

TAMOXIFEN/ENDOXIFENE IN PLASMA BY LC/MS – Code LC92010

INTRODUCTION

In recent decades, Tamoxifen has been the most important therapeutic agent for the treatment of estrogen-receptor-positive breast cancers. Tamoxifen binds to the estrogen receptor by inhibiting the binding of estrogen and the subsequent proliferation of the tumor. The drug is metabolized in the liver from isoform CYP2D6 cytochrome P450, resulting in the formation of active metabolites. The endoxifene and the 4-OH-tamoxifen have the same binding affinity for the estrogen receptor ER- α and the same capacity to inhibit the proliferation of estrogen-dependent breast tumor cells that express this receptor.

In some individuals a lower formation of active metabolites, due to reduced or absent enzyme CYP2D6 function, may lead to a worse therapeutic response to the drug. In fact, despite the therapeutic benefit tamoxifen has shown in more than three decades of clinical use, with reduction in tumor recurrence (47%) and mortality rates (26%) in patients with ER+ breast cancer, approximately 30% -50% of women treated with this chemotherapy experience relapse and death.

Several clinical studies have shown that patients with zero or reduced metabolism, treated with tamoxifen, have a worse treatment outcome, both in terms of increased risk of recurrence, and in terms of disease-free / recurrence time, than carriers of functional CYP2D6 alleles.

Recent clinical studies have shown that patients with wild-type CYP2D6, simultaneously treated with tamoxifen and strong antidepressant drugs CYP2D6 inhibitors, have lower plasma levels of endoxifene, compared to patients not taking such inhibitors and therefore receive less therapeutic benefit from treatment with tamoxifen.

With these conditions it is clear that Therapeutic Drug Monitoring (TDM) of tamoxifen and endoxifene represents a very timely topic. The analytical methods available to date (UV-VIS HPLC / FLD) make the TDM of these analytes time consuming and poorly aligned to the normal request of routine care in terms of sensitivity, reliability and rapidity of reporting.

This method allows the analysis of both tamoxifen and its main active metabolite (endoxifene) by means of LC-MS / MS reference technique. It provides a natural response to diagnostic and clinical needs to determine, in a reliable, robust, sensitive and rapid manner, the plasma levels and / or serum tamoxifen and its active metabolites.

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This product meets all the requirements of Directive 98/79 / EC on in vitro diagnostic medical devices (IVD). The EC declaration of conformity is available on request.

N° 005	Tamoxifen/Endoxifene in plasma by LC/MS	10/2024
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TECHNICAL FEATURES

Principle of the Method:

Tamoxifen and its primary active metabolite are analyzed by treating the plasma with a suitable deproteinization reagent. The solution is directly injected into the LC / MS.

Recovery:

Endoxifene 99,5%
Tamoxifen 102,7%

Sensitivity (LLOD) :

Endoxifene 0,17 ng/ml
Tamoxifen 0,033 ng/ml

Minimum Concentration analyzable (LLOQ):

Endoxifene 0,5 ng/ml
Tamoxifen 0,1 ng/ml

Linearity:

Endoxifene 0,5 – 100 ng/ml
Tamoxifen 0,1 – 1000 ng/ml

Therapeutic Range in serum :

Endoxifene normal values > 5,6 ng/ml
Tamoxifen normal values >130 ng/ml

1) N. G. L. Jager, H. Rosing, J. H. M. Schellens, S. C. Linn, J. H. Beijnen Tamoxifen dose and serum concentrations of tamoxifen and six of its metabolites in routine clinical outpatient care. Breast Cancer Res Treat (2014) 143:477–483 DOI 10.1007/s10549-013-2826-1

2) Lisa Madlensky, Loki Natarajan, Simone Tchu, Minya Pu, Joanne Mortimer, Shirley W. Flatt, D. Michele Nikoloff, Grantland Hillman, Marcel R. Fontecha, H. Jeffrey Lawrence, Barbara A. Parker, Alan H.B. Wu, and John P. Pierce. Tamoxifen Metabolite Concentrations, CYP2D6 Genotype and Breast Cancer Outcomes. Clin Pharmacol Ther. 2011 May ; 89(5): 718–725. doi:10.1038/clpt.2011.32.

