and its active metabolites can be seen in fig. 2.



**Fig. 1: Tamoxifen metabolic pathway**  
Orange: compounds which have antiestrogenic activity



**Fig. 2: The structure of tamoxifen and its active metabolites (4-hydroxytamoxifen and endoxifen)**

The phase II reactions of tamoxifen are the sulfation and glucuronidation reaction catalyzed by sulfotransferase (SULT) and uridine 5'-diphospho-glucuronosyltransferase (UGT). SULT and UGT play a vital role in tamoxifen and its metabolite elimination [37].

### Tamoxifen pharmacogenetics

Many studies reported that there are more than 100 polymorphisms of CYP2D6. CYP3A4 has fewer polymorphisms compared to CYP2D6. SULT and UGT, the vital enzymes in tamoxifen elimination, also have polymorphisms. These polymorphisms are associated with variation

of catalytic activity in the tamoxifen metabolism that significantly correlates with interpatient variability in the concentration of endoxifen and 4-hydroxy tamoxifen. The therapy using tamoxifen should be optimized by identifying genetic variation in CYP2D6, CYP3A4, SULT, and UGT [37-40].

### Therapeutic Drug Monitoring (TDM)

In the last few years, many studies reported the relationship between pharmacokinetics and pharmacogenetics, introducing the concept of "individualized", "personalized" or "precision" medicine.

The high genetic variability of drug-metabolizing enzymes causes various therapeutic effects among patients. This concept suggested therapeutic drug monitoring (TDM) which measures the drug concentrations in biological matrices to ensure that the concentrations are in the therapeutic window. Measured concentration can be used to decide the individualized dose to reach the minimum effective concentration (MEC) and prevent achieving the minimum toxic concentration (MTC). Therefore, the treatments would be more accurate and precise for each patient [9, 41-44].

#### TDM of tamoxifen

TDM of tamoxifen can be carried out by measuring the level of its active metabolites. The recommended threshold concentration of endoxifen is 5.97 ng/ml in serum [10, 11] and 3.3 ng/ml in dried blood spot samples [12]. The initial dose of tamoxifen is 20 mg per day for 8 w. The dose can be increased to 40 or 60 mg per day when the measured level is lower than the threshold level [13]. Therapeutic drug monitoring is proposed to select the right dose of tamoxifen for each breast cancer patient [6, 37].

#### Biological matrices for TDM

There are many biological matrices used for TDM. Currently, the gold standard matrices are serum and plasma. The other matrices, such as urine, saliva, cerebrospinal fluid, hair, peripheral blood, tissue biopsies, sweat, tears, and breast milk, also can be alternative matrices for TDM. The considerations in the matrix selection are the physicochemical properties of the analyte. Moreover, minimizing the stress level of breast cancer patients is important to be considered. These two critical requirements can be fulfilled by the microsampling technique using peripheral blood as the matrices. The microsampling technique needs very low sample volumes (<50  $\mu$ l) which is less invasive than the serum or plasma sampling technique [43, 45].

#### Bioanalytical method of tamoxifen and its metabolites

##### Urine

The bioanalytical method of tamoxifen in urine has been developed by Rathi *et al.*, 2016 [19]. Tamoxifen is rapidly metabolized to 3-hydroxy-4-methoxy tamoxifen, which can be found in urine. The urine sample was collected in a container, then added azide sodium as a preservative. The sample was extracted with a solid-phase extraction procedure. 50  $\mu$ l of internal standard (17 $\alpha$ -methyltestosterone, chlorotestosterone acetate, d3-testosterone, and d3-epitestosterone) and the sample were poured into the XAD-2 column. The first step was washing the XAD-2 column with distilled water, followed by elution with 0.5 ml methanol repeated 5 times. The remaining methanol in the screw test tube was appropriately squeezed out of the XAD-2 column. The hydrolysis procedure was conducted at 60  $^{\circ}$ C for one hour. The solution was cooled to room temperature and its pH was adjusted to range between 9 and 10. 5 ml of tertiary-butyl methyl ether was added into the mixture, shaken for 10 min, and centrifugated at 3000 rpm for 5 min. The organic layer was separated, added 1 mg of Na<sub>2</sub>SO<sub>4</sub>, then evaporated under nitrogen at 60  $^{\circ}$ C.

