Extraction of Tamoxifen and Metabolites from Plasma Using ISOLUTE® SLE+

Introduction

This application note describes the extraction of Tamoxifen and metabolites from plasma using ISOLUTE SLE+ supported liquid extraction plates followed by LC-MS/MS analysis.

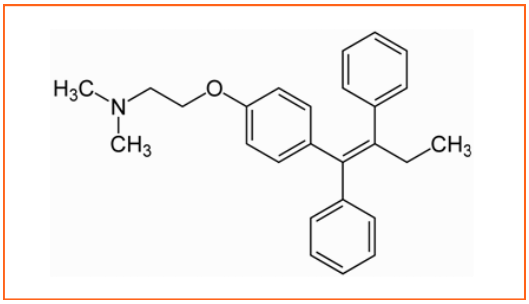

Figure 1. Structure of Tamoxifen

Tamoxifen is an important estrogen receptor antagonist primarily used in breast cancer therapy. More recently its action has also been shown to inhibit prostate cancer. It's mode of action also reduces the secondary effects linked to adsorption of androgen anabolic steroids. As a result the International Olympic Committee designated Tamoxifen as a 'banned substance'. This widespread use along with various forms of misuse has led to the necessity of rapid and reliable methods for its analysis and quantification. Here we demonstrate a rapid and reliable 96-well supported liquid extraction assay for the extraction of Tamoxifen and metabolites from plasma.

ISOLUTE SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation time.

Analytes

Tamoxifen, Endoxifen, 4-OH-Tamoxifen and Des-methyl-tamoxifen.

Procedure

ISOLUTE SLE + Configuration: ISOLUTE SLE+ 200 Supported Liquid Extraction Plate, part number 820-0200-P01

Sample pre-treatment: Dilute 100 µL of plasma 1:1 (v/v) with 0.5 M NH_4OH.

Sample loading: Load pre-treated plasma (200 µL) onto the plate and apply a pulse of vacuum to initiate flow. Leave the samples to absorb for 5 minutes.

ISOLUTE SLE+ procedure: Elution 1: Apply 500 µL chlorobutane, apply a short pulse of vacuum and wait for 5 minutes.
Elution 2: Apply a further 500 µL chlorobutane, apply a short pulse of vacuum and wait for 5 minutes.

Post Extraction: The eluate was evaporated to dryness and reconstituted in 500 µL of 0.1% formic acid 50:50 (v/v) H_2O/MeOH prior to analysis.

HPLC Conditions

Instrument: Waters Acquity UPLC (Waters Assoc., Milford, MA, USA).

Column: Acquity UPLC BEH C18 column (1.7µ, 50 x 2.1 mm id) (Waters Assoc., Milford, MA, USA).

Mobile Phase: 0.1% formic acid aq and 0.1% formic acid/acetonitrile at a flow rate of 0.6 mL/min.

Gradient: The gradient conditions are set to 75%, 0.1% (v/v) formic acid aq and 25% 0.1% (v/v) formic acid/acetonitrile increasing to 65% 0.1% (v/v) formic acid/ acetonitrile over 1.6 minutes. Initial starting conditions are resumed at 1.7 minutes.

Injection Volume: 5 µL.

Temperature: 35 °C.

Application Note

Mass Spectrometry Conditions

Instrument: Quattro Premier XE triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. *Table 1.* shows the positive ions acquired in the multiple reaction monitoring (MRM) mode.

Desolvation Temperature: 450 °C

Ion Source Temperature: 150 °C

Collision Gas Pressure: 3.46 x 10^{-3} mbar

Scan Function	Compound	Transition	Cone Voltage V	Collision Energy eV
1	Endoxifen	374.2 > 58.0	38	22
	4-OH-Tamoxifen	388.2 > 72.0	42	23
2	N-desmethyltamoxifen	358.2 > 58.0	37	21
	Tamoxifen	372.2 > 72.0	36	25

Table 1. MRM transitions for Tamoxifen and metabolites

Results

All results show recoveries above 95% with %RSDs below 10%. It should be noted that LOQ values can be improved if by increasing the injection volume or through concentrating the sample by using a smaller reconstitution volume.