Accuracy intra serie (relative error %)

Endoxifene:

C1	C2	C3
2 ng/ml	6 ng/ml	12 ng/ml
7,7%	7,3%	3,7%

Accuracy inter serie (relative error %)

Endoxifene:

C1	C2	C3
2 ng/ml	6 ng/ml	12 ng/ml
5,3%	5,7%	3,1%

Reproducibility intra serie (relative error %)

Endoxifene:

Ci	Cm	Cs	
1 ng/ml	4 ng/ml	8 ng/ml	
13,9%	11,0%	3,6%	

Reproducibility inter serie (coefficient of variation %)

Endoxifene:

Ci	Cm	Cs	
1 ng/ml	4 ng/ml	8 ng/ml	
11,6%	5,6%	5,0%	

Coefficient of Correlation R2 + Dev Std

0,9937 ± 0,0053 Endoxifene

Accuracy intra serie (relative error %) Tamoxifen :

C1	C2	C3
10 ng/ml	100 ng/ml	250 ng/ml
19,5%	3,4%	3,4%

Accuracy inter serie (relative error %) Tamoxifen :

C1	C2	C3	
10 ng/ml	100 ng/ml	250 ng/ml	
6,3%	7,8%	4,3%	

Reproducibility intra serie (relative error %) Tamoxifen :

Ci	Cm	Cs
1 ng/ml	50 ng/ml	150 ng/ml
3,8%	7,7%	2,3%

Reproducibility inter serie (coefficient of variation %) Tamoxifen :

Ci	Cm	Cs	
1 ng/ml	50 ng/ml	150 ng/ml	
8,8%	6,3%	2,7%	

Coefficient of Correlation R2 + Dev Std

0,9922 ± 0,0074 Tamoxifen

Components of the kit :All Reagents are ready to use and stable 3 years at 2–8 °C, except **Reagent A**.**Reagent A** – Deproteinization Solution + Internal Standard, **1 x 30 ml****Store at -20 °C****Reagent B** – Stabilization Solution, **1 x 20 ml****Calibrator in plasma – Level 1, 1 x 1 ml****Code LC92016 (packed separately – see data sheets)****Calibrator in plasma – Level 2, 1 x 1 ml****Code LC92016 (packed separately – see data sheets)****Calibrator in plasma – Level 3, 1 x 1 ml****Code LC92016 (packed separately – see data sheets)****Calibrator in plasma – Level 4, 1 x 1 ml****Code LC92016 (packed separately – see data sheets)****Calibrator in plasma – Level 5, 1 x 1 ml****Code LC92016 (packed separately – see data sheets)****Reagent M1** – Mobile Phase M1, **2 x 500 ml****Reagent M2** – Mobile Phase M2, **2 x 500 ml****Minimum Instrumental equipment required:**LC-MS triple quadrupole
MRM, ESI positive**Optional Equipment:**

Autosampler

Plasma Collection Procedure:Collect 3 ml of blood with EDTA. Centrifuge **immediately** at 4.000 rpm for 5 min. Plasma samples should be stored at **–20° C** for no longer than **1 month**.**Bibliography**

- 1- Tamoxifen dose and serum concentrations of tamoxifen and six of its metabolites in routine clinical outpatient care. N. G. L. Jager • H. Rosing • J. H. M. Schellens • S. C. Linn • J. H. Beijnen
Breast Cancer Res Treat (2014) 143:477–483 DOI 10.1007/s10549-013-2826-1
- 2- Tamoxifen Metabolite Concentrations, CYP2D6 Genotype and Breast Cancer Outcomes. Lisa Madlensky1, Loki Natarajan1, Simone Tchu2, Minya Pu1, Joanne Mortimer3, Shirley W. Flatt1, D. Michele Nikoloff4, Grantland Hillman4, Marcel R. Fontecha4, H. Jeffrey Lawrence4, Barbara A. Parker1, Alan H.B. Wu2, and John P. Pierce1.
Clin Pharmacol Ther. 2011 May ; 89(5): 718–725. doi:10.1038/clpt.2011.32.

ANALYTICAL PROCEDURE

STEP 1 :

Dispense in eppendorf.

	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Sample	Controls
Calibrator – Level 1	100 µl						
Calibrator – Level 2		100 µl					
Calibrator – Level 3			100 µl				
Calibrator – Level 4				100 µl			
Calibrator – Level 5					100 µl		
Sample						100 µl	
Controls							100 µl
Reagent A – Deproteinization Solution + internal Standard	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl

Vortex for 20 sec.

STEP 2: Centrifuge at 13.000 rpm for 5 minutes.