The second step was the derivatization of the dried sample by adding 50  $\mu$ l of derivatizing agent (N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), iodotrimethylsilane (iodo-TMS), and dithioerythritol (DTE) 1000:2:2). The sample was derivatized at 60  $^{\circ}$ C for 30 min. 20  $\mu$ l of aliquot was injected into GC-MS.

The GC-MS system was performed using an ultra-1-dimethylpolymer polysiloxane fused silica capillary (17 m x 0.22 mm, 0.11  $\mu$ m), operated in SIM and scan modes. Helium was flowing at a rate of 0.2 ml/min. The oven temperature was set to rise from 180  $^{\circ}$ C for one minute, hold, to 229  $^{\circ}$ C at a rate of 3  $^{\circ}$ C per minute, then from 229  $^{\circ}$ C to 300  $^{\circ}$ C at a rate of 40  $^{\circ}$ C per minute. The total time for running was 23.1 min.

The bioanalytical method was applied to analyze the urine samples of one healthy subject taking a single dose of tamoxifen (20 mg). The range of the tamoxifen metabolite concentrations in the urine samples was 3–90 ng/ml.

##### Scalp hair

The bioanalytical method of tamoxifen in scalp hair has been developed by Drooger *et al.*, 2015 [23]. The scalp hair sample was

extracted with a liquid-liquid extraction procedure. The samples were collected from around 150-300 hair strands, and the root ends were then cut into pieces measuring around 1 mm long. Tamoxifen-d<sub>5</sub>, N-desmethyl-tamoxifen-d<sub>5</sub>, 4-hydroxy-tamoxifen-d<sub>5</sub>, endoxifen-d<sub>5</sub> as the internal standard (50  $\mu$ l), methanol (950  $\mu$ l), and hair samples (10-30 mg) were combined, then agitated for 24 h at room temperature while being shielded from light. The organic phase was separated and then evaporated under nitrogen at 60  $^{\circ}$ C. The dried sample was reconstituted with sodium carbonate buffer pH 8.8 (200  $\mu$ l) and a combination of hexane/2-propanol (95:5) (1 ml). The mixture was vortexed and centrifugated at 18,000 x g for ten minutes. The organic layer (800  $\mu$ l) was dried up under nitrogen at 60  $^{\circ}$ C. The dried sample was reconstituted in the mixture of water/acetonitrile/formic acid, 60:40:0.1 v/v/v (100  $\mu$ l), and then centrifugated at 4,000 x g for five minutes. 5  $\mu$ l of aliquot was injected into the ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

The UPLC-MS/MS system was performed using an Acquity UPLC<sup>®</sup> BEH C<sub>18</sub> column (100 mm x 2.1 mm, 1.7  $\mu$ m). The mixture of ammonium format 0.2 mmol and acetonitrile, both containing 0.1% formic acid, was used as the mobile phase. The flow rate was 0.3 ml/min with a linear gradient. The run time was 10 min.

The bioanalytical method was used in the determination of tamoxifen and its active metabolites from 8 breast cancer women taking 20 mg of tamoxifen every day for a period of 6 mo to 5 y. Tamoxifen, endoxifen, 4-hydroxytamoxifen, and N-desmethyltamoxifen had average concentrations of 1.15 pmol/mg, 0.0931 pmol/mg, 0.0471 pmol/mg, and 1.46 pmol/mg, respectively. The different hair pigmentation was related to the measured concentrations of the analyte. In comparison to samples of blond and brown hair, the concentrations in black hair were higher. In addition, UV irradiation also influenced the analytical result.

##### Serum

The bioanalytical method of tamoxifen in serum has been developed by Teunissen *et al.*, 2011 [20] and applied by Pistilli *et al.*, 2020 [21]. The serum sample was extracted with a protein precipitation procedure. 50  $\mu$ l of the serum sample was added to 150  $\mu$ l of internal standard (tamoxifen-d<sub>5</sub>, N-desmethyltamoxifen-d<sub>5</sub>, N-desmethyl-4-hydroxytamoxifen-d<sub>5</sub>, and 4-hydroxytamoxifen-d<sub>5</sub>), vortexed for 10 s and then centrifugated at 11,300 x g for 10 min. 120  $\mu$ l of the supernatant was separated and dried up under nitrogen at 30  $^{\circ}$ C. The dried sample was reconstituted in 60  $\mu$ l of the mobile phase. 15  $\mu$ l of aliquot was injected into the high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

The HPLC-MS/MS system was performed using a C18 column (150 mm x 2.1 mm, 2.6  $\mu$ m). The mixture of acetonitrile–5 mmol ammonium formate buffer pH 3.5 (3:7) was used as the mobile phase. The flow rate was 0.4 ml/min with a gradient. The run time was 10 min.