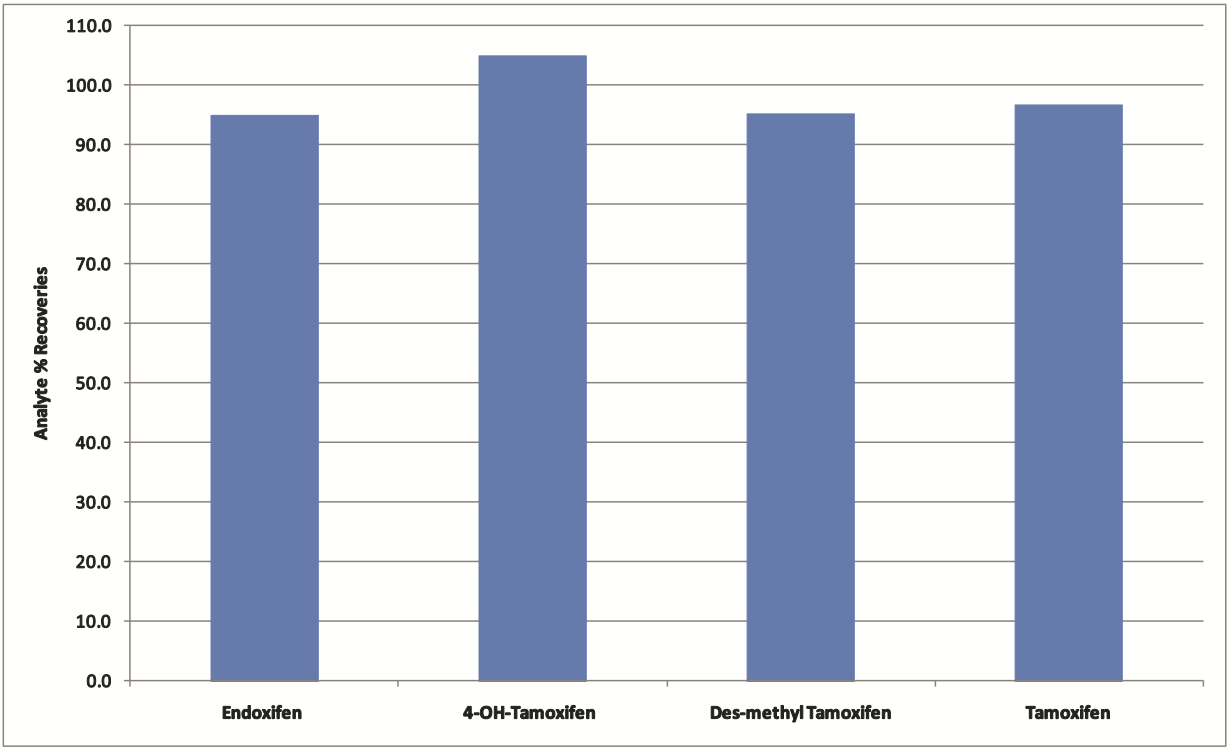

Figure 2. Tamoxifen and metabolites % recoveries from plasma

References

This application note is based on the poster 'Extraction of Tamoxifen and Metabolites from Urine and Plasma using Supported Liquid Extraction (SLE) prior to LC-MS/MS Analysis', L Williams et al, presented at SOFT, **Richmond, VA, October 18-22, 2010.**

Additional assistance

To assist with the blowing down and subsequent concentration of samples, the TurboVap 96 Concentration Workstation is a high speed concentrator designed to work with 96-well microplates and deep-well plates. It is an efficient alternative to the constant monitoring and long evaporation times that are characteristic of conventional techniques with the added bonus of unattended operation.

Ordering information

Part number	Description	Quantity
820-0200-P01	ISOLUTE SLE+ 200 Supported Liquid Extraction Plate	1
820-0400-P01	ISOLUTE SLE+ 400 Supported Liquid Extraction Plate	1
C103263	TurboVap 96 100/120 V, 50/60 Hz	1
C103264	TurboVap 96 220/240 V, 60/60 Hz	1

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