STEP 3: Take 200 µl of supernatant in a vial and add 200 µl of **Reagent B – Stabilization Solution** (or an adequate volume for the instrument sensitivity)

N.B.: At this step, the sample is stable 1 day at 2-8 °C

INJECTION:

- Inject 20 µl of supernatant in LC system

TAMOXIFEN/ENDOXIFENE IN PLASMA - Warnings

COLUMN CONDITIONING

Install a new analytical RRHD Eclipse Plus C18 (50 x 2,1 mm, 1,8 µm), thermostated at 40 °C. Disconnect the detector and flush a solution of Acetonitrile : H₂O (95 : 5 v/v) set flow at 0,6 ml / min for 15 minutes. **Don't recycle the washing solutions.** Condition further on the column with a solution of Mobile Phase M2 : H₂O (60 : 40 v/v) set flow at 0,3 ml / min for 15 minutes. Finally inject H₂O HPLC grade for 2 times and verify the quality of the HPLC run.

It is NOT possible to make analysis at recycling phase.

If Room Tem. is > 20° C is better to preserve the Mobile Phase at 2 – 8° C among runs.

COLUMN CLEANING

Wash the column with a solution of Mobile Phase M2 : Mobile Phase M1 (95 : 5 v/v) at flow of 600 µl / minutes for 15 minutes. **It recommends flushing the Analytical Column at the end of each run.** Store the column in a solution of Mobile Phase M2 : Mobile Phase M1 (95 : 5 v/v).

INJECTION WASH NEEDLE

Wash with a solution of Water / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).

ESI Sciex 4500 PARAMETERS

Curtain Gas (CUR): 40
Collision Gas (CAD): 8
Ion Spray Voltage: (IS): 4500 V
Temperature (TEM): 550°C
Ion Source Gas 1 (GS1): 60
Ion Source Gas 2 (GS2): 60

FLOW SET

GRADIENT UHPLC			
Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0.01	98	2	300
0.50	98	2	300
6.00	2	98	300
7.00	2	98	300
7.01	98	2	300
8.00	98	2	300

GRADIENT HPLC			
Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0	98	2	300
0.50	98	2	300
6.00	2	98	300
7.00	2	98	300
7.1	98	2	300
13.00	98	2	300

FRAGMENTATIONS (optimized on TRIPLE QUADRUPOLE Sciex 4500)

Analyte	Q1	Q3	COLLISION ENERGY
ENDOXIFENE	374.3	223.0 – 145.0	25.0 – 33.0
TAMOXIFEN	372.3	72.1 – 129.2	50.0 – 35.0
ENDOXIFENE-D5	379.5	379.5	10.0

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC92016	Calibrator in plasma for Tamoxifen/Endoxifene	5 x 2 x 1 ml
LC92019	Control in plasma for Tamoxifen/Endoxifene – Levels 1 and 2	2 x 5 x 1 ml
Analytical Column for UHPLC		
Z959757902	Analytical Column RRHD Eclipse Plus C 18 (50 x 2.1 mm - 1.8 µm)	1 Pc
Analytical Column for HPLC		
Z959793902	Analytical Column Eclipse Plus C 18 (100 x 2.1 mm - 3.5 µm)	1 Pc
S51843550	Clear glass vials with reduced volume	1 x 100 Pcs
S51820717	Caps for clear glass vials with reduced volume	1 x 100 Pcs
SK92010	Starter kit for Tamoxifen/Endoxifene	3 x 1 ml

TAMOXIFEN/ENDOXIFENE IN PLASMA

(Reference Chromatograms for UHPLC systems)

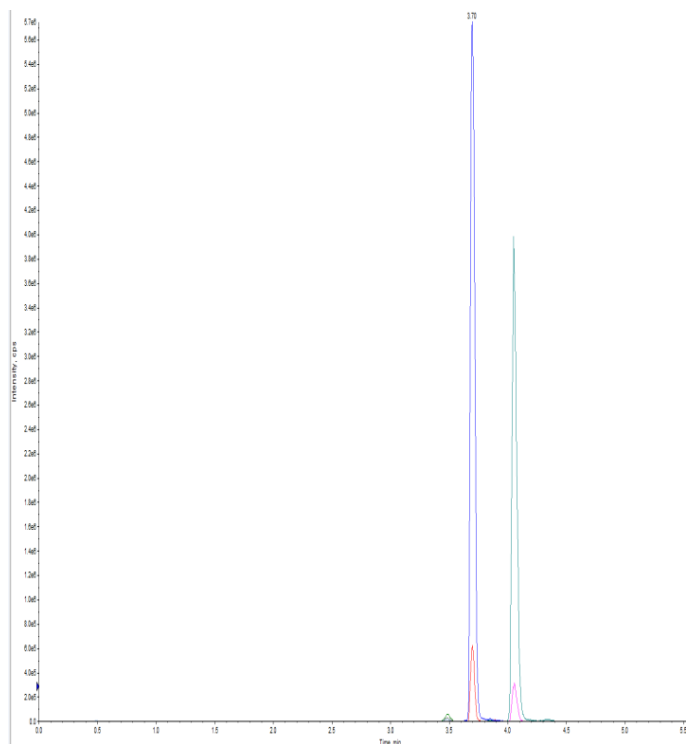


Fig. 1 : Calibrator in plasma		
R.T.	3.48 Endoxifene	30 ng/ml
R.T.	3.70 Tamoxifen	50 ng/ml
R.T.	4.05 IS	