The bioanalytical method was applied to determine the level of tamoxifen and its active metabolites in serum to assess the rate of nonadherence in 188 breast cancer patients. The tamoxifen serum level <60 ng/ml was defined as the adherence threshold. 16.0% of patients had a serum level below the adherence threshold. However, there were only 12.3% of patients reported nonadherence through interviews. Therapeutic drug monitoring may be required to identify patients who did not adhere and were at risk for poorer outcomes.

##### Plasma

Several bioanalytical methods of tamoxifen in plasma have been developed, including by Antunes *et al.*, 2013 using high-performance liquid chromatography with photodiode array detector (HPLC-PDA) with a run time of 16 min [17], Arellano *et al.*, 2014 using UPLC-MS/MS with a run time of 6 min [16], Antunes *et al.*, 2015 using LC-MS/MS with a run time of 8 min [18], and Bobin-Dubigeon *et al.*, 2019 using UPLC-MS/MS with a run time of 4.5 min [8]. The method by Bobin-Dubigeon *et al.*, 2019 was the fastest one, suitable for time- and cost-efficiency.

The plasma sample (100  $\mu$ l) was added to formic acid 1% (100  $\mu$ l), and then vortexed for 30 s. N-desmethyltamoxifen-d<sub>5</sub>, 4-

hydroxytamoxifen-d5, and endoxifen-d5 as the internal standard (400 µl), and methanol (100 µl) were added to the mixture, followed by shaking and centrifugation at 18,000 x g for ten minutes at 4 °C. The separated supernatant (300 µl) was combined with 300 µl of ammonium format 2 mmol containing formic acid 0.2%. 7 µl of aliquot was injected into UPLC-MS/MS.

The UPLC-MS/MS system was carried out using a C18 (50 x 2.1 mm, 1.7 µm) column. The mobile phase was ammonium format 2 mmol-acetonitrile, both containing formic acid 0.5%. The flow rate was 0.6 ml/min with a gradient.

The application of the bioanalytical method was in the analysis of tamoxifen and its active metabolites in 10 breast cancer patients. The measured concentrations of tamoxifen, N-desmethyltamoxifen, endoxifen, and 4-hydroxytamoxifen were 108–330 ng/ml, 152–329 ng/ml, 3.55–15.21 ng/ml, and 0.91–2.63 ng/ml, respectively. Interpatient variability of the concentration was associated with CYP2D6 polymorphisms. Therapeutic drug monitoring was needed to individualize the dose of tamoxifen according to plasma concentration.

#### Dried blood spot (DBS)

Several bioanalytical methods of tamoxifen in DBS samples have been developed by Antunes *et al.*, 2015 [18], Tré-Hardy *et al.*, 2016 [11], Harahap *et al.*, 2019 [12], and Maggadani *et al.*, 2021 [25]. All of them used LC-MS/MS, with the run time of 8 min, 6.5 min, 4 min, and 5 min, respectively. The method by Harahap *et al.*, 2019 was the fastest one, suitable for time-and cost-efficiency.

Whole blood (20 µl) was spotted on the DBS card and allowed to dry for 2 h. The blood spot was cut, added methanol (1 ml), and vortexed for one minute, followed by sonication for 25 min. The sample (850 µl) was evaporated under nitrogen at 55 °C. The dried sample was reconstituted in 0.1% formic acid in water–0.1% formic acid in acetonitrile (35:65), vortexed, and centrifugated at 3000 rpm for ten minutes. 10 µl of aliquot was injected into LC-MS/MS.

The LC-MS/MS system was performed using a C18 (2.1 x 100 mm, 1.7 µm) column. The mobile phase was 0.1% formic acid in water–0.1% formic acid in acetonitrile (35:65), flowing at a rate of 0.25 ml/min with an isocratic system.

The application of the bioanalytical method was in the determination of tamoxifen and its active metabolites in 29 breast cancer patients. The measured concentrations of tamoxifen,

endoxifen, and 4-hydroxytamoxifen were 30.29–188.63 ng/ml, 1.45–28.77 ng/ml, 0.21–11.28 ng/ml, respectively. Interpatient variability was associated with polymorphisms of CYP2D6 and CYP3A4 and drug interactions. There were 2 patients with endoxifen concentrations below the clinical threshold (<3.30 ng/ml).

#### Volumetric absorptive microsampling (VAMS)

The bioanalytical method of tamoxifen in the VAMS sample has been developed by Maggadani *et al.*, 2021 [25]. The tip of the VAMS sampler was dipped into the whole blood for 2 s and dried for 1-3 h. The dried tip was removed from the plastic handle and then extracted with 1 ml of methanol (containing 100 ng/ml of propranolol), vortexed for 1 min, followed by sonication for 25 min. The sample (850 µl) was evaporated under nitrogen at 50 °C. The dried sample was reconstituted in 100 µl of 0.1% formic acid in water–0.1% formic acid in acetonitrile (5:95), vortexed, and centrifugated at 805 x g for 10 min. 10 µl of aliquot was injected into LC-MS/MS.

The LC-MS/MS system was carried out using a C18 (2.1 x 100 mm, 1.7 µm) column. 0.1% formic acid in water and 0.1% formic acid in acetonitrile was used as the mobile phase, eluted with a gradient system. The composition of 5:95 was held for 3 min, then altered to the composition of 70:30 and held for 2 min. The flow rate was 0.20 ml/min.

The application of the bioanalytical method was in the analysis of tamoxifen and its metabolite concentration from 30 breast cancer patients. The measured concentrations were 36.14–233.31 ng/ml for tamoxifen, 3.78–30.03 ng/ml for endoxifen, 1.55–6.56 ng/ml for 4-hydroxytamoxifen, and 48.80–385.01 ng/ml for N-desmethyltamoxifen. There was interpatient variability related to polymorphisms of CYP2D6 and CYP3A4 and drug interactions.

#### Bioanalytical method of tamoxifen for TDM in breast cancer patients

Various bioanalytical methods of tamoxifen and its active metabolites in several matrices are summarized in table 2. Endoxifen and 4-hydroxytamoxifen are biomarkers in therapeutic drug monitoring of tamoxifen; however, the method by Rathi *et al.*, 2016 [19] was unable to determine them. Moreover, there was derivatization, which required more reagents for derivatizing agents and a longer time for sample preparation so it would be more expensive.

Table 2: Summary of several bioanalytical method of tamoxifen and its metabolites

Matrices	Sample preparation	Analytical instruments	Run time (min)	References
Urine	Solid-phase extraction	GC-MS	23.1	Rathi <i>et al.</i> , 2016 [19]
Scalp hair	Liquid-liquid extraction	UPLC-MS/MS	10	Drooger <i>et al.</i> , 2015 [23]
Serum	Protein precipitation	HPLC-MS/MS	10	Teunissen <i>et al.</i> , 2011 [20] and Pistilli <i>et al.</i> , 2020 [21]
Plasma	Liquid-liquid extraction	HPLC-PDA	16	Antunes <i>et al.</i> , 2013 [17]
Plasma	Liquid-liquid extraction	UPLC-MS/MS	6	Arellano <i>et al.</i> , 2014 [16]
Plasma	Liquid-liquid extraction	LC-MS/MS	8	Antunes <i>et al.</i> , 2015 [18]
Plasma	Protein precipitation	UPLC-MS/MS	4.5	Bobin-Dubigeon <i>et al.</i> , 2019 [8]
DBS	Liquid-liquid extraction	LC-MS/MS	8	Antunes <i>et al.</i> , 2015 [18]
DBS	Protein precipitation	LC-MS/MS	6.5	Tré-Hardy <i>et al.</i> , 2016 [11]
DBS	Protein precipitation	LC-MS/MS	4	Harahap <i>et al.</i> , 2019 [12]
DBS	Protein precipitation	LC-MS/MS	5	Maggadani <i>et al.</i> , 2021 [25]
VAMS	Protein precipitation	LC-MS/MS	5	Maggadani <i>et al.</i> , 2021 [25]

The simplicity of the sample preparation procedure is important to be considered. The method by Drooger *et al.*, 2015 [23] was relatively difficult to be applied due to the cutting process into very small samples (1 mm). In addition, liquid-liquid extraction is needed a longer time and is more expensive than protein precipitation. The methods in serum, plasma, and DBS use protein precipitation as sample preparation with methanol.