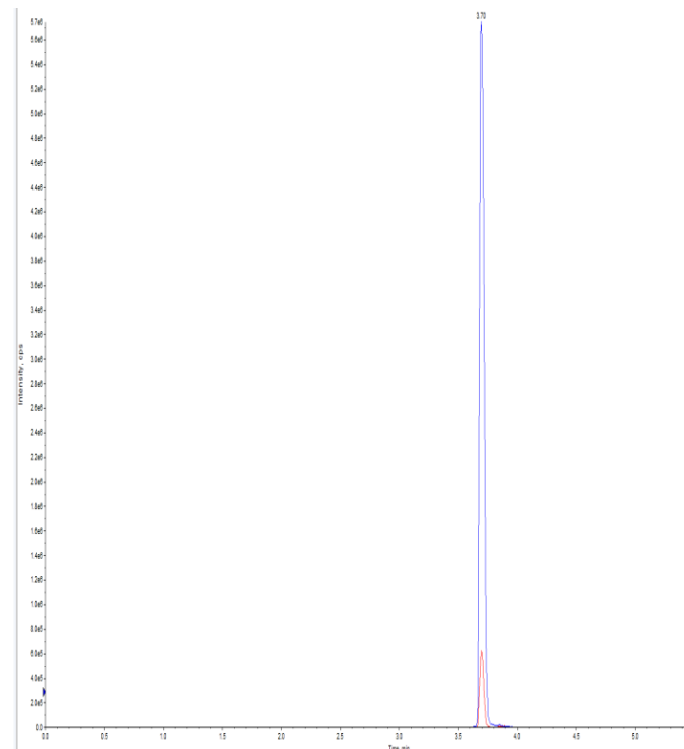


Fig. 2 : Calibrator in plasma	
R.T.	3.70 Tamoxifen 50 ng/ml

TAMOXIFEN/ENDOXIFENE IN PLASMA

(Reference Chromatograms for UHPLC systems)

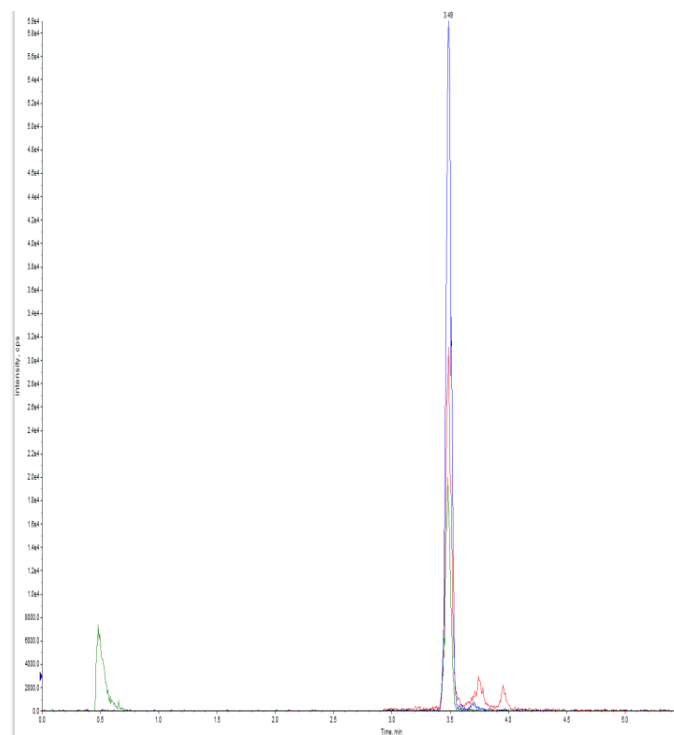


Fig. 3 :	Calibrator in plasma
R.T. 3.49	Endoxifene 30 ng/ml

TAMOXIFEN/ENDOXIFENE IN PLASMA

(Reference Chromatograms for HPLC systems)