Therapeutic drug monitoring will be done repeatedly so that the method should be comfortable for breast cancer patients. Serum and plasma sampling techniques are more invasive. In addition, the methods in serum and plasma require the phlebotomist to take

samples and the refrigerator to freeze samples so they would be more expensive.

DBS and VAMS are the newer biosampling techniques that collect the microvolume sample from finger capillary blood, which is less invasive than venipuncture. The sample is easy to be taken by the patient themselves without a phlebotomist. The collected sample is dried thus, it is more stable and can be stored at room temperature without the refrigerator requirement [10, 11, 45]. According to table 2, the shortest run time was the method by Harahap *et al.*, 2020 which used DBS as the biosampling technique. The method can be used to quantify the concentration of tamoxifen and its active

metabolites, which are 4-hydroxytamoxifen and endoxifen [12]. The comparative study conducted by Maggadani *et al.*, 2021 showed that there is no statistically significant difference between using VAMS and DBS in the analysis of tamoxifen and its metabolites [25]. In addition, the cost of the Whatman™ 903 DBS card is 7 times lower than the Mitra™ VAMS sampler. The card also takes less space in storage [46].

## CONCLUSION

Several bioanalytical methods of tamoxifen and its metabolites have been developed in various biological matrices: urine, scalp hair, serum, plasma, DBS, and VAMS samples, with different sample preparations. The most frequently used analytical instrument was LC-MS/MS. The bioanalytical method of tamoxifen and its metabolites in the DBS sample was more suitable in the TDM application due to the low invasive sampling technique, more stable sample, and rapid sample preparation. Therefore, it is more time- and cost-efficient than the other methods. This method can be applied for therapeutic drug monitoring in breast cancer patients.

## FUNDING

Nil

## AUTHORS CONTRIBUTIONS

All authors have contributed equally

## CONFLICT OF INTERESTS

Declared none

## REFERENCES

1. The Global Cancer Observatory. Indonesia Cancer Fact Sheet; c2020. Available from: <https://gco.iarc.fr/today/data/factsheets/populations/360-indonesia-fact-sheets.pdf>. [Last accessed on 10 Nov 2023]
2. The Global Cancer Observatory. World Cancer Fact Sheet; c2020. Available from: <https://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf>. [Last accessed on 10 Nov 2023]
3. Costa B, Amorim I, Gartner F, Vale N. Understanding Breast cancer: from conventional therapies to repurposed drugs. *Eur J Pharm Sci.* 2020;151:105401. doi: 10.1016/j.ejps.2020.105401. PMID 32504806.
4. McDonald ES, Clark AS, Tchou J, Zhang P, Freedman GM. Clinical diagnosis and management of breast cancer. *J Nucl Med.* 2016;57Suppl 1:9S-16S. doi: 10.2967/jnumed.115.157834, PMID 26834110.
5. Fang X, Cao J, Shen A. Advances in anti-breast cancer drugs and the application of nano-drug delivery systems in breast cancer therapy. *J Drug Deliv Sci Technol.* 2020;57:101662. doi: 10.1016/j.jddst.2020.101662.
6. Jager NGL, Linn SC, Schellens JHM, Beijnen JH. Tailored tamoxifen treatment for breast cancer patients: a perspective. *Clin Breast Cancer.* 2015;15(4):241-4. doi: 10.1016/j.clbc.2015.04.005, PMID 25997856.
7. Sanchez Spitman AB, Swen JJ, Dezentje VO, Moes DJAR, Gelderblom H, Guchelaar HJ. Clinical pharmacokinetics and pharmacogenetics of tamoxifen and endoxifen. *Expert Rev Clin Pharmacol.* 2019;12(6):523-36. doi: 10.1080/17512433.2019.1610390, PMID 31008668.
8. Bobin Dubigeon C, Campone M, Rossignol E, Salaun E, Amiard MB, Bard JM. New UPLC-MS/MS assay for the determination of tamoxifen and its metabolites in human plasma, application to patients. *Future Sci OA.* 2019;5(5):FSO374. doi: 10.2144/foa-2018-0113, PMID 31245038.
9. Groenland SL, Mathijssen RHJ, Beijnen JH, Huitema ADR, Steeghs N. Individualized dosing of oral targeted therapies in oncology is crucial in the era of precision medicine. *Eur J Clin Pharmacol.* 2019;75(9):1309-18. doi: 10.1007/s00228-019-02704-2, PMID 31175385.
10. Maggadani BP, Harahap Y, Harmita HSJ, Haryono SJ, Sitorus TRJ. Simple and rapid method for the simultaneous analysis of tamoxifen, endoxifen, and 4-hydroxytamoxifen in dried blood spot using liquid chromatography-tandem mass spectrometry. *Int J App Pharm.* 2020;12(3):112-20. doi: 10.22159/ijap.2020v12i3.36434.
11. Tre Hardy M, Capron A, Antunes MV, Linden R, Wallemacq P. Fast method for simultaneous quantification of tamoxifen and metabolites in dried blood spots using an entry-level LC-MS/MS system. *Clin Biochem.* 2016;49(16-17):1295-8. doi: 10.1016/j.clinbiochem.2016.07.018, PMID 27498307.
12. Harahap Y, Maggadani BP, Sitorus TRJ, Mulyadi CA, Purwanto DJ. Clinical application of dried blood spot for monitoring studies of tamoxifen, endoxifen, and 4-hydroxytamoxifen in breast cancer patient using liquid chromatography-tandem mass spectrometry. *Int J App Pharm.* 2019;11(2):59-63. doi: 10.22159/ijap.2019v11i2.30310.
13. Martinez de Dueñas E, Ochoa Aranda E, Blancas Lopez Barajas I, Ferrer Magdalena T, Bandres Moya F, Chicharro Garcia LM. Adjusting the dose of tamoxifen in patients with early breast cancer and CYP2D6 poor metabolizer phenotype. *Breast.* 2014;23(4):400-6. doi: 10.1016/j.breast.2014.02.008, PMID 24685597.
14. Jager NGL, Rosing H, Linn SC, Schellens JHM, Beijnen JH. Importance of highly selective LC-MS/MS analysis for the accurate quantification of tamoxifen and its metabolites: focus on endoxifen and 4-hydroxytamoxifen. *Breast Cancer Res Treat.* 2012;133(2):793-8. doi: 10.1007/s10549-012-2000-1, PMID 22388692.
15. Teunissen SF, Rosing H, Seoane MD, Brunsveld L, Schellens JHM, Schinkel AH. Investigational study of tamoxifen phase I metabolites using chromatographic and spectroscopic analytical techniques. *J Pharm Biomed Anal.* 2011;55(3):518-26. doi: 10.1016/j.jpba.2011.02.009, PMID 21392921.
16. Arellano C, Allal B, Goubaa A, Roche H, Chatelut E. An UPLC-MS/MS method for separation and accurate quantification of tamoxifen and its metabolites isomers. *J Pharm Biomed Anal.* 2014;100:254-61. doi: 10.1016/j.jpba.2014.07.033, PMID 25173109.
17. Antunes MV, Rosa DD, Viana Tdos S, Andreolla H, Fontanive TO, Linden R. Sensitive HPLC-PDA determination of tamoxifen and its metabolites N-desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen in human plasma. *J Pharm Biomed Anal.* 2013;76:13-20. doi: 10.1016/j.jpba.2012.12.005, PMID 23291438.
18. Antunes MV, Raymundo S, De Oliveira V, Staudt DE, Gosling G, Peteffi GP. Ultra-high performance liquid chromatography tandem mass spectrometric method for the determination of tamoxifen, N-desmethyl tamoxifen, 4-hydroxytamoxifen and endoxifen in dried blood spots—development, validation and clinical application during breast cancer adjuvant therapy. *Talanta.* 2015;132:775-84. doi: 10.1016/j.talanta.2014.10.040, PMID 25476377.
19. Rathi A, Jain DS, Beotra DA, Trivedi DM, Nimker V, Kumar DR. Urine excretion study of tamoxifen metabolite, 3-hydroxy-4-methoxy tamoxifen by GC-MS. *J Drug Deliv Ther.* 2016;6(1):19-24. doi: 10.22270/jddt.v6i1.1194.
20. Teunissen SF, Jager NGL, Rosing H, Schinkel AH, Schellens JHM, Beijnen JH. Development and validation of a quantitative assay for the determination of tamoxifen and its five main phase I metabolites in human serum using liquid chromatography coupled with tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2011;879(19):1677-85. doi: 10.1016/j.jchromb.2011.04.011, PMID 21543272.
21. Pistilli B, Paci A, Ferreira AR, Di Meglio A, Poinsignon V, Bardet A. Serum detection of nonadherence to adjuvant tamoxifen and breast cancer recurrence risk. *J Clin Oncol.* 2020;38(24):2762-72. doi: 10.1200/JCO.19.01758, PMID 32568632.
22. Jager NGL. Bioanalysis and clinical pharmacology of tamoxifen in breast cancer; c2014. Available from: <https://dSPACE.library.uu.nl/handle/1874/297733> [Last accessed on 10 Nov 2023]
23. Drooger JC, Jager A, Lam MH, den Boer MD, Sleijfer S, Mathijssen RHJ. Development and validation of an UPLC-MS/MS method for the quantification of tamoxifen and its main metabolites in human scalp hair. *J Pharm Biomed Anal.* 2015;114:416-25. doi: 10.1016/j.jpba.2015.06.018, PMID 26119504.
24. Ximenez JPB, De Andrade JM, Marques MP, Coelho EB, Suarez Kurtz G, Lanchote VL. Hormonal status affects plasma exposure of tamoxifen and its main metabolites in tamoxifen-treated

- breast cancer patients. *BMC Pharmacol Toxicol.* 2019;20Suppl 1:81. doi: 10.1186/s40360-019-0358-y, PMID 31852530.
25. Maggadani BP, Harahap Y, Harmita, Haryono SJ, Untu CWP. Analysis of tamoxifen and its metabolites in dried blood spot and volumetric absorptive microsampling: comparison and clinical application. *Heliyon Heliyon.* 2021;7(6):e07275. doi: 10.1016/j.heliyon.2021.e07275, PMID 34179536.
  26. Hanf V, Hanf D. Reproduction and breast cancer risk. *Breast Care (Basel).* 2014;9(6):398-405. doi: 10.1159/000369570, PMID 25759622.
  27. Wielsøe M, Gudmundsdottir S, Bonefeld-Jørgensen EC. Reproductive history and dietary habits and breast cancer risk in Greenlandic Inuit: a case-control study. *Public Health.* 2016;137:50-8. doi: 10.1016/j.puhe.2016.06.016, PMID 27450442.
  28. Shah R, Rosso K, Nathanson SD. Pathogenesis, prevention, diagnosis and treatment of breast cancer. *World J Clin Oncol.* 2014;5(3):283-98. doi: 10.5306/wjco.v5.i3.283, PMID 25114845.
  29. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L. Breast cancer development and progression: risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes Dis.* 2018;5(2):77-106. doi: 10.1016/j.gendis.2018.05.001, PMID 30258937.
  30. Mehdi S, Chauhan A, Dhutty A. Cancer and new prospective to treat cancer. *Int J Curr Pharm Sci.* 2023;15(6):16-22. doi: 10.22159/ijcpr.2023v15i6.3078.
  31. Mansouri S, Farahmand L, Hosseinzade A, Eslami S Z, Majidzadeh AK. Estrogen can restore tamoxifen sensitivity in breast cancer cells amidst the complex network of resistance. *Biomed Pharmacother.* 2017;93:1320-5. doi: 10.1016/j.biopha.2017.07.057, PMID 28747013.
  32. Venugopal J, SP, AR. A review on screening methods of breast cancer, cancer biomarkers and phytoconstituents against breast cancer. *Asian J Pharm Clin Res* 2017;10(12):17-21. doi: 10.22159/ajpcr.2017.v10i12.21039.
  33. Suganya J, Radha M, Naorem DL, Nishandhini M. *In silico* docking studies of selected flavonoid-natural healing agents against breast cancer. *Asian Pac J Cancer Prev.* 2014;15(19):8155-9. doi: 10.7314/apjcp.2014.15.19.8155, PMID 25338999.
  34. Goetz MP, Sangkuhl K, Guchelaar HJ, Schwab M, Province M, Whirl Carrillo M. Clinical pharmacogenetics implementation consortium (CPIC) guideline for CYP2D6 and tamoxifen therapy. *Clin Pharmacol Ther.* 2018;103(5):770-7. doi: 10.1002/cpt.1007, PMID 29385237.
  35. Clifford RE, Bowden D, Blower E, Kirwan CC, Vimalachandran D. Does tamoxifen have a therapeutic role outside of breast cancer? A systematic review of the evidence. *Surg Oncol.* 2020;33:100-7. doi: 10.1016/j.suronc.2020.02.006, PMID 32561074.
  36. KS. Tamoxifen: pharmacokinetics and pharmacodynamics. *Open Access J Pharm Res.* 2017;1(8).
  37. Wigle TJ, Jansen LE, Teft WA, Kim RB. Pharmacogenomics guided personalization of warfarin and tamoxifen. *J Pers Med.* 2017;7(4):20. doi: 10.3390/jpm7040020, PMID 29236081.
  38. Lee CI, Low SK, Maldonado R, Fox P, Balakrishnar B, Coulter S. Simplified phenotyping of CYP2D6 for tamoxifen treatment using the N-desmethyl-tamoxifen/ endoxifen ratio. *Breast.* 2020;54:229-34. doi: 10.1016/j.breast.2020.10.008, PMID 33161337.
  39. Binkhorst L, Mathijssen RHJ, Jager A, Van Gelder T. Individualization of tamoxifen therapy: much more than just CYP2D6 genotyping. *Cancer Treat Rev.* 2015;41(3):289-99. doi: 10.1016/j.ctrv.2015.01.002, PMID 25618289.
  40. Gjerde J, Hauglid M, Breilid H, Lundgren S, Varhaug JE, Kisanga ER. Effects of CYP2D6 and SULT1A1 genotypes, including SULT1A1 gene copy number on tamoxifen metabolism. *Ann Oncol.* 2008;19(1):56-61. doi: 10.1093/annonc/mdm434, PMID 17947222.
  41. Mao X, Cheung YK. Sequential designs for individualized dosing in phase I cancer clinical trials. *Contemp Clin Trials.* 2017;63:51-8. doi: 10.1016/j.cct.2016.08.018, PMID 27592121.
  42. Hertz DL, Rae JM. Individualized tamoxifen dose escalation: confirmation of feasibility, question of utility. *Clin Cancer Res.* 2016;22(13):3121-3. doi: 10.1158/1078-0432.CCR-16-0370, PMID 27012810.
  43. Avataneo V, D'Avolio A, Cusato J, Cantù M, De Nicolò A. LC-MS application for therapeutic drug monitoring in alternative matrices. *J Pharm Biomed Anal.* 2019;166:40-51. doi: 10.1016/j.jpba.2018.12.040, PMID 30609393.
  44. Iyer RK, Athreya AP, Wang L, Weinshilboum RM. Artificial intelligence and pharmacogenomics. *Adv Mol Pathol.* 2019;2(1):111-8. doi: 10.1016/j.yamp.2019.08.003.
  45. Londhe V, Rajadhyaksha M. Opportunities and obstacles for microsampling techniques in bioanalysis: special focus on DBS and VAMS. *J Pharm Biomed Anal.* 2020;182:113102. doi: 10.1016/j.jpba.2020.113102, PMID 32014628.
  46. Paniagua Gonzalez L, Lendoiro E, Otero Anton E, Lopez Rivadulla M, de-Castro-Rios A, Cruz A. Comparison of conventional dried blood spots and volumetric absorptive microsampling for tacrolimus and mycophenolic acid determination. *J Pharm Biomed Anal.* 2022;208:114443. doi: 10.1016/j.jpba.2021.114443, PMID 34735994